A turn-on NIR fluorescent probe for the detection of homocysteine over cysteine

Jian Zhang, Xin-Dong Jiang, Xiangmin Shao, Jiuli Zhao, Yajun Su, Dongmei Xi, Haifeng Yu, Shuai Yue, Lin-jiu Xiao and Weili Zhao

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1 General

$^1$H NMR spectra were recorded on a VARIAN Mercury 400 MHz spectrometer. $^1$H NMR chemical shifts ($\delta$) are given in ppm downfield from Me$_4$Si, determined by chloroform ($\delta = 7.26$ ppm) and dimethyl sulfoxide ($\delta = 2.5$ ppm). $^{13}$C NMR spectra were recorded on a VARIAN Mercury 100 MHz spectrometer. $^{13}$C NMR chemical shifts (\(\delta\)) are reported in ppm with the internal CDCl$_3$ and $d_6$-DMSO at $\delta$ 77.0 and 39.4 ppm as standard, respectively. Mass spectrometric measurements were performed by the mass spectrometry service of the ETHZ on a Bruker Reflex MALDI as matrix (20 kV). The refractive index of the medium was measured by 2 W Abbe’s refractometer at 20 °C.

Tetrahydrofuran (THF) was freshly distilled from Na/benzophenone, n-hexane was distilled over Na, and other solvents were distilled over CaH$_2$. Merck silica gel 60 was used for the column chromatography.

Fluorescence spectra were recorded on FluoroSENS spectrophotometer. UV/Vis spectra were recorded on Perkin-Elmer Lambda 35 UV/Vis spectrophotometer at room temperature.

The fluorescence quantum yields (\(\Phi_f\)) of the BODIPY systems were calculated using the following relationship (equation 1):

\[
\Phi_f = \Phi_{ref}\frac{F_{sampl} A_{sampl} n_{sampl}^2}{F_{ref} A_{ref} n_{ref}^2}\]

Here, $F$ denotes the integral of the corrected fluorescence spectrum, $A$ is the absorbance at the excitation wavelength, and $n$ is the refractive index of the medium ($n = 1.4448$ in CHCl$_3$; $n = 1.3461$ in MeCN/H$_2$O (v/v = 80/20)), ref and sampl denote parameters from the reference and unknown experimental samples, respectively. The reference systems used was boronazadipyrromethene compound aza-BODIPY ($\Phi_f = 0.36$ in chloroform).\(^1\)
2 Synthesis

BODIPY 2

under N₂, Acetoxycetyl chloride (0.14 mL, 1.3 mmol) was added to a solution of 7-methoxy-4,5-dihydro-1H-benzo[g]indole² (0.40 g, 2.0 mmol) in dry CH₂Cl₂ (2 mL) and stirred for 2 h at 40 °C. The reaction was cooled to room temperature and Triethylamine (0.73 mL, 4.2 mmol) was added, followed by dropwise addition of BF₃·OEt₂ (0.53 mL, 4.2 mmol). After the mixture was stirred for 0.5 h, the solvent was evaporated under reduced pressure, and the resulting crude mixture was purified by chromatography on silica gel followed by recrystallization from CH₂Cl₂/n-hexane to afford 2 (0.10 g, 0.19 mmol, 19%) as coppery solids. M.p.: 238–239 °C. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.71 (d, J = 8.80, 2H), 6.99 (s, 2H), 6.97 (dd, J = 8.80, 2.40, 2H), 6.80 (d, J = 2.40, 2H), 5.26 (s, 2H), 3.88 (s, 6H), 2.90 (t, J = 6.80, 4H), 2.74(t, J = 6.80, 4H), 2.12 (s, 3H). ¹³C NMR (100 MHz, CDCl₃), δ (ppm) =170.2, 160.5, 152.2, 142.7, 135.7, 132.1, 129.9, 127.8, 121.6, 121.0, 114.0, 112.1, 59.4, 55.0, 30.5, 22.1, 20.7. HRMS-MALDI (m/z): [M]⁺ calcd for C₃₀H₂₇BF₂N₂O₄: 528.2026; found 528.2017.

BODIPY 3

Compound 2 (52.8 mg, 0.10 mmol) was added to K₂CO₃ (50 mg, 0.35 mmol) in a solution of CH₂Cl₂ (25 mL), MeOH (10 mL) and water (1.0 mL) at room temperature and stirred overnight. After removing the solvents by evaporation, the crude solid was
dissolved in water (40 mL), and neutralized with dilute NaH₂PO₄ to a pH about 5. The solution was stirred until the solid was formed, and the precipitated solid was filtered and washed. The resulting crude mixture was purified by chromatography on silica gel followed by recrystallization from CH₂Cl₂/n-hexane to afford 3 (24.8 mg, 0.050 mmol, 50%) as a coppery solid. M.p.: 194–195 °C. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 9.43 (br s, 1H), 8.71 (d, J = 8.80, 1H), 7.33 (d, J = 9.20, 1H), 7.04 (s, 1H), 6.97 (dd, J = 9.20, 2.80, 1H), 6.82 (s, 2H), 6.80 (d, J = 2.80, 1H), 6.77 (d, J = 2.00, 1H) 4.68 (s, 2H), 3.88 (s, 3H), 3.83 (s, 3H), 2.91 (m, 4H), 2.74 (t, J = 7.20, 4H). HRMS-MALDI (m/z): [M]+ calcd for C₂₈H₂₅BF₂N₂O₃: 486.1921; found 486.1929.

BODIPY 1

Dess Martin periodinane (61.8 mg, 0.15 mmol) was dissolved in dry dichloromethane. To the suspension was slowly added a solution of BODIPY 3 (48.6 mg, 0.1 mmol) in dry dichloromethane at 0 °C under argon. After 10 min, the ice bath was removed and the reaction mixture was left stirring at room temperature for extended periods of time as detailed below. The reaction mixture was extracted with saturated aqueous Na₂S₂O₃ followed by saturated aqueous NaHCO₃ and brine. The combined organic solutions were dried over MgSO₄. The solvent was evaporated, and the resulting crude mixture was purified by chromatography on silica gel followed by recrystallization from CH₂Cl₂/n-hexane to afford 1 (30.1 mg, 0.062 mmol, 62%) as a coppery solid. M.p.>250 °C ¹H NMR (400 MHz, CDCl₃): δ (ppm) 10.36 (s, 1H), 8.73 (d, J = 8.80, 2H), 7.38 (s, 2H), 6.98 (d, J = 8.80, 2H), 6.82 (s, 2H), 3.90 (s, 6H), 2.93 (t, J = 6.80, 4H), 2.78 (t, J = 6.80, 4H) ¹³C NMR (100 MHz, CDCl₃), δ (ppm) =189.5, 161.5, 153.2, 144.0, 137.0, 134.7, 131.0, 122.9, 121.7, 121.2, 114.5, 112.9, 55.4, 30.9, 22.5. HRMS-MALDI (m/z): [M]+ calcd for C₂₈H₂₃BF₂N₂O₃: 484.1764; found 484.1763.
Absorption and Fluorescence response of 20 μM probe 1 (MeCN/H₂O = 8 : 2, v/v) upon reacting with Cys in 1, 2, 10, 20, 40, 80, 120, 160, 200, 400, 800, 1200, 1600, 2000 μM concentration after 4 h of incubation at 20 °C. The excitation wavelength was 620 nm. The inner panel displays the fluorescence enhancement of probe 1 toward Cys of 1, 2, 10, 20, 40, 80, 120, 160, 200 μM.
4 Spectroscopic data
5 X-ray crystal structure determinations of compound 1

Crystals suitable for the X-ray structural determination were mounted on a Mac Science DIP2030 imaging plate diffractometer and irradiated with graphite monochromated Mo-K\(\alpha\) radiation (\(\lambda = 0.71073\) Å) for the data collection. The unit cell parameters were determined by separately autoindexing several images in each data set using the DENZO program (MAC Science).\(^3\) For each data set, the rotation images were collected in 3° increments with a total rotation of 180° about the \(\phi\) axis. The data were processed using SCALEPACK. The structures were solved by a direct method with the SHELX-97 program.\(^4\) Refinement on \(F^2\) was carried out using the full-matrix least-squares by the SHELX-97 program.\(^4\) All non-hydrogen atoms were refined using the anisotropic thermal parameters. The hydrogen atoms were included in the refinement along with the isotropic thermal parameters.

Crystal data for 1. \(\text{C}_{28}\text{H}_{23}\text{BF}_2\text{N}_2\text{O}_3\), \(M = 484.29\), triclinic, \(a = 7.704(2)\), \(b = 12.3376(2)\), \(c = 13.3334(5)\) Å, \(\alpha = 63.617(3)\), \(\beta = 81.760(3)\), \(\gamma = 88.163(3)°\), \(V = 1122.8(3)\) Å\(^3\), \(T = 298(2)\) K, space group \(P\)-1, \(Z = 2\), Data/restraints/parameters = 3855/0/327, \(R_1 = 0.0468\) \((I > 2\sigma(I))\), \(wR_2\) (all data) = 0.1506, \(GOF = 1.033\). CCDC reference number 902488 for 1.

CCDC reference number 902488 for 1 contains the supplementary crystallographic data. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336033; or deposit@ccdc.cam.uk).
6 References


