Electronic Supplementary Material (ESI) for Analyst.

Unique Development of a New Dual Application Probe for Selective Detection of Antiparallel G-Quadrauplex

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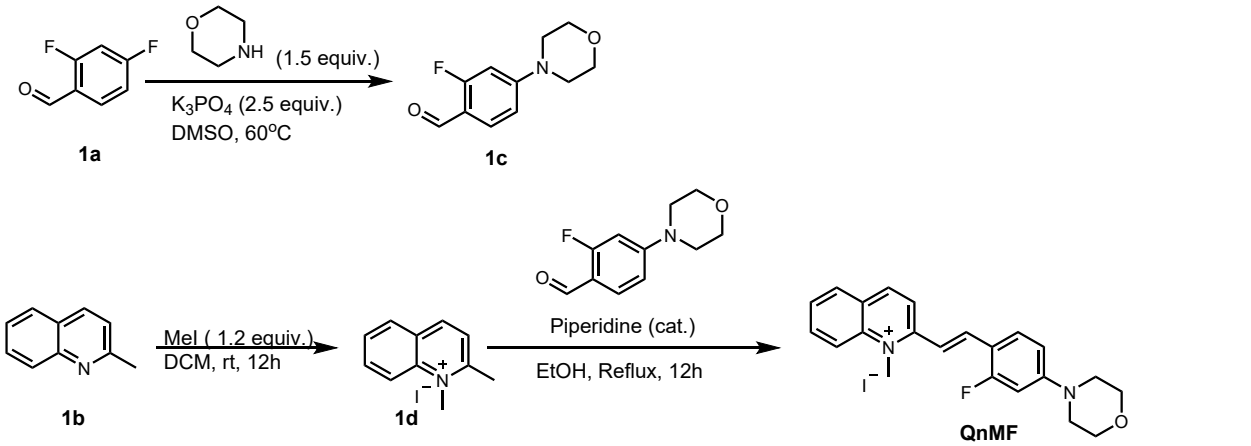
Contents

1. General Information

All the chemicals including organic solvents were obtained from commercial vendors and used without further purification. The oligonucleotides used in this work were purchased from Barcode Biosciences Biotechnology (Bangalore, India) and the sequences were listed in Table 2. Oligonucleotides were dissolved in 10 mM Tris-HCl buffer (containing 100mM KCl or NaCl, pH 7.5). Prior to use, all oligonucleotides were pre-treated by heating at 95 °C for 5 min, followed by gradual cooling to room temperature and kept at this temperature for 30 min. The UV-vis spectra were recorded at room temperature using a Cary Series UV–Vis spectrophotometer (Agilent Technologies) and a 1-cm path-length quartz cuvette. Fluorescence emission spectra were recorded at room temperature using a PF–65000 spectrofluorometer. CD spectra were performed on a JASCO-J815 circular dichroism spectrophotometer. The ^{1}H NMR ^{13}C NMR and ^{19}F NMR spectra were measured on a 400 MHz spectrometer using TMS(^{1}H and ^{13}C) and TFT (^{19}F) as internal standard and Jeol JNMECZ 600S Nuclear Magnetic Resonance Spectrometer using $CDCl_3$, DMSO-d_6, CD_3OD and D_2O as the solvent. A Xevo G2-XS QT of spectrometer and Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer was used to measure the mass-spectra of the synthesized compounds.

2. Synthesis of (E)-2-(2-fluoro-4-morpholinostyryl)-1-methylquinolin-1-ium iodide (QnMF)



(*E*)-2-(2-fluoro-4-morpholinostyryl)-1-methylquinolin-1-ium iodide

Synthesis of 1,2-dimethylquinolin-1-ium iodide (1d). The solution of 2-methylquinoline (1.00 g, 9.35 mmol) and MeI (1.19 g, 8.38 mmol) in DCM (20 mL) was stirred under a N_2 atmosphere at room temperature for 12 h. The solid that formed was filtered off, washed with ether (3 × 15 mL), and dried under vacuum to give 1,2-dimethylquinolin-1-ium iodide (**2**, 1.4g, 70%). **^{1}H NMR (400 MHz, DMSO-d_6):**δ 9.11 (d, *J* = 8.53 Hz, 1H), 8.59 (d, *J* = 8.99 Hz, 1H), 8.41 (d, *J* = 8.07 Hz, 1H), 8.21–8.25 (m, 1H), 8.13 (d, *J* = 8.25 Hz, 1H), 8.00 (t, *J* = 7.86 Hz, 1H), 4.45 (s, 3H), 3.30 (s, 1H).**^{13}C NMR ($CDCl_3$, 100 MHz):**δ (ppm) 161.65, 145.91, 139.7, 135.54, 130.80, 129.48, 128. 28, 125.61, 119.47, 40.21, 23.57.

Synthesis of 2-fluoro-4-morpholinobenzaldehyde (1c). The solution of 2,4-difluorobenzaldehyde (**1a**, 1.00 g, 7.03 mmol), Morpholine (0.910mL, 10.55 mmol) and K_3PO_4 (3.73 g, 17.6 mmol) were suspended in DMSO in a round bottom flask, heated to 60°C with stirring for 16 hours, cooled to RT. The reaction mixture was poured into ice water (50 mL) and was extracted with

ethyl acetate (3X 30 mL) the combined fractions were collected, dried over anhydrous sodium sulfate, and evaporated to dryness to yield crude solid. The solid was recrystallized to obtain the product as pale yellow solid. (**1c**, 0.9 g, 61%).

Synthesis of (E)-2-(2-fluoro-4-morpholinostyryl)-1-methylquinolin-1-ium iodide (QnMF). Piperidine (1 drop) was added to a solution of 1,2-dimethylquinolin-1-ium iodide (**2**, 0.3 g, 1.89 mmol) and 2-fluoro-4-morpholinobenzaldehyde (**1c**, 0.396 g, 1.89 mmol) in dry ethanol and then the mixture was heated under reflux for 16 h. After cooling to room temperature, the solid was filtered off, washed with cold EtOH, and recrystallized from diethyl ether to obtain QnMF as a brown solid (0.25 g, 38%). **^{1}H NMR (400 MHz, DMSO-d_6):** δ 9.08 (d, *J* = 8.75 Hz, 1H), 8.58 (d, *J* = 9.35 Hz, 1H), 8.49 (d, *J* = 8.75 Hz, 1H), 8.42–8.39 (m, 1H), 8.23–8.19 (m, 1H), 8.17–8.05 (m, 2H), 7.98(t, *J* = 7.48 Hz, 1H), 7.90(d, *J* = 16.2 Hz, 1H), 7.12–7.06 (m, 2H), 4.57 (s, 3H), 3.83(t, *J* = 4.38 Hz, 4H), 3.00(t, *J* = 4.4 Hz, 4H). **^{13}C NMR ($CDCl_3$, 100 MHz):** δ (ppm) 166.26, 163.77, 156.91, 155.36 (*J* = 9.05 Hz), 145.02, 142.48, 139.68, 135.5, 131.79 (*J* = 9.87 Hz), 130.62, 129.51, 128.33, 125.33 (*J* = 2.70 Hz), 121.70, 119.77, 119.71, 110.68 (*J* = 22.03 Hz),107.27 (*J* = 22.93 Hz), 66.62, 53.35. **MS (LCMS, FAB$^+$):** Calculated for $C_{22}H_{22}FN_2O^+$ ([M]$^+$): *m*/*z*: 349.17; found: 349.20

Table 2: DNA/Oligonucleotide sequences used in this study.

ODN	Sequence 5´→ 3´	Topology/ structure	Molar extinction coefficient (L mol^{-1} cm^{-1})
cMyc	TGGGGAGGGTGGGGAGGGTGGGGAAGG	Intramolecular parallel	279,900
c-KIT-1	AGGGAGGGCGCTGGGAGGAGGG	Parallel	226,700
c-KIT-2	CCCGGGCGGGCGCGAGGGAGGGGAGG	Parallel	253,400
TBA	GGTTGGTGTGGTTGG	Intramolecular anti-parallel	143,300
22AG ($Na^{+/}$ K^+)	AGGGTTAGGGTTAGGGTTAGGG	Intramolecular anti-parallel/hybrid	228,500
HRAS	TCGGGTTGCGGGCGCAGGGCACGGGCG	Intramolecular anti-parallel	250,400
BOM17	GGTTAGGTTAGGTTAGG	Anti-parallel	174,600
Hairpin	ACGTGCCACGATTCAACGUGGCACAG	Not specified	249,700
ssDNA	CCAGTTCGTAGTAACCC	Single-stranded	160,900
dsDNA	GGGTTACTACGAACTGG & CCAGTTCGTAGTAACCC	Double-stranded	167,400 & 160,900
TWJ	CGC AAG CGA CAG GAA CCT CGA GGA ATT CAA CCA CCG GAC G GCA GGC TAG GAC GGA TCC CTC GAG GTT CCT GTC GCT TGC G	Not specified	757,800

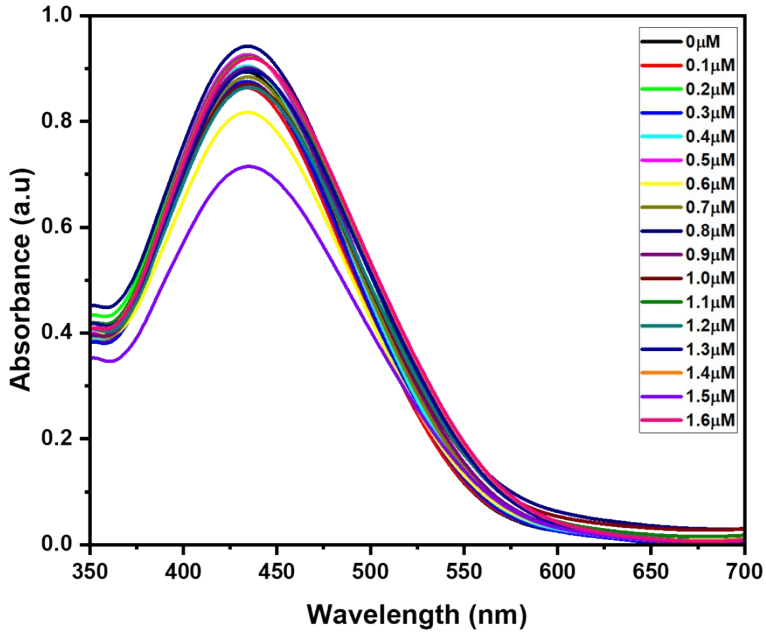


Figure S1: a) UV–Vis spectra of **QnMF**(2μM) in the presence of various concentrations of 22AG (0–1.6 mM) in buffer solution containing 10 mM Tris–HCl at pH 7.5 and 100 mM of NaCl

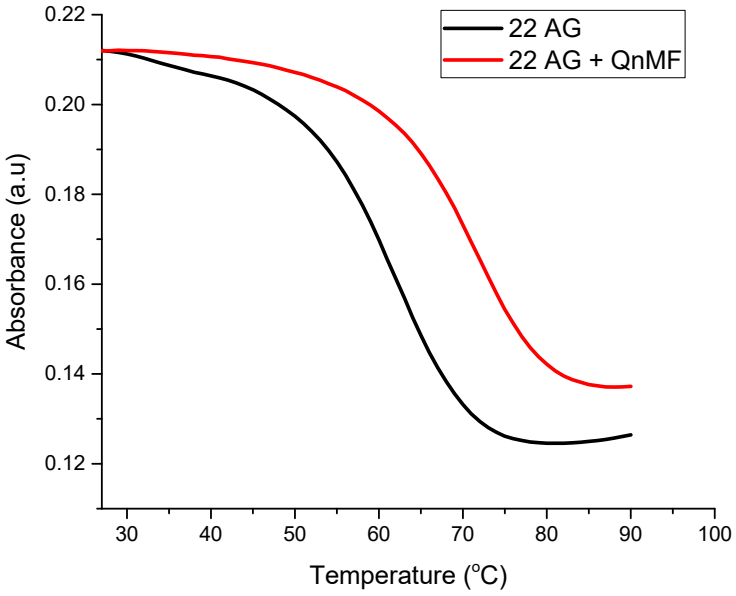


Figure S2: Melting temperatures of 22AG in the presence and absence of QnMF in buffer solution containing 10 mM Tris–HCl buffer and 100 mM of NaCl. The spectra were recorded at 295 nm.

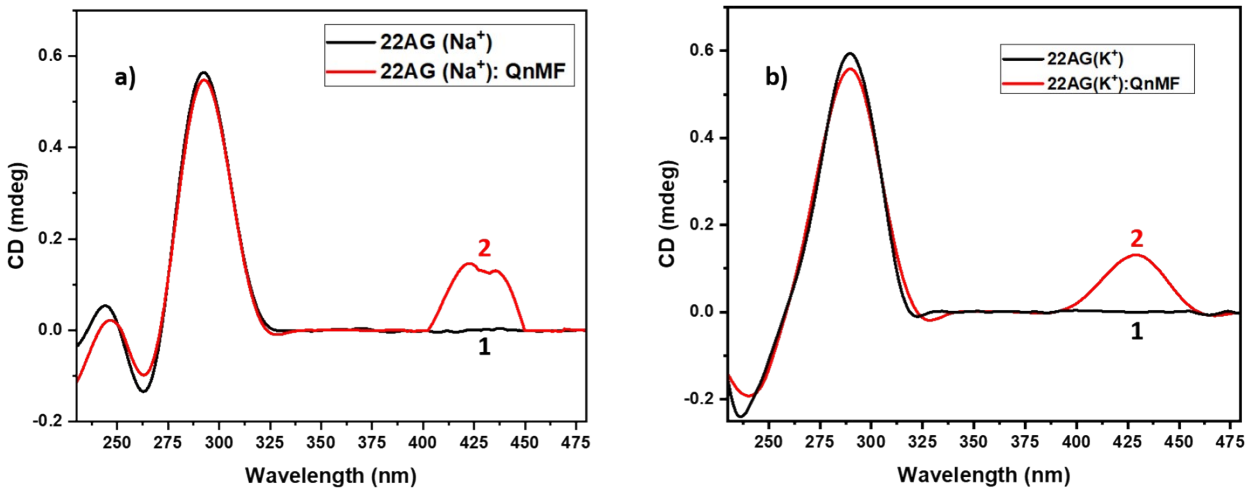


Figure S3 CD spectra recorded for 22AG DNA (10µM) solutions in 100 mM Tris HCl 7.5. **a)** NaCl (100 mM) **b)** KCl (100 mM) With QnMF: (1) 0 equivalent (2) 6 equivalent.

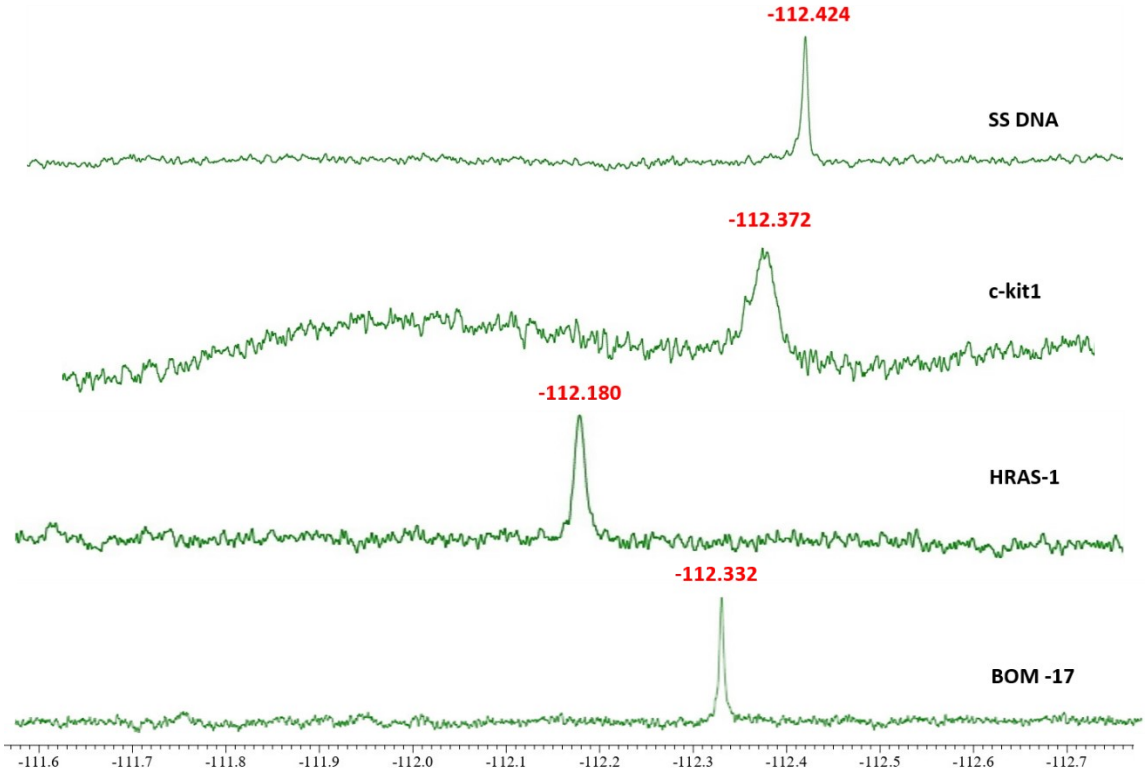


Figure S4:

^{19}F NMR spectrum of QnMF gives a distinct chemical shift for different DNA G4 topologies. Fluorine- 19 NMR spectrum of QnMF (100 µM) and BOM 17 containing 100 mM KCl. Fluorine- 19 NMR spectrum of QnMF (100 µM) and HRAS in 100 mM KCl. Fluorine- 19 NMR spectrum of QnMF (100 µM) and c-kit1 in 100 mM KCl. Fluorine- 19 NMR spectrum of QnMF (100 µM) and ssDNA. All

samples contain 0.1 mM TrisHCl Buffer(7.5 pH) and 20% D_2O was added before recording NMR spectra. All samples recorded at a frequency of 564.9 MHz for ^{19}F on a JEOL 600 MHz Spectrometer.

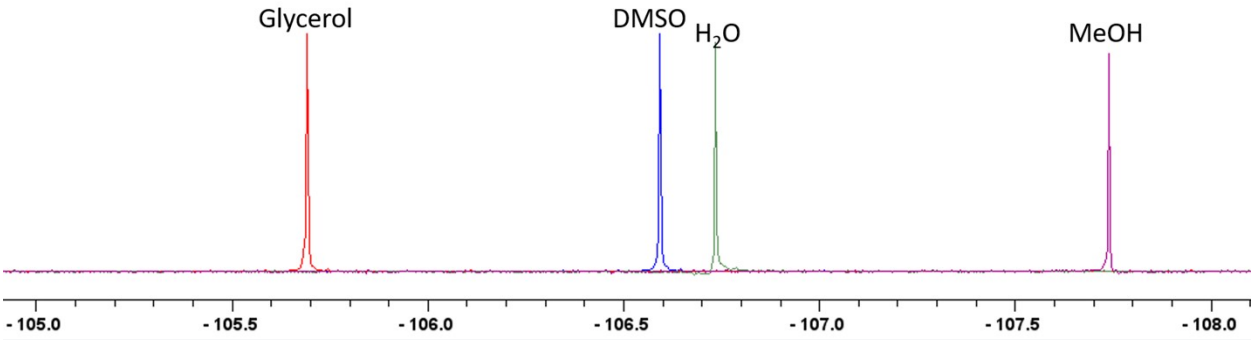
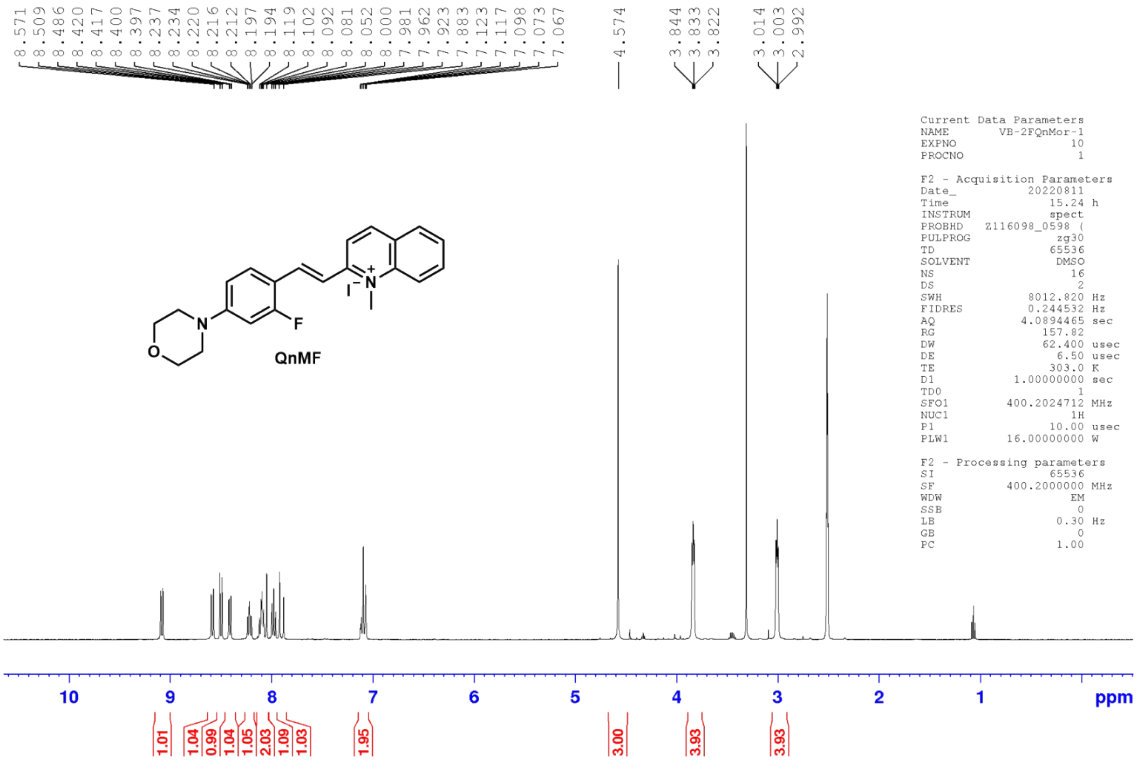


Figure S5:

^{19}F NMR spectrum of QnMF in different solvents like methanol, glycerol, water, and DMSO gives a distinct chemical shifts. All samples recorded in 400 MHz Bruker NMR using an external standard trifluorotoluene (TFT, −63.72 ppm).



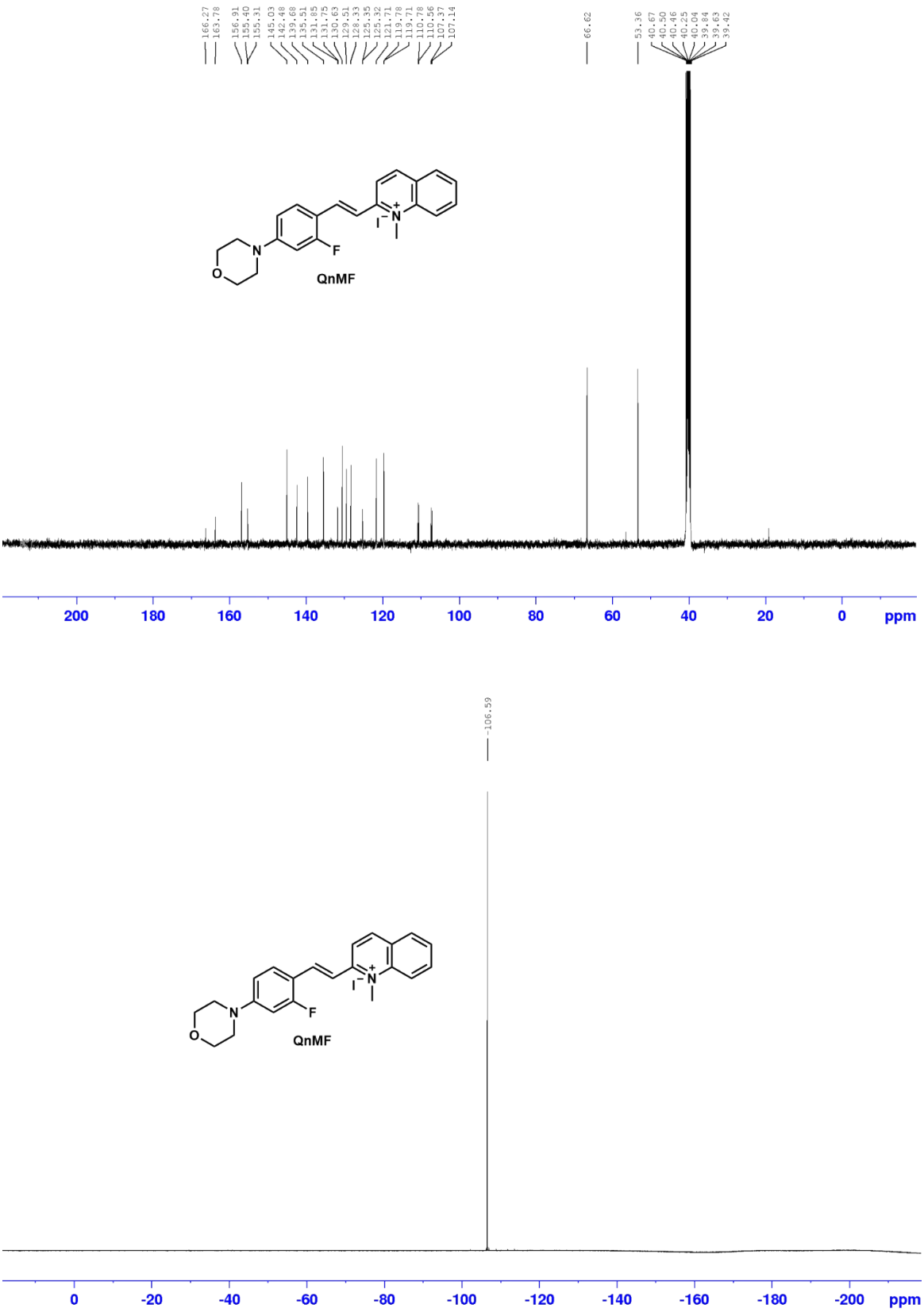
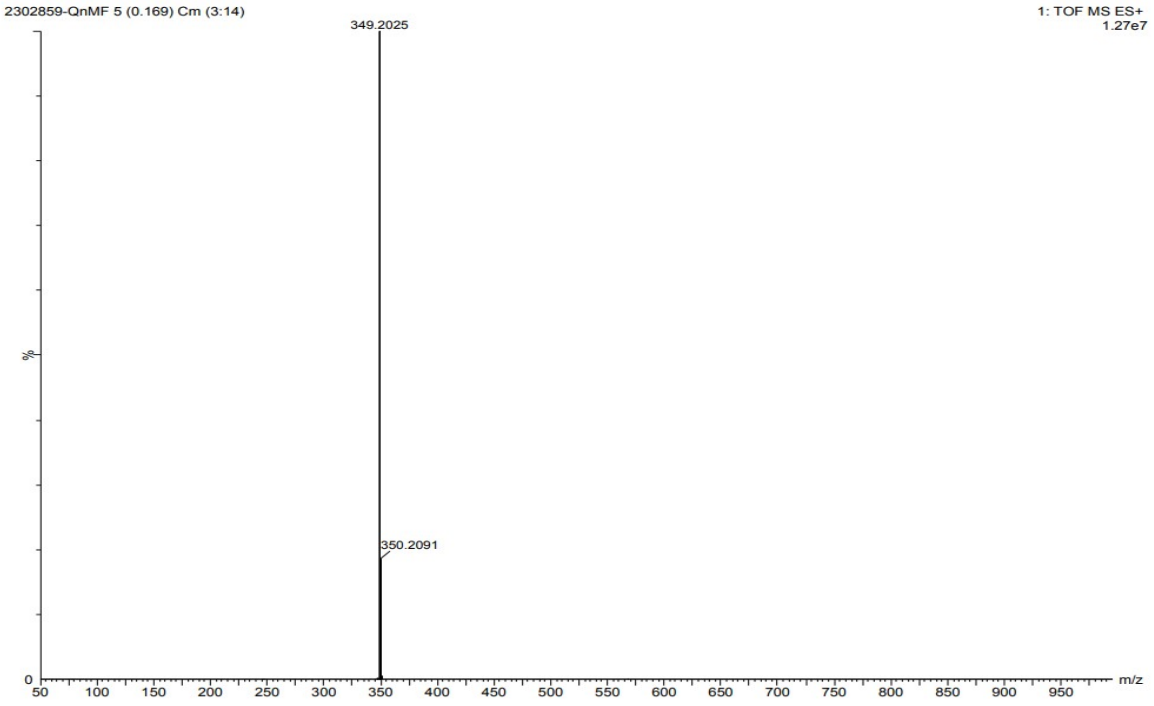


Figure S6: ^{1}H,^{13}C ,^{19}F NMR spectra of QnMF in DMSO-d_6



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igure S7: LCMS; Calculated mass for $C_{22}H_{22}FN_2O^+$ ([M]$^+$): *m*/*z*: 349.17; found: 349.20.

References

1. Guralamatta Siddappa Ravi Kumara, Anup Pandith, Young Jun Seo, Bioorganic & Medicinal Chemistry Letters., 2022, **55**, 128462.
2. S. Manna, D. Sarkar and S. G. Srivatsan, *J Am Chem Soc*, 2018, **140**, 12622-12633.
3. A. Karimi, R. Börner, G. Mata and N. W. Luedtke, *J Am Chem Soc*, 2020, **142**, 14422-14426.