Supporting information

A spirooxazine derivative as a highly sensitive cyanide sensor by means of UV-visible difference spectrum

Shaoyin Zhu\textsuperscript{a,b}, Minjie Li\textsuperscript{a*}, Lan Sheng\textsuperscript{a}, Peng Chen\textsuperscript{a}, Yumo Zhang\textsuperscript{a} and Sean Xiao-An Zhang\textsuperscript{a*}

\textsuperscript{a} State Key Lab of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, People’s Republic of China. Fax: +86-431-85153812; E-mail: seanzhang@jlu.edu.cn

\textsuperscript{b} State Key Laboratory of Fine Chemicals, School of Chemical Engineering, Dalian University of Technology, Dalian, 116024, People’s Republic of China

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Electronic Supplementary Material (ESI) for Analyst

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1. Synthesis and characterization of P1, P2 and P3

1-1 Synthesis of 2-nitro-5a-(2-(4-dimethylaminophenyl)-ethylene)-6,6-dimethyl-5a,6-dihydro-12H-indolo[2,1-b][1,3]benzooxazine (P1)

P1 were synthesized according to literature S1 with improved procedures and higher yield. Detailed procedures were as follows:

1-(2-hydroxy-5-nitrobenzyl)-2,3,3-trimethylindoleninium chloride (0.51 mmol, 176.9 mg) and 4-dimethylaminobenzaldehyde (0.5 mmol, 74.5 mg) were refluxed in 10 ml ethanol solution for 3.5 h. Then the solvent was removed with rotary vacuum evaporator and treated with NaHCO₃ aqueous solution. Acetic ether was added to extract the product. Then the organic phase was separated and the solvent was distilled off under reduced pressure. The residue was recrystallized by acetic ether/hexane to get the product, orange solid (196 mg, 89%). ¹H NMR (300 MHz, DMSO-d₆): δ=1.35 (6H, s), 2.92 (6H, s), 4.8 (2H, s), 6.39 (1H, d, J=18 Hz), 6.67 (2H, d, J=9 Hz), 6.84 (1H, m), 6.86 (1H, d, J=18 Hz), 6.87 (1H, d, J=9 Hz), 6.94 (1H, d, J=6 Hz), 7.11 (1H, m), 7.24 (1H, d, J=9 Hz), 7.42 (2H, d, J=9 Hz), 7.93 (1H, dd, J=6 Hz), 8.09 (1H, d, J=3 Hz); ¹³C NMR (75 MHz, CDCl₃): δ=40.3, 40.6, 49.9, 108.7, 112.1, 117.6, 118.4, 120.1, 120.6, 122.3, 123.2, 123.7, 123.9, 127.5, 127.9, 136.2, 138.4, 140.2, 146.5, 150.7, 159.7. LC-HRMS: m/z 442.2121 [M+H], calculated 442.2125.

1-2 Synthesis of 10-[2-(4-dimethylaminophenyl)ethylene]-9,9-trimethyl-7-nitroindolino[2,1-b] oxazolidine (P2)

P2 was synthesized and purified as described in reference S2. Pale yellow solid was gained. ¹H NMR (300 MHz, CDCl₃): δ= 1.19 (s, 3H), 1.47 (s, 3H), 2.98 (s, 6H), 3.67 (m, 4H), 5.98 (1H, d, J = 16.2 Hz), 6.75 – 6.65 (m, 4H), 7.35 (2H, d, J = 8.1 Hz), 7.96 (s, 1H), 8.13 (1H, d, J = 7.5 Hz); LC-HRMS: m/z 380.1971 [M+H]⁺, calculated 380.1974. Melting point: 193.3-195.2 °C.
1-3 Synthesis of 2,8-Nitro-5a-(2-(4-dimethylaminophenyl)-ethylene)-6,6-dimethyl-5a,6-dihydro-12H-indolo[2,1-b][1,3]benzooxazine (P3)

2,8-dinitro-5a,6,6-trimethyl-5a,6-dihydro-12H-indolo-[2,1-b][1,3]benzooxazine (0.7 mmol, 247.8 mg) and 4-(N,N-dimethyl)benzaldehyde (1.4 mmol, 208.6 mg) were refluxed in 10 ml ethanol solution with CH$_3$SO$_3$H as catalyst (200 mg) for 12 h under N$_2$ atmosphere. The solvent was distilled off under reduced pressure. The residue was washed with saturated aqueous Na$_2$CO$_3$ solution until the pH of the aqueous solution was neutral, treated with aqueous KOH solution and extracted by ethyl acetate. Then, the organic phase was separated and dried by anhydrous Na$_2$SO$_4$. Ethyl acetate was evaporated to get the crude product. The crude product was purified by column chromatography to give P3 (190 mg, 56%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$= 1.58 (6H, s), 3.25 (6H, s), 5.76 (2H, s), 6.95 (2H, d, $J=8$ Hz), 7.04 (1H, d, $J=8$ Hz), 7.37 (1H, d, $J=16$ Hz), 7.72 (1H, d, $J=8$ Hz), 8.11 (3H, d, $J=8$ Hz), 8.24 (1H, s), 8.36 (1H, d, $J=8$ Hz), 8.49 (1H, d, $J=16$ Hz), 8.72 (1H, s); $^{13}$C NMR (75 MHz, D$_6$MSO): $\delta$=27.8, 43.9, 54.3, 95.1, 108.8, 113.6, 117.1, 122.1, 123.1, 123.7, 125.7, 128.1, 128.7, 129.1, 130.8, 133.3, 141.2, 144.1, 145.6, 145.9, 155.8, 158.0, 163.5; LC-HRMS: $m/z$ 487.1962 [M+H]$^+$, calculated 487.1976. Melt point:128-131$^\circ$C.
2. Characterization and Solution preparation details

2-1 Characterizations:
NMR spectra (¹H and ¹³C) were obtained using Varian 300M and Varian INOVA 400M spectrometer. Spectra were referenced to the residual proton solvent peaks using shifts reported by Gregory R. Fulmer et al. LC-HRMS was obtained by Agilent 1290-microTOF Q II. Melting point was determined using a SGW X-4B microscopy melting point apparatus (Shanghai). UV-visible absorption spectra were recorded with a Shimadzu UV-2550 PC double-beam spectrophotometer, path length was 1 cm. pH values were measured with Sartorius PB-21.

2-2 Preparation of NaH₂PO₄/Na₃PO₄ buffer solution
0.2 M aqueous solution of NaH₂PO₄ and Na₃PO₄ were prepared separately. And the two solutions were diluted to 0.02 M with water. The two diluted solutions were mixed and monitored with pH-meter until the pH value got 9.4.

2-3 Preparation of P₁ in buffered CH₃CH₂OH/H₂O (v/v, 2/8) solution
200 μl ethanol solution of P₁ was added into a flask and then 3.8 ml ethanol and 6 ml buffer solution (NaH₂PO₄/Na₃PO₄, pH 9.4) were added into another flask. The two samples were kept at 25 °C in water bath. After temperature of the samples was stabilized at 25 °C, they were mixed and shaken to get NaH₂PO₄/Na₃PO₄ buffered CH₃CH₂OH/H₂O (v/v, 2/8).

2-4 Difference UV-Vis absorption spectra measurements:
3 ml above P₁ solution was added to the reference cell and sample cell respectively. UV-visible absorption was measured subsequently to check the base line. Then 15μl or 30 μl tetrabutylammonium cyanide with different concentration in NaH₂PO₄/Na₃PO₄ buffer solution was added to the sample cell and shaken to mix them well. Then UV-Vis absorption spectra were recorded with time until the absorption stabilized. Other measurements with the interference anions were done in the same way.
3. UV-vis absorption spectra of P2 in CH₃CH₂OH/H₂O and in NaH₂PO₄/Na₃PO₄ buffered CH₃CH₂OH/H₂O solution

Fig. S1 UV-vis absorption spectra of molecule P2 (10 μM) in CH₃CH₂OH/H₂O (v/v, 2/8) and buffered CH₃CH₂OH/H₂O mixture (v/v, 2/8; NaH₂PO₄/Na₃PO₄ buffer, pH 9.4). Note: spectra of P2 in buffered solution were measured soon after it was prepared and we defined the first measurement in buffered CH₃CH₂OH/H₂O as 0 min, and 12 min were relative to this time.

4. UV-vis absorption spectra of P3 in CH₃CH₂OH/H₂O and in NaH₂PO₄/Na₃PO₄ buffered CH₃CH₂OH/H₂O solution

Fig. S2 UV-vis absorption spectra of molecule P3 (10 μM) in CH₃CH₂OH/H₂O (v/v, 4/6) and buffered CH₃CH₂OH/H₂O mixture (v/v, 2/8; NaH₂PO₄/Na₃PO₄ buffer, pH 9.4). Note: spectra of P3 in buffered solution were measured soon after it was prepared and we defined the first measurement in buffered CH₃CH₂OH/H₂O as 0 min, and other times were relative to this time.
5. UV-vis difference absorption spectra of P3 in CH₃CH₂OH/H₂O and in NaH₂PO₄/Na₃PO₄ buffered CH₃CH₂OH/H₂O solution for the selectivity to cyanide

![Absorption spectra of P1](image)

Fig. S3 Absorption spectra of P1 (10 μM) measured with only interference anions, such as NO₂⁻, Cl⁻, CH₃COO⁻, F⁻, NO₃⁻, N₃⁻, SO₄²⁻, SCN⁻, Br⁻, I⁻, ClO₄⁻, ClO₃⁻) (30 equiv) and S²⁻ (3equiv), and CN⁻ (3 equiv) together with other interference anions (30 equiv or 3equiv). The spectra were obtained after the absorption reached stable state.

6. ¹H NMR spectra and ¹³C NMR spectra

5-1 ¹H NMR spectrum of P1 (DMSO-d₆)

![Full spectra](image)

![Enlarged view](image)
5-2 $^{13}$C NMR spectrum of P1 (DMSO-$d_6$)
5-3 $^1$H NMR spectrum of P1-CN$^-$ (DMSO-$d_6$)

Full spectra

Enlarged view
$^1$H NMR spectrum of P2 (CDCl$_3$)
For there are both SP form and MC form of P3 in CD3CN, it is hard to resolve the spectra. 5-6 $^1$H NMR spectrum of P3 in presence of CH$_3$SO$_3$H was measured in (DMSO-$_d_6$) as show in 5-6.
5-6 $^1$H NMR spectrum of [P3-CH$_3$SO$_3^-$](DMSO-$d_6$)

Full spectra

Enlarged view
\[5-7^{13}C\text{ NMR spectrum of P3 (DMSO-}d_6)\]
7. LC-HRMS spectra of P1, P1-CN, P2 and P3

LC-HRMS of P1

LC-HRMS of P1-CN

LC-HRMS of P2

LC-HRMS of P3

8. References

