Fluorescent Ratiometric Sensing of Pyrophosphate via Induced

Aggregation of a Conjugated Polyelectrolyte

Xiaoyong Zhao* and Kirk S. Schanze*

Department of Chemistry, University of Florida

Gainesville, FL, 32603

Materials

 $Pd(PPh_3)_4$ were purchased from Strem Chemical Company and used as received. Triethylamine and THF used in the polymerization were purified by distillation over CaH₂. 4-(2-Carboxyethyl)-4-nitroheptanedioic acid was purchased from Aldrich Chemical Company. All the other chemicals were supplied by either Acros Co. or Aldrich Chemical Company and used without further purification.

Instrumentation

NMR spectra were recorded on a Varian VXR-300 or Gemin-300 FT-NMR, operating at 300 MHz for ¹H-NMR and at 75.4 MHz for ¹³C-NMR. High temperature NMR spectra were recorded on a Varian Mercury 300 FT-NMR. Chemical shifts were reported in ppm using CHCl₃ or C₂HD₅SO as internal reference. FT-IR spectra were taken on a Perkin-Elmer 1600 spectrometer. Size exclusion chromatography (SEC) analyses were carried out on a system comprised of a Rainin Dynamax SD-200 pump and a Beckman Instruments Spectroflow 757 absorbance detector using polystyrene standards. UV-Visible absorption spectra were recorded with a Lambda 25 spectrophotometer from PerkinElmer. Steady-state fluorescence spectra were obtained with a Fluorolog-3 spectrofluorometer from Jobin Yvon.

Sonogashira Polycondensation. Monomer **20** (340.8 mg, 0.25 mmol) and 0.25 mmol of the 1,4diethynylbenzene were dissolved in 25 ml of THF/Et₃N (v/v, 4/1). The resulting solution was deoxygenated with argon for 15 minutes. Then 17.3 mg of Pd(PPh₃)₄ (15 μ M) and 5.7 mg of CuI (30 μ M) were added to the stirred solution under the protection of argon. The reaction mixture was then heated up to 55 °C - 60 °C and stirred for 20 hr. The viscous solution was then poured into 200 ml of hexane. The precipitate was collected by vacuum filtration and washed with hexane (200 ml). After dried under vacuum, the polymer was stored as a solid. Typical reaction yields for the polymerization are 80% - 90%. For the hydrolysis, the organic polymer was dissolved in 20 ml of dioxane. The polymer solution was then cooled to 0 °C - 5 °C using an ice/water bath. Concentrated HCl (7 ml, 12 N) was added to the stirred solution dropwisely. Upon the completion of the addition, the reaction mixture was allowed to warm to room temperature and stirred for another 12 hr. The polymer was then precipitated by pouring the solution into a large amount of acetone (200 ml). The precipitate was collected, washed with acetone (100 ml) and finally dried under vacuum (yield: 90% - 100%). No further purification was done on these polymers and they were stored as solid powders in a desiccator and can be redissolved in water easily.



Scheme S-1. Synthesis of monomer carrying polyamine side groups.

4-(3-Chloro-3-oxopropyl)-4-nitroheptanedioyl dichloride (1). A 100 ml round bottom flask was charged with 4.44 g of 4-(2-carboxyethyl)-4-nitroheptanedioic acid (16 mmol) and 30 ml thionyl chloride (SOCl₂). Two drops of DMF was added to the suspension and the mixture was then slowly heated up to reflux. After 1 hr, the solution became clear and there was no more gas evolution. The excess SOCl₂ was removed by vacuum distillation. The yellow residue solidified after flushing with nitrogen and used without further purification. ¹H NMR (CDCl₃, δ_{ppm}): 2.96 (t, 6H), 2.30 (t, 6H). ¹³C NMR (CDCl₃, δ_{ppm}): 172.46, 89.91, 41.29, 30.16.

3-(2-Aminoethyl)-3-nitropentane-1,5-diamine-HCl salt (2). The acid chloride obtained from last step was dissolved in 30 ml dioxane in a three-necked round bottom flask protected with argon. Trimethylsilyl azide (6.3 ml, 48 mmol) was added to the solution at room temperature. The solution was then slowly heated up to 80 °C. When there was no more gas evolution, the reaction mixture was allowed to cool down to 45 °C and then added 20 ml of acetone. Concentrated HCl (12 ml) was added to the mixture drop-wise. White precipitate formed immediately following the addition. After 1 hr, the reaction mixture was allowed to cool down to room temperature. The white precipitate was collected by vacuum filtration and washed with 200 ml cold acetone. After drying in the hood overnight, a slightly yellow solid was obtained (yield: 3.86 g, 81%). ¹H NMR (DMSO-d₆): 8.39 (s, 9 H), 2.81 (t, 6 H), 2.33 (t, 6 H).

Compound (3). 3-(2-Aminoethyl)-3-nitropentane-1,5-diamine•HCl salt (3.0 g, 10.0 mmol)was dissolved in 100 ml of Et₃N/CH₃CN (v/v, 1/3). Then 10.2 g of di-*tert*-butyl dicarbonate (46.8 mmol) was added. The mixture was heated at reflux for 7 hr and then diluted with 150 ml of ethyl acetate. The mixture was washed with H₂O (250 ml × 1). Then the aqueous phase was extracted with ethyl acetate (150 ml × 1). The organic phase was combined and dried with anhydrous MgSO₄. After the filtration, the solvent was removed *in vacuo*, affording a yellow oil which solidified under vacuum (yield: 4.2 g, 86%). ¹H NMR (CDCl₃, δ_{ppm}): 4.81 (s, 3H), 3.13 (m, 6H), 2.16 (t, 6H), 1.40 (s, 27H). ¹³C NMR (CDCl₃, δ_{ppm}): 155.84, 90.59, 79.63, 35.79, 35.59, 28.34. LR-MS: calcd for C₂₂H₄₂N₄O₈[M+H] = 491.6, found 491. Elemental analysis: Calcd for C₂₂H₄₂N₄O₈: C, 57.86; H, 8.63; N, 11.42. Found: C, 53.78; H, 9.03; N, 11.24.

Compound (4). A solution of 3.1 g of compound **3** (6.3 mmol) in 200 ml of ethanol with T1 Raney Nickel (3.0 g) was hydrogenated at 100 psi and 70 °C for 36 hr. The catalyst was removed by filtering the reaction mixture through a bed of celite. The solvent was removed *in vacuo*, affording a slightly yellow oil, which solidified as a fluffy white solid under vacuum (yield:

90%). ¹H NMR (CDCl₃, δ_{ppm}): 5.06 (s, 3H), 3.18 (m, 6H), 1.78 (s, 2H), 1.56 (t, 6H), 1.41 (s, 27H). ¹³C NMR (CDCl₃, δ_{ppm}): 155.99, 79.22, 52.96, 39.37, 36.14, 28.41. LR-MS: calcd for C₂₂H₄₄N₄O₆ [M+H] = 461.6, found 461.

2,2'-(2,5-Diiodo-1,4-phenylene)bis(oxy)diacetic acid (5). In a 100 ml of three-necked round bottom flask purged with argon, a solution of 2.6 g of NaOH (66 mmol) in 10 ml of water was added drop-wise to a stirred solution of 5.4 g of 2, 5-Diiodohydroquinone and 5.0 g of bromoacetic acid (36 mmol) in 25 ml of water. The reaction mixture was heated at reflux for 4 hr. At which time, there was a lot of white precipitate formed. The reaction flask was then placed in a refrigerator overnight. The settled precipitate was collected by vacuum filtration, then redissolved in 60 ml of water and acidified with 20 ml of 6 N HCl. The white solid obtained was collected by vacuum filtration, dried under the vacuum. Yield: 6.0 g, 84%. ¹H NMR (DMSO-d₆, δ_{ppm}): 13.07 (s, br, 2H), 7.25 (s, 2H), 4.74 (s, 4H). ¹³C NMR (DMSO-d₆, δ_{ppm}): 169.76, 151.78, 122.28, 86.13, 66.03.

2,2'-(2,5-Diiodo-1,4-phenylene)bis(oxy)diacetyl chloride (6). 2,2'-(2,5-Diiodo-1,4-phenylene)bis(oxy)diacetic acid (5.8 g, 12 mmol) was suspended in 30 ml of SOCl₂. After adding 2 drops of DMF, the reaction mixture was heated up and stirred at reflux for 2 hr. then the excess SOCl₂ was removed by vacuum distillation and the resulting off-white solid was crystallized from 200 ml of heptane/toluene (v/v, 10/1), affording a slightly yellow crystalline solid (yield: 5.0 g, 80%). ¹H NMR (CDCl₃, δ_{ppm}): 7.15 (s, 2H), 4.92 (s, 4H). ¹³C NMR (CDCl₃, δ_{ppm}): 152.58, 124.41, 113.62, 86.44, 74.05.

Monomer (7). Compound **4** (1.5 g, 3.3 mmol), 0.45 ml of Et₃N (3.2 mmol) and 30 ml of dry CH₂Cl₂ were placed in a 50 ml round bottom flask, which was cooled in an ice/water bath. To the mixture, a solution of 0.76 g of 2,2'-(2,5-diiodo-1,4-phenylene)bis(oxy)diacetyl chloride (1.5 mmol) was added via a syringe. After 2 hr, the reaction mixture was allowed to warm to room temperature and further stirred for 24 hr. The solvent was removed *in vacuo*, the crude product was purified by flash chromatography on silica gel with EtOAc/hexane (1/1) to give a colorless oil, which solidified as a white solid under vacuum (yield: 1.0 g, 50%). The ¹H NMR (CDCl₃, δ_{ppm}): 7.15 (s, 2H), 6.71 (s, 2H), 4.80 (s, 6H), 4.36 (s, 4H), 3.19 (m, 12H), 2.03 (m, 12H), 1.42 (s, 54H). ¹³C NMR (CDCl₃, δ_{ppm}): 166.38, 155.94, 151.61. 122.72, 86.43, 79.33, 68.71, 59.92, 35.76, 35.60, 28.39. HR-MS: calcd for C₅₄H₉₂I₂N₈O₁₆ [M+Na] = 1385.4613, found 1385.4613.

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Fig. S1 ¹H NMR spectra of monomer (7), the neutral polymer (**PPE-NHBoc**) and the final polymer, **PPE-NH₃Cl**. The asterisk indicates the signal due to the trace water.



Fig. S2 FT-IR spectra of monomer (7), the neutral polymer (PPE-NHBoc) and the final polymer, PPE-NH₃Cl. The arrows indicate the absorption associated with ammonium groups.

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Fig. S3 Absorption (top) and fluorescence (bottom) spectra of **PPE-NH₃Cl** at different pH conditions (MES buffered solution). The inset shows the image of solutions with varied pH.



Fig. S4 Potentiometric titration curves of **PPE-NH₃Cl** in water ([**PPE-NH₃Cl**] = \sim 50 µM). It is apparent that a three-step deprotonation occurs with increasing concentration of NaOH.



Fig. S5 Absorption (top) and fluorescence (bottom) spectra of PPE-NH₃Cl with increasing Pi concentration ([PPE-NH₃Cl] = 10μ M).

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Fig. S6 Fluorescence response of **PPE-NH₃Cl** in MES buffer (pH = 6.5, [**PPE-NH₃Cl**] = 10 μ M) to different inorganic anions (F⁻, Cl⁻, Br⁻, I⁻, CO₃²⁻, SO₄²⁻, PO₄³⁻ and P₂O₇⁴⁻) and biological important anions including adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP).



Fig. S7. Ratiometric response of PPE-NH₃Cl ([PPE-NH₃Cl] = ~ 1.5 μ M) to PPi in the low concentration range (0 – 1 μ M). The analytical detection limit (D.L.) was calculated to be 340 nM using the equation D.L. = 3 δ_{bk}/m , where δ_{bk} is the standard deviation of the blank and m is the slope of the calibration plot¹.

1. Valcarcel, M. Principles of Analytical Chemistry: A textbook; Springer-Verlag: New York, 2000.