Supplementary Information

Covid-19 Detection using AIE-Active Iridium Complexes

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EXPERIMENTAL SECTION

Materials. The iridium(III) dimer ($[Ir(PQ)_2Cl]_2$) was synthesized as reported in the literature.[1] Unless otherwise stated, all reactions were performed under an argon atmosphere using oven-dried glassware and commercially available chemicals and solvents. Ethanolamine, 2-phenylquinoline (PQ), 2,2′-bipyridine-4,4′-dicarboxylic acid, dithiothreitol (DTT) and ethidium bromide were purchased from Sigma-Aldrich. $IrCl_3 \cdot 3H_2O$ was obtained from Alfa-Aesar. Maleic anhydride, *N*,*N*'-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) were purchased from Central Drug House (CDH), India. Furan was obtained from Glr Innovations, India. Thin layer chromatography (TLC) was sourced from Merck, Germany. Agarose gel, polyacrylamide gel, and TBE buffer were obtained from HiMedia. qPCR and genomic control RNA of SARS-CoV-2 (isolate USA-WA1/2020) were obtained from BEI Resources, NIAID, NIH, USA (NR-52347 and NR-

52285). The qPCR RNA was extracted from heat-inactivated SARS-CoV-2 (NR-52286 lot 70033321) using a QIAamp® viral RNA mini kit (Qiagen® 52906) and vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) while the genomic RNA was extracted from SARS-CoV-2-infected cell lysate and supernatant of *Cercopithecus aethiops* kidney epithelial cells (Vero E6, ATCC® CRL-1586™) using QIAamp® viral RNA mini kit (Qiagen 52904). Black nunc 384-well microtitre plates (Greiner) were used for recording all the fluorescence intensities. Dynamic light scattering (DLS) measurements were performed using Zetasizer Nano ZS90 (Malvern Instrument Ltd., Worcestershire, UK). RNAase-free water (Qiagen, India) was used to prepare all aqueous solutions. All experiments and measurements were carried out at room temperature.

N-gene sequence. Target gene sequence of SARS-CoV-2 isolate 2019-nCoV/USA-WA1-A12/2020 gene="N" (1260 number of nucleotide bases)[2]

Accession MT020880

CDS 28274..29533

/product="nucleocapsid phosphoprotein"

/protein_id="QHU79201.1"

/translation="MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQG
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LPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGTSPARMAGNGGDAA
LALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAYNVTQAFGR
RGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYT
GAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTV
TLLPAADLDDFSKQLQQSMSSADSTQA"

Table S1 ASO sequences targeted against two selective portions of the SARS-CoV-2 N-gene.

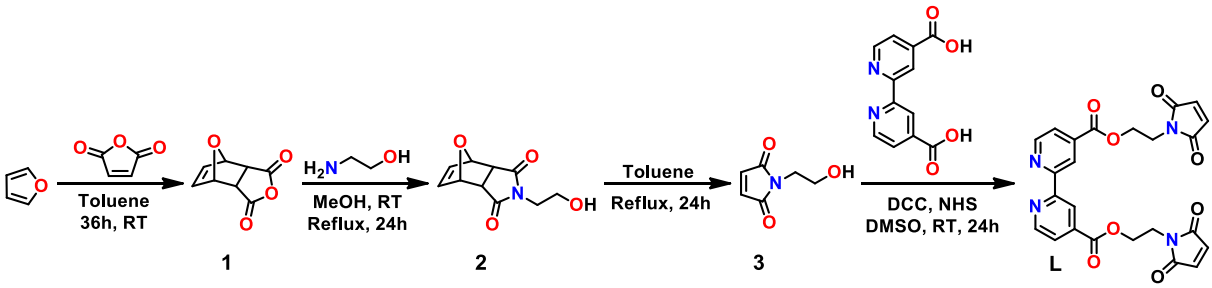
Starting target position	End target position	target sequence (5p → 3p)	antisense oligo (5p → 3p)	GC content	binding energy (kcal/mol)[2]
421	440	ACACCAAAAGAUCAC AUUGG	CCAATGTGATCTTTTGGTGT (**ASO1**)	40%	-15.8
443	462	CCCGCAAUCCUGCUAA CAAU	ATTGTTAGCAGGATTGCGGG (**ASO2**)	50%	-10.4

Instrumentation and Methods

TLC was carried out on aluminum plates coated with silica gel mixed with a fluorescence indicator. The synthesized ligands and complexes were purified with silica gel (60-120 mesh) column chromatography. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker 500 MHz spectrometer in $CDCl_3$ or DMSO-d_6 at room temperature with tetramethylsilane (TMS) as an internal standard. NMR standards used were as follows: (^{1}H-NMR) $CDCl_3$ = 7.26 ppm; DMSO-d_6 = 2.50 ppm. (^{13}C-NMR) $CDCl_3$ = 77.00 ppm; DMSO-d_6 = 39.52 ppm. All chemical shifts (δ) were reported in ppm relative to TMS. Spin multiplicities were reported as a singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), triplet of doublets (td), doublet of doublet of doublets (ddd), multiplet (m) and broad (br) with coupling constant (J) reported in Hz. Electrospray ionization mass spectra (ESI-MS) were obtained using a Waters make ESI-MS model synapt G2 high definition mass spectrometry. UV-vis absorption spectra were recorded using Agilent Technologies Carry 100 spectrophotometer at 298 K. Emission spectra was measured on Edinburgh Instruments F900 fluorescence spectrophotometer and SpectraMax M2 plate reader (Molecular Devices).

Synthesis and Characterization

Ligand synthesis



Scheme S1 Synthetic routes of 2,2′-bipyridine-4,4′-dicarboxylic acid functionalized with bis-*N*-(2-hydroxyethyl)maleimide (**L**).

Compound 1: This was synthesized after slightly modifying a method reported in literature.[3,4] Maleic anhydride (1.0 g, 10.20 mmol) was taken in an oven dried schlenk flask with 20 mL of toluene and stirred for 30 min under argon atmosphere. To this, furan (1.60 mL, 21.42 mmol) was added slowly over 15 min and the reaction mixture was stirred for the next 36 h at room temperature. After the reaction was complete, the solution was stored at 4 °C for 12 h to obtain precipitation. The solvent was then removed by vacuum filtration and the precipitate was washed with diethyl ether (3 × 20 mL), dried in vacuum to give a white crystalline powder as a pure product (yield: 80%).

^{1}H NMR (500 MHz, $CDCl_3$): **δ** 6.58 (s, 2H), 5.46 (s, 2H), 3.18 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): **δ** 170.06, 137.12, 82.34, 48.84.

Compound 2: Compound **1** (1.0 g, 6.02 mmol) was suspended in dry methanol (2.5 mL) and stirred under inert atmosphere. To this, ethanolamine (364 µL, 6.02 mmol) was added dropwise and refluxed for 24 h. The solution was then cooled to room temperature and kept at 4 ºC for 6 h to give an off-white precipitate. This precipitate was collected in a cooling condition by filtration and then washed with chilled ether (3 × 10 mL) to obtain a white microcrystals as a product (yield: 83%).

^{1}H NMR (500 MHz, DMSO): **δ** 6.55 (s, 2H), 5.12 (s, 2H), 4.79 (s, 1H), 3.41 (m, 4H), 2.92 (s, 2H). ^{13}C NMR (126 MHz, DMSO): **δ** 176.53, 136.50, 80.30, 57.28, 47.16, 40.63.

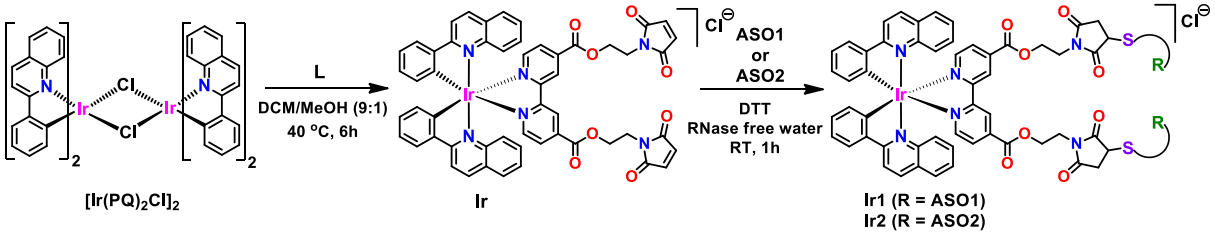
Compound 3: Compound **2** (0.6 g, 2.87 mmol) was dissolved in 10 mL of toluene and refluxed for 24 h under inert atmosphere. The reaction mixture was kept at 0 °C for 12 h to obtain a precipitate. The solvent was removed in a cooling condition via filtration and the precipitate was washed with pet ether (3 × 5 mL), and recrystallized by hot toluene to yield colourless crystals (yield: 64 %).

^{1}H NMR (500 MHz, DMSO): **δ** 7.01 (s, 2H), 4.79 (s, 1H), 3.46 (m, 4H). ^{13}C NMR (126 MHz, DMSO): **δ** 171.62, 134.98, 58.39, 40.02.

Synthesis of ligand (L): 2,2′-Bipyridine-4,4′-dicarboxylic acid (10 mg, 0.041 mmol) was dissolved in DMSO (0.4 mL). To this, DCC (21.1 mg, 0.102 mmol) and NHS (11.8 mg, 0.102 mmol) were added and the reaction mixture was stirred at room temperature under argon atmosphere for 1 h. Then, *N*-(2-hydroxyethyl)maleimide (12.1 mg, 0.086 mmol) was added to the above reaction mixture and stirred for 24 h at room temperature. The reaction was diluted with EtOAc (10 mL) and the insoluble DCU was removed via vacuum filtration. The organic phase was washed with water (3 × 20 mL) and brine (50 mL), dried over $MgSO_4$ and evaporated on a rotary evaporator to give an off-white crude material. This crude was purified by column chromatography on silica using CH_2Cl_2/MeOH (20:1, v/v) as the eluent to afford a white powdered product (yield: 89%).

^{1}H NMR (500 MHz,): **δ** 8.98 (d, *J* = 3.6 Hz, 2H), 8.91 (s, 2H), 7.98 (d, *J* = 3.4 Hz, 2H), 7.07 (d, *J* = 3.6 Hz, 4H), 3.85 (m, 4H), 2.93 (m, 4H).^{13}C NMR (126 MHz,): **δ** 171.34, 165.26, 156.57, 150.19, 139.13, 134.54, 123.41, 120.70, 62.05, 40.02. ESI-MS (*m/z*): calculated 491.1203 $[M+H]^+$, found 491.1162.

Synthesis of probes



Scheme S2 Synthesis of **Ir1** and **Ir2** probes for SARS-CoV-2 detection. Here, PQ = 2-phenylequinoline and **L** = 2,2′-bipyridine-4,4′-dicarboxylic acid functionalized with bis-*N*-(2-hydroxyethyl)maleimide.

Synthesis of Ir complex: The iridium(III) dimer, ($[Ir(PQ)_2Cl]_2$) (10 mg, 7.85 µmol) and the ligand **L** (8.5 mg, 0.017 mmol) were placed in an oven-dried round bottom-flask under argon atmosphere and to this, degassed CH_2Cl_2/MeOH (1.5 mL; 9:1 v/v) was introduced. The resulting mixture was heated at 40 °C and stirred for 6 h. Thereafter, the solution was cooled to room temperature, and the solvent was removed on a rotary evaporator to give a red-orange solid. The solid was washed with degassed diethyl ether (3 × 10 mL) and purified by preparative TLC with CH_2Cl_2/MeOH (20:1, v/v) to yield a red-orange powder (yield: 90%).

^{1}H NMR (500 MHz,): **δ** 8.94 (d, *J* = 15.9 Hz, 1H), 8.58 (dd, *J* = 11.3, 6.0 Hz, 5H), 8.31 (d, *J* = 17.5 Hz, 4H), 8.11 (m, 3H), 7.93 (t, *J* = 8.3 Hz, 3H), 7.43 (t, *J* = 7.2 Hz, 3H), 7.16 (d, *J* = 15.4 Hz, 5H), 7.07 (d, *J* = 15.5 Hz, 2H), 6.83 (d, *J* = 5.2 Hz, 2H), 6.37 (d, *J* = 5.4 Hz, 2H), 3.87 (m, 4H), 3.06 (m, 4H).^{13}C NMR (126 MHz, DMSO): **δ** 173.36, 169.98, 163.91, 155.85, 150.40, 146.95, 146.06, 141.17, 140.23, 136.92, 135.12, 134.01, 131.92, 131.28, 130.00, 129.52, 129.36, 128.36, 127.92, 127.69, 127.41, 124.31, 118.86, 53.79, 45.96. ESI-MS (*m/z*): calculated 1091.2380 $[M]^+$, found 1091.2304.

Synthesis of Ir1 and Ir2 probes: 25 µL of ASO1 or ASO2 oligomers were taken from the master stock solution of 200 µM in a 1.5 ml vial. 5 µL DTT (from 1 mM stock solution) was added to the above solution and stirred for 15 min at room temperature. Subsequently, 2.5 µL of iridium maleimide complex (**Ir**) was taken from 1 mM stock solution and along with 67.5 µL RNase-free water was added to make up the final volume as 100 µL and stirred for the next 30 min under inert atmosphere. The reaction mixture was centrifuged at 1000 rpm for 5 min and the supernatant was carefully collected as a product (**Ir1** or **Ir2**). The supernatant solution was stored at 4 ºC in a refrigerator for several weeks.

Agarose gel electrophoresis

For the gel electrophoresis experiments, agarose gel (2.8 % w/v) was prepared in 1X Tris/Borate/EDTA (TBE) buffer. A 0.8 µg of samples were loaded on to gel after mixing with 6X DNA loading dye. The electrophoresis was carried out in 1X TBE buffer for 1.5 h at 80 V. The gel images were taken and bands were visualized under UV light using a transilluminator at 254 nm wavelength while the fluorescence photograph of the bands of iridium probes (**Ir1** and **Ir2**) were recorded under UV light of 365 nm wavelength.

Polyacrylamide gel electrophoresis (PAGE)[5]

For this experiment, the gel was prepared using 20 % denaturing 8.0 M urea polyacrylamide gel having 1X TBE buffer. The samples were prepared by mixing of 1.2 µg of ASO's (ASO1 and ASO2) or Ir-ASO's conjugates (**Ir1** and **Ir2**) with 2X RNA loading dye containing 1.0 µg/mL ethidium bromide. Before loading to the gel, samples were denatured at 70 °C in dry bath for 10 minutes, then immediately chilled on ice followed by loading on gel and the electrophoresis was carried out using

1X TBE buffer at 50 V for 4 h. The gel images were recorded using Gel Imaging System (Bio-Red ChemiDoc).

Probe characterization

The UV-visible absorption and fluorescence emission spectra of 10 µM **Ir1** and **Ir2** probes in RNase-free water (Qiagen) were recorded at room temperature using the Shimadzu UV-2450 and Edinburgh Instruments F900 fluorescence spectrophotometers, respectively. All the measurements were performed in quartz cuvettes with an optical path length of 10 mm and the excitation wavelength used for fluorescence measurements was 430 nm. The dynamic light scattering (DLS) experiments were performed with Malvern Nano ZS90 Zetasizer by taking 100 nM **Ir1** or **Ir2** probes in 200 µL of RNase-free water with and without 3300 copies of qPCR control RNA. The DLS was operated at 25 ºC using a 4 mW laser at 632.8 nm wavelength and 90º detector angle.

Sample preparation

25 µM master stocks of **Ir1** and **Ir2** probes were prepared in RNase-free water and serially diluted with RNase-free water as desired. Quantitative PCR (qPCR) control RNA containing 50,000 copies/µL (or ~ 83 fM) in pH 8 TE buffer (10 mM Tris-HCl, 1 mM EDTA) and genomic RNA containing 18400 copies/µL (or ~ 30.6 fM) in cellular nucleic acid and carrier RNA matrix were obtained from BEI Resources, USA (NR-52347 and NR-52285). The master stocks were serially diluted with RNase-free water as required.

Assay procedure

Reactions were performed at RT in 30 µL assay volumes. For this, 1 µL of RNA sample containing 500 or 5 copies was added to 29 µL of **Ir1/Ir2** complex in a 384 well microtitre plate (Greiner) and the fluorescence spectra of this mixture was recorded after 10 min using the SpectraMax M2 plate reader (λ_{ex} = 430 nm, λ_{em} = 610 nm). The final data was reported as normalized fluorescence intensity after subtracting the fluorescence intensity of the non-RNA template control (NTC). All experiments were performed in duplicates and repeated twice. The results were reported.as the mean of four readings ± 1 SD.

Clinical study

Nasopharyngeal-oropharyngeal swabs were collected from 10 COVID-19 positive and 10 COVID-19 negative patients in a commercial viral transport medium (VTM). The VTM containing two swabs was briefly vortexed and 150 µL of the VTM was subjected to viral RNA extraction process using commercial viral RNA/DNA purification kits as prescribed by the manufacturer (Macharey-Nagel GmbH and Co. KG-Düren, Germany). The final elution volume was 50 µL. The RT-PCR of these samples was performed as reported earlier.[6]

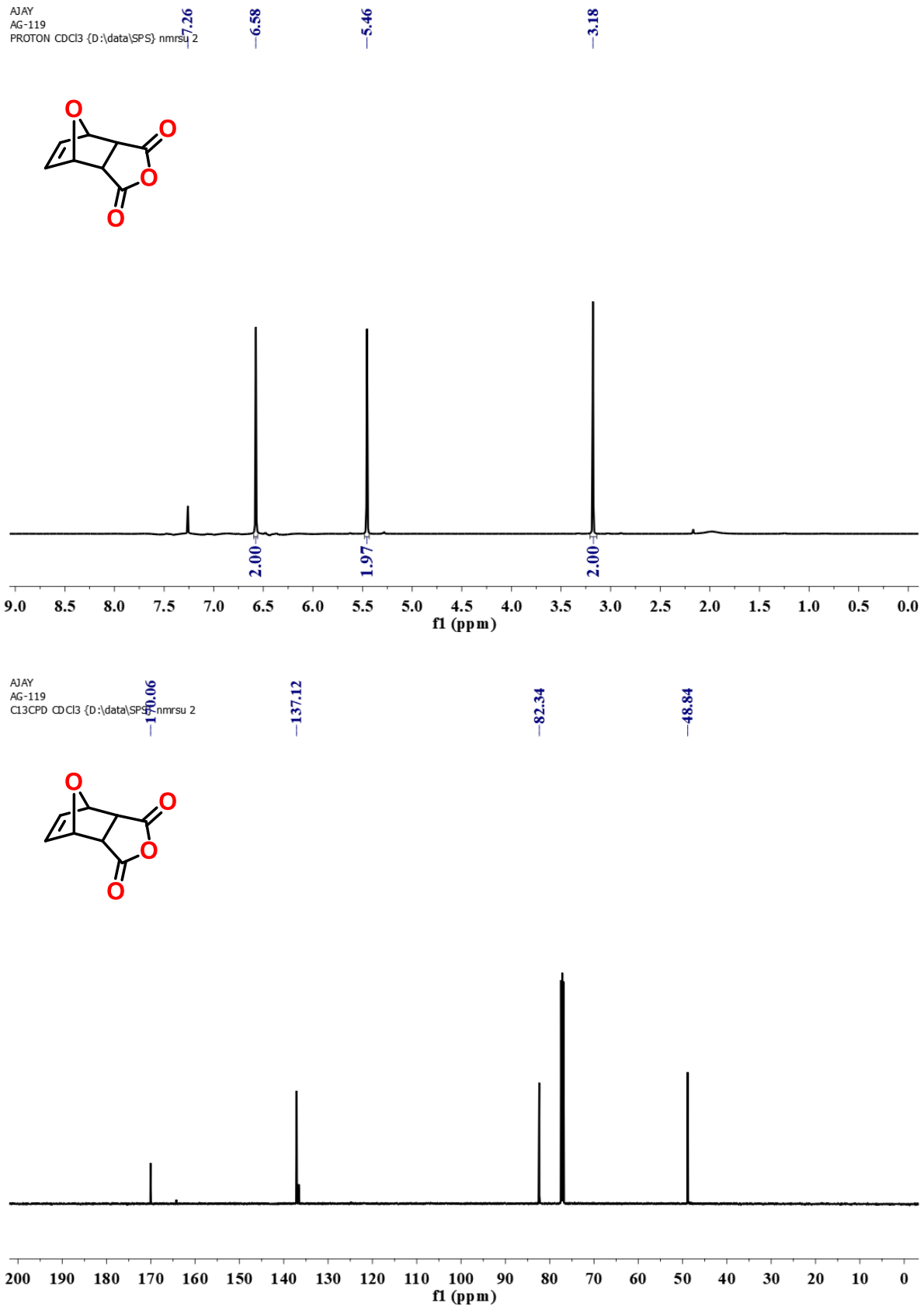


Fig. S1 ^{1}H and ^{13}C NMR of **1** in $CDCl_3$ at 298K.

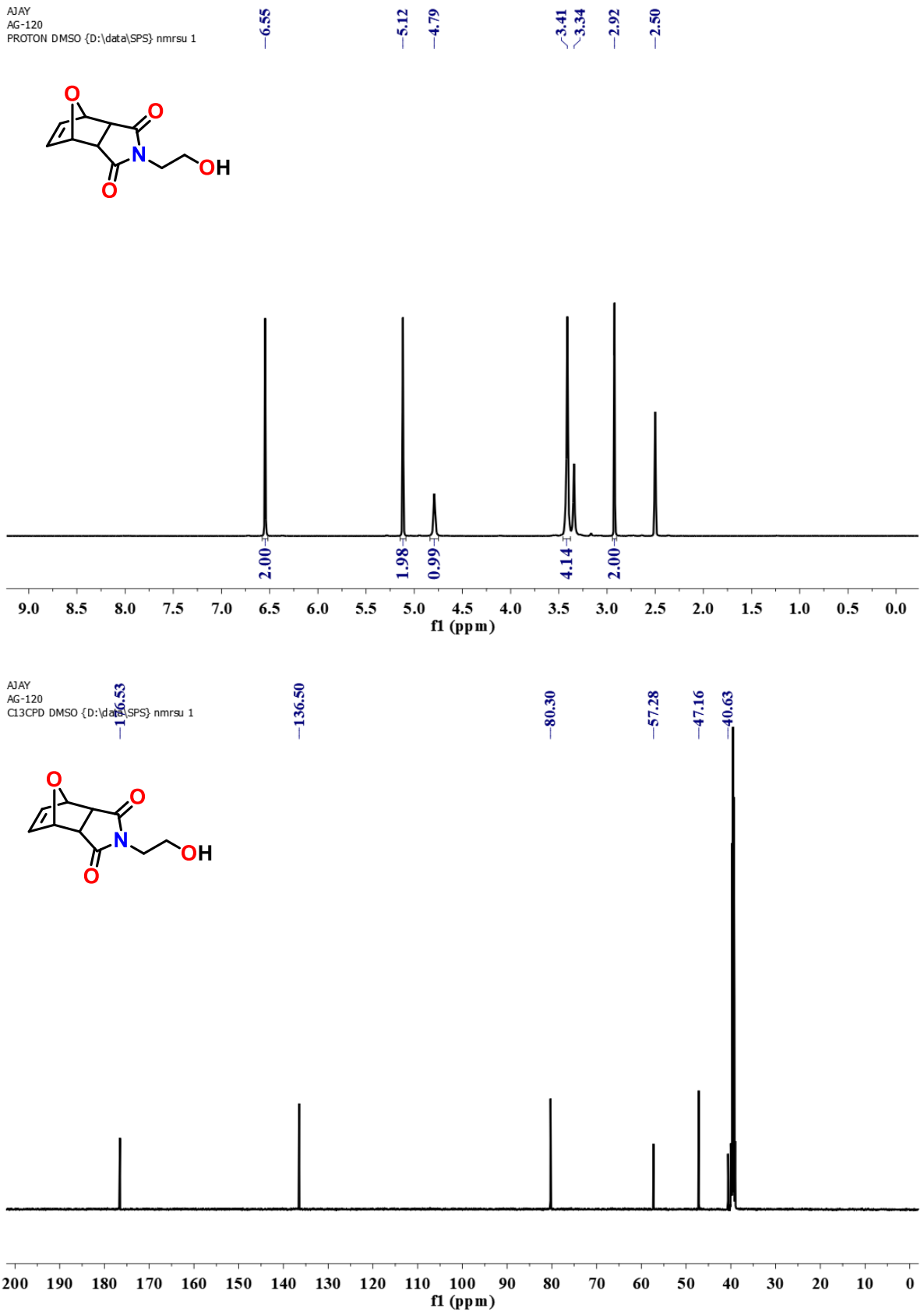


Fig. S2 ^{1}H and ^{13}C NMR of **2** in DMSO at 298K.

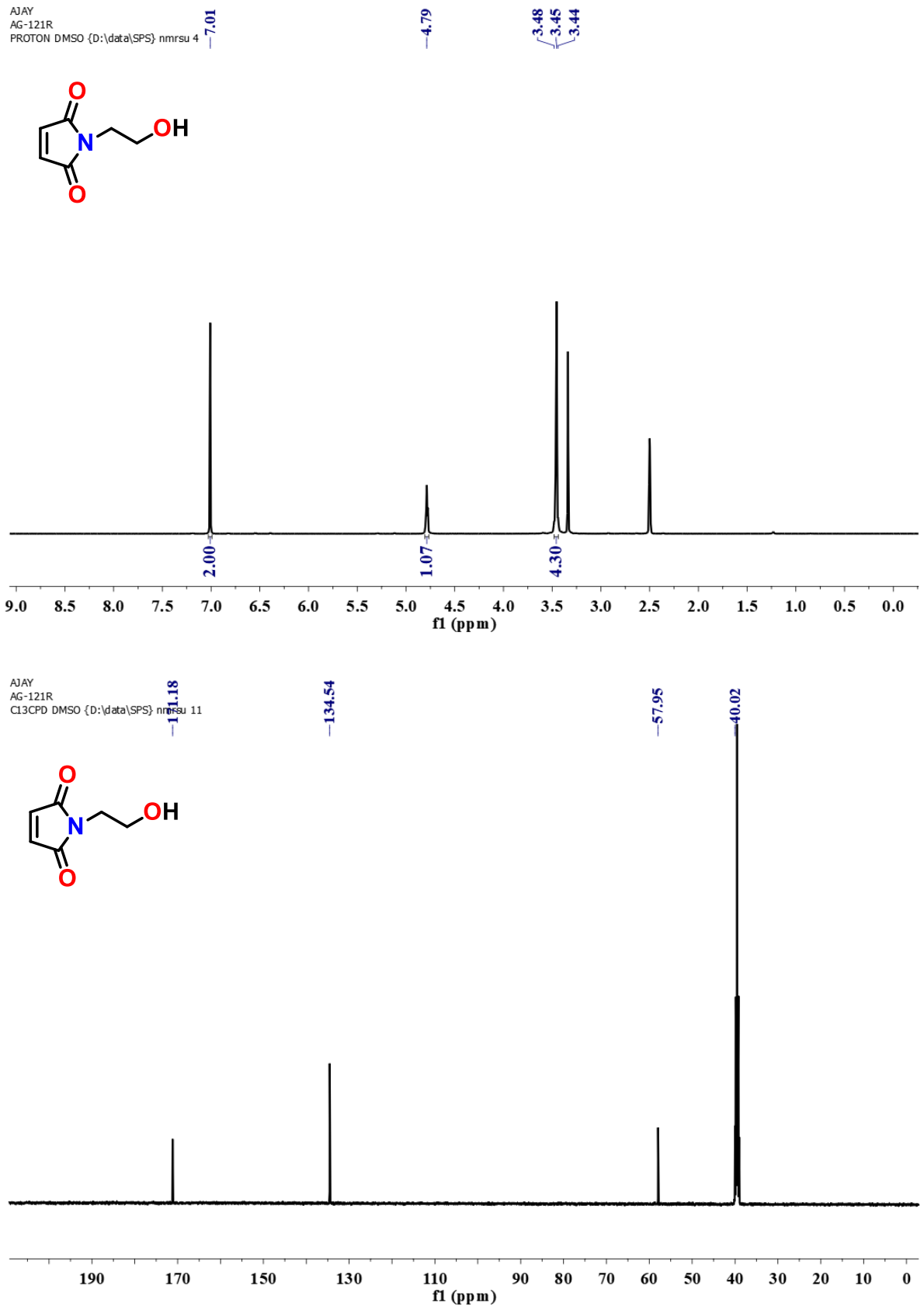


Fig. S3 ^{1}H and ^{13}C NMR of **3** in DMSO at 298K.

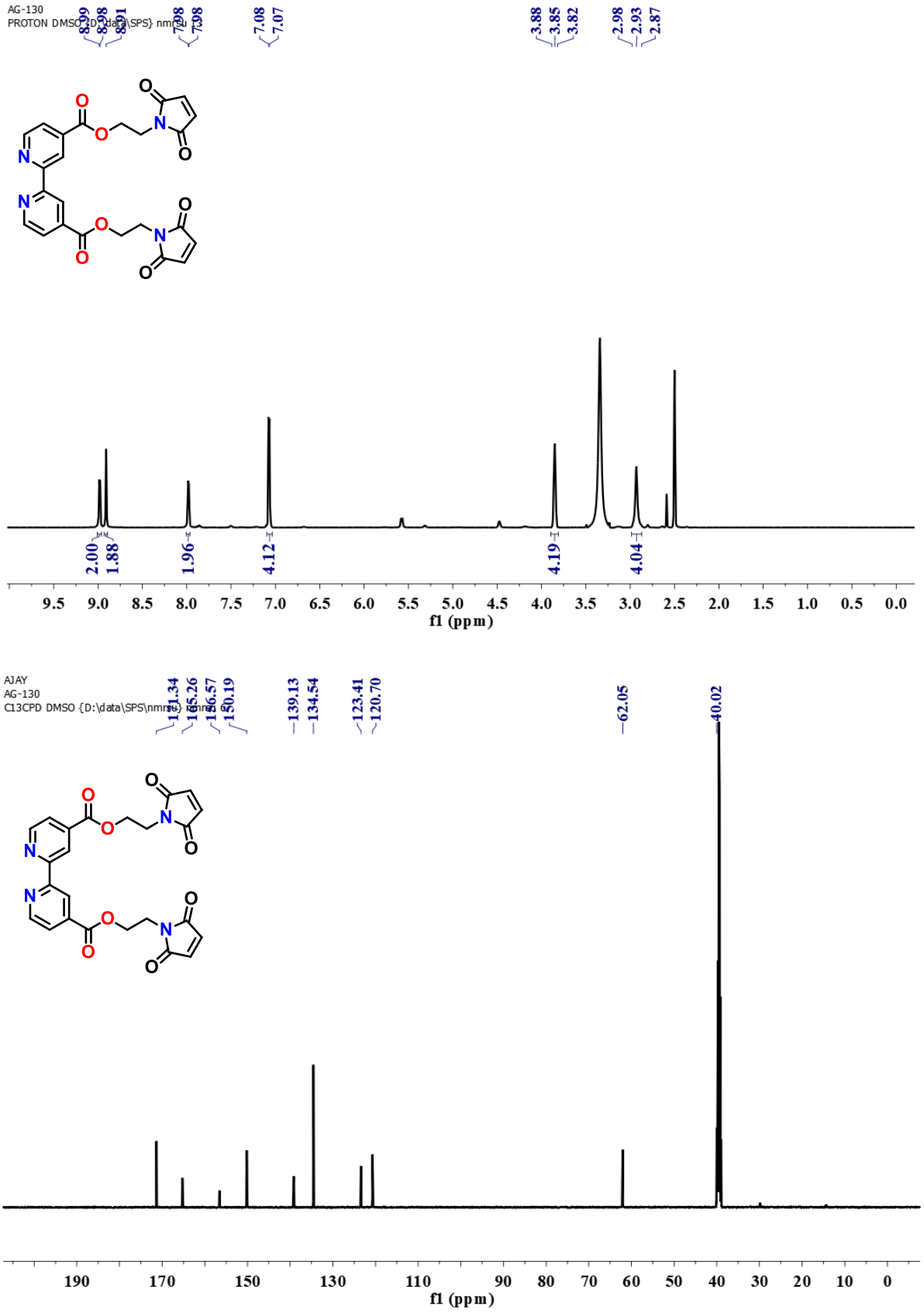


Fig. S4 ^{1}H and ^{13}C NMR of **L** in DMSO at 298K.

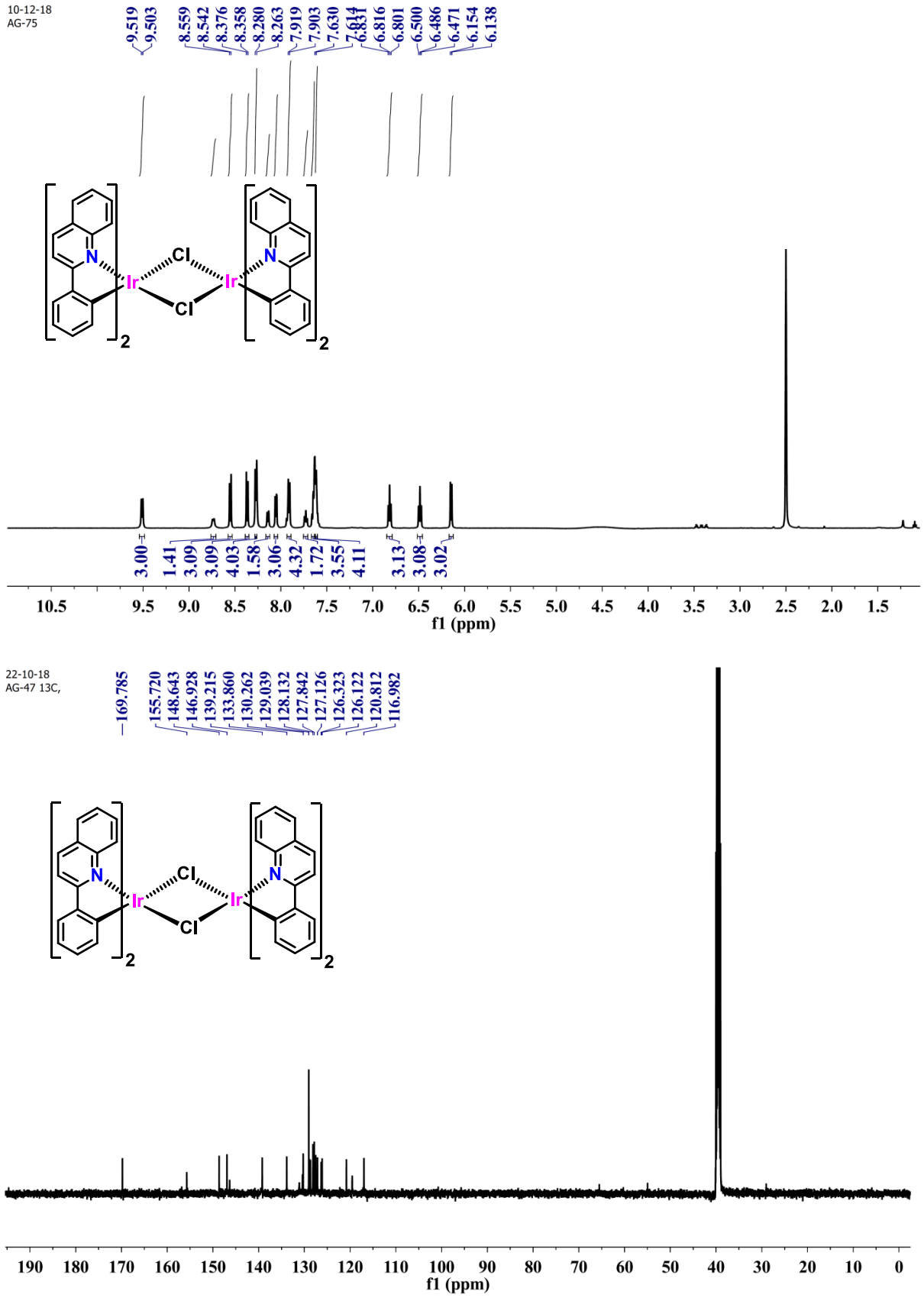


Fig. S5 ^{1}H and ^{13}C NMR of bis-[μ-chloro-bis(2-phenylquinolinato)iridium(III)] dimer in DMSO.

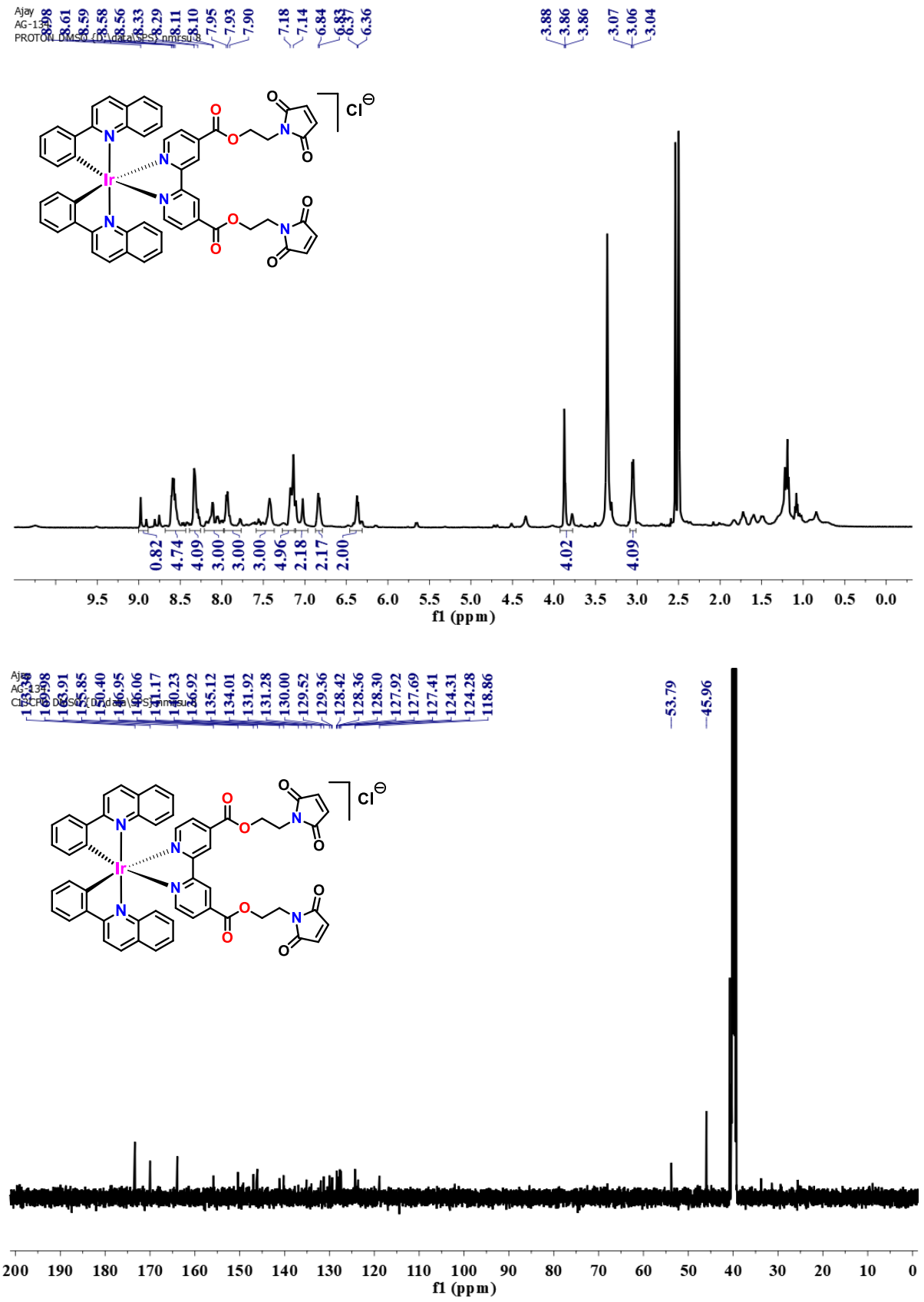


Fig. S6 ^{1}H and ^{13}C NMR of **[Ir(PQ)$_2$(L)]Cl** in DMSO at 298K.

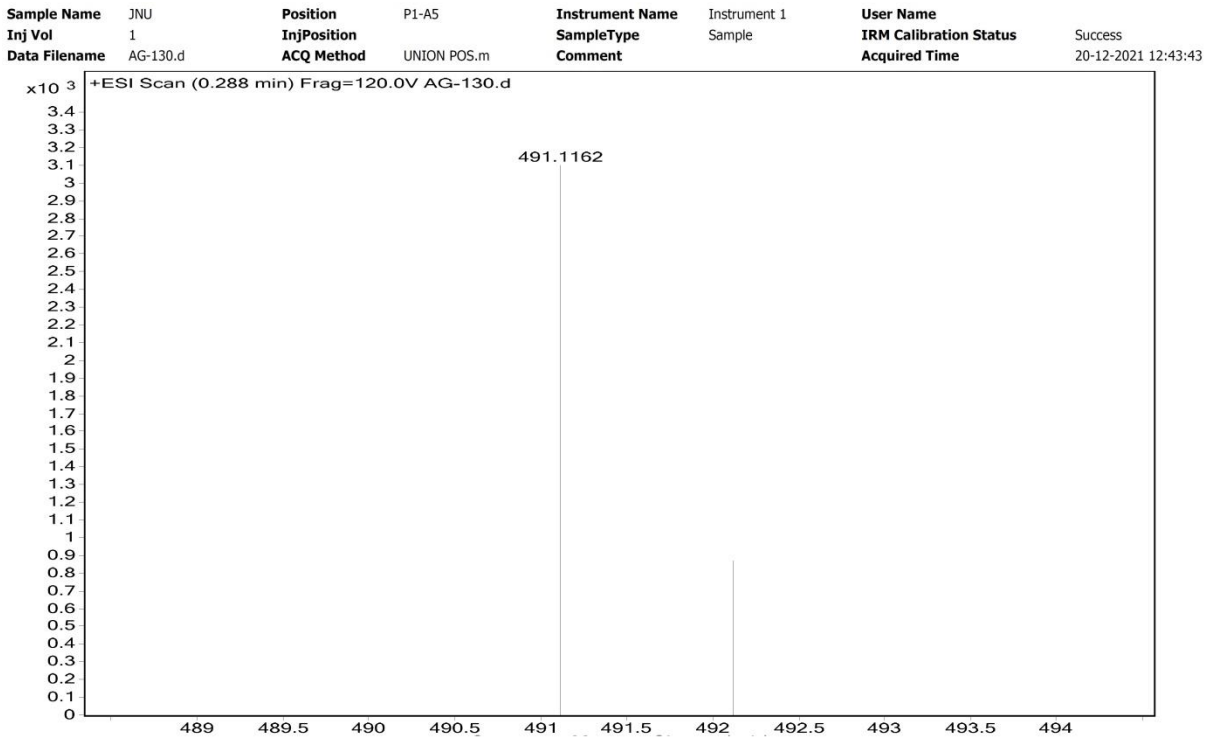


Fig. S7 The ESI mass spectrum of **L** in MeCN showing the peak at 491.1162 (m/z) assignable to $[M+H]^+$ at 298K.

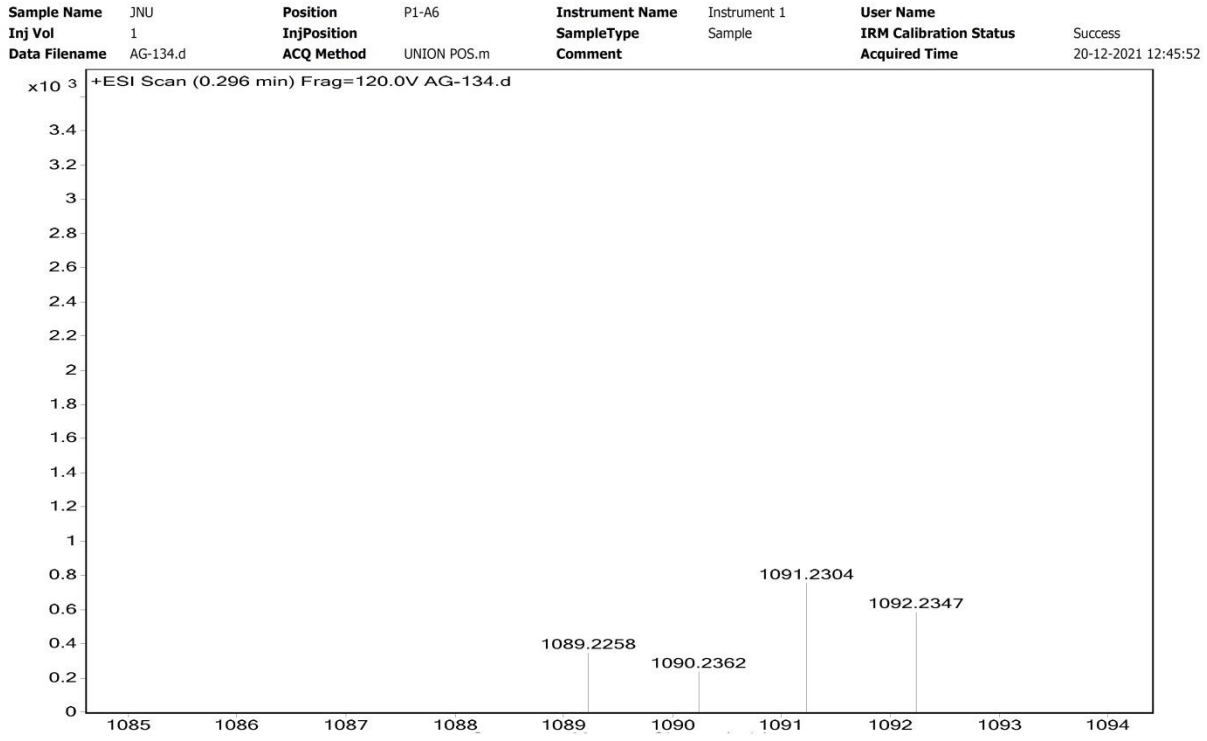


Fig. S8 The ESI mass spectrum of **$[Ir(PQ)_2(L)]Cl$** in MeCN showing the peak at 1091.2304 (m/z) assignable to $[M]^+$ at 298K.

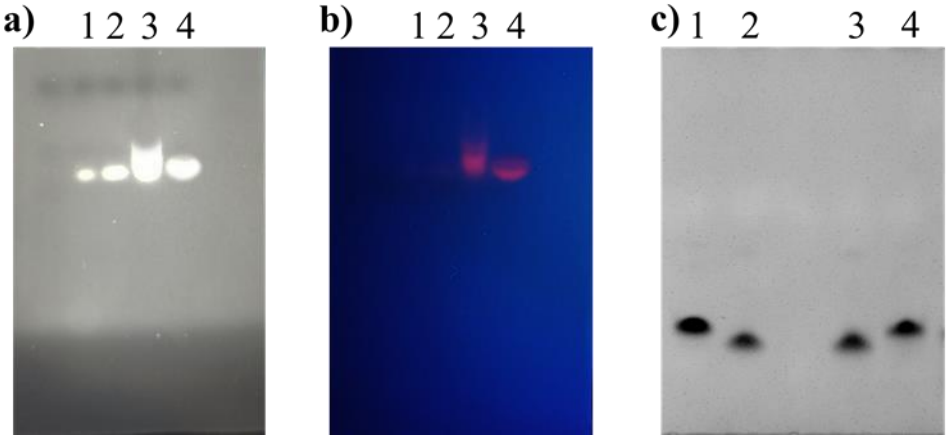


Fig. S9 Gel electrophoresis of iridium probes (**Ir1** and **Ir2**) in 1X TBE buffer at 80 V for 1.5 h and visualized under UV light at (a) λ = 254 nm and (b) λ = 365 nm. Lane 1: ASO1, Lane 2: ASO2, Lane 3: **Ir1** and Lane 4: **Ir2**. (c) Denaturing PAGE analysis of iridium probes (**Ir1** and **Ir2**) in 1X TBE buffer at 50 V for 4 h and visualized under UV light (λ = 254 nm). Lane 1: **Ir1**, Lane 2: ASO1, Lane 3: ASO2, Lane 4: **Ir2**.

The bands in agarose gels were directly observed under UV light at 254 nm (**Fig. S9a**) and 365 nm (**Fig. S9b**), which were convenient to discriminate ASO's (ASO1 and ASO2) and Ir–ASO's conjugates (**Ir1** and **Ir2**). Indeed, the reddish luminescence of bands for **Ir1** and **Ir2** probes were visualized at 365 nm,[5] which was not observed for ASO1 and ASO2 (**Fig. S9b**). These results confirmed the successful conjugation of iridium moiety with ASO's. Further, we validated these results by PAGE analysis in which the fast-moving band corresponds to the ASO's sequence while the slow moving band is confirmed as the ASO's-functionalized iridium complexes (**Ir1** and **Ir2**) shown in **Fig. S9c**.

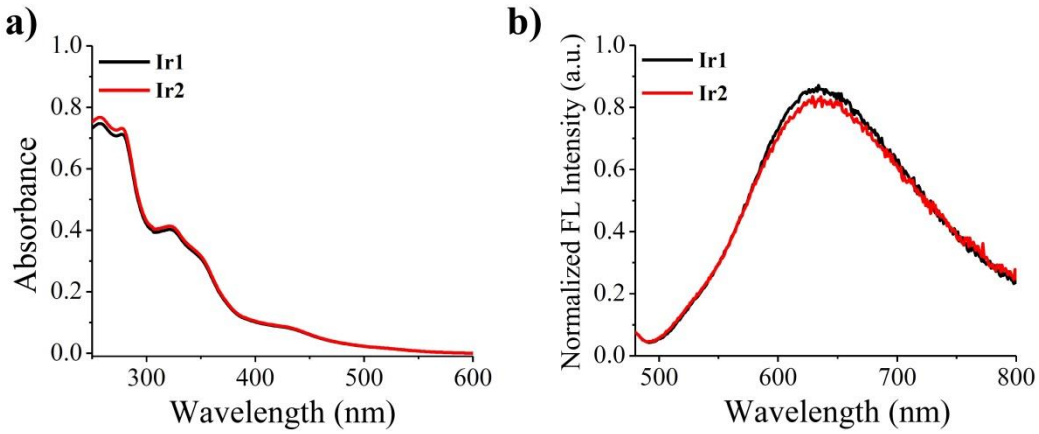


Fig. S10 (a) Absorption and (b) normalized emission spectra (λ_{ex} = 430 nm, λ_{em} = 610 nm) of 10 µM solution of **Ir1** and **Ir2**.

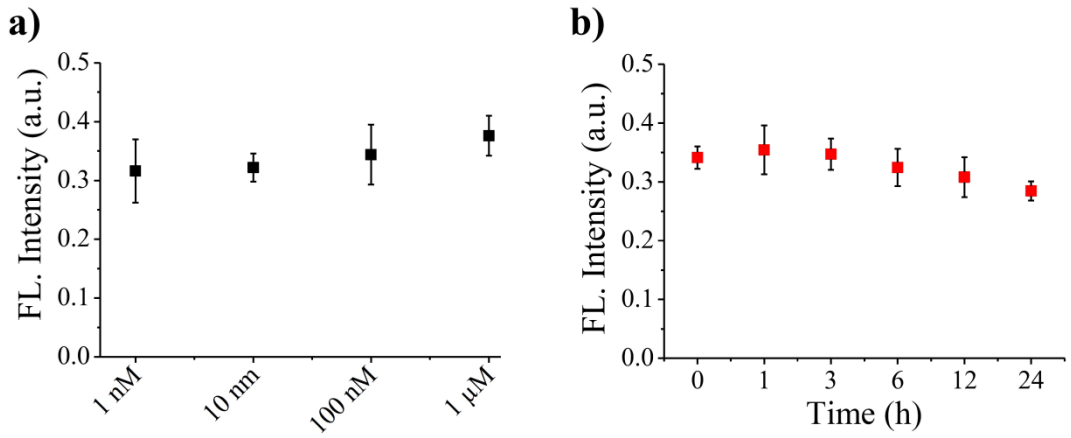


Fig. S11 (a) Fluorescence emission intensity (λ_{ex} = 430 nm, λ_{em} = 610 nm) of **Ir1** probe as a function of concentration. (b) The stability of 100 nM solution of **Ir1** probes measured by fluorescence spectroscopy in RNA-free water at RT at different time points (λ_{ex} = 430 nm, λ_{em} = 610 nm).

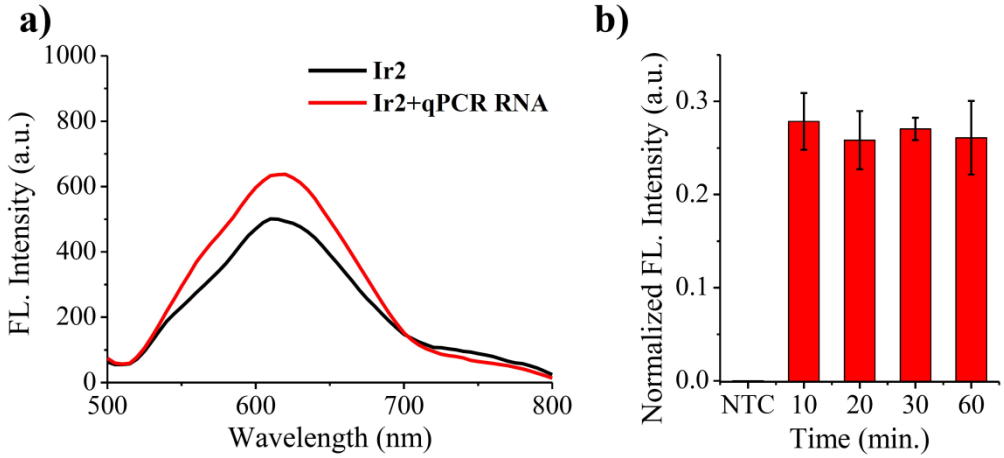


Fig. S12 (a) Emission spectra (λ_{ex} = 430 nm, λ_{em} = 610 nm) of **Ir2** (10 μM) in the absence (—) and presence (—) of qPCR control RNA in RNase-free water at room temperature. (b) Normalized emission intensity of **Ir1** (100 nM) with qPCR control RNA (500 copies) of SARS-CoV-2 RNA at different time point.

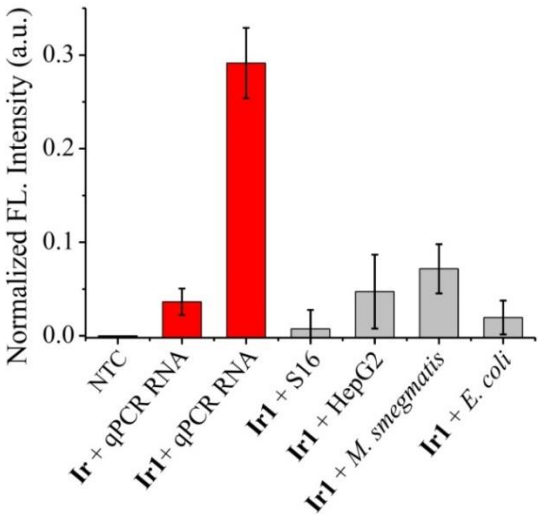


Fig. S13 Emission intensity of 100 nM **Ir** (without ASO) and **Ir1** with qPCR control SARS-CoV-2 RNA (500 copies), and **Ir1** with non-COVID RNAs from HepG2, *M. smegmatis* and *E. coli*, and S16 synthetic bacterial RNA (weight equivalent to 500 copies of SARS-CoV-2 RNA). NTC stands for non-template control.

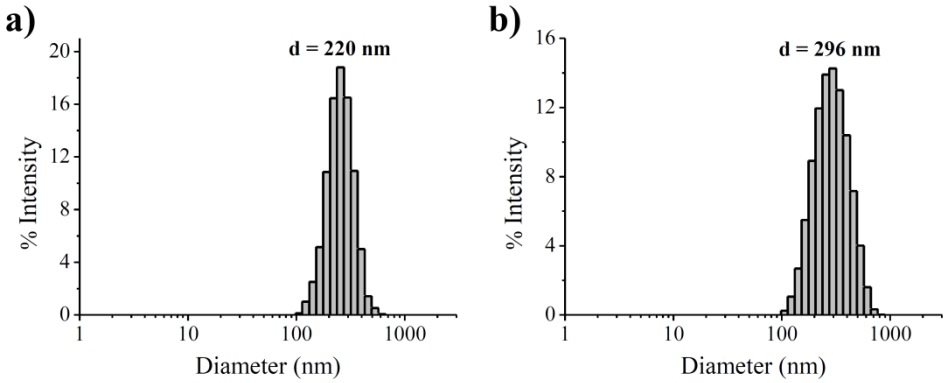


Fig. S14 Size distribution of **Ir1** (100 nM) (a) in RNA free water and (b) in the presence of qPCR control RNA (500 copies per 30 µl reaction mixture).

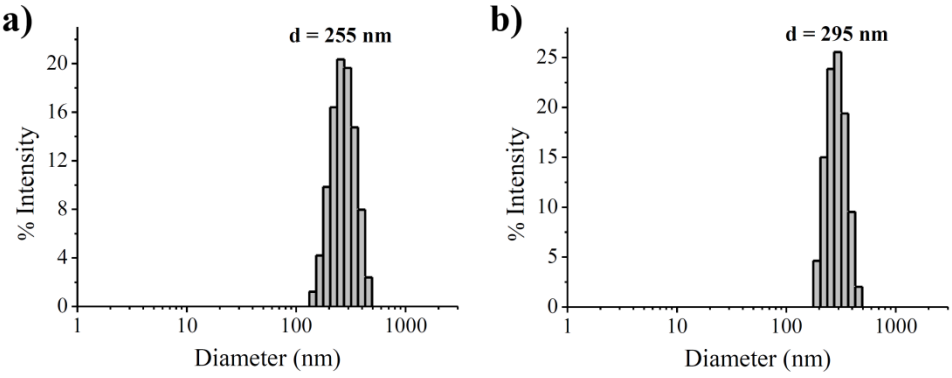


Fig. S15 Size distribution of **Ir2** (100 nM) (a) in RNA free water and (b) in the presence of qPCR control RNA (500 copies per 30 µl reaction mixture).

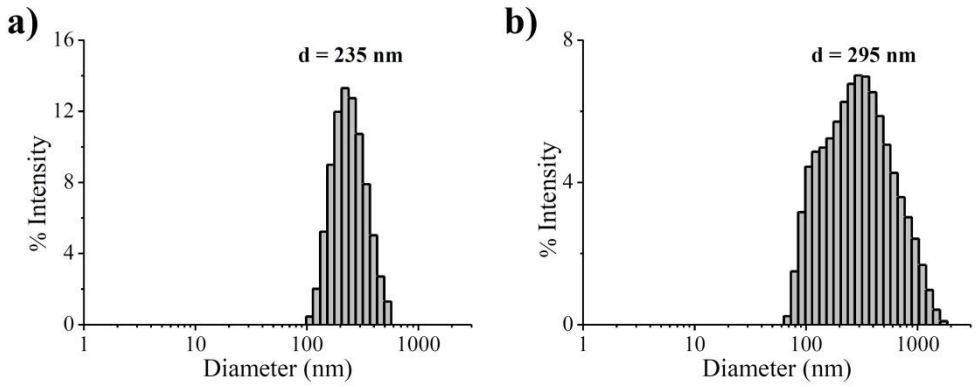


Fig. S16 Size distribution of **Ir1:Ir2** (1:1) 50 nM each **(**a) in RNAse free water and (b) in the presence of qPCR control RNA (500 copies per 30 µl reaction mixture).

Supplementary References

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