Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Isothiocyanate intermediates facilitate divergent synthesis of *N*-heterocycles for DNA-encoded libraries

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1. Materials and general methods

1.1 Materials

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used as received. Headpiece (**HP**, 5'-/5Phos/GAGTCA/iSp9/iUniAmM/i-Sp9/TGACTCCC-3', MW = 4937), Headpiece-primer(**HP-P**,5'/5Phos/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, and EtOH, depending on solubility and optimized reaction conditions. T4 DNA ligase and 10× ligation buffer (500 mM Tris pH 7.5, 500 mM NaCl, 100 mM $MgCl_2$, 100 mM DTT, and 25 mM ATP) were purchased from HitGen Inc.. Aqueous solutions, including NaCl (5 M), basic borate buffer (250 mM, sodium borate/boric acid, pH 9.4), and acetate buffer (3 M, sodium acetate/acetic acid, pH 5.2) were prepared in-house. Cestbon water was used in the reactions unless otherwise stated. All the gel images were captured by a Bio-Rad Chemidoc^{TM} 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.

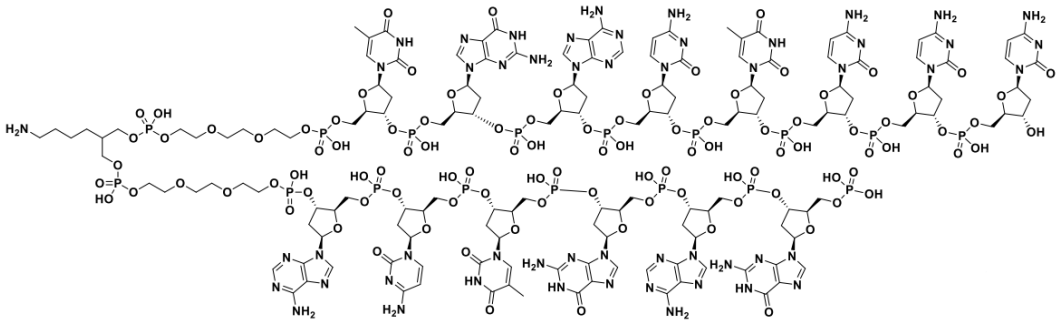


Figure S1. Structure of **HP**. (MW = 4937)

1.2 General methods for DNA analysis

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

Table G1. Analytical method for **4aa-4ka** and **4a'a-4f'a**

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

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

Table G2. Analytical method for **4ab**-**4aj**

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	15
12	0.3	25
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 G3. Analytical method for **6aa-6ka, 6a'a** and **6ab-6aj**

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	17
5.5	0.3	40
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 G4. Analytical method for **9aa-9ma** and **9ab-9am**

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 G5. Analytical method for co-injection experiment (**S1, S1', S2, 4ia, 6ia**)

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 G6. Analytical method for co-injection experiment (**S3, 9na**)

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	15
12	0.3	50
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 G7. Analytical method for DNA ligation analysis **(4ob, 6ob, 9ob**)

Time (min)	Flow (mL/min)	%B
0	0.3	3
1	0.3	12
2.5	0.3	18
4	0.3	20
6	0.3	22
9	0.3	30
10	0.3	85
11	0.3	85
12	0.3	3

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

Conversion calculation. The 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].

1.3 General methods for DNA conjugates purification

Ethanol precipitation. To an on-DNA reaction mixture was added 10% volume of NaCl solution (5 M) and 3 times volume of absolute cold ethanol. Alternatively, to a DNA ligation mixture was added 10% volume of acetate buffer (3 M, pH 5.2) and 3 times volume of absolute cold ethanol. After swirling and centrifuging, the solution was maintained at -80 °C for 2 h and then was centrifuged at 13500 rpm for 30 minutes at 4 °C by Eppendorf 5424R centrifuge. The supernatant was discarded, and the pellet was rinsed with 200 μL cold 75% ethanol. After centrifuging at 13500 rpm for 10 minutes at 4 °C, the supernatant was discarded again and the DNA pellet was dried by Speedvac (CV200, JM company, Beijing, China), which was equipped with cryotrap (JM86, JM company, Beijing, China). The recovered sample was dissolved in ddH_2O for subsequent experiments.

HPLC purification. Preparative reversed-phase high-performance liquid chromatography (RP-HPLC) for the DNA conjugate was performed on Waters 1575EF Series with the column (Eclipse-XDB C18, 5 μM, 9.4 × 250 mm). Fractions containing the product were combined and lyophilized.

Table G8. 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

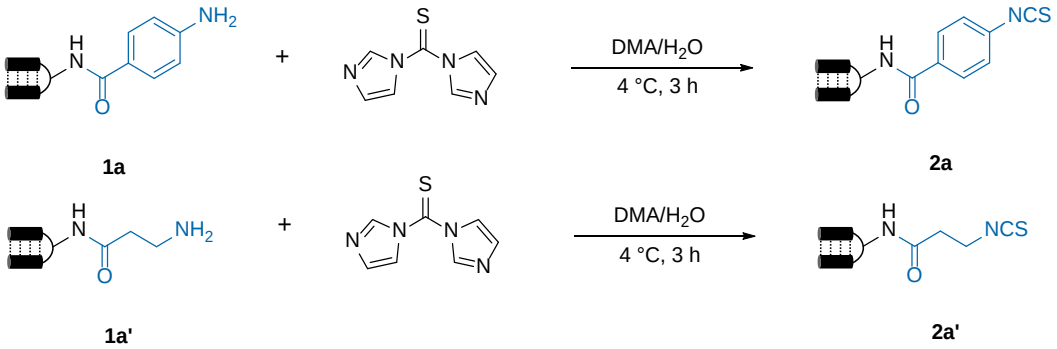
This reaction contained variably-derivatized **HP-P** starting material (10 nmol in H_2O, 1 equiv), code (12 nmol in H_2O, 1.2 equiv), 10× ligation buffer (4 μL), T4 DNA ligase (2 μL, 2000 units/μL) and nuclease-free water (to the total volume of 40 μL). The reaction was incubated at 20 °C overnight before performing gel analysis. The crude product was purified by ethanol precipitation and used for the next step.

1.5 General information for off-DNA synthesis

Off-DNA reactions were monitored by TLC. Analytical TLCs were performed with 0.25 mm silica gel HSGF254. The TLC plates were visualized by ultraviolet light. Flash chromatography was conducted on silica gel 60 (SiO_2, 100–200 mesh). All the new compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, and HRMS. ^{1}H and ^{13}C NMR spectra were recorded on an Agilent 400 MHz spectrometer. [DMSO-d_6] (H δ = 2.50; C δ = 40.0) was used as solvents. Multiplicity abbreviations are as follows: s = singlet, brs = broad singlet, d = doublet (dd = doublet of doublets), t = triplet, q = quartet, m = multiplet.

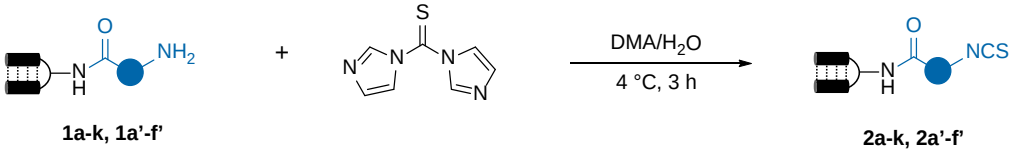
2. Preparation of DNA-conjugated isothiocyanates, scale-up reaction and stability experiment

2.1 Preparation of DNA-conjugated isothiocyanates



Reaction conditions: DNA-conjugated aniline **1a** or propanoic amine **1a'** were prepared according to procedures reported in our previous work[2]. To the solution of **1a** or **1a'** (1 nmol, 10 μL, 100 μM in H_2O, 1 equiv.) was added 1,1′-thiocarbonyldiimidazole (4 uL, 200 mM in DMA, 800 equiv.) and 6 uL DMA. The reaction mixture was vortexed, centrifuged, and incubated at 4 °C for 3 h. The products were obtained by ethanol precipitation and analyzed by UPLC-MS(Conversion: **1a** >95%, **1a'** >95%). Deconvoluted molecular mass of **2a**: calculated: 5098 Da; observed: 5098 Da. Deconvoluted molecular mass of **2a'**: calculated: 5050 Da; observed: 5050 Da.

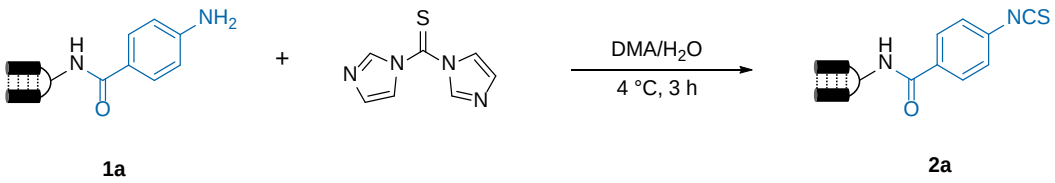
2.2 General procedure for on-DNA synthesis of isothiocyanates (2a-k, 2a'-f')



1a-k, 1a'-f' **2a-k, 2a'-f'**

Standard condition: DNA-conjugated amines **1a-k, 1a'-f'** (10 μL, 100 μM in H_2O, 1 nmol, 1 equiv.), 1,1′-thiocarbonyldiimidazole (4 uL, 200 mM in DMA, 800 equiv.) and 6 uL DMA were added sequentially. The reaction mixture was vortexed, centrifuged, and allowed to proceed at 4 °C for 3 h. After purification by ethanol precipitation, the crude product **2a-k, 1a'-f'** can be directly used for the next step without HPLC purification.

2.3 Scale-up reaction



1a **2a**

To the solution of DNA-conjugated aniline **1a** (30 μL, 1 mM in H_2O, 30 nmol,) was added 1,1′-thiocarbonyldiimidazole (20 μL, 600 mM in DMA) and 10 μL DMA. The reaction mixture was vortexed, centrifuged, and incubated at 4 °C for 3 h. The product was obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5098 Da; observed: 5098 Da.

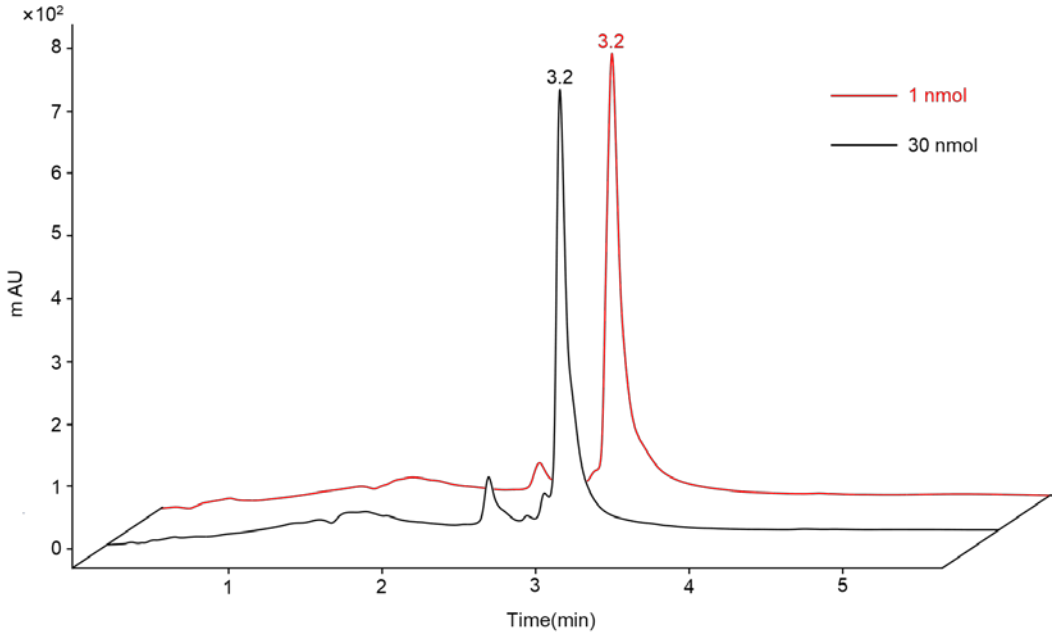


Figure S2. UPLC chromatogram of **2a** at 1 nmol and 30 nmol scales

2.4 Stability testing of DNA-conjugated isothiocyanates

The crude product **2a**, obtained through ethanol precipitation, was redissolved in water and placed at a concentration of 50 μM in a refrigerator at 4°C. The samples were analyzed using HPLC before and after one week. It was observed that DNA conjugate **2a** demonstrated stability in water at 4°C for up to one week without notable degradation.

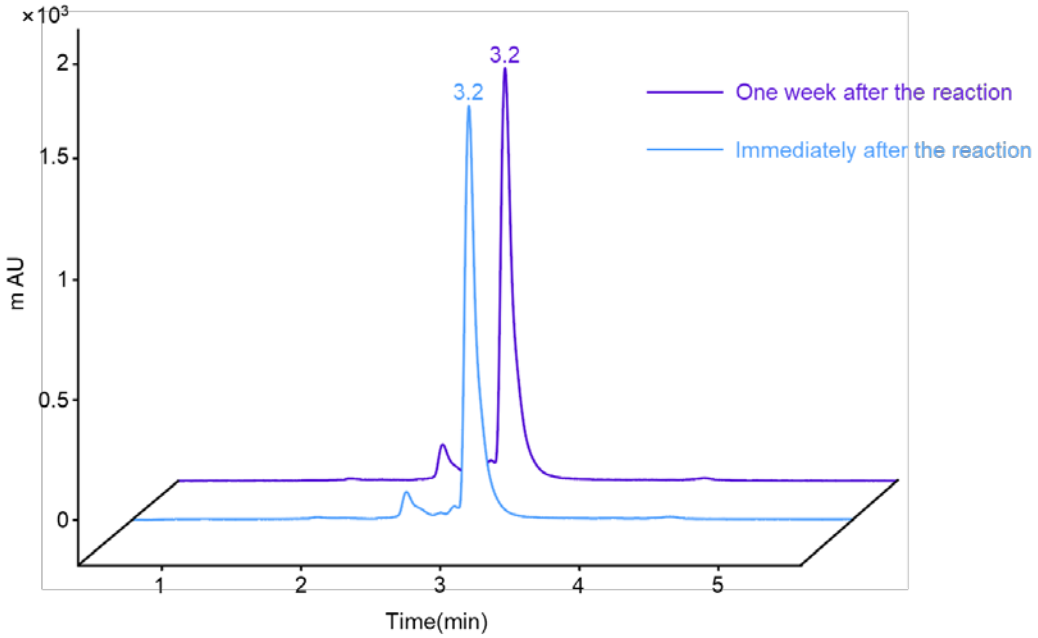


Figure S3. The UPLC chromatography showed that **2a** exhibit stability at 4 °C in water (deep blue curve).

To further evaluate the stability of DNA conjugates **2a** and **2a'** under different temperature and time conditions, stability tests were conducted. A quantity of 200 pmol each of **2a** and **2a'** was dissolved in water and PB buffer (pH 5.5), respectively to prepare 15 μM solutions. These solutions were then subjected to the conditions specified in **Table G9**, and their stability was assessed by measuring recovery rates.

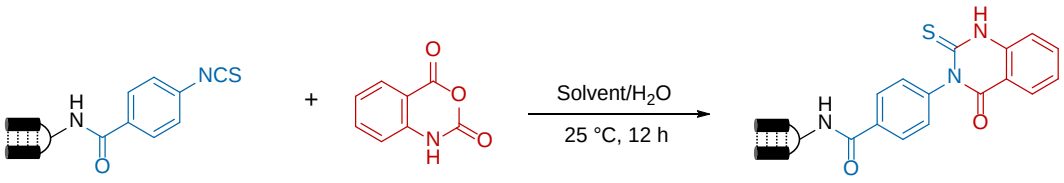
Table G9. Stability experiments across temp. & time conditions

Entry	Time(h)	Temp.(°C)	Stability[a]	
			2a	**2a'**
1	2/6/12/24	25	91%, 87%, 84%, 83%	98%, 98%, 96%, 95%
2	2/6/12/24	25[b]	94%, 93%, 93%, 88%	98%, 98%, 98%, 97%
3	2/6/12/24	60	47%, 15%, 7%, 0%	89%, 81%, 71%, 50%
4	2/6/12/24	60[b]	51%, 21%, 8%, 0%	89%, 79%, 68%, 50%
5	2/6/12/24	90	0%, 0%, 0%, 0%, 0%	39%, 19%, 0%, 0%, 0%
6	2/6/12/24	90[b]	0%, 0%, 0%, 0%, 0%	29%, 8%, 0%, 0%, 0%

[a]Calculated based on recovery rate. recovery rate (%) = UV peak area (recovered **2a** or **2a'**)/UV peak area (total DNA recovered); [b]PB buffer pH=5.5, final concentration: 125 mM

3. Reaction optimization and general procedure

3.1 Reaction optimization for on-DNA synthesis of 2-thioxo-quinazolinones



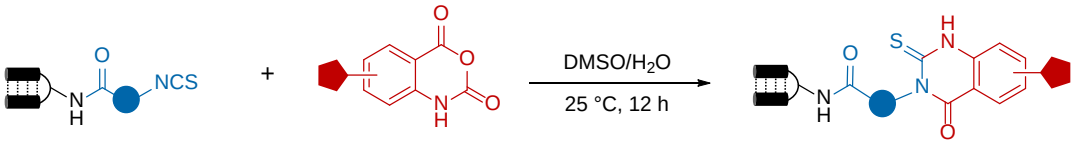
2a **3a** **4aa**

Table S1. Optimization of conditions

Entry	Co-solvent	Buffer	Conversion(%)[b]
1	ACN/H_2O	PB buffer (pH 8.0)	85%
2	DMA/H_2O	PB buffer (pH 8.0)	83%
3	DMSO/H_2O	PB buffer (pH 8.0)	87%
4	DMSO/H_2O	PB buffer (pH 4.2)	68%
5	DMSO/H_2O	MOPS buffer (pH 5.8)	66%
6	DMSO/H_2O	PB buffer (pH 5.5)	>90%
7	DMSO/H_2O	PB buffer (pH 7.4)	82%
8	DMSO/H_2O	PB buffer (pH 8.5)	88%
9	DMSO/H_2O	BBS buffer (pH 9.4)	84%
10	DMSO/H_2O	------	40%

Reaction conditions: To the solution of DNA conjugate **2a** (2 μL, 100 μM in H_2O, 0.2 nmol, 1 equiv.) was sequentially added isatoic anhydride **3a** (4 μL, 200 mM in corresponding solvent, 4000 equiv.), 16 μL corresponding solvent and 18 μL indicated buffer. The reaction mixture was vortexed, centrifuged, and proceeded at 25 °C for 12 h. [b]Conversions were determined by UPLC-MS.

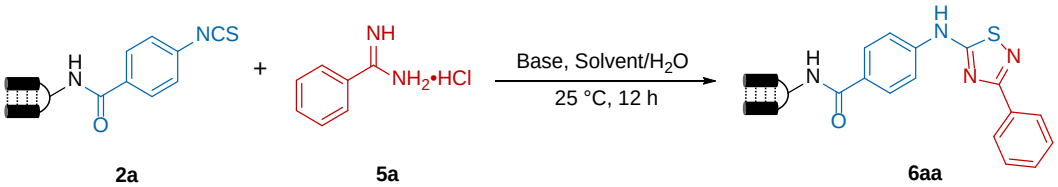
3.2 General procedure for on-DNA synthesis of 2-thioxo-quinazolinones (4aa-ka and 4ab-aj，4a'a-f'a)



2a-k, 2a'-f' **3a-j** **4aa-4ka, 4ab-4aj, 4a'a-f'a**

Standard condition: In each eppendorf tube, to the solution of DNA conjugate isothiocyanates (**2a-k, 2a'-f'**) (2 μL, 100 μM in PB buffer, 0.2 nmol, 1 equiv.) was added corresponding isatoic anhydrides (**3a-j**) (4 μL, 200 mM in DMSO, 4000 equiv.), 16 μL DMSO and 18 μL PB buffer (250 mM in H_2O, pH=5.5). The reaction mixture was vortexed, centrifuged, and proceeded at 25 °C for 12 h. The product was obtained by ethanol precipitation and analyzed by UPLC-MS.

3.3 Reaction optimization for on-DNA synthesis of 1,2,4-thiadiazoles



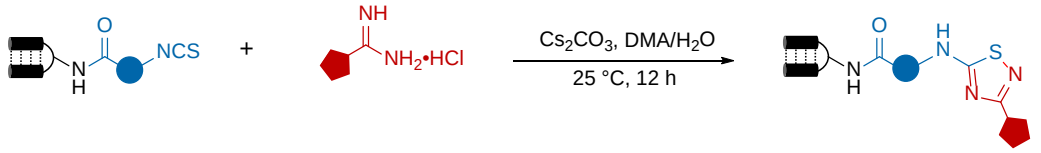
2a **5a** **6aa**

Table S2. Optimization of conditions

Entry	Co-solvent	Base	Conversion(%)[b]
1	THF/H_2O	K_2CO_3	0%
2	DMSO/H_2O	K_2CO_3	27%
3	DMA/H_2O	K_2CO_3	64%
4	ACN/H_2O	K_2CO_3	35%
5	DMA/H_2O	KOH	60%
6	DMA/H_2O	NaOAc	0%
7	DMA/H_2O	DIPEA	0%
8	DMA/H_2O	K_3PO_4	47%
9	DMA/H_2O	Cs_2CO_3	78%
10[c]	DMA/H_2O	Cs_2CO_3	29%
11[d]	DMA/H_2O	Cs_2CO_3	35%

Reaction conditions: To the solution of amidine hydrochloride **5a** (2 μL, 200 mM in organic solvent, 400 nmol, 2000 equiv.) was added indicated base (5 μL, 100 mM in H_2O, 500 nmol, 2500 equiv.) and 10 μL the same solvent. Then DNA conjugate **2a** (2 μL, 100 μM in H_2O, 200 pmol, 1 equiv.) was added to the solution and incubated at 25 °C for 12 h. [b]Conversions were determined by UPLC-MS. Add additional oxidants: [c]H_2O_2 (2 μL, 100 μM in H_2O, 1000 equiv.) [d]I_2 (2 μL, 100 μM in DMA, 1000 equiv.)

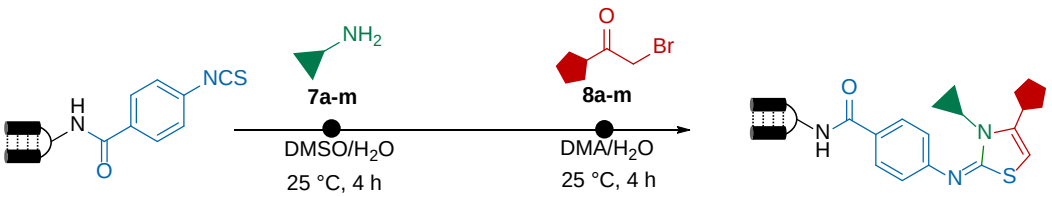
3.4 General procedure for on-DNA synthesis of 1,2,4-thiadiazoles (6aa-ka and 6ab-aj, 6aa')



2a-k, 2a' **5a-j** **6aa-ka, 6ab-aj, 6aa'**

Standard condition: In each eppendorf tube, to the solution of amidine hydrochlorides **5a-j** (2 μL, 200 mM in DMA, 400 nmol, 2000 equiv.) was added Cs_2CO_3 (5 μL, 100 mM in H_2O, 500 nmol, 2500 equiv.), and 10 μL DMA. Then DNA-conjugated isothiocyanates **2a-k, 2a'** (2 μL, 100 μM in H_2O, 200 pmol, 1 equiv.) was added to the mixture solution and incubated at 25 °C for 12 h. The product was obtained by ethanol precipitation and analyzed by UPLC-MS. Unless otherwise noted, on-DNA synthesis of 1,2,4-thiadiazoles described in the main article were synthesized under this standard condition.

3.5 General procedure for on-DNA synthesis of 2-Imino thiazolines (9aa-ma and 9ab-am)



2a **9aa-ma, 9ab-am**

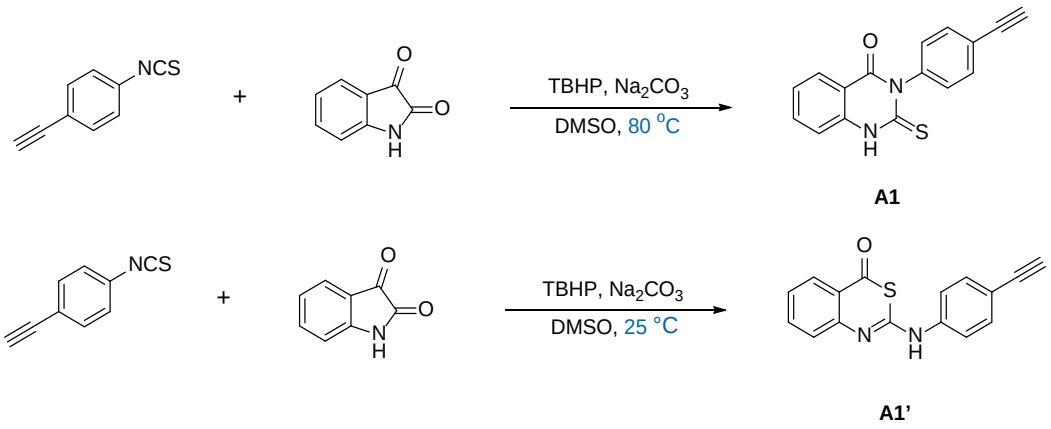
Step 1: In each eppendorf tube, to the solution of DNA conjugate **2a** (10 μL, 20 μM in H_2O, 0.2 nmol,1 equiv.) was added amines **7a-m** (2 μL, 300 mM in

DMSO, 600 nmol, 3000 equiv.) and 8 μL DMSO. The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 4 h. The thiourea product was obtained through ethanol precipitation, subsequently vacuum-dried, and redissolved in water for the next step.

Step 2: To the solution of collected thiourea (10 μL, 20 μM in H_2O, 0.2 nmol, 1 equiv.) was added α-bromoketones **8a-m** (2 μL, 300 mM in DMSO, 600 nmol, 3000 equiv.) and 8 μL DMSO. The entire mixture was vortexed, centrifuged, and incubated at 25 °C for 4 h. After purification by ethanol precipitation, the product was analyzed by UPLC-MS.

4. Structural validation

4.1 Off-DNA synthesis of 2-thioxo-quinazolinone A1 and benzothiazinone A1'

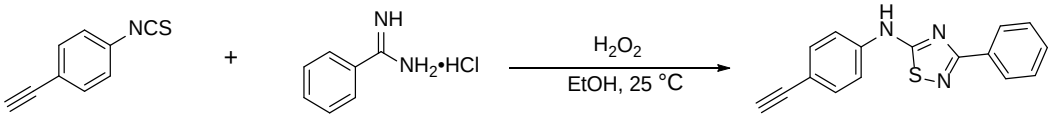


Authentic **A1** and **A1'** were prepared according to procedures reported in the literature[3].

3-(4-ethynylphenyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (A1) (Yellow powder, 63% yield): **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 13.10 (s, 1H), 7.99 – 7.92 (m, 1H), 7.83 – 7.76 (m, 1H), 7.61 – 7.56 (m, 2H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.39 – 7.28 (m, 3H), 4.30 (s, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 176.3, 160.2, 140.3, 140.2, 136.1, 132.8, 130.1, 127.9, 124.9, 122.0, 116.7, 116.3, 83.5, 81.9. **HRMS (TOF ESI):** m/z: $[M - H]^-$ Calcd for $C_{16}H_9N_2OS^-$ 277.0441; Found: 277.0447.

2-((4-ethynylphenyl)amino)-4H-benzo[d][1,3]thiazin-4-one(A1') (White solid, 72% yield): **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 10.39 (s, 1H), 7.99 (d, *J* = 7.7 Hz, 1H), 7.93 – 7.84 (m, 2H), 7.81 – 7.74 (m, 1H),7.55 (d, *J* = 8.0 Hz, 1H), 7.50 – 7.45 (m, 2H), 7.35 (t, *J* = 7.4 Hz, 1H), 4.11 (s, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 183.9, 151.6, 149.7, 140.4, 136.8, 132.9, 129.1, 125.6, 124.9, 120.3, 117.9, 116.6, 84.0, 80.6. HRMS (TOF ESI): m/z: $[M + H]^+$ Calcd for $C_{16}H_{11}N_2OS^+$ 279.0587; Found: 279.0634.

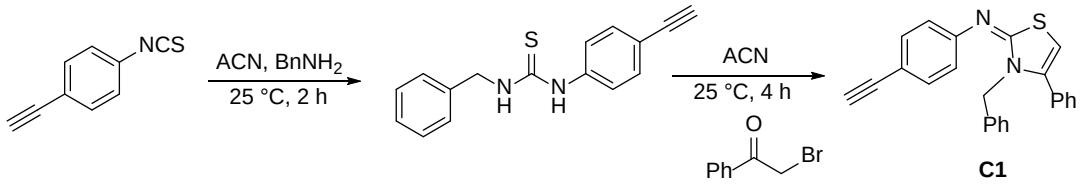
4.2 Off-DNA synthesis of 1,2,4-thiadiazole B1



B1

Authentic **B1** was prepared according to procedures reported in the literature[4]. ***N*-(4-ethynylphenyl)-3-phenyl-1,2,4-thiadiazol-5-amine (B1)** (White solid, 87%): **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 11.25 (s, 1H), 8.23 – 8.14 (m, 2H), 7.72 – 7.67 (m, 2H), 7.57 – 7.49 (m, 5H), 4.13 (s, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 179.1, 169.1, 140.6, 133.5, 133.1, 130.8, 129.3, 128.1, 118.0, 116.0, 84.1, 80.6. **HRMS (TOF ESI):** m/z: $[M - H]^-$ Calcd for $C_{16}H_{10}N_3S^-$ 276.0601; Found: 276.0601.

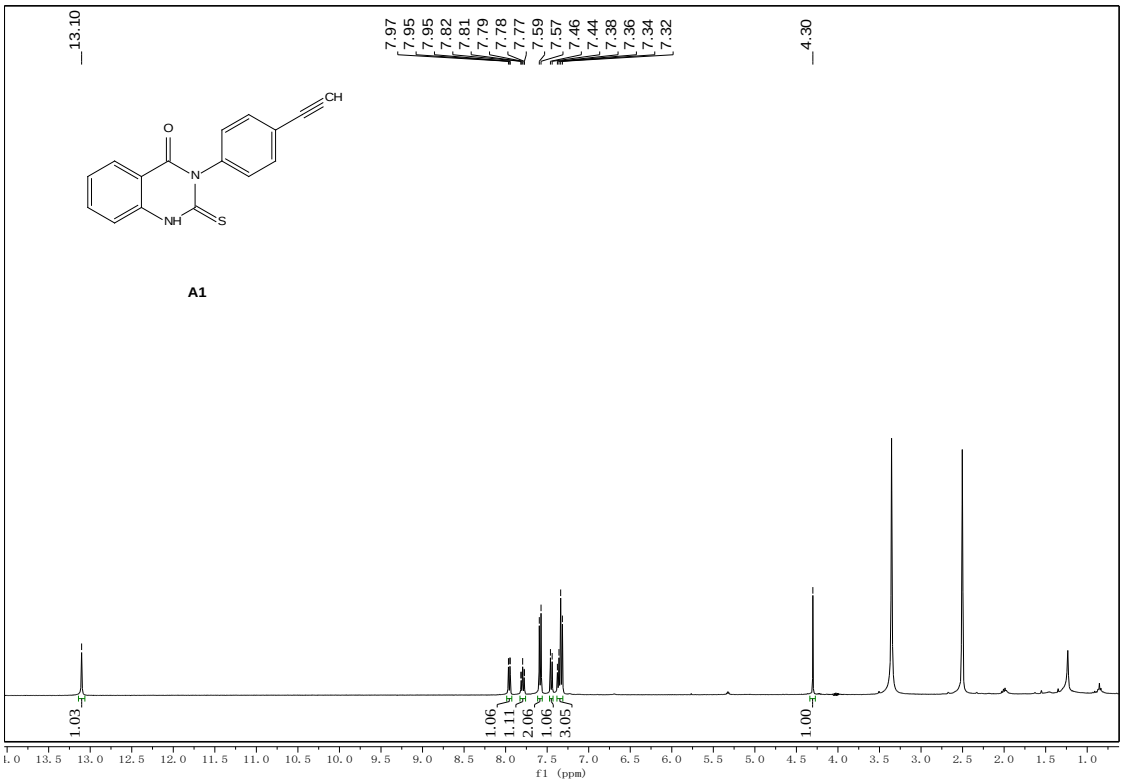
4.3 Off-DNA synthesis of 2-Imino thiazoline C1



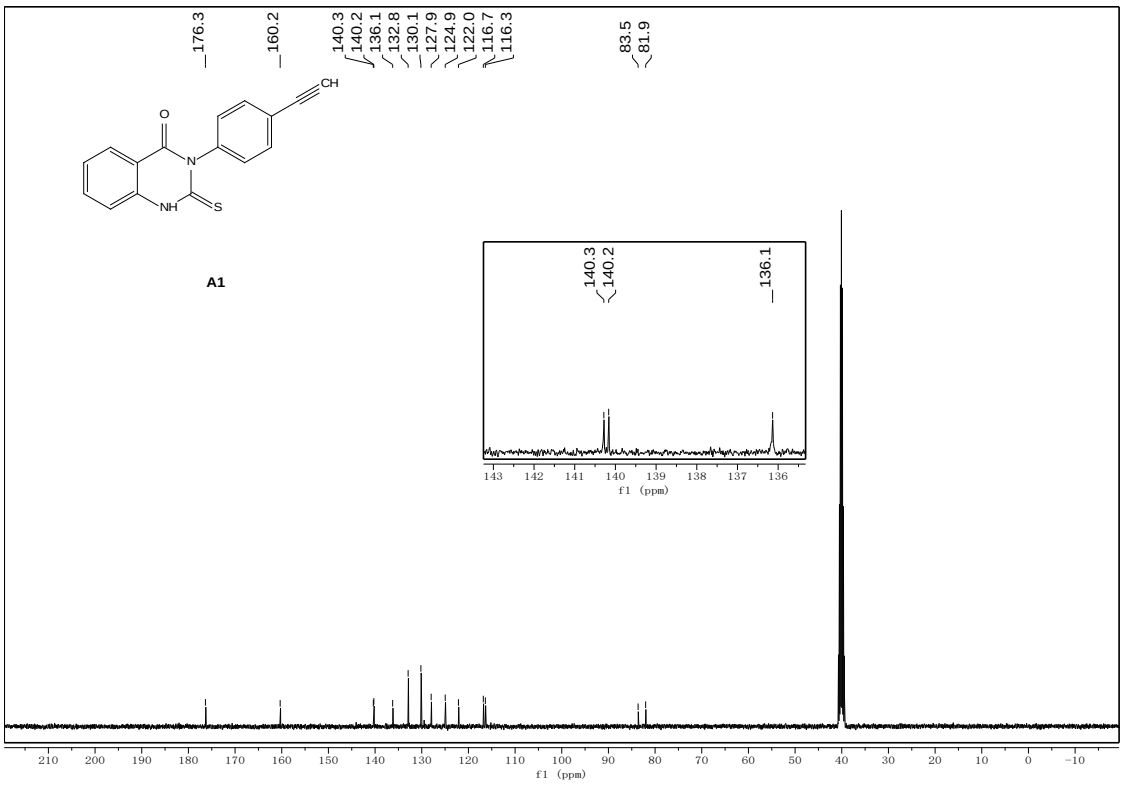
C1

Authentic **C1** was prepared according to procedures reported in the literature[5]. **(*E*)-3-benzyl-*N*-(4-ethynylphenyl)-4-phenylthiazol-2(3H)-imine (C1)** (Yellow solid, 76% yield): **^{1}H NMR (600 MHz, DMSO)** δ 7.45 – 7.38 (m, 5H), 7.36 – 7.31 (m, 2H), 7.28 – 7.23 (m, 2H), 7.23 – 7.18 (m, 1H), 7.01 (d, *J* = 7.5 Hz, 2H), 6.97 (d, *J* = 7.9 Hz, 2H), 6.35 (s, 1H), 5.07 (s, 2H), 4.05 (s, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 159.7, 151.8, 140.2, 137.6, 133.5, 131.3, 129.7, 129.2, 129.1, 128.8, 127.5, 126.9, 121.8, 115.9, 97.3, 84.4, 80.0, 48.3. **HRMS (TOF ESI):** m/z: $[M - H]^+$ Calcd for $C_{24}H_{19}N_2S^+$ 367.1263; Found: 367.1260.

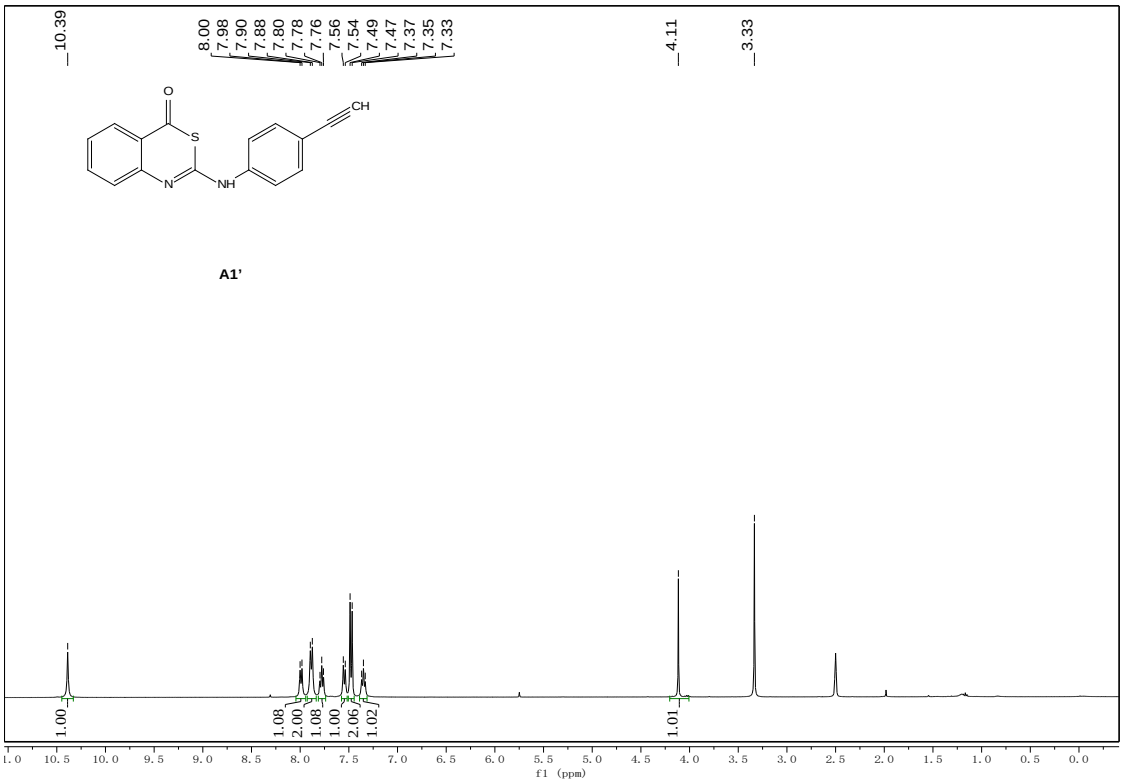
^{1}H NMR (400 MHz, DMSO-d_6) of **A1**



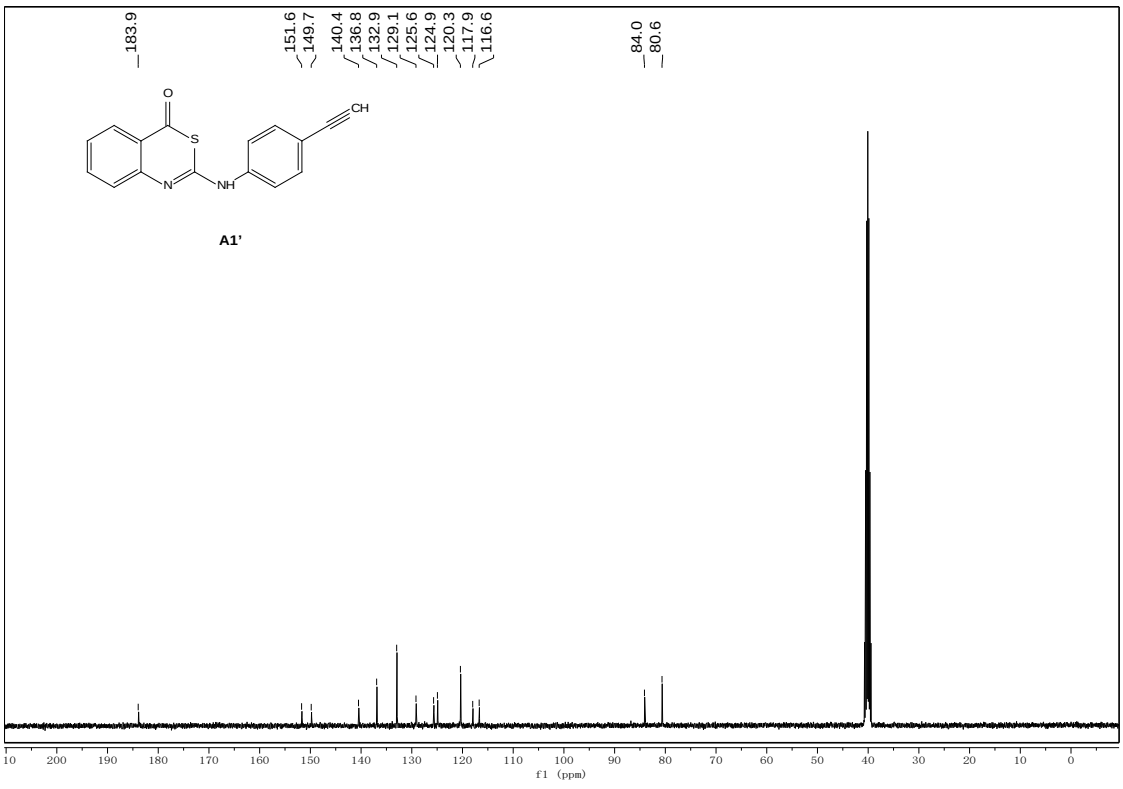
^{13}C NMR (101 MHz, DMSO-d_6) of **A1**



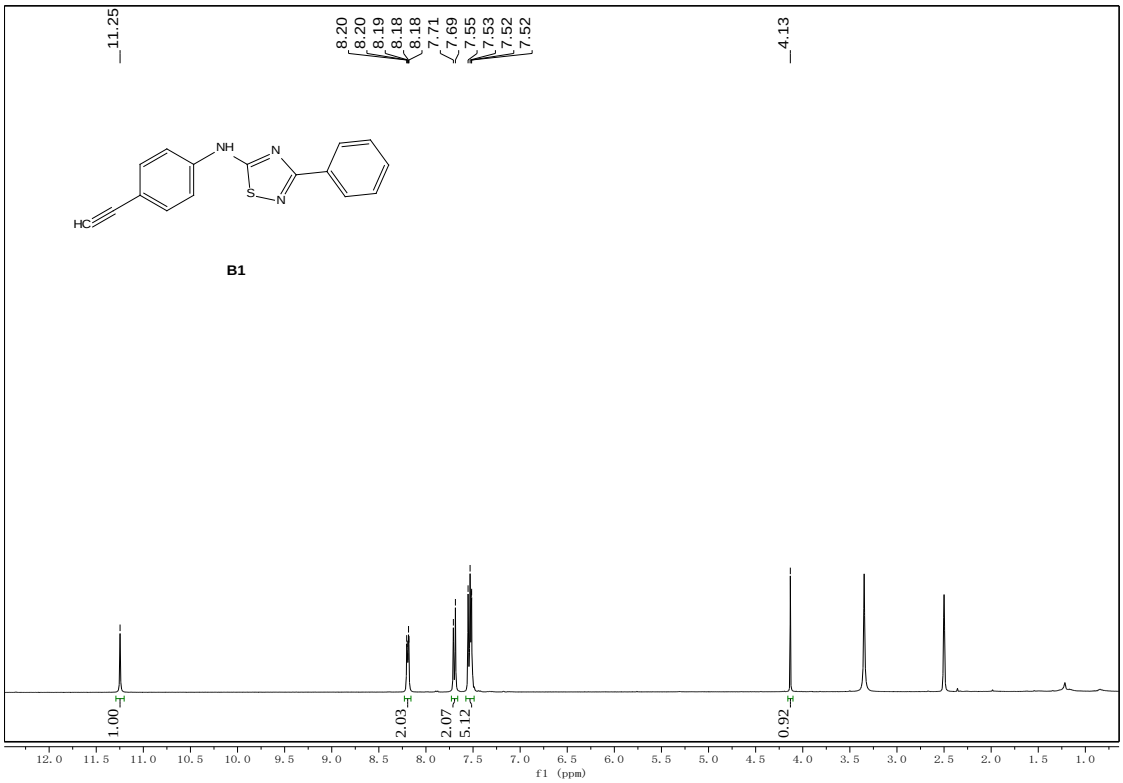
^{1}H NMR (400 MHz, DMSO-d_6) of **A1'**



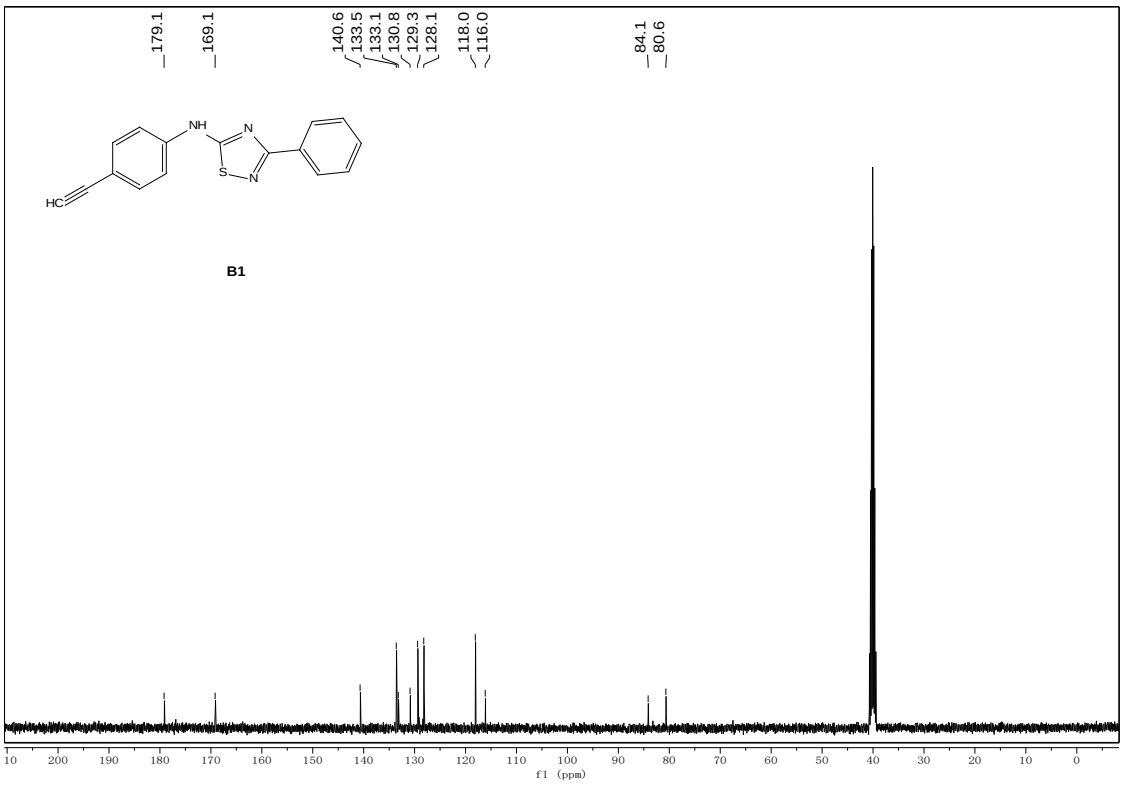
^{13}C NMR (101 MHz, DMSO-d_6) of **A1'**



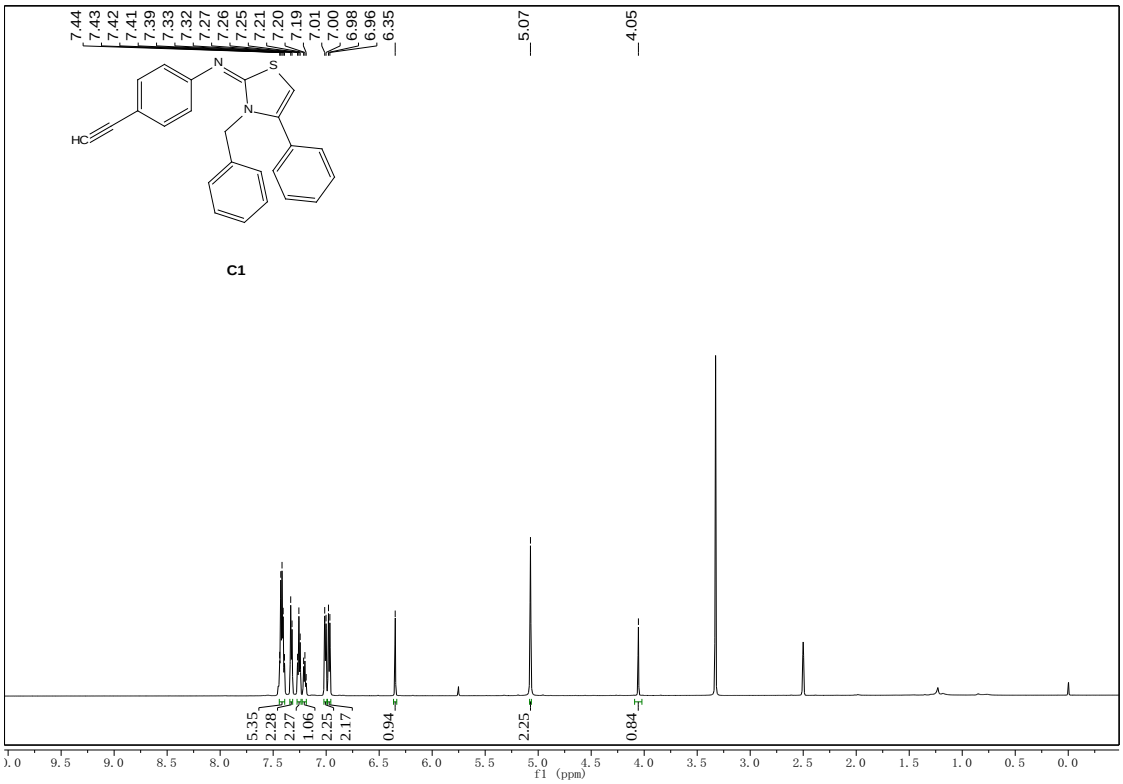
^{1}H NMR (400 MHz, DMSO-d_6) of **B1**



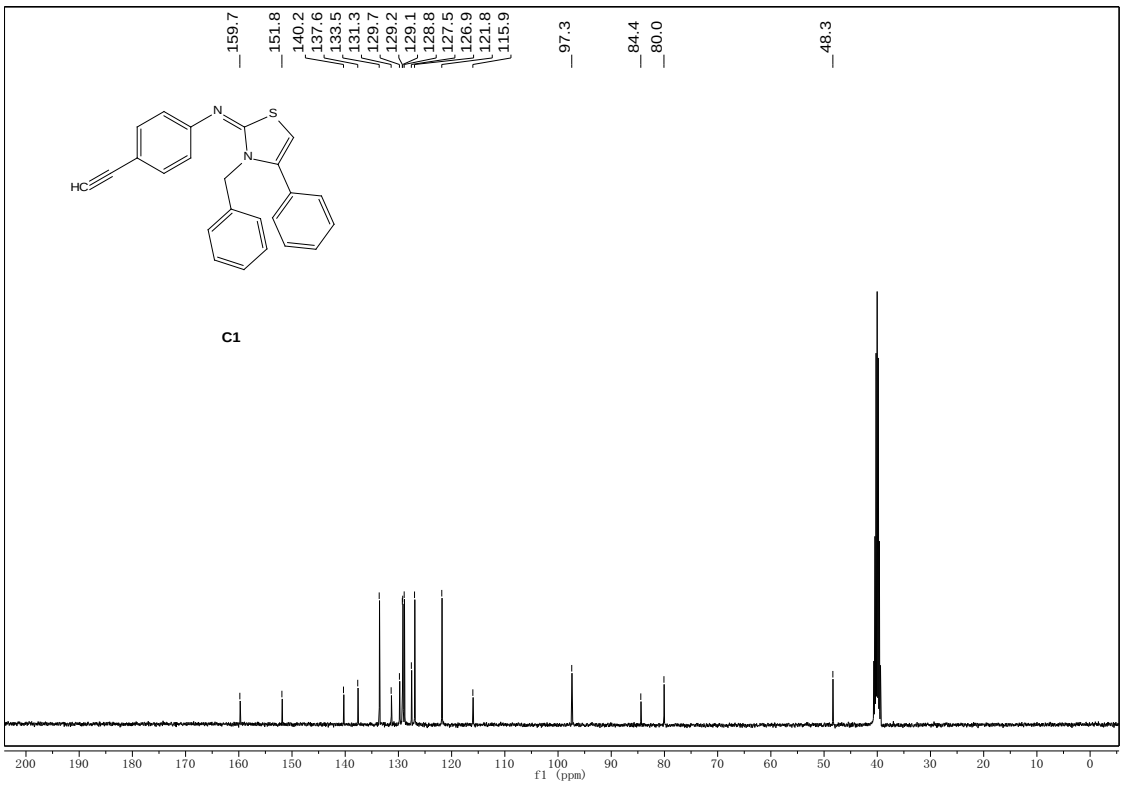
^{13}C NMR (101 MHz, DMSO-d_6) of **B1**



^{1}H NMR (600 MHz, DMSO-d_6) of **C1**



^{13}C NMR (101 MHz, DMSO-d_6) of **C1**

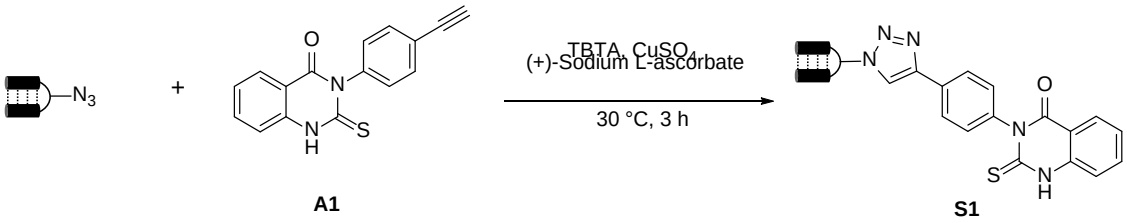


4.4 Co-injection experiment

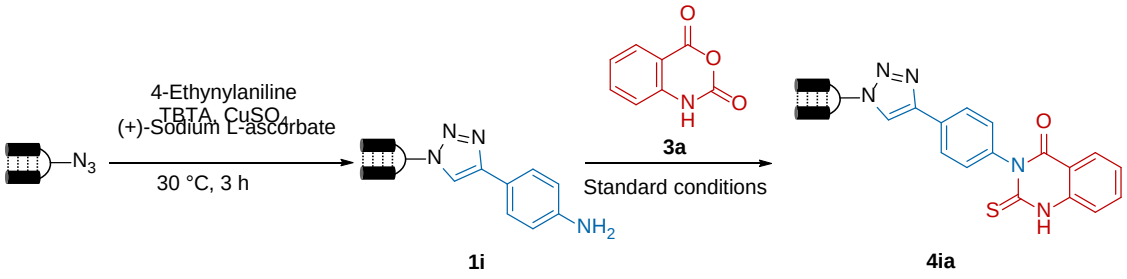
4.4.1 Co-injection experiment of DNA-conjugated 2-thioxo-quinazolinones

General procedure for click reaction：HP-N$_3$ (2 nmol) was dissolved in sodium borate buffer (16 μL, 250 mM, pH 9.4). Phenylacetylene **A1, A1', B1, C1** (4 μL, 40 mM in DMSO), TBTA (4 μL, 60 mM in DMSO), $CuSO_4 \cdot 5H_2O$ (4 μL, 50 mM in H_2O), L-sodium ascorbate (4 μL, 70 mM in H_2O) and 8 μL DMSO were added sequentially to the DNA solution. The reaction was allowed to proceed at 30 °C for 3 h. Then 30 equiv. of sodium diethyldithiocarbamic acid compared with $CuSO_4 \cdot 5H_2O$ were added to the mixture to scavenge the cupric, and the reaction mixture was stood at 25 °C for 30 minutes. The mixture was centrifuged at 25 °C for 10 min at 13,500 rpm. The resultant supernatant was collected and precipitated with ethanol. The reaction was analyzed by UPLC-MS.

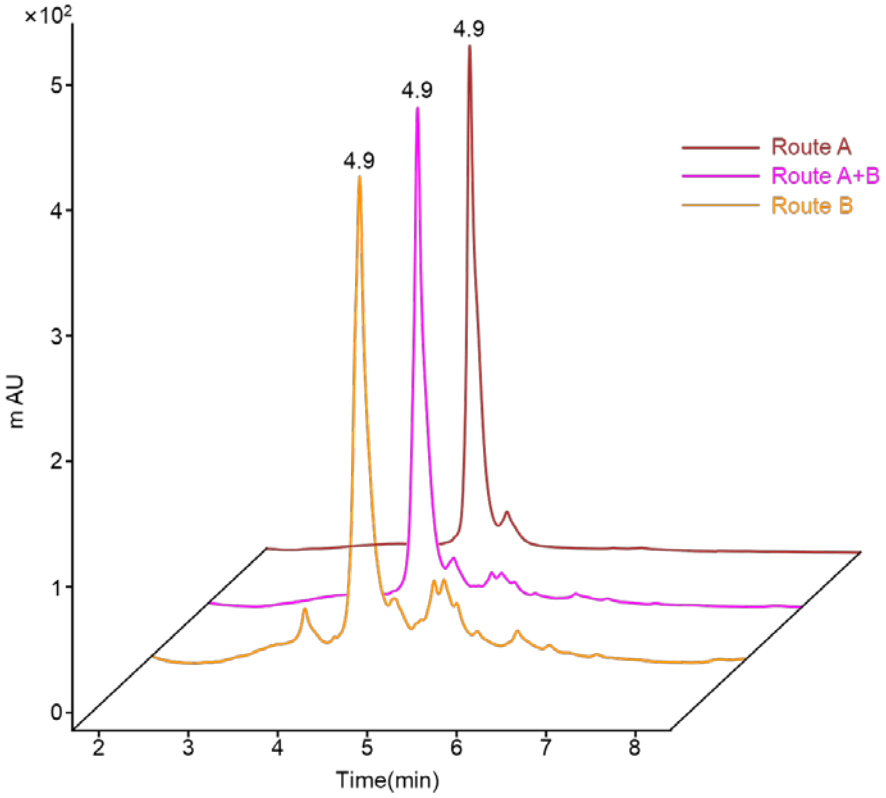
Route A



Route B

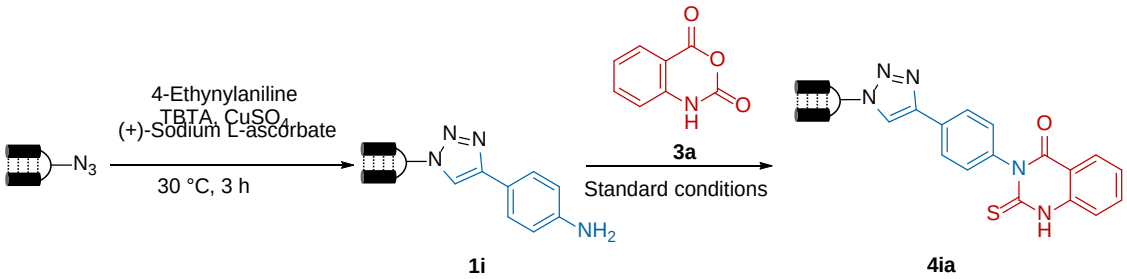


DNA conjugate **S1** and **1i** were prepared according to the general procedure for click reaction. DNA conjugate **4ia** was prepared according to the general procedure for synthesis of 2-thioxo-quinazolinones.

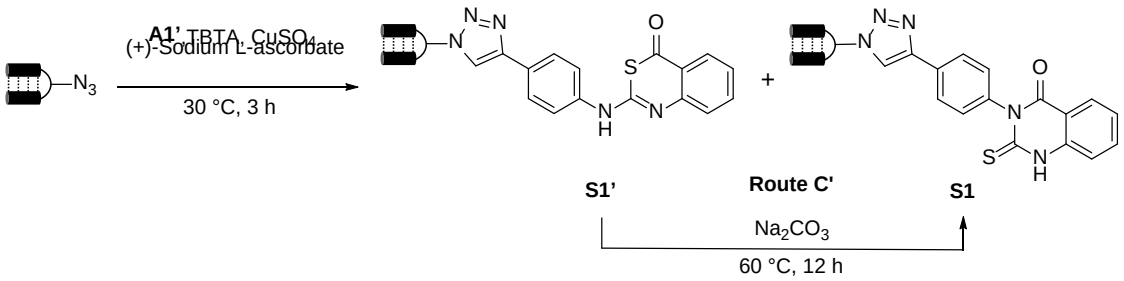


Scheme S1. Co-injection experiment of **S1** and **4ia** from two independent synthetic routes. HPLC chromatography showed that the peak from the co-injection (purple curve) had the same retention time as the other two peaks (**S1** from route A, brown curve; **4ia** from route B, yellow curve).

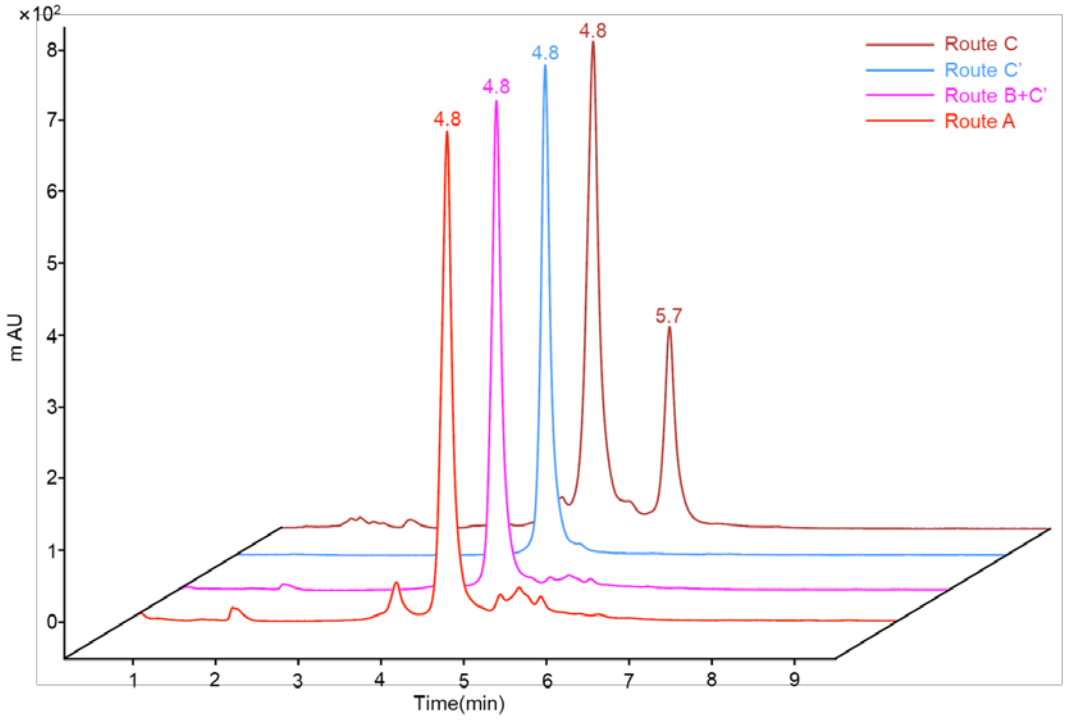
Route A



Route C



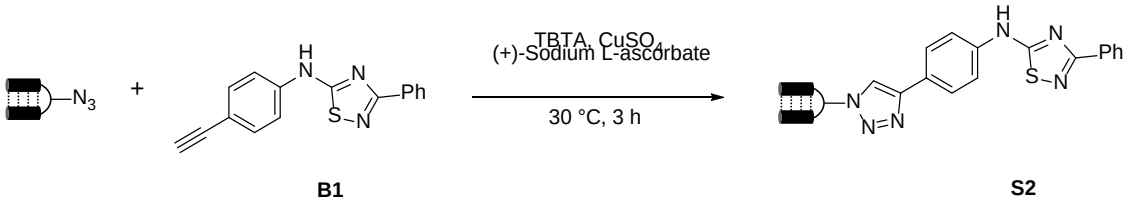
Upon linking benzothiazinone **A1'** with **HP-N_3** via a click reaction, we observed the chromatogram displaying two peaks of identical molecular weight (as shown in **Scheme S1'**, **Route C**, brown curve). According to the researched literature[3] it is known that **S1'** could be converted into the more stable product **S1** under the base condition. To achieve complete isomerization, we redissolved the collected isomer mixture in a sodium carbonate solution (40 mM in water) and heated it at 60 °C for 12 hours, resulting in almost complete isomerization (As shown in **Scheme S1', Route C'** blue curve). We then conducted co-injection experiments with the converted DNA product **S1** (As shown in **Scheme S1'**).



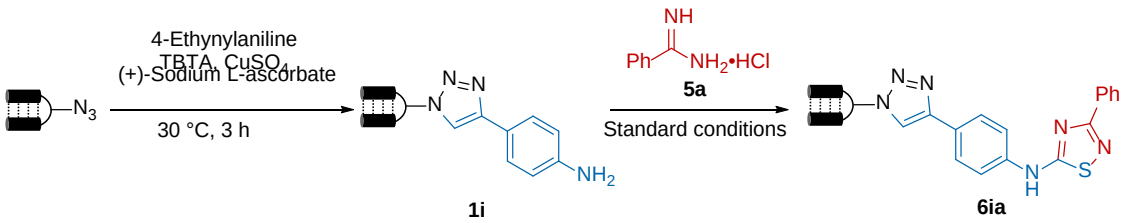
Scheme S1'. HPLC chromatography showed that the peak from the route B+C' (purple curve) had the same retention time as the other two peaks (**S1** from route C', blue curve; **4ia** from route A, red curve).

4.4.2 Co-injection experiment of DNA-conjugated 1,2,4-thiadiazoles

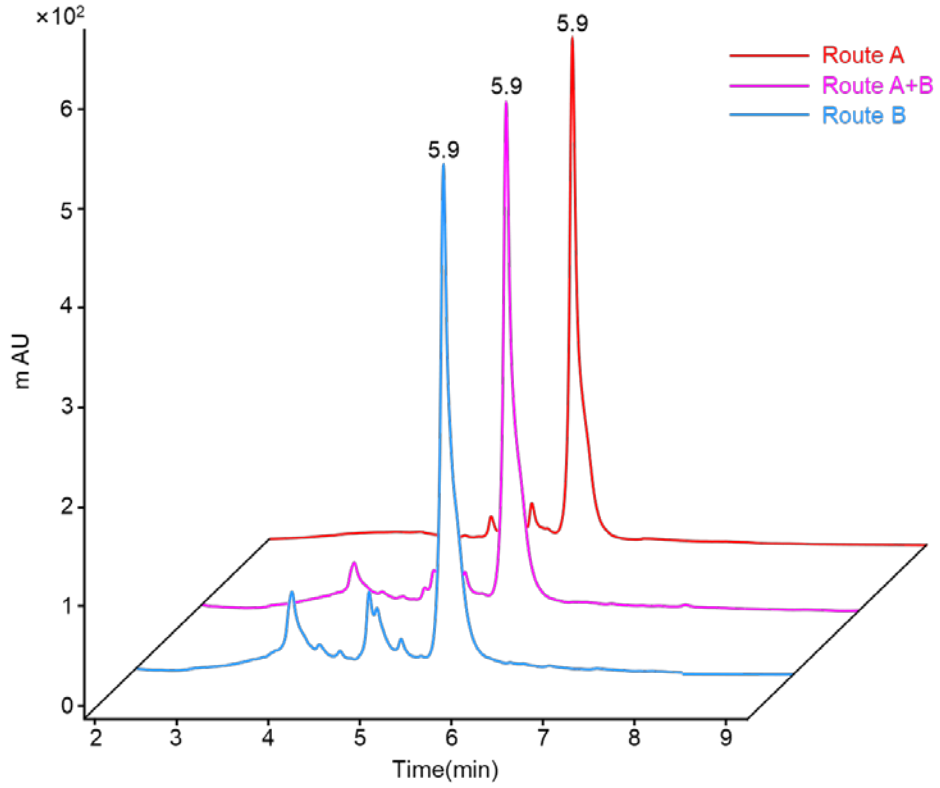
Route A



Route B



DNA conjugate **S2** and **1i** were prepared according to the general procedure for click reaction. DNA conjugate **6ia** was prepared according to the general procedure for synthesis of 1,2,4-thiadiazoles.

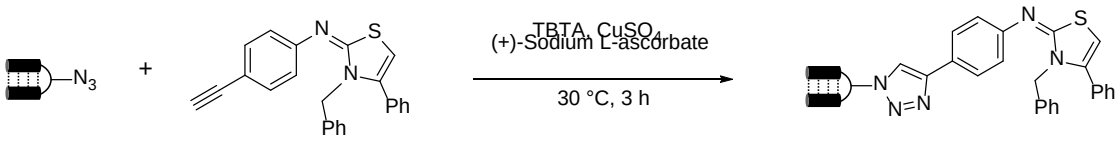


Scheme S2. Co-injection experiment of **S2** and **6ia** from two independent synthetic routes. HPLC chromatography showed that the peak from the co-

injection (purple curve) had the same retention time as the other two peaks (**S2** from route A, red curve; **6ia** from route B, blue curve).

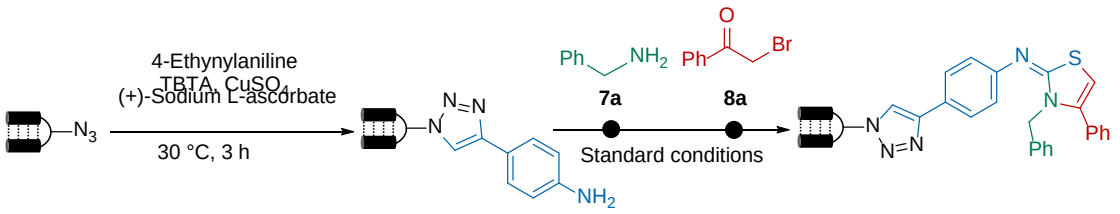
4.4.3 Co-injection experiment of DNA-conjugated 2-Imino thiazolines

Route A



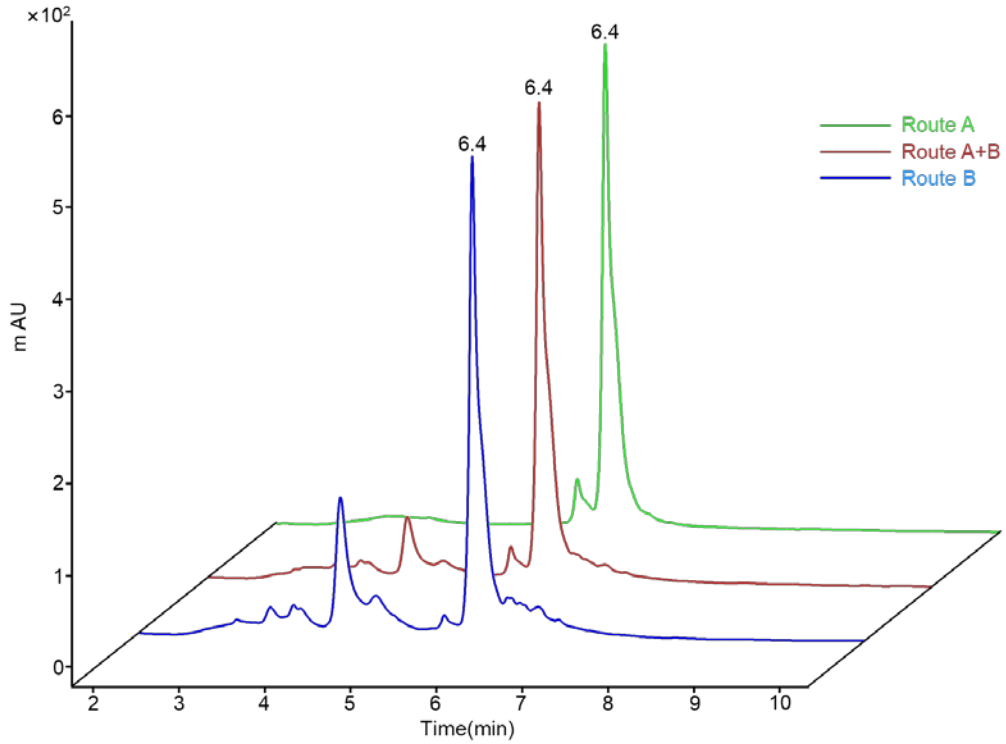
C1 **S3**

Route B



1i **9na**

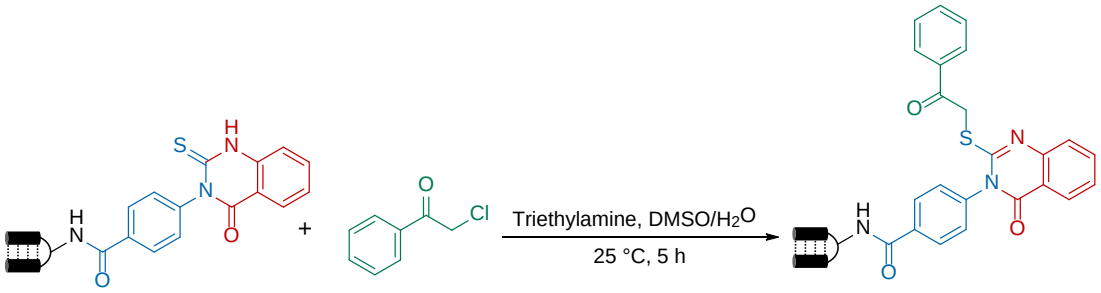
DNA conjugate **S3** and **1i** were prepared according to the general procedure for click reaction. DNA conjugate **9na** was prepared according to the general procedure for synthesis of 2-imino thiazolines.



Scheme S3. Co-injection experiment of **S3** and **9na** from two independent synthetic routes. HPLC chromatography showed that the peak from the co-injection (brown curve) had the same retention time as the other two peaks (**S3** from route A, green curve; **9na** from route B, blue curve).

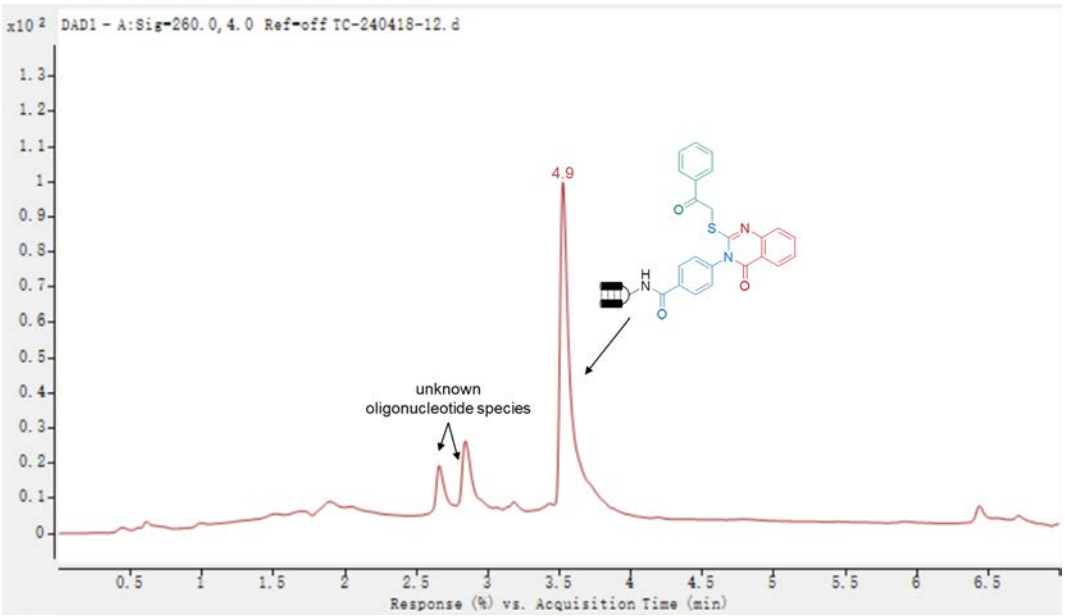
5. Diversification of 2-thioxoquinazolinone and preparation of mock library

5.1 Diversification of 2-thioxo-quinazolinones



4aa **5aa**

Reaction conditions: To the solution of DNA conjugate **4aa** (8 μL, 12.5 μM in H_2O, 200 pmol, 1 equiv.) was added 2-chloroacetophenone (2 μL, 200 mM in DMSO, 400 nmol, 2000 equiv.), Triethylamine (2 μL, 200 mM in H_2O, 400 nmol, 2000 equiv.), and 8 μL DMSO. The reaction mixture was vortexed, centrifuged, and incubated at 25 °C for 5 h. After purification by ethanol precipitation, the reaction was analyzed by UPLC-MS (Conversion: 72%). Deconvoluted molecular mass: calculated: 5335 Da; observed: 5335 Da.



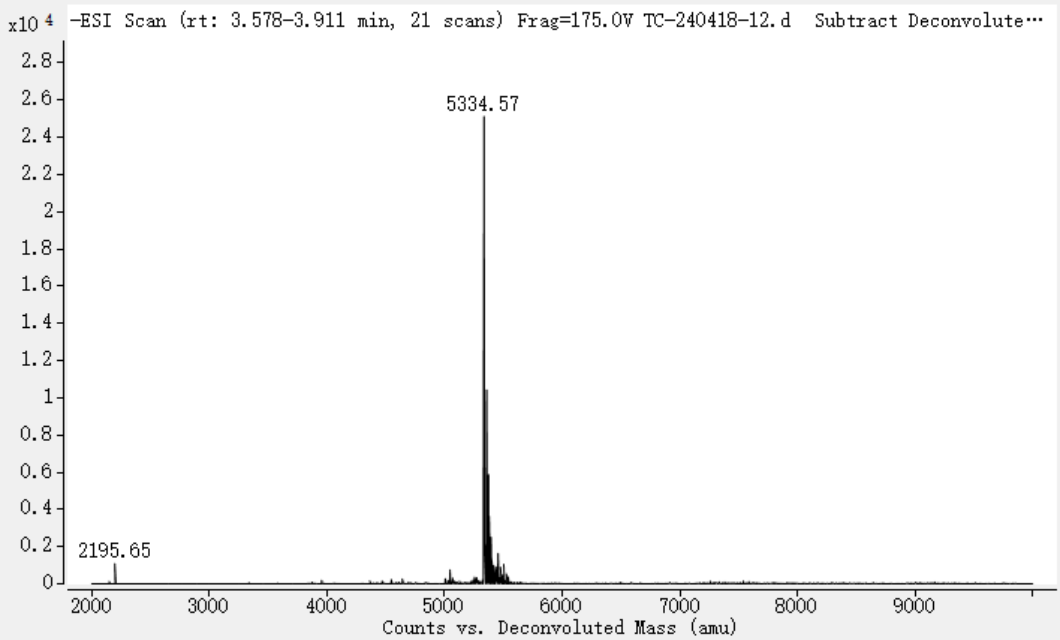
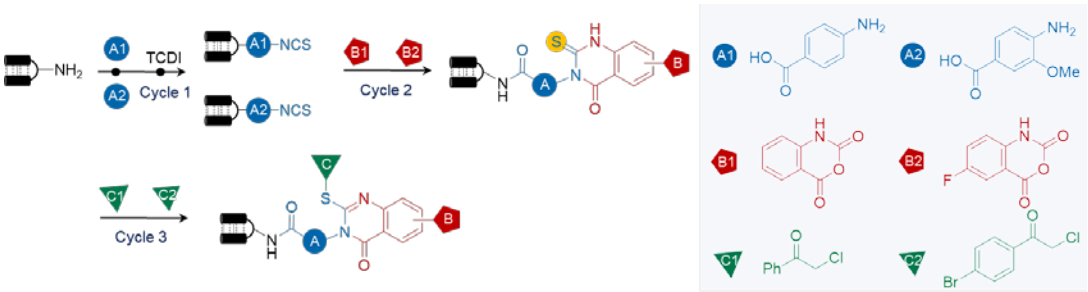


Figure S4. UPLC chromatogram and deconvoluted MS of **5aa**.

5.2 Synthesis of a three-dimensional mock library containing quinazolinone core



Number	Calc.(Da)	Obs.(Da)
A1-B1-C1	5335	5335
A1-B1-C2	5413	5414
A1-B2-C1	5353	5355
A1-B2-C2	5430	5433
A2-B1-C1	5365	5366
A2-B1-C2	5443	5445
A2-B2-C1	5383	5384
A2-B2-C2	5461	5463

Scheme S4. Three-dimensional mock pool containing quinazolinone core.

Headpiece 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

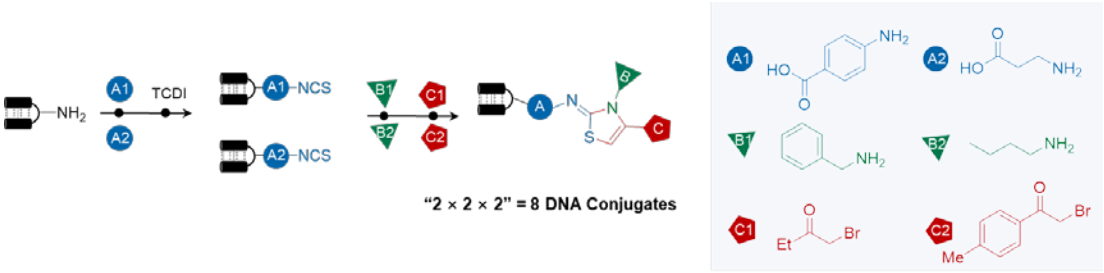
to pre-activate for 10 minutes at 25 °C, and then the mixture was transferred to **HP** solution (10 μL, 2 nmol). The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 2 h. After purification by ethanol precipitation, the reaction was analyzed by UPLC-MS.

The pooled cycle 1 product redissolved in 40 μL H_2O, followed by added TCDI (16 μL, 200 mM in DMA) and 24 μL DMA. The reaction mixture was vortexed, centrifuged, and incubated at 4 ºC for 3 h. After purification by ethanol precipitation the product divided into two tubes and separately added solution of the isatoic anhydrides **B1** and **B2** (10 μL, 200 mM in DMSO), DMSO (40 μL) and 50 μL PB buffer (250 mM in H_2O, pH=5.5). The reaction mixture was vortexed, centrifuged, and proceeded at 25 °C for 12 h.

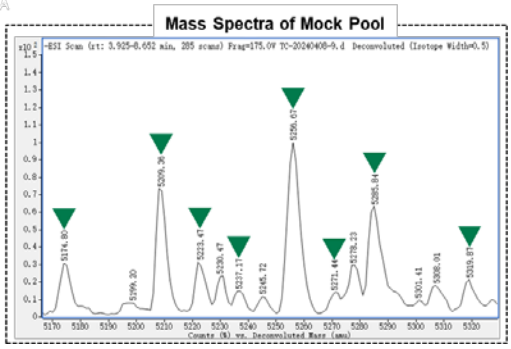
After purification by ethanol precipitation, the pooled cycle 2 product were divided into two tubes and separately dissolved in H_2O (16 μL) and DMSO (16 μL), followed by addition of the 2-chloroacetophenones **C1** and **C2** (4 μL, 200 mM in DMSO), Triethylamine (4 μL, 200 mM in H_2O). The reaction mixture was vortexed, centrifuged, and incubated at 25 °C for 5 h. After pooled and purification by ethanol precipitation, the reaction mixture was analyzed by UPLC-MS.

As illustrated in **Scheme S4**, we verified the two-round synthetic crude mixture by MS to find all the eight DNA conjugates.

5.3 Synthesis of a three-dimensional mock library containing 2-aminothiazole core



Number	Calc.(Da)	Obs.(Da)
A1-B1-C1	5257	5257
A1-B1-C2	5319	5320
A1-B2-C1	5223	5223
A1-B2-C2	5285	5286
A2-B1-C1	5209	5209
A2-B1-C2	5271	5271
A2-B2-C1	5175	5175
A2-B2-C2	5237	5237



Scheme S5. Three-dimensional mock pool containing 2-aminothiazole core.

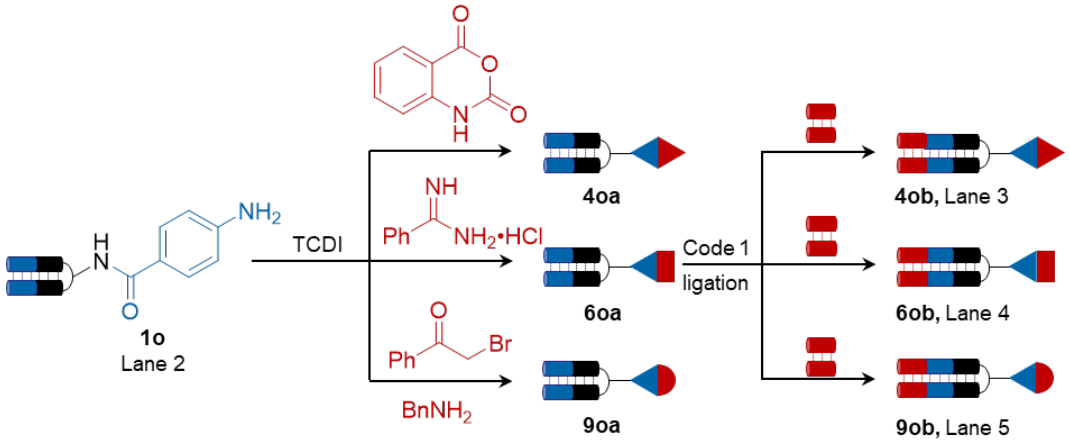
DNA conjugate **A1** and **A2** were prepared by the aforementioned amide coupling method and equivalent pooled (20 μL, 20 μM in H_2O).

The pooled cycle 1 product divided into two tubes (10 μL per tube), then added amines **B1** and **B2** (2 μL, 300 mM in DMSO) and 8 μL DMSO respectively. The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 4 h. After purification by ethanol precipitation, the product was pooled and analyzed by UPLC-MS.

The pooled cycle 2 product are divided into two tubes and separately dissolved in H_2O (20 μL), followed by added α-bromoketones **C1** and **C2** (4 μL, 300 mM in DMSO) and 16 μL DMSO. The entire mixture was vortexed, centrifuged, and incubated at 25 °C for 4 h. After pooled and purification by ethanol precipitation, the reaction mixture was analyzed by UPLC-MS.

As illustrated in **Scheme S5**, we verified the two-round synthetic crude mixture by MS to find all the eight DNA conjugates.

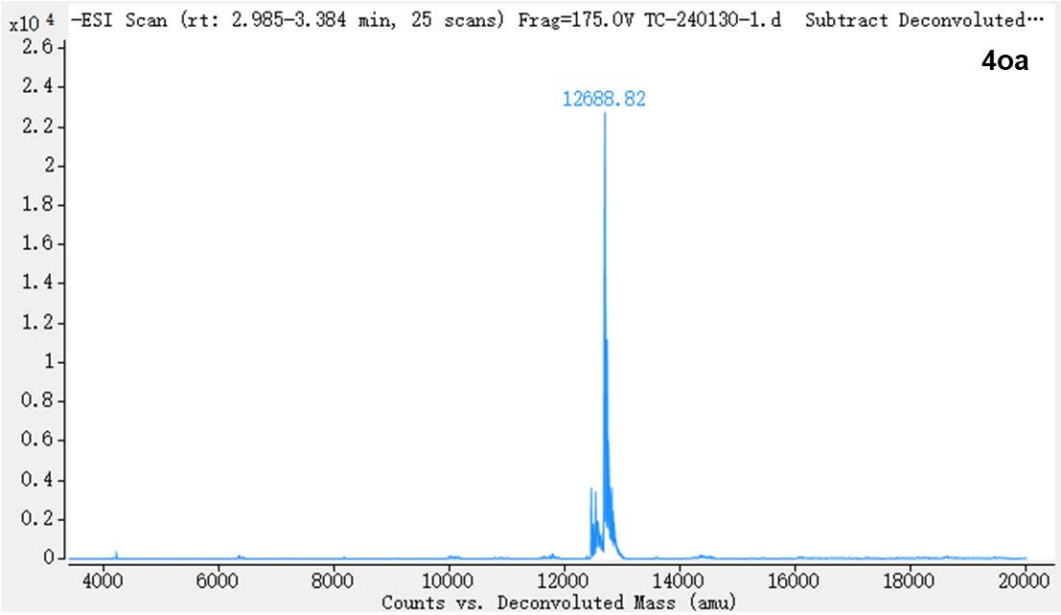
6. Enzymatic ligation

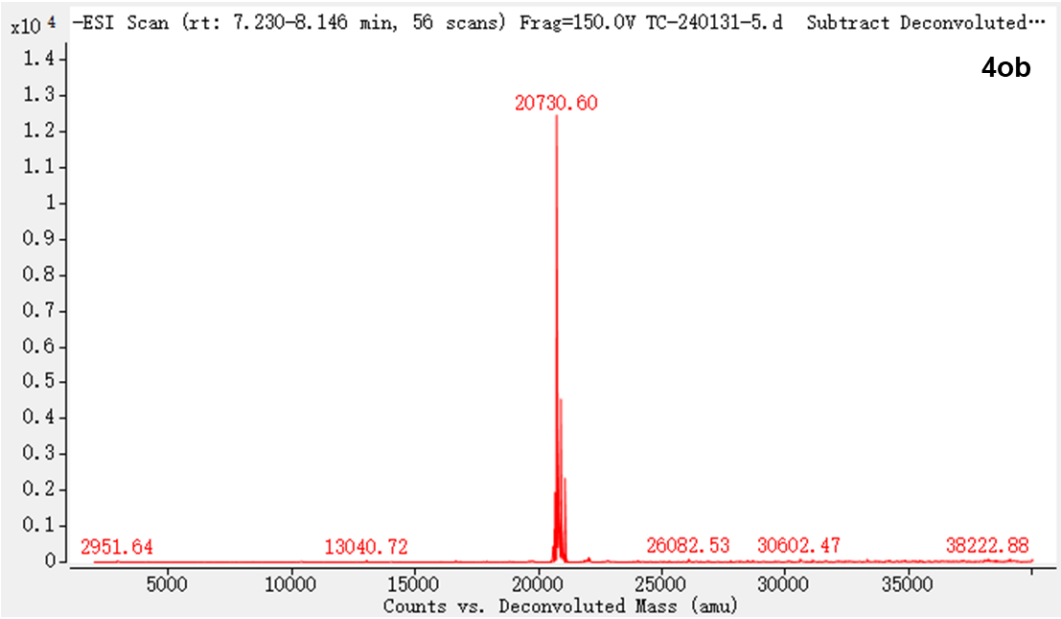


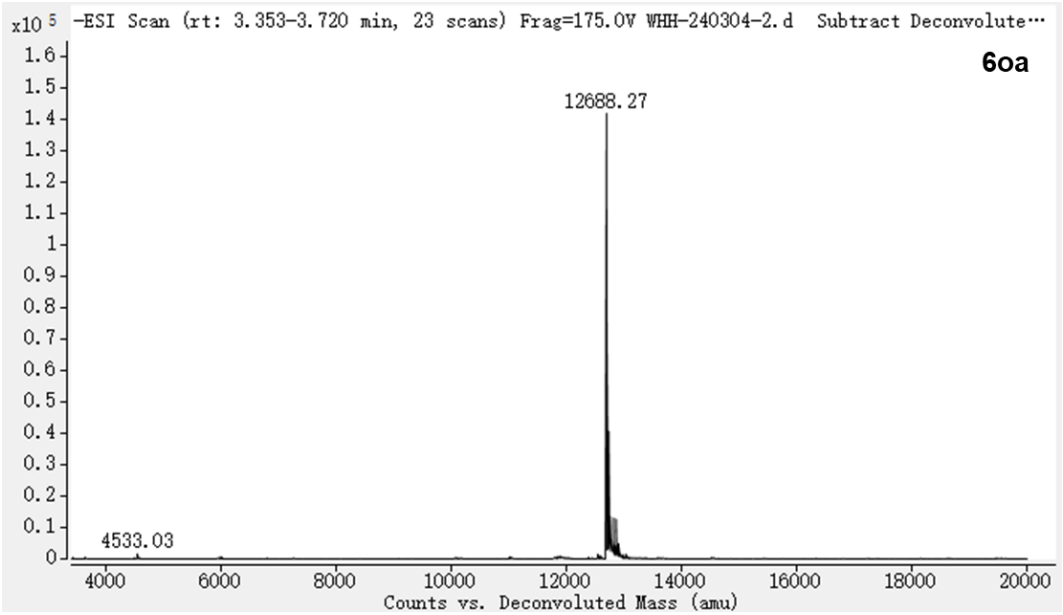
Scheme S5. Protocol of enzymatic ligation.

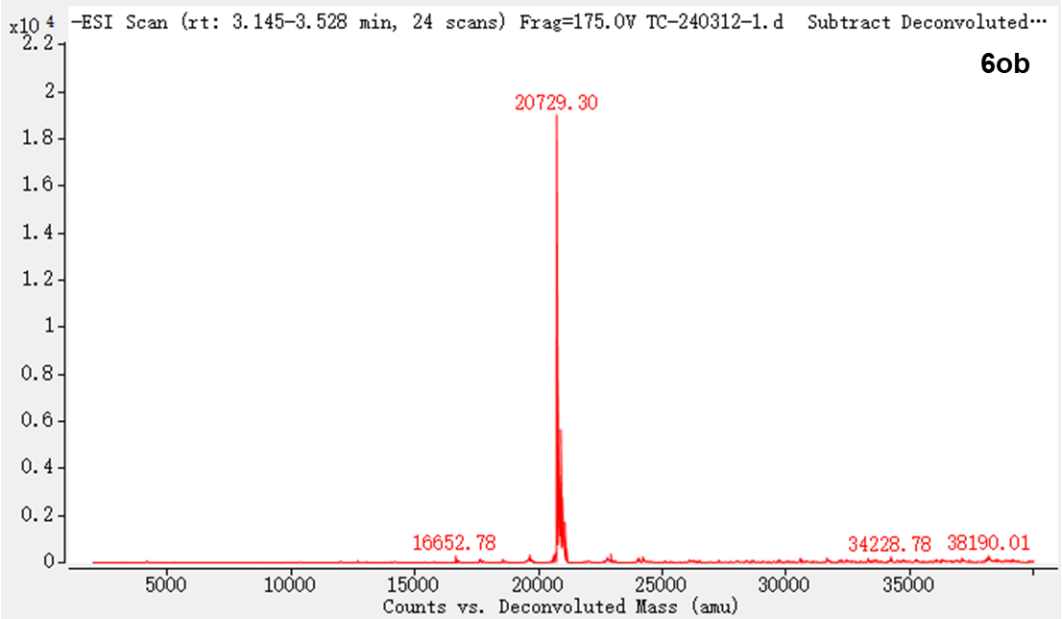
DNA conjugate **4oa, 6oa, 9oa** were prepared according to the general procedure (mentioned above). The products were obtained by ethanol precipitation and analyzed by UPLC-MS (Conversion of **4oa**: >90%; Conversion of **6oa**: 72%; Conversion of **9oa**: 84%).

Enzymatic ligation conditions: DNA conjugate **4oa, 6oa, 9oa** (200 pmol), code 13 nt (220 pmol, 1.1 equiv.), and 10× ligation buffer (2 μL) were added into a 0.6 mL tube and mixed by vortex. Then, T4 DNA ligase (2 μL, 350 units/μL) was added and mixed gently. The reaction mixture was vortexed, centrifuged, and incubated at 20 °C for 3 h. After ligation confirmation by UPLC-MS analysis, the reaction system was denatured by incubating at 95 °C in a dry bath for 10 min, and the ligation product was isolated by ethanol precipitation. The resulting pellets were vacuum-dried and dissolved in nuclease-free water.


-ESI Scan (rt: 2.985-3.384 min, 25 scans) Frag=175.0V TC-240130-1.d Subtract Deconvoluted…
4oa
12688.82
Counts vs. Deconvoluted Mass (amu)


-ESI Scan (rt: 7.230-8.146 min, 56 scans) Frag=150.0V TC-240131-5.d Subtract Deconvoluted…
4ob
20730.60
2951.64
13040.72
26082.53
30602.47
38222.88
Counts vs. Deconvoluted Mass (amu)


-ESI Scan (rt: 3.353-3.720 min, 23 scans) Frag=175.0V WHH-240304-2.d Subtract Deconvolute…
x10 5
6oa
12688.27
4533.03
Counts vs. Deconvoluted Mass (amu)


-ESI Scan (rt: 3.145-3.528 min, 24 scans) Frag=175.0V TC-240312-1.d Subtract Deconvoluted…
x10 4
6ob
20729.30
16652.78
34228.78
38190.01
Counts vs. Deconvoluted Mass (amu)

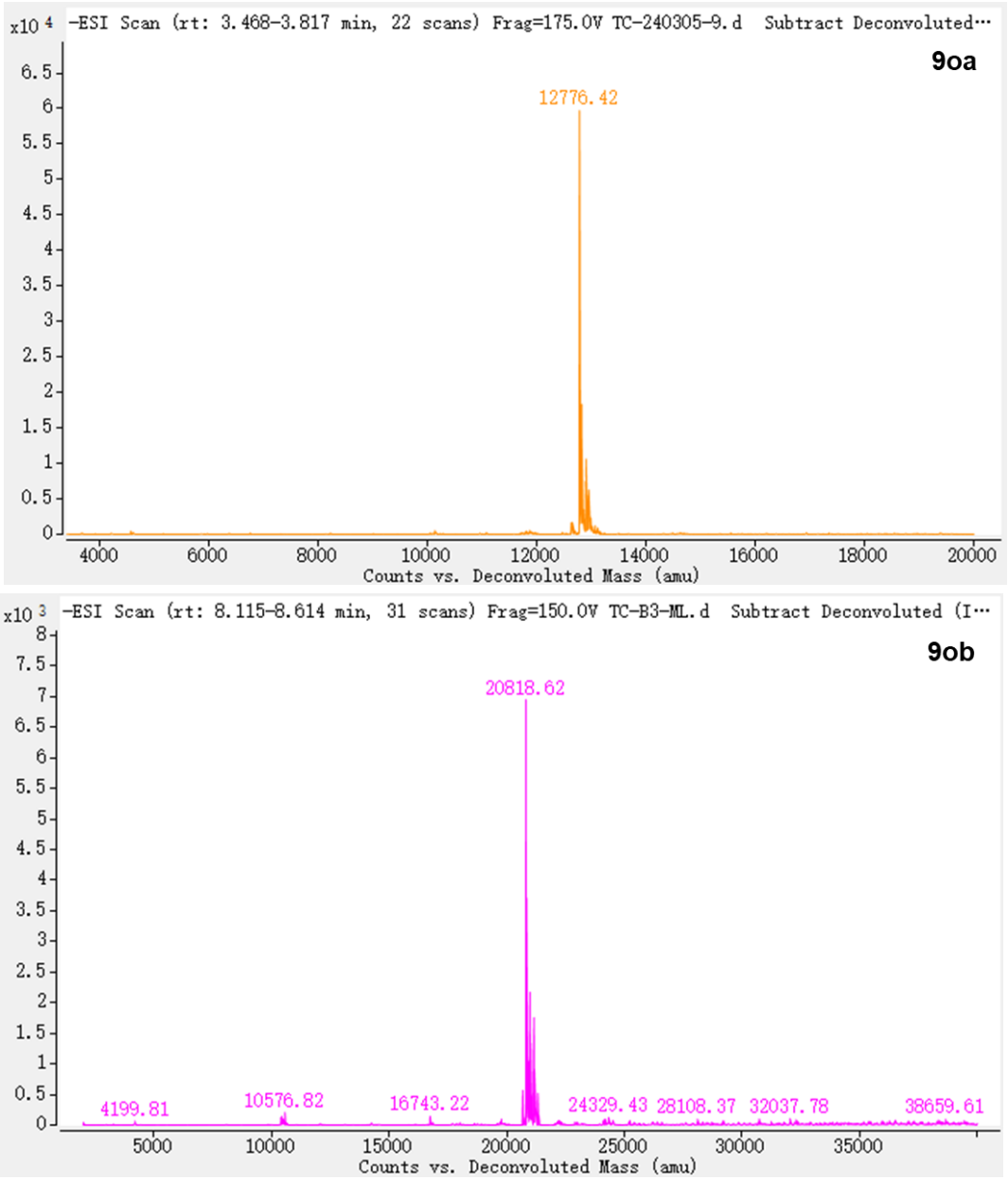


Figure S5. Deconvoluted MS of **4oa, 4ob, 6oa, 6ob, 9oa, 9ob**.

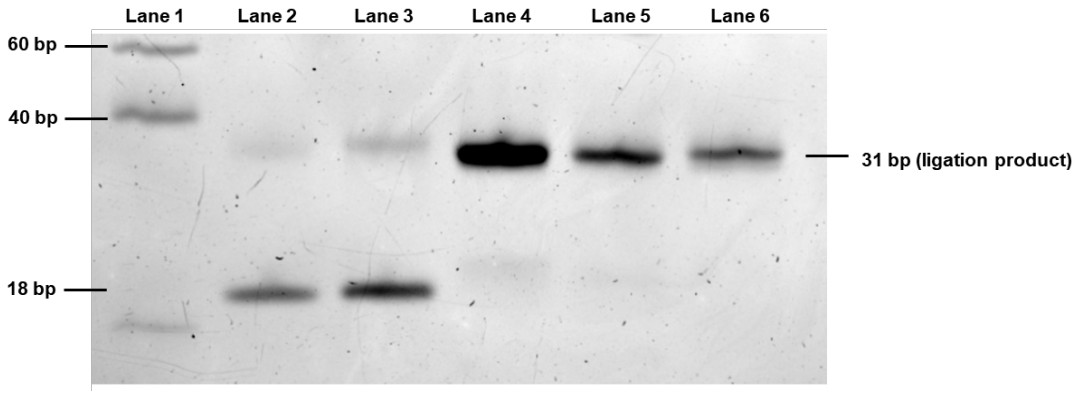
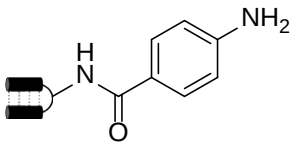
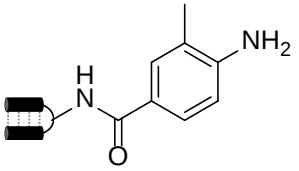
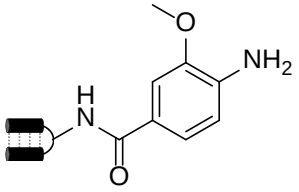
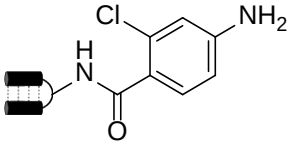
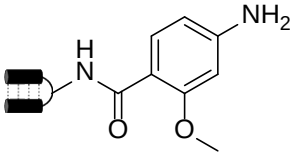
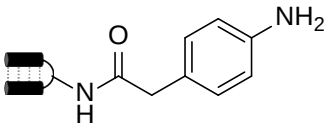
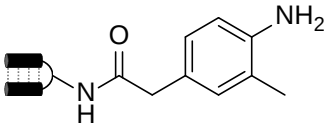
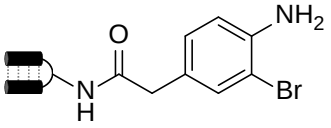
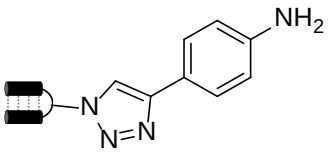
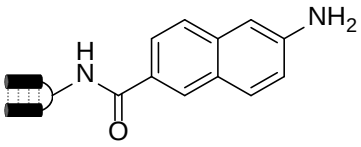
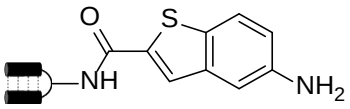
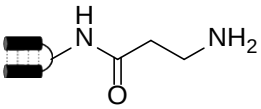
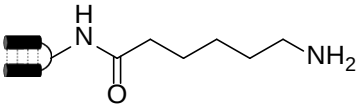
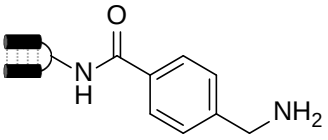
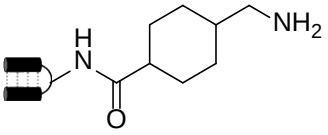
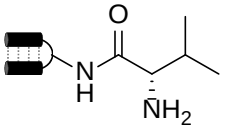
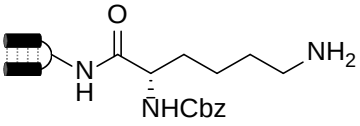


Figure S6. 20% denatured PAGE analysis of DEL-encoding compatibility. Lane 1, **Ladder**; Lane 2, **HP-P**; Lane 3, DNA conjugate **1o**; Lane 4, DNA conjugate **4ob**; Lane 5, DNA conjugate **6ob**; Lane 6, DNA conjugate **9ob**.

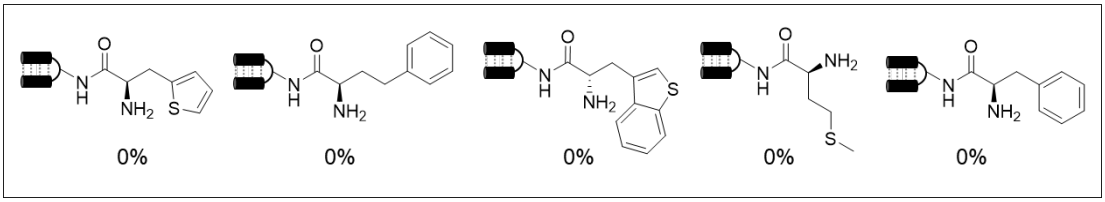
7. UPLC chromatogram and deconvoluted MS

7.1 Substrate scope of DNA-conjugated amines for on-DNA synthesis of 2-thioxo-quinazolinones

Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
1a		**4aa**	5217	5217	> 90%
1b		**4ba**	5231	5231	75%
1c		**4ca**	5247	5247	86%
1d		**4da**	5251	5251	61%
1e		**4ea**	5247	5247	80%
1f		**4fa**	5231	5231	88%
1g		**4ga**	5245	5245	> 90%
1h		**4ha**	5309	5309	78%

1i		**4ia**	5241	5241	83%
1j		**4ja**	5267	5267	> 90%
1k		**4ka**	5273	5273	> 90%
1a'		**4a'a**	5169	5169	86%
1b'		**4b'a**	5197	5197	87%
1c'		**4c'a**	5231	5231	90%
1d'		**4d'a**	5237	5237	85%
1e'		**4e'a**	5197	5078	0%
1f'		**4f'a**	5360	5360	81%

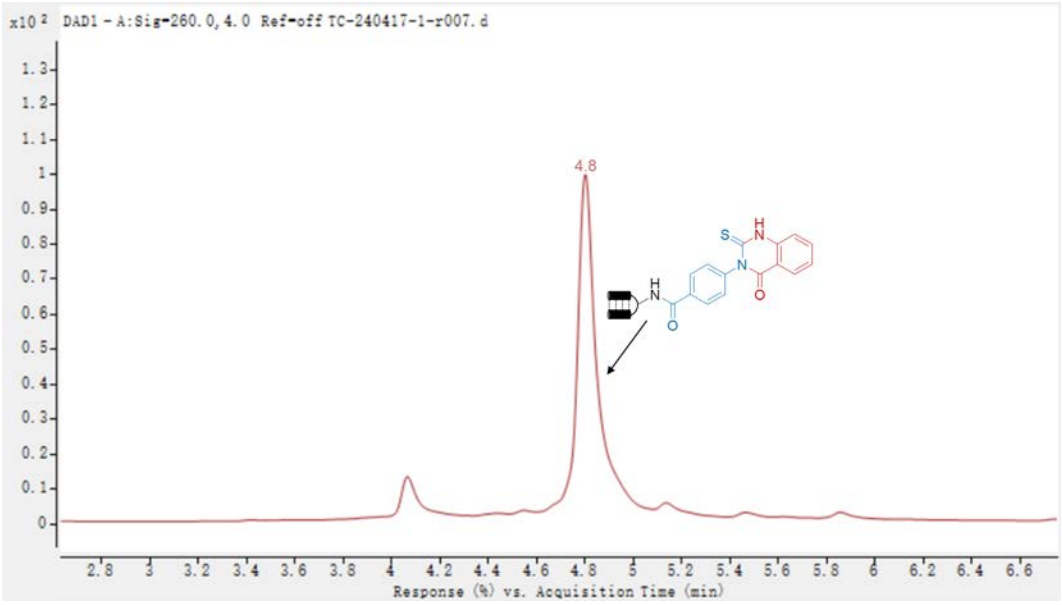
Other tested α-amino acids

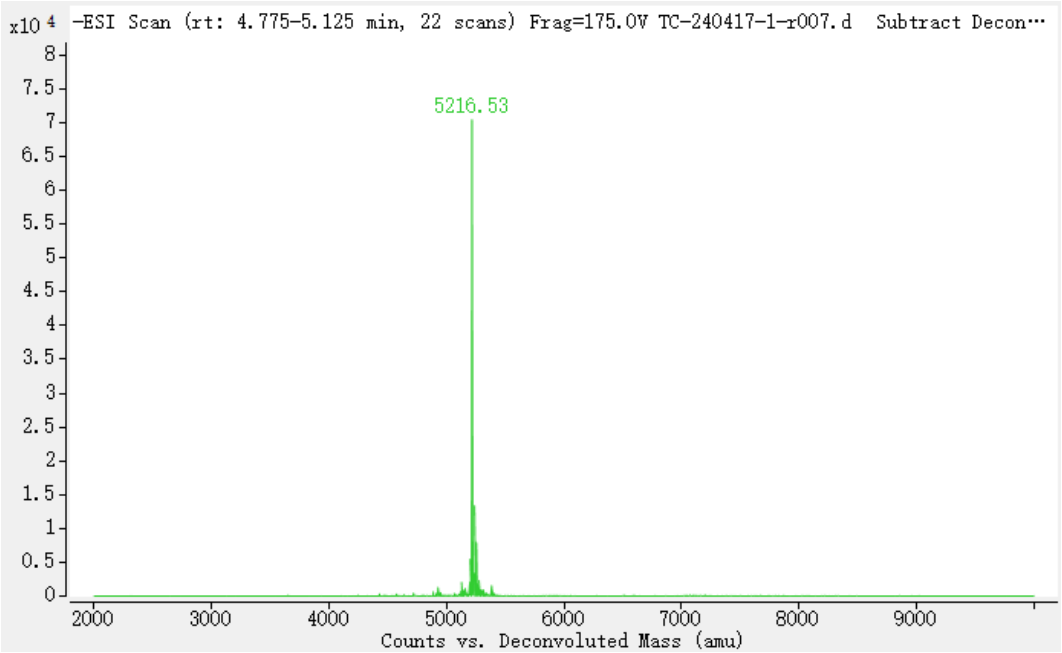


UPLC chromatogram and deconvoluted MS of **4aa**

Conversion: ＞90%

Calculated Mass: 5217 Da; Observed Mass: 5217 Da

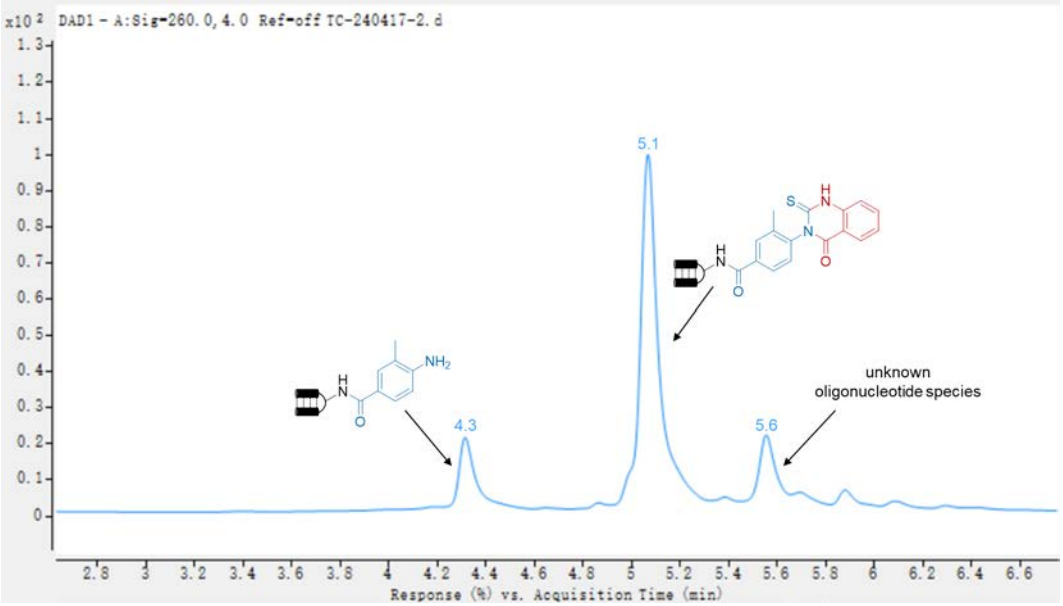


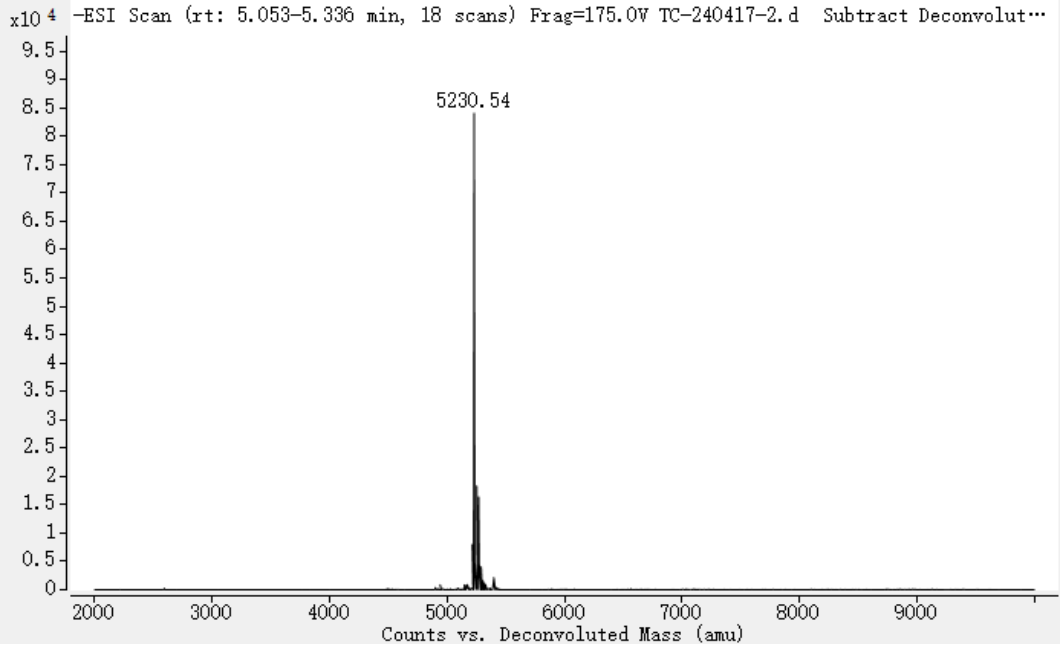


UPLC chromatogram and deconvoluted MS of **4ba**

Conversion: 75%

Calculated Mass: 5231 Da; Observed Mass: 5231 Da

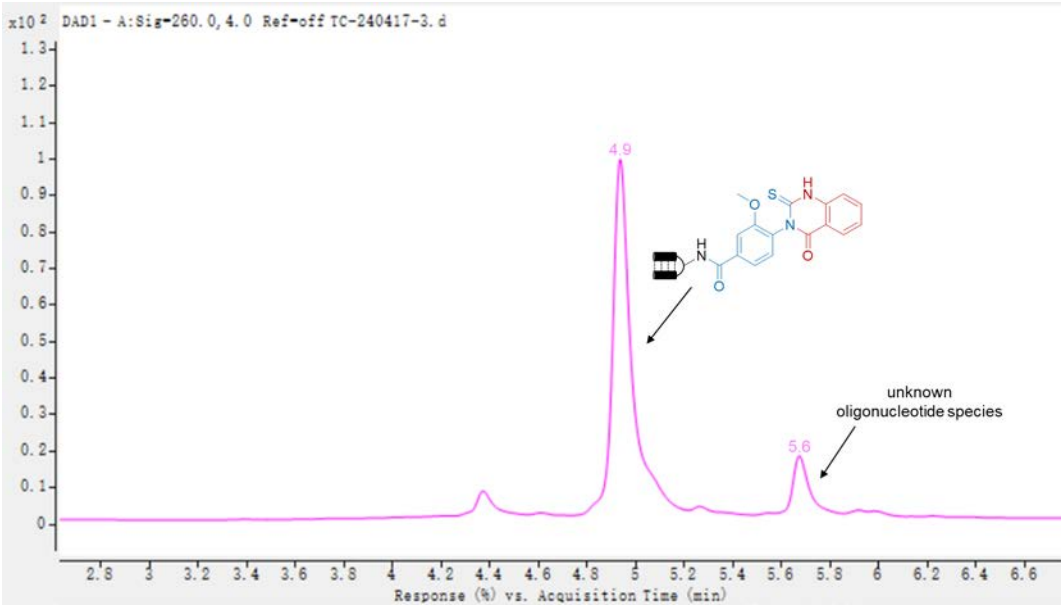


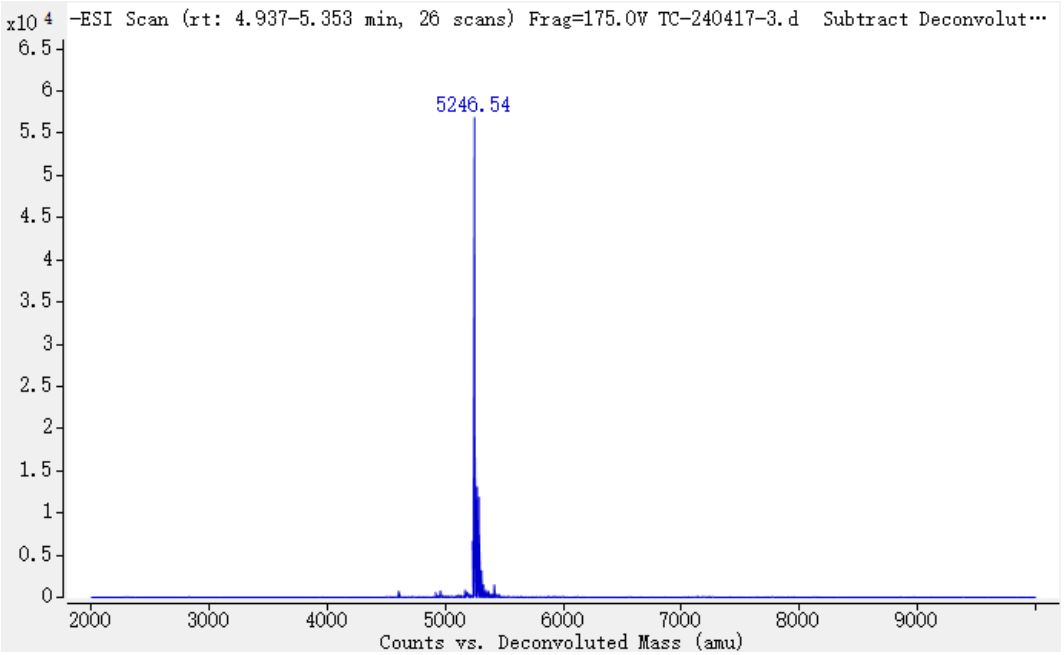


UPLC chromatogram and deconvoluted MS of **4ca**

Conversion: 86%

Calculated Mass: 5247 Da; Observed Mass: 5247 Da

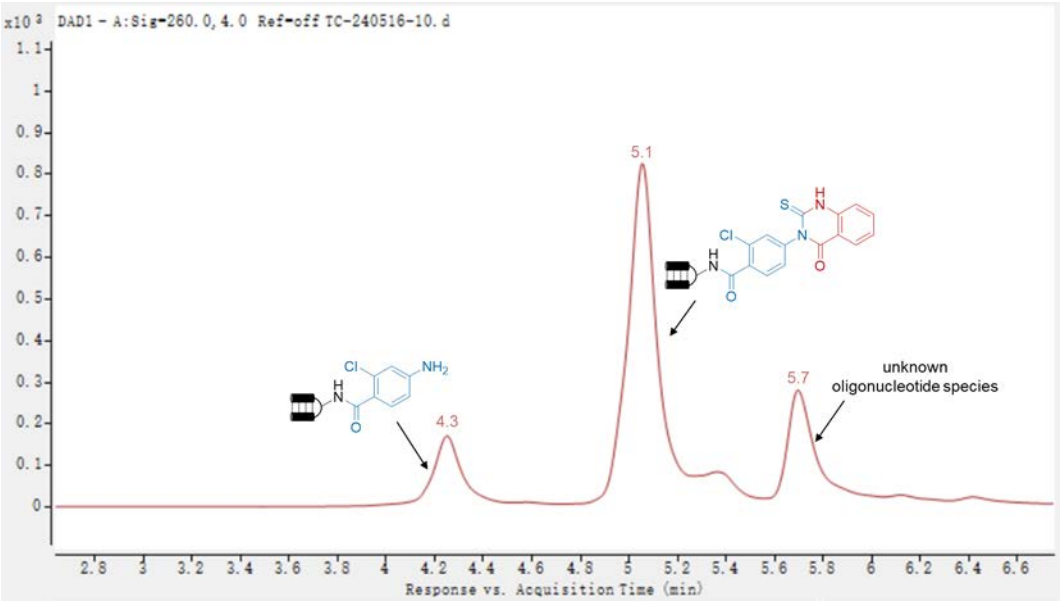


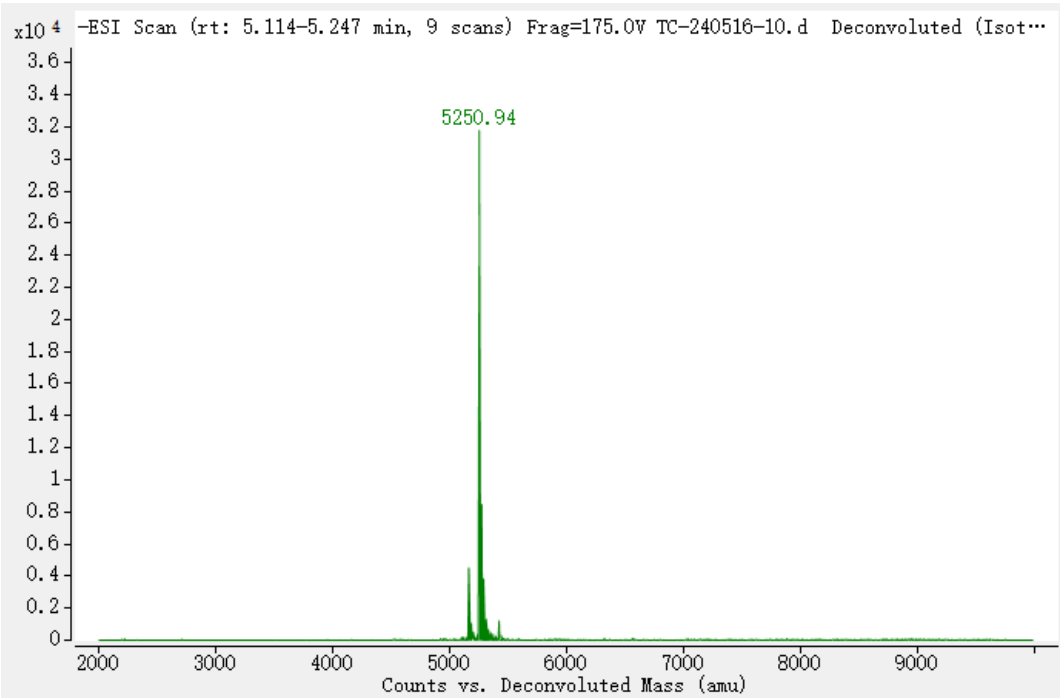


UPLC chromatogram and deconvoluted MS of **4da**

Conversion: 61%

Calculated Mass: 5251 Da; Observed Mass: 5251 Da

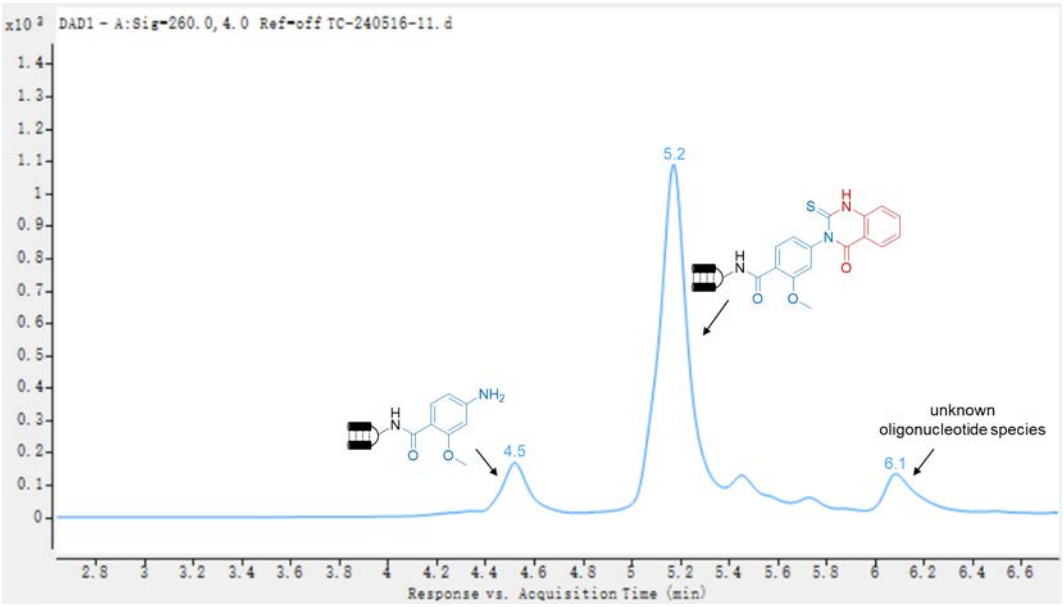


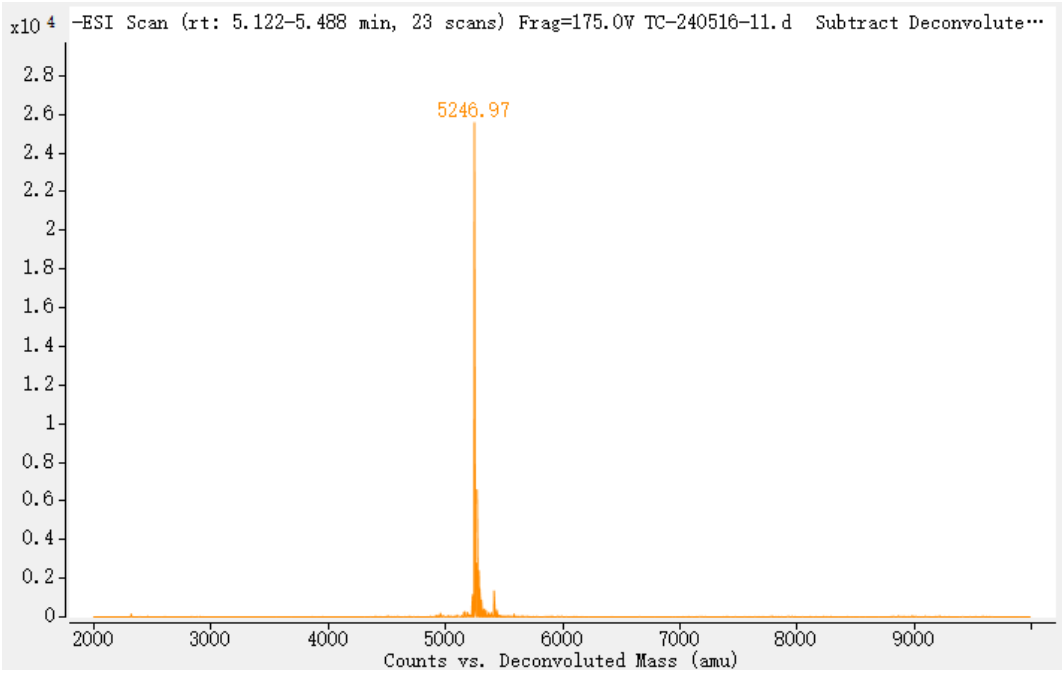


UPLC chromatogram and deconvoluted MS of **4ea**

Conversion: 80%

Calculated Mass: 5247 Da; Observed Mass: 5247 Da

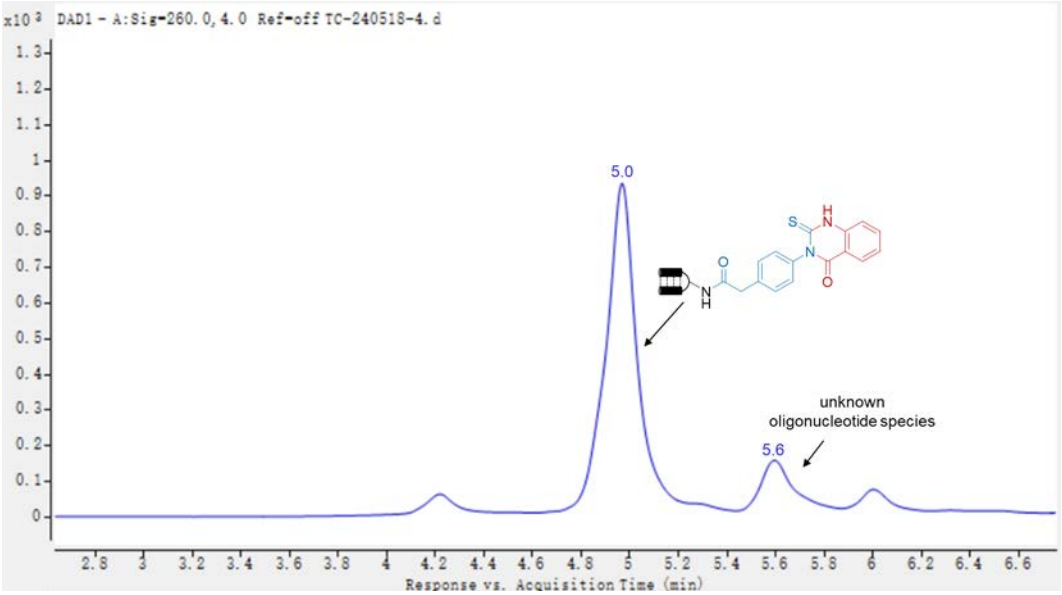


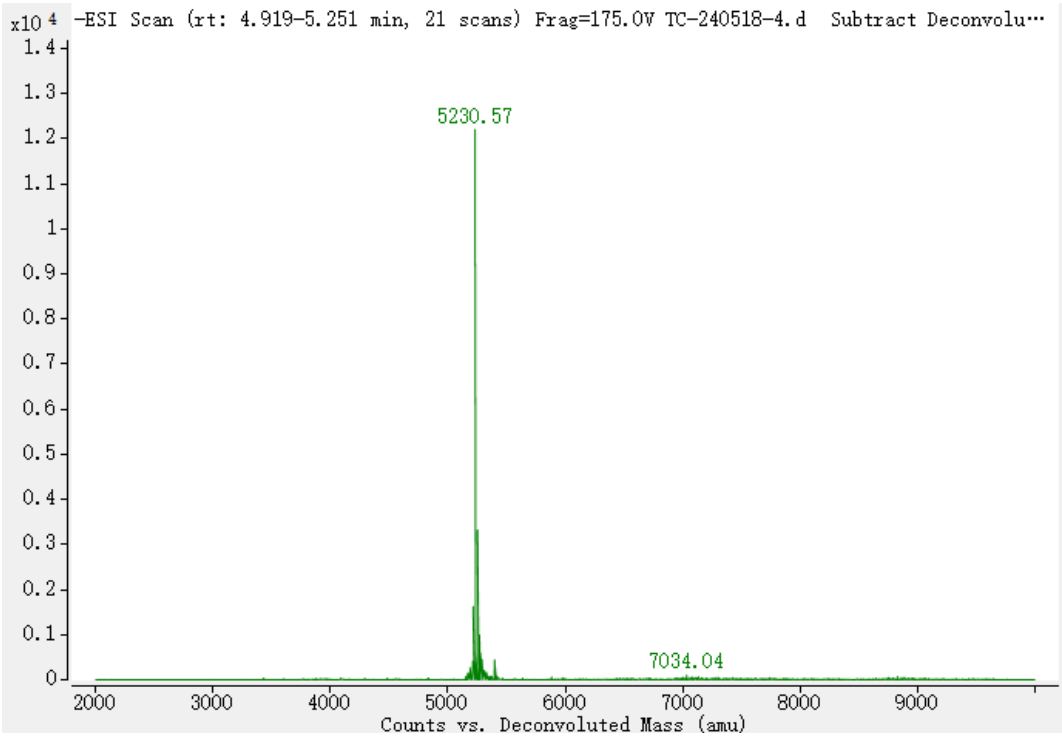


UPLC chromatogram and deconvoluted MS of **4fa**

Conversion: 88%

Calculated Mass: 5231 Da; Observed Mass: 5231 Da

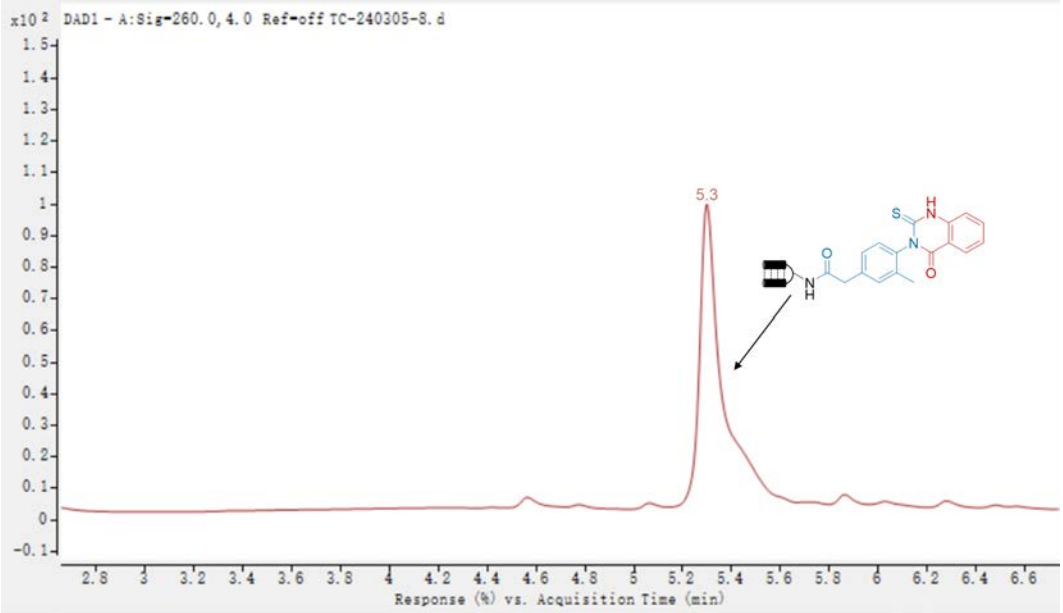


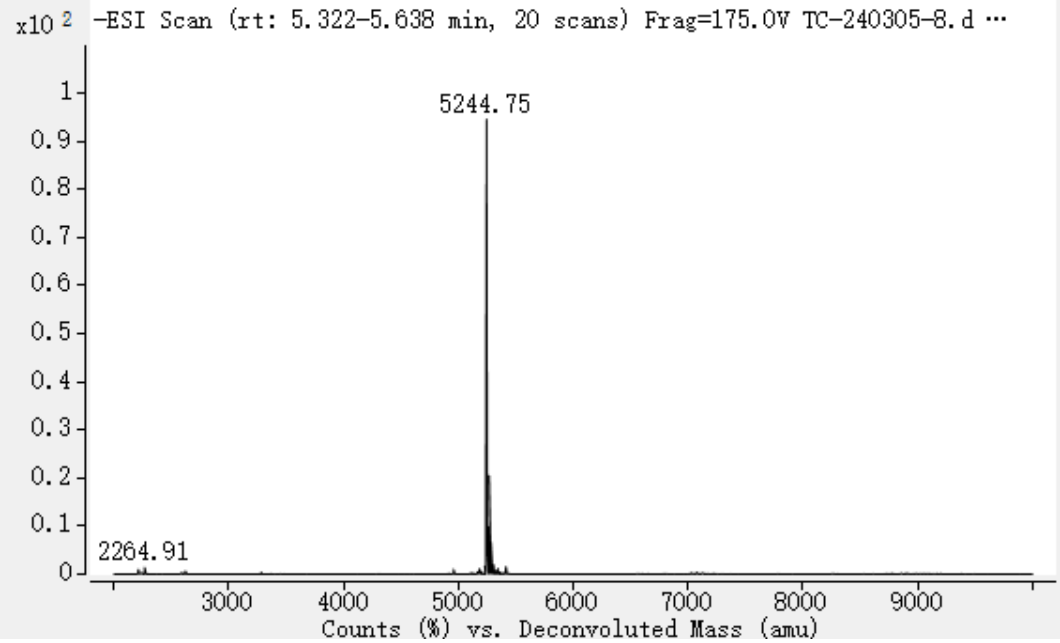


UPLC chromatogram and deconvoluted MS of **4ga**

Conversion: ＞90%

Calculated Mass: 5245 Da; Observed Mass: 5245 Da

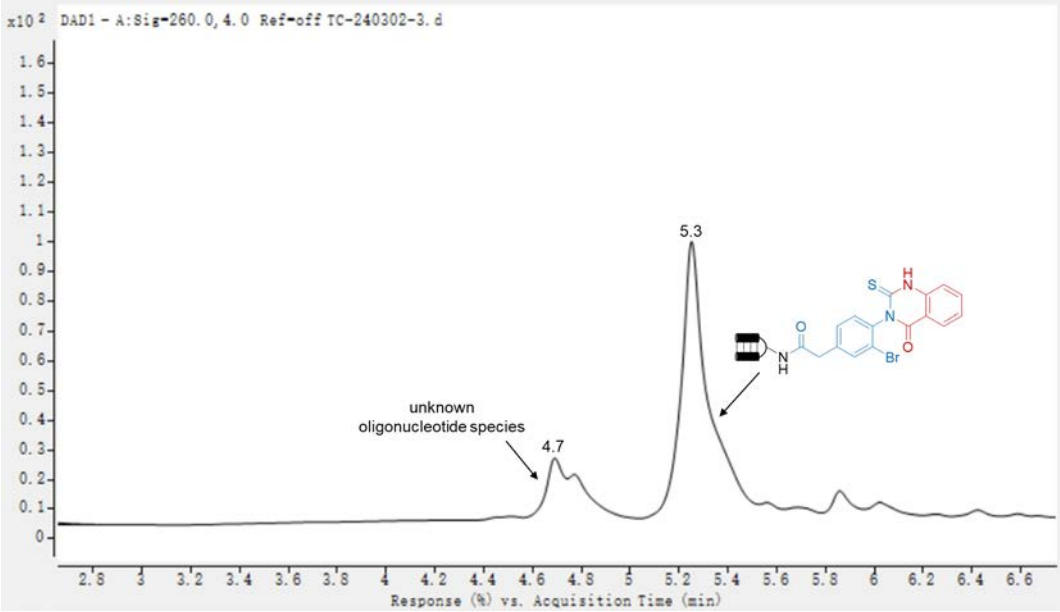


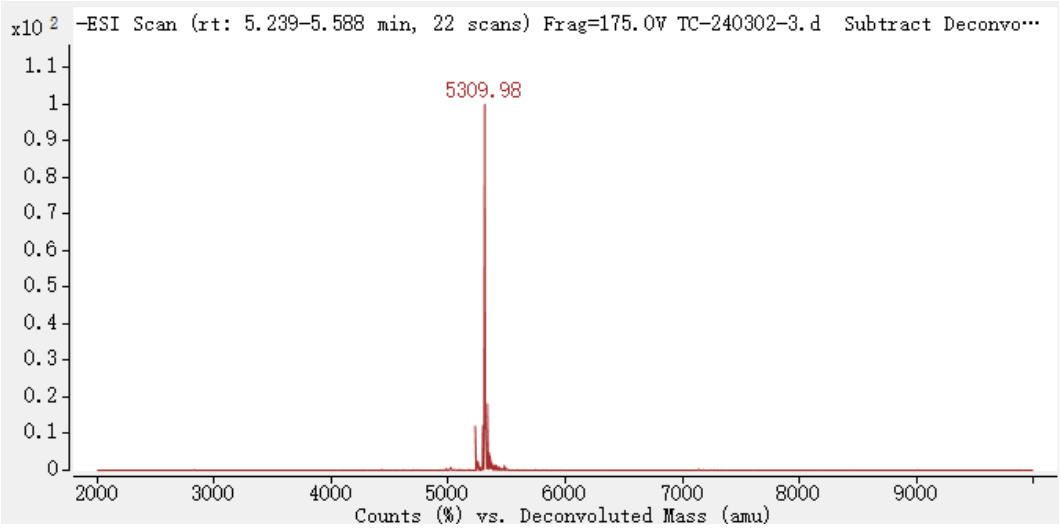


UPLC chromatogram and deconvoluted MS of **4ha**

Conversion: 78%

Calculated Mass: 5310 Da; Observed Mass: 5310 Da

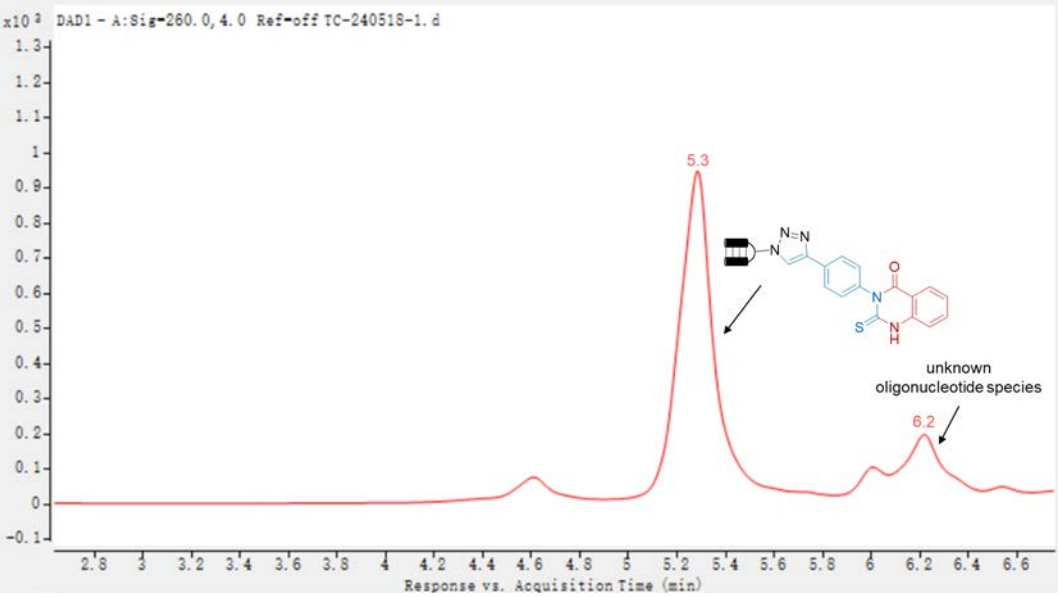


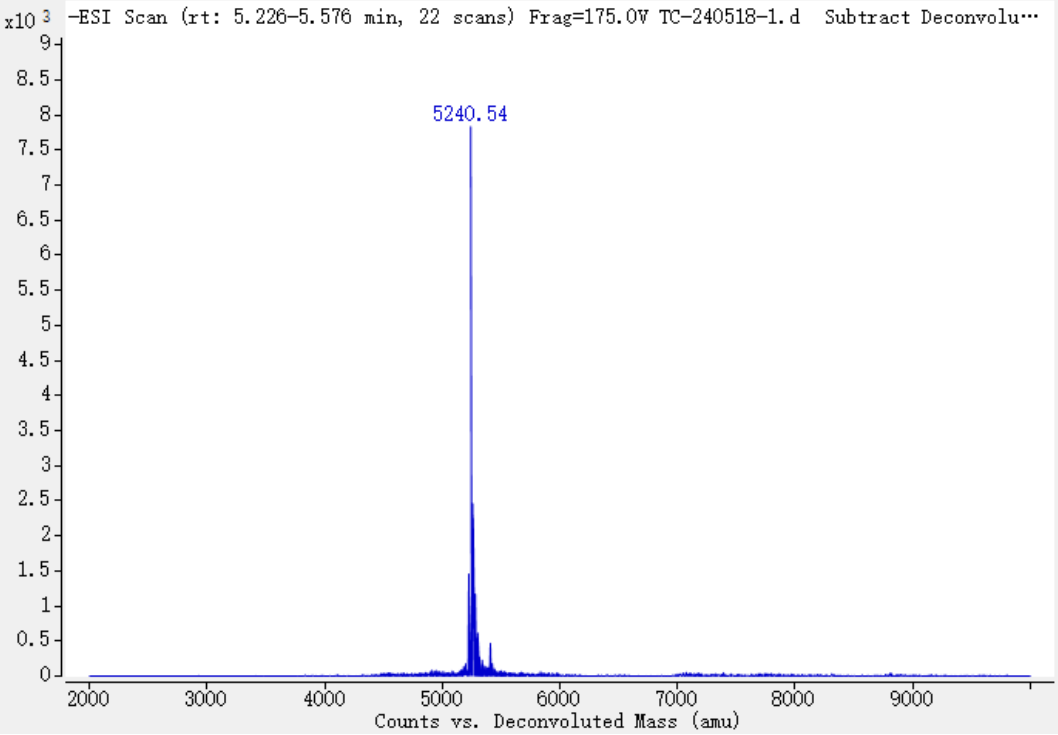


UPLC chromatogram and deconvoluted MS of **4ia**

Conversion: 83%

Calculated Mass: 5241 Da; Observed Mass: 5241 Da

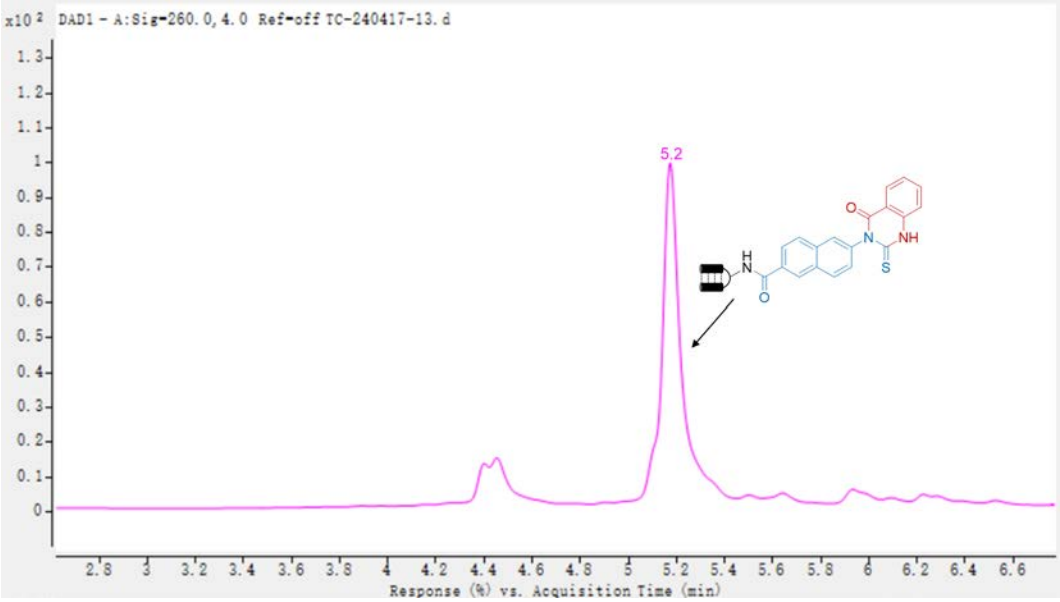


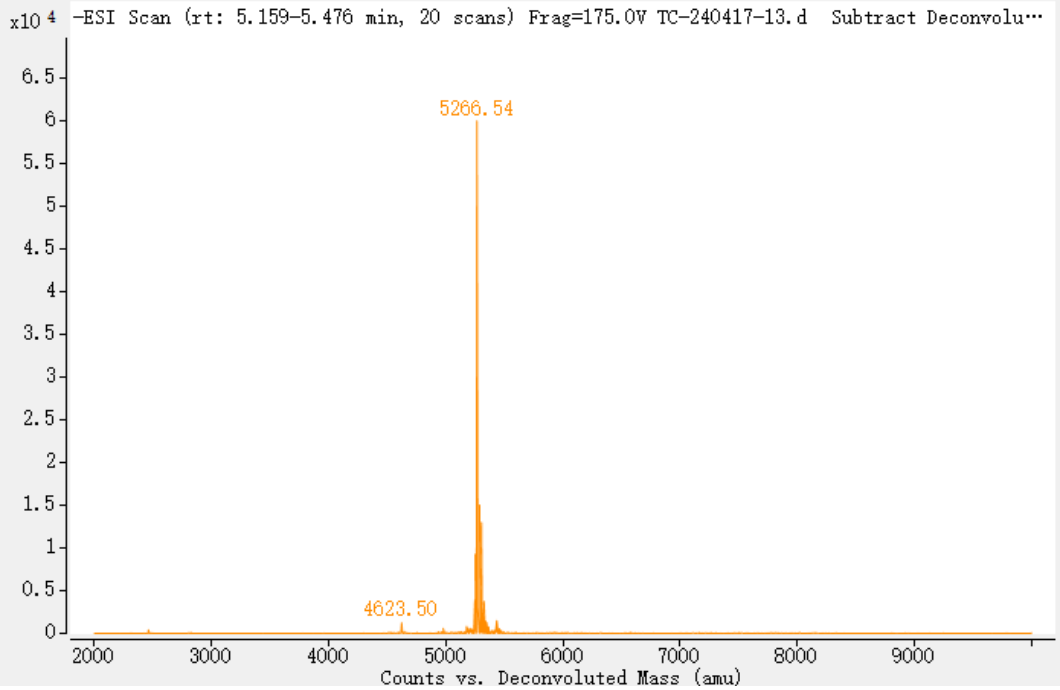


UPLC chromatogram and deconvoluted MS of **4ja**

Conversion: ＞90%

Calculated Mass: 5267 Da; Observed Mass: 5267 Da

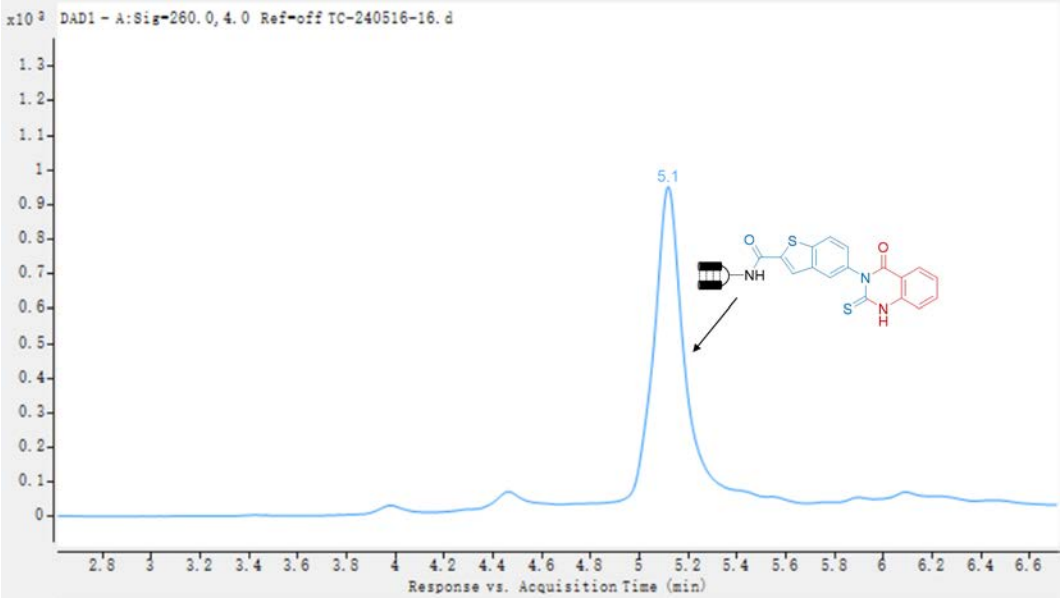


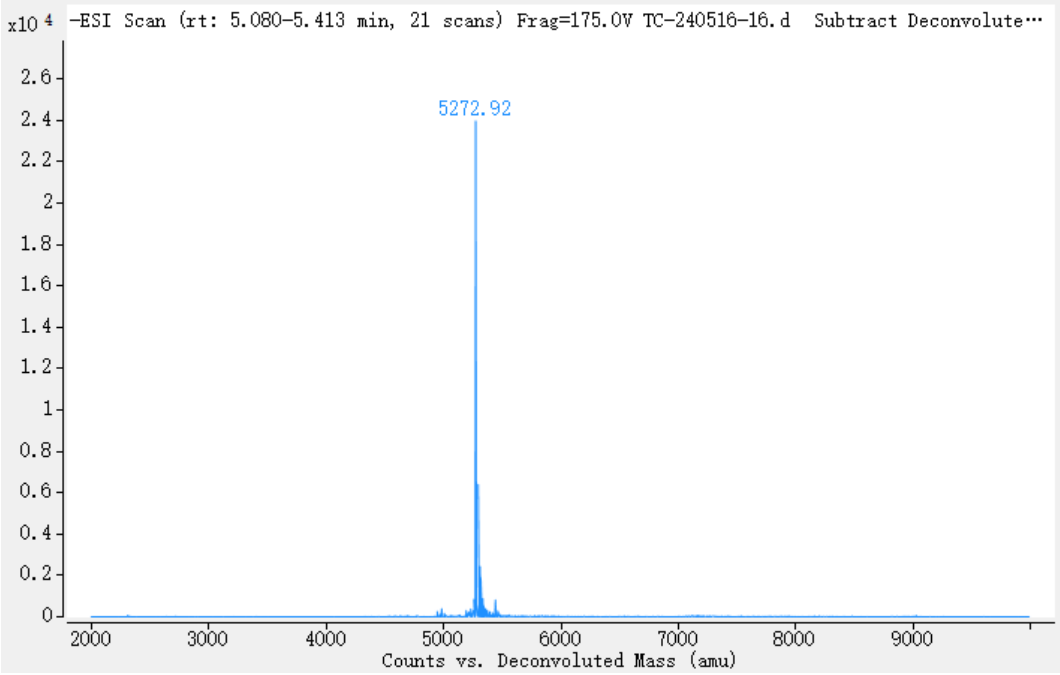


UPLC chromatogram and deconvoluted MS of **4ka**

Conversion: ＞90%

Calculated Mass: 5273 Da; Observed Mass: 5273 Da

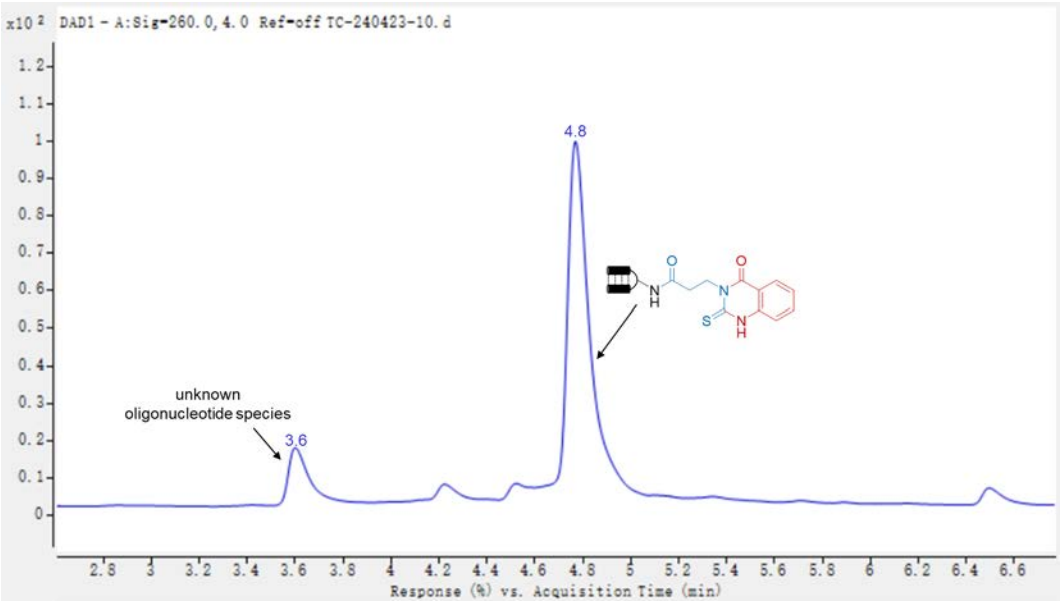


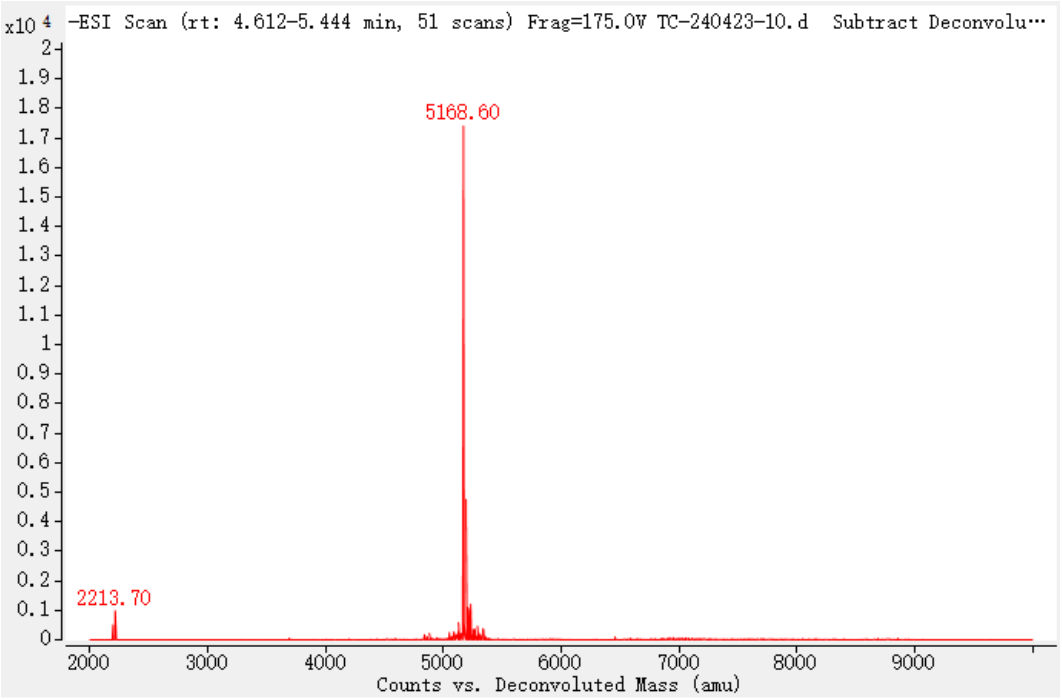


UPLC chromatogram and deconvoluted MS of **4a'a**

Conversion: 86%

Calculated Mass: 5169 Da; Observed Mass: 5169 Da

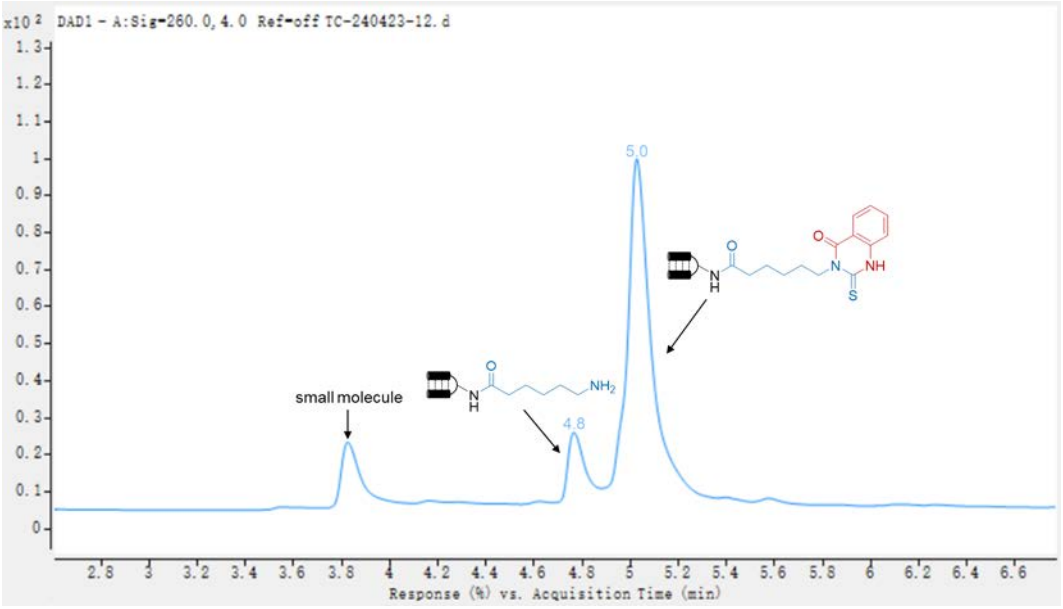


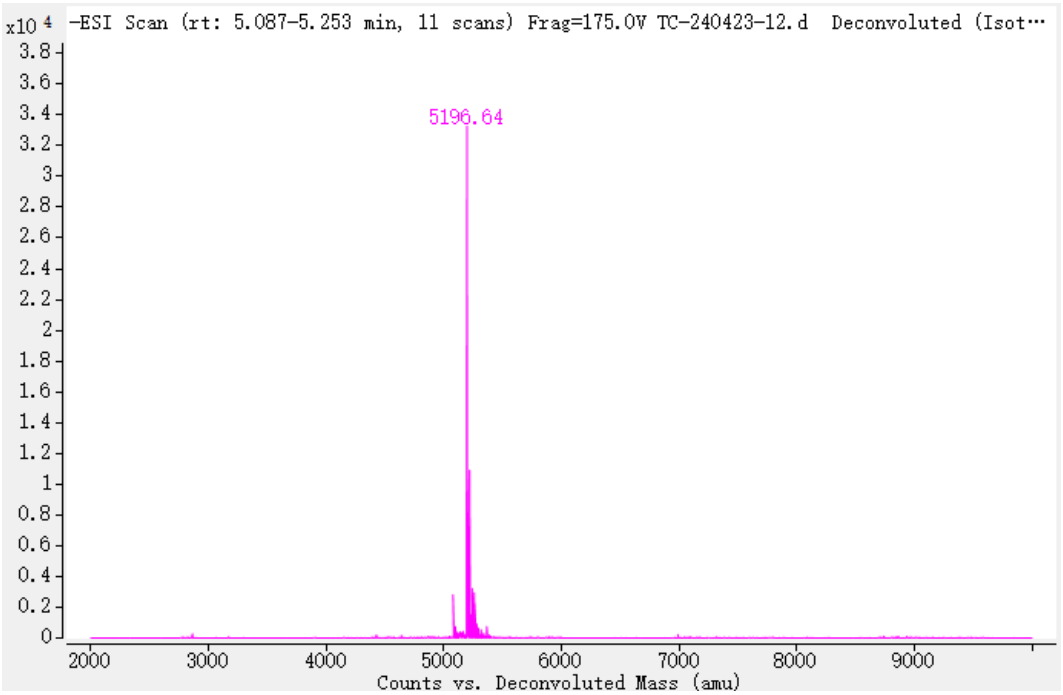


UPLC chromatogram and deconvoluted MS of **4b'a**

Conversion: 87%

Calculated Mass: 5197 Da; Observed Mass: 5197 Da

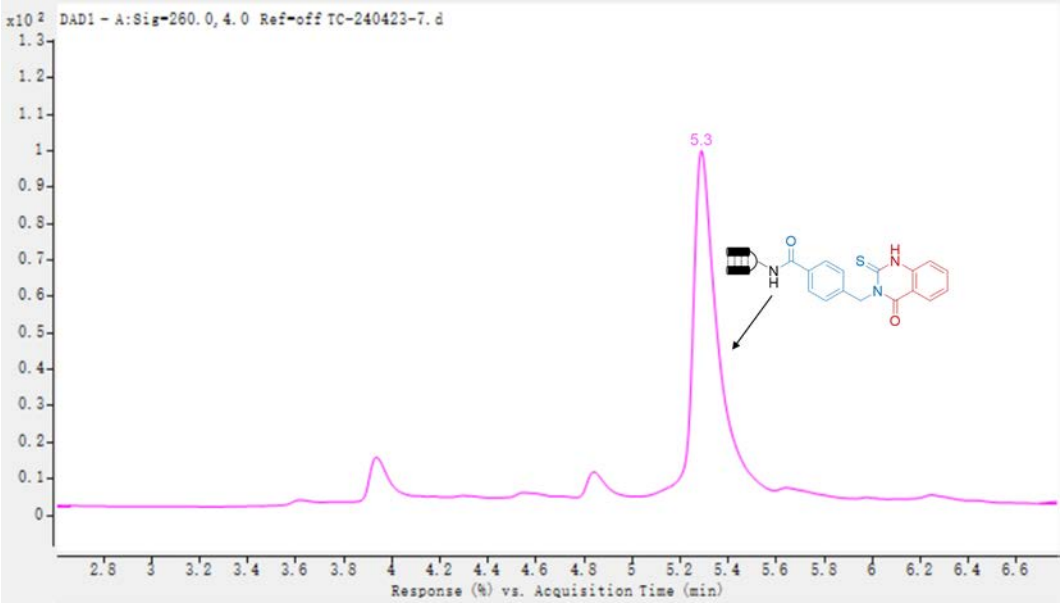


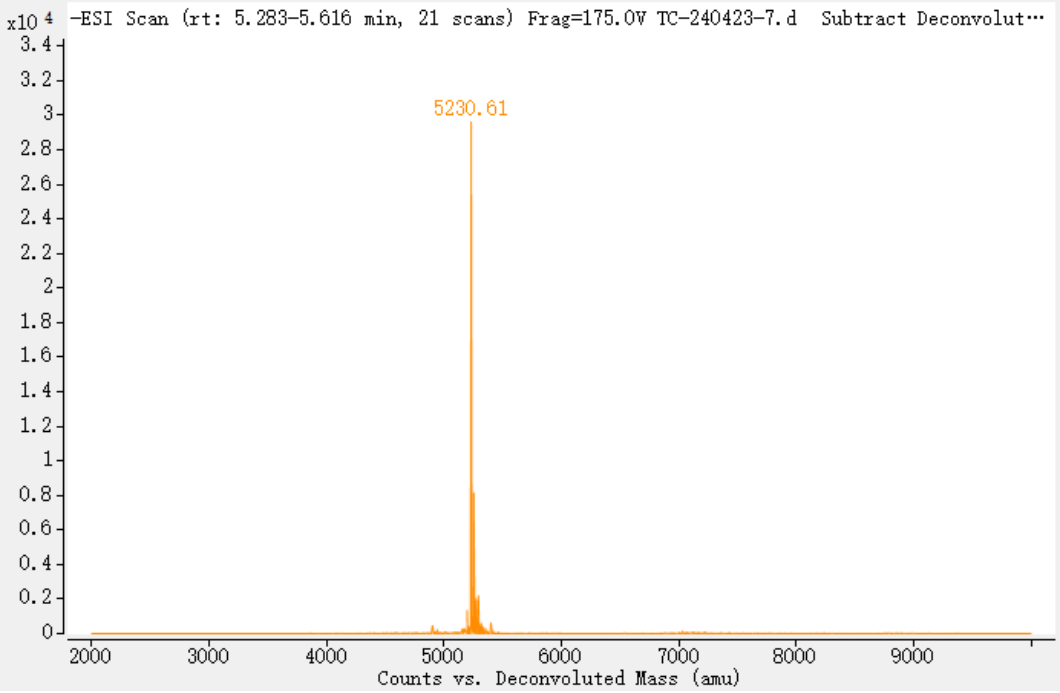


UPLC chromatogram and deconvoluted MS of **4c'a**

Conversion: 90%

Calculated Mass: 5231 Da; Observed Mass: 5231 Da

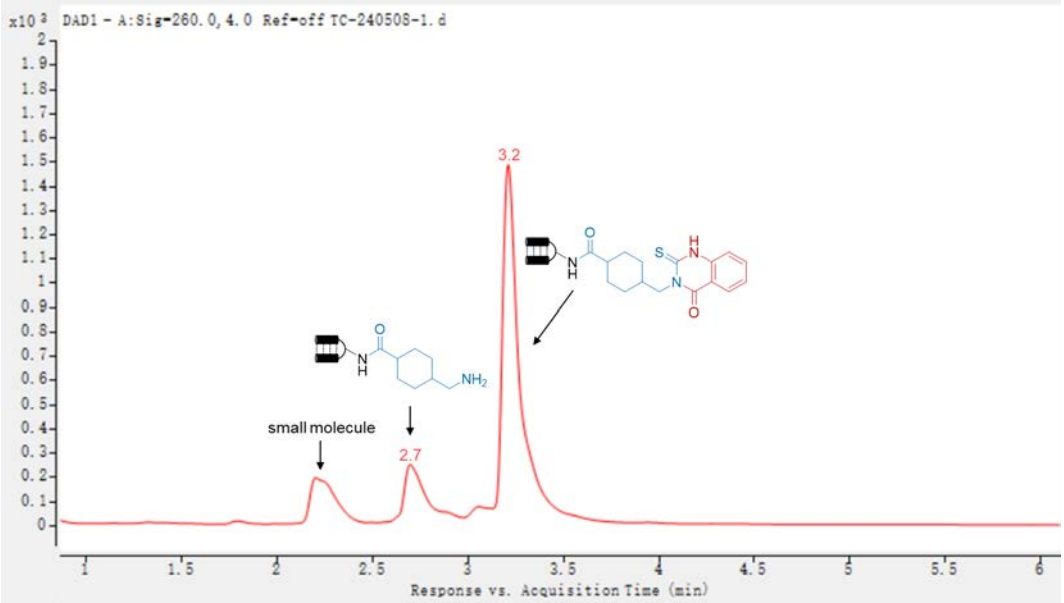


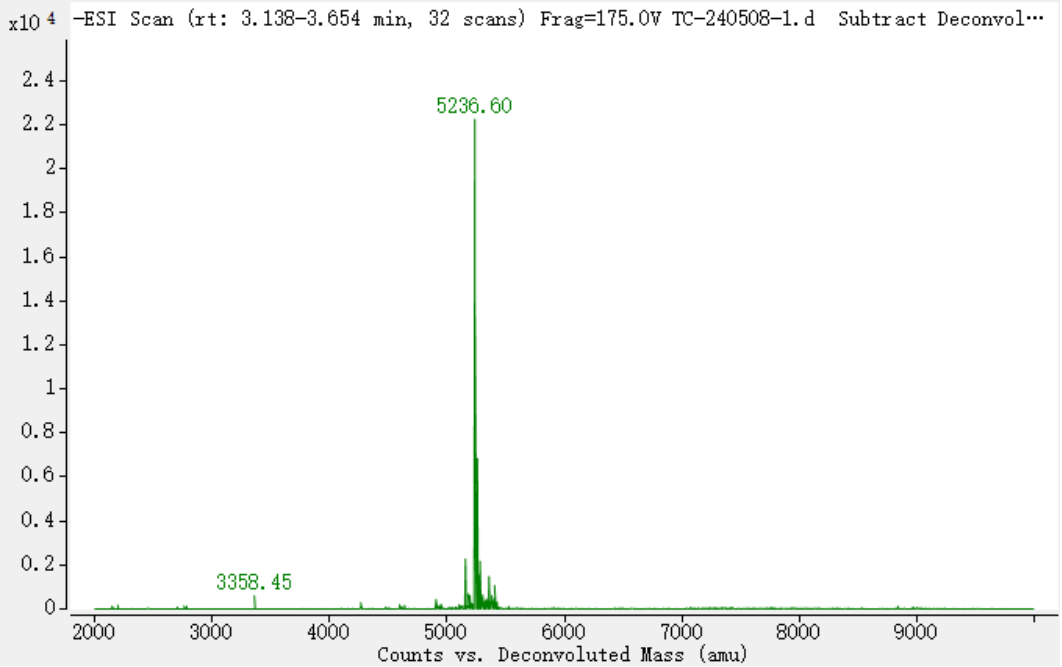


UPLC chromatogram and deconvoluted MS of **4d'a**

Conversion: 85%

Calculated Mass: 5237 Da; Observed Mass: 5237 Da

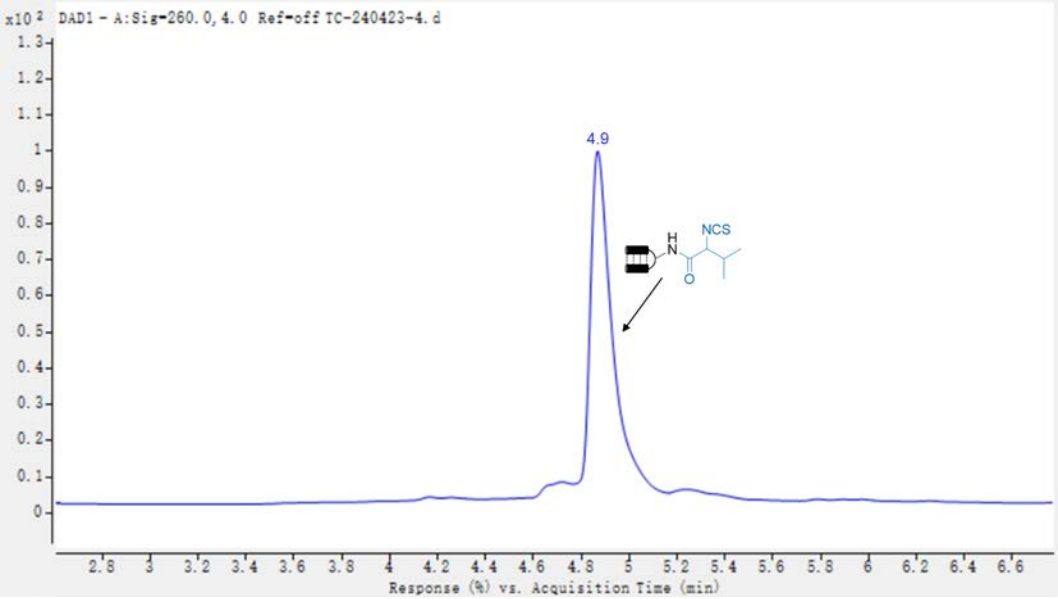


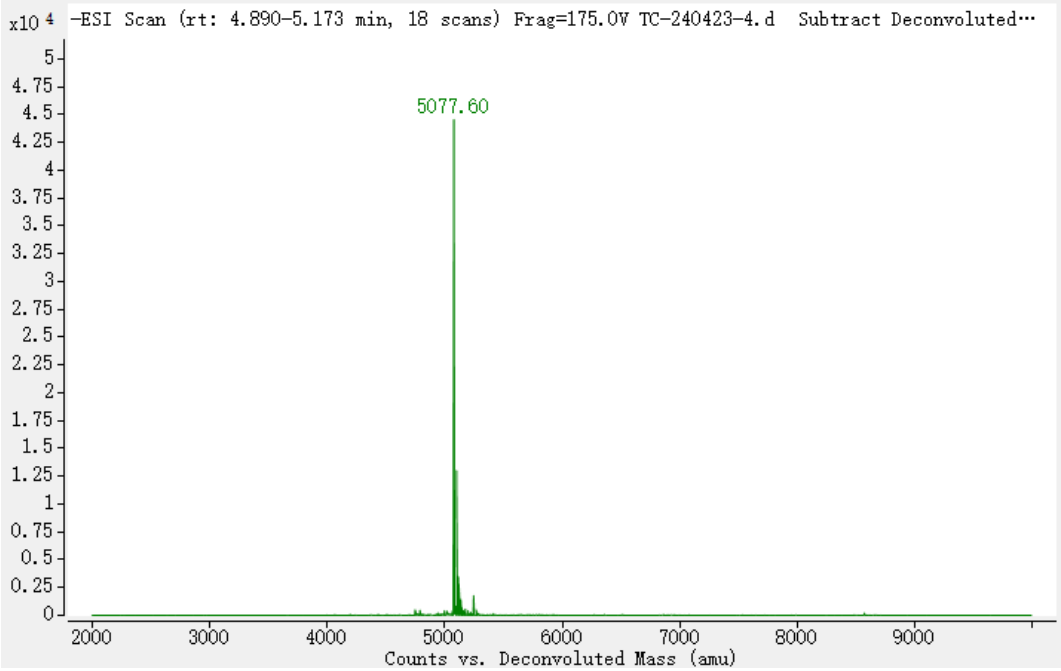


UPLC chromatogram and deconvoluted MS of **4e'a**

Conversion: 0%

Calculated Mass: 5197 Da; Observed Mass: 5078 Da

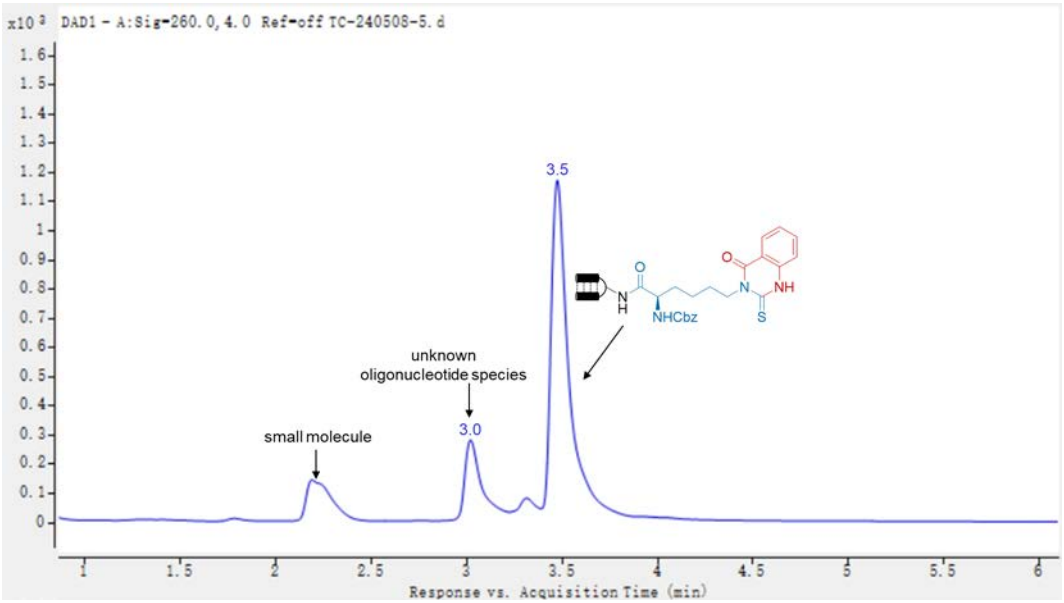


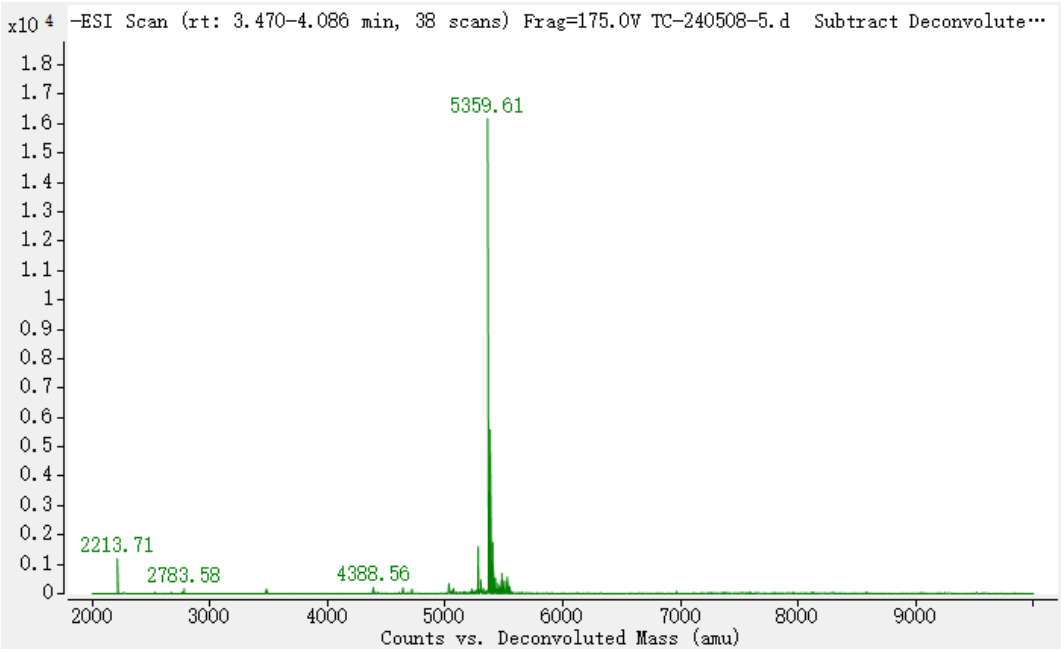


UPLC chromatogram and deconvoluted MS of **4f'a**

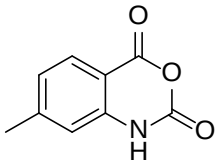
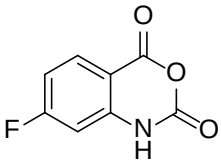
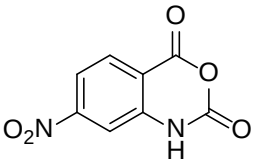
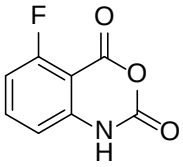
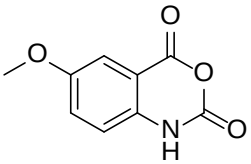
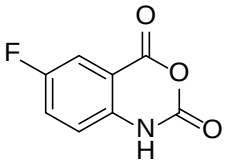
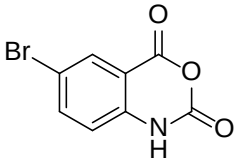
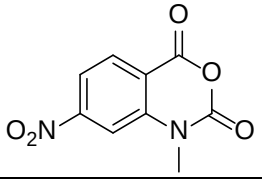
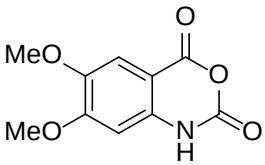
Conversion: 81%

Calculated Mass: 5360 Da; Observed Mass: 5360 Da





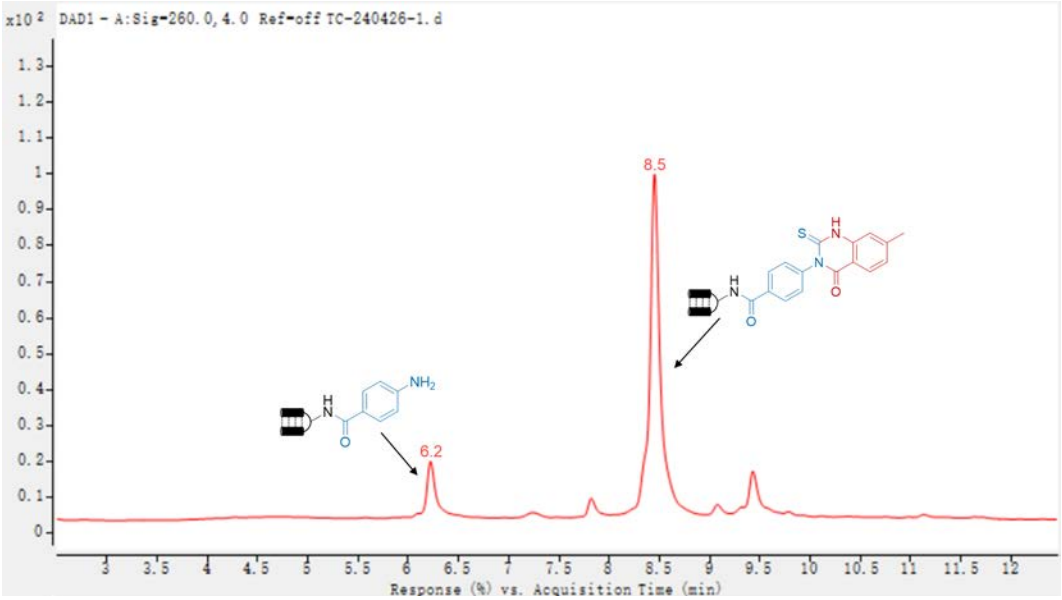
7.2 Substrate scope of isatoic anhydrides

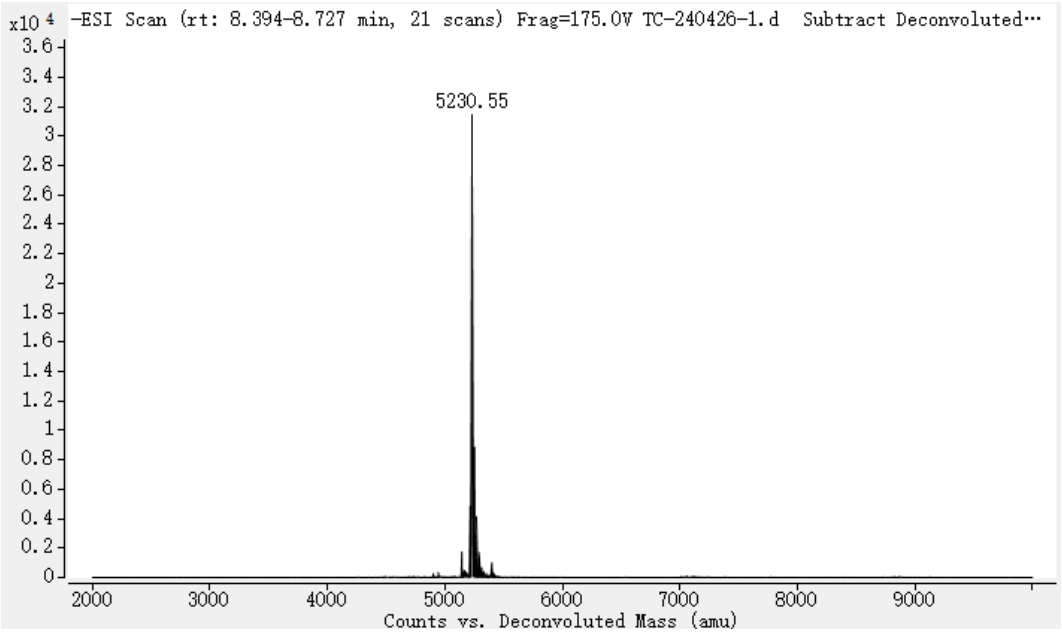
Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
3b		**4ab**	5231	5231	78%
3c		**4ac**	5235	5235	74%
3d		**4ad**	5262	5262	82%
3e		**4ae**	5235	5235	61%
3f		**4af**	5247	5247	70%
3g		**4ag**	5235	5235	> 90%
3h		**4ah**	5295	5295	65%
3i		**4ai**	5276	5276	75%
3j		**4aj**	5277	5277	74%

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

Conversion: 78%

Calculated Mass: 5231 Da; Observed Mass: 5231 Da

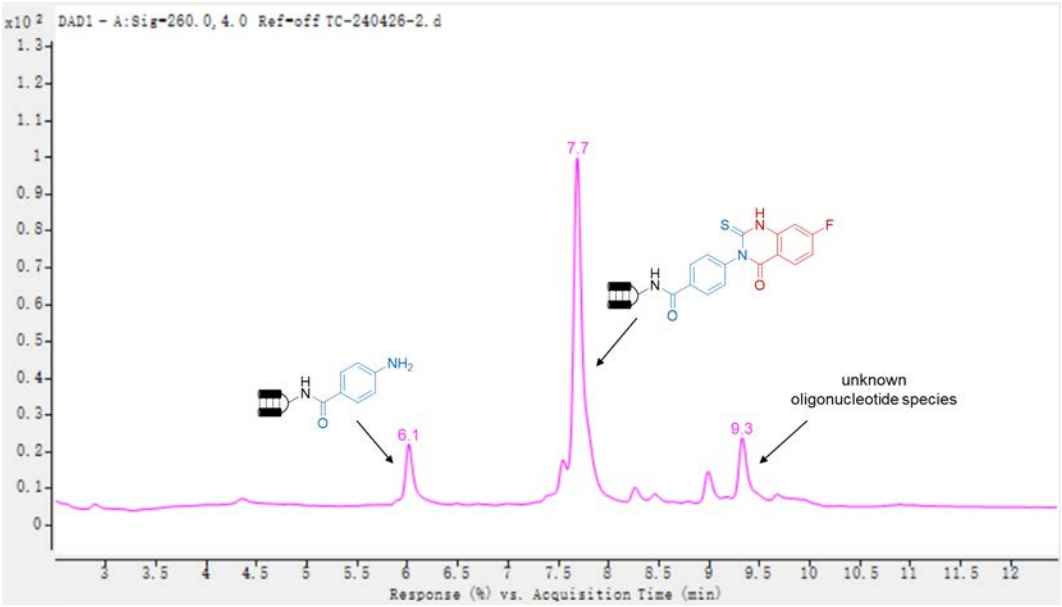


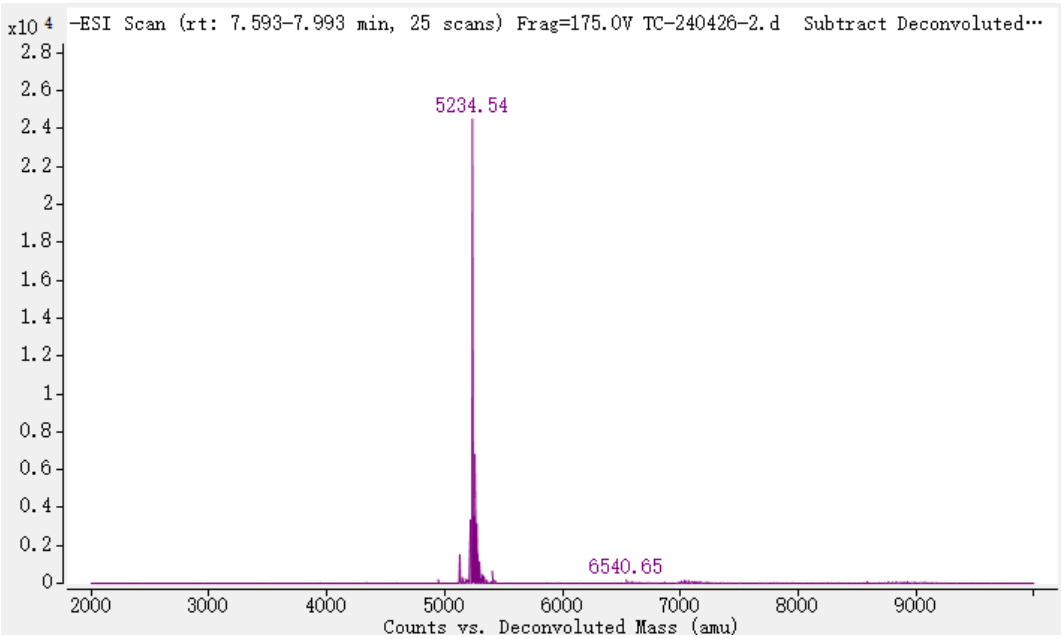


UPLC chromatogram and deconvoluted MS of **4ac**

Conversion: 74%

Calculated Mass: 5235 Da; Observed Mass: 5235 Da

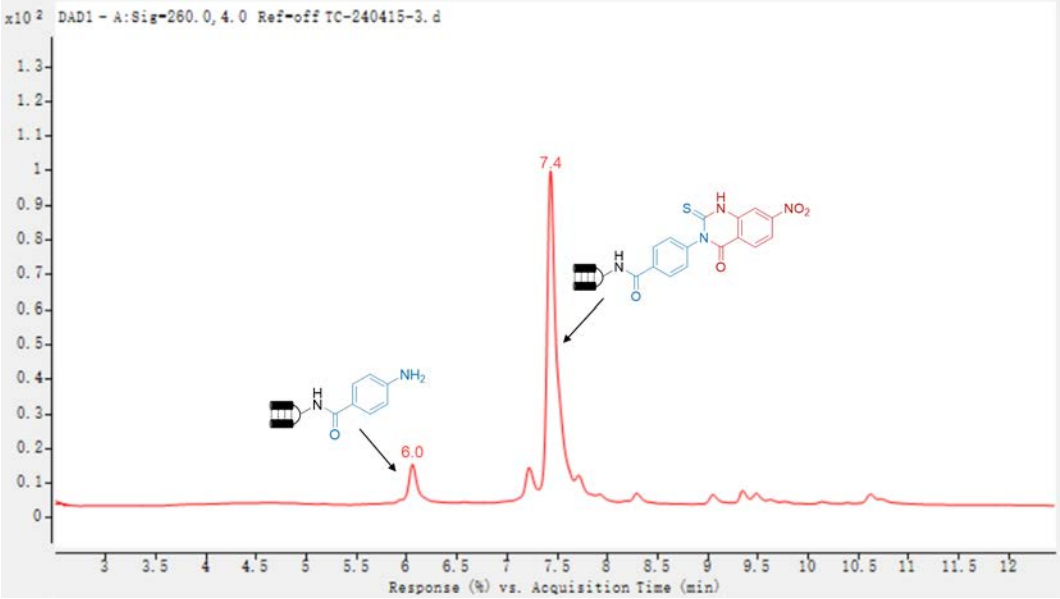


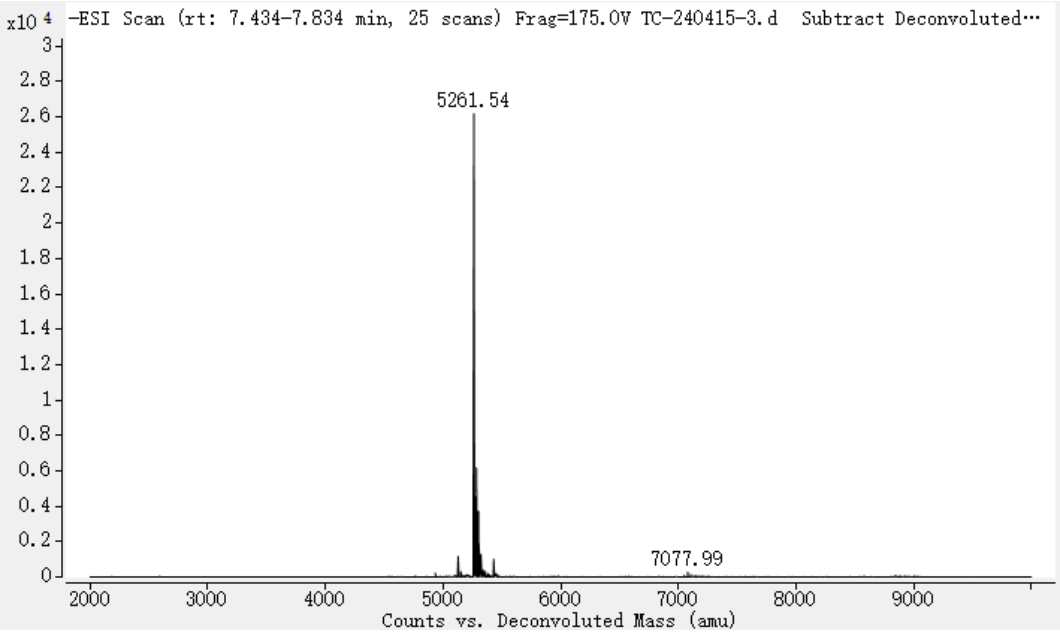


UPLC chromatogram and deconvoluted MS of **4ad**

Conversion: 82%

Calculated Mass: 5262 Da; Observed Mass: 5262 Da

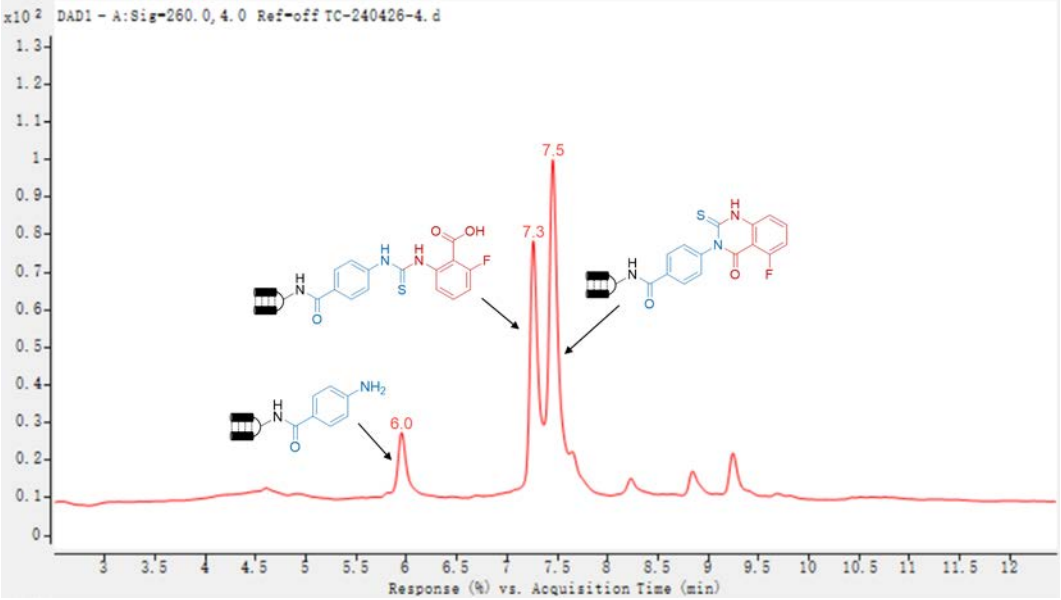


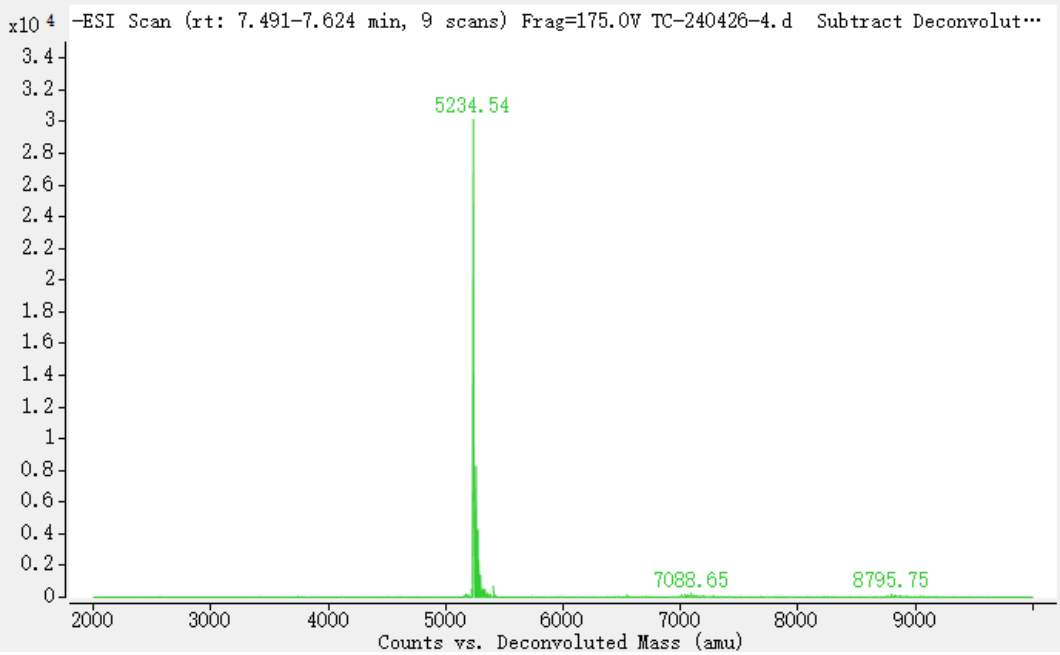


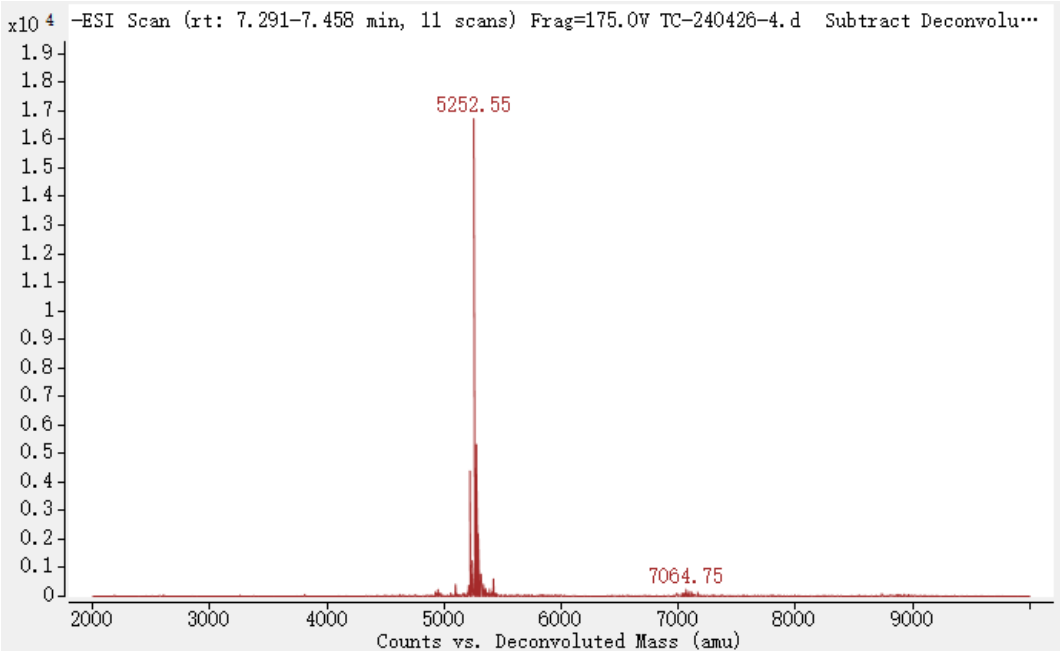
UPLC chromatogram and deconvoluted MS of **4ae**

Conversion: 61%

Calculated Mass: 5235 Da; Observed Mass: 5235 Da



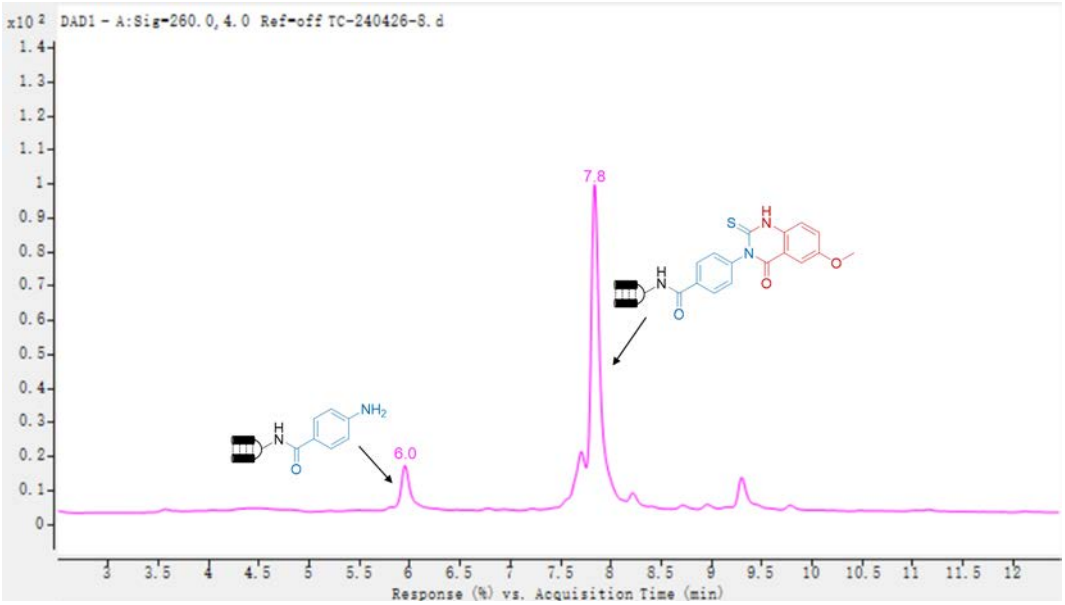


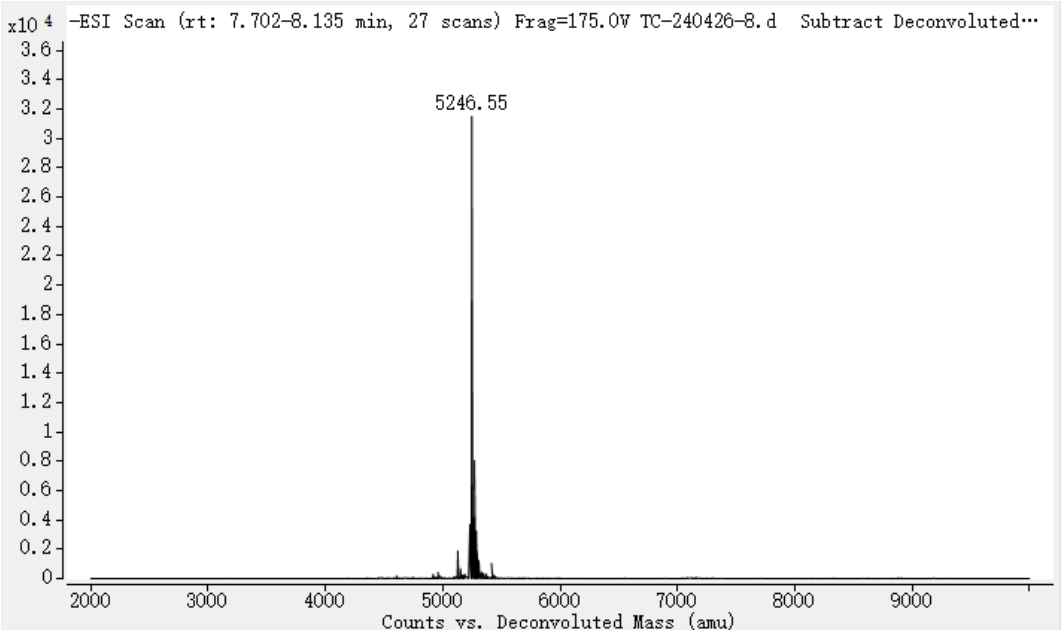

x10 4
-ESI Scan (rt: 7.291-7.458 min, 11 scans) Frag=175.0V TC-240426-4.d Subtract Deconvolu…
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5252.55
7064.75
2000
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

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

Conversion: 70%

Calculated Mass: 5247 Da; Observed Mass: 5247 Da

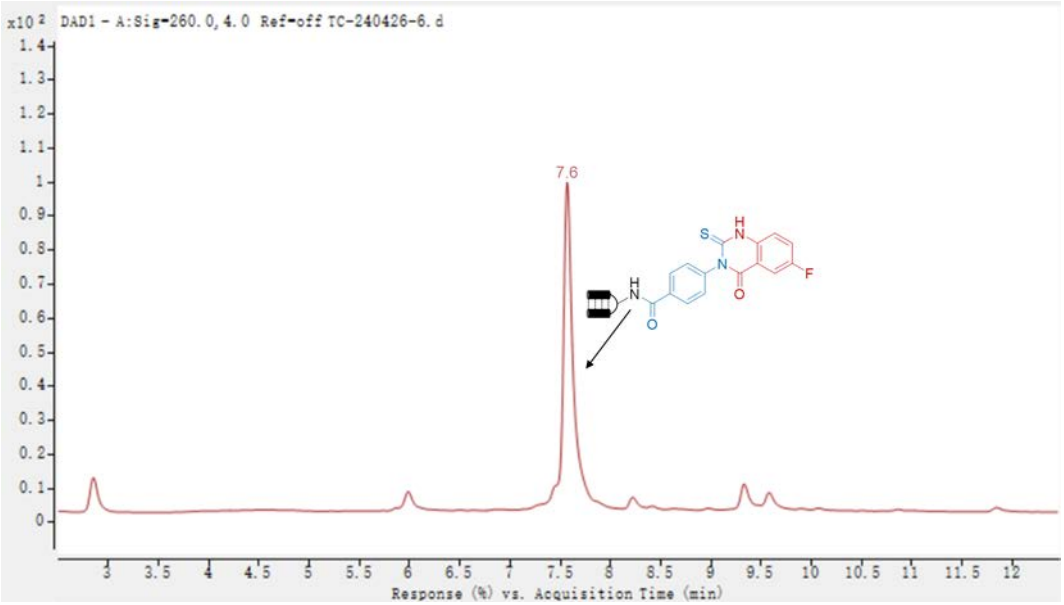


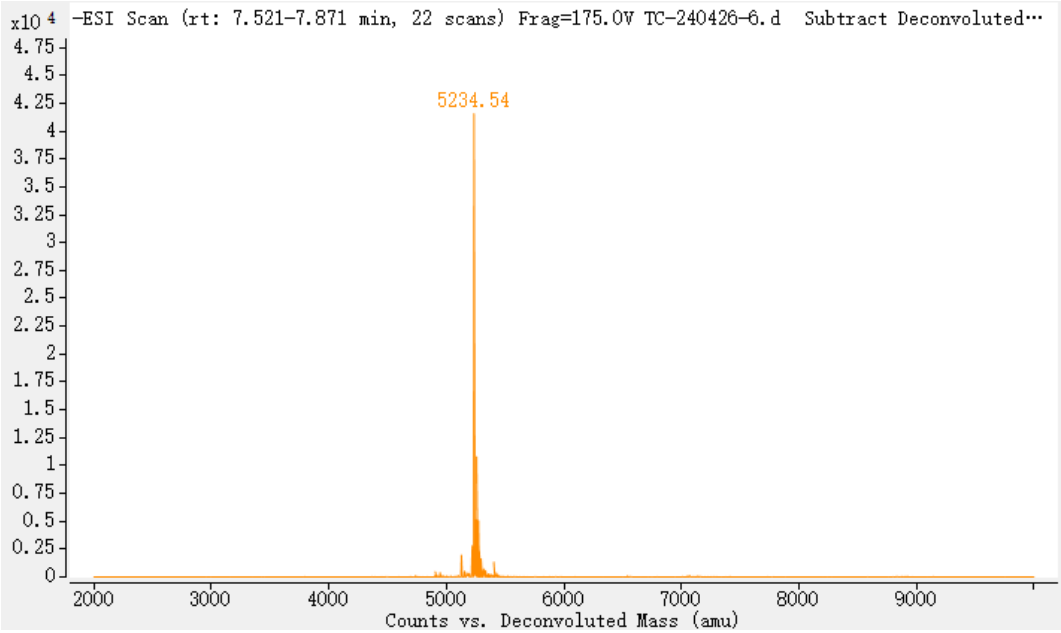


UPLC chromatogram and deconvoluted MS of **4ag**

Conversion: ＞90%

Calculated Mass: 5235 Da; Observed Mass: 5235 Da

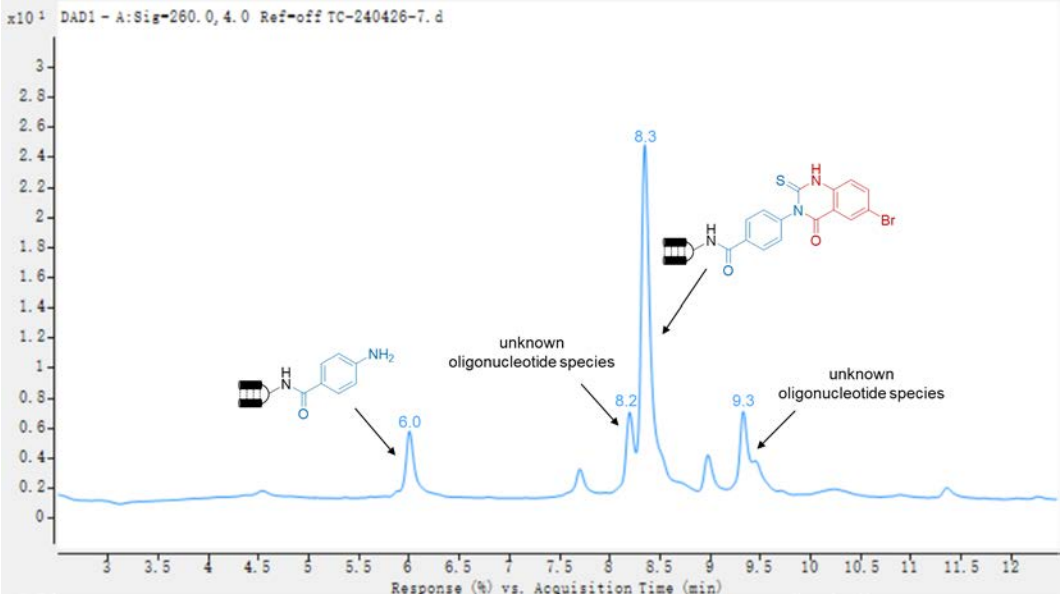


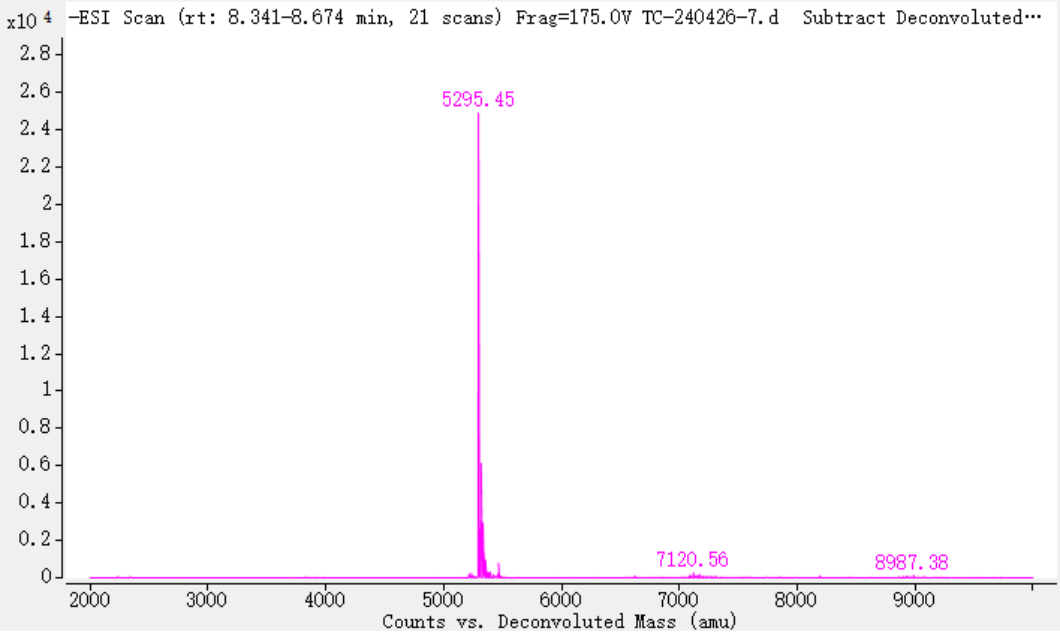


UPLC chromatogram and deconvoluted MS of **4ah**

Conversion: 65%

Calculated Mass: 5295 Da; Observed Mass: 5295 Da

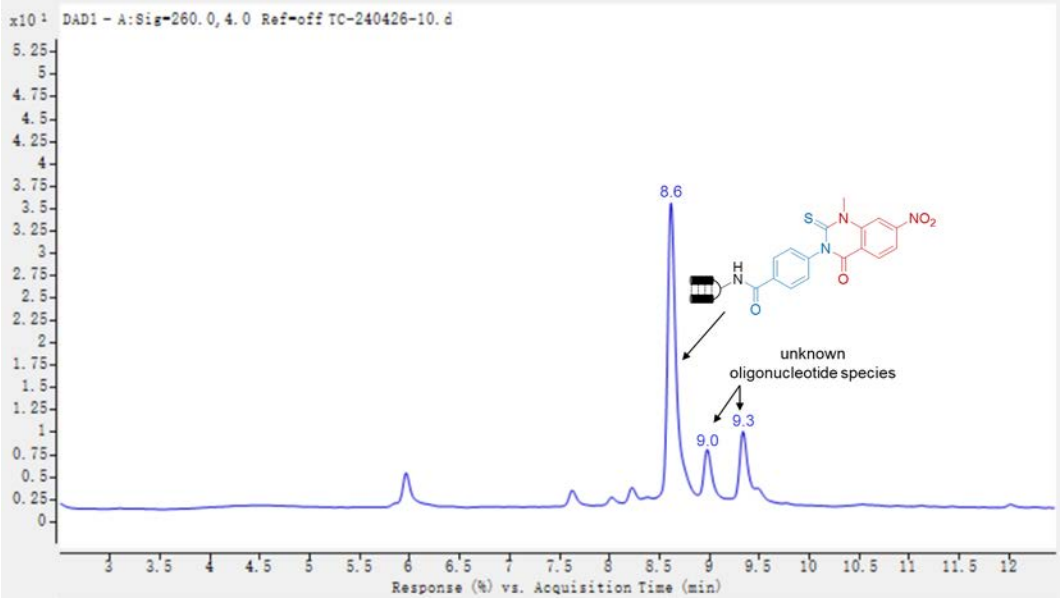


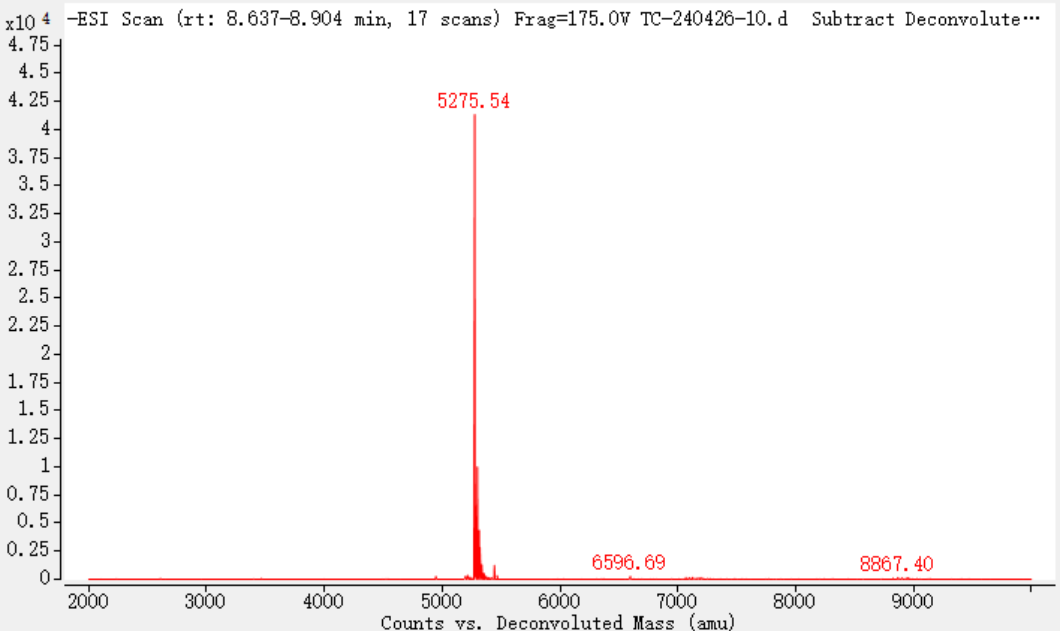


UPLC chromatogram and deconvoluted MS of **4ai**

Conversion: 75%

Calculated Mass: 5276 Da; Observed Mass: 5276 Da

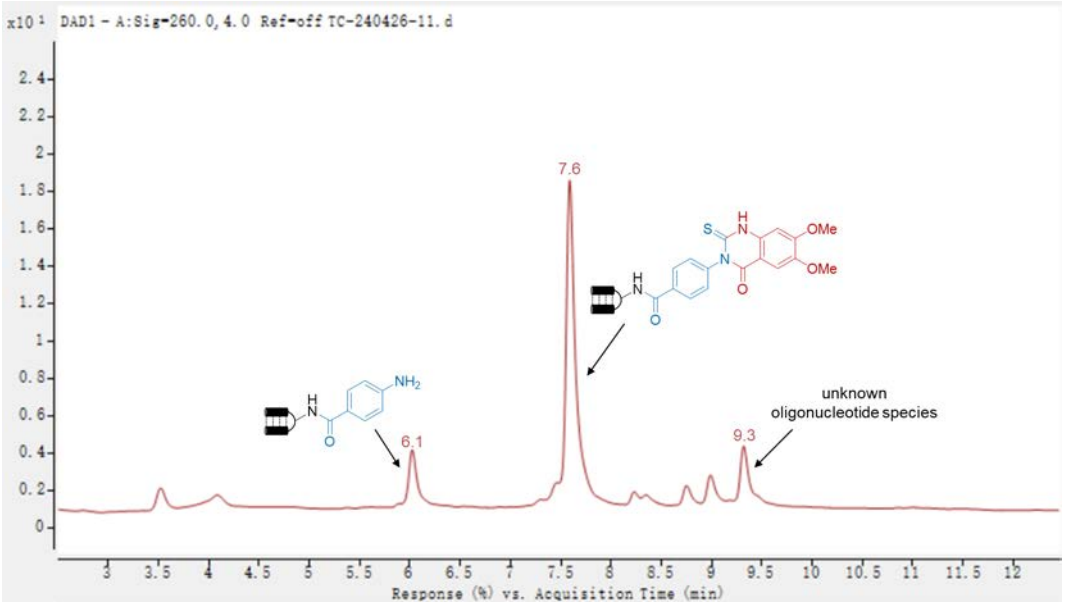


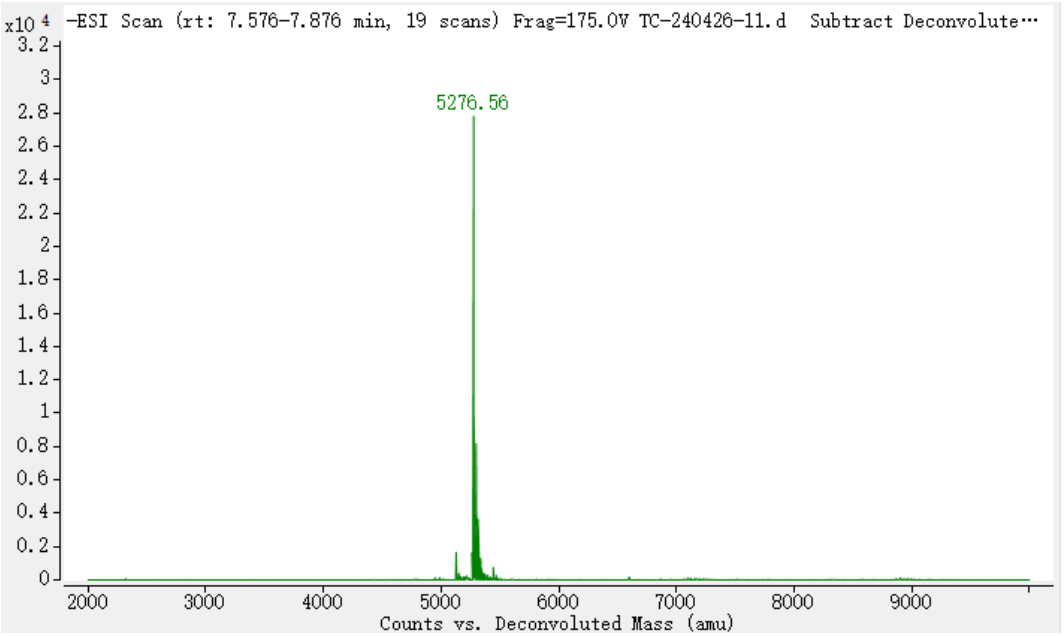


UPLC chromatogram and deconvoluted MS of **4aj**

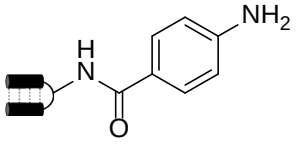
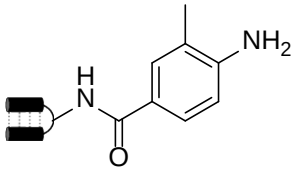
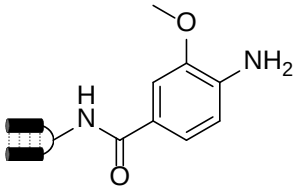
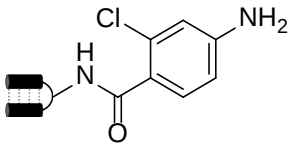
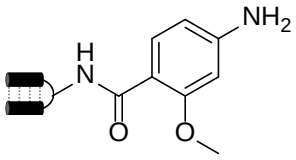
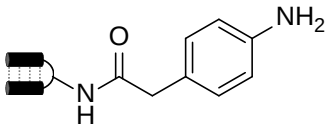
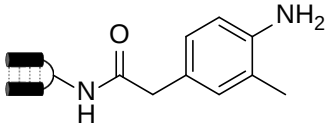
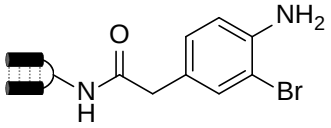
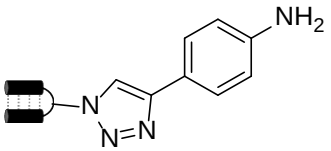
Conversion: 74%

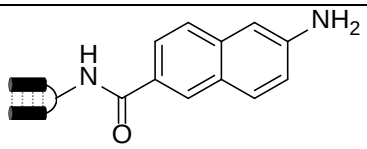
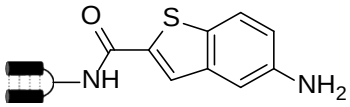
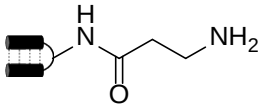
Calculated Mass: 5277 Da; Observed Mass: 5277 Da





7.3 Substrate scope of DNA-conjugated amines for on-DNA synthesis of 1,2,4-thiadiazoles

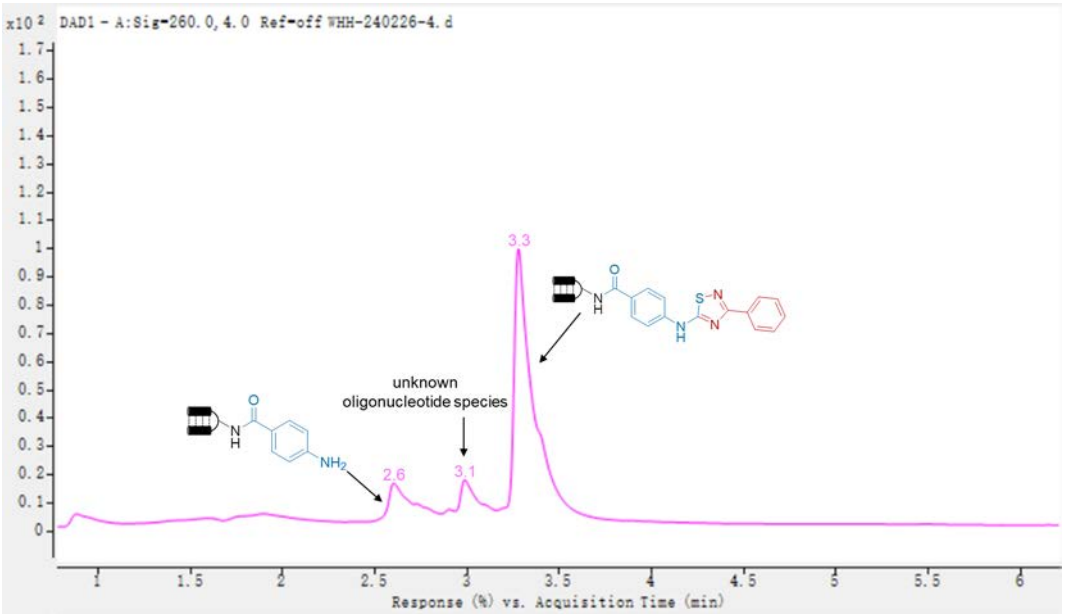
Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
1a		**6aa**	5216	5216	78%
1b		**6ba**	5230	5230	72%
1c		**6ca**	5246	5246	> 90%
1d		**6da**	5250	5250	78%
1e		**6ea**	5246	5246	81%
1f		**6fa**	5230	5230	> 90%
1g		**6ga**	5244	5244	> 90%
1h		**6ha**	5308	5308	79%
1i		**6ia**	5240	5240	72%

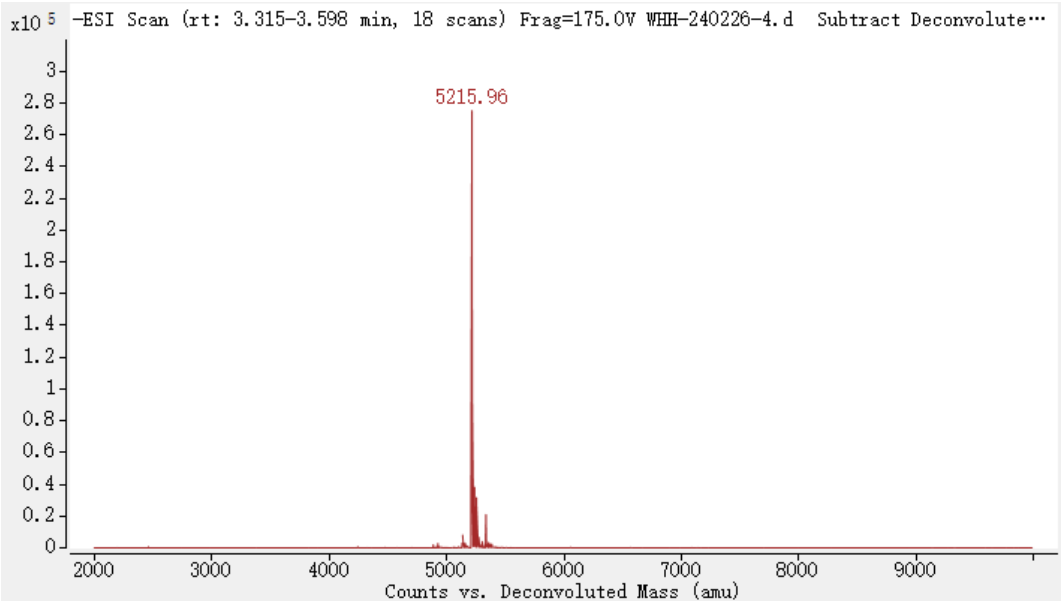
1j		**6ja**	5266	5266	85%
1k		**6ka**	5272	5272	87%
1a'		**6a'a**	5168	5168	12%

UPLC chromatogram and deconvoluted MS of **6aa**

Conversion: 78%

Calculated Mass: 5216 Da; Observed Mass: 5216 Da

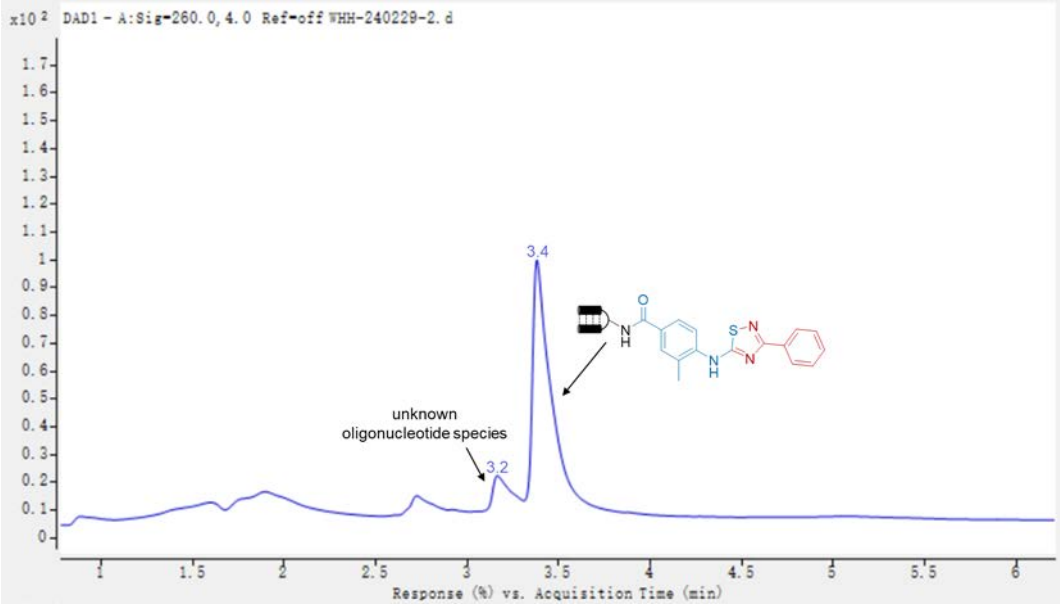


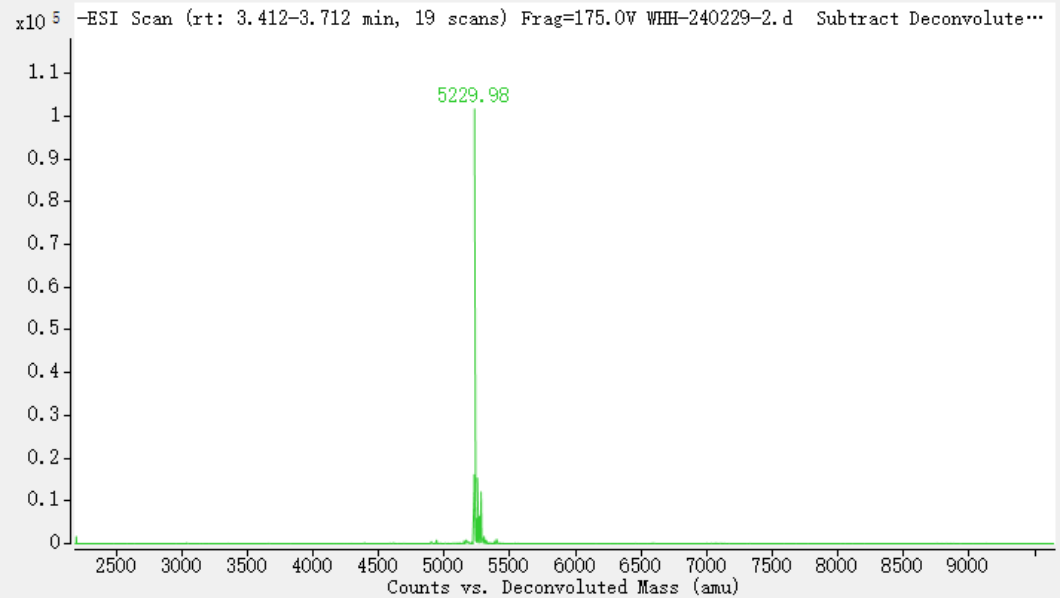


UPLC chromatogram and deconvoluted MS of **6ba**

Conversion: 72%

Calculated Mass: 5230 Da; Observed Mass: 5230 Da

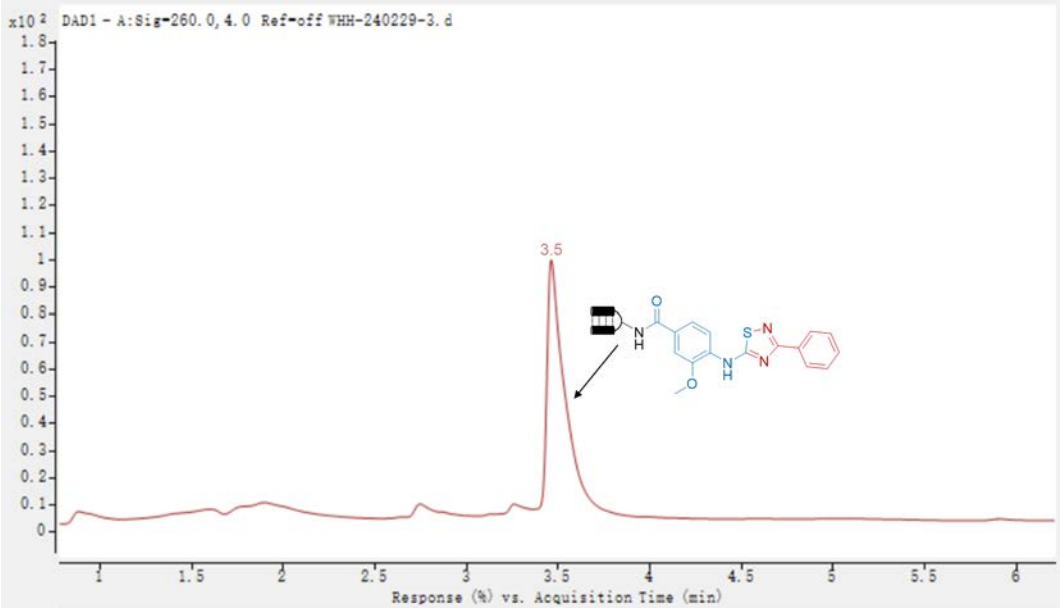


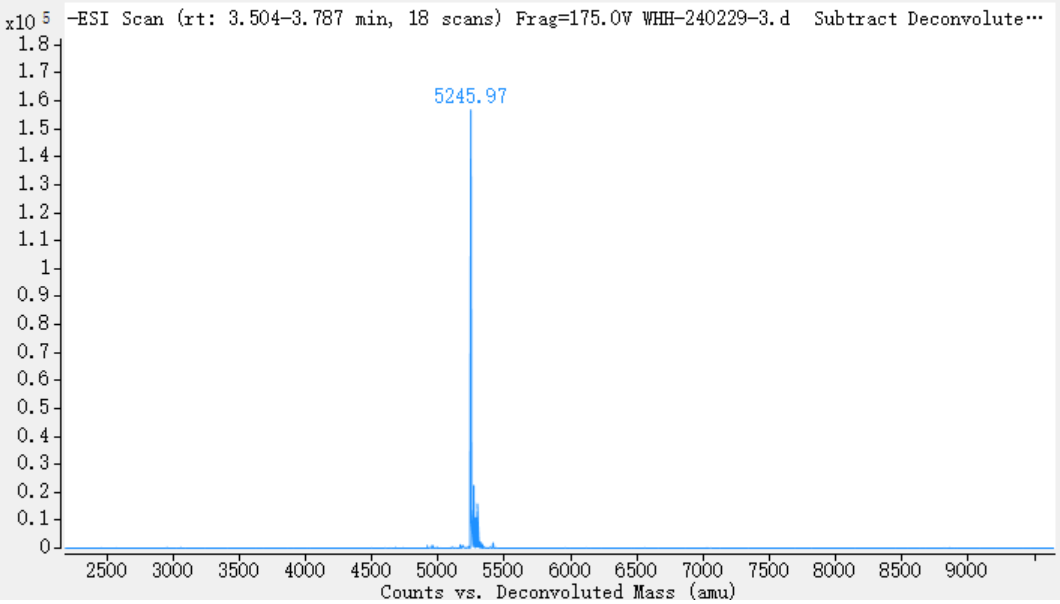


UPLC chromatogram and deconvoluted MS of **6ca**

Conversion: ＞90%

Calculated Mass: 5246 Da; Observed Mass: 5246 Da

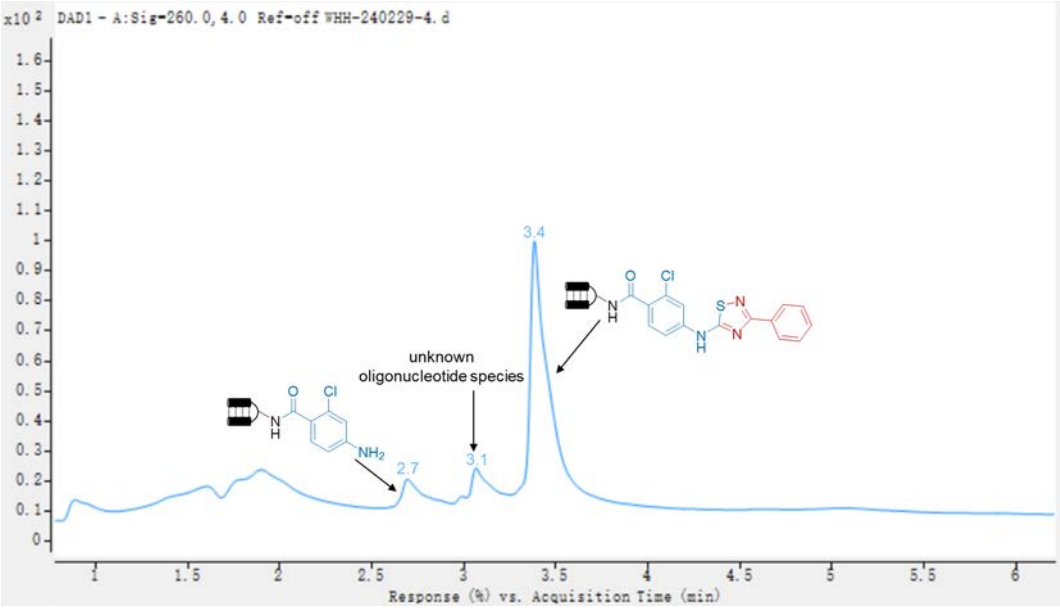


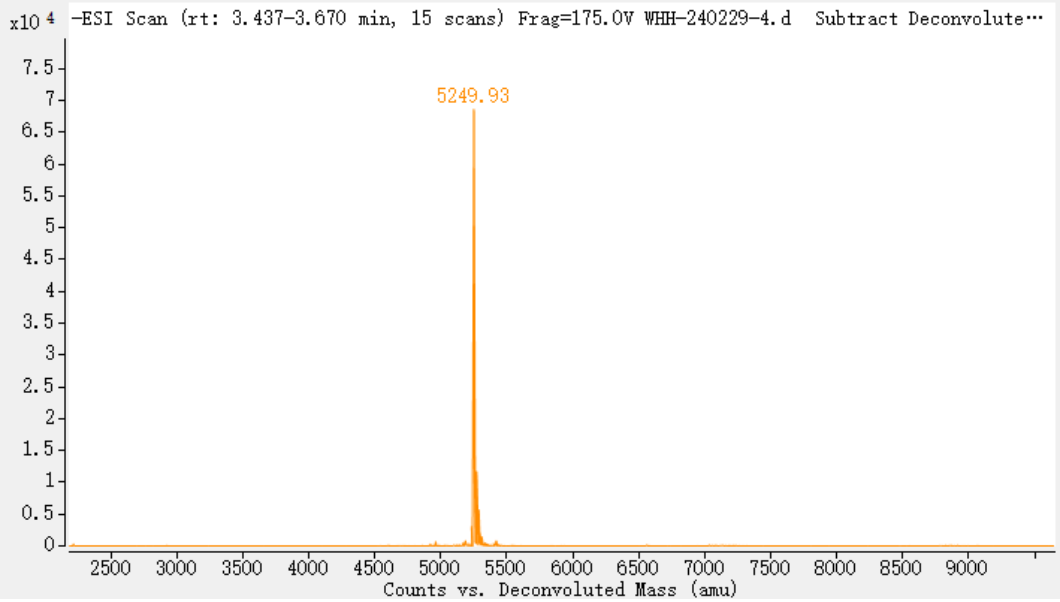


UPLC chromatogram and deconvoluted MS of **6da**

Conversion: 78%

Calculated Mass: 5250 Da; Observed Mass: 5250 Da

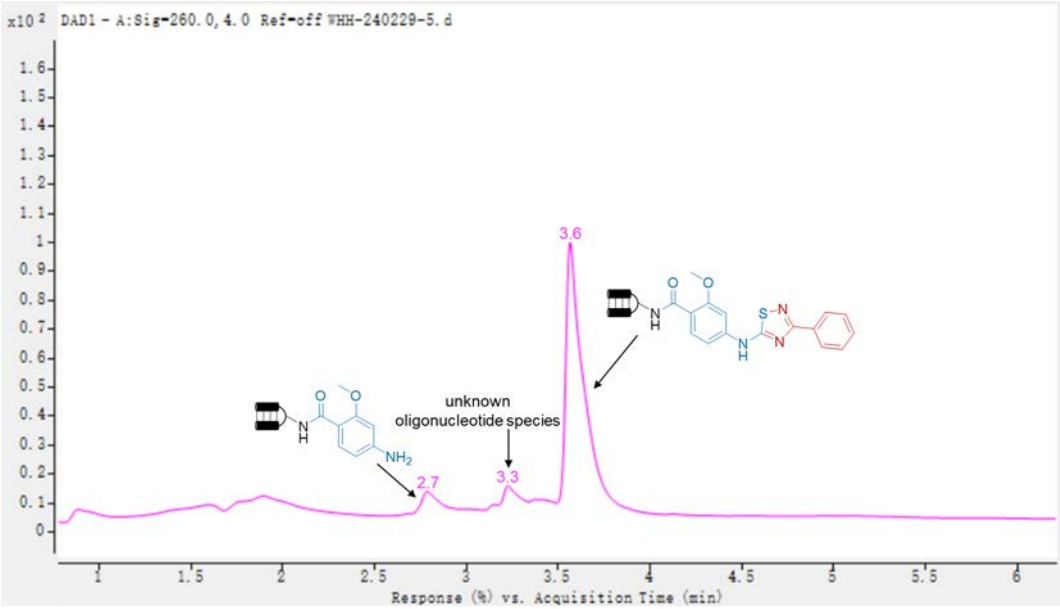


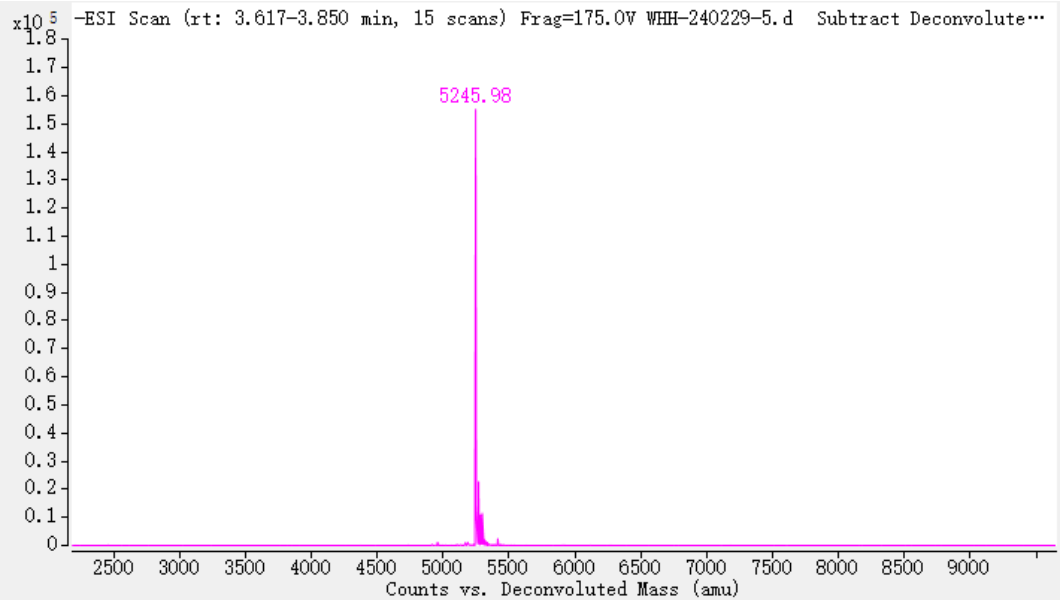


UPLC chromatogram and deconvoluted MS of **6ea**

Conversion: 81%

Calculated Mass: 5246 Da; Observed Mass: 5246 Da

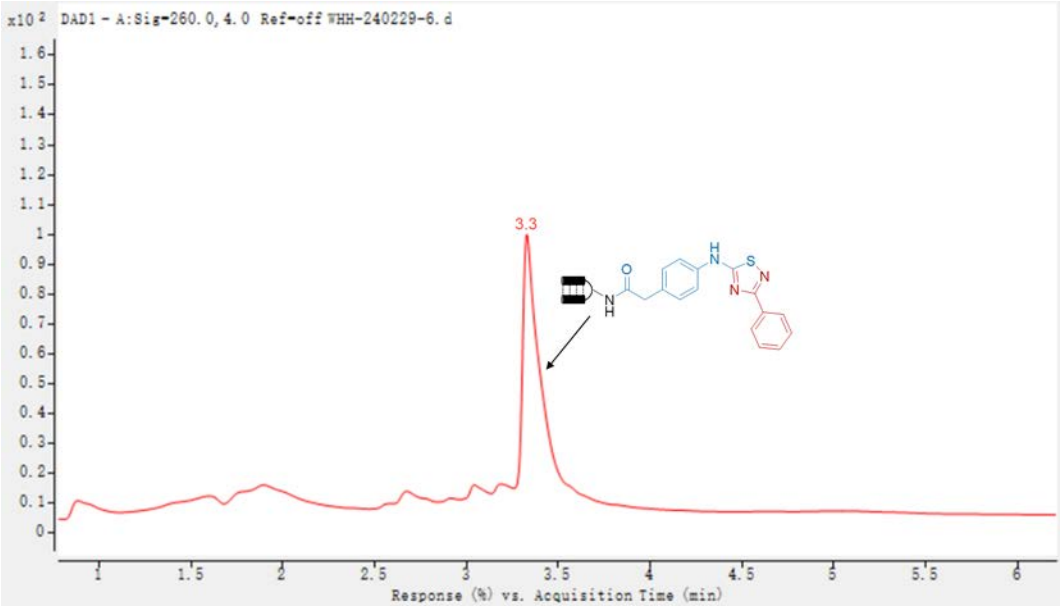


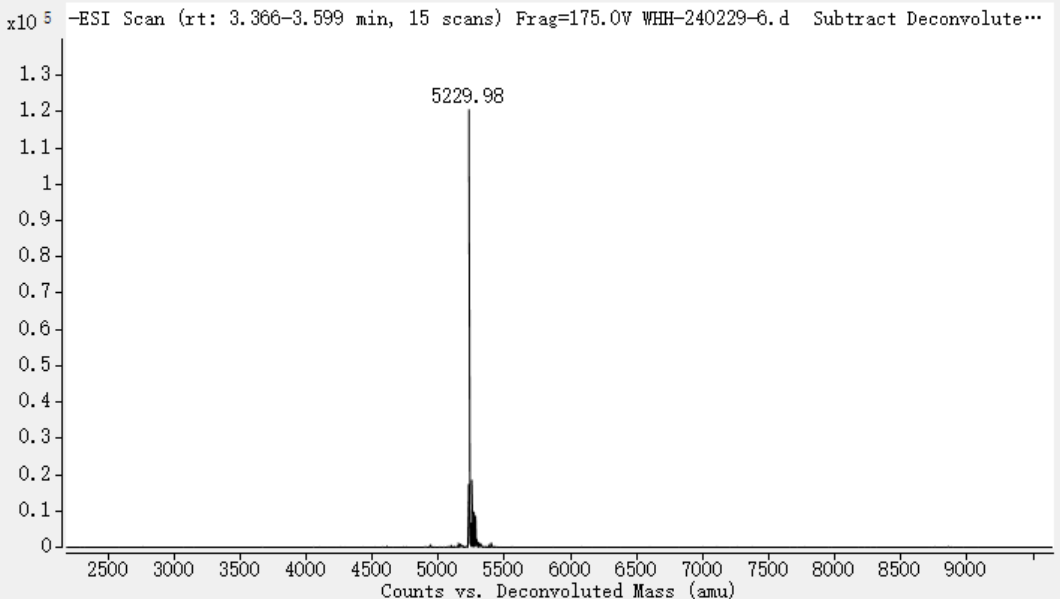


UPLC chromatogram and deconvoluted MS of **6fa**

Conversion: ＞90%

Calculated Mass: 5230 Da; Observed Mass: 5230 Da

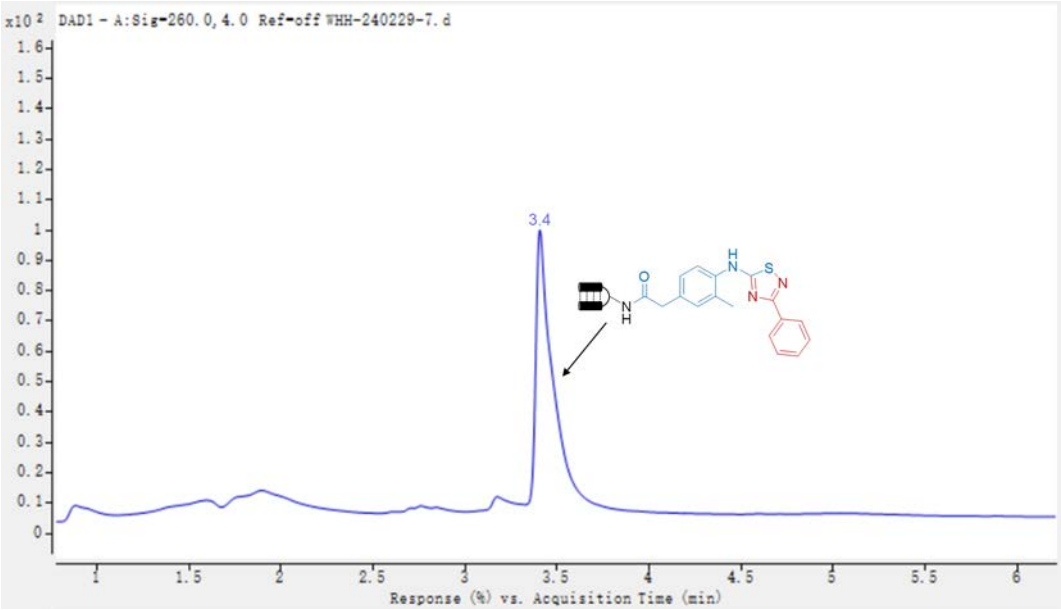


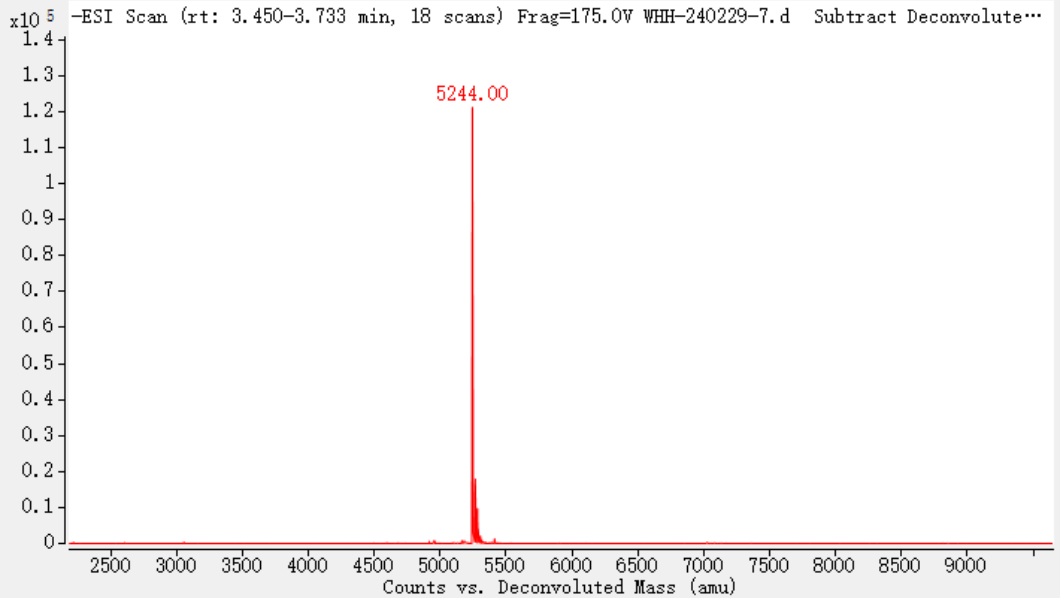


UPLC chromatogram and deconvoluted MS of **6ga**

Conversion: ＞90%

Calculated Mass: 5244 Da; Observed Mass: 5244 Da

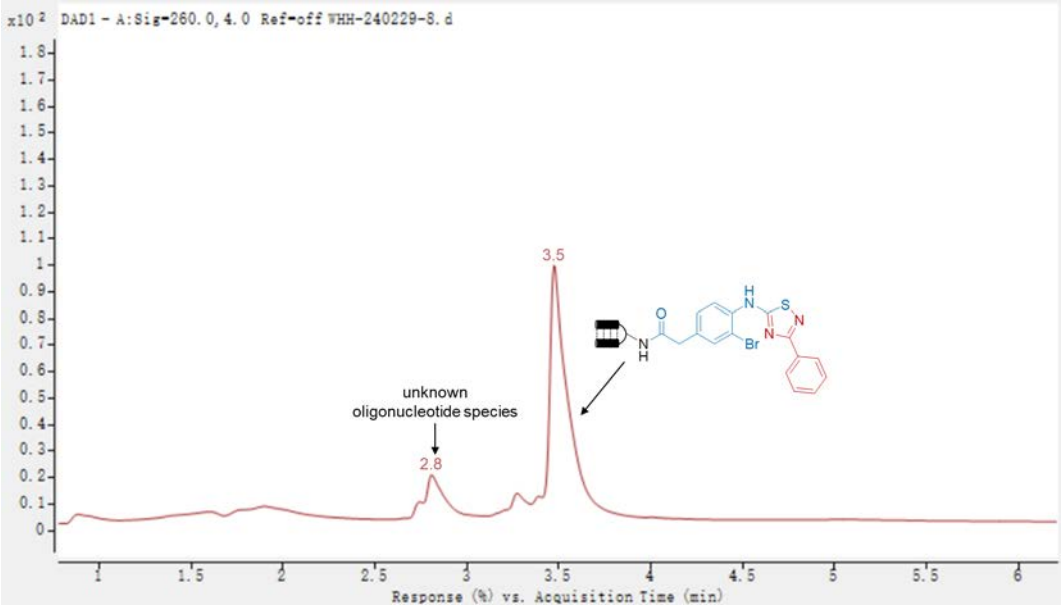


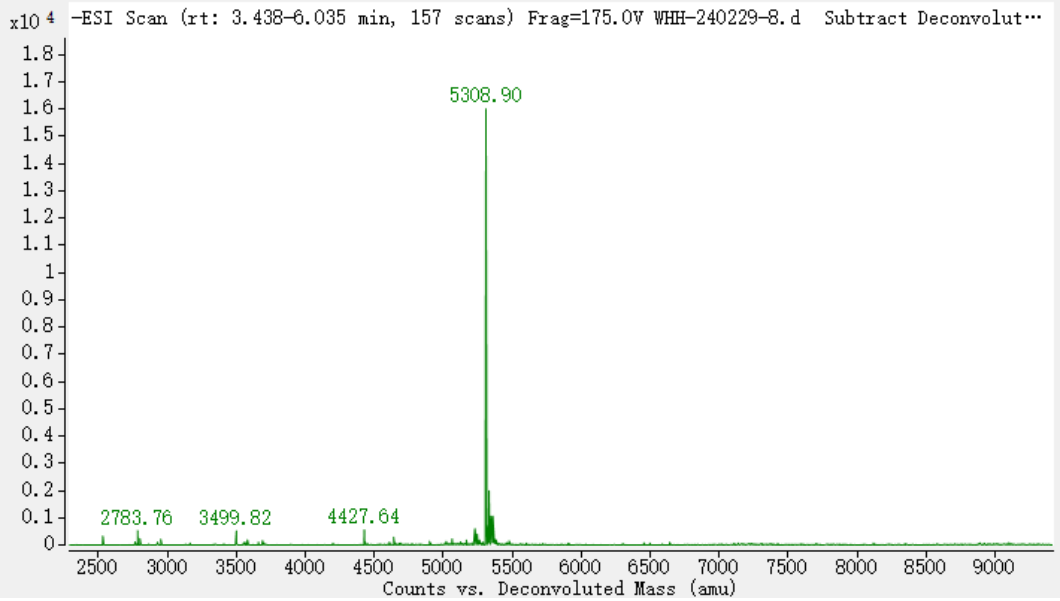


UPLC chromatogram and deconvoluted MS of **6ha**

Conversion: 79%

Calculated Mass: 5309 Da; Observed Mass: 5309 Da

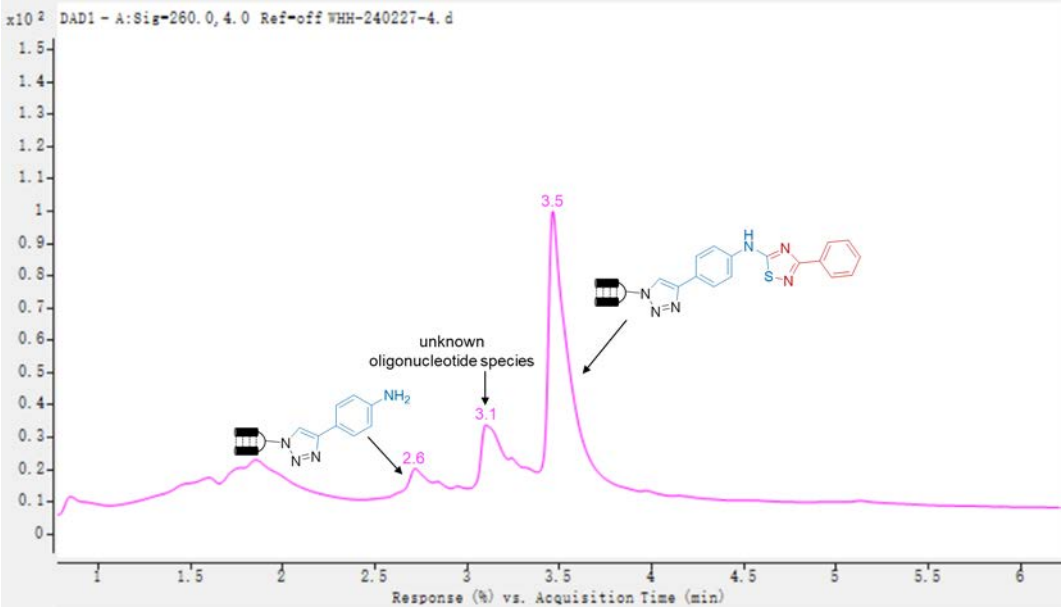


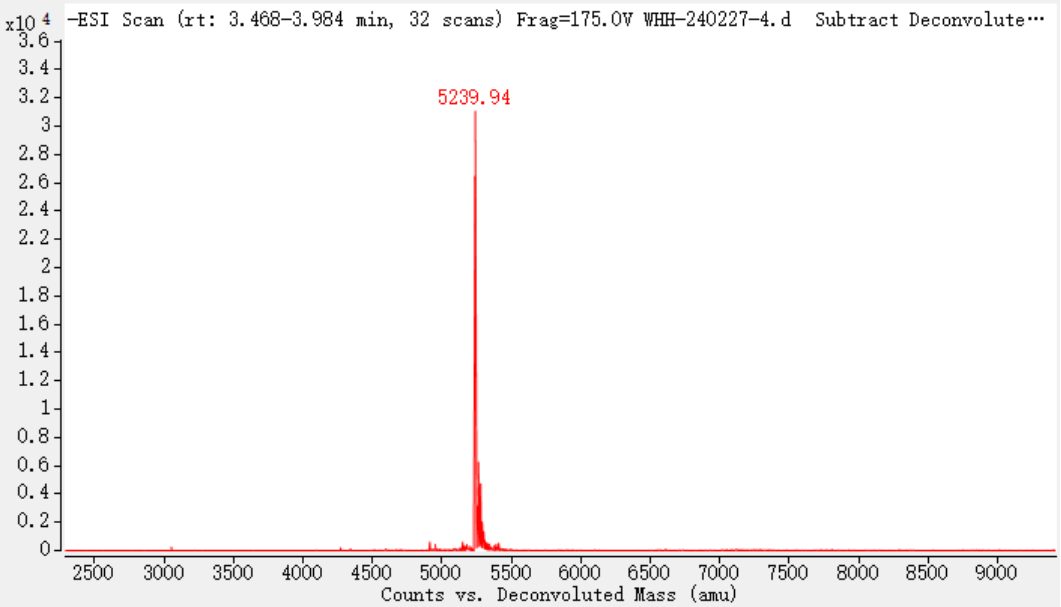


UPLC chromatogram and deconvoluted MS of **6ia**

Conversion: 72%

Calculated Mass: 5240 Da; Observed Mass: 5240 Da

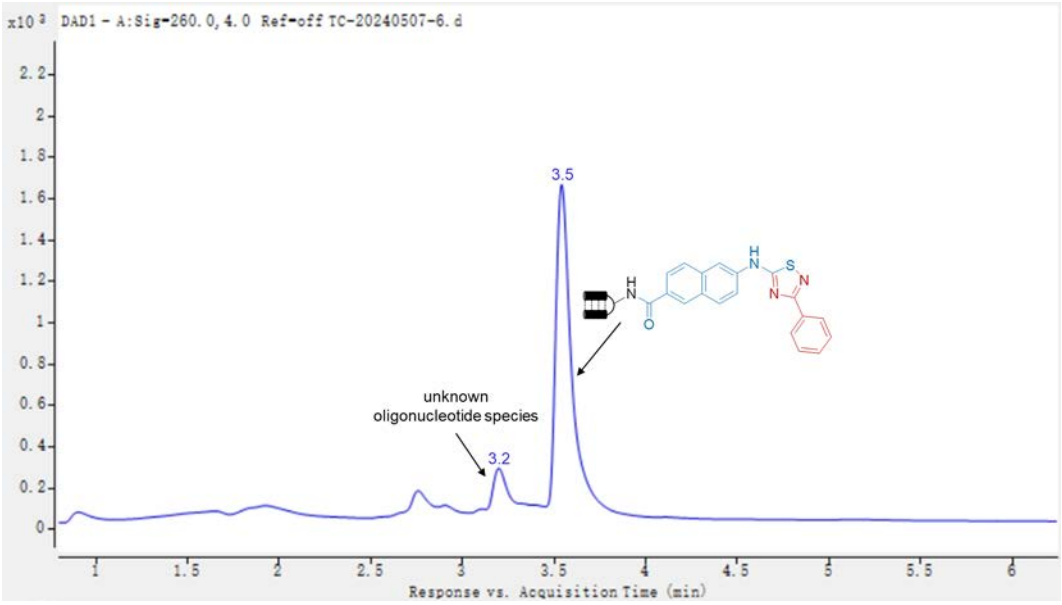


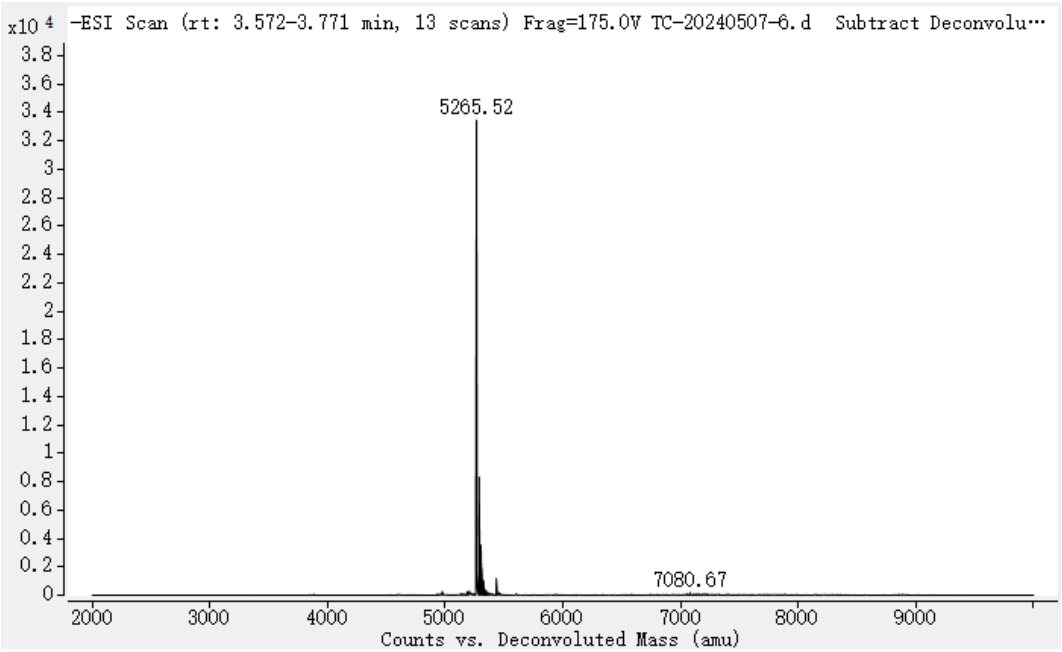


UPLC chromatogram and deconvoluted MS of **6ja**

Conversion: 85%

Calculated Mass: 5266 Da; Observed Mass: 5266 Da

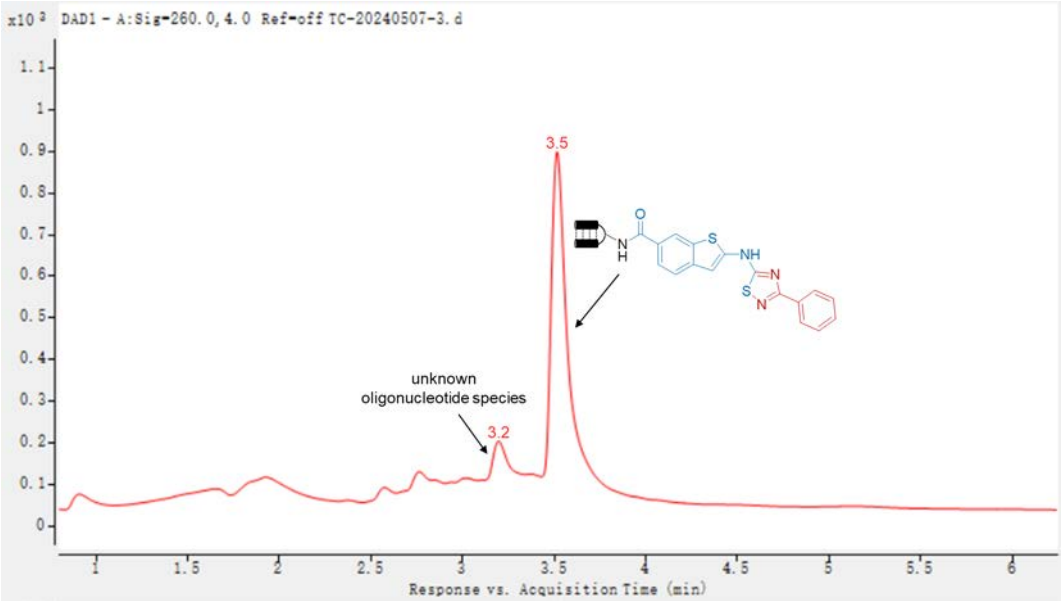


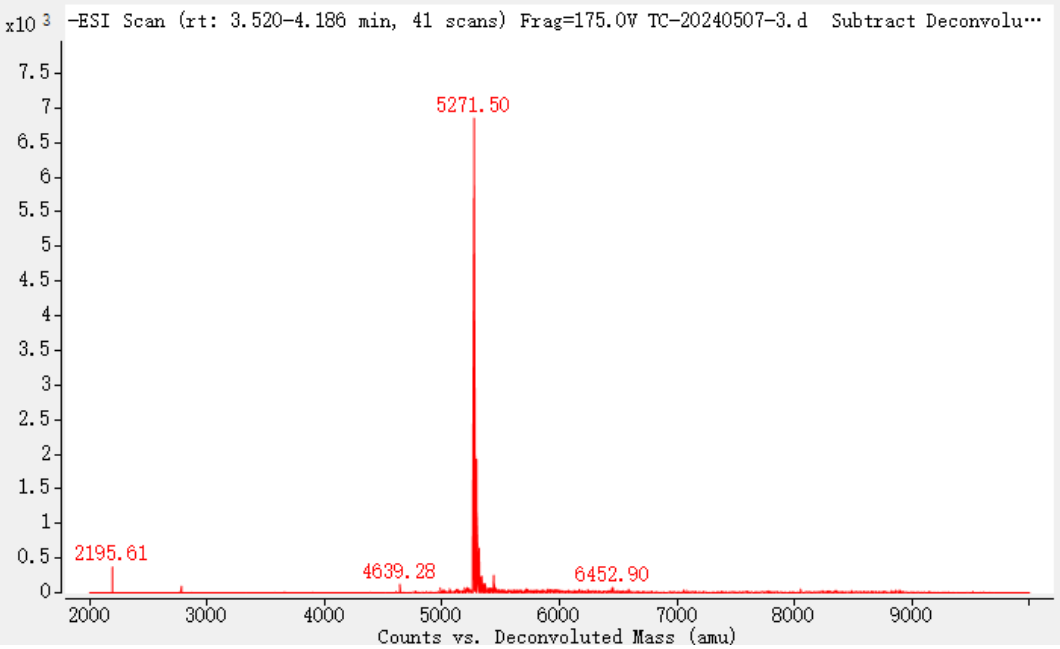


UPLC chromatogram and deconvoluted MS of **6ka**

Conversion: 87%

Calculated Mass: 5272 Da; Observed Mass: 5272 Da

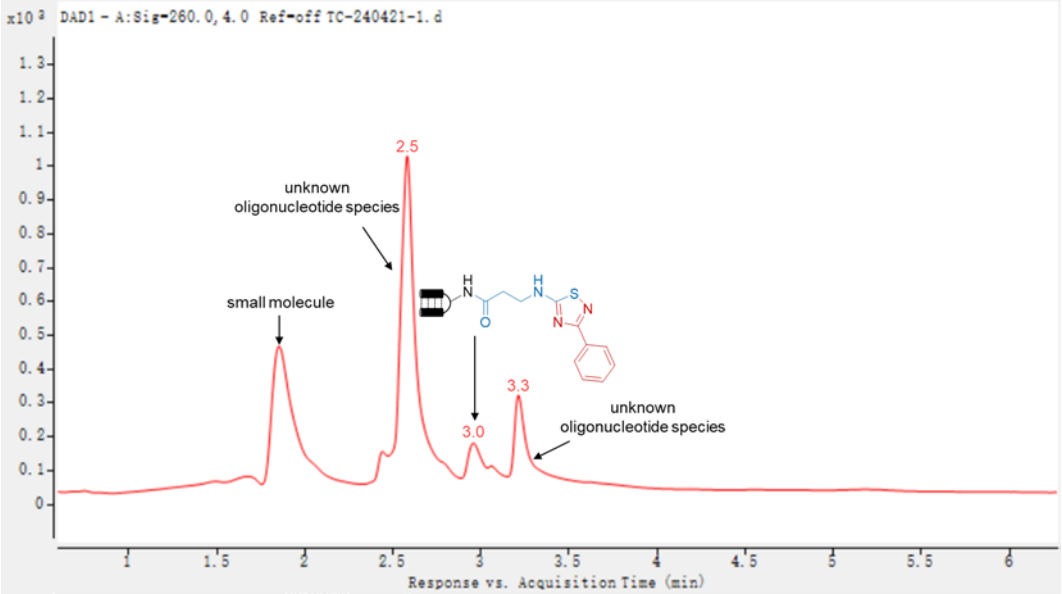


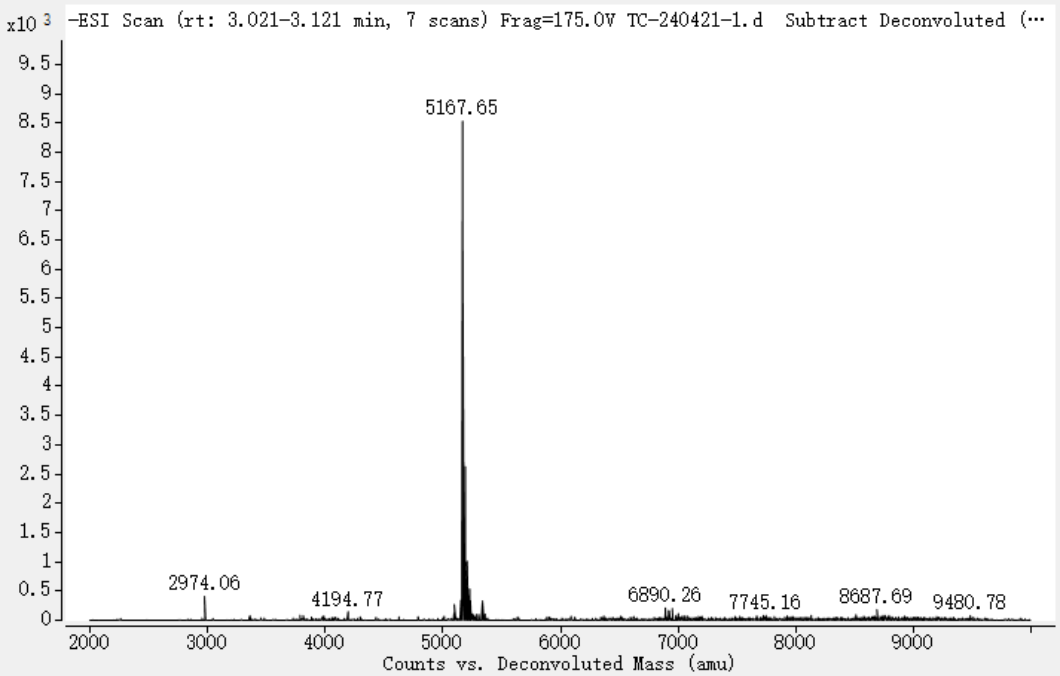


UPLC chromatogram and deconvoluted MS of **6a'a**

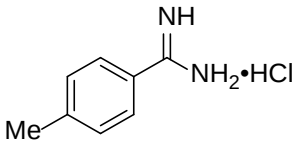
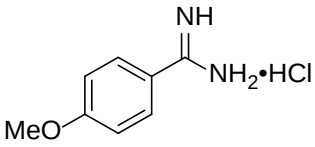
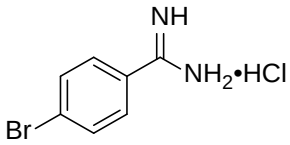
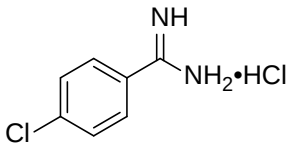
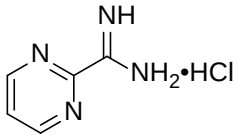
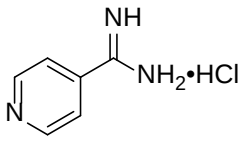
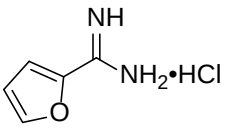
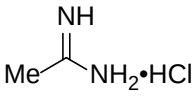
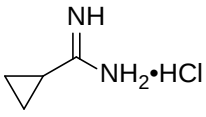
Conversion: 12%

Calculated Mass: 5168 Da; Observed Mass: 5168 Da





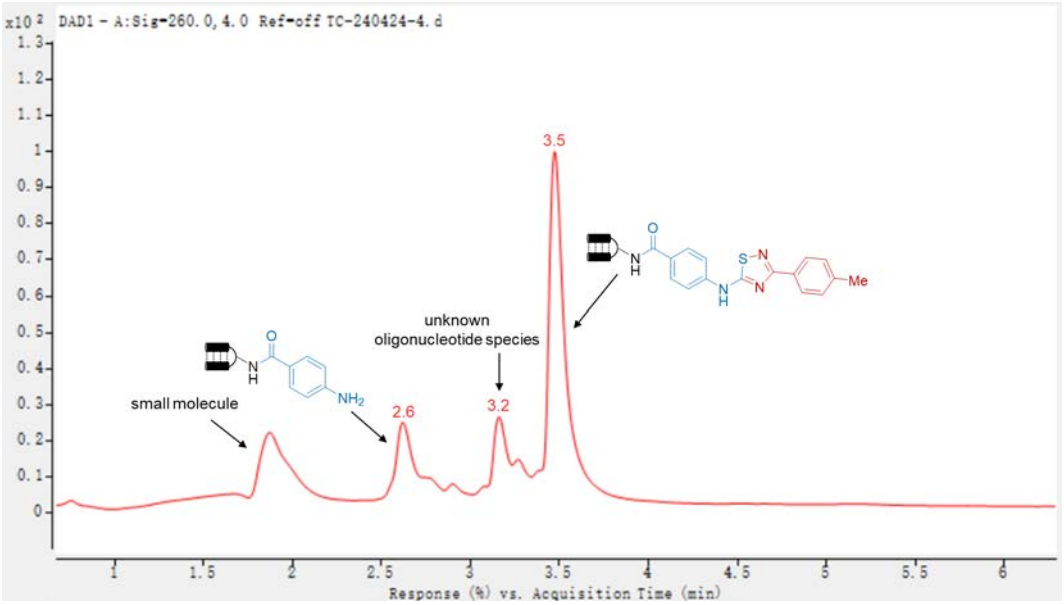
7.4 Substrate scope of amidines

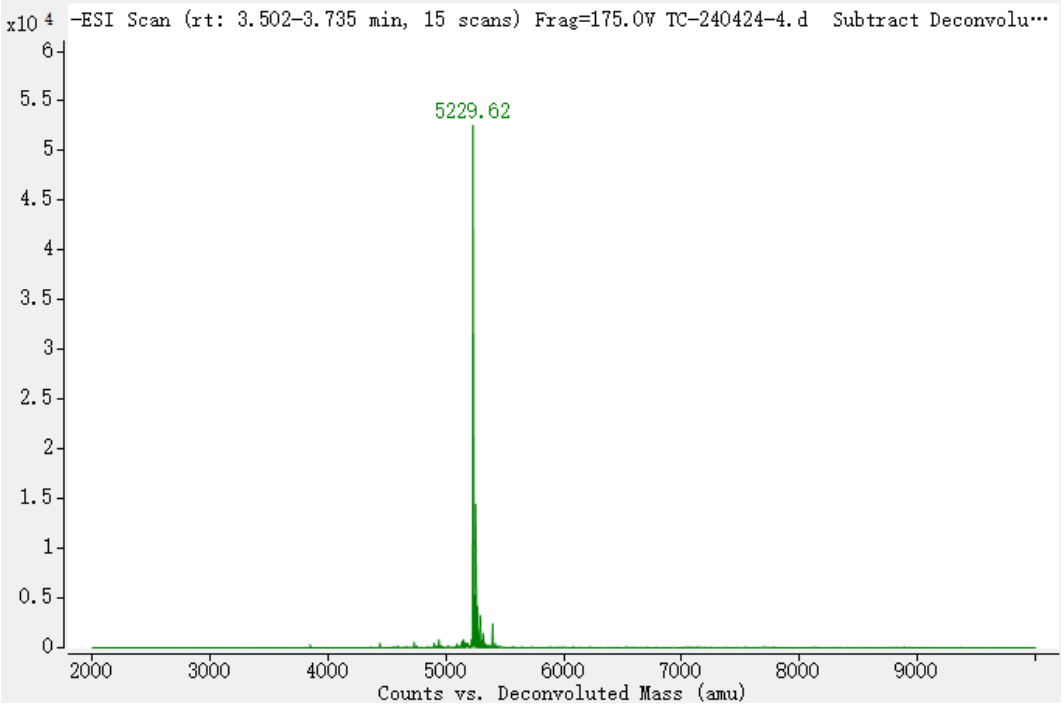
Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
5b		**6ab**	5230	5230	73%
5c		**6ac**	5246	5246	66%
5d		**6ad**	5294	5295	71%
5e		**6ae**	5250	5251	78%
5f		**6af**	5218	5218	74%
5g		**6ag**	5217	5217	61%
5h		**6ah**	5206	5206	78%
5i		**6ai**	5154	5154	67%
5j		**6aj**	5180	5180	66%

UPLC chromatogram and deconvoluted MS of **6ab**

Conversion: 73%

Calculated Mass: 5230 Da; Observed Mass: 5230 Da

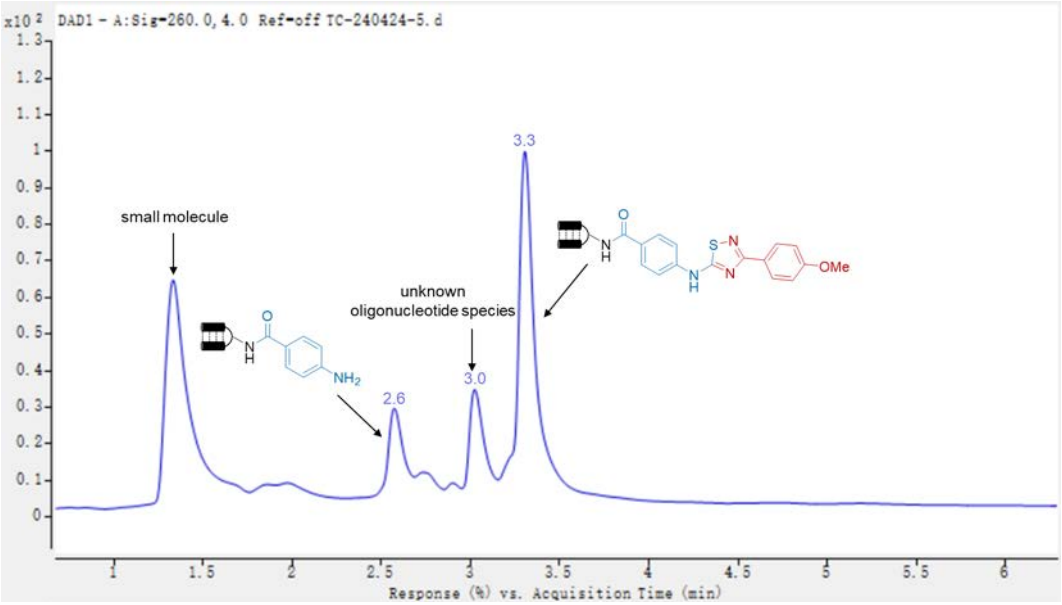


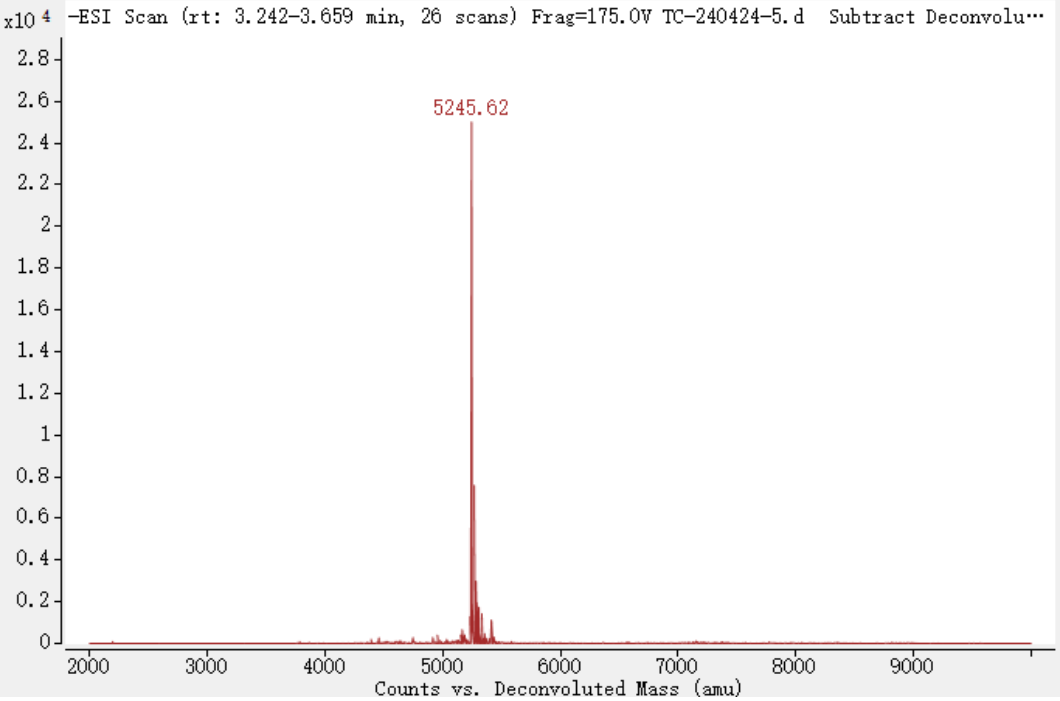


UPLC chromatogram and deconvoluted MS of **6ac**

Conversion: 66%

Calculated Mass: 5246 Da; Observed Mass: 5246 Da

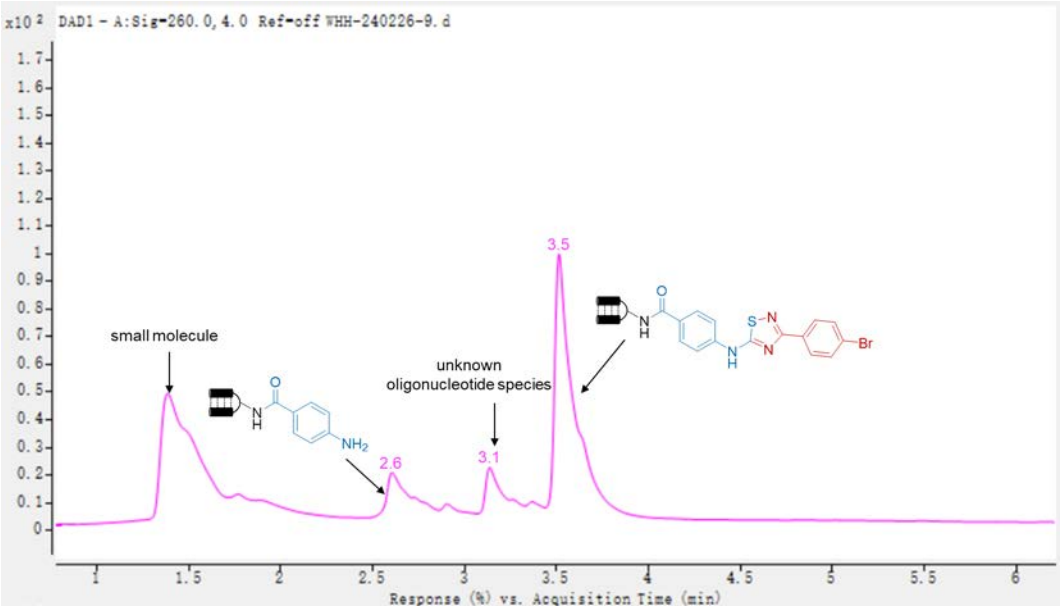


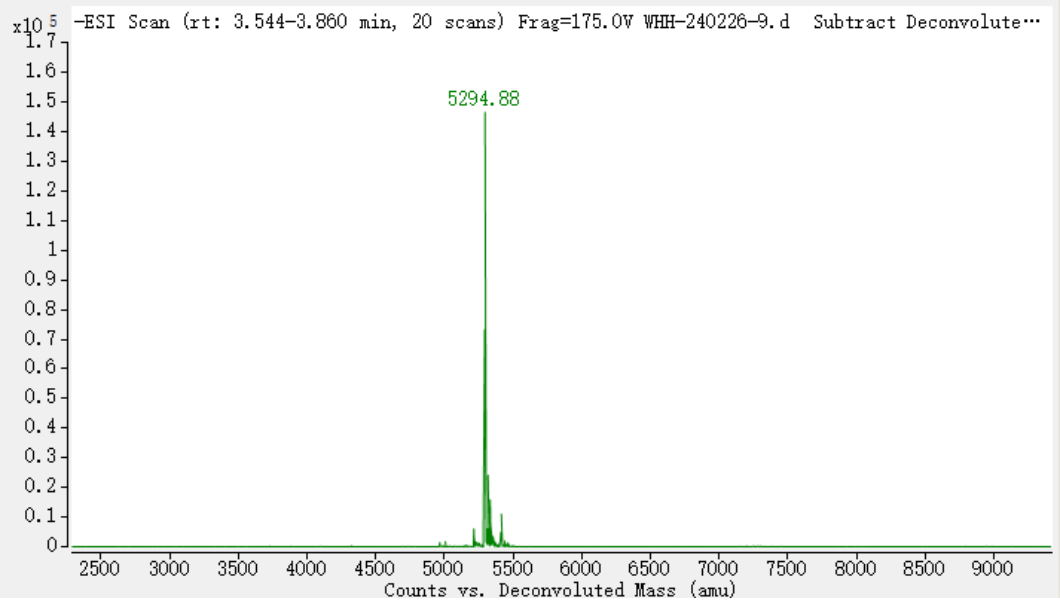


UPLC chromatogram and deconvoluted MS of **6ad**

Conversion: 71%

Calculated Mass: 5294 Da; Observed Mass: 5295 Da

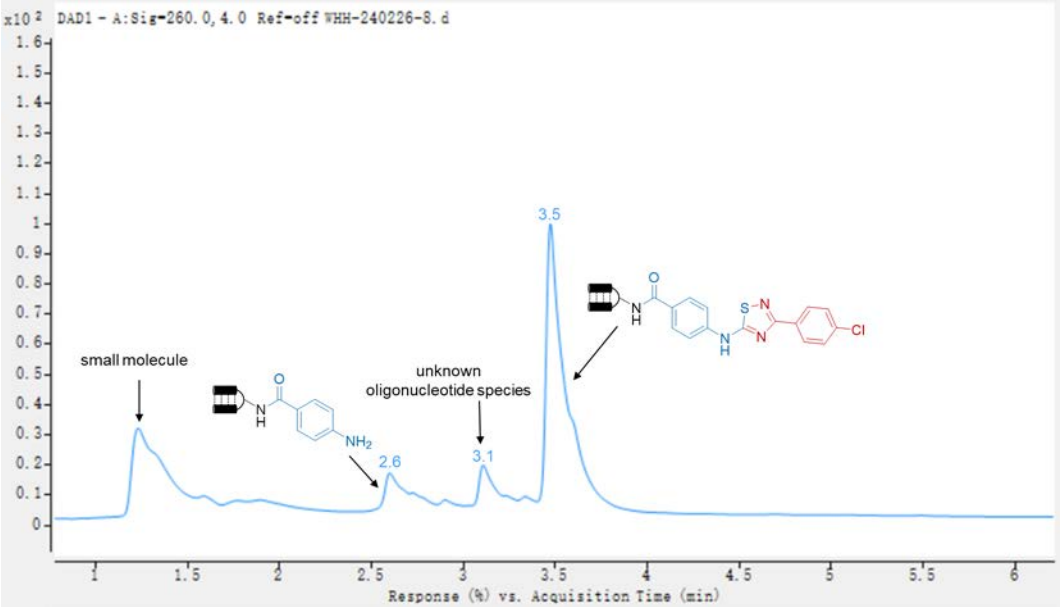


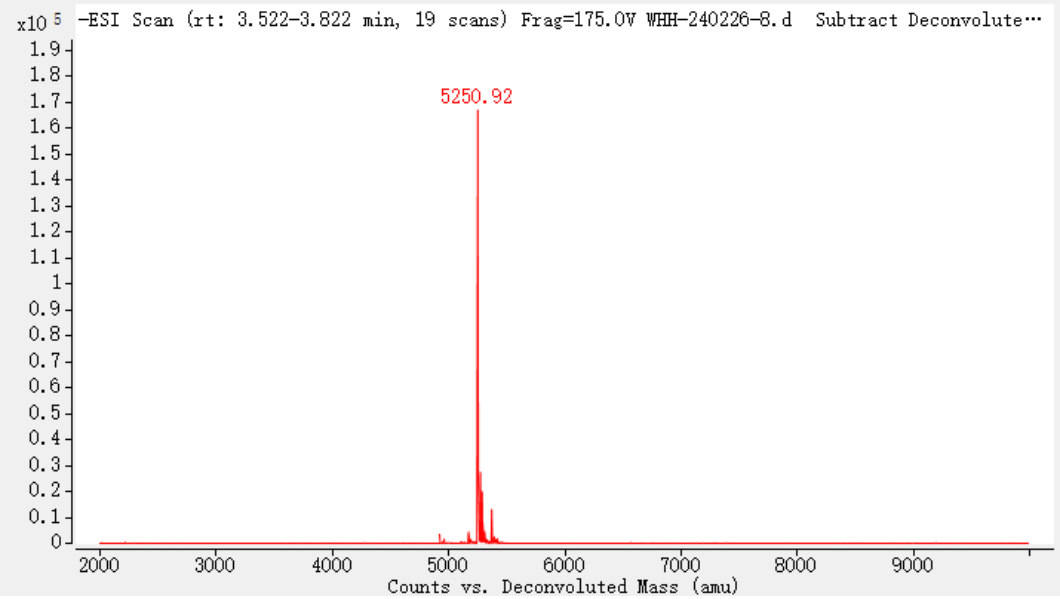


UPLC chromatogram and deconvoluted MS of **6ae**

Conversion: 78%

Calculated Mass: 5250 Da; Observed Mass: 5251 Da

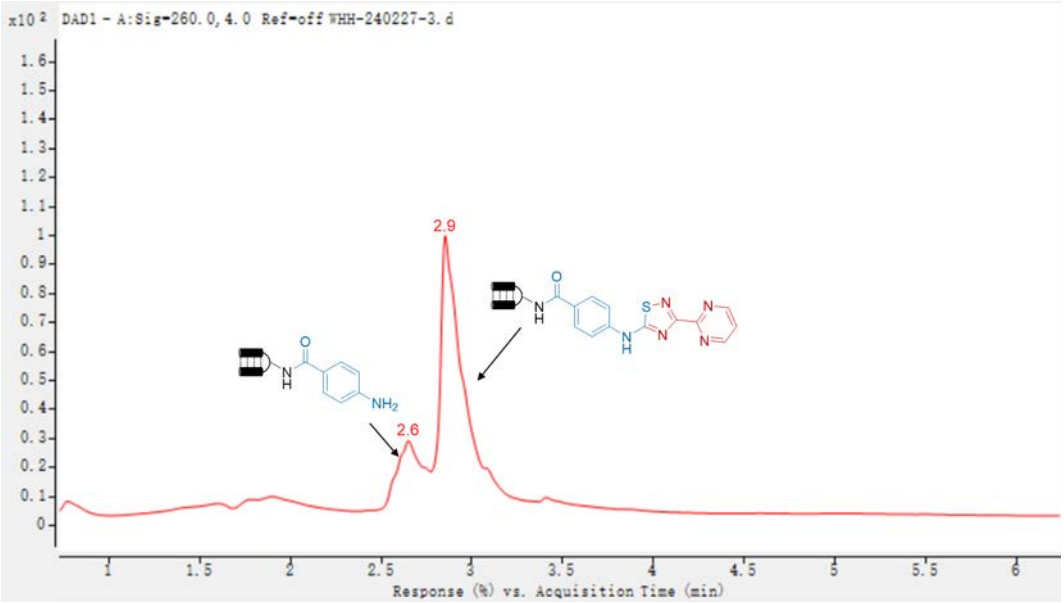


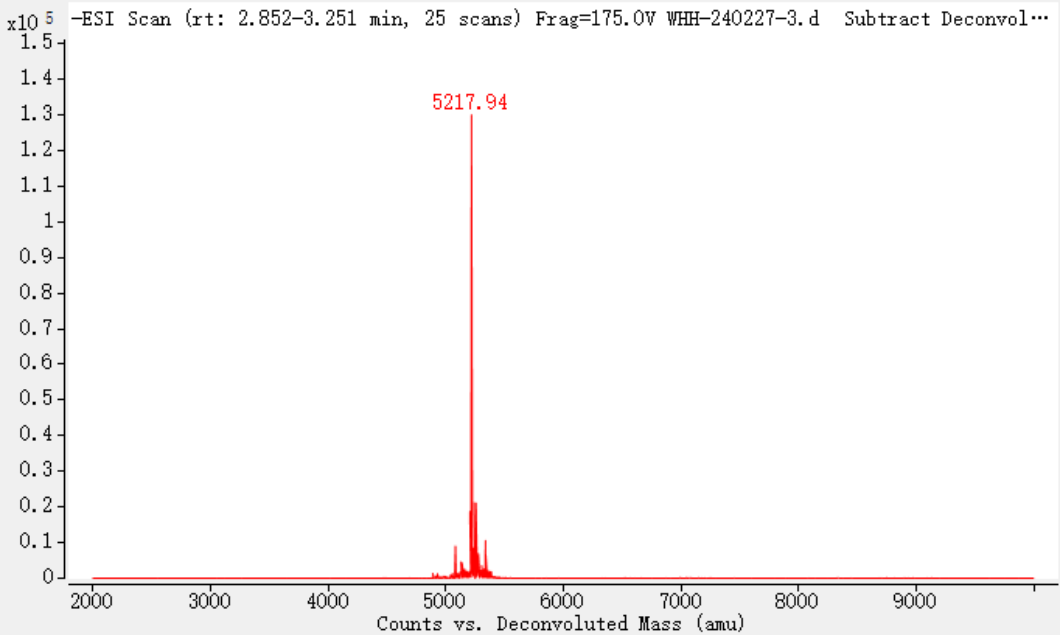


UPLC chromatogram and deconvoluted MS of **6af**

Conversion: 70%

Calculated Mass: 5218 Da; Observed Mass: 5218 Da

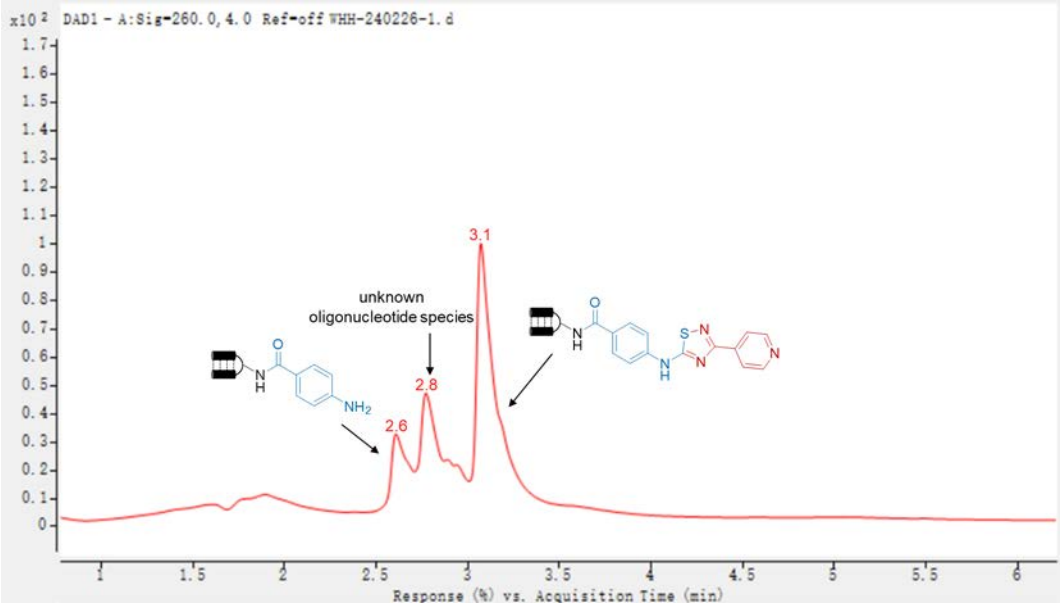


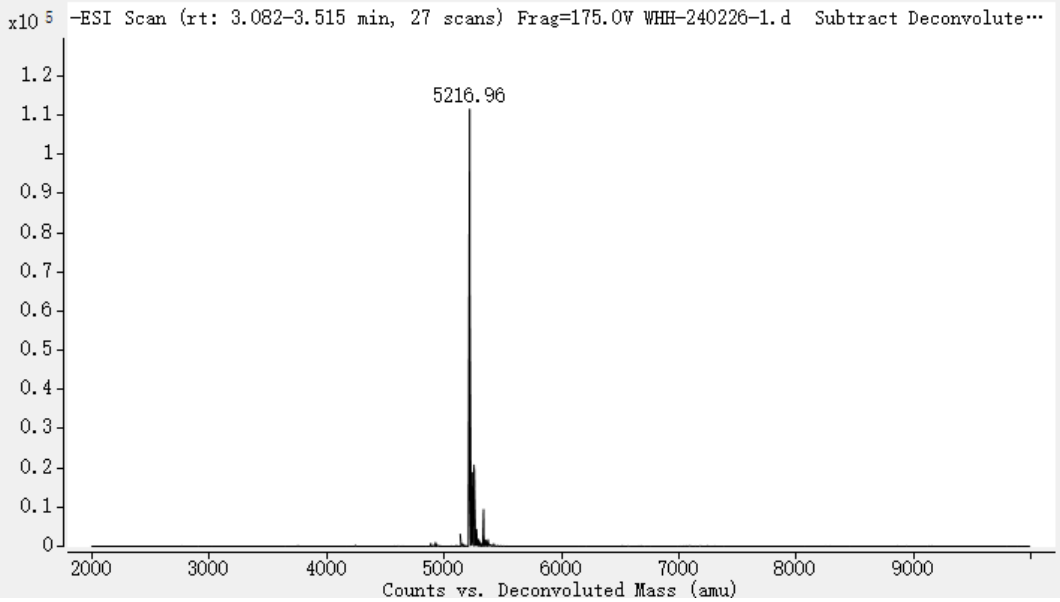


UPLC chromatogram and deconvoluted MS of **6ag**

Conversion: 61%

Calculated Mass: 5217 Da; Observed Mass: 5217 Da

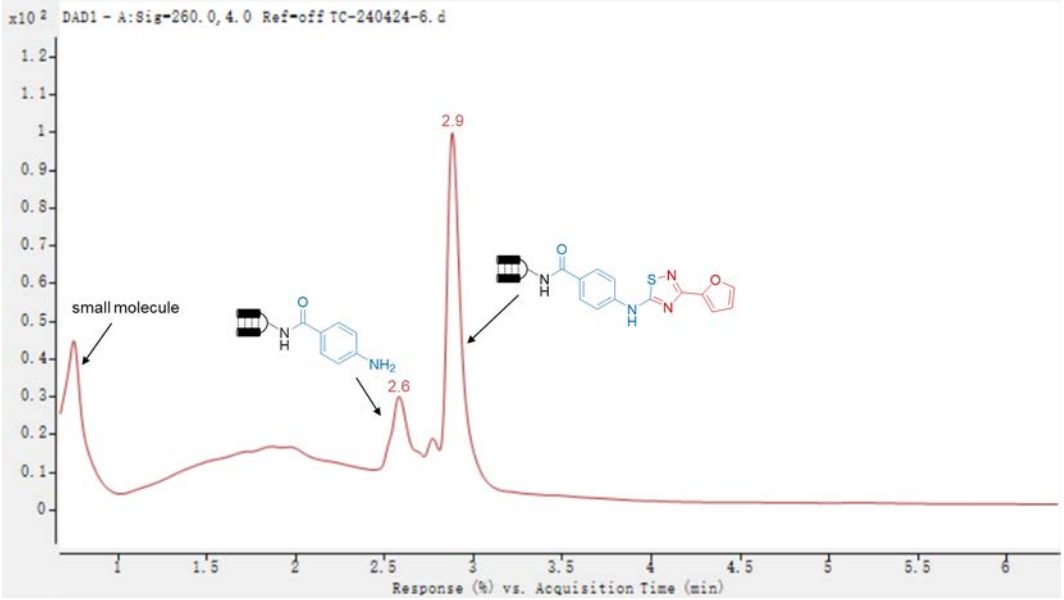


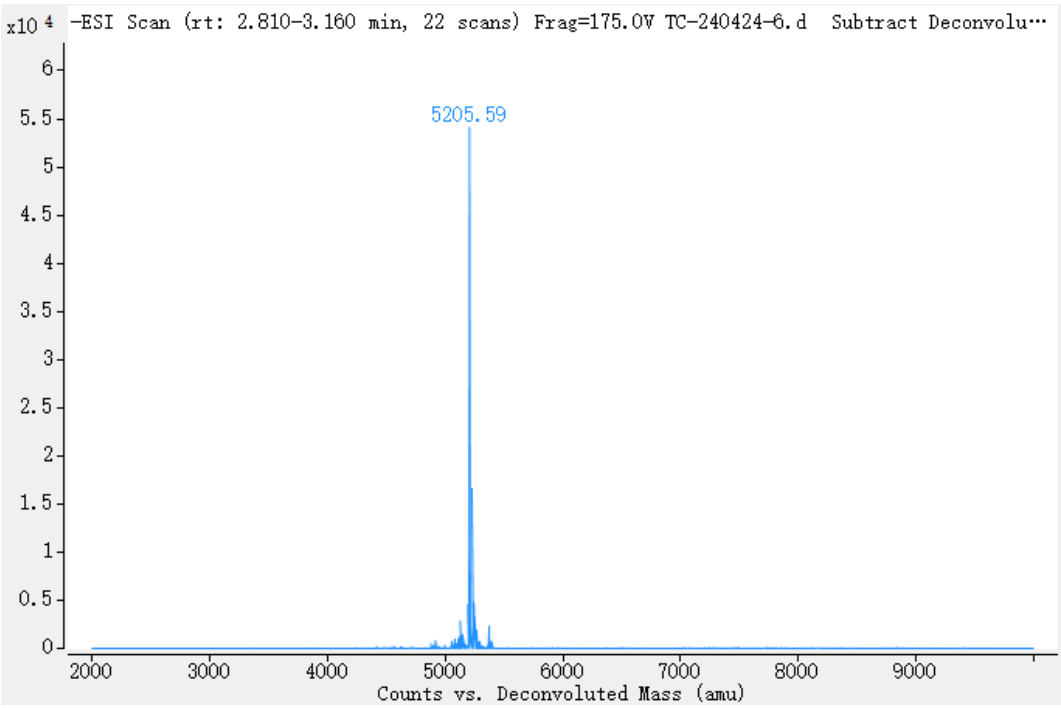


UPLC chromatogram and deconvoluted MS of **6ah**

Conversion: 78%

Calculated Mass: 5206 Da; Observed Mass: 5206 Da

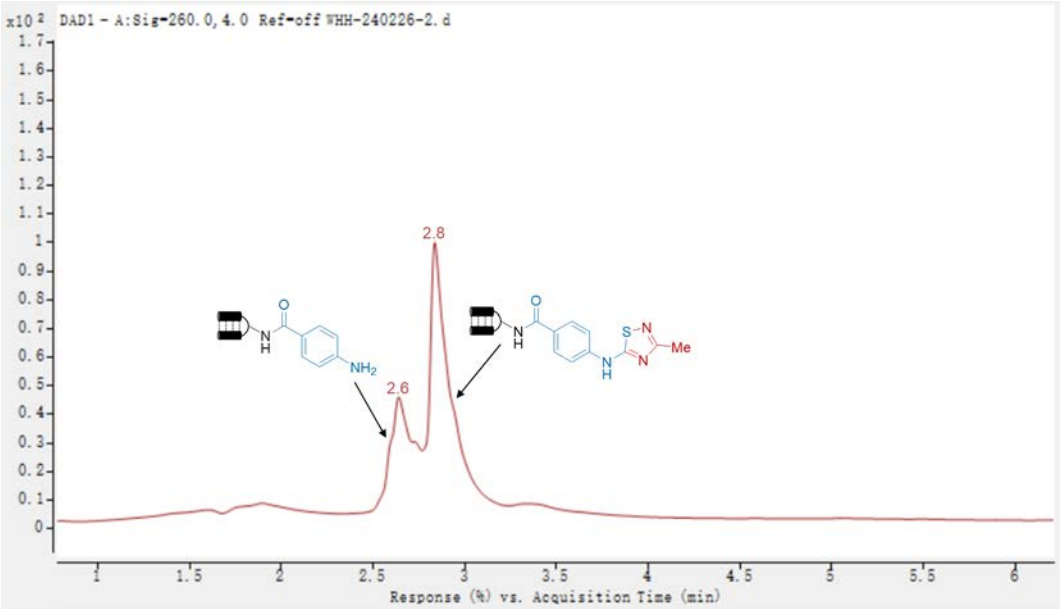


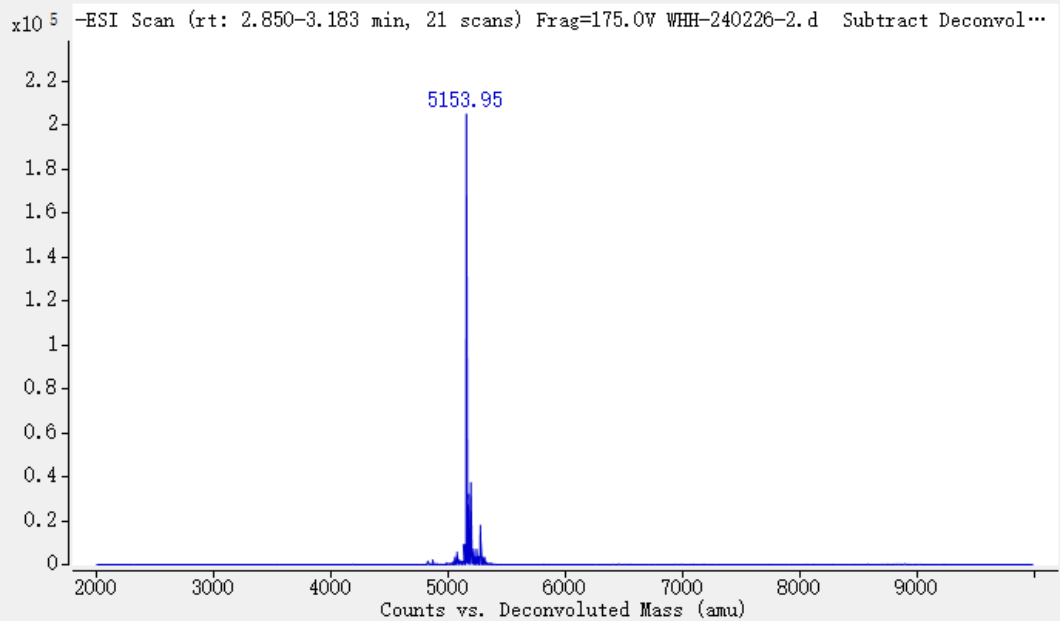


UPLC chromatogram and deconvoluted MS of **6ai**

Conversion: 67%

Calculated Mass: 5154 Da; Observed Mass: 5154 Da

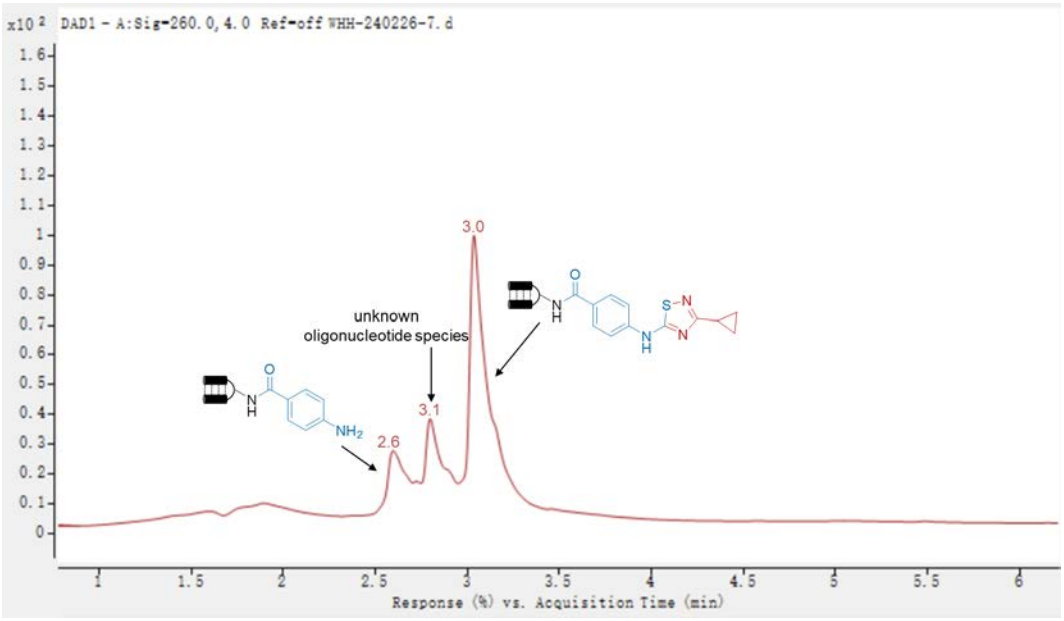


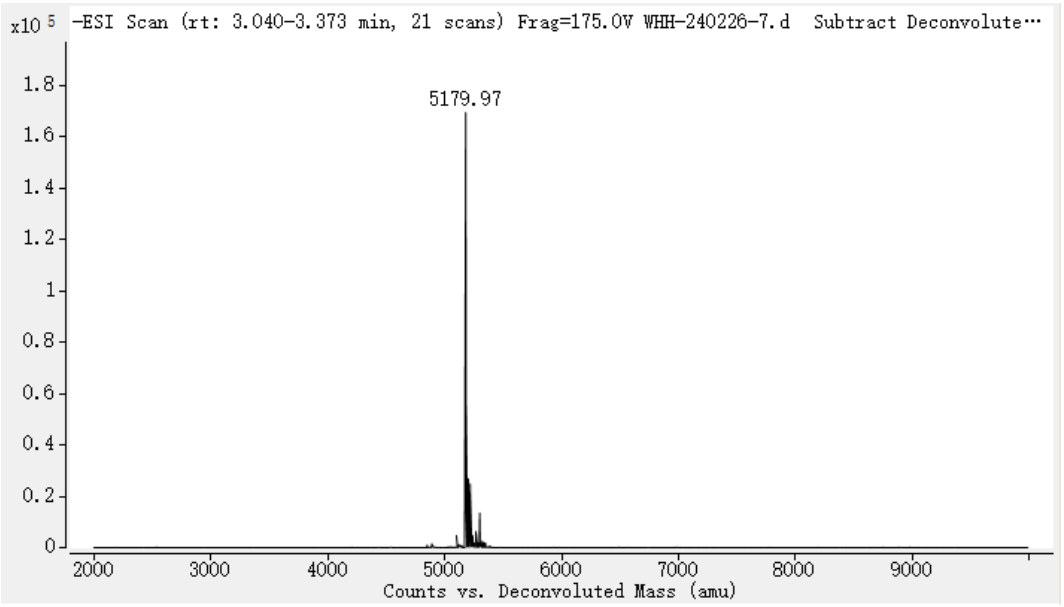


UPLC chromatogram and deconvoluted MS of **6aj**

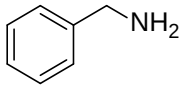
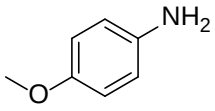
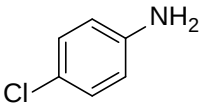
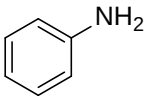
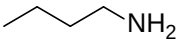
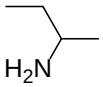
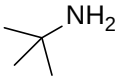
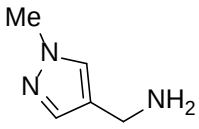
Conversion: 66%

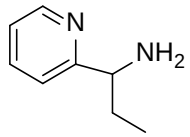
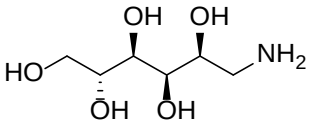
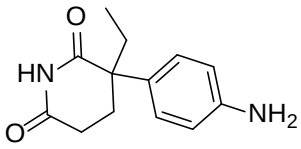
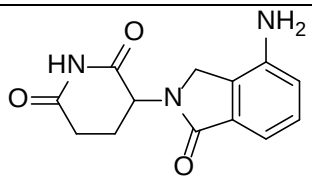
Calculated Mass: 5180 Da; Observed Mass: 5180 Da





7.5 Substrate scope of amines for on-DNA synthesis of 2-Imino thiazolines

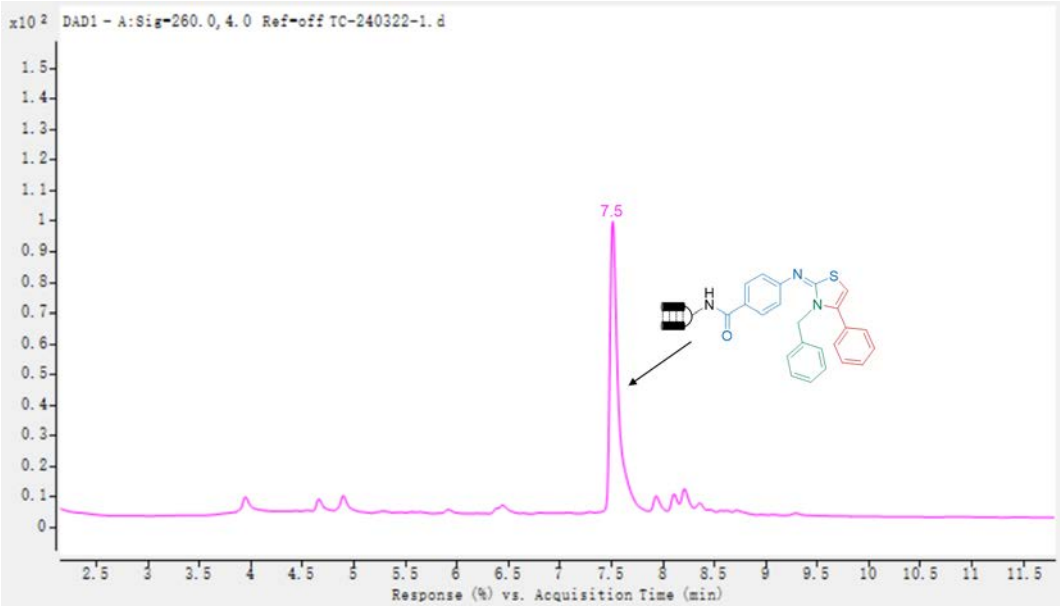
Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
7a		**9aa**	5305	5305	90%
7b		**9ba**	5321	5321	81%
7c		**9ca**	5325	5325	72%
7d		**9da**	5291	5291	79%
7e		**9ea**	5271	5271	82%
7f		**9fa**	5271	5271	62%
7g		**9ga**	5271	5289	0%
7h	NH_4OH	**9ha**	5215	5215	87%
7i		**9ia**	5309	5309	> 90%

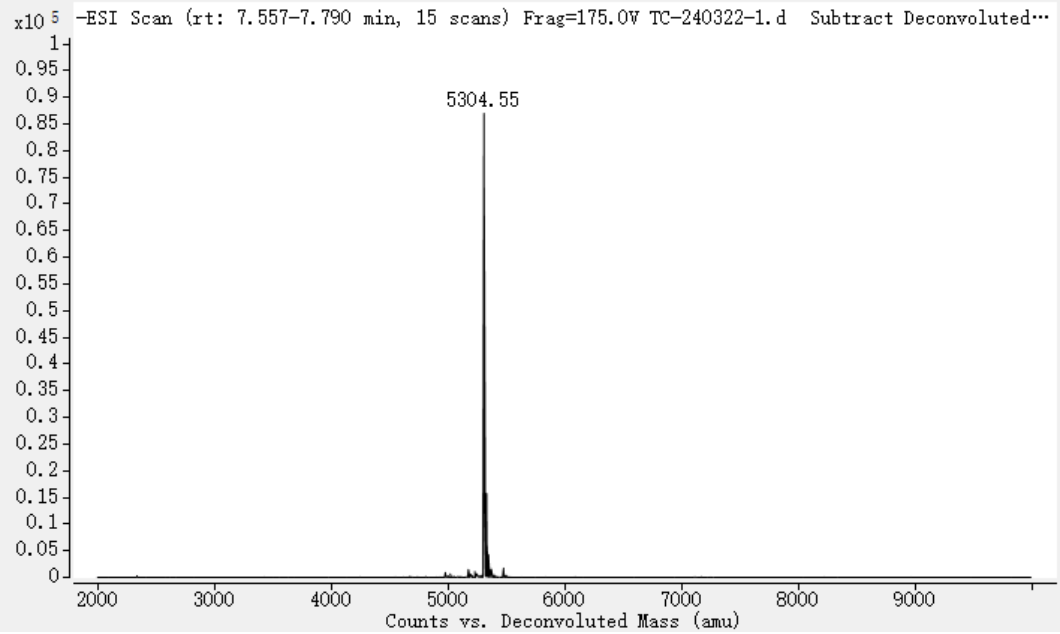
7j		**9ja**	5334	5334	81%
7k		**9ka**	5379	5379	83%
7l		**9la**	5430	5430	85%
7m		**9ma**	5457	5457	84%

UPLC chromatogram and deconvoluted MS of **9aa**

Conversion: 90%

Calculated Mass: 5305 Da; Observed Mass: 5305 Da

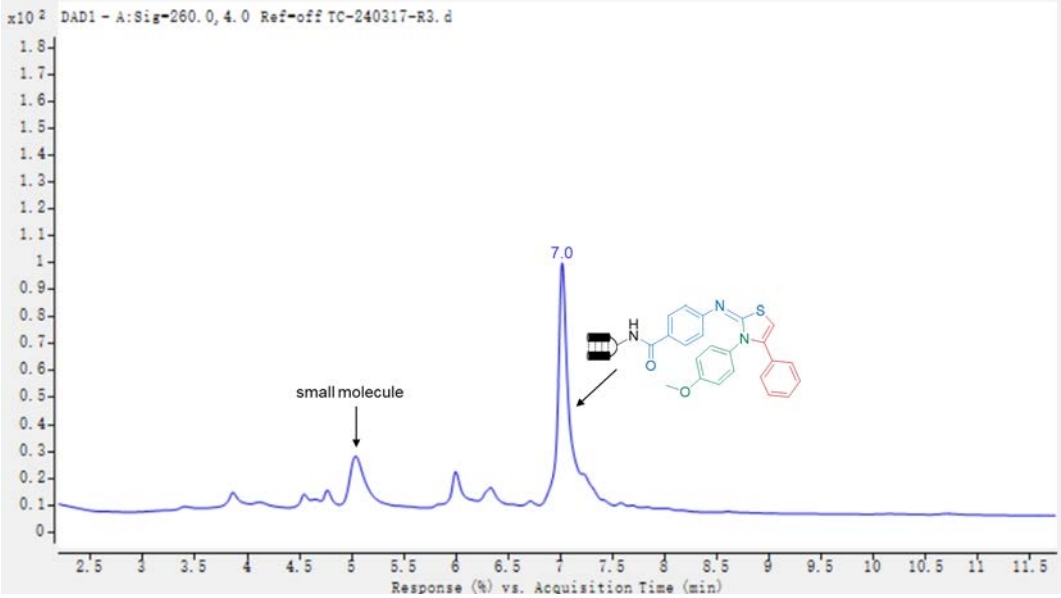


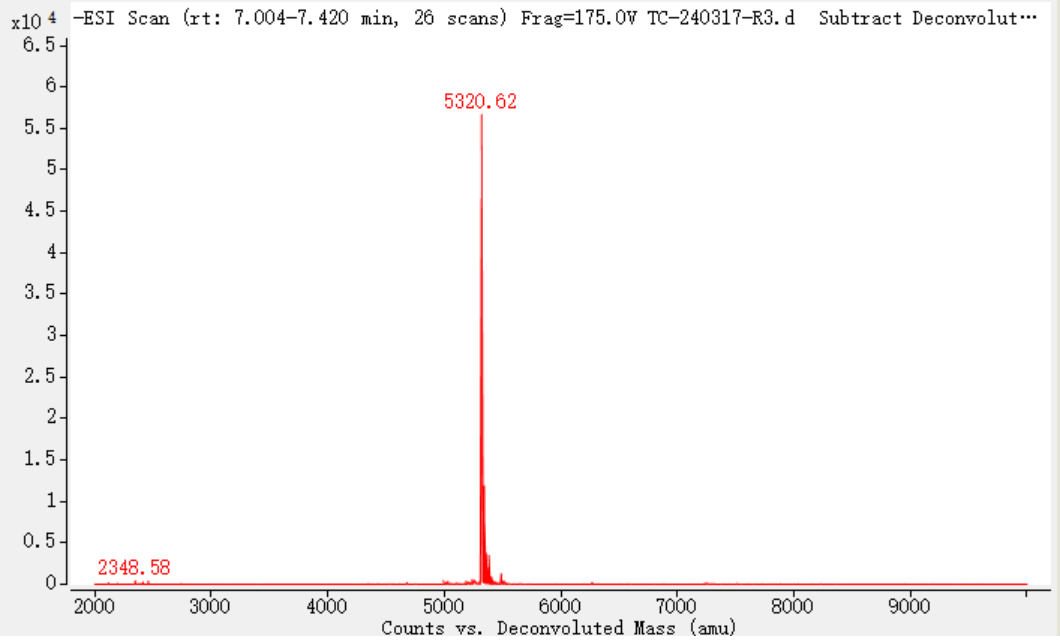


UPLC chromatogram and deconvoluted MS of **9ba**

Conversion: 81%

Calculated Mass: 5321 Da; Observed Mass: 5321 Da

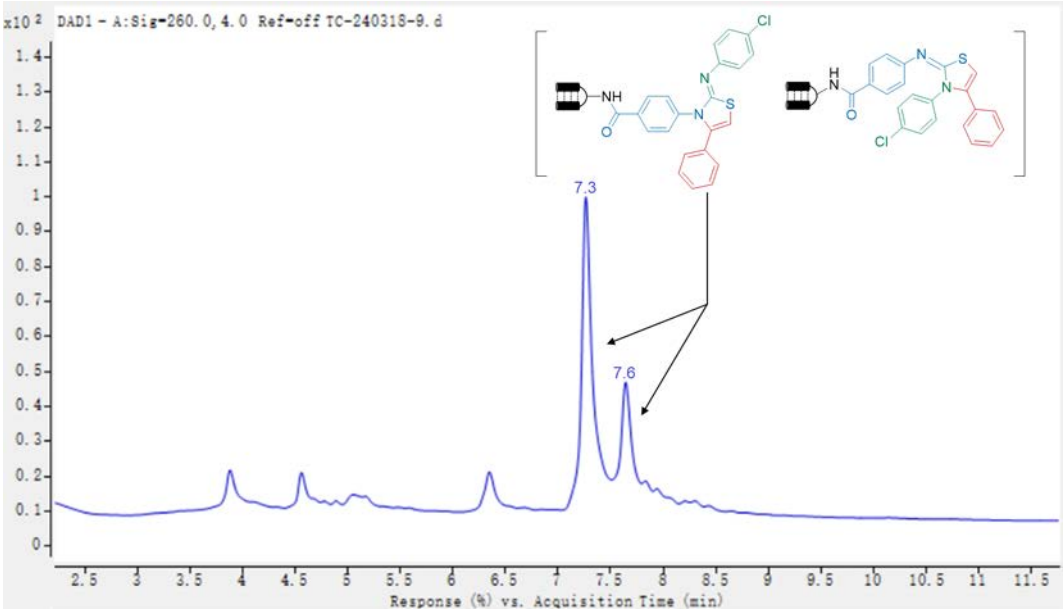


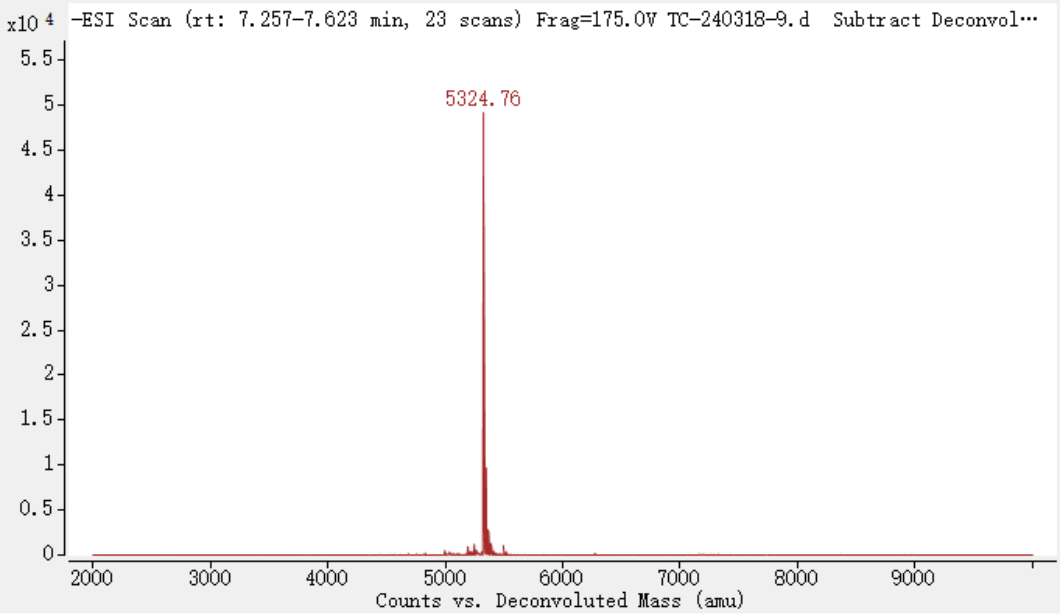


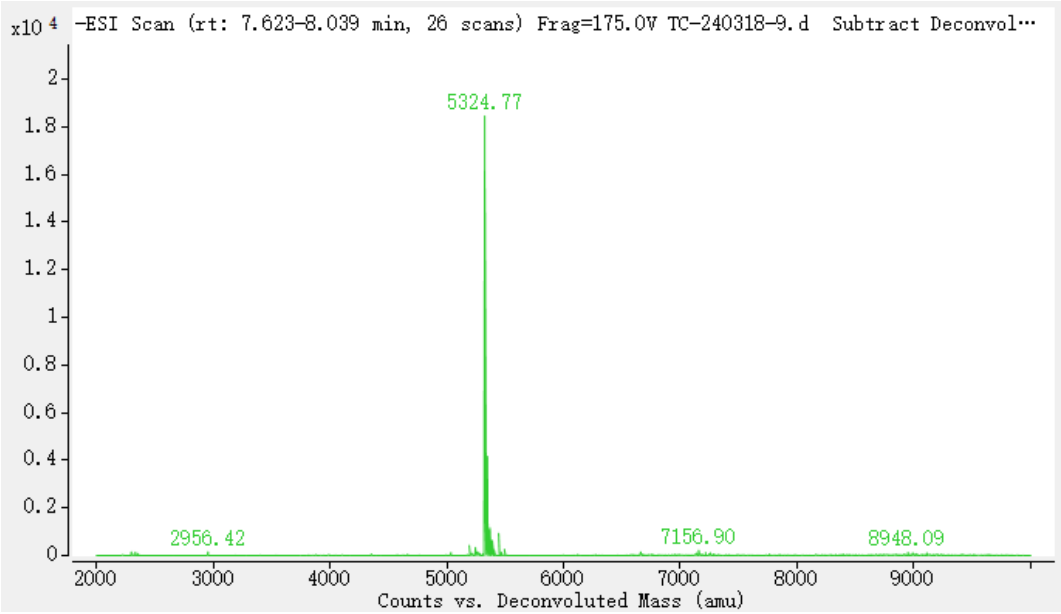
UPLC chromatogram and deconvoluted MS of **9ca**

Conversion: 72%

Calculated Mass: 5325 Da; Observed Mass: 5325 Da



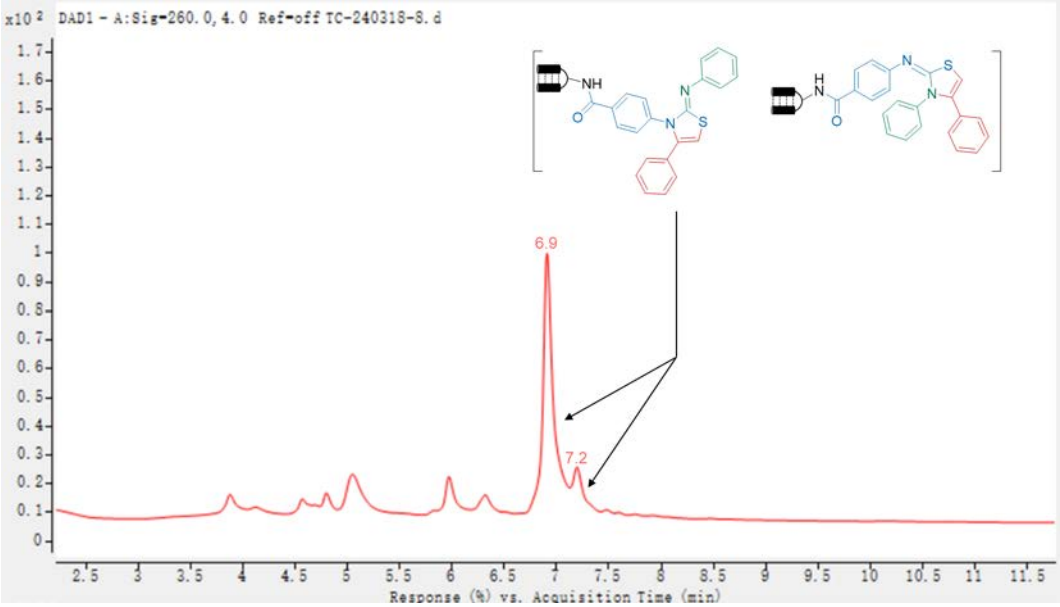


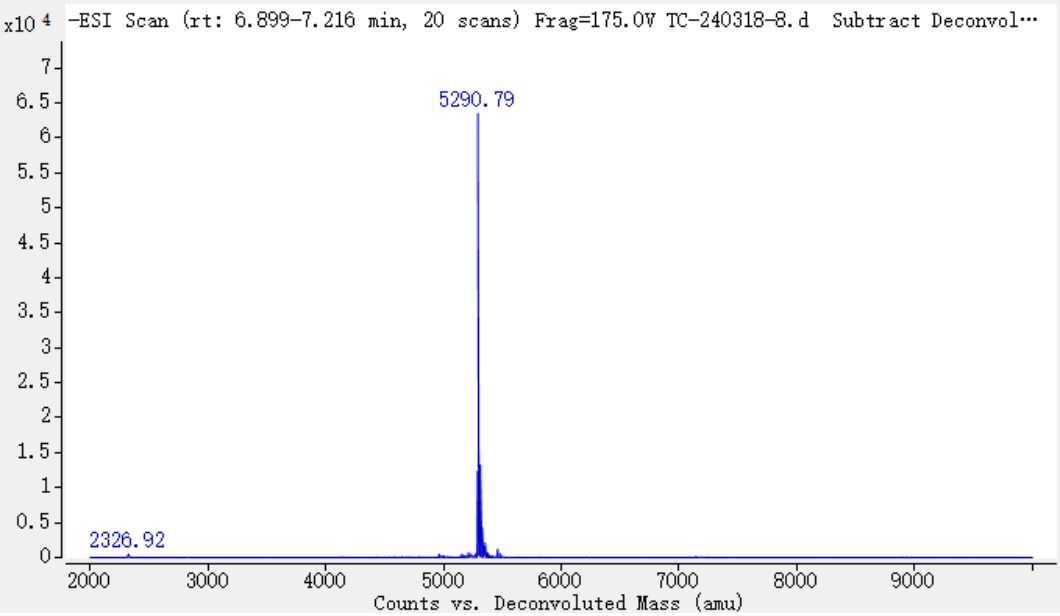

x10 4
-ESI Scan (rt: 7.623-8.039 min, 26 scans) Frag=175.0V TC-240318-9.d Subtract Deconvol…
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5324.77
2956.42
7156.90
8948.09
2000
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

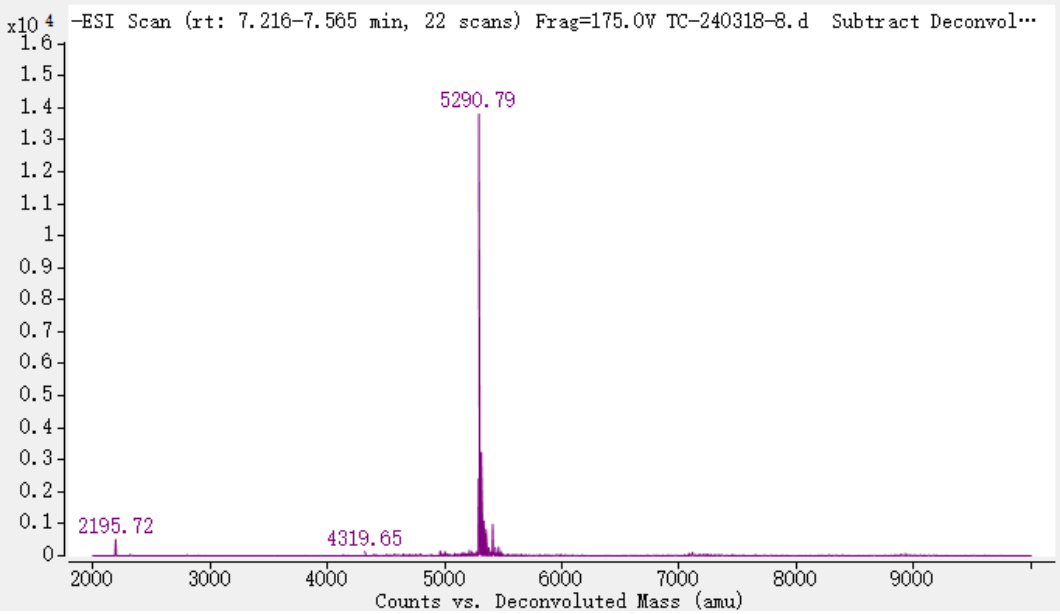
UPLC chromatogram and deconvoluted MS of **9da**

Conversion: 76%

Calculated Mass: 5291 Da; Observed Mass: 5291 Da



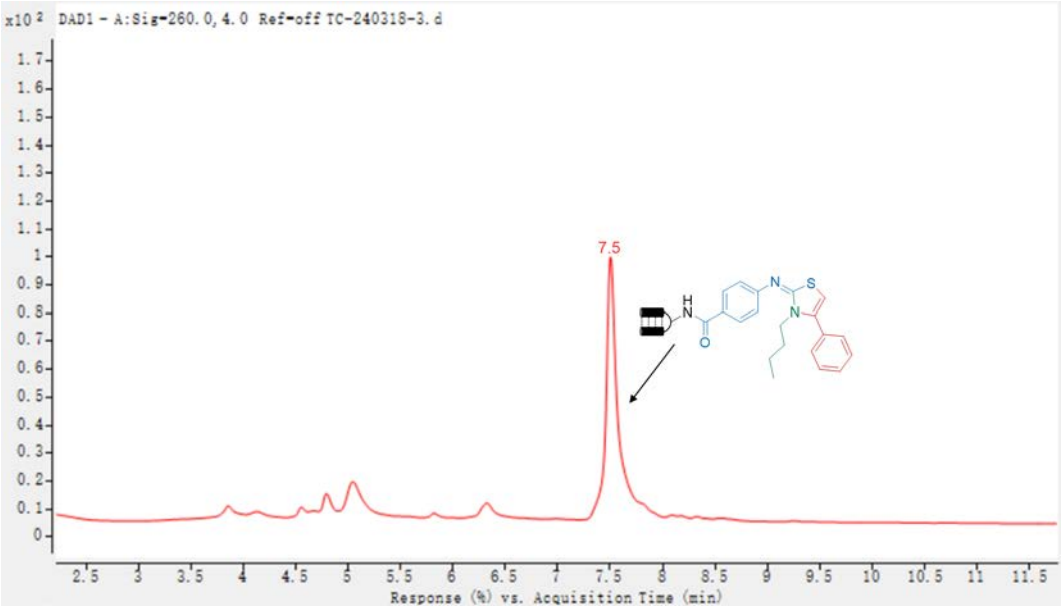


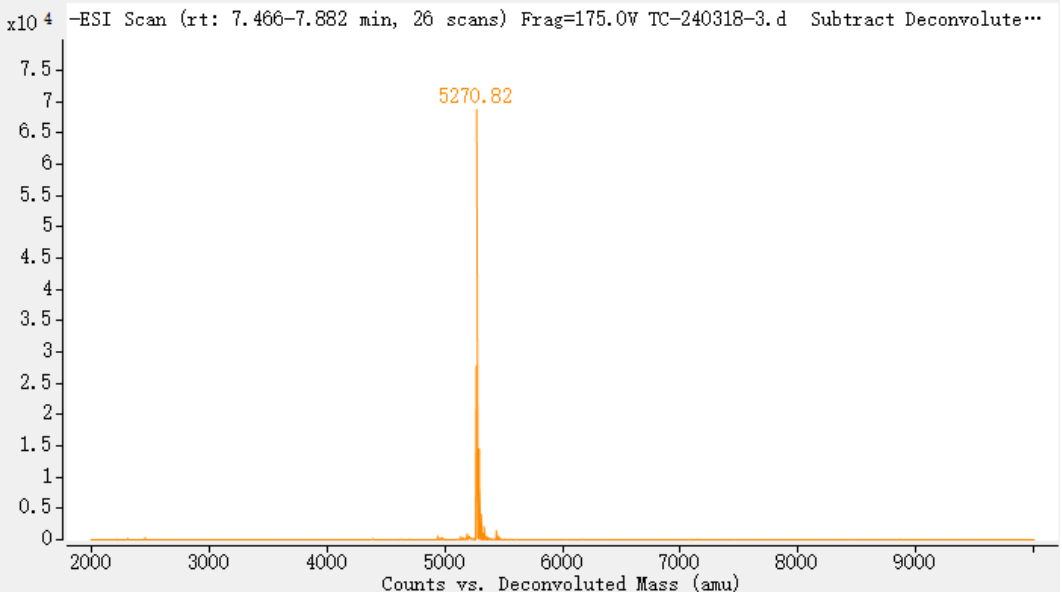

x10 4
-ESI Scan (rt: 7.216-7.565 min, 22 scans) Frag=175.0V TC-240318-8.d Subtract Deconvol…
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5290.79
2195.72
4319.65
2000
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

UPLC chromatogram and deconvoluted MS of **9ea**

Conversion: 82%

Calculated Mass: 5271 Da; Observed Mass: 5271 Da

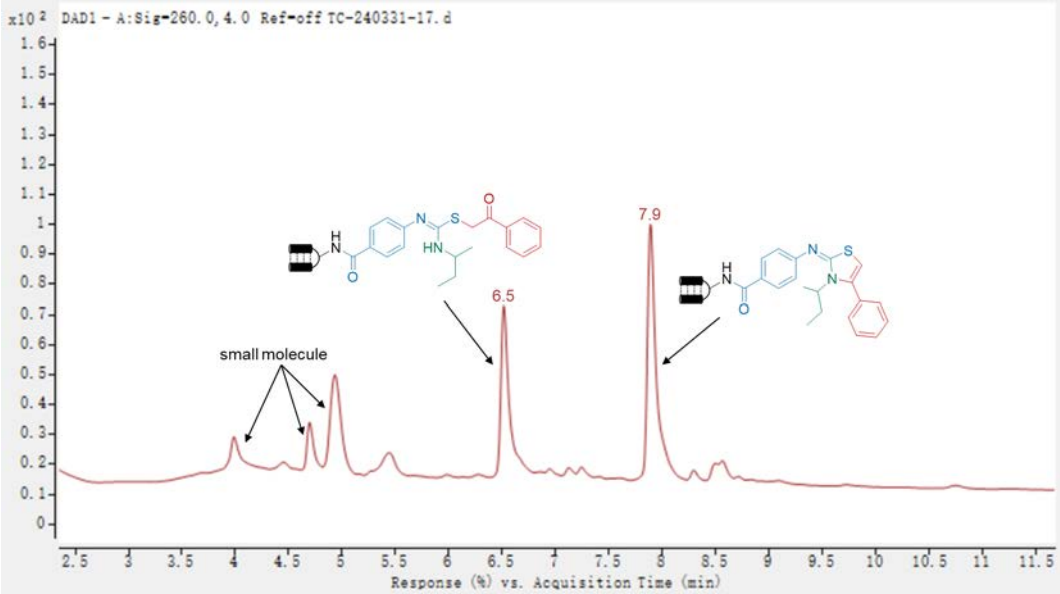


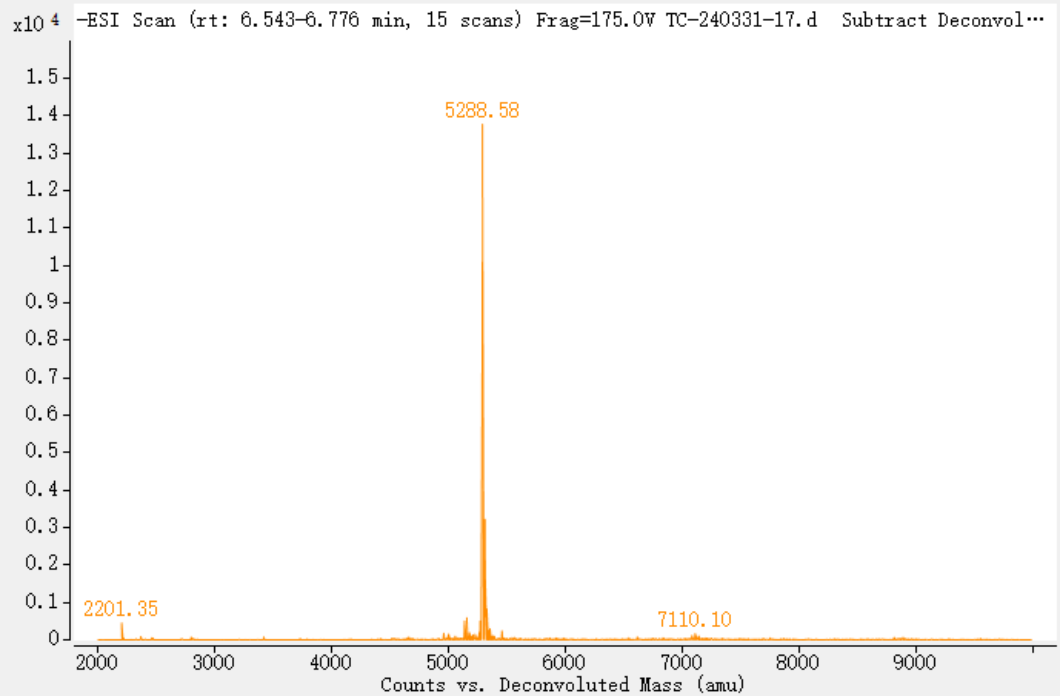


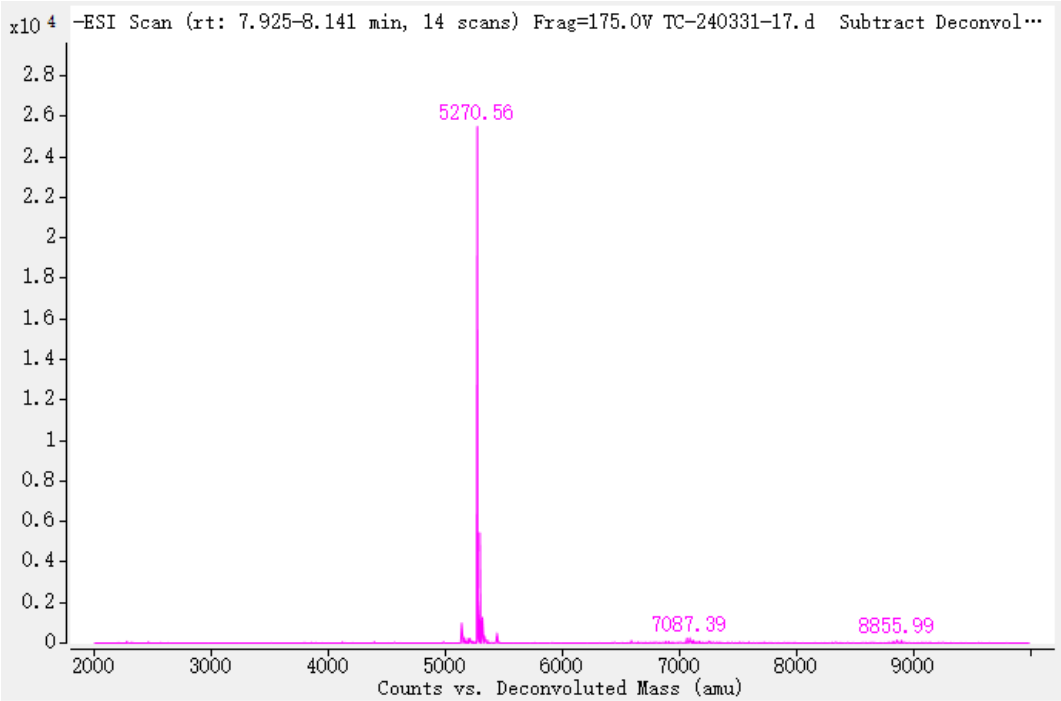
UPLC chromatogram and deconvoluted MS of **9fa**

Conversion: 62%

Calculated Mass: 5271 Da; Observed Mass: 5271 Da



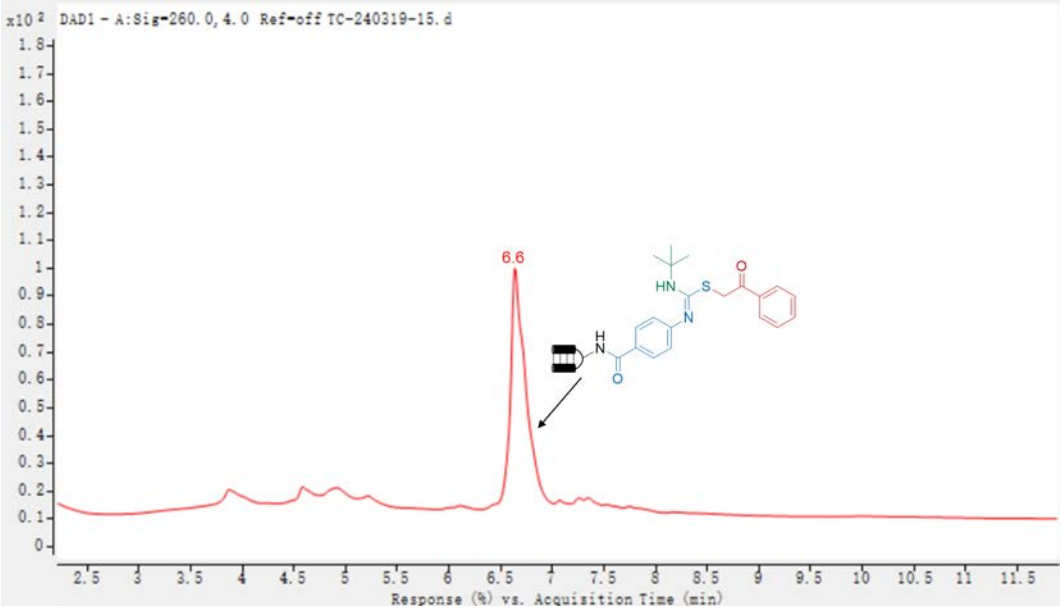


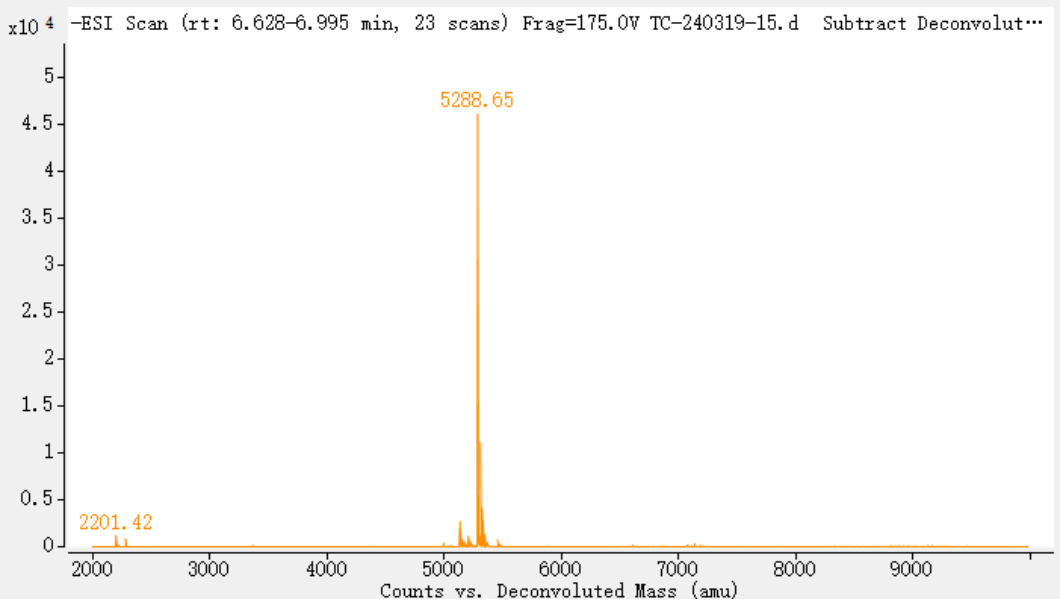

x10 4
-ESI Scan (rt: 7.925-8.141 min, 14 scans) Frag=175.0V TC-240331-17.d Subtract Deconvol…
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5270.56
7087.39
8855.99
2000
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

UPLC chromatogram and deconvoluted MS of **9ga**

Conversion: 0%

Calculated Mass: 5271 Da; Observed Mass: 5289 Da

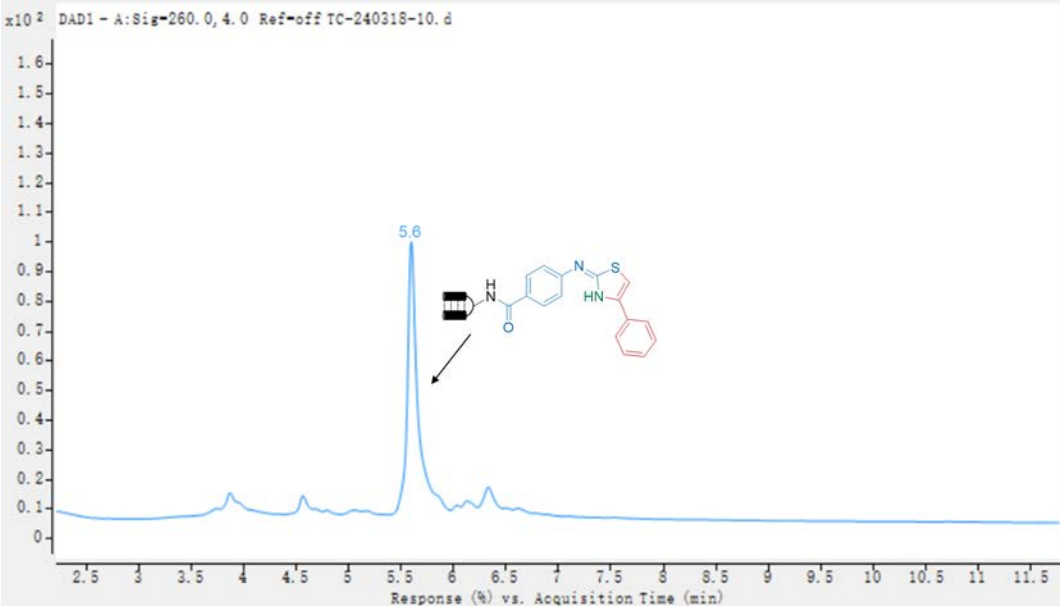


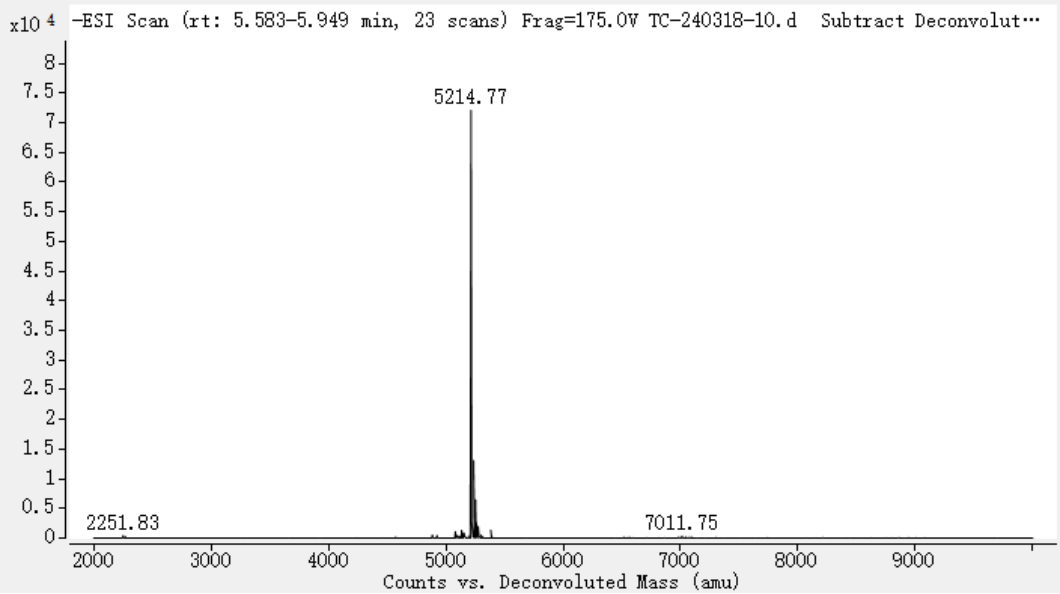


UPLC chromatogram and deconvoluted MS of **9ha**

Conversion: 87%

Calculated Mass: 5215 Da; Observed Mass: 5215 Da

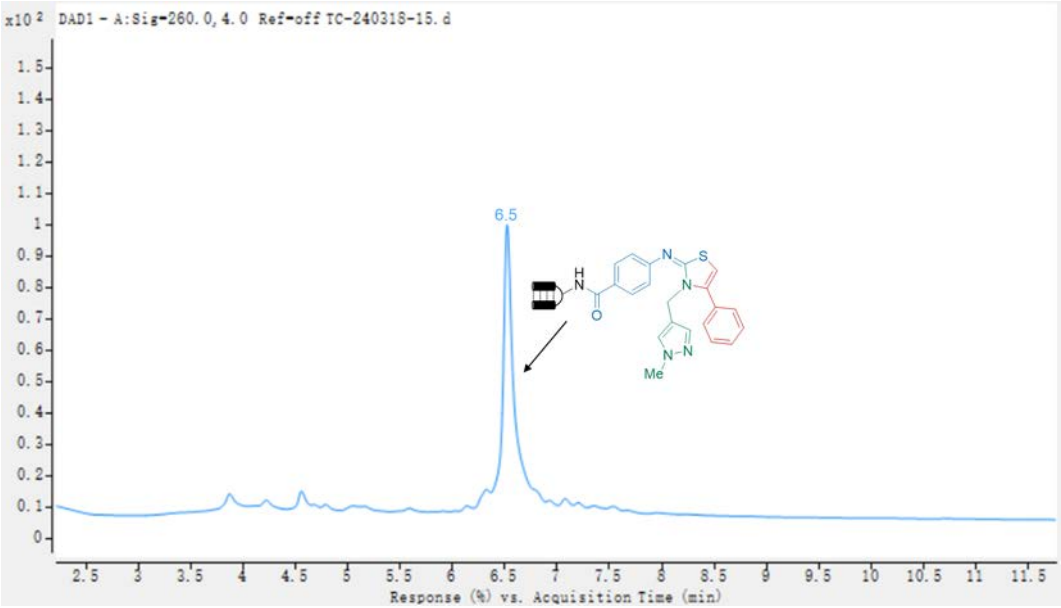


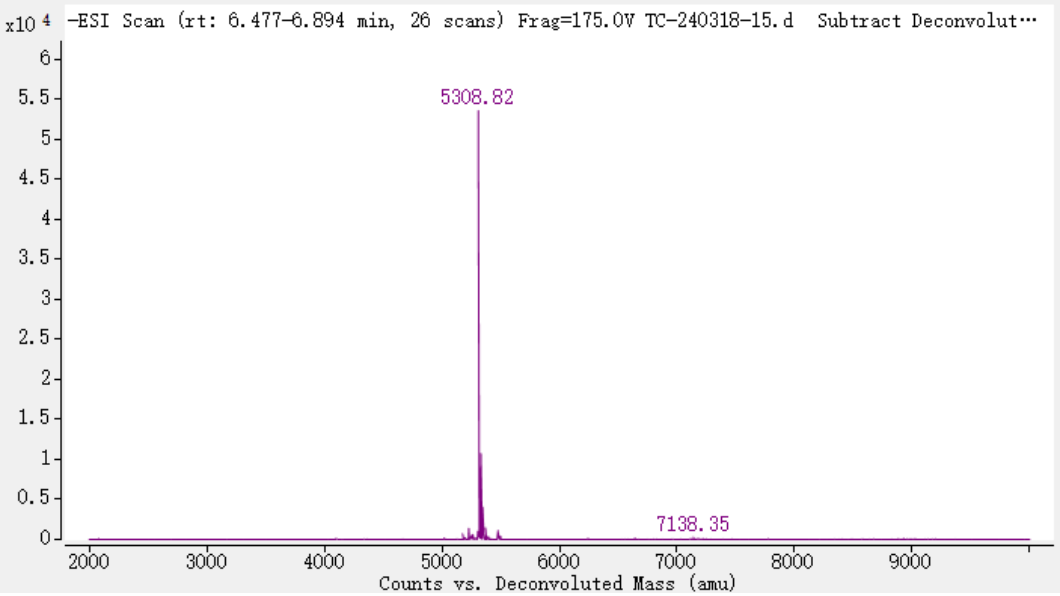


UPLC chromatogram and deconvoluted MS of **9ia**

Conversion: ＞90%

Calculated Mass: 5309 Da; Observed Mass: 5309 Da

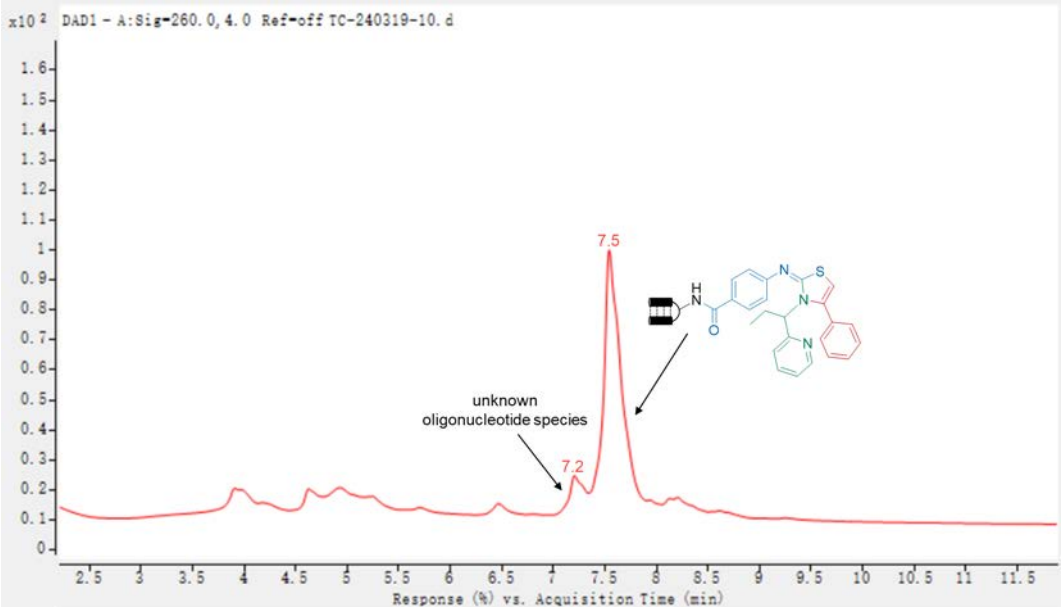


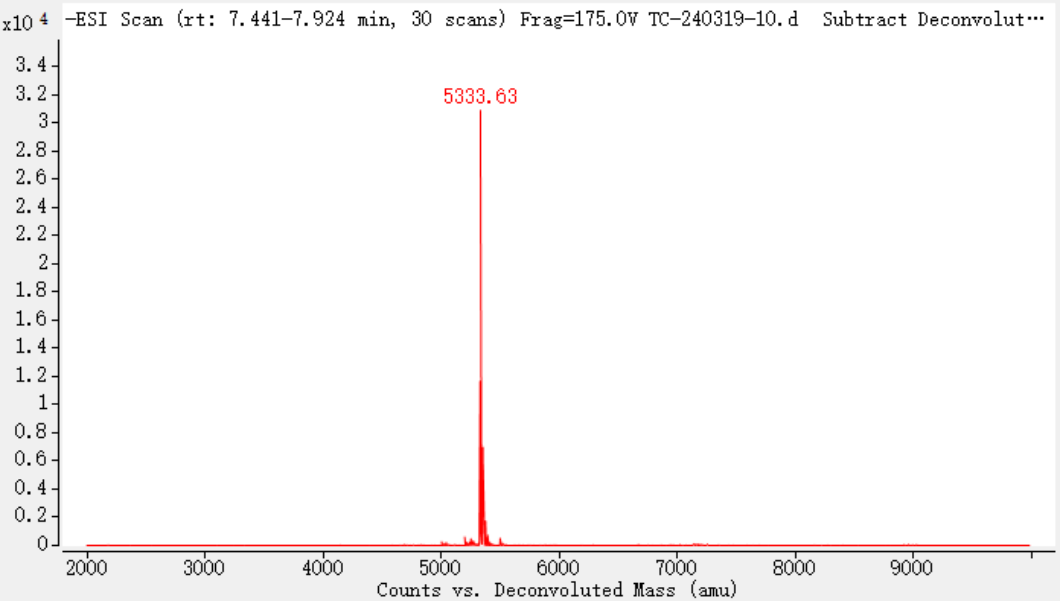


UPLC chromatogram and deconvoluted MS of **9ja**

Conversion: 81%

Calculated Mass: 5334 Da; Observed Mass: 5334 Da

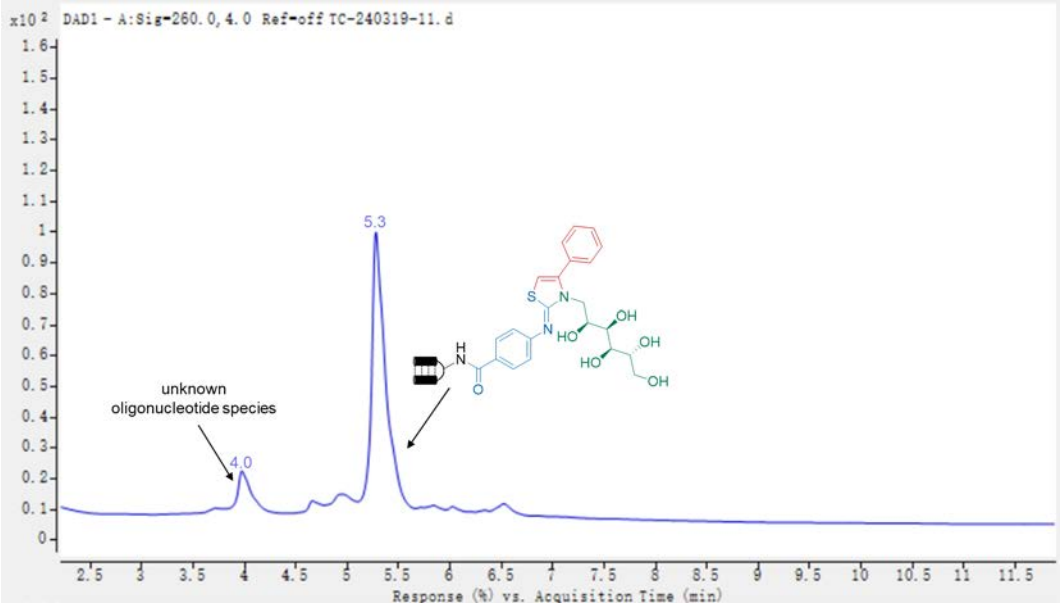


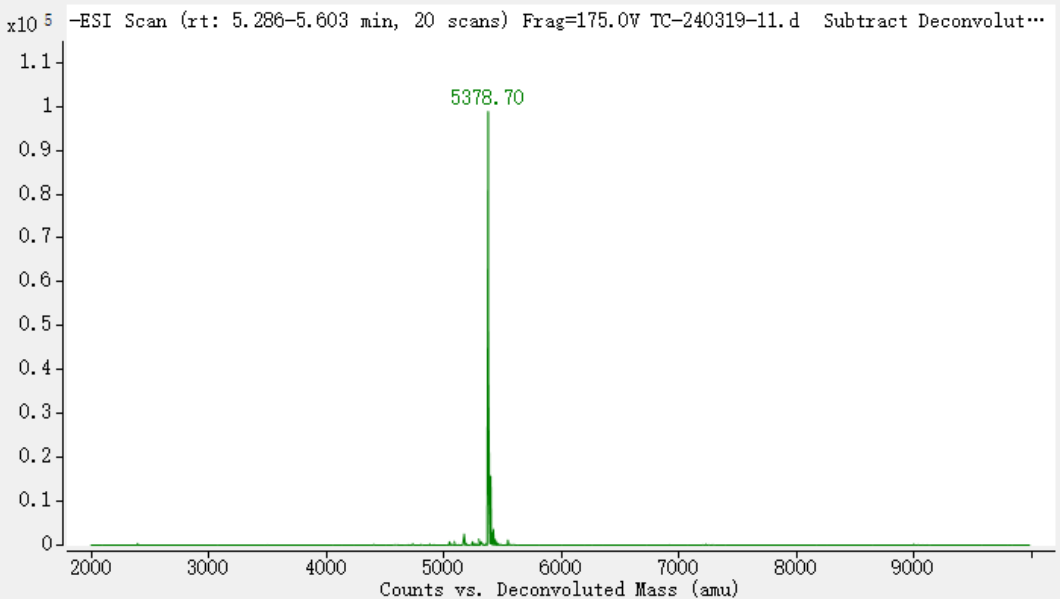


UPLC chromatogram and deconvoluted MS of **9ka**

Conversion: 83%

Calculated Mass: 5379 Da; Observed Mass: 5379 Da

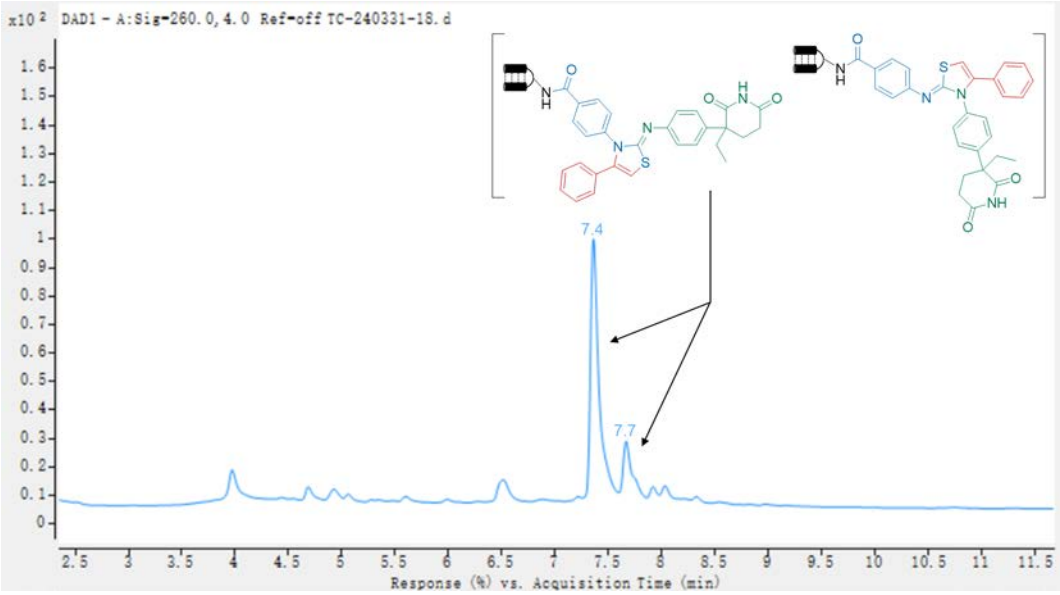


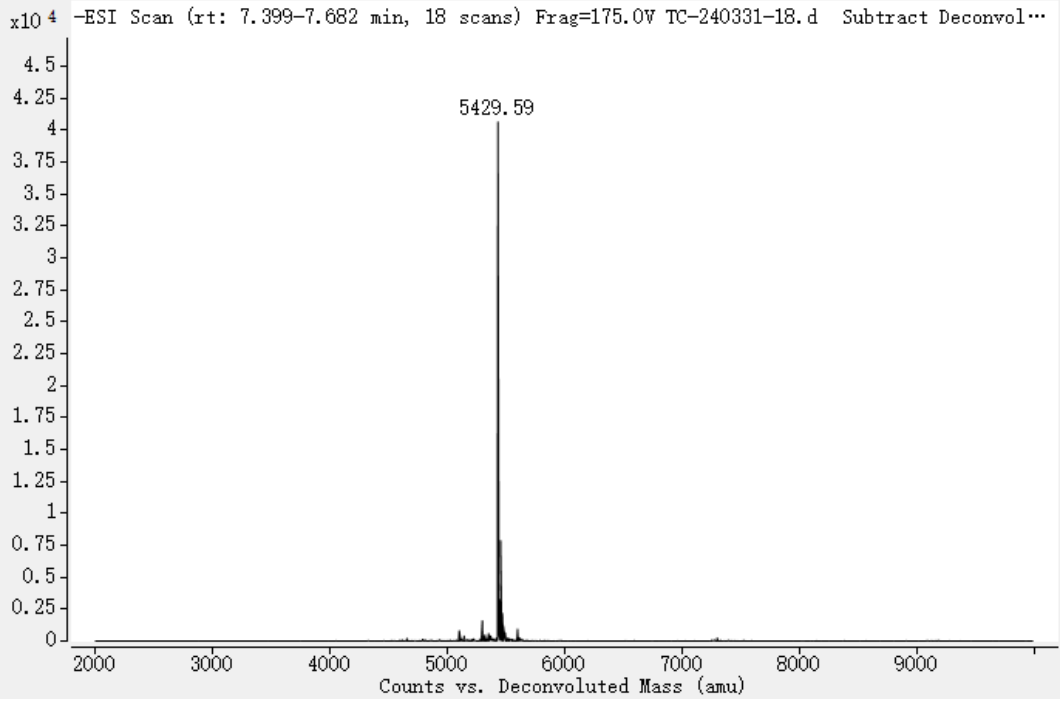


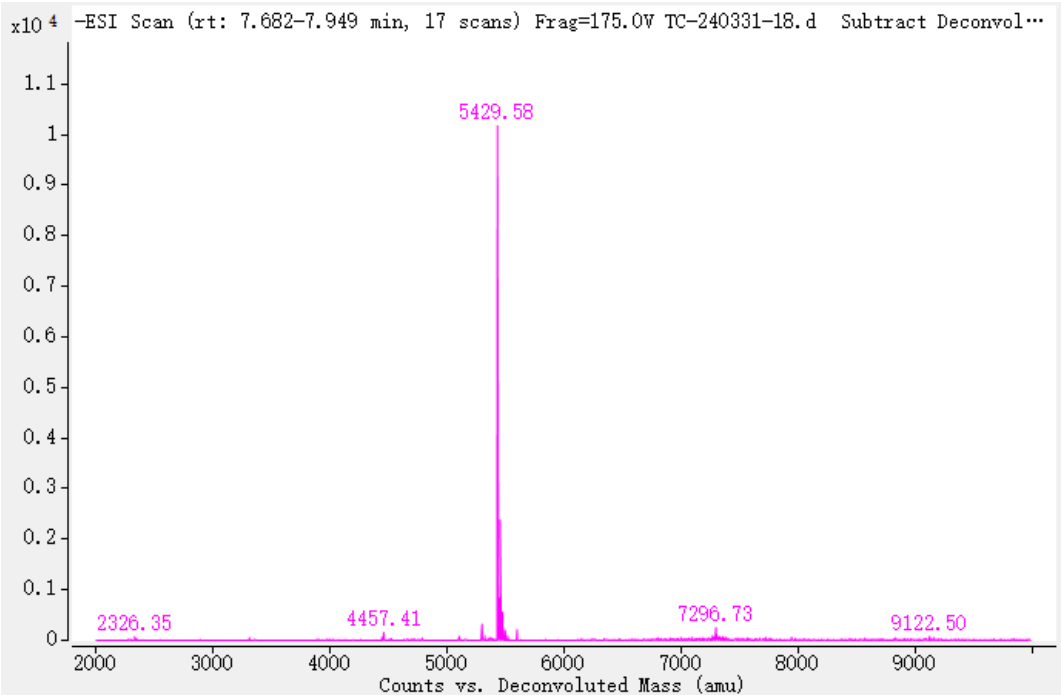
UPLC chromatogram and deconvoluted MS of **9la**

Conversion: 85%

Calculated Mass: 5430 Da; Observed Mass: 5430 Da



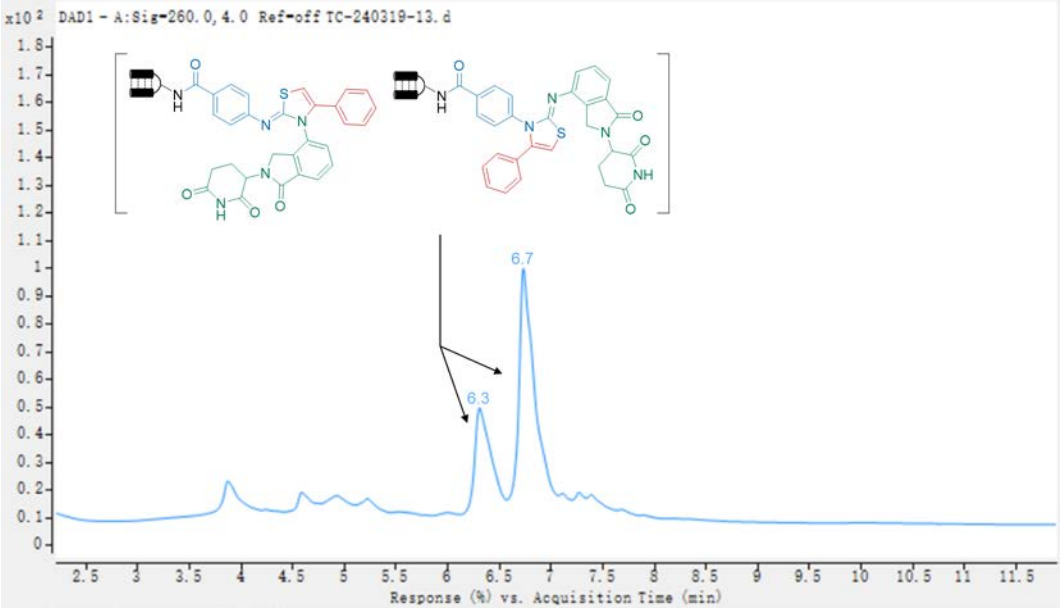


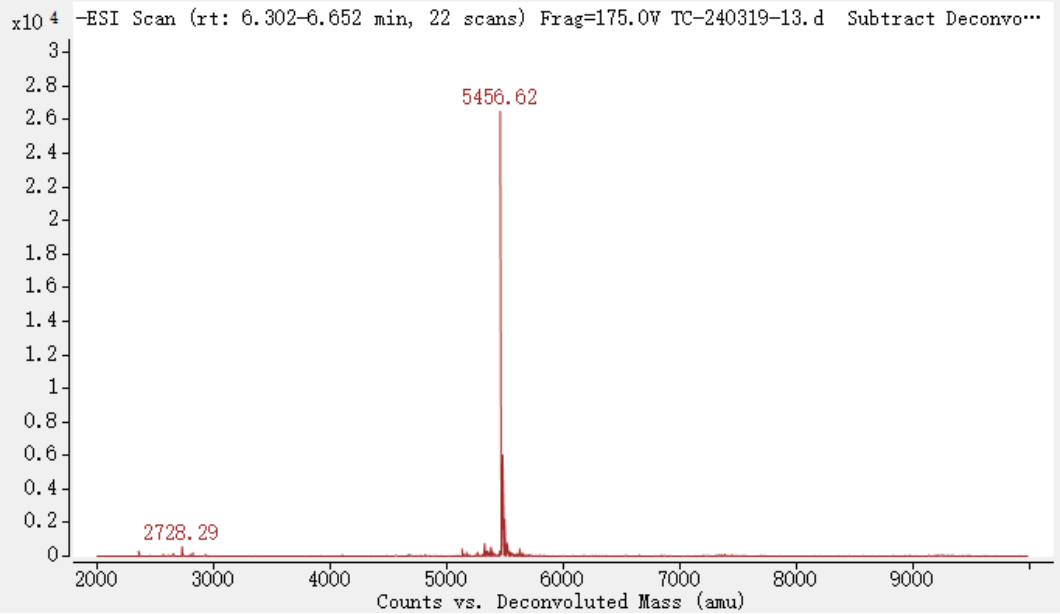

x10 4
-ESI Scan (rt: 7.682-7.949 min, 17 scans) Frag=175.0V TC-240331-18.d Subtract Deconvol…
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5429.58
2326.35
4457.41
7296.73
9122.50
2000
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

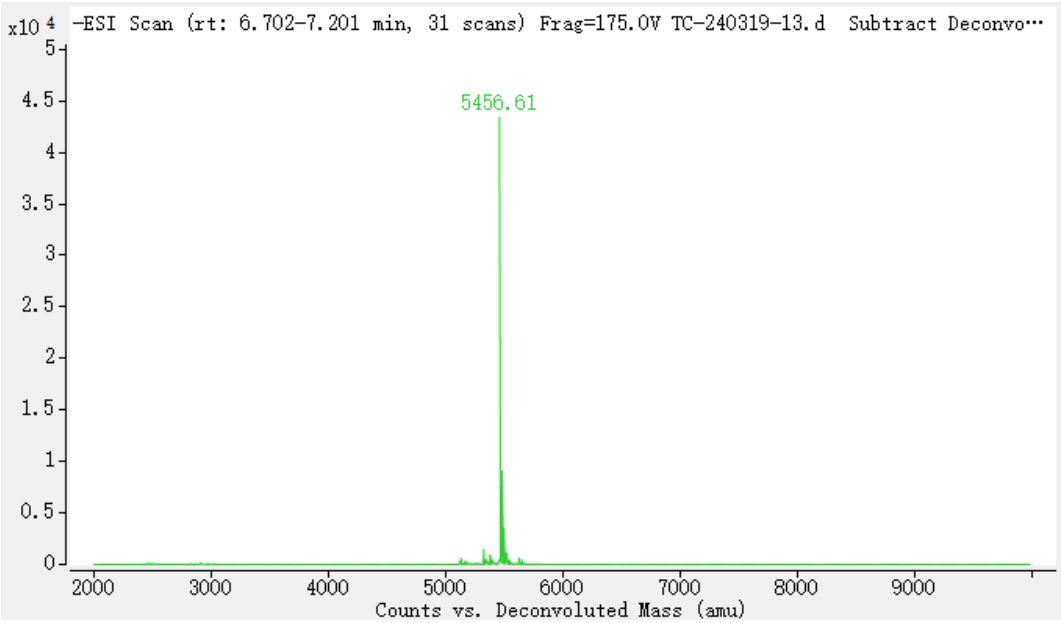
UPLC chromatogram and deconvoluted MS of **9ma**

Conversion: 84%

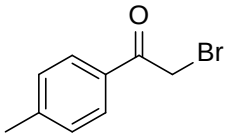
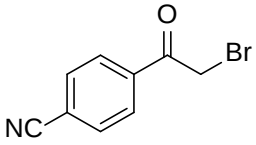
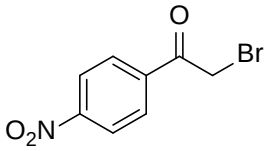
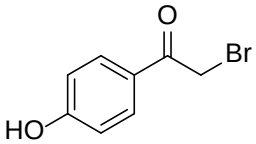
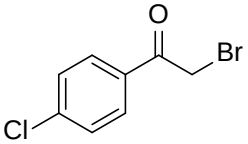
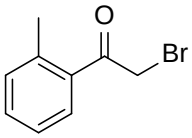
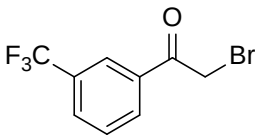
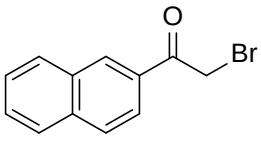
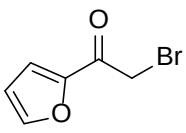
Calculated Mass: 5457 Da; Observed Mass: 5457Da

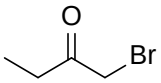
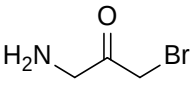
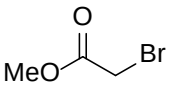





-ESI Scan (rt: 6.702-7.201 min, 31 scans) Frag=175.0V TC-240319-13.d Subtract Deconvo…
x10 4
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
5456.61
2000
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

7.6 Substrate scope of α-bromoketones

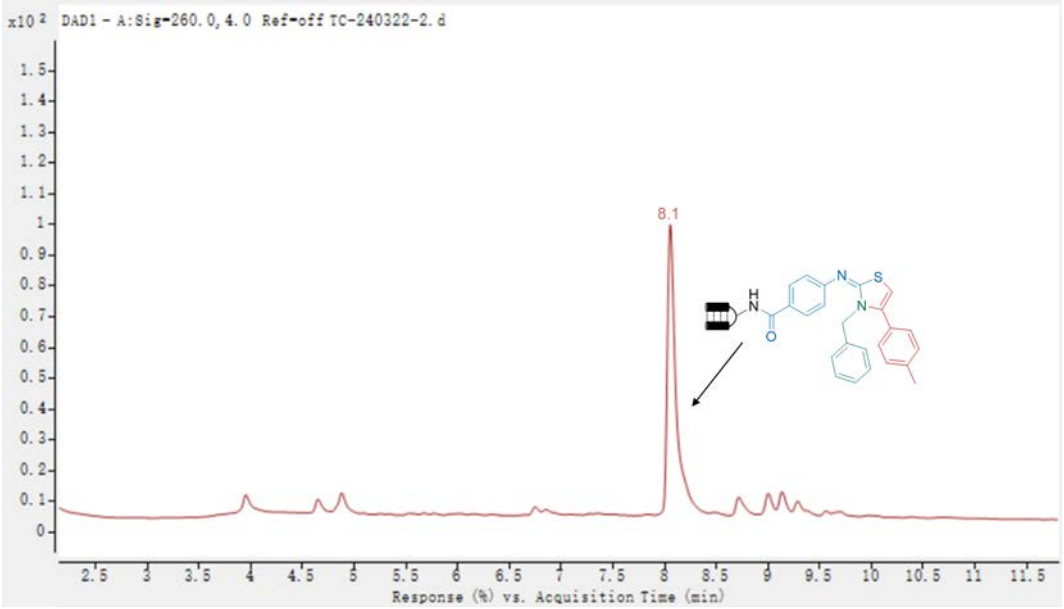
Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
8b		**9ab**	5319	5319	82%
8c		**9ac**	5330	5330	61%
8d		**9ad**	5350	5350	65%
8e		**9ae**	5321	5321	66%
8f		**9af**	5339	5339	> 90%
8g		**9ag**	5319	5319	> 90%
8h		**9ah**	5373	5373	88%
8i		**9ai**	5355	5355	84%
8j		**9aj**	5295	5295	87%

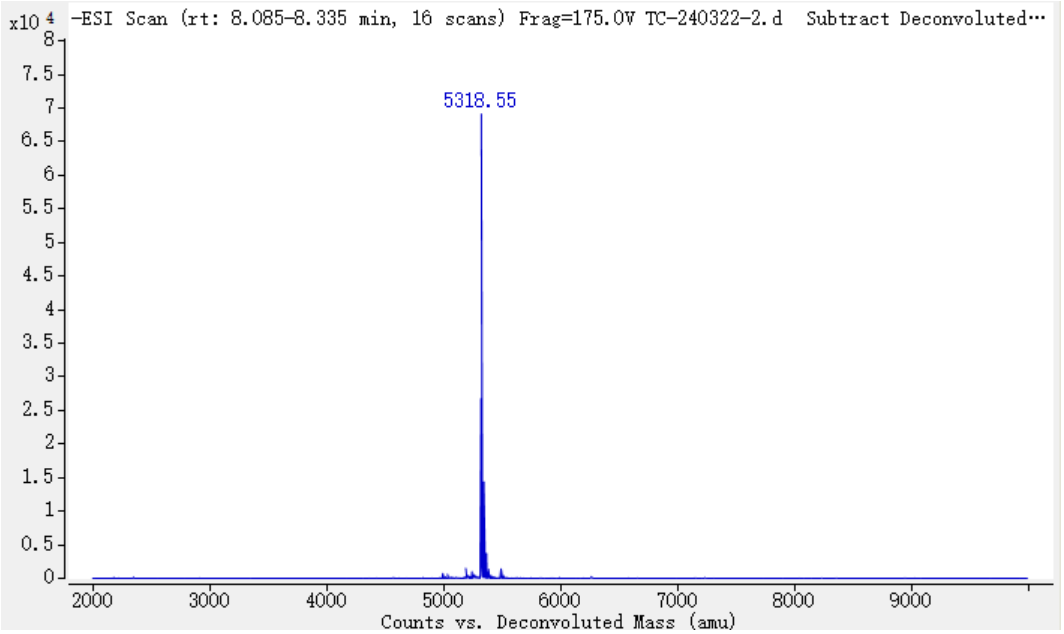
8k		**9ak**	5257	5257	83%
8l		**9al**	5244	5244	64%
8m		**9am**	5245	5245	74%

UPLC chromatogram and deconvoluted MS of **9ab**

Conversion: 82%

Calculated Mass: 5319 Da; Observed Mass: 5319 Da

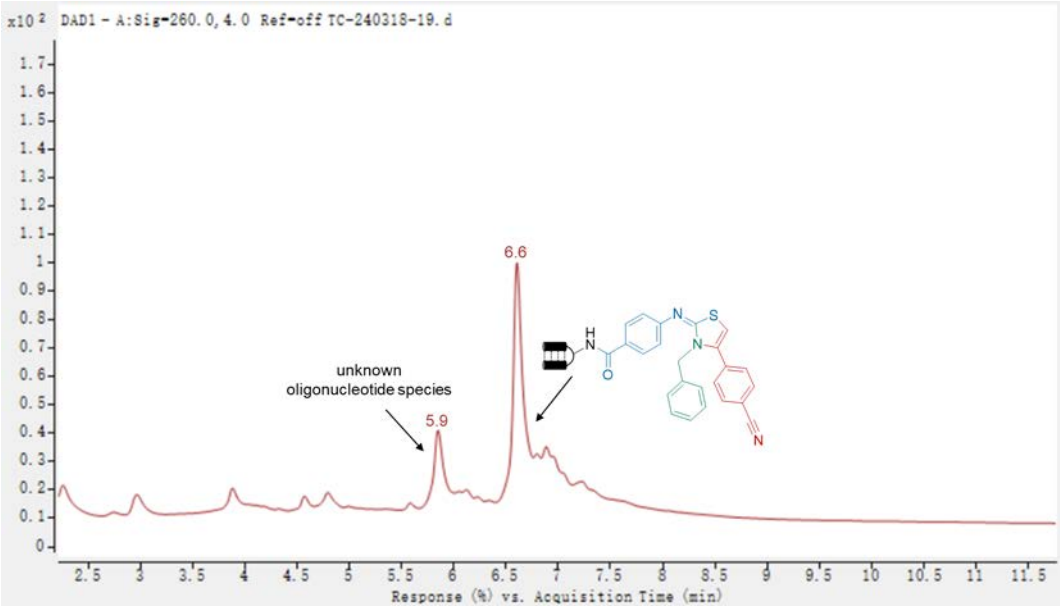


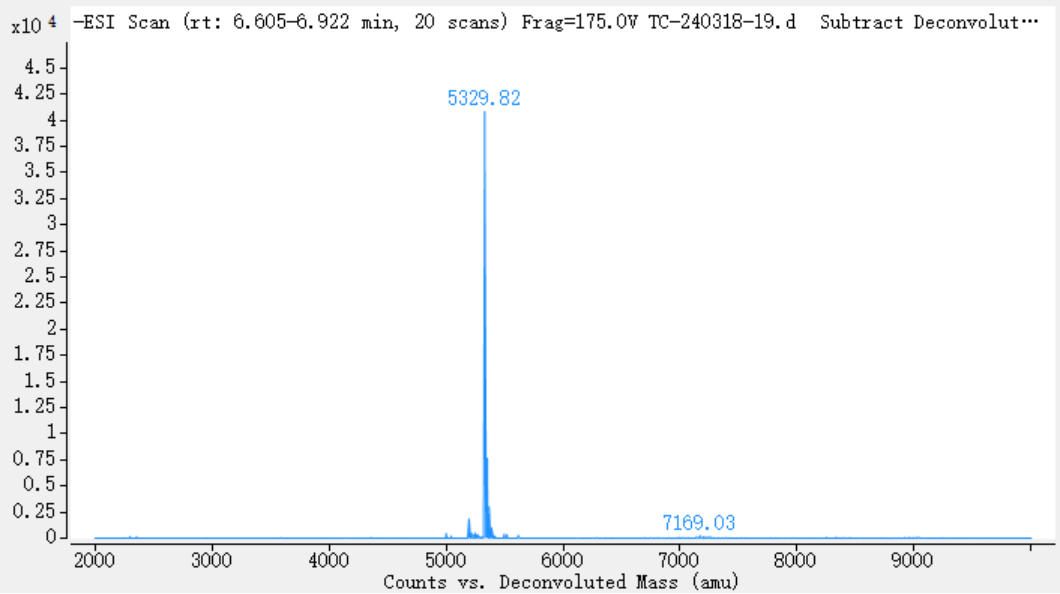


UPLC chromatogram and deconvoluted MS of **9ac**

Conversion: 61%

Calculated Mass: 5330 Da; Observed Mass: 5330 Da

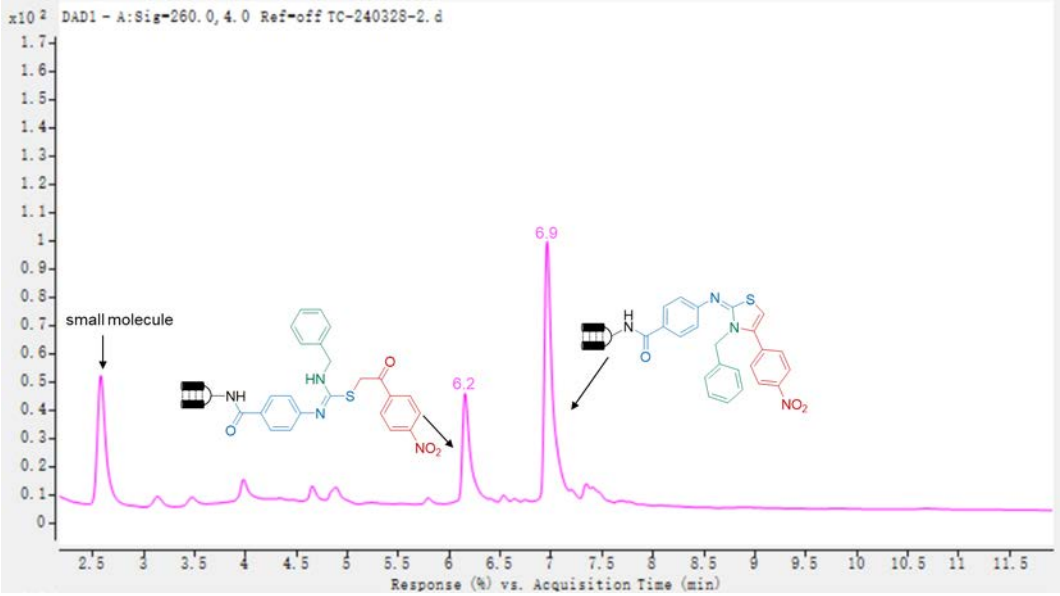


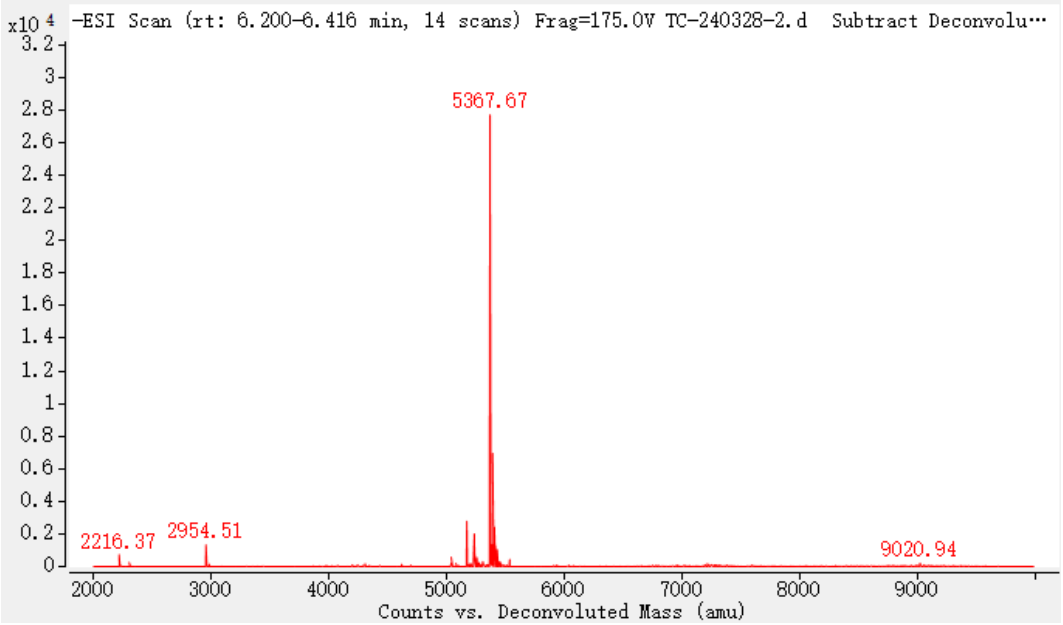


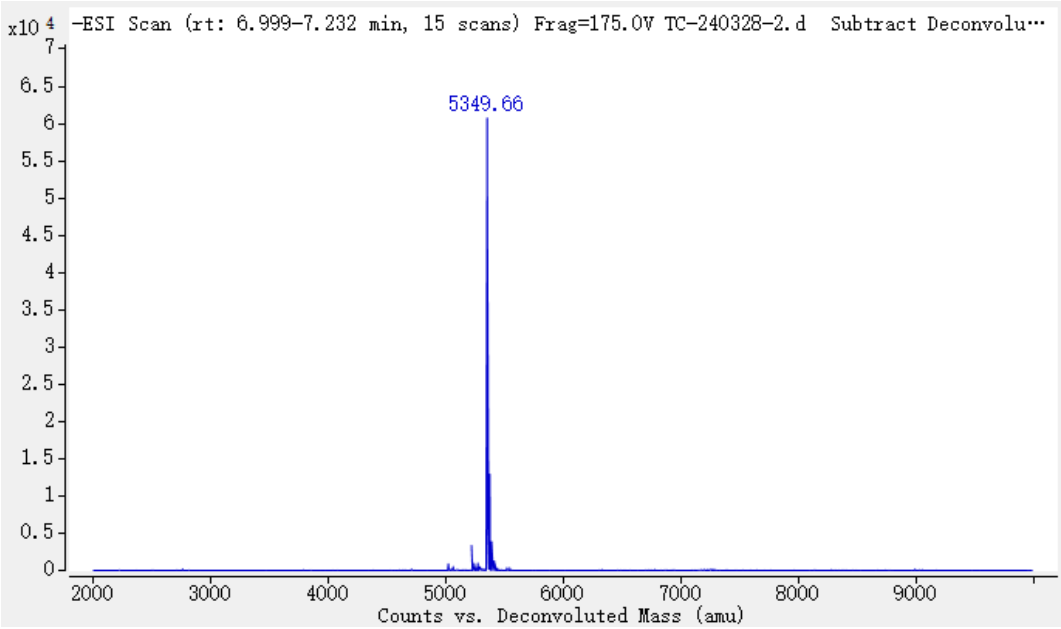
UPLC chromatogram and deconvoluted MS of **9ad**

Conversion: 65%

Calculated Mass: 5350 Da; Observed Mass: 5350 Da



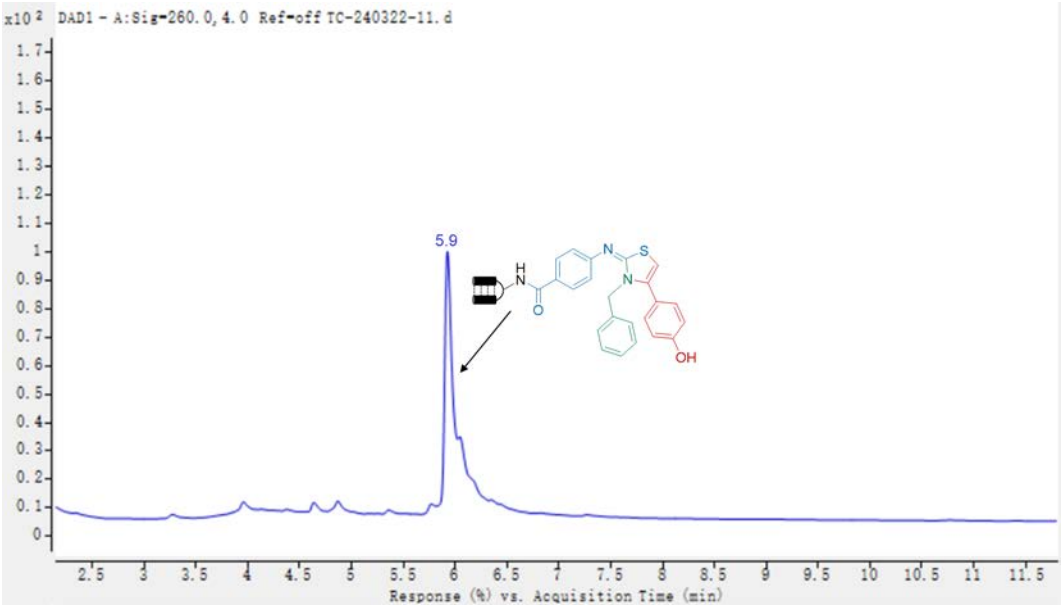


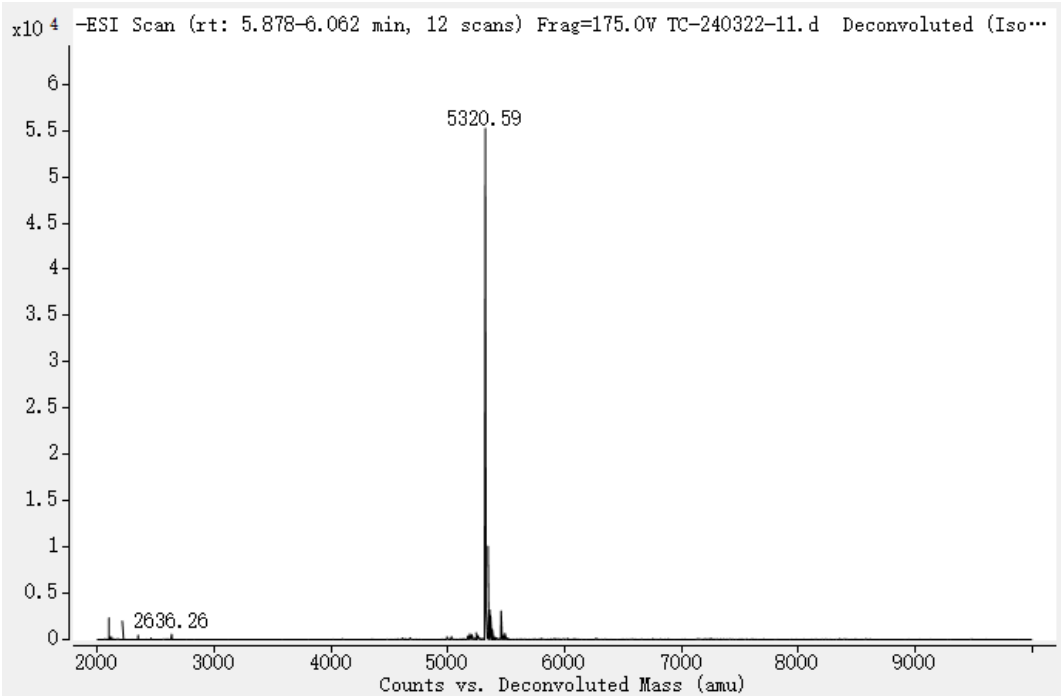

-ESI Scan (rt: 6.999-7.232 min, 15 scans) Frag=175.0V TC-240328-2.d Subtract Deconvolu…
x10 4
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
5349.66
2000
3000
4000
5000
6000
7000
8000
9000
Counts vs. Deconvoluted Mass (amu)

UPLC chromatogram and deconvoluted MS of **9ae**

Conversion: 66%

Calculated Mass: 5321 Da; Observed Mass: 5321 Da

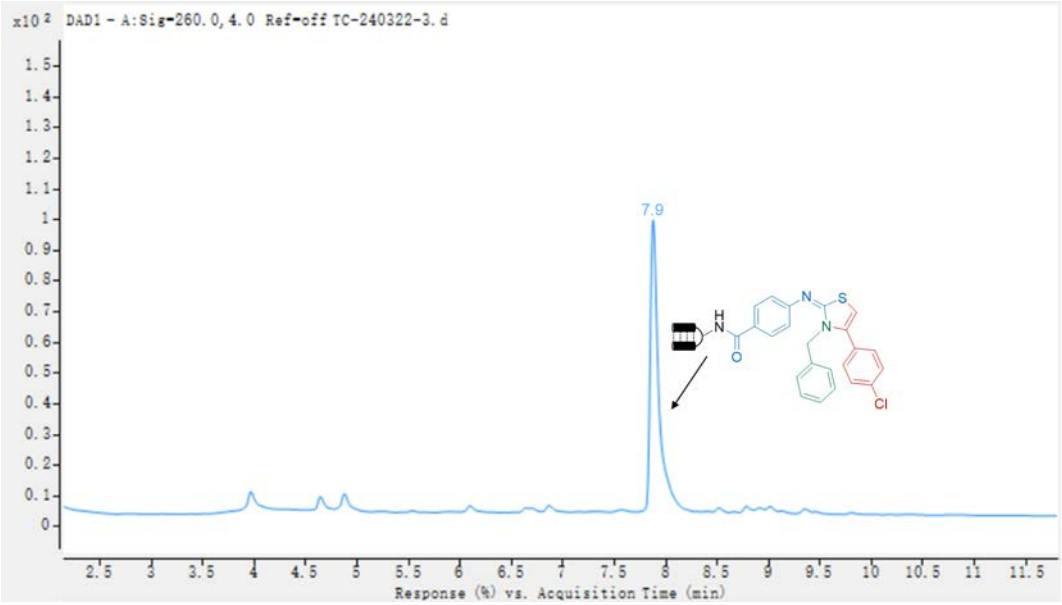


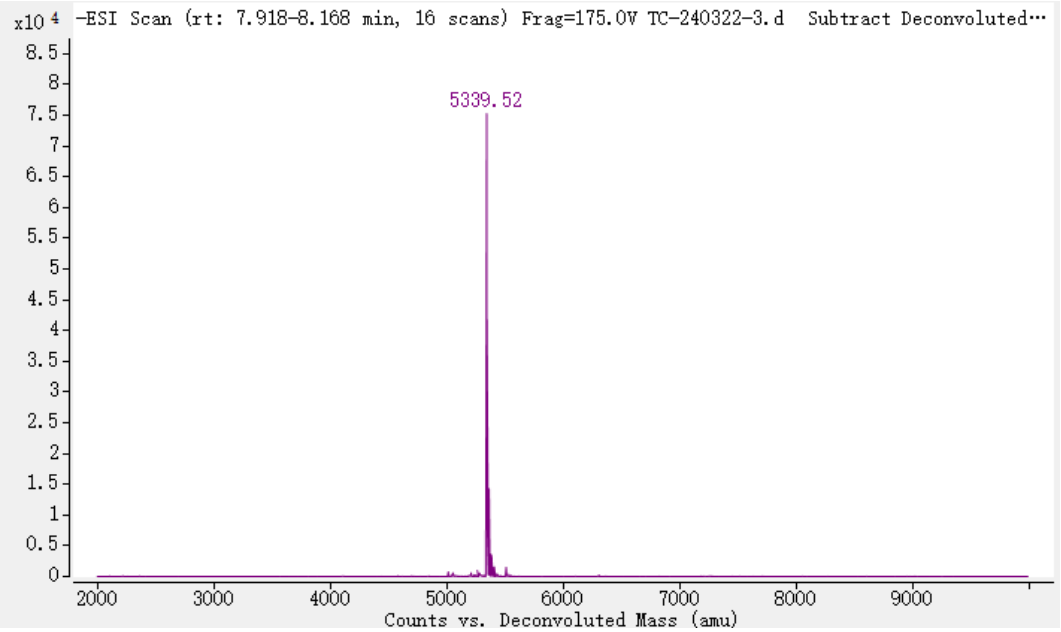


UPLC chromatogram and deconvoluted MS of **9af**

Conversion: ＞90%

Calculated Mass: 5340 Da; Observed Mass: 5340 Da

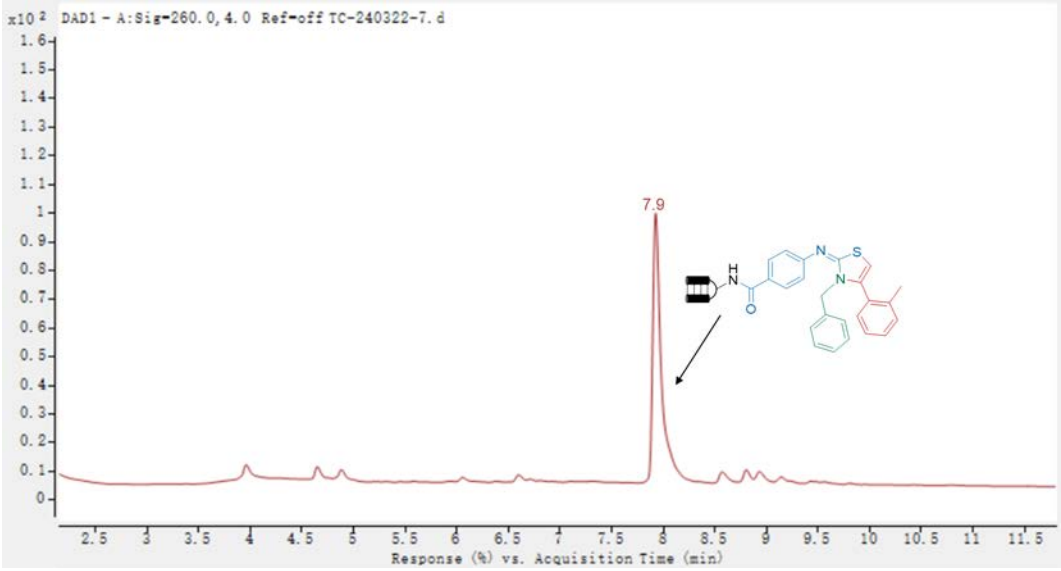


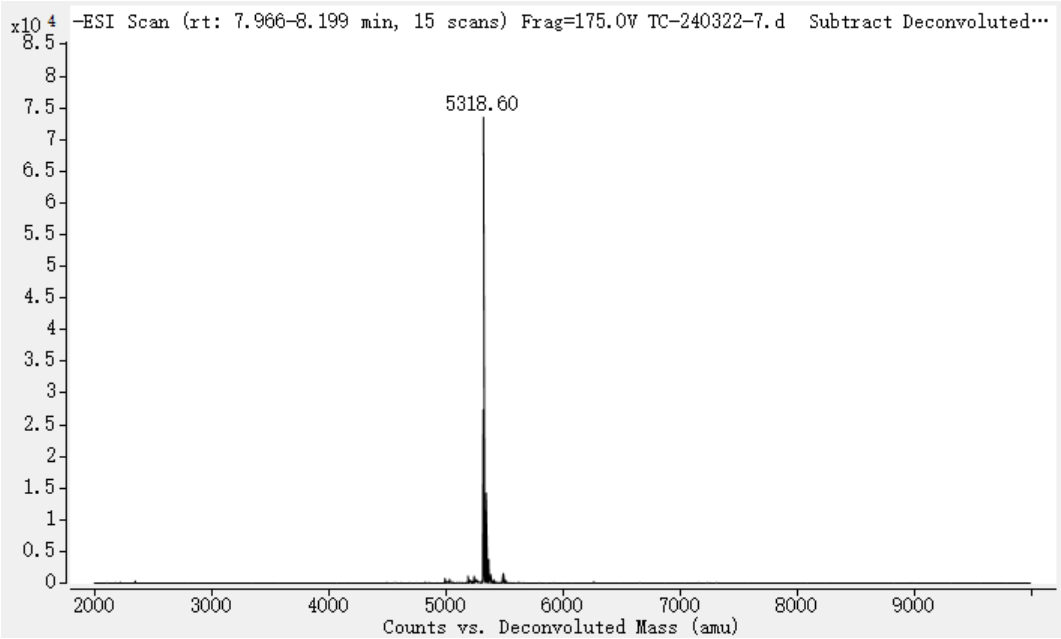


UPLC chromatogram and deconvoluted MS of **9ag**

Conversion: ＞90%

Calculated Mass: 5319 Da; Observed Mass: 5319 Da

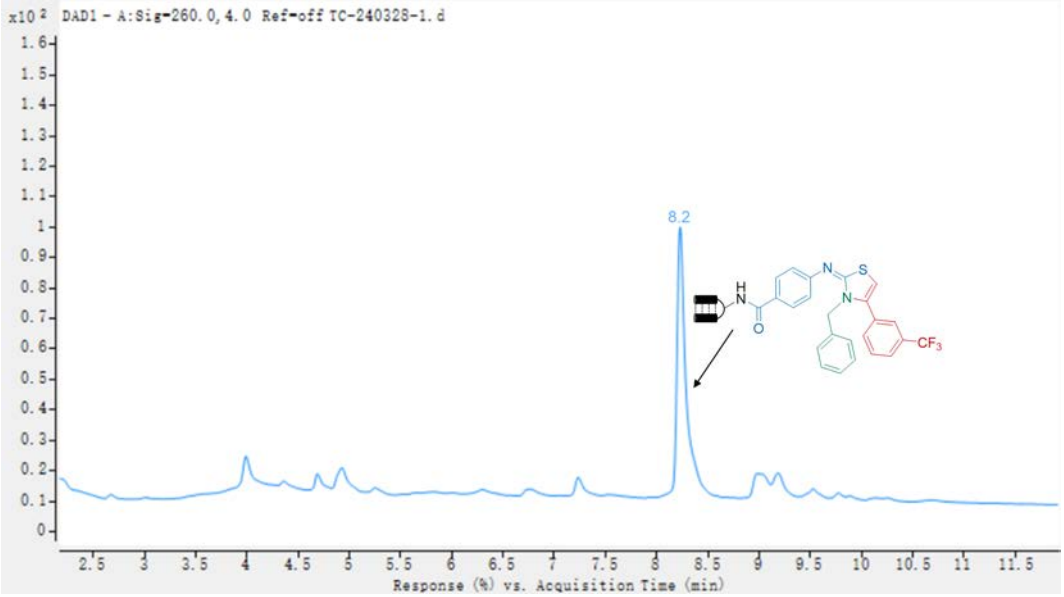


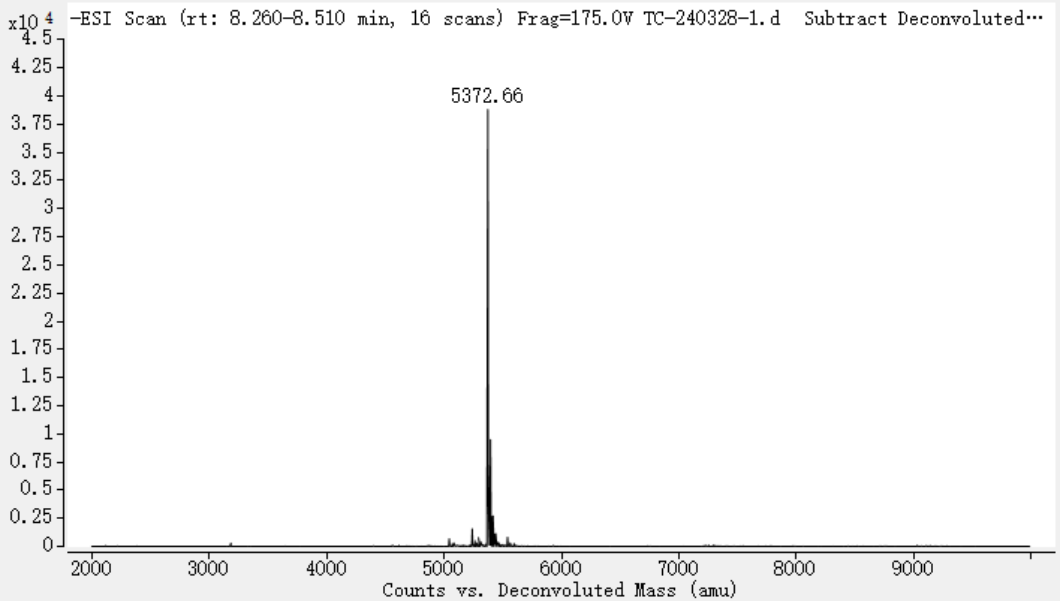


UPLC chromatogram and deconvoluted MS of **9ah**

Conversion: 88%

Calculated Mass: 5373 Da; Observed Mass: 5373 Da

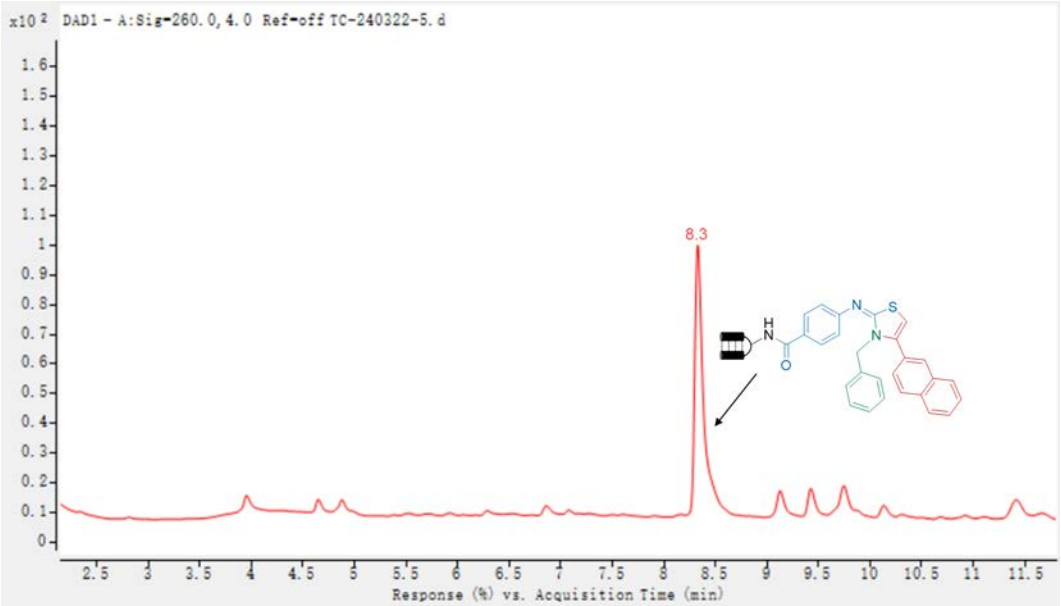


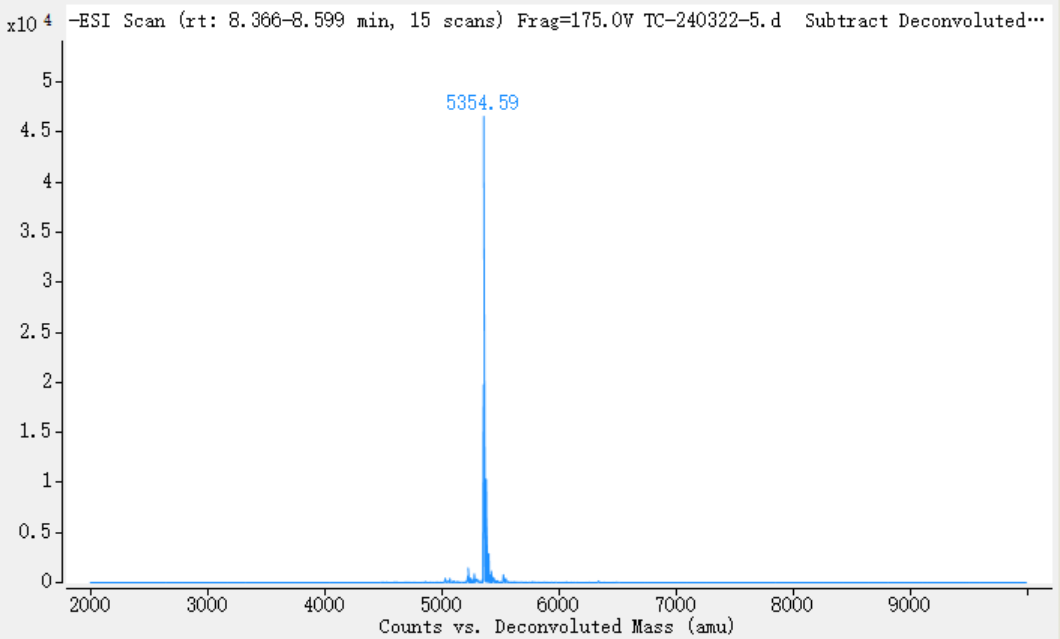


UPLC chromatogram and deconvoluted MS of **9ai**

Conversion: 84%

Calculated Mass: 5355 Da; Observed Mass: 5355 Da

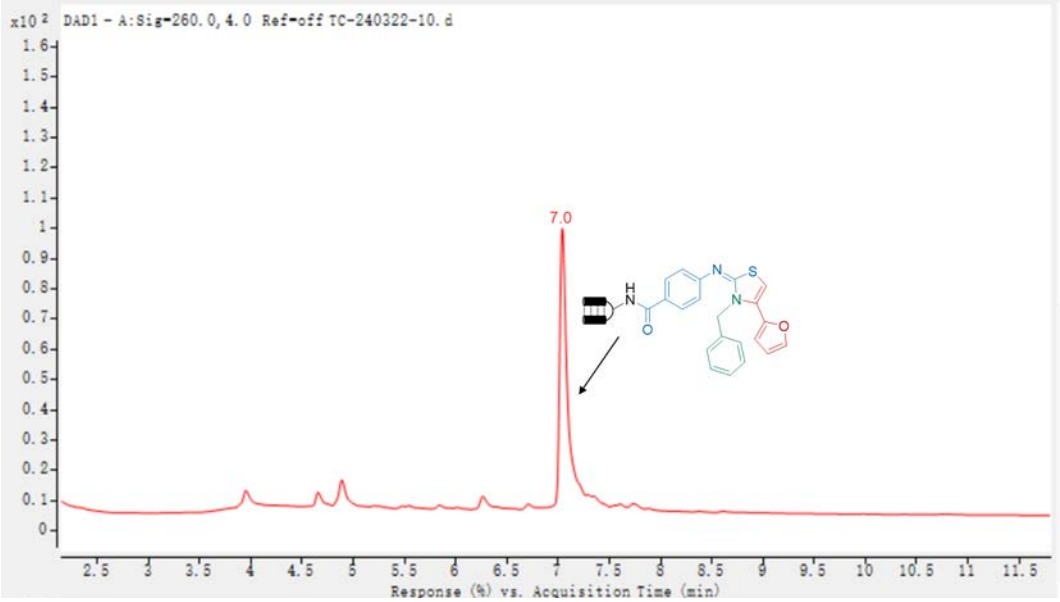


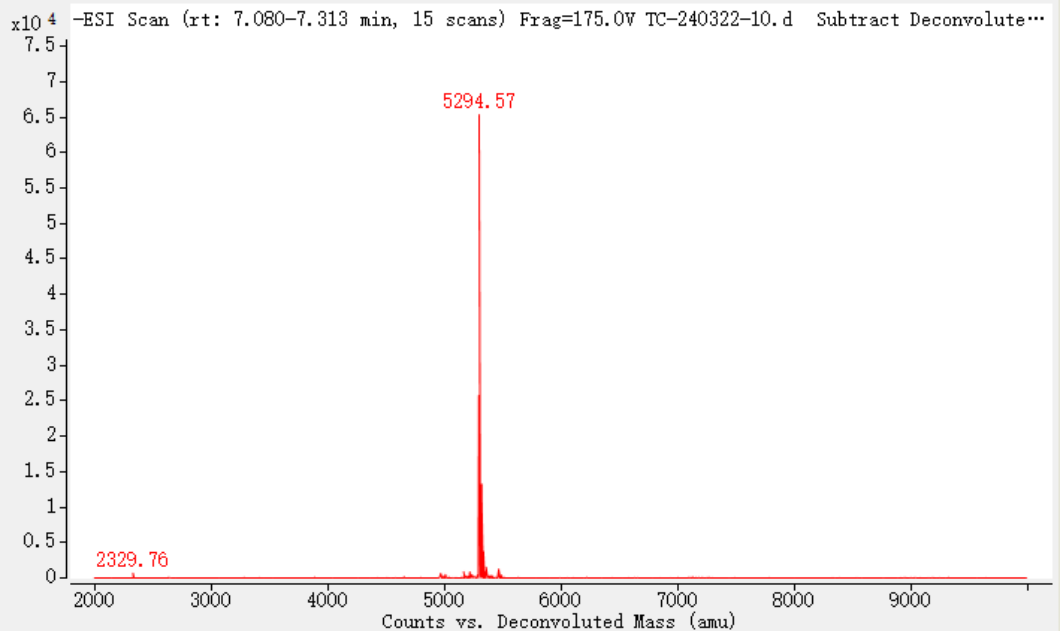


UPLC chromatogram and deconvoluted MS of **9aj**

Conversion: 87%

Calculated Mass: 5295 Da; Observed Mass: 5295 Da

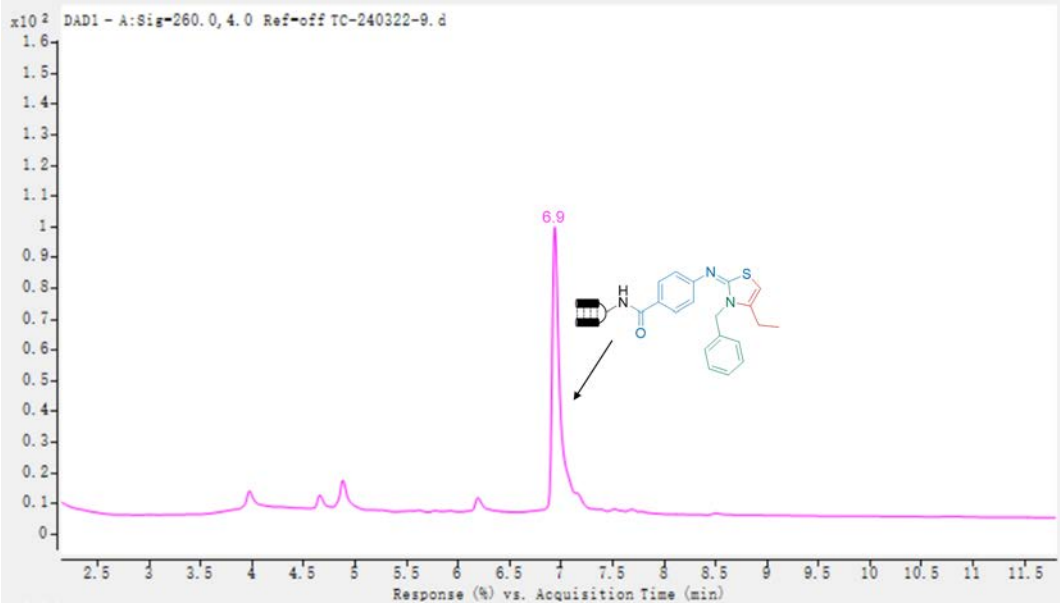


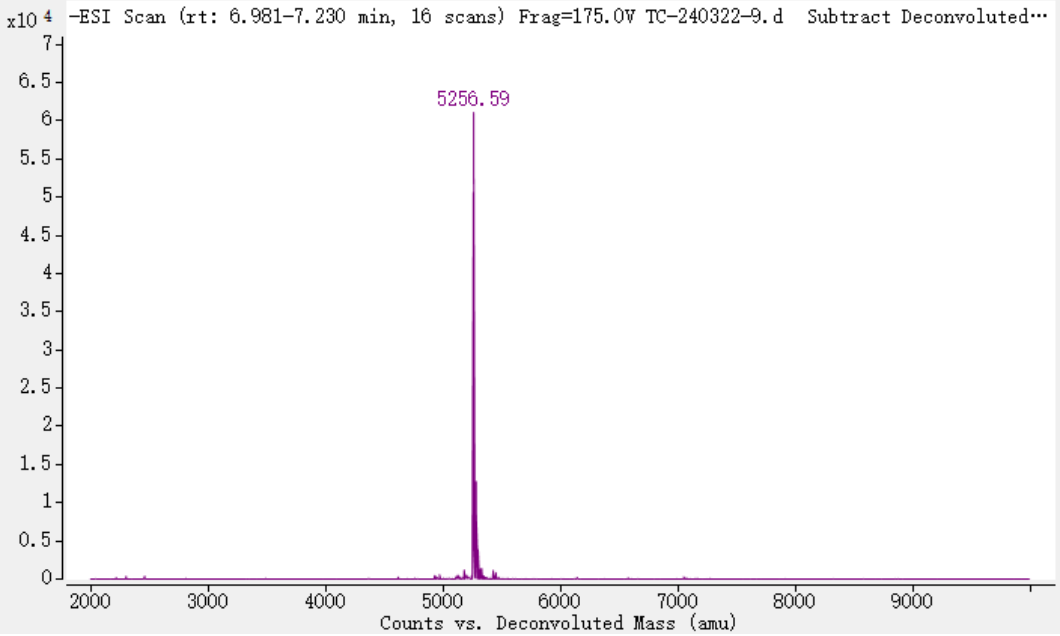


UPLC chromatogram and deconvoluted MS of **9ak**

Conversion: 83%

Calculated Mass: 5257 Da; Observed Mass: 5257 Da

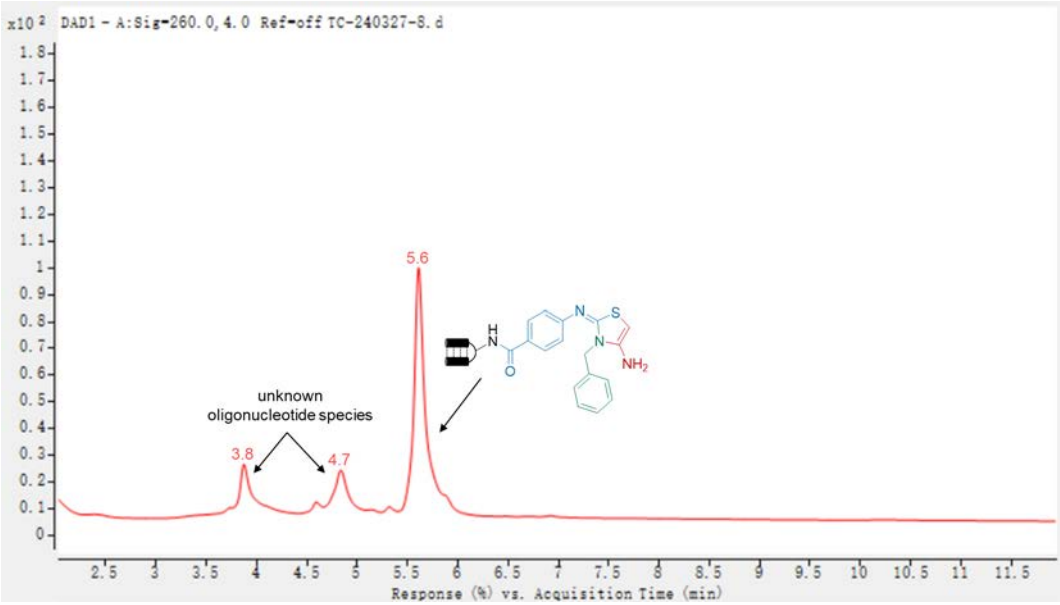


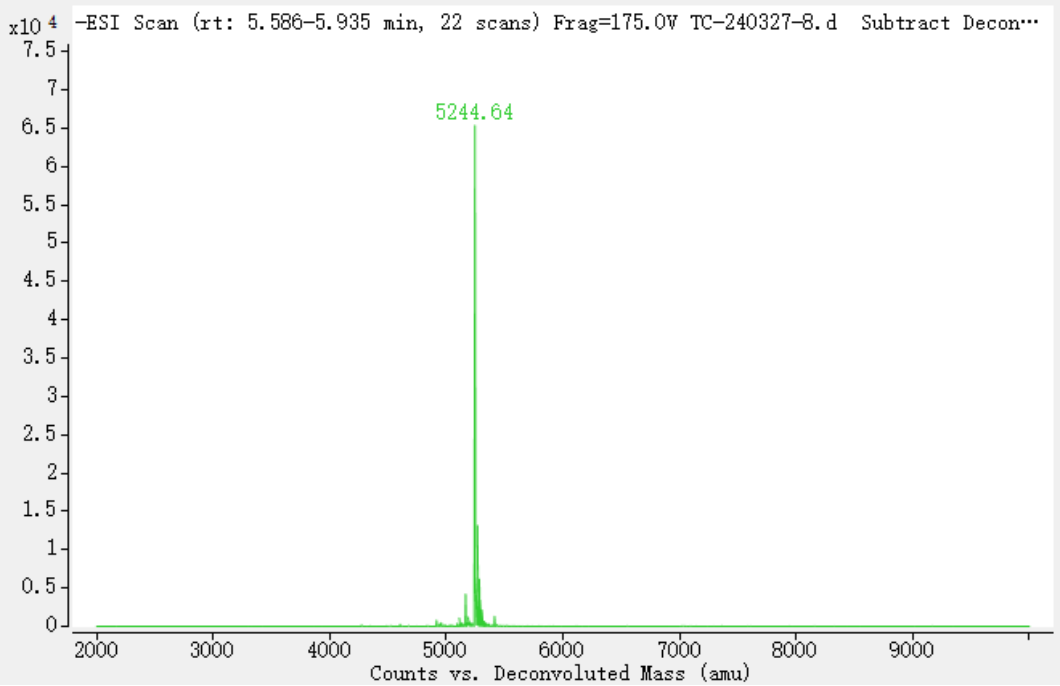


UPLC chromatogram and deconvoluted MS of **9al**

Conversion: 64%

Calculated Mass: 5245 Da; Observed Mass: 5245 Da

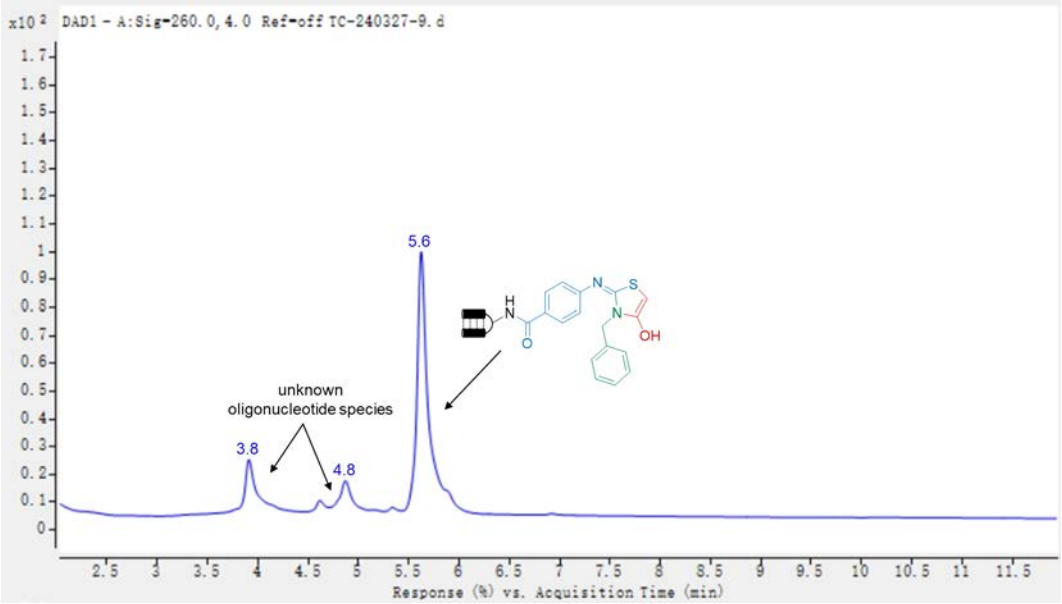


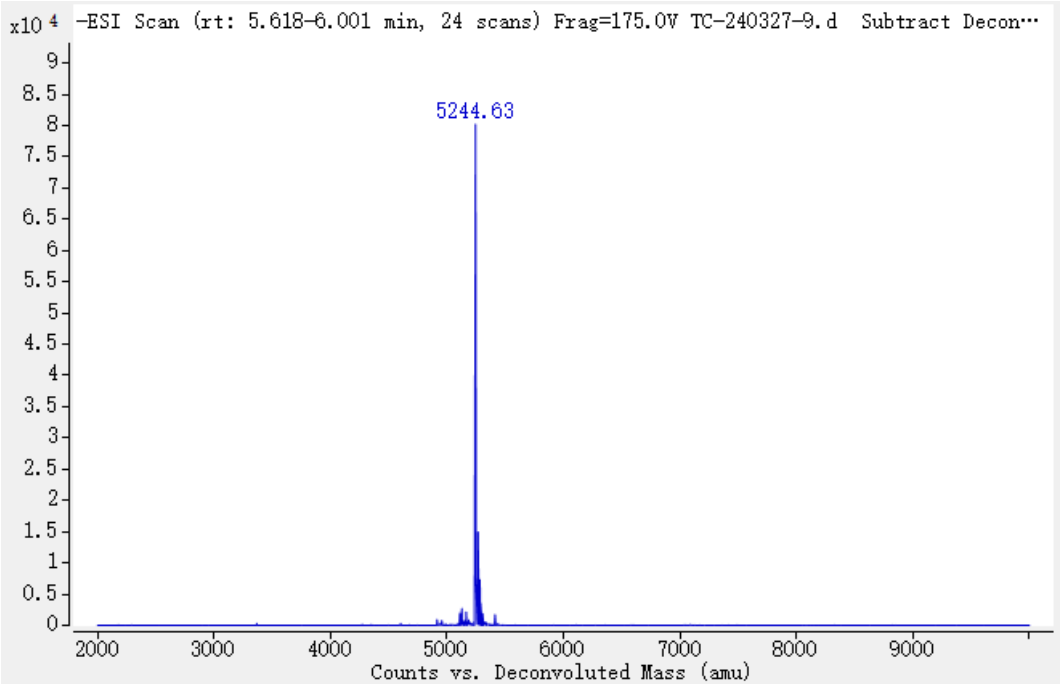


UPLC chromatogram and deconvoluted MS of **9am**

Conversion: 74%

Calculated Mass: 5245 Da; Observed Mass: 5245 Da





8. References

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