Electronic Supplementary Information

Luminescent iridium(III) 2-cyanobenzothiazole complexes as site-specific labels to afford peptide-based phosphorogenic probes and hydrogels for enzyme activity sensing, cancer imaging and photodynamic therapy

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Table S1 Electronic absorption spectral data of complexes **1** – **3** at 298 K.

Complex	Solvent	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
1	CH_2Cl_2	256 sh (48,300), 309 (43,000), 364 sh (7,550)
	CH_3CN	259 sh (37,130), 308 (27,000), 360 sh (4,590)
2	CH_2Cl_2	258 (67,070), 308 (46,005), 385 sh (7,655), 417 sh (3,560)
	CH_3CN	257 (63,425), 309 (42,925), 379 sh (7,355), 414 sh (3,750)
3	CH_2Cl_2	261 (59,325), 293 (63,290), 349 (28,125), 370 sh (27,490), 472 sh (4,920)
	CH_3CN	261 (53,550), 291 (53,110), 346 (25,075), 366 sh (23,275), 475 sh (3,920)

Table S2 Singlet oxygen ($^1O_2$) generation quantum yields (Φ_Δ) of complexes **1** – **3** and conjugates **2-MMP** and **2-MMP-QSY7** in aerated CH_3CN at 298 K.

Complex/Conjugate	Φ_Δ[a]
1	0.85
2	0.59
3	0.66
2-MMP	0.52
2-MMP-QSY7	0.06

[a] $[Ru(bpy)_3]Cl_2$ was used (Φ_Δ = 0.57 in aerated CH_3CN, λ_{ex} = 450 nm).

Table S3 Photophysical data of conjugates **2-MMP**, **2-MMP-QSY7** and **2-VPMS** at 298 K.

Conjugate	Solvent	λ_{em}/nm[a]	τ_o/μs[b]	Φ_{em}[c]
2-MMP	H_2O/CH_3CN[d]	608	0.15	0.07
2-MMP-QSY7	H_2O/CH_3CN[d]	623	0.13	< 0.005
2-VPMS	H_2O/CH_3CN[d]	590	0.19	0.08

[a] λ_{ex} = 350 nm.

[b] The lifetimes were measured at the emission maxima (λ_{ex} = 355 nm).

[c] The emission quantum yields were determined using $[Ru(bpy)_3]Cl_2$ (Φ_{em} = 0.04 in aerated H_2O, λ_{ex} = 455 nm) as a reference.[1]

[d] H_2O/CH_3CN (1:1, *v*/*v*).

Table S4 Förster resonance energy transfer (FRET) parameters of conjugate **2-MMP-QSY7**.

Donor	Acceptor	$J(\lambda)$/nm^4 M^{-1} cm^{-1}[a]	R_o/Å	D/Å[b]	E_{calc}	E_{expt}
2-MMP	QSY-7	3.20×10^{15}	40.0	12.9	0.99	0.93

[a] Overlap integral of the emission spectrum of the QSY-7-free conjugate **2-MMP** and the absorption spectrum of QSY-7 (acceptor).

[b] Distance between the iridium(III) metal centre and centroid of QSY-7 in conjugate **2-MMP-QSY7**.

Table S5 (Photo)cytotoxicity (IC_{50}) of conjugate **2-MMP-QSY7** towards MDA-MB-231 and HEK-293 cells. Photocytotoxicity index (PI) = $IC_{50,drak}/IC_{50,light}$.

Cell line	$IC_{50,dark}/\mu M$	$IC_{50,light}/\mu M$	PI
MDA-MB-231	> 40	1.93 ± 0.18	> 20.7
HEK-293	> 40	10.17 ± 0.86	> 3.9

Table S6 Cellular uptake of conjugate **2-MMP-QSY7**.

Conjugate	Amount of iridium per cell/fmol[a]	
	MDA-MB-231	HEK-293
2-MMP-QSY7	0.38 ± 0.03	0.03 ± 0.004

[a] Amount of iridium associated with an average MDA-MB-231 or HEK-293 cell upon incubation with the conjugate (10 μM) at 37°C for 4 h, as determined by ICP-MS.

Fig. S1 Electronic absorption spectra of complexes **1** – **3** in CH_2Cl_2 (black) and CH_3CN (red) at 298 K.

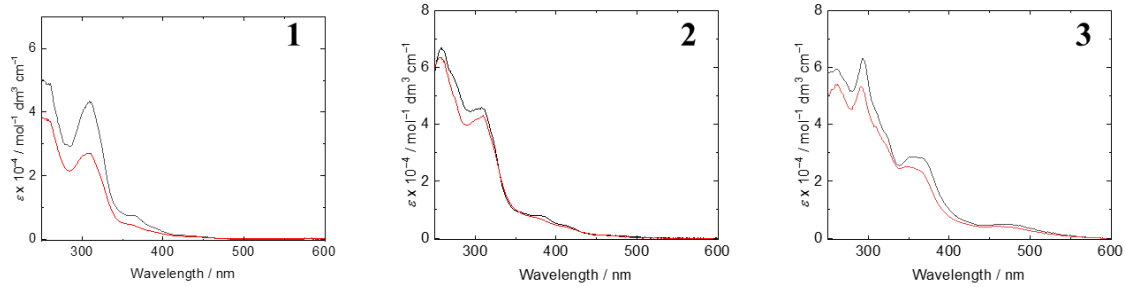


Fig. S2 Normalised emission spectra of complexes **1** – **3** in degassed CH_2Cl_2 (black) and CH_3CN (red) at 298 K and in alcohol glass at 77 K (blue) (λ_{ex} = 350 nm).

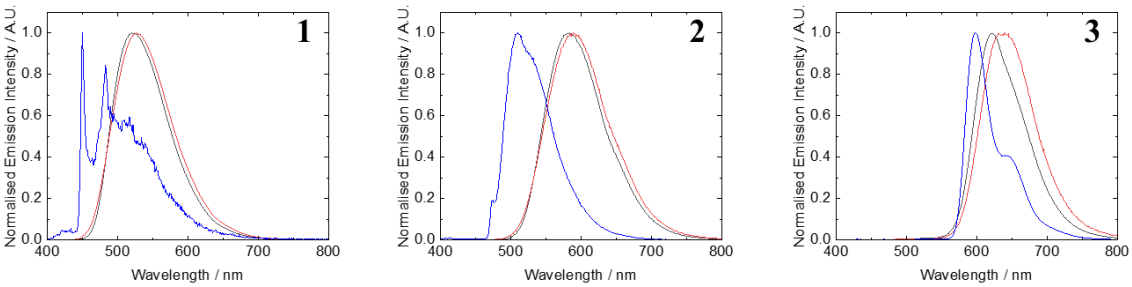


Fig. S3 HPLC chromatograms (λ_{abs} = 350 nm) of complexes **1** – **3** (100 μM) (black) and the reaction mixtures of complexes **1** – **3** (100 μM) and L-cysteine (L-Cys) (250 μM) in phosphate-buffered saline (PBS) (pH 7.4)/CH_3CN (9:1, *v*/*v*) containing tris(2-carboxyethyl) phosphine (TCEP) (100 μM) after incubation at 298 K for 4 h (red).

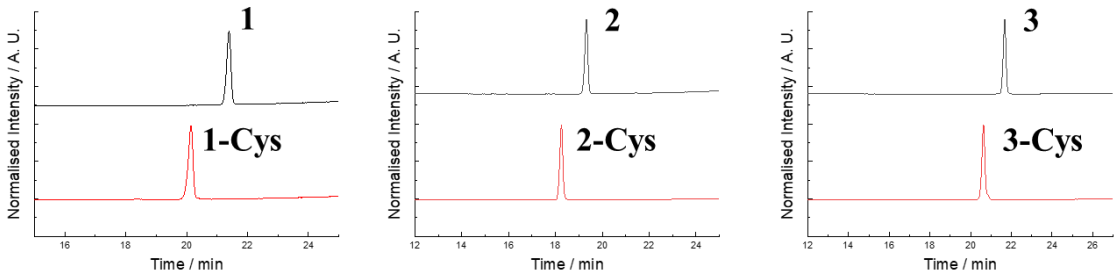


Fig. S4 ESI mass spectra of the eluent collected at t_R = 20.2 min (**1-Cys**), 18.2 min (**2-Cys**) and 20.9 min (**3-Cys**).

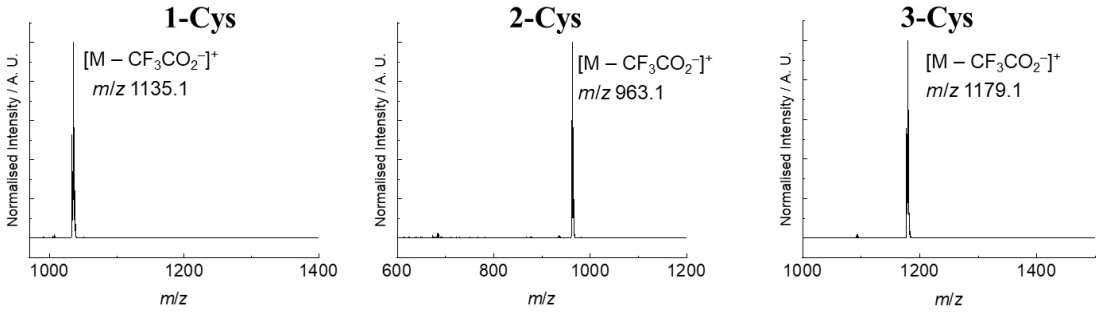


Fig. S5 ESI mass spectra of a mixture of complex **2** (100 μM) and glutathione (250 μM), histidine (250 μM), lysine (250 μM), serine (250 μM), or threonine (250 μM) in PBS (pH 7.4)/CH_3CN (9:1, *v*/*v*) after incubation at 37°C for 12 h.

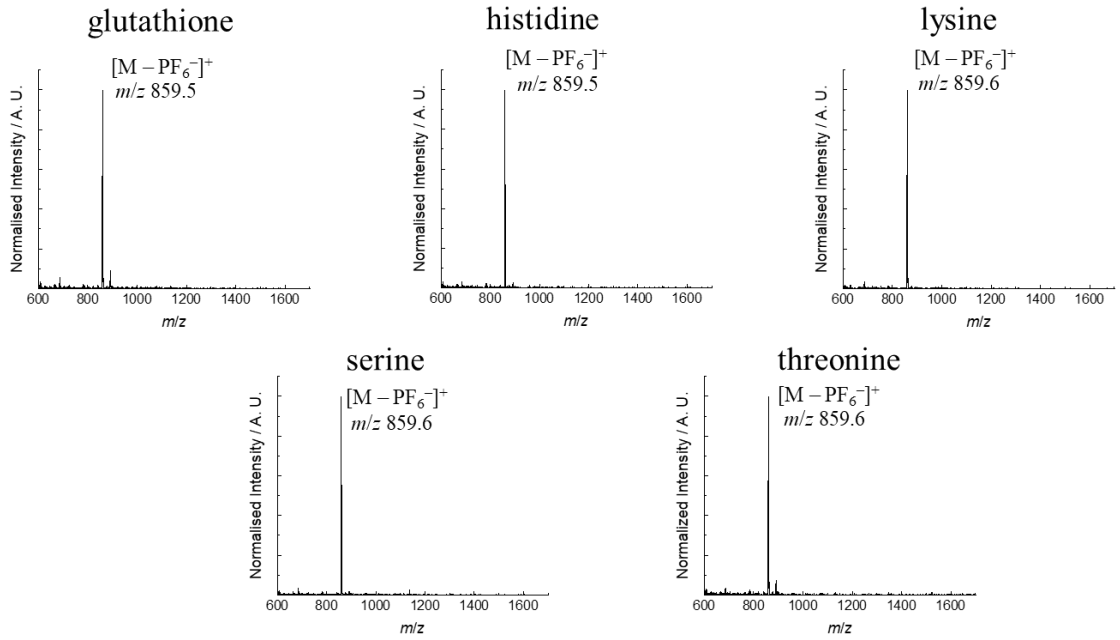


Fig. S6 HPLC chromatograms (λ_{abs} = 350 nm) of conjugates **2-MMP** (t_R = 15.56 min), **2-MMP-QSY7** (t_R = 15.83 min) and **2-VPMS** (t_R = 14.12 min).

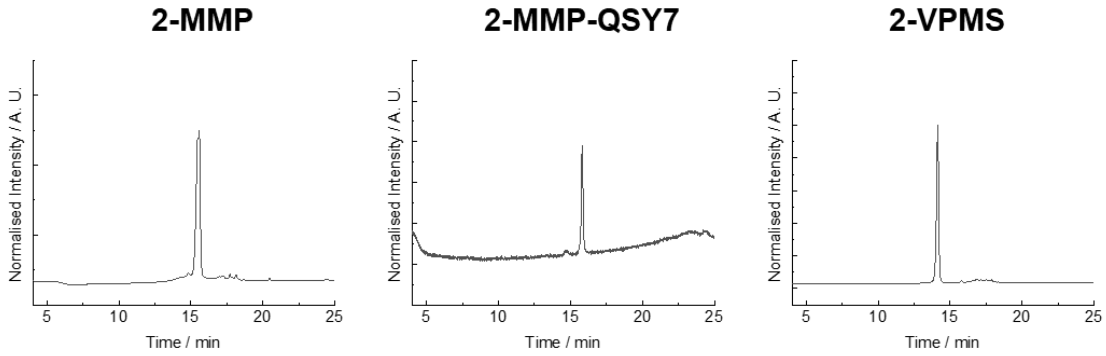


Fig. S7 ESI mass spectra of purified conjugates **2-MMP**, **2-MMP-QSY7** and **2-VPMS** in CH_3OH at 298 K.

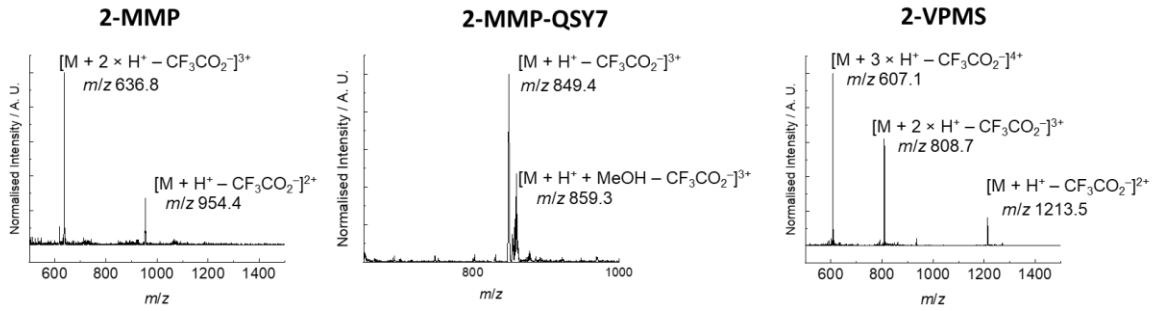


Fig. S8 Spectral overlap of the absorption spectrum of the acceptor QSY-7 (black) and normalised emission spectrum of the donor conjugate **2-MMP** (red) in H_2O/CH_3CN (1:1, *v*/*v*) at 298 K.

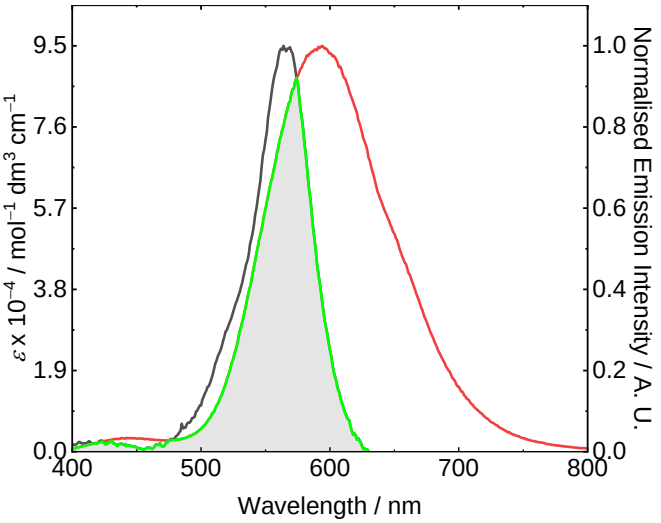


Fig. S9 HPLC chromatograms (λ_{abs} = 350 and 560 nm) of (a) conjugate **2-MMP-QSY7** (5 μM) before (black) and after (red) incubation with matrix metalloproteinase (MMP)-2 (0.002 mg mL^{-1} in PBS) in aerated MMP reaction buffer/DMSO (99:1, *v*/*v*) at 37°C for 12 h. ESI mass spectra of cleavage products (b) **2-CVPMS** and (c) MRGGK-QSY7.

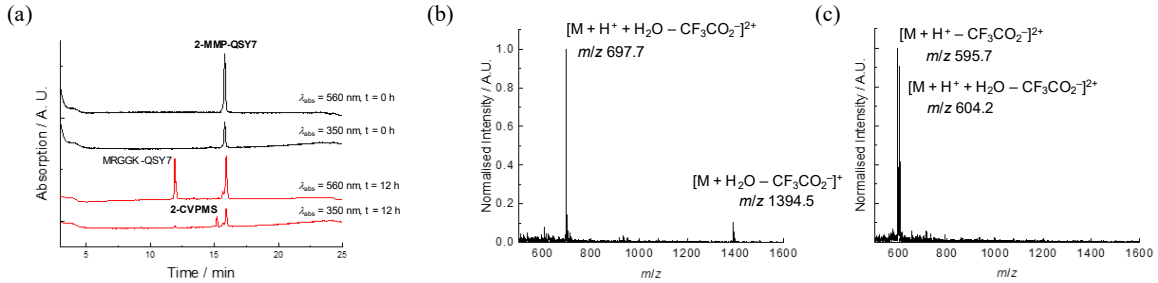


Fig. S10 (a) SEM image and (b) EDS images and spectrum of **Gel-1**. Scale bar = 100 μm.

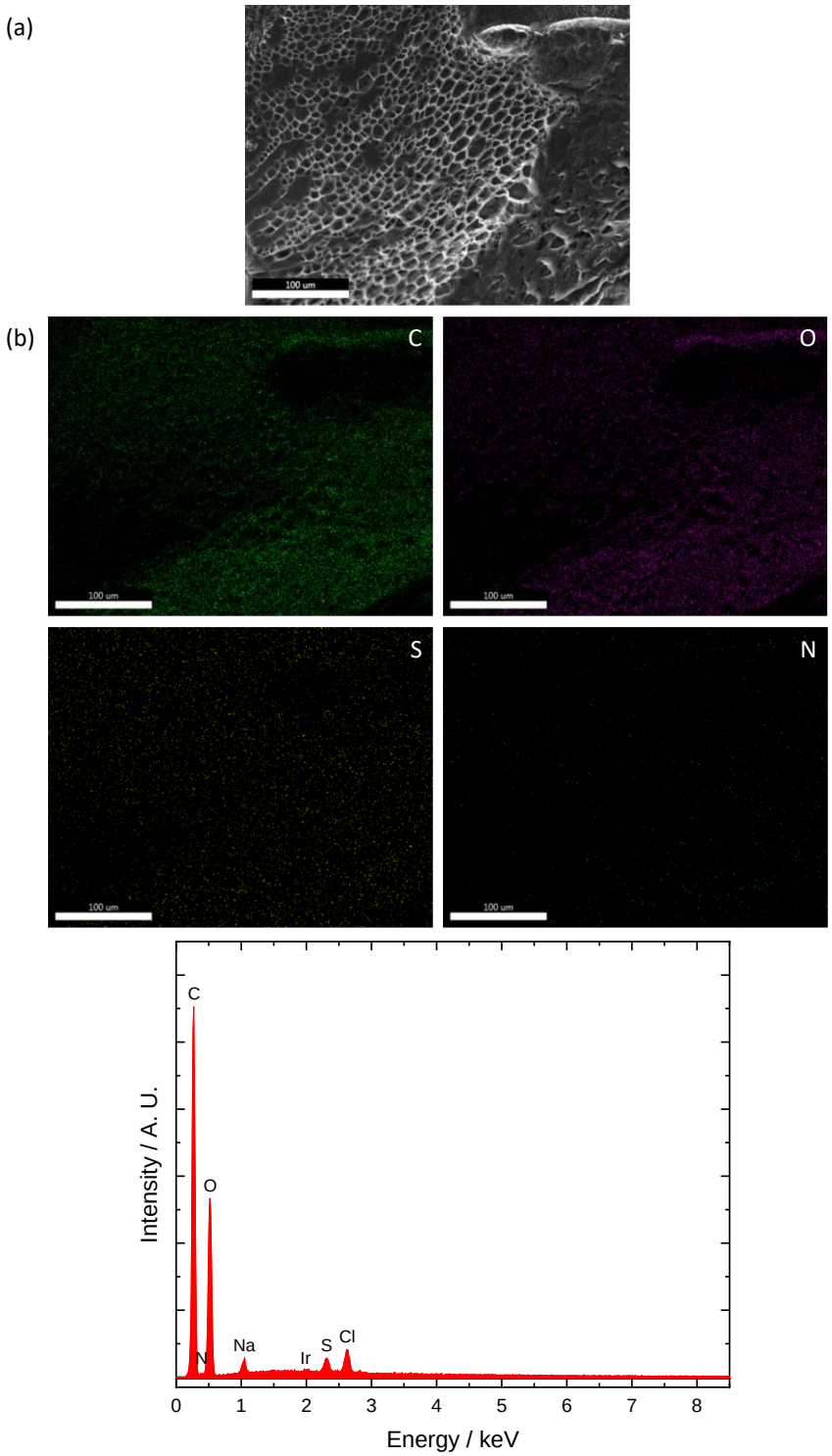


Fig. S11 Photographs of (a) **Gel-1** ([Ir] = 80 μM, 100 μL) and (b) **Gel-2** ([Ir] = 40 μM, 100 μL) upon addition of Dulbecco's Modified Eagle Medium (DMEM) (150 μL) and incubation for 24, 48 and 72 h.

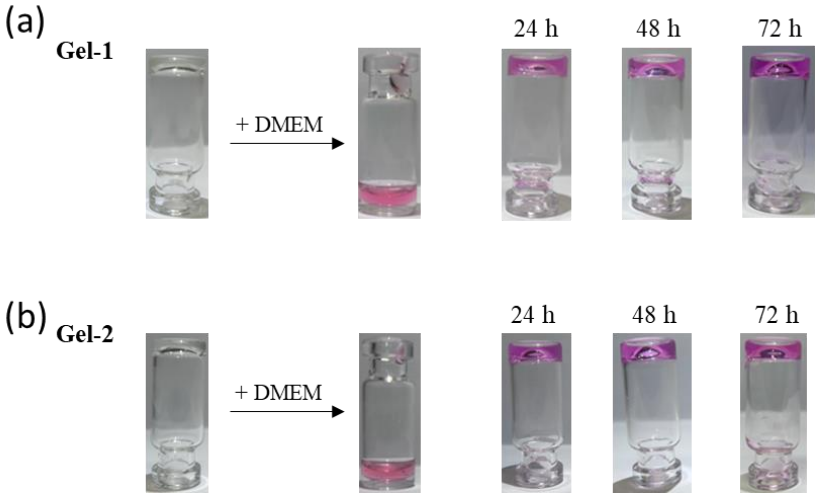


Fig. S12 Optical microscopy images of MDA-MB-231 cells encapsulated by **Gel-1** ([Ir] = 40 μM, 100 μL) at different focal lengths.

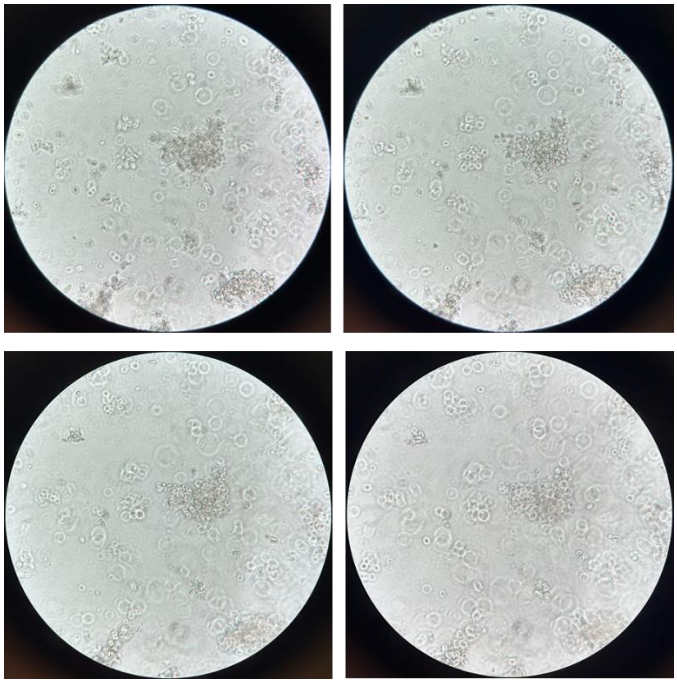


Fig. S13 Analysis of live/dead MDA-MB-231 and HEK-293 cells using Calcein-AM (1 μM, 30 min; λ_{ex} = 488 nm, λ_{em} = 510 – 540 nm) and propidium iodide (PI) (3 μM, 30 min; λ_{ex} = 532 nm, λ_{em} = 610 – 640 nm). The cells were encapsulated by **Gel-1** ([Ir] = 40 μM) for 72 h. Scale bar = 100 μm.

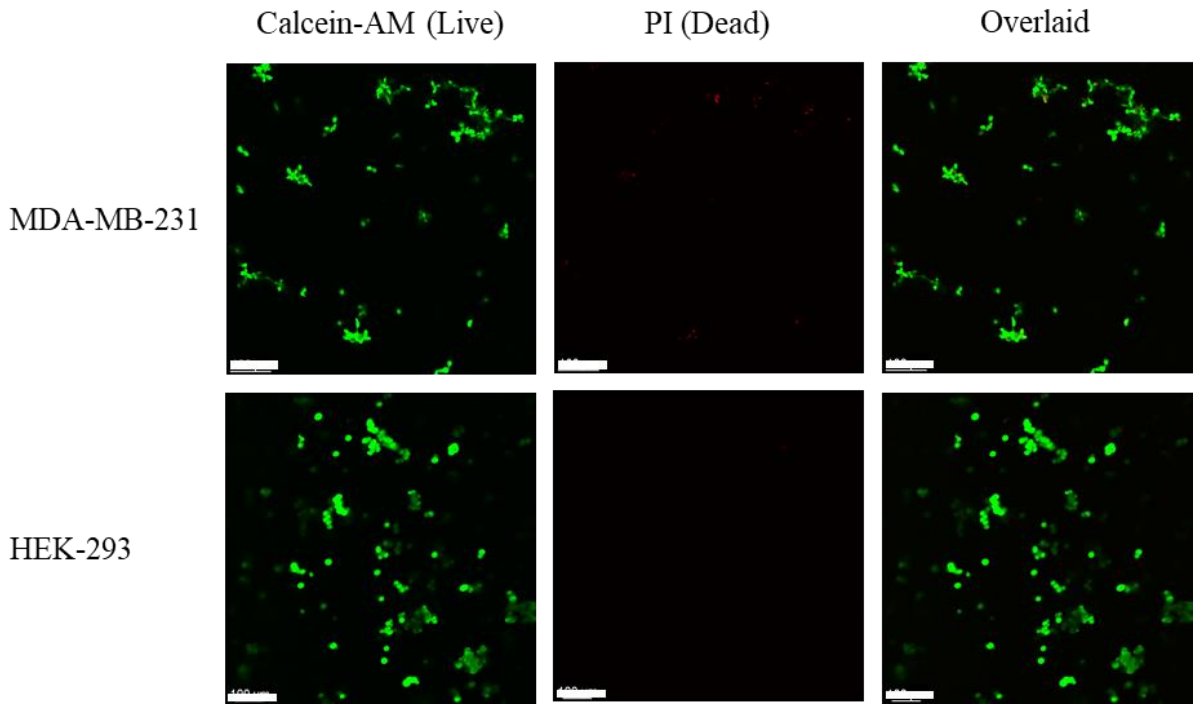


Fig. S14 Laser-scanning confocal microscopy (LSCM) images of MDA-MB-231 cells encapsulated by **Gel-1** ([Ir] = 40 μM; λ_{ex} = 405 nm, λ_{em} = 570 – 620 nm) at 0, 6 and 18 h at 37°C. Scale bar = 20 μm.

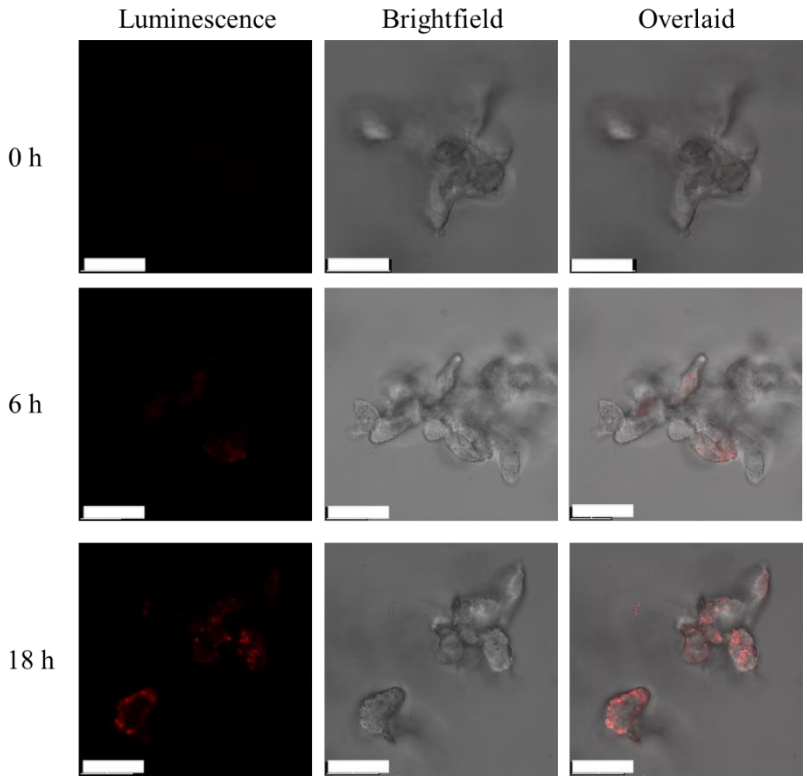


Fig. S15 (a) SEM image and (b) EDS images and spectrum of **Gel-2**. Scale bar = 100 μm.

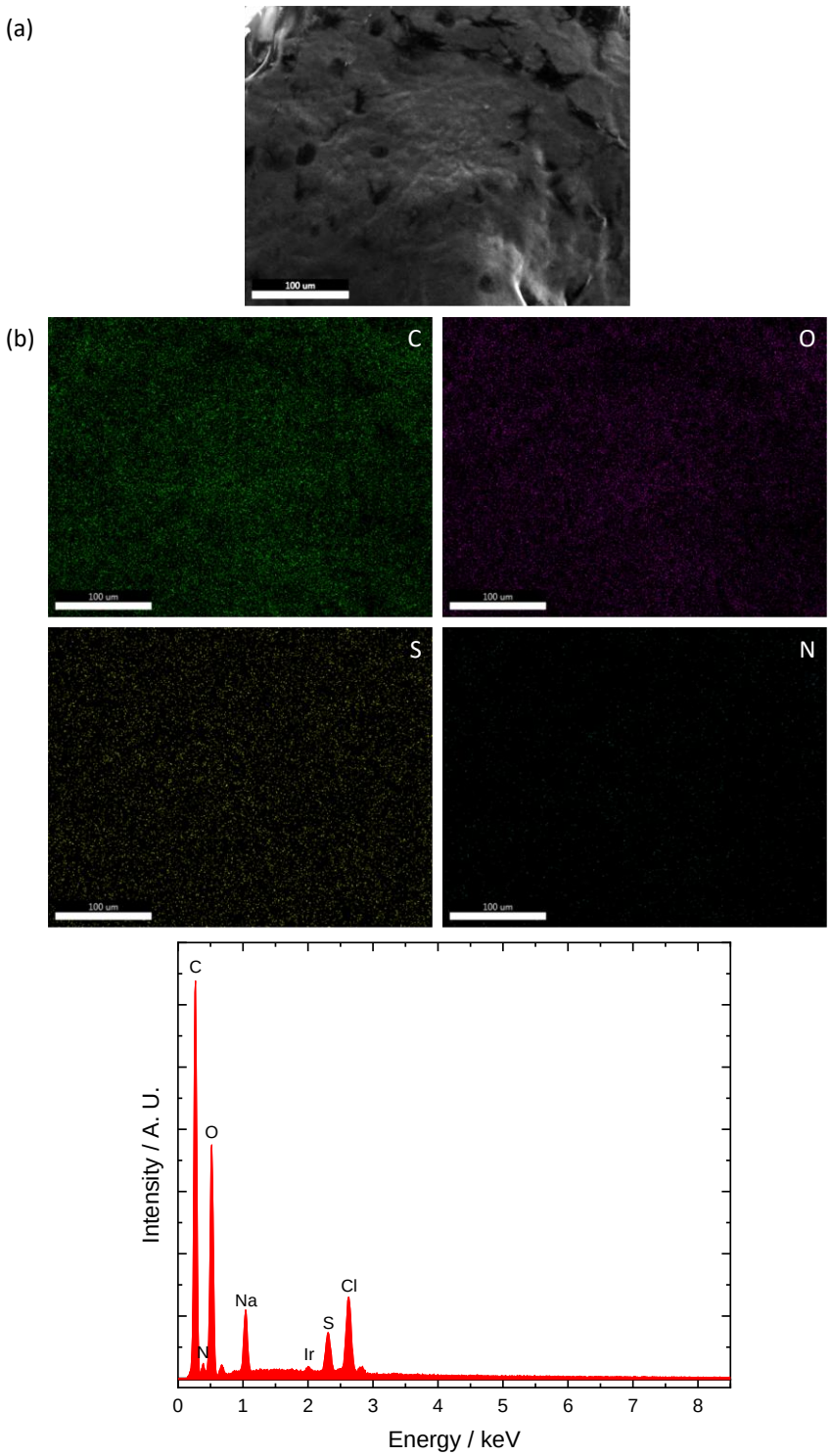


Fig. S16 Intracellular reactive oxygen species (ROS) levels of **Gel-2** ([Ir] = 80 μM, 24 h)-pretreated MDA-MB-231 cells incubated with chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) (10 μM, 30 min; λ_{ex} = 488 nm, λ_{em} = 500 – 550 nm) without (left) or with (right) photoirradiation at 450 nm (15 mW cm^{-2}) for 30 min. Scale bar = 100 μm.

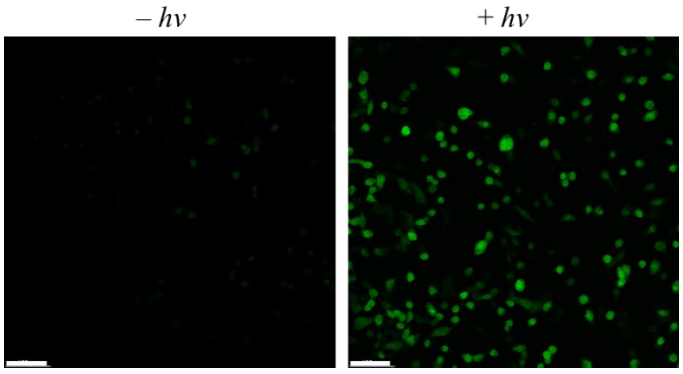


Fig. S17 ^{1}H NMR spectrum of the ligand bpy-CBT in $(CD_3)_2SO$ at 298 K.

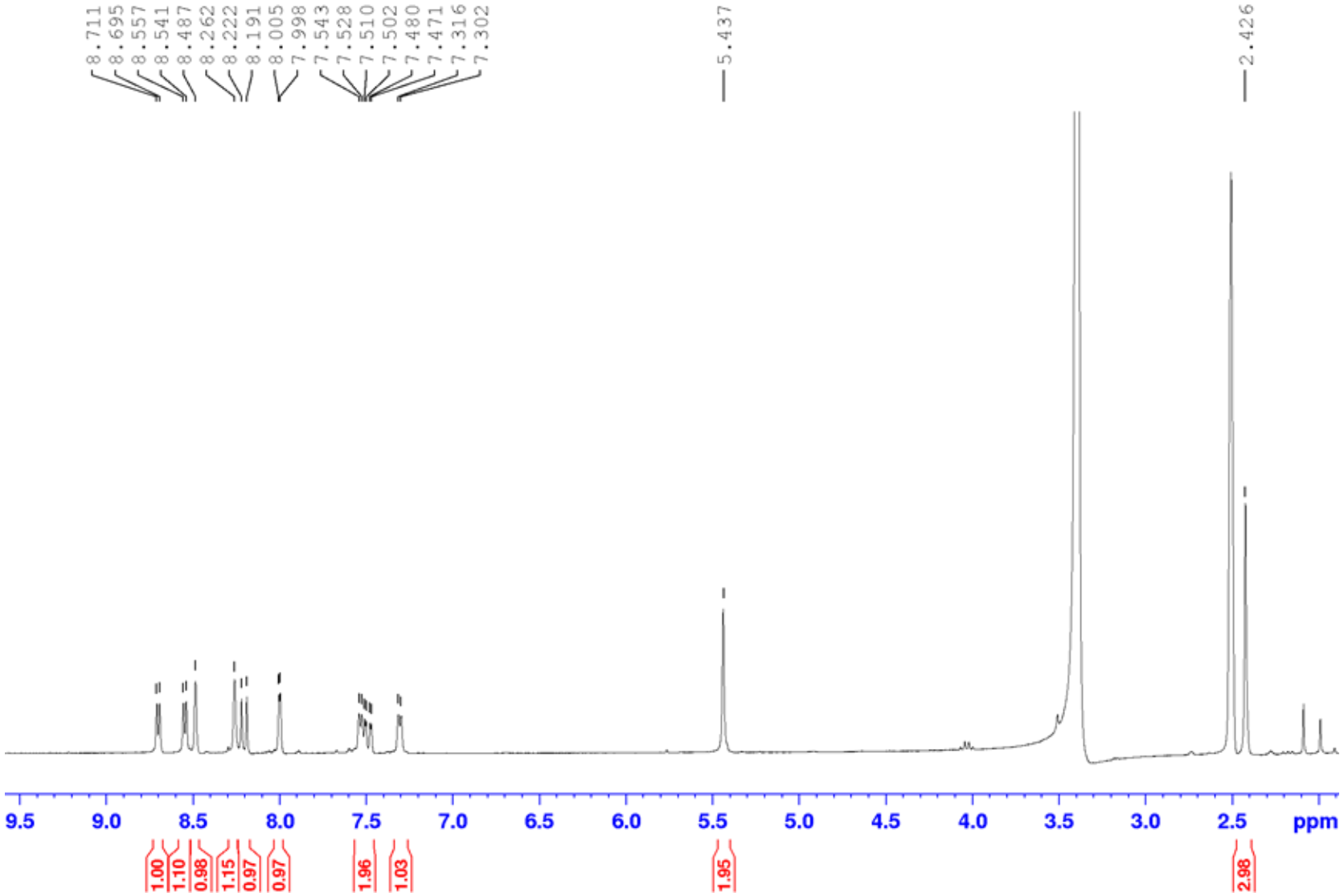


Fig. S18 ^{1}H NMR spectrum of complex **1** in $(CD_3)_2CO$ at 298 K.

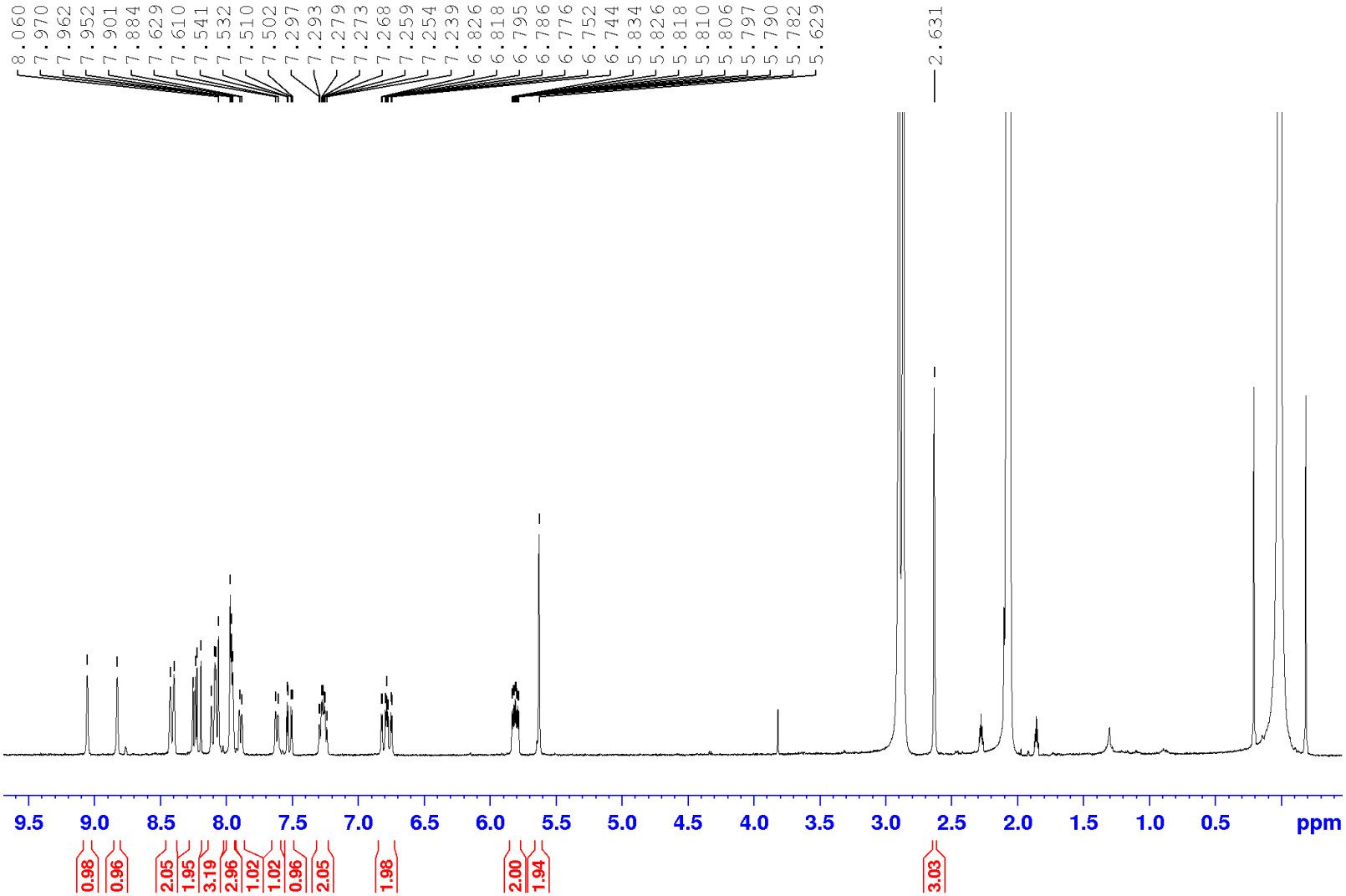


Fig. S19 ^{13}C NMR spectrum of complex **1** in $(CD_3)_2SO$ at 298 K.

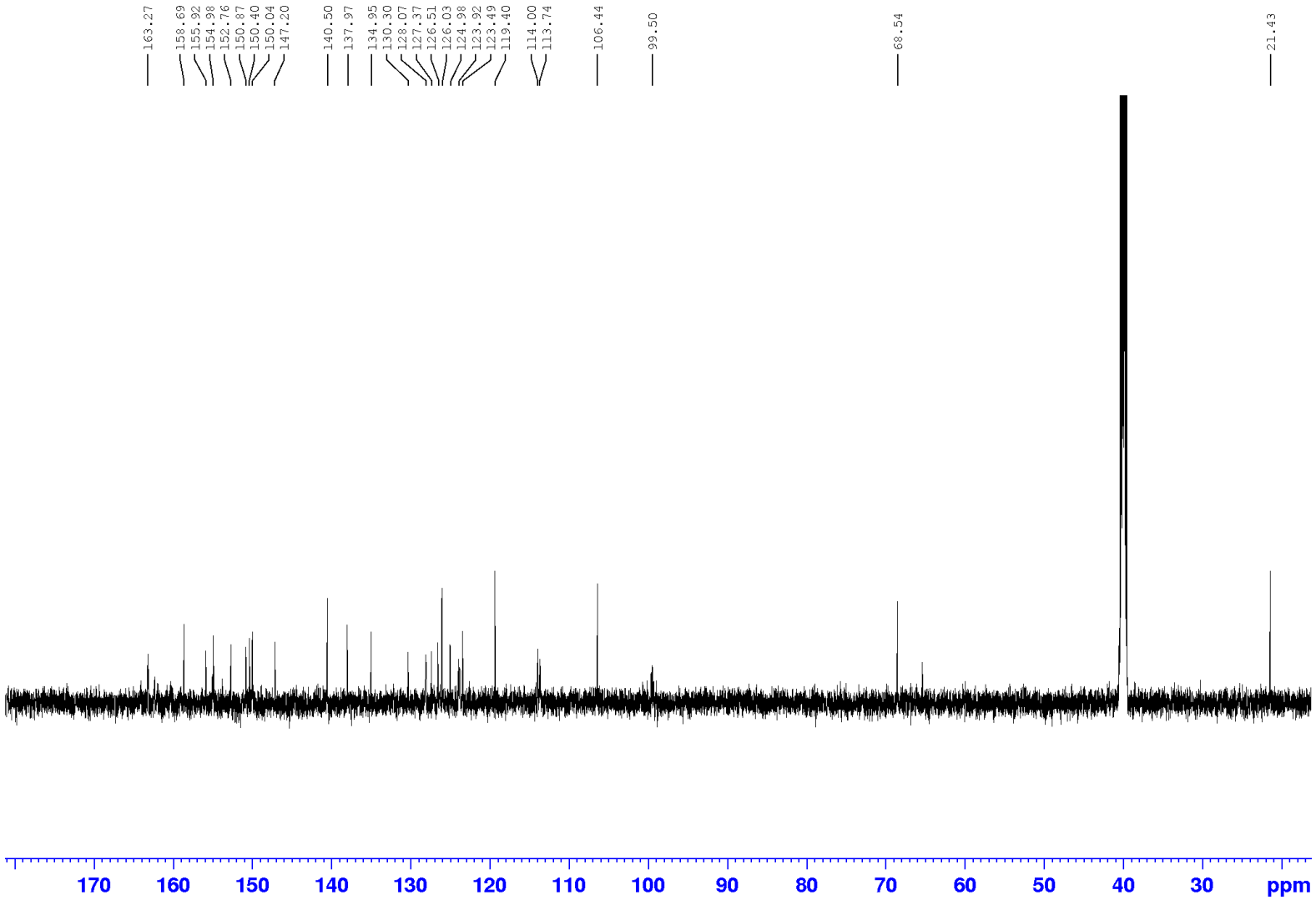


Fig. S20 HR-ESI mass spectrum of complex **1** in CH_3OH at 298 K.

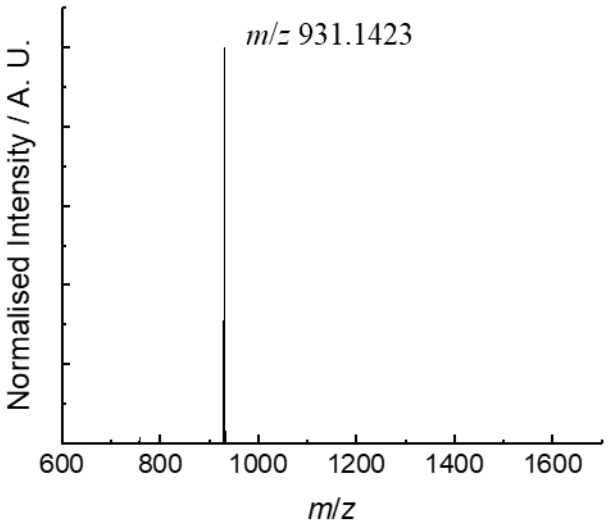


Fig. S21 ^{1}H NMR spectrum of complex **2** in $(CD_3)_2CO$ at 298 K.

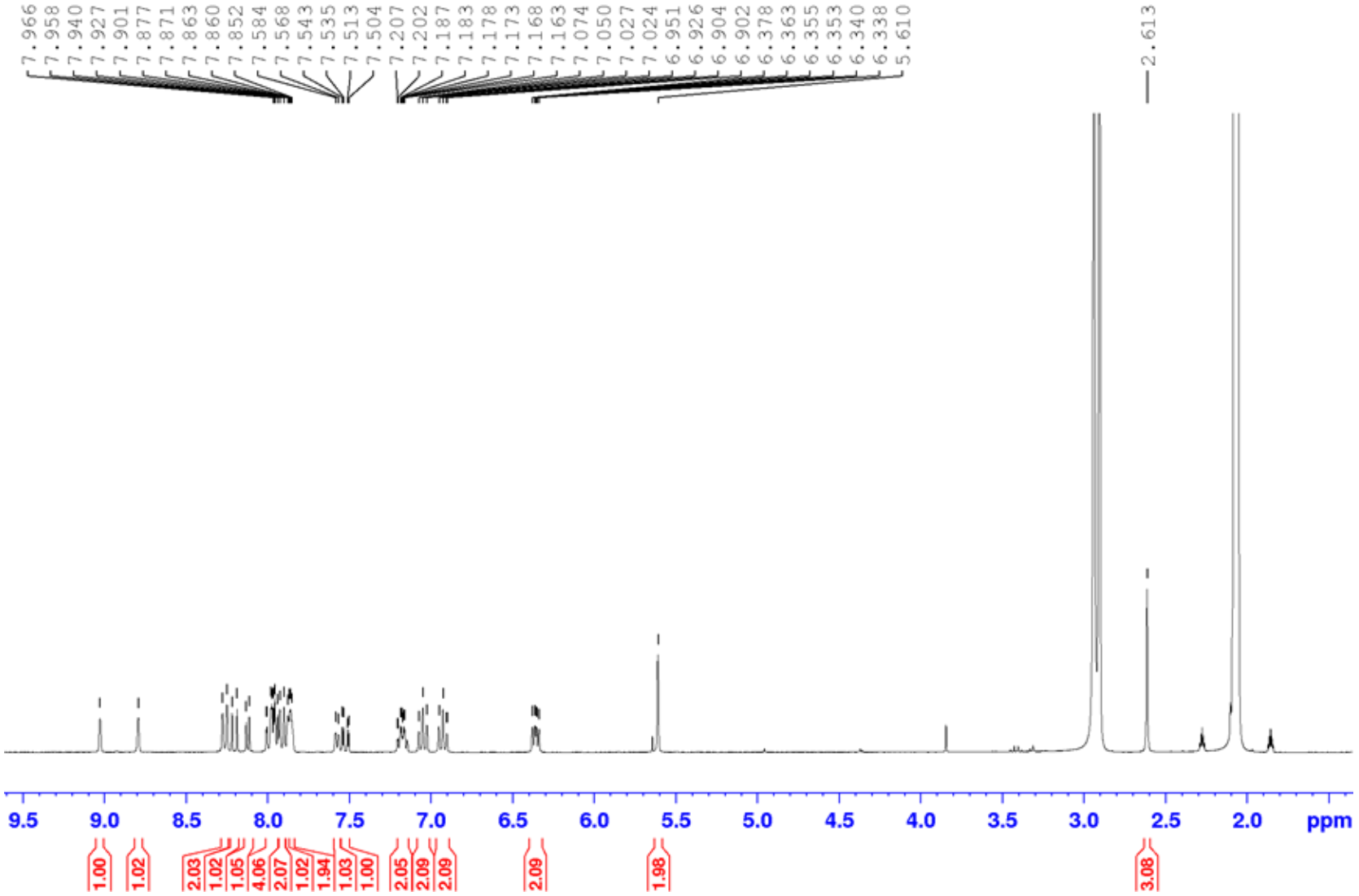


Fig. S22 ^{13}C NMR spectrum of complex **2** in $(CD_3)_2SO$ at 298 K.

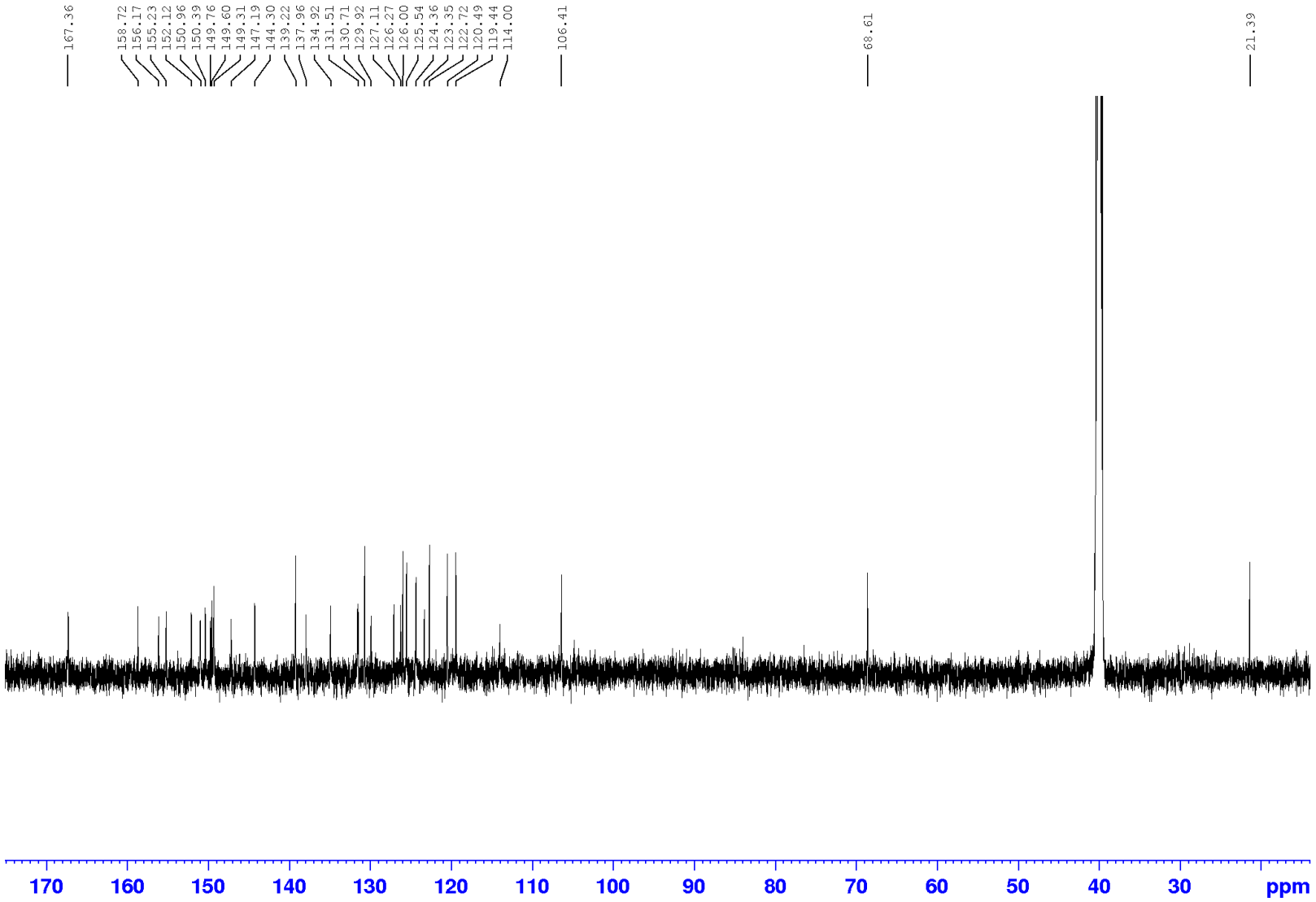


Fig. S23 HR-ESI mass spectrum of complex **2** in CH_3OH at 298 K.

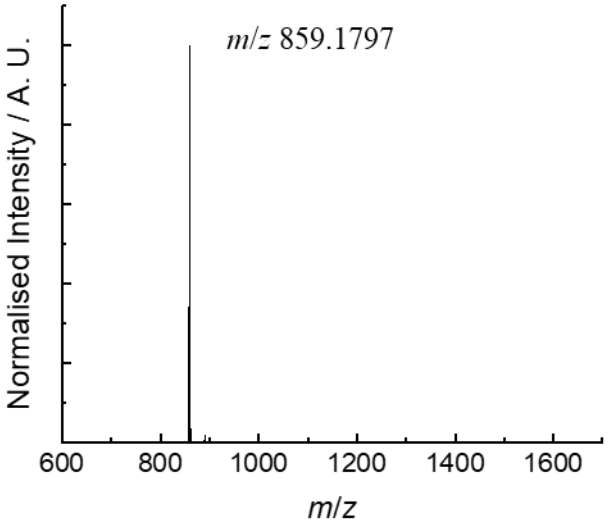


Fig. S24 ^{1}H NMR spectrum of complex **3** in $(CD_3)_2CO$ at 298 K.

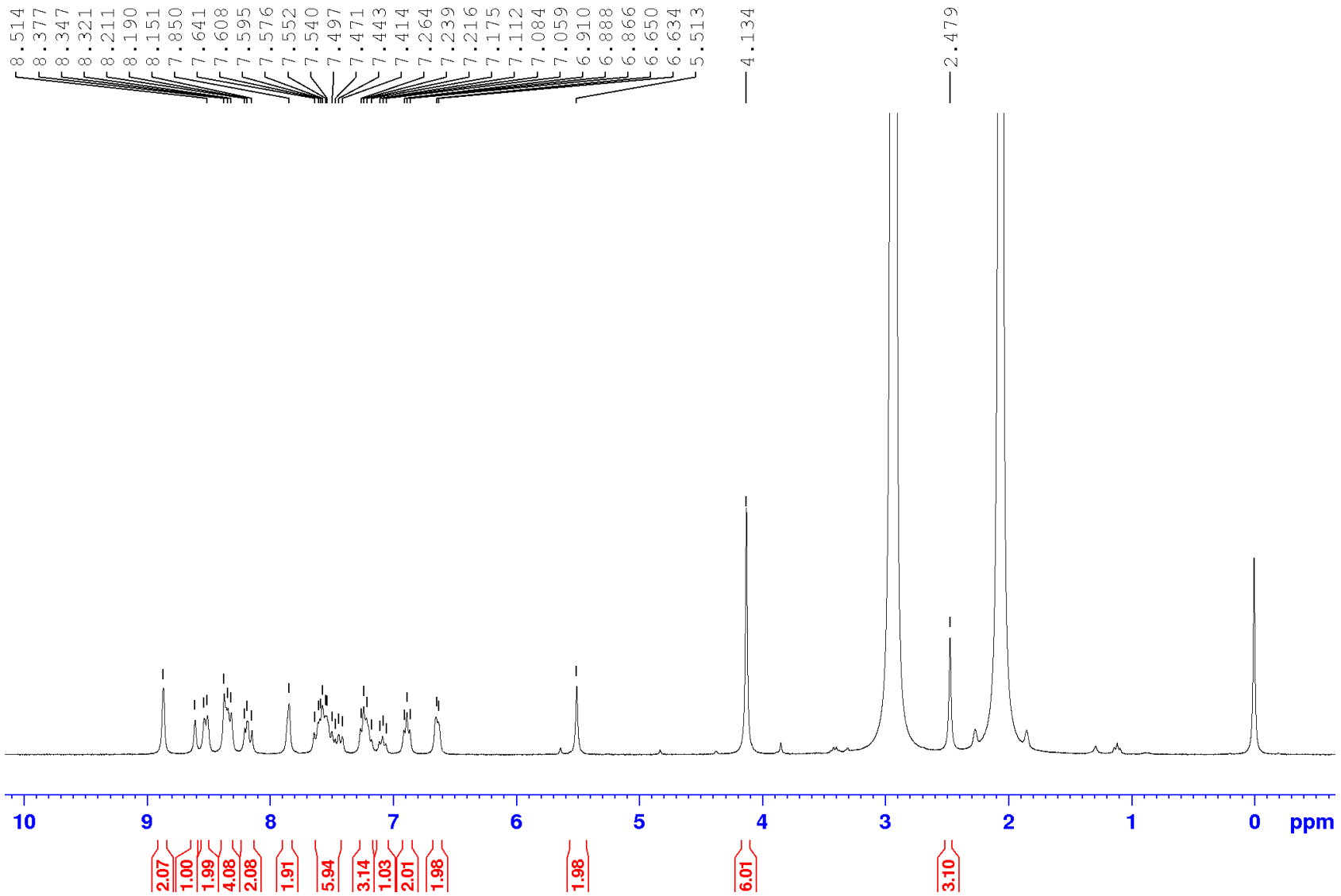


Fig. S25 ^{13}C NMR spectrum of complex **3** in $(CD_3)_2SO$ at 298 K.

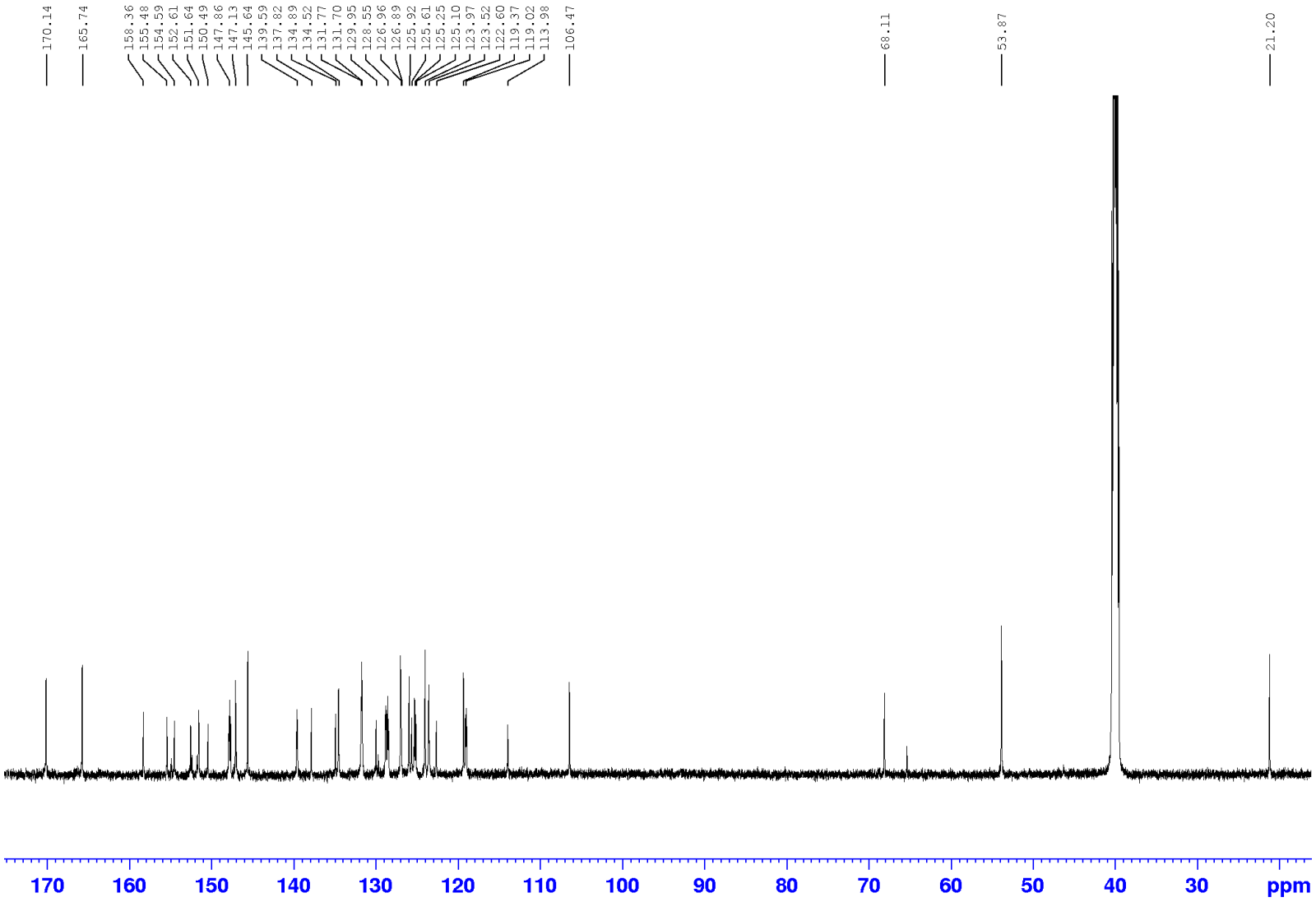
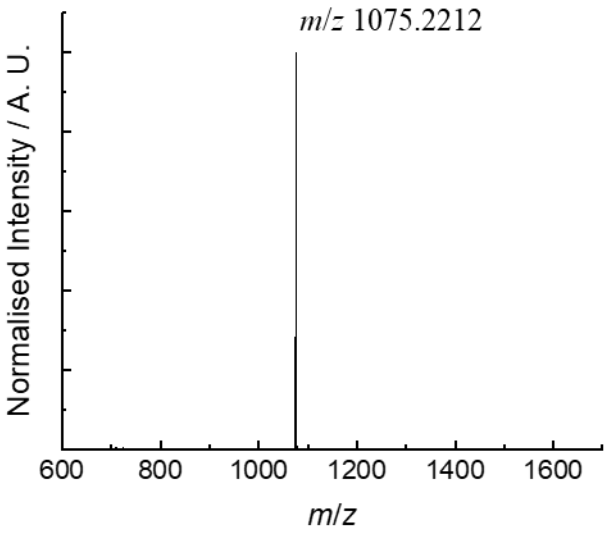


Fig. S26 HR-ESI mass spectrum of complex **3** in CH_3OH at 298 K.



Reference

1. K. Suzuki, A. Kobayashi, S. Kaneko, K. Takehira, T. Yoshihara, H. Ishida, Y. Shiina, S. Oishi and S. Tobita, *Phys. Chem. Chem*. *Phys*., 2009, **11**, 9850–9860.