Fluorescent Ratiometric Sensing of Pyrophosphate via Induced Aggregation of a Conjugated Polyelectrolyte

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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 CaH$_2$. 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 $^1$H-NMR and at 75.4 MHz for $^{13}$C-NMR. High temperature NMR spectra were recorded on a Varian Mercury 300 FT-NMR. Chemical shifts were reported in ppm using CHCl$_3$ or C$_2$H$_5$SO 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 1,4-diethynylbenzene were dissolved in 25 ml of THF/Et$_3$N (v/v, 4/1). The resulting solution was deoxygenated with argon for 15 minutes. Then 17.3 mg of Pd(PPh$_3$)$_4$ (15 µM) and 5.7 mg of CuI (30 µM) were added to the stirred solution under the protection of argon. The reaction mixture was then heated up to 55 ºC - 60 ºC 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 dropwise. 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 re-dissolved in water easily.

Scheme S-1. Synthesis of monomer carrying polyamine side groups.
4-(3-Chloro-3-oxopropyl)-4-nitroheptanediol 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, δppm): 2.96 (t, 6H), 2.30 (t, 6H). ¹³C NMR (CDCl₃, δppm): 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 ºC. When there was no more gas evolution, the reaction mixture was allowed to cool down to 45 ºC 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, 9H), 2.81 (t, 6H), 2.33 (t, 6H).

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, δppm): 4.81 (s, 3H), 3.13 (m, 6H), 2.16 (t, 6H), 1.40 (s, 27H). ¹³C NMR (CDCl₃, δppm): 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 ºC 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%). $^1$H NMR (CDCl$_3$, $\delta$ppm): 5.06 (s, 3H), 3.18 (m, 6H), 1.78 (s, 2H), 1.56 (t, 6H), 1.41 (s, 27H). $^{13}$C NMR (CDCl$_3$, $\delta$ppm): 155.99, 79.22, 52.96, 39.37, 36.14, 28.41. LR-MS: calcd for C$_{22}$H$_{44}$N$_4$O$_6$ [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 re-dissolved 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%. $^1$H NMR (DMSO-d$_6$, $\delta$ppm): 13.07 (s, br, 2H), 7.25 (s, 2H), 4.74 (s, 4H).

$^{13}$C NMR (DMSO-d$_6$, $\delta$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 SOCl$_2$. After adding 2 drops of DMF, the reaction mixture was heated up and stirred at reflux for 2 hr. then the excess SOCl$_2$ 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%). $^1$H NMR (CDCl$_3$, $\delta$ppm): 7.15 (s, 2H), 4.92 (s, 4H). $^{13}$C NMR (CDCl$_3$, $\delta$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 Et$_3$N (3.2 mmol) and 30 ml of dry CH$_2$Cl$_2$ 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 $^1$H NMR (CDCl$_3$, $\delta$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). $^{13}$C NMR (CDCl$_3$, $\delta$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 C$_{54}$H$_{92}$I$_2$N$_8$O$_{16}$ [M+Na] = 1385.4613, found 1385.4613.
**Fig. S1** $^1$H NMR spectra of monomer (7), the neutral polymer (PPE-NHBoc) and the final polymer, PPE-NH$_3$Cl. 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-NH$_3$Cl. The arrows indicate the absorption associated with ammonium groups.
Fig. S3 Absorption (top) and fluorescence (bottom) spectra of PPE-NH$_3$Cl at different pH conditions (MES buffered solution). The inset shows the image of solutions with varied pH.
**Fig. S4** Potentiometric titration curves of PPE-NH$_3$Cl in water ([PPE-NH$_3$Cl] = ~ 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-NH$_3$Cl with increasing Pi concentration ([PPE-NH$_3$Cl] = 10 µM).
**Fig. S6** Fluorescence response of PPE-NH$_3$Cl in MES buffer (pH = 6.5, [PPE-NH$_3$Cl] = 10 µM) to different inorganic anions (F$^-$, Cl$^-$, Br$^-$, I$^-$, CO$_3^{2-}$, SO$_4^{2-}$, PO$_4^{3-}$ and P$_2$O$_7^{4-}$) and biological important anions including adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP).

**Fig. S7.** Ratiometric response of PPE-NH$_3$Cl ([PPE-NH$_3$Cl] = ~ 1.5 µM) to PPi in the low concentration range (0 ─ 1 µM). The analytical detection limit (D.L.) was calculated to be 340 nM using the equation D.L. = 3 $\delta_{bk}$/m, where $\delta_{bk}$ is the standard deviation of the blank and m is the slope of the calibration plot$^1$.