Supplementary information

Identification and detection of conserved G-quadruplex in monkeypox virus using using conformation specific fluorogenic probe

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1. General experimental information

Chemical reagents and Instruments

All chemical reagents and solvents were purchased from Merck. Single-stranded oligos were purchased from Integrated DNA Technologies (IDT). High-resolution mass spectra (HRMS) were recorded for molecular weight confirmation on Agilent Technologies 6538 UHD Accurate-Mass Q-TOF LC/MS spectrometer. NMR data were recorded in Bruker AVANCE 400 Fourier Transform NMR spectrometer or JEOL 600 MHz NMR spectrometer. Fluorescence screening was done in Thermo Fisher Scientific Varioskan LUX 3020-973. CD spectra were recorded on a Jasco-815 spectropolarimeter (Japan). Fluorescence lifetime measurements were performed Horiba Delta Flex time-correlated single photon counting (TCSPC) instrument with a 560 nm laser with a pulse repetition of 1 MHz. Fluorescence Imaging of PAGE images was captured in the Bio-Rad ChemiDoc MP imaging system.

Preparation of DNA samples

Single-stranded Oligonucleotides (oligos) were purchased from IDT. Oligo stocks were prepared using nuclease-free water and stored at -20 °C. Substocks were prepared in G Quadruplex (GQ) stabilizing buffer (Tris. HCl 20 mM Tris. HCl, 100 mM KCl, pH 7.4) by annealing the oligos at 95 °C for five minutes followed by slow cooling them at room temperature.

Circular dichroism (CD) measurements

CD measurements were performed using a Jasco-815 spectropolarimeter (Japan) in GQ stabilizing buffer (20 mM Tris. HCl, 100 mM KCl, pH 7.4). 800 µL solution of 4 µM was taken in a 1 cm path length cuvette and the spectra were recorded from 235 nm to 350 nm at a scanning rate of 100 nm/min and plotted as an average of 3 accumulations. The corresponding buffer spectra were used for baseline corrections.

NMR characterization

^{1}H and ^{13}C NMR spectra were recorded in a Bruker AVANCE 400 Fourier Transform NMR spectrometer or JEOL 600 MHz NMR spectrometer. Tetra methylene silane was used as an internal standard. For DNA samples, 200 µM of the DNA solutions were prepared in GQ stabilization buffer containing 10% DMSO -d_6. Water suppression was performed using WATERGATE.

Fluorescent screening of the probes.

Fluorescence screening was done in Thermo Fisher Scientific Varioskan LUX 3020-973 instrument. 160 µL of 1:1 (2 µM: 2 µM) solution of the probe and oligonucleotides were prepared. By taking 50 µL of the solution in each well, data points were collected in triplicates and plotted using GraphPad Prism /

Origin software. Screening the lead probe with other nucleic acid conformations follows the same procedure.

Electrophoretic mobility shift assay

A 20 µL of 4 µM 1:1 (DNA: Lead Probe) was prepared and incubated at -4 °C for 12 h. Electrophoretic Mobility Shift Assay was performed in 16% Polyacrylamide gel in TBE buffer containing 100mM KCl for 2 h at 80 V. The gel was imaged in the channel corresponding to the lead probe emission. Then the gel was stained with SYBR SAFE (dilution 1:10,000) for 10 minutes followed by destaining for 5 minutes and imaged in the SYBR SAFE channel using a Bio-Rad ChemiDoc MP imaging system.

Determination of lifetime

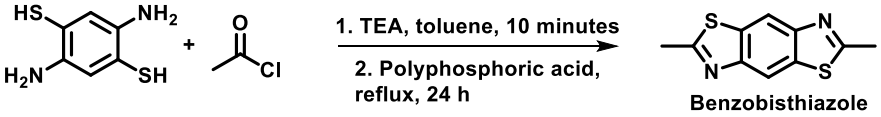
Fluorescence Lifetime was measured in a Horiba Delta Flex time-correlated single photon counting (TCSPC) instrument. A 560 nm lased pulse was used as a source. Ludox (AS40 COLLOIDAL SILICA, Sigma-Aldrich) was used as the standard to record the Instrument Response Function (IRF). A 1:1 solution of lead probe (2 µM) with DNA (2 µM) was used for measuring the lifetime.

Job's plot analysis

The binding stoichiometry of BBJL and MP2 was determined by the continuous variation method. A series of BBJL and MP2 solutions were prepared by sequentially varying the molar fraction of both, keeping the total concentration to 4 µM. The fluorescence output of each of the solutions was plotted against the mole fraction. The mole fraction corresponding to the maxima gave the binding stoichiometry.

2. Synthesis and characterization of molecular probes

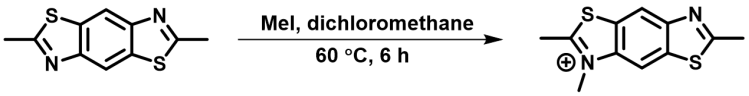
Synthesis of benzobisthiazole



Benzobisthiazole

Benzobisthiazole was synthesized following reported procedure[1]. 2, 5-diaminobenzene-1,4-dithiol dihydrochloride (1 mmol) was dissolved in toluene, and triethyl amine (3 mmol) was added and stirred at RT for 10 min. This was followed by the addition of acetyl chloride (5 mmol) and polyphosphoric acid (10 mmol) and the solution was refluxed for 24 h. The progress of the reaction was monitored by thin-layer chromatography (TLC). After the completion of the reaction, the reaction mixture was extracted using water: DCM mixture and the organic layer was dried over anhydrous sodium sulphate and concentrated by evaporation using a rotary evaporator. The crude product was further purified by column chromatography (ethyl acetate: hexane 50%). Pale yellow solid. Yield 60%

Synthesis of N-Methylbenzobisthiazole



N-Methyl Benzobisthiazole

Excess methyl iodide was added to a suspension of benzobisthiazole in DCM (2 ml) in a sealed tube, and the reaction mixture was stirred at 60 °C for 6 h. The white precipitate formed after 6 h was filtered and washed with diethyl ether. The monomethylated product was obtained as a white powder. Yield 95%.

General procedure for the synthesis of benzothiazole derivatives

In a sealed tube, 2-methyl N-methyl benzothiazole (1 mmol) and 0.2 equivalent of piperidine were dissolved in ethanol and stirred for 10 min. To this, the aldehyde (1.2 mmol) dissolved in ethanol was added dropwise and the mixture was stirred at 60 °C. After the completion of the reaction, the excess solvent was evaporated and the crude product was purified by reverse-phase chromatography (C18 column, grad. 70-80% acetonitrile: water).

General procedure for the synthesis of benzobisthiazole derivatives

In a sealed tube N-Methyl Benzobisthiazole (1 mmol) and 0.2 equiv. of piperidine was dissolved in ethanol and stirred for 10 min. The corresponding aldehyde (1.2 mmol) was added dropwise and stirred at 60 °C. After the completion of the reaction, the excess solvent was removed by evaporation, and the crude mixture was purified by reverse phase chromatography (C18 column, grad. 75-85% acetonitrile: water).

Benzobisthiazole

Pale yellow solid,(132.6 mg, 60% yield), ^{1}H NMR (600 MHz, Chloroform-d) δ 8.29 (s, 2H), 2.80 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-d) δ 168.0, 151.2, 134.6, 114.5, 20.5. HRMS (ESI-Q-TOF, m/z) calculated for $[C_{10}H_9N_2S_2]^+$ 221.0202, observed 221.0198.

Probe 1 (E)-2-(4-(diethylamino)styryl)-3-methylbenzo[d]thiazol-3-ium

Red solid, (210 mg, 65% yield), ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.6 Hz, 1H), 8.04 (d, J = 15.3 Hz, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.78 (t, J = 7.8 Hz, 1H), 7.67 (t, J = 7.7 Hz, 1H), 7.58 (d, J = 15.2 Hz, 1H), 6.82 (d, J = 8.6 Hz, 2H), 4.22 (s, 3H), 3.51 (q, J = 6.7 Hz, 4H), 1.16 (t, J = 7.0 Hz, 6H).^{13}C NMR (151 MHz, DMSO-d_6) δ 171.6, 152.0, 150.5, 142.4, 133.8, 129.3, 127.8, 127.2, 124.2,

121.5, 116.3, 112.1, 106.1, 44.7, 36.0, 13.0. HRMS (ESI-Q-TOF, m/z) calculated for $[C_{20}H_{23}N_2S]^+$ 323.1576, observed 323.1587.

Probe 2, (E)-2-(4-(dimethylamino)styryl)-3-methylbenzo[d]thiazol-3-ium

Red Solid, (222.6 mg, 73% yield), ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (d, J = 8.0 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 8.05 (d, J = 15.3 Hz, 1H), 7.90 (d, J = 9.0 Hz, 2H), 7.78 (t, J = 7.3 Hz, 1H), 7.68 (t, J = 7.7 Hz, 1H), 7.62 (d, J = 15.3 Hz, 1H), 6.83 (d, J = 9.1 Hz, 2H), 4.22 (s, 3H), 3.10 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.8, 150.6, 142.4, 129.3, 127.9, 127.3, 124.3, 122.0, 116.4, 112.4, 106.7, 40.3, 36.1. HRMS (ESI-Q-TOF, m/z) calculated for $[C_{18}H_{19}N_2S]^+$ 295.1263, observed 295.1290.

Probe 3 (E)-2-(2-(1-hydroxynaphthalen-2-yl)vinyl)-3-methylbenzo[d]thiazol-3-ium

Purple solid, (222.6 mg, 70% yield), ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.61 (d, J = 15.5 Hz, 1H), 8.36 (d, J = 8.1 Hz, 1H), 8.30 (d, J = 8.6 Hz, 1H), 8.23 (s, 1H), 8.21 (d, J = 8.3 Hz, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 7.8 Hz, 1H), 7.74 (t, J = 7.7 Hz, 1H), 7.61 (t, J = 7.7 Hz, 1H), 7.41 (t, J = 7.4 Hz, 1H), 7.33 (d, J = 9.0 Hz, 1H), 4.22 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.4, 160.4, 142.6, 142.1, 135.6, 133.1, 129.8, 129.7, 128.9, 128.6, 128.5, 128.1, 124.5, 124.4, 122.7, 117.1, 116.0, 113.1, 36.4.HRMS (ESI-Q-TOF, m/z) calculated for $[C_{20}H_{16}NOS]^+$ 318.0947, observed 318.0948.

Probe 4 (E)-2-(4-hydroxy-2,6-dimethoxystyryl)-3-methylbenzo[d]thiazol-3-ium

Yellow Solid, (147.6 mg, 45% yield), ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 – 8.23 (m, 2H), 8.20 – 8.15 (m, 2H), 7.79 (t, *J* = 7.8 Hz, 1H), 7.74 (s, 1H), 7.71 – 7.66 (m, 2H), 4.15 (s, 3H), 3.94 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.2, 165.7, 162.6, 142.0, 139.7, 128.9, 127.5, 126.7, 123.8, 116.1, 109.4, 104.1, 92.6, 56.3, 35.4. HRMS (ESI-Q-TOF, m/z) calculated for $[C_{18}H_{18}NO_3S]^+$ 328.1002, observed 328.0998.

Probe5, (E)-2-(2-(8-hydroxy-2,3,6,7-tetrahydro-1H,5H-pyrido[3,2,1-ij]quinolin-9-yl)vinyl)-3-methyl benzo [d]thiazol-3-ium was synthesized following the reported procedure.[1]

Probe 6 (E)-3-methyl-2-(2-(2,3,6,7-tetrahydro-1H,5H-pyrido[3,2,1-ij]quinolin-9-yl)vinyl) benzo [d] thiazol-3-ium

Purple solid, (208.2 mg, 60% yield), ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.19 (d, *J* = 8.0 Hz, 1H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 15.0 Hz, 1H), 7.70 (t, *J* = 8.3 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.46 (s, 2H), 7.40 (d, *J* = 15.0 Hz, 1H), 4.12 (s, 3H), 3.33 (t, *J* = 5.7 Hz, 4H), 2.69 (t, *J* = 6.1 Hz, 4H), 1.86 (p, *J* = 6.3 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.8, 150.6, 148.3, 142.4, 131.0, 129.2, 127.5, 126.9, 124.1, 121.7, 121.1, 115.9, 104.7, 50.2, 35.7, 27.4, 21.1. HRMS (ESI-Q-TOF, m/z) calculated for $[C_{22}H_{23}N_2S]^+$ 347.1576, observed 347.1581.

Probe 7 (E)-2-(2-(8-methoxy-2,3,6,7-tetrahydro-1H,5H-pyrido[3,2,1-ij]quinolin-9-yl)vinyl)-3-methyl benzo[d]thiazol-3-ium

Purple solid, (301.7 mg, 80% yield), ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 – 8.19 (m, 1H), 8.03 (d, *J* = 8.3 Hz, 1H), 7.94 (d, *J* = 15.1 Hz, 1H), 7.74 (ddd, *J* = 8.5, 7.4, 1.2 Hz, 1H), 7.71 (s, 1H), 7.65 – 7.60 (m, 1H), 7.47 (d, *J* = 15.1 Hz, 1H), 4.16 (s, 3H), 3.76 (s, 3H), 3.37 (q, *J* = 5.3 Hz, 4H), 2.76 – 2.66 (m, 4H), 1.88 (dp, *J* = 11.8, 6.6 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.1, 158.7, 149.7, 143.9, 142.5, 129.1, 127.9, 127.5, 126.6, 124.0, 119.2, 115.9, 114.0, 113.3, 104.7, 62.4, 50.2, 49.8, 35.6, 27.2, 21.1, 20.4. HRMS (ESI-Q-TOF, m/z) calculated for $[C_{23}H_{25}N_2OS]^+$ 377.1682, observed 377.1676.

Probe 8 (E)-3-ethyl-2-(2-(8-hydroxy-2,3,6,7-tetrahydro-1H,5H-pyrido[3,2,1-ij]quinolin-9-yl)vinyl) benzo[d]thiazol-3-ium

Purple Solid, (207.4 mg, 55% yield), ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 8.20 (d, J = 14.6 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.68 (t, J = 7.8 Hz, 1H), 7.60 (s, 1H), 7.56 (t, J = 7.9 Hz, 1H), 7.29 (d, J = 14.6 Hz, 1H), 4.67 (q, J = 7.0 Hz, 2H), 3.36 (m, 2H), 3.32 (m, 2H), 2.69 (t, J = 6.0 Hz, 2H), 2.60 (t, J = 6.2 Hz, 2H), 1.93 – 1.80 (m, 4H), 1.39 (t, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.6, 156.5, 150.4, 145.4, 141.4, 129.0, 127.6, 126.9, 126.7, 124.1, 117.0, 115.1, 112.1, 106.7, 101.4, 50.5, 49.7, 43.0, 27.2, 21.5, 21.2, 20.5, 13.9. HRMS (ESI-Q-TOF, m/z) calculated for $[C_{23}H_{25}N_2OS]^+$ 377.1682, observed 377.1678.

Probe 9 (E)-2-(2-(8-hydroxy-2,3,6,7-tetrahydro-1H,5H-pyrido[3,2,1-ij]quinolin-9-yl)vinyl)-3,6-dimethyl benzo[1,2-d:4,5-d']bis(thiazole)-3-ium

Purple solid, (303.8 mg, 70% yield, ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.44 (s, 1H), 7.95 (d, J = 14.5 Hz, 1H), 7.38 (s, 1H), 7.07 (d, J = 14.6 Hz, 1H), 3.95 (s, 3H), 3.21 (t, J = 5.7 Hz, 2H), 3.15 (t, J = 5.5 Hz, 2H), 2.75 (s, 3H), 2.55 (t, J = 6.4 Hz, 2H), 2.44 (t, J = 6.4 Hz, 2H), 1.79 (m, 2H), 1.73 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.9, 155.5, 150.3, 149.5, 144.0, 138.9, 135.5, 126.4, 123.8, 116.1, 115.4, 111.2, 107.1, 105.6, 100.9, 49.4, 48.6, 34.2, 26.0, 20.4, 20.0, 19.5, 19.3. HRMS (ESI-Q-TOF, m/z) calculated for $[C_{24}H_{24}N_3OS_2]^+$ 434.1355, observed 434.1353.

3. Tables

Table S1. Photophysical properties of the probes 1-9

Probe	Absorbance Max λ_{ex} (nm)	Emission Max λ_{em} (nm)	Stokes Shift (nm)
1	520	590	70
2	510	590	80
3	540	580	40
4	505	530	25
5	570	600	30
6	560	615	55
7	555	610	55
8	570	605	35
9	530	605	75

Table S2. List of oligonucleotide sequences used in the study.

Name	Length	Sequence	Conformation
MP1	20	GGTCTAGGATGAGGATCAGG	Parallel GQ
MP2	20	GGATGAGGATCAGGTATCGG	Parallel GQ
MP3	20	GGATCAGGTATCGGAGTAGG	Antiparallel GQ
MP4	20	GGTATCGGAGTAGGTTTTGG	Antiparallel GQ
M1	30	GGGCTTGATGTGGGGAGGGGTGTTTAAGGG	Parallel GQ
M2	26	AGGGACGCGGGCGGGGGATATAGGGT	Parallel GQ
M3	13	GGGAGGTAGGTGG	Parallel GQ
M4	20	GGCGTAGGTTTGGTCTAGGG	Antiparallel GQ
M5	30	GGGTGGGAGTAGTTCCCTGCTAAGGGAGGG	Parallel GQ
M6	30	GGGCCAGGGGATTAATTAGTAGTACGGGAA	Hybrid GQ
M7	30	GAAGCGGGGGAGGGGGGGTTTGGTGGAAAT	Parallel GQ
C-MYC	27	TGGGGAGGGTGGGGAGGGTGGGGAAGG	Parallel GQ
BCL2	23	GGGCGCGGGAGGAAGGGGGCGGG	Hybrid GQ
KRAS	28	GGGAGGGAGGGAAGGAGGGAGGGAGGGA	Parallel GQ

VEGF	36	GGGGGCGGGCCGGGGGCGGGGTCCCGGCGGGGCGGA	Parallel GQ
TELL22	22	AGGGTTAGGGTTAGGGTTAGGG	Antiparallel GQ
MAPK1	21	TGGGTCGGGGTCGGGCCGGGG	Parallel GQ
MAPK2	21	AGGGAAGGGCCGGGAGCGGGA	Parallel GQ
MAPK12	56	TGGGTCGGGGTCGGGCCQGGGTCGGCACCTGGGACATCCCTG AGGGAAGGGCCGGG	Parallel GQ
Thrombin	17	GGGTTGGTGTGGTTGGA	Antiparallel GQ
DM7	14	GCGCGAATTCGCGC	dsDNA
Drew AT	14	GCGCAAATTTGCGC	dsDNA
A20T20	20	AAAAAAAAAAAAAAAAAAAA : TTTTTTTTTTTTTTTTTTTT	dsDNA
C20G20	20	CCCCCCCCCCCCCCCCCCCC : GGGGGGGGGGGGGGGGGGGG	dsDNA
Tell22 I	22	CCCTAACCCTAACCCTAACCCT	i-motif
C-MYC i	27	CCTTCCCCACCCTCCCCACCCTCCCCA	i-motif
MP1C	20	CCAGATCCTACTCCTAGTCC	dsDNA
MP2C	20	CCTACTCCTAGTCCATAGCC	dsDNA
MP3C	20	CCTAGTCCATAGCCTCATCC	dsDNA
MP4C	20	CCATAGCCTCATCCAAAACC	dsDNA

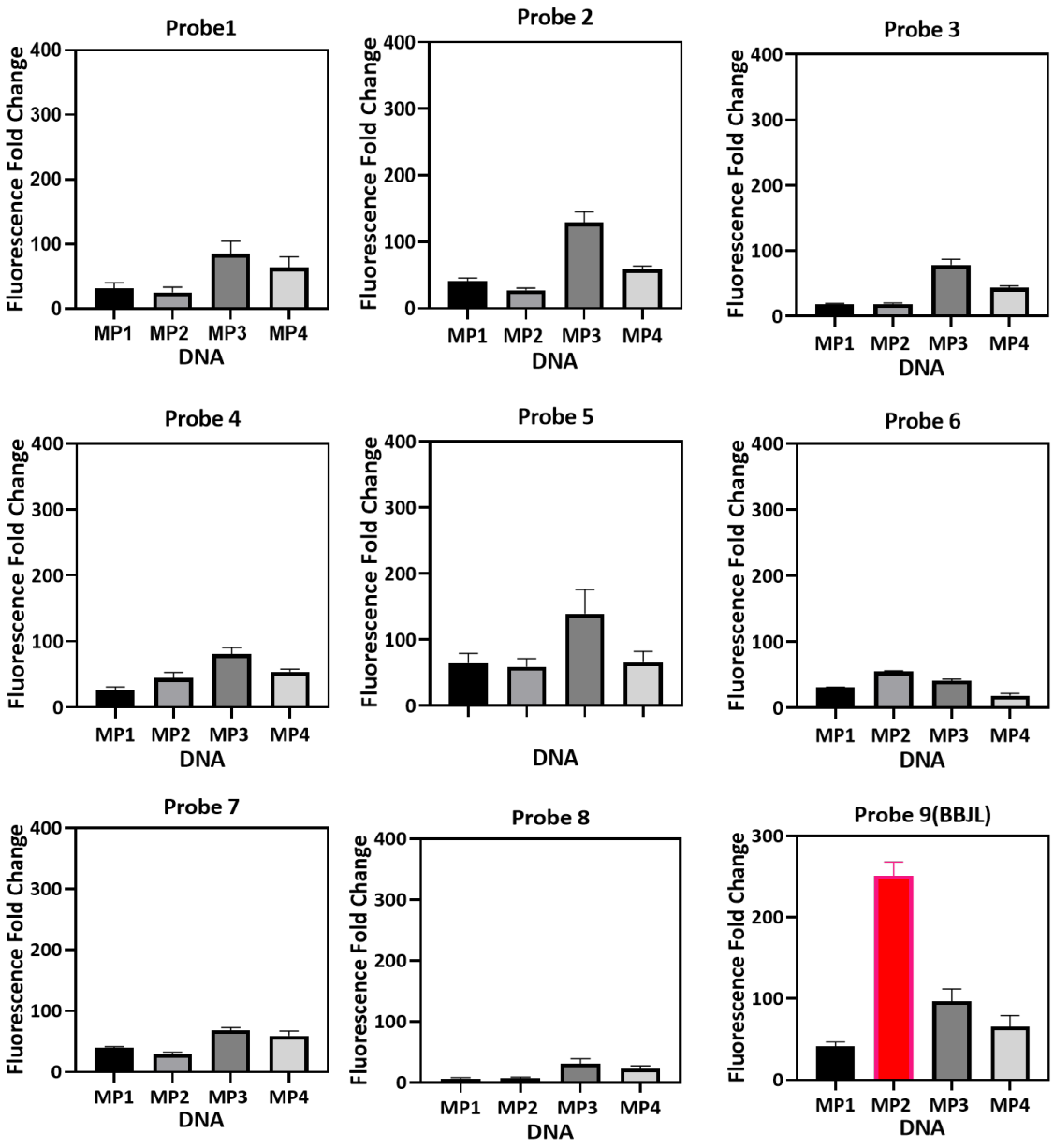


Fig. S1. Fluorescence Screening of the Probes **1**-**9** (2 µM) in the presence of GQ sequences MP 1-4 (2 µM) derived from monkeypox virus.

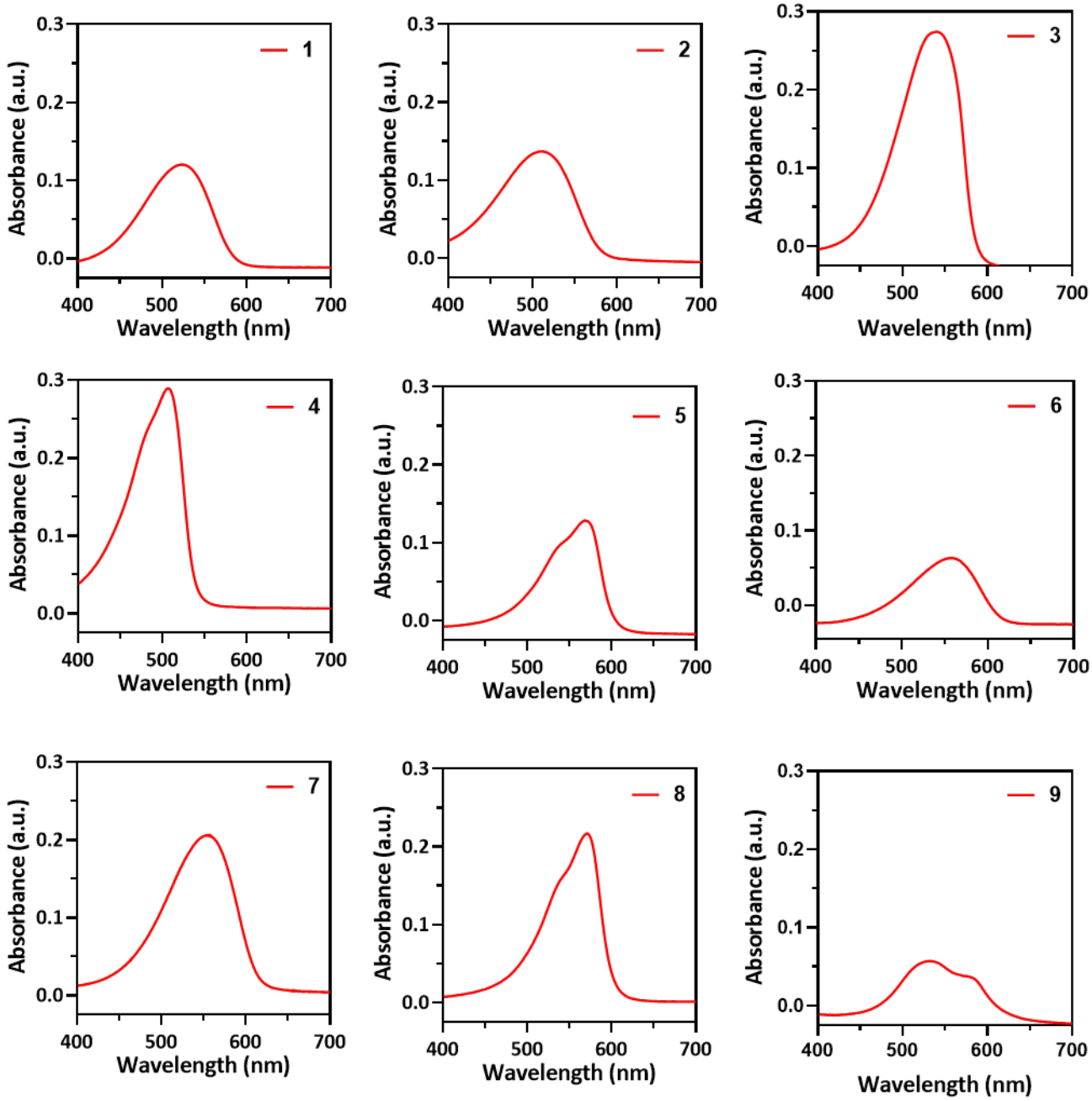


Fig. S2. Absorbance spectra of probes 1-9 (5 µM) in buffer Tris.HCl (20 mM, pH 7.4, KCl 100 mM).

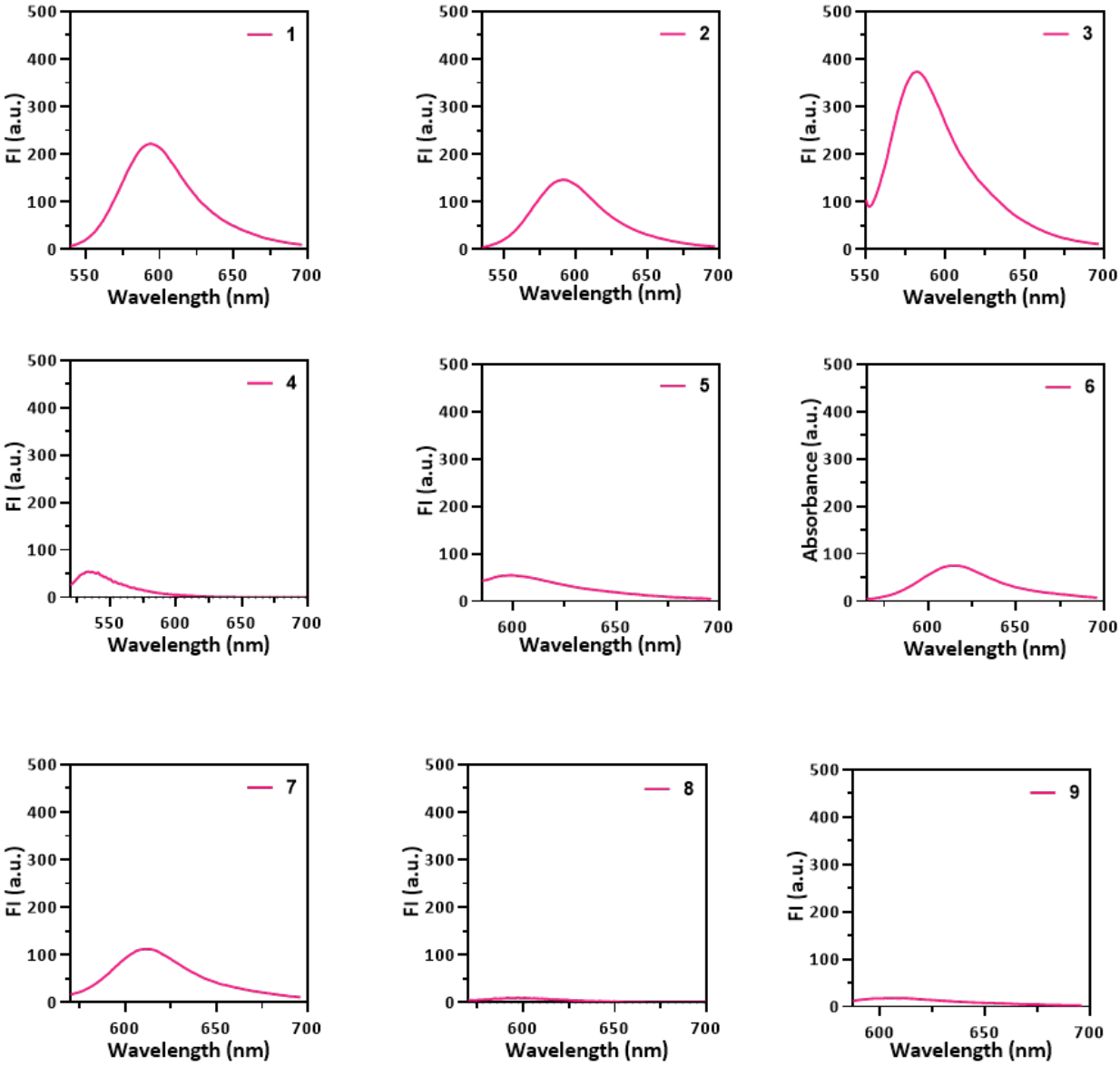
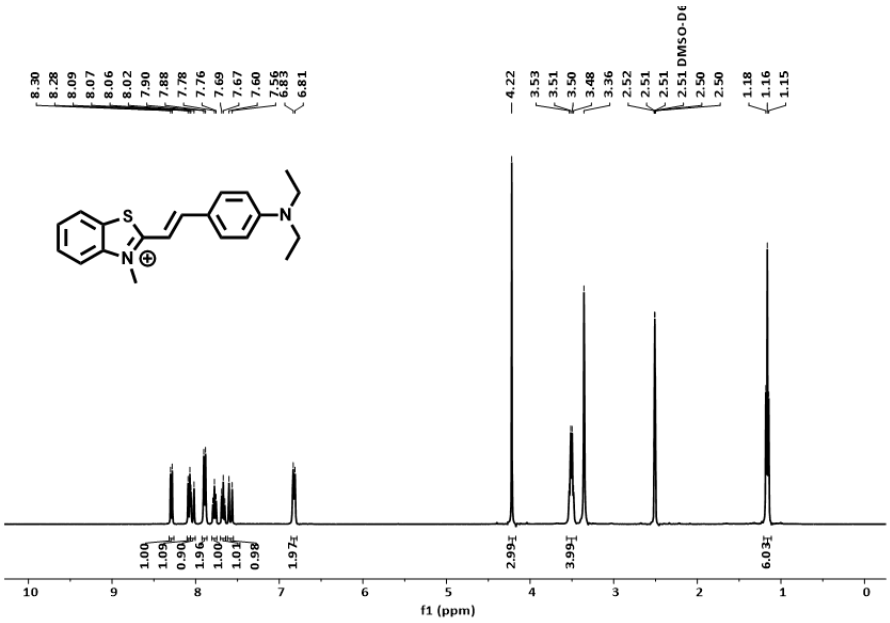
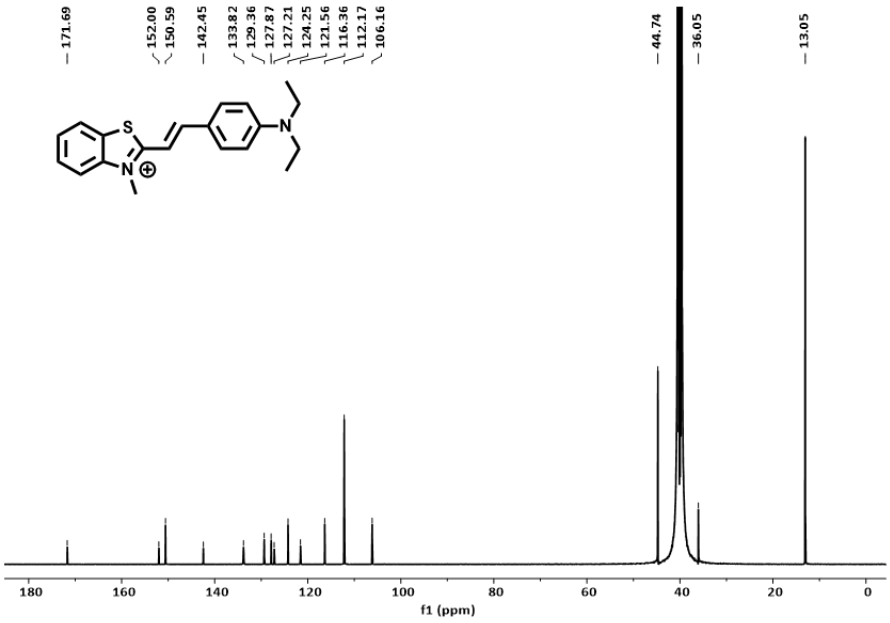


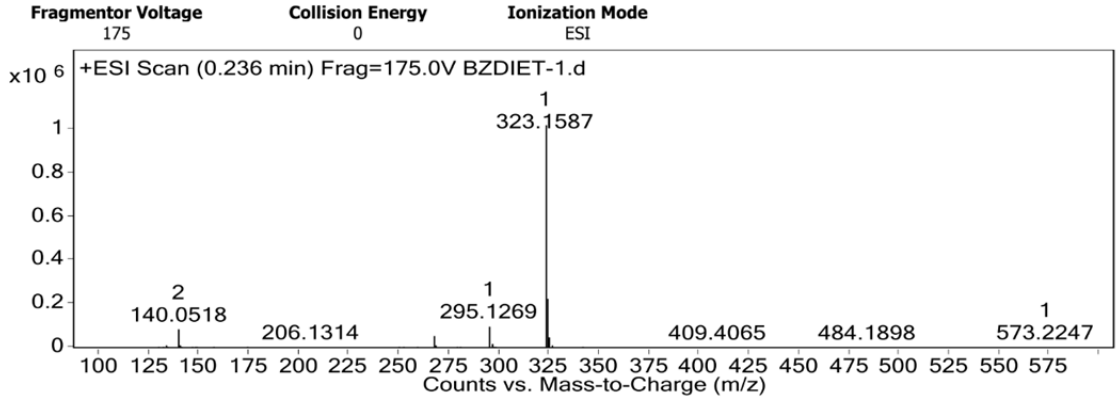
Fig. S3. Fluorescence spectra of probes **1**-**9** (5 µM) in buffer Tris.HCl (20 mM, pH 7.4, KCl 100 mM)

7. Compound characterization data

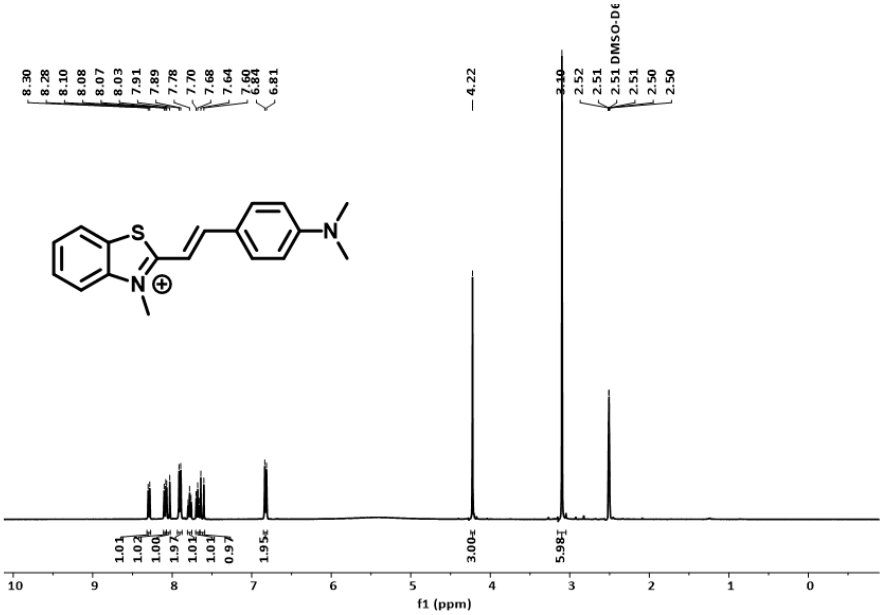
^{1}H, ^{13}C and HRMS spectra of probe **1**

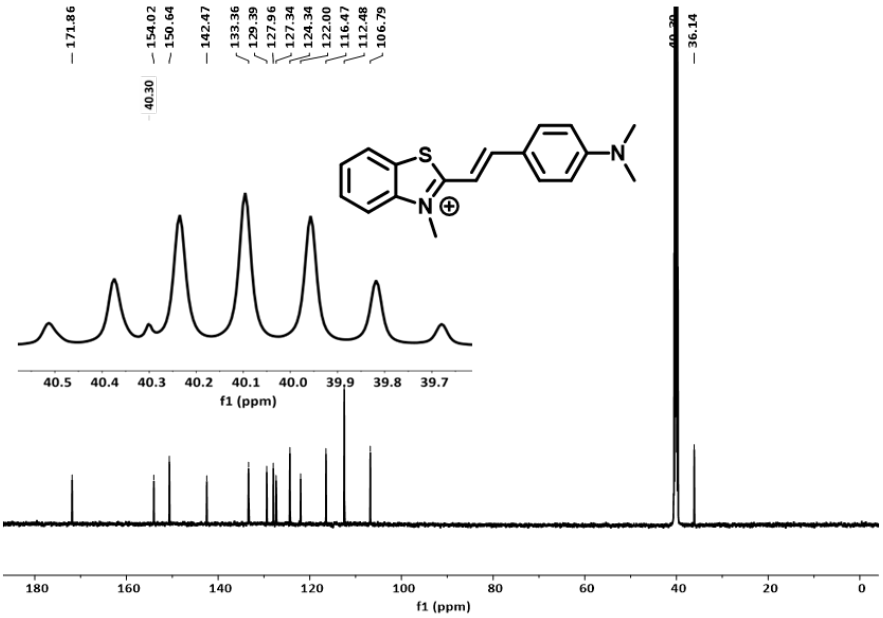


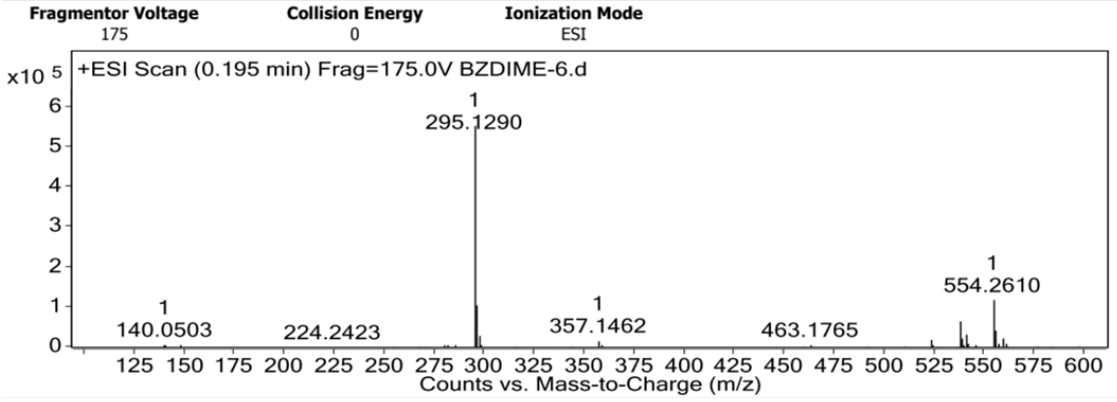




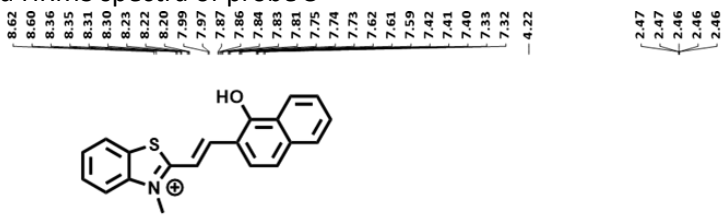
^{1}H, ^{13}C, and HRMS spectra of probe **2**

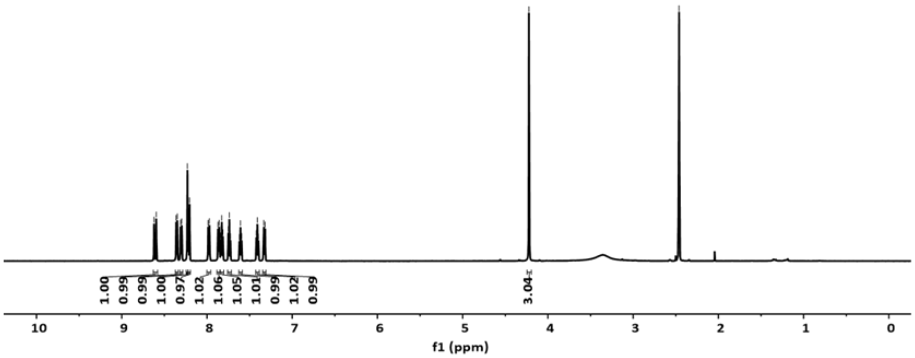


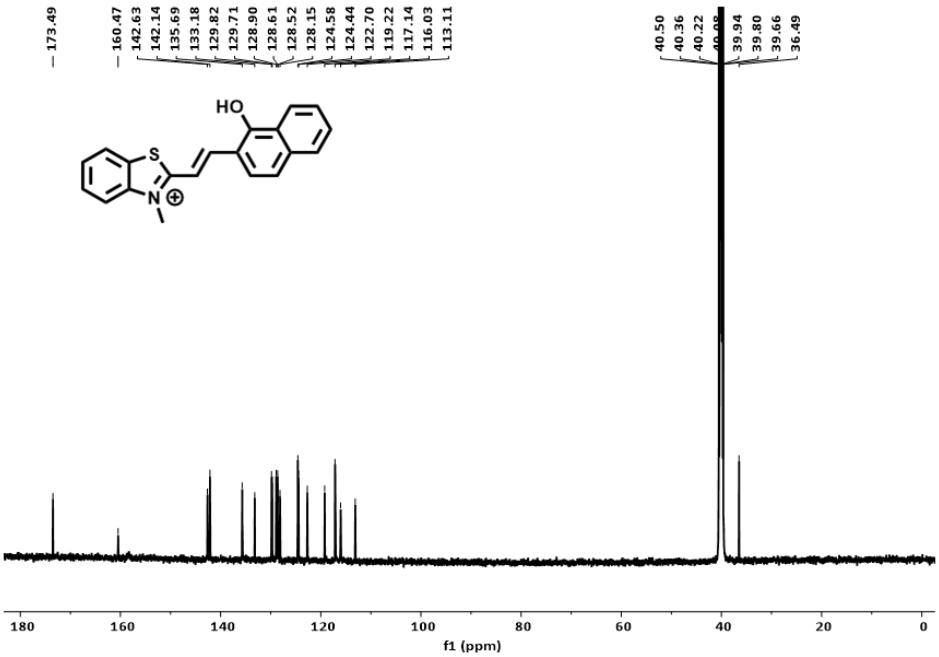


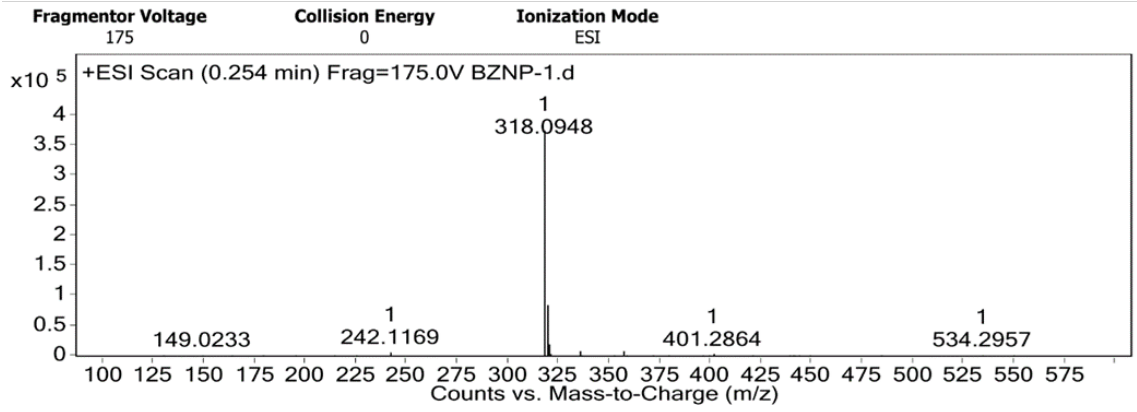


^{1}H, ^{13}C and HRMS spectra of probe **3**

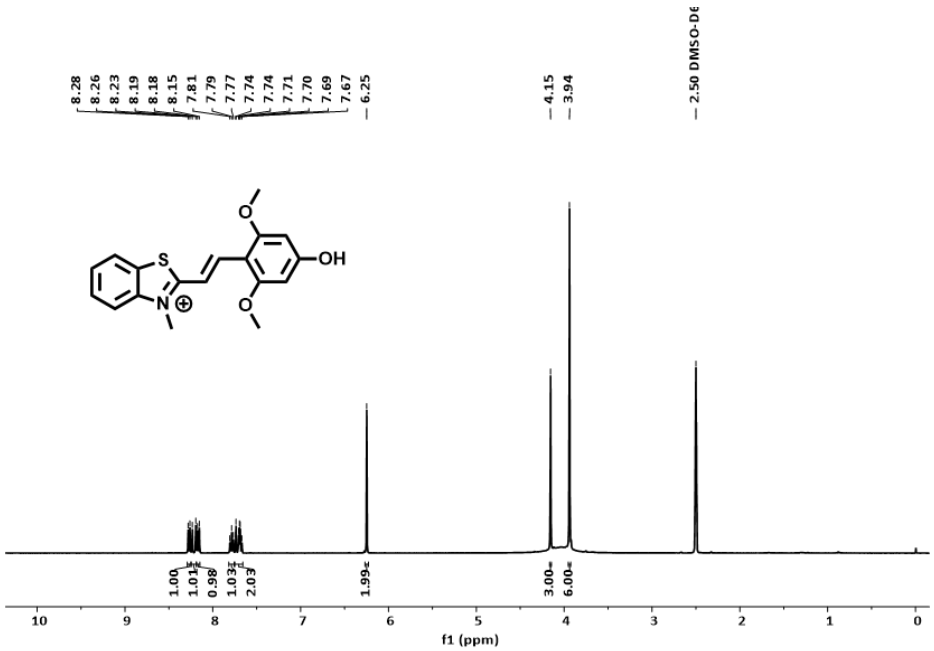


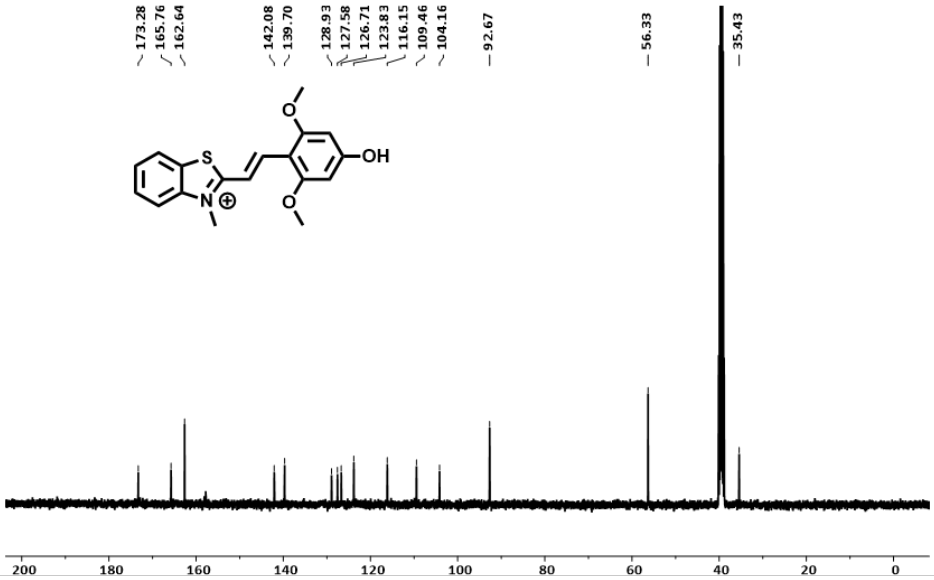


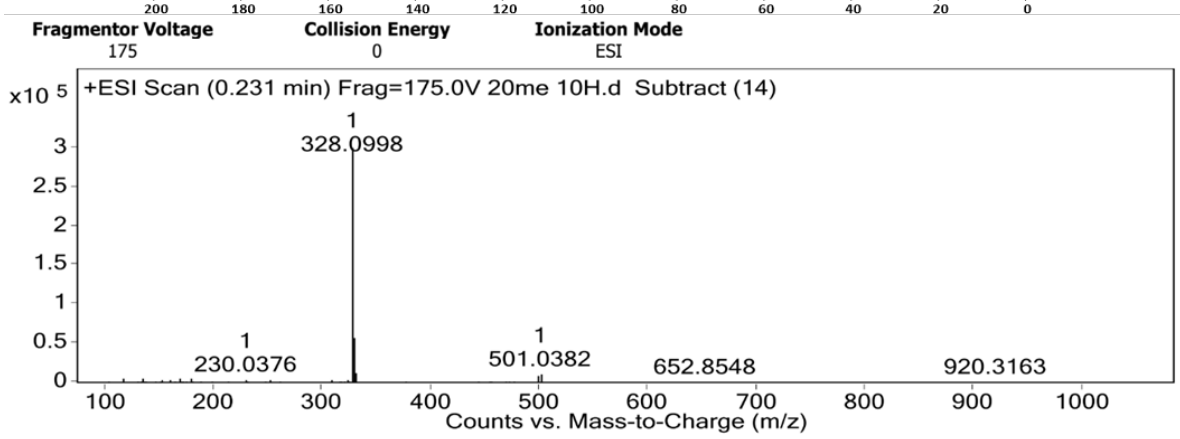




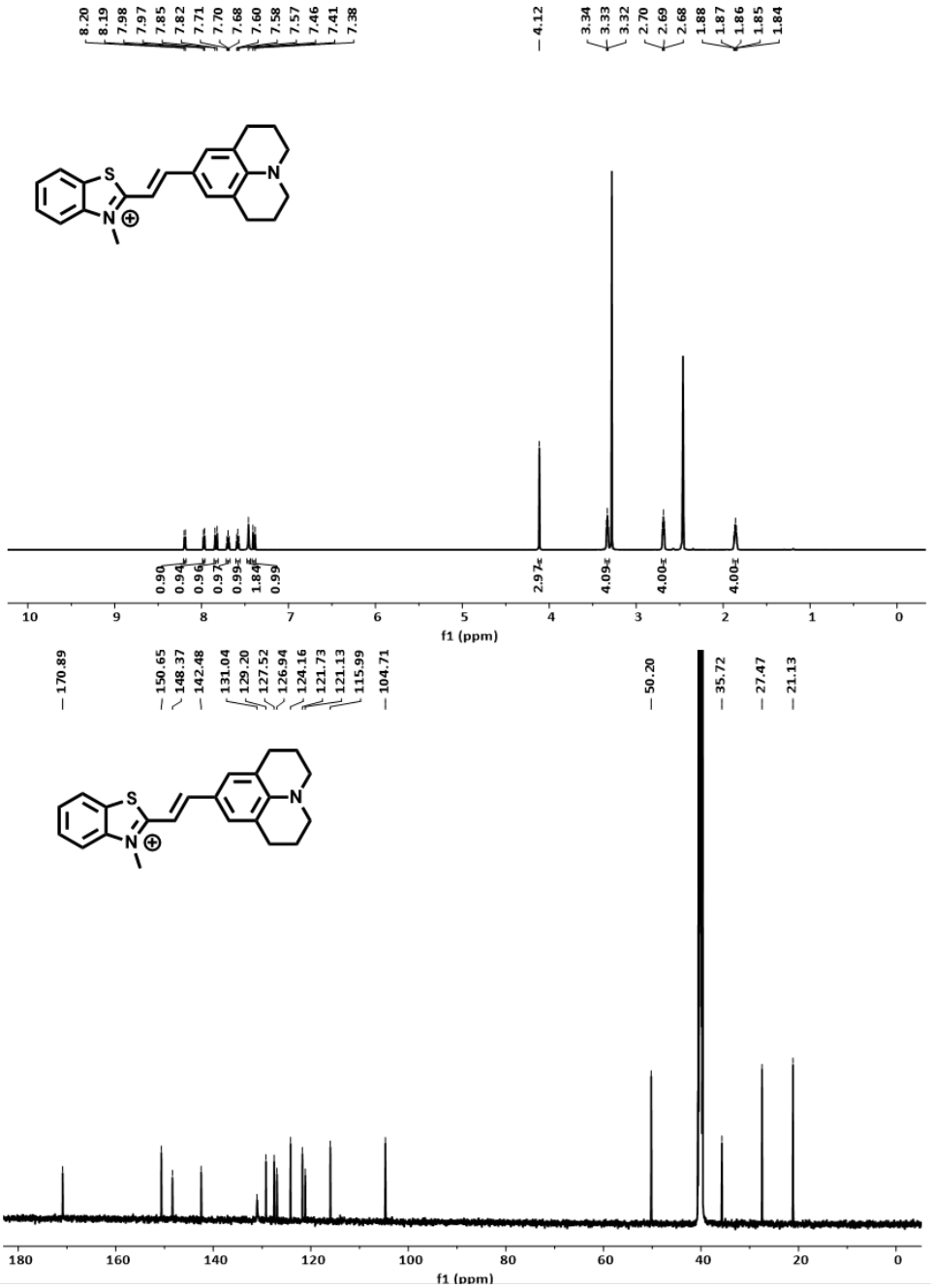
^{1}H, ^{13}C and HRMS spectra of probe **4**

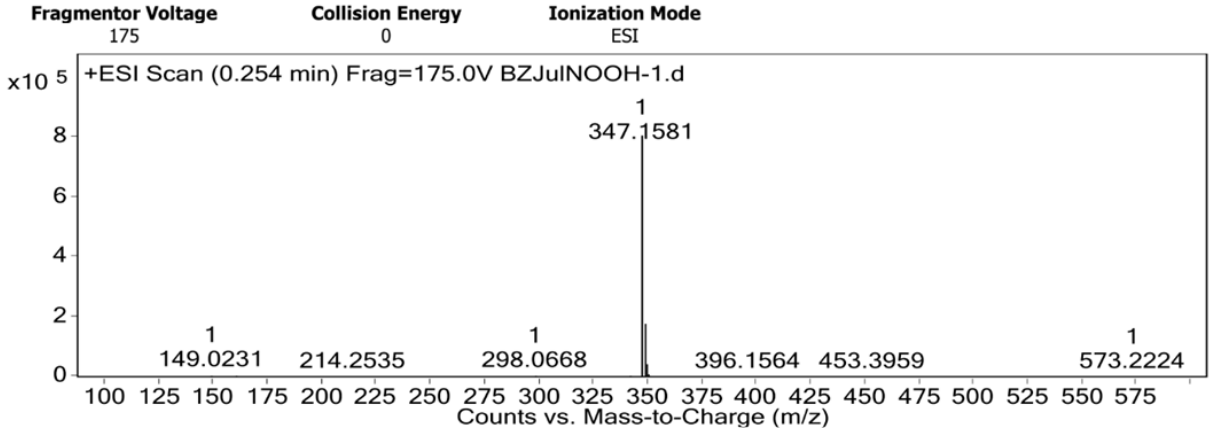




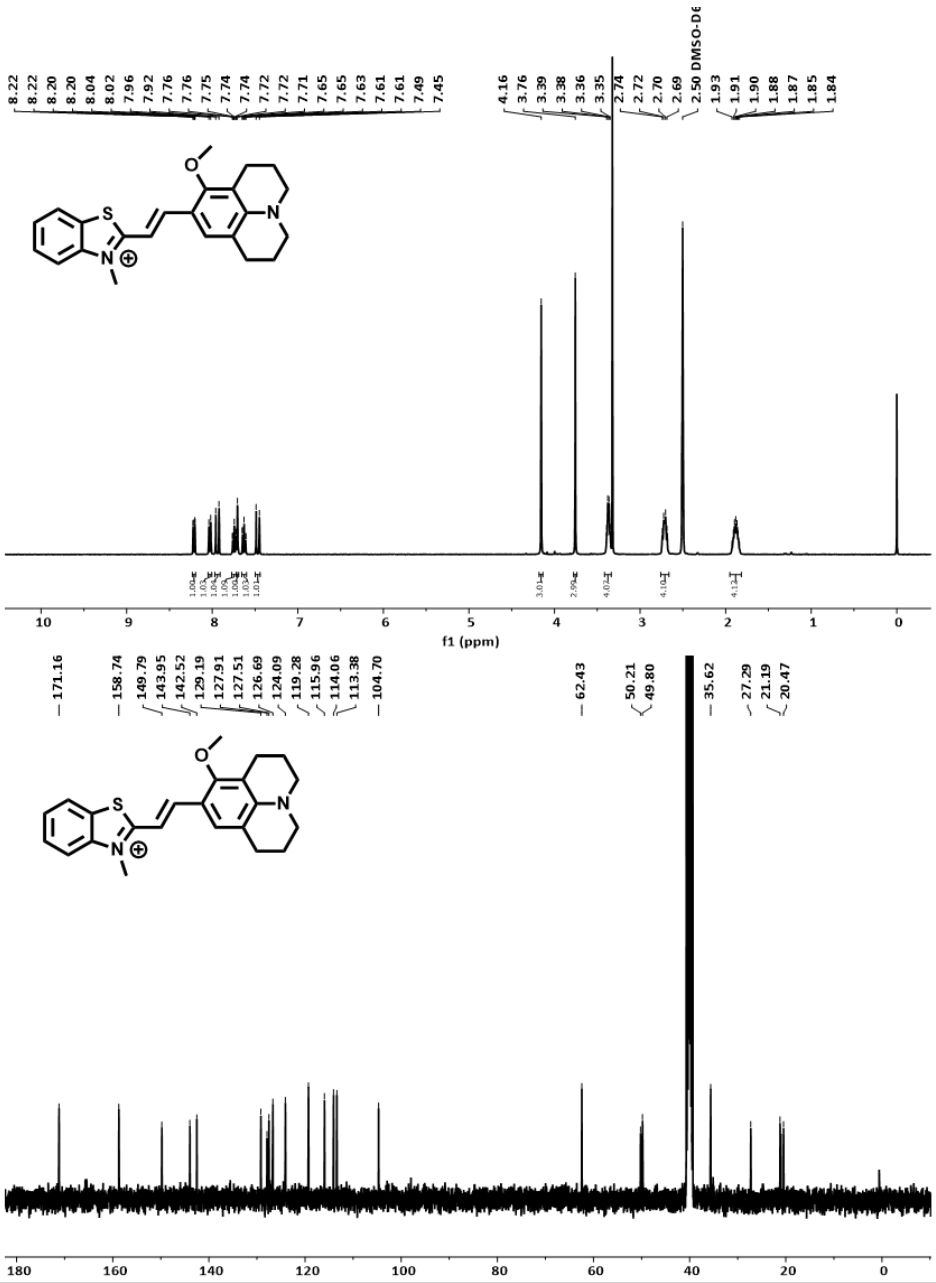


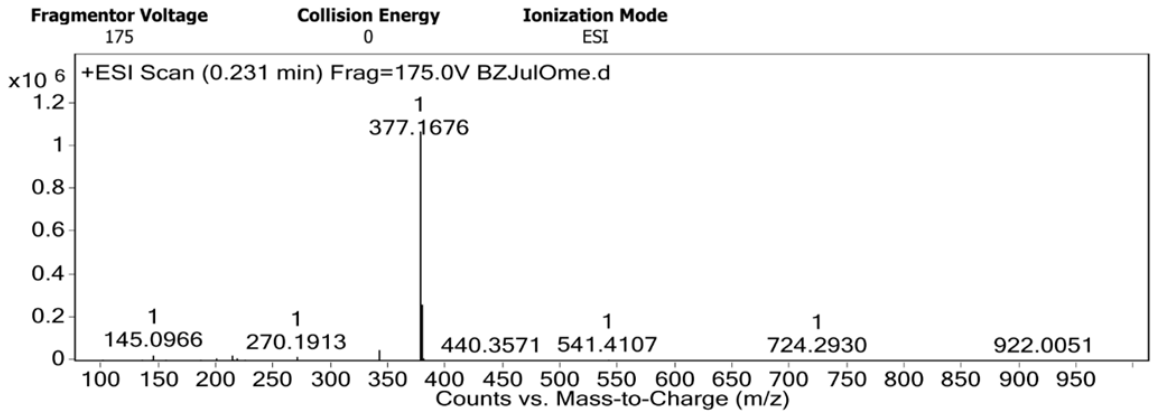
^{1}H, ^{13}C and HRMS spectra of probe **6**



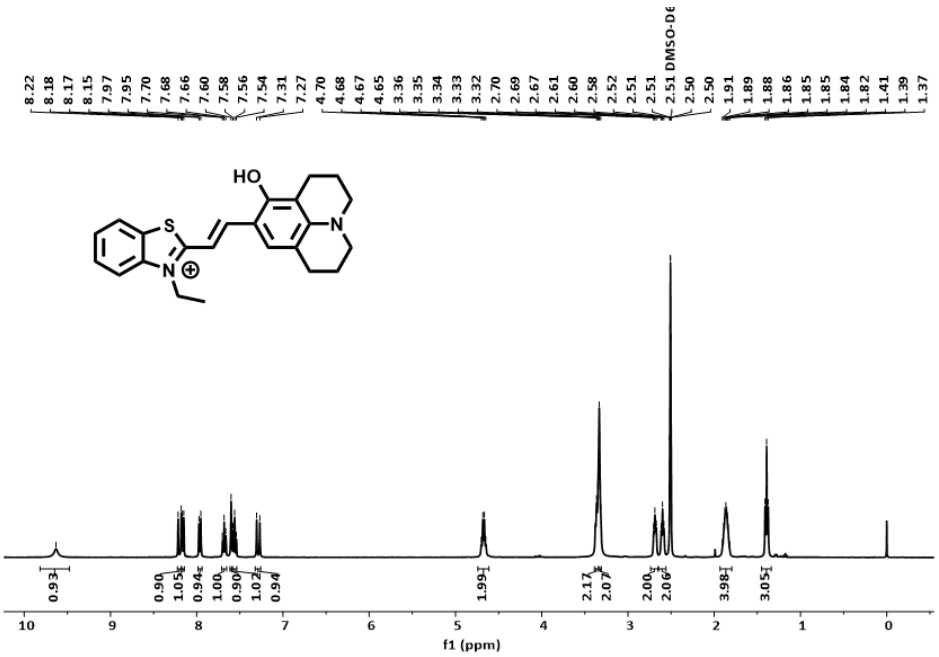


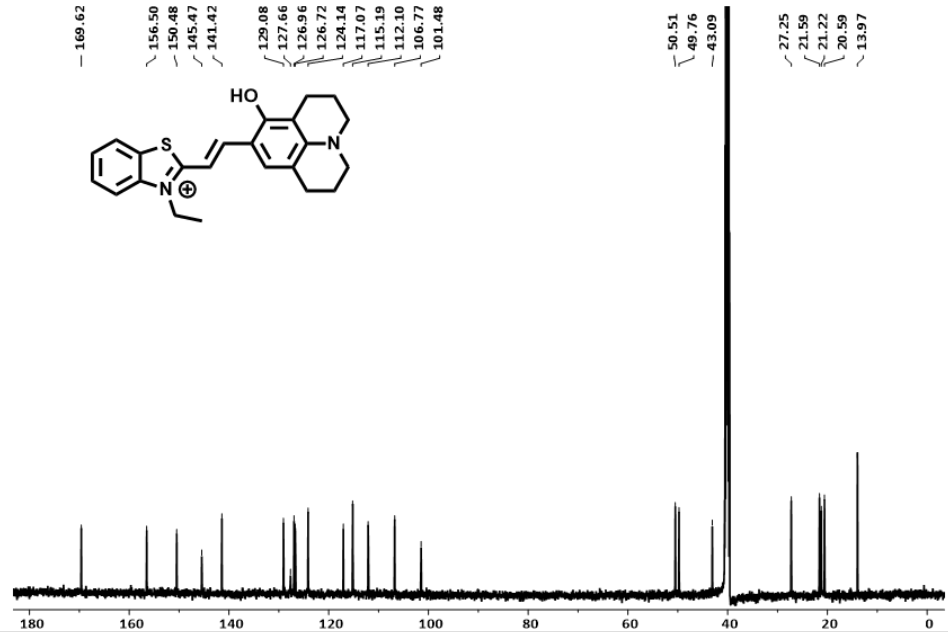
^{1}H, ^{13}C and HRMS spectra of probe **7**

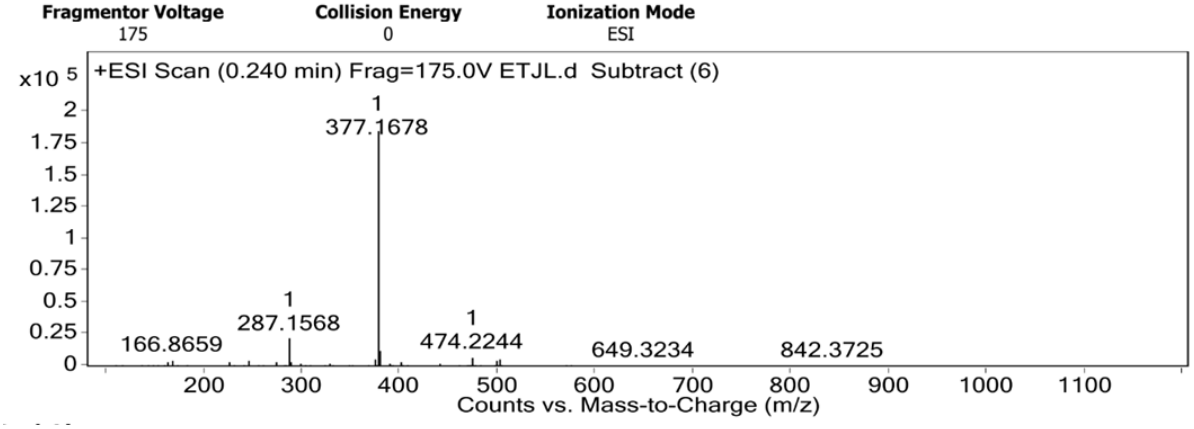




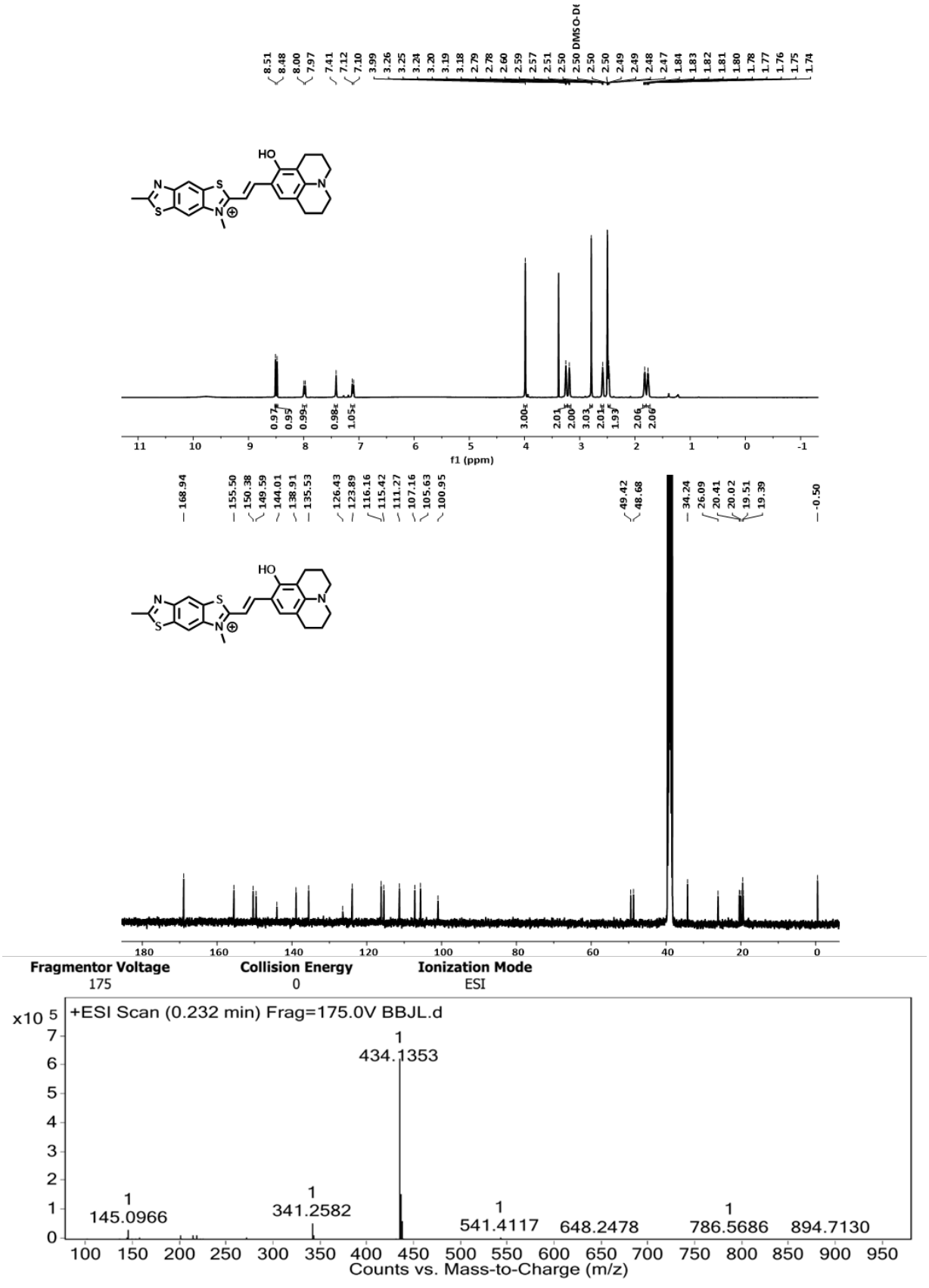
^{1}H, ^{13}C and HRMS spectra of probe **8**



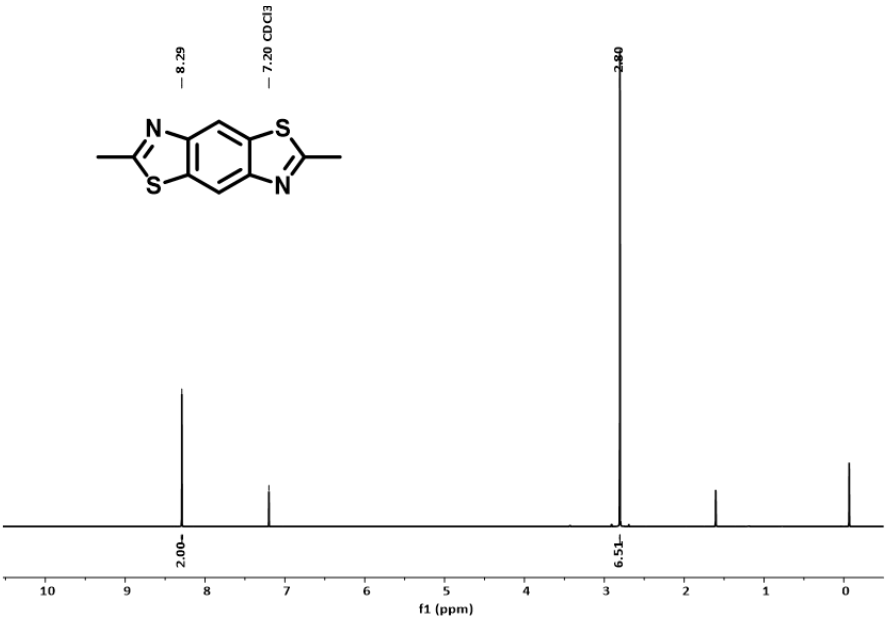


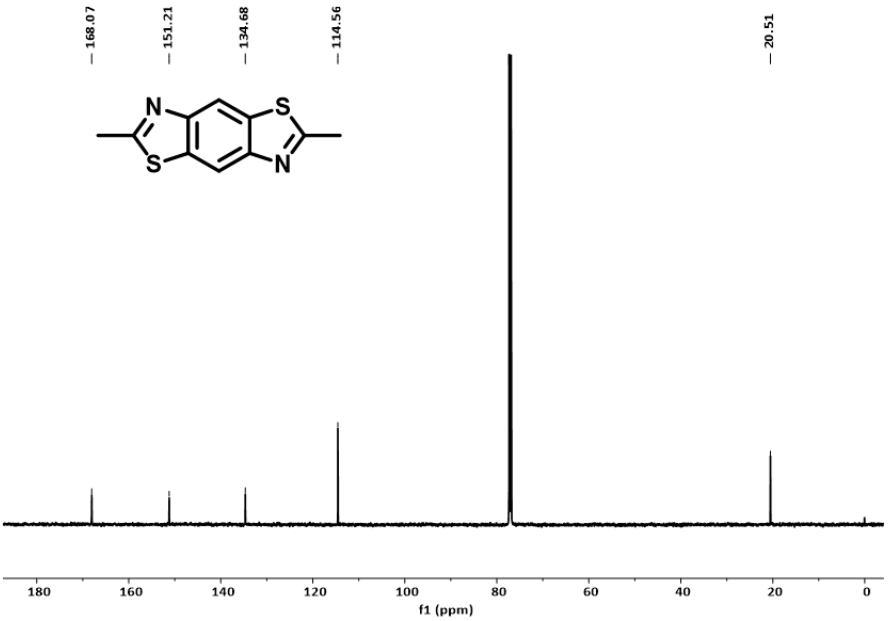


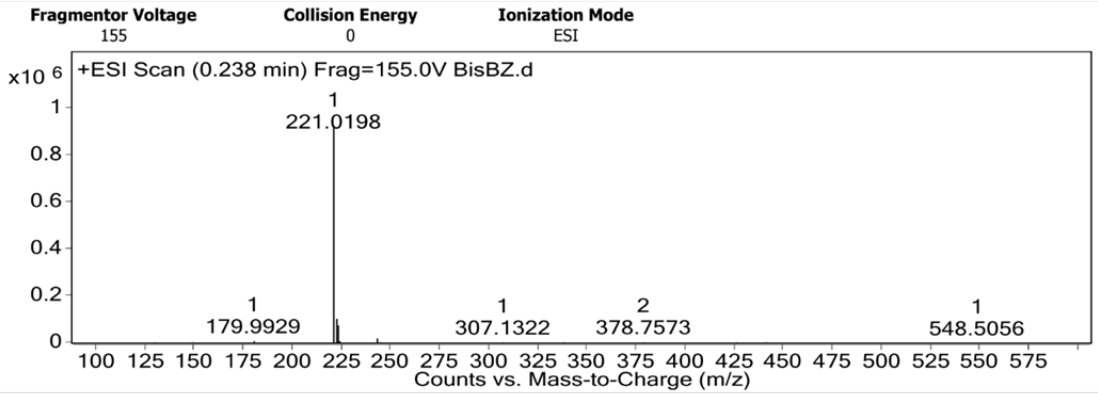
^{1}H, ^{13}C and HRMS spectra of probe **9**



^{1}H, ^{13}C and HRMS data of Benzobisthiazole







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