Supporting information for

A Blue Fluorescent Antibody-Cofactor Sensor for Mercury

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Synthesis

Synthesis of **3**

To a solution of 4-bromostilbene (85.0 mg, 0.328 mmol) in THF (2.5 mL) was added *n*-BuLi (2N in THF, 0.328 mL, 0.656 mmol) at -78°C , and the mixture was allowed to warm to -20°C . After stirring at -20°C for 1 hr, the reaction mixture was cooled to -78°C and a solution of phosphodithioate[§] (228 mg, 0.984 mmol) in THF (2 mL) was added. The mixture was stirred at -78°C to r.t. for 18 hr. The mixture was diluted with EtOAc (20 mL), washed with sat. NH_4Cl (2 x 5 mL) and brine (5 mL), dried over Na_2SO_4 and concentrated. Flash chromatography of the residue (MeOH in EtOAc, 10 ~ 20 %) gave **3** (26 mg, 22 %). ^1H NMR (400 MHz, CD_3OD) δ 8.06-7.78 (2H, m), 7.55-7.49 (2H, m), 7.37-7.23 (2H, m), 7.21-7.07 (10H, m); ^{31}P NMR (100 MHz, CD_3OD) δ 62.82; ESI-TOF MS m/z = 351.0436 $[\text{M}-\text{H}]^-$, calcd. for $\text{C}_{20}\text{H}_{16}\text{PS}_2$ = 351.0436.

[§] Martin, S. F.; Wagman, A. S. *J. Org. Chem.* **1996**, *61*, 8016.

Synthesis of **4**

To a solution of 4-stilbenylbromomethane (36.7 mg, 0.133 mmol) in EtOH (20 mL) was added thioacetic acid potassium salt (39.9 mg, 0.35 mmol) and stirred at r.t. for 18 hr. The mixture was concentrated and the residue was purified by prep TLC (toluene-hexane, 1:1) to give thioester (14.2 mg, 38 %). ^1H NMR (400 MHz, CDCl_3) δ 7.51-7.27 (9H, m), 7.08 (2H, br. S), 4.13 (2H, s), 2.36 (3H, s); ESI MS m/z = 285.1 $[\text{M}+\text{H}]^+$, calcd. for $\text{C}_{17}\text{H}_{16}\text{S}_2$ = 285.1.

Synthesis of thiourea **5**

To a solution of 4-stilbenylmethanol (2.0 g, 9.52 mmol) and DIPEA (2 mL) in CH_2Cl_2 (100 mL) was added MsCl (1.0 mL, 13.0 mmol) at 0°C and the mixture was stirred at r.t. for 3 hr. The mixture was diluted with EtOAc (300 mL), washed with sat. NH_4Cl (2 x 20 mL) and brine (20 mL), dried over Na_2SO_4 and concentrated. The resulted mesylate was used for the following reaction without further purification. A mixture of mesylate (97.5 mg, 0.338 mmol) and thiourea (38 mg, 0.50 mmol) in THF-DMF (1:1, 4 mL) was stirred at r.t. for 18 hr. The mixture was diluted with EtOAc (20 mL), washed with sat. NH_4Cl (2 x 5 mL) and brine (5 mL), dried over Na_2SO_4 and concentrated. Flash chromatography of the residue (MeOH in EtOAc, 10 ~ 20 %) gave thiourea (36 mg, 40 %) ^1H NMR (400 MHz, CD_3OD) δ 7.57 (4H, dd, $J = 8.4, 10.4$ Hz), 7.41 (2H, 8.0 Hz), 7.35 (2H, t, $J = 7.6$ Hz), 7.27-7.23 (1H, m), 7.21 (1H, d, $J = 16.4$ Hz), 7.16 (1H, d, $J = 16.4$ Hz).; ESI-TOF MS $m/z = 296.1110$ $[\text{M}+\text{H}]^+$, calcd. for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{S} = 269.1107$.

Synthesis of **11**

To a solution of 4-stilbenylboric acid (21.9 mg, 0.098 mmol) in CH_2Cl_2 (5 mL) and MeOH (1 mL) was added HgCl_2 (61.1 mg, 0.225 mmol) and the mixture was stirred at r.t. for 18 hr. The mixture was concentrated and the residue was purified by prep TLC (5 % MeOH in EtOAc) to give **11** (10.2 mg, 25 %). ^1H NMR (400 MHz, CDCl_3) δ 7.51-7.27 (9H, m), 7.08 (2H, br. S), 4.13 (2H, s), 2.36 (3H, s); LC-ESI MS $m/z = 417.0$ $[\text{M}+\text{H}]^+$, calcd. for $\text{C}_{14}\text{H}_{11}\text{ClHg} = 417.1$. CG MS $m/z = 416$ $[\text{M}]^+$, calcd. for $\text{C}_{14}\text{H}_{10}\text{ClHg} = 416$.

Figure S1. Changes in fluorescence intensity of biphenyl boronic acid **12** as a function of Hg^{2+} ions. $[\mathbf{12}] = 20\ \mu\text{M}$. $[\text{HgCl}_2] = 0, 4, 8, 12, 20, 40, 80\ \mu\text{M}$ in 300 μL PBS with 5% DMF cosolvent. $\lambda_{\text{exc}} = 260\ \text{nm}$. The fluorescence intensities were recorded on a Spectra Max Gemini apparatus (Molecular Devices) using 100 μL solutions in a 96-well plate (Costar 3916) at 25 $^\circ\text{C}$.

Figure S2. LC-MS analysis of the formation of (E)-(4-styrylphenyl)mercury(II) chloride **11**. Conditions: Flowrate: 1.0 mL / min; Column: VYDAC RPC18 #201TP54 (4.6 x 250 mm); Eluent: CH₃CN in H₂O, 0 min: 50 %, 5 min: 50 %, 15 min: 98 % 20 min: 98 %; Detection: 280 nm; Temperature: 25 °C. a) Compound **6**. b) **6** (200 μM), HgCl₂ (200 μM, 1.0 equiv.), PBS buffer (5 % DMF as co-solvent), r.t. 30 min. c) **6** (200 μM), HgCl₂ (2.0 mM, 10 equiv.), PBS buffer (5 % DMF as co-solvent), r.t. 30 min. d) Compound **11**.

Figure S3. Fluorescence spectra of 19G2-EP2 (**1**) in the presence of **11**. [19G2] = 0.1 μM , [EP2 (**1**)] = 0.2 μM , [**11**] = 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000 μM in PBS (10 mM sodium phosphate, 10 mM NaCl, pH 7.4) with 5% DMF cosolvent. λ_{exc} = 327 nm.

Figure S4. Fluorescence intensity of 19G2-EP2 (**1**) in the presence of **11**. [19G2] = 0.1 μM , [EP2 (**1**)] = 0.2 μM , [**11**] = 7.8, 15.6, 31.3, 62.5, 125, 250, 500 μM in PBS (10 mM sodium phosphate, 10 mM NaCl, pH 7.4) with 5% DMF cosolvent. λ_{exc} = 327 nm, λ_{em} = 410 nm.