

## Supplementary Information

### A (Z)-Fluoro-Alkene Mimic of Gly-*trans*-Pro Produces a Stable Collagen Triple Helix

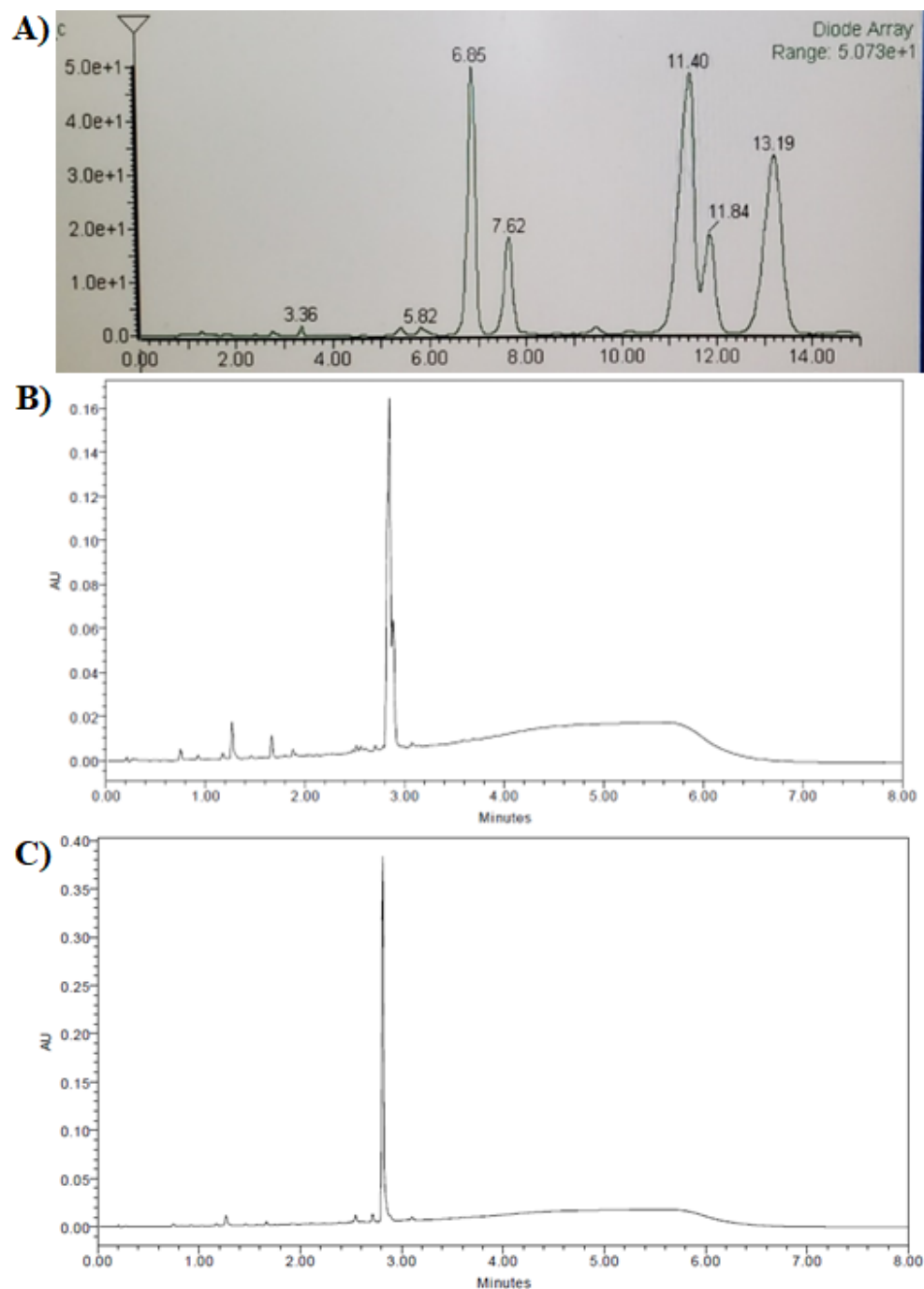
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\*Email: [fetzkorn@vt.edu](mailto:fetzkorn@vt.edu)

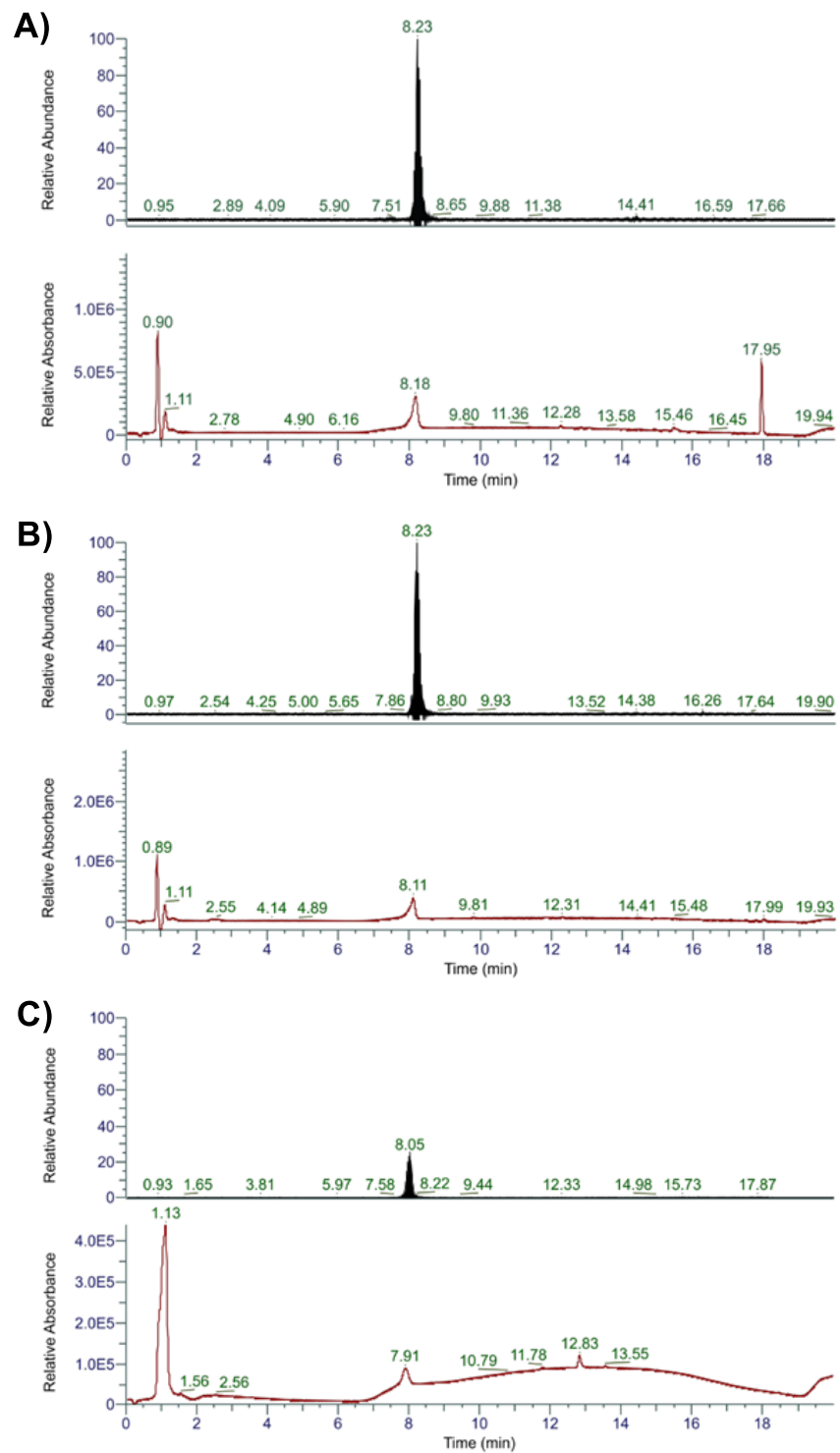
## Table of Contents

Figure S1.....	2
Figure S2.....	3
<b>Table S1. ....</b>	<b>4</b>
Figure S3.....	4
Figure S4.....	5
<b>Data for four-parameter Hill equation fitting of <math>T_m</math> for fluoro-alkene peptide 2-(R,Z) ...</b>	<b>6</b>
<b>Data for four-parameter Hill equation fitting of <math>T_m</math> for control peptide 3 .....</b>	<b>8</b>



**Figure S1.**

HPLC chromatograph of benzyl ester **13-(Z)** for separation of diastereomers. A) The mixture of diastereomers. The peaks at 11.40 min and 11.84 min correspond to the two rotamers of the diastereomer **13-(S,Z)**. The peak at 13.19 min corresponds to the diastereomer **13-(R,Z)** (rotamer separation not observed). B) UPLC chromatograph of the separated diastereomer **13-(S,Z)**. C) UPLC chromatograph of the separated diastereomer **13-(R,Z)**.



**Figure S2.**

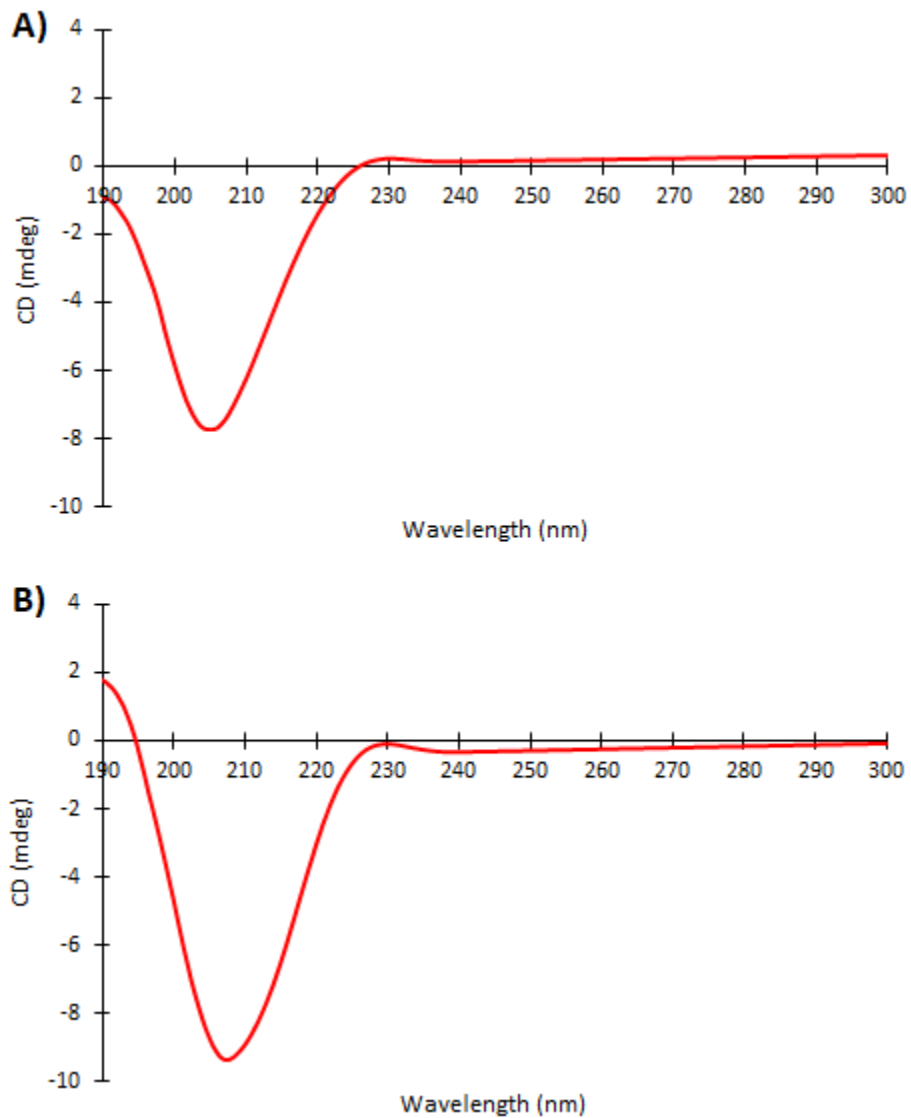
LC-MS chromatograms showing total ion count and UV absorption at 280 nm of A) 2-(*R,Z*), B)

2-(*S,Z*) and C) control 3.

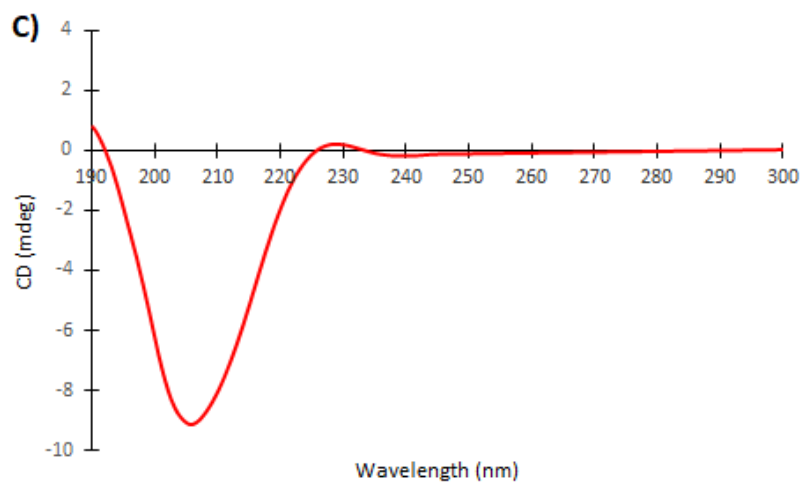
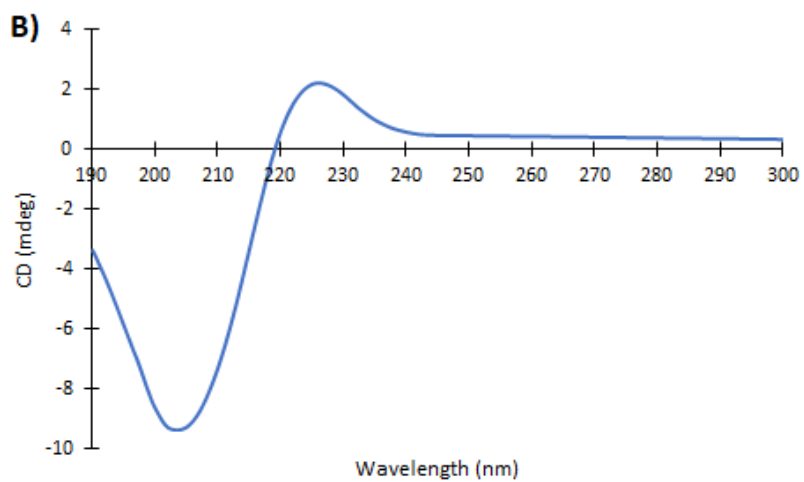
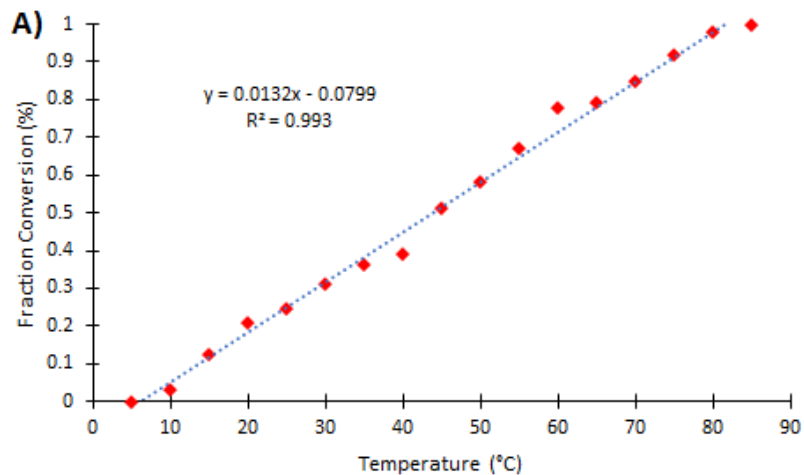
**Table S1.** MALDI-TOF results of peptides **2-(R,Z)**, **2-(S,Z)**, and **3**.

	[M + H] <sup>+</sup>		[M + Na] <sup>+</sup>		[M + K] <sup>+</sup>	
	Calcd <sup>a</sup>	Found	Calcd <sup>a</sup>	Found	Calcd <sup>a</sup>	Found
<b>2-(R,Z)</b>	2474.1190	N.D.	2497.1088	2497.0881	2513.0827	2514.0687
<b>2-(S,Z)</b>	2474.1190	2475.1099	2497.1088	2497.0918	2513.0827	2513.0670
<b>3</b>	2474.1259	2474.1081	2496.1084	2496.0927	2512.0823	2512.0674

<sup>a</sup>Calcd mass for the most abundant isotopes. N.D. = not detected.



**Figure S3.** Full-range CD spectra at 85 °C of **A)** peptide **2-(R,Z)** and **B)** control **3**.



**Figure S4.** **A)** linear thermal melting curve of fluoro-alkene peptide **2-(S,Z)**, **B)** Full-range CD spectrum of peptide **2-(S,Z)** at 5 °C and **C)** Full-range CD spectrum of peptide **2-(S,Z)** at 85 °C.

## Data for four-parameter Hill equation fitting of $T_m$ for fluoro-alkene peptide 2-(R,Z)

### Nonlinear Regression

Data Source: Data 1 in Notebook1

Equation: Sigmoidal, Sigmoid, 4 Parameter

$$f = y_0 + a / (1 + \exp(-(x - x_0)/b))$$

<b>R</b>	<b>Rsqr</b>	<b>Adj Rsqr</b>	<b>Standard Error of Estimate</b>
0.9993	0.9986	0.9982	0.0173

	<b>Coefficient</b>	<b>Std. Error</b>	<b>t</b>	<b>P</b>	<b>VIF</b>
a	1.0539	0.0202	52.1638	<0.0001	9.8801<
b	9.0676	0.4366	20.7676	<0.0001	3.3070
x0	42.2261	0.4427	95.3859	<0.0001	2.6643
y0	-0.0261	0.0133	-1.9650	0.0712	9.9823<

### Analysis of Variance:

Uncorrected for the mean of the observations:

	<b>DF</b>	<b>SS</b>	<b>MS</b>
Regression	4	7.5839	1.8960
Residual	13	0.0039	0.0003
Total	17	7.5878	0.4463

Corrected for the mean of the observations:

	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Regression	3	2.7248	0.9083	3025.9605	<0.0001
Residual	13	0.0039	0.0003		
Total	16	2.7287	0.1705		

### Statistical Tests:

**PRESS** 0.0066

**Durbin-Watson Statistic** 0.8654 Failed

**Normality Test** Passed (P = 0.9602)

K-S Statistic = 0.1184 Significance Level = 0.9602

**Constant Variance Test** Passed (P = 0.1600)

**Power of performed test with alpha = 0.0500: 1.0000**

### Regression Diagnostics:

<b>Row</b>	<b>Std. Res.</b>	<b>Stud. Res.</b>	<b>Stud. Del. Res.</b>
2	0.5193	0.6509	0.6358
3	0.1085	0.1280	0.1230
4	-0.2300	-0.2579	-0.2485
5	-1.1969	-1.3089	-1.3496
6	-0.2054	-0.2278	-0.2193

7	0.6546	0.7572	0.7441
8	0.9849	1.1623	1.1797
9	0.4900	0.5666	0.5512
10	-0.7653	-0.8818	-0.8738
11	-1.5080	-1.7691	-1.9506
12	-0.4225	-0.4878	-0.4730
13	1.0814	1.1977	1.2199
14	1.4663	1.5861	1.6969
15	1.0834	1.1797	1.1995
16	-0.0556	-0.0622	-0.0597
17	-0.9384	-1.0864	-1.0947
18	-1.0664	-1.2756	-1.3103

**Influence Diagnostics:**

Row	Cook's Dist	Leverage	DFFITS
2	0.0605	0.3635	0.4804
3	0.0016	0.2818	0.0771
4	0.0043	0.2047	-0.1260
5	0.0839	0.1638	-0.5973
6	0.0030	0.1869	-0.1051
7	0.0484	0.2526	0.4326
8	0.1327	0.2820	0.7394
9	0.0270	0.2520	0.3199
10	0.0637	0.2467	-0.5001
11	0.2943	0.2734	-1.1964
12	0.0198	0.2499	-0.2730
13	0.0812	0.1847	0.5806
14	0.1069	0.1453	0.6997
15	0.0646	0.1567	0.5170
16	0.0002	0.2010	-0.0300
17	0.1005	0.2540	-0.6388
18	0.1753	0.3011	-0.8600

**95% Confidence:**

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	-0.0090	-0.0316	0.0136	-0.0527	0.0347
3	0.0032	-0.0166	0.0231	-0.0391	0.0456
4	0.0238	0.0068	0.0407	-0.0173	0.0649
5	0.0575	0.0424	0.0727	0.0172	0.0979
6	0.1111	0.0949	0.1272	0.0703	0.1518
7	0.1912	0.1724	0.2100	0.1493	0.2331
8	0.3013	0.2815	0.3212	0.2590	0.3437
9	0.4365	0.4177	0.4553	0.3946	0.4784
10	0.5808	0.5622	0.5994	0.5390	0.6226
11	0.7139	0.6943	0.7334	0.6716	0.7561
12	0.8208	0.8021	0.8395	0.7789	0.8626
13	0.8977	0.8816	0.9138	0.8570	0.9384
14	0.9487	0.9344	0.9630	0.9087	0.9888
15	0.9807	0.9659	0.9956	0.9405	1.0210
16	1.0002	0.9834	1.0170	0.9592	1.0412
17	1.0117	0.9928	1.0306	0.9698	1.0536
18	1.0185	0.9979	1.0390	0.9758	1.0612

**Fit Equation Description:**

[Variables]

x = col(1)

```

y = col(3)
reciprocal_y = 1/abs(y)
reciprocal_ysquare = 1/y^2
[Parameters]
a = max(y)-min(y) "Auto" {{previous: 1.0539}}
b = xwtr(x,y-min(y),.5)/4 "Auto" {{previous: 9.06758}}
x0 = x50(x,y-min(y),.5) "Auto" {{previous: 42.2261}}
y0 = min(y) "Auto" {{previous: -0.0260873}}
[Equation]
f= y0+a/(1+exp(-(x-x0)/b))
fit f to y
"fit f to y with weight reciprocal_y
"fit f to y with weight reciprocal_ysquare
[Constraints]
[Options]
tolerance=1e-10
stepsize=1
iterations=200

```

Number of Iterations Performed = 9

### Data for four-parameter Hill equation fitting of $T_m$ for control peptide 3

#### Nonlinear Regression

Data Source: Data 1 in Notebook1

Equation: Sigmoidal, Sigmoid, 4 Parameter

$f = y_0 + a / (1 + \exp(-(x - x_0) / b))$

**R**      **Rsq**    **Adj Rsqr**      **Standard Error of Estimate**

0.9994   0.9987   0.9984      0.0158

	<b>Coefficient</b>	<b>Std. Error</b>	<b>t</b>	<b>P</b>	<b>VIF</b>
a	1.0958	0.0253	43.2320	<0.0001	14.7409<
b	11.1588	0.5468	20.4063	<0.0001	5.3052<
x0	48.3899	0.5132	94.2895	<0.0001	3.7774
y0	-0.0434	0.0129	-3.3716	0.0050	11.2828<

#### Analysis of Variance:

Uncorrected for the mean of the observations:

	<b>DF</b>	<b>SS</b>	<b>MS</b>
Regression	4	6.1540	1.5385
Residual	13	0.0032	0.0002
Total	17	6.1572	0.3622

Corrected for the mean of the observations:

	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Regression	3	2.5140	0.8380	3361.5246	<0.0001
Residual	13	0.0032	0.0002		
Total	16	2.5173	0.1573		

#### Statistical Tests:



**PRESS** 0.0058

**Durbin-Watson Statistic** 1.0081 Failed

**Normality Test** Passed (P = 0.5103)

K-S Statistic = 0.1923 Significance Level = 0.5103

**Constant Variance Test** Passed (P = 0.4314)

**Power of performed test with alpha = 0.0500: 1.0000**

**Regression Diagnostics:**

Row	Std. Res.	Stud. Res.	Stud. Del. Res.
2	1.3540	1.6803	1.8246
3	0.6190	0.7244	0.7105
4	-0.5299	-0.5914	-0.5760
5	-1.6408	-1.7823	-1.9698
6	-0.7299	-0.7931	-0.7811
7	-0.5955	-0.6632	-0.6483
8	0.5107	0.5843	0.5689
9	1.8018	2.0645<	2.4194<
10	0.4401	0.4963	0.4815
11	-0.7379	-0.8340	-0.8236
12	-0.6542	-0.7543	-0.7411
13	-0.7328	-0.8442	-0.8343
14	0.2685	0.3005	0.2897
15	0.2626	0.2880	0.2776
16	0.9319	1.0401	1.0437
17	0.2367	0.2813	0.2711
18	-0.8043	-1.0603	-1.0659

**Influence Diagnostics:**

Row	Cook's Dist	Leverage	DFFITs
2	0.3811	0.3506	1.3407
3	0.0485	0.2697	0.4318
4	0.0214	0.1970	-0.2853
5	0.1428	0.1525	-0.8354
6	0.0284	0.1530	-0.3320
7	0.0264	0.1937	-0.3177
8	0.0264	0.2361	0.3162
9	0.3335	0.2383	1.3535
10	0.0167	0.2138	0.2511
11	0.0482	0.2171	-0.4338
12	0.0468	0.2477	-0.4253
13	0.0583	0.2466	-0.4773
14	0.0057	0.2017	0.1456
15	0.0042	0.1684	0.1249
16	0.0665	0.1973	0.5174
17	0.0082	0.2918	0.1740
18	0.2074	0.4246	-0.9156

**95% Confidence:**

Row	Predicted	95% Conf-L	95% Conf-U	95% Pred-L	95% Pred-U
2	-0.0214	-0.0416	-0.0012	-0.0610	0.0183

3	-0.0093	-0.0271	0.0084	-0.0478	0.0291
4	0.0090	-0.0062	0.0241	-0.0283	0.0463
5	0.0364	0.0231	0.0497	-0.0002	0.0730
6	0.0766	0.0632	0.0899	0.0400	0.1132
7	0.1335	0.1185	0.1485	0.0962	0.1707
8	0.2103	0.1937	0.2269	0.1724	0.2482
9	0.3077	0.2911	0.3244	0.2698	0.3457
10	0.4219	0.4062	0.4377	0.3844	0.4595
11	0.5440	0.5281	0.5599	0.5063	0.5816
12	0.6622	0.6452	0.6792	0.6241	0.7003
13	0.7663	0.7494	0.7833	0.7283	0.8044
14	0.8506	0.8353	0.8659	0.8132	0.8880
15	0.9143	0.9003	0.9283	0.8774	0.9512
16	0.9600	0.9448	0.9751	0.9227	0.9973
17	0.9915	0.9731	1.0099	0.9527	1.0303
18	1.0127	0.9905	1.0349	0.9720	1.0534

### Fit Equation Description:

[Variables]

x = col(1)

y = col(2)

reciprocal\_y = 1/abs(y)

reciprocal\_ysquare = 1/y^2

[Parameters]

a = max(y)-min(y) "Auto {{previous: 1.09577}}

b = xwtr(x,y-min(y),.5)/4 "Auto {{previous: 11.1588}}

x0 = x50(x,y-min(y),.5) "Auto {{previous: 48.3899}}

y0 = min(y) "Auto {{previous: -0.0433683}}

[Equation]

$\hat{f} = y_0 + a / (1 + \exp(-(x - x_0) / b))$

fit f to y

"fit f to y with weight reciprocal\_y

"fit f to y with weight reciprocal\_ysquare

[Constraints]

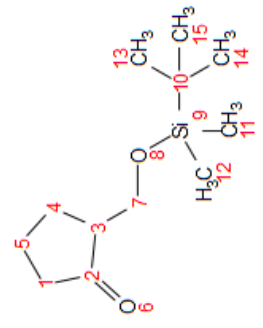
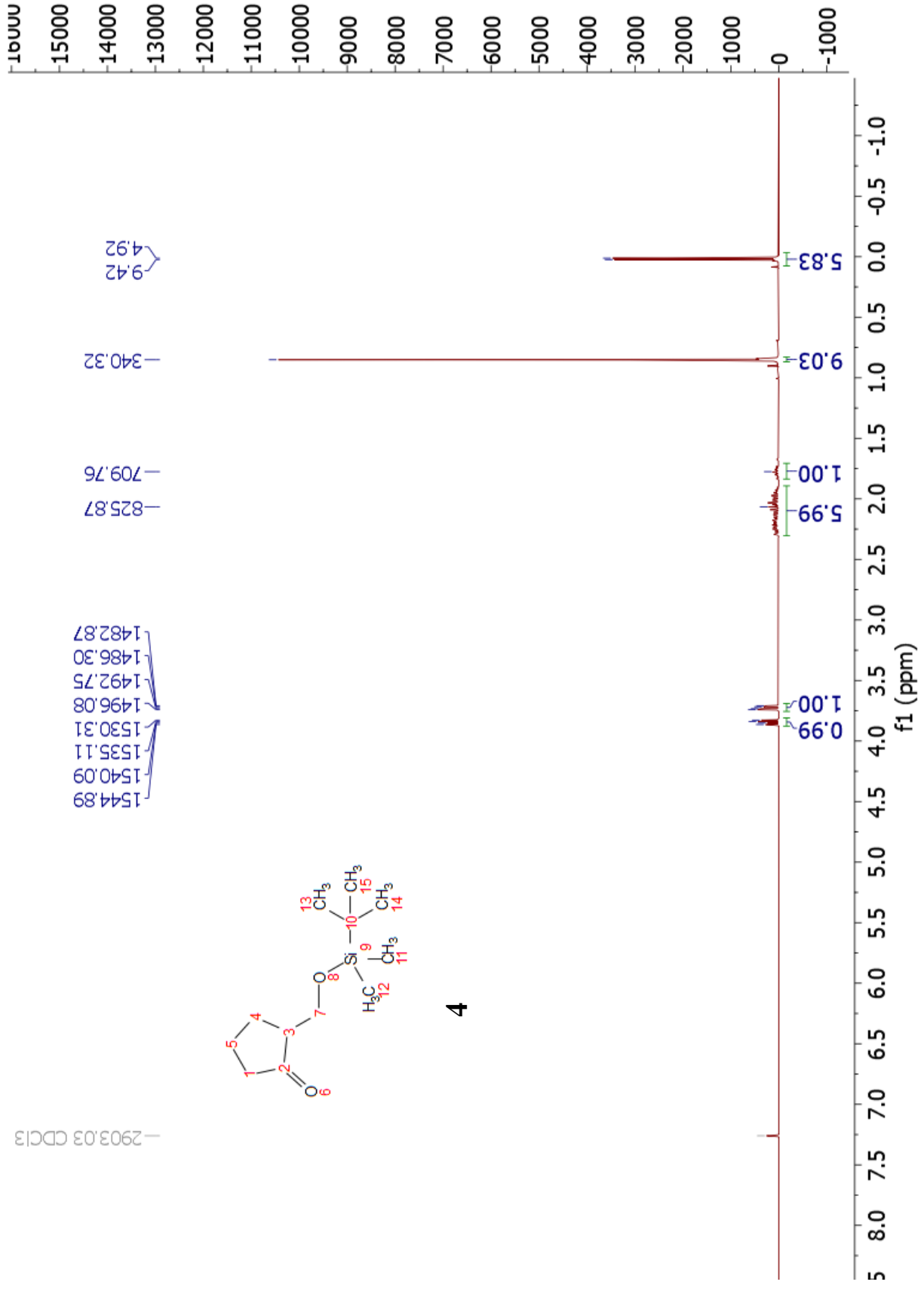
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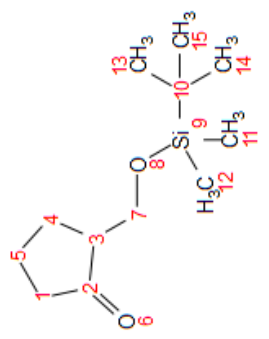
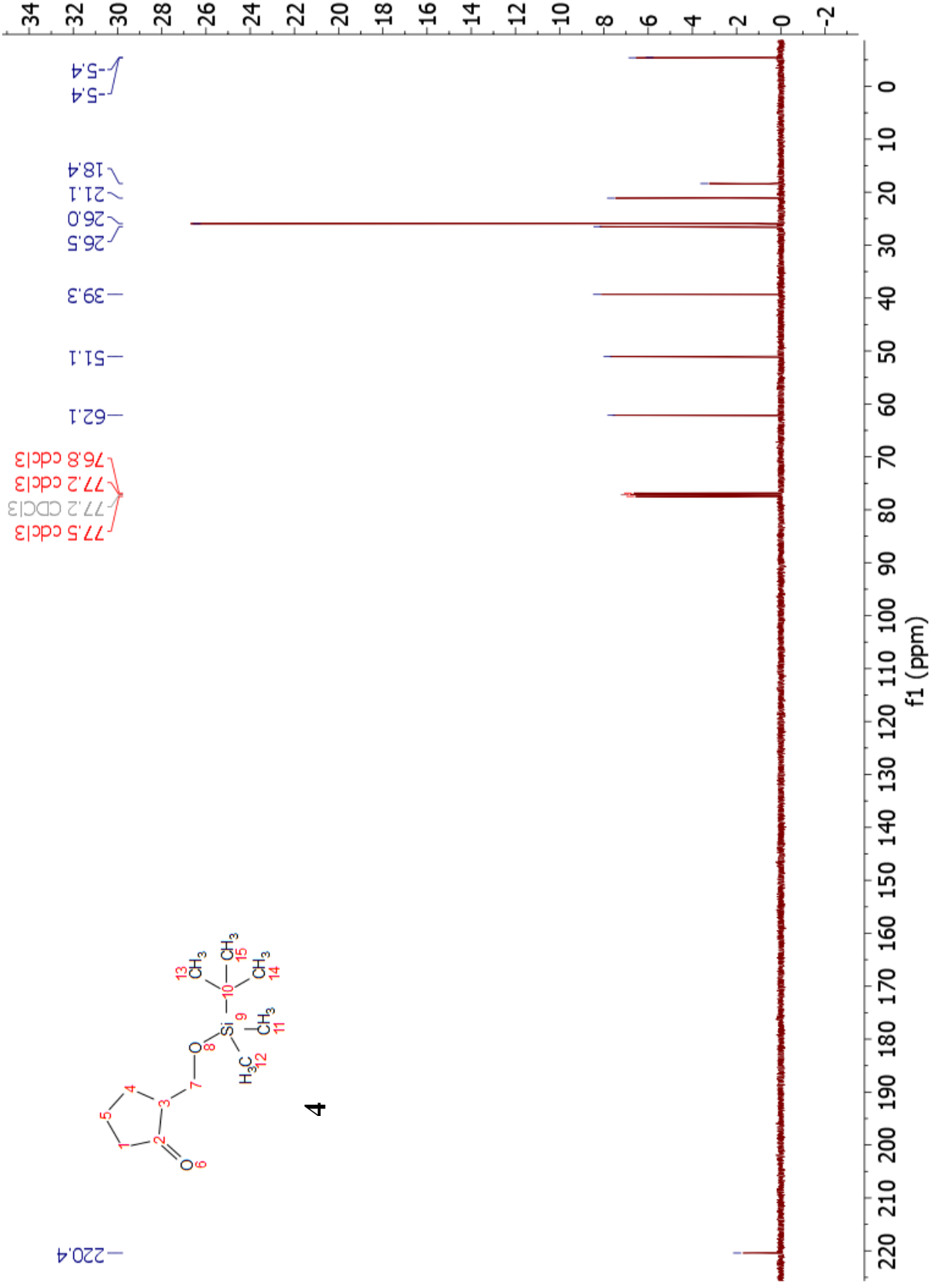
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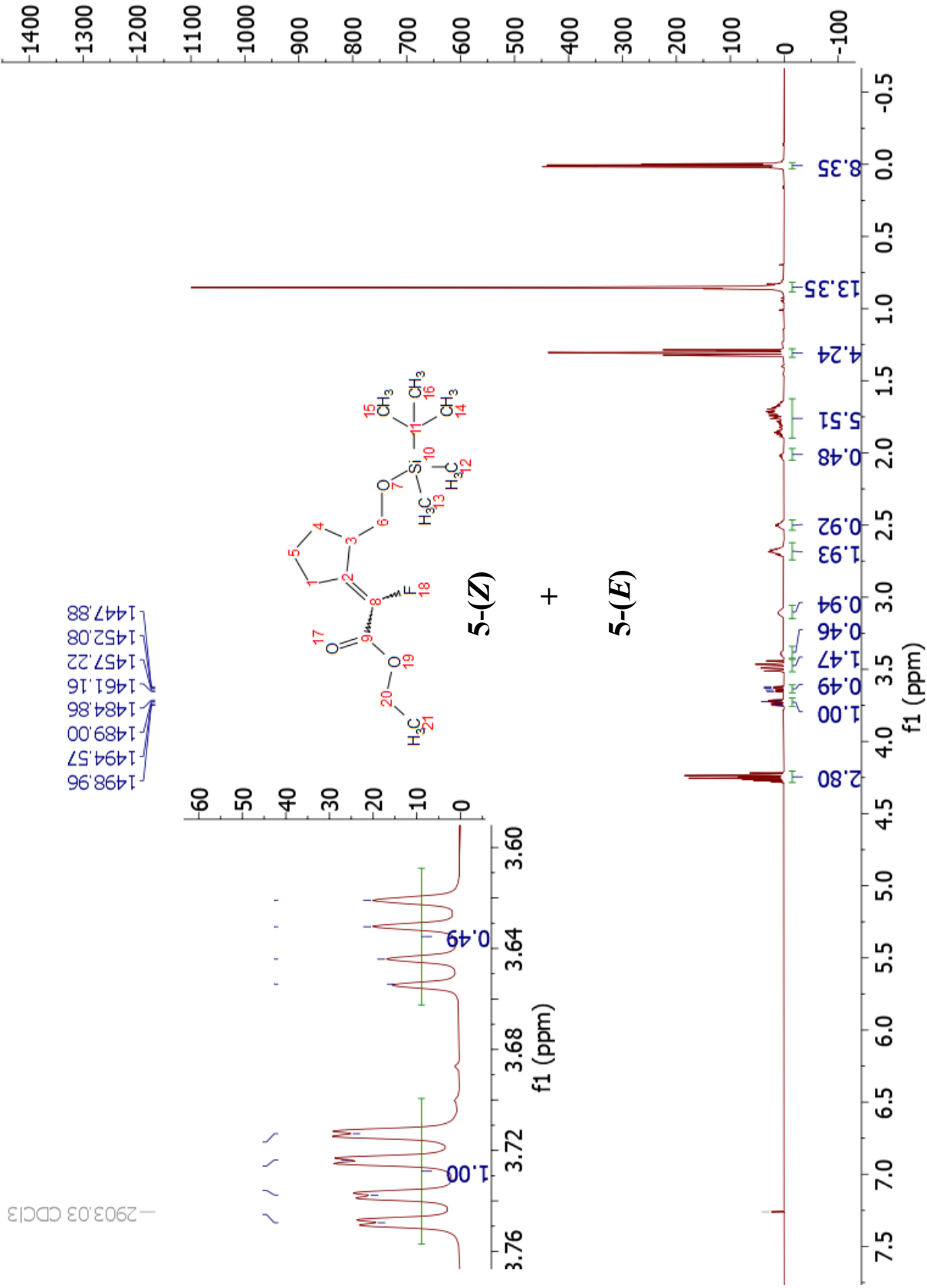
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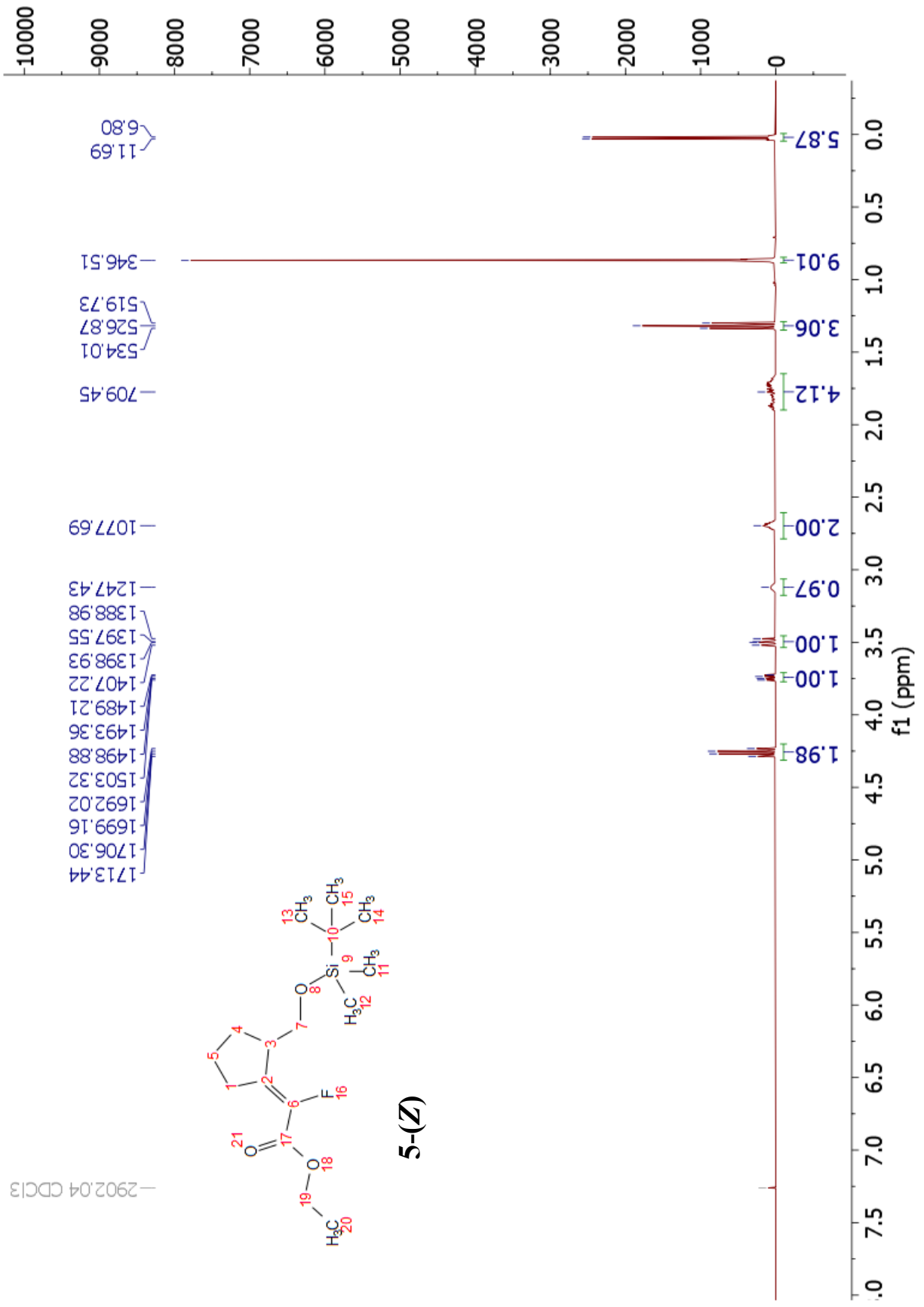
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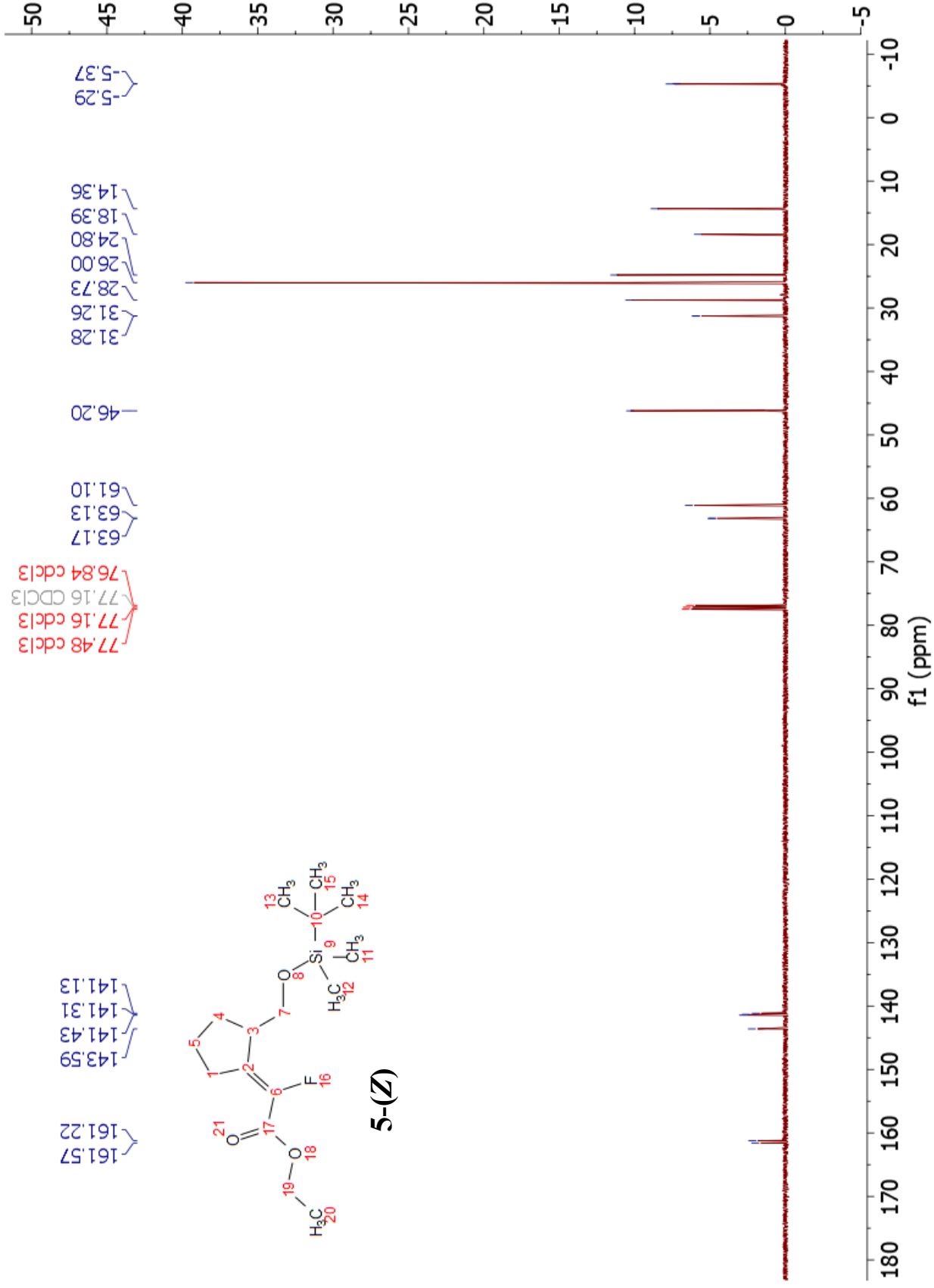


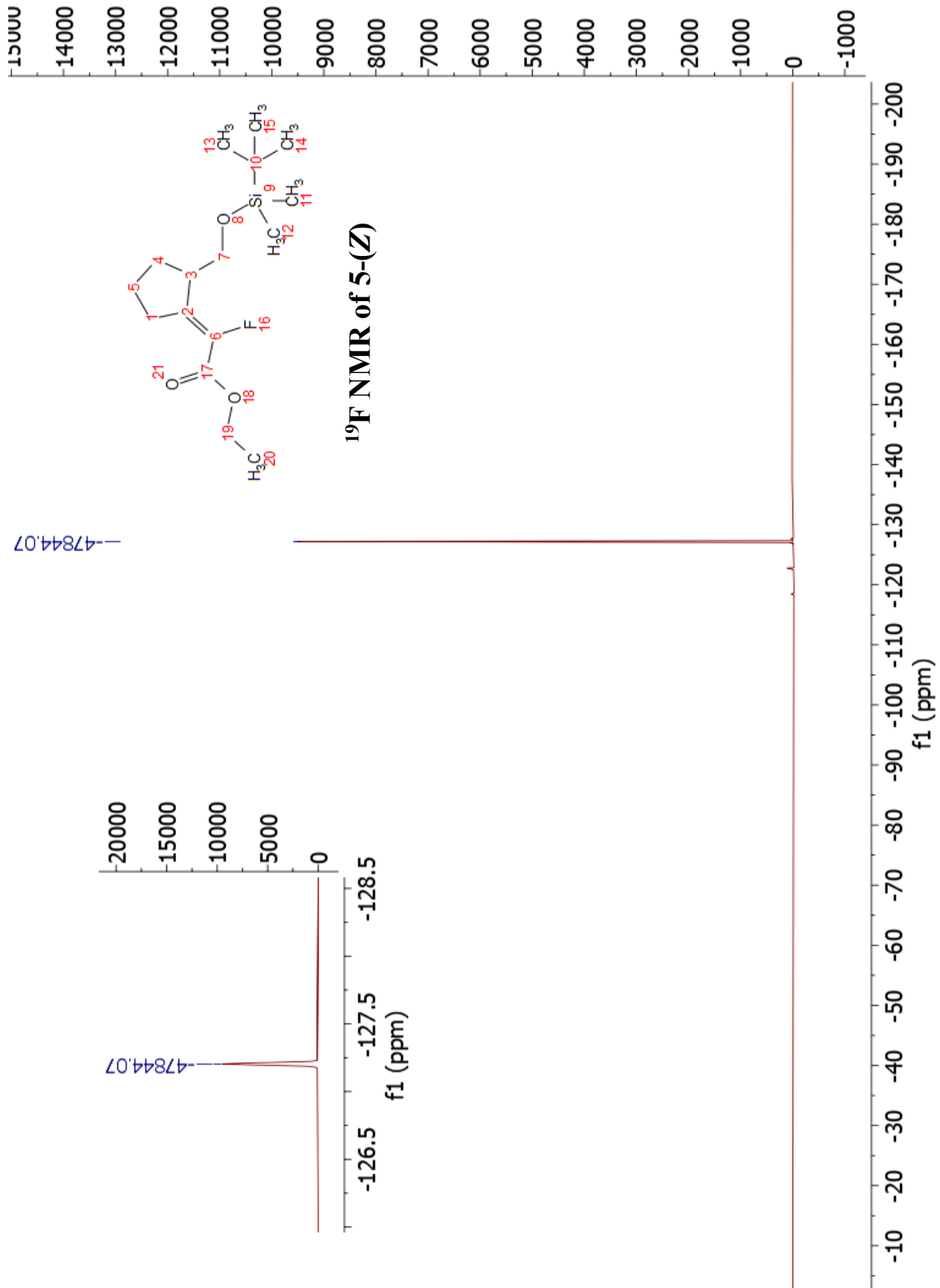
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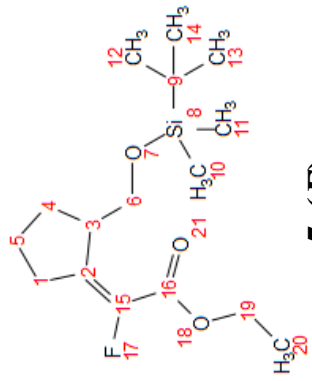
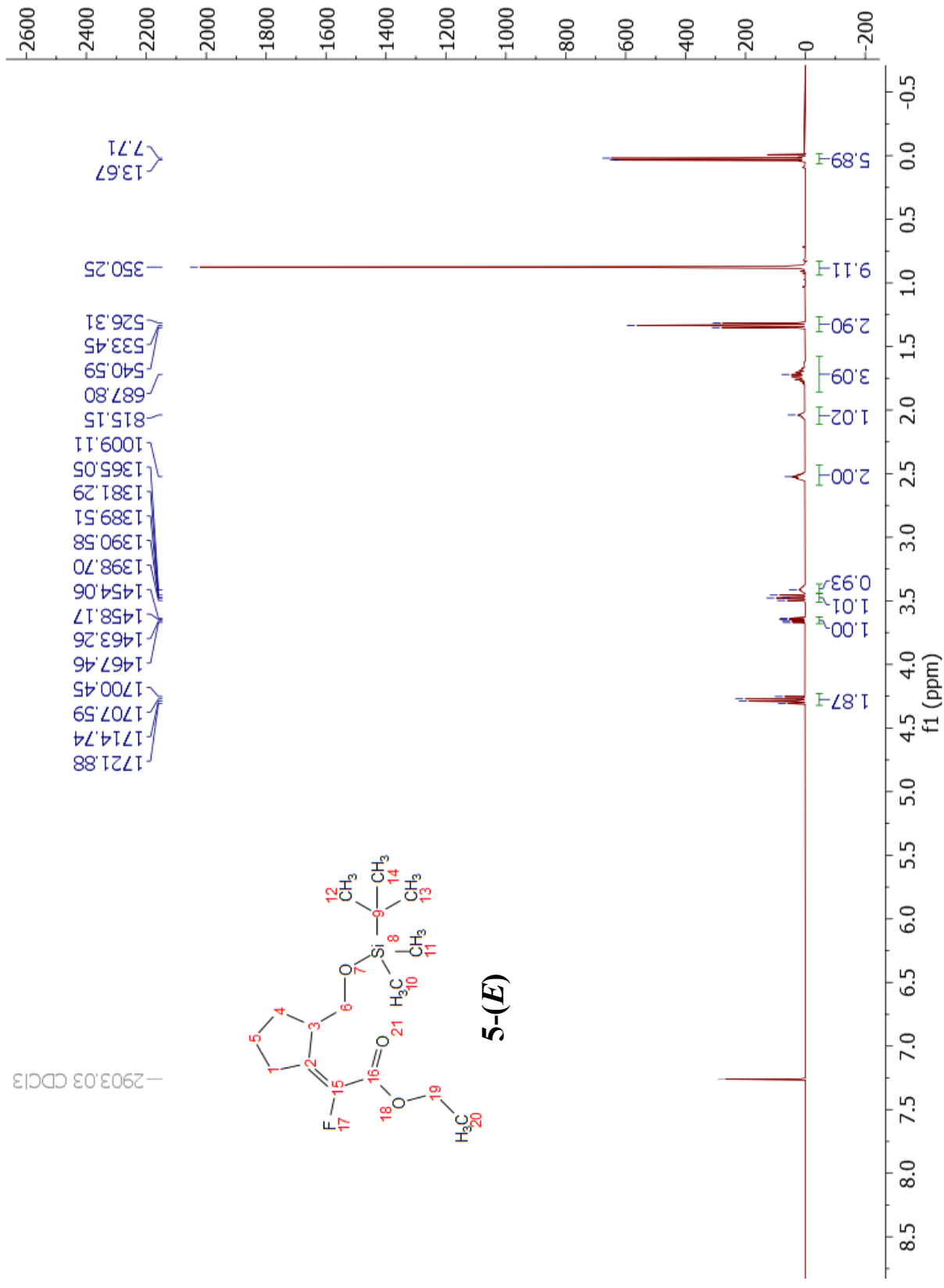
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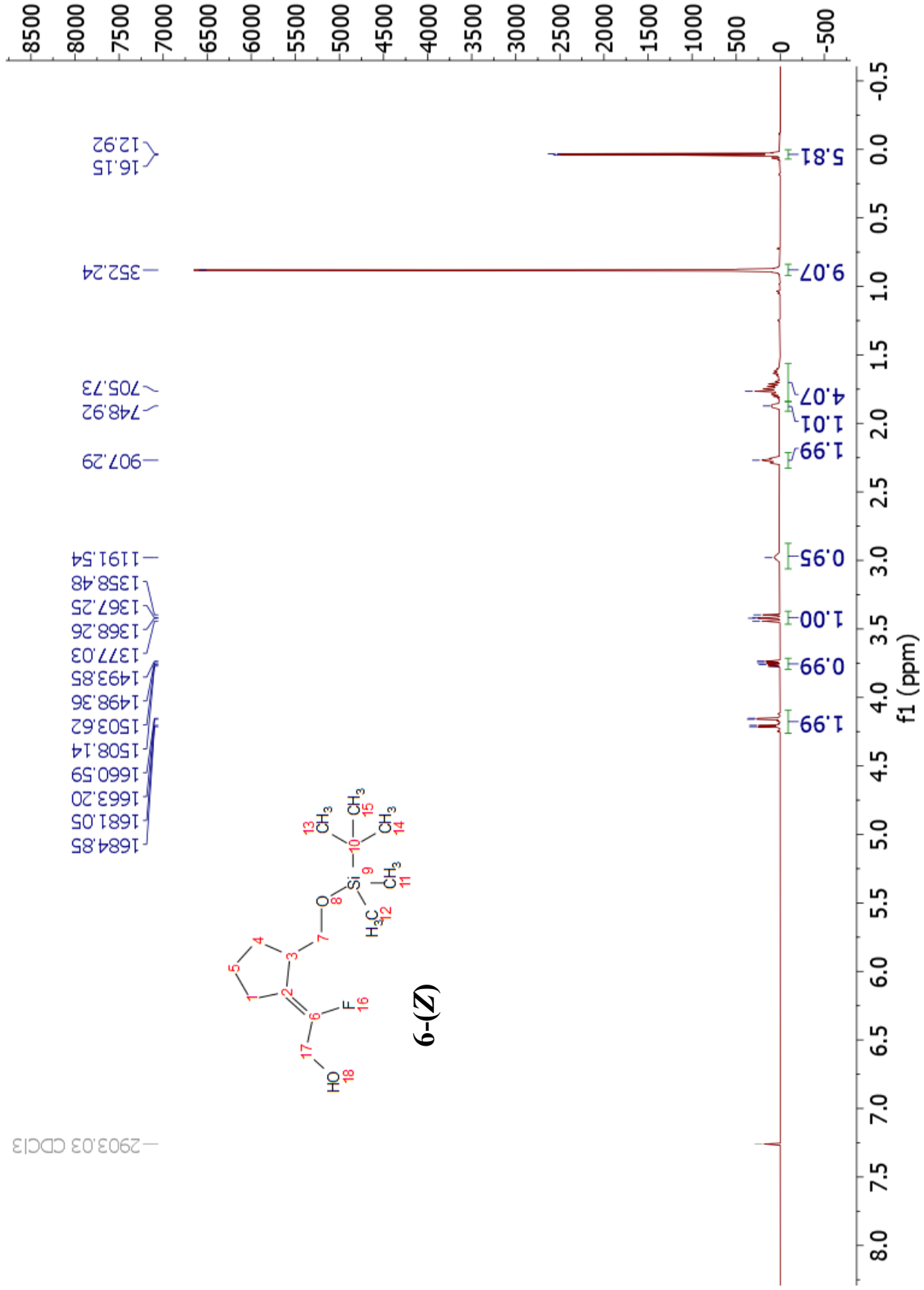


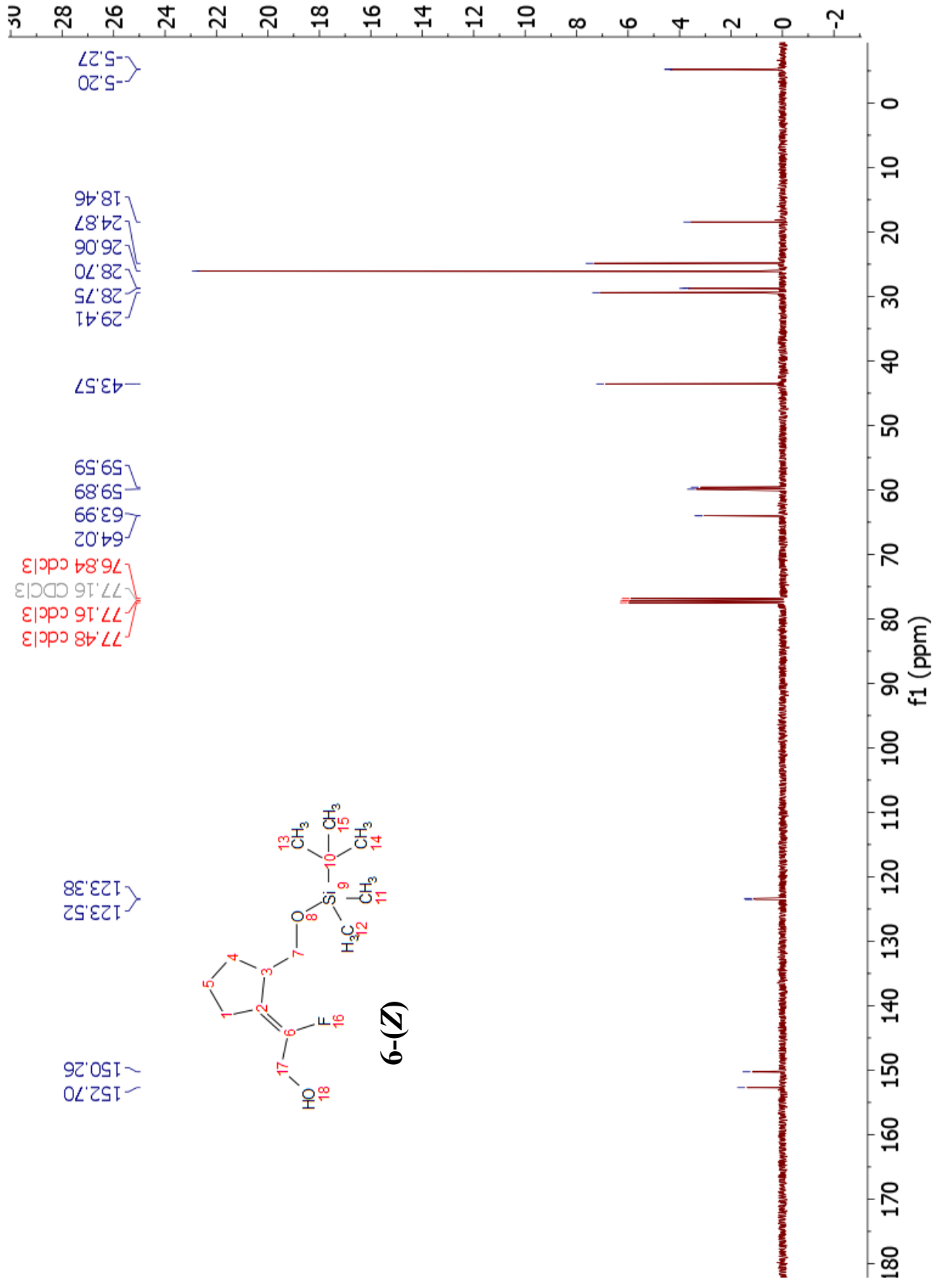


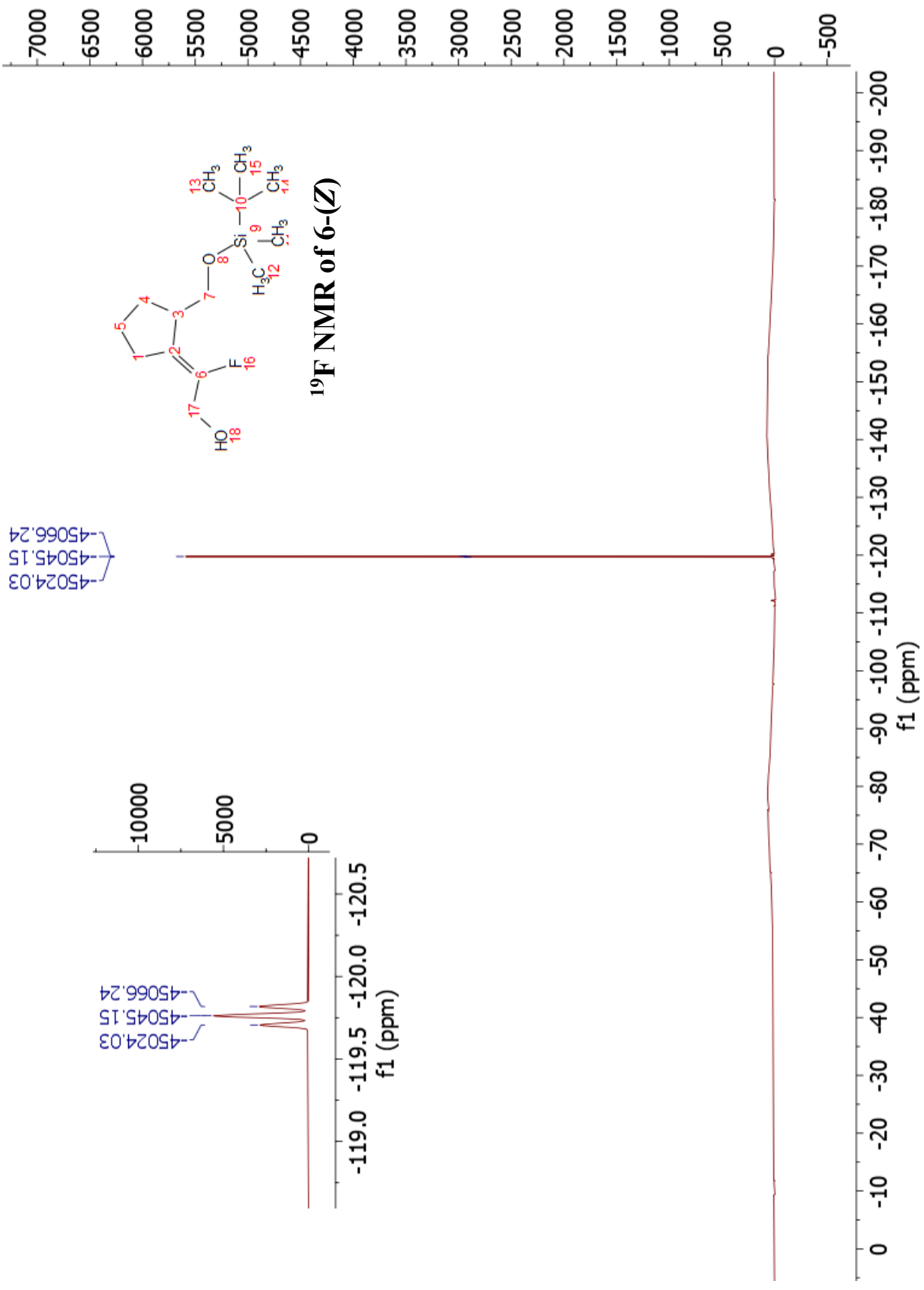


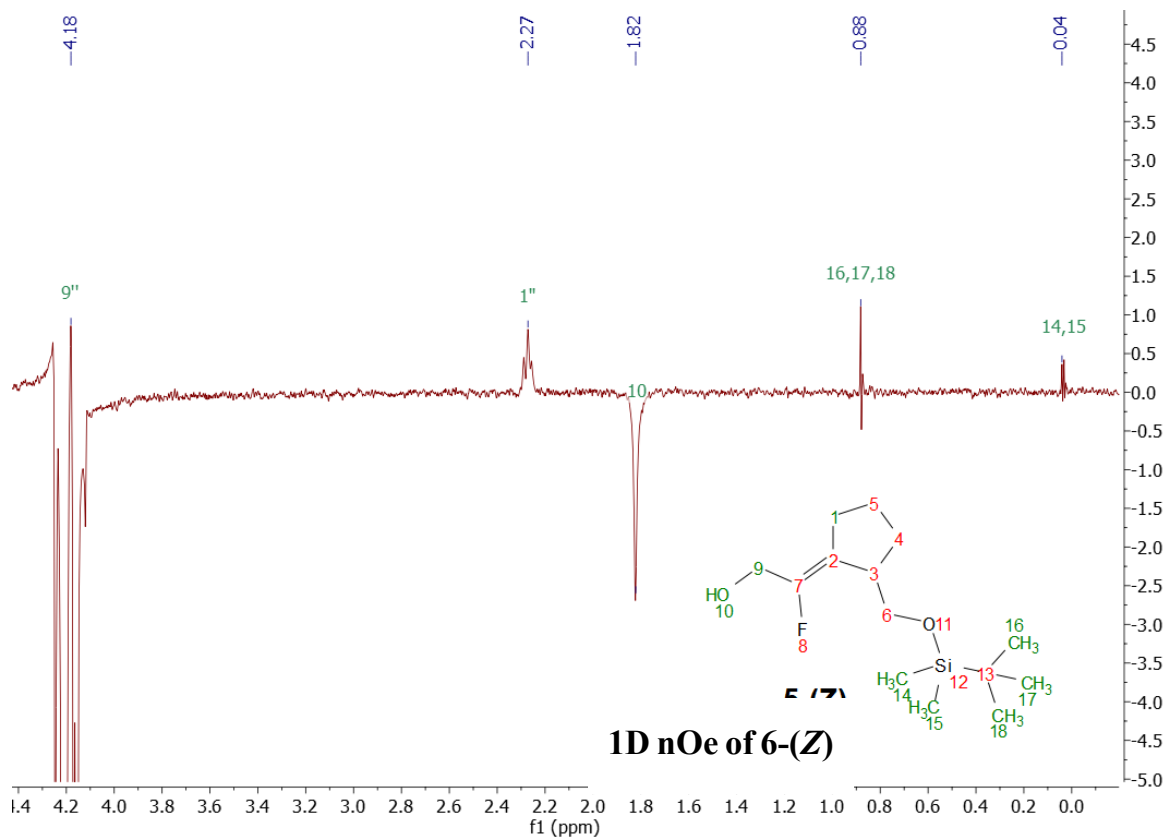
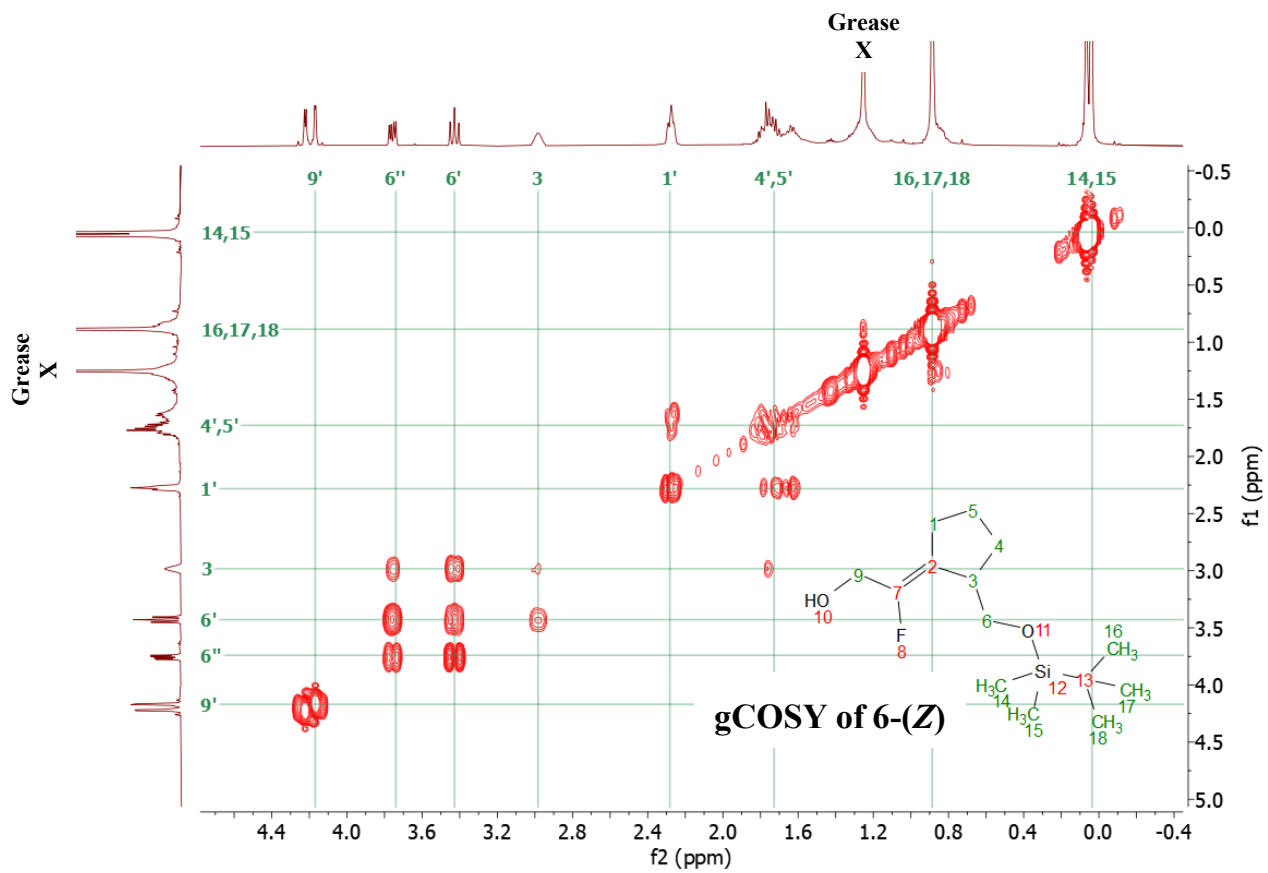
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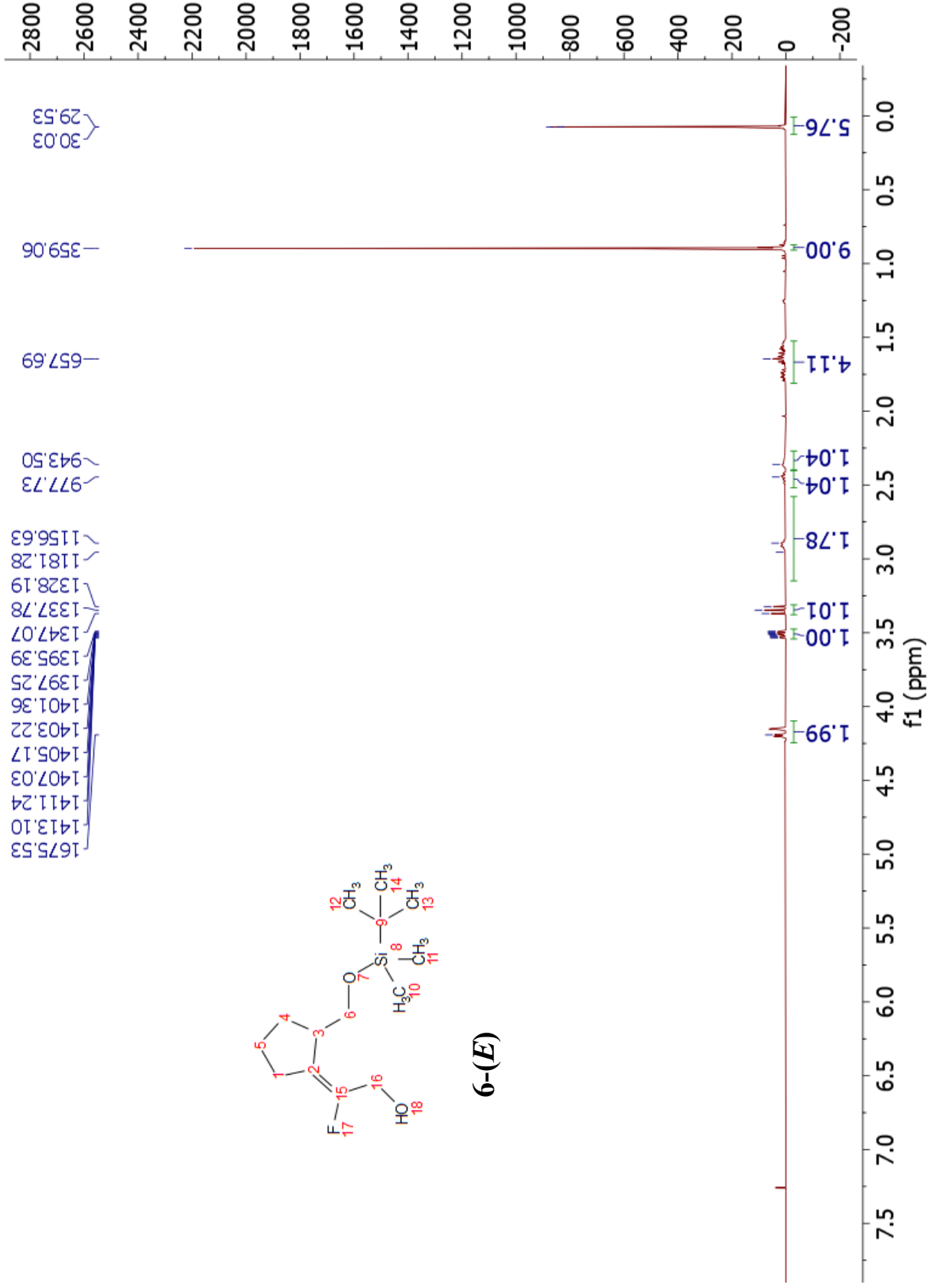
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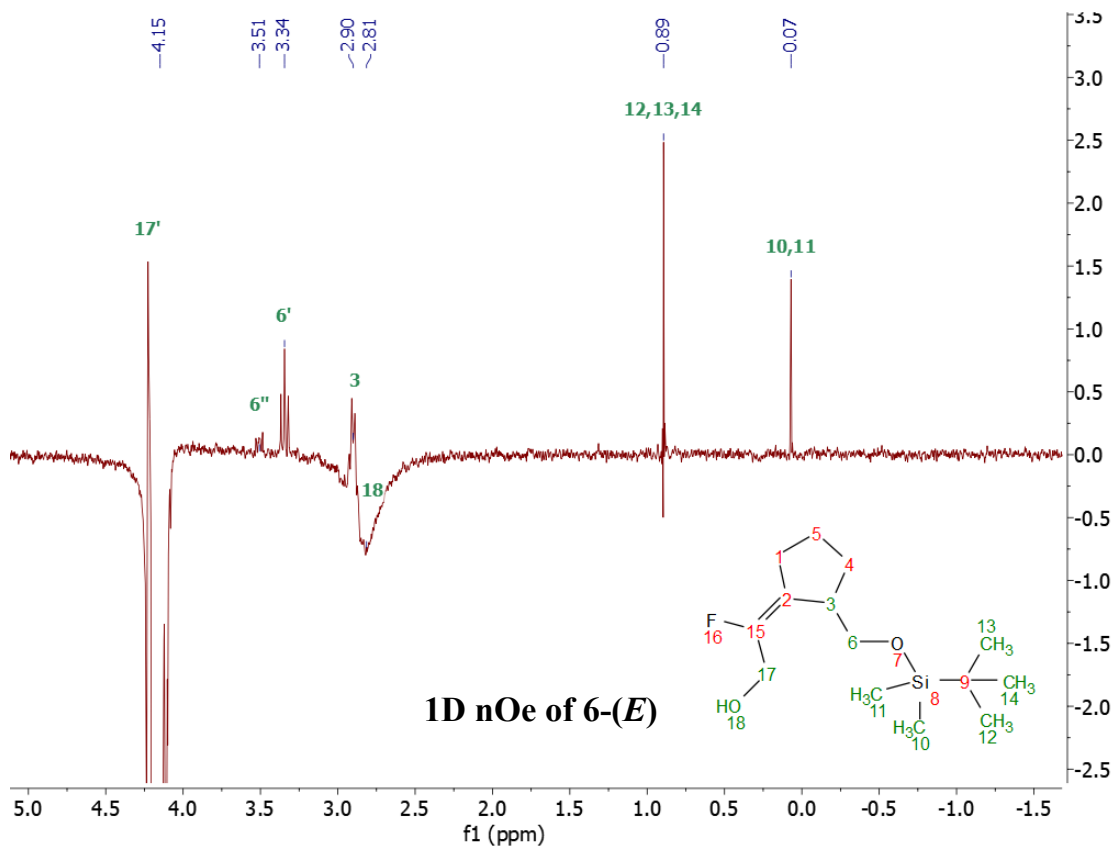
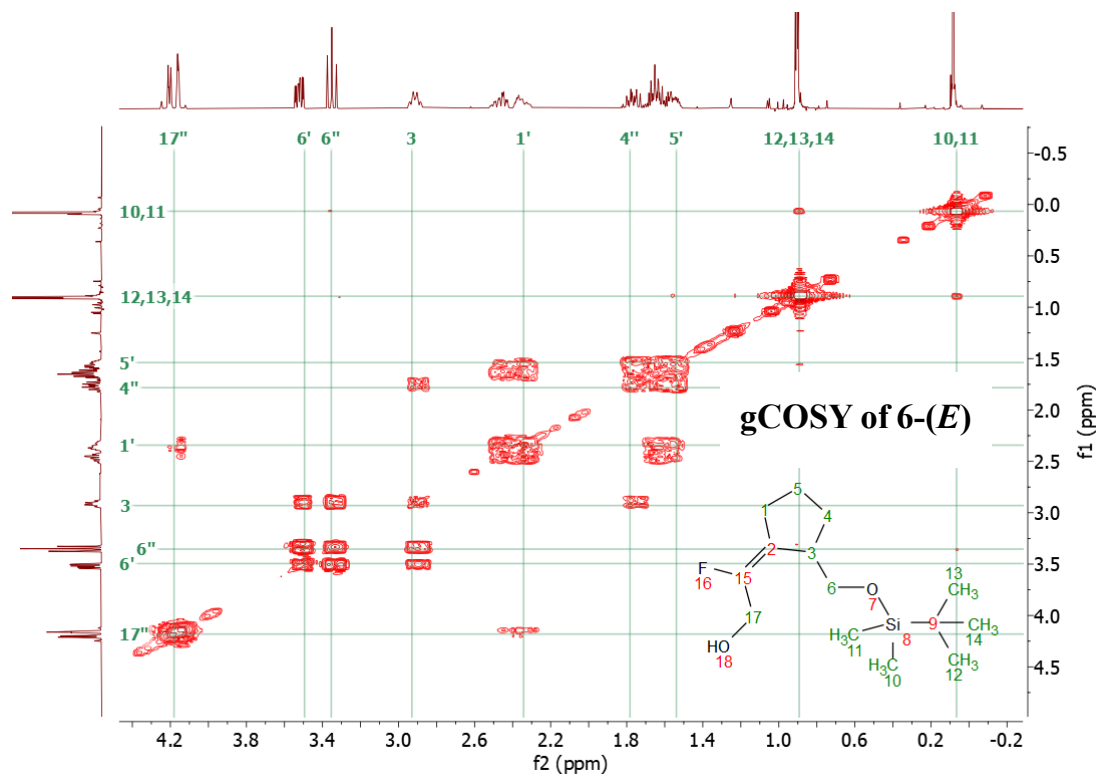


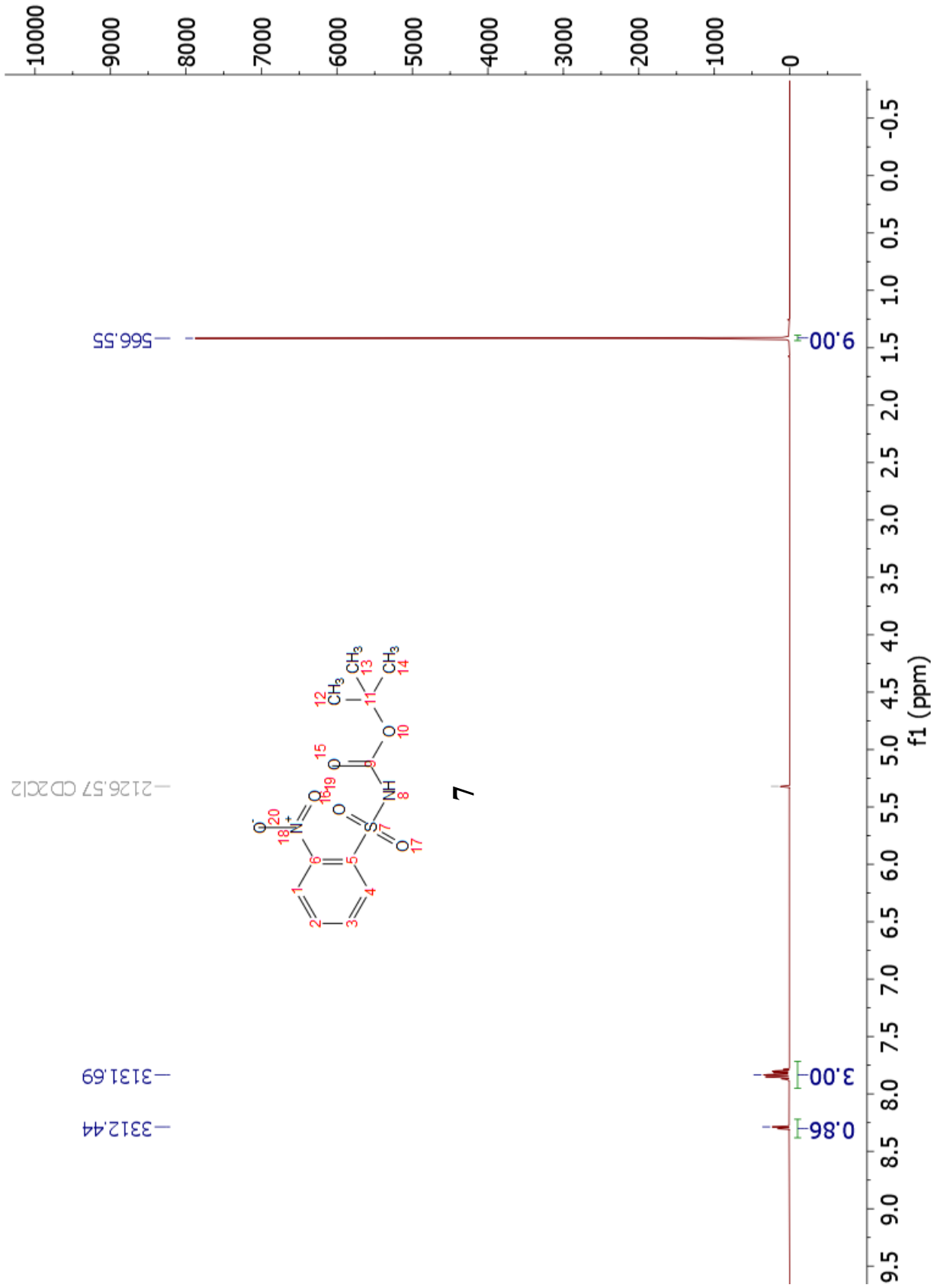












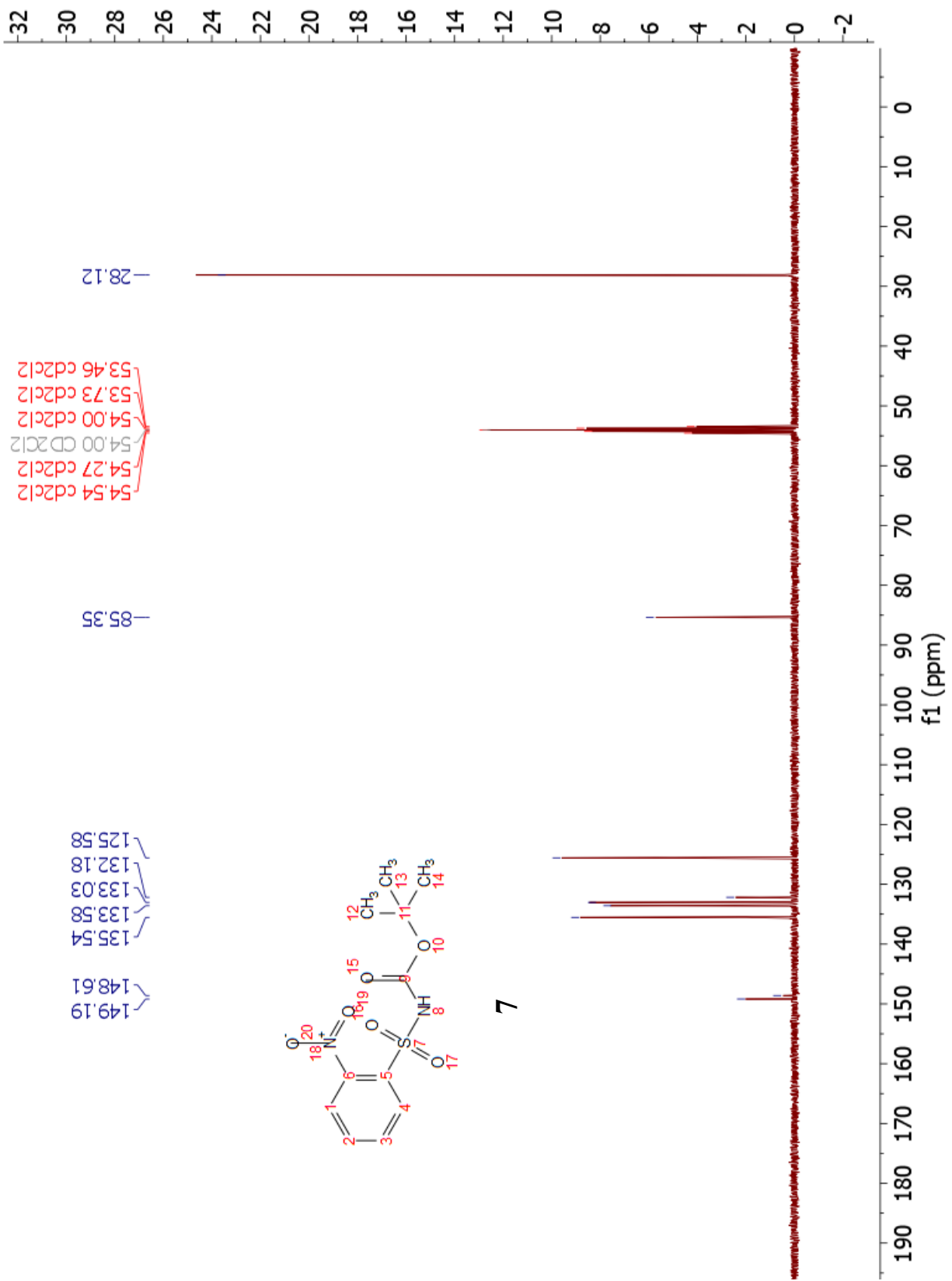
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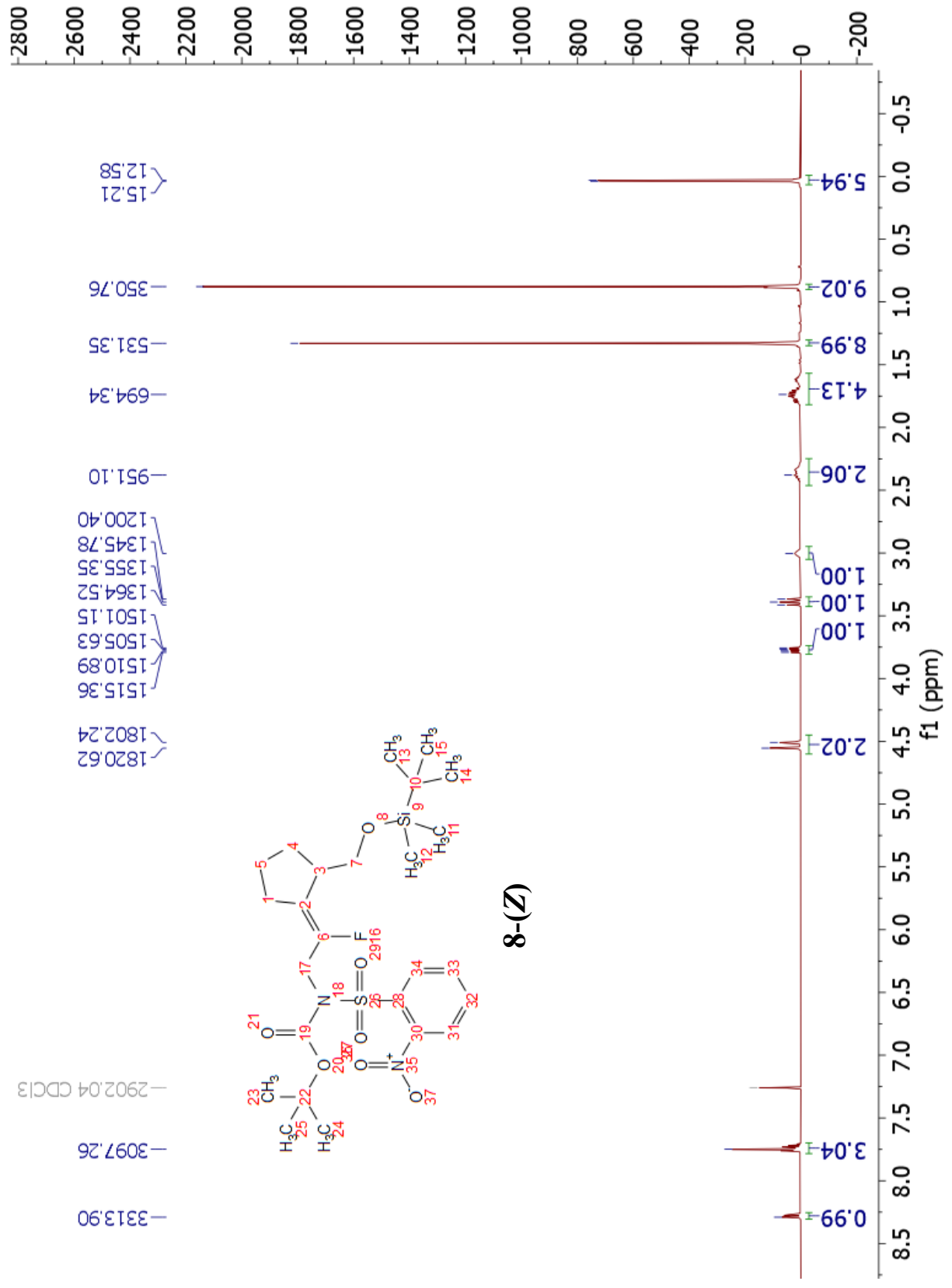
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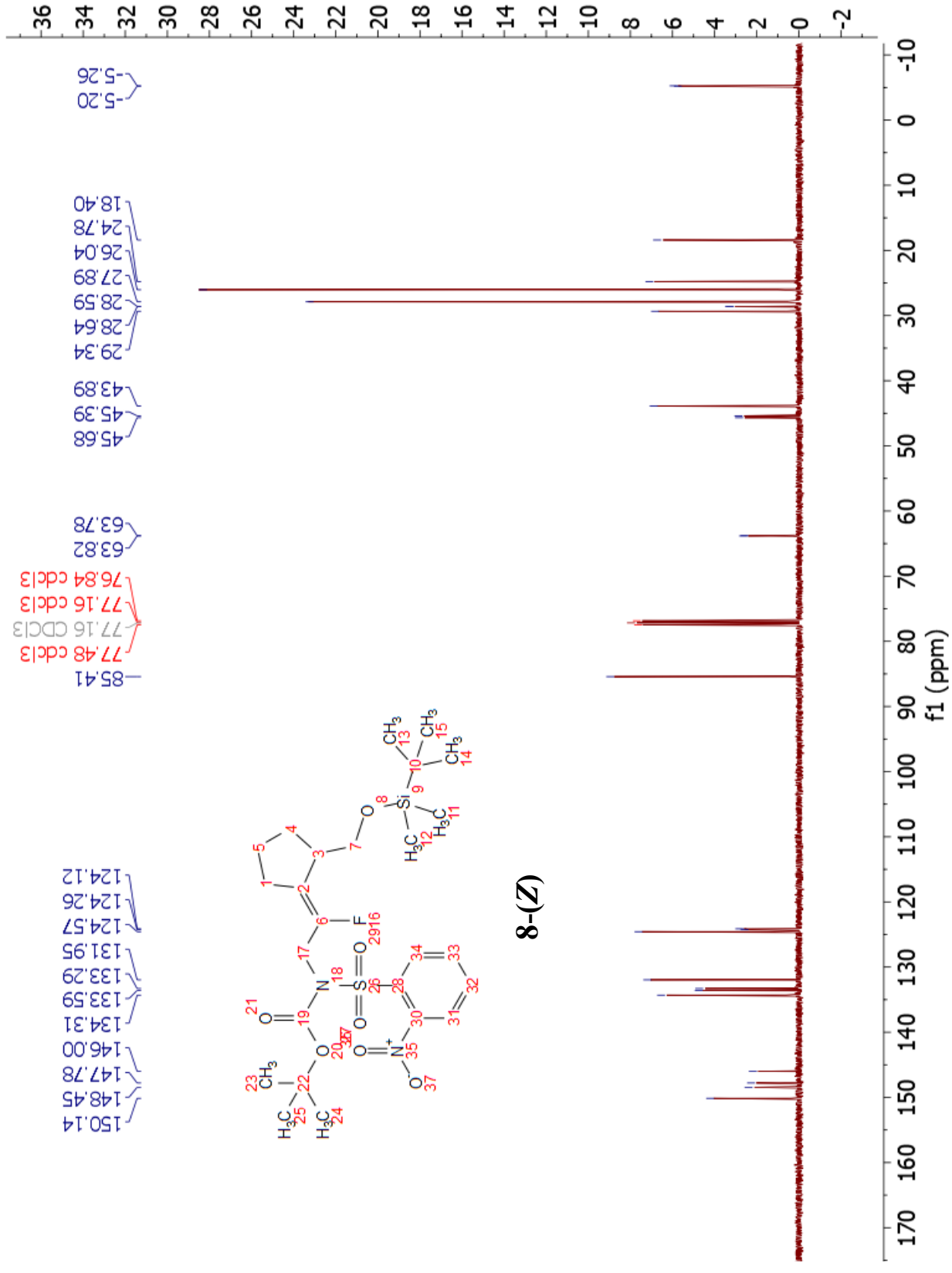
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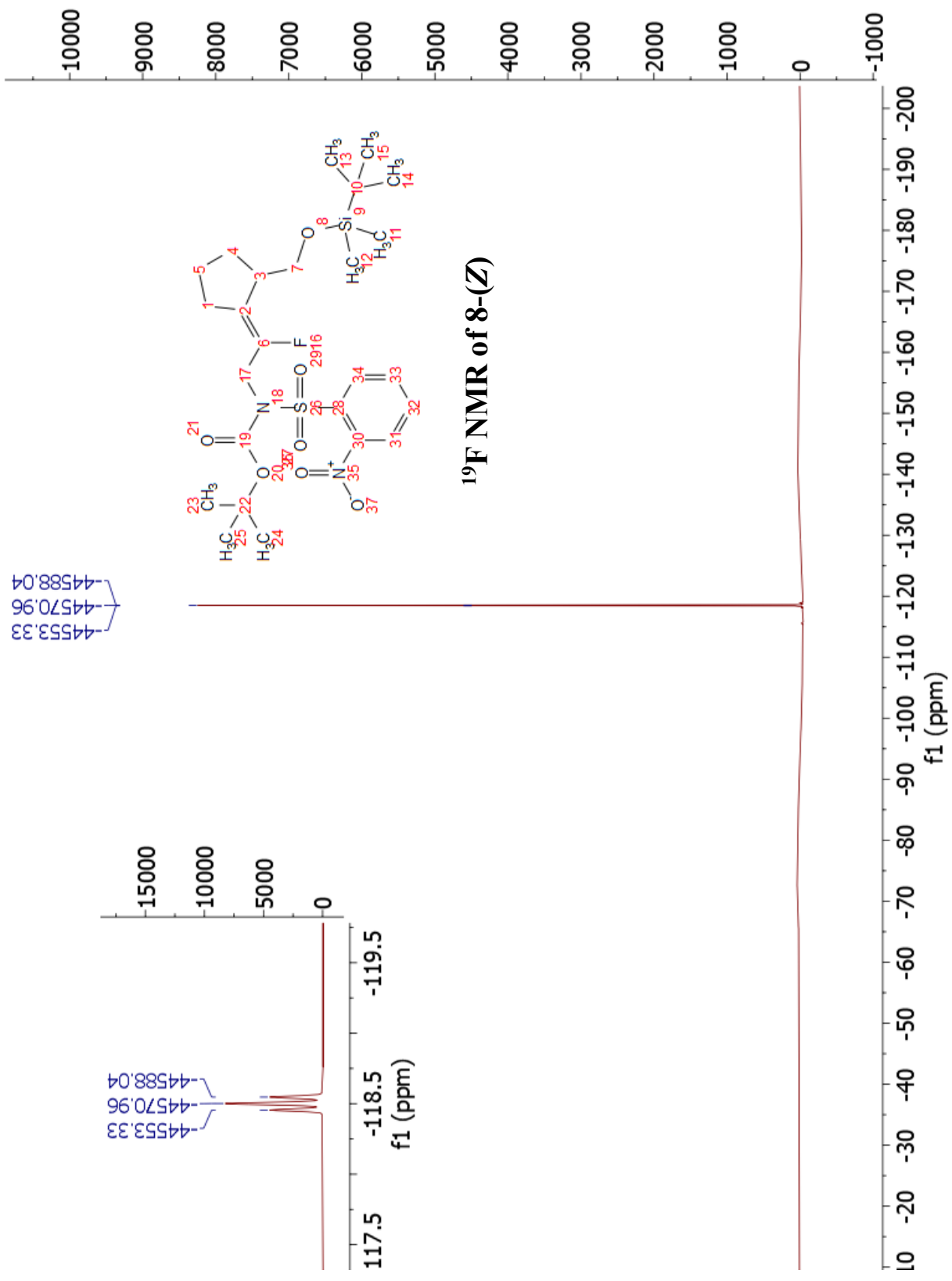
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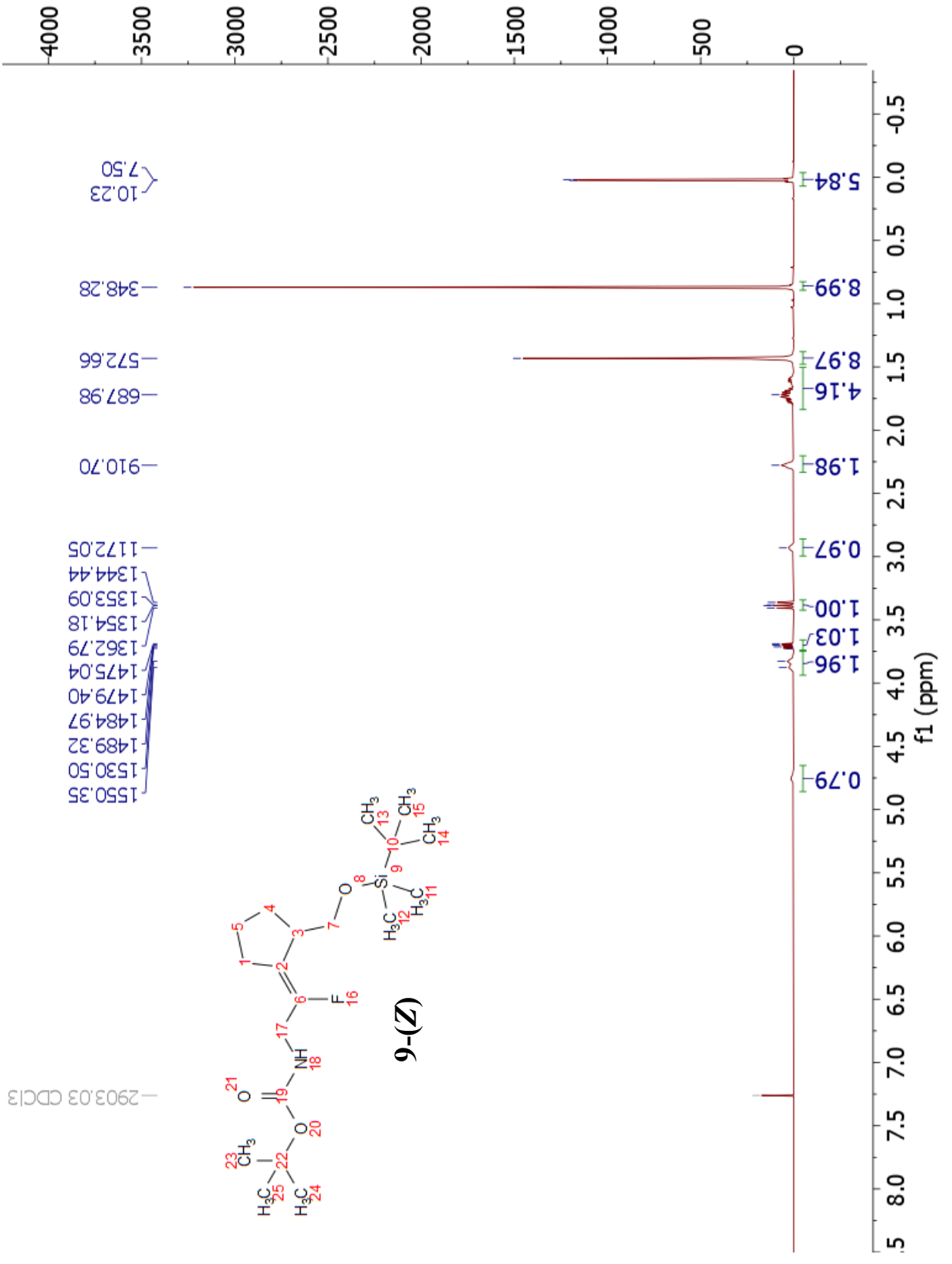


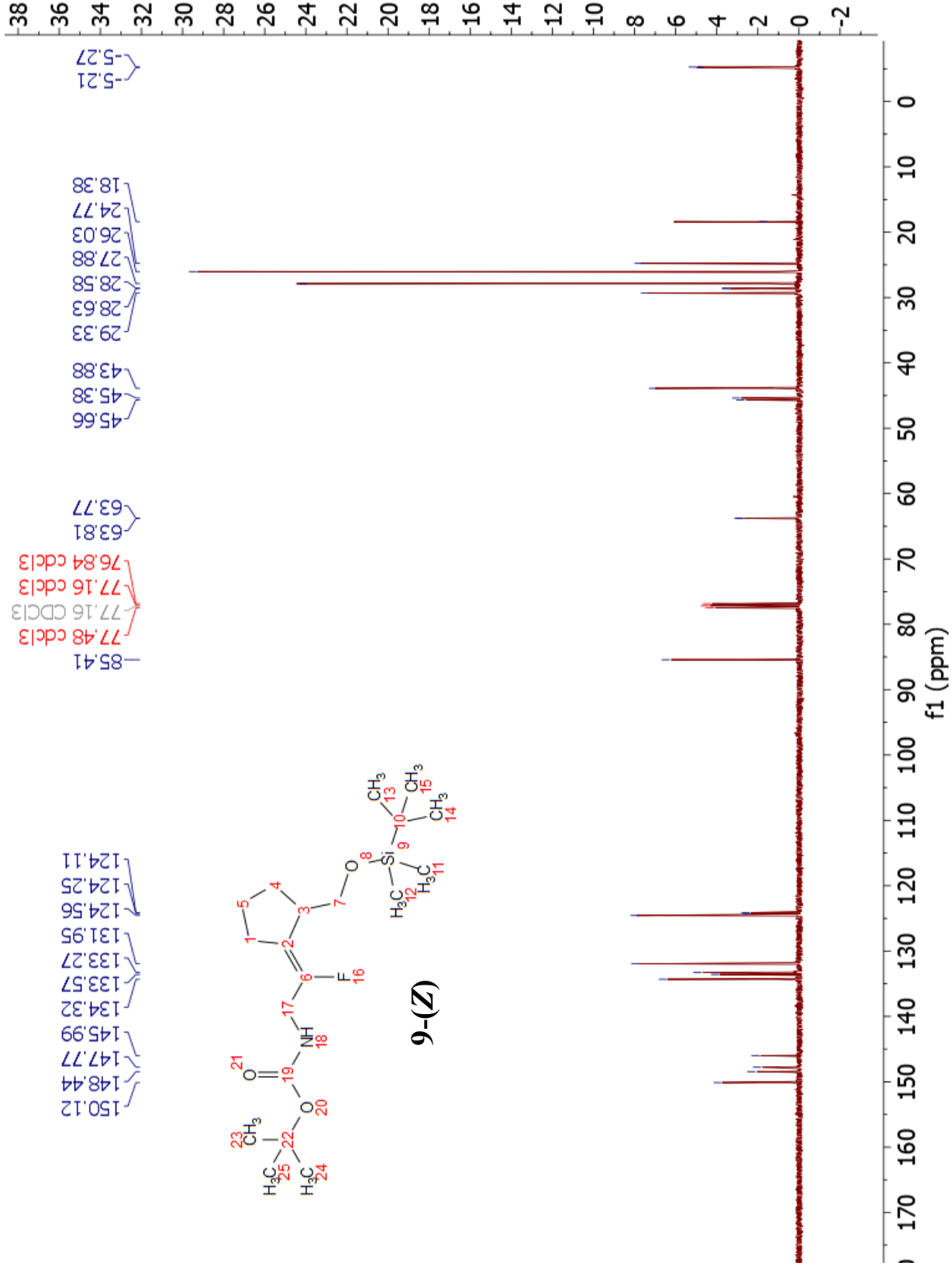


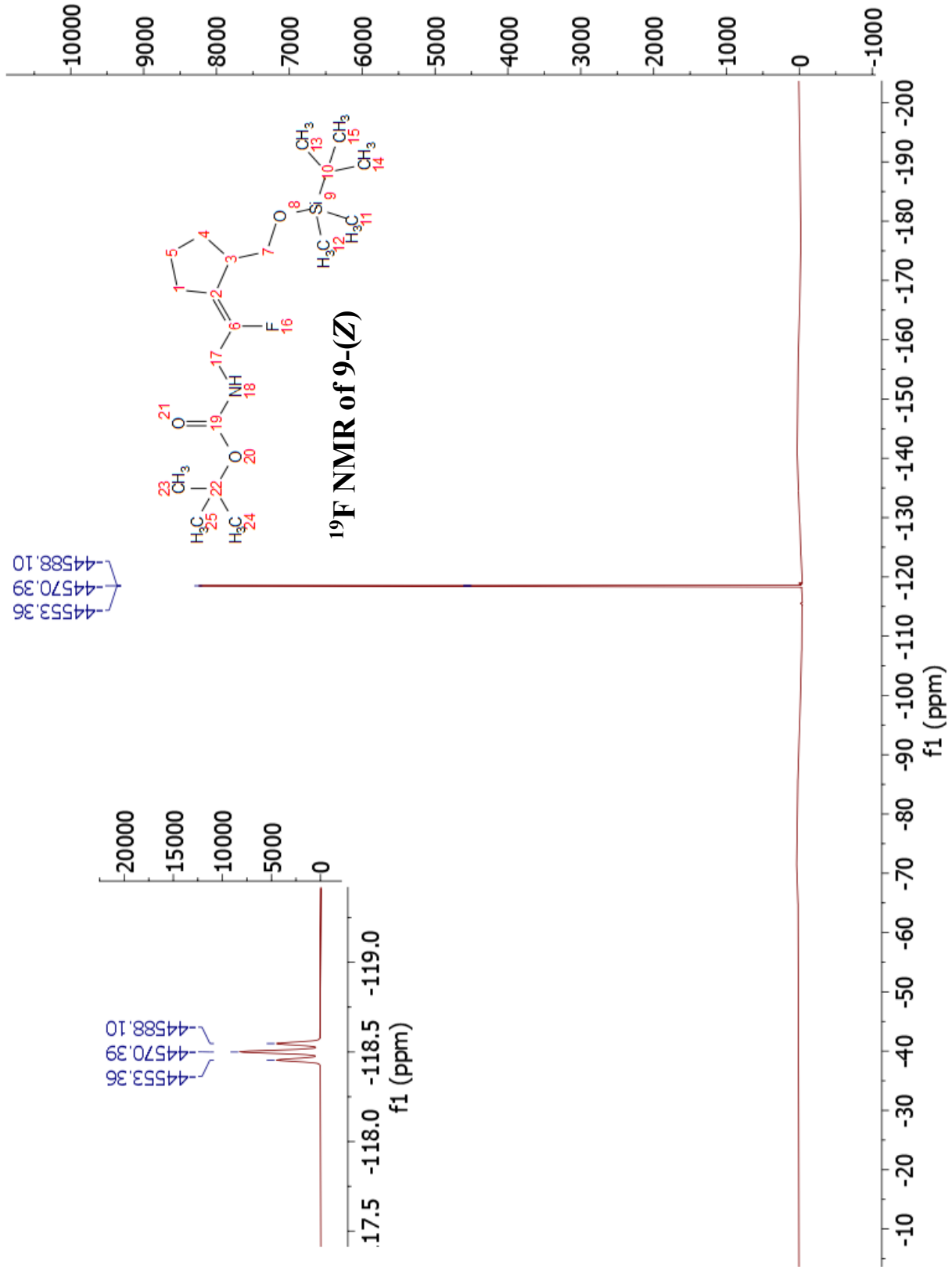


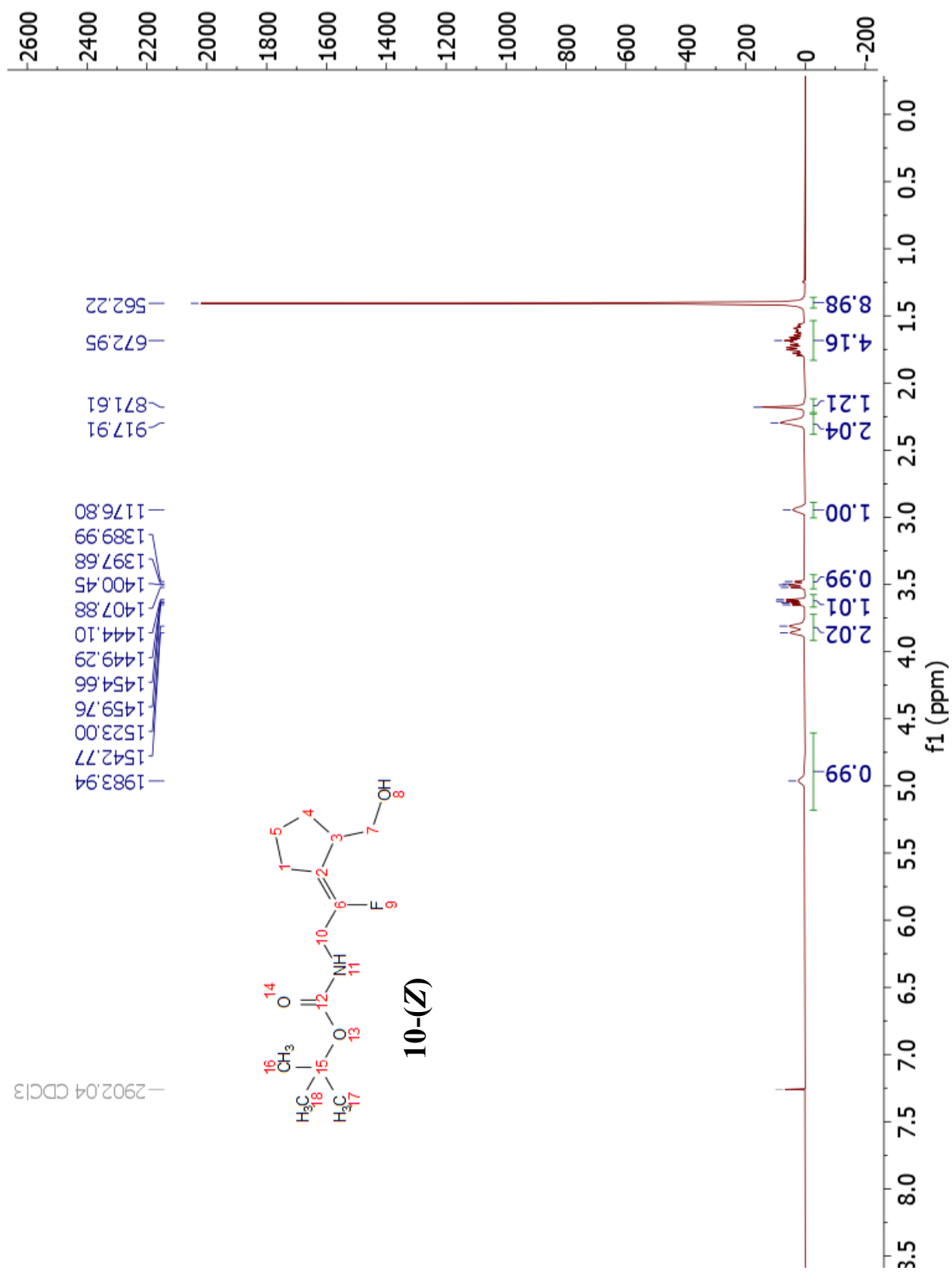


$^{19}\text{F}$  NMR of 8-(Z)

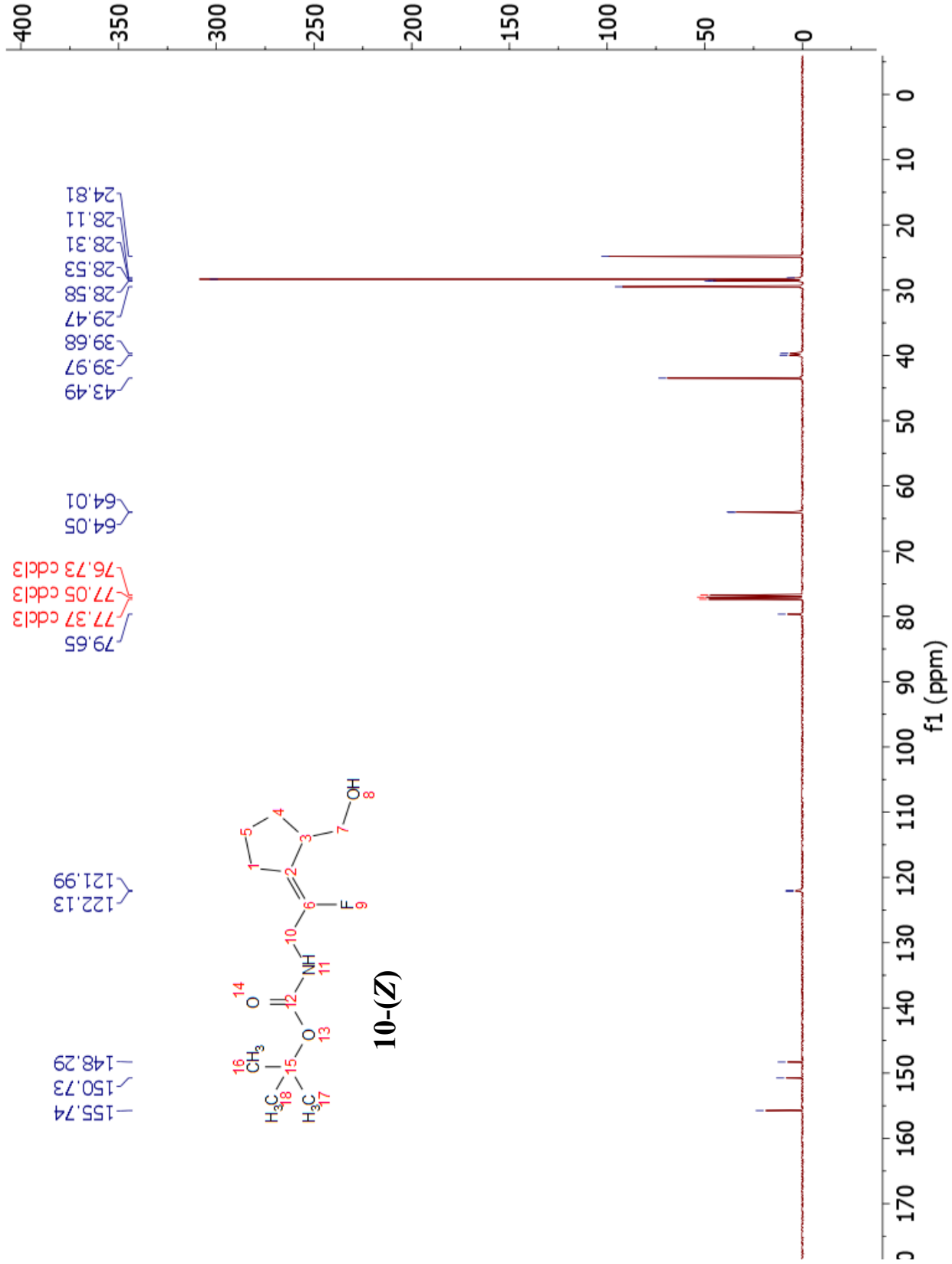






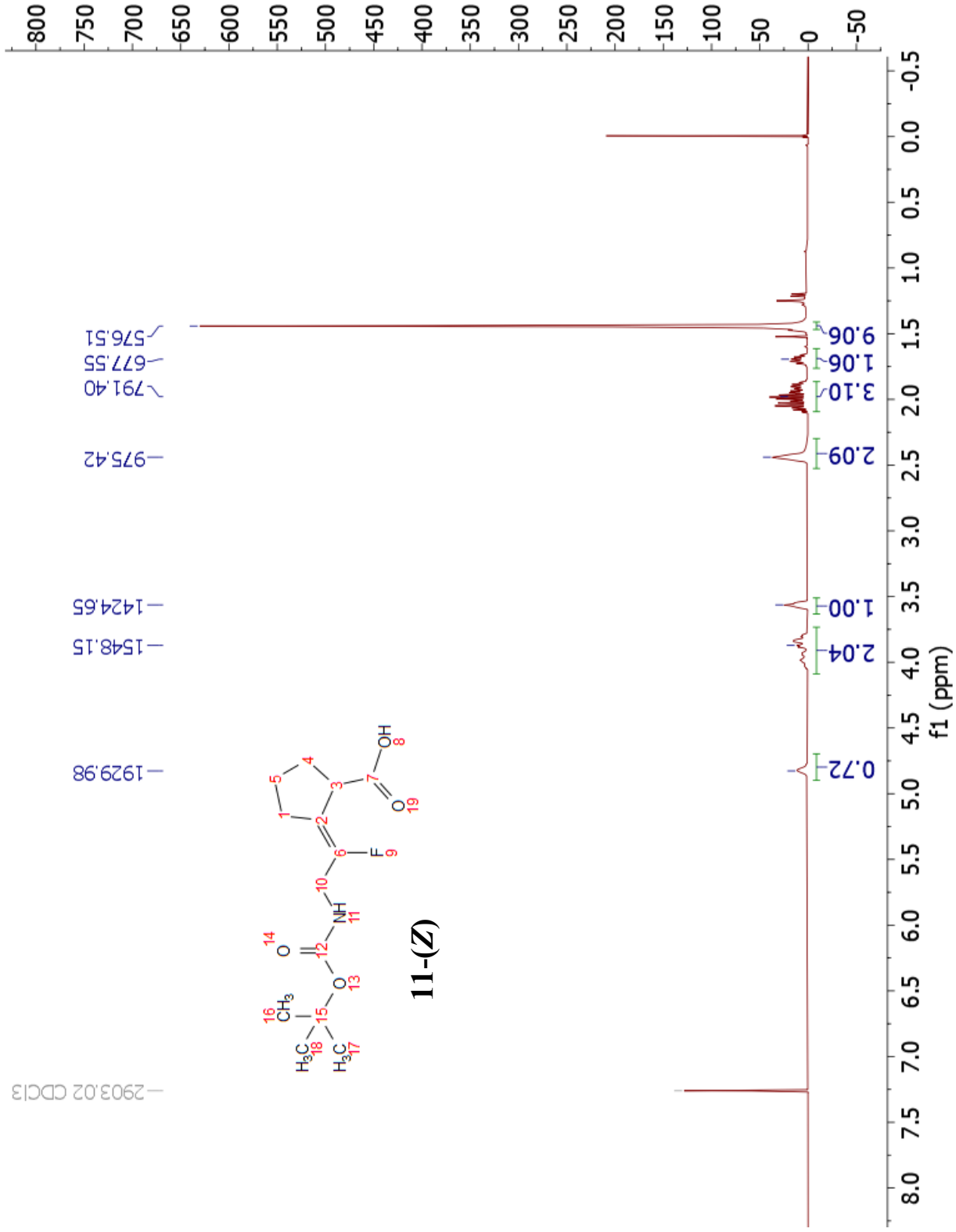


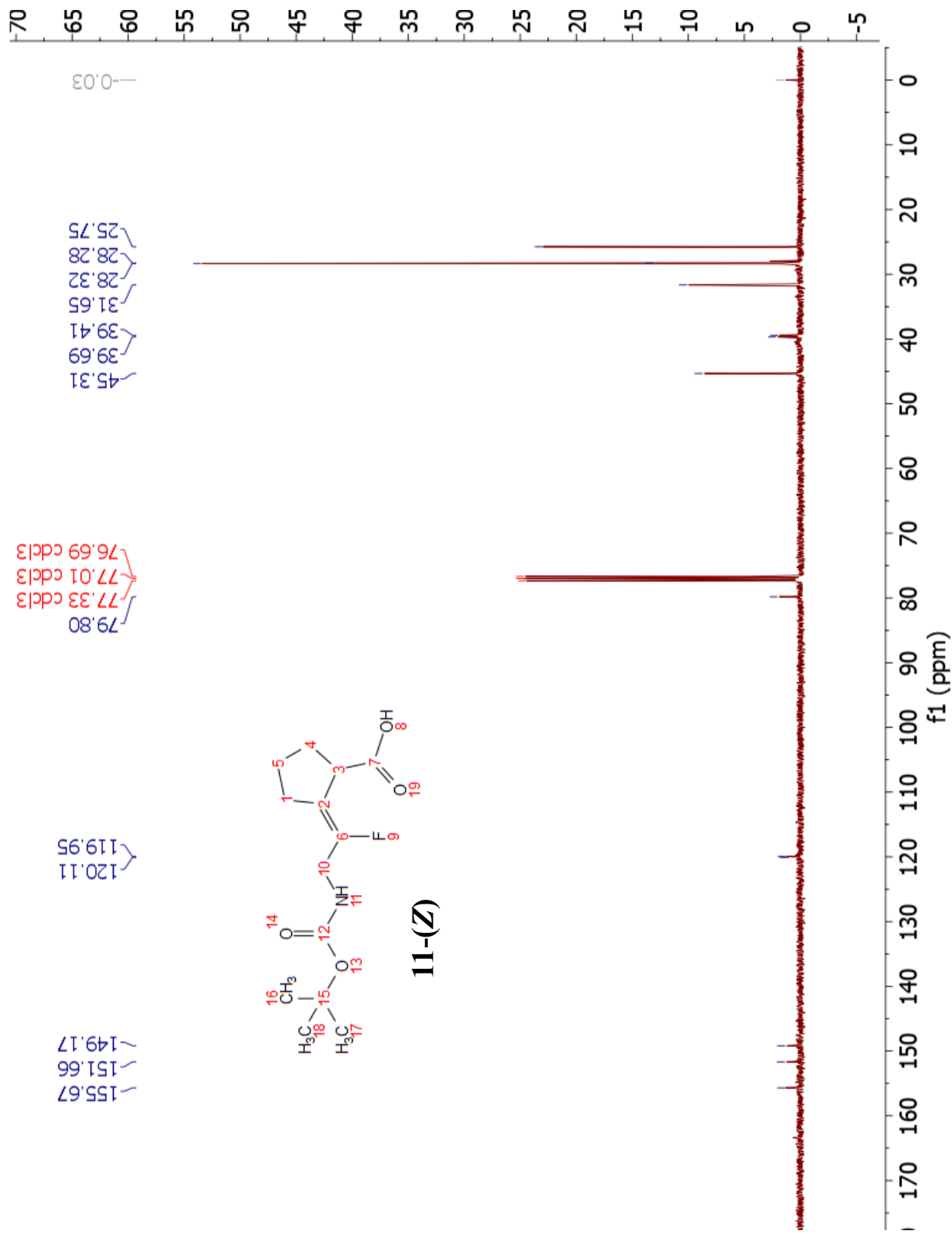


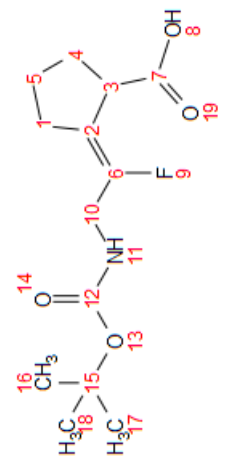
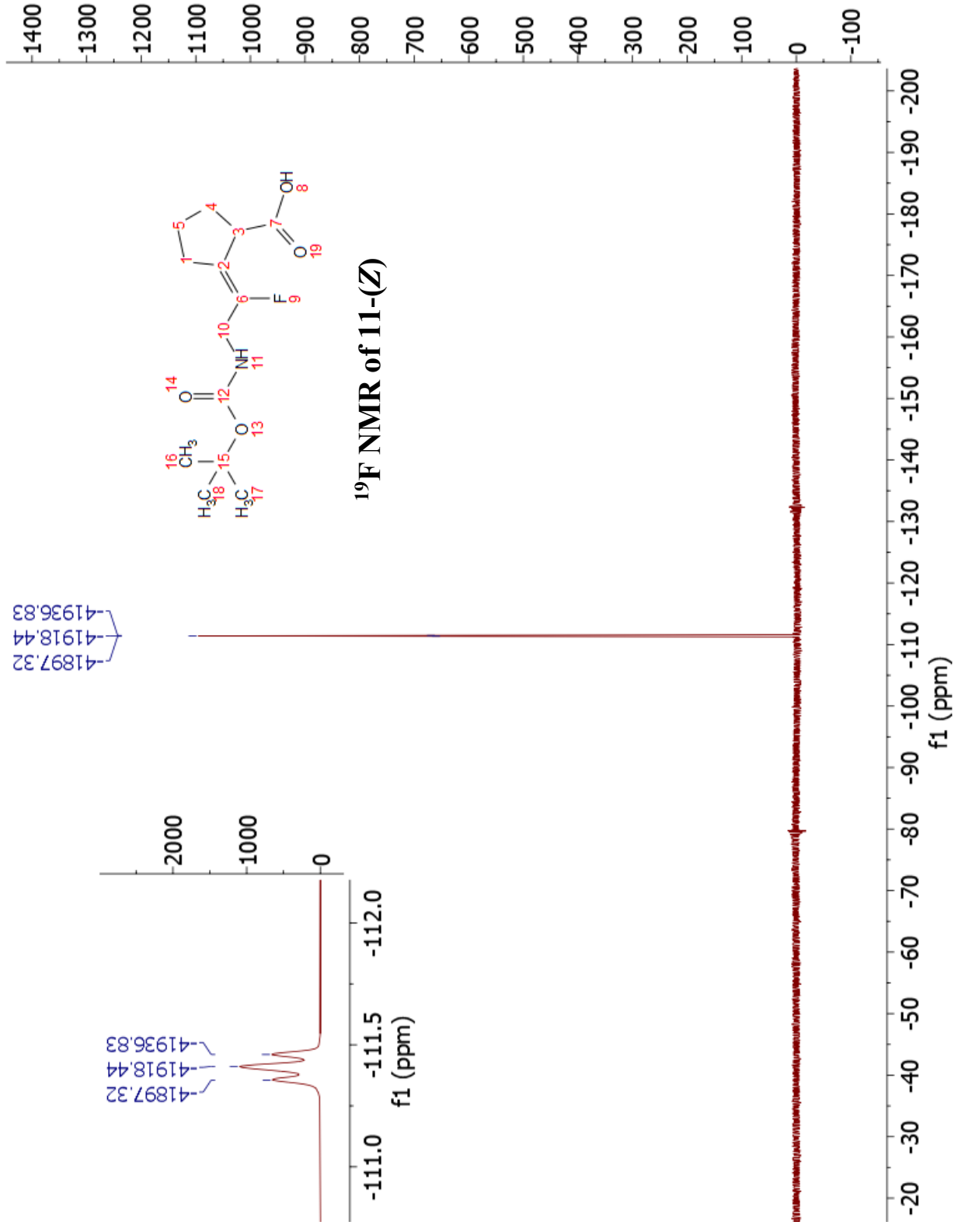


10-(Z)

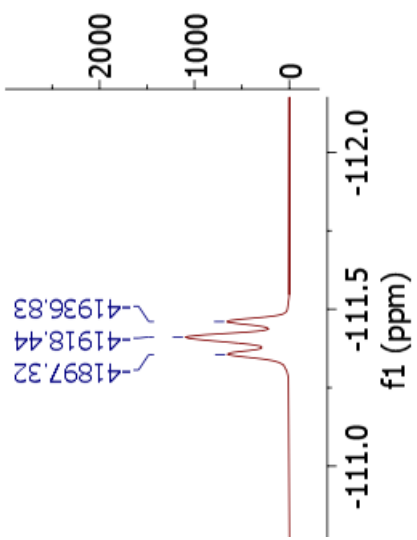




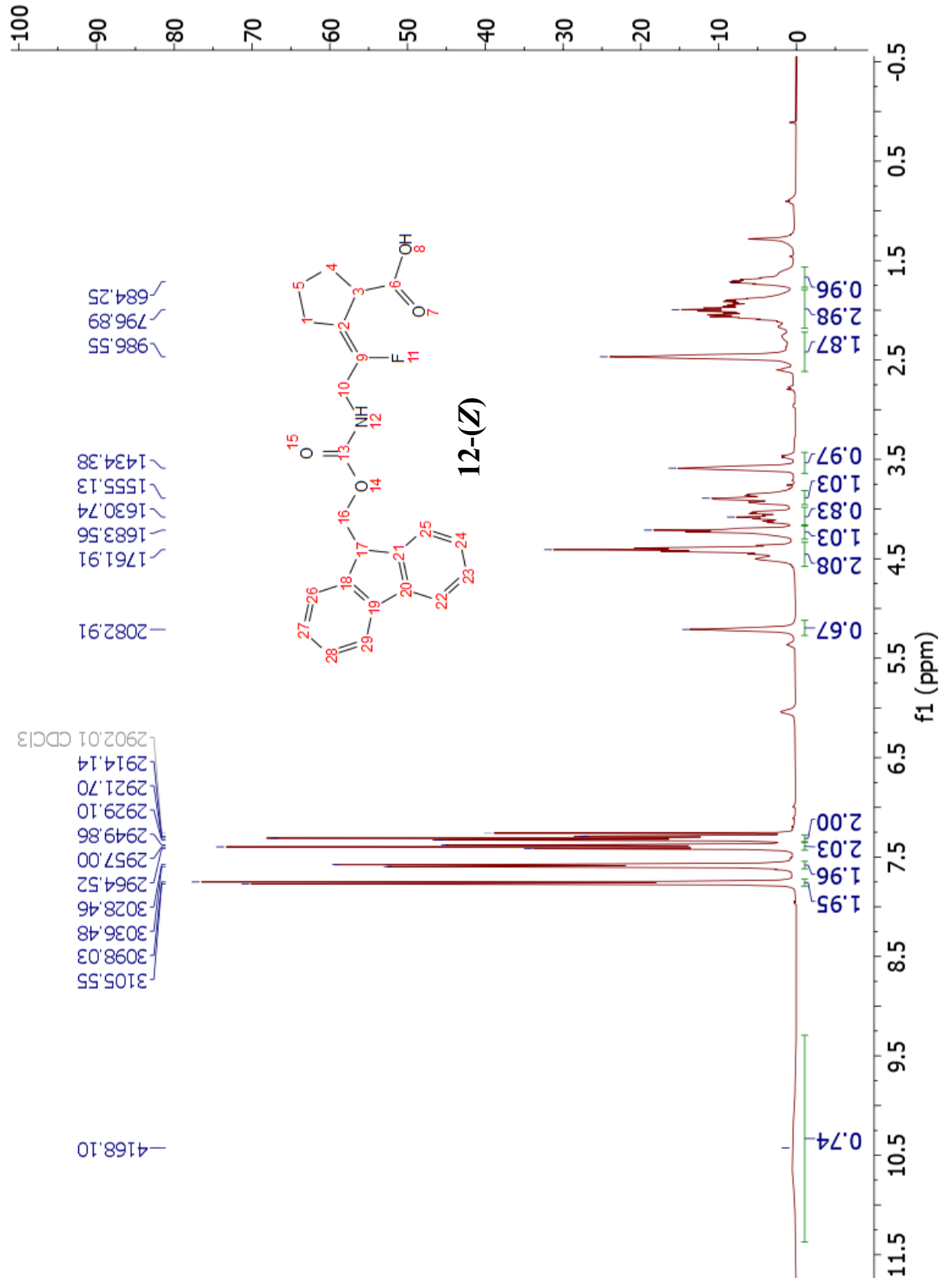


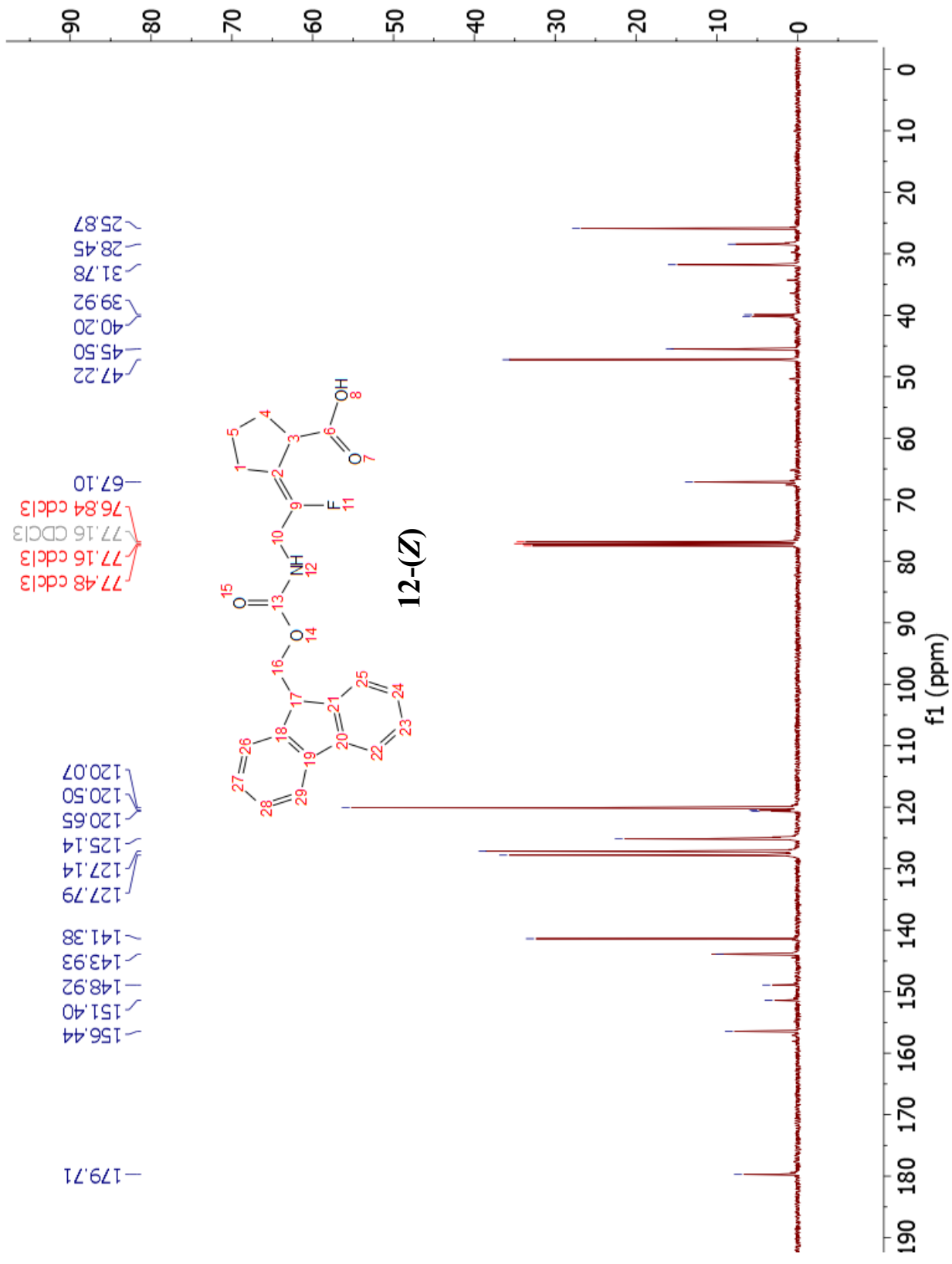


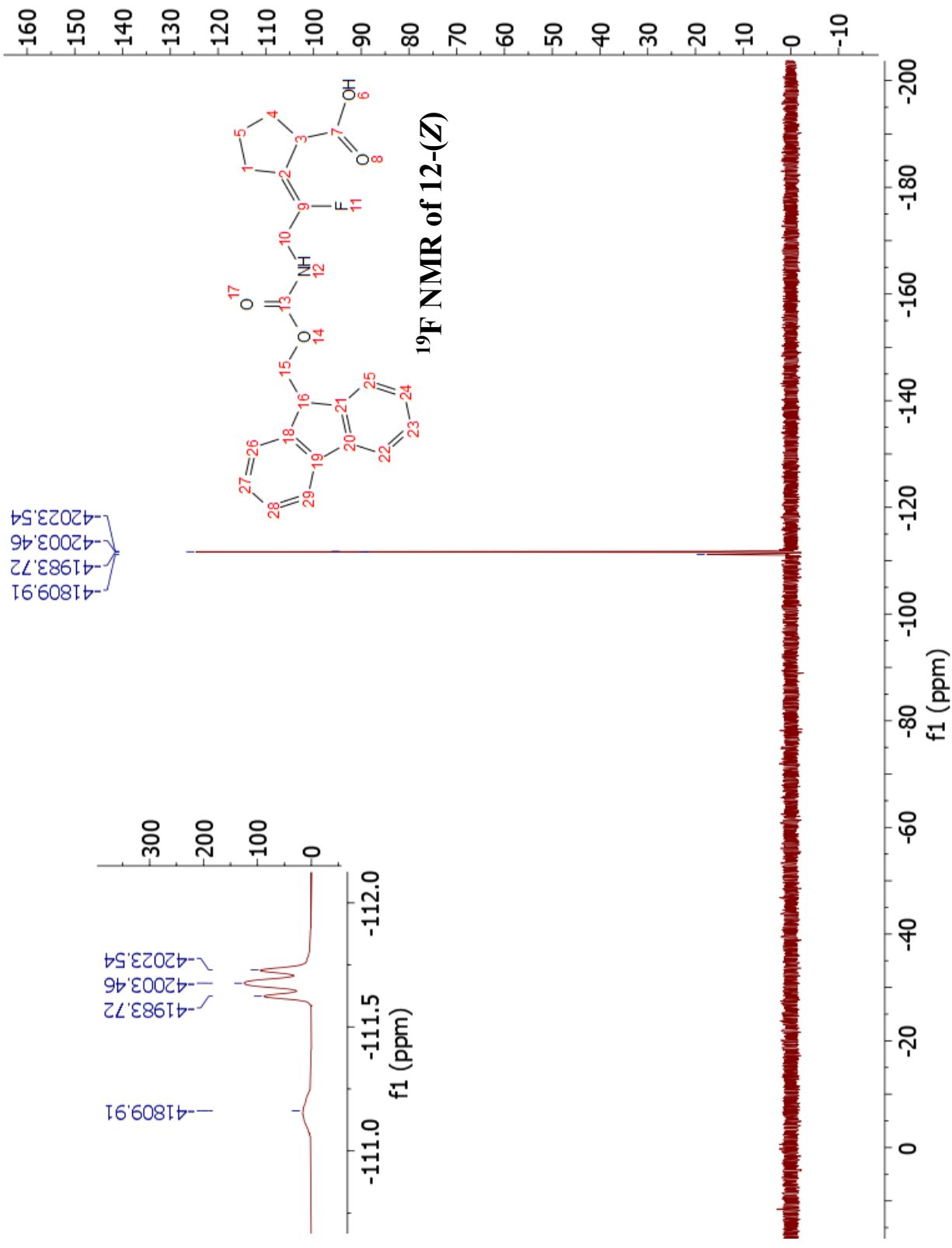
-41897.32  
-41918.44  
-41936.83



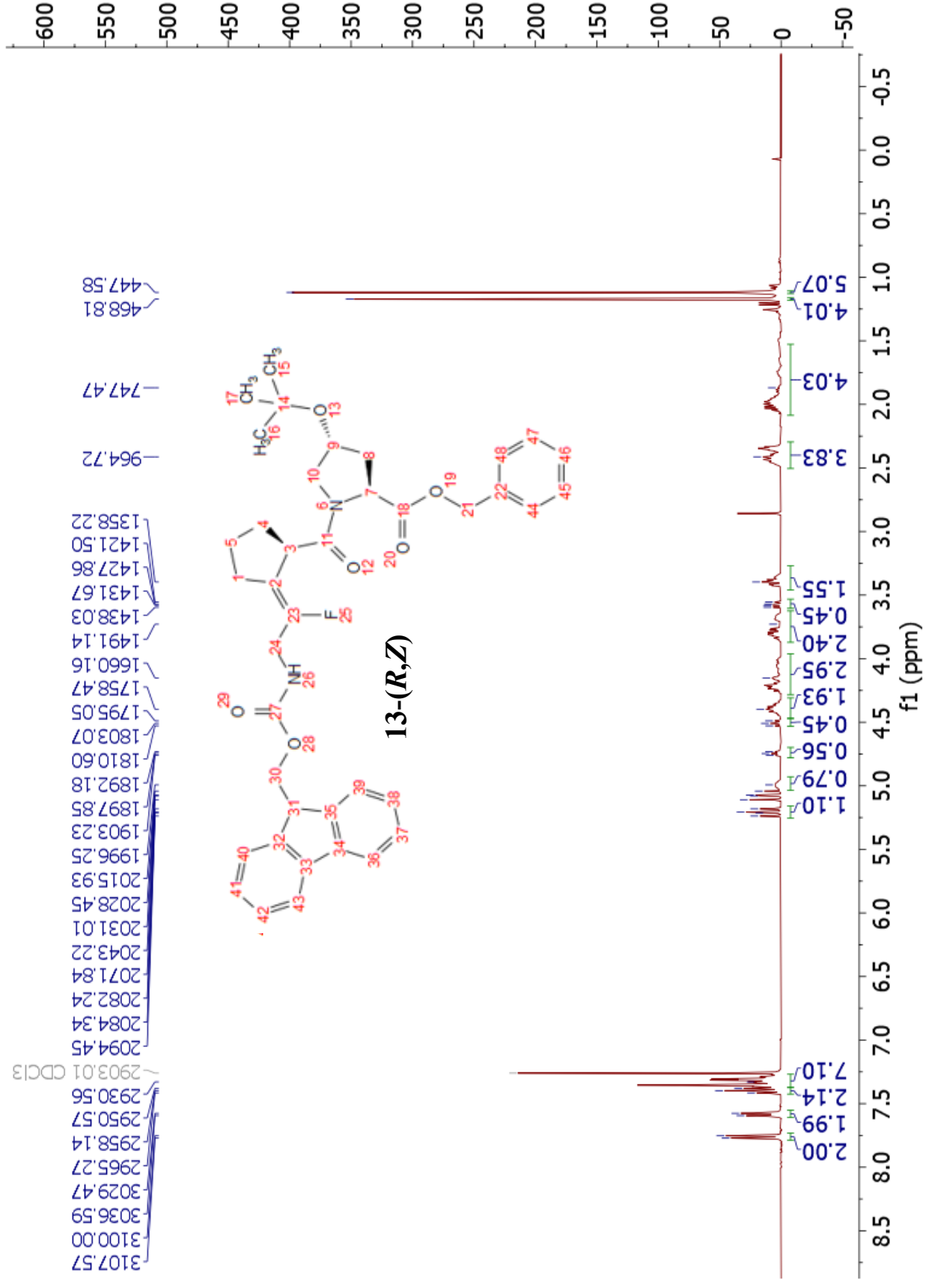
-41897.32  
-41918.44  
-41936.83

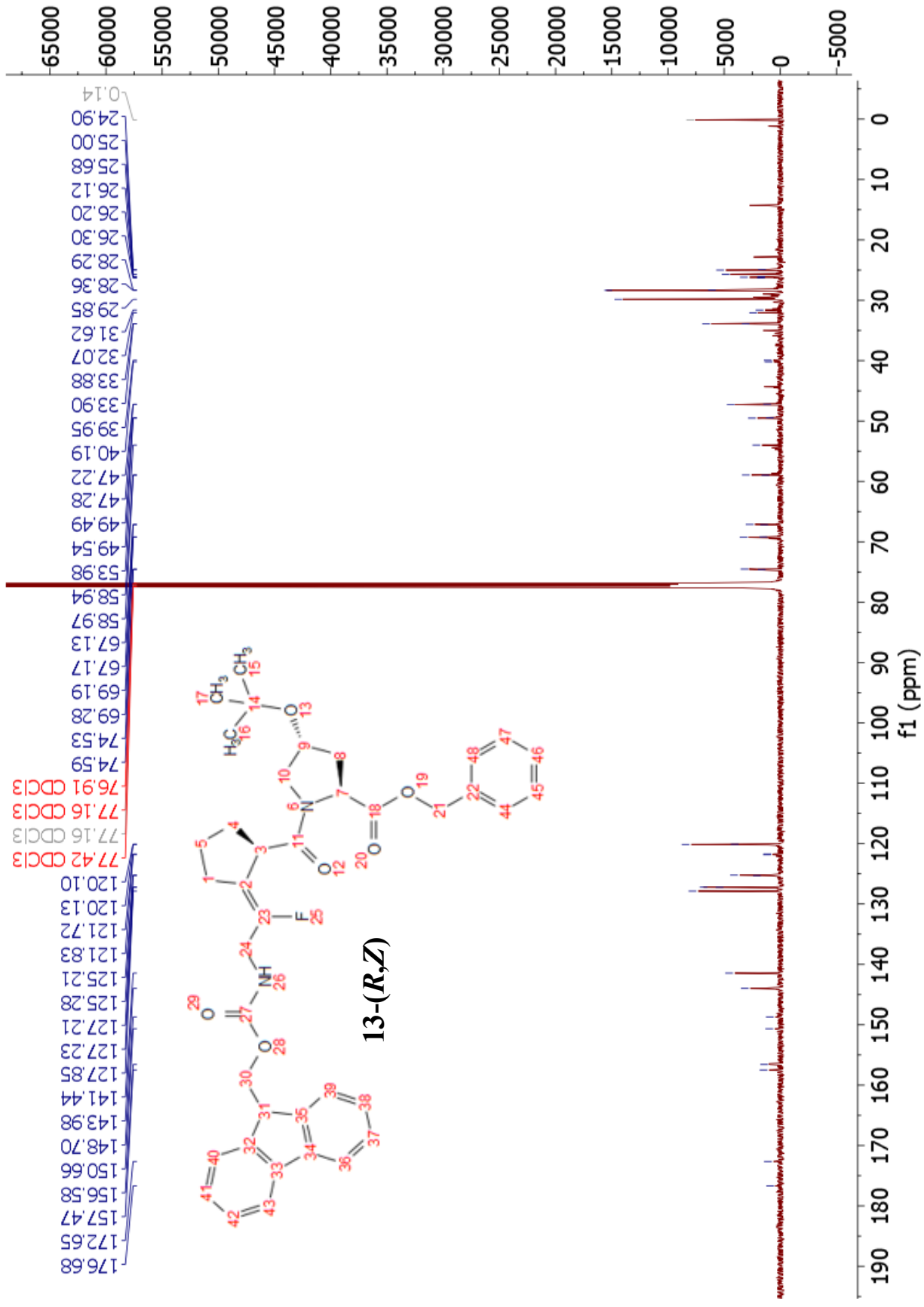


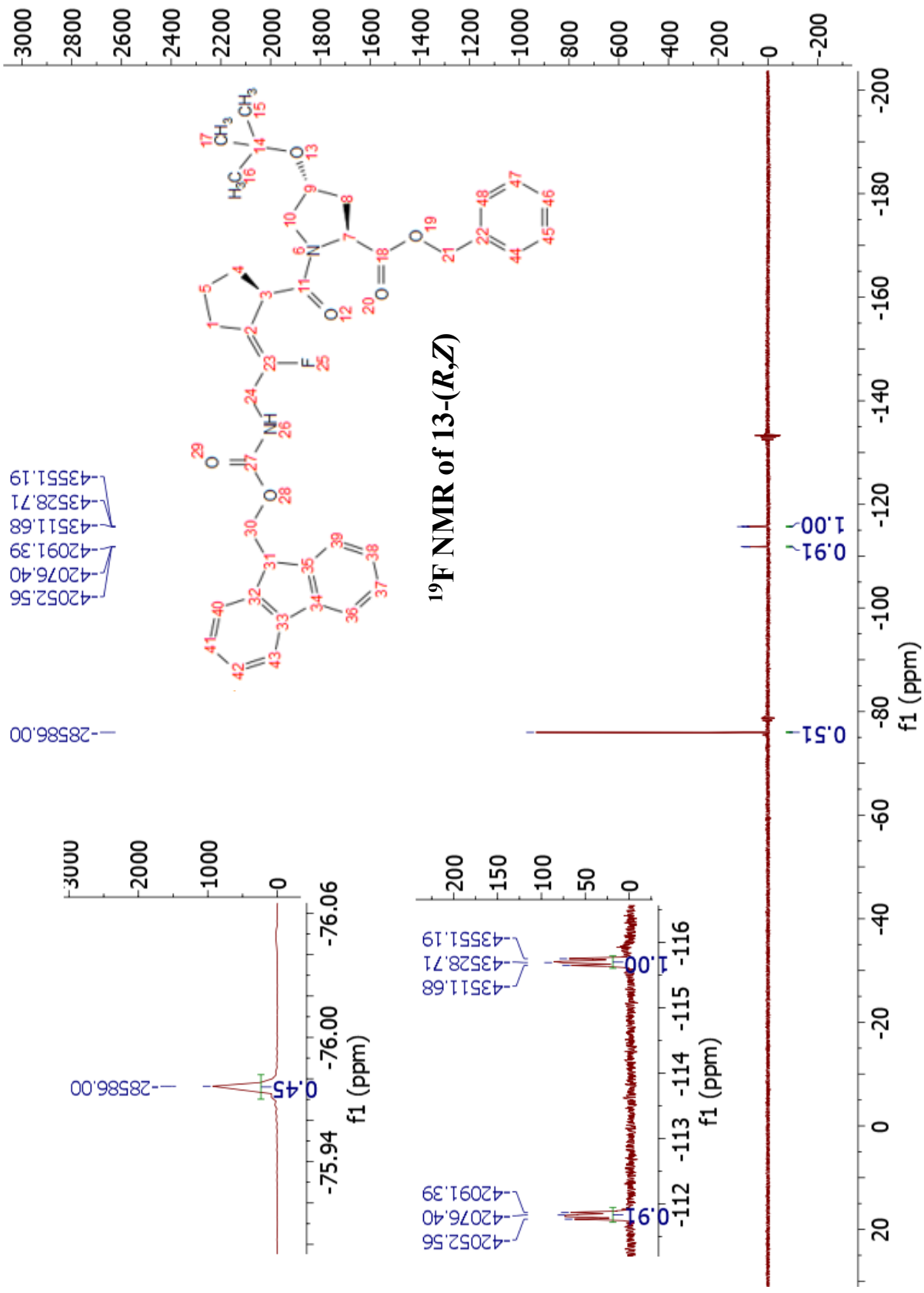




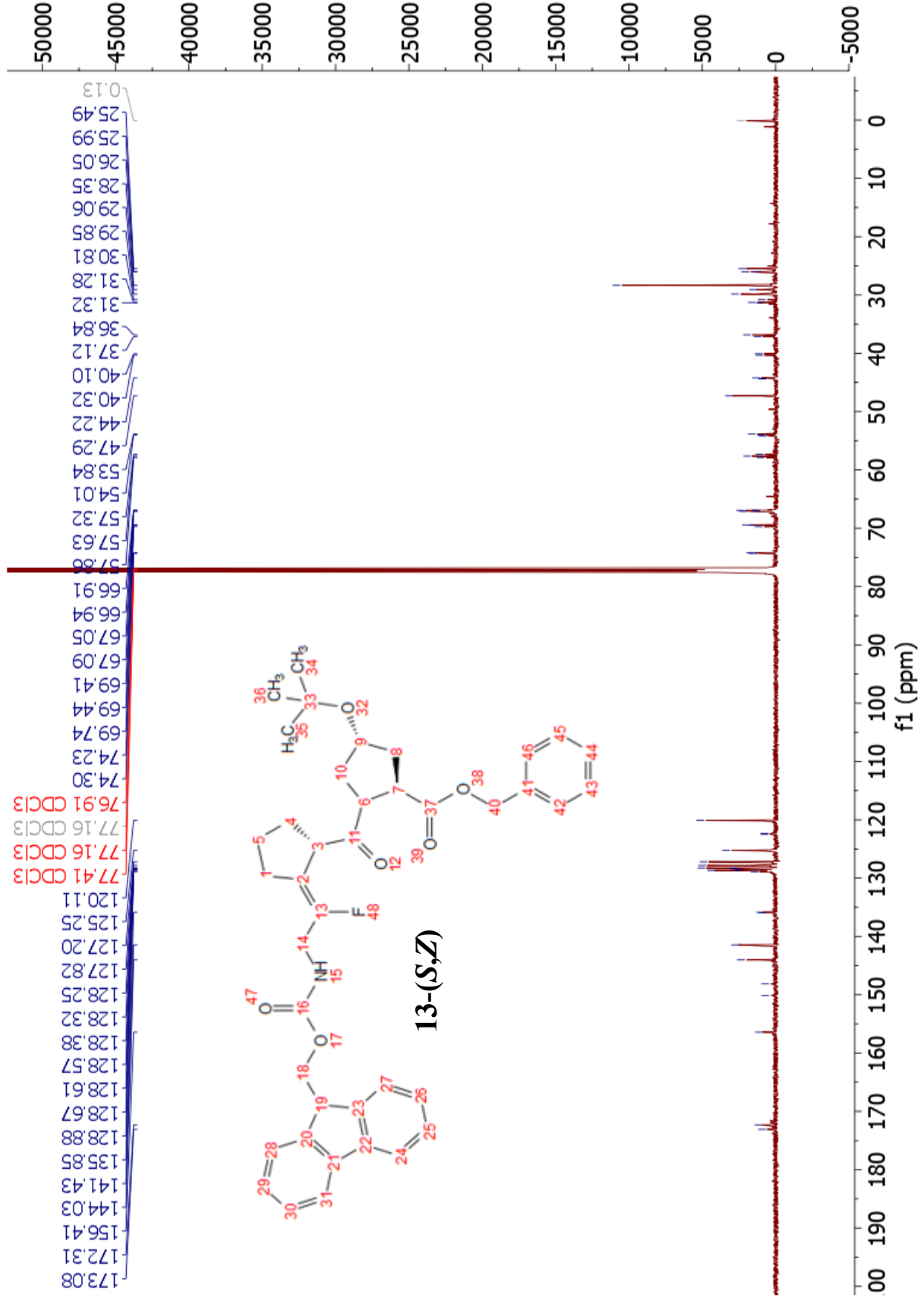


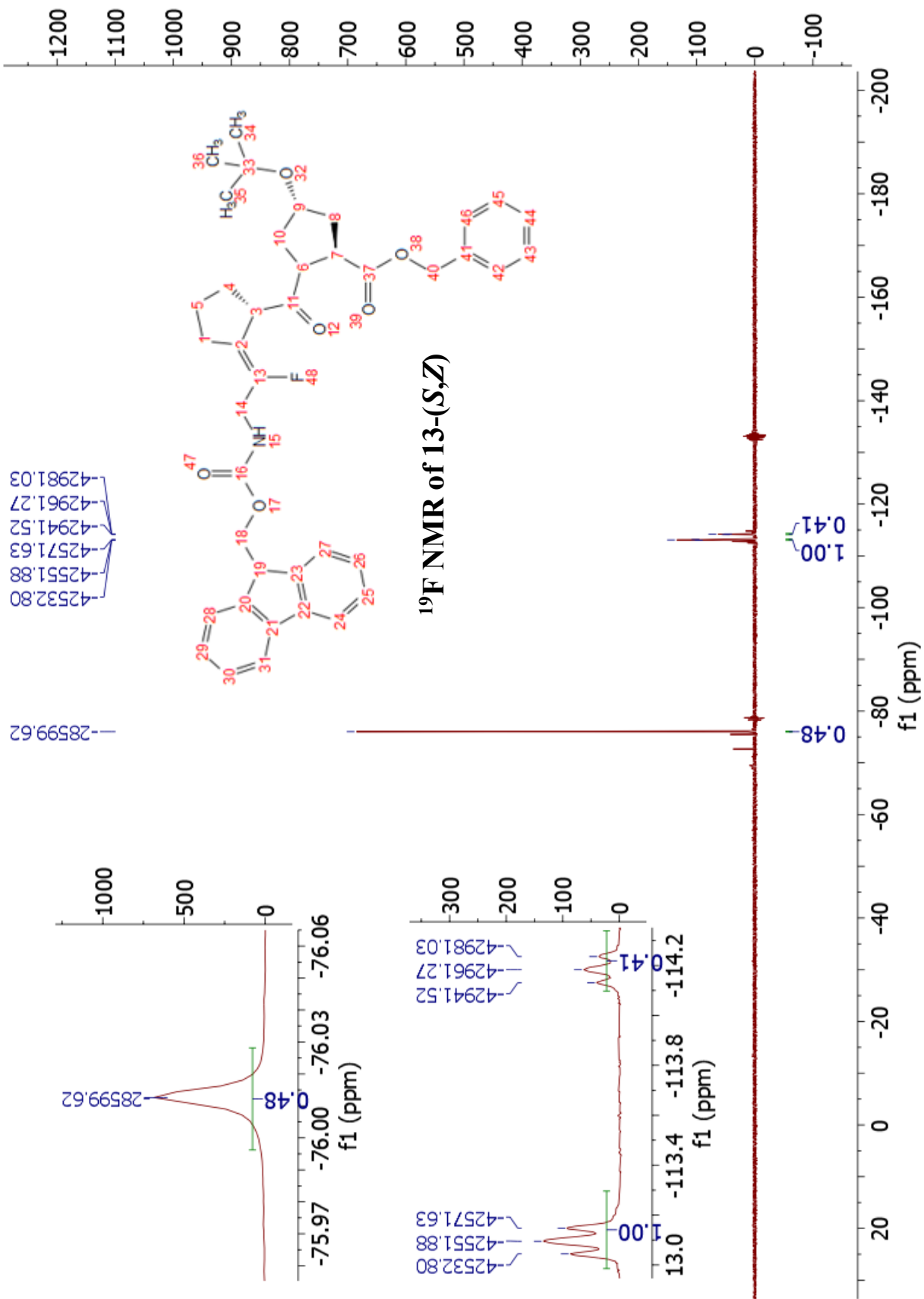


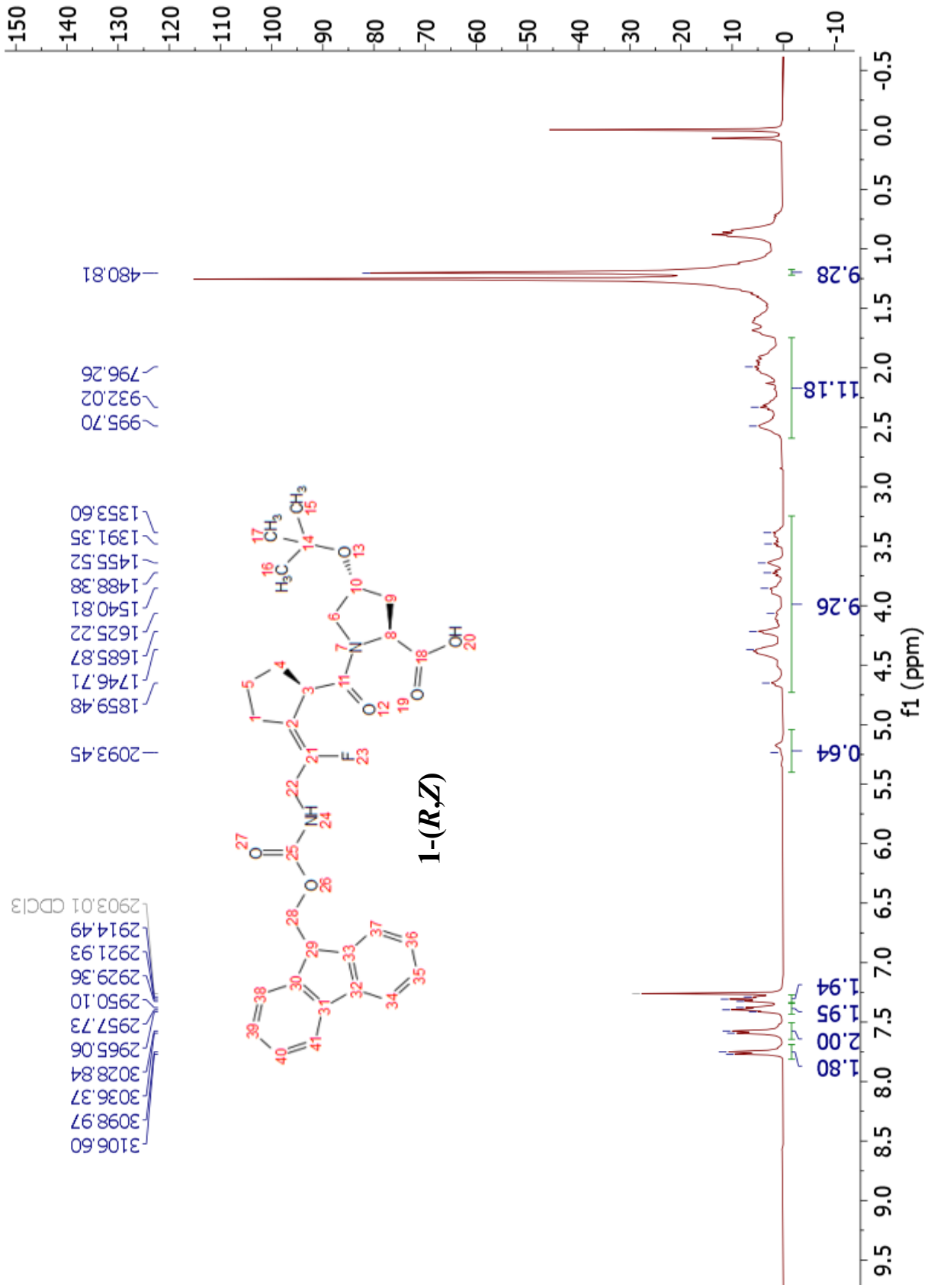


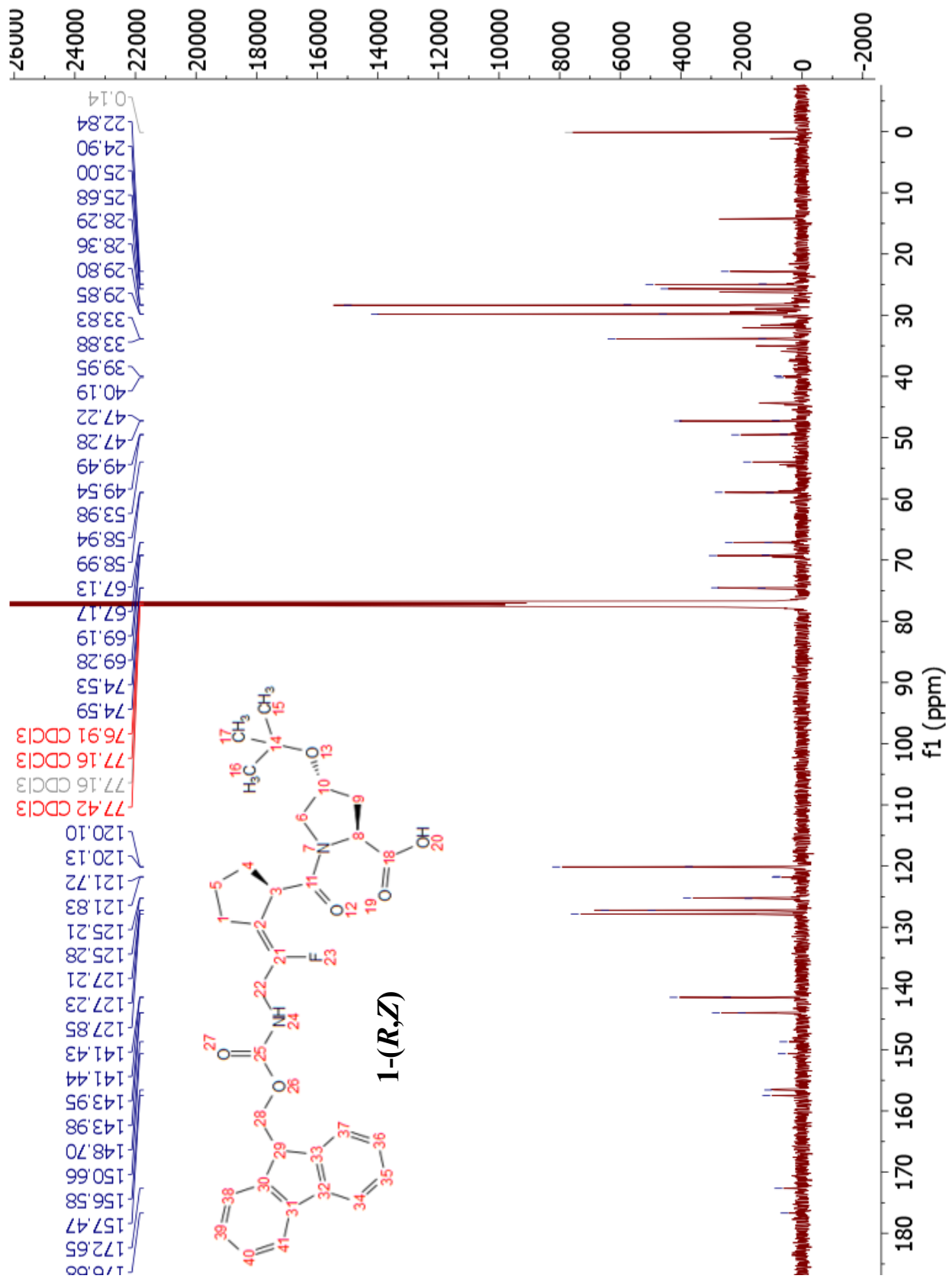




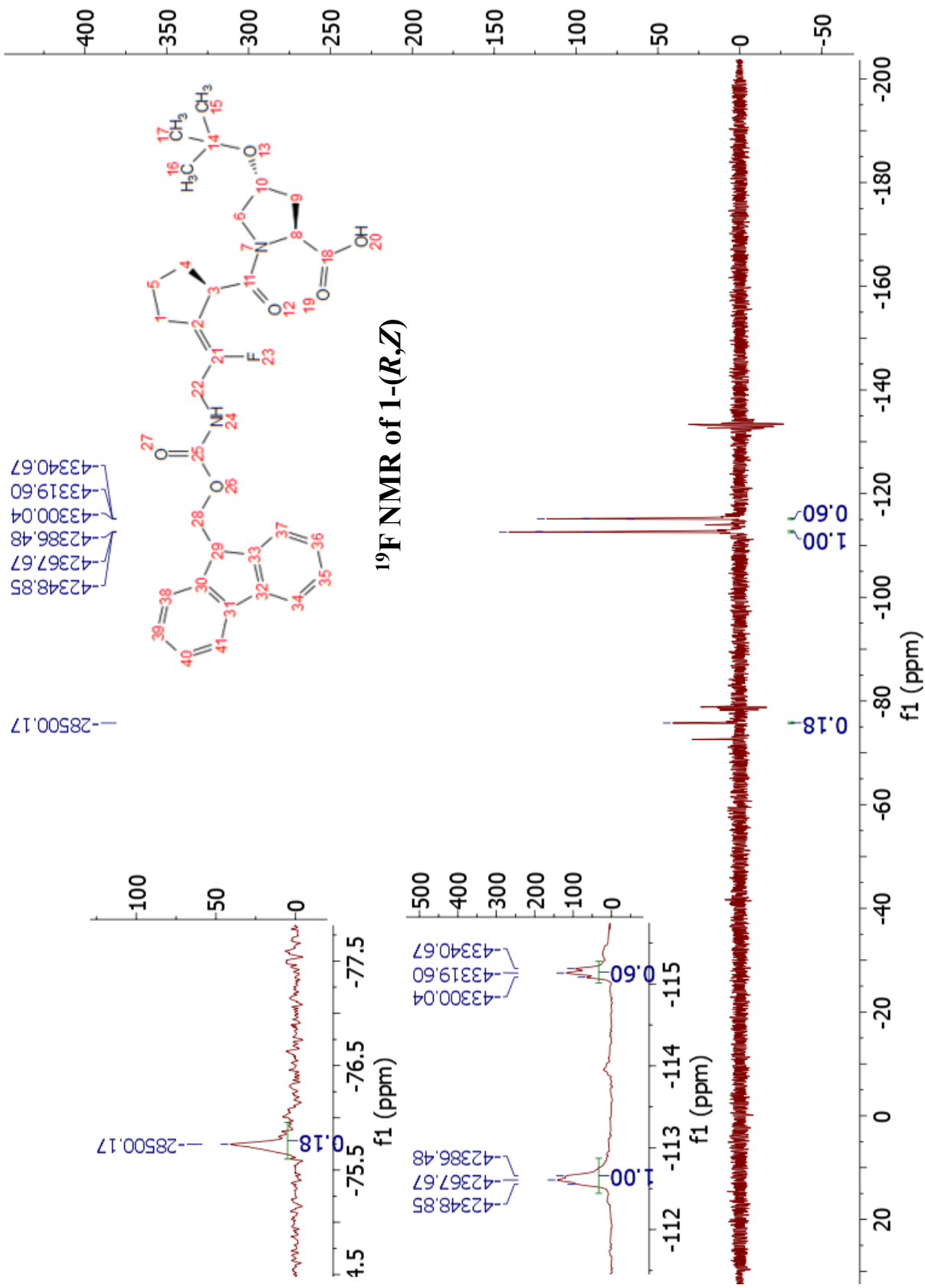


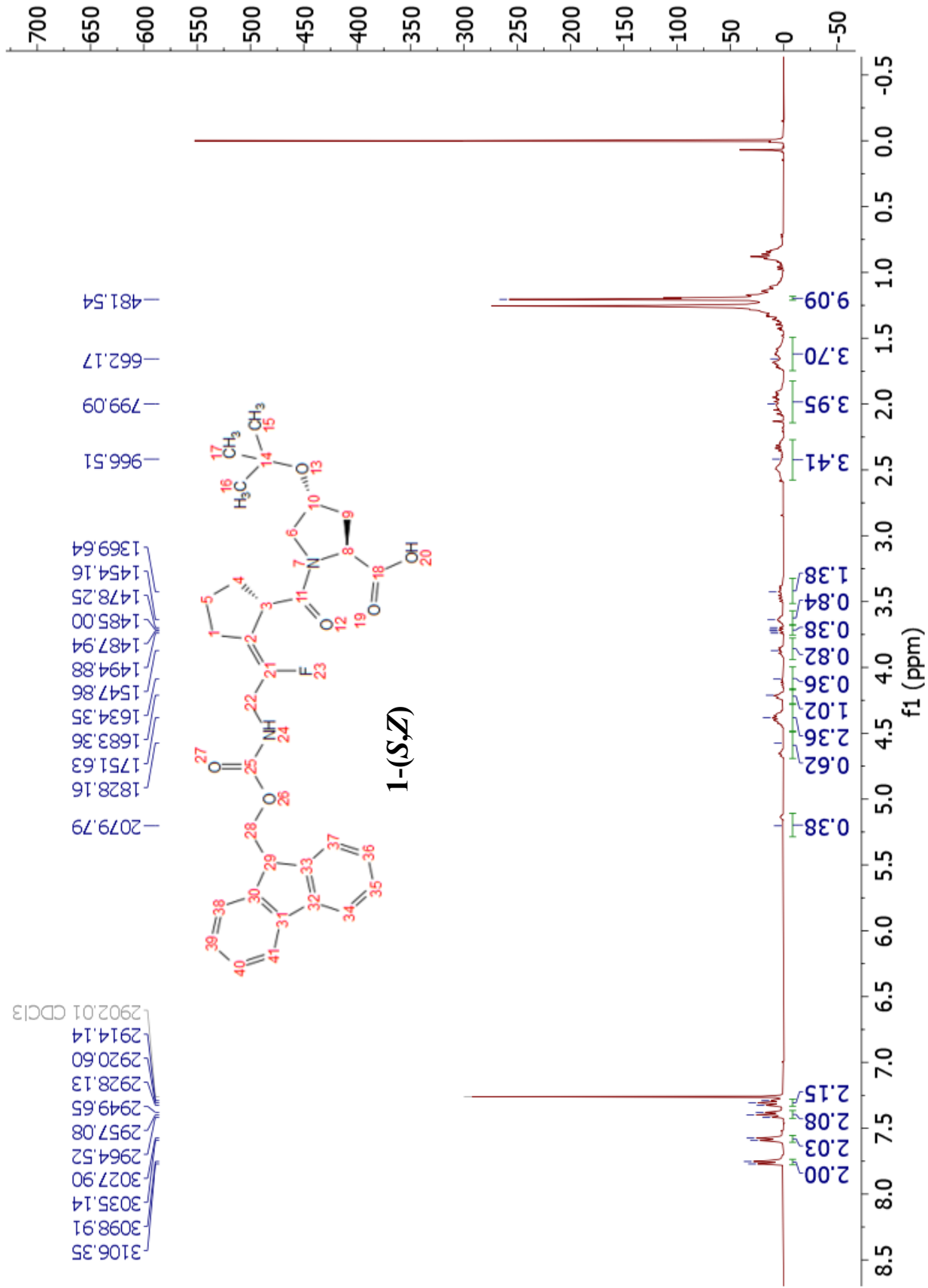


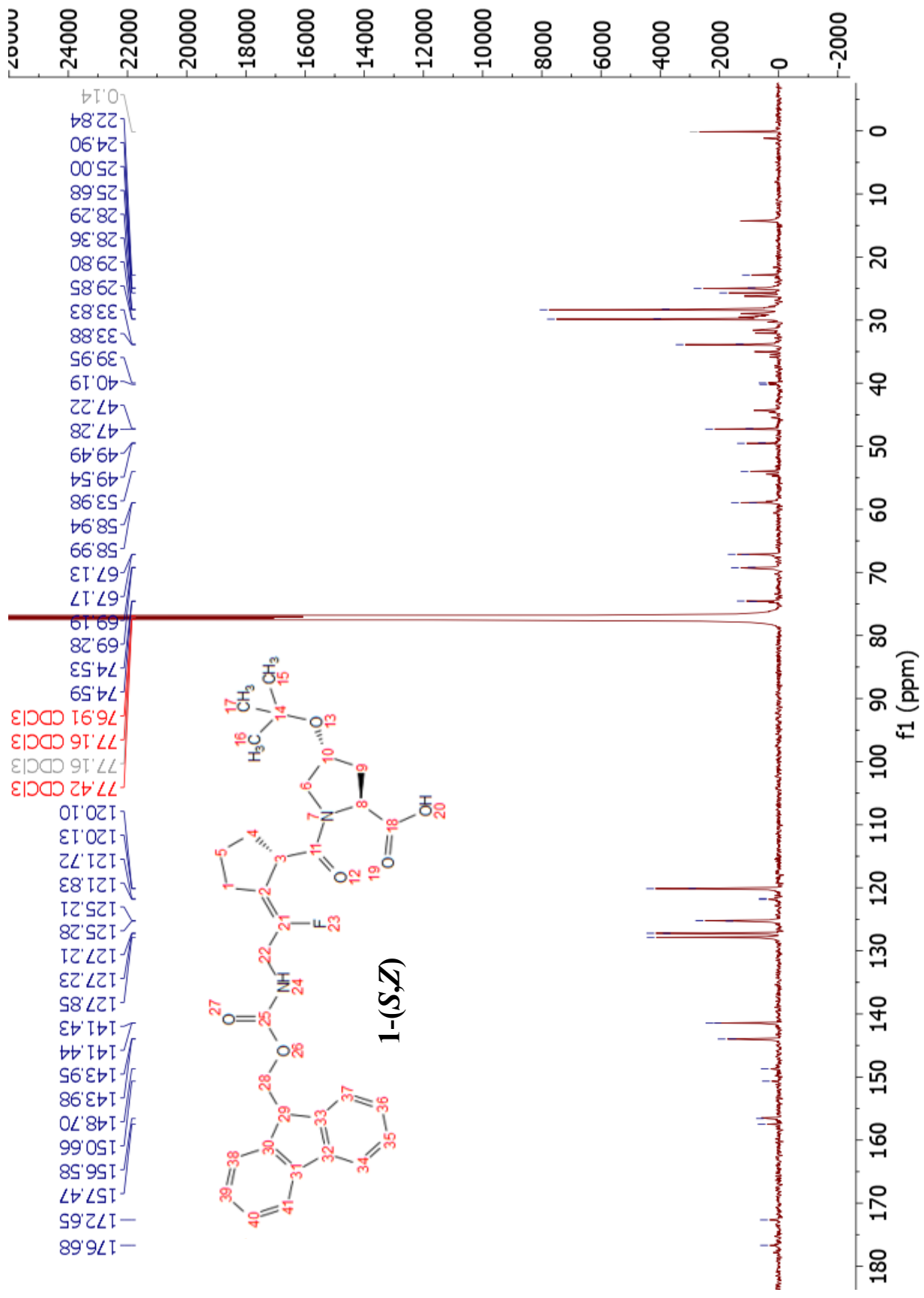


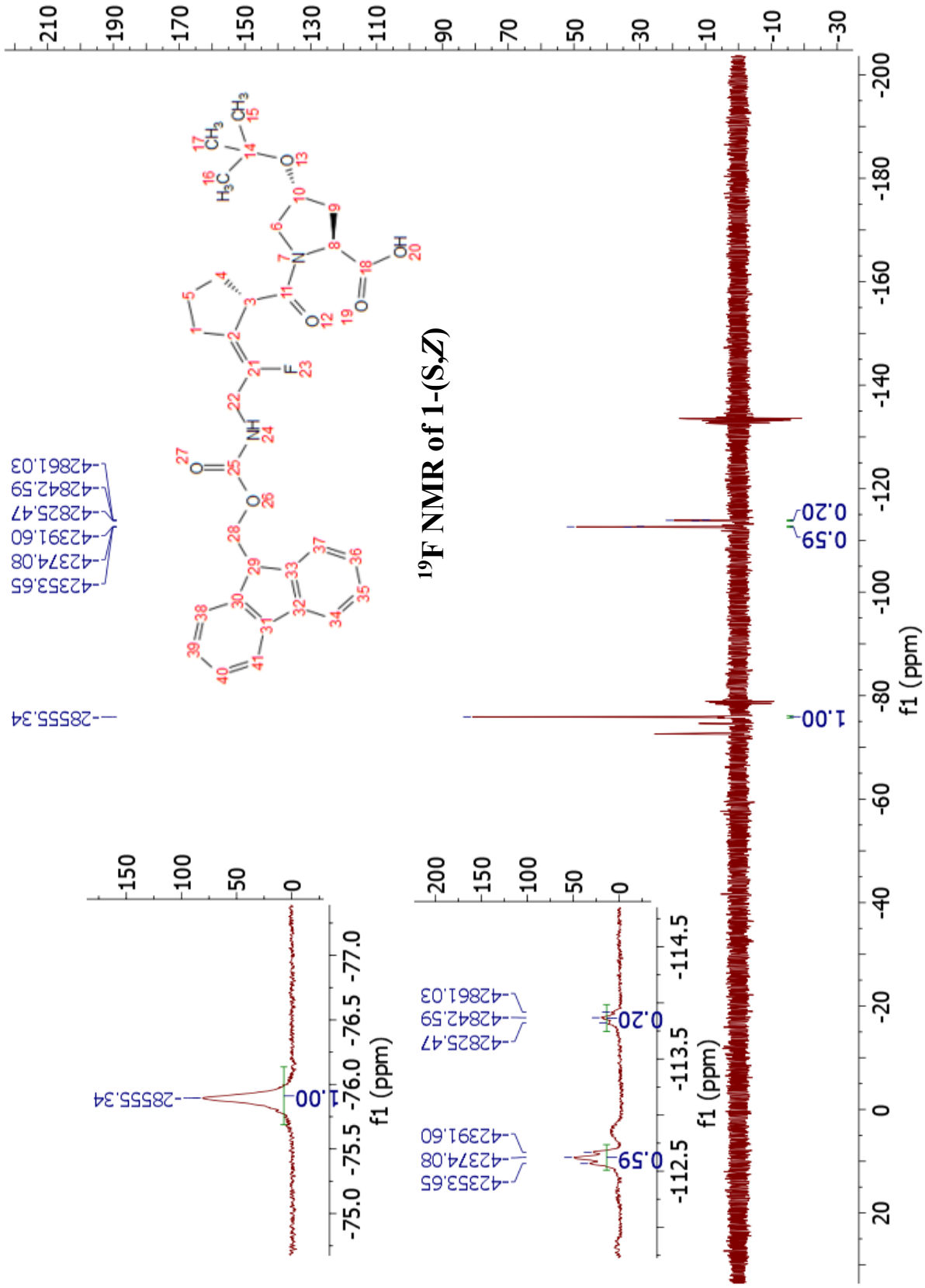




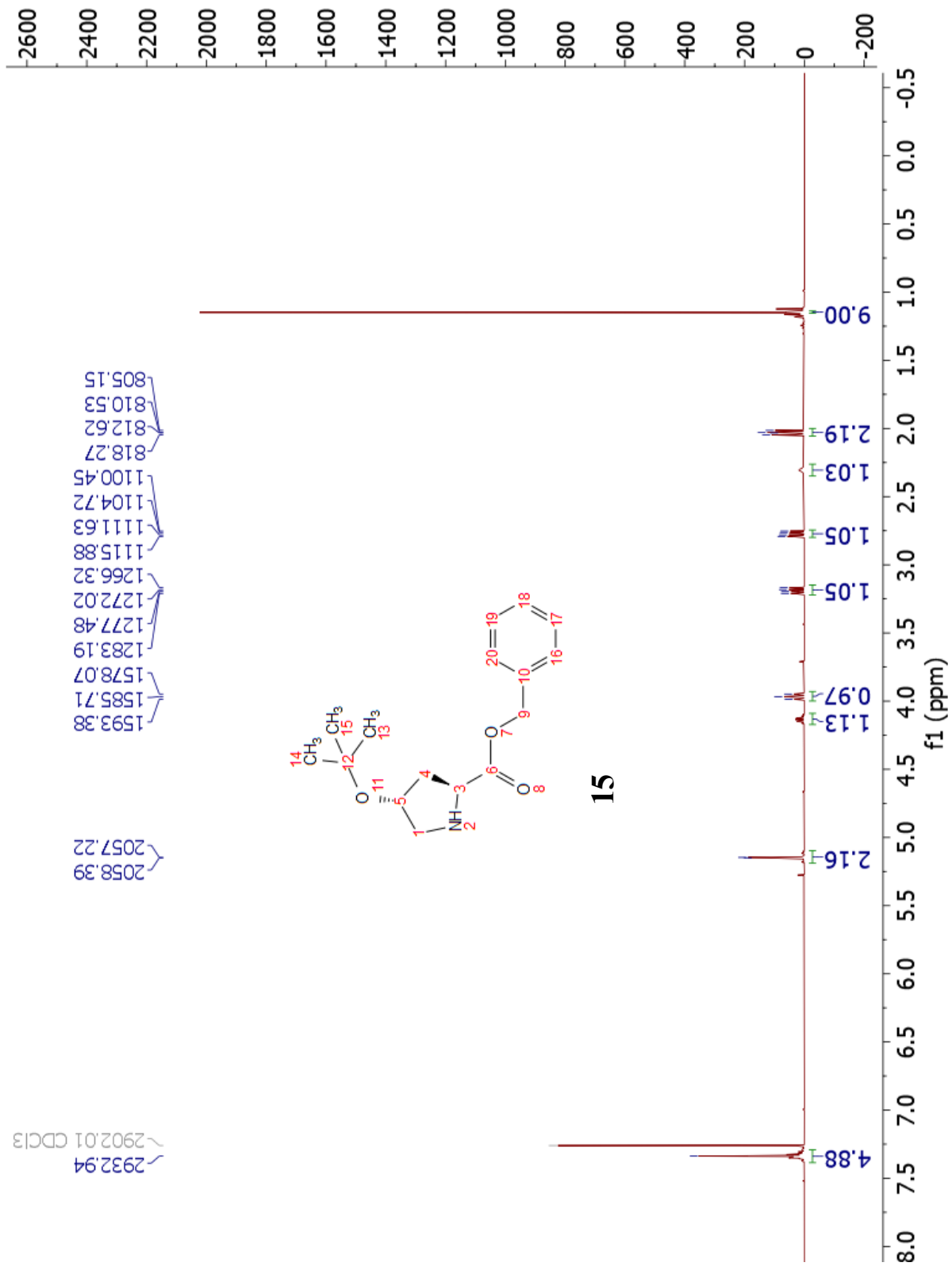


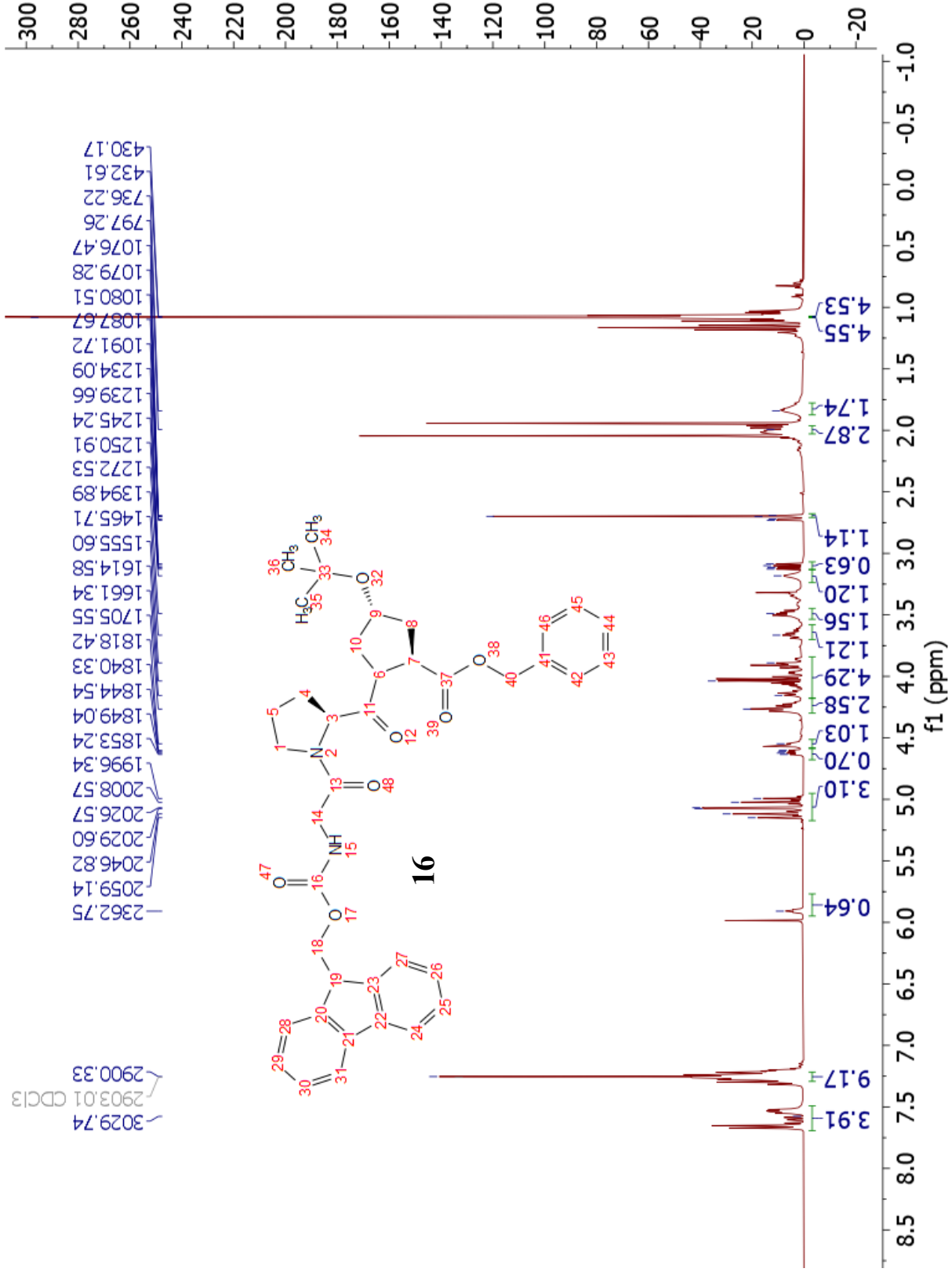


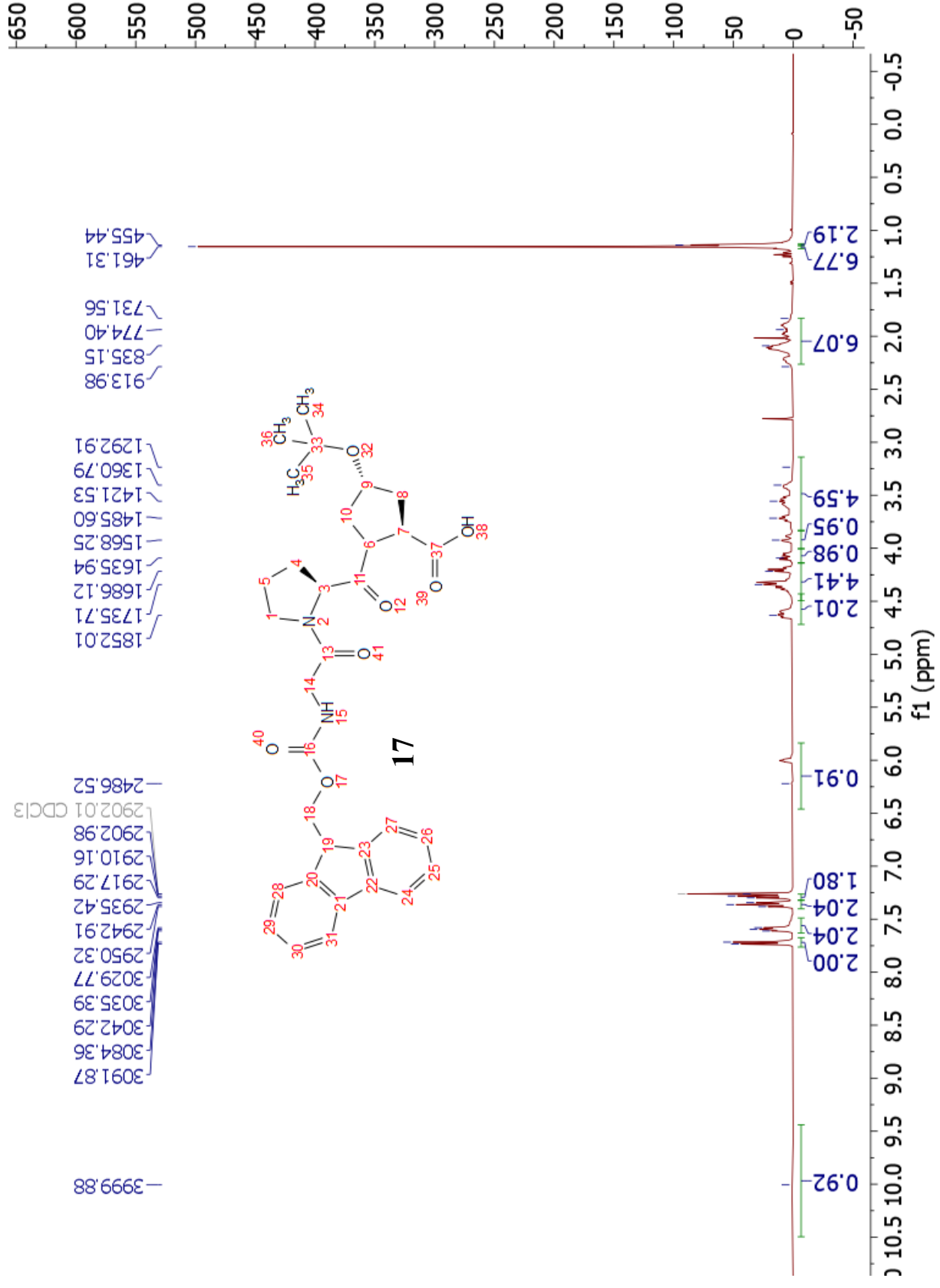




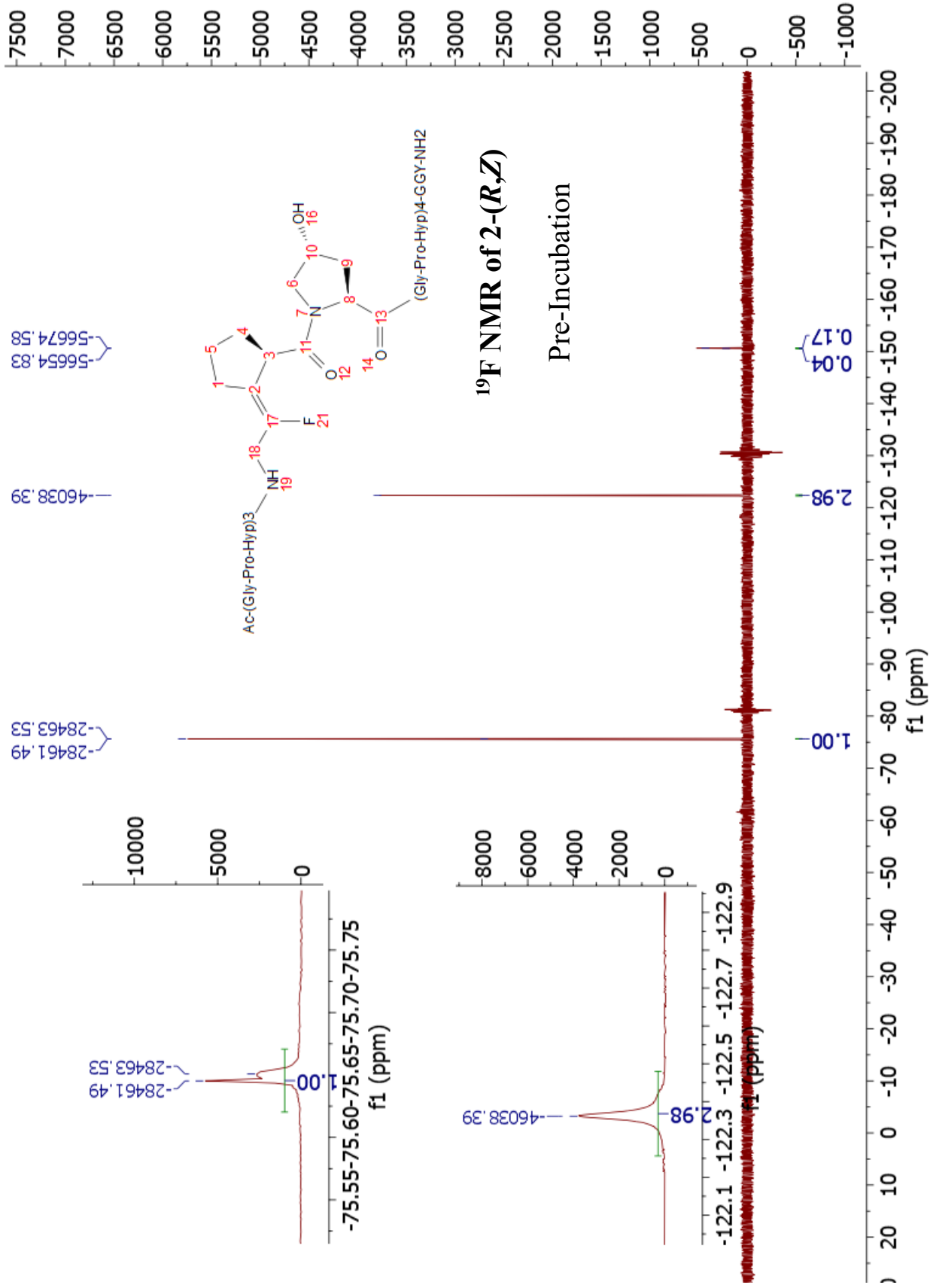


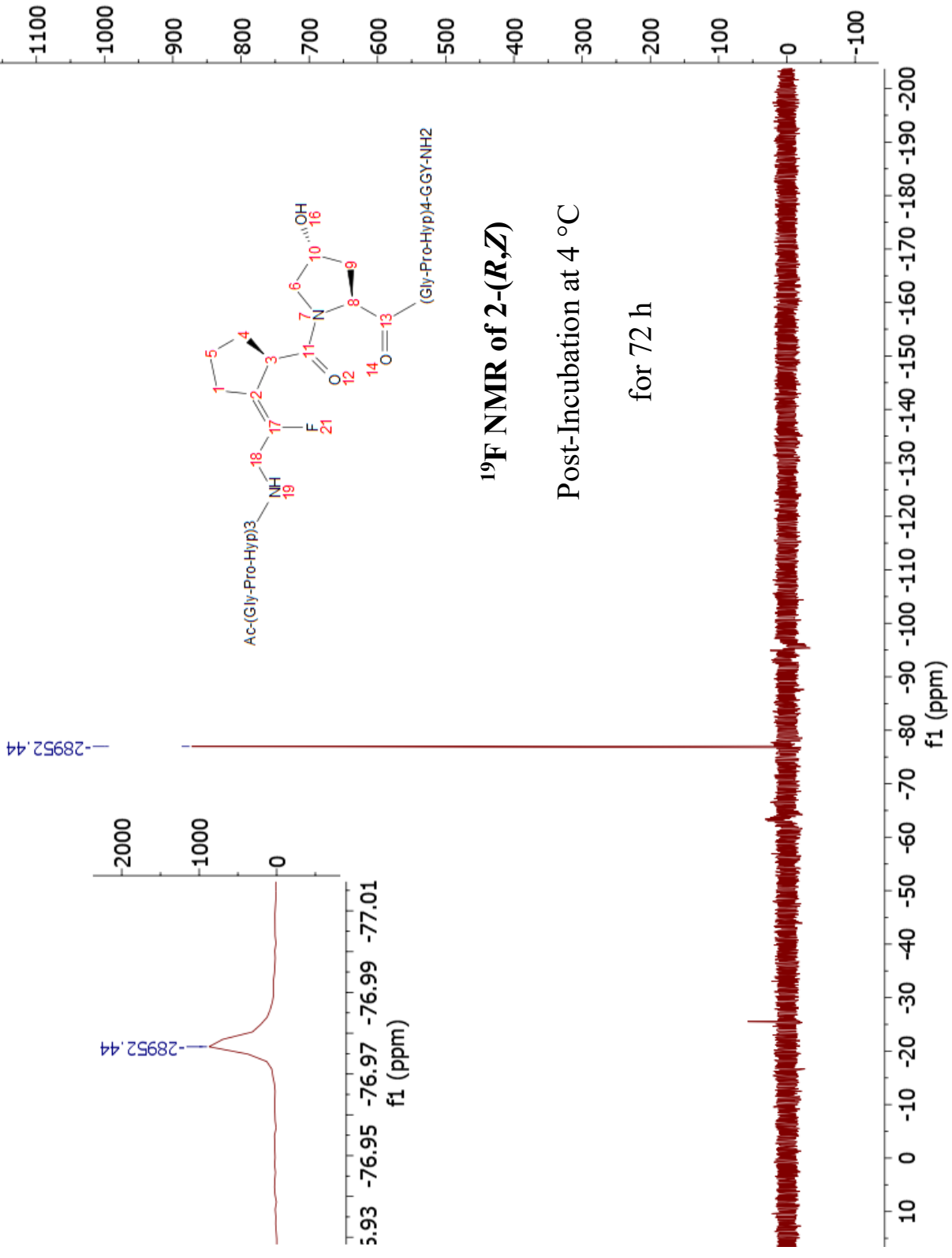


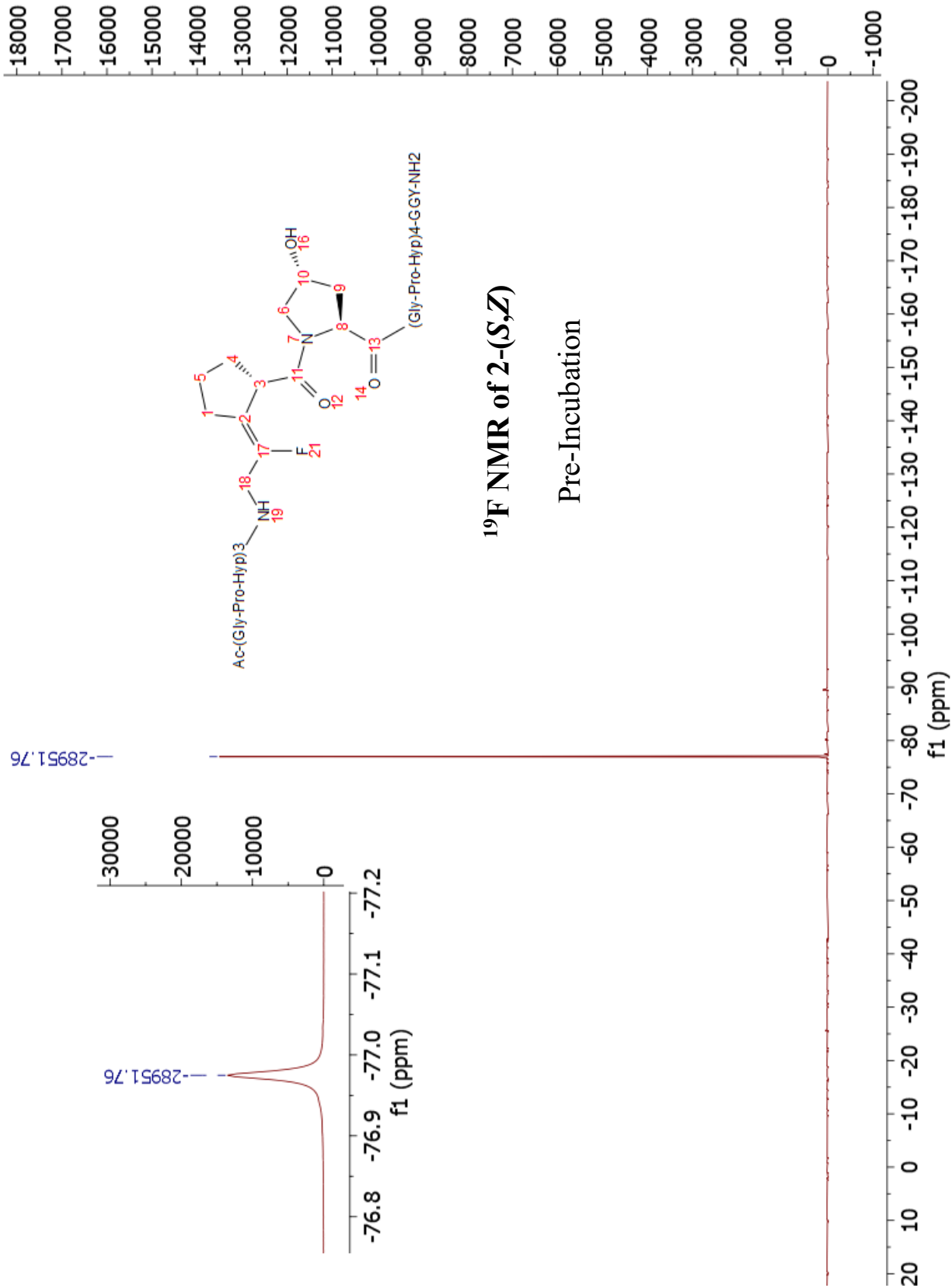






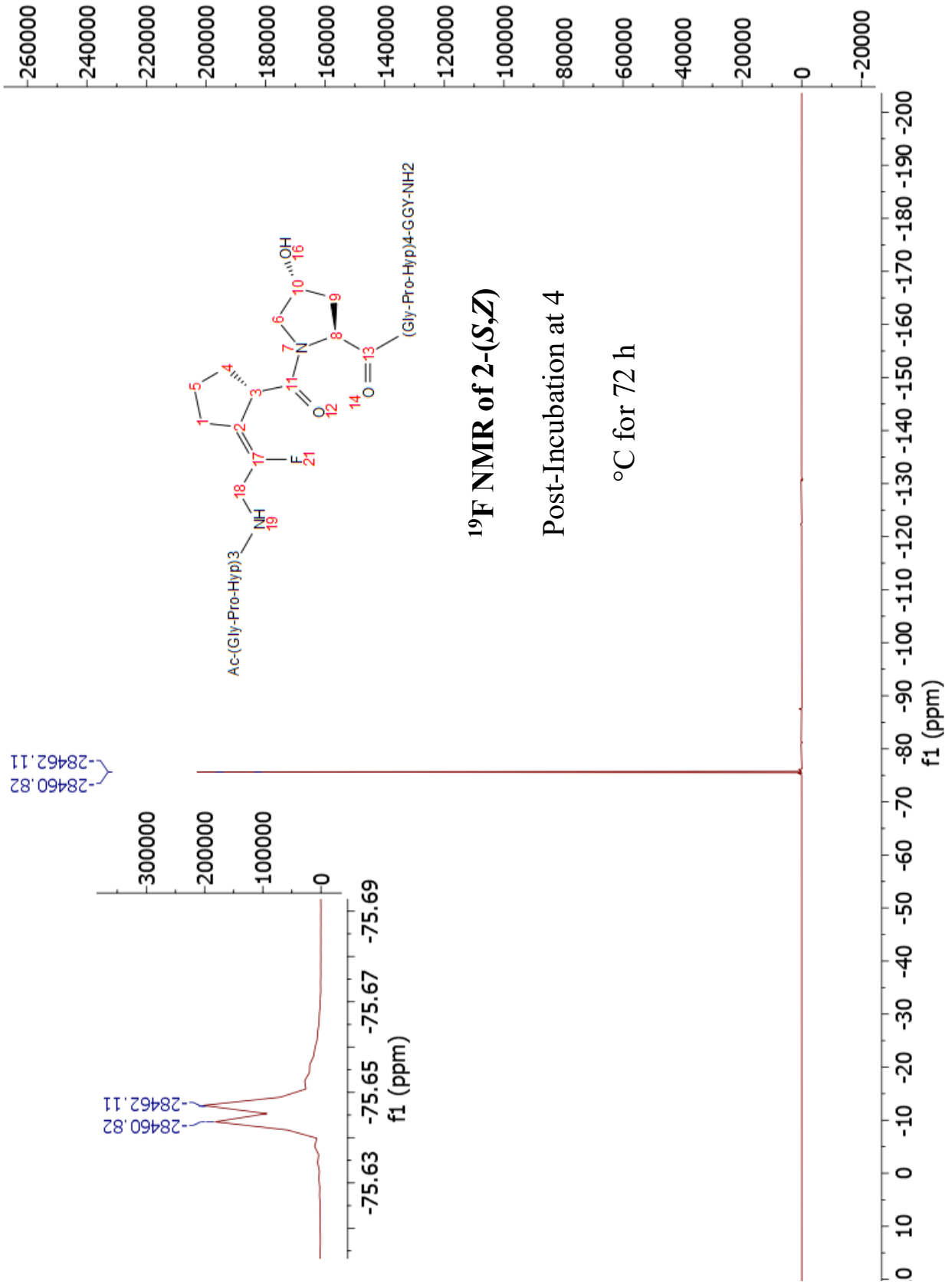


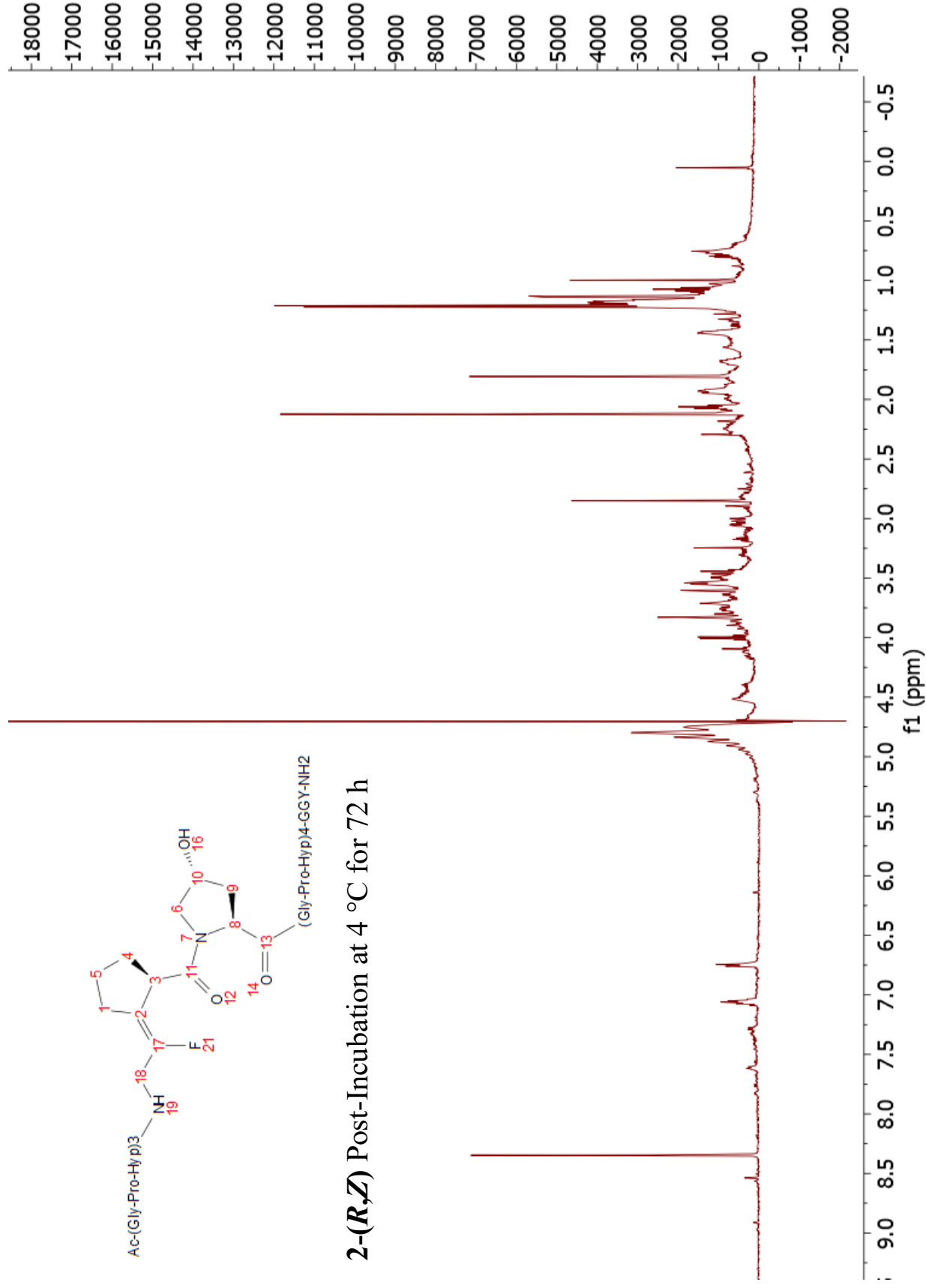




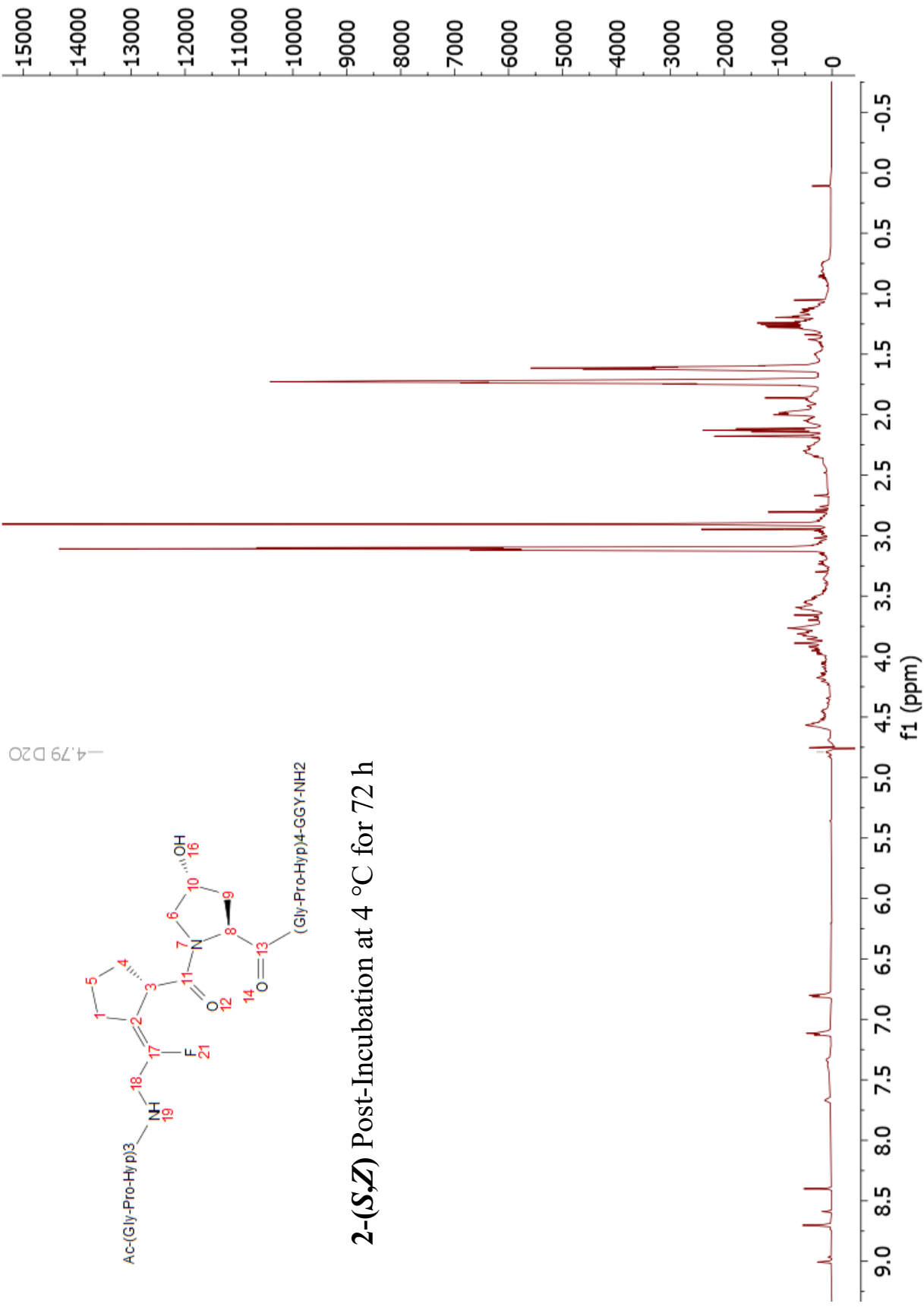
### $^{19}\text{F}$ NMR of 2-(S,Z)

Pre-Incubation

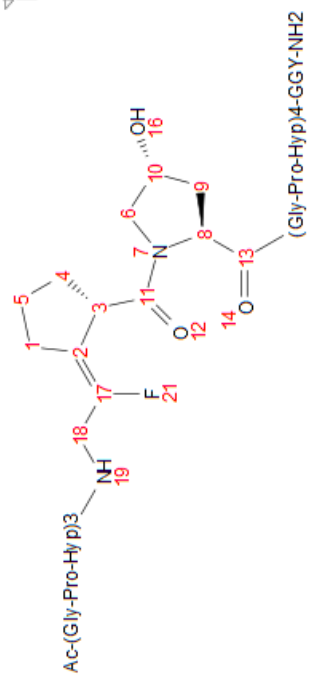




**2-(*R,Z*)** Post-Incubation at 4 °C for 72 h



-4.79 D20



2-(*S,Z*) Post-Incubation at 4 °C for 72 h

