Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information

DNA-Compatible Functional Group Transformations via K_2RuO_4-mediated Oxidation

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1. Abbreviations

DIPEA: *N*, *N*-diisopropylethylamine

DMA: *N*, *N*-dimethylacetamide

DMSO: dimethyl sulfoxide

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

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

HP: headpiece

HP-P: headpiece primer

HPLC: high-performance liquid chromatography

$KRuO_4$: potassium perruthenate

K_2RuO_4: potassium ruthenate

MW: molecular weight

PAGE: polyacrylamide gel electrophoresis

TBE: tris-borate-EDTA

TEAA: triethylammonium acetate

TEA: trimethylamine

UHPLC-MS: ultra-high performance liquid chromatography-mass spectrometry

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, MW = 4937), and code sequences were received from HitGen Inc. (Shuangliu District, Chengdu, China). All the DNA sequences were written in 5'- to 3'- orientation unless otherwise noted. Chemicals and reagents were purchased from several commercial suppliers including J&K Scientific, Bidepharm, Adamas, and Sigma-Aldrich, and were generally used from aliquots dissolved in DMA, EtOH, 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. Aqueous solutions, including NaCl (5 M), basic borate buffer (250 mM, sodium borate/boric acid, pH 9.4), and acetate buffer (3 M, sodium acetate/acetic acid, pH 5.2) were prepared in-house. Cestbon water was used in the reactions unless otherwise stated. All the gel images were captured by a Bio-Rad Chemidoc^{TM} image system.

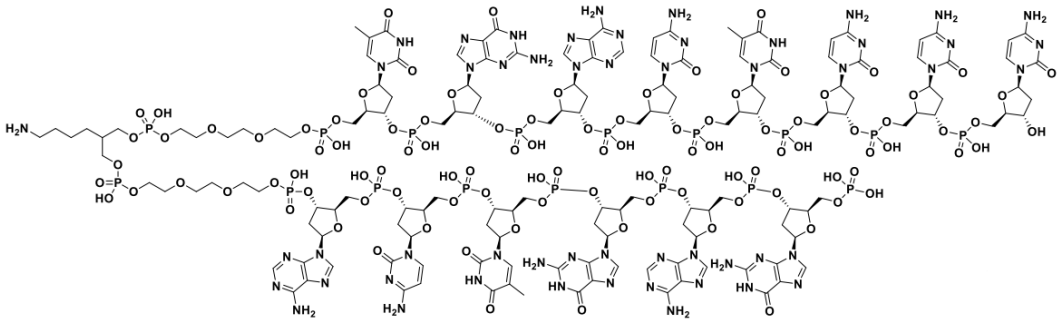


Figure S1. Structure of **HP**.

(5/5Phos/GAGTCA/iSp9/iUniAmM/iSp9/TGACTCCC-3), MW = 4937.

DNA oligonucleotide sequences:

RCHP$_1$-T: NH_2-5'-TGAKTCCCGACCGAAGGTTG-3'

RCHP$_1$-D: 5'-ACCTTCGGTCGGGAGTCA-3'-NH_2

2.2 General methods for DNA analysis

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

UHPLC-MS method of on-DNA synthesis of DNA-Conjugated Acid and Cyanide:

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	15
2	0.3	25
5.5	0.3	30
6	0.3	90
6.5	0.3	90
7	0.3	5
8	0.3	5

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

UHPLC-MS method of co-injection analysis:

Time (min)	Flow (mL/min)	%B
0	0.3	5
1	0.3	15
12	0.3	30
12.1	0.3	90
13	0.3	90
13.1	0.3	5
14	0.3	5

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

UHPLC-MS method of DNA ligation analysis:

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

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

Conversion calculation. The conversion of on-DNA product was determined by UV absorbance (260 nm) peak area integration using the following equation: conversion% = UV (product)/UV (total DNA recovered), ignoring the UV extinction coefficient difference for DNA species and assuming 100% DNA recovery. Any non-oligo material with UV absorbance (260 nm) was subtracted from the conversion calculation.[1]

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

2.3 General methods for DNA conjugates purification

Ethanol precipitation. To an on-DNA reaction mixture add 10% volume of NaCl solution (5 M) and 3 times volume of absolute cold ethanol. Alternatively, a DNA ligation mixture was added 10% volume of acetate buffer (3 M, pH 5.2) and 3 times the volume of absolute cold ethanol. After swirling and centrifuging, the solution was maintained at -80 °C for 2 h and then was centrifuged at 13500 rpm for 30 minutes at 4 °C by Eppendorf 5425R centrifuge. The supernatant was discarded and the pellet was rinsed with 200 μL cold 75% ethanol. After centrifuging at 13500 rpm for 10 minutes at 4 °C, the supernatant was discarded again and the DNA pellet was dried by Speedvac (CV200, JM company, Beijing, China), which was equipped with cryotrap (JM86, JM company, Beijing, China). The recovered sample was dissolved in ddH_2O for subsequent experiments.

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

RP-HPLC method of purification:

Time (min)	Flow (mL/min)	%B
0	4	10
1	4	10
11	4	30
11.1	4	100
12	4	100
12.1	4	10
16	4	10

Solvent A: 100 mM TEAA in H_2O; Solvent B: 100 mM TEAA in 80% ACN

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 was incubated at 20 °C overnight before performing gel analysis. The crude product was purified by ethanol precipitation and used for the next step.

2.5 General procedure for polyacrylamide gel

The ligation reaction was monitored by gel electrophoresis with 20% urea polyacrylamide gel in 1× TBE buffer (89 mM Tris-Borate, 2 mM EDTA, pH 8.3) system referenced by a 20 bp DNA ladder (Takara, Japan). First, the DNA samples were denatured at 95 °C in a dry bath for 10 min and mixed with loading buffer. Then, 5 pmol of treated DNA samples were loaded on the gel, and the gel was run at 200 V for 50 - 60 min. DNA fragments were visualized and analyzed by the Bio-Rad Chemidoc™ Image System (Bio-Rad, CA, USA).

3. General Preparation of potassium ruthenate (K_2RuO_4)

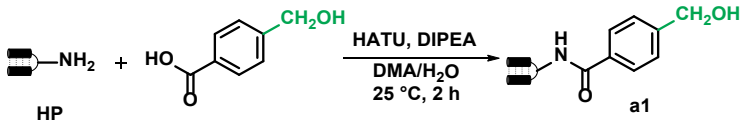
0.15 mmol potassium perruthenate was added to 0.5 M NaOH solution (1 mL) and vortexed to make sure all solid was dissolved. The color of the solution should be yellow/brown (Figure S1). The solution was incubated at 25 °C for 2 days to produce potassium ruthenate (K_2RuO_4). Bubbles were generated during the incubation, and a vortex was performed to get rid of the bubble twice a day. After 2 days, the color should change into a very deep red color (Figure S2). The color of the oxidant solution is indicative of the successful preparation of K_2RuO_4. Next, the K_2RuO_4 solution was aliquoted into 100 parts and stored at -20 °C refrigerator as the 10x stock of oxidant. The solution remains stable for at least two months under storage conditions. If the color changes or precipitation is found, it indicates that the oxidant may have deteriorated and should be freshly prepared again.[2]

Figure s1

Figure s2

4. Preparation and standard reactions of the DNA-conjugated model substrates

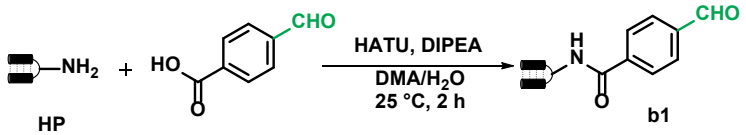
4.1 Preparation of the DNA-conjugated alcohol by amide coupling



HP a1

HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 1 mM solution. 4-(hydroxymethyl)benzoic acid (17 μL, 200 mM in DMA, 170 equiv.), HATU (17 μL, 200 mM in DMA, 170 equiv.), and DIPEA (17 μL, 200 mM in DMA, 170 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 (20 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 analyzed by UHPLC-MS. Deconvoluted molecular mass: calculated: 5071 Da; observed: 5071 Da.

4.2 Preparation of the DNA-conjugated aldehyde by amide coupling

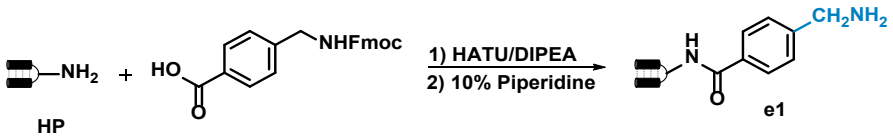


HP b1

HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 1 mM solution. 4-formylbenzoic acid (17 μL, 200 mM in DMA, 170 equiv.), HATU (17 μL, 200 mM in DMA, 170 equiv.), and DIPEA (17 μL, 200 mM in DMA, 170 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 (20 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 analyzed by UHPLC-MS. Deconvoluted molecular mass: calculated: 5069 Da; observed: 5069 Da.

4.3 Preparation of the DNA-conjugated amine by amide coupling

Scheme S1. Synthesis of compound **a1**.

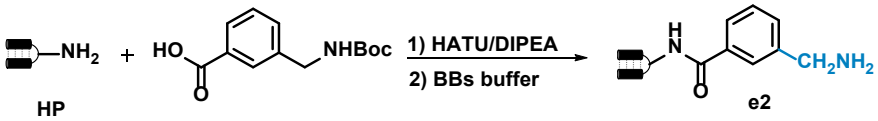


HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 1 mM solution. Fmoc-4-(aminomethyl) benzoic acid (17 μL, 200 mM in DMA, 170 equiv.), HATU (17 μL, 200 mM in DMA, 170 equiv.), and DIPEA (17 μL, 200 mM in DMA, 170 equiv.) were mixed by vortex and allowed to pre-activate for 10 minutes at 25 °C, and then the mixture was transferred to **HP** solution (20 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 analyzed by UHPLC-MS.

The lyophilized pellet of DNA (20 nmol) was then deprotected by exposure to 100 mL of 10% v/v piperidine in water. The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 20 min. After ethanol precipitation, the reaction was analyzed by UHPLC-MS. The separated and collected conjugates were purified by preparative HPLC and vacuum-dried overnight, redissolved in H_2O for subsequent experiments. Deconvoluted molecular mass: calculated: 5070 Da; observed: 5070 Da.

Unless otherwise noted, on-DNA amidation and de-Fmoc described in the supporting information were synthesized under this standard condition.

Scheme S2. Synthesis of compound **a2**.



HP was dissolved in sodium borate buffer (250 mM, pH 9.4) to make 1 mM solution. Boc-3-(aminometmyl) benzoic acid (17 μL, 200 mM in DMA, 170 equiv.), HATU (17 μL, 200 mM in DMA, 170 equiv.), and DIPEA (17 μL, 200 mM

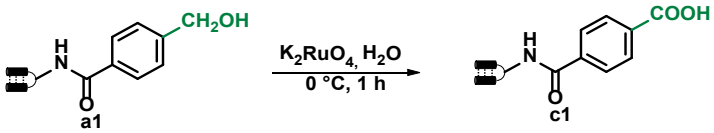
in DMA, 170 equiv.) were mixed by vortex and allowed to pre-activate for 10 minutes at 25 °C, and then the mixture was transferred to **HP** solution (20 μL, 20 nmol). The reaction mixture was vortexed, centrifuged, and allowed to proceed at 25 °C for 2 h. After ethanol precipitation, the reaction was analyzed by UHPLC-MS.

The lyophilized pellet of DNA (20 nmol) was reconstituted in pH 9.4 sodium borate buffer (20 μL, 250 mM, 250 eq.) and heated at 90 °C for 16 hours. After ethanol precipitation, the reaction was analyzed by UHPLC-MS. The separated and collected conjugates were purified by preparative HPLC and vacuum-dried overnight, redissolved in H_2O for subsequent experiments. Deconvoluted molecular mass: calculated: 5070 Da; observed: 5070 Da.

Unless otherwise noted, on-DNA amidation and de-Boc described in the supporting information were synthesized under this standard condition.

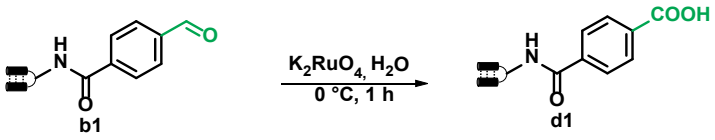
4.4 Preparation of the DNA-conjugated carboxylic acid and nitrile by the standard oxidation protocol

Type A



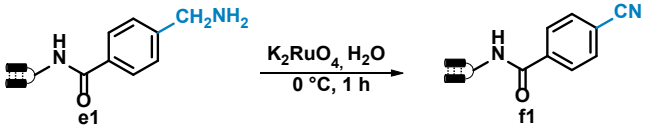
To the solution of **a1** (0.2 nmol, 2 μL, 100 μM in H_2O, 1 equiv.) was added K_2RuO_4(15nmol, 1 μL, 15 mM in 500 mM NaOH, 75 equiv.), H_2O (17 μL). The reaction mixture was vortexed, centrifuged, and incubated at 0 °C for 1 h. After the reaction, 30 equiv. of sodium diethyldithiocarbamic acid (compared with K_2RuO_4) was added to the mixture, and the reaction mixture was stood at 25 °C for 30 minutes. The mixture was centrifuged at 4 °C for 30 min at 13,500 rpm, and the resultant supernatant was collected. The product was obtained by ethanol precipitation and analyzed by UHPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5085 Da; observed: 5085 Da.

Type B



To the solution of **b1** (0.2 nmol, 2 μL, 100 μM in H_2O, 1 equiv.) was added K_2RuO_4(15nmol, 1 μL, 15 mM in 500 mM NaOH, 75 equiv.), H_2O (17 μL). The reaction mixture was vortexed, centrifuged, and incubated at 0 °C for 1 h. After the reaction, 30 equiv. of sodium diethyldithiocarbamic acid (compared with K_2RuO_4) was added to the mixture, and the reaction mixture was stood at 25 °C for 30 minutes. The mixture was centrifuged at 4 °C for 30 min at 13,500 rpm, and the resultant supernatant was collected. The product was obtained by ethanol precipitation and analyzed by UHPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5085 Da; observed: 5085 Da.

Type C

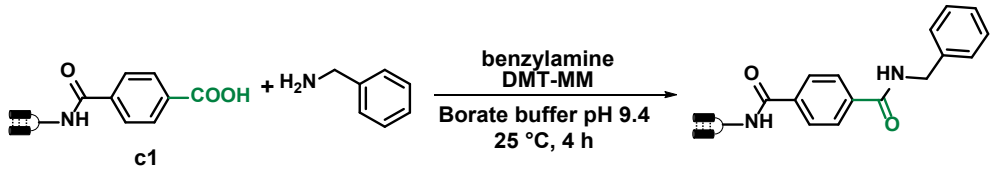


To the solution of **e1** (0.2 nmol, 2 μL, 100 μM in H_2O, 1 equiv.) was added K_2RuO_4 (15nmol, 1 μL, 15 mM in 500 mM NaOH, 75 equiv.), H_2O (17 μL). The reaction mixture was vortexed, centrifuged, and incubated at 0 °C for 1 h. After the reaction, 30 equiv. of sodium diethyldithiocarbamic acid (compared with K_2RuO_4) was added to the mixture, and the reaction mixture was stood at 25 °C for 30 minutes. The mixture was centrifuged at 4 °C for 30 min at 13,500 rpm, and the resultant supernatant was collected. The product was obtained by ethanol precipitation and analyzed by UHPLC-MS (Conversion: >90%). Deconvoluted molecular mass: calculated: 5065 Da; observed: 5065 Da.

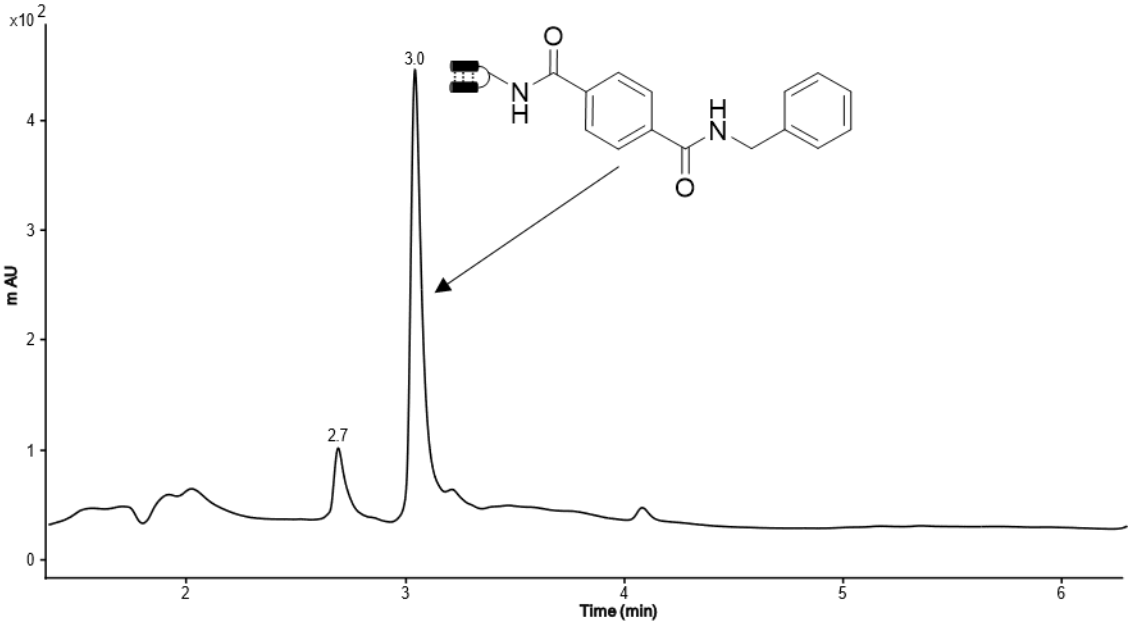
5. Structural confirmation of on-DNA K_2RuO_4-mediated oxidation products

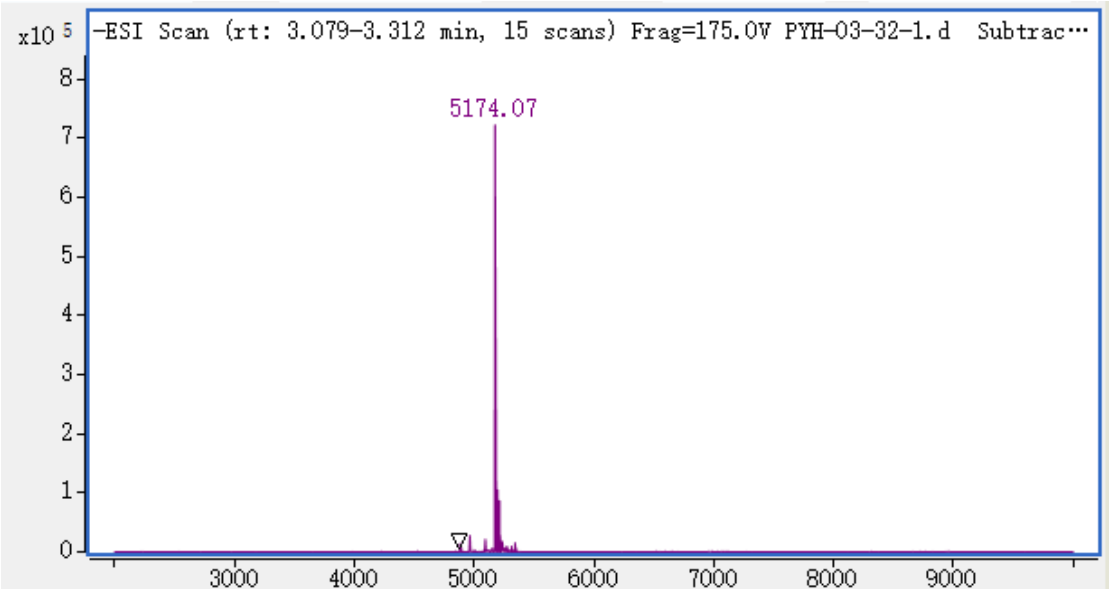
5.1 Structural confirmation of the DNA-conjugated carboxylic acid

5.1.1 General procedure for amide coupling



c1

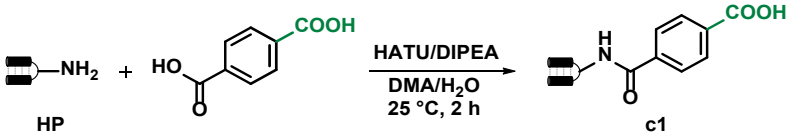




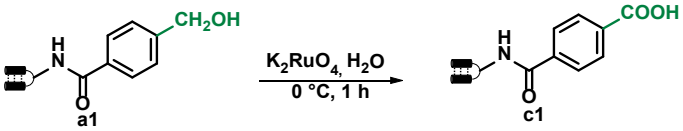
To the solution of **c1** (0.2 nmol, 1 μL, 200 μM in H_2O, 1 equiv.) was added benzylamine (1500 nmol, 5 μL, 300 mM in ACN/H_2O, 1:1, 750 equiv.), Borate buffer pH 9.4 (2250 nmol, 9 μL, 250 mM in H_2O, 11250 equiv.), DMT-MM (4500 nmol, 5 μL, 900 mM in H_2O, 22500 equiv.). The reaction mixture was vortexed, centrifuged, and incubated at 25 °C for 12 h. The product was obtained by ethanol precipitation and analyzed by UHPLC-MS. Deconvoluted molecular mass: calculated: 5174 Da; observed: 5174 Da.[3]

5.1.2 Co-injection experiment

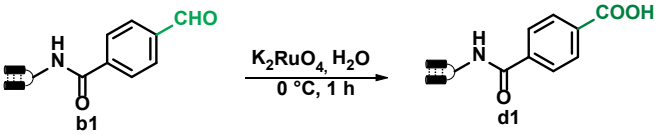
Route A



Route B



Route C



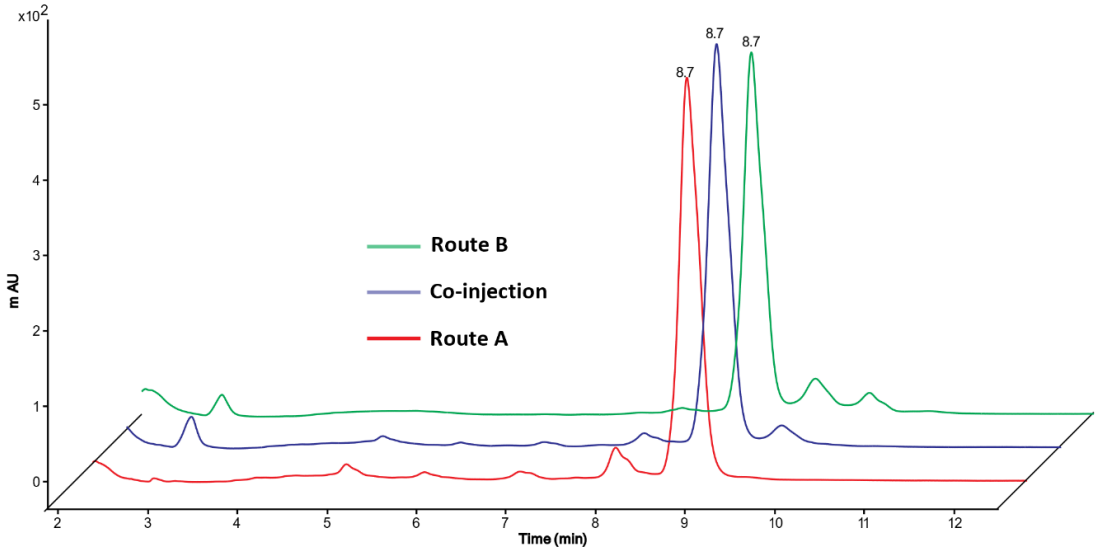


Figure S3. Co-injection experiment from two independent synthetic routes.

HPLC chromatography showed that the peak of the co-injection (blue curve) had the same retention time as the other two samples (route A, red curve; route B, green curve).

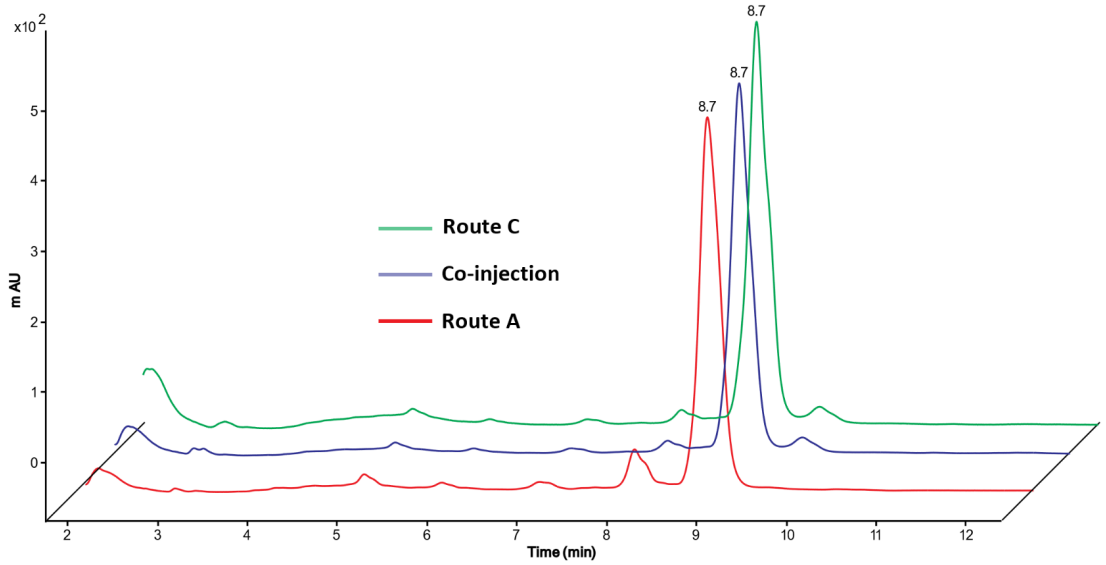
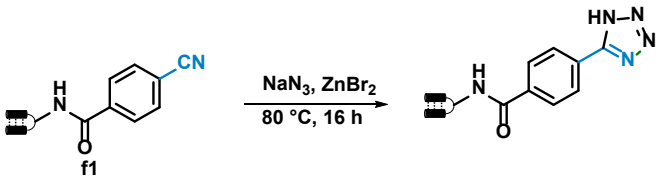
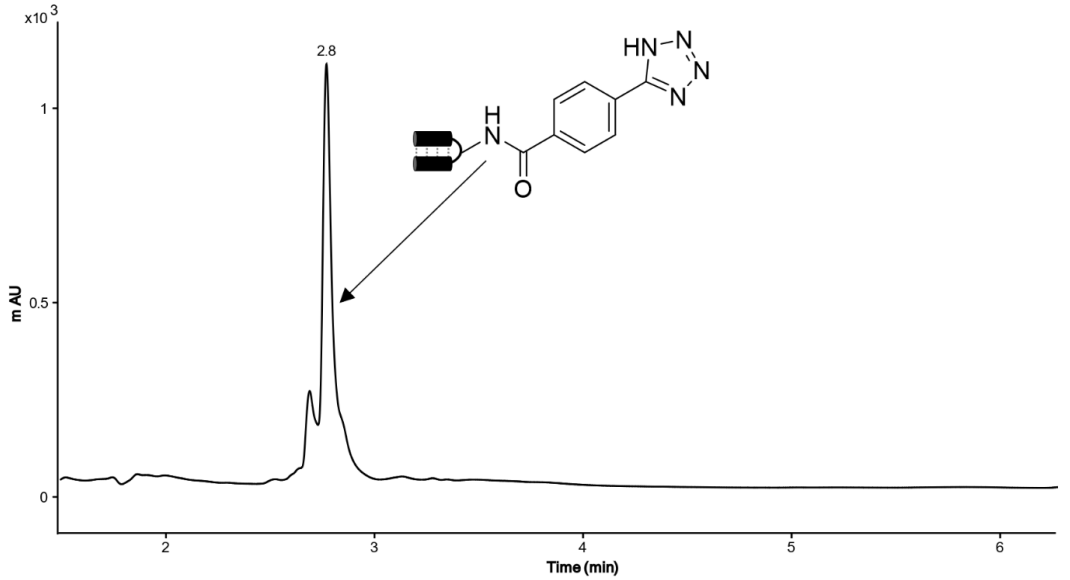


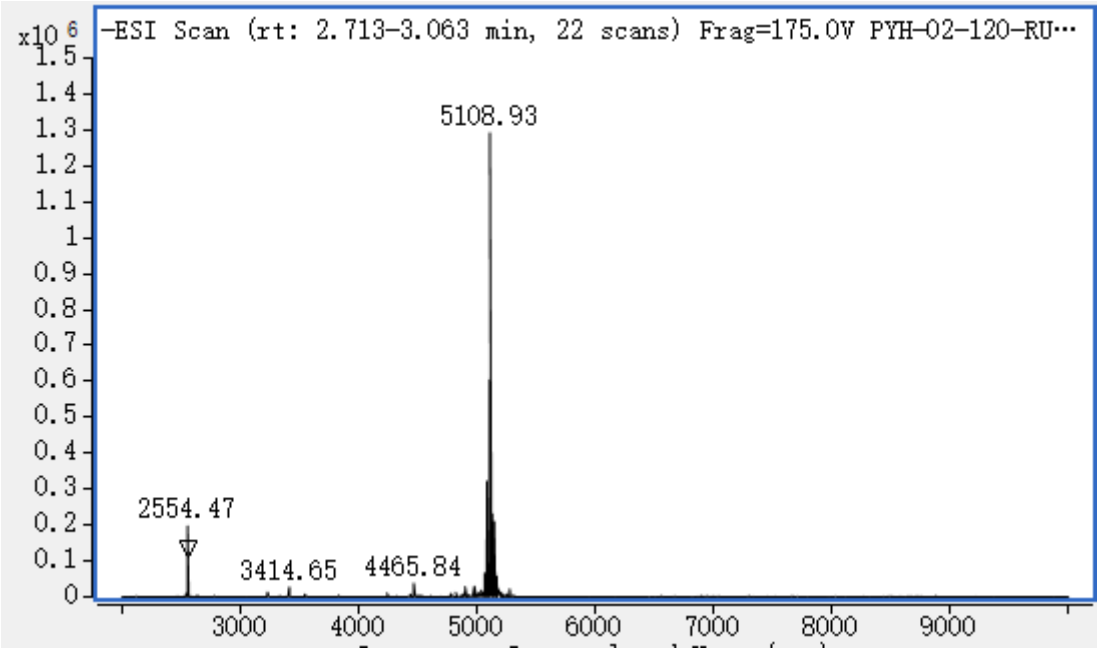
Figure S4. Co-injection experiment from two independent synthetic routes. HPLC chromatography showed that the peak of the co-injection (blue curve) had the same retention time as the other two samples (route A, red curve; route C, green curve).

5.2 Structural confirmation of the DNA-conjugated nitrile

5.2.1 General procedure for 5-substituted tetrazole formation





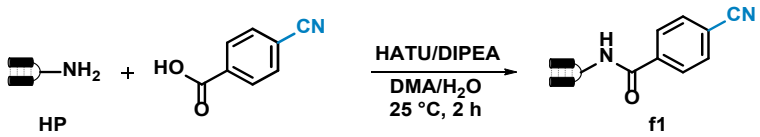


DNA-conjugated nitriles were reconstituted to the concentration of 1.0 mM after the attaching procedure. pH 5.8 Mes buffer (2500 nmol, 10 μL, 250 mM, 250 equiv) was added to DNA conjugates (10 nmol, 10 μL, 1.0 mM in H_2O, 1 equiv) followed by the addition of 1,4-dioxane (18 μL), NaN_3(2000 nmol, 5 μL, 400 mM in H_2O, 200 equiv), and $ZnBr_2$ (500 nmol, 2 μL, 250 mM in H_2O, 50 equiv). The reaction mixture was heated at 80 °C for 16 h. After the reaction mixture was cooled down to room temperature, sodium cysteinate (1000 nmol, 5 μL, 200 mM in H_2O, 100 equiv) was added and then heated at 80 °C for 15 min before being quenched by EtOH precipitation. The product was obtained by ethanol

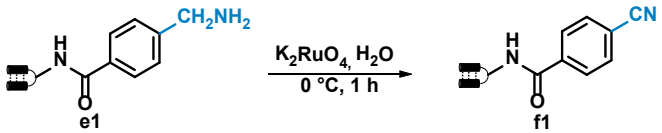
precipitation and analyzed by UHPLC-MS. Deconvoluted molecular mass: calculated: 5109 Da; observed: 5109 Da..[4]

5.2.2 Co-Injection experiment

Route A



Route B



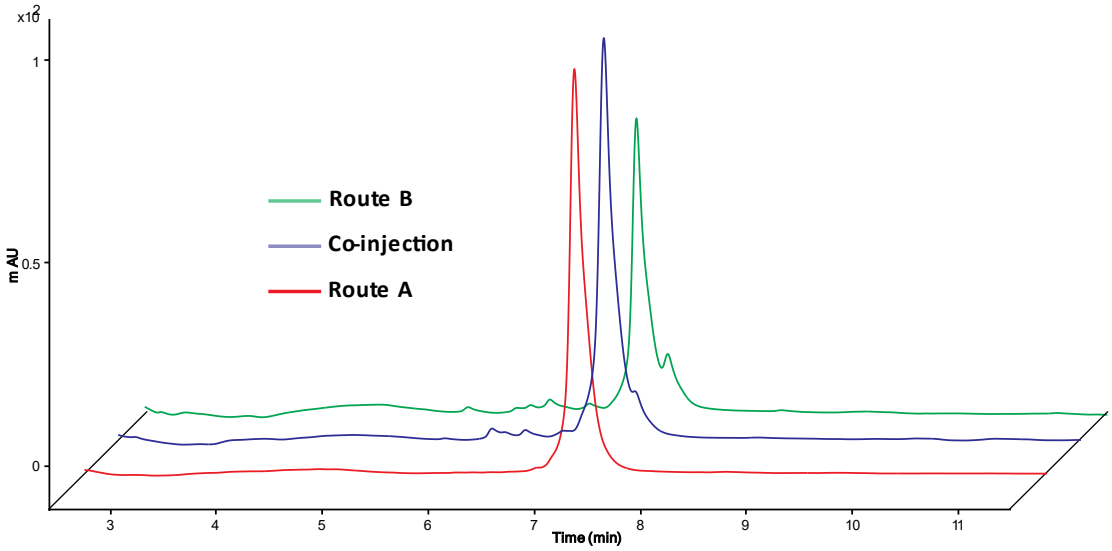


Figure S5. Co-injection experiment from two independent synthetic routes. HPLC chromatography showed that the peak of the co-injection (blue curve) had the same retention time as the other two samples (route A, red curve; route B, green curve).

6. Scale-up reactions

Type A

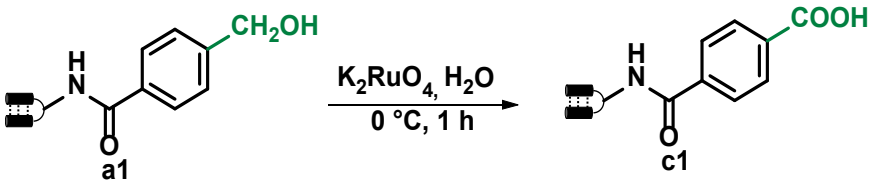
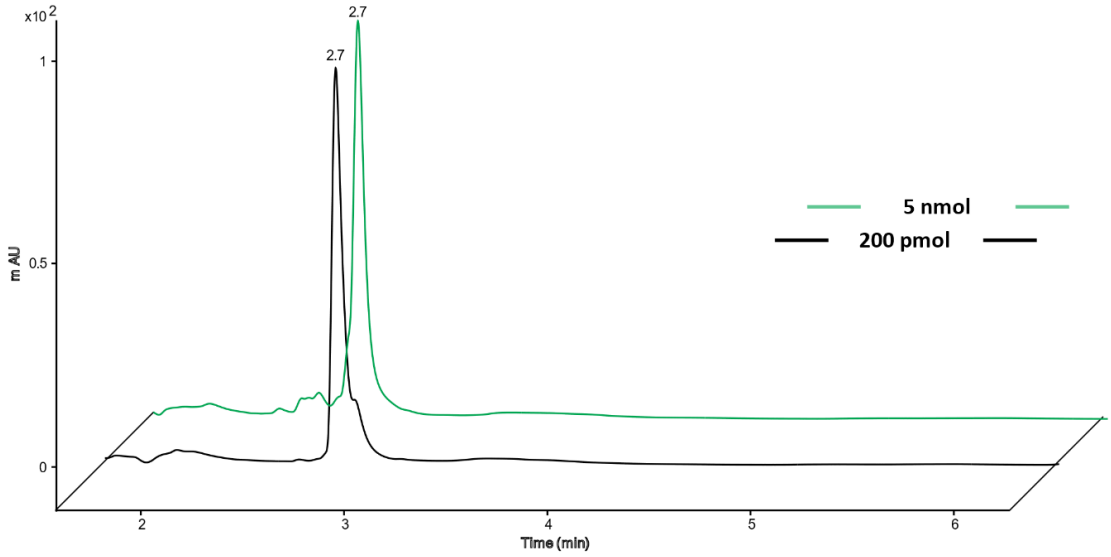


Figure S6. UHPLC chromatogram of **a1** at 200 pmol and 5 nmol scales.



To the solution of **a1** (5 nmol, 5 μL, 1 mM in H_2O, 1 equiv.) was added K_2RuO_4 (75nmol, 5 μL, 15 mM in 500 mM NaOH, 25 equiv.), H_2O (10 μL). The reaction mixture was vortexed, centrifuged, and incubated at 0 °C for 1 h. The product was obtained by ethanol precipitation and analyzed by UHPLC-MS (Conversion: 85%). Deconvoluted molecular mass: calculated: 5085 Da; observed: 5085 Da.

Type B

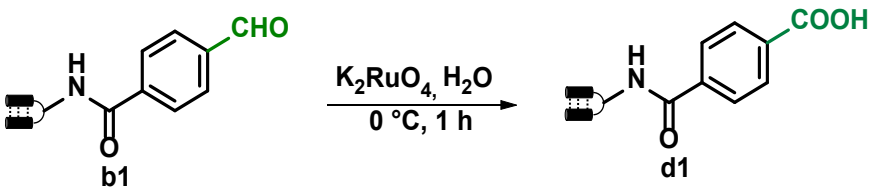
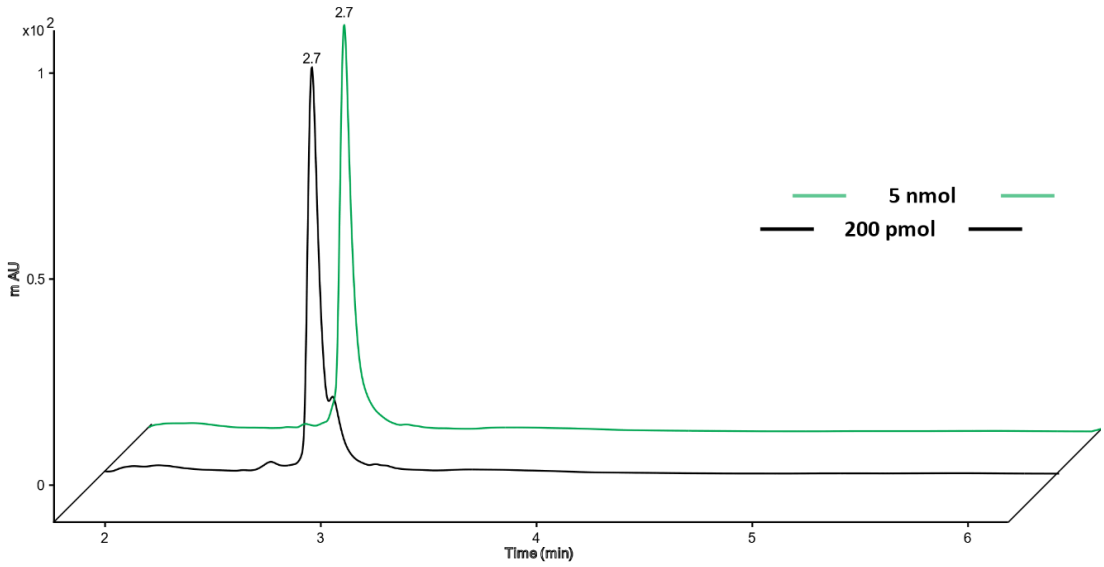


Figure S7. UHPLC chromatogram of **b1** at 200 pmol and 5 nmol scales.



To the solution of **b1** (5 nmol, 5 μL, 1 mM in H_2O, 1 equiv.) was added K_2RuO_4 (75nmol, 5 μL, 15 mM in 500 mM NaOH, 25 equiv.), H_2O (10 μL). The reaction mixture was vortexed, centrifuged, and incubated at 0 °C for 1 h. The product was obtained by ethanol precipitation and analyzed by UHPLC-MS (Conversion: 85%). Deconvoluted molecular mass: calculated: 5085 Da; observed: 5085 Da.

Type C

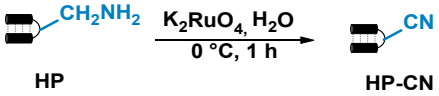
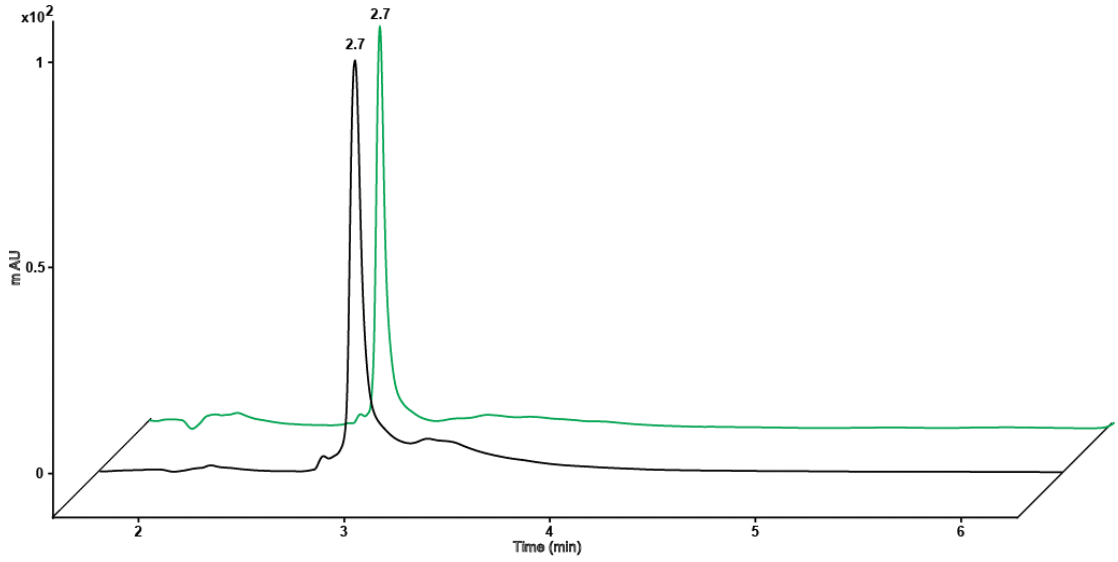


Figure S8. UHPLC chromatogram of **HP** at 200 pmol and 5 nmol scales.



To the solution of **HP** (5 nmol, 5 μL, 1 mM in H_2O, 1 equiv.) was added K_2RuO_4

(75nmol, 5 μL, 15 mM in 500 mM NaOH, 25 equiv.), H_2O (10 μL). The reaction mixture was vortexed, centrifuged, and incubated at 0 °C for 1 h. The product was obtained by ethanol precipitation and analyzed by UHPLC-MS (Conversion: 85%). Deconvoluted molecular mass: calculated: 4933 Da; observed: 4933 Da.

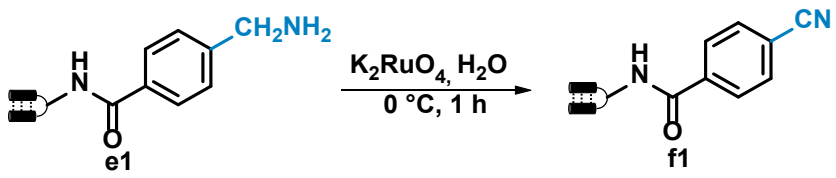
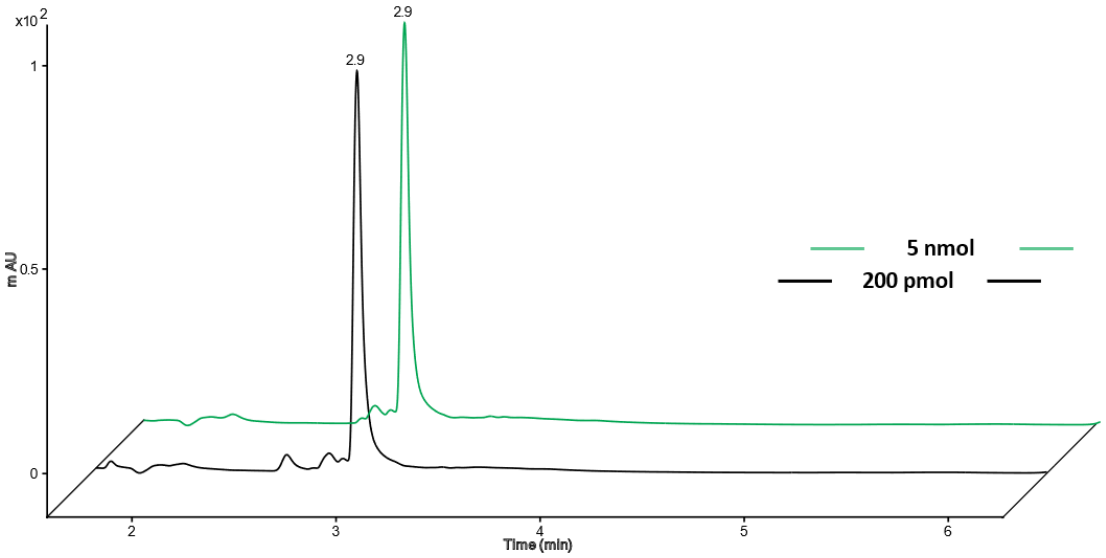


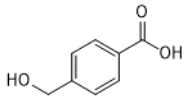
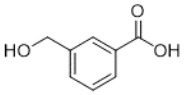
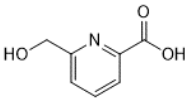
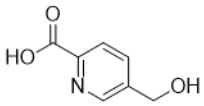
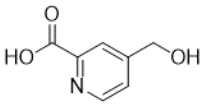
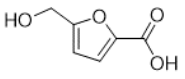
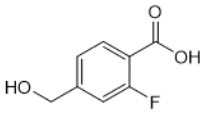
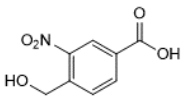
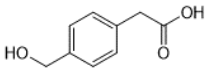
Figure S9. UHPLC chromatogram of **e1** at 200 pmol and 5 nmol scales.

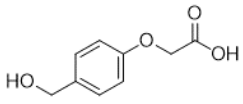
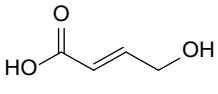
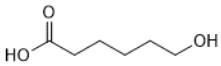
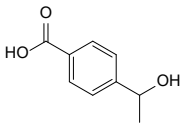


To the solution of **e1** (5 nmol, 5 μL, 1 mM in H_2O, 1 equiv.) was added K_2RuO_4 (75nmol, 5 μL, 15 mM in 500 mM NaOH, 25 equiv.), H_2O (10 μL). The reaction mixture was vortexed, centrifuged, and incubated at 0 °C for 1 h. The product was obtained by ethanol precipitation and analyzed by UHPLC-MS (Conversion: 85%). Deconvoluted molecular mass: calculated: 5066 Da; observed: 5066 Da.

7. UPLC chromatogram and deconvoluted MS

7.1 Substrate scope of DNA-conjugated alcohols

Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
a1		**c1**	5085	5085	**>90%**
a2		**c2**	5085	5085	**>90%**
a3		**c3**	5086	5086	**>90%**
a4		**c4**	5086	5086	**>90%**
a5		**c5**	5086	5086	**>90%**
a6		**c6**	5075	5075	**>90%**
a7		**c7**	5103	5103	**>90%**
a8		**c8**	5130	5130	**>90%**
a9		**c9**	5099	5099	**>90%**

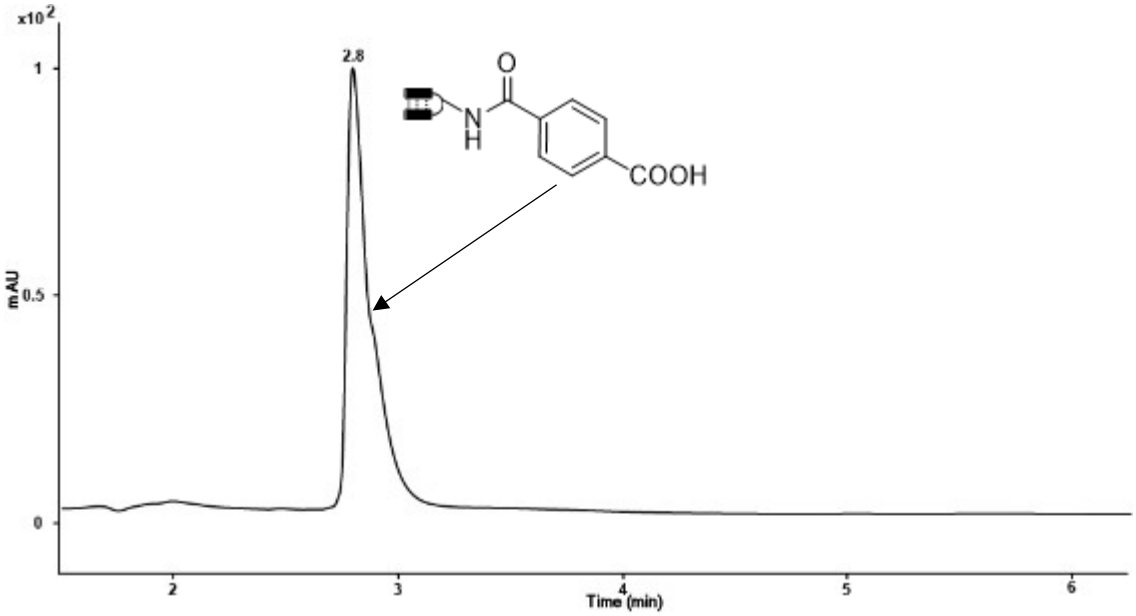
a10		**c10**	5115	5115	**>90%**
a11		**c11**	5035	5035	**>90%**
a12		**c12**	5065	5051	**0%**
a13		**c13**	5083	5083	**>90%***

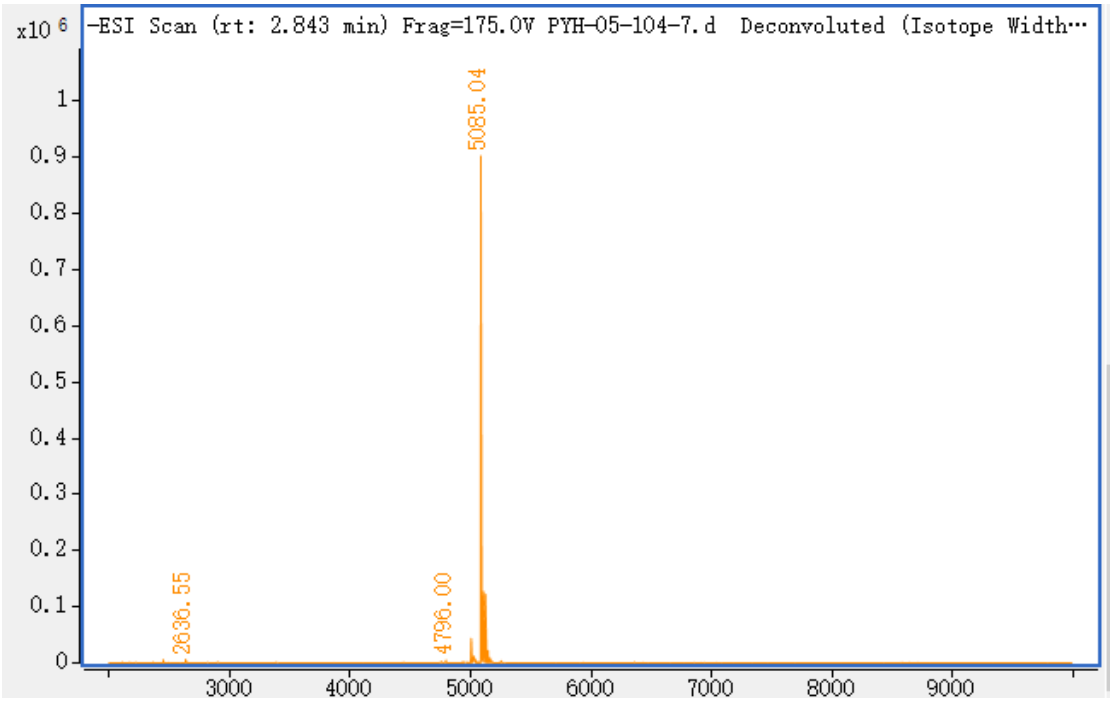
* The α-substituted benzyl alcohol produced the oxidation product as the ketone. Further oxidation could not occur.

UPLC chromatogram and deconvoluted MS of **a1**

Conversion: ＞90%

Calculated Mass: 5085 Da; Observed Mass: 5085 Da

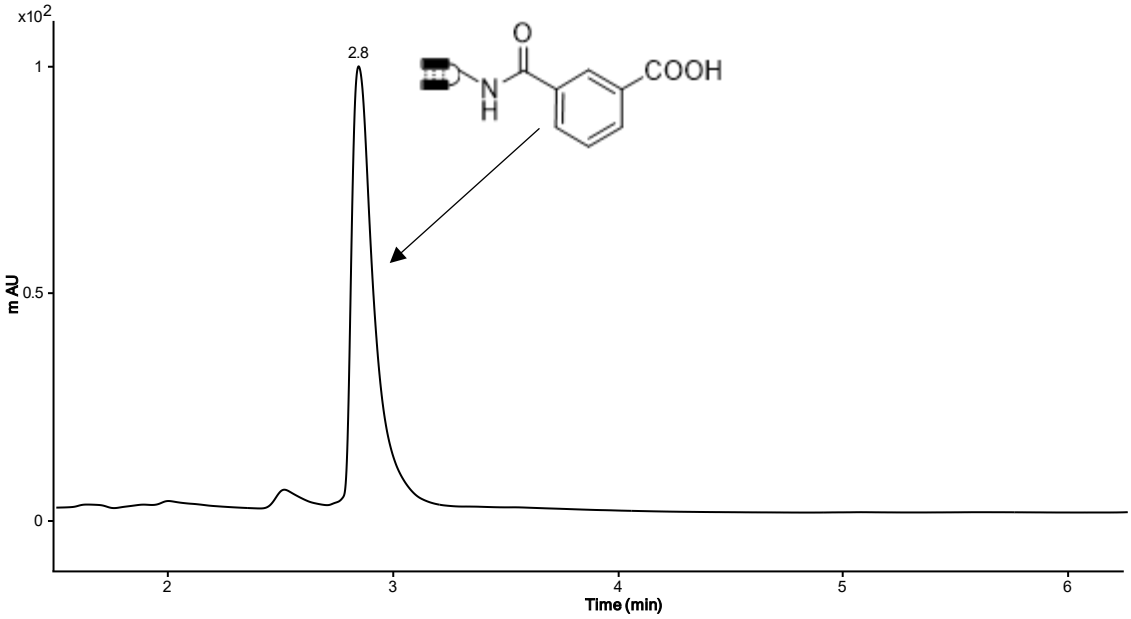


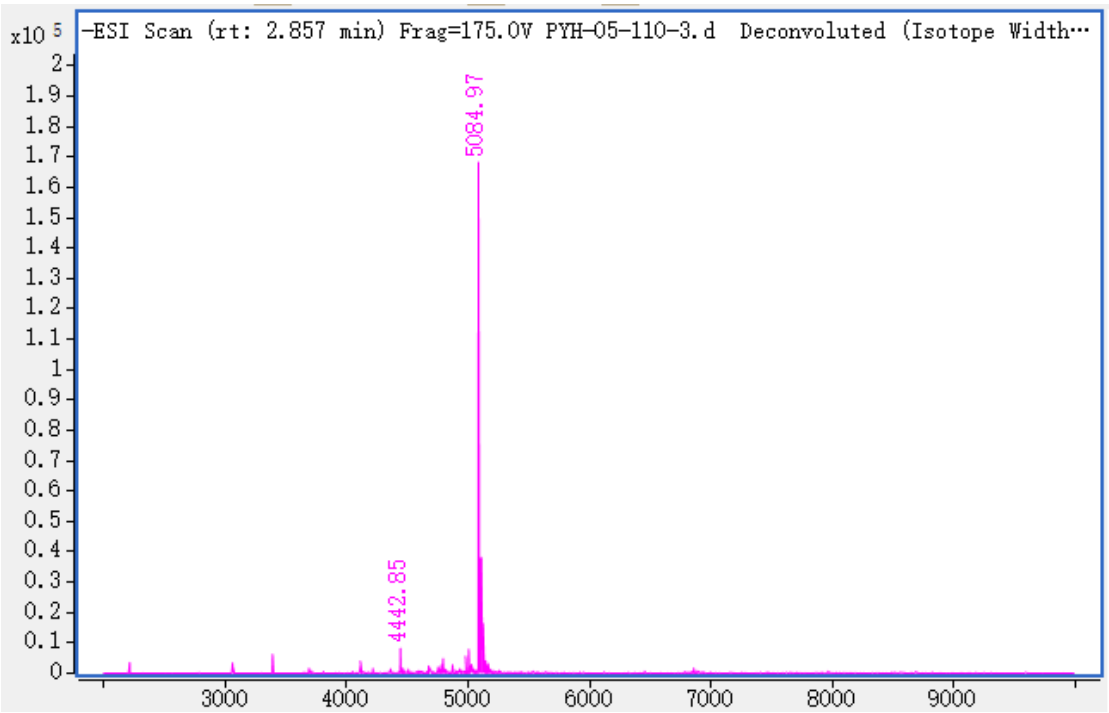


UPLC chromatogram and deconvoluted MS of **a2**

Conversion: ＞90%

Calculated Mass: 5085 Da; Observed Mass: 5085 Da

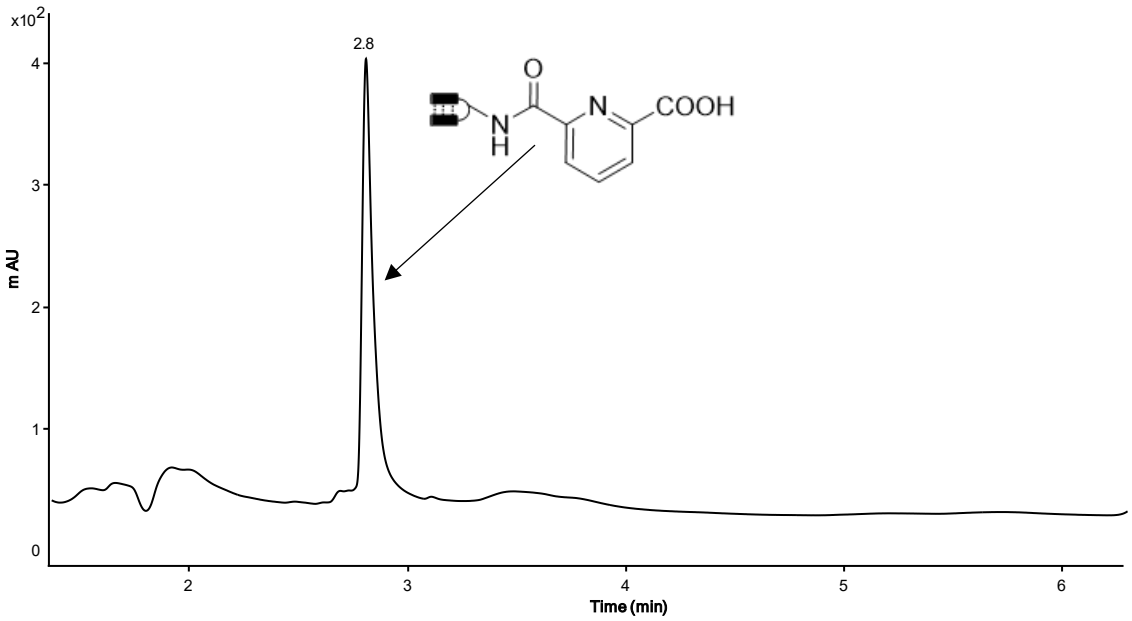


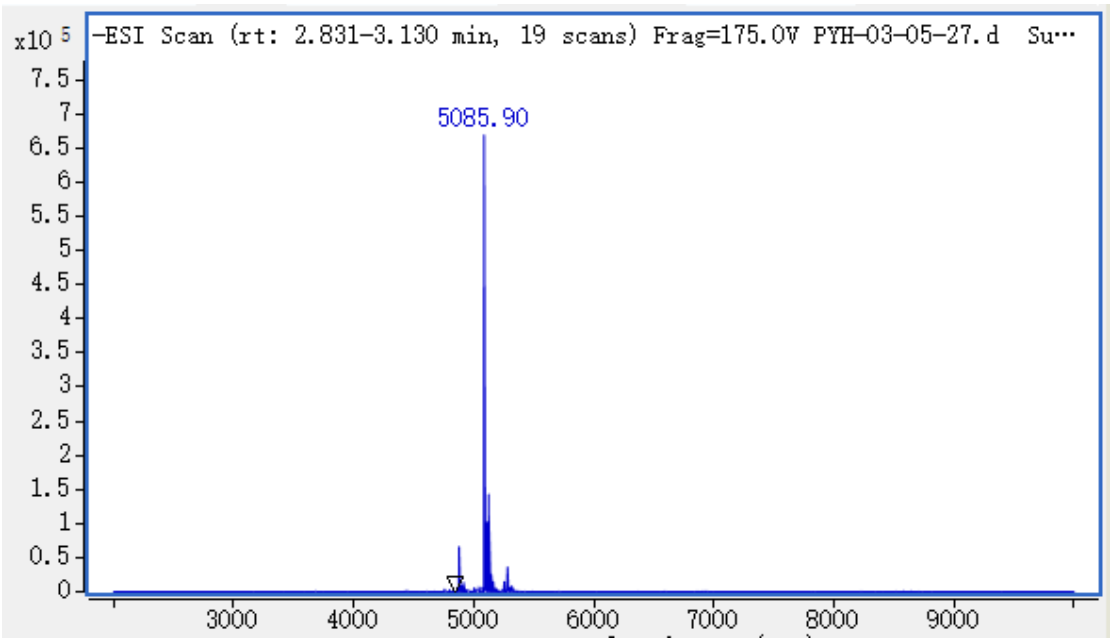


UPLC chromatogram and deconvoluted MS of **a3**

Conversion: ＞90%

Calculated Mass: 5086 Da; Observed Mass: 5086 Da

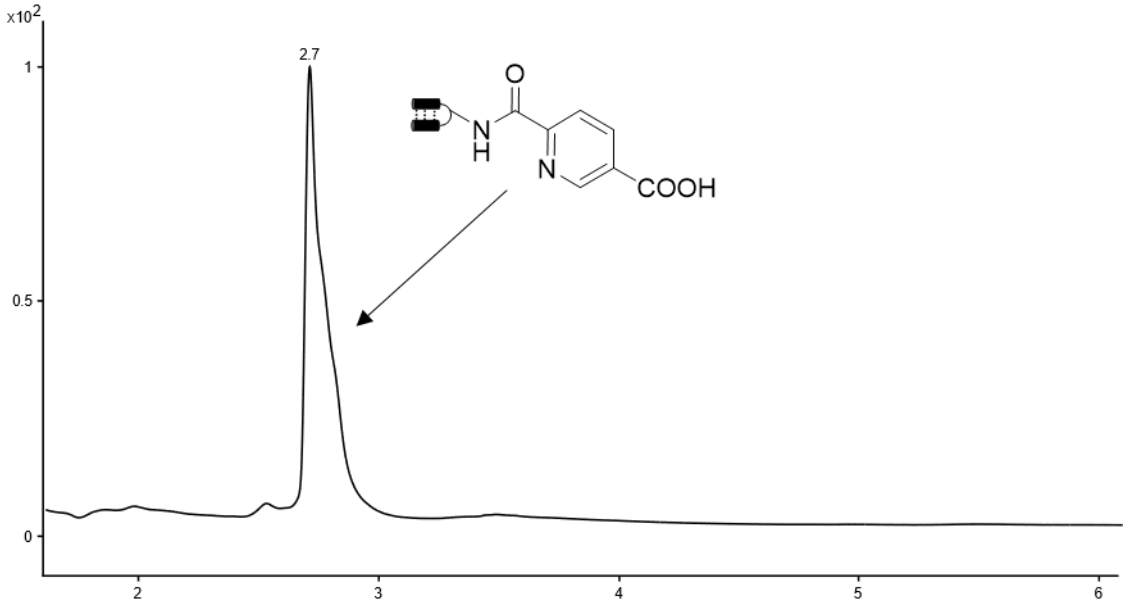


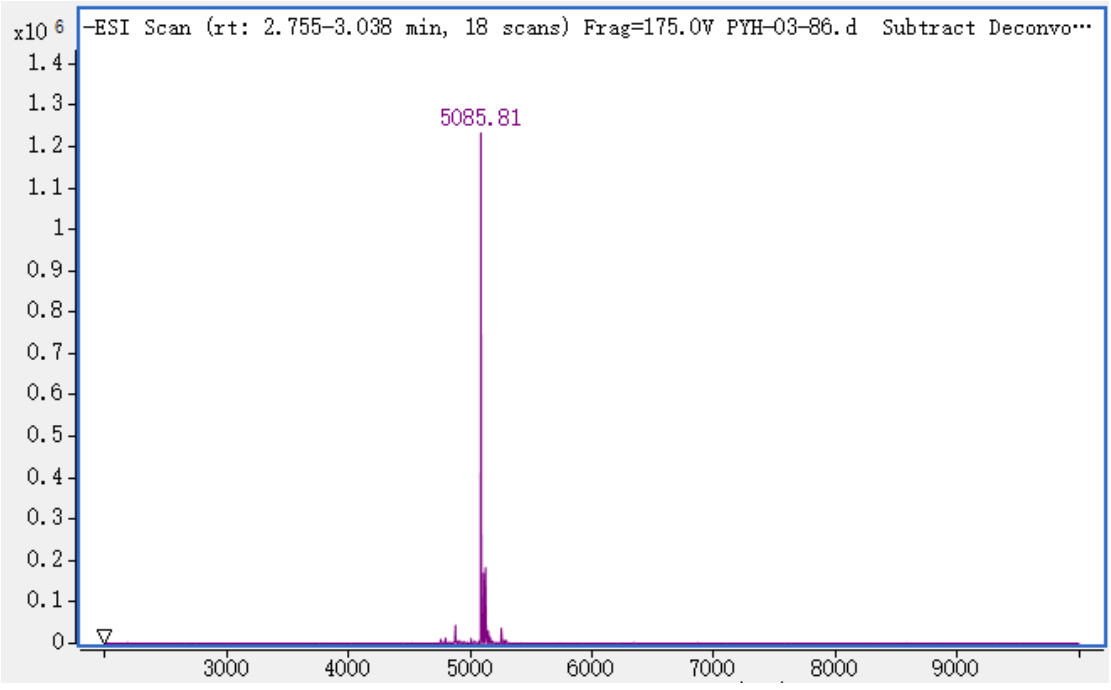


UPLC chromatogram and deconvoluted MS of **a4**

Conversion: ＞90%

Calculated Mass: 5086 Da; Observed Mass: 5086 Da

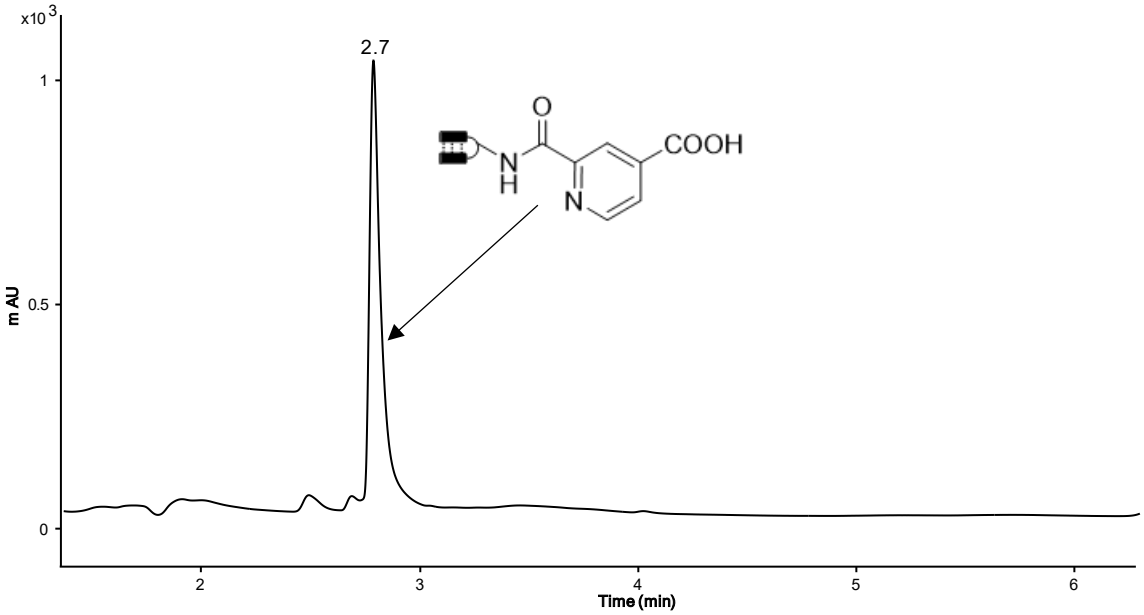


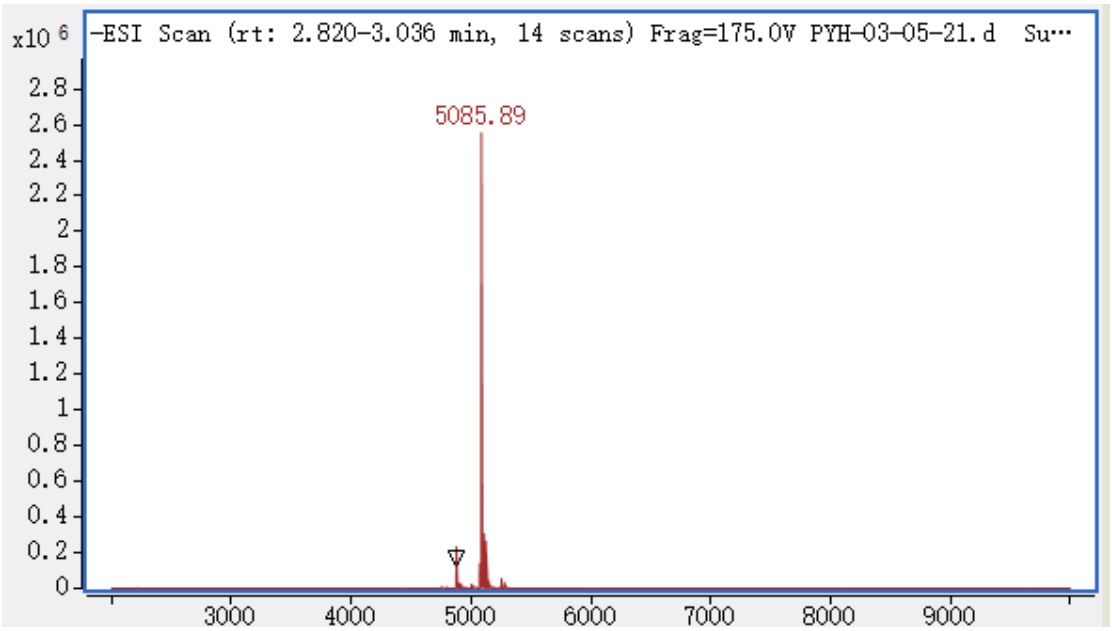


UPLC chromatogram and deconvoluted MS of **a5**

Conversion: ＞90%

Calculated Mass: 5086 Da; Observed Mass: 5086 Da

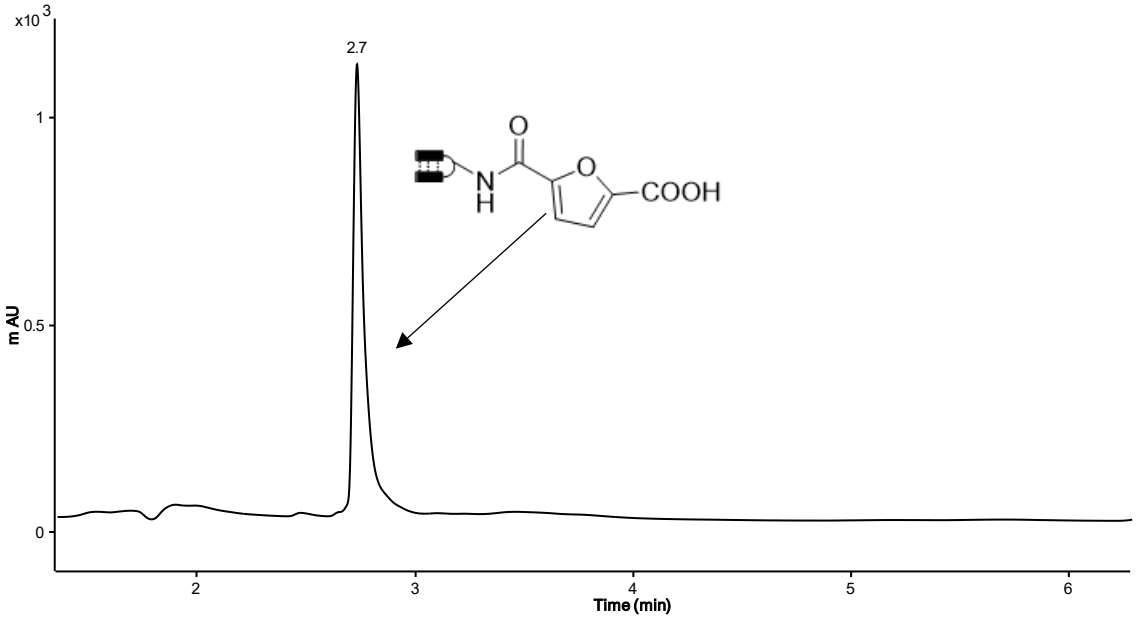


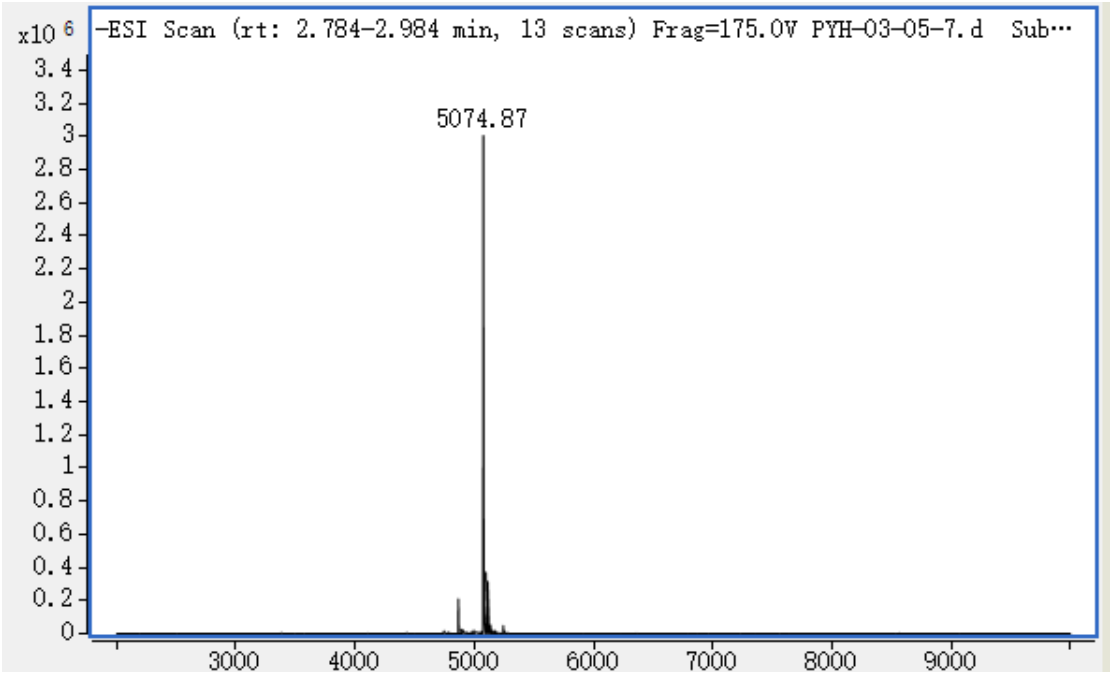


UPLC chromatogram and deconvoluted MS of **a6**

Conversion: ＞90%

Calculated Mass: 5075 Da; Observed Mass: 5075 Da

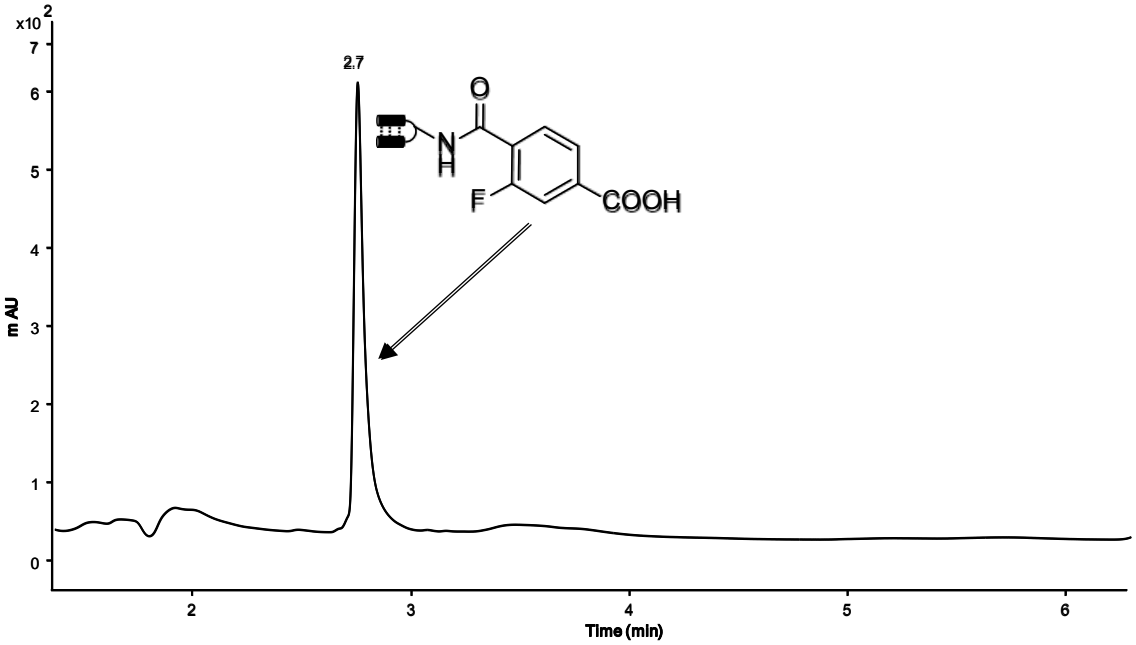


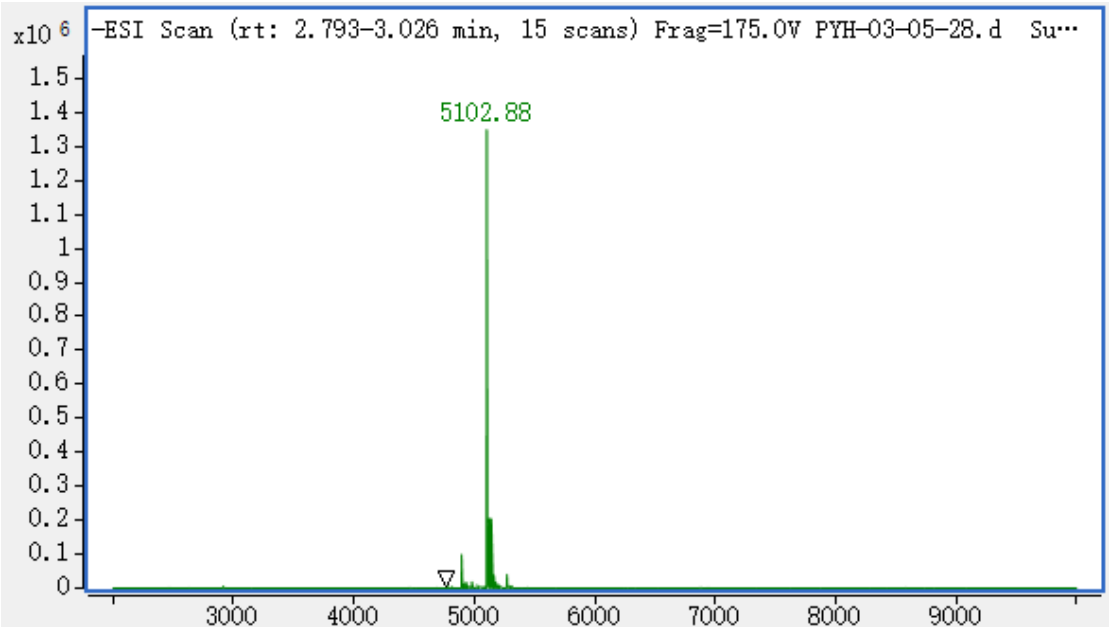


UPLC chromatogram and deconvoluted MS of **a7**

Conversion: ＞90%

Calculated Mass: 5103 Da; Observed Mass: 5103 Da

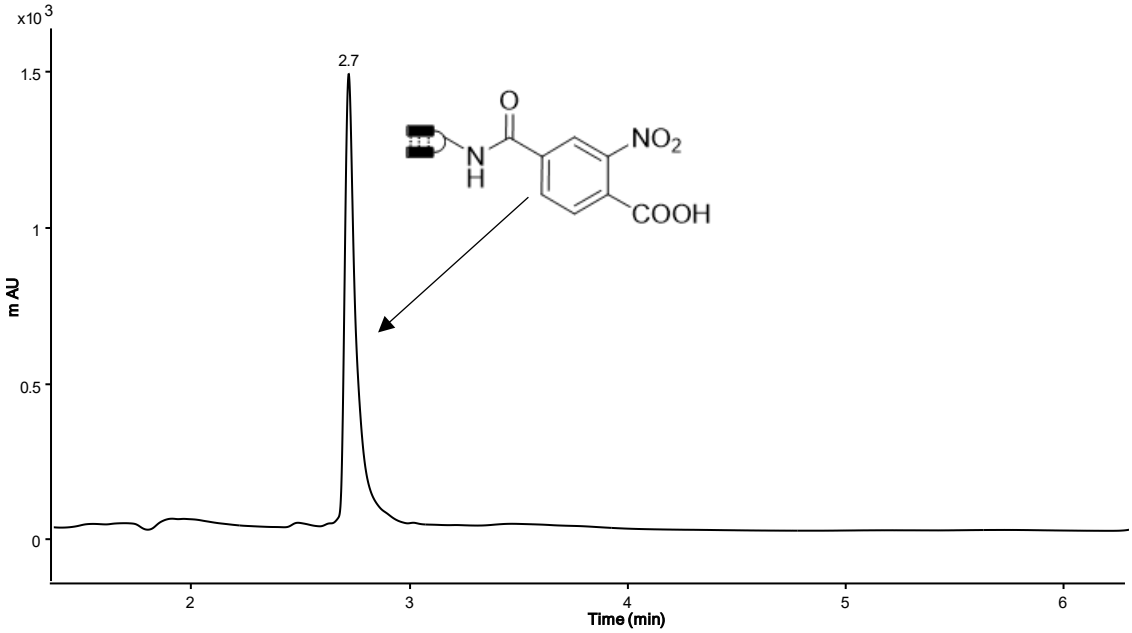


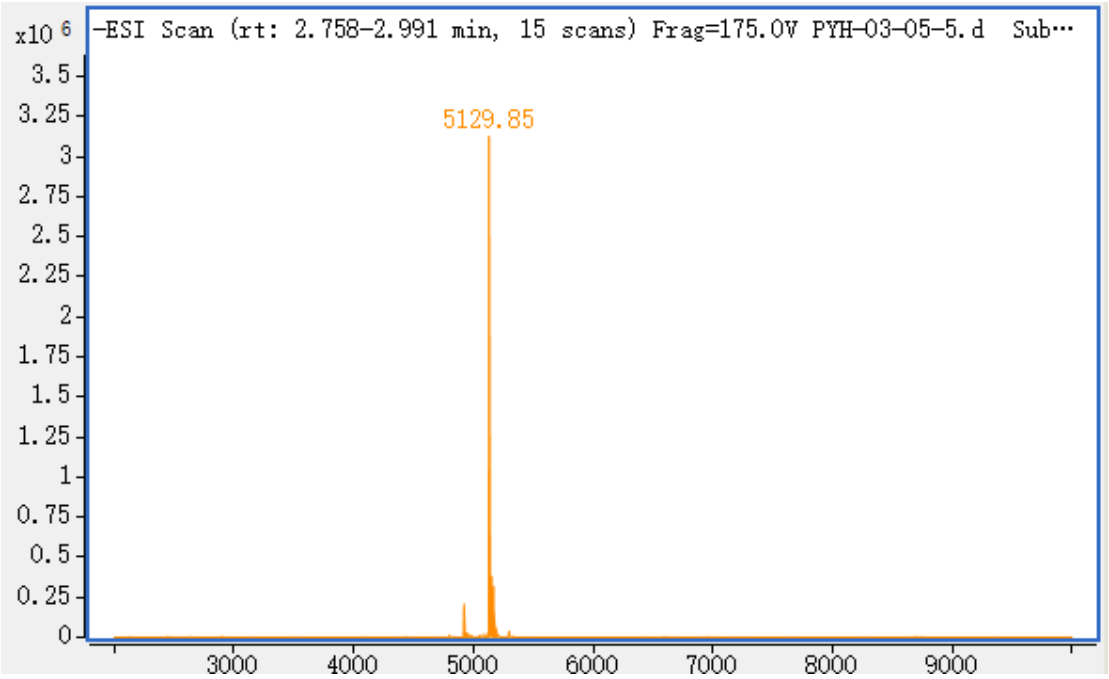


UPLC chromatogram and deconvoluted MS of **a8**

Conversion: ＞90%

Calculated Mass: 5130 Da; Observed Mass: 5130 Da

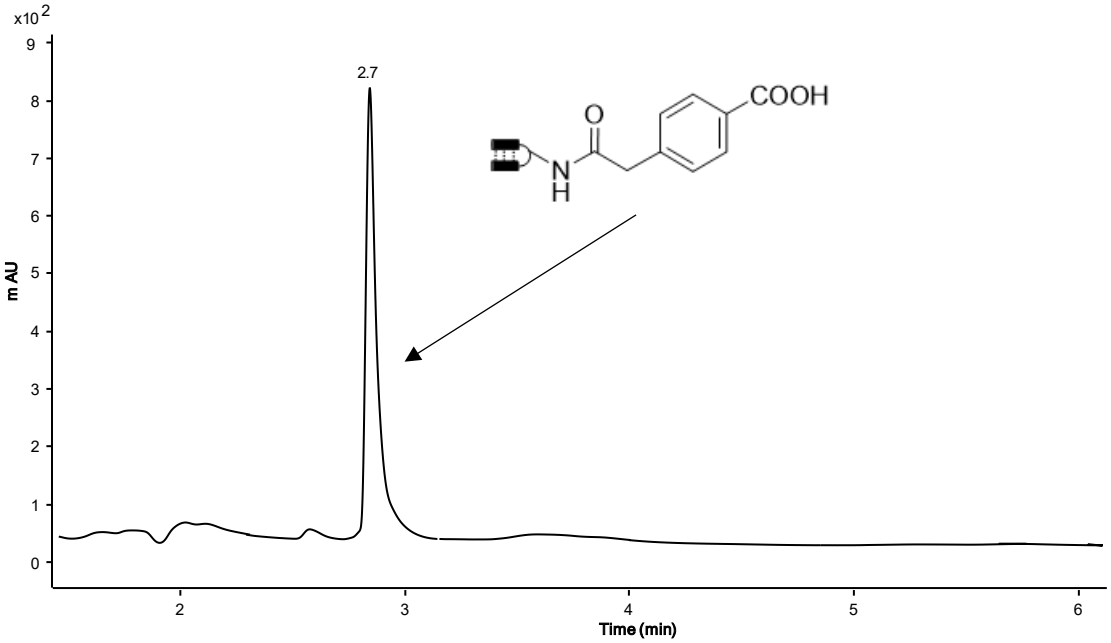


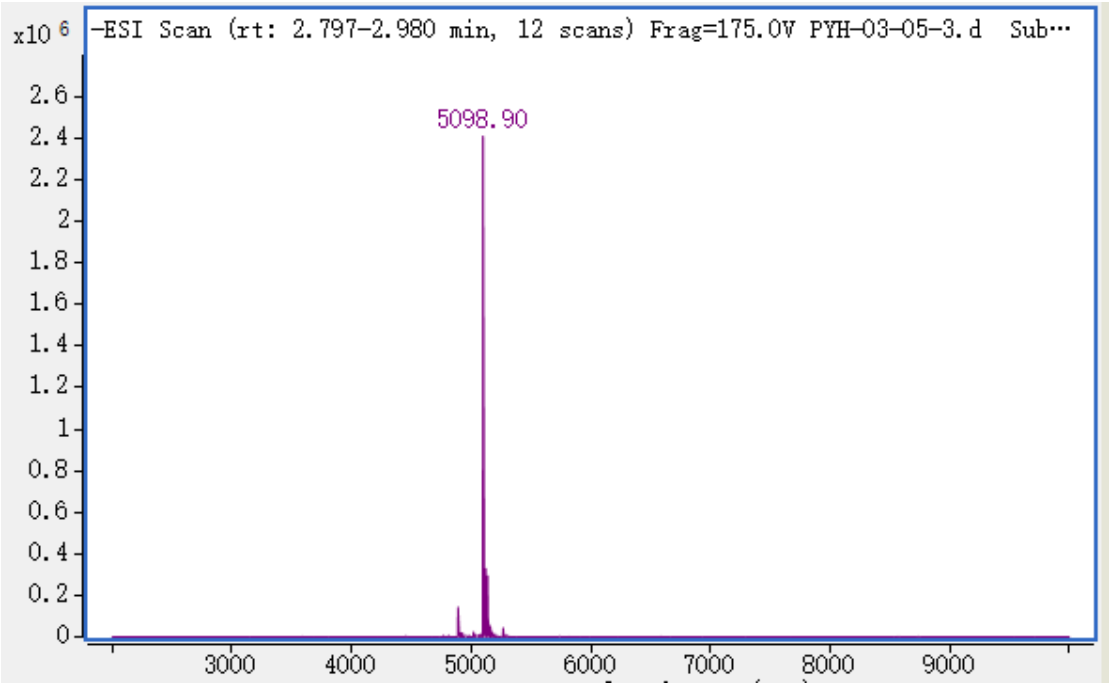


UPLC chromatogram and deconvoluted MS of **a9**

Conversion: ＞90%

Calculated Mass: 5099 Da; Observed Mass: 5099 Da

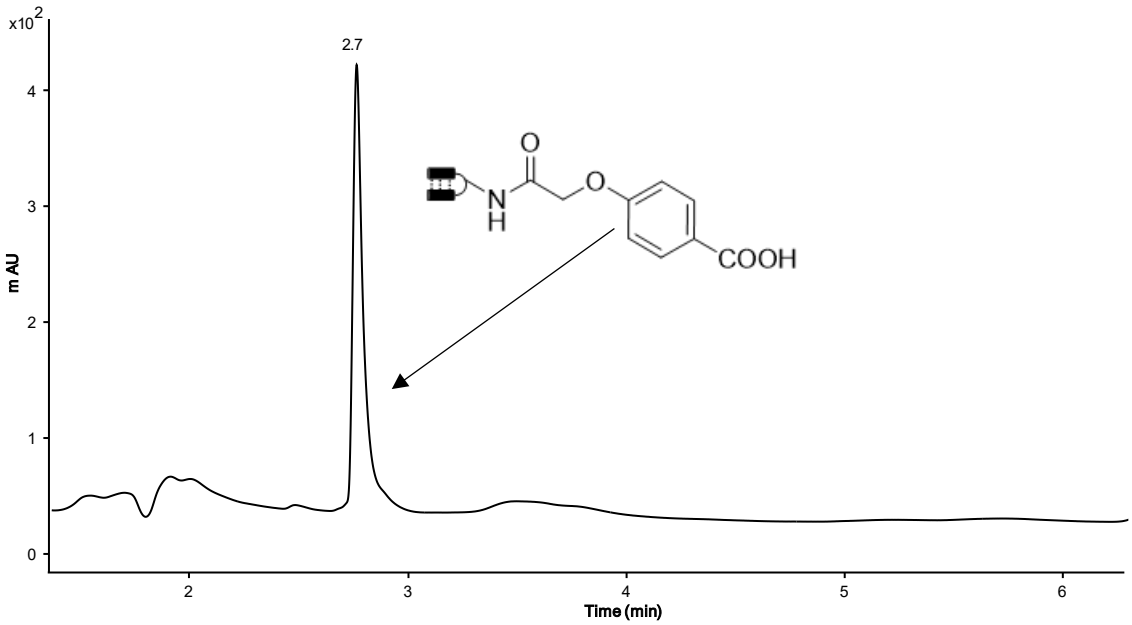


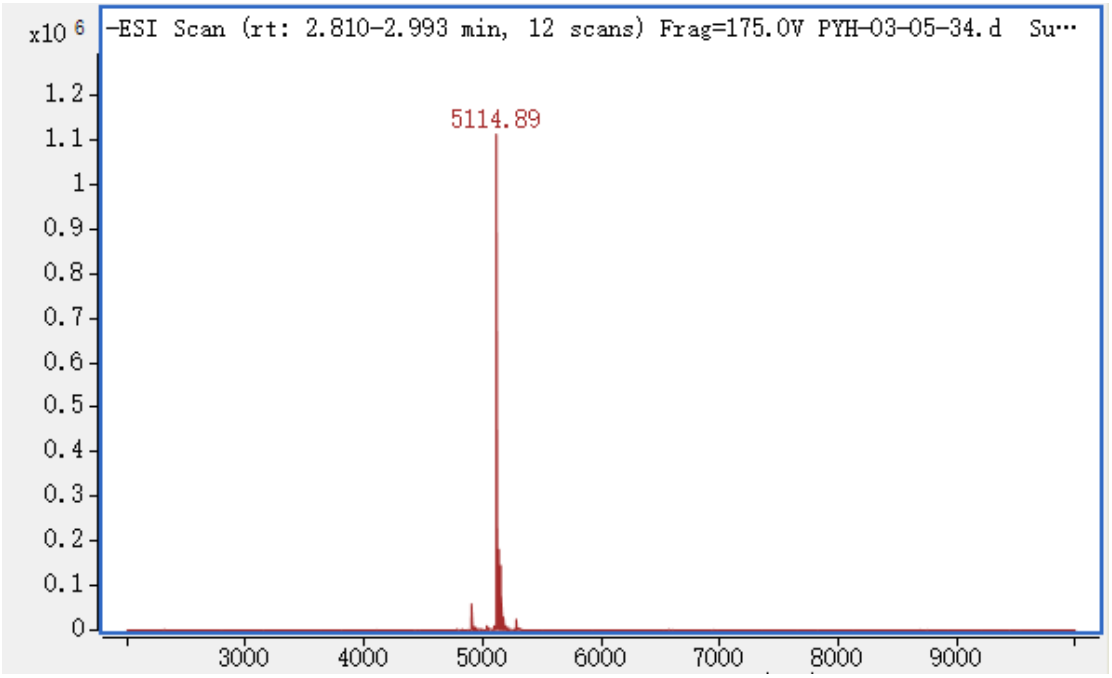


UPLC chromatogram and deconvoluted MS of **a10**

Conversion: ＞90%

Calculated Mass: 5115 Da; Observed Mass: 5115 Da

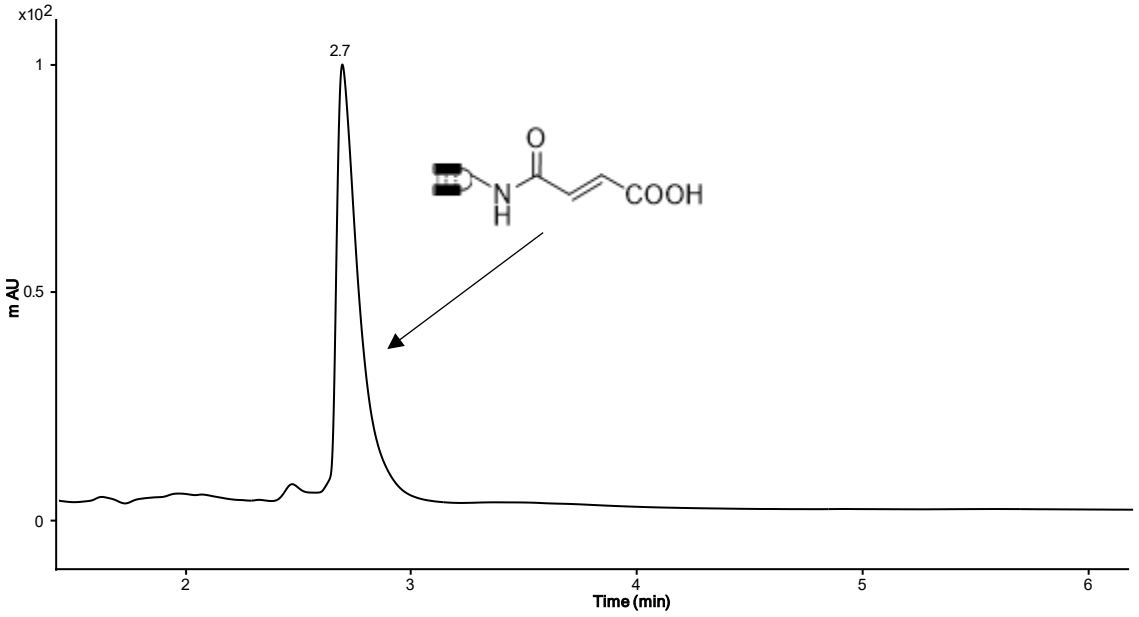


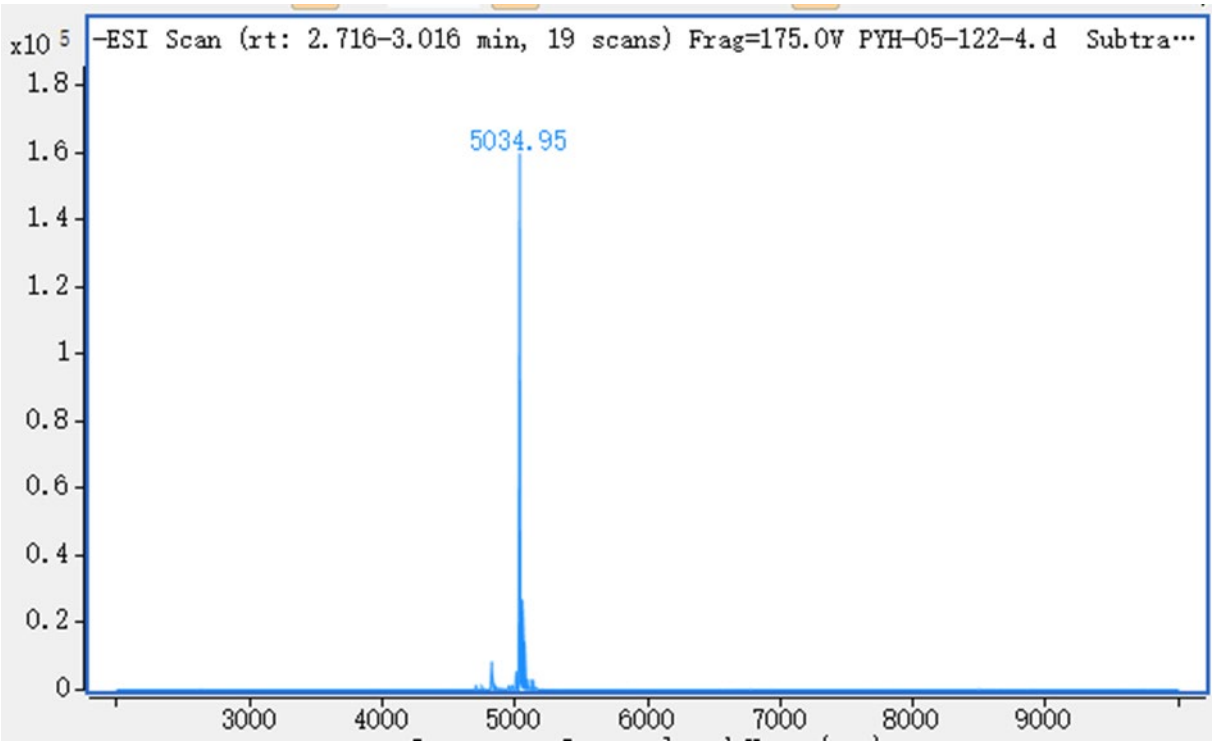


UPLC chromatogram and deconvoluted MS of **a11**

Conversion: ＞90%

Calculated Mass: 5035 Da; Observed Mass: 5035 Da

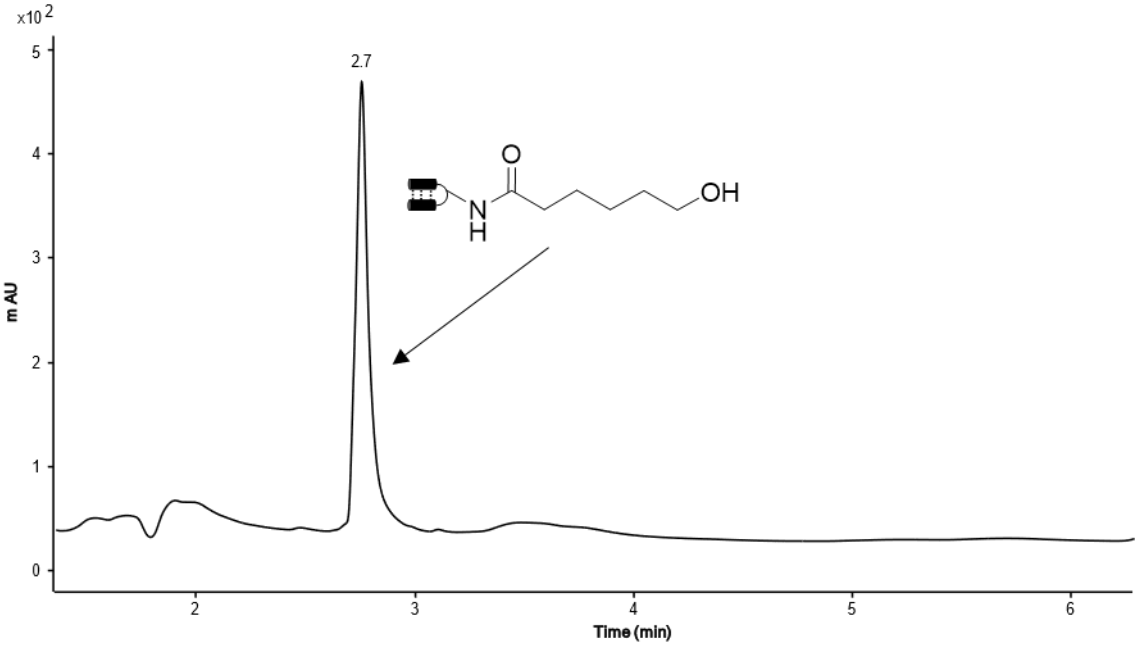


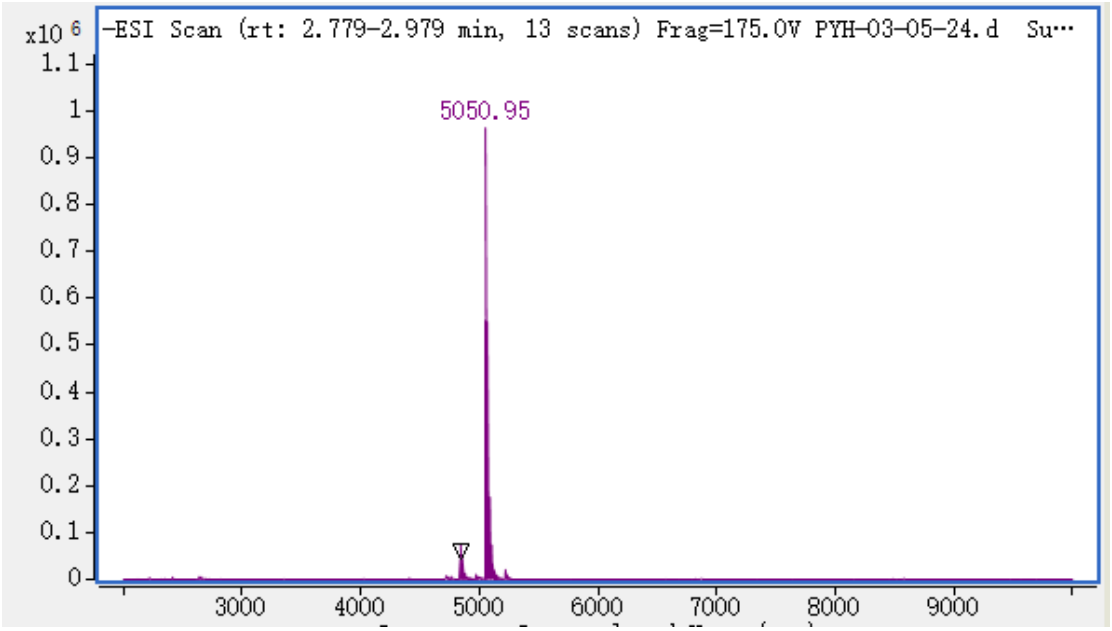


UPLC chromatogram and deconvoluted MS of **a12**

Conversion: 0%

Calculated Mass: 5065 Da; Observed Mass: 5051 Da

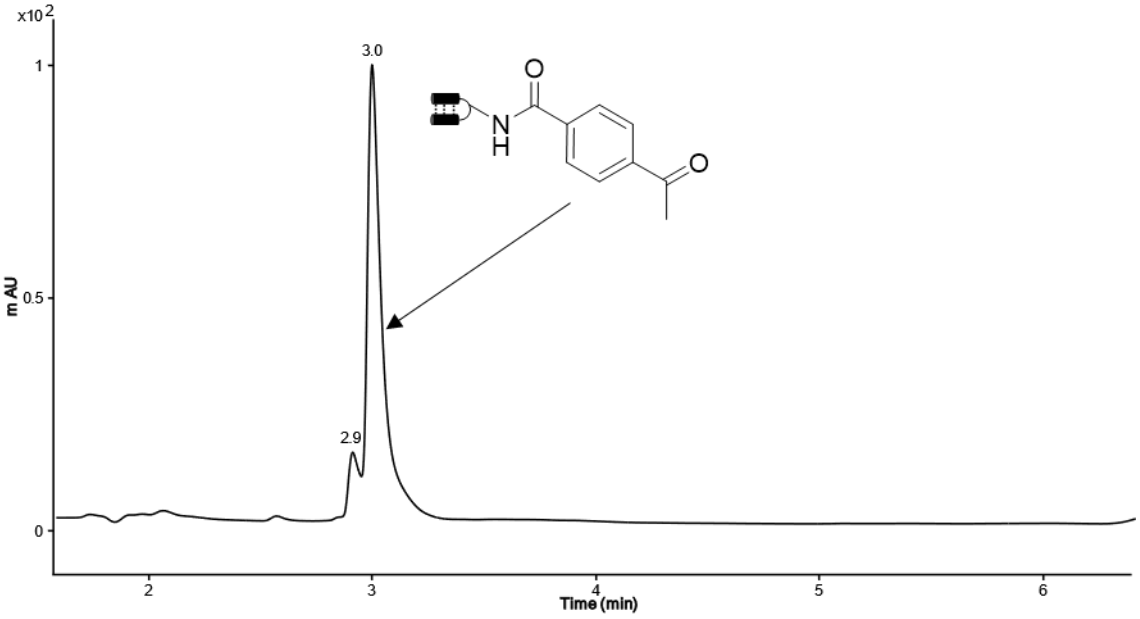


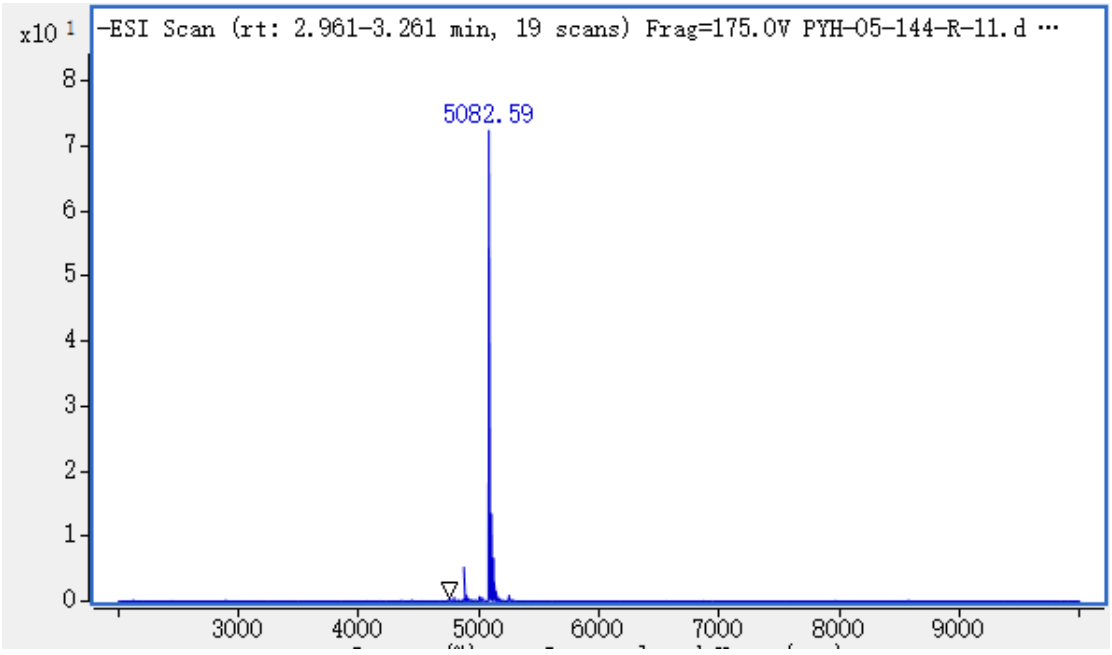


UPLC chromatogram and deconvoluted MS of **a13**

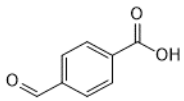
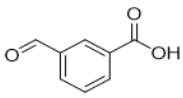
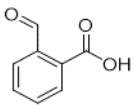
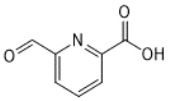
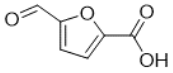
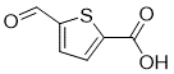
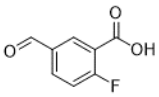
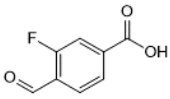
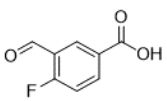
Conversion: >90%

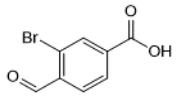
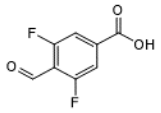
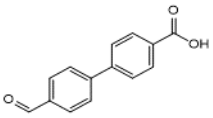
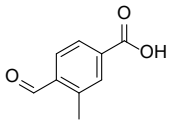
Calculated Mass: 5085 Da; Observed Mass: 5085 Da





7.2 Substrate scope of DNA-conjugated aldehydes

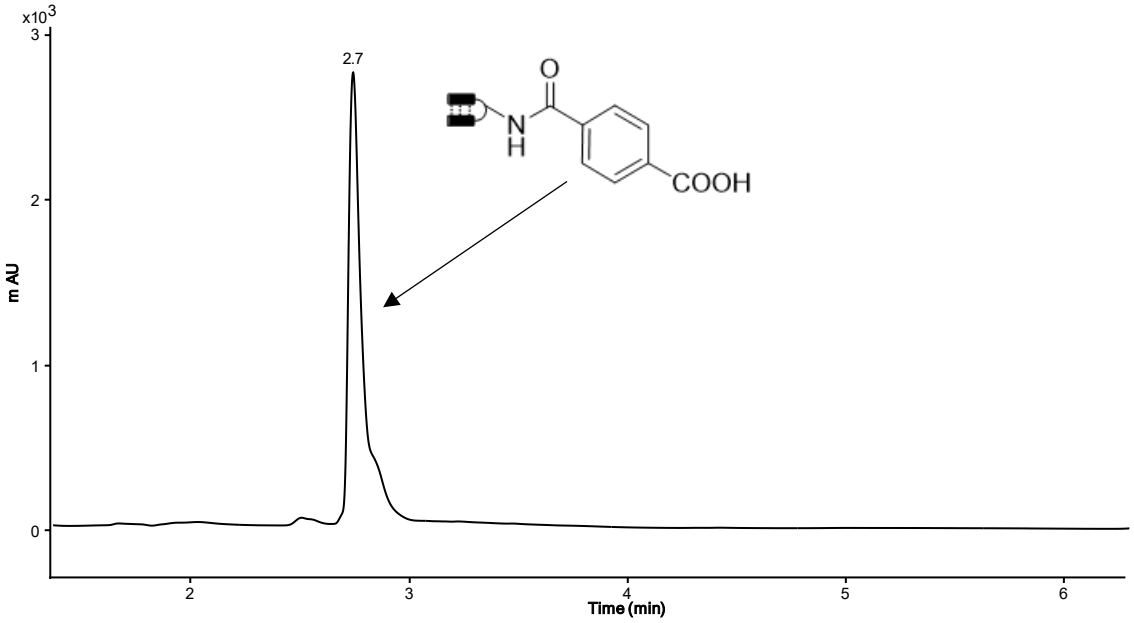
Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
b1		d1	**5085**	**5085**	>90%
b2		d2	**5085**	**5085**	>90%
b3		d3	**5085**	**5085**	>90%
b4		d4	**5086**	**5086**	>90%
b5		d5	**5075**	**5075**	>90%
b6		d6	**5091**	**5091**	>90%
b7		d7	**5103**	**5103**	>90%
b8		d8	**5103**	**5103**	>90%
b9		d9	**5103**	**5103**	>90%

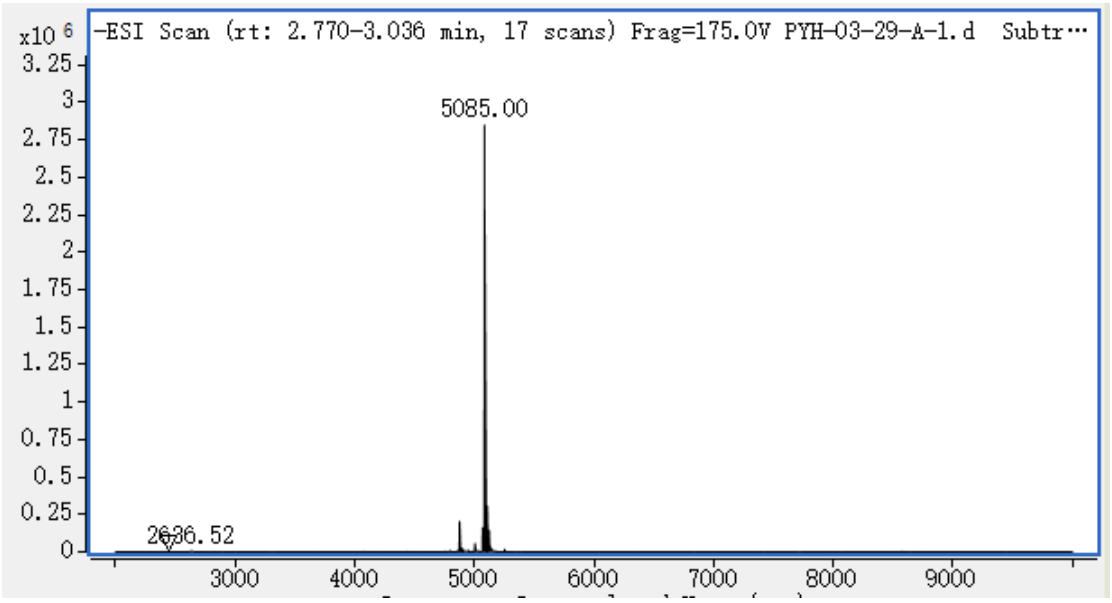
b10		**d10**	**5164**	**5164**	**>90%**
b11		**d11**	**5121**	**5121**	**55%**
b12		**d12**	**5161**	**5161**	**48%**
b13		**d13**	**5099**	**5099**	**>90%**

UPLC chromatogram and deconvoluted MS of **b1**

Conversion: ＞90%

Calculated Mass: 5085 Da; Observed Mass: 5085 Da

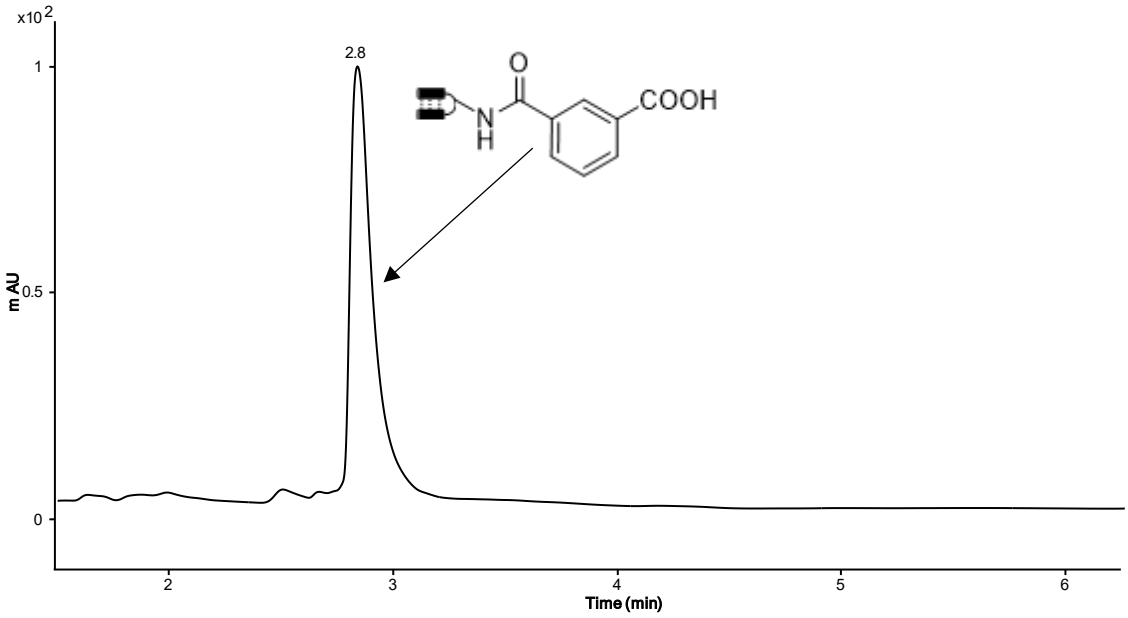


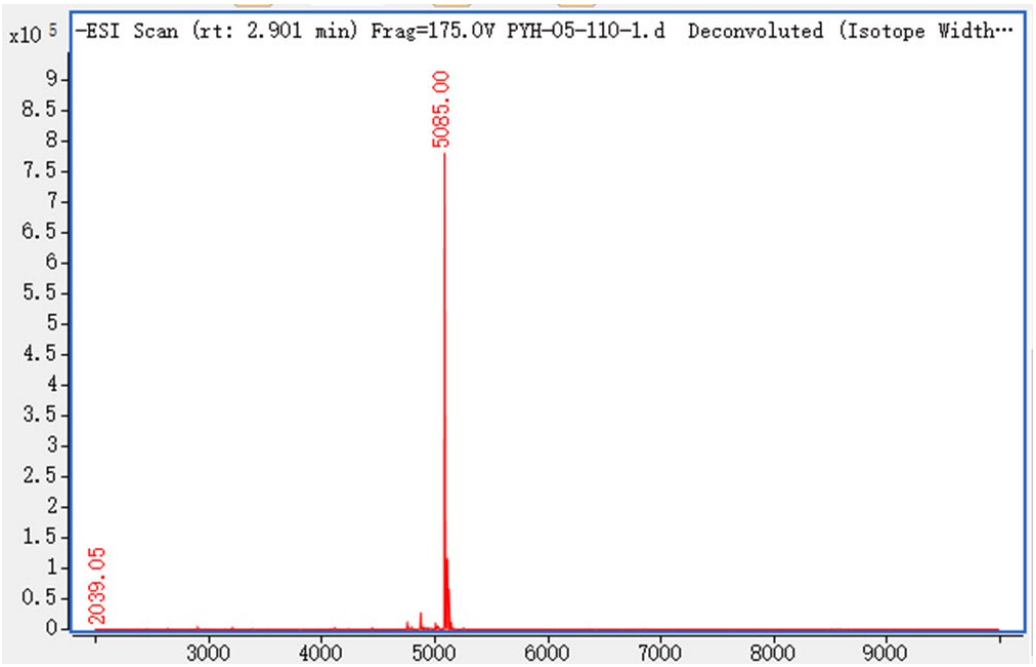


UPLC chromatogram and deconvoluted MS of **b2**

Conversion: ＞90%

Calculated Mass: 5085 Da; Observed Mass: 5085 Da

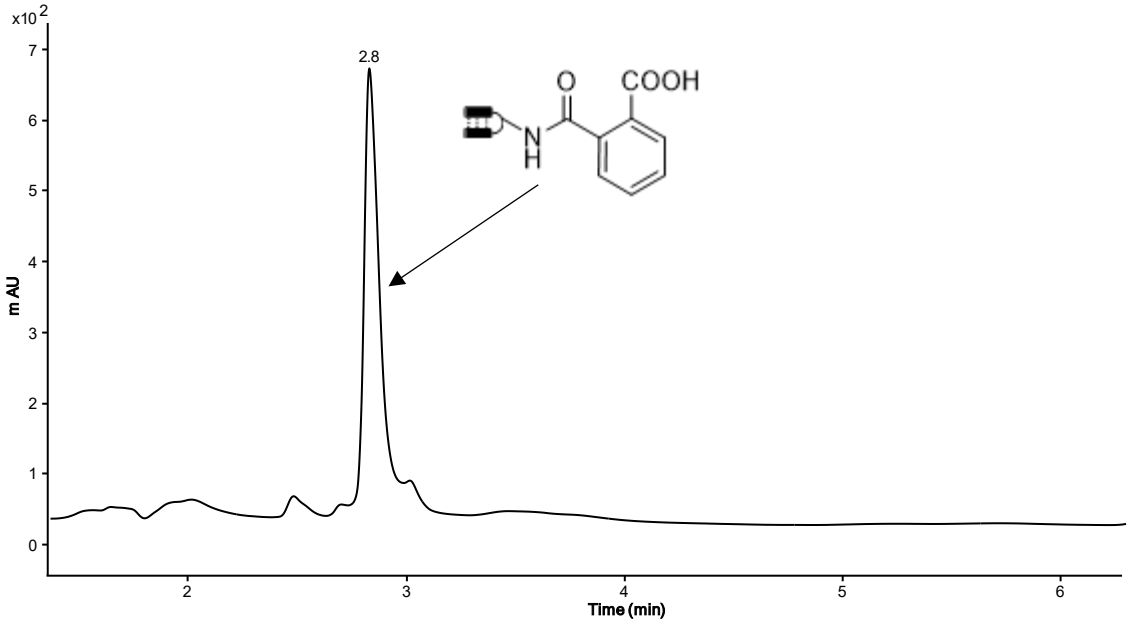


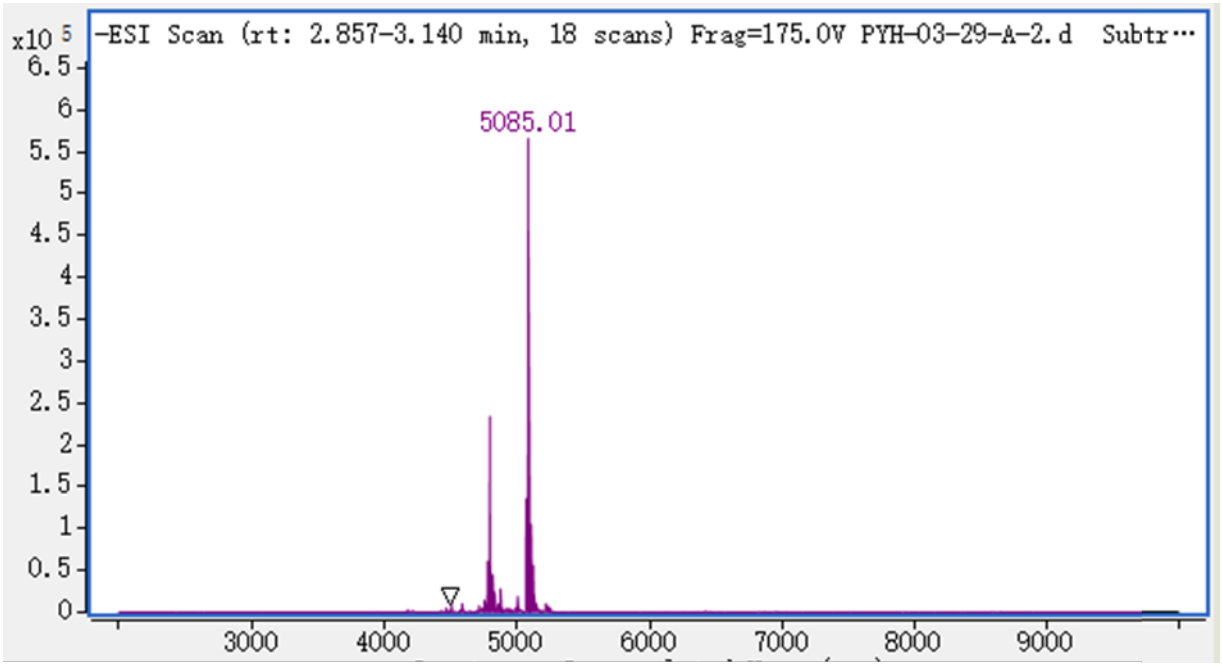


UPLC chromatogram and deconvoluted MS of **b3**

Conversion: ＞90%

Calculated Mass: 5085 Da; Observed Mass: 5085 Da

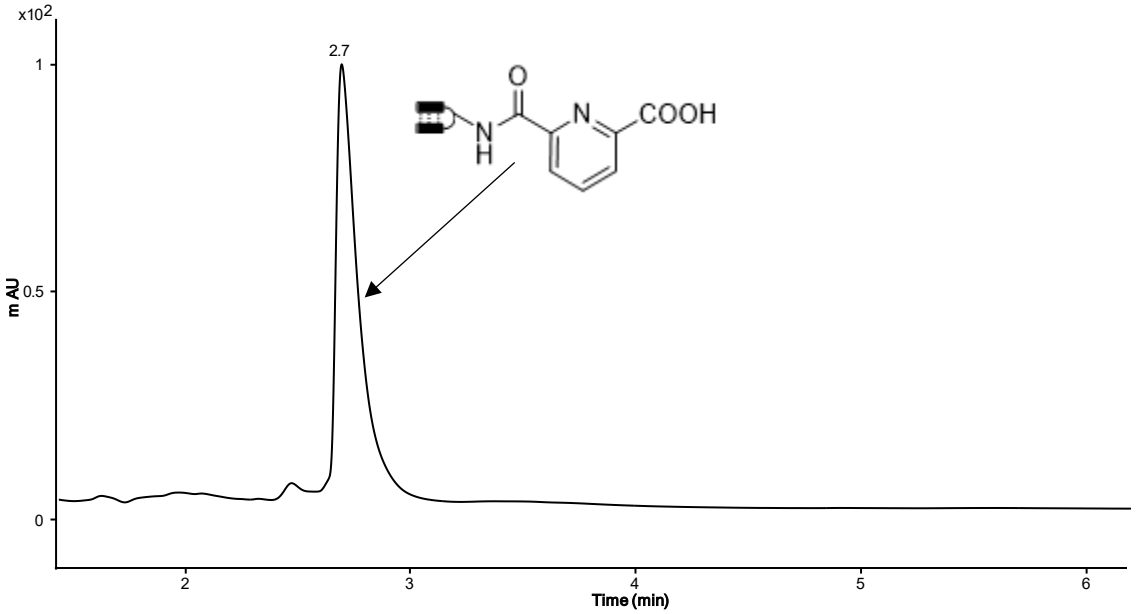


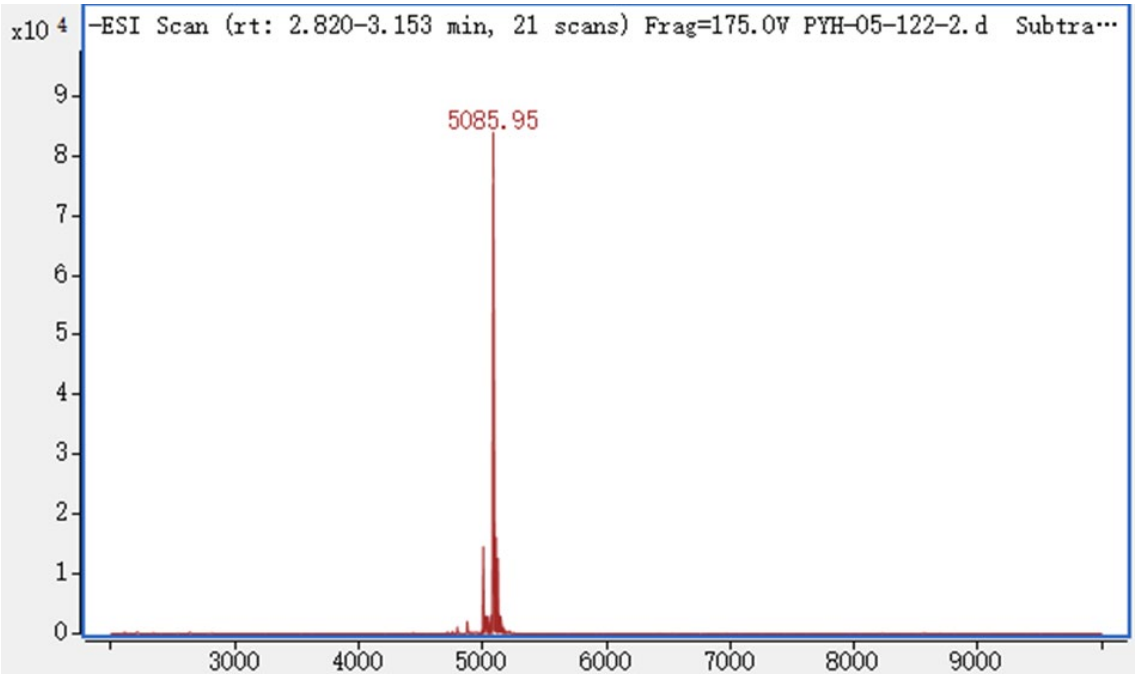


UPLC chromatogram and deconvoluted MS of **b4**

Conversion: ＞90%

Calculated Mass: 5086 Da; Observed Mass: 5086 Da

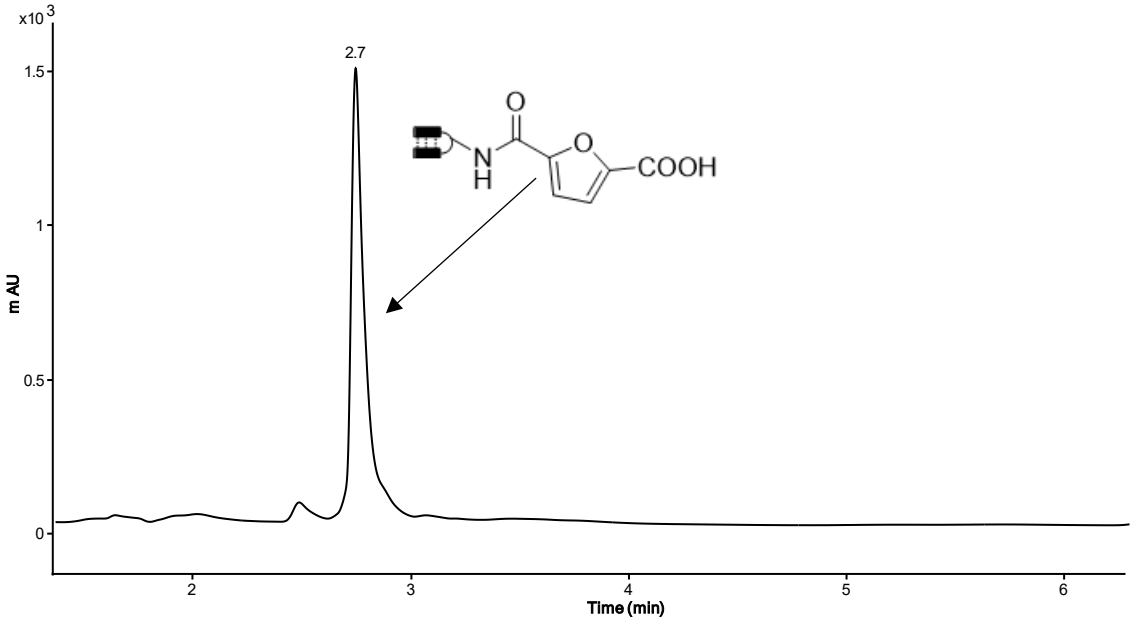


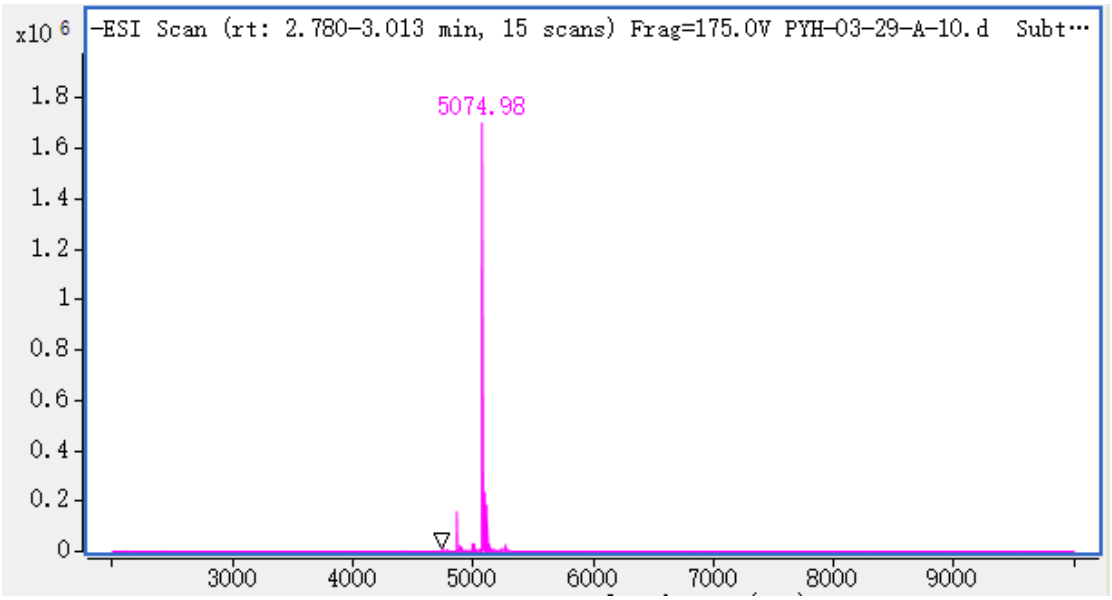


UPLC chromatogram and deconvoluted MS of **b5**

Conversion: ＞90%

Calculated Mass: 5075 Da; Observed Mass: 5075 Da

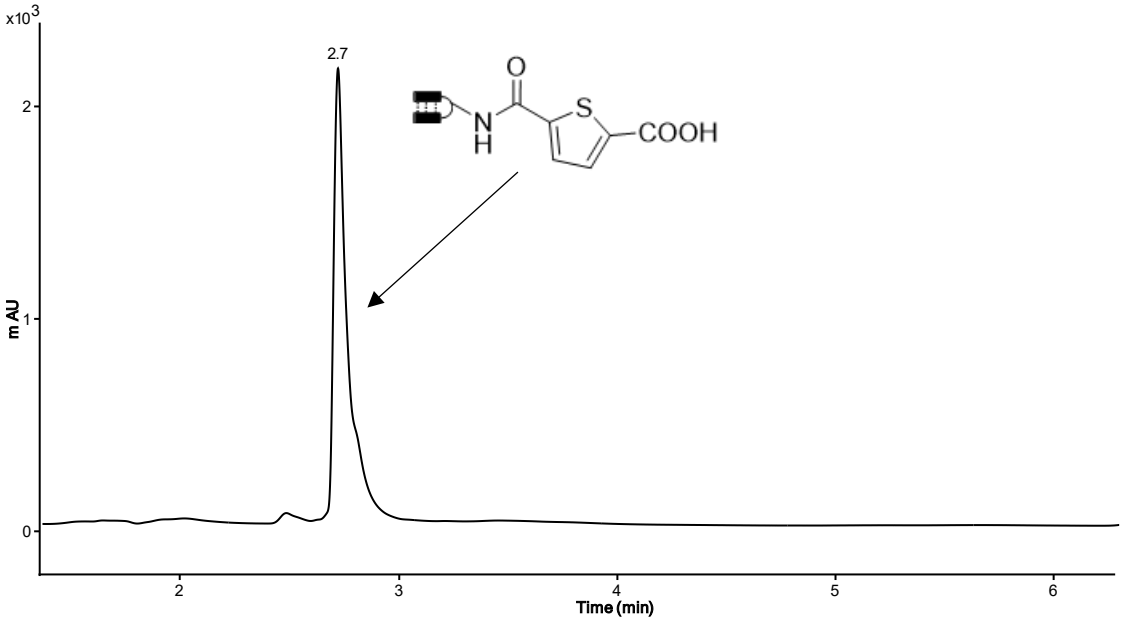


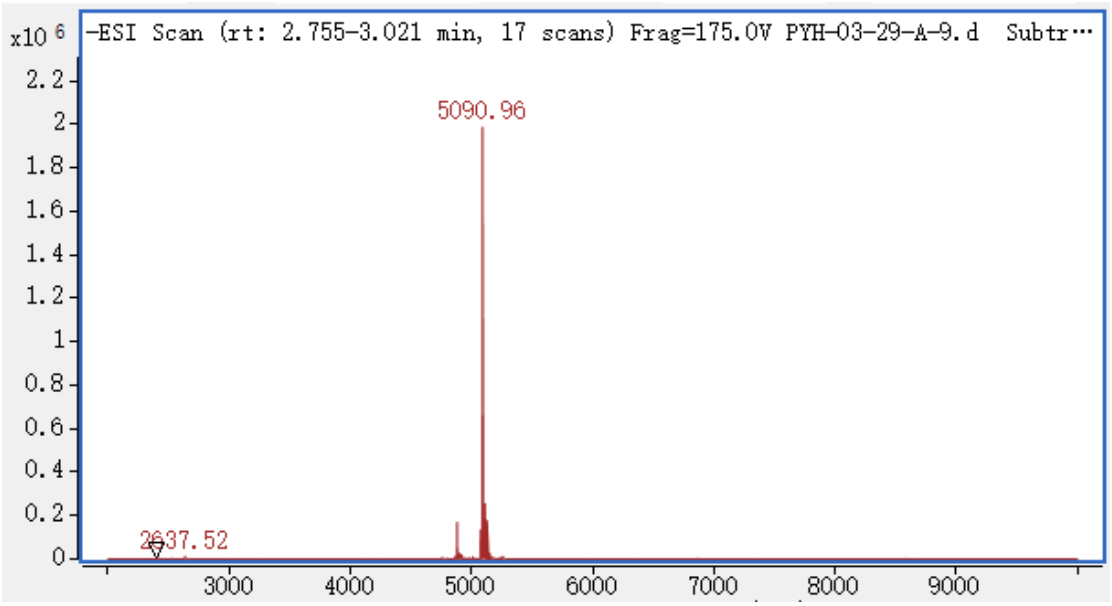


UPLC chromatogram and deconvoluted MS of **b6**

Conversion: ＞90%

Calculated Mass: 5091 Da; Observed Mass: 5091 Da

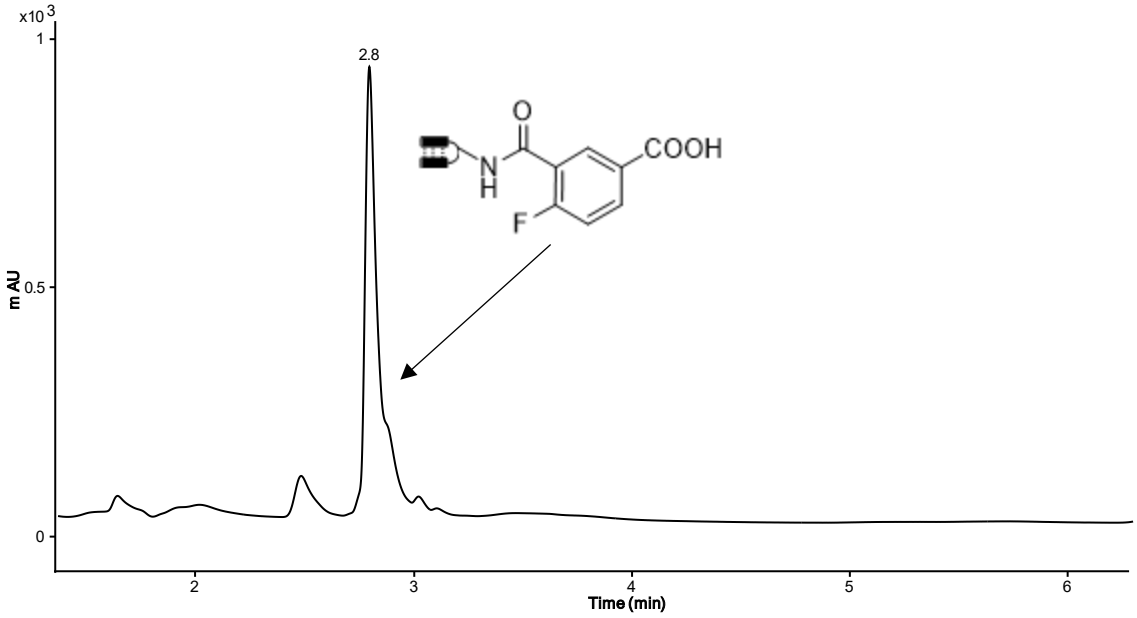


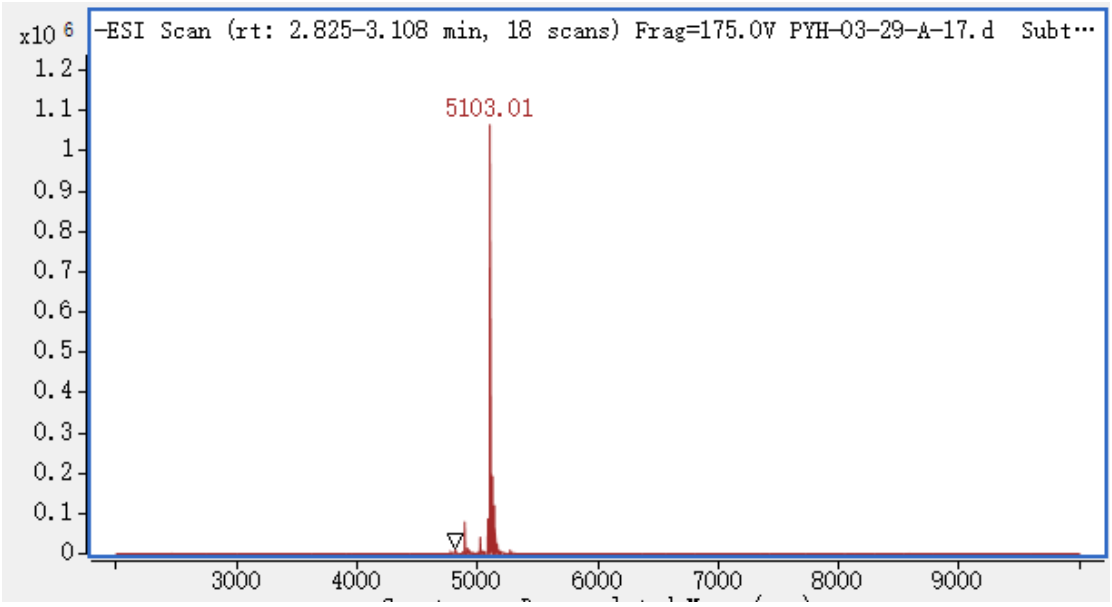


UPLC chromatogram and deconvoluted MS of **b7**

Conversion: ＞90%

Calculated Mass: 5103 Da; Observed Mass: 5103 Da

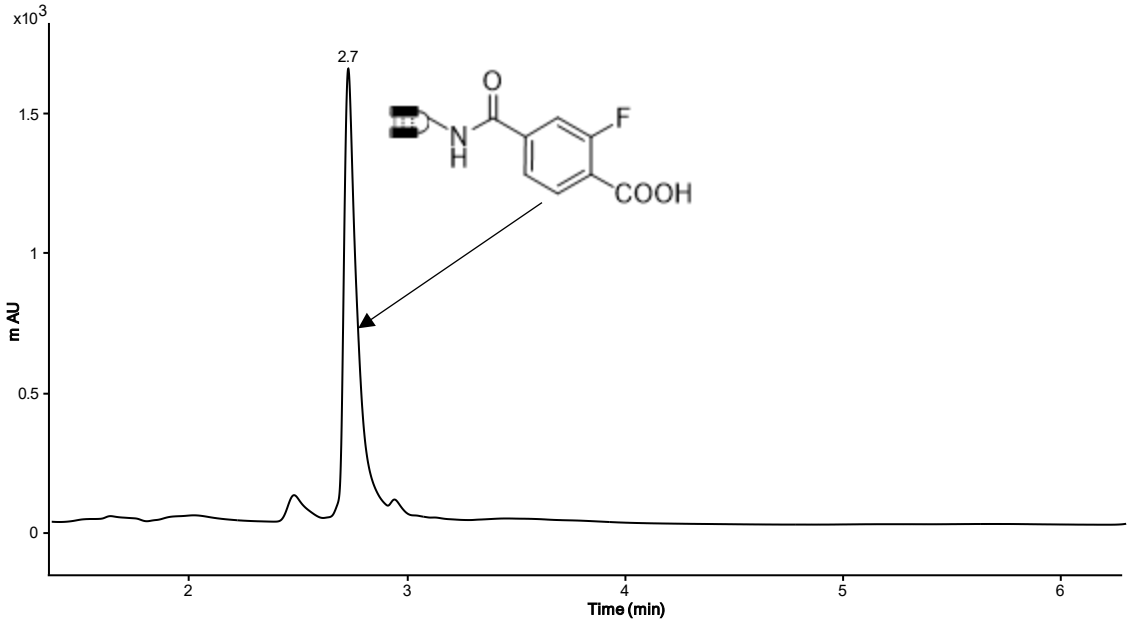


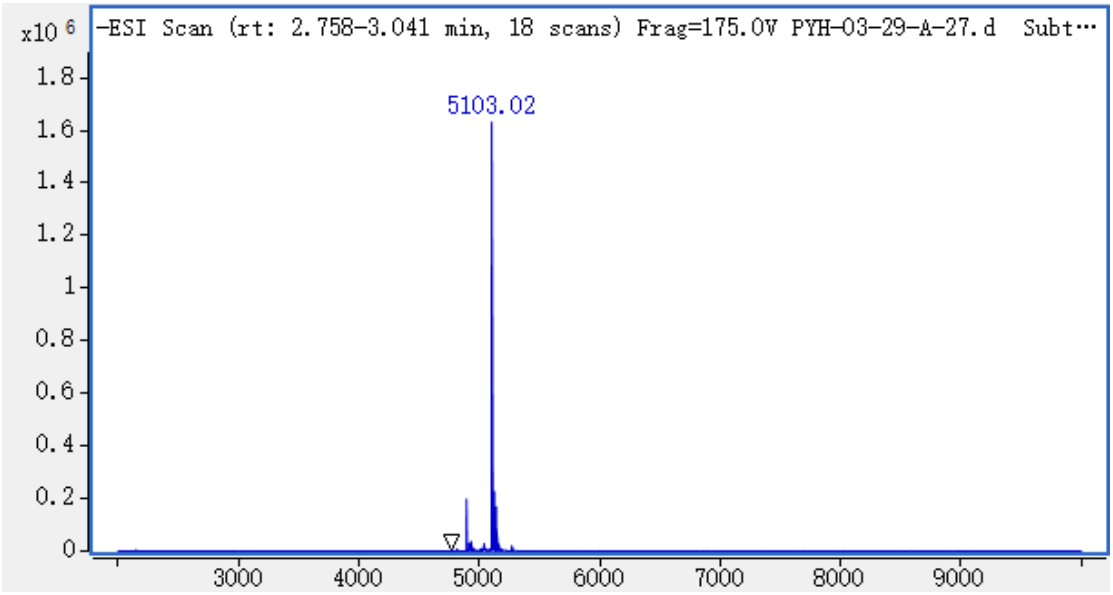


UPLC chromatogram and deconvoluted MS of **b8**

Conversion: ＞90%

Calculated Mass: 5103 Da; Observed Mass: 5103 Da

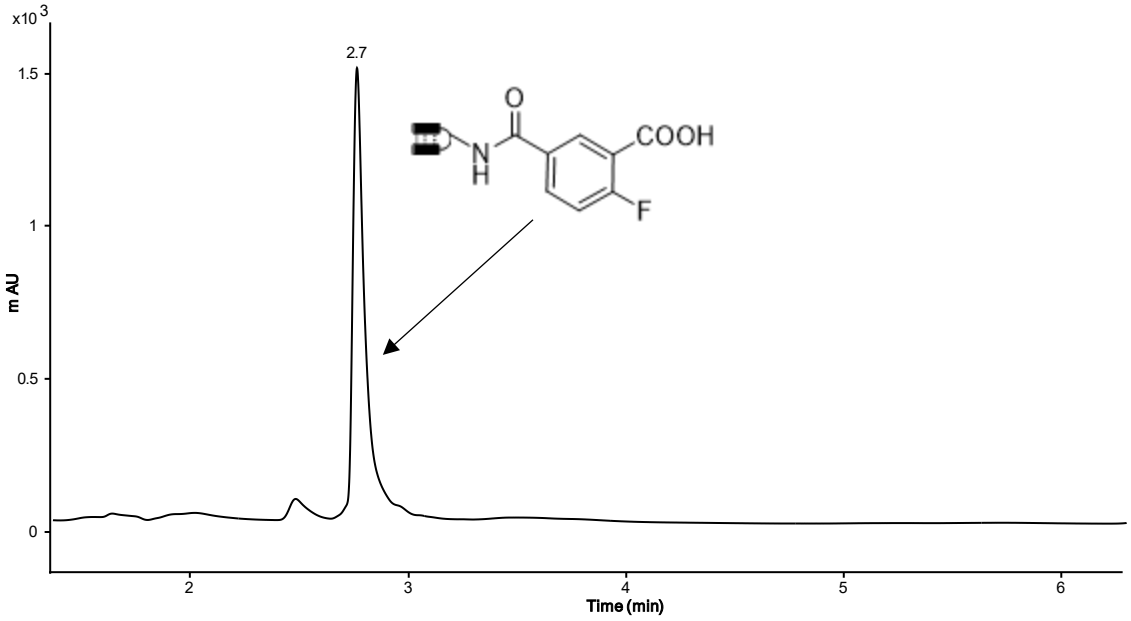


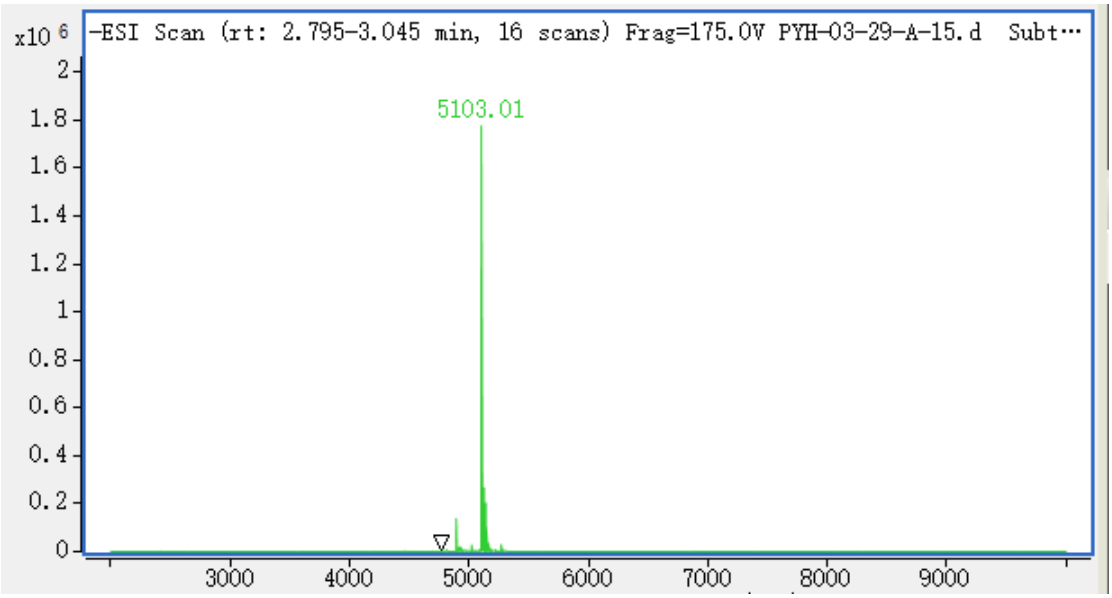


UPLC chromatogram and deconvoluted MS of **b9**

Conversion: ＞90%

Calculated Mass: 5103 Da; Observed Mass: 5103 Da

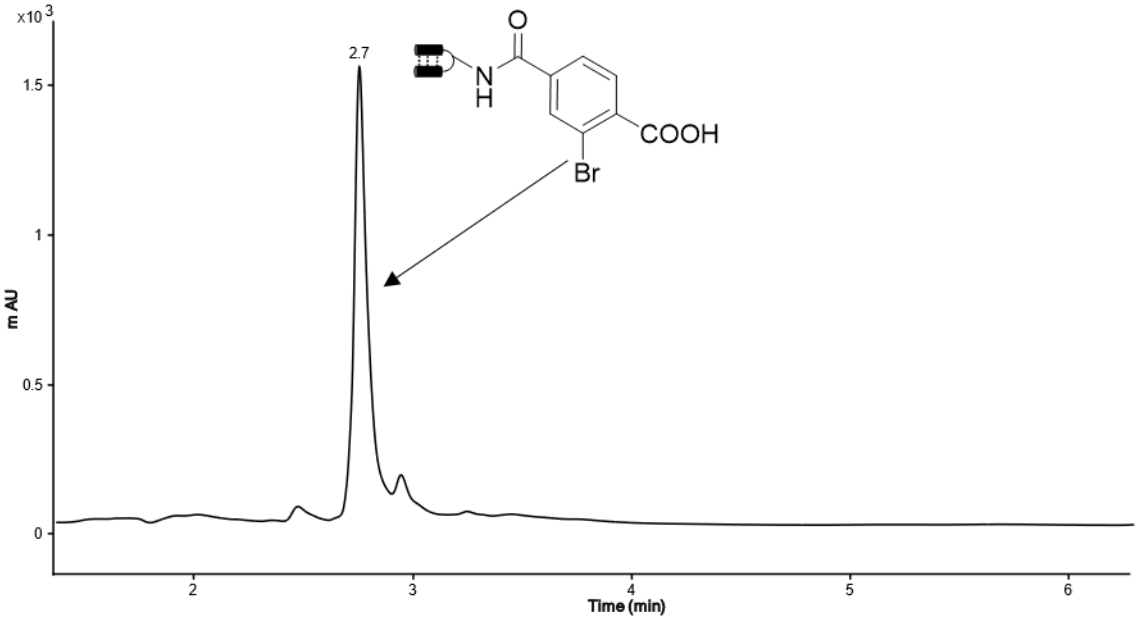


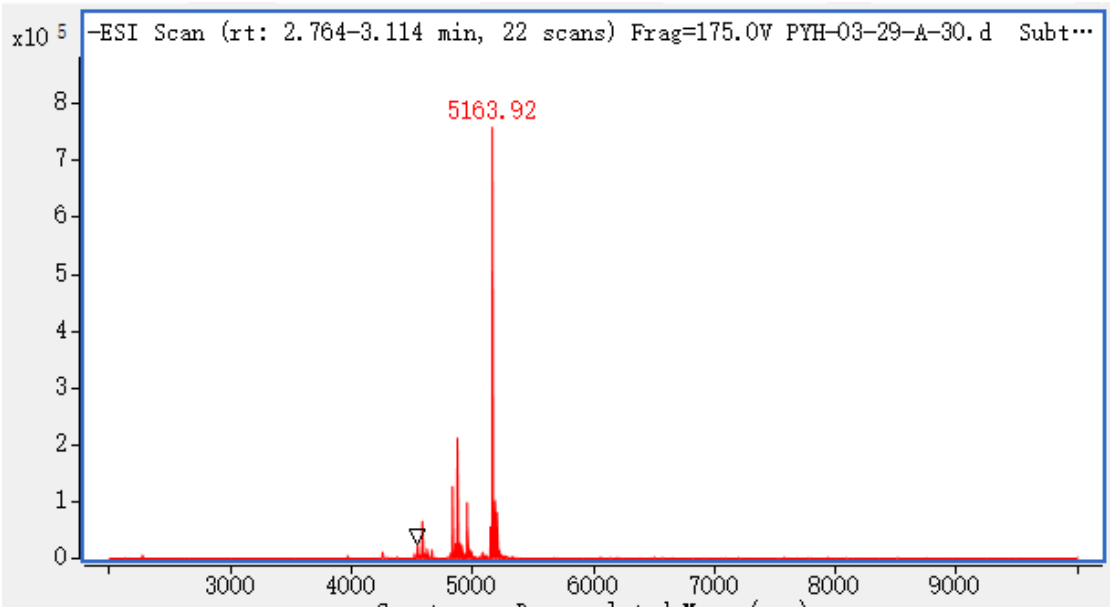


UPLC chromatogram and deconvoluted MS of **b10**

Conversion: ＞90%

Calculated Mass: 5164 Da; Observed Mass: 5164 Da

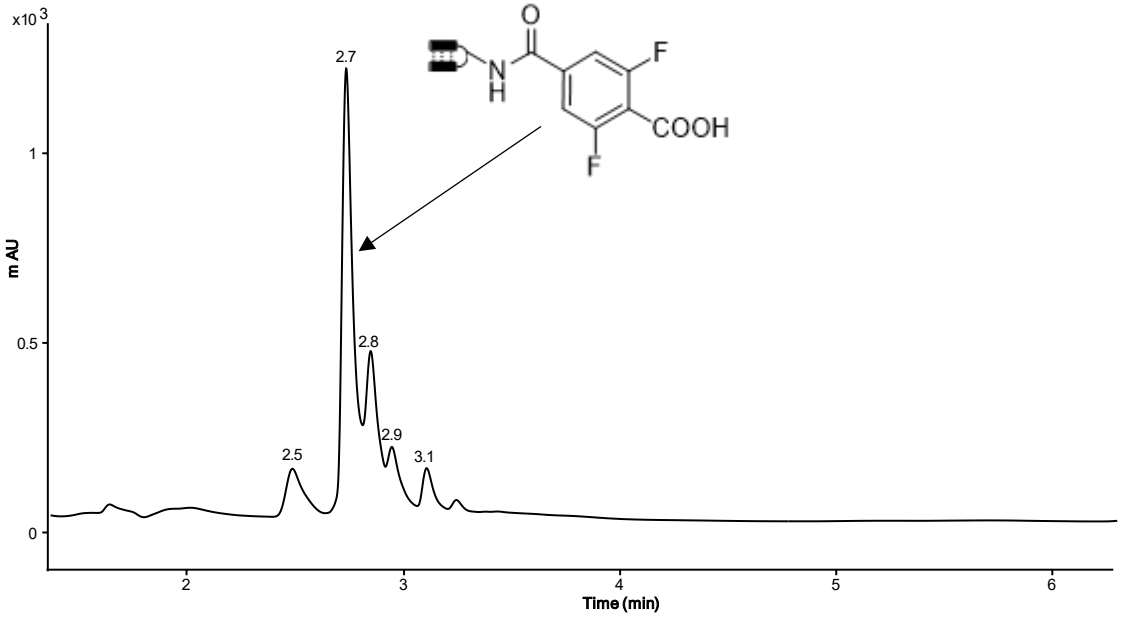


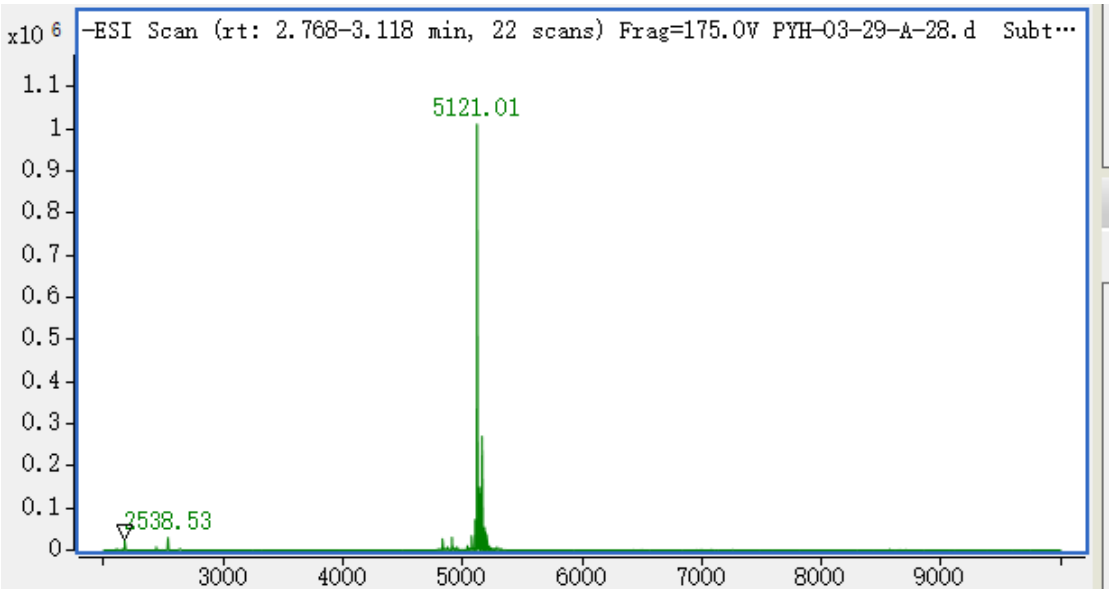


UPLC chromatogram and deconvoluted MS of **b11**

Conversion: 55%

Calculated Mass: 5121 Da; Observed Mass: 5121 Da

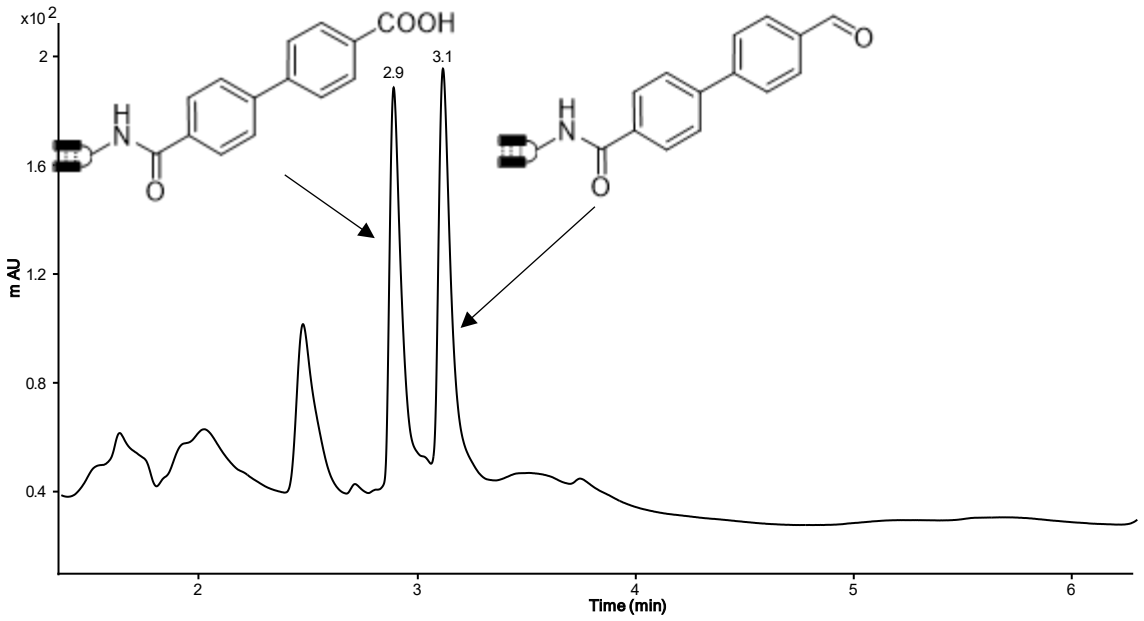


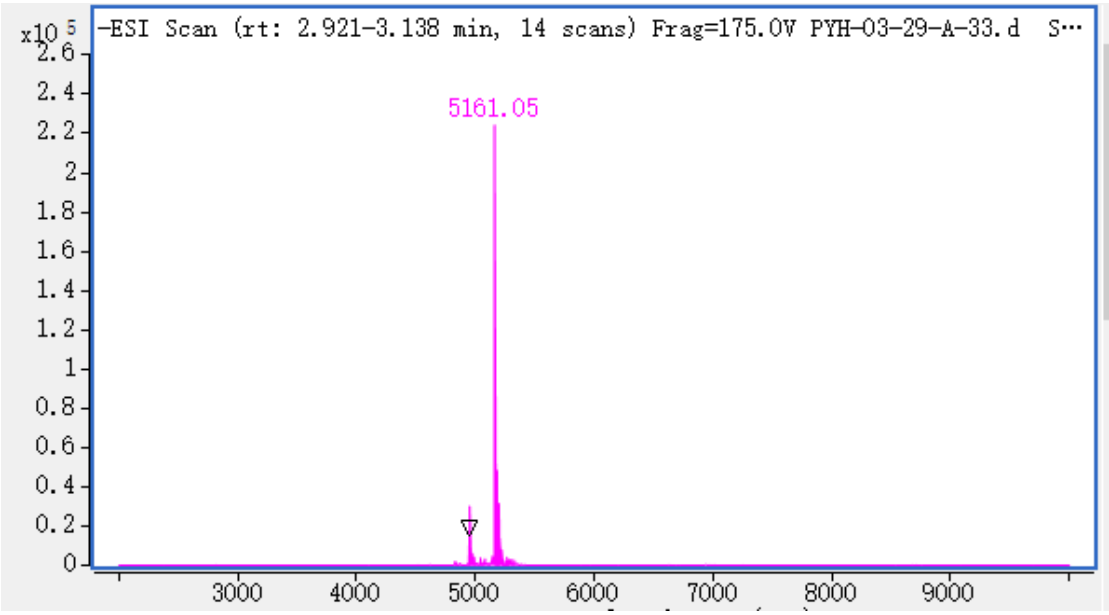


UPLC chromatogram and deconvoluted MS of **b12**

Conversion: 48%

Calculated Mass: 5161 Da; Observed Mass: 5161 Da

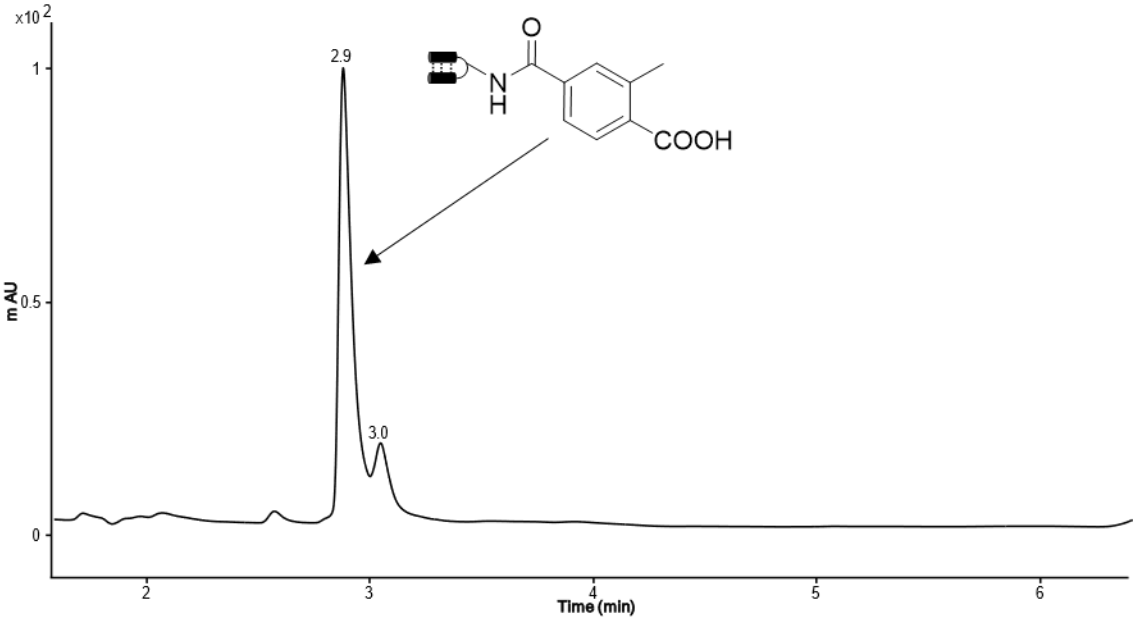


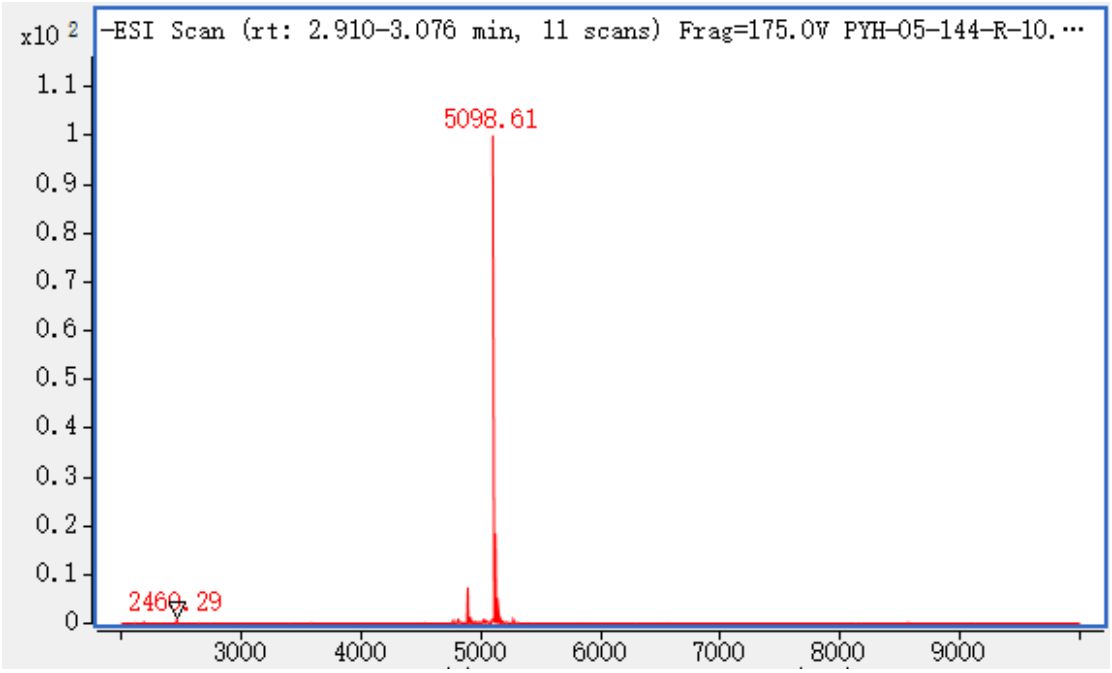


UPLC chromatogram and deconvoluted MS of **b13**

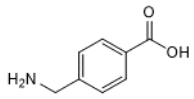
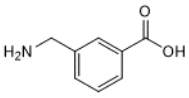
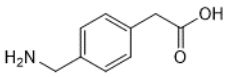
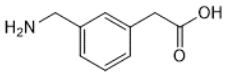
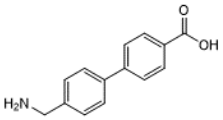
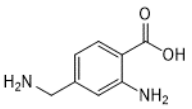
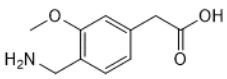
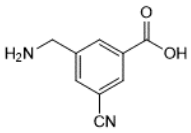
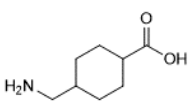
Conversion: 81%

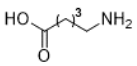
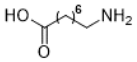
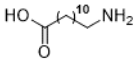
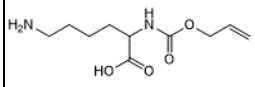
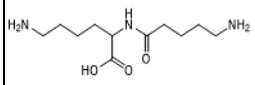
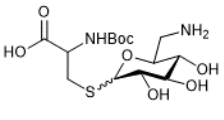
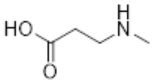
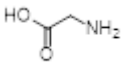
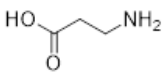
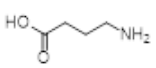
Calculated Mass: 5099 Da; Observed Mass: 5099 Da

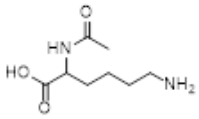
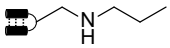
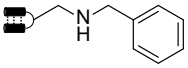




7.3 Substrate scope of DNA-conjugated amines

Compound	Structure	Product	Calculated mass [Da]	Observed mass [Da]	Conversion [%]
e1		**f1**	5066	5066	**>90%**
e2		**f2**	5066	5066	**>90%**
e3		**f3**	5080	5080	**>90%**
e4		**f4**	5080	5080	**>90%**
e5		**f5**	5142	5142	**>90%**
e6		**f6**	5081	5081	**70%**
e7		**f7**	5110	5110	**70%**
e8		**f8**	5091	5091	**70%**
e9		**f9**	5072	5072	**>90%**

e10		**f10**	5032	5032	**>90%**
e11		**f11**	5074	5074	**>90%**
e12		**f12**	5130	5130	**>90%**
e13		**f13**	5145	5145	**>90%**
e14		**f14**	5156	5156	**>90%**
e15		**f15**	5297	5297	**>90%**
e16		**f16**	5009	5009	**>90%***
e17		**f17**	5008	5008	**>90%***
e18		**f18**	5009	5009	**>90%***
e19		**f19**	5018	5018	**>90%**

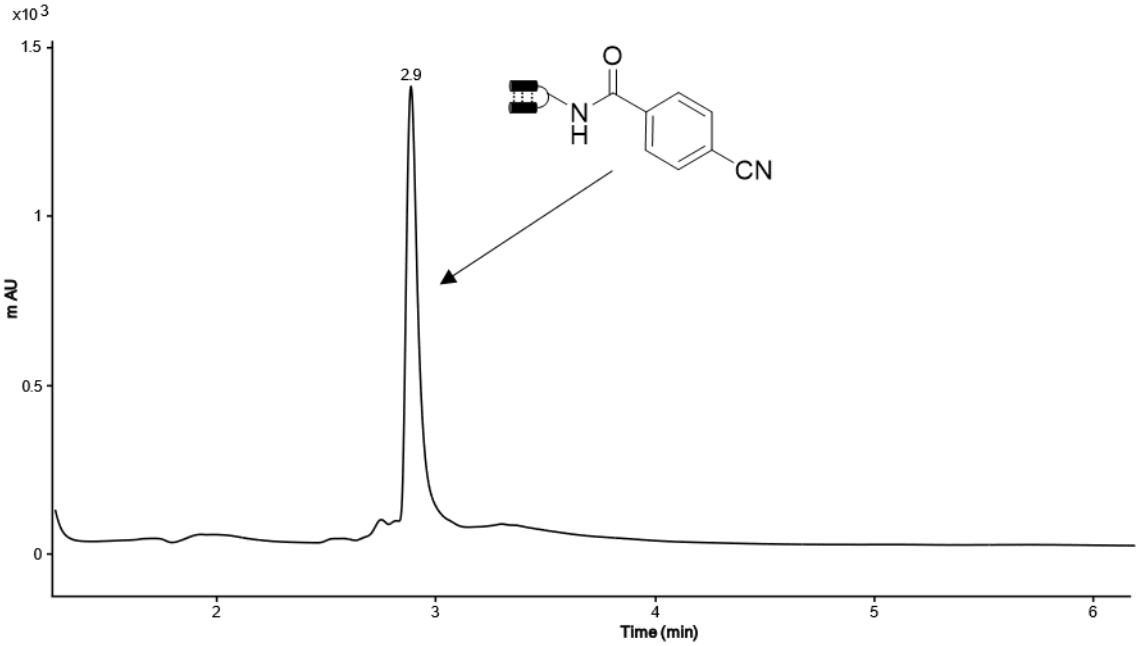
e20		**f20**	5103	5103	**>90%**
e21		**f21**	4952	4952	**76%***
e22		**f22**	4952	4952	**54%***

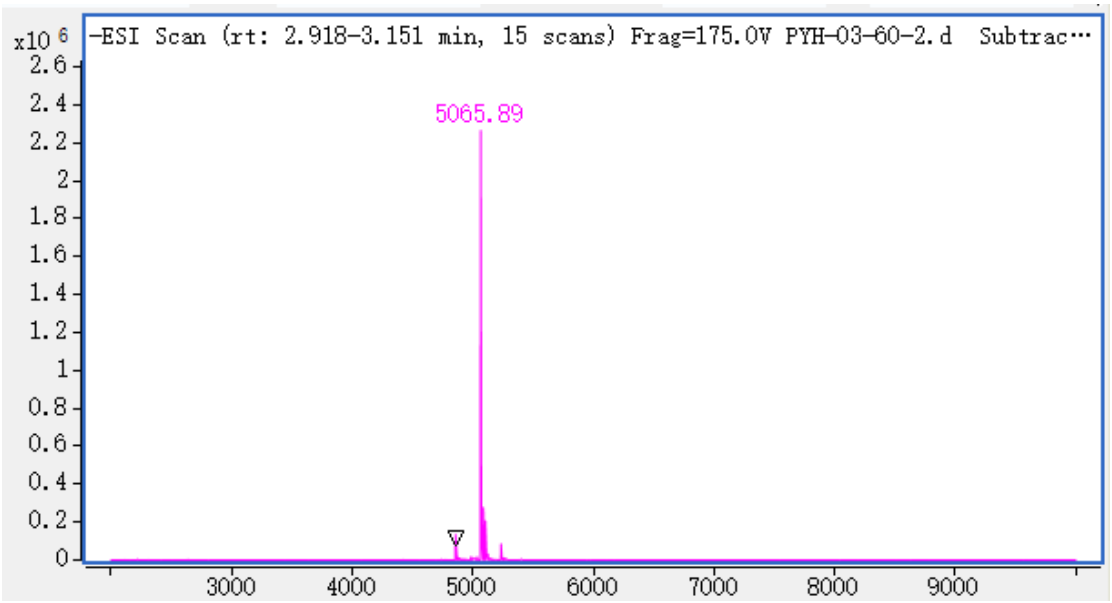
* The K_2RuO_4-mediated oxidation reactions of amino acids and secondary amines were different from benzylamines and aliphatic amines with long alkyl chains (≥3). We speculate this is due to different transformations of the imine intermediate during the oxidation process. For glycine and the beta amino-acid, according to the chain length, they resulted in oxamide (**e17**) or carboxylic acid structures (**e18**). A long alkyl chain between the primary amine and the amide bond (≥3) ensured the successful transformation into nitriles (**e19**, **e20**). For secondary amines, a mixture of carboxylic acids and nitriles were generated (**e21**, **e22**). In sum, benzyl amines and aliphatic amines with long alkyl chains (≥3) could be converted into nitriles by K_2RuO_4-mediated oxidation.

UPLC chromatogram and deconvoluted MS of **e1**

Conversion: ＞90%

Calculated Mass: 5066 Da; Observed Mass: 5066 Da

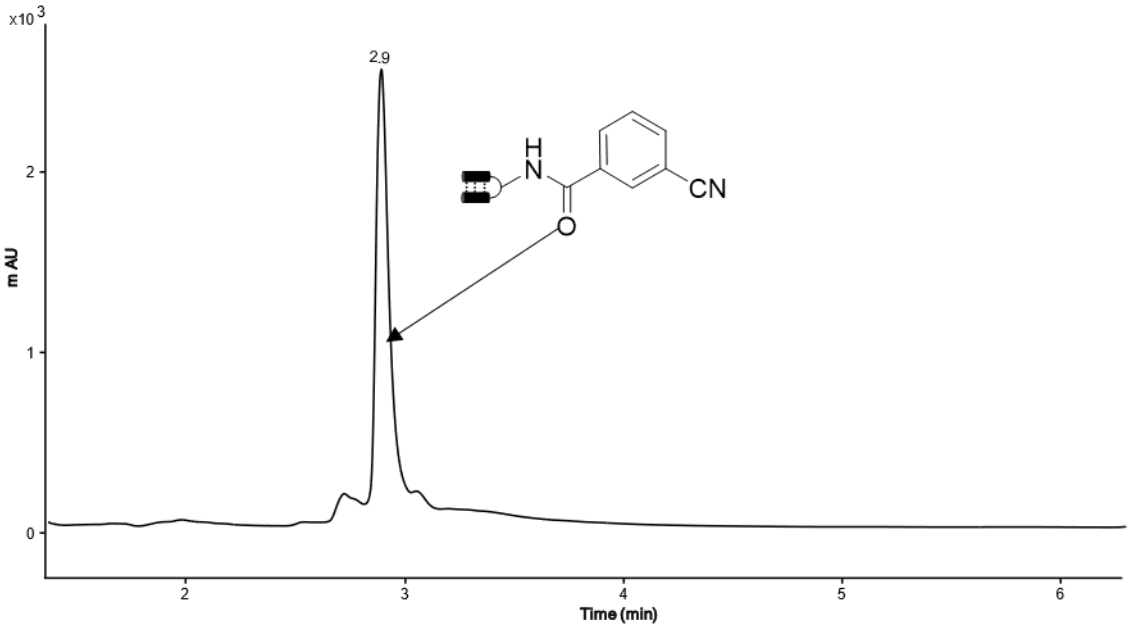


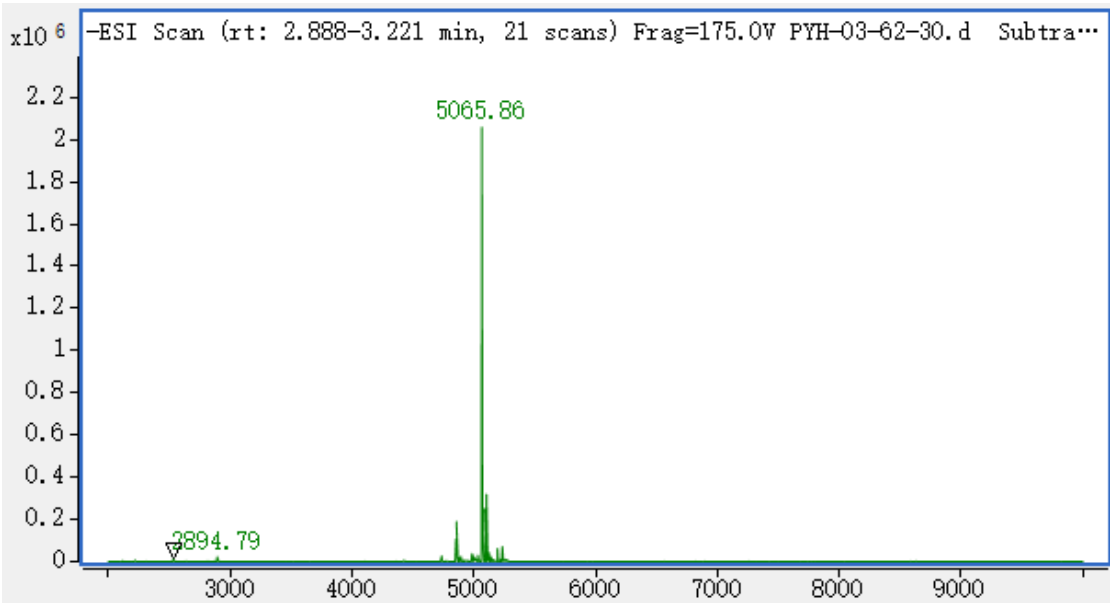


UPLC chromatogram and deconvoluted MS of **e2**

Conversion: ＞90%

Calculated Mass: 5066 Da; Observed Mass: 5066 Da

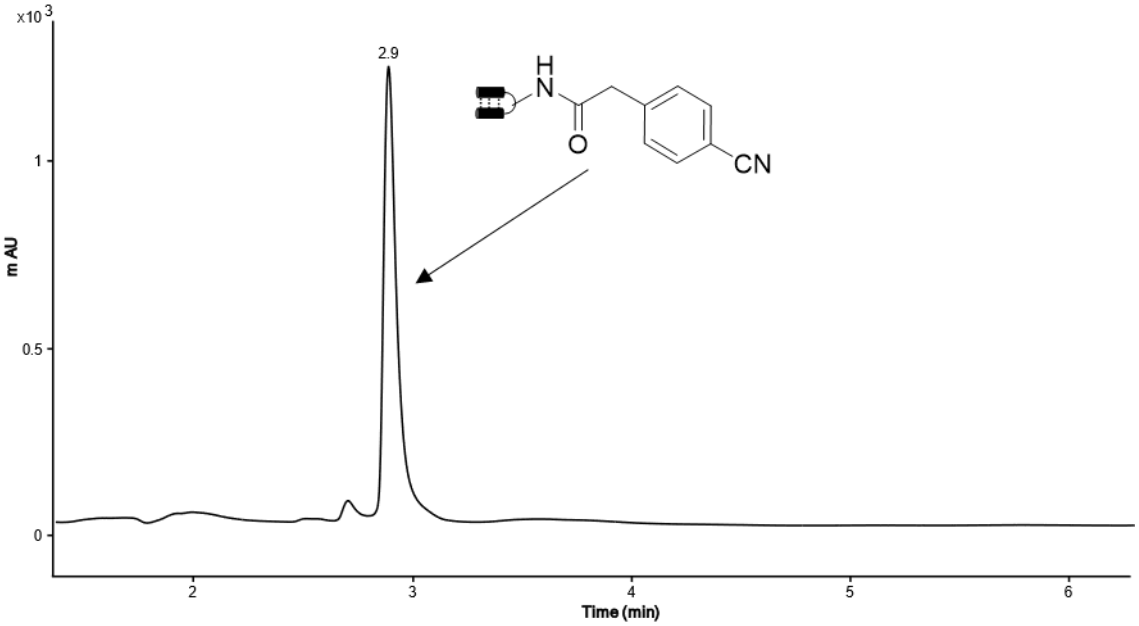


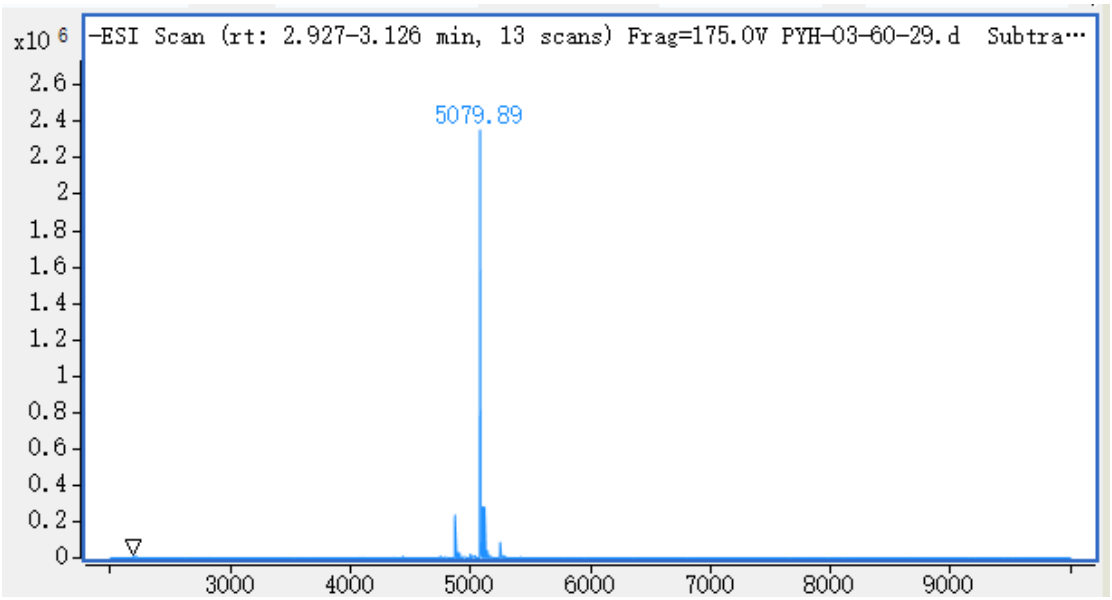


UPLC chromatogram and deconvoluted MS of **e3**

Conversion: ＞90%

Calculated Mass: 5080 Da; Observed Mass: 5080 Da

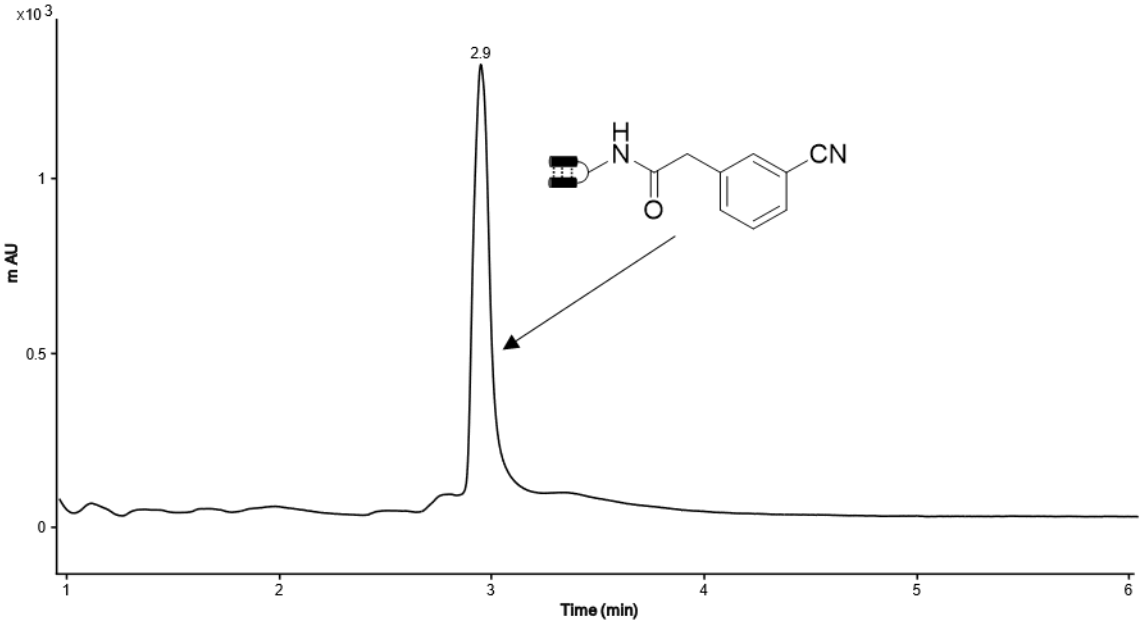


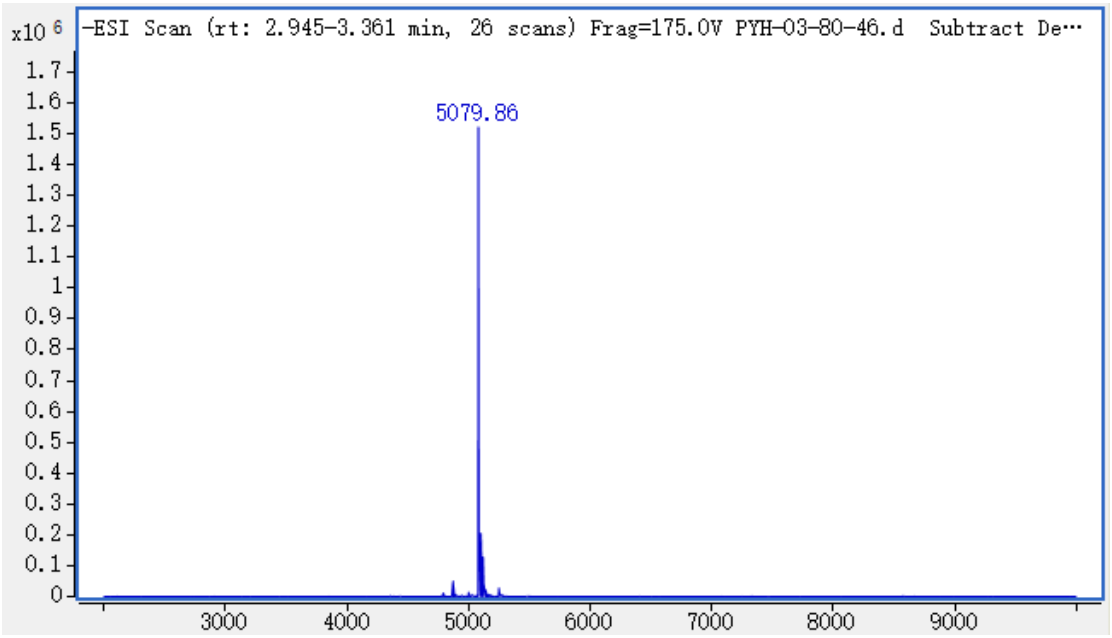


UPLC chromatogram and deconvoluted MS of **e4**

Conversion: ＞90%

Calculated Mass: 5080 Da; Observed Mass: 5080 Da

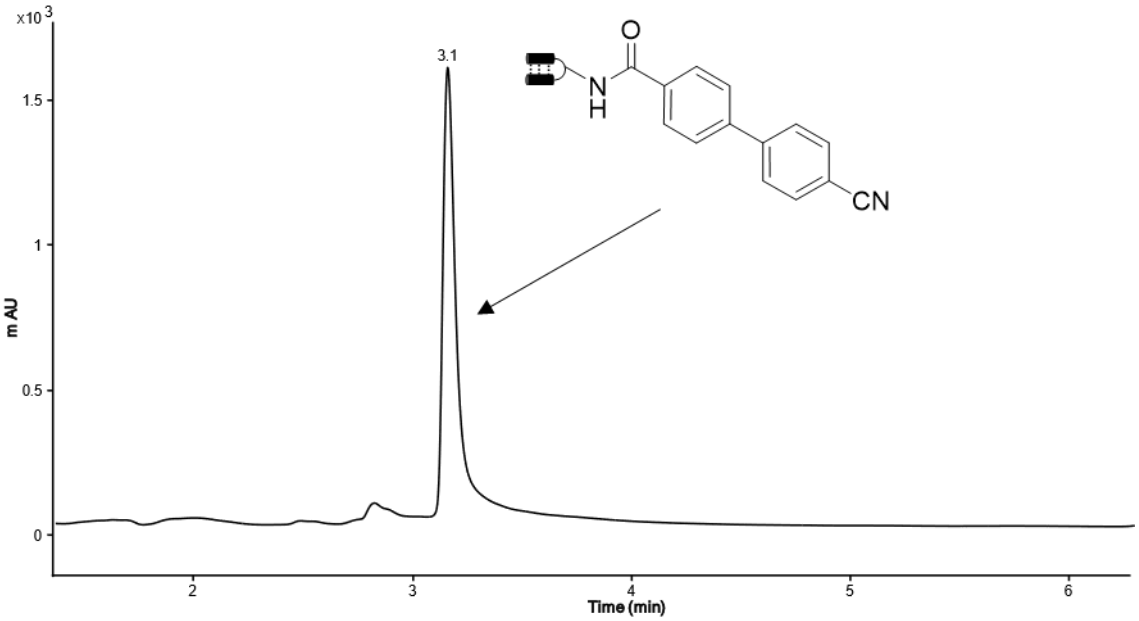


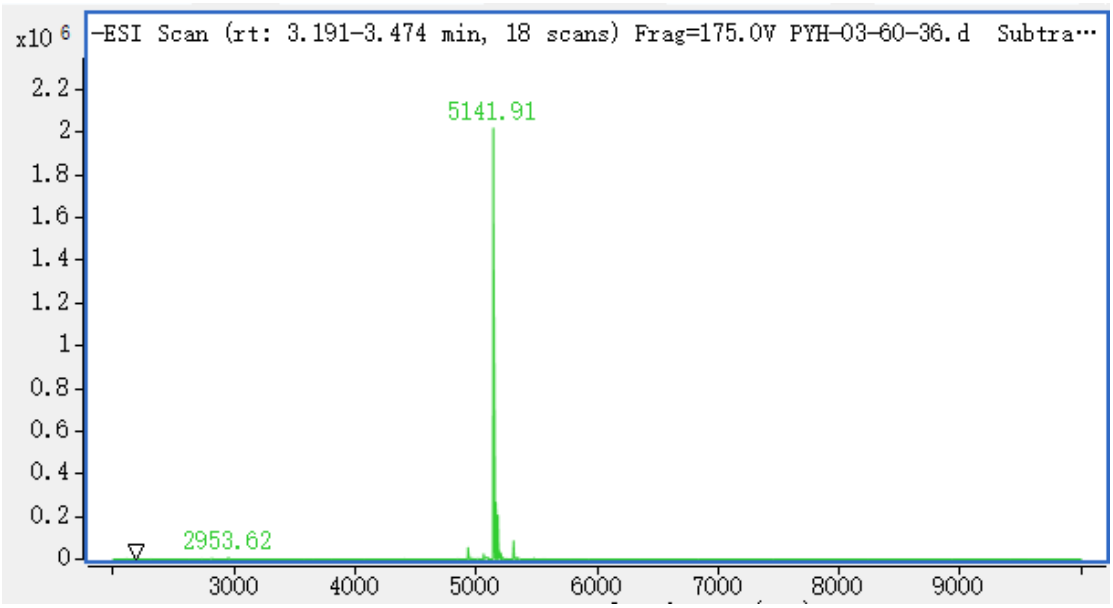


UPLC chromatogram and deconvoluted MS of **e5**

Conversion: ＞90%

Calculated Mass: 5142 Da; Observed Mass: 5142 Da

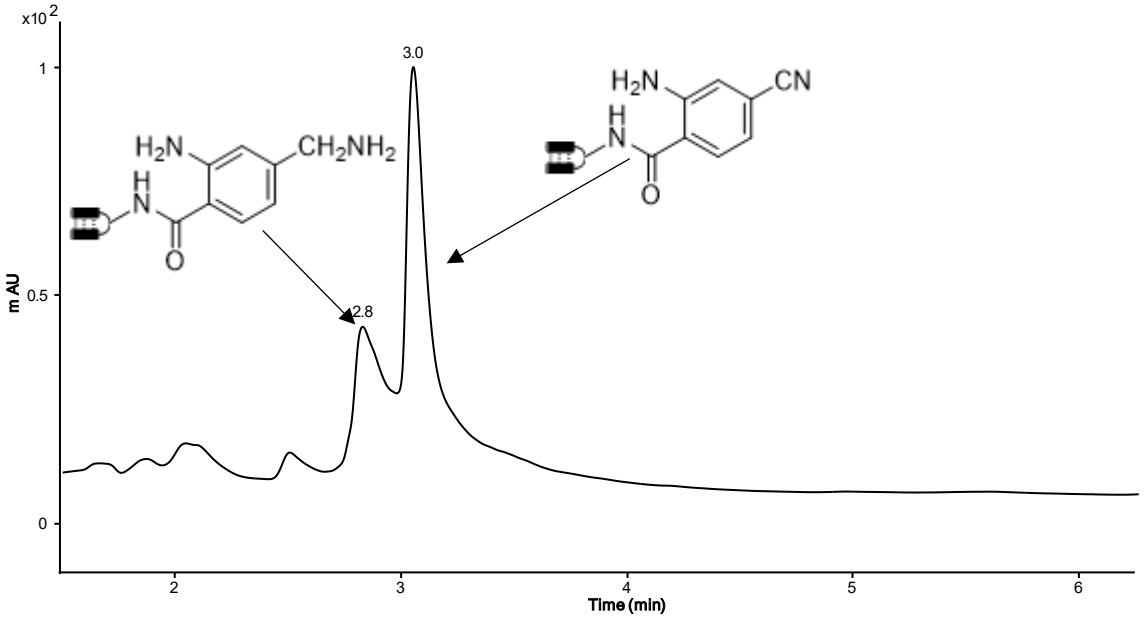


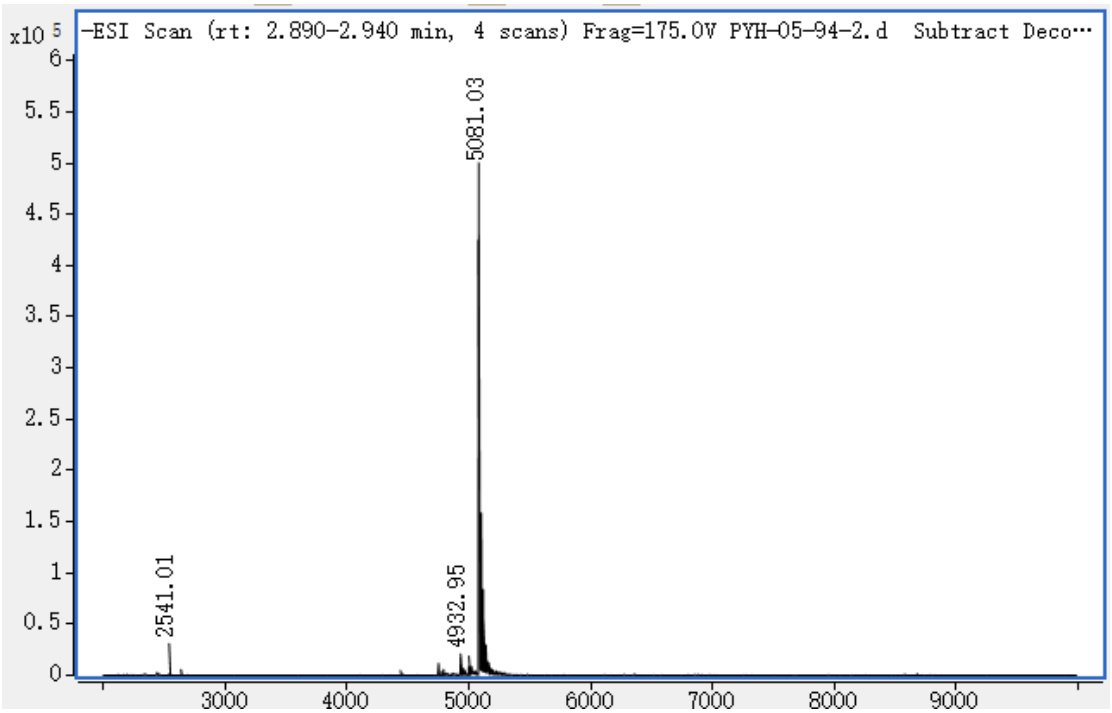


UPLC chromatogram and deconvoluted MS of **e6**

Conversion: 70%

Calculated Mass: 5081 Da; Observed Mass: 5081 Da

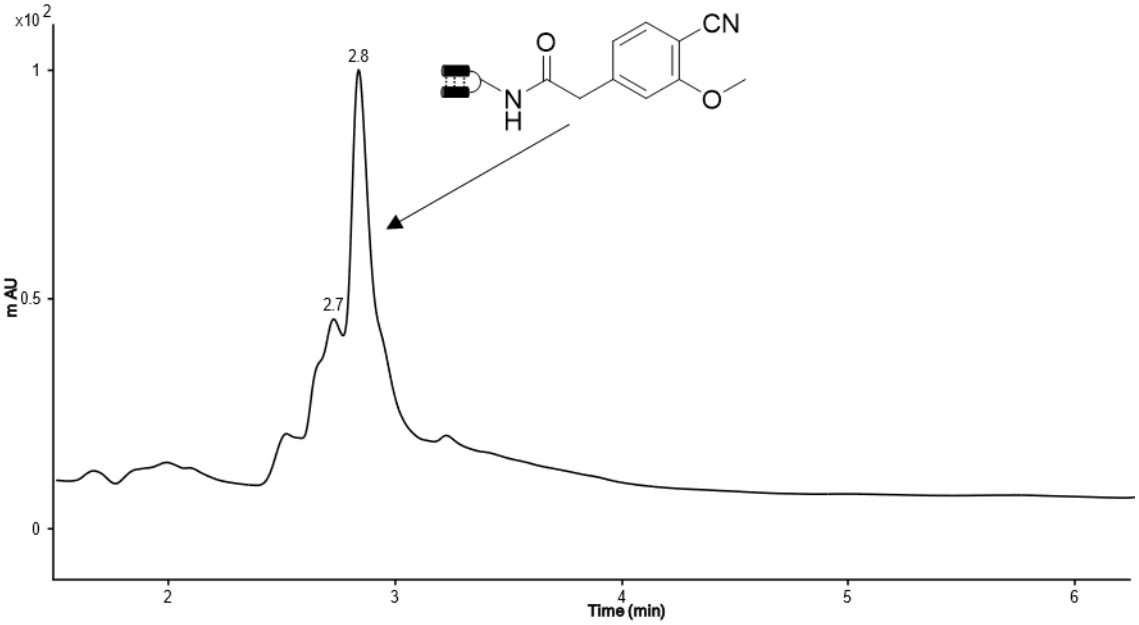


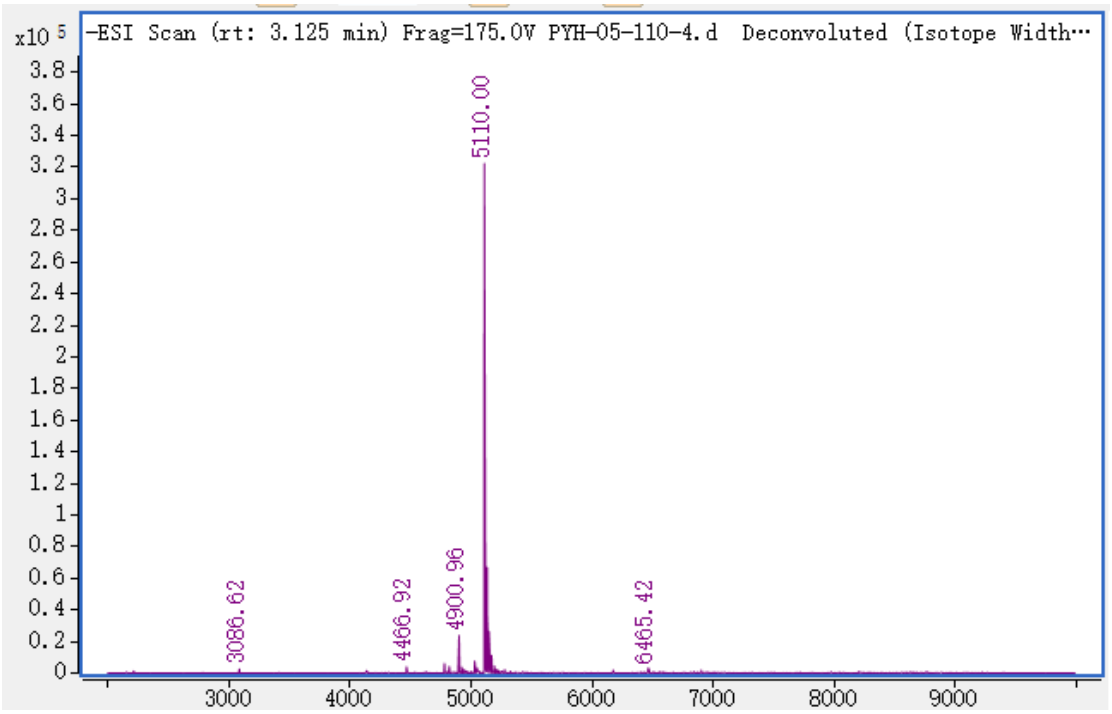


UPLC chromatogram and deconvoluted MS of **e7**

Conversion: 70%

Calculated Mass: 5110 Da; Observed Mass: 5110 Da

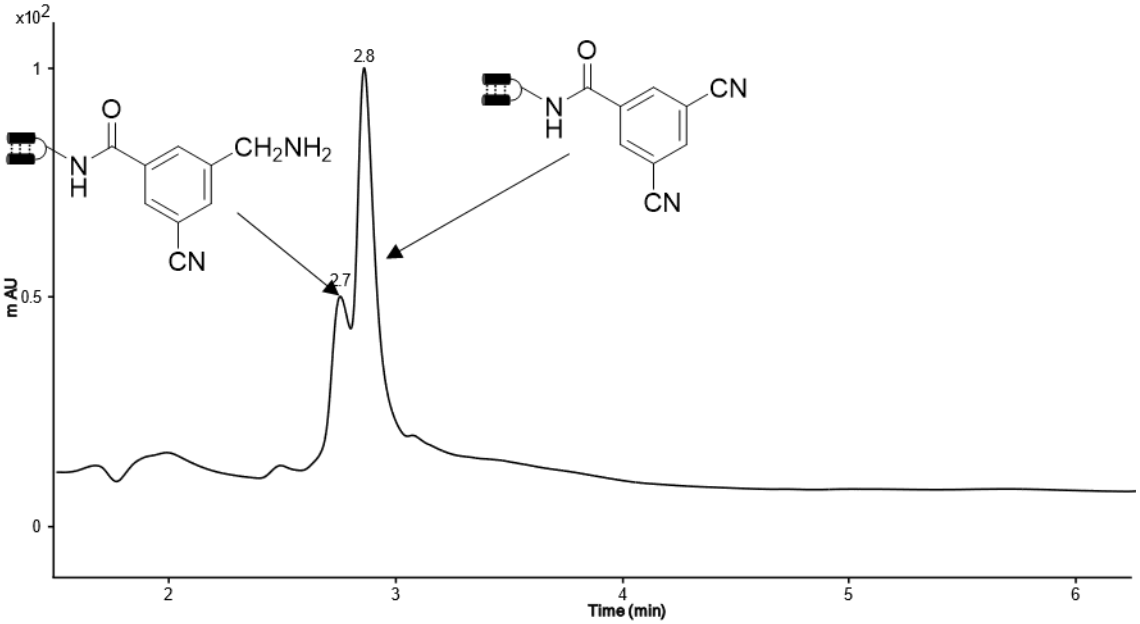


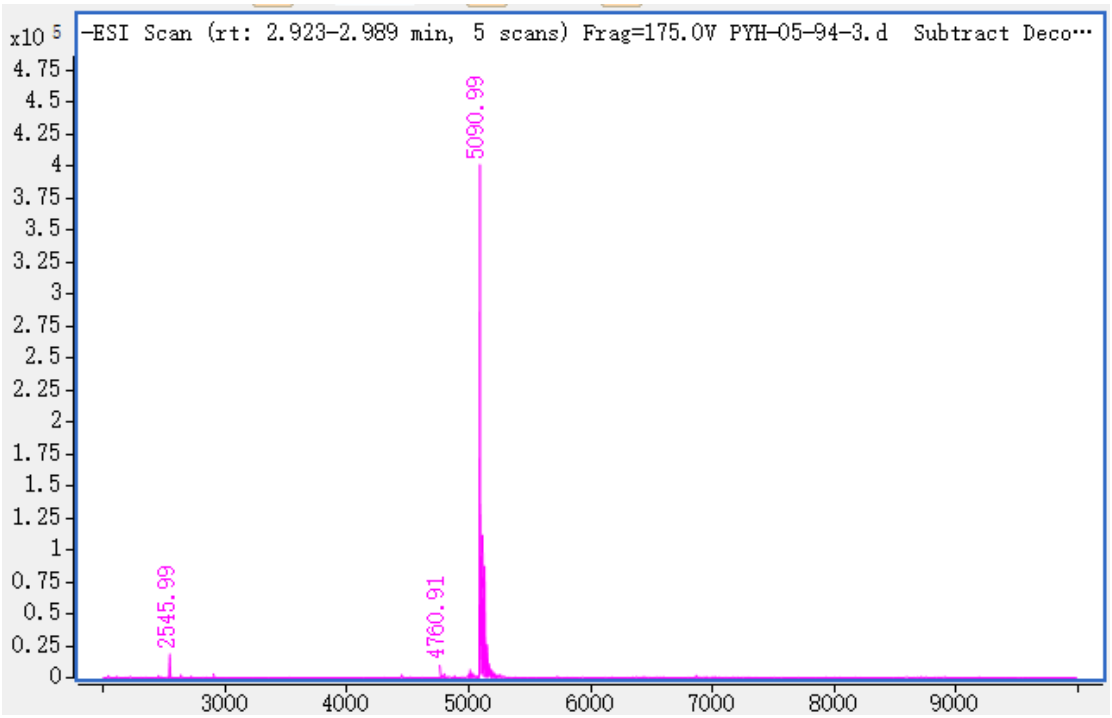


UPLC chromatogram and deconvoluted MS of **e8**

Conversion: 70 %

Calculated Mass: 5091 Da; Observed Mass: 5091 Da

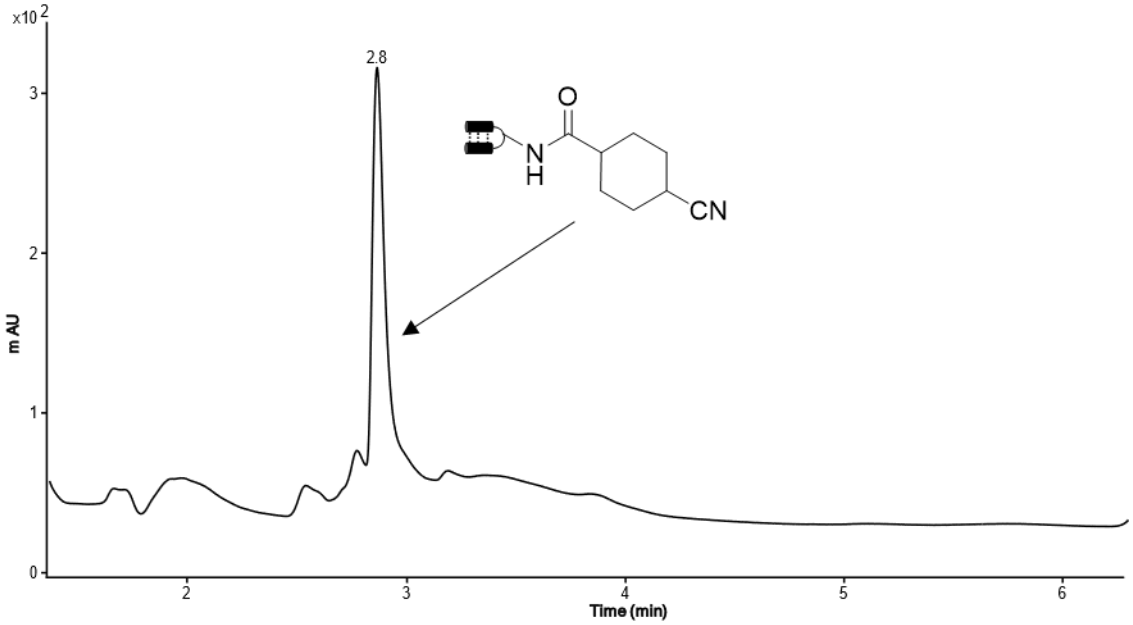


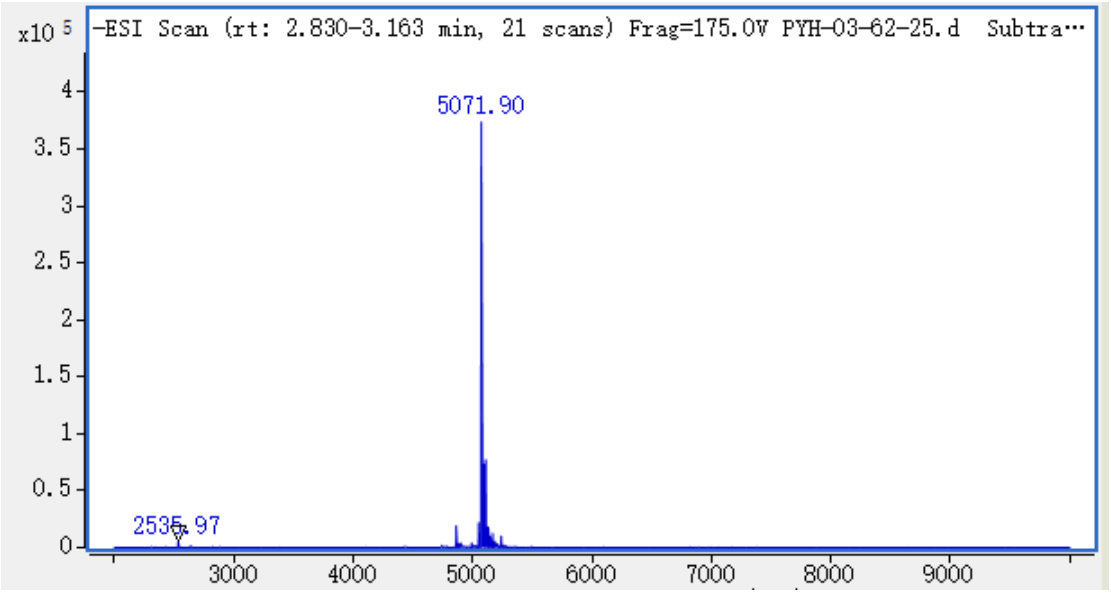


UPLC chromatogram and deconvoluted MS of **e9**

Conversion: ＞90%

Calculated Mass: 5072 Da; Observed Mass: 5072 Da

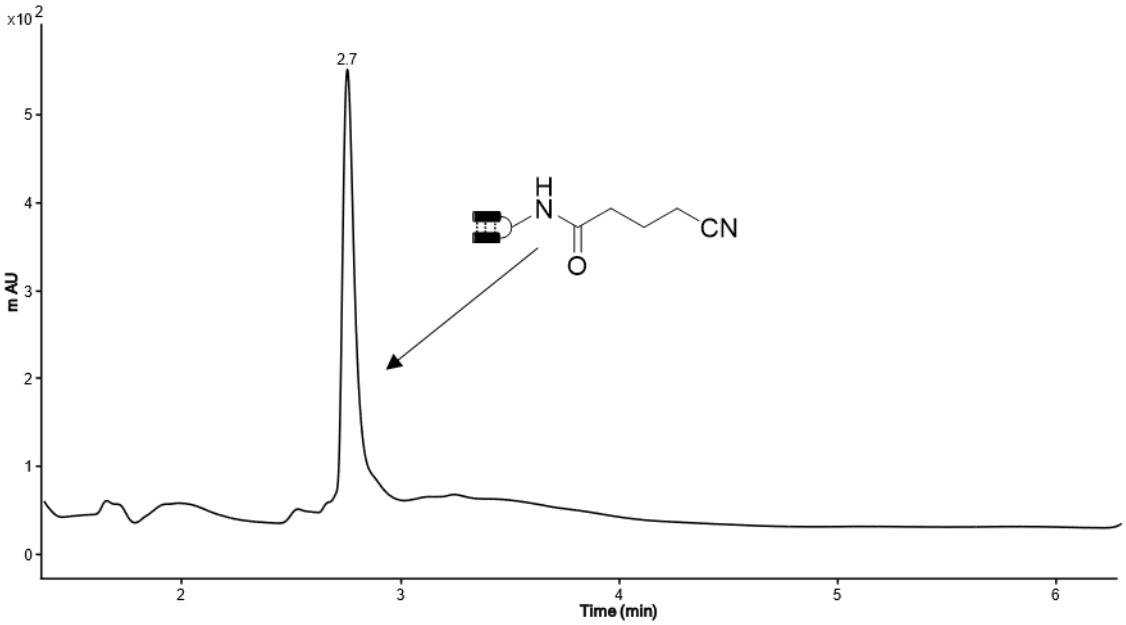


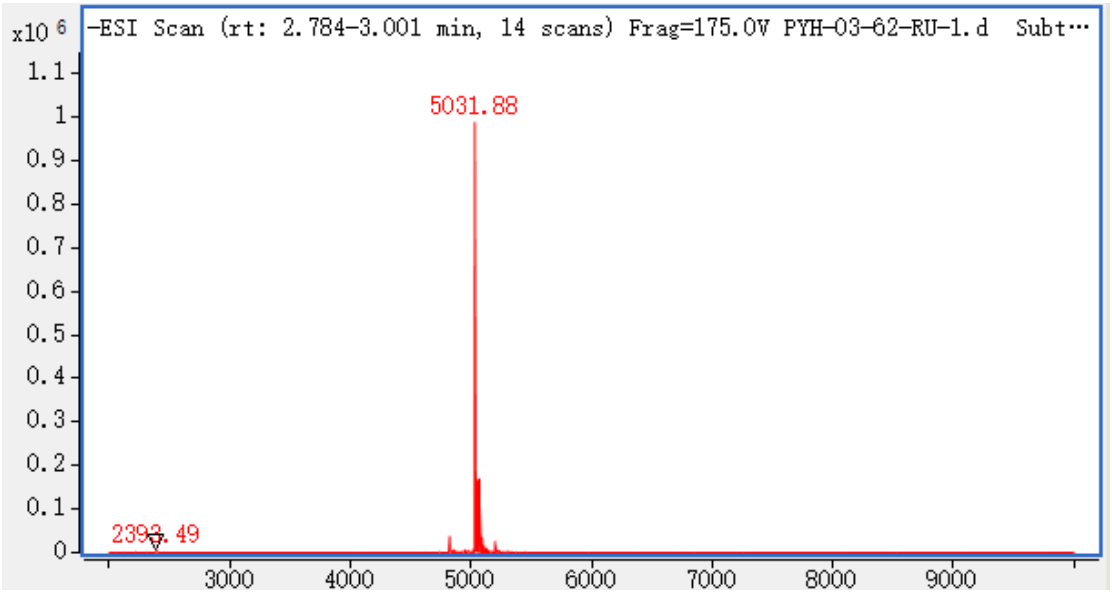


UPLC chromatogram and deconvoluted MS of **e10**

Conversion: ＞90%

Calculated Mass: 5032 Da; Observed Mass: 5032 Da

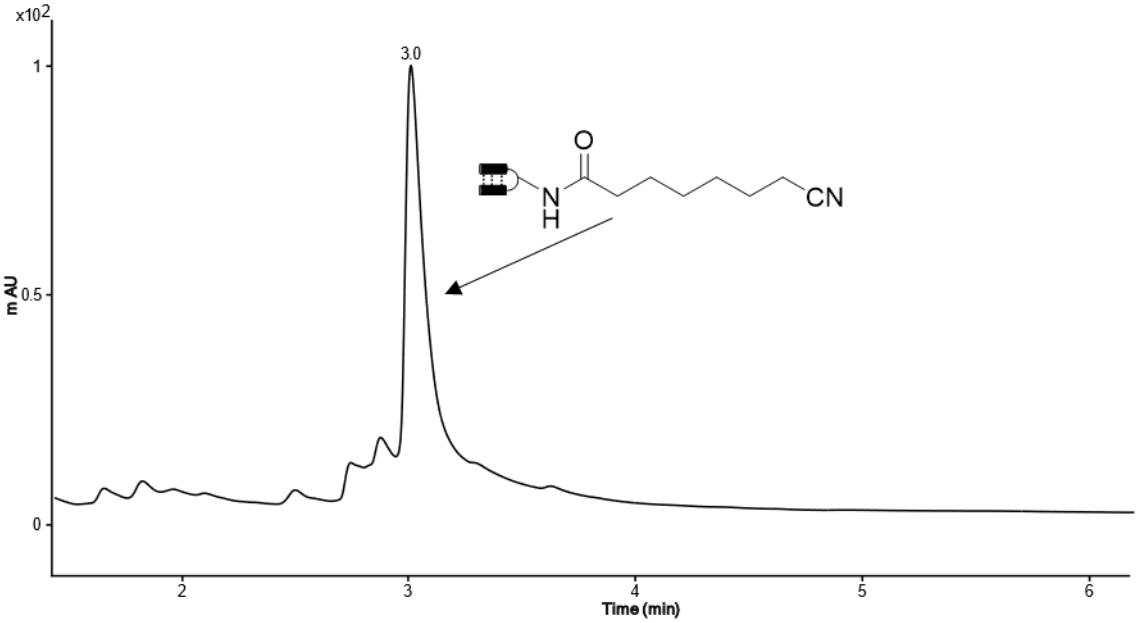


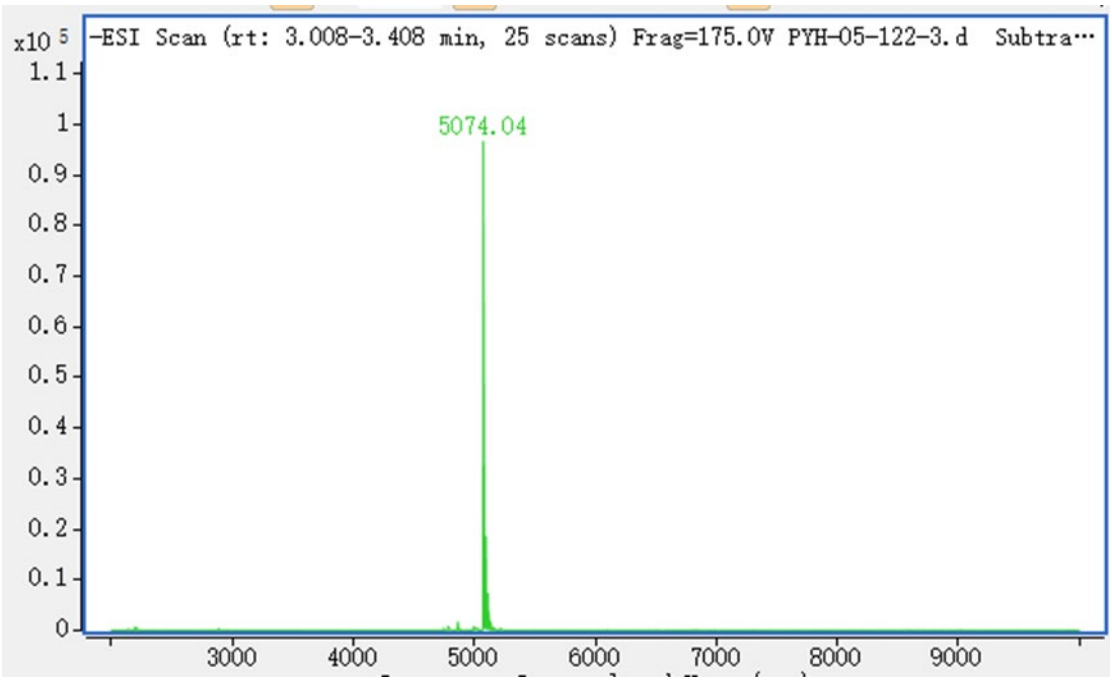


UPLC chromatogram and deconvoluted MS of **e11**

Conversion: ＞90%

Calculated Mass: 5074 Da; Observed Mass: 5074 Da

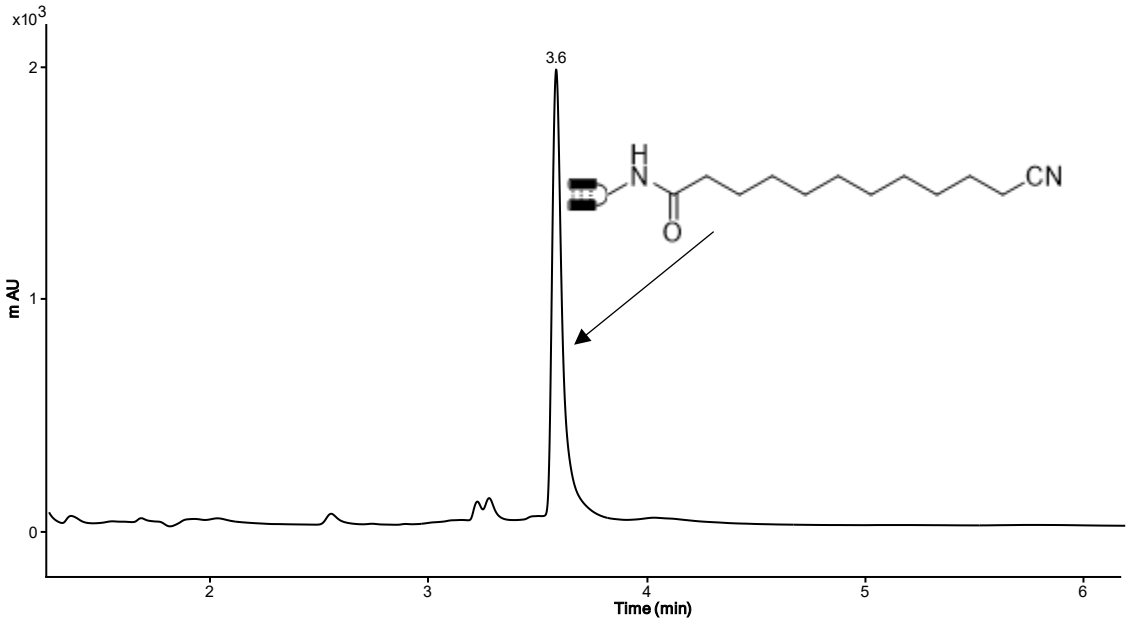


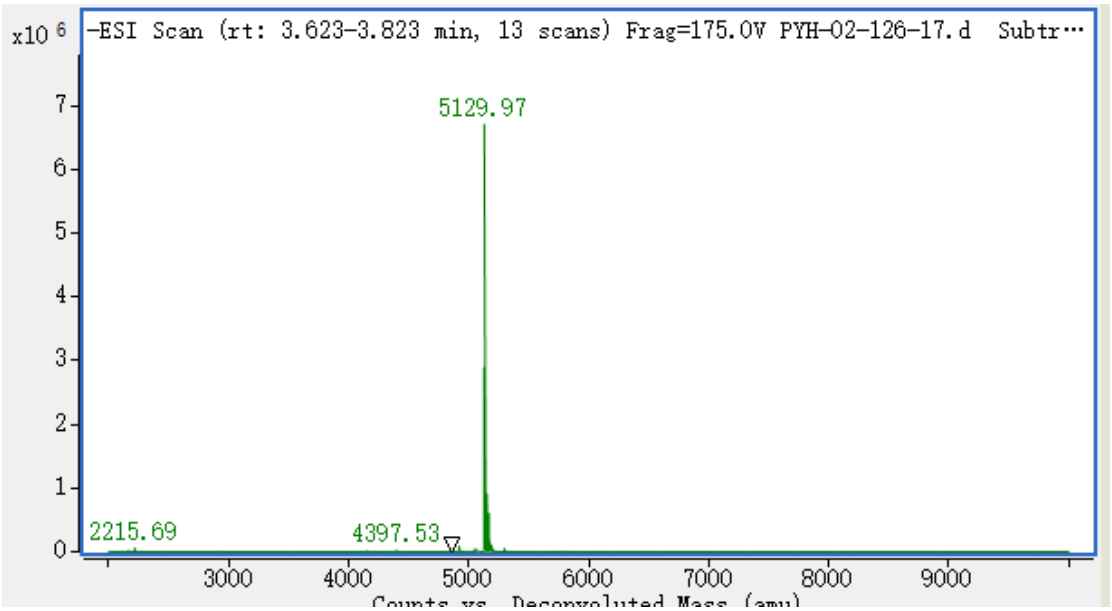


UPLC chromatogram and deconvoluted MS of **e12**

Conversion: ＞90%

Calculated Mass: 5130 Da; Observed Mass: 5130 Da

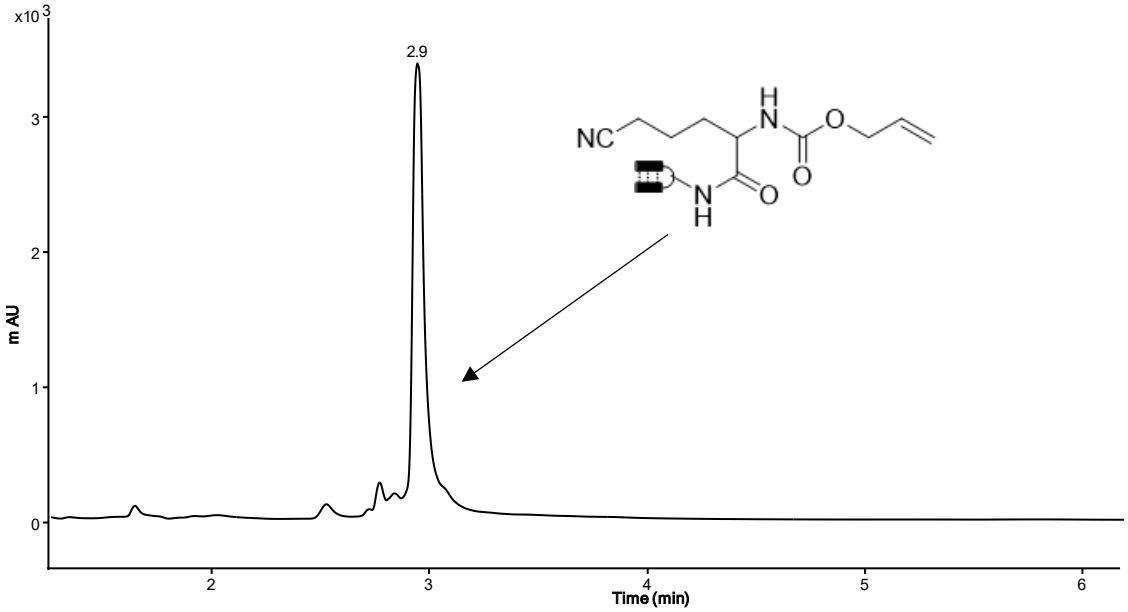


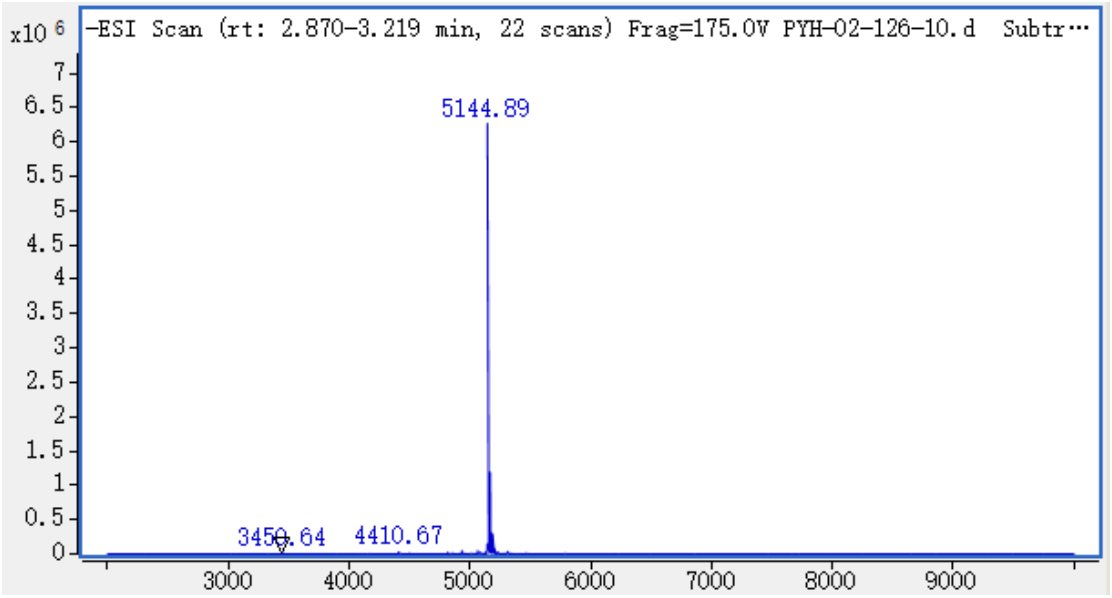


UPLC chromatogram and deconvoluted MS of **e13**

Conversion: ＞90%

Calculated Mass: 5145 Da; Observed Mass: 5145 Da

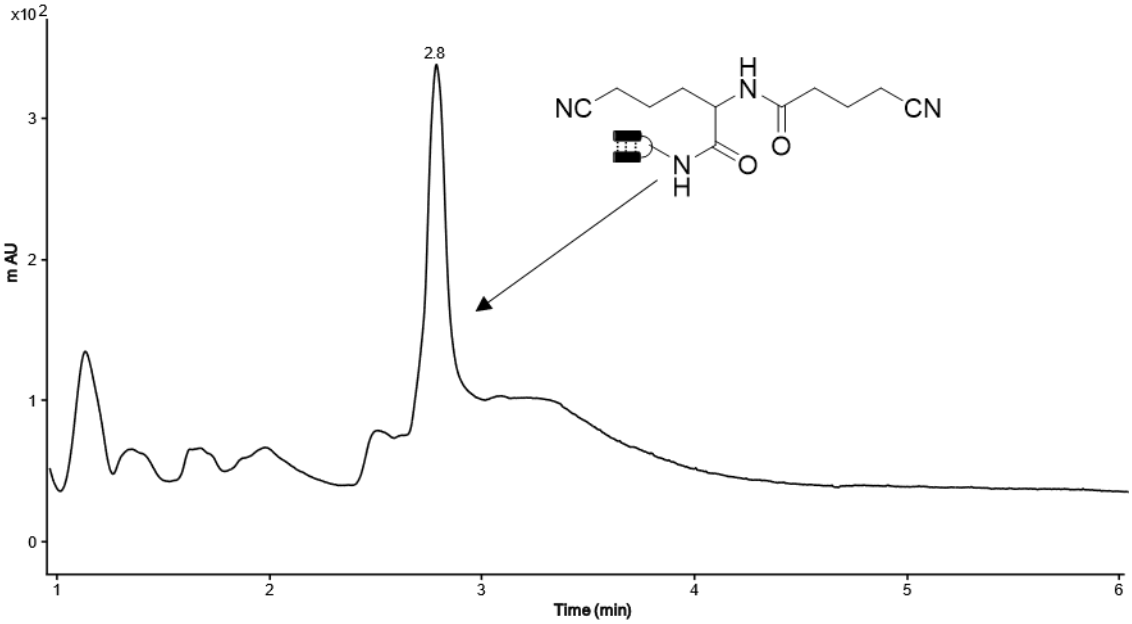


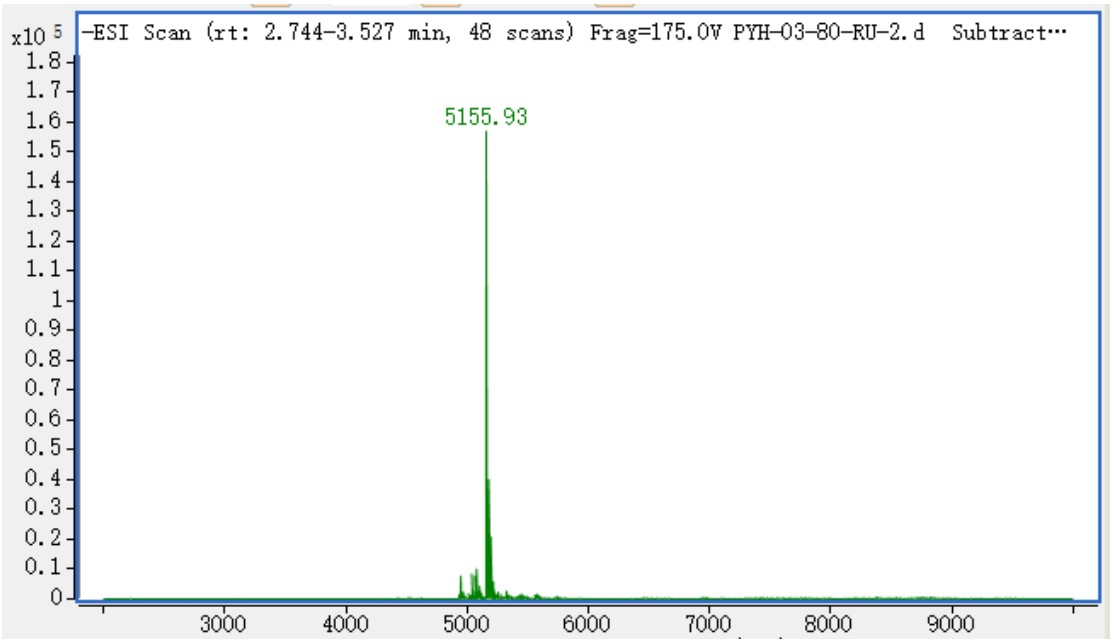


UPLC chromatogram and deconvoluted MS of **e14**

Conversion: ＞90%

Calculated Mass: 5156 Da; Observed Mass: 5156 Da

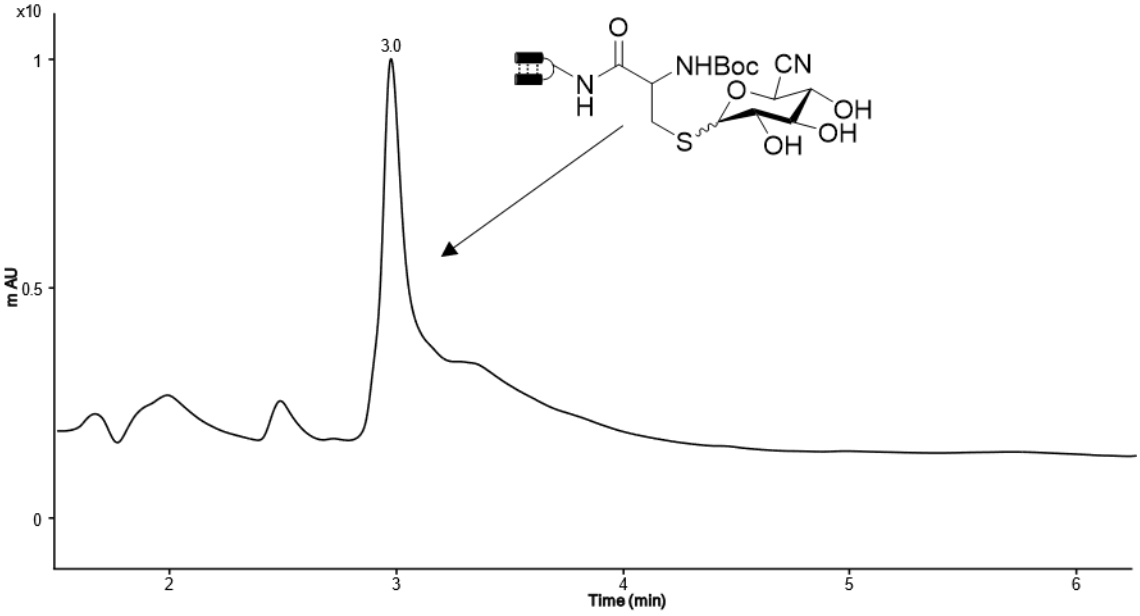


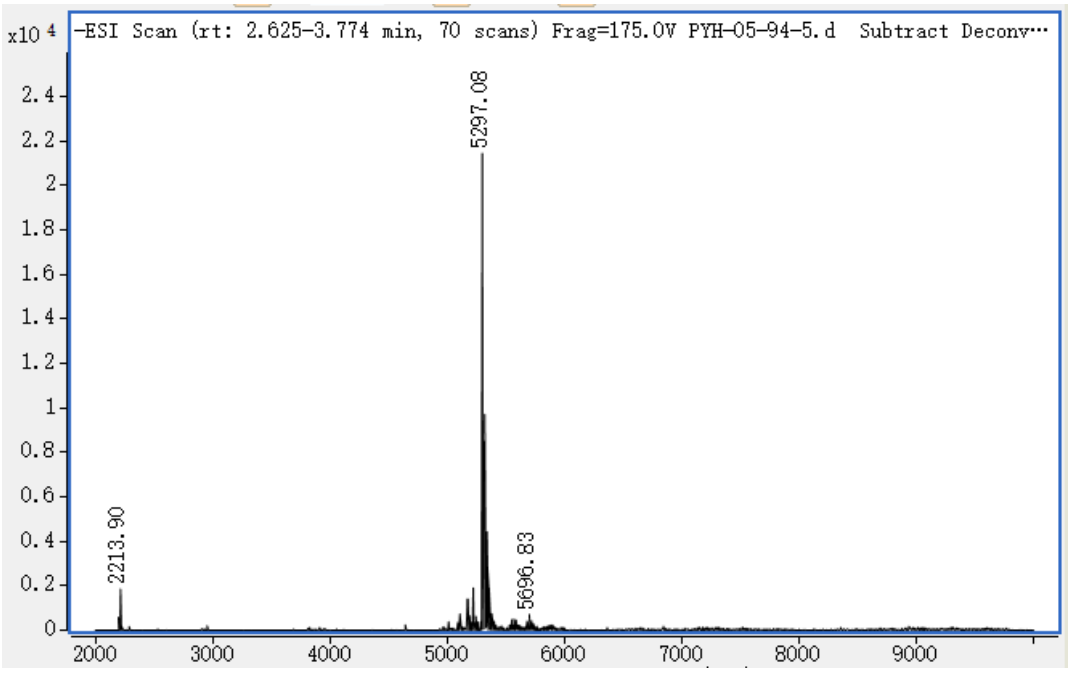


UPLC chromatogram and deconvoluted MS of **e15**

Conversion: ＞90%

Calculated Mass: 5297 Da; Observed Mass: 5297 Da



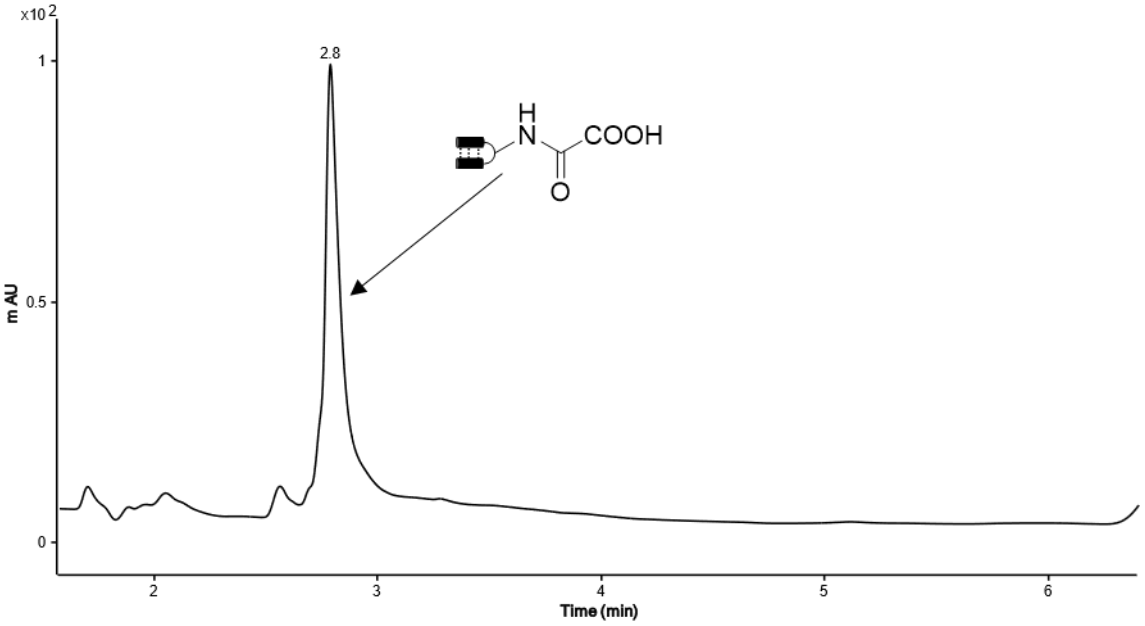


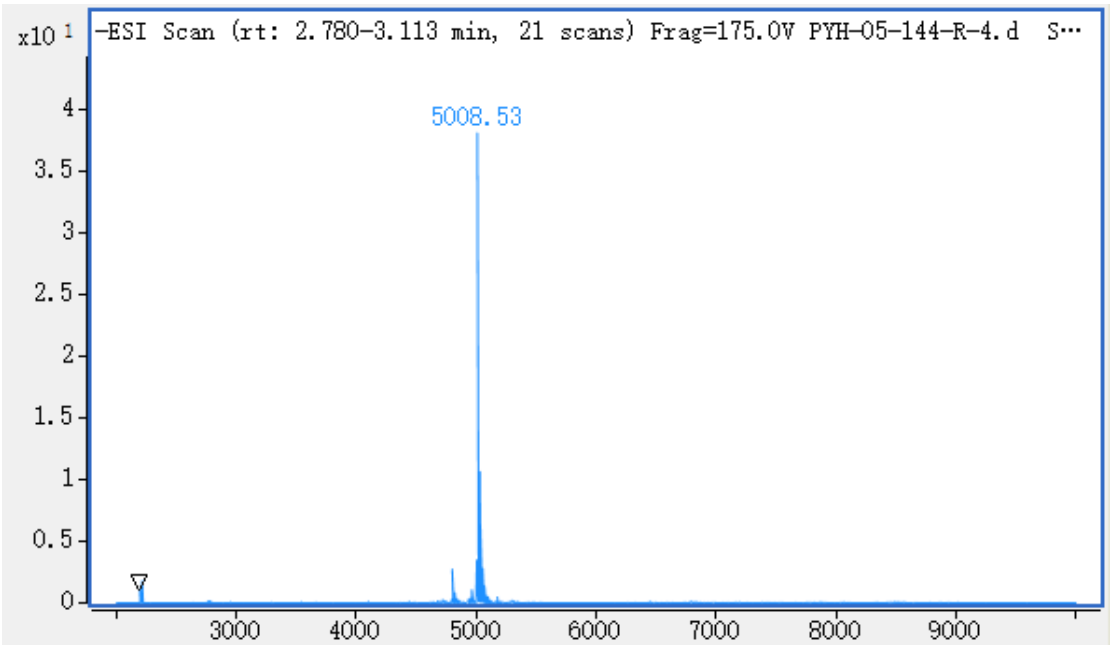
UPLC chromatogram and deconvoluted MS of **e16**

Conversion: ＞90%

Calculated Mass for nitrile: 5004 Da;

Calculated Mass for carboxylic acid: 5009 Da; Observed Mass: 5009 Da



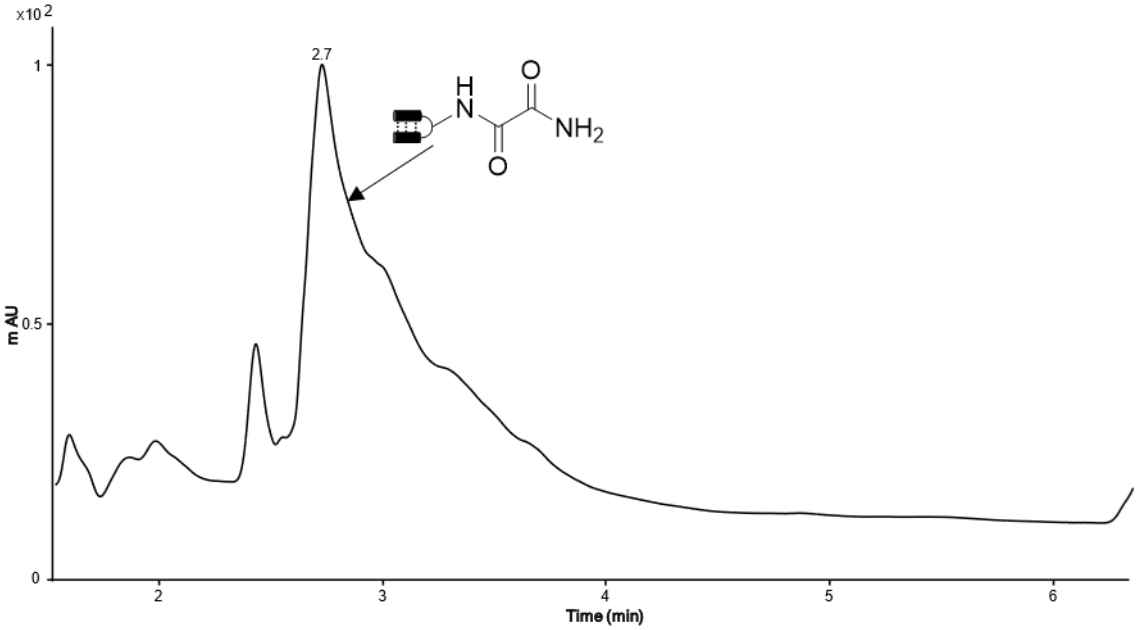


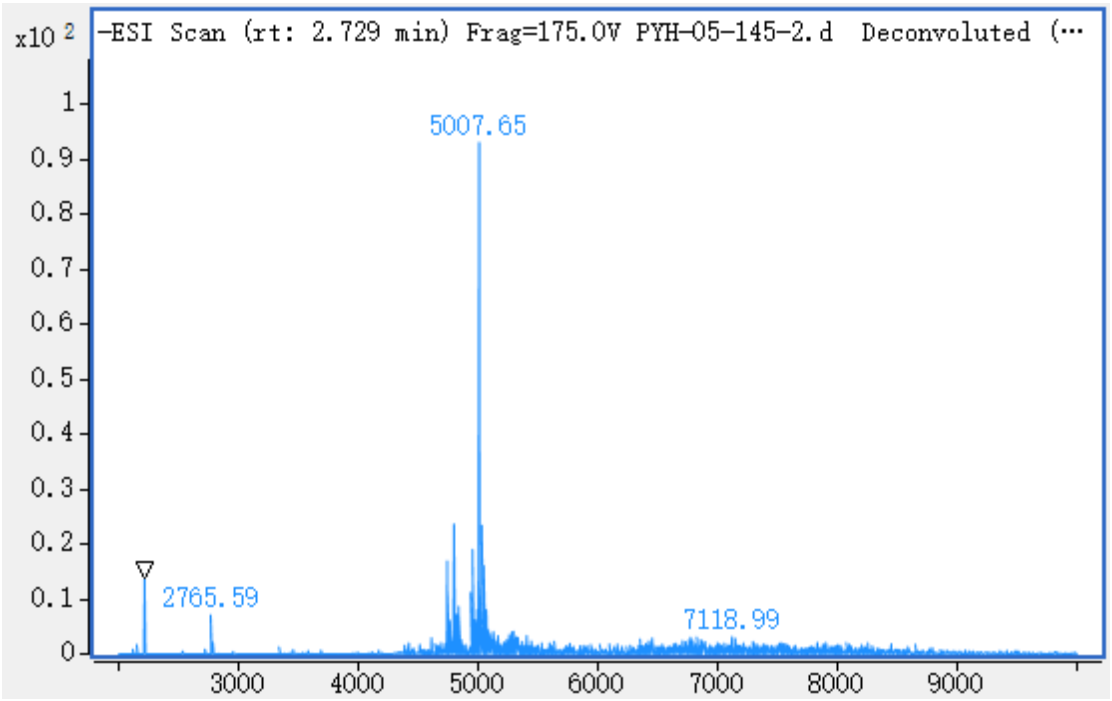
UPLC chromatogram and deconvoluted MS of **e17**

Conversion: ＞90%

Calculated Mass for nitrile : 4990 Da;

Calculated Mass for amide: 5008; Observed Mass: 5008 Da



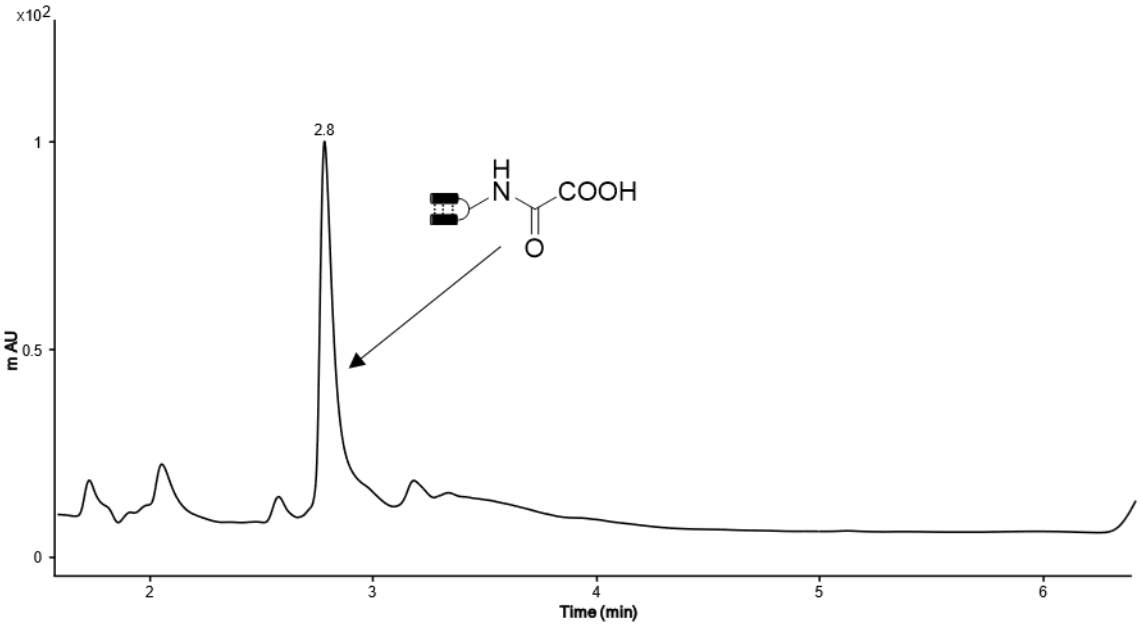


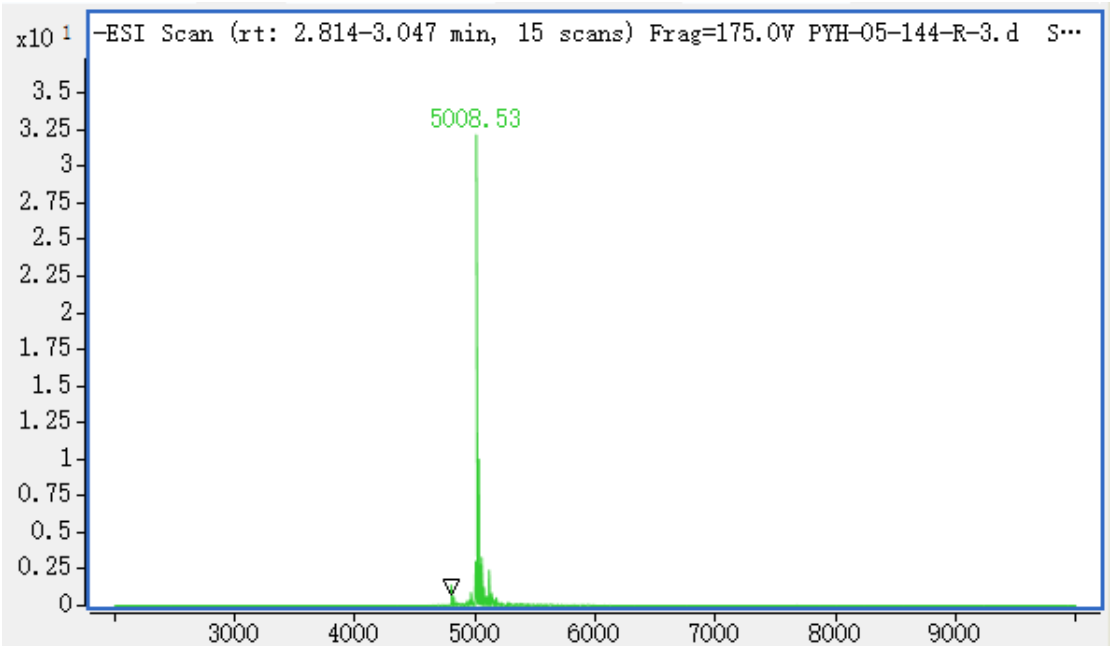
UPLC chromatogram and deconvoluted MS of **e18**

Conversion: ＞90%

Calculated Mass for nitrile: 5004 Da;

Calculated Mass for carboxylic acid: 5009 Da; Observed Mass: 5009 Da

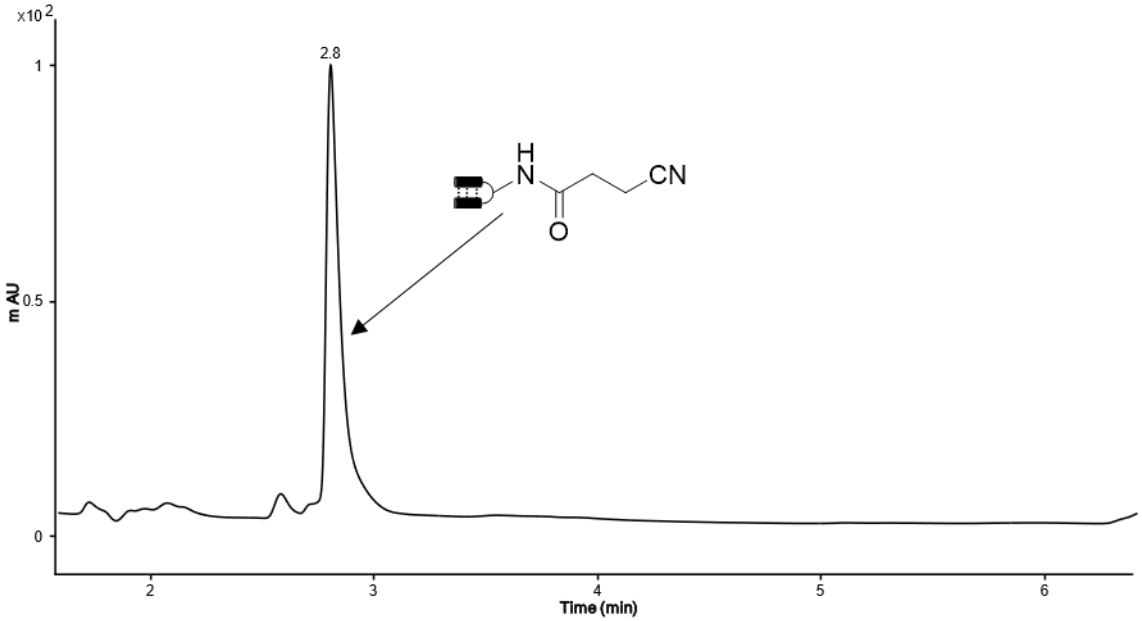


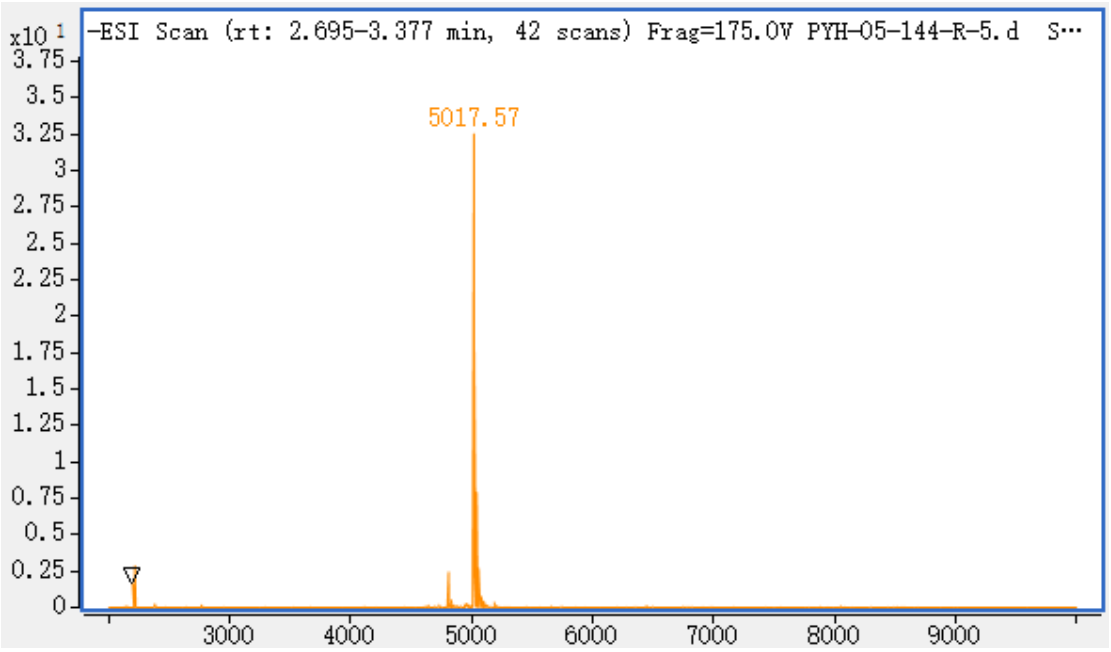


UPLC chromatogram and deconvoluted MS of **e19**

Conversion: ＞90%

Calculated Mass: 5018 Da; Observed Mass: 5018 Da

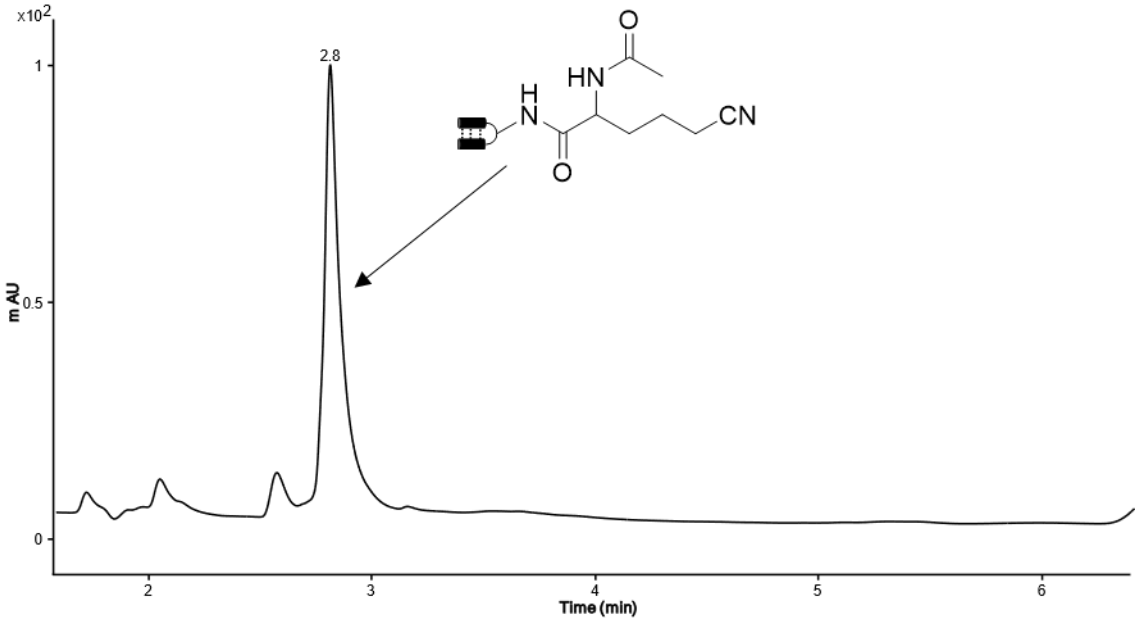


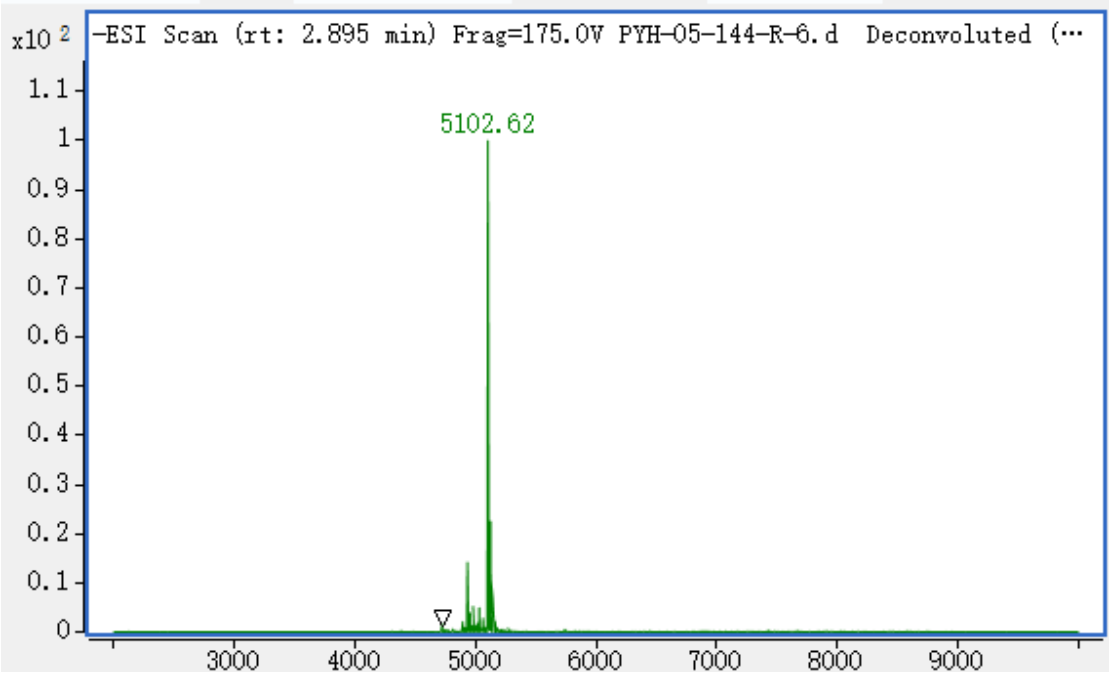


UPLC chromatogram and deconvoluted MS of **e20**

Conversion: ＞90%

Calculated Mass: 5103 Da; Observed Mass: 5103 Da





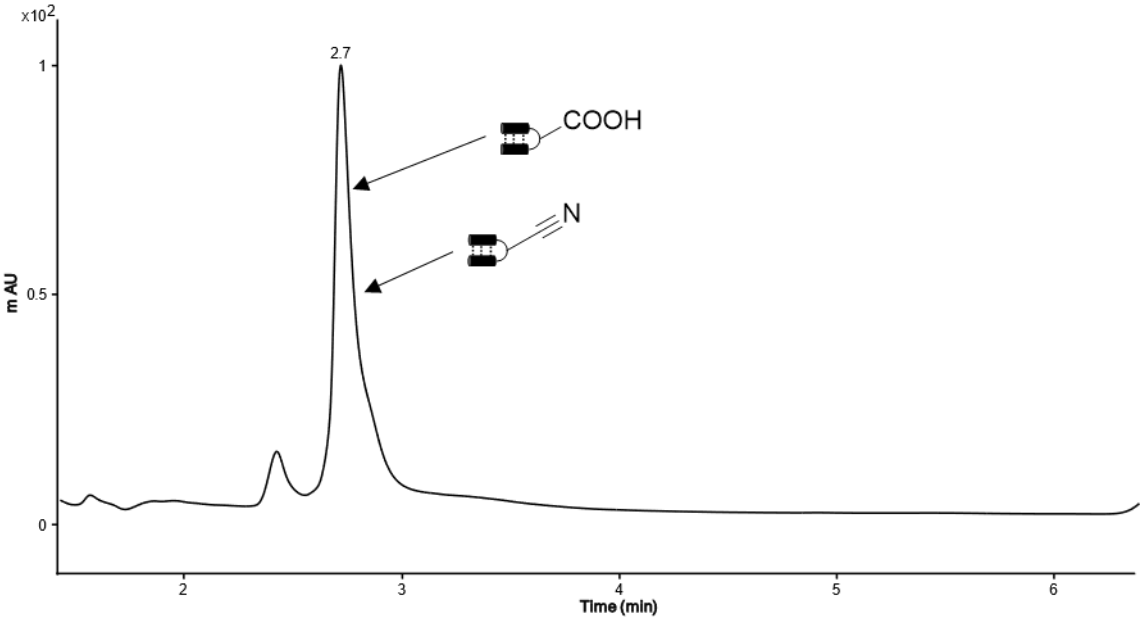
This reactant is prepared from DNA Headpiece by the reductive amination reaction.

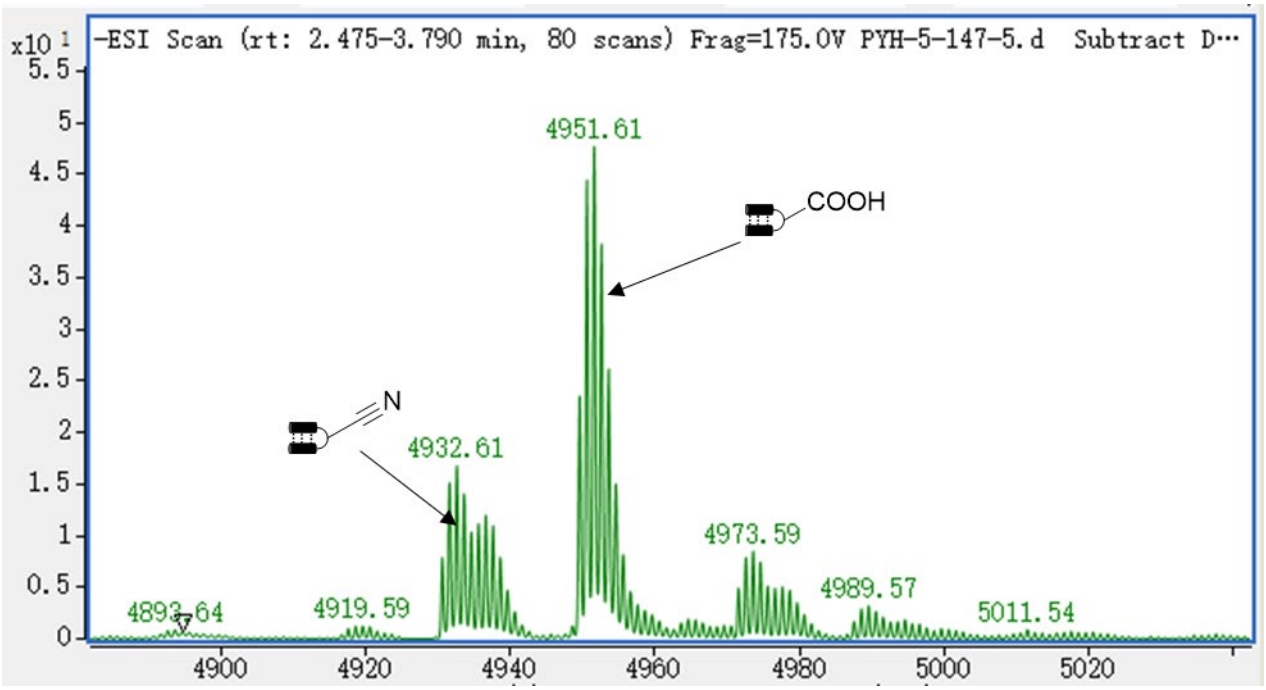
UPLC chromatogram and deconvoluted MS of **e21**

Conversion: 76%

Calculated Mass for nitrile : 4933 Da; Observed Mass: 4933 Da

Calculated Mass for carboxylic acid: 4952 Da; Observed Mass: 4952 Da





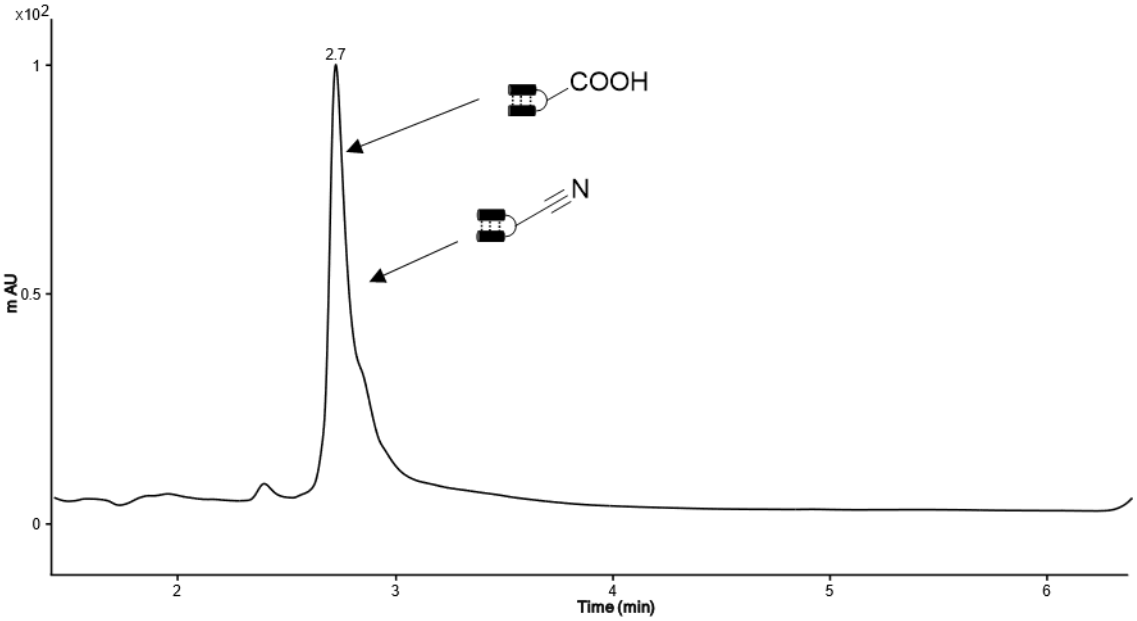
This reactant is prepared from DNA Headpiece by the reductive amination reaction.

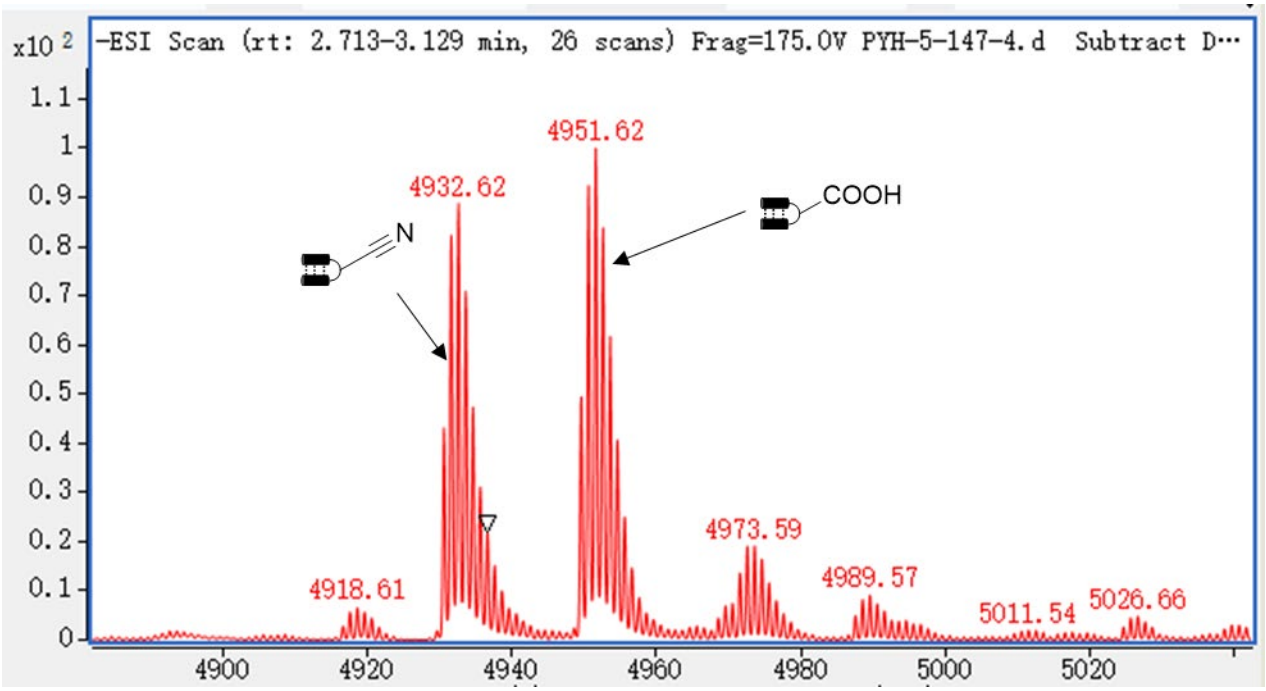
UPLC chromatogram and deconvoluted MS of **e22**

Conversion: 54%

Calculated Mass for nitrile : 4933 Da; Observed Mass: 4933 Da

Calculated Mass for carboxylic acid: 4952 Da; Observed Mass: 4952 Da

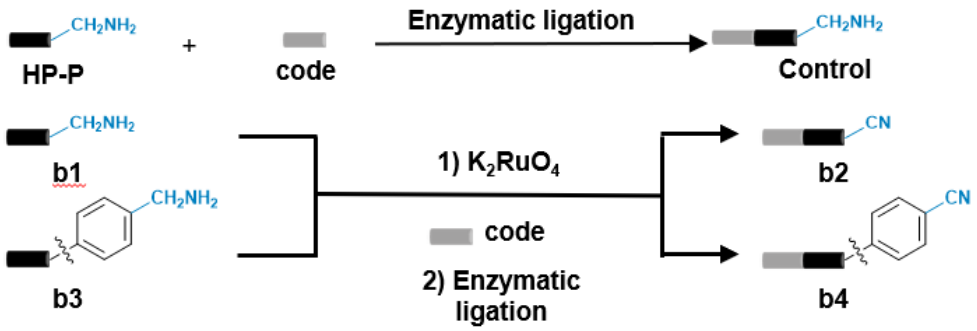




8. Experiments to validate the DNA barcode integrity

Enzymatic ligation

Scheme S3. Protocol of enzymatic ligation.



DNA conjugate **b1** and **b3** (200 pmol), code 13 nt (220 pmol, 1.1 equiv.), and 10× ligation buffer (2 μL) were added into a 0.6 mL tube and mixed by the 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 16 h. After ligation confirmation by UHPLC-MS analysis, the reaction system was denatured by incubating at 95 °C in a dry bath for 10 min, and the ligation product was isolated by ethanol precipitation. The resulting pellets were vacuum-dried and dissolved in nuclease-free water. Meanwhile, the raw headpiece-primer (**HP-P**) was treated in the same way as a positive control for the enzymatic ligation assay.

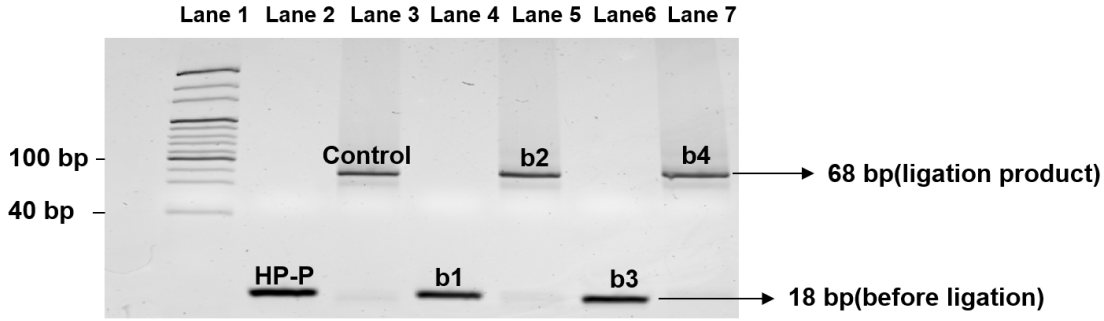
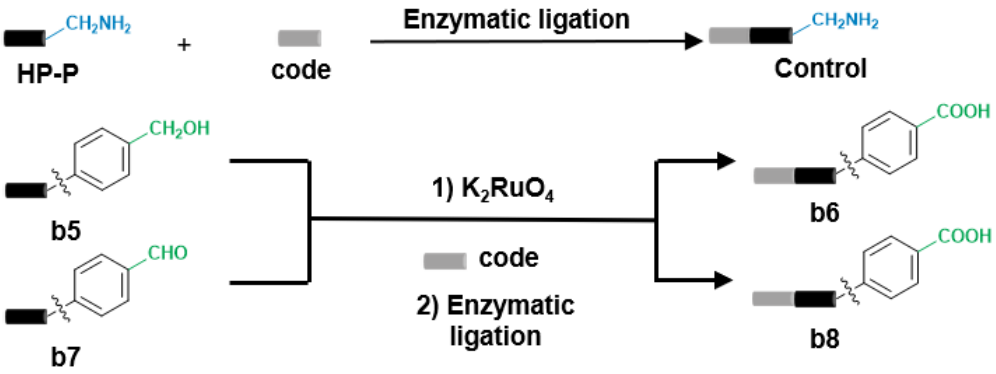


Figure S10. 20% denatured PAGE analysis of DEL-encoding compatibility. Lane 1, DNA ladder; Lane 2, **HP-P** starting material; Lane 3, control ligation product; Lane 4, reaction product **b1**; Lane 5, ligation product **b2**. Lane 6, reaction product **b3**; Lane 7, ligation product **b4.**

Scheme S3. Protocol of enzymatic ligation.



DNA conjugate **b5** and **b7** (200 pmol), code 13 nt (220 pmol, 1.1 equiv.), and 10× ligation buffer (2 μL) were added into a 0.6 mL tube and mixed by vortex. Then, T4 DNA ligase (1 μL, 350 units/μL) was added and mixed gently. The reaction mixture was vortexed, centrifuged, and incubated at 20 °C for 16 h. After ligation confirmation by UHPLC-MS analysis, the reaction system was denatured by incubating at 95 °C in a dry bath for 10 min, and the ligation product was isolated by ethanol precipitation. The resulting pellets were vacuum-dried and dissolved in nuclease-free water. Meanwhile, the raw headpiece-primer (**HP-P**) was treated in the same way as a positive control for the enzymatic ligation assay.

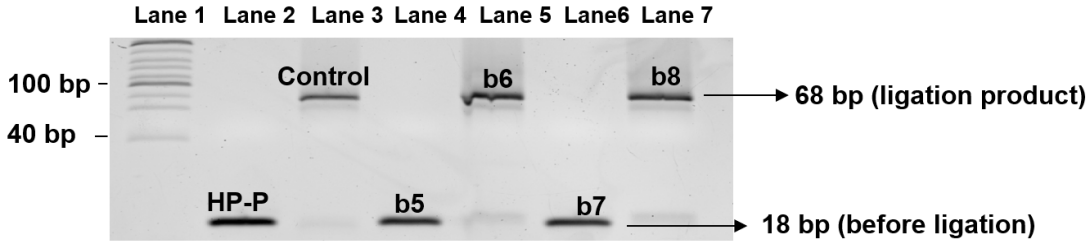


Figure S10. 20% denatured PAGE analysis of DEL-encoding compatibility. Lane 1, DNA ladder; Lane 2, **HP-P** starting material; Lane 3, control ligation product; Lane 4, reaction product **b5**; Lane 5, ligation product **b6**. Lane 6, reaction product **b7**; Lane 7, ligation product **b8.**

HP-P: 5′/5Phos/ACCTTCGGGAGTCA/iSp9/iUniAmM/iSp9/TGACTCCCGACCG AAGGTTG -3′

Code 60 nt:

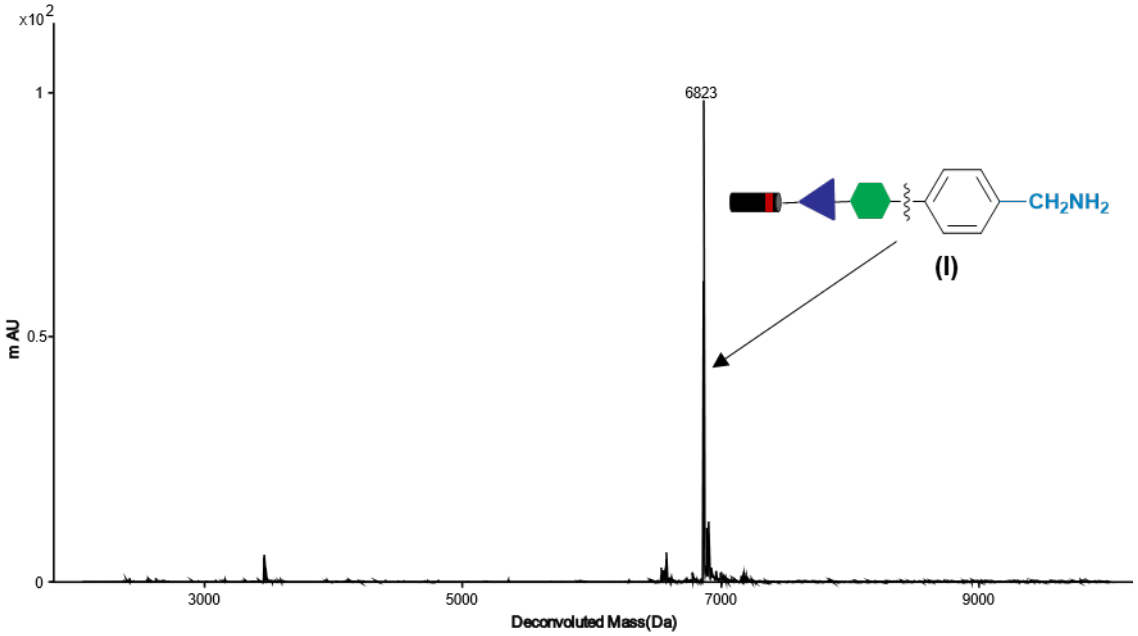
5’-AAC ACA GGC TTT GCT CGT ACA TAA AGC TCT TGC GTG GTC GTC TGA TGG CGC GAG GGA GGC-3’

5’-CTC CCT CGC GCC ATC AGA CGA CCA CGC AAG AGC TTT ATG TAC GAG CAA AGC CTG TGT TCA-3’

Oxidation experiments of $RCHP_1$-T and $RCHP_1$-D crosslinking products in K_2RuO_4-mediated oxidation system

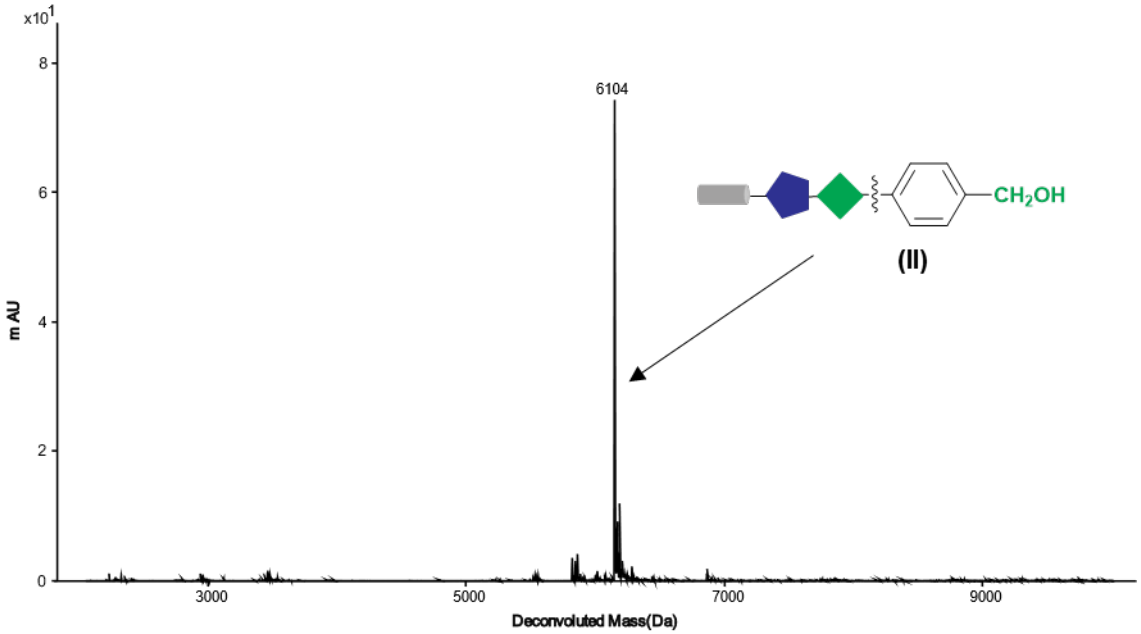
Structure of the chain of amine

$RCHP_1$-T+Val+Trp+4-(Aminomethyl)benzoic acid

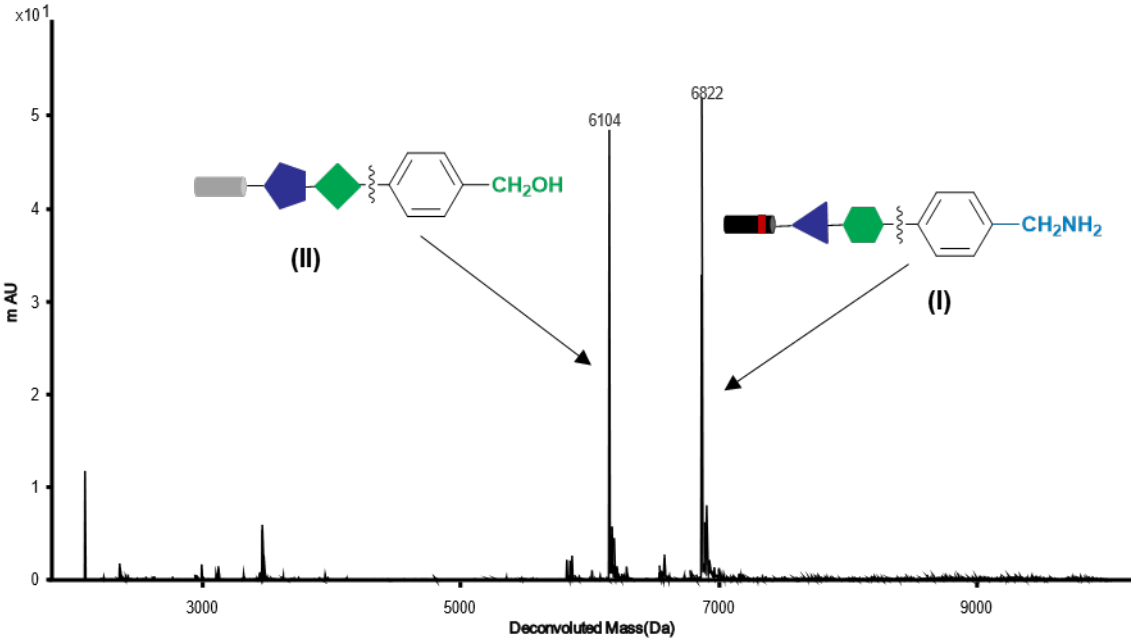


Structure of the chain of alcohol

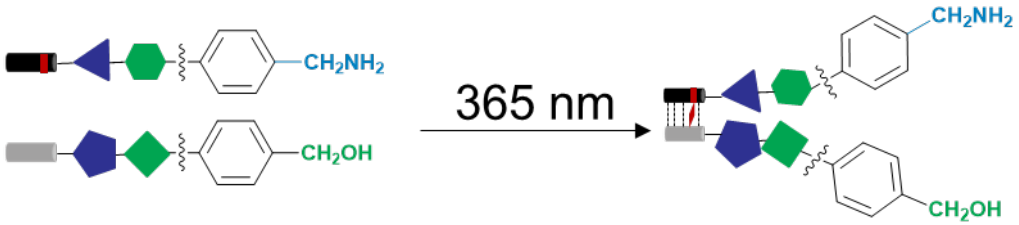
$RCHP_1$-D+Leu+2-Amino-2-phenylacetic acid+4-(Hydroxymethyl)benzoic acid



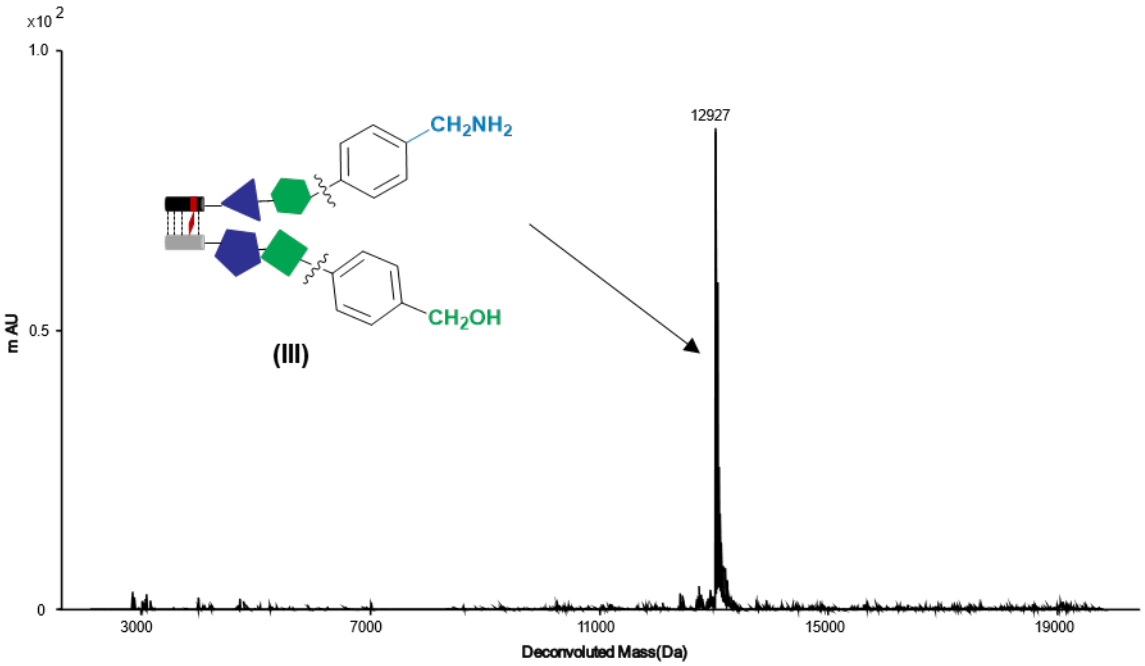
Structure of the chain of alcohol and amine（mixed for test）



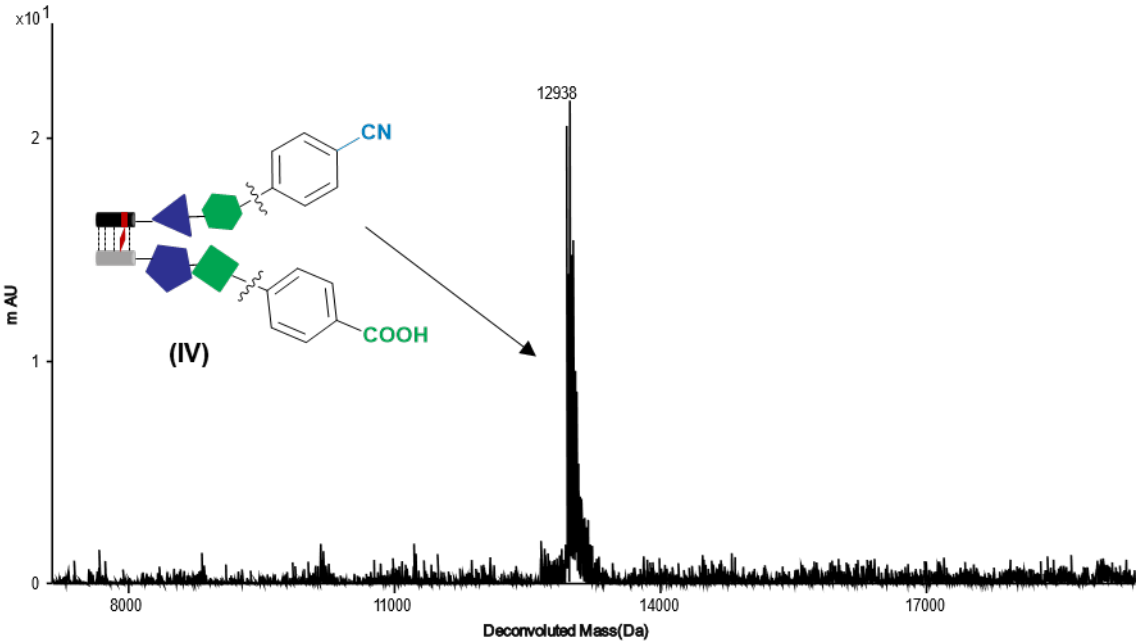
Methods for RCHP1-T and RCHP1-D Crosslinking: The reaction mixture (total volume 30 μL) containing I (200 pmol) and II (200 pmol) in 0.1 M NaCl was irradiated with a UVP CL-1000 Ultraviolet crosslinker (365 nm) at 0 °C for 3 min. After irradiation, the reaction mixture was monitored by UHPLC-MS.[5]



Structure of the chain of alcohol and amine after photo-crosslinking



Structure of the final K_2RuO_4-mediated oxidation product of the crosslinked DNA



9. References

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