Supporting Information

Fluorene Based Chemodosimeter for "Turn-on" Sensing of Cyanide by Hampering ESIPT and Live Cell Imaging

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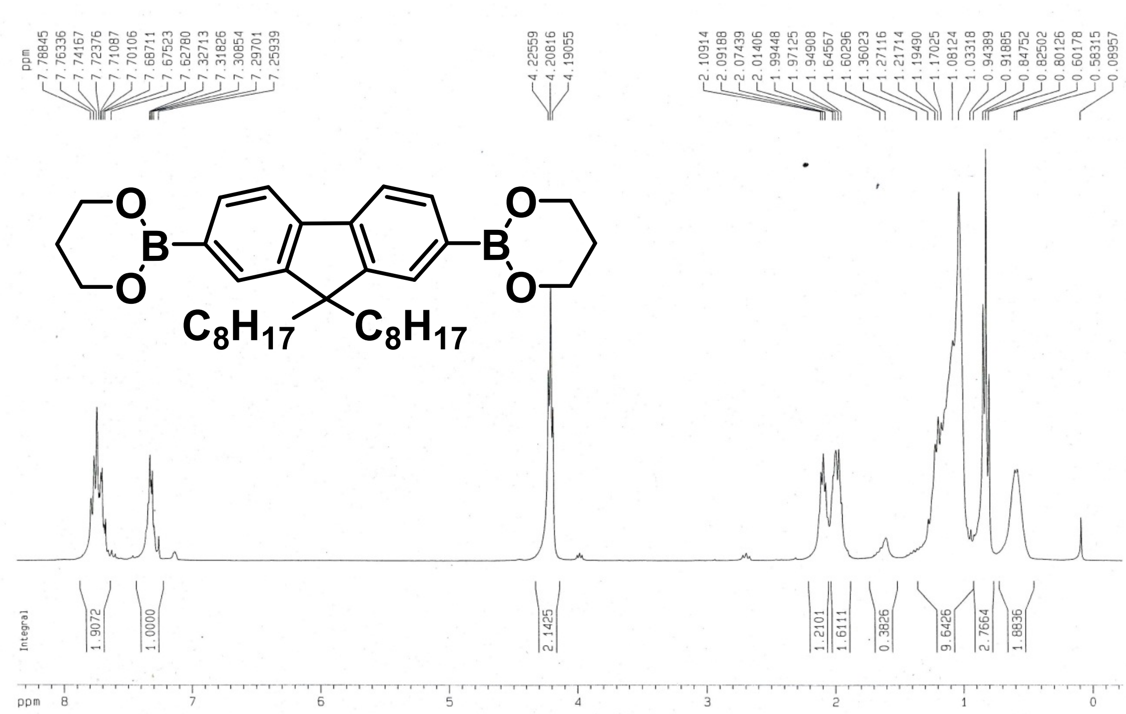


Fig. S1 ^{1}H-NMR of compound 2

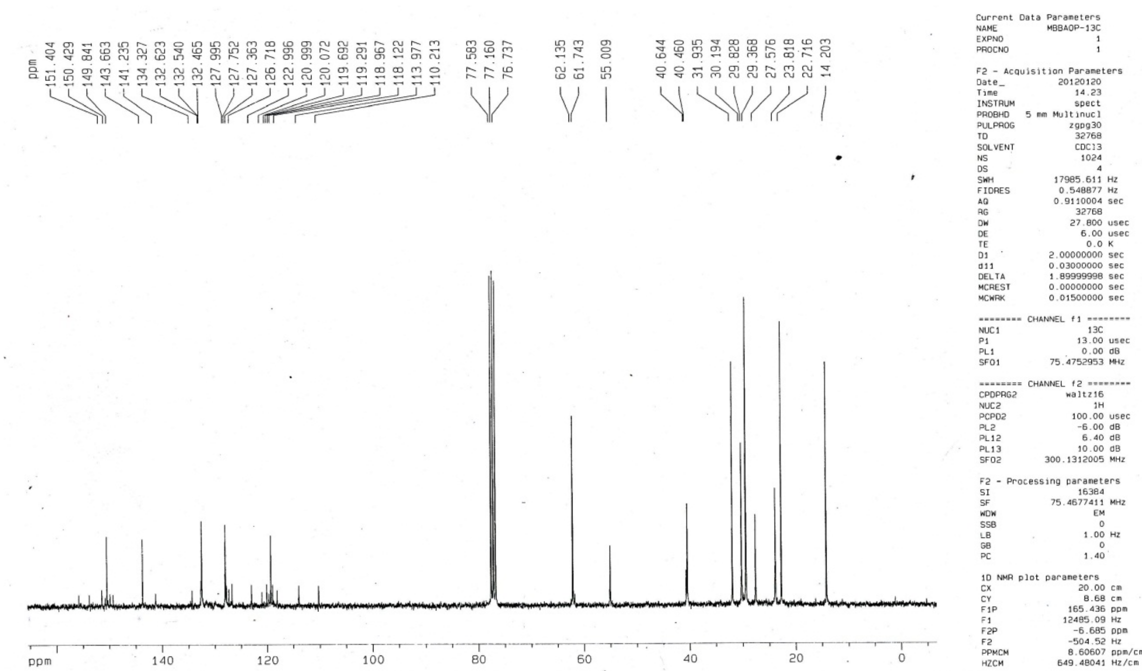


Fig. S2 ^{13}C-NMR of compound 2

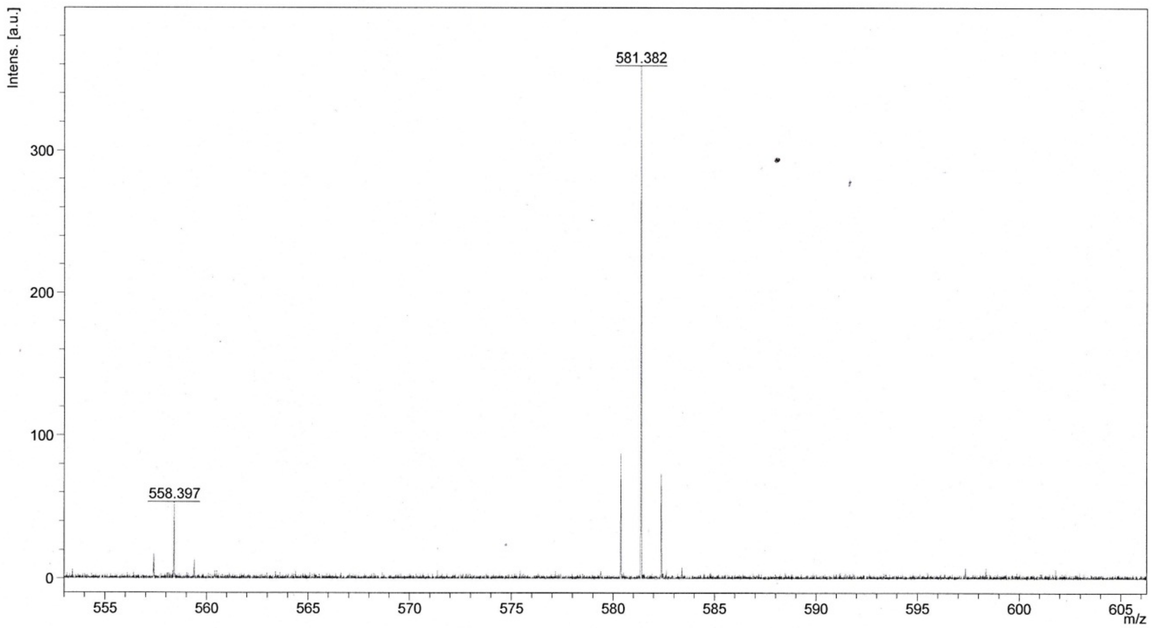


Fig. S3 MALDI-TOF of compound 2

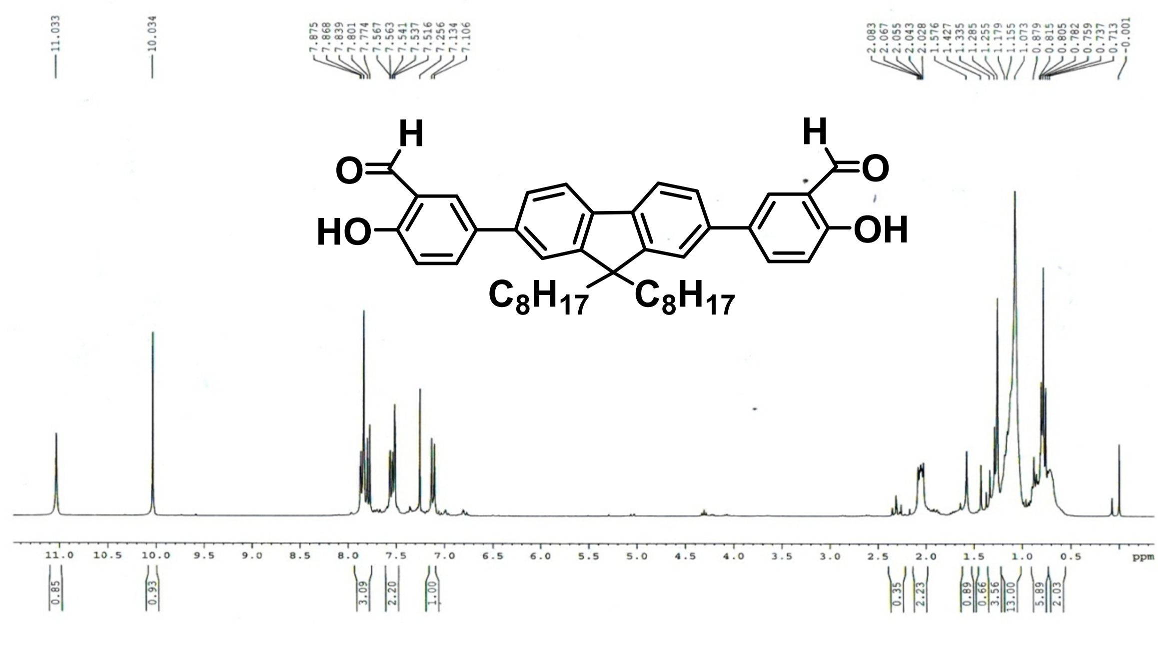


Fig. S4 ^{1}H-NMR of compound FSal

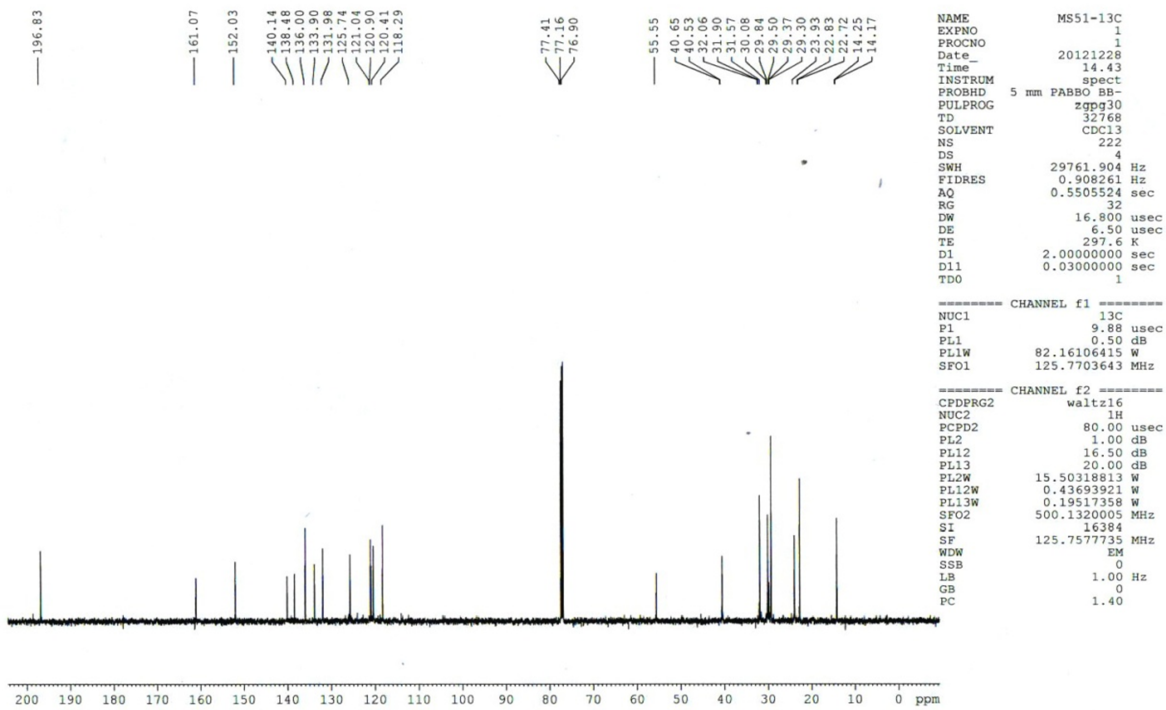


Fig. S5 ^{13}C-NMR of compound FSal

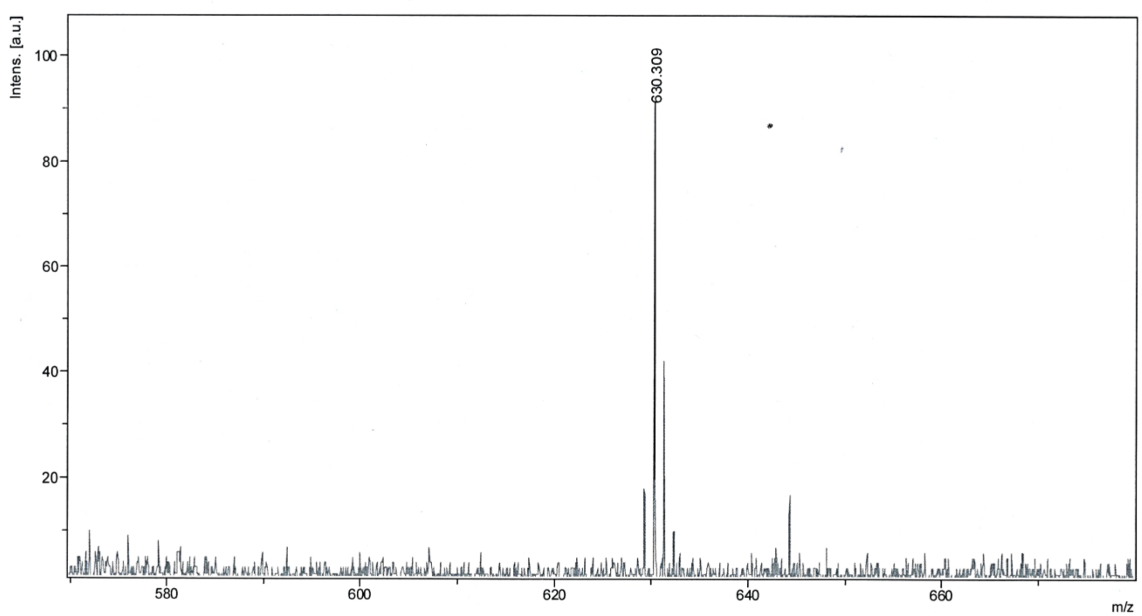


Fig. S6 MALDI-TOF of compound FSal

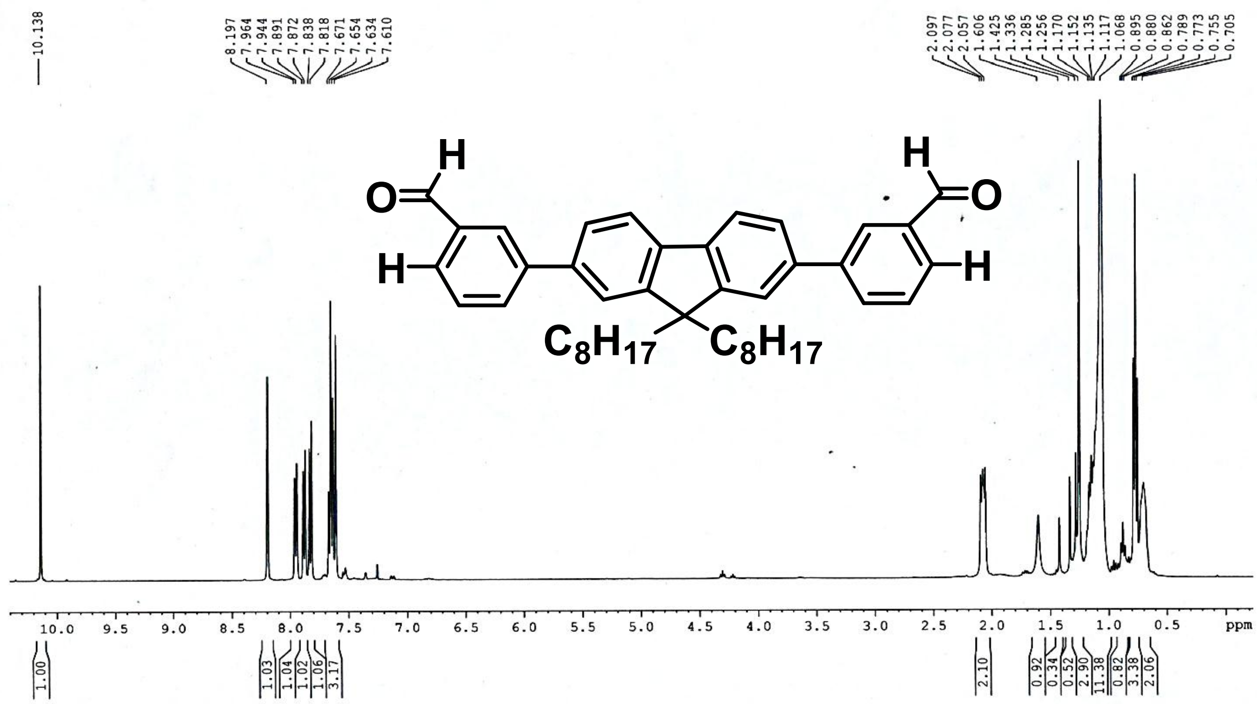


Fig. S7 ^{1}H-NMR of compound FBal

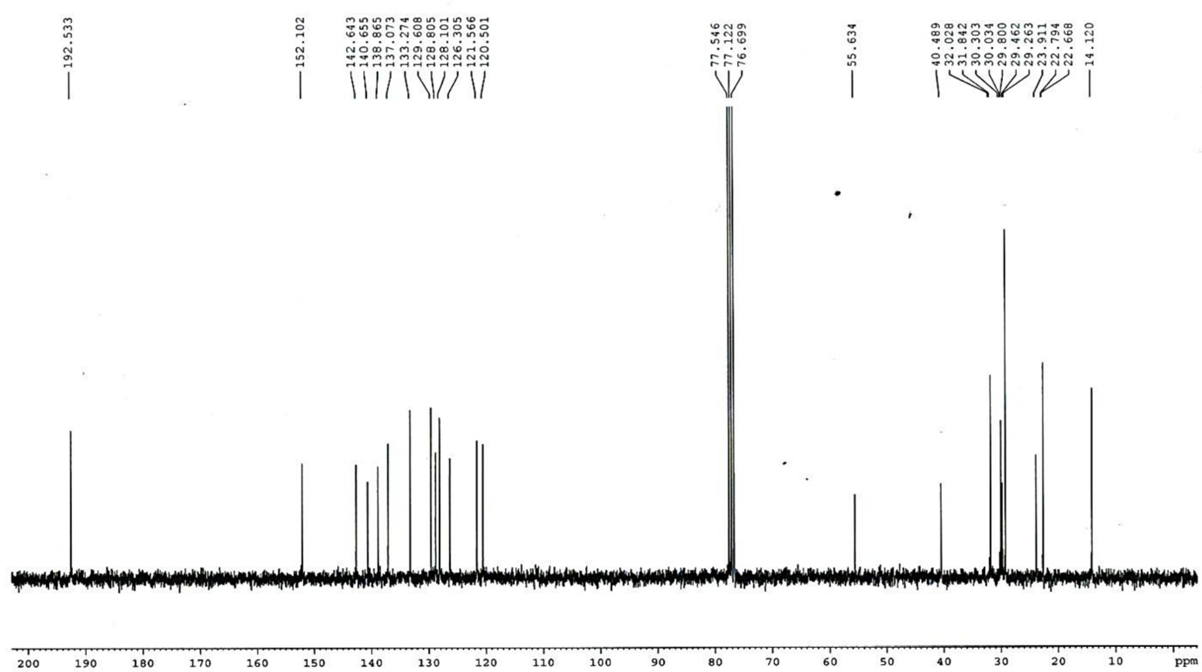


Fig. S8 ^{13}C-NMR of compound FBal

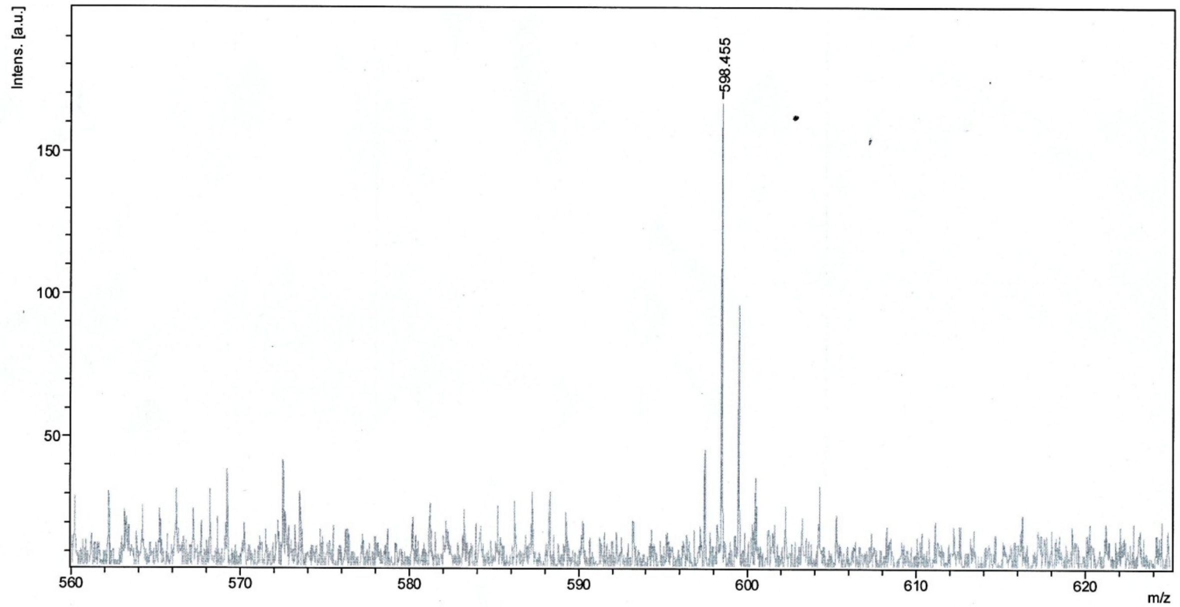


Fig. S9 MALDI-TOF of compound FBal

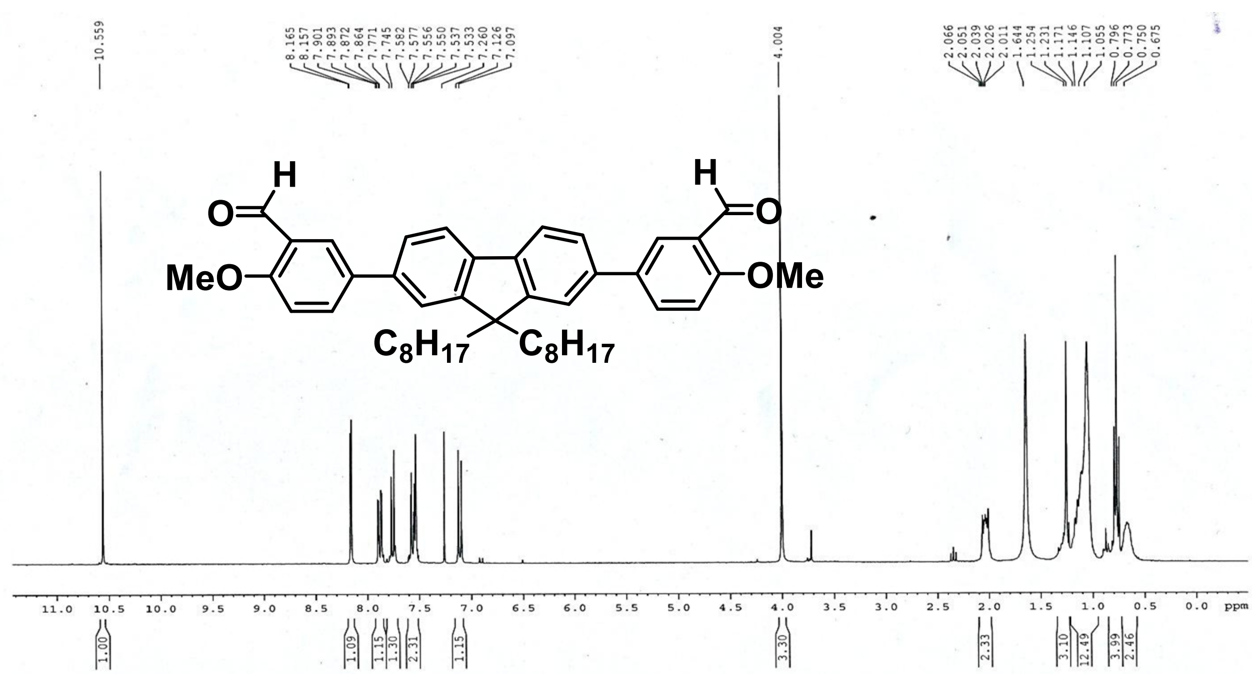


Fig. S10 ^{1}H-NMR of compound FMBal

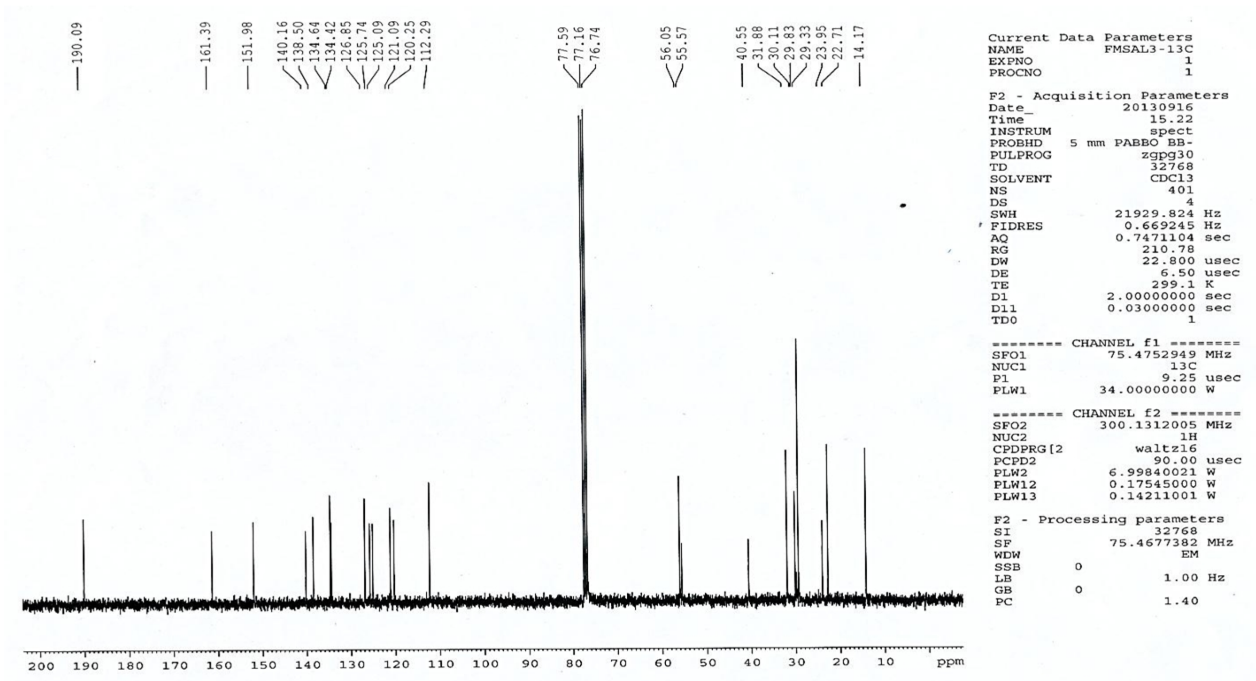


Fig. S11 ^{13}C-NMR of compound FMBal

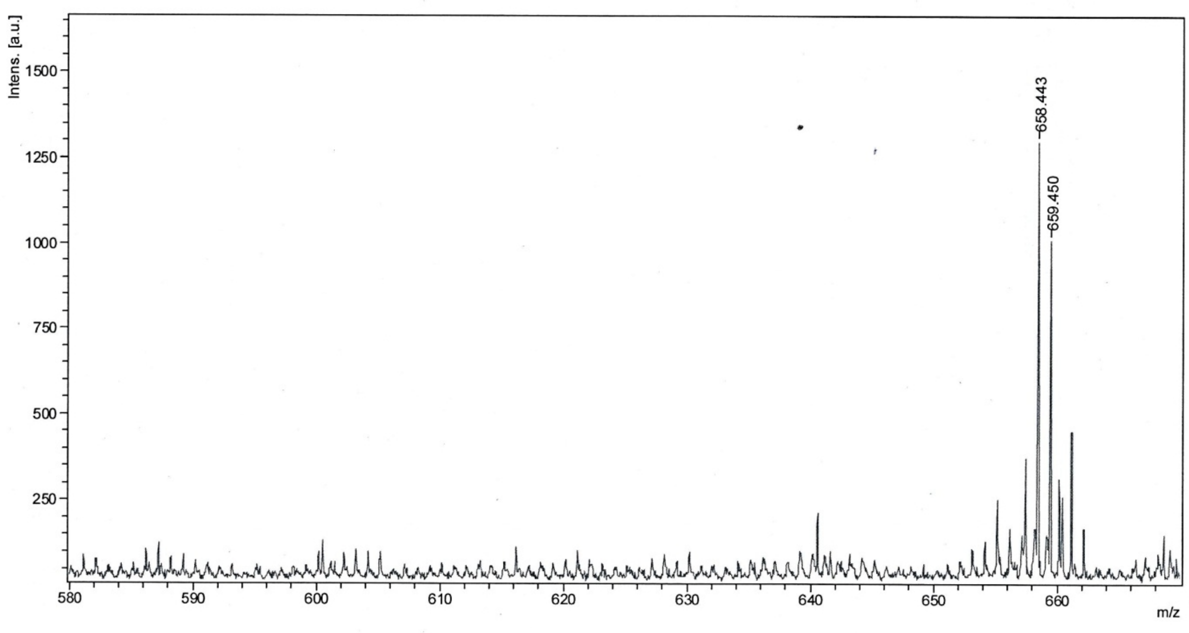


Fig. S12 MALDI-TOF of compound FMBal

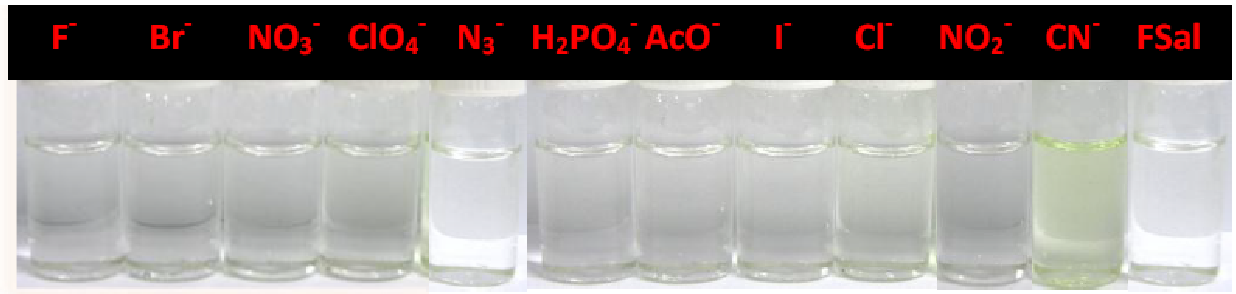


Fig. S13 A) Colorimetric response of FSal (40 μM in CH_3CN) in presence of 5 equiv. of cyanide and 10 equiv. of other anions in H_2O.

Compound	State	Excitation	λ = nm (eV)	*f*
FSal	S1	H to L+2 (95%)	338.17 (3.66)	1.4584
	S2	H-1 to L+ 4 (44%)	228.43 (5.4276)	0.7541
	S3	H-3 to L+3 (45%)	216.02 (5.7394)	0.2447
	S4	H to L+ 6 (35%)	240.92 (5.1463)	0.1934
	S5	H-10 to L (56%)	229.22 (5.409)	0.1681

Table ST1. Low-lying transition calculation for FSal at B3LYP/TZVP using optimized geometries at B3LYP/6-31G(d) (H and L stand for HOMO and LUMO).

Compound	State	Excitation	λ = nm (eV)	*f*
FSal-CN	S1	H to L (98%)	410.4 (3.0211)	1.5178
	S2	H-2 to L (40%)	275.87 (4.4943)	0.2613
		H-1 to L+3 (41%)		
	S3	H-6 to L + 1 (32.72%)	218.6 (5.6718)	0.2164
	S4	H-2 to L + 2 (79.38%)	226.78 (5.4617)	0.0512
	S5	H - 1 to L + 7 (60.50%)	253.76 (4.8859)	0.0471

Table ST2. Low-lying transition calculation for FSal-CN at B3LYP/TZVP using optimized geometries at B3LYP/6-31G(d) (H and L stand for HOMO and LUMO).

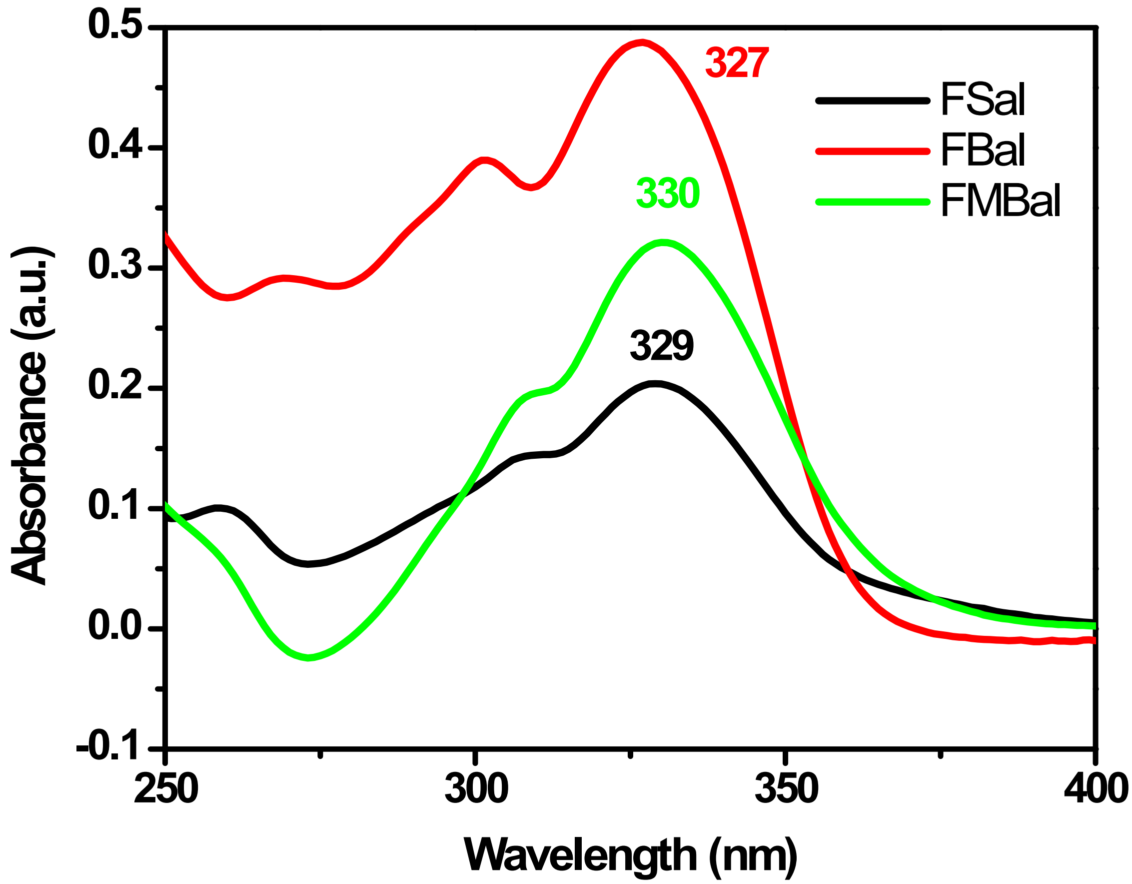


Fig. S14 UV-Vis spectra of FSal, FBal, FMBal (5 μM) in CH_3CN.

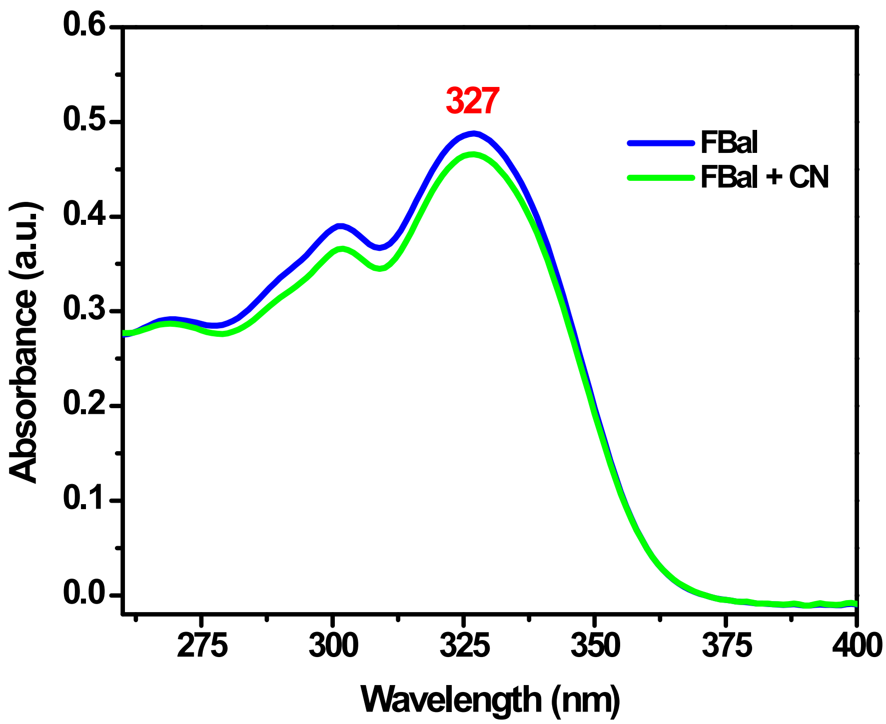


Fig. S15 UV-Vis spectra of FBal (5 μM in CH_3CN) followed by addition of 20 eqiv. CN^- in H_2O.

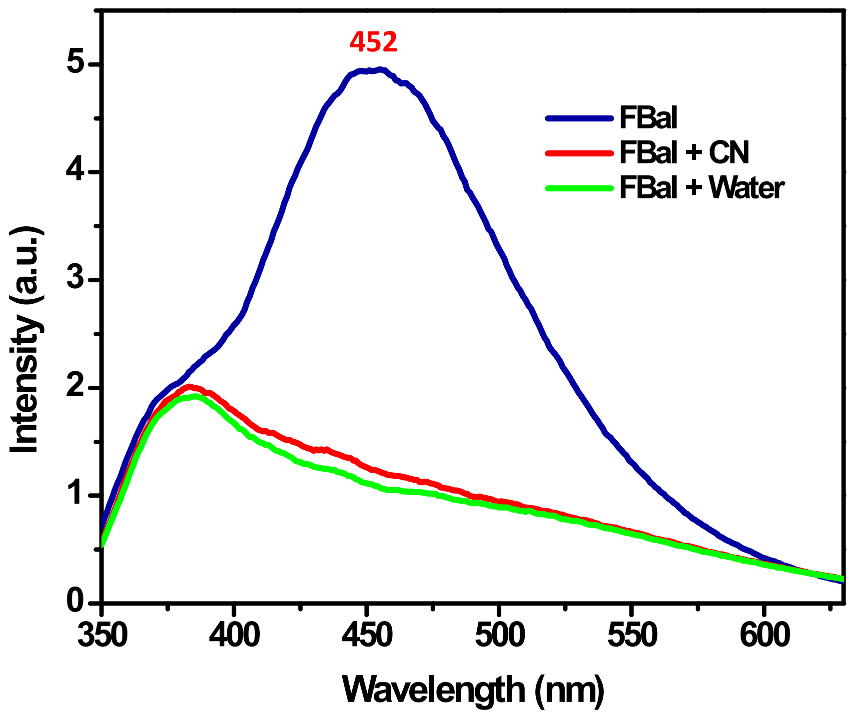


Fig. S16 Emission spectra of FBal (5 μM in CH_3CN) followed by addition of 20 eqiv. CN^- in H_2O.

From the above study we see that FBal shows absorbance at 327 nm and emission (λ_{ex} = 327 nm) at 452 nm in acetonitrile. After addition of CN^- in water absorbance maxima does not altered but intensity of emission maxima greatly decreased. To claque, weather this decrease of intensity is due to CN^- or due to dilution effect of water we further measure the emission of FBal followed by addition of same volume of water as like CN^- solution. But here also intensity decreased by same amount as in case of CN^-.

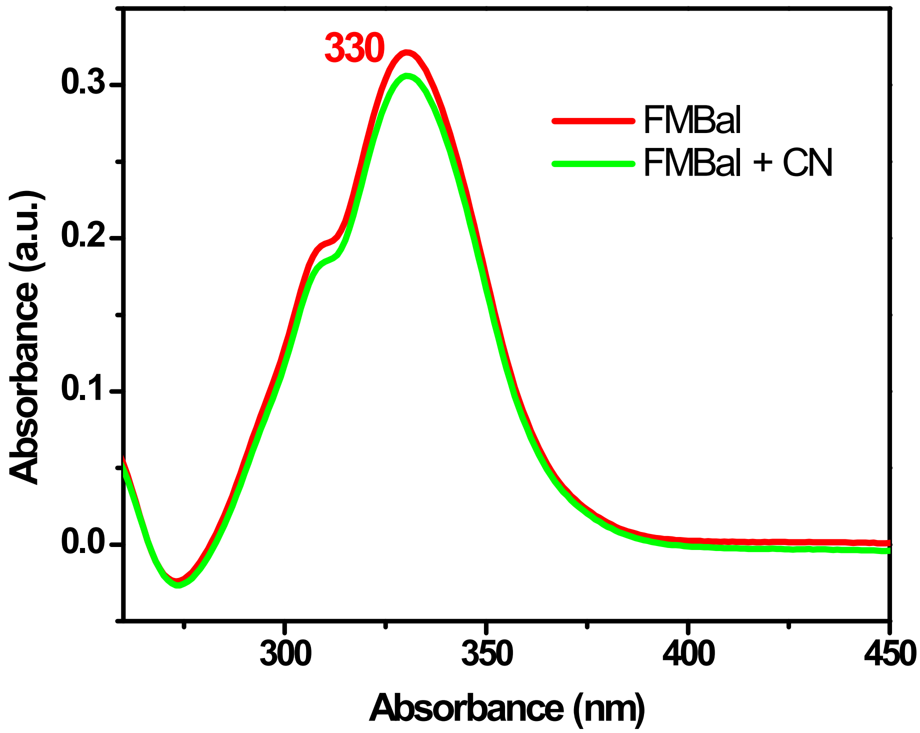


Fig. S17 UV-Vis spectra of FMBal (5 μM in CH_3CN) followed by addition of 20 eqiv. CN^- in H_2O.

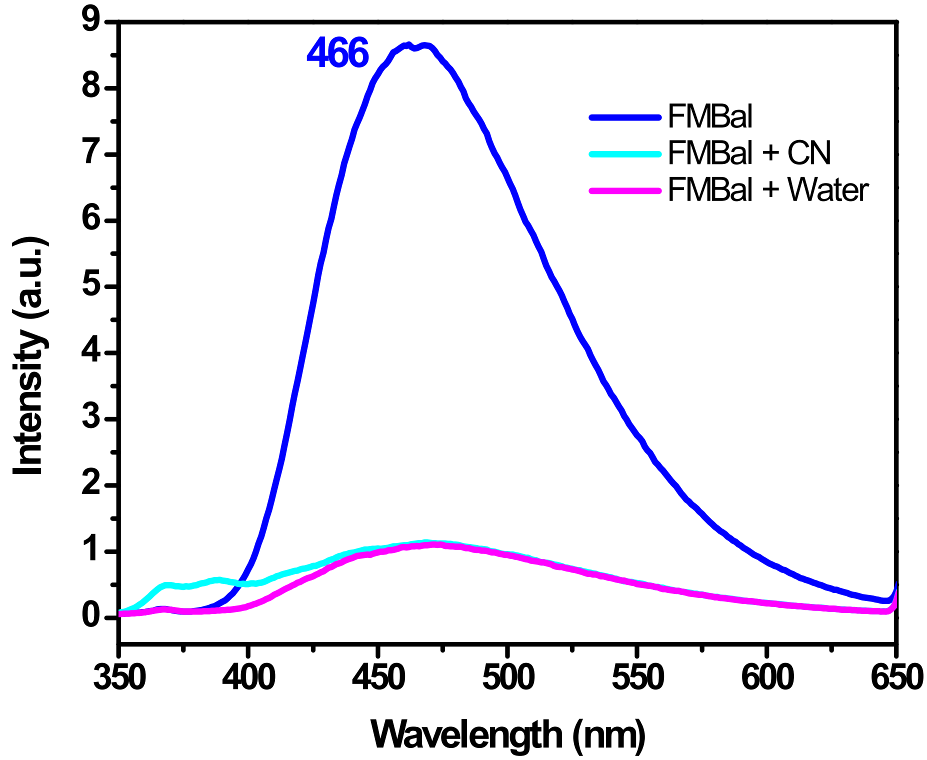
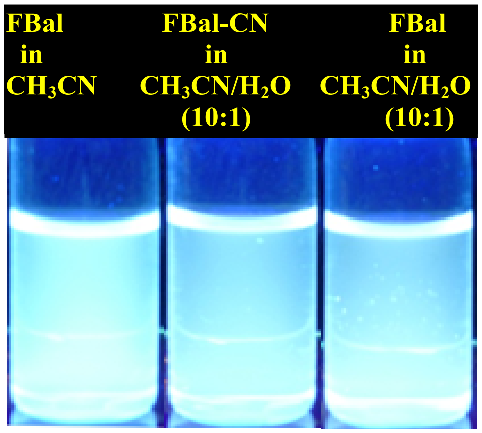


Fig. S18 Emission spectra of FMBal (5 μM in CH_3CN) followed by addition of 20 eqiv. CN^- in H_2O.

FMBal shows absorbance at 330 nm and emission (λ_{ex} = 330nm) at 466 nm in acetonitrile. After addition of CN^- in water absorption peak does not alter but intensity of emission is greatly decreased. To check, weather this decrease of intensity is due to CN^- or due to dilution effect of solvent (water) we further measured the emission of FMBal followed by addition of same volume of water as like CN^- solution. The decrease of intensity is solely due the effect of water.



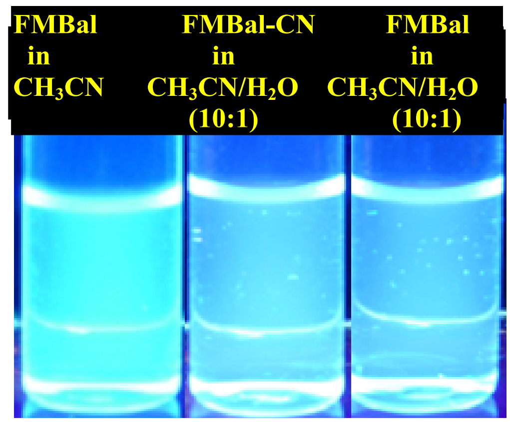


Fig. S19 Fluorescence color of FBal, FMBal (10 μM in CH_3CN) and corresponding cyanide complex.

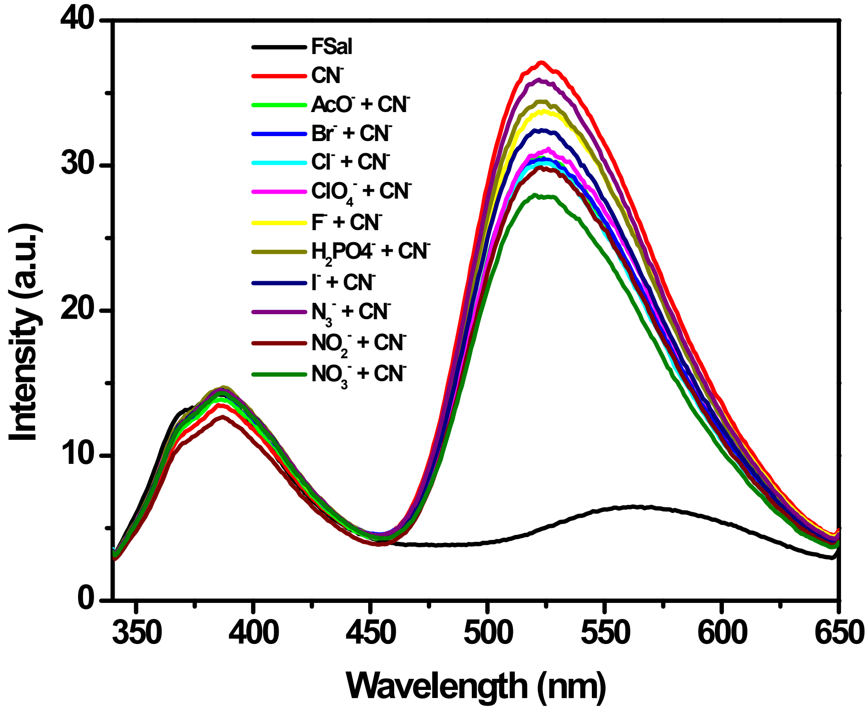


Fig. S20 Selectivity response of FSal (5 μM in CH_3CN) towards cyanide (26 equiv.) in presence of excess other anions (100 equiv.) in H_2O.

Cell Study and Fluorescence Imaging. To evaluate the any toxic effect of FSal on human cell line, MTT assay was performed. FSal stock solution (3mM) was prepared in acetonitrile in sterile conditions and used in the study. In brief, SH-SY5Y neuronal cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Himedia, India) supplemented with 10% FBS (Invitrogen, USA), 100 units⁄ml penicillin and 100 µg⁄mL streptomycin. Cells were seeded in 96-well plate (Nunc, USA) at a cell density of 10,000 cells per well in 100 µL media and incubated for 24 hr in a 5% CO_2 humidified environment at 37°C. After 24 hr of incubation, the old media was replaced with fresh media containing increasing concentrations (3, 6 and 12 µM) of FSal. The FSal was added to cells in such a way so that the final residual concentration of acetonitrile became less than 0.5%. Similar percentage of acetonitrile in cell culture media was used as a control. To test the effect of FSal on cells viability, cells were incubated for 20 hr in a 5% CO_2 humidified environment at 37°C. After incubation, 10 µl of a 5 mg/ml MTT (prepared in PBS) was added to each well and the incubation was continued for 4 hr. After 4 hr, 100 µl of a solution containing 50% dimethylformamide and 20% SDS (pH 4.8) was added to each well and incubated for overnight at 37°C. Finally, the absorption values at 560 nm were determined with spectraMax M2 microplate reader (Molecular Devices, USA).

Fluorescence imaging

For fluorescence imaging of FSal in presence and absence of TBACN in neuronal cells, SH-SY5Y cells were cultured as described above and seeded in 24-well plate (Nunc, USA) at a cell density of 10,000 cells per well in 1 mL media. After 24 hr, the old media was discarded and cells were gently washed with PBS. FSal was diluted in 1 mL cell culture media to a final concentration of 12 µM and added to cells. The cells were incubated for 35 mins in a 5% CO_2 humidified environment at 37°C. After incubation, FSal containing media was discarded and cells were gently washed with PBS. After this 200 μL of 3mM TBACN (prepared in PBS) was diluted in 1 mL cell culture media and added to the cells (previously incubated with FSal). The cells were further incubated for 35 mins. After 35 mins cells were washed with PBS and imaged immediately. Cells were incubated with only FSal and only media were also imaged in similar conditions. The imaging was done using Zeiss Axio Observer Z1 microscope (40X Plan-Neofluar objective, NA = 0.75; Carl Zeiss MicroImaging Inc.) equipped with Axiocam camera. Images were processed using the Axiovision software.

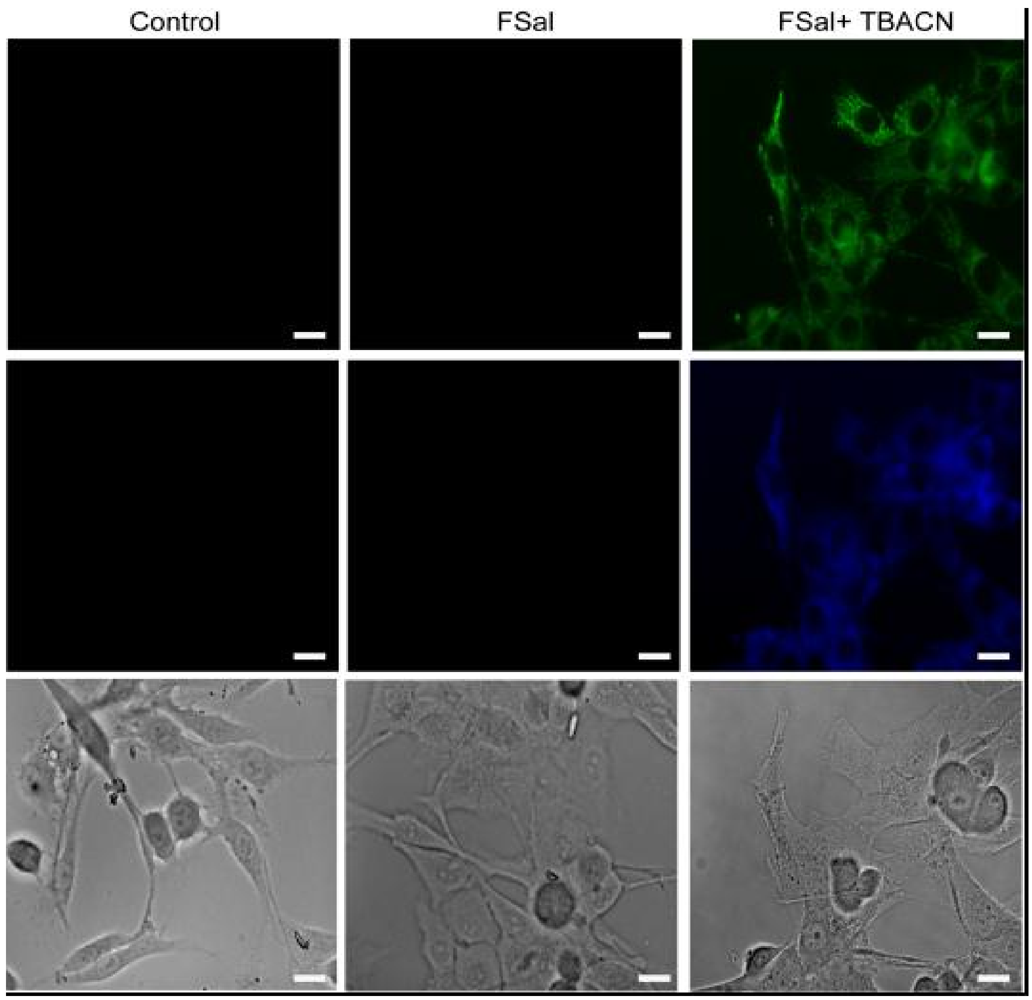


Fig. S21 Fluorescence microscope image of SH-SY5Y neuronal cells a) Under control condition b) Cells were incubated with FSal (12μM) c) Cells after treatment with 3mM Tetrabutyl ammonium cyanide. Scale bars 20 microns.

Coordinates of optimized geometry for FSal

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-2.201656	-2.362597	-1.475789
2	6	0	-1.529054	-1.289049	-0.856588
3	6	0	-0.143674	-1.299131	-0.726201
4	6	0	0.590534	-2.400324	-1.219816
5	6	0	-0.059616	-3.472086	-1.833269
6	6	0	-1.446288	-3.445594	-1.959911
7	1	0	-2.114567	-0.470112	-0.454714
8	1	0	0.505208	-4.315311	-2.222578
9	1	0	-1.951506	-4.263205	-2.466506
10	6	0	2.013206	-2.172246	-0.969530
11	6	0	3.135760	-2.951396	-1.257210
12	6	0	2.158337	-0.930395	-0.324917
13	6	0	4.396708	-2.485234	-0.891741
14	1	0	3.035112	-3.916250	-1.747717
15	6	0	3.418580	-0.469942	0.031805
16	6	0	4.562240	-1.245281	-0.245256
17	1	0	5.268692	-3.103984	-1.083887
18	1	0	3.537008	0.501600	0.504718
19	6	0	5.912874	-0.767140	0.139454
20	6	0	6.130172	-0.067876	1.340437
21	6	0	7.030872	-0.990674	-0.671692
22	6	0	7.395567	0.378549	1.703913
23	1	0	5.297391	0.107757	2.015428
24	1	0	6.904209	-1.504302	-1.622079
25	6	0	8.504269	0.142938	0.881201
26	1	0	7.533179	0.907220	2.645742
27	6	0	0.797772	-0.249492	-0.114671
28	6	0	0.815998	1.082162	-0.935688
29	1	0	1.690671	1.657020	-0.600595
30	1	0	1.014901	0.816226	-1.982507
31	6	0	-0.407552	2.007071	-0.886897
32	1	0	-1.313949	1.454340	-1.164349
33	1	0	-0.563531	2.373000	0.135650
34	6	0	-0.266517	3.217281	-1.823233
35	1	0	0.642254	3.779607	-1.561933
36	1	0	-0.120132	2.865446	-2.854939
37	6	0	-1.478671	4.155912	-1.771031
38	1	0	-1.623059	4.502908	-0.736928
39	1	0	-2.385710	3.588267	-2.028015
40	6	0	-1.365856	5.371955	-2.699879
41	1	0	-0.461862	5.943994	-2.442970
42	1	0	-1.222922	5.027863	-3.734968
43	6	0	-2.587129	6.297857	-2.637076
44	1	0	-3.490608	5.724464	-2.893757
45	1	0	-2.730613	6.639516	-1.600950

46	6	0	-2.485496	7.518477	-3.561029
47	1	0	-1.584869	8.093939	-3.303239
48	1	0	-2.341715	7.178079	-4.596418
49	6	0	-3.712685	8.432831	-3.490175
50	1	0	-3.608703	9.295215	-4.158494
51	1	0	-4.624648	7.895807	-3.778908
52	1	0	-3.863464	8.815791	-2.473255
53	6	0	0.599463	-0.024520	1.423029
54	1	0	1.202987	0.851721	1.702151
55	1	0	1.055697	-0.876551	1.942919
56	6	0	-0.820620	0.144778	1.987181
57	1	0	-1.366077	0.938402	1.463164
58	1	0	-1.388672	-0.779297	1.824825
59	6	0	-0.809040	0.462198	3.490386
60	1	0	-0.251319	-0.321775	4.023943
61	1	0	-0.256979	1.398248	3.663100
62	6	0	-2.215032	0.584102	4.091931
63	1	0	-2.774166	1.363199	3.552535
64	1	0	-2.763492	-0.354138	3.921834
65	6	0	-2.218904	0.907478	5.591837
66	1	0	-1.659236	0.129762	6.132570
67	1	0	-1.673402	1.847626	5.762810
68	6	0	-3.627820	1.024185	6.186987
69	1	0	-4.188297	1.800902	5.645332
70	1	0	-4.172949	0.083774	6.016211
71	6	0	-3.638588	1.348189	7.686609
72	1	0	-3.078232	0.572879	8.228379
73	1	0	-3.096796	2.289399	7.857790
74	6	0	-5.050823	1.458937	8.270218
75	1	0	-5.025126	1.688415	9.341628
76	1	0	-5.624107	2.251488	7.773500
77	1	0	-5.606193	0.521425	8.143348
78	8	0	9.746259	0.565035	1.217822
79	1	0	9.697861	1.026848	2.070604
80	6	0	9.409747	-0.851212	-1.290890
81	1	0	9.049906	-1.401825	-2.191616
82	6	0	8.319951	-0.555769	-0.334457
83	8	0	10.583075	-0.560933	-1.176718
84	6	0	-3.679345	-2.348483	-1.610432
85	6	0	-4.439665	-3.507689	-1.467112
86	6	0	-4.380540	-1.151226	-1.889838
87	6	0	-5.840519	-3.500375	-1.594526
88	1	0	-3.950686	-4.449061	-1.226144
89	6	0	-5.759957	-1.109271	-2.018771
90	1	0	-3.815808	-0.233819	-2.031165
91	6	0	-6.515609	-2.282558	-1.874324
92	1	0	-6.277901	-0.182310	-2.242748
93	8	0	-7.848184	-2.218931	-2.004826
94	1	0	-8.198691	-3.136301	-1.869717
95	6	0	-6.597050	-4.730930	-1.427545
96	1	0	-6.005262	-5.639622	-1.201695
97	8	0	-7.824388	-4.814013	-1.522322

Coordinates of optimized geometry for FSal-CN

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-2.469256	-2.212804	-0.704749
2	6	0	-1.730827	-1.042498	-0.397623
3	6	0	-0.346363	-1.070859	-0.264079
4	6	0	0.351952	-2.289803	-0.425227
5	6	0	-0.356141	-3.458433	-0.717684
6	6	0	-1.741299	-3.412941	-0.859312
7	1	0	-2.279535	-0.120773	-0.238204
8	1	0	0.169388	-4.402041	-0.851935
9	1	0	-2.275890	-4.321364	-1.122223
10	6	0	1.782311	-2.051330	-0.242909
11	6	0	2.889645	-2.903119	-0.302760
12	6	0	1.980449	-0.685340	0.033944
13	6	0	4.166694	-2.391440	-0.080581
14	1	0	2.761992	-3.964440	-0.507721
15	6	0	3.255287	-0.179318	0.241995
16	6	0	4.392247	-1.022973	0.195538
17	1	0	5.015761	-3.069028	-0.094903
18	1	0	3.400576	0.883788	0.417957
19	6	0	5.749981	-0.493760	0.426064
20	6	0	5.981148	0.626766	1.254806
21	6	0	6.889384	-1.074959	-0.174221
22	6	0	7.250944	1.129740	1.477963
23	1	0	5.137609	1.087774	1.766909
24	1	0	6.752743	-1.917644	-0.849906
25	6	0	8.417377	0.555717	0.879305
26	1	0	7.404977	1.981874	2.137130
27	6	0	0.638738	0.070835	0.046949
28	6	0	0.707483	1.134238	-1.095159
29	1	0	1.584239	1.768087	-0.899765
30	1	0	0.933751	0.588577	-2.021049
31	6	0	-0.497028	2.049414	-1.358946
32	1	0	-1.404750	1.450241	-1.496463
33	1	0	-0.677122	2.702621	-0.495316
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36	1	0	-0.134251	2.289002	-3.479350
37	6	0	-1.470666	3.877273	-2.869489
38	1	0	-1.630707	4.516355	-1.987741
39	1	0	-2.390995	3.286272	-2.984874
40	6	0	-1.281827	4.765244	-4.106553
41	1	0	-0.359071	5.354648	-3.994271
42	1	0	-1.128524	4.128777	-4.990820
43	6	0	-2.459887	5.713471	-4.364366

44	1	0	-3.382014	5.124495	-4.479033
45	1	0	-2.615580	6.348398	-3.478863
46	6	0	-2.275437	6.606630	-5.598073
47	1	0	-1.353243	7.194899	-5.484984
48	1	0	-2.123665	5.973302	-6.483890
49	6	0	-3.456287	7.551779	-5.844423
50	1	0	-3.295725	8.175630	-6.732205
51	1	0	-4.386965	6.990614	-5.994977
52	1	0	-3.611883	8.221937	-4.989469
53	6	0	0.462866	0.690708	1.472369
54	1	0	1.157619	1.540995	1.547821
55	1	0	0.829521	-0.053143	2.191565
56	6	0	-0.929204	1.134949	1.949682
57	1	0	-1.369439	1.873793	1.269287
58	1	0	-1.606073	0.273039	1.946840
59	6	0	-0.883905	1.730834	3.364799
60	1	0	-0.452783	0.991040	4.055888
61	1	0	-0.198739	2.592568	3.379850
62	6	0	-2.258775	2.169304	3.886094
63	1	0	-2.686633	2.915395	3.199678
64	1	0	-2.945842	1.311043	3.863778
65	6	0	-2.221215	2.751602	5.305168
66	1	0	-1.803244	2.001918	5.993761
67	1	0	-1.526568	3.605204	5.330468
68	6	0	-3.593912	3.201944	5.821417
69	1	0	-4.010920	3.955424	5.136216
70	1	0	-4.290260	2.350715	5.792609
71	6	0	-3.559808	3.777653	7.243016
72	1	0	-3.147676	3.023678	7.929024
73	1	0	-2.862554	4.627598	7.273600
74	6	0	-4.934730	4.228264	7.748033
75	1	0	-4.878444	4.633507	8.765820
76	1	0	-5.357547	5.007577	7.101517
77	1	0	-5.644740	3.391939	7.760680
78	8	0	9.602504	1.018778	1.078250
79	6	0	9.403972	-1.124751	-0.702120
80	6	0	8.170865	-0.586373	0.027648
81	8	0	10.554592	-1.168428	0.119970
82	6	0	-3.936742	-2.173129	-0.855749
83	6	0	-4.747977	-3.288771	-0.549393
84	6	0	-4.608410	-1.016628	-1.310692
85	6	0	-6.127665	-3.267611	-0.682218
86	1	0	-4.275129	-4.189303	-0.160918
87	6	0	-5.983790	-0.971573	-1.457914
88	1	0	-4.023277	-0.142267	-1.593060
89	6	0	-6.822627	-2.090386	-1.152971
90	1	0	-6.475389	-0.074850	-1.830093
91	8	0	-8.103278	-2.057867	-1.284121
92	6	0	-7.035783	-4.421192	-0.249395
93	8	0	-8.124384	-4.624092	-1.130082
94	6	0	9.200110	-2.480173	-1.267134
95	7	0	9.061333	-3.533224	-1.737593

96	1	0	9.593632	-0.469537	-1.578206
97	1	0	10.461084	-0.285090	0.605268
98	6	0	-6.315554	-5.709995	-0.115461
99	7	0	-5.772013	-6.728070	0.017163
100	1	0	-7.414866	-4.192027	0.768704
101	1	0	-8.390775	-3.664085	-1.308564

Table ST3: Comparison of FSal with previously reported literature

S.No.	Publications	Interference by other anions	Detection limit of CN^- ions	CN^- detection in biological system
1	***Macromolecules***, **2008,** ***41***, **7433.**	I^-, F^-, SO_4^{2-}	0.31 ppm	NO
2	***Macromol. Chem. Phys.*** **2010,** ***211***, **18.**	I^-, AcO^-, SO_3^{2-}	0.47 ppm	NO
3	***Journal of Polymer Science Part A: Polymer Chemistry***, **2011,** ***49***, **3314.**	I^-	0.05 ppm	NO
4	***Chem. Commun.***, **2008, 1094.**	HPO_4^{2-}, PO_4^{3-}	1.82 ppm	NO
5	***Org. Biomol. Chem.***, **2012,** ***10***, **555.**	$H_2PO_4^{2-}$, HSO_4^-	0.001 ppm	NO
6	***Dalton Trans.***, **2012,** ***41***, **11413.**	NO	0.15 ppm	NO
7	***Chem. Commun.***, **2013,** ***49***, **2912.**	NO	1.6 µM	NO
8	***Chem. Asian J.*** **2013,** ***8***, **1271.**	NO	25 µM	NO
9	***Org. Lett.***, **2013,** ***15***, **10, 2386.**	NO	0.2 ppm	NO
10	***New J. Chem.***, **2013,** ***37***, **3222.**	NO	0.5 µM	NO
11	***Tetrahedron***, **2014,** ***70***, **1889.**	NO	0.4 µM	NO
12	FSal	NO	0.06 ppm	YES