

Supporting Information

Title: A novel self-assembled fluorescent organic nanoprobe and its application for detection of sulfite in food samples and living system

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1. Synthesis

4-(1,4,5-triphenyl-1H-imidazol-2-yl)benzaldehyde (TIB). Terephthalaldehyde (1.34 g, 10.00 mmol) and aniline (930 mg, 10.00 mmol) were dissolved in acetic acid (100 mL) and stirred for 1 h at room temperature. Benzil (2.10 g, 10.00 mmol) and ammonium acetate (5.40 g, 70.00 mmol) were added subsequently. The mixture was heated at 120 °C overnight. After termination of the reaction, the solution was poured into copious amounts of water. After neutralization, the mixture was filtered and washed with water. The organic compounds were reprecipitated in methanol from dichloromethane solution, which was dried over anhydrous MgSO₄ and concentrated. Silica gel column purification with EtOAc: CHCl₃:n-hexane (1:10:50, v/v/v) gave a yellowish green powder (1.62 g, yield = 40.5%). **¹H NMR (500 MHz, CDCl₃):** δ = 9.98 (s, 1H), 7.76 - 7.78 (d, 2H, J = 10.0 Hz), 7.62 - 7.65 (m, 4H), 7.36 - 7.23 (m, 9H), 7.16 (m, 2H), 7.09 - 7.11 (d, 2H, J = 10.0 Hz). **¹³C NMR (125 MHz, CDCl₃):** δ = 191.70, 145.34, 135.47, 130.23, 129.39, 128.78, 128.37, 128.26, 126.91. **HRMS (m/z):** Calcd for C₂₈H₂₀N₂O, 400.1576; found [M+H]⁺, 401.1654.

2-(4-(1,4,5-triphenyl-1H-imidazol-2-yl)benzylidene)malononitrile (TIBM). To a solution of 4-(1,4,5-triphenyl-1H-imidazol-2-yl)benzaldehyde (800 mg, 2.00 mmol) and malononitrile (132 mg, 2.0 mmol) in EtOH (20 mL) was added 2–3 drops of piperidine. The solution was further stirred at rt for 3–5 hours to afford yellow precipitates, which were filtered off, and washed with EtOH. After recrystallization from EtOH, the yellow product was obtained (761.6 mg, yield = 85%). **¹H NMR (500 MHz, CDCl₃):** δ = 7.80-7.79 (d, 1H, J = 8.4 Hz), 7.69 (s, 1H), 7.65 - 7.61 (m, 4H), 7.42 - 7.34 (m, 3H), 7.29 - 7.23 (m, 6H), 7.17 - 7.12 (dd, 4H, J = 6.7 Hz, J = 6.9 Hz). **¹³C NMR (125 MHz, CDCl₃):** δ = 159.26, 137.18, 133.20, 130.55, 129.57, 128.87, 127.49, 114.27, 113.14, 82.57, 77.74, 77.48, 77.23. **HRMS (m/z):** calcd for C₃₁H₂₀N₄, 448.1688; found [M + H]⁺, 449.1772.

2. Computational details

The molecule geometry, HOMO and LUMO energy levels were calculated by MOE (Molecular Operating Environment, 2014.10). Gaussian is the engine. STO-3G* was chosen as the basis set.

3. Solvatochromism

Table S1. Photophysical properties of TIBM in various solvents.

Solvent ^a	Δf^b	TIBM			
		λ_{abs}^c (nm)	λ_{em}^d (nm)	$\Delta\nu^e$ (cm^{-1})	Φ_{F}^f (%)
Cyc	-0.0016	420	494	3567	19
Tol	0.0132	421	515	4335	17
Dio	0.0223	405	538	6104	12
THF	0.2096	412	566	6604	14
DCM	0.2172	420	572	6327	14
DMSO	0.2630	400	581	7789	6.0
BuOH	0.2644	383	592	9218	4.5
MeCN	0.3055	402	616	8642	1.9
CH ₃ OH	0.3087	388	631	9926	3.4

^a Abbreviations: Cyc, Cyclohexane; Tol, Toluene; Dio, Dioxane; Et₂O, ethylether; THF, Tetrahydrofuran; DCM, Dichloromethane; DMSO, Dimethyl sulfoxide; BuOH, n-Butanol; MeCN, Acetonitrile. ^b $\Delta f = (\epsilon - 1) / (2\epsilon + 1) - (n^2 - 1) / (2n^2 + 1)$ accounts for the spectral shifts due to reorientation of the solvent molecules, called the orientation polarizability, where ϵ is solvent dielectric constant and n is index of refraction. ^c λ_{abs} , absorption maximum wavelength. ^d λ_{em} , emission maximum wavelength. ^e $\Delta\nu$, Stokes shift. ^f Φ_{F} , fluorescence quantum yield.

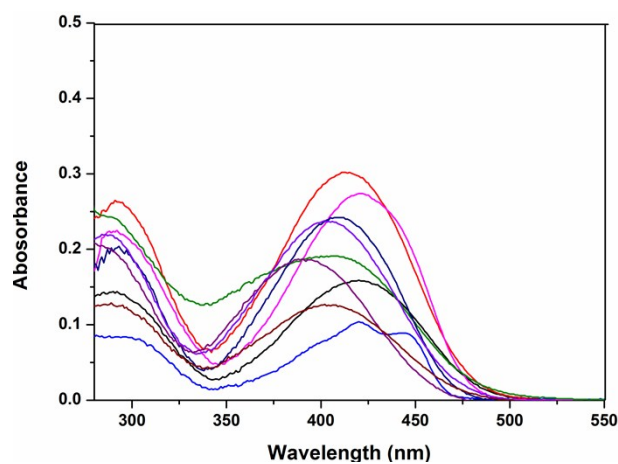


Figure. S1 Absorption spectra of TIBM in different solvents.

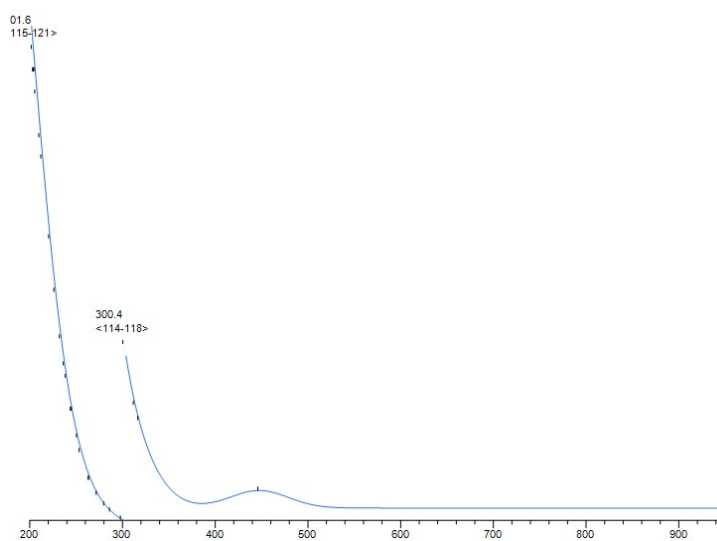


Figure. S2 Computer calculated absorption spectra of **TIBM**.

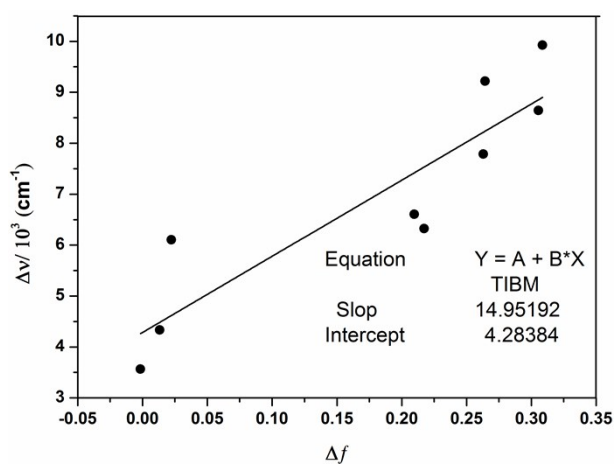


Figure. S3 Plot of Stokes shift (Δf) of **TIBM** versus $\Delta \nu$ in different solvent.

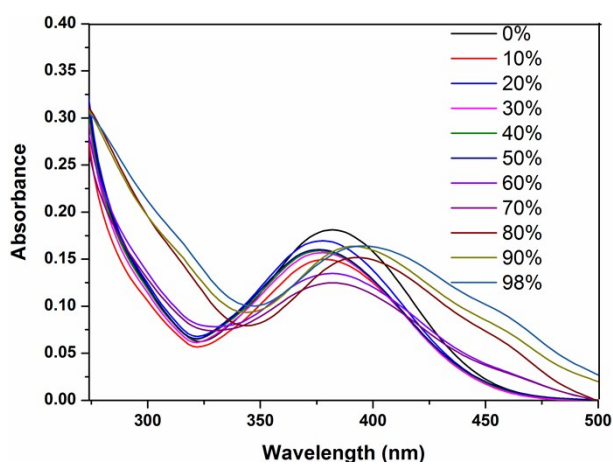


Figure. S4 Absorption spectra of **TIBM** in CH_3CN solution in the presence of increasing amount of water.

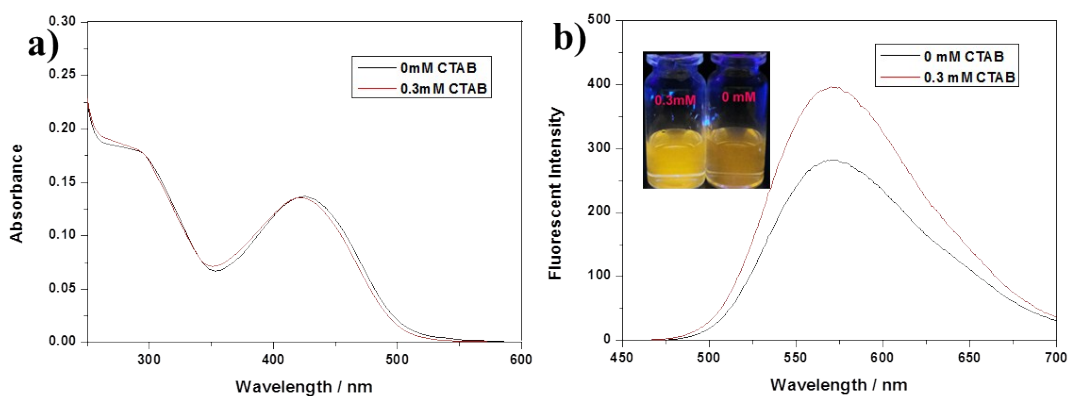


Figure. S5 (a) Absorption spectra of **TIBM** in aqueous solution and in the presence of 0.3 mM CTAB; (b) Fluorescence emission spectra of **TIBM** in aqueous and in the presence of 0.3 mM CTAB, inset: fluorescence images of **TIBM** in aqueous solution and in the presence of 0.3 mM CTAB under portable UV light excitation.

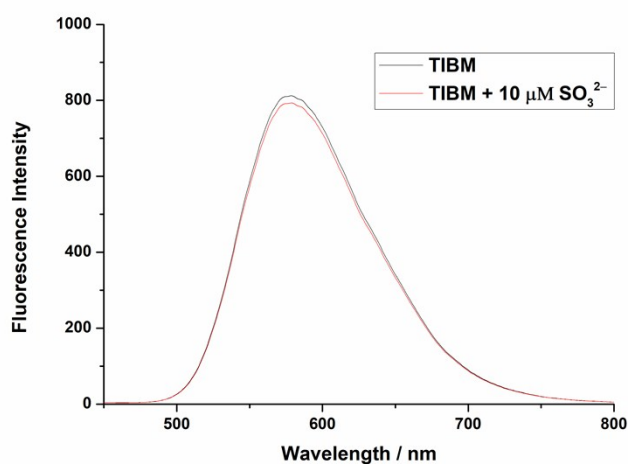


Figure. S6 Fluorescence emission spectra of **TIBM** (10 μM) in the absence and presence of SO_3^{2-} (10 μM).

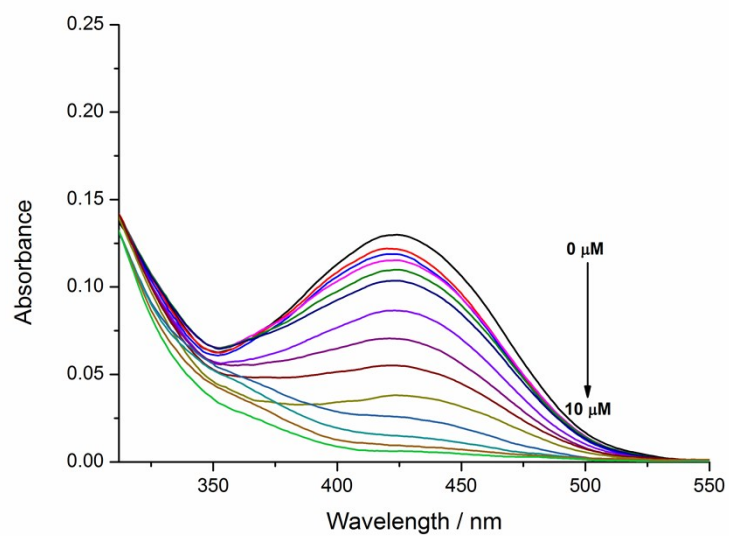


Figure. S7 Absorption spectral changes of CTAB-FONs (10 μM) upon addition of SO_3^{2-} (0 – 10 μM) in buffered at pH 7.4 (PBS, 10 mM).

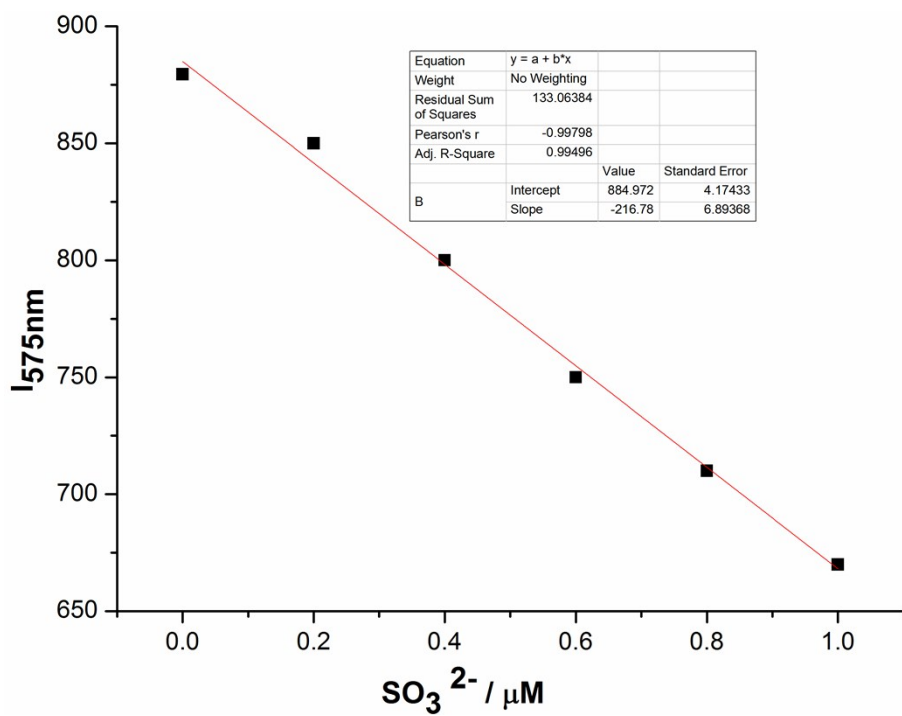


Figure. S8 Plots of emission intensity against the concentration of SO_3^{2-} .

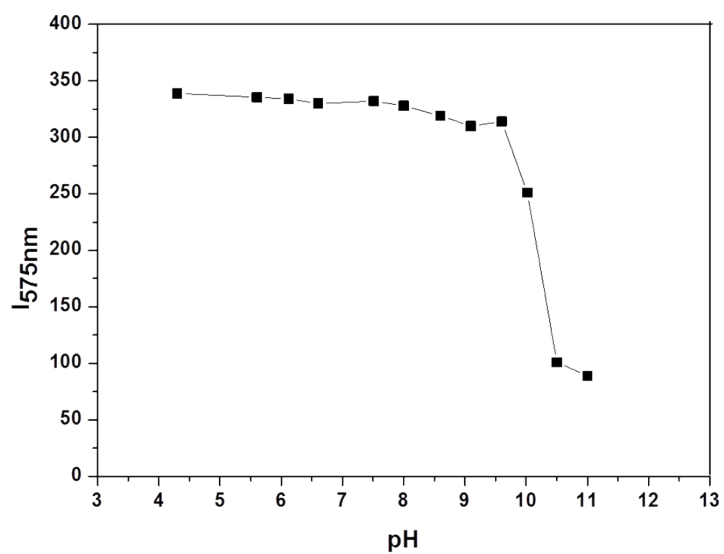
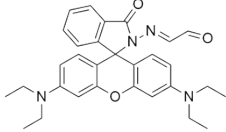
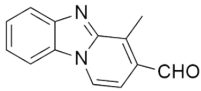
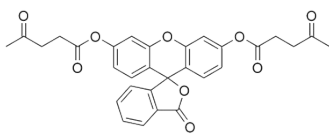
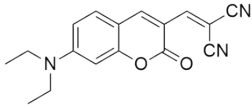
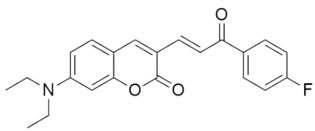
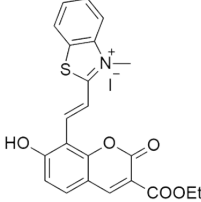
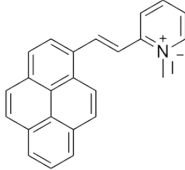
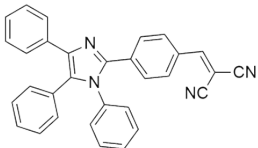


Figure. S9 The fluorescence intensity of CTAB-FONs (10 μM) in the presence of sulfite (10 μM) as a function of pH in PBS (10 mM) solutions.

Table S1. Comparison of fluorescent probes for the detection of SO_3^{2-}

probes	$\lambda_{\text{ex}} / \lambda_{\text{em}}$ (nm)	Detection medium	Detection limit	Response time	Reference
	563/580	water-ethanol (80/20, v/v) buffered at pH 4.8	8.9×10^{-7} M	10 min	18
	350/428	DMSO:PBS = 1:1, v/v, pH = 5	7.6×10^{-8} M	30 s	23
	490/520	1% (v/v) CH ₃ CN) 20 mM HEPES buffer pH = 7.4	10×10^{-6} M	25 min	27
	446/578	20% DMF buffer solution (HEPES, pH = 7.4)	5.8×10^{-5} M	30 s	29
	410/465	20 mM PBS pH =7.4	0.2×10^{-6} M	60 min	30
	415/460	MeOH buffer (Na ₂ HPO ₄ /citric acid, 30 mM, 1:1 v/v)	NA	20 min	34
	340/468	phosphate buffer pH 7.4, 30 mM, containing 30% ethanol	2.76×10^{-6} M	20 min	35
	420/ 575	PBS bu ffer pH 7.4	7.4×10^{-9} M	15 s	This work

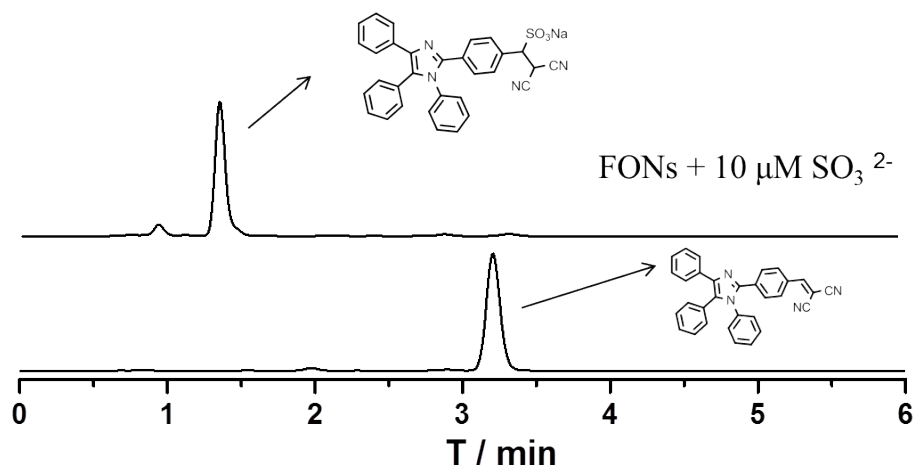


Figure. S10 HPLC chromatograms of CTAB-FONs without sulfite (bottom); CTAB-FONs with sulfite treatment in PBS for 1 min at 25 °C (top).

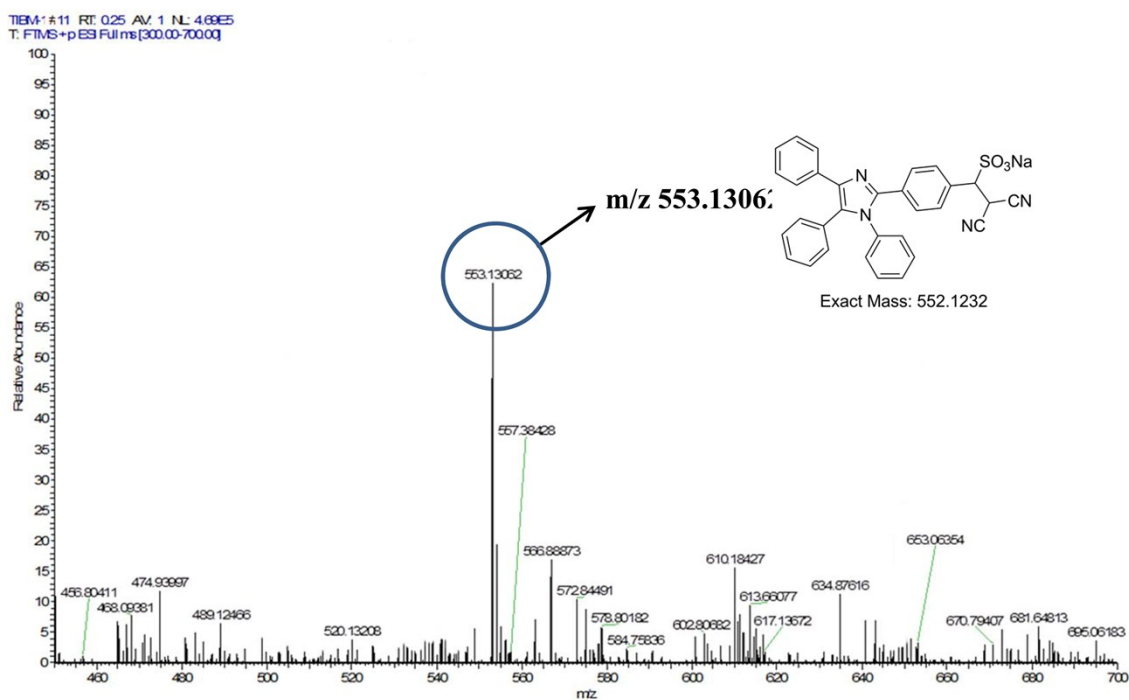


Figure. S11 HRMS of reaction product of CTAB-FONs with SO₃²⁻.

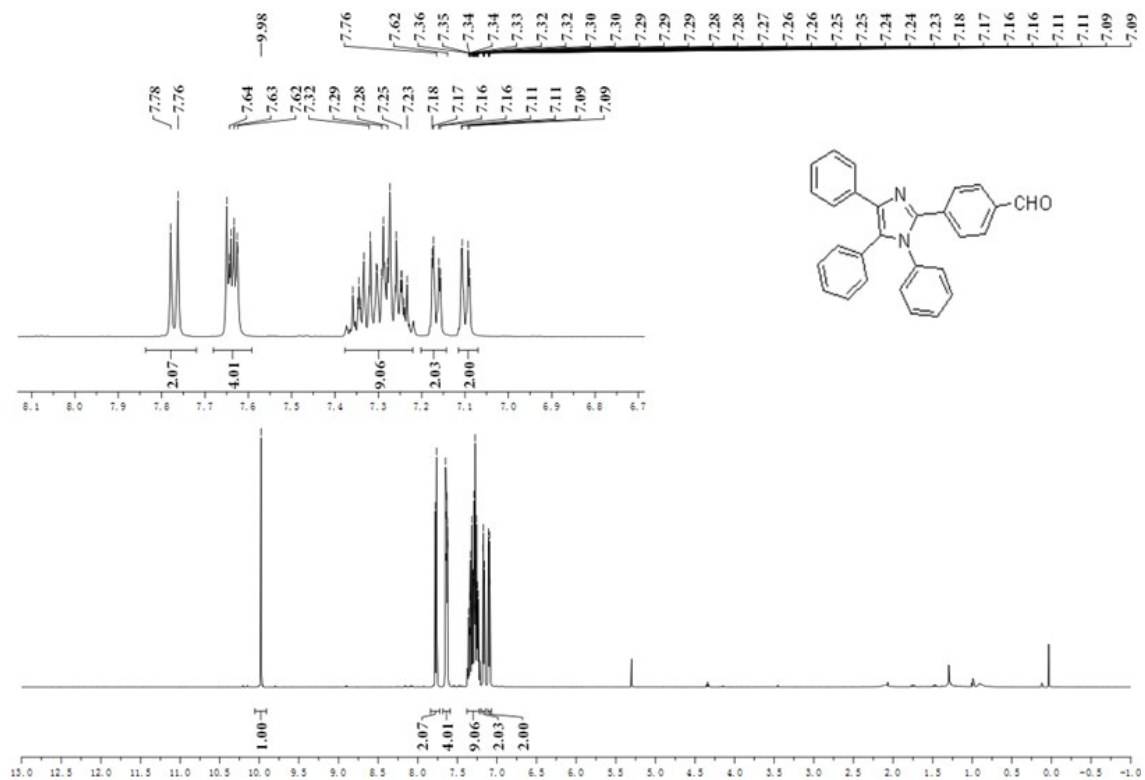


Figure. S12 ¹H NMR (500 MHz, CDCl₃) of TIB

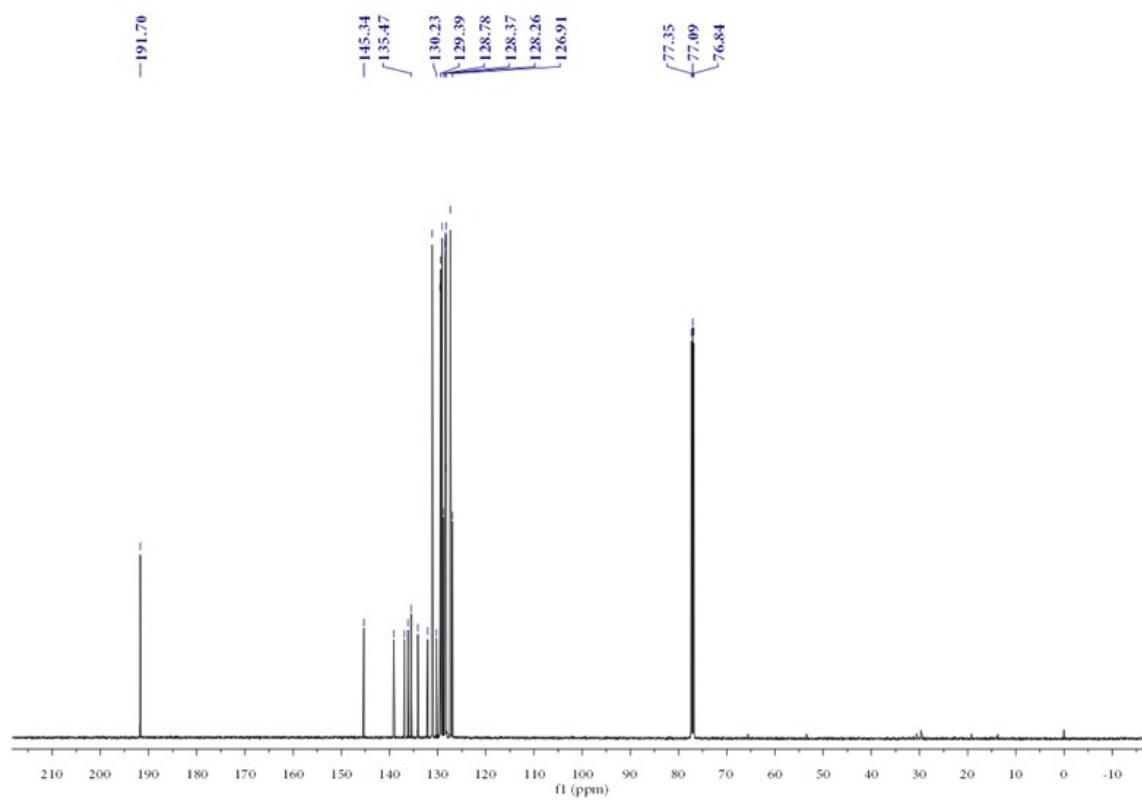


Figure. S13 ¹³C NMR (125 MHz, CDCl₃) of TIB

J-1#37 RF: 027 AV: 1 NL: 206E7
T: FTMS+pESI Full ms [200.00-700.00]

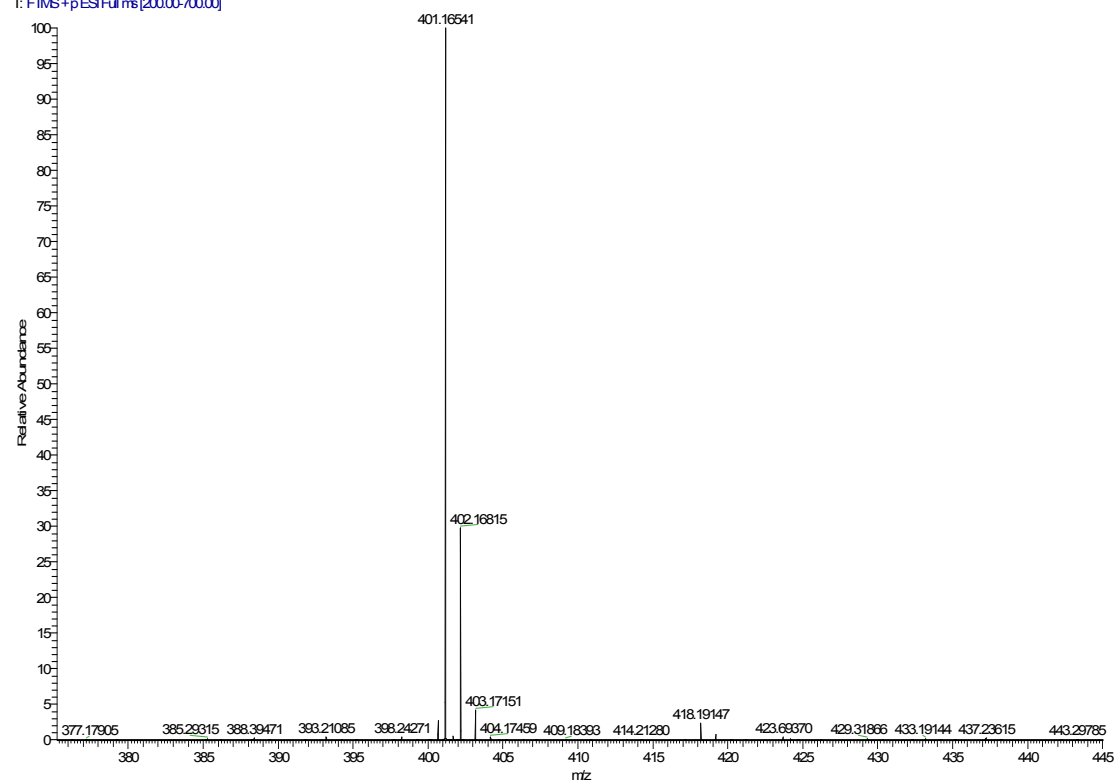


Figure. S14 HRMS spectra of TIB

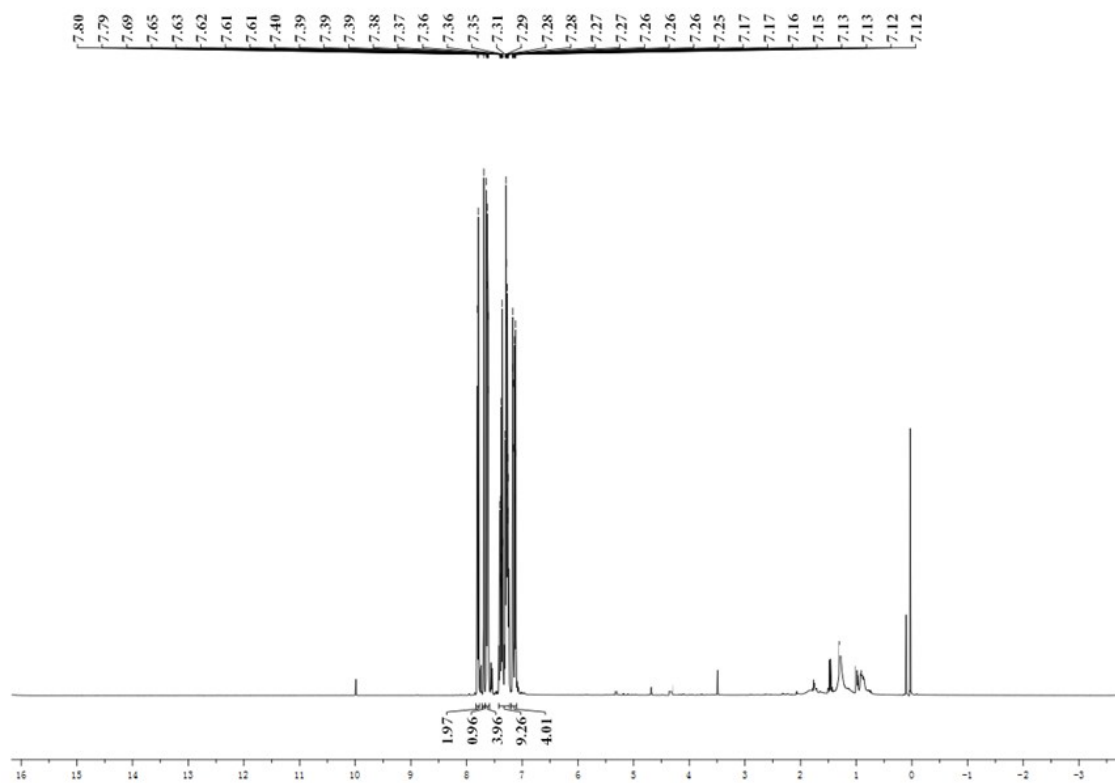


Figure S15. ¹H NMR (500 MHz, CDCl₃) of TIBM

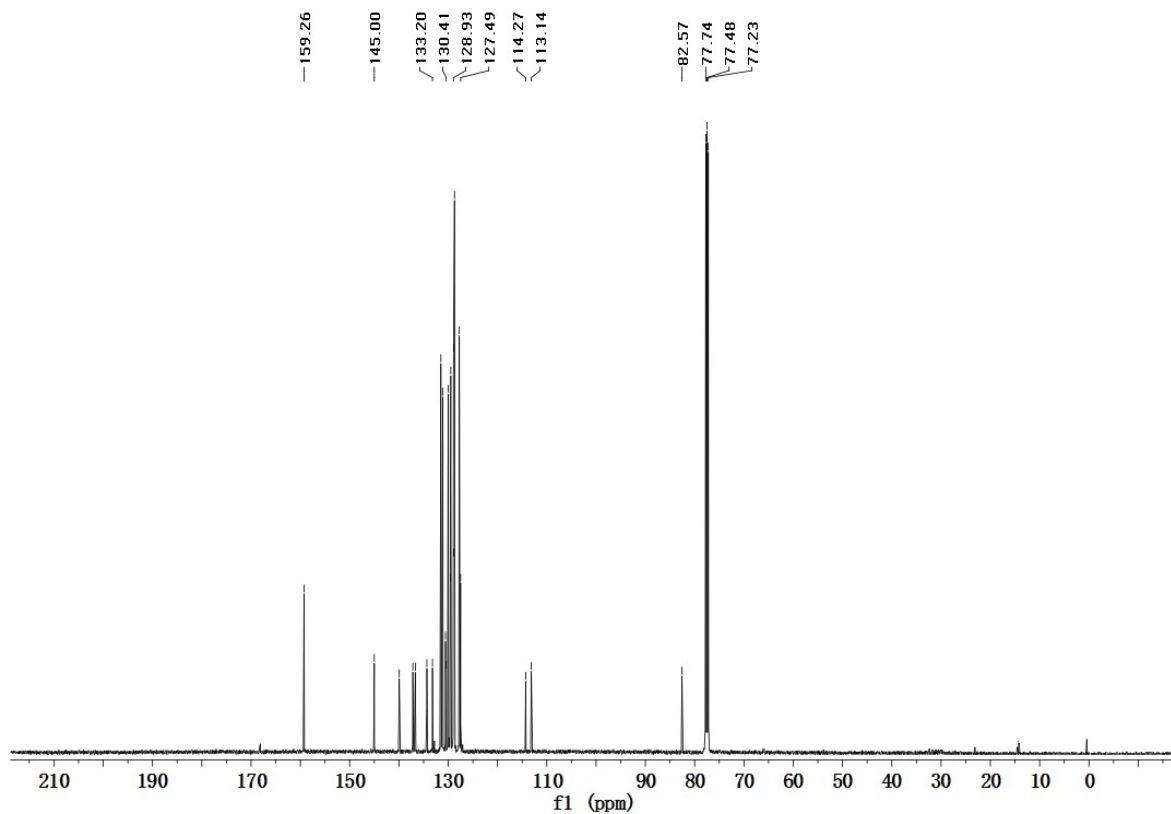


Figure S16. ^{13}C NMR (125 MHz, CDCl_3) of TIBM

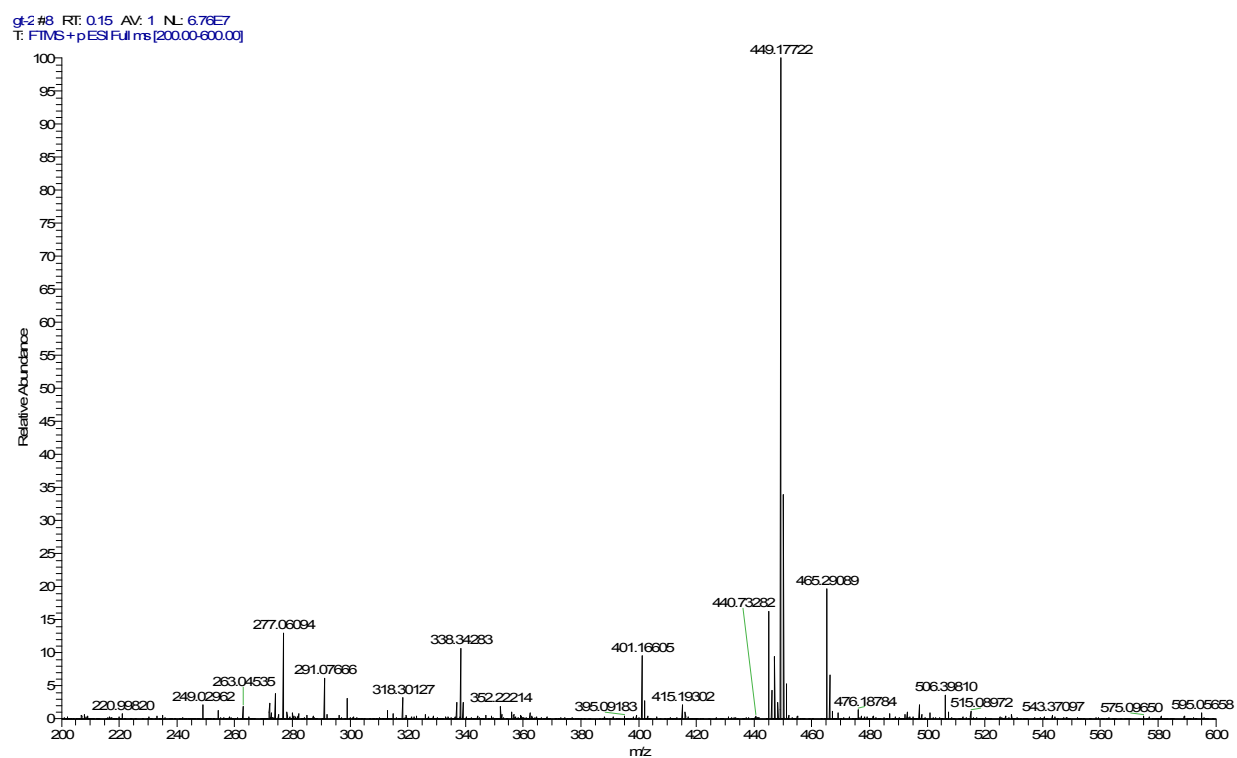


Figure. S17 HRMS spectra of TIBM