Supporting information

A simple and sensitive fluorescent sensor platform for Al^{3+} sensing in aqueous media and monitoring through combined PET and ESIPT mechanisms: Practical applications in drinking water and bio–imaging

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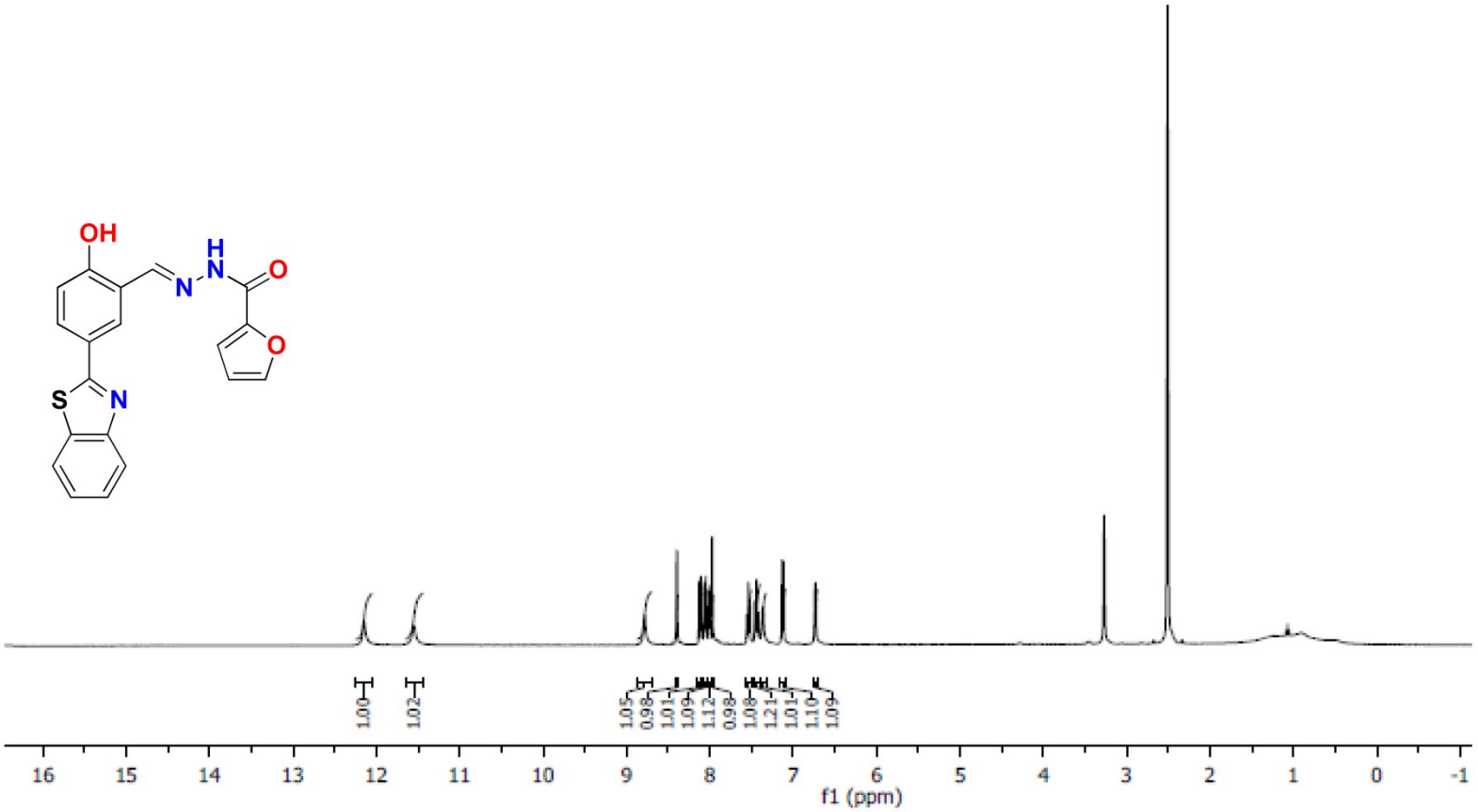

Fig S1. ^{1}H–NMR spectra of the probe **BOTH**

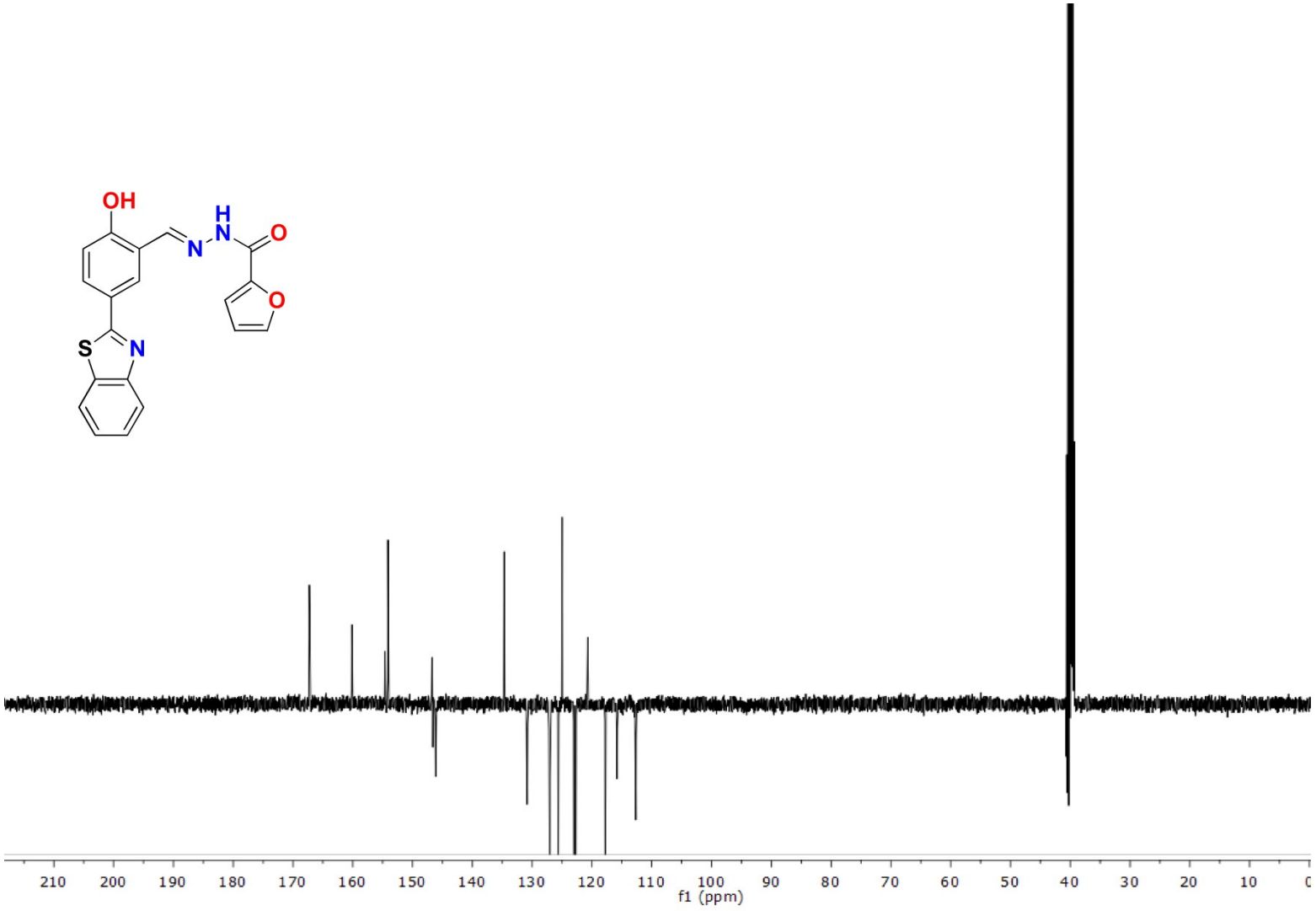

Fig S2. ^{13}C–APT– NMR spectra of the probe **BOTH**

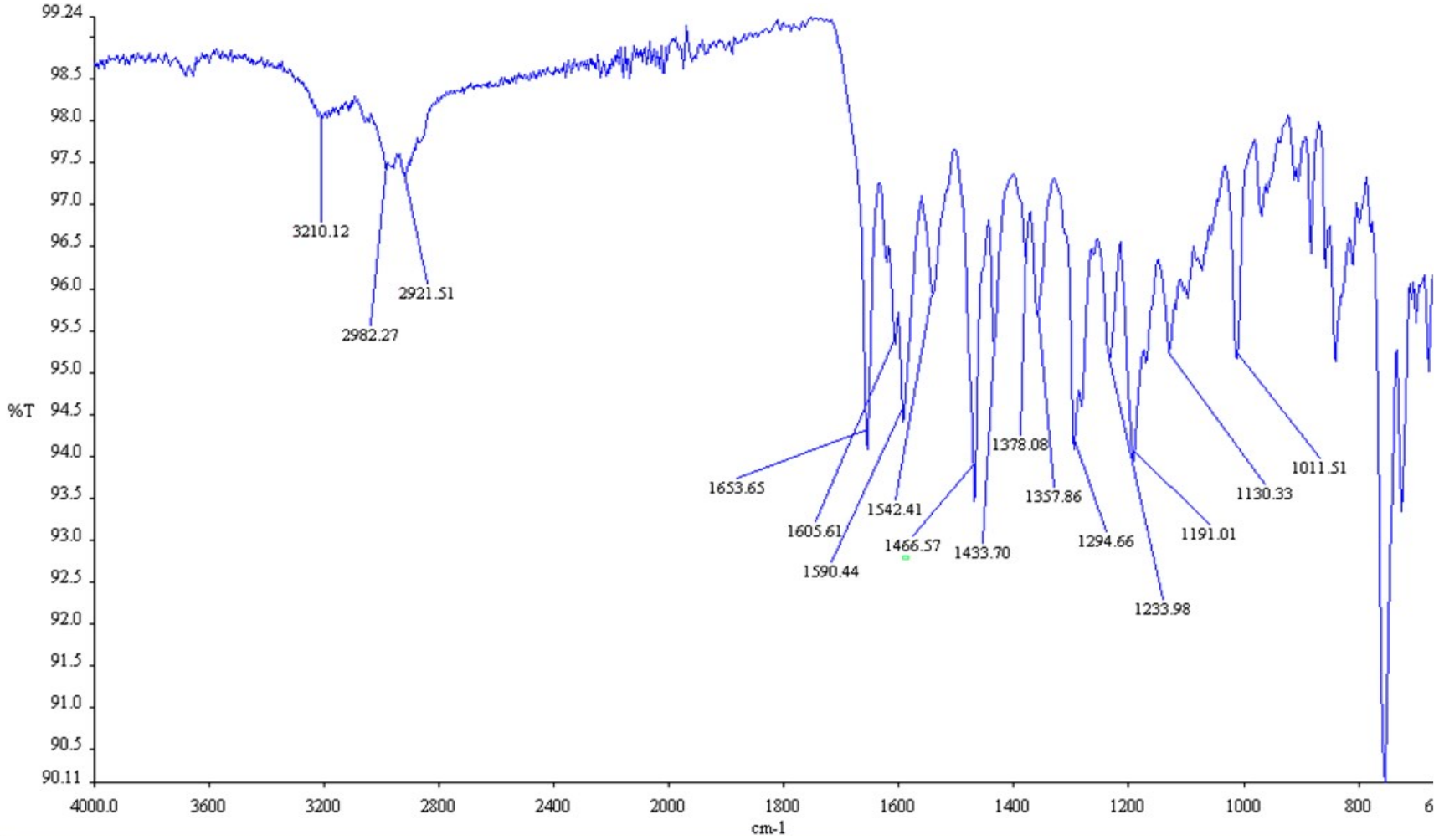

Fig S3. FT–IR spectra of the probe **BOTH**

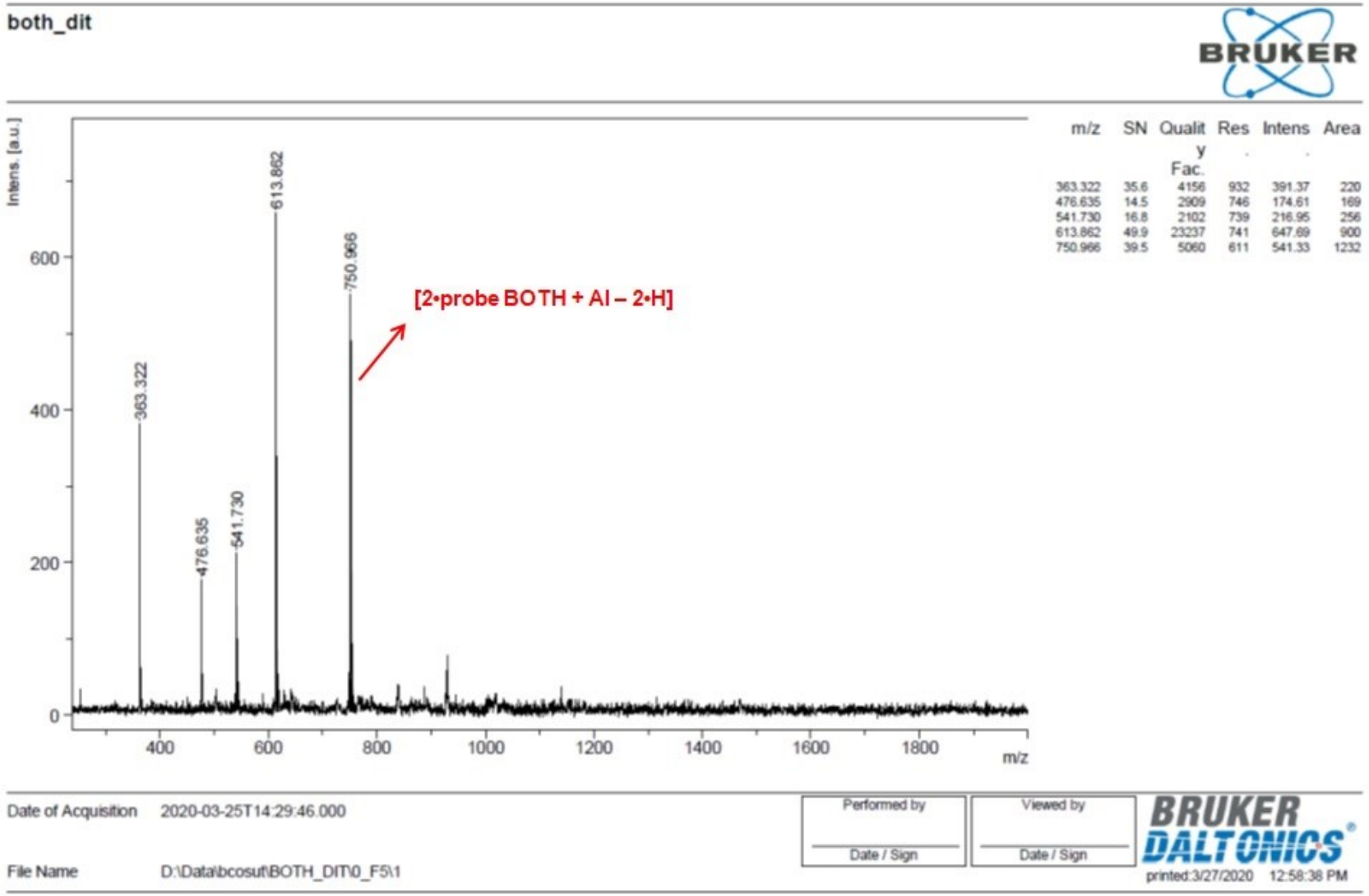

Fig S4. MALDI–TOF MS spectra of the **BOTH –Al^{3+}** complex

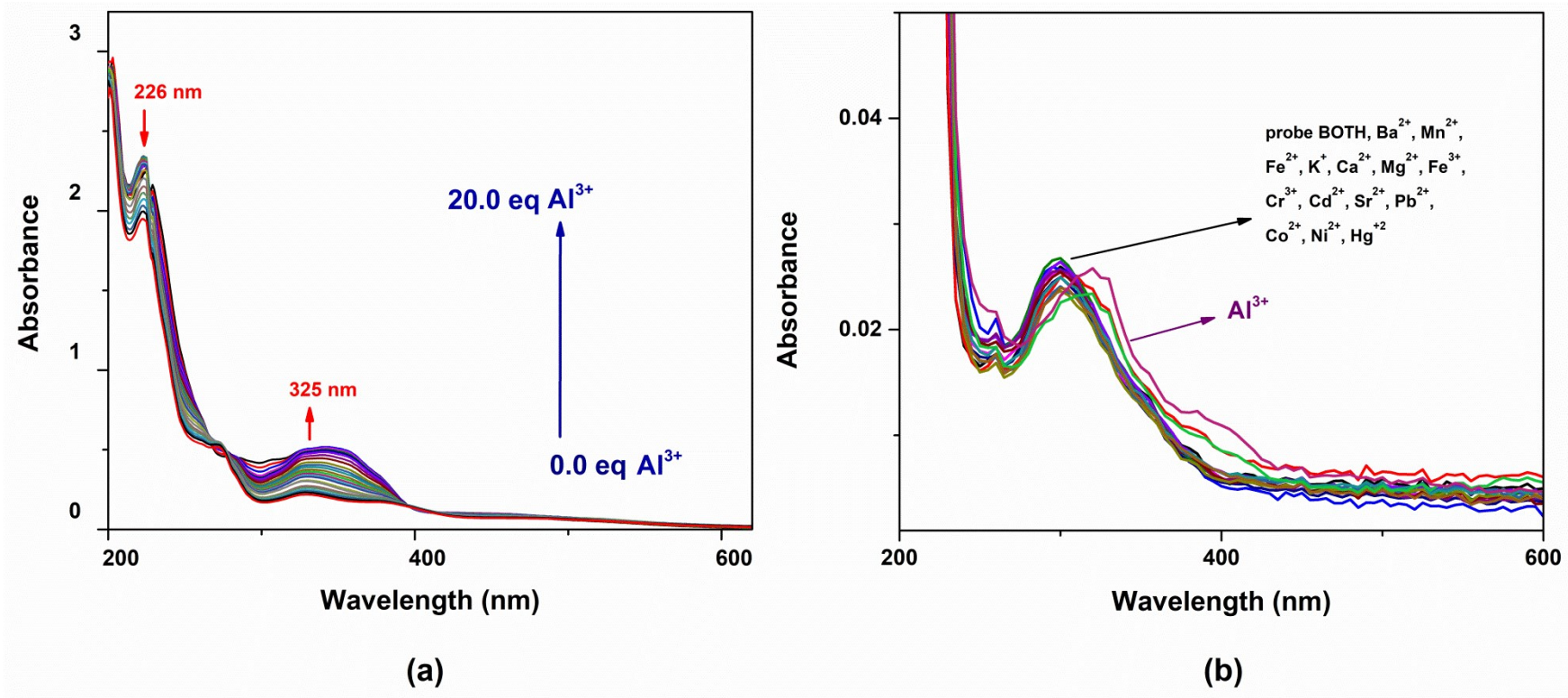

Fig S5. UV–Vis **(a)** titration of the probe **BOTH** toward Al^{3+} and **(b)** spectral behaviors of the probe **BOTH** toward Al^{3+} and other cations

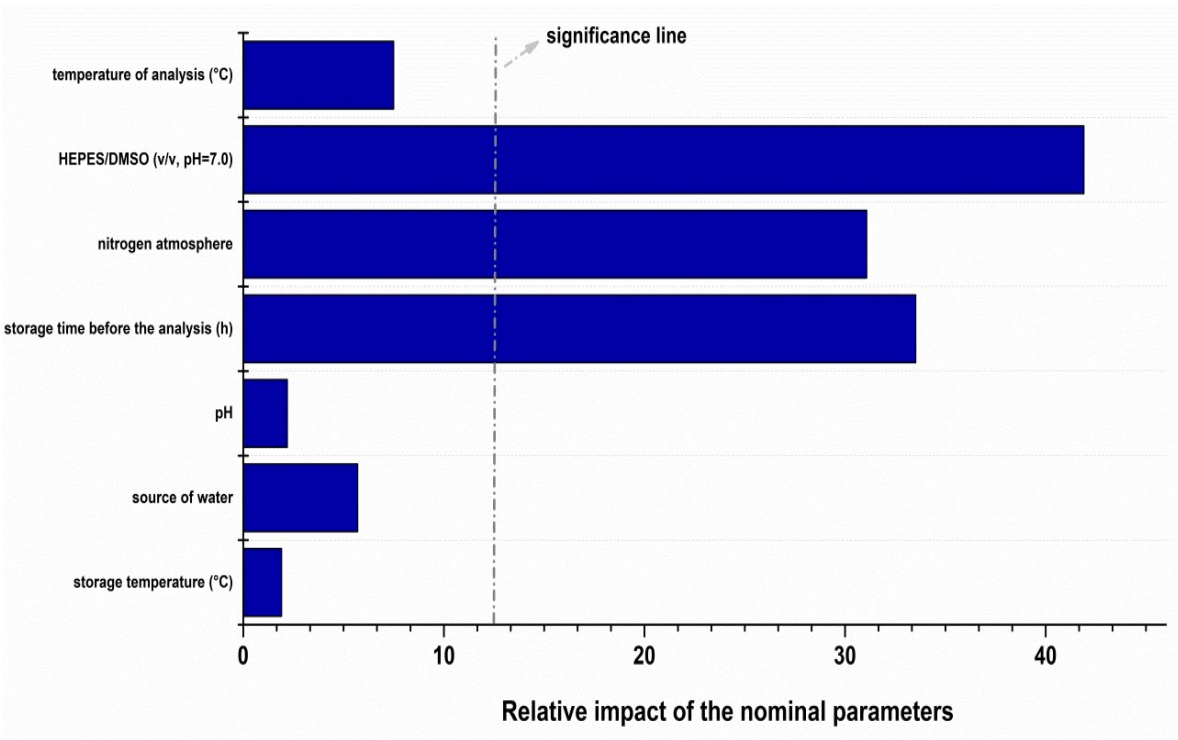

Fig S6. Pareto's graph of our fluorescence method

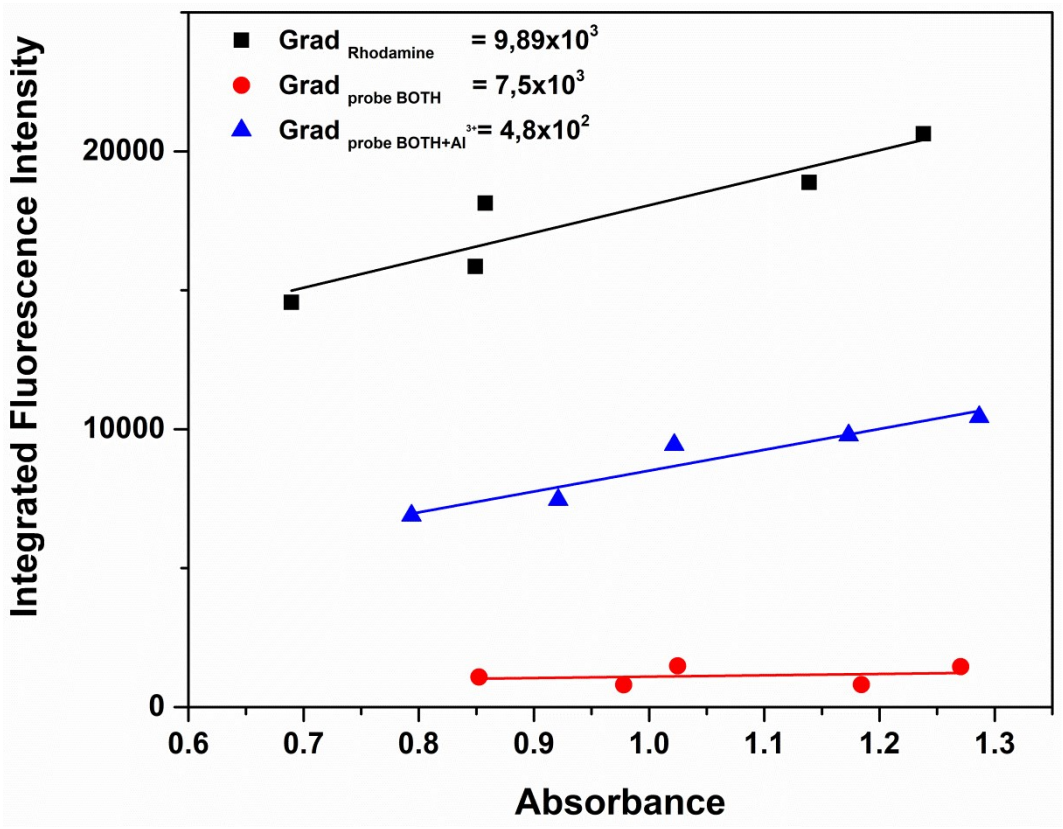

Fig S7. Quantum yields of the probe **BOTH** and probe **BOTH** toward Al^{3+} (λ_{ex}= 327 nm, λ_{em}= 468 nm, probe conc. 5.0 μM).

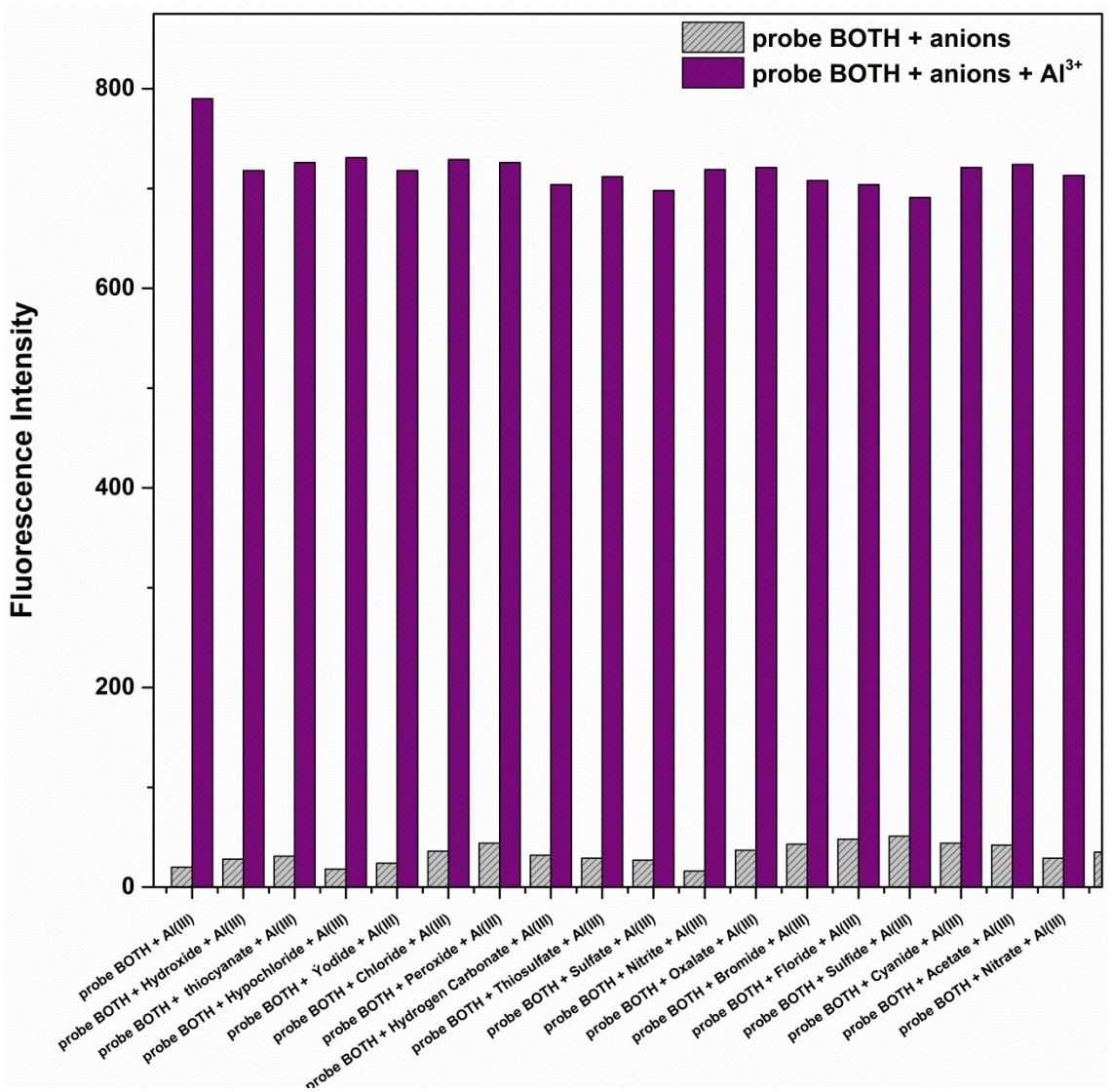

Fig S8. Competitive selectivity of probe **BOTH** toward Al^{3+} (20.0 equiv) in the presence of anions (20.0 equiv) in HEPES/DMSO (v/v, 99.95/0.05, pH=7.0) (λ_{ex}= 327 nm, λ_{em}= 468 nm, probe conc. 5.0 μM).

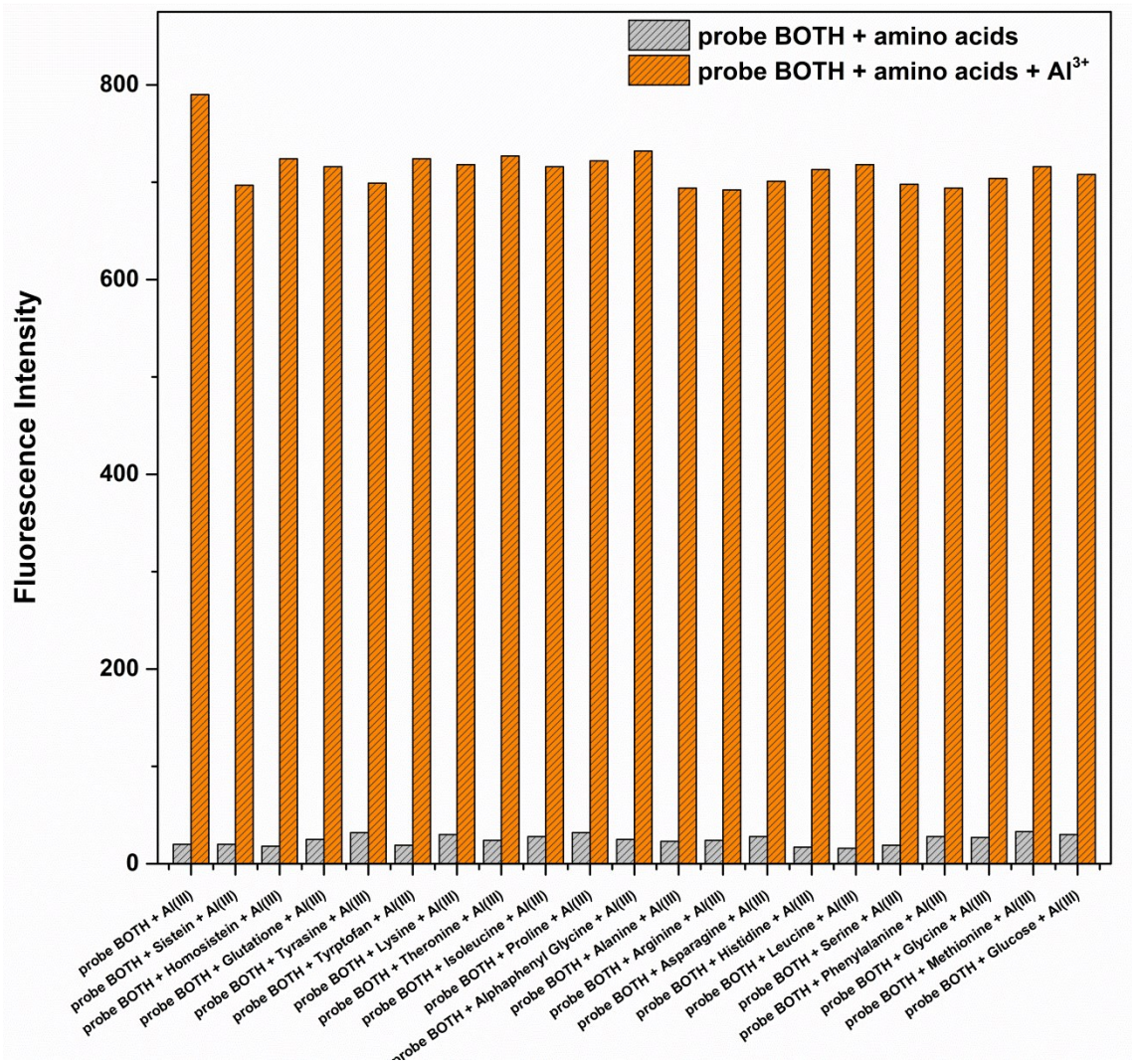

Fig S9. Competitive selectivity of probe **BOTH** toward Al^{3+} (20.0 equiv) in the presence of amino acids (20.0 equiv) in HEPES/DMSO (v/v, 99.95/0.05, pH=7.0) (λ_{ex}= 327 nm, λ_{em}= 468 nm, probe conc. 5.0 μM).

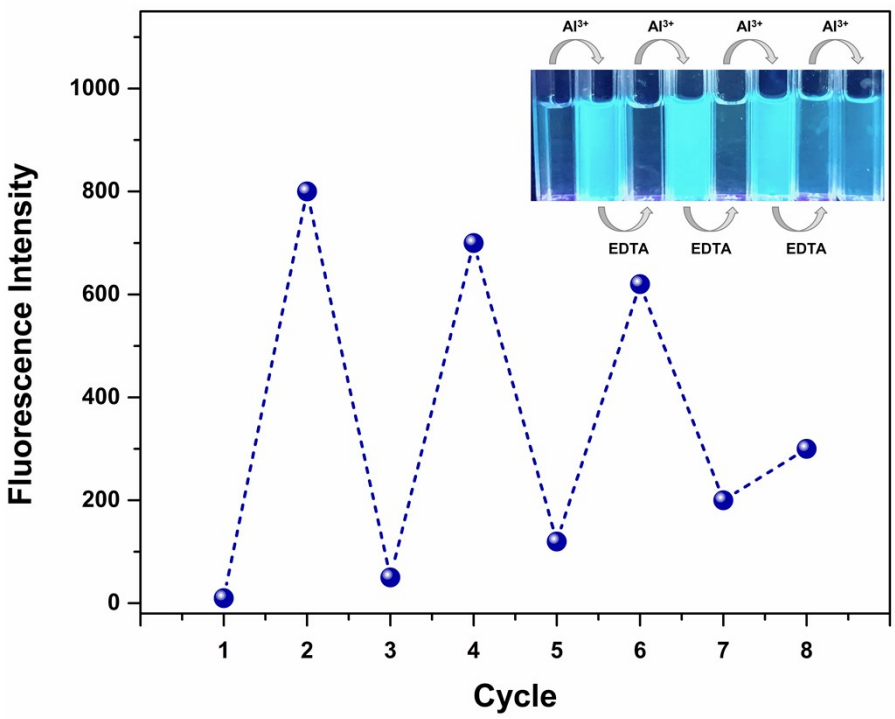

Fig S10 Reversible visual fluorescence changes after sequential addition of Al^{3+} to probe **BOTH** solutions (λ_{ex}= 327 nm, λ_{em}= 468 nm, probe conc. 5.0 μM).

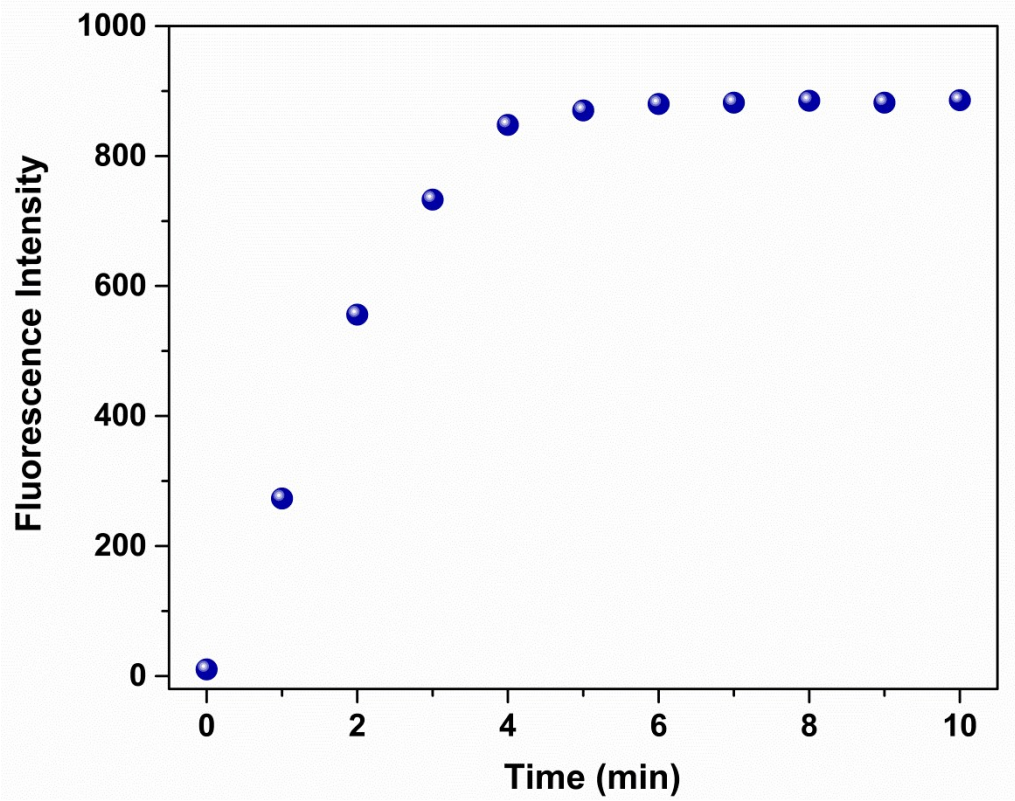

Fig S11. Response time of the **BOTH –Al^{3+}** complex (λ_{ex}= 327 nm, λ_{em}= 468 nm, probe conc. 5.0 μM).

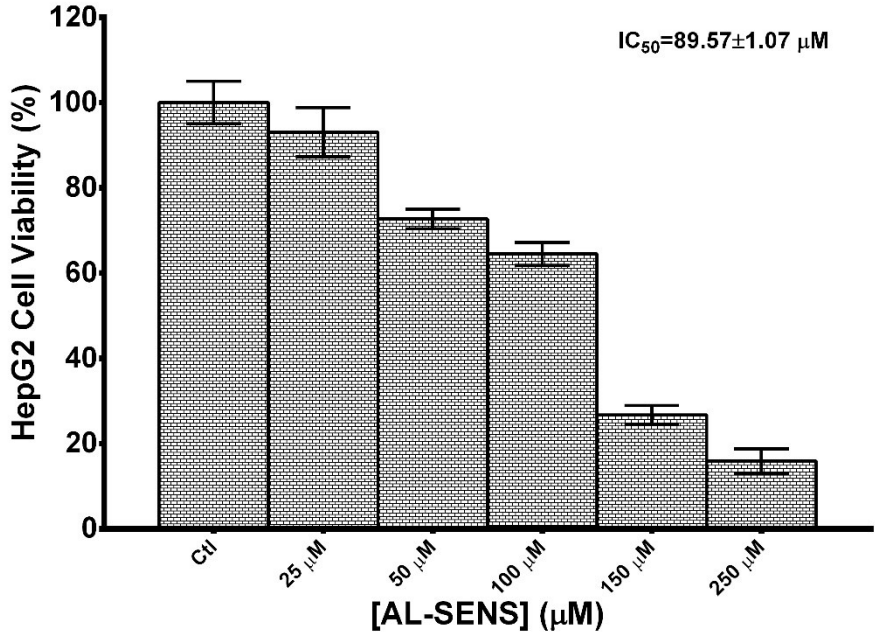

Fig S12. In vitro cytotoxic effects of the probe **BOTH** on HepG2 cells for 24–h incubation. Data presented the mean of at least triplicate measurements and given as mean ± standard deviation

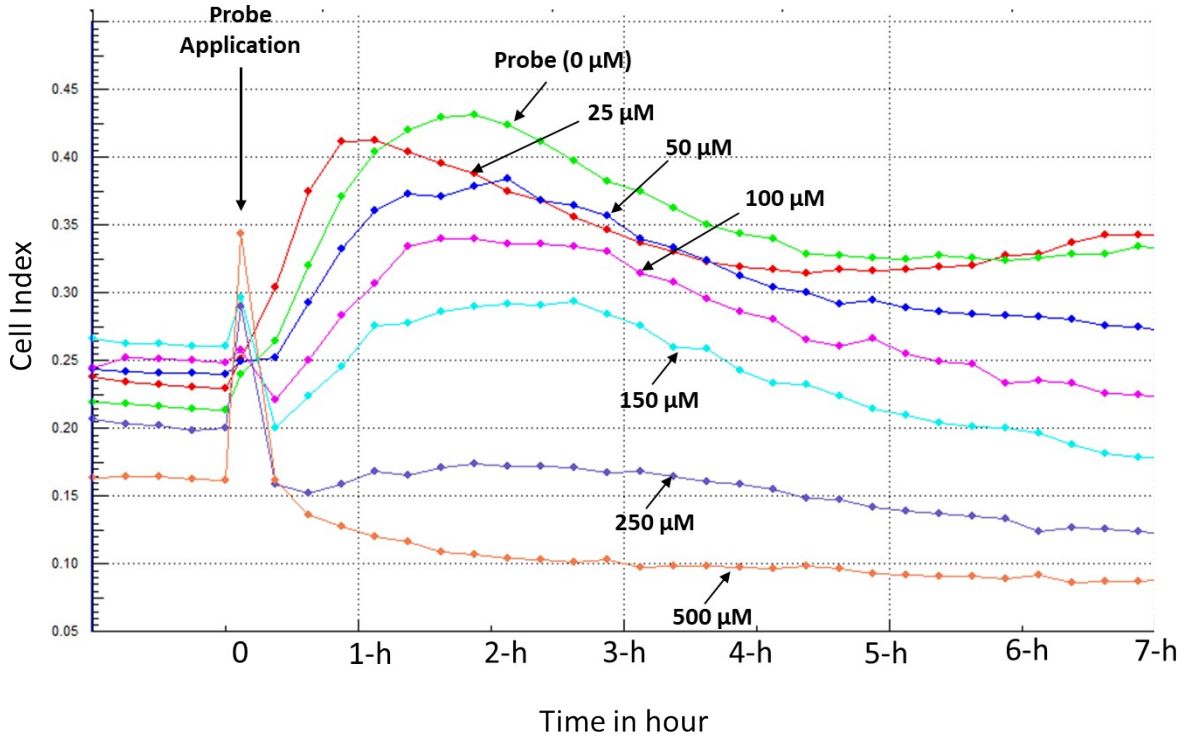

Fig S13. Real–time growth dynamics of the HepG2 cells in the presence of different doses of the synthesized the probe **BOTH**

Table S1. Nominal parameters specified during the assessment of our fluorescence method

slit of excitation	10 nm
slit of emission	10 nm
monitored wavelength	λ_{ex}=327 nm, λ_{em}=468 nm
photomultiplier tube (PMT) voltage	600 Volt
temperature	room temperature
pH	pH=7.0 [HEPES/DMSO (v/v, 99.95/0.05) media]
time of storage after preparation of the samples	20 h at room temperature

Table S2. Parameters employed in the robustness analysis of our fluorescence method

	parameters	situation	
		nominal (+)	**Changed (–)**
1	storage temperature (°C)	rt	4
2	source of water	ultrapure	distilled
3	pH	7.0	5.0
4	storage time before the analysis (h)	16	24
5	nitrogen atmosphere	no	yes
6	HEPES/DMSO (v/v, pH=7.0)	99.95/0.05	99.90/0.10
7	temperature of analysis (°C)	25	15

Table S3. Factorial combinations employed in the Youden test of robustness analysis of our fluorescence method

Parameter \ C_i	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
1	+	+	+	+	–	–	–	–
2	+	+	–	–	+	+	–	–
3	+	–	+	–	+	–	+	–
4	+	+	–	–	–	–	+	+
5	+	–	+	–	–	+	–	+
6	+	–	–	+	+	–	–	+
7	+	–	–	+	–	+	+	–

Table S4. Dixon's test employed to the repeatability of our fluorescence method

concentration levels	average values ($\bar{x}$)			highest value of fluorescence	Q	lowest value of fluorescence	Q
	16h	20h	24h				
1×10^{-4} M	430.08	547.20	556.16	556.16	0.07	430.08	0.93
2×10^{-4} M	583.97	388.69	427.27	583.97	0.80	388.69	0.20

Table S5. Intermediate precision analysis of our fluorescence method confirmed by the HorRat ratio

		analyst (1)	analyst (2)	$F_{[analyst\ (2);analyst\ (1)]}$
16 h 1×10^{-4} M	**Average value ($\bar{x}$)**	583.97	592.44	5.02 $F_{calculated} > F_{critical}$
	SD	2.87	6.53	
	RSD (%)	0.49	1.10	
	RSD Horwitz (%)	5.36	5.36	
	HorRat ratio	**0.09**	**0.21**	
16 h 2×10^{-4} M	**Average value ($\bar{x}$)**	430.08	479.14	0.29 $F_{calculated} < F_{critical}$
	SD	8.41	5.07	
	RSD (%)	1.96	1.06	
	RSD Horwitz (%)	4.17	4.17	
	HorRat ratio	**0.47**	**0.25**	
20 h 1×10^{-4} M	**Average value ($\bar{x}$)**	547.20	580.76	0.31 $F_{calculated} < F_{critical}$
	SD	6.44	3.82	
	RSD (%)	1.18	0.66	
	RSD Horwitz (%)	5.36	5.36	
	HorRat ratio	**0.22**	**0.12**	
20 h 2×10^{-4} M	**Average value ($\bar{x}$)**	388.69	392.80	0.19 $F_{calculated} < F_{critical}$
	SD	14.14	6.24	
	RSD (%)	3.64	1.59	
	RSD Horwitz (%)	4.17	4.17	
	HorRat ratio	**0.87**	**0.38**	
24 h 1×10^{-4} M	**Average value ($\bar{x}$)**	556.16	573.43	0.52 $F_{calculated} < F_{critical}$
	SD	5.57	4.13	
	RSD (%)	1.00	0.72	
	RSD Horwitz (%)	5.36	5.36	
	HorRat ratio	**0.19**	**0.13**	
24 h 2×10^{-4} M	**Average value ($\bar{x}$)**	427.27	420.75	0.31 $F_{calculated} < F_{critical}$
	SD	6.08	3.33	
	RSD (%)	1.42	0.79	
	RSD Horwitz (%)	4.17	4.17	
	HorRat ratio	**0.34**	**0.19**	

† $F_{critical}$=2.98