Synthesis, characterization and biological evaluation of *N*-(2,3-dimethyl-5-oxo-1-phenyl-2,5-dihydro-1*H*-pyrazol-4-yl)benzamides

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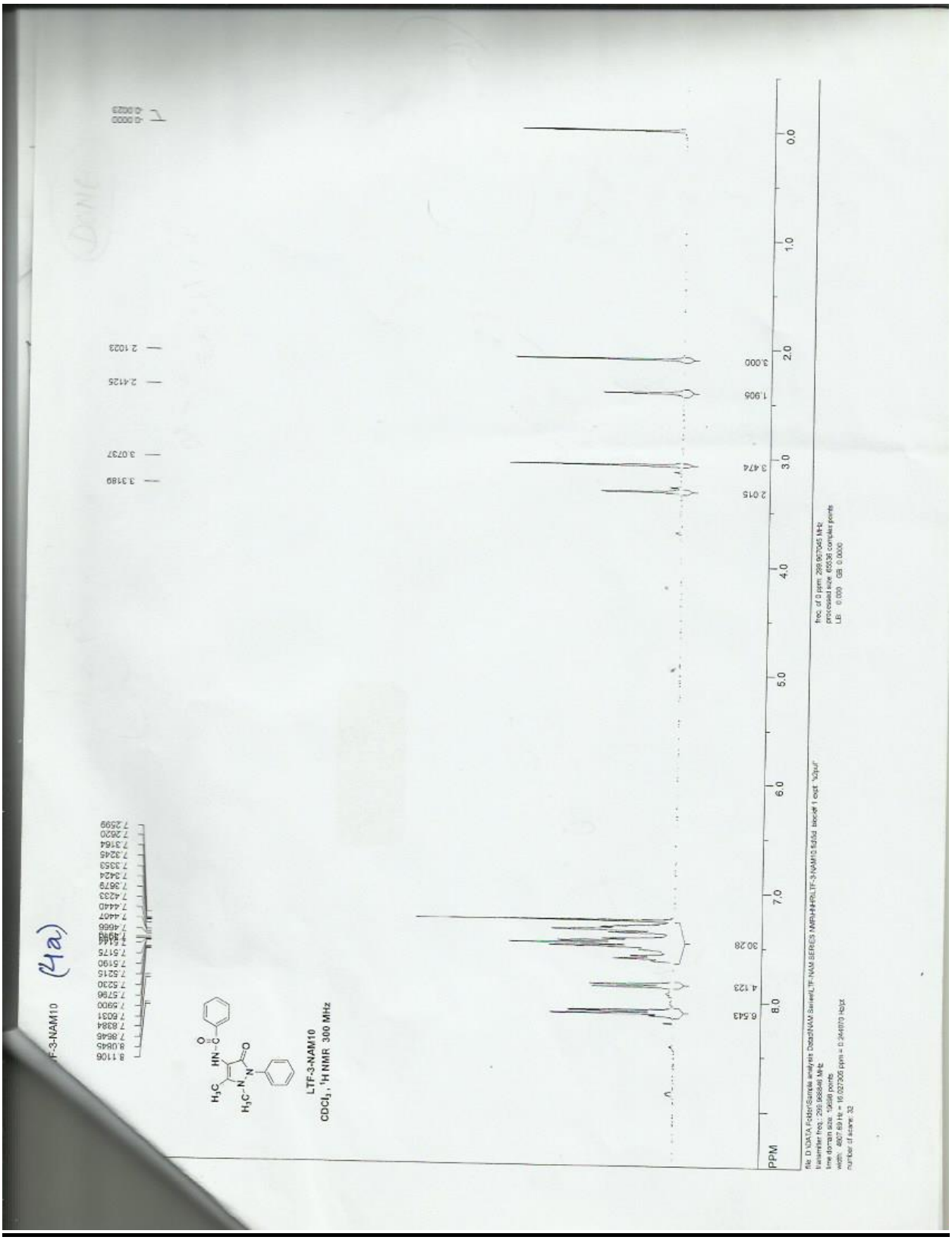
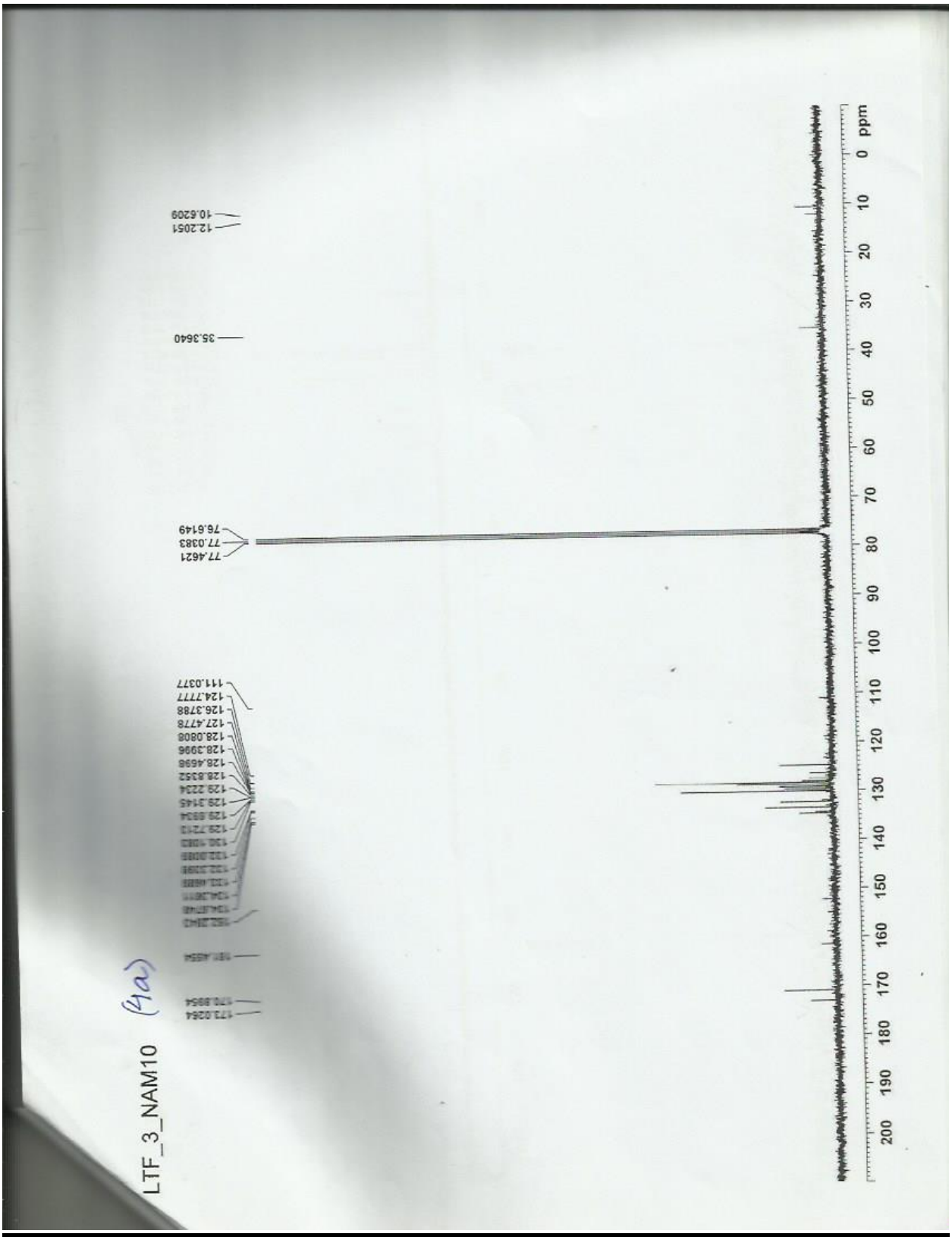
[f] *Centre de Recherche du CHU de Québec – Université Laval, Québec, QC, Canada*

SUPPORTING INFORMATION

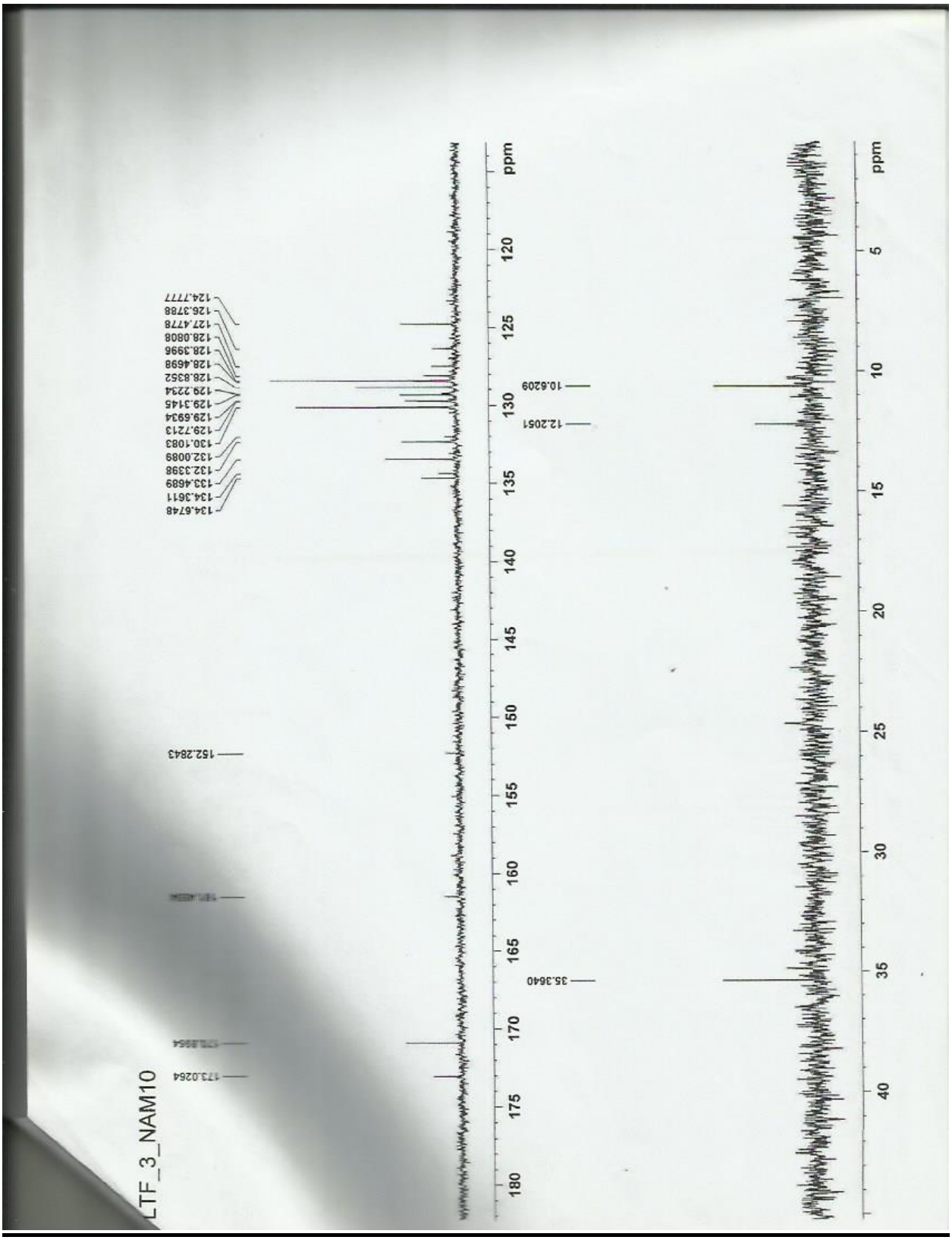
4a -^{1}H-NMR a:



4a-^{13}C-NMR a:



4a-^{13}C-NMR-b: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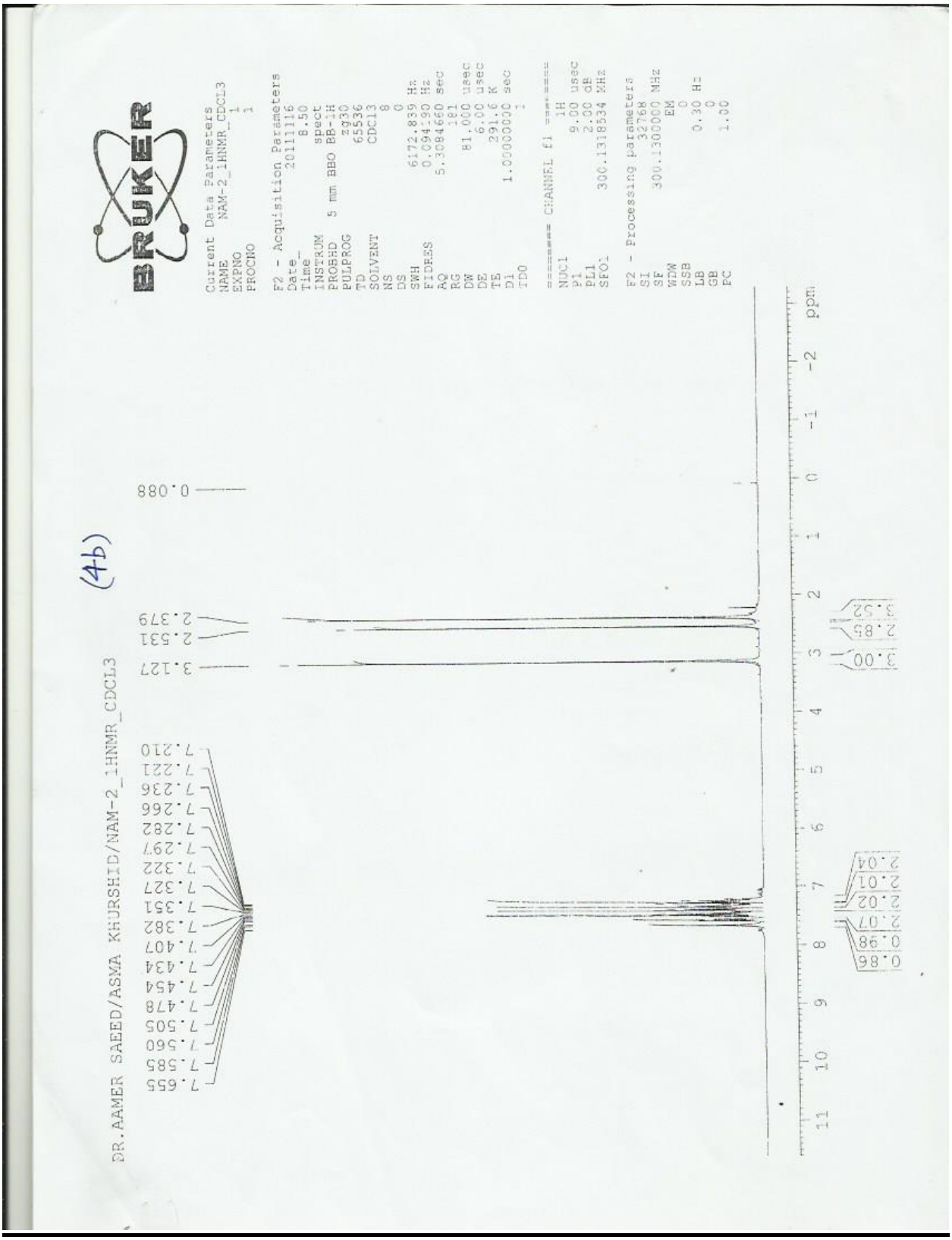
129.6934
129.3145
129.2234
128.8352
128.4698
128.3996
128.0808
127.4778
126.3788
124.7777
35.3640
12.2051
10.6209
ppm
ppm

4b-^{1}H-NMR-a: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(4b)
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PROCNO 1
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Time 8.50
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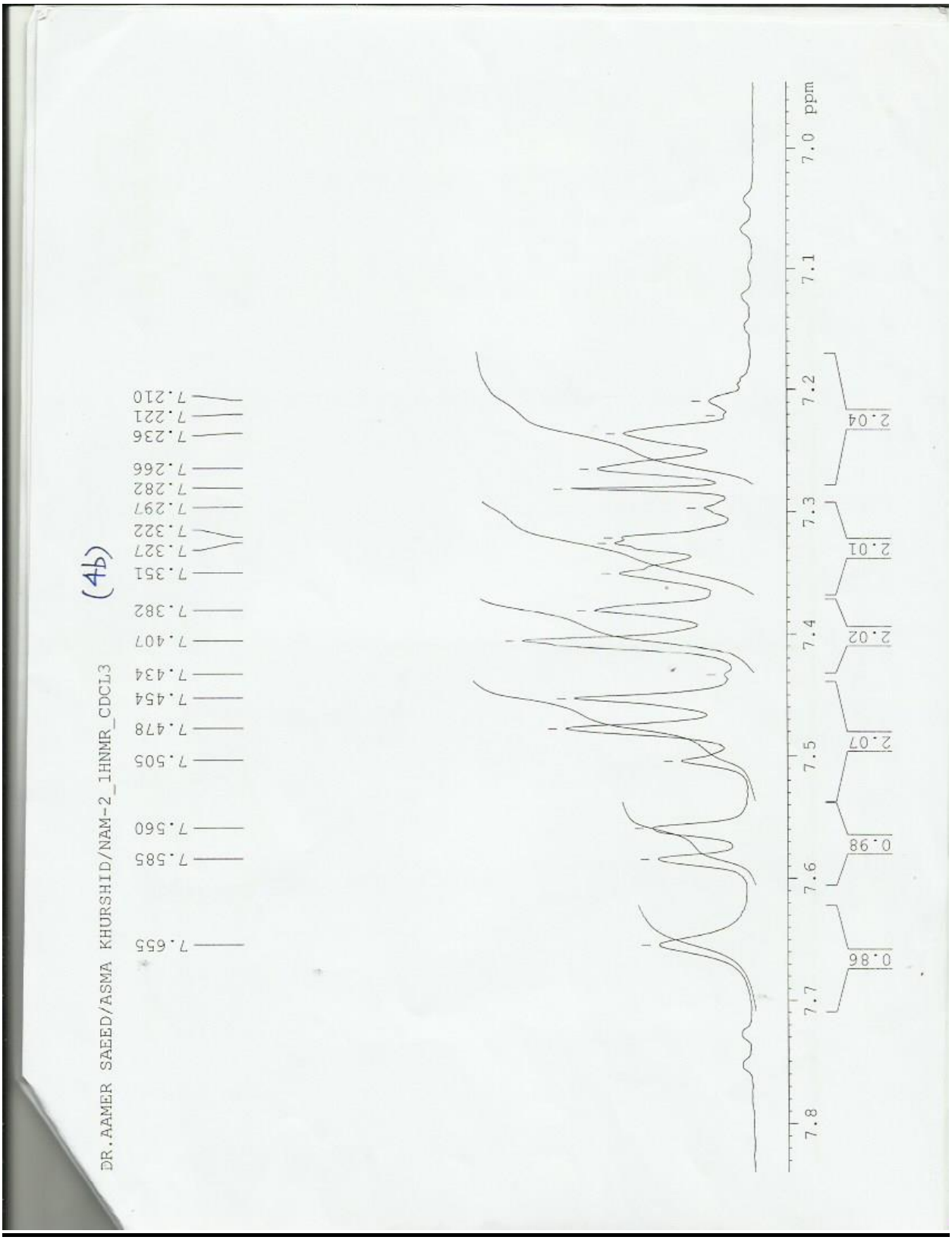
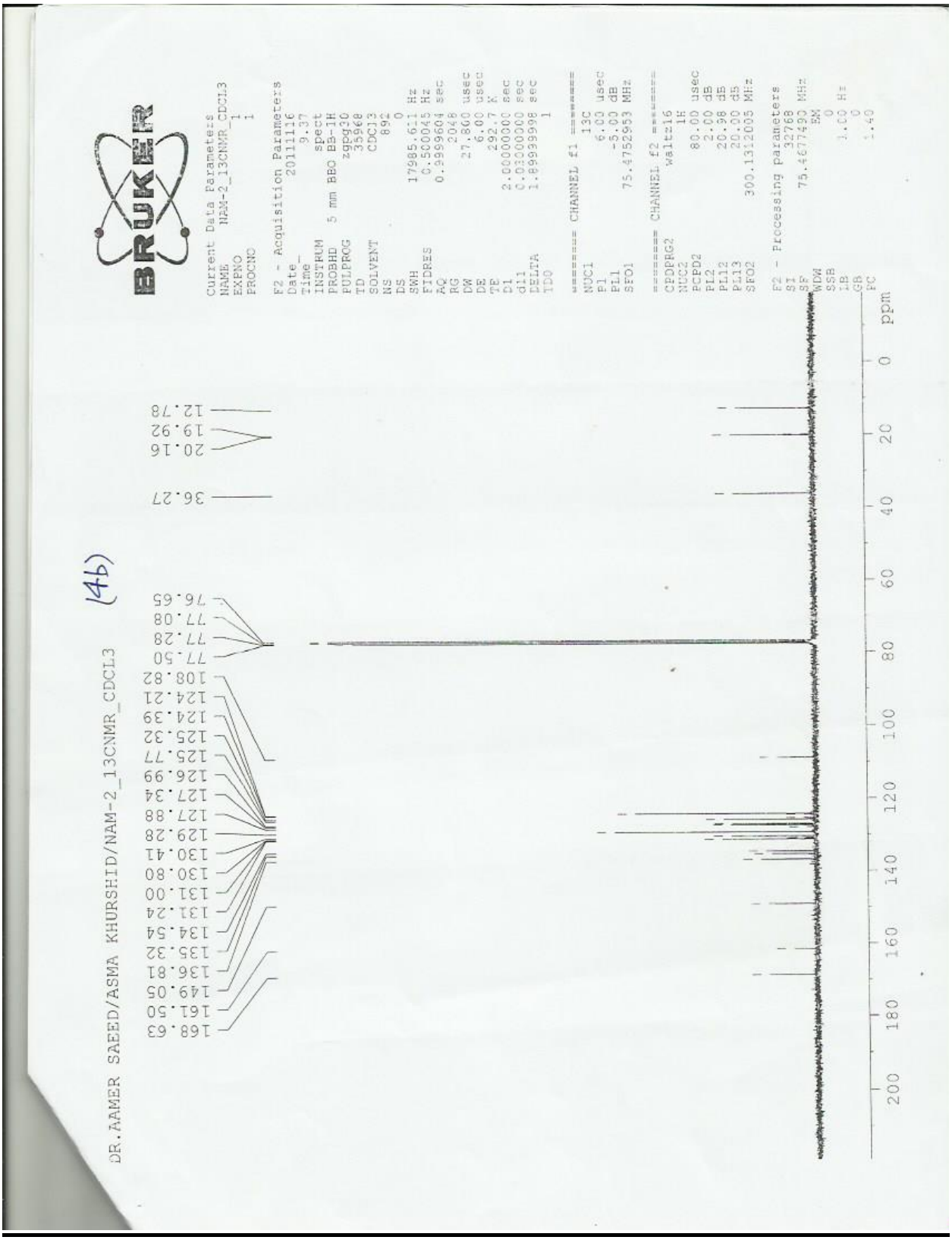
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 0
SWH 6172.839 Hz
FIDRES 0.094190 Hz
AQ 5.3084660 sec
RG 181
DW 81.000 usec
DE 6.00 usec
TE 291.6 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 2.00 dB
SFO1 300.1318534 MHz
F2 - Processing parameters
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
7.655
7.585
7.560
7.505
7.478
7.454
7.434
7.407
7.382
7.351
7.327
7.322
7.297
7.282
7.266
7.236
7.221
7.210
3.127
2.531
2.379
0.088
3.00
2.85
3.52
0.86
0.98
2.07
2.02
2.01
2.04
ppm

4b-^{1}H-NMR-b:


DR.AAMER SAEED/ASMA KHURSHID/NAM-2_1HNMR_CDCL3
(4b)
7.210
7.221
7.236
7.266
7.282
7.297
7.322
7.327
7.351
7.382
7.407
7.434
7.454
7.478
7.505
7.560
7.585
7.655
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0 ppm
2.04
2.01
2.02
2.07
0.98
0.86

4b-^{13}C-NMR-a:


DR.AAMER SAEED/ASMA KHURSHID/NAM-2_13CNMR_CDCL3
(4b)
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149.05
136.81
135.32
134.54
131.24
131.00
130.80
130.41
129.28
127.88
127.34
126.99
125.77
125.32
124.39
124.21
108.82
77.50
77.28
77.08
76.65
36.27
20.16
19.92
12.78
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PROCNO 1
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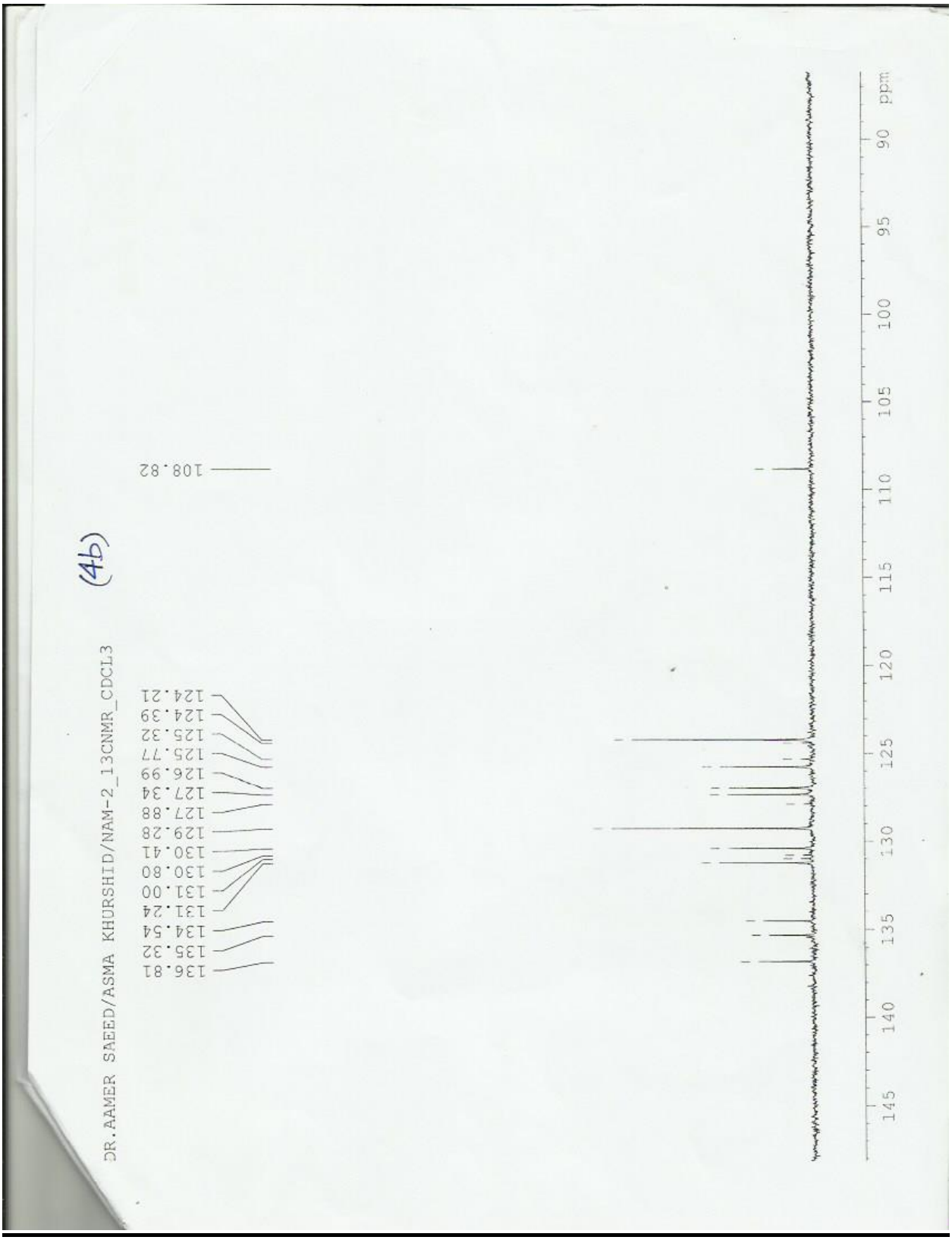
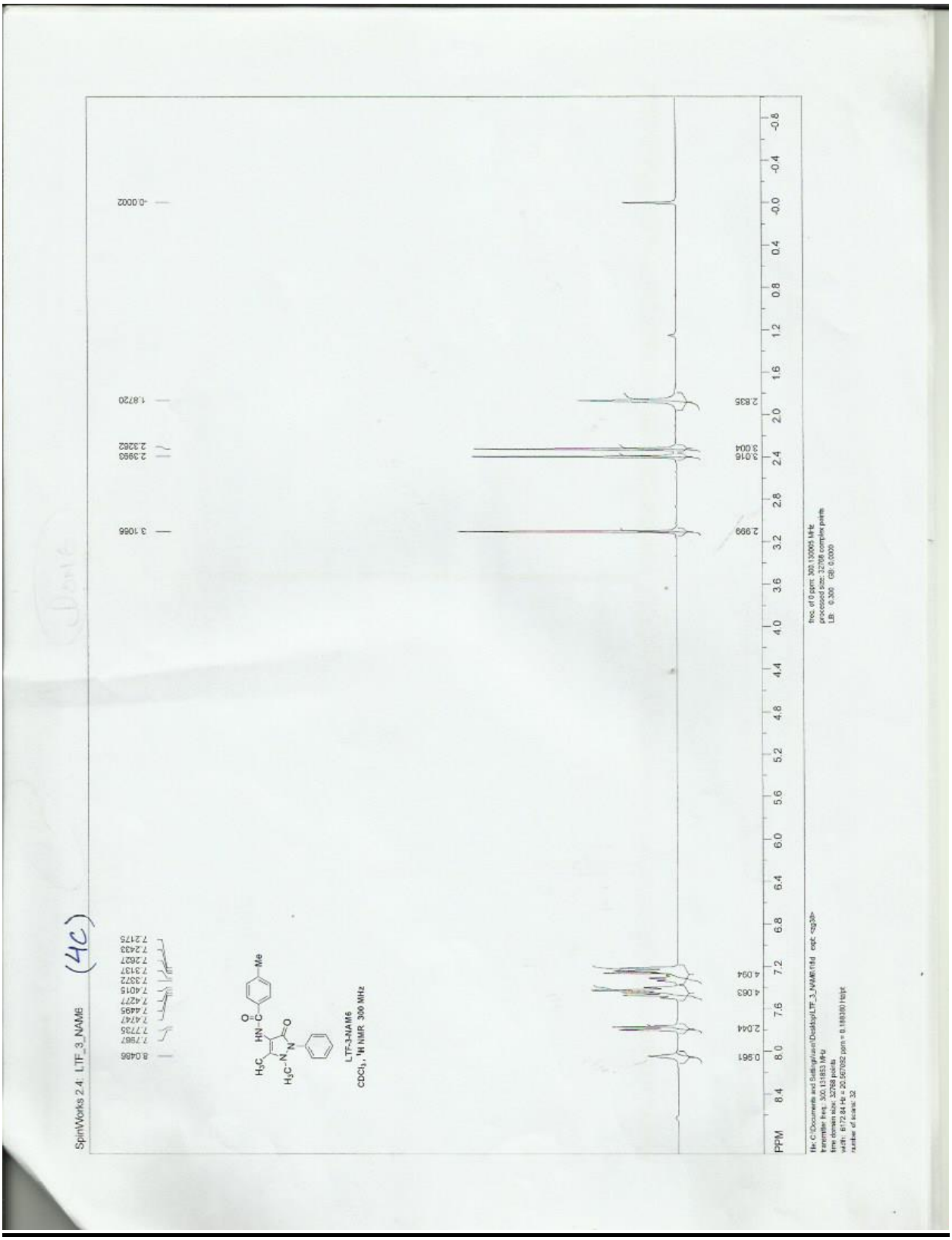
PULPROG zgpg30
TD 35968
SOLVENT CDCl3
NS 892
DS 0
SWH 17985.611 Hz
FIDRES 0.500045 Hz
AQ 0.9999604 sec
RG 2048
DW 27.860 usec
DE 6.00 usec
TE 292.7 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 6.00 usec
PL1 -5.00 dB
SFO1 75.4752953 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 2.00 dB
PL12 20.98 dB
PL13 20.00 dB
SFO2 300.1312005 MHz
F2 - Processing parameters
SI 32768
SF 75.4677490 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

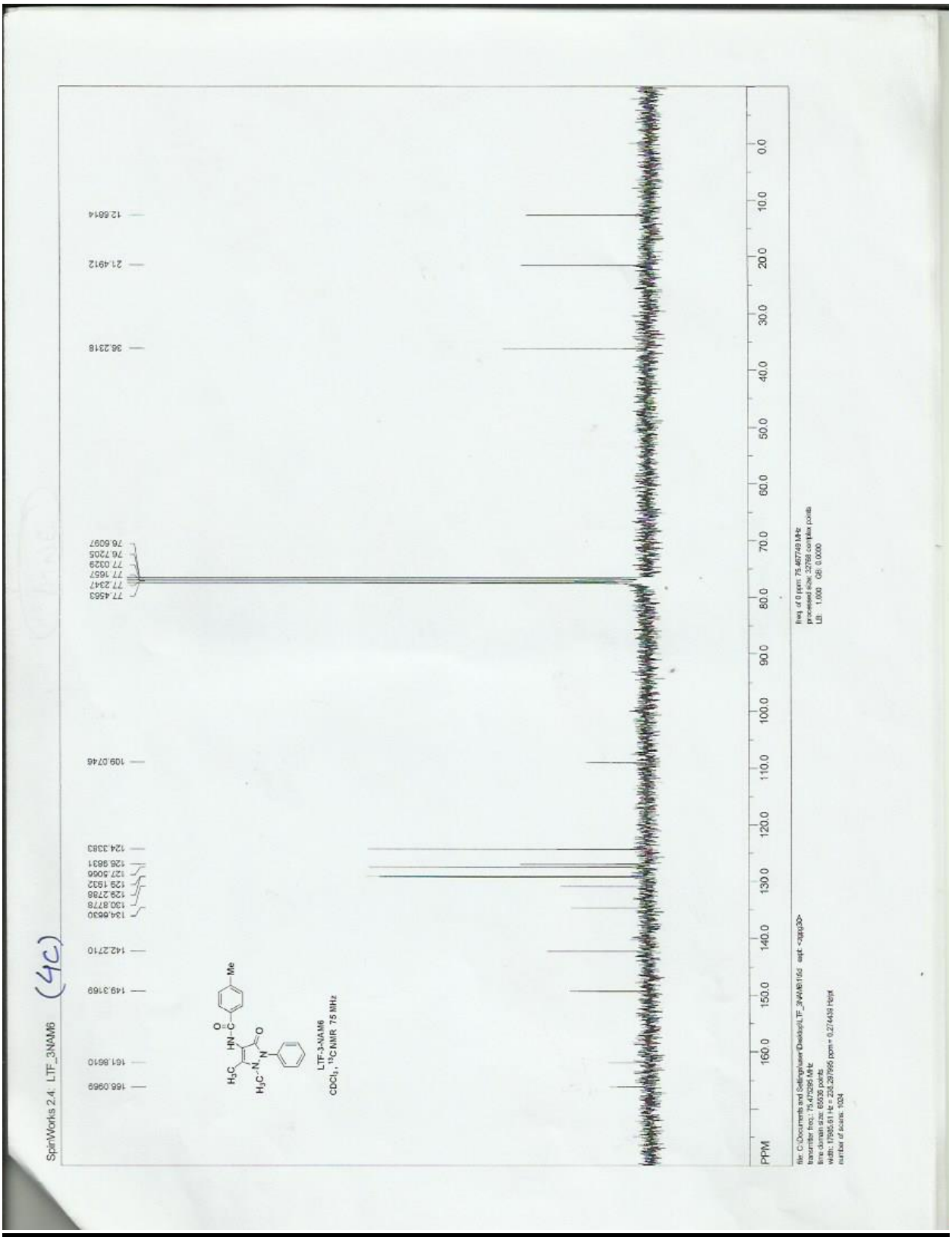
4b-^{13}C-NMR-b:


DR.AAMER SAEED/ASMA KHURSHID/NAM-2_13CNMR_CDCL3
(4b)
108.82
136.81
135.32
134.54
131.24
131.00
130.80
130.41
129.28
127.88
127.34
126.99
125.77
125.32
124.39
124.21
145
140
135
130
125
120
115
110
105
100
95
90
ppm

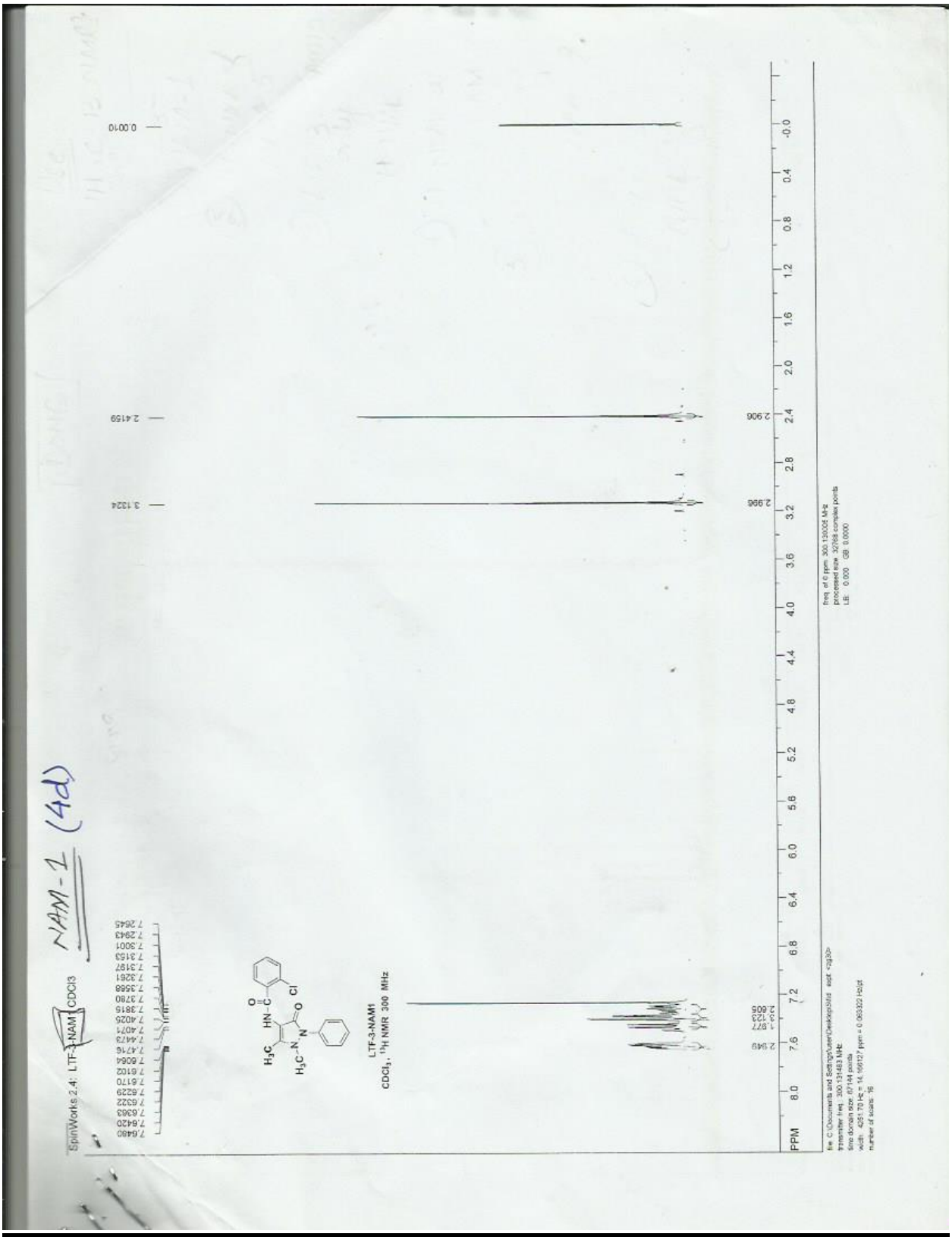
4c-^{1}H-NMR-a:



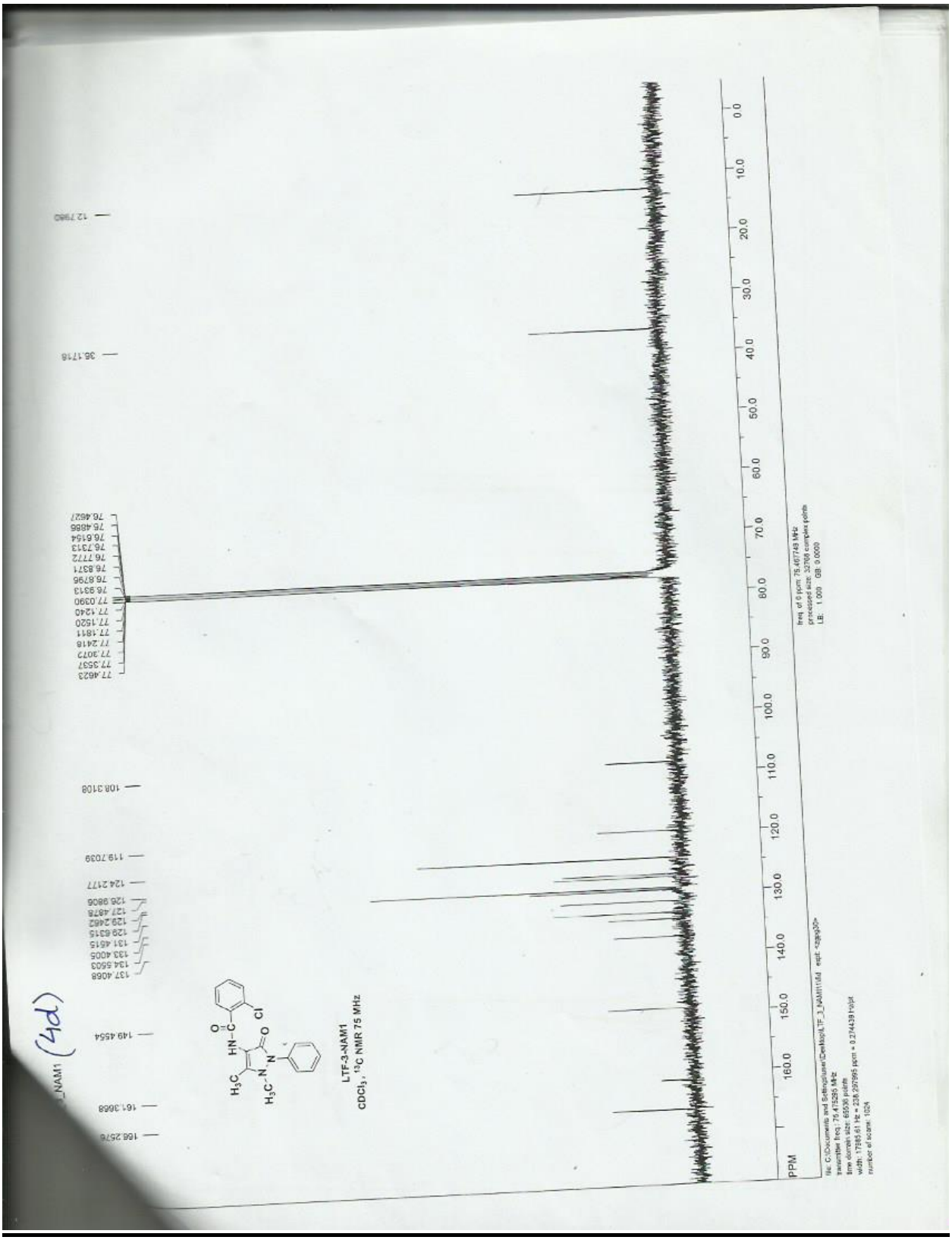
4c-^{13}C-NMR-a:


SpinWorks 2.4: LTF_3NAM6
(4c)
LTF-3-NAM6
CDCl3, 13C NMR 75 MHz
12.8814
21.4912
36.2318
76.6097
76.7205
77.0329
77.1657
77.2347
77.4563
109.0746
124.3383
126.9831
127.5656
128.1932
129.2788
130.8778
134.6630
142.2710
149.3169
161.8610
166.0969
PPM
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0

4d-^{1}H-NMR-a:

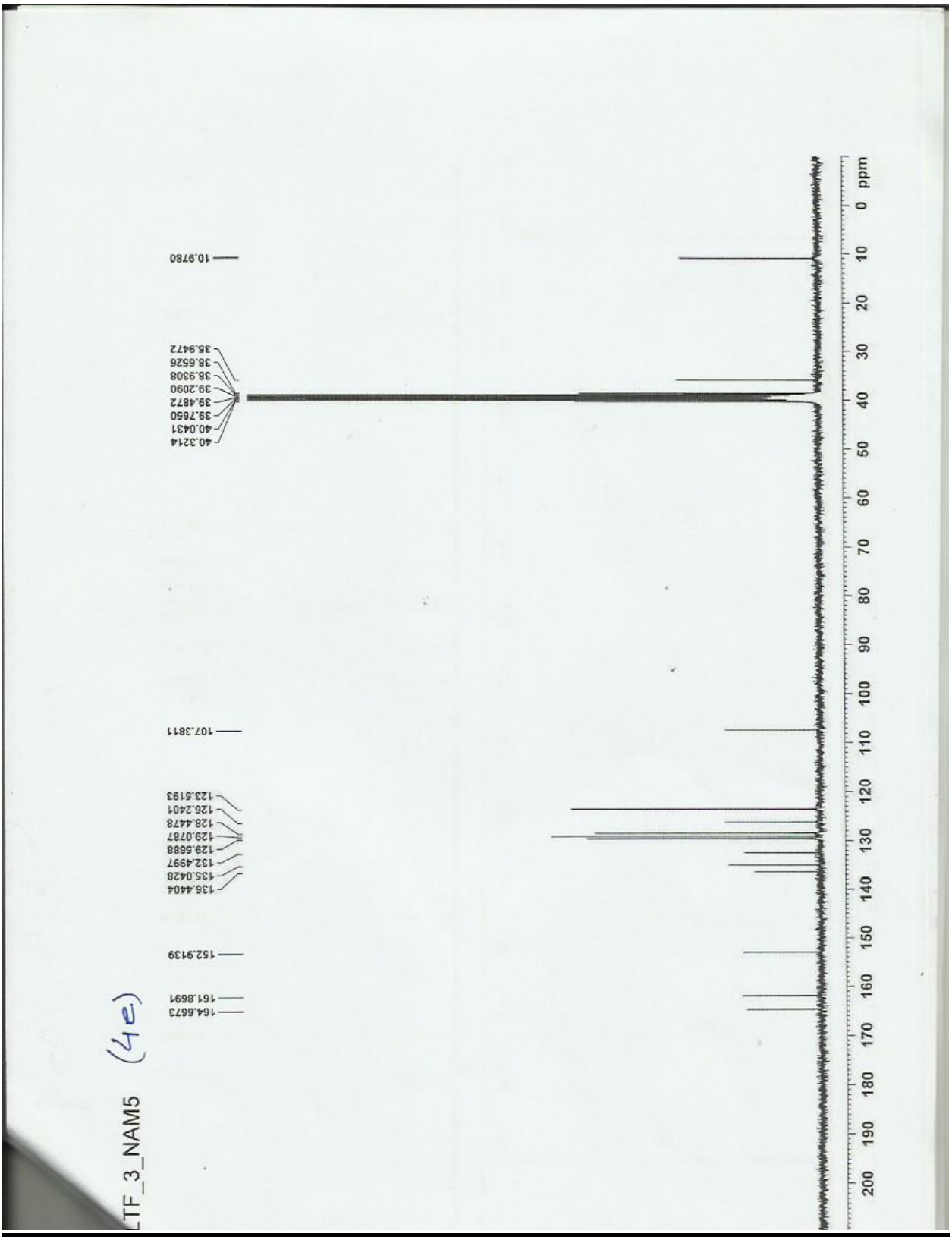

SpinWorks 2.4: LTF-3-NAM1 CDCl3
NAM-1 (4d)
LTF-3-NAM1
CDCl3, 1H NMR 300 MHz
PPM

4d-^{13}C-NMRa:



4e-^{1}H-NMR-a:

4e-^{13}C-NMR-a: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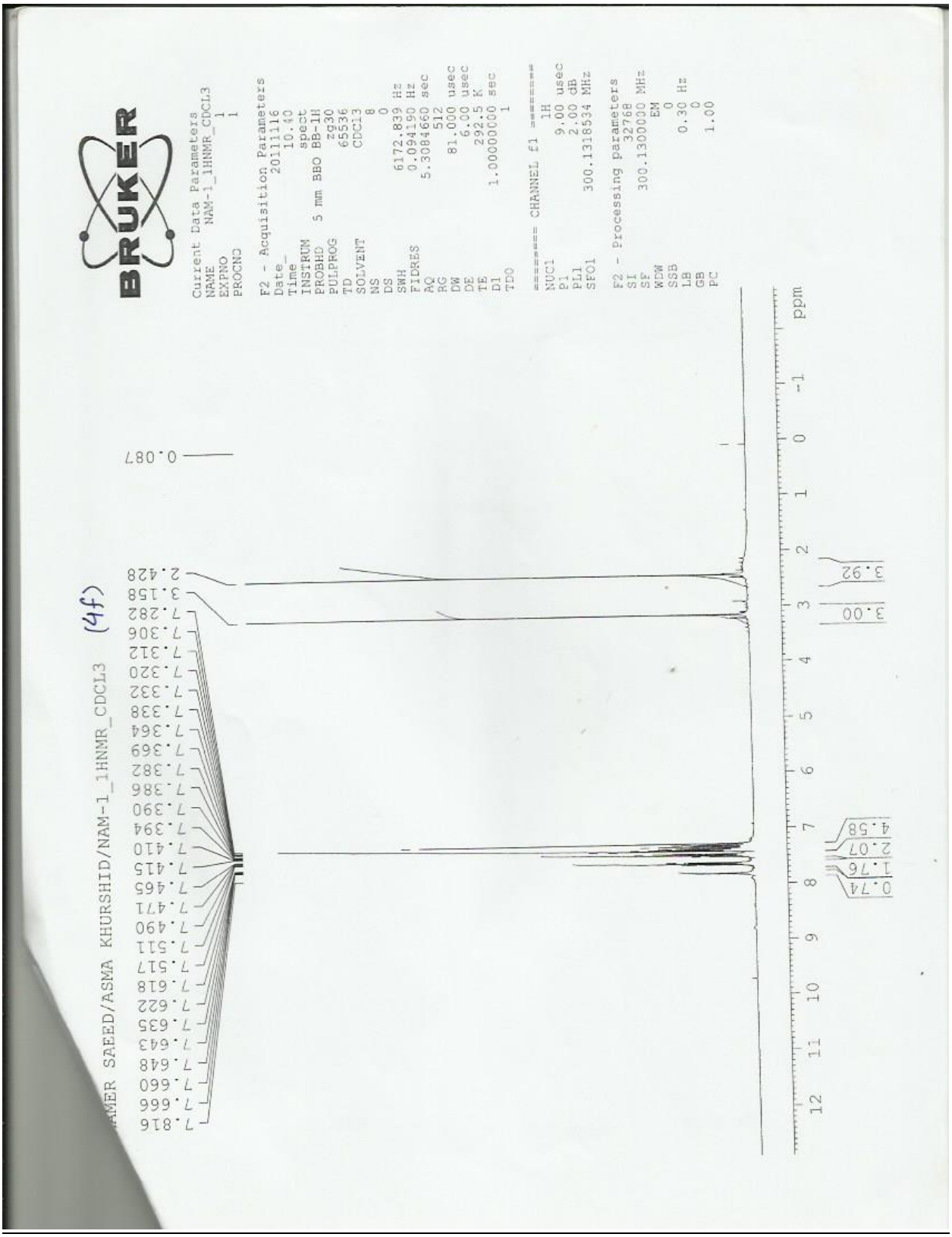
164.6673
161.8691
152.9139
136.4404
135.0428
132.4997
129.5688
129.0787
128.4478
126.2401
123.5193
107.3811
40.3214
40.0431
39.7650
39.4872
39.2090
38.9308
38.6526
35.9472
10.9780
ppm

4f-^{1}H-NMR-a: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PROCNO 1
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Time_ 10.40
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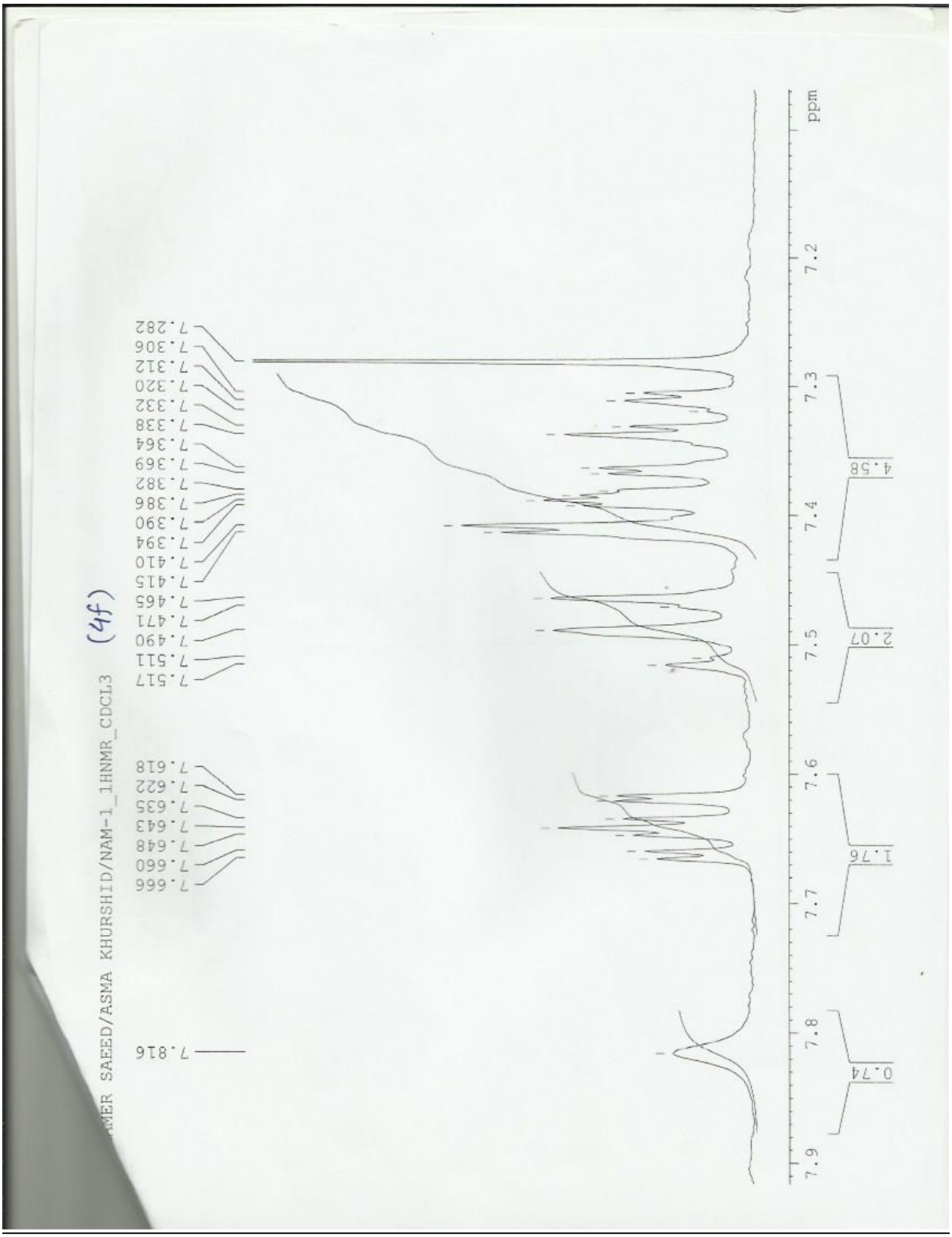
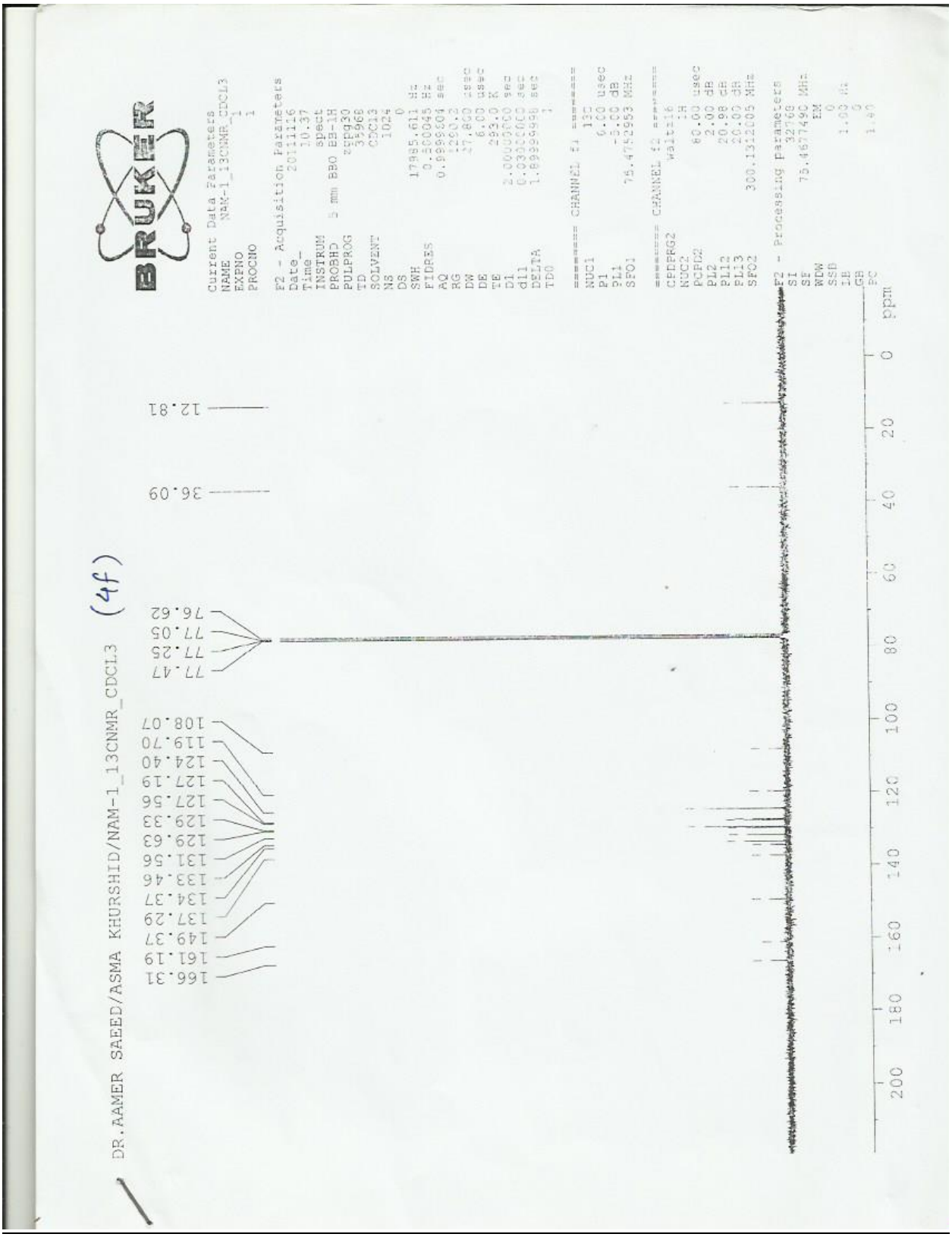
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 0
SWH 6172.839 Hz
FIDRES 0.094190 Hz
AQ 5.3084660 sec
RG 512
DW 81.000 usec
DE 6.00 usec
TE 292.5 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 2.00 dB
SFO1 300.1318534 MHz
F2 - Processing parameters
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
0.087
2.428
3.158
7.282
7.306
7.312
7.320
7.332
7.338
7.364
7.369
7.382
7.386
7.390
7.394
7.410
7.415
7.465
7.471
7.490
7.511
7.517
7.618
7.622
7.635
7.643
7.648
7.660
7.666
7.816
(4f)
NAM-1_1HNMR_CDCl3
KHURSHID/ASMA
SAEED/ASMA
3.92
3.00
0.74
1.76
2.07
4.58
ppm

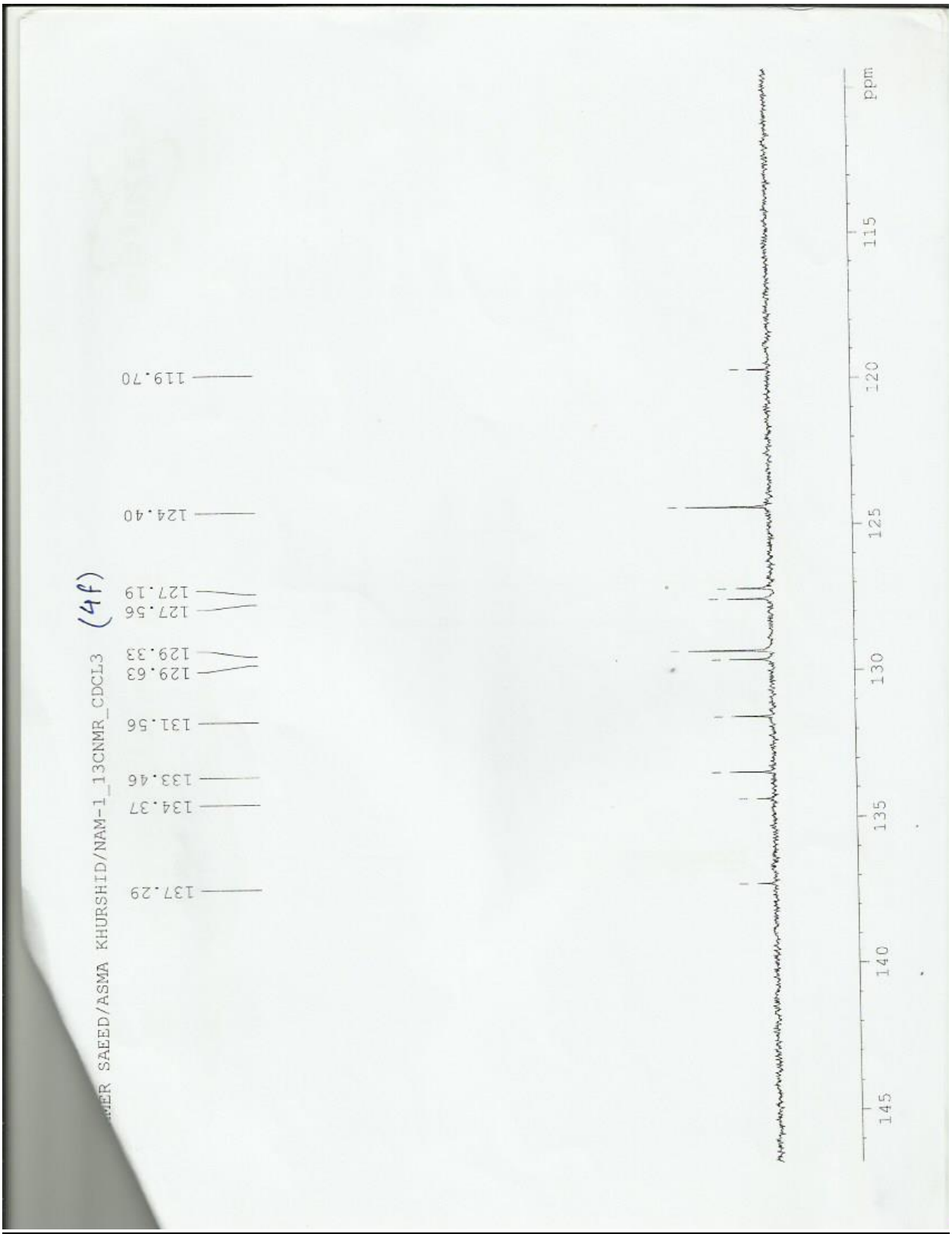
4f-^{1}H-NMR-b:


SAEED/ASMA KHURSHID/NAM-1_1HNMR_CDCL3 (4f)
7.816
7.666
7.660
7.648
7.643
7.635
7.622
7.618
7.517
7.511
7.490
7.471
7.465
7.415
7.410
7.394
7.390
7.386
7.382
7.369
7.364
7.338
7.332
7.320
7.312
7.306
7.282
ppm
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
0.74
1.76
2.07
4.58

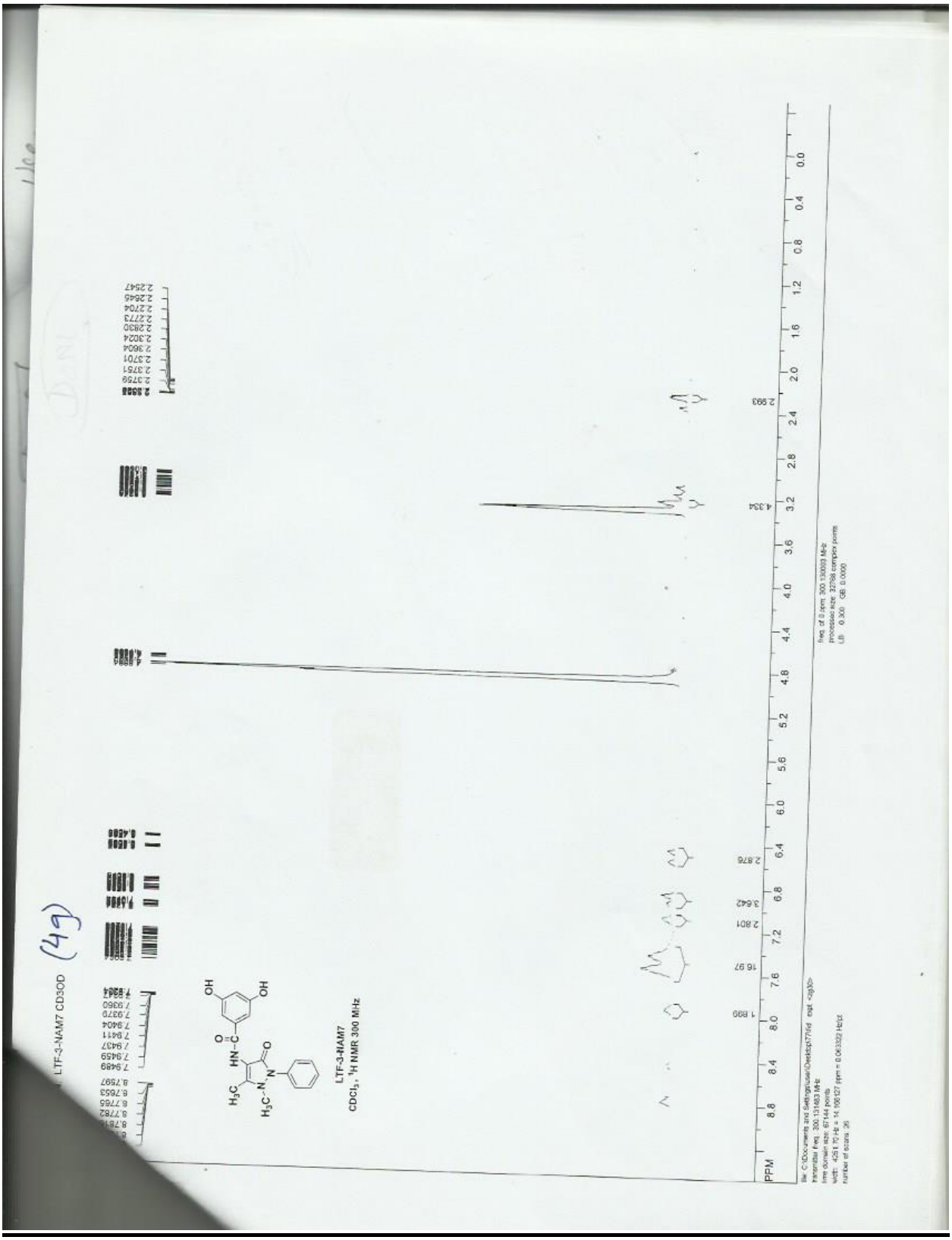
4f-^{13}C-NMRa:


DR.AAMER SAEED/ASMA KHURSHID/NAM-1_13CNMR_CDCL3
(4f)
166.31
161.19
149.37
137.29
134.37
133.46
131.56
129.63
129.33
127.56
127.19
124.40
119.70
108.07
77.47
77.25
77.05
76.62
36.09
12.81
ppm

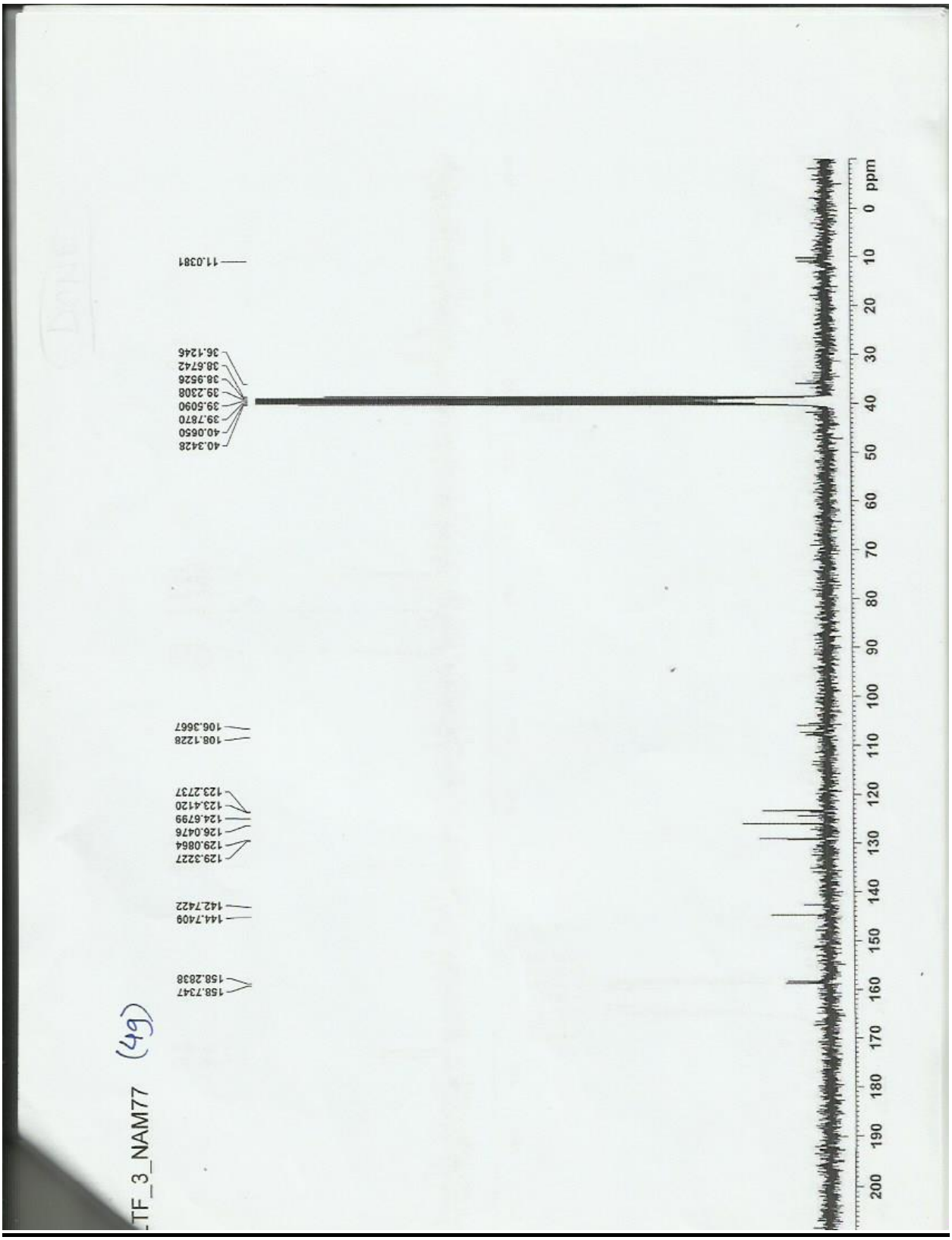
4f-^{13}C-NMR-b:


ER SAEED/ASMA KHURSHID/NAM-1_13CNMR_CDCL3
(4f)
137.29
134.37
133.46
131.56
129.63
129.33
127.56
127.19
124.40
119.70
145
140
135
130
125
120
115
ppm

4g-^{1}H-NMR-a:



4g-^{13}C-NMR-a: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38.5742
38.9526
39.2308
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40.0650
40.3428
106.3667
108.1228
123.2737
123.4120
124.6799
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129.0864
129.3227
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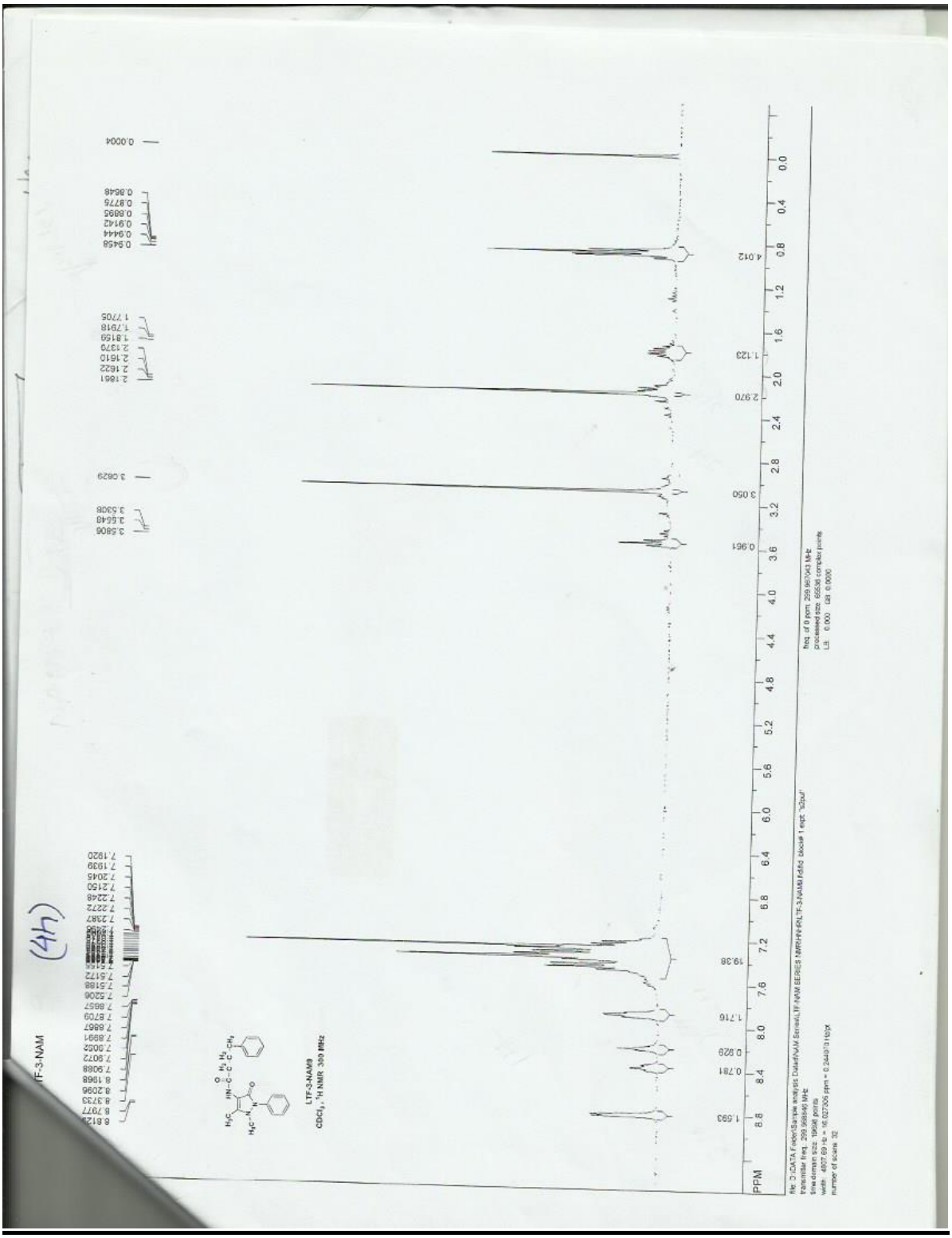
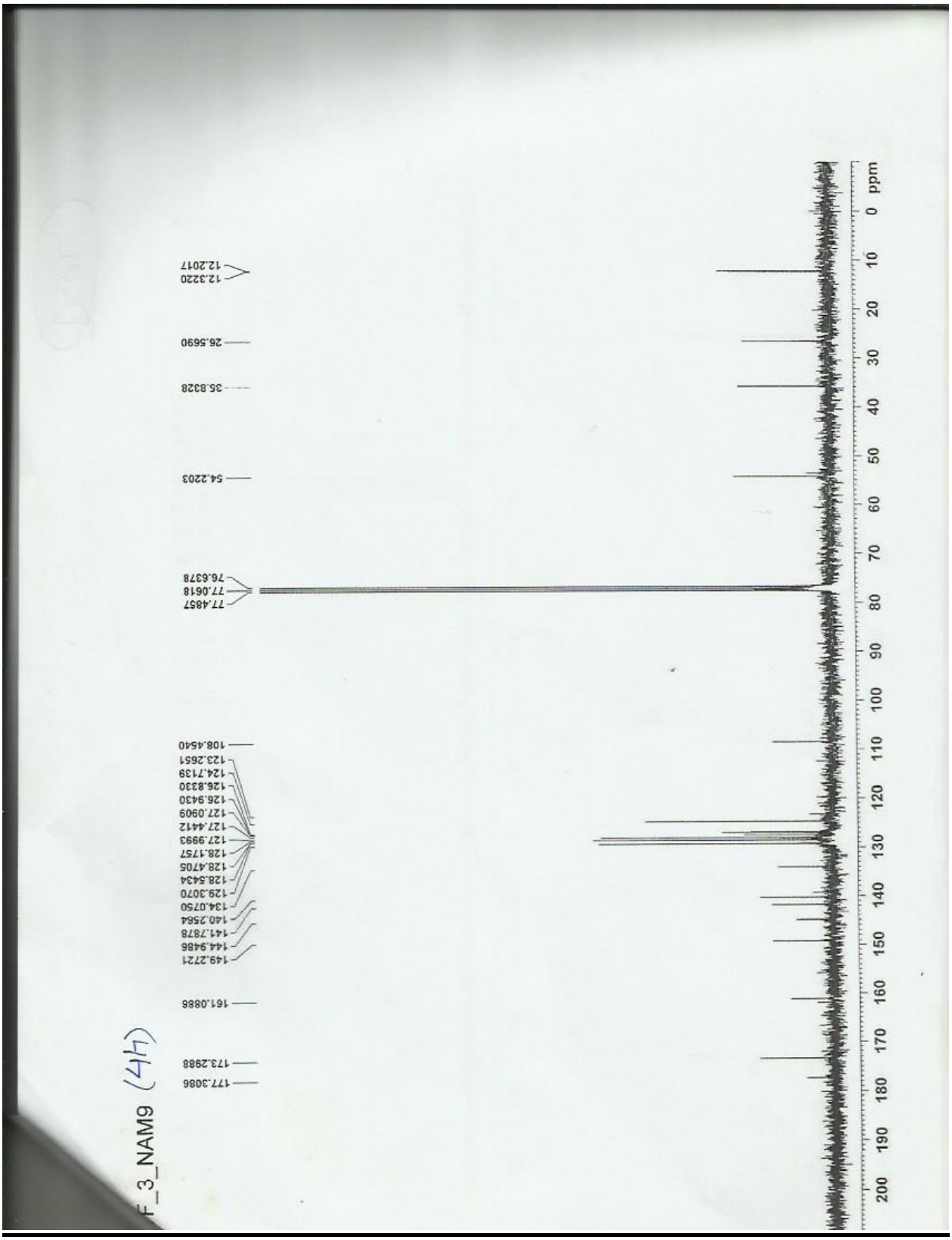
158.7347
ppm

4h-^{1}H-NMR-a:



4h-^{13}C-NMR-a: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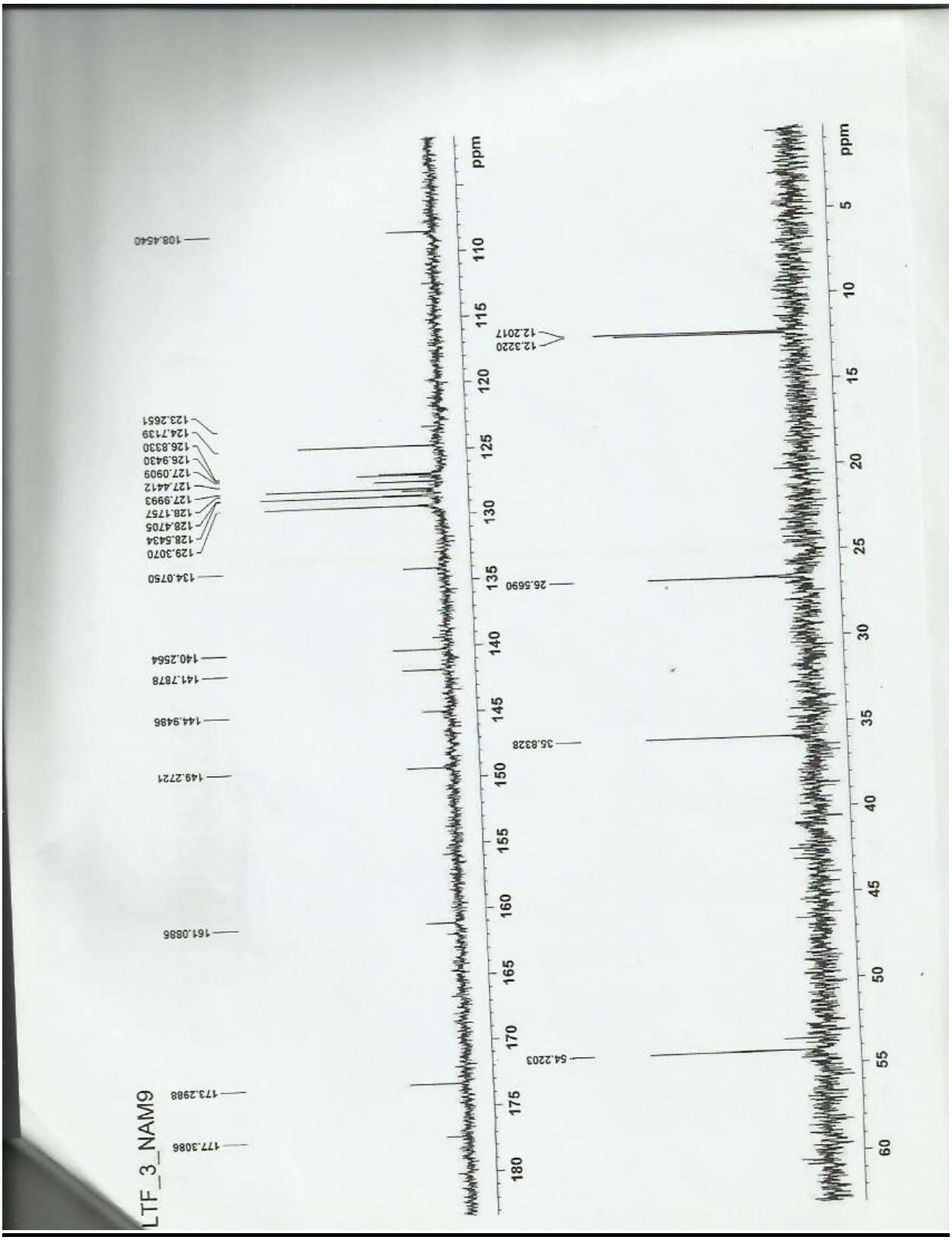
173.2988
161.0886
149.2721
144.9486
141.7878
140.2564
134.0750
129.3070
128.5434
128.4705
128.1757
127.9993
127.4412
127.0909
126.9430
126.8330
124.7139
123.2651
108.4540
77.4857
77.0618
76.6378
54.2203
35.8328
26.5690
12.3220
12.2017
ppm

4h-^{13}C-NMR-b:


LTF_3_NAM9
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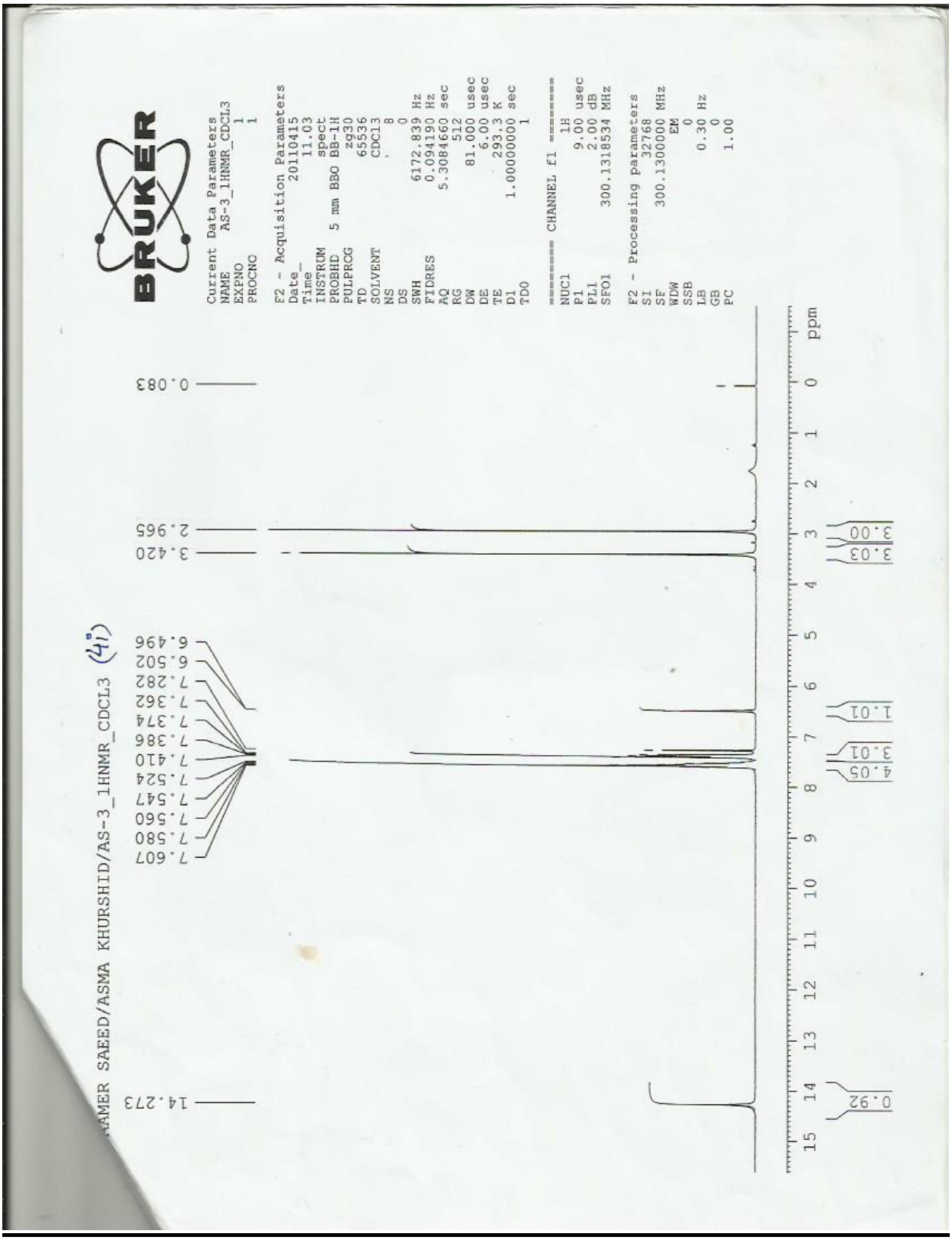
173.2988
161.0886
149.2721
144.9486
141.7878
140.2564
134.0750
129.3070
128.5434
128.4705
128.1757
127.9993
127.4412
127.0909
126.9430
126.8330
124.7139
123.2651
108.4540
54.2203
35.8328
26.5690
12.3220
12.2017
ppm

4i-^{1}H-NMR-a: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PROCNO 1
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Time 11.03
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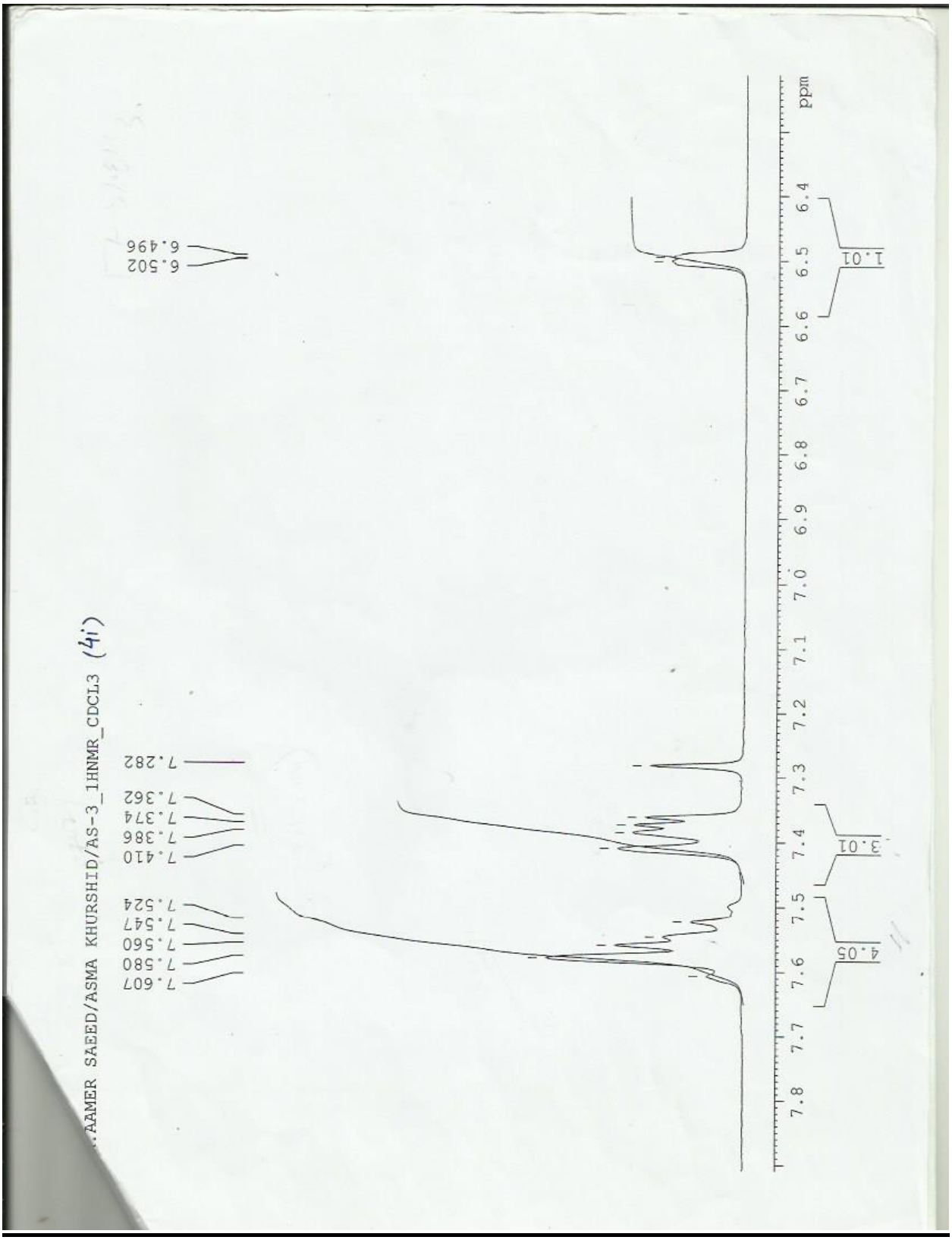
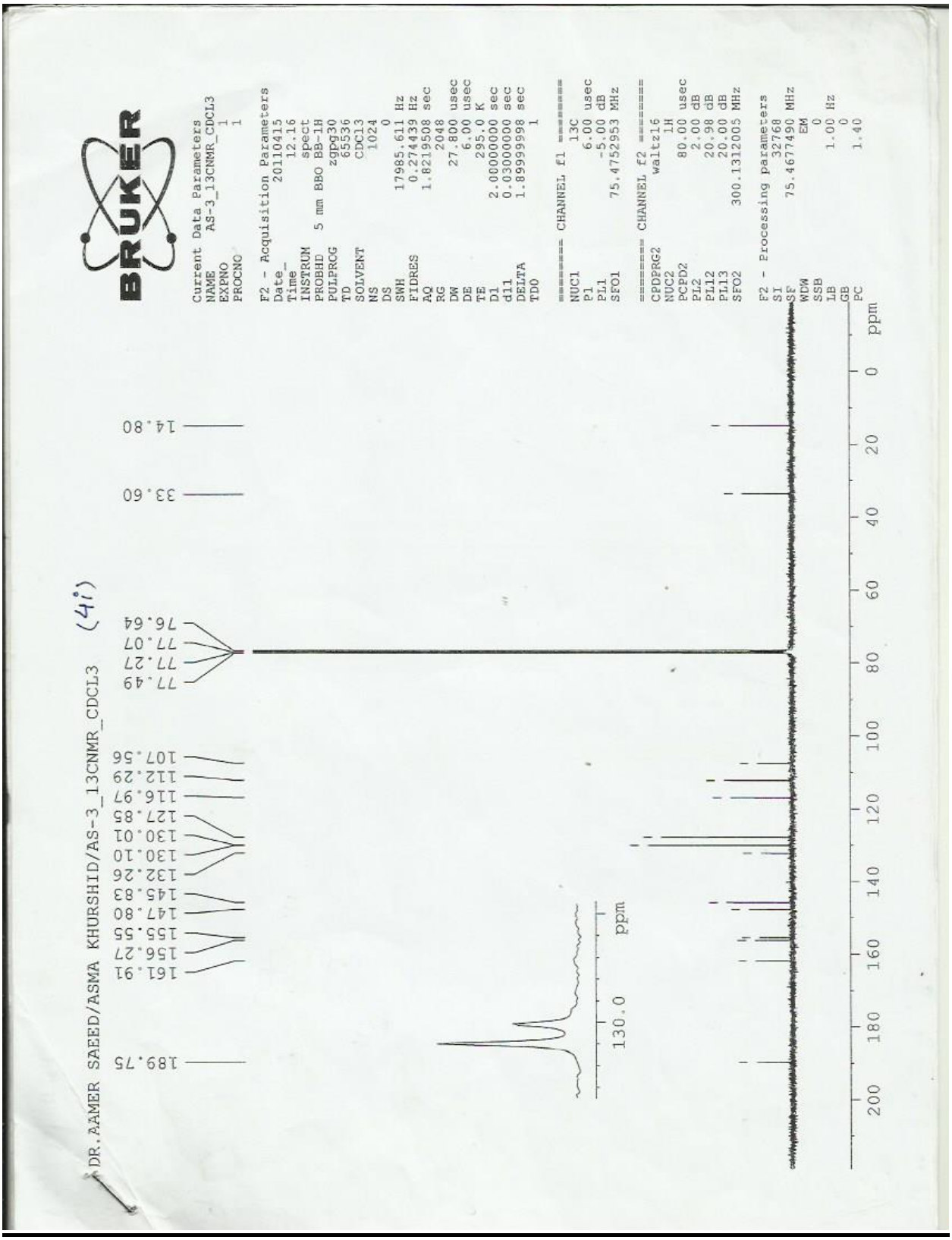
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 8
DS 0
SWH 6172.839 Hz
FIDRES 0.094190 Hz
AQ 5.3084660 sec
RG 512
DW 81.000 usec
DE 6.00 usec
TE 293.3 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 2.00 dB
SFO1 300.1318534 MHz
F2 - Processing parameters
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
AMER SAEED/ASMA KHURSHID/AS-3_1HNMR_CDCL3 (4i)
14.273
7.607
7.580
7.560
7.547
7.524
7.410
7.386
7.374
7.362
7.282
6.502
6.496
3.420
2.965
0.083
0.92
3.00
3.03
1.01
3.01
4.05
ppm

4i-^{1}H-NMR-b:


AAMER SAEED/ASMA KHURSHID/AS-3_1HNMR_CDCL3 (4i')
6.496
6.502
7.282
7.362
7.374
7.386
7.410
7.524
7.547
7.560
7.580
7.607
1.01
3.01
4.05
ppm

4i-^{13}C-NMR-a:


DR.AAMER SAEED/ASMA KHURSHID/AS-3_13CNMR_CDCL3 (4i)
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PROCNO 1
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Date_ 20110415
Time_ 12.16
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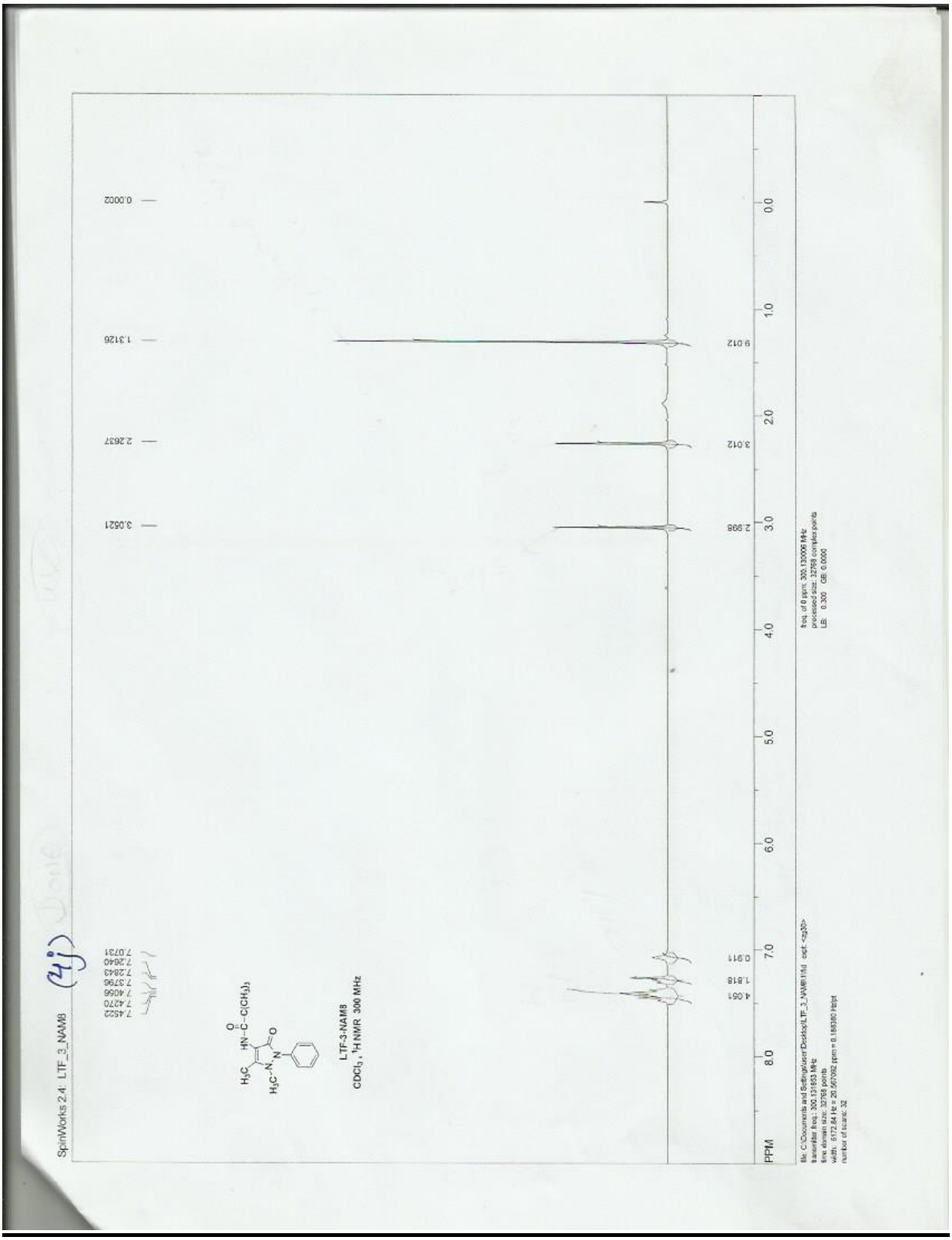
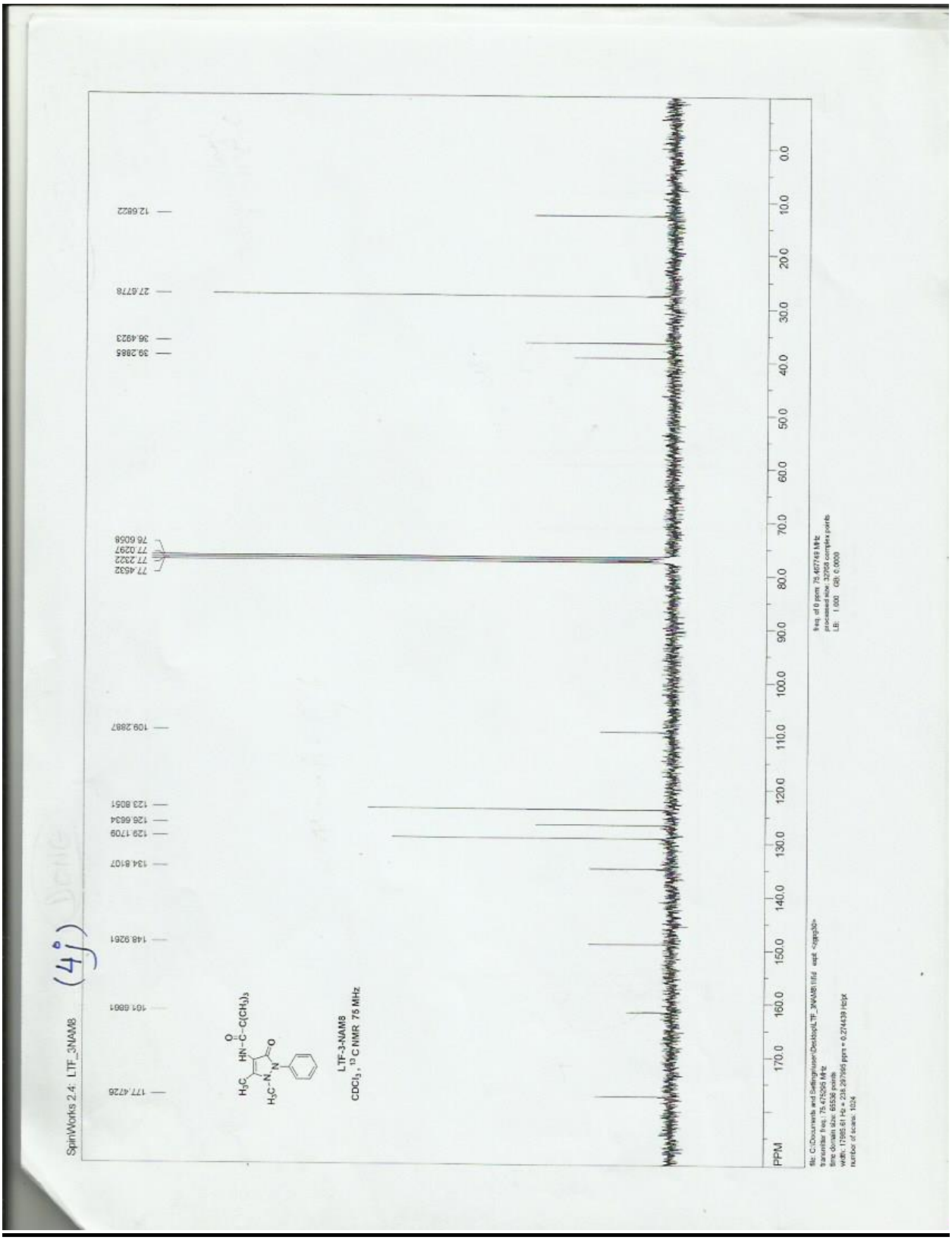
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 0
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 2048
DW 27.800 usec
DE 6.00 usec
TE 295.0 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 6.00 usec
PL1 -5.00 dB
SFO1 75.4752953 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 2.00 dB
PL12 20.98 dB
PL13 20.00 dB
SFO2 300.1312005 MHz
F2 - Processing parameters
SI 32768
SF 75.4677490 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
189.75
161.91
156.27
155.55
147.80
145.83
132.26
130.10
130.01
127.85
116.97
112.29
107.56
77.49
77.27
77.07
76.64
33.60
14.80
130.0
ppm
200 180 160 140 120 100 80 60 40 20 0 ppm

4j-^{1}H-NMR-a:



4j-^{13}C-NMR-a:


SpinWorks 2.4: LTF_3NAM8
(4j)
LTF-3-NAM8
CDCl3 , 13C NMR 75 MHz
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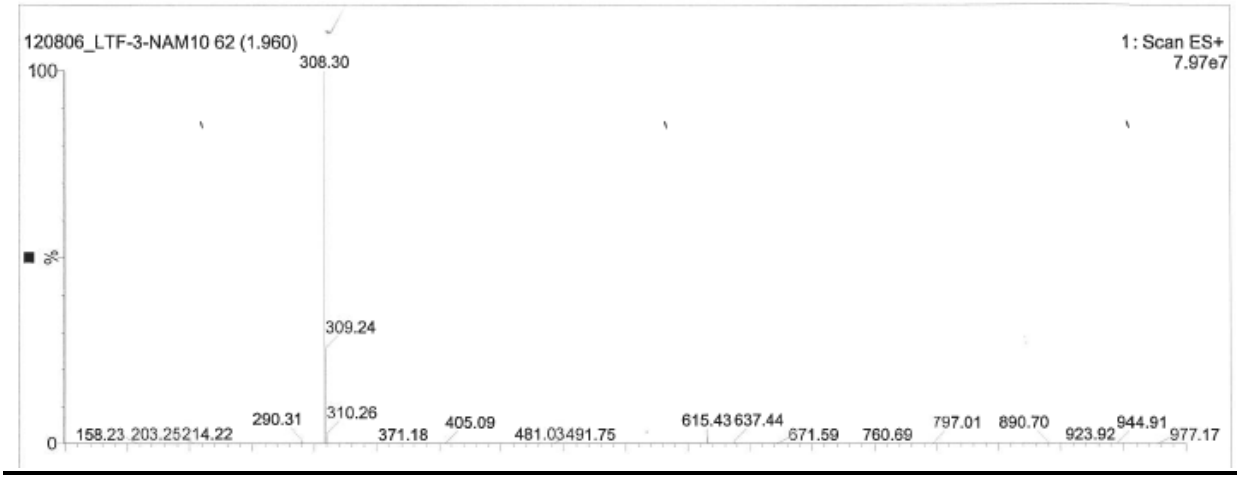
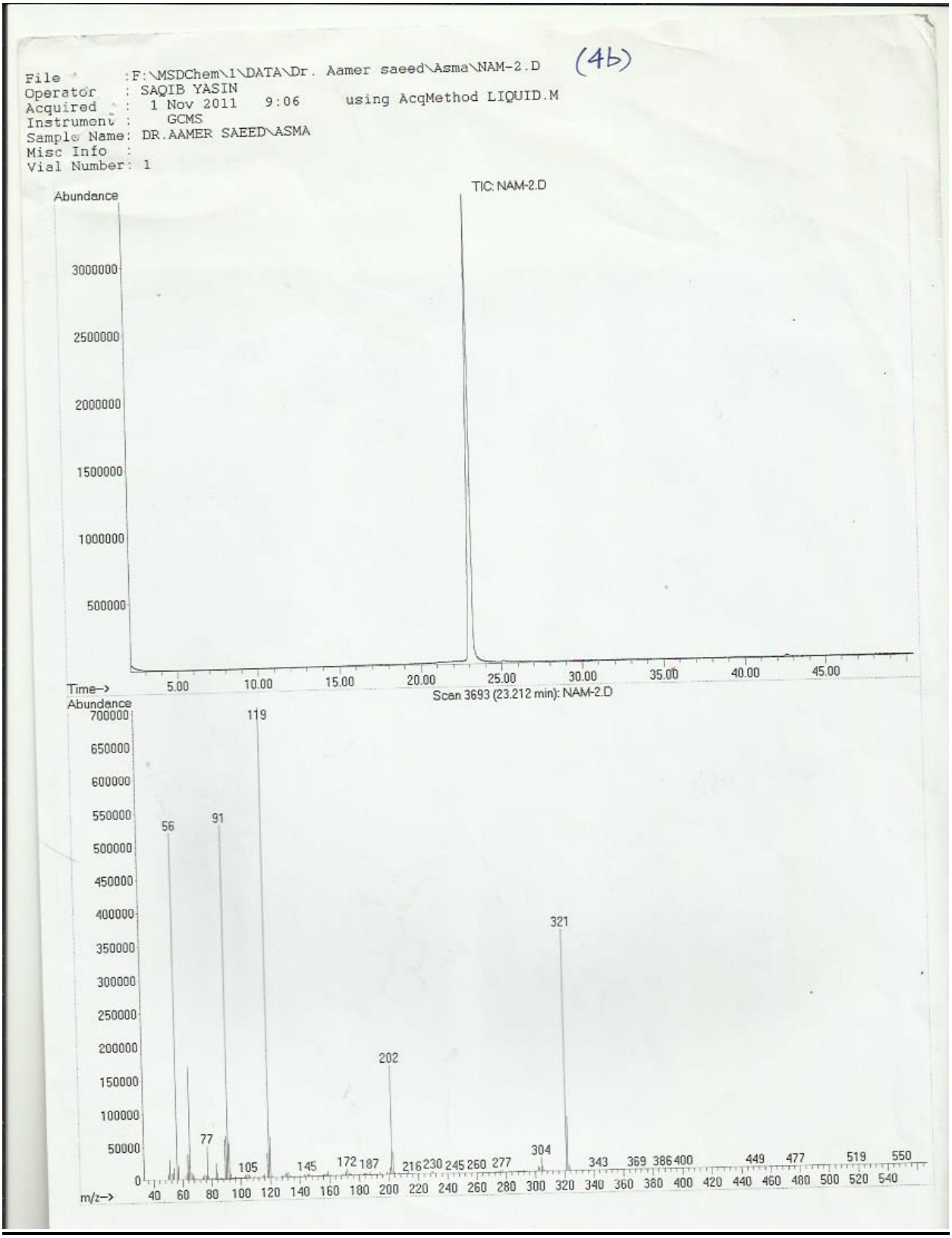
161.6861
148.9261
134.8107
129.1709
126.6634
123.8051
109.2887
77.4532
77.2322
77.0297
76.6058
39.2885
36.4923
27.6778
12.6822
PPM
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0

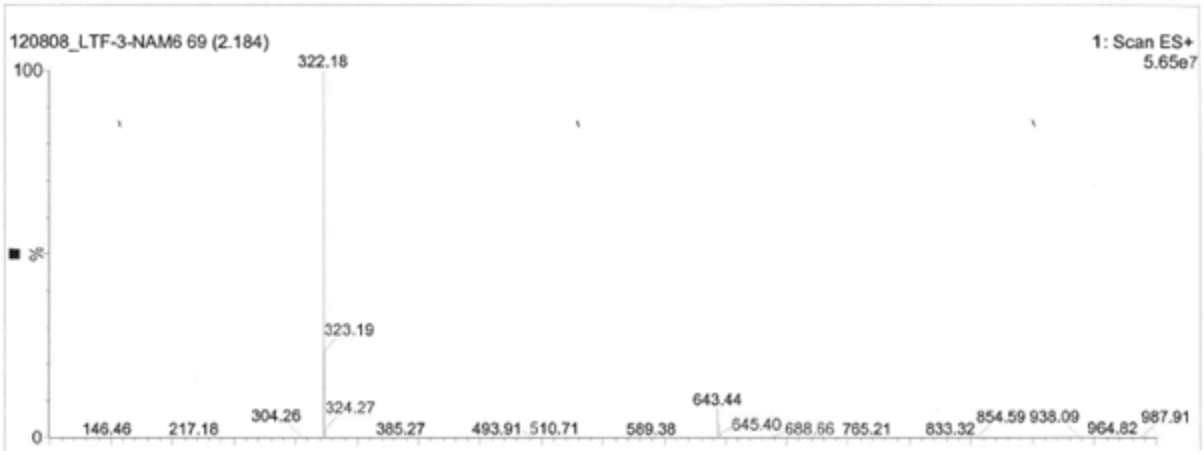
4a (LC/MS)


120806_LTF-3-NAM10 62 (1.960)
1: Scan ES+
7.97e7
100
%
0
308.30
309.24
158.23
203.25
214.22
290.31
310.26
371.18
405.09
481.03
491.75
615.43
637.44
671.59
760.69
797.01
890.70
923.92
944.91
977.17

4b (GC/MS)

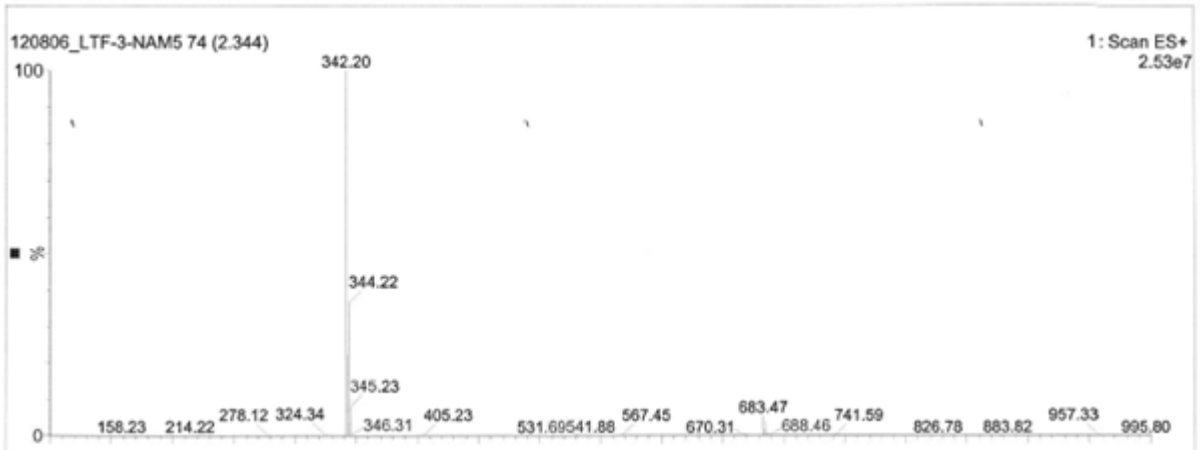

File :F:\MSDChem\1\DATA\Dr. Aamer saeed\Asma\NAM-2.D
(4b)
Operator : SAQIB YASIN
Acquired : 1 Nov 2011 9:06 using AcqMethod LIQUID.M
Instrument : GCMS
Sample Name: DR.AAMER SAEED\ASMA
Misc Info :
Vial Number: 1
Abundance
TIC: NAM-2.D
3000000
2500000
2000000
1500000
1000000
500000
Time-->
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00
Scan 3693 (23.212 min): NAM-2.D
Abundance
700000
650000
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
119
56
91
321
202
77
105
145
172
187
216
230
245
260
277
304
343
369
386
400
449
477
519
550
m/z-->
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540

4c (LC/MS)

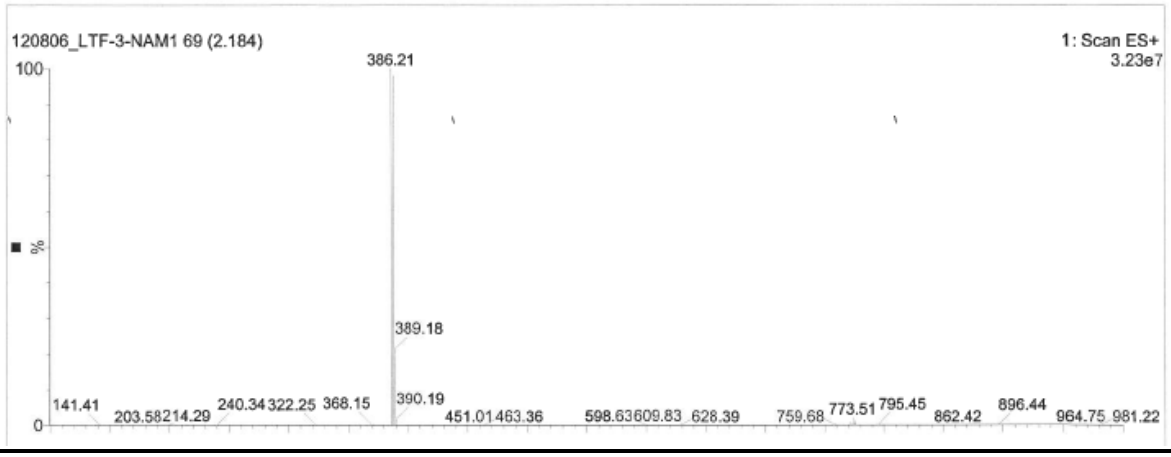


4d (LC/MS)

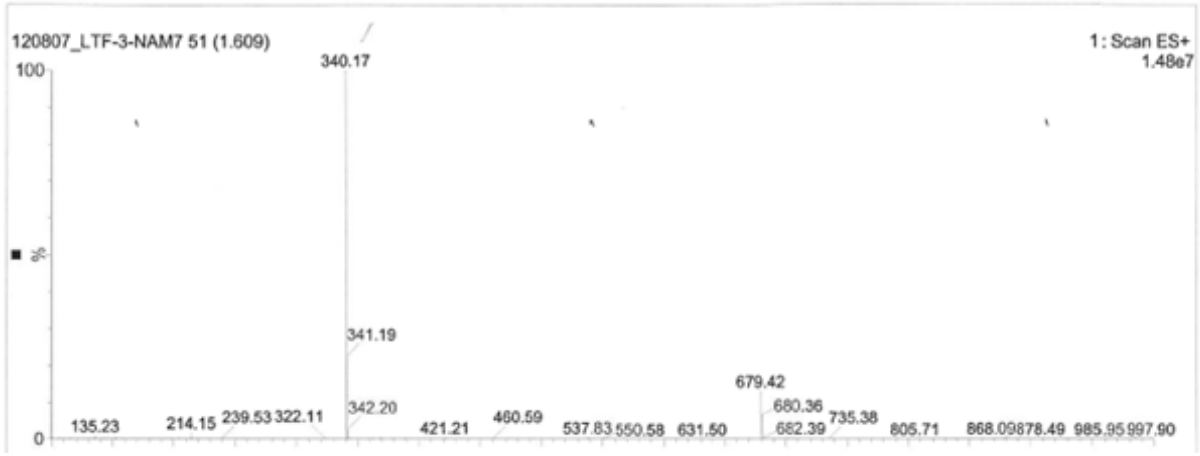
4e (LC/MS)



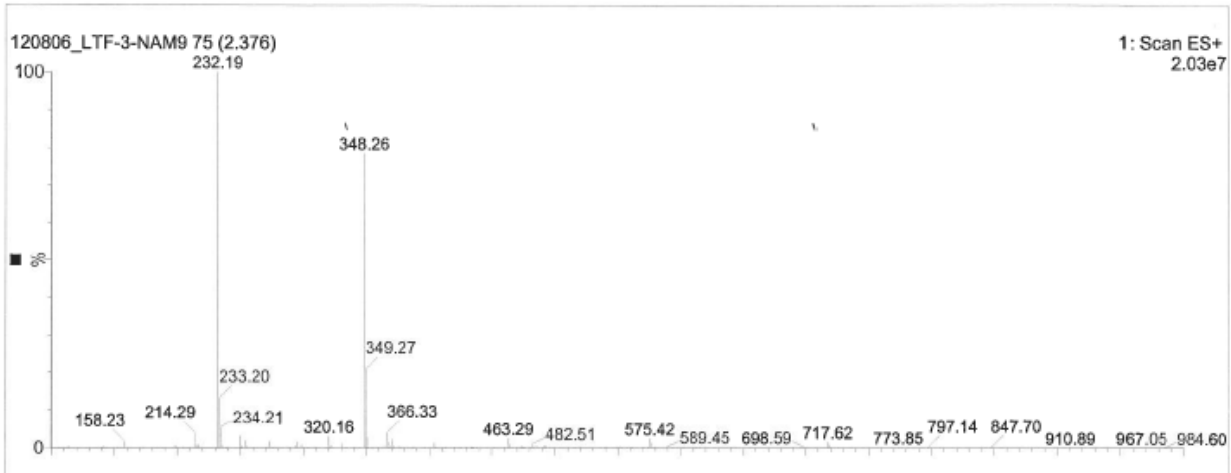
4f (LC/MS)



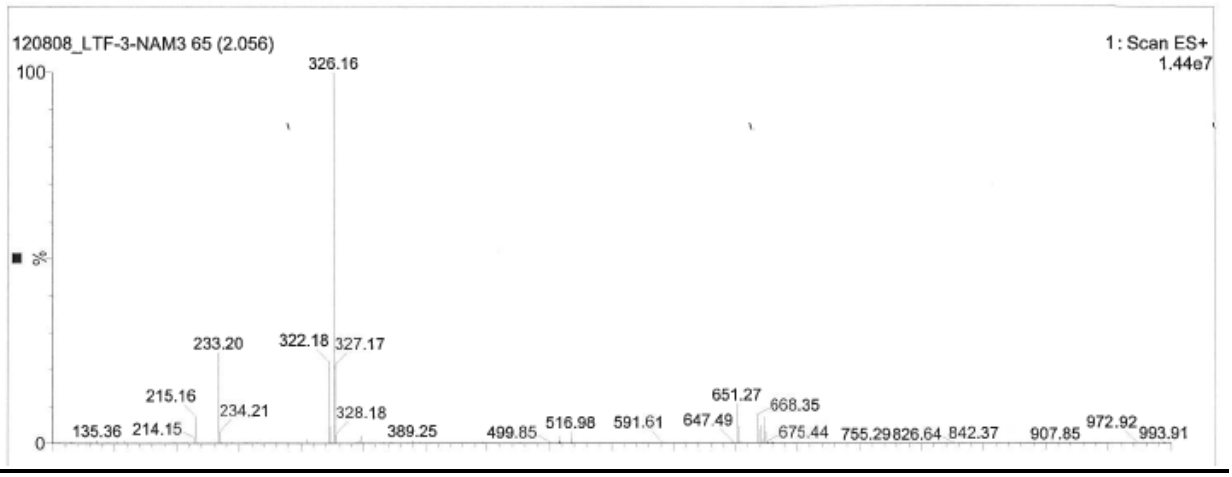
4g (LC/MS)



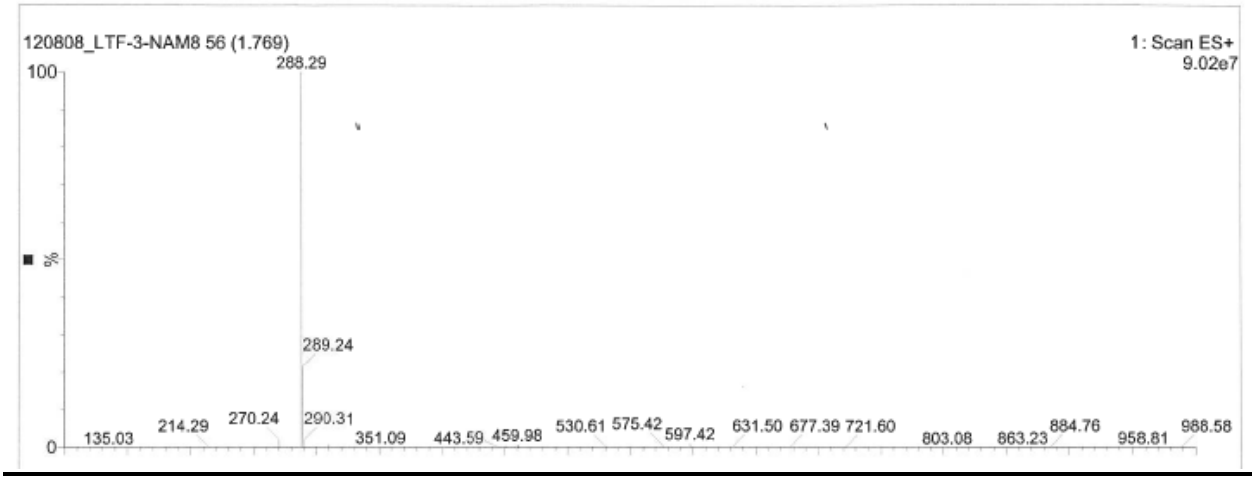
4h (LC/MS)



4i (LC/MS)



4j (LC/MS)



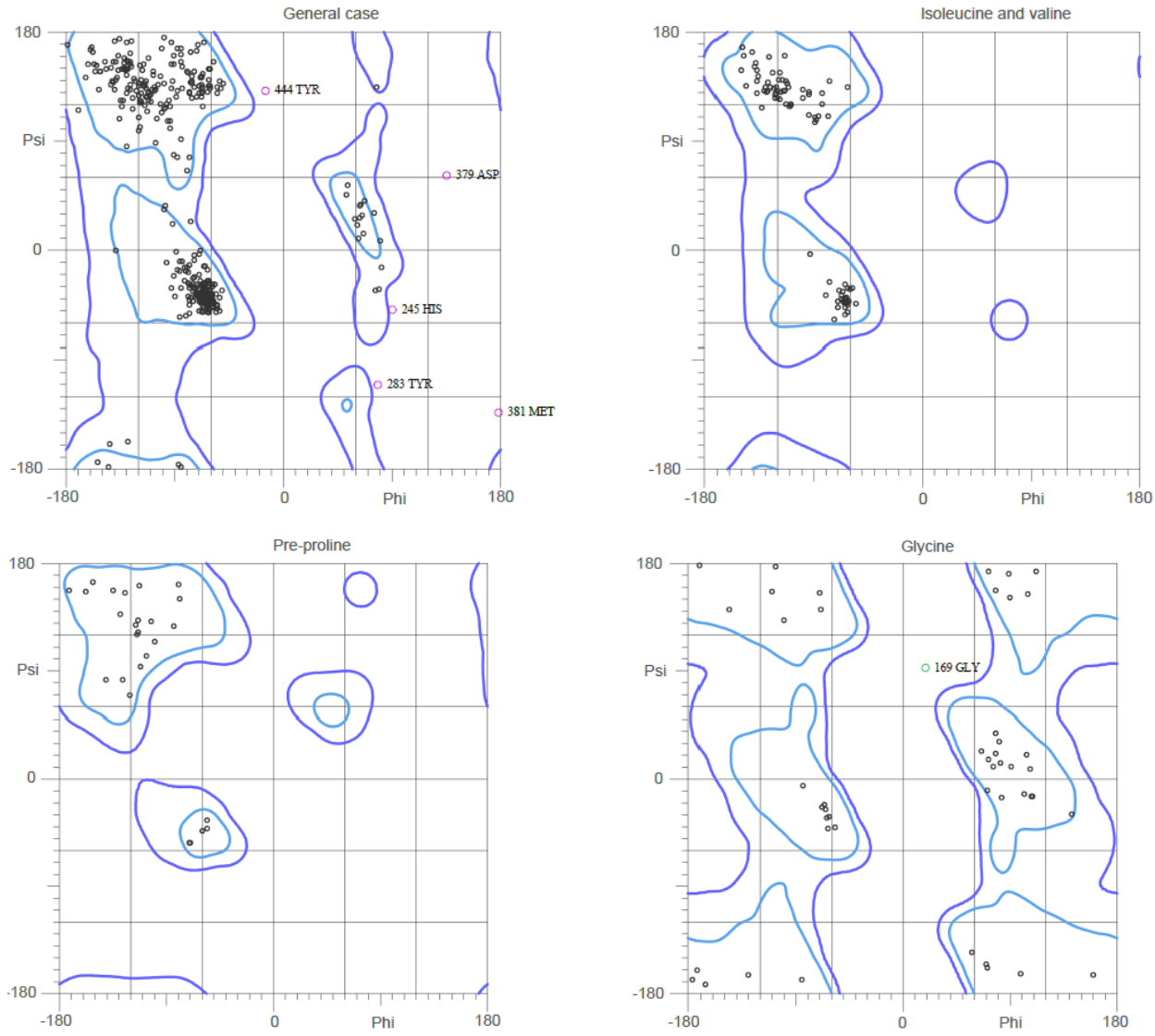


Figure S1. Ramachandran Plot of re5NT, (A) General; (B) Isoleucine & Valine; (C) Pre-Proline; (D) Glycine. 96.5% (554/574) of all residues are in favored (98%) regions. 99.0% (568/574) of all residues are in allowed (>99.8%) regions.

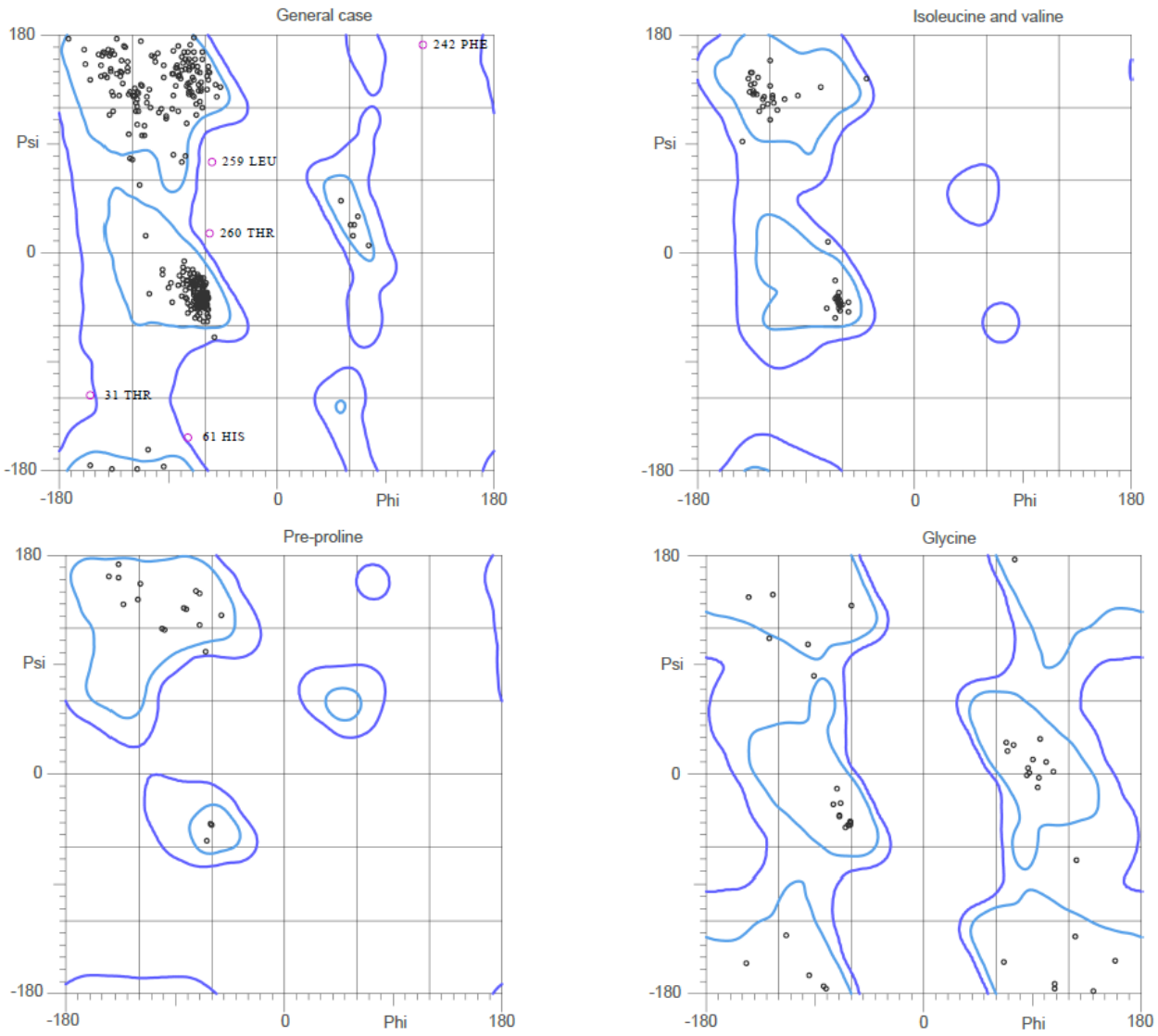


Figure S2. Ramachandran Plot of hTNAP, (A) General; (B) Isoleucine & Valine; (C) Pre-Proline; (D) Glycine. 96.0% (459/478) of all residues are in favored (98%) regions. 98.5% (471/478) of all residues are in allowed (>99.8%) regions.

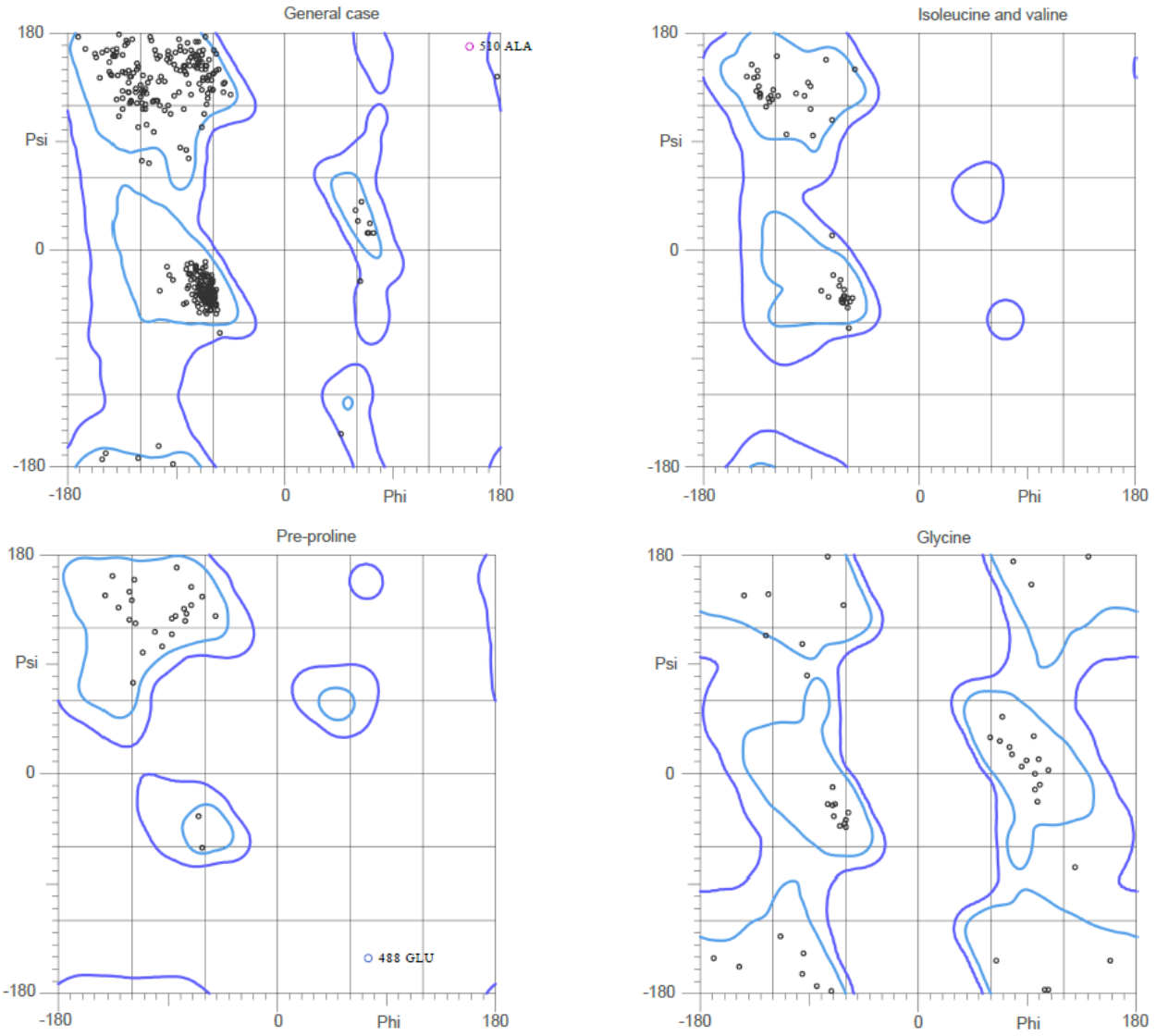


Figure S3. Ramachandran Plot of hIALP, (A) General; (B) Isoleucine & Valine; (C) Pre-Proline; (D) Glycine. 96.8% (509/526) of all residues are in favored (98%) regions. 99.4% (523/526) of all residues are in allowed (>99.8%) regions.

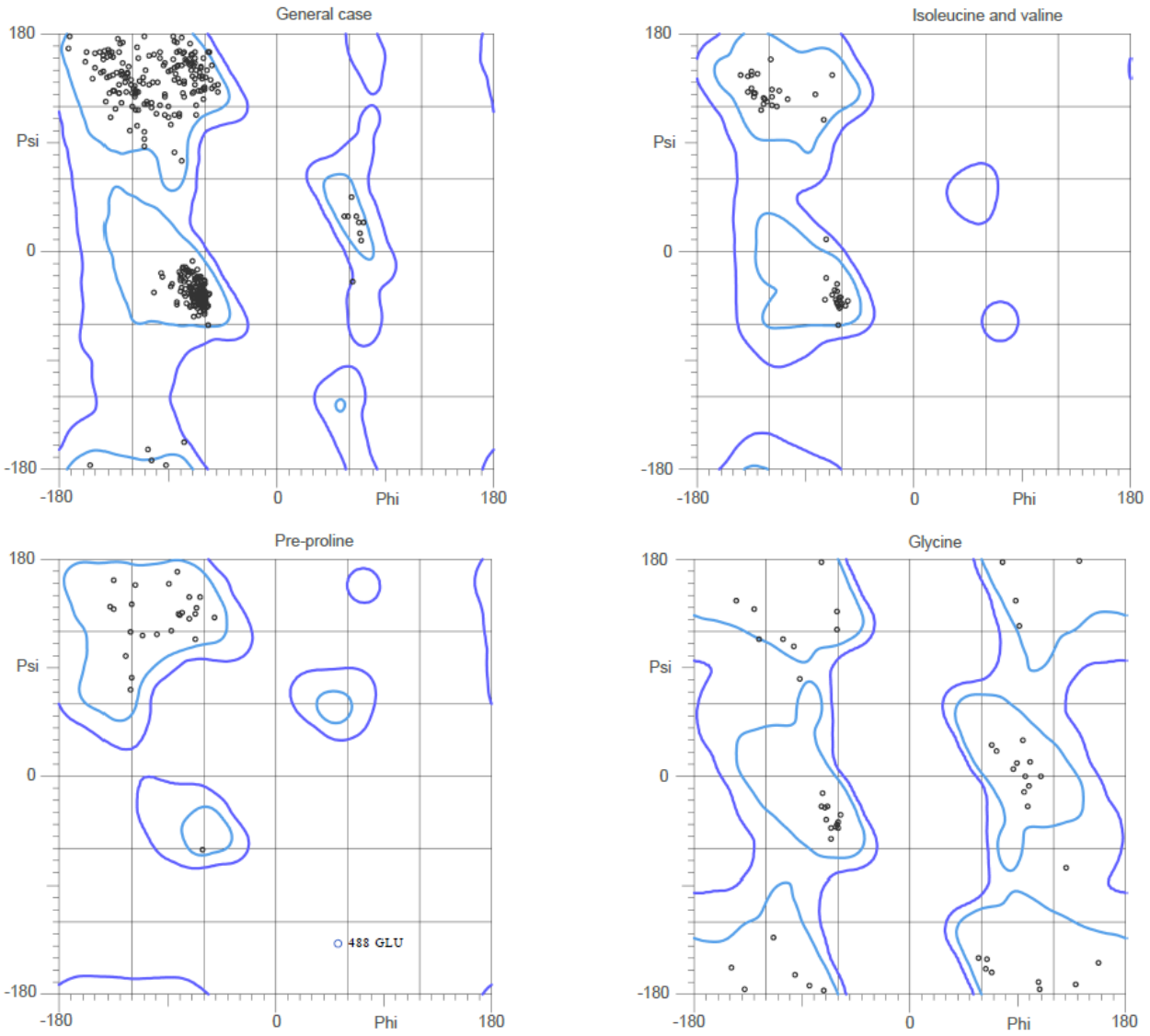


Figure S4. Ramachandran Plot of hGCALP, (A) General; (B) Isoleucine & Valine; (C) Pre-Proline; (D) Glycine. 97.9% (519/530) of all residues are in favored (98%) regions. 99.8% (529/530) of all residues are in allowed (>99.8%) regions.

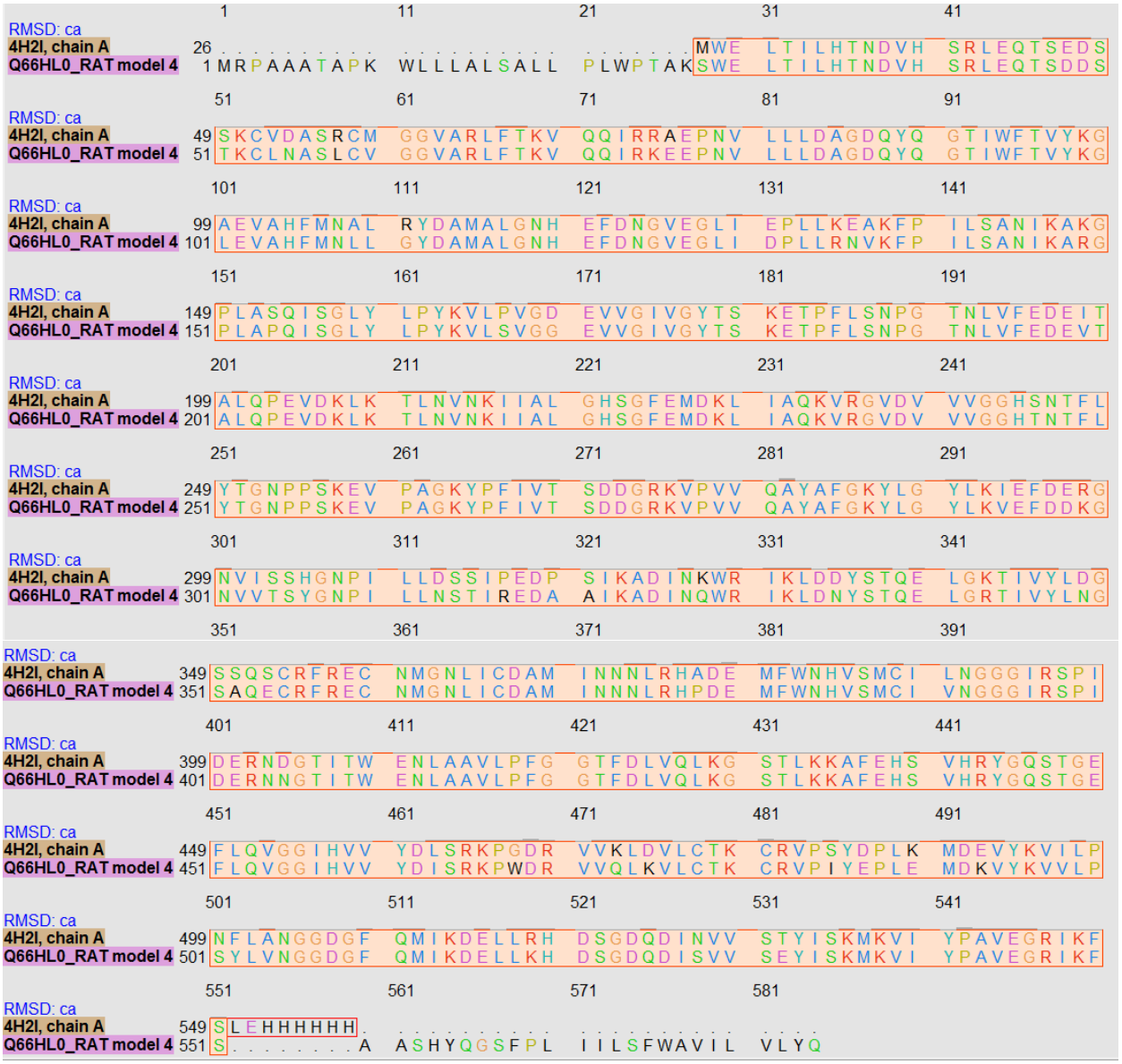


Figure S5. Sequence alignment of target rat ecto-5'-Nucleotidase (re5NT) with the template protein human ecto-5'-Nucleotidase (he5NT).

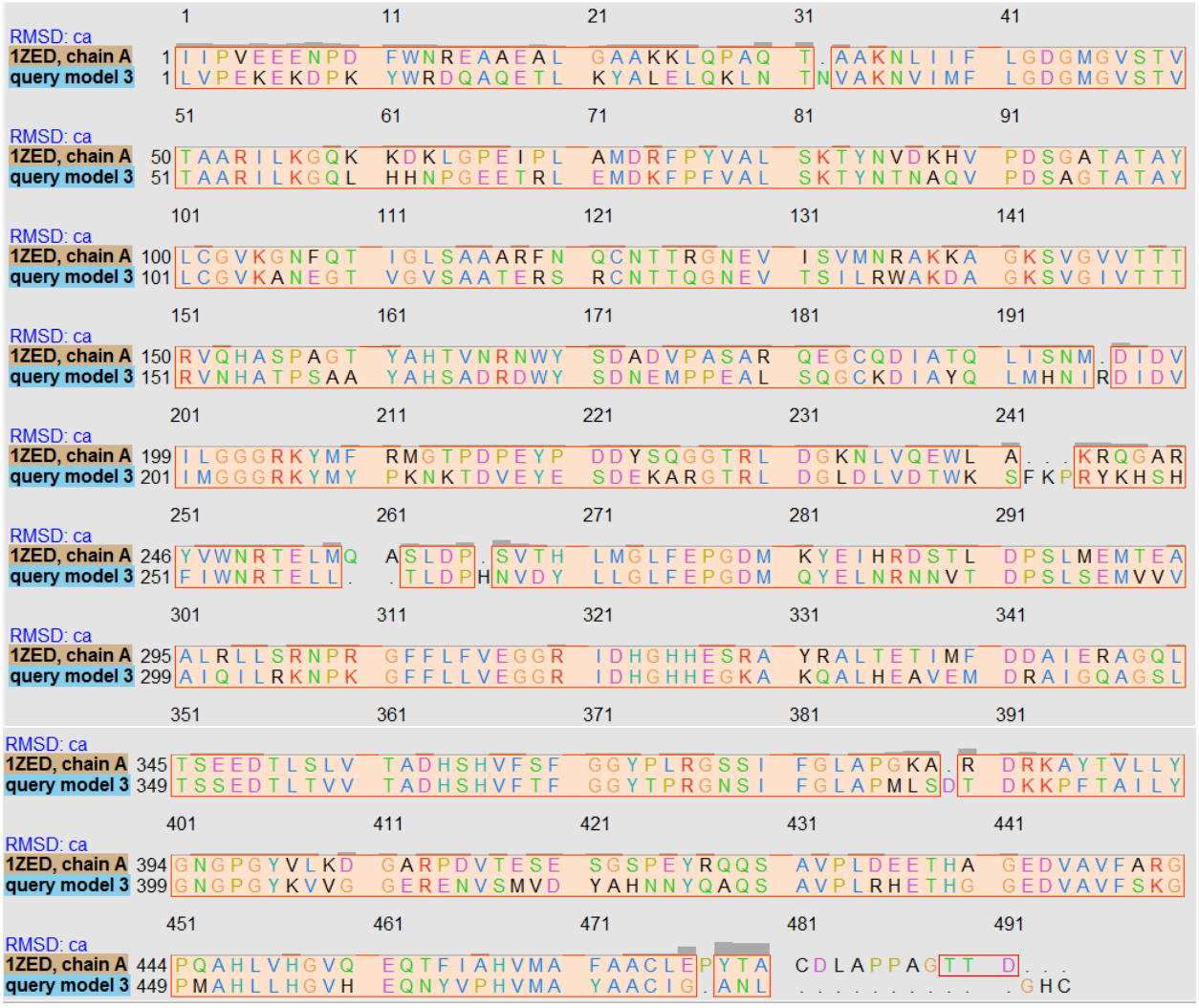


Figure S6. Sequence alignment of target human tissue non-specific alkaline phosphatase (hTNAP) with the template protein human placental alkaline phosphatase (hPALP).

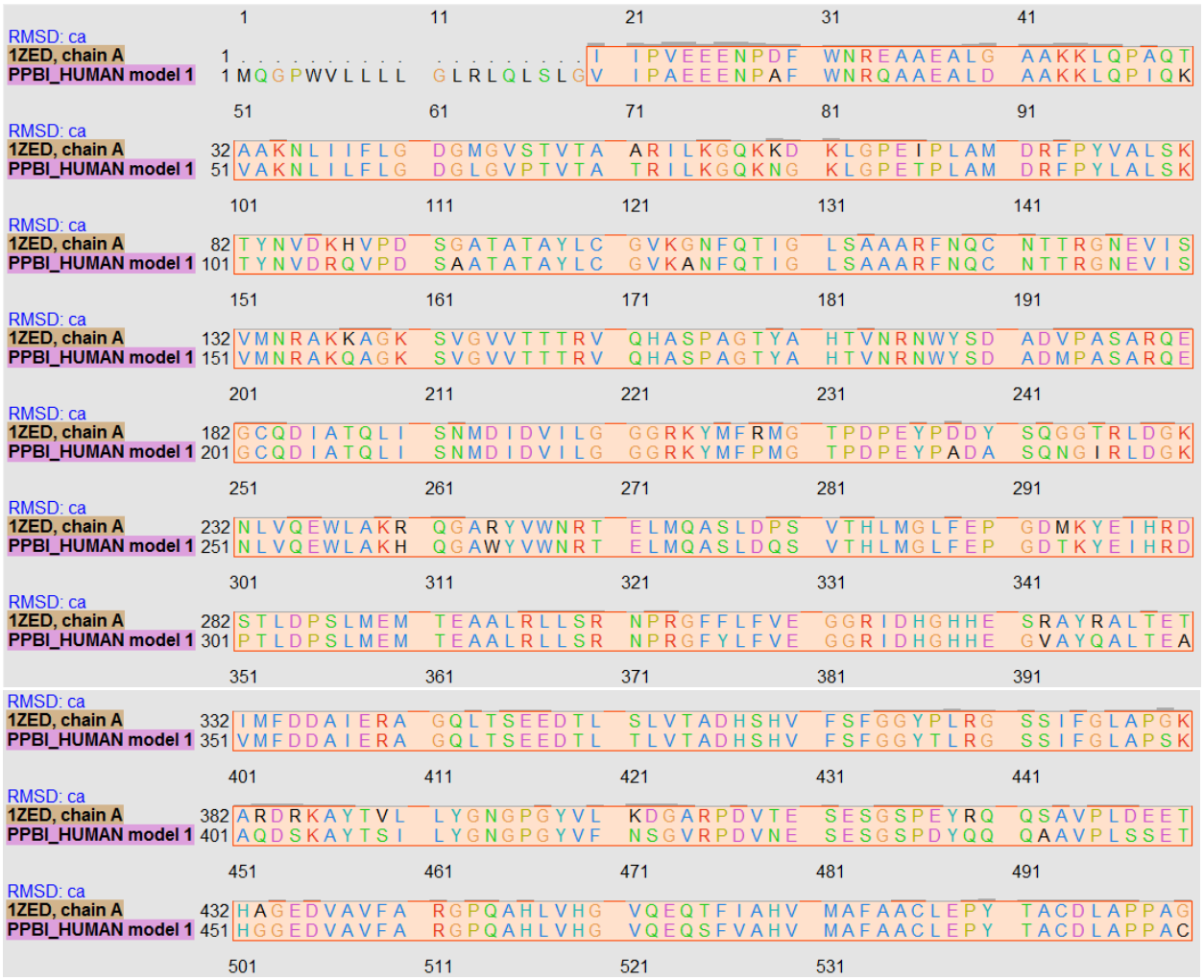


Figure S7. Sequence alignment of target human intestinal alkaline phosphatase (hIALP) with the template protein human placental alkaline phosphatase (hPALP).

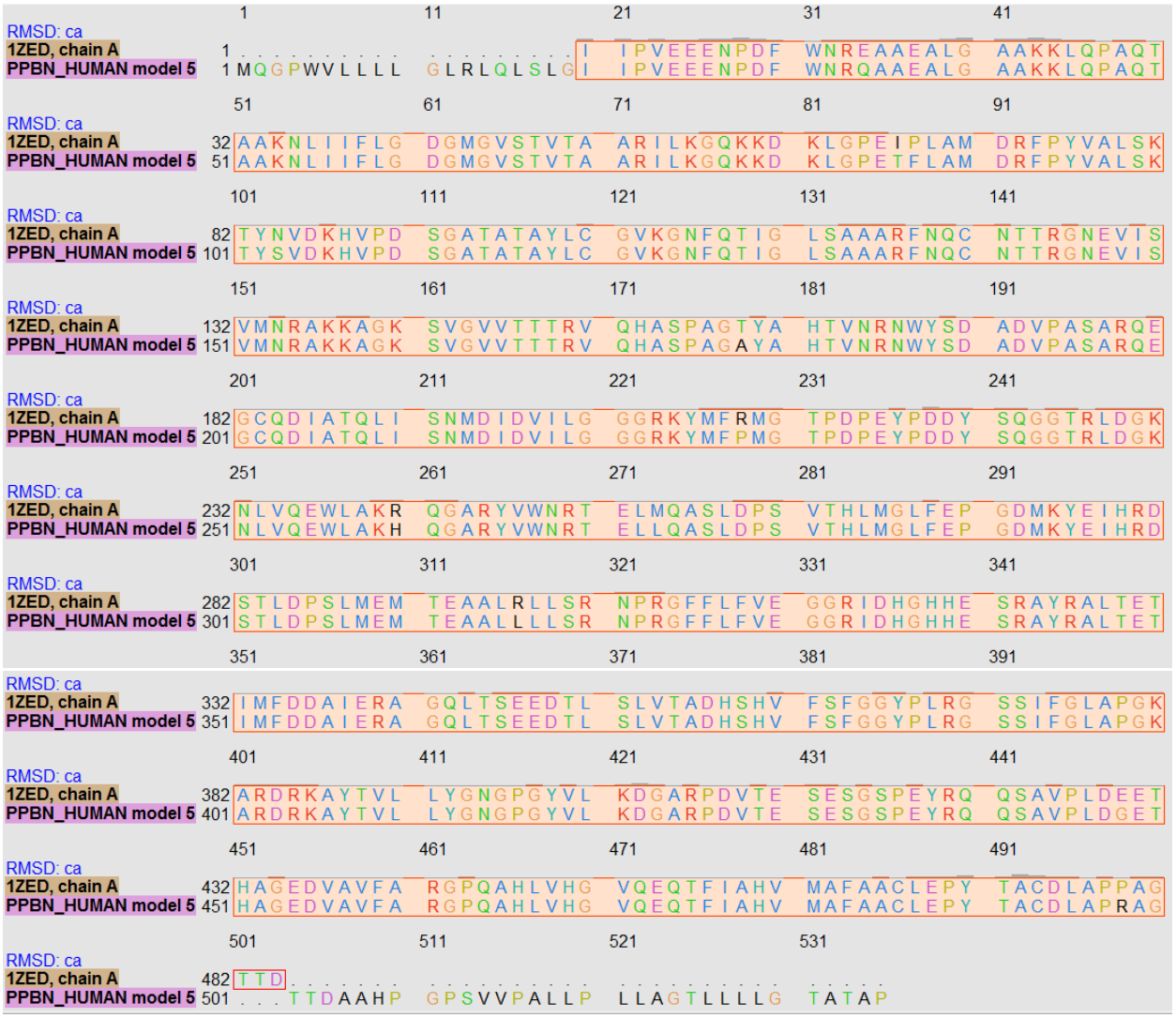


Figure S8. Sequence alignment of target human germ cell alkaline phosphatase (hGCAP) with the template protein human placental alkaline phosphatase (hPALP).

Table S1. Comparison of active site residues of hTNALP, hIALP and hGCALP with hPALP (PDB id: 1ZED). Conserved residues (with respect to hPALP) are in purple font color, the residues which are not conserved are in red font color.

hPALP	**hTNALP**	**hIALP**	**hGCALP**
Asp42	Asp43	Asp61	Asp61
Asp91	Asp92	Asp110	Asp110
Ser92	Ser93	Ser111	Ser111
Phe107	Glu108	Phe126	Phe126
Gln108	Gly109	Gln127	Gln127
His153	His154	His172	His172
Ser155	Thr156	Ser174	Ser174
Arg166	Arg167	Arg185	Arg185
Glu311	Glu315	Glu330	Glu330
Asp316	Asp320	Asp335	Asp335
His320	His324	His339	His339
Asp357	Asp361	Asp376	Asp376
His358	His362	His377	His377
Glu429	His434	Ser448	Gly448
His432	His437	His451	His451