Supporting Information

Development of Chromene Based Fluorescent and Colorimetric Sensor for Sensitive Detection of Calcium Ions in Complex Medium: A Detailed DFT and Experimental Approach

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SI-1. Instruments and reagents

The targeted furyl and nitrile substituted chromene sensor **CFN** was efficiently synthesized and characterized through ^{1}H, ^{13}C and DEPT 135 NMR experiments at 400 and 100 MHz, respectively, by utilizing a Bruker Advance III HD MHz spectrometer, taking DMSO-d_6 as solvent. The ^{1}H NMR titration assay was also performed in DMSO-d_6 at 400 MHz. To execute the optical investigations, FluoroMax-Plus-P-C, Horiba Jobin Yvon Technology, USA, was employed, and UV-*Vis.* absorption spectra were recorded using UV-Visible Spectrophotometer Double Beam, Shimadzu 1601, Japan. The reagents and chemicals required for all studies were purchased from Alfa Aesar (UK), Sigma Aldrich (USA), Daejung Chemicals & Metals (Korea), and Oakwood Chemicals (USA). These chemicals include 5-methyl-2-furaldehyde malononitrile, resorcinol, *n*-hexane (HX), methanol, *N,N*-dimethylformamide (DMF), ethanol dichloromethane (DCM), distilled H_2O, acetone, ethylene glycol, glycerol, acetonitrile, dimethyl sulfoxide (DMSO), thin layer chromatographic (TLC) plates, ethyl acetate, different metal salts, silica gel, hot plates, column, condensers, round bottom flasks, stirrer, micro pipettes (10-1000 μL), UV lamp, and oven.

SI-2. Optical experiments

A 1.4 mL volume quartz cuvette having 10 mm path length and transparent window was considered for the optical studies by exciting the sensor **CFN** at 330 nm excitation wavelength in aqueous media (DMF: H_2O; 1:9, v/v). A 300 μM stock solution of sensor **CFN** and all analytes was prepared with further diluted according to experimental requirements. Fluorescence and UV-*Vis* studies were performed in triplicate, and the best collected results are provided here.

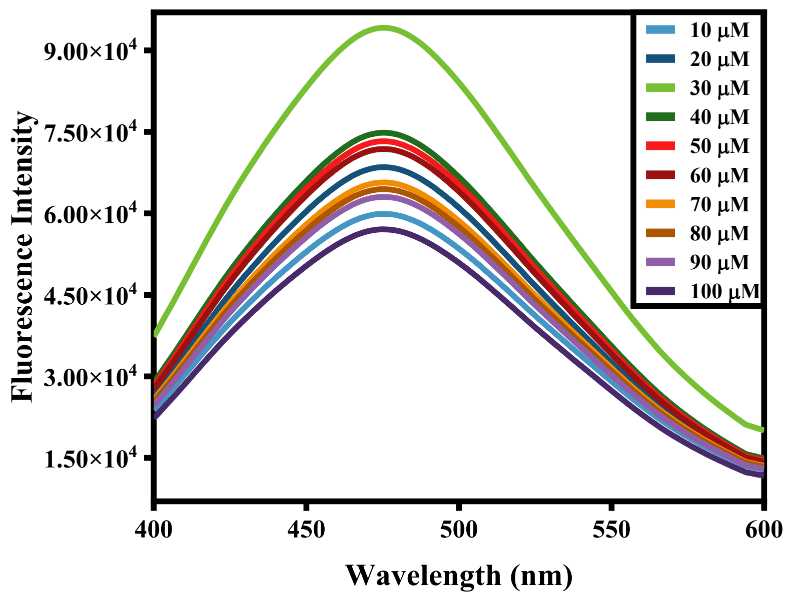

Figure S1. Emission spectra of DMF solution of sensor **CFN** at different concentrations (0–100 μM).

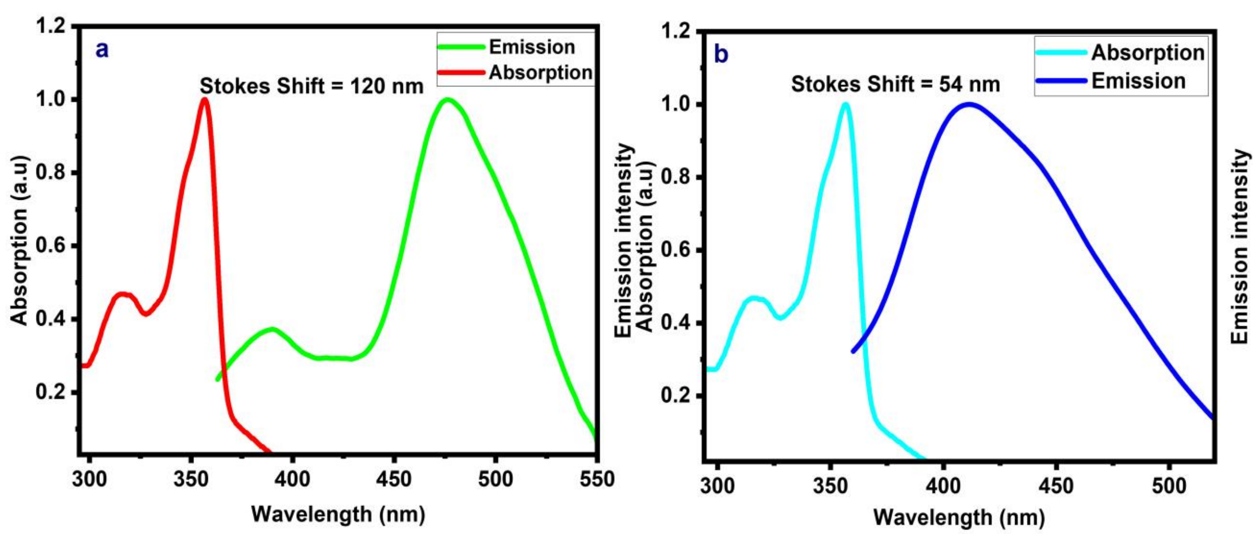

Figure S2. Emission and absorption spectra of sensor **CFN** in pure DMF (a) and in DMF/water (1:90, v/v) (b)

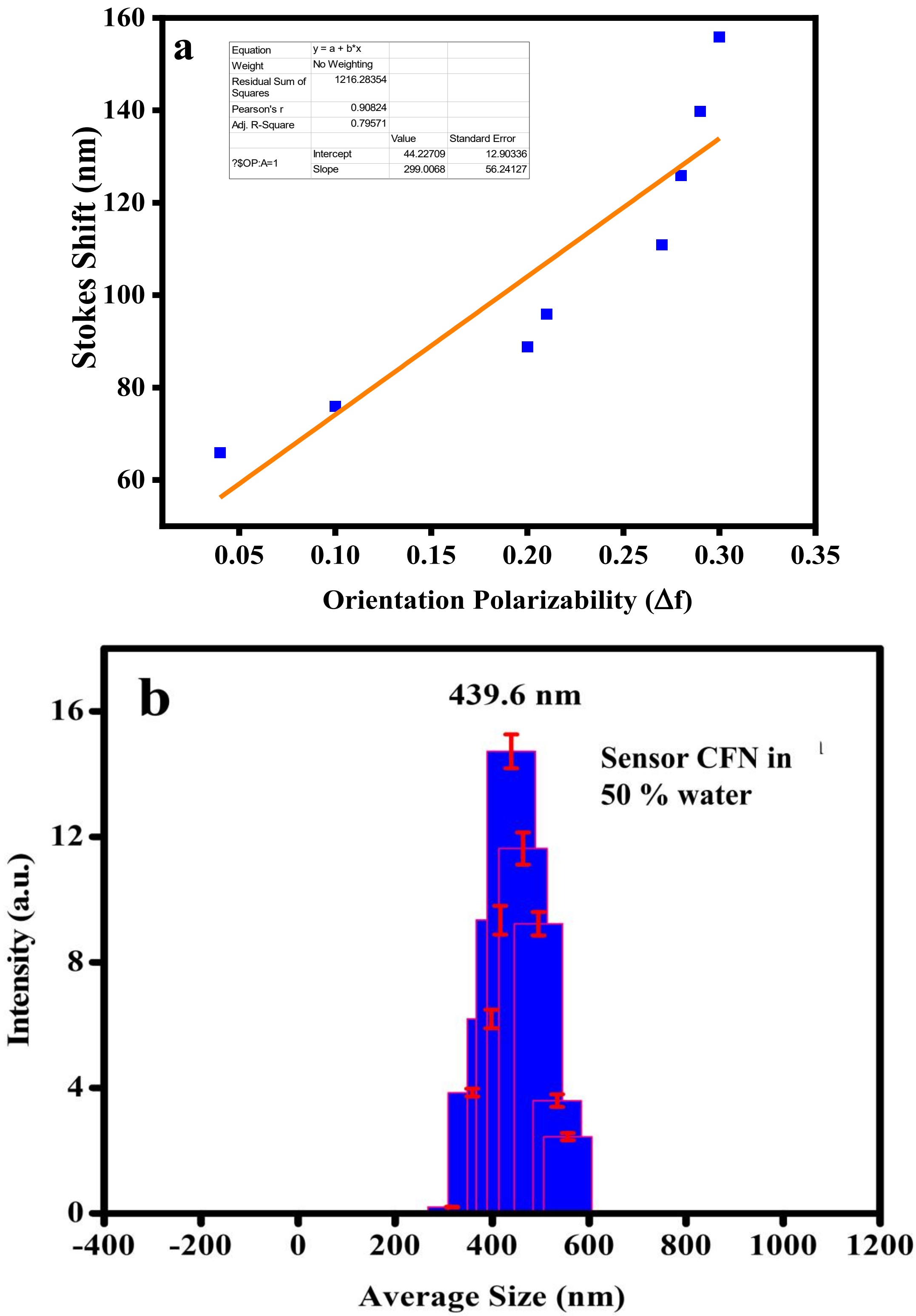

Figure S3. Depiction of Lippert-Mataga graph of compound **CFN** in diversified polarity solvents (a), DLS size distribution analysis of sensor **CFN** after adding 50% water portion into its 30 µM DMF solution (b).

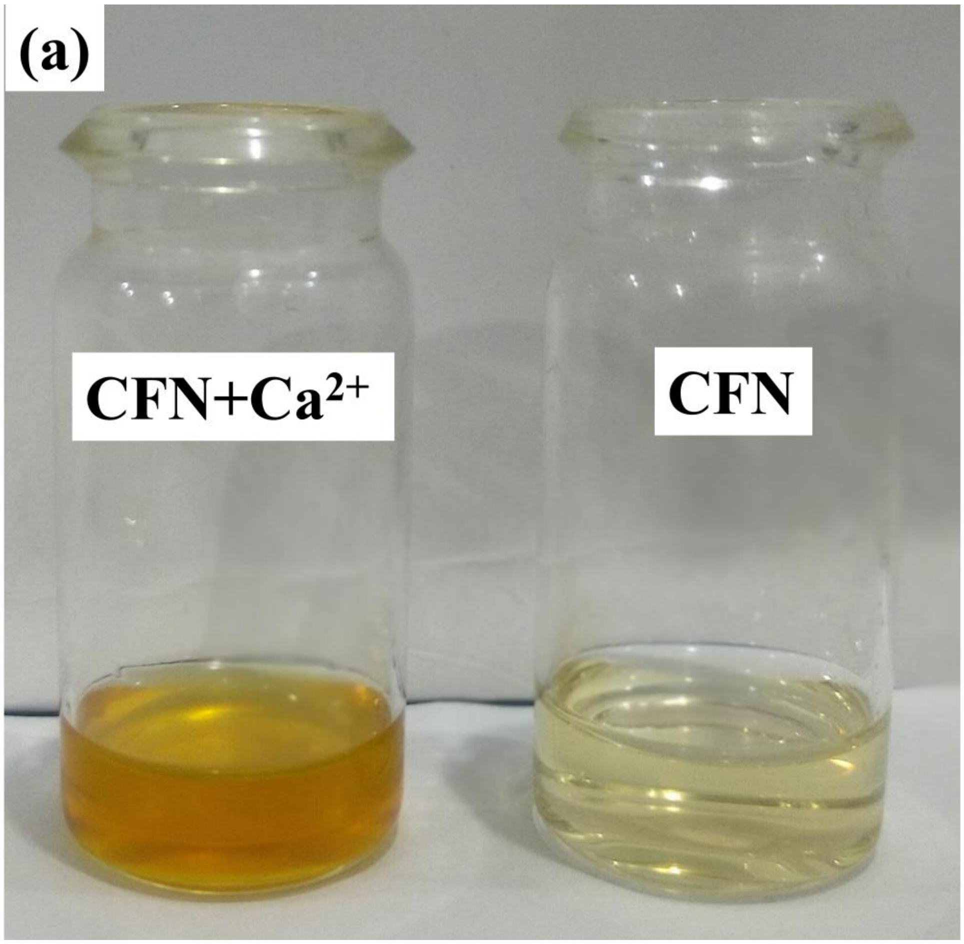

Figure S4. Visible color change of 30 μM **CFN** in DMF upon addition of 1 μM Ca^{2+}.

SI-3. Association constant (K_a)

The association constant (K_a) was calculated using a non-linear least squares analysis, analyzing the relationship between C_m and C_o concentrations and the emission decline, using in Eq. S1. This approach validates the 1:2 probe-analyte binding ratio [1].

$$F = F_o + \frac{F_{lim}-F}{2C_o}\left[C_o + C_m + \frac{1}{K} - \sqrt{\left\{\left(C_o + C_m + \frac{1}{K}\right)^2 - 4C_oC_m\right\}}\right] \quad \text{(S1)}$$

Here, F_o and F denote the emission intensity of the pristine sensor **CFN** in the absence and presence of Ca^{2+}, respectively. Similarly, C_o and C_m represent the concentrations of the original probe and Ca^{2+}. F_{lim} is designated as the highest emission enhancement of the probe at maximum Ca^{2+} ion concentration. The equilibrium constant of the probe-analyte solution is represented by the symbol K. Consequently, a linear regression line was plotted between the fluorescence enhancement and progressively added fractions of Ca^{2+} (Figure S5).

SI-4. Calculation of limit of detection (LOD)

The LOD for the Ca^{2+} ions by sensor **CFN** was estimated by following the IUPAC published rules [2]. In pursuit of this, data was collected from the fluorescence titration of sensor **CFN** against analyte Ca^{2+} and a linear fit plot was established. Decline in the emission intensity was plotted as a function of Ca^{2+} concentration (0-1000 nM) (Figure S5). The standard deviation and S/N ratio was recorded after four times repeating the titration experiment.

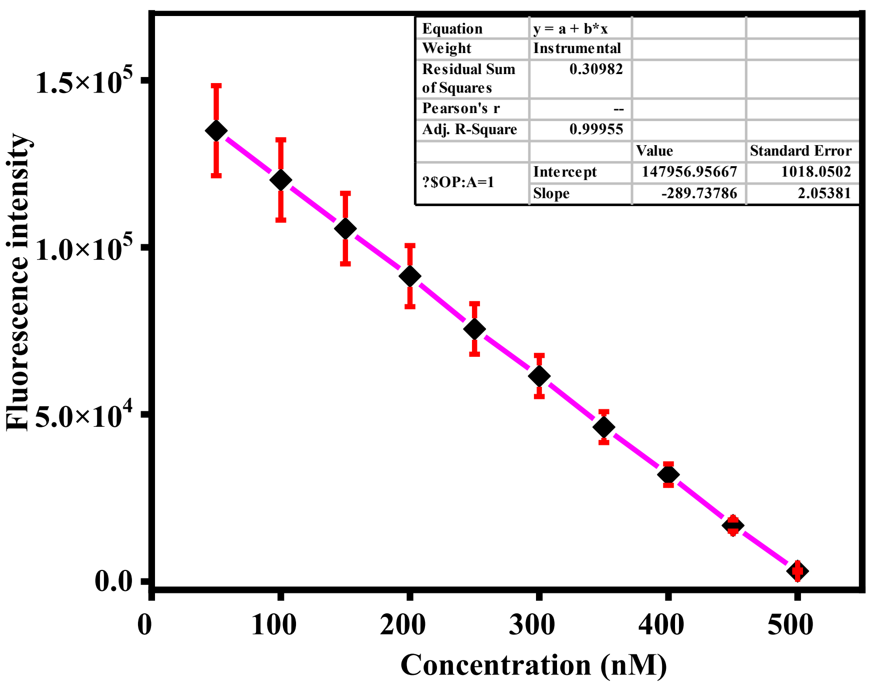

Figure S5 The standard linear-fit plot to assess the LOD of sensor **CFN** for the sensitive tracing of Ca^{2+}.

LOD was calculated by the results of the blank scan, and the slopes of the above presented (Figure S1) calibration curve by employing 3σ/S formula. Here, σ represents the standard deviation of the blank measurement of the sensor **CFN**, and S denotes the slope between the fluorescence decline and Ca^{2+} concentration.

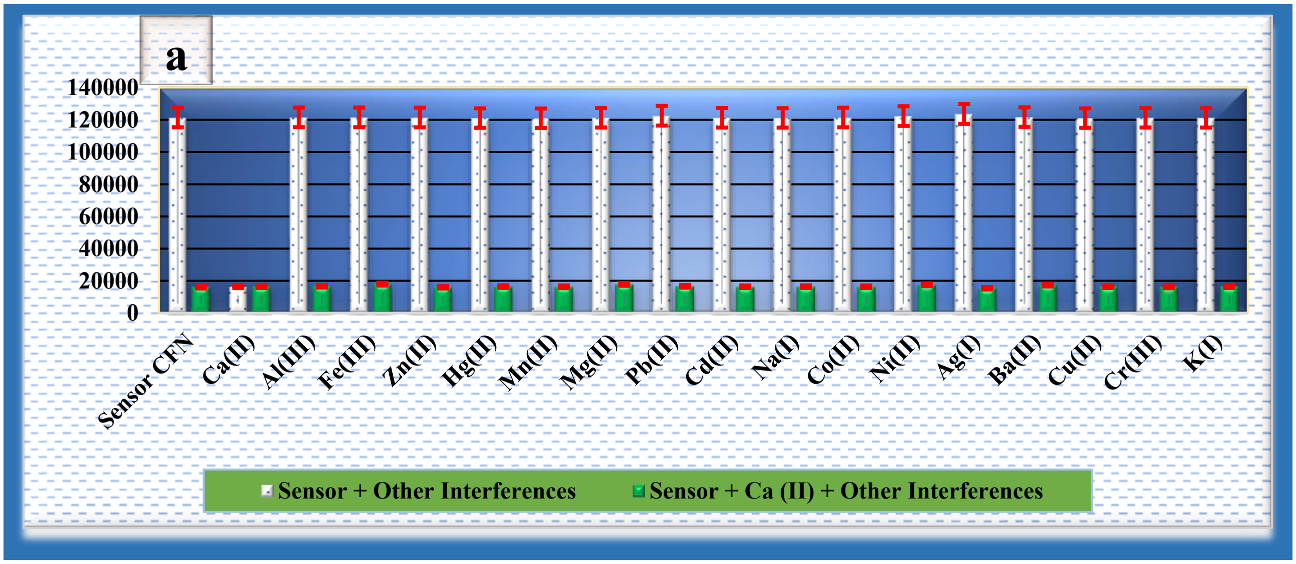

Figure S6. Interference experiments by collecting the spectra after simultaneous blending of sensor **CFN** (30 µM) against the 100.0 equivalents of potential interferences into pure sensor solution.

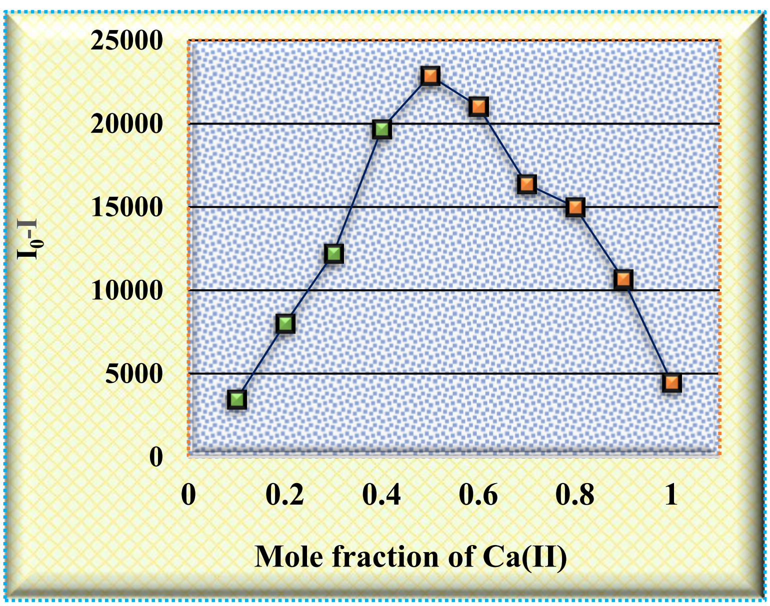

Figure S7. Graphical demonstration of binding stoichiometry (1:1) calculated for sensors **CFN** (30 µM) when its sensing potential was tested in changed mole fractions (0-1) of Ca^{2+}.

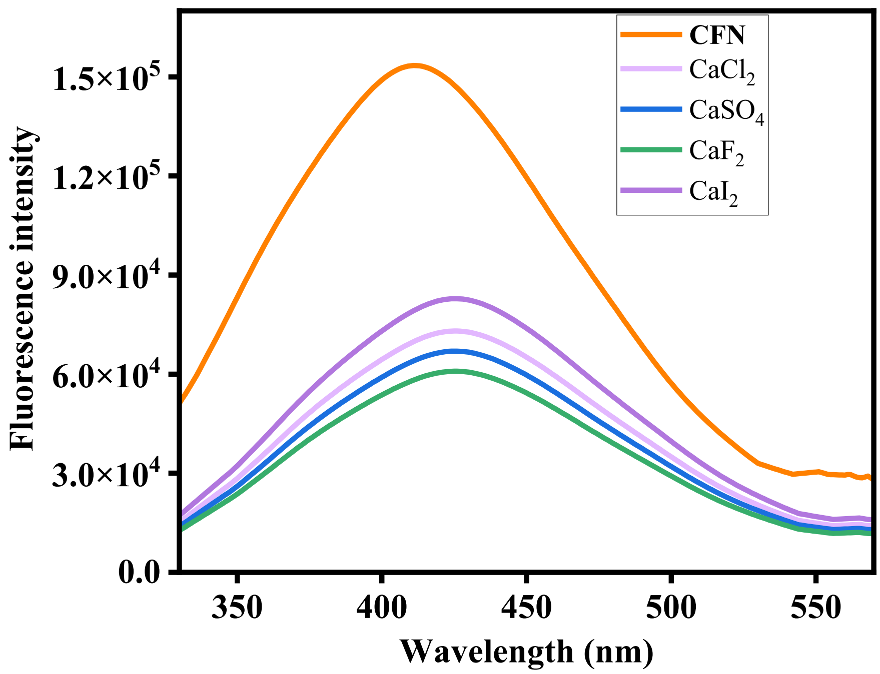

Figure S8. Study of counter ion influence on the selective detection of Ca^{2+} sensor **CFN**.

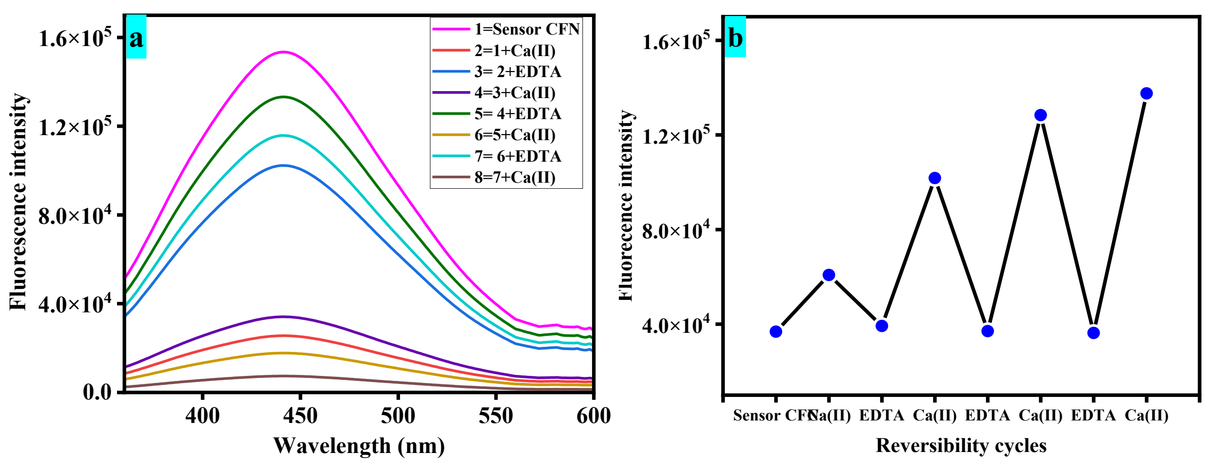

Figure S9. Fluorescence spectrum scanned to reveal the reversibility response of the sensor **CFN** (a and b) and through concomitant mixing of Ca^{2+} (1 μM) and EDTA showcasing the reversibility cycle pattern.

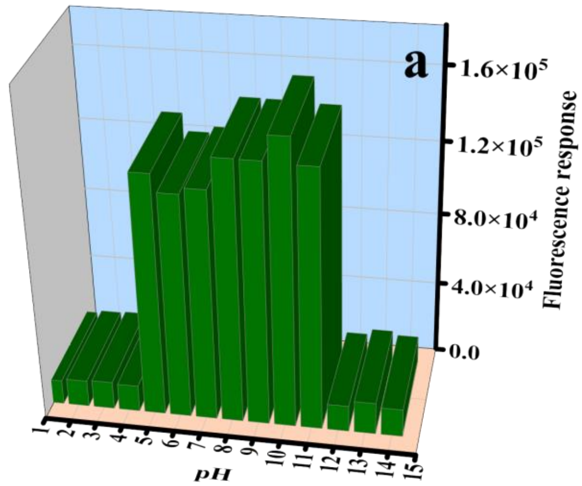

Figure S10. Demonstration of change in fluorescence of sensor **CFN** (10 μM) at varying pH.

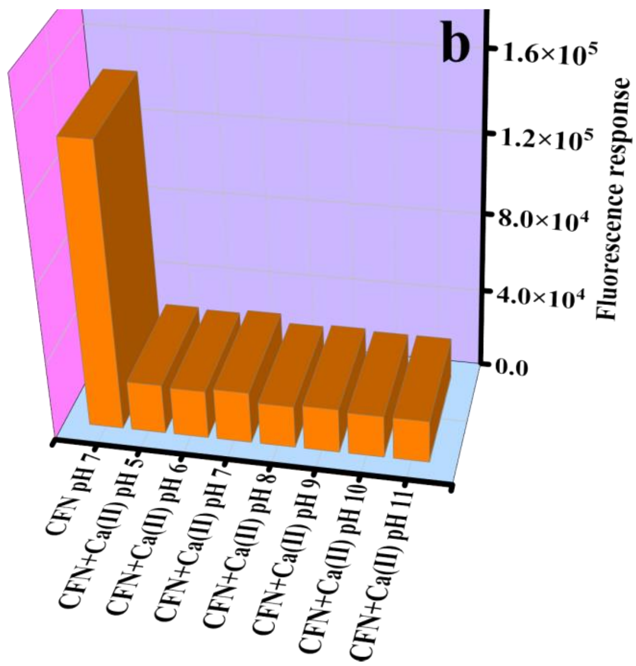

Figure S11. Fluorescence response of sensor **CFN** (30 μM) against Ca^{2+} at pH 5 to 11.

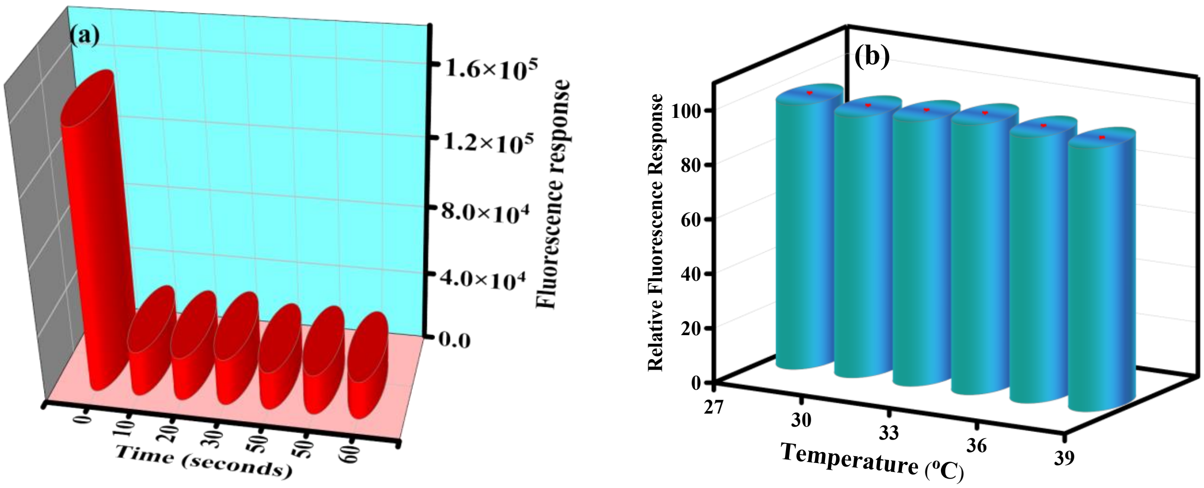

Figure S12. Fluorescence measurement of sensor **CFN** (30 μM) at different time spans (0-60 seconds) after mixing with analyte Ca^{2+} (a) and Fluorescence measurement of sensor **CFN** (30 μM) at varying temperature (28-38 °C) after mixing with analyte Ca^{2+} (b)

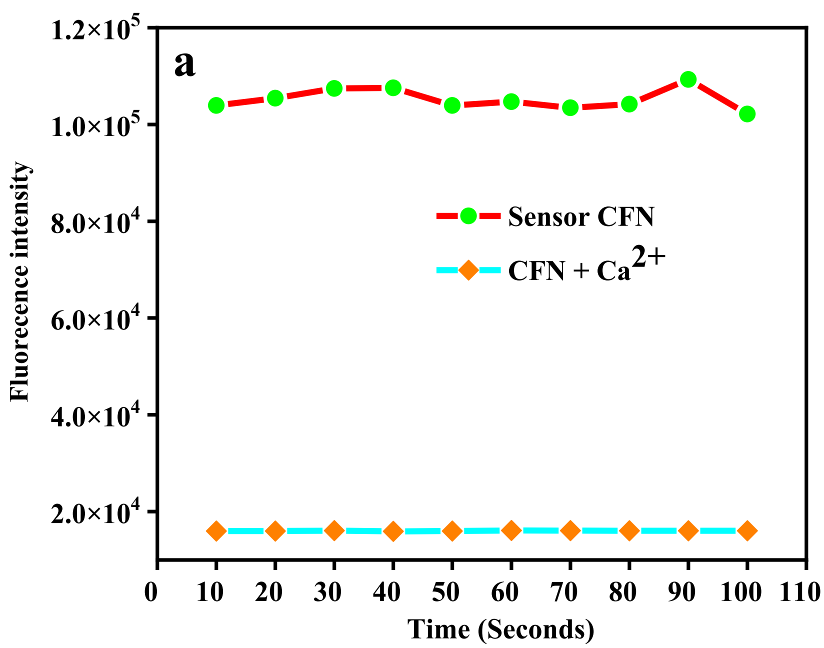

Figure S13. Photostability investigation of sensor **CFN** (30 μM) when pristine sensor solution and sensor-analyte complex were constantly exposed to high energy radiations after continuous time intervals and fluorescence spectra were collected (0–100 *sec.*).

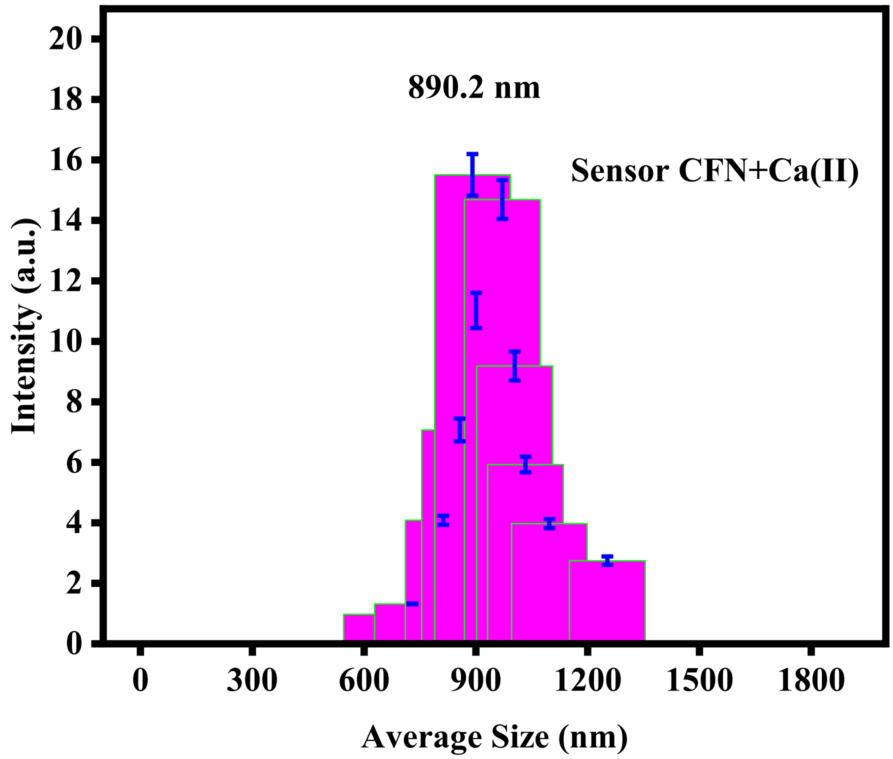

Figure S14. DLS particle size distribution data of sensor **CFN** (30 μM) collected after mixing with 500 nM Ca^{2+} in its 4 mL DMF solution.

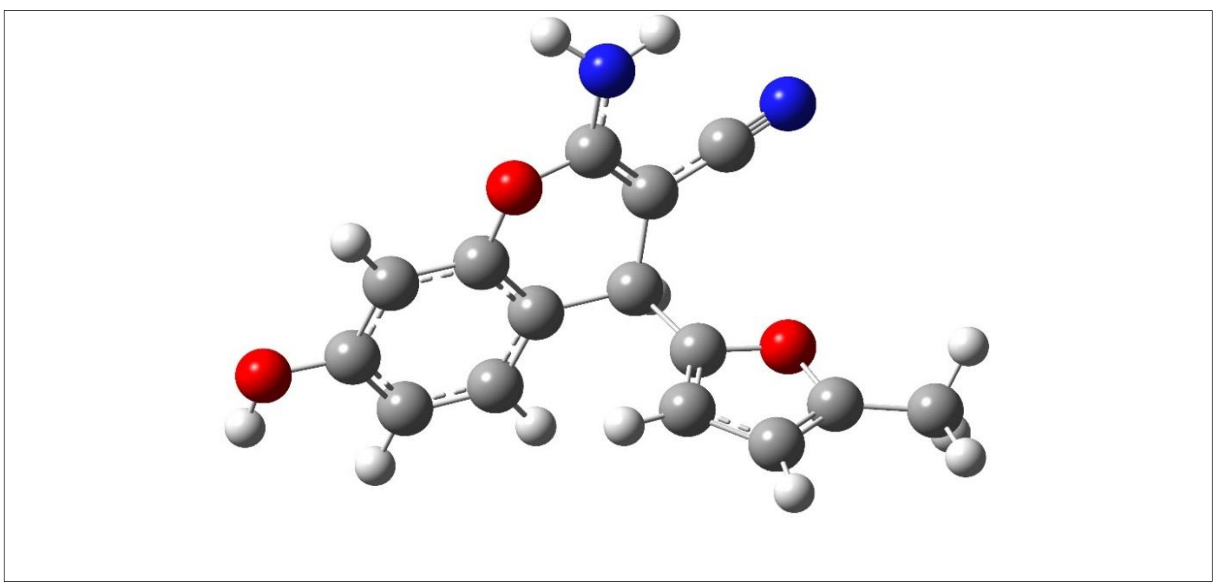

Figure S15. Optimized geometry of sensors **CFN**

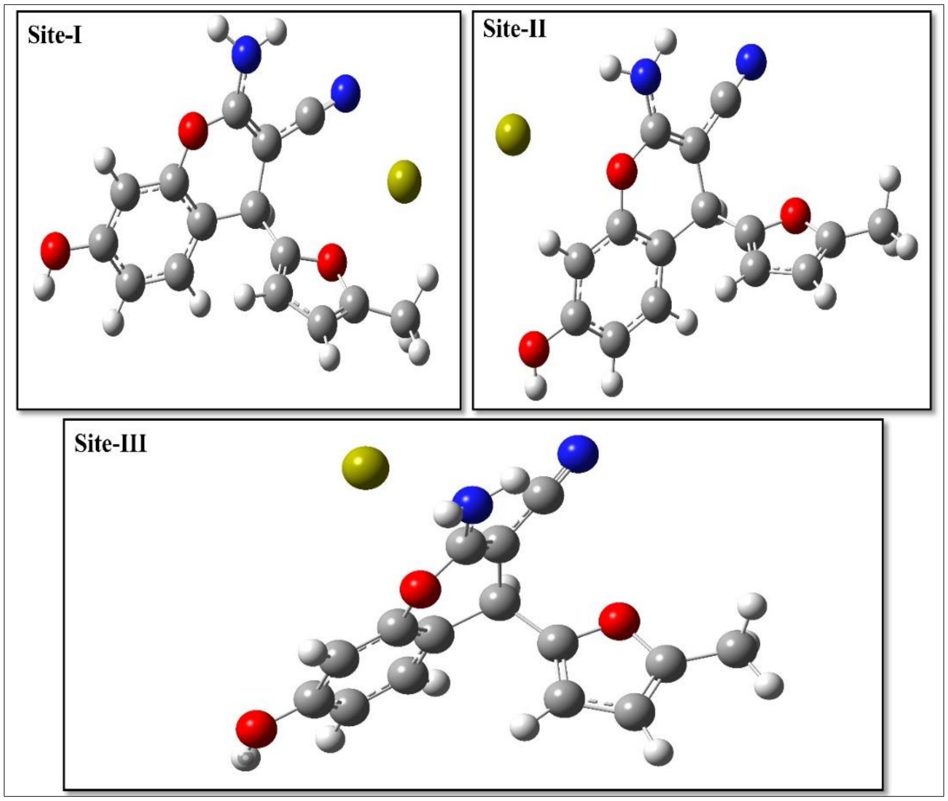

Figure S16. Optimized interaction sites of sensors **CFN** against Ca^{2+}

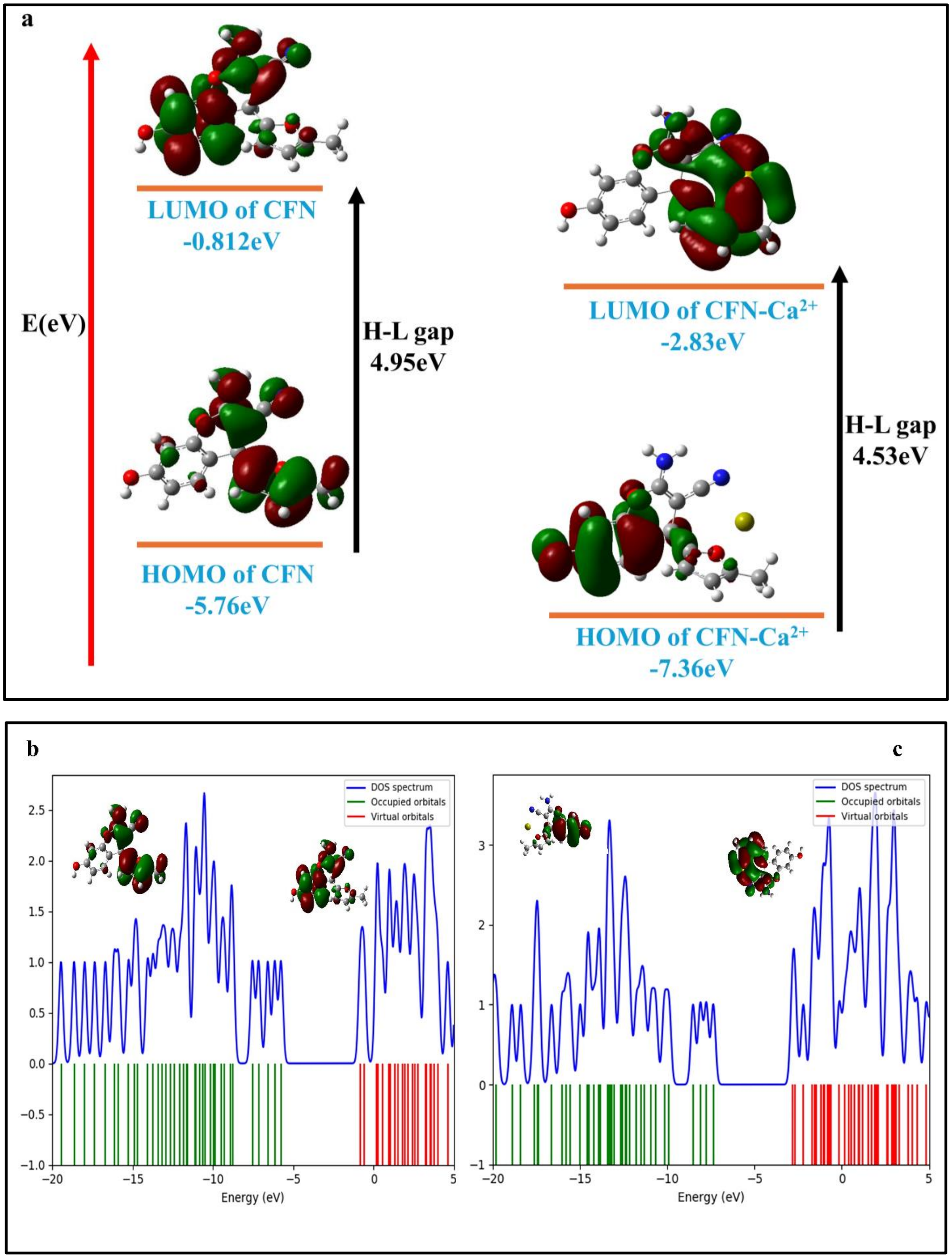

Figure S17. Distribution of electron over HOMO-LUMO of the sensor **CFN** and Complex **CFN**-Ca^{2+} (a) and DOS spectra of sensors **CFN** (b) and Complex **CFN**-Ca^{2+} (c).

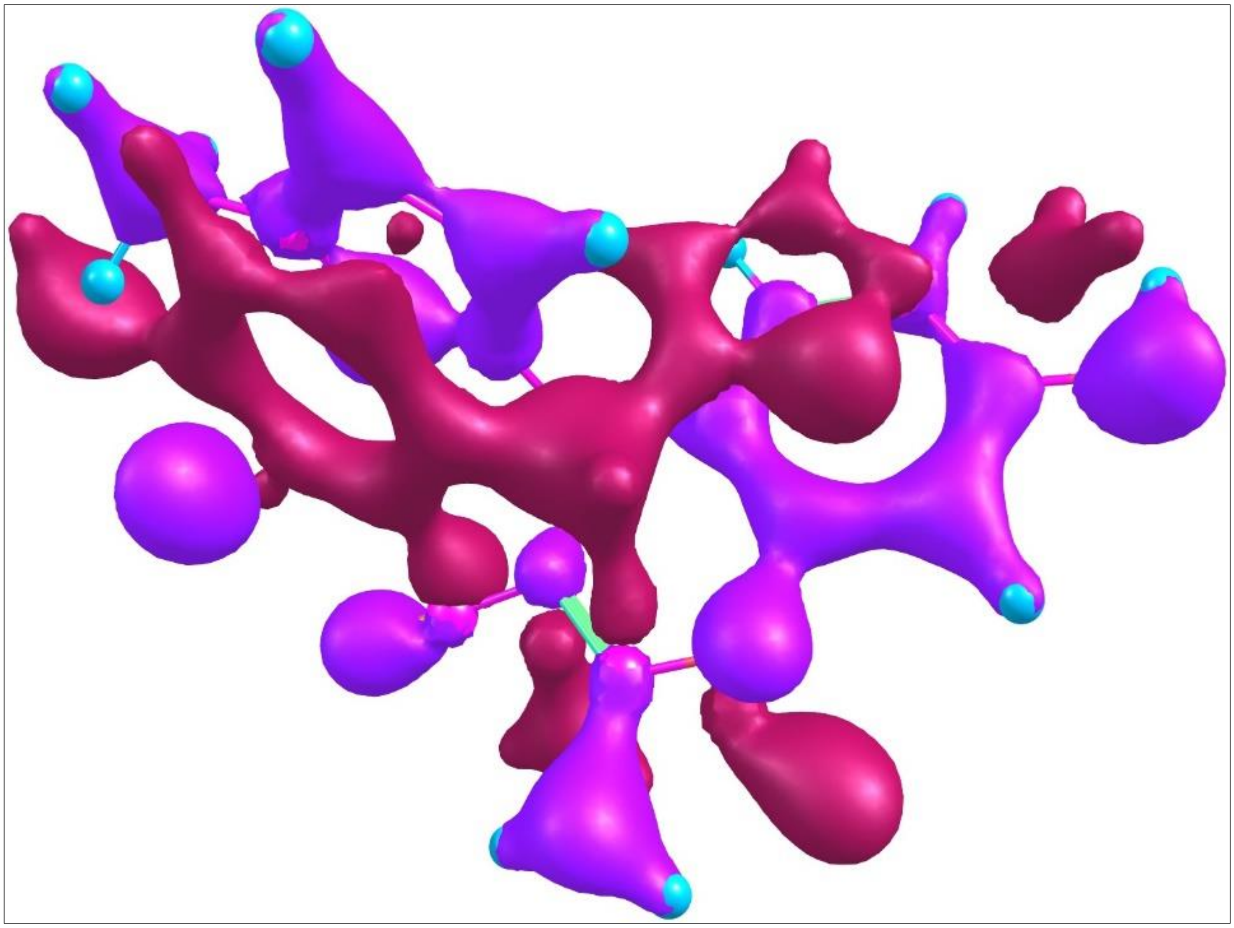

Figure S18. Pictorial representation of EDD analysis of **CFN**-Ca^{2+}

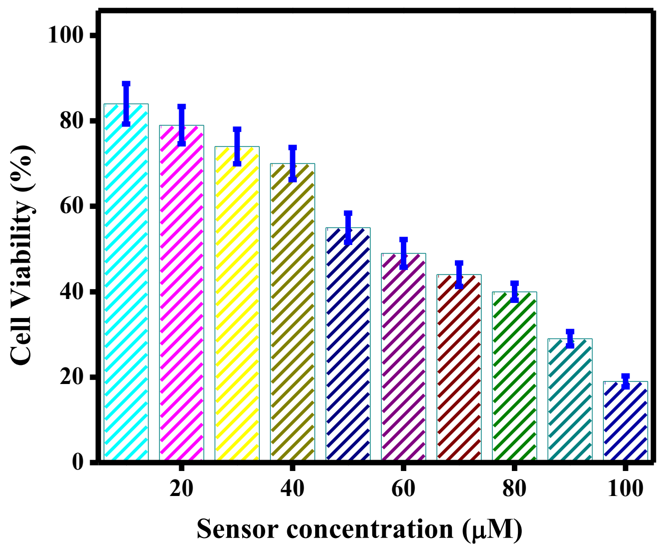

Figure S19. Cell viability assay at varying concentrations of sensor **CFN**

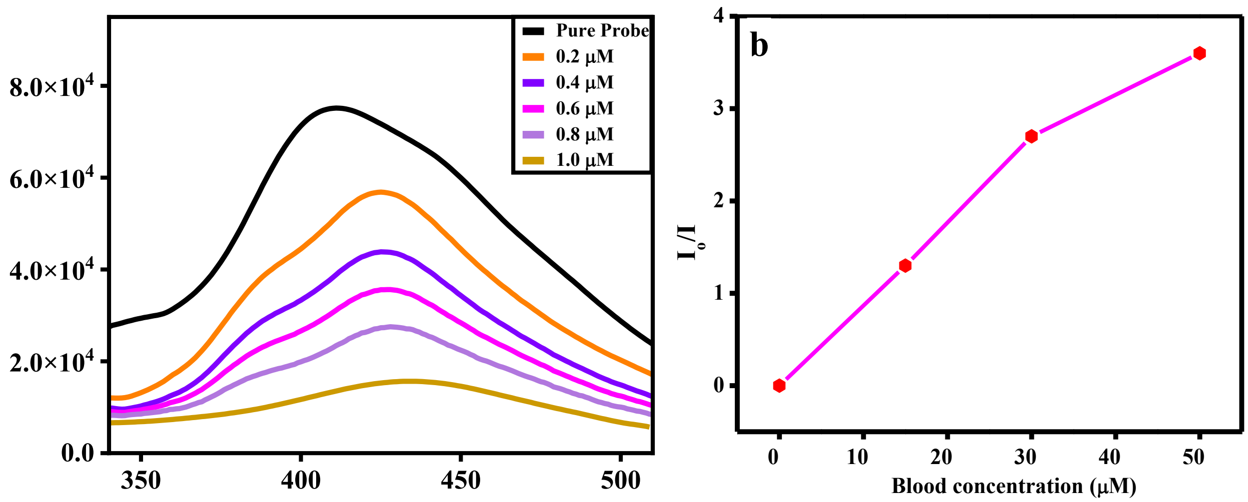

Figure S20. Fluorescence sensing spectra collected after the sequential treatment against different human plasma samples.

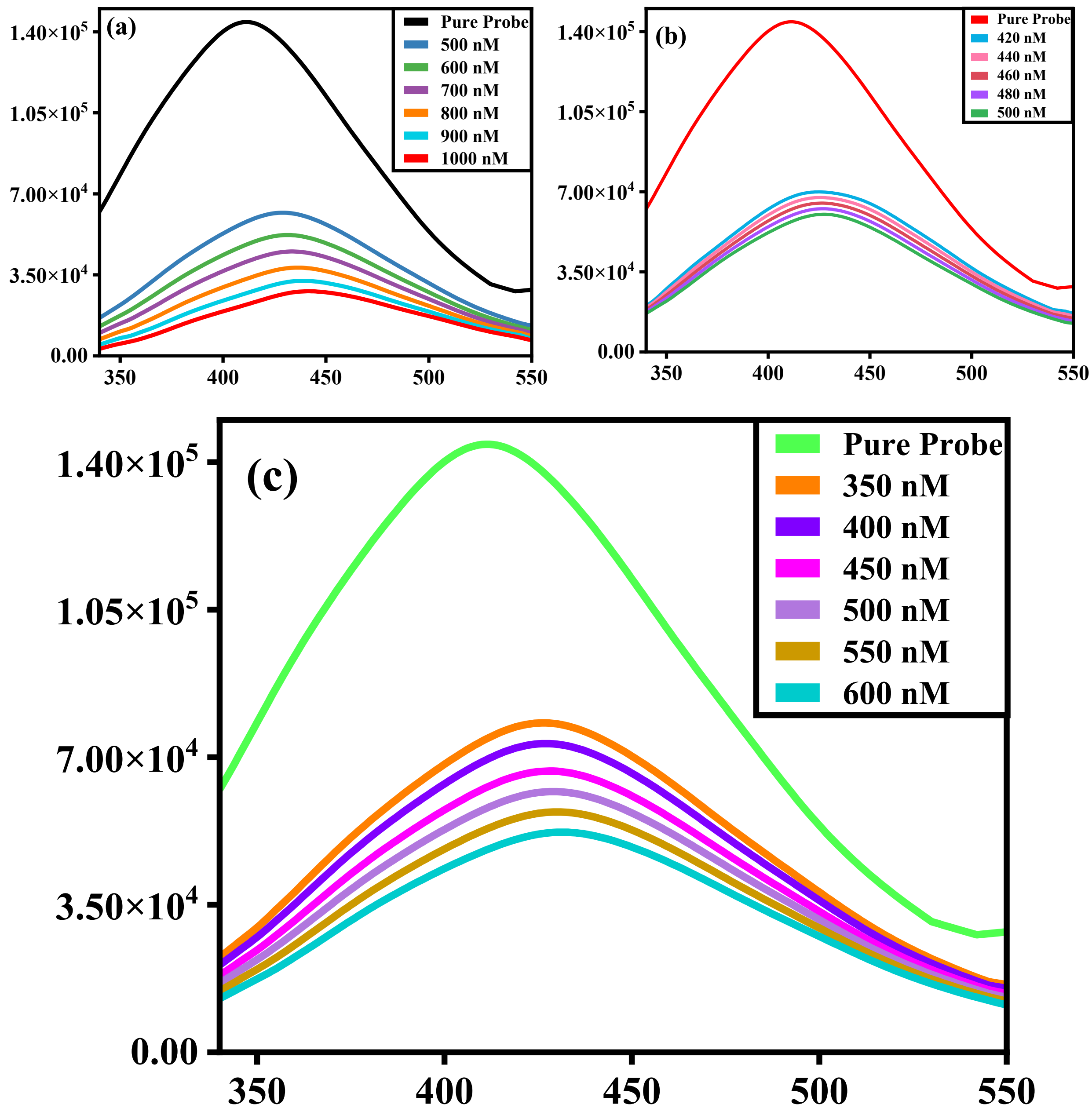

Figure S21. Spike recovery-based fluorescence analysis of 30 μM sensor **CFN** against Ca^{2+} in milk sample (a) river water (b) and tap water (c) samples.

Table S1. Comparison of our proposed sensor **CFN** against previously reported work

Reported sensors	**Solvato-chromism**	**AIEE**	**H-aggregates**	**DFT studies**	**Multi-response**	**LOD**	**Linear range**	**Binding Constant**	**Ref.**
Our proposed sensor **CFN**	Reported	Reported	Reported	Reported	Reported	10.5 nM	50 – 1000 nM	1.76×10^{-7} M-1	This work
Fluorescent ECDs biosensor	No	No	No	No	No	77 pM	0.077 – 10 nM	Not mentioned	3
Organelle-specific blue-emitting two-photon (TP) probes	No	No	No	No	No	0.137 μM	0.137 - 39 μM	Not mentioned	4
Simple fluorescence chemosensors	No	No	No	No	No	0.01 ng/mL^{-1}	0.5 – 2.5 μM	Not mentioned	5
Ca^{2+} selective fluoroionophore Bis- 2,2'-(1,2-phenylenebis nitrilomethylylidene) diphenol (DMK)	Yes	No	No	No	No	0.016 nM	$1.0 \times 10^{-10} – 1.0 \times 10^{-4}$ M	14.4 nM	6
PBTMA/CPPs sensor	No	No	No	No	No	8.4 μM	0.01 – 3.0 mM	Not mentioned	7
"Turn-on" fluorescent probe detection of Ca^{2+} ions	No	No	No	No	No	2.70×10^{-7} M	Not mentioned	1.03×10^{5} M^{-1}	8

Fluorescent chemosensor 1 based on a coumarin-modified rhodamine derivative	No	No	No	No	No	0.03 μM	Not mentioned	Not mentioned	9
Coumarin-3-carboxamide derivatives	No	No (Quenching reported)	No	Yes	Yes	1.21 μM	0.1 – 25 μM	4.8×10^4 M^{-1}	10
CdS quantum dots (QDs) with hexametaphosphate	No	No	No	No	yes	4 μmol/L	10 – 400 μM	Not mentioned	11

Table S2. Photophysical properties of reported sensor **CFN**

Solvents polarizability (Δ*f*)		**CFN**		
Solvent	Δf*	λ_{abs} (nm)	λ_{em} (nm)	Φ* (%)
ACT	0.21	352.5	373	36
EtOAc	0.22	348	409	37
ACN	0.28	352	370	40
MeOH	0.29	352	411	43
DMF	**0.31**	**356**	**476**	**44**
DMSO	0.33	352	462	45

Table S3. QTAIM analysis metrics calculated for **CFN**-Ca^{2+} complex

BCPs	CFN-Ca2+	ρ(r) (a.u)	$\nabla^2\rho(r)$ (a.u)	G(r) (a.u)	V(r) (a.u)	H(r) (a.u)	-V/G	E_{int} (kcal/mol)
1	H…Ca	0.0171	0.091	0.019	-0.016	-0.00307	0.84	-5.02
2	Ca…N	0.0158	0.081	0.017	-0.014	0.00313	0.82	-4.39
3	O…Ca	0.0149	0.081	0.016	-0.013	-0.00331	0.81	-4.07

Table S4. Recovery measurements of analysis of Ca^{2+} in river water samples.

Sr. No.	Spiked (nM)	Recovered (nM)	Recovery (%)	RSD % n=3
1	420	434	103.3	1.21
2	440	449	102	1.09
3	460	471	102.3	1.23
4	480	504	105	2.29
5	500	519	103.8	1.18

Table S5. Recovery measurements of analysis of Ca^{2+} in tap water samples.

Sr. No.	**Spiked (nM)**	**Recovered (nM)**	**Recovery (%)**	**RSD % n=3**
1	350	368	105.1	1.67
2	400	439	109.7	1.56
3	450	469	104.2	1.54
4	500	521	104.2	1.61
5	550	574	104.3	1.47
6	600	633	105.5	2.33

Table S6. Recovery measurements of analysis of Ca^{2+} in milk samples.

Sr. No.	**Estimated (nM)**	**Recovered (nM)**	**Recovery (%)**	**RSD % n=3**
1	500	540	108	1.44
2	600	647	107.8	1.6

3	700	762	108.8	2.37
4	800	849	106.1	1.38
5	900	989	109.8	1.53
6	1000	1087	108.7	1.31

Table S7 The truth table of logic gates designed for Ca^{2+} sensing by **CFN**

IN-1 (**CFN**)	IN-2 (Ca^{2+})	Out (Above 420 nm)
1	0	1
0	1	1
0	0	1
1	1	0
1	0	1
0	1	1
0	0	1

Complete Reference for Gaussian 09 Software:

Gaussian 09, Revision D.01,M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega,J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo,R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma,V. G.

Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2013.

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11. S. Liu, H. Wang, Z. Cheng and H. Liu, *Sensors and Actuators B: Chemical*, 2016, **232**, 306-312.

1H NMR of Sensor CFN (DMSO-d6, 400 MHz)

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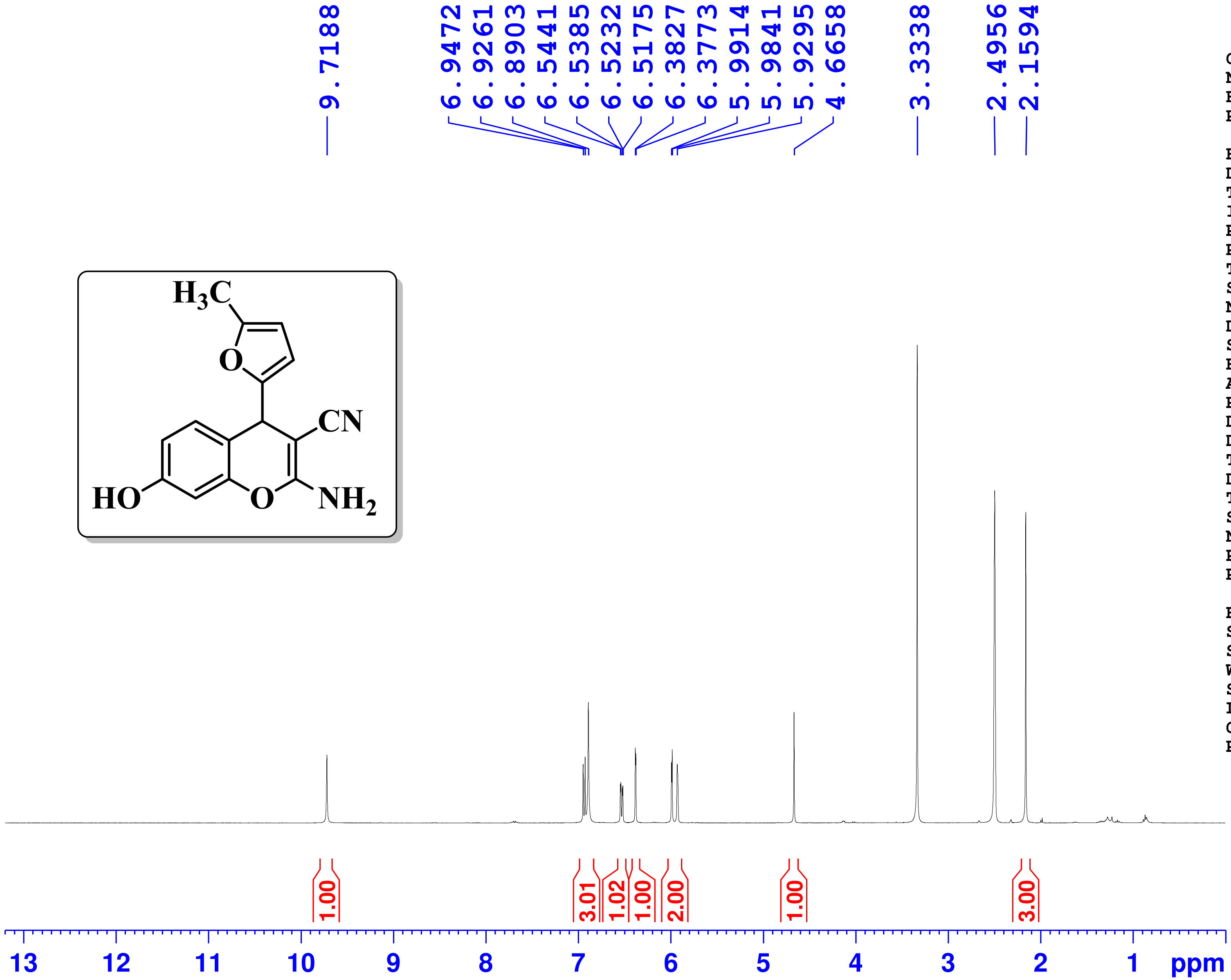

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1H NMR of Sensor CFN (DMSO-d6, 400 MHz)

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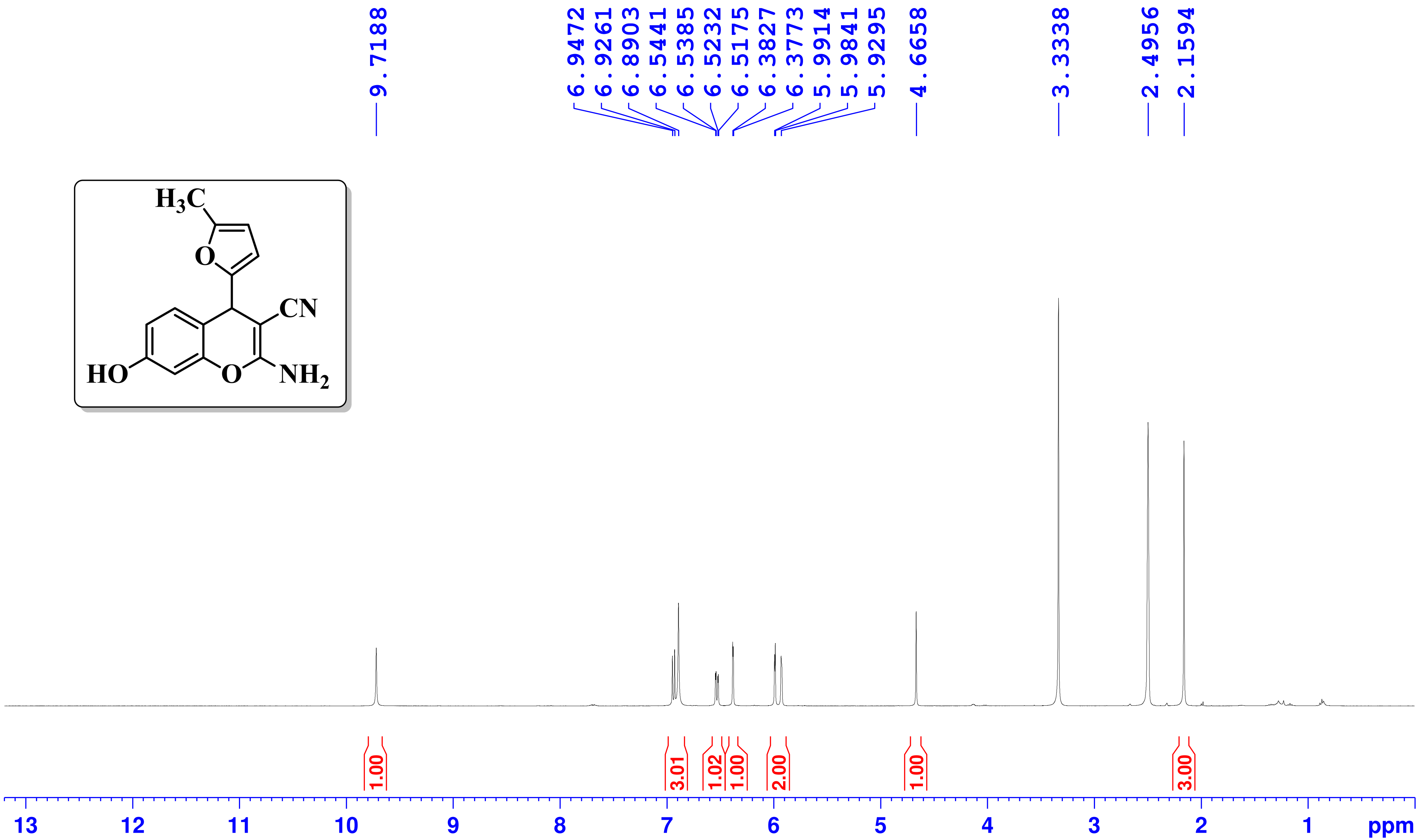

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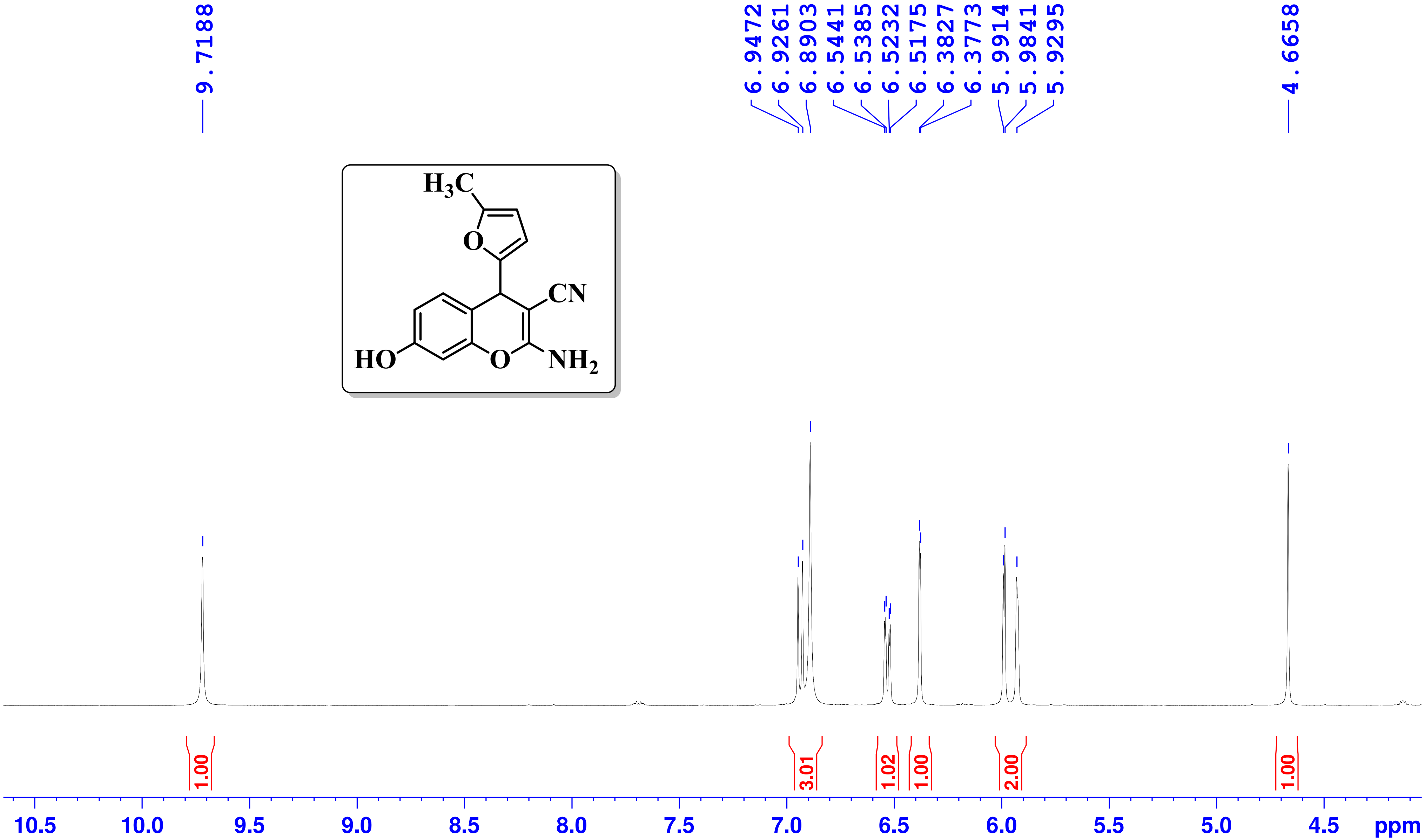

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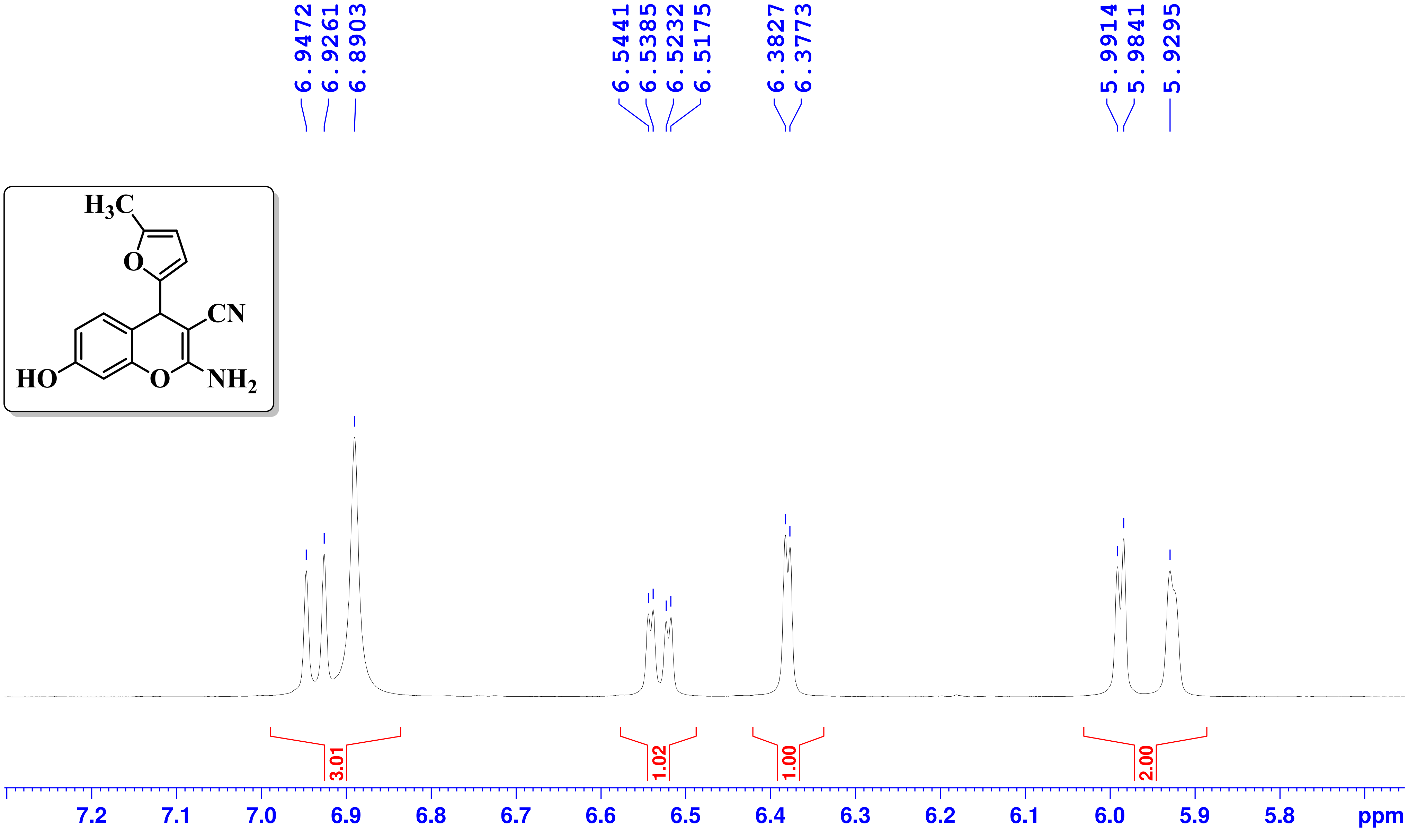

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1H NMR of Sensor CFN (DMSO-d6, 400 MHz)

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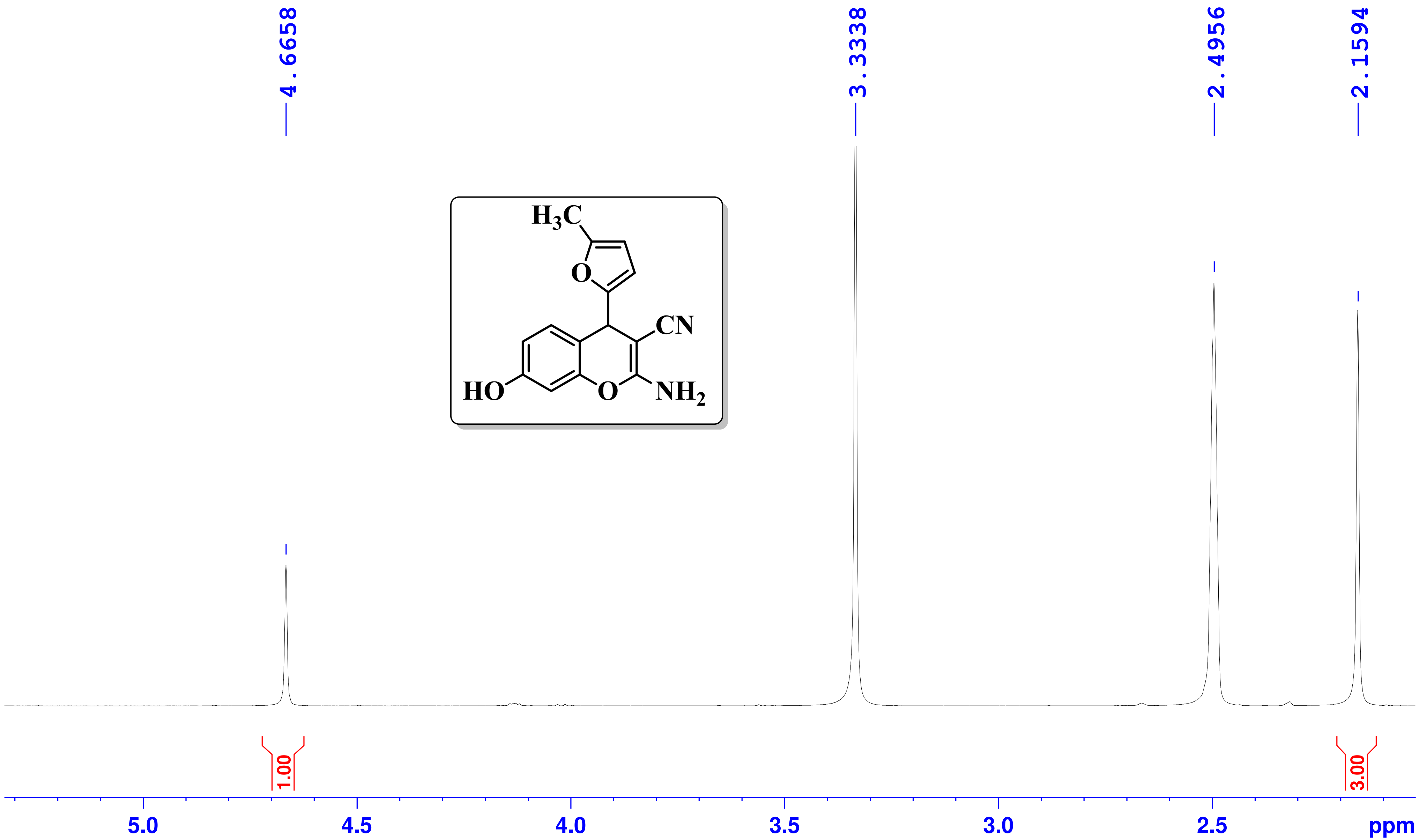

13C NMR of Sensor CFN (DMSO-d6, 100 MHz)

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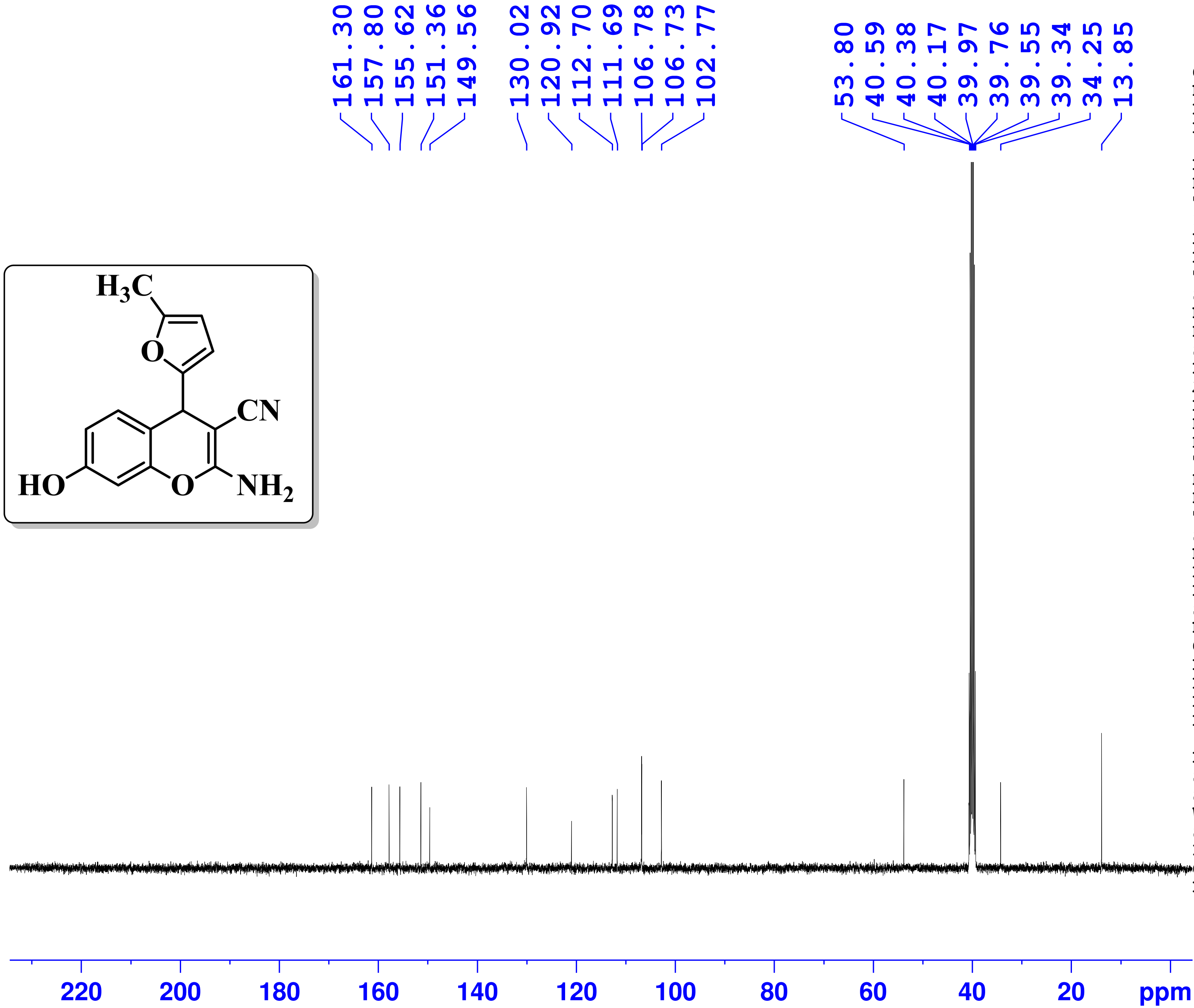

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F2 - Processing parameters
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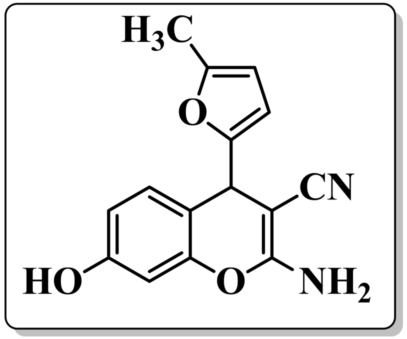
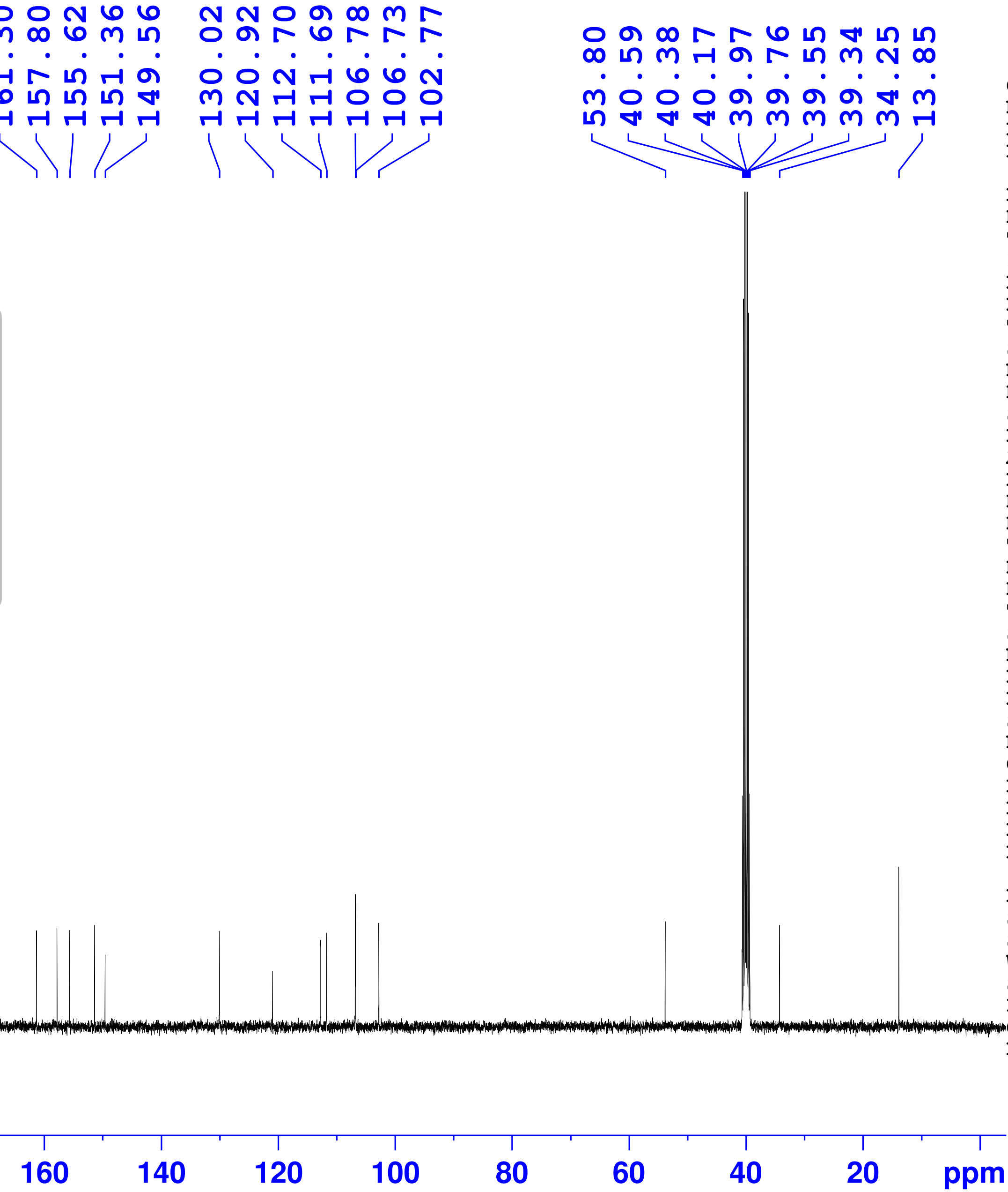
SF 100.6127685 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

13C NMR of Sensor CFN (DMSO-d6, 100 MHz)

KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)





Current Data Parameters	
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EXPNO	45
PROCNO	1
F2 - Acquisition Parameters	
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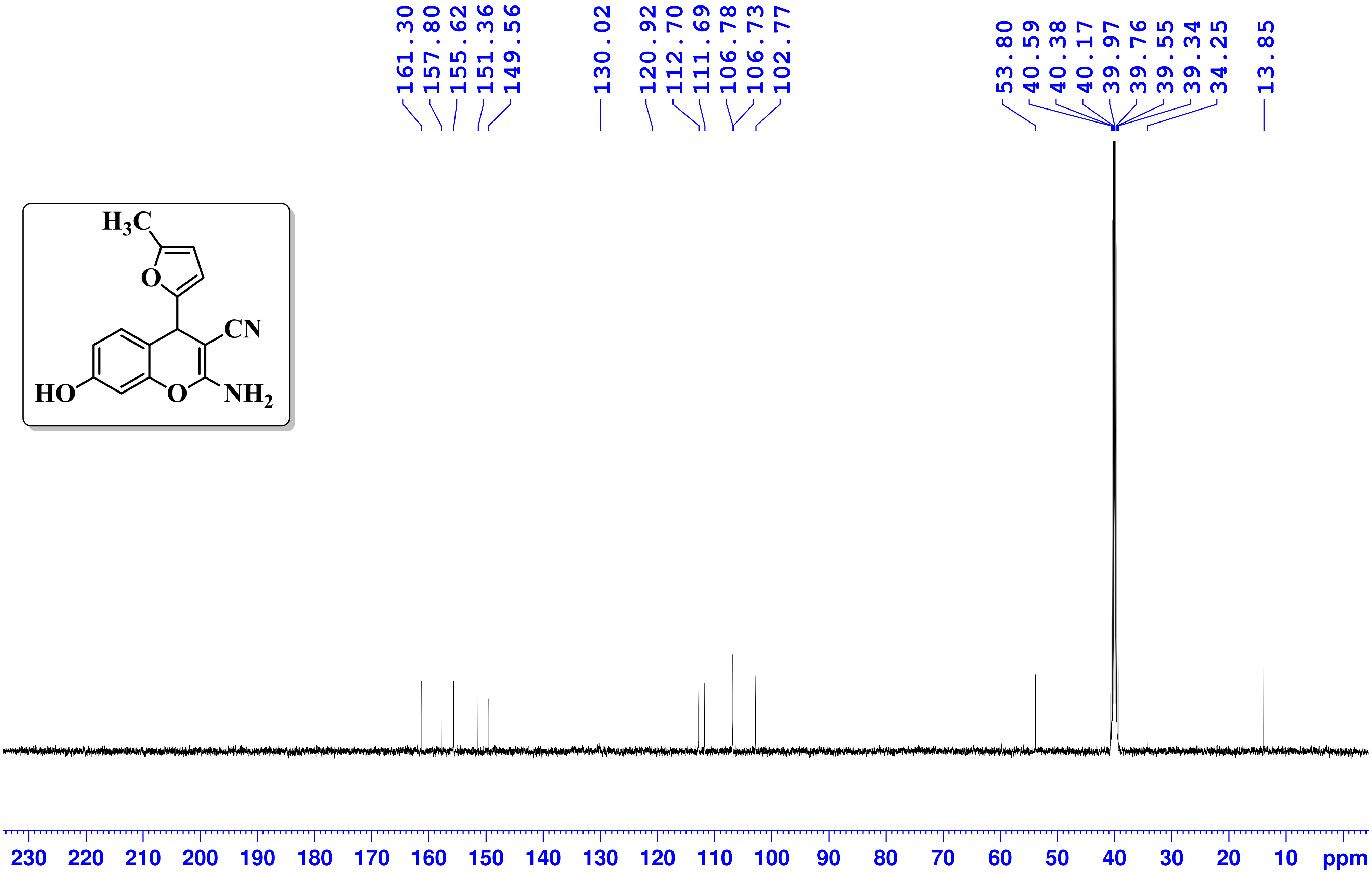
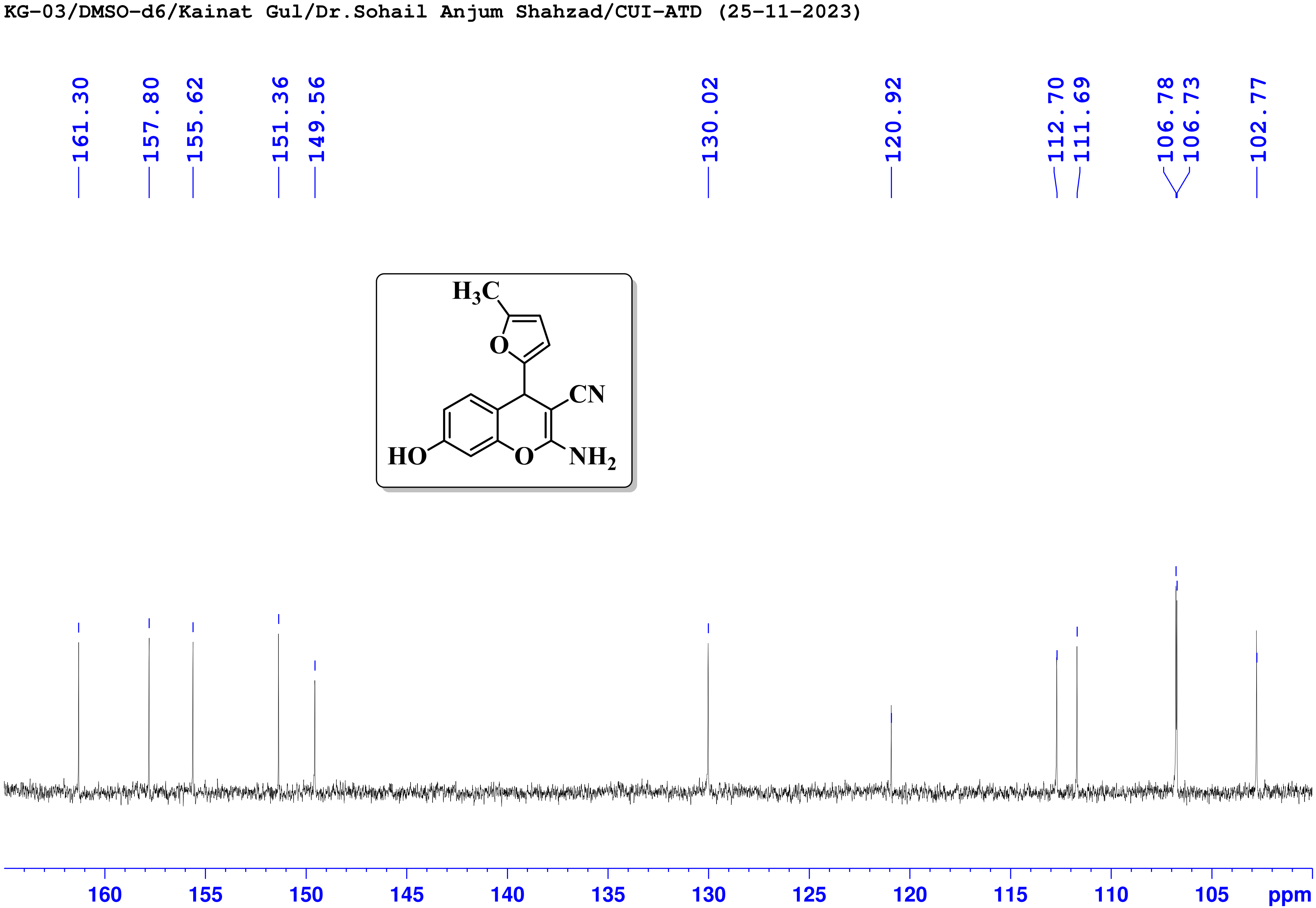
Time	19.11 h
INSTRUM	spect
PROBHD	Z116098_0621 (
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	400
DS	2
SWH	24038.461 Hz
FIDRES	0.733596 Hz
AQ	1.3631488 sec
RG	199.48
DW	20.800 usec
DE	6.50 usec
TE	298.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
SFO1	100.6243390 MHz
NUC1	13C
P1	10.00 usec
PLW1	72.56700134 W
SFO2	400.1316005 MHz
NUC2	1H
CPDPRG[2	waltz16
PCPD2	90.00 usec
PLW2	16.68099976 W
PLW12	0.20593999 W
PLW13	0.10342000 W
F2 - Processing parameters	
SI	32768
SF	100.6127685 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

13C NMR of Sensor CFN (DMSO-d6, 100 MHz)

KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)

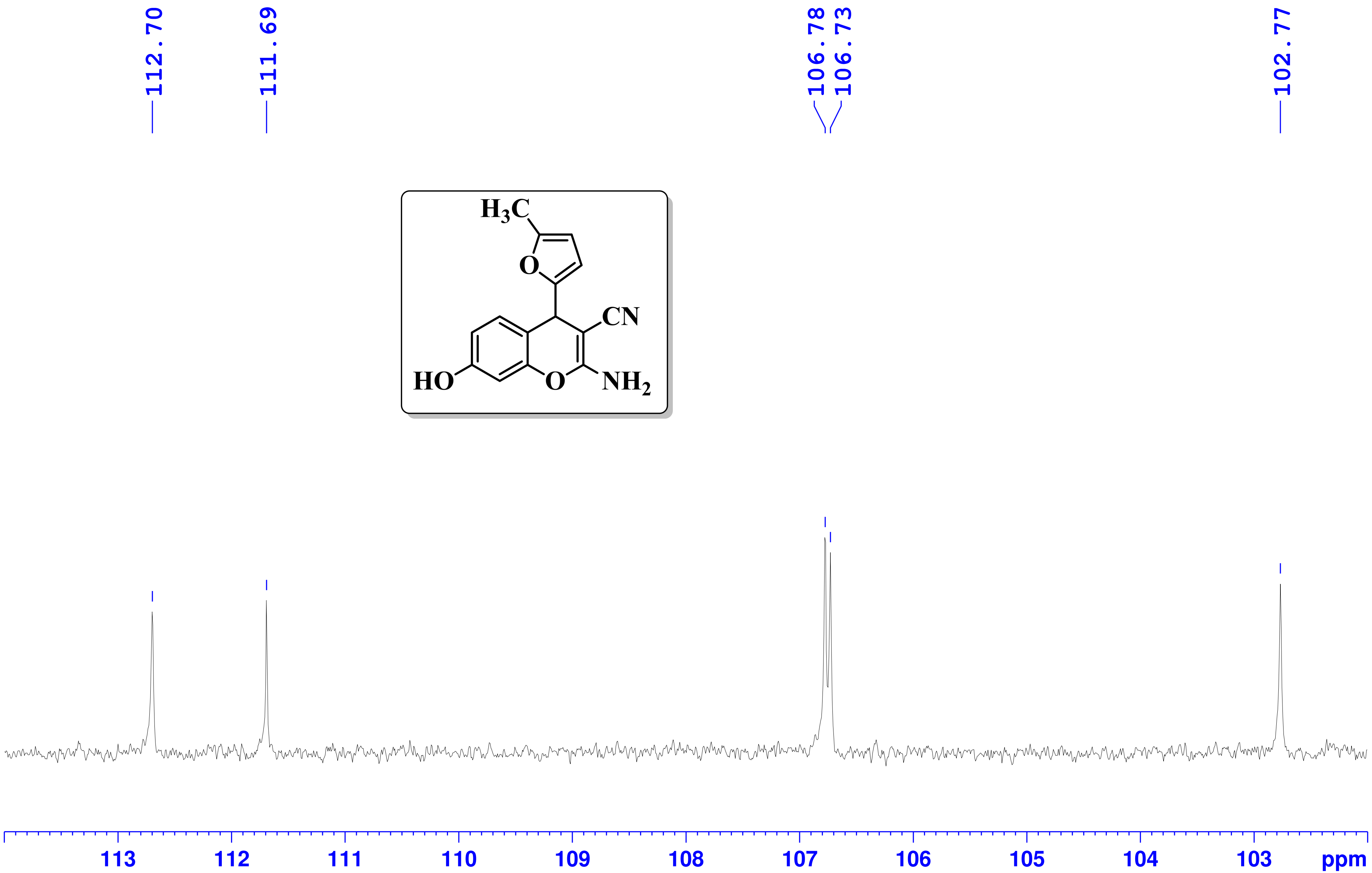


13C NMR of Sensor CFN (DMSO-d6, 100 MHz)



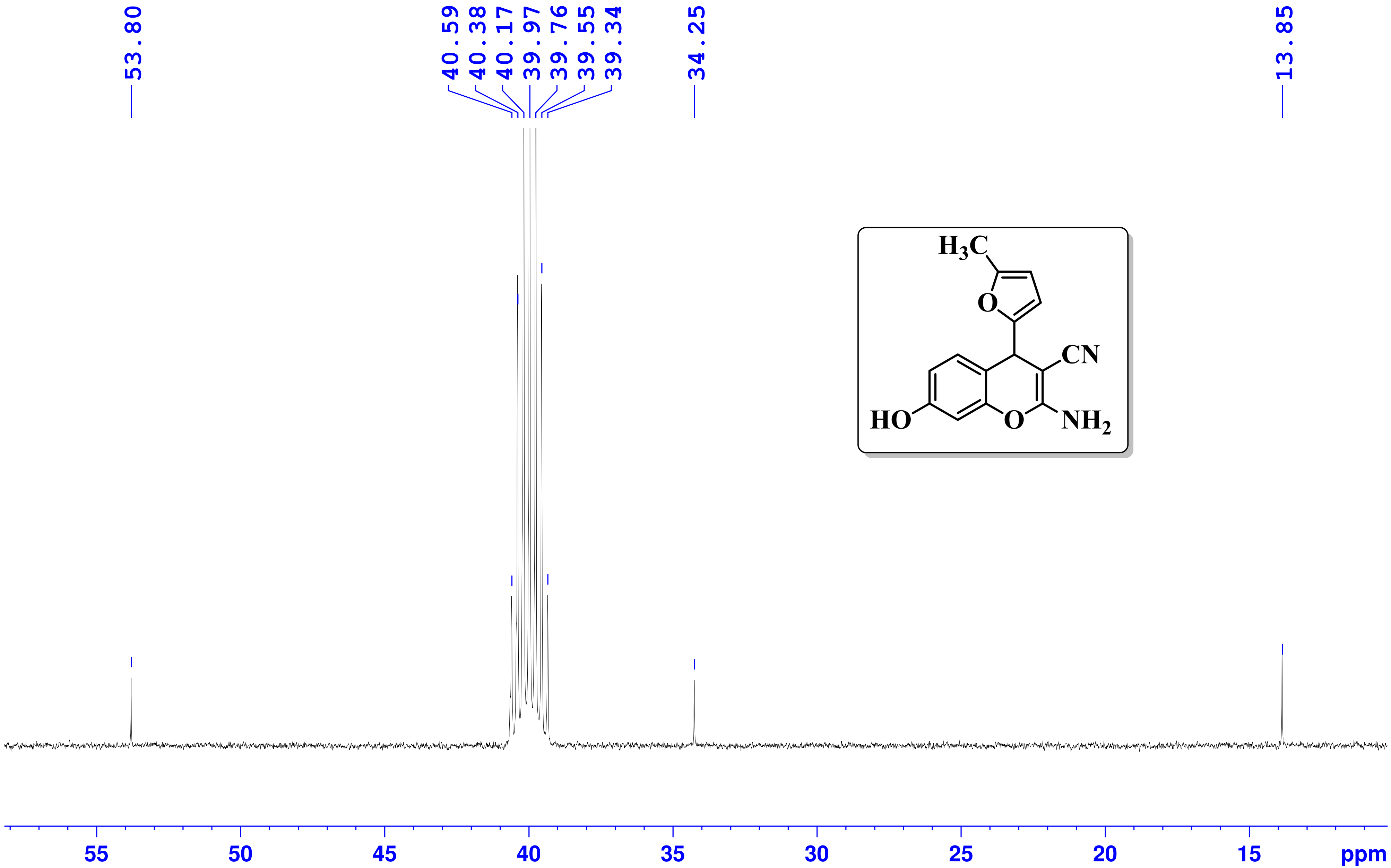
13C NMR of Sensor CFN (DMSO-d6, 100 MHz)

KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)



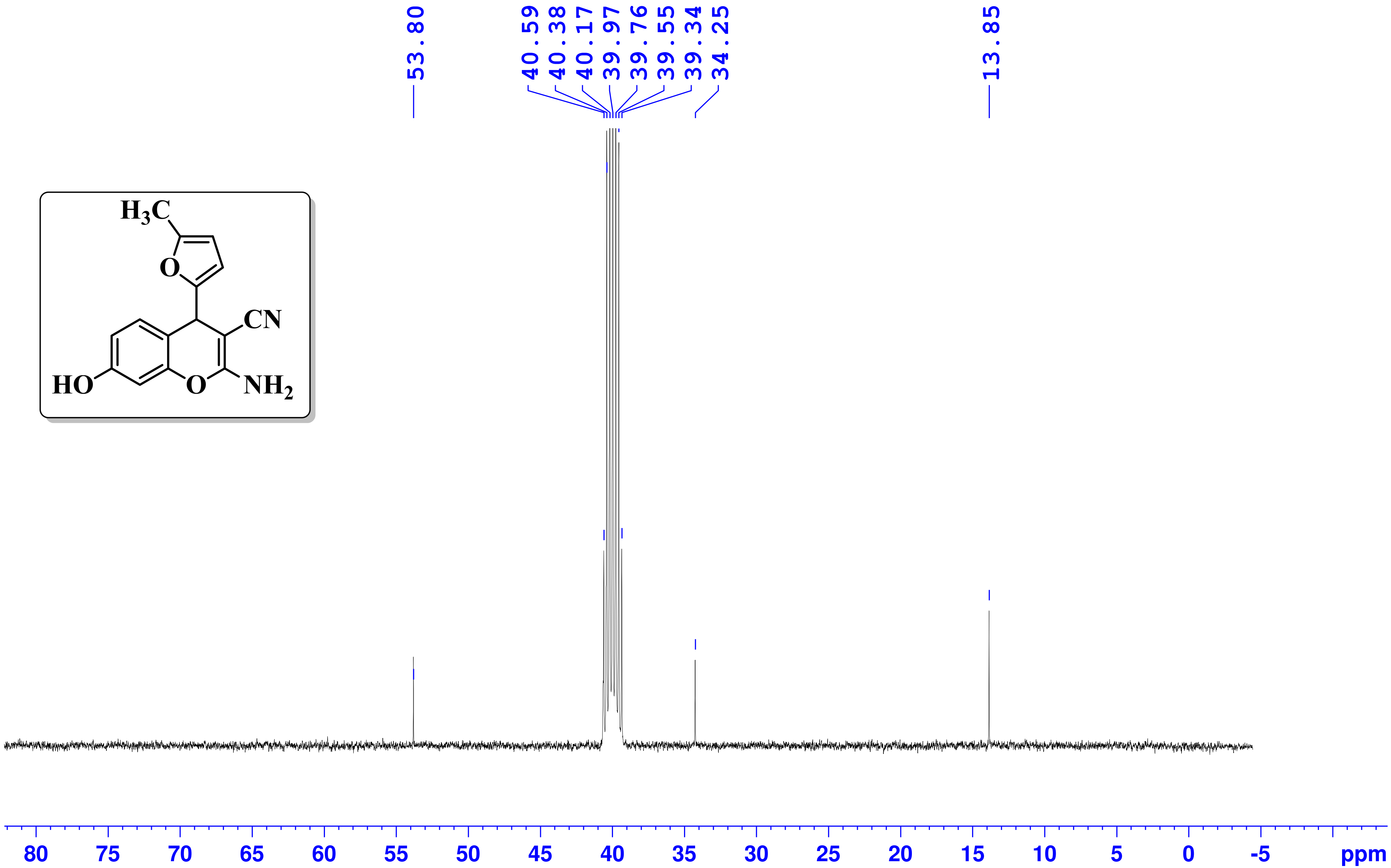
13C NMR of Sensor CFN (DMSO-d6, 100 MHz)

KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)



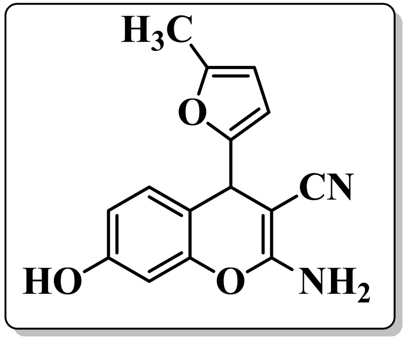
13C NMR of Sensor CFN (DMSO-d6, 100 MHz)

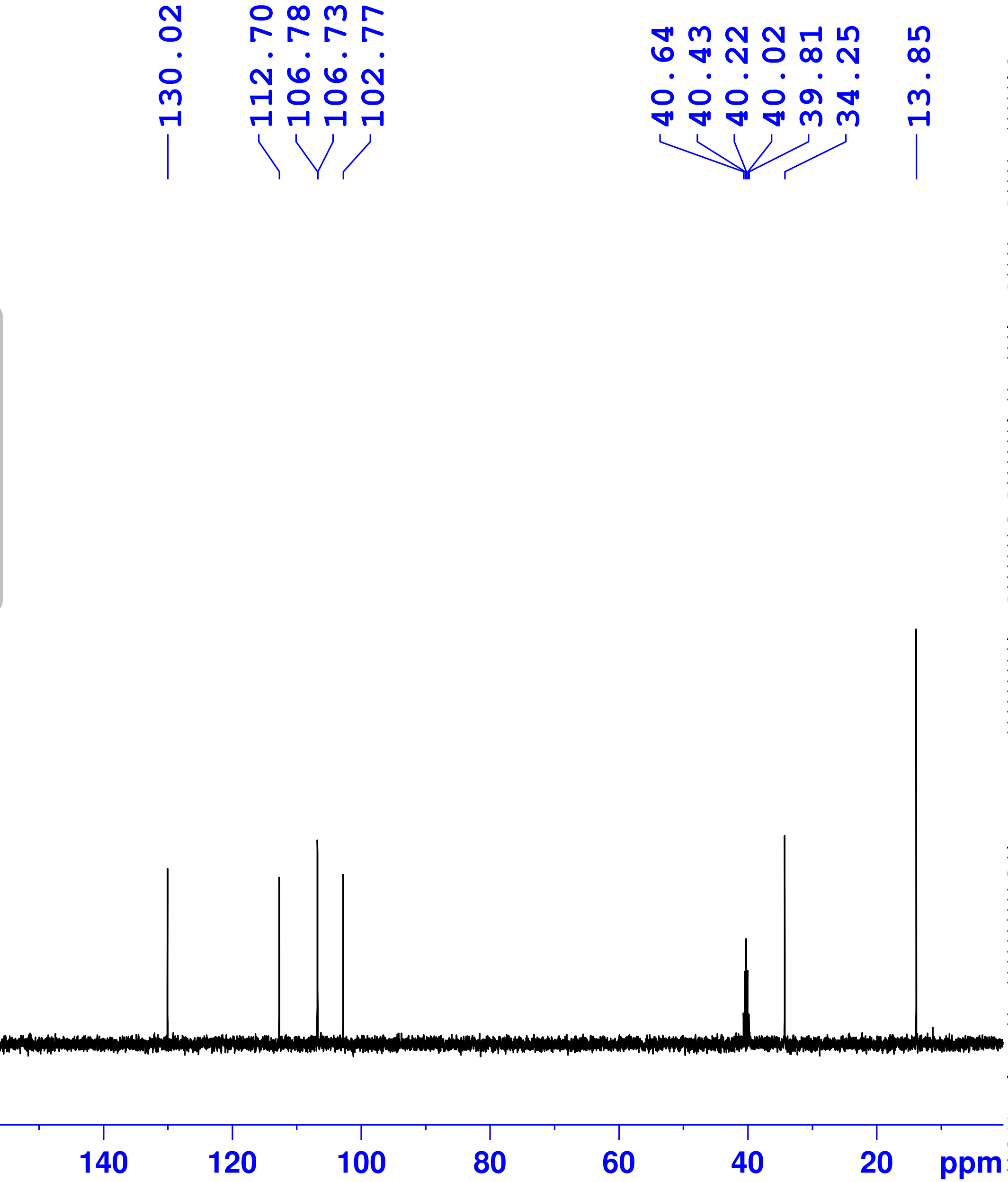
KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)



DEPT 135 NMR of Sensor CFN (DMSO-d6, 100 MHz)

KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)



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Current Data Parameters
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EXPNO                  46
PROCNO                  1

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Time                19.27 h
INSTRUM             spect
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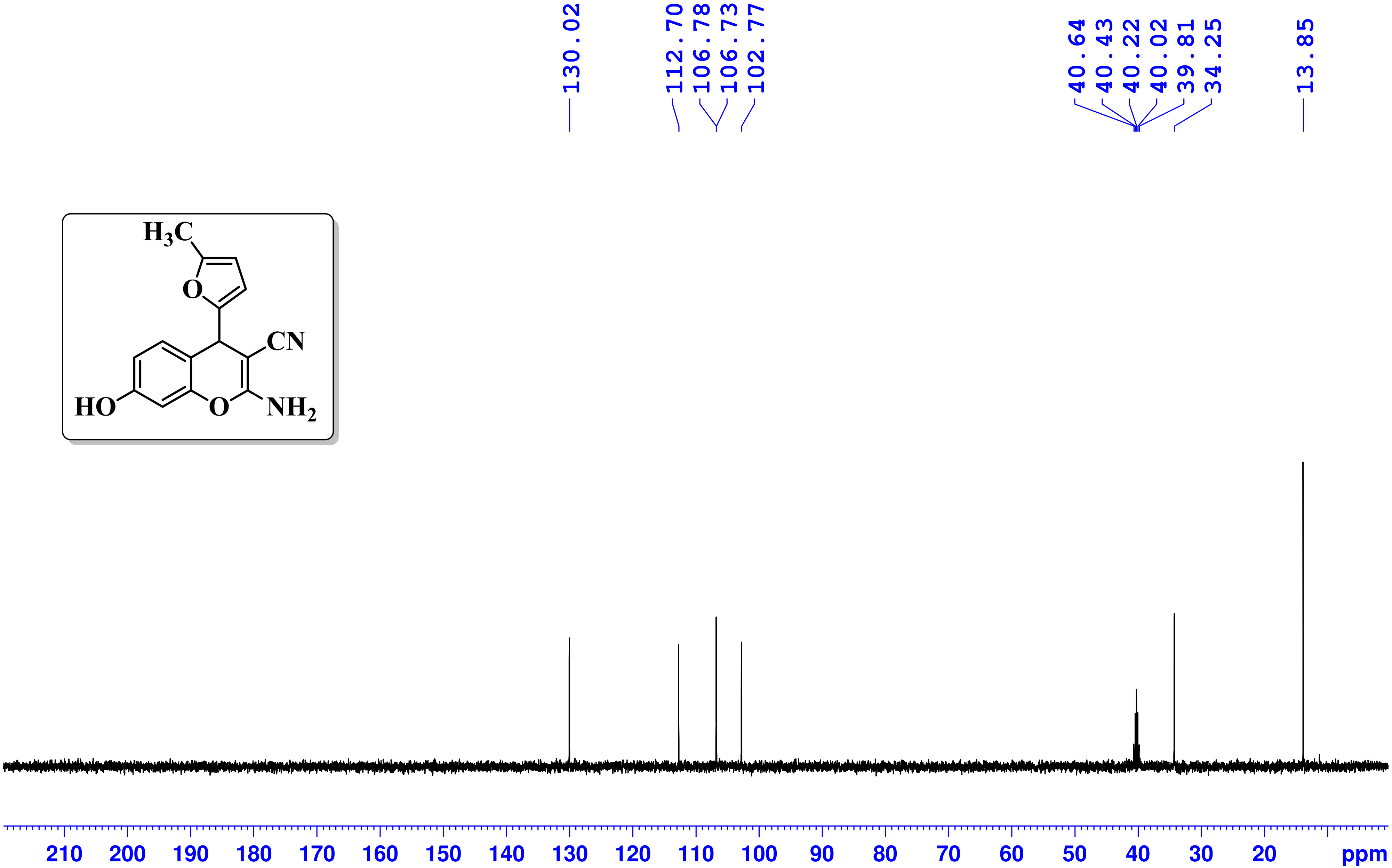
PULPROG         deptsp135
TD                  65536
SOLVENT              DMSO
NS                    256
DS                      4
SWH             22058.824 Hz
FIDRES           0.673182 Hz
AQ              1.4854827 sec
RG                 199.48
DW                 22.667 usec
DE                   6.50 usec
TE                  298.0 K
CNST2         145.0000000
D1             2.00000000 sec
D2             0.00344828 sec
D12            0.00002000 sec
TD0                     1
SFO1          100.6238359 MHz
NUC1                  13C
P1                  10.00 usec
P13               2000.00 usec
PLW0                    0 W
PLW1          72.56700134 W
SPNAM[5]     Crp60comp.4
SPOAL5              0.500
SPOFFS5                 0 Hz
SPW5          11.08699989 W
SFO2          400.1316005 MHz
NUC2                   1H
CPDPRG[2          waltz16
P3                  10.00 usec
P4                  20.00 usec
PCPD2               90.00 usec
PLW2          16.68099976 W
PLW12          0.20593999 W

F2 - Processing parameters
SI                  32768
SF            100.6127685 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```

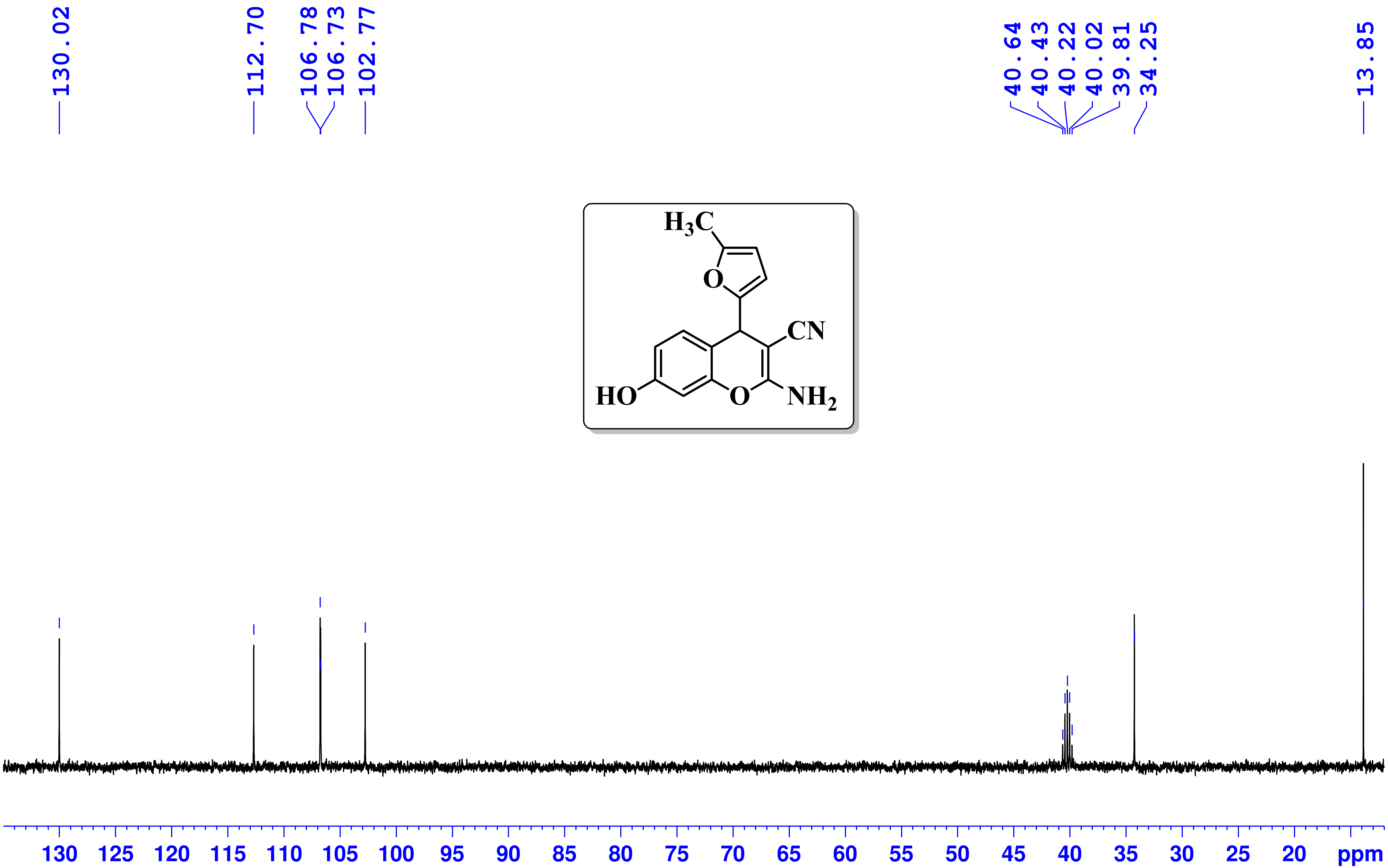
DEPT 135 NMR of Sensor CFN (DMSO-d6, 100 MHz)

KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)



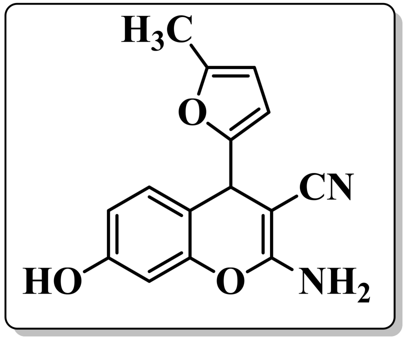
DEPT 135 NMR of Sensor CFN (DMSO-d6, 100 MHz)

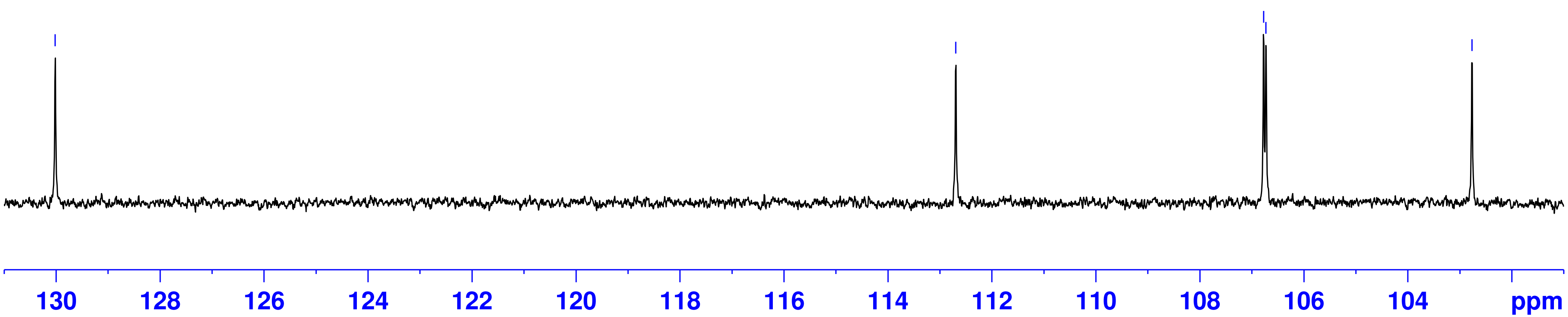
KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)



DEPT 135 NMR of Sensor CFN (DMSO-d6, 100 MHz)

KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)

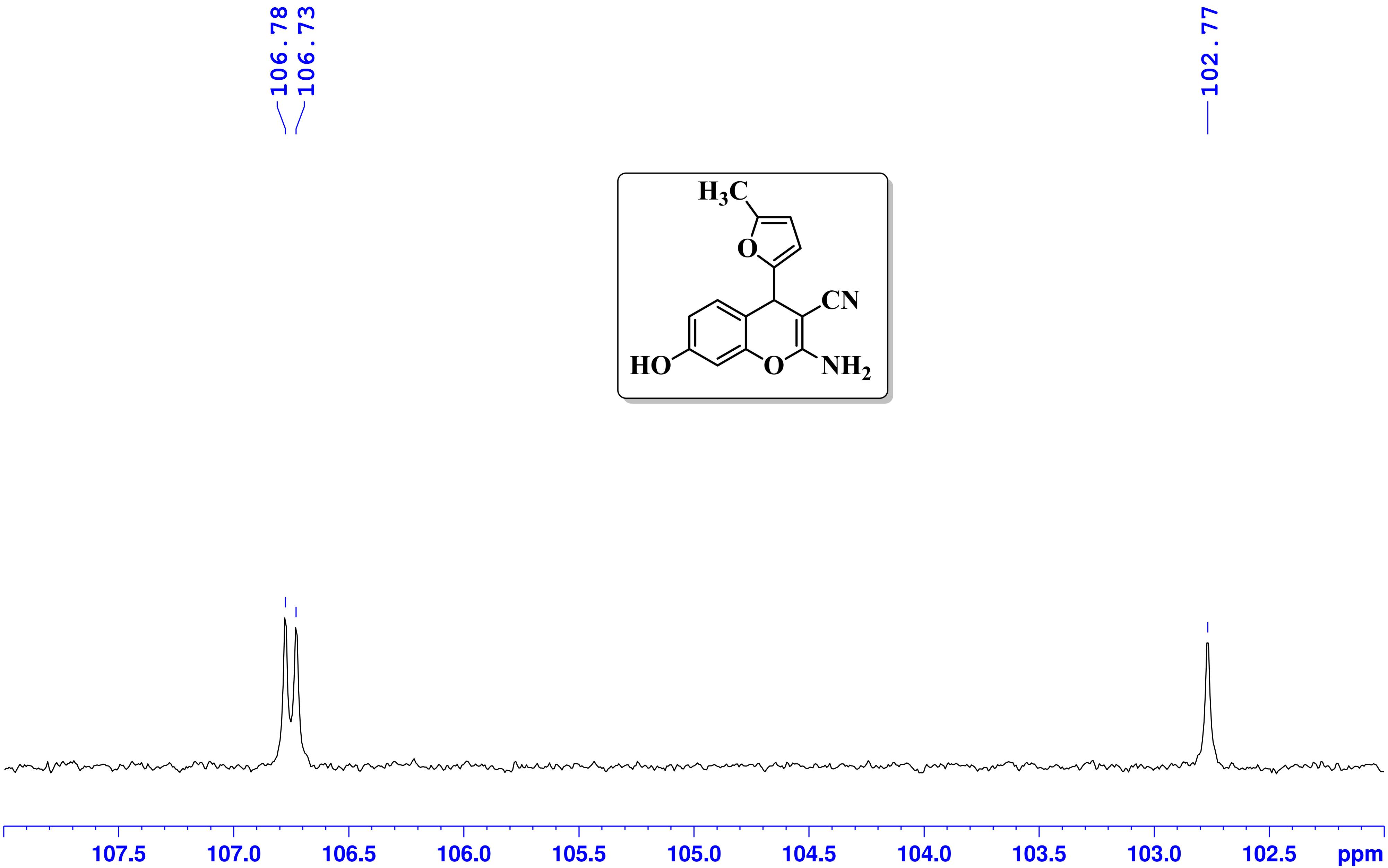




S40

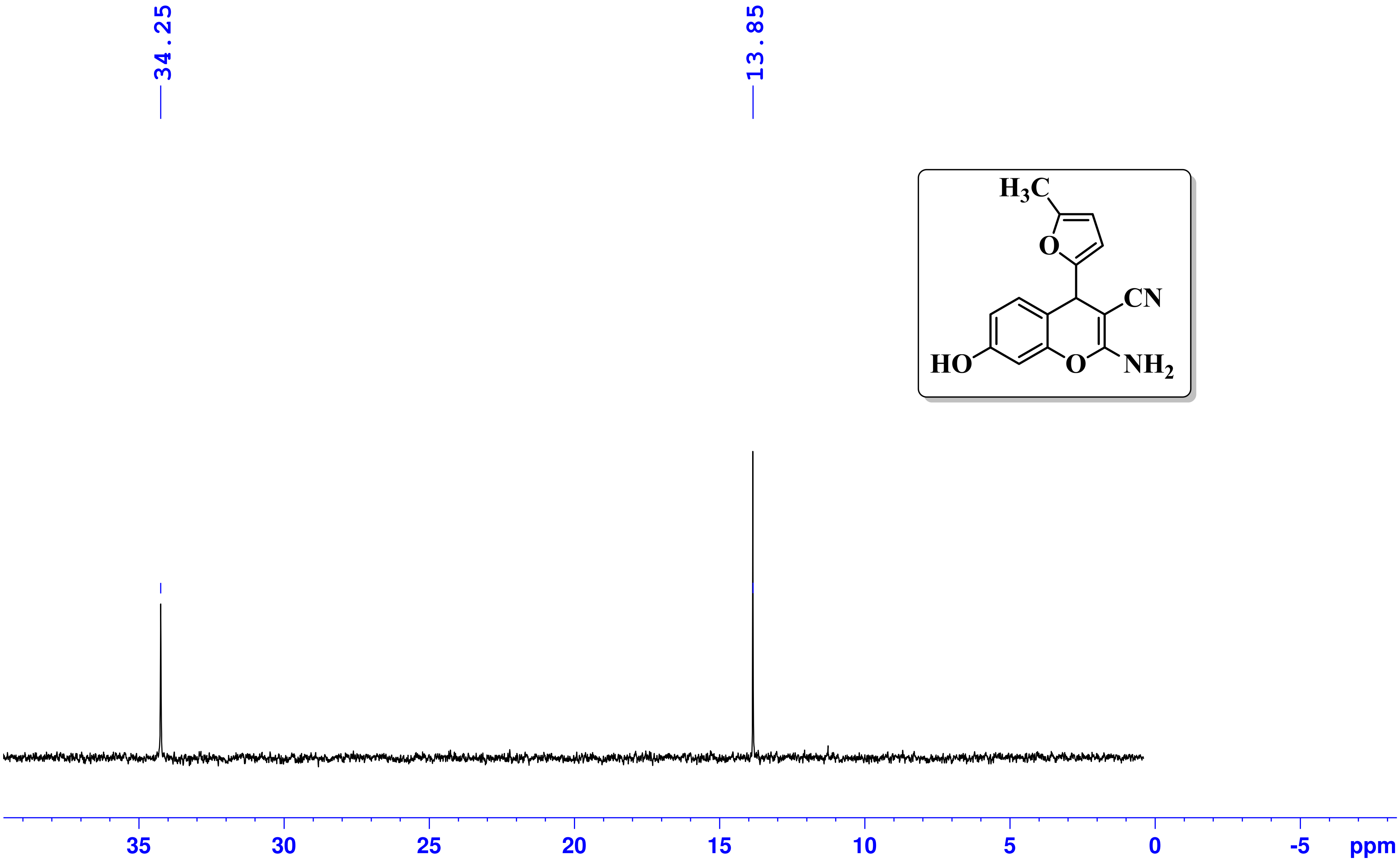
DEPT 135 NMR of Sensor CFN (DMSO-d6, 100 MHz)

KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)

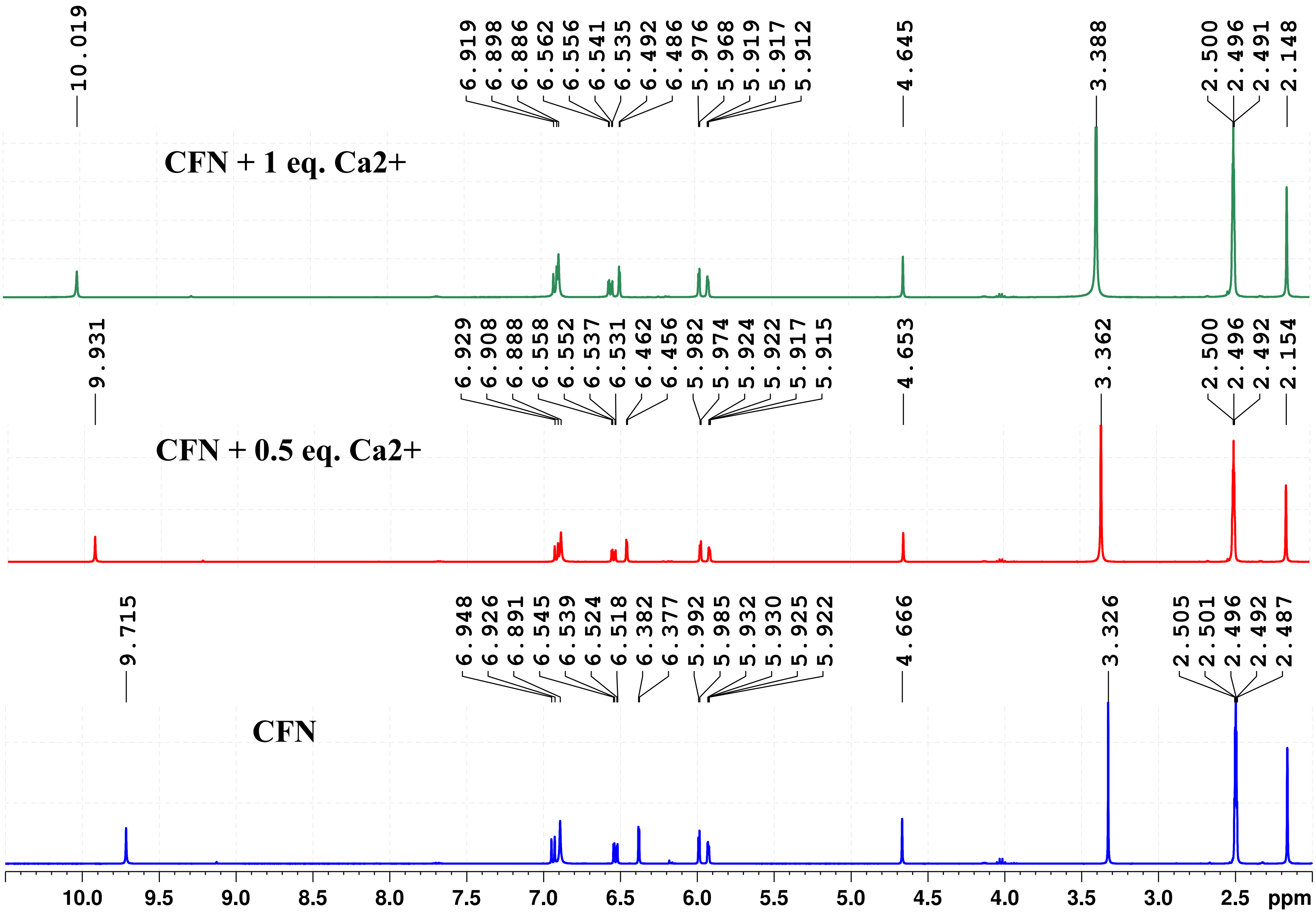


DEPT 135 NMR of Sensor CFN (DMSO-d6, 100 MHz)

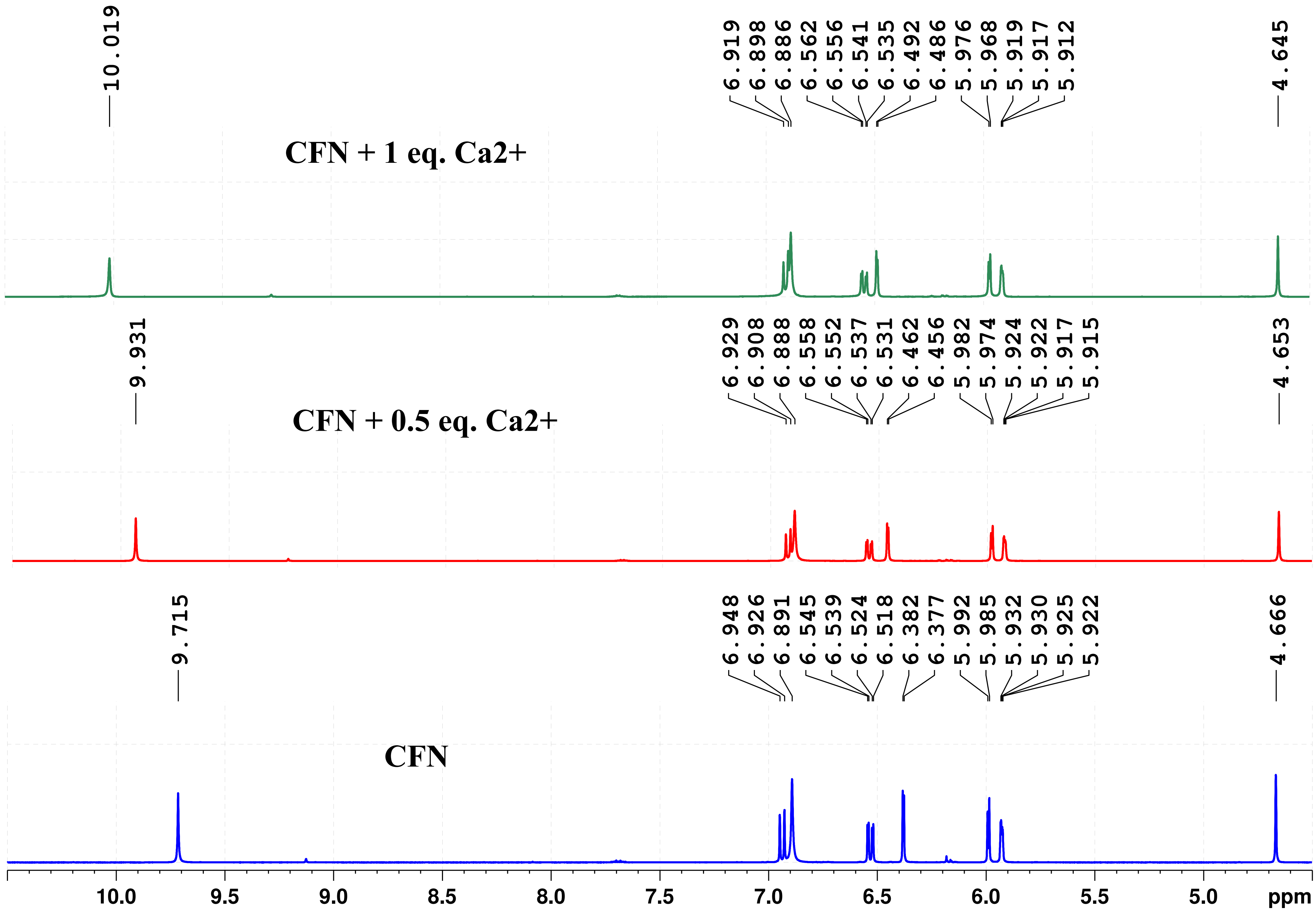
KG-03/DMSO-d6/Kainat Gul/Dr.Sohail Anjum Shahzad/CUI-ATD (25-11-2023)



1H NMR Titration of Sensor CFN (DMSO-d6, 400 MHz)



1H NMR Titration of Sensor CFN (DMSO-d6, 400 MHz)



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1H NMR Titration of Sensor CFN (DMSO-d6, 400 MHz)