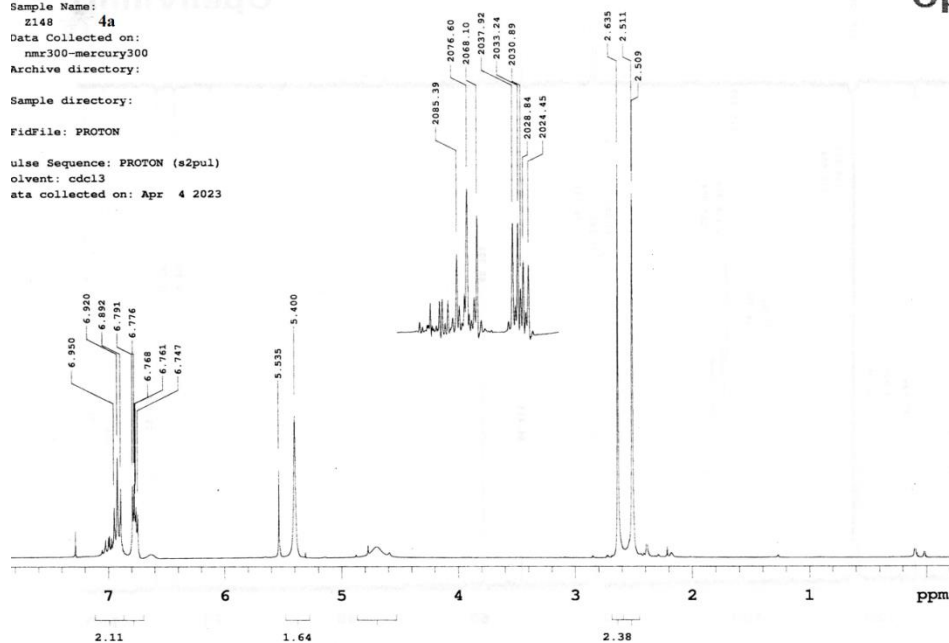


4a ¹H-NMR

Sample Name:
Z148 4a
Data Collected on:
nmr300-mercury300
Archive directory:
Sample directory:
FidFile: PROTON
ulse Sequence: PROTON (s2pul)
olvent: cdcl3
ata collected on: Apr 4 2023

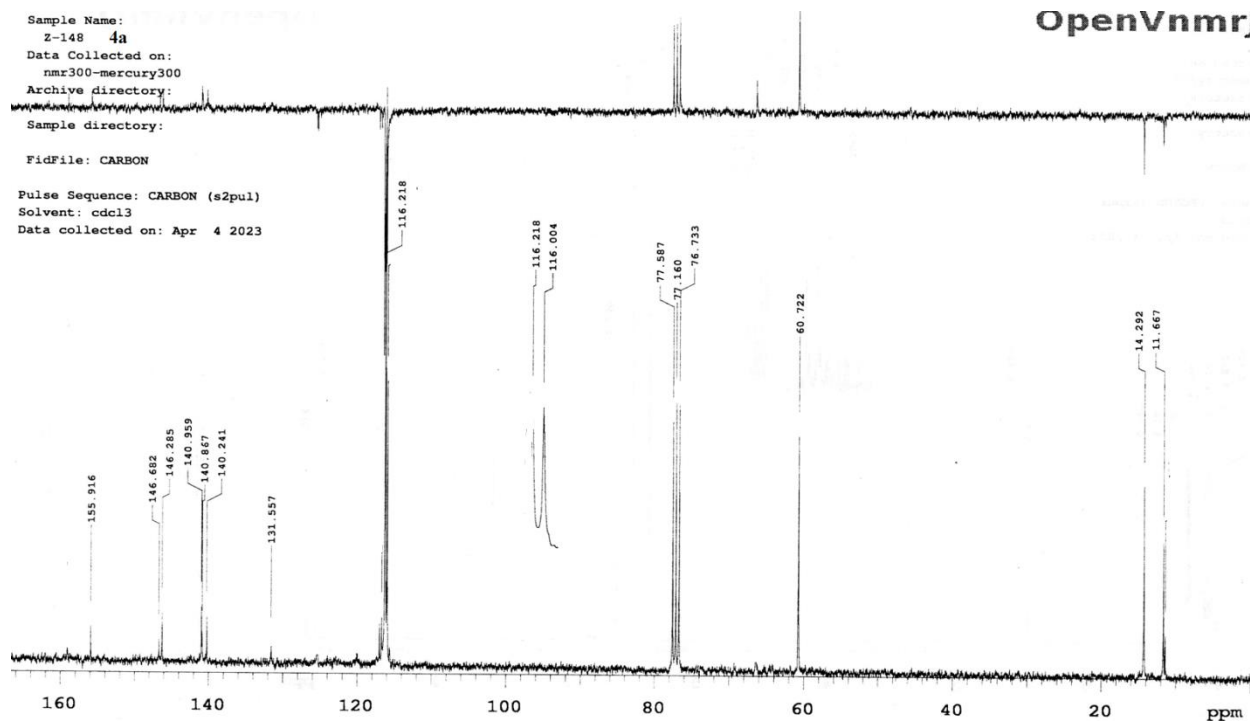
OpenVnmrj



4a ¹³C-NMR

Sample Name:
Z-148 4a
Data Collected on:
nmr300-mercury300
Archive directory:
Sample directory:
FidFile: CARBON
Pulse Sequence: CARBON (s2pul)
Solvent: cdcl3
Data collected on: Apr 4 2023

OpenVnmrj

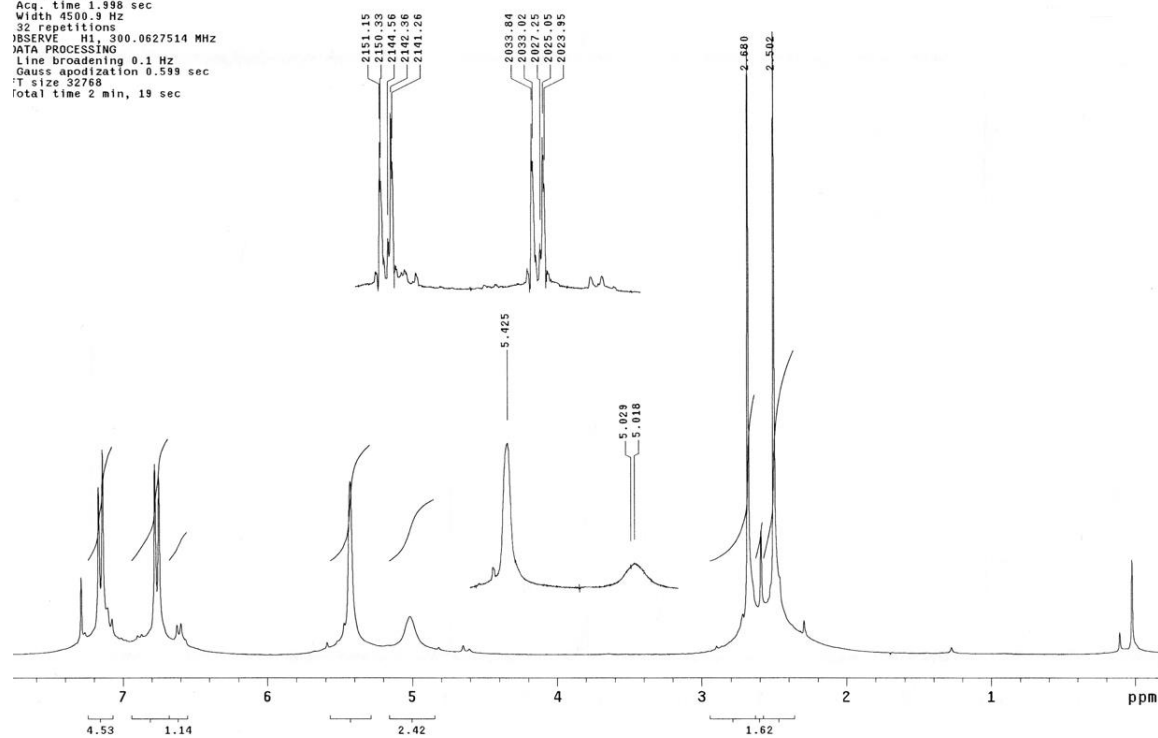


4b ¹H-NMR

137 4b

Pulse Sequence: s2pu1
Solvent: CDCl₃
Ambient temperature
JEKINI-30085 "gemin1300"

Relax. delay 2.000 sec
Pulse 25.4 degrees
Acq. time 1.998 sec
Width 4500.9 Hz
32 repetitions
OBSERVE H1, 300.0627514 MHz
DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.599 sec
F1 size 32768
Total time 2 min, 19 sec



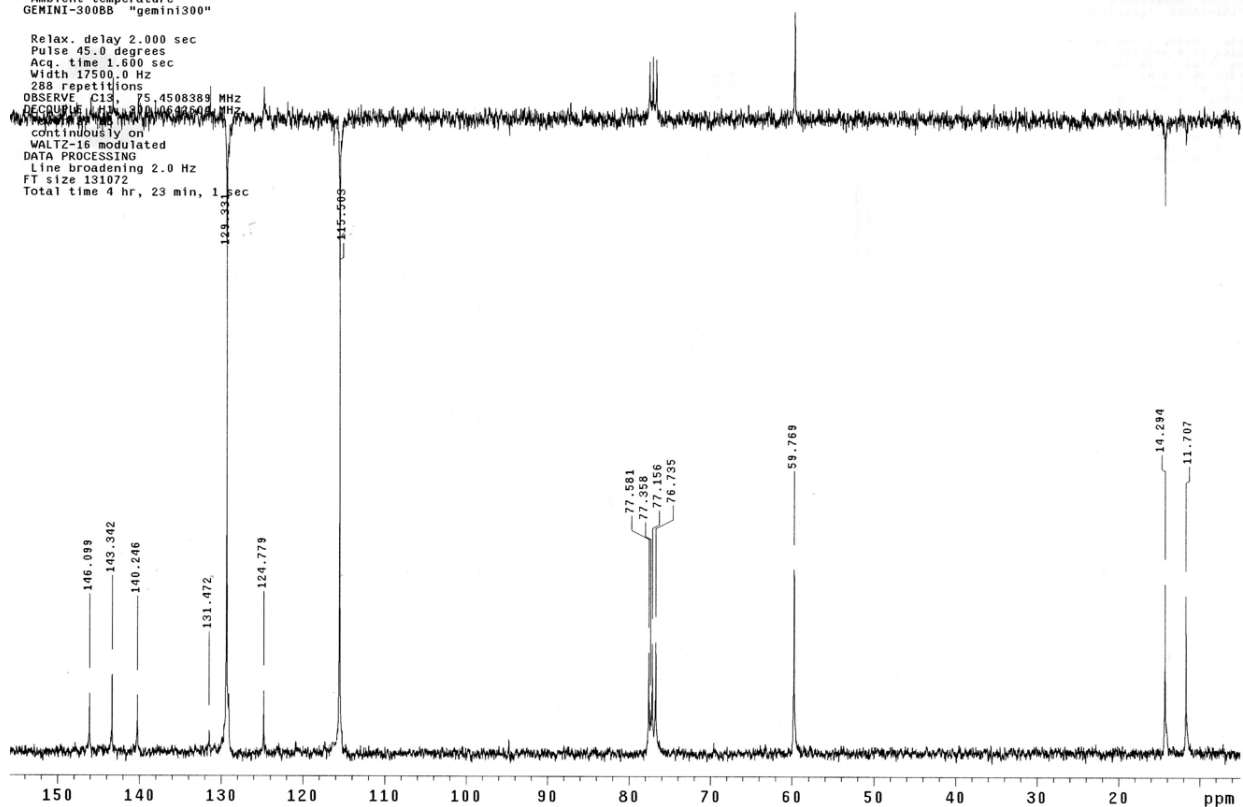
4b ¹³C-NMR

Z137

4b

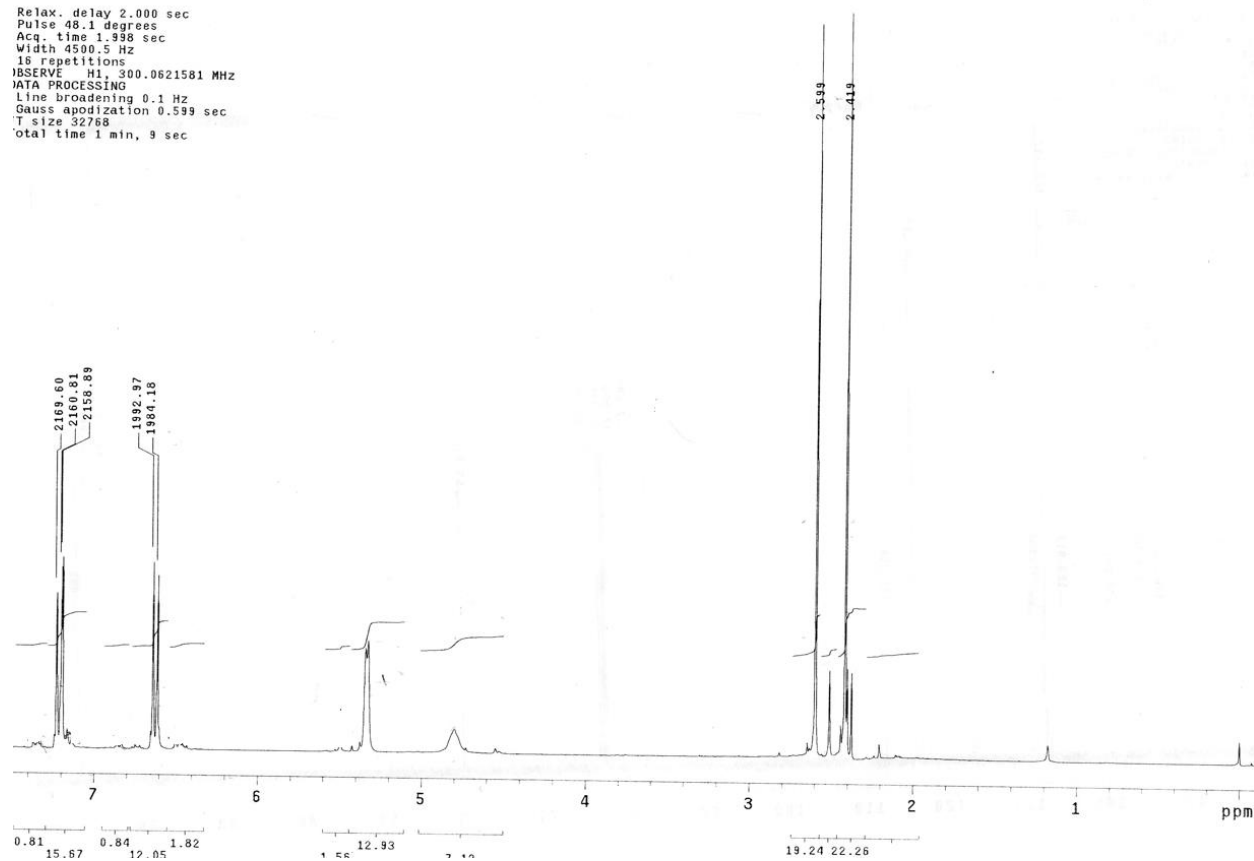
Pulse Sequence: s2pul
Solvent: CDCl₃
Ambient temperature
GEMINI-300BB "gemini300"

Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.600 sec
Width 17500.0 Hz
288 repetitions
OBSERVE C13, 75.4508389 MHz
RECUPIRE C13, 75.4508389 MHz
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 131072
Total time 4 hr, 23 min, 1 sec

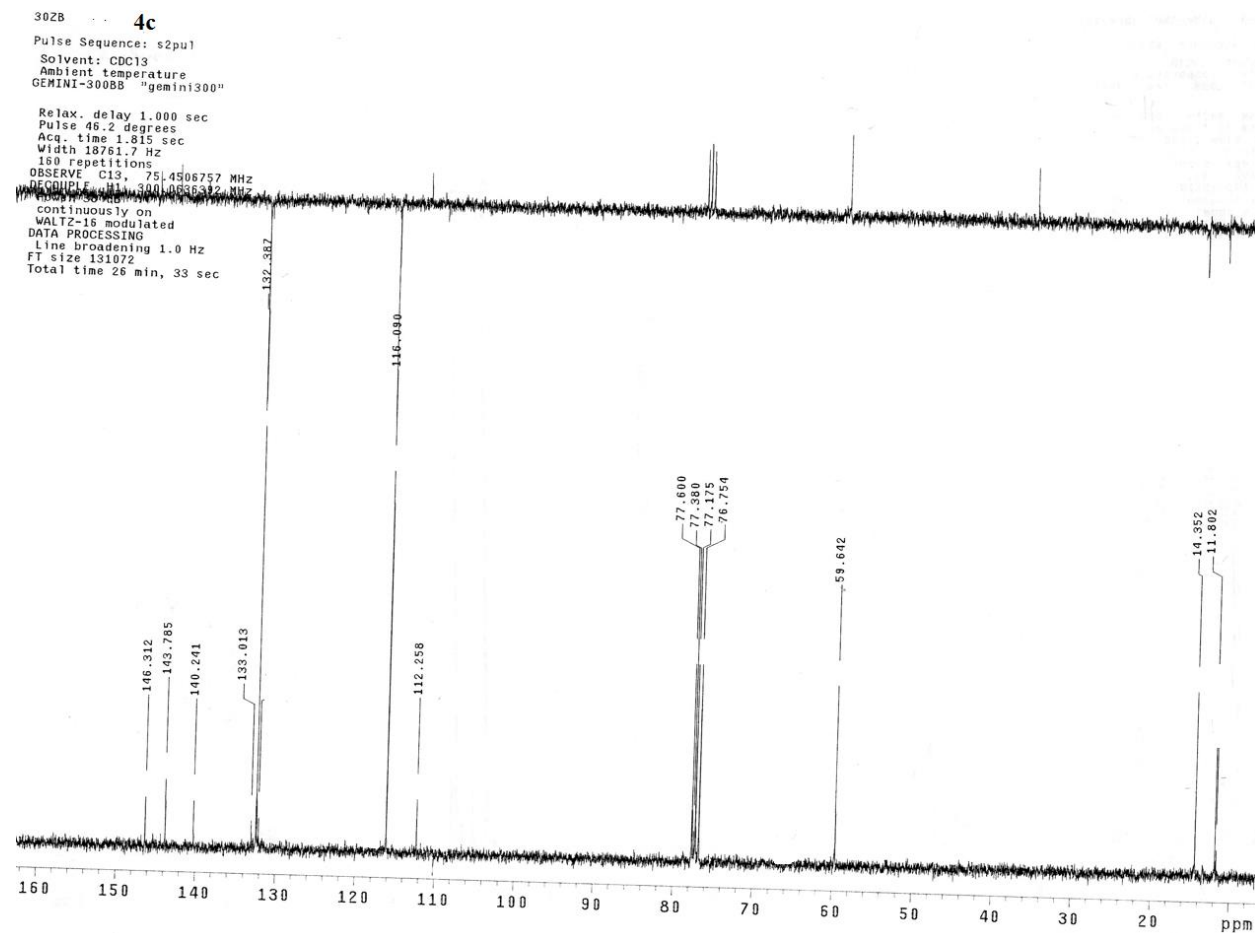


4c ¹H-NMR

302B 4c
Pulse Sequence: s2pu1
Solvent: CDCl3
Ambient temperature
JEMINI-300BB "gemin1300"
Relax. delay 2.000 sec
Pulse 48.1 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0621581 MHz
DATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.599 sec
F size 32768
Total time 1 min, 9 sec



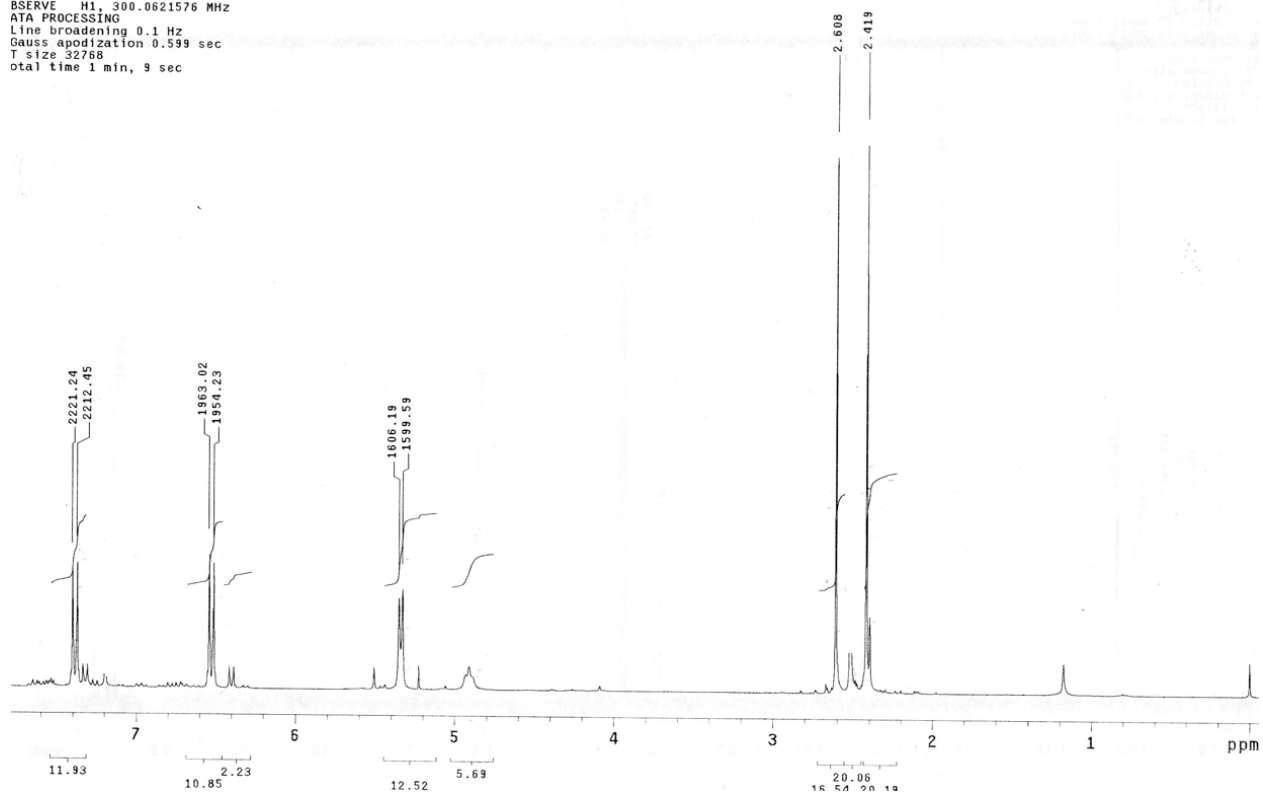
4c ¹³C-NMR



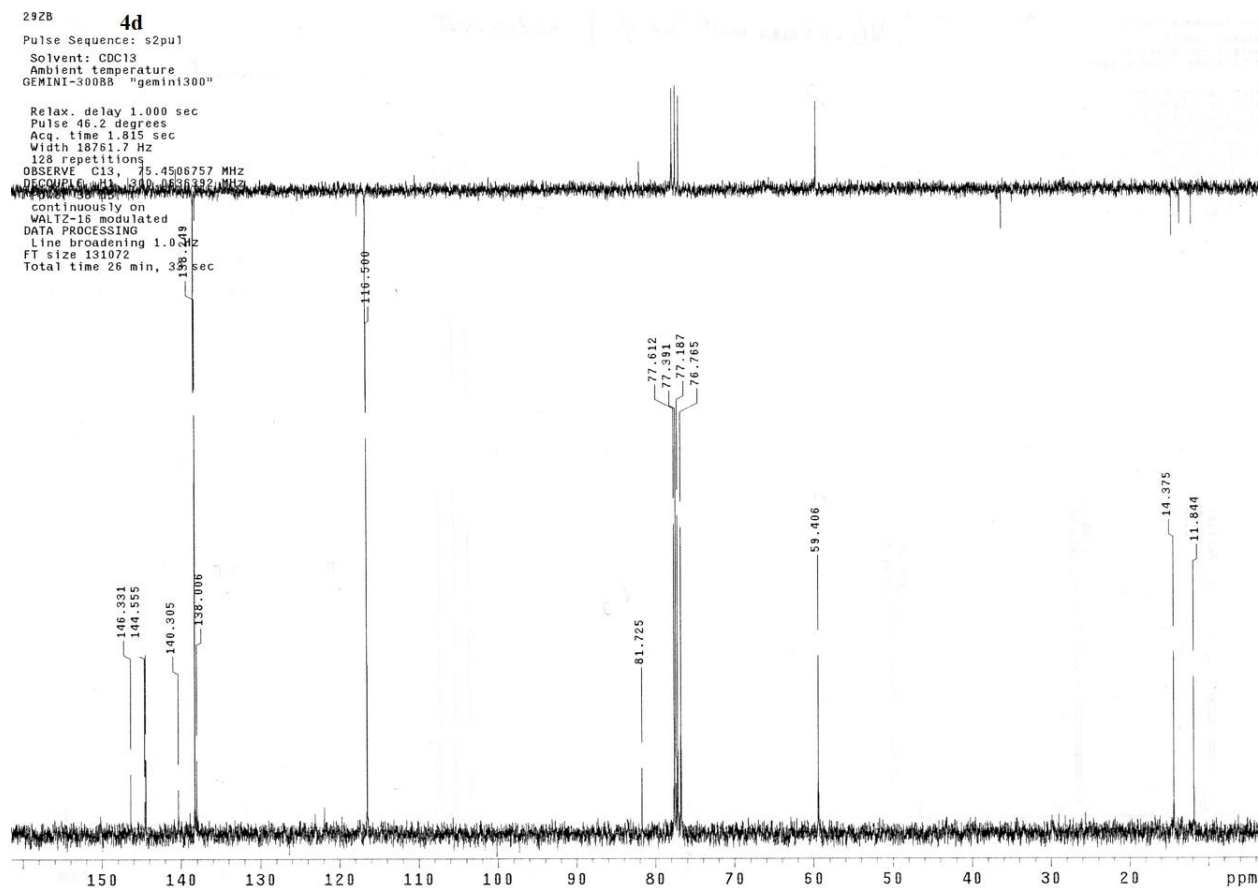
4d ¹H-NMR

928 **4d**
ulse Sequence: s2pu1
Solvent: CDCl3
Ambient temperature
EMINI-300BB "gemin1300"

Relax. delay 2.000 sec
Pulse 48.1 degrees
Acq. time 1.958 sec
Width 4500.5 Hz
16 repetitions
DSERVE H1, 300.0621576 MHz
ATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.599 sec
T size 32768
otal time 1 min, 9 sec

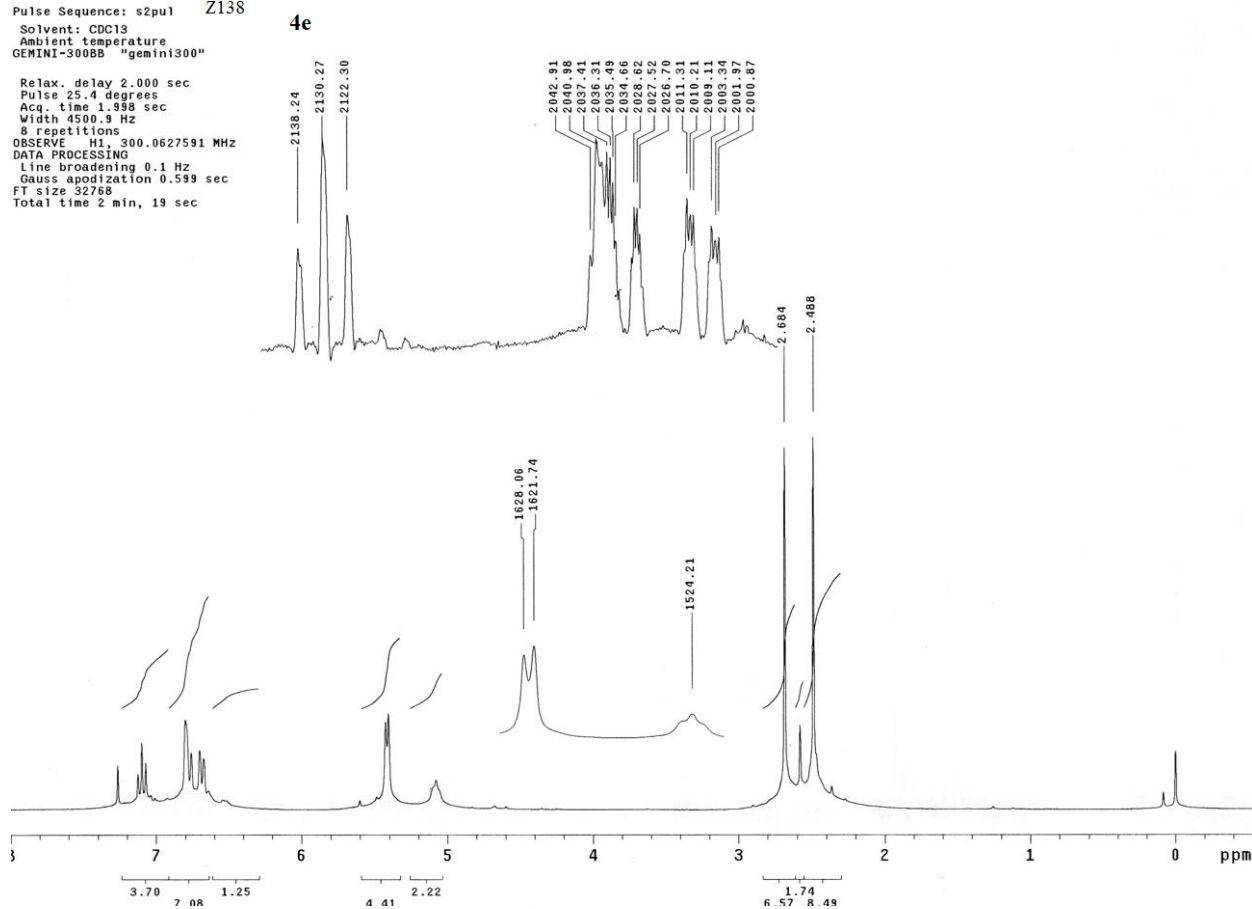


4d ¹³C-NMR

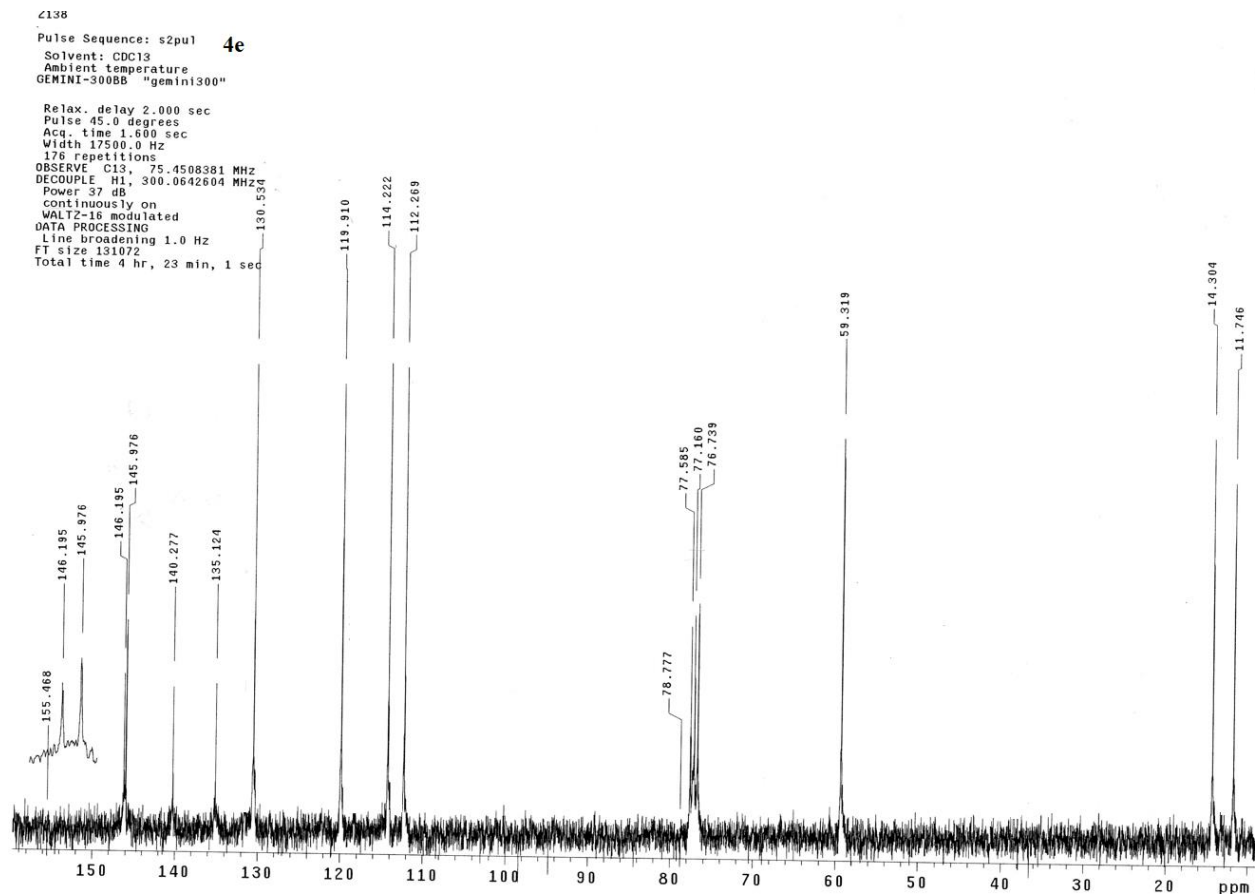


4e ¹H-NMR

Pulse Sequence: s2pu1 Z138
 Solvent: CDCl₃
 Ambient temperature
 GEMINI-300BB "gemini300"
 Relax. delay 2.000 sec
 Pulse 25.4 degrees
 Acq. time 1.998 sec
 Width 4500.9 Hz
 8 repetitions
 OBSERVE H1, 300.0627591 MHz
 DATA PROCESSING
 Line broadening 0.1 Hz
 Gauss apodization 0.599 sec
 FT size 32768
 Total time 2 min, 19 sec



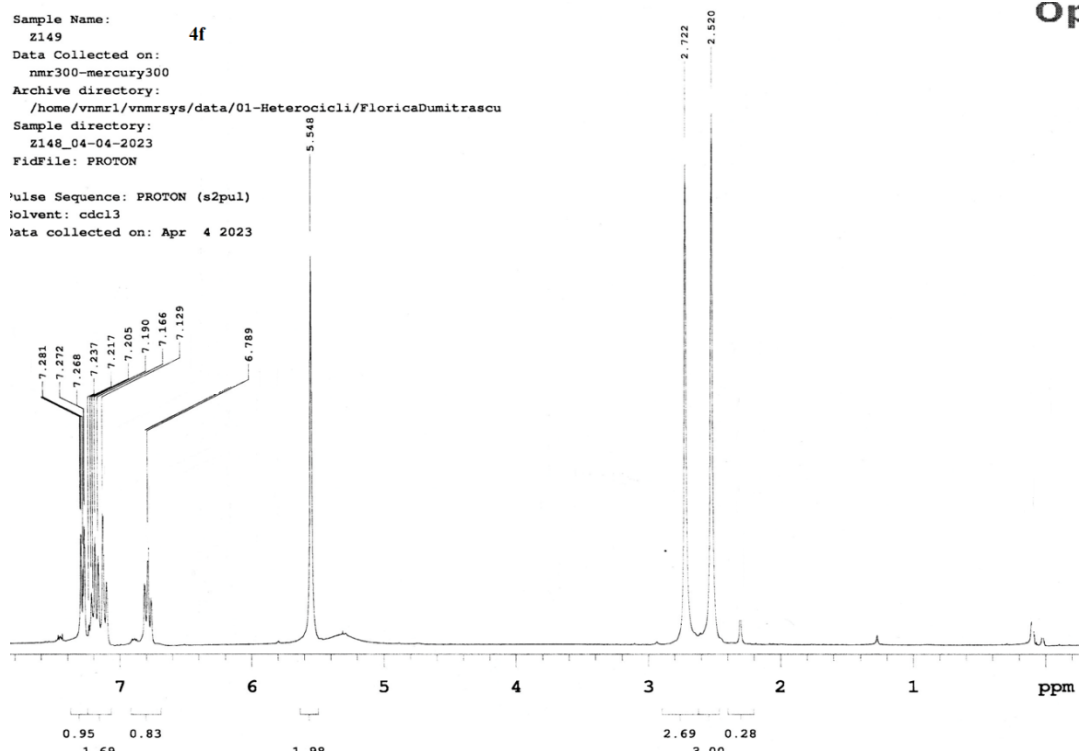
4e ¹³C-NMR



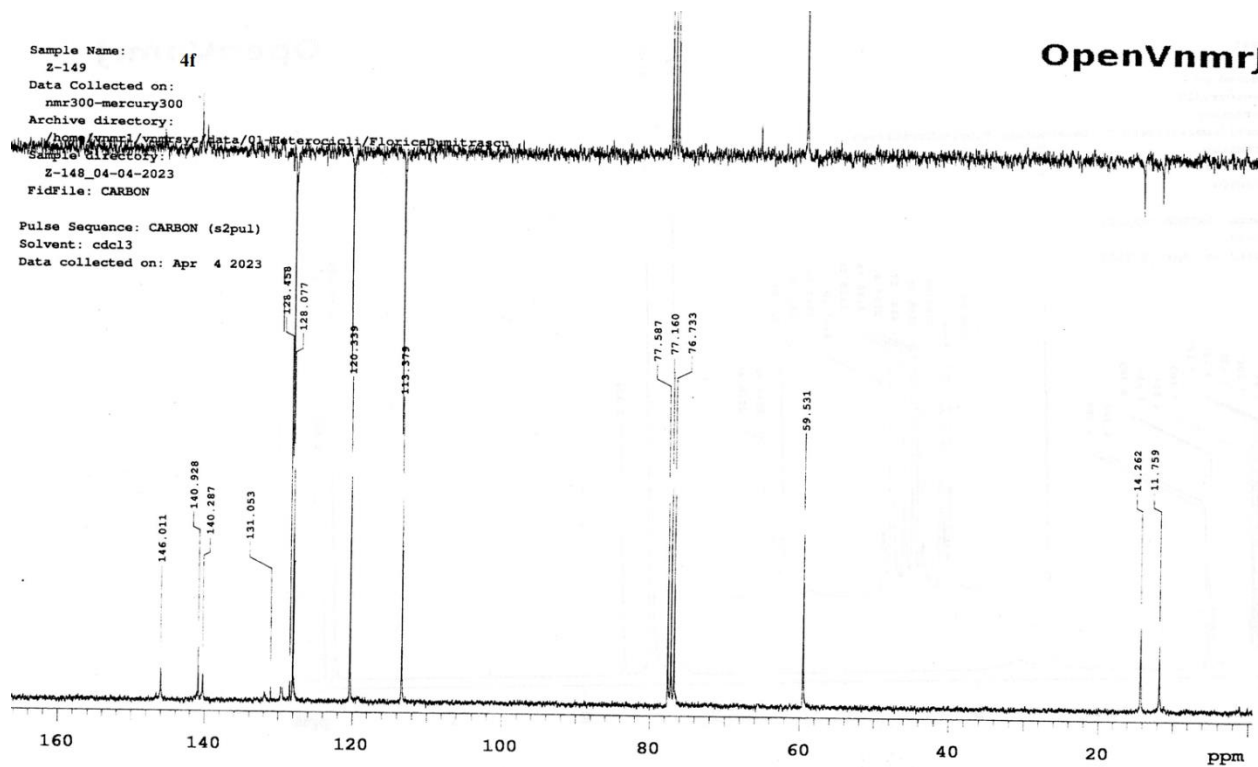
4f ^1H -NMR

OpenVnmrj

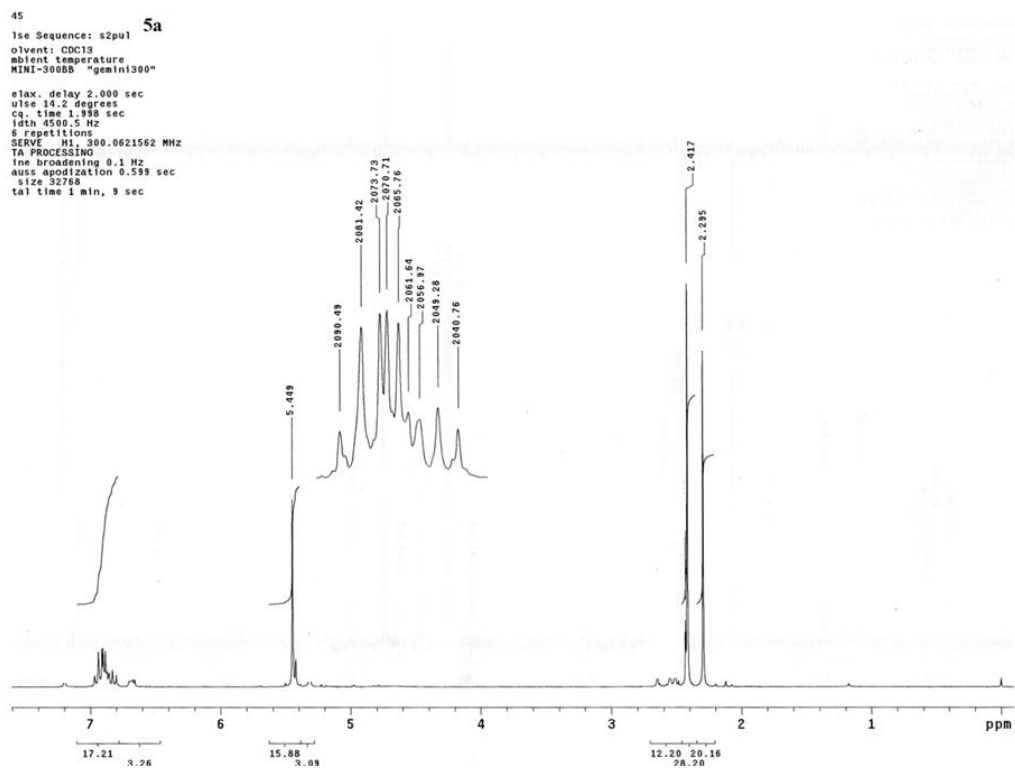
Sample Name: 4f
Z149
Data Collected on: nmr300-mercury300
Archive directory: /home/vnmr1/vnmr300/data/01-Heterocicli/FloricaDumitrascu
Sample directory: Z148_04-04-2023
FidFile: PROTON
Pulse Sequence: PROTON (s2pul)
Solvent: cdcl3
Data collected on: Apr 4 2023



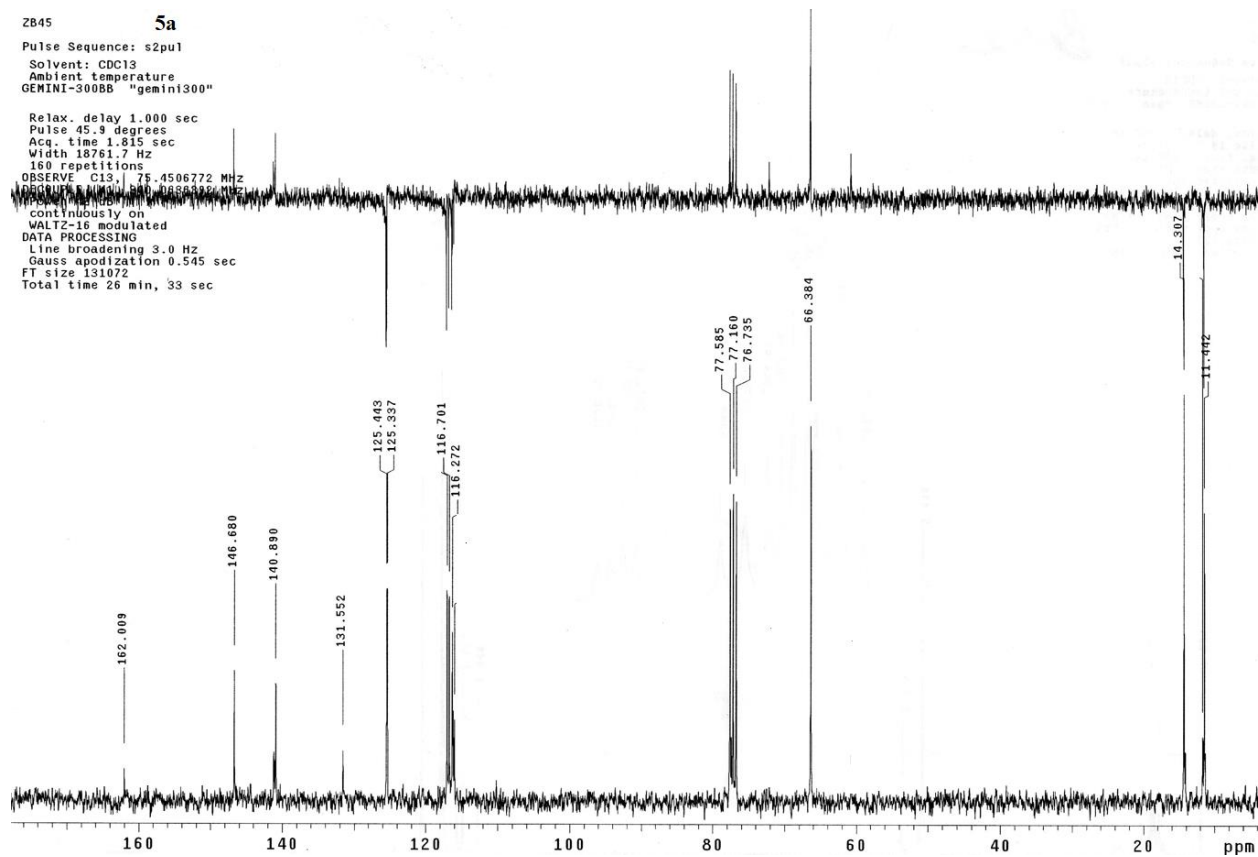
$4f$ ^{13}C -NMR



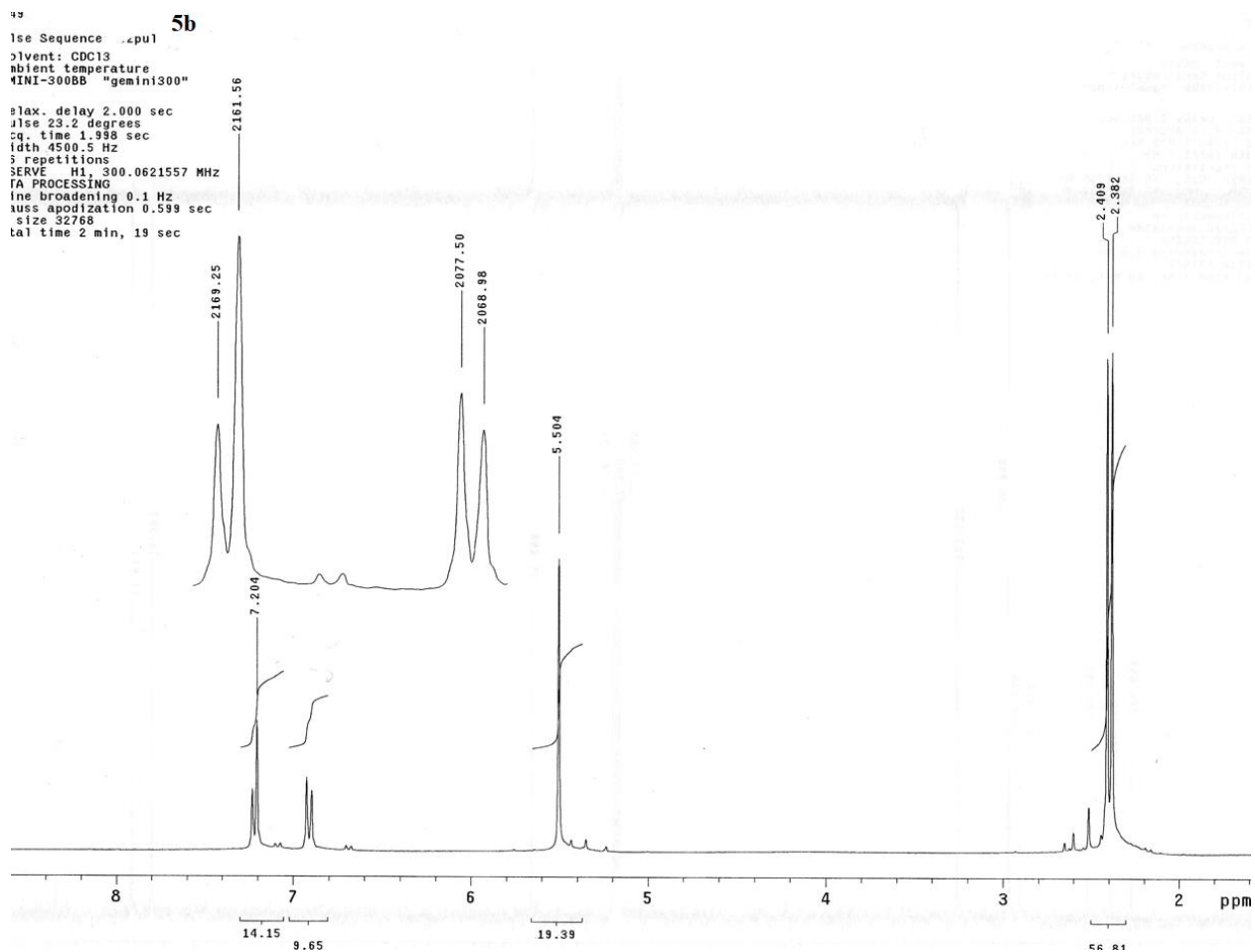
5a ¹H-NMR



5a ¹³C-NMR



5b ¹H-NMR

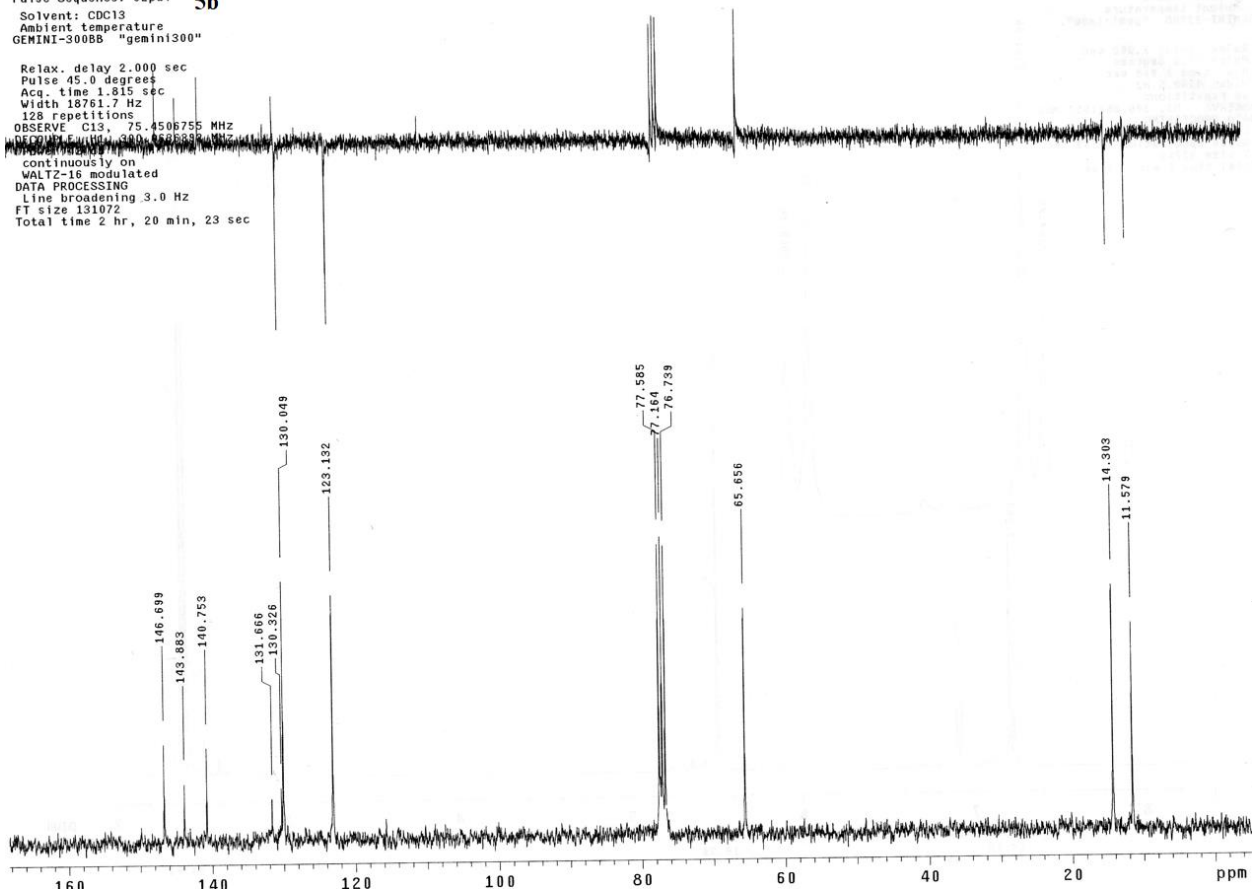


5b ¹³C-NMR

ZB49

Pulse Sequence: s2pul **5b**
Solvent: CDCl₃
Ambient temperature
GEMINI-300BB "gemin300"

Relax. delay 2.000 sec
Pulse 45.0 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
128 repetitions
OBSERVE C13, 75.4506755 MHz
DECODE F1H1H, 300.1362633 MHz
Modulated continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 3.0 Hz
FT size 131072
Total time 2 hr, 20 min, 23 sec

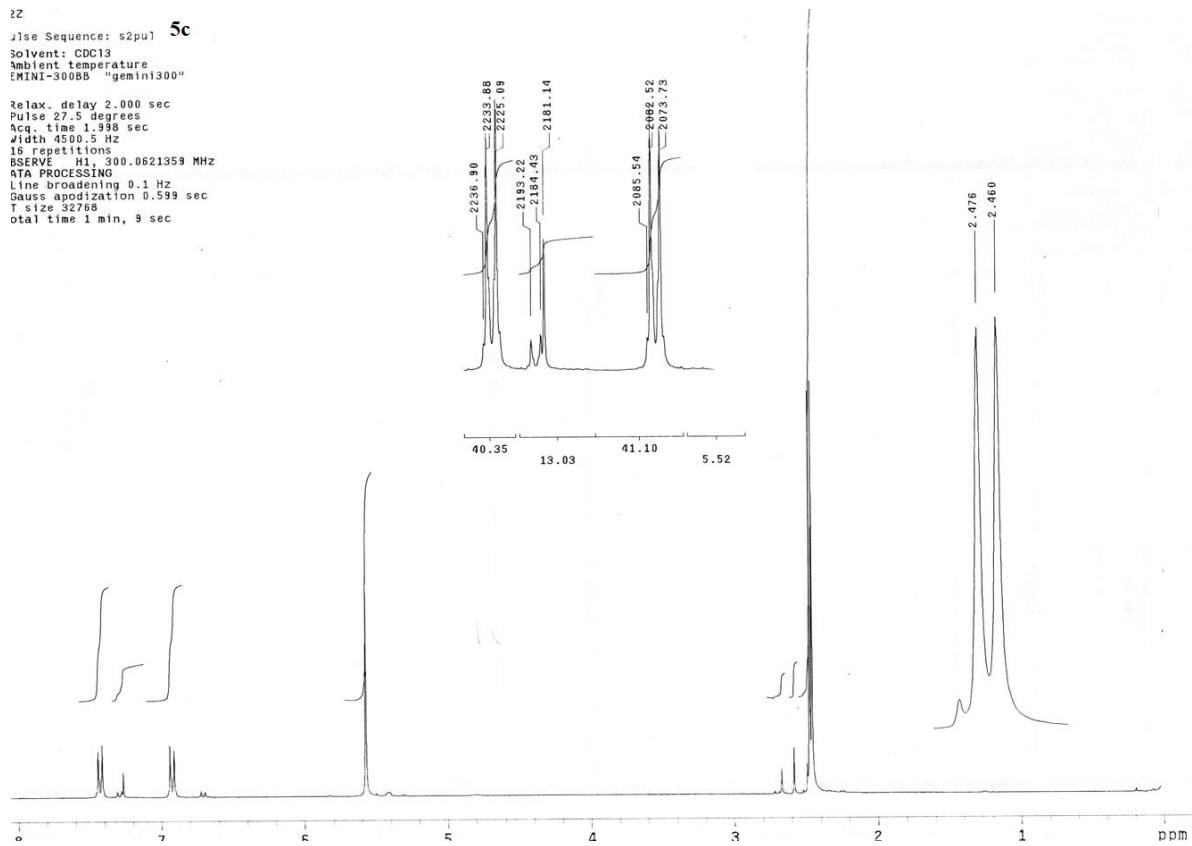


5c ¹H-NMR

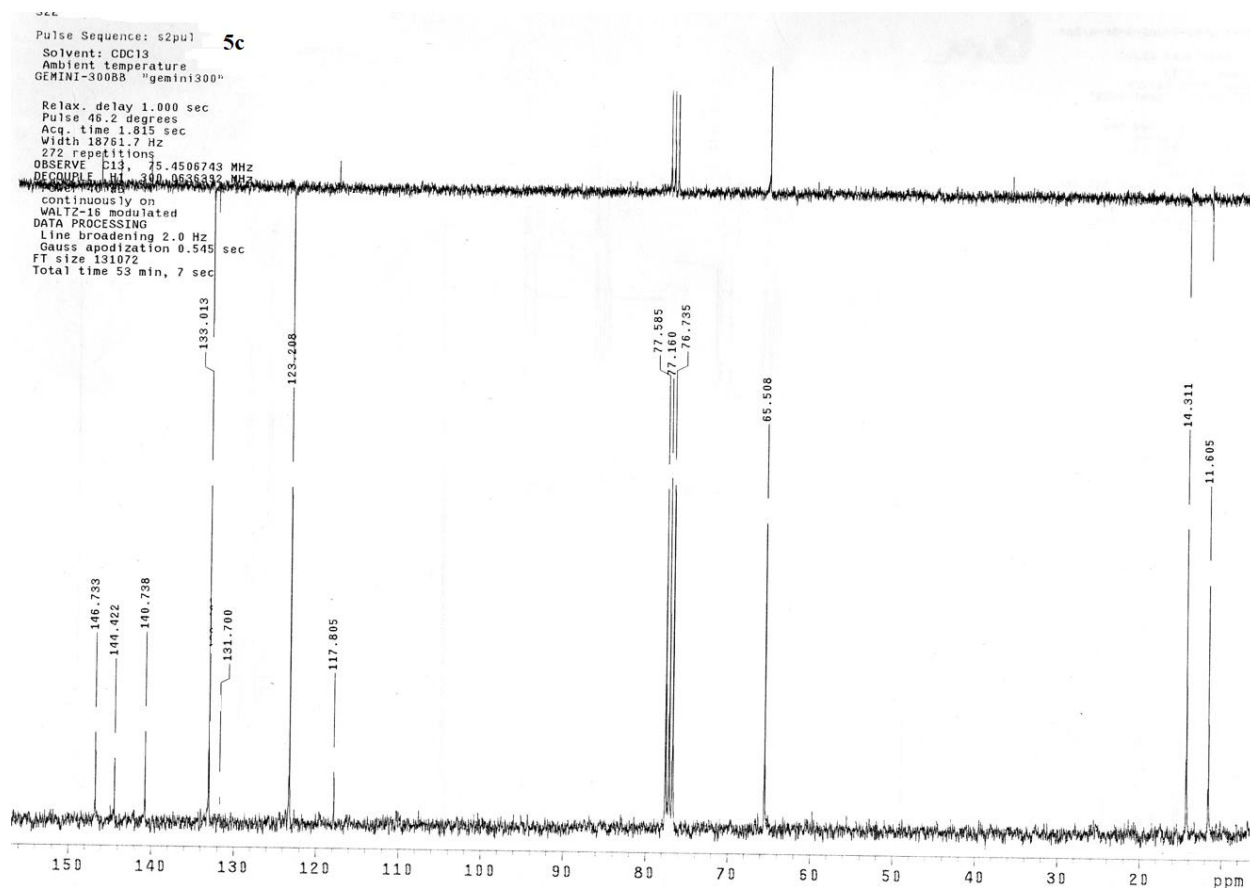
22

File Sequence: s2pu1 **5c**
Solvent: CDCl₃
Ambient temperature
EMINI-300BB "geminis300"

Relax. delay 2.000 sec
Pulse 27.5 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
16 repetitions
RSERVE H1, 300.0621359 MHz
ATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.599 sec
T size 32768
Total time 1 min, 9 sec



5c ¹³C-NMR

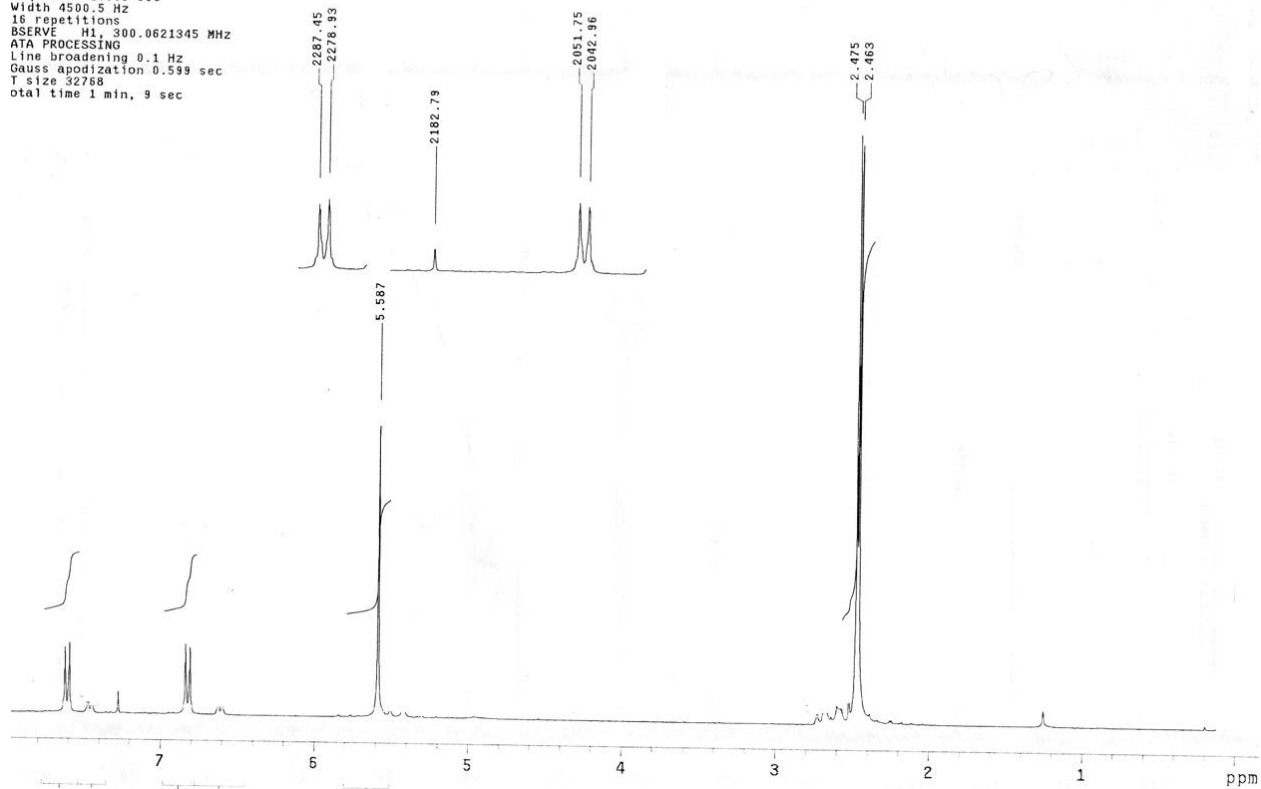


5d ¹H-NMR

12B

Pulse Sequence: s2pu1 **5d**
Solvent: CDCl₃
Ambient temperature
EMINI-300BB "geminis300"

Relax. delay 2.000 sec
Pulse 27.5 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
16 repetitions
BSERVE H1, 300.0621345 MHz
ATA PROCESSING
Line broadening 0.1 Hz
Gauss apodization 0.599 sec
T size 32768
otal time 1 min, 9 sec



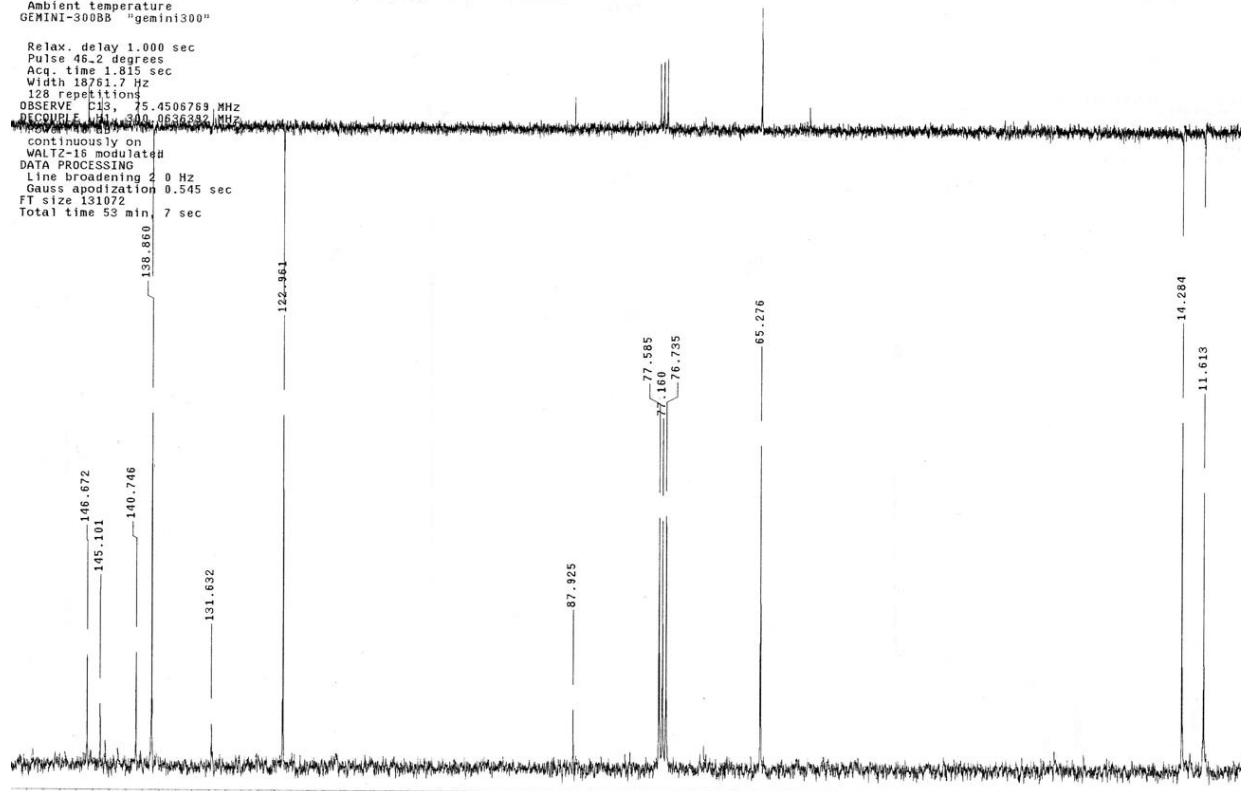
5d ¹³C-NMR

312B

Pulse Sequence: s2pu1 **5d**

Solvent: CDCl₃
Ambient temperature
GEMINI-300BB "gemini300"

Relax. delay 1.000 sec
Pulse 46.2 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
128 repetitions
OBSERVE C13, 75.4508763 MHz
DECOUPLE H1, 300.0836392 MHz
Hetero J 149.0 Hz
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
Gauss apodization 0.545 sec
FT size 131072
Total time 53 min, 7 sec



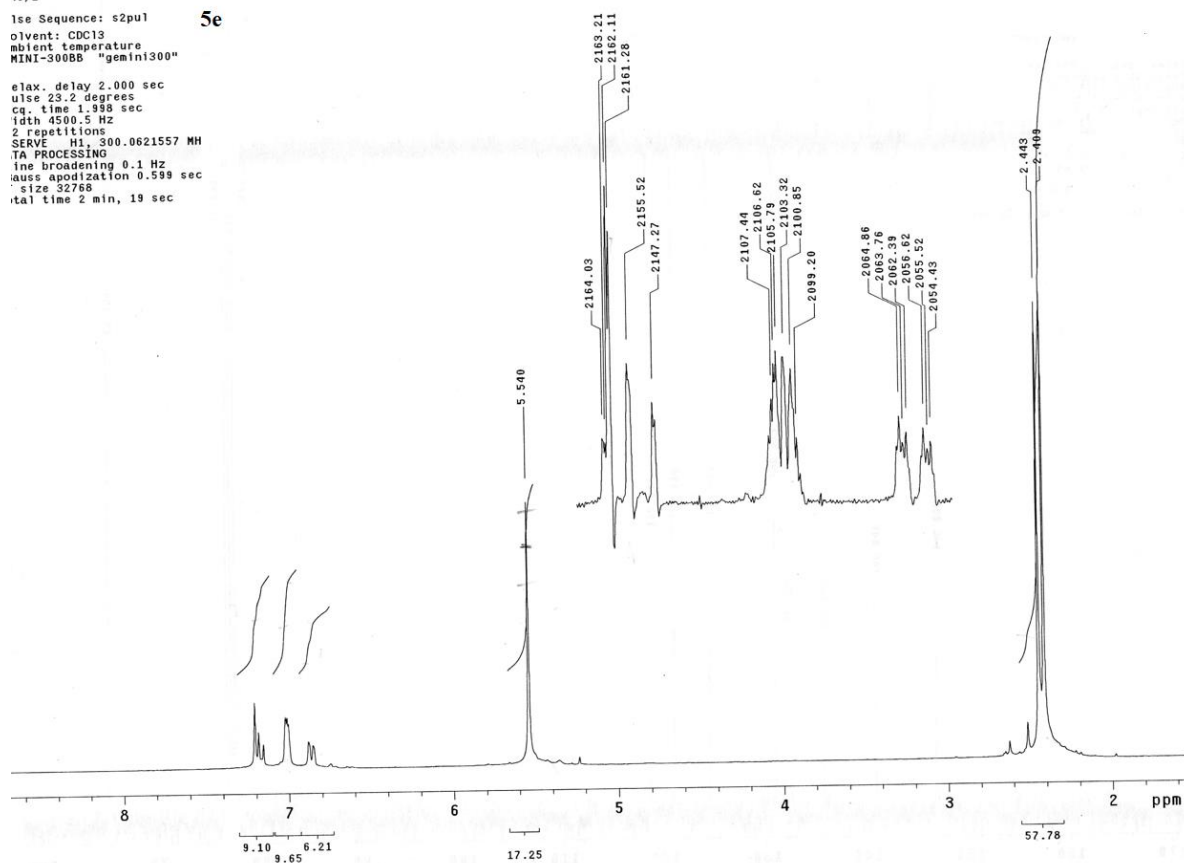
5e ¹H-NMR

46/2

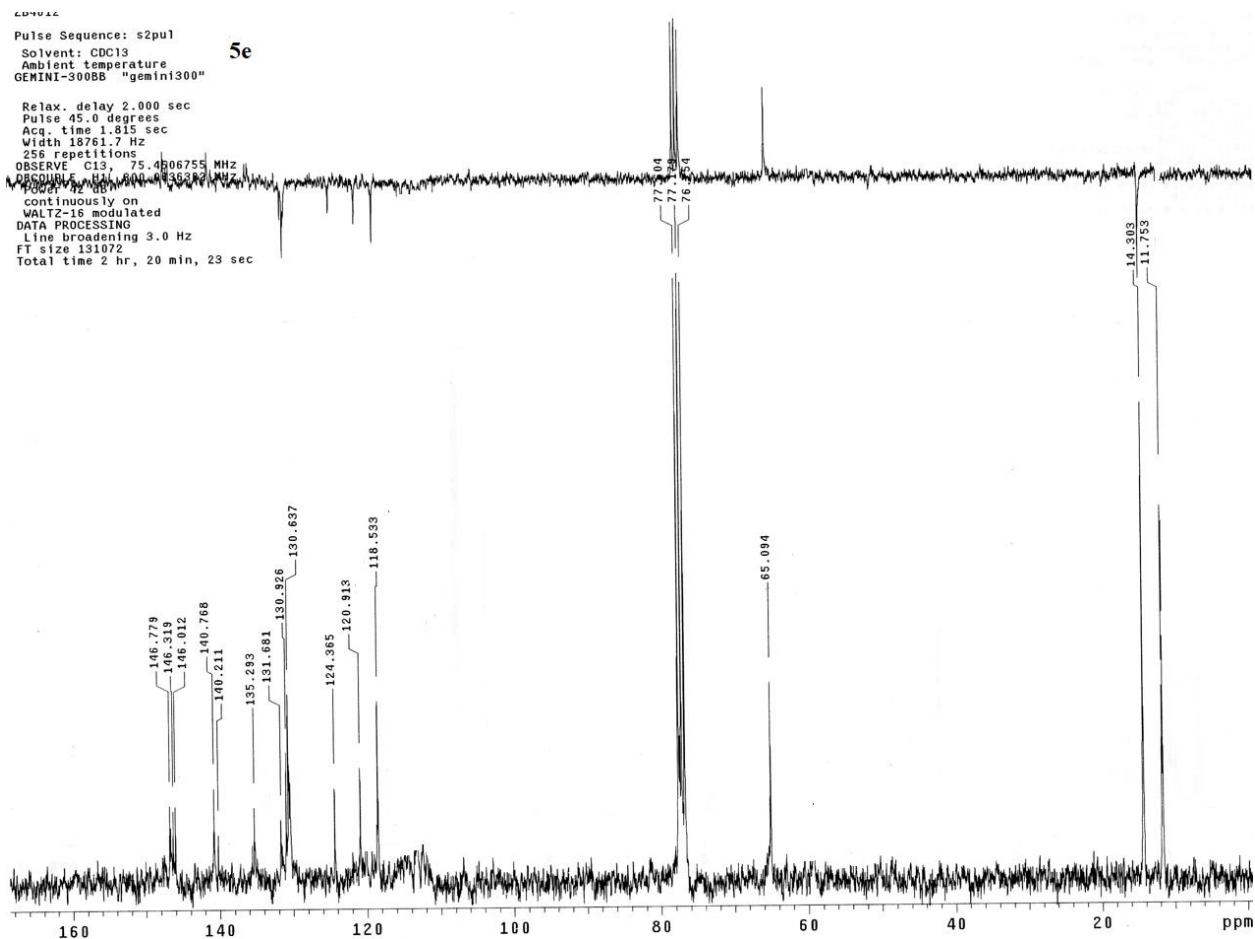
1se Sequence: s2pu1
 olvent: CDC13
 mbient temperature
 MINI-300BB "gemin1300"

5e

elax. delay 2.000 sec
 ulse 23.2 degrees
 cq. time 1.998 sec
 fath 4500.5 Hz
 2 repetitions
 SERVE H1, 300.0621557 MH
 TA PROCESSING
 ine broadening 0.1 Hz
 iauss apodization 0.599 sec
 size 32768
 tal time 2 min, 19 sec



5e ¹³C-NMR



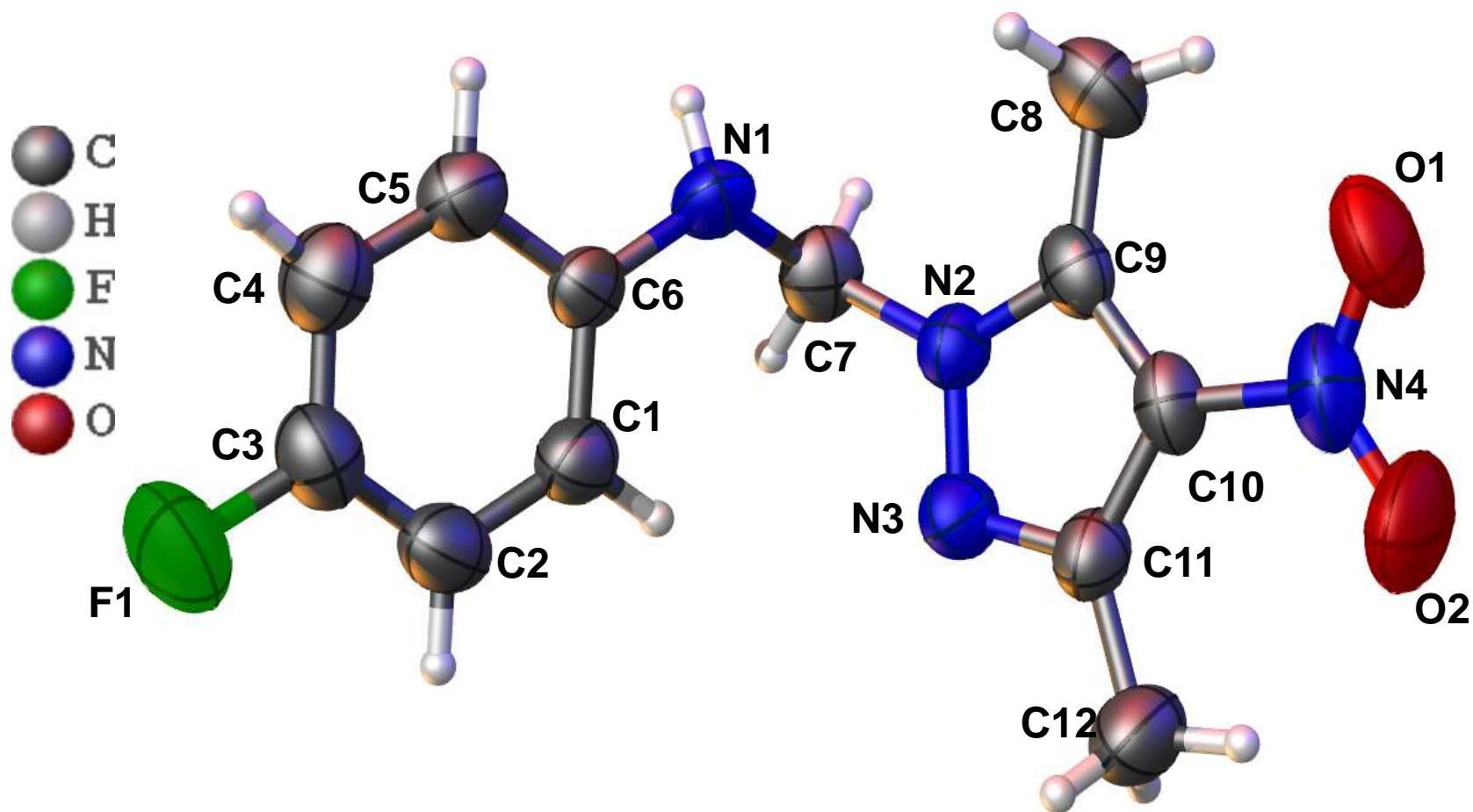


Figure S1. X-ray molecular structure of (**4a**) with color and atoms numbering scheme. Thermal ellipsoids representation with 50% probability.

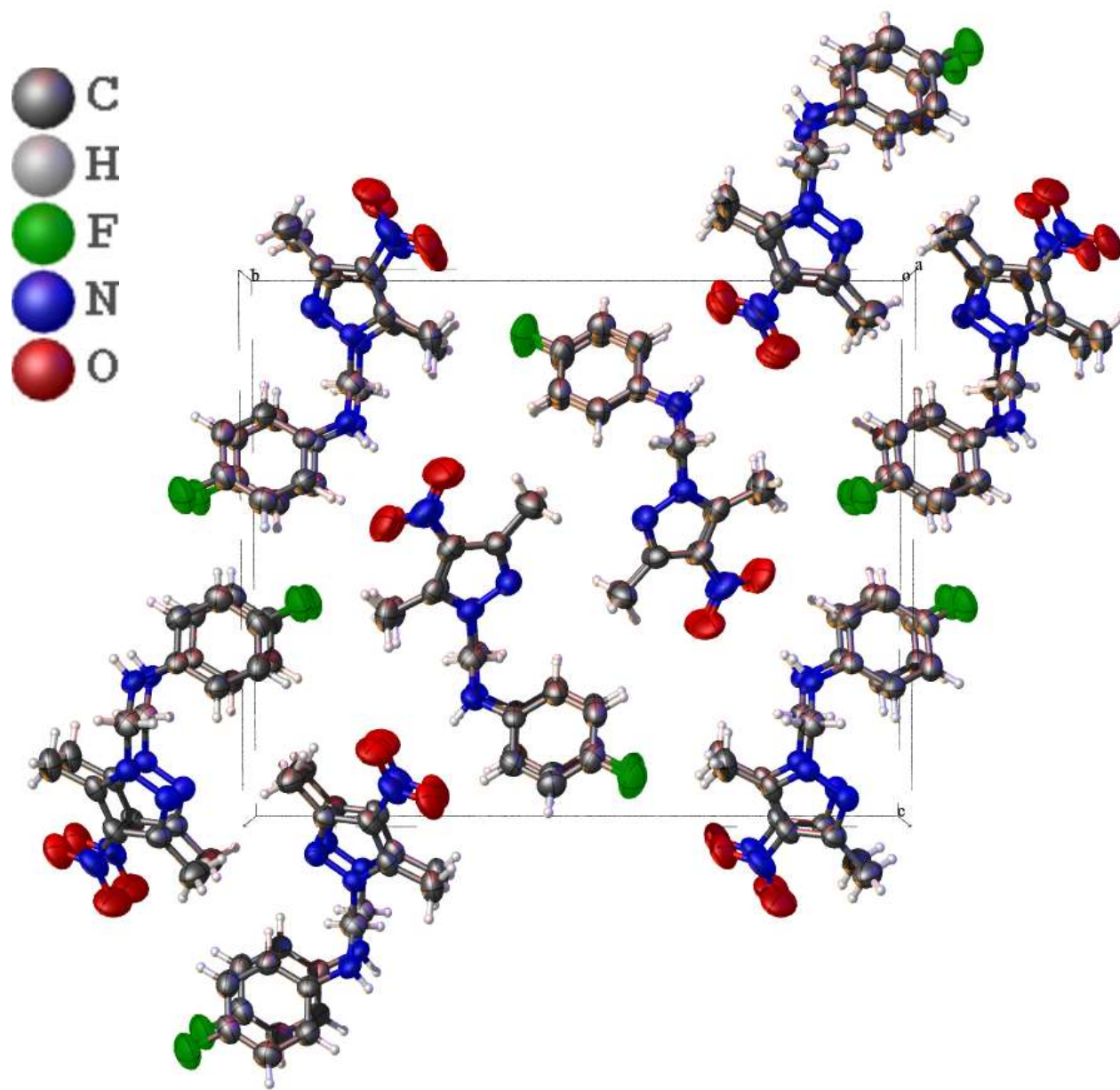


Figure S1.1. Crystal packing for (4a) along *a* axis.

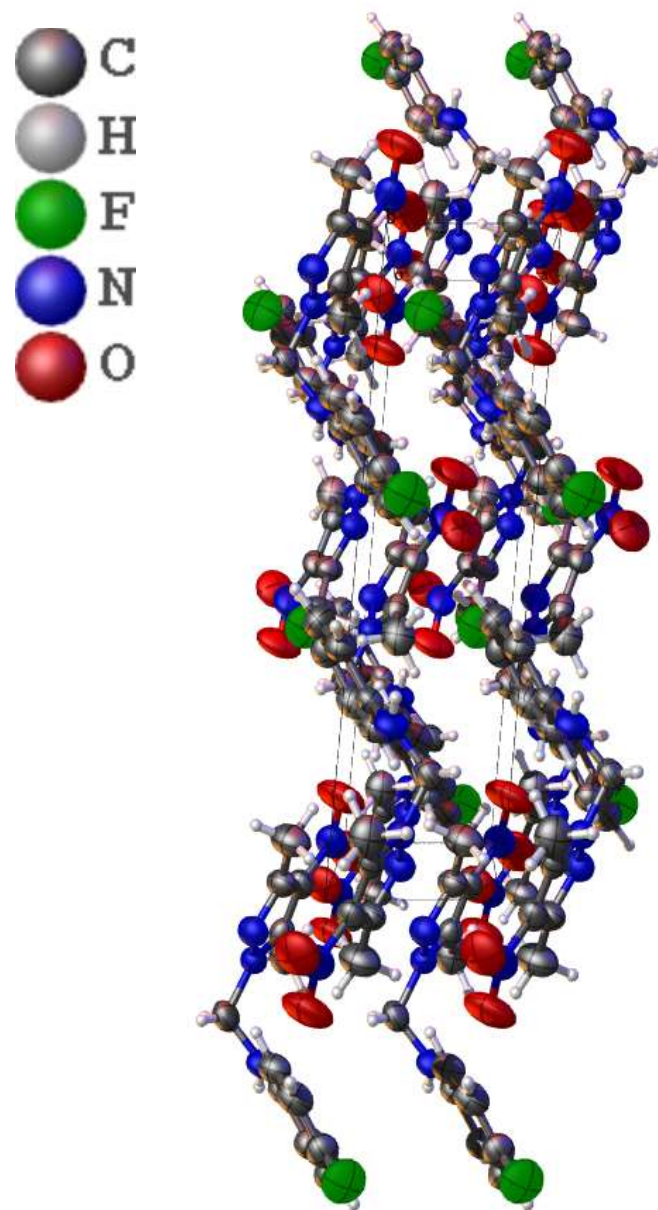


Figure S1.2. Crystal packing for (4a) along *b* axis.

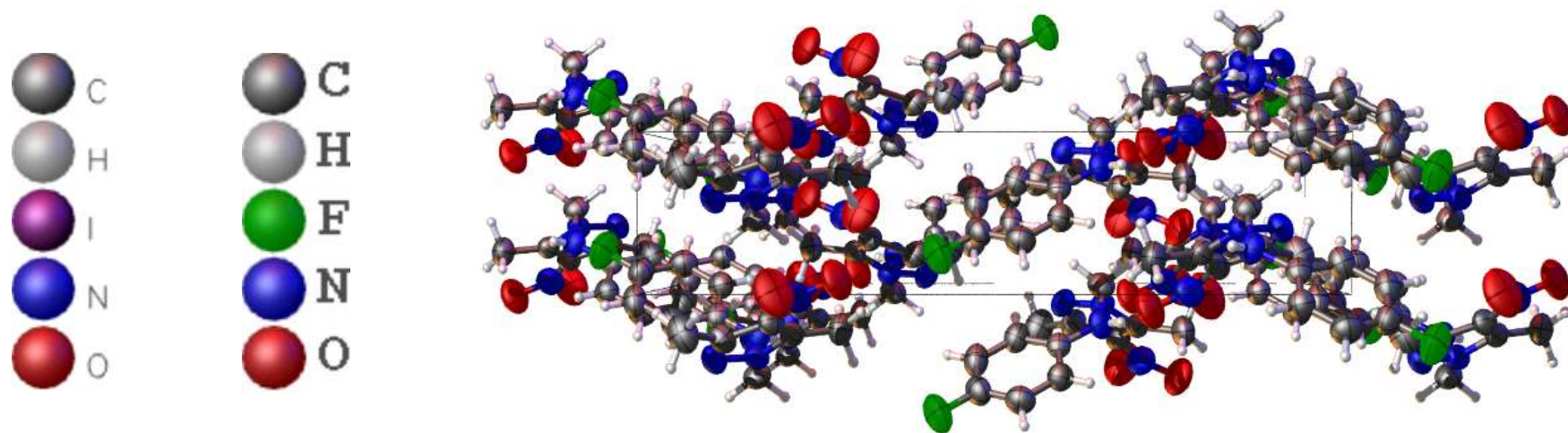
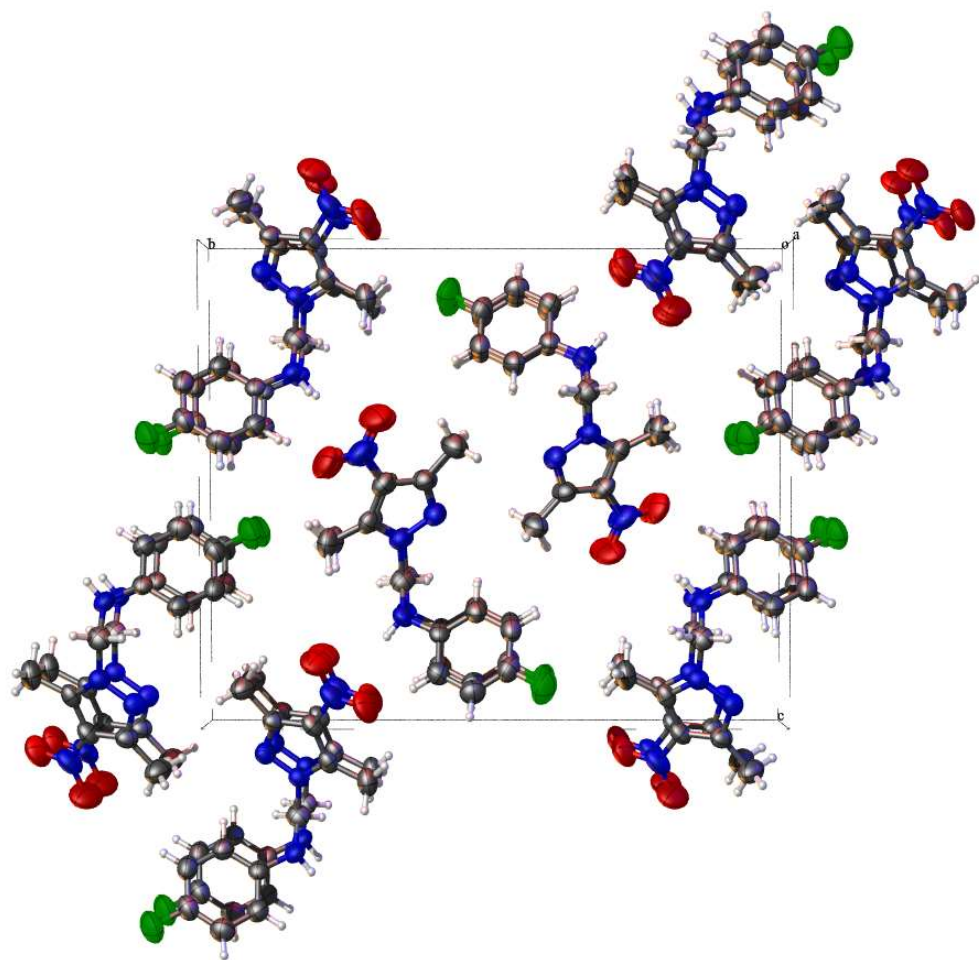
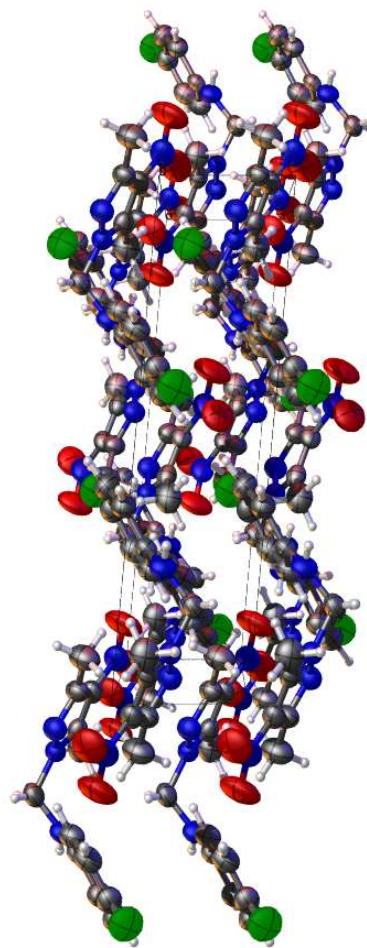


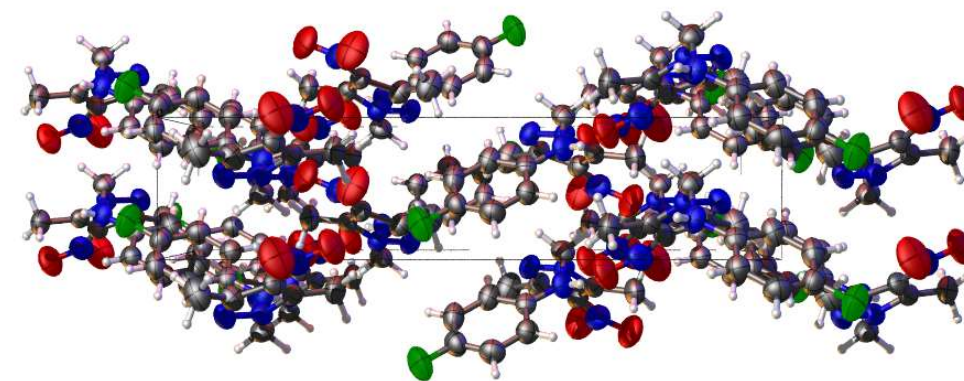
Figure S1.3. Crystal packing for (**4a**) along *c* axis.



a



b



c

Figure S1.4. Crystal packing for (**4a**) along *a*, *b* and *c* axes.

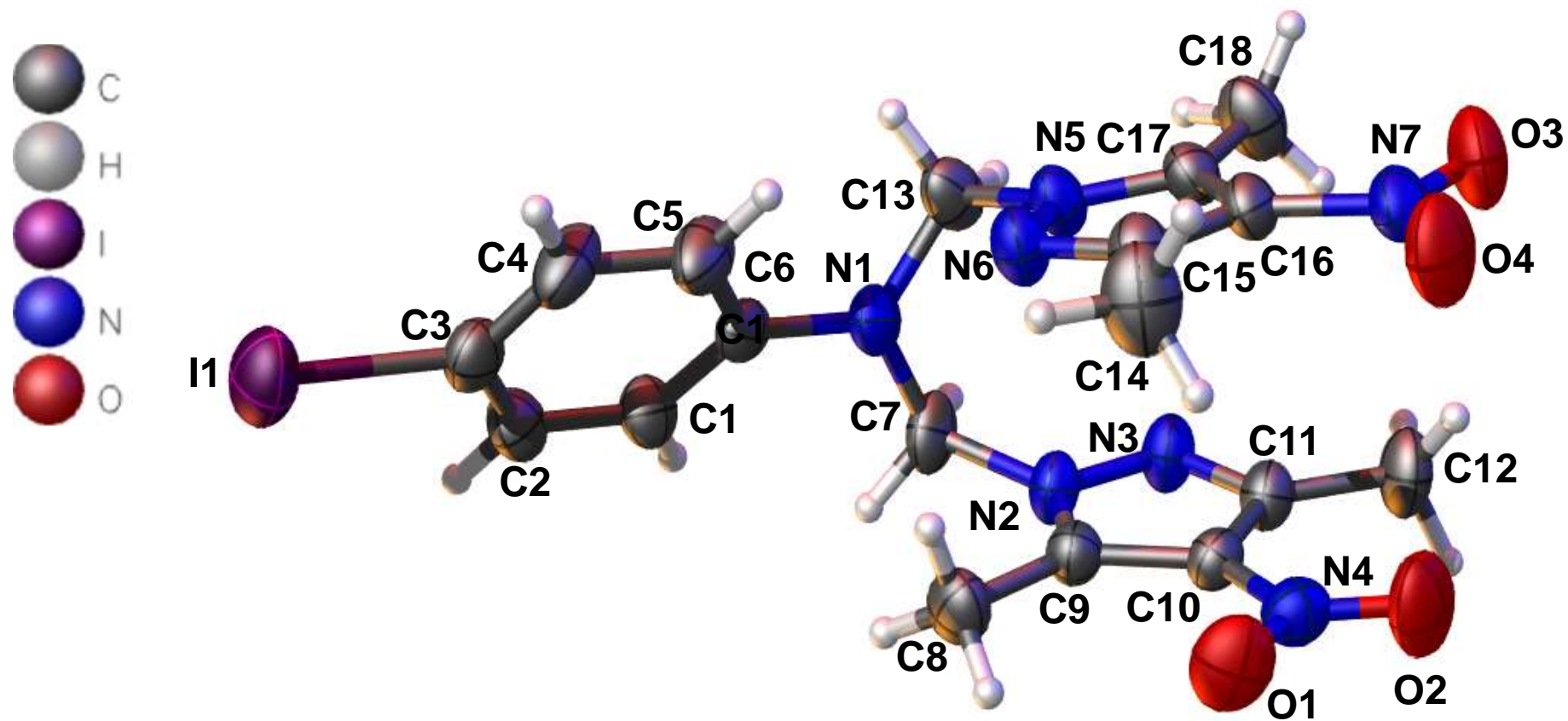


Figure S2. X-ray molecular structure of (**5d**) with color and atoms numbering scheme. Thermal ellipsoids representation with 50% probability.

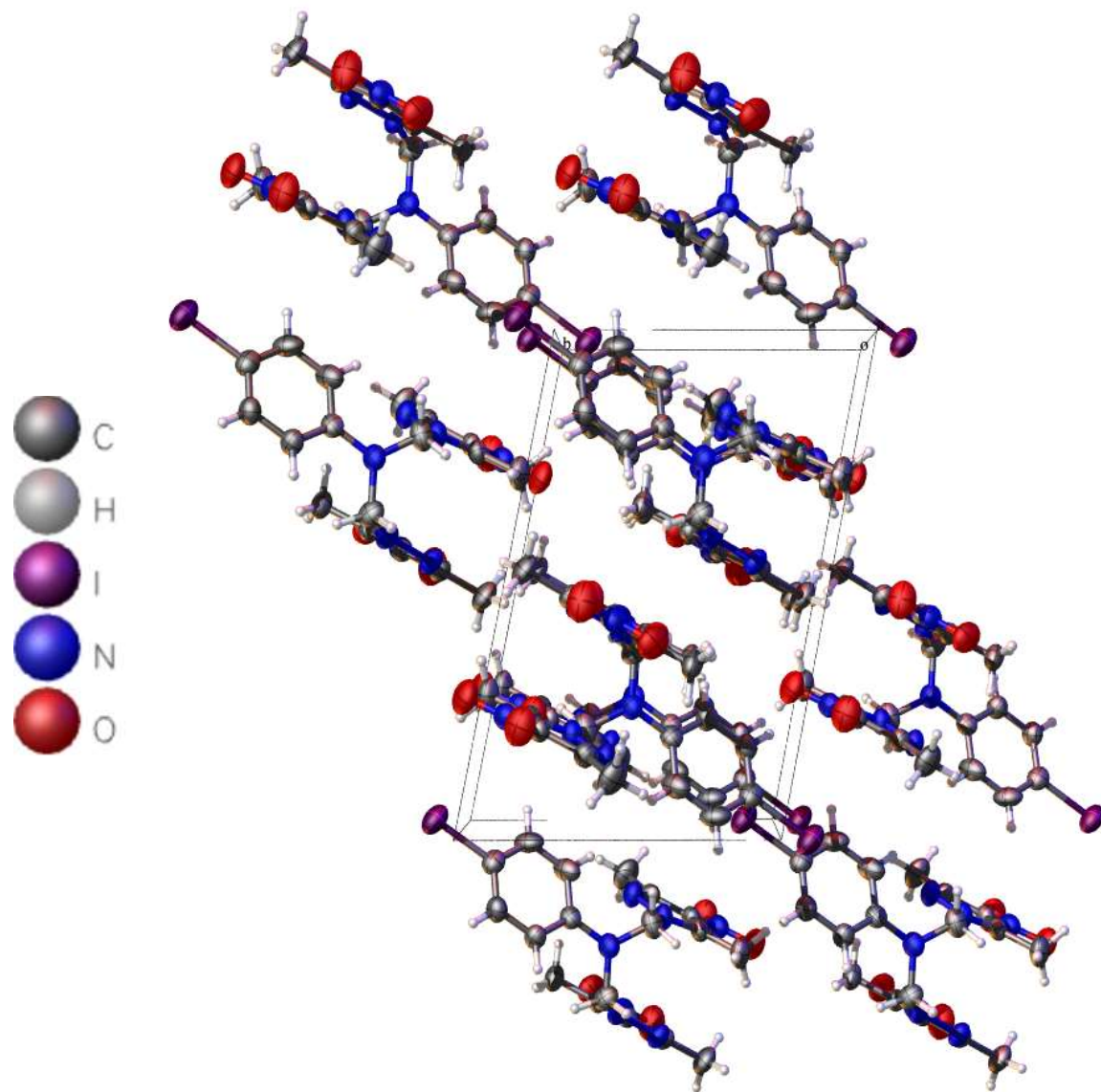


Figure S2.1. Crystal packing for **(5d)** along *a* axis.

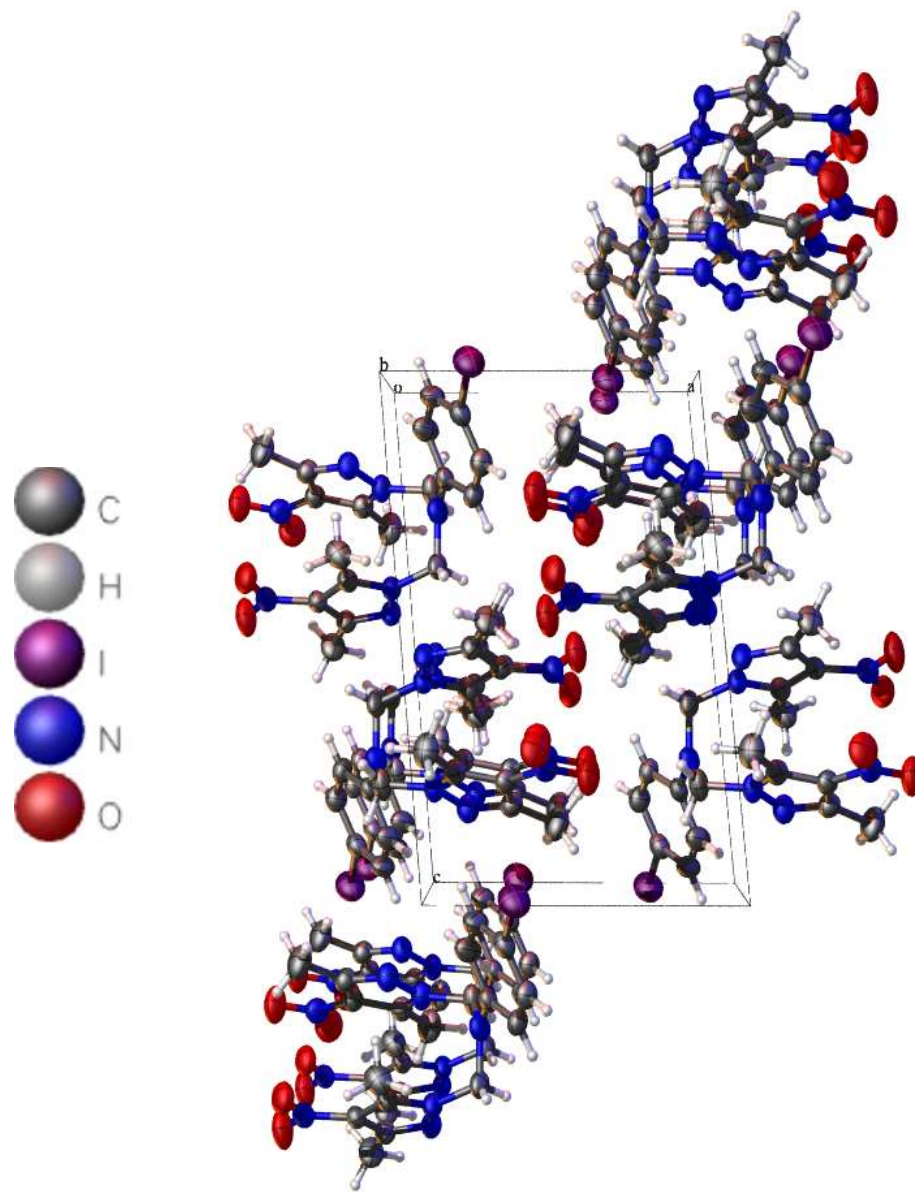


Figure S2.2. Crystal packing for (5d) along *b* axis.

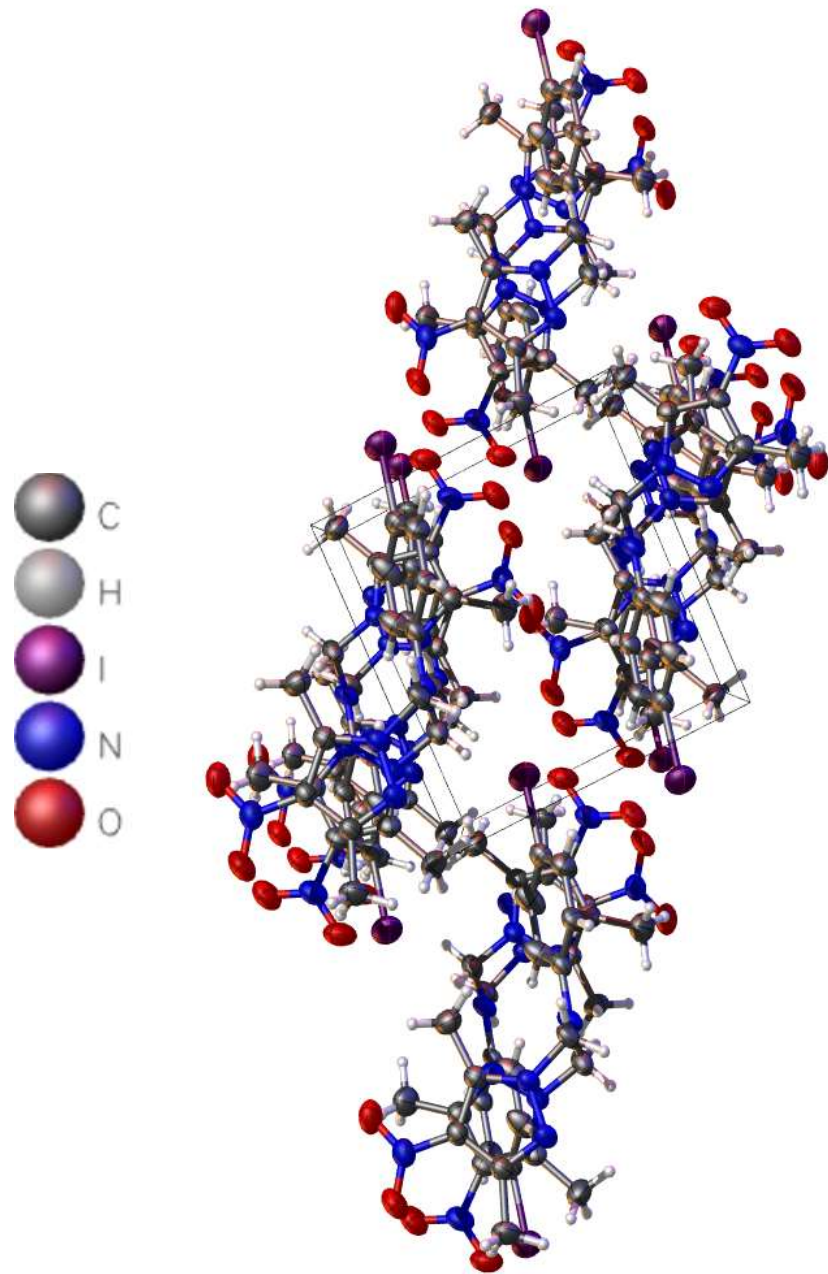


Figure S2.3. Crystal packing for (5d) along c axis.

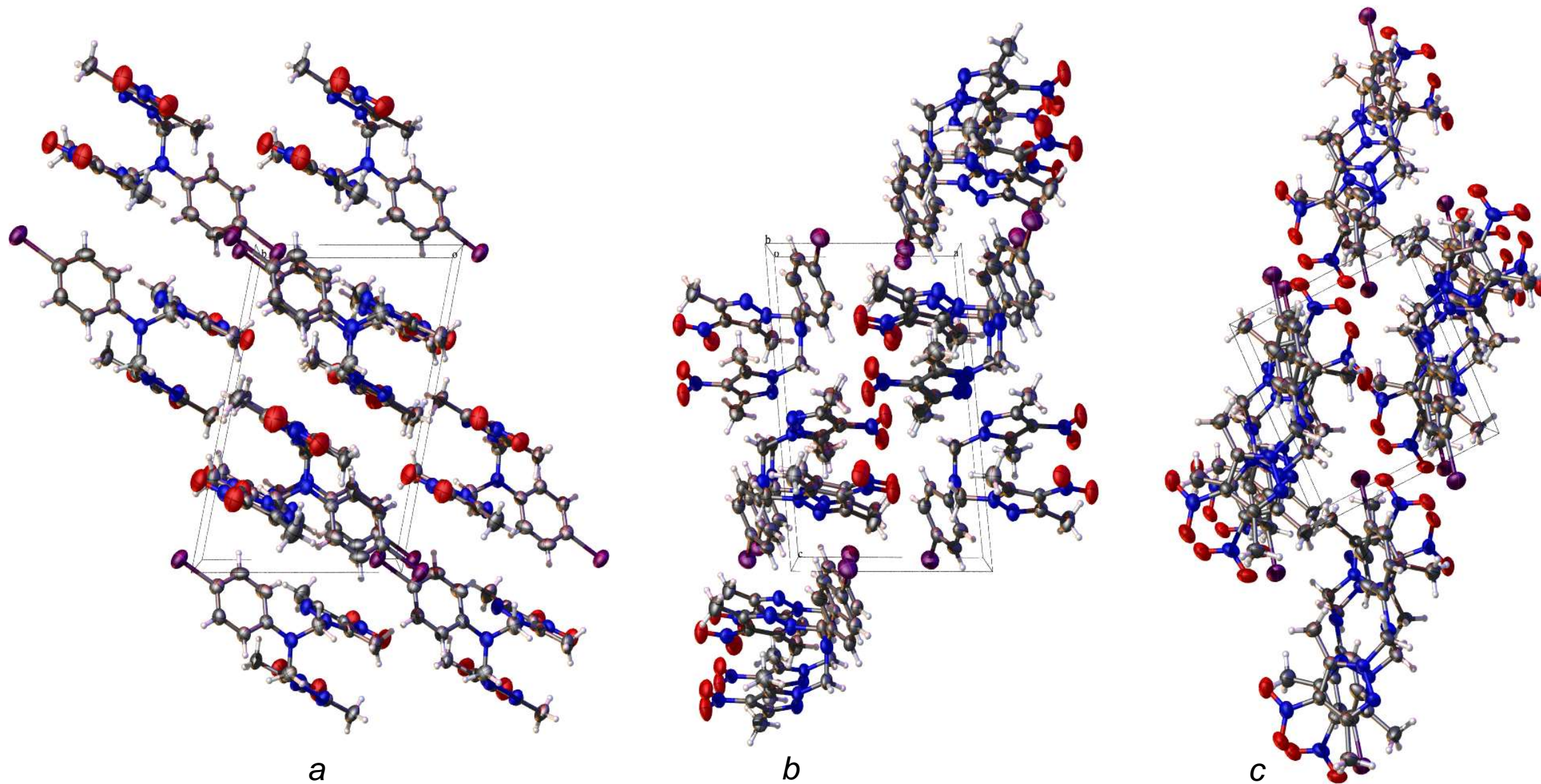


Figure S2.4. Crystal packing for (5d) along *a*, *b* and *c* axes.

Table S1. Summary of crystallographic data and refinement for **4a** and **5d**.

Crystal data	4a	5d
Empirical formula	C ₁₂ H ₁₃ FN ₄ O ₂	C ₁₈ H ₂₀ IN ₇ O ₄
Formula weight	264.261	525.309
Crystal size (mm)	0.300 x 0.100 x 0.100	0.450 x 0.400 x 0.300
Crystal system	Monoclinic	Triclinic
Space group	P2 ₁ /n	P-1
<i>a</i> (Å)	4.2196(3)	8.5652(2)
<i>b</i> (Å)	19.7021(13)	9.0137(2)
<i>c</i> (Å)	15.3651(10)	13.5854(9)
α (°)	90	78.245(6)
β (°)	95.511(7)	84.279(6)
γ (°)	90	87.549(6)
Volume (Å ³)	1271.47(15)	1021.47(8)
<i>Z</i>	4	2
D calc.(g/cm ³)	1.380	1.708
F(000)	552.3	523.438
μ MoK α (cm ⁻¹)	0.107	1.609
Temperature (K)	293	293.15
Range of <i>h</i> , <i>k</i> , <i>l</i>	5, 23, 19	9, 11, 17
θ min/max	3.40/27.40	3.02/27.49
Reflections collected/unique/observed	7698/2914/2914	14337/4682/4239
Data/restraints/parameters	2914/0/174	4682/30/275
Goodness of fit on <i>F</i> ²	0.981	1.0255
Final <i>R</i> indices [<i>I</i> > 2 σ (<i>I</i>)]	<i>R</i> ₁ =0.0576, <i>wR</i> ₂ =0.1243	<i>R</i> ₁ =0.0388, <i>wR</i> ₂ =0.1130
<i>R</i> indices (all data)	<i>R</i> ₁ =0.1452, <i>wR</i> ₂ =0.1672	<i>R</i> ₁ =0.0416, <i>wR</i> ₂ =0.1169

Table S2. Selected bond lengths (Å) and angles (°) for **4a** and **5d**.

4a		5d	
Atoms/Bond lengths (Å)	Atoms/Bond angles (°)	Atoms/Bond lengths (Å)	Atoms/Bond angles (°)
N2-N3 1.373(3)	C11-N2-N3	I1-C3 2.096(3)	C9-N2-N3 113.3(2)
N4-O2 1.226(3)	113.5(2)C10-	N7-C16 1.432(3)	C4-C5-C6 121.1(3)
N2-C11 1.333(3)	C11-N2 104.0(2)	N2-N3 1.372(3)	C7-N2-N3 117.8(2)
N4-O1 1.228(3)	C7-N2-N3	C10-C9 1.388(4)	C10-C9-N2 104.1(2)
N2-C7 1.476(3)	117.7(2)C12-	N2-C9 1.341(4)	C7-N2-C9 128.0(2)
C10-C11 1.381(4)	C11-N2 122.8(3)	C10-C11 1.412(4)	C8-C9-N2 122.8(3)
F1-C3 1.372(3)	C7-N2-C11 128.7(2)	N2-C7 1.470(3)	C10-N4-O1 117.8(3)
C10-C9 1.402(4)	C12-C11-C10 133.2(3)	C6-C5 1.383(5)	C8-C9-C10 133.1(3)
N1-C6 1.402(3)	C7-N1-C6 123.0(2)	N4-O1 1.225(4)	O2-N4-O1 124.0(3)
C11-C12 1.485(4)	C1-C2-C3 118.6(3)	C6-C1 1.391(4)	N1-C7-N2 111.9(2)
N1-C7 1.421(3)	C9-N3-N2 105.4(2)	N4-C10 1.436(3)	O2-N4-C10 118.2(3)
C2-C3 1.362(4)	C3-C4-C5 118.7(3)	N6-C15 1.321(4)	C2-C1-C6 121.2(3)
N3-C9 1.325(3)	C5-C6-N1 122.8(3)	N4-O2 1.198(4)	C11-N3-N2 105.9(2)
C2-C1 1.365(4)	C10-C9-N3 108.6(2)	C14-C15 1.491(4)	C18-C17-N5 124.5(3)
C6-C5 1.379(4)	C1-C6-N1	N3-C11 1.318(3)	N6-N5-C13 117.9(2)
C4-C5 1.380(4)	118.6(2)C8-C9-	C5-C4 1.386(5)	C16-C17-N5 104.3(2)
C6-C1 1.380(4)	N3 120.3(3)	O4-N7 1.219(4)	C17-N5-C13 129.1(3)
C4-C3 1.362(4)	C1-C6-C5	C9-C8 1.487(4)	C16-C17-C18 131.2(3)
N4-C10 1.419(3)	118.6(3)C8-C9-	N5-C13 1.486(3)	C17-N5-N6 113.1(2)
C9-C8 1.485(4)	C10 131.0(2)	C1-C2 1.383(4)	C3-C4-C5 119.8(3)

	O2-N4-C10 118.9(3)C4-C5- C6120.5(3) O1-N4-C10 119.1(3)C2-C3- F1 118.9(3) O1-N4-O2122.0(3) C4-C3-F1 118.9(3) C11-C10-N4125.7(3) C4-C3-C2122.2(3) C9-C10-N4125.8(3) C2-C1-C6121.4(3) C9-C10-C11108.5(2) N1-C7-N2 113.0(2)	N5-N6 1.377(3) C17-C181.486(4) N5-C17 1.337(4) C17-C161.388(4) N1-C13 1.428(4) C4-C3 1.385(5) N1-C6 1.415(4) C11-C12 1.489(4) N1-C7 1.445(4) C15-C161.404(4) N7-O3 1.216(4) C2-C3 1.376(5)	C6-N1-C13120.9(3) C10-C11-N3108.9(2) C7-N1-C13117.7(3) C12-C11-N3120.6(3) C7-N1-C6121.4(2) C12-C11-C10130.4(3) O3-N7-O4123.1(3) C14-C15-N6120.6(3)
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Table S3. Cell viability assay results of series **4a-f** tested *in vitro* on NCTC normal fibroblasts and HEp-2 epithelial tumor cells, at 24 and 48h of treatment

Sample	Concentration ($\mu\text{g/mL}$)	Cell viability on NCTC cells (%)		Cell viability on Hep2 cells (%)	
		24h	48h	24h	48h
	Control	100.00	100.00	100.00	100.00
4a	3.1	99.56 \pm 1.38	110.87 \pm 3.28	99.14 \pm 3.42	93.05 \pm 0.78
	6.25	99.35 \pm 2.20	109.13 \pm 3.80	94.44 \pm 1.19	89.00 \pm 1.50
	12.5	99.02 \pm 2.51	100.63 \pm 1.48	93.38 \pm 0.92	83.79 \pm 2.30
	25	90.22 \pm 1.71	73.46 \pm 2.96	91.35 \pm 3.73	72.26 \pm 1.99
	50	62.72 \pm 1.88	47.48 \pm 1.31	73.18 \pm 2.71	36.37 \pm 1.45
4b	3.1	100.17 \pm 1.63	95.08 \pm 0.82	115.84 \pm 2.48	112.57 \pm 1.03
	6.25	100.53 \pm 3.31	93.03 \pm 1.35	105.08 \pm 2.91	104.00 \pm 1.08
	12.5	98.60 \pm 2.62	89.70 \pm 3.15	100.30 \pm 2.49	98.02 \pm 1.42
	25	96.31 \pm 2.22	61.85 \pm 1.38	88.79 \pm 1.65	79.53 \pm 1.49
	50	64.91 \pm 0.94	31.57 \pm 1.27	65.62 \pm 2.78	46.82 \pm 3.35
4c	3.1	99.20 \pm 0.97	99.67 \pm 0.71	101.36 \pm 2.04	116.32 \pm 2.55
	6.25	97.10 \pm 3.04	98.85 \pm 1.92	102.66 \pm 2.31	104.22 \pm 0.88
	12.5	95.60 \pm 0.62	97.21 \pm 3.50	100.48 \pm 3.15	99.57 \pm 2.32
	25	95.50 \pm 2.14	95.64 \pm 2.93	98.70 \pm 1.83	82.62 \pm 3.28
	50	91.11 \pm 3.04	81.53 \pm 3.26	92.64 \pm 0.90	70.23 \pm 1.91
4d	3.1	99.89 \pm 2.31	95.69 \pm 0.80	104.97 \pm 1.93	100.60 \pm 3.72
	6.25	100.53 \pm 2.24	96.26 \pm 0.78	109.78 \pm 2.64	110.14 \pm 3.49
	12.5	102.74 \pm 3.44	92.64 \pm 1.40	113.02 \pm 3.48	110.79 \pm 2.80
	25	101.67 \pm 1.07	91.55 \pm 1.41	109.40 \pm 2.75	101.56 \pm 0.56
	50	101.37 \pm 2.55	88.16 \pm 2.10	106.24 \pm 1.63	68.97 \pm 0.81
4e	3.1	93.11 \pm 2.76	89.55 \pm 2.24	106.54 \pm 0.73	98.12 \pm 2.88
	6.25	94.28 \pm 2.92	98.33 \pm 2.03	103.71 \pm 0.28	97.11 \pm 1.78
	12.5	97.81 \pm 0.99	93.18 \pm 2.94	99.70 \pm 0.32	94.75 \pm 2.84
	25	95.63 \pm 2.19	92.20 \pm 0.89	89.15 \pm 0.88	81.69 \pm 2.38
	50	63.53 \pm 3.18	63.21 \pm 2.56	56.46 \pm 0.42	54.91 \pm 2.76
4f	3.1	107.06 \pm 1.61	99.61 \pm 1.20	98.29 \pm 1.16	98.26 \pm 3.38
	6.25	107.50 \pm 1.60	105.98 \pm 1.70	98.61 \pm 0.72	97.74 \pm 0.62
	12.5	103.04 \pm 1.05	103.07 \pm 0.68	95.51 \pm 2.45	93.63 \pm 0.75
	25	93.69 \pm 2.77	97.79 \pm 2.35	94.34 \pm 2.78	86.05 \pm 0.93
	50	87.72 \pm 1.77	80.47 \pm 1.16	93.91 \pm 1.03	72.84 \pm 2.33

Table S4. Cell viability assay results of series **5a-e** tested *in vitro* on NCTC normal fibroblasts and HEP-2 epithelial tumor cells, at 24 and 48h of treatment

Sample	Concentration (µg/mL)	Cell viability on NCTC cells (%)		Cell viability on Hep2 cells (%)	
		24h	48h	24h	48h
Control		100.00	100.00	100.00	100.00
5a	3.1	97.69±1.80	87.19±0.70	90.85±1.10	90.09±2.22
	6.25	98.35±2.22	88.74±2.40	104.37±0.32	85.25±3.13
	12.5	100.00±2.07	98.18±2.04	96.62±3.10	80.09±1.54
	25	97.36±2.40	93.71±1.17	82.68±1.85	67.37±3.23
	50	88.56±2.66	92.55±1.32	57.18±1.13	38.80±0.71
5b	3.1	99.08±3.42	100.38±2.53	105.41±1.52	110.12±1.25
	6.25	103.36±3.06	94.93±2.14	103.69±2.25	94.67±3.80
	12.5	98.78±3.47	84.05±3.77	101.68±3.76	87.08±0.92
	25	70.03±2.51	55.16±1.60	90.80±2.84	74.80±0.33
	50	48.62±1.35	28.14±0.35	65.27±2.02	54.56±1.17
5c	3.1	104.64±1.96	96.74±2.96	111.76±2.32	96.94±1.38
	6.25	106.01±2.30	101.14±3.63	113.54±2.18	96.48±1.58
	12.5	105.90±3.74	97.37±1.99	116.63±2.83	89.21±2.02
	25	105.37±1.88	96.63±2.02	112.94±1.52	65.31±2.09
	50	83.98±2.12	68.00±1.61	101.73±2.71	50.25±2.02
5d	3.1	102.67±1.49	93.58±0.71	88.45±0.28	96.17±0.13
	6.25	107.86±2.40	103.10±3.22	89.15±0.73	78.61±0.67
	12.5	106.29±2.05	101.77±3.03	91.41±0.69	61.51±0.34
	25	98.43±3.87	95.80±2.12	89.86±0.48	45.75±0.33
	50	83.02±2.90	88.27±1.55	89.86±0.16	20.84±0.13
5e	3.1	101.94±0.73	95.71±0.50	103.69±0.73	102.47±0.48
	6.25	98.57±0.27	89.47±0.34	102.40±0.28	96.06±0.34
	12.5	88.26±0.32	69.25±0.30	82.29±0.32	77.40±0.63
	25	77.65±0.88	40.30±0.68	70.51±0.88	60.32±0.67
	50	65.79±0.42	26.45±0.46	59.94±0.42	37.74±0.46
dioscin	3.1	94.29±2.49	94.17±1.66	97.09±0.43	95.99±0.53
	6.25	93.04±3.24	72.63±1.67	76.68±2.01	61.97±1.78
	12.5	38.74±2.55	37.02±2.55	58.92±0.30	38.03 ±2.09
	25	14.93±2.63	8.55±1.26±	13.86±0.18	7.49±1.32
	50	13.73±2.06	7.14±1.28	11.53±0.14	7.27±1.14

Legend: the green color represents the non-cytotoxic effect, cell viability percentage>80%. the blue color represents the slightly cytotoxic effect, percentage of cell viability between 50 and 80%. the red color represents the severe cytotoxic effect, percentage of cell viability < 50 %.