

Supporting Information

Photophysics and Phosphate Fluorescence Sensing by poly(phenylene ethynylene) Conjugated Polyelectrolytes with Branched Ammonium Side Groups

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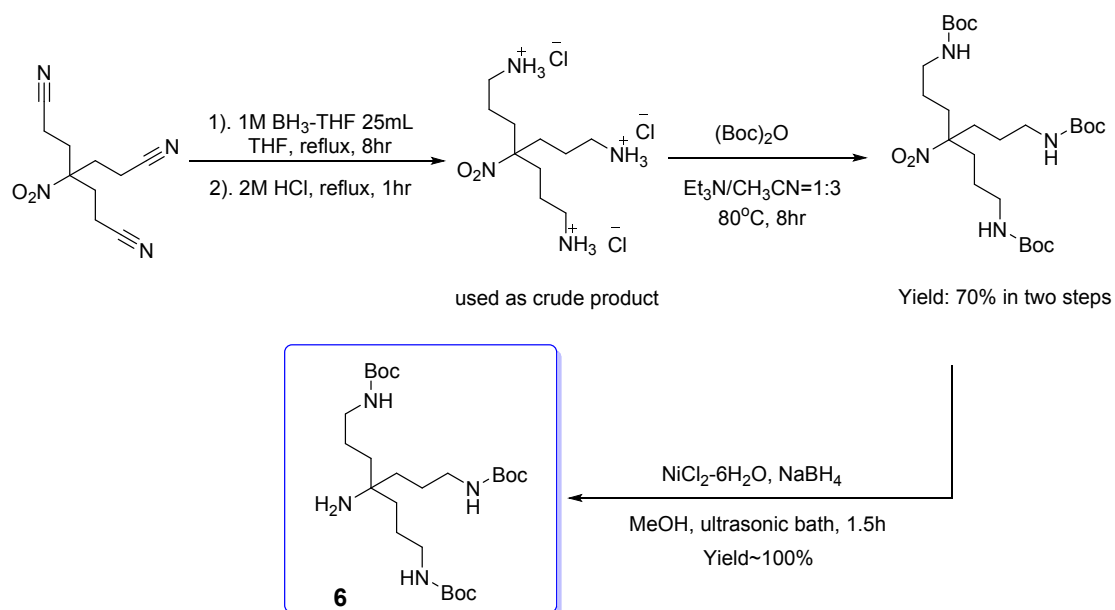
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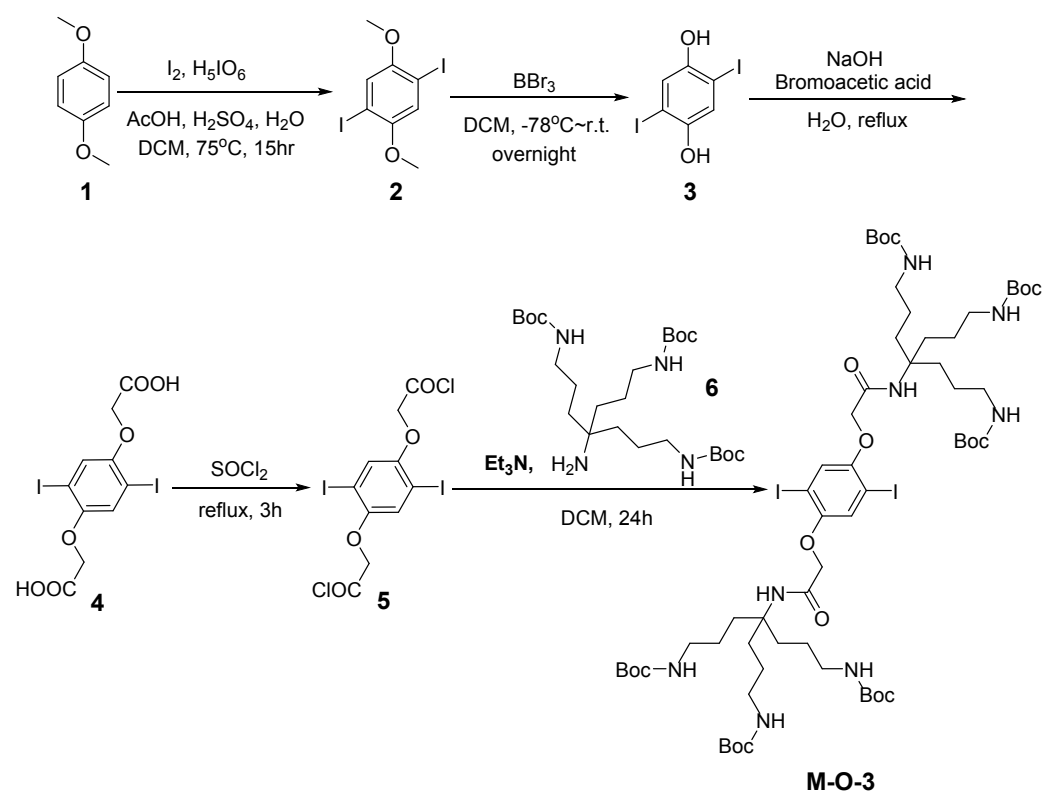
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A. Synthetic Schemes:

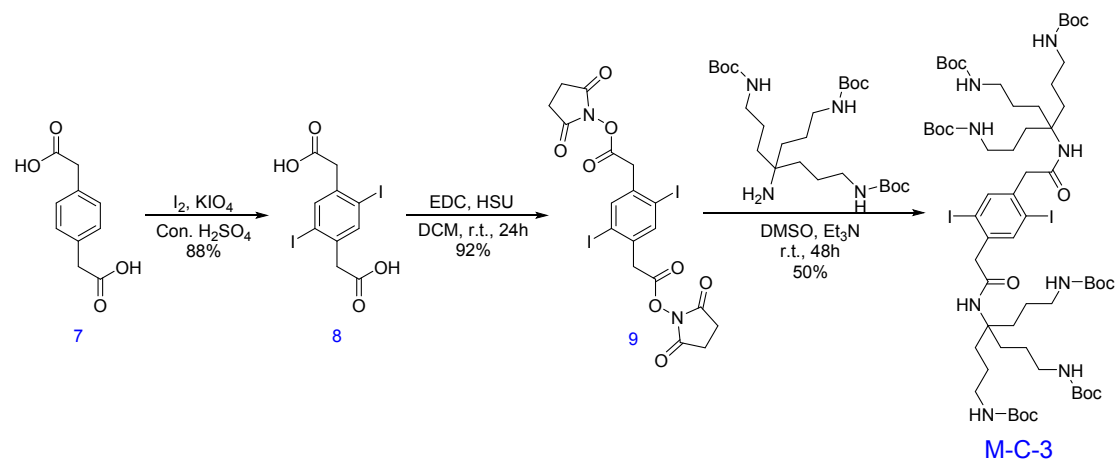
Scheme S1. Synthesis of compound **6**. [S1]



Scheme S2. Synthesis of Monomer M-O-3. [S2]



Scheme S3. Synthesis of Monomer M-C-3.



B. Supporting Figures and Tables:

Table S1. GPC data of **P-O-3-Boc** and **P-C-3-Boc**.

	Mn (kDa)	Mw (kDa)	Mw/Mn (PDI)
P-O-3-Boc	21.6	40.9	1.89
P-C-3-Boc	12	26	2.17

Table S2. K_{sv} data of **P-O-3** and **P-C-3** to different quenchers.

K _{sv} (M ⁻¹)					
Quencher	PPI	ATP	ADP	AMP	Pi
P-O-3	2.3x10 ⁵	1.1x10 ⁵	4.0x10 ⁴	2.6x10 ³	8.5x10 ³
P-C-3	1.5x10 ⁵	8.3x10 ⁴	1.8x10 ⁴	1.1x10 ³	3x10 ³

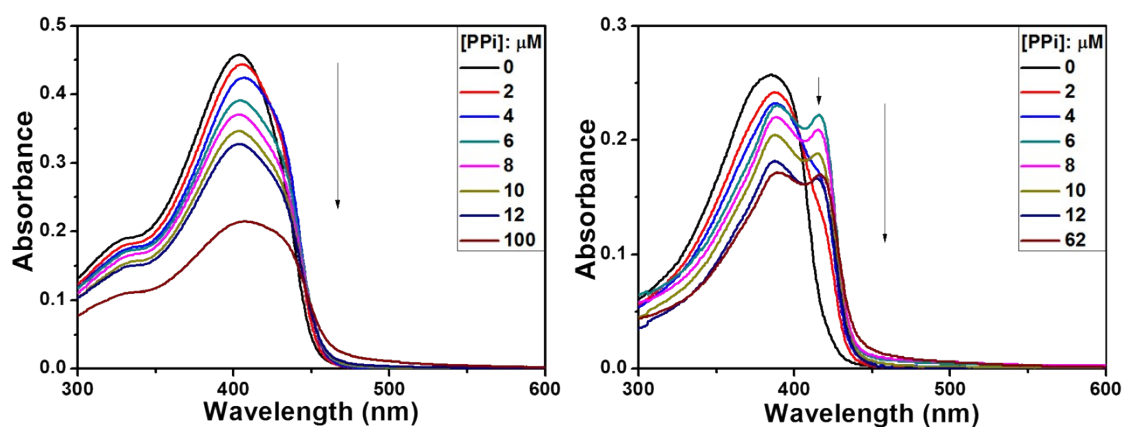


Figure S1. Absorption spectra of P-O-3 (left) and P-C-3 (right) in buffered solutions (pH = 6.5) with increasing the concentrations of PPI. (polymer concentration = 10 μM in both cases).

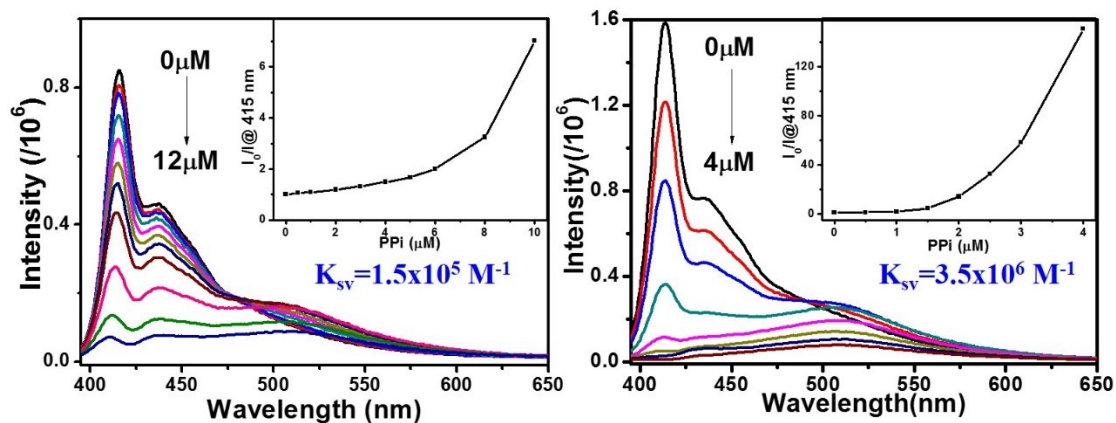


Figure S2. Fluorescence spectra of 10 μM (left) and 1 μM (right) of P-C-3 with increasing the concentration of PPI.

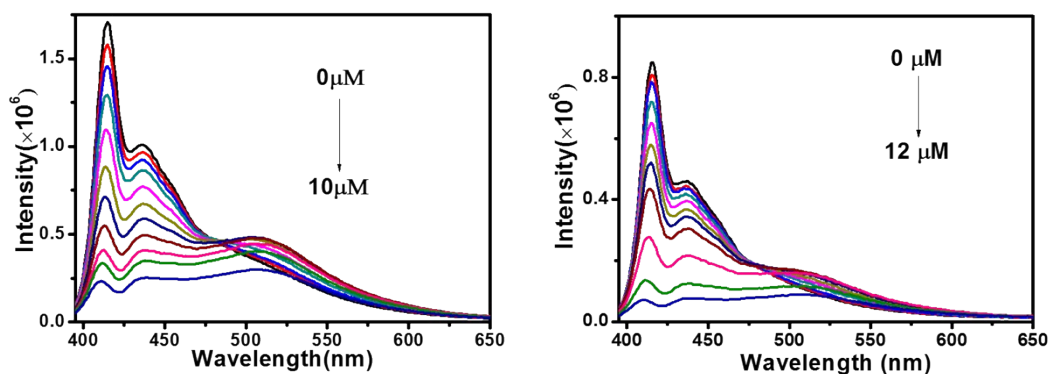


Figure S3. Fluorescence quenching spectra of P-C-3 in 10 mM of sodium chloride solution (left) and 10 mM of MES pH=6.5 buffered solutions (right) with increasing concentration of PPI. (polymer solutions' concentration = 10 μM in both cases).

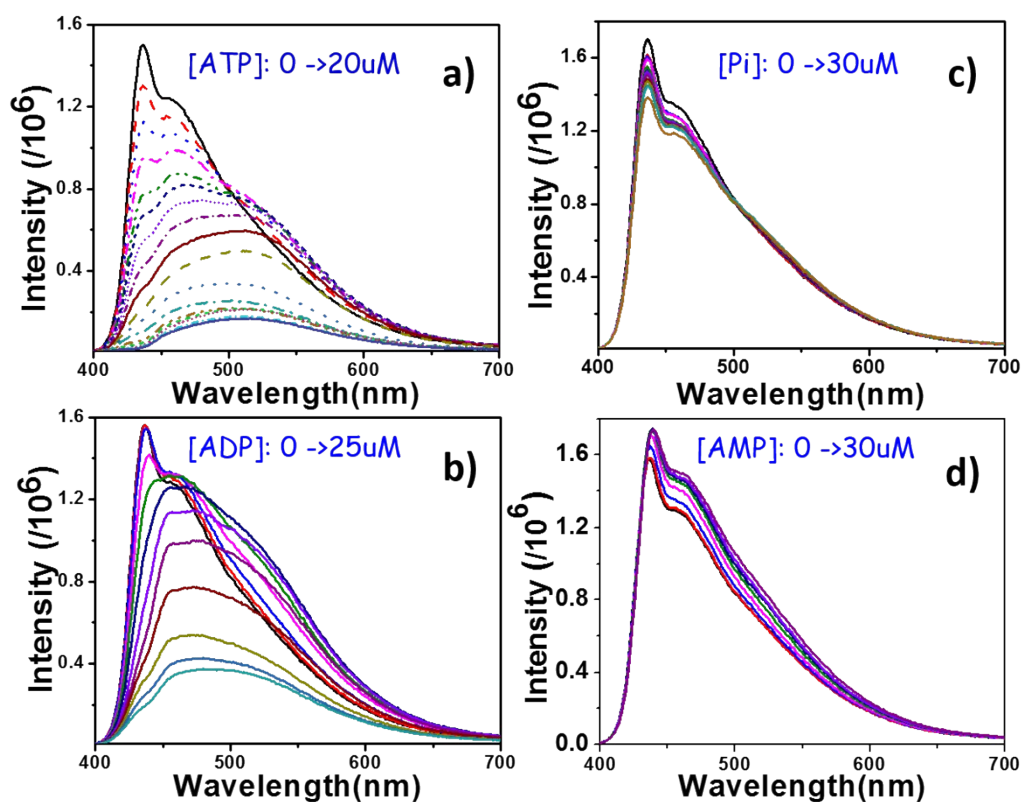


Figure S4. Fluorescence spectra of P-O-3 in buffered solutions (pH = 6.5) with increasing quenchers' concentrations: a) ATP; B) ADP; c) Pi; d) AMP. (polymer concentration = 10 μ M in all cases).

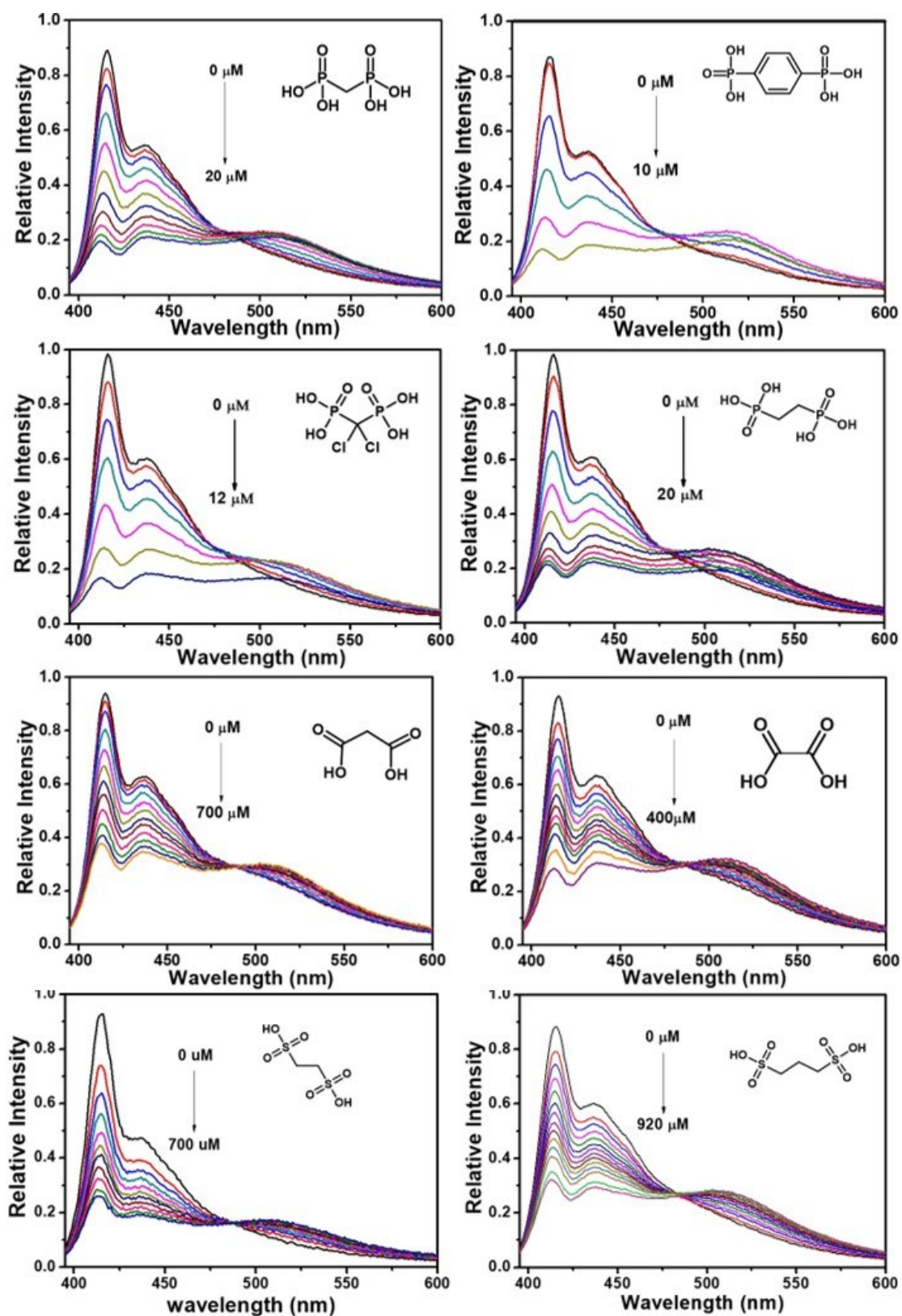


Figure S5. Absorption spectra of P-C-3 in buffered solutions (pH = 6.5) with increasing quenchers' concentrations: (polymer concentration = 10 μ M in both cases).

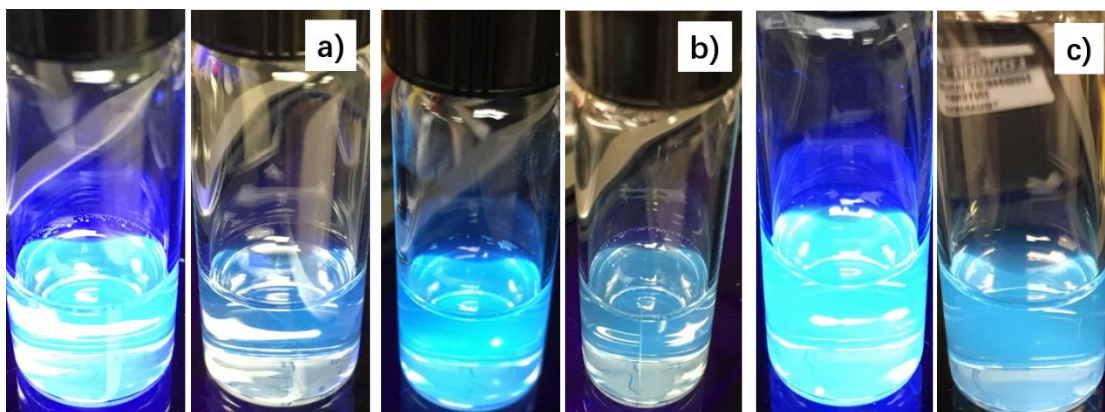


Figure S6. Pictures showing the polymer fluorescence under UV light before (left) and after (right) adding a quencher. a) P-C-3; Quencher: PPI. b) P-O-3; Quencher: PPI. c) P-C-3; Quencher: ATP. The polymer concentration is 10 μM in buffer (pH=6.5), and the quencher concentration is 12 μM .

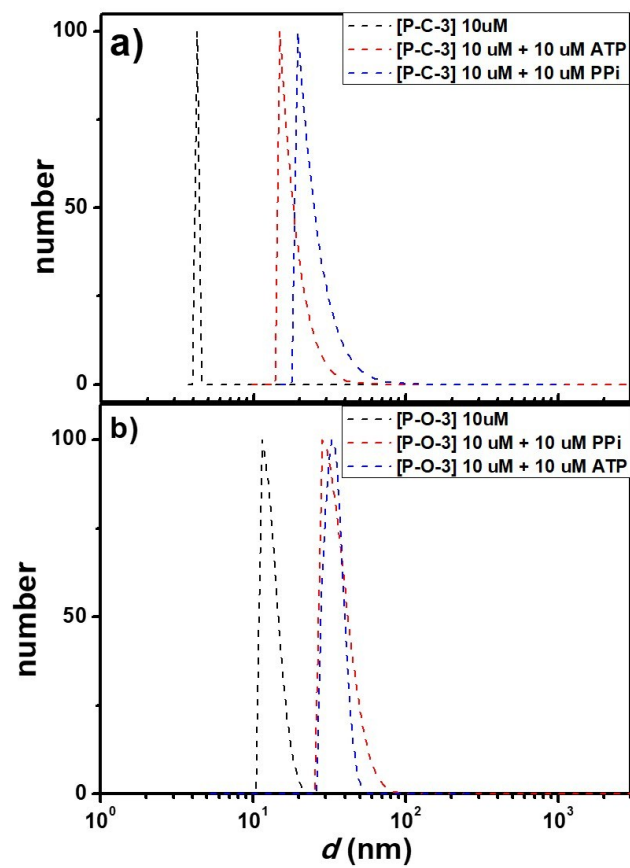


Figure S7. DLS results for polymer P-C-3 (a) and P-O-3 (b) and aggregates formed from polymer solution (10 uM) with PPI or ATP concentration of 10 μ M.

Calculation of Analytical Limit of Detection (LOD) for PPI Sensing by P-C-3:

Method: The intensity (I^0) of 5 freshly prepared 10 uM of P-C-3 solution was recorded. Then the standard deviation (σ) of these five measurements was calculated as shown in the table below.

Measurement	1	2	3	4	5	Mean (I^0_{mean})
Intensity (I^0)	643212.1	611910.5	621364.4	613003.2	631325.4	624163.1
Standard deviation (σ)	13197					

Limit of Detection of P-C-3 to PPI by Stern-Volmer Measurement:

The analytical limit of detection (LOD) is defined as the point where the measurement signal exceeds three times the error in the measurement, e.g., $3 \times \sigma$.

In the experiments to detect pyrophosphate, we used Stern-Volmer equation,
 $I^0/I - 1 = K_{SV} [Q]$

where ($I^0/I - 1$) is measured signal intensity ratio. In the quenching experiment, I^0 is the intensity at the beginning, and I is the intensity after addition of amount of analyte (quencher).

$I^0/I - 1 = (I^0 - I)/I = \Delta I/I$ where ΔI is the difference in the signal intensity in the presence (I) and absence of analyte (I^0).

For LOD calculation, the standard deviation for the blank samples (σ) represents the change in signal intensity in absence of analyte, and the corresponding change in intensity ratio is given by σ/I^0_{mean} .

$$\sigma/I^0_{mean} = 13197 / 624163 = 0.021;$$

This value represents the fluctuations in the signal intensity ratio in absence of analyte concentration. Therefore, limit of detection is given approximately by $3(\sigma/I^0_{mean}) = 3 \times 0.021 = 0.063$.

In the Stern-Volmer plot of PPI quenching of P-C-3 (Figure S8), 0.063 on the y-axis corresponds to the 0.65 μM concentration of PPI. Therefore, the estimated LOD of P-C-3 sensor for PPI is 0.65 μM .

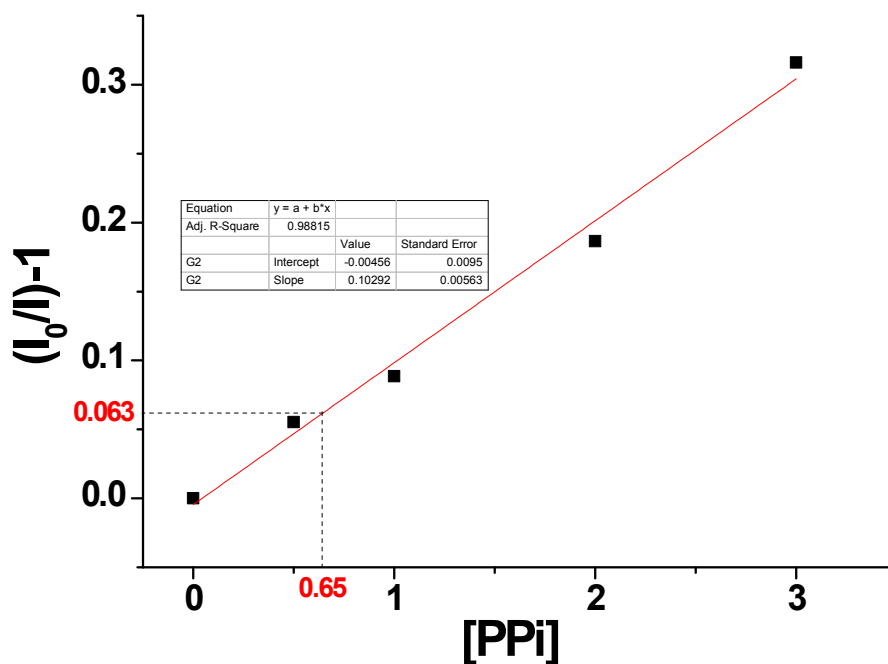


Figure S8: Stern-Volmer Plot showing the estimated LOD of P-C-3 for PPI.

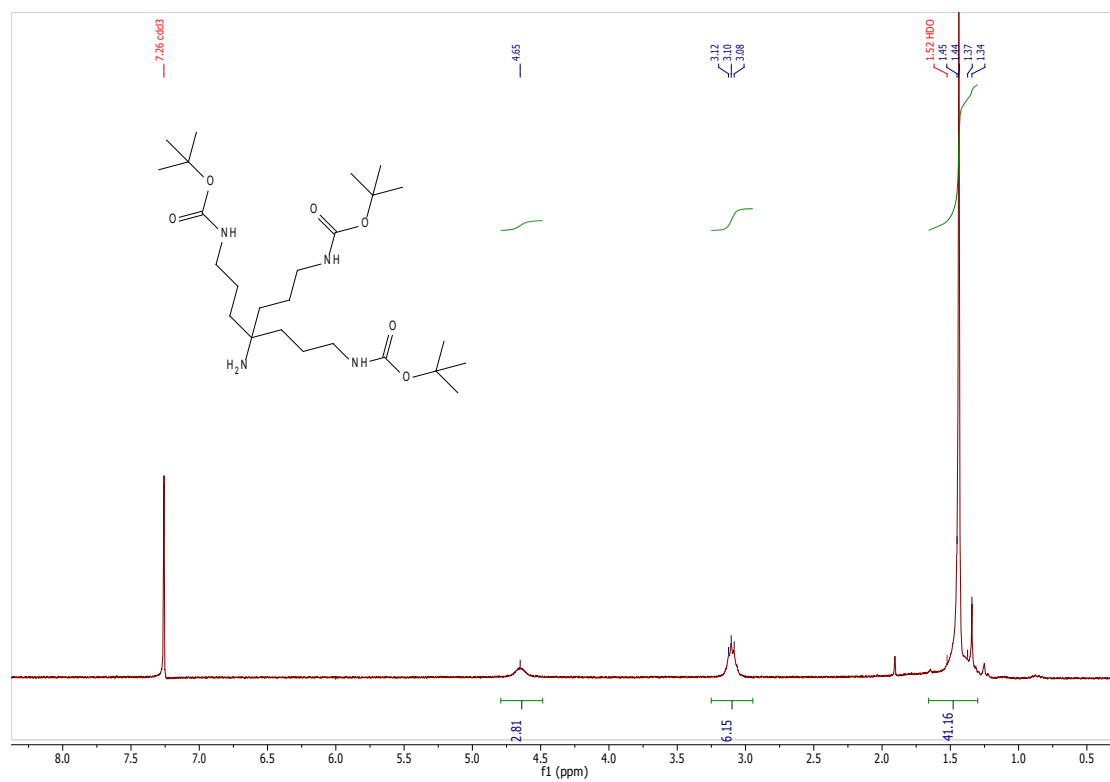


Figure S9: ¹H NMR spectrum of Compound 6 in CDCl₃.

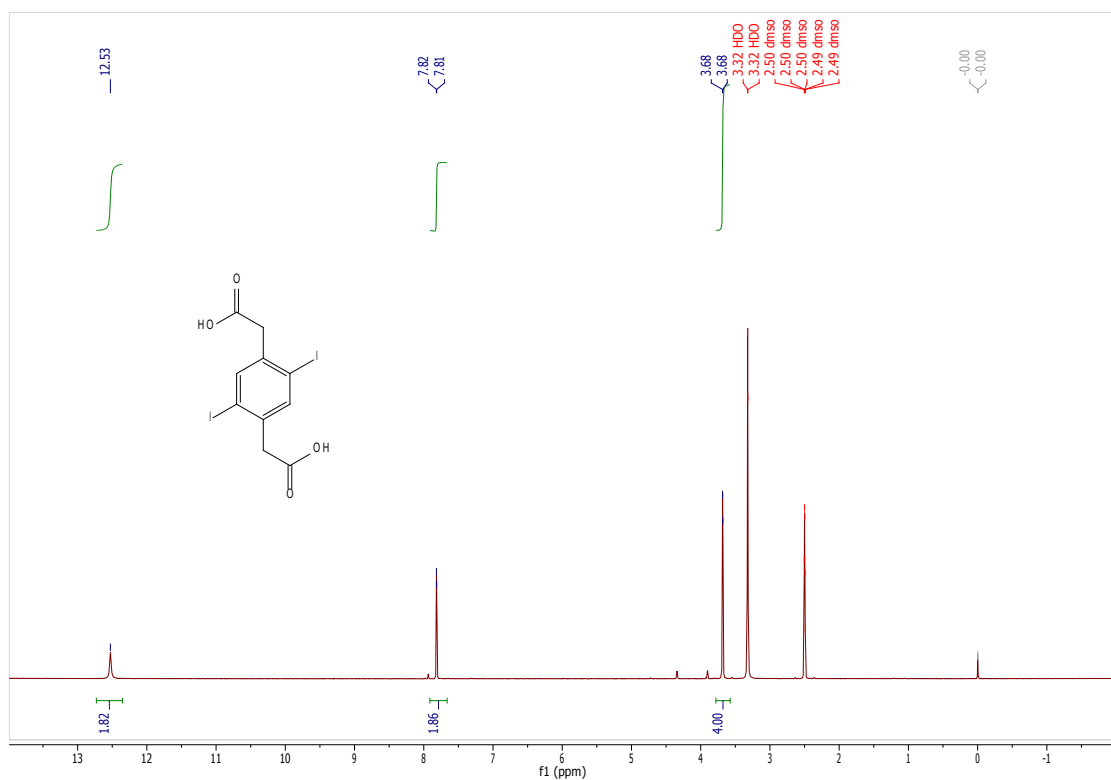


Figure S10: ^1H NMR spectrum of Compound 8 in $\text{DMSO-}d_6$.

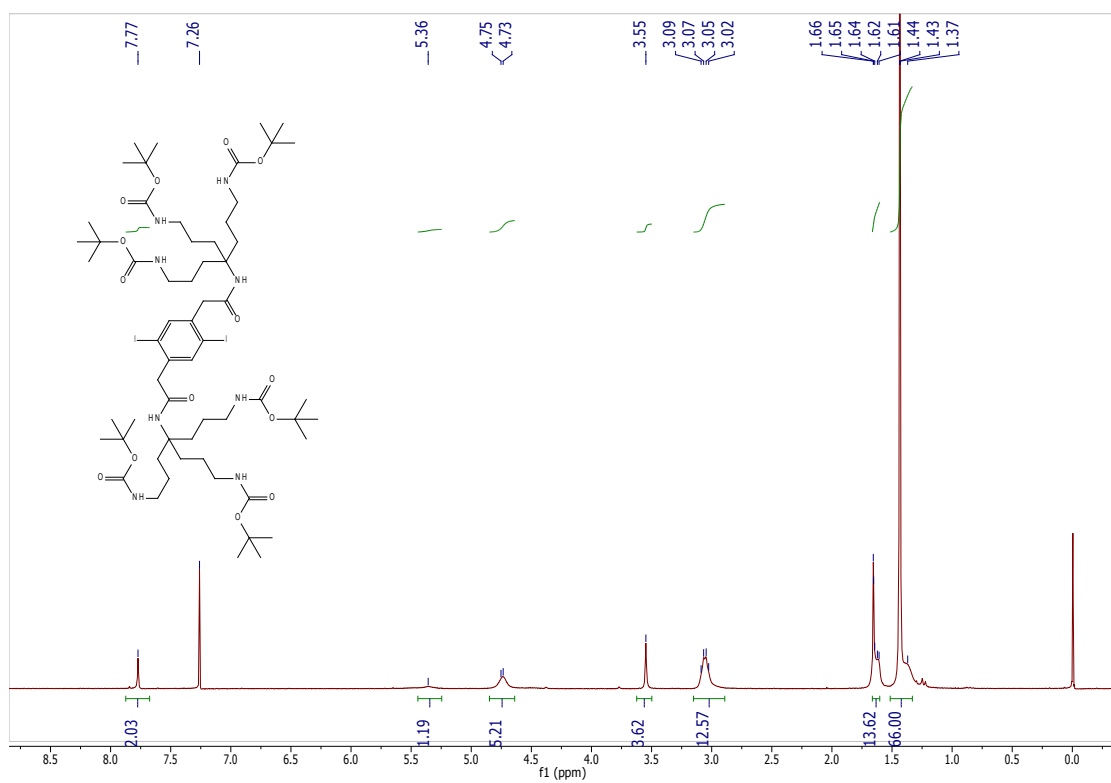


Figure S11: ^1H NMR spectrum of M-C-3 in CDCl_3 .

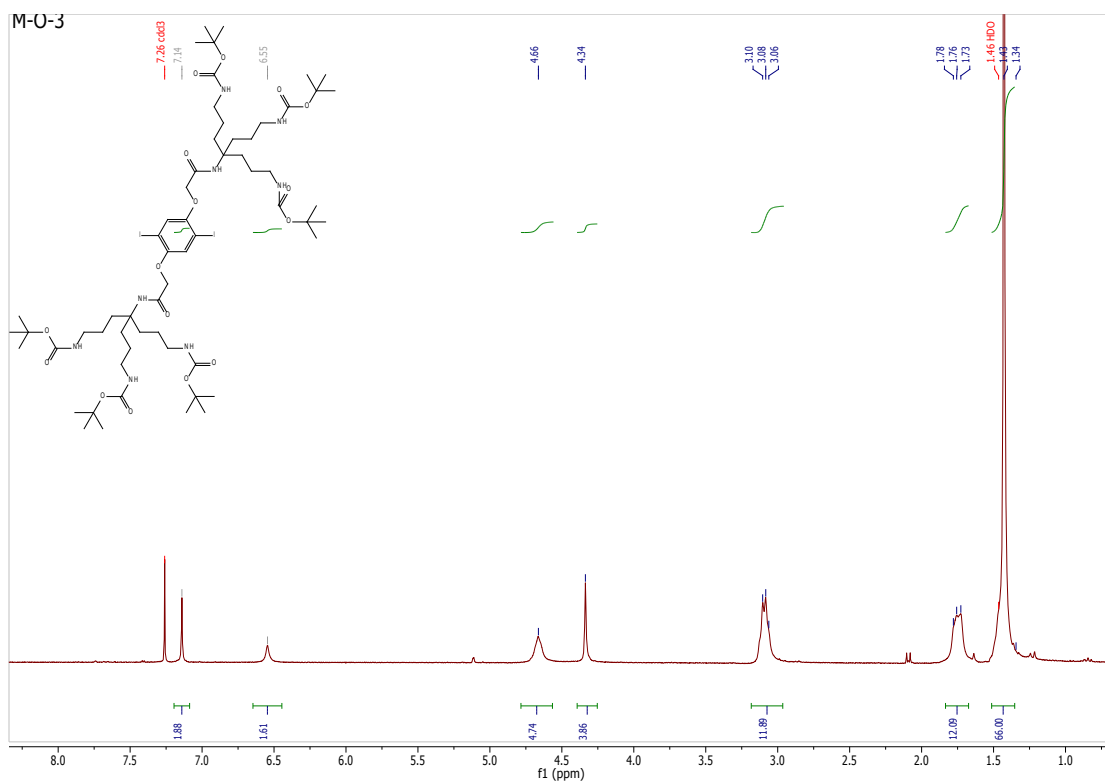


Figure S12: ^1H NMR spectrum of M-O-3 in CDCl_3 .

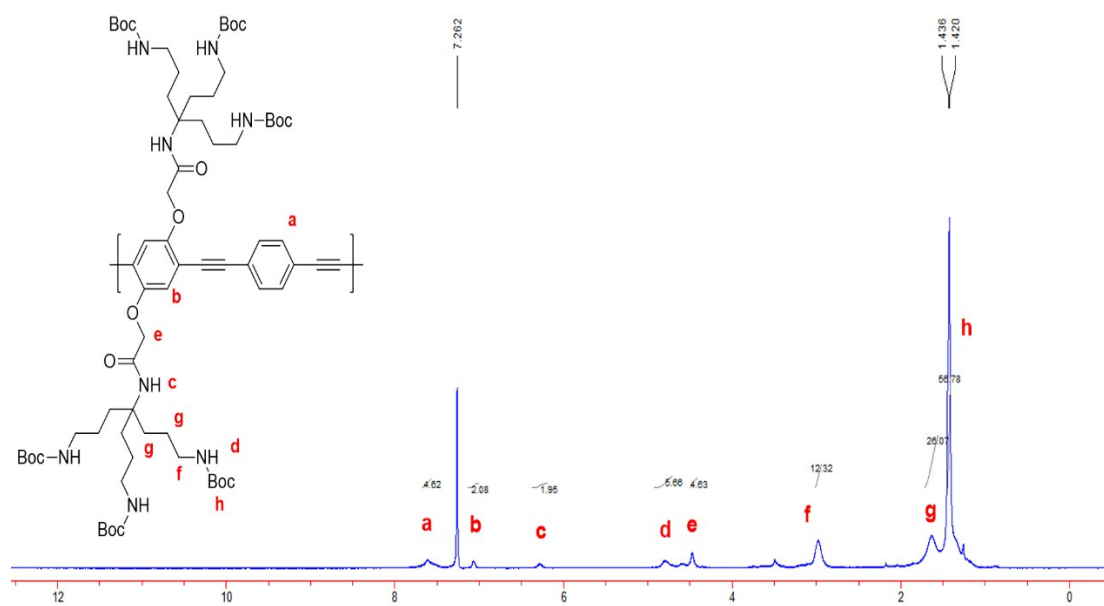


Figure S13: ^1H NMR spectrum of P-O-3-Boc in CDCl_3 .

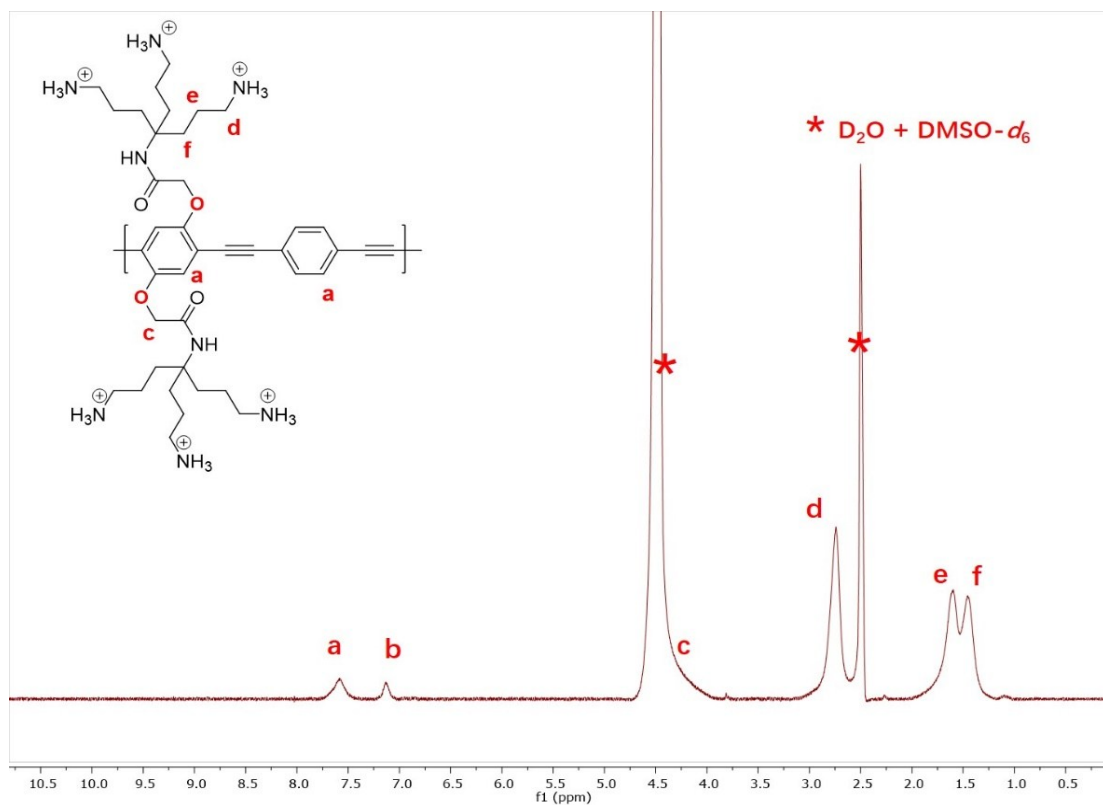


Figure S14: ¹H NMR spectrum of P-O-3 in D₂O and DMSO-*d*₆.

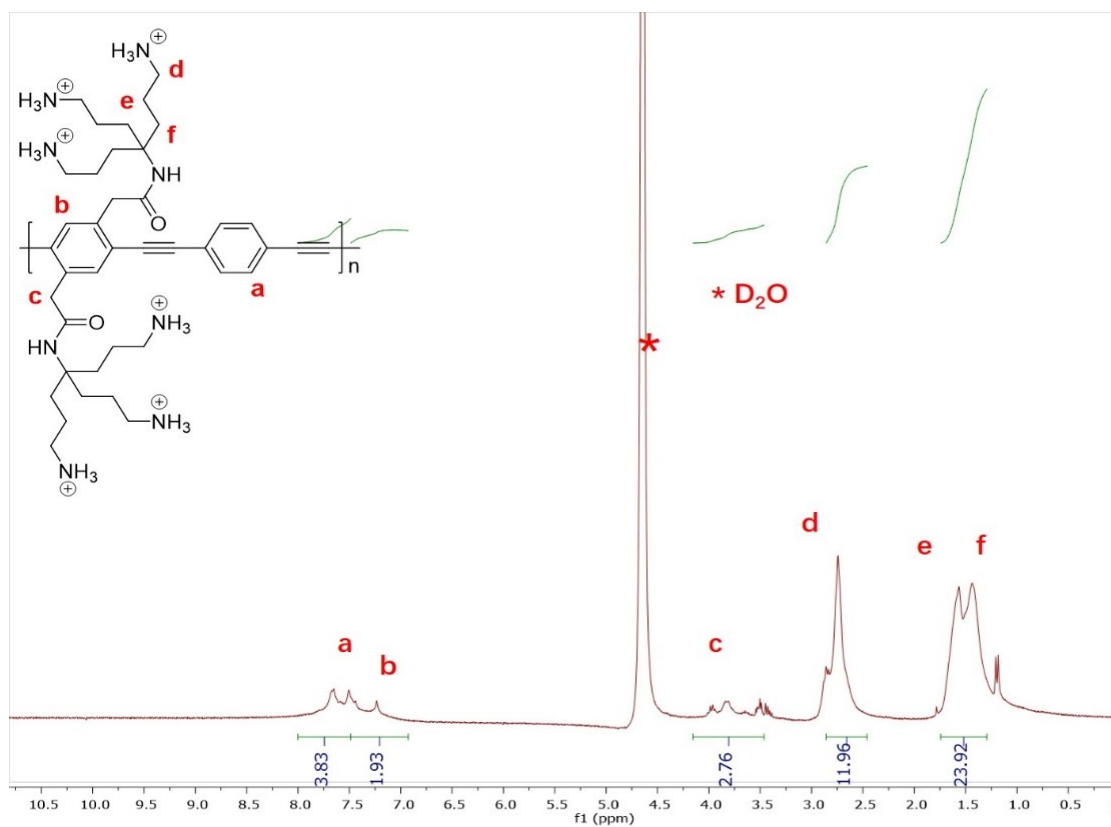


Figure S15: ¹H NMR spectrum of P-C-3 in D₂O.

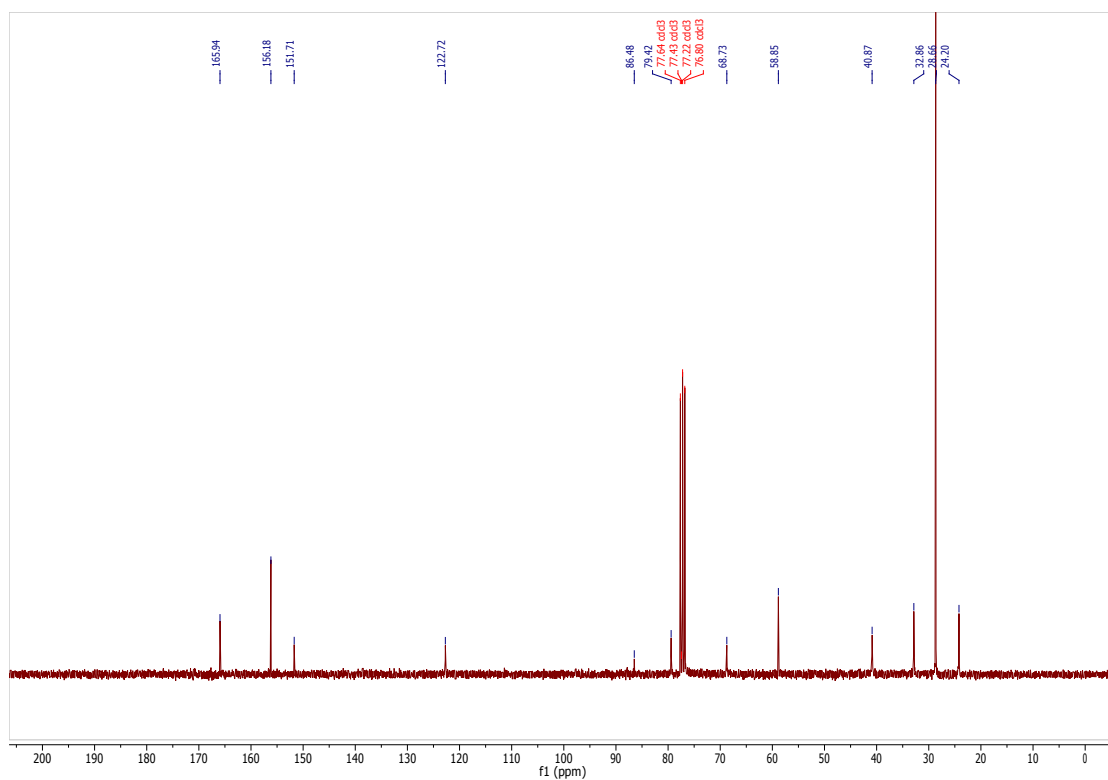


Figure S16: ^{13}C NMR spectrum of M-O-3 in CDCl_3 .

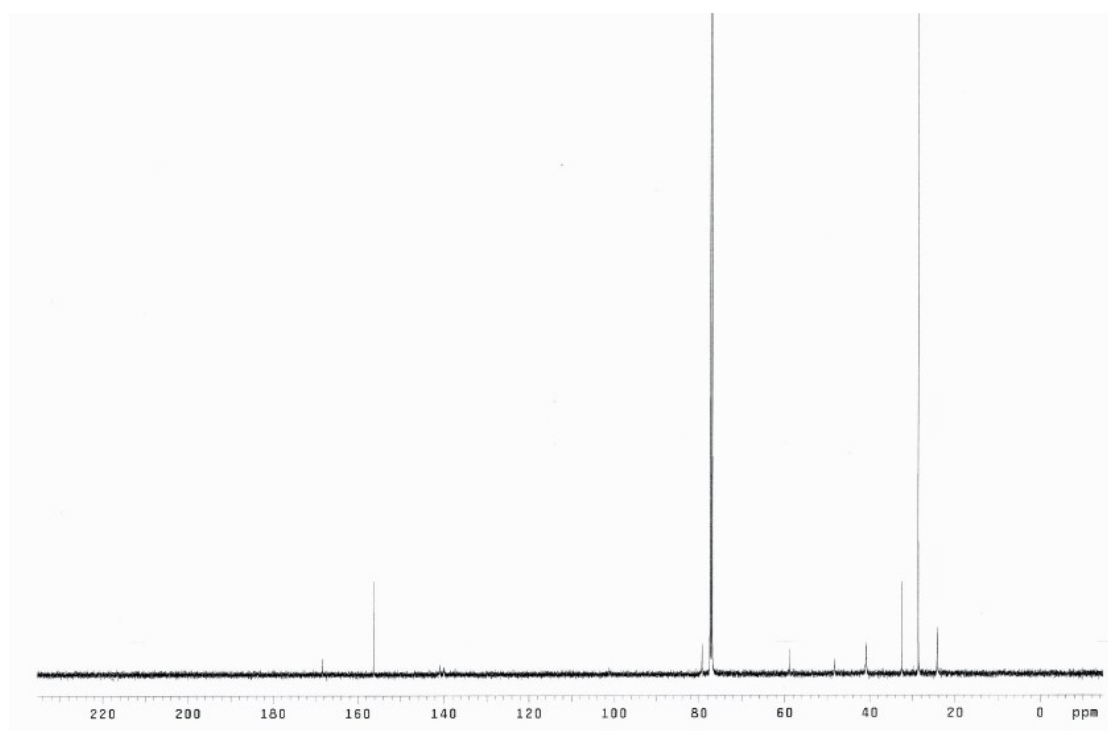


Figure S17: ^{13}C NMR spectrum of M-C-3 in CDCl_3 .

HRMS:

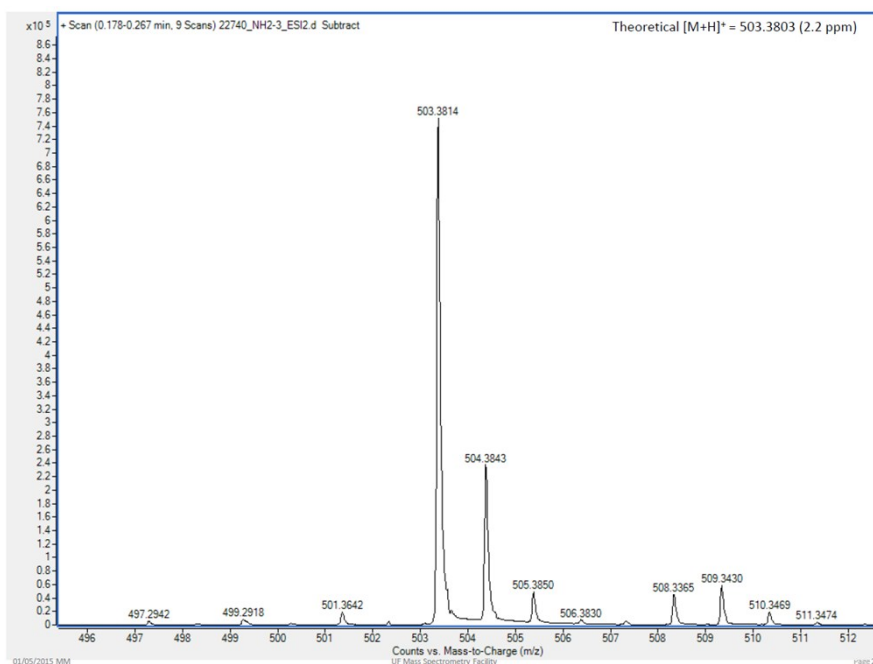


Figure S18: ESI high-resolution mass spectrum of Compound 6.

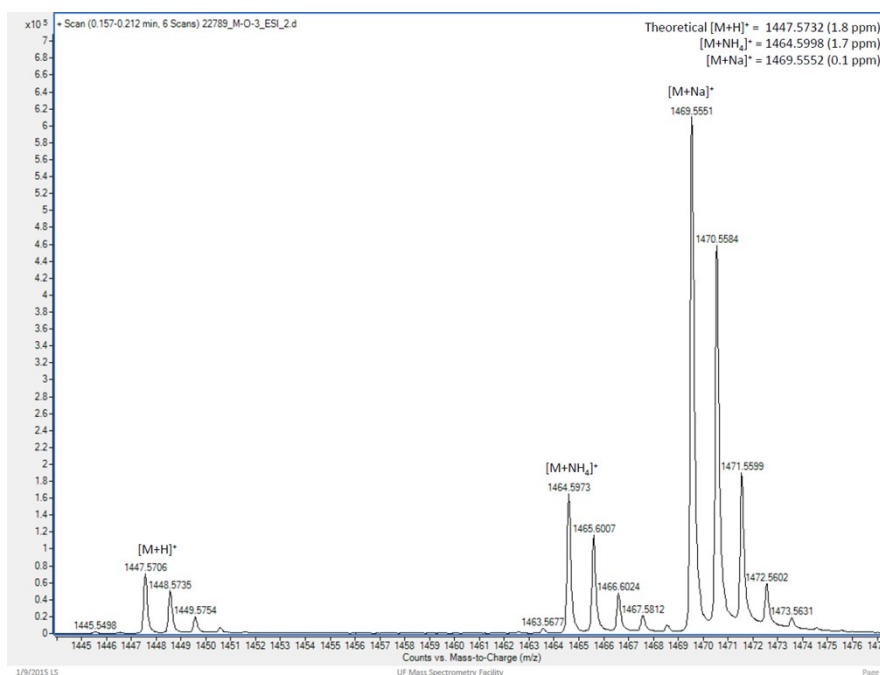


Figure S19: ESI high-resolution mass spectrum of M-O-3.

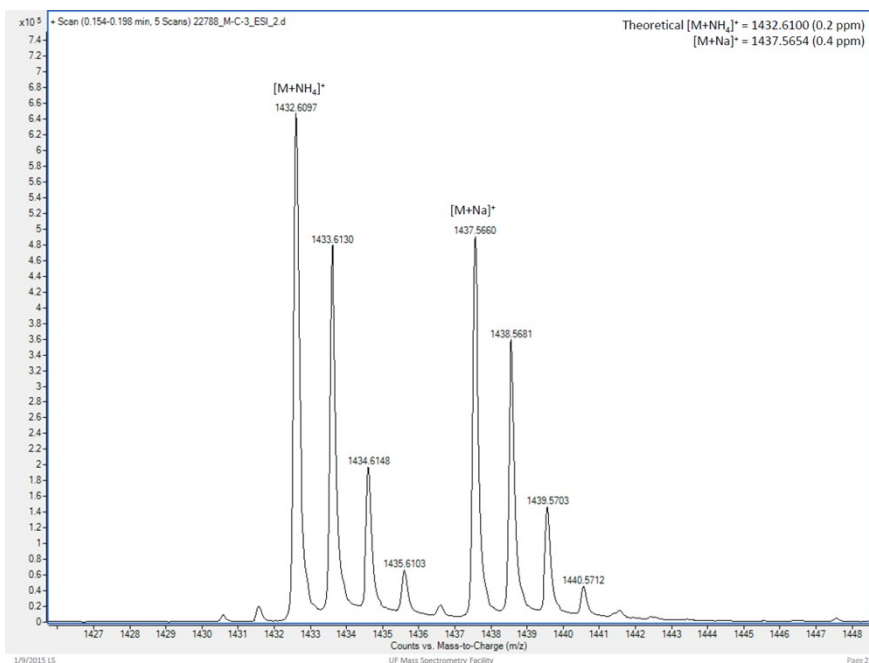


Figure S20: ESI high-resolution mass spectrum of M-C-3.

References:

- (S1) Ji, C.; Miller, P. A.; Miller, M. J., Iron transport-mediated drug delivery: practical syntheses and in vitro antibacterial studies of tris-catecholate siderophore-aminopenicillin conjugates reveals selectively potent antipseudomonal activity. *J. Am. Chem. Soc.* **2012**, *134*, 9898-9901.
- (S2) Zhao, X.; Schanze, K. S., Fluorescent ratiometric sensing of pyrophosphate via induced aggregation of a conjugated polyelectrolyte. *Chem. Commun.* **2010**, *46*, 6075-6077.