Electronic Supplementary Information

Coronavirus genomic cDNA derived G-Quadruplex as selective target for fluorometric detection

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1. General Information

All chemical reagents and solvents were purchased from Merck. Single stranded oligos were procured from Integrated DNA Technologies (IDT). ^{1}H and ^{13}C NMR for molecular characterization were recorded on a Bruker AV-400 MHz spectrometer and chemical shifts in the spectra are reported as parts per million (*ppm*) with TMS (tetramethylsilane) as the internal standard. For confirmation of molecular weight, high resolution mass spectra (HRMS) were recorded on Agilent Technologies 6538 UHD Accurate-Mass Q-TOF LC/MS spectrometer. CD spectra were recorded in a quartz cuvette of 1 cm path length on a Jasco-815 spectropolarimeter (Japan). For fluorescence lifetime measurements a 560 nm laser with pulse repetition of 1 MHz was used in a Horiba Delta Flex time-correlated single photon counting (TCSPC) instrument. Fluorescence Imaging of PAGE images of DNA-probe solutions were captured in Bio Rad ChemiDoc MP imaging system.

2. Synthesis of molecular probe TPAMB and TPAEB

To a solution of 2-methyl benzothiazole (5 mmol) dissolved in dichloromethane (5 mL), excess methyl iodide (20 mmol) was added, and refluxed in a sealed tube for 6 h. A white precipitate indicated formation of product. Completion of reaction was monitored by thin layer chromatography (TLC). After completion of reaction, the precipitate was filtered and washed with diethyl ether to remove the (unreacted starting material) excess methyl iodide and unreacted 2-methylbenzothazole, and dried to obtain the product (N-methyl-2-methylbenzothiazole) in quantitative yield.

To a stirred solution of N-methyl-2-methylbenzothiazole in ethanol, piperidine was added. After stirring for 10 min, 4-(N, N-diphenylamino)benzaldehyde was dissolved in ethanol and added

dropwise. The reaction mixture was stirred over a magnetic stirrer stabilized at 60 °C. Following completion of the reaction, the solvent was evaporated *in vacuo* and purification was done using semipreparative RP-HPLC (grad. 70-85% acetonitrile in water, 12 min) to obtain TPAMB as pure product. A dark violet powder was obtained after lyophilization. Yield 56%.

^{1}H NMR (400 MHz, DMSO-d_6) 8.37 (d, J= 8.1, 1H), 8.18(d, J =8.4, 1H), 8.12 (d, J = 15.6, 1H), 7.91 (d, J = 8.84, 2H), 7.83 (t, J = 8.32, 1H), 7.78(d, J = 15.64, 1H), 7.74 (t, J = 8, 1H), 7.4348 (t, J = 7.6, 4H), 7.24 (t, J = 7.44, 2H), 7.2 (d, J = 7.48, 4H), 6.9 (d, J = 8.76, 2H), 4.29 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): 171.7, 151.3, 148.6, 145.5, 141.9, 137.1, 131.8, 129.9, 129.1, 127.9, 127.3, 126.1, 125. 4, 124, 118.9, 116.5, 110.1, 36.1. HRMS (ESI, m/z) calculated for $[C_{28}H_{23}N_2S]^+$ 419.1576, observed 419.1576.

TPAEB was synthesized following similar synthetic protocols as in TPAMB.

Dark pink solid, Yield 74 %. ^{1}H NMR (400 MHz, DMSO-d_6) 8.39 (d, J = 8.88, 1H), 8.24 (d, J = 8.44, 1H), 8.15 (d, J = 15.4, 1H), 7.95 (d, J = 8.92, 2H), 7.84 (t, J = 8.52, 1H) , 7.78 (d, J = 15.52, 1H), 7.75 (t, J = 8, 1H), 7.44 (t, J = 7.6, 4H), 7.25 (t, J = 7.4, 2H), 7.20 (d, J = 7.4, 4H), 6.91 (d, J = 8.9, 2H), 4.91 (q, J = 7, 2H), 1.45 (t, J = 7.12, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): 171.3, 151.4, 149.2, 145.5, 140.8, 131.9, 129.9, 129.3, 127.8, 126.1, 125.4, 124.2, 118.9, 116.2, 109.5, 44.1, 14. HRMS (ESI, m/z) calculated for $[C_{29}H_{25}N_2S]^+$ 433.1733, observed 433.1741.

3. Fluorescence screening of molecular probes with cDNA GQs

The fluorescence screening for identification of lead molecular probe and target cDNA GQ was performed in a 384 transparent bottom microplate. 200 µL of DNA (1 µM) in Tris. HCl (100 mM KCl) was treated with molecular probes to a final concentration of 4 µM. 50 µL of the solution

was added per well and the emission maxima were recorded and plotted in Graph Pad Prism software.

4. Jobs plot analysis for interaction of TPAMB with cDNA5

Continuous variation method was used for determination of binding stoichiometry between TPAMB and cDNA5. Briefly, a series of solution with sequential decrease in the mole fraction of TPAMB was prepared. Total concentration of [TPAMB + cDNA] was kept constant at 0.5 μM. The fluorescence intensities were recorded, and values were plotted against corresponding mole fractions. The mole fraction corresponding to the maxima was used to determine the stoichiometry of binding.

5. Gel electrophoresis of TPAMB with cDNA GQ

15 μL cDNA (1 to 6) solutions of 2 μM were prepared in GQ stabilizing buffer. 5 μM of TPAMB was added and the solutions were incubated for 10 min. 15 % PAGE gel was prepared with 1X TBE (supplemented with 100 mM KCl) as the running buffer. A constant voltage of 80 V was applied for 60 min for electrophoretic separation. Next the gel was stained with SYBR gold and imaged independently in the appropriate excitation of SYBR gold and TPAMB in a Bio Rad ChemiDoc MP imaging system.

6. Tables

Table S1. List of identified sequences with –CC- repeats and their length, and position in the genome of SARS-CoV-2.

Name	Position	Length	Sequence
C1	10015	25	CCAACCACCACAAACCTCTATCACC
C2	15924	18	CCTTCCTTACCCAGATCC
C3	13255	27	CCGTTGCCACATAGATCATCCAAATCC
C4	15924	28	CCCAGATCCATCAAGAATCCTAGGGGCC
C5	15	23	CCTTCCCAGGTAACAAACCAACC
C6	15924	37	CCTTCCTTACCCAGATCCATCAAGAATCCTAGGGGCC

Table S2. Table for the photophysical properties of the probes TPAEB and TPAMB.

Molecular code	Absorbance max λ_{ex} (nm)	Emission max λ_{ex} (nm)	Stokes Shift (nm)
TPAMB	497	580	83
TPAEB	480	637	157

Table S3. Summary of fluorescence decay of TPAMB with respect to different DNA targets.

Target DNA	λ_{ex} (nm)	$\lambda_{monitored}$ (nm)	t_1 (ns)	$<t_{avg.}>$ (ns)
cDNA3	510	585	3.9 (100%)	3.9
cDNA5	510	585	3.8 (100%)	3.8
BCL2	510	585	2.6 (100%)	2.6

Table S4. List of DNA and RNA sequences used in the work.

Name	Length	Sequence	Conformation
KRAS	28	GGGAGGGAGGGAAGGAGGGAGGGAGGGA	Parallel GQ
Thrombin	17	GGGTTGGTGTGGTTGGA	Antiparallel GQ
VEGF	37	GGGGGCGGGCCGGGGGCGGGGTCCCGGCGGGGC GGAG	Parallel GQ
Tel 22	22	AGGGTTAGGGTTAGGGTTAGGG	Antiparallel GQ
BCL2	27	CGGGCGCGGGAGGAATTGGGCGGGAGC	Hybrid GQ
c-MYC	27	TGGGGAGGGTGGGGAGGGTGGGGAAGG	Parallel GQ
R1	20	GGTATGTGGAAAGGTTATGG	Antiparallel GQ
R2	15	GGCTGGCAATGGCGG	Antiparallel GQ
R3	20	GGTTGGACCTTTGGTGCAGG	Antiparallel GQ
R4	26	GGTGTTGTTGGAGAAGGTTCCGAAGG	no GQ
R1 RNA	20	GGUAUGUGGAAAGGUUAUGG	Parallel GQ
mt9438	20	GGCGTAGGTTTGGTCTAGGG	Antiparallel GQ
mt6363	26	AGGGACGCGGGCGGGGGATATAGGGT	Hybrid GQ
Rb i-motif	18	GCCGCCCAAAACCCCCCG	*i*-motif
RO1C	30	AGACGCCATACACCTTTCCAATACCGACAT	no *i*-motif
A20T20	20	AAAAAAAAAAAAAAAAAAAA : TTTTTTTTTTTTTTTTTTTT	dsDNA
R1:R1C	20	GGTATGTGGAAAGGTTATGG : CCATACACCTTTCCAATACC	dsDNA
cDNA1	25	GGTTGGTGGTGTTTGGAGATAGTGG	Antiparallel GQ
cDNA2	18	GGAAGGAATGGGTCTAGG	-
cDNA3	27	GGCTTCGGTGTATCTAGTAGGTTTAGG	Antiparallel GQ
cDNA4	28	GGGTCTAGGTAGTTCTTAGGATCCCCGG	Hybrid
cDNA5	23	GGAAGGGTCCATTGTTTGGTTGG	Antiparallel GQ
cDNA6	37	GGAAGGAATGGGTCTAGGTAGTTCTTAGGATCCC CGG	Hybrid
Telo GQ	21	AGGGTTAGGGTTAGGGTTAGGG	Antiparallel

7. Figures

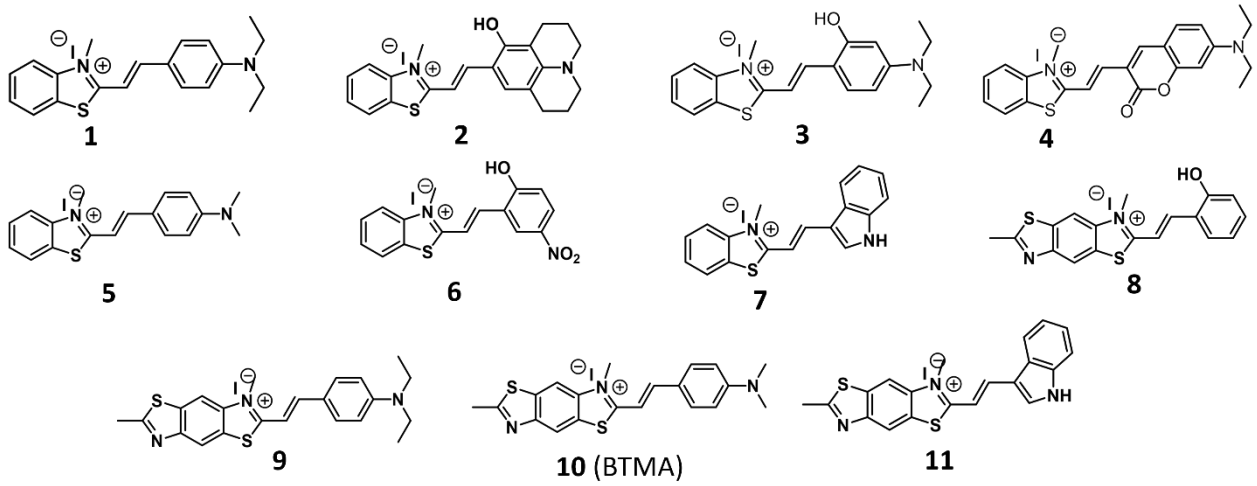


Fig. S1. Chemical structure of probes **1-11** used in fluorescence screening studies.

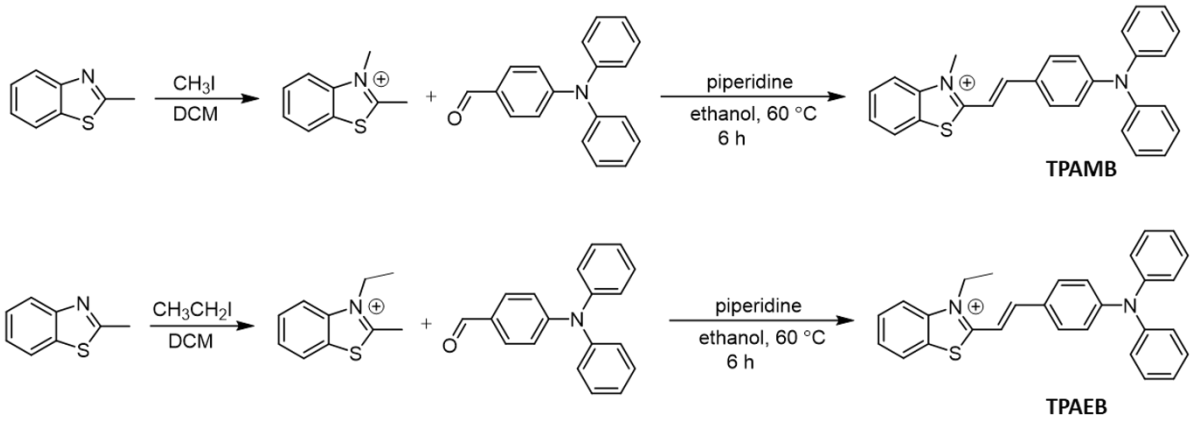


Fig. S2. Synthesis of molecular probes TPAMB and TPAEB.

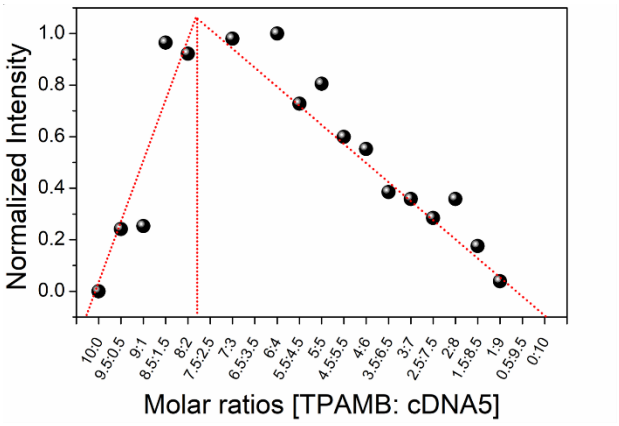


Fig. S3. Determination of binding stoichiometry of TPAMB with cDNA5.

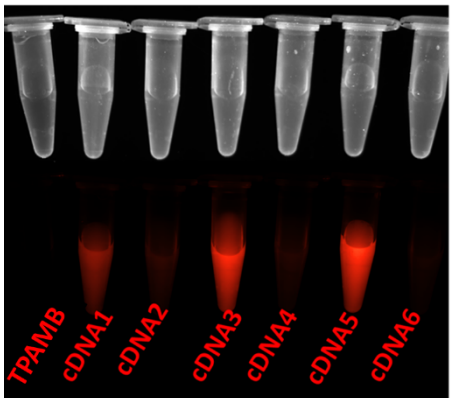


Fig. S4. Photograph showing fluorescence emission of TPAMB with the selective cDNA sequences.

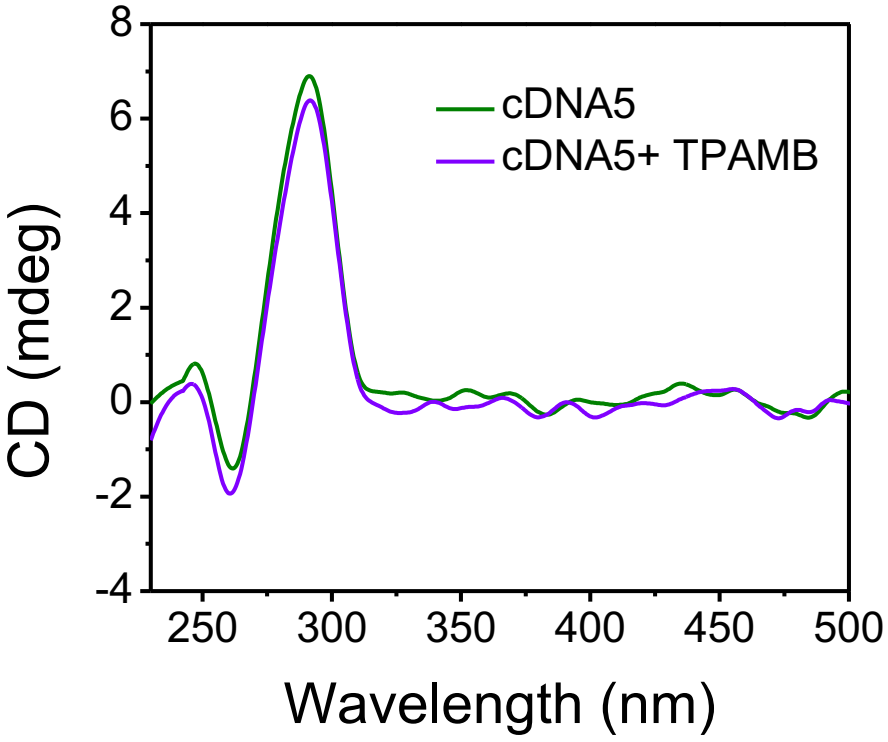


Fig. S5. CD spectra of cDNA5 (2 μM), and upon incubation with TPAMB.

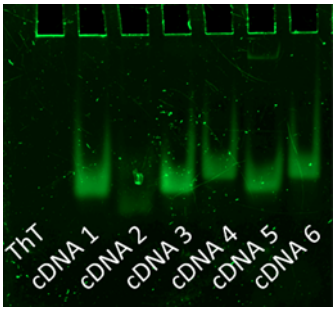
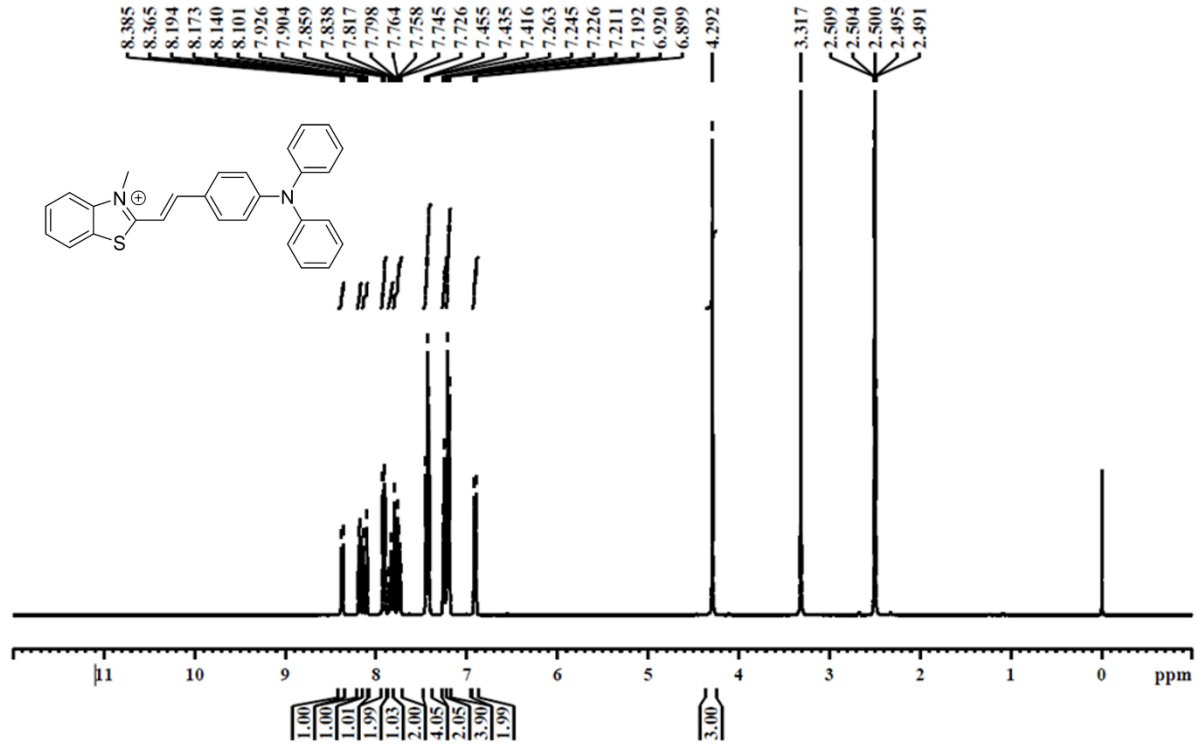
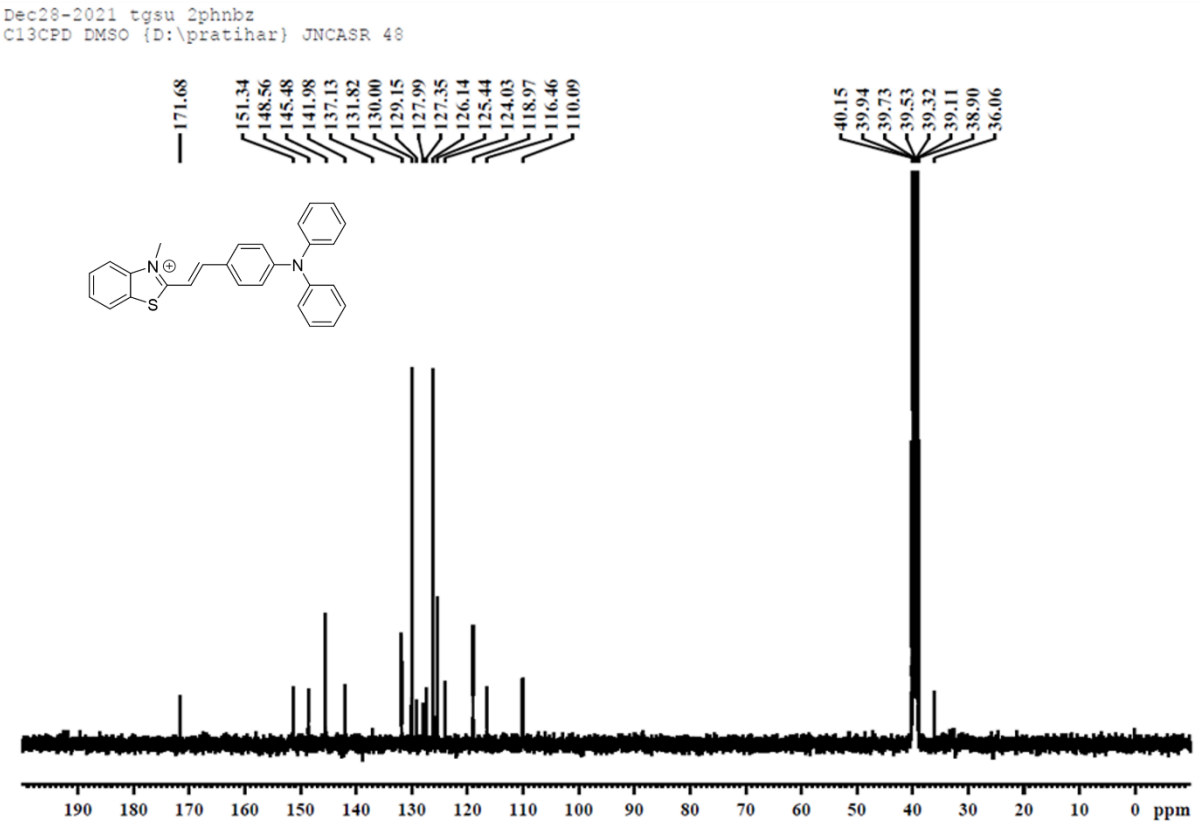


Fig. S6. PAGE image of cDNA sequences poststained with ThT (2 μM).

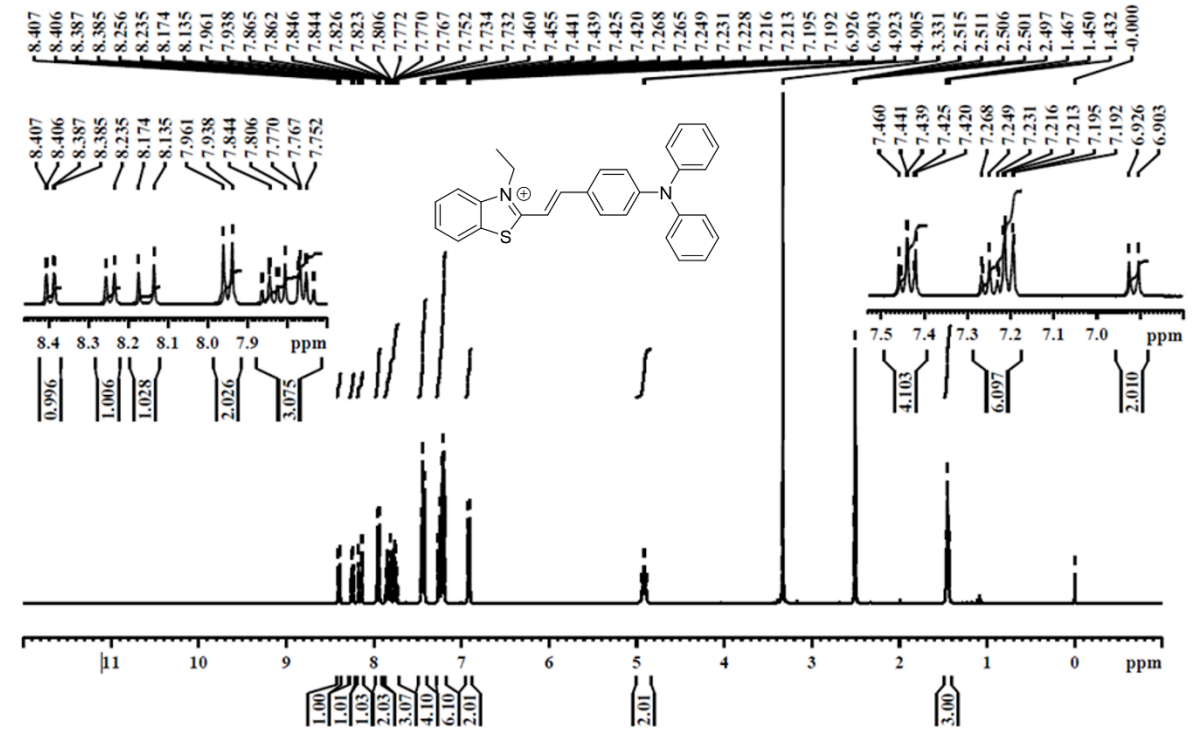
8. Compound characterization data

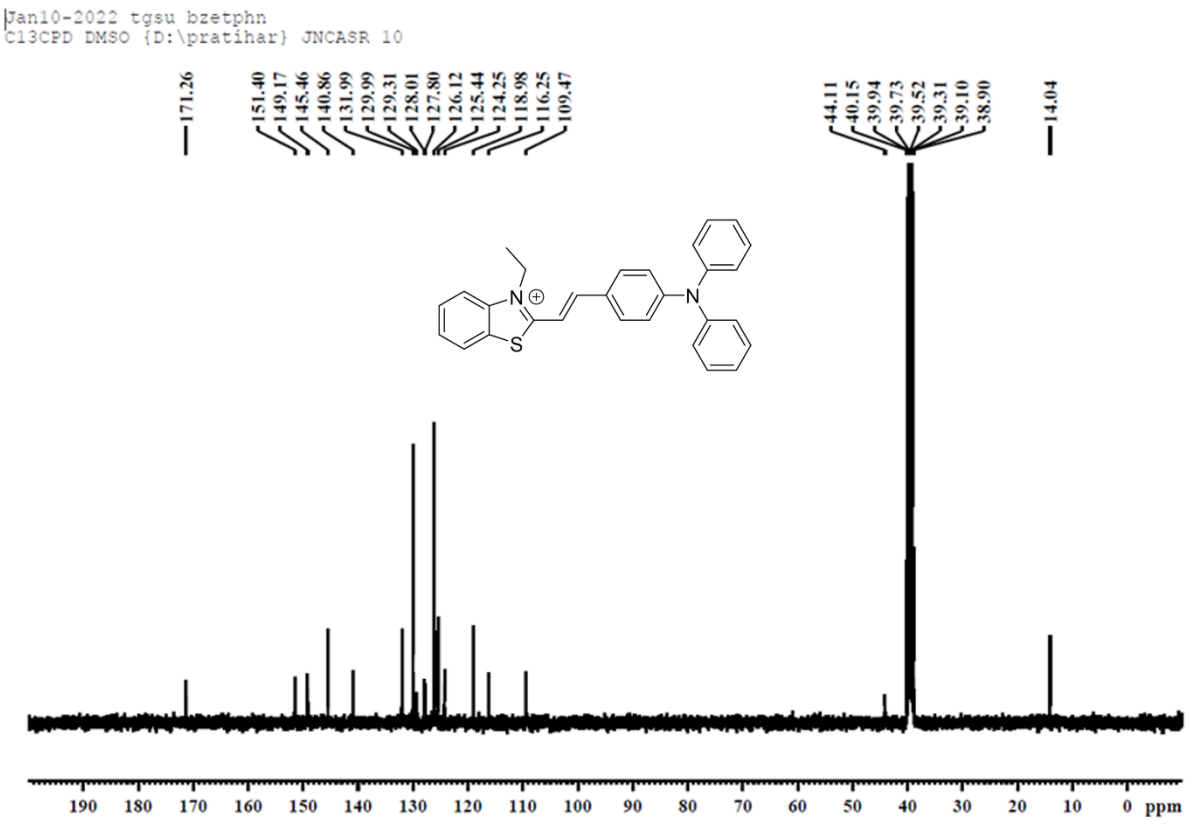
^{1}H and ^{13}C NMR data for TPAMB



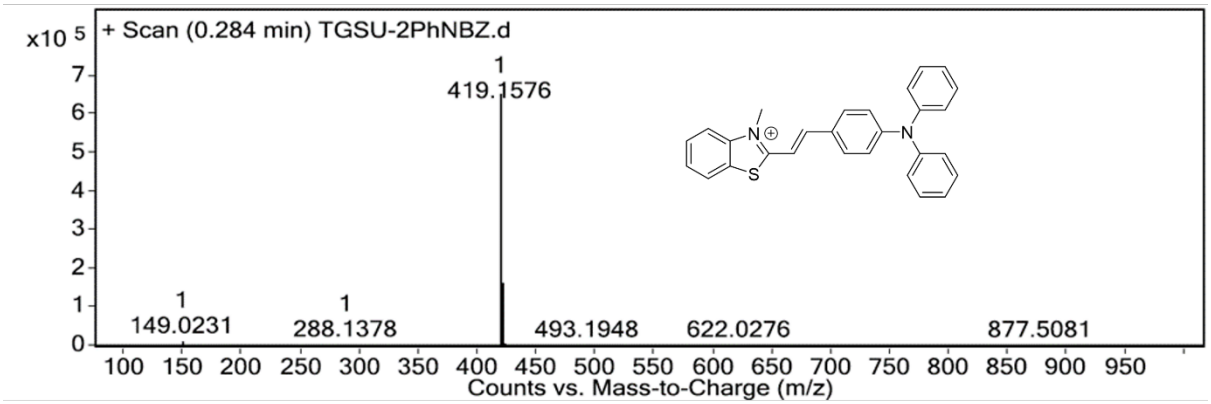


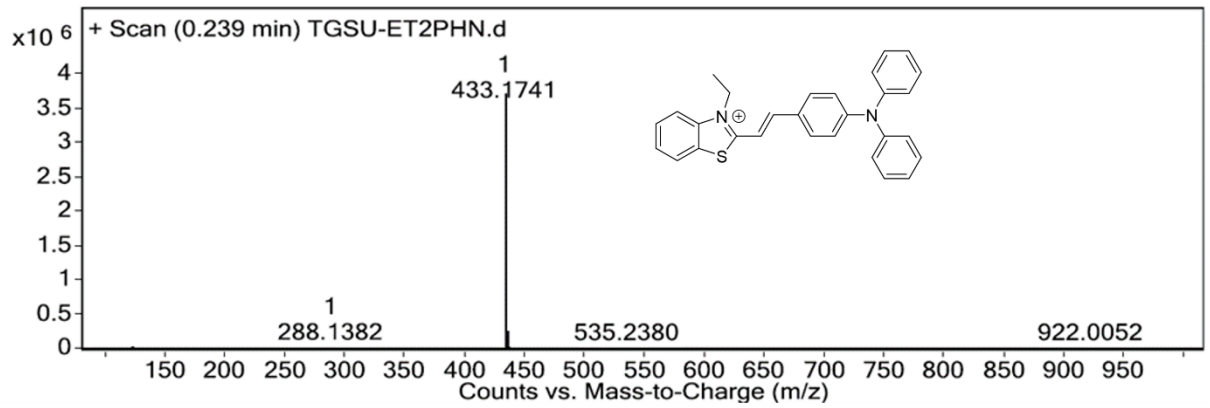
^{1}H and ^{13}C NMR data for TPAEB



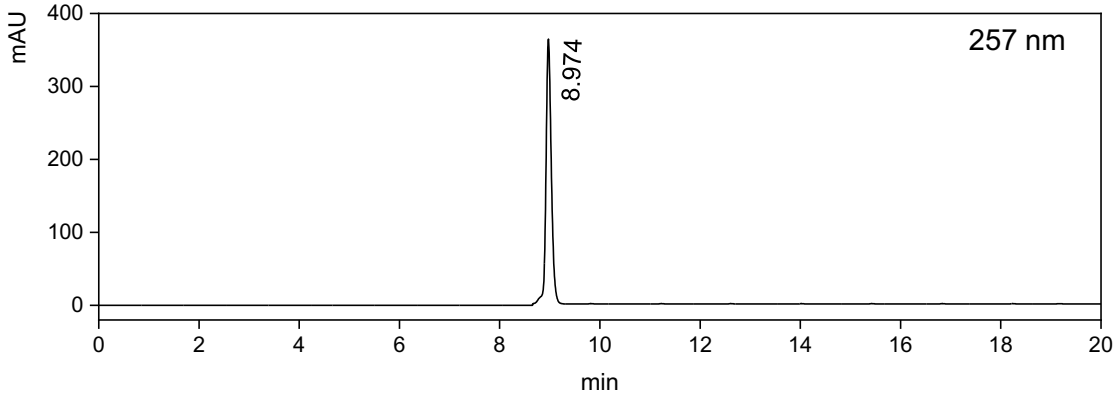


HRMS data for TPAMB and TPAEB.





HPLC trace for TPAMB



HPLC trace for TPAEB