Electronic Supplementary Material (ESI) for New Journal of Chemistry.

7-azaspiroketal as a Unique and Effective Auxochrome Moiety: Demonstration in a Fluorescent Coumarin Dyes and Application in Cell Imaging

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1. Supplemental Experimental Procedure

Materials and instruments

All chemicals were purchased from Sigma-Aldrich, TCI Chemicals, SRL Chemicals, and Avra, and used as received. Molychem silica gel (60–120 mesh) was used for column chromatography, and thin-layer chromatography was performed on Merck pre-coated silica gel 60-F254 plates. All other chemicals and solvents were obtained from commercial sources and purified using standard methods. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance spectrometers. Fluorescence data were recorded on a Horiba Scientific FluoroMax-4. Data are represented as follows: chemical shift, integration, multiplicity (br = broad, s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, pent = pentet and m = multiplet), and coupling constants in hertz (Hz).

Photophysical Characterization: UV absorbance was measured on Agilent Cary UV 60 UV-Visible Spectrophotometer. Molar absorption coefficients (ε) were determined by direct application of the Beer−Lambert's law using solutions of compounds **7** to **11** in PBS (1X) with concentrations ranging from 10^{-6} to 10^{-5} M. Fluorescence data were recorded on a Horiba Scientific FluoroMax-4. Solvent used for measuring fluorescence spectra: PBS, 1X (SRL Chemicals). GraphPad Prism ver. 7.0a (GraphPad Software, Inc.) and Origin ver. 6.0 were used to analyse data and generate graphs.

Absolute Quantum Yield Measurement: Absorbance spectra were recorded for each sample at a single concentration with an absorbance ranging between 0.07-0.09. The absolute quantum yields (QYs) of the synthesized compounds were obtained with the use of a PTI K – Sphere petite Integrating sphere and quantum yield calculations were done through Horiba PTI Fluorescence Quanta Master 400 Systems. For evaluating the quantum yield of different sample, the quartz cuvette located at the centre of the integrating sphere filled with different sample solutions (in 1X PBS) to ensure the maximum interaction of light with the sample regardless of its direction or angle. The integrating sphere allows the collection of all the light emitted and scattered by the fluorescent sample. Samples were excited with different excitation with help of 75 W Xe PTI arc lamp housing (A-1010B).

Lyophilized solubility assay (LYSA): Compounds are prepared in duplicate from MeOH stock solution (10 mM). For one portion, after evaporation of MeOH, dyes are dissolved in water (pH 7.2), stirred, and shaken for 1-2 h. The solution was allowed to stand for about 20

h and filtered before UV analysis. The other portion was used to prepare a six-point calibration curve by dilution of the MeOH stock solution using the same water mentioned above. This four-point calibration curve (R^2 > 0.99) is used for the solubility determination of the compounds. The results are in µg/mL.

Cell culture and conditions:

Breast cancer cell line (MDA-MB-231) was procured from the National Centre for Cell Science (NCCS), Pune, India. DMEM (Dulbecco's Modified Eagle Medium) and 12 well cell culture plate was purchased from Genetix Private Limited. The 96 well plates and T-25 flasks were purchased from Eppendorf. Penicillin-streptomycin, Trypsin-EDTA, and FBS (Fetal Bovine Serum) were purchased from Gibco. PBS (Phosphate Buffer Saline) was prepared in the laboratory. The MDA-MB-231 cells were cultured in DMEM, supplemented with FBS and penicillin-streptomycin solution and grown in a humidified CO_2 incubator at 37 °C.

***In vitro* Cytotoxicity assay:**

For analysis of the cytotoxicity of the compounds (**7**, **8**, **9** and **4**) against MDA-MB-231 cell line, cells were seeded in the density of 10,000 cells per well on a 96-well cell culture plate followed by overnight incubation for allowing the adherence of the cells. After 24 hours of incubation of the cells with different concentrations of the synthesized compounds (10 µM, 25µM, 50 µM, 75 µM and 100 µM), the media from each well was aspirated and replaced with fresh media comprising of MTT-containing solution. The MTT-containing media was removed after 2 hours of incubation, followed by addition of 100 µl of DMSO in each well and incubation for another 30 minutes. The absorbance was determined at 570 nm using a multiplate reader

Intracellular uptake studies in cells and evaluation of light emission at different time intervals:

Intracellular uptake study was performed in MDA-MB-231 cell lines. Cells were seeded at the density of 0.5 x 10^5 cells per well in a 12-well plate, followed by overnight incubation for allowing the adherence of the cells. The cells were then treated with a 5 µM concentration of each treatment and then incubated. Prior to imaging, the media were aspirated, washed with chilled PBS, and images were captured *via* phase contrast microscope (400 X magnification) as well as fluorescence microscope, equipped with blue (λex: 357 nm; λem: 447–460 nm) and green channels (λex: 470 nm; λem: 510–542 nm) with a 3 s exposure. After the completion of

incubation period, fluorescent images were captured at different time intervals (0 minute, 15 minutes and 30 minutes).

Computational methods: Density functional theory (DFT) was performed using Gaussian 09. Geometry optimizations of the ground state were performed in water. These geometry optimizations employ the B3LYP functional and the 6-31+G (d, p) basis set.

2. Supplementary Data

Table 1 Absorption and emission wavelength of compounds **7**-**11** and **4** in various solvents.

C. No.	λ_{abs} (nm)[a]				λ_{em} (nm)[b]			
	DCM	PBS	EtOH	DMSO	DCM	PBS	EtOH	DMSO
7	360	345	340	360	420	460	440	440
8	310	330	325	320	420	470	440	440
9	360	340	330	360	420	460	440	440
10	360	340	330	360	420	460	440	440
11	360	350	340	360	430	470	450	450
4	360	350	370	370	430	470	450	445

[a]λabs = absorbance maxima in PBS, [b]λem = emission maxima in PBS

Intracellular uptake studies in cells at different time intervals

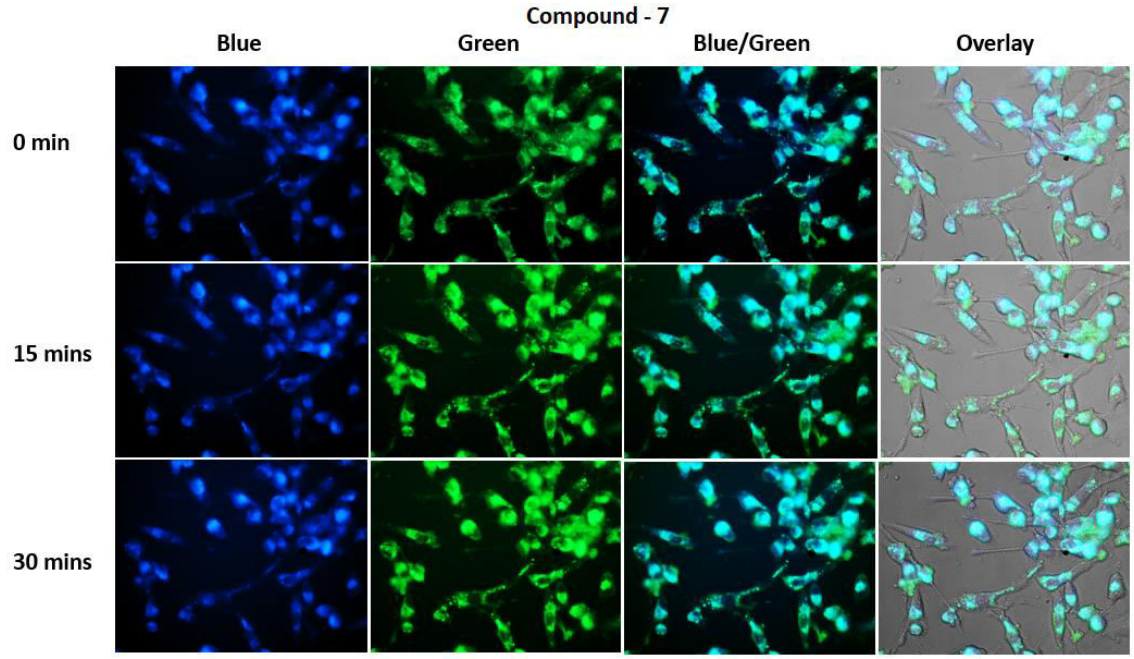


Figure S1: Fluorescent microscope images of MDA-MB-231 cells after incubation with dye **7** at a concentration of 5 μM at 37 ^{o}C. The images were captured after indicated incubation period by phase contrast microscope as well as fluorescence at 400 X magnification using blue (λex: 357 nm; λem: 447–460 nm) and green channels (λex: 470 nm; λem: 510–542 nm) with a 3 s exposure. Bar= 100 μm.

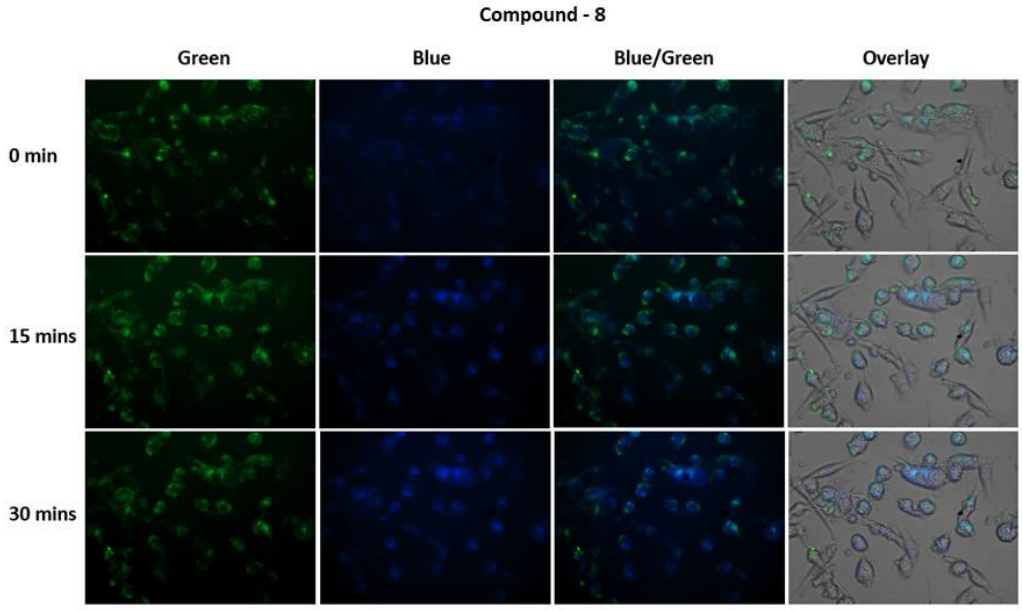


Figure S2: Fluorescent microscope images of MDA-MB-231 cells after incubation with dye **8** at a concentration of 5 μM at 37 ^{o}C. The images were captured after indicated incubation

period by phase contrast microscope as well as fluorescence at 400 X magnification using blue (λex: 357 nm; λem: 447–460 nm) and green channels (λex: 470 nm; λem: 510–542 nm) with a 3 s exposure. Bar = 100 μm.

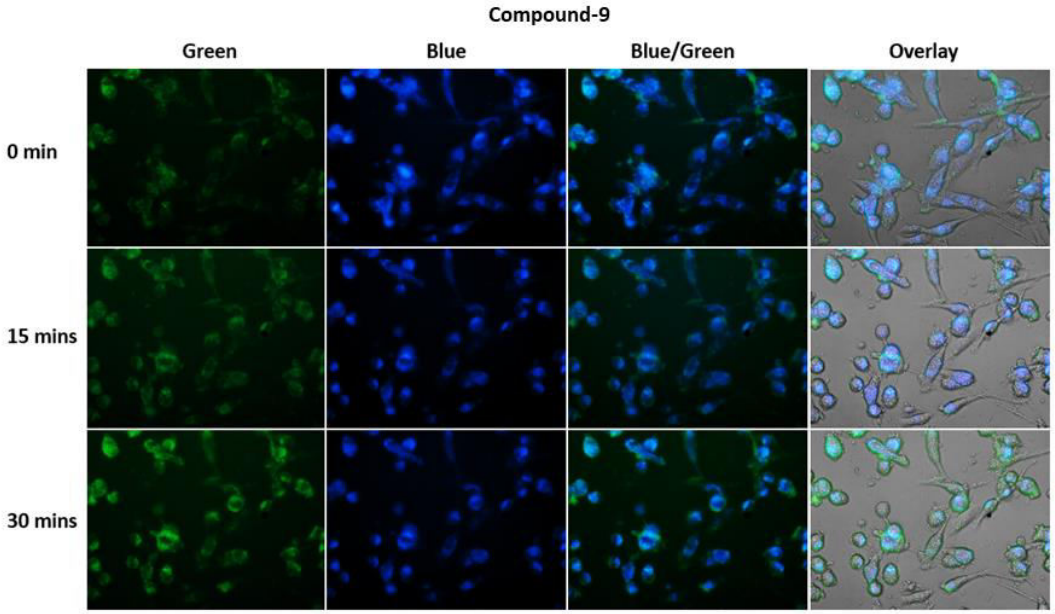


Figure S3: Fluorescent microscope images of MDA-MB-231 cells after incubation with dye **9** at a concentration of 5 μM at 37 ^{o}C. The images were captured after indicated incubation period by phase contrast microscope as well as fluorescence at 400 X magnification using blue (λex: 357 nm; λem: 447–460 nm) and green channels (λex: 470 nm; λem: 510–542 nm) with a 3 s exposure. Bar= 100 μm.

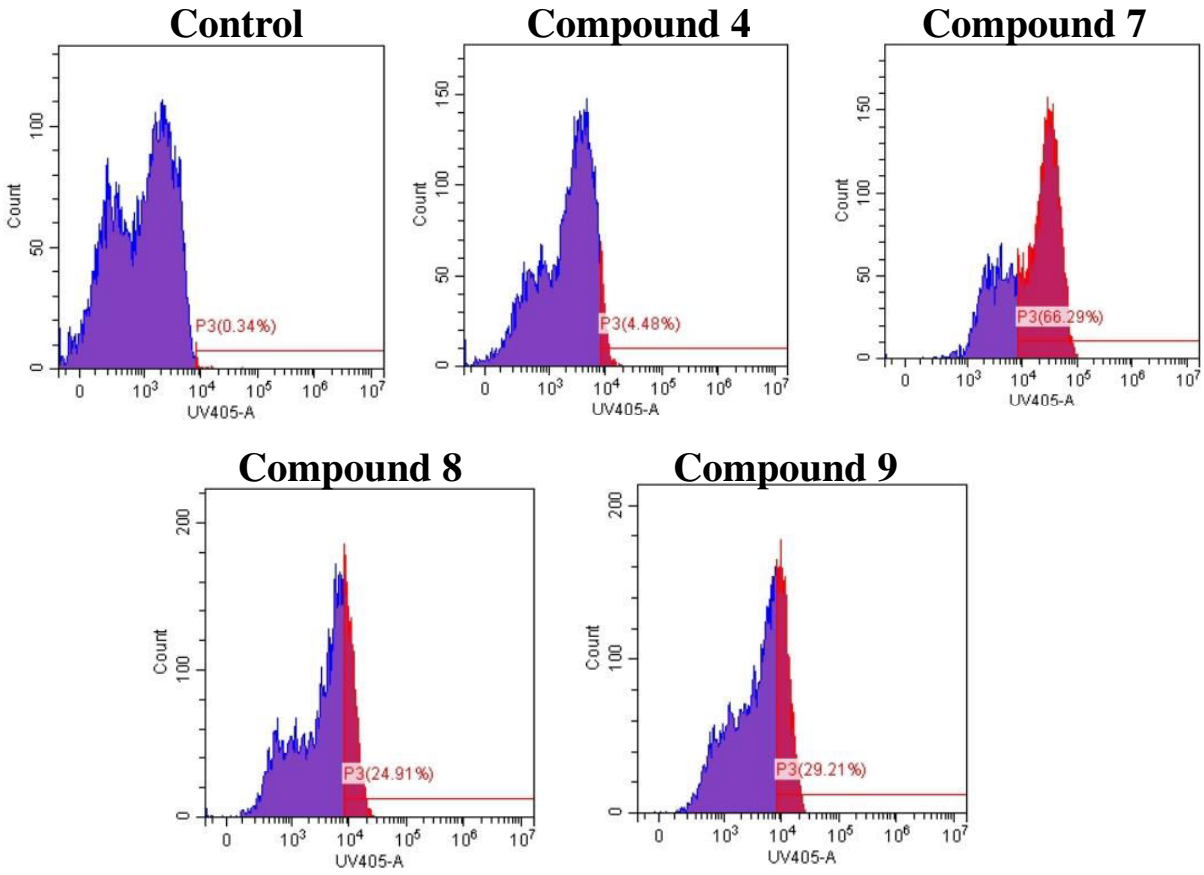


Figure S4: Average fluorescence intensity of compounds **4**, **7**, **8**, and **9** were determined through flow cytometry against MDA-MB-231 cell lines after 30 minutes of incubation.

Compound 7

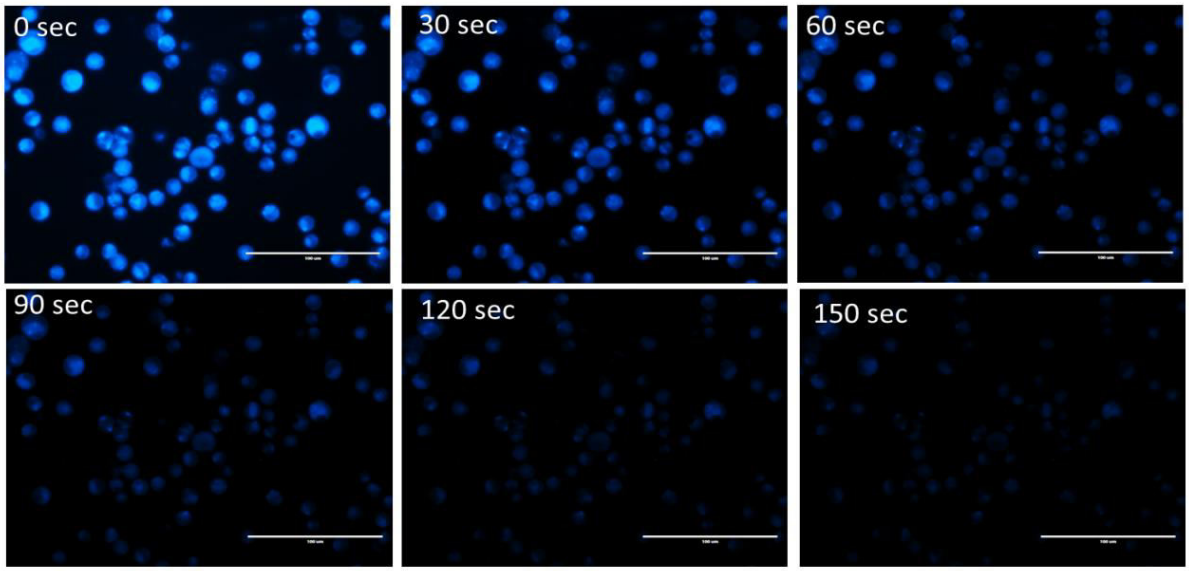


Figure S5: Fluorescence photobleaching images of compound **7** at a concentration of 5 µM in T-cell lymphoma cells at 400X magnification. The study was performed by continuous exposure of ultraviolet excitation wavelength: 357/44 and emission wavelength: 447/60 nm from 0 to 150 seconds and a time dependent photobleaching were been observed**.** Bar = 100 µm.

Compound **8**

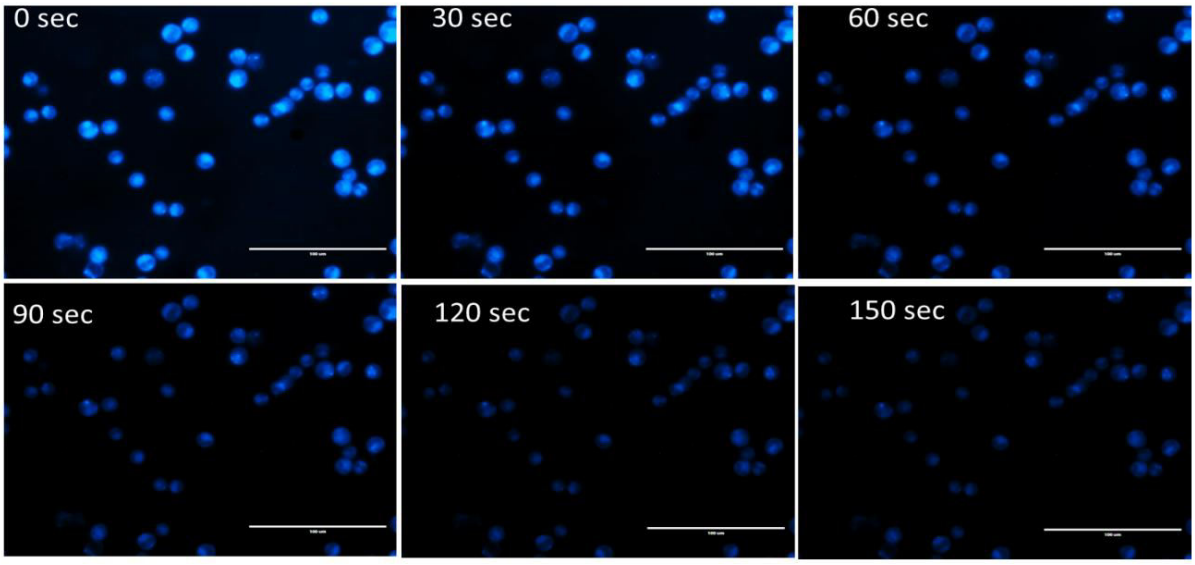


Fig S6: Fluorescence photobleaching images of compound **8** at a concentration of 5 µM in T-cell lymphoma cells at 400X magnification. The study was performed by continuous

exposure of ultraviolet excitation wavelength: 357/44 and emission wavelength: 447/60 nm from 0 to 150 seconds and a time dependent photobleaching were been observed. Bar = 100 µm.

Compound 9

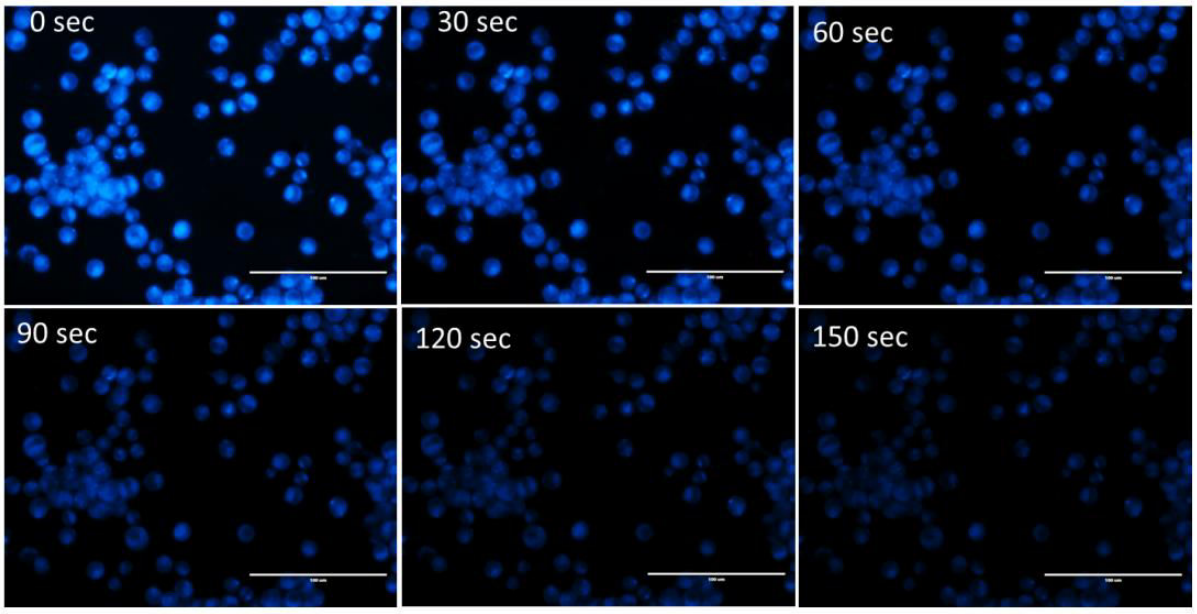


Figure S7: Fluorescence photobleaching images of compound **9** at a concentration of 5 µM in T-cell lymphoma cells at 400X magnification. The study was performed by continuous exposure of ultraviolet excitation wavelength: 357/44 and emission wavelength: 447/60 nm from 0 to 150 seconds and a time dependent photobleaching were been observed. Bar = 100 µm.

Compound 4

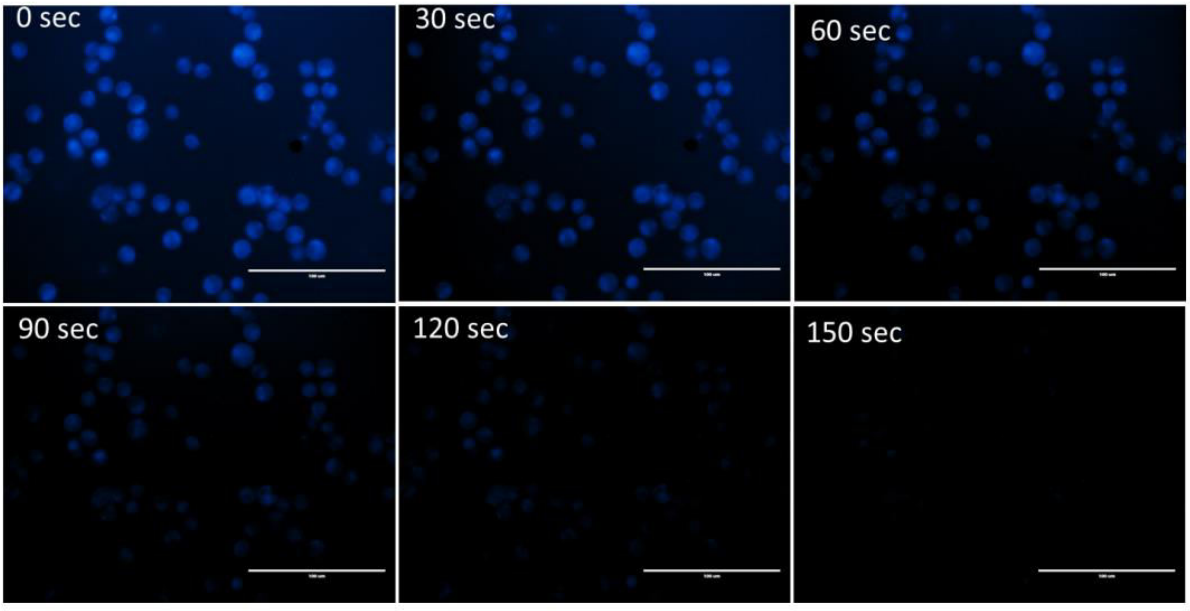


Figure S8: Fluorescence photobleaching images of compound **4** at a concentration of 5 μM in T-cell lymphoma cells at 400X magnification. The study was performed by continuous exposure of ultraviolet excitation wavelength: 357/44 and emission wavelength: 447/60nm from 0 to 150 seconds and a time dependent photobleaching were been observed. Bar = 100 μm.

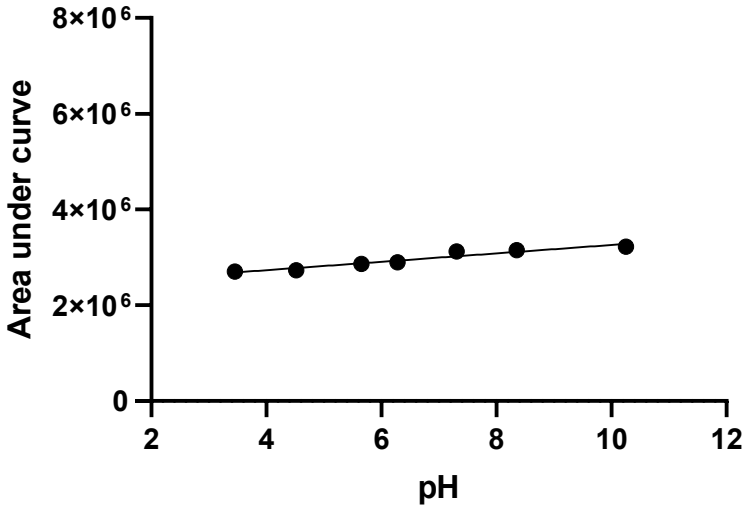
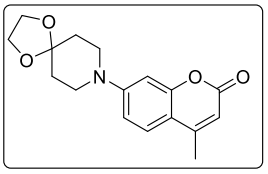


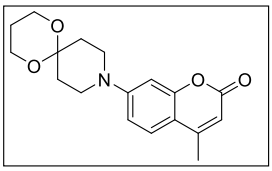
Figure S9: pH dependent stability study of compound **7.**

3. Synthesis

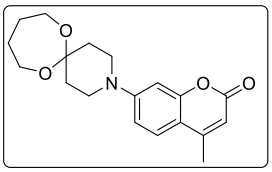
General Procedure for synthesis of compounds 7-11: An oven-dried screw cap vial was charged with 4-methyl-7-((perfluorobutyl)sulfonyl)-2H-chromen-2-one (0.45 mmol), cyclic amines derivatives (2 equiv.), pd_2dba_3 (10 mol%), xant-phos (15 mol%), potassium *tert*-Butoxide (2equiv.), Toluene (4 mL), 110 ^oC, 12 h. After 12 h The reaction mixture was diluted with water (20 mL), then extracted with ethyl acetate (15 mL × 3). After drying with anhydrous Na_2SO_4, the organic phase was evaporated to dryness and purified by column chromatography using ethyl acetate : hexane.



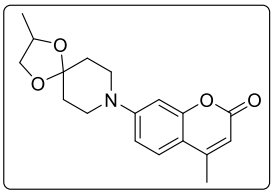
4-methyl-7-(1,4-dioxa-8-azaspiro [4.5] decan-8-yl)-2H-chromen-2-one (7): The representative general procedure mentioned above was followed. The compound was purified by column chromatography using 30% ethyl acetate:hexane. Compound 1 was obtained as a yellow semi solid with 30 % yield. ^1H NMR (500 MHz, CD_3OD) δ 7.55 (d, *J* = 9 Hz, 1H), 6.97 (dd, *J* = 9 Hz, *J*=3 Hz, 1H), 6.77 (d, *J* = 2.5 Hz, 1H), 6.02 (d, *J* = 1 Hz, 1H), 4.00 (s, 4H), 3.54-3.51 (m, 4H), 2.40 (d, *J* = 1 Hz, 3H), 1.80-1.77 (m, 4H). ^{13}C NMR (125 MHz, CD_3OD) δ 162.9, 155.4, 154.5, 153.2, 125.6, 111.6, 110.6, 108.7, 106.7, 100.2, 64.0, 45.5, 33.8, 17.0. HRMS (ESI) m/z calculated for $C_{17}H_{19}NO_4$ $[M + H]^+$ calculated as 302.1387, found 302.1386.



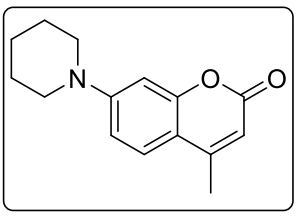
4-methyl-7-(1,5-dioxa-9-azaspiro[5.5]undecan-9-yl)-2H-chromen-2-one (8) : The representative general procedure mentioned above was followed**.** The compound was purified by column chromatography using 22% ethyl acetate:hexane. Compound 2 was obtained as a yellow semi solid with 20% yield. ^1H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, *J* = 9 Hz, 1H), 6.84 (dd, *J* = 9 Hz, *J* = 2.5 Hz, 1H), 6.74 (d, *J* = 2.5 Hz, 1H), 6.05 (d, *J* = 1 Hz, 1H), 3.97 (t, *J* = 5.5 Hz, 4H), 3.44-3.41 (m, 4H), 2.34 (d, *J* = 1 Hz, 3H), 2.03-2.01 (m, 4H), 1.79 (pent, *J* = 5.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.9, 155.5, 153.1, 152.6, 125.3, 111.5, 111.1. 110.4, 101.3, 96.0, 59.4, 44.7, 32.1, 25.5, 18.4. HRMS (ESI) m/z calculated for $C_{18}H_{21}NO_4$ $[M + H]^+$ calculated as 316.1543, found 316.1543.



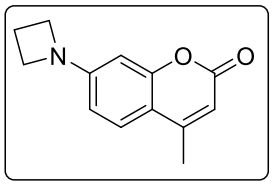
4-methyl-7-(7,12-dioxa-3-azaspiro[5.6]dodecan-3-yl)-2H-chromen-2-one (9): The representative general procedure mentioned above was followed**.** The compound was purified by column chromatography using 25% ethyl acetate:hexane. Compound 3 was obtained as a yellow semi solid with 16% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, *J* = 9 Hz, 1H), 6.84 (dd, *J* = 9 Hz, *J* = 2.5 Hz, 1H), 6.73 (d, *J* = 2.5 Hz, 1H),6.04 (d, *J* = 1 Hz, 1H), 3.75 (bs, 4H), 3.44-3.41 (m, 4H), 2.37 (d, *J* = 1 Hz, 3H), 1.86-1.84 (m, 4H), 1.65 (bs, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.2, 155.5, 153.2, 152.9, 125.3, 111.6, 111.0, 110.1, 101.1, 99.3, 61.9, 45.3, 33.1, 29.6, 18.5. HRMS (ESI) m/z calculated for $C_{19}H_{23}NO_4$ $[M + H]^+$ calculated as 330.1700, found 330.1700.



4-methyl-7-(2-methyl-1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-2H-chromen-2-one (10): The representative general procedure mentioned above was followed**.** The compound was purified by column chromatography using 30% ethyl acetate:hexane. Compound 10 was obtained as a yellow semi solid with 15% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, *J* = 9 Hz, 1H), 6.84 (dd, *J* = 8.5 Hz, *J* = 2.5 Hz, 1H), 6.74 (d, *J* = 2.5 Hz, 1H), 6.04 (s, 1H), 4.31-4.27 (m, 1H), 4.13-4.10 (m, 1H), 3.65-3.43 (m, 5H), 2.37 (s, 3H), 1.87-1.80 (m, 4H), 1.32 (d, *J* = 6 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.0, 155.5, 153.0, 152.7, 125.3, 111.6, 111.1, 110.3, 107.1, 101.2, 72.1, 70.6, 46.0, 35.5, 34.3, 18.5. HRMS (ESI) m/z calculated for $C_{18}H_{21}NO_4$ $[M + H]^+$ calculated as 316.1543, found 316.1543.



4-methyl-7-(piperidin-1-yl)-2H-chromen-2-one (11): The representative general procedure mentioned above was followed**.** The compound was purified by column chromatography using 30% ethyl acetate:hexane. Compound 11 was obtained as a yellow solid with 35% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, *J* = 9 Hz, 1H), 6.84 (dd, *J* = 9 Hz, *J* = 2.5 Hz, 1H), 6.74 (d, *J* = 2.5 Hz, 1H), 6.02 (d, *J* = 1 Hz, 1H), 3.36-3.33 (m, 4H), 2.36 (d, *J* = 1 Hz, 1H), 1.71-1.67 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.0, 155.6, 153.9, 152.7, 125.2, 111.4, 110.8, 110.0, 100.9, 48.8, 25.2, 24.3, 18.4. HRMS (ESI) m/z calculated for $C_{15}H_{17}NO_2$ $[M + H]^+$ calculated as 244.1332, found 244.1342.



7-(azetidin-1-yl)-4-methyl-2H-chromen-2-one (4): The compound was purified by column chromatography using 20% ethyl acetate:hexane. Compound 4 was obtained as a brownish yellow solid with 88% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.4 Hz, 1H), 6.32 (dd, *J* = 2.4 Hz, 1.8 Hz, 1H), 6.23 (d, *J* = 1.8 Hz, 1H), 5.99 (s, 1H), 4.01 (t, *J* = 7.2 Hz, 4H), 2.48 - 2.43 (m, 2H), 2.36 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.0, 155.6, 154.0, 153.0, 125.4, 110.3, 109.4, 107.7, 97.1, 51.8, 18.6, 16.5. HRMS (ESI) m/z calculated for $C_{13}H_{13}O_2N$ $[M + H]^+$ calculated as 216.1019, found 216.1039.

4. Cartesian Coordinates

Cartesian coordinates for compound-**4**

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-3.207230	2.404539	0.102148
2	6	0	-2.646022	1.007855	0.051809
3	6	0	-1.215703	0.798225	-0.023277
4	6	0	-0.248573	1.826226	-0.053369
5	6	0	1.105946	1.553622	-0.124769
6	6	0	1.563647	0.210663	-0.172496
7	7	0	2.903411	-0.064364	-0.275740
8	6	0	3.597088	-1.315356	0.064042
9	6	0	4.905413	-0.495061	0.234259
10	6	0	4.038070	0.788055	0.109885
11	6	0	0.619446	-0.831268	-0.141007
12	6	0	-0.736552	-0.529876	-0.069948
13	8	0	-1.591522	-1.595083	-0.044095
14	6	0	-3.470083	-0.077507	0.075418
15	6	0	-2.978091	-1.441455	0.029690
16	8	0	-3.650426	-2.451408	0.049191
17	1	0	-2.918581	2.977800	-0.786513
18	1	0	-4.297872	2.384441	0.155878
19	1	0	-2.831376	2.949479	0.975926

20	1	0	-0.572592	2.861345	-0.018705
21	1	0	1.821971	2.368058	-0.153316
22	1	0	3.586841	-2.070038	-0.730268
23	1	0	3.229366	-1.771848	0.994752
24	1	0	5.425927	-0.624755	1.184238
25	1	0	5.611763	-0.625073	-0.587934
26	1	0	4.340366	1.514915	-0.652547
27	1	0	3.878819	1.314575	1.062450
28	1	0	0.920764	-1.871765	-0.179802
29	1	0	-4.547826	0.023377	0.130951

Cartesian coordinates for compound-**7**

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	8	0	-5.518923	-2.453166	-0.141690
2	6	0	-4.761739	-1.513476	-0.087675
3	6	0	-5.267590	-0.113750	0.086415
4	6	0	-4.461252	0.948790	0.150222
5	6	0	-5.011520	2.330013	0.324684
6	6	0	-3.029282	0.716423	0.044185
7	6	0	-2.126468	1.784682	0.103131

8	6	0	-0.752868	1.552492	0.000360
9	6	0	-0.275651	0.253059	-0.161641
10	7	0	0.969099	0.041707	-0.254834
11	6	0	1.832220	1.014788	-0.939695
12	6	0	3.088773	1.289080	-0.123994
13	6	0	3.759466	-0.030494	0.234370
14	8	0	4.893721	0.268019	1.002458
15	6	0	5.885057	-0.578635	0.486674
16	6	0	5.716808	-0.134185	-0.785587
17	8	0	4.375982	-0.518909	-0.926216
18	6	0	2.821586	-0.856762	1.104539
19	6	0	1.555137	-1.178766	0.322267
20	6	0	-1.171906	-0.814217	-0.220922
21	6	0	-2.545489	-0.584743	-0.118435
22	8	0	-3.343102	-1.692279	-0.187514
23	1	0	-6.353088	0.045856	0.165280
24	1	0	-4.174201	3.062739	0.352581
25	1	0	-5.685974	2.570458	-0.527411
26	1	0	-5.583218	2.383736	1.278122
27	1	0	-2.500322	2.811281	0.230920
28	1	0	-0.047679	2.395406	0.047300
29	1	0	1.271582	1.966402	-1.077110
30	1	0	2.130965	0.595440	-1.926441
31	1	0	2.814203	1.830587	0.808825

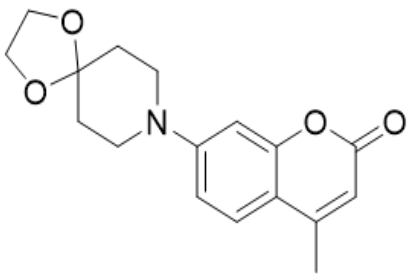
32	1	0	3.790965	1.911900	-0.722150
33	1	0	6.898902	-0.369548	0.895539
34	1	0	5.806996	-1.671314	0.683466
35	1	0	6.385791	-0.652060	-1.508800
36	1	0	5.949353	0.937962	-0.973182
37	1	0	3.326756	-1.804056	1.398149
38	1	0	2.556731	-0.278410	2.017845
39	1	0	0.814454	-1.649601	1.006718
40	1	0	1.816411	-1.873988	-0.506691
41	1	0	-0.795430	-1.839845	-0.348806

Cartesian coordinates for Compound-**8**

Center	Atomic	Atomic	Coordinates (Angstroms)		
Number	Number	Type	X	Y	Z
1	8	0	0.000000	0.000000	0.000000
2	6	0	0.000000	0.000000	1.208000
3	6	0	1.279742	0.000000	1.987544
4	6	0	1.318767	-0.000124	3.322354
5	6	0	2.621594	-0.000377	4.059681
6	6	0	0.050411	-0.000006	4.034429
7	6	0	0.017483	-0.000055	5.433944
8	6	0	-1.205049	-0.000035	6.109715
9	6	0	-2.399050	0.000016	5.390781

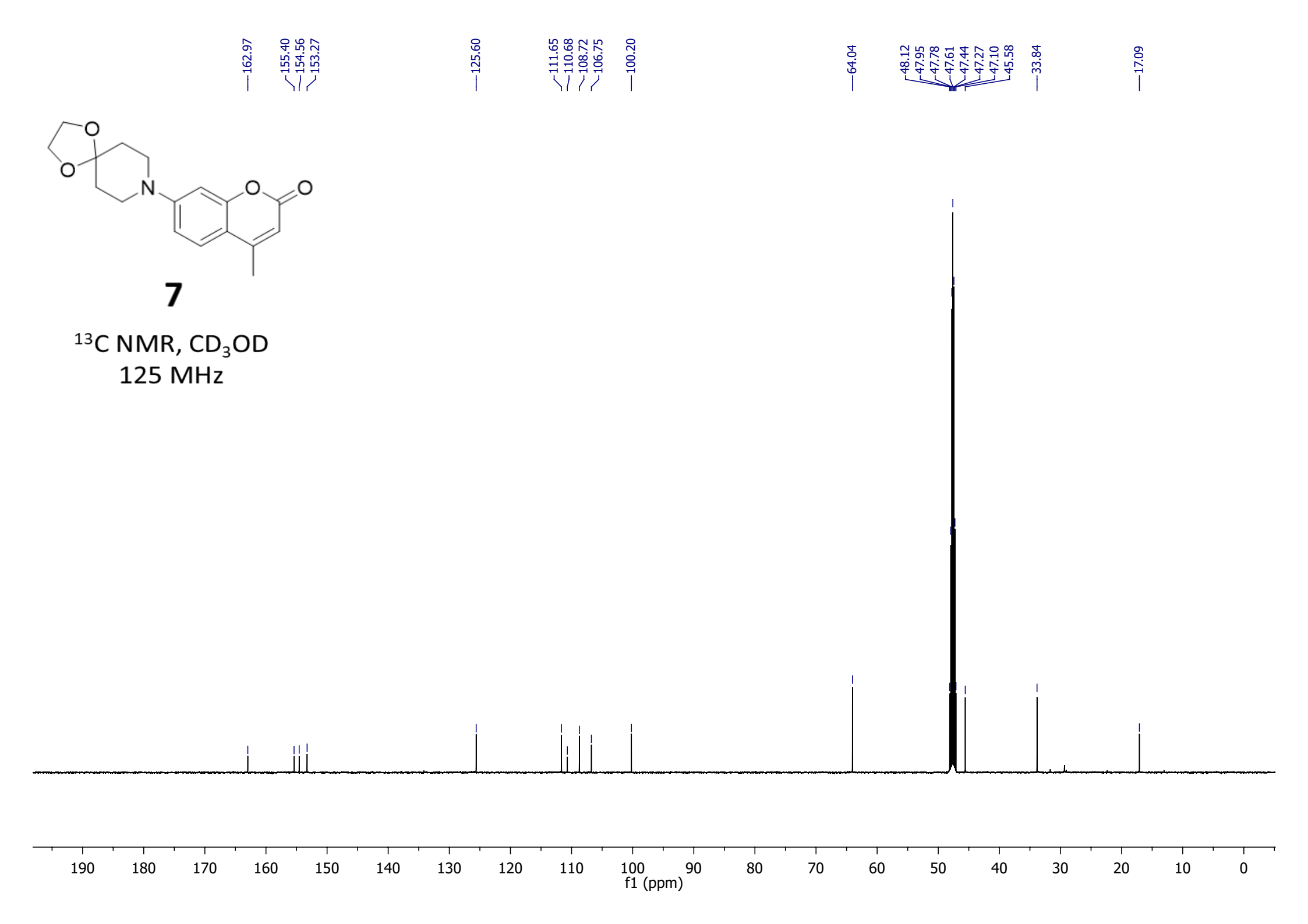
10	7	0	-3.687419	0.000079	6.101708
11	6	0	-3.825358	-0.723571	7.373795
12	6	0	-4.544888	0.133705	8.406696
13	6	0	-5.844943	0.659823	7.812906
14	8	0	-6.457822	1.462935	8.785015
15	6	0	-7.485463	2.166050	8.140664
16	6	0	-8.526457	1.148168	7.693701
17	6	0	-7.827984	0.003675	6.973583
18	8	0	-6.753194	-0.407511	7.774429
19	6	0	-5.533351	1.547677	6.615344
20	6	0	-4.837169	0.723829	5.540108
21	6	0	-2.370647	0.000005	3.996127
22	6	0	-1.149842	-0.000017	3.318241
23	8	0	-1.224115	-0.000000	1.953661
24	1	0	2.230795	0.000108	1.434825
25	1	0	2.428182	-0.000447	5.155747
26	1	0	3.202057	-0.909695	3.785866
27	1	0	3.202892	0.908374	3.785757
28	1	0	0.958306	-0.000111	6.003901
29	1	0	-1.225406	-0.000058	7.209526
30	1	0	-2.813338	-0.983872	7.756975
31	1	0	-4.421740	-1.646566	7.197226
32	1	0	-3.895408	0.990533	8.694434
33	1	0	-4.769955	-0.480658	9.307068

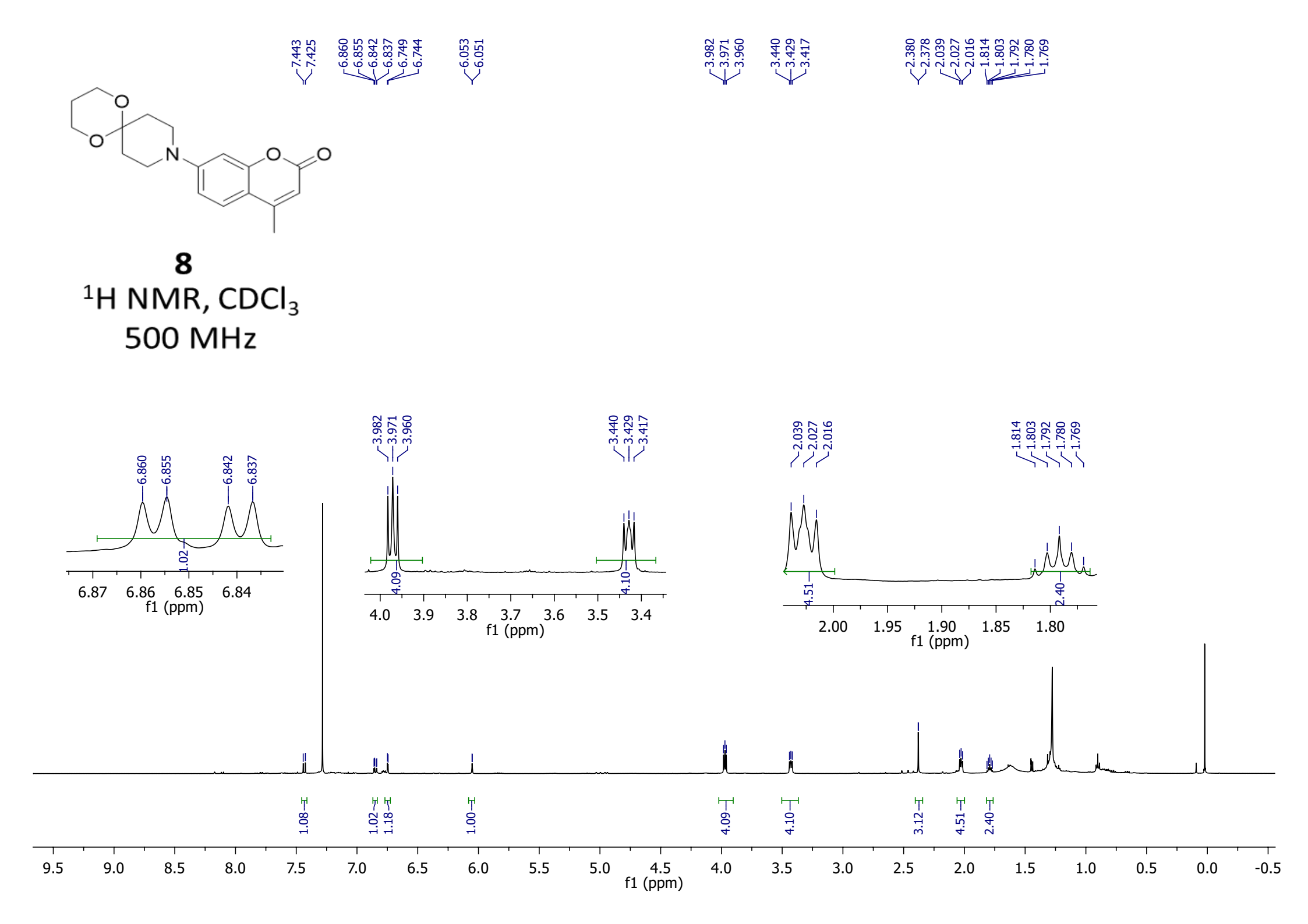
34	1	0	-7.945477	2.895417	8.844355
35	1	0	-7.087318	2.729092	7.267031
36	1	0	-9.250845	1.635362	7.003285
37	1	0	-9.071699	0.755466	8.580982
38	1	0	-8.537229	-0.841574	6.827650
39	1	0	-7.467107	0.332974	5.973534
40	1	0	-6.480855	1.965092	6.206969
41	1	0	-4.867396	2.380550	6.934089
42	1	0	-4.481857	1.402999	4.733106
43	1	0	-5.561169	-0.016586	5.132213
44	1	0	-3.312915	0.000016	3.428563

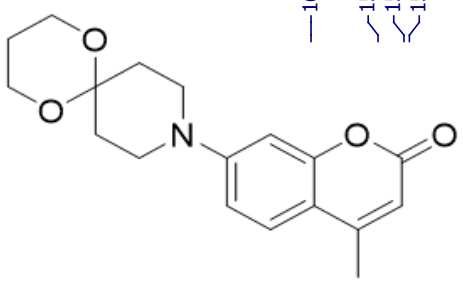


7

^{1}H NMR, CD_3OD
500 MHz


162.97
155.40
154.56
153.27
125.60
111.65
110.68
108.72
106.75
100.20
64.04
48.12
47.95
47.78
47.61
47.44
47.27
47.10
45.58
33.84
17.09
O
O
N
O
O
7
13C NMR, CD3OD
125 MHz
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.443
7.425
6.860
6.855
6.842
6.837
6.749
6.744
6.053
6.051
3.982
3.971
3.960
3.440
3.429
3.417
2.380
2.378
2.039
2.027
2.016
1.814
1.803
1.792
1.780
1.769
O
O
N
O
O
8
1H NMR, CDCl3
500 MHz
1.02
4.09
4.10
4.51
2.40
1.08
1.02
1.18
1.00
4.09
4.10
3.12
4.51
2.40
f1 (ppm)



8

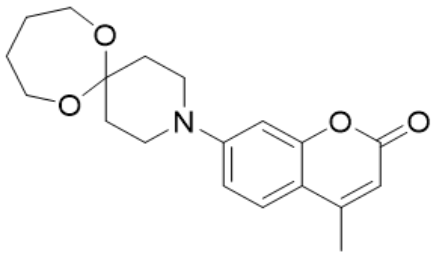
^{13}C NMR, $CDCl_3$

125 MHz

161.91
155.58
153.14
152.62
125.31
111.53
111.18
110.45
101.30
96.07
59.45
44.71
32.18
25.56
18.47

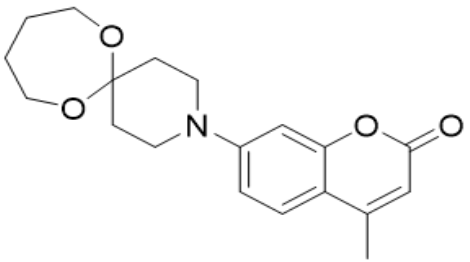
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

f1 (ppm)



9

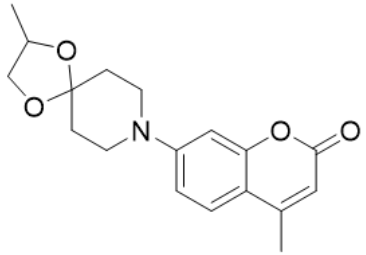
^{1}H NMR, $CDCl_3$
500 MHz



9

^{13}C NMR, $CDCl_3$

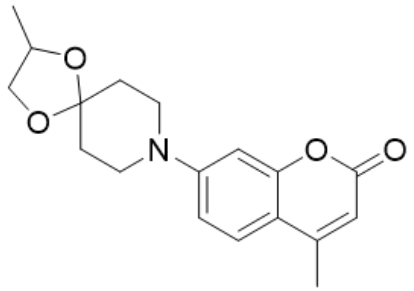
125 MHz



10

^{1}H NMR, $CDCl_3$

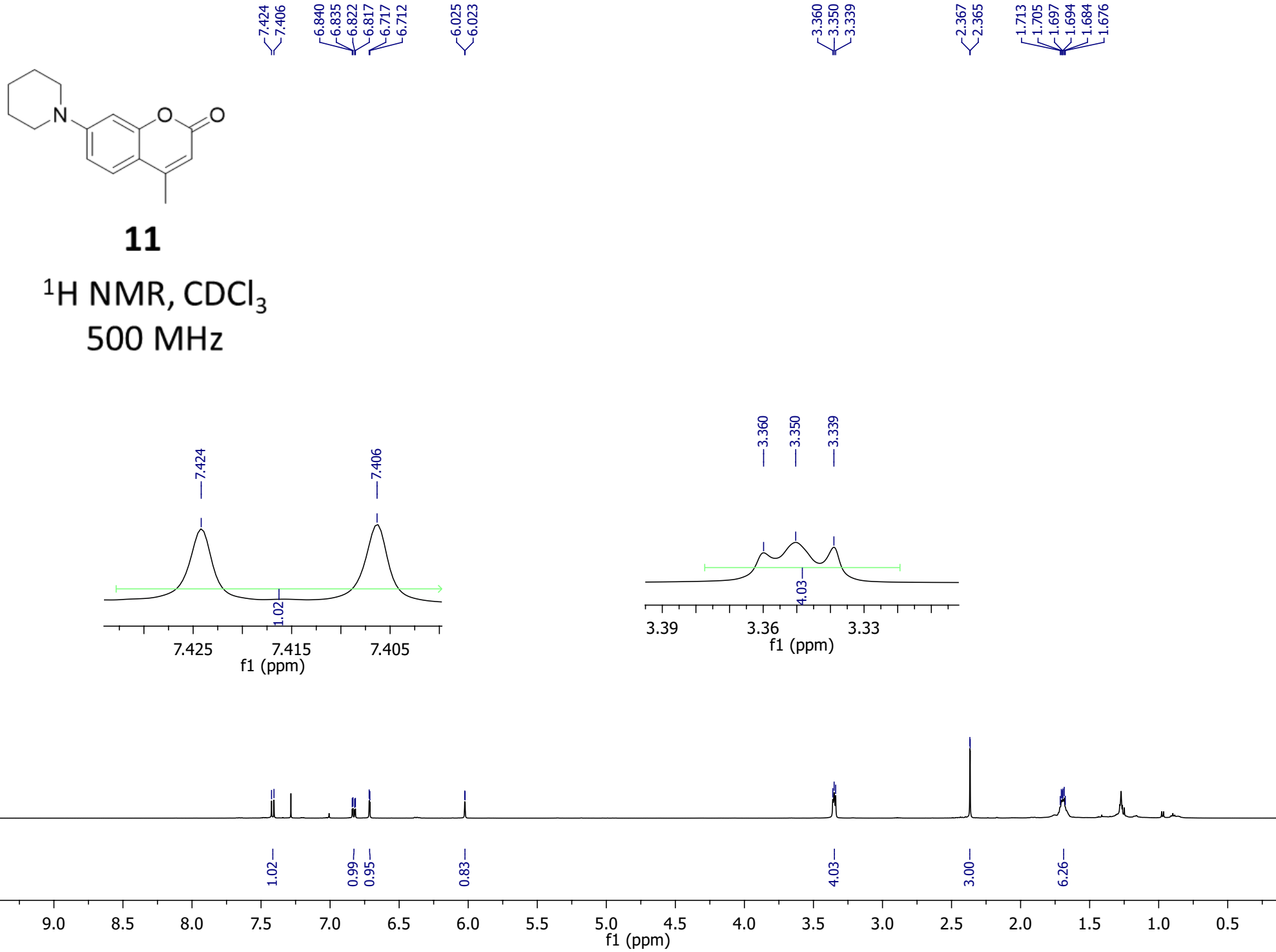
500 MHz

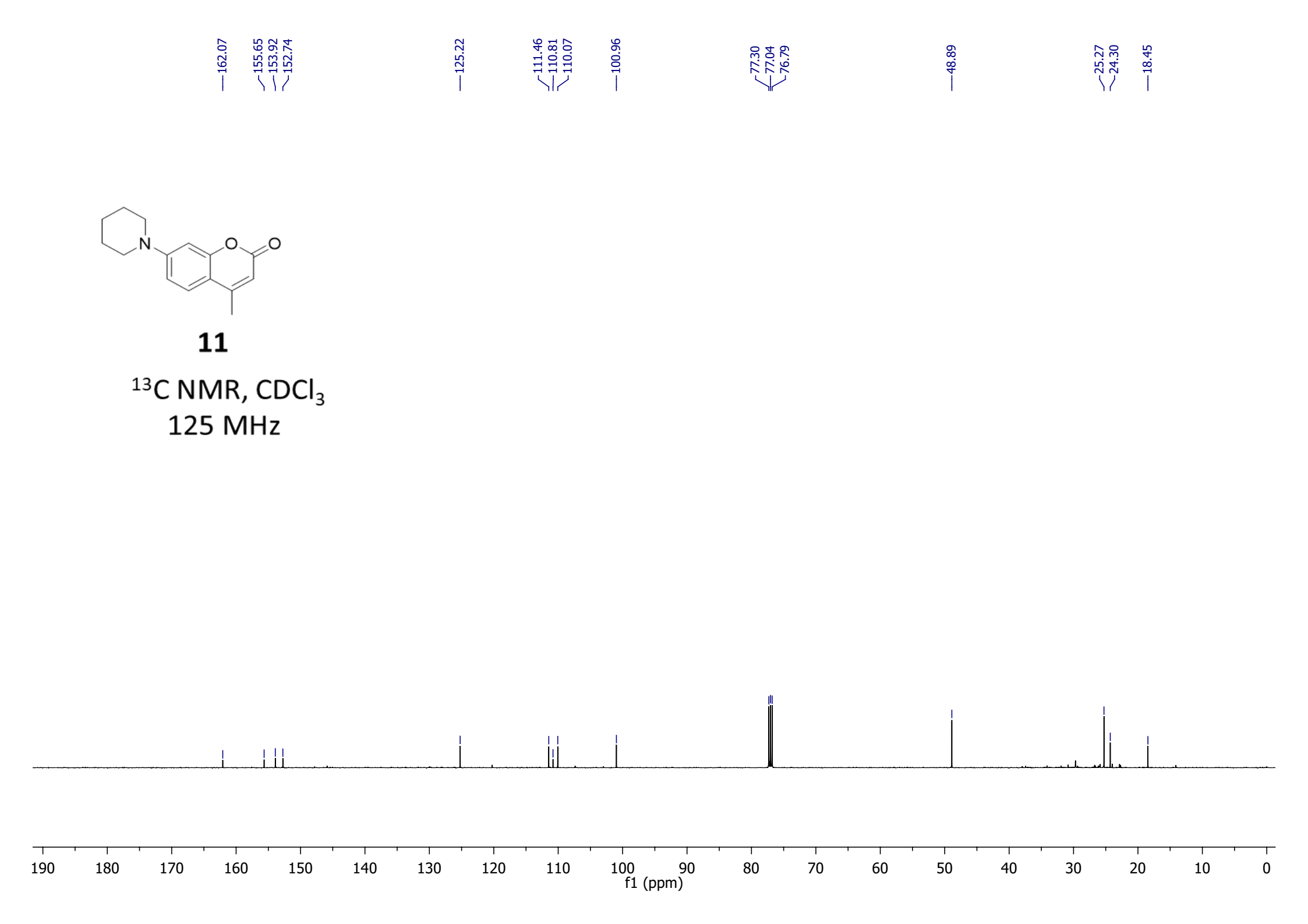


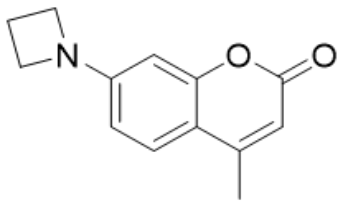
10

^{13}C NMR, $CDCl_3$

125 MHz


7.424
7.406
6.840
6.835
6.822
6.817
6.717
6.712
6.025
6.023
3.360
3.350
3.339
2.367
2.365
1.713
1.705
1.697
1.694
1.684
1.676
N
O
O
11
1H NMR, CDCl3
500 MHz
7.424
7.406
1.02
7.425
7.415
7.405
f1 (ppm)
3.360
3.350
3.339
4.03
3.39
3.36
3.33
f1 (ppm)
1.02
0.99
0.95
0.83
4.03
3.00
6.26
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)

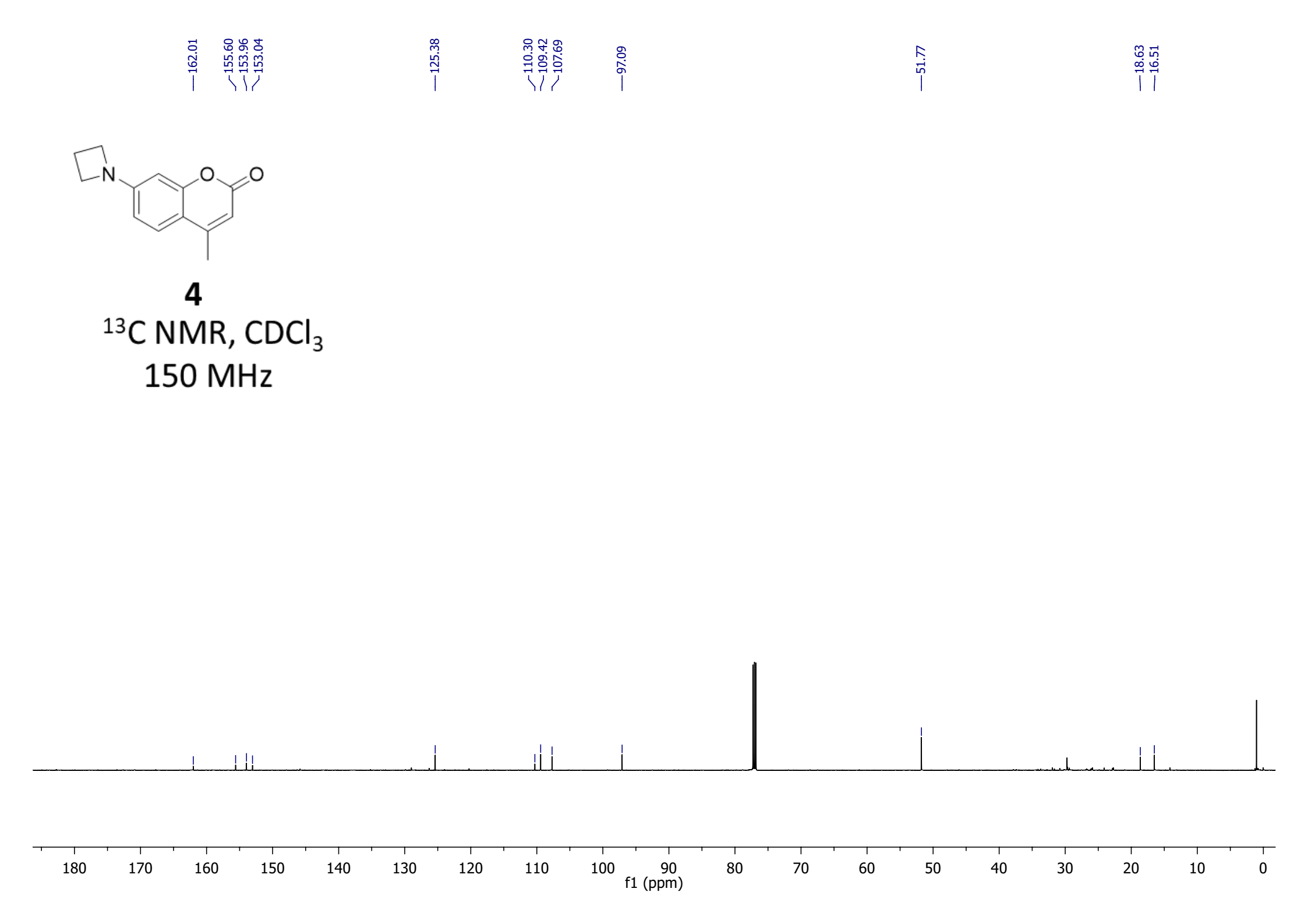

162.07
155.65
153.92
152.74
125.22
111.46
110.81
110.07
100.96
77.30
77.04
76.79
48.89
25.27
24.30
18.45
N
O
O
11
13C NMR, CDCl3
125 MHz
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



4

^{1}H NMR, $CDCl_3$

600 MHz


162.01
155.60
153.96
153.04
125.38
110.30
109.42
107.69
97.09
51.77
18.63
16.51
N
O
O
4
13C NMR, CDCl3
150 MHz
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)