“Turn-on” FRET-based Luminescent Iridium(III) Probes for the Detection of Cysteine and Homocysteine

Hoi-Yan Shiu, Man-Kin Wong, Chi-Ming Che

a Department of Chemistry and Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Pokfulam Road, Hong Kong (China)

b Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong (China)

Supporting Information
General

Chemicals purchased from commercial sources were used without further purification. Stock solutions of analytes for analyses were freshly prepared prior use. TLC analyses were performed on silica gel plates and flash column chromatography was conducted over silica gel 60 (230–400 mesh ASTM) with ethyl acetate/n-hexane or methanol/dichloromethane as eluent. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker DPX-300 or DPX-400 spectrometer. Chemical shifts (ppm) are referenced to TMS. Mass spectra were measured by Finnigan MAT 95 or LCQ mass spectrometer.

General Spectral Measurements

Electronic absorption spectra were recorded with a HP Agilent 8453 UV/Vis spectrophotometer. The emission spectra were measured on a SPEX Fluorolog-3-21 spectrofluorometer.
Synthesis of 1

1. 3 → 4
   - CrO$_3$ in acetic acid, acetone, 0 °C, 78%

2. HS-NH$_2$•HCl → TrtS-NH$_2$•HCl
   - TFA, rt, 100%

3. 5 → 6
   - EDC, DMAP, CH$_2$Cl$_2$, rt, 71%

4. 4 → 7
   - TFA, triisopropylsilane, H$_2$O, CH$_2$Cl$_2$, rt, 84%

5. 7 → 8
   - DMF, H$_2$O, rt, 34%

6. 8 → 9
   - N=N

7. BocHN→N
   - DCC, CH$_2$Cl$_2$, rt, 74%

8. 10 → 11
   - TFA, CH$_2$Cl$_2$, rt, 95%
9 + 11 $\xrightarrow{EDC}$
$\text{CH}_2\text{Cl}_2$, rt, 96%

12

$\text{[Ir}_2(ppy)_4\text{Cl}_2]$ $\xrightarrow{}$
$\text{CH}_2\text{Cl}_2$, $\text{CH}_3\text{OH}$, reflux 98%

1
Synthesis of 2a–d

\[ \text{BocHN} \text{STrt} \xrightarrow{\text{RH amine}} \text{BocHN} \text{STrt} \xrightarrow{\text{TFA triisopropylsilane}} \]

\[ \xrightarrow{\text{EDC, DMAP, CH}_2\text{Cl}_2, \text{rt}} \; \xrightarrow{\text{H}_2\text{O, CH}_2\text{Cl}_2, \text{rt}} \]

13a \( R = \) 96%
13b \( R = \) 98%
13c \( R = \) 88%
13d \( R = \) 89%

14a \( R = \) 96%
14b \( R = \) 98%
14c \( R = \) 95%
14d \( R = \) 97%

15a \( R = \) 100%
15b \( R = \) 100%
15c \( R = \) 100%
15d \( R = \) 100%

6

\[ \text{EDC, DMAP, CH}_2\text{Cl}_2, \text{rt} \]

16

16a \( R = \) 75%
16b \( R = \) 74%
16c \( R = \) 80%
16d \( R = \) 76%

16

17

\[ \xrightarrow{\text{TFA}} \; \xrightarrow{\text{triisopropylsilane, H}_2\text{O, CH}_2\text{Cl}_2, \text{rt}} \]

18

17a \( R = \) 83%
17b \( R = \) 78%
17c \( R = \) 83%
17d \( R = \) 79%
[Ir(ppy)$_2$Cl]$_2$ →  
CH$_2$Cl$_2$, CH$_3$OH reflux

2a R = 89%  
2b R = 98%  
2c R = 93%  
2d R = 96%
Literature Reference of Known Compounds 3, 5, 6, 18, 20 and [Ir₂(ppy)₄Cl₂]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.png" alt="5" /></td>
<td>Commercial available</td>
</tr>
</tbody>
</table>
Preparation and Characterization of 4, 7, 8, 9, 10, 11, 12 and 1

4: 3 (200 mg, 1.1 mmol), chromium(VI) oxide (227 mg, 2.2 mmol) and acetic acid (0.6 mL, 11 mmol) in 10 mL of acetone was stirred at 0 °C for 3 h. After evaporation of solvent, the residue was purified by flash column chromatography (70% EtOAc in n-hexane) to give product 4 (154 mg, 78% isolated yield).

4: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.22 (d, $J = 6.4$ Hz, 2H), 8.14 (d, $J = 6.8$ Hz, 2H), 4.05 (s, 1H); $^{13}$C NMR $\delta$ 177.8, 166.8, 140.2, 136.2, 130.6, 120.6, 120.0, 84.2, 80.5 (125 MHz, CDCl$_3$); EIMS: $m/z = 174$ (M$^+$); HRMS (EI) for C$_{10}$H$_6$O$_3$, calcd 174.0317, found 174.0313.

7: 5 (29 mg, 0.09 mmol), 6 (30 mg, 0.08 mmol), 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (17.6 mg, 0.09 mmol), 4-di(methylamino)pyridine (DMAP) (1 mg, catalytic amount) and triethylamine (9.3 mg, 0.09 mmol) in 2 mL of anhydrous dimethylformamide (DMF) was stirred at room temperature for 24 h. After evaporation of solvent, the residue was purified by flash column chromatography (2% MeOH in CH$_2$Cl$_2$) to give product 7 (38 mg, 71% isolated yield).

7: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.04 (d, $J = 8.2$ Hz, 1H), 8.68 (d, $J = 8.4$ Hz,
1H), 8.33 (d, J = 8.2 Hz, 1H), 8.00 (d, J = 8.5 Hz, 2H), 7.78–7.70 (m, 3H), 7.40–7.38 (m, 6H), 7.29–7.20 (m, 9H), 6.76 (d, J = 8.4 Hz, 2H), 5.46 (s, 1H), 3.76 (t, J = 6.8 Hz, 2H), 3.49 (q, J = 7.1 Hz, 2H), 3.10 (q, J = 6.2 Hz, 2H), 2.45–2.42 (m, 4H), 1.21 (t, J = 7.1 Hz, 1H); 13C NMR (125 MHz, CDCl3) δ 170.4, 152.1, 150.8, 145.9, 144.7, 144.6, 131.5, 129.5, 128.0, 127.9, 127.2, 126.9, 126.4, 125.0, 124.6, 123.2, 111.5, 109.8, 67.0, 46.7, 45.8, 38.3, 34.6, 31.9, 31.8, 29.7, 29.7, 29.4, 22.7, 14.1, 12.4; ESIMS m/z 694 ([M + H]+); HRMS (ESI) for C42H40N5O3S, calcd 694.2852, found 694.2850.

8: 7 (43.6 mg, 0.063 mmol), triisopropylsilane (0.12 mL), trifluoroacetic acid (1.3 mL) and H2O (0.05 mL) was stirred in 0.5 mL of CH2Cl2 at room temperature for 1 h. After evaporation of solvent, the residue was purified by flash column chromatography (2% MeOH in CH2Cl2) to give product 8 (23.8 mg, 84% isolated yield).

8: 1H NMR (400 MHz, CDCl3) δ 9.04 (d, J = 8.2 Hz, 1H), 8.68 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 8.2 Hz, 1H), 8.00 (d, J = 8.5 Hz, 2H), 7.78–7.70 (m, 3H), 6.76 (d, J = 8.4 Hz, 2H), 5.91 (s, 1H), 3.83 (t, J = 6.8 Hz, 2H), 3.56 (q, J = 7.1 Hz, 2H), 3.46 (q, J = 6.2 Hz, 2H), 2.67 (d, J = 8.4 Hz, 2H), 2.57 (t, J = 6.8 Hz, 2H), 1.21 (t, J = 7.1 Hz, 3H); 13C NMR (125 MHz, CDCl3) δ 170.7, 152.0, 150.8, 145.9, 144.7, 131.5, 129.5, 127.2, 126.6, 126.4, 125.0, 124.6, 123.2, 111.5, 109.8, 46.7, 45.8, 42.4, 34.7, 31.9, 29.7, 29.4, 24.6, 22.7, 14.1, 12.4; ESIMS m/z 452 ([M
+ H\]^+)\); HRMS (ESI) for C\textsubscript{23}H\textsubscript{26}N\textsubscript{5}O\textsubscript{3}S, calcd 452.1756, found 452.1751.

9: 8 (20.2 mg, 0.05 mmol), 4 (9.4 mg, 0.05 mmol) and H\textsubscript{2}O (1.5 mL) was stirred in 0.5 mL of DMF at room temperature for 24 h. After evaporation of solvent, the residue was purified by flash column chromatography (5% MeOH in CH\textsubscript{2}Cl\textsubscript{2}) to give product 9 (9.6 mg, 34% isolated yield, Z/E = 4/6).

9: \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.93–8.97 (m, 1H), 8.66–8.62 (m, 1H), 8.31–8.25 (m, 1H), 8.12–8.10 (m, 1H), 8.04–8.01 (m, 2H), 7.97–7.95 (m, 1H), 7.92–7.86 (m, 2H), 7.75 (t, \(J = 7.5\) Hz, 1H), 7.70–7.61 (m, 2H), 7.36 (d, \(J = 9.6\) Hz, 0.4H), 7.14 (d, \(J = 14.8\) Hz, 0.6H), 6.78 (t, \(J = 9.8\) Hz, 2H) 6.95 (d, \(J = 9.7\) Hz, 0.4H), 6.35 (t, \(J = 5.8\) Hz, 0.6H), 6.29 (t, \(J = 5.7\) Hz, 0.4H), 3.83 (t, \(J = 6.2\) Hz, 2H), 3.73 (q, \(J = 7.0\) Hz, 1H), 3.52–3.50 (m, 2H), 3.55–3.50 (m, 2H), 3.11 (t, \(J = 6.7\) Hz, 1H), 3.00–2.97 (m, 1H), 2.63–2.58 (m, 2H), 1.21 (t, \(J = 7.1\) Hz, 3H); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) \(\delta\) 186.0, 184.3, 171.4, 168.0, 151.9, 150.8, 148.6, 141.7, 132.4, 131.5, 130.9, 130.4, 130.3, 129.6, 128.8, 128.4, 128.1, 127.9, 127.2, 126.6, 126.4, 125.0, 123.1, 118.7, 111.5, 109.8, 85.2, 85.1, 83.3, 83.0, 79.3, 79.0, 77.6, 68.2, 46.8, 46.7, 45.8, 38.8, 38.7, 34.6, 31.9, 31.8, 30.9, 30.4, 29.7, 29.3, 28.9, 23.8, 23.3, 23.2, 23.0, 22.7, 16.5, 14.1, 12.4, 11.0; ESIMS \textit{m/z} 626 ([M + H\]^+); HRMS (ESI) for C\textsubscript{33}H\textsubscript{32}N\textsubscript{5}O\textsubscript{6}S, calcd 626.2073, found 626.2071.
10: A solution of 6-tert-butoxycarbonylamino-hexanoic acid (1060 mg, 4.6 mmol) and N,N'-Dicyclohexylcarbodiimide (DCC) (473 mg, 2.3 mmol) in acetonitrile (30 mL) was stirred at room temperature for 1 h and filtered to remove the dicyclohexylurea (DCU) precipitate. The resulting anhydride was used without further purification and added to a solution of 5-amino-1,10-phenanthroline (70.8 mg, 0.36 mmol) and triethylamine (255 mg, 2.5 mmol) in acetonitrile (30 mL). The solution was stirred at room temperature for 6 days under argon. After evaporation of solvent, the residue was purified by flash column chromatography on Al₂O₃ (2% MeOH in CH₂Cl₂) to give product 10 (107.8 mg, 74% isolated yield).

10: ¹H NMR (400 MHz, CDCl₃) δ 9.14 (d, J = 3.0 Hz, 1H), 9.08 (d, J = 2.5 Hz, 1H), 8.51 (s, 1H), 8.46 (d, J = 8.2 Hz, 1H), 8.21 (s, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.59 (m, 2H), 4.64 (s, 1H), 3.08–3.07 (m, 2H), 2.49–2.46 (m, 2H), 1.74–1.65 (m, 2H), 1.42 (m, 11H), 1.33–1.26 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 174.2, 172.6, 156.2, 156.1, 150.1, 149.8, 146.5, 144.5, 135.9, 130.9, 130.3, 128.4, 124.4, 123.4, 122.8, 122.3, 120.0, 105.6, 79.3, 79.2, 78.2, 78.0, 77.9, 53.4, 40.3, 40.2, 37.2, 37.1, 36.4, 29.8, 29.7, 29.7, 28.6, 28.6, 28.5, 28.5, 28.4, 28.3, 26.3, 26.2, 25.3, 25.1; ESIMS m/z 409 ([M + H]⁺); HRMS (ESI) for C₂₃H₂₉N₄O₃, calcd 409.2240, found 409.2241.
11: 10 (100 mg, 0.25 mmol) and trifluoroacetic acid (1 mL) was stirred in 0.5 mL of CH₂Cl₂ at room temperature for 30 min. After evaporation of solvent, the crude mixture 11 was used for synthesis without further purification (75 mg, 96% yield).

12: 9 (40 mg, 0.064 mmol), 11 (70.3 mg, 0.23 mmol), 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (49 mg, 0.26 mmol), 4-di(methylamino)pyridine (DMAP) (1 mg, catalytic amount) and triethylamine (33 mg, 0.32 mmol) in 2 mL of anhydrous dimethylformamide (DMF) was stirred at room temperature for 24 h. After evaporation of solvent, the residue was purified by flash column chromatography (2% MeOH in CH₂Cl₂) to give product 12 (56 mg, 96% isolated yield).

12: ¹H NMR (400 MHz, CDCl₃) δ 9.13–9.06 (m, 2H), 9.05–9.00 (m, 3H), 8.62 (d, J = 8.6 Hz, 1H), 8.53–8.49 (m, 2H), 8.33–8.29 (m, 2H), 8.24–8.21 (m, 1H), 8.11–8.10 (m, 1H), 8.08–8.05 (m, 1H), 8.01–7.98 (m, 1H), 7.97–7.95 (m, 1H), 7.94–7.93 (m, 1H), 7.90–7.83 (m, 3H), 7.80–7.74 (m, 1H), 7.72–7.68 (m, 4H),
7.67–7.63 (m, 1H), 7.09 (d,  $J = 14.8$ Hz, 0.5H), 7.05 (d,  $J = 9.7$ Hz, 0.5H), 6.88–6.83 (m, 2H), 6.55 (d,  $J = 6.4$ Hz, 0.5H), 3.81–3.80 (m, 2H), 3.58–3.50 (m, 4H), 3.46–3.45 (m, 2H), 3.10–3.08 (m, 1H), 3.04 (s, 1H), 2.95 (t,  $J = 6.8$ Hz, 1H), 2.63–2.58 (m, 5H), 1.90–1.87 (m, 2H), 1.75–1.72 (m, 2H), 1.62–1.57 (m, 2H), 1.21 (t,  $J = 7.1$ Hz, 3H); ESIMS $m/z$ 916 ([M + H]$^+$); HRMS (ESI) for C$_{51}$H$_{50}$N$_9$O$_6$S, calcd 916.3605, found 916.3604.

1: A mixture of [Ir$_2$(ppy)$_4$Cl$_2$] (7.5 mg, 0.007 mmol) and 12 (12.7 mg, 0.014 mmol) in 30 mL MeOH/CH$_2$Cl$_2$ = 1:1 (v/v) was refluxed under an inert atmosphere of N$_2$ in the dark for 4 h. The solution was then evaporated to dryness and the solid was dissolved in CH$_2$Cl$_2$ and purified by column chromatography on Al$_2$O$_3$ (8% MeOH in CH$_2$Cl$_2$) to give product 1 (9.7 mg, 98% isolated yield).

1: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 11.38–11.23 (m, 1H), 9.66–9.53 (m, 1H), 9.01 (d,  $J = 8.4$ Hz, 1H), 8.65 (d,  $J = 8.7$ Hz, 1H), 8.54 (m, 1H), 8.32–8.28 (m, 2H), 8.21–8.18 (m, 1H), 8.14–8.11 (m, 1H), 8.10–8.06 (m, 1H), 8.01–7.90 (m, 6H), 7.86–7.79 (m, 3H), 7.77–7.68 (m, 8H), 7.63–7.60 (m, 1H), 7.29–7.26 (m, 2H), 7.11–7.07 (m, 2H), 7.02–6.96 (m, 3H), 6.87–6.82 (m, 2H), 6.80–6.76 (m, 2H), 6.42–6.36 (m, 2H), 3.80 (q,  $J = 6.4$ Hz, 2H), 3.54–3.47 (m, 6H), 3.26–3.24 (m, 0.5H), 3.19–3.16 (m, 0.5H), 3.05 (t,  $J = 6.8$ Hz, 1H), 3.01–2.93 (m, 3H), 2.56 (t,  $J$
= 6.6 Hz, 2H), 2.36 (s, 1H), 1.98–1.93 (m, 2H), 1.84–1.79 (m, 2H), 1.21 (t, $J = 7.1$ Hz, 3H); ESIMS $m/z$ 1416 ([M + H]⁺).
**Preparation and Characterization of 13a–d, 14a–d, 15a–d, 16a–d, 17a–d, 19a–d, 21, 22a–d, 2a–d**

**General Procedure for 13a–d**

Boc-Cys(Trt)-OH (100 mg, 0.22 mmol), amine, 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (50 mg, 0.26 mmol), 4-di(methylamino)pyridine (DMAP) (1 mg, catalytic amount) and triethylamine (26 mg, 0.26 mmol) in 2 mL of anhydrous dimethylformamide (DMF) was stirred at room temperature for 24 h. After evaporation of solvent, the residue was purified by flash column chromatography (50–80% EtOAc in n-hexane) to give product 13.

**13a**: According to the general procedure for 13a–d using methylamine hydrochloride (17.5 mg, 0.26 mmol), 13a was obtained (100.5 mg, 96% isolated yield).

**13a**: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.56–7.40 (m, 7H), 7.28–7.24 (m, 4H), 7.25–7.20 (m, 4H), 6.13 (s, 1H), 4.96 (d, $J = 7.8$ Hz, 1H), 3.88 (s, 1H), 2.72–2.65 (m, 4H), 2.53 (dd, $J = 12.8$, 5.2 Hz, 1H), 1.41 (s, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.9, 144.4, 129.5, 128.0, 126.8, 100.3, 80.2, 67.1, 53.5, 34.0, 28.3, 26.2; EIMS $m/z$ 476 (M$^+$); HRMS (EI) for C$_{28}$H$_{32}$N$_2$O$_3$S, calcld 476.2134, found 476.2132.

**13b**: According to the general procedure for 13a–d using isopropylamine (15 mg, 0.26 mmol), 13b was obtained (88 mg, 80% yield).
13b: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.56–7.40 (m, 7H), 7.28–7.25 (m, 4H), 7.25–7.20 (m, 4H), 5.75 (d, $J = 7.2$ Hz, 1H), 4.82 (s, 1H), 4.02–3.93 (m, 1H), 3.78 (s, 1H), 2.68 (dd, $J = 12.4$, 6.6 Hz, 1H), 2.50 (dd, $J = 12.8$, 5.0 Hz, 1H), 1.41 (s, 9H), 1.10–1.08 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 169.5, 155.4, 129.9, 129.6, 129.3, 128.8, 128.3, 128.0, 127.8, 127.0, 126.8, 80.1, 67.1, 53.7, 41.5, 34.0, 30.2, 28.3, 22.6; EIMS $m/z$ 504 (M$^+$); HRMS (EI) for C$_{30}$H$_{36}$N$_2$O$_3$S, calcd 504.2447, found 504.2445.

13c: According to the general procedure for 13a–d using piperidine (22 mg, 0.26 mmol), 13c was obtained (103 mg, 88% yield).

13c: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.42–7.39 (m, 7H), 7.28–7.25 (m, 4H), 7.25–7.18 (m, 4H), 5.39 (d, $J = 8.6$ Hz, 1H), 4.56 (s, 1H), 3.88 (s, 1H), 3.48–3.46 (m, 2H), 3.14–3.13 (m, 1H), 3.07–3.05 (m, 1H), 2.48–2.46 (m, 1H), 2.35 (dd, $J = 16.2$, 9.9 Hz, 1H), 1.56–1.54 (m, 2H), 1.47 (m, 2H), 1.43 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 144.6, 129.6, 127.9, 126.7, 49.3, 46.4, 43.3, 35.2, 28.3, 26.3, 25.4, 24.4; EIMS $m/z$ 476 (M$^+$); HRMS (EI) for C$_{32}$H$_{38}$N$_2$O$_3$S, calcd 530.2603, found 530.2601.

13d: According to the general procedure for 13a–d using t-butylamine (19 mg, 0.26 mmol), 13d was obtained (101 mg, 89% yield).

13d: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.44–7.41 (m, 7H), 7.31–7.24 (m, 4H), 7.23–7.21 (m, 4H), 5.84 (s, 1H), 4.79 (s, 1H), 3.72 (s, 1H), 2.65–2.64 (m, 1H), 2.46 (dd, $J = 7.5$, 5.3 Hz, 1H), 1.47 (s, 9H), 1.26 (s, 9H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 169.5, 144.4, 129.6, 128.0, 127.9, 126.8, 67.1, 53.9, 51.3, 33.8, 31.7, 28.6, 28.2, 22.6, 14.1; EIMS $m/z$ 518 (M$^+$); HRMS (EI) for C$_{34}$H$_{38}$N$_2$O$_3$S, calcd 518.2603, found 518.2601.
General Procedure for 14a–d

13, triisopropylsilane (0.12 mL), trifluoroacetic acid (1.3 mL) and H₂O (0.05 mL) was stirred in 0.5 mL of CH₂Cl₂ at room temperature for 0.5 h. After evaporation of solvent, CH₂Cl₂ (30 mL) was added to the resulting residues. The organic layer was extracted with H₂O (10 mL). The aqueous layer was then evaporated to give the transparent oil product. The crude mixture 14 was used for synthesis without further purification.

14a: According to the general procedure for 14a–d using 13a (50 mg, 0.11 mmol), 14a was obtained (14 mg, 96% yield).

14b: According to the general procedure for 14a–d using 13b (50 mg, 0.10 mmol), 14b was obtained (16 mg, 98% yield).

14c: According to the general procedure for 14a–d using 13c (50 mg, 0.094 mmol), 14c was obtained (16.8 mg, 95% yield).

14d: According to the general procedure for 14a–d using 13d (50 mg, 0.097 mmol), 14d was obtained (17.0 mg, 97% yield).
General Procedure for 15a–d

14, triphenylmethanol in 2 mL of trifluoroacetic acid was stirred at room temperature for 3 h. After evaporation of solvent, the crude mixture was washed by hexane for several times to give product 15.

15a: According to the general procedure for 15a–d using 14a (15 mg, 0.112 mmol) and triphenylmethanol (29 mg, 0.112 mmol), 15a was obtained (42.1 mg, 100% yield).

15b: According to the general procedure for 15a–d using 14b (15 mg, 0.093 mmol) and triphenylmethanol (24 mg, 0.093 mmol) was used, 15b was obtained (37.8 mg, 100% yield).

15c: According to the general procedure for 15a–d using 14c (15 mg, 0.079 mmol) and triphenylmethanol (21 mg, 0.079 mmol) was used, 15c was obtained (34.0 mg, 100% yield).

15d: According to the general procedure for 15a–d using 14d (15 mg, 0.085 mmol) and triphenylmethanol (22 mg, 0.085 mmol), 15d was obtained (35.5 mg, 100% yield).
General Procedure for 16a–d

16a–d: 15, 6 (30 mg, 0.08 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (61.4 mg, 0.32 mmol), 4-di(methylamino)pyridine (DMAP) (1 mg, catalytic amount) and triethylamine (40.4 mg, 0.40 mmol) in 2 mL of anhydrous dimethylformamide (DMF) was stirred at room temperature for 24 h. After evaporation of solvent, the residue was purified by flash column chromatography (2–6% MeOH in CH₂Cl₂) to give product 16.

16a: According to the general procedure for 16a–d using 15a (90 mg, 0.24 mmol), 16a was obtained (45 mg, 75% isolated yield).

16a: ¹H NMR (400 MHz, CDCl₃) δ 9.04 (d, J = 8.2 Hz, 1H), 8.68 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 8.2 Hz, 1H), 8.00 (d, J = 8.5 Hz, 2H), 7.78–7.70 (m, 3H), 7.40–7.38 (m, 6H), 7.29–7.20 (m, 9H), 6.74 (d, J = 8.4 Hz, 2H), 6.04 (d, J = 7.6 Hz, 1H), 5.89–5.87 (m, 1H), 4.13–4.08 (m, 1H), 3.73 (t, J = 6.8 Hz, 2H), 3.46 (q, J = 7.1 Hz, 2H), 2.75–2.71 (m, 0.6 H), 2.70 (m, 3H), 2.69 – 2.65 (m, 1H), 2.68 (dd, J = 13.3, 7.6 Hz, 1H), 2.60 (dd, J = 13.3, 5.6 Hz, 1H), 2.46–2.45 (m, 2H), 1.19 (t, J = 7.1 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 170.7, 170.3, 150.7, 144.6, 144.3, 131.5, 129.6, 129.5, 128.1, 127.9, 127.9, 127.2, 126.9, 126.8, 126.6, 126.4, 125.0, 124.6, 123.1, 111.5, 109.8, 52.3, 46.5, 45.6, 34.4, 33.5, 26.3, 14.2, 12.3; ESIMS
m/z 751 ([M + H]$^+$); HRMS (ESI) for C$_{44}$H$_{43}$N$_6$O$_4$S, calcd 751.3066, found 751.3065.

**16b**: According to the general procedure for 16a–d using 15b (97 mg, 0.24 mmol), 16b was obtained (46.1 mg, 74% isolated yield).

16b: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.04 (d, $J$ = 8.2 Hz, 1H), 8.68 (d, $J$ = 8.4 Hz, 1H), 8.33 (d, $J$ = 8.2 Hz, 1H), 8.00 (d, $J$ = 8.5 Hz, 2H), 7.78–7.70 (m, 3H), 7.40–7.38 (m, 6H), 7.29–7.20 (m, 9H), 6.72 (d, $J$ = 8.4 Hz, 2H), 6.29 (d, $J$ = 7.8 Hz, 1H), 5.70 (d, $J$ = 7.8 Hz, 1H), 4.03–3.98 (m, 1H), 3.96–3.88 (m, 1H), 3.77–3.68 (m, 2H), 3.46–3.41 (m, 2H), 2.68–2.63 (m, 1H), 2.55–2.50 (m, 1H), 2.49–2.44 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.6, 168.9, 150.8, 144.6, 144.4, 131.5, 129.6, 129.6, 129.5, 128.1, 128.0, 127.2, 126.9, 126.6, 126.4, 125.1, 124.6, 123.1, 111.4, 109.7, 67.2, 60.4, 54.1, 52.5, 46.5, 45.6, 41.8, 41.0, 37.5, 34.4, 34.0, 31.5, 30.9, 30.1, 29.7, 22.7, 22.6, 21.1, 14.2, 12.3; ESIMS m/z 779 ([M + H]$^+$); HRMS (ESI) for C$_{46}$H$_{47}$N$_6$O$_4$S, calcd 779.3379, found 779.3380.

**16c**: According to the general procedure for 16a–d using 15c (103.2 mg, 0.24 mmol), 16c was obtained (51.4 mg, 80% isolated yield).

16c: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.04 (d, $J$ = 8.2 Hz, 1H), 8.68 (d, $J$ = 8.4 Hz, 1H), 8.33 (d, $J$ = 8.2 Hz, 1H), 8.00 (d, $J$ = 8.5 Hz, 2H), 7.75–7.73 (m, 3H), 7.39–7.37 (m, 6H), 7.29–7.21 (m, 9H), 6.76 (d, $J$ = 8.9 Hz, 2H), 6.50 (d, $J$ = 8.1 Hz, 1H), 4.93–4.92 (m, 1H), 3.80–3.77 (m, 2H), 3.51–3.47 (m, 3H), 3.42–3.41 (m, 1H), 3.12–3.08 (m, 1H), 3.02–2.99 (m, 1H), 2.59–2.52 (m, 3H), 2.46–2.43 (m, 1H), 1.67 (m, 2H), 1.56 (m, 2H), 1.20 (t, $J$ = 7.0 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.1, 168.0, 152.1, 150.9, 145.7, 144.6, 144.4, 131.4, 130.0, 129.6, 129.5, 129.3, 128.0, 127.7, 127.2, 127.0, 126.8, 126.6, 126.4, 125.1, 124.6, 123.1,
111.5, 111.2, 109.8, 66.9, 60.4, 47.9, 46.6, 46.4, 45.6, 43.4, 34.8, 34.5, 31.5, 30.8, 30.1, 29.7, 26.3, 25.6, 25.4, 25.4, 24.3, 14.2, 12.4; ESIMS m/z 805 ([M + H]+);
HRMS (ESI) for C₄₈H₄₉N₆O₄S, calcd 805.3536, found 805.3533.

16d: According to the general procedure for 16a–d using 15d (100.3 mg, 0.24 mmol), 16d was obtained (48.2 mg, 76% isolated yield).

16d: ¹H NMR (400 MHz, CDCl₃) δ 9.04 (d, J = 8.2 Hz, 1H), 8.68 (d, J = 8.4 Hz, 1H), 8.33 (d, J = 8.2 Hz, 1H), 8.00 (d, J = 8.5 Hz, 2H), 7.75–7.73 (m, 3H), 7.42–7.40 (m, 6H), 7.31–7.22 (m, 9H), 6.74 (d, J = 9.2 Hz, 2H), 5.97 (d, J = 7.7 Hz, 1H), 5.61 (s, 1H), 3.94–3.92 (m, 1H), 3.74–3.71 (m, 2H), 3.46–3.43 (m, 2H), 2.62 (dd, J = 7.2, 5.8 Hz, 1H), 2.51–2.46 (m, 1H), 2.45–2.43 (m, 1H), 1.25 (s, 9H), 1.20 (t, J = 7.0 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 170.4, 168.8, 150.7, 144.6, 144.4, 132.6, 129.6, 128.1, 128.0, 127.2, 126.9, 126.6, 126.4, 125.0, 124.6, 123.1, 111.5, 109.8, 52.8, 51.7, 46.5, 45.6, 34.5, 33.9, 28.6, 12.3, 5.6; ESIMS m/z 793 ([M + H]+); HRMS (ESI) for C₄₇H₄₉N₆O₄S, calcd 793.3536, found 793.3533.

General Procedure for 17a–d

17a–d: 16, triisopropylsilane (0.12 mL), trifluoroacetic acid (1.3 mL) and H₂O (0.05 mL) was stirred in 0.5 mL of CH₂Cl₂ at room temperature for 1 h. After evaporation of solvent, the residue was purified by flash column chromatography
(2–6% MeOH in CH₂Cl₂) to give product 17.

**17a:** According to the general procedure for **17a–d** using **16a** (50 mg, 0.067 mmol), **17a** was obtained (28.1 mg, 83% isolated yield).

**17a:** $^1$H NMR (400 MHz, CDCl₃) δ 9.03 (d, $J = 8.4$ Hz, 1H), 8.67 (d, $J = 8.6$ Hz, 1H), 8.33 (d, $J = 8.4$ Hz, 1H), 8.01 (d, $J = 8.9$ Hz, 2H), 7.80–7.70 (m, 3H), 7.05–7.02 (m, 1H), 6.82 (d, $J = 9.0$ Hz, 2H), 4.53–4.48 (m, 1H), 3.80 (t, $J = 6.9$ Hz, 2H), 3.54 (t, $J = 7.1$ Hz, 2H), 2.90–2.80 (m, 1H), 2.74–2.64 (m, 1H), 2.78–2.76 (m, 3H), 2.62–2.58 (m, 2H), 1.26 (t, $J = 7.0$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl₃) δ 171.2, 170.3, 152.0, 150.8, 145.8, 144.6, 131.4, 129.5, 127.2, 126.6, 126.3, 125.0, 124.5, 123.1, 111.5, 109.8, 54.6, 54.5, 50.1, 49.9, 49.7, 49.5, 49.3, 46.6, 45.5, 34.5, 34.4, 30.8, 29.7, 26.4, 26.3, 26.2, 12.3; ESIMS m/z 509 ([M + H]$^+$); HRMS (ESI) for C₂₅H₂₉N₆O₄S, calcd 509.1971, found 509.1970.

**17b:** According to the general procedure for **17a–d** using **16b** (50 mg, 0.064 mmol), **17b** was obtained (26.9 mg, 78% isolated yield).

**18b:** $^1$H NMR (400 MHz, CDCl₃) δ 9.04 (d, $J = 8.2$ Hz, 1H), 8.68 (d, $J = 8.4$ Hz, 1H), 8.33 (d, $J = 8.2$ Hz, 1H), 8.00 (d, $J = 8.5$ Hz, 2H), 7.75–7.73 (m, 3H), 6.83 (d, $J = 9.3$ Hz, 2H), 4.41 (dd, $J = 6.7$, 5.8Hz, 1H), 4.06–3.96 (m, 1H), 3.82–3.78 (m, 2H), 3.57–3.52 (m, 2H), 2.79–2.76 (m, 2H), 2.64–2.60 (m, 2H), 1.26 (t, $J = 7.1$ Hz, 3H), 1.19–1.15 (m, 6H); $^{13}$C NMR (100 MHz, CDCl₃) δ 171.2, 168.7, 152.0, 150.9, 145.8, 144.6, 131.4, 129.5, 127.2, 126.6, 126.3, 125.0, 124.5, 123.1, 111.5, 109.8, 54.7, 50.0, 49.8, 49.6, 49.4, 49.2, 49.0, 48.8, 46.6, 45.4, 41.7, 34.4, 26.4, 22.4, 22.2, 12.3; ESIMS m/z 537 ([M + H]$^+$); HRMS (ESI) for C₂₇H₃₉N₆O₄S, calcd 537.2284, found 537.2283.

**17c:** According to the general procedure for **17a–d** using **16c** (50 mg, 0.062
mmol), 17c was obtained (29.0 mg, 83% isolated yield).

17c: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.04 (d, \(J = 8.2\) Hz, 1H), 8.68 (d, \(J = 8.4\) Hz, 1H), 8.33 (d, \(J = 8.2\) Hz, 1H), 8.00 (d, \(J = 8.5\) Hz, 2H), 7.75–7.72 (m, 3H), 6.81 (d, \(J = 9.2\) Hz, 2H), 6.77 (m, 1H), 5.13–5.11 (m, 1H), 3.81 (t, \(J = 7.0\) Hz, 2H), 3.57–3.52 (m, 3H), 3.51–3.48 (m, 3H), 2.94–2.87 (m, 1H), 2.76–2.70 (m, 1H), 2.62–2.59 (m, 2H), 1.65–1.45 (m, 6H), 1.24 (t, \(J = 7.0\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 170.2, 167.5, 152.0, 150.9, 145.8, 144.6, 131.5, 129.5, 127.2, 126.6, 126.4, 125.0, 124.6, 123.1, 111.5, 111.3, 109.8, 50.1, 46.9, 46.6, 45.6, 45.4, 43.6, 42.7, 34.6, 30.7, 29.7, 26.5, 25.5, 24.4, 24.3, 14.1, 12.4; ESIMS m/z 563 ([M + H])\(^+\); HRMS (ESI) for C\(_{29}\)H\(_{35}\)N\(_6\)O\(_4\)S, calcld 563.2440, found 563.2442.

17d: According to the general procedure for 17a–d using 16d (50 mg, 0.063 mmol), 17d was obtained (27.4 mg, 79% isolated yield).

17d: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.04 (d, \(J = 8.2\) Hz, 1H), 8.68 (d, \(J = 8.5\) Hz, 1H), 8.33 (d, \(J = 8.4\) Hz, 1H), 8.01 (d, \(J = 9.1\) Hz, 2H), 7.75–7.71 (m, 3H), 6.80 (d, \(J = 9.1\) Hz, 2H), 6.64 (d, \(J = 7.2\) Hz, 1H), 6.02 (s, 1H), 4.41–4.39 (m, 1H), 3.83–3.78 (m, 2H), 3.53 (q, \(J = 7.0\) Hz, 2H), 2.93–2.91 (m, 1H), 2.70–2.66 (m, 1H), 2.61 (t, \(J = 6.8\) Hz, 2H), 1.27 (s, 9H), 1.23 (t, \(J = 7.1\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 170.6, 168.4, 152.0, 150.8, 145.9, 144.7, 131.5, 129.5, 127.2, 126.6, 126.3, 125.0, 124.6, 123.1, 111.5, 109.8, 55.0, 52.0, 46.6, 45.6, 34.6, 30.9, 29.7, 28.7, 26.8, 12.4; ESIMS m/z 793 ([M + H])\(^+\); HRMS (ESI) for C\(_{28}\)H\(_{33}\)N\(_6\)O\(_4\)S, calcld 551.2440, found 551.2443.
General Procedure for 19a–d

19a–d: 17, 18 and H₂O (1.5 mL) was stirred in 0.5 mL of DMF at room temperature for 24 h. After evaporation of solvent, the residue was purified by flash column chromatography (5–10% MeOH in CH₂Cl₂) to give product 19.

19a: According to the general procedure for 19a–d using 17a (20 mg, 0.039 mmol), 18 (8.2 mg, 0.039 mmol), 19a was obtained (10.1 mg, 36% isolated yield, Z/E = 1/1).

19a: ¹H NMR (400 MHz, CDCl₃) δ 9.03–9.00 (m, 1H), 8.67–8.62 (m, 1H), 8.34–8.28 (m, 1H), 8.03–7.97 (m, 2H), 7.95–7.93 (m, 1H), 7.90–7.88 (m, 1H), 7.87–7.85 (m, 1H), 7.80–7.78 (m, 1H), 7.75–7.69 (m, 2H), 7.48 (d, J = 9.7 Hz, 0.5H), 7.12 (d, J = 15.0 Hz, 0.5H), 7.07 (d, J = 9.7 Hz, 0.5H), 6.86–6.80 (m, 2H), 4.22–4.17 (m, 2H), 3.79 (q, J = 7.2 Hz, 2H), 3.54 (q, J = 7.2 Hz, 2H), 3.22 (dd, J = 17.4, 6.8 Hz, 1H), 3.08 (dd, J = 14.5, 6.8 Hz, 1H), 2.84–2.77 (m, 3H), 2.77–2.64 (m, 2H), 2.31 (t, J = 2.5 Hz, 1H), 1.26–1.23 (m, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 188.6, 186.6, 171.5, 170.0, 153.5, 152.0, 150.8, 149.3, 145.7, 139.9, 131.3, 129.5, 128.5, 128.0, 127.6, 127.5, 127.5, 127.2, 126.5, 126.2, 124.9, 124.5, 123.0, 118.7, 116.8, 111.6, 109.7, 71.5, 53.2, 52.1, 49.7, 49.5, 49.3, 49.1, 48.8, 48.6, 48.4,
19b: According to the general procedure for 19a–d using 17b (20 mg, 0.037 mmol) and 18 (7.8 mg, 0.037 mmol), 19b was obtained (10.5 mg, 38% isolated yield, Z/E = 1/1).

19b: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.03–9.00 (m, 1H), 8.66 (d, $J = 8.6$ Hz, 1H), 8.33 (d, $J = 8.4$ Hz, 1H), 8.03–7.99 (m, 3H), 7.96–7.88 (m, 3.5H), 7.82–7.79 (m, 1H), 7.74–7.71 (m, 2H), 7.52 (d, $J = 9.7$ Hz, 0.5 H), 7.13 (d, $J = 14.9$ Hz, 0.5H), 7.08 (d, $J = 9.7$ Hz, 0.5H), 6.85 (d, $J = 8.5$ Hz, 2H), 4.65 (t, $J = 6.5$ Hz, 0.5H), 4.58 (t, $J = 6.7$ Hz, 0.5H), 4.00–3.95 (m, 1H), 3.81–3.80 (m, 4H), 3.56–3.54 (m, 2H), 3.30–3.25 (m, 0.5H), 3.21–3.11 (m, 1H), 3.06–3.04 (m, 0.5H), 2.64–2.63 (m, 2H), 2.35 (t, $J = 2.5$ Hz, 1H), 1.26–1.23 (m, 3H), 1.16–1.13 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 192.6, 190.7, 175.7, 172.4, 171.0, 157.9, 156.0, 155.0, 153.6, 149.6, 148.4, 143.9, 141.3, 141.1, 135.3, 133.5, 132.4, 131.9, 131.5, 131.2, 130.4, 130.2, 128.9, 128.4, 126.9, 122.6, 120.6, 115.4, 113.6, 83.1, 75.1, 57.2, 56.1, 50.5, 49.2, 45.7, 45.6, 42.6, 38.5, 38.1, 33.5, 33.3, 25.9, 16.0; ESIMS m/z 748 ([M + H]$^+$); HRMS (ESI) for C$_{40}$H$_{42}$N$_7$O$_6$S, calcd 748.2917, found 748.2919.

19c: According to the general procedure for 19a–d using 17c (20 mg, 0.036 mmol) and 18 (7.6 mg, 0.036 mmol), 19c was obtained (11.7 mg, 42% isolated yield, Z/E = 3/2).

19c: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.02–8.99 (m, 1H), 8.66 (d, $J = 8.7$ Hz, 1H), 8.31 (d, $J = 8.4$ Hz, 1H), 8.07 (d, $J = 8.3$ Hz, 1H), 8.01–7.96 (m, 2H), 7.90–7.84 (m, 2H), 7.79–7.76 (m, 2H), 7.74–7.68 (m, 2H), 7.33 (d, $J = 9.7$ Hz, 0.6H), 7.25 (d, $J = 13.9$ Hz, 0.4H), 6.99–6.94 (m, 2H), 6.83–6.80 (m, 2H), 6.45–6.44 (m,
0.4H), 6.42–6.40 (m, 0.6H), 5.25–5.23 (m, 0.4H), 5.23–5.21 (m, 0.6H), 4.26–4.22 (m, 2H), 3.85–3.78 (m, 2H), 3.55–3.51 (m, 3H), 3.49–3.47 (m, 3H), 3.24–3.21 (m, 1H), 3.12–3.03 (m, 2H), 2.63–2.59 (m, 2H), 2.30–2.29 (m, 1H), 1.71 (m, 4H) 1.62–1.56 (m, 6H), 1.26–1.27 (m, 3H); 13C NMR (100 MHz, CDCl3) δ 187.9, 185.6, 170.4, 170.2, 167.6, 167.4, 166.2, 166.1, 153.3, 151.9, 150.9, 150.9, 148.4, 145.8, 144.6, 140.1, 140.1, 137.2, 137.0, 131.4, 129.6, 128.8, 128.1, 127.4, 127.3, 127.2, 126.6, 126.3, 125.0, 124.5, 123.1, 118.5, 116.8, 111.6, 111.5, 109.8, 79.1, 76.7, 72.1, 48.6, 47.4, 47.2, 46.5, 45.4, 43.7, 43.6, 39.6, 34.9, 34.6, 34.5, 29.9, 29.7, 26.4, 26.3, 25.3, 24.2, 12.4, 12.3; ESIMS m/z 774 ([M + H]+); HRMS (ESI) for C42H44N7O6S, calcd 774.3074, found 774.3071.

19d: According to the general procedure for 19a–d using 17d (20 mg, 0.036 mmol) and 18 (7.6 mg, 0.036 mmol), was used, 19d was obtained (8.6 mg, 31% isolated yield, Z/E = 3/2).

19d: 1H NMR (400 MHz, CDCl3) δ 8.99–8.96 (m, 1H), 8.63 (d, J = 8.6 Hz, 1H), 8.29–8.27 (dd, J = 8.4, 1.9 Hz, 1H), 7.48 (d, J = 9.7 Hz, 0.6H), 7.12 (d, J = 13.8 Hz, 0.4H), 7.00–6.97 (m, 2H), 6.79 (d, J = 9.1 Hz, 2H), 6.31 (d, J = 7.4 Hz, 1H), 4.25–4.23 (m, 2H), 3.82–3.77 (m, 2H), 3.54–3.51 (m, 2H), 3.32–2.95 (m, 2H), 2.63–2.62 (m, 2H), 2.31–2.29 (m, 1H), 2.04 (m, 1H), 1.33 (s, 9H), 1.23 (t, J = 7.1 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 188.2, 188.0, 185.7, 170.9, 168.3, 168.0, 166.4, 166.3, 166.1, 162.6, 153.1, 151.9, 150.9, 147.7, 145.8, 144.6, 140.0, 138.5, 137.7, 137.3, 137.1, 131.9, 131.5, 129.6, 128.8, 128.5, 128.1, 127.6, 127.5, 127.4, 127.3, 126.6, 126.3, 125.0, 124.6, 123.1, 118.8, 117.1, 116.5, 111.6, 111.5, 109.9, 109.8, 79.3, 79.2, 72.2, 72.1, 60.4, 54.1, 52.6, 52.5, 52.2, 52.0, 46.6, 45.5, 39.0, 36.5, 34.6, 34.3, 34.1, 31.5, 30.1, 30.0, 29.9, 29.7, 28.7, 28.6, 21.1, 14.2, 12.4; ESIMS m/z 793 ([M + H]+); HRMS (ESI) for C41H44N7O6S, calcd 762.3074, found
(100 mg, 0.28 mmol) and sodium azide (268.9 mg, 0.84 mmol) was stirred in 2 mL of DMF at room temperature for 24 h. After evaporation of solvent, the residue was purified by flash column chromatography on Al₂O₃ (1% MeOH in CH₂Cl₂) to give product 21 (67.2 mg, 75% isolated yield).

21: ¹H NMR (400 MHz, CDCl₃) δ 9.22 (s, 1H), 8.92 (d, J = 7.7 Hz, 2H), 8.31 (d, J = 8.2 Hz), 7.94 (d, J = 7.8 Hz, 1H), 7.83 (s, 1H), 7.48–7.45 (m, 1H), 7.35–7.32 (m, 1H), 3.26 (t, J = 6.6 Hz, 2H), 2.50 (t, J = 6.9 Hz, 2H), 1.82–1.75 (m, 2H), 1.64–1.59 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 172.5, 149.6, 149.4, 148.7, 146.0, 144.1, 135.8, 130.9, 130.7, 128.1, 124.6, 123.3, 122.5, 120.5, 106.6, 51.1, 36.1, 28.3, 22.7; EIMS m/z 476 (M⁺); HRMS (EI) for C₁₇H₁₆N₆O, calcd 320.1386, found 320.1386.
**General Procedure for 22a–d**

22: 19, 21, sodium ascorbate, CuSO$_4$•5H$_2$O, was stirred in a solvent mixture of 1 mL of H$_2$O and 2 mL of DMF at room temperature for 24 h. After evaporation of solvent, the residue was purified by flash column chromatography on Al$_2$O$_3$ (5–10% MeOH in CH$_2$Cl$_2$) to give product 22.

22a: According to the general procedure for 22a–d using 19a (40 mg, 0.056 mmol), 21 (17.9 mg, 0.056 mmol), sodium ascorbate (1.1 mg, 0.0056 mmol), CuSO$_4$•5H$_2$O (0.14 mg, 0.00056 mmol), 22a was obtained (32.6 mg, 56% isolated yield).

22a: $^1$H NMR (400 MHz, CDCl$_3$) δ 9.15 (m, 2H), 9.07 (m, 2H), 9.01 (d, $J = 8.4$ Hz, 1H), 8.65 (d, $J = 8.4$ Hz, 1H), 8.59–8.57 (m, 1H), 8.49–8.43 (m, 2H), 8.32 (d, $J = 8.3$ Hz, 1H), 8.29–8.25 (m, 2H), 8.17–8.12 (m, 2H), 8.00 (d, $J = 9.2$ Hz, 2H), 7.95–7.90 (m, 2H), 7.86 (s, 3H), 7.82 (s, 2H), 7.79–7.74 (m, 1H), 7.72–7.67 (m, 6H), 7.47 (d, $J = 9.6$ Hz, 1H), 7.00 (d, $J = 9.7$ Hz, 1H), 6.84 (d, $J = 9.2$ Hz, 2H), 4.63 (t, $J = 5.7$ Hz, 3H), 4.48–4.45 (m, 2H), 4.04–4.03 (m, 2H), 3.81–3.78 (m, 3H), 3.55–3.53 (m, 2H), 3.43–3.41 (m, 3H), 3.22–3.19 (m, 2H), 3.17–3.11 (m, 1H), 3.07–3.01 (m, 1H), 2.99 (m, 1H), 2.77–2.76 (m, 2H), 2.66–2.60 (m, 6H), 4.63 (t, $J = 5.7$ Hz, 3H), 4.48–4.45 (m, 2H), 4.04–4.03 (m, 2H), 3.81–3.78 (m, 3H), 3.55–3.53 (m, 2H), 3.43–3.41 (m, 3H), 3.22–3.19 (m, 2H), 3.17–3.11 (m, 1H), 3.07–3.01 (m, 1H), 2.99 (m, 1H), 2.77–2.76 (m, 2H), 2.66–2.60 (m, 6H),
2.12–2.08 (m, 3H), 1.95–1.91 (m, 3H), 1.83–1.77 (m, 5H), 1.45–1.35 (m, 4H), 1.24 (m, 3H); ESIMS m/z 1040 ([M + H]^+); HRMS (ESI) for C_{55}H_{54}N_{13}O_{7}S, calcd 1040.3990, found 1040.3991.

22b: According to the general procedure for 22a–d using 19b (40 mg, 0.054 mmol), 21 (17.3 mg, 0.054 mmol), sodium ascorbate (1.07 mg, 0.0054 mmol), CuSO_{4}·5H_{2}O (0.14 mg, 0.00054 mmol) was used, 22b was obtained (33.4 mg, 58% isolated yield).

22b: ^1^H NMR (400 MHz, CDCl₃) δ 9.14–9.12 (m, 2H), 9.06–9.00 (m, 3H), 8.65–8.63 (d, J = 8.8 Hz, 1H), 8.50–8.46 (m, 2H), 8.33–8.31 (m, 1H), 8.28–8.27 (m, 2H), 8.13–8.12 (m, 2H), 8.02–8.00 (m, 2H), 7.90–7.77 (m, 7H), 7.74–7.66 (m, 6H), 7.49 (d, J = 9.8 Hz, 0.5H), 7.06 (d, J = 14.9 Hz, 0.5 H), 7.02 (d, J = 9.8 Hz, 0.5H), 6.85 (d, J = 9.2 Hz, 2H), 5.69 (d, J = 12.2 Hz, 1H), 4.63 (d, J = 7.1 Hz, 3H), 4.60–4.56 (m, 1H), 4.47 (t, J = 6.8 Hz, 3H), 4.03–3.98 (m, 1H), 3.97–3.86 (m, 1H), 3.86–3.80 (m, 4H), 3.65 (q, J = 7.0, 3H), 3.57–3.52 (m, 2H), 2.63–2.61 (m, 6H), 2.09 (t, J = 7.3Hz, 4H), 1.91–1.79 (m, 4H), 1.26–1.23 (m, 3H), 1.16–1.13 (m, 6H); ESIMS m/z 1068 ([M + H]^+); HRMS (ESI) for C_{57}H_{58}N_{13}O_{7}S, calcd 1068.4303, found 1068.4300.

22c: According to the general procedure for 22a–d using 19c (40 mg, 0.052 mmol), 21 (16.6 mg, 0.052 mmol), sodium ascorbate (1.03 mg, 0.0052 mmol), CuSO_{4}·5H_{2}O (0.13 mg, 0.00052 mmol) was used, 22c was obtained (30.1 mg, 53% isolated yield).

22c: ^1^H NMR (400 MHz, CDCl₃) δ 9.20–9.17 (m, 0.3H), 9.10–9.07 (m, 2H), 9.04–9.00 (m, 1H), 8.92–8.86 (m, 0.5H), 8.68–8.61 (m, 1H), 8.63–8.61 (m, 1H), 8.44–8.27 (m, 2H), 8.19–8.11 (m, 3H), 8.03–7.97 (m, 2H), 7.83–7.78 (m, 4H),
7.75 (m, 2H), 7.73–7.62 (m, 5H), 7.61 (m, 3H), 7.18–7.16 (m, 0.4H), 6.86 (d, J = 9.6 Hz, 0.6H), 6.80 (d, J = 9.2 Hz, 2H), 5.61 (m, 1H), 4.72–4.65 (m, 3H), 4.38 (m, 3H), 4.06–4.00 (m, 0.5H), 3.88–3.81 (m, 2H), 3.71 (q, J = 7.0 Hz, 2H), 3.64 (m, 1H), 3.57–3.46 (m, 2H), 3.04–2.88 (m, 7H), 2.88–2.85 (m, 2H), 2.61–2.58 (m, 2H), 2.57–2.53 (m, 3H), 2.09–1.97 (m, 3H), 1.91–1.79 (m, 3H), 1.71 (m, 15H), 1.66 (m, 6H), 1.55 (m, 6H); ESIMS m/z 1094 ([M + H]+); HRMS (ESI) for C_{59}H_{60}N_{13}O_{7}S, calcd 1094.4459, found 1094.4461.

22d: According to the general procedure for 22a–d using 19d (40 mg, 0.053 mmol), 21 (17.0 mg, 0.053 mmol), sodium ascorbate (1.05 mg, 0.0053 mmol), CuSO_{4}·5H_{2}O (0.13 mg, 0.00053 mmol) was used, 22d was obtained (33.8 mg, 59% isolated yield).

22d: \(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)) \(\delta\) 9.15–9.03 (m, 2H), 9.02–8.98 (m, 1H), 8.64 (d, J = 8.6 Hz, 1H), 8.41–8.38 (m, 1H), 8.34–8.30 (m, 1H), 8.23–8.16 (m, 1H), 8.13–8.11 (m, 1H), 8.00–7.95 (m, 2H), 7.90–7.84 (m, 3H), 7.81–7.72 (m, 4H), 7.71–7.68 (m, 2H), 7.62–7.58 (m, 2H), 7.49–7.44 (m, 1H), 7.00–6.93 (m, 2H), 6.83–6.79 (m, 2H), 4.62 (m, 2H), 4.55–4.50 (m, 1H), 4.46–4.42 (m, 2H), 4.35–4.31 (m, 1H), 3.80–3.75 (m, 2H), 3.66–3.65 (m, 0.6H), 3.55–3.51 (m, 2H), 3.43–3.40 (m, 2H), 3.20–3.19 (m, 1H), 3.06–3.03 (m, 1H), 2.63–2.54 (m, 3H), 2.09–2.06 (m, 2H), 1.84–1.80 (m, 2H), 1.68–1.65 (m, 3H), 1.32 (s, 9H), 1.23 (t, J = 7.1 Hz, 3H); ESIMS m/z 1082 ([M + H]^+); HRMS (ESI) for C_{58}H_{60}N_{13}O_{7}S, calcd 1082.4459, found 1082.4455.
**General Procedure for 2a–d**

2a–d: A mixture of [Ir2(ppy)4Cl2] (7.5 mg, 0.007 mmol) and 22 in 30 mL MeOH/CH2Cl2 = 1:1 (v/v) was refluxed under an inert atmosphere of N2 in the dark for 4 h. The solution was then evaporated to dryness and the solid was dissolved in CH2Cl2 and purified by column chromatography on Al2O3 (8% MeOH in CH2Cl2) to give product 2.

2a: According to the general procedure for 2a–d using 22a (14.5 mg, 0.014 mmol), 2a was obtained (9.6 mg, 89% isolated yield).

2a: 1H NMR (400 MHz, CDCl3) δ 9.66 (d, J = 8.7 Hz, 0.3H), 9.05–9.00 (m, 0.5H), 8.70–8.64 (m, 1H), 8.58–8.53 (m, 0.3H), 8.45–8.28 (m, 1H), 8.22–8.19 (m, 1H), 8.12–8.11 (m, 1H), 7.98–7.96 (m, 1H), 7.93–7.89 (m, 3H), 7.85–7.82 (m, 2H) 7.73–7.70 (m, 6H), 7.66–7.64 (m, 2H), 7.33–7.30 (m, 1H), 7.09–7.07 (m, 2H), 7.00–6.95 (m, 2H), 6.86–6.79 (m, 4H), 6.40–6.37 (m, 2H), 4.73–4.62 (m, 1H), 4.73–4.62 (m, 1H), 4.11–4.01 (m, 0.2H), 3.85–3.75 (m, 1H), 3.73–3.71 (m, 0.6H), 3.66–3.64 (m, 1H), 3.54–3.49 (m, 2H), 3.35 (t, J = 6.8 Hz, 1H), 3.27–2.99 (m,
1H), 2.94–2.90 (m, 1H), 2.76–2.72 (m, 2H), 2.62 (s, 1H), 2.24–2.17 (m, 0.4H),
2.08–2.04 (m, 1H), 1.93–1.86 (m, 1H), 1.81–1.76 (m, 2H), 1.24 (m, 3H); ESIMS
\( m/z \) 1539 ([\( M + H \)]\(^+\)).

2b: According to the general procedure for 2a–d using 22b (14.9 mg, 0.014 mmol), 2b was obtained (10.7 mg, 98% isolated yield).

2b: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 9.60–9.51 (m, 1H), 9.00 (d, \( J = 8.3 \) Hz, 1H),
8.63 (d, \( J = 8.7 \) Hz, 1H), 8.55–8.52 (m, 1H), 8.40–8.35 (m, 1H), 8.28–8.26 (m, 1H), 8.19–8.18 (m, 1H), 8.12–8.11 (m, 1H), 7.96–7.89 (m, 6H), 7.87–7.83 (m, 2H), 7.75–7.69 (m, 7H), 7.67–7.63 (m, 2H), 7.32–7.30 (m, 1H), 7.09–7.05 (m, 2H), 6.97–6.94 (m, 2H), 6.86–6.77 (m, 4H), 6.40–6.37 (m, 2H), 5.68–5.65 (m, 0.3H), 4.74–4.69 (m, 2H), 4.48–4.44 (m, 2H), 4.06–4.01 (m, 2H), 4.00–3.94 (m, 0.4H), 3.77–3.76 (m, 1H), 3.50–3.48 (m, 1H), 3.21–3.14 (m, 1H), 3.09–3.01 (m, 1H), 2.95–2.88 (m, 3H), 2.66–2.63 (m, 1H), 2.12–2.05 (m, 2H), 1.88–1.84 (m, 2H), 1.68–1.64 (m, 2H), 1.44–1.39 (m, 2H), 1.26–1.22 (m, 3H), 1.20–1.13 (m, 3H), 1.12–1.09 (m, 3H); ESIMS \( m/z \) 1567 ([\( M + H \)]\(^+\)).

2c: According to the general procedure for 2a–d using 22c (15.3 mg, 0.014 mmol) was used, 2c was obtained (10.0 mg, 93% isolated yield).

2c: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 9.56–9.54 (m, 1H), 9.02 (d, \( J = 8.2 \) Hz, 1H),
8.67–8.57 (m, 2H), 8.41–8.30 (m, 2H), 8.19–8.12 (m, 2H), 8.02–7.97 (m, 5H), 7.92–7.85 (m, 6H), 7.85–7.75 (m, 3H), 7.77–7.69 (m, 6H), 7.65–7.63 (m, 1H), 7.54–7.52 (m, 1H), 7.32–7.26 (m, 2H), 7.09–7.07 (m, 2H), 7.00–6.96 (m, 3H), 6.86–6.77 (m, 6H), 6.41–6.36 (m, 2H), 5.22–5.17 (m, 1H), 4.76–4.75 (m, 2H), 4.49–4.46 (m, 2H), 4.25–4.22 (m, 1H), 4.11 (m, 1H), 4.06–4.03 (m, 2H), 3.91–3.88 (m, 0.6H), 3.86–3.79 (m, 3H), 3.75–3.70 (m, 2H), 3.65 (m, 5H),
3.59–3.48 (m, 8H), 3.08–3.05 (m, 2H), 2.88–2.83 (m, 2H), 2.62–2.59 (m, 3H),
2.24–2.20 (m, 2H), 2.17–2.09 (m, 2H), 1.89–1.85 (m, 3H), 1.42–1.32 (m, 6H);
ESIMS m/z 1539 ([M + H]^+).

2d: According to the general procedure for 2a–d using 22d (15.1 mg, 0.014 mmol)
was used, 2d was obtained (10.6 mg, 96% isolated yield).

2d: ^1H NMR (400 MHz, CDCl3) δ 9.56–9.54 (m, 1H), 9.02–9.00 (m, 1H),
8.65–8.63 (m, 1H), 8.56–8.53 (m, 1H), 8.45–8.37 (m, 1H), 8.33–8.28 (m, 1H),
8.24–8.19 (m, 2H), 8.13–8.12 (m, 1H), 7.98–7.91 (m, 7H), 7.87–7.82 (m, 2H),
7.78–7.75 (m, 2H), 7.73–7.69 (m, 6H), 7.68–7.65 (m, 1H), 7.64–7.61 (m, 1H),
7.45–7.39 (m, 1H), 7.31–7.30 (m, 2H), 7.26 (m, 1H), 7.09–7.06 (m, 2H),
6.98–6.96 (m, 2H), 6.93–6.90 (m, 1H), 6.85–6.75 (m, 4H), 6.46–6.36 (m, 2H),
4.80–4.75 (m, 1H), 4.71–4.65 (m, 1H), 4.59–4.53 (m, 1H), 4.92–4.46 (m, 2H),
3.80–3.78 (m, 2H), 3.54–3.49 (m, 2H), 3.24–3.09 (m, 1H), 3.06–2.97 (m, 1H),
2.93–2.90 (m, 2H), 2.65–2.61 (m, 2H), 2.65–2.61 (m, 2H), 2.12–2.09 (m, 2H),
1.93–1.86 (m, 2H), 1.31 (s, 9H), 1.23 (t, J = 7.1 Hz, 3H); ESIMS m/z 1581 ([M +
H]^+).
Time Course of the Reaction between 1 and Hcy/Cys

Stock solutions of Hcy and Cys were freshly prepared in double distilled water (100 mM). Probe 1 was dissolved in CH$_3$CN at room temperature to afford the probe stock solution (1 mM). 10 μL of the probe stock and 5 μL of Hcy or Cys stock were added in a solvent mixture of 5 μL H$_2$O, 80 μL of pH 8.1 PBS buffer and 300 μL of CH$_3$CN. The resulting solution was shaken well before the emission spectra were recorded. The excitation wavelength was at 590 nm and the excitation and emission slit widths were 3.5 nm. The emission intensity of the reaction mixture was monitored for 30 min. (Fig. S1)

Fig. S1 Time course of the reaction between 1 and Hcy and Cys in a molar ratio of 1:50
Titration Curve for the Detection of Hcy by 1

Two stock solutions of Hcy were freshly prepared in double distilled water (1.4 mg in 1 mL H₂O, 10 mM) and (14 mg in 1 mL H₂O, 100 mM). Probe 1 was dissolved in CH₃CN at room temperature to afford the probe stock solution (1 mg in 1 mL CH₃CN, 1 mM). 10 μL of the probe stock, 80 μL of pH 8.1 PBS buffer, appropriate aliquots of Hcy stock were mixed with different volumes of H₂O, (for details, see Table S1). The resulting solution was diluted with 300 μL of CH₃CN and was shaken well. After 15 min, the emission spectra were recorded. The excitation wavelength was 590 nm and the excitation and emission slit widths were 3.5 nm. Different concentrations of Hcy (0–2.5 mM) were prepared in order to obtain an overlaid emission spectrum. A titration curve of the emission intensity against different concentrations of Hcy was obtained, with correlation coefficient R = 0.99 (Fig. S2).

Table S1 Various conditions for setting a titration curve of Hcy and 1

<table>
<thead>
<tr>
<th>[Hcy]</th>
<th>Hcy stock (10 mM)</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM</td>
<td>0 μL</td>
<td>10 μL</td>
</tr>
<tr>
<td>0.025 mM</td>
<td>1 μL</td>
<td>9 μL</td>
</tr>
<tr>
<td>0.075 mM</td>
<td>3 μL</td>
<td>7 μL</td>
</tr>
<tr>
<td>0.125 mM</td>
<td>5 μL</td>
<td>5 μL</td>
</tr>
<tr>
<td>0.175 mM</td>
<td>7 μL</td>
<td>3 μL</td>
</tr>
<tr>
<td>0.25 mM</td>
<td>10 μL</td>
<td>0 μL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[Hcy]</th>
<th>Hcy stock (100 mM)</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75 mM</td>
<td>3 μL</td>
<td>7 μL</td>
</tr>
<tr>
<td>1.25 mM</td>
<td>5 μL</td>
<td>5 μL</td>
</tr>
<tr>
<td>1.75 mM</td>
<td>7 μL</td>
<td>3 μL</td>
</tr>
<tr>
<td>2.25 mM</td>
<td>9 μL</td>
<td>1 μL</td>
</tr>
<tr>
<td>2.5 mM</td>
<td>10 μL</td>
<td>0 μL</td>
</tr>
</tbody>
</table>

The above procedures were followed to obtain a titration curve of Hcy and 2d.
Fig. S2 Emission spectral traces of 1 (25 µM) in CH₃CN/PBS buffer with [Hcy]/[1] ratios from 0 to 100 at room temperature. Each spectrum was acquired 15 mins after Hcy addition. (Detection limit: 0.13 mM)
Fig. S3 Emission spectral traces of \(\textit{2d} \) (25 µM) in CH\(_3\)CN/PBS buffer with [Hcy]/[\textit{2d}] ratios from 0 to 100 at room temperature. Each spectrum was acquired 15 mins after Hcy addition. (Detection limit: 0.12 mM)
Titration Curve for the Detection of Cys by 1

Two stock solutions of Cys were freshly prepared in double distilled water (1.2 mg in 1 mL H₂O, 10 mM) and (12 mg in 1 mL H₂O, 100 mM). Probe 1 was dissolved in CH₃CN at room temperature to afford the probe stock solution (1 mg in 1 mL CH₃CN, 1 mM). 10 μL of the probe stock, 80 μL of pH 8.1 PBS buffer, appropriate aliquots of Cys stock were mixed with different volumes of H₂O, (for details, see Table S2). The resulting solution was diluted with 300 μL of CH₃CN and was shaken well. After 15 min, the emission spectra were recorded. The excitation wavelength was 590 nm and the excitation and emission slit widths were 3.5 nm. Different concentrations of Cys (0–2.5 mM) were prepared in order to obtain an overlaid emission spectrum. A titration curve of the emission intensity against different concentrations of Cys was obtained, with correlation coefficient \( R = 0.99 \) (Fig. S4).

Table S2 Various conditions for setting a titration curve of Cys and 1

<table>
<thead>
<tr>
<th>[Cys]</th>
<th>Cys stock (10 mM)</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM</td>
<td>0 μL</td>
<td>10 μL</td>
</tr>
<tr>
<td>0.025 mM</td>
<td>1 μL</td>
<td>9 μL</td>
</tr>
<tr>
<td>0.075 mM</td>
<td>3 μL</td>
<td>7 μL</td>
</tr>
<tr>
<td>0.125 mM</td>
<td>5 μL</td>
<td>5 μL</td>
</tr>
<tr>
<td>0.175 mM</td>
<td>7 μL</td>
<td>3 μL</td>
</tr>
<tr>
<td>0.25 mM</td>
<td>10 μL</td>
<td>0 μL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[Cys]</th>
<th>Cys stock (100 mM)</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75 mM</td>
<td>3 μL</td>
<td>7 μL</td>
</tr>
<tr>
<td>1.25 mM</td>
<td>5 μL</td>
<td>5 μL</td>
</tr>
<tr>
<td>1.75 mM</td>
<td>7 μL</td>
<td>3 μL</td>
</tr>
<tr>
<td>2.25 mM</td>
<td>9 μL</td>
<td>1 μL</td>
</tr>
<tr>
<td>2.5 mM</td>
<td>10 μL</td>
<td>0 μL</td>
</tr>
</tbody>
</table>

The above procedures were followed to obtain a titration curve of Cys and 2d (Fig. S5).
Fig. S4 Emission spectral traces of 1 (25 µM) in CH$_3$CN/PBS buffer with [Cys]/[1] ratios from 0 to 100 at room temperature. Each spectrum was acquired 15 mins after Cys addition. (Detection limit: 0.15 mM)
Figure S5 Emission spectral traces of 2d (25 μM) in CH₃CN/PBS buffer with [Cys]/[2d] ratios from 0 to 100 at room temperature. Each spectrum was acquired 15 mins after Cys addition. (Detection limit: 0.15 mM)
Mass Spectrometry Analysis of the Mixtures of Probes 1, 2a–d and Hcy/Cys

A stock solution of Hcy was freshly prepared in double distilled water (1.4 mg in 1 mL H₂O, 10 mM). Probe 1 was dissolved in CH₃CN at room temperature to afford the probe stock solution (1 mg in 1 mL CH₃CN, 1 mM). 10 μL of the probe stock and 10 μL of Hcy stock were added to a solvent mixture 80 μL of pH 8.1 PBS buffer and 300 μL of CH₃CN. After 15 min, the reaction mixture was analyzed by ESI-MS (Fig. S6)

Fig S6 ESI-MS of the reaction mixture upon addition of Hcy to 1 in CH₃CN/PBS buffer
Mass spectrometry analyses of the reaction mixture of 2a–d and Hcy followed the same procedures (Fig. S7–S10).

Fig. S7 ESI-MS of a reaction mixture containing Hcy and 2a in CH$_3$CN/PBS buffer

Fig. S8 ESI-MS of a reaction mixture containing Hcy and 2b in CH$_3$CN/PBS buffer
Fig. S9 ESI-MS of a reaction mixture containing Hcy and 2c in CH$_3$CN/PBS buffer

Fig. S10 ESI-MS of a reaction mixture containing Hcy and 2d in CH$_3$CN/PBS buffer
A stock solution of Cys was freshly prepared in double distilled water (1.2 mg in 1 mL H₂O, 10 mM). Probe 1 was respectively dissolved in CH₃CN at room temperature to afford the probe stock solution (1 mg in 1 mL CH₃CN, 1 mM). 10 μL of the probe stock and 10 μL of Cys stock were added to a solvent mixture 80 μL of pH 8.1 PBS buffer and 300 μL of CH₃CN. After 15 min, the reaction mixture was analyzed by ESI-MS (Fig. S11).

Fig. S11 ESI-MS of the reaction mixture upon addition of Cys to 1 in CH₃CN/PBS buffer
Mass spectrometry analyses of the reaction mixture of 2a–d and Cys followed the same procedures (Fig. S12–S15).

Fig. S12 ESI-MS of a reaction mixture containing Cys and 2a in CH$_3$CN/PBS buffer

Fig. S13 ESI-MS of a reaction mixture containing Cys and 2b in CH$_3$CN/PBS buffer
Fig. S14 ESI-MS of a reaction mixture containing Cys and 2c in CH$_3$CN/PBS buffer

Fig. S15 ESI-MS of a reaction mixture containing Cys and 2d in CH$_3$CN/PBS buffer
Mass Spectrometry Analysis of the Mixtures of Probe 2d and BSA

A stock solution of Bovine Serum Albumin (BSA) was freshly prepared in double distilled water (66 mg in 1 mL H₂O, 1 mM). Probe 2d was dissolved in CH₃CN at room temperature to afford the probe stock solution (1 mg in 1 mL CH₃CN, 1 mM). 10 μL of the probe stock and 10 μL of BSA stock were added to a solvent mixture 80 μL of pH 8.1 PBS buffer and 300 μL of CH₃CN. As a control, probe 2d was dissolved in CH₃CN at room temperature to afford the probe stock solution (1 mg in 1 mL CH₃CN, 1 mM). 10 μL of the probe stock was added to a solvent mixture of 10 μL of H₂O, 80 μL of pH 8.1 PBS buffer and 300 μL of CH₃CN. After 15 min, the reaction mixtures were analyzed by LC-MS (Fig. S16). BSA is a protein (MW = 66 kDa) which contains a single and surface-exposed cysteine residue. This experiment demonstrated that the probe is not able to react with steric bulk cysteine-containing biomacromolecules.

![Fig. S16 LC-MS of a reaction mixture containing (a) BSA only (b) BSA and probe 2d](image-url)
Emission Intensity Enhancement in the Detection of Different Analytes by Probe 1

The amino acids (Cys, Hcy, Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val) and GSH stock solutions were freshly prepared in double distilled water (100 mM). Probe 1 was dissolved in CH$_3$CN at room temperature to afford the probe stock solution (1 mM). 10 μL of the probe stock and 5 μL of analyte stock were added to a solvent mixture of 5 μL H$_2$O, 80 μL of pH 8.1 PBS buffer and 300 μL of CH$_3$CN. The resulting solution was shaken well. After 15 min, the emission spectra were recorded. For all the measurement, the excitation wavelength was 590 nm and the excitation and emission slit widths were 3.5 nm. The emission intensity obtained after reacting 1 with each analyte in the aforementioned conditions was measured and compared (Fig. S17).
Fig. S17 Enhancement of emission intensity in the detection of different analytes by probe I

Quantum Yield Determination

Williams et al.\textsuperscript{1} stated the comparative method for recording $\Phi_F$. The relative quantum yield of sample was related to that of a solution of a standard by the equation

$$\Phi_{F(\text{sample})} = \left( \frac{A_{\text{standard}}}{A_{\text{sample}}} \right) \left( \frac{F_{\text{sample}}}{F_{\text{standard}}} \right) \left( \frac{n_{\text{sample}}}{n_{\text{standard}}} \right)^2 \Phi_{F(\text{standard})} \quad (1)$$

$\Phi_F$ is the fluorescence quantum yield, $A$ is the absorbance, $F$ is the area under the corrected emission curve and $n$ is the refractive index. The concentration of standard was adjusted so that the absorbance of the sample at the wavelength of excitation was equal to that of the standard, which made the absorbance ratio to be
equal to 1. Acetonitrile was used for both the standard and the sample solution; the refractive index ratio was therefore also equal to 1.

So equation (1) is reduced to

$$\Phi_{F(\text{sample})} = \left( \frac{F_{\text{sample}}}{F_{\text{standard}}} \right) \Phi_{F(\text{standard})}$$

(2)

Here, $[\text{Ru(bipy)}_3\text{Cl}_2]$ was used as the standard; the quantum yield of $[\text{Ru(bipy)}_3\text{Cl}_2]$ in acetonitrile is 0.062. The quantum yield of 1 was calculated to be 0.0028.

Literature reference for quantum yield determination:


**Emission Intensity Enhancement in the Detection of Hcy/Cys by Probes 1, 2a–d**

A stock solution of Hcy was freshly prepared in double distilled water (14 mg in 10 mL H$_2$O, 100 mM). Cys stock was freshly prepared in the same way (12 mg in 10 mL H$_2$O, 100mM). Probe 1, 2a–d was respectively dissolved in CH$_3$CN at room temperature to afford the probe stock solution (1 mg in 1 mL CH$_3$CN, 1 mM). 10 μL of the probe stock and 7 μL of Hcy or Cys stock were added to a solvent mixture of 3 μL of H$_2$O, 80 μL of pH 8.1 PBS buffer and 300 μL of CH$_3$CN. After 15 min, the emission spectra were recorded. The excitation
wavelength was 590 nm and the excitation and emission slit widths were 3.5 nm. The emission intensity obtained after reacting 1, 2a–d with either Hcy or Cys in the aforementioned conditions was measured and compared (Fig. S18).

Fig. S18 Emission intensity enhancement in the detection of Hcy/Cys by probes 1, 2a–d
Detection of Hcy and Cys in Human Blood Plasma by Probe 2d

Probe 2d was dissolved in CH$_3$CN at room temperature to afford the probe stock solution (1 mM). Commercial lyophilized human blood plasma is reconstituted with distilled water (5.0 mL). The thiols assay of human blood plasma requires prior reduction of disulfides to free thiols. This can be accomplished by using tris[2-carboxyethyl]phosphine hydroxide (TCEP) (1.5 mM). This is followed by deproteinization upon addition of MeOH which contains triphenylphosphine (PPh$_3$) (1.5 mM) at room temperature for 30 min. After centrifugation (5.0 min, 3000 g) the supernatant is used for the analysis.

Literature reference for preparation of human blood plasma sample:

The amount of thiols in the reduced human blood plasma was estimated by using the standard addition method with Hcy (10 mM stock) as the standard. Different volumes of the freshly prepared Hcy stock (0, 1, 3, 5, 7 or 10 µL) was added directly to 10 µL of probe 2d (25 µM) and 50 µL of reduced human blood plasma in a solvent mixture of 30 µL of pH 8.1 PBS buffer and appropriate volume of H$_2$O (for details, see Table S3). The resulting solution was shaken well and diluted with 300 µL of CH$_3$CN. After 15 min, the emission at 590 nm was recorded. A titration curve of the emission intensity against different volumes of Hcy was obtained, with correlation coefficient R = 0.99 (Fig. S19). The
concentration of thiols in the reduced human blood plasma sample detected by 2d was found to be 0.31 mM, using the equation:

$$C_X = \frac{X C_S}{V_X^A}$$

where $C_X$ is the concentration of the sample; $C_S$ is the concentration of the standard, i.e. 10 mM; $V_X$ is the volume of the sample aliquot, i.e. 50 µL; $X$ is the absolute value of x-intercept, i.e. 1.5 µL.

Table S3 Various conditions for setting a titration curve of Hcy and 2d

<table>
<thead>
<tr>
<th>Hcy stock (10 mM)</th>
<th>H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µL</td>
<td>10 µL</td>
</tr>
<tr>
<td>1 µL</td>
<td>9 µL</td>
</tr>
<tr>
<td>3 µL</td>
<td>7 µL</td>
</tr>
<tr>
<td>5 µL</td>
<td>5 µL</td>
</tr>
<tr>
<td>7 µL</td>
<td>3 µL</td>
</tr>
<tr>
<td>10 µL</td>
<td>0 µL</td>
</tr>
</tbody>
</table>
Fig. S19 Titration curve obtained when 2d (25 μM) reacts with 50 μL of the reduced human blood plasma including different volumes (0, 1, 3, 5, 7, 10 μL) of standard Hcy stock (10 mM)

**Lifetime Determination**

FRET is the energy transfer from a donor luminophore to an acceptor which may not have to re-emit in the case of dark quenching. The FRET efficiency relates to the lifetimes of the donor molecule in the presence and absence of an acceptor:

\[
B = 1 - \frac{\tau_D^f}{\tau_D},
\]

where \(\tau_D^f\) and \(\tau_D\) are the donor fluorescence lifetimes in the presence and absence of an acceptor. In order to validate our Ir(III) probe is built on a FRET-based system, the metal part was synthesized and the corresponding
lifetime was measured as $\tau_D$ whereas the lifetime of probe 1 was measured as $\tau_D^I$. The lifetime of the metal part $\tau_D$ was measured as 0.6 µs; $\tau_D^I$ was too short to be measured by our instrument. The reduction in lifetime of the quenched probe 1 can be considered as the result of energy transfer from the Ir(III) complex to the diarylazo quencher.

Fig. S20 Luminescence emission spectrum (left) and lifetime (right) spectrum of the metal part (inset)

Fig. S21 Luminescence emission spectrum (left) and lifetime (right) spectrum of probe 1 (inset)
Supplementary Material (ESI) for Chemical Communications
This journal is (c) The Royal Society of Chemistry 2011