Supplementary Information

A Highly Selective Colorimetric Sensor for Cysteine Detection

Mengjiao Peng, *,[a] ***Hui Wang,[b] Gregory L. Gibson,[c] Hua Shang,[a] Jianmin Yang,[a] Yan Chen[a] and Yin Lu[a]***

[a]*Department of Chemical Engineering and Textile, Shaanxi Polytechnic Institute, 712000 Xianyang, Shaanxi, P. R. China*

[b]*Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, College of Chemistry and Materials Science, Northwest University, 710069 Xi'an, Shaanxi, P. R. China*

[c]*Faculty of Science and Technology, Douglas College, V3M 5Z5 New Westminster, British Columbia, Canada*

Figures of sensing mechanism

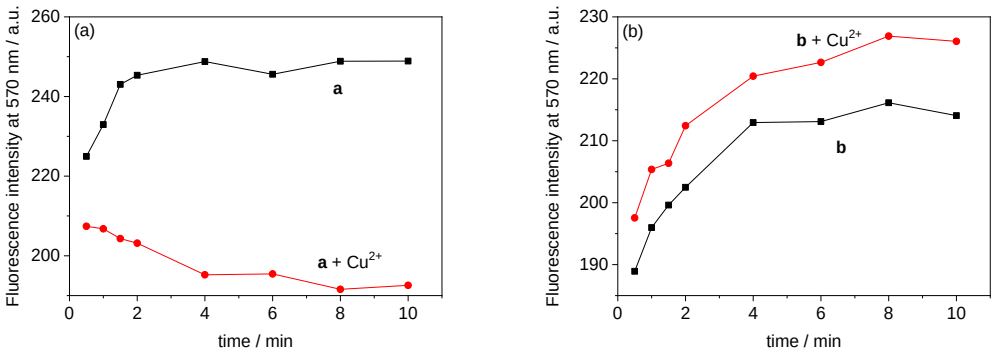

Figure S1. Time dependence of the fluorescence response of (a) **a**, **a**-Cu^{II}; and (b) **b**, **b**-Cu^{II} (2 μmol L^{-1}, respectively) in DMF-buffer (tris-HCl, 1.0 mmol L^{-1}, pH 7.4, 1:1, v/v) (ex: 540 nm; em: 570 nm; slit = 2.5 / 5).

*e-mail:dream_vivi@163.com

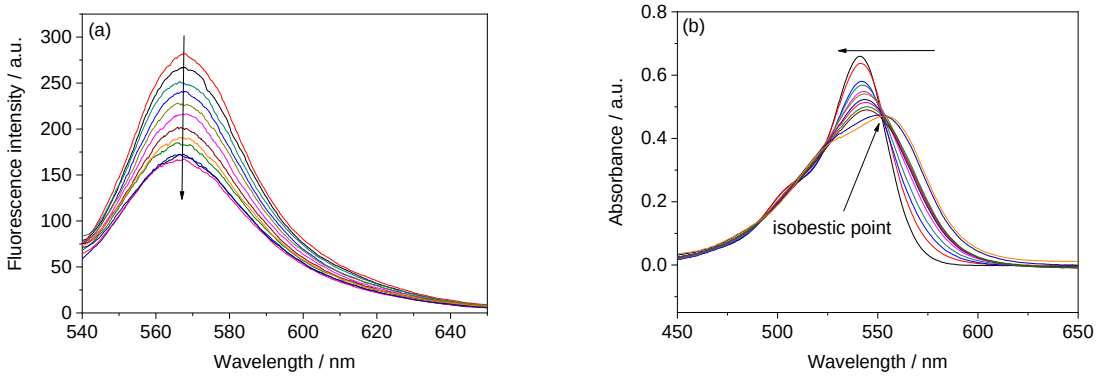

Figure S2. (a) Fluorescence (2.0 μmol L^{-1}) and (b) UV-Vis (2.0 μmol L^{-1}) spectra of sensor **a** in DMF-buffer (tris-HCl, 1.0 mmol L^{-1}, pH 7.4, 1:1, v/v) upon addition of different concentrations of Cu^{II} (0-ca. 1.2 equiv.) (ex: 540 nm; slit = 2.5 / 5).

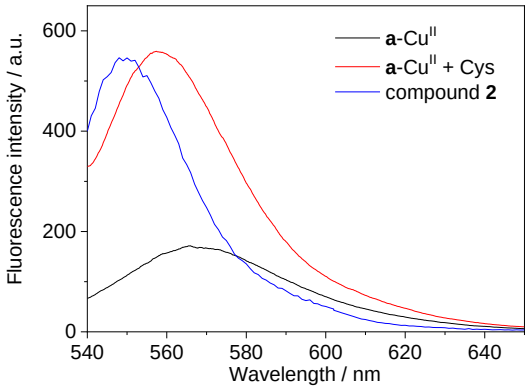

Figure S3. Fluorescence emission spectrum comparison of **a**-Cu^{II} (2.0 μmol L^{-1}), **a**-Cu^{II} + Cys and compound **2** in DMF-buffer (tris-HCl, 1.0 mmol L^{-1}, pH 7.4, 1:1, v/v) (ex: 530 nm; em: 560 nm; slit = 2.5 / 5).

Effect of pH value

As the experimental results are based on the chemical reaction between compound **a**-Cu^{II} and Cys, we hypothesized that the reaction rate might impact the observed spectrum changes. We therefore studied the impact of the reaction time on the sensing effect (Figure S4). The fluorescence intensity reached a plateau after approximately 20 min, showing that the sensor can detect Cys in real-time.

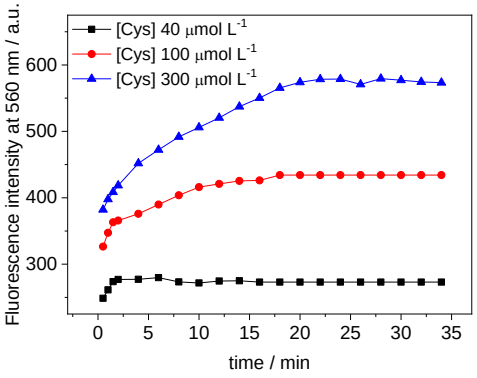

Figure S4. Time-dependent fluorescent intensity of **a**-Cu^{II} (2.0 μmol L^{-1}) upon addition of Cys in DMF-buffer (tris-HCl, 1.0 mmol L^{-1}, pH 7.4, 1:1, v/v) (ex: 530 nm; em: 560 nm; slit = 2.5 / 5).

Kinetic studies

To confirm the biological application of **a**-Cu^{II} as a Cys sensor, the fluorescence change upon Cys addition was monitored with respect to pH (Figure S5). Compound **a**-Cu^{II} + Cys achieved consistent fluorescence between pH 7 and 10.

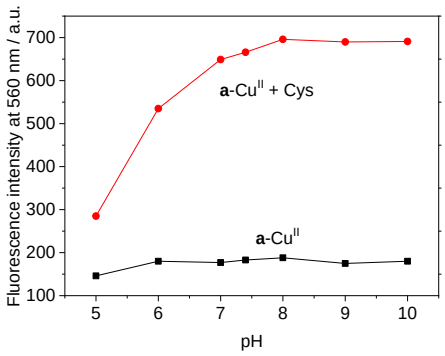

Figure S5. pH-dependent fluorescence profiles of **a**-Cu^{II} (2.0 μmol L^{-1}) and the corresponding Cys (300 μmol L^{-1}) in DMF-buffer (tris-HCl, 1.0 mmol L^{-1}, pH 7.4, 1:1, v/v) (ex: 530 nm; em: 560 nm; slit = 2.5 / 5).

Biocompatible studies

The ability of molecules to selectively recognize guest objects in living tissues is of great significance for biological applications. First of all, we chose breast carcinoma cell lines (MCF-7) to test the toxicity of **a**-Cu^{II}. The cell viability data were described as a percentage bar graph (Figure S6). The viability of untreated cells was considered to be 100%. When the concentration of **a**-Cu^{II} was 5 μmol L^{-1}, more than 96% of the MCF-7 cells were kept alive. Even when the concentration of **a**-Cu^{II} rose to 50 μmol L^{-1}, approximately 91% of the MCF-7 cells remained alive. Overall, the sensor **a**-Cu^{II} shows low toxicity to MCF-7 cells even the concentration reached 50 μmol L^{-1} and the incubation time reached 24 h, indicating that **a**-Cu^{II} is highly biocompatible.

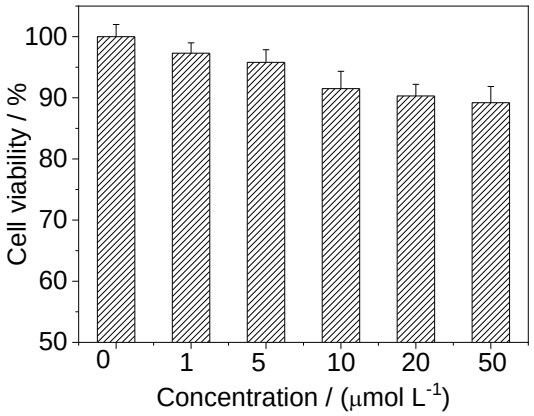

Figure S6. Percentage of MCF-7 cell viability after cell treatment with **a**-Cu^{II} (untreated cells were considered 100% surviving).

Synthetic procedure of sensor **a** and compound **b**

Synthesis of compound **1** [2-(4-(diethylamino)-2-hydroxybenzoyl)benzoic acid]

Compound **1** was synthesized on the basis of a reported procedure.[1] Briefly, a mixture of *o*-phthalic anhydride (8.25 g, 0.05 mol) and 3-diethylaminophenol (9.5 g, 0.064 mol) in methylbenzene (35 mL) was stirred for 18 h by programmed heating. The subsidence was filtered off, washed with methyl alcohol and dried to give the crude product, which was further purified by recrystallization from *n*-butyl alcohol to give **1** as a light pink solid (12.3 g, yield 78%). mp 200-202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.083 (t, 6H, *J* 12.0, $2CH_3$), 3.369 (q, 4H, *J* 12.0, $2CH_2$), 6.078 (s, 1H, ArH), 6.170 (d, 1H, *J* 8.0, ArH), 6.786 (d, 1H, *J* 8.0, ArH), 7.368 (d, 1H, *J* 8.0, ArH), 7.620 (m, 2H, ArH), 7.967 (d, 1H, *J* 8.0, ArH), 12.592 (s, 1H, COOH), 13.108 (s, 1H, Ar-OH) (according to Nakata *et al.*[1]).

Synthesis of compound **2** [2-(6-(diethylamino)-2-formyl-3-oxo-3*H*-xanthen-9-yl)benzoic acid]

A mixture of compound **1** (0.15 g, 0.48 mmol) and 2,4-dihydroxybenzaldehyde (0.066 g, 0.48 mmol) in methylsulfonic acid (35 mL) was stirred for 1 h at 90 °C. The reaction mixture was cooled to room temperature and then poured into ice-cold water (20 mL). The subsidence was filtered off, washed with ice-cold water, and then dried under vacuum to get the crude product, which was further purified by silica gel column chromatography (CH_2Cl_2-CH_3OH, 300:1, v/v) to produce compound **2** as a pink solid (23 mg, yield 31%). IR (KBr) ν / cm^{-1} 3672, 1750, 1625, 1521, 1405, 875, 801, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (t, 6H, *J* 16.8, $2CH_3$), 3.37 (q, 4H, *J* 16.8, $2CH_2$), 6.39 (d, 1H, *J* 8.4, ArH), 6.48 (s, 1H, ArH), 6.57 (d, 1H, *J* 8.8, ArH), 6.80 (s, 1H, ArH), 7.01 (s, 1H, ArH), 7.22 (d, 1H, *J* 7.4, ArH), 7.68 (m, 2H, ArH), 8.05 (d, 1H, *J* 7.2, ArH), 9.57 (s, 1H, ArH), 11.19 (s, 1H, COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.38, 44.45, 82.99, 97.67, 104.37, 108.86, 113.24, 117.80, 123.86, 125.07, 125.92, 126.94, 128.72, 129.83, 135.07, 135.49, 149.71, 152.19, 152.47, 157.83, 162.91, 169.28, 194.62; HRMS (ESI) *m/z*, calcd. for $C_{25}H_{22}NO_5$ $[M]^+$: 416.1492, found: 416.1483.

Synthesis of sensor **a** [(*E*)-2-(6-(diethylamino)-2-((2-hydroxyphenylimino)methyl)-3-oxo-3*H*-xanthen-9-yl)benzoic acid]

A mixture of **2** (0.1371 g, 0.33 mmol) and 2-aminophenol (0.0546 g, 0.50 mmol) in absolute ethanol (10 mL) was stirred for 5 h at 85 °C. The subsidence was filtered off, washed with ethanol and dried to give the crude product, which was further purified by silica gel column chromatography (CH_2Cl_2-CH_3OH, 50:1, v/v) to produce sensor **a** as a

dark purple solid (0.1344 g, yield 81%). IR (KBr) ν / cm^{-1} 3417, 3060, 2971, 2919, 2895, 1760, 1691, 1585, 1467, 1344, 1137, 1076, 931, 879, 825, 752; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (t, 6H, *J* 8.0, $2CH_3$), 1.25 (q, 4H, *J* 8.0, $2CH_2$), 5.76 (s, 1H, OH), 6.47 (s, 2H, ArH), 6.79 (s, 2H, ArH), 6.93 (d, 1H, *J* 8.0, ArH), 7.12 (m, 2H, ArH), 7.33 (m, 2H, ArH), 7.77 (m, 2H, ArH), 8.01 (d, 1H, *J* 8.0, CH=N), 8.87 (s, 1H, ArH), 9.57 (s, 1H, ArH), 11.19 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.93, 44.47, 96.79, 103.73, 105.00, 109.17, 117.26, 119.48, 124.60, 125.12, 126.88, 128.03, 128.60, 129.06, 129.76, 130.29, 133.59, 134.27, 134.70, 136.07, 140.50, 149.77, 151.57, 152.42, 153.91, 154.75, 160.67, 169.27, 199.07; HRMS (ESI) *m/z*, calcd. for $C_{31}H_{27}N_2O_5$ $[M]^+$: 507.1914, found: 507.1908.

Synthesis of compound **b** [(*E*)-2-(6-(diethylamino)-3-oxo-2-((phenylimino)methyl)-3*H*-xanthen-9-yl)benzoic acid]

A mixture of **2** (0.1371g, 0.33 mmol) and phenylamine (0.0316 g, 0.50 mmol) in absolute ethanol (10 mL) was stirred for 5 h at 85 °C. The subsidence was filtered off, washed with ethanol and dried to give the crude product, which was further purified by silica gel column chromatography (CH_2Cl_2-CH_3OH, 100:1, v/v) to produce compound **b** as a pink solid (0.1194 g, yield 75%). IR (KBr) ν / cm^{-1} 3401, 3060, 2964, 2908, 2856, 1754, 1623, 1560, 1494, 1434, 1334, 1008, 966, 871, 808, 761, 696; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (t, 6H, *J* 8.0, $2CH_3$), 3.37 (q, 4H, *J* 8.0, $2CH_2$), 6.38 (s, 1H, ArH), 6.48 (s, 1H, ArH), 6.57 (d, 1H, *J* 12.0, ArH), 6.84 (d, 2H, *J* 8.0, ArH), 7.25 (m, 4H, ArH), 7.37 (m, 2H, ArH), 7.65 (m, 2H, ArH), 8.04 (d, 1H, *J* 8.0, CH=N), 8.37 (s, 1H, ArH), 13.66 (s, 1H, COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.25, 44.14, 83.80, 97.82, 104.16, 104.93, 108.62, 111.80, 116.44, 121.09, 121.40, 124.03, 124.96, 126.93, 127.18, 128.82, 129.19, 129.36, 129.61, 133.09, 134.93, 147.97, 149.70, 152.62, 153.08, 155.23, 161.28, 162.85, 169.55; HRMS (ESI) *m/z*, calcd. for $C_{31}H_{27}N_2O_4$ $[M]^+$: 491.1965, found: 491.1943.

NMR spectra of the compounds

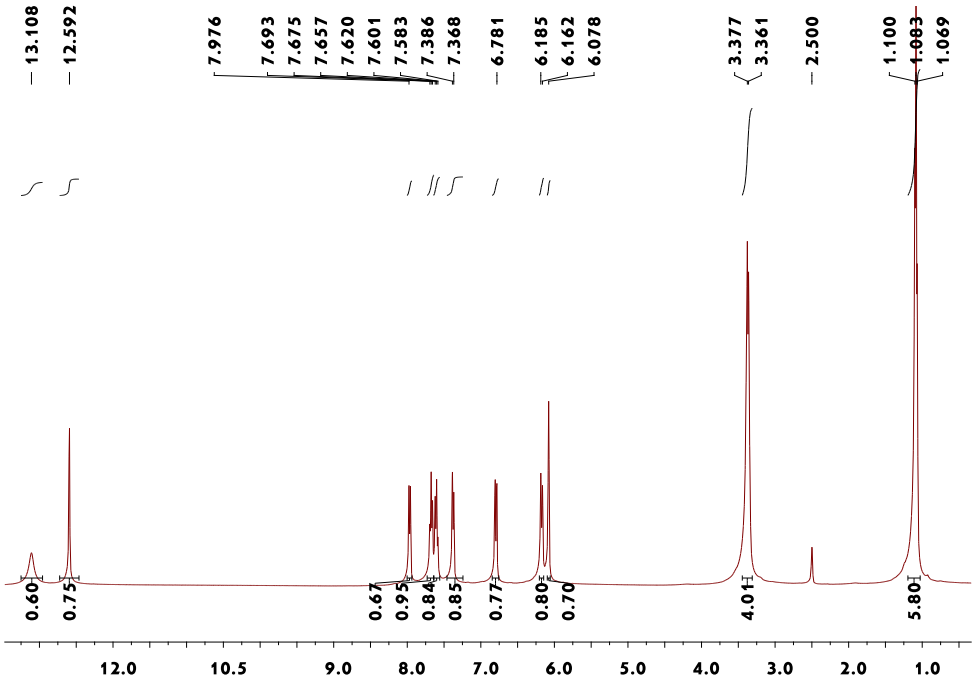

Figure S7. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of compound **1**.

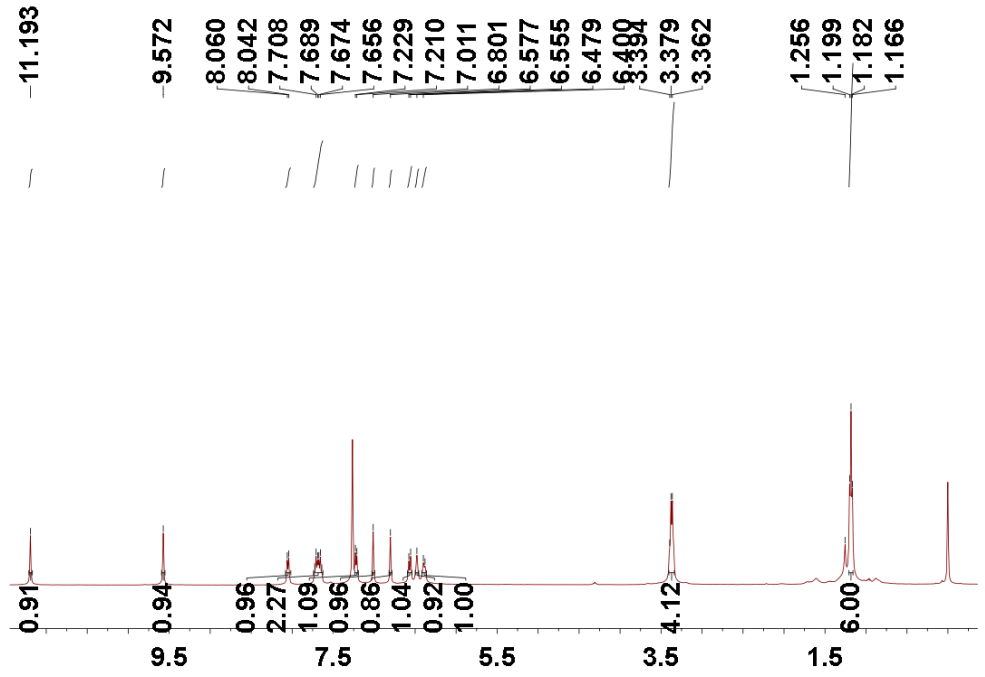

Figure S8. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **2**.

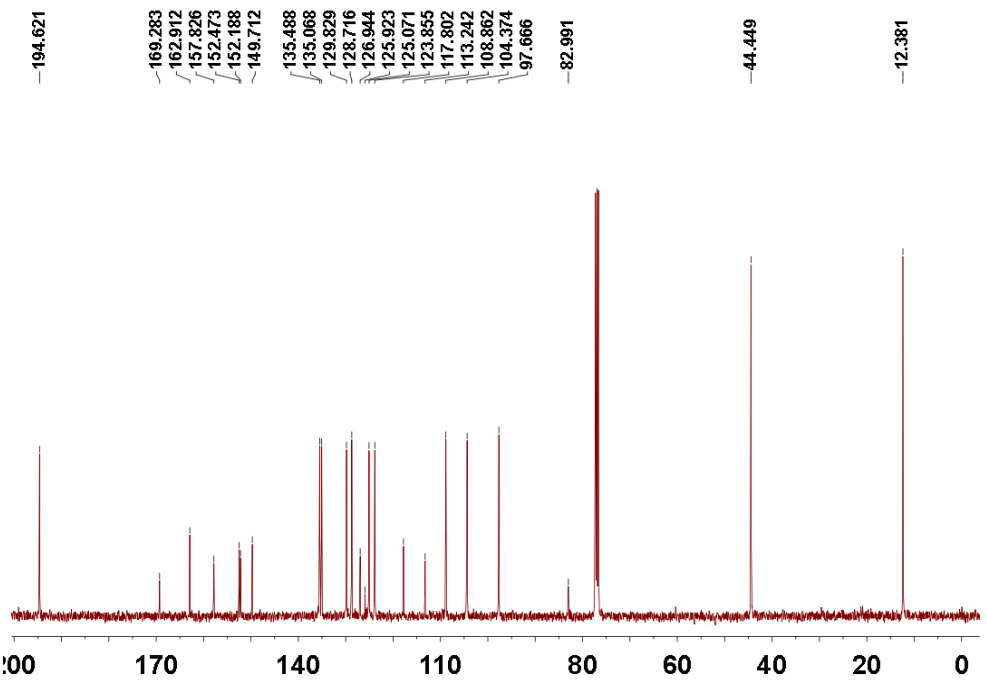

Figure S9. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **2**.

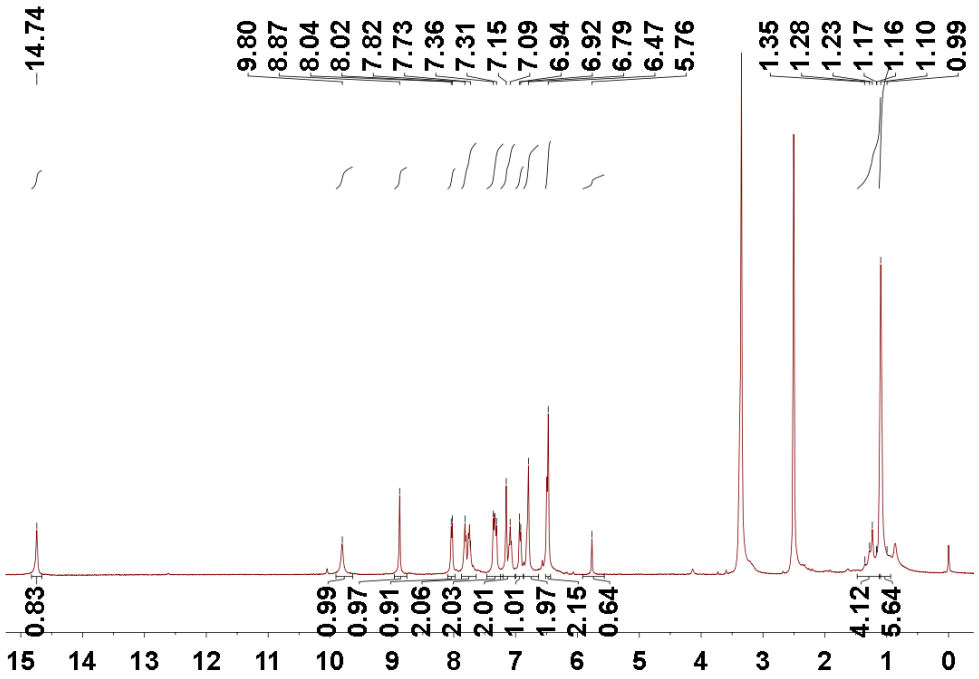

Figure S10. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of sensor **a**.

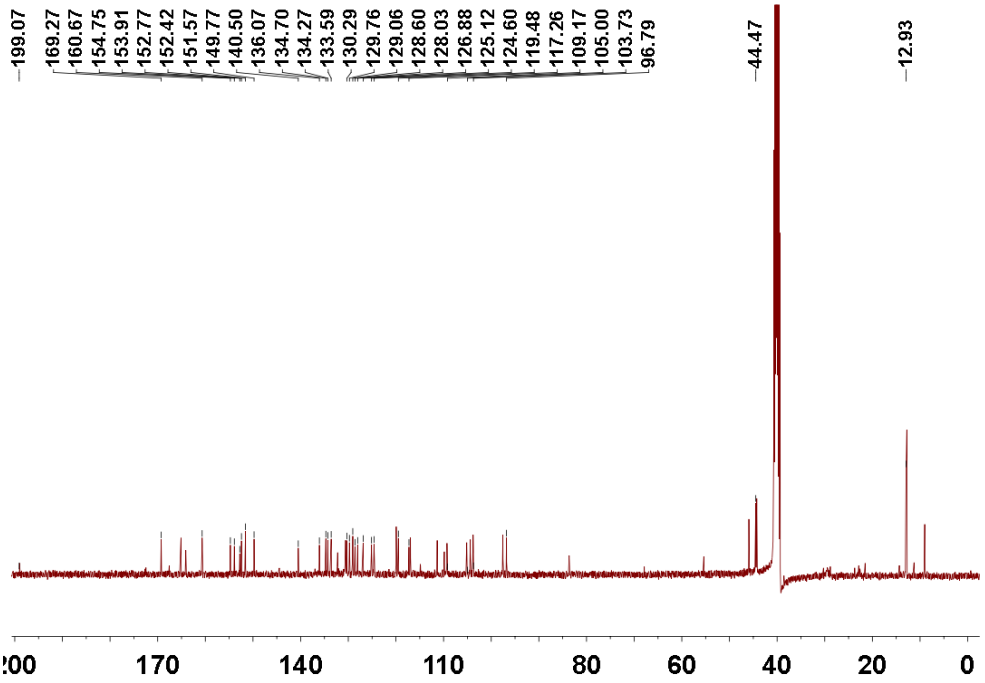

Figure S11. ^{13}C NMR spectrum (100 MHz, DMSO-d_6) of sensor **a**.

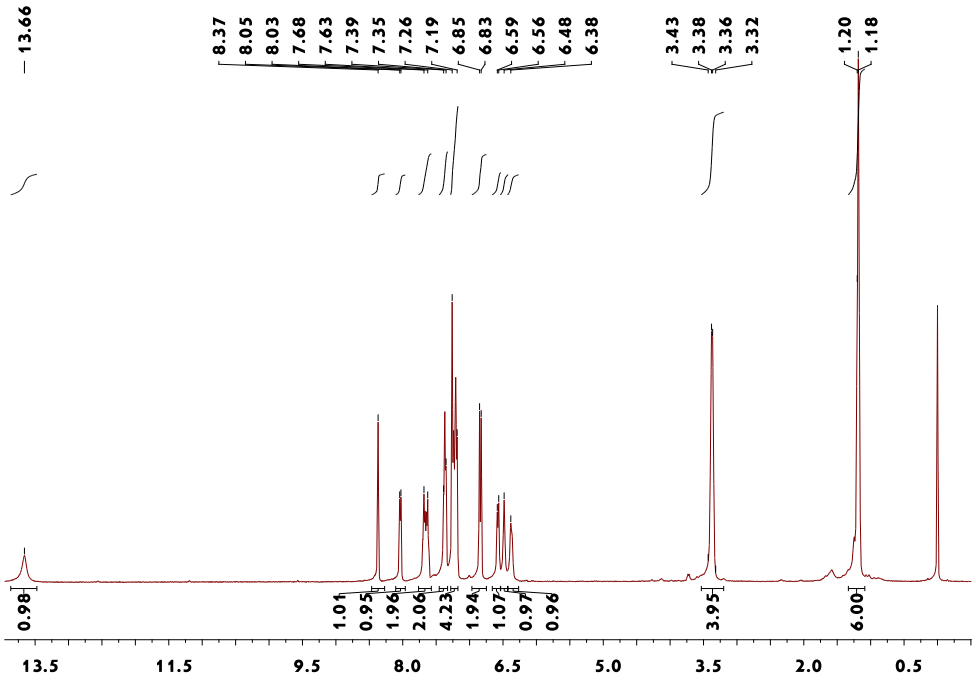

Figure S12. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **b**.

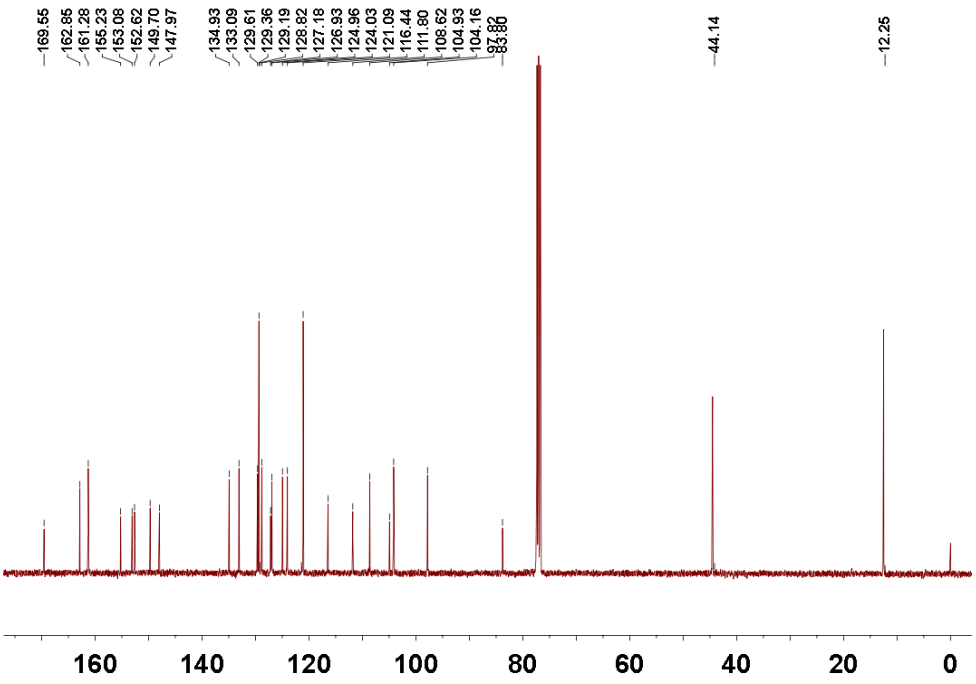

Figure S13. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of compound **b**.

Mass spectra of the compounds

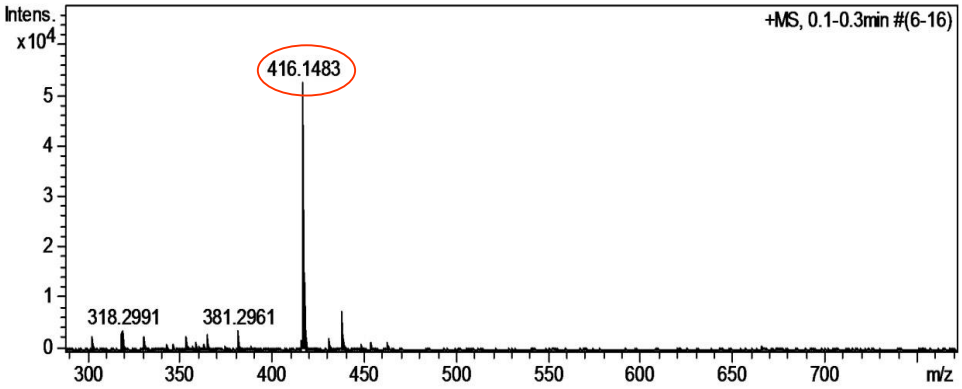

Figure S14. Mass spectrum of compound **2**.

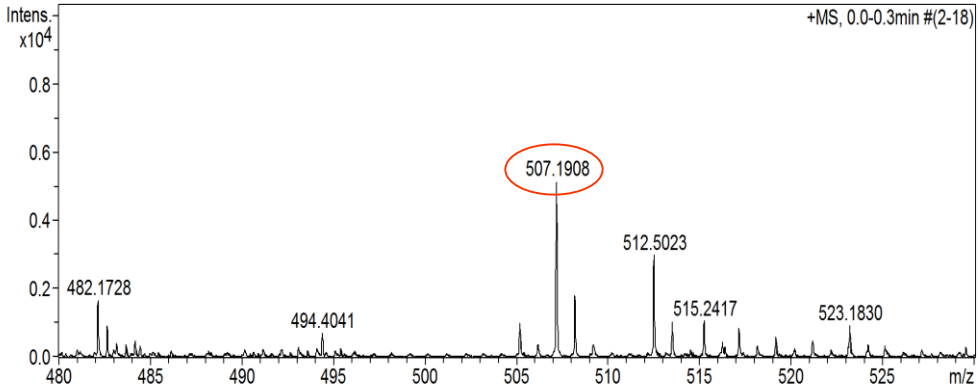

Figure S15. Mass spectrum of sensor **a**.

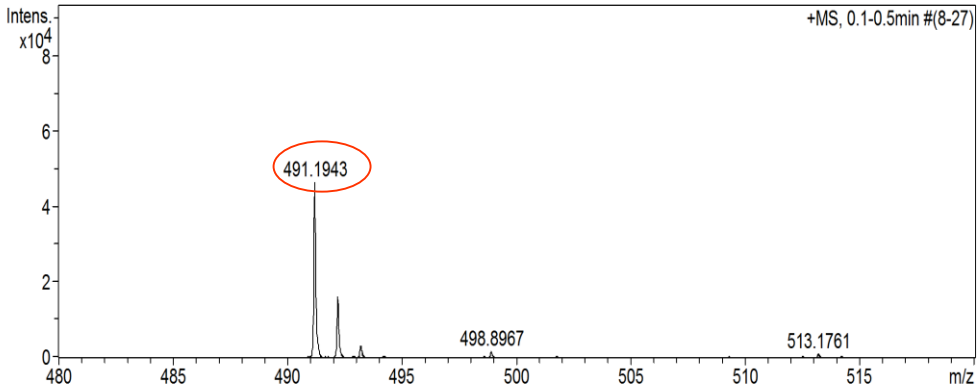

Figure S16. Mass spectrum of compound **b**.

IR of the compounds

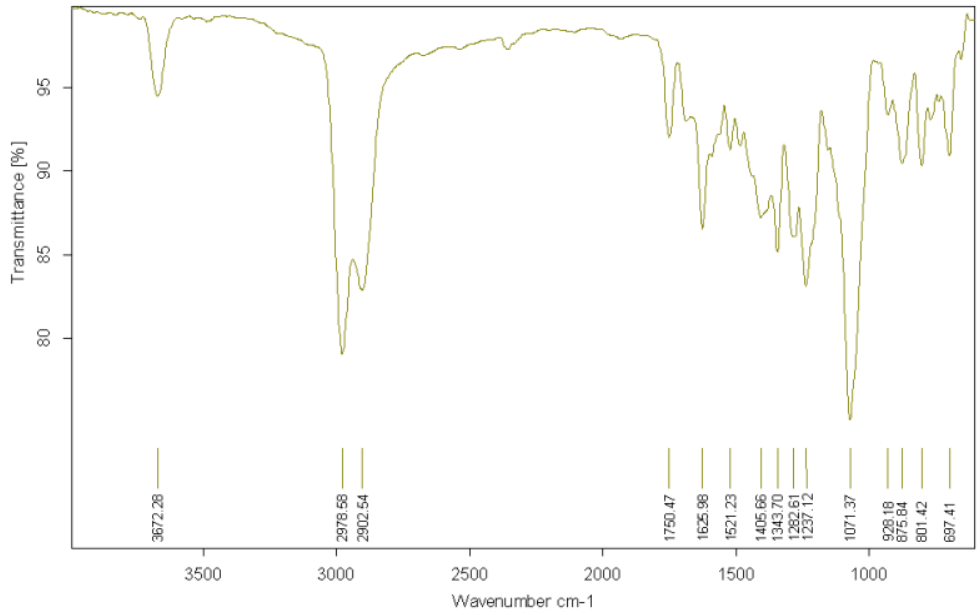

Figure S17. FTIR (KBr) spectrum of compound **2**.

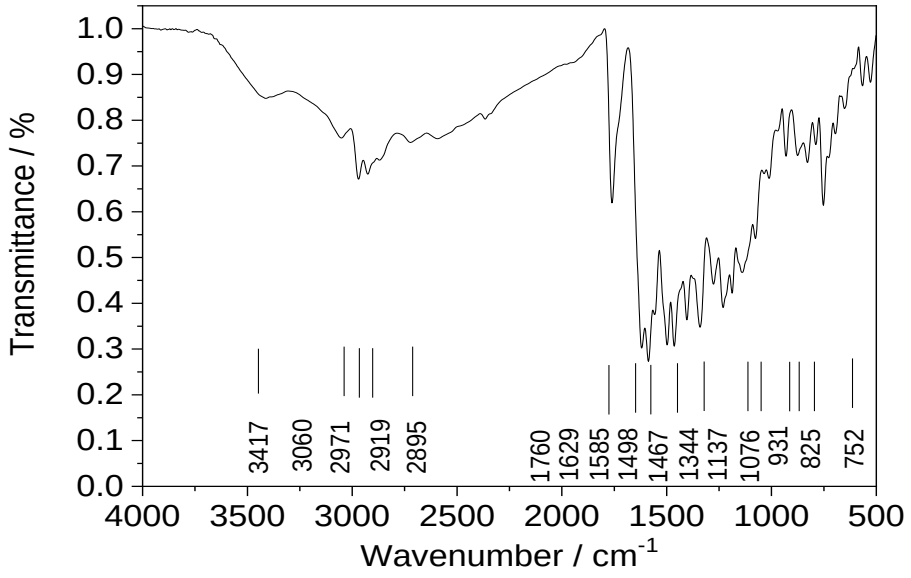

Figure S18. FTIR (KBr) spectrum of sensor **a**.

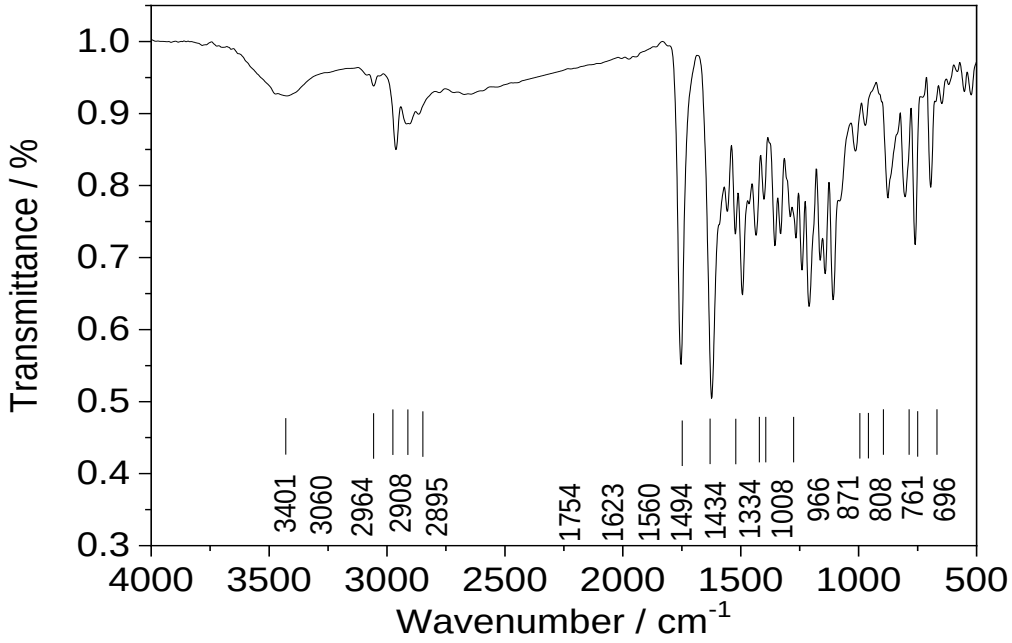

Figure S19. FTIR (KBr) spectrum of compound **b**.

Reference

1. Nakata, E.; Yukimachi, Y.; Kariyazono, H.; Im, S.; Abe, C.; Uto, Y.; Maezawa, H.; Hashimoto, T.; Okamoto, Y.; Hori, H.; *Bioorg. Med. Chem.* **2009**, *17*, 6952.