Electronic Supplementary Information (ESI†)

Naphthalimide–coumarin conjugate: ratiometric fluorescent receptor for self-calibrating quantification of cyanide anion in cells

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Table S1. Calculated excitation energy (*E*), wavelength (λ), and oscillator strength (*f*) for low-laying singlet state (S_n) of **1′** and [**1′**–HCN]$^-$.

compound		Main orbital transition (CIC[a])	*E* (eV) [λ (nm)]	*f*
1'	$S_0 \rightarrow S_1$	HOMO–1→LUMO (0.1274) HOMO→LUMO (0.66351)	2.0822 eV [595.45 nm]	0.2709
	$S_0 \rightarrow S_2$	HOMO–2→LUMO (–0.13519) HOMO–1→LUMO (0.65002) HOMO–1→LUMO+2 (0.11064)	2.4022 eV [516.12 nm]	0.1303
	$S_0 \rightarrow S_3$	**HOMO–2→LUMO (0.63054)** HOMO–1→LUMO (0.11524) HOMO–1→LUMO+2 (–0.118) HOMO→LUMO+1 (–0.1104)	2.9877 eV [414.99 nm]	0.3544
	$S_0 \rightarrow S_4$	HOMO–3→LUMO (–0.14082) HOMO–2→LUMO (0.11213) HOMO→LUMO+1 (0.54394) HOMO→LUMO+2 (0.3419)	3.1157 eV [397.93 nm]	0.1277
	$S_0 \rightarrow S_5$	HOMO–2→LUMO (0.13045) HOMO–2→LUMO+2 (–0.10679) HOMO–1→LUMO+2 (0.59954) HOMO→LUMO+1 (0.12527) HOMO→LUMO+2 (–0.18121)	3.2612 eV [380.17 nm]	0.0172
	$S_0 \rightarrow S_6$	HOMO–3→LUMO (–0.18455) HOMO–2→LUMO+2 (–0.12133) HOMO–1→LUMO+2 (0.16452) HOMO→LUMO+1 (–0.31593) HOMO→LUMO+2 (0.48233)	3.3726 eV [367.62 nm]	0.1713
[**1'**–HCN]$^-$	$S_0 \rightarrow S_1$	HOMO → LUMO (0.70568)	2.2116 eV [560.61 nm]	0.0003
	$S_0 \rightarrow S_2$	**HOMO–1 → LUMO (0.64000)** HOMO–1 → LUMO+3 (–0.11607)	2.8779 eV [430.82 nm]	0.3315
	$S_0 \rightarrow S_3$	HOMO–2 → LUMO (0.68930) HOMO → LUMO+1 (–0.11225)	3.2806 eV [377.93 nm]	0.0067
	$S_0 \rightarrow S_4$	HOMO–2 → LUMO (0.12200) **HOMO → LUMO+1 (0.61102)**	3.4332 eV [361.13 nm]	0.2942
	$S_0 \rightarrow S_5$	HOMO–3 → LUMO (0.70271)	3.6106 eV [343.39 nm]	0.0021
	$S_0 \rightarrow S_6$	HOMO → LUMO+2 (0.70471)	3.7164 eV [333.61 nm]	0.0032

[a] CI expansion coefficients for the main transitions.

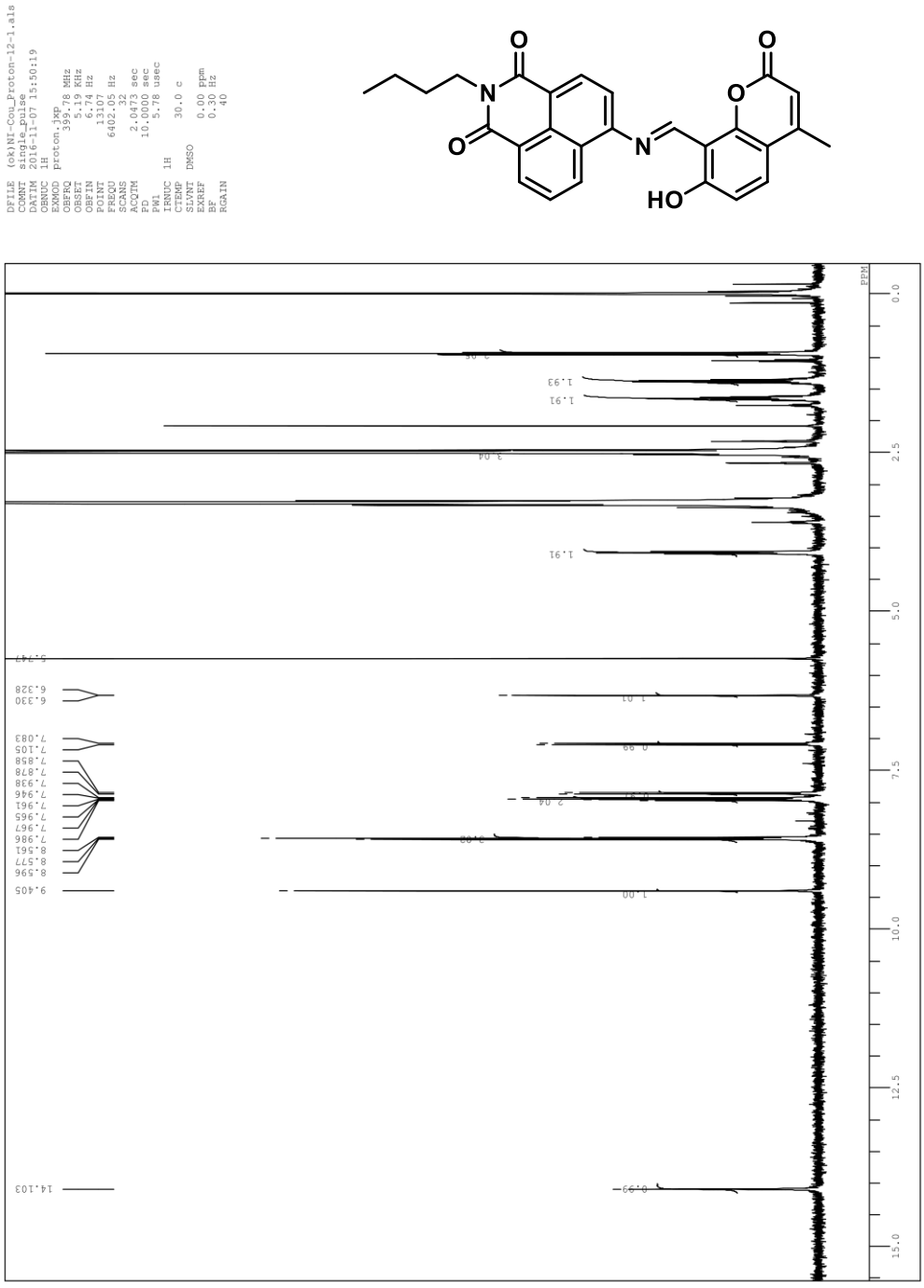


Fig. S1 ^{1}H NMR chart of **1** (10 mM, DMSO–d_6, 400 MHz).

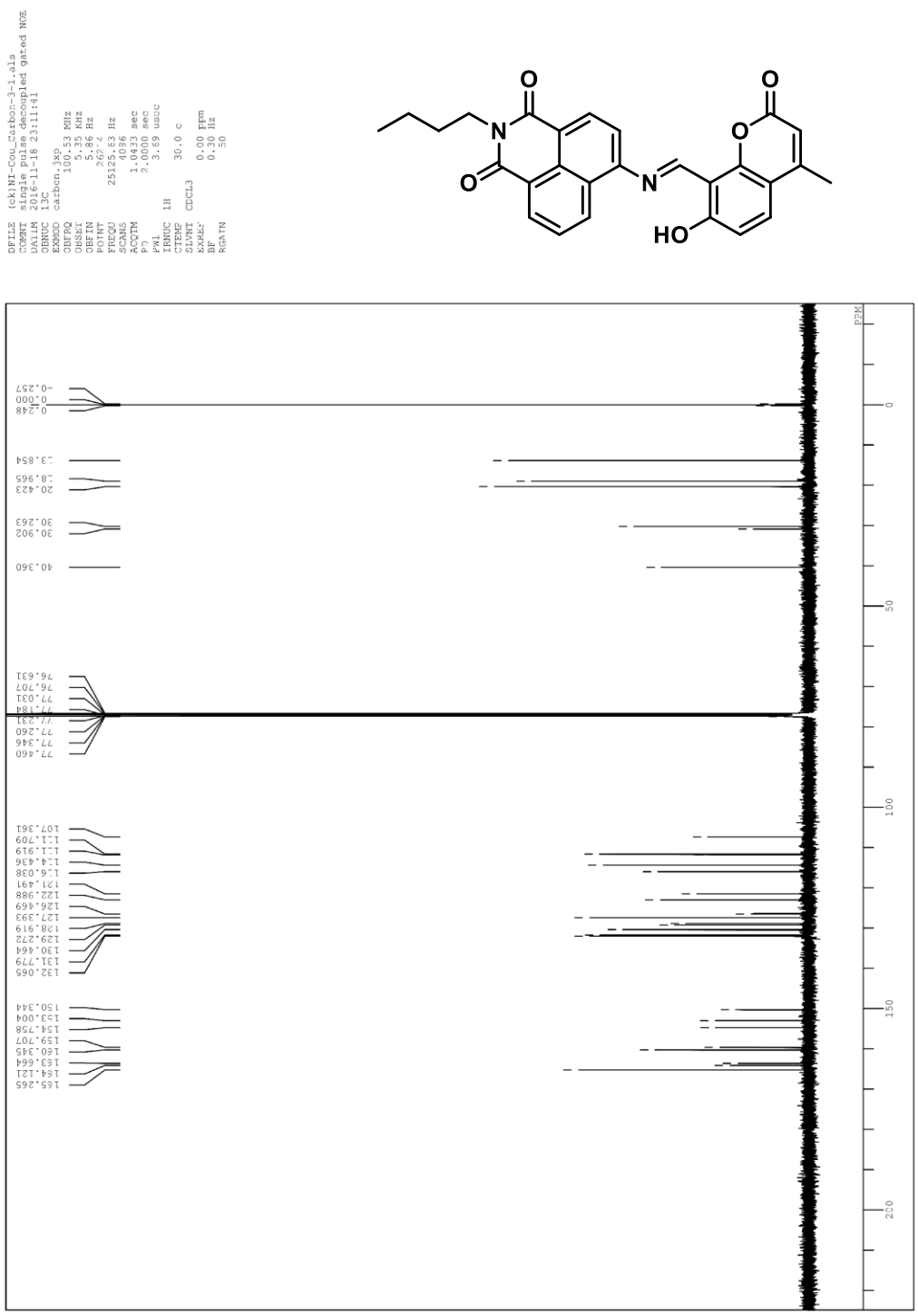


Fig. S2 ^{13}C NMR chart of **1** (30 mM, $CDCl_3$, 100 MHz).

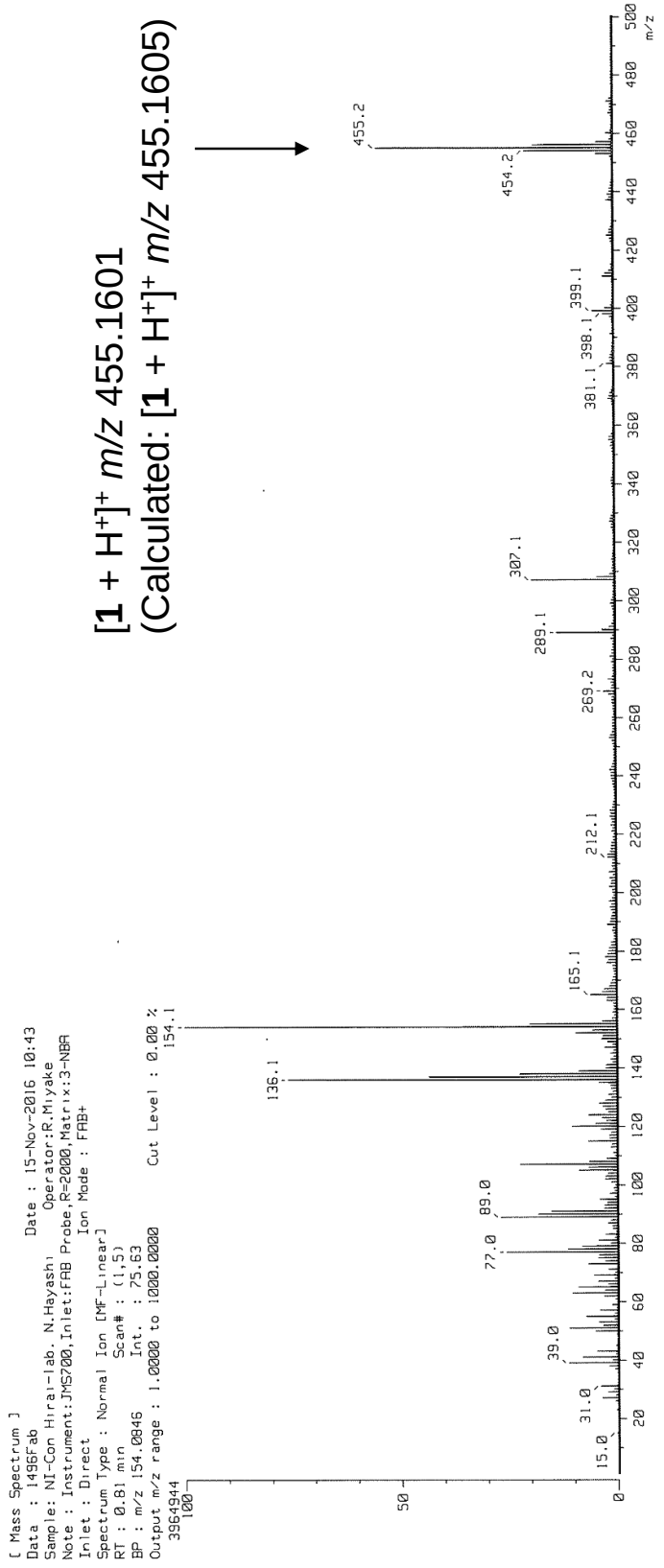


Fig. S3 FAB–MS chart of 1.

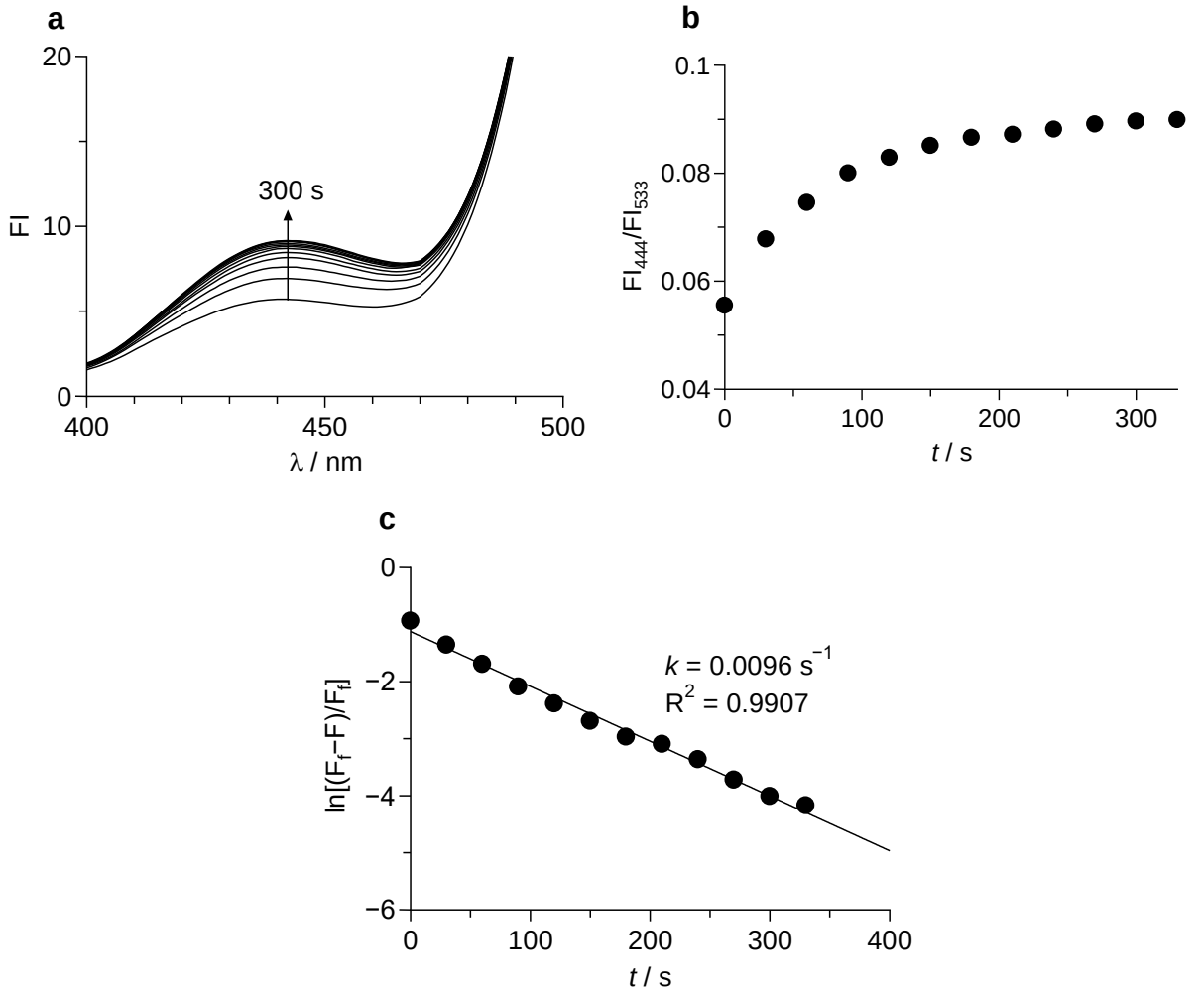


Fig. S4 (a) Time-dependent change in fluorescence spectra of **1** (10 μM), measured with 40 μM of CN^- in a buffered water/MeCN mixture (1/1 v/v; HEPES 0.1 M, pH 7.0) at 25°C. (b) Change in the ratio of fluorescence intensity (FI_{444}/FI_{533}), where FI_{444} is the intensity at λ_{em} = 444 nm and FI_{533} is the intensity at λ_{em} = 533 nm), respectively. (c) Pseudo-first-order kinetic plot of the normalized fluorescence increase of **1** (10 μM) with 40 μM of CN^-.

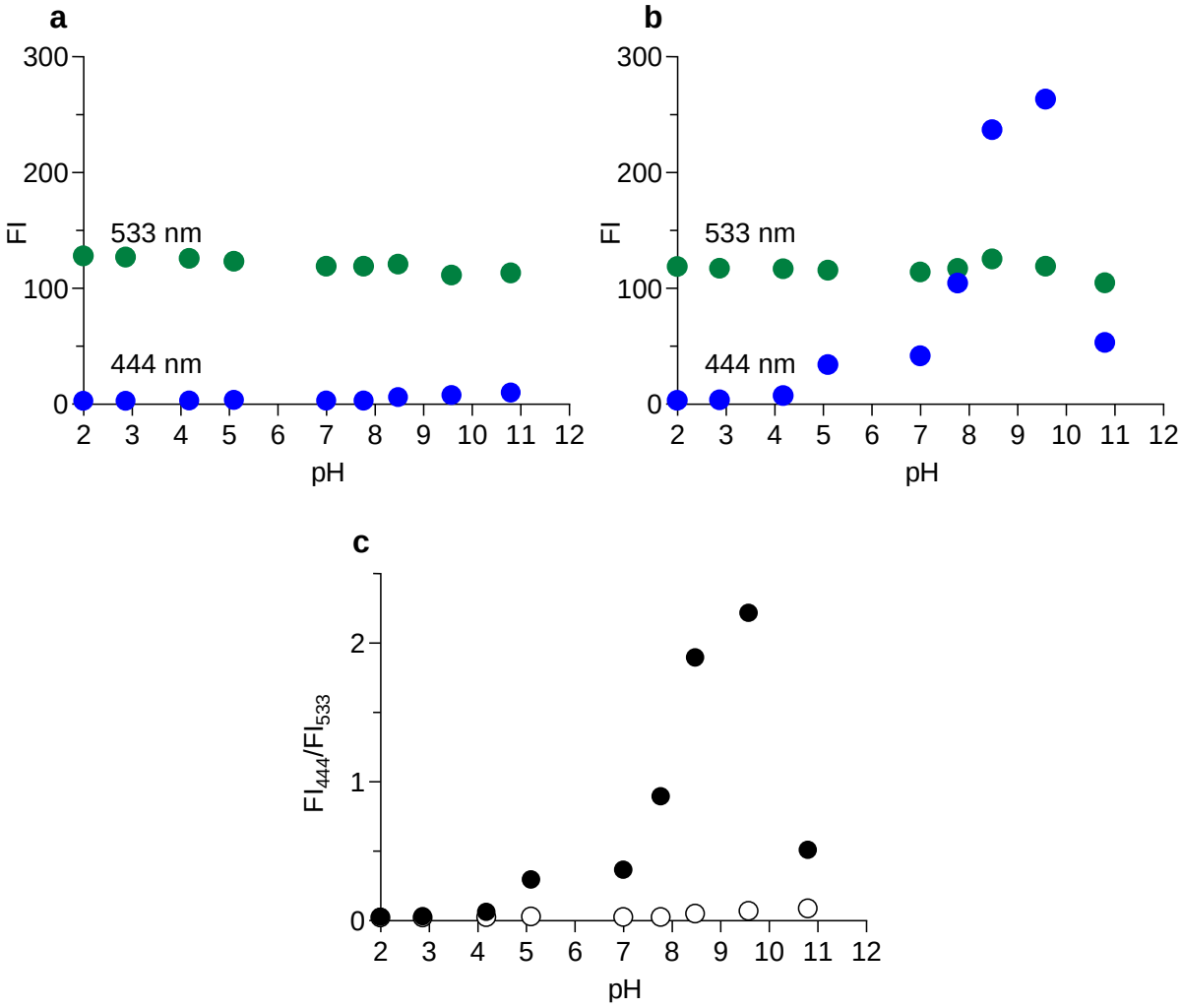


Fig. S5 pH-Dependent change in the fluorescence intensity at 444 nm and 533 nm of **1** (10 μM) measured in water/MeCN mixtures (1/1 v/v) at 25 °C with different pH (a) without and (b) with 200 equiv of CN^-. (c) Change in the ratio of the fluorescence intensity (FI_{444}/FI_{533}) of **1** (10 μM) measured in water/MeCN mixtures (1/1 v/v) with different pH at 25 °C, (white) without and (black) with CN^-.

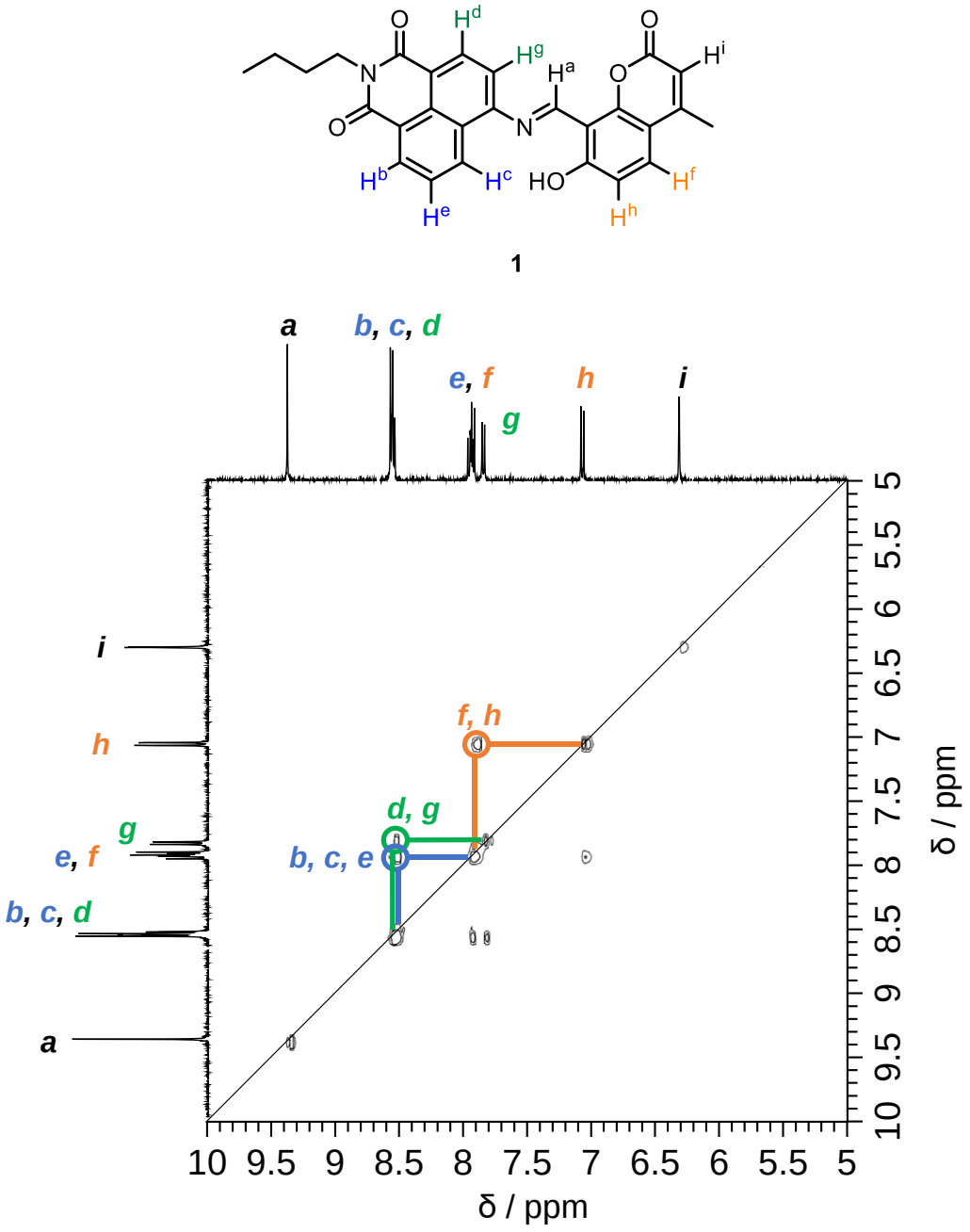


Fig. S6 ^{1}H-^{1}H COSY chart of **1** (30 mM, DMSO–d_6, 400 MHz). Colored circles indicate the observed cross peaks. The texts next to the circle mean the coupling protons.

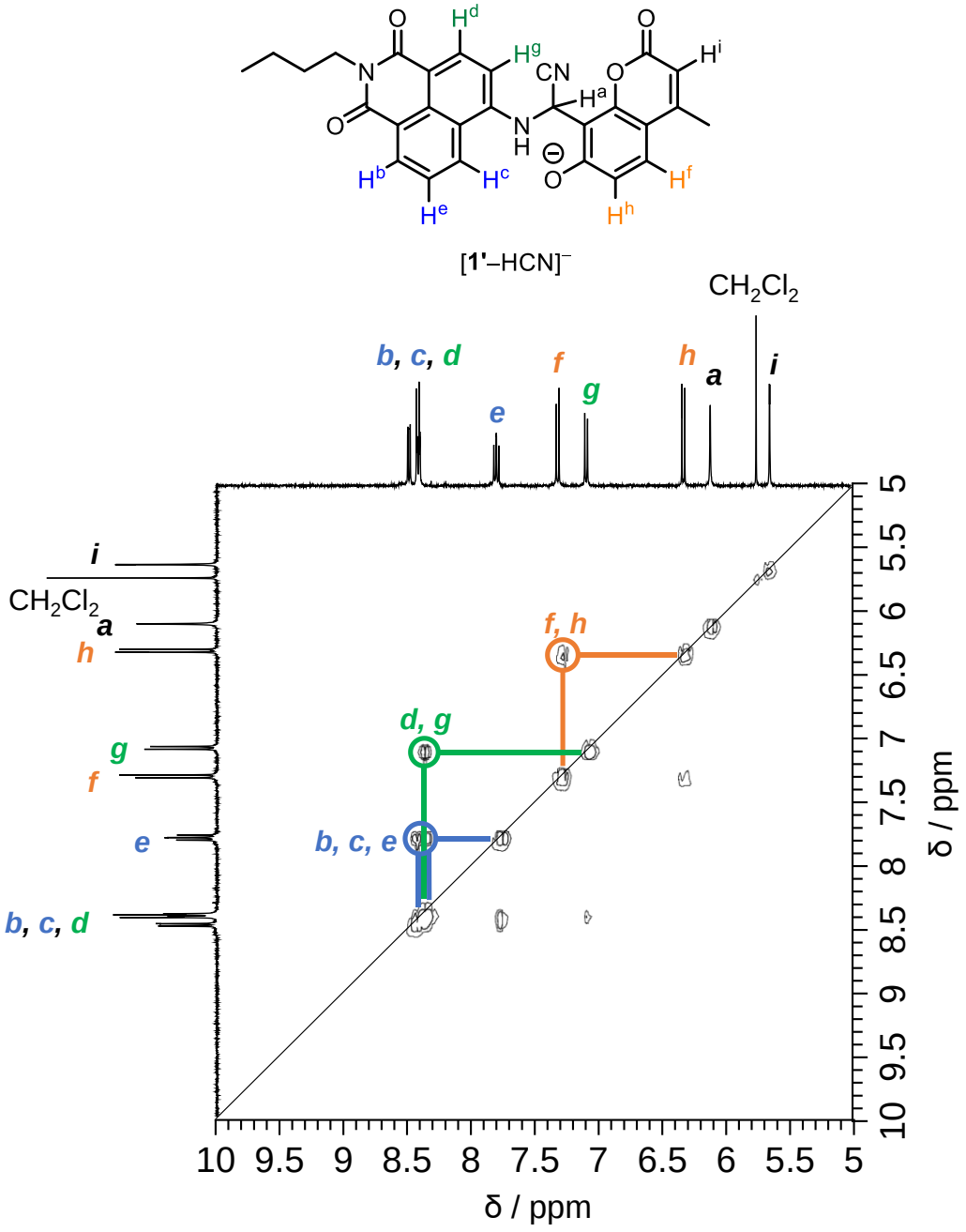


Fig. S7 ^{1}H-^{1}H COSY chart of 1:1 association species for **1** and CN^{-} (30 mM, DMSO–d_6, 400 MHz). Colored circles indicate the observed cross peaks. The texts next to the circle mean the coupling protons.

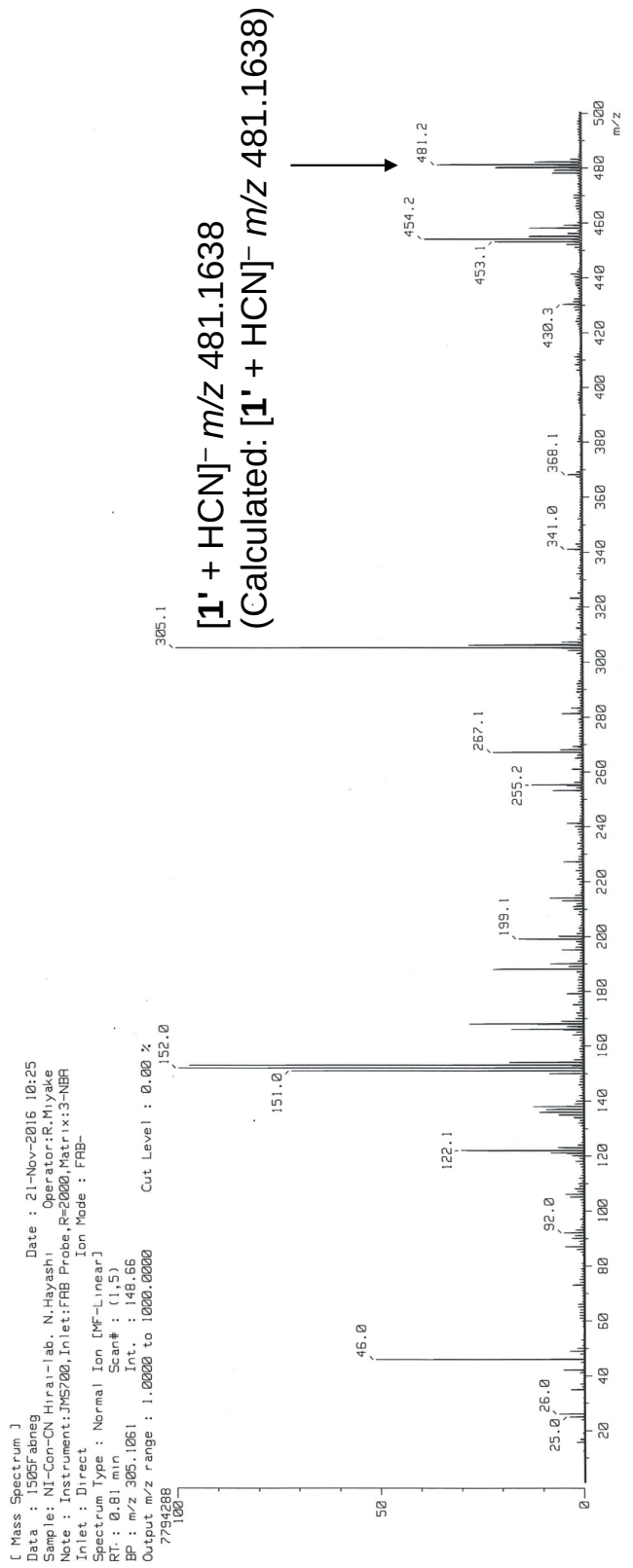


Fig. S8 FAB–MS chart of 1:1 association species for **1** and CN^-.

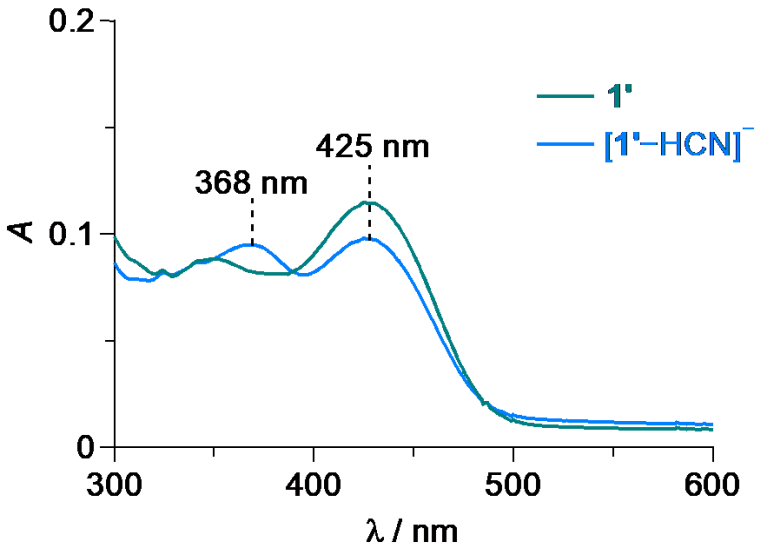


Fig. S9 Change in the absorption spectra of **1** (10 μM) in a buffered water/MeCN mixture (1/1 v/v; HEPES 0.1 M, pH 7.0) at 25°C.

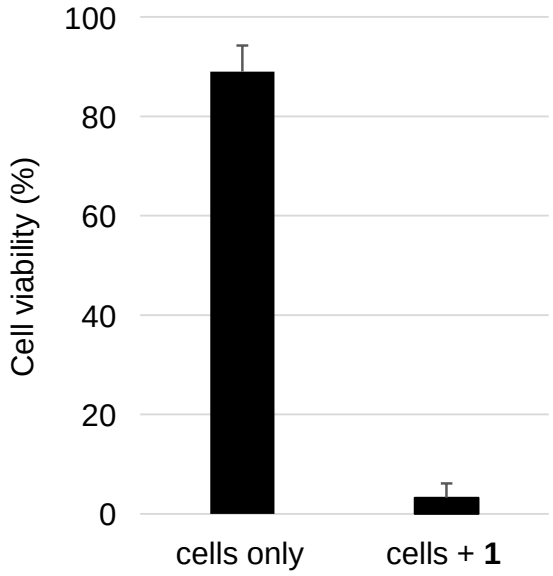
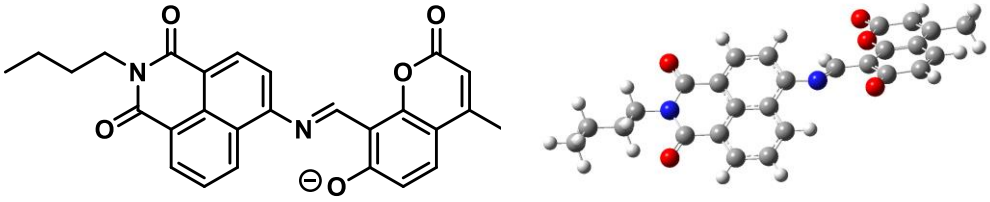


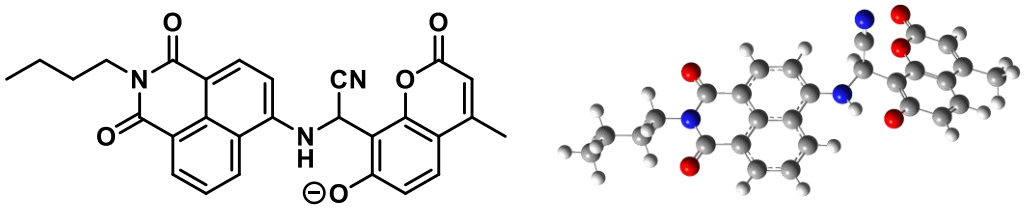
Fig. S10 Viability of HeLa cells determined before and after 20 min incubation with DMF containing **1** (100 μM) at 37°C.

Cartesian Coordinates (in Å) of **1′** (DFT/B3LYP/6–31+G*)



C	3.097334	2.92449	-0.88103	O	-4.62723	-3.85619	-0.5609
C	1.772245	3.3787	-0.99741	O	-3.43792	2.964953	0.540858
C	0.721157	2.601123	-0.53239	H	3.929279	3.52102	-1.24323
C	0.963309	1.341692	0.06247	H	1.571855	4.347546	-1.4478
C	2.309548	0.888234	0.203195	H	-0.30831	2.941663	-0.59522
C	3.369618	1.698579	-0.28455	H	-0.608	-1.31205	1.578261
C	-0.12671	0.51098	0.531723	H	1.742919	-2.06963	1.79237
C	0.193089	-0.70296	1.170293	H	-2.22744	-0.8518	-0.01816
C	1.517496	-1.12466	1.306038	H	-5.97579	3.288196	0.33817
C	2.5817	-0.35764	0.829152	H	-7.61754	1.496872	-0.06512
C	3.958726	-0.82762	0.985532	H	-7.22412	-3.31805	-0.71158
N	4.98023	0.015239	0.485087	H	-8.92758	-0.49415	0.433966
C	4.769491	1.2432	-0.15856	H	-8.77716	-0.25257	-1.30801
O	5.727005	1.89259	-0.58616	H	-9.08087	-1.87892	-0.6656
O	4.264314	-1.89689	1.519495	C	6.369769	-0.44365	0.638025
N	-1.39494	1.001802	0.388567	H	6.992679	0.448724	0.726154
C	-2.40941	0.210392	0.185319	H	6.415018	-1.01688	1.566216
C	-3.78506	0.612631	0.152331	C	6.843402	-1.30602	-0.53907
C	-4.21684	2.01629	0.3249	H	6.754647	-0.72375	-1.4658
C	-5.65807	2.255645	0.218509	H	6.179915	-2.17558	-0.63163
C	-6.55799	1.257958	-0.00461	C	8.292335	-1.77827	-0.36213
C	-6.15235	-0.10798	-0.16271	H	8.95088	-0.90308	-0.25967
C	-4.77186	-0.37902	-0.08078	H	8.37833	-2.34182	0.578608
C	-7.05358	-1.19378	-0.39549	C	8.783828	-2.65145	-1.52345
C	-6.56864	-2.47192	-0.53521	H	8.739244	-2.10536	-2.47472
C	-5.16521	-2.76508	-0.45407	H	9.821561	-2.97504	-1.37185
O	-4.32796	-1.66804	-0.22366	H	8.164691	-3.55161	-1.62914
C	-8.54017	-0.94394	-0.48917				

Cartesian Coordinates (in Å) of [**1′**–HCN]$^{-}$ (DFT/B3LYP/6–31+G*)



C	3.208333	2.962385	-0.46945	O	-2.88919	2.761697	0.332131
C	1.877622	3.404258	-0.52087	H	4.031708	3.622184	-0.72497
C	0.840279	2.542783	-0.19196	H	1.654728	4.424806	-0.81987
C	1.094	1.208945	0.20369	H	-0.17825	2.916087	-0.24501
C	2.450855	0.758399	0.256073	H	-0.38903	-1.72934	1.238273
C	3.495509	1.658612	-0.08677	H	1.95852	-2.44742	1.280952
C	0.02828	0.280532	0.552424	H	-2.31684	-1.11494	0.483674
C	0.381245	-1.02604	0.943233	H	-5.1703	3.464648	-0.68409
C	1.712684	-1.43409	0.977378	H	-6.9812	1.919768	-1.33534
C	2.757831	-0.57356	0.642116	H	-7.40572	-2.94597	-1.16691
C	4.140175	-1.04239	0.695939	H	-8.77565	0.263585	-1.0282
N	5.145974	-0.10123	0.362076	H	-8.07767	0.139346	-2.64485
C	4.907242	1.21546	-0.0427	H	-8.87516	-1.26959	-1.91682
O	5.846107	1.956792	-0.34313	H	-1.5189	1.691219	0.383982
O	4.467222	-2.18904	1.010999	C	-2.56786	-0.16547	2.325124
N	-1.25715	0.694915	0.465742	N	-2.67195	-0.23624	3.48062
C	-2.42784	-0.08533	0.83878	C	6.545761	-0.55053	0.427047
C	-3.68263	0.507245	0.205779	H	6.598427	-1.30861	1.210652
C	-3.81492	1.936587	0.022947	H	7.146299	0.315799	0.712642
C	-5.06108	2.391696	-0.55058	C	7.046972	-1.12789	-0.90277
C	-6.06183	1.523686	-0.91023	H	6.410147	-1.97684	-1.18415
C	-5.92959	0.117825	-0.74676	H	6.942845	-0.36455	-1.685
C	-4.71421	-0.34311	-0.17997	C	8.50941	-1.58438	-0.81908
C	-6.92132	-0.84953	-1.1052	H	9.139157	-0.73384	-0.51891
C	-6.6778	-2.18602	-0.90379	H	8.61013	-2.33889	-0.0251
C	-5.44607	-2.66722	-0.33623	C	9.03303	-2.16291	-2.13941
O	-4.5118	-1.68882	0.017777	H	10.07972	-2.48098	-2.05151
C	-8.23408	-0.40802	-1.70646	H	8.443457	-3.03528	-2.44975
O	-5.13239	-3.82868	-0.13186	H	8.975768	-1.42051	-2.94605