

Supporting Information for

A Novel Fluorescent “Turn-On” Chemodosimeter for Cyanide Based on Dual Reversible and Irreversible Deprotonation of NH and CH Group

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1. ^1H , ^{13}C NMR, IR and HRMS-ESI copies of the compound 2.

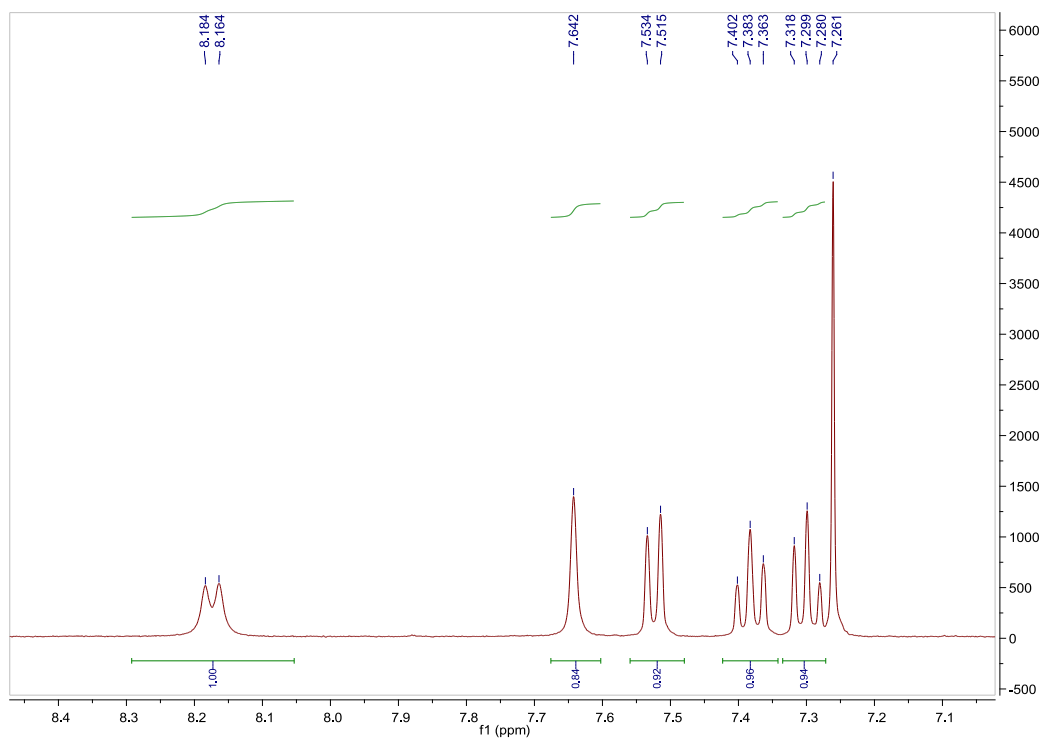
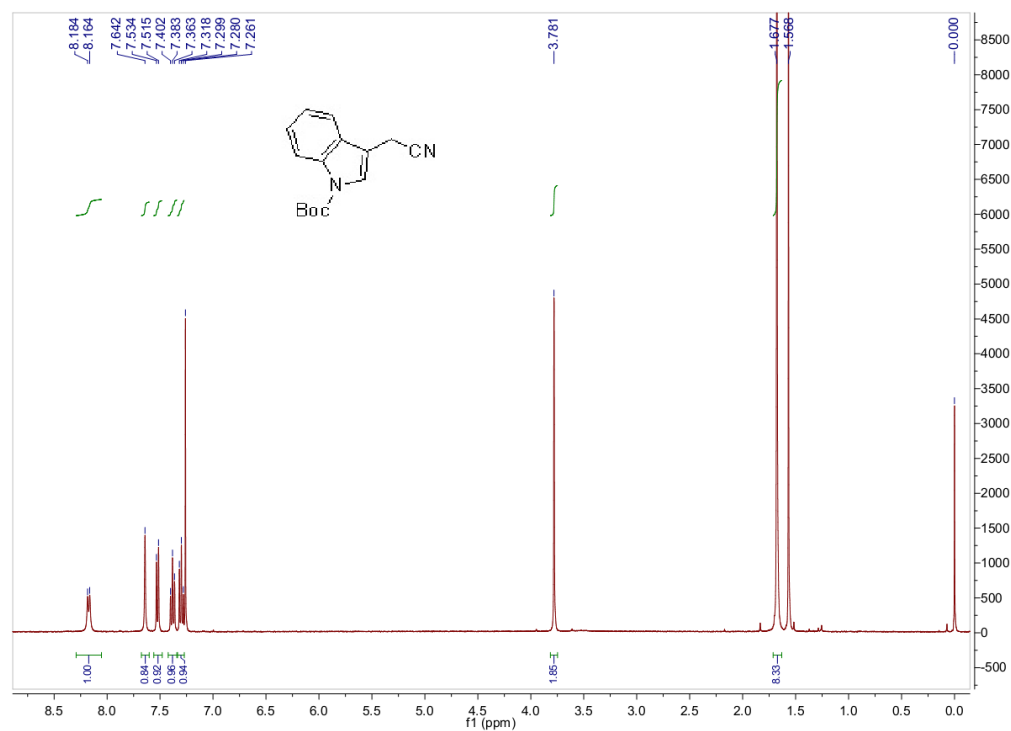


Fig. S1. ^1H NMR (CDCl_3 , 400 MHz) spectra of compound 2.

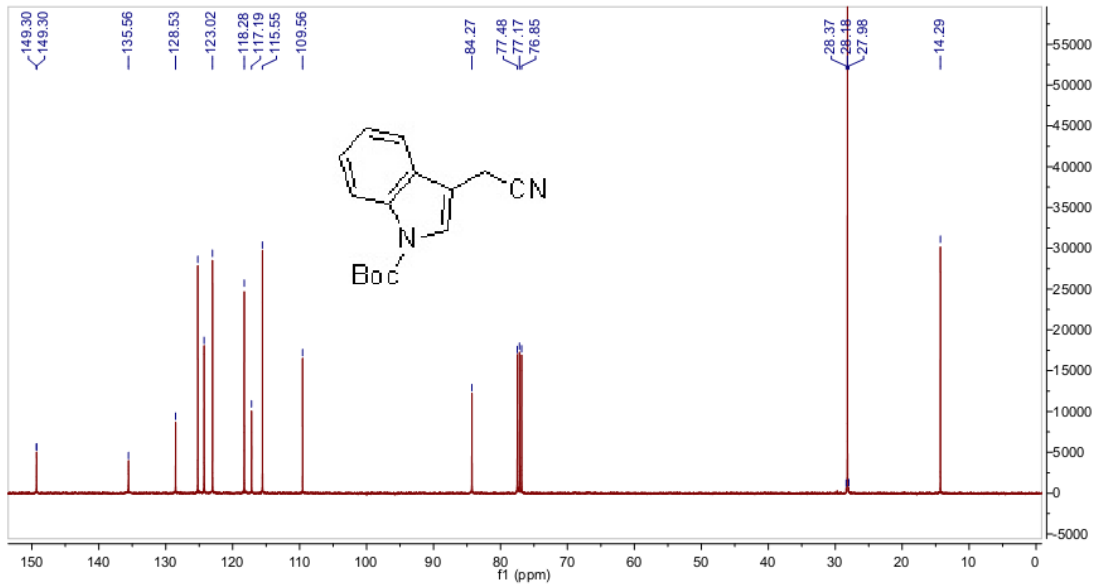


Fig. S2. ^{13}C NMR (DMSO- d_6 , 100 MHz) spectra of compound 2.

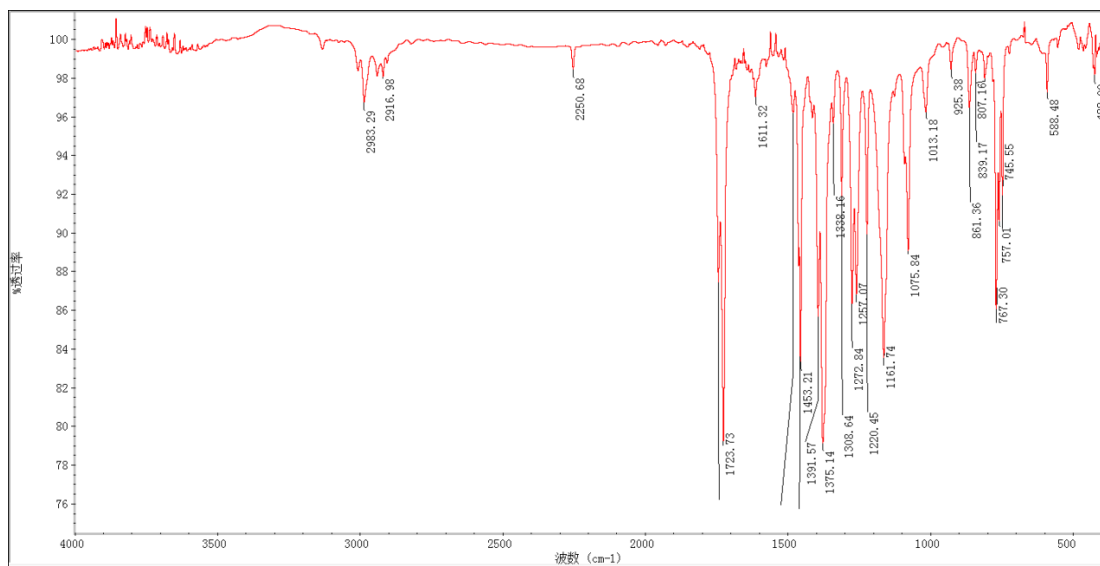


Fig. S3. IR spectra of compound 2.

Mass Spectrum List Report

Analysis Info

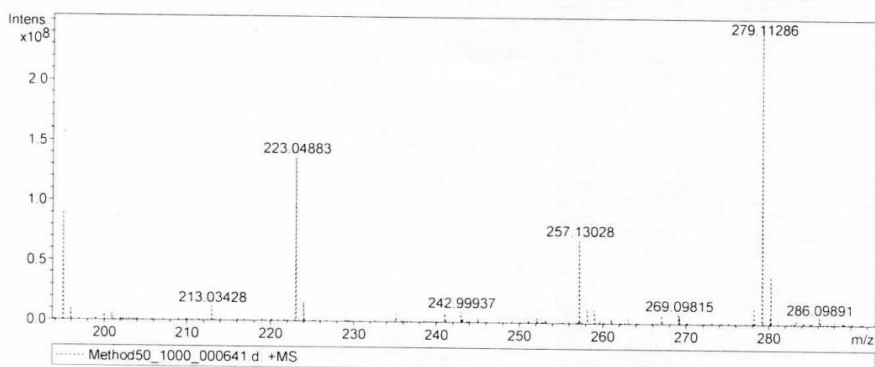
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 Sample Name 95 2
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Acquisition Date 1/12/2014 7:13:36 PM

Operator
 Instrument solariX

Acquisition Parameter

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n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 lp
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1000.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Tue Jan 8 02:14:05 2013
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Ion Accumulation Time	0.200 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization



m/z	z	I	Res.
108.02675		14929178	178462
159.45604		12804844	98565
174.05474		9658163	98978
195.02328		91060976	85793
196.02673		10277756	85042
213.03428		12175473	77228
223.04531		9883213	123622
223.04883		137198672	70631
224.05224		16098314	69800
257.13028		69990152	59549
258.13369		12131103	59794
259.01842		11489305	59198
278.09886		14172003	55689
279.10744		17484222	69809
279.11286		244547008	54666
280.11634		41053980	54941
308.10664		21800516	46177
313.19416		26393118	46346
316.21504		10694091	44825
324.06946		10216936	57930
324.07662		43253996	61074
324.08434		630857984	44677
325.08782		133778040	44739
326.09125		13170176	42986

Fig. S4. ESI mass spectra of compound **2**.

2. ^1H , ^{13}C NMR, IR and HRMS-ESI copies of the dosimeter 4

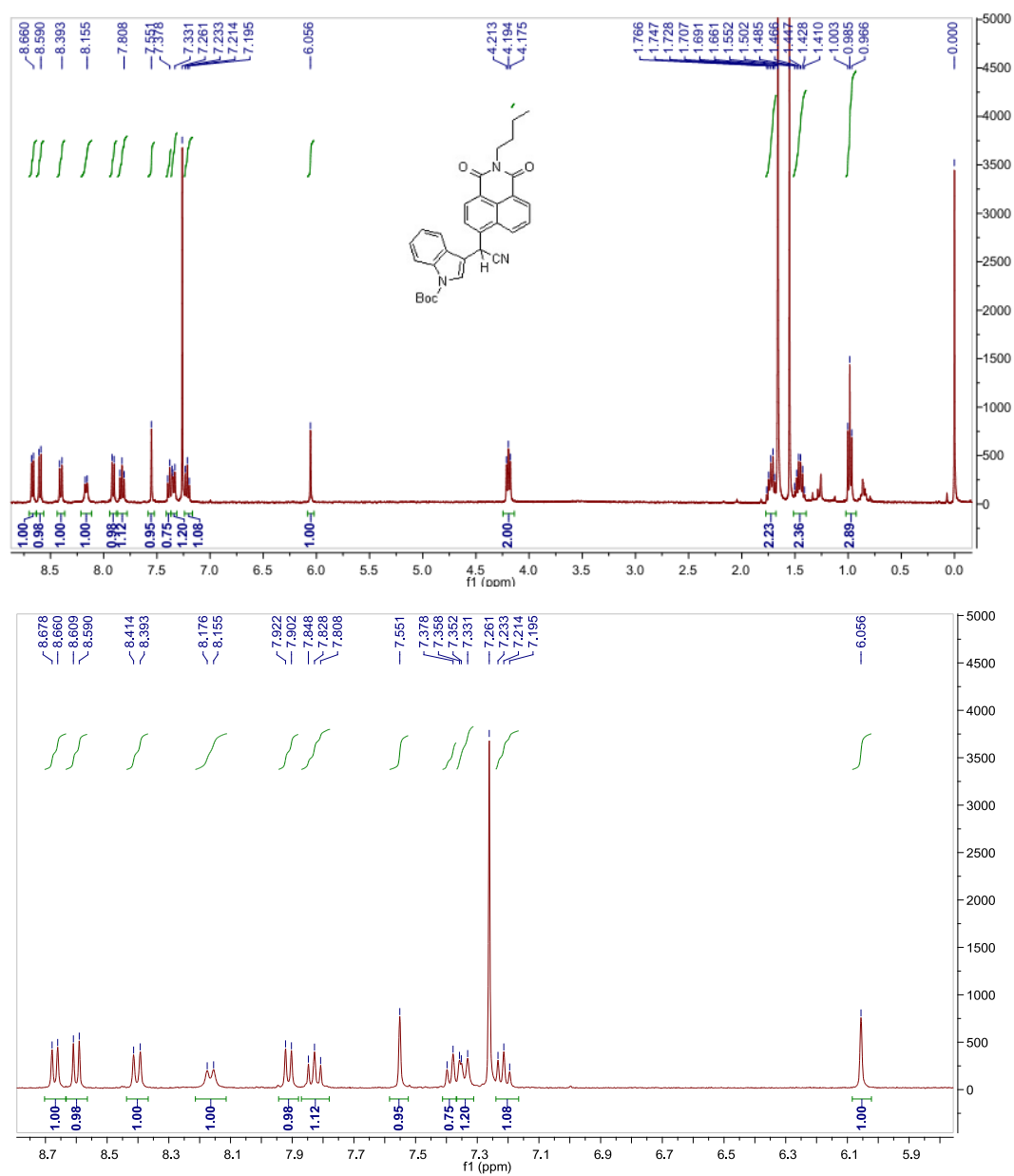


Fig. S5. ^1H NMR (CDCl_3 , 400 MHz) spectra of dosimeter 4.

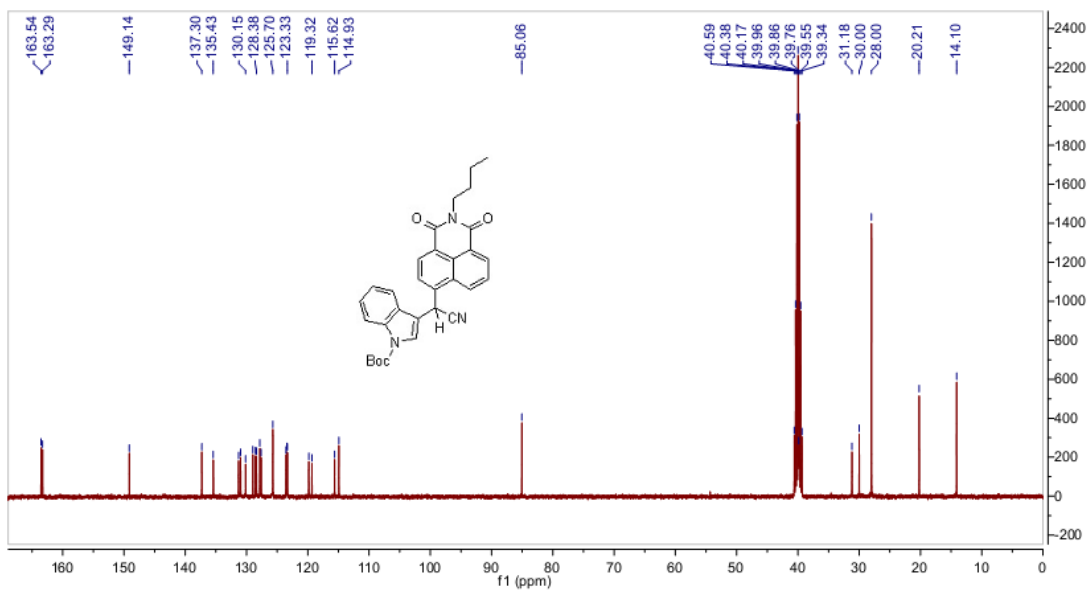


Fig. S6. ^{13}C NMR (DMSO- d_6 , 100 MHz) spectra of dosimeter 4.

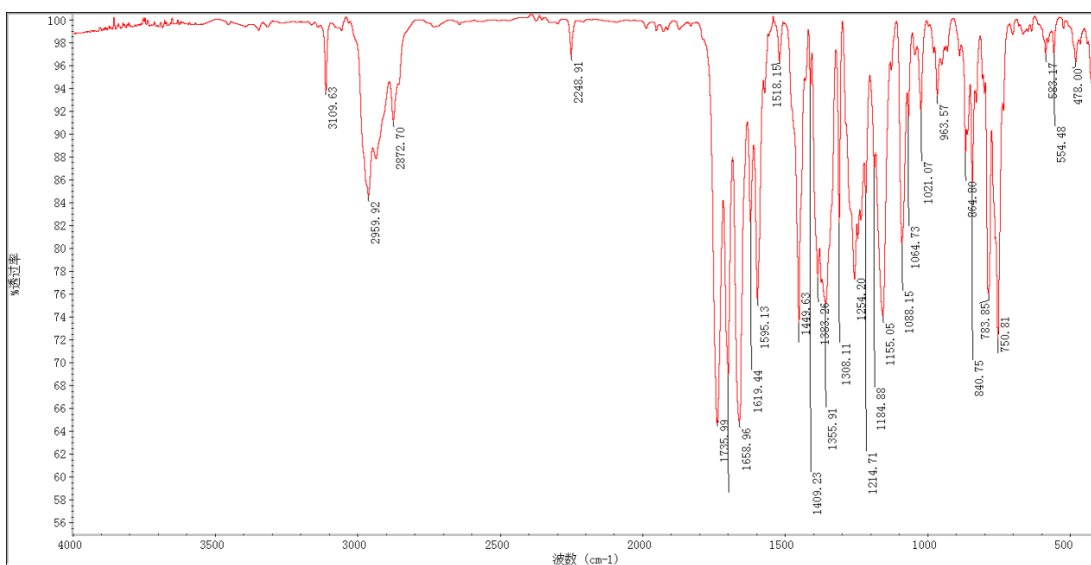


Fig. S7. IR spectra of dosimeter 4.

Mass Spectrum List Report

Analysis Info

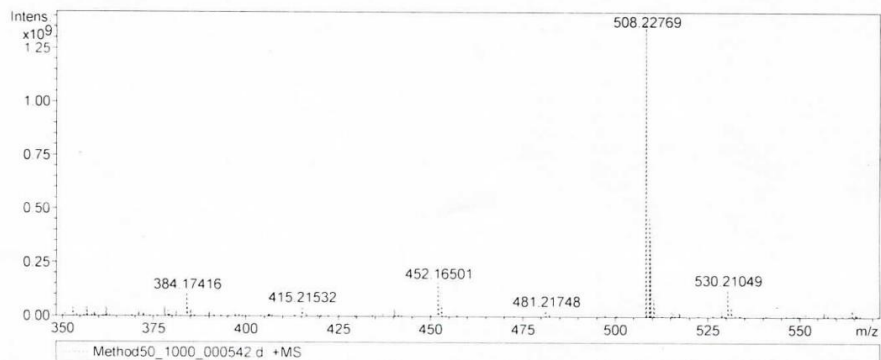
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 Method 4_19_MassAccuNeg
 Sample Name 3
 Comment

Acquisition Date 11/6/2013 4:29:22 PM

Operator
 Instrument solarix

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 Ip
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1000.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a		
Pulse Program	basic	n/a	n/a	Calibration Date	Tue Jan 8 02:14:05 2013
Source Accumulation	0.010 sec	n/a	n/a	Data Acquisition Size	1048576
Ion Accumulation Time	0.200 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Flight Time to Acq. Cell	0.001 sec	n/a	n/a	Apodization	Apodization



m/z	z	I	Res.
169.41104		38419920	104037
234.20670		17608852	67770
242.28464		13608340	67951
245.08746		17312574	66586
261.06546		46066160	58905
262.23864		28541882	58491
275.08159		11902311	56976
278.23400		18734024	57310
290.27062		232309584	51936
291.27396		35576108	52574
301.12021		25117170	48732
306.26563		18819096	49462
313.27549		45407292	47054
318.30209		58993304	46254
328.10975		10848316	46253
331.28616		15654663	44730
333.61177		17399304	43653
334.29722		125242496	44589
335.30037		24744620	44265
341.30727		21226426	43264
346.33378		22328502	43338
351.11357		11633705	42705
353.26863		36937180	41664
357.18347		40895064	40598

Fig. S8. ESI mass spectra of dosimeter 4.

3. ^1H , ^{13}C NMR, IR and HRMS-ESI copies of the dosimeter 5

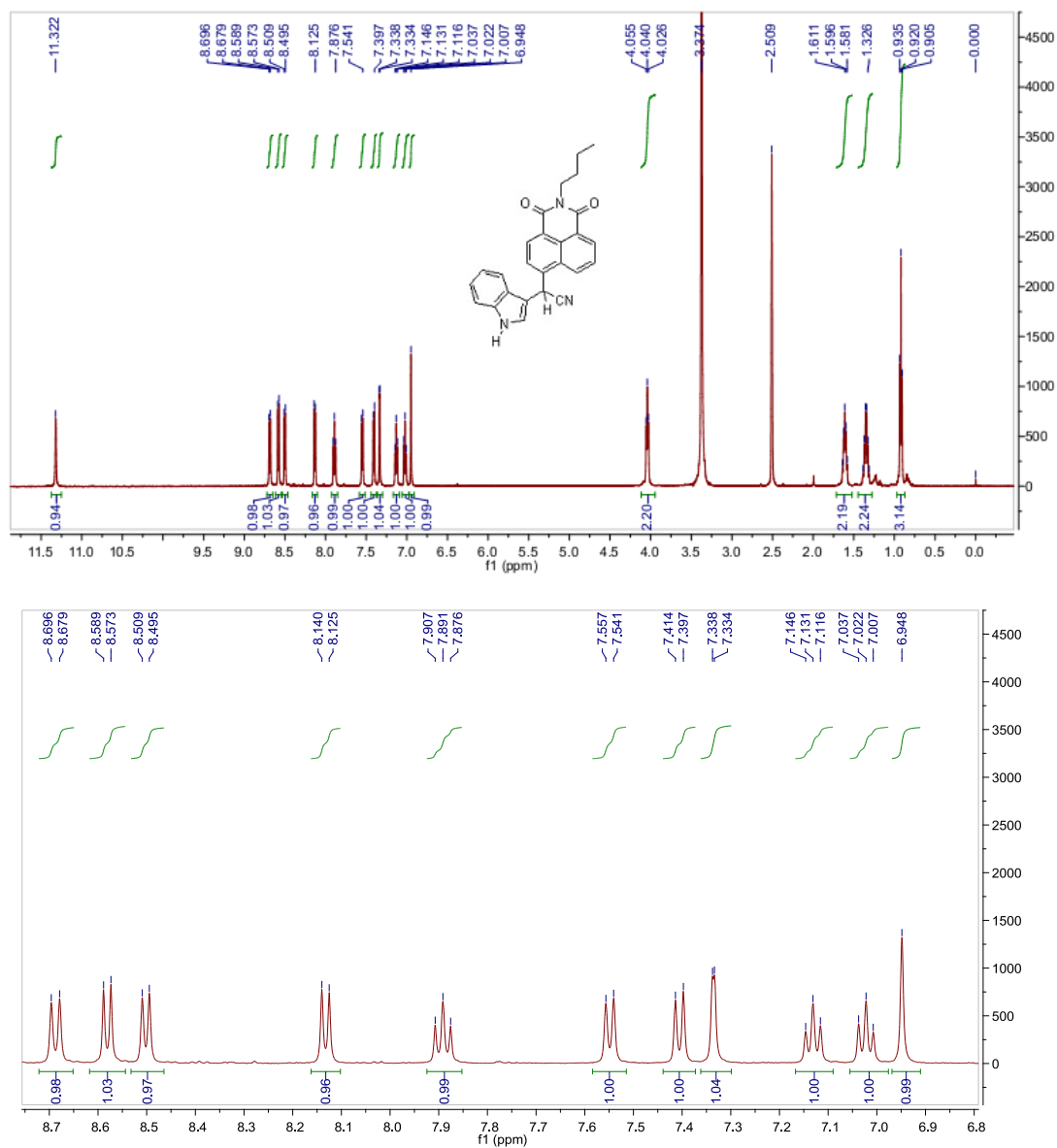


Fig. S9. ^1H NMR ($\text{DMSO-}d_6$, 500 MHz) spectra of dosimeter 5.

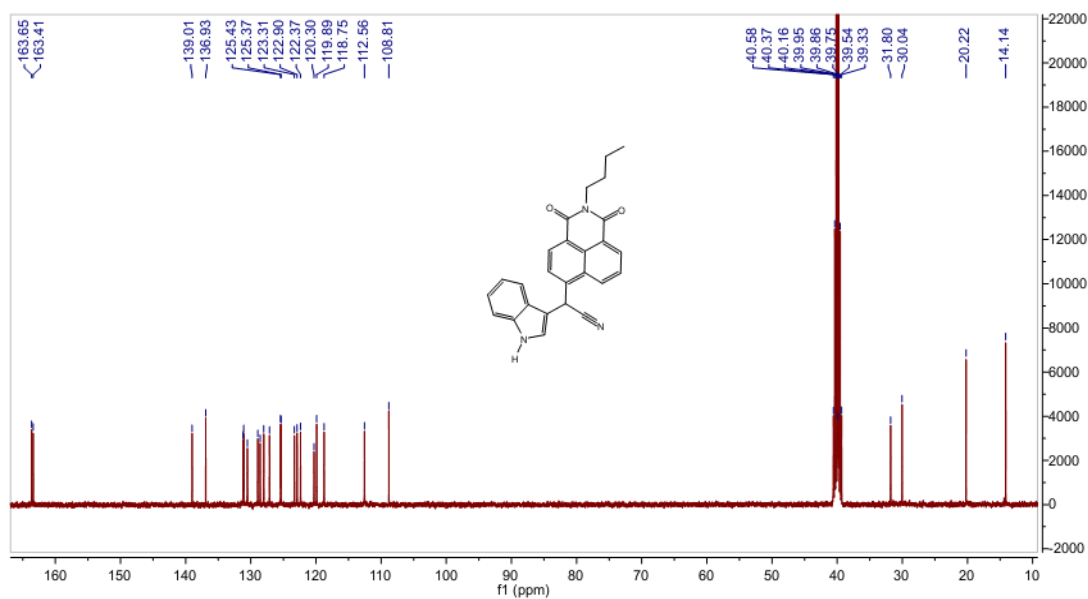


Fig. S10. ^{13}C NMR (DMSO- d_6 , 100 MHz) spectra of dosimeter 5.

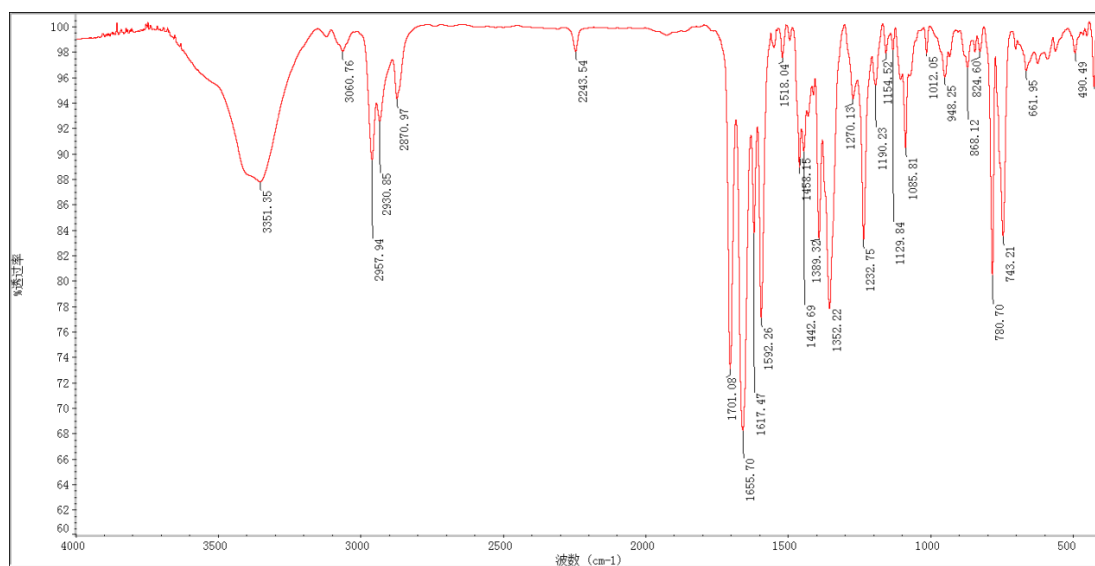


Fig. S11. IR spectra of dosimeter 5.

Mass Spectrum List Report

Analysis Info

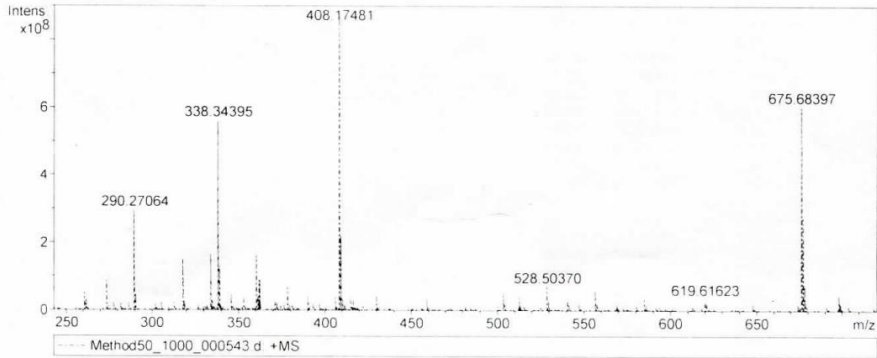
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 Method 4_19_MassAccuNeg
 Sample Name 3
 Comment

Acquisition Date 11/6/2013 4:32:20 PM

Operator
 Instrument solarix

Acquisition Parameter

Polarity	Positive	n/a	n/a	No. of Laser Shots	200
n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 Ip
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1000.0 m/z	n/a	n/a	n/a	n/a
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Ion Accumulation Time	0.200 sec	n/a	n/a	Apodization	Apodization
Flight Time to Acq. Cell	0.001 sec	n/a	n/a		



m/z	z	I	Res.
136.05796		15992886	140450
234.20668		18767520	69157
242.28469		45517596	66044
261.06541		53432144	59994
262.23868		35828124	58726
274.27541		88077472	56504
278.23391		21556706	56923
282.28054		24240928	54988
287.21355		20749252	53531
290.27064		297158912	51968
291.27395		49805000	52699
302.30718		21966778	49202
306.26575		25646234	49724
313.27566		25702160	48273
318.30216		148867664	46245
319.30549		29180466	47991
327.20344		15398042	45413
332.28168		15467297	42150
334.29732		165022320	43741
335.30088		32063658	43935
338.34395		563168128	43420
339.34729		132560328	43427
346.33400		51510872	42406
350.29262		15287574	42570

Fig. S12. ESI mass spectra of dosimeter 5.

4. ^1H , ^{13}C NMR, IR and HRMS-ESI copies of the compound 6.

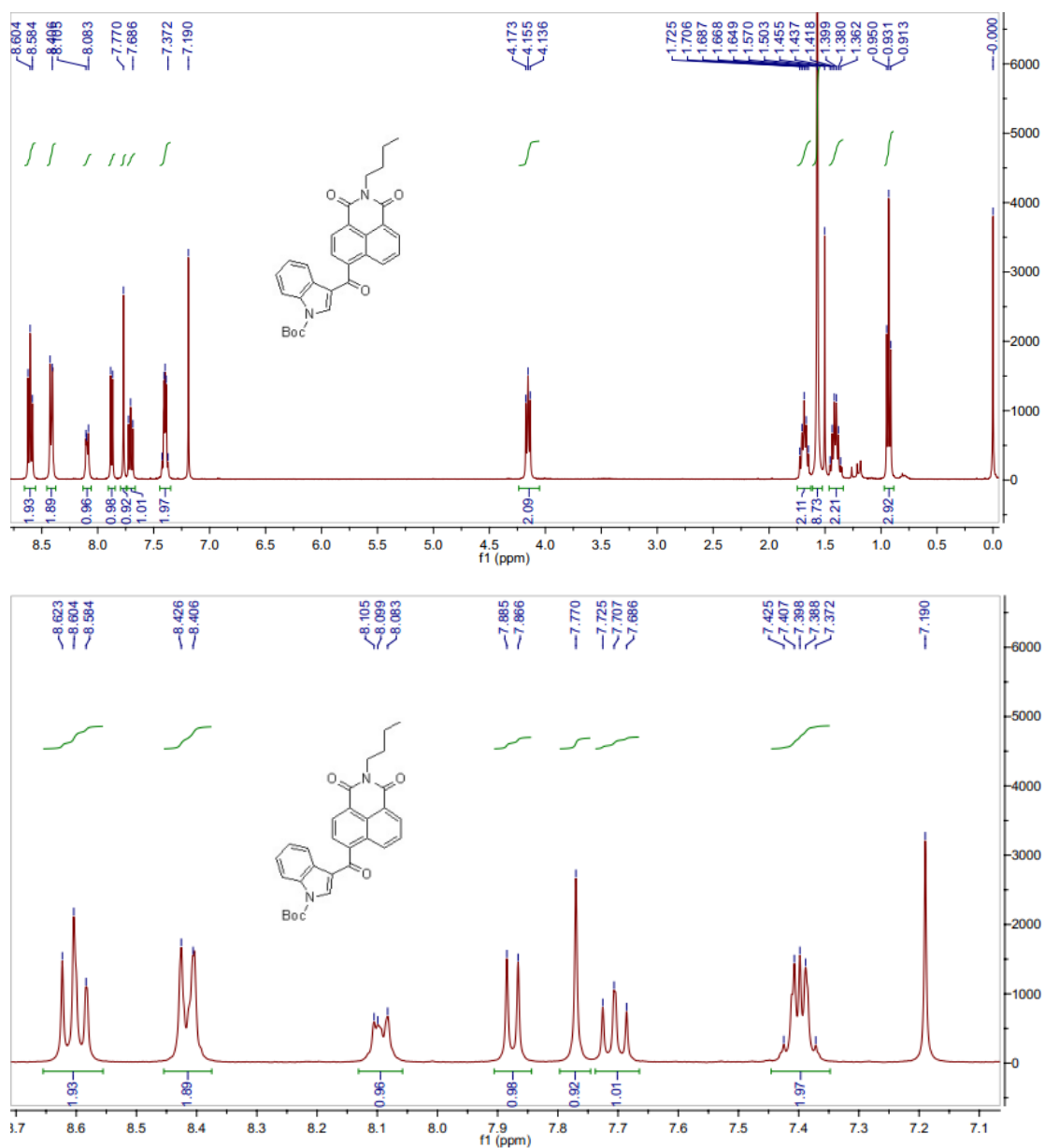


Fig. S13. ^1H NMR (CDCl_3 , 400 MHz) spectra of compound 6.

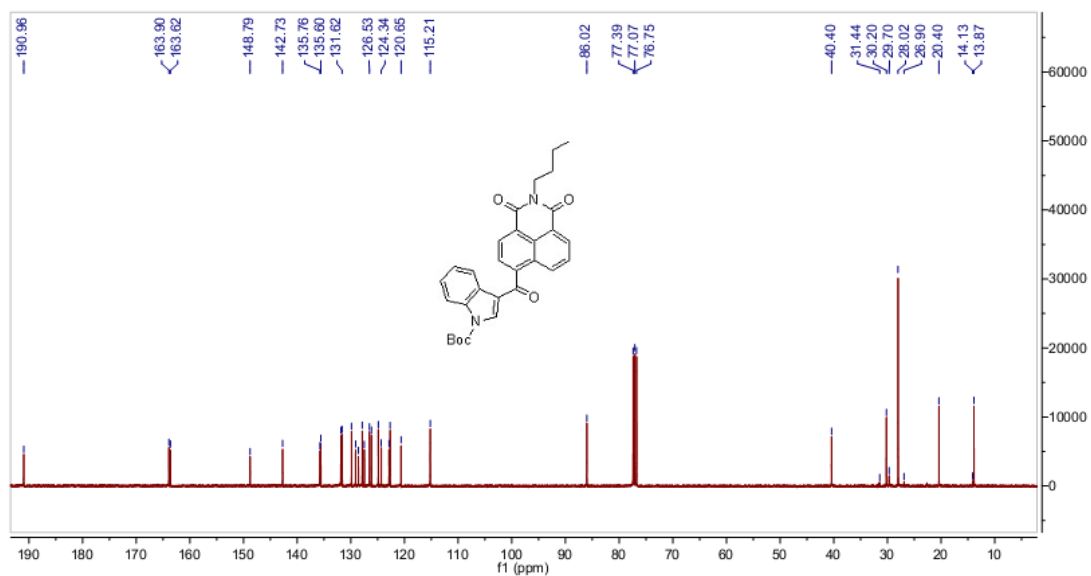


Fig. S14. ^{13}C NMR (DMSO- d_6 , 100 MHz) spectra of compound **6**.

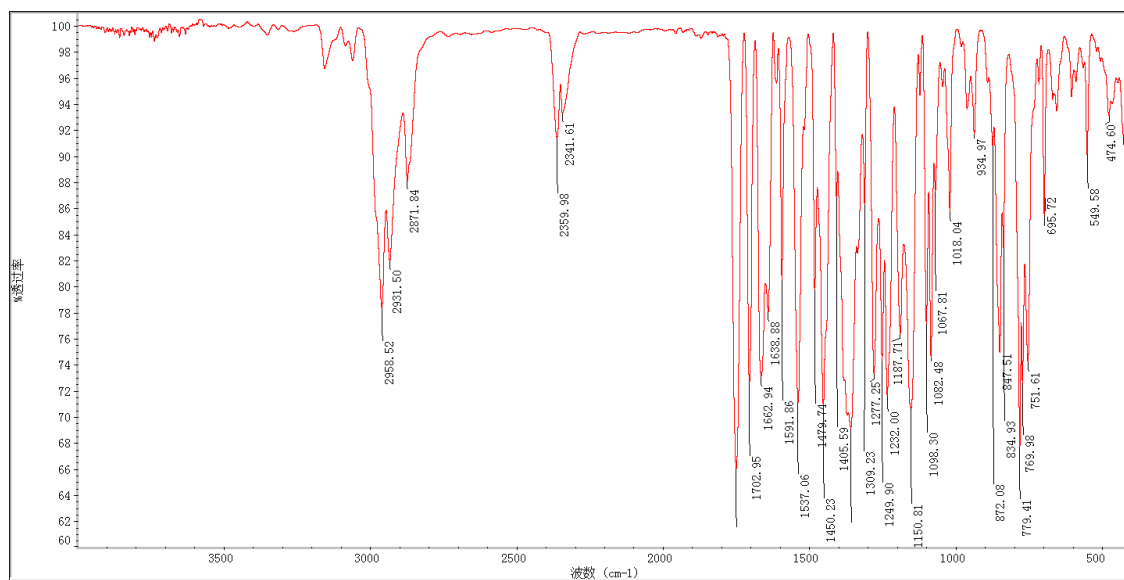
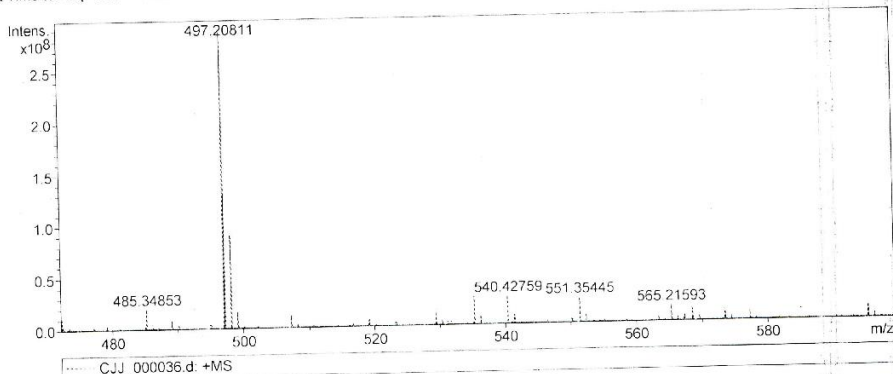


Fig. S15. IR spectra of compound **6**.

Mass Spectrum List Report

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Sample Name	NaTFA		
Comment			

Acquisition Parameter				No. of Laser Shots	200
Polarity	Positive	n/a	n/a	Laser Power	20.0 lp
n/a	n/a	No. of Cell Fills	1	n/a	n/a
Broadband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Broadband High Mass	1200.0 m/z	n/a	n/a		
Acquisition Mode	Single MS	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Pulse Program	basic	n/a	n/a	Data Acquisition Size	1048576
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Ion Accumulation Time	0.200 sec	n/a	n/a	Apodization	Apodization
Flight Time to Acq. Cell	0.001 sec	n/a	n/a		



m/z	z	I	Res.
157.45187		11513903	109067
165.73875		9311901	114822
261.05937		30511410	101238
261.06409		376113472	59618
262.06738		32451198	60645
275.08003		58617804	52381
279.05375		19813714	56485
282.27917		13236452	53010
285.03130		18594012	52268
287.02810		9878214	54185
300.19870		13634037	51850
316.21177		16552447	45654
325.05939		30607434	43178
338.34170		68011648	43205
339.07503		15579393	43645
339.34492		14382479	44770
362.29762		12409971	42003
367.09667		15062013	70219
367.10634		172827648	38021
368.10983		28071992	38973
369.16970		15158669	36446
371.28098		20687158	37338
383.24701		24846236	33407
387.18099		35045952	32556

Fig. S16. ESI mass spectra of compound **6**.

5. ^1H , ^{13}C NMR, IR and HRMS-ESI copies of the compound 7.

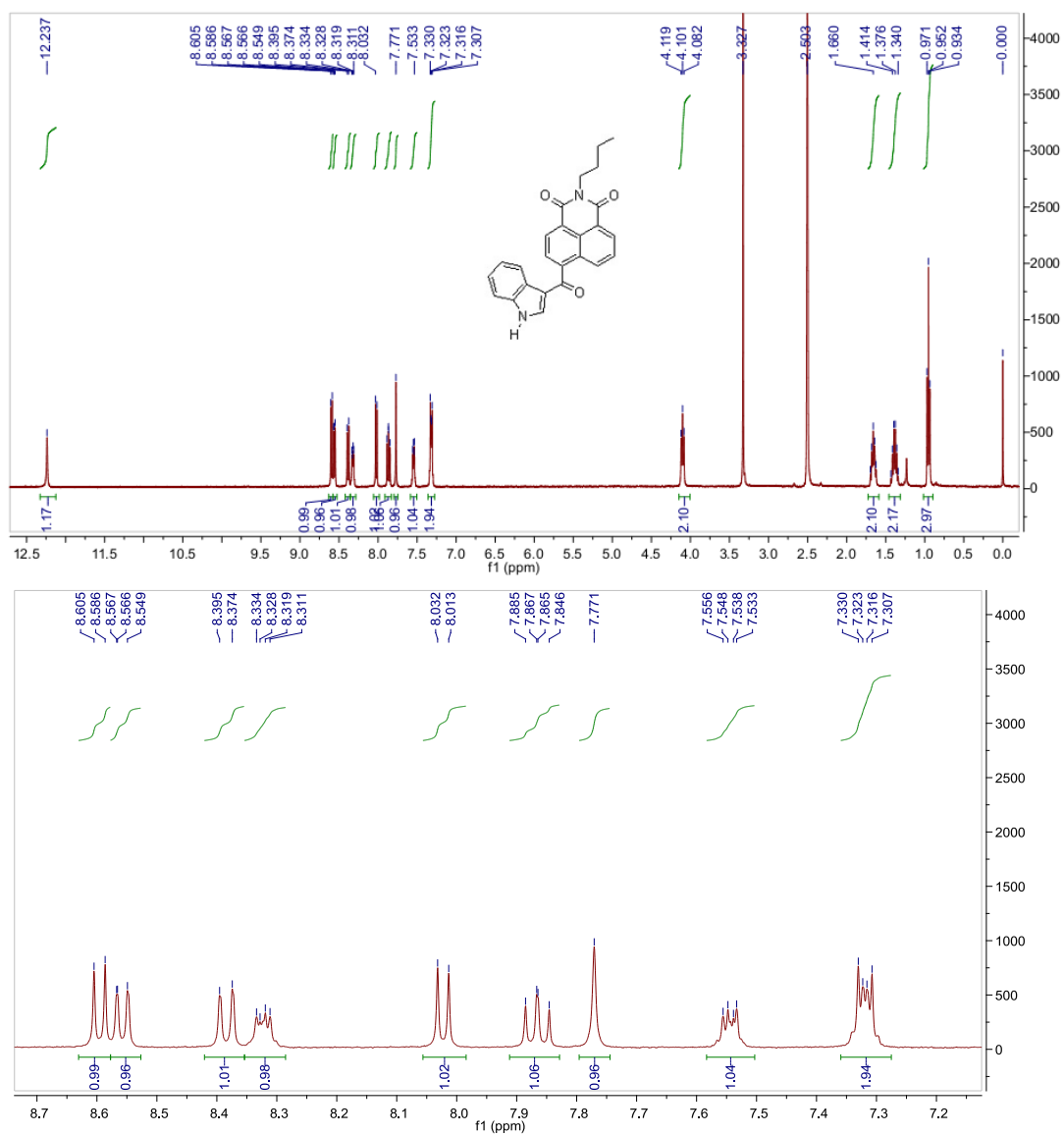


Fig. S17. ^1H NMR (CDCl_3 , 400 MHz) spectra of compound 7.

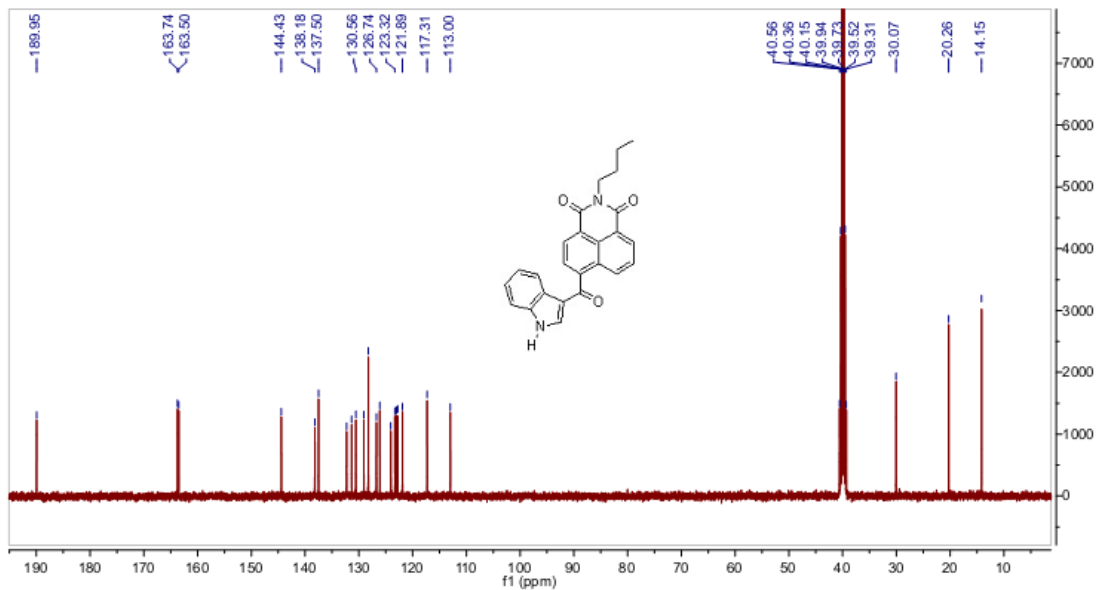


Fig. S18. ^{13}C NMR (DMSO- d_6 , 100 MHz) spectra of compound **7**

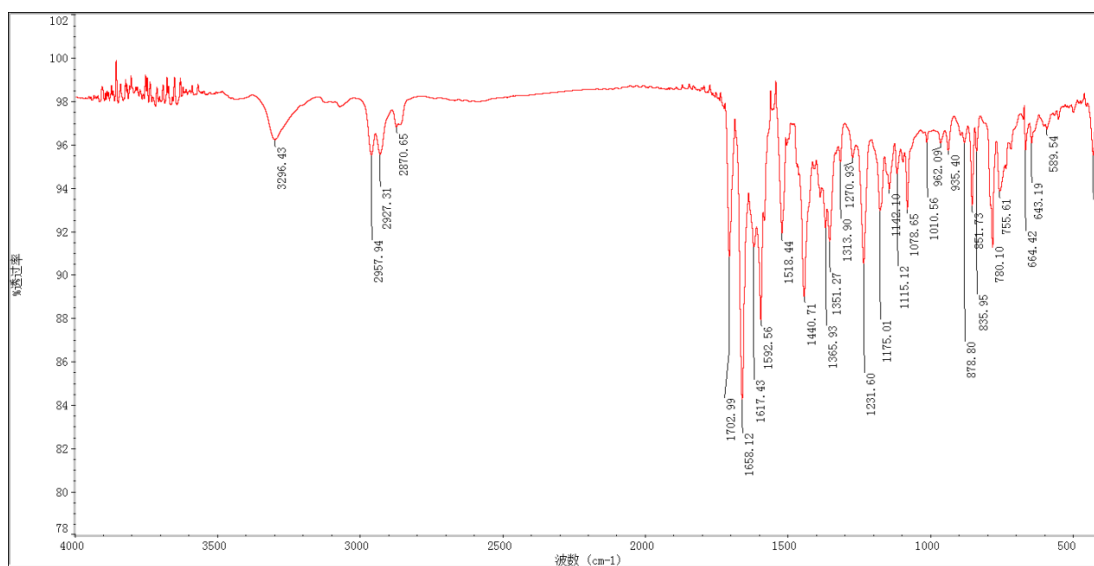


Fig. S19. IR spectra of compound **7**

Mass Spectrum List Report

Analysis Info

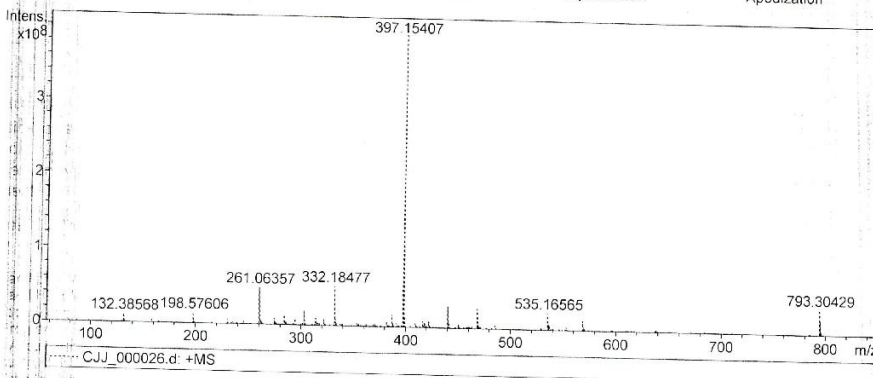
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 Method 4_19_MassAccuNeg
 Sample Name NaTFA
 Comment

Acquisition Date 4/24/2014 1:10:33 PM

Operator
 Instrument solariX

Acquisition Parameter

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n/a	n/a	No. of Cell Fills	1	Laser Power	20.0 lp
Bandband Low Mass	53.8 m/z	n/a	n/a	n/a	n/a
Bandband High Mass	1200.0 m/z	n/a	n/a	n/a	n/a
Acquisition Mode	Single MS	n/a	n/a	Calibration Date	Fri Feb 21 02:36:54 2014
Pulse Program	basic	n/a	n/a	Data Acquisition Size	1048576
Source Accumulation	0.010 sec	n/a	n/a	Apodization	Sine-Bell Multiplication
Ion Accumulation Time	0.200 sec	n/a	n/a	Apodization	Apodization
Flight Time to Acq. Cell	0.001 sec	n/a	n/a		



m/z	z	I	Res.
132.38568		11626266	138945
158.77817		3380649	60870
198.57606		14399388	86862
198.57874		7347356	90943
198.58130		2976861	102006
199.07766		3634269	87905
231.06398		6031502	69414
238.22368		3977453	69591
248.06376		3864465	67264
261.06357		49759772	61176
262.06698		4915223	61518
263.09926		4114455	61345
275.07931		8288082	57924
278.59891		2574379	59632
284.59629		13249763	54940
285.09832		5747763	59520
285.59483		5251074	55683
288.17936		4150880	54423
293.10799		2625682	54649
295.08745		8835277	54211
302.05383		3253227	54075
303.19472		19620812	51265
304.19789		4157205	50467
314.17429		10368756	49619

Bruker Compass DataAnalysis 4.0

printed: 4/24/2014 4:52:43 PM

Page 1 of 2

Fig. S20. ESI mass spectra of compound 7

6. Interference experiments of **5** toward cyanide.

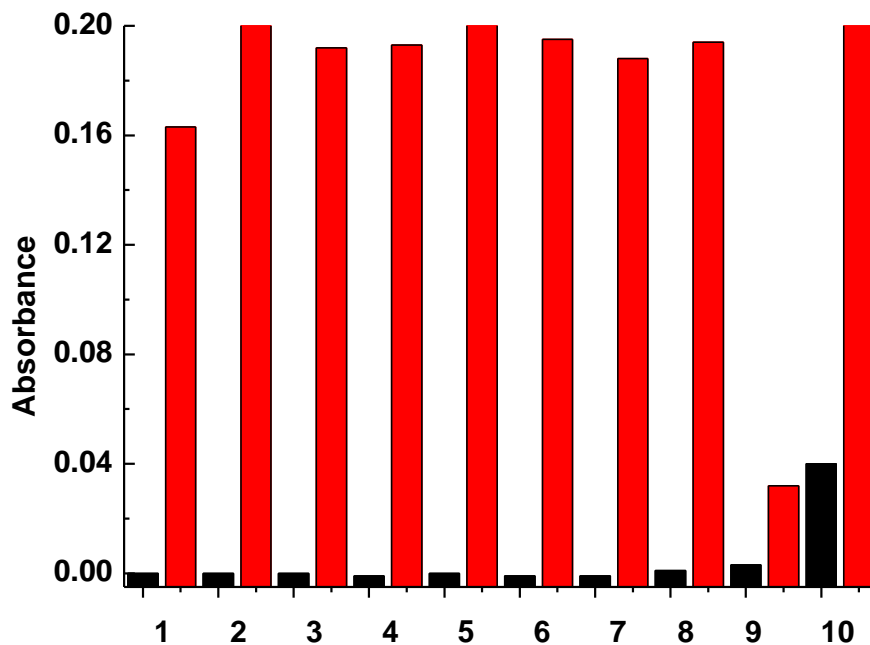


Fig. S21 Absorbance responses of **5** toward cyanide and other anions (30 eq, from left to right: HSO_4^- , H_2PO_4^- , Cl^- , Br^- , I^- , NO_3^- , BF_4^- , ClO_4^- , AcO^- , F^-) in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (9:1, v/v). Black and red bars represent the absorbance of **5** ($20 \mu\text{M}$) in the presence of various analytes before and after addition of CN^- , respectively.

7. The UV detection limit of the probe 5 with CN⁻.

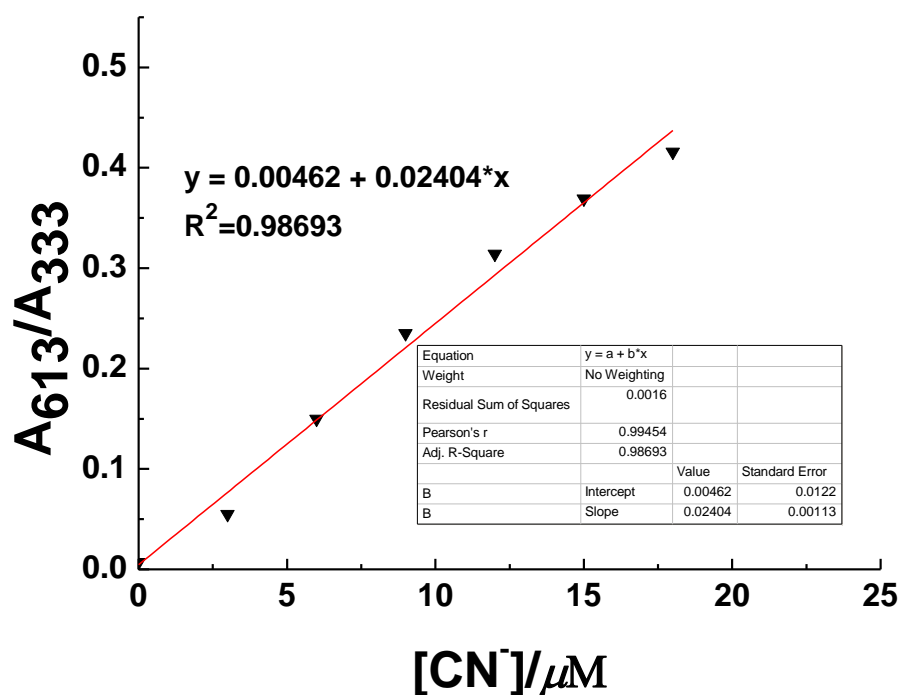


Fig. S22. Absorbance intensity ratio (A_{610}/A_{333}) of dosimeter **5** ($20 \mu\text{M}$) as a function of CN⁻ concentration from 0- $42 \mu\text{M}$ (0–2.1 equiv),

SD	N
0.01791	7

The result of the analysis as follows:

Linear Equation : $y = 0.00462 + 0.02404 * x$, $R^2 = 0.98693$

$S = 2.404 * 10^4$, $K = 3$, $\delta = 0.01791$

$\text{LOD} = K * \delta/S = 2.235 \mu\text{M}$

8. The UV detection limit of the probe 4 with CN⁻.

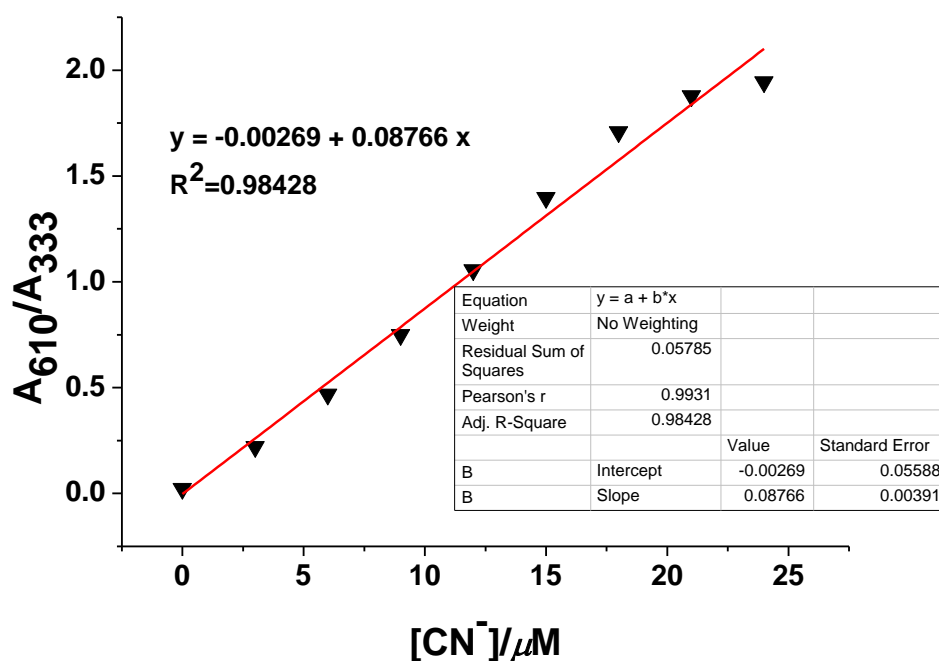


Fig. S23. Absorbance intensity ratio (A_{610}/A_{333}) of dosimeter 4 ($20 \mu\text{M}$) as a function of CN^- concentration from 0- $27 \mu\text{M}$ (0–1.35 equiv),

SD	N
0.09091	9

The result of the analysis as follows:

Linear Equation : $y = -0.00269 + 0.08766 * x$, $R^2 = 0.98428$

$S = 8.766 * 10^{-4}$, $K = 3$, $\delta = 0.09091$

$\text{LOD} = K * \delta/S = 3.11 \mu\text{M}$

9. UV-visible titration of 5 with $\text{Bu}_4\text{N}^+\text{CN}^-$ in CH_3CN .

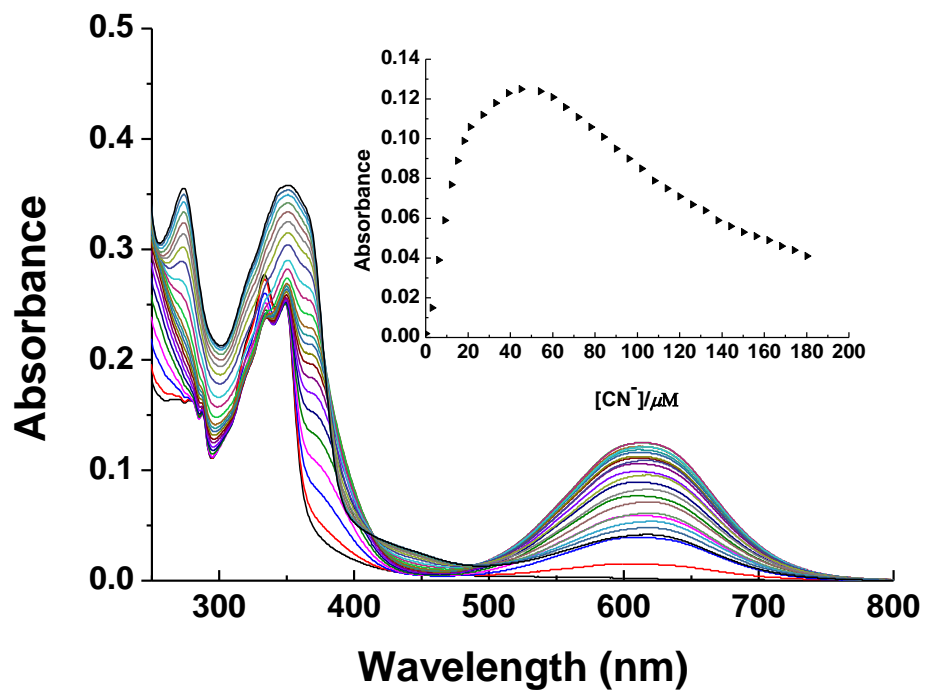


Fig. S24. UV-visible titration of 5 (20 μM) with $\text{Bu}_4\text{N}^+\text{CN}^-$ in CH_3CN . The inset shows the absorbance at 610 nm as a function of $[\text{CN}^-]$.

10. UV-visible titration of 4 with $\text{Bu}_4\text{N}^+\text{CN}^-$ in CH_3CN .

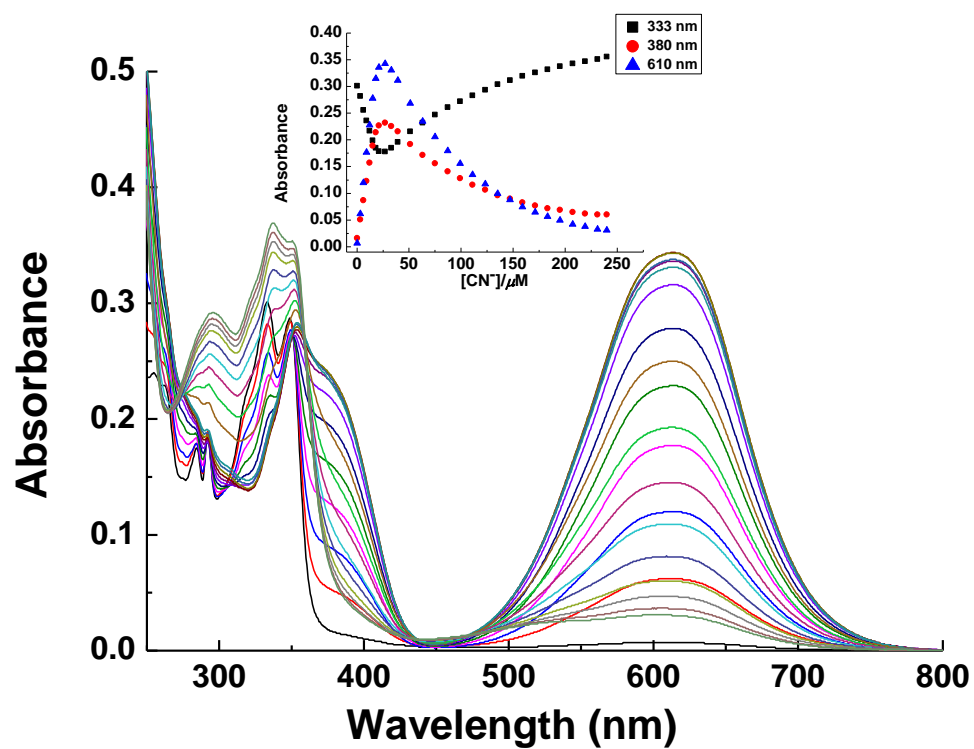


Fig. S25. UV-visible titration of 4 (20 μM) with $\text{Bu}_4\text{N}^+\text{CN}^-$ in CH_3CN . The inset shows the absorbance at 333, 380 and 610 nm as a function of $[\text{CN}^-]$.

11. Fluorescence spectra of 5 in the presence of different anions in CH₃CN.

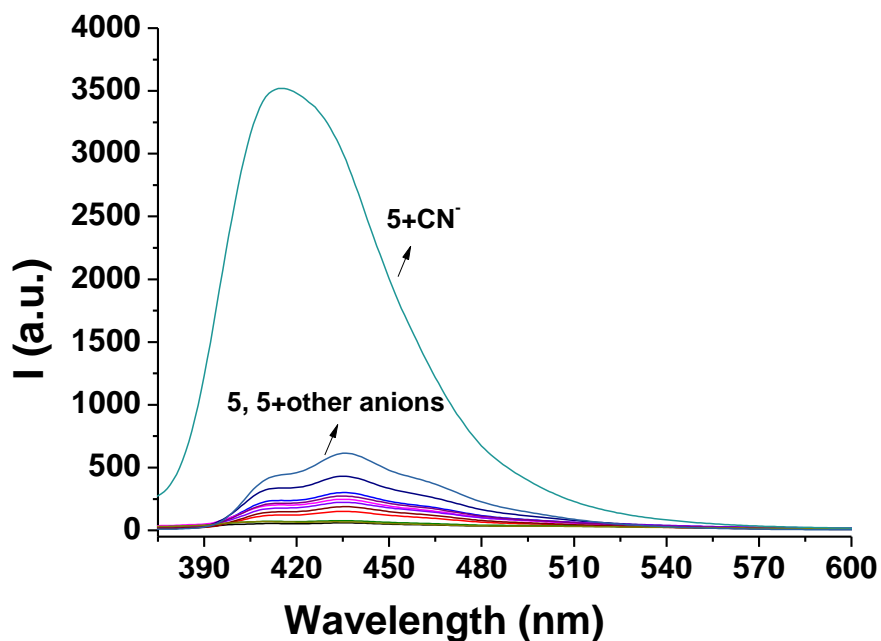


Fig. S26. Fluorescence spectra of 5 (20 μ M) in the presence of different anions (ca. 24 equiv) in CH₃CN.

12. Comparative table of this sensor with others.

Table S1. Comparison of this sensor with others

	Fluorescence change	Recognition site
J. Org. Chem. (2011) 76, 6962	On-Off	Single: C=C double bonds
Org. Lett. (2006) 8, 5721	On-Off	Single: C=C double bonds
Chem. Lett. (2011) 40, 623	On-Off	Single: C=C double bonds
J. Org. Chem. (2009) 74, 7496	On-Off	Single: C=N double bonds
Org. Lett. (2008) 10, 461	On-Off	Single: C=O double bonds
Tetrahedron Lett. (2008) 49, 5544	On-Off	Single: C=O double bonds
This sensor	Off-On	Dual deprotonation: CH (irreversible) and NH (reversible)

13. UV-visible titration of 7 with $\text{Bu}_4\text{N}^+\text{CN}^-$ in CH_3CN .

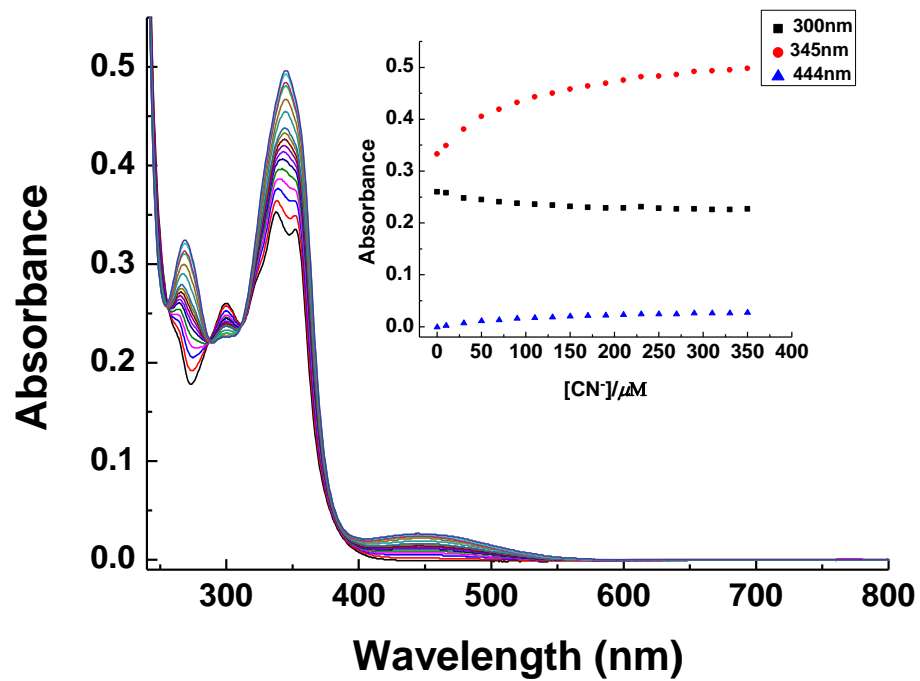


Fig. S27. UV-visible titration of 7 (20 μM) with $\text{Bu}_4\text{N}^+\text{CN}^-$ in CH_3CN . The inset shows the absorbance at 300, 345 and 444 nm as a function of $[\text{CN}^-]$.

14. The fluorescence detection limit of probe 5 with CN^- .

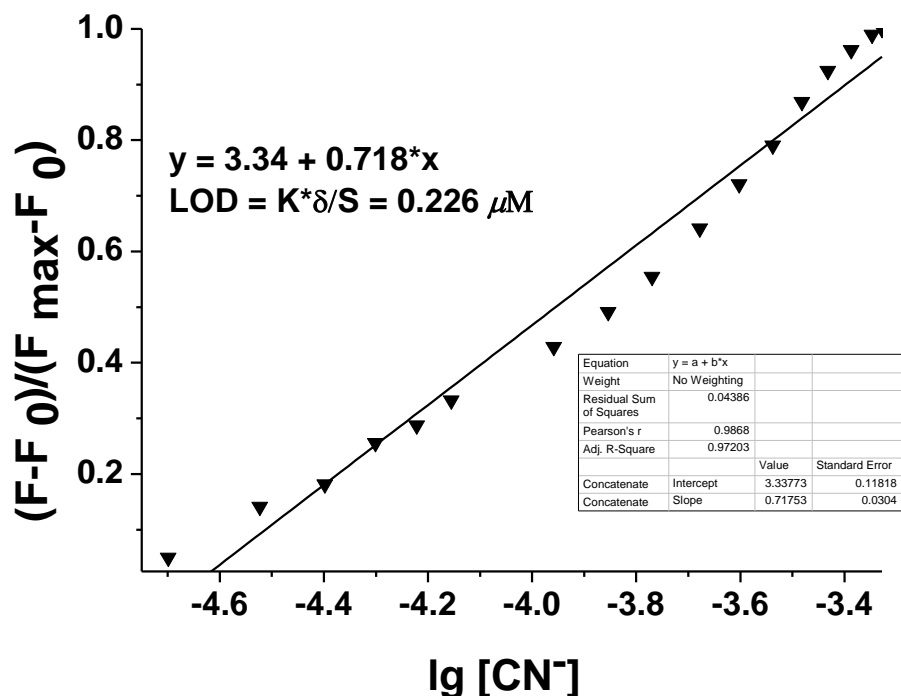


Fig. S28. Response of fluorescence intensity to changing CN^- concentrations in CH_3CN . ($[\text{5}] = 20 \mu\text{M}$, $[\text{CN}^-] = 5000 \mu\text{M}$, $\lambda_{\text{ex}} = 353 \text{ nm}$, $\lambda_{\text{em}} = 373 \text{ nm}$).

SD	N
0.05407	16

The result of the analysis as follows:

Linear Equation : $y = 3.34 + 0.718 * x$, $R^2 = 0.97203$

$S = 7.18 * 10^5$, $K = 3$, $\delta = 0.05407$

$\text{LOD} = K * \delta/S = 0.226 \mu\text{M}$

15. Linear fluorescence response of probe 5 to CN^- .

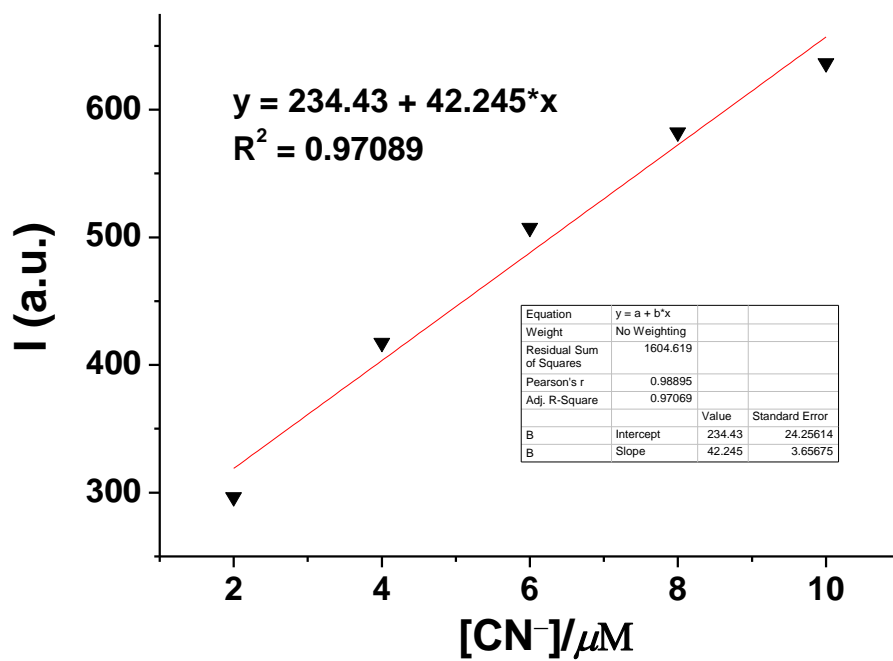


Fig. S29. Linear fluorescence response of probe 5 to CN^- concentration ranging from 0 to 10 μM .

16. Fluorescence spectra of compound 5 with NaCN in mixture solvents

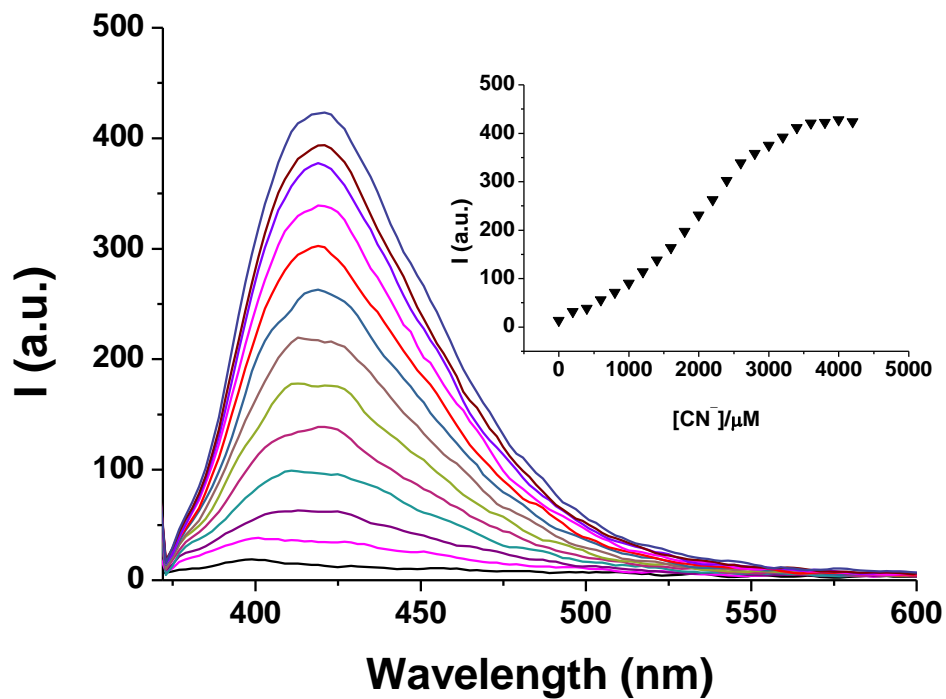


Fig. S30. Fluorescence spectra of compound **5** (20 μM) upon gradual addition of NaCN (0-200 equiv) in CH₃CN/H₂O (95/5; v/v) with excitation at 353 nm. Inset: Plot of fluorescence intensity ($\lambda_{\text{ex}}=420\text{nm}$).

17. Fluorescence spectra of compound 5 with $\text{Bu}_4\text{N}^+\text{CN}^-$ in mixture solvents

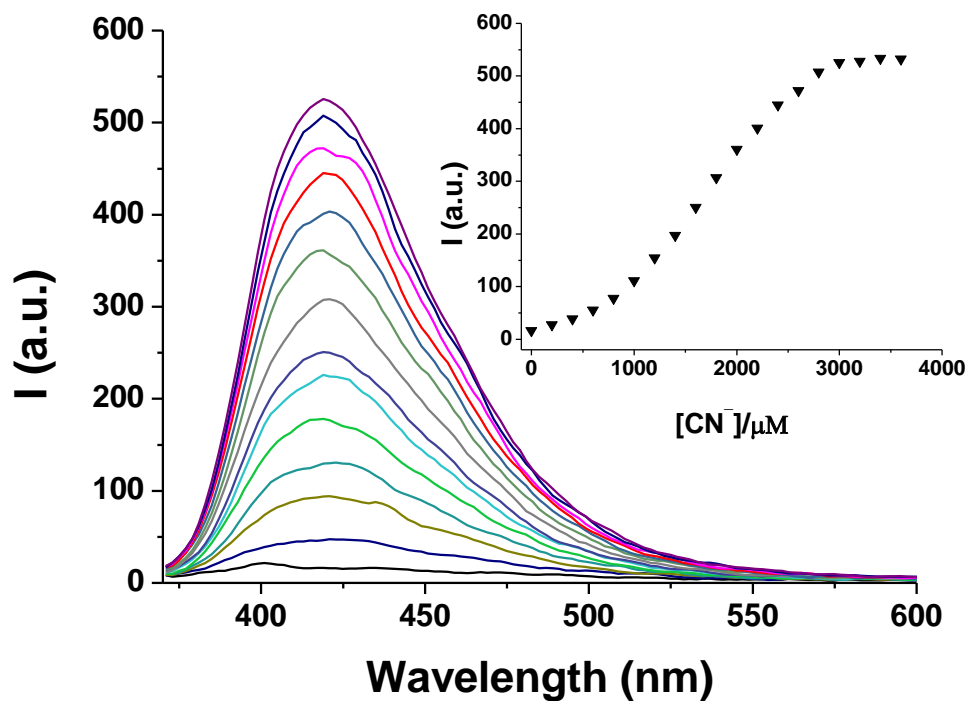


Fig. S31. Fluorescence spectra of compound 5 (20 μM) upon gradual addition of $\text{Bu}_4\text{N}^+\text{CN}^-$ (0-200 equiv) in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (95/5; v/v) with excitation at 353 nm. Inset: Plot of fluorescence intensity ($\lambda_{\text{ex}}=420\text{nm}$).

18. The pH-dependent behaviour of the probe 5 in different pH values.

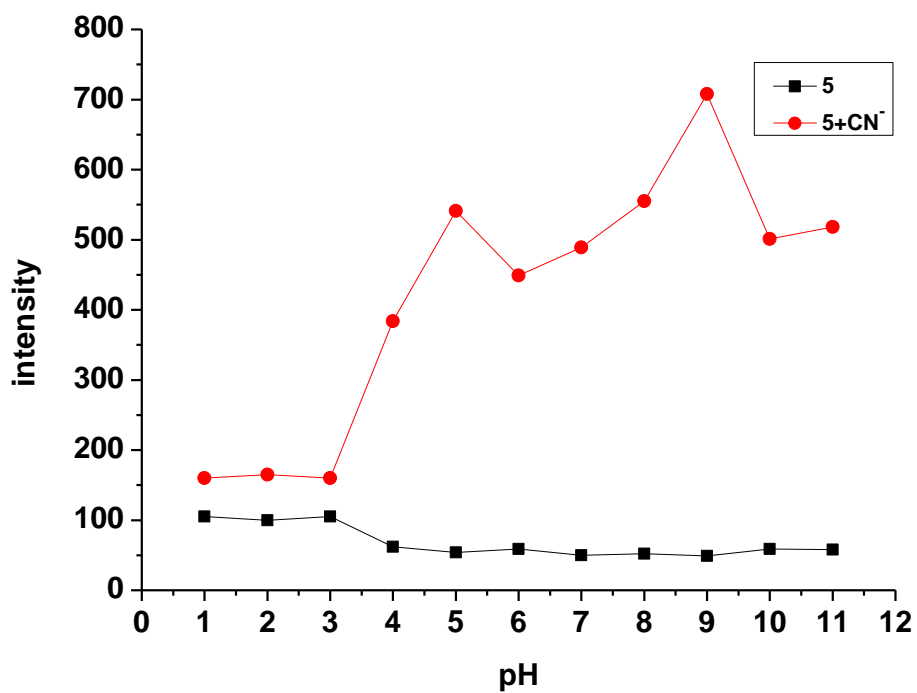


Fig. S32 Before and after the addition of cyanide ions respectively, the effect of pH on the fluorescence responses (415 nm) of **5** (20 μ M) in CH₃CN/H₂O (9:1, v/v). The pH of solution was adjusted by aqueous solution of NaOH (aq, 1 M) or HCl (aq, 1 M); λ_{ex} = 353 nm.

19. Confocal microscopic images of RAW 264.7 Macrophages cells

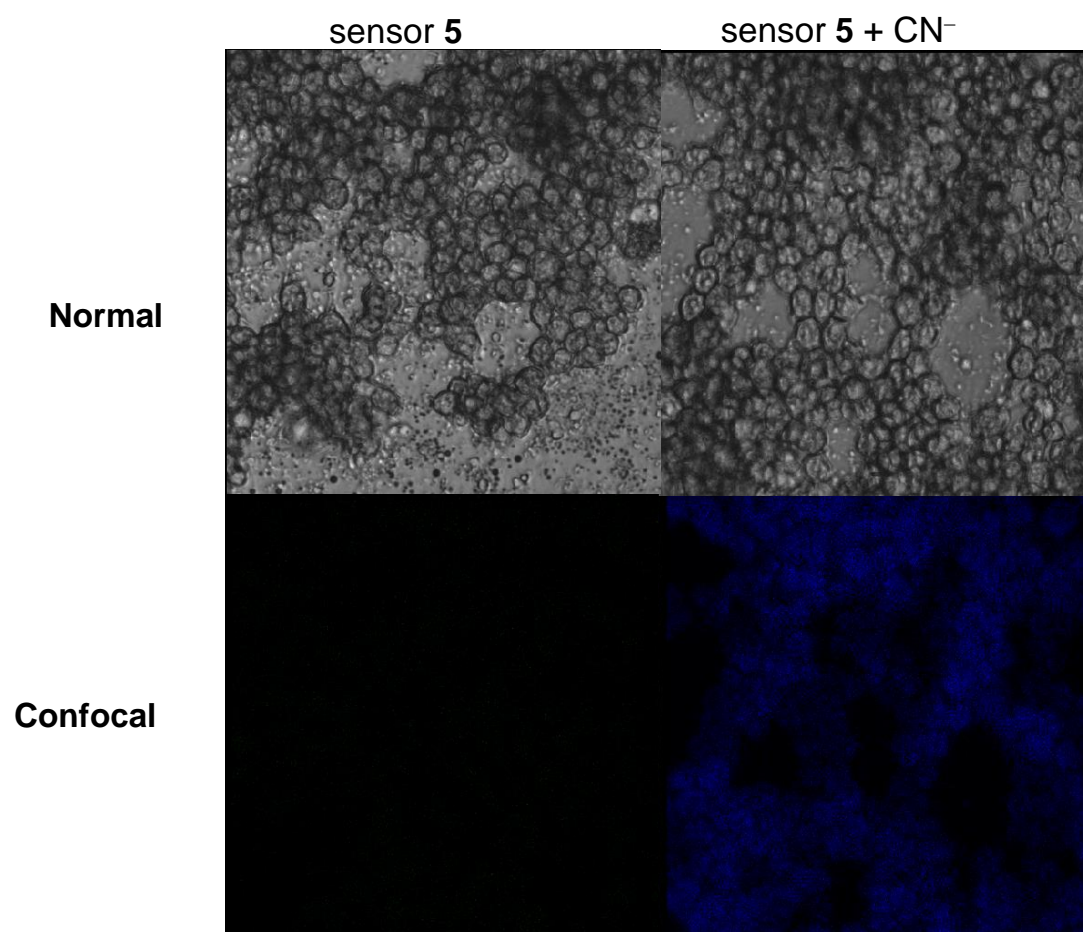


Fig. S33. Confocal fluorescence microscope images of RAW 264.7 Macrophages cells in the presence of sensor **5** ($100\ \mu\text{M}$). The fluorescence images were recorded after 10 min of treatment of CN⁻ ($100\ \mu\text{M}$) at $37\ ^\circ\text{C}$.

20. The detailed ^1H NMR titration of **7** and **5** with F^- (6–10 ppm)

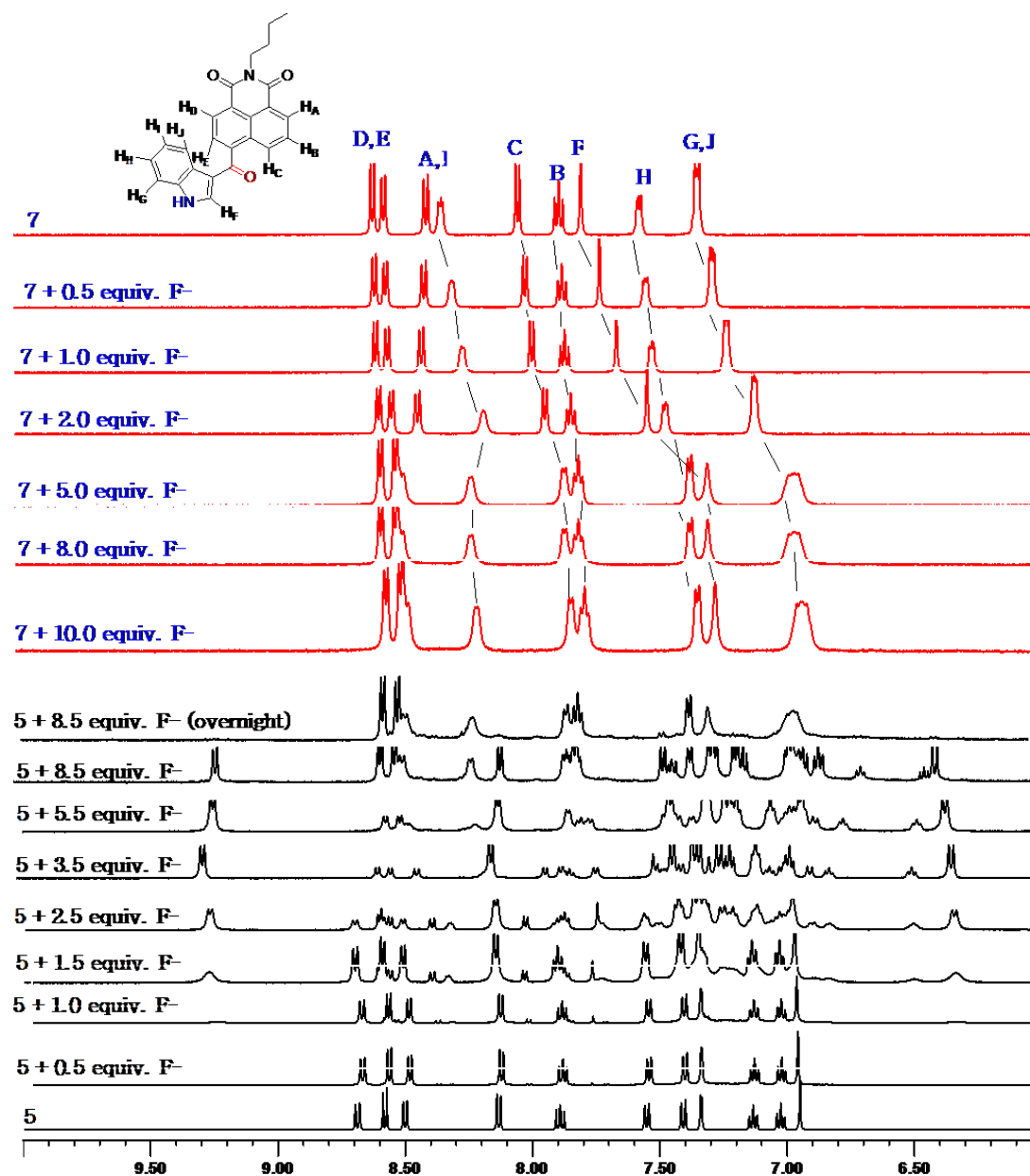


Fig. S34. Plots of ^1H NMR spectra of **7** (red) and **5** (black) on addition of F^- in $\text{DMSO}-d_6$ (expanded the region of 6–10 ppm).

21. The ^1H NMR titration of **5** with CN^-

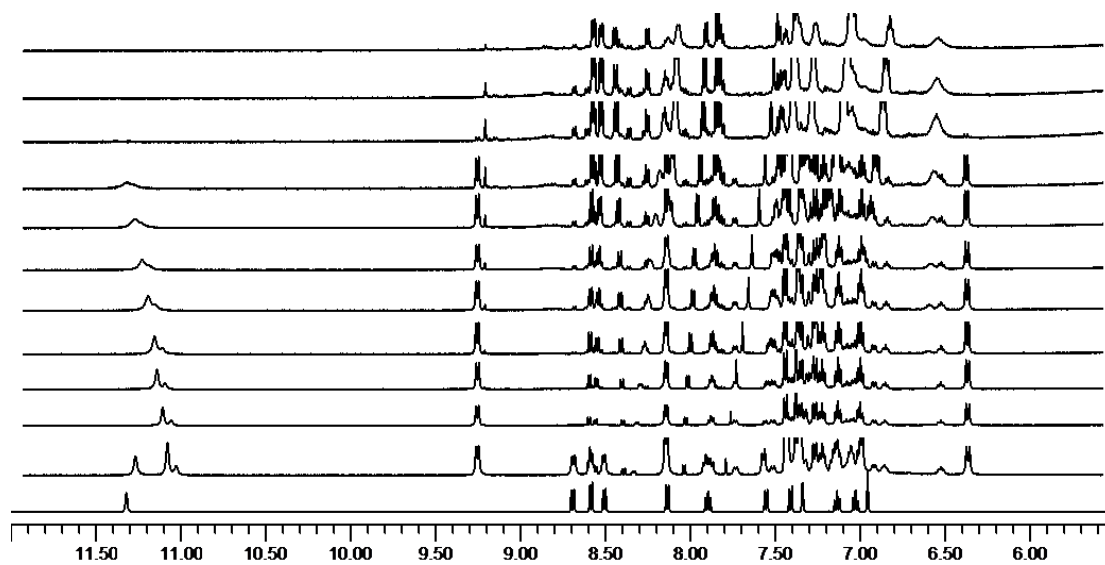


Fig. S35. Plots of ^1H NMR spectra of **5** (red, 3.0×10^{-2} mol/L) on addition of CN^- in $\text{DMSO-}d_6$ (from bottom to top, CN^- equiv. = 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 11.0 (overnight), 11.0 (after 24 hours).

22. The ^1H NMR titration of **7** with CN^-

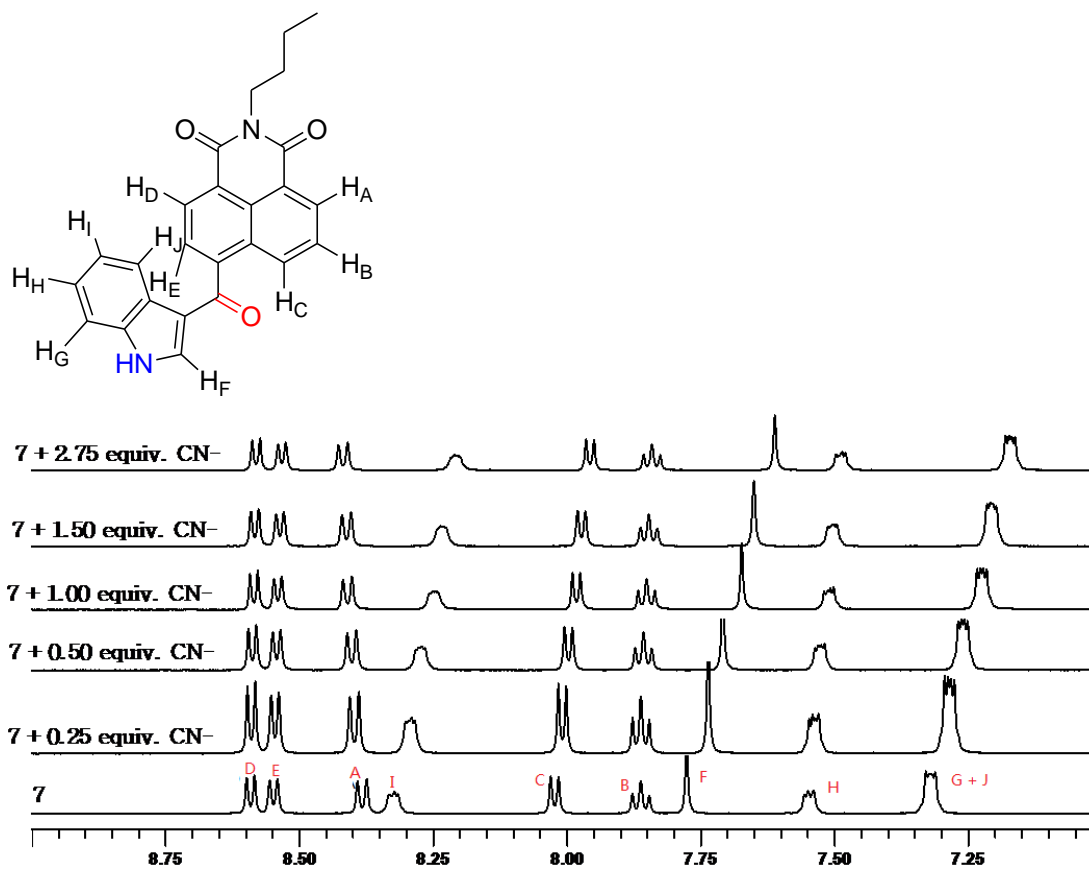


Fig. S36. Plots of ^1H NMR spectra of **7** (3.0×10^{-2} mol/L) on addition of CN^- in $\text{DMSO-}d_6$.

23. The final state of compound 5 and 7 with F^- and CN^- .

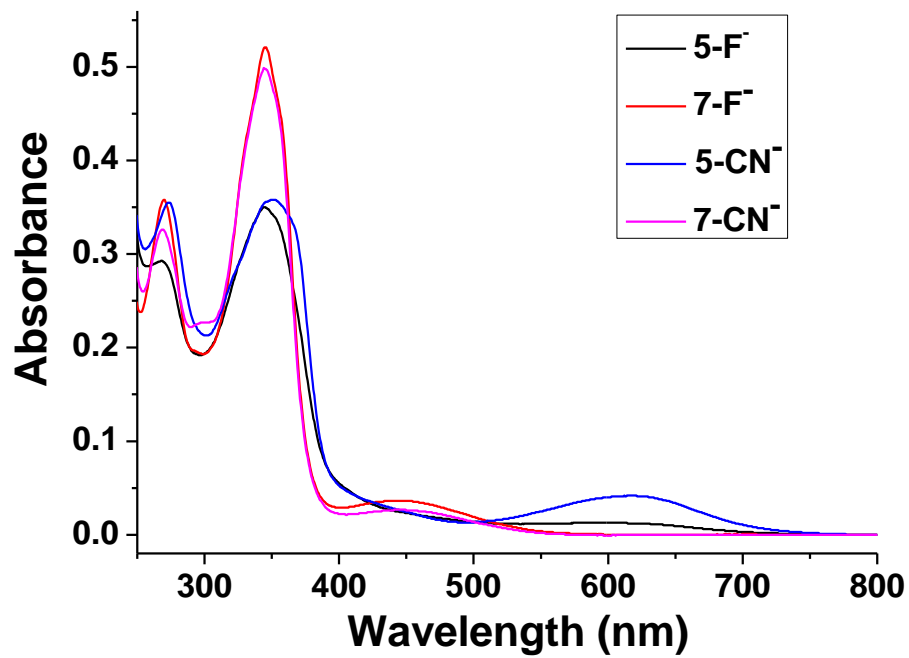


Fig. S37. UV-vis spectra of 5 and 7 after addition of F^- and CN^- in CH_3CN (The final state).