Iridium (III) Complexes of 1,2,4-Triazines as Potential Biorthogonal Reagents: Metal Coordination Facilitates Luminogenic Reaction with Strained Cyclooctyne

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Supporting Information

Synthesis. All solvents and reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. NMR spectra were recorded on a JEOL ECS400FT Delta spectrometer (399.78 MHz for ^{1}H NMR, 100.53 MHz for ^{13}C NMR). Chemical shifts are reported in parts per million (ppm) relative to a tetramethylsilane internal standard.

3-mercapto-5-(pyridin-2-yl)-1,2,4-triazine 6

48% Aqueous HBr (100 mL) was added to a stirred solution of 2-acetylpyridine (12.1 g, 100 mmol) in DMSO (100 mL). The mixture was stirred at 50-55°C for 2 days. Water (100 mL) was added and the mixture was basified by addition of Na_2CO_3*$10H_2O$ (*ca*.160 g, careful CO_2 evolution!). To this solution, thiosemicarbazide (9.1 g, 100 mmol) was added as a solid and the mixture was stirred at RT for 30 minutes. The mixture was then warmed up to 50-60°C and stirred at this temperature for 1 hour. The mixture was then neutralised by addition of an excess of acetic acid causing a formation of the solid. The solid was filtered off, washed with water and dried in an oven at 100°C. Yield 11.3 g (59%). ^{1}H NMR (400 MHz, DMSO-D_6): δ 9.08 (s, 1H), 8.81 (br.d, *J* = 4.6 Hz, 1H), 8.34 (d, *J* = 7.7 Hz, 1H), 8.06 (br.t , *J* = 7.7 Hz, 1H) 7.68 (dd, *J* =7.7, 4.6 Hz, 1 H).

3-mercapto-5-(pyridin-2-yl)-1,2,4-triazine 7

A mixture of **6** (11.1 g, 58.4 mmol) and hydrazine hydrate (14.6 g, 292 mmol, 5 eq) was heated under reflux for 15 minutes. Methanol (20 mL), water (20 mL) and acetic acid (10 mL) were added. The solid was filtered off, washed with water (30 mL) and methanol (30 mL). Yield 5.96 g (54%). ^{1}H NMR (400 MHz, DMSO-D_6): δ 9.36 (s, 1H), 8.88 (br.s, 1H), 8.74 (br.d, *J* = 4.6 Hz, 1H), 8.40 (br.s, 1H), 8.02 (ddd, J = 7.8, 7.8, 1.8, 1H), 7.59 (m, 1H), 4.45 (s, 2H).

5-(2-pyridyl)-1,2,4-triazine 1

A mixture of **7** (940 mg, 5 mmol), methanol (100 mL) and sodium methoxide (2.7 g, 50 mmol) was stirred at 75°C (bath temperature) for 14 hours. Acetic acid (5 mL) and water (20 mL) were added and the mixture was evaporated to a volume of approximately 15 mL. The mixture was basified by addition of 33% aqueous ammonia (5 mL). Precipitated solid was filtered off, washed with water (20 mL) and dried. The product was purified by recrystallization from methanol. Yield 526 mg (67%). ^{1}H (400 Hz, $CDCl_3$): δ 10.30 (d, *J* = 1.8 Hz 1H), 9.71 (d, *J* = 1.8 Hz 1H), 8.79 (br.d, *J* = 4.6 Hz, 1H), 8.54 (d, *J* = 7.8 Hz, 1H), 7.92 (ddd, J = 7.8, 7.8, 1.8, 1H), 7.49 (dd, *J* =7.4, 4.6 Hz, 1 H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 157.3, 154.0, 151.7, 150.1, 147.4, 137.5, 126.7, 122.9.

Iridium complex 8Cl

A mixture of $[Ir(2,4\text{-diF-py})_2]Cl_2$ (608 mg, 1 mmol for Ir), ligand **1** (164 mg, 1.04 mmol), chloroform (10 mL) and methanol (10 mL) was heated under reflux for 18 hours. The solvent was evaporated to dryness and the product was purified by silica column chromatography using DCM/methanol (4/1 v/v) as an eluent. Yield 260 mg (34%). ^{1}H NMR (400 Hz, CD_3CN): δ 10.75 (br.s, 1H), 9.22 (d, *J* = 7.6 Hz, 1H), 9.08 (br.s, 1H), 8.29 (m, 3H), 8.10 (d, *J* = 6.4 Hz, 1H), 7.91 (m, 2H), 7.73 (m, 2H), 7.50 (d, *J* = 6.4 Hz, 1H), 7.07 (m, 2H), 6.70 (m, 2H), 5.72 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.60 (dd, *J* = 8.8, 2.4 Hz, 1H); ^{19}F NMR (100 Hz, CD_3CN): δ – 106.62, -106.73, -109.14, -109.31.

Iridium complex $8PF_6$

A mixture of $[Ir(2,4\text{-diF-py})_2]Cl_2$ (460 mg, 0.76 mmol for Ir), VNK-408R3 (120 mg, 0.76 mmol), methanol (15 mL) and chloroform (15 mL) was heated under reflux for 16 hours. The solvent was evaporated to a volume of 5 mL. Water (30 mL) and methanol (30 mL) were added, followed by 1M aqueous solution of KPF_6 (10 mL, 10 mmol). The product was extracted with chloroform (40 mL). The mixture was separated, the organic layer was dried over anhydrous magnesium sulfate. During this drying stage the product precipitates and covers the crystals of magnesium sulfate. The mixture was filtered by suction. The chloroform phase was discarded. The filter cake was washed with methanol (10 mL). The methanol filtrate was also discarded. The filter cake was then transferred in a beaker and stirred with acetone (20 mL) for 5 minutes (the product is soluble in acetone). The suspension was gravity filtered. The filtrate was evaporated to dryness, treated with diethyl ether. The solid was filtered off to give pure product **$8PF_6$**. Yield 130 mg (20%). ^{1}H NMR (400 Hz, CD_3CN): δ 10.32 (d, *J* = 1.6 Hz, 1H), 9.10 (d, *J* = 1.6 Hz, 1H), 8.78 (d, *J* = 7.6 Hz, 1H), 8.29 (m, 3H), 8.10 (d, *J* = 6.4 Hz, 1H), 7.90 (m, 2H), 7.71 (m, 2H), 7.45 (d, *J* = 6.4 Hz, 1H), 7.06 (m, 2H), 6.71 (m, 2H), 5.72 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.60 (dd, *J* = 8.8, 2.4 Hz, 1H).

Reaction kinetics

The reaction between the dienophiles and BCN were carried out in methanol at 25°C under pseudo-first order conditions, with BCN in at least 10-fold excess. Reactions were followed by monitoring the loss of the substrate absorbance spectrophotometrically at a suitable wavelength (see Figures S1, S3, S5, S7, S9, S11 and S13 for details). Observed pseudo-first-order rate constants, k_{obs}, were obtained using nonlinear regression of the monoexponential loss of absorbance with time. Second order rate constants, k_2, were obtained from linear regression of plots of k_{obs} against BCN concentration. Pseudo-first-order and second order plots are shown in Figures S1 to S6.

Stock solutions of dienophiles (range 7 to 10 x 10^{-4} M) were prepared in methanol and kept in the dark until use. BCN stock solutions in the range 4 to 7 x 10^{-3} M were also prepared in methanol. Reaction solutions of BCN were prepared by mixing the stock BCN solution with methanol to a total of 1 mL in a quartz cuvette. The reaction was started by pipetting 0.1 mL of the dienophile into the cuvette, shaking three times and quickly placing in a Pharmacia Biotech Ultraspec 2000 spectrophotometer with thermostatted cell-holder. The reactions of iridium complexes were measured at 5 s intervals until completion. The reactions of uncoordinated ligand was measured at 120 s intervals. Suitable wavelengths at which to carry out the reaction kinetics were determined using a similar procedure to that outlined above but measuring sequential wavelength scans (300 to 600 nm) so as to determine the wavelength at which the maximum absorbance change took place during the reaction.

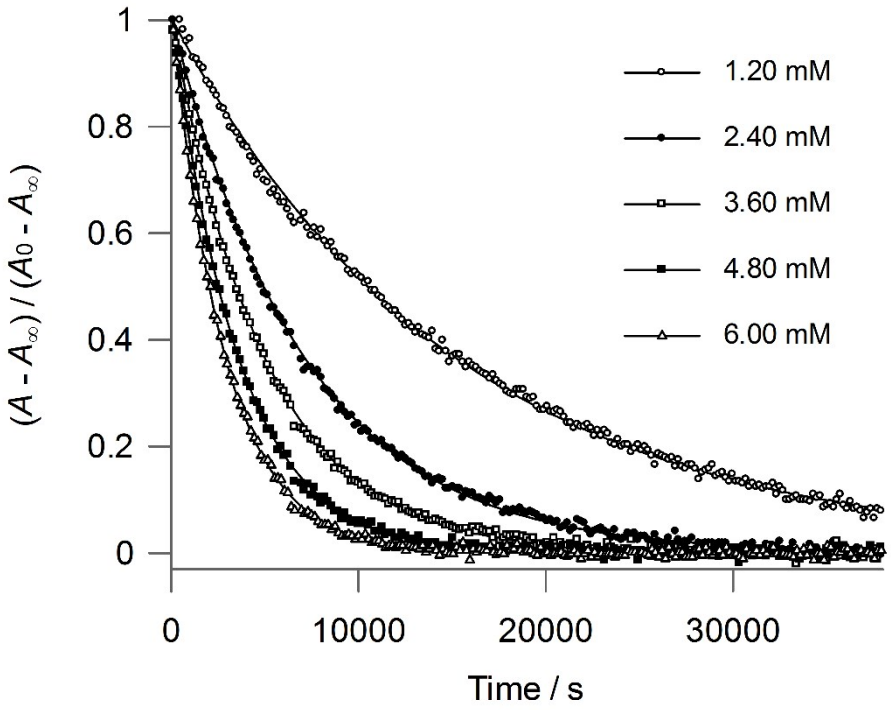


Figure S1: Reaction of ligand **1** with BCN. Plots show **1** absorbance loss (normalised) at 386 nm with time. The reactions were carried out in methanol at 25°C under pseudo-first-order conditions, with $[\mathbf{1}]_0$ = 1.20 x 10^{-4} M and BCN concentrations as stated in the legend. Lines are the best fit to a monoexponential decay using non-linear least squares analysis and the rate data shown in Table S1.

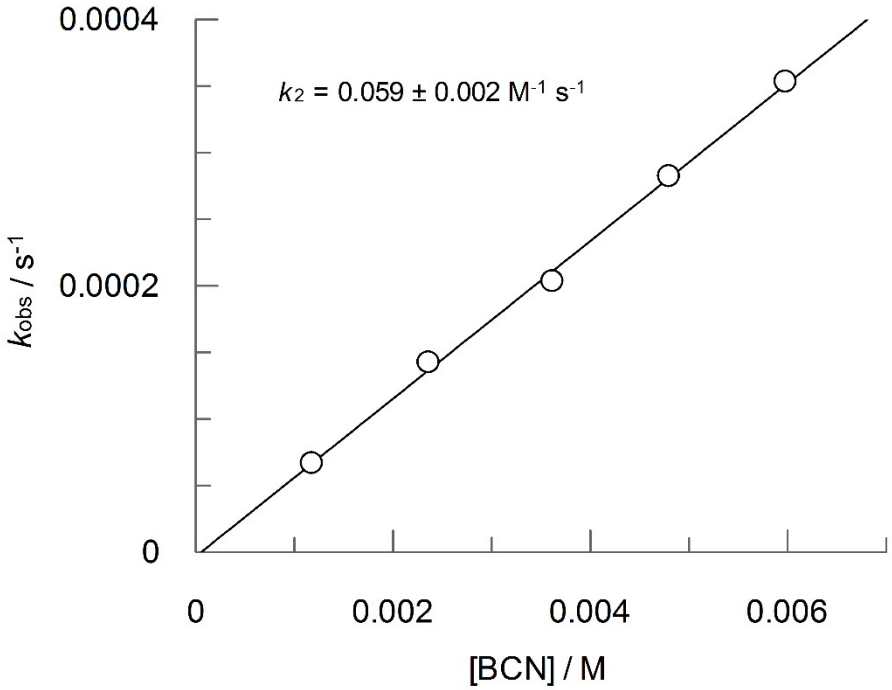


Figure S2: Plot of observed pseudo-first-order rate constant, k_{obs}, against BCN concentration for the reaction with **1**. The slope, which is equal to the second order rate constant, k_2, is shown on the plot. Conditions are as stated in Figure S1.

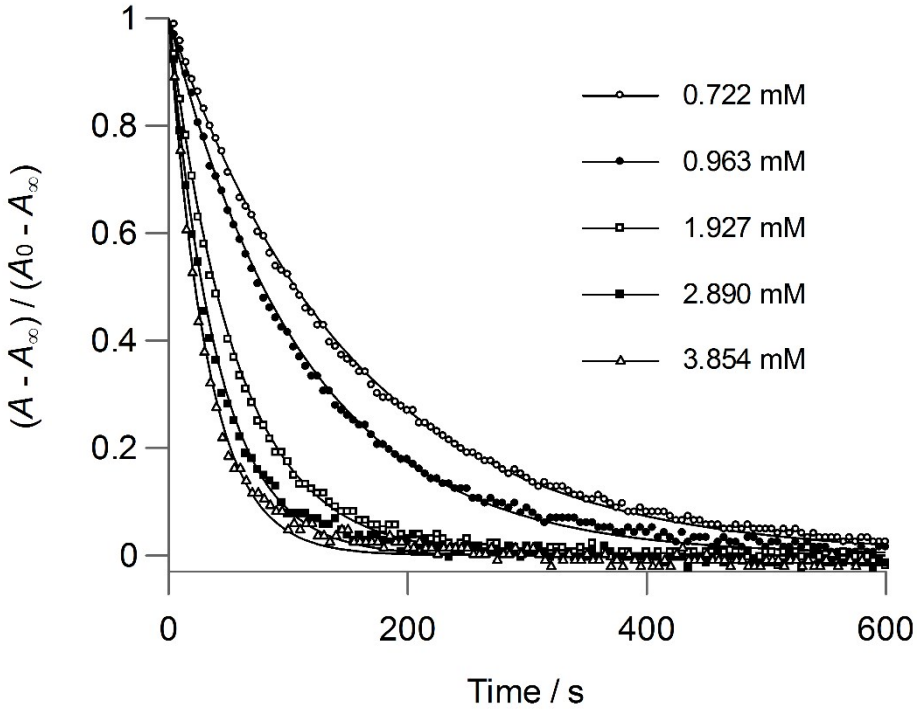


Figure S3: Reaction of **8Cl** with BCN. Plots show **8Cl** absorbance loss (normalised) at 377 nm with time. The reactions were carried out in methanol at 25°C under pseudo-first-order conditions, with $[\mathbf{8Cl}]_0 = 6.36 \times 10^{-5}$ M and BCN concentrations as stated in the legend. Lines are the best fit to a monoexponential decay using non-linear least squares analysis and the rate data shown in Table S1.

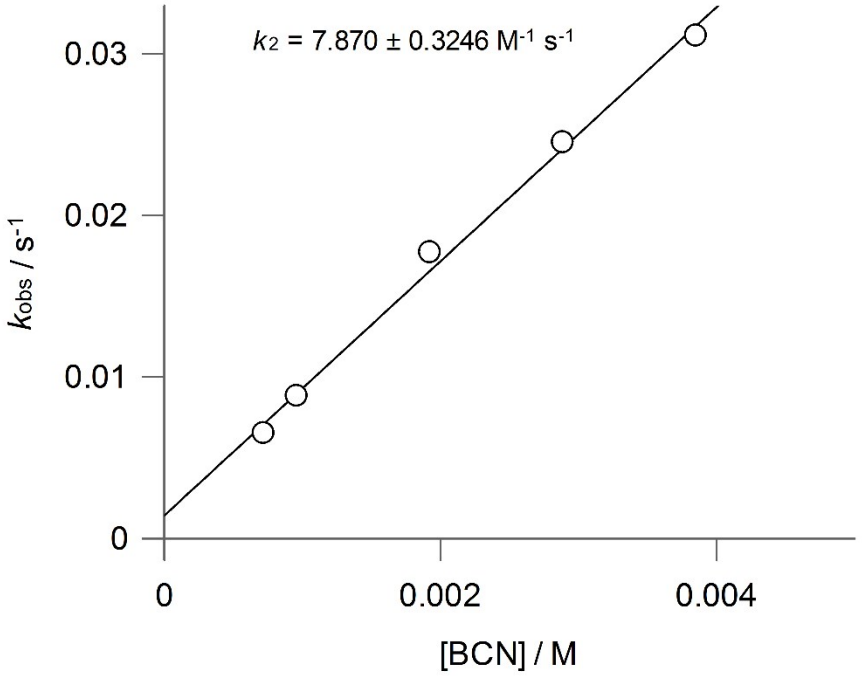


Figure S4: Plot of observed pseudo-first-order rate constant, k_{obs}, against BCN concentration for the reaction with **8Cl**. The slope, which is equal to the second order rate constant, k_2, is shown on the plot. Conditions are as stated in Figure S3.

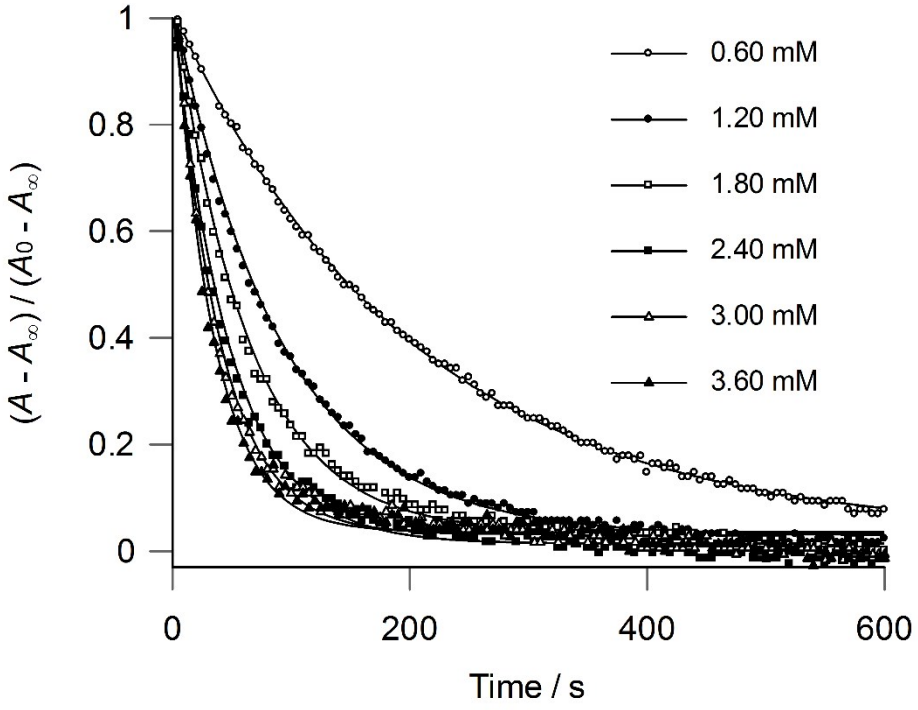


Figure S5: Reaction of $\mathbf{8PF_6}$ with BCN. Plots show $\mathbf{8PF_6}$ absorbance loss (normalised) at 377 nm with time. The reactions were carried out in methanol at 25°C under pseudo-first-order conditions, with $[\mathbf{8PF_6}]_0$ = 5.48 x 10^{-5} M and BCN concentrations as stated in the legend. Lines are the best fit to a monoexponential decay using non-linear least squares analysis and the rate data shown in Table S1.

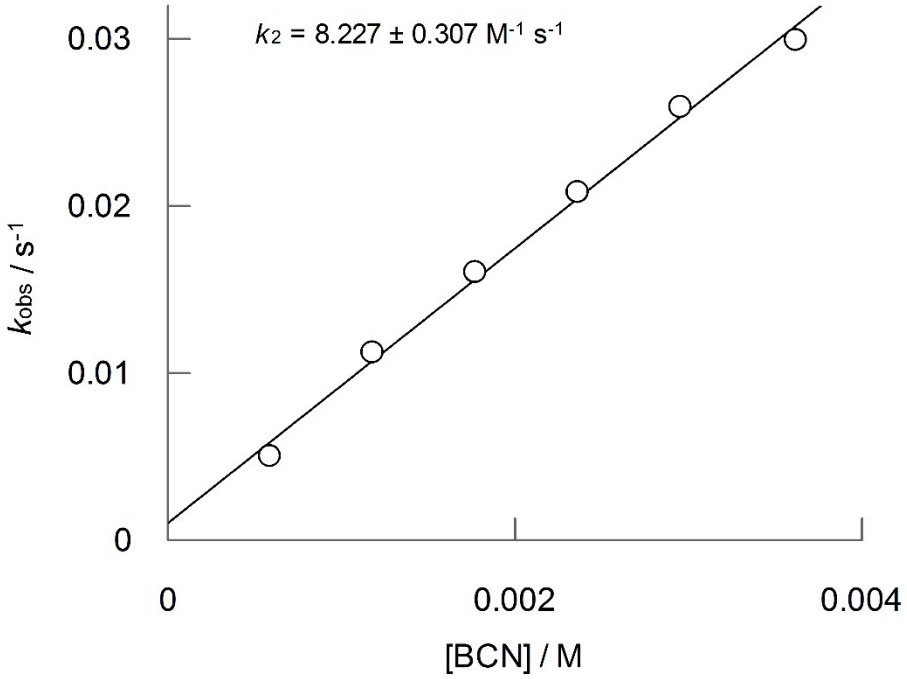


Figure S6: Plot of observed pseudo-first-order rate constant, k_{obs}, against BCN concentration for the reaction with **8PF$_6$**. The slope, which is equal to the second order rate constant, k_2, is shown on the plot. Conditions are as stated in Figure S5.

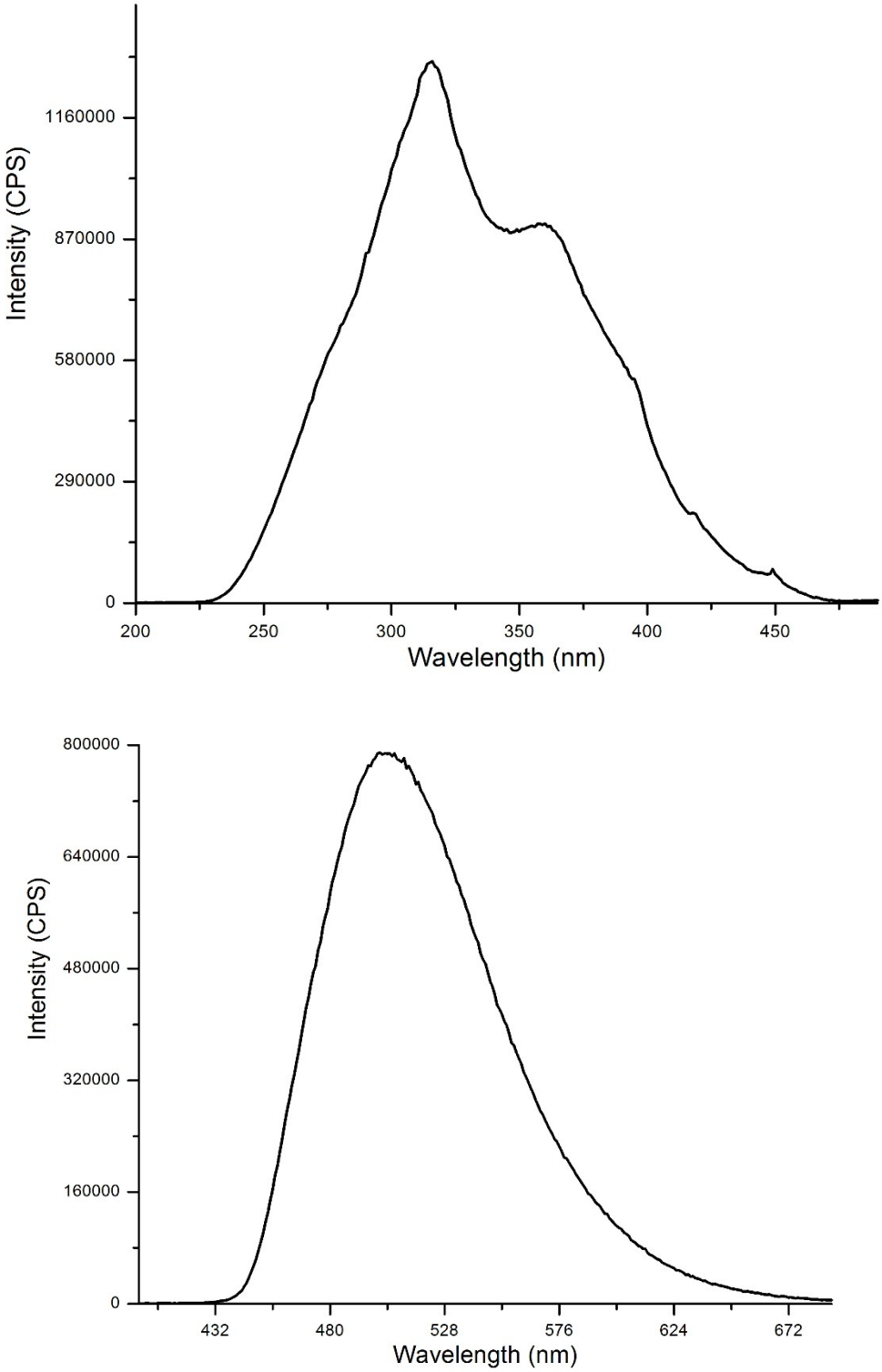


Figure S7: Excitation spectrum (top, for the emission at 504 nm) and emission spectrum (bottom, the sample was excited at 350 nm) of **8PF6** in acetonitrile.

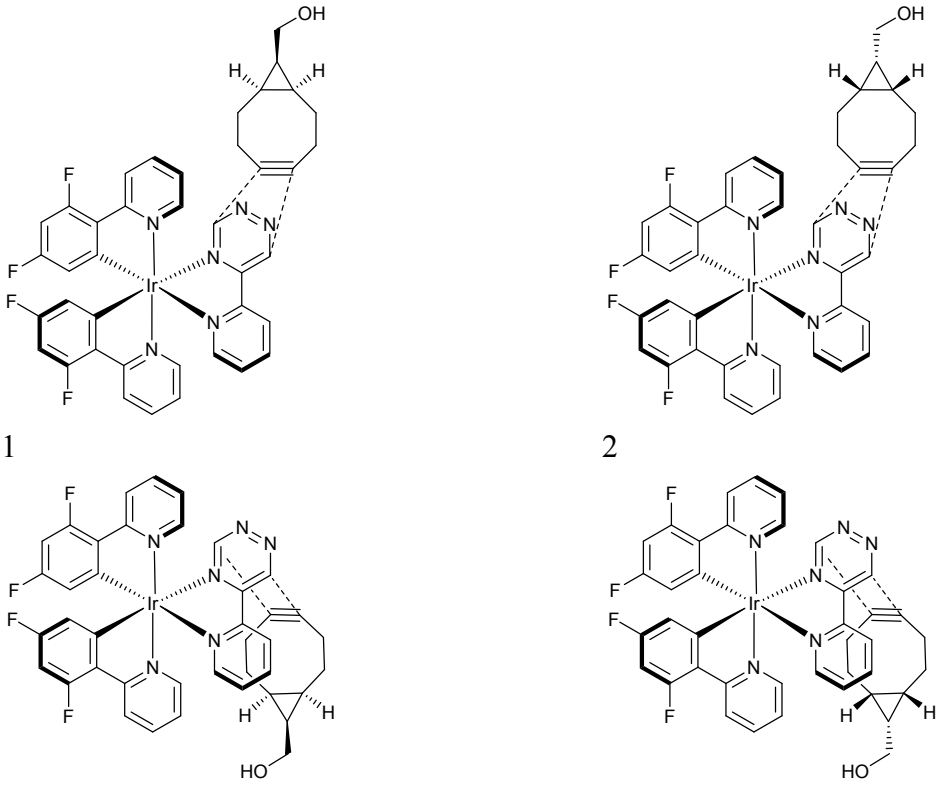
Computational methods

All electronic structure calculations were performed using Gaussian 09[14] using the M06 functional,[15] the 6-311G(d,p) basis set[16] for non-metallic atoms, and the LANL2DZ basis set[17] with included ECP and augmented with one f-polarisation function for Ir. This level of theory has been used successfully in previous studies calculating the energies of Ir-centred organometallic compounds,[18] and is also comparable to previous computational studies of transition states in Diels-Alder reactions.[3a, 12-13]

Each isolated molecule and transition-state geometry was optimised without solvent, and the correction to obtain the Gibbs energy was determined using a quasi-harmonic correction for the entropy calculation[13b, 19]with a temperature of 298.15 K. Each transition state was verified by checking for a single negative frequency corresponding to vibrations of the reacting groups in the diene and dienophile. Energies of solvated species were calculated using the IEFPCM solvation model, and the Gibbs energies of the solvated structures were determined by adding the previously calculated correction to the energy of the solvated structure.[13b, 19a]

For each reaction, multiple transition state geometries were shown to be possible, an example of which is shown in Figure S7 in the Supporting Information, so the effective activation energy, $\Delta G^{\ddagger}_{calc}$, was determined for each reaction from the activation energies of the different transition state geometries.[20] Experimental activation energies were determined using the Eyring equation.[12]

Each distortion energy was determined as the difference between the calculated energy of the individual molecular geometry taken from the transition state and the energy of the respective optimised geometry. Each interaction energy was determined as the difference between the sum of the fragment energies and the optimised transition state energy.[13c]



1 2

3 4

Figure S8: Representations of the 4 possible transition state geometries for the reaction between **8** and BCN, from which the calculated parameters were determined.

Table S1: calculated energies of gas-phase optimised structures and transition state geometries, along with Gibbs correction energies and solvation energies.

Dienophile	Diene	Geometry	Gas phase SCF energy / Ha	Gibbs correction energy / Ha	Solvation energy / Ha
1	-	Optimised	-527.16835	0.098697	0.00916377
8	-	Optimised	-1985.68667	0.365853	0.06785441
-	BCN	Optimised	-464.38169	0.182428	0.00731441
1	BCN	TS conformation 1	-991.53359	0.305876	0.01549673
1	BCN	TS conformation 2	-991.53336	0.305406	0.01556631
8	BCN	TS conformation 1	-2450.07442	0.576202	0.06421940
8	BCN	TS conformation 2	-2450.06910	0.575897	0.06892663
8	BCN	TS conformation 3	-2450.06759	0.575736	0.06834110
8	BCN	TS conformation 4	-2450.06791	0.573839	0.06839961

Coordinates of computed stationary points:

BCN

```
C    0.664499   -1.676291  -0.328174
C    2.203025   -1.583842  -0.244128
C    -0.025365  -0.950833  0.806706
C    2.499310   -0.160685  -0.257668
C    -0.365866  0.522463   0.884092
C    2.229529   1.009158   -0.205862
C    -0.086933  1.582853   -0.159096
C    1.344963   2.153916   -0.067727
H    0.359578   -2.732077  -0.309729
H    0.357129   -1.280584  -1.302371
H    2.664946   -2.127097  -1.076177
H    2.563567   -2.054896  0.680268
H    1.492347   2.652785   0.899676
H    -1.651372  -0.486789  -1.393051
C    -1.461840  -0.509749  0.756195
H    -2.731335  -1.608265  -0.571657
H    -0.804103  2.407877   -0.031676
H    -0.232036  1.194426   -1.174108
H    1.501800   2.914326   -0.841236
C    -2.279254  -0.612604  -0.497716
O    -3.353526  0.302488   -0.498780
H    0.274430   -1.355548  1.774539
H    -0.254427  0.919668   1.893999
H    -2.059162  -0.682662  1.649815
H    -2.985560  1.172627   -0.327782
```

1

```
C    1.488763   -1.172424  0.000001
C    0.750346   0.019287   0.000000
C    2.716559   1.096851   -0.000003
H    0.990328   -2.135845  0.000001
H    3.274159   2.031078   0.000002
```

C	-0.726609	0.018769	0.000000
C	-1.431609	1.218765	-0.000001
C	-2.814339	1.168833	-0.000001
H	-0.885803	2.155021	-0.000001
C	-2.646653	-1.208518	0.000001
C	-3.439862	-0.067738	0.000000
H	-3.397852	2.084053	-0.000001
H	-3.105386	-2.195827	0.000001
H	-4.520960	-0.154018	0.000000
N	3.448952	-0.006598	0.000001
N	2.808847	-1.172262	0.000002
N	1.387666	1.180387	-0.000001
N	-1.321760	-1.176872	0.000001

8

C	3.168533	-0.709142	0.502146
N	1.940462	-1.099842	0.865423
N	4.113915	-2.487110	1.772704
C	3.255645	0.457558	-0.384191
N	2.068569	0.949094	-0.803842
C	4.458073	1.027719	-0.779101
H	5.401616	0.626350	-0.427937
C	4.438314	2.125318	-1.624521
C	3.218125	2.621430	-2.052824
C	2.054885	2.006416	-1.617884
H	1.073666	2.358800	-1.926505
H	5.366622	2.586311	-1.943208
H	3.158407	3.477431	-2.714567
Ir	0.274816	-0.013771	0.006694
C	-1.229179	-1.053957	0.836105
C	-1.874445	-0.752338	2.028850
C	-1.603988	-2.216225	0.116907
C	-2.866262	-1.595889	2.499223
H	-1.642329	0.132457	2.614416
C	-2.614096	-3.026195	0.643293
C	-3.254846	-2.740419	1.827092
H	-4.033489	-3.388413	2.210059
C	-0.883640	-2.454165	-1.122700
C	0.820699	-1.617345	-2.500134
C	-1.111121	-3.480607	-2.042959
C	0.629223	-2.605890	-3.437533
H	1.567805	-0.841855	-2.641713
C	-0.359428	-3.552870	-3.197293
H	-1.884087	-4.208508	-1.845555
H	1.236511	-2.628366	-4.333980
H	-0.545244	-4.345909	-3.913737
C	-1.106594	1.146957	-0.875573
C	-1.419079	2.330382	-0.160722
C	-1.718860	0.909795	-2.100435
C	-2.338741	3.220878	-0.719613
C	-2.619923	1.832894	-2.602878
H	-1.530393	0.015919	-2.687659
C	-2.948221	2.997564	-1.933466
H	-3.658123	3.707203	-2.340050

C	-0.732963	2.505584	1.108769
C	0.851580	1.537357	2.539945
C	-0.928741	3.533325	2.034536
C	0.690462	2.525715	3.483843
H	1.535920	0.710292	2.702857
C	-0.222131	3.539607	3.219470
H	-1.645522	4.312481	1.819691
H	1.259701	2.493965	4.404675
H	-0.385935	4.332354	3.941633
N	0.091630	-1.536460	-1.376240
N	0.164922	1.519302	1.387336
F	-2.652439	4.348239	-0.079350
F	-3.192903	1.595762	-3.774588
F	-2.988629	-4.131706	-0.001564
F	-3.471379	-1.297756	3.639972
C	4.259038	-1.439627	0.981565
H	5.282899	-1.186140	0.722892
C	1.864588	-2.174384	1.667735
H	0.870521	-2.497817	1.967779
N	2.891557	-2.862652	2.124517

1 BCN (TS conformation 1)

C	-1.530343	-0.836091	-1.342684
C	-2.269210	-0.748061	-0.134524
C	-1.124719	-2.567252	0.414992
H	-1.633936	-0.071845	-2.106128
H	-0.787630	-3.278316	1.165918
C	-3.247386	0.326980	0.106167
C	-4.031842	0.320568	1.257406
C	-4.939650	1.346766	1.441421
H	-3.909590	-0.483423	1.974479
C	-4.191820	2.270590	-0.622571
C	-5.027206	2.345983	0.483223
H	-5.572628	1.369088	2.323160
H	-4.229723	3.041102	-1.391005
H	-5.726071	3.168864	0.587067
N	-0.978339	-2.974500	-0.881357
N	-1.217875	-2.090028	-1.782892
N	-2.042914	-1.653419	0.784251
N	-3.321884	1.288189	-0.818791
C	2.880788	-0.970768	1.388303
C	1.635298	-1.857252	1.255784
C	3.578556	-0.759325	0.067324
C	0.691198	-1.225275	0.333950
C	3.237969	0.332919	-0.916262
C	0.406051	-0.350259	-0.484294
C	2.147978	1.356982	-0.712494
C	0.785253	0.865285	-1.214425
H	3.579230	-1.438042	2.095962
H	2.578080	-0.018627	1.838387
H	1.179116	-2.019599	2.240793
H	1.910662	-2.851929	0.874409
H	0.835592	0.648498	-2.291629
H	3.971331	1.626819	1.476873

C	4.552472	0.358477	-0.173736
H	5.635351	1.055528	1.535834
H	2.402783	2.278933	-1.255805
H	2.050992	1.636738	0.343218
H	0.023361	1.646900	-1.095820
C	4.852774	1.409102	0.854823
O	5.356506	2.587170	0.263678
H	3.851623	-1.702638	-0.406409
H	3.318945	0.021629	-1.958002
H	5.429235	0.110573	-0.769466
H	4.731348	2.854478	-0.414164

1 BCN (TS conformation 2)

N	-3.200447	-1.173389	0.862438
C	-3.106850	-0.313078	-0.155176
C	-3.791122	-0.480711	-1.357153
C	-4.614556	-1.582263	-1.496494
C	-4.718869	-2.480760	-0.444723
C	-3.987616	-2.230887	0.708115
C	-2.208288	0.838678	0.033365
C	-1.635371	1.149867	1.293763
N	-1.413381	2.471500	1.558181
N	-1.073486	3.207811	0.560838
C	-1.045790	2.596026	-0.659521
N	-1.886467	1.595959	-0.985917
C	0.378487	0.588571	0.782516
C	0.771739	1.322007	-0.125090
C	1.785261	1.746969	-1.089068
C	3.068137	0.930794	-0.873335
C	2.839343	-0.551840	-1.036832
C	3.762360	-1.604925	-0.472362
C	4.974035	-1.233065	0.331098
O	6.069689	-0.910608	-0.497467
C	0.627279	-0.557934	1.666398
C	2.070074	-1.037957	1.478833
C	2.364848	-1.470141	0.063128
H	-1.822821	0.514491	2.153078
H	-0.623897	3.188250	-1.468498
H	-3.655876	0.251700	-2.145430
H	-5.169331	-1.741415	-2.416089
H	-4.041997	-2.917755	1.551182
H	-5.352465	-3.358439	-0.512061
H	2.254810	-1.881533	2.158039
H	2.742245	-0.234395	1.800457
H	0.454443	-0.286601	2.715542
H	-0.088847	-1.361780	1.437960
H	1.412107	1.597922	-2.113368
H	4.752883	-0.413718	1.031720
H	5.296549	-2.084996	0.940325
H	3.819611	1.270910	-1.601146
H	3.466776	1.169419	0.119509
H	1.993476	2.820090	-0.989542
H	1.675596	-2.241903	-0.281040
H	2.428001	-0.797834	-2.016336

H	3.948300	-2.457697	-1.122831
H	5.770782	-0.239135	-1.115276

8 BCN (TS conformation 1)

C	0.645863	2.602627	0.711766
N	0.356082	1.398271	1.183603
N	1.702953	3.140539	2.758883
C	-0.063315	3.043030	-0.491563
N	-0.883471	2.121219	-1.044880
C	0.077663	4.310015	-1.042147
H	0.724494	5.042474	-0.572581
C	-0.636675	4.633398	-2.184003
C	-1.480548	3.685992	-2.740904
C	-1.577280	2.442019	-2.139698
H	-2.224749	1.665232	-2.539780
H	-0.542251	5.618643	-2.627388
H	-2.063493	3.901392	-3.628528
Ir	-1.020315	0.200013	0.010926
C	-0.932144	-1.473704	1.107106
C	-1.740870	-1.792802	2.192612
C	0.125351	-2.348361	0.751651
C	-1.481725	-2.939843	2.921427
H	-2.577911	-1.172267	2.499679
C	0.351480	-3.477385	1.542963
C	-0.433713	-3.795147	2.626736
H	-0.235507	-4.677394	3.222364
C	0.904084	-1.963307	-0.414148
C	1.213451	-0.259740	-1.990475
C	1.886565	-2.724979	-1.052202
C	2.194445	-0.969776	-2.648184
H	0.896611	0.719664	-2.337718
C	2.532095	-2.230476	-2.167545
H	2.135021	-3.702583	-0.664281
H	2.672086	-0.547151	-3.524839
H	3.310635	-2.816594	-2.645957
C	-2.382516	-0.682995	-1.171403
C	-3.725862	-0.387577	-0.825523
C	-2.125597	-1.485049	-2.278081
C	-4.747084	-0.904629	-1.625566
C	-3.182079	-1.969669	-3.029290
H	-1.118502	-1.759062	-2.579435
C	-4.502972	-1.691841	-2.728365
H	-5.316914	-2.079554	-3.328269
C	-3.913031	0.444226	0.352401
C	-2.768103	1.636861	2.015510
C	-5.127005	0.787780	0.952070
C	-3.938646	2.000803	2.641282
H	-1.801463	1.934880	2.410486
C	-5.137891	1.560226	2.095790
H	-6.051594	0.432446	0.520944
H	-3.906020	2.605691	3.539052
H	-6.081932	1.815682	2.565469
N	0.579709	-0.734468	-0.907513
N	-2.747852	0.880286	0.907619

F	-6.023580	-0.633736	-1.341737
F	-2.922178	-2.730104	-4.085460
F	1.386193	-4.281077	1.276167
F	-2.257234	-3.230657	3.957127
C	1.615449	3.339643	1.415938
H	2.014746	4.283709	1.058531
C	1.077972	1.032764	2.275955
H	0.925739	0.023115	2.649832
N	1.422271	1.975543	3.208103
C	4.982497	1.675195	-0.959999
C	4.081028	2.616427	-0.145251
C	5.985421	0.937455	-0.106505
C	3.310206	1.807124	0.796245
C	5.712013	-0.332476	0.662939
C	3.003542	0.765208	1.378045
C	4.412584	-1.091102	0.642597
C	3.389874	-0.624556	1.680940
H	5.516897	2.269910	-1.712541
H	4.330454	0.987989	-1.511756
H	3.421084	3.171745	-0.826283
H	4.681026	3.364038	0.391586
H	3.802104	-0.671699	2.698556
H	5.269210	-0.690959	-2.181045
C	6.627672	-0.360642	-0.531001
H	6.967282	-0.896387	-2.574791
H	4.614271	-2.159575	0.790425
H	3.946864	-1.016122	-0.343122
H	2.522325	-1.297851	1.665955
C	6.216480	-1.076917	-1.791094
O	5.998329	-2.461182	-1.606049
H	6.662798	1.619659	0.406940
H	6.220605	-0.378997	1.625679
H	7.688929	-0.451393	-0.305296
H	6.826027	-2.866742	-1.336513

8 BCN (TS conformation 2)

C	0.790739	-2.415543	-0.828493
N	0.445416	-1.188197	-1.190578
N	1.894162	-2.703251	-2.900321
C	0.088010	-2.999642	0.316010
N	-0.811960	-2.184770	0.910849
C	0.318737	-4.286741	0.782499
H	1.028923	-4.931906	0.277604
C	-0.381636	-4.738576	1.888919
C	-1.301956	-3.897360	2.493782
C	-1.491587	-2.628957	1.970922
H	-2.202918	-1.931300	2.406484
H	-0.215747	-5.740502	2.269390
H	-1.874284	-4.214243	3.357540
Ir	-1.069786	-0.208271	-0.012849
C	-1.087875	1.559691	-0.958928
C	-1.870627	1.903258	-2.055654
C	-0.156809	2.500454	-0.452156
C	-1.714689	3.147143	-2.641435

H	-2.612902	1.232108	-2.477689
C	-0.033457	3.732439	-1.098832
C	-0.797638	4.080067	-2.188385
H	-0.681937	5.043473	-2.669227
C	0.607545	2.075398	0.707276
C	1.047880	0.246646	2.104652
C	1.464486	2.864997	1.479797
C	1.906593	0.980747	2.891490
H	0.837060	-0.797105	2.319083
C	2.102244	2.320463	2.575956
H	1.607675	3.904316	1.218605
H	2.394554	0.516674	3.740280
H	2.751851	2.939822	3.186121
C	-2.559111	0.451871	1.164164
C	-3.853221	0.069419	0.729222
C	-2.424500	1.177865	2.342547
C	-4.951176	0.429183	1.513642
C	-3.552298	1.505486	3.075058
H	-1.461087	1.514508	2.715633
C	-4.828022	1.140910	2.685607
H	-5.699399	1.406091	3.271177
C	-3.911853	-0.671644	-0.521209
C	-2.593010	-1.603258	-2.221108
C	-5.062492	-1.071182	-1.204509
C	-3.698002	-2.014086	-2.931948
H	-1.587670	-1.775395	-2.594338
C	-4.954623	-1.737593	-2.407977
H	-6.032628	-0.842044	-0.788173
H	-3.571945	-2.529268	-3.876251
H	-5.850451	-2.036528	-2.941885
N	0.400492	0.773802	1.054728
N	-2.688846	-0.950867	-1.052319
F	-6.184246	0.074834	1.143593
F	-3.408926	2.194874	4.199982
F	0.870532	4.620000	-0.673082
F	-2.465820	3.461820	-3.687357
C	1.795358	-3.039432	-1.586904
H	2.231324	-3.994977	-1.312499
C	1.158497	-0.683240	-2.230918
H	0.955350	0.348802	-2.507703
N	1.560891	-1.514321	-3.242487
C	4.792681	1.315349	-1.047838
C	3.377599	1.027420	-1.561453
C	4.923856	1.050802	0.431366
C	3.066914	-0.392569	-1.323402
C	5.307892	-0.276101	1.046568
C	3.462792	-1.460373	-0.856738
C	5.616841	-1.521911	0.257334
C	4.354556	-2.330056	-0.097027
H	5.036024	2.366128	-1.251535
H	5.498779	0.717135	-1.633928
H	3.297452	1.259700	-2.631014
H	2.641928	1.666120	-1.047260
H	3.856083	-2.676238	0.821954

H	7.468819	0.503096	-0.613968
C	6.244448	0.902863	1.128029
H	7.885836	1.965203	0.268120
H	6.282066	-2.176523	0.836921
H	6.145670	-1.288709	-0.673501
H	4.630461	-3.224943	-0.667189
C	7.557835	0.928322	0.397907
O	8.571688	0.290267	1.134932
H	4.170781	1.593130	1.003444
H	4.784068	-0.496999	1.978253
H	6.321360	1.363160	2.111213
H	8.294441	-0.613781	1.299909

8 BCN (TS conformation 3)

C	-0.985535	-1.294821	2.095506
N	-0.525111	-1.378956	0.856690
N	-2.035040	-3.401346	1.841064
C	-0.363672	-0.301097	2.974426
N	0.658410	0.391524	2.422985
C	-0.773560	-0.069747	4.280742
H	-1.583810	-0.648557	4.710062
C	-0.127999	0.901335	5.028540
C	0.915262	1.610419	4.455029
C	1.278512	1.324581	3.149468
H	2.092838	1.849745	2.655993
H	-0.433783	1.098129	6.050252
H	1.448201	2.375715	5.006720
Ir	1.082116	-0.030599	0.304284
C	1.281574	-0.596785	-1.608854
C	0.656876	-0.012061	-2.705446
C	2.104054	-1.734468	-1.801818
C	0.836724	-0.561293	-3.963417
H	0.033845	0.874711	-2.618367
C	2.248968	-2.237720	-3.096294
C	1.625834	-1.674328	-4.187580
H	1.756595	-2.088548	-5.179502
C	2.719389	-2.280043	-0.602410
C	2.855172	-2.019992	1.723018
C	3.622573	-3.342487	-0.530054
C	3.748759	-3.059537	1.845087
H	2.526596	-1.450463	2.587155
C	4.136222	-3.729170	0.690997
H	3.917151	-3.851437	-1.436312
H	4.132556	-3.333178	2.820185
H	4.841567	-4.551629	0.743814
C	2.558455	1.298193	0.008312
C	2.119040	2.602625	-0.329135
C	3.921196	1.051982	0.137593
C	3.078046	3.601017	-0.515956
C	4.822184	2.084459	-0.057123
H	4.316024	0.068701	0.377068
C	4.427874	3.369857	-0.383189
H	5.147832	4.164461	-0.534400
C	0.681613	2.775892	-0.442704

C	-1.373309	1.676641	-0.205208
C	0.008700	3.934791	-0.840788
C	-2.079077	2.793504	-0.593864
H	-1.882192	0.751449	0.053988
C	-1.367778	3.940997	-0.923032
H	0.578469	4.817442	-1.092785
H	-3.162165	2.762758	-0.640301
H	-1.886529	4.838562	-1.242742
N	2.354143	-1.634991	0.540105
N	-0.032984	1.655803	-0.138890
F	2.701186	4.843932	-0.826765
F	6.118925	1.837735	0.073888
F	3.009833	-3.311456	-3.316252
F	0.225571	-0.000783	-5.000327
C	-2.030598	-2.175436	2.436840
H	-2.561013	-2.118544	3.382536
C	-1.130090	-2.321652	0.088293
H	-0.827946	-2.372765	-0.954792
N	-1.571147	-3.486821	0.652488
C	-4.362169	-0.270362	-1.907363
C	-3.181430	-1.204296	-1.619096
C	-5.679855	-0.815605	-1.414084
C	-3.059165	-1.385124	-0.164021
C	-6.242061	-0.609035	-0.025448
C	-3.545880	-1.204507	0.953119
C	-5.556978	0.163386	1.073500
C	-4.561174	-0.693478	1.872627
H	-4.421727	-0.105844	-2.991068
H	-4.140487	0.707822	-1.463411
H	-2.255854	-0.788906	-2.042797
H	-3.328230	-2.182692	-2.097830
H	-5.081704	-1.528205	2.362526
H	-5.873419	1.961616	-1.114931
C	-6.888117	0.052819	-1.217686
H	-6.982875	1.783736	-2.469622
H	-6.306987	0.551521	1.776489
H	-5.023695	1.037296	0.680476
H	-4.106458	-0.093059	2.672770
C	-6.850692	1.542422	-1.409104
O	-7.899766	2.183629	-0.727780
H	-5.896893	-1.799631	-1.829334
H	-6.786112	-1.472726	0.356521
H	-7.840185	-0.372535	-1.527914
H	-7.899277	1.869540	0.179408

8 BCN (TS conformation 4)

C	0.903575	-0.081739	2.548862
N	0.522968	0.665027	1.522798
N	1.961636	1.798858	3.516338
C	0.207926	-1.357939	2.741884
N	-0.810550	-1.586469	1.882927
C	0.541803	-2.275066	3.729363
H	1.354673	-2.067806	4.416520
C	-0.183017	-3.451492	3.830014

C	-1.227496	-3.677348	2.947590
C	-1.508505	-2.719569	1.987594
H	-2.317306	-2.848903	1.272441
H	0.062593	-4.180946	4.593956
H	-1.822374	-4.581561	2.996774
Ir	-1.070891	-0.108525	0.274043
C	-1.106074	1.359072	-1.090811
C	-0.404691	1.377779	-2.292040
C	-1.888566	2.482016	-0.726065
C	-0.478149	2.495271	-3.106010
H	0.196108	0.537364	-2.629354
C	-1.926277	3.576078	-1.592781
C	-1.231489	3.608135	-2.781469
H	-1.280837	4.471884	-3.432668
C	-2.574734	2.386061	0.552145
C	-2.877726	1.007335	2.422414
C	-3.454589	3.319507	1.103921
C	-3.752224	1.895665	3.006861
H	-2.620757	0.066668	2.900060
C	-4.043934	3.071649	2.326959
H	-3.672698	4.228629	0.562647
H	-4.194794	1.667046	3.968461
H	-4.732458	3.794747	2.750928
C	-2.563784	-0.997241	-0.731679
C	-2.146810	-1.989527	-1.653092
C	-3.922006	-0.742192	-0.574727
C	-3.123752	-2.683811	-2.371660
C	-4.841284	-1.464051	-1.315199
H	-4.299457	0.016154	0.104883
C	-4.469998	-2.442399	-2.220200
H	-5.204050	-2.995833	-2.792365
C	-0.712513	-2.196245	-1.752271
C	1.355828	-1.515265	-0.884900
C	-0.055004	-3.074559	-2.617701
C	2.045441	-2.369471	-1.717189
H	1.880445	-0.867252	-0.185042
C	1.321246	-3.158633	-2.601659
H	-0.634216	-3.678916	-3.300170
H	3.127814	-2.409940	-1.672654
H	1.829657	-3.837220	-3.278338
N	-2.307084	1.236366	1.230757
N	0.016791	-1.421336	-0.899387
F	-2.770290	-3.632606	-3.242659
F	-6.133705	-1.213033	-1.154401
F	-2.648371	4.654055	-1.281317
F	0.203896	2.503722	-4.245355
C	1.920914	0.445618	3.364952
H	2.392545	-0.124453	4.159272
C	1.171249	1.852337	1.411706
H	0.931270	2.461997	0.543778
N	1.570910	2.522013	2.536331
C	5.677306	-0.653969	1.205085
C	4.466559	-0.617484	2.152123
C	5.296774	-0.997906	-0.213843

C	3.535610	0.389732	1.649038
C	4.859376	0.004451	-1.261871
C	3.119836	1.182476	0.803809
C	4.721900	1.488097	-1.025784
C	3.341232	1.869566	-0.478072
H	6.392096	-1.397407	1.580258
H	6.184716	0.314420	1.264807
H	4.792442	-0.393321	3.174512
H	3.971639	-1.602047	2.182609
H	2.550551	1.588688	-1.192575
H	7.444355	0.807256	-0.476148
C	6.185025	-0.700875	-1.386751
H	8.276619	-0.671457	-0.944383
H	4.871728	2.024703	-1.972576
H	5.485984	1.861566	-0.334785
H	3.263270	2.956078	-0.346155
C	7.493317	0.029010	-1.253793
O	7.912742	0.560969	-2.485160
H	4.782177	-1.959812	-0.270520
H	4.091123	-0.369416	-1.941145
H	6.231255	-1.468367	-2.157222
H	7.242341	1.177204	-2.789091

Materials and Methods

Cell lines

The human malignant melanoma, A375, cell line was obtained from Sigma-Aldrich (St. Louis, MO, USA). The normal human immortalized keratinocyte, HaCaT, cell line was kindly provided by Dr. Sharon Broby (Dermal Toxicology and Effects Group; Centre for Radiation, Chemical and Environmental Hazards; Public Health England, UK). Both cell lines were cultured in Dulbeccos's Modified Eagle Medium (DMEM), high glucose, supplemented with 10% fetal bovine serum, 2mM L-glutamine and 1% pen/strep (100u/ml penicillin, 100ug/ml streptomycin). Cells were maintained in a humidified atmosphere at 37^{o}C and 5% CO_2. All cell culture media and reagents were purchased from Labtech International Ltd (East Sussex, UK) whereas all plasticware from Corning (NY, USA).

Treatment with 8Cl

A375 and HaCaT cells were plated into 96-well plates, at appropriate cell densities, over different incubation periods and cultured overnight at 37^{o}C. On the following day, media was discarded and cells were exposed to various concentrations of VNK-477 at 24, 48 and 72hrs of exposure. **8Cl** was dissolved in Dimethylsulfoxide (DMSO) and prepared fresh for each experiment. Control cells were incubated with media only while additional DMSO controls were used for higher concentrations of **8Cl** (i.e. 0.1% DMSO for less than 50μM of **8Cl**, 0.2% DMSO for 50μM of **8Cl** and 0.4% DMSO for 100μM of **8Cl**).

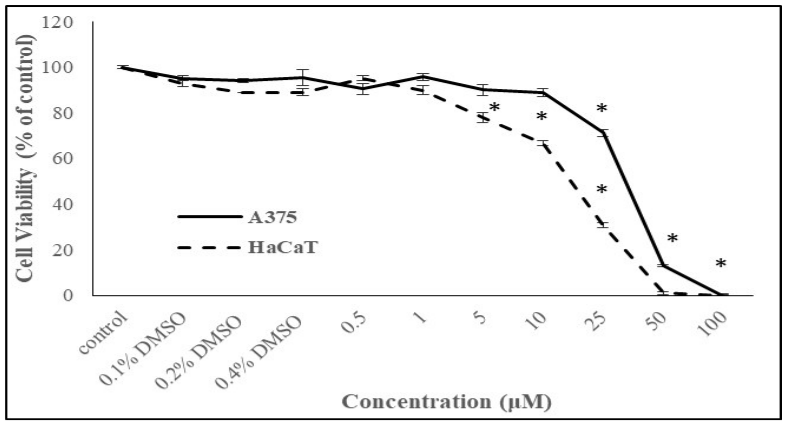
Determination of cell viability

Cell viability levels were determined by utilizing the Alamar blue assay that is based on the reduction of resasurin to resorufin, by metabolically-active cells, which can then be monitored spectrophotometrically. More specifically, following exposure to various concentrations of **8Cl** (over different time courses) 10μl of 1mg/ml of resazurin reagent (dissolved in PBS) was added into each well, mixed by gentle shaking and incubated at 37^{o}C for 4h in both A375 and HaCaT cells respectively. Absorbance was recorded at 570 and 600nm by using the Spark multimode plate reader (Tecan UK Ltd., Reading, UK). Rates of viable cells were estimated according to the amount of the oxidized/reduced forms of the reagent. Cell viability was expressed as percentage of control cells and five replicates (n=5) of each experimental condition were used in each experiment.

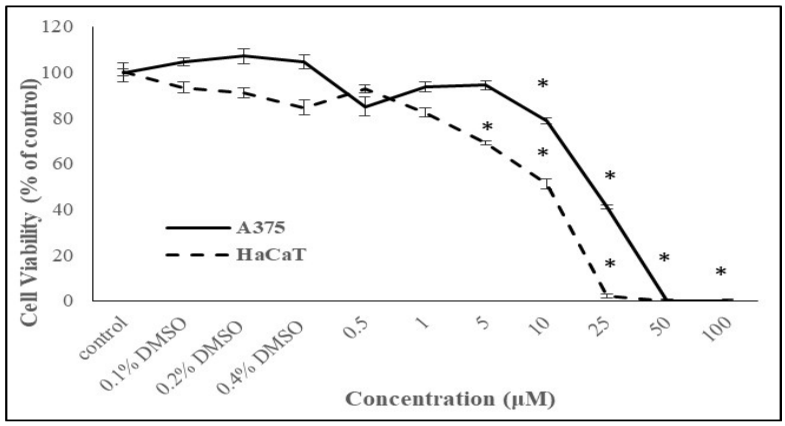
Statistical analysis

Comparisons were made between control and **8Cl** exposed groups while each experiment was repeated two independent times. Means were compared by one-way ANOVA with post hoc Bonferoni test. SPSS 22.0 software was used for all statistical analyses. A value of $p<0.05$ was considered statistically significant.

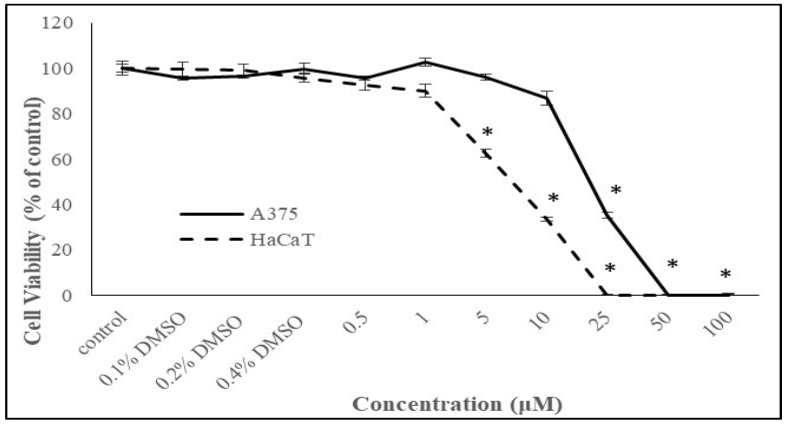
A.



B.



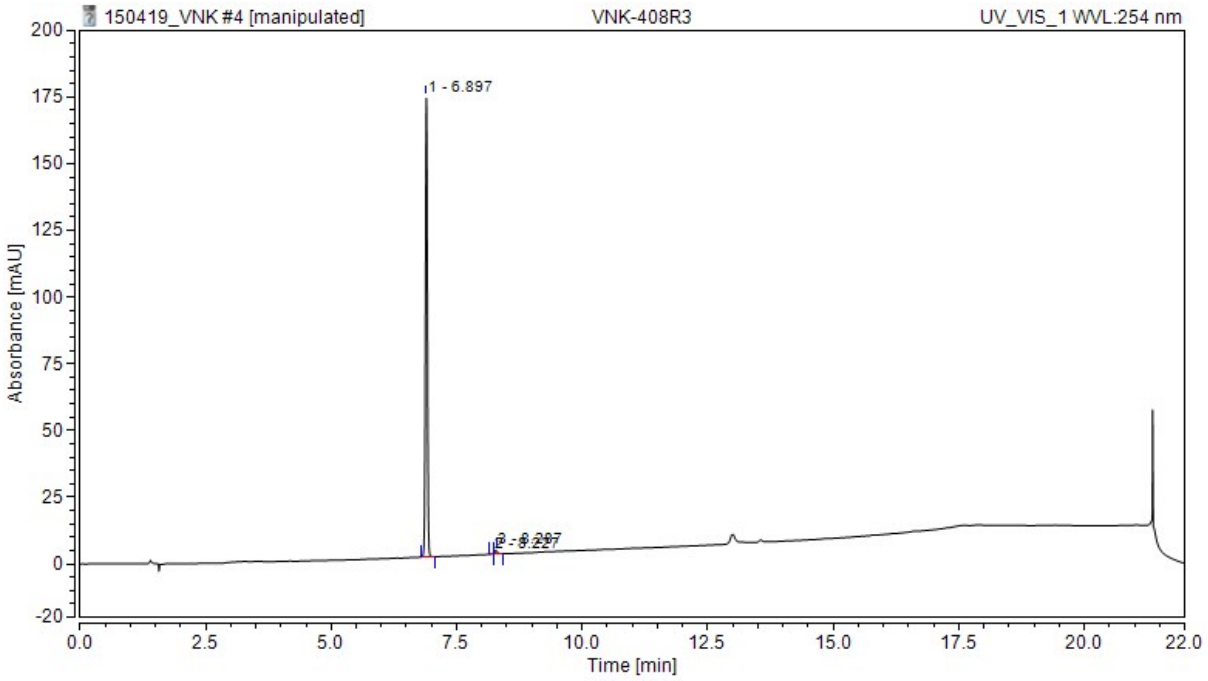
C.



D.

	A375	**HaCaT**
$EC50_{24h}$	32.93 ± 2.47	17.52 ± 3.43
$EC50_{48h}$	22.8 ± 2.98	10.22 ± 1.87
$EC50_{72h}$	20.7 ± 1.22	7.27 ± 0.65

Figure S9: Cytotoxicity profile of 8Cl in human malignant melanoma (A375) and keratinocyte (HaCaT) cells. Cytotoxic effect of increasing concentrations of **8Cl** over 24 **(A)**, 48 **(B)** and 72hrs **(C)** of exposure on A375 and HaCaT cells; Table showing EC_{50} values ± SEM of **8Cl** in A375 and HaCaT cell lines following different incubation periods **(D)**. Briefly, cells were seeded in 96-well plates and incubated overnight at 37°C. On the following day, cells were exposed to various concentrations of **8Cl** over different incubation periods (e.g. 24, 48 and 72hrs). Cell viability levels were determined by using the Alamar blue assay and were expressed as percentage of control cells. Data are representative of two independent experiments. Data shown are means ± SEM (n=5) whereas asterisks indicate statistically significant differences at $p<0.05$.



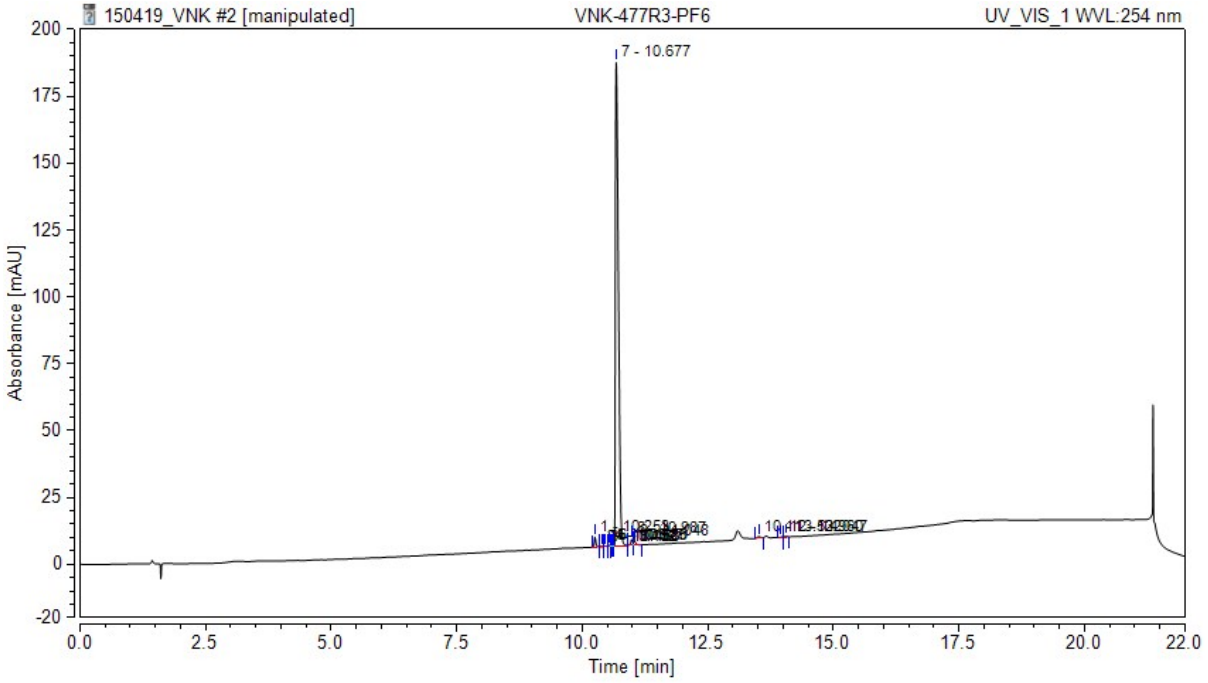


Figure S10. HPLC traces for samples of ligand **1** and of complex **8 PF6**.

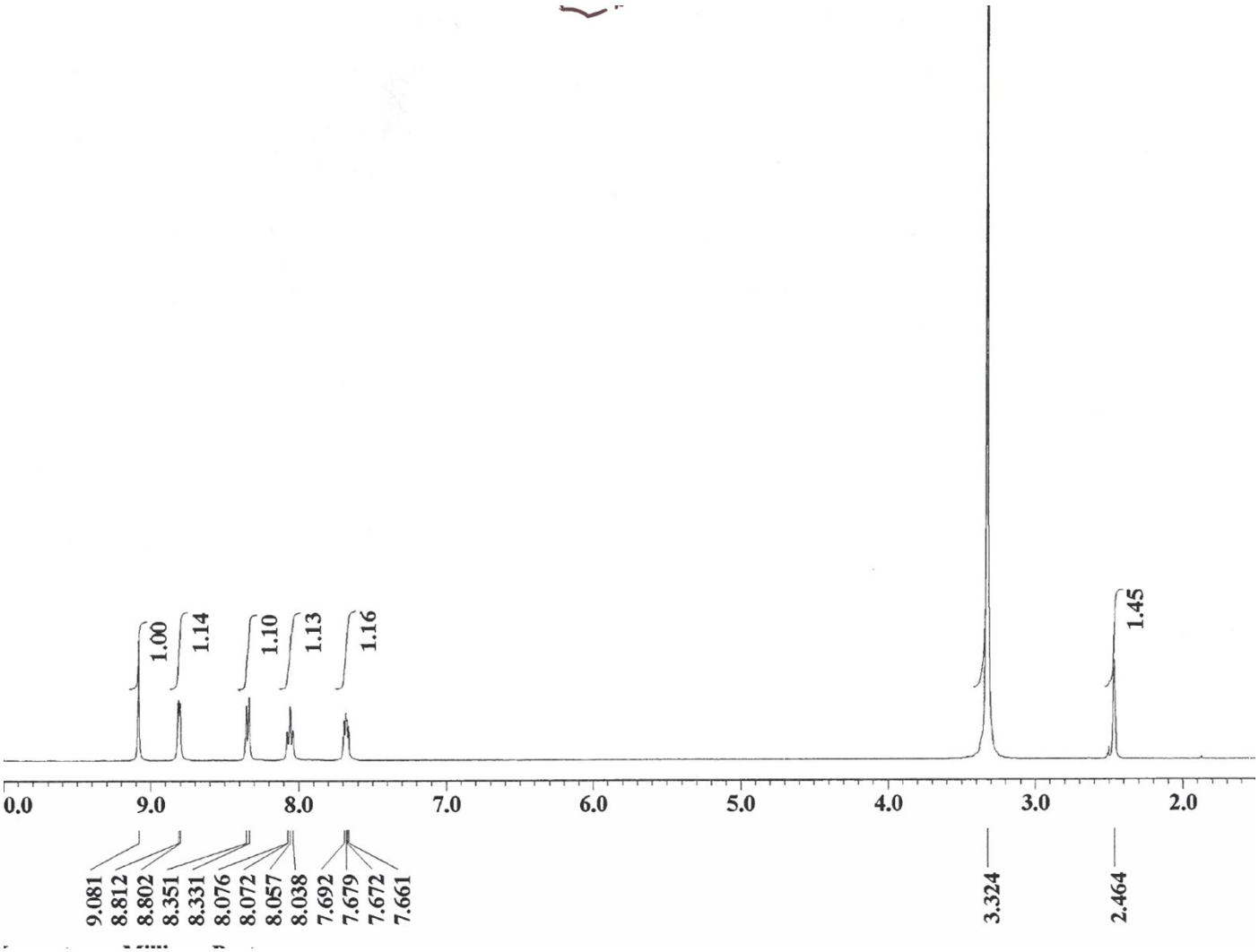


Figure S11 ^{1}H NMR spectrum of compound **6** in DMSO-D_3

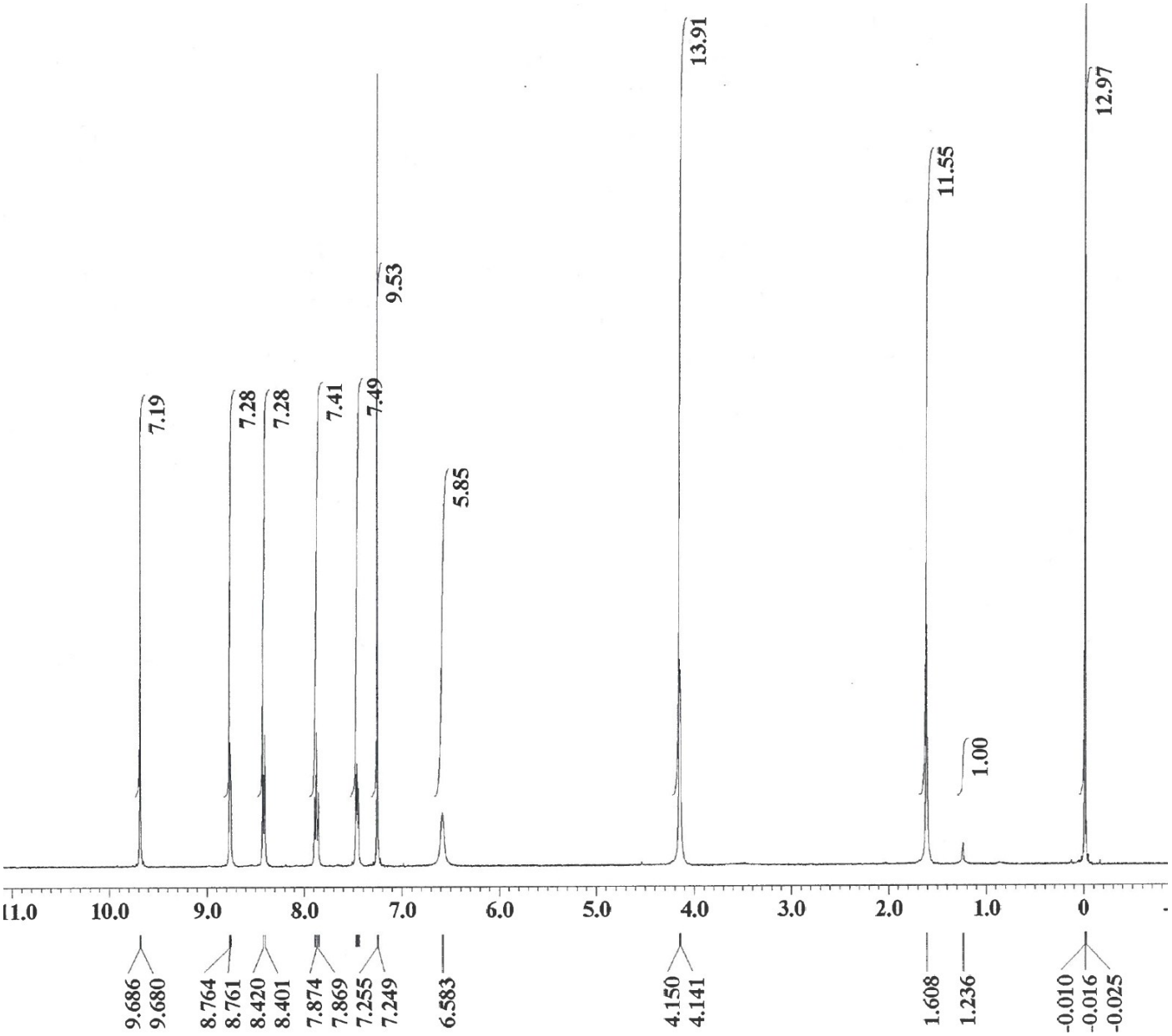


Figure S12 ^{1}H NMR spectrum of compound **7** in $CDCl_3$

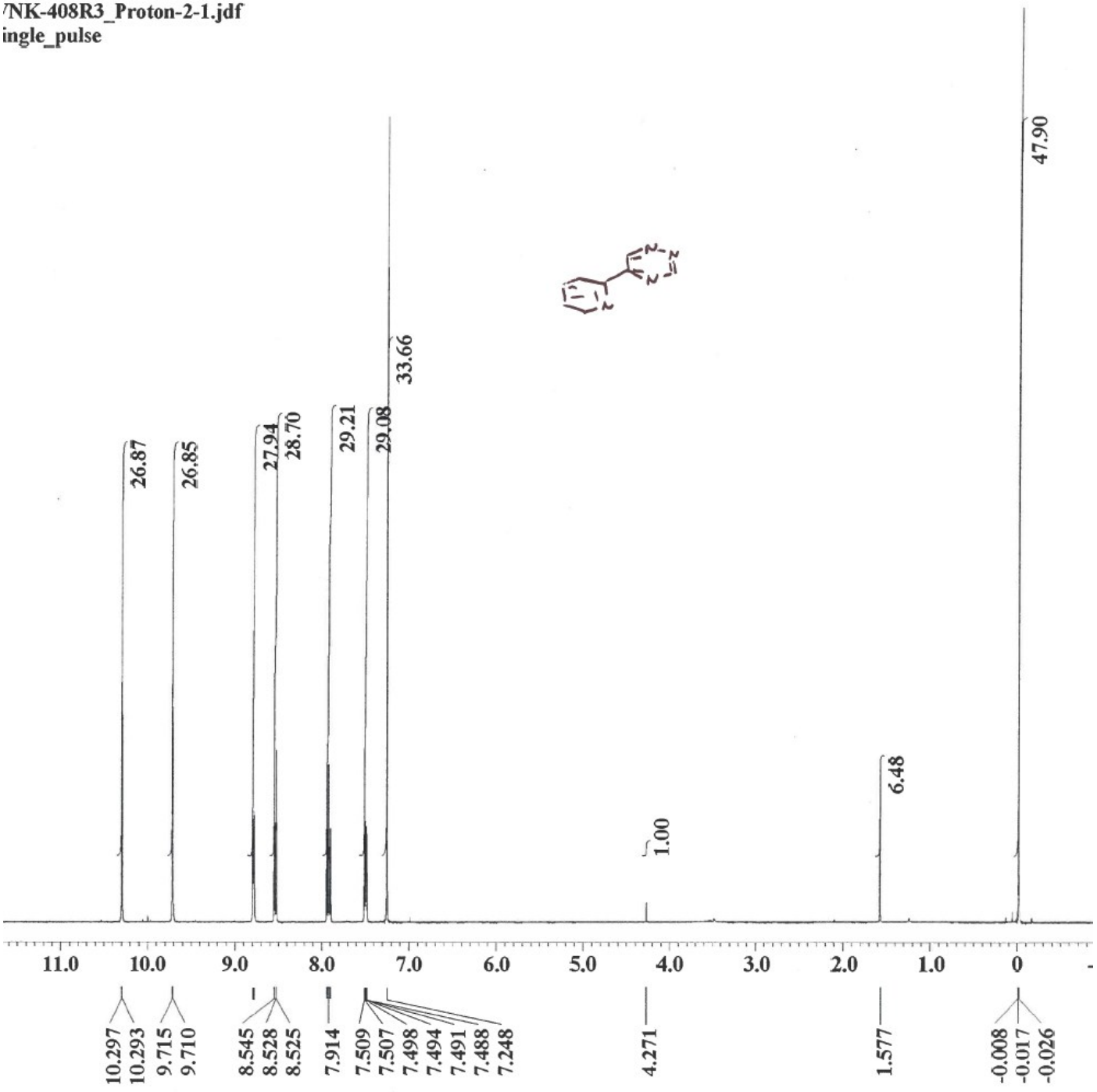


Figure S13 ^{1}H NMR spectrum of compound **1** in $CDCl_3$

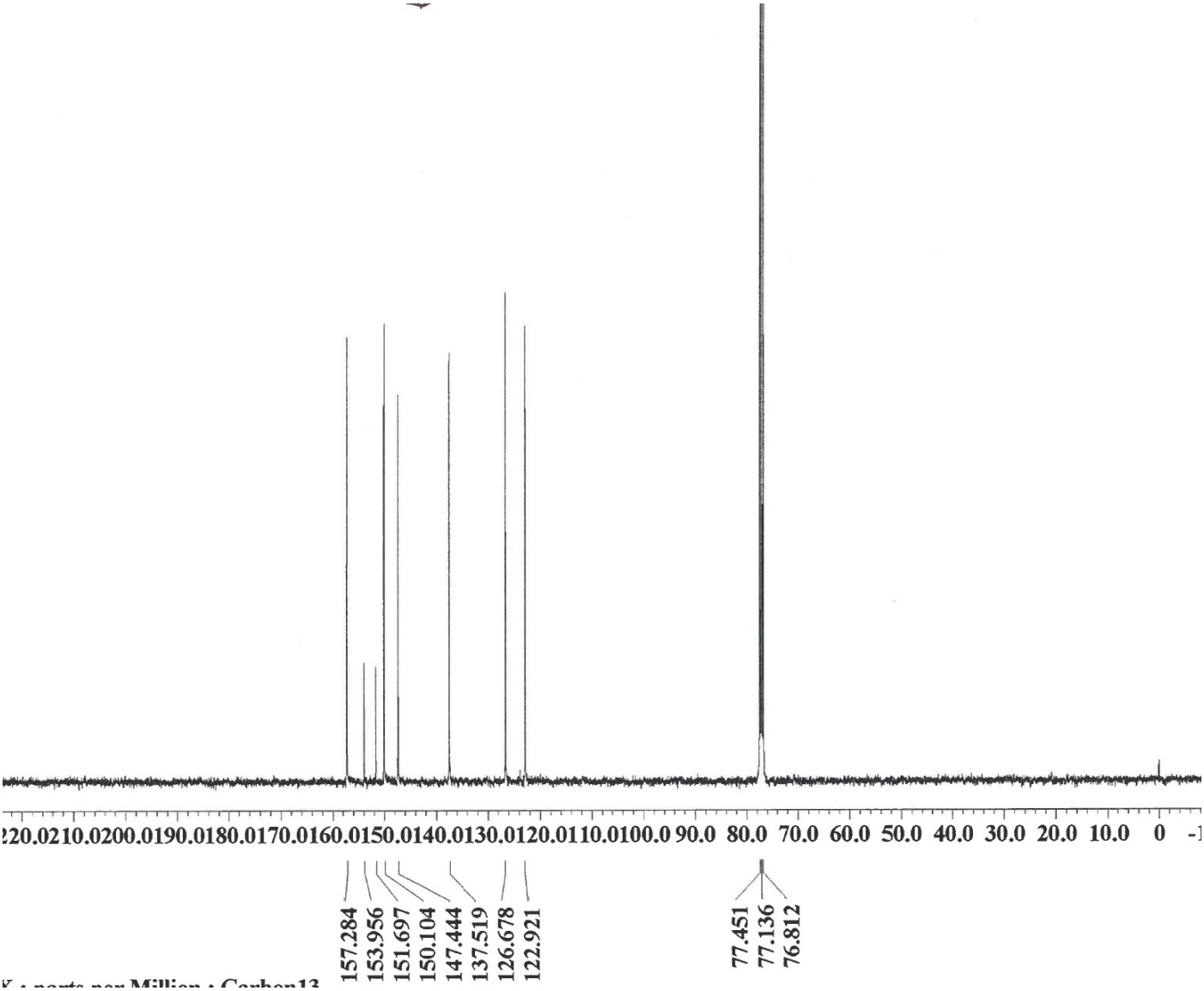


Figure S14 ^{13}C NMR spectrum of compound **1** in $CDCl_3$

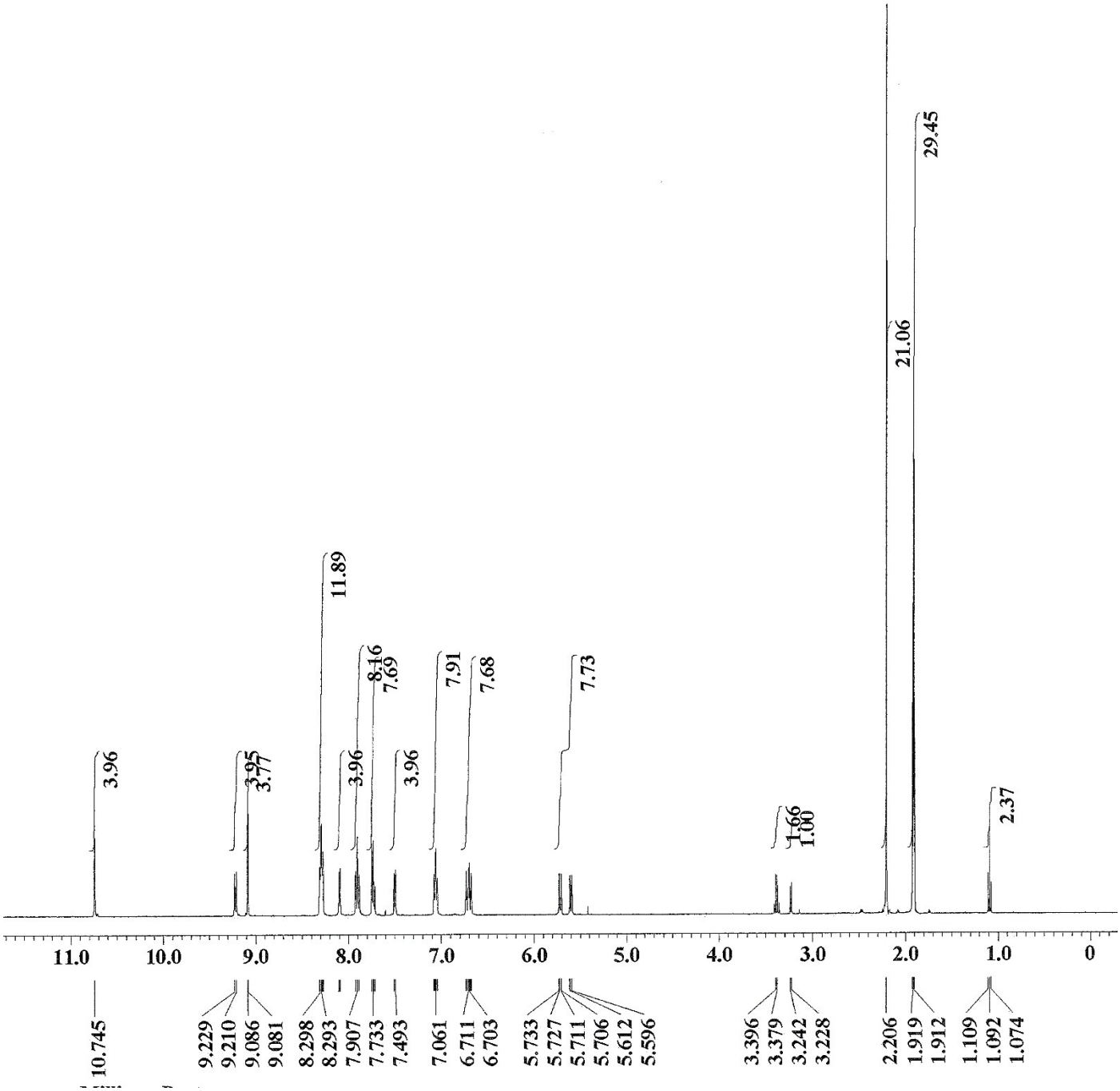


Figure S15 ^{1}H NMR spectrum of compound **8Cl** in acetonitrile-D_3

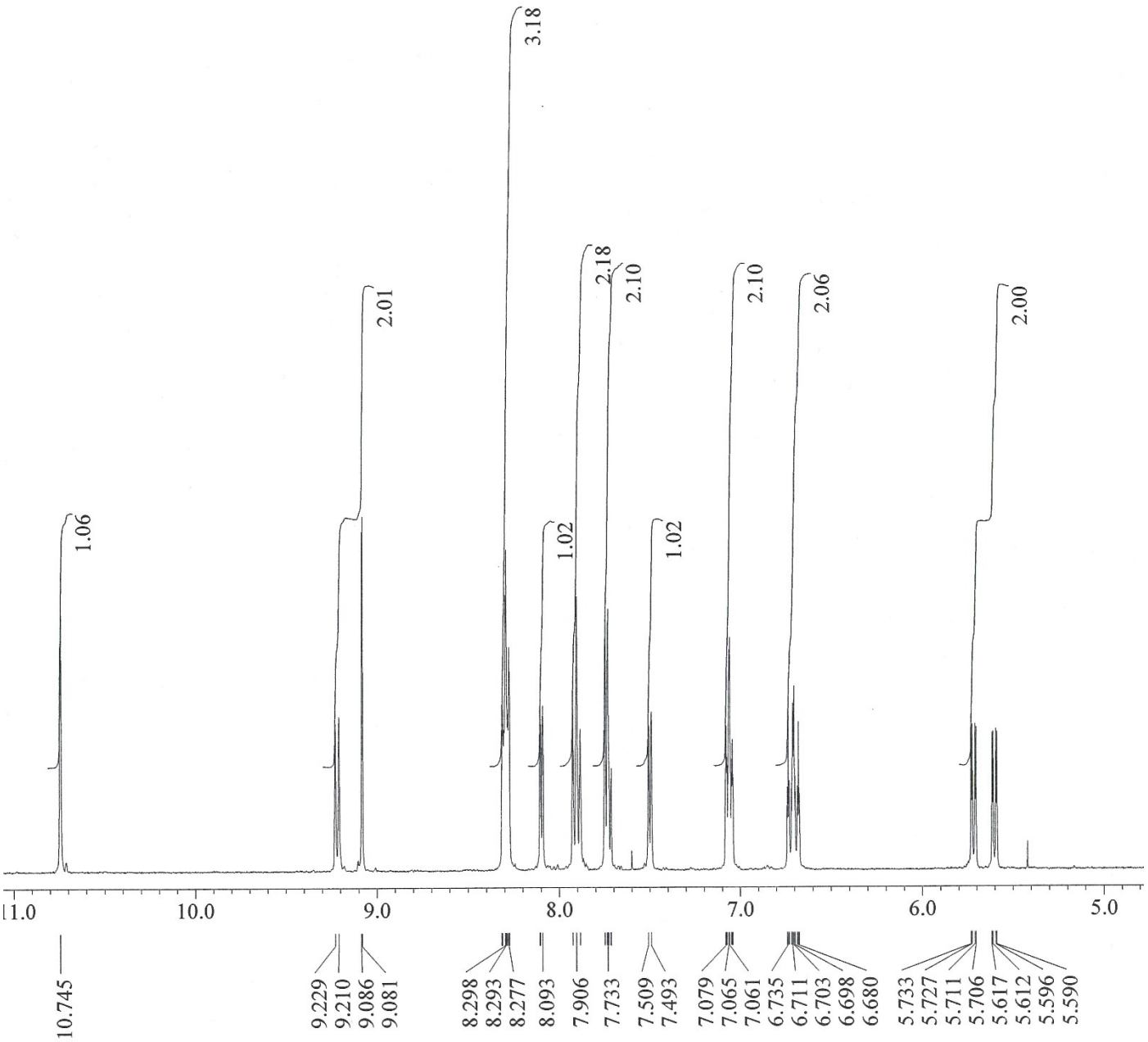


Figure S16 Zoom of ^{1}H NMR spectrum of compound **8Cl** in acetonitrile-D_3

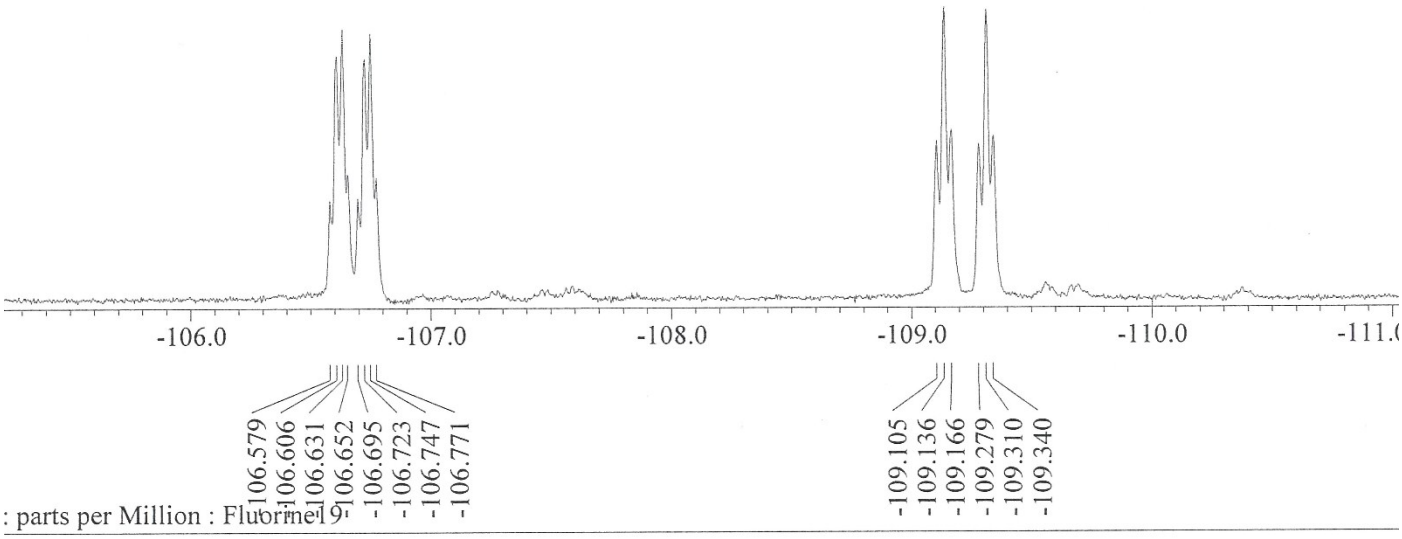


Figure S17 ^{19}F NMR spectrum of compound **8Cl** in acetonitrile-D_3

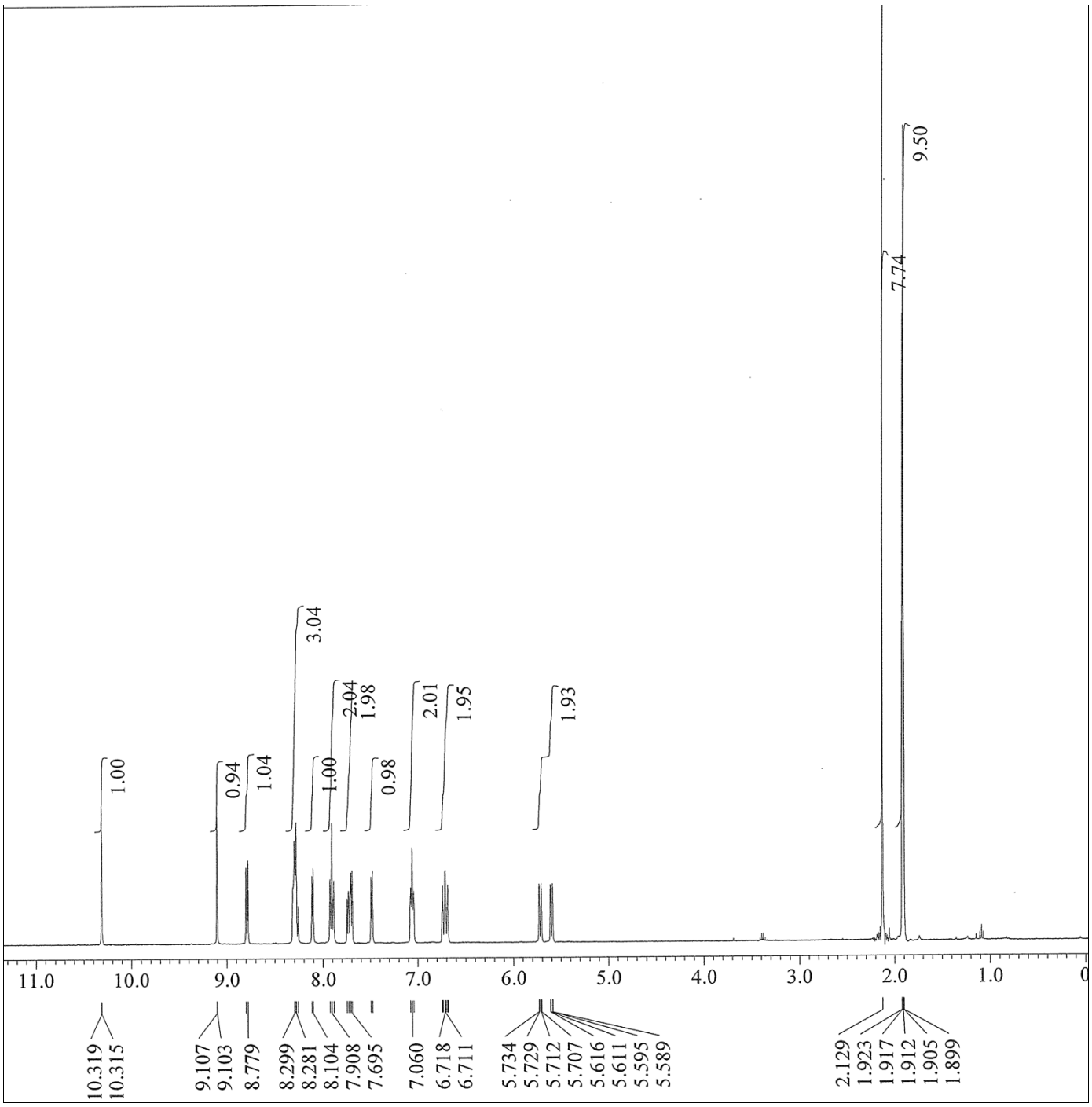


Figure S18 1H NMR spectrum of compound **8PF6** in acetone-D_6

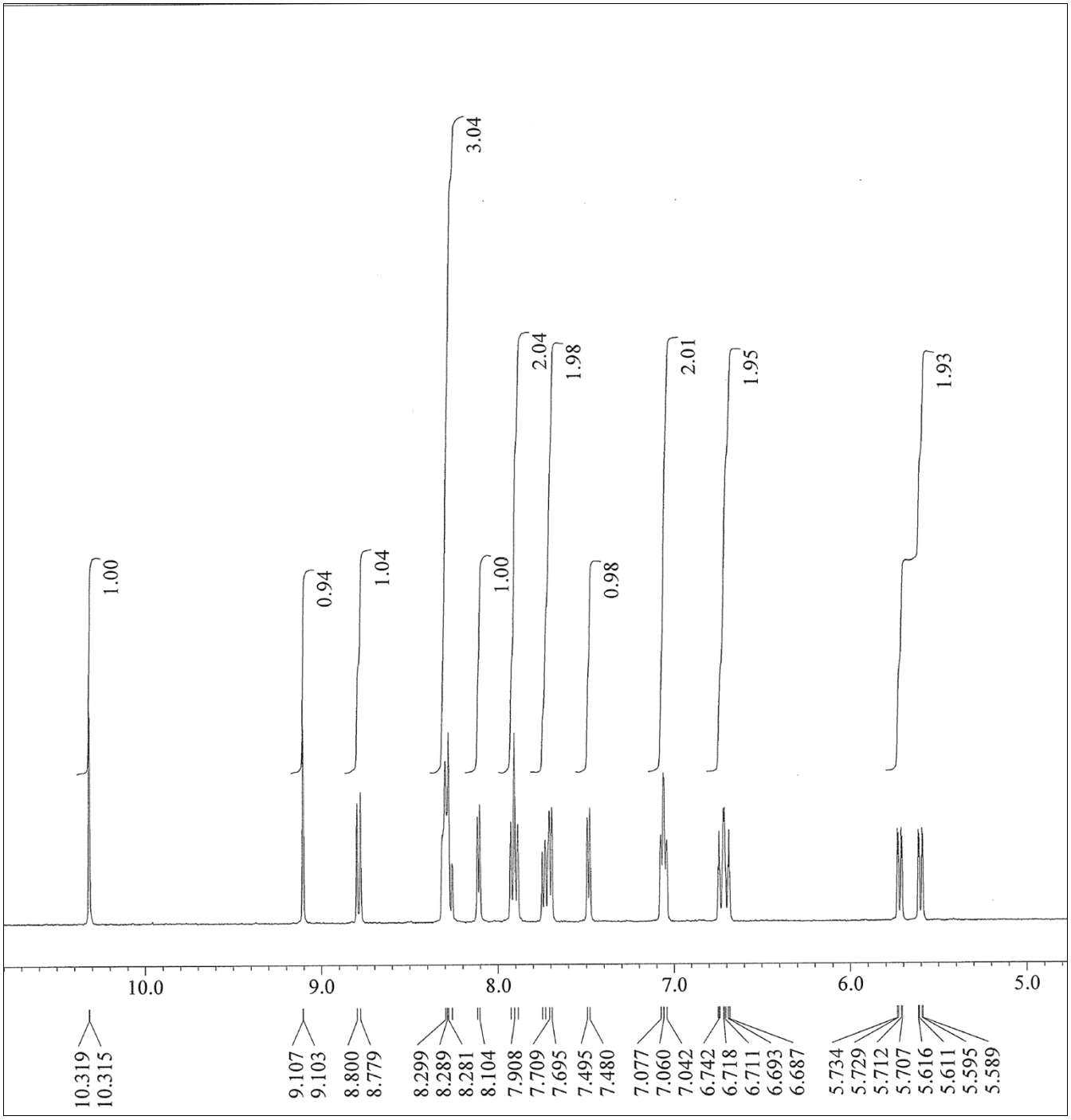


Figure S19 Zoom of ^{1}H NMR spectrum of compound **8PF6** in acetone-D_6

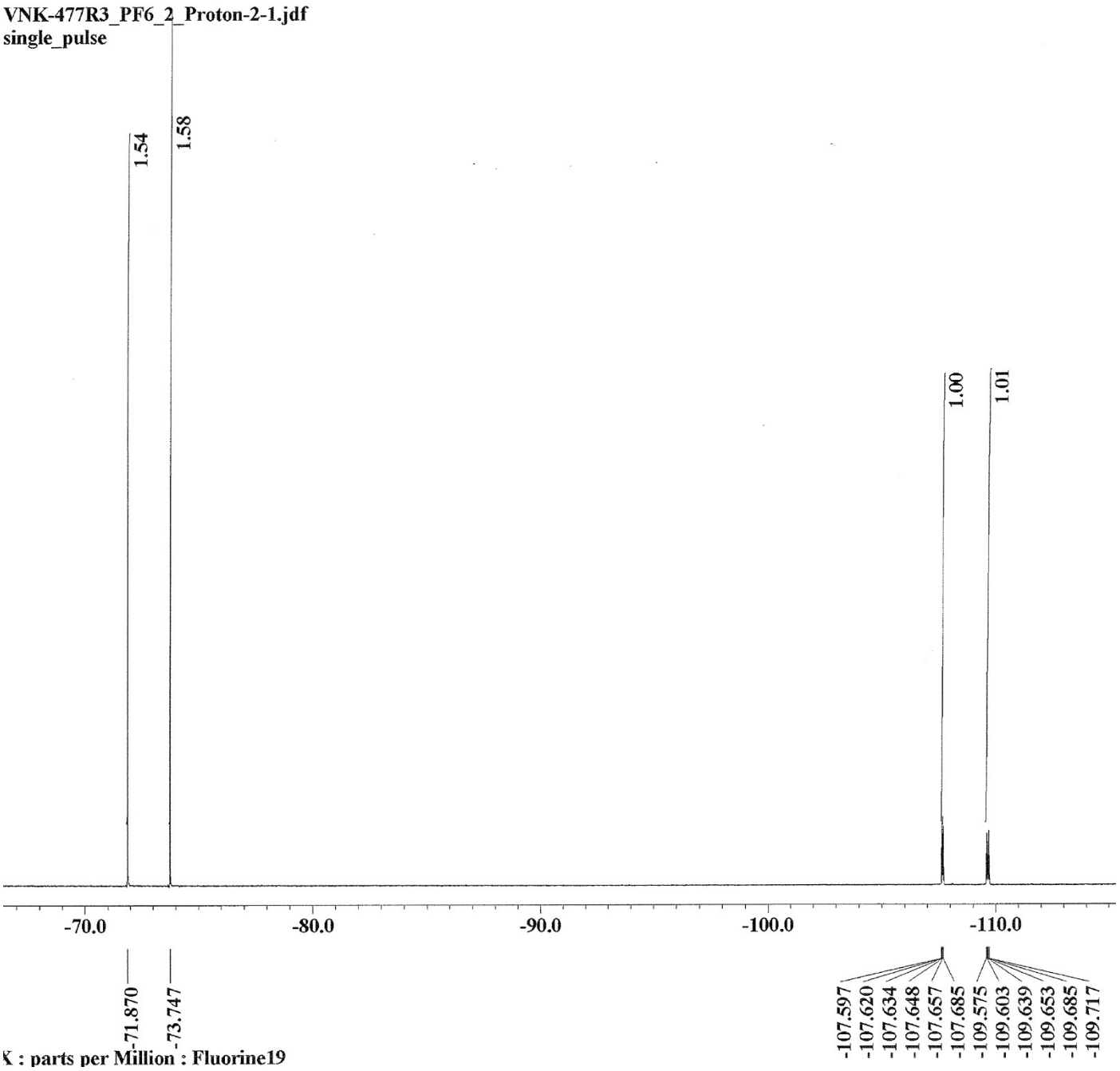


Figure S20 ^{19}F NMR spectrum of compound **8PF6** in acetone-D_6