Supporting Information

Vinyl azide as a synthon for DNA-compatible divergent transformations into N-heterocycles

Jie Sun, [a†] Qigui Nie, [a†] Xianfu Fang,[a] Zhiwei He,[a] Gong Zhang,[ab] Yangfeng Li,*[ab] and Yizhou Li*[abcd]

[a] Chongqing Key Laboratory of Natural Product Synthesis and Drug Research, Innovative Drug Research Center, School of Pharmaceutical Sciences, Chongqing University, 401331 Chongqing, P. R. China

[b] Chemical Biology Research Center, School of Pharmaceutical Sciences, Chongqing University, 401331 Chongqing, P. R. China

[c] Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, 400044 Chongqing, P. R. China

[d] Beijing National Laboratory for Molecular Sciences, 100190 Beijing, P. R. China

* E-mail: yfli3@cqu.edu.cn.

* E-mail: yizhouli@cqu.edu.cn.

† J. Sun and Q. Nie contributed equally to this work.

Table of Contents

1. Abbreviations

BBS: boric acids buffer

DIPEA: *N*, *N*-diisopropylethylamine

DMA: *N*, *N*-dimethylacetamide

DMF: dimethylformamide

DMSO: dimethyl sulfoxide

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

HP: headpiece

HP-P: headpiece primer

HPLC: high performance liquid chromatography

MW: molecular weight

NMR: nuclear magnetic resonance

PAGE: polyacrylamide gel electrophoresis

PE: petroleum ether

TBHP: *tert*-butyl hydroperoxide

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/TGACTC CCGACCGAAGGTTG-3') and the sequence of code 1: 5'-/5Phos/TGTAGG CGAACAG-3'. All the DNA sequences were written in 5'- to 3'- orientation unless otherwise noted. Chemicals and reagents were purchased from several commercial suppliers including J&K Scientific, Bidepharm, Adamas, and Sigma-Aldrich, and were generally used from aliquots dissolved in DMA, DMF, 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), 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. All the gel images were captured by a Bio-Rad Chemidoc™ image system.

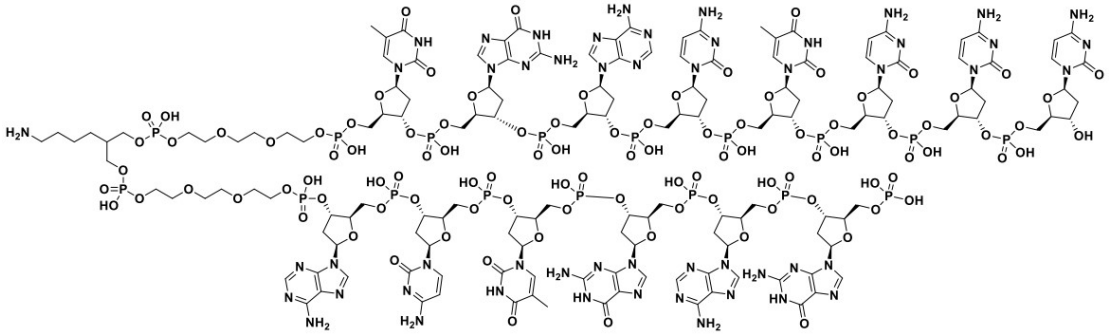


Figure S1. Structure of **HP** (5'-/5Phos/GAGTCA/iSp9/iUniAmM/iSp9/TGACTC CC-3'), MW = 4937.

2.2. General Methods for DNA Analysis (UPLC-MS Method)

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 reaction, an aliquot of the reaction mixture was diluted with water to make the sample approximately 1 μM, and 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 ^{o}C). The effluent was detected by UV absorbance at 260 nm and analysed on Agilent TOF (6230 B) in negative ion mode.

LCMS 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 was performed on BioConfirm 10.0 software (Agilent, v10.0).

2.3. General Methods for DNA Conjugates Purification

General procedure for ethanol precipitation. To an on-DNA reaction mixture was added 10% (V/V) 5 M NaCl solution and 3 times volume of absolute cold ethanol. Alternatively, to a DNA ligation 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 ^{o}C for 2 h and then was centrifuged at 13500 rpm for 30 minutes at 4 ^{o}C by using Eppendorf 5424R centrifuge. The supernatant was discarded and the pellet was rinsed with 200 μL cold 75% ethanol. After centrifuging at 13500 rpm for 10 minutes at 4 ^{o}C, the supernatant was discarded again and the DNA pellet was dried by Speedvac (CV200, JM company, Beijing, China), which was equipped with cryotrap (JM86, JM company, Beijing, China). The recovered sample was dissolved in the appropriate solvent for subsequent experiments.

General method for HPLC purification. Preparative reversed-phase high-pressure liquid chromatography (RP-HPLC) for the DNA conjugate was performed on Waters 1575EF Series with the column (Eclipse-XDB C18, 5 μM, 9.4 × 250 mm) using eluent A (100 mM TEAA in H_2O) and eluent B (100 mM TEAA in 80% ACN) with gradient: 10% B (0 - 1 min), 10% to 30% B (1 - 11min), 30% to 100% B (11 - 11.1 min), 100% B (11.1 - 12 min), 100% to 10% B (12 - 12.1 min), 10% B (12.1 - 16 min). 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 ^{o}C for overnight before performing gel analysis. The crude product was purified by ethanol precipitation and used for the next step of synthesis without further purification.

2.5. General Procedure for Polyacrylamide Gel

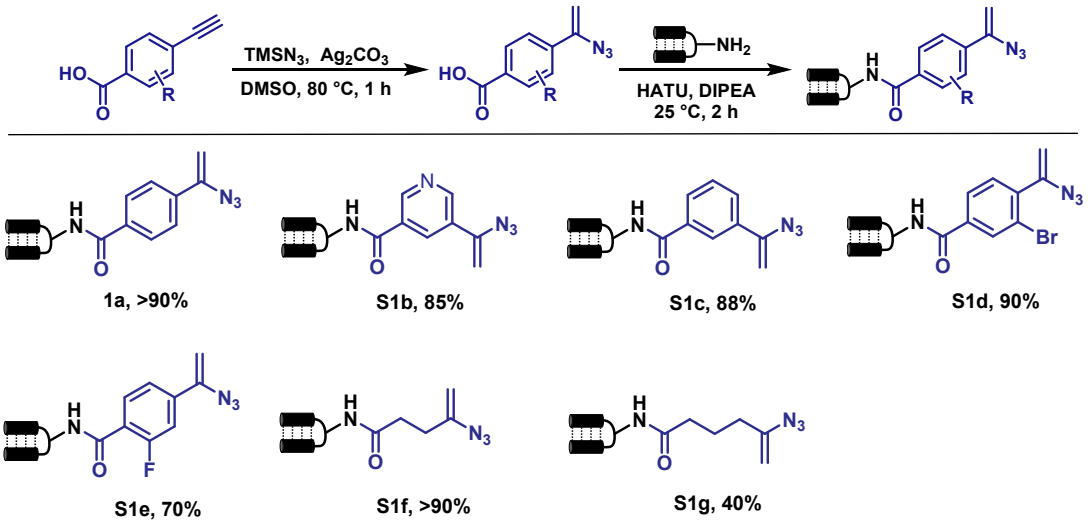
Ligation reaction was monitored by gel electrophoresis with 20% urea polyacrylamide gel in 1× TBE buffer (89 mM Tris-Borate, 2 mM EDTA, pH 8.3) system referenced by a 20 bp DNA ladder (Takara, Japan). First, the DNA samples were denatured at 95 ^{o}C for 10 min and mixed with loading buffer. Then, 10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

2.6. General Methods for Monitoring Reaction and Characterizing Small Molecules

Reactions were monitored by TLC and general staining reagents were used to analyze TLC intuitively. All the new compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, and HRMS. 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. Preparation of DNA-Conjugated Vinyl Azides

Scheme S1. Substrate scope of on-DNA vinyl azides



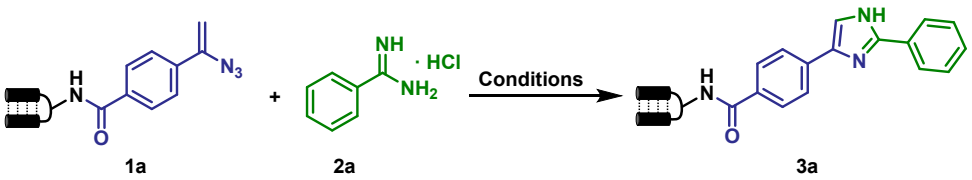
Standard Condition[1]: To the solution of 4-acetylene benzoic acid (50 mg, 0.34 mmol) were added $TMSN_3$ (90 µL, 0.68 mmol), Ag_2CO_3 (18 mg, 0.068 mmol), H_2O (10 µL, 0.68 mmol), and DMSO (2 mL) at 80 °C. The mixture was then stirred for 1 h. Upon completion, the mixture was cooled down and filtered to give a light-yellow solution, which was then concentrated. The residue was used for the next step without further purification.

HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 0.5 mM solution. 4-Vinyl azide benzoic acid (20 µL, 200 mM in DMA, 200 equiv), HATU (10 µL, 400 mM in DMA, 200 equiv), and DIPEA (10 µL, 400 mM in DMA, 200 equiv) were mixed by vortex and allowed to pre-activate for 10 minutes at 25 °C, and then the mixture was transferred to **HP** solution (40 µL, 20 nmol). The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 2 h. After ethanol precipitation, the reaction was analysed by UPLC-MS. The separated and collected conjugates were vacuum-dried overnight, redissolved in H_2O for subsequent experiments. Deconvoluted molecular mass: calculated: 5109 Da; observed: 5109 Da.

4. Experimental Conditions for on-DNA Reactions

4.1. On-DNA 2,4-Disubstituted Imidazoles Formation

Table S1. Reaction optimization for DNA-conjugated **3a** synthesis



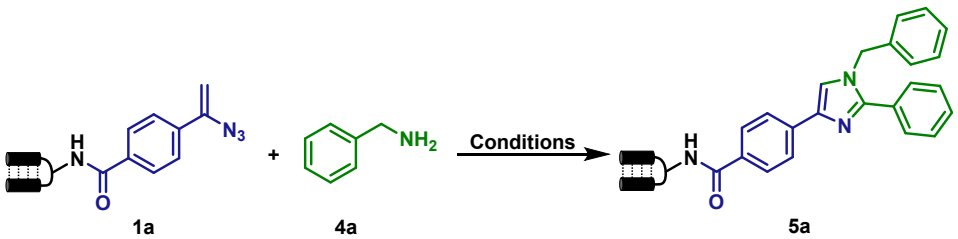
1a 2a 3a

Entry	Base (mM)	Solvent	Temperature (°C)	Conversion (%)
1	-	MeCN	60	0
2	K_2CO_3 (26)	MeCN	60	30
3	K_2CO_3 (26)	DMF	60	26
4	Cs_2CO_3 (26)	MeCN	60	23
5	DBU (26)	MeCN	100	>90
6	DBU (26)	DMSO	80	70

Standard Condition[2]: To a solution of DNA-conjugated **1a** (2 μL, 100 μM in H_2O, 200 pmol, 1 equiv) was added **2a** (4 μL, 200 mM in DMA, 800 nmol, 4000 equiv), DBU (4 μL, 200 mM in DMA, 800 nmol, 4000 equiv) and MeCN (20 μL). The reaction mixture was heated at 100 °C for 2 h. The product was obtained by ethanol precipitation and analysed by UPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5183 Da; observed: 5183 Da. Unless otherwise noted, on-DNA 2,4-disubstituted imidazoles described in the supporting information were synthesized under this standard condition.

4.2. On-DNA 1,2,4-Trisubstituted Imidazoles Formation

Table S2. Reaction optimization for DNA-conjugated **5a** synthesis



1a 4a 5a

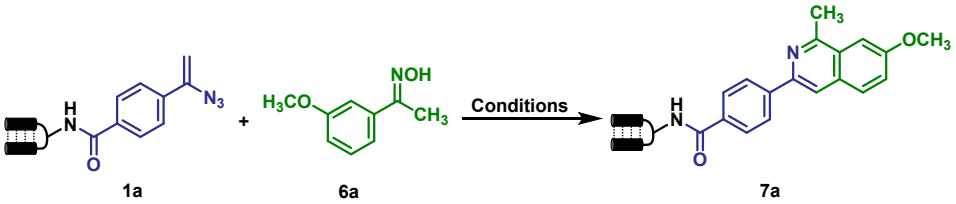
Entry	**4a** (mM)	Catalyst (mM)	Additive (mM)	Solvent	Temperature (°C)	Time (h)	Conversion (%)[a]
1	20	I_2 (2)	TBHP (25)	DMA	40	6	10
2	20	I_2 (2)	TBHP (25)	DMSO	60	6	10
3	20	I_2 (2)	TBHP (25)	DMF	60	6	3
4	20	I_2 (2)	-	DMA	60	6	28
5[b]	20	I_2 (2)	-	DMA	80	2	75
6[b]	20	I_2 (2)	-	DMA	90	1	70
7[b]	20	I_2 (2)	-	DMA	100	1	78
8[b]	20	I_2 (2)	-	DMA	100	1.5	82
9[b]	20	I_2 (2)	-	DMA	100	2	75

[a]Conversions determined by UPLC-MS. [b]**4a** and I_2 were premixed at 80 °C for 30 minutes.

Standard Condition[3]: **4a** (5 μL, 200 mM in DMA, 1000 nmol, 5000 equiv) and I_2 (5 μL, 8 mM in DMA, 40 nmol, 200 equiv) were premixed at 80 °C for 30 minutes, then DNA-conjugated **1a** (2 μL, 100 μM in H_2O, 200 pmol, 1 equiv) was added to the premix, 10 μL DMA, 100 °C, 1.5 h . The product was obtained by ethanol precipitation and analysed by UPLC-MS (Conversion: 82%). Deconvoluted molecular mass: calculated: 5274 Da; observed: 5274 Da. Unless otherwise noted, on-DNA 1,2,4-trisubstituted imidazoles described in the supporting information were synthesized under this standard condition.

4.3. On-DNA Isoquinolines Formation

Table S3. Reaction optimization for DNA-conjugated **7a** synthesis



Entry	**6a** (mM)	$Pd(OAc)_2$ (mM)	Solvent	Temperature (°C)	Time (h)	Conversion (%)[a]
1	20	20	DMSO	80	8	46

2	20	20	DMA	80	8	40
3	40	10	DMSO	80	8	45
4	80	30	DMSO	80	12	43
5[b]	80	30	DMSO	80	6	75
6[b]	20	20	DMSO	90	3	58

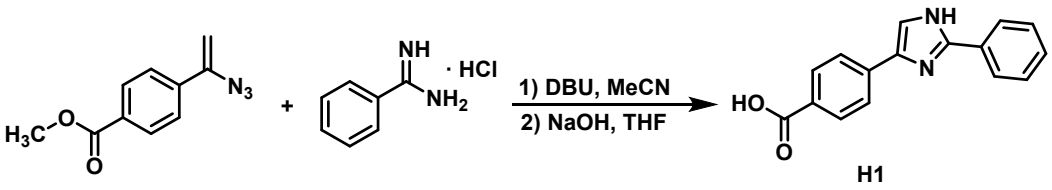
[a]Conversions determined by UPLC-MS. [b]**6a** and $Pd(OAc)_2$ were premixed at 80 ^oC for 30 minutes.

Standard Condition[4]: **6a** (8 μL, 200 mM in DMA, 1600 nmol, 8000 equiv) and $Pd(OAc)_2$ (6 μL, 100 mM in DMA, 600 nmol, 3000 equiv) were premixed at 80 ^oC for 30 minutes, then DNA-conjugated **1a** (2 μL, 100 μM in H_2O, 200 pmol, 1 equiv) was added to the premix, 2 μL BBS buffer (pH 9.4), 3 μL DMSO, 80 ^oC, 6 h. The product was obtained by ethanol precipitation and characterized by UPLC-MS. The product was obtained by ethanol precipitation and analysed by UPLC-MS (Conversion: 75%). Deconvoluted molecular mass: calculated: 5213 Da; observed: 5213 Da. Unless otherwise noted, on-DNA isoquinolines described in the supporting information were synthesized under this standard condition.

5. Structural Confirmation

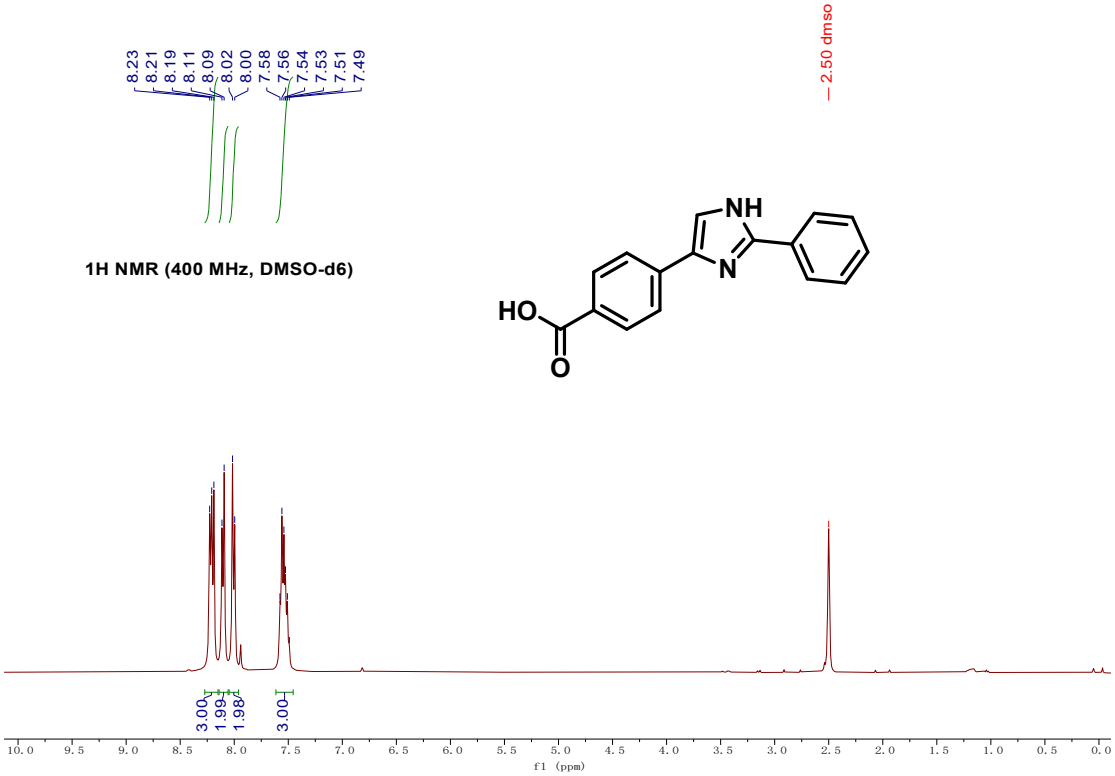
5.1. Synthetic Scheme of 2,4-Disubstituted Imidazoles

Scheme S2. Synthesis of authentic **H1**

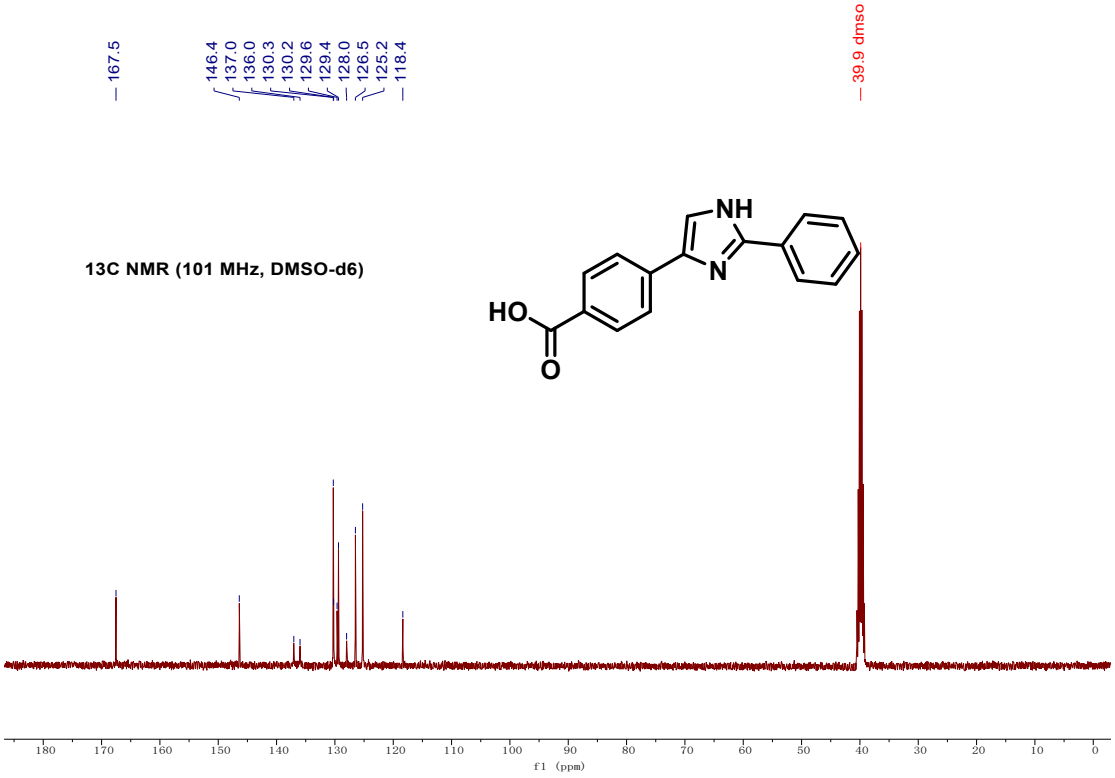


4-(2-phenyl-1*H*-imidazol-4-yl) benzoic acid (H1). Benzimidamide hydrochloride (578 mg, 3.7 mmol), 1-(1-azidovinyl)-4-methyl benzoate (500 mg, 2.5 mmol), and DBU (0.55 mL, 3.7 mmol) were taken in a 10 mL reaction tube; to the mixture, 3.0 mL of acetonitrile was added. The reaction tube was placed in a preheated oil bath at 80 °C for 8 h (progress of the reaction was monitored by TLC). Then, the reaction mixture was allowed to attain room temperature; 10 mL of brine solution was extracted with ethyl acetate (3×15 mL) and dried with anhydrous Na_2SO_4. After the removal of solvent, the crude mixture was used in the next step without further purification. Then, a 20 mL tube was charged with previous product (250 mg, 0.94 mmol), NaOH (75 mg, 1.88 mmol), H_2O (0.5 mL), and THF (2 mL). The mixture was stirred at 90 °C for 3 h. Upon completion, water was added to the solution, and the mixture was extracted with ethyl acetate three times. The organic phase was combined and dried with Na_2SO_4. The solvent was removed in vacuo to give a residue, which was purified by flash column chromatography on silica gel using dichloromethane/methanol (20:1) as eluent, affording **H1** as a white amorphous solid (215 mg, 34%).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 – 8.15 (m, 3H), 8.10 (d, J = 8.0 Hz, 2H), 8.01 (d, J = 8.0 Hz, 2H), 7.54 (dt, J = 12.9, 7.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.5, 146.4, 137.0, 136.0, 130.3, 130.2, 129.6, 129.4, 128.0, 126.5, 125.3, 118.4. HRMS (ESI) calculated for $C_{16}H_{12}N_2O_2$ $[M + H]^+$, 265.0972; observed, 265.0968.

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

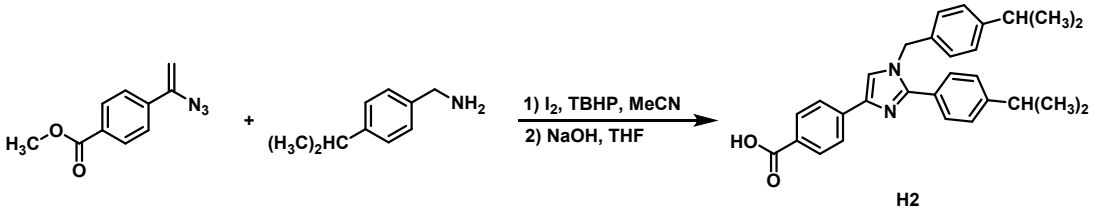


^{13}C-NMR of **H1**



5.2. Synthetic Scheme of 1,2,4-Trisubstituted Imidazoles

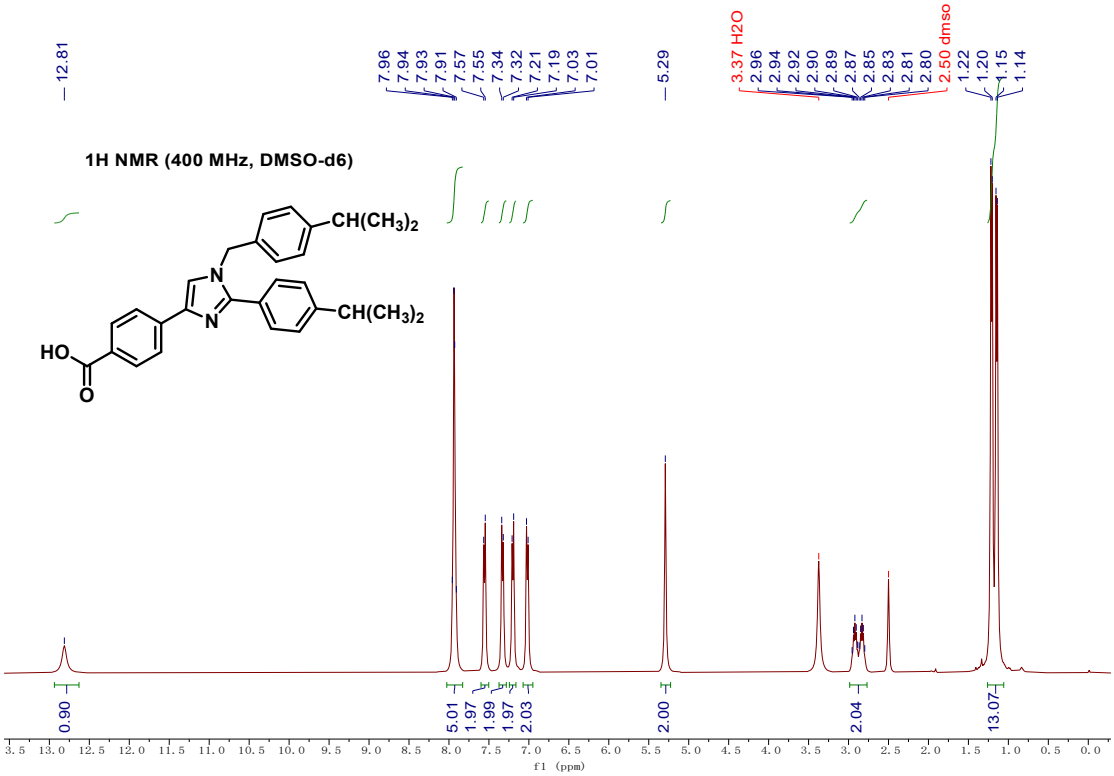
Scheme S3. Synthesis of authentic **H2**



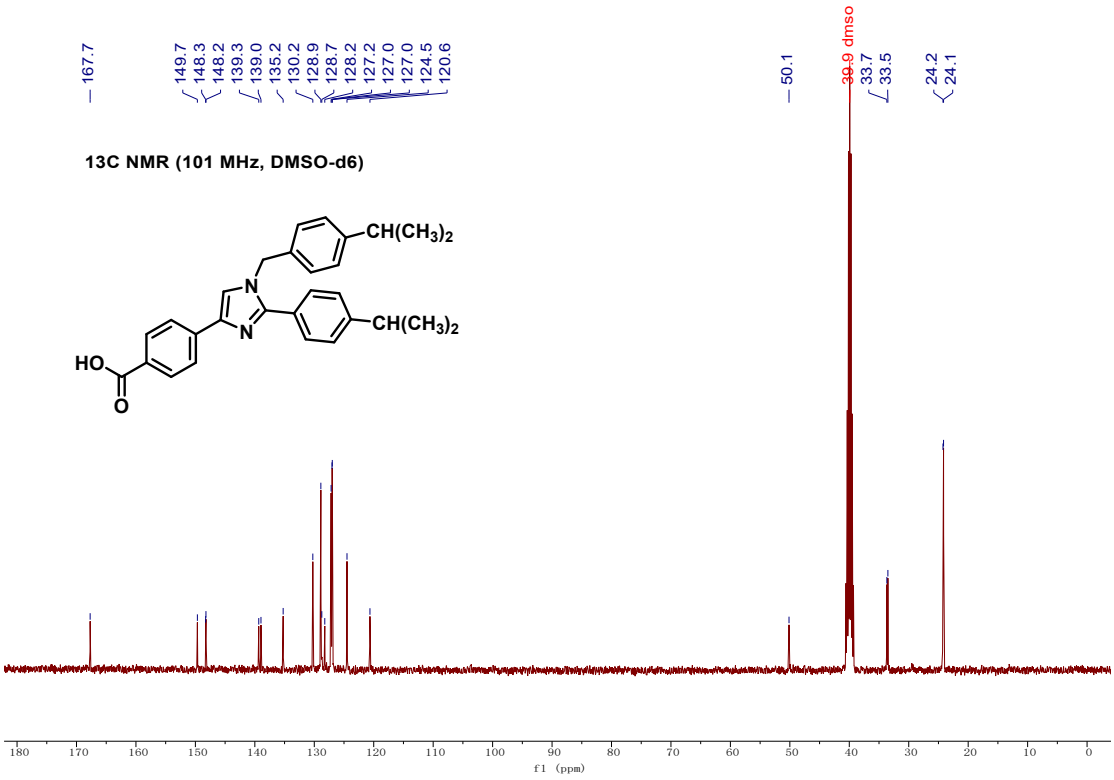
H2

4-(1-(4-isopropylbenzyl)-2-(4-isopropylphenyl)-1*H*-imidazol-4-yl) benzoic acid (H2). The 1-(1-azidovinyl)-4-methyl benzoate (0.5 mmol), phenylmethanamine (1.5 mmol), I_2 (0.025 mmol) and TBHP (1.5 mmol) were mixed in DMA (2 mL) and this mixture was at 100 ^{o}C for 10 h. The reaction mixture was cooled down to room temperature and then extracted with ethyl acetate (3×15 mL). The combined organic phase was dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the crude product was used in the next step without further purification. Then, a 20 mL tube was charged with previous product (120 mg, 0.43 mmol), NaOH (12.8 mg, 0.53 mmol), H_2O (0.5 mL), and THF (0.5 mL). The mixture was stirred at 90 ^{o}C for 3 hours. Upon completion, water was added to the solution, and the mixture was extracted with ethyl acetate three times. The organic phase was combined and dried with Na_2SO_4. The solvent was removed in vacuo to give a residue, which was purified by flash column chromatography on silica gel using dichloromethane/methanol (20:1) as eluent, affording **H2** as a yellow amorphous solid (217 mg, 85%).^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 1H), 7.93 (d, J = 4.1 Hz, 5H), 7.56 (d, J = 7.8 Hz, 2H), 7.33 (d, J = 7.8 Hz, 2H), 7.20 (d, J = 7.7 Hz, 2H), 7.02 (d, J = 7.9 Hz, 2H), 5.29 (s, 2H), 2.88 (dp, J = 35.5, 6.9 Hz, 2H), 1.18 (dd, J = 25.5, 6.9 Hz, 13H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7, 149.7, 148.3, 148.2, 139.34, 139.0, 135.2, 130.3, 128.9, 128.7, 128.2, 127.2, 127.0, 127.0, 124.5, 120.6, 50.1, 33.7, 33.5, 24.2, 24.1. HRMS (ESI) calculated for $C_{29}H_{30}N_2O_2$ $[M + H]^+$, 439.2380; observed, 439.2370.

^{1}H-NMR of **H2**

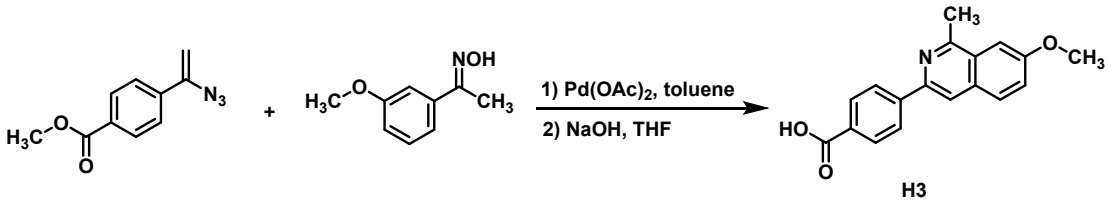


^{13}C-NMR of **H2**



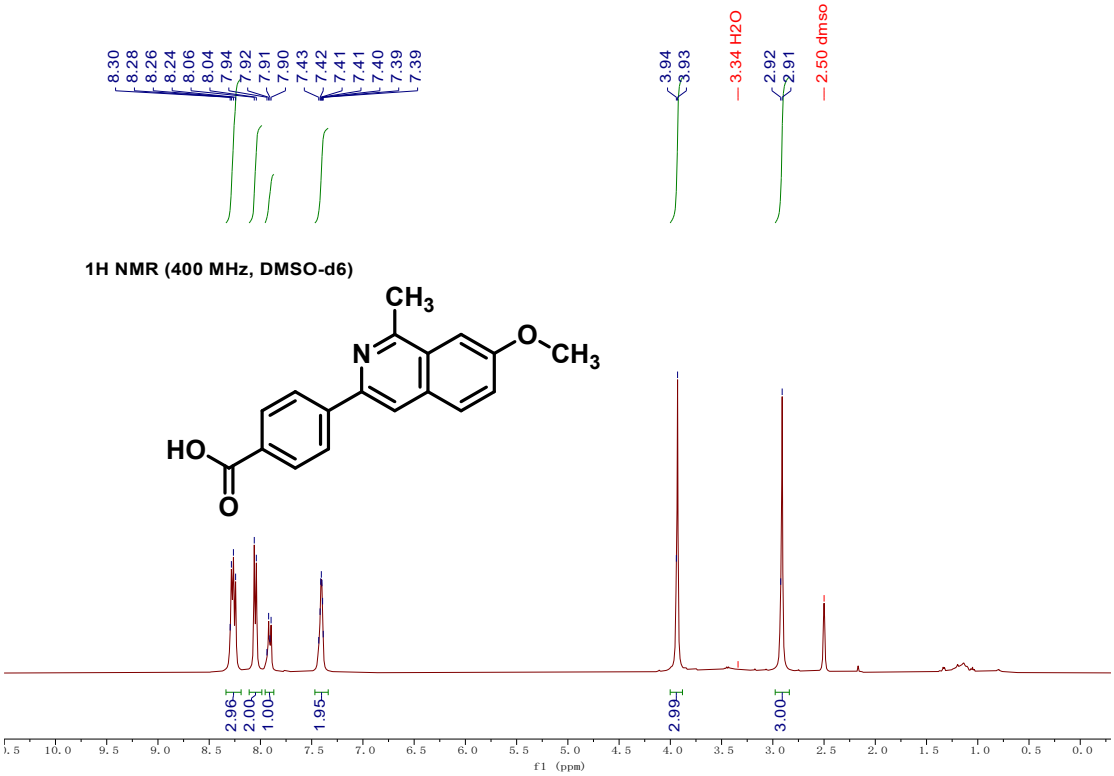
5.3. Synthetic Scheme of Isoquinolines

Scheme S4. Synthesis of authentic **H3**

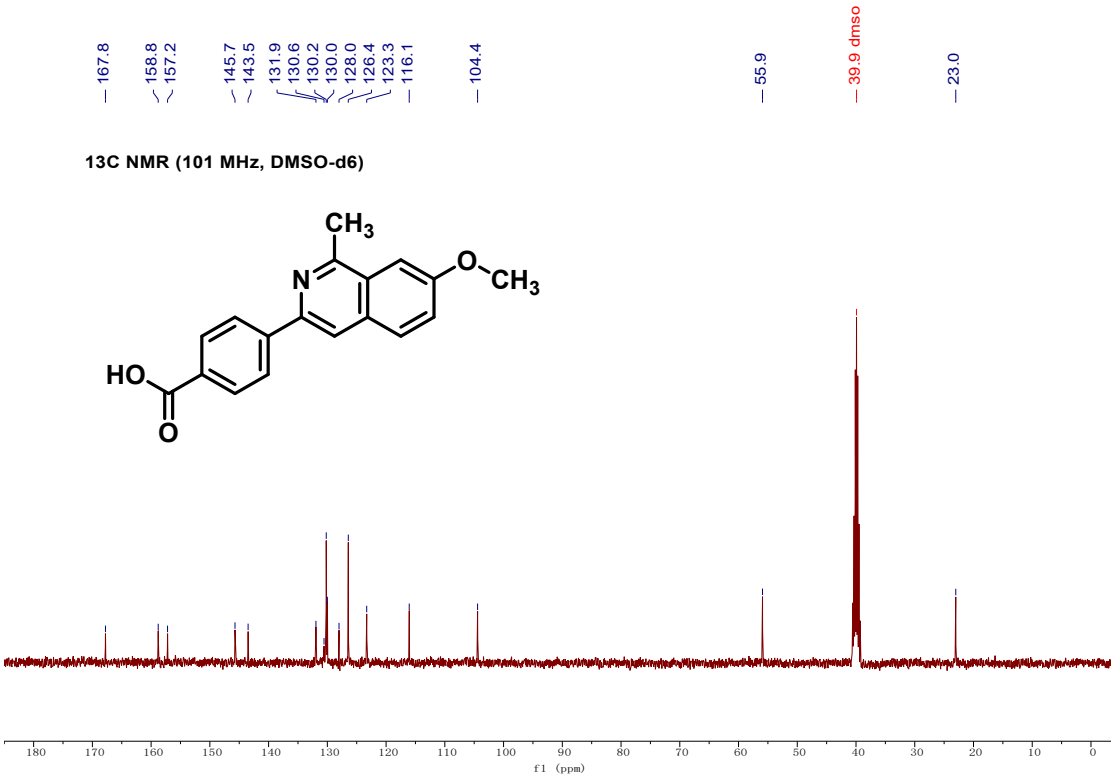


4-(7-methoxy-1-methylisoquinolin-3-yl)benzoic acid (H3). Oximes (495 mg, 3 mmol), 1-(1-azidovinyl)-4-methyl benzoate (500 mg, 2.5 mmol), $Pd(OAc)_2$ (56 mg, 0.25 mmol), and toluene (2 mL) were placed in a 10 mL screw-capped tube. The reaction vessel was closed with the cap, and the reaction mixture was stirred at 90 ^oC for 4 h. The crude product was cooled to room temperature and concentrated under vacuum to give a residue, which was used in the next step without further purification. Then, a 20 mL tube was charged with previous product (454 mg, 1.48 mmol), NaOH (120 mg, 3.0 mmol), H_2O (1 mL), and THF (4 mL). The mixture was stirred at 90 ^oC for 2 hours. Upon completion, water was added to the solution, and the mixture was extracted with ethyl acetate three times. The organic phase was combined and dried with Na_2SO_4. The solvent was removed in vacuo to give a residue, which was purified by flash column chromatography on silica gel using dichloromethane/methanol (20:1) as eluent, affording **H3** as a yellow amorphous solid (186 mg, 25%).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (q, J = 8.5, 6.7 Hz, 3H), 8.05 (d, *J* = 8.1 Hz, 2H), 7.92 (dd, *J* = 10.5, 5.6 Hz, 1H), 7.47 – 7.34 (m, 2H), 3.94 (d, *J* = 4.6 Hz, 3H), 2.92 (d, *J* = 5.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.8, 158.8, 157.2, 145.7, 143.5, 131.9, 130.6, 130.2, 130.0, 128.0, 126.4, 123.3, 116.1, 104.4, 55.9, 23.0. HRMS (ESI) calculated for $C_{18}H_{15}NO_3$ $[M + H]^+$, 294.1125; observed, 294.1121.

^{1}H-NMR of **H3**

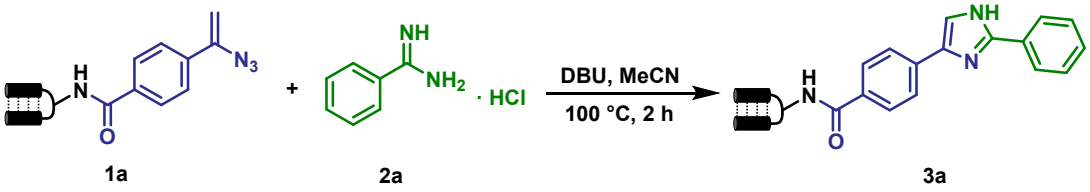


^{13}C-NMR of **H3**

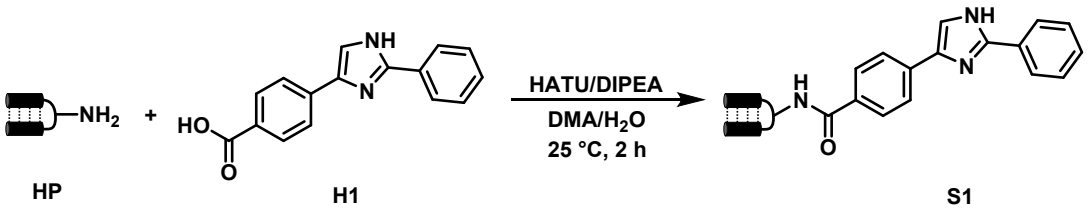


6. Co-Injection Experiment

Route A



Route B



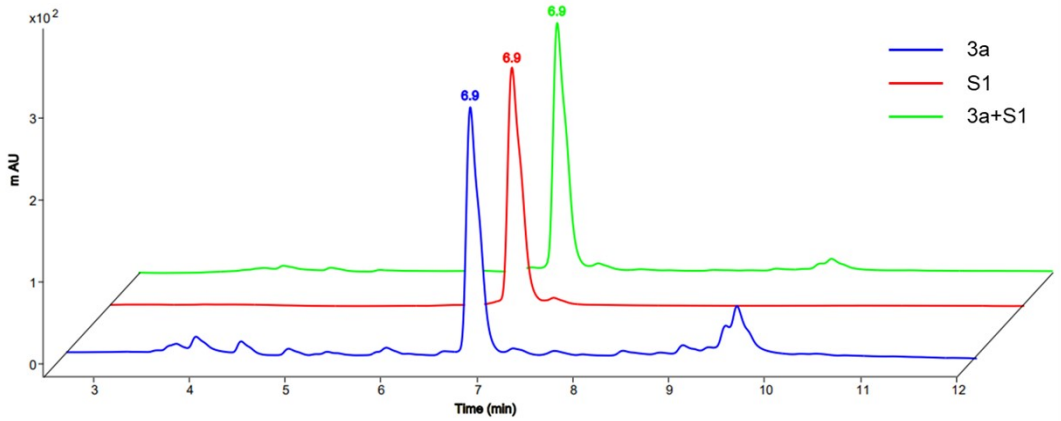
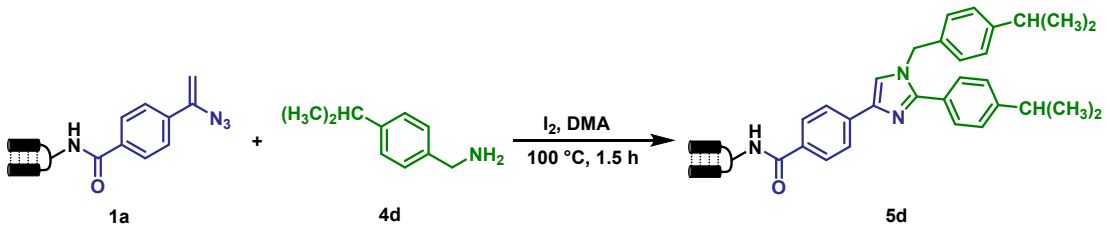
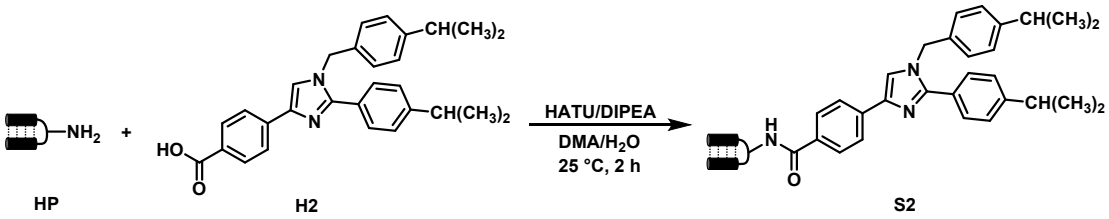


Figure S2. Co-injection experiment of **3a** and **S1** from two independent synthetic routes. HPLC chromatography showed that the peak from the co-injection (curve in green) had the same retention time with the other two peaks (**3a** from route A, curve in blue; **S1** from route B, curve in red)

Route A



Route B



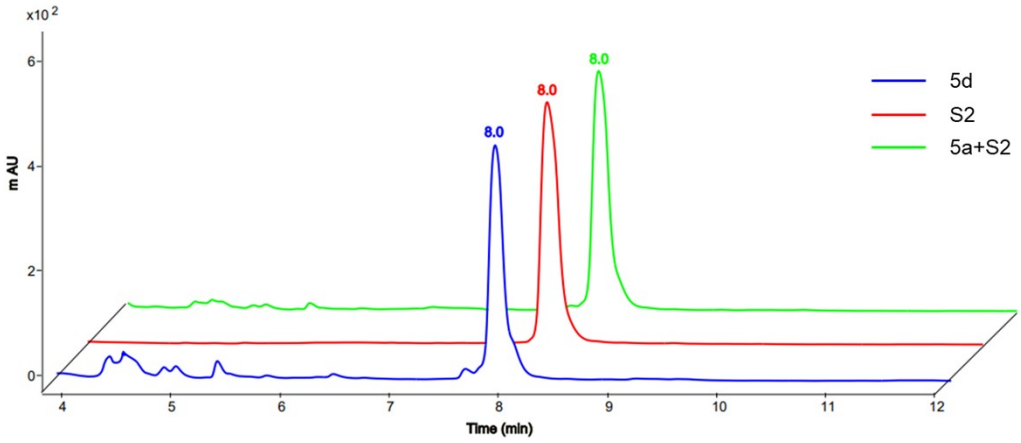
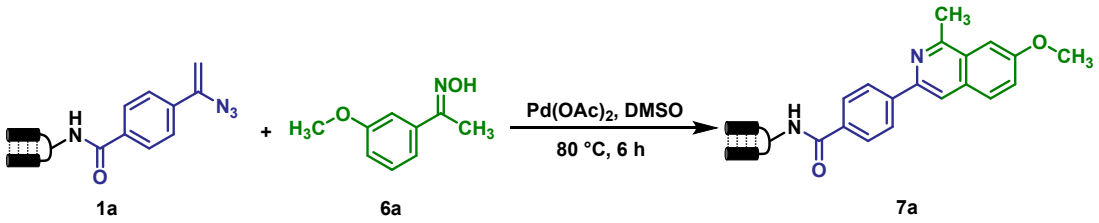
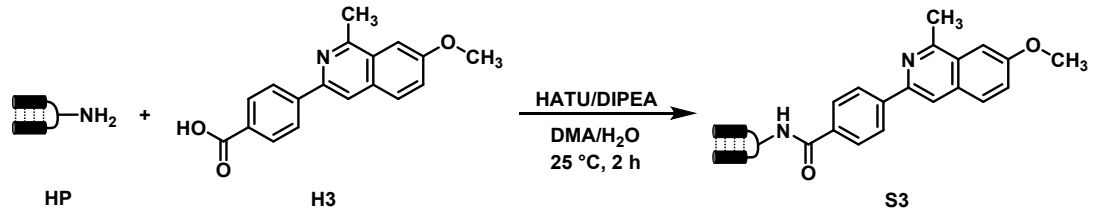


Figure S3. Co-injection experiment of **5d** and **S2** from two independent synthetic routes. HPLC chromatography showed that the peak from the co-injection (curve in green) had the same retention time with the other two peaks (**5d** from route A, curve in blue; **S2** from route B, curve in red)

Route A



Route B



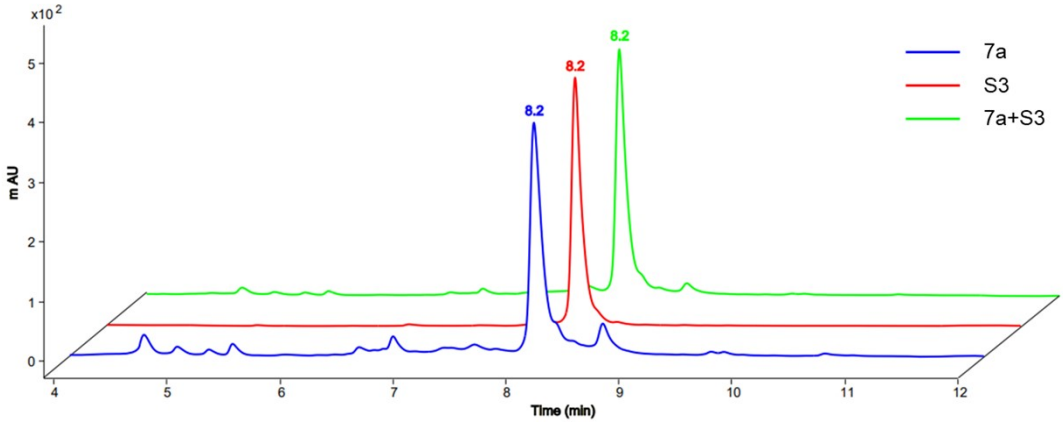


Figure S4. Co-injection experiment of **7a** and **S3** from two independent synthetic routes. HPLC chromatography showed that the peak from the co-injection (curve in green) had the same retention time with the other two peaks (**7a** from route A, curve in blue; **S3** from route B, curve in red)

7. Scale-Up Reaction

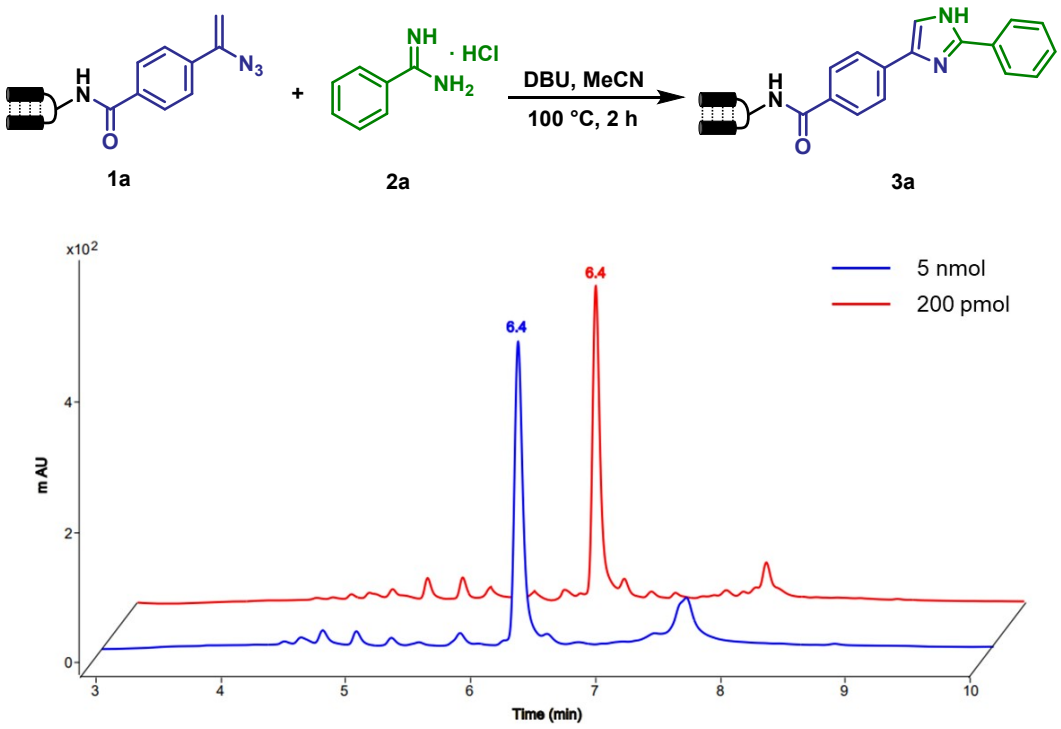


Figure S5. UPLC chromatogram of **3a** at 200 pmol and 5 nmol scale.

To the solution of DNA-conjugated substrate **1a** (2 μL, 2.5 mM in H_2O, 5 nmol, 1 equiv) was added **2a** (4 μL, 200 mM in DMA, 800 nmol, 160 equiv), DBU (4 μL, 200 mM in DMA, 800 nmol, 160 equiv) and 20 μL MeCN. The reaction mixture was heated at 100 °C for 2 h. The product was obtained by ethanol precipitation and analysed by UPLC-MS (Conversion: 90%). Deconvoluted molecular mass: calculated: 5183 Da; observed: 5183 Da.

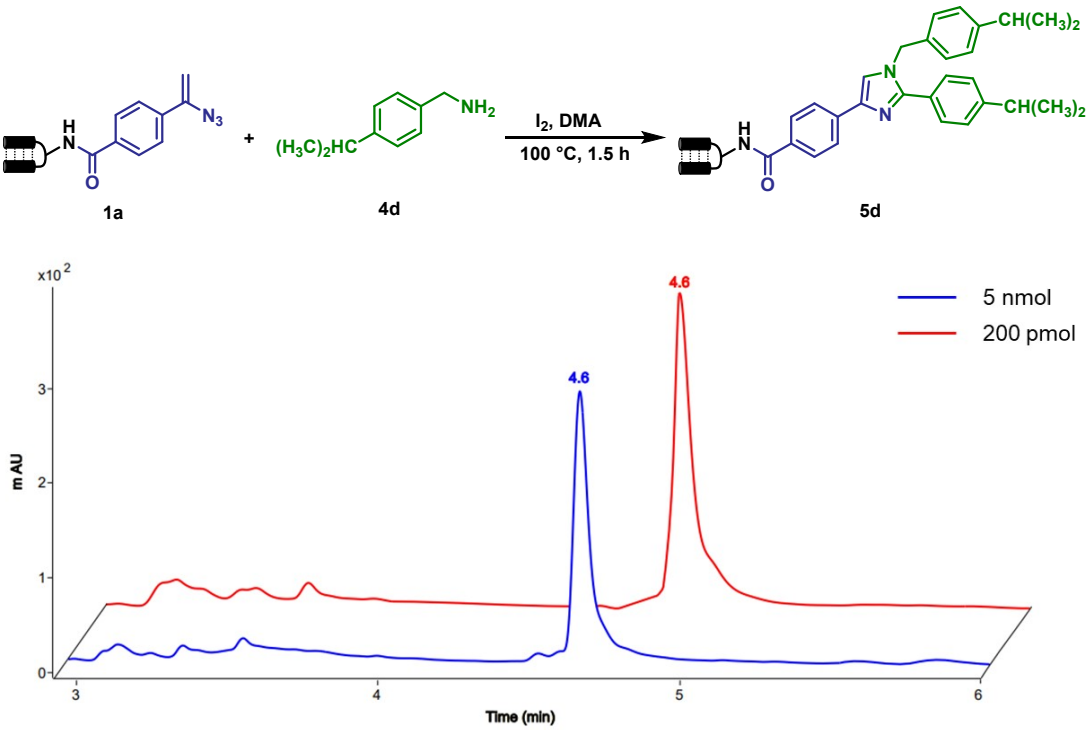


Figure S6. UPLC chromatogram of **5d** at 200 pmol and 5 nmol scale, respectively.

4a (5 μL, 200 mM in DMA, 1000 nmol, 200 equiv) and I_2 (5 μL, 8 mM in DMA, 40 nmol, 8 equiv) were premixed at 80 °C for 30 minutes, then DNA-conjugated **1a** (2 μL, 2.5 mM in H_2O, 5 nmol, 1 equiv) was added to the premix, 10 μL DMA, 100 °C, 1.5 h . The product was obtained by ethanol precipitation and analysed by UPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5357 Da; observed: 5357 Da.

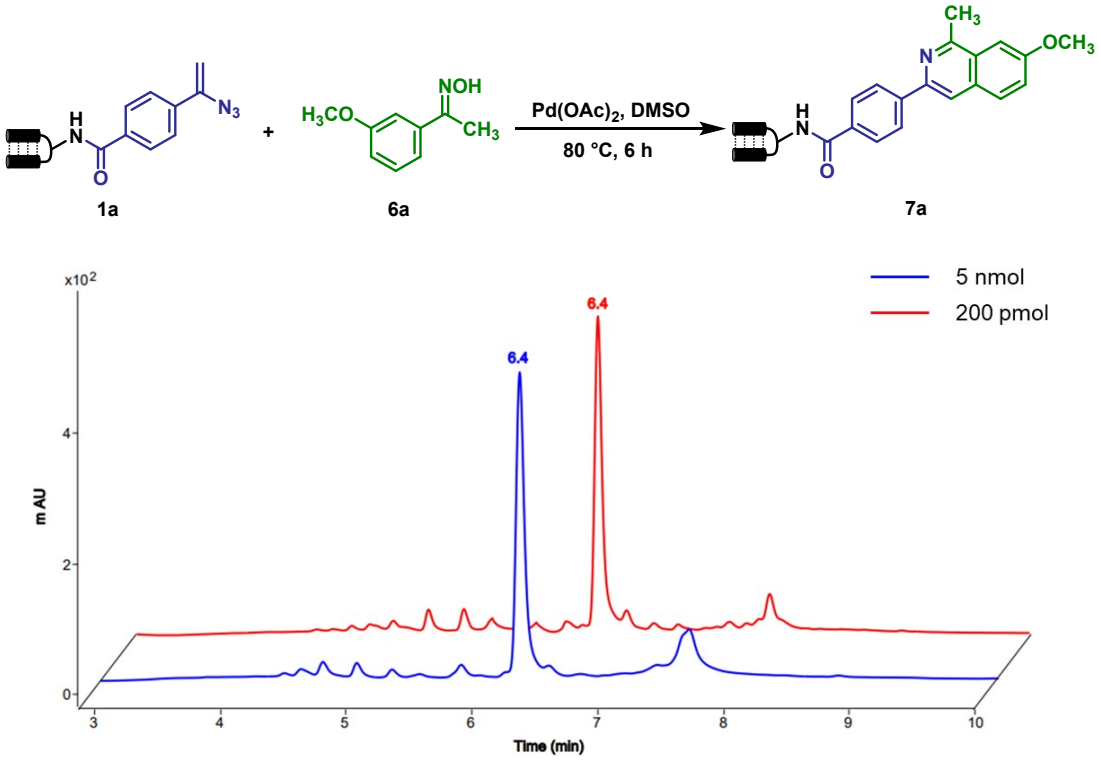
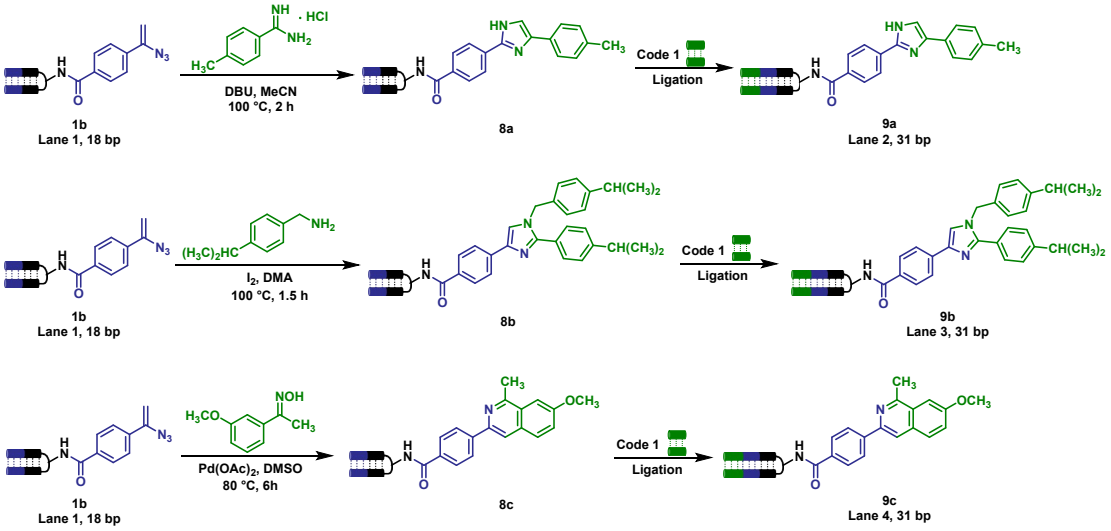


Figure S7. UPLC chromatogram of **7a** at 200 pmol and 5 nmol scale, respectively.

6a (8 µL, 200 mM in DMA, 1600 nmol, 320 equiv) and $Pd(OAc)_2$ (6 µL, 100 mM in DMA, 600 nmol, 120 equiv) were premixed at 80 °C for 30 minutes, then DNA-conjugated **1a** (2 µL, 2.5 mM in H_2O, 5 nmol, 1 equiv) was added to the premix, 2 µL BBS buffer (pH 9.4), 3 µL DMSO, 80 °C, 6 h. The product was obtained by ethanol precipitation and analysed by UPLC-MS (Conversion: 75%). Deconvoluted molecular mass: calculated: 5213 Da; observed: 5213 Da.

8. Enzymatic Ligation



Headpiece-primer (**HP-P**, 20 nmol**)** was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 1 mM solution. 4-Vinyl azide benzoic acid (10 μL, 200 mM in DMA, 100 equiv), HATU (5 μL, 400 mM in DMA, 200 equiv), and DIPEA (5 μL, 400 mM in DMA, 200 equiv) were mixed by vortex and allowed to preactivated for 10 minutes at 25 °C, and then the mixture was transferred to **HP-P** solution in a 0.6 mL tube and mixed. The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 2 hours. After ethanol precipitation, the resulting pellet was vacuum-dried and dissolved in nuclease-free water (200 μL). The reaction was analysed by UPLC-MS.

To the solution of **1b** (10 μL, 500 μM in H_2O, 5 nmol, 1 equiv) reacted with **2b/4d/6a** respectively according to the standard conditions. The product was obtained by ethanol precipitation and analysed by UPLC-MS.

8a/8b/8c (10 μL, 1 nmol), code 1 (1 μL, 1.2 nmol, 1.2 equiv) and 10× ligation buffer (2 μL) was added into 0.6 mL tube, mixed by vortex, then T4 DNA ligase (1 μL, 350 units/μL) was added and mixed gently. The reaction mixture was vortexed, centrifuged, and incubated at 20 °C for 3 h. After ligation confirmation by UPLC-MS analysis, the reaction system was denatured by incubating at 95 °C for 10 min, and the ligation product was isolated by ethanol precipitation. The resulting pellets were vacuum-dried and dissolved in nuclease-free water.

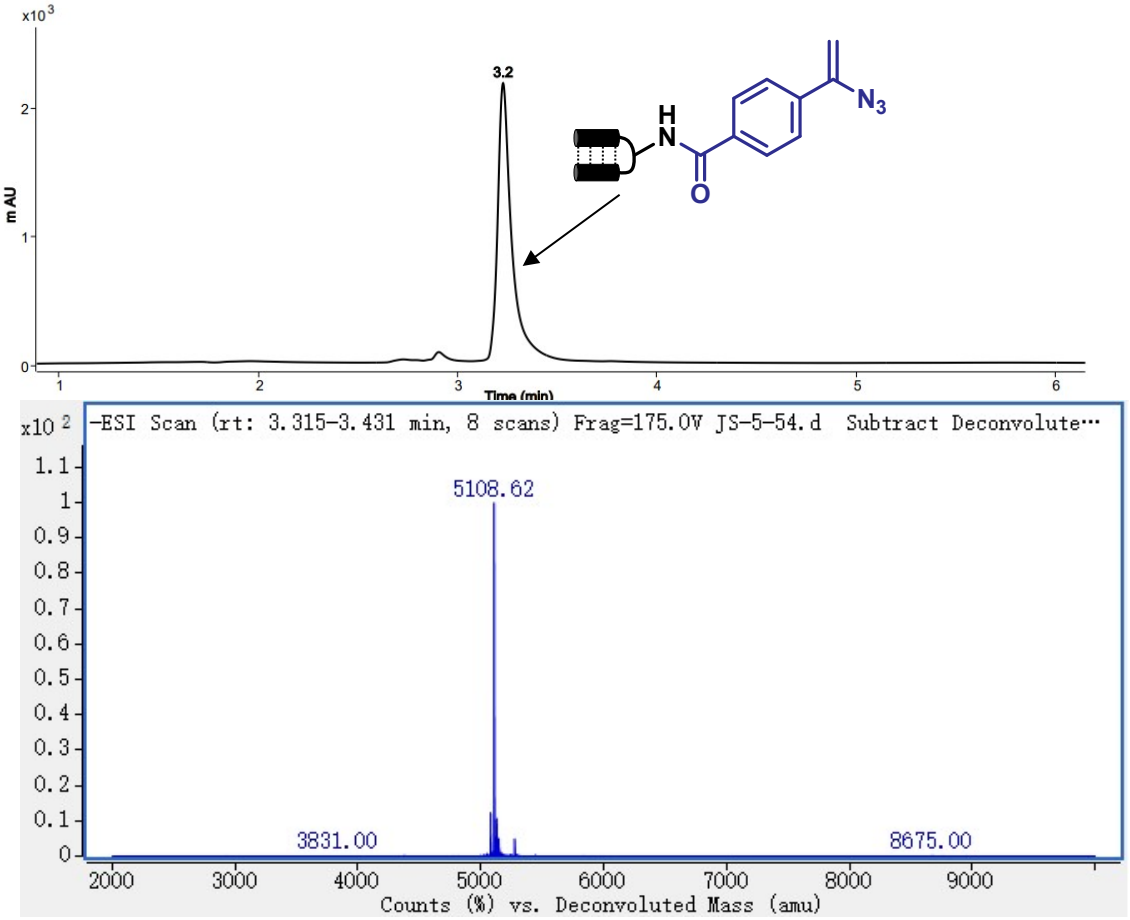
9. UPLC Chromatogram and Deconvoluted MS

9.1. Substrate Scope of on-DNA Conjugated Vinyl Azide

UPLC chromatogram and deconvoluted MS of **1a**

Conversion: >90%

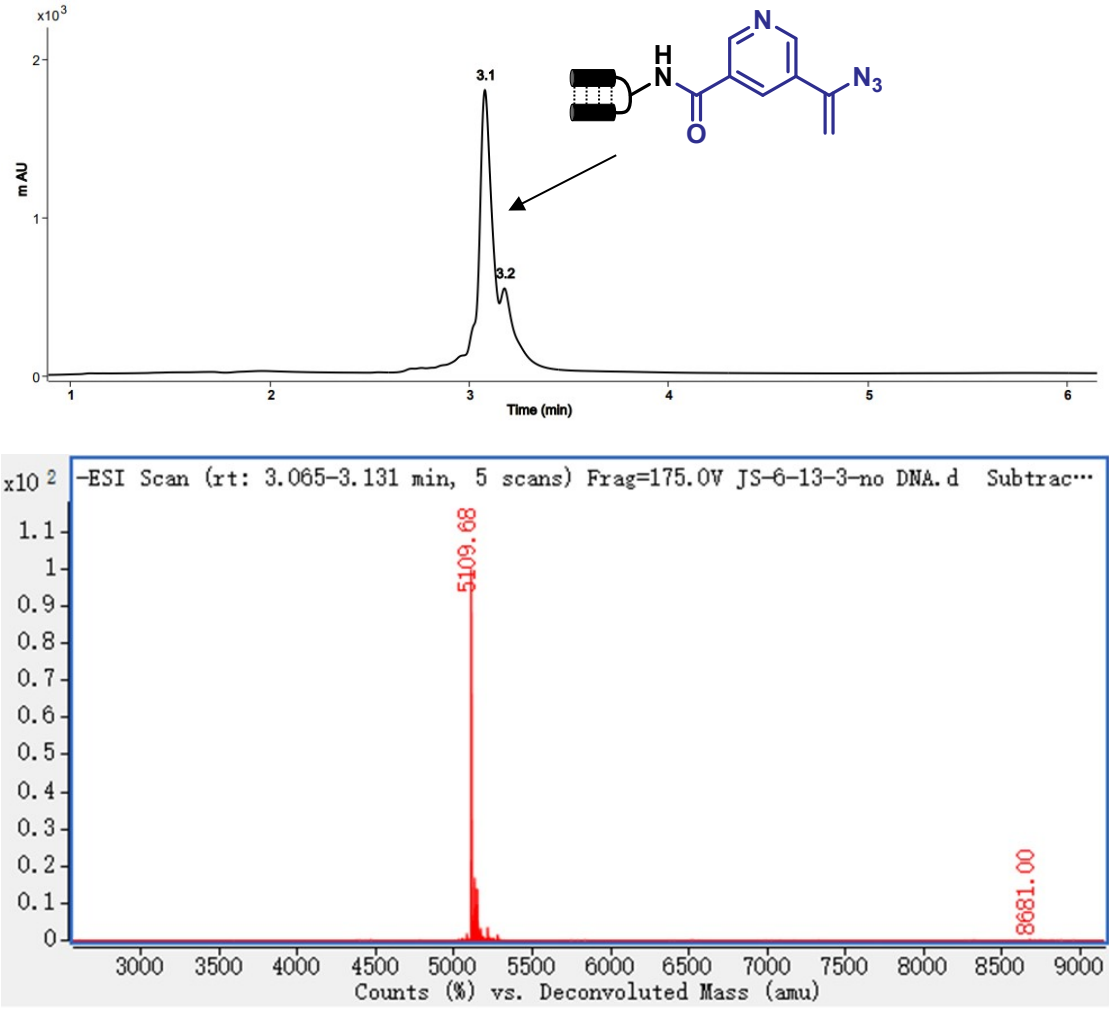
Calculated Mass: 5109 Da; Observed Mass: 5109 Da



UPLC chromatogram and deconvoluted MS of **S1b**

Conversion: 85%

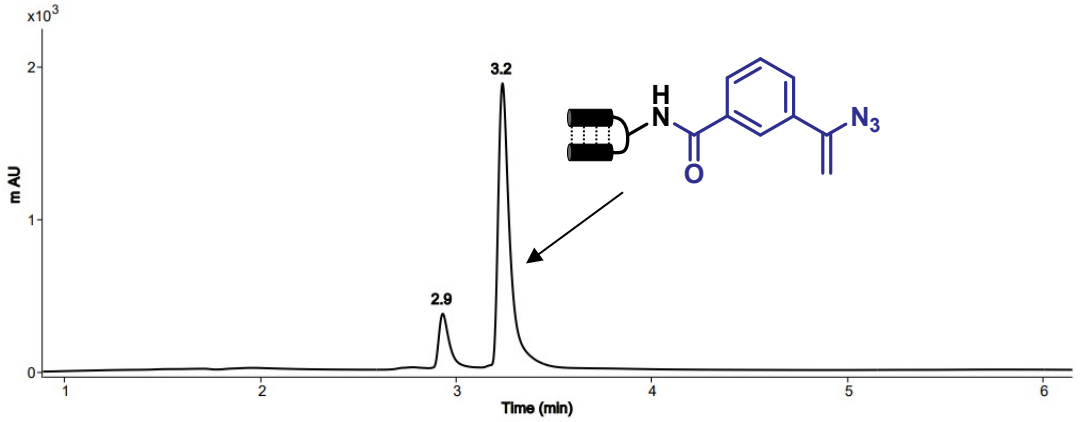
Calculated Mass: 5110 Da; Observed Mass: 5110 Da

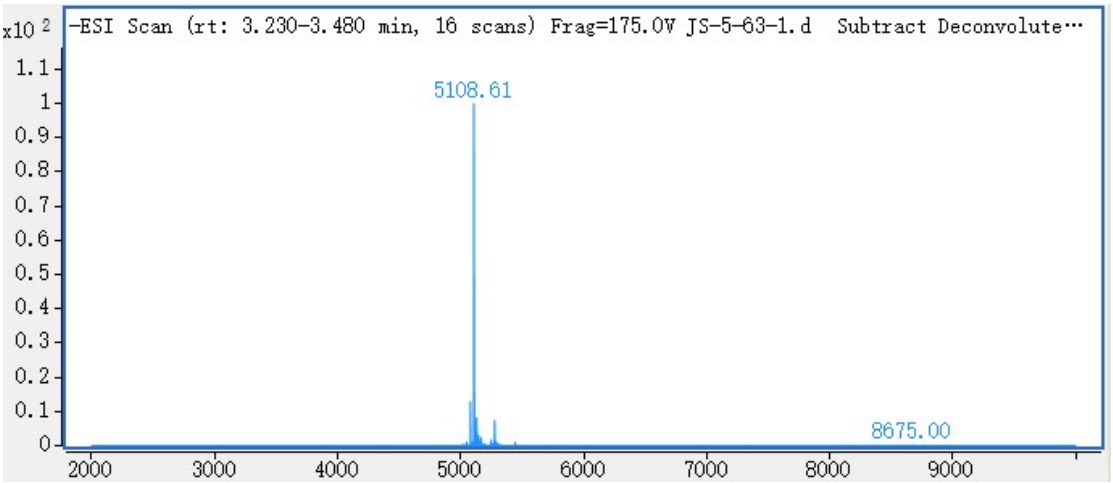


UPLC chromatogram and deconvoluted MS of **S1c**

Conversion: 88%

Calculated Mass: 5109 Da; Observed Mass: 5109 Da

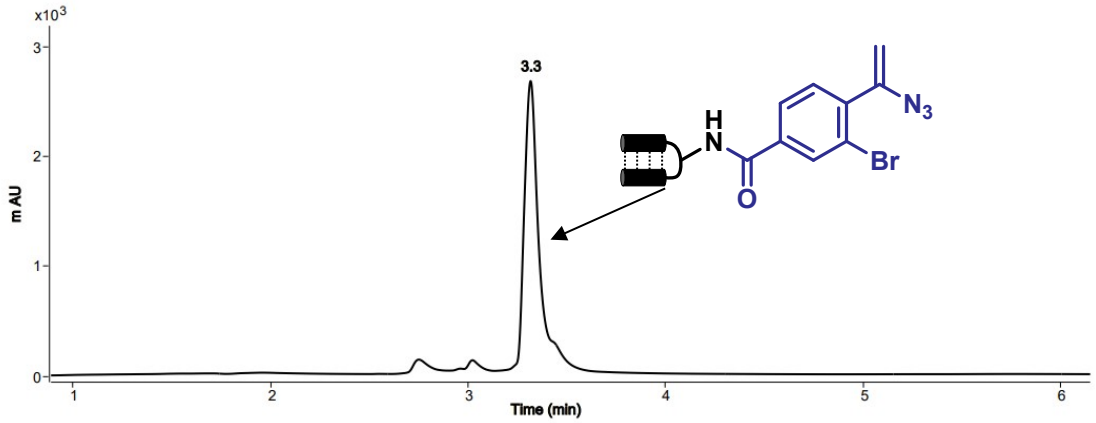


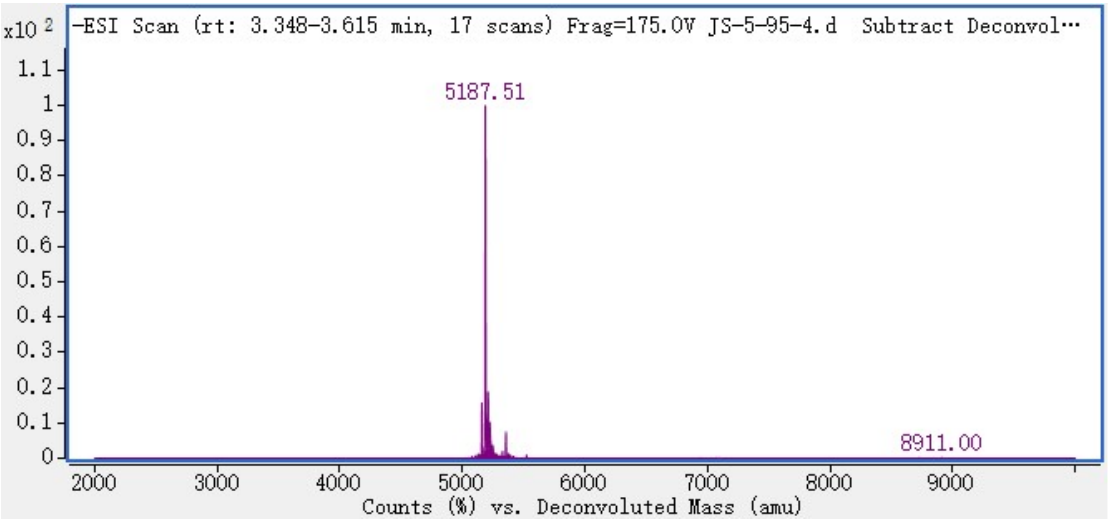


UPLC chromatogram and deconvoluted MS of **S1d**

Conversion: 90%

Calculated Mass: 5187 Da; Observed Mass: 5187 Da

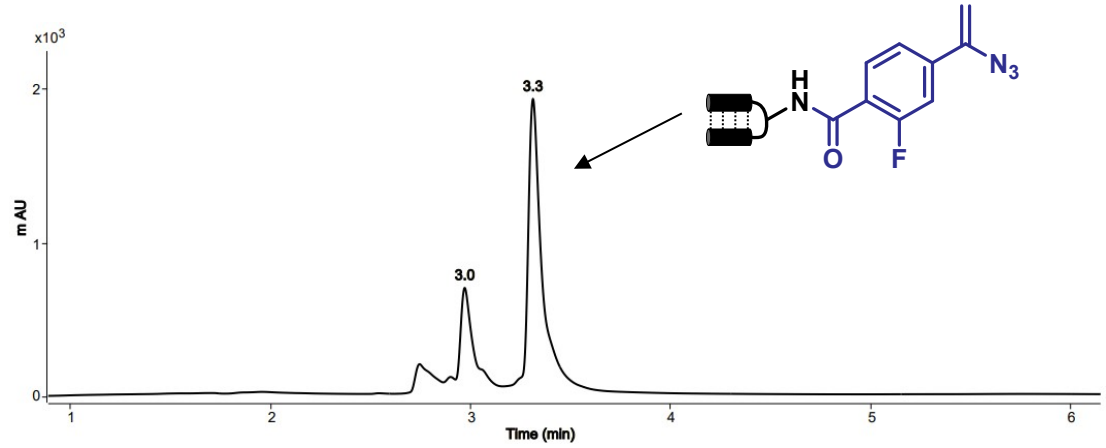


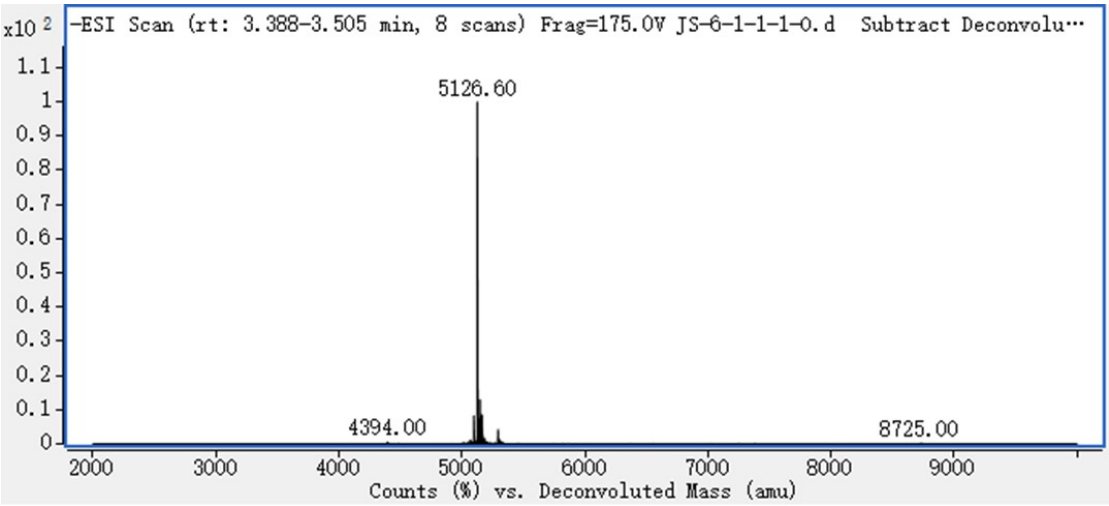


UPLC chromatogram and deconvoluted MS of **S1e**

Conversion: 70%

Calculated Mass: 5126 Da; Observed Mass: 5126 Da

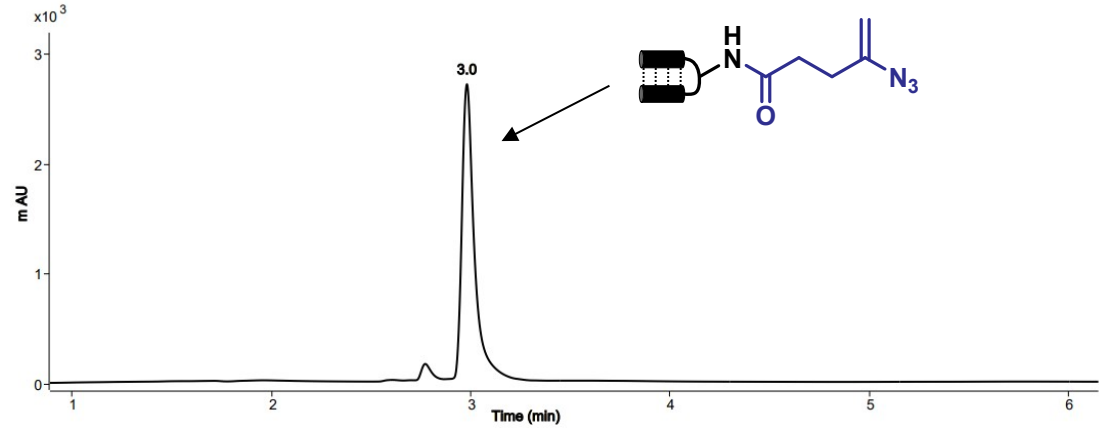


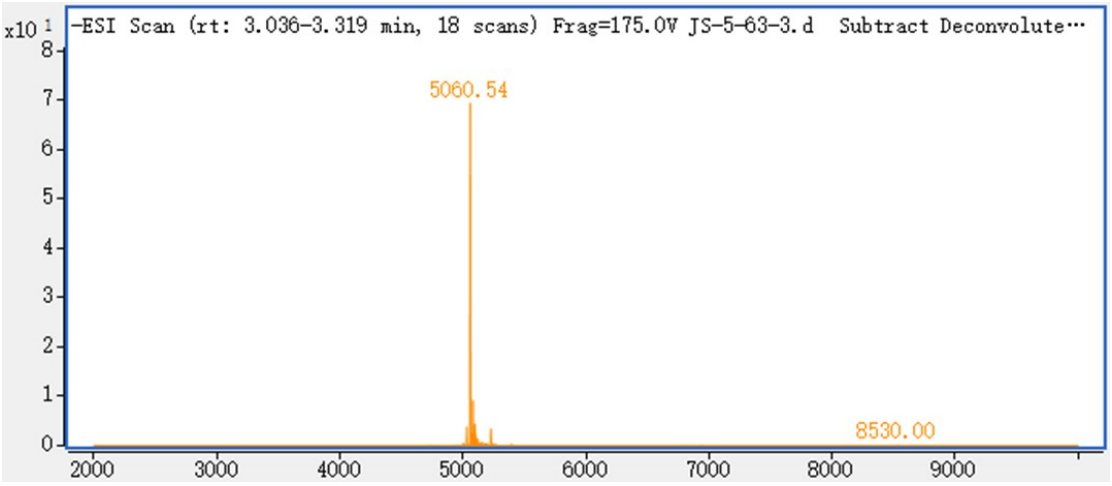


UPLC chromatogram and deconvoluted MS of **S1f**

Conversion: >90%

Calculated Mass: 5060 Da; Observed Mass: 5060 Da

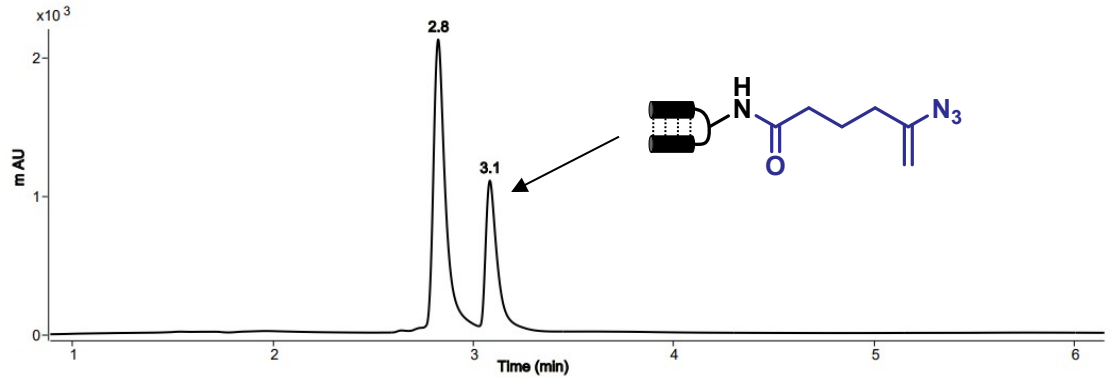


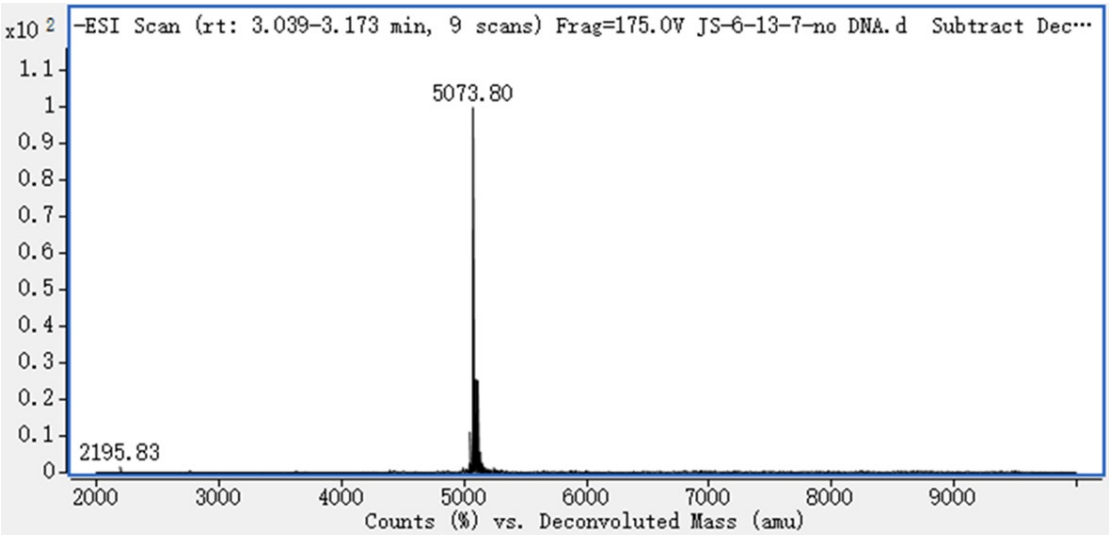


UPLC chromatogram and deconvoluted MS of **S1g**

Conversion: 40%

Calculated Mass: 5074 Da; Observed Mass: 5074 Da



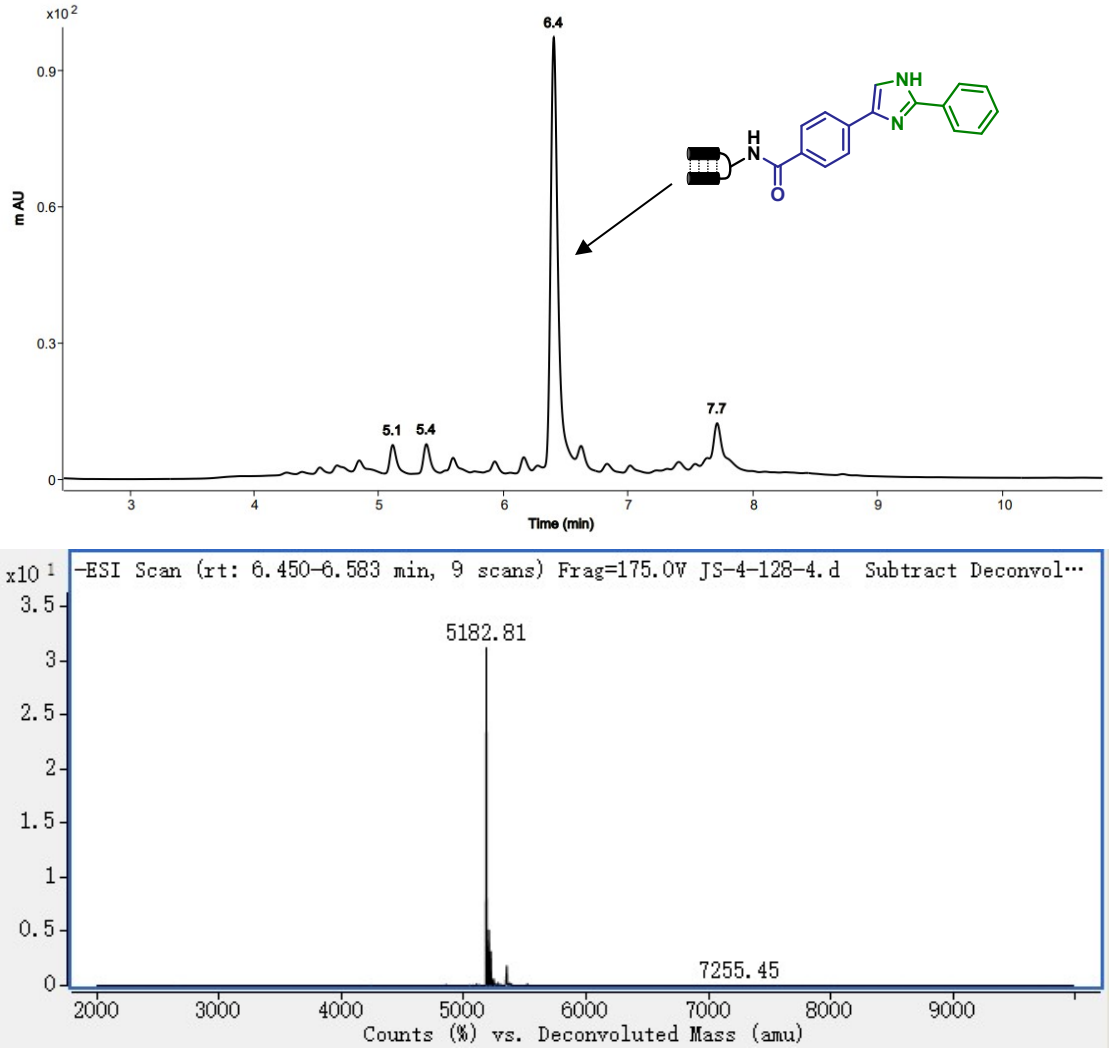


9.2. Substrate Scope of on-DNA 2,4-Disubstituted Imidazoles Formation

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

Conversion: 90%

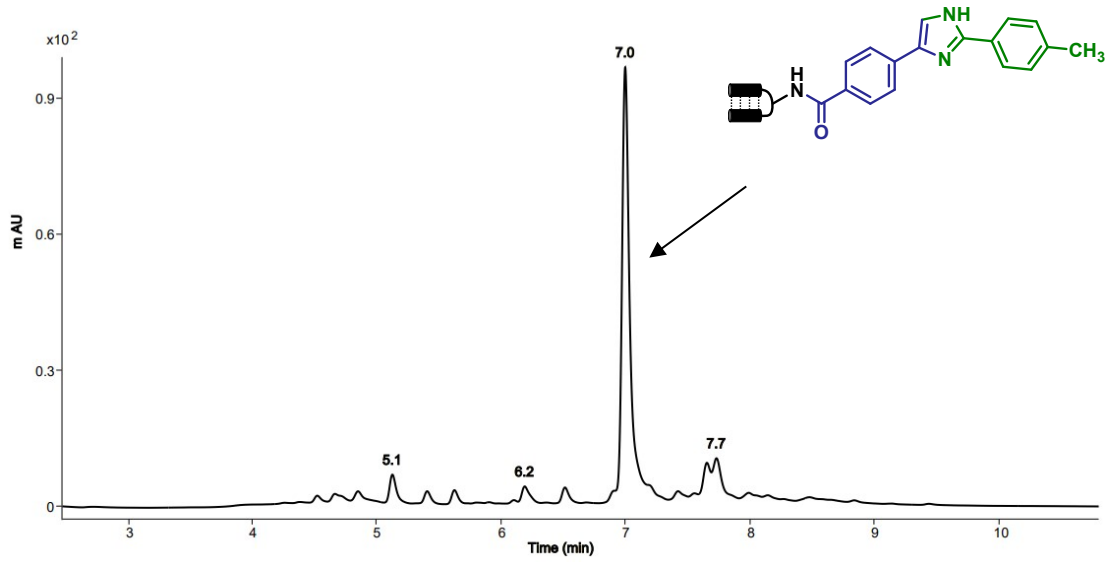
Calculated Mass: 5182 Da; Observed Mass: 5183 Da

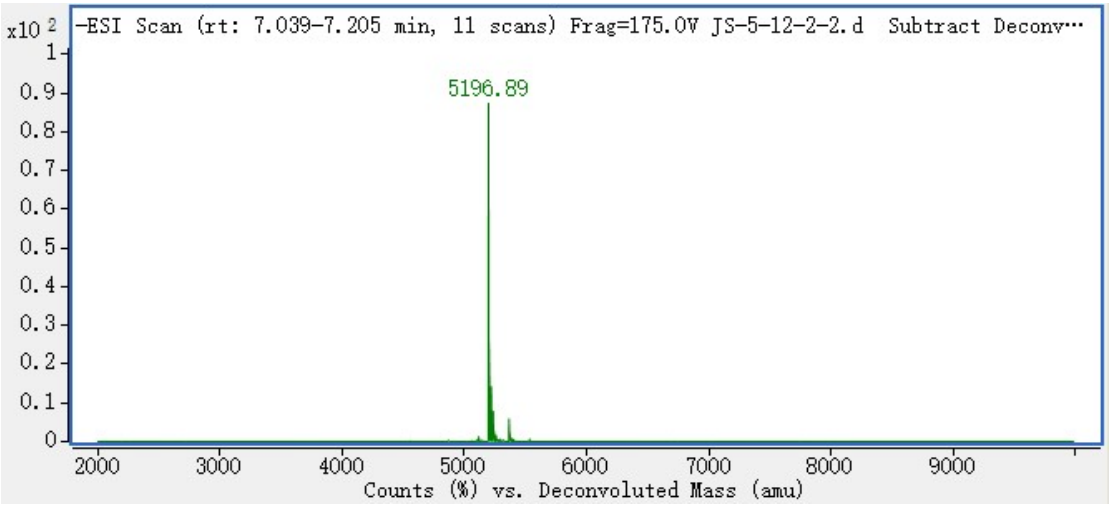


UPLC chromatogram and deconvoluted MS of **3b**

Conversion: 91%

Calculated Mass: 5196 Da; Observed Mass: 5197 Da

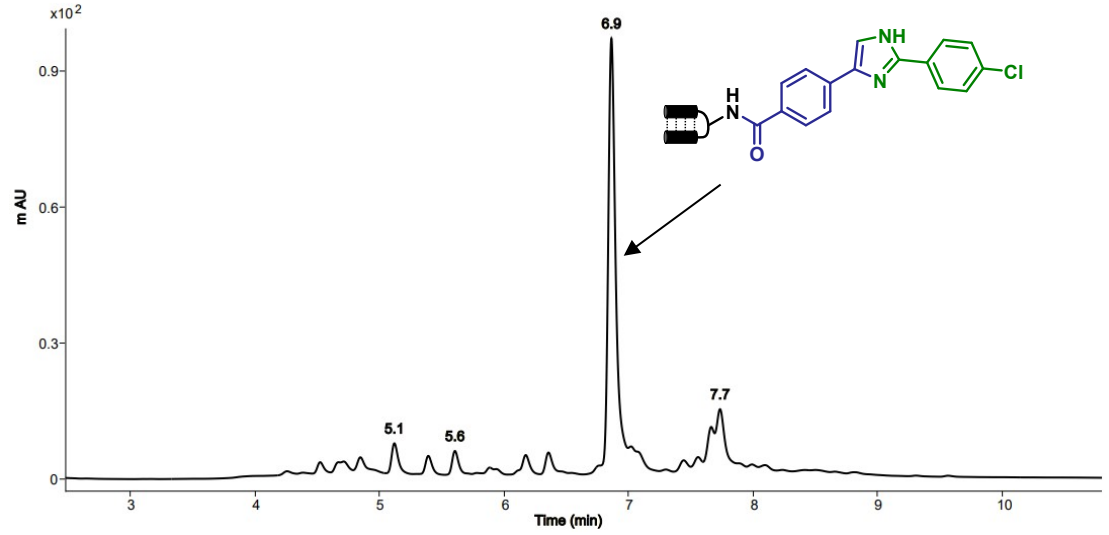


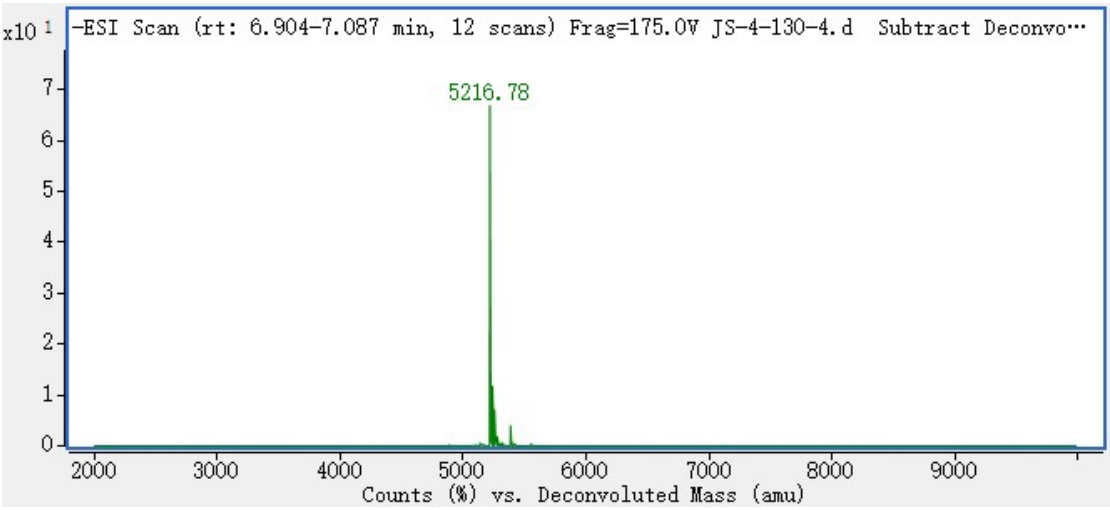


UPLC chromatogram and deconvoluted MS of **3c**

Conversion: 80%

Calculated Mass: 5216 Da; Observed Mass: 5217 Da

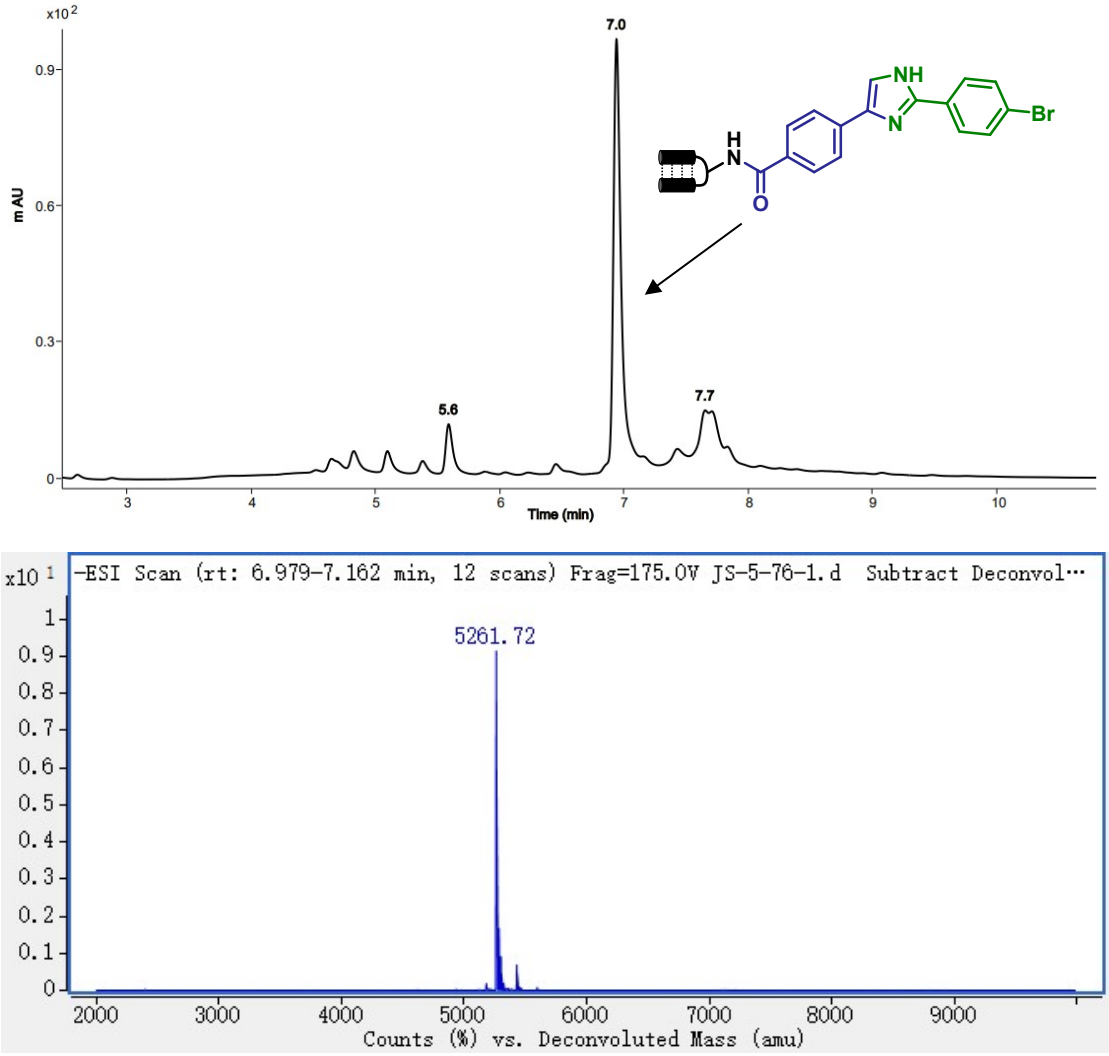




UPLC chromatogram and deconvoluted MS of **3d**

Conversion: 82%

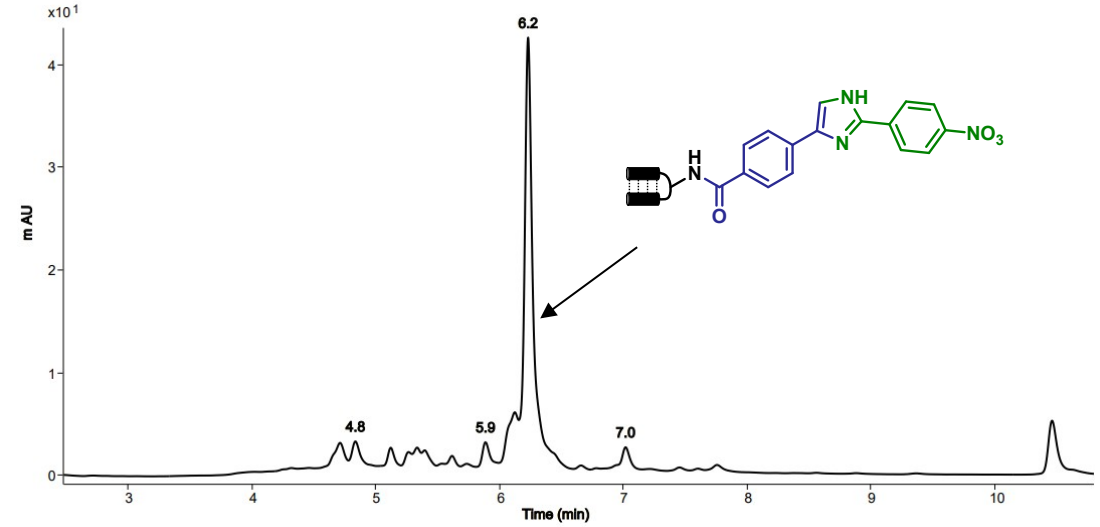
Calculated Mass: 5261 Da; Observed Mass: 5262 Da

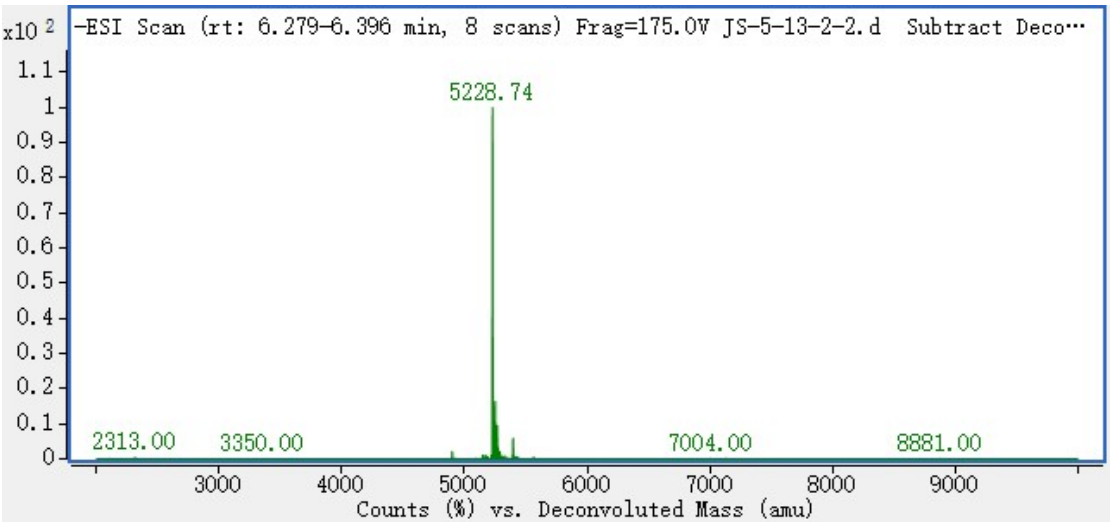


UPLC chromatogram and deconvoluted MS of **3e**

Conversion: 86%

Calculated Mass: 5229 Da; Observed Mass: 5229 Da

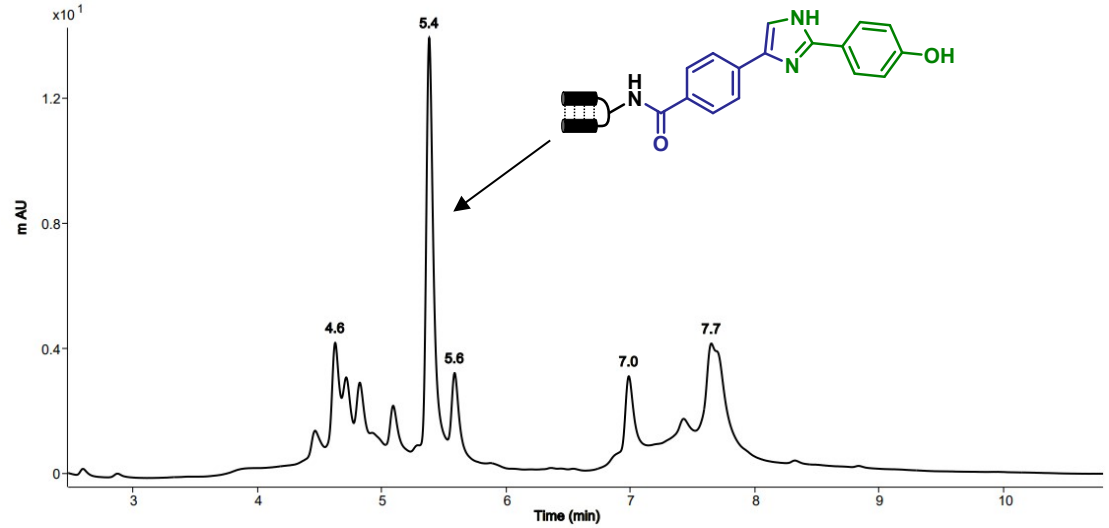


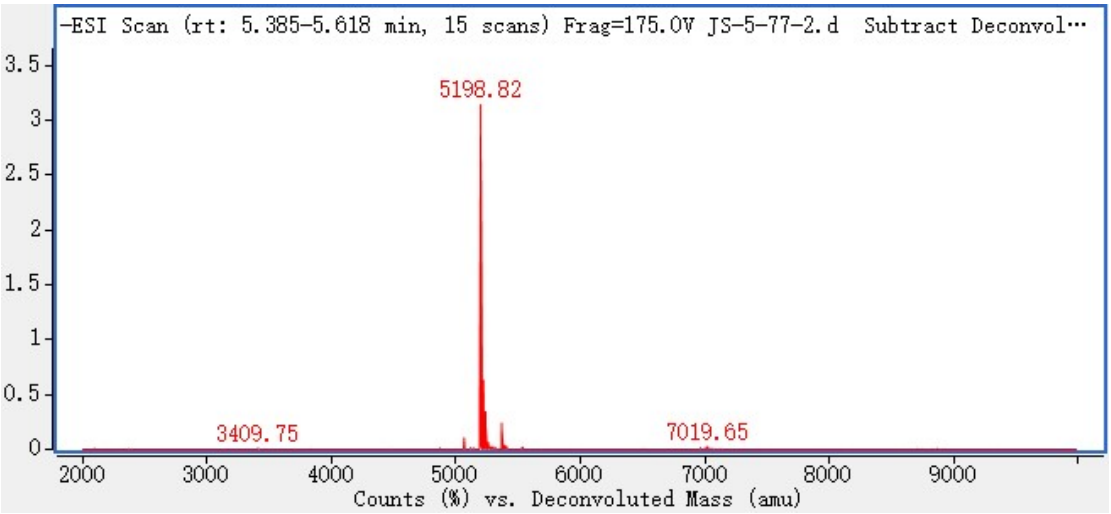


UPLC chromatogram and deconvoluted MS of **3f**

Conversion: 32%

Calculated Mass: 5198 Da; Observed Mass: 5199 Da

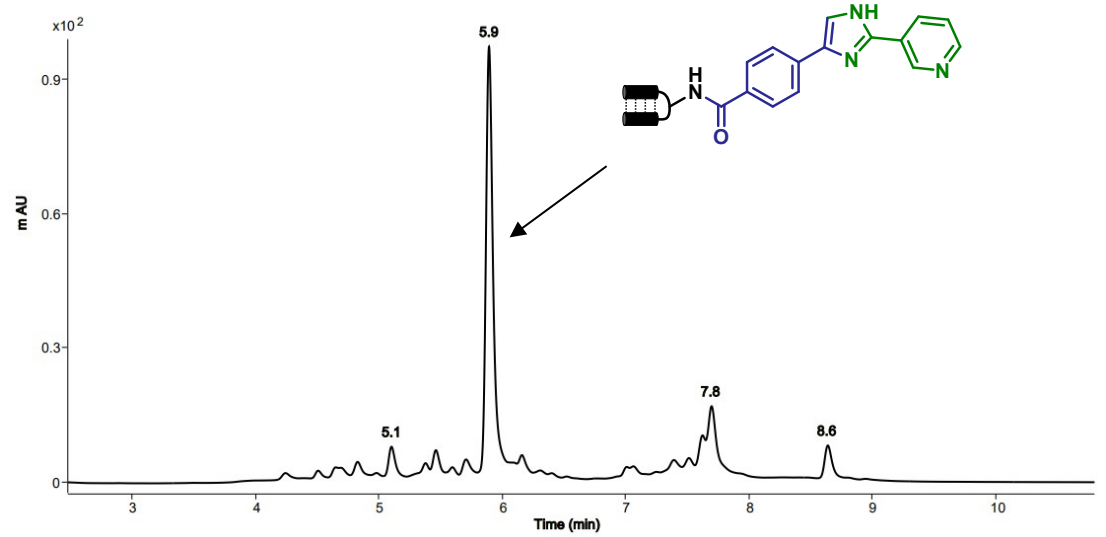


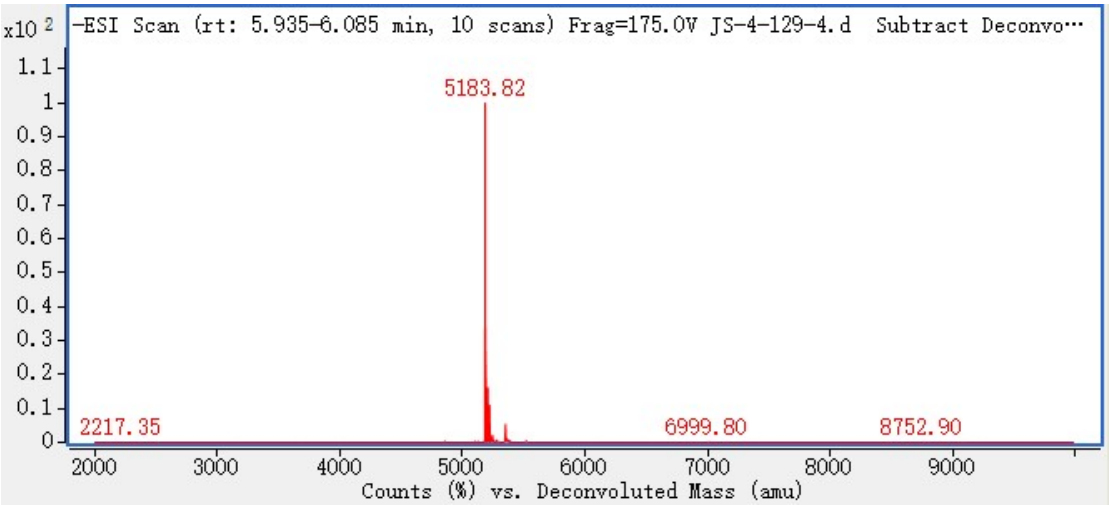


UPLC chromatogram and deconvoluted MS of **3g**

Conversion: 81%

Calculated Mass: 5184 Da; Observed Mass: 5184 Da

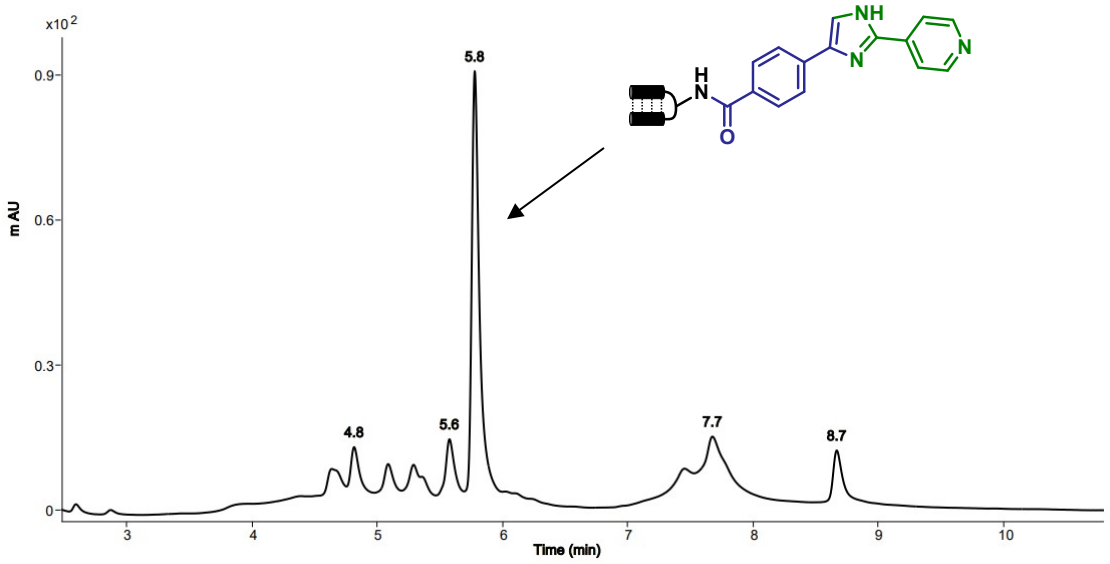


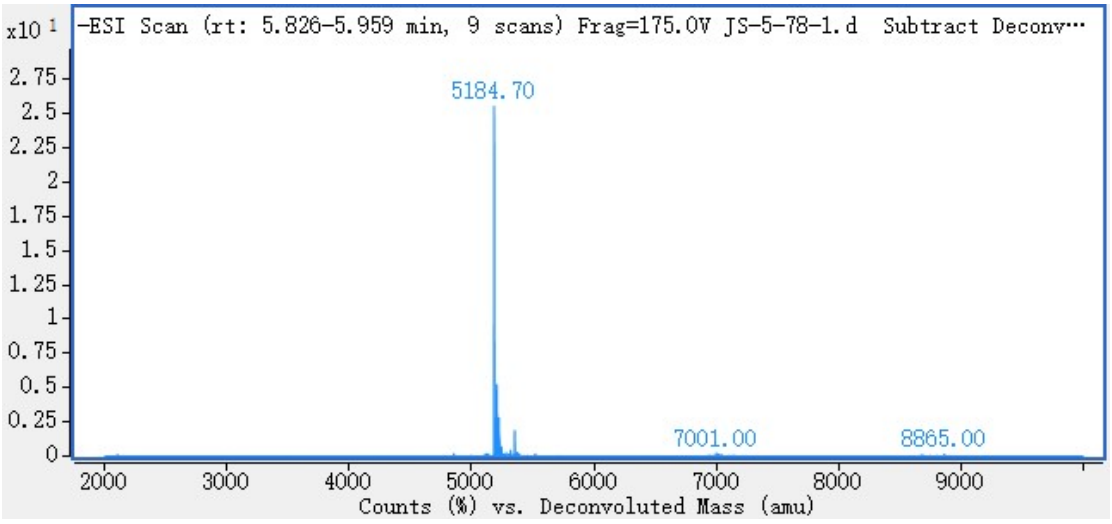


UPLC chromatogram and deconvoluted MS of **3h**

Conversion: 60%

Calculated Mass: 5184 Da; Observed Mass: 5185 Da

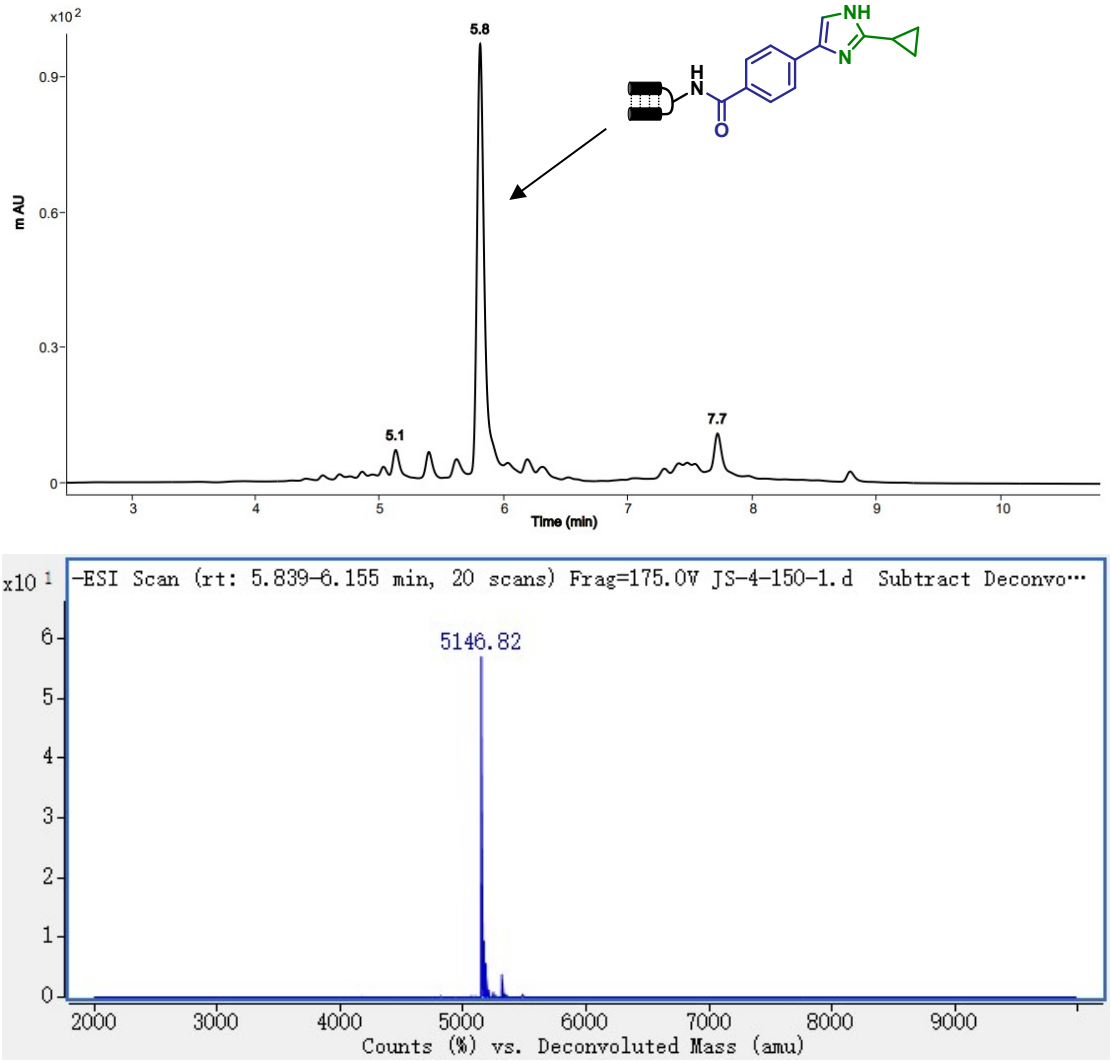




UPLC chromatogram and deconvoluted MS of **3i**

Conversion: 90%

Calculated Mass: 5147 Da; Observed Mass: 5147 Da

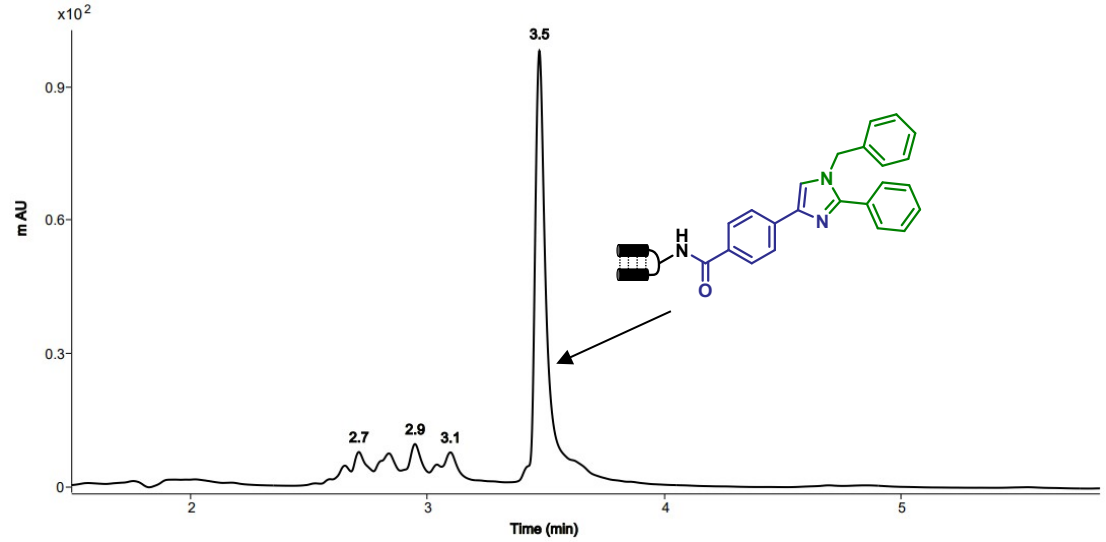


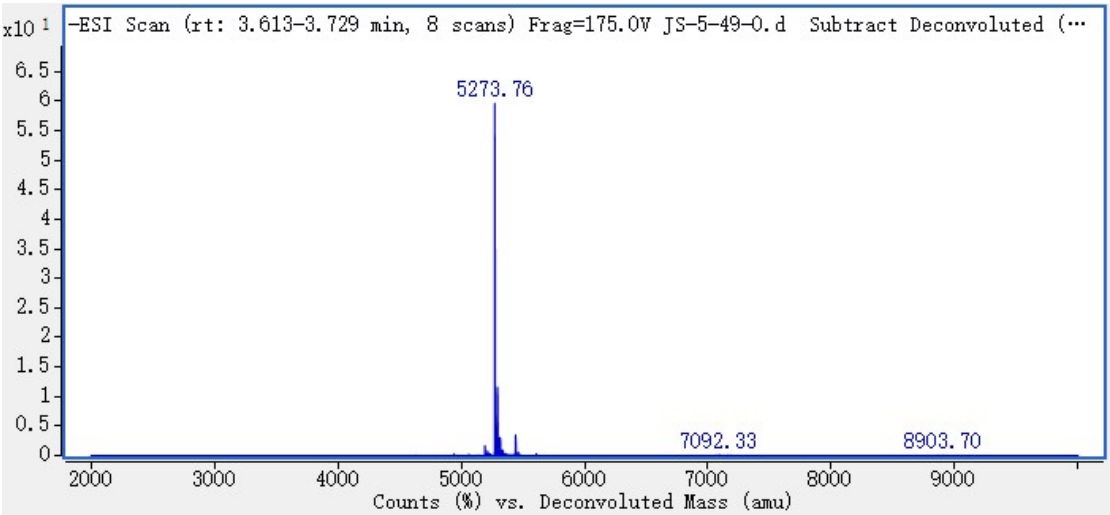
9.3. Substrate Scope of on-DNA 1,2,4-Trisubstituted Imidazoles Formation

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

Conversion: 82%

Calculated Mass: 5274 Da; Observed Mass: 5274 Da

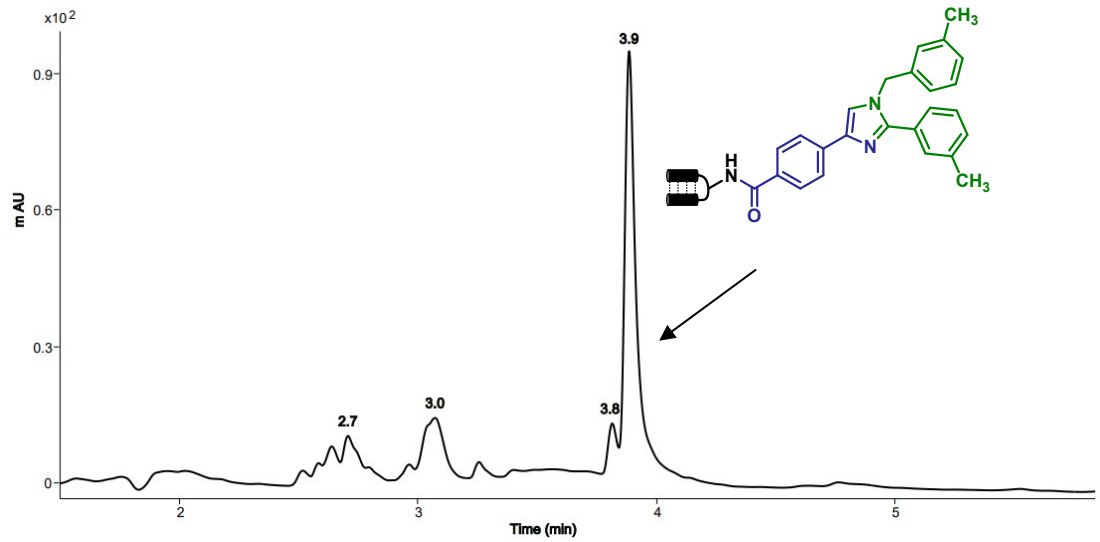


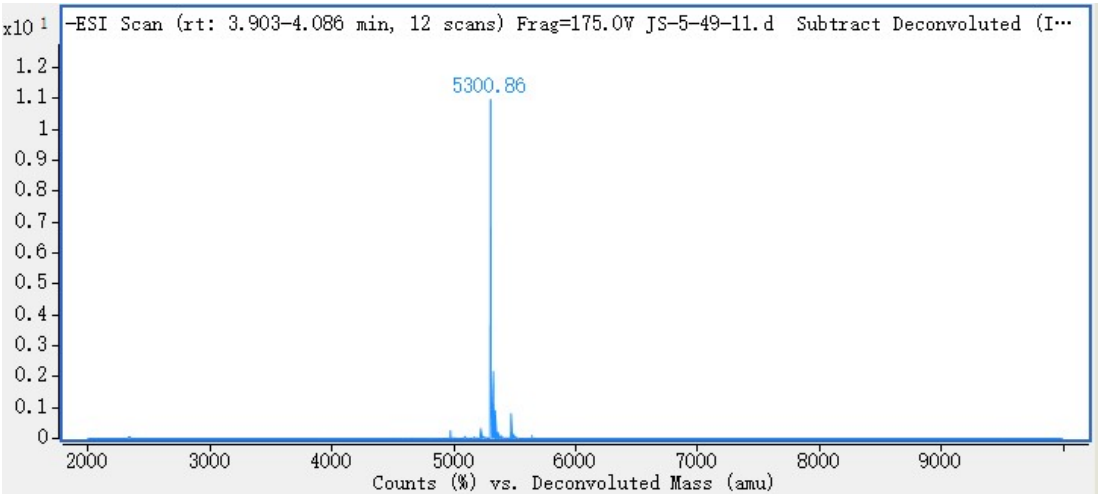


UPLC chromatogram and deconvoluted MS of **5b**

Conversion: 71%

Calculated Mass: 5300 Da; Observed Mass: 5301 Da

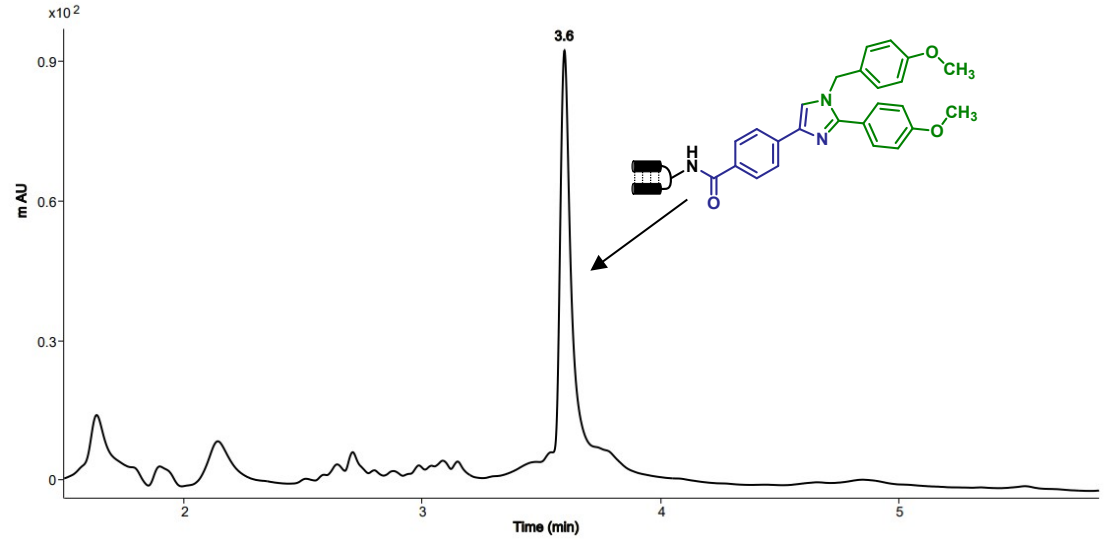


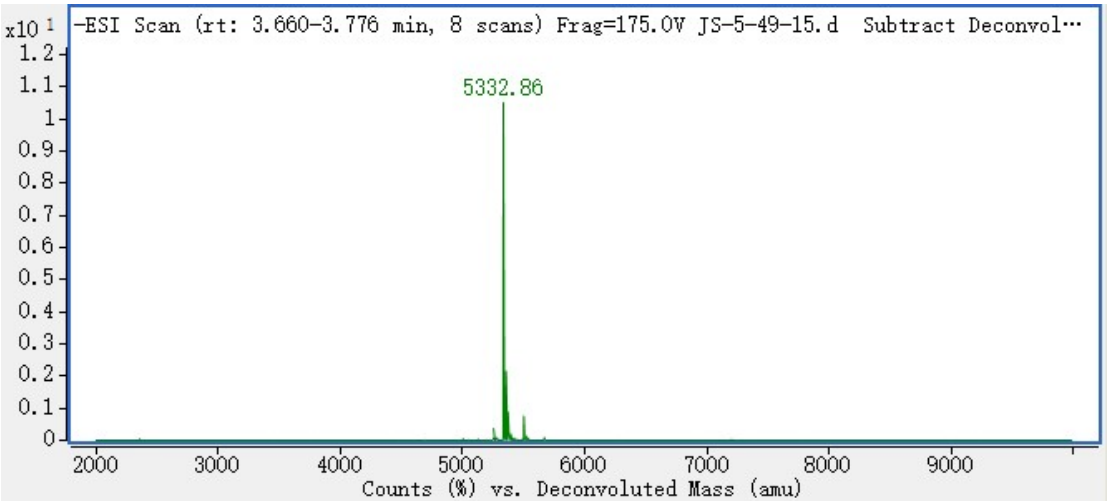


UPLC chromatogram and deconvoluted MS of **5c**

Conversion: 90%

Calculated Mass: 5332 Da; Observed Mass: 5333 Da

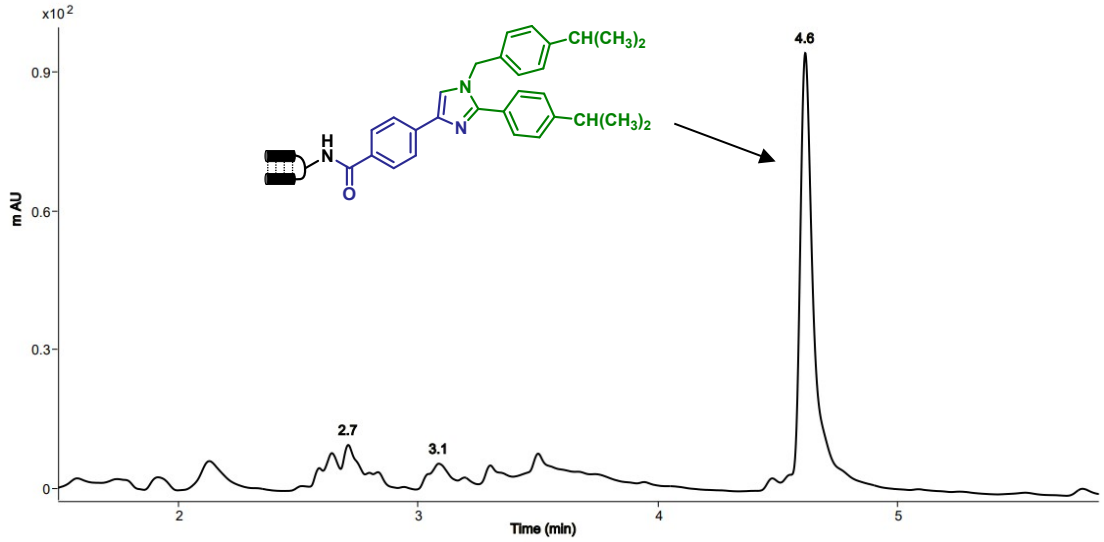


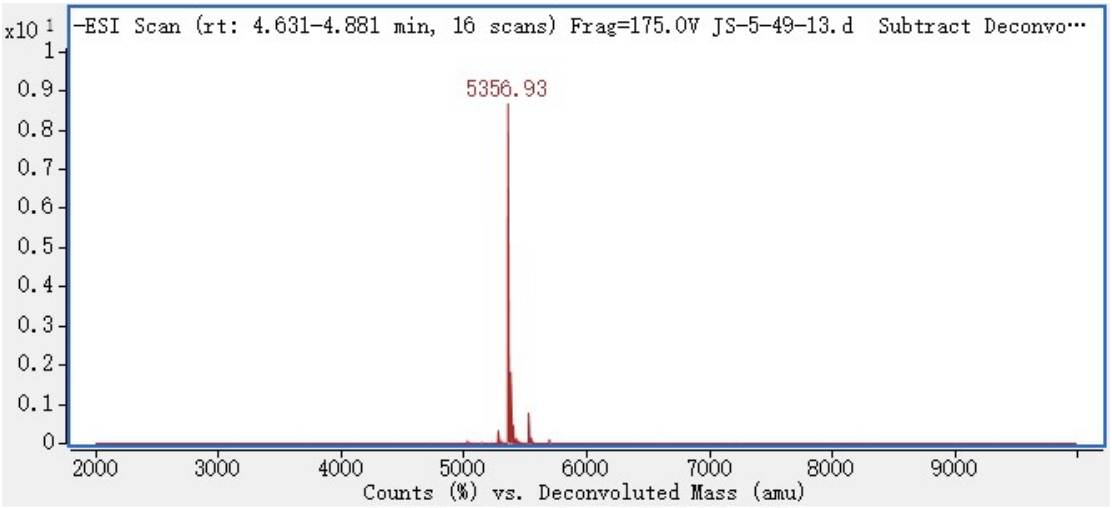


UPLC chromatogram and deconvoluted MS of **5d**

Conversion: >90%

Calculated Mass: 5356 Da; Observed Mass: 5357 Da

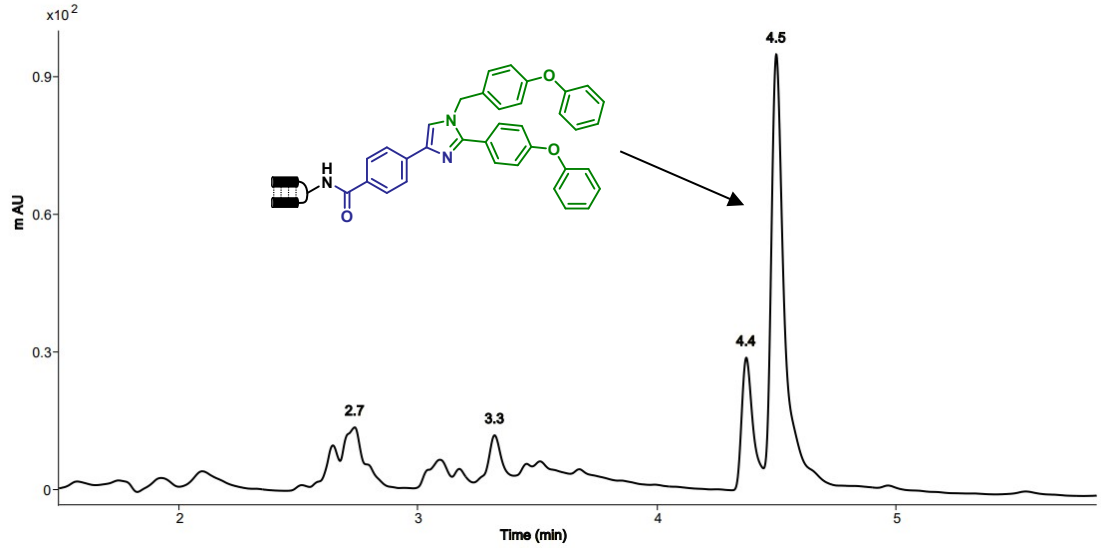


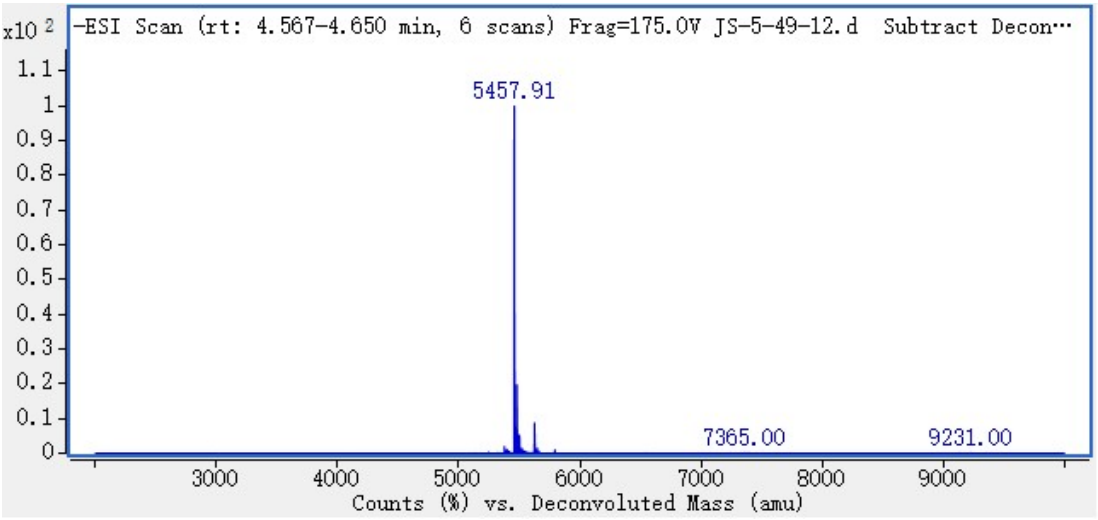


UPLC chromatogram and deconvoluted MS of **5e**

Conversion: 63%

Calculated Mass: 5458 Da; Observed Mass: 5458 Da

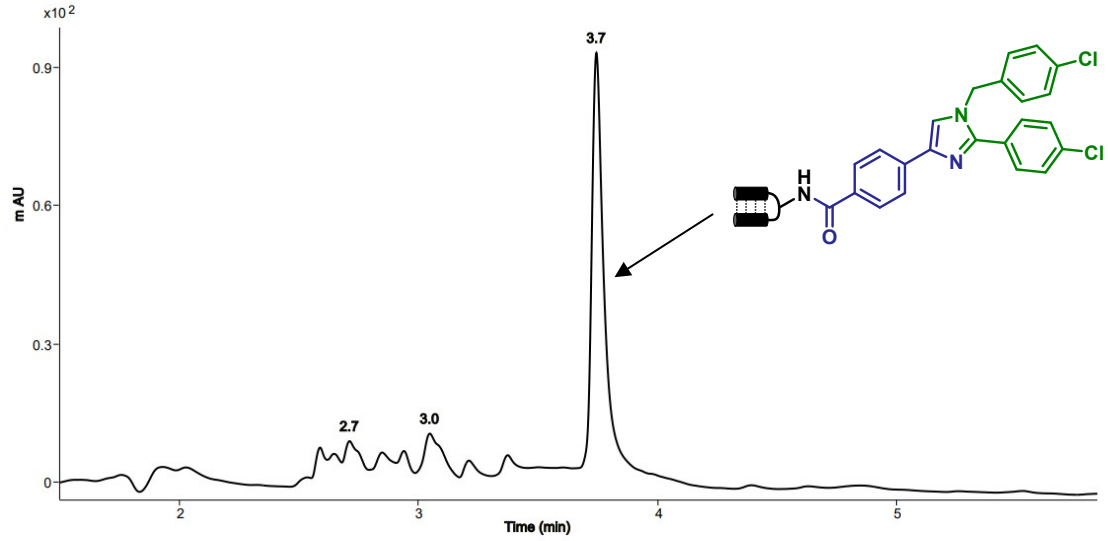


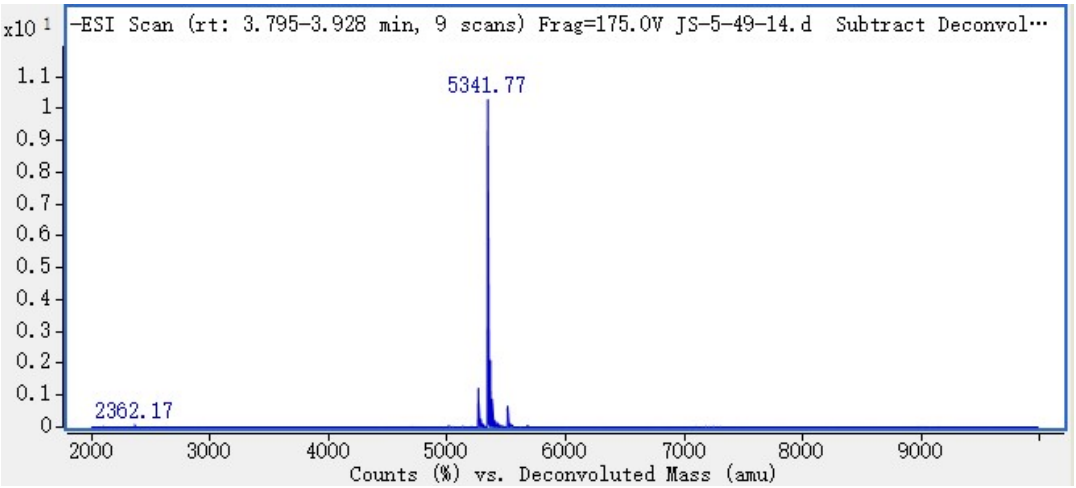


UPLC chromatogram and deconvoluted MS of **5f**

Conversion: 90%

Calculated Mass: 5341 Da; Observed Mass: 5342 Da

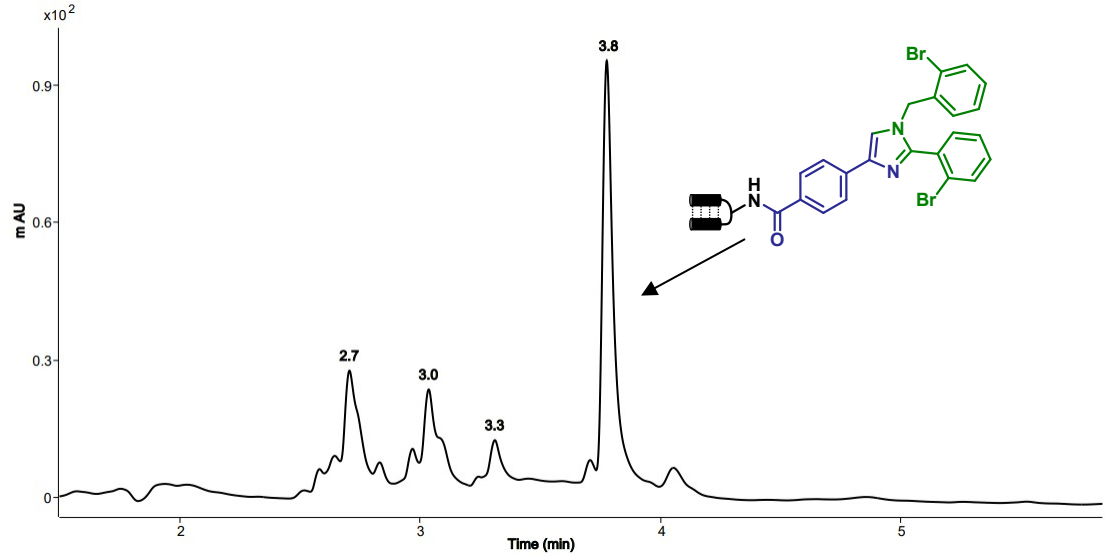


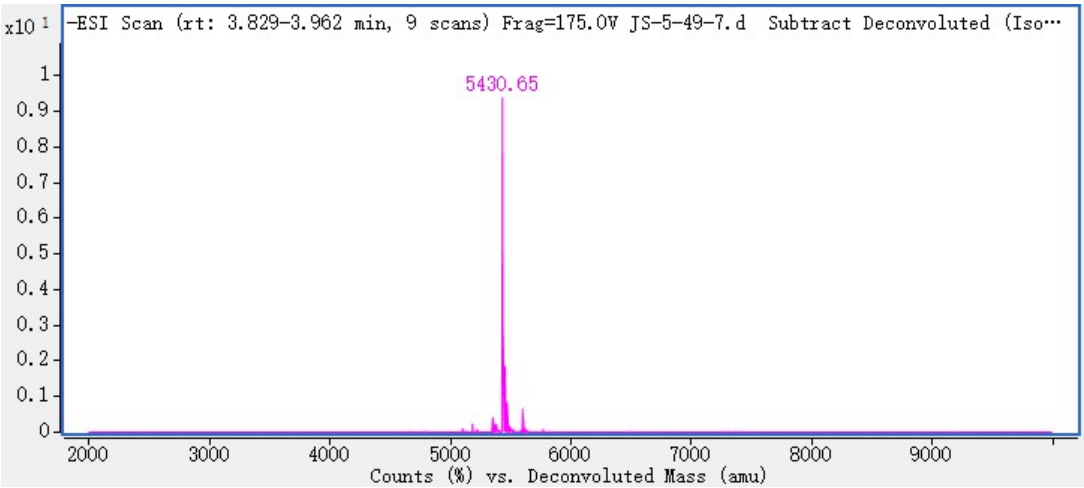


UPLC chromatogram and deconvoluted MS of **5g**

Conversion: 62%

Calculated Mass: 5430 Da; Observed Mass: 5431 Da

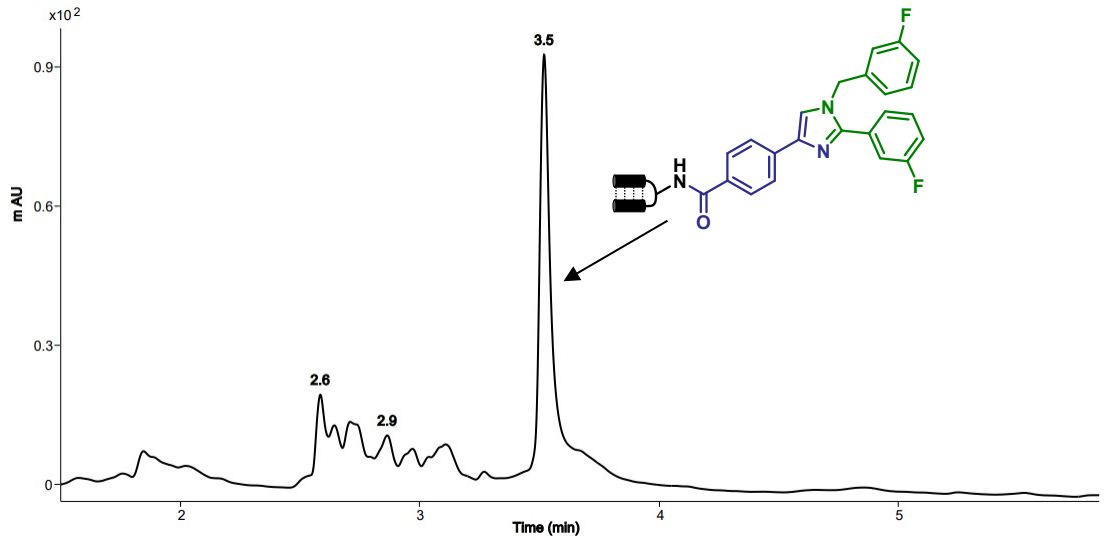


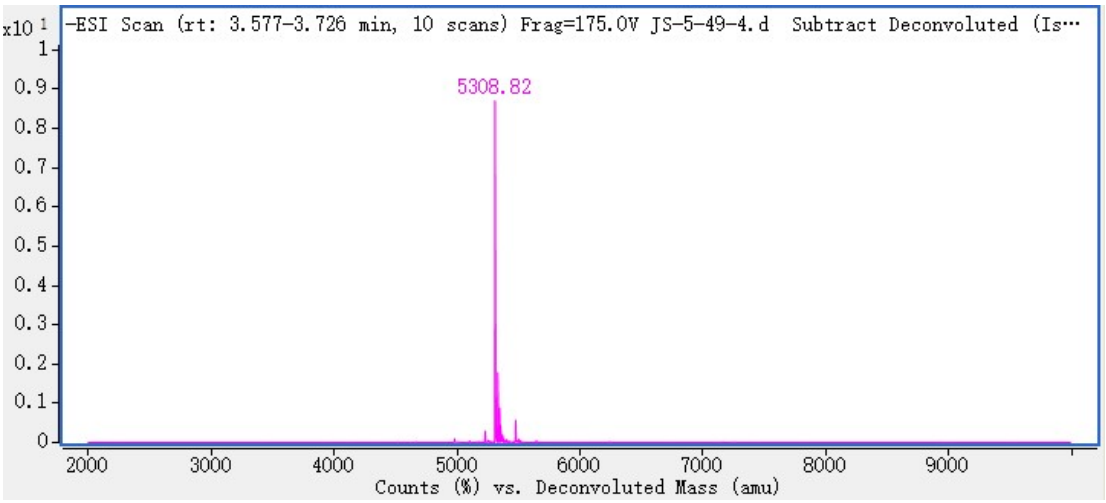


UPLC chromatogram and deconvoluted MS of **5h**

Conversion: 72%

Calculated Mass: 5309 Da; Observed Mass: 5309 Da

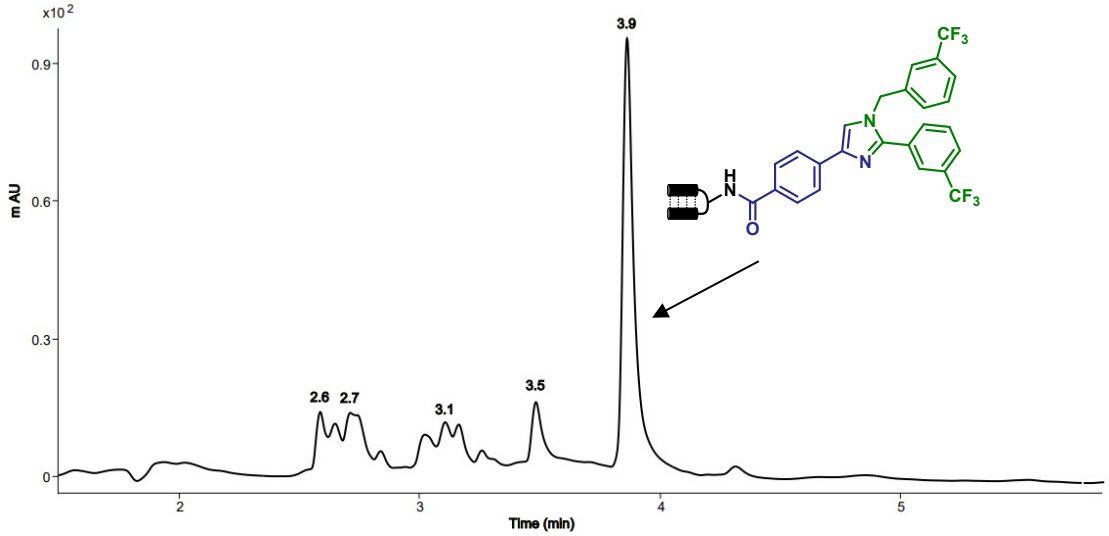


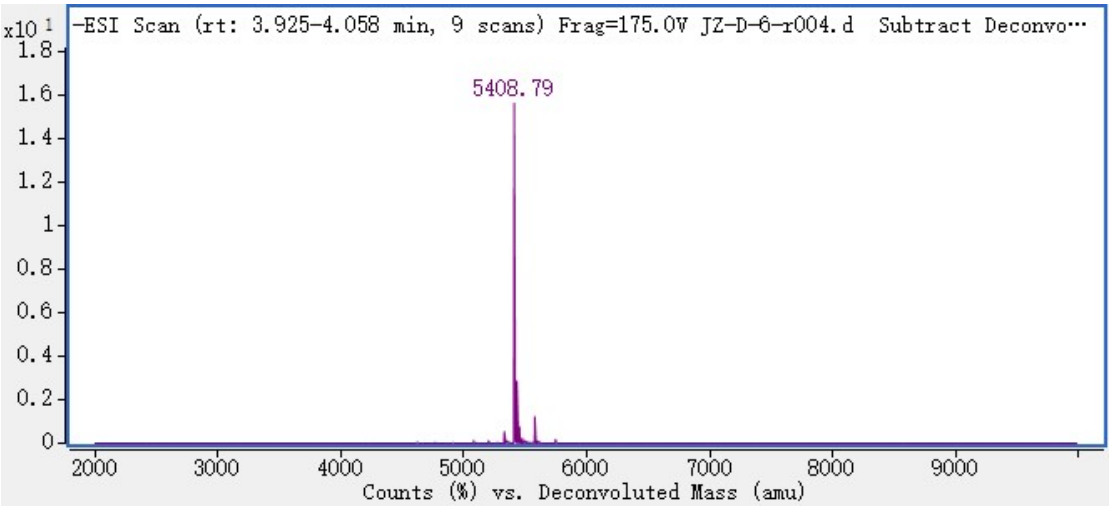


UPLC chromatogram and deconvoluted MS of **5i**

Conversion: 72%

Calculated Mass: 5408 Da; Observed Mass: 5409 Da

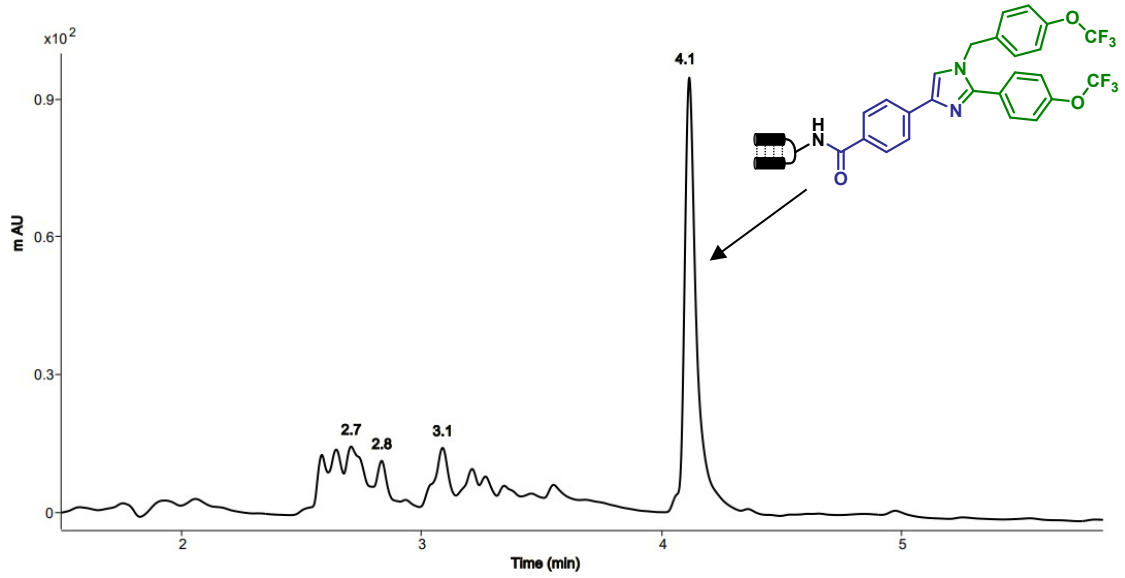


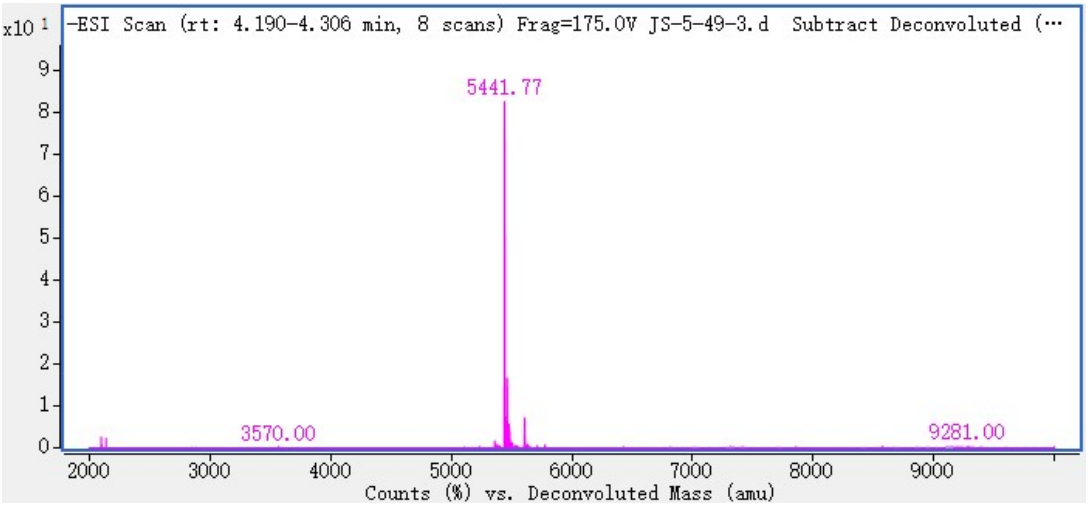


UPLC chromatogram and deconvoluted MS of **5j**

Conversion: 70%

Calculated Mass: 5442 Da; Observed Mass: 5442 Da

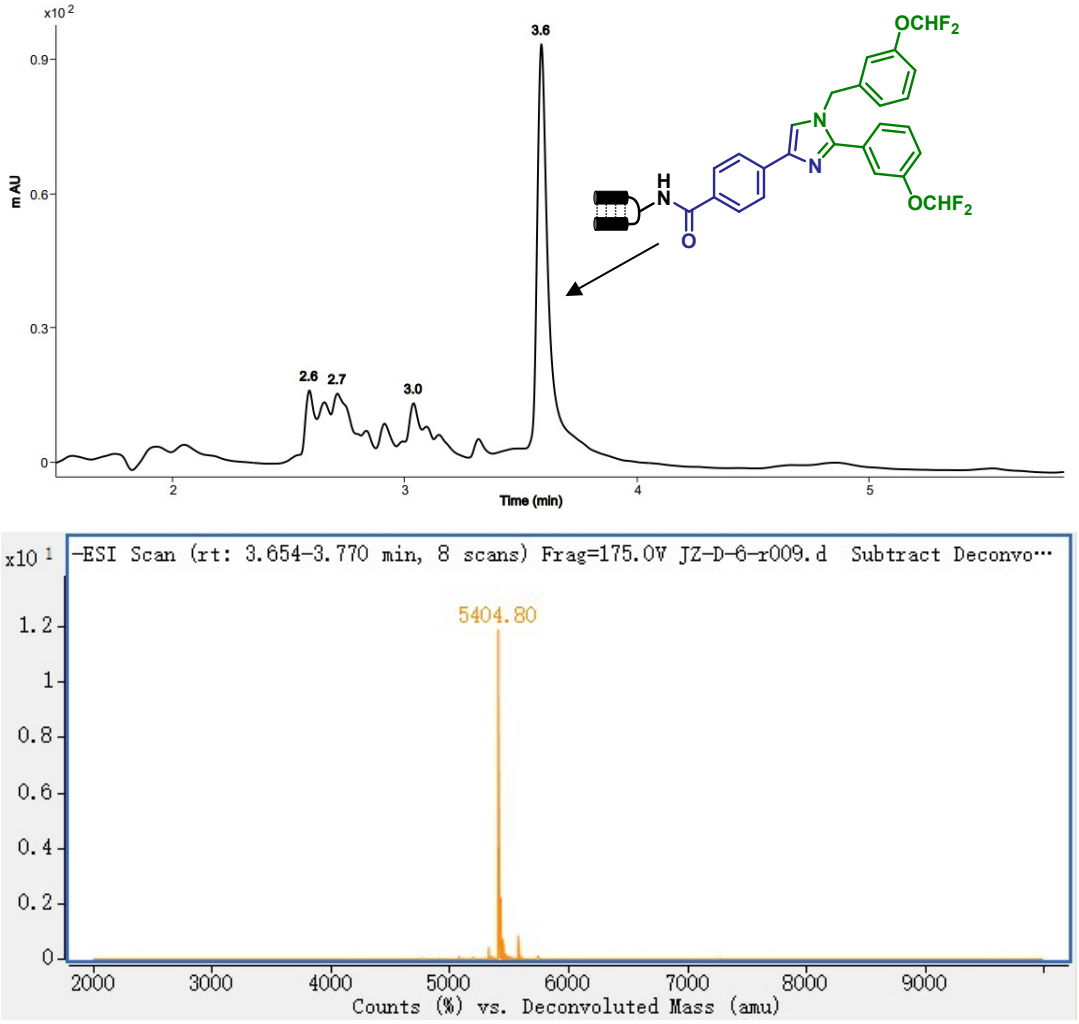




UPLC chromatogram and deconvoluted MS of **5k**

Conversion: 80%

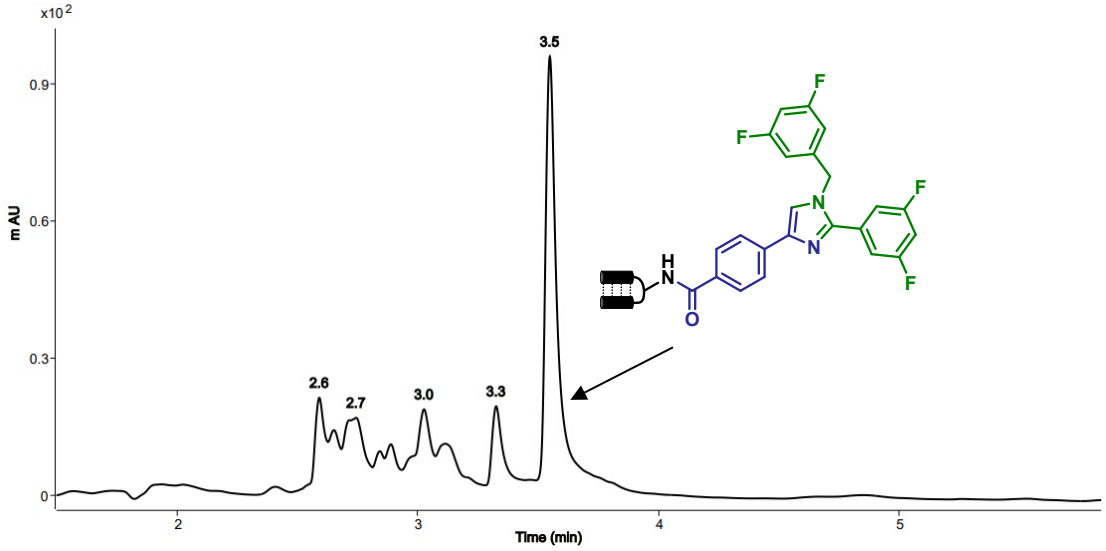
Calculated Mass: 5404 Da; Observed Mass: 5405 Da

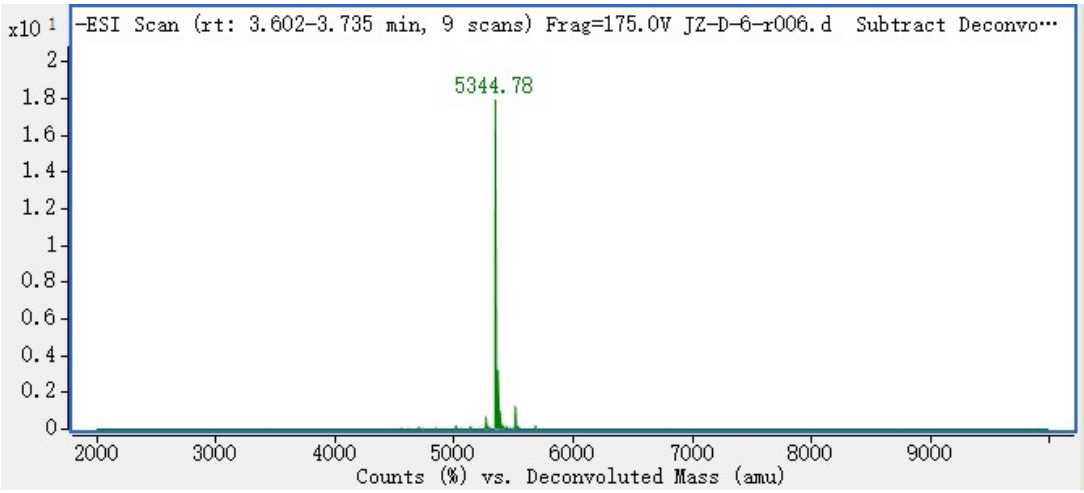


UPLC chromatogram and deconvoluted MS of **5l**

Conversion: 59%

Calculated Mass: 5344 Da; Observed Mass: 5345 Da

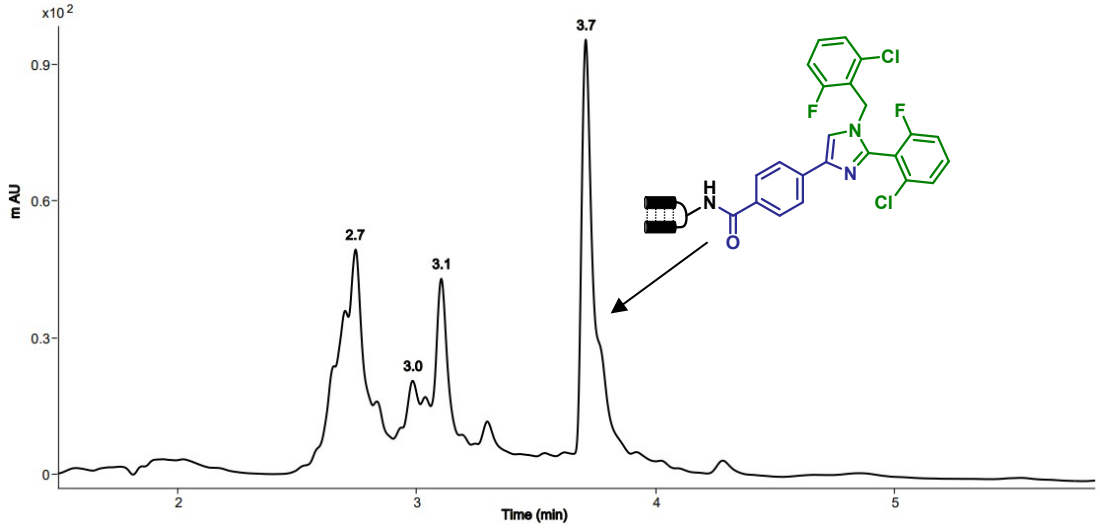


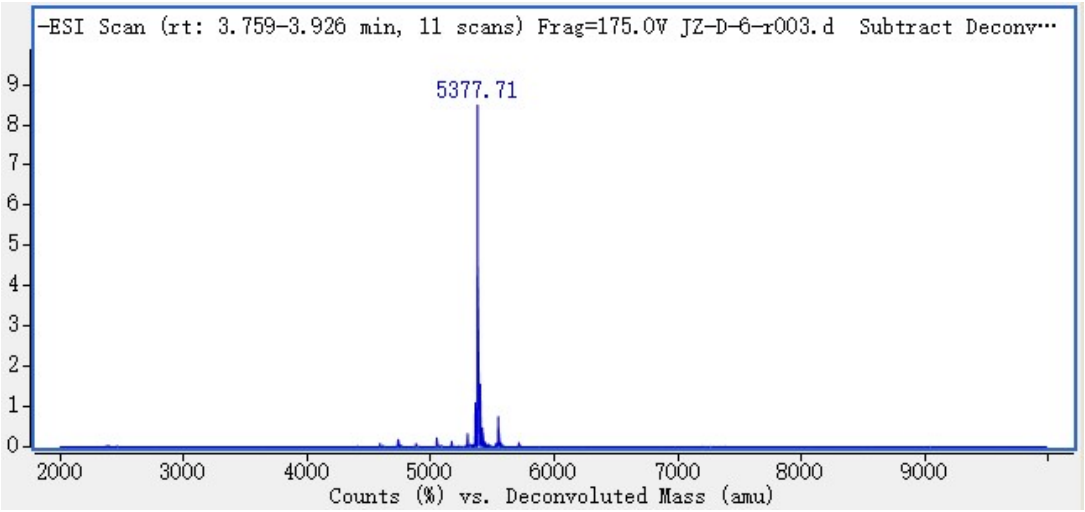


UPLC chromatogram and deconvoluted MS of **5m**

Conversion: 48%

Calculated Mass: 5377 Da; Observed Mass: 5378 Da

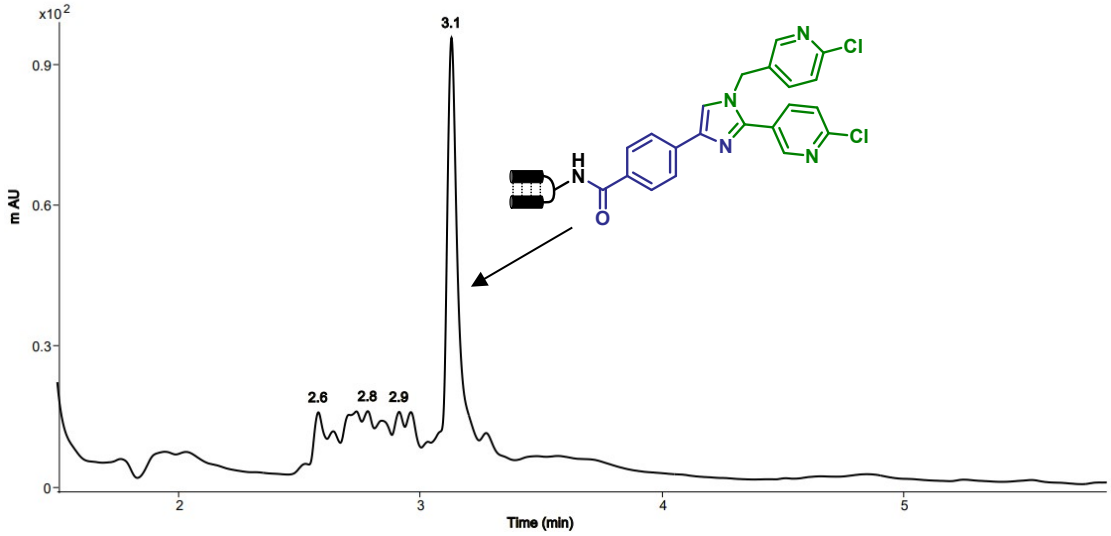


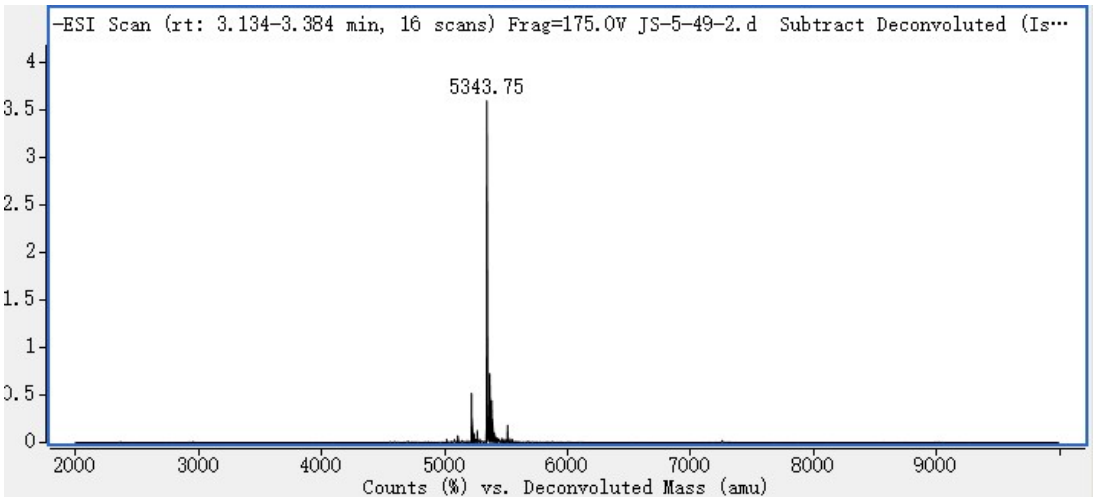


UPLC chromatogram and deconvoluted MS of **5n**

Conversion: 65%

Calculated Mass: 5344 Da; Observed Mass: 5344 Da

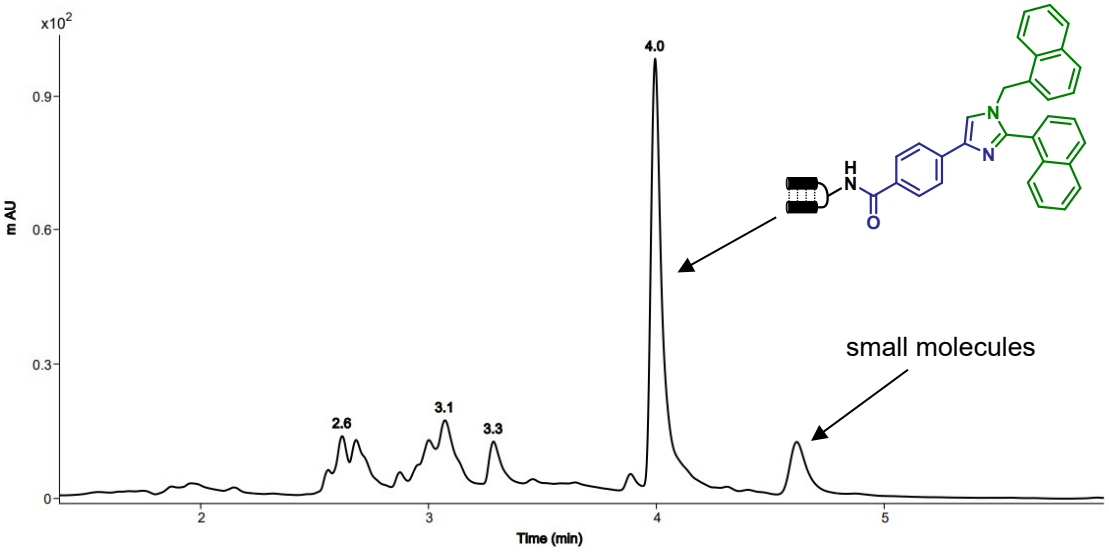


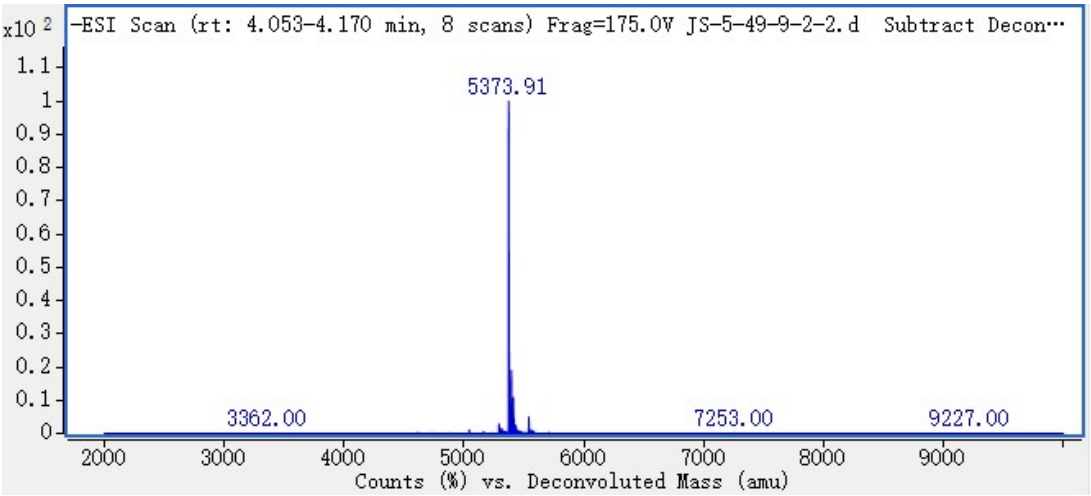


UPLC chromatogram and deconvoluted MS of **5o**

Conversion: 75%

Calculated Mass: 5374 Da; Observed Mass: 5374 Da

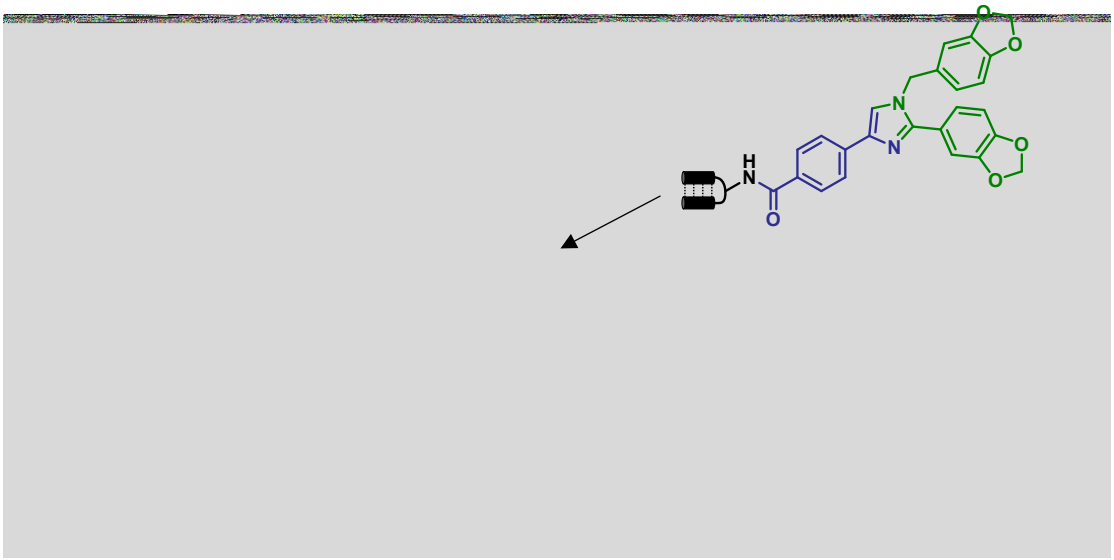


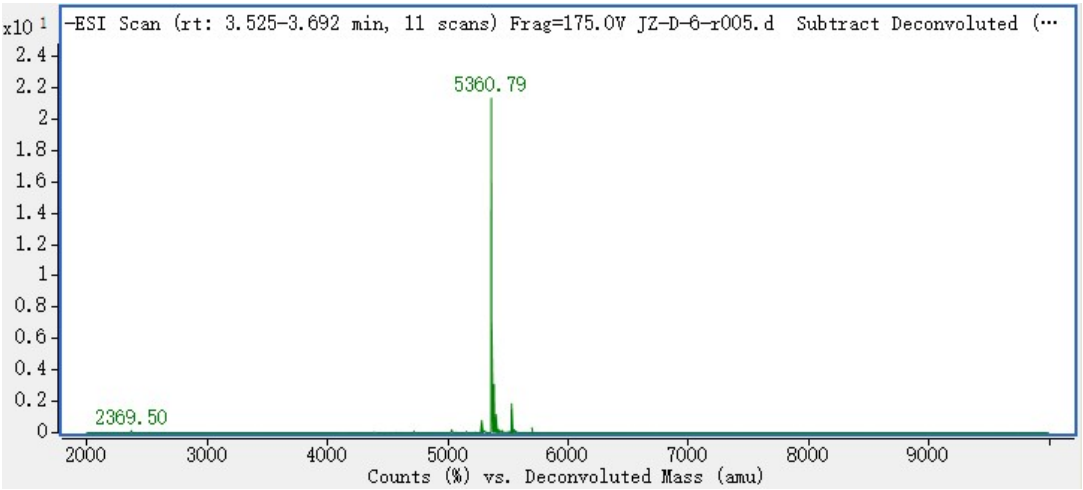


UPLC chromatogram and deconvoluted MS of **5p**

Conversion: 90%

Calculated Mass: 5360 Da; Observed Mass: 5361 Da

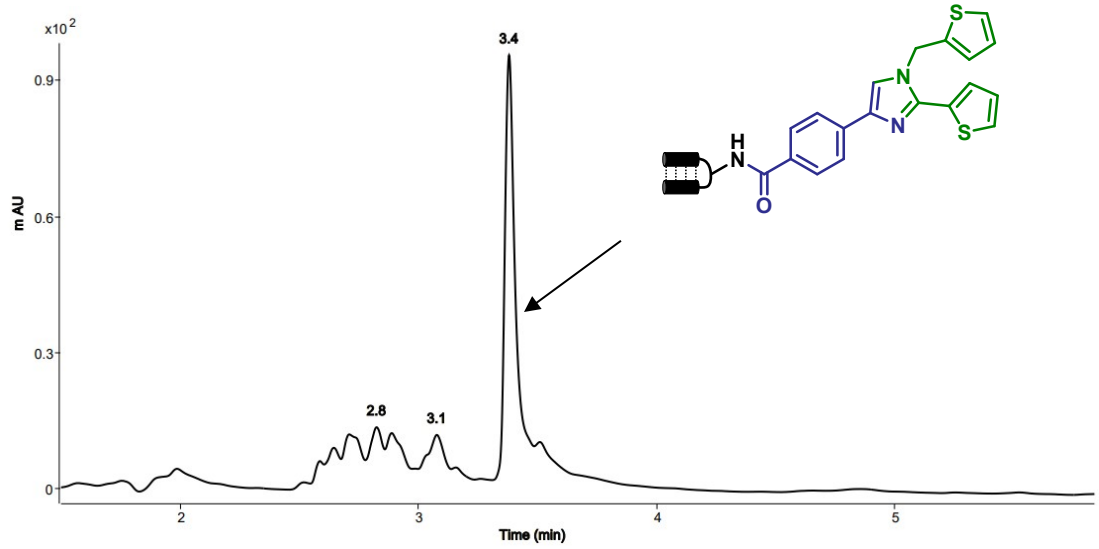


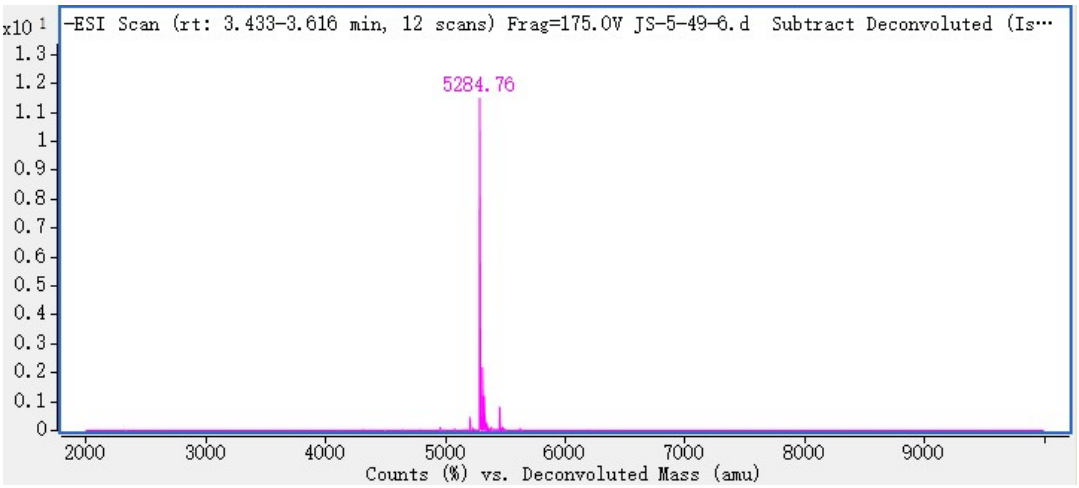


UPLC chromatogram and deconvoluted MS of **5q**

Conversion: 83%

Calculated Mass: 5284 Da; Observed Mass: 5285 Da



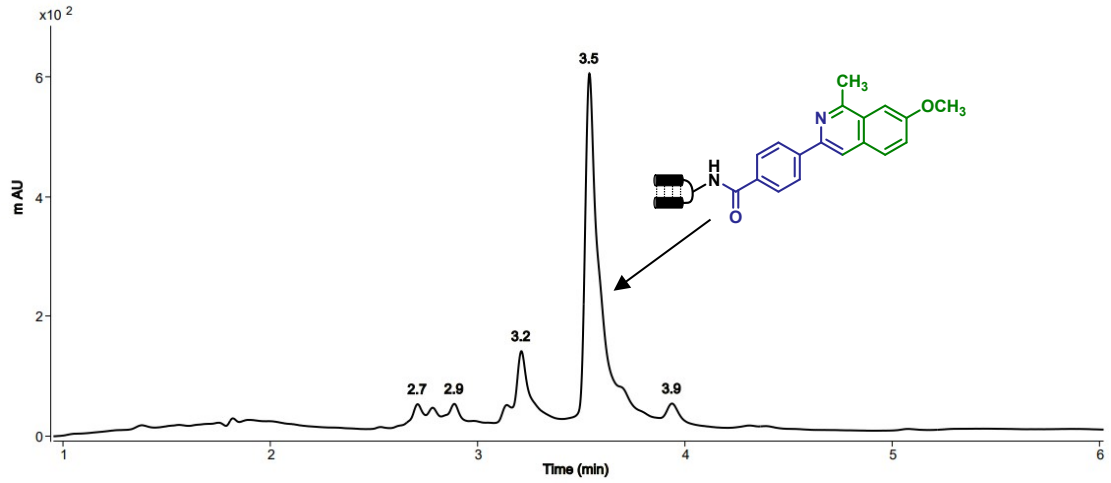


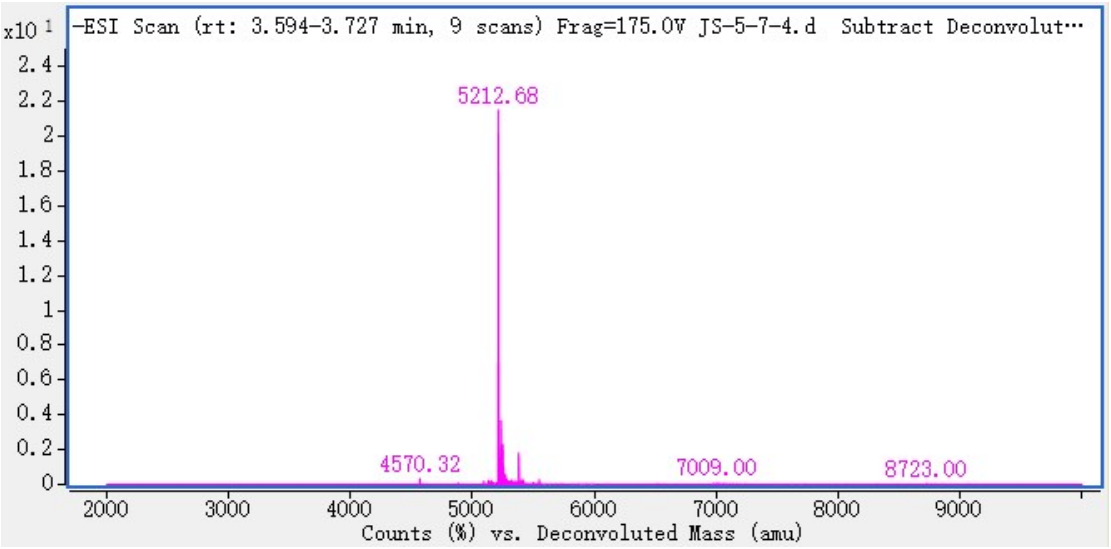
9.4. Substrate Scope of on-DNA Isoquinolines Formation

UPLC chromatogram and deconvoluted MS of **7a**

Conversion: 75%

Calculated Mass: 5212 Da; Observed Mass: 5213 Da

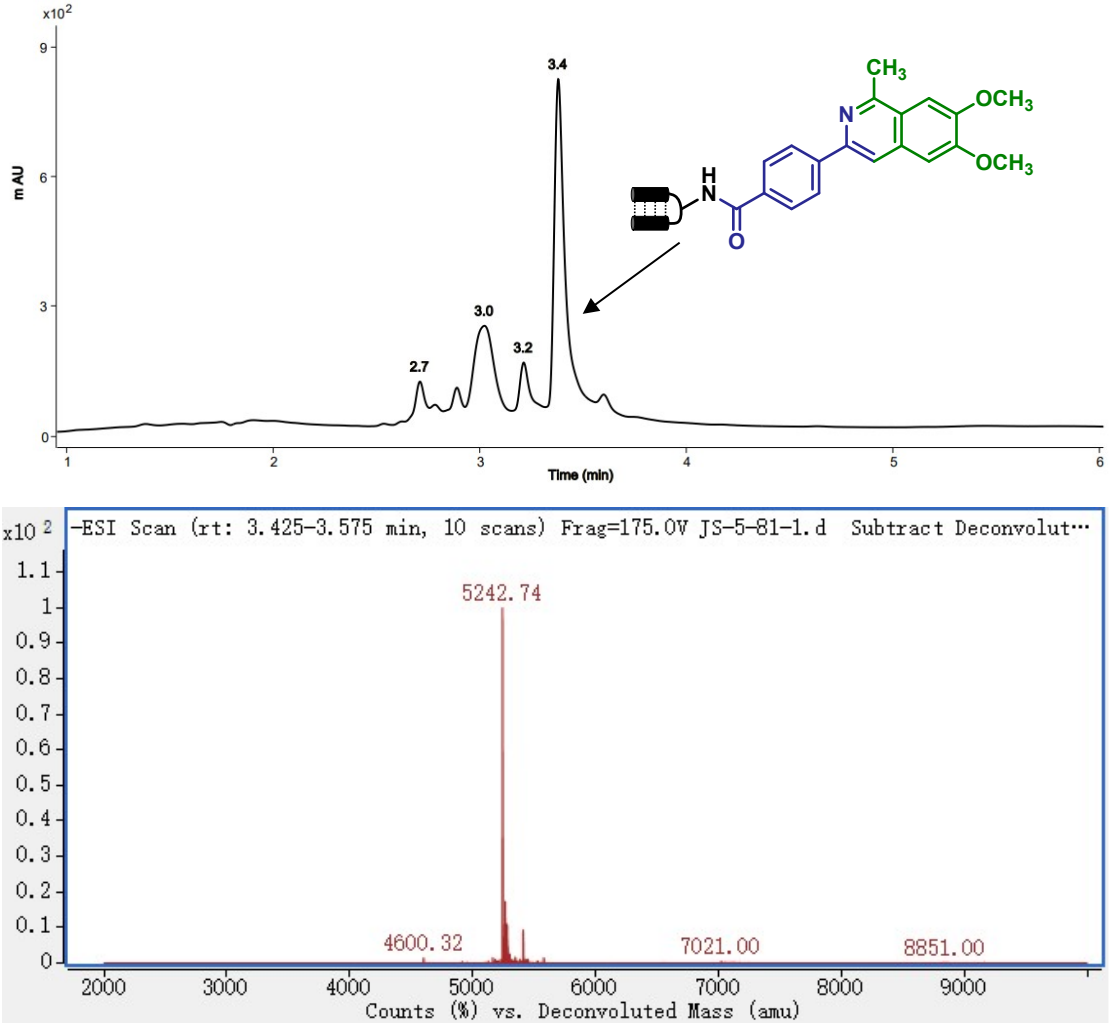




UPLC chromatogram and deconvoluted MS of **7b**

Conversion: 56%

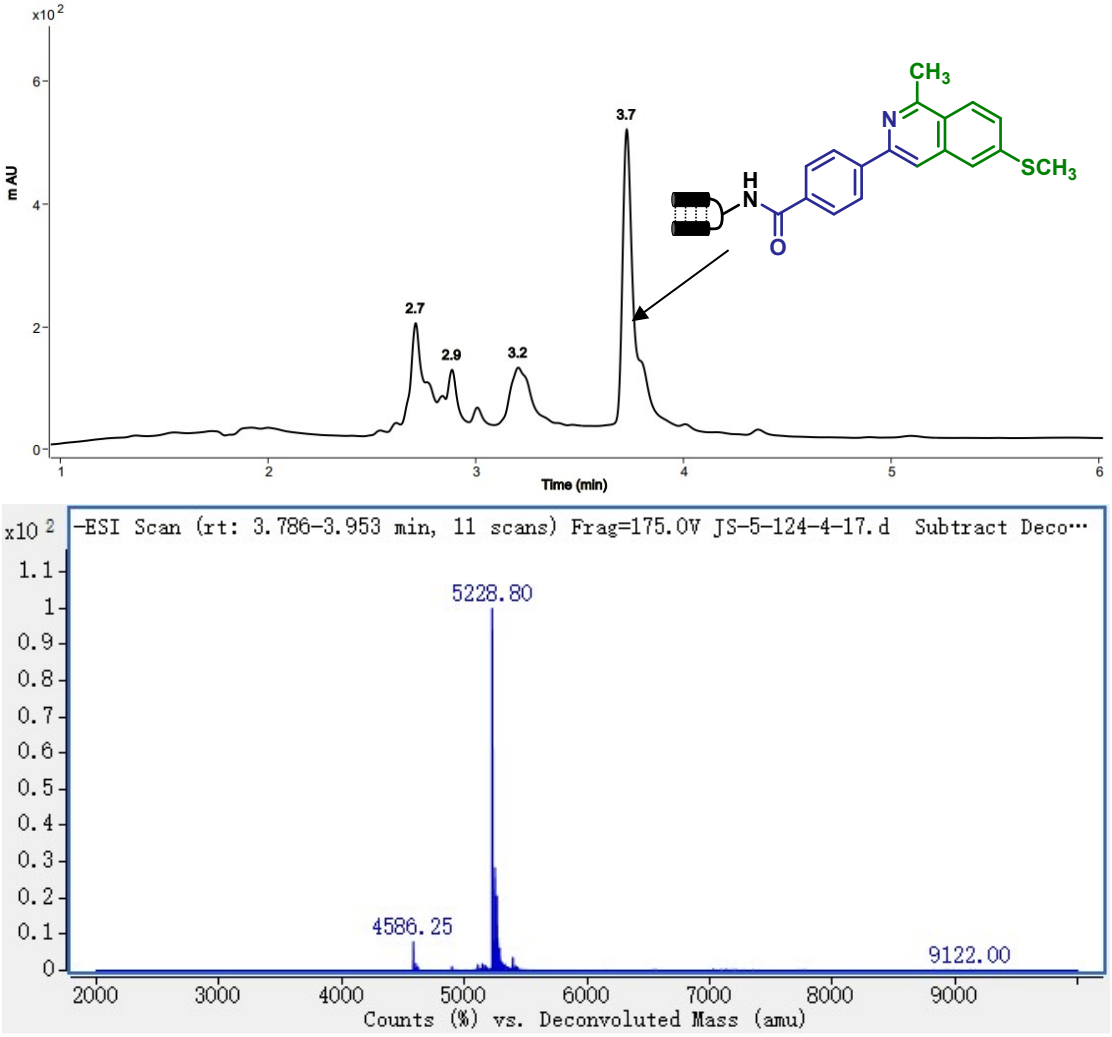
Calculated Mass: 5242 Da; Observed Mass: 5243 Da



UPLC chromatogram and deconvoluted MS of **7c**

Conversion: 53%

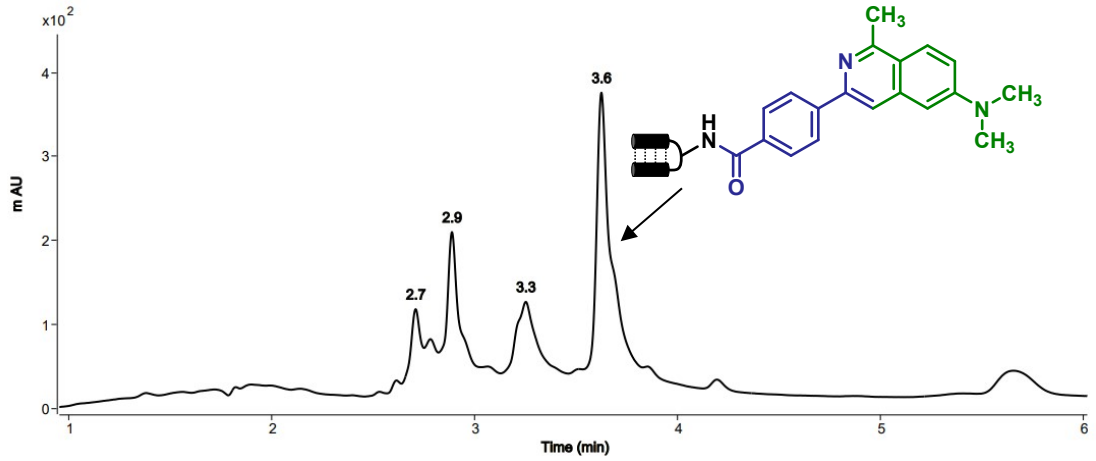
Calculated Mass: 5228 Da; Observed Mass: 5229 Da

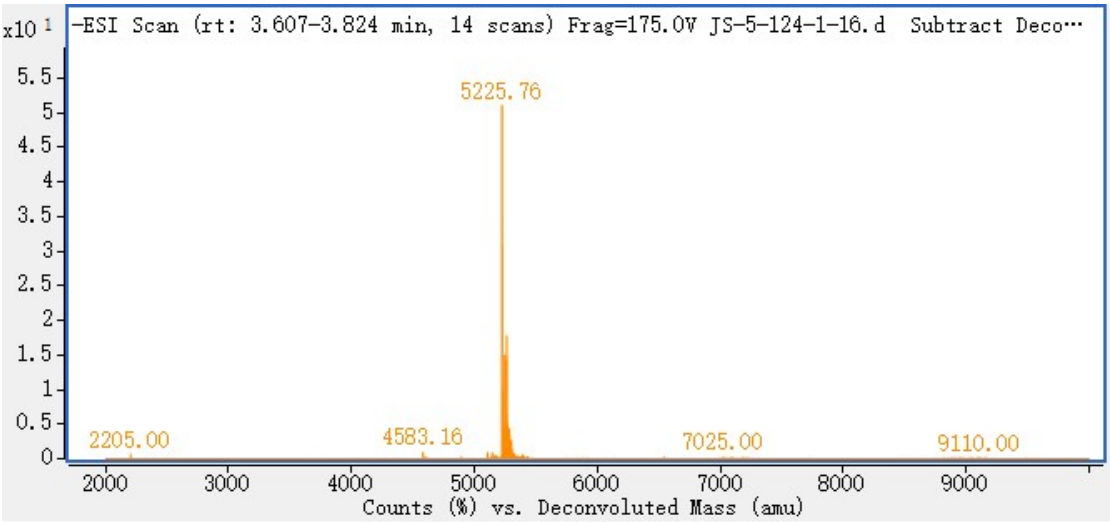


UPLC chromatogram and deconvoluted MS of **7d**

Conversion: 51%

Calculated Mass: 5225 Da; Observed Mass: 5226 Da

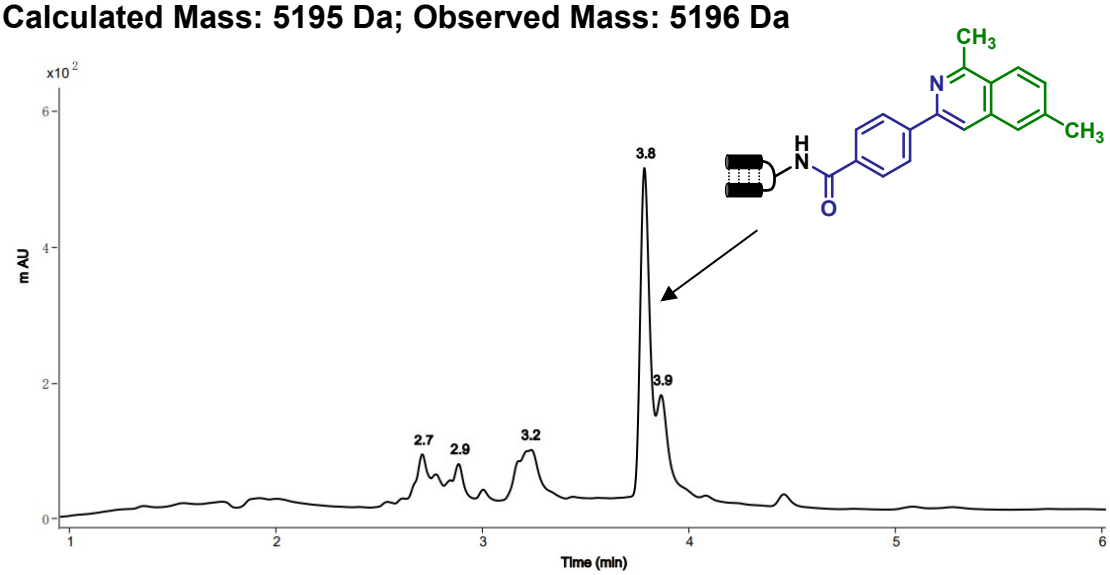


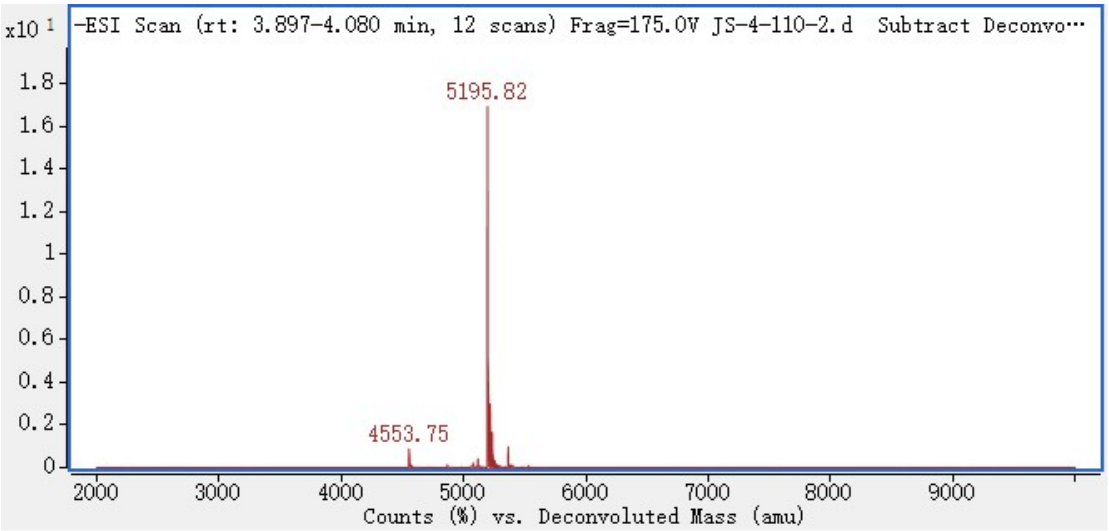


UPLC chromatogram and deconvoluted MS of **7e**

Conversion: 54%

Calculated Mass: 5195 Da; Observed Mass: 5196 Da

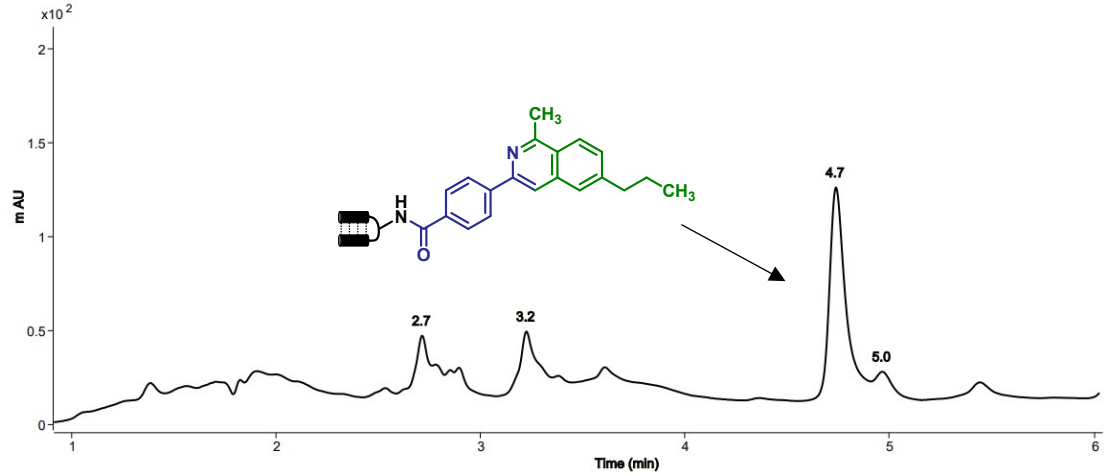


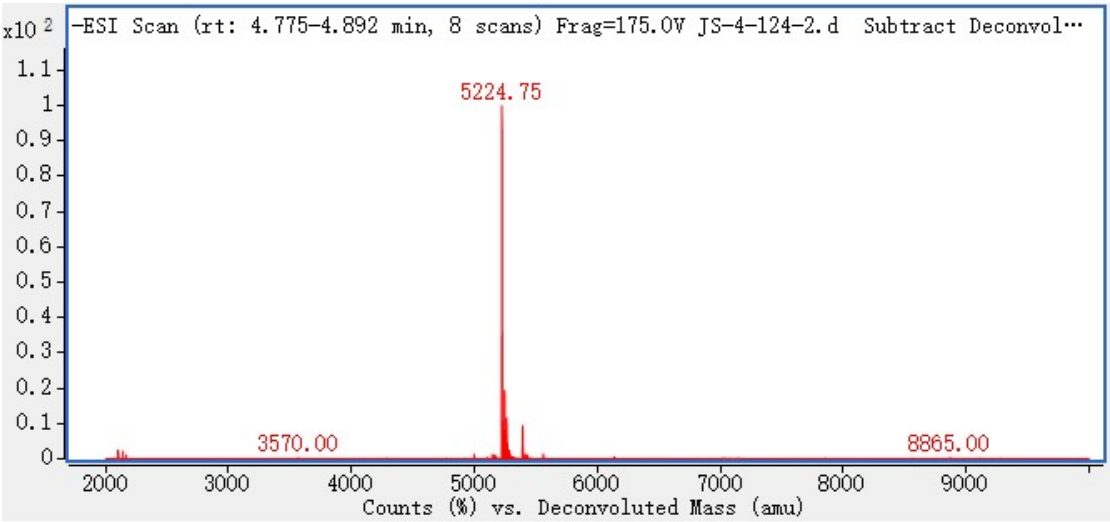


UPLC chromatogram and deconvoluted MS of **7f**

Conversion: 60%

Calculated Mass: 5224 Da; Observed Mass: 5225 Da

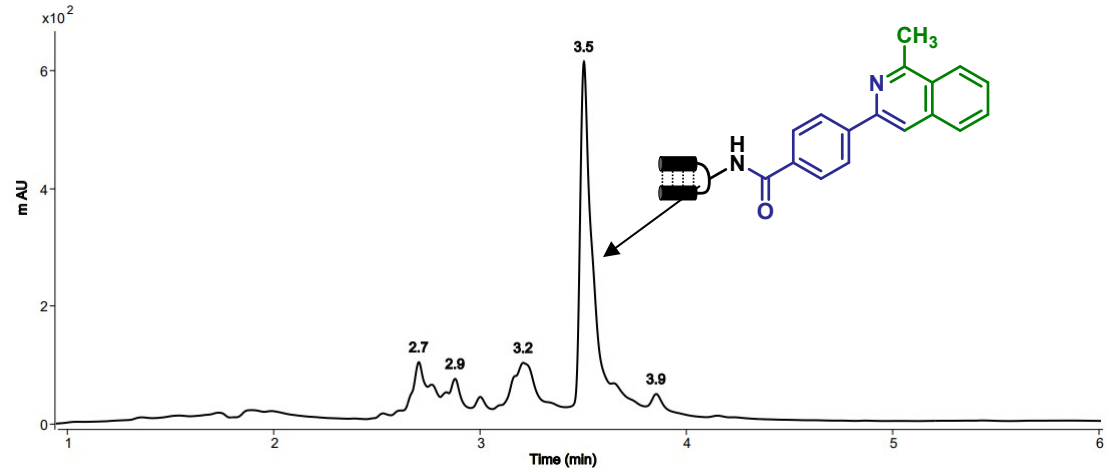


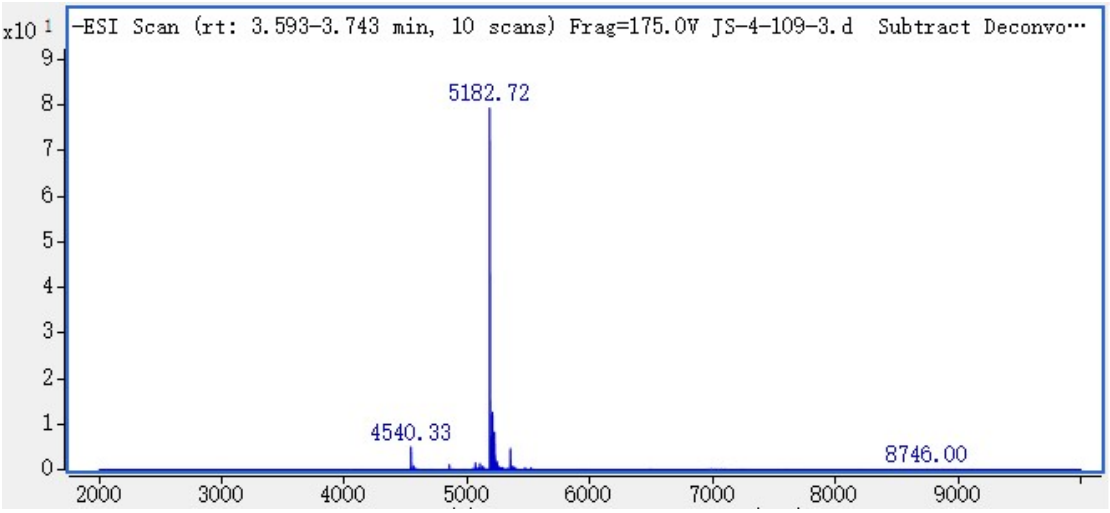


UPLC chromatogram and deconvoluted MS of **7h**

Conversion: 64%

Calculated Mass: 5182 Da; Observed Mass: 5183 Da

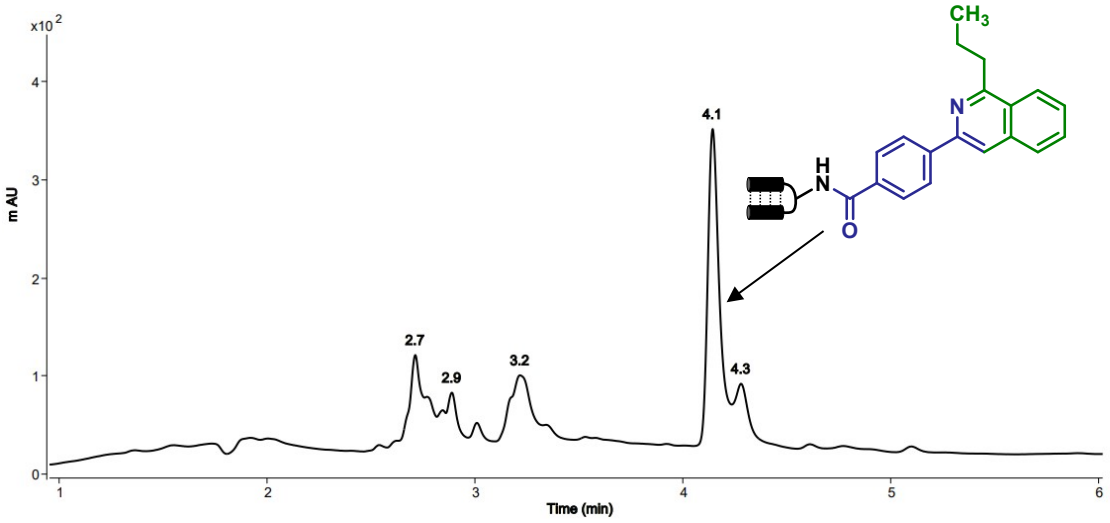


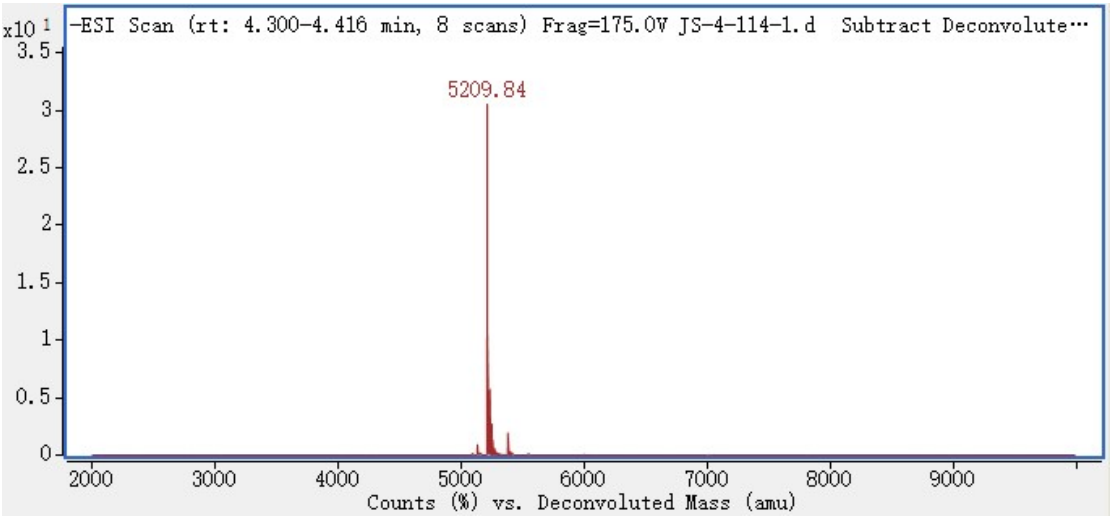


UPLC chromatogram and deconvoluted MS of **7i**

Conversion: 51%

Calculated Mass: 5209 Da; Observed Mass: 5210 Da

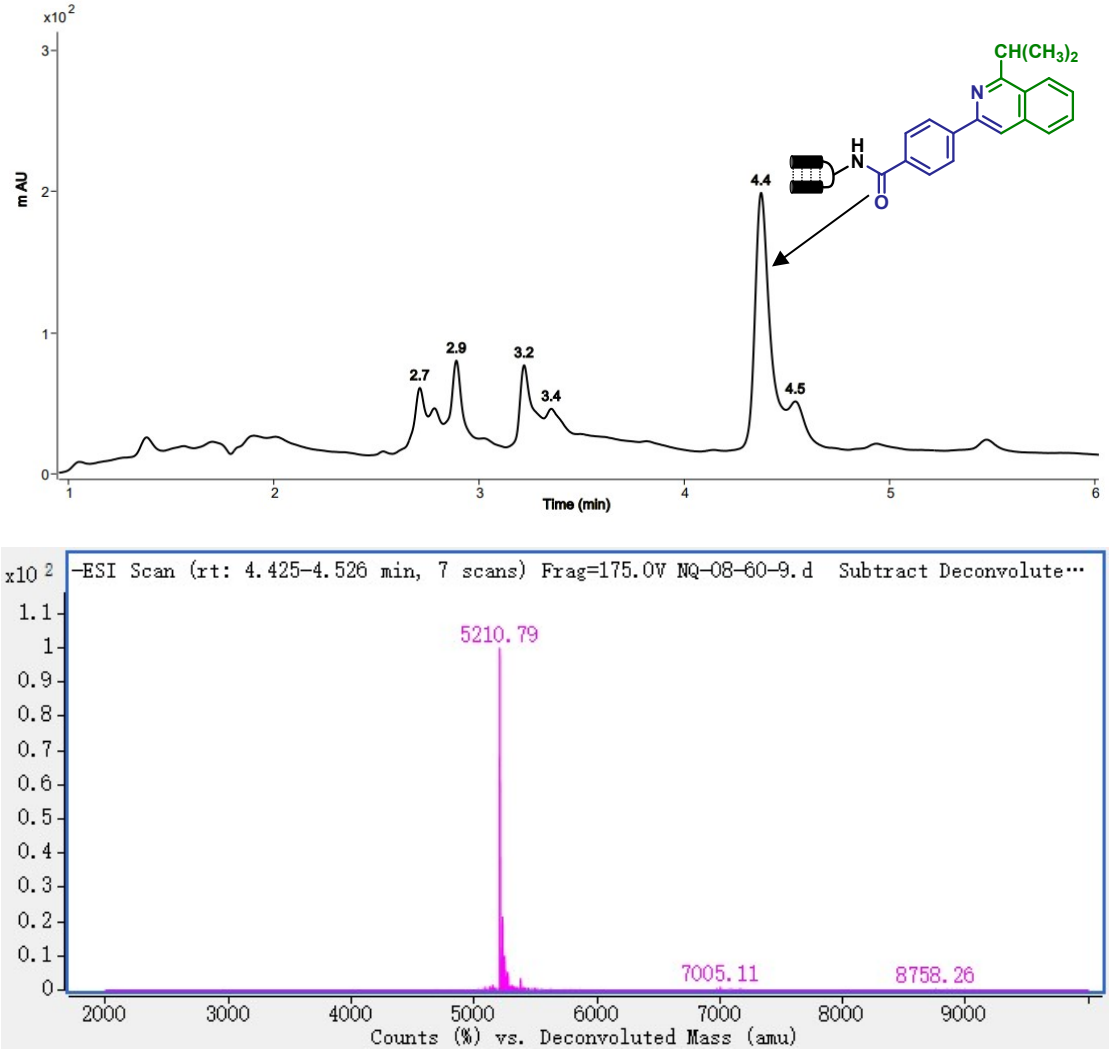




UPLC chromatogram and deconvoluted MS of **7j**

Conversion: 51%

Calculated Mass: 5209 Da; Observed Mass: 5210 Da



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4. Z. Zhu, X. Tang, X. Li, W. Wu, G. Deng and H. Jiang, *J. Org. Chem.*, 2016, **81**, 1401-1409.