

Supporting information

Structure-activity Relationship of SPOP Inhibitors Against Kidney Cancer

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Table S1. T_m shift when SPOP^{MATH} was incubated with compounds

Compound	ΔT_m (°C)		Compound	ΔT_m (°C)	
	10 ×	20 ×		10 ×	20 ×
DMSO	-1.7	-3.8	6lc	3.1	3.5
6b	2.8	4.0	6ld	3.5	3.7
6c	0	0.2	6lf	5.3	4.5
6d	1.8	2.1	6lg	1.6	1.7
6e	2.5	3.6	6lh	2.7	2.4
6f	2.5	2.9	6li	2.7	2.7
6g	2.4	3.5	6lj	4.0	3.8
6h	2.3	3.5	6lk	1.4	2.5
6i	3.7	5.3	6ll	2.2	2.6
6k	0.6	1.4	7a	0.8	2.1
6l	3.2	5.1	7b	1.1	2.1
6m	0.5	1.1	7c	3.7	4.0
6n	1.4	2.3	7d	0.8	1.6
6o	1.1	2.3	7f	4.9	5.3
6p	0.1	1.5	7g	1.7	1.9
6q	0.4	1.4	7h	0.6	0.9
6r	4.3	6.1	7i	1.0	1.0
6s	0.3	0.8	7j	0.9	1.3
6t	1.4	1.3	8c	2.3	3.7
6u	1.6	1.6	8a	0.2	1.6
6v	0.9	0.8	8b	3.5	5.3
6w	1.6	1.8	11b	0.2	1.3
6x	0.4	0.9	12a	0.9	2.1
6y	0.4	0.8	12b	-7.4	1.4
6la	2.8	2.6	14a	-0.2	2.0
6lb	3.1	3.3	14b	-0.4	1.2

ΔT_m (°C) for DMSO-treated SPOP^{MATH} was calculated using naive SPOP^{MATH} sample as the reference, while ΔT_m data for compound-treated SPOP^{MATH} were reported using DMSO-treated SPOP^{MATH} as the reference.

Table S2. Antiproliferative activities of compounds on A498 and OS-RC-2 cell lines

Compound	Inhibition @ 30 μ M (%)		Compound	Inhibition @ 30 μ M (%)	
	A498	OS-RC-2		A498	OS-RC-2
6b	88	90	6li	90	90
6c	32	22	6lj	83	81
6d	91	94	6lk	75	77
6e	90	89	6ll	62	56
6f	90	88	7a	27	23
6g	90	97	7b	38	35
6h	89	96	7c	24	20
6i	89	92	7d	42	48
6j	61	74	7e	51	49
6k	86	89	7f	38	49
6l	90	82	7g	59	56
6m	35	40	7h	24	26
6n	90	91	7i	51	10
6o	50	36	7j	33	16
6p	27	35	8a	18	22
6q	58	46	8b	21	29
6r	48	34	8c	13	28
6s	12	23	8d	35	46
6t	37	22	8e	51	32
6u	59	62	8f	43	40
6v	11	15	8g	39	39
6w	57	66	8h	4	22
6x	37	37	8i	24	23
6y	55	51	8j	32	31
6z	17	38	11a	15	19
6la	62	58	11b	14	15
6lb	86	82	12a	33	24
6lc	85	90	12b	21	15
6ld	85	52	13a	8	14
6le	12	21	13b	13	30
6lf	87	75	14a	25	16
6lg	60	50	14b	38	11
6lh	86	83			

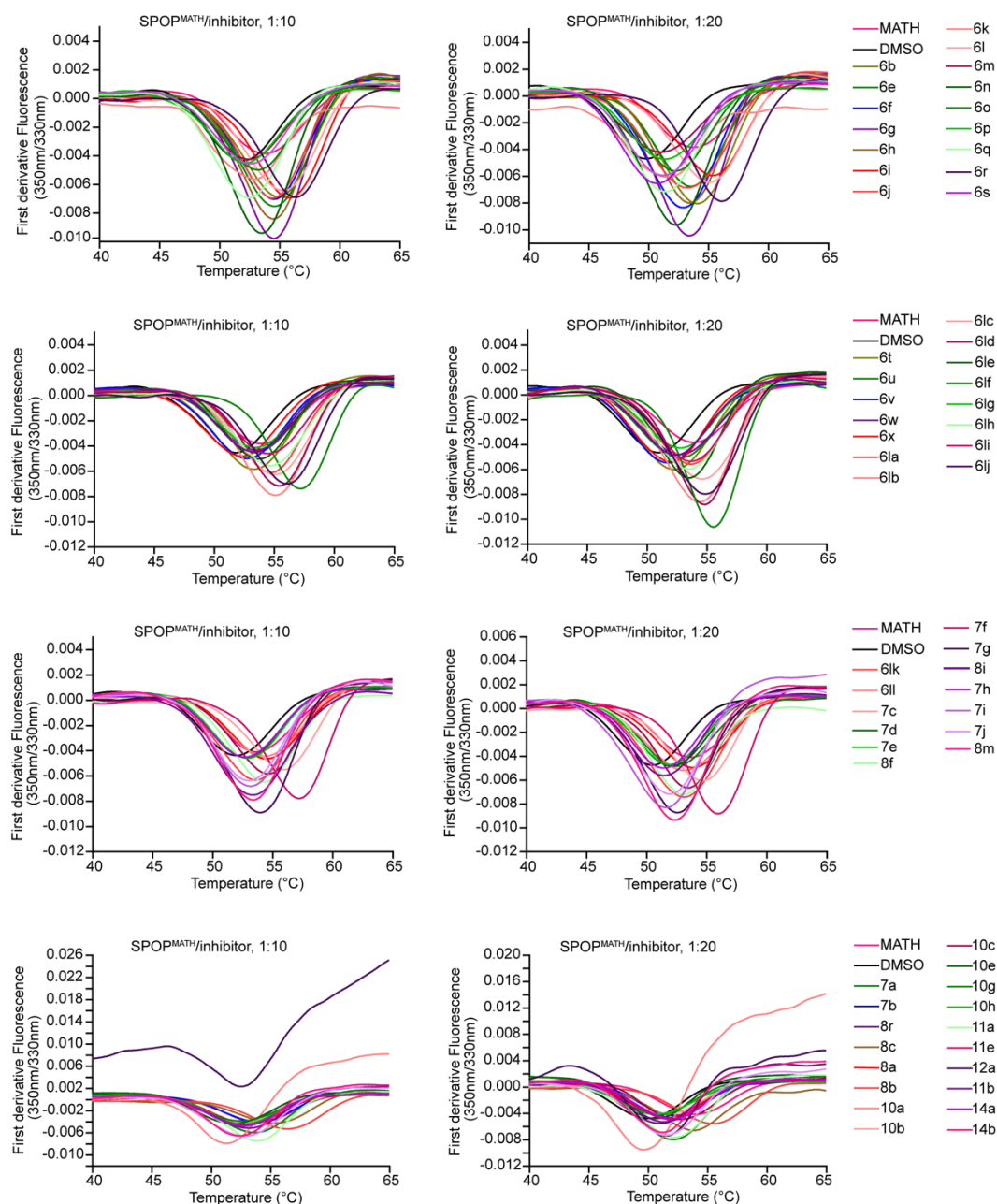


Figure S1 Representative nanoDSF traces for all compounds.

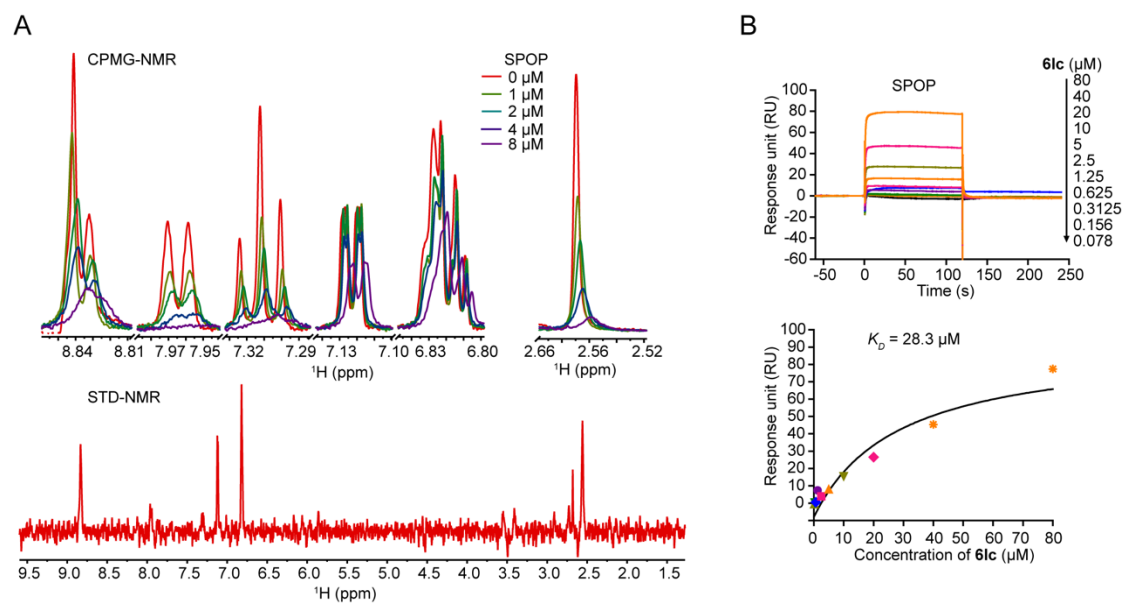
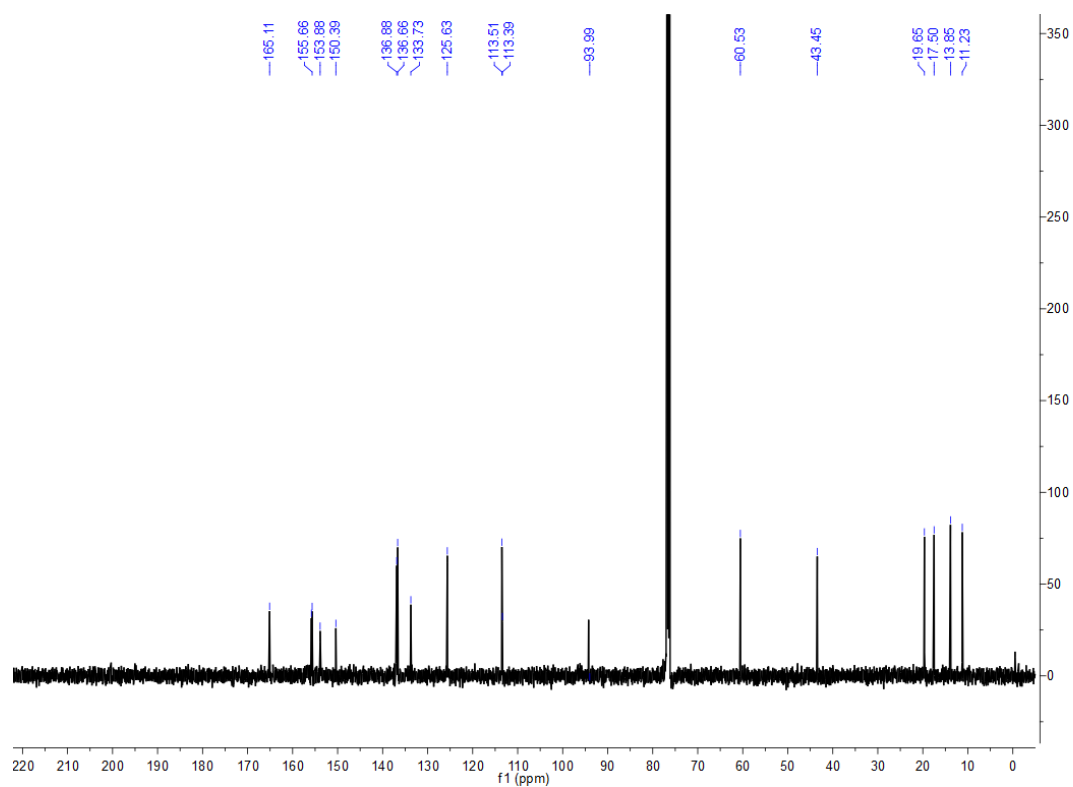
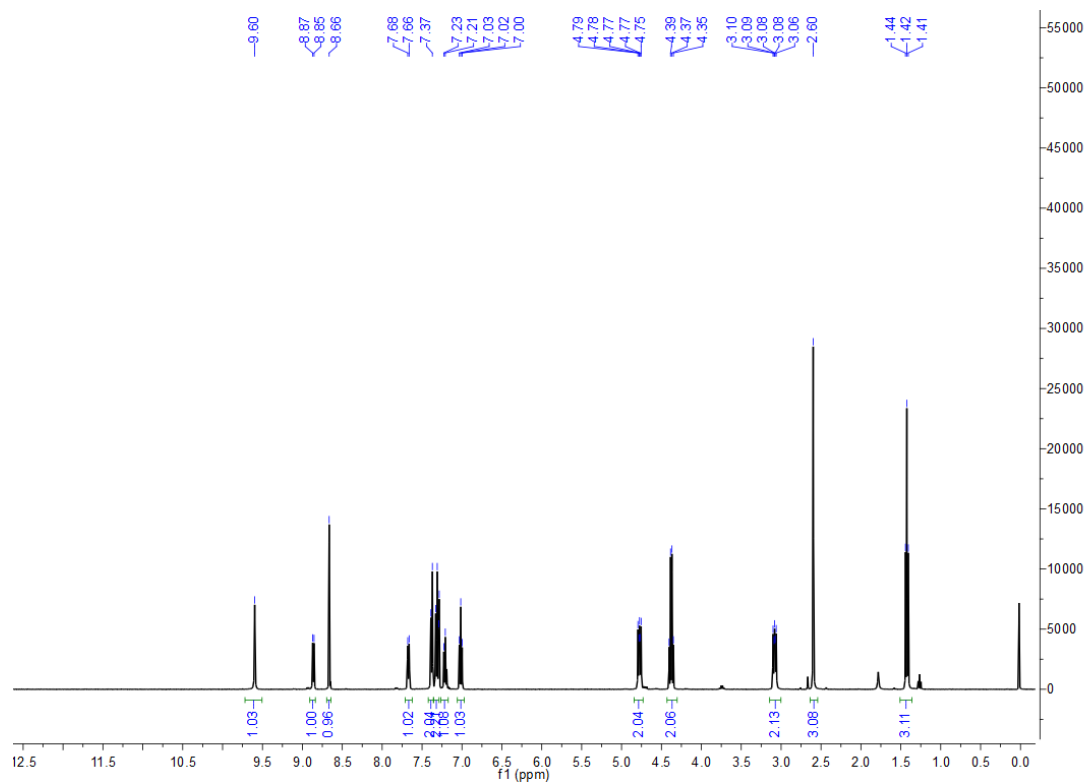
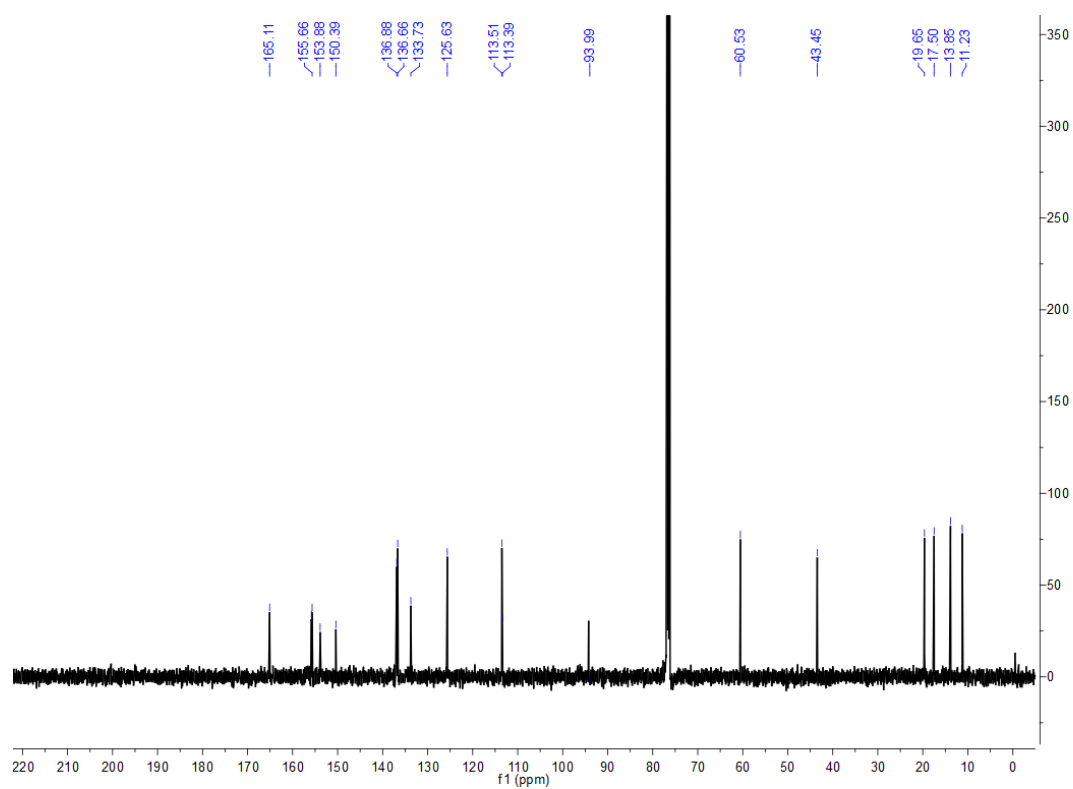
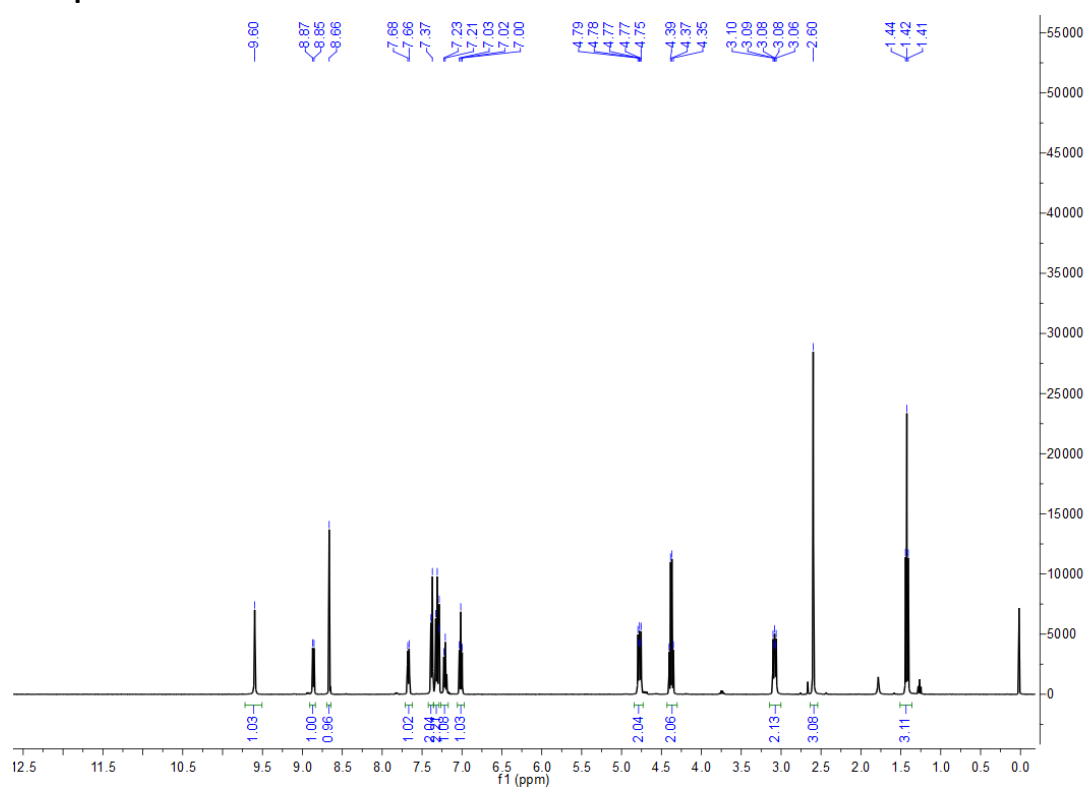


Figure S2 Interaction of **61c** and full-length SPOP. (A) NMR measurement of **61c** interaction with SPOP. The STD-NMR spectrum is recorded for **61c** at 200 μM with SPOP. (B) SPR measurement of compound **61c** binding to SPOP. Graphs of equilibrium RU responses versus **61c** concentrations are plotted. The estimated K_D is 28.3 μM .

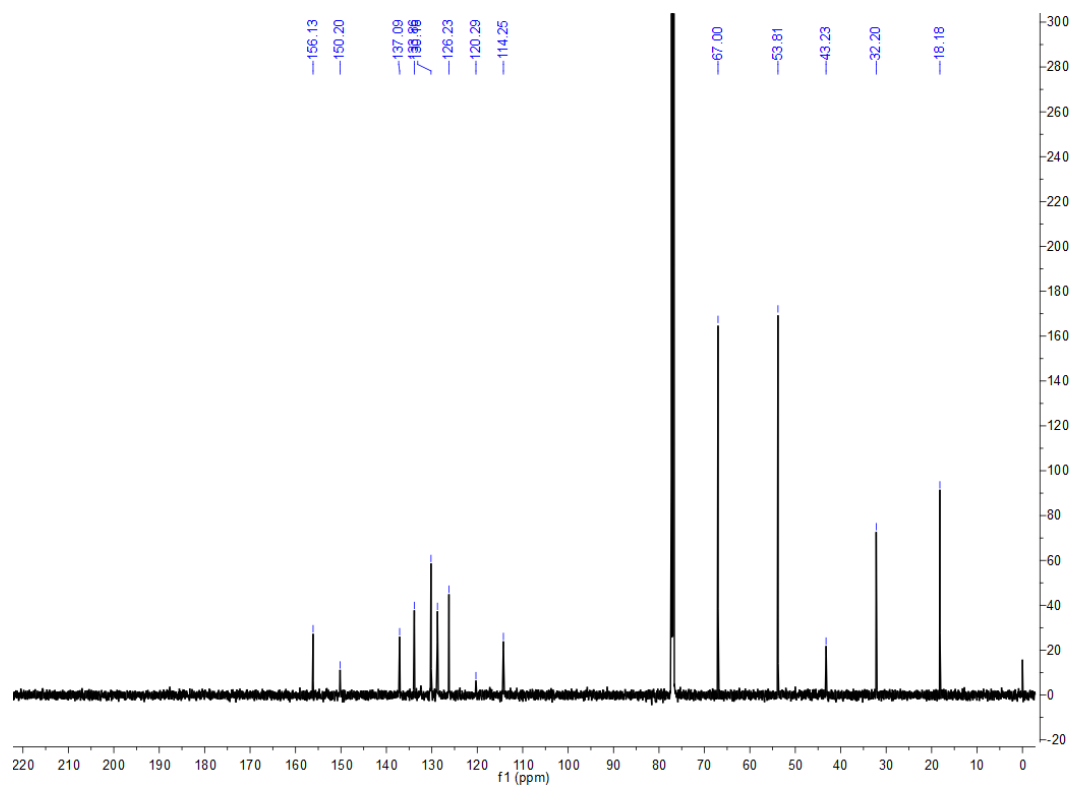
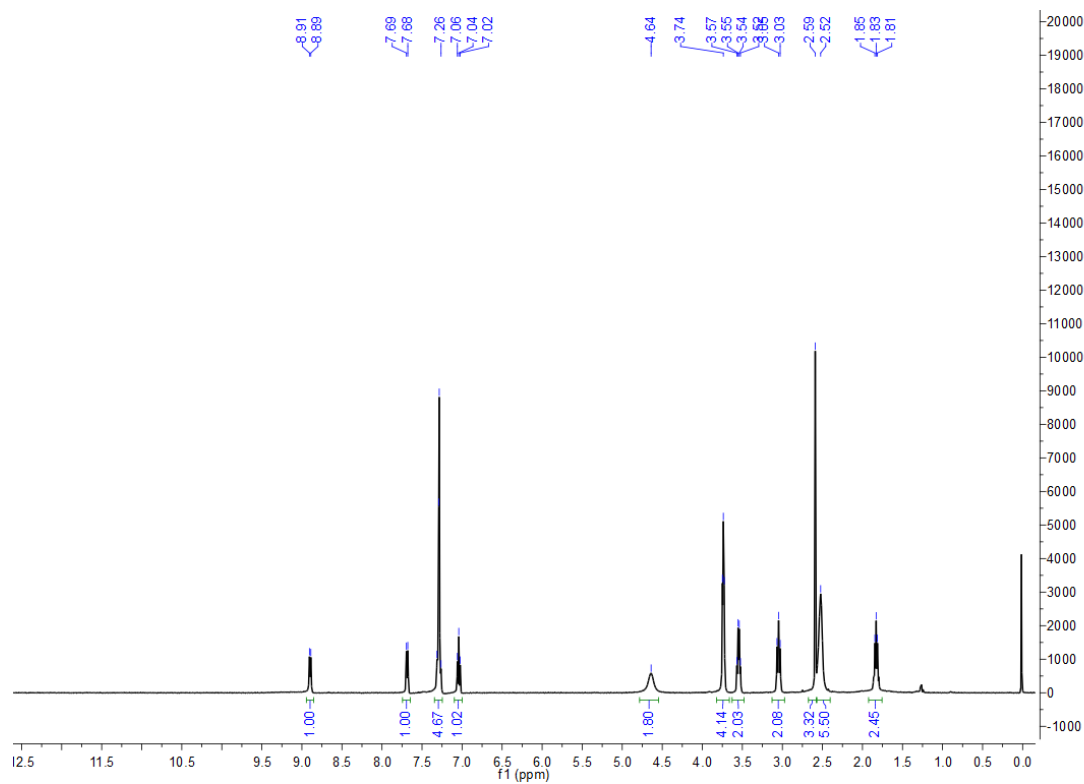
NMR spectrum
Compound 6b



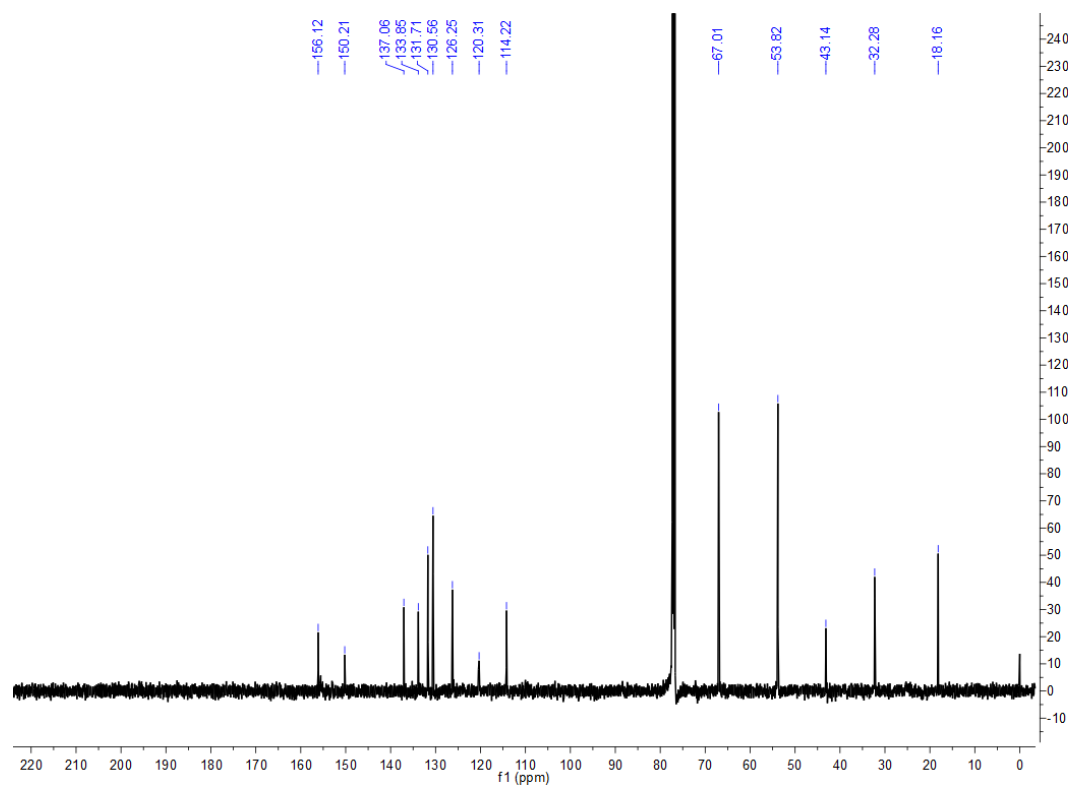
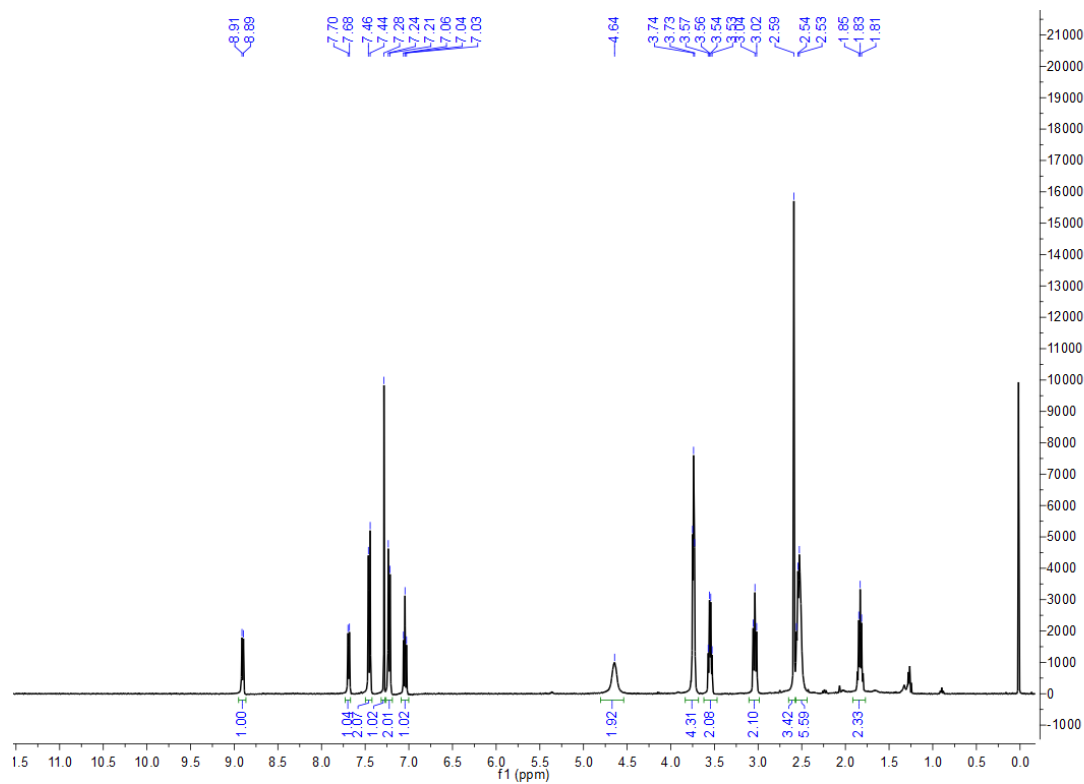
Compound 6d



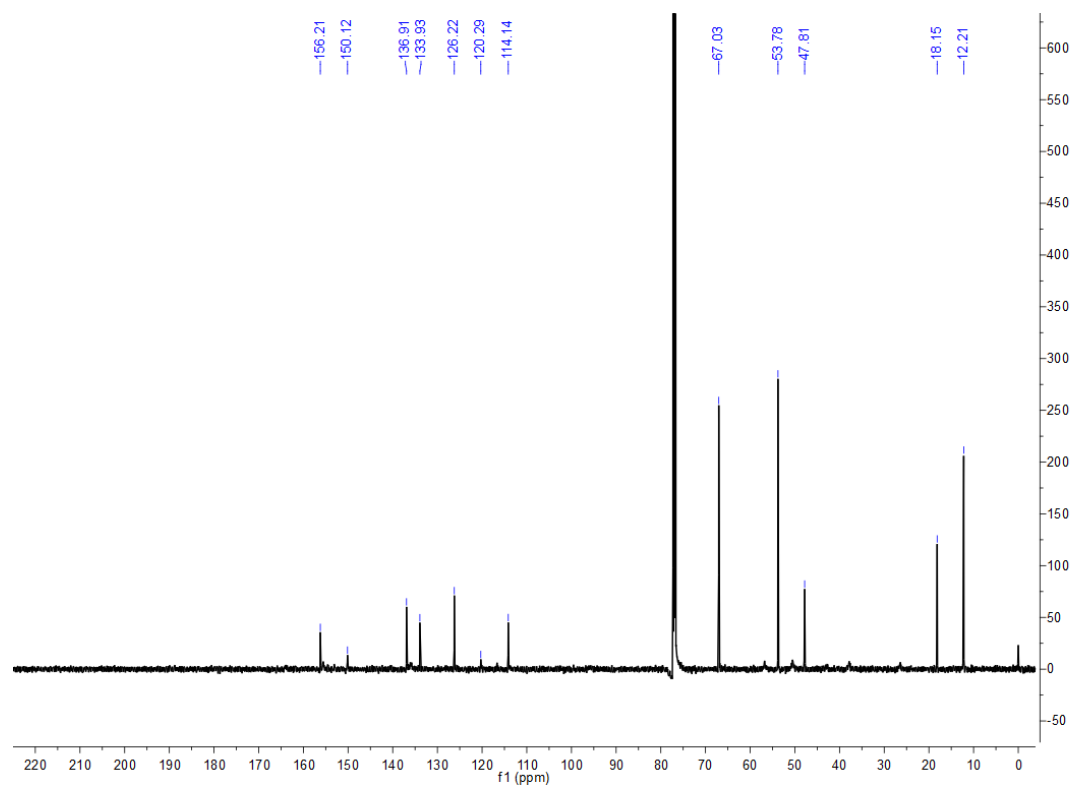
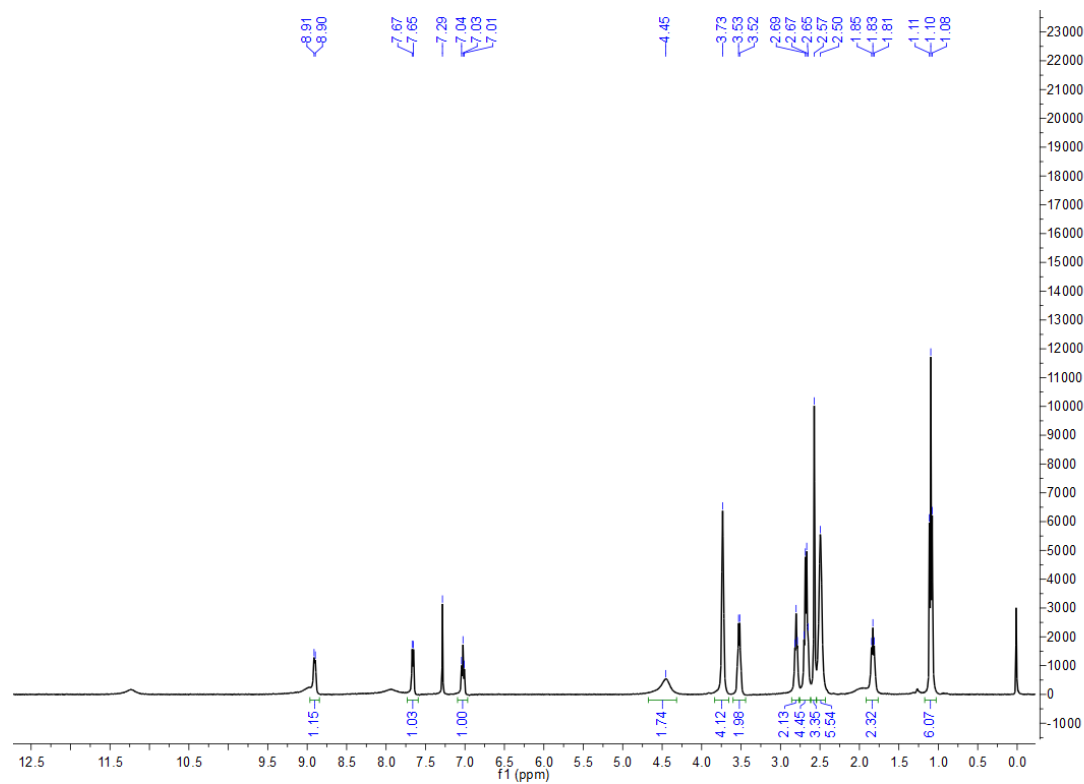
Compound 6g



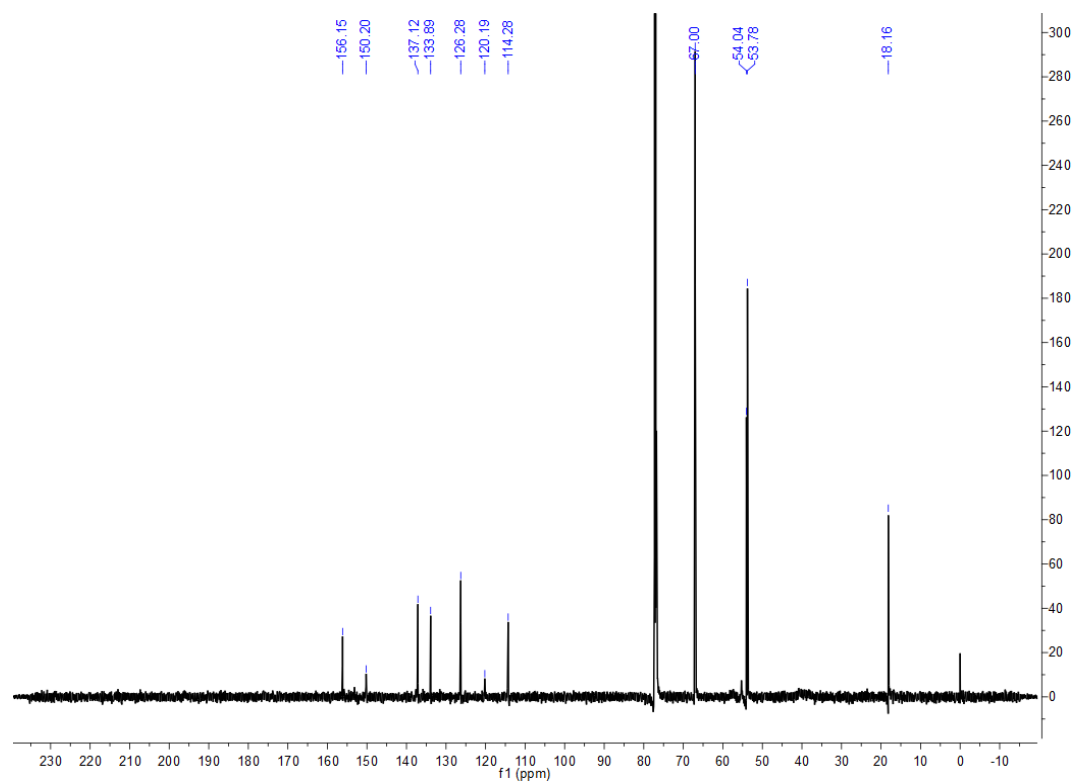
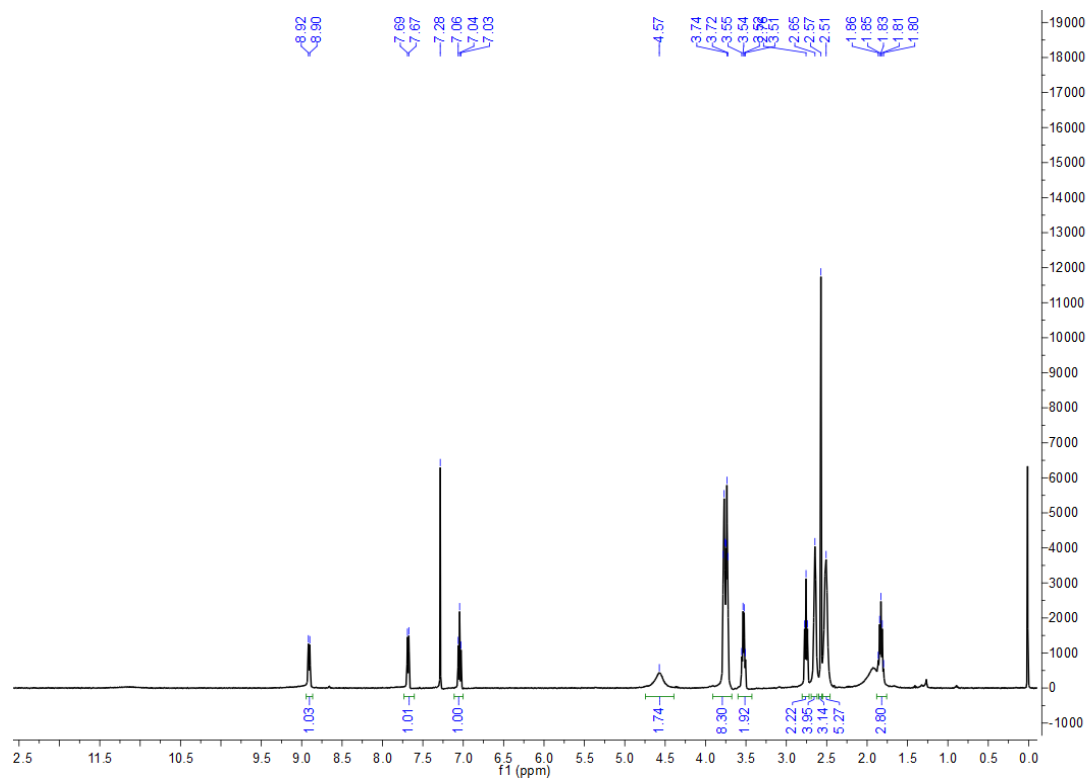
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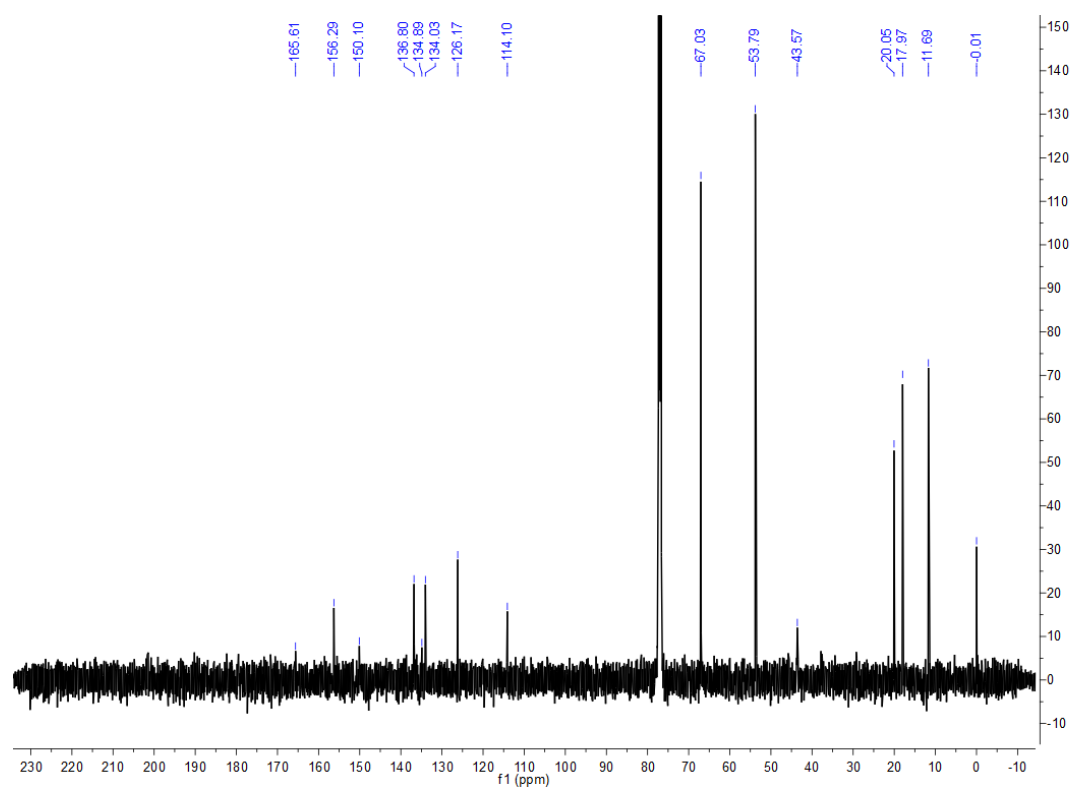
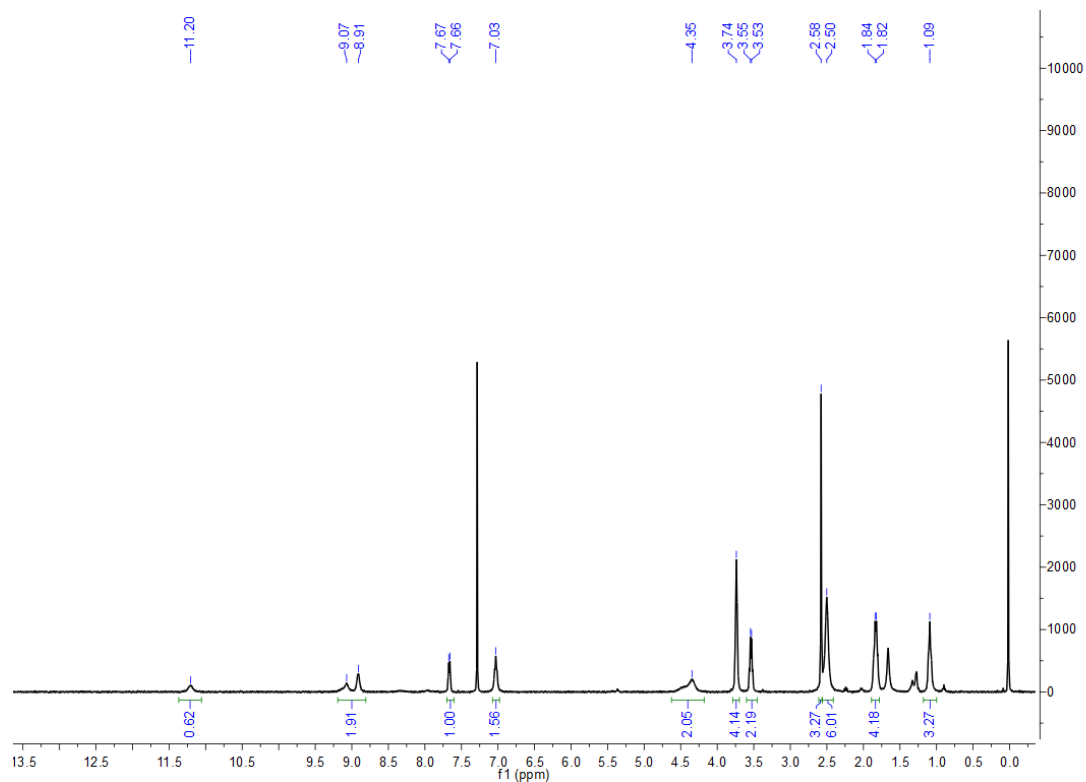
Compound 6k



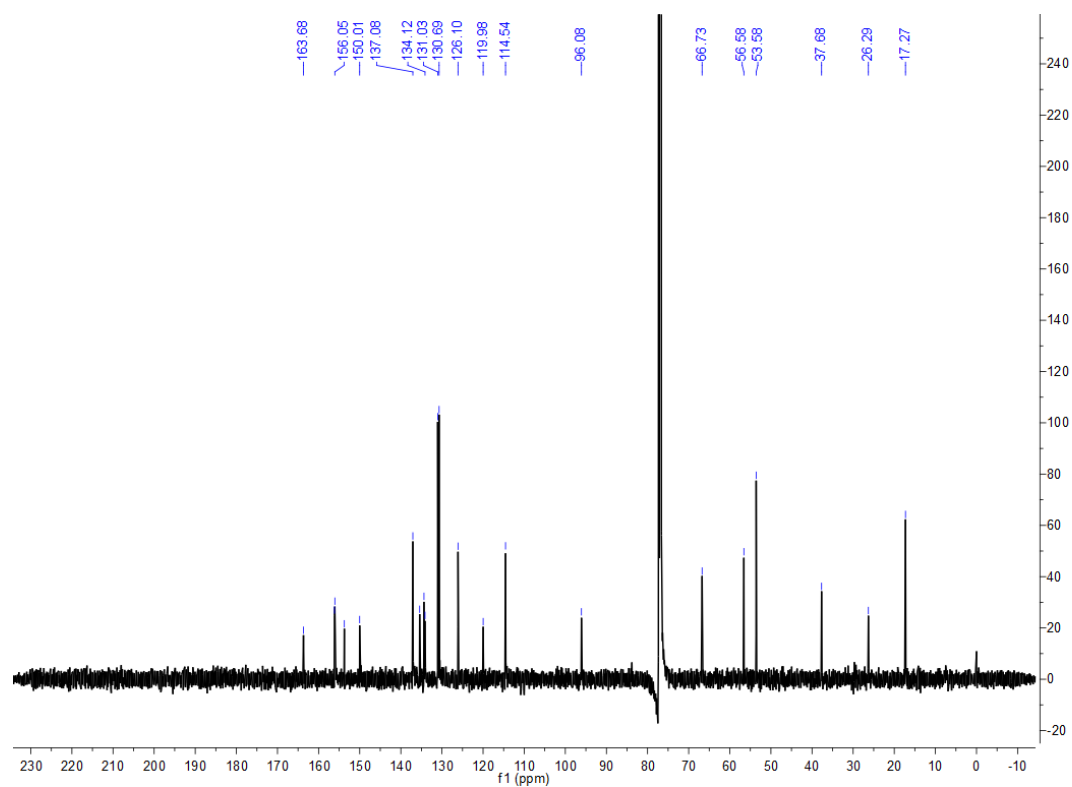
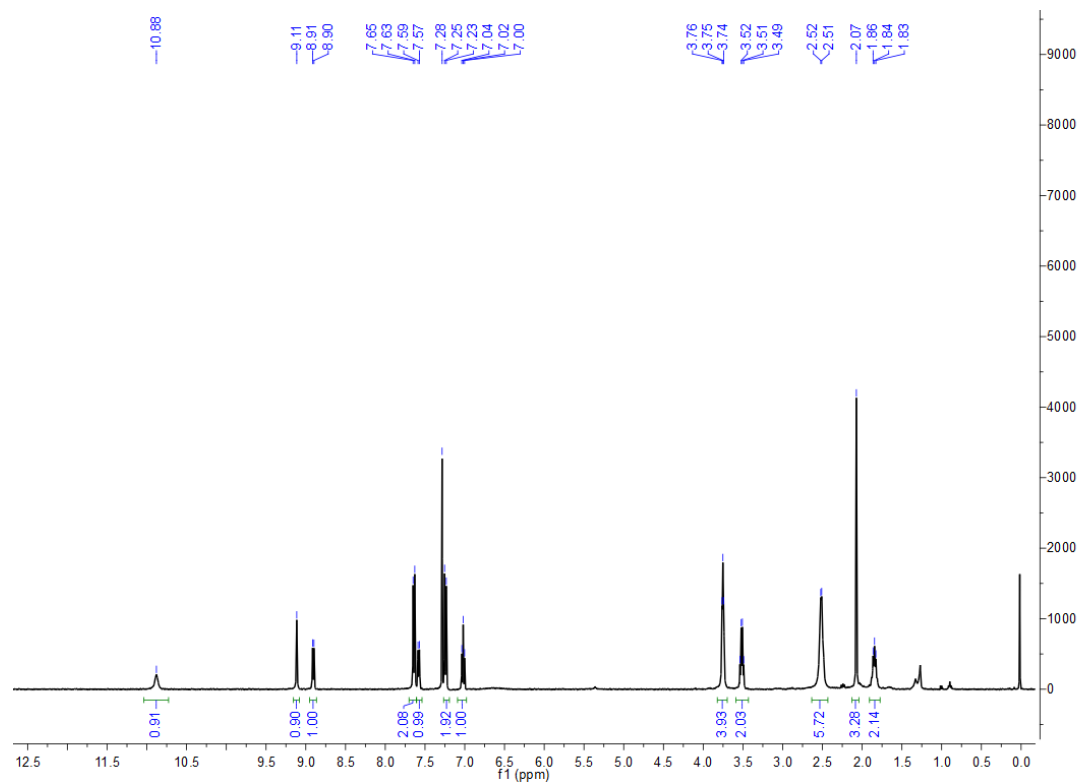
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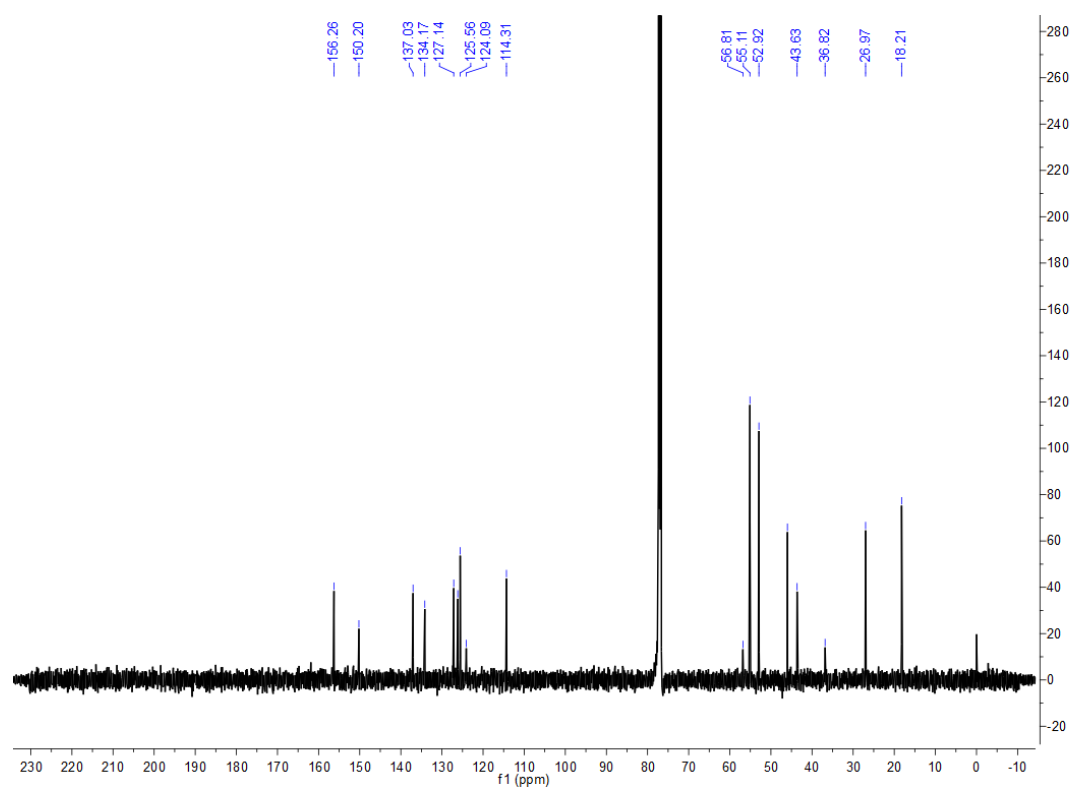
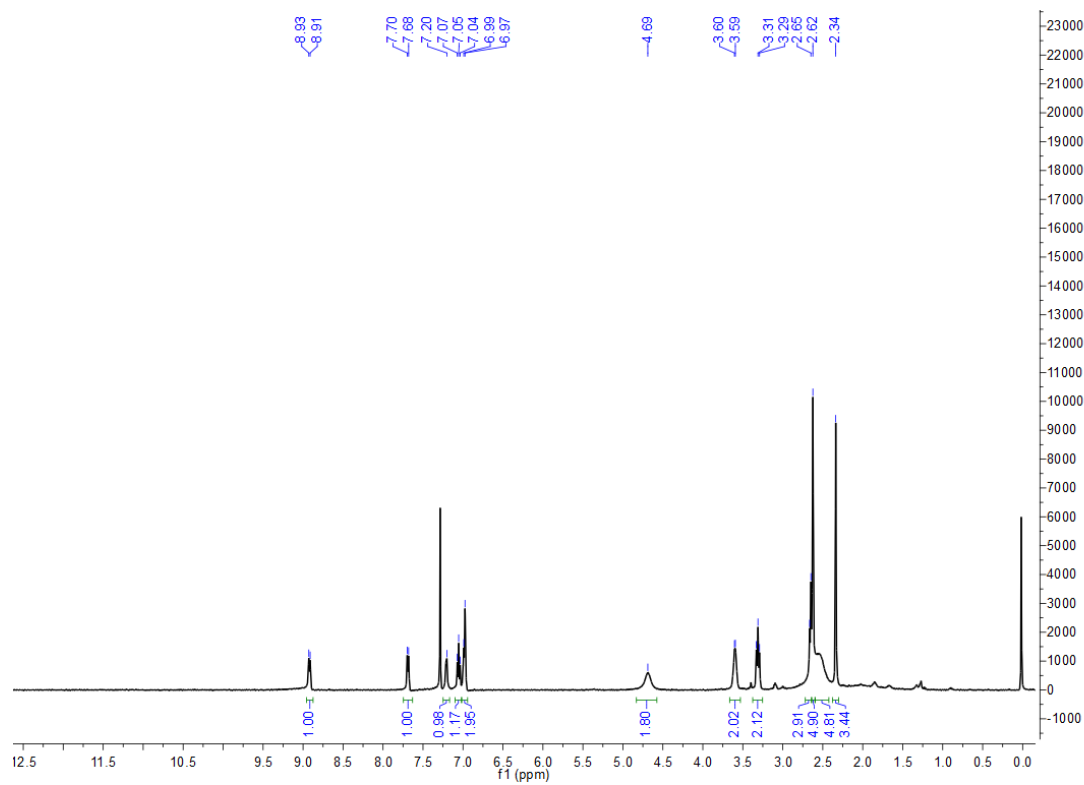
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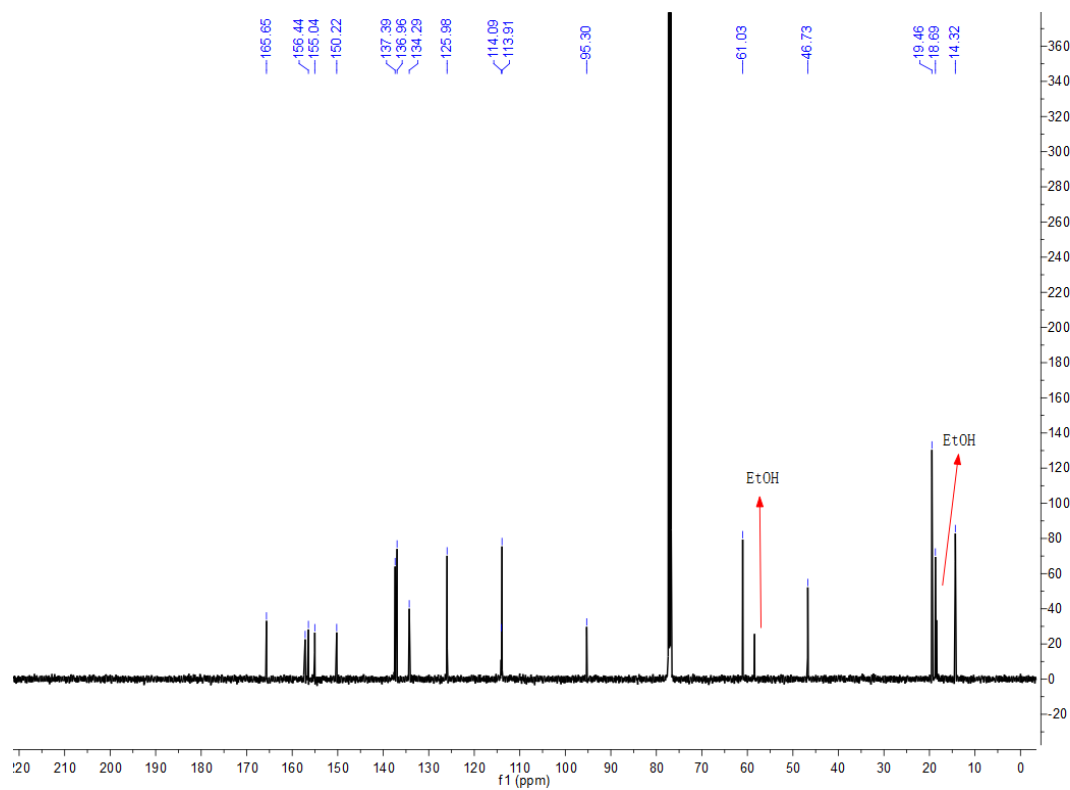
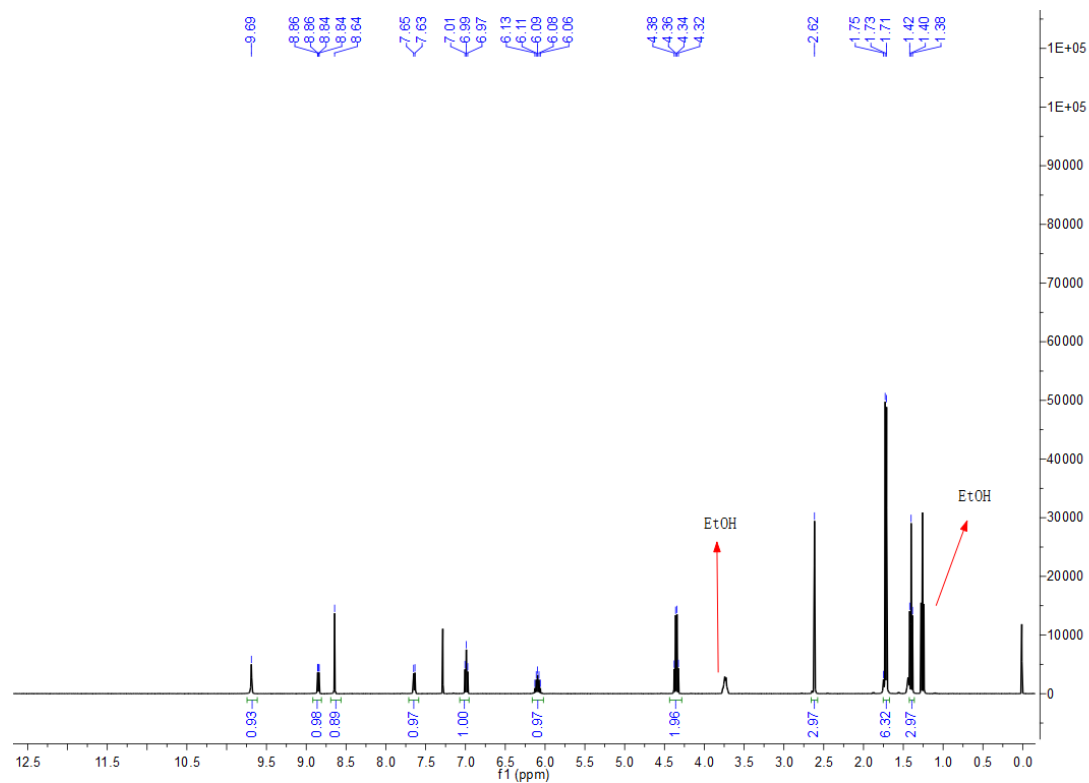
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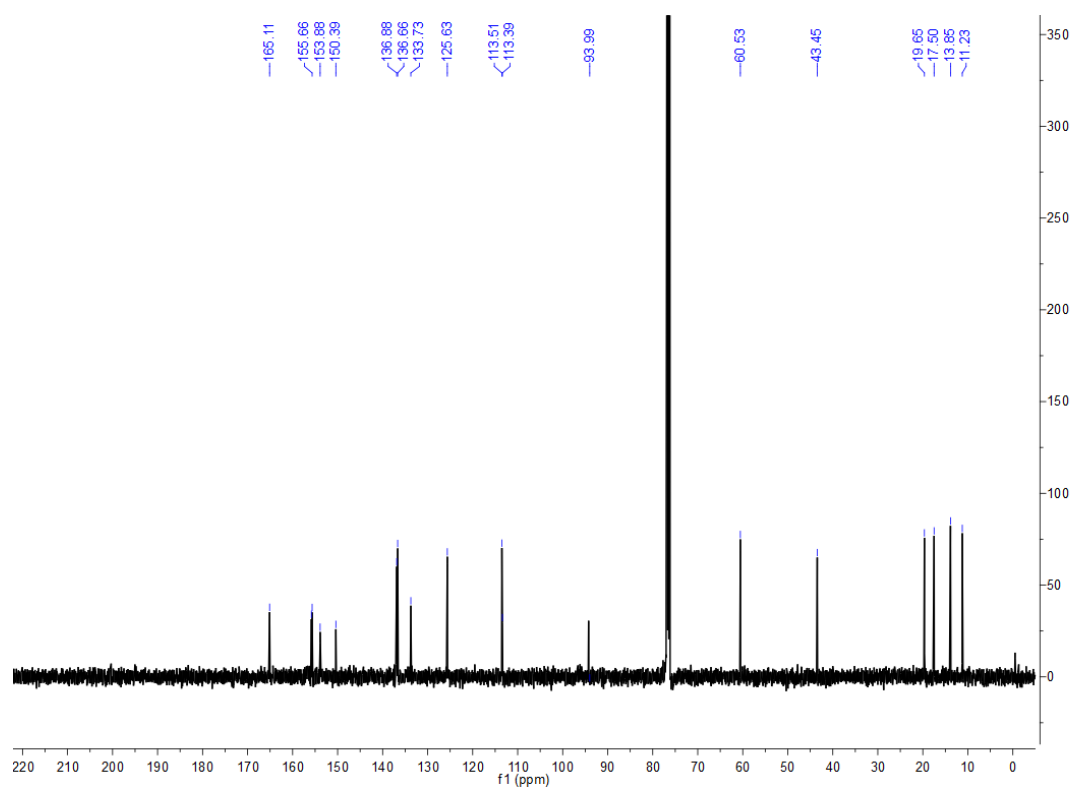
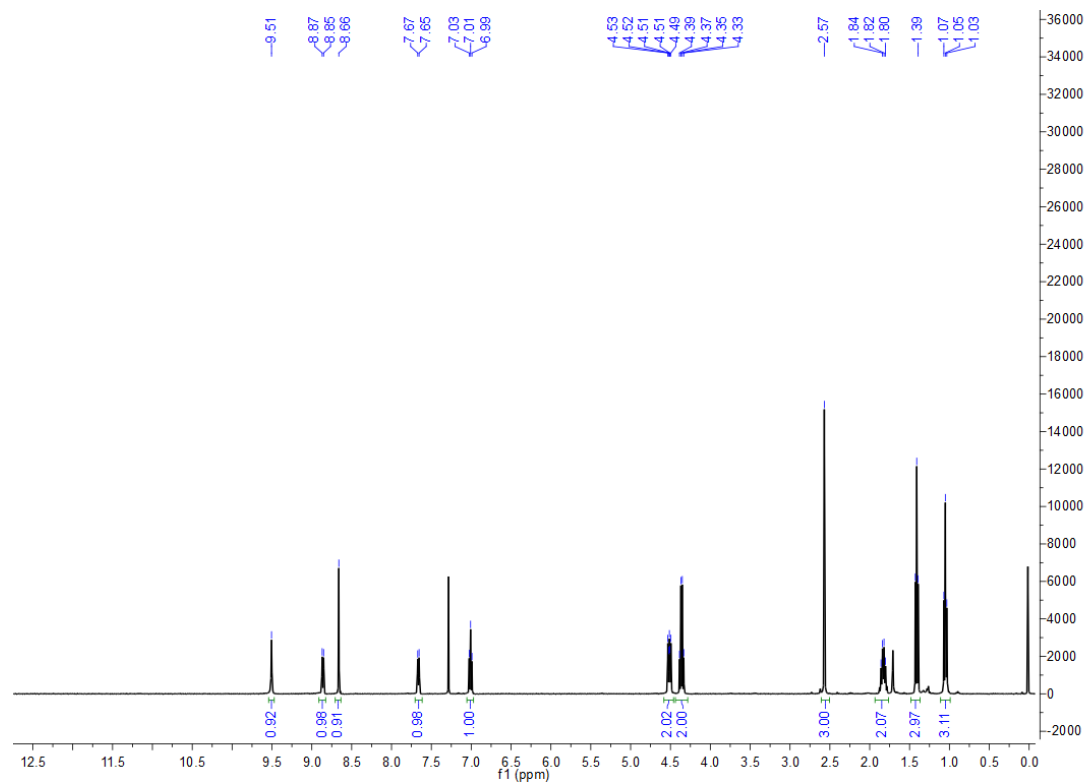
Compound 6lc



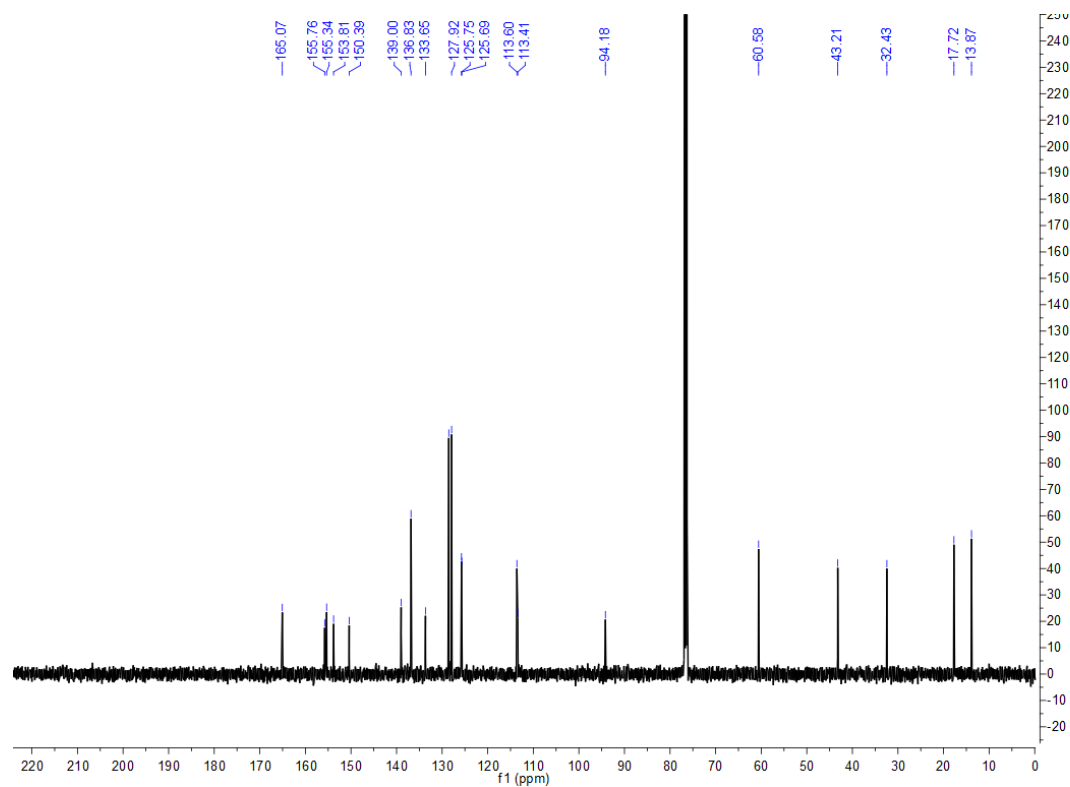
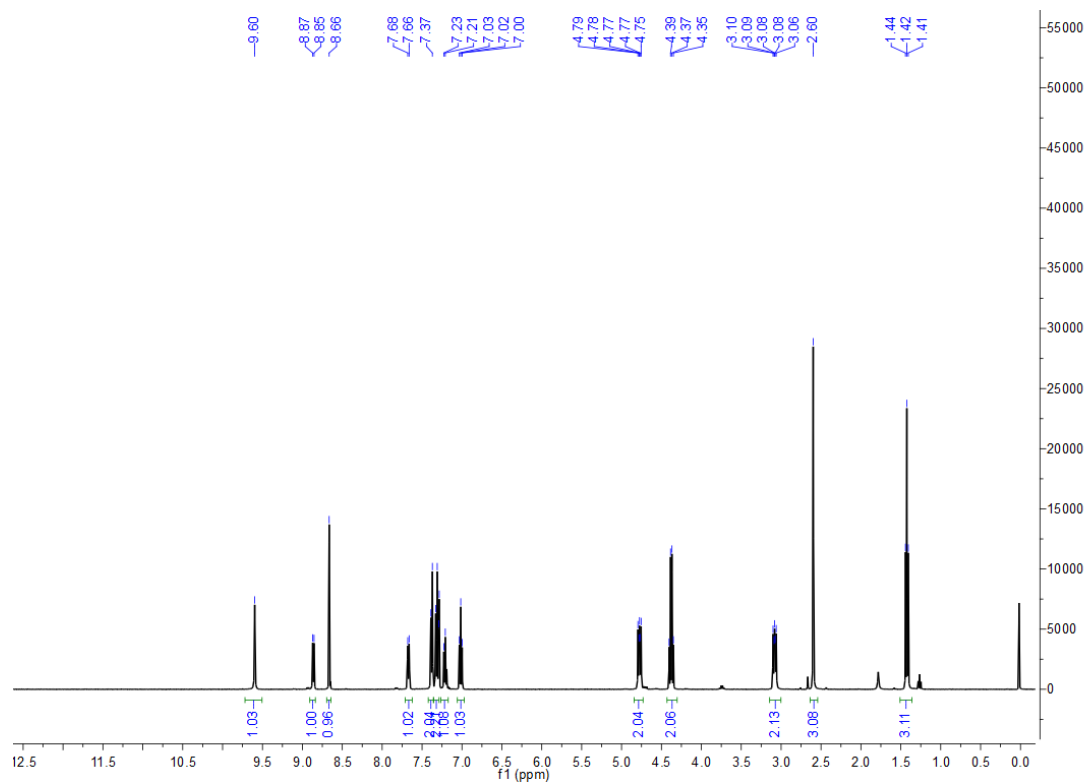
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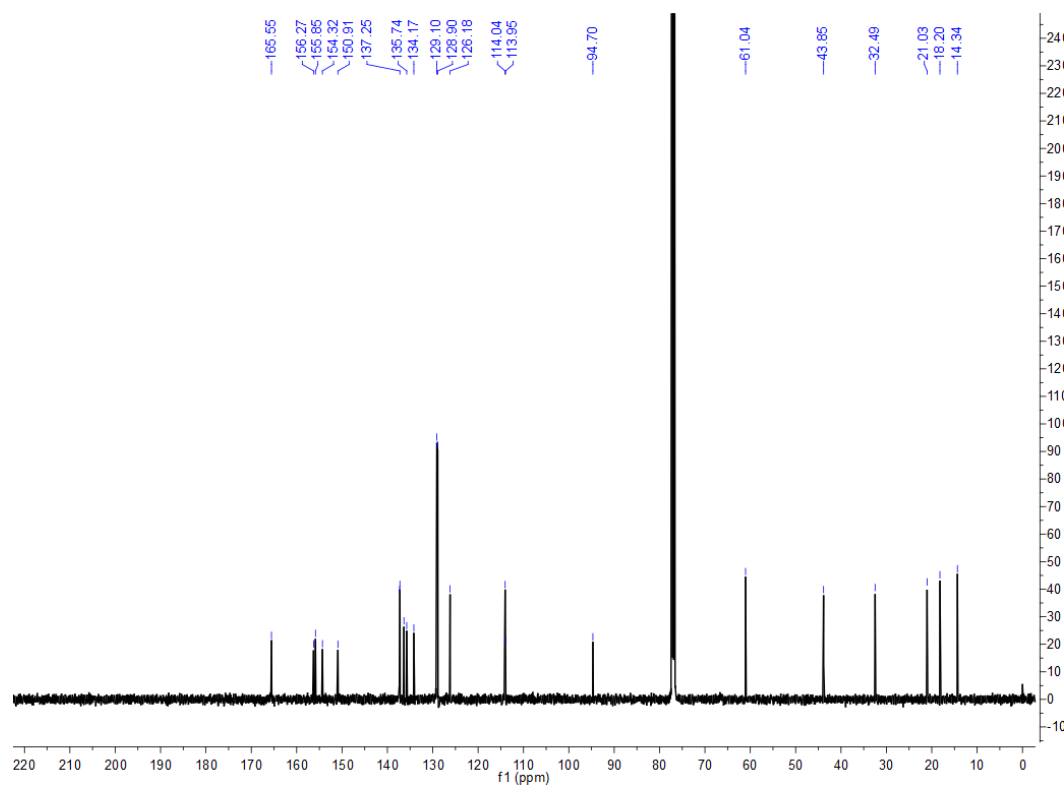
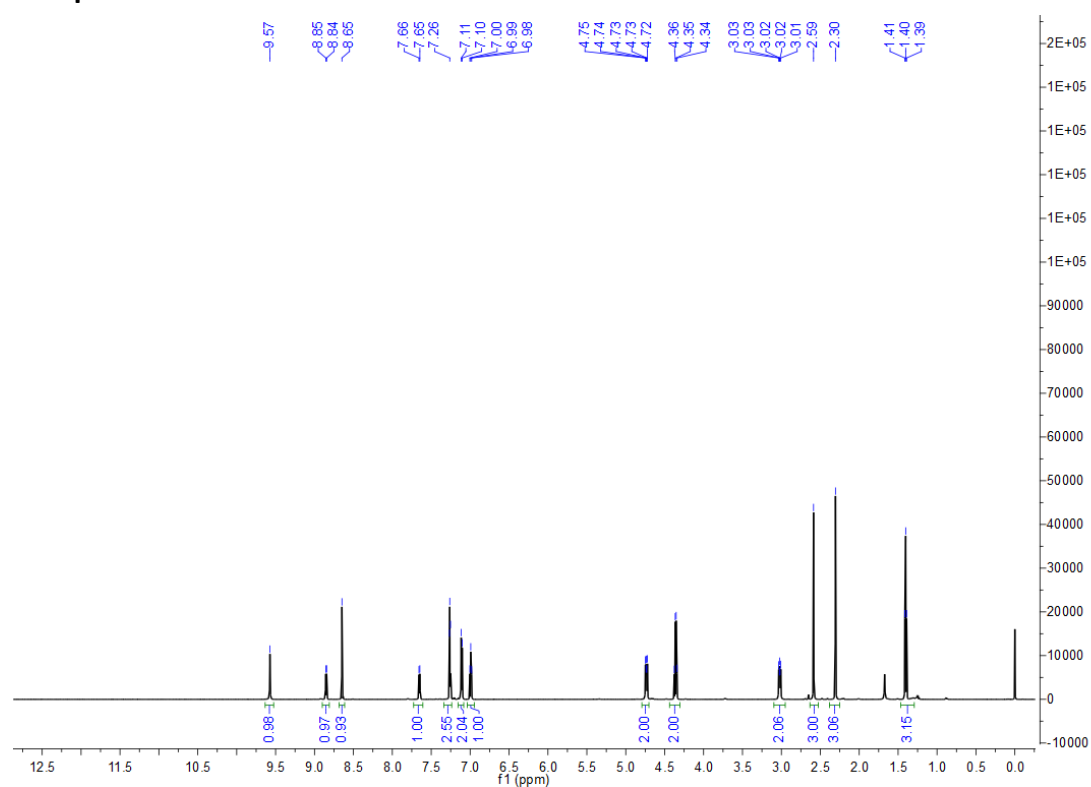
Compound 7b



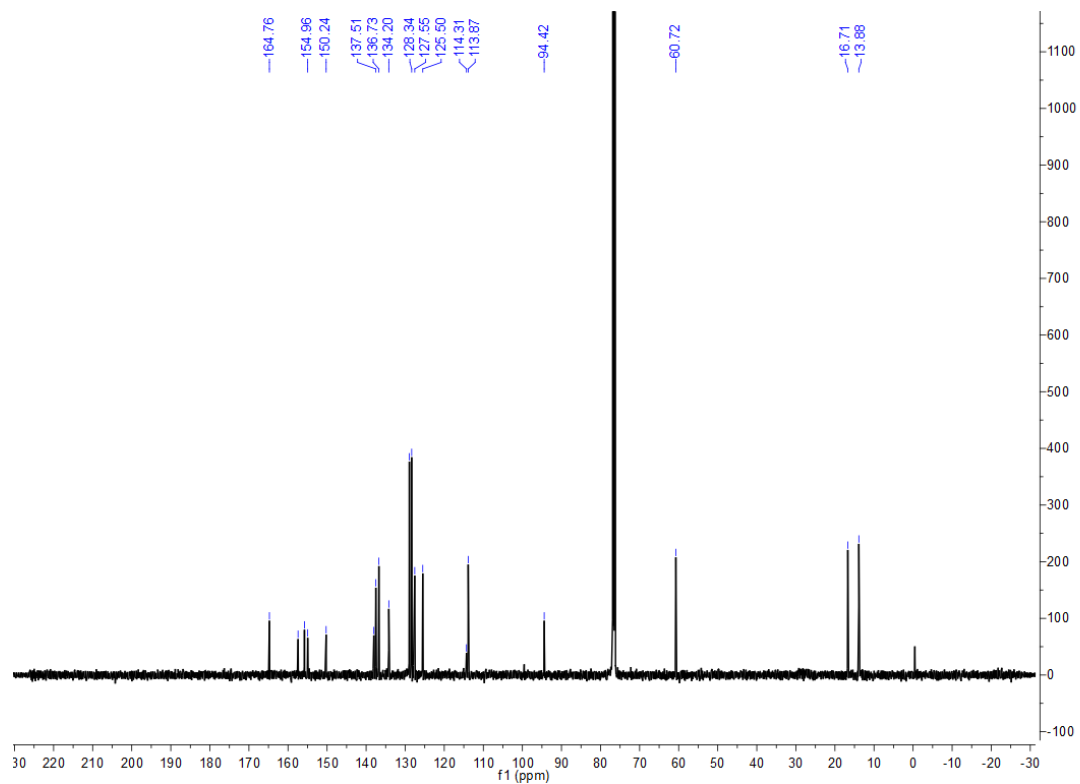
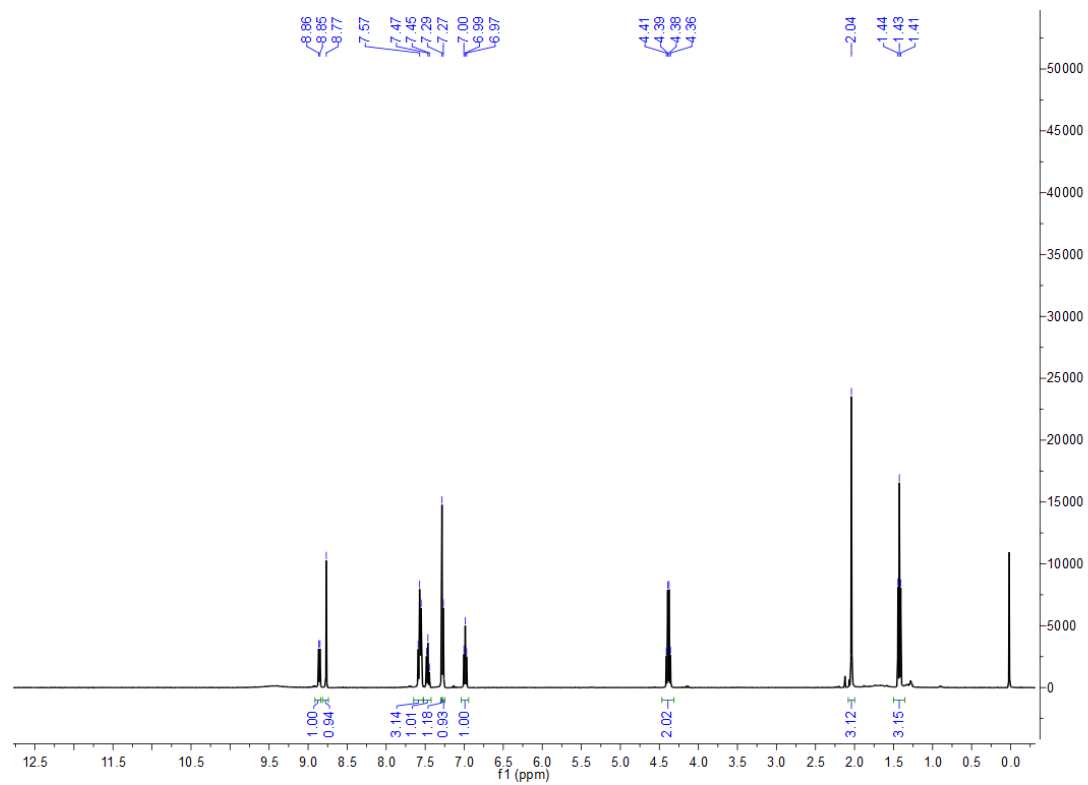
Compound 7c



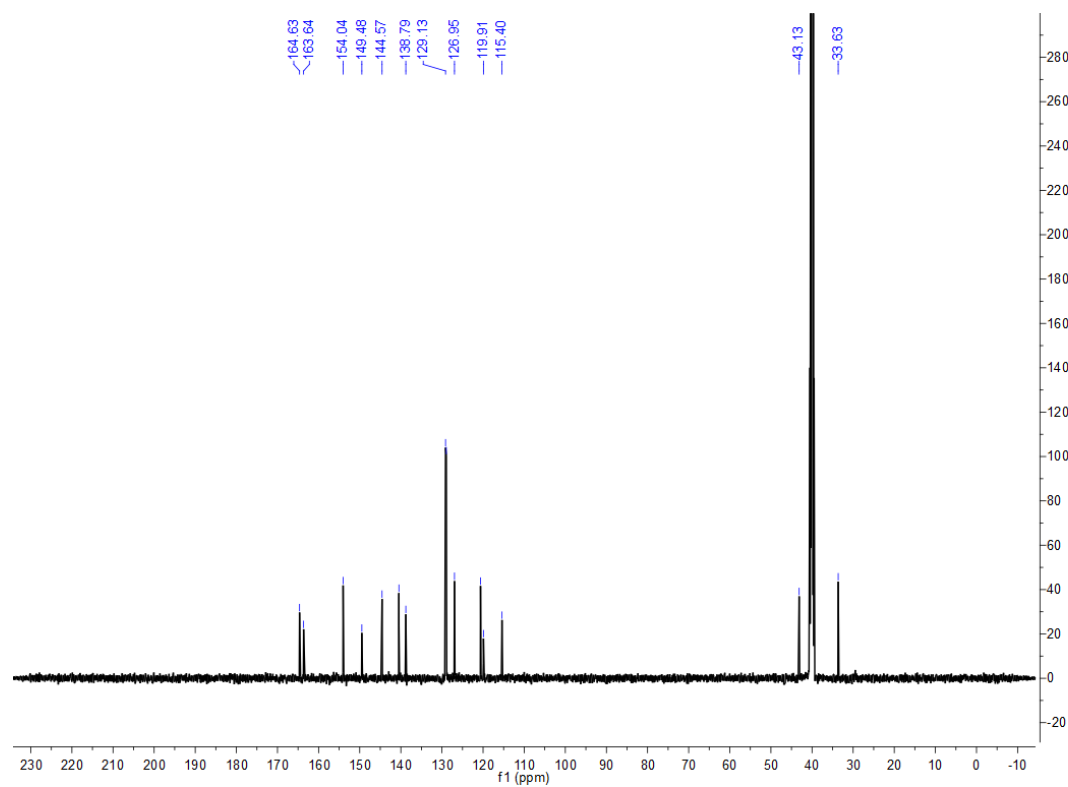
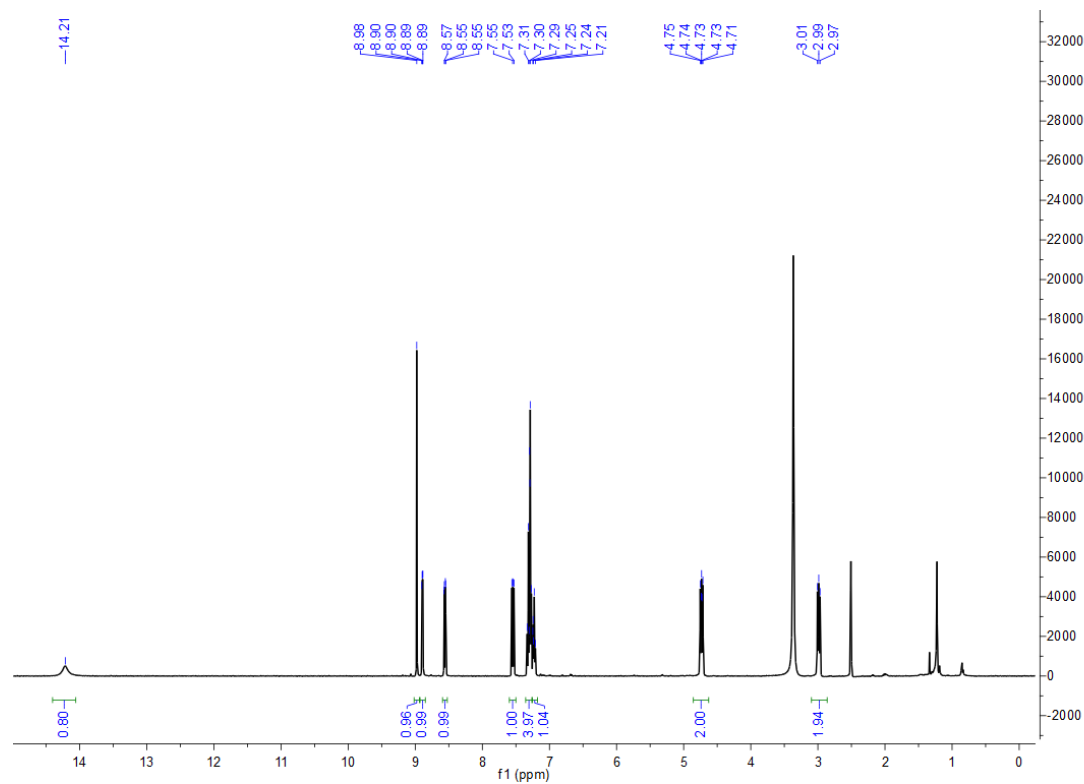
Compound 7e



Compound 7i



Compound 12a

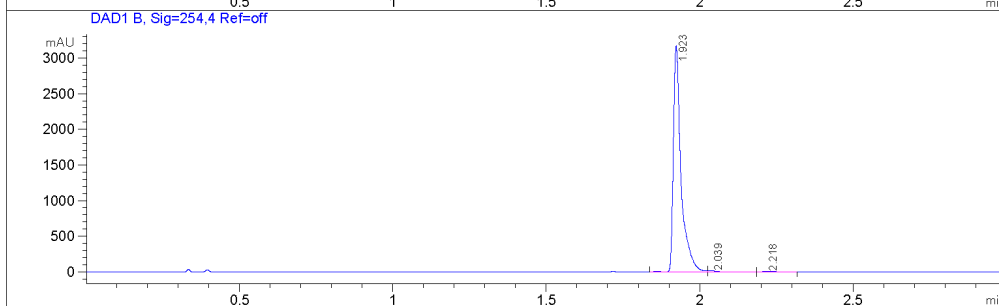
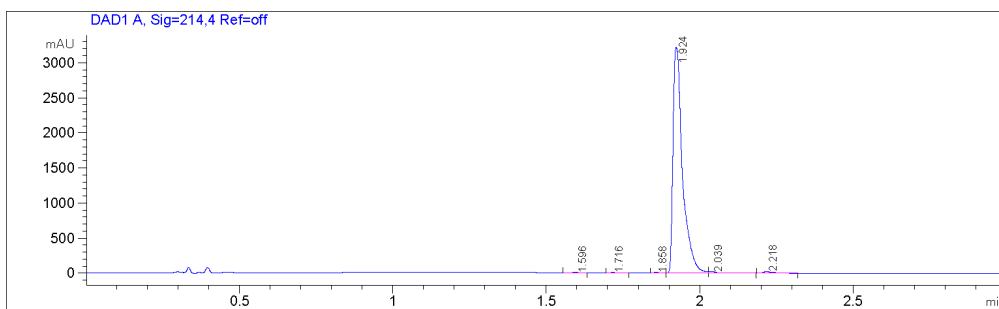


Representative HPLC

Compound 6b

LC/MS Report

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Injection Date : 23 May 19 3:19 pm +0800 Tgt Mass(EZX) :
Sample Name : DZ-7A Location : P1-C-03
Acq. Operator : A02-Monitor Inj : 1
Spec. Reported : MS Integration Inj Volume : 5 ul
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column:XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range:100-1000



Integration Results for DAD1 A, Sig=214.4 Ref=off

RetTim	Width	Area	Height	Area%
1.60	0.02	7.85	5.41	0.11
1.72	0.02	9.00	6.15	0.13
1.86	0.03	9.11	4.89	0.13
1.92	0.03	6871.33	3221.23	98.24
2.04	0.03	42.46	20.99	0.61
2.22	0.03	54.75	24.12	0.78

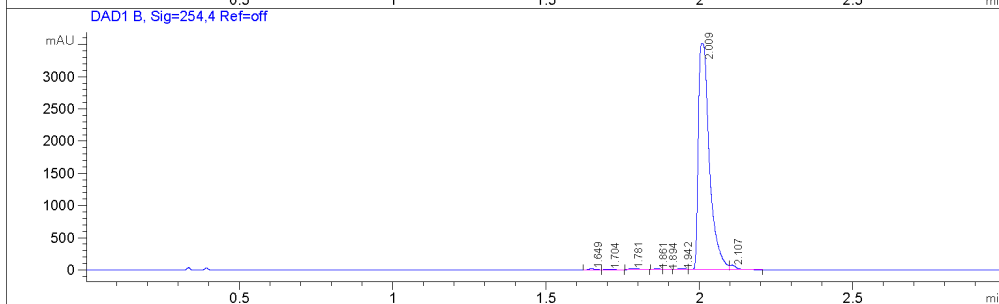
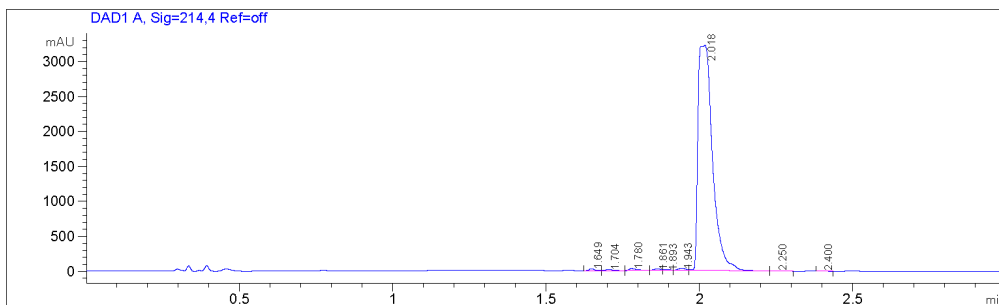
Integration Results for DAD1 B, Sig=254.4 Ref=off

RetTim	Width	Area	Height	Area%
1.92	0.03	5509.22	3176.04	98.78
2.04	0.03	37.94	20.51	0.68
2.22	0.03	29.82	13.29	0.53

Compound 6d

LC/MS Report

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Injection Date : 23 May 19 3:23 pm +0800 Tgt Mass(EZX) :
Sample Name : DZ-7B Location : P1-C-04
Acq. Operator : A02-Monitor Inj : 1
Spec. Reported : MS Integration Inj Volume : 5 ul
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column: XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range: 100-1000



Integration Results for DAD1 A, Sig=214.4 Ref=off

RetTim	Width	Area	Height	Area%
1.65	0.02	36.79	29.46	0.34
1.70	0.03	27.05	14.60	0.25
1.78	0.02	53.10	30.47	0.49
1.86	0.02	28.82	20.95	0.27
1.89	0.02	11.93	9.91	0.11
1.94	0.02	41.79	26.61	0.39
2.02	0.04	10543.50	3224.01	97.96
2.25	0.03	12.64	6.98	0.12
2.40	0.02	7.08	5.37	0.07

Integration Results for DAD1 B, Sig=254.4 Ref=off

RetTim	Width	Area	Height	Area%
1.65	0.02	22.75	18.54	0.25
1.70	0.03	15.84	8.59	0.18
1.78	0.03	37.47	19.09	0.42
1.86	0.02	14.32	10.60	0.16
1.89	0.02	6.36	5.36	0.07

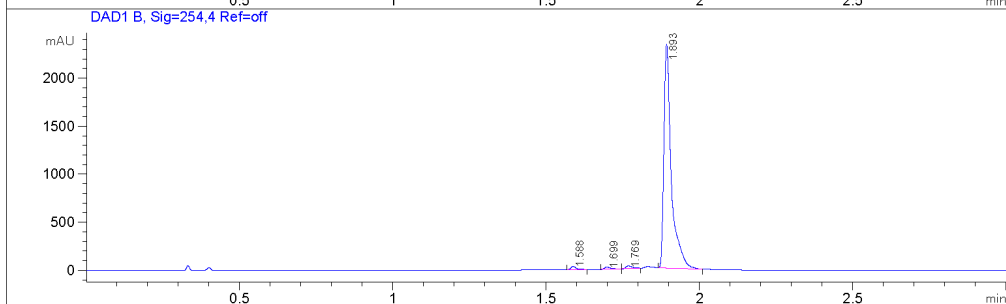
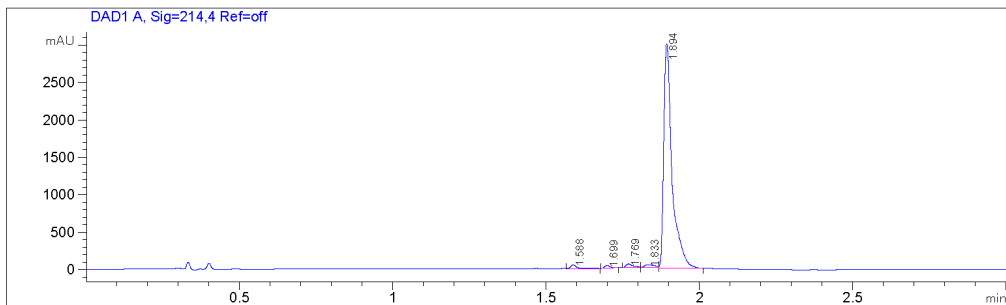
LC/MS Report

1.94	0.02	16.87	12.50	0.19
2.01	0.04	8769.60	3504.43	97.62
2.11	0.02	100.26	69.29	1.12

Compound 6e

LC/MS Report

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Injection Date : 23 May 19 3:27 pm +0800 Tgt Mass(EZX) :
Sample Name : DZ-7C Location : P1-C-05
Acq. Operator : A02-Monitor Inj : 1
Spec. Reported : MS Integration Inj Volume : 5 ul
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column:XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range:100-1000



Integration Results for DAD1 A, Sig=214.4 Ref=off

RetTim	Width	Area	Height	Area%
1.59	0.02	60.72	47.75	1.03
1.70	0.02	45.05	35.88	0.76
1.77	0.03	88.46	50.88	1.50
1.83	0.04	100.87	43.33	1.71
1.89	0.03	5611.69	3001.12	95.00

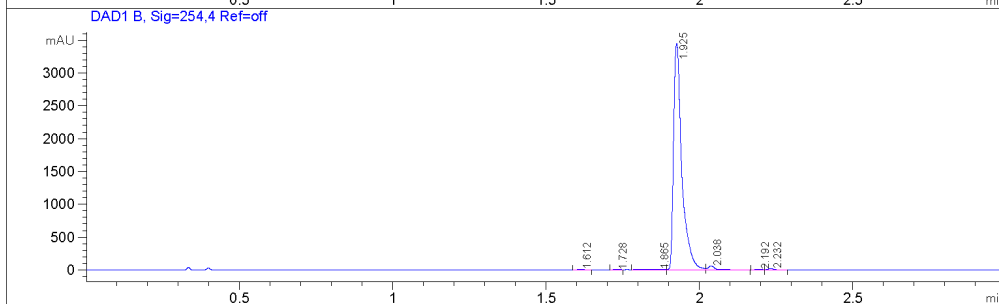
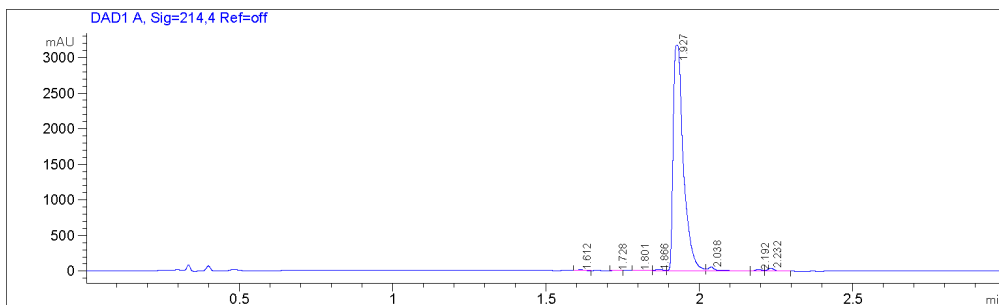
Integration Results for DAD1 B, Sig=254.4 Ref=off

RetTim	Width	Area	Height	Area%
1.59	0.02	37.06	31.95	0.92
1.70	0.02	34.19	24.46	0.85
1.77	0.02	45.57	28.53	1.14
1.89	0.02	3897.72	2333.72	97.09

Compound 6f

LC/MS Report

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Sample Name : DZ-7D Location : P1-C-06
Acq. Operator : A02-Monitor Inj : 1
Spec. Reported : MS Integration Inj Volume : 5 ul
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column:XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range:100-1000



Integration Results for DAD1 A, Sig=214.4 Ref=off

RetTim	Width	Area	Height	Area%
1.61	0.02	12.63	12.36	0.17
1.73	0.02	9.79	8.66	0.13
1.80	0.04	19.42	7.01	0.26
1.87	0.03	25.87	13.67	0.34
1.93	0.03	7305.35	3172.71	96.84
2.04	0.02	86.34	51.30	1.14
2.19	0.02	27.05	19.96	0.36
2.23	0.02	57.30	41.57	0.76

Integration Results for DAD1 B, Sig=254.4 Ref=off

RetTim	Width	Area	Height	Area%
1.61	0.02	10.73	9.89	0.16
1.73	0.02	7.38	6.47	0.11
1.80	0.04	31.68	9.57	0.48
1.93	0.03	6433.33	3449.72	97.19
2.04	0.02	91.42	60.32	1.38
2.19	0.02	17.32	12.71	0.26

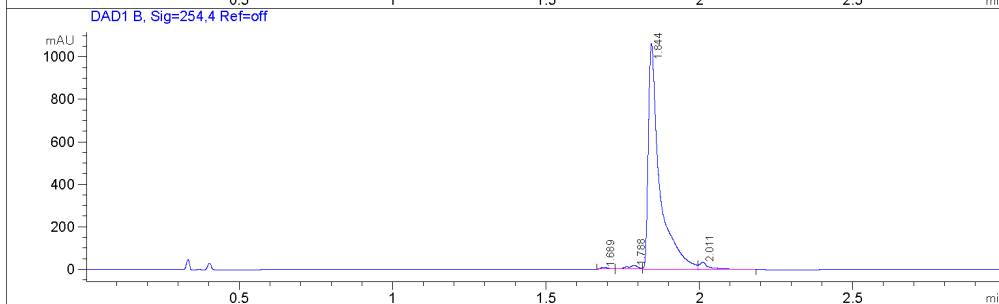
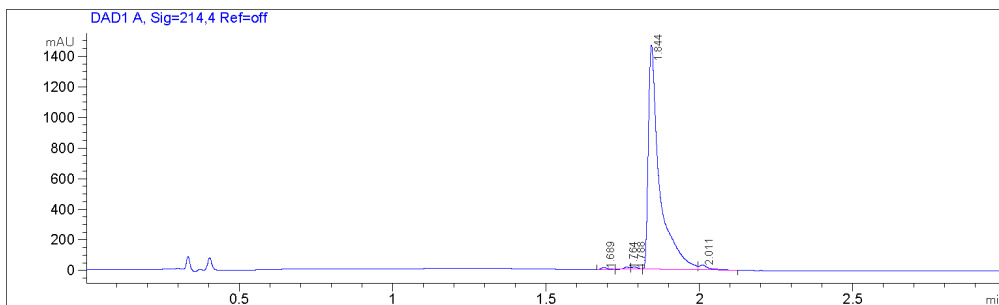
LC/MS Report



Compound 6lc

LC/MS Report

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Spec. Reported : MS Integration Inj Volume : 5 ul
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column:XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range:100-1000



Integration Results for DAD1 A, Sig=214.4 Ref=off

RetTim	Width	Area	Height	Area%
1.69	0.02	17.20	12.58	0.44
1.76	0.02	17.09	13.69	0.44
1.79	0.02	18.69	13.39	0.48
1.84	0.04	3743.67	1468.42	96.82
2.01	0.03	69.85	32.24	1.81

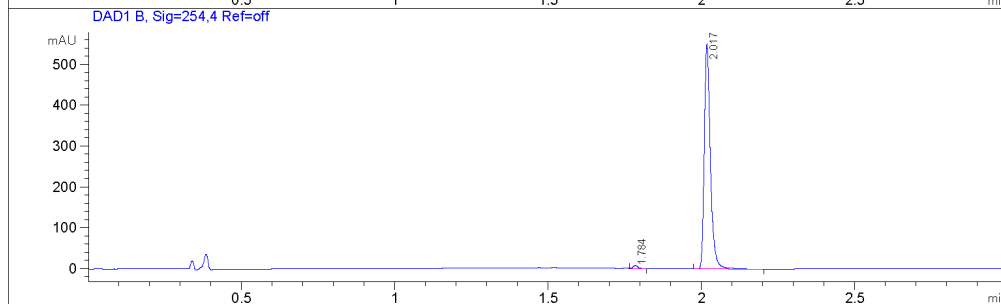
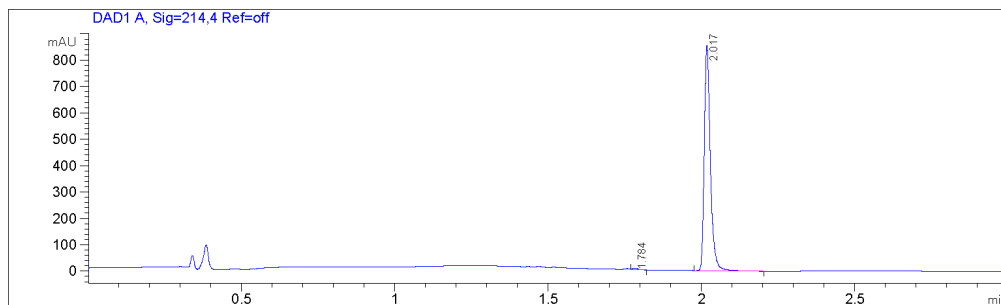
Integration Results for DAD1 B, Sig=254.4 Ref=off

RetTim	Width	Area	Height	Area%
1.69	0.02	13.00	9.43	0.46
1.79	0.03	37.84	17.09	1.33
1.84	0.04	2723.94	1063.40	95.59
2.01	0.03	74.87	33.50	2.63

Compound 7g

LC/MS Report

File D:\DATABASE\RAWDATA\A02\LCMS\MONITOR\2019-06\2019-06-21\7g-83249-LCMSA042.D
Injection Date : 21 Jun 19 3:39 pm +0800 Tgt Mass(EZX) :
Sample Name : 8H Location : P2-A-05
Acq. Operator : A02-Monitor Inj : 1
Spec. Reported : MS Integration Inj Volume : 2 ul
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column:XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range:100-1000



Integration Results for DAD1 A, Sig=214.4 Ref=off

RetTim	Width	Area	Height	Area%
1.78	0.02	7.38	6.55	0.62
2.02	0.02	1173.36	856.01	99.38

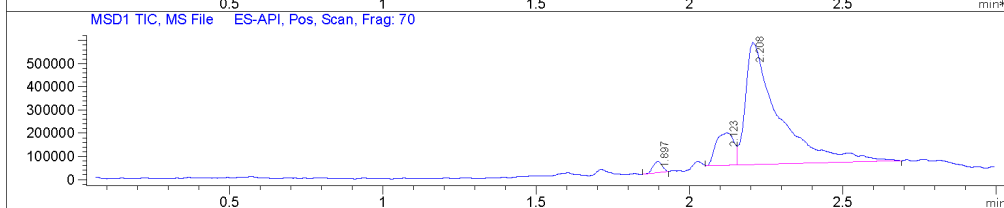
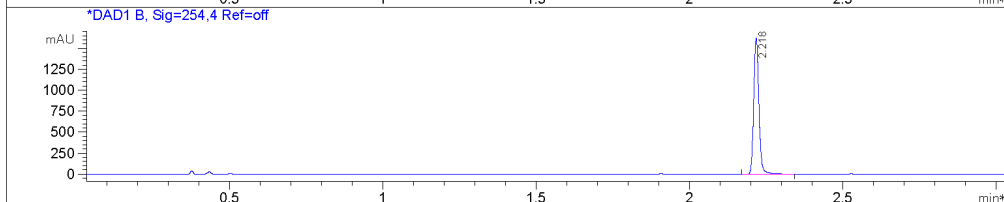
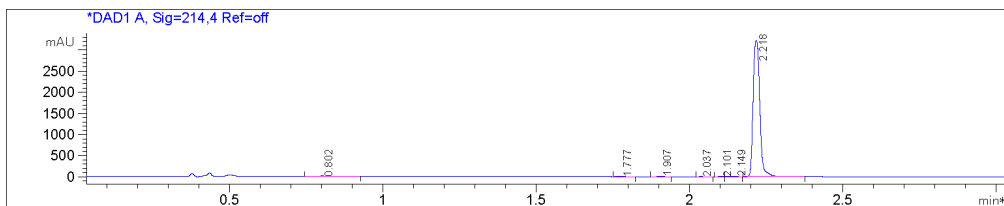
Integration Results for DAD1 B, Sig=254.4 Ref=off

RetTim	Width	Area	Height	Area%
1.78	0.02	7.43	7.13	0.97
2.02	0.02	761.14	552.63	99.03

Compound 13a

LC/MS Report

File D:\DATA\A02\MONITOR\13a-83256-LCMSA042.D
Injection Date : 21 Jun 19 4:08 pm +0800 Tgt Mass(EZX) :
Sample Name : 11C Location : P2-B-03
Acq. Operator : A02-Monitor Inj : 1
Spec. Reported : MS Integration Inj Volume : 5 ul
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column:XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range:100-1000



Integration Results for DAD1 A, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
0.80	0.02	10.94	7.67	0.22
1.78	0.02	8.51	7.28	0.17
1.91	0.02	15.39	13.32	0.31
2.04	0.02	8.32	6.13	0.17
2.10	0.02	11.76	10.05	0.24
2.15	0.03	21.50	11.84	0.44
2.22	0.02	4830.27	3226.36	98.44

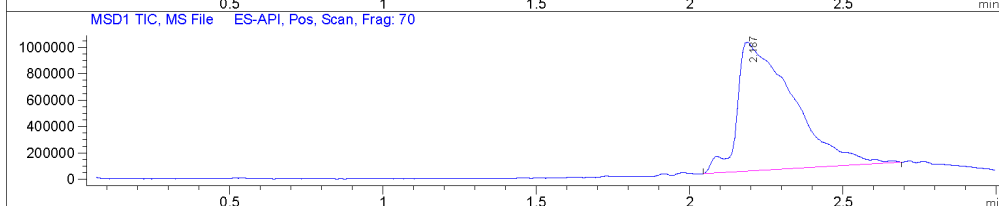
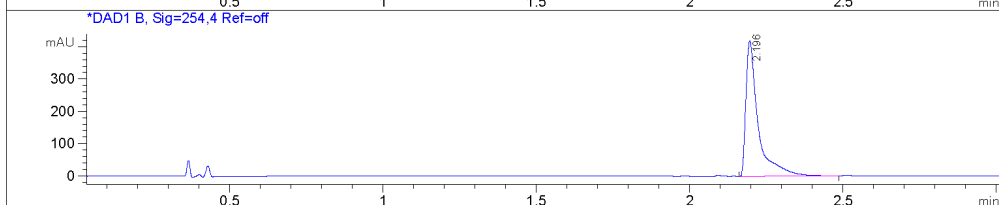
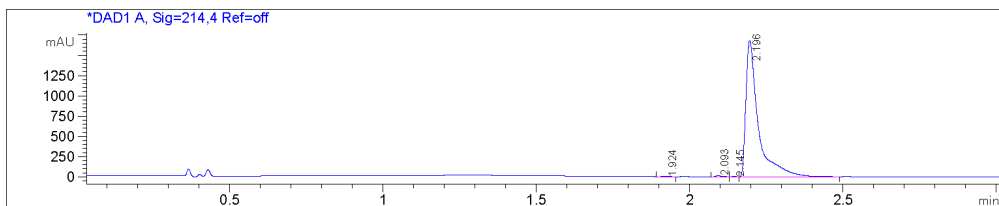
Integration Results for DAD1 B, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
2.22	0.02	1882.03	1627.06	100.00

Compound 13b

LC/MS Report

File D:\DATA\A02\MONITOR\13b-83257-LCMSA042.D
Injection Date : 21 Jun 19 4:12 pm +0800 Tgt Mass(EZX) :
Sample Name : 11G Location : P2-B-04
Acq. Operator : A02-Monitor Inj : 1
Spec. Reported : MS Integration Inj Volume : 5 ul
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column:XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range:100-1000



Integration Results for DAD1 A, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
1.92	0.02	6.12	5.35	0.13
2.09	0.02	22.12	16.32	0.47
2.14	0.02	5.24	5.70	0.11
2.20	0.04	4702.02	1685.49	99.29

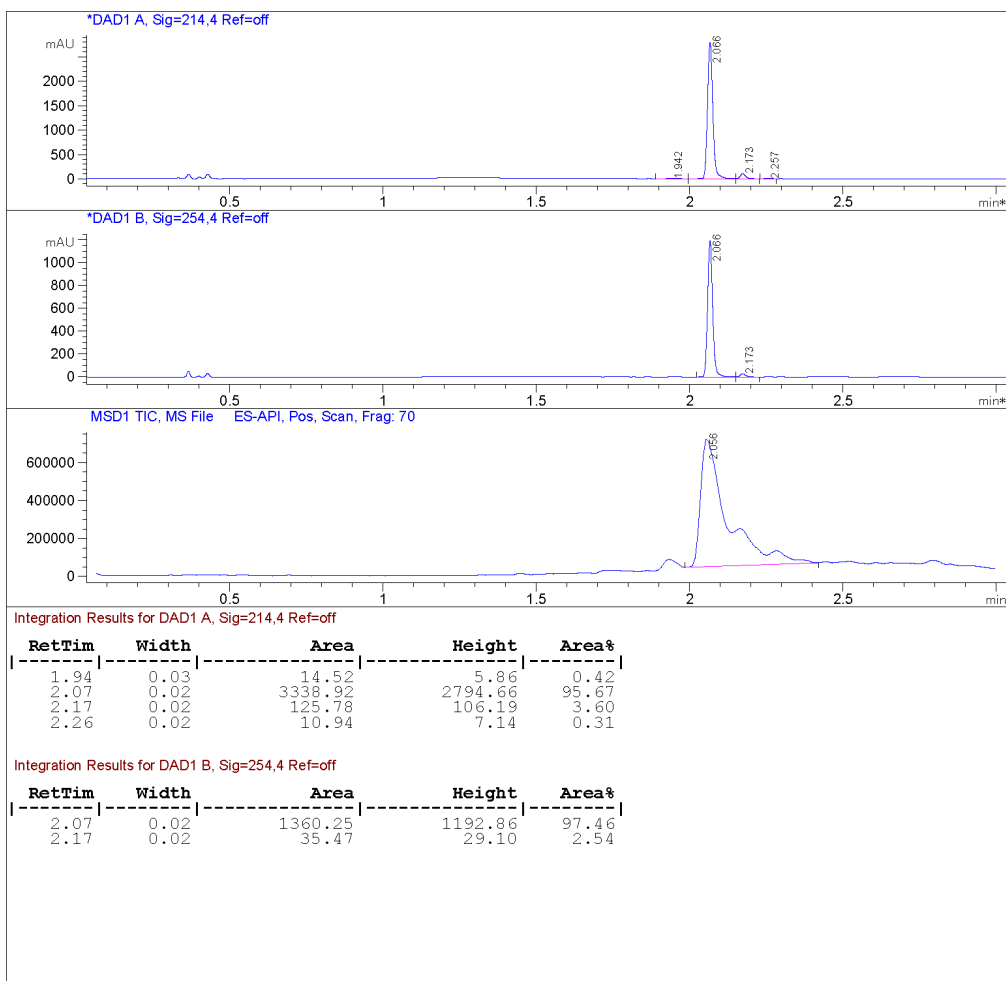
Integration Results for DAD1 B, Sig=254,4 Ref=off

RetTim	Width	Area	Height	Area%
2.20	0.04	1170.77	420.79	100.00

Compound 14a

LC/MS Report

File D:\DATA\A02\MONITOR\14a-83260-LCMSA042.D
Injection Date : 21 Jun 19 4:25 pm +0800 Tgt Mass(EZX) :
Sample Name : 12C Location : P2-B-07
Acq. Operator : A02-Monitor Inj : 1
Spec. Reported : MS Integration Inj Volume : 5 ul
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column:XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range:100-1000



Compound 14b

LC/MS Report

File D:\DATA\A02\MONITOR\14b-83261-LCMSA042.D
Injection Date : 21 Jun 19 4:29 pm +0800 Tgt Mass(EZX) :
Sample Name : 12G Location : P2-B-08
Acq. Operator : A02-Monitor Inj : 1
Spec. Reported : MS Integration Inj Volume : 5 uL
Acq. Method : D:\METHODS\2-POS-MON-1.M
Analysis Method : D:\METHODS\2-POS-MON-1.M
Sample Info : Easy-Access Method: '2-POS-MON-1'
Method Info : Mobile Phase: A: water(10mM Ammonium hydrogen carbonate) B: ACN
Gradient: 5%-95% B in 1.5min,95%B for 1.5 min
Flow Rate: 1.8ml/min
Column:XBridge ,4.6*50mm,3.5um
Oven Temperature: 50 C
MASS Range:100-1000

