Electronic Supplementary Material (ESI) for Chemical Communications.

DNA-compatible combinatorial synthesis of functionalized 2-thiobenzazole scaffolds

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Supplementary Information

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1. Abbreviations

ACN: acetonitrile

DIPEA: *N*, *N*-diisopropylethylamine

DMA: *N*, *N*-dimethylacetamide

DMF: *N*, *N*- dimethylformamide

DMSO: dimethylsulfoxide

DCM: dichloromethane

MeOH: methanol

TBTA: tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine

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

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

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

TCDI: 1,1'-thiocarbonyldiimi

Bpy: 2,2'-bipyridine

Tris-HCl: trometamol hydrochloride

HP: headpiece

HP-P: headpiece primer

HPLC: high-performance liquid chromatography

MW: molecular weight

NMR: nuclear magnetic resonance

PAGE: polyacrylamide gel electrophoresis

TBE: tris-borate-EDTA

TEAA: triethylammonium acetate

TEA: trimethylamine

TLC: thin layer chromatography

TIC: total ion chromatogram

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

UV: ultraviolet

2. Materials and general methods

2.1. Materials

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used as received. Headpiece (HP, 5'-/5Phos/GAGTCA/iSp9/iUniAmM/iSp9/TGACTCCC-3'), Headpiece-primer (HP-P, 5'-/5Phos/ACCTTCGGTCGGGAGTCA/iSp9/iUniAmM/iSp9/TGACTCCCGACCGAAGGTTG-3') and code sequences were received from HitGen Inc. (Shuangliu District, Chengdu, China). All the DNA sequences were written in 5'- to 3'- orientation unless otherwise noted. Chemicals and reagents were purchased from several commercial suppliers including Bidepharm, Adamas and Sigma-Aldrich, *etc.*, and were generally used from aliquots dissolved in DMA, DMSO, ACN or other solvents, depending on solubility and optimized reaction conditions. T4 DNA ligase and 10× ligation buffer (500 mM Tris pH 7.5, 500 mM NaCl, 100 mM $MgCl_2$, 100 mM DTT and 25 mM ATP) were purchased from HitGen Inc.. All the buffer and aqueous solutions, 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 all the reactions unless otherwise stated. A Bio-Rad Chemidoc™ image system captured all the gel images.

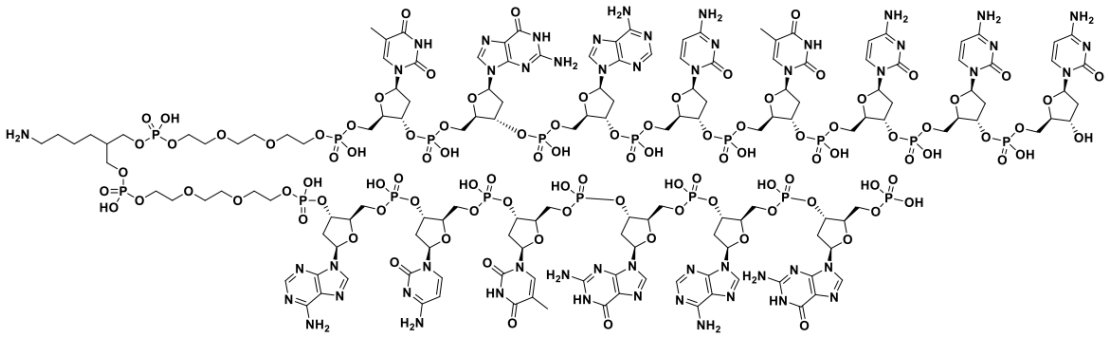


Figure S1. Structure of HP (5'-/5Phos/GAGTCA/iSp9/iUniAmM/iSp9/TGACTCCC-3'), MW = 4937.

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

Analysis of on-DNA reactions by UPLC-MS: The detection was performed by a high-resolution mass spectrometry-Agilent 6230 Time-of-Flight (TOF) mass spectrometer connected to an Agilent 1290 UPLC. After 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 at 260 nm and analyzed on Agilent TOF (6230 B) in negative ion mode.

LC-MS method of DNA reaction analysis:

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

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

LCMS method of DNA ligation analysis:

Time (min)	Flow (mL/min)	%B
initial	0.3	3.0
1	0.3	12.0
2.5	0.3	18.0
4	0.3	20.0
6	0.3	22.0
9	0.3	30.0
10	0.3	85.0
11	0.3	85.0
12	0.3	3.0

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

Conversion calculation: The conversion of on-DNA products was determined by UV absorbance (260 nm) peak area integration using the following equation: Conversion%

= UV (desired products)/UV (total products), ignoring UV extinction coefficient difference for DNA species and assuming 100% DNA recovery. Any non-oligo material with UV absorbance at 260 nm was subtracted from the conversion calculation.

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

2.3. General methods for DNA conjugates purification

General method for ethanol precipitation: To a DNA chemical reaction mixture was added 10% (V/V) 5 M NaCl solution and 3 times volume of absolute cold ethanol. Or to a DNA ligation reaction mixture was added 10% (V/V) 3 M acetate buffer (pH 5.2) and 3 times volume of absolute cold ethanol. After swirling and centrifuging, the solution was maintained at -80 °C for 2 h and then was centrifuged at 13500 rpm for 30 minutes at 4 °C by using an Eppendorf 5424 R centrifuge. The supernatant was discarded and the pellet was rinsed with 200 µL cold 70% ethanol. After centrifuging at 13500 rpm for 10 minutes at 4 °C, the supernatant was discarded again and the DNA pellet was dried by a Speedvac (CV200, JM company, Beijing, China), which was equipped with cryotrap (JM86, JM company, Beijing, China). The recovered sample was dissolved in the appropriate buffer for subsequent analysis or experiments.

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

2.4. General procedure for DNA ligation

This reaction contained variably derivatized HP-P starting material (10 nmol in H_2O, 1 equiv.), Code (12 nmol in H_2O, 1.2 equiv.), 10× ligation buffer (4 µL), T4 DNA ligase (1

μL, 1000 units/μL) and nuclease-free water (to the total volume of 40 μL). The reaction mixture was incubated at 20 °C for overnight before performing gel analysis. The crude material was purified by ethanol precipitation and taken on to the next step of synthesis without further purification.

2.5. General procedure for polyacrylamide gel

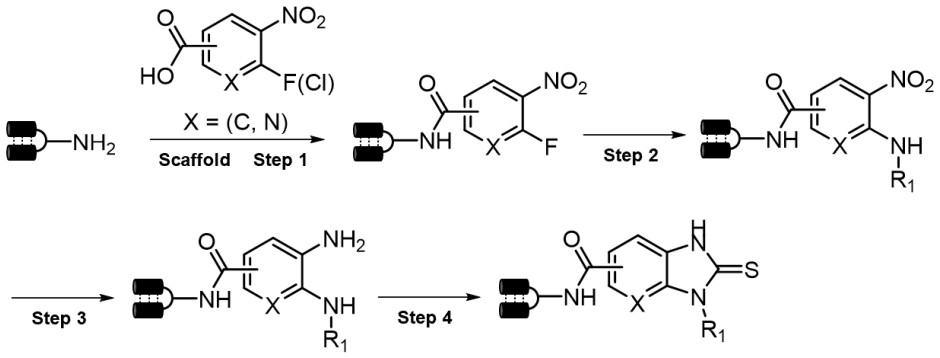
Ligation reactions were monitored by gel electrophoresis with 20% urea polyacrylamide gel in 1× TBE buffer (89 mM Tris-Borate, 2 mM EDTA，pH 8.3) system referenced by a 20 bp DNA ladder (Takara, Japan). Before gel loading, the DNA samples were denatured at 95°C for 10 min and mixed with 0.20 volume of the 6× gel-loading buffer. Then, 10 pmol of treated DNA samples were loaded on the gel, and the gel was run at 200 V for 50-60 min. DNA fragments were visualized and analyzed by Bio-Rad ChemidocTM Image System (Bio-Rad, CA, USA).

2.6. General methods for monitoring and characterization of small molecule

Reactions were monitored by TLC and general staining reagents were used to analyze TLC intuitively. The novel synthetic compound was characterized by ^{1}H-NMR and ^{13}C-NMR. NMR spectrum was recorded on Agilent 400 MHz spectrometer using residual non-deuterated solvent (DMSO-d_6) as the internal standard. Multiplicity abbreviations are as follows: s = singlet, brs = broad singlet, d = doublet (dd = doublet of doublets), t = triplet, q = quartet, m = multiplet. Unless otherwise noted, all deuterated solvents were purchased from Adamas.

3. General procedure for On-DNA 2-thiobenzazole formation

3.1. Preparation of DNA-conjugated *N*-substituted 2-mercaptobenzimidazole 3a-3f, 3i-3aj



Step 1-amide formation. Scaffold (**1a**-**1e**, 4 μL, 200 mM in DMA, 80 equiv), HATU (4 μL, 200 mM in DMA, 80 equiv), DIPEA (4 μL, 200 mM in DMA, 80 equiv) were mixed by vortex, and 6 μL of them were added to a solution of headpiece (10 μL, 1 M in 250mM pH 9.4 borate buffer). The reaction was allowed to stand at room temperature for ten minutes. Then 4 μL of pre-mixture containing scaffold, HATU, DIPEA were added to the DNA solution again. The reaction was agitated at room temperature for 1 h and precipitated with ethanol to give the DNA conjugates.

Scaffold (**1f**, 12.5 μL, 200 mM in DMSO, 250 equiv), EDCI (7.5 μL, 400 mM in DMSO, 300 equiv), S-NHS (5 μL, 400 mM in DMSO/H_2O = 1 : 1, 200 equiv) were mixed by vortex and all of them were added to a solution of headpiece (25 μL, 0.4 M in 250 mM pH 9.4 borate buffer). The reaction was agitated at room temperature for 2 h and precipitated with ethanol to give the DNA conjugate.

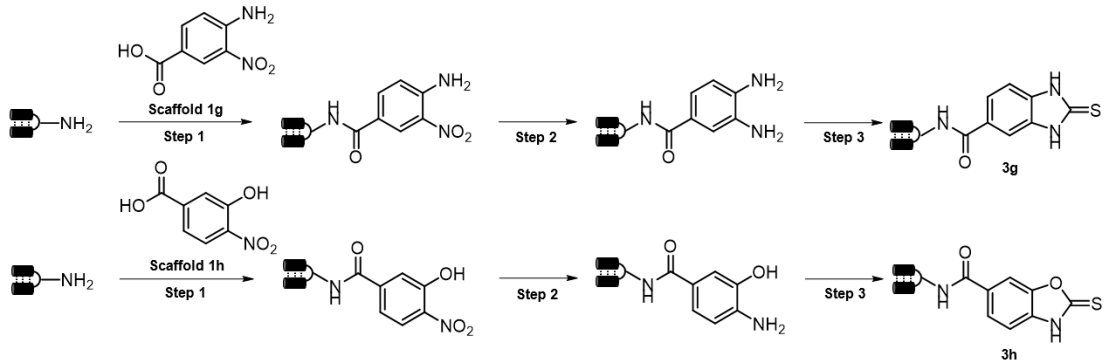
Step 2-nucleophilic substitution. Product of step 1 was dissolved in sodium borate buffer (20 μL, 250 mM, pH 9.4). Amine (10 μL, 200 mM in DMSO, 200 equiv) was added to the DNA solution. The reaction was allowed to proceed at 75 °C in incubator overnight and precipitated with ethanol to give the DNA conjugates.

Step 3-nitro reduction. Product of step 2 was dissolved in sodium borate buffer (20 μL, 250 mM, pH 9.4). $FeSO_4 \cdot 7H_2O$ (4 μL, 200 mM in H_2O, 80 equiv) and NaOH (4 μL, 800 mM in H_2O, 320 equiv) were added into the DNA solution, then vortex. The reaction

was allowed to proceed at 80 °C by metal bath for 2 hours. Then 30 equiv. of sodium diethyldithiocarbamic acid compared with $FeSO_4 \cdot 7H_2O$ were added to the mixture to scavenge the iron, 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 to give the DNA conjugates.

Step 4-thiocarbonylation reaction. Product of step 3 was dissolved in H_2O (80 µL). TCDI (10 µL, 200 mM in DMSO, 200 equiv) was added to the DNA solution. The reaction was allowed to proceed at 25 °C for 1 h. Then the newly prepared TCDI (10 µL, 200 mM in DMSO, 200 equiv) was added to the solution again and the reaction continued for 1h. After ethanol precipitation, the DNA conjugates were dissolved in H_2O and further purified by HPLC.

3.2. Preparation of DNA-conjugated *N*-substituted 2-mercaptobenzimidazole 3g and 3h



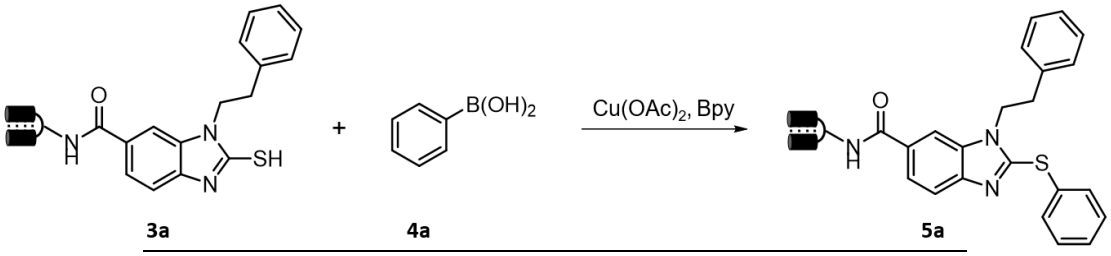
Step 1-amide formation. Scaffold (**1g**-**1h**, 4 µL, 200 mM in DMA, 80 equiv), HATU (4 µL, 200 mM in DMA, 80 equiv), DIPEA (4 µL, 200 mM in DMA, 80 equiv) were mixed by vortex, and 6 µL of them were added to a solution of headpiece (10 µL, 1 M in 250 mM pH 9.4 borate buffer). The reaction was allowed to stand at room temperature for ten minutes. Then 4 µL of pre-mixture containing scaffold, HATU, DIPEA were added to the DNA solution again. The reaction was agitated at room temperature for 1 h and precipitated with ethanol to give the DNA conjugates.

Step 2-nitro reduction. Product of step 1 was dissolved in sodium borate buffer (20 µL, 250 mM, pH 9.4). $FeSO_4 \cdot 7H_2O$ (4 µL, 200 mM in H_2O, 80 equiv) and NaOH (4 µL, 800 mM in H_2O, 320 equiv) were added into the DNA solution, then vortex. The reaction

was allowed to proceed at 80 °C by metal bath for 2 hours. Then 30 equiv. of sodium diethyldithiocarbamic acid compared with $FeSO_4 \cdot 7H_2O$ were added to the mixture to scavenge the iron, 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 to give the DNA conjugates.

Step 3-thiocarbonylation reaction. Product of step 2 was dissolved in H_2O (80 μL). TCDI (10 μL, 200 mM in DMSO, 200 equiv) was added to the DNA solution. The reaction was allowed to proceed at 25 °C for 1 h. Then the newly prepared TCDI (10 μL, 200 mM in DMSO, 200 equiv) was added to the solution again and the reaction continued for 1h. After ethanol precipitation, the DNA conjugates were dissolved in H_2O and further purified by HPLC.

3.3. Optimization of the model reaction

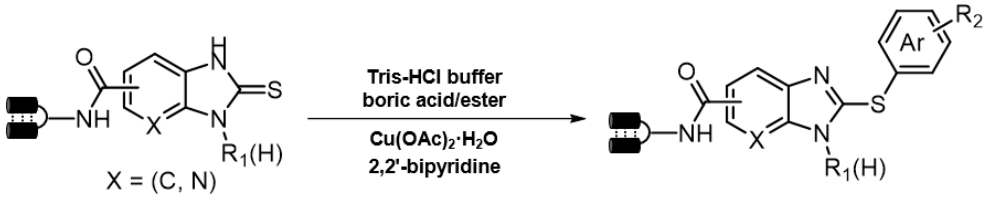


3a **4a** **5a**

Entry	Additive	Reaction system	Conv. (%)
1	H_2O	pH ≈ 7	35
2	NaOH	pH ≈ 10	58
3	$NaHCO_3$	pH ≈ 8	75
4	Et_3N	pH ≈ 8	56
5	MOPS Buffer	pH = 8.0	81
6	**Tris-HCl Buffer**	**pH = 8.0**	**>90**
7	BB Buffer	pH = 8.0	48
8	HEPES Buffer	pH = 8.0	76
9[a]	Tris-HCl Buffer	pH = 8.0	0
10[b]	Tris-HCl Buffer	pH = 8.0	0

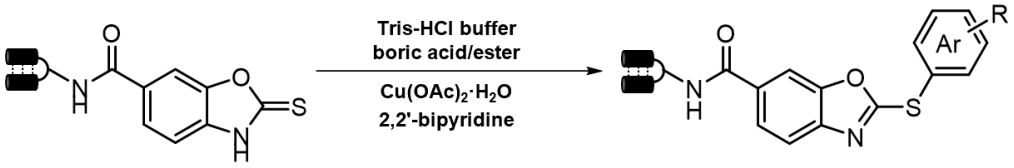
Standard reaction condition: **3a** (2 μL, 100 μM in H_2O), Tris-HCl (16 μL, 500 mM, pH 8.0), $Cu(OAc)_2 \cdot H_2O$ (2 μL, 10 mM in H_2O), Bpy (2 μL, 20 mM in DMF), phenylboronic acid **4a** (2 μL, 200 mM in DMF), and H_2O to a total volume of 50 μL, 25 °C, 3 h. [a] no $Cu(OAc)_2$, [b] no Bpy.

3.4. On-DNA 2-thiobenzimidazole synthesis



To the solution of the DNA conjugates in chapter 3.1 step 4 above (2 μL, 100 μM in H_2O) was added H_2O (26 μL), Tris-HCl (16 μL, 500 mM, pH 8.0), $Cu(OAc)_2·H_2O$ (2 μL, 10 mM in H_2O, 100 equiv), Bpy (2 μL, 20 mM in DMA, 200 equiv) and boric acid/ester (2 μL, 200 mM in DMA, 2000 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 3 h (Some boric acid/ester substrates need to be placed in a metal bath at 40 °C). Then 30 equiv. of sodium diethyldithiocarbamic acid compared with $Cu(OAc)_2·H_2O$ 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 products were obtained by ethanol precipitation as described above. Unless otherwise noted, on-DNA thiobenzimidazoles described in the supplementary information were synthesized under this standard condition.

3.5. On-DNA 2-thiobenzoxazole synthesis

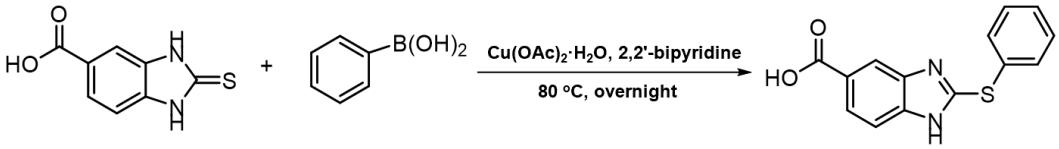


To the solution of the DNA conjugate **3h** (2 μL, 100 μM in H_2O) was added H_2O (26 μL), Tris-HCl (16 μL, 500 mM, pH 8.0), $Cu(OAc)_2·H_2O$ (2 μL, 10 mM in H_2O, 100 equiv), Bpy (2 μL, 20 mM in DMA, 200 equiv) and boric acid/ester (2 μL, 200 mM in DMA, 2000 equiv). The mixture was vortexed, centrifuged, and placed at 25 °C for 3 h (Some boric acid/ester substrates need to be placed in a metal bath at 40 °C). Then 30 equiv. of sodium diethyldithiocarbamic acid compared with $Cu(OAc)_2·H_2O$ 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 products were obtained

by ethanol precipitation as described above. Unless otherwise noted, on-DNA thiobenzimidazoles described in the supplementary information were synthesized under this standard condition.

4. Structure Validation of the On-DNA Synthesized Product

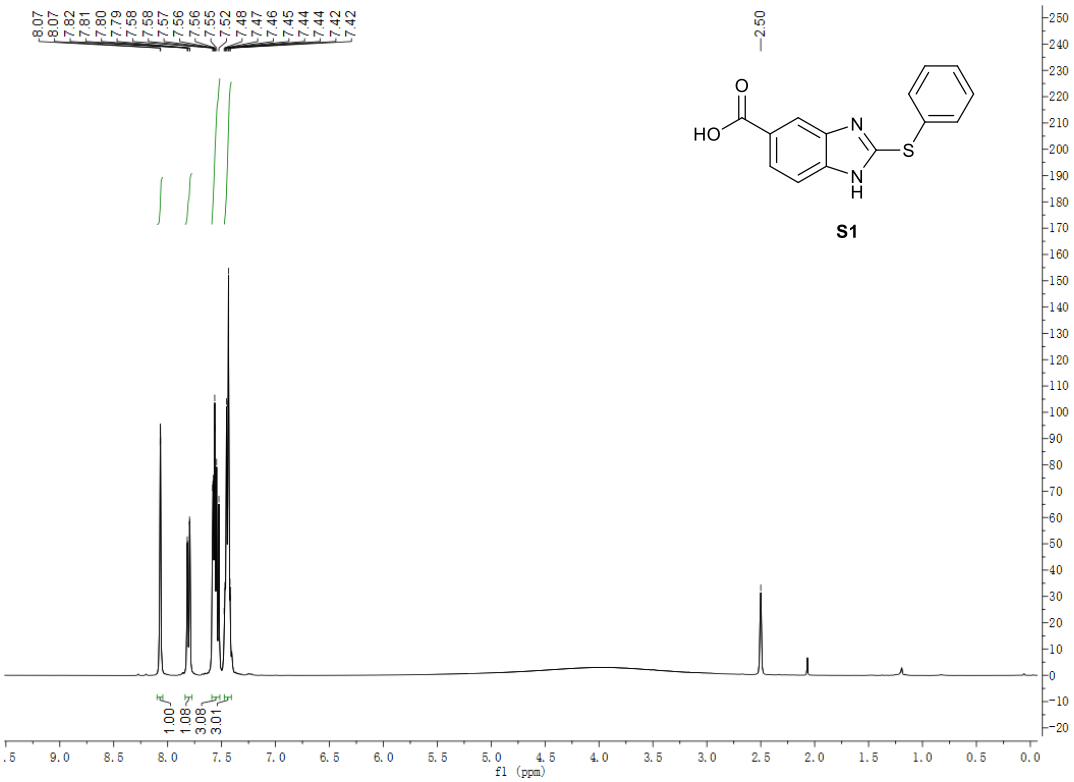
4.1. Off-DNA Synthesis of S1



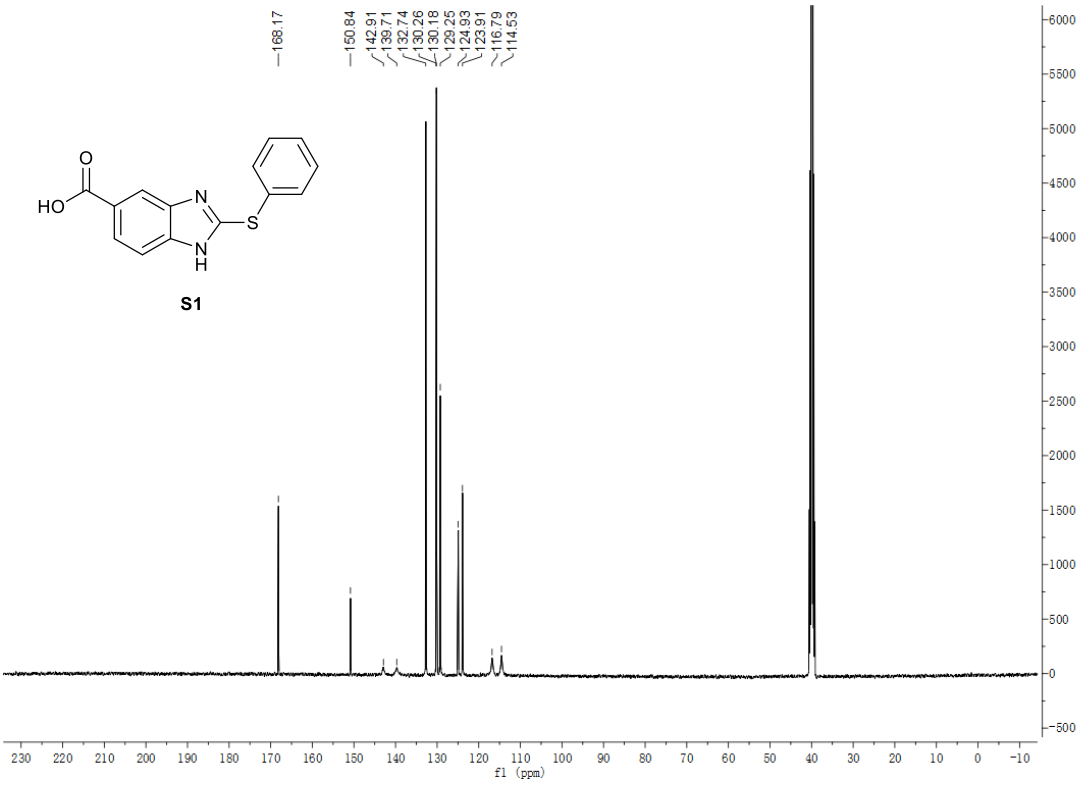
Reaction was performed in a 25 mL Schlenk flask equipped with a stirring bar. 2-mercapto-5-benzimidazolecarboxylic acid (100 mg, 0.515 mmol, 1 equiv), phenylboronic acid (94.2 mg, 0.772 mmol, 1.5 equiv), $Cu(OAc)_2 \cdot H_2O$ (5.20 mg, 0.026 mmol, 0.05 equiv) and 2,2'-bipyridine (8.12 mg, 0.052 mmol, 0.1 equiv) were dissolved with DMF (0.75 mL) and H_2O (2.25 mL) in the flask. Then the reaction system reacted overnight in an oil bath at 80 °C, monitored using TLC (DCM : MeOH = 20 : 1). Next, the reaction was terminated with a saturated ammonium chloride solution and the pH of the reaction system was adjusted to 2-4 with HCl (6 M). The reaction mixture was extracted twice with ethyl acetate (10 mL) and then concentrated. The crude product was purified by a flash chromatography with DCM and MeOH to give the desired product **S1**.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (d, *J* = 1.7 Hz, 1H), 8.07 (d, *J* = 1.7 Hz, 1H), 7.81 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.81 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.60 – 7.55 (m, 2H), 7.56 (ddd, *J* = 15.4, 10.1, 5.3 Hz, 3H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.48 – 7.41 (m, 3H), 7.47 – 7.41 (m, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 168.17 (s), 150.84 (s), 142.91 (s), 139.71 (s), 132.74 (s), 130.26 (s), 130.18 (s), 129.25 (s), 124.93 (s), 123.91 (s), 116.79 (s), 114.53 (s). **HRMS (ESI) m/z**: Calculated for $C_{14}H_{10}N_2O_2S$ $[M - H]^-$, 270.0463; Found: 269.0396.

^{1}H-NMR spectrum of **S1**



^{13}C{1H} NMR spectrum of **S1**



4.2. Validation of On-DNA thiobenzimidazole product by co-injection assay

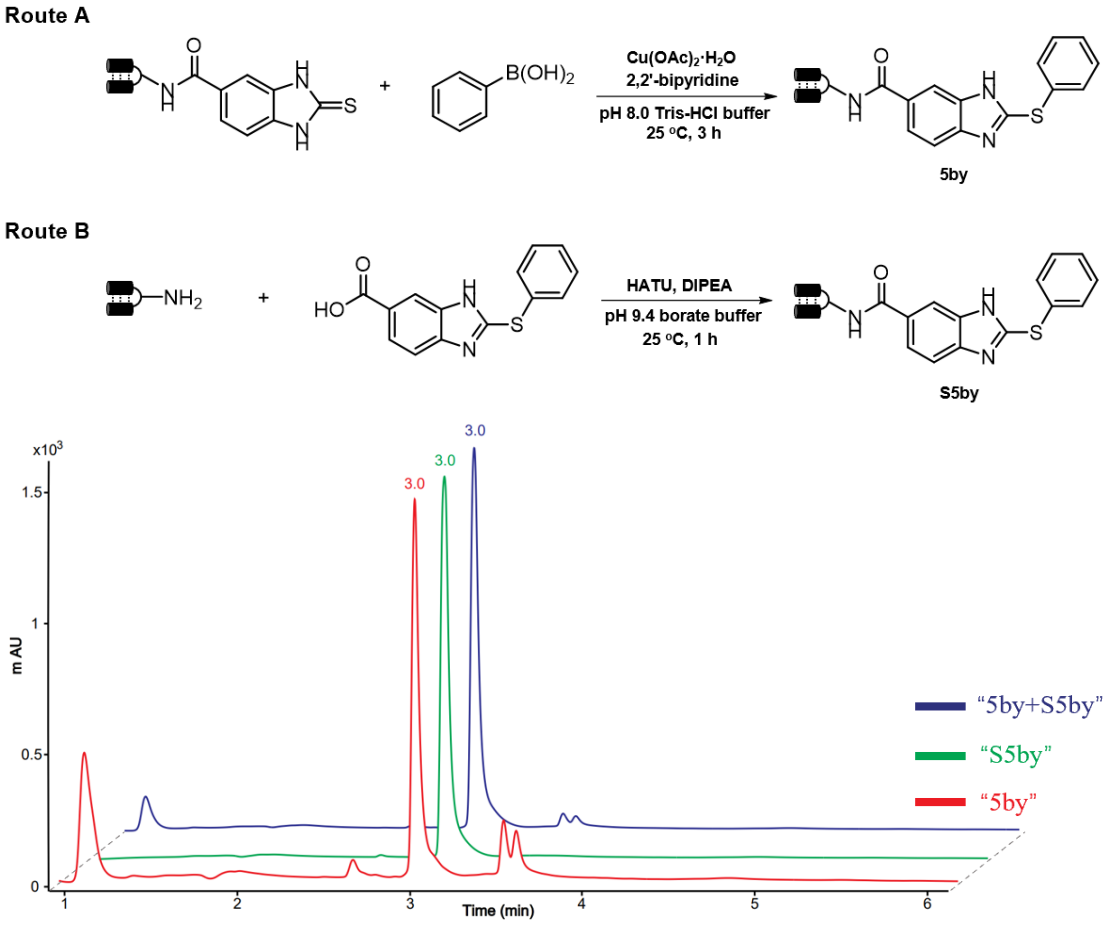
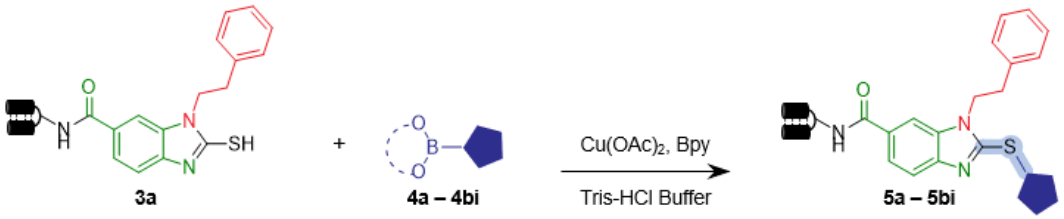


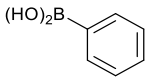
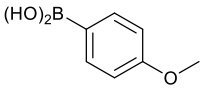
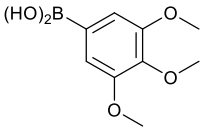
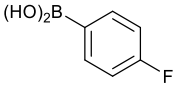
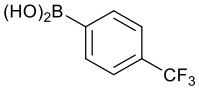
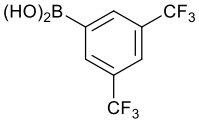
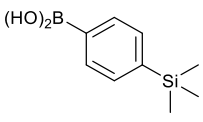
Figure S2. **The co-injection experiment of 5by and S5by by UPLC-MS.** The "blue" curve is the co-injection sample mixing **5by** and **S5by**; the "red" curve is **5by**; the "green" curve is **S5by**. The on-DNA synthesized **5by** sample was co-eluted with the off-DNA synthesized and characterized **S5by** sample, indicating their structures were identical.

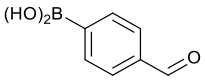
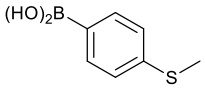
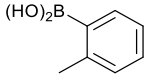
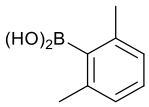
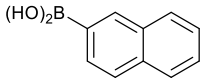
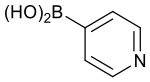
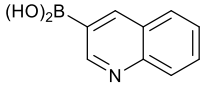
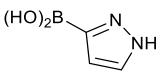
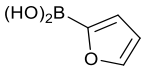
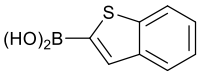
5. Substrate scopes of On-DNA thiobenzazoles

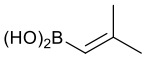
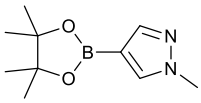
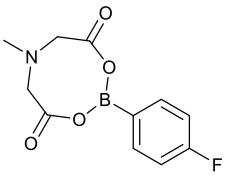
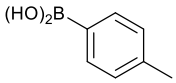
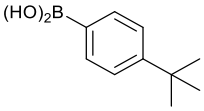
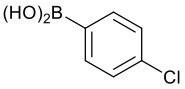
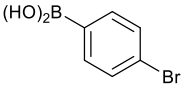
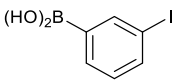
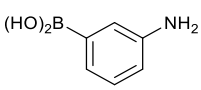
5.1. Substrate scope of boronic acids for the on-DNA thiobenzimidazole synthesis

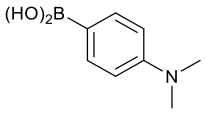
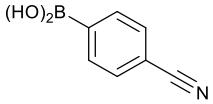
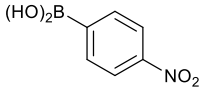
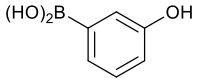
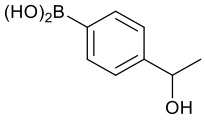
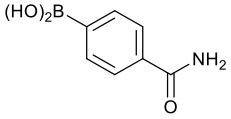
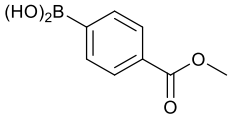
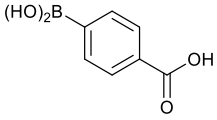
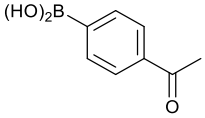
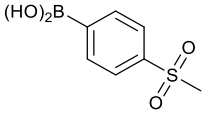


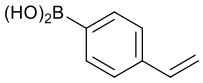
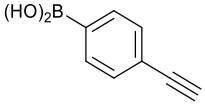
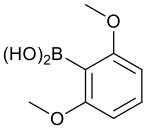
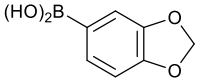
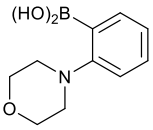
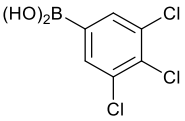
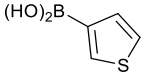
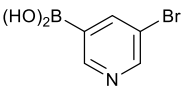
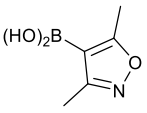
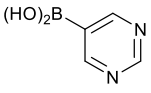
3a + 4a – 4bi → 5a – 5bi

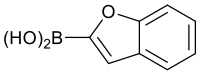
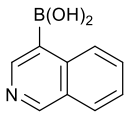
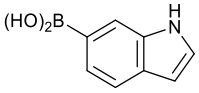
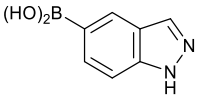
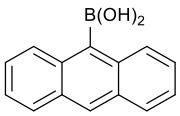
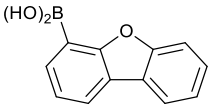
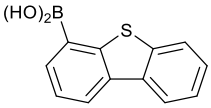
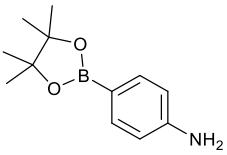
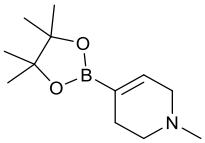
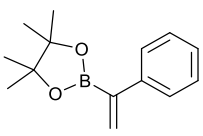
Compound	Boronic acid	Product	Predicted MW	Observed MW	Conversion (%)
4a		**5a**	5293	5293	>90
4b		**5b**	5323	5323	>90
4c		**5c**	5383	5383	>90
4d		**5d**	5311	5311	>90
4e		**5e**	5361	5361	>90
4f[a]		**5f**	5429	5429	>90
4g		**5g**	5365	5365	>90

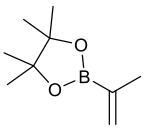
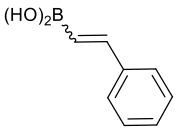
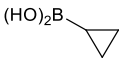
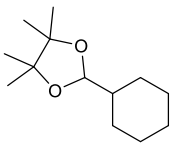
Compound	Boronic acid	Product	Predicted MW	Observed MW	Conversion (%)
4h		**5h**	5321	5321	>90
4i		**5i**	5339	5339	>90
4j		**5j**	5307	5307	>90
4k[a]		**5k**	5321	5321	76
4l		**5l**	5343	5343	>90
4m[a]		**5m**	5294	5294	>90
4n		**5n**	5344	5344	>90
4o		**5o**	5283	5283	>90
4p		**5p**	5283	5283	>90
4q		**5q**	5349	5349	>90

Compound	Boronic acid	Product	Predicted MW	Observed MW	Conversion (%)
4r[a]		**5r**	5271	5271	>90
4s		**5s**	5243	5243	>90
4t[a]		**5t**	5297	5297	>90
4u[a]		**5u**	5311	5311	>90
4v		**5v**	5307	5307	>90
4w		**5w**	5349	5349	>90
4x		**5x**	5327	5327	>90
4y		**5y**	5372	5372	>90
4z		**5z**	5419	5419	>90
4aa		**5aa**	5308	5308	>90

Compound	Boronic acid	Product	Predicted MW	Observed MW	Conversion (%)
4ab		**5ab**	5336	5336	>90
4ac		**5ac**	5318	5318	>90
4ad		**5ad**	5338	5338	>90
4ae		**5ae**	5309	5309	>90
4af		**5af**	5337	5337	>90
4ag		**5ag**	5336	5336	>90
4ah		**5ah**	5351	5351	>90
4ai		**5ai**	5337	5337	>90
4aj		**5aj**	5335	5335	>90
4ak		**5ak**	5371	5371	>90

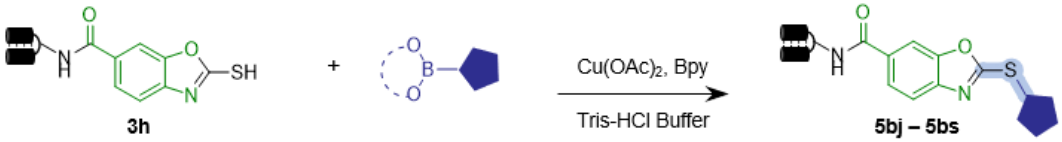
Compound	Boronic acid	Product	Predicted MW	Observed MW	Conversion (%)
4al		**5al**	5319	5319	>90
4am		**5am**	5317	5317	>90
4an		**5an**	5353	5353	>90
4ao		**5ao**	5337	5337	>90
4ap[a]		**5ap**	5378	5378	81
4aq		**5aq**	5396	5396	>90
4ar		**5ar**[a]	5299	5299	>90
4as[a]		**5as**	5373	5373	>90
4at[a]		**5at**	5312	5312	80
4au[a]		**5au**	5295	5295	>90

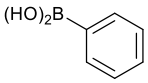
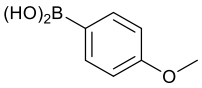
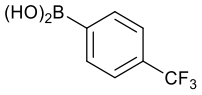
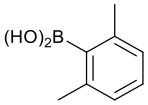
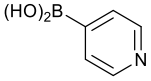
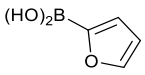
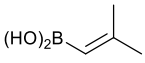
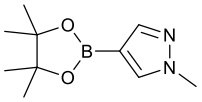
Compound	Boronic acid	Product	Predicted MW	Observed MW	Conversion (%)
4av		**5av**	5333	5333	>90
4aw		**5aw**	5344	5344	>90
4ax		**5ax**	5332	5332	>90
4ay		**5ay**	5333	5333	>90
4az		**5az**	5393	5393	>90
4ba		**5ba**	5383	5383	>90
4bb		**5bb**	5399	5399	>90
4bc[a]		**5bc**	5308	5308	>90
4bd[a]		**5bd**	5312	5312	>90
4be[a]		**5be**	5319	5319	31

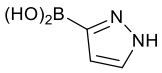
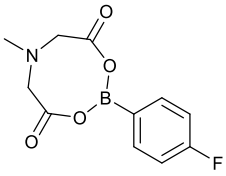
Compound	Boronic acid	Product	Predicted MW	Observed MW	Conversion (%)
4bf[a]		**5bf**	5257	5257	>90
4bg[a]		**5bg**	5319	5319	73
4bh[a]		**5bh**	5257	5257	12
4bi[a]		**5bi**	5299	-	0

[a] The reaction temperature is 40 °C.

5.2. Substrate scope of boronic acids for the on-DNA thiobenzoxazole synthesis

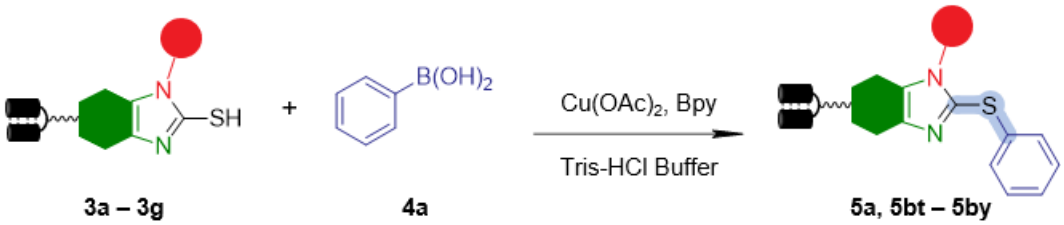


Compound	Boronic acid	Product	Predicted MW	Observed MW	Conversion (%)
4a		**5bj**	5190	5190	>90
4b		**5bk**	5220	5220	>90
4e		**5bl**	5258	5258	>90
4k[a]		**5bm**	5218	5218	>90
4m[a]		**5bn**	5191	5191	88
4p		**5bo**	5180	5180	>90
4r[a]		**5bp**	5168	5168	>90
4t[a]		**5bq**	5194	5194	>90

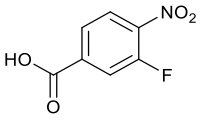
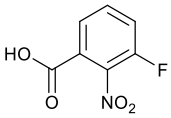
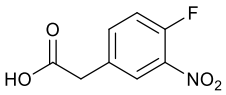
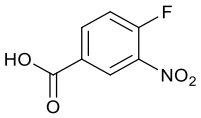
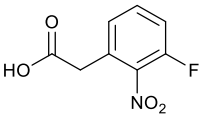
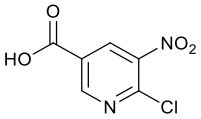
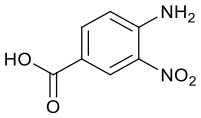
Compound	Boronic acid	Product	Predicted MW	Observed MW	Conversion (%)
4o		**5br**	5180	5180	>90
4u[a]		**5bs**	5208	5208	>90

[a] The reaction temperature is 40 °C.

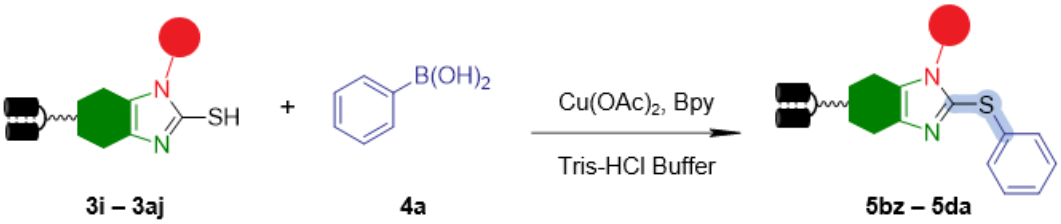
5.3. Substrate scope of scaffolds for the on-DNA thiobenzimidazole synthesis



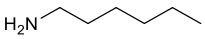
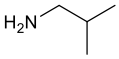
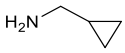
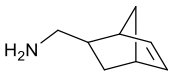
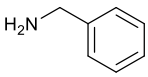
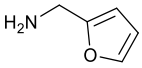
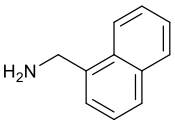
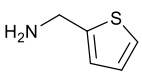
3a – 3g 4a 5a, 5bt – 5by

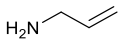
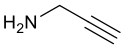
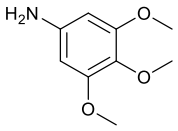
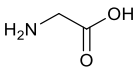
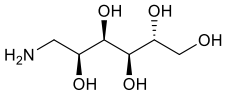
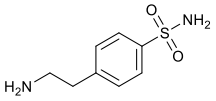
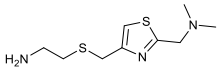
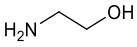
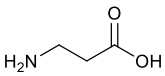
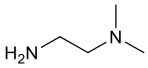
Compound	Scaffold	Product	Predicted MW	Observed MW	Conversion (%)
1a		**5a**	5293	5293	>90
1b		**5bt**	5293	5293	>90
1c		**5bu**	5307	5307	>90
1d		**5bv**	5293	5293	>90
1e		**5bw**	5307	5307	>90
1f		**5bx**	5294	5294	>90
1g		**5by**	5189	5189	>90

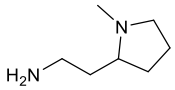
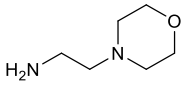
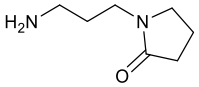
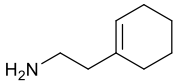
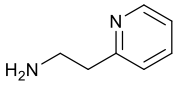
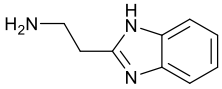
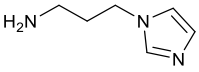
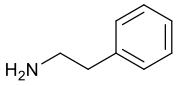
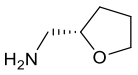
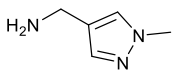
5.4. Substrate scope of amines for the on-DNA thiobenzimidazole synthesis

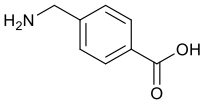


3i – 3aj 4a 5bz – 5da

Compound	Amine	Product	Predicted MW	Observed MW	Conversion (%)
2a		**5bz**	5273	5273	80
2b		**5ca**	5245	5245	>90
2c		**5cb**	5243	5243	>90
2d		**5cc**	5295	5295	>90
2e		**5cd**	5279	5279	>90
2f		**5ce**	5269	5269	>90
2g		**5cf**	5329	5329	>90
2h		**5cg**	5285	5285	>90

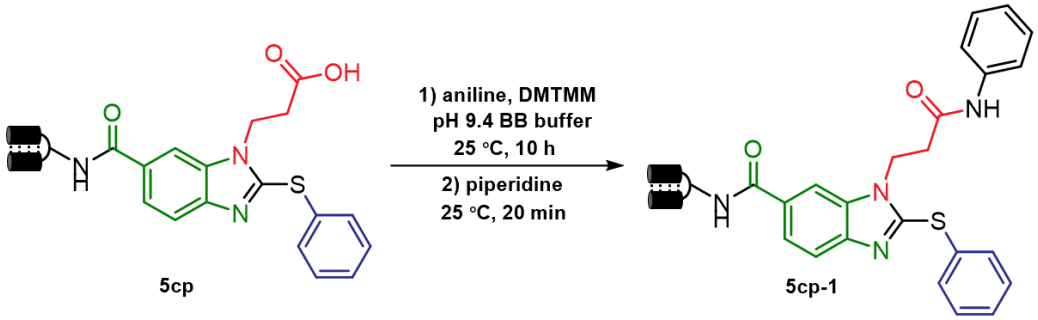
Compound	Amine	Product	Predicted MW	Observed MW	Conversion (%)
2i		**5ch**	5229	5229	>90
2j		**5ci**	5227	5227	60
2k		**5cj**	5355	5355	>90
2l		**5ck**	5247	5247	83
2m		**5cl**	5353	5353	87
2n		**5cm**	5372	5372	>90
2o		**5cn**	5403	5403	>90
2p		**5co**	5233	5233	81
2q		**5cp**	5261	5261	79
2r		**5cq**	5260	5260	62

Compound	Amine	Product	Predicted MW	Observed MW	Conversion (%)
2s		**5cr**	5300	5300	>90
2t		**5cs**	5302	5302	>90
2u		**5ct**	5314	5314	>90
2v		**5cu**	5297	5297	>90
2w		**5cv**	5294	5294	81
2x		**5cw**	5333	5333	85
2y		**5cx**	5297	5297	82
2z		**5a**	5293	5293	>90
2aa		**5cy**	5273	5273	>90
2ab		**5cz**	5283	5283	>90

Compound	Amine	Product	Predicted MW	Observed MW	Conversion (%)
2ac		**5da**	5323	5323	>90

6. General procedure for subsequent diversification of thiobenzimidazoles

6.1. Amide coupling



The DNA conjugate **5cp** (200 pmol) was dissolved in sodium borate buffer (20 µL, 250 mM, pH 9.4). Aniline (5 µL, 500 mM in DMA) and DMTMM (5 µL, 500 mM in H_2O) were added to the DNA solution. The reaction was allowed to proceed at 25 °C for 10 h. Then the DMTMM adduct was removed by adding piperidine (3 µL) to the reaction solution. The reaction was precipitated with ethanol to give the DNA conjugate **5cp-1**. After ethanol precipitation, the DNA conjugate **5cp-1** was redissolved in H_2O (60 µL) and characterized by UPLC-MS for 77% conversion. Calculated Mass: 5398 Da; Observed Mass: 5398 Da.

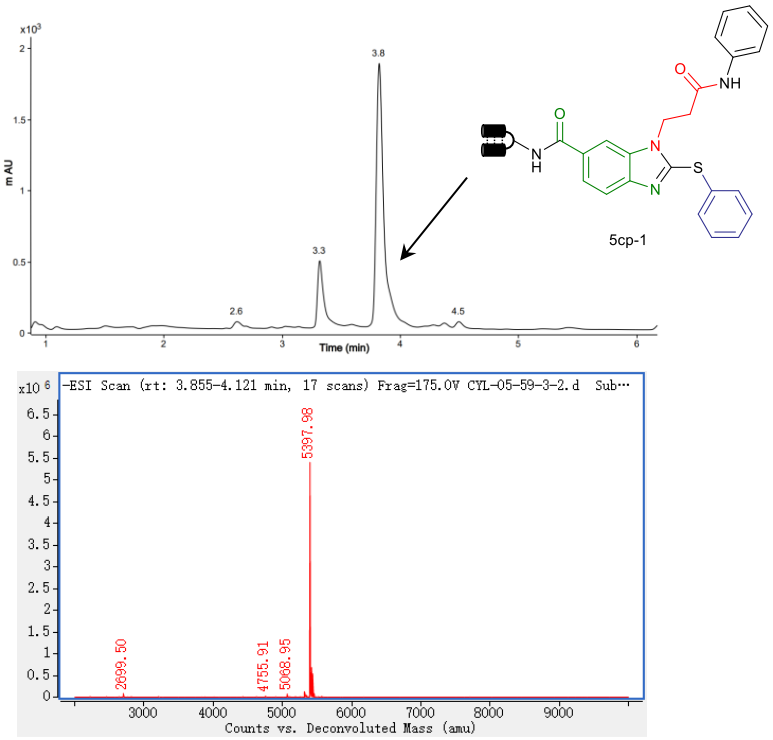
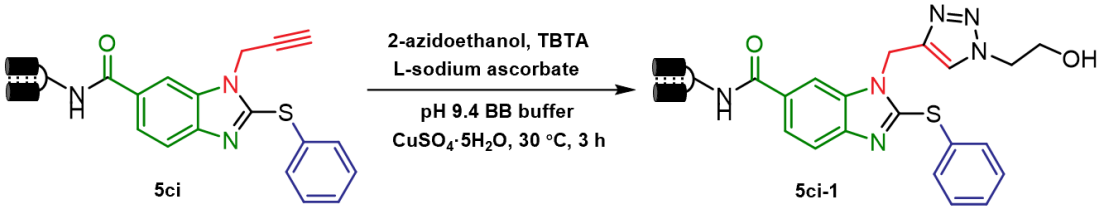


Figure S3. UPLC chromatogram and deconvoluted MS of **5cp-1**

6.2. Click Reaction



The DNA conjugate **5ci** (200 pmol) was dissolved in sodium borate buffer (8 µL, 250 mM, pH 9.4). 2-azidoethanol (4 µL, 40 mM in DMSO), TBTA (4 µL, 60 mM in DMSO), $CuSO_4·5H_2O$ (4 µL, 50 mM in H_2O) and L-sodium ascorbate (4 µL, 70 mM in H_2O) were added 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 $CuSO_4·5H_2O$ 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 to give the DNA conjugate **5ci-1**. After ethanol precipitation, the DNA conjugate **5ci-1** was redissolved in H_2O (60 µL) and characterized by UPLC-MS for 60% conversion. Calculated Mass: 5314 Da; Observed Mass: 5314 Da.

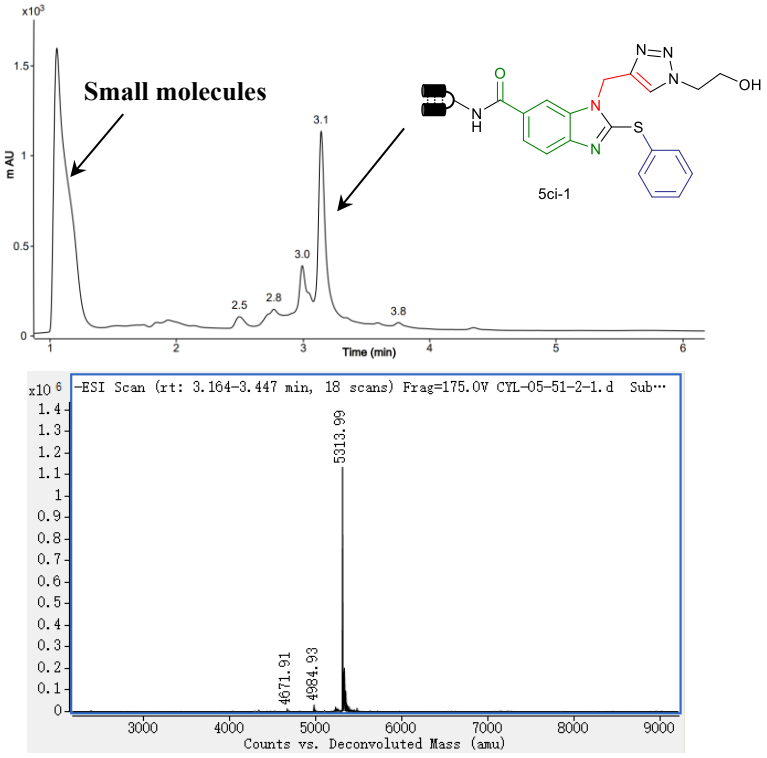
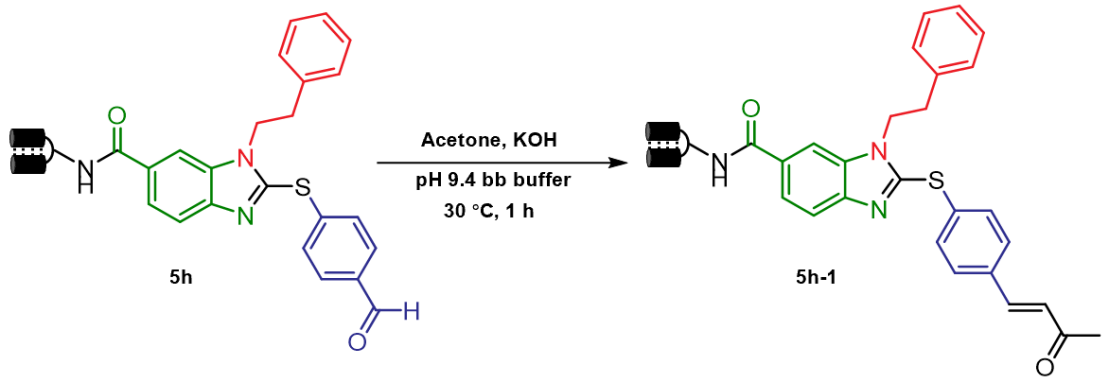


Figure S4. UPLC chromatogram and deconvoluted MS of **5ci-1**

6.3. Aldol Reaction



The DNA conjugate **5h** (200 pmol) was dissolved in sodium borate buffer (10 µL, 250 mM, pH 9.4). KOH (5 µL, 500 mM in H_2O) and acetone (10 µL, 200 mM in DMSO) were added to the DNA solution. The reaction was allowed to proceed at 30 °C for 1 h. Then the reaction was precipitated with ethanol to give the DNA conjugate **5h-1**. After ethanol precipitation, the DNA conjugate **5h-1** was redissolved in H_2O (60 µL) and characterized by UPLC-MS for 92% conversion. Calculated Mass: 5361 Da; Observed Mass: 5361 Da.

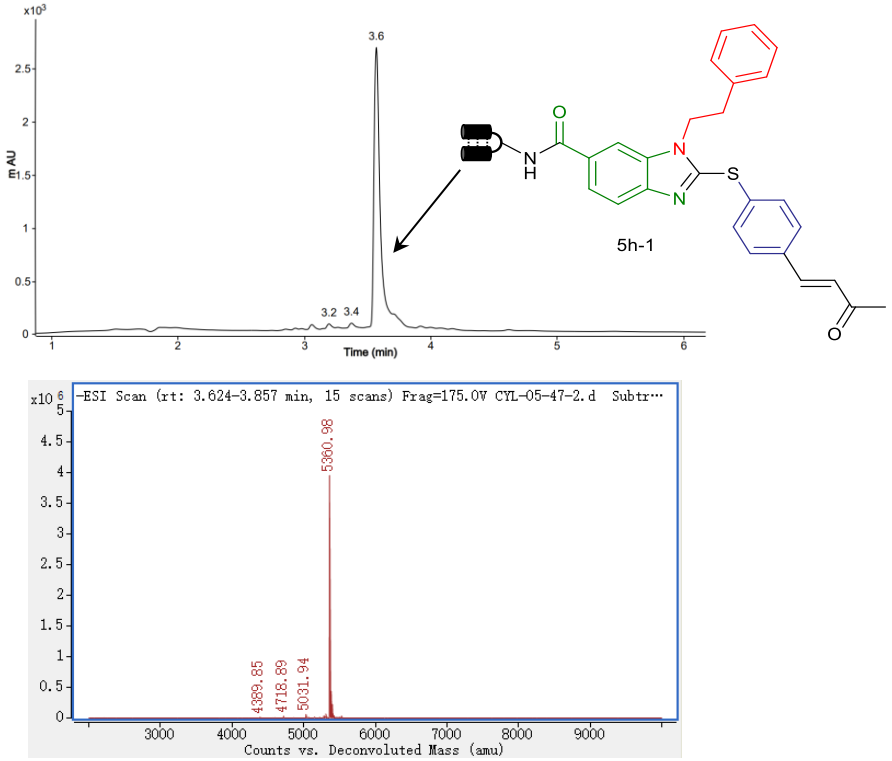
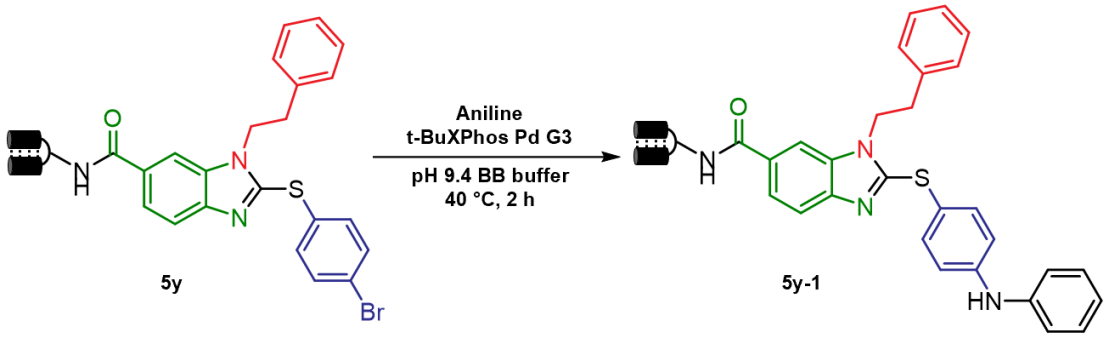


Figure S5. UPLC chromatogram and deconvoluted MS of **5ch-1**

6.4. Buchwald-Hartwig Amination



The DNA conjugate **5y** (200 pmol) was dissolved in H_2O (7 µL). Sodium borate buffer (2 µL, 250 mM, pH 9.4), aniline (4 µL, 400 mM in DMA) and t-BuXPhos Pd G3 (2 µL, 100 mM in DMA) were added to the DNA solution. The reaction was allowed to proceed at 40 °C for 2 h. Then 30 equiv. of sodium diethyldithiocarbamic acid compared with t-BuXPhos Pd G3 were added to the mixture to scavenge the palladium, 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 to give the DNA conjugate **5y-1**. After ethanol precipitation, the DNA conjugate **5y-1** was redissolved in H_2O (60 µL) and characterized by UPLC-MS for 62% conversion. Calculated Mass: 5384 Da; Observed Mass: 5384 Da.

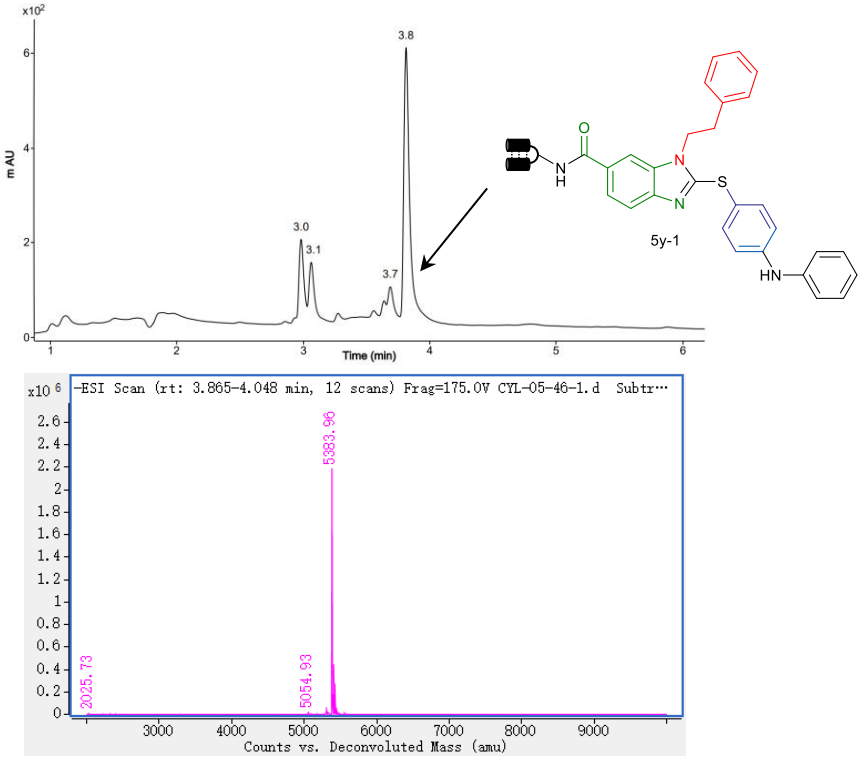
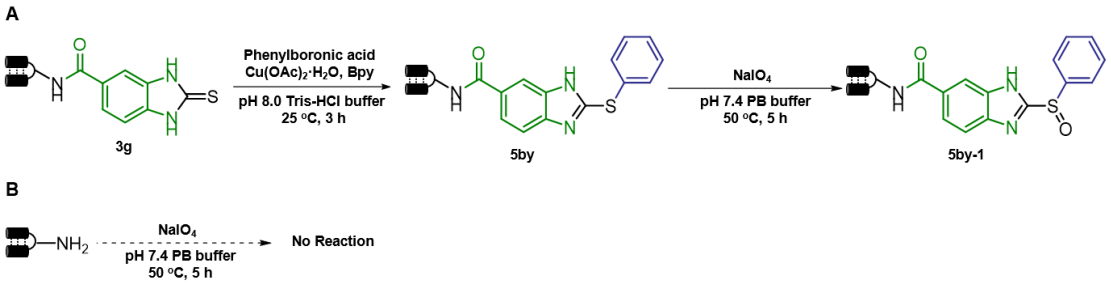


Figure S6. UPLC chromatogram and deconvoluted MS of **5y-1**

6.5. On-DNA sulfoxide formation



To the solution of the DNA conjugate **3g** (5 μL, 100 μM in H_2O) was added H_2O (23 μL), Tris-HCl (16 μL, 500 mM, pH 8.0), $Cu(OAc)_2·H_2O$ (2 μL, 10 mM in H_2O), 2,2'-bipyridine (2 μL, 20 mM in DMA) and phenylboronic acid (2 μL, 200 mM in DMA). The mixture was vortexed, centrifuged, and placed at 25 °C for 3 h. Then 30 equiv. of sodium diethyldithiocarbamic acid compared with $Cu(OAc)_2·H_2O$ 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 to give the DNA conjugate **5by**. Next, to the solution of the DNA conjugate **5by** (15 μL, about 200 pmol in H_2O) was added phosphate buffer (5 μL, 250 mM, pH 7.4) and $NaIO_4$ (5 μL, 800 mM in H_2O). The reaction mixture was stood at 50 °C for 5 h in metal bath and then precipitated with ethanol to give the DNA conjugate **5by-1**. After ethanol precipitation, the DNA conjugate **5by-1** was redissolved in H_2O (60 μL) and characterized by UPLC-MS for 76% conversion. Calculated Mass: 5205 Da; Observed Mass: 5205 Da. Meanwhile, HP was tested under the same oxidation conditions, and the result showed no reaction.

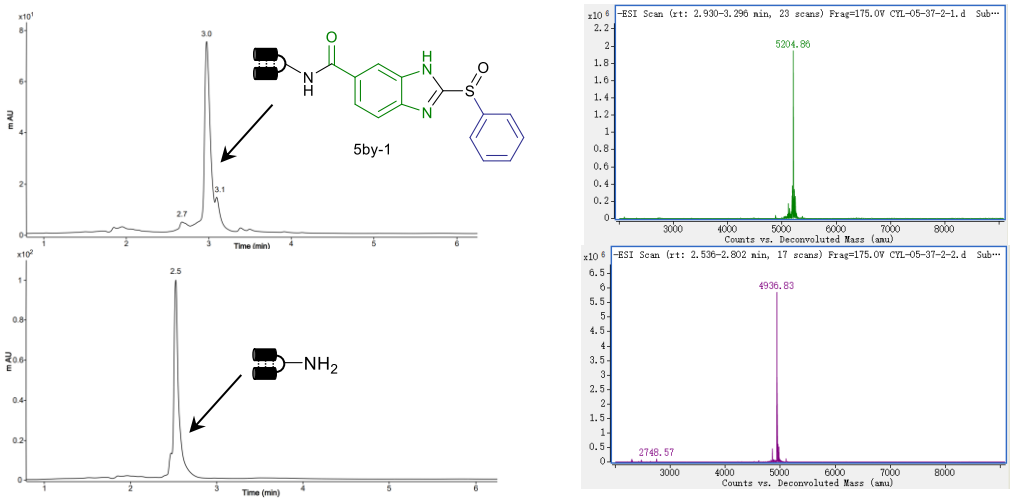
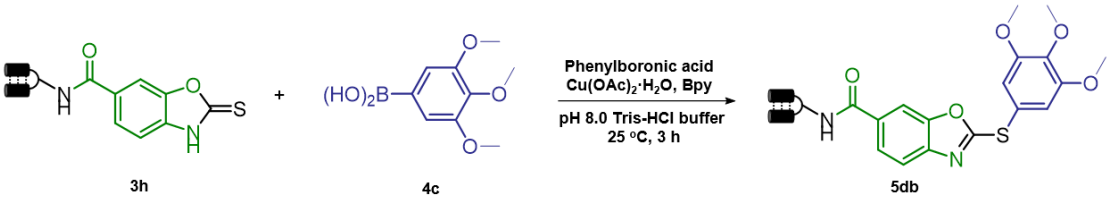


Figure S7. UPLC chromatogram and deconvoluted MS of **5by-1** and **HP**

6.6. Synthesis of the bioactive molecule



To the solution of the DNA conjugate **3h** (2 μL, 100 μM in H_2O) was added H_2O (26 μL), Tris-HCl (16 μL, 500 mM, pH 8.0), $Cu(OAc)_2·H_2O$ (2 μL, 10 mM in H_2O), 2,2'-bipyridine (2 μL, 20 mM in DMA) and 3,4,5-trimethoxyphenylboronic acid (2 μL, 200 mM in DMA). The mixture was vortexed, centrifuged, and placed at 25 °C for 3 h. Then 30 equiv. of sodium diethyldithiocarbamic acid compared with $Cu(OAc)_2·H_2O$ 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 to give the DNA conjugate **5db**. After ethanol precipitation, the DNA conjugate **5db** was redissolved in H_2O (60 μL) and characterized by UPLC-MS for over 90% conversion. Calculated Mass: 5281 Da; Observed Mass: 5281 Da.

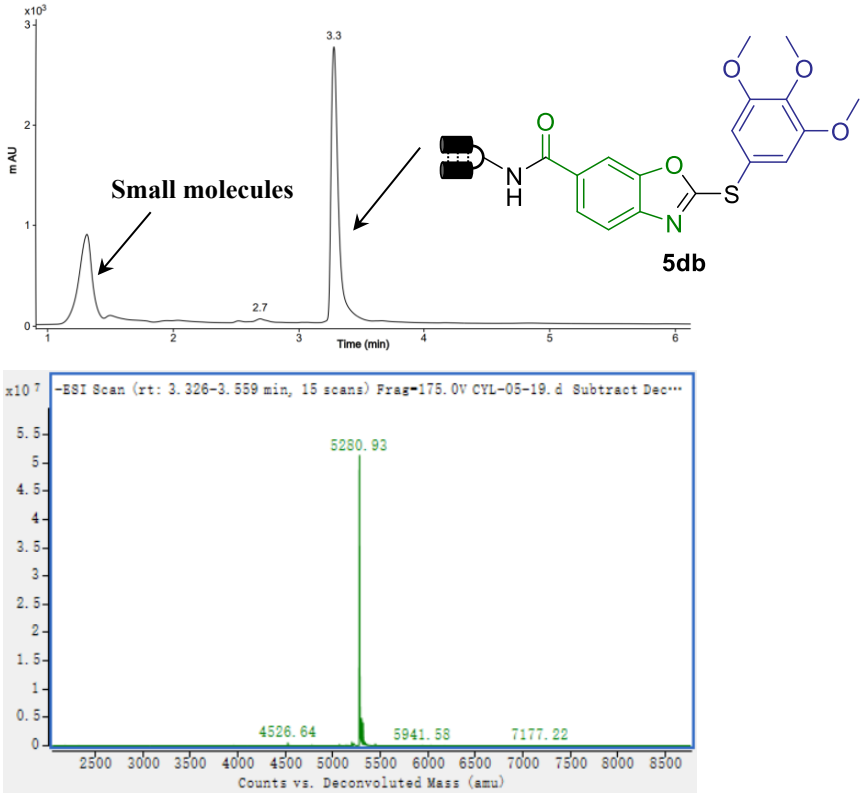
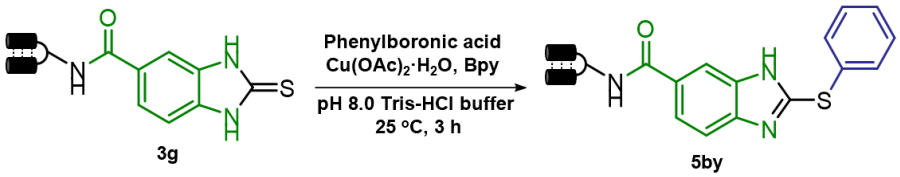


Figure S8. UPLC chromatogram and deconvoluted MS of **5db**

6.7. Scale-up reaction



To the solution of the DNA conjugate **3g** (25 µL, 200 µM in H_2O) was added H_2O (3 µL), Tris-HCl (16 µL, 500 mM, pH 8.0), $Cu(OAc)_2·H_2O$ (2 µL, 10 mM in H_2O), 2,2'-bipyridine (2 µL, 20 mM in DMA) and phenylboronic acid (2 µL, 200 mM in DMA). The mixture was vortexed, centrifuged, and placed at 25 °C for 3 h. Then 30 equiv. of sodium diethyldithiocarbamic acid compared with $Cu(OAc)_2·H_2O$ 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 to give the DNA conjugate **5by**. After ethanol precipitation, the DNA conjugate **5by** was characterized by UPLC-MS for 90% conversion. Calculated Mass: 5189 Da; Observed Mass: 5190 Da.

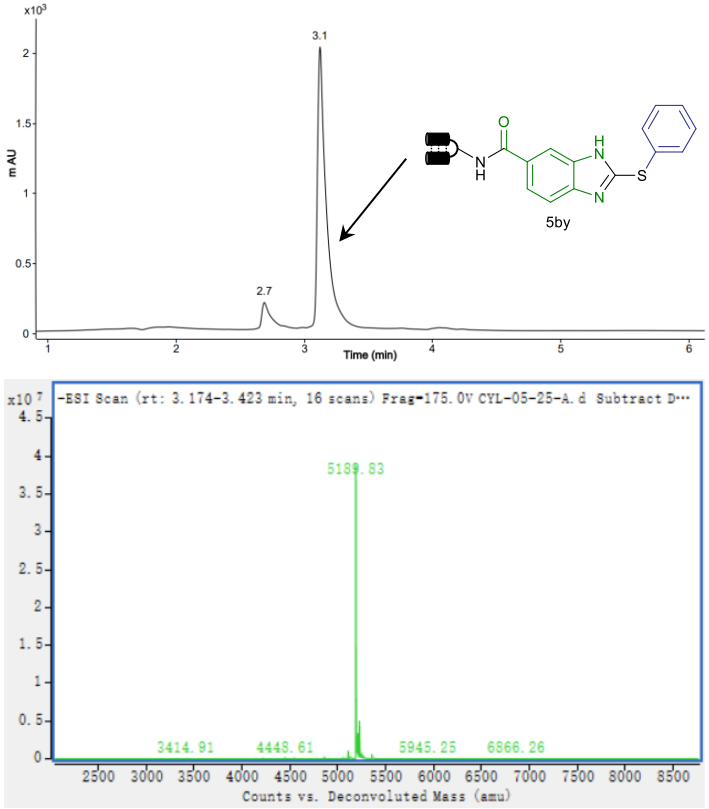
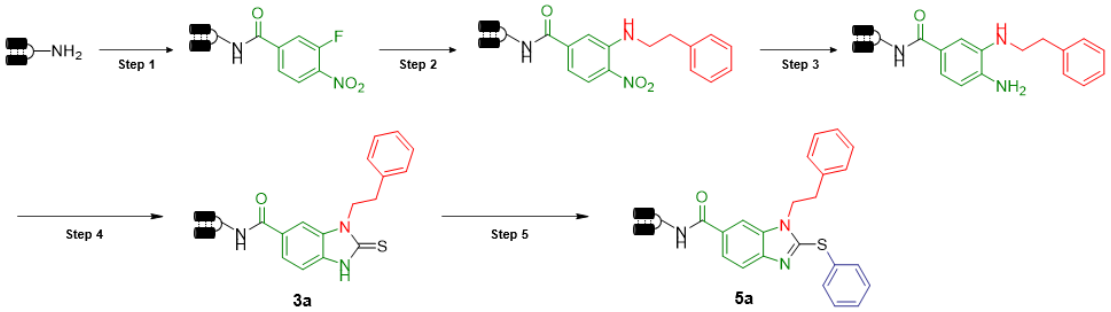


Figure S9. UPLC chromatogram and deconvoluted MS of **5by**

6.8. Overall conversion



The preparation of the DNA conjugate **5a** was carried out in accordance with the procedure described above. The multistep reactions were performed continuously and didn't involve the purification of DNA. The DNA conjugate **5a** was characterized by UPLC-MS for over 80% conversion. Calculated Mass: 5293 Da; Observed Mass: 5293 Da.

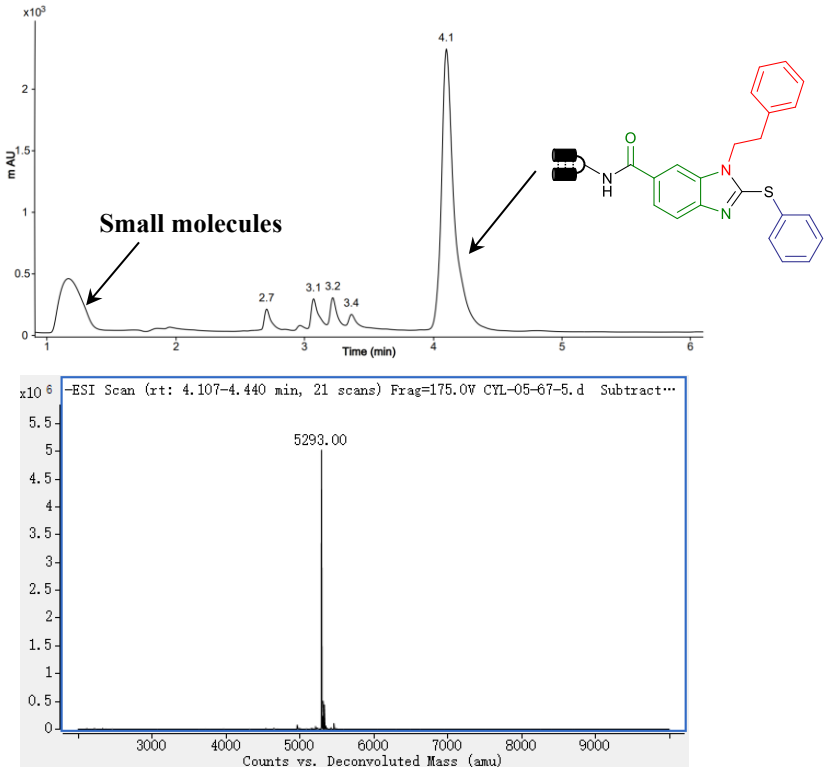


Figure S10. UPLC chromatogram and deconvoluted MS of **5a**

7. Enzymatic ligation and sequencing

7.1. Enzymatic ligation

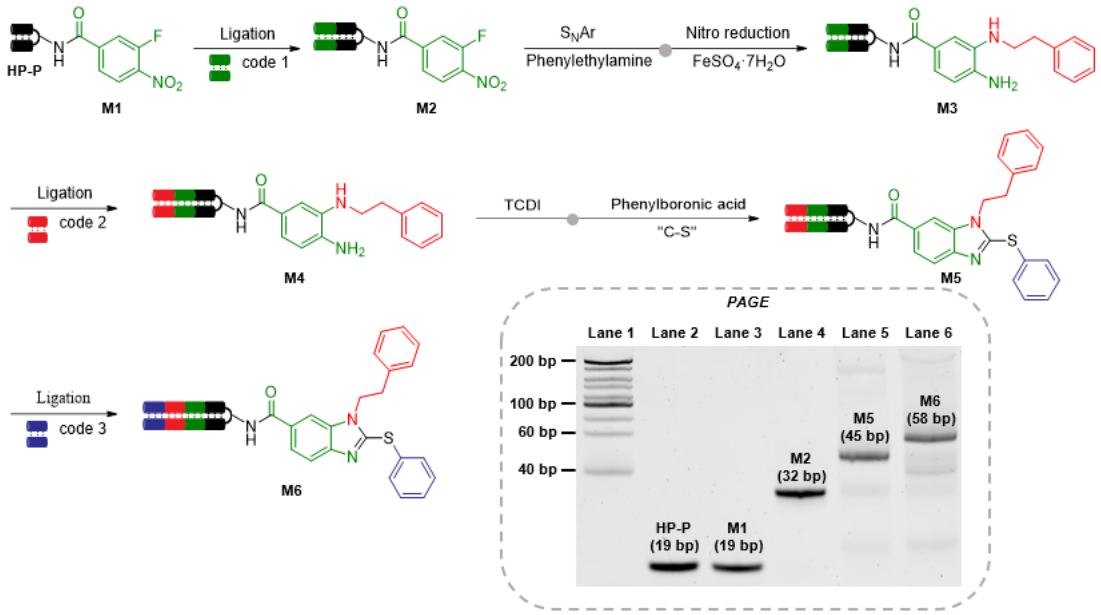


Figure S11. Enzymatic ligation and polyacrylamide gel analysis

The preparation of the DNA conjugates was carried out in accordance with the procedure described above. (The General procedure for On-DNA thiobenzimidazoles, General procedure for DNA ligation).**M2**, Calculated Mass: 20615 Da; Observed Mass: 20619 Da. **M5**, Calculated Mass: 28825 Da; Observed Mass: 28832 Da. **M6**, Calculated Mass: 36865 Da; Observed Mass: 36874 Da.

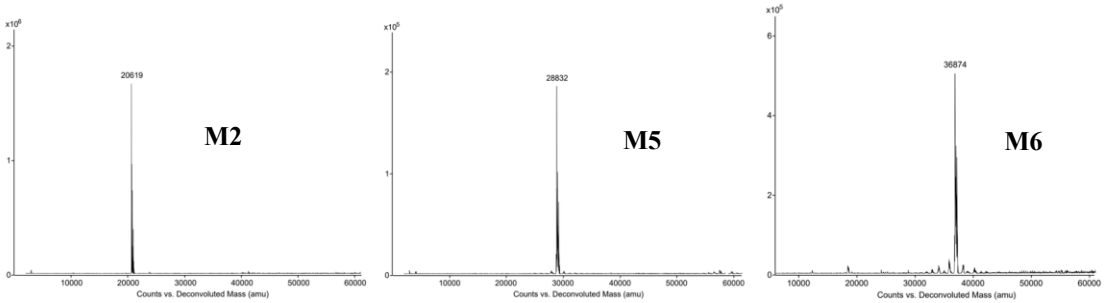


Figure S12. Deconvoluted MS of **M2, M5** and **M6**

7.2. Sequencing

7.2.1 DNA integrity verification of copper/bipyridine catalyst system

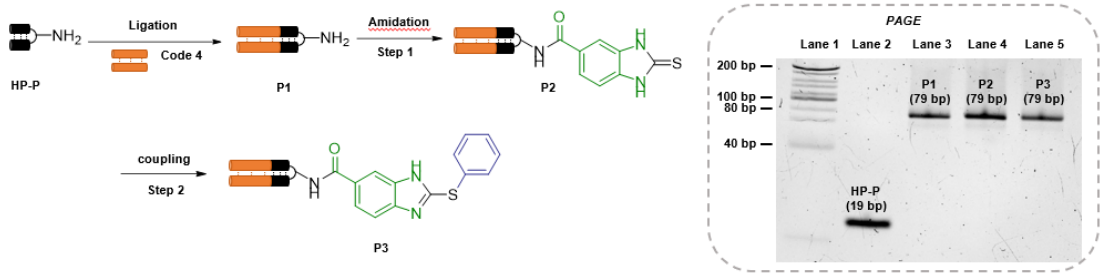


Figure S13. Preparation of sequencing substrate and polyacrylamide gel analysis

The preparation of the DNA conjugates was carried out in accordance with the procedure described above.

Sanger sequencing: The template (DNA conjugate **P3**) was diluted to 1.2 μM, and further diluted 10^4 times to obtain 0.1 nM template solution. Upstream primer 1 (2 μL, 10 μM), downstream primer 1 (2 μL, 10 μM), Es Taq MasterMix (10 μL, 2×), diluted template (2μL) and H_2O (4μL) were mixed in the centrifuge tube and placed on the PCR apparatus. PCR procedure: predenaturation (94 °C, 2 min); denaturation (94 °C, 0.5 min); annealing primer (56 °C, 0.5 min); primer extension (72°C, 0.5 min); 30 cycles; standing (72 °C, 5 min). After the primary PCR, the secondary PCR was performed according to the conditions of the primary PCR. The secondary PCR template was the primary PCR product (0.2μL). After the secondary PCR, the sample and sequencing primer were ready for sequencing.

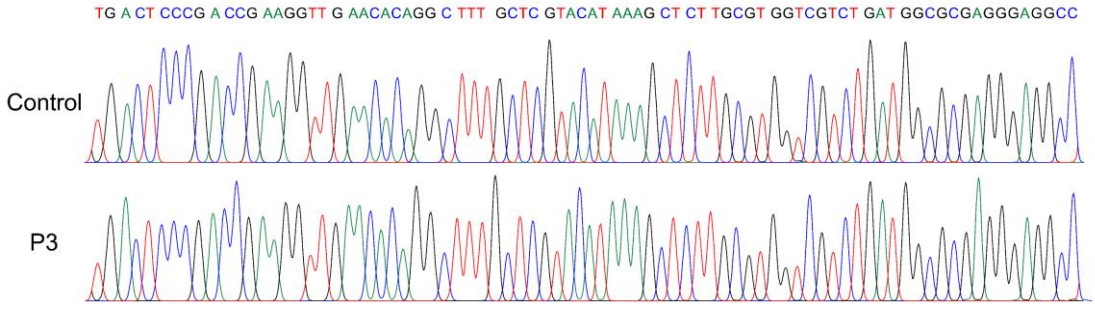


Figure S14. Sanger sequencing results of samples before and after chemical reactions

By comparing the sequencing results of the starting material before the reaction (P1) with the chemical reaction products (P3), we could conclude that the method was compatible with PCR and sequencing involved in DEL synthesis and selection.

7.2.2 DNA integrity verification of the whole procedure of chemical synthesis

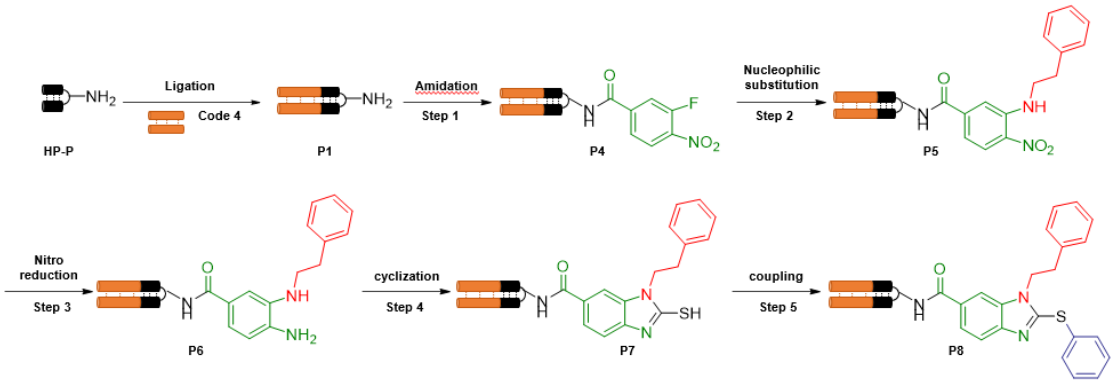


Figure S15. Preparation of sequencing substrate **P8**

The preparation of the DNA conjugates was carried out in accordance with the procedure described above.

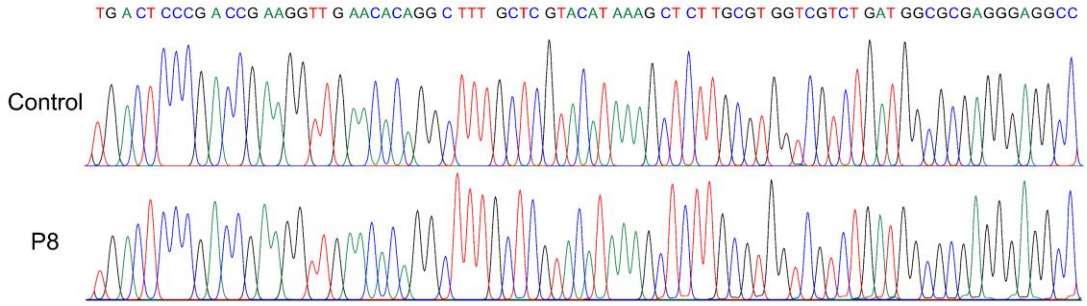


Figure S16. Sanger sequencing results of samples before and after chemical reactions

By comparing the sequencing results of the starting material before the reaction (P1) with the chemical reaction products (P8), we could conclude that the method was compatible with PCR and sequencing involved in DEL synthesis and selection.

Upstream primer for the primary PCR (from 5' to 3'):

GTTGGAAGCCAGCCCTCAGTGACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG

Downstream primer for the primary PCR (from 5' to 3'):

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCTCCCTCGCGCCATCAGAC

Upstream primer for the secondary PCR (from 5' to 3'):

AATGATACGGCGACCACCGAGATCTACACTCTTTCGTCTCGTGGGCTCGGAGATG

Downstream primer for the secondary PCR (from 5' to 3'):

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCTCCCTCGCGCCATCAGAC

Upstream primer for sequencing (from 5' to 3'):

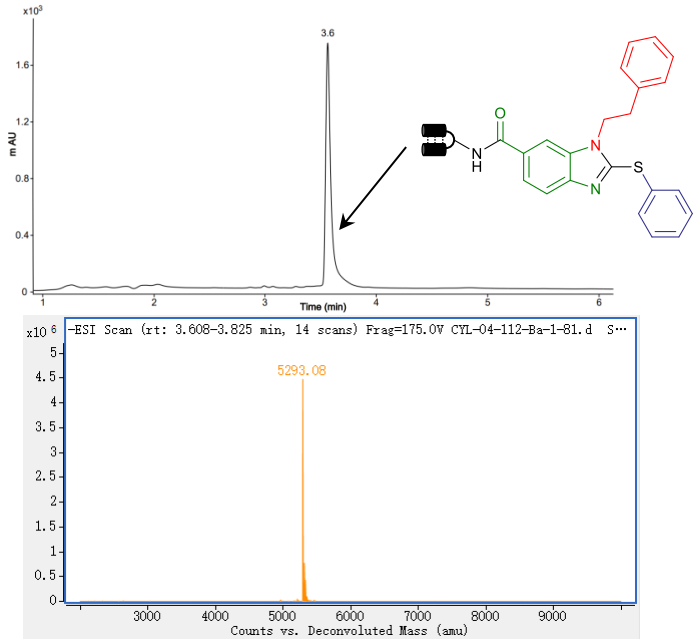
AATGATACGGCGACCACCGAGATCTACACTCTTTCGTCTCGTGGGCTCGGAGATG

8. UPLC chromatogram and deconvoluted MS

8.1. Substrate scope of boronic acids

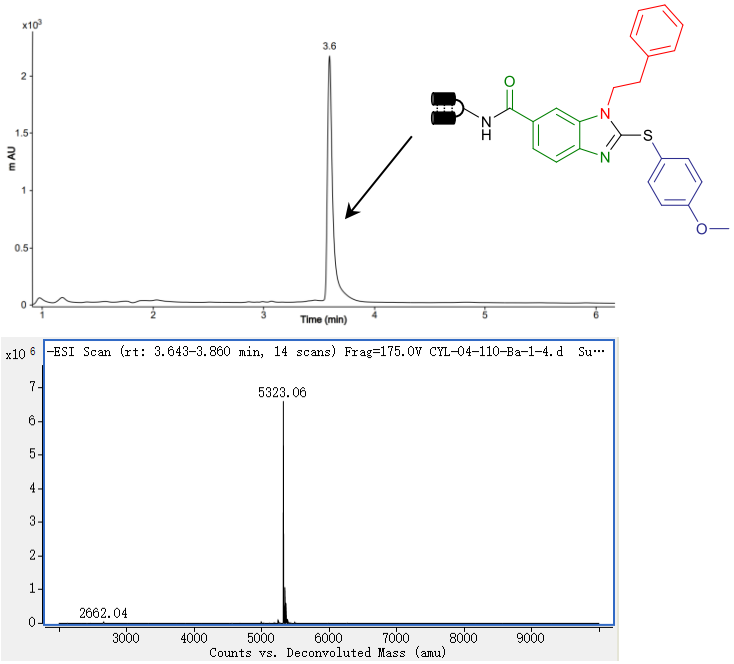
UPLC chromatogram and deconvoluted MS of **5a**

Conversion: **>90%**. Calculated Mass: **5293 Da**; Observed Mass: **5293 Da**



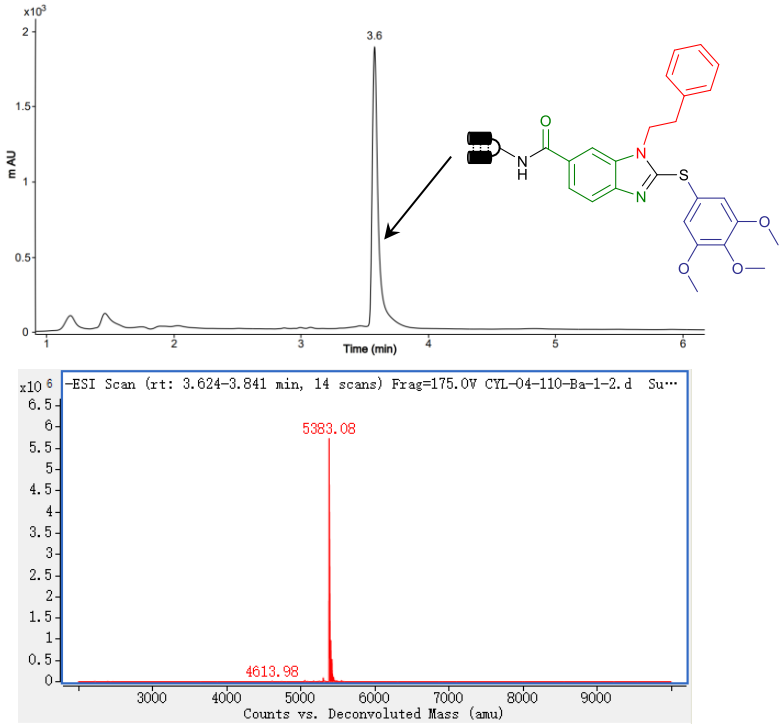
UPLC chromatogram and deconvoluted MS of **5b**

Conversion: **>90%**. Calculated Mass: **5323 Da**; Observed Mass: **5323 Da**



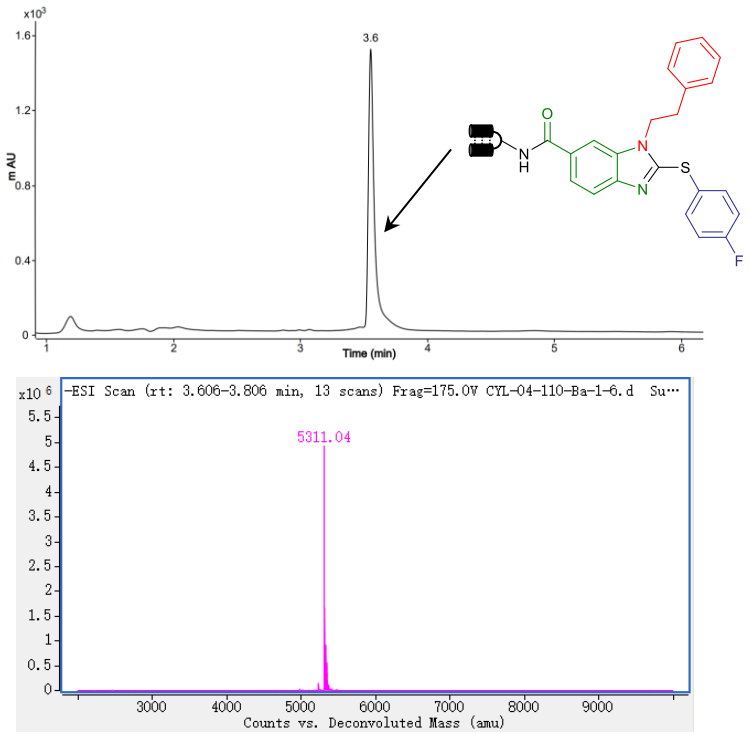
UPLC chromatogram and deconvoluted MS of **5c**

Conversion: **>90%**. Calculated Mass: **5383 Da**; Observed Mass: **5383 Da**



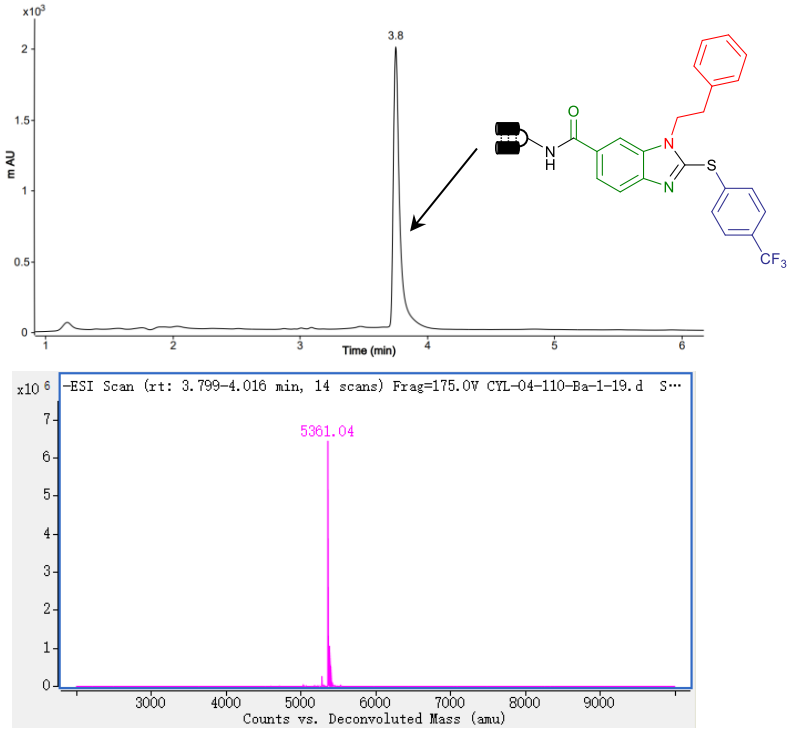
UPLC chromatogram and deconvoluted MS of **5d**

Conversion: **>90%**. Calculated Mass: **5311 Da**; Observed Mass: **5311 Da**



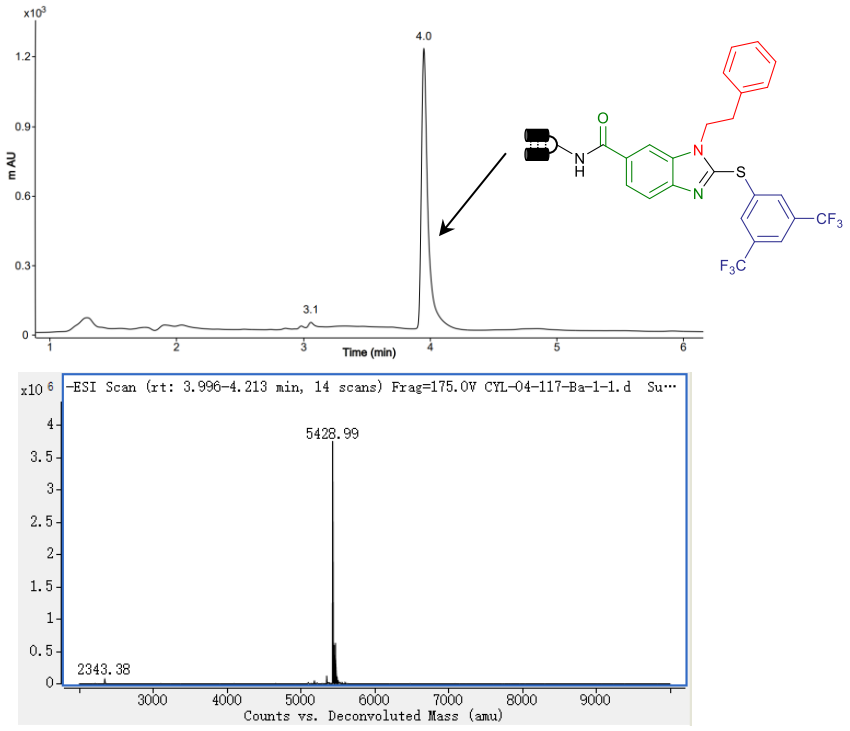
UPLC chromatogram and deconvoluted MS of **5e**

Conversion: **>90%**. Calculated Mass: **5361 Da**; Observed Mass: **5361 Da**



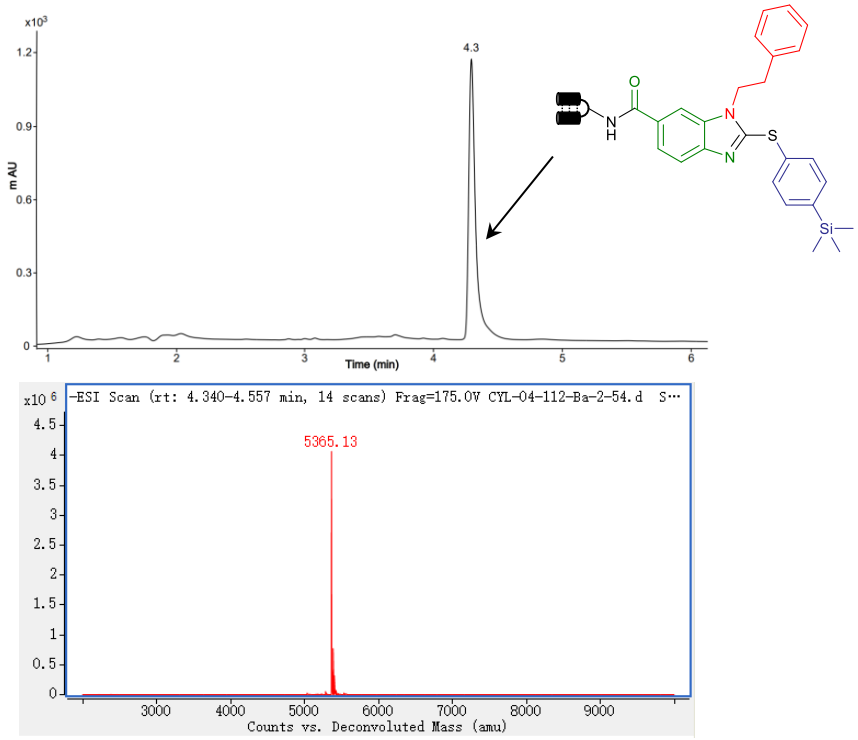
UPLC chromatogram and deconvoluted MS of **5f**

Conversion: **>90%**. Calculated Mass: **5429 Da**; Observed Mass: **5429 Da**



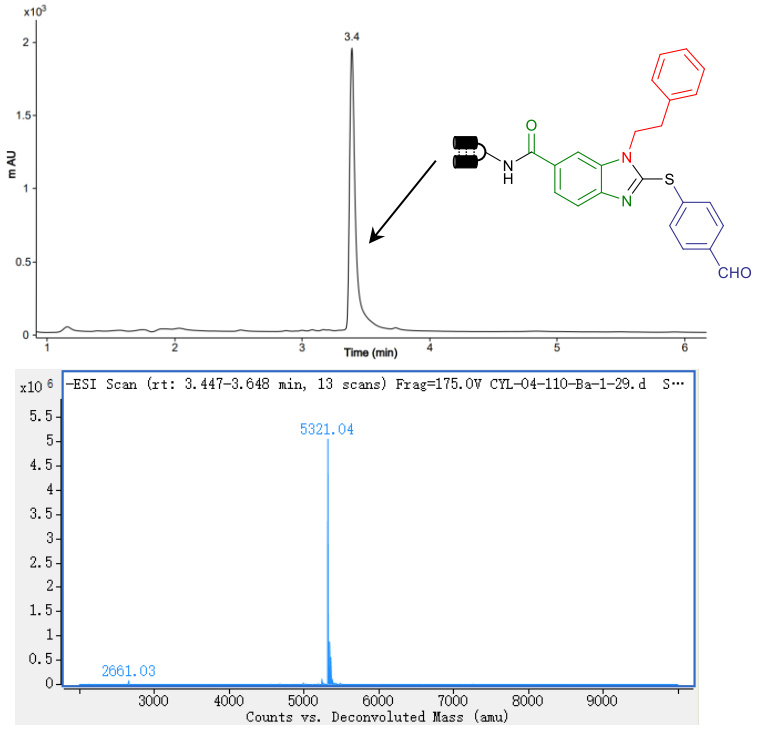
UPLC chromatogram and deconvoluted MS of **5g**

Conversion: **>90%**. Calculated Mass: **5365 Da**; Observed Mass: **5365 Da**



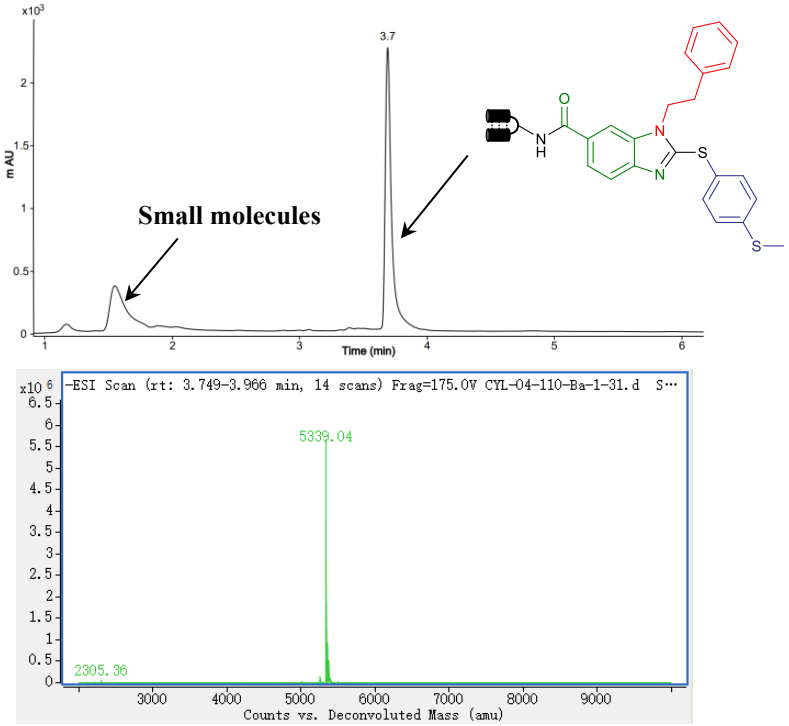
UPLC chromatogram and deconvoluted MS of **5h**

Conversion: **>90%**. Calculated Mass: **5321 Da**; Observed Mass: **5321 Da**



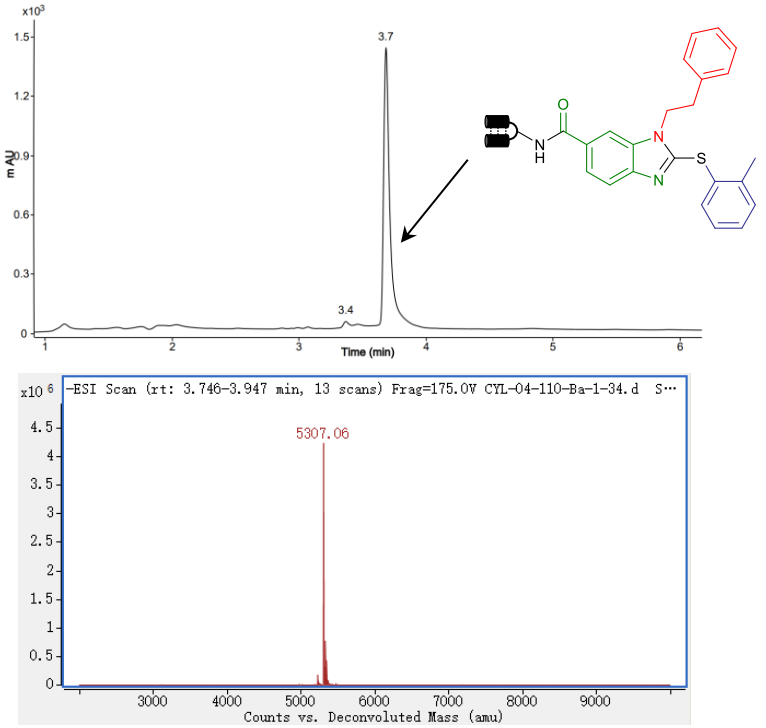
UPLC chromatogram and deconvoluted MS of **5i**

Conversion: **>90%**. Calculated Mass: **5339 Da**; Observed Mass: **5339 Da**



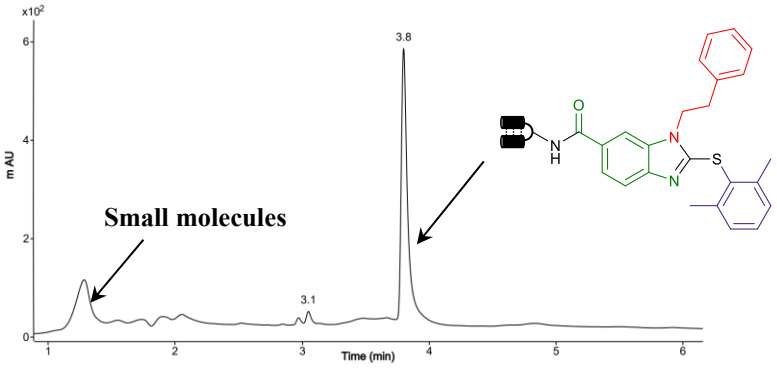
UPLC chromatogram and deconvoluted MS of **5j**

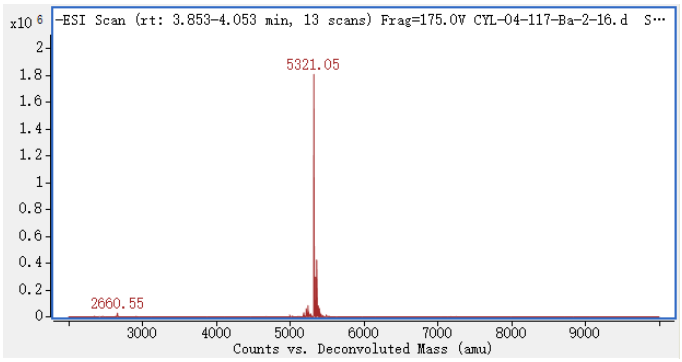
Conversion: **>90%**. Calculated Mass: **5307 Da**; Observed Mass: **5307 Da**



UPLC chromatogram and deconvoluted MS of **5k**

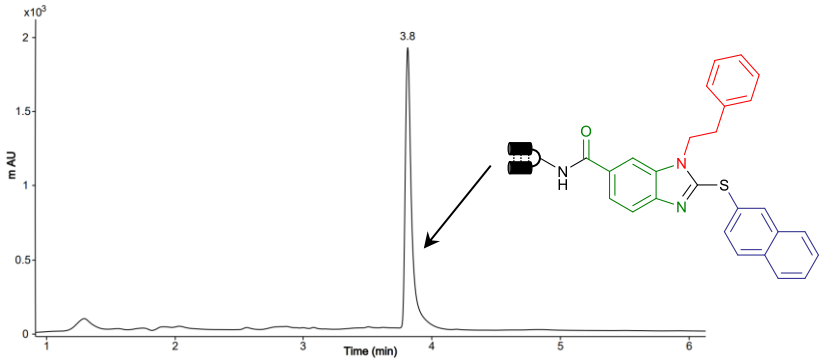
Conversion: **76%**. Calculated Mass: **5321 Da**; Observed Mass: **5321 Da**

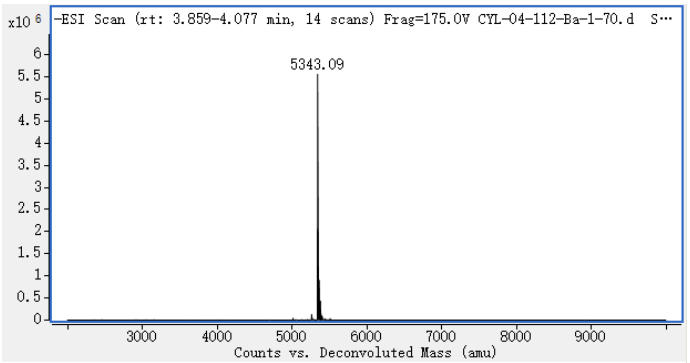




UPLC chromatogram and deconvoluted MS of **5l**

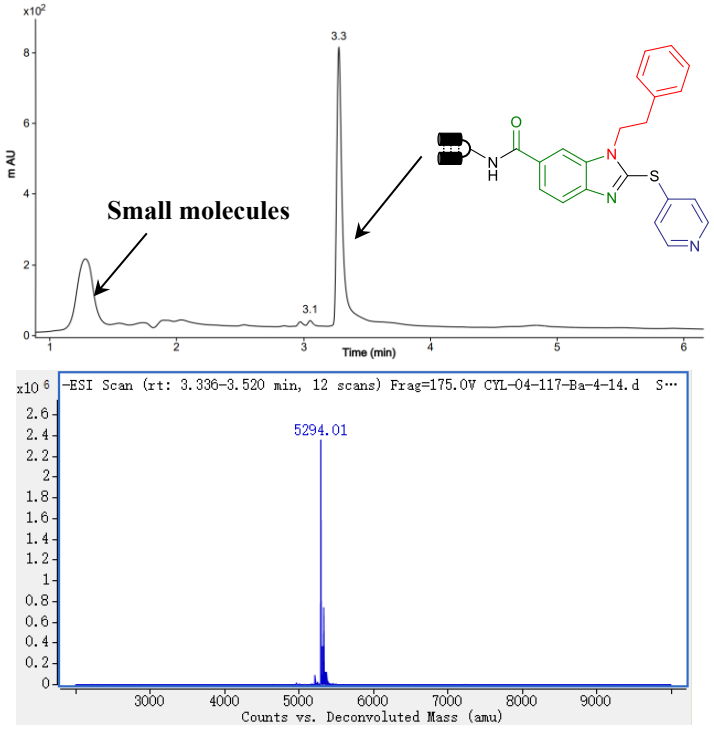
Conversion: **>90%**. Calculated Mass: **5343 Da**; Observed Mass: **5343 Da**





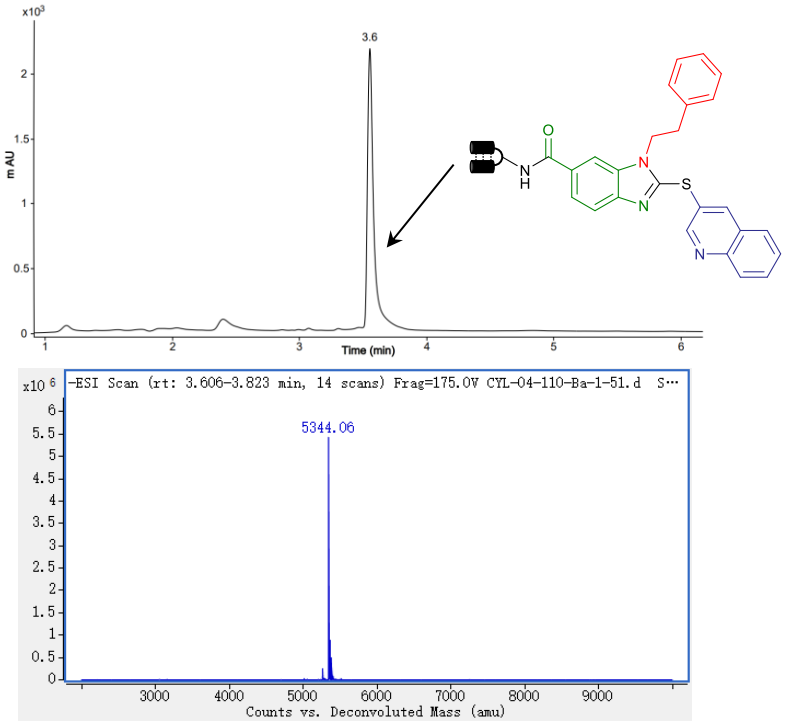
UPLC chromatogram and deconvoluted MS of **5m**

Conversion: **>90%**. Calculated Mass: **5294 Da**; Observed Mass: **5294 Da**



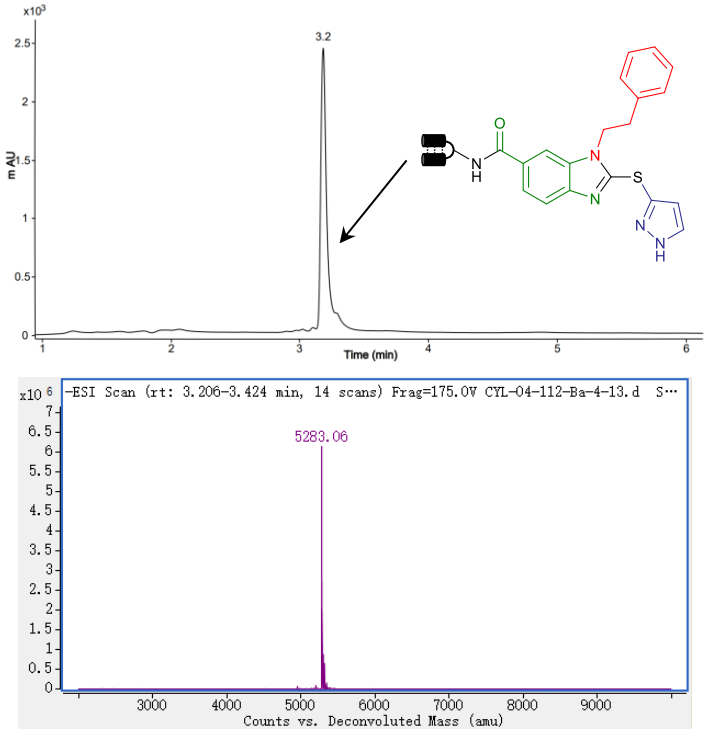
UPLC chromatogram and deconvoluted MS of **5n**

Conversion: **>90%**. Calculated Mass: **5344 Da**; Observed Mass: **5344 Da**



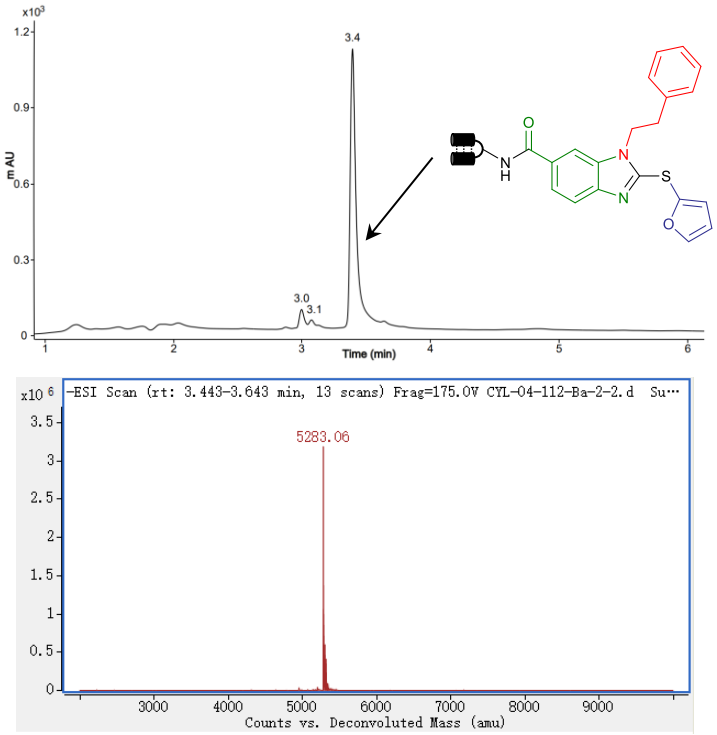
UPLC chromatogram and deconvoluted MS of **5o**

Conversion: **>90%**. Calculated Mass: **5283 Da**; Observed Mass: **5283 Da**



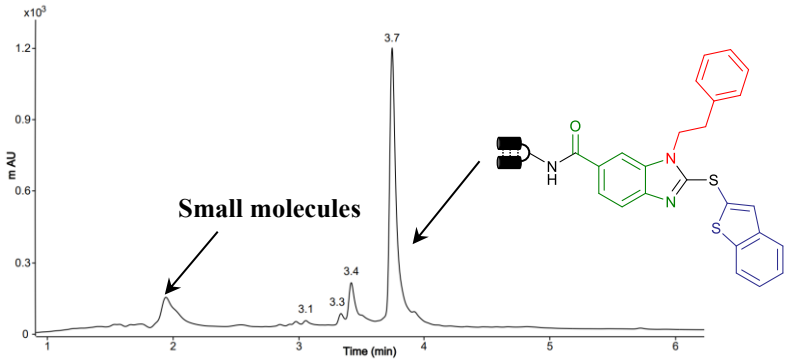
UPLC chromatogram and deconvoluted MS of **5p**

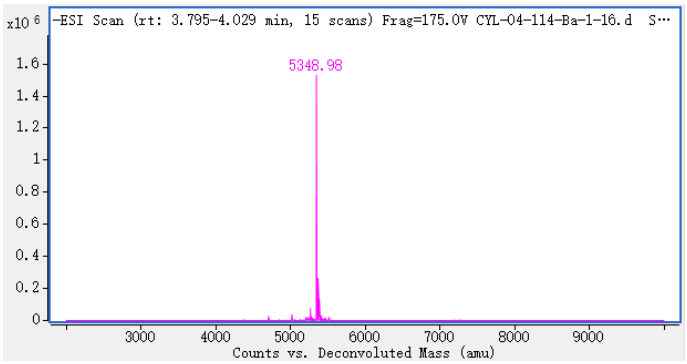
Conversion: **>90%**. Calculated Mass: **5283 Da**; Observed Mass: **5283 Da**



UPLC chromatogram and deconvoluted MS of **5q**

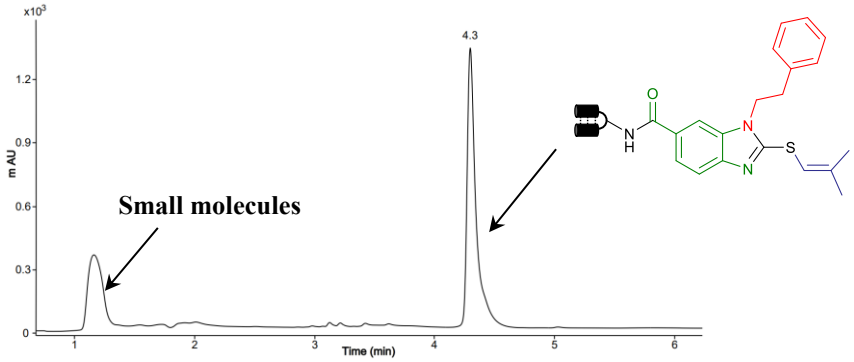
Conversion: **77%**. Calculated Mass: **5349 Da**; Observed Mass: **5349 Da**

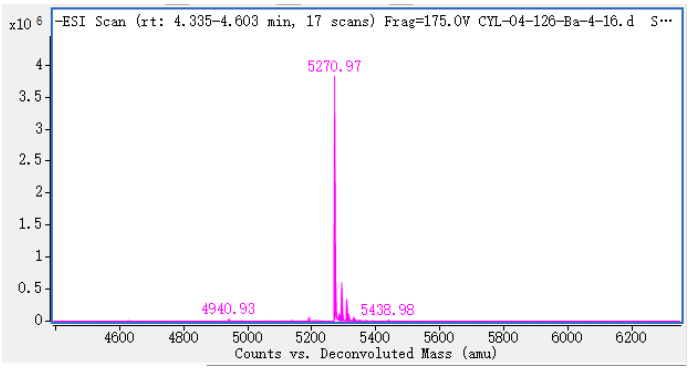




UPLC chromatogram and deconvoluted MS of **5r**

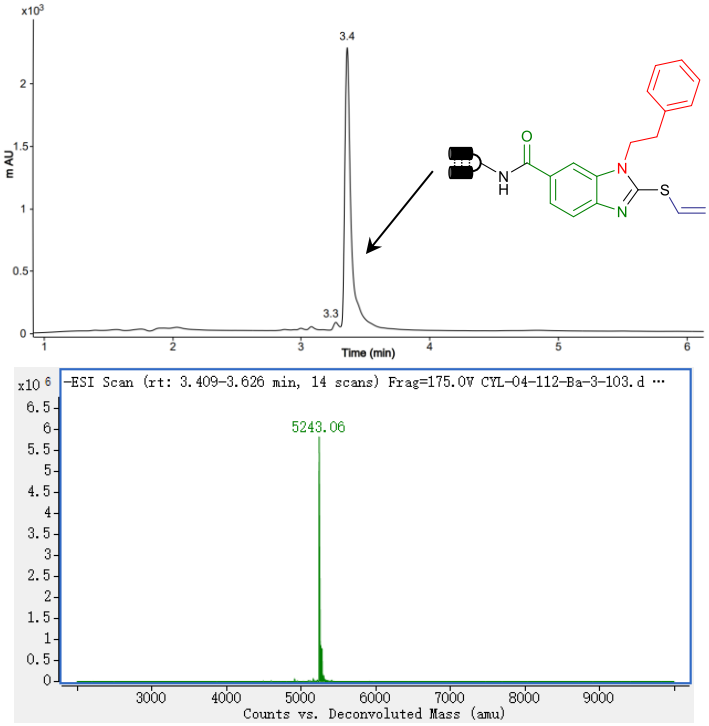
Conversion: **>90%**. Calculated Mass: **5271 Da**; Observed Mass: **5271 Da**





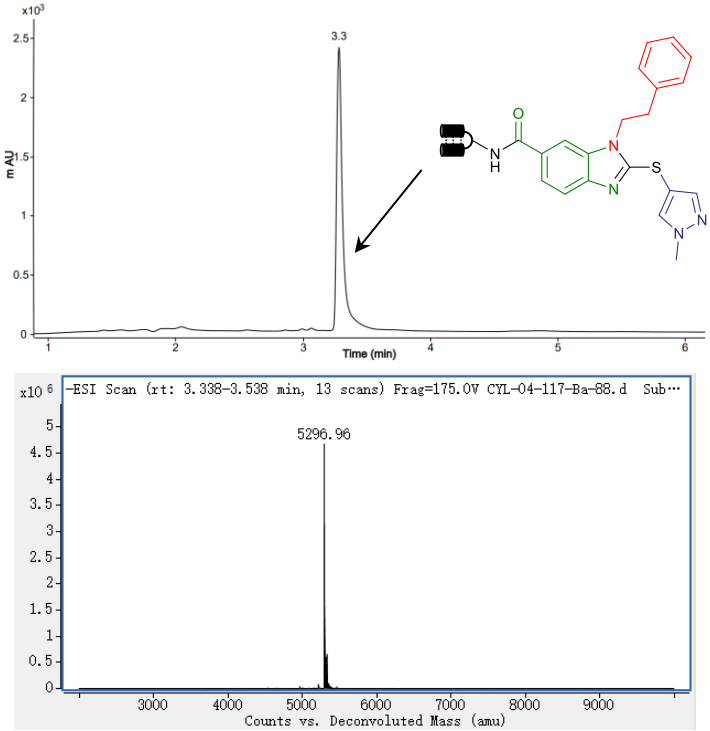
UPLC chromatogram and deconvoluted MS of **5s**

Conversion: **>90%**. Calculated Mass: **5243 Da**; Observed Mass: **5243 Da**



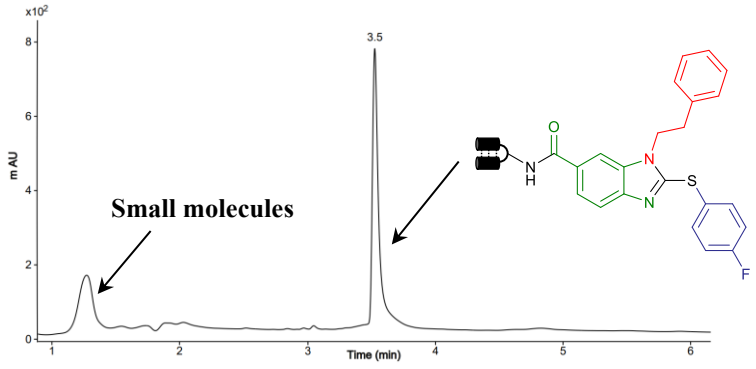
UPLC chromatogram and deconvoluted MS of **5t**

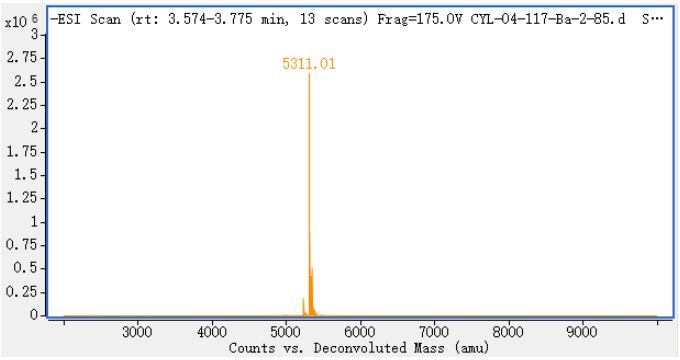
Conversion: **>90%**. Calculated Mass: **5297 Da**; Observed Mass: **5297 Da**



UPLC chromatogram and deconvoluted MS of **5u**

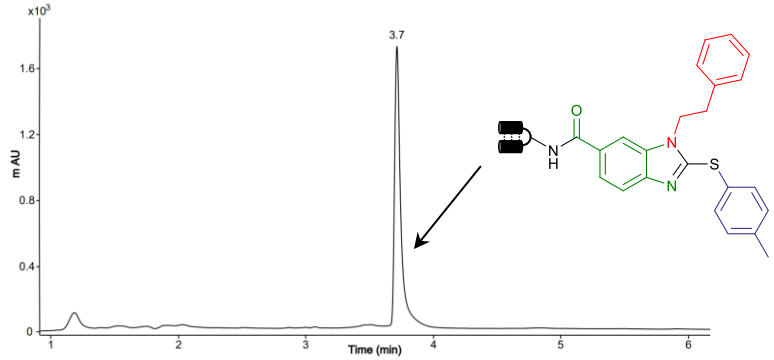
Conversion: **>90%**. Calculated Mass: **5311 Da**; Observed Mass: **5311 Da**

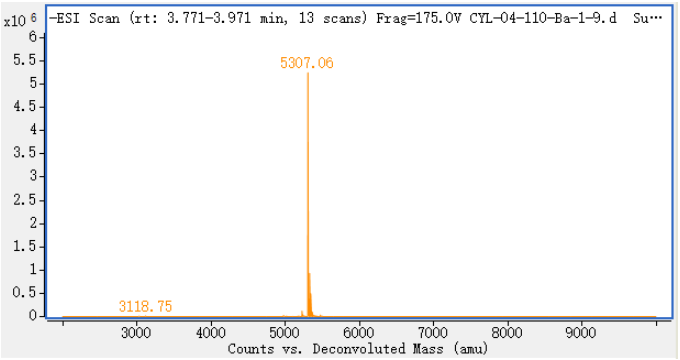




UPLC chromatogram and deconvoluted MS of **5v**

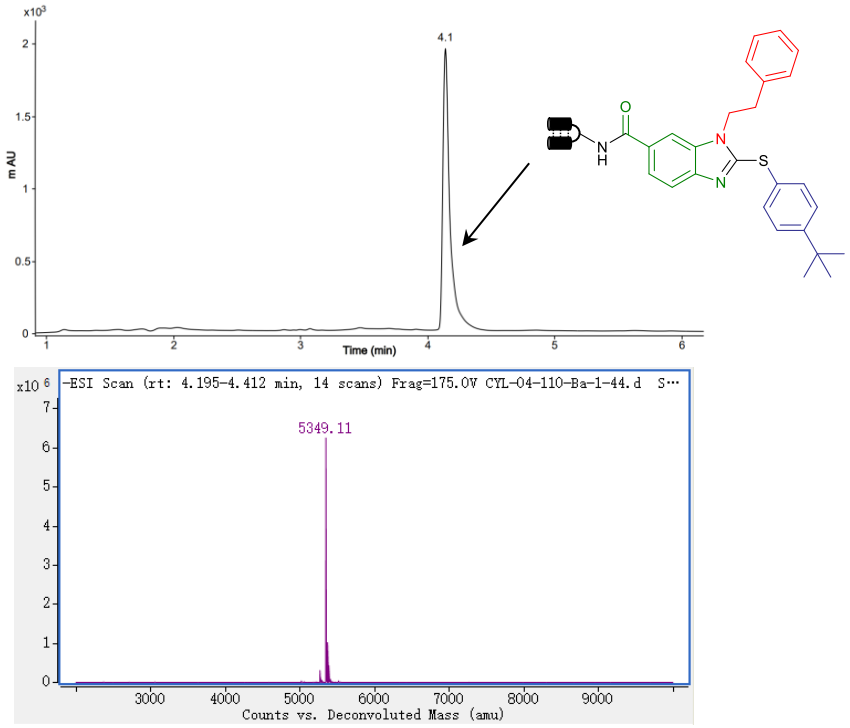
Conversion: **>90%**. Calculated Mass: **5307 Da**; Observed Mass: **5307 Da**





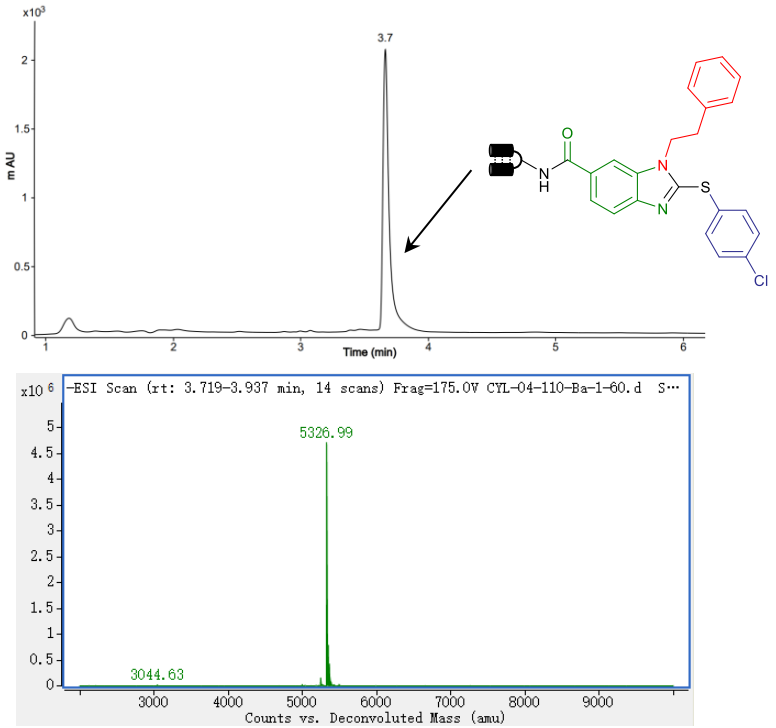
UPLC chromatogram and deconvoluted MS of **5w**

Conversion: **>90%**. Calculated Mass: **5349 Da**; Observed Mass: **5349 Da**



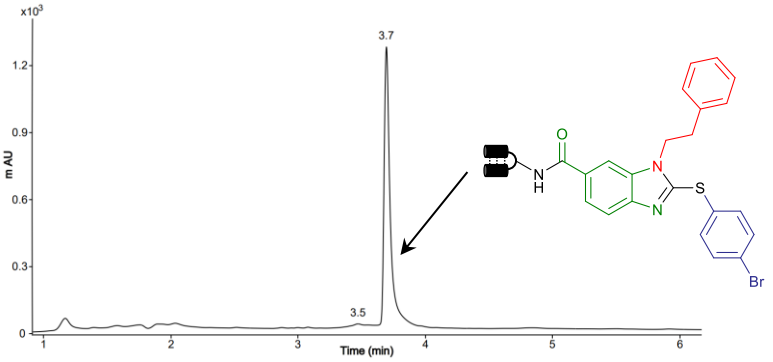
UPLC chromatogram and deconvoluted MS of **5x**

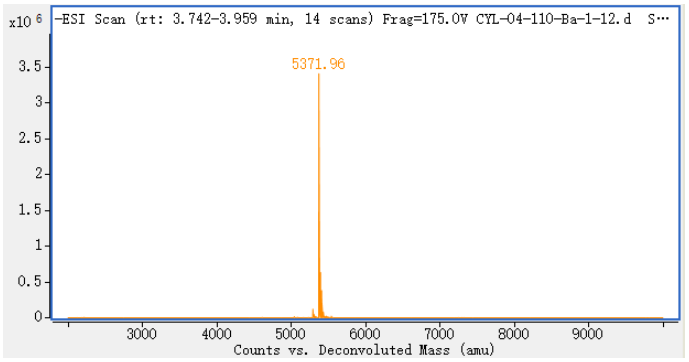
Conversion: **>90%**. Calculated Mass: **5327 Da**; Observed Mass: **5327 Da**



UPLC chromatogram and deconvoluted MS of **5y**

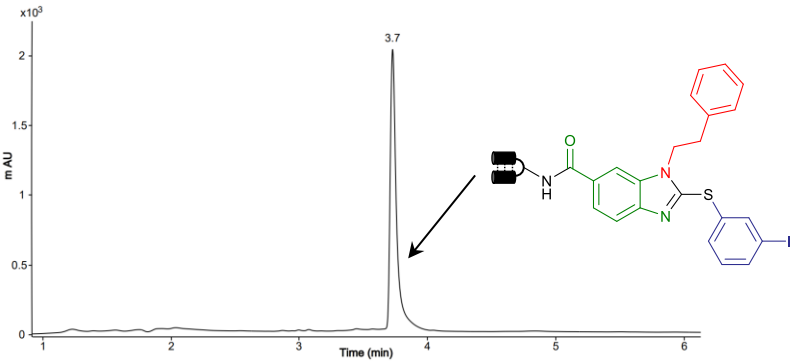
Conversion: **>90%**. Calculated Mass: **5372 Da**; Observed Mass: **5372 Da**

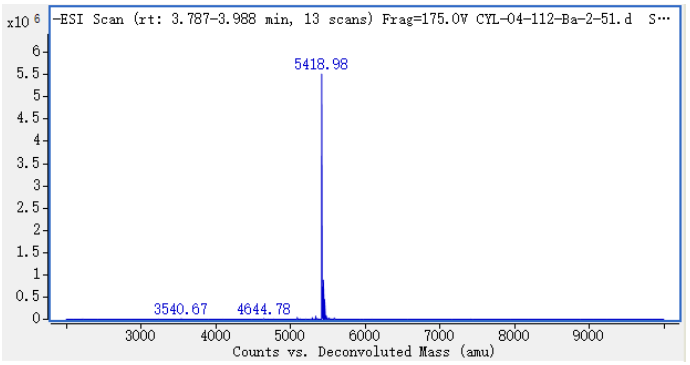




UPLC chromatogram and deconvoluted MS of **5z**

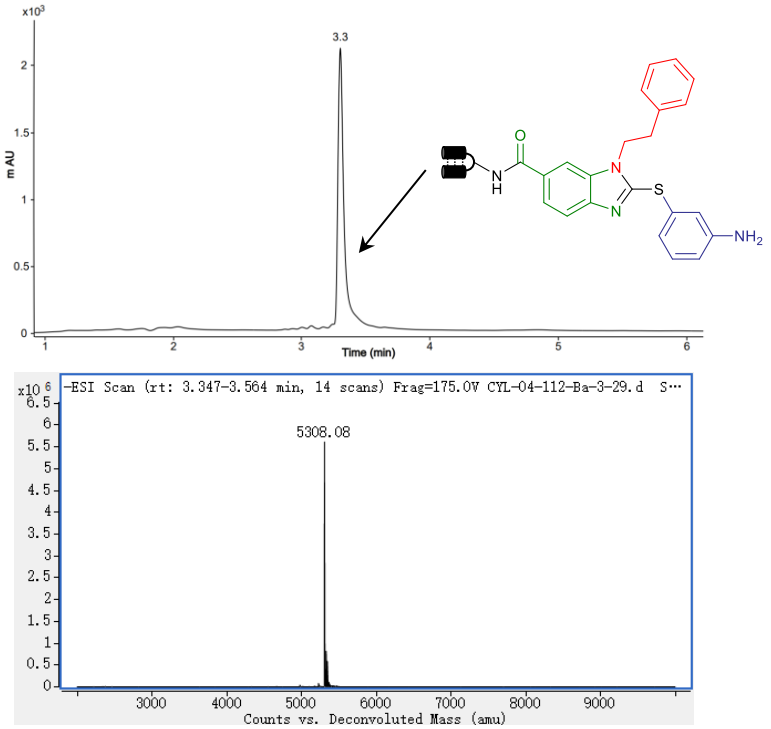
Conversion: **>90%**. Calculated Mass: **5419 Da**; Observed Mass: **5419 Da**





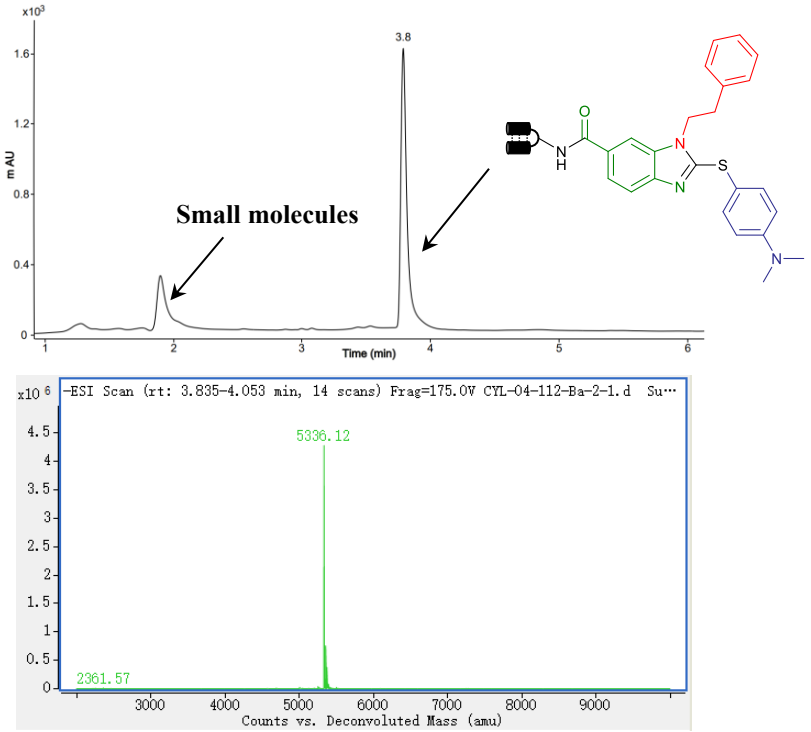
UPLC chromatogram and deconvoluted MS of **5aa**

Conversion: **>90%**. Calculated Mass: **5308 Da**; Observed Mass: **5308 Da**



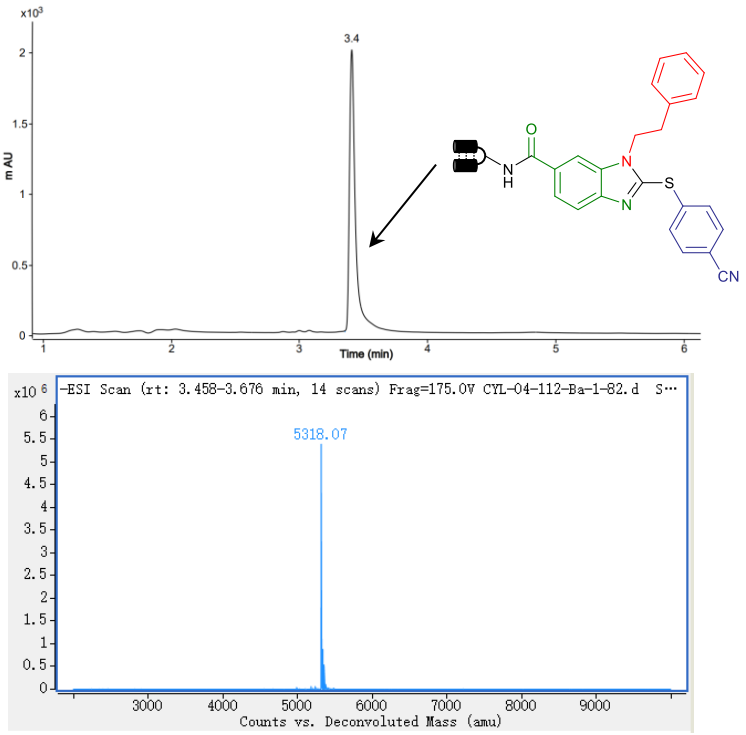
UPLC chromatogram and deconvoluted MS of **5ab**

Conversion: **>90%**. Calculated Mass: **5336 Da**; Observed Mass: **5336 Da**



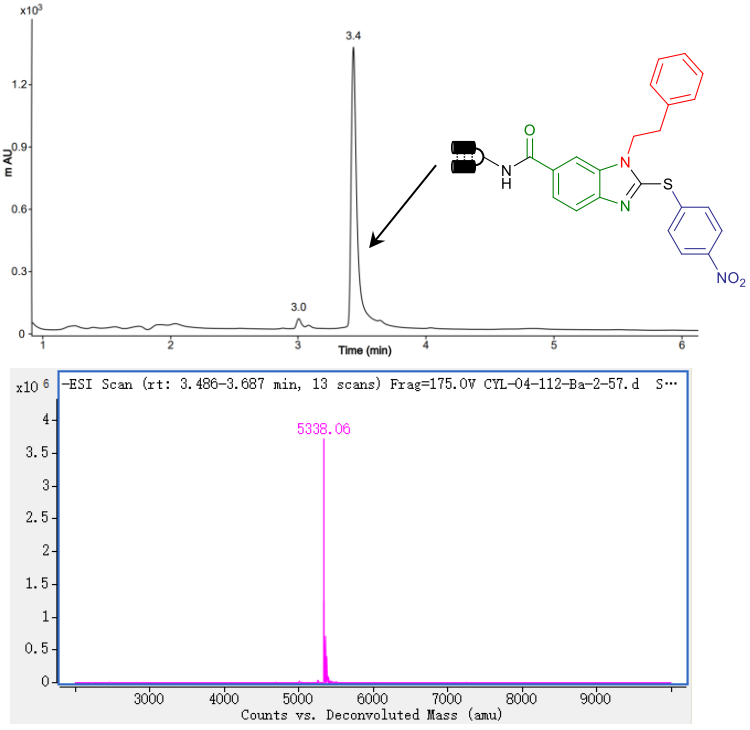
UPLC chromatogram and deconvoluted MS of **5ac**

Conversion: **>90%**. Calculated Mass: **5318 Da**; Observed Mass: **5318 Da**



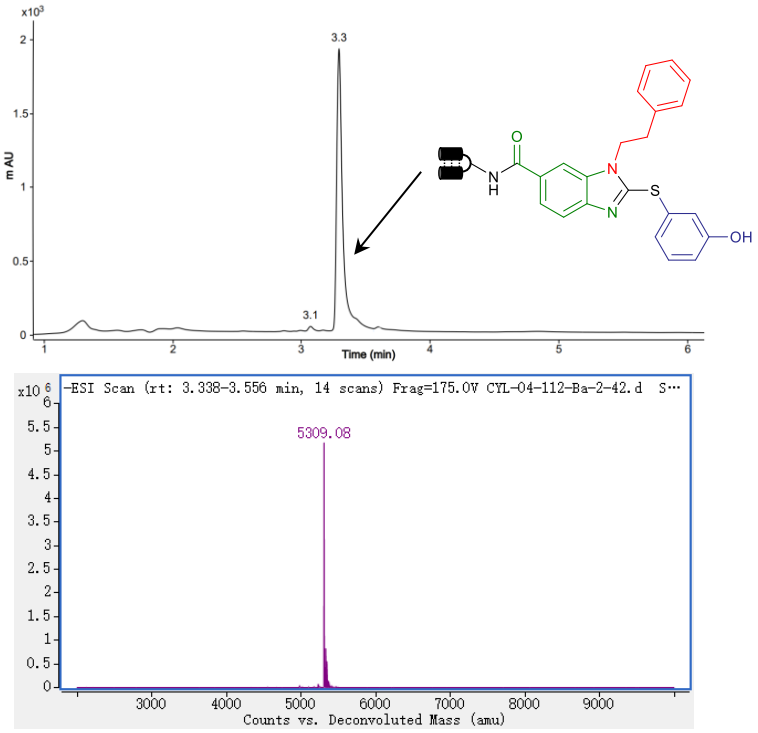
UPLC chromatogram and deconvoluted MS of **5ad**

Conversion: **>90%**. Calculated Mass: **5338 Da**; Observed Mass: **5338 Da**



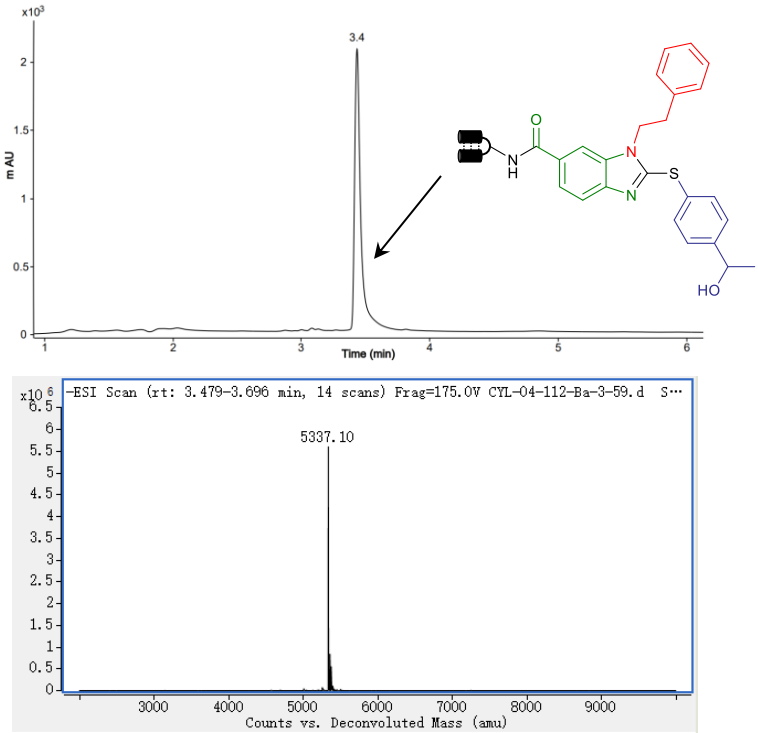
UPLC chromatogram and deconvoluted MS of **5ae**

Conversion: **>90%**. Calculated Mass: **5309 Da**; Observed Mass: **5309 Da**



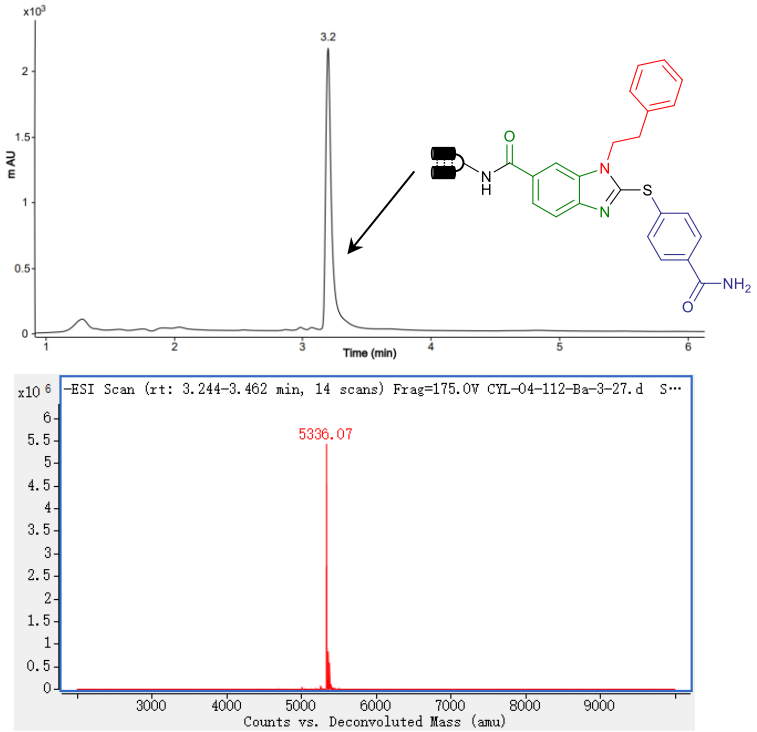
UPLC chromatogram and deconvoluted MS of **5af**

Conversion: **>90%**. Calculated Mass: **5337 Da**; Observed Mass: **5337 Da**



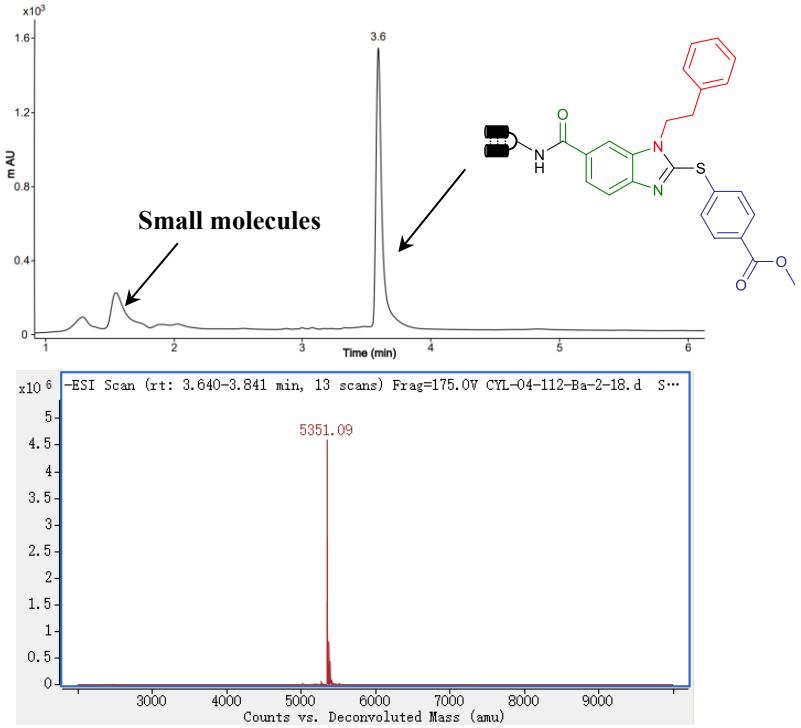
UPLC chromatogram and deconvoluted MS of **5ag**

Conversion: **>90%**. Calculated Mass: **5336 Da**; Observed Mass: **5336 Da**



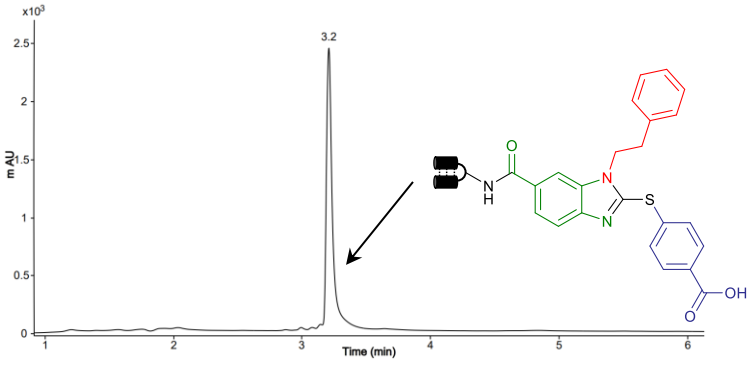
UPLC chromatogram and deconvoluted MS of **5ah**

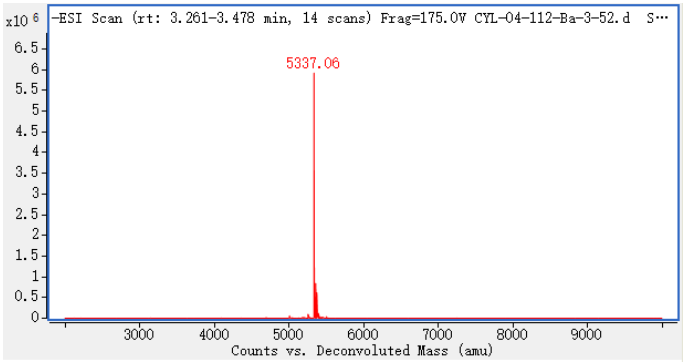
Conversion: **>90%**. Calculated Mass: **5351 Da**; Observed Mass: **5351 Da**



UPLC chromatogram and deconvoluted MS of **5ai**

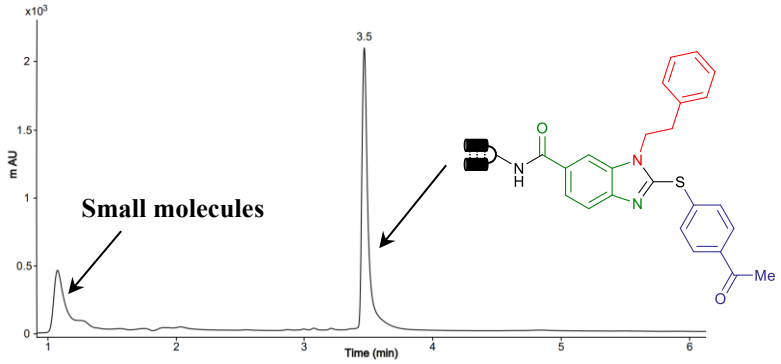
Conversion: **>90%**. Calculated Mass: **5337 Da**; Observed Mass: **5337 Da**

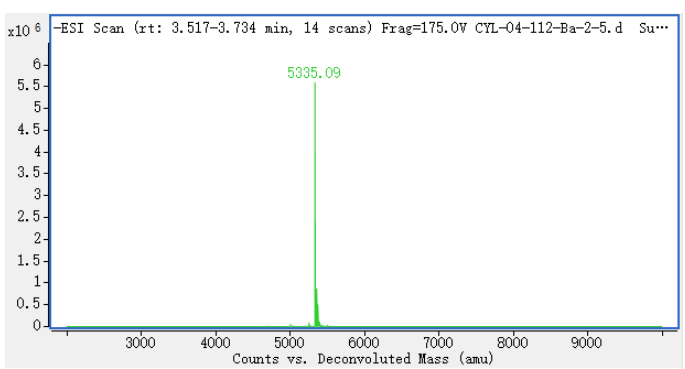




UPLC chromatogram and deconvoluted MS of **5aj**

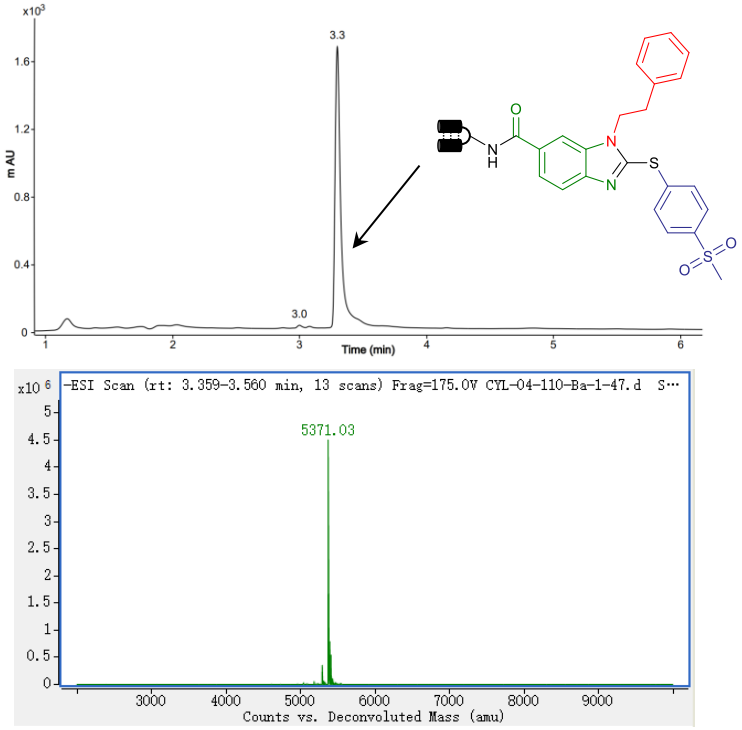
Conversion: **>90%**. Calculated Mass: **5335 Da**; Observed Mass: **5335 Da**





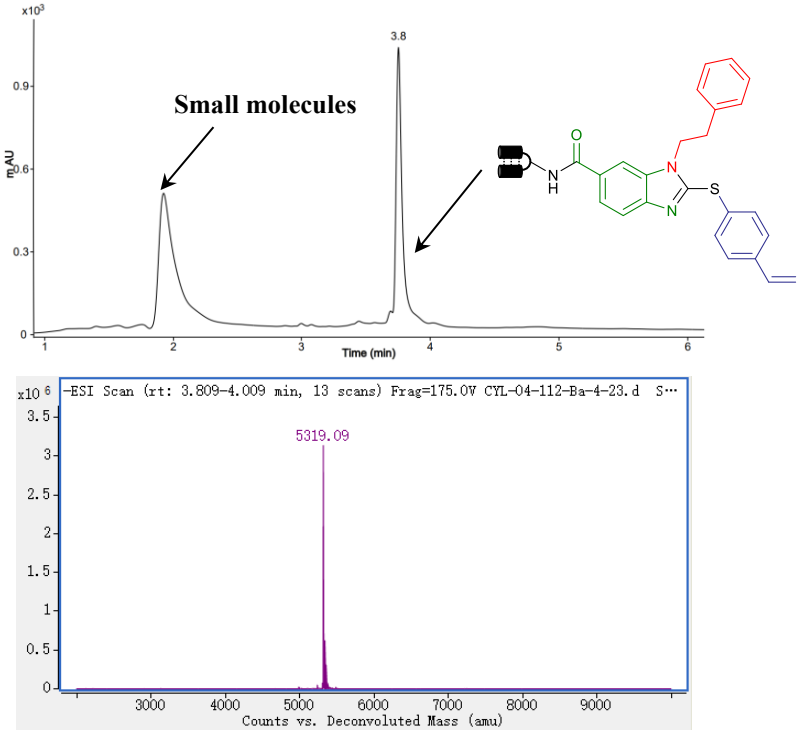
UPLC chromatogram and deconvoluted MS of **5ak**

Conversion: **>90%**. Calculated Mass: **5371 Da**; Observed Mass: **5371 Da**



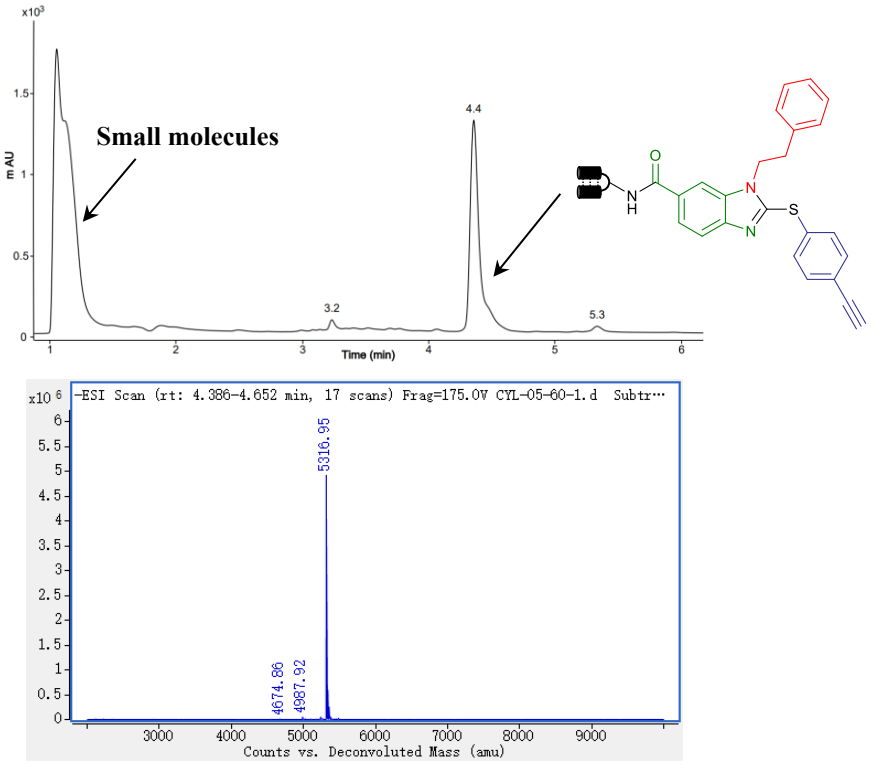
UPLC chromatogram and deconvoluted MS of **5al**

Conversion: **>90%**. Calculated Mass: **5319 Da**; Observed Mass: **5319 Da**



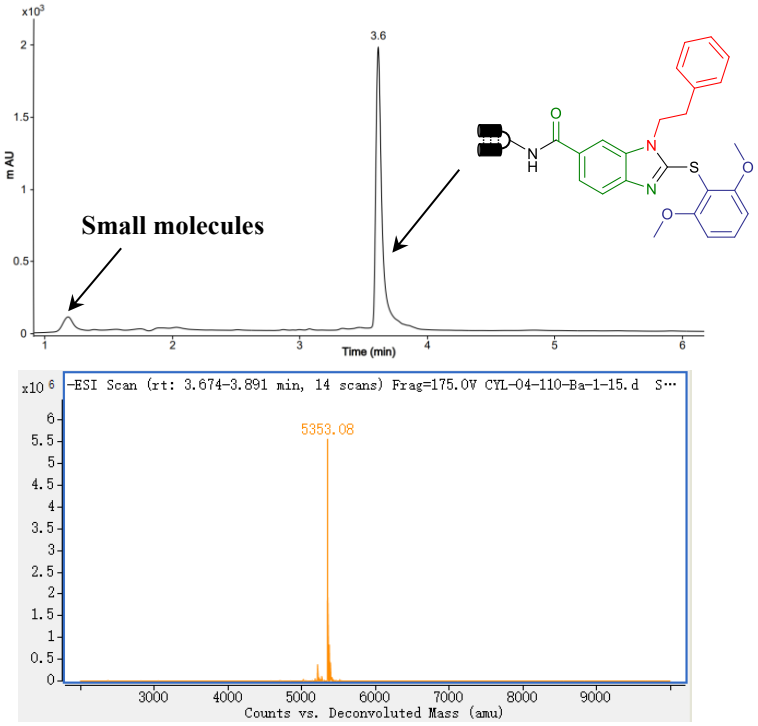
UPLC chromatogram and deconvoluted MS of **5am**

Conversion: **>90%**. Calculated Mass: **5317 Da**; Observed Mass: **5317 Da**



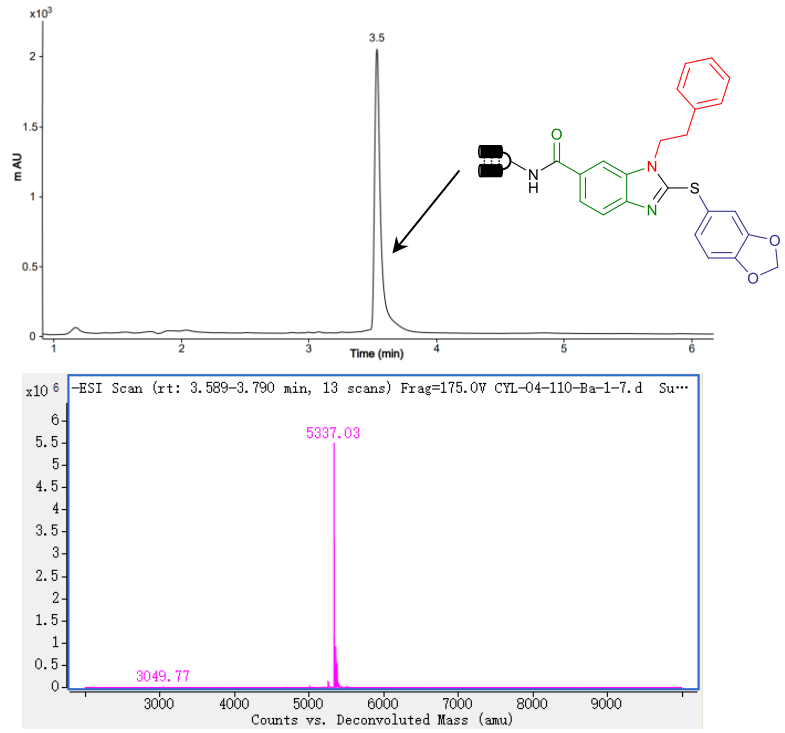
UPLC chromatogram and deconvoluted MS of **5an**

Conversion: **>90%**. Calculated Mass: **5353 Da**; Observed Mass: **5353 Da**



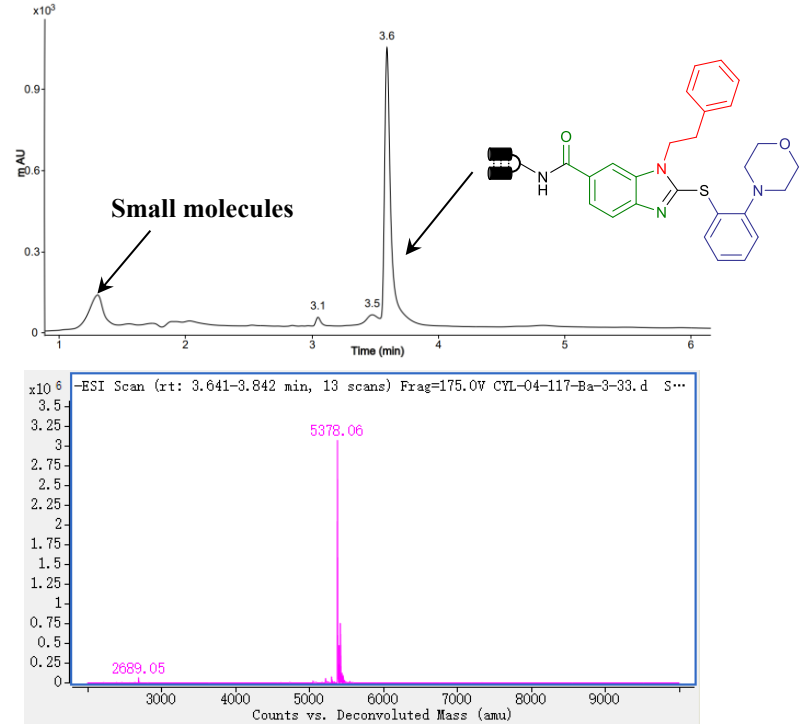
UPLC chromatogram and deconvoluted MS of **5ao**

Conversion: **>90%**. Calculated Mass: **5337 Da**; Observed Mass: **5337 Da**



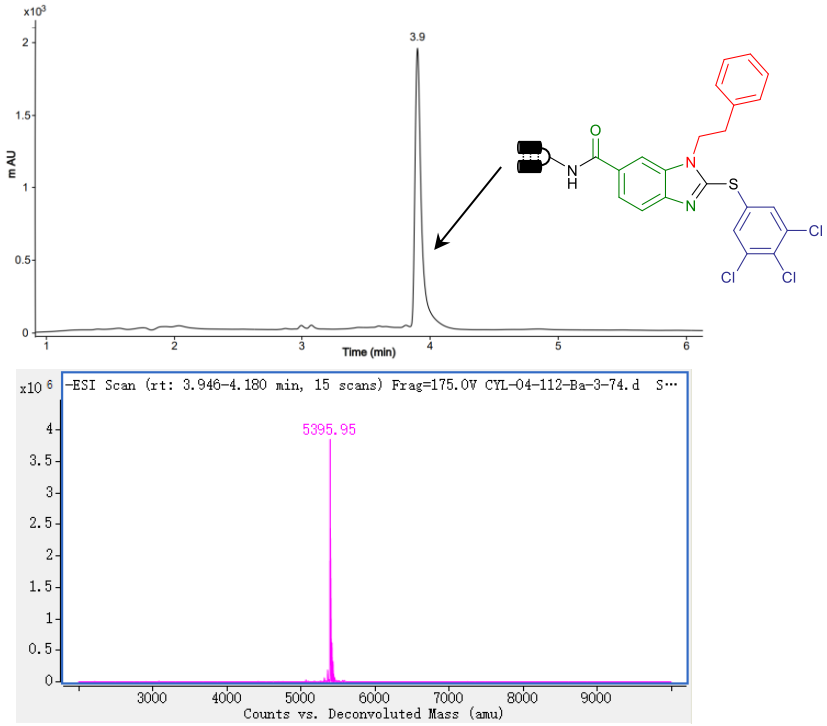
UPLC chromatogram and deconvoluted MS of **5ap**

Conversion: **81%**. Calculated Mass: **5378 Da**; Observed Mass: **5378 Da**



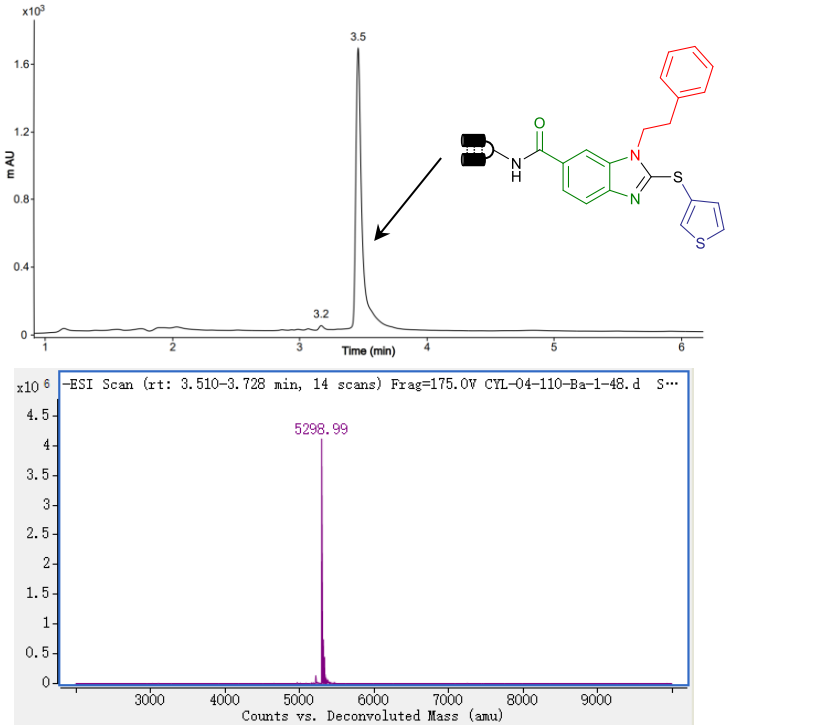
UPLC chromatogram and deconvoluted MS of **5aq**

Conversion: **>90%**. Calculated Mass: **5396 Da**; Observed Mass: **5396 Da**



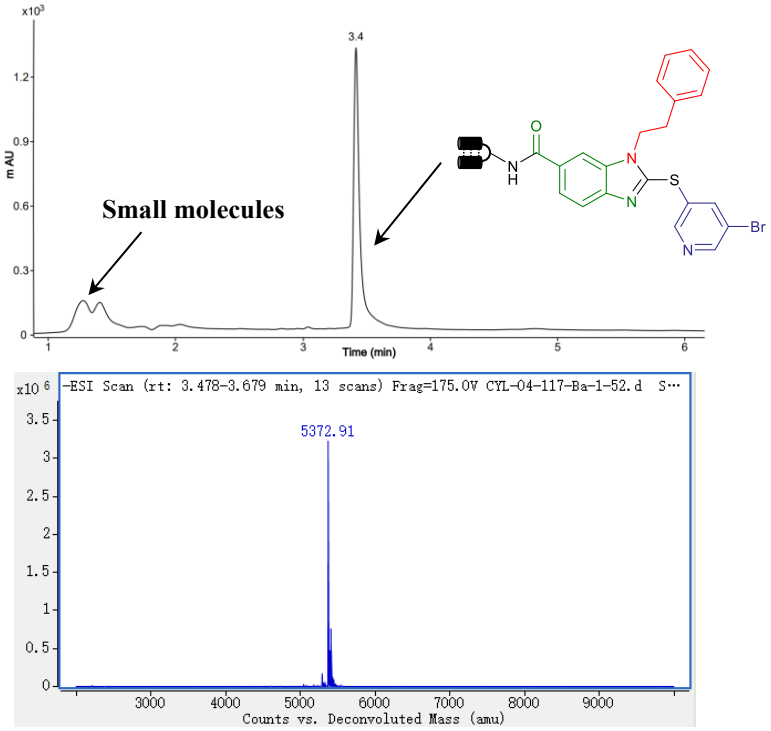
UPLC chromatogram and deconvoluted MS of **5ar**

Conversion: **>90%**. Calculated Mass: **5299 Da**; Observed Mass: **5299 Da**



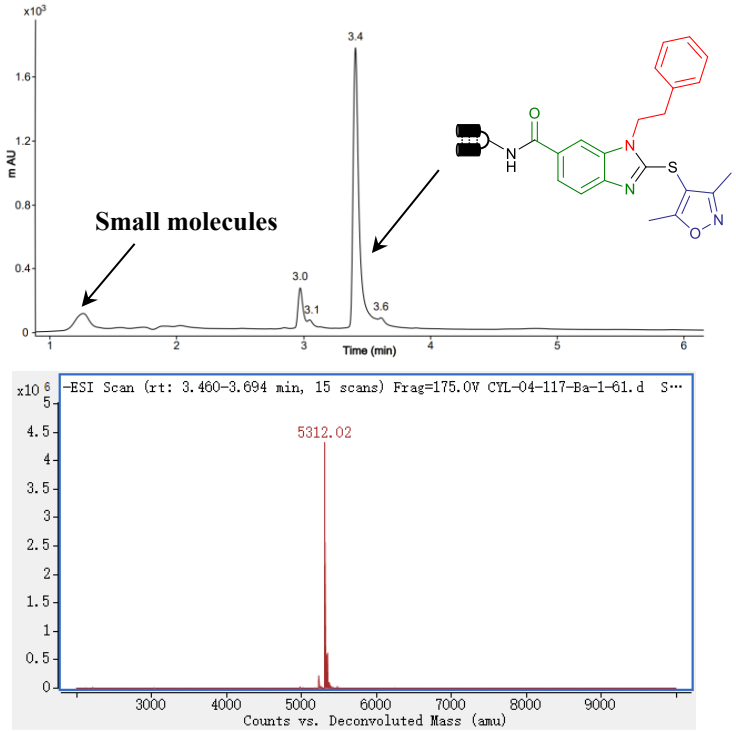
UPLC chromatogram and deconvoluted MS of **5as**

Conversion: **>90%**. Calculated Mass: **5373 Da**; Observed Mass: **5373 Da**



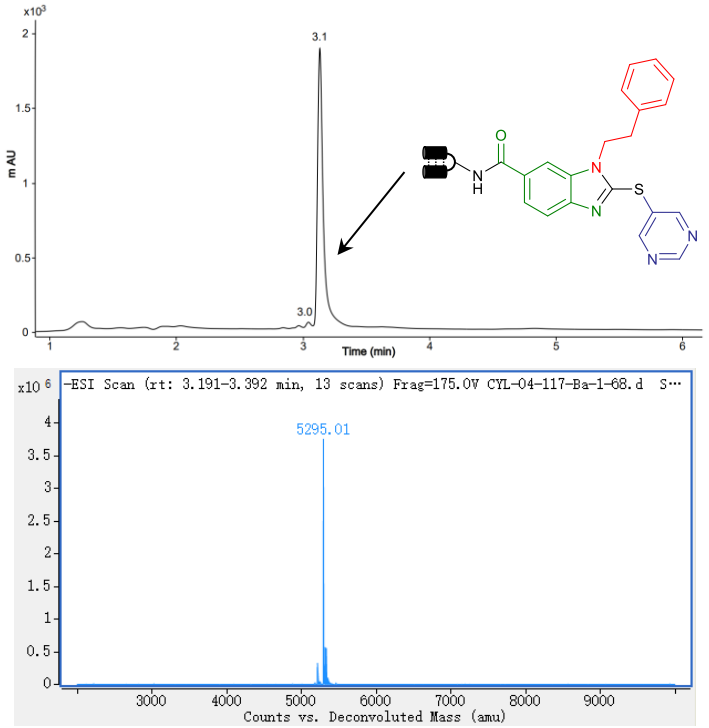
UPLC chromatogram and deconvoluted MS of **5at**

Conversion: **80%**. Calculated Mass: **5312 Da**; Observed Mass: **5312 Da**



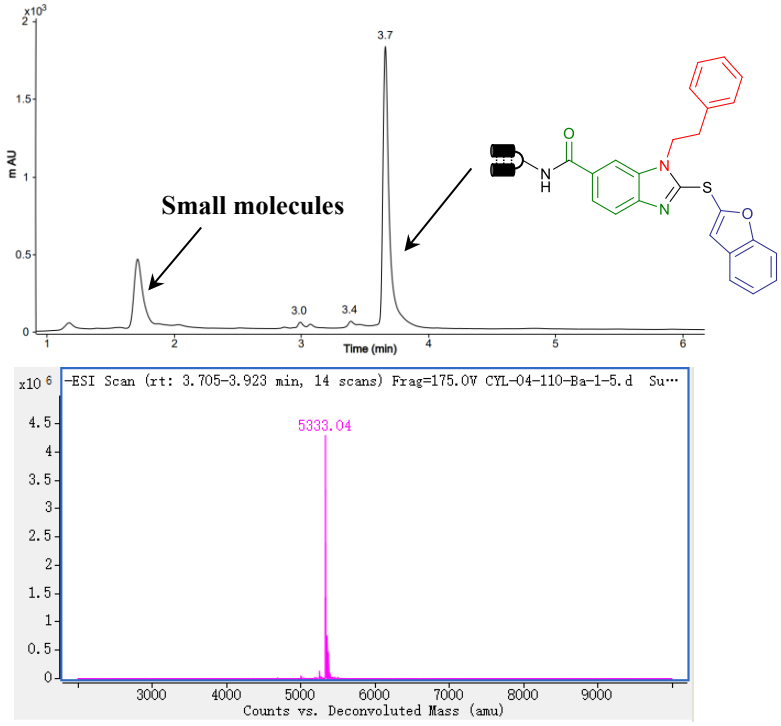
UPLC chromatogram and deconvoluted MS of **5au**

Conversion: **>90%**. Calculated Mass: **5295 Da**; Observed Mass: **5295 Da**



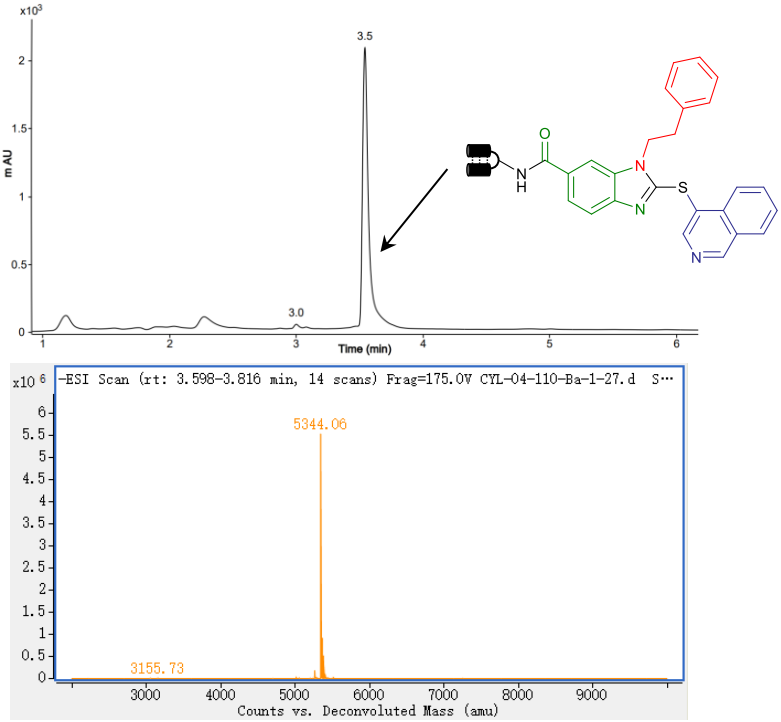
UPLC chromatogram and deconvoluted MS of **5av**

Conversion: **>90%**. Calculated Mass: **5333 Da**; Observed Mass: **5333 Da**



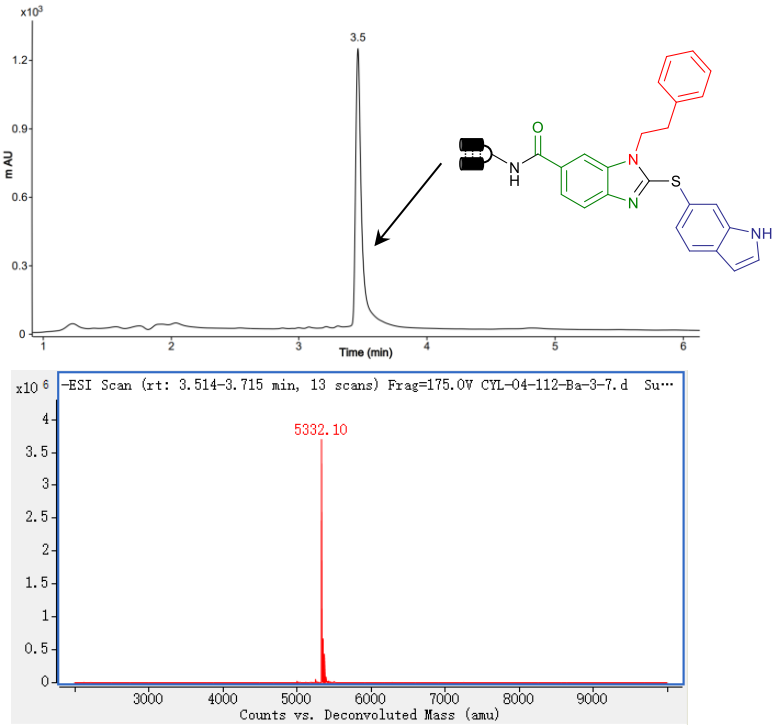
UPLC chromatogram and deconvoluted MS of **5aw**

Conversion: **>90%**. Calculated Mass: **5344 Da**; Observed Mass: **5344 Da**



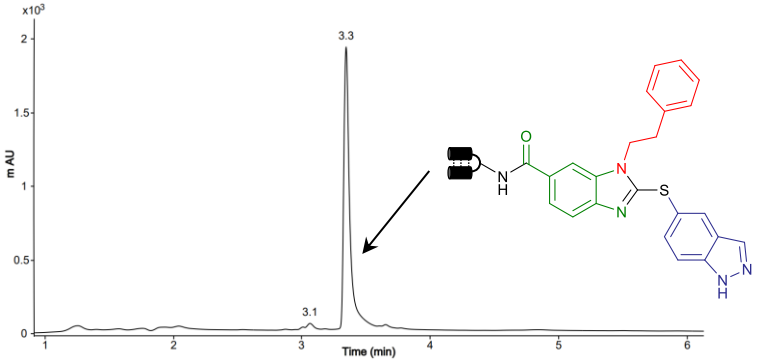
UPLC chromatogram and deconvoluted MS of **5ax**

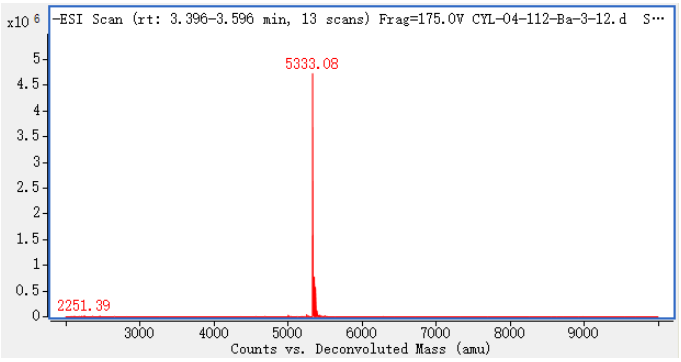
Conversion: **>90%**. Calculated Mass: **5332 Da**; Observed Mass: **5332 Da**



UPLC chromatogram and deconvoluted MS of **5ay**

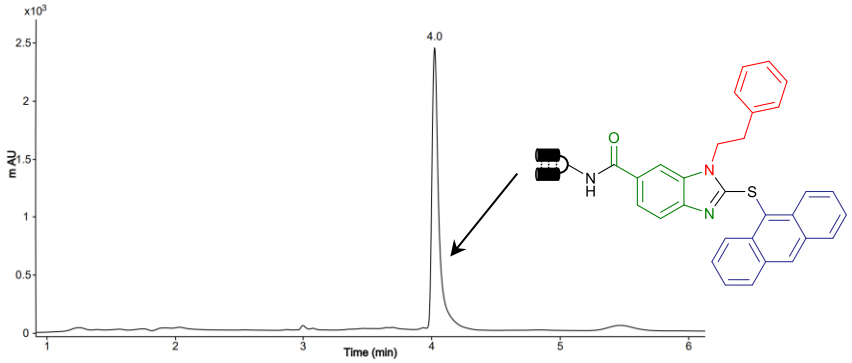
Conversion: **>90%**. Calculated Mass: **5333 Da**; Observed Mass: **5333 Da**

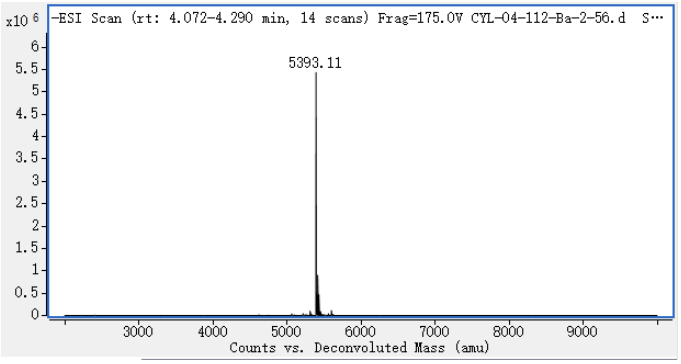




UPLC chromatogram and deconvoluted MS of **5az**

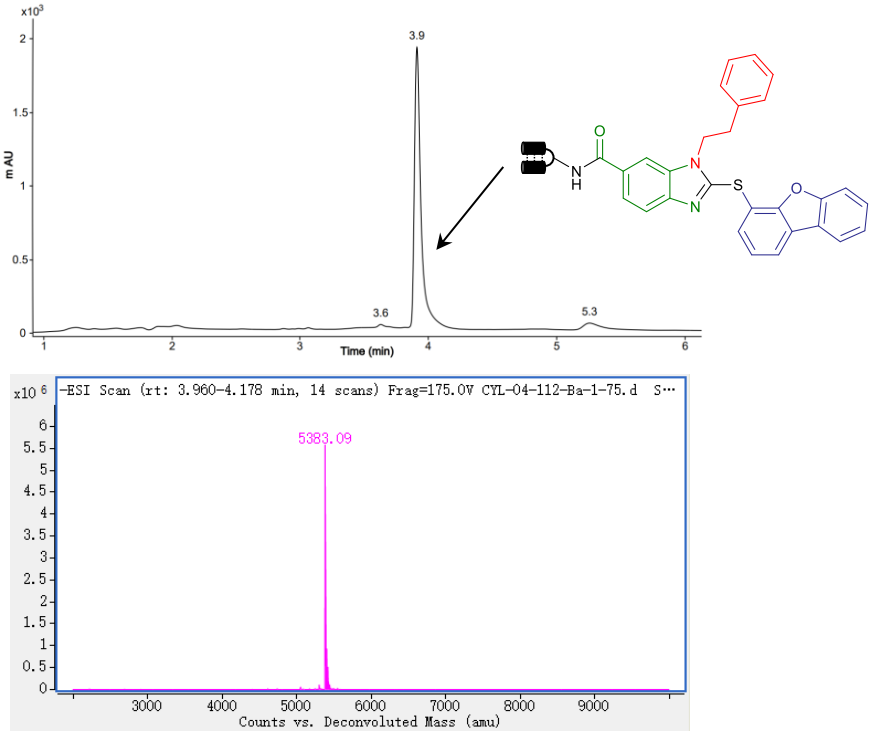
Conversion: **>90%**. Calculated Mass: **5393 Da**; Observed Mass: **5393 Da**





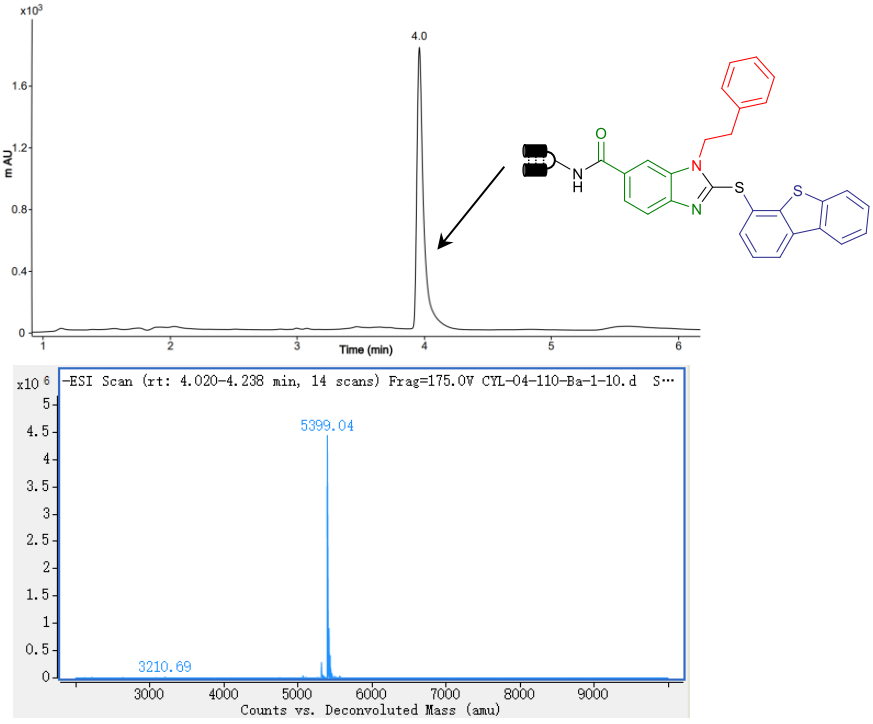
UPLC chromatogram and deconvoluted MS of **5ba**

Conversion: **>90%**. Calculated Mass: **5383 Da**; Observed Mass: **5383 Da**



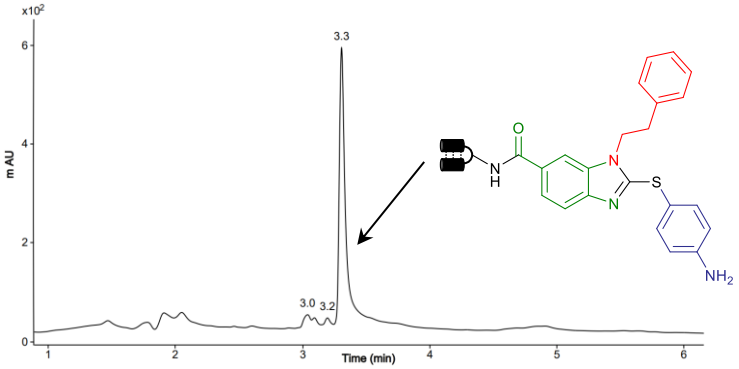
UPLC chromatogram and deconvoluted MS of **5bb**

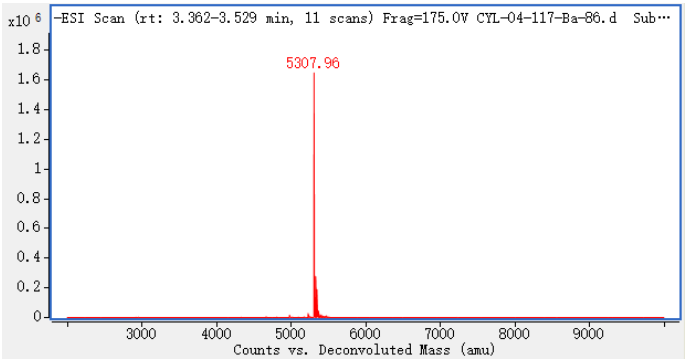
Conversion: **>90%**. Calculated Mass: **5399 Da**; Observed Mass: **5399 Da**



UPLC chromatogram and deconvoluted MS of **5bc**

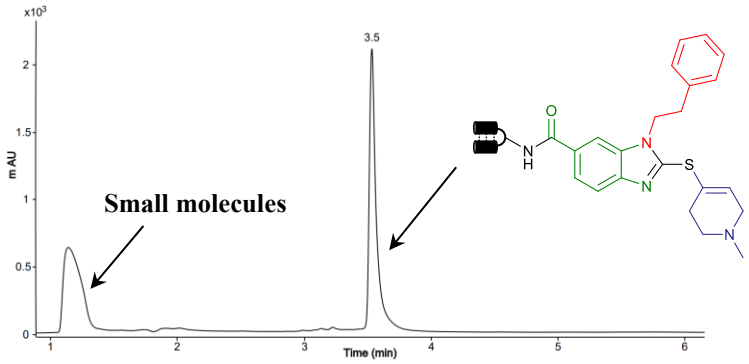
Conversion: **>90%**. Calculated Mass: **5308 Da**; Observed Mass: **5308 Da**

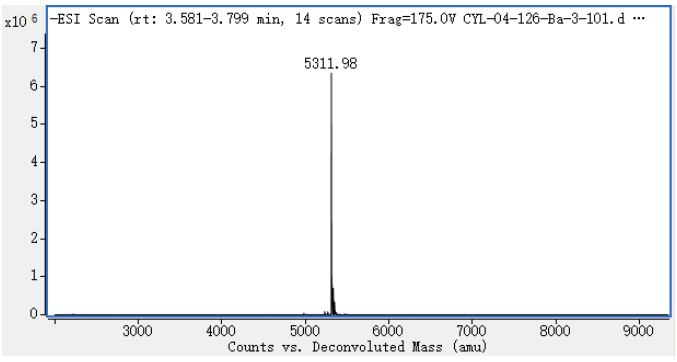




UPLC chromatogram and deconvoluted MS of **5bd**

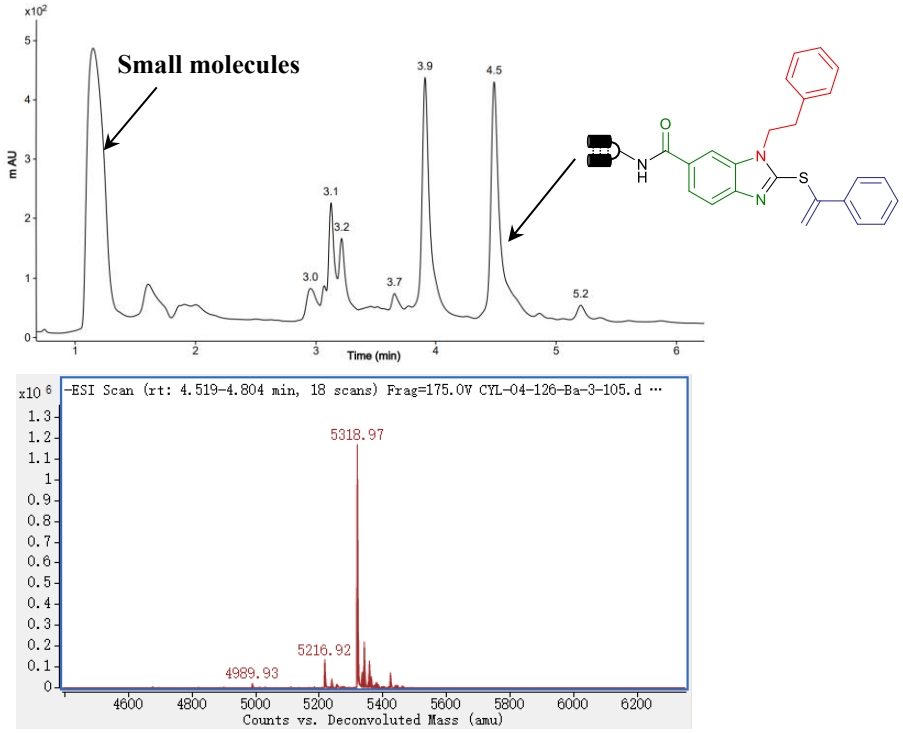
Conversion: **>90%**. Calculated Mass: **5312 Da**; Observed Mass: **5312 Da**





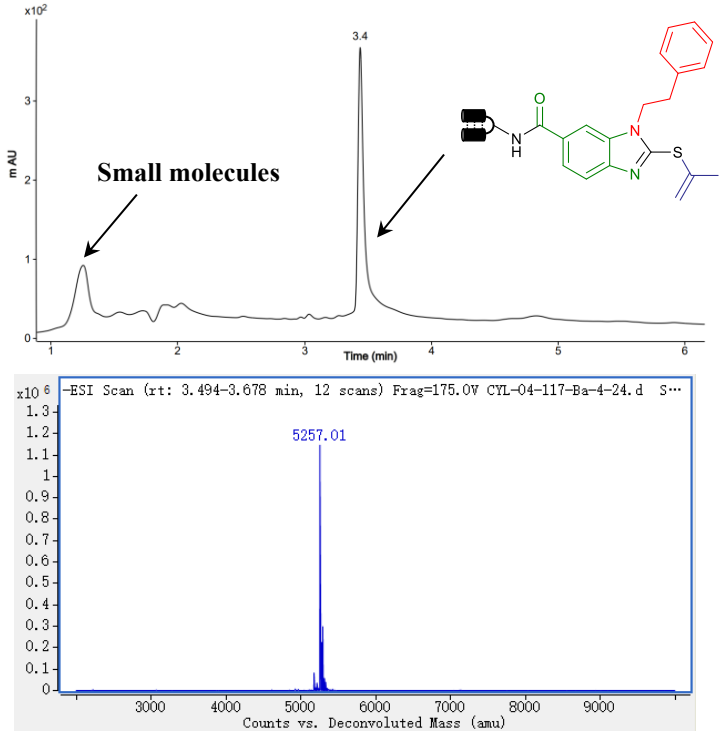
UPLC chromatogram and deconvoluted MS of **5be**

Conversion: **31%**. Calculated Mass: **5319 Da**; Observed Mass: **5319 Da**



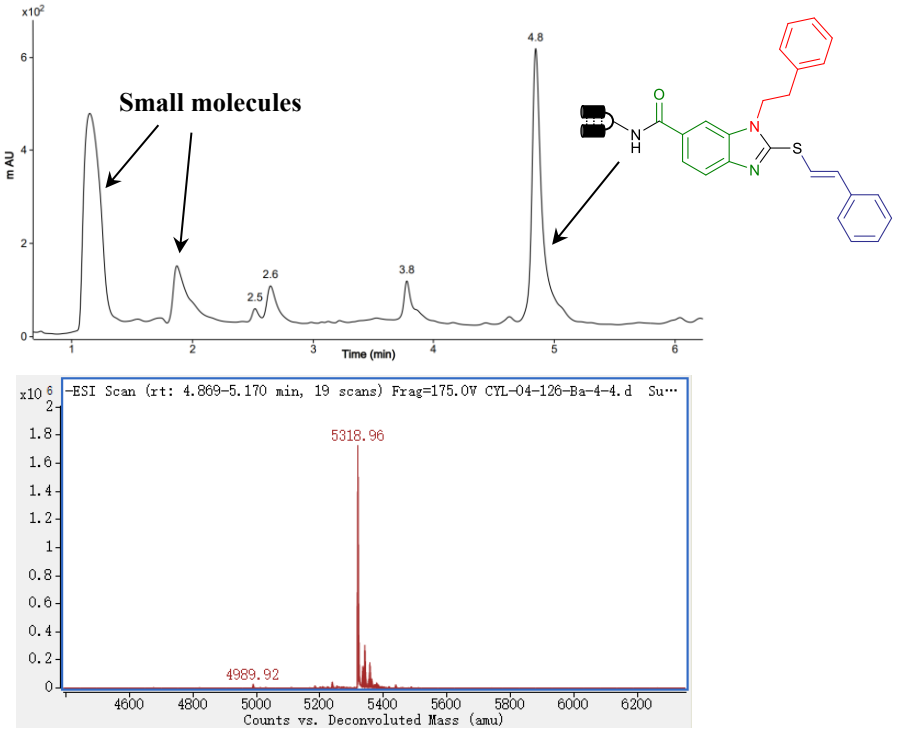
UPLC chromatogram and deconvoluted MS of **5bf**

Conversion: **>90%**. Calculated Mass: **5257 Da**; Observed Mass: **5257 Da**



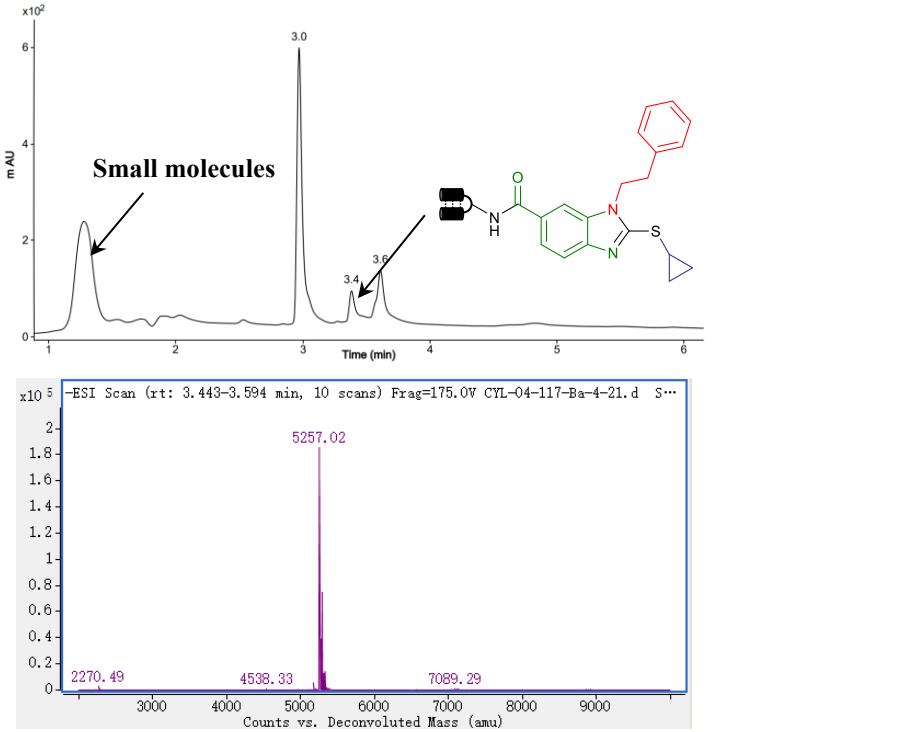
UPLC chromatogram and deconvoluted MS of **5bg**

Conversion: **73%**. Calculated Mass: **5319 Da**; Observed Mass: **5319 Da**



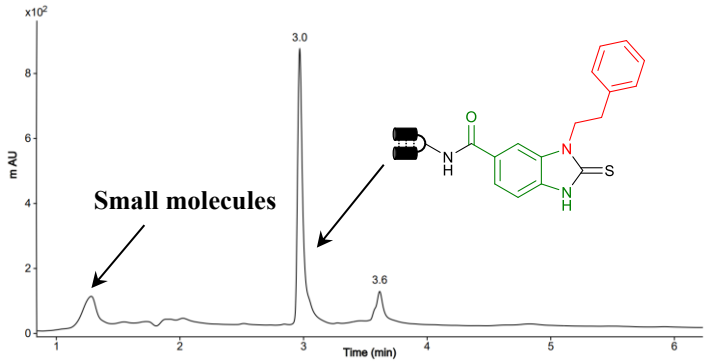
UPLC chromatogram and deconvoluted MS of **5bh**

Conversion: **12%**. Calculated Mass: **5257 Da**; Observed Mass: **5257 Da**



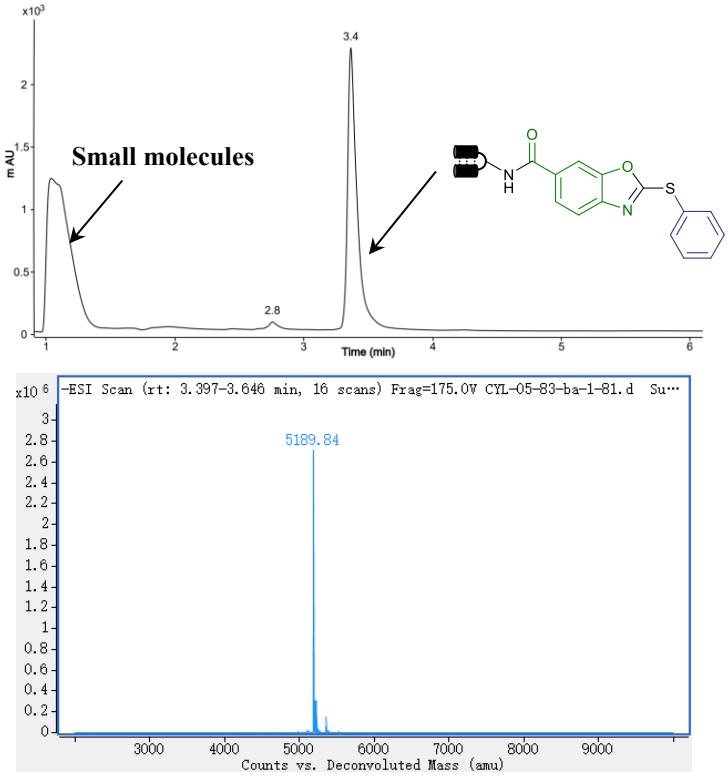
UPLC chromatogram and deconvoluted MS of **5bi**

Conversion: **0%**. Calculated Mass: **5299 Da**; Observed Mass: **5217 Da**



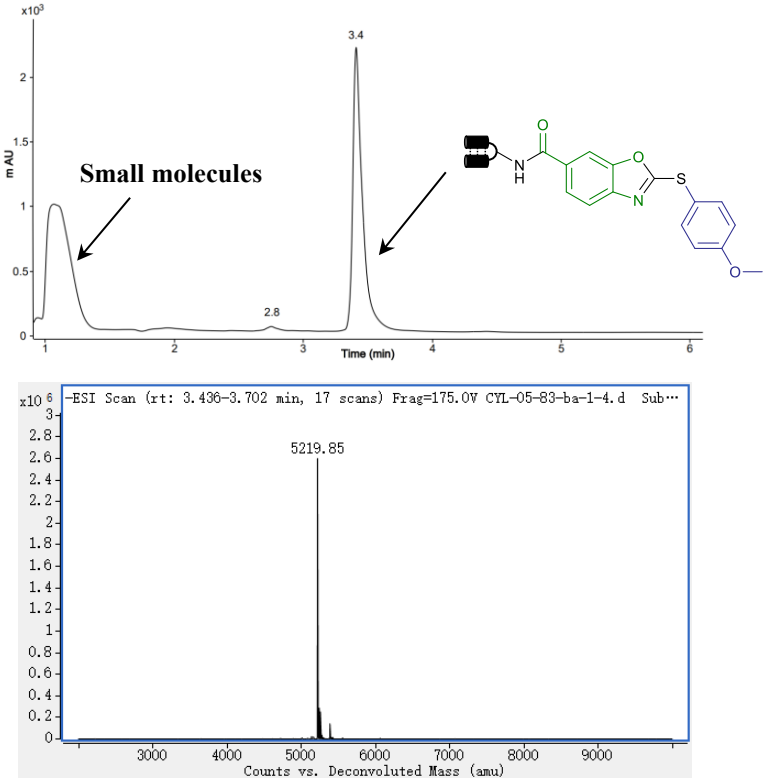
UPLC chromatogram and deconvoluted MS of **5bj**

Conversion: **>90%**. Calculated Mass: **5190 Da**; Observed Mass: **5190 Da**



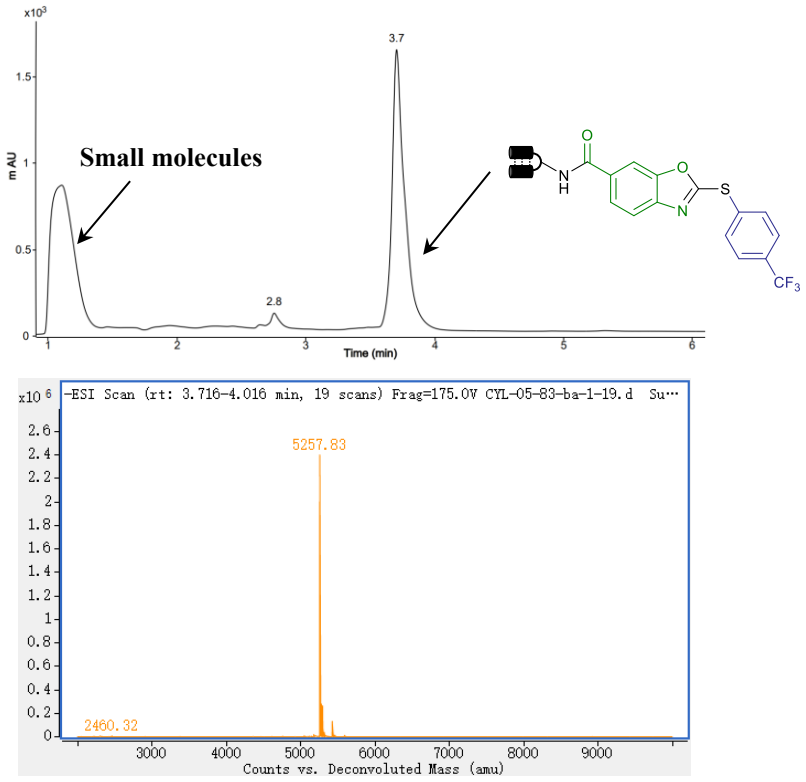
UPLC chromatogram and deconvoluted MS of **5bk**

Conversion: **>90%**. Calculated Mass: **5220 Da**; Observed Mass: **5220 Da**



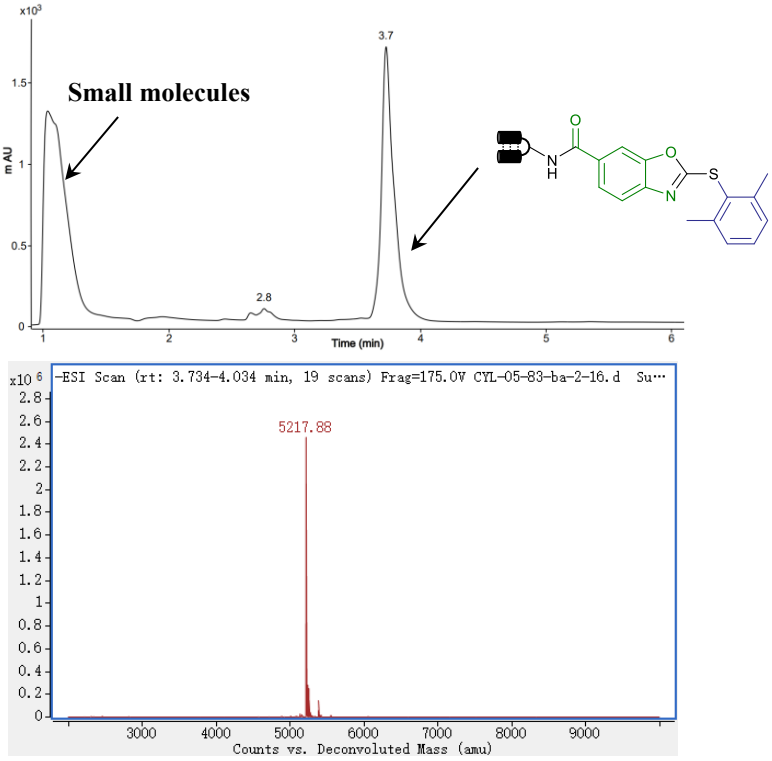
UPLC chromatogram and deconvoluted MS of **5bl**

Conversion: **>90%**. Calculated Mass: **5258 Da**; Observed Mass: **5258 Da**



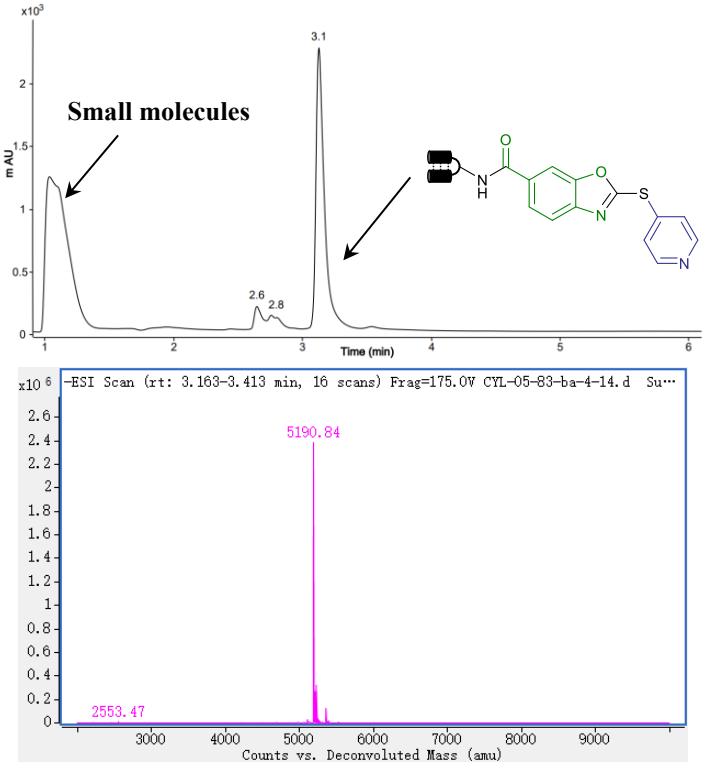
UPLC chromatogram and deconvoluted MS of **5bm**

Conversion: **>90%**. Calculated Mass: **5218 Da**; Observed Mass: **5218 Da**



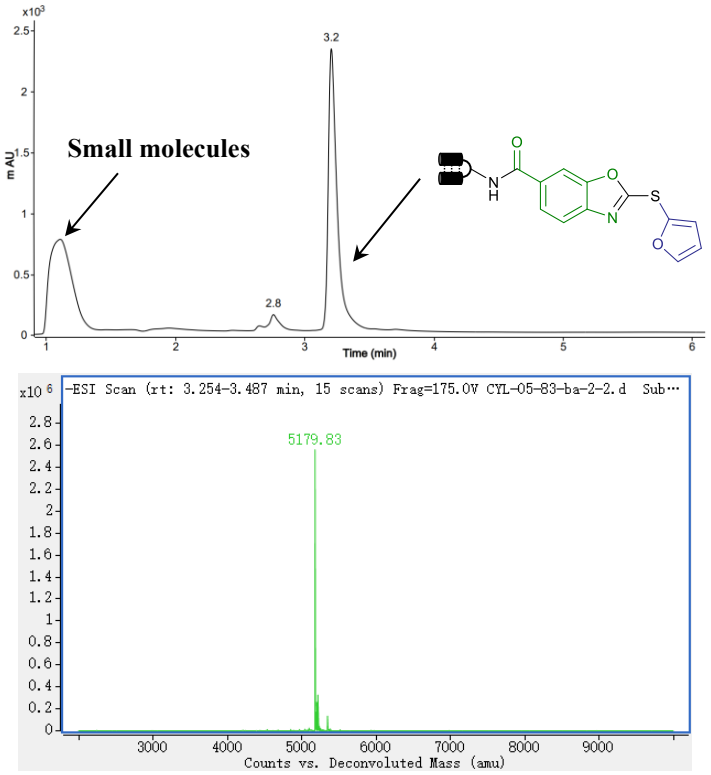
UPLC chromatogram and deconvoluted MS of **5bn**

Conversion: **88%**. Calculated Mass: **5191 Da**; Observed Mass: **5191 Da**



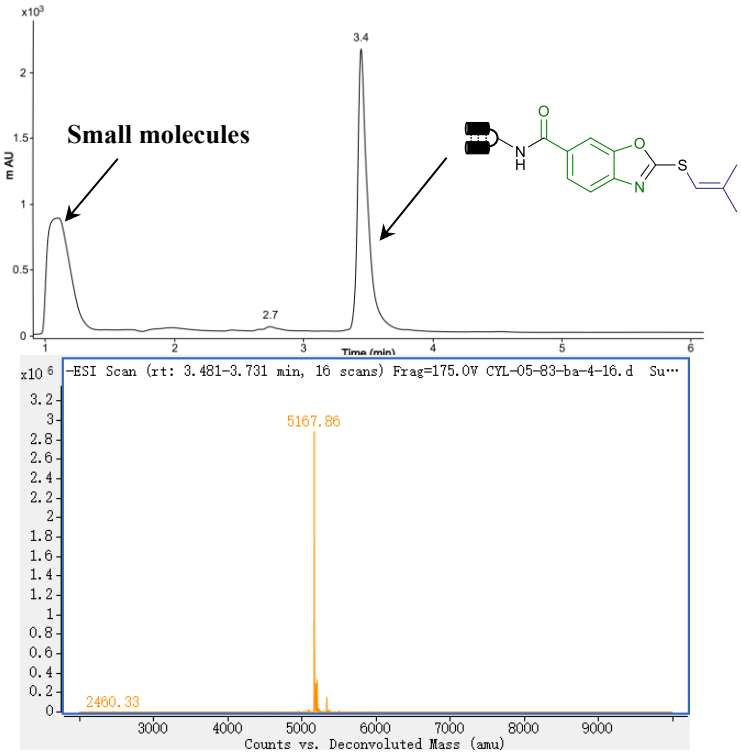
UPLC chromatogram and deconvoluted MS of **5bo**

Conversion: **>90%**. Calculated Mass: **5180 Da**; Observed Mass: **5180 Da**



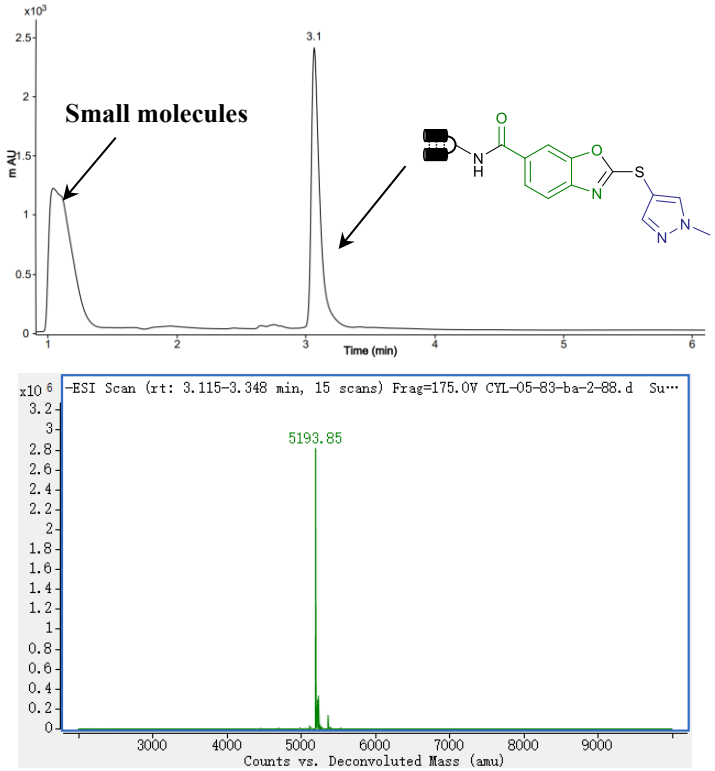
UPLC chromatogram and deconvoluted MS of **5bp**

Conversion: **>90%**. Calculated Mass: **5168 Da**; Observed Mass: **5168 Da**



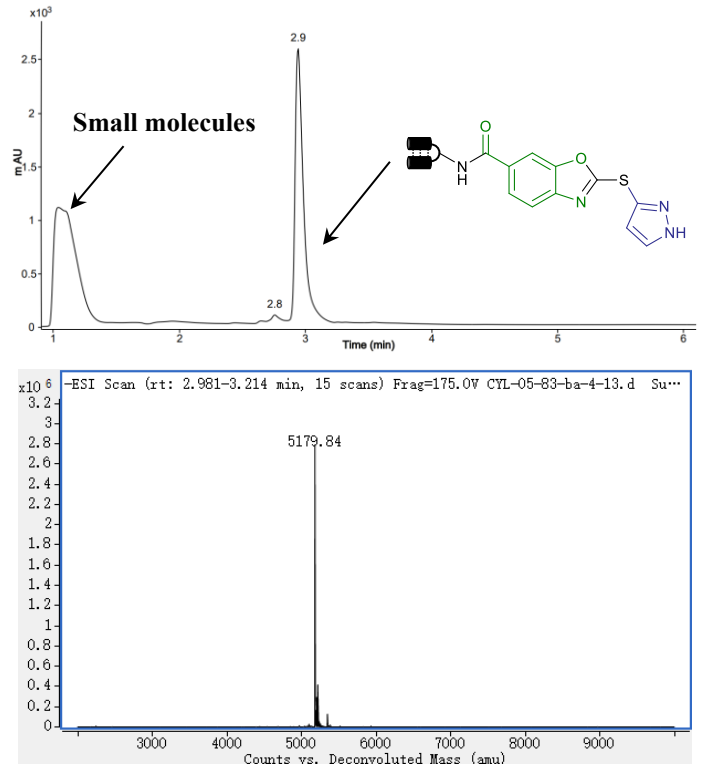
UPLC chromatogram and deconvoluted MS of **5bq**

Conversion: **>90%**. Calculated Mass: **5194 Da**; Observed Mass: **5194 Da**



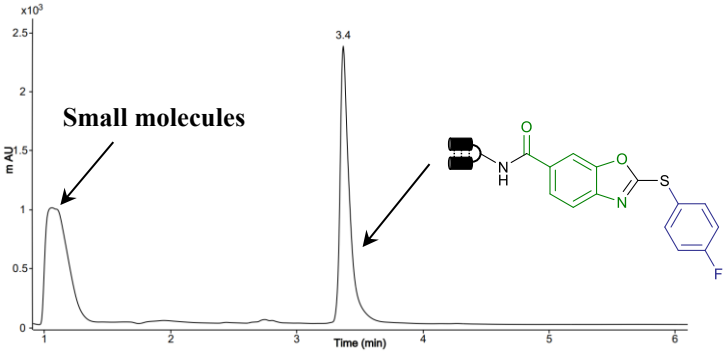
UPLC chromatogram and deconvoluted MS of **5br**

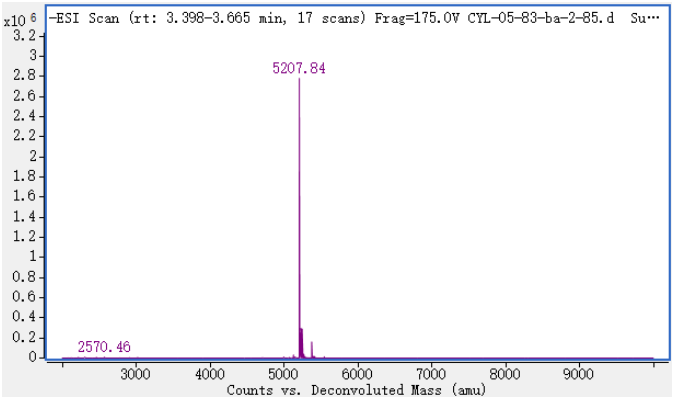
Conversion: **>90%**. Calculated Mass: **5180 Da**; Observed Mass: **5180 Da**



UPLC chromatogram and deconvoluted MS of **5bs**

Conversion: **>90%**. Calculated Mass: **5208 Da**; Observed Mass: **5208 Da**

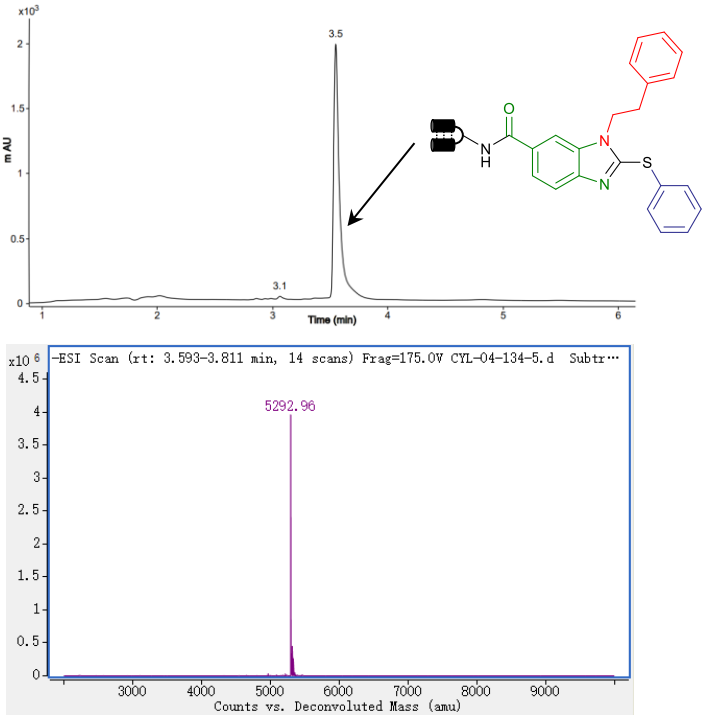




8.2. Substrate scope of scaffolds

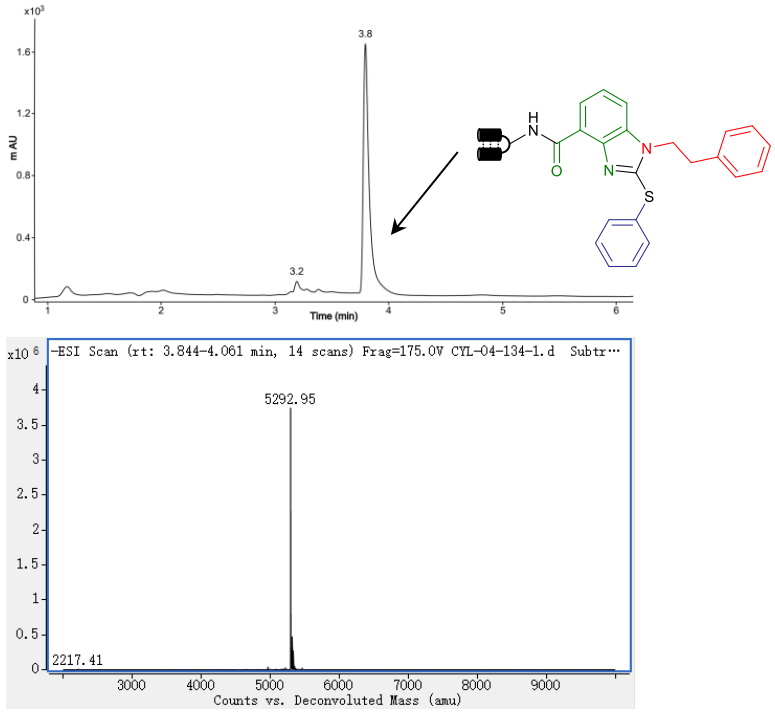
UPLC chromatogram and deconvoluted MS of **5a**

Conversion: **>90%**. Calculated Mass: **5293 Da**; Observed Mass: **5293 Da**



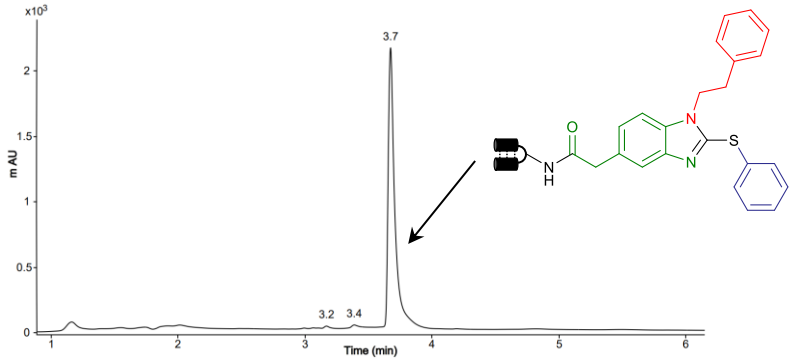
UPLC chromatogram and deconvoluted MS of **5bt**

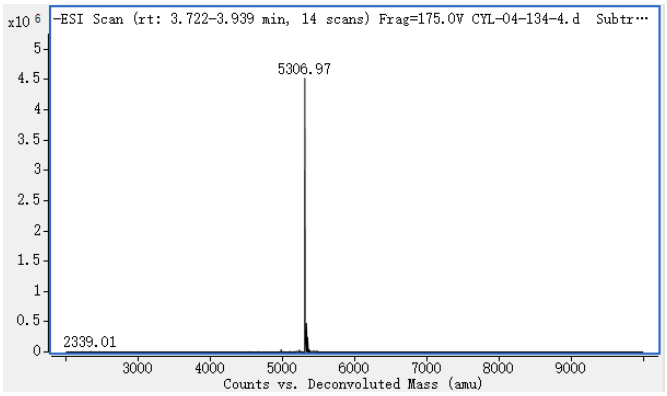
Conversion: **>90%**. Calculated Mass: **5293 Da**; Observed Mass: **5293 Da**



UPLC chromatogram and deconvoluted MS of **5bu**

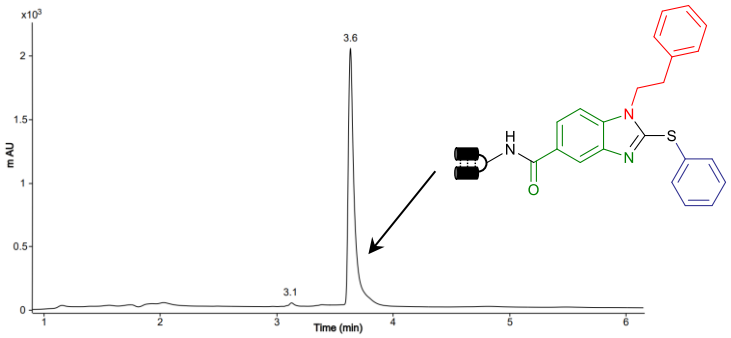
Conversion: **>90%**. Calculated Mass: **5307 Da**; Observed Mass: **5307 Da**

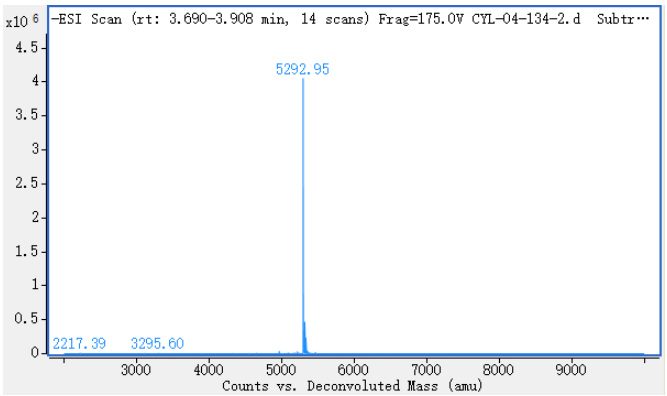




UPLC chromatogram and deconvoluted MS of **5bv**

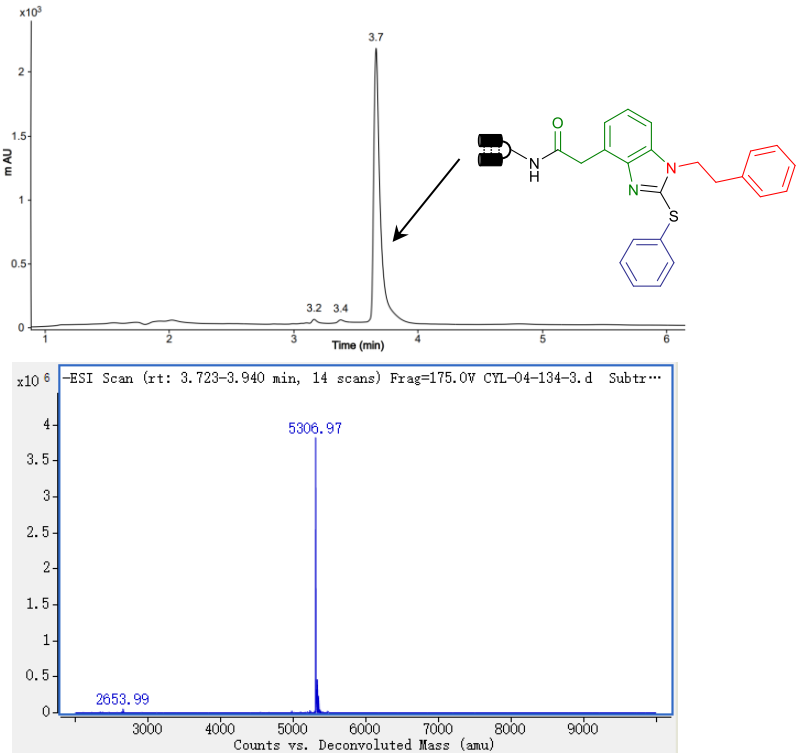
Conversion: **>90%**. Calculated Mass: **5293 Da**; Observed Mass: **5293 Da**





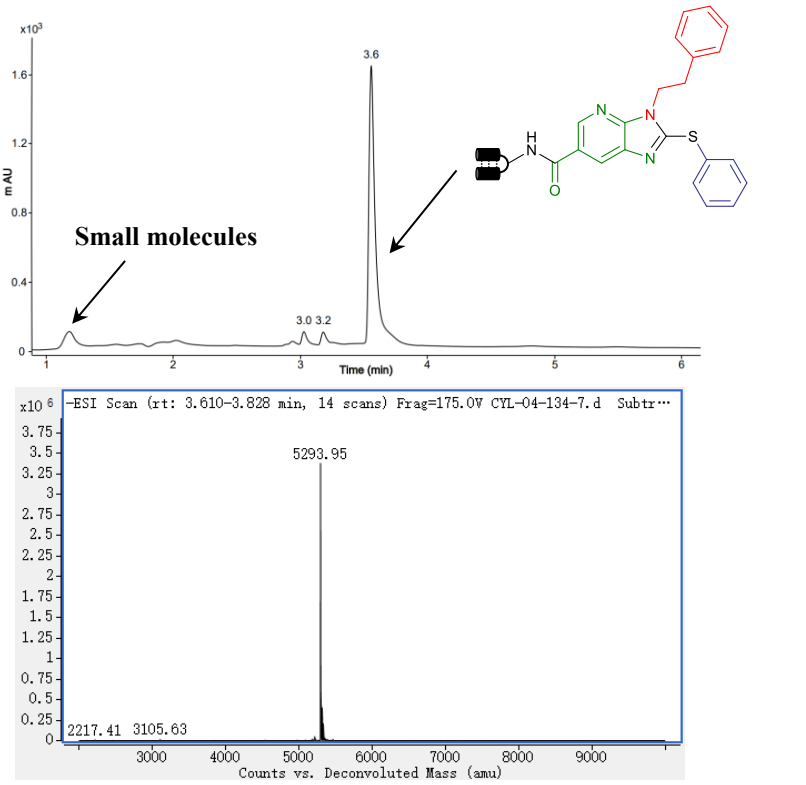
UPLC chromatogram and deconvoluted MS of **5bw**

Conversion: **>90%**. Calculated Mass: **5307 Da**; Observed Mass: **5307 Da**



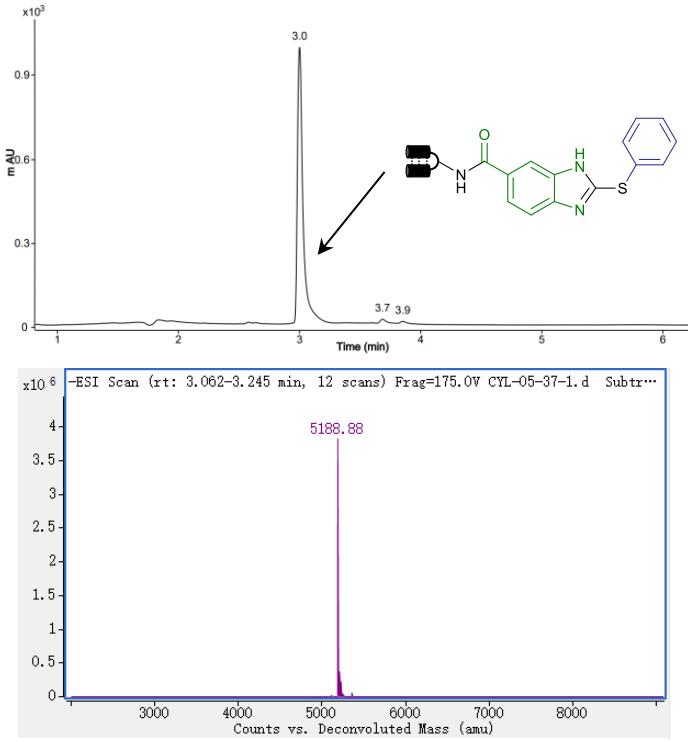
UPLC chromatogram and deconvoluted MS of **5bx**

Conversion: **>90%**. Calculated Mass: **5294 Da**; Observed Mass: **5294 Da**



UPLC chromatogram and deconvoluted MS of **5by**

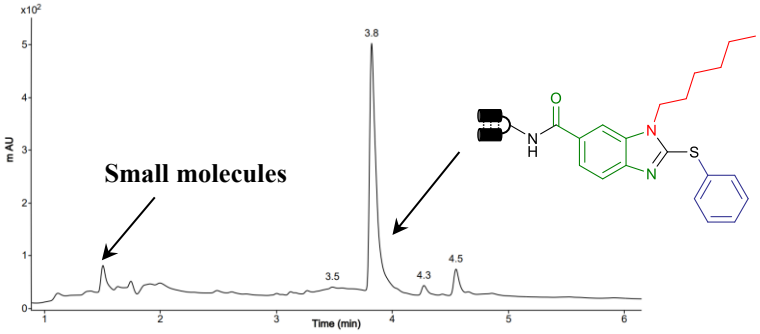
Conversion: **>90%**. Calculated Mass: **5189 Da**; Observed Mass: **5189 Da**

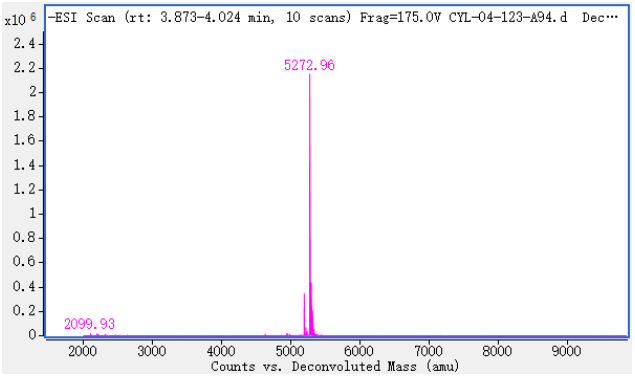


8.3. Substrate scope of amines

UPLC chromatogram and deconvoluted MS of **5bz**

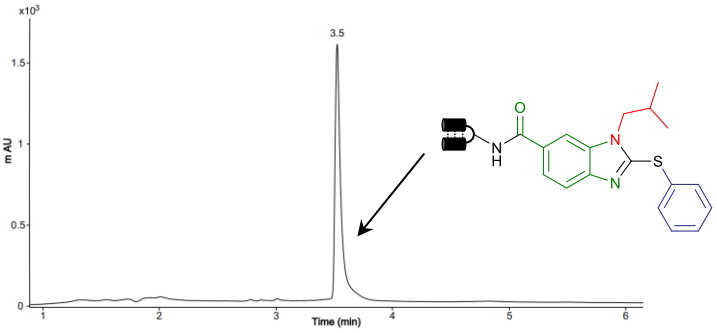
Conversion: **80%**. Calculated Mass: **5273 Da**; Observed Mass: **5273 Da**

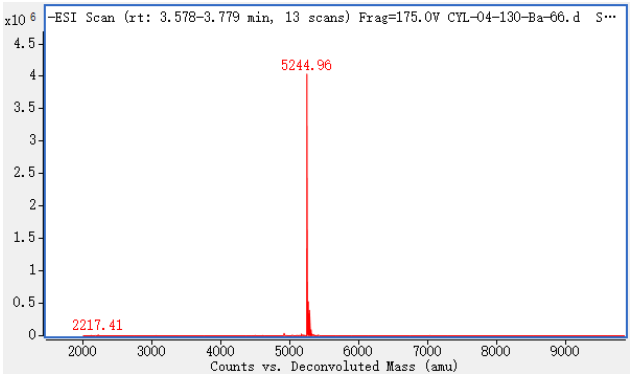




UPLC chromatogram and deconvoluted MS of **5ca**

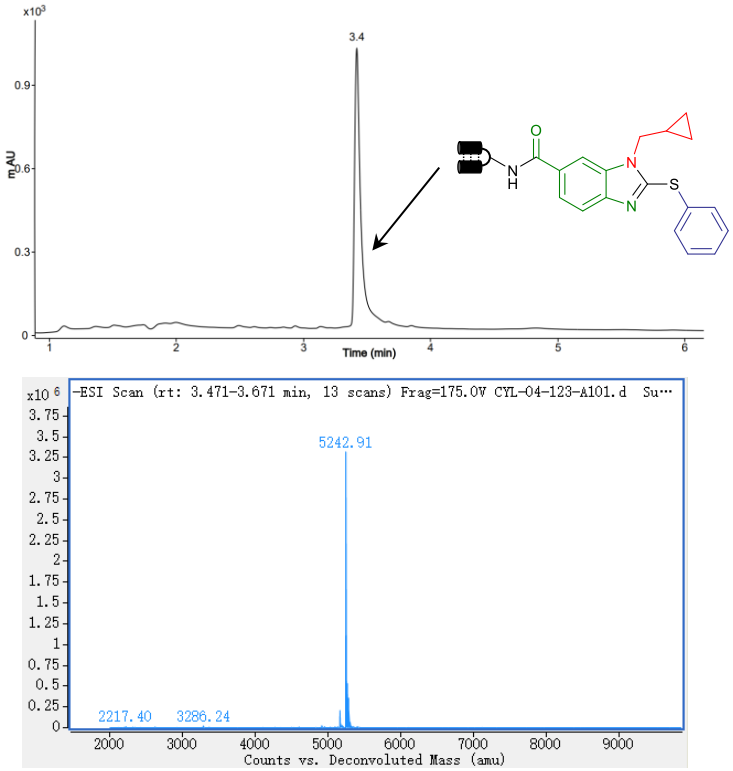
Conversion: **>90%**. Calculated Mass: **5245 Da**; Observed Mass: **5245 Da**





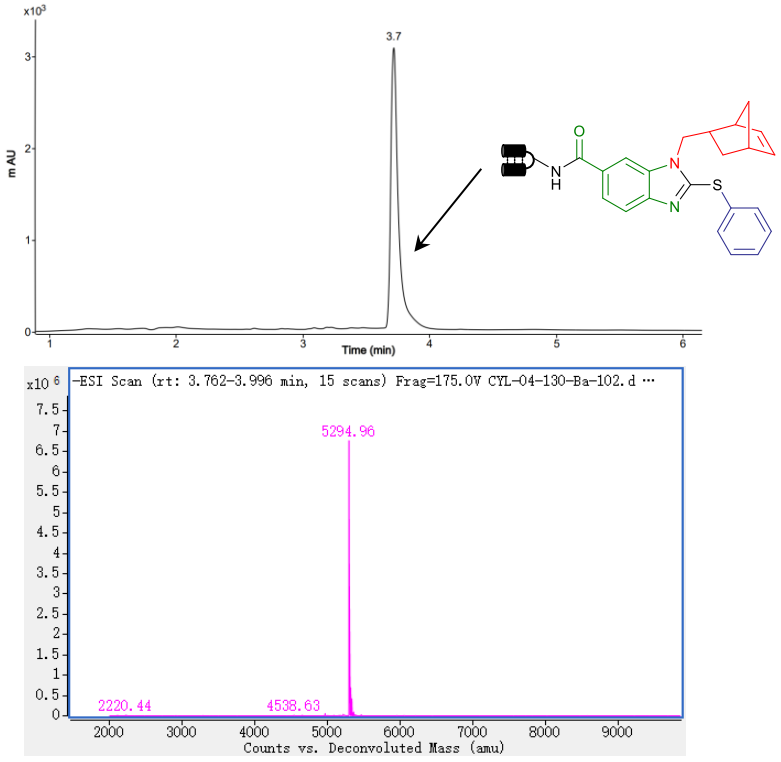
UPLC chromatogram and deconvoluted MS of **5cb**

Conversion: **>90%**. Calculated Mass: **5243 Da**; Observed Mass: **5243 Da**



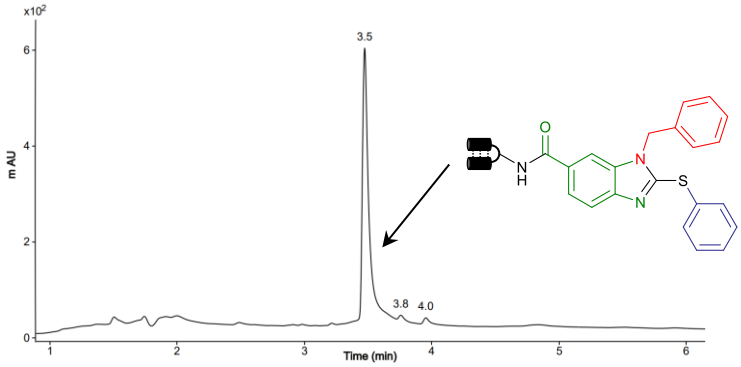
UPLC chromatogram and deconvoluted MS of **5cc**

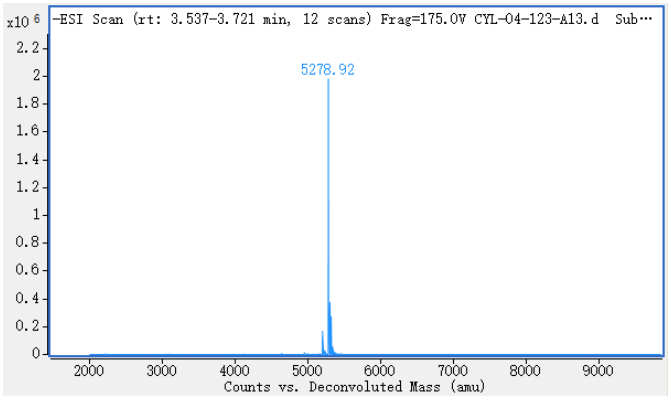
Conversion: **>90%**. Calculated Mass: **5295 Da**; Observed Mass: **5295 Da**



UPLC chromatogram and deconvoluted MS of **5cd**

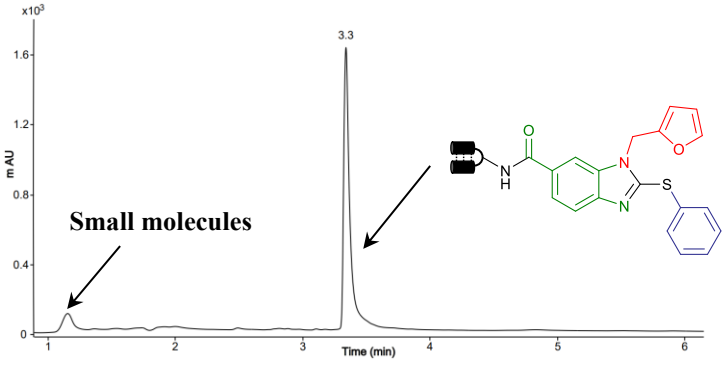
Conversion: **>90%**. Calculated Mass: **5279 Da**; Observed Mass: **5279 Da**

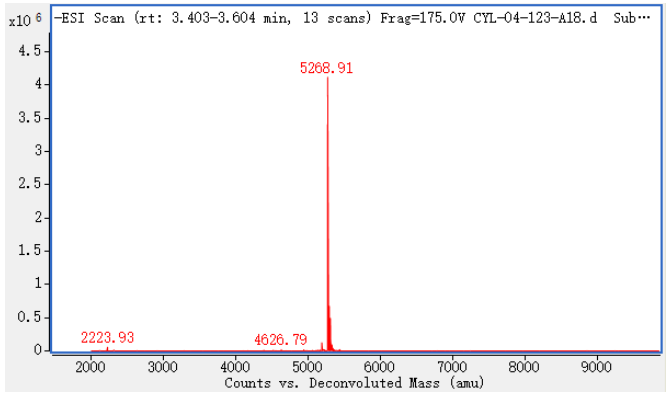




UPLC chromatogram and deconvoluted MS of **5ce**

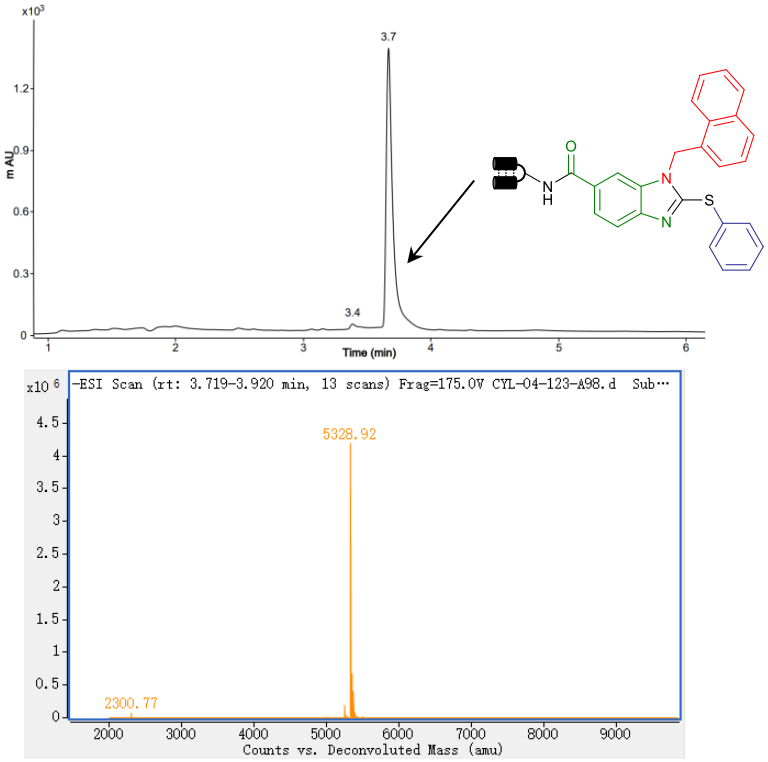
Conversion: **>90%**. Calculated Mass: **5269 Da**; Observed Mass: **5269 Da**





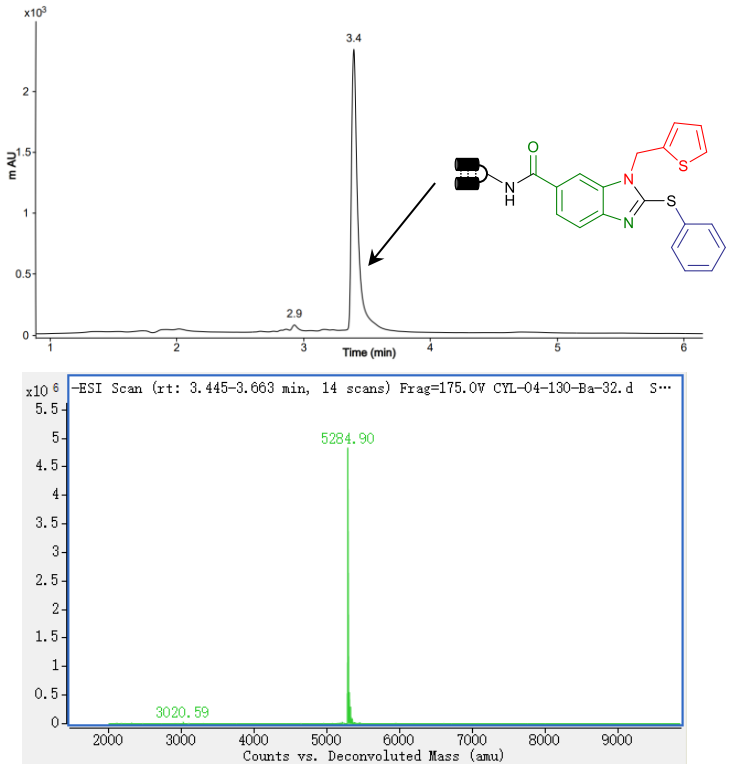
UPLC chromatogram and deconvoluted MS of **5cf**

Conversion: **>90%**. Calculated Mass: **5329 Da**; Observed Mass: **5329 Da**



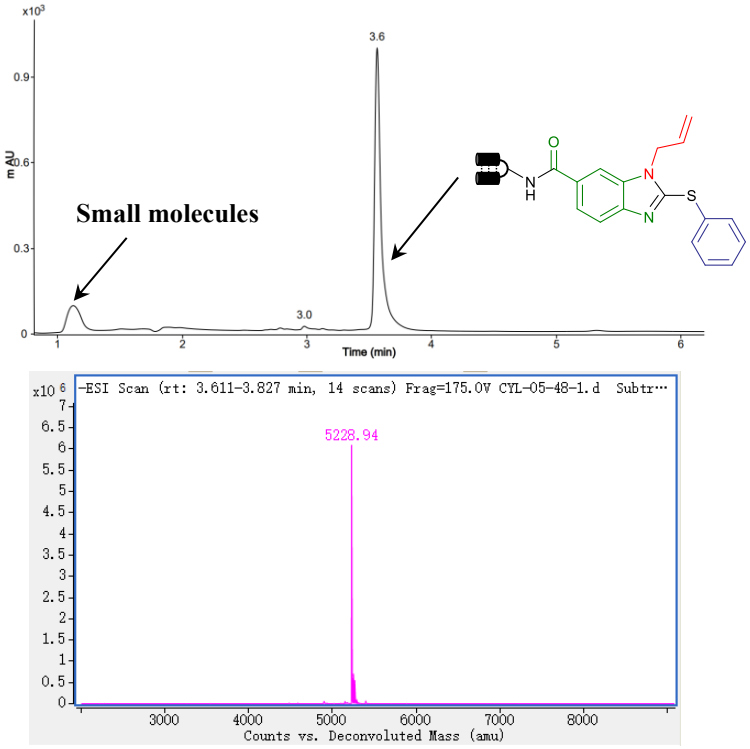
UPLC chromatogram and deconvoluted MS of **5cg**

Conversion: **>90%**. Calculated Mass: **5285 Da**; Observed Mass: **5285 Da**



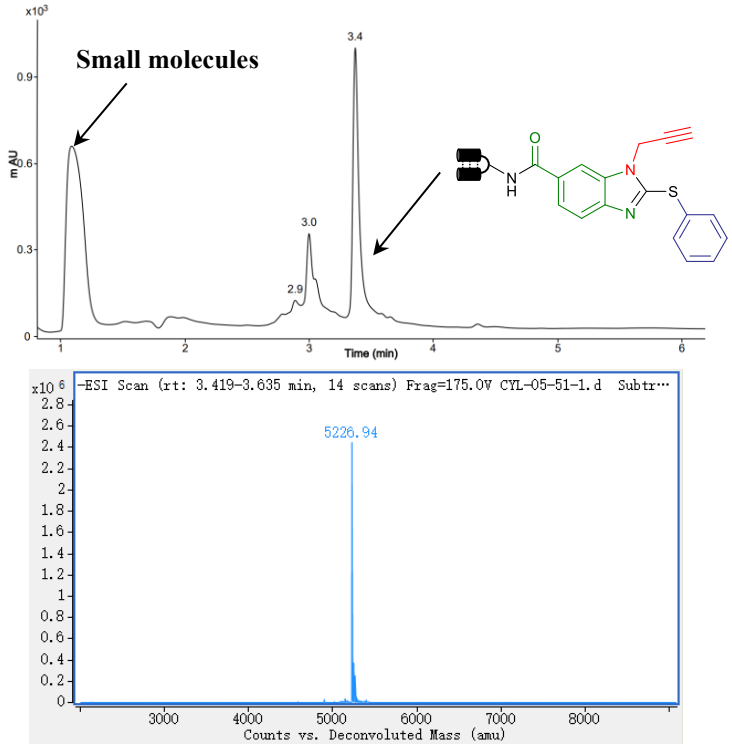
UPLC chromatogram and deconvoluted MS of **5ch**

Conversion: **>90%**. Calculated Mass: **5229 Da**; Observed Mass: **5229 Da**



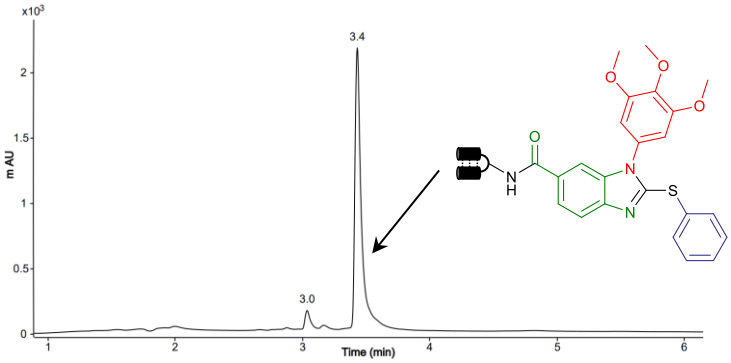
UPLC chromatogram and deconvoluted MS of **5ci**

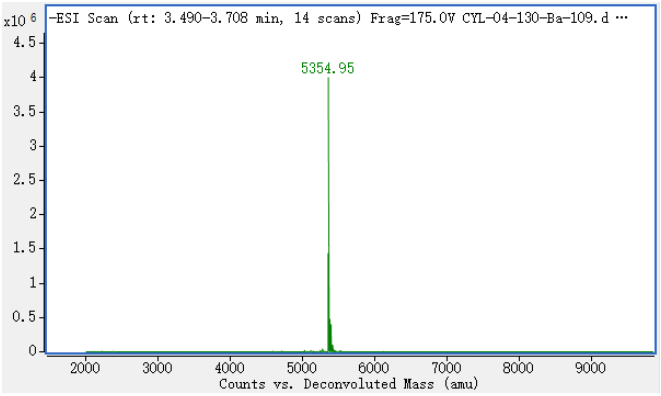
Conversion: **60%**. Calculated Mass: **5227 Da**; Observed Mass: **5227 Da**



UPLC chromatogram and deconvoluted MS of **5cj**

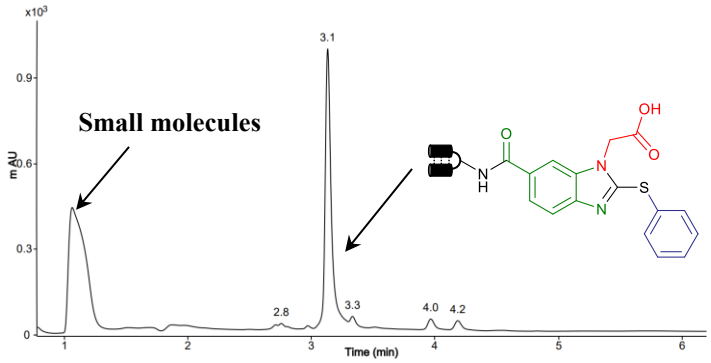
Conversion: **>90%**. Calculated Mass: **5355 Da**; Observed Mass: **5355 Da**

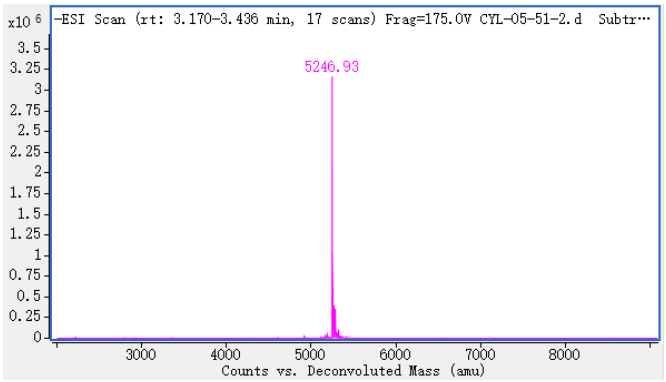




UPLC chromatogram and deconvoluted MS of **5ck**

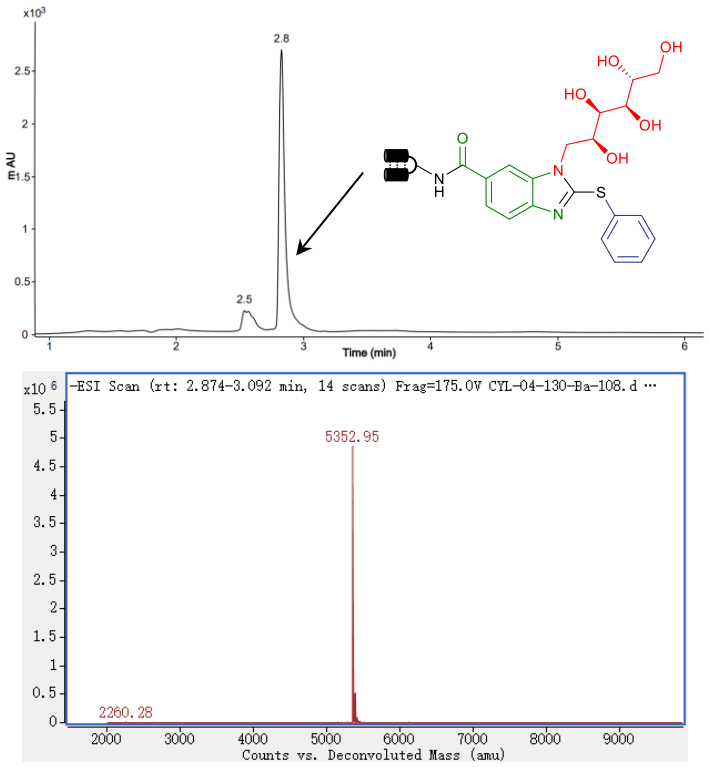
Conversion: **83%**. Calculated Mass: **5247 Da**; Observed Mass: **5247 Da**





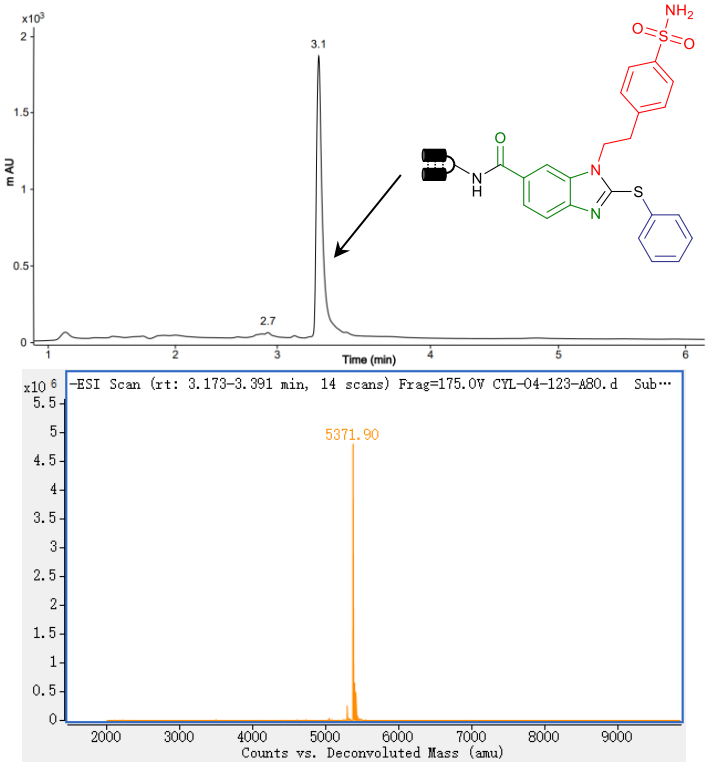
UPLC chromatogram and deconvoluted MS of **5cl**

Conversion: **87%**. Calculated Mass: **5353 Da**; Observed Mass: **5353 Da**



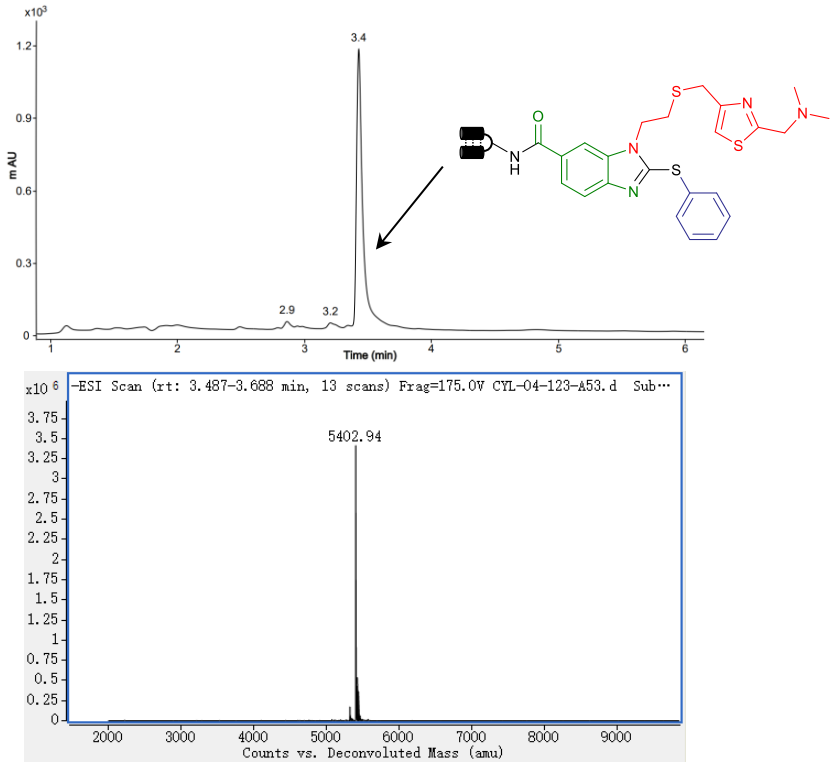
UPLC chromatogram and deconvoluted MS of **5cm**

Conversion: **>90%**. Calculated Mass: **5372 Da**; Observed Mass: **5372 Da**



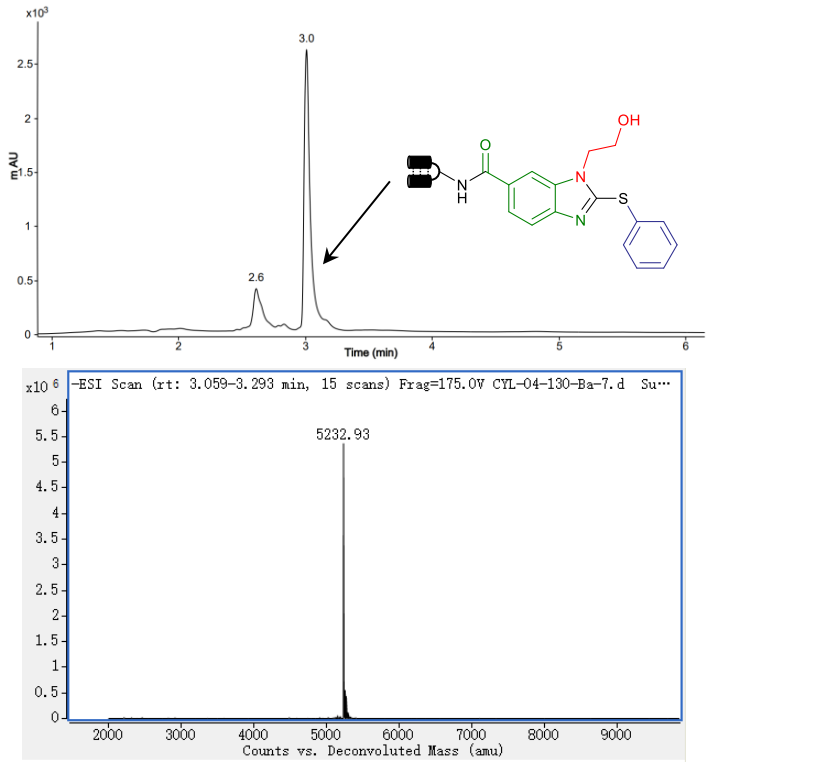
UPLC chromatogram and deconvoluted MS of **5cn**

Conversion: **>90%**. Calculated Mass: **5403 Da**; Observed Mass: **5403 Da**



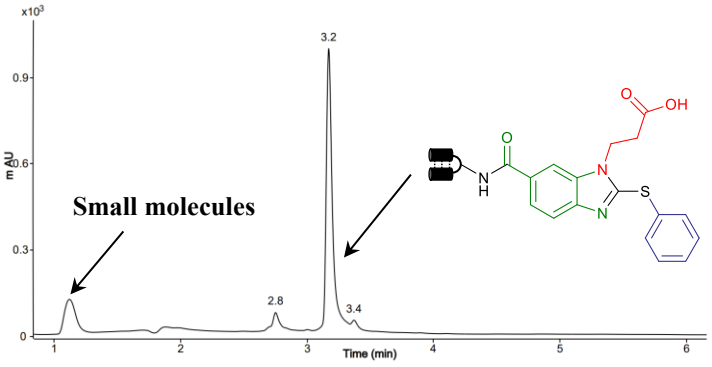
UPLC chromatogram and deconvoluted MS of **5co**

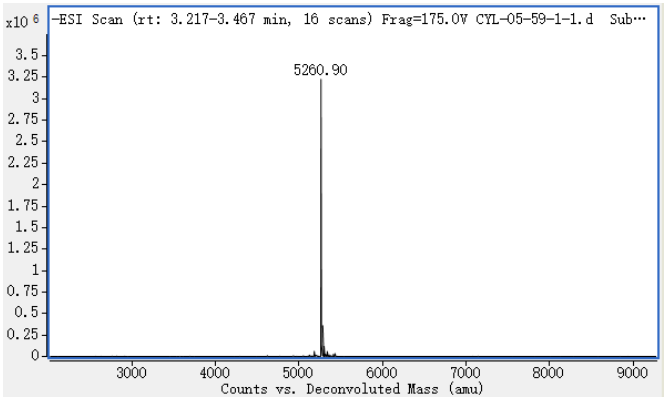
Conversion: **81%**. Calculated Mass: **5233 Da**; Observed Mass: **5233 Da**



UPLC chromatogram and deconvoluted MS of **5cp**

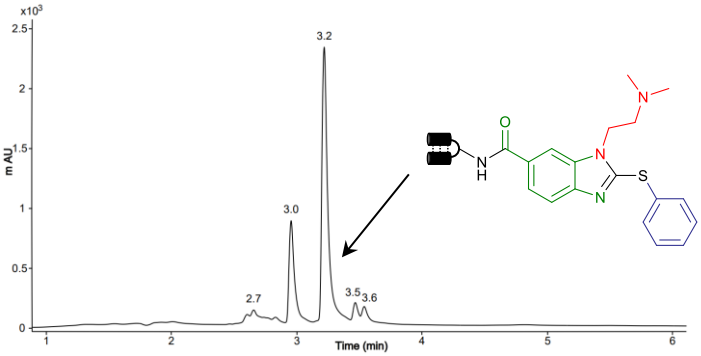
Conversion: **79%**. Calculated Mass: **5261 Da**; Observed Mass: **5261 Da**

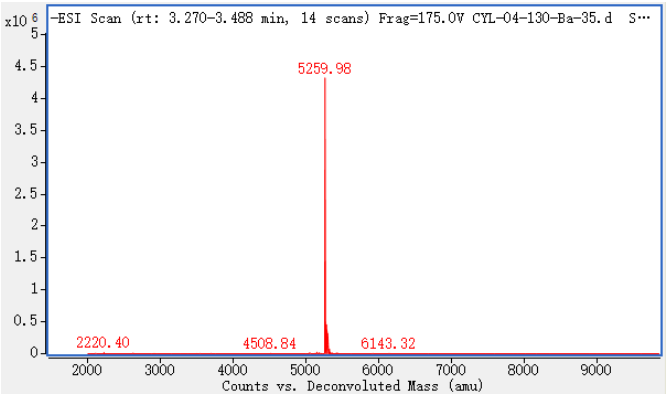




UPLC chromatogram and deconvoluted MS of **5cq**

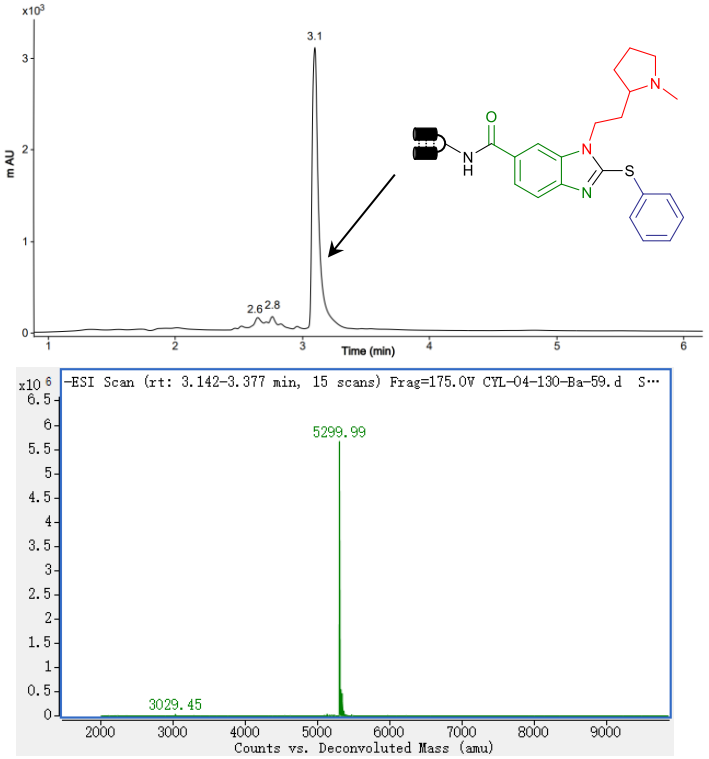
Conversion: **62%**. Calculated Mass: **5260 Da**; Observed Mass: **5260 Da**





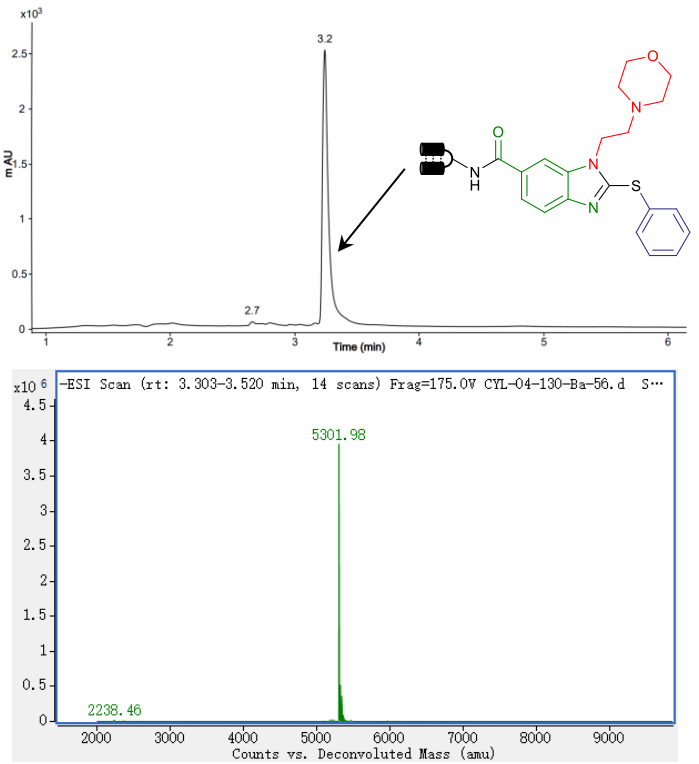
UPLC chromatogram and deconvoluted MS of **5cr**

Conversion: **>90%**. Calculated Mass: **5300 Da**; Observed Mass: **5300 Da**



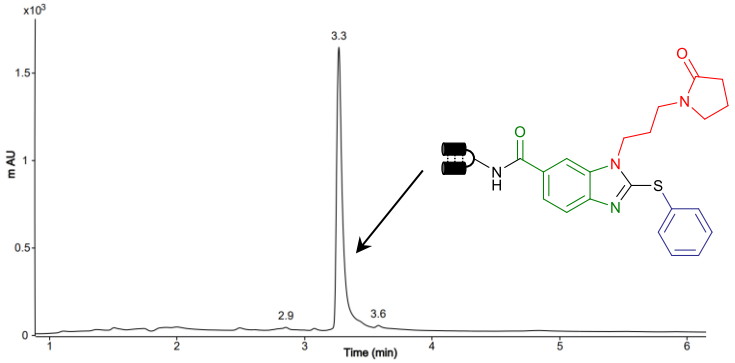
UPLC chromatogram and deconvoluted MS of **5cs**

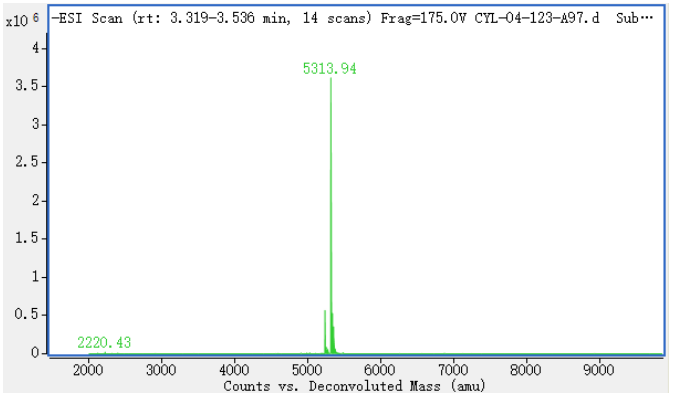
Conversion: **>90%**. Calculated Mass: **5302 Da**; Observed Mass: **5302 Da**



UPLC chromatogram and deconvoluted MS of **5ct**

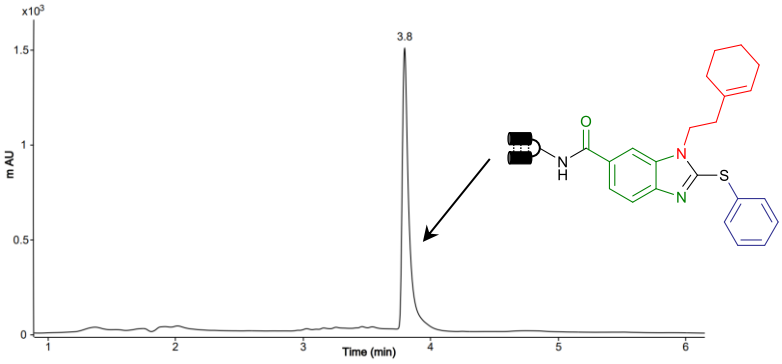
Conversion: **>90%**. Calculated Mass: **5314 Da**; Observed Mass: **5314 Da**

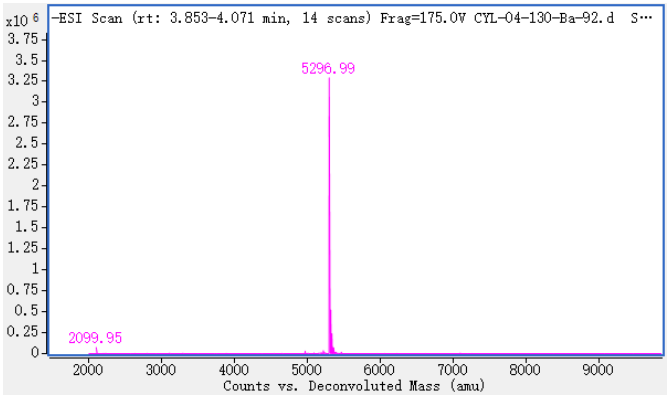




UPLC chromatogram and deconvoluted MS of **5cu**

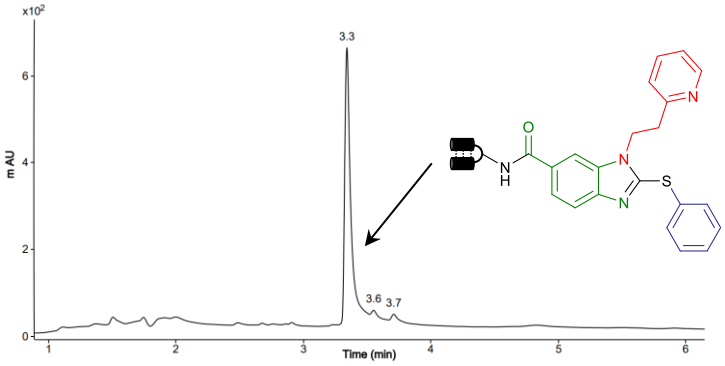
Conversion: **>90%**. Calculated Mass: **5297 Da**; Observed Mass: **5297 Da**

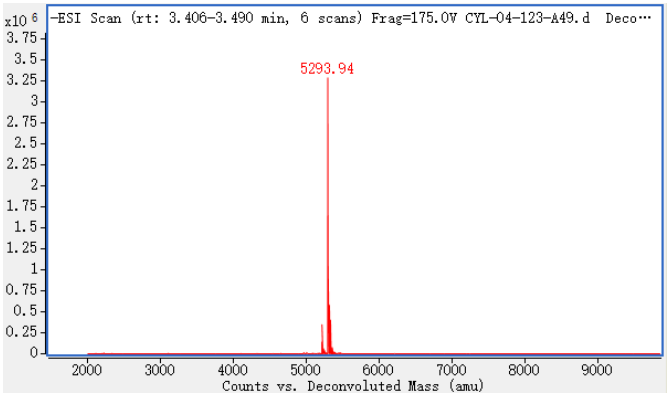




UPLC chromatogram and deconvoluted MS of **5cv**

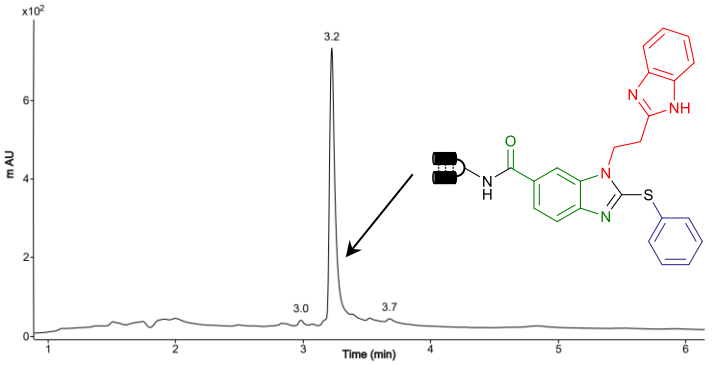
Conversion: **81%**. Calculated Mass: **5294 Da**; Observed Mass: **5294 Da**

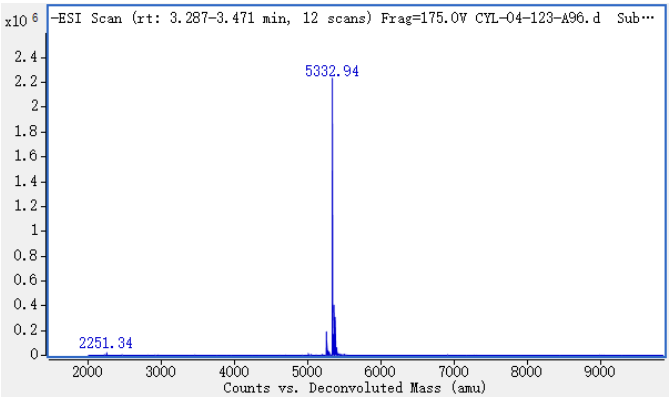




UPLC chromatogram and deconvoluted MS of **5cw**

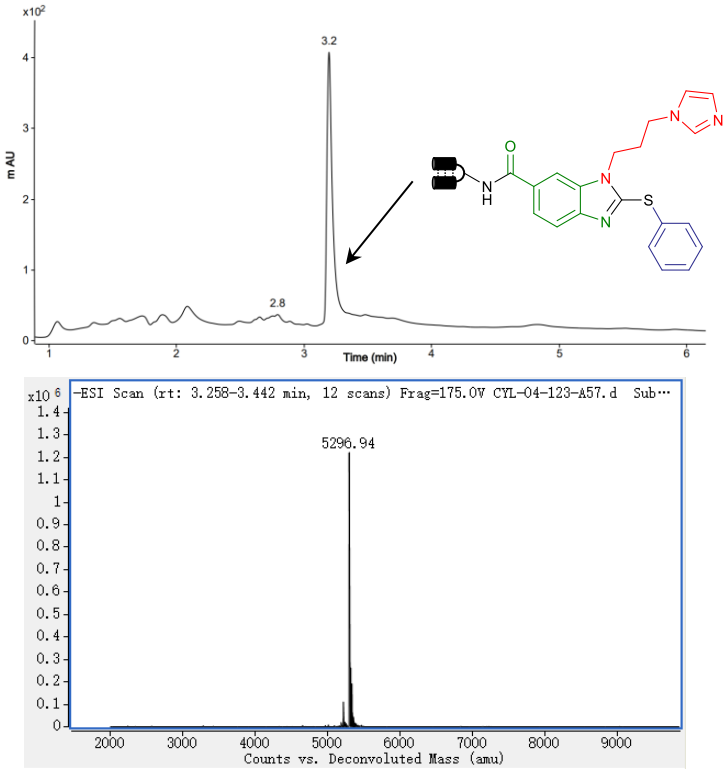
Conversion: **85%**. Calculated Mass: **5333 Da**; Observed Mass: **5333 Da**





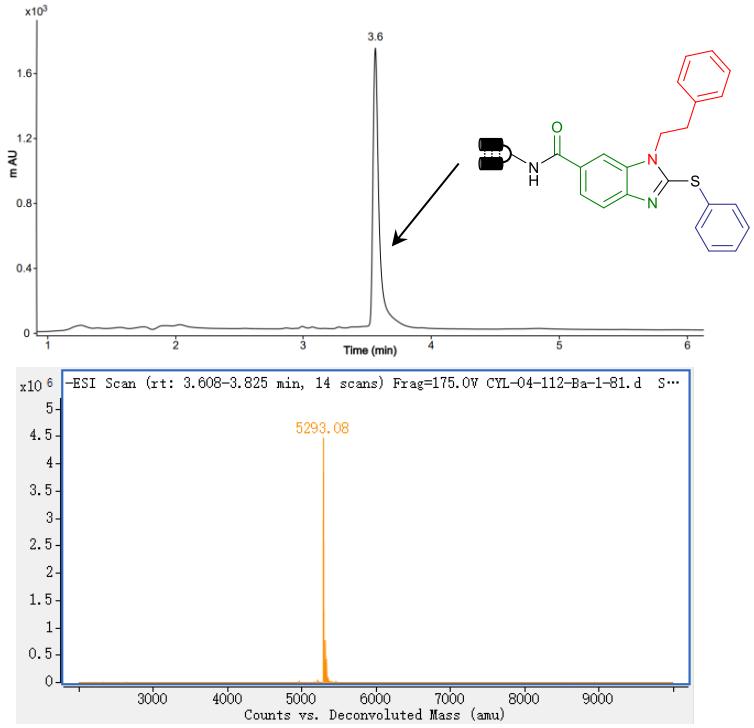
UPLC chromatogram and deconvoluted MS of **5cx**

Conversion: **82%**. Calculated Mass: **5297 Da**; Observed Mass: **5297 Da**



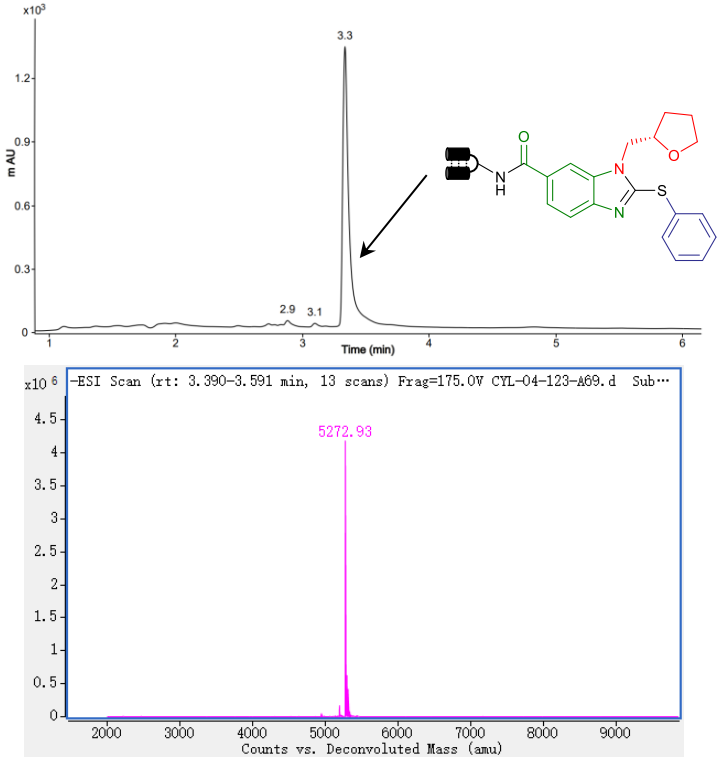
UPLC chromatogram and deconvoluted MS of **5a**

Conversion: **>90%**. Calculated Mass: **5293 Da**; Observed Mass: **5293 Da**



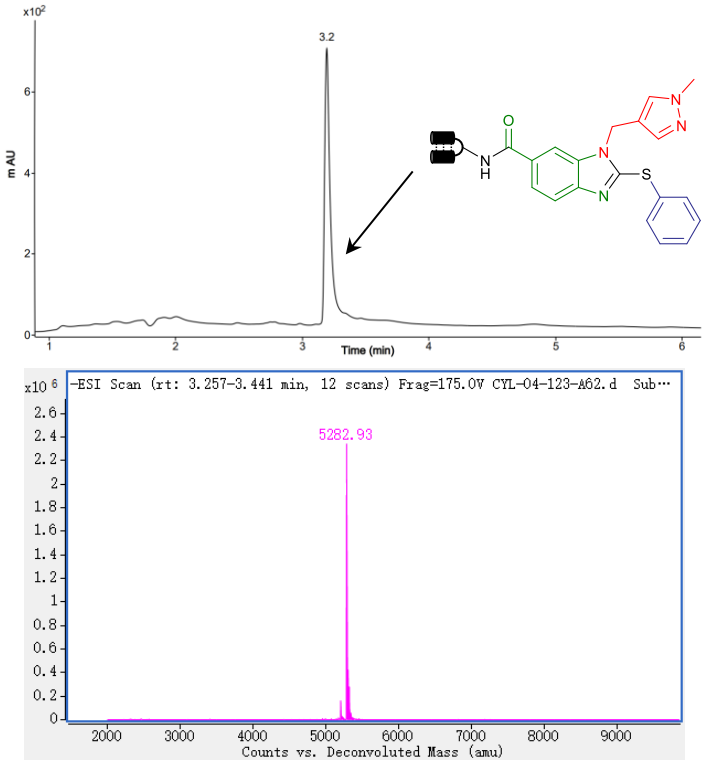
UPLC chromatogram and deconvoluted MS of **5cy**

Conversion: **>90%**. Calculated Mass: **5273 Da**; Observed Mass: **5273 Da**



UPLC chromatogram and deconvoluted MS of **5cz**

Conversion: **>90%**. Calculated Mass: **5283 Da**; Observed Mass: **5283 Da**



UPLC chromatogram and deconvoluted MS of **5da**

Conversion: **>90%**. Calculated Mass: **5323 Da**; Observed Mass: **5323 Da**