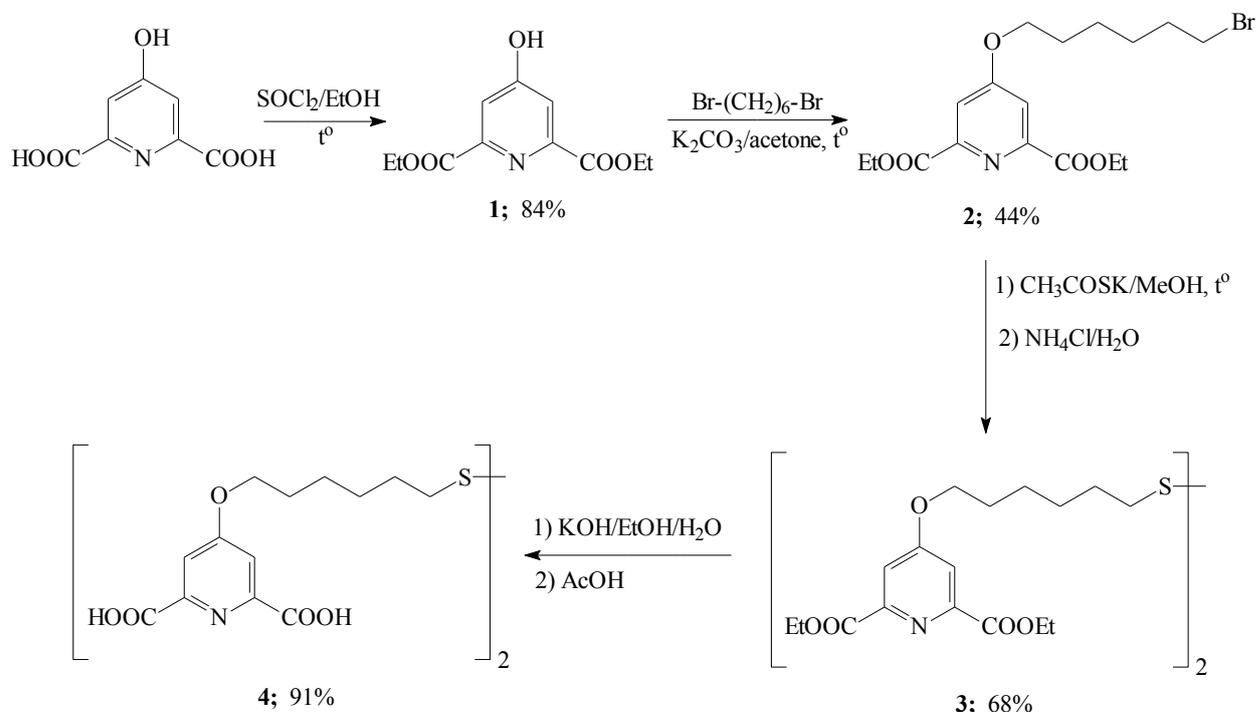


Direct reagentless detection of the affinity binding of recombinant His-tagged firefly luciferase with a nickel-modified gold electrode

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Experimental

Reaction control was carried out with chromatography with Silufol sheets. NMR spectra were obtained with a Bruker Avance spectrometer (400 MHz). Deuteriochloroform and DMSO- d_6 were used as solvents. Chemical shifts are expressed in δ -scale at parts per million referenced against hexamethyldisilozane. C^{13} NMR spectra were recorded on a Bruker Avance spectrometer (100 MHz). IR spectra were obtained with UR-20 Carl Zeiss in Vaseline oil or with Termo Nicolet IR200 FTIR at KBr. Melting points were estimated with open capillary. Elemental analysis was performed with Vario MICRO cube (Elementar).



Scheme of disulfide synthesis.

Steps of disulfide synthesis

Synthesis of diethyl 4-hydroxypyridine-2,6-dicarboxylate (1)

Thionyl chloride (6.2 ml, 85 mmol) was dropwise added to 25 ml of dry ethanol at 0 °C. 4-Hydroxypyridine-2,6-dicarboxylic acid (2.5 g, 14 mmol) was added in one portion to this solution. The solution was stirred at room temperature for 12 h and then refluxed for 2 h. The solvent was removed under reduced pressure and 20 ml of water was added to crude product at 0 °C. The mixture was treated by 5 ml of 10% solution of sodium carbonate and 5 ml of 50% aqueous ethanol. The white precipitate was filtered and dried under vacuum to afford 2.74 g (84%) of product **1**.

Mp 114–115 °C (lit., 115–116 °C).

¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 7.58 (s, 1H, HC-Py), 4.35 (q, J= 6.9 Hz, 2H, CH₂-O), 1.33 (t, J= 7.1 Hz, 3H, CH₃).

Found (%): C, 55.68; H, 5.67; N, 5.59. Calc. for C₁₁H₁₃NO₅ (%): C, 55.23; H, 5.44; N, 5.86.

Synthesis of diethyl 4-[(6-bromohexyl)oxy]pyridine-2,6-dicarboxylate (2)

Diethyl 4-hydroxypyridine-2,6-dicarboxylate (**1**) (2 g, 8.4 mmol) and potassium carbonate (2.32 g, 16.8 mmol) were dissolved in dry acetone and 6.15 g (25.2 mmol) of 1,6-dibromohexane was added. The reaction was conducted under argon atmosphere. The solution was refluxed for 48 h. The solvent was removed under reduced pressure, the residue was dissolved in dichloromethane and filtered. The solvent was removed under reduced pressure. The final product was purified by column chromatography using light petroleum–ethyl acetate (6:1).

Diethyl 4-[(6-bromohexyl)oxy]pyridine-2,6-dicarboxylate (**2**) (1.48 g, 44 %) was obtained as yellowish oil.

¹H NMR (400 MHz, CDCl₃, δ, ppm): 7.77 (s, 2H, HC₃, HC₅-Ar), 4.47 (q, J=7.2 Hz, 2H, CH₂-OC(O)), 4.14 (t, J=6.4 Hz, 2H, CH₂-O), 3.43 (t, J=6.6 Hz, 2H, CH₂-Br), 1.88 (m, 4H, (CH₂)₂), 1.53 (m, 4H, (CH₂)₂), 1.45 (t, J=7.1 Hz, 3H, CH₃).

¹³C NMR (100 MHz, CDCl₃, δ, ppm): 166.9; 164.8; 150.1; 114.3; 68.7; 62.4; 33.7; 32.5; 28.6; 27.8; 25.1; 14.2.

Found (%): C, 51.03; H, 6.18; N, 3.19. Calc. for C₁₇H₂₄NO₅Br (%): C, 50.75; H, 5.97; N, 3.48.

Synthesis of 1,16-di[4-(2,6-diethoxycarbonyl)pyridine]-1,16-dioxa-8,9-dithiahexadecane (3)

Diethyl 4-[(6-bromohexyl)oxy]pyridine-2,6-dicarboxylate (**2**) (0.9 g, 2.2 mmol) was dissolved in 15 ml of dry methanol and 0.51 g (4.4 mmol) of potassium thioacetate was added. The mixture was refluxed for 12 h. Then 30 ml of saturated ammonium chloride aqueous solution was added, the organic layer was extracted by diethyl ether (3x20 ml). Combined organic layers were dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, which resulted in obtaining 0.53 g (68%) of 1,16-di[4-(2,6-diethoxycarbonyl)pyridine]-1,16-dioxa-8,9-dithiahexadecane (**3**).

^1H NMR (400 MHz, CDCl_3 , δ , ppm): 7.71 (s, 2H, HC_3 , HC_5 -Ar), 4.20 (q, $J=7.1$ Hz, 2H, $\text{CH}_2\text{-OC(O)}$), 4.10 (t, $J=6.5$ Hz, 2H, $\text{CH}_2\text{-O}$), 2.70 (t, $J=6.8$ Hz, 2H, $\text{CH}_2\text{-S}$), 1.75 (m, 2H, CH_2), 1.64 (m, 2H, CH_2), 1.55 (m, 4H, $(\text{CH}_2)_2$), 1.45 (t, $J=6.8$ Hz, 3H, CH_3).

Found (%): C, 57.48; H, 6.53; N, 3.58; S, 9.47. Calc. for $\text{C}_{34}\text{H}_{48}\text{N}_2\text{O}_{10}\text{S}_2$ (%): C, 57.63; H, 6.78; N, 3.95; S, 9.04.

Synthesis of 1,16-di[4-(2,6-dihydroxycarbonyl)pyridine]-1,16-dioxa-8,9-dithiahexadecane (disulfide) (4)

1,16-Di[4-(2,6-diethoxycarbonyl)pyridine]-1,16-dioxa-8,9-dithiahexadecane (**3**) (0.23 g, 0.32 mmol) was dissolved in 2M KOH in 70% ethanol. The mixture was stirred under room temperature for 3 h. Ethanol was removed under reduced pressure, 10 ml of water was added and the mixture was treated by glacial acetic acid to pH 5 at 0 °C. The white precipitate was filtered, washed with water and ethanol and dried under vacuum to afford 0.19 g (91%) of 1,16-di[4-(2,6-dihydroxycarbonyl)pyridine]-1,16-dioxa-8,9-dithiahexadecane (**4**).

Mp > 340 °C. ^1H NMR (400 MHz, DMCO-d_6 , δ , ppm): 7.62 (s, 2H, HC_3 , HC_5 -Ar), 4.23 (t, $J=6.5$ Hz, 2H, CH_2O), 2.67 (t, $J=6.9$ Hz, 2H, CH_2S), 1.75 (m, 2H, CH_2), 1.62 (m, 2H, CH_2), 1.28 (m, 4H, $(\text{CH}_2)_2$). IR: 3400 cm^{-1} (OH), 1720 cm^{-1} (C=O).

Found (%): C, 51.98; H, 4.97; N, 4.36; S, 10.33. Calc. for $\text{C}_{26}\text{H}_{32}\text{N}_2\text{O}_{10}\text{S}_2$ (%): C, 52.35; H, 5.37; N, 4.70; S, 10.74.