Electronic Supplementary Material (ESI) for Analyst.

Imaging Nucleus Viscosity and G-quadruplex DNA in Living Cells by a Nucleus-Targeting Two-Photon Fluorescent Probe

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Contents

1. Materials and methods

All chemicals were purchased as reagent grade materials and used without further purification. The solvents were dried and distilled according to standard procedures.

Table S1. Names and sequences of different GQ DNA, ssDNA, and dsDNA

Name	Sequence (5'→3')	Structure in K^+ solution
c-kit3	GGCGAGGAGGGGCGTGGCCGGC	Antiparallel G4 DNA
htg21	GGGTTAGGGTTAGGGTTAGGG	Antiparallel G4 DNA
c-kit1	AGGGAGGGCGCTGGGAGGAGGG	Parallel G4 DNA
c-kit2	CGGGCGGGCGCGAGGGAGGGT	Parallel G4 DNA
c-myc	GAGGGTGGGGAGGGTGGGGAAG	Parallel G4 DNA
htg22	AGGGTTAGGGTTAGGGTTAGGG	Hybrid-type G4 DNA
telo24	TTAGGGTTAGGGTTAGGGTTAGGG	Hybrid-type G4 DNA
mpu22	TGAGCGTGGCGAGCGTGGCGAA	Single-stranded DNA
ct-DNA		Double- stranded DNA

The G-quadruplex DNA solutions were annealed by heating telo24 in 50 mM Tris buffer (pH 7.2) and 50 mM KCl, at 95 °C for 5 min and by cooling at 2 – 4 ^{o}C for 10 min.

^{1}H and ^{13}C NMR spectra were recorded using a Bruker Avance III 400 MHz spectrometer and 500MHz spectrometer (Bruker Co., Germany). Coupling constants *J* are given in Hz. High resolution mass spectra were acquired using a waters LCT Premier XE spectrometer (Waters, Milford, MA, USA).

Absorption spectra were recorded with a Varian Cary-50 UV–Vis spectrophotometer (Agilent Technologies, USA). Fluorescence spectra were recorded with a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, USA) equipped with a 1 cm × 1 cm quartz cell.

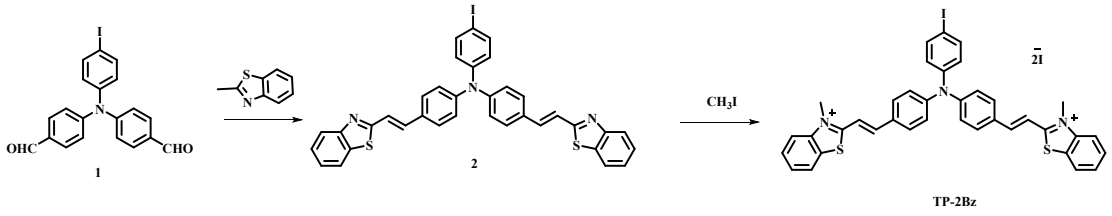
TPA cross-sections (δ) of the samples were obtained by the two-photon excited fluorescence (TPEF) method with a femtosecond laser pulse and a Ti : sapphire system (760 – 880 nm, 80 MHz, SP-5W, Spectra-Physics, America), as the light source. The concentration of the sample solution was $1.0*10^{-5}$ M. Thus, the δ values of samples were determined using Equation (1).

$$\delta s = \delta r \cdot Fs \cdot \Phi r \cdot Cr \cdot nr / Fr \cdot \Phi s \cdot Cs \cdot ns \qquad (1)$$

where the subscripts "s" and "r" represent the sample and reference (here, Rhodamine B in ethanol solution at a concentration of $1.0*10^{-5}$mol/L was used as a reference), respectively. F is the overall fluorescence collection efficiency intensity of the fluorescence signal collected by the Fluorescence Spectrophotometer. Φ, n and c are the quantum yield of the fluorescence, the refractive index of solvent, and the concentration of the solution, respectively.

Fluorescent images were acquired on Olympus FV1200 and Olympus FV1200MPE. Image data acquisition and processing was performed using Olympus LSM Image Browser, Olympus LSM Image Expert and Image *J*. All measurements were in air at room temperature.

2. Scheme of TP-2Bz Synthesis



Scheme S1 The synthetic pathway toward **TP-2Bz**

3. Fig. S1

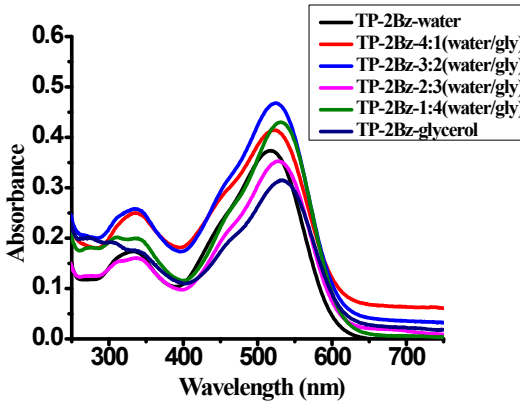


Fig. S1 Absorption spectra of **TP-2Bz** in different water/glycerol solvent (c = 1.0×10^{-5})

4. Fig. S2

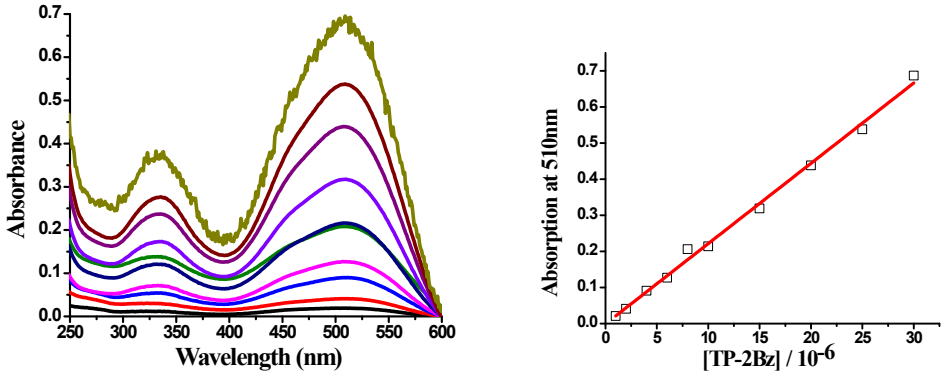


Fig. S2 absorption spectra and plot of the intensity at 510 nm against the concentration of **TP-2Bz** in pure PBS buffer (pH = 7.4), respectively.

5. Fig. S3

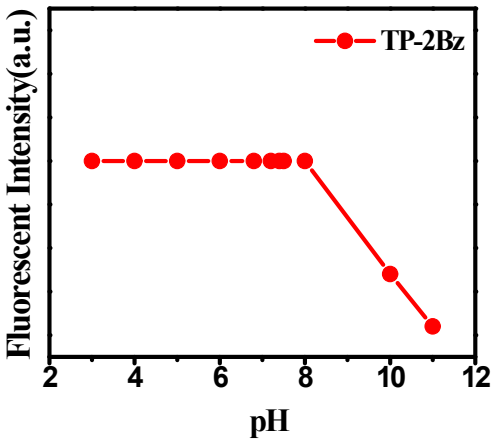


Fig. S3 The fluorescent intensities of **TP-2Bz** at various pH values at 650nm

6. Fig. S4

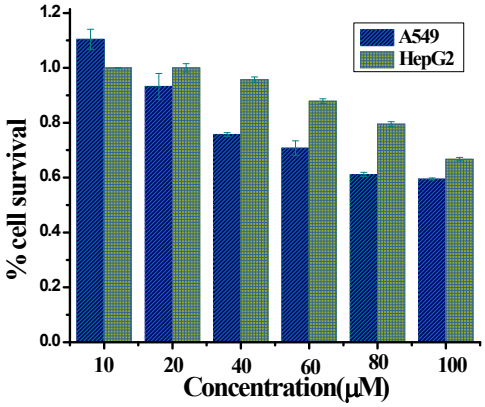


Fig. S4 Cytotoxicity of **TP-2Bz** in HepG2 and A549 cell lines (The data are given as mean ± SD (n = 6)).

7. Fig. S5

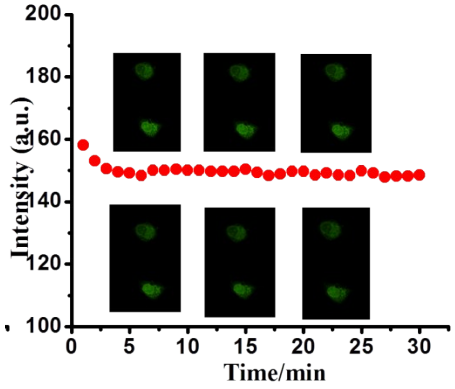


Fig. S5 Two-photo excited fluorescence images of **TP-2Bz** by 30 min of laser radiation (λ_{ex} = 800 nm, λ_{em} = 600-670 nm).

8. Molecular docking studies

Molecular docking calculations were performed using the Auto dock 4.0 software, which has been reported to be of high accuracy of prediction [1]. The crystal structure (NDB: 2JPZ) of telo 24 GQ DNA fragment (base sequences TTAGGGTTAGGGTTAGGGTTAGGGTT) were downloaded from RCSB Protein Data Bank.[2] The redundant solvent molecules and ions were removed from the crystal structure.

The initial structure of **TP-2Bz** is modeled in Gauss03, and optimized at B3LYP/6-31g (d, p).

Table S2 The docked energy of docking probes

Molecule	docked energy (Kcal/mol)
TP-2Bz	-7.96
	-7.35
	-7.62
	-7.37
	-7.32
	-7.34
	-7.32
	-7.29
	-7.27
	-7.01

Docked energy optimized: docked interaction score (negative of the energy) after optimizing the hydrogen position at the ligand and on the receptor in the vicinity of the ligand.

9. References

[1] O. Trott, A.J. Olson, *J. Comput. Chem.* 2010, **31**, 455–461.

[2] J. Dai, M. Carver, C. Punchihewa, R. A. Jones, D. Yang, *Nucleic Acids Res.* 2007, **35**, 4927–4940.

10. Spectra data of TP-2Bz

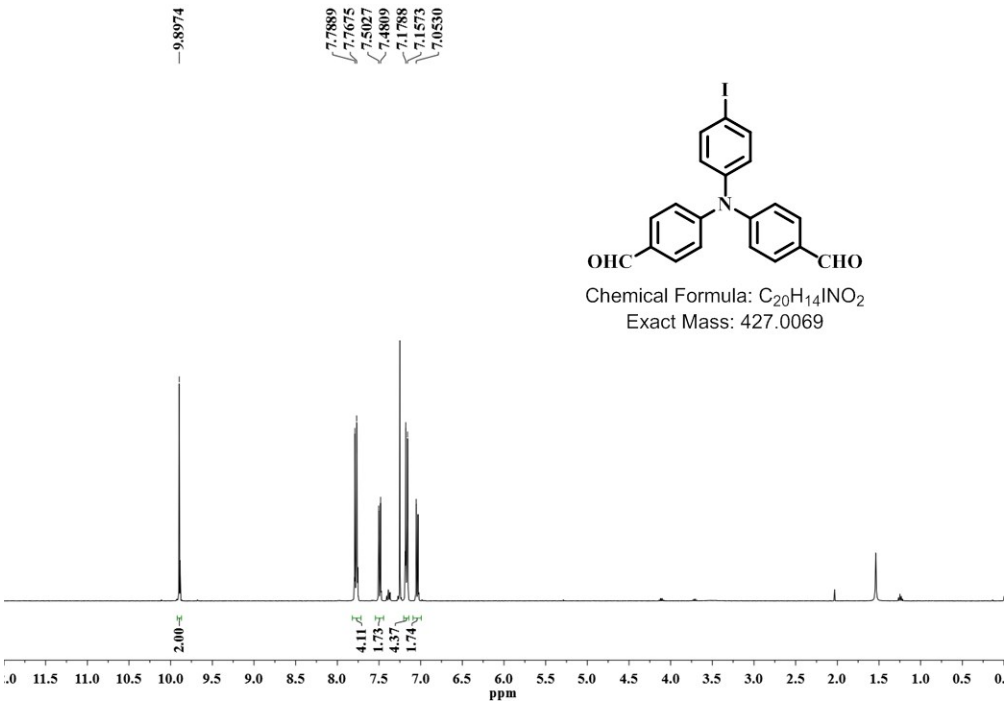


Fig S6 [1]H NMR (400 MHz, CDCl3) spectrum of **1**

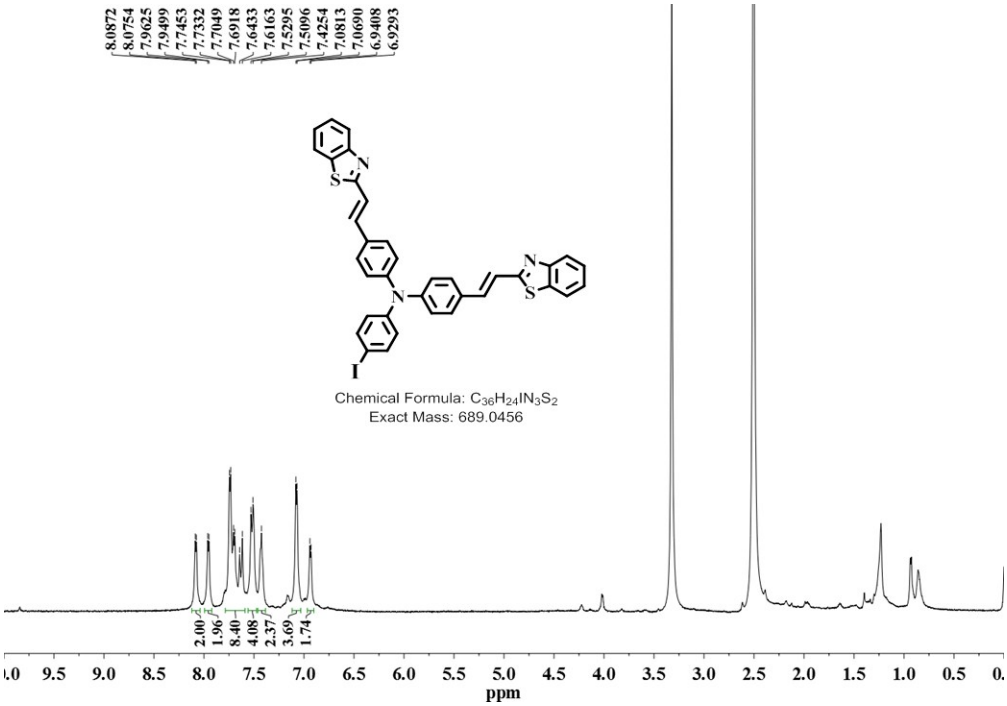


Fig S7 [1]H NMR (400 MHz, DMSO-*d6*) spectrum of **2**

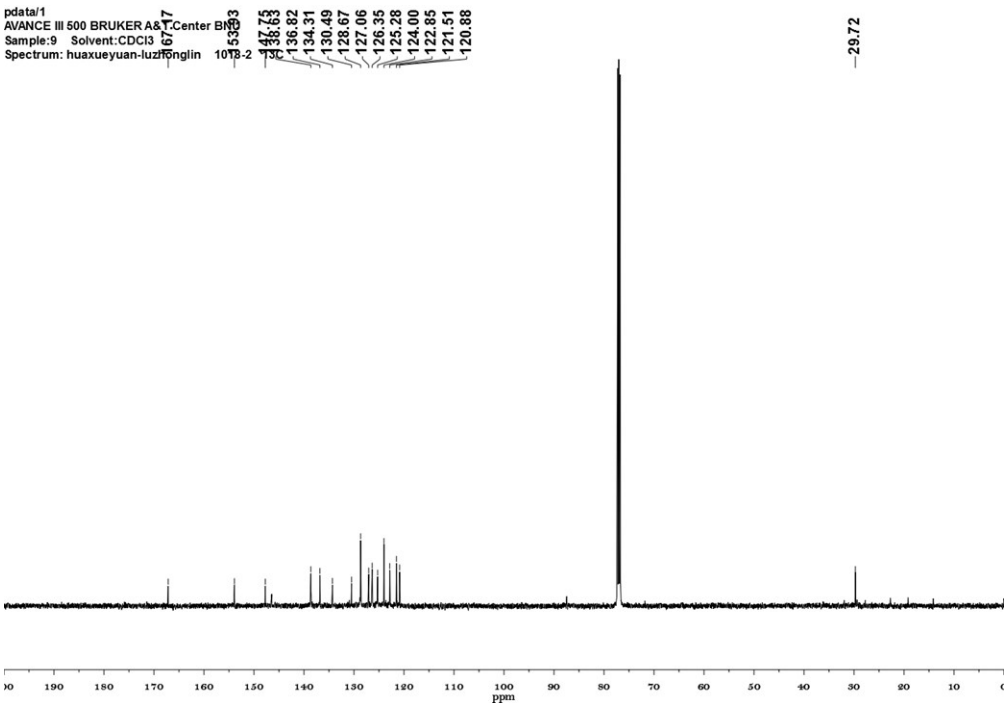


Fig S8 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **2**

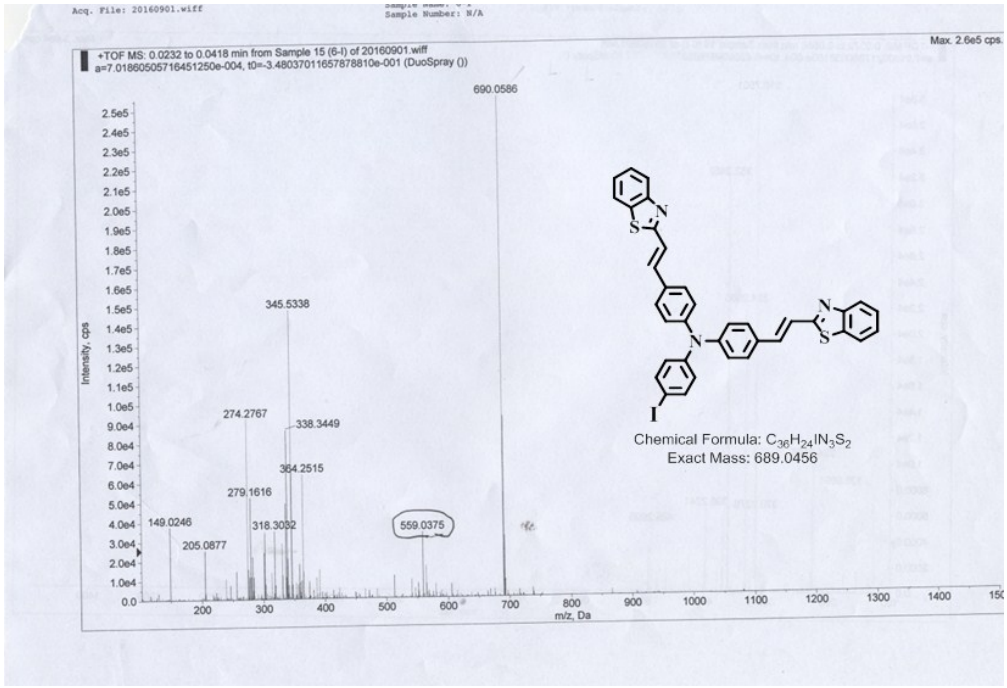


Fig S9 The MS-ESI spectrum of **2**

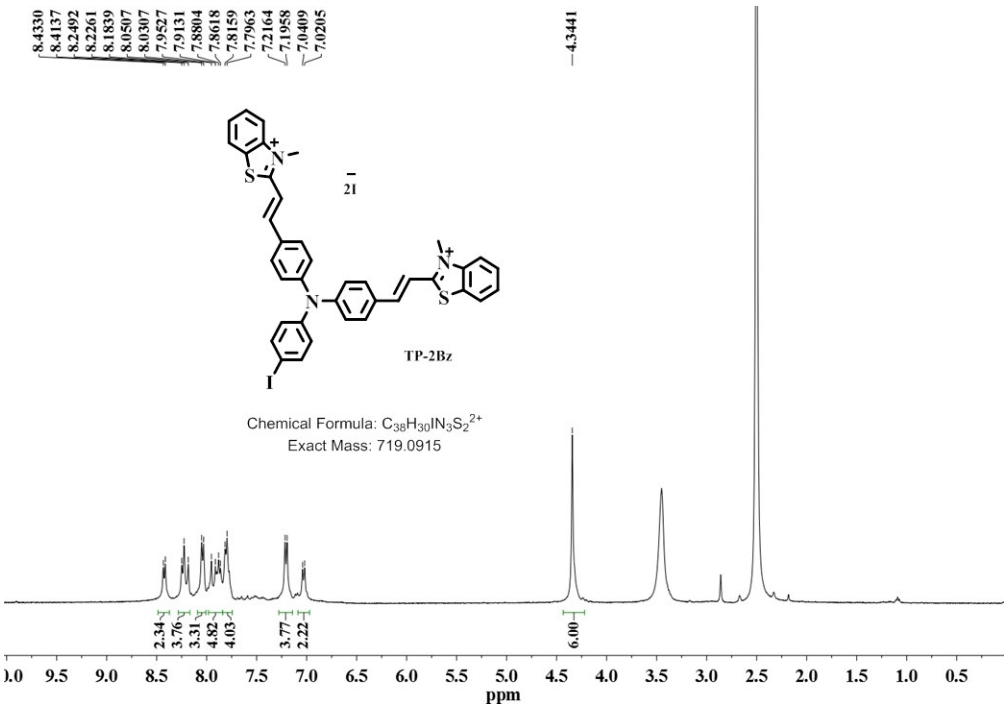


Fig S10 ^{1}H NMR (400 MHz, DMSO-*d6*) spectrum of **TP-2Bz**

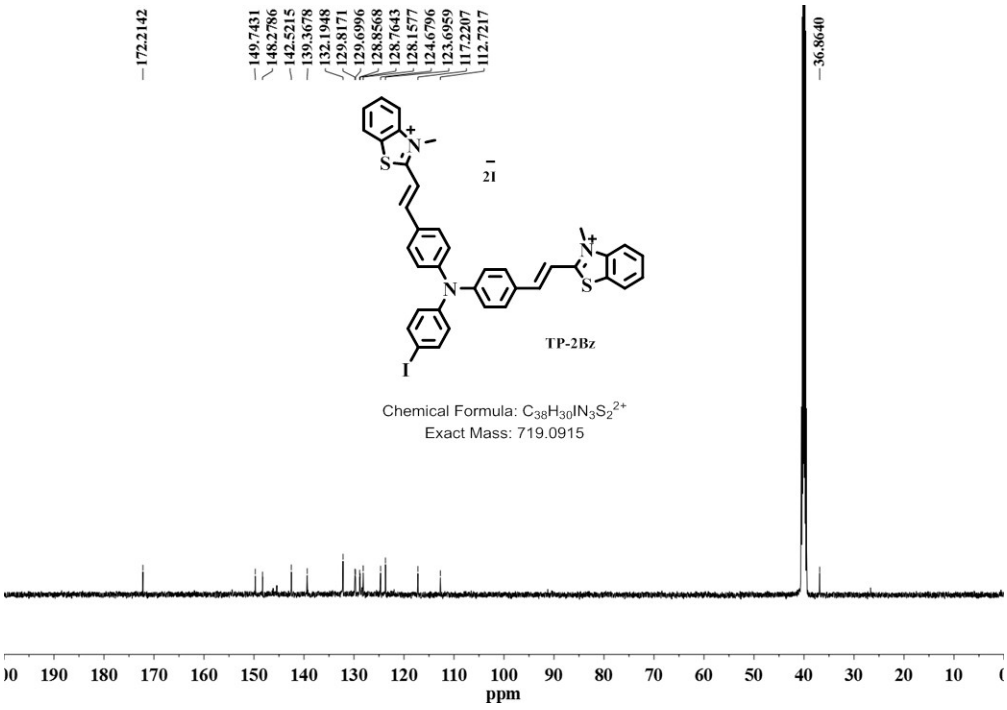


Fig S11 ^{13}C NMR (400 MHz, DMSO-*d6*) spectrum of **TP-2Bz**

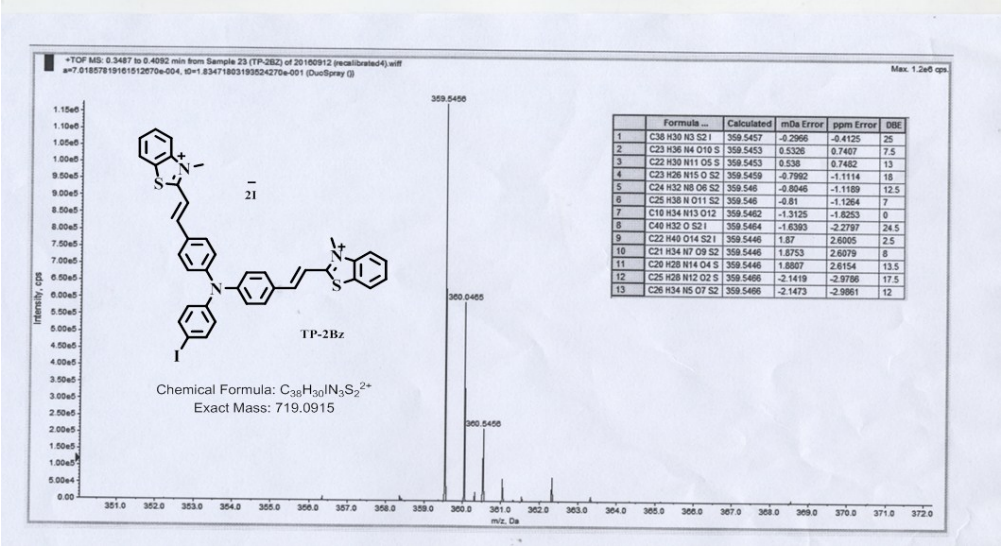


Fig S12 The HRMS-ESI spectrum of **TP-2Bz**