Supporting Information for

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

Chuanxiu Zhang, a,b Chuanxiang Liu,* a Baiyun Li, a,b Jinju Chen, a Hua Zhang,* a Zhou Hu a and Fengping Yi,* b

a School of Chemical and Environmental Engineering, Shanghai Institute of Technology, 201418 Shanghai, China
b School of Perfume and Aroma Technology, Shanghai Institute of Technology, 201418 Shanghai, China
e-mail: cxliu@sit.edu.cn; yifengping@sit.edu.cn; zhanghua@sit.edu.cn

Table of Contents

1. 1H, 13C NMR, IR and HRMS-ESI copies of the compound 2 (Fig.S1-S4)..........................S1
2. 1H, 13C NMR, IR and HRMS-ESI copies of the dosimeter 4 (Fig.S5-S8)..........................S4
3. 1H, 13C NMR, IR and HRMS-ESI copies of the dosimeter 5 (Fig.S9-S12)..........................S7
4. 1H, 13C NMR, IR and HRMS-ESI copies of the compound 6 (Fig.S13-S16)......................S10
5. 1H, 13C NMR, IR and HRMS-ESI copies of the compound 7 (Fig.S17-S20)......................S13
6. Interference experiments of 5 toward cyanide (Fig.S21)........................................S16
7. The UV detection limit of the probe 5 with CN− (Fig.S22)........................................S17
8. The UV detection limit of the probe 4 with CN− (Fig.S23)........................................S18
9. UV-visible titration of 5 with Bu₄N⁺CN− in CH₃CN (Fig.S24).....................................S19
10. UV-visible titration of 4 with Bu₄N⁺CN− in CH₃CN (Fig.S25)...................................S20
11. Fluorescence spectra of 5 in the presence of different anions in CH₃CN (Fig.S26)..........S21
12. Comparative table of this sensor with others (Table.S1)........................................S21
13. UV-visible titration of 7 with Bu₄N⁺CN− in CH₃CN (Fig.S27)...................................S21
14. The fluorescence detection limit of probe 5 with CN− (Fig.S28).................................S23
15. Linear fluorescence response of probe 5 to CN− (Fig.S29)......................................S24
16. Fluorescence spectra of compound 5 with NaCN in mixture solvents (Fig.S30)..........S25
17. Fluorescence spectra of compound 5 with in Bu₄N⁺CN− mixture solvents (Fig.S31)......S26
18. The pH-dependent behaviour of the probe 5 in different pH values (Fig.S32).............S27
19. Confocal microscopic images of RAW 264.7 Macrophages cells (Fig.S33)..................S28
20. The detailed 1H NMR titration of 7 and 5 with F− (6–10 ppm) (Fig.S34).....................S29
21. The 1H NMR titration of 5 with CN− (Fig.S35)..................................................S30
22. The 1H NMR titration of 7 with CN− (Fig.S36)..................................................S31
23. The final state of compound 5 and 7 with F− and CN− (Fig.S37)...............................S32
1. $^1$H, $^{13}$C NMR, IR and HRMS-ESI copies of the compound 2.

Fig. S1. $^1$H 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.
Fig. S4. ESI mass spectra of compound 2.
2. $^1$H, $^{13}$C NMR, IR and HRMS-ESI copies of the dosimeter 4

Fig. S5. $^1$H 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.
Fig. S8. ESI mass spectra of dosimeter 4.
3. $^1$H, $^{13}$C NMR, IR and HRMS-ESI copies of the dosimeter 5

![NMR Spectra](image)

Fig. S9. $^1$H NMR (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.
Fig. S12. ESI mass spectra of dosimeter 5.
4. $^1$H, $^{13}$C NMR, IR and HRMS-ESI copies of the compound 6.

**Fig. S13.** $^1$H 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.
Fig. S16. ESI mass spectra of compound 6.
5. $^1$H, $^{13}$C NMR, IR and HRMS-ESI copies of the compound 7.

Fig. S17. $^1$H 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
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$_2$PO$_4^-$, Cl$^-$, Br$^-$, I$^-$, NO$_3^-$, BF$_4^-$, ClO$_4^-$, AcO$^-$, F$^-$) in CH$_3$CN/H$_2$O (9:1, v/v). Black and red bars represent the absorbance of 5 (20 μM) in the presence of various analytes before and after addition of CN$^-$, respectively.
7. The UV detection limit of the probe 5 with CN⁻.

![Graph showing absorbance intensity ratio (A613/A333) as a function of CN⁻ concentration.]

**Figure S22.** Absorbance intensity ratio ($A_{613}/A_{333}$) of dosimeter 5 (20 μM) as a function of CN⁻ concentration from 0-42 μM (0–2.1 equiv).

<table>
<thead>
<tr>
<th>SD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01791</td>
<td>7</td>
</tr>
</tbody>
</table>

The result of the analysis as follows:

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

**Regression:**
- $S = 2.404 \times 10^4$, $K = 3$, $\delta = 0.01791$
- LOD = $K \times \delta/S = 2.235 \mu$M
8. The UV detection limit of the probe 4 with CN⁻.

**Fig. S23.** Absorbance intensity ratio (A₆₁₀/A₃₃₃) of dosimeter 4 (20 μM) as a function of CN⁻ concentration from 0–27 μM (0–1.35 equiv),

<table>
<thead>
<tr>
<th>SD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.09091</td>
<td>9</td>
</tr>
</tbody>
</table>

The result of the analysis as follows:

Linear Equation : \( y = -0.00269 + 0.08766 \times x \), \( R^2 = 0.98428 \)

\( S = 8.766 \times 10^4 \), \( K = 3 \), \( \delta = 0.09091 \)

LOD = \( K \times \delta / S = 3.11 \mu \text{M} \)
9. UV-visible titration of 5 with Bu₄N⁺CN⁻ in CH₃CN.

Fig. S24. UV-visible titration of 5 (20 μM) with Bu₄N⁺CN⁻ in CH₃CN. The inset shows the absorbance at 610 nm as a function of [CN⁻].
10. UV-visible titration of 4 with Bu₄N⁺CN⁻ in CH₃CN.

Fig. S25. UV-visible titration of 4 (20 μM) with Bu₄N⁺CN⁻ in CH₃CN. The inset shows the absorbance at 333, 380 and 610 nm as a function of [CN⁻].
11. Fluorescence spectra of 5 in the presence of different anions in CH$_3$CN.

**Fig. S26.** Fluorescence spectra of 5 (20 μM) in the presence of different anions (ca. 24 equiv) in CH$_3$CN.

12. Comparative table of this sensor with others.

**Table S1.** Comparison of this sensor with others

<table>
<thead>
<tr>
<th></th>
<th>Fluorescence change</th>
<th>Recognition site</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>This sensor</strong></td>
<td><strong>Off-On</strong></td>
<td><strong>Dual deprotonation: CH (irreversible) and NH (reversible)</strong></td>
</tr>
</tbody>
</table>

521
13. UV-visible titration of 7 with Bu₄N⁺CN⁻ in CH₃CN.

**Fig. S27.** UV-visible titration of 7 (20 μM) with Bu₄N⁺CN⁻ in CH₃CN. The inset shows the absorbance at 300, 345 and 444 nm as a function of [CN⁻].
The fluorescence detection limit of probe 5 with CN$^-$. The result of the analysis as follows:

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

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

$LOD = K \times \frac{\delta}{S} = 0.226 \mu M$

**Fig. S28.** Response of fluorescence intensity to changing CN$^-$ concentrations in CH$_3$CN. ([5] = 20 μM, [CN$^-$] = 5000 μM, $\lambda_{ex} = 353$ nm, $\lambda_{em} = 373$ nm).
15. Linear fluorescence response of probe 5 to CN$^-$.

\[ y = 234.43 + 42.245x \]

\[ R^2 = 0.97089 \]

Fig. S29. Linear fluorescence response of probe 5 to CN$^-$ concentration ranging from 0 to 10 $\mu$M.
16. Fluorescence spectra of compound 5 with NaCN in mixture solvents

**Fig. S30.** Fluorescence spectra of compound 5 (20μM) upon gradual addition of NaCN (0-200 equiv) in CH₃CN/H₂O (95/5; v/v) with excitation at 353nm. Inset: Plot of fluorescence intensity ($\lambda_{ex}=420$nm).
Fig. S31. Fluorescence spectra of compound 5 (20μM) upon gradual addition of Bu₄N⁺CN⁻ (0-200 equiv) in CH₃CN/H₂O (95/5; v/v) with excitation at 353nm. Inset: Plot of fluorescence intensity (λₑₓ=420nm).
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 μM) 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); λₑₓ = 353 nm.
Fig. S33. Confocal fluorescence microscope images of RAW 264.7 Macrophages cells in the presence of sensor 5 (100 μM). The fluorescence images were recorded after 10 min of treatment of CN\(^{-}\) (100 μM) at 37 °C.
20. The detailed $^1$H NMR titration of 7 and 5 with F$^-$ (6–10 ppm)

Fig. S34. Plots of $^1$H NMR spectra of 7 (red) and 5 (black) on addition of F$^-$ in DMSO-$d_6$ (expanded the region of 6–10 ppm).
21. The $^1$H NMR titration of 5 with CN$^-$

**Fig. S35.** Plots of $^1$H NMR spectra of 5 (red, $3.0 \times 10^{-2}$ mol/L) on addition of CN$^-$ in 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, 11.0 (overnight), 11.0 (after 24 hours).
22. The $^1$H NMR titration of 7 with CN$^-$

Fig. S36. Plots of $^1$H NMR spectra of 7 ($3.0 \times 10^{-2}$ mol/L) on addition of CN$^-$ in 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 CH₃CN (The final state).