

Electronic Supporting Information for:

Fluorescence Ratiometric Assays of Hydrogen Peroxide and Glucose in Serum Using Conjugated Polyelectrolytes

FangHe, Fude Feng, Shu Wang,* Yuliang Li and Daoben Zhu

Monomers Synthesis

1-(6'-azidohexyloxy)-2,5-dibromo-4-methoxybenzene (2).

A solution of 1 (890 mg, 2 mmole) and NaN_3 (260 mg, 4 mmole) in 25 mL of anhydrous DMF was heated to 100°C for 10 min under N_2 . After cooling down to room temperature the solution was poured into 100 mL ethyl acetate and the organic phase was washed three times with 300 mL of water. Then the organic layer was dried over anhydrous MgSO_4 . The solvent was removed and the residue was purified by the silica gel column with 40:1 (v/v) petroleum ether/ethyl acetate as eluent to afford a colorless oil (773 mg, 95%). ^1H NMR (CDCl_3 , 400 MHz): δ (ppm) 7.09 (s, 2H), 3.96 (t, 2H), 3.84 (s, 3H), 3.29 (t, 2H), 1.82 (m, 2H), 1.63 (m, 2H), 1.52 (m, 2H), 1.46 (m, 2H). ^{13}C NMR (CDCl_3 , 100 MHz): δ (ppm) 150.8, 150.3, 118.9, 117.3, 111.5, 110.7, 70.3, 57.3, 51.6, 29.3, 29.1, 26.6, 25.9. MS (EI): 407 (M). Anal. Calcd. for $\text{C}_{13}\text{H}_{17}\text{Br}_2\text{N}_3\text{O}_2$: C, 38.35; H, 4.21; N, 10.32. Found: C, 39.04; H, 4.54; N, 12.10%.

1-(6'-tert-butoxycarbonylaminohexyloxy)-2,5-dibromo-4-methoxybenzene (3).

A solution of 2 (610 mg, 1.5 mmole) and PPh_3 (786 mg, 3 mmole) in 50 mL of THF/ H_2O (v/v: 44/6) was stirred overnight at room temperature, then $(\text{Boc})_2\text{O}$ (654 mg, 3 mmole) was added and then stirred for another 4 h at room temperature. The solvent was removed under vacuum, and the residue was purified by the silica gel column with with 10:1 (v/v) petroleum ether/ethyl acetate as eluent to give a white solid (600 mg, 86%). ^1H NMR (CDCl_3 , 400 MHz): δ (ppm)

7.09 (s, 2H), 4.51 (s, 1H), 3.95 (t, 2H), 3.84 (s, 3H), 3.13 (t, 2H), 1.81 (m, 2H), 1.52 (m, 4H), 1.44 (s, 9H), 1.40 (m, 2H). ^{13}C NMR (CDCl_3 , 100 MHz): δ (ppm) 155.9, 150.4, 150.0, 118.5, 116.9, 111.2, 110.3, 79.0, 70.1, 56.9, 55.8, 40.4, 30.0, 29.0, 28.4, 26.4, 25.7. MS (EI): 481 (M). Anal. Calcd. for $\text{C}_{18}\text{H}_{27}\text{Br}_2\text{NO}_4$: C, 44.93; H, 5.66; N, 2.91. Found: C, 45.02; H, 5.75; N, 2.88%.

3',6'-Diiodo-6-carboxy-fluoran pyridinium salt (6).

A solution of 3-Iodophenol (4) (2.21 g, 10 mmole) and benzene-1,2,4-tricarboxylic acid (5) (1.05 g, 5 mmole) in 10 mL of methanesulfonic acid was stirred at 135 °C for 2 days. After cooling down to room temperature, the solution was poured into 100 mL of ice water and stirred vigorously for about 30 min. A black solid was filtered, dried and crystallized from acetic/pyridine to afford a white solid (1.2 g, 36%). ^1H NMR (DMSO-d_6 , 400 MHz): δ (ppm) 8.57 (d, 2H), 8.24 (d, 1H), 8.15 (d, 1H), 7.76-7.83 (m, 4H), 7.48 (d, 2H), 7.38 (t, 2H), 6.66 (d, 2H). ^{13}C NMR (DMSO-d_6 , 100 MHz): δ (ppm) 167.6, 166.0, 152.3, 150.4, 149.6, 137.8, 136.2, 133.3, 131.4, 129.8, 128.6, 125.7, 125.6, 124.7, 123.9, 117.8, 97.2, 81.1. MS (ESI): 595.8. Anal. Calcd. for $\text{C}_{26}\text{H}_{15}\text{I}_2\text{NO}_5$: C, 46.18; H, 2.38; N, 2.07. Found: C, 46.05; H, 2.33; N, 2.46%.

3',6'-Bis(pinacolatoboron)fluoran-6-carboxylic acid (7). Pyridinium salt 6 (338 mg, 0.5 mmole), bis(pinacolato)diboron (508 mg, 2 mmole), potassium acetate (200 mg, 2 mmole), and $\text{Pd}(\text{dppf})\text{Cl}_2$ (90 mg, 0.4 mmole) were dried under vacuum overnight in a 25-mL flask before use. Then 20 mL of fresh DMSO were added and the resulting solution was heated at 80 °C for 3 hours under a nitrogen atmosphere. After cooling down to room temperature, the solution was poured into 100 mL of stirring ice/water slurry to precipitate a black solid which was then dissolved in dichloromethane and dried over anhydrous MgSO_4 . The resulting solution was concentrated and eluted through a short silica gel column with methanol/dichloromethane (v/v: 1/99) to give a yellow solid (80 mg, 27%). ^1H

NMR (CDCl₃, 400 MHz): δ (ppm) 8.34 (d, 1H), 8.12 (d, 1H), 7.78 (m, 3H), 7.45 (t, 2H), 6.82 (t, 2H), 1.35 (s, 24H). MS (ESI): 597.3.

Polymers Synthesis

Boc-Br-PF.

A mixture of monomer 3 (90 mg, 0.2 mmole), 8 (130 mg, 0.2 mmole) and 9 (101 mg, 0.4 mmole) in 8 mL of THF and 2 mL of 2.0 M potassium carbonate was degassed and then Pd(dppf)Cl₂ was added. The resulting mixture was stirred at 80 °C for 2 days under nitrogen. After cooling down to room temperature, it was diluted with 100 mL CHCl₃. The organic layer was dried over anhydrous MgSO₄ and the solvent was removed. The crude product was redissolved in about 1 mL of CHCl₃ and then added into 100 mL of acetone. The precipitate was collected by centrifugation. The procedure was repeated twice to obtain a gray solid (80 mg, 43%). ¹H NMR (CDCl₃, 400 MHz): δ (ppm) 7.83-7.65 (br, 19H), 7.10 (s, 2H), 4.51 (br, 1H), 4.00 (br, 2H), 3.88 (m, 3H), 3.30 (t, 6H), 3.09 (br, 2H), 2.14 (br, 6H), 1.77 (br, 2H), 1.73-1.68 (br, 6H), 1.43 (br, 13H), 1.35 (m, 2H), 1.25 (br, 6H), 1.14 (br, 6H), 0.78 (br, 6H). GPC: $M_w = 60210$, $M_n = 24660$, PDI = 2.44.

Boc-NMe₃-PF: Boc-Br-PF (20 mg) was dissolved in 5 mL of dichloromethane and then 1 mL of 30% trimethylamine aqueous solution was added. The resulting solution was stirred for 24 hours at room temperature. The solvent was removed under reduced pressure to give a solid. ¹H NMR (DMSO-d₆, 400 MHz): δ (ppm) 7.91-7.74 (br, 19H), 7.14 (br, 2H), 6.77 (br, 1H), 4.05 (br, 2H), 3.84 (m, 3H), 3.15 (br, 8H), 2.94 (br, 27H), 1.70 (br, 6H), 1.48 (br, 8H), 1.35 (m, 15H), 1.30-1.05 (br, 12H), 0.78 (br, 6H).

NMe₃-NH₂-PF.

To a solution of Boc-NMe₃-PF in 5 mL of methanol was added 1 mL of trifluoroacetic acid (TFA) and then stirred at room temperature overnight. The solvent was removed under reduced pressure. The solid was redissolved in 5 mL of

methanol and 1 mL of triethylamine was added. The resulting solution was stirred overnight and the solvent was removed to yield a solid. ^1H NMR (DMSO- d_6 , 400 MHz): δ (ppm) 7.91-7.76 (br, 19H), 7.14 (br, 2H), 6.77 (br, 1H), 4.05 (br, 2H), 3.85 (m, 3H), 3.15-3.10 (br, 8H), 2.94 (br, 27H), 1.74 (br, 6H), 1.58 (br, 2H), 1.49 (br, 6H), 1.35 (br, 4H), 1.18-1.07 (br, 12H), 0.78 (br, 6H).

PF-FB.

To a solution of NMe₃-NH₂-PF (20 mg), **7** (20 mg, 0.034 mmole) and N-hydroxysuccinimide (6 mg, 0.05 mmole) in 5 mL of DMSO was dropwise added a solution of EDCI (10 mg, 0.05 mmole) in 1 mL of DMSO. The resulting solution was stirred at room temperature for 2 days in dark. The solvent was removed under reduced pressure. A gray solid was obtained after dialysis for 2 days in water under nitrogen. ^1H NMR (DMSO- d_6 , 400 MHz): δ (ppm) 8.37-7.39 (m), 7.14 (m), 6.92 (m), 6.79 (m), 6.55 (m), 4.04 (m), 3.85 (m), 3.14 (m), 2.95 (m), 1.69 (m), 1.47 (m), 1.36 (m), 1.23-1.07 (m), 0.80 (m).