SUPPORTING INFORMATION

Design, Synthesis, and Biological Evaluation of Indole-Modified Tamoxifen Relatives as Anticancer Agents

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[c]Molecular Cancer Biology Laboratory, High Technology Application and Research Center, Erzurum Technical University, Erzurum, 25050, Türkiye

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Comparisons of ^{1}H NMR spectra

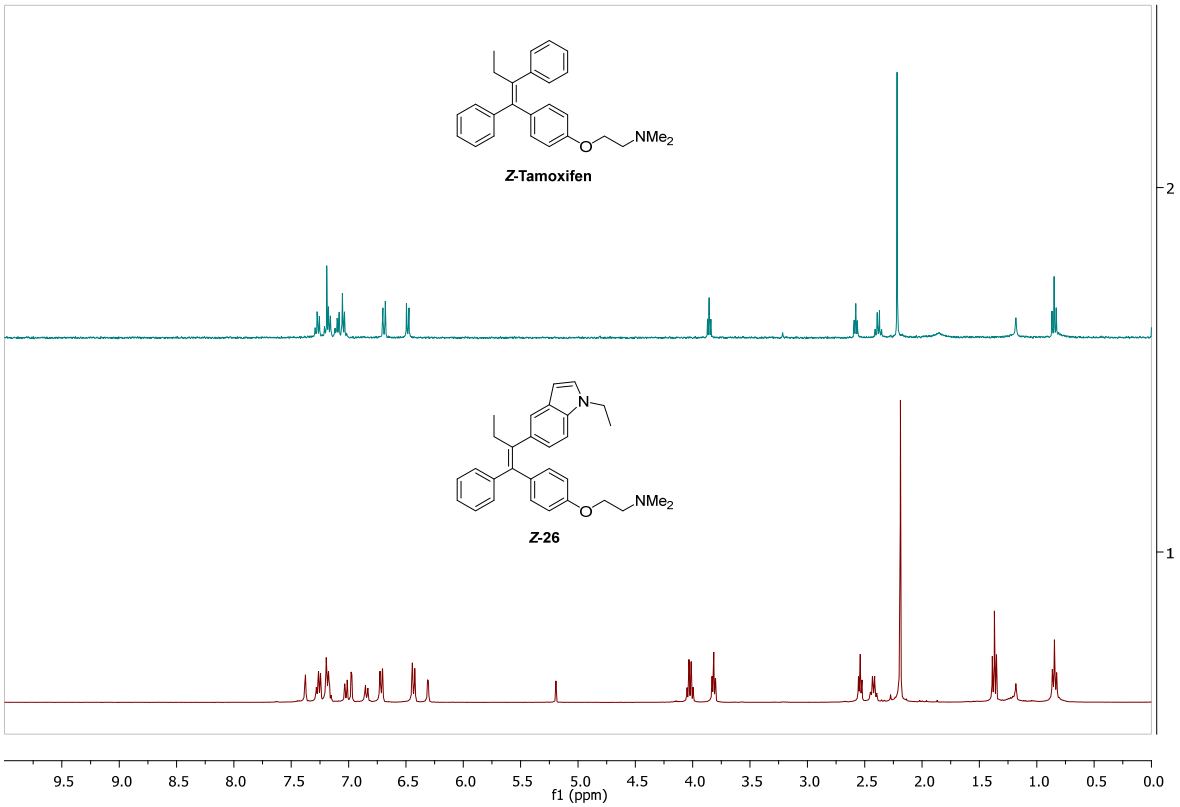


Figure S1. Comparison of ^{1}H NMR spectra of ***Z*-Tamoxifen** and ***Z*-26** compounds.

NOE Experiments

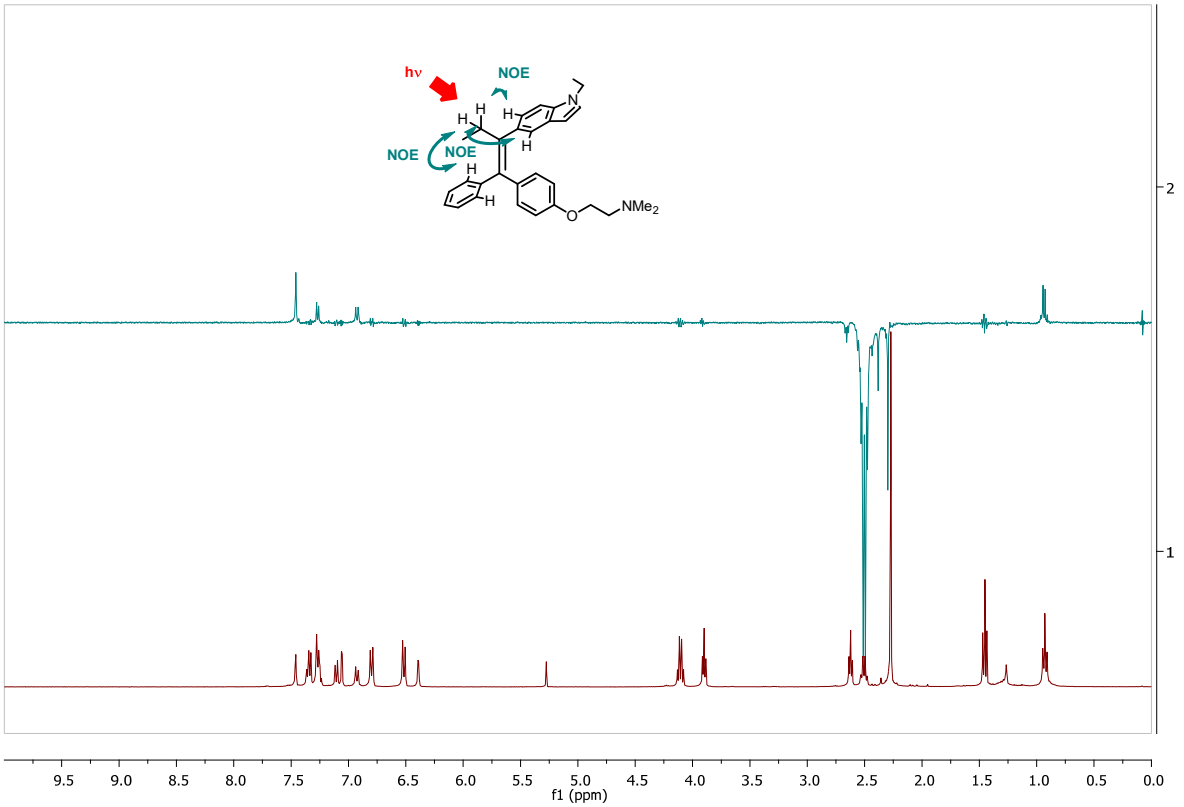


Figure S2. Correlations in compound ***Z*-26**.

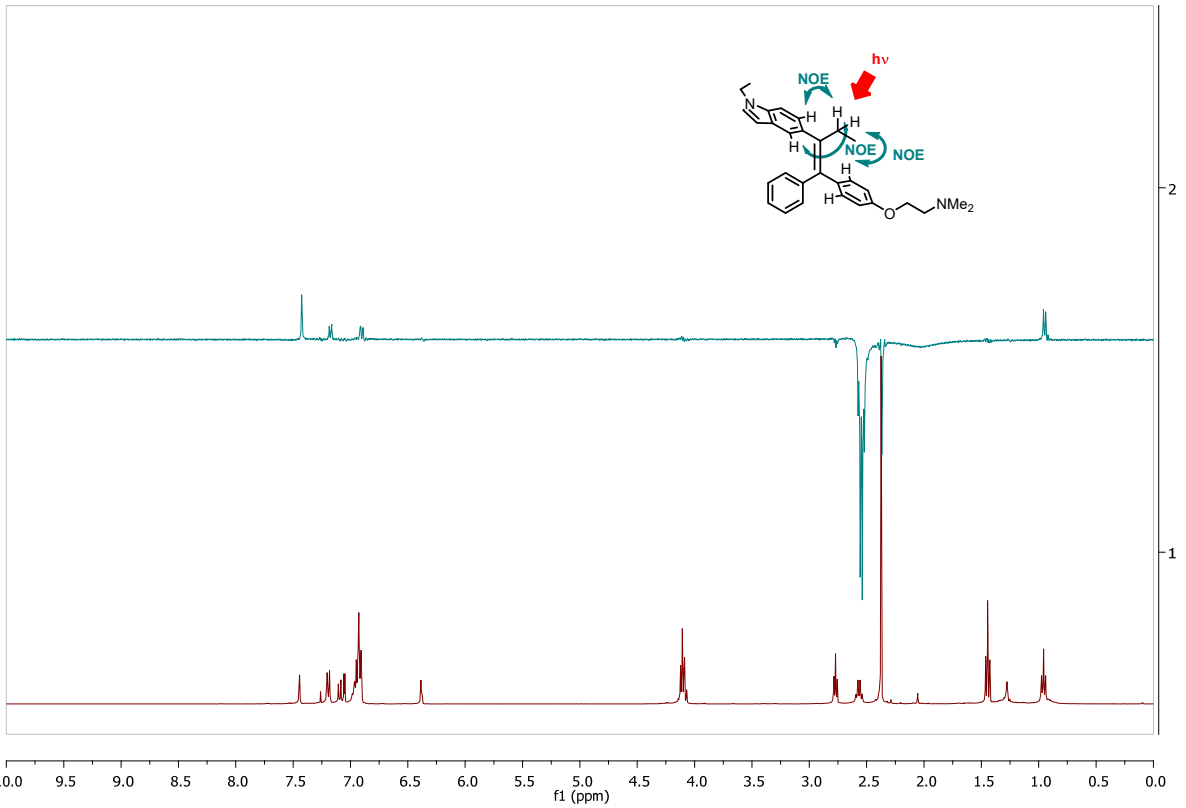


Figure S3. Correlations in compound ***E*-26**.

Figures for cell viability of compounds

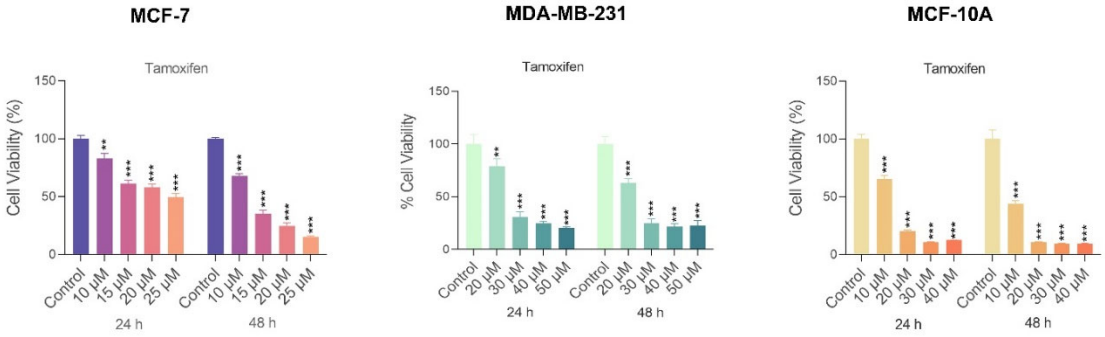


Figure S4. Cell viability of MCF-7, MDA-MB-231, and MCF10A cells after treatment at different concentrations of TMX. The bar graph represents the mean ± S.E.M. of four independent experiments. The significance between groups was tested using the Student t-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

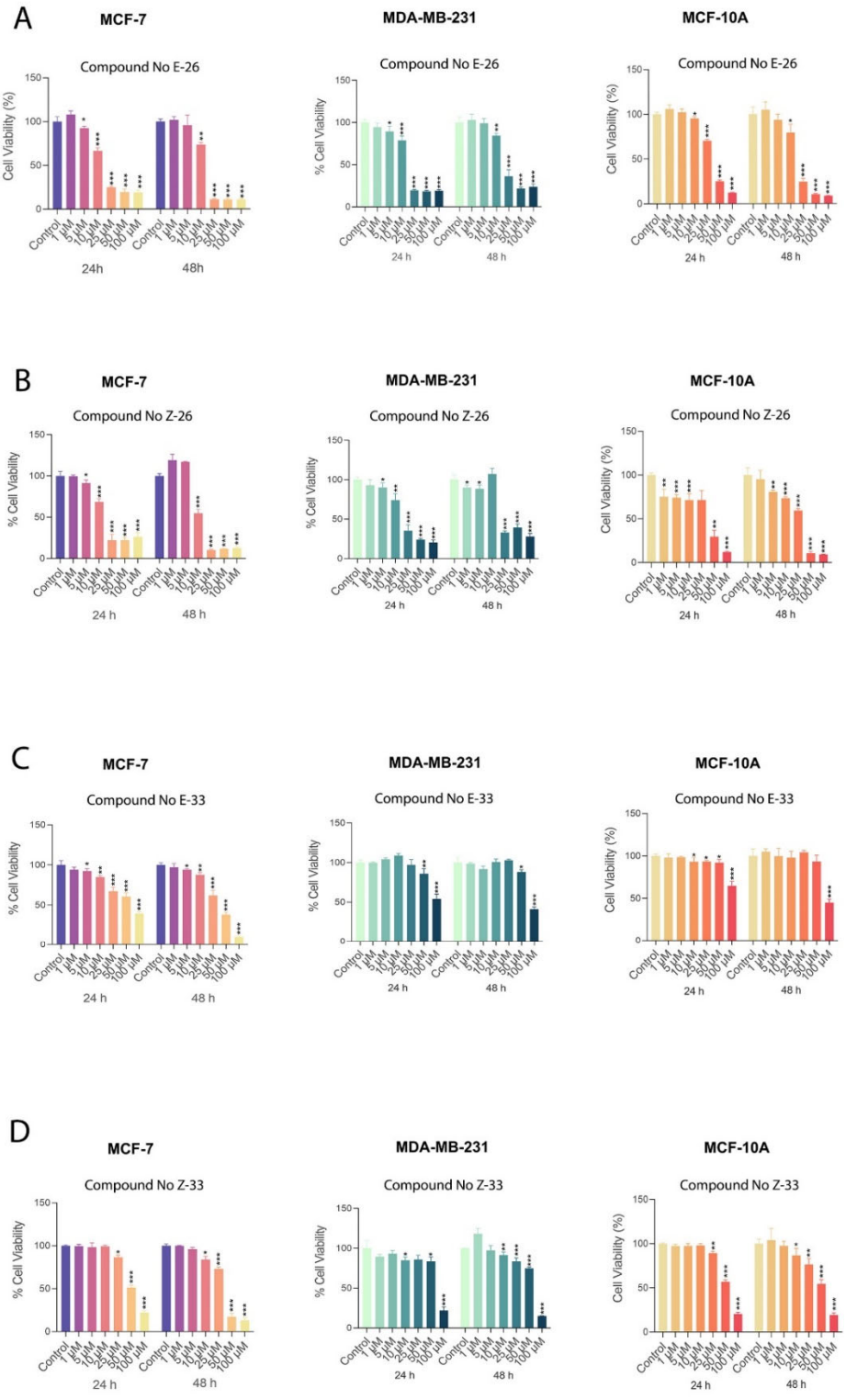


Figure S5A-D. Cell viability of MCF-7, MDA-MB-231, and MCF10A cells after treatment at different concentrations of TMX indole-derivatives ***E*-26**, ***Z*-26**, ***E*-33**, and ***Z*-33**. The bar graph represents the mean ± S.E.M. of four independent experiments. The significance between groups was tested using the Student t-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

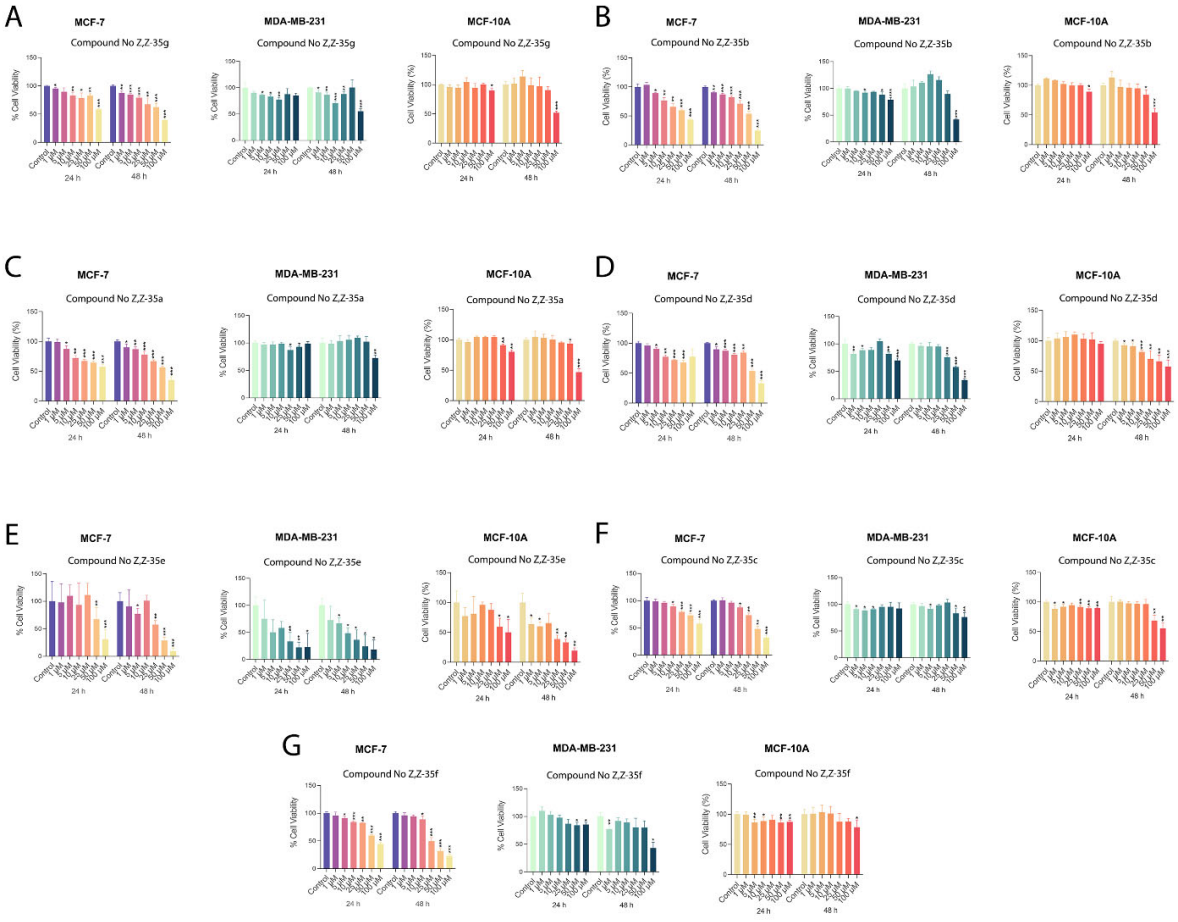


Figure S6A-G. Cell viability of MCF-7, MDA-MB-231, and MCF10A cells after treatment at different concentrations of **BIM *Z,Z*-isomers 35a-g**. The bar graph represents the mean ± S.E.M. of four independent experiments. The significance between groups was tested using the Student t-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

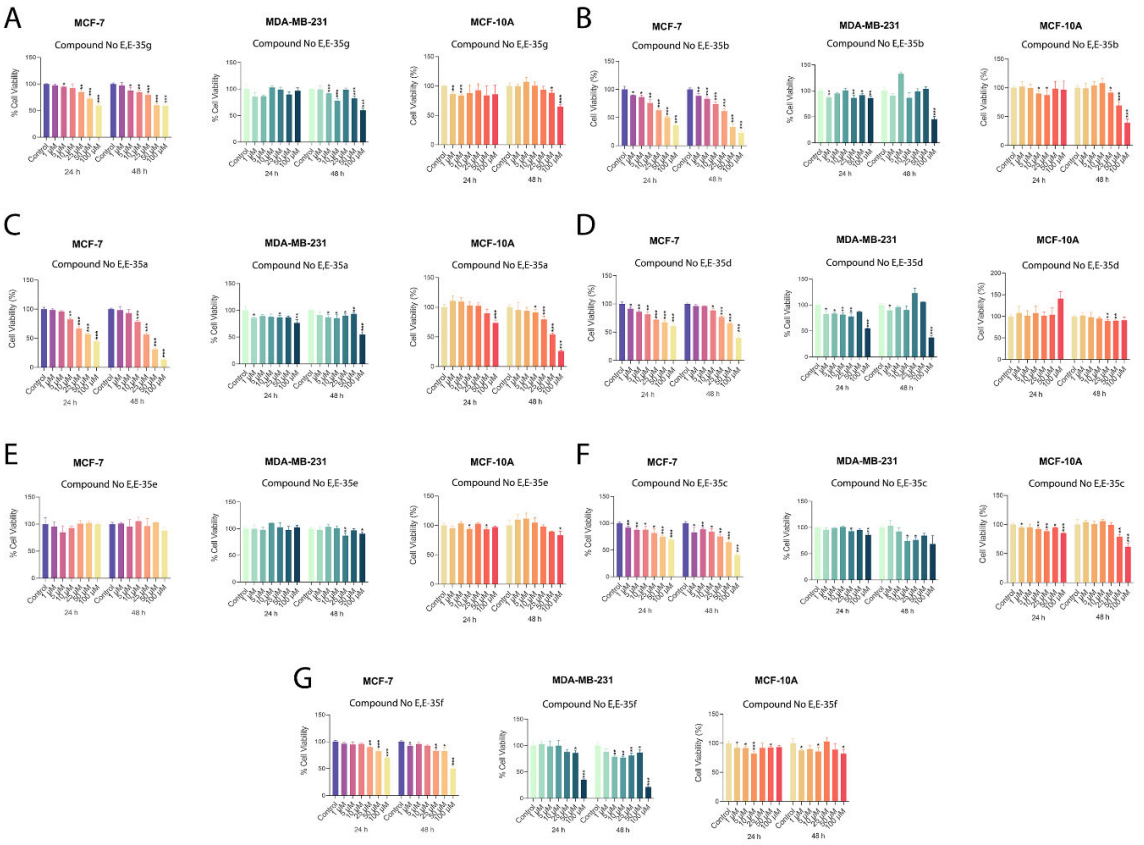


Figure S7A-G. Cell viability of MCF-7, MDA-MB-231, and MCF10A cells after treatment at different concentrations of **BIM *E,E*-isomers 35a-g**. The bar graph represents the mean ± S.E.M. of four independent experiments. The significance between groups was tested using the Student t-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

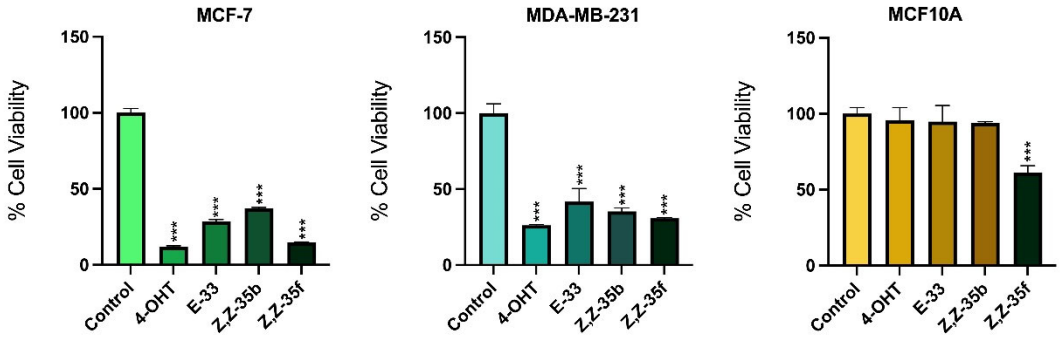


Figure S8. Cell viability of MCF-7, MDA-MB-231, and MCF10A cells after treatment with IC_{50} concentrations of 4-OHT and TMX-relative compounds. The bar graph represents the mean ± S.E.M. of three independent experiments. Significance between groups was tested using Student t-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

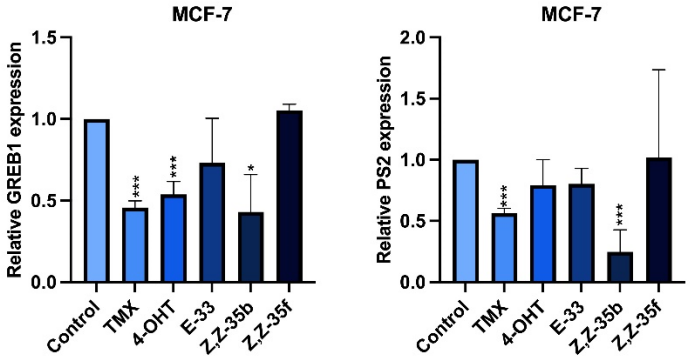
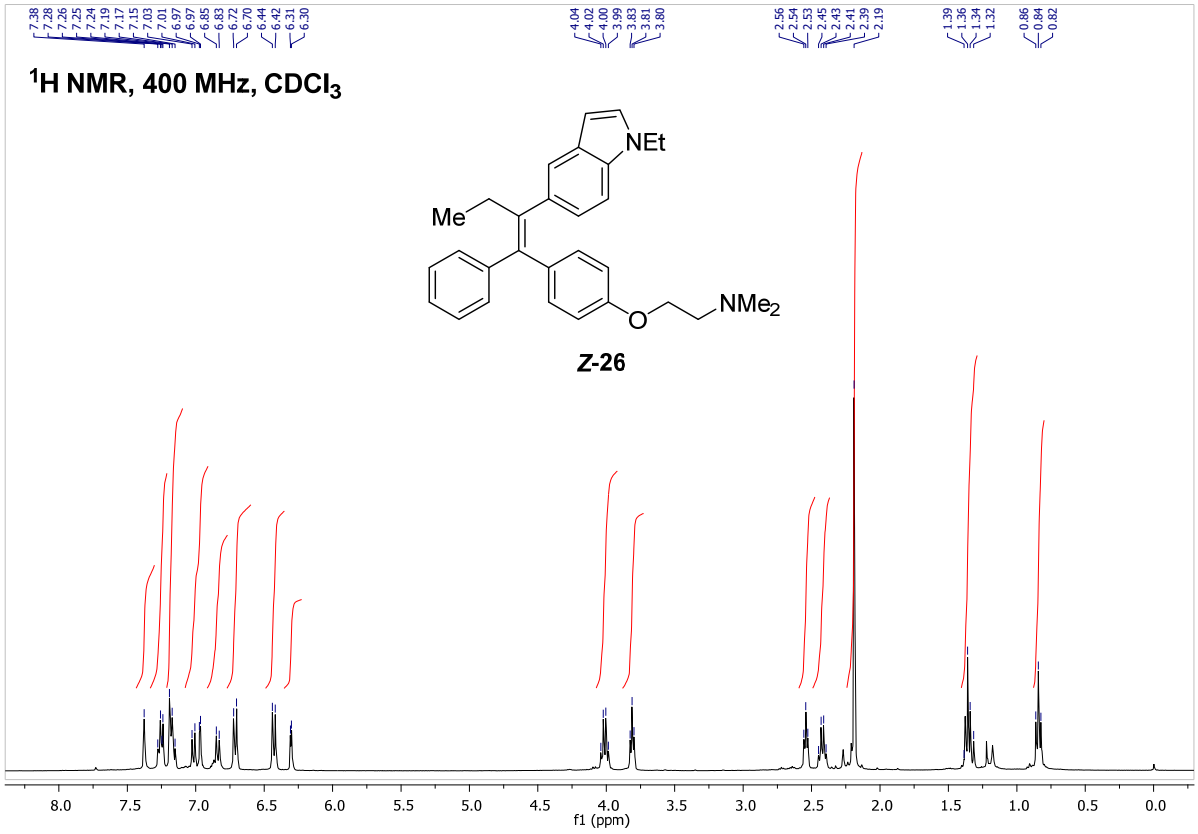
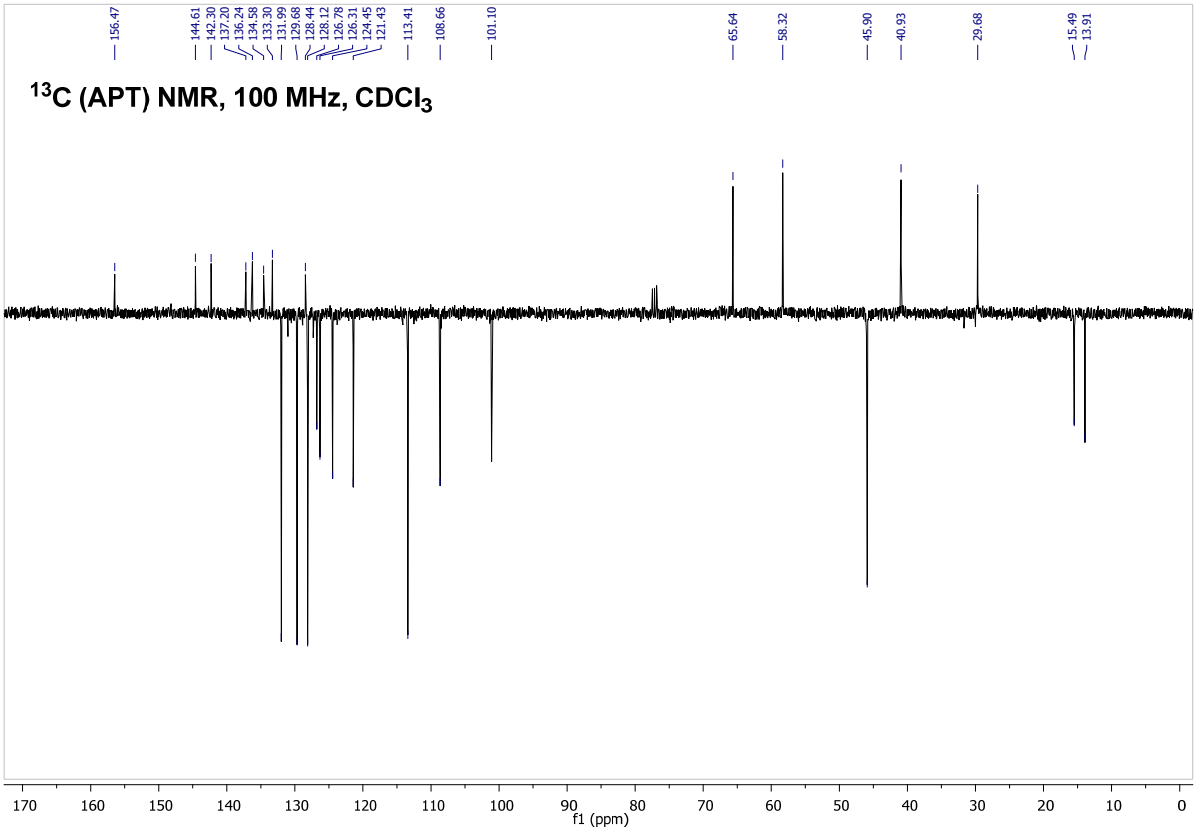
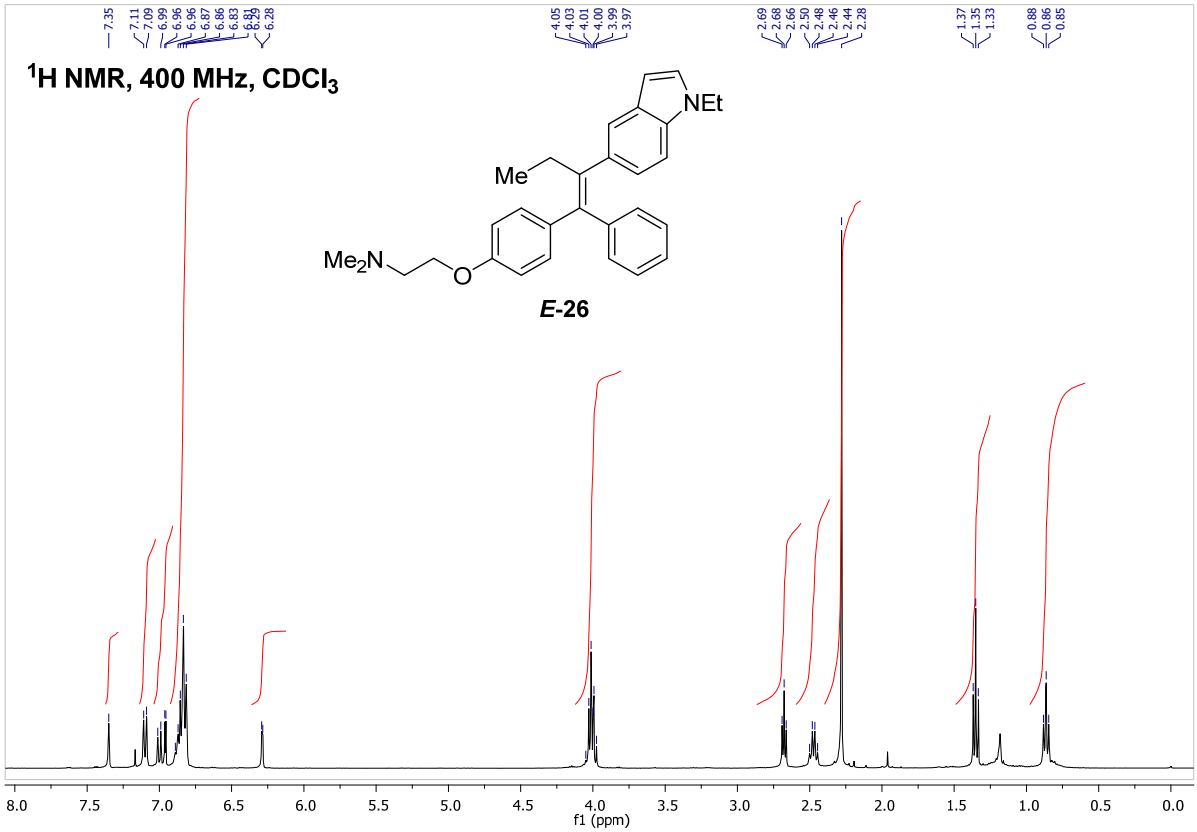


Figure S9. Relative expression levels of GREB1 and PS2 genes in MCF-7 cells. The levels of GREB1 and PS2 were normalized to GAPDH. The mean ± SEM is shown in Figure 6. *p < 0.05, **p < 0.01, ***p < 0.001; t-test.

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra of compounds



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4.01
4.00
3.99
3.97
2.69
2.68
2.66
2.50
2.48
2.46
2.44
2.28
1.37
1.35
1.33
0.88
0.86
0.85
^{1}H NMR, 400 MHz, CDCl$_3$
NEt
Me
Me$_2$N
O
E-26
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7.5
7.0
6.5
6.0
5.5
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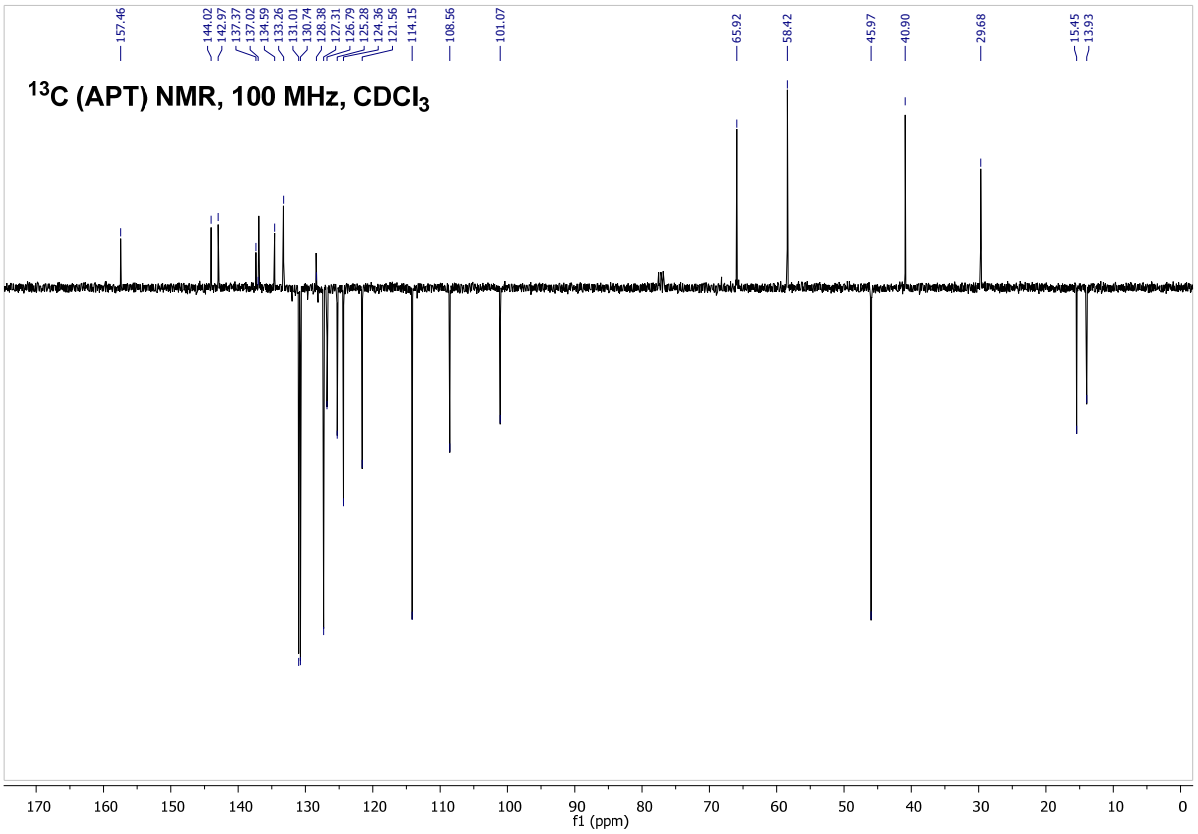
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125.28
124.36
121.56
114.15
108.56
101.07
65.92
58.42
45.97
40.90
29.68
15.45
13.93
^{13}C (APT) NMR, 100 MHz, CDCl$_3$
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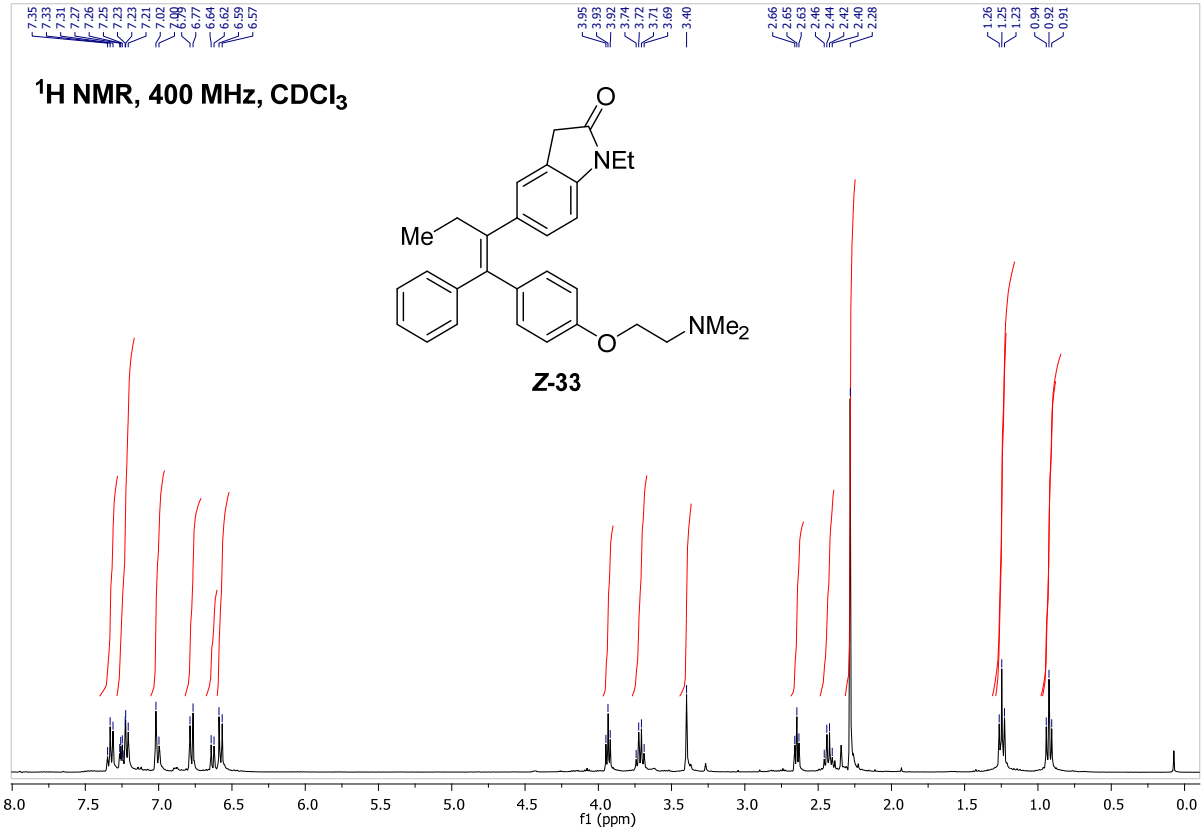
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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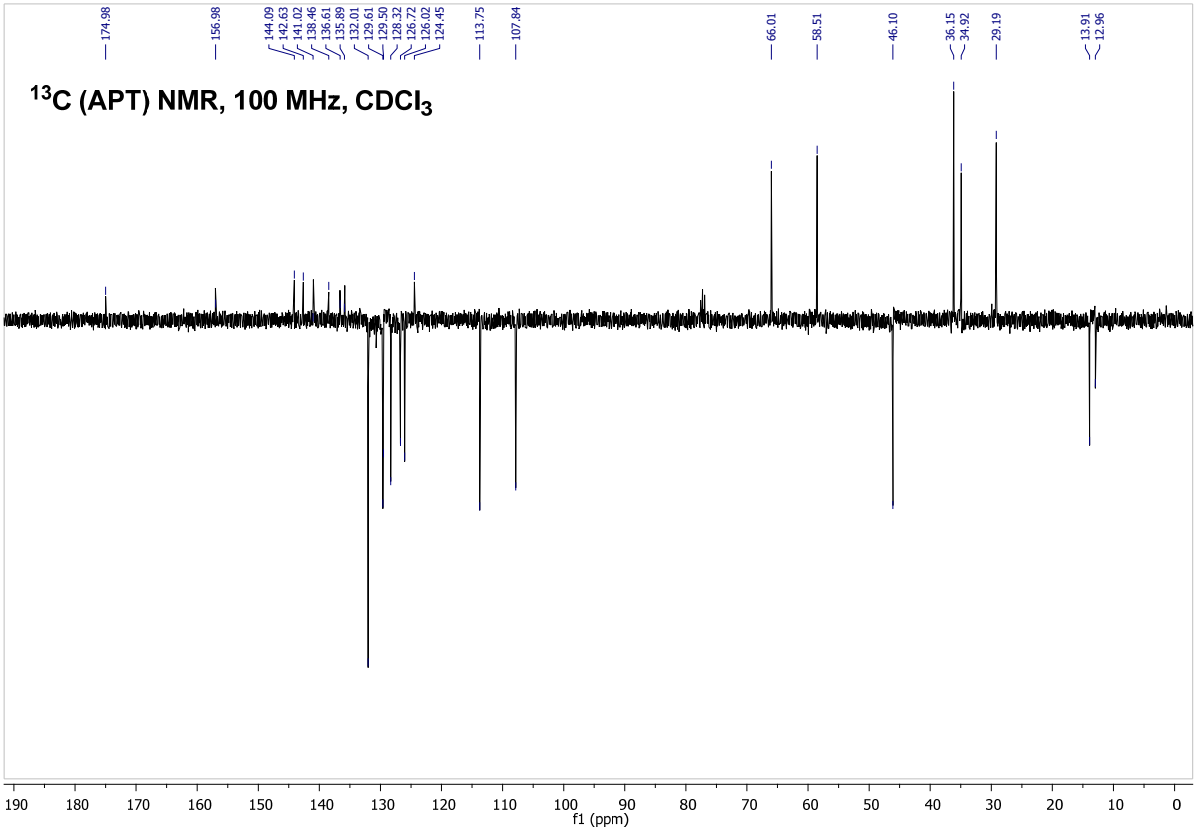
NEt
Me
NMe2
O
Z-33
f1 (ppm)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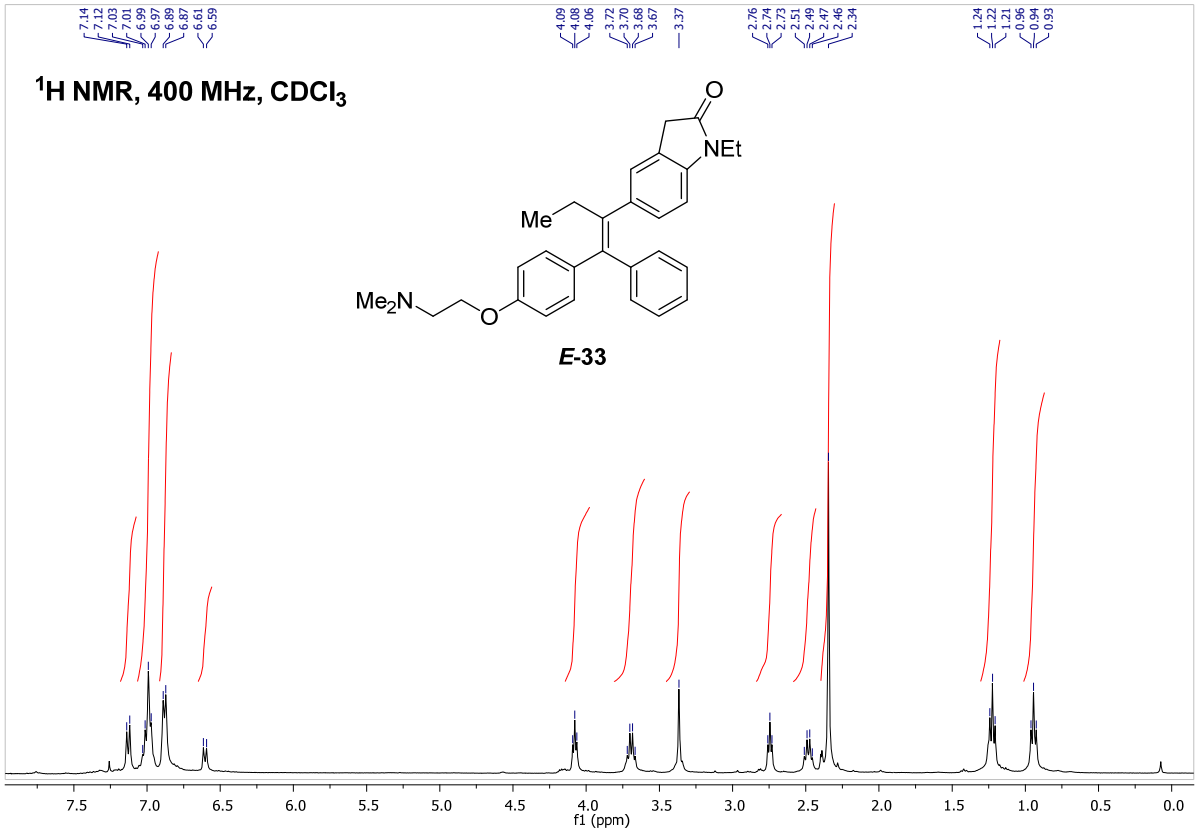
f1 (ppm)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2.74
2.73
2.51
2.49
2.47
2.46
2.34
1.24
1.22
1.21
0.96
0.94
0.93
1H NMR, 400 MHz, CDCl3
O
NEt
Me
Me2N
O
E-33
7.5
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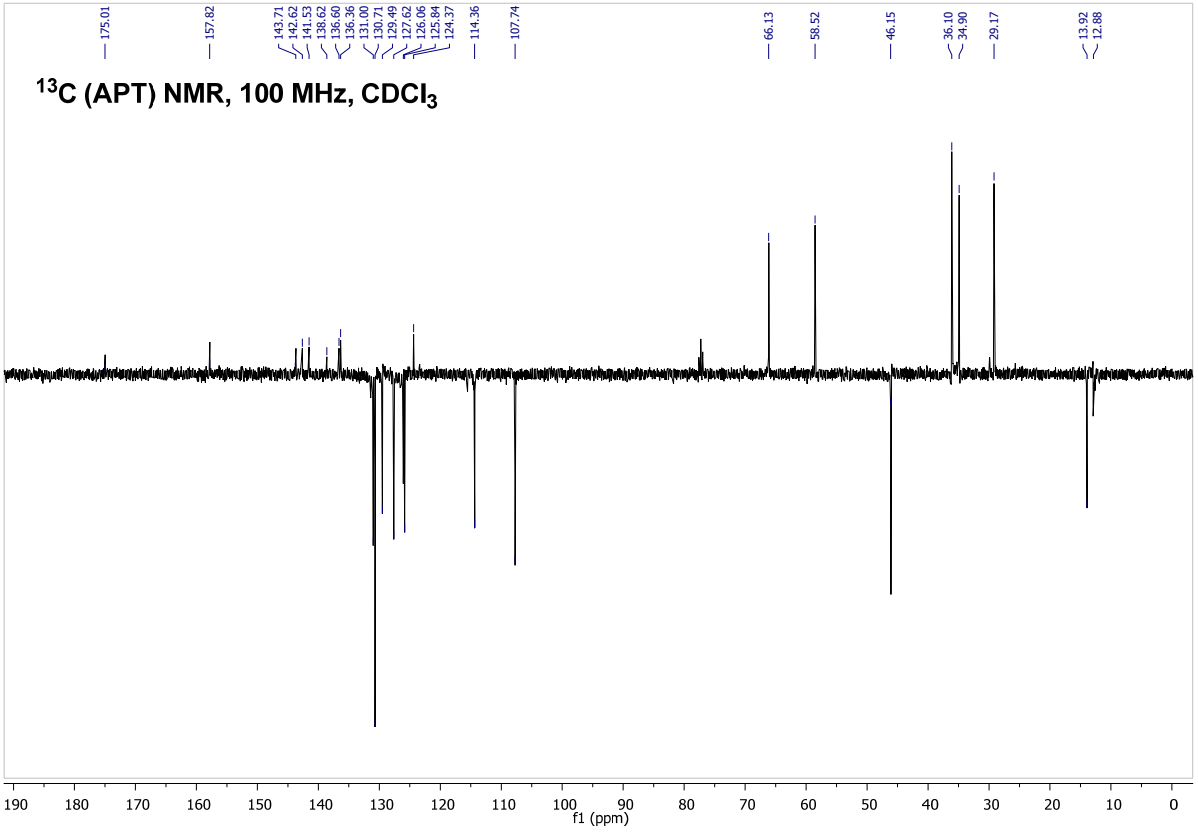
f1 (ppm)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124.37
114.36
107.74
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36.10
34.90
29.17
13.92
12.88
13C (APT) NMR, 100 MHz, CDCl3
190
180
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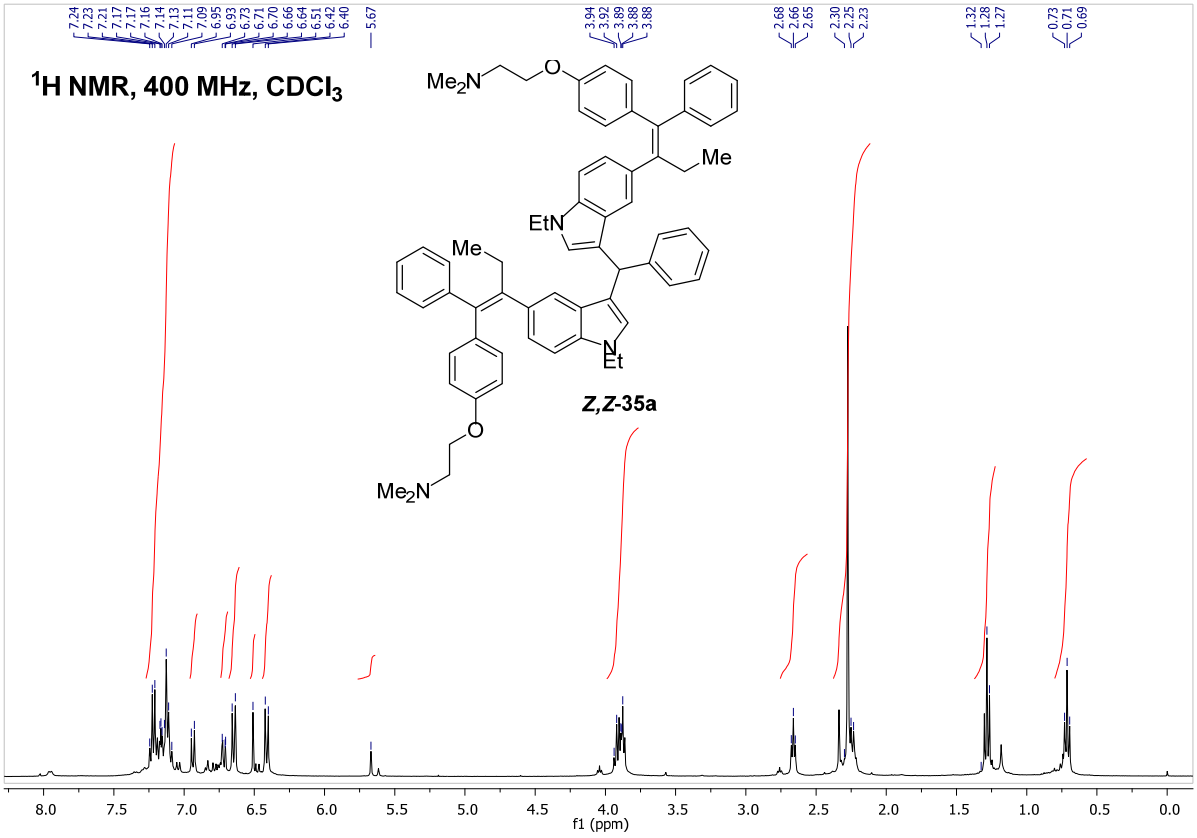
0
f1 (ppm)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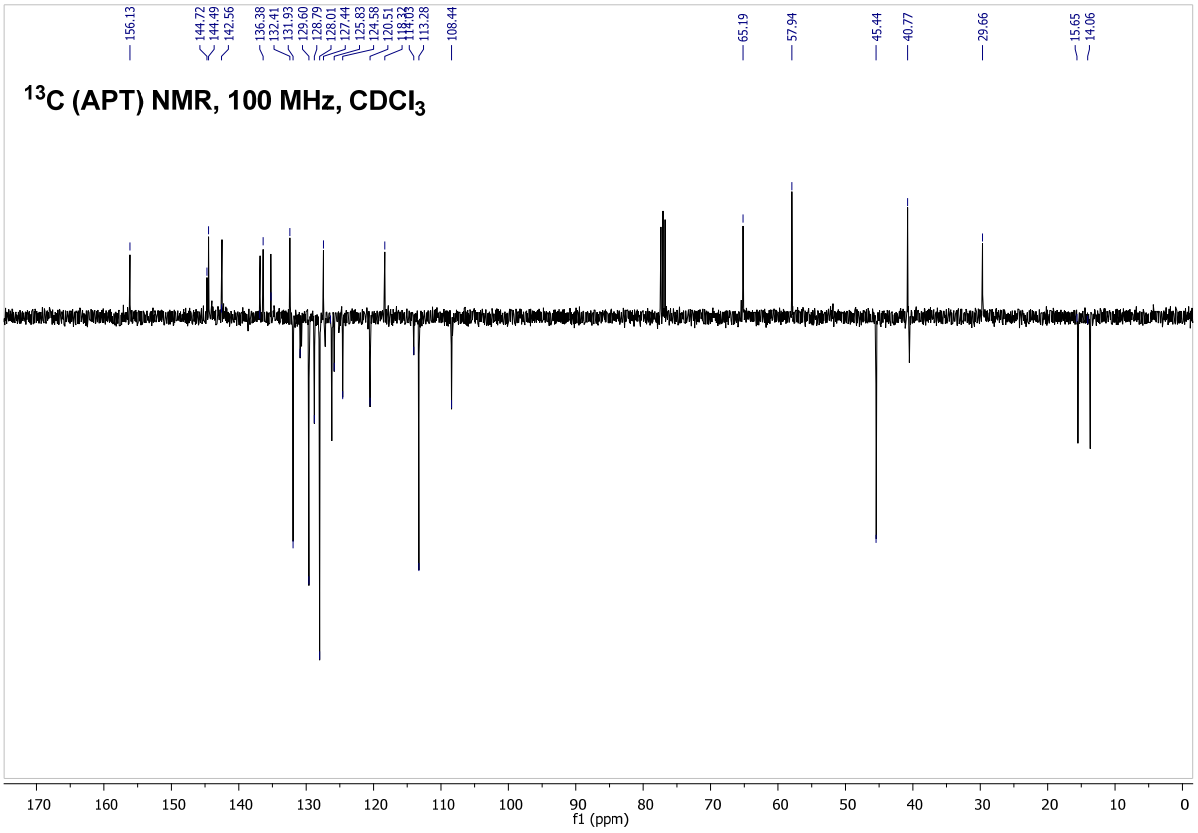
Me
EtN
Me
N
Et
O
Me$_2$N
Z,Z-35a
f1 (ppm)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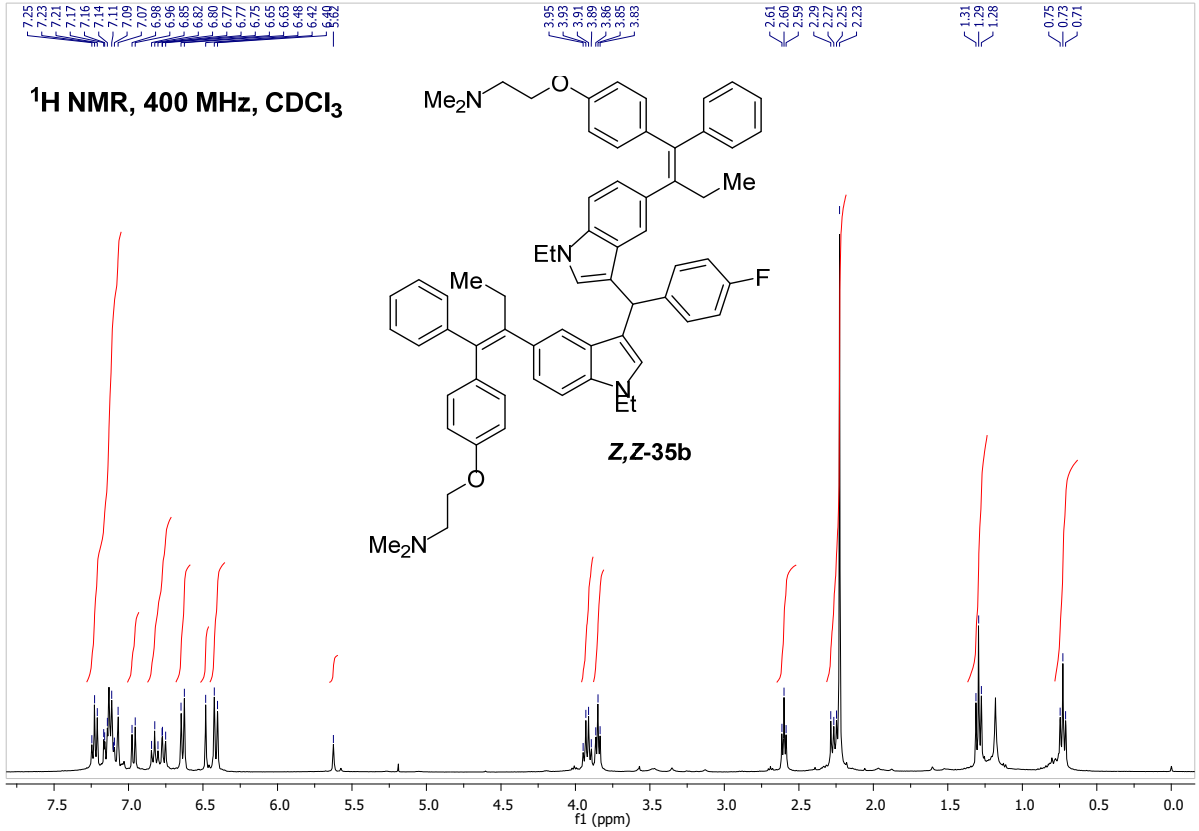
f1 (ppm)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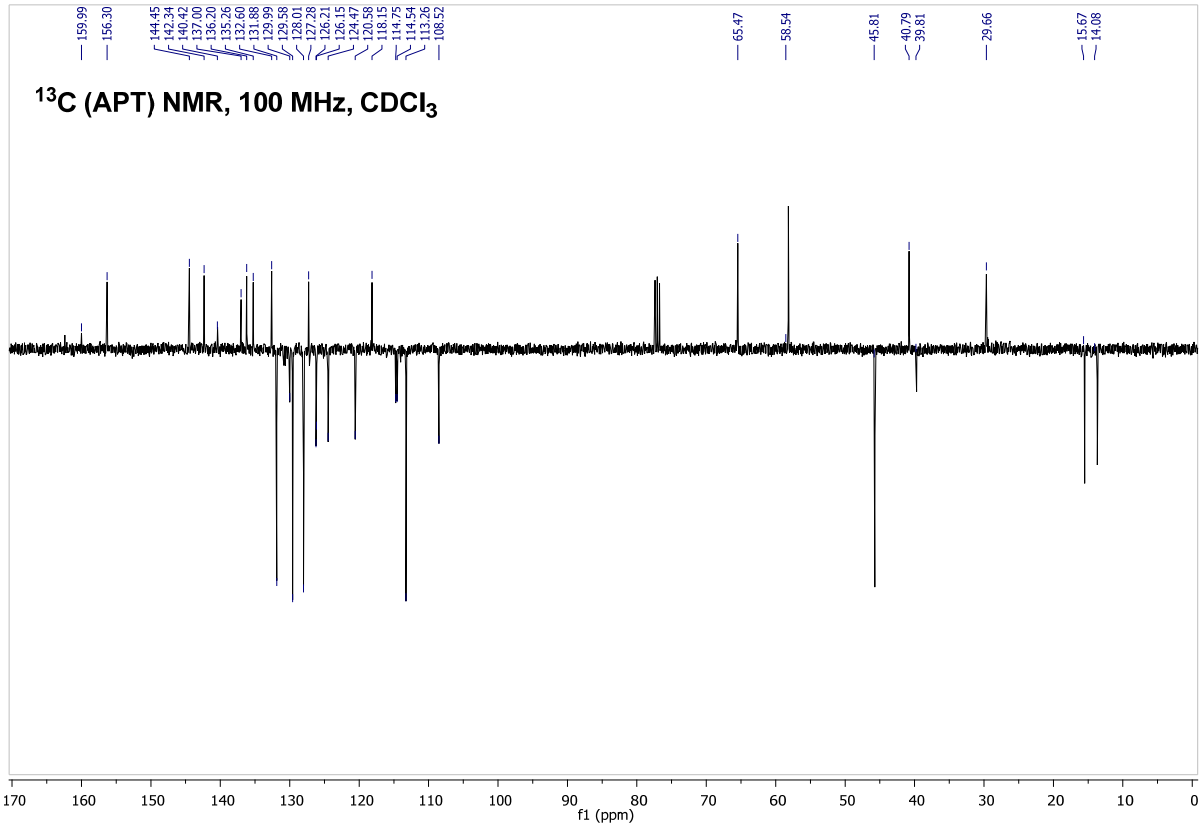
Me
EtN
F
Me
N
Et
O
Me$_2$N
Z,Z-35b
f1 (ppm)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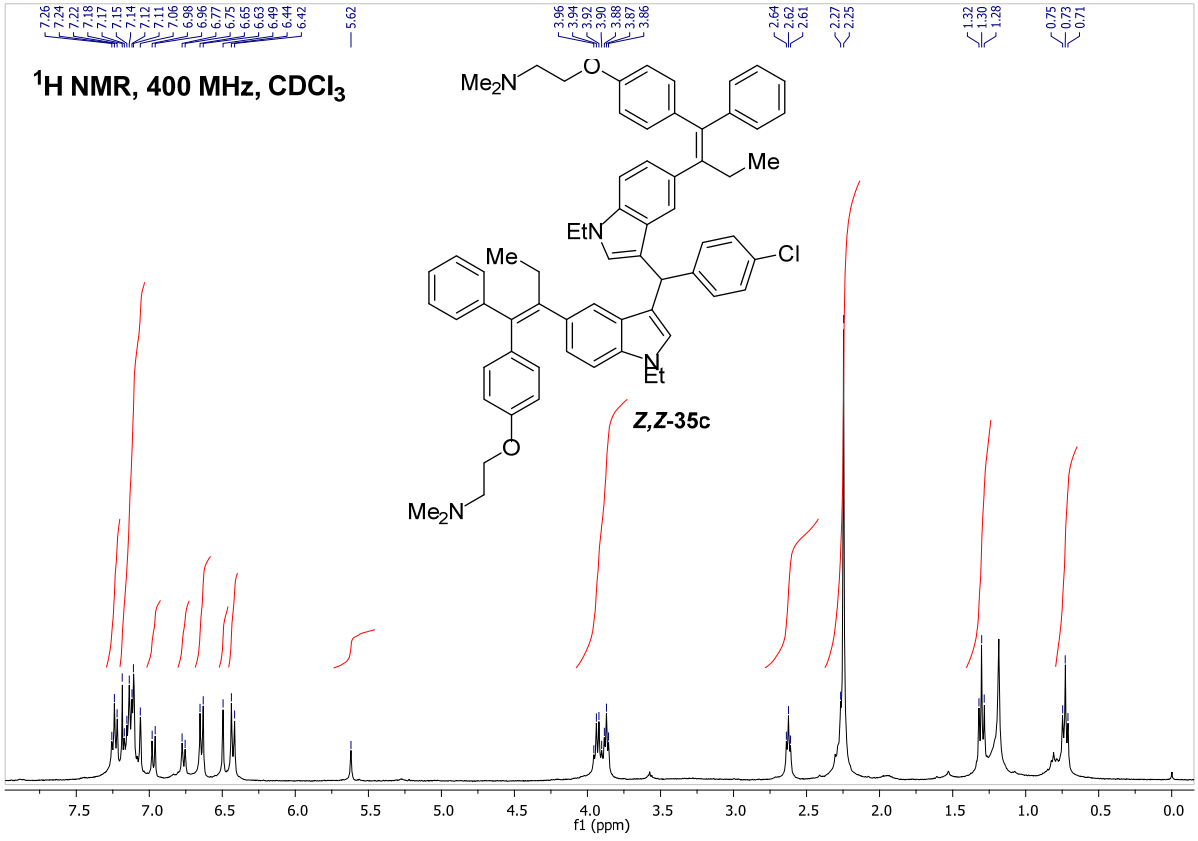
f1 (ppm)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EtN
Cl
Me
N
Et
O
Me$_2$N
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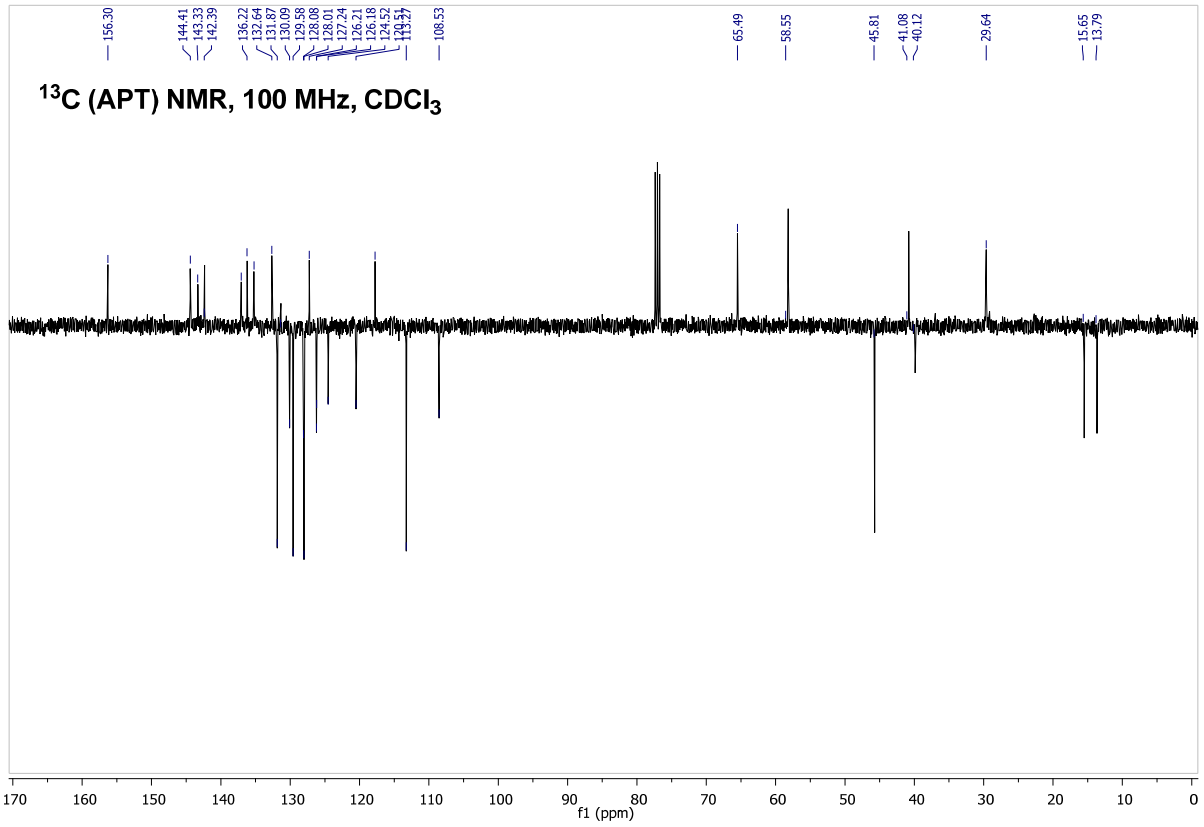
f1 (ppm)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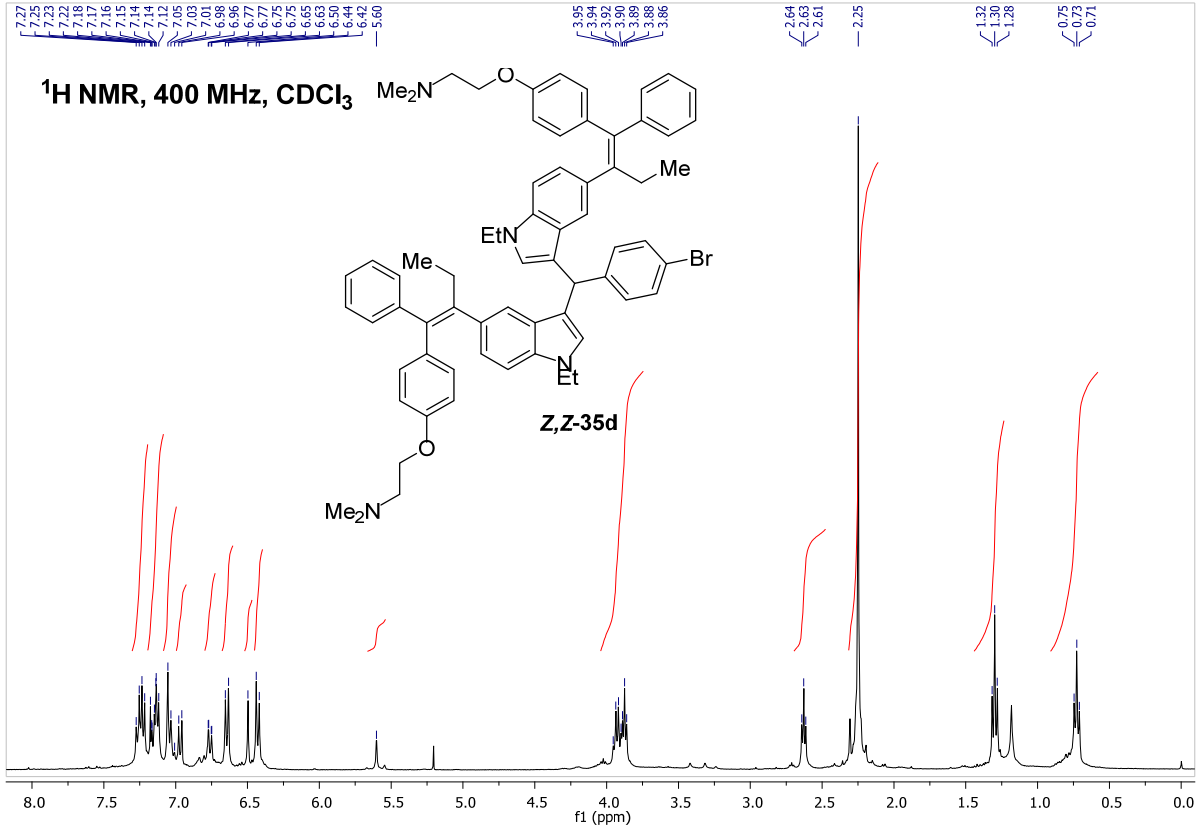
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3.88
3.86
2.64
2.63
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1.30
1.28
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0.73
0.71
1H NMR, 400 MHz, CDCl3
Me2N
O
Me
EtN
Br
Me
N
Et
O
Me2N
Z,Z-35d
8.0
7.5
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6.0
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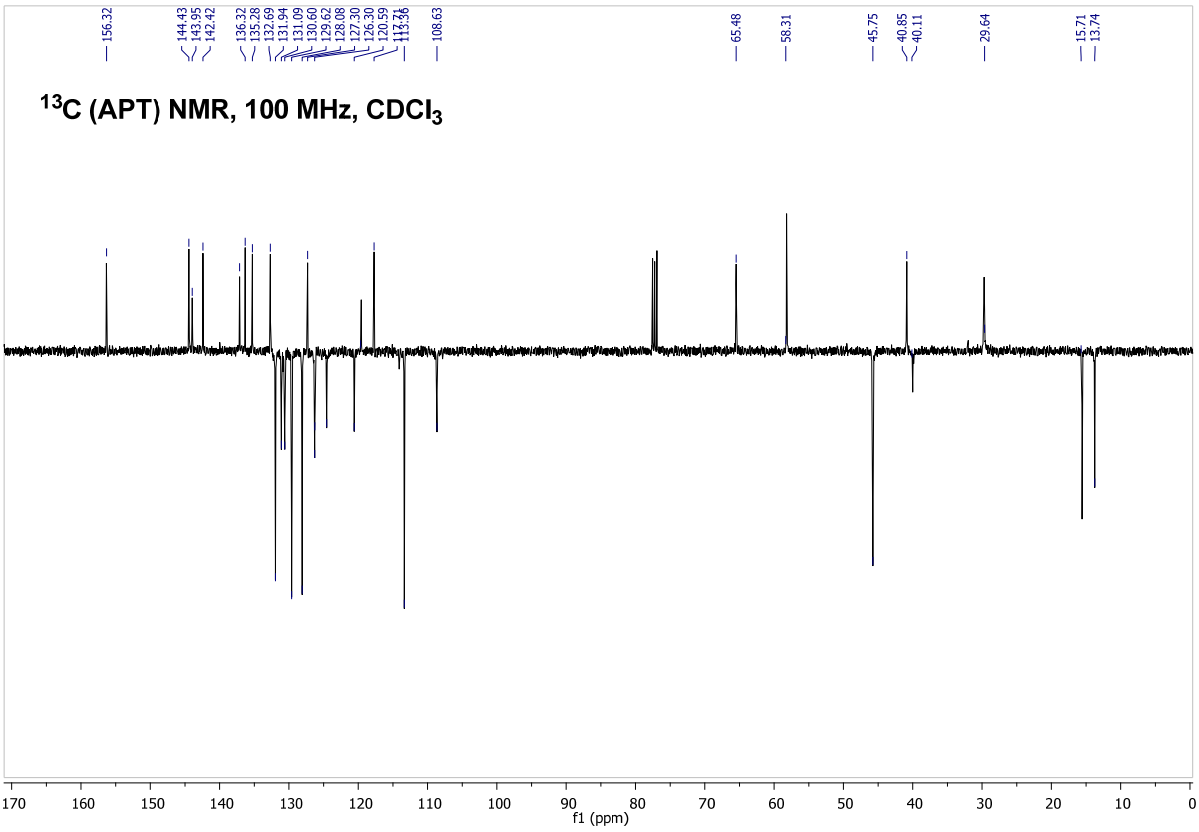
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65.48
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45.75
40.85
40.11
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15.71
13.74
13C (APT) NMR, 100 MHz, CDCl3
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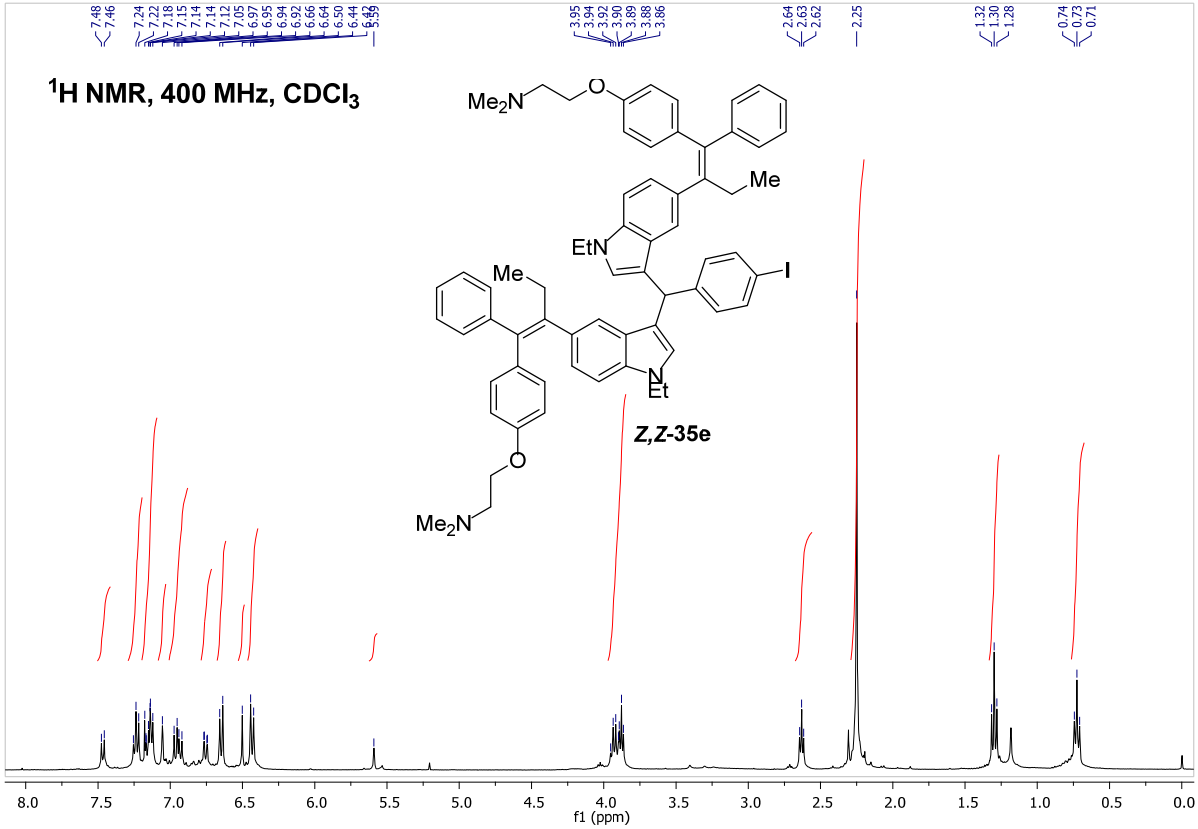
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Me2N
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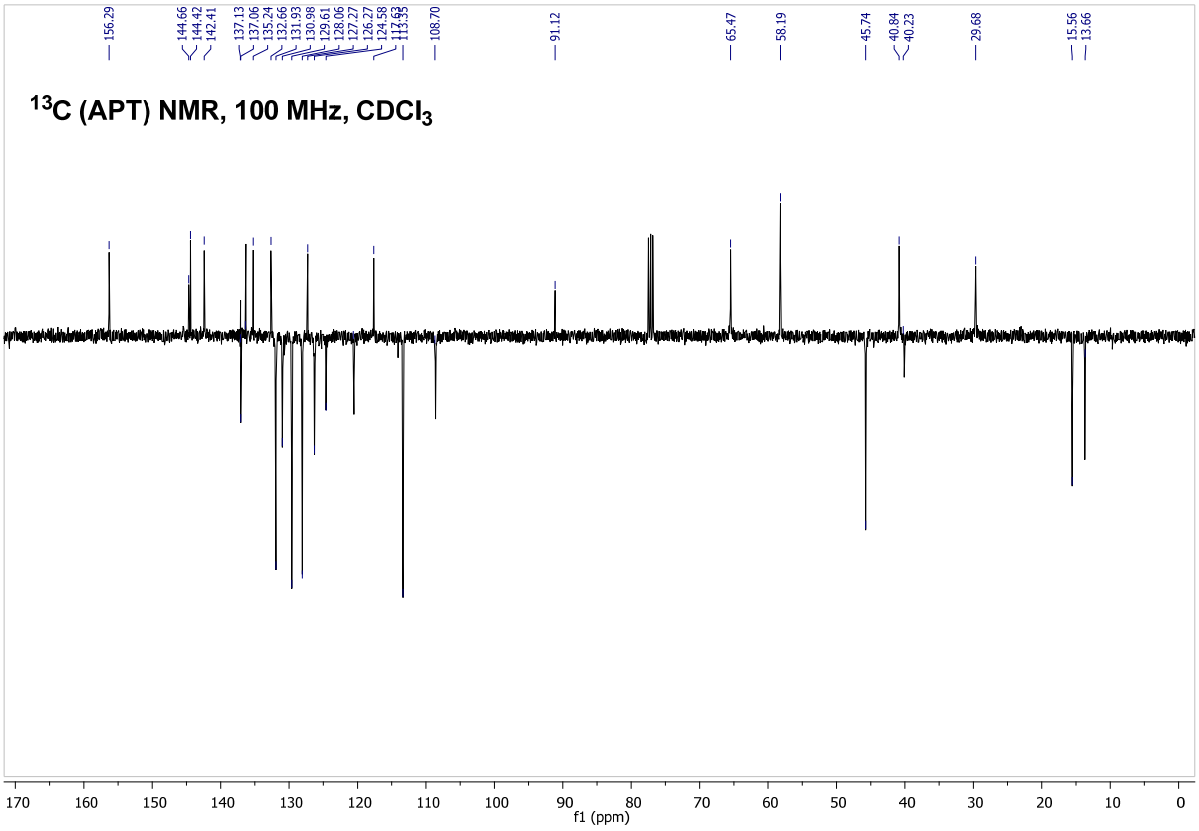
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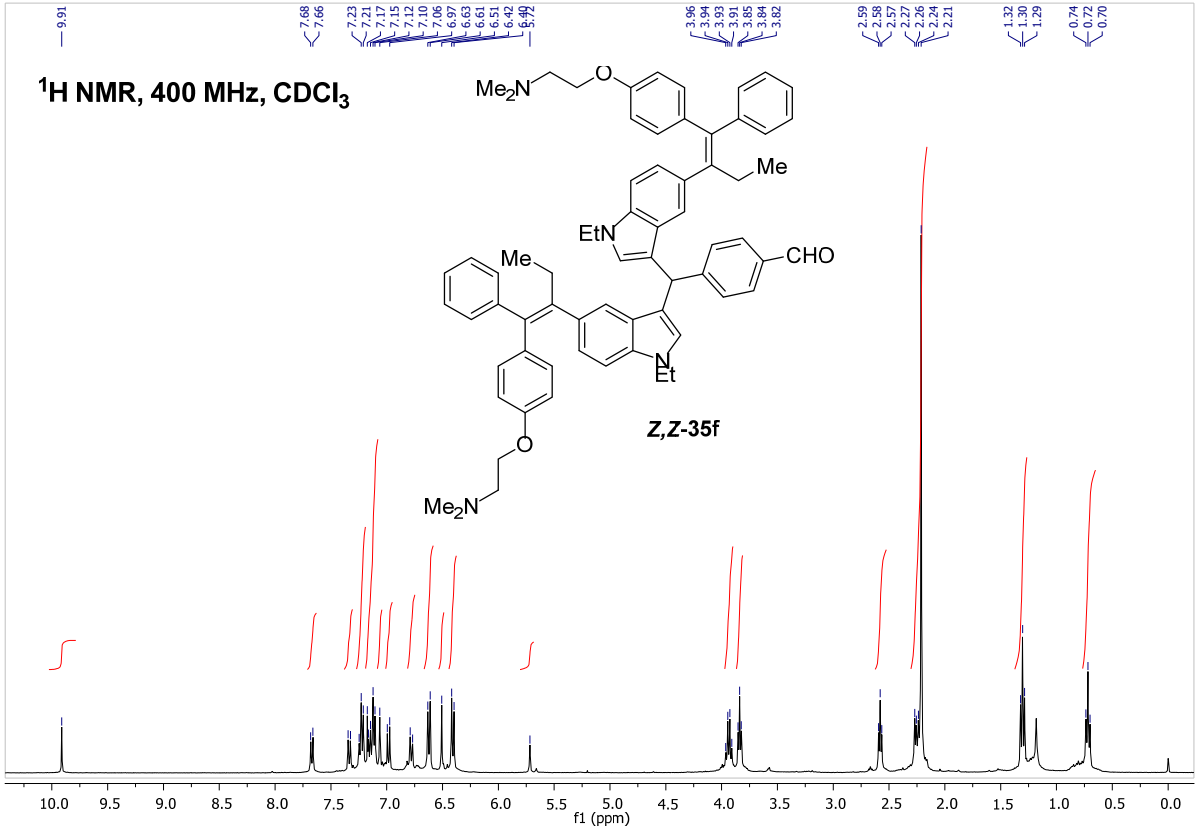
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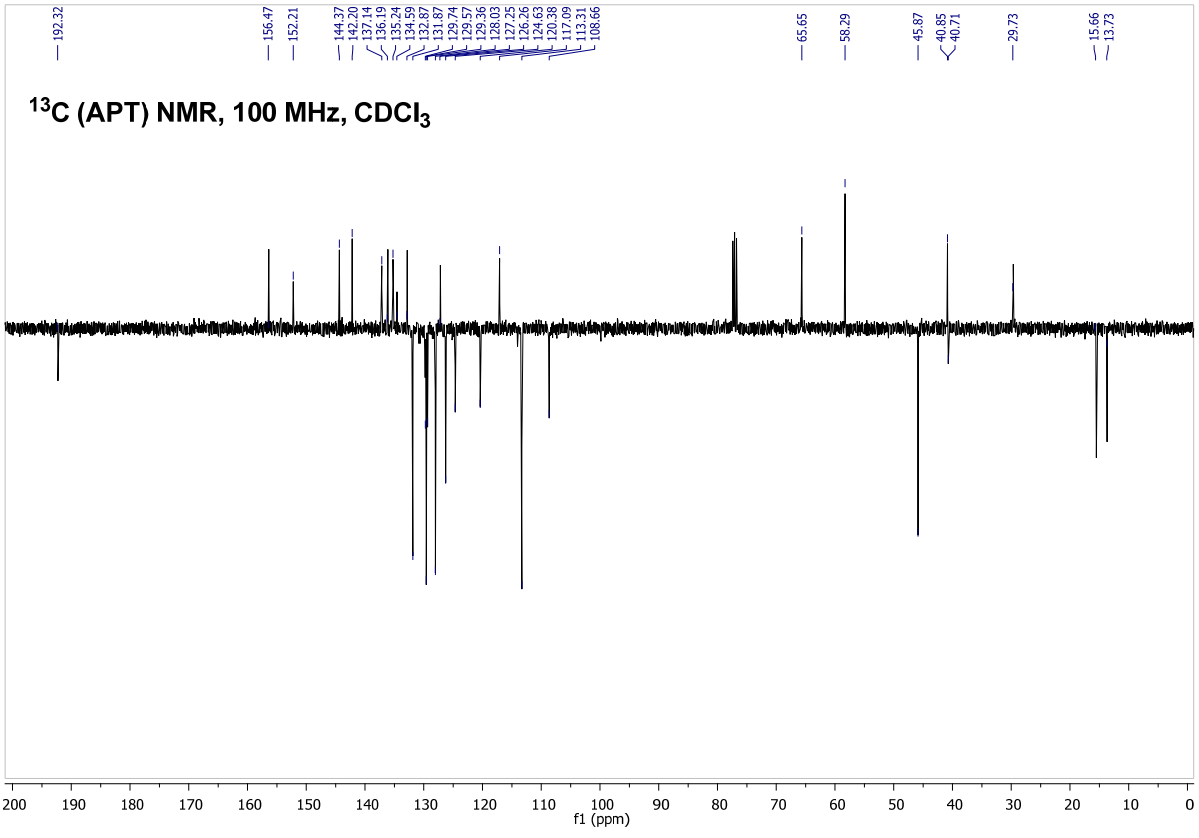
f1 (ppm)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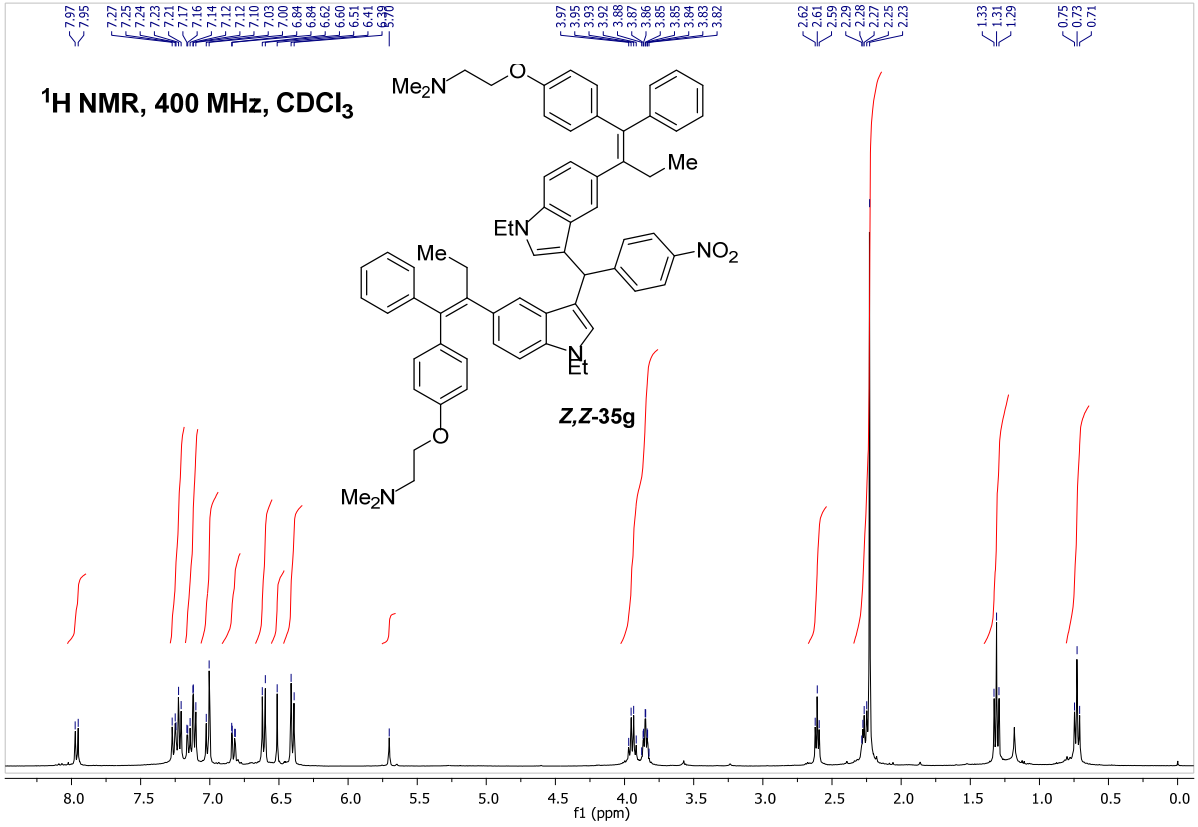
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1.31
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0.73
0.71
1H NMR, 400 MHz, CDCl3
Me2N
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Me
EtN
NO2
Me
N
Et
Z,Z-35g
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Me2N
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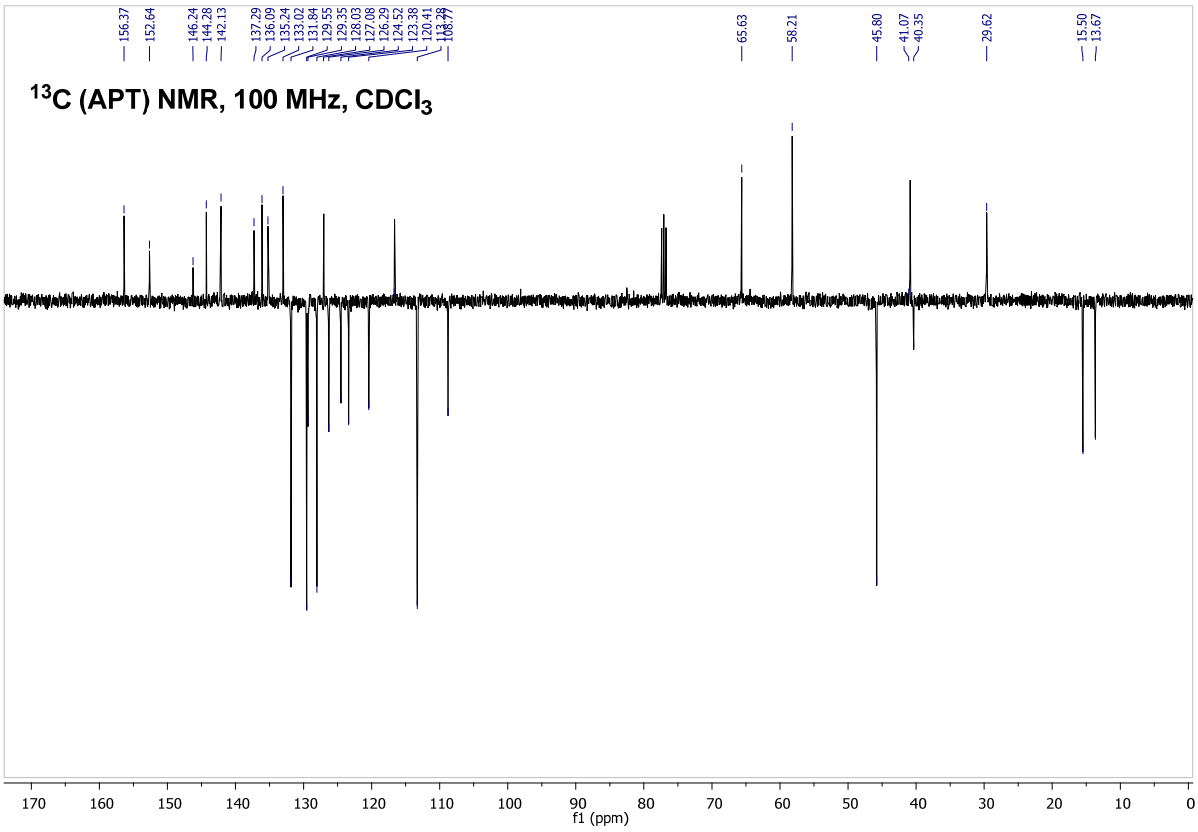
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13C (APT) NMR, 100 MHz, CDCl3
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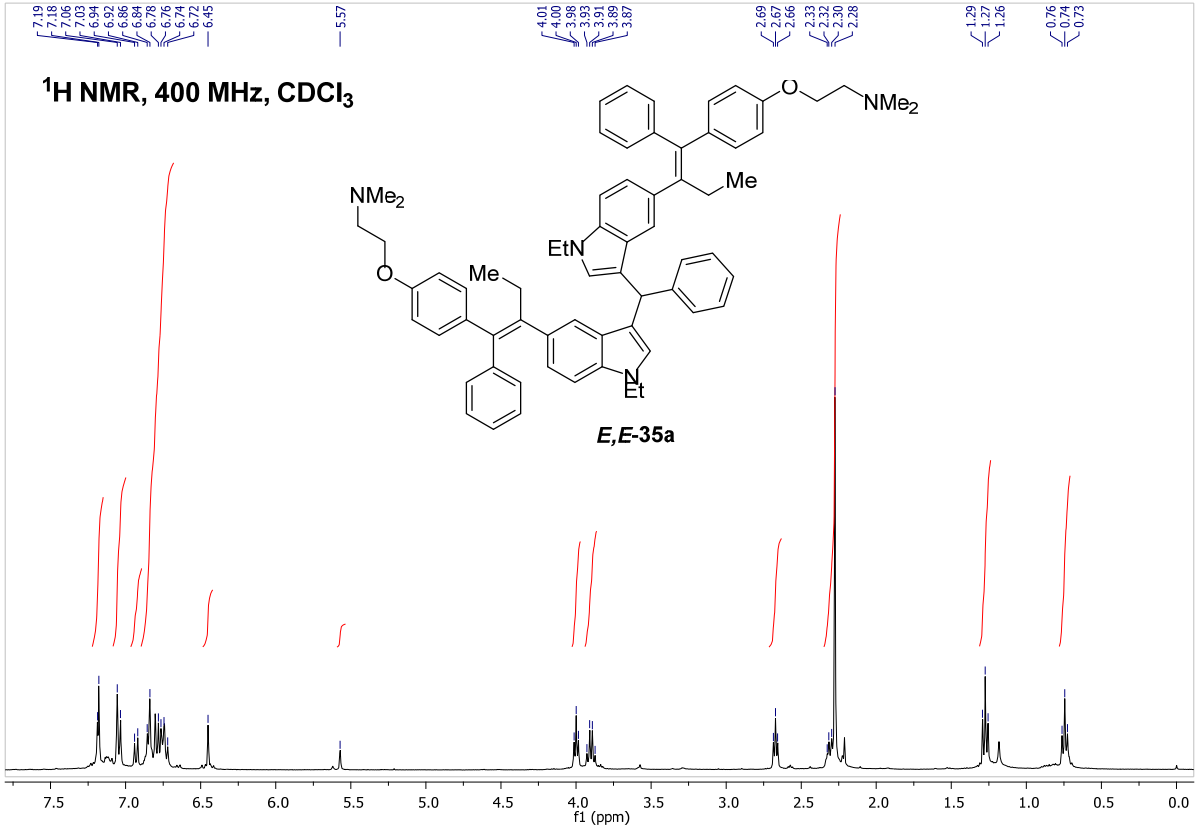
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3.87
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2.66
2.33
2.32
2.30
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1.27
1.26
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0.74
0.73
1H NMR, 400 MHz, CDCl3
NMe2
O
Me
EtN
Me
O
NMe2
N
Et
E,E-35a
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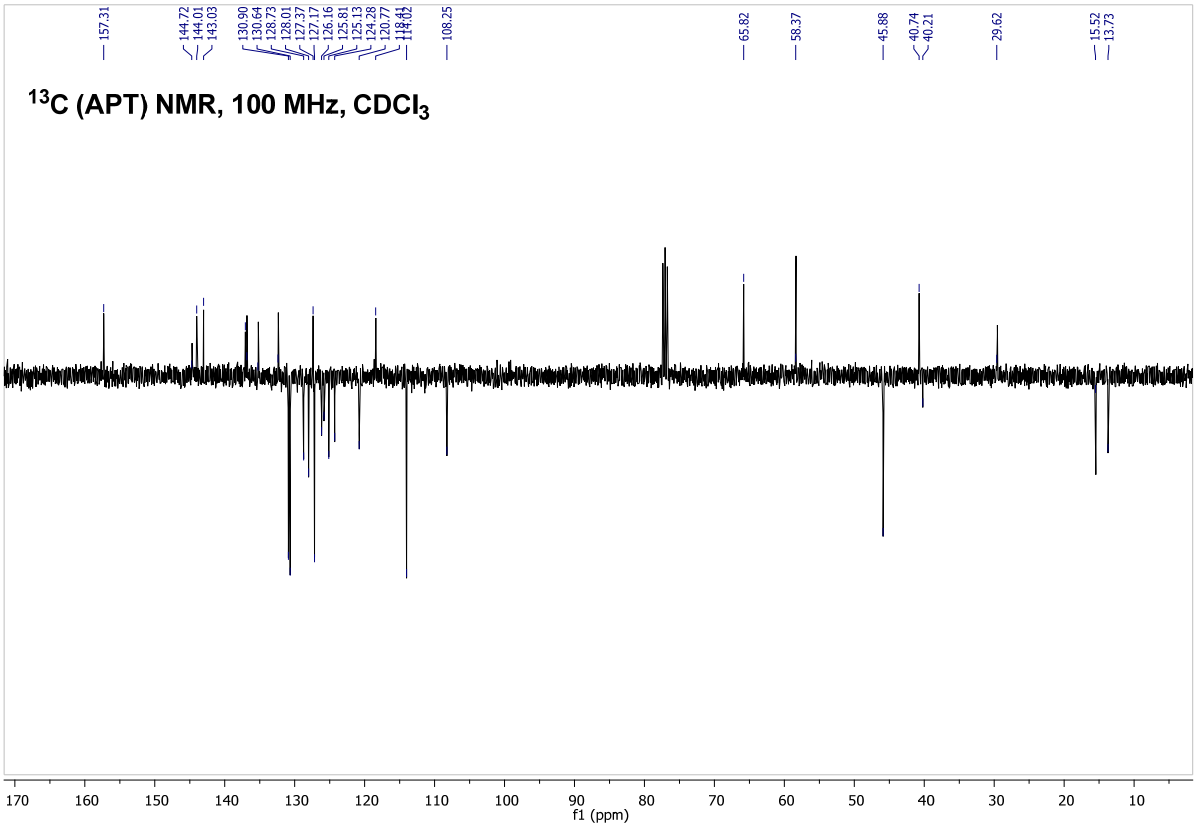
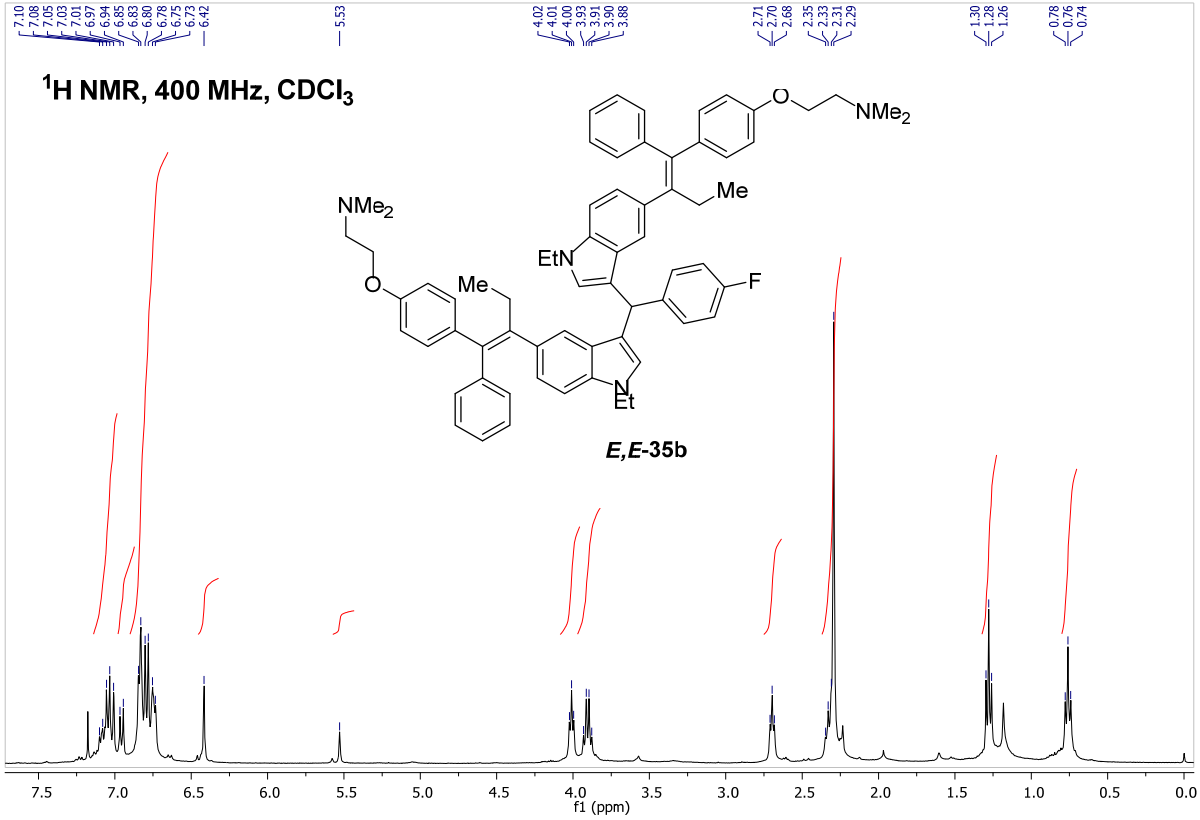
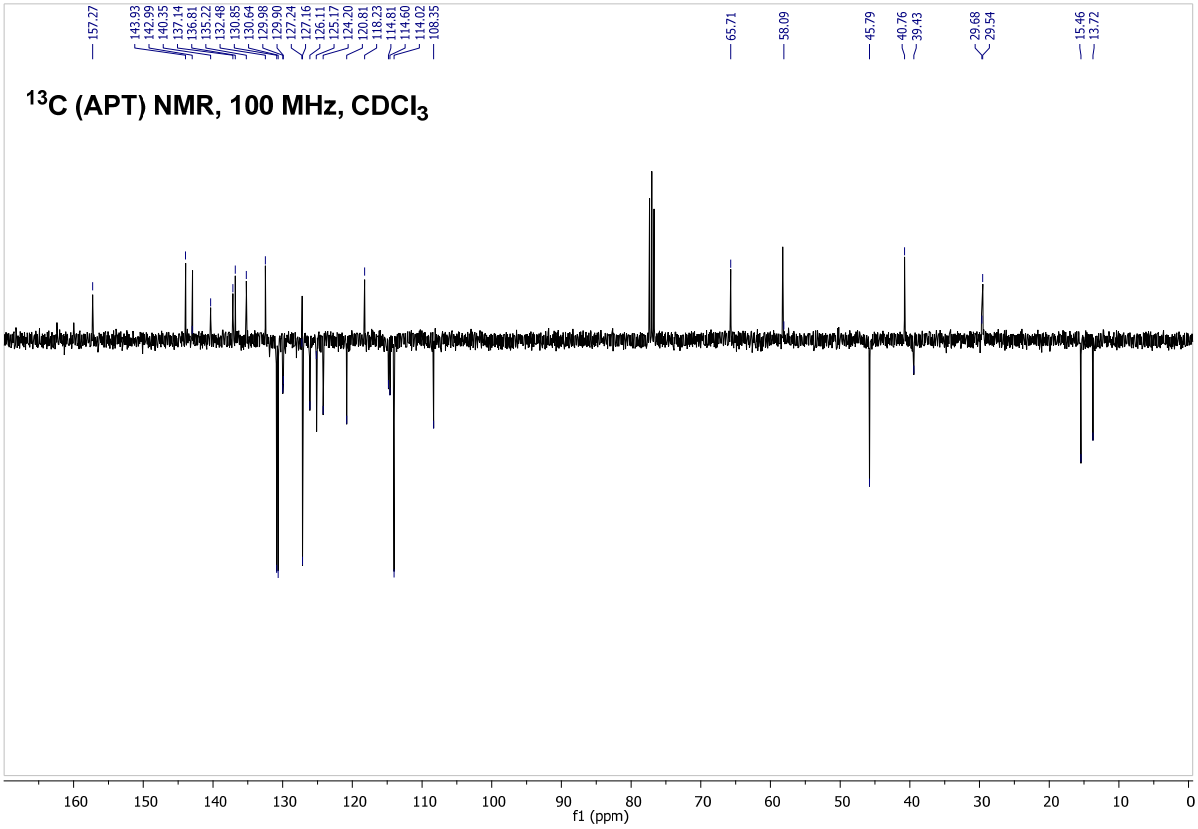
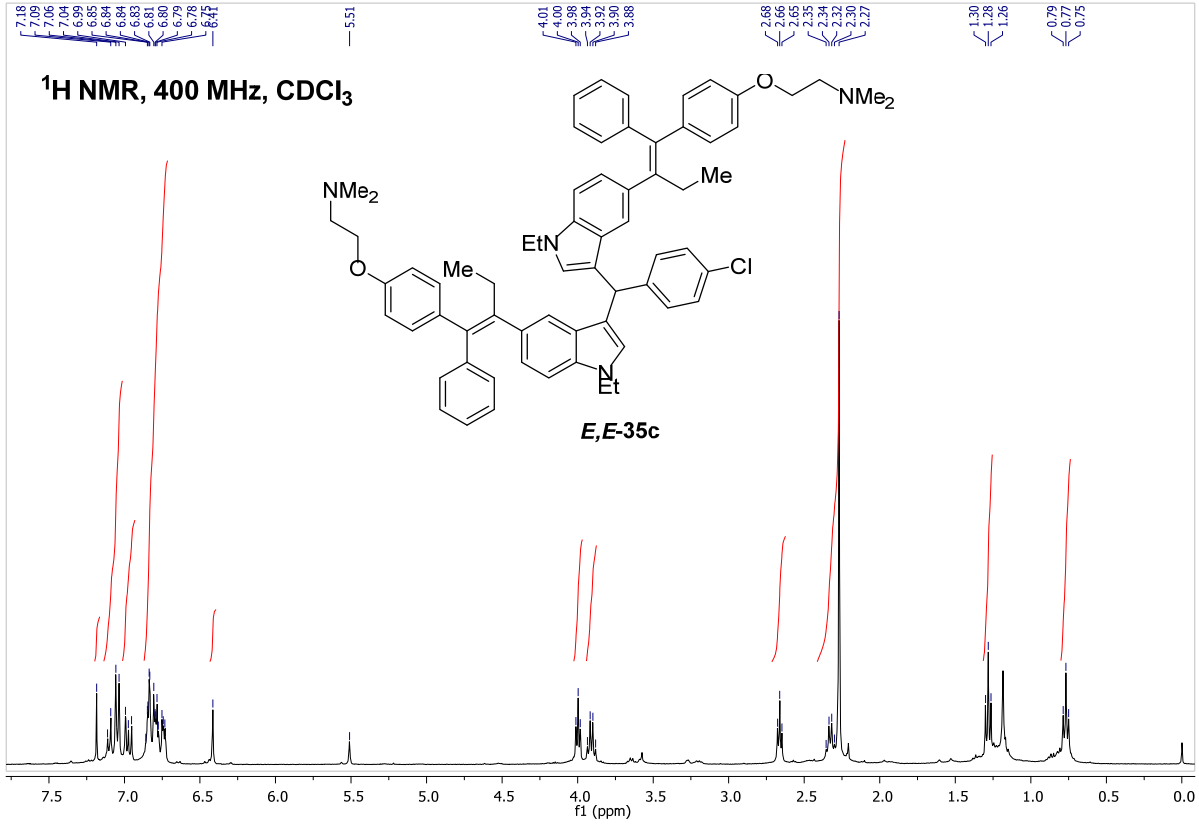
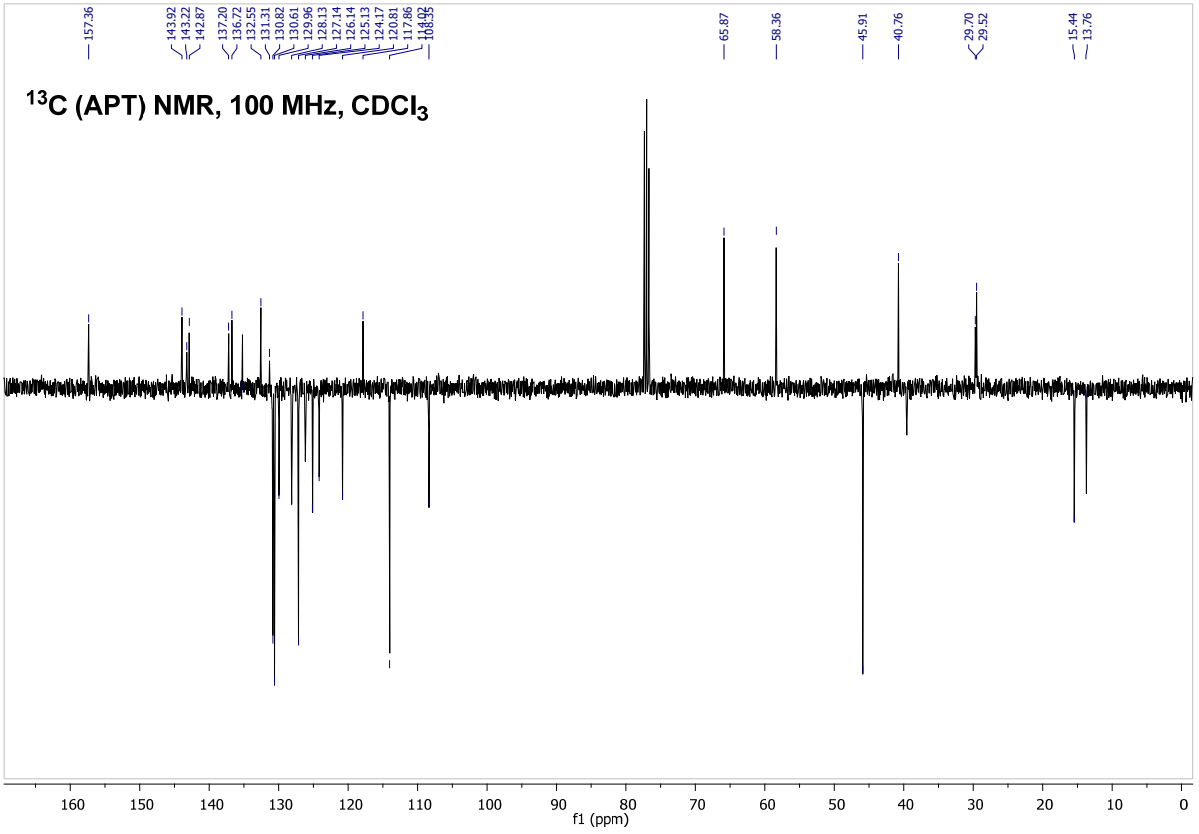
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

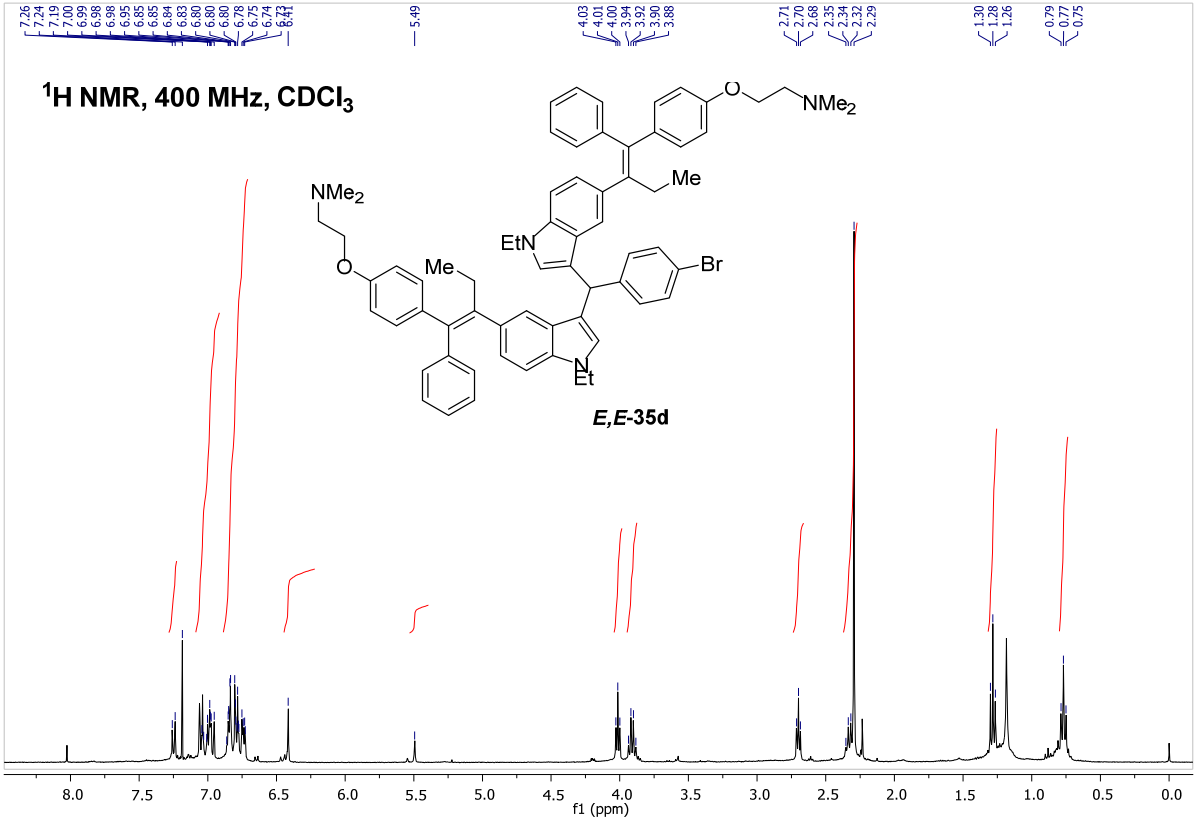

157.31
144.72
144.01
143.03
130.90
130.64
128.73
128.01
127.37
127.17
126.16
125.81
125.13
124.28
120.77
118.41
114.02
108.25
65.82
58.37
45.88
40.74
40.21
29.62
15.52
13.73
13C (APT) NMR, 100 MHz, CDCl3
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

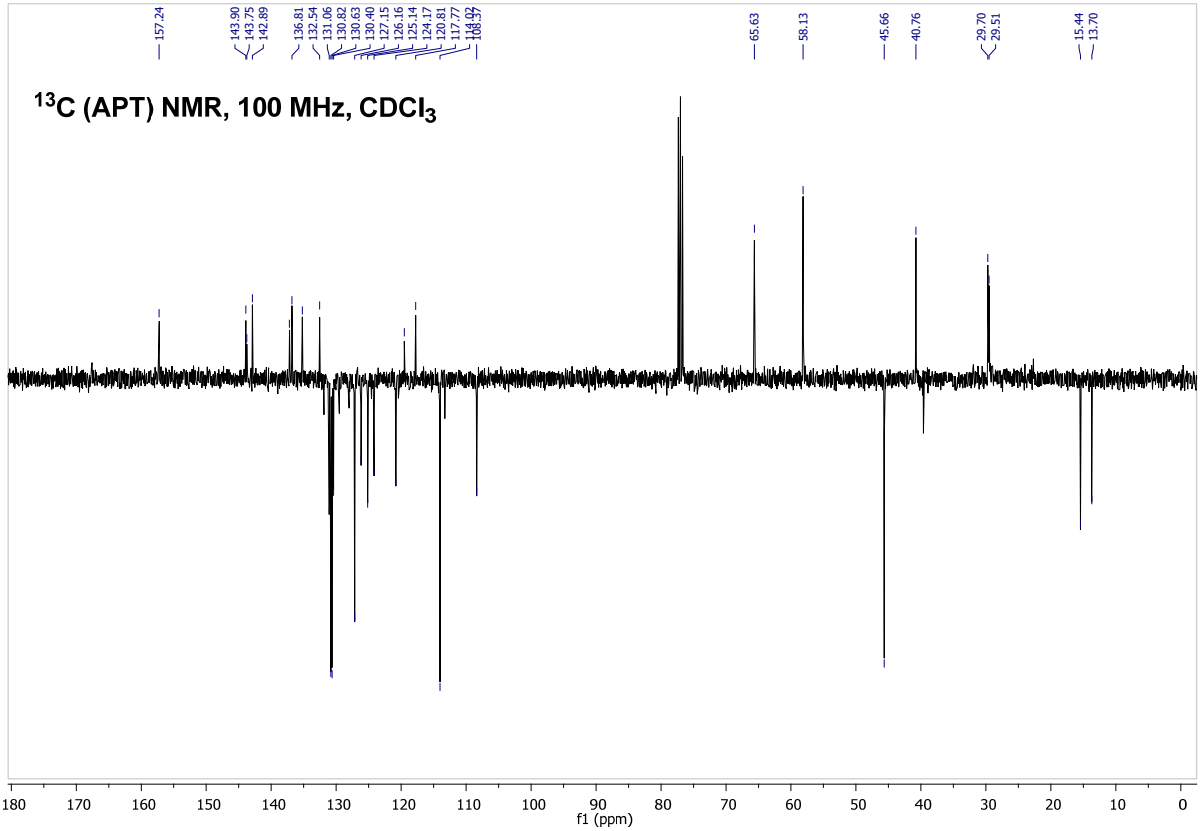

7.10
7.08
7.05
7.03
7.01
6.97
6.94
6.85
6.83
6.80
6.78
6.75
6.73
6.42
5.53
4.02
4.01
4.00
3.93
3.91
3.90
3.88
2.71
2.70
2.68
2.35
2.33
2.31
2.29
1.30
1.28
1.26
0.78
0.76
0.74
^{1}H NMR, 400 MHz, CDCl$_3$
NMe$_2$
O
Me
EtN
F
N
Et
NMe$_2$
O
Me
E,E-35b
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

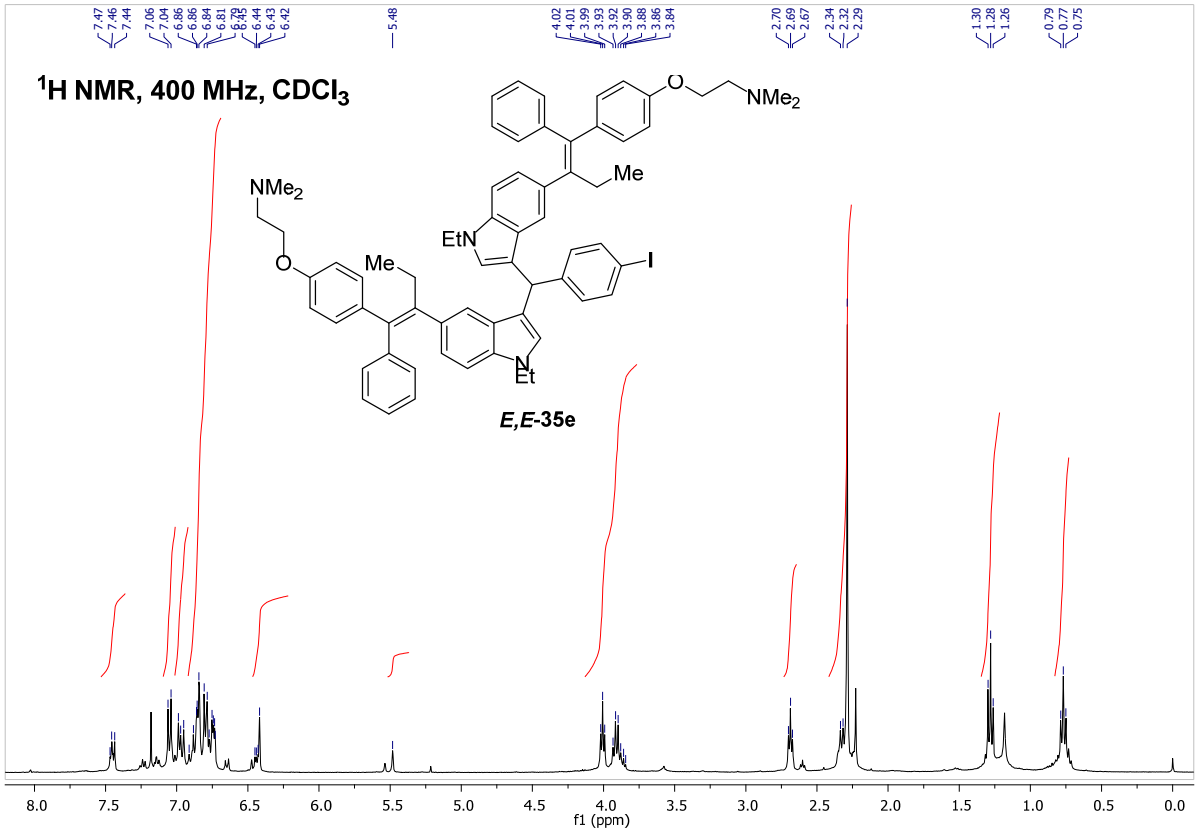

157.27
143.93
142.99
140.35
137.14
136.81
135.22
132.48
130.85
130.64
129.98
129.90
127.24
127.16
126.11
125.17
124.20
120.81
118.23
114.81
114.60
114.02
108.35
65.71
58.09
45.79
40.76
39.43
29.68
29.54
15.46
13.72
^{13}C (APT) NMR, 100 MHz, CDCl$_3$
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

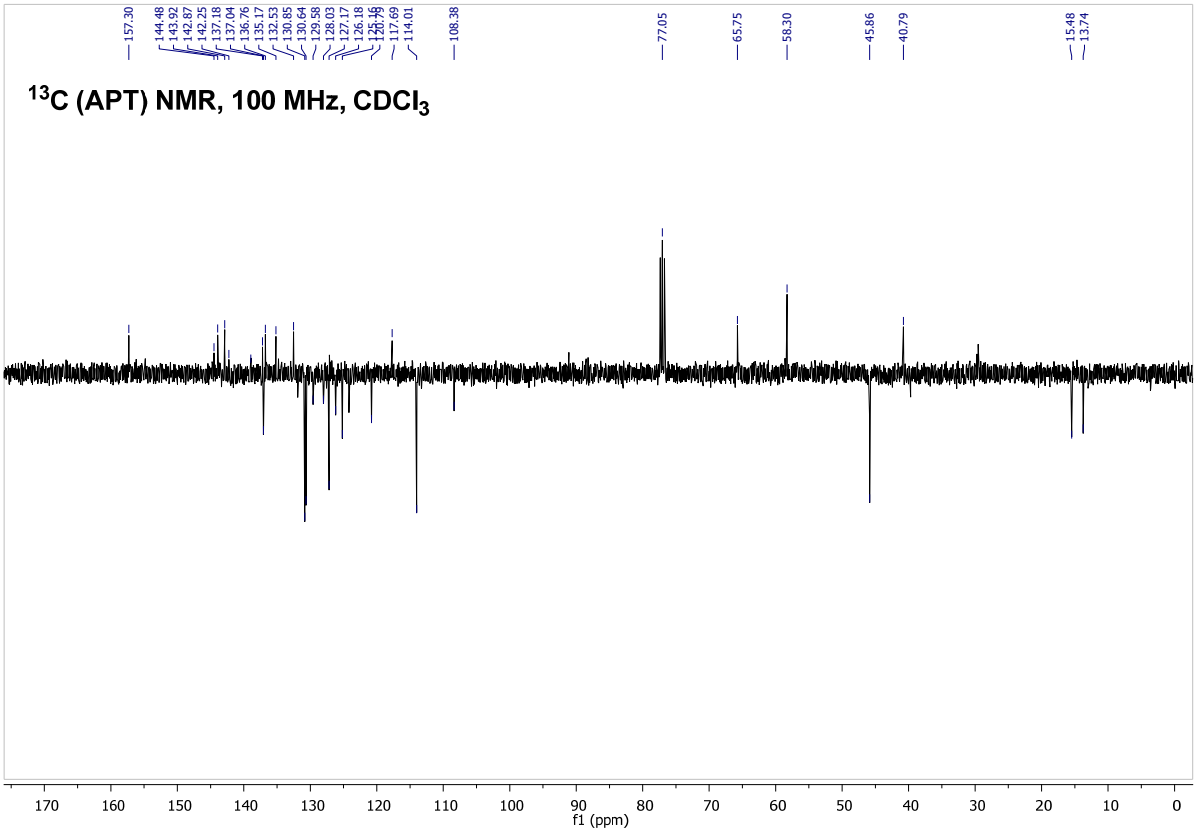

^{1}H NMR, 400 MHz, $CDCl_3$
NMe2
O
Me
EtN
Cl
Et
E,E-35c
f1 (ppm)

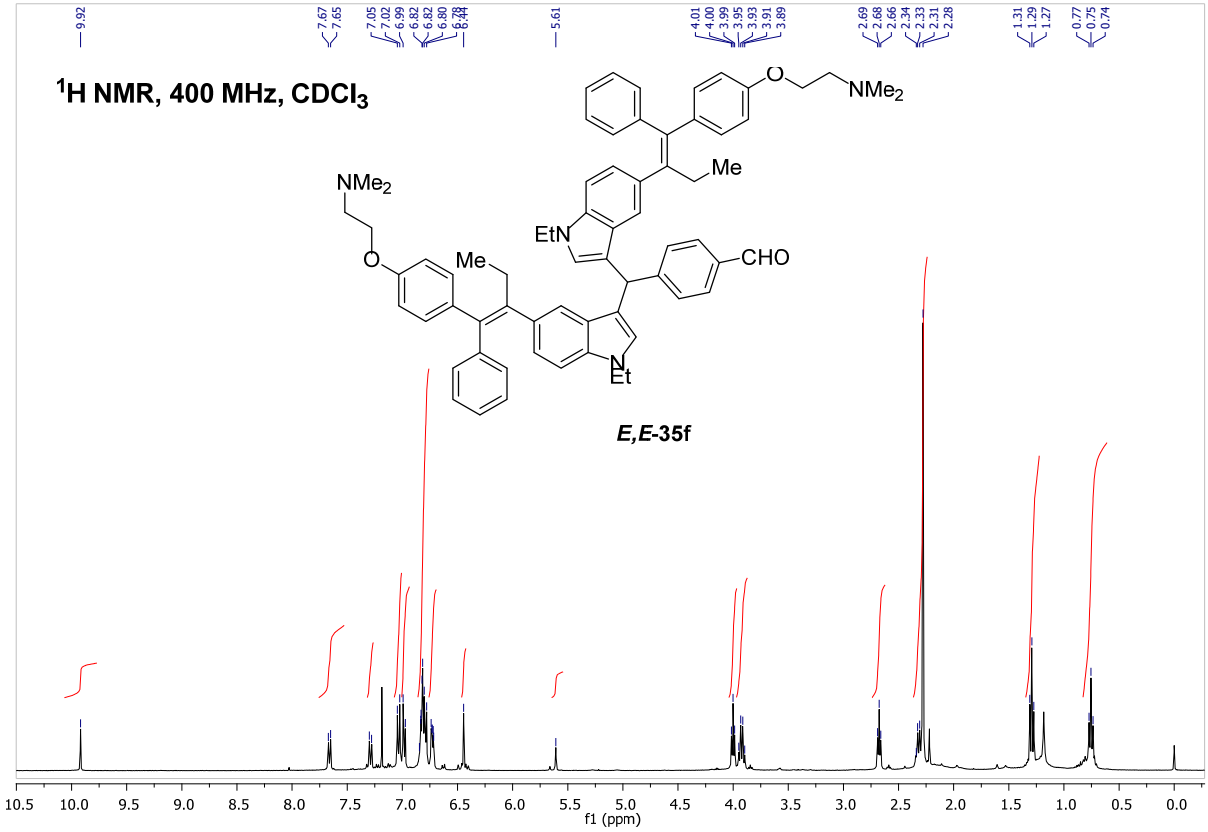

^{13}C (APT) NMR, 100 MHz, $CDCl_3$
f1 (ppm)

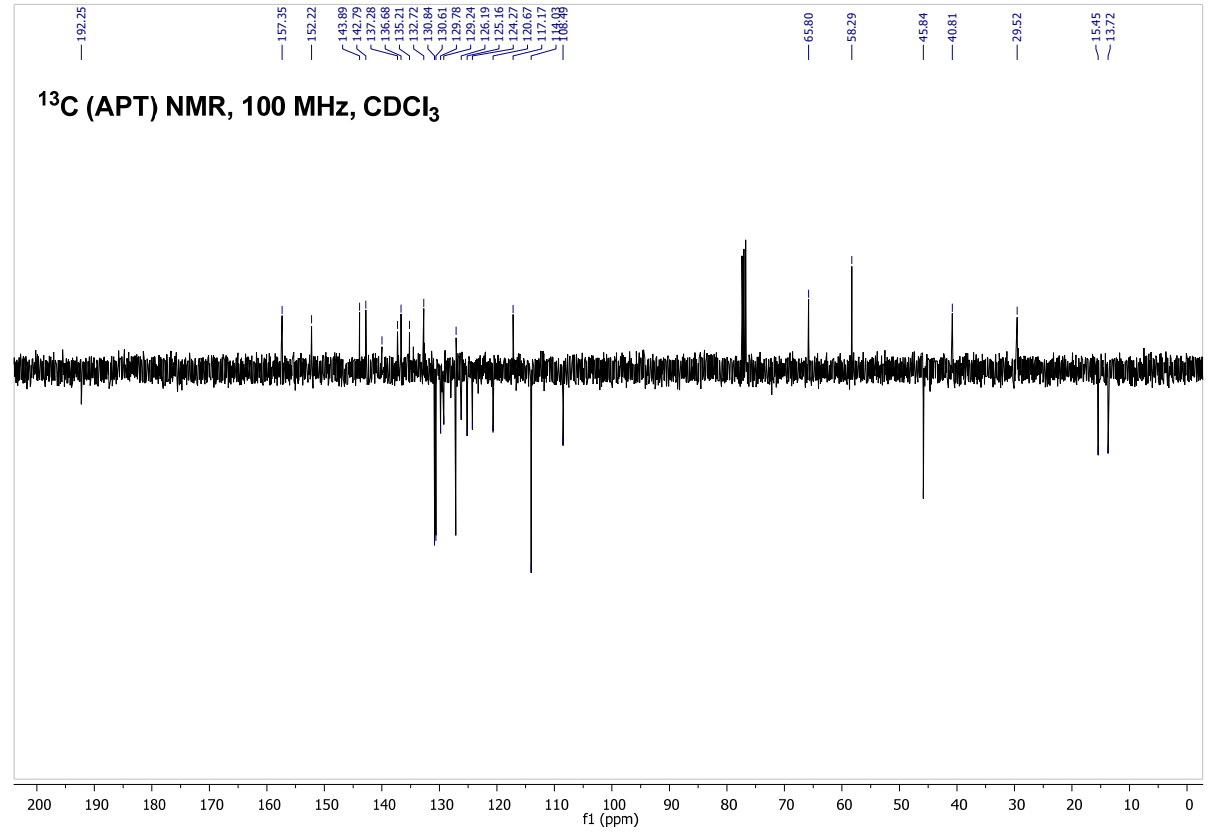

^{1}H NMR, 400 MHz, $CDCl_3$
NMe2
O
Me
EtN
Br
N
Et
E,E-35d
f1 (ppm)

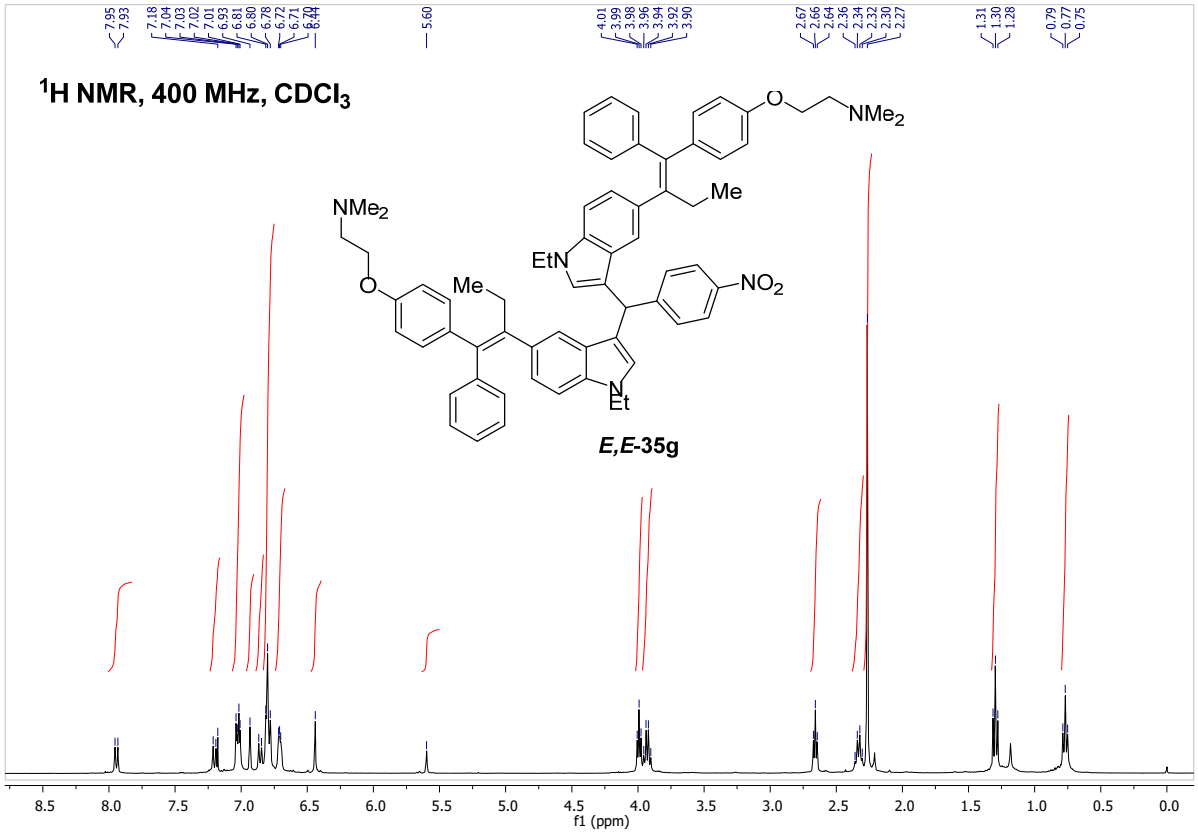

^{13}C (APT) NMR, 100 MHz, $CDCl_3$
f1 (ppm)

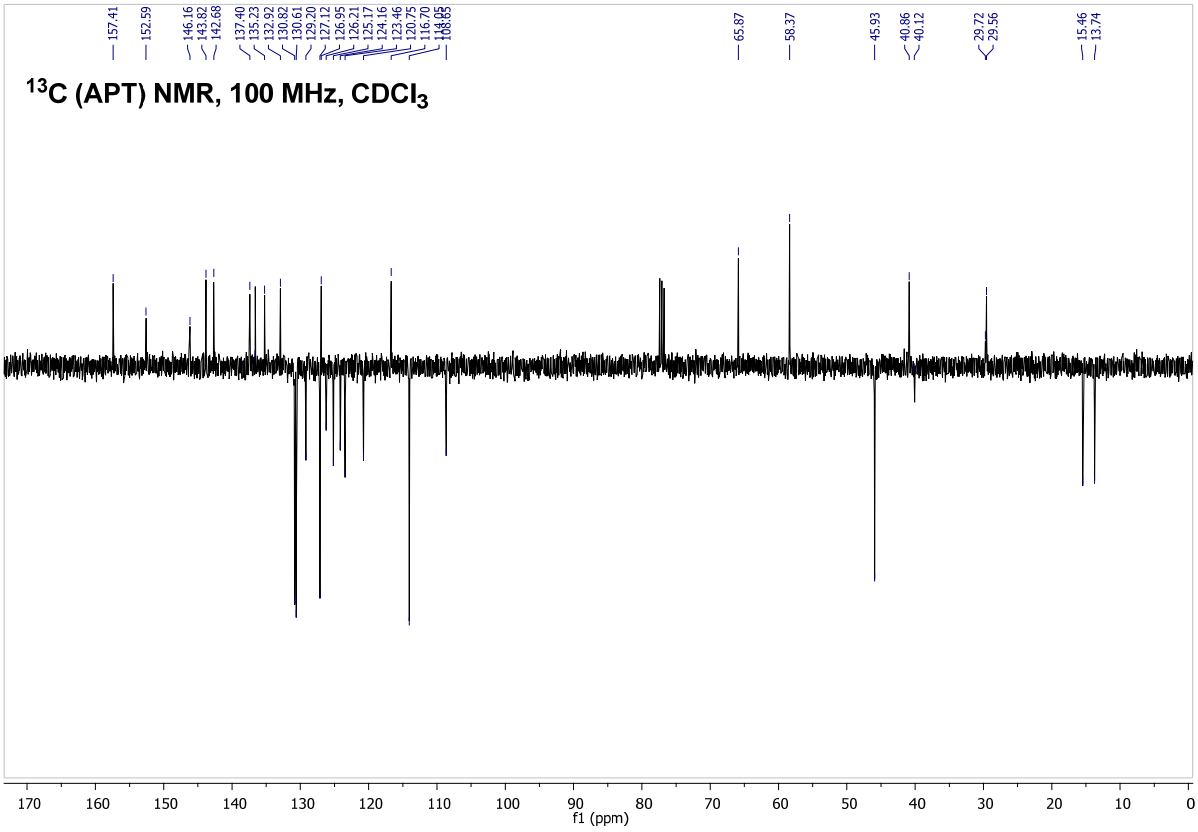

7.47
7.46
7.44
7.06
7.04
6.86
6.86
6.84
6.81
6.79
6.45
6.44
6.43
6.42
5.48
4.02
4.01
3.99
3.93
3.92
3.90
3.88
3.86
3.84
2.70
2.69
2.67
2.34
2.32
2.29
1.30
1.28
1.26
0.79
0.77
0.75
1H NMR, 400 MHz, CDCl3
NMe2
O
Me
EtN
I
Me
O
NMe2
N
Et
E,E-35e
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


157.30
144.48
143.92
142.87
142.25
137.18
137.04
136.76
135.17
132.53
130.85
130.64
129.58
128.03
127.17
126.18
117.69
114.01
108.38
77.05
65.75
58.30
45.86
40.79
15.48
13.74
13C (APT) NMR, 100 MHz, CDCl3
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


9.92
7.67
7.65
7.05
7.02
6.99
6.82
6.82
6.80
6.78
6.44
5.61
4.01
4.00
3.99
3.95
3.93
3.91
3.89
2.69
2.68
2.66
2.34
2.33
2.31
2.28
1.31
1.29
1.27
0.77
0.75
0.74
1H NMR, 400 MHz, CDCl3
NMe2
O
Me
EtN
CHO
Me
O
NMe2
N
Et
E,E-35f
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

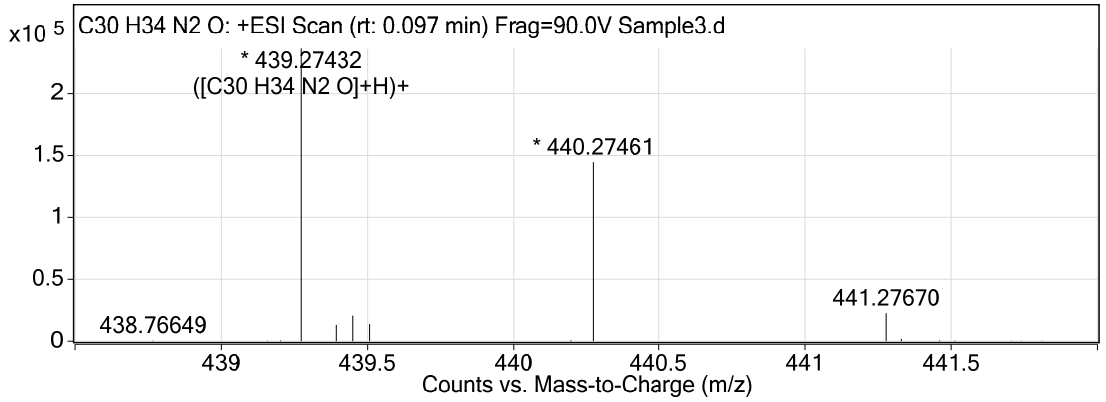

192.25
157.35
152.22
143.89
142.79
137.28
136.68
135.21
132.72
130.84
130.61
129.78
129.24
126.19
125.16
124.27
120.67
117.17
65.80
58.29
45.84
40.81
29.52
15.45
13.72
13C (APT) NMR, 100 MHz, CDCl3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.95
7.93
7.18
7.04
7.03
7.02
7.01
6.93
6.81
6.80
6.78
6.72
6.71
5.60
4.01
3.99
3.98
3.96
3.94
3.92
3.90
2.67
2.66
2.64
2.36
2.34
2.32
2.30
2.27
1.31
1.30
1.28
0.79
0.77
0.75
1H NMR, 400 MHz, CDCl3
NMe2
O
Me
EtN
NO2
N
Et
E,E-35g
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

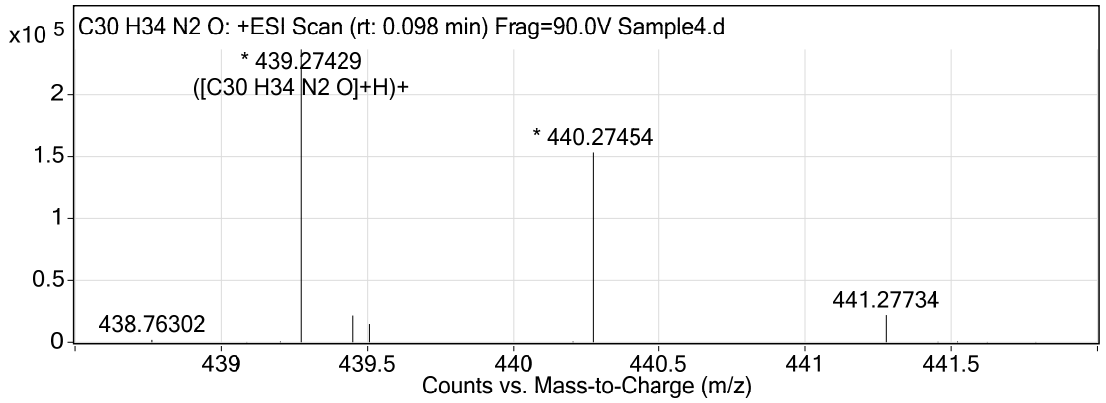

157.41
152.59
146.16
143.82
142.68
137.40
135.23
132.92
130.82
130.61
129.20
127.12
126.95
126.21
125.17
124.16
123.46
120.75
116.70
65.87
58.37
45.93
40.86
40.12
29.72
29.56
15.46
13.74
13C (APT) NMR, 100 MHz, CDCl3
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

HRMS spectra of compounds

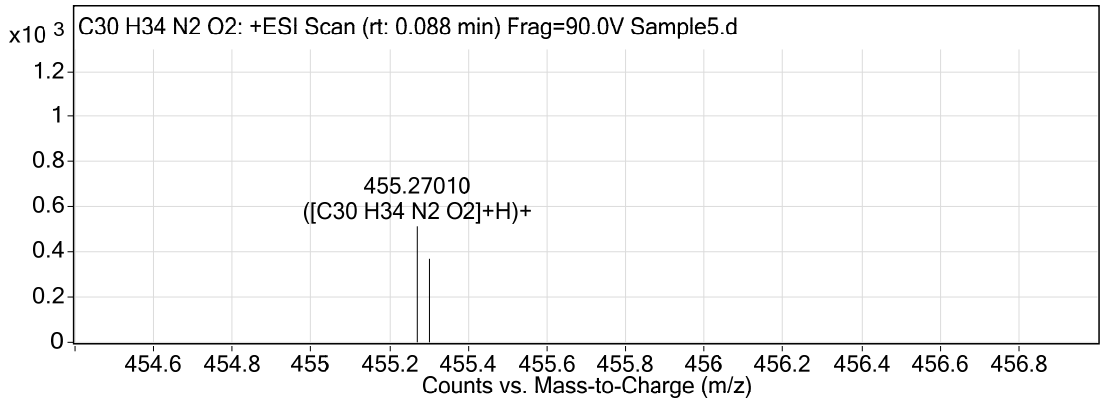
HRMS spectrum of ***E*-26**



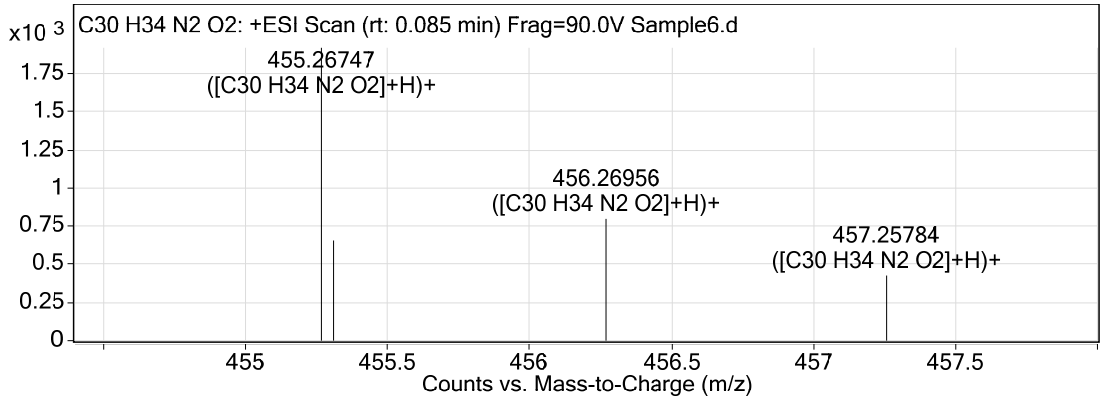
HRMS spectrum of ***Z*-26**



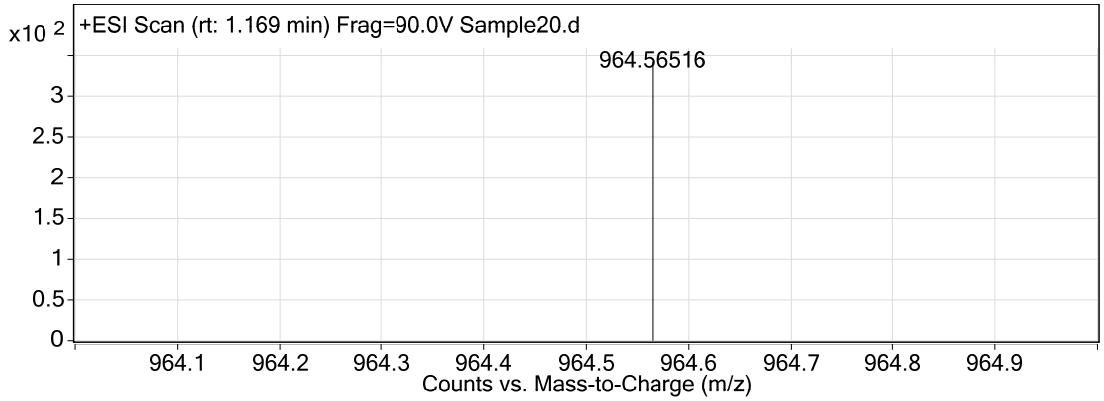
HRMS spectrum of ***E*-33**



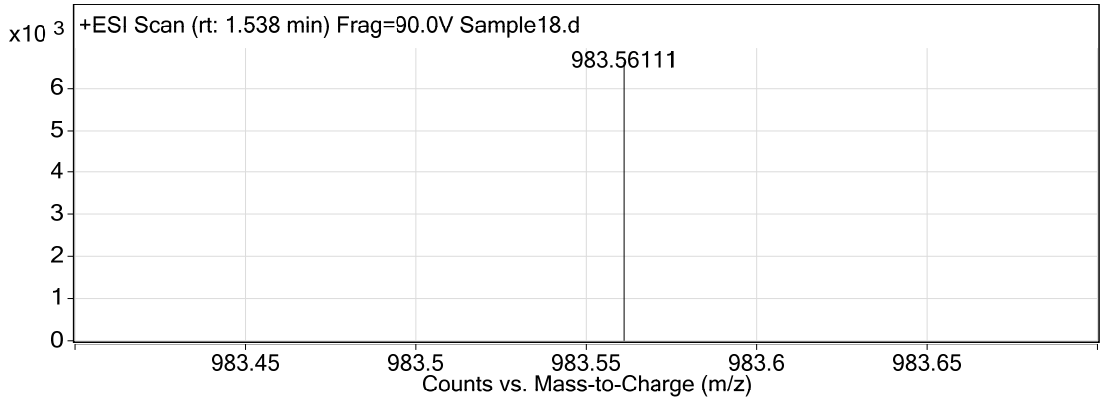
HRMS spectrum of ***Z*-33**



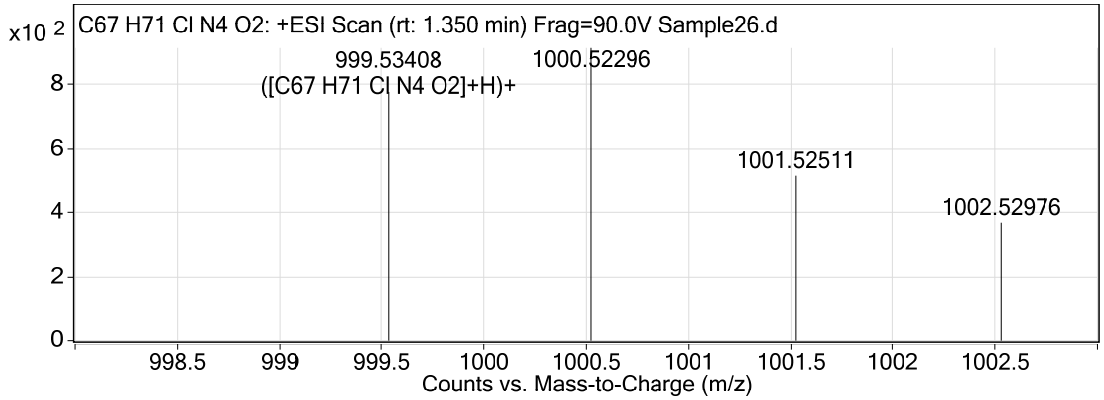
HRMS spectrum of ***Z,Z*-35a**



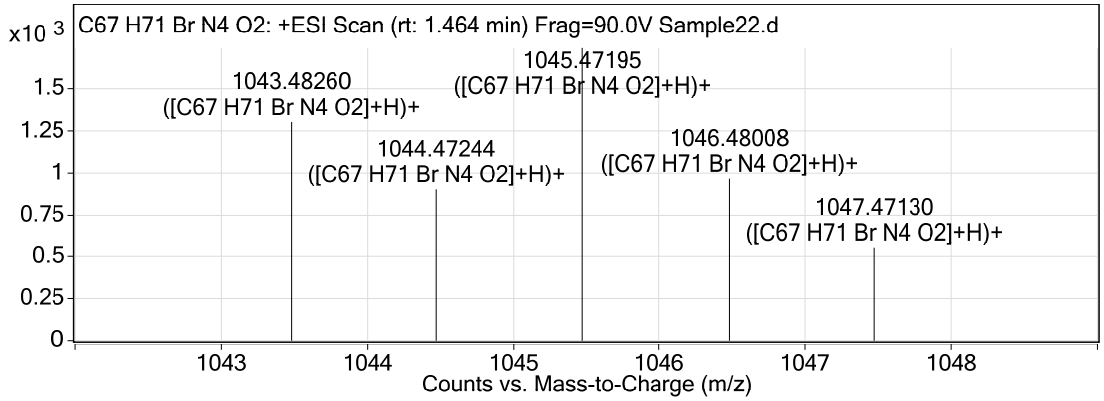
HRMS spectrum of ***Z,Z*-35b**



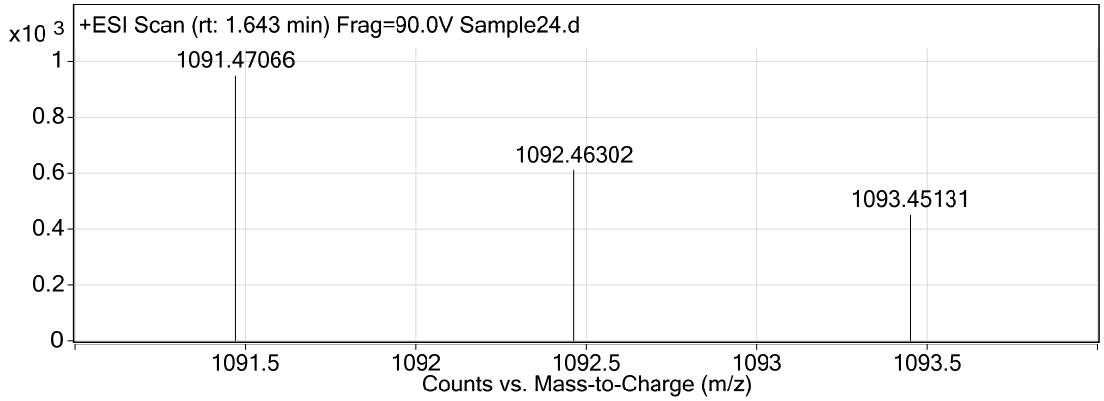
HRMS spectrum of ***Z,Z*-35c**



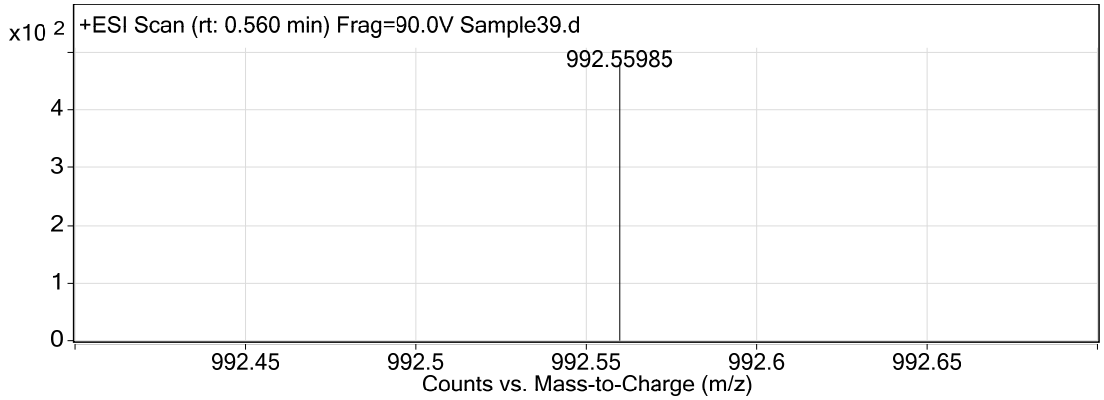
HRMS spectrum of ***Z,Z*-35d**



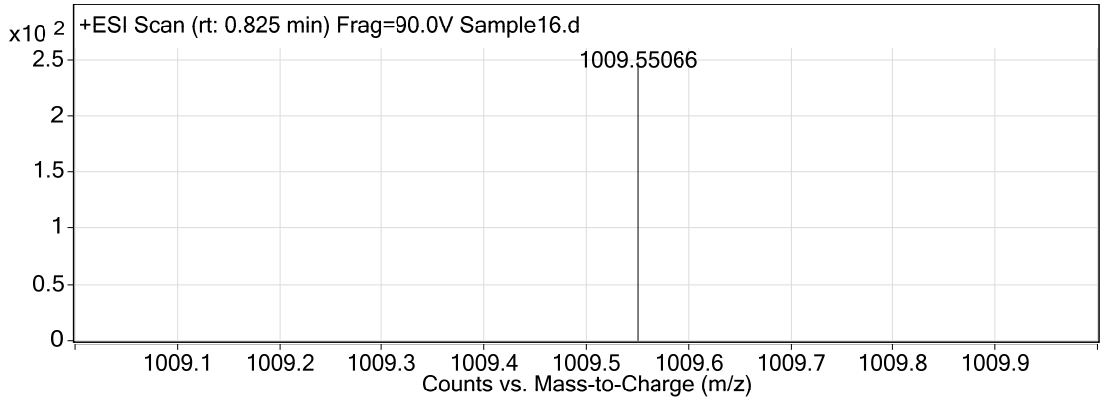
HRMS spectrum of ***Z,Z*-35e**



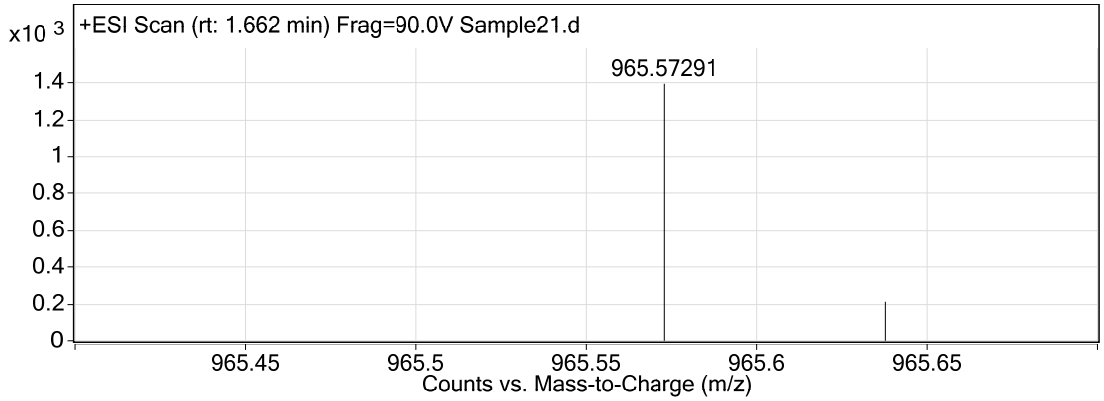
HRMS spectrum of ***Z,Z*-35f**



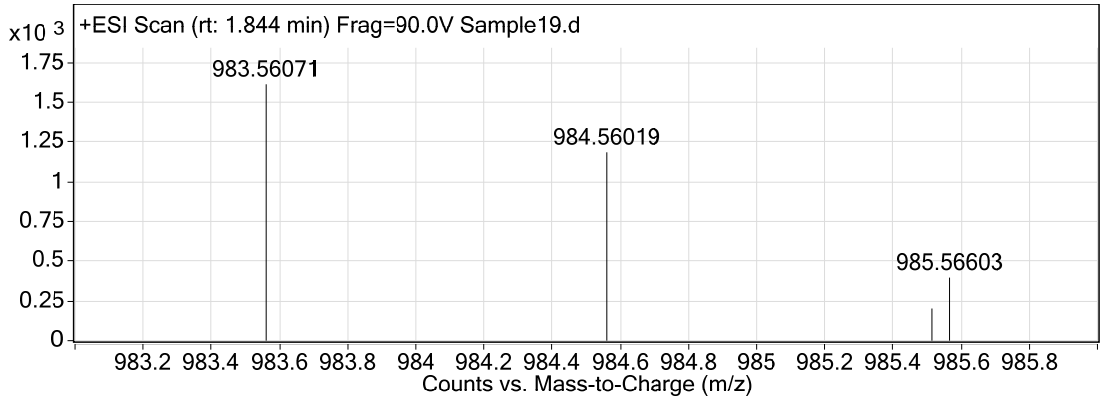
HRMS spectrum of ***Z,Z*-35g**



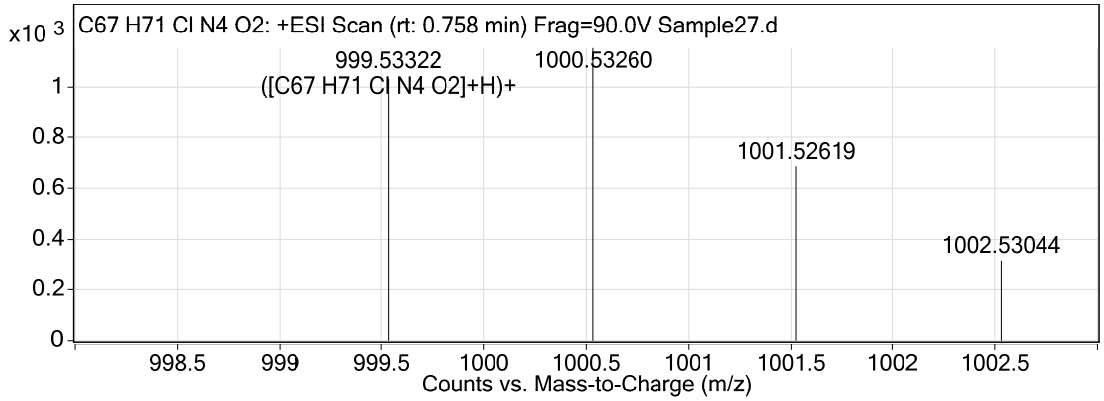
HRMS spectrum of ***E,E*-35a**



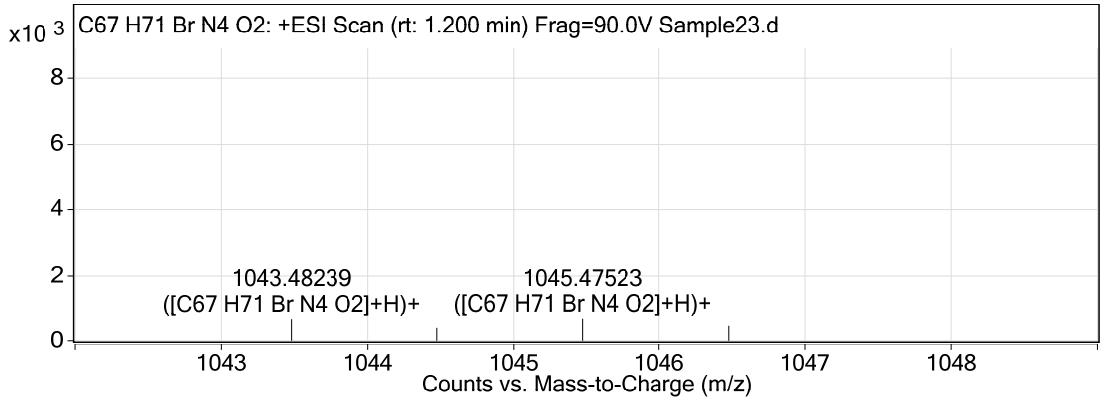
HRMS spectrum of ***E,E*-35b**



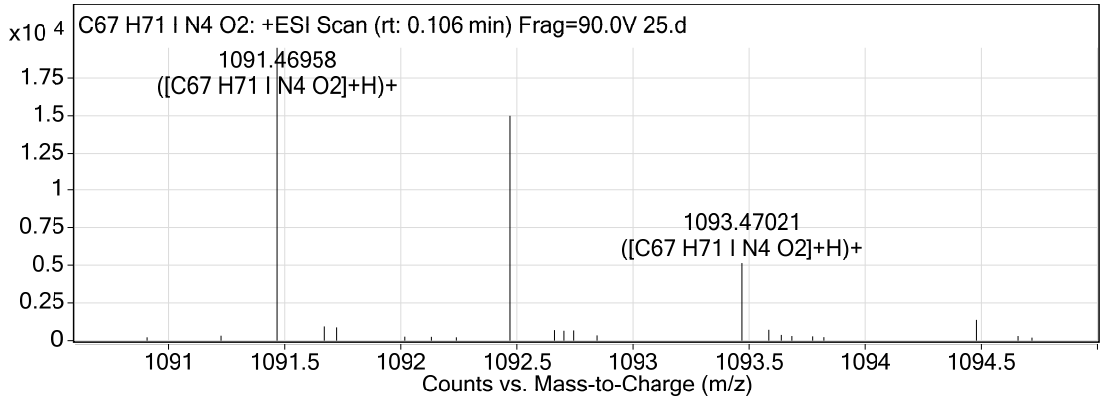
HRMS spectrum of ***E,E*-35c**



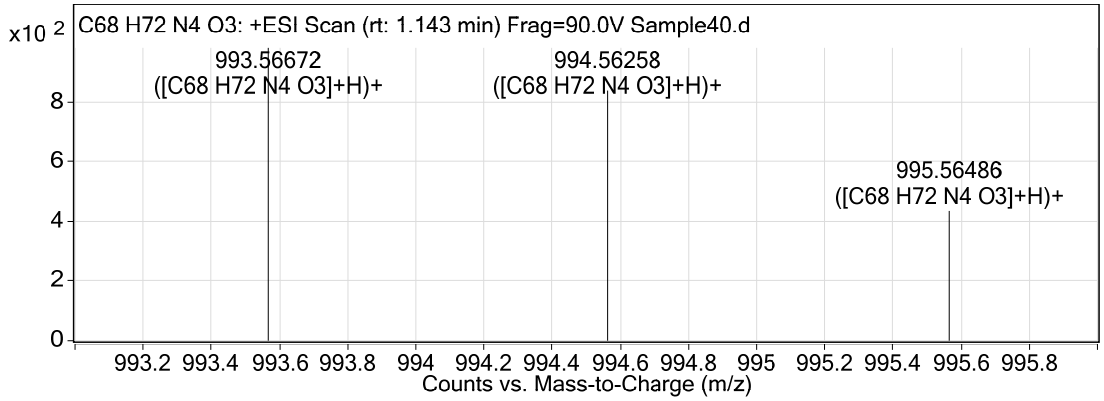
HRMS spectrum of ***E,E*-35d**



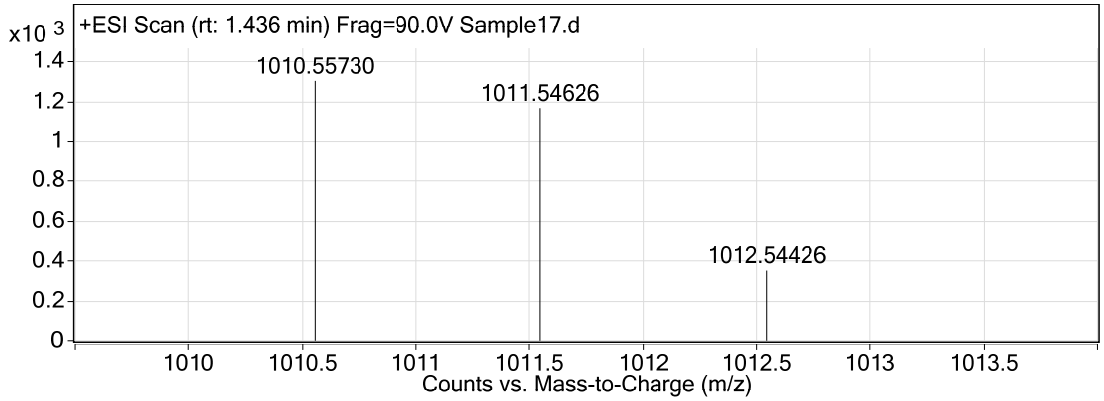
HRMS spectrum of ***E,E*-35e**



HRMS spectrum of ***E,E*-35f**

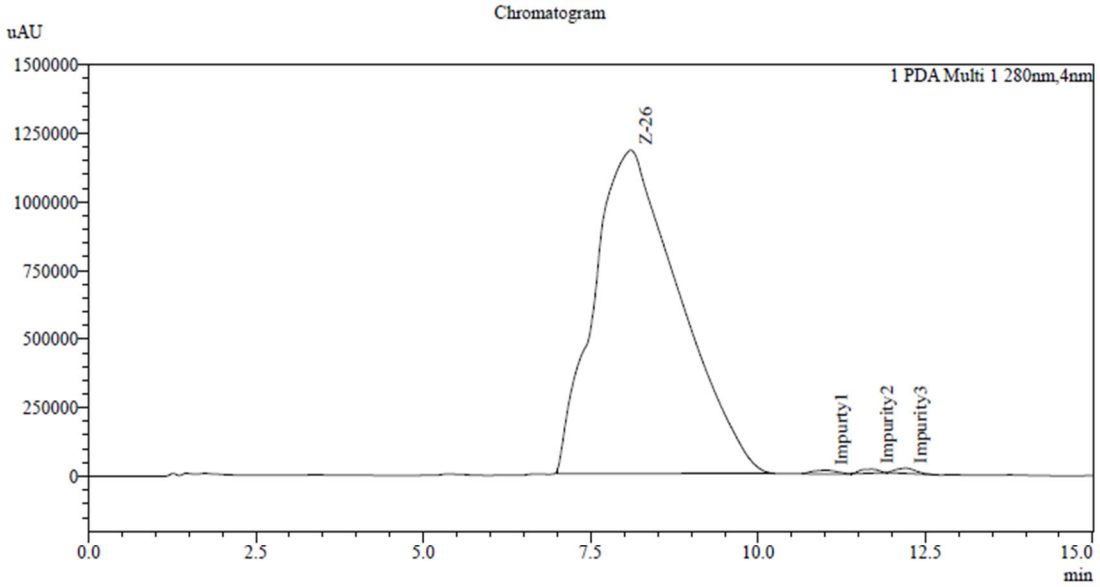


HRMS spectrum of ***E,E*-35g**



HPLC trace for final compounds

Compound ***Z*-26**

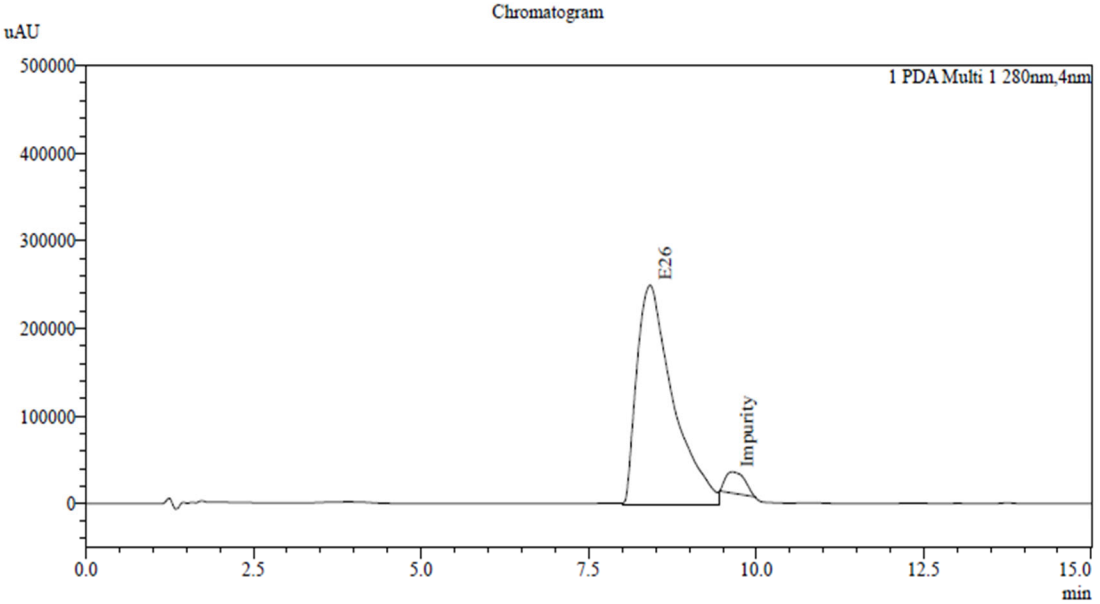


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Z-26	8.095	105187792	99.069
2	Impurity1	11.013	329171	0.310
3	Impurity2	11.701	277342	0.261
4	Impurity3	12.204	381705	0.360
Total			106176010	100.000

Compound ***E*-26**

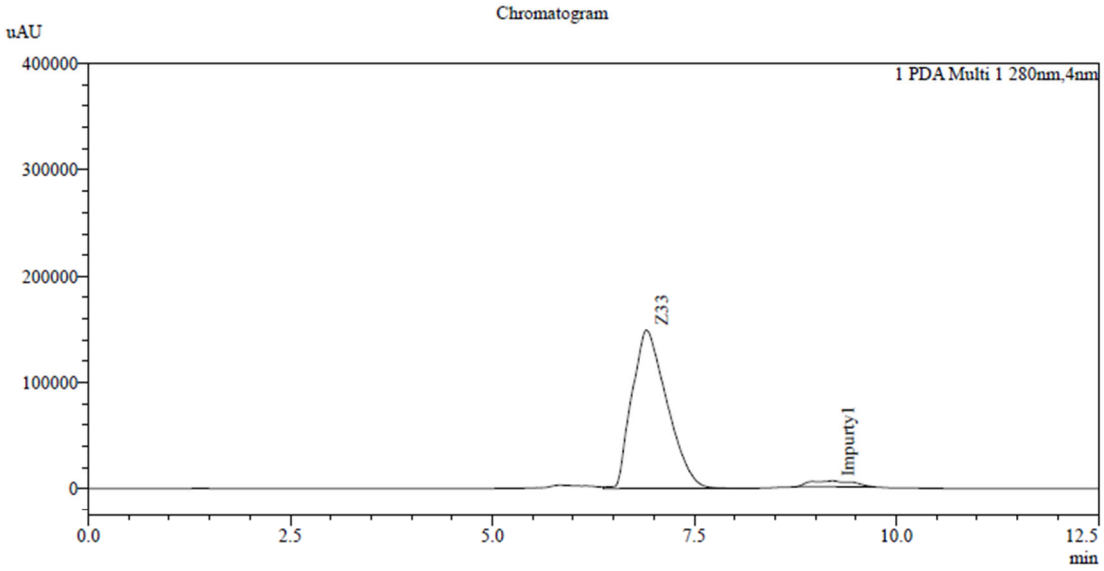


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	E26	8.417	9424276	95.028
2	Impurity	9.638	493106	4.972
Total			9917382	100.000

Compound ***Z*-33**

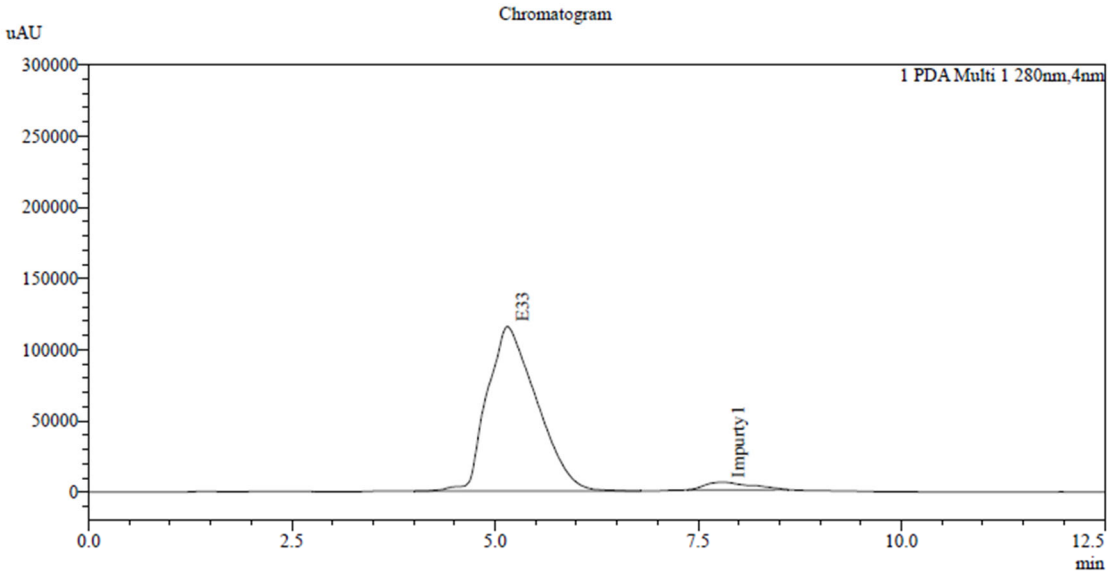


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Z33	6.908	4504497	95.627
2	Impurty1	9.213	206013	4.373
Total			4710510	100.000

Compound *E*-33

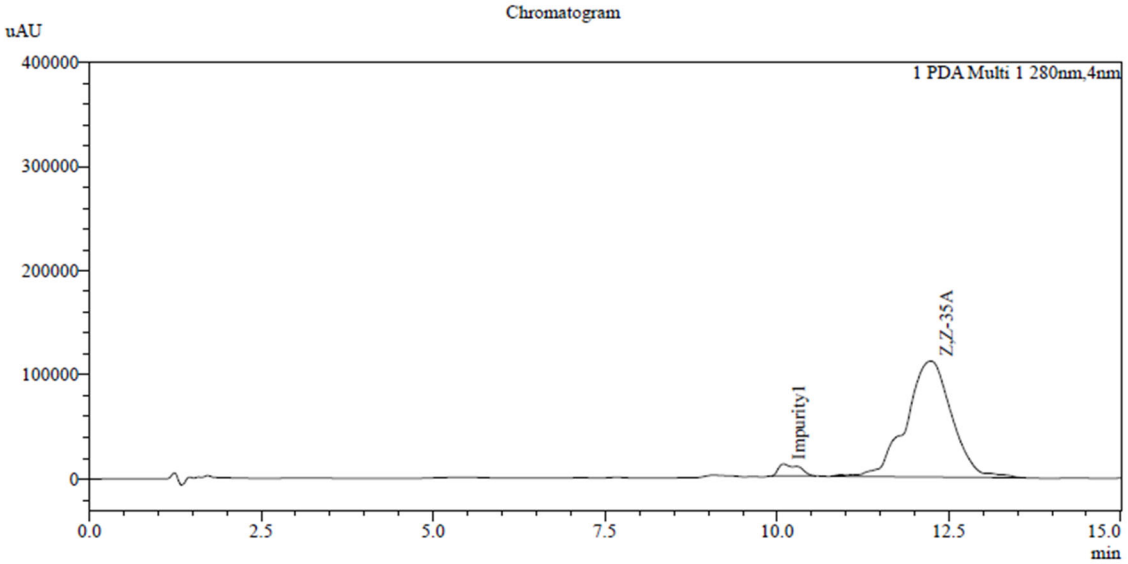


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	E33	5.153	4895245	95.886
2	Impurty1	7.807	210014	4.114
Total			5105260	100.000

Compound ***Z,Z*-35a**

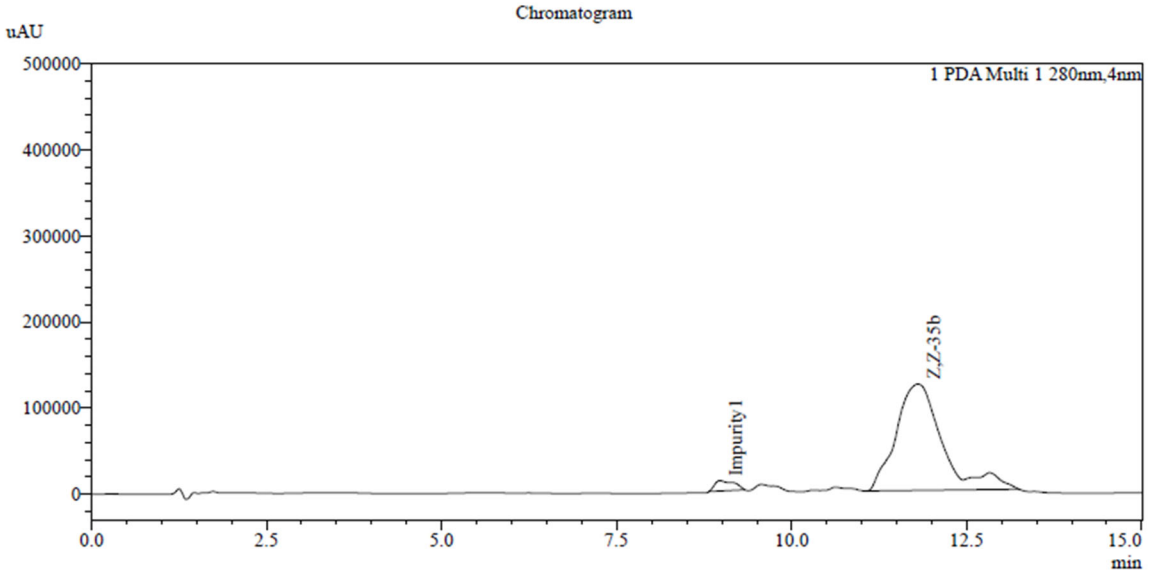


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurity1	10.094	235575	4.380
2	Z,Z-35A	12.236	5143203	95.620
Total			5378778	100.000

Compound ***Z,Z*-35b**

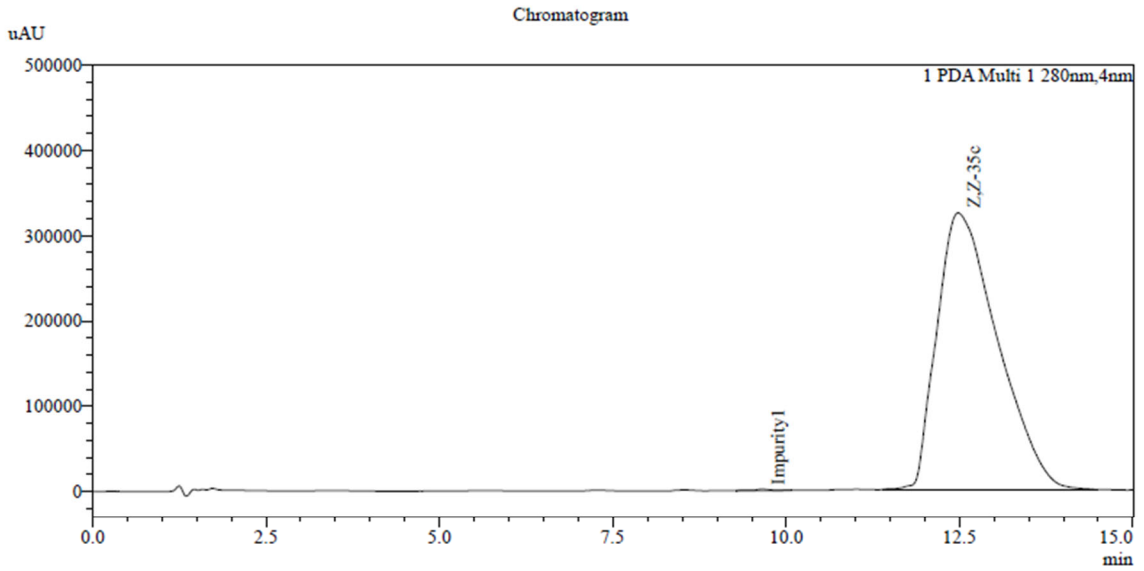


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurity1	8.975	232153	3.918
2	Z,Z-35b	11.805	5693774	96.082
Total			5925927	100.000

Compound ***Z,Z*-35c**

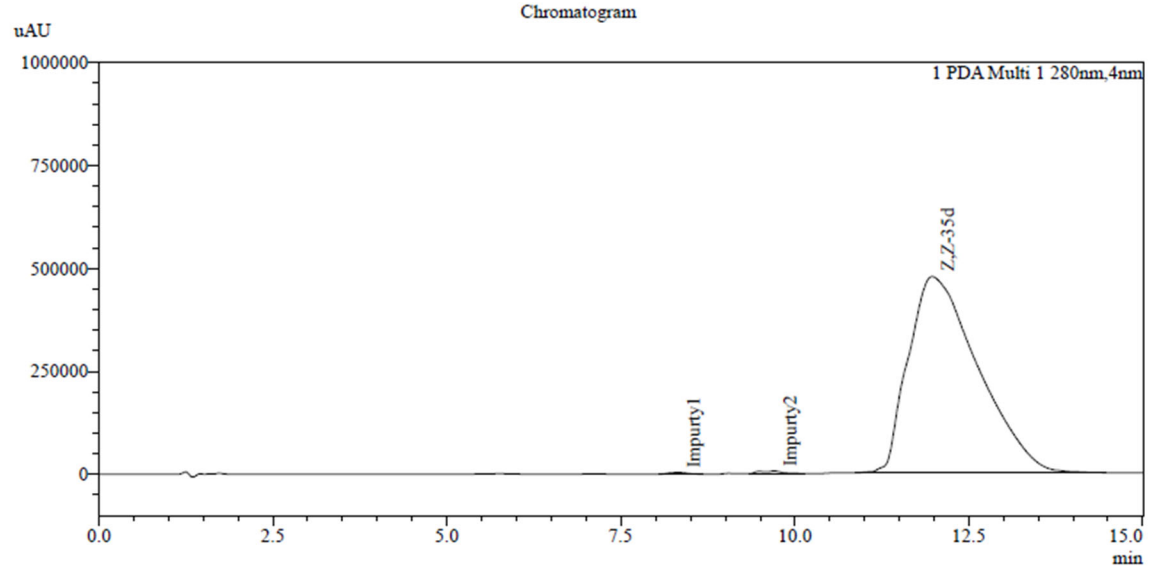


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurity1	9.653	28700	0.145
2	Z,Z-35c	12.479	19705150	99.855
Total			19733849	100.000

Compound ***Z,Z*-35d**

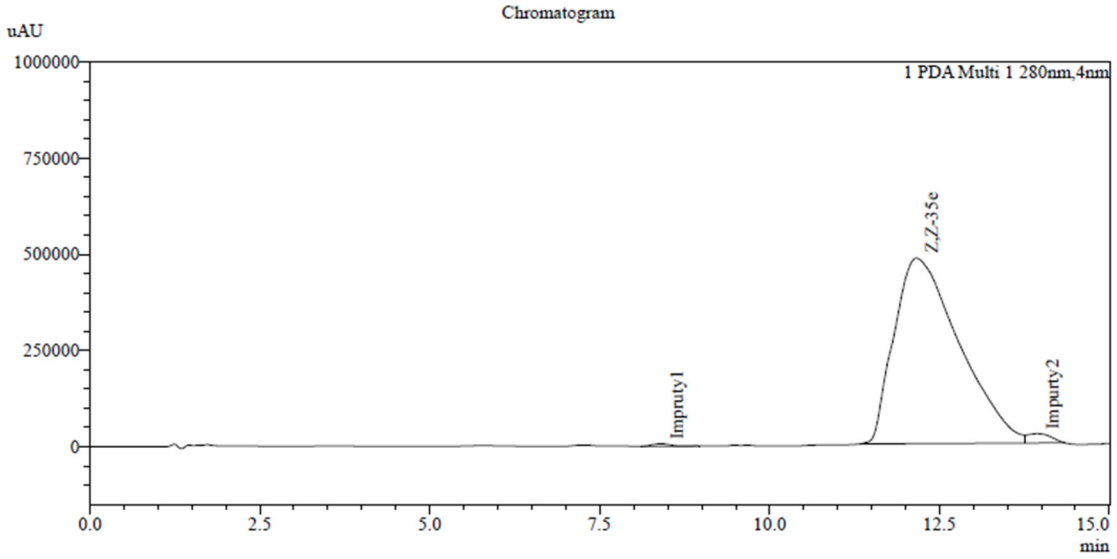


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurty1	8.318	72500	0.217
2	Impurty2	9.703	136579	0.410
3	Z,Z-35d	11.973	33141624	99.373
Total			33350703	100.000

Compound ***Z,Z*-35e**

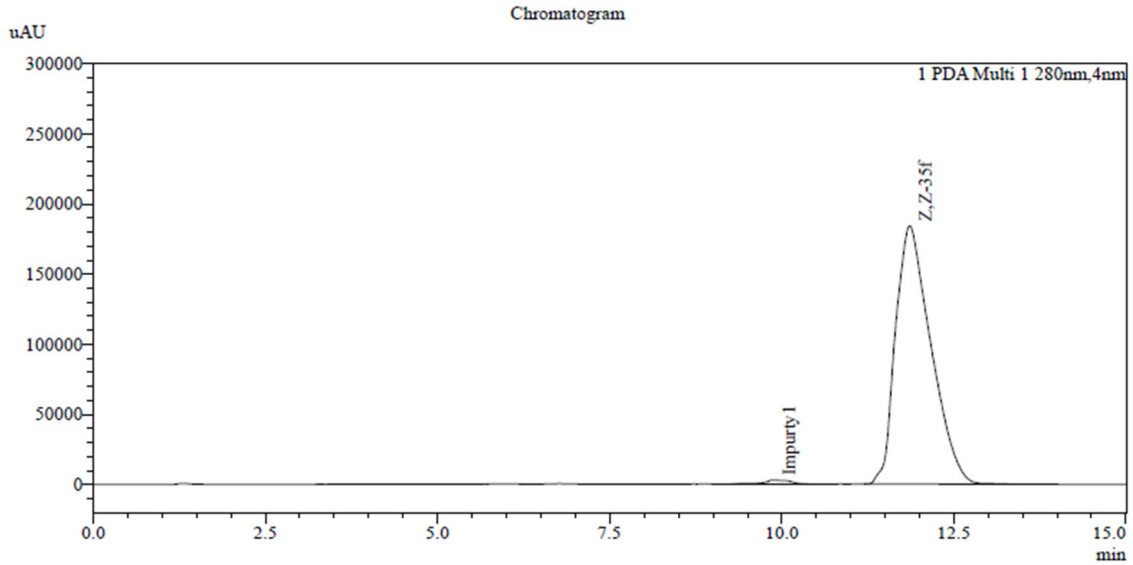


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impruty1	8.406	104782	0.319
2	Z,Z-35e	12.160	32131487	97.904
3	Impurty2	13.936	583077	1.777
Total			32819346	100.000

Compound ***Z,Z*-35f**

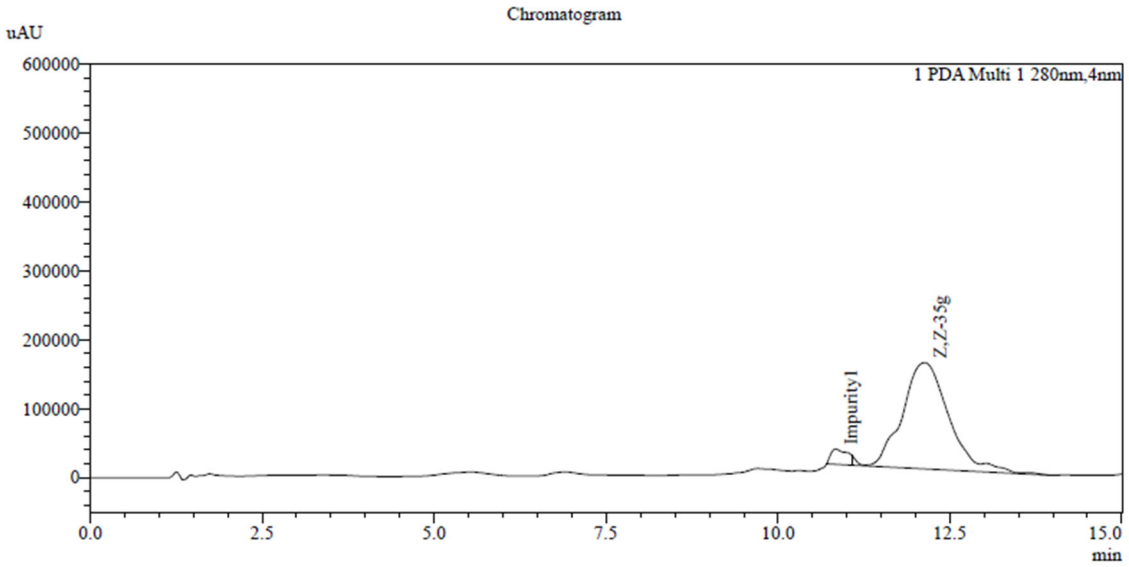


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurty1	9.882	78642	1.183
2	Z,Z-35f	11.858	6568687	98.817
Total			6647329	100.000

Compound ***Z,Z*-35g**

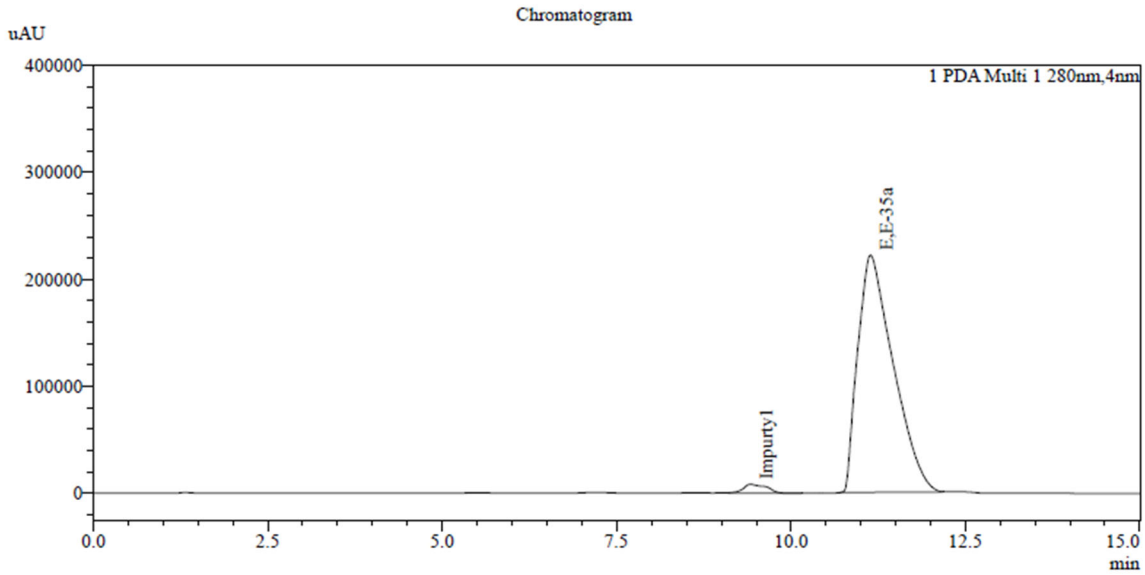


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurity1	10.839	374647	4.875
2	Z,Z-35g	12.128	7311104	95.125
Total			7685751	100.000

Compound ***E,E*-35a**

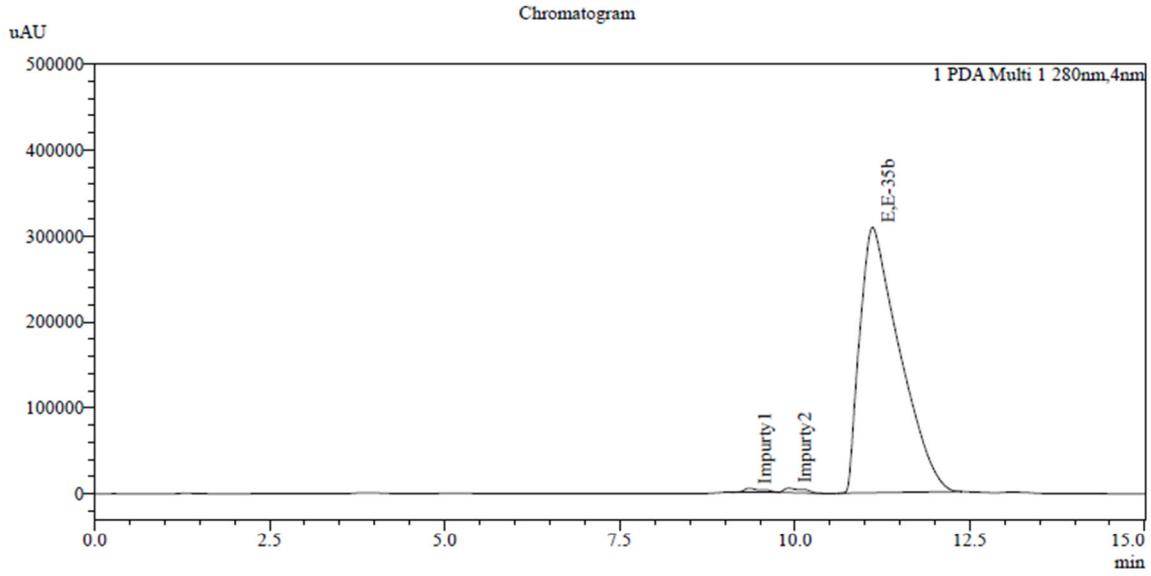


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurty1	9.420	194434	2.424
2	E,E-35a	11.140	7825703	97.576
Total			8020137	100.000

Compound ***E,E*-35b**

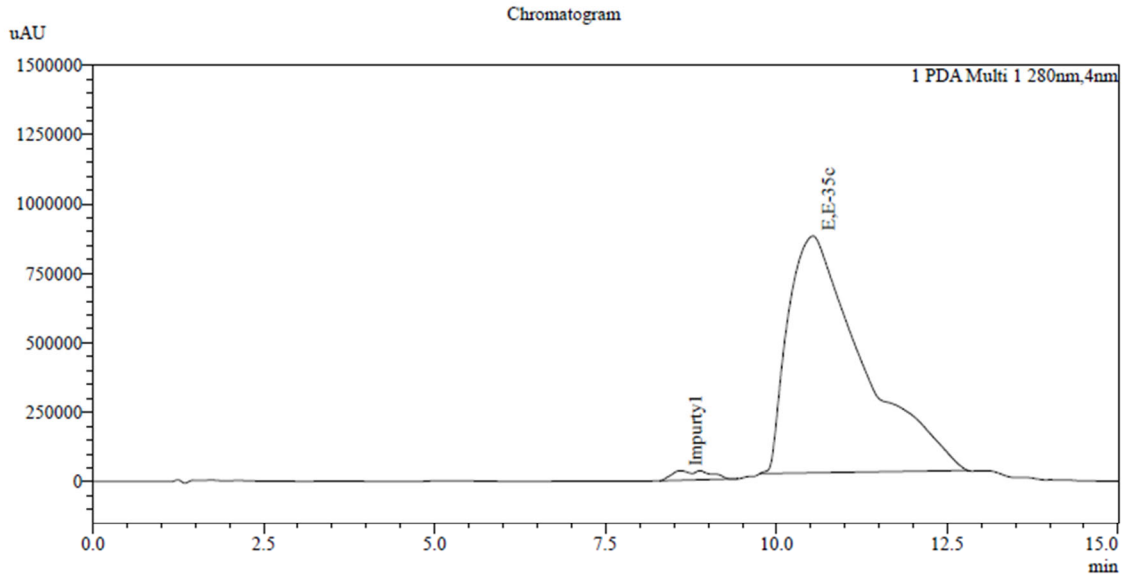


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurity1	9.346	84237	0.681
2	Impurity2	9.912	101323	0.820
3	E,E-35b	11.111	12177886	98.499
Total			12363446	100.000

Compound ***E,E*-35c**

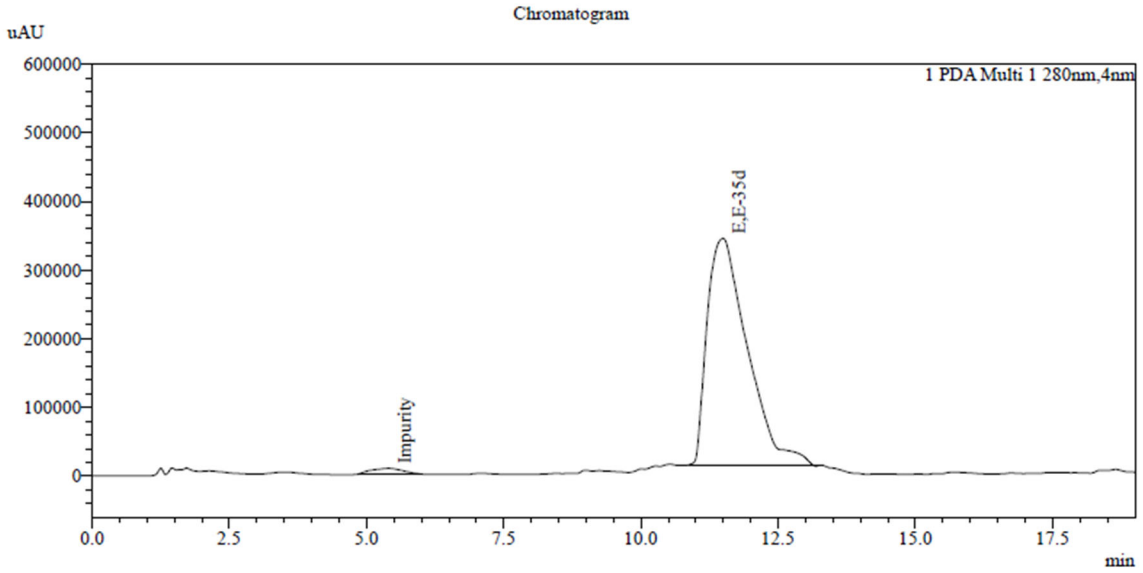


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurity1	8.593	1262289	1.911
2	E,E-35c	10.533	64793343	98.089
Total			66055632	100.000

Compound ***E,E*-35d**

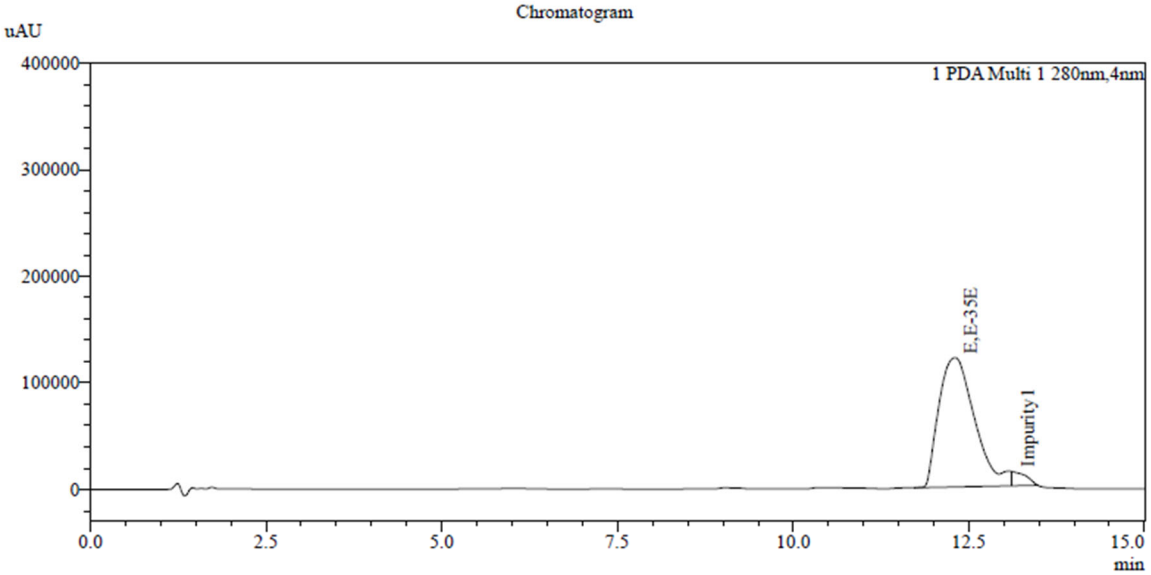


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurity	5.408	327165	1.893
2	E,E-35d	11.495	16956242	98.107
Total			17283407	100.000

Compound ***E,E*-35e**

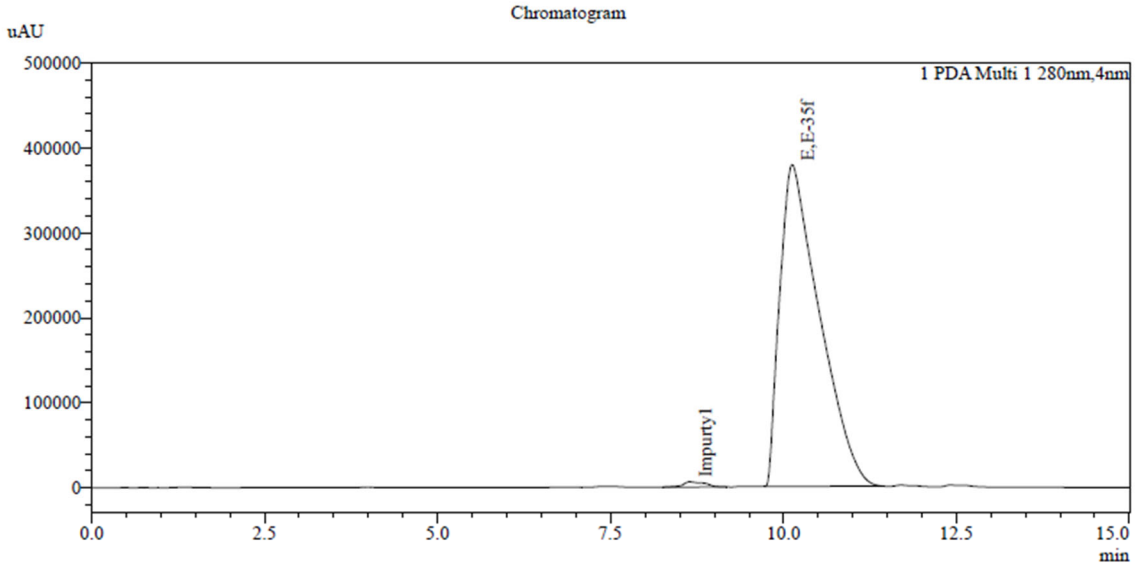


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	E,E-35E	12.302	4311501	95.948
2	Impurity1	13.115	182064	4.052
Total			4493564	100.000

Compound ***E,E*-35f**

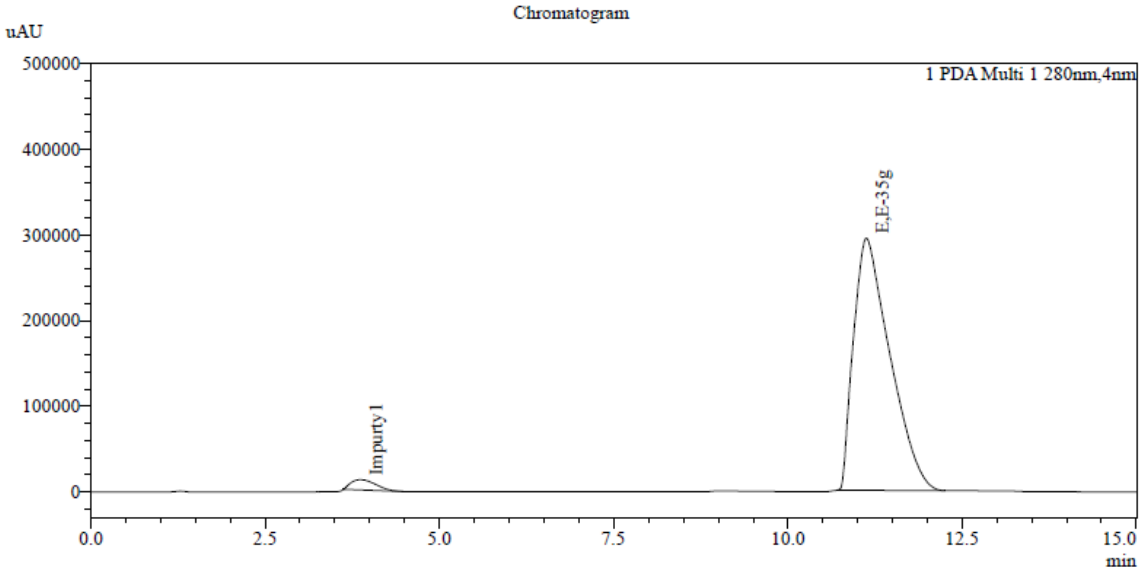


QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurty1	8.646	133792	0.878
2	E,E-35f	10.126	15107923	99.122
Total			15241715	100.000

Compound ***E,E*-35g**



QuantitativeResult

PDA

ID#	Name	Ret. Time	Area	Area%
1	Impurty1	3.868	292914	2.673
2	E,E-35g	11.126	10663518	97.327
Total			10956432	100.000