Supporting information for

pH-Responsive Self-assembled Polymer-Photosensitizer Conjugate for Activable Photodynamic Therapy

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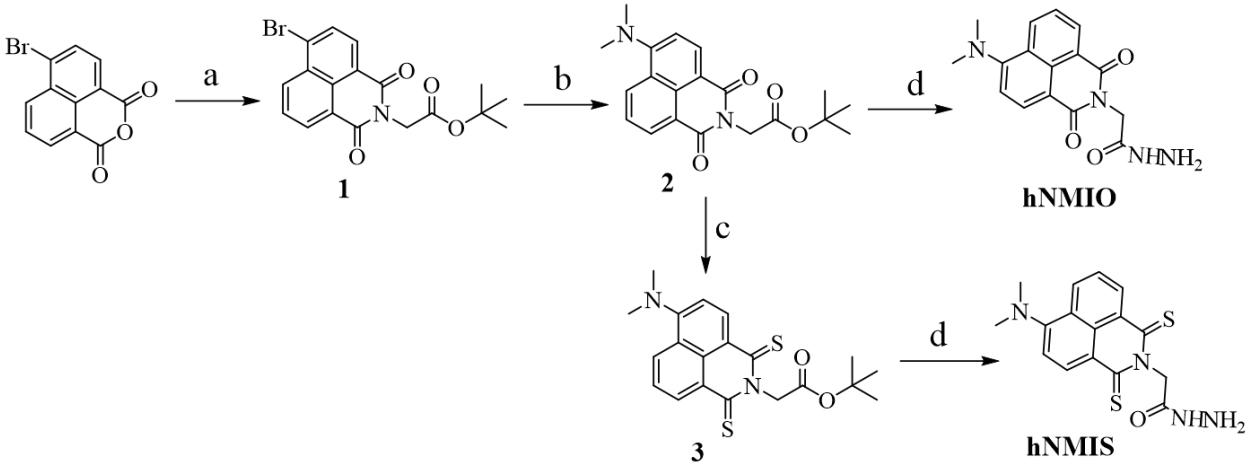
Materials and methods: Solvents and reagents were purified by standard methods.[1] ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DPX- 400 MHz spectrometer using TMS as internal standard. Mass spectrometry was recorded by electron spray ionization (ESI) technique in a Q-tof-micro quadruple mass spectrometer. For the GPC measurements, polymer concentration was 5.0 mg/ mL, and the flow rate of the solvent was 0.6 mL/ min. UV /Visible spectra were recorded in a JASCO V-750 spectrometer. Photoluminescence spectra were recorded in a FluoroMax-3 spectrophotometer from Horiba Jobin Yvon. FTIR studies were done from ATR diamond tip on a Thermo Scientific Nicolet 8700 FTIR instrument. TEM experiment was performed using JEOL-2010EX machine operating at an accelerating voltage of 200KV. DLS experiment was performed in Malvern instrument. X-ray diffraction (XRD) experiment was done by Seifert XRD3000P diffractometer with Cu Kα radiation (a = 0.154 nm) and working S2 voltage and current of 40 kV and 30 mA, respectively. Microscopy images were obtained from an inverted fluorescence microscope (Olympus, IX73, Tokyo, Japan) and for MTT assay (VARIOSKAN, Thermo Fisher) the microplate reader was used.

Scheme S1: Synthesis of hNMIO and hNMIS



Reagents and conditions: a) Glycine tertiary butyl ester hydrochloride, Et_3N, Ethanol, 90 °C, 12h, Yield = 60%, b) NMe_2, 2-methoxyethanol, 126 °C, 12 h, Yield = 80%, c) Lawesson's reagent, toluene, 110 °C, 24 h, Yield = 65%, d) Hydrazine hydrate, t-Butanol, 70 °C, 12 h, Yield = 70 %, for **hNMIO**) / 80 % (**hNMIS**)

Synthesis of compound 1: 4-bromo-1,8-napthalic anhydride (2.0 g, 7.2 mmol), glycine tertiary butyl ester hydrochloride (2.4 g, 14.4 mmol) and triethyl amine (3 ml, 21.6mmol) were dissolved in 10 ml ethanol and refluxed overnight. Subsequently the reaction was stopped, cooled to room temperature and it was extracted using ethyl acetate and brine (2 x 15 ml). Excess water was removed after passing through anhydrous Na_2SO_4. The crude product was further purified by silica gel column chromatography using 10% ethyl acetate and hexane as eluent. Yield= 1.7 g (60 %). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ(ppm): 8.68 (d, 1H), 8.60 (d, 1H), 8.43 (d, 1H), 8.06 (d, 1H), 7.87 (t, 1H), 4.83 (s, 2H), 1.48 (s, 9H).

Synthesis of compound 2: Compound **1** (1.7 g, 4 mmol), N, N-dimethylamine hydrochloride (1.78 g, 20 mmol) and triethylamine (4.2 ml, 30 mmol) were dissolved in 10 ml of 2-methoxyethanol and stirred at 130 °C for 12 h. The reaction was then stopped, and solvent was removed under reduced pressure and the crude product was extracted using ethyl acetate and brine (2 x 15 ml) and dried over anhydrous Na_2SO_4. The organic part was removed, and the product was further purified by column chromatography using silica gel as the stationary phase and 15% ethyl

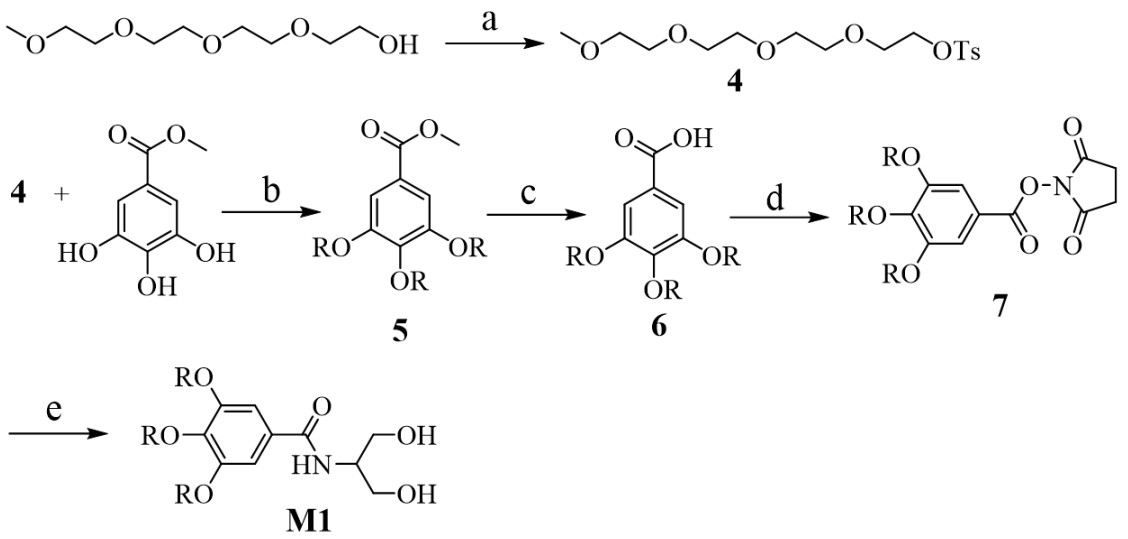
acetate/hexane as the eluent to get the pure product as yellow semi solid. Yield = 1.2 g (80 %). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm): 8.59 (d, 1H), 8.50 (d, 1H), 8.43 (d, 1H), 7.68 (t, 1H), 7.12 (d, 1H), 4.83 (s, 2H), 3.10 (s, 6H), 1.57 (s, 9H).

Synthesis of compound 3: Compound **2** (700 mg, 1.975 mmol) and Lawesson's reagent (1.6 g, 3.95 mmol) were dissolved in 10 mL toluene and refluxed for 24 h. The reaction was stopped and the solvent was removed to get the crude product, which was further purified by silica gel column chromatography using 8% ethyl acetate/hexane as the eluent to get the pure product as a purple semi solid. Yield = 500 mg (65 %). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm): 8.97 (d, 1H), 8.88 (d, 1H), 8.35 (d, 1H), 7.54 (t, 1H), 7.01 (d, 1H), 6.17 (s, 2H), 3.18 (s, 6H), 1. 47 (s, 9H).

Synthesis of hNMIO: Compound **2** (250 mg, 0.7 mmol) and hydrazine hydrate (2ml, 35.27 mmol) were dissolved in 5 mL *t*-butanol and refluxed for 12 h. The reaction was stopped and cooled to room temperature to obtain yellow precipitate. It was filtered and washed with minimum amount of *t*-butanol to obtain a yellow solid product in pure form. Yield = 134 mg (67 %). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm): 8.97 (d, 1H), 8.88 (d, 1H), 8.35 (d, 1H), 7.52 (t, 1H), 7. 01 (d, 1H), 6.17 (s, 2H), 3.18 (s, 6H). HRMS (ESI): $[M+H]^+$ calculated for $C_{16}H_{16}N_4O_3$: 313.30; $[M+H]^+$ found: 313.29.

Synthesis of hNMIS: Similar procedure was followed for NMIS synthesis (starting from compound **3**) like hNMIO. Yield: 95 mg (80 %). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm): 8.65 (d, 1H), 8.57 (m, 2H), 8.24 (d, 1H), 7.53 (t, 1H), 6.25 (s, 2H), 3.13 (s, 6H). HRMS (ESI): m/z calculated for $C_{26}H_{36}N_2S_2$: 344.07; $[M+H]^+$ found: 344.2867.

Scheme S2: Synthesis of monomer **M1**



Reagents and Conditions: a) TsCl, THF, NaOH, H_2O, 0 °C, 6 h, Yield = 75%, b) K_2CO_3, CH_3CN, 80 °C, 48 h, Yield = 80%, c) KOH, H_2O, EtOH, 90 °C, 6 h, 90%, d) NHS, EDC, DCM, 0 °C-RT, 24 h, 80%, e) Serinol, ACN, 90 °C, 12 h, Yield = 72%

Synthesis of compound 4: Tetra-ethylene glycol monomethyl ether (5 g, 20 mmol) in 5 mL THF was mixed with 5 mL aqueous NaOH (1.2 g, 30 mmol) solution and placed in an ice bath and stirred for 15 min. Subsequently, *p*- toluene sulfonyl chloride (3.8 g, 20 mmol) solution in THF was added dropwise to this ice-cold reaction mixture and stirred at ~ 0 ^{o}C for 6 h. The reaction was stopped, and 50 mL dichloromethane was added to it. The organic part was washed with brine solution for three times and the passed through anhydrous Na_2SO_4. After concentrating the organic part at low pressure, the crude product was collected as a viscous colorless oil. It was further purified by column chromatography using silica gel (100-200 mesh) as a stationary phase and 30 % ethyl acetate/hexane as eluent to obtain the colorless oil with 75 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 7.81 (d, 2H); 7.35 (d, 2H); 4.16 (t, 2H); 3.69-3.60 (m, 14H); 3.36 (s, 3H); 2.44 (s, 3H).

Synthesis of compound 5: Compound **4** (2 g, 5.5 mmol), methyl-3, 4, 5-trihydroxybenzoate (339 mg, 1.8 mmol), anhydrous K_2CO_3 (1.24 g, 9 mmol) and catalytic amount of KI were dissolved in

10 mL of dry CH_3CN and placed in an oil bath to reflux for 48 h under inert atmosphere. After that, the reaction was stopped, cooled to room temperature and CH_3CN solvent was evaporated. To this crude reaction mixture, 30 mL ethyl acetate was added, and it was washed with brine solution (3×30 mL). The organic part was collected and evaporated under reduced pressure to obtain light brown viscous oil with 80 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 7.28 (s, 2H); 4.18 (m, 6H); 3.87-3.55 (m, 42 H); 3.36 (s, 9H).

Synthesis of compound 6: Compound **5** (1.3 g, 2 mmol) solution in ethanol (10 ml) was mixed with aqueous KOH (11 mg, 0.2 mmol) solution and reflux for 12 h. After that the reaction was stopped, cooled to room temperature and the solvent was evaporated. Further, 30 mL ethyl acetate was added to this crude reaction mixture and washed with brine solution and 30 mL 1(N) HCl solution. The organic part was collected and evaporated under reduced pressure to get the pure product with quantitative yield. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ (ppm) = 7.34 (s, 2H); 4.12 (m, 6H); 3.86–3.50 (m, 42H); 3.36 (s, 9H).

Synthesis of compound 7: Compound **6** (1.3 g, 2 mmol), N-hydroxy succinimide (345 mg, 3 mmol) and N, N-dimethyl amino pyridine (DMAP) (12 mg, 0.1 mmol) was dissolved in 10 mL dry dichloromethane and placed in an ice bath under inert atmosphere. Further, 10 mL of 1-ethyl-3(3-dimethylamino propyl carbodiimide hydrochloride (575 mg, 3 mmol) solution in dry dichloromethane was dropwise added to the ice-cold reaction mixture and it was stirred overnight at room temperature under inert atmosphere. After that the reaction mixture was washed with 1(N) HCl solution and brine solution. The organic part was passed through anhydrous sodium sulfate and concentrated under low pressure to get the light yellow colored viscous oil with 80 % yield. ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ (ppm) = 7.40 (s, 2H); 4.22 (m, 6H); 3.88–3.65 (m, 42H); 3.39 (s, 9H) and 2.91 (s, 4H).

Synthesis of M1: compound **4** (1 g, 1.3 mmol), 280 μL of triethyl amine (2 mmol) and serinol (182 mg, 2 mmol) were dissolved in anhydrous CH_3CN and placed in an oil bath and refluxed for 12 h under inert atmosphere. After that the reaction was stopped and solvent was evaporated, and the product was purified by column chromatography using silica gel (100-200 mesh) as stationary phase and 4% MeOH in ethyl acetate as eluent to collect the light brown colored viscous product with 72% yield. ^{1}H NMR (400 MHz, DMSO-D_6, TMS): δ (ppm) = 7.94 (d, 1H), 7.19 (s, 2H); 4.66 (s, 2H); 4.16 (m, 6H); 4.07–3.50 (m, 47H); 3.24 (s, 9H).

Synthesis of P1: M1 (100 mg, 0.138 mmol, 0.7 equiv.), 1,3-dihydroxy acetone (**M2**) (5 mg, 0.059 mmol, 0.3 equiv.) and (*S*)-(-)-2-methyl 1-butanol (MFI-1) (4 mg, 0.039 mmol, 0.02 equiv.) were dissolved in dimethyl-acetamide (DMAc) and the reaction mixture was purged with dry argon gas for 10 min. Then, a solution of 1,4-diisocyanato butane (**M3**) (31 mg, 0.22 mmol, 1.1 equiv.) in 200 μL anhydrous DMAc and 1,4-diazabicyclo [2.2.2] octane (DABCO) (1.1 mg, 0.05 mmol) in 50 μL anhydrous DMAc were added, and the mixture was degassed for another 10 min with constant stirring. Then this homogeneous reaction mixture was stirred at 65 °C for 12 h under inert atmosphere. After that the polymerization was stopped and the crude polymer was dissolved with 0.3 mL THF and precipitated from cold excess diethyl ether and dried under high vacuum to obtain the desired polymer as a white sticky solid. Yield = 79 %. SEC: Mn = 9000 g/ mol ($Đ$ = 1.17). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 8.27 (NH amide); 7.18 (2H, benzene)); 5.75 (NH urethane proton); 4.15 (m, 4H, Serinol Block);4.06 (m, 4H, DHA block) 3.76 (t, 6H); 3.75-3.48 (OEG chain); 3.31 (s, 9H); 2.92 (4H, broad); 1.32 (4H, broad); 0.84 (12H, end group methyl).

Synthesis of P1S / P1O: Polymer **P1** (35 mg, 0.135 mmol) with hNMIS / hNMIO (21 mg, 0. 068 mmol) were dissolved in 1.0 ml dry DMF and heated at 70 °C for 12 h. After that the reaction was stopped and cooled to rt. Then it was purified by precipitation from cold excess diethyl ether

followed by dialysis using 3500 MWCO membrane against water for 3 days. Then the solution from the dialysis bag was lyophilized and dried over vacuum for 4-5 hours to obtain the polymers as Red (for **P1S**) / Yellow (for **P1O**) sticky solid with quantitative yield. It was further characterized by the ^{1}H NMR spectra.

^{1}H-NMR spectra of **P1S** (DMSO-d_6, 400 MHz) δ (ppm): 8.32 (d, 1H), 8.13 (m, 2H), 8.07 (d, 1H), 7.53 (t, 1H), 7.18 (S, 2H, benzene) ,6.89 (d, 1H), 5.75 (NH urethane), 4.15 (m, 4H, Serinol Block), 4.06 (m, 4H, DHA block) 3.76 (t, 6H), 3.75-3.48 (OEG chain), 3.31 (s, 9H), 3.13 (s, 6H) 2.92 (4H, broad), 1.32 (4H, broad), 0.84 (12H, end group methyl).

^{1}H-NMR spectra of **P1O** (DMSO-d_6, 400 MHz) δ (ppm): 8.52 (d, 1H), 8.33 (m, 2H), 8.20 (d, 1H), 7.52 (t, 1H), 7.20 (S, 2H, benzene) , 6.91 (d, 1H), 5.75 (NH urethane) , 4.15 (m, 4H, Serinol Block), 4.06 (m, 4H, DHA block) 3.76 (t, 6H), 3.75-3.48 (OEG chain), 3.31 (s, 9H), 3.13 (s, 6H) 2.92 (4H, broad), 1.32 (4H, broad), 0.84 (12H, end group methyl).

EXPERIMENTAL

Solution preparation and self-assembly studies: P1S/P1O was first dissolved in THF (c = 1.0 mg/mL) and then THF was evaporated to get a thin film. It was then added with measured amount of water and dissolved to get the aqueous solution of desired concentration, which was used from UV/Vis or fluorescence spectroscopy studies. For transmission electron microscopy (TEM) experiment, aqueous solution of P1S (c = 0.1 mg/ml) was drop cast on carbon coated Cu grid. After 5 min the surface solvent on the grid was removed by tapping a filter paper on it. Then the grid was kept overnight for air drying before the TEM experiment. For Dynamic light scattering experiment, aqueous solution of P1S/P1O (c = 0.1 mg/ml) was used and measurements were done

at a scattering angle of 173°. The X-ray diffraction pattern of a dried film was generated from aqueous solution of P1S (1 mg/ml).

pH-responsive disassembly and ROS generation: P1S/P1O was dissolved in two different phosphate buffer solution having pH= 5.5 or pH 7. Then, it was dialyzed against the respective phosphate buffer solutions using 7000 MWCO dialysis bags. Aliquot was taken out from the dialysis bag at every 10-min time interval for UV-Visible absorbance spectral measurement. Total ROS generation efficiency of aqueous P1S solution as well as hNMIS in different pH (pH=7, pH=5.5) was determined using 2,7- dichlorofluorescein diacetate (DCFH-DA) as a probe. Initially, DCFH-DA was activated by treating with aqueous NaOH solution (1.0 M) to produce DCFH (75 μM). After that 1.0 ml of P1S solutions at pH = 7 was mixed with equal volume of DCFH solution and fluorescence spectra were measured in dark as well as after light irradiation (λ = 560-590 nm) for 1 min intervals of total 10 minutes. Both hNMIS and P1S were treated with pH 5 buffer for 2 h prior to the experiment. DCF fluorescence is pH sensitive and at lower pH its emission intensity gets reduced. Therefore, we performed a modified DCFH assay at pH 5 where aqueous solution of hNMIS or P1S was incubated with pH 5 buffer for 2 h and then the solution pH was turned back to 7 by adding few drops of concentrated NaOH solution to observe the maximum efficiency of ROS generation.

Biological studies: HeLa cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% Glutamine-penicillin-streptomycin. The cells were grown at 37 °C in 5 % CO_2 containing incubator. For uptake assay, equal number of HeLa cells were treated with P1O **(**1.0 mg/mL**)** and incubated for different time points (6 h and 12h). For photoluminescence study, the P1O-treated cells were lyzed with the lysis buffer and fluorescence spectra of the cell lysates were measured. For inverted fluorescence microscopy

measurement, only P1O-treated cells (for 6 h) were used. In this case, the polymer solution was removed, and the cells were washed with PBS buffer (pH=7.2) for three times. Further, fresh complete media was added to the cells and imaged under inverted fluorescence microscope. For cell viability assay, HeLa cells were seeded in 96 well plate at 10,000 cells in each well and incubated for 24 h. Then P1S solution (c = 1mg/mL) was added to it and incubated for 6 h. The supernatant solution was removed, cells were washed with PBS buffer and fresh complete media was added. Then cells were irradiated with light (7W, λ = 560-590 nm) for 1h and placed in the incubator for further 24 h. After that, cells were treated with the MTT reagent (0.5 mg/mL) and incubated for 4 h. The supernatant was removed and 200 µL DMSO was added to dissolve all the precipitates to produce violet colour solutions and absorbance were checked at 580 nm by a microplate reader (VARIOSKAN, Thermo Fisher). For dead cell assay, HeLa cells were treated with P1S (1.0 mg/mL) for 6 h. The supernatant solution was removed and washed with phosphate buffer (pH = 7.2). Cells were then irradiated with 560-590 nm light (LED bulb) for 1h. Then the cells are stained with 4 µM propidium iodide (PI) and kept in dark for 10 min. After that cells were washed with PBS and imaged under fluorescence microscopes (Olympus IX73) in red channel. In a control experiment P1S treated HeLa cells were incubated in dark for 6 h, stained with PI (4 µM) and imaged under fluorescence microscope. For ROS generation assay HeLa cells were incubated with P1S (1.0 mg/ml) for 6 h. Subsequently, it was washed with PBS buffer (pH=7.2) and treated with 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA, 10 µM) in complete media for 30 min. Cells were again washed with phosphate buffer and irradiated for 1h (560-590 nm LED bulb) and imaged in green channel under fluorescence microscope. For nuclear staining Hoechst 33342 was used (Concentration = 10 µM)

Additional figures:

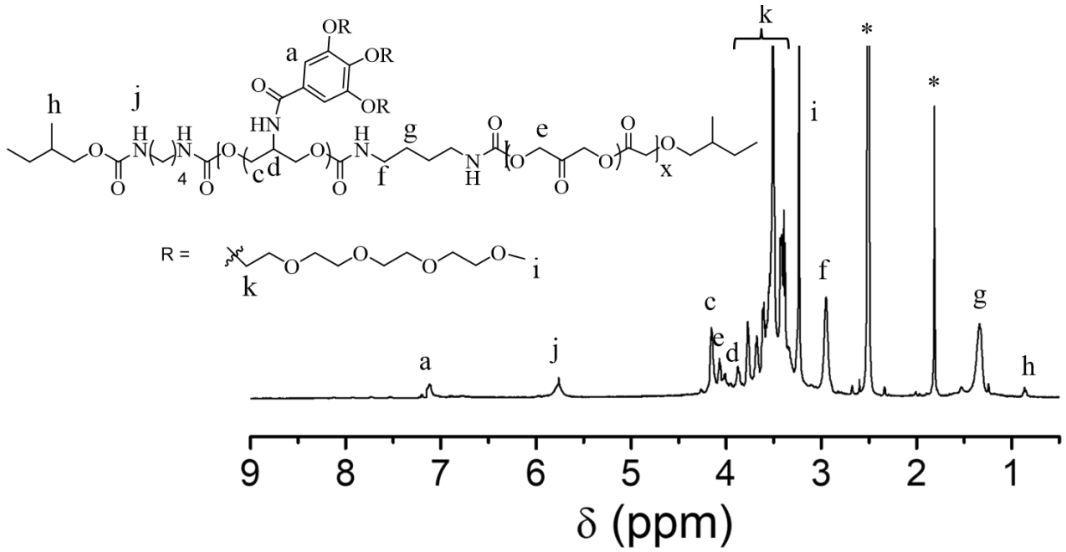
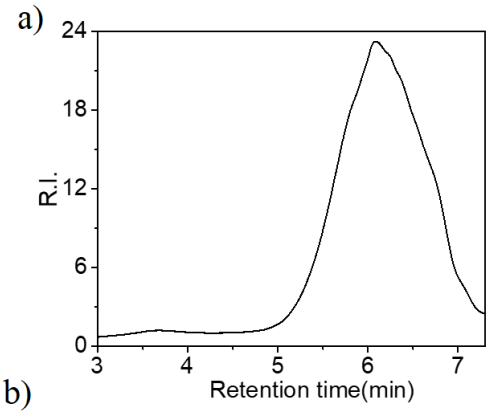


Figure S1: ^{1}H-NMR spectra of **P1** in DMSO-d_6 (* indicates peaks from residual solvent).



b)

Polymer	Mol wt. (GPC)	Mol wt. (Theo)	Mol wt. (NMR)	Đ
PU	9000	10000	9200	1.17

Figure S2: a) Size exclusion chromatogram (SEC) of **P1** in DMF; b) Table depicts the theoretical and estimated molecular weight (NMR, GPC) of **P1** and its polydispersity index.

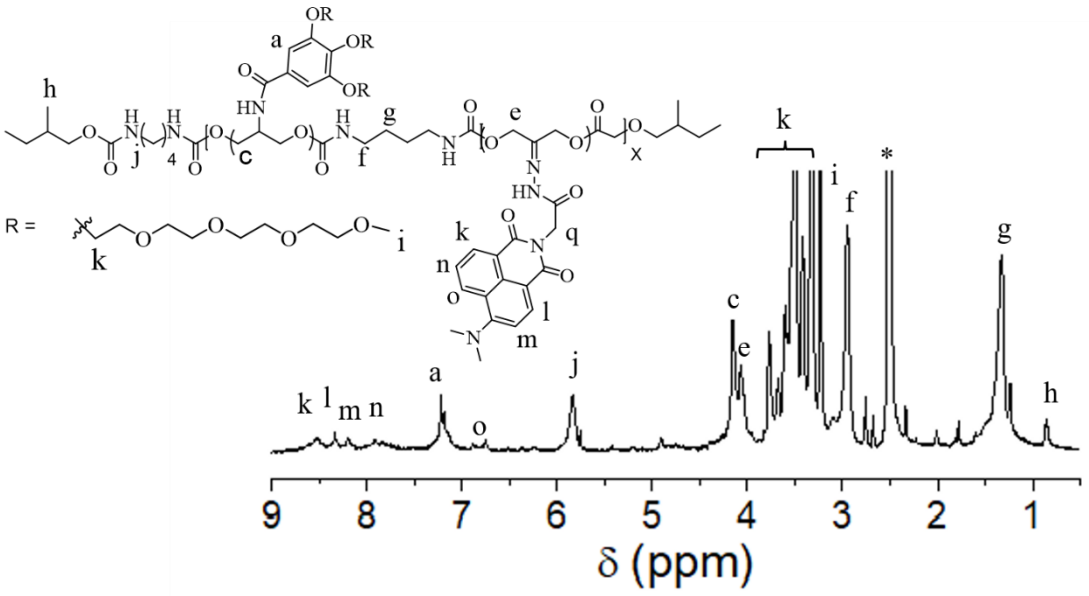


Figure S3: ^{1}H-NMR spectra of **P1O** in DMSO-d_6 (* indicates peaks from residual solvent).

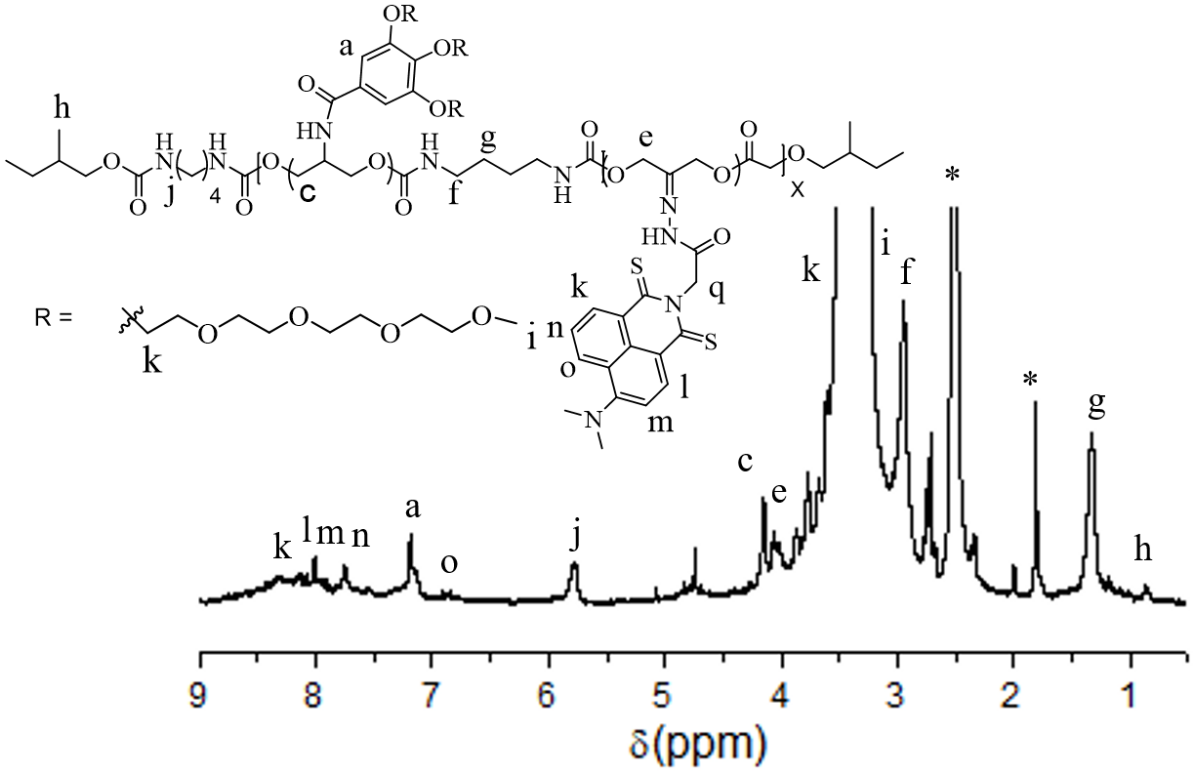


Figure S4: ^{1}H-NMR spectra of **P1S** in DMSO-d_6 (* indicates peaks from residual solvent).

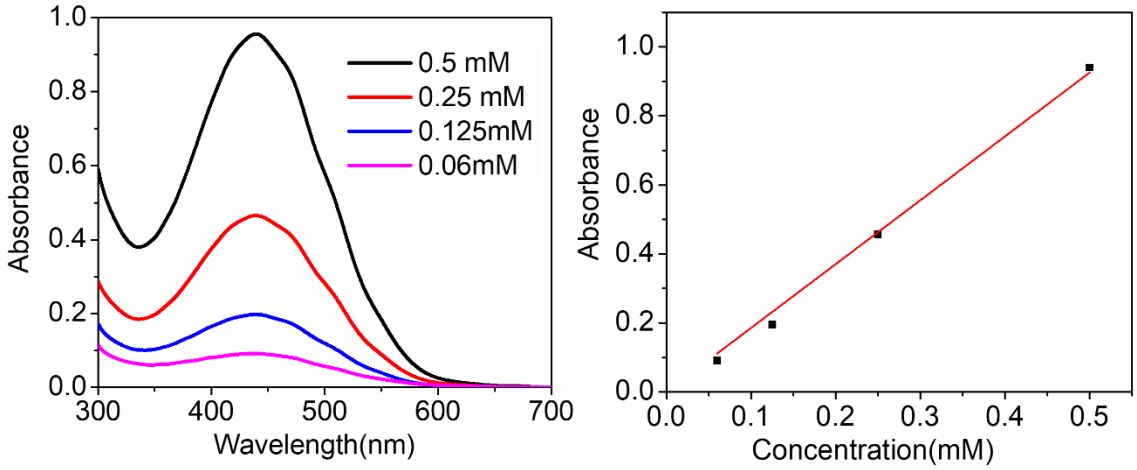


Figure S5: Left- Concentration dependent UV/Vis spectra of hNMIS in THF and right- plot of absorbance (450 nm) vs. concentration. From these data, molar extinction coefficient of hNMIS was estimated to be 1852 $M^{-1}cm^{-1}$.

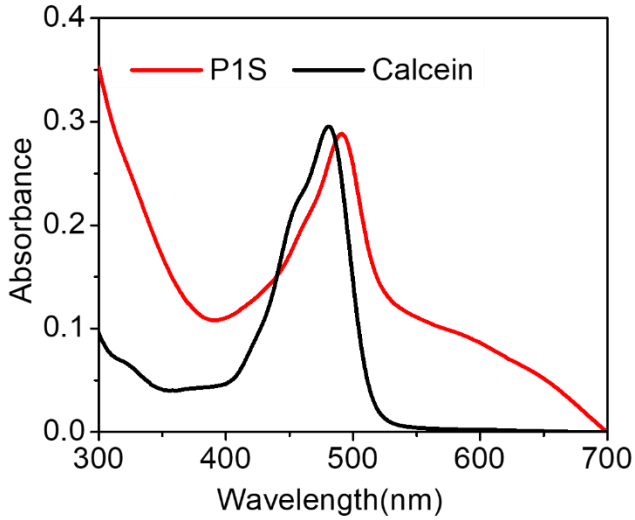


Figure S6: UV/ Vis absorption spectra of calcein encapsulated in P1S (*c* = 1.0 mg/ mL) and free calcein (c = 0.015 mM) .

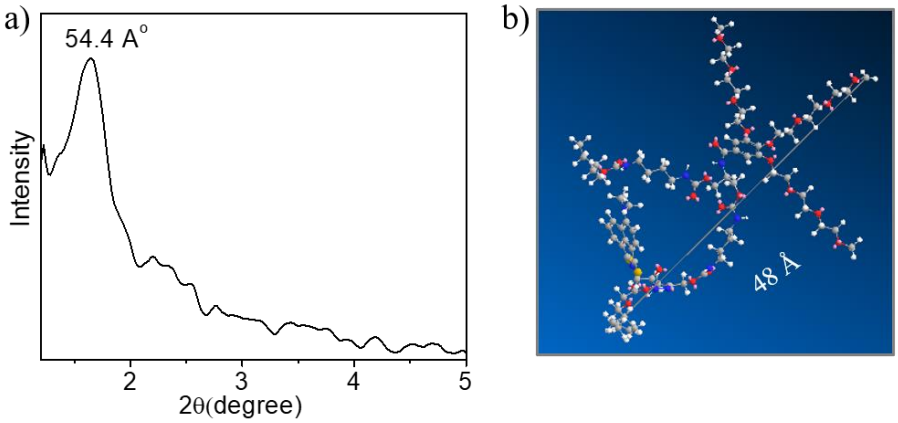


Figure S7: a) X-ray diffraction pattern of the dried film produced from aqueous solution of P1S (*c* = 1.0 mg/ mL); b) Energy minimized structure of a repeating unit of P1S by molecular modelling done in Chem 3D 18.0 using MM2 for energy minimization.

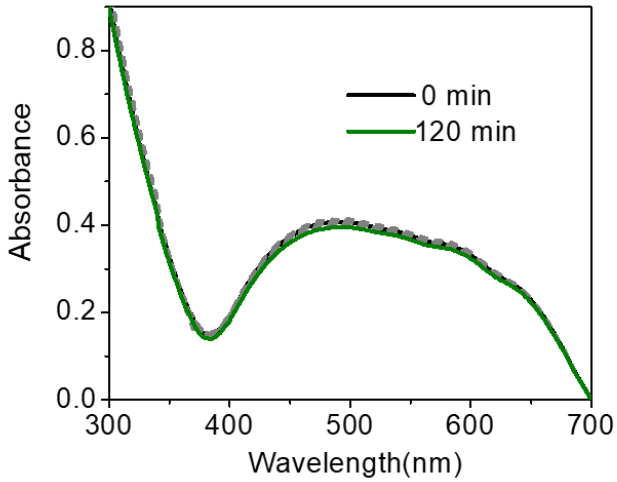


Figure S8: Time dependent absorbance spectra of P1S treated at pH=7 buffer.

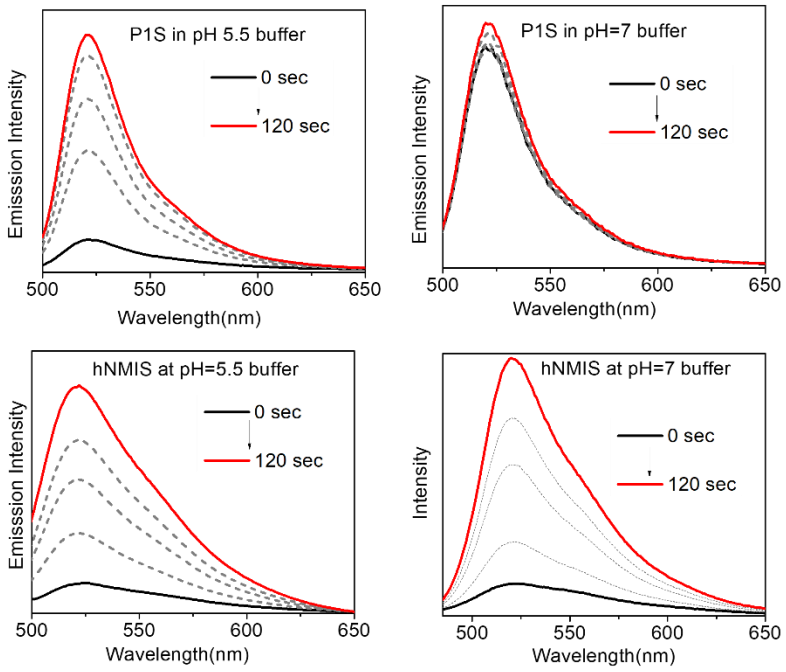


Figure S9: Time dependent emission spectra of in situ generated DCF in DCFH assay of aqueous solution of P1S and hNMIS in pH=5.5 and pH=7 buffer.

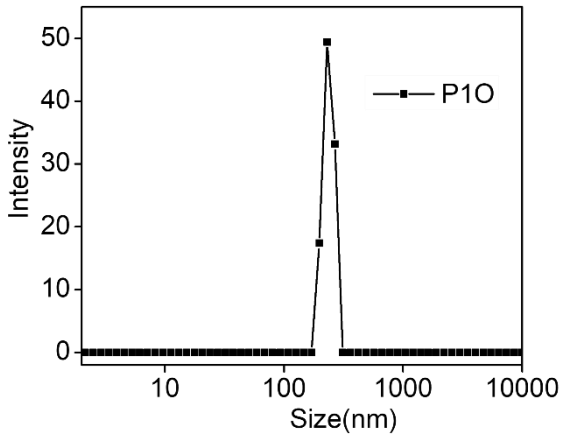


Figure S10: DLS of P1O (concentration = 0.1 mg/ml) in water.

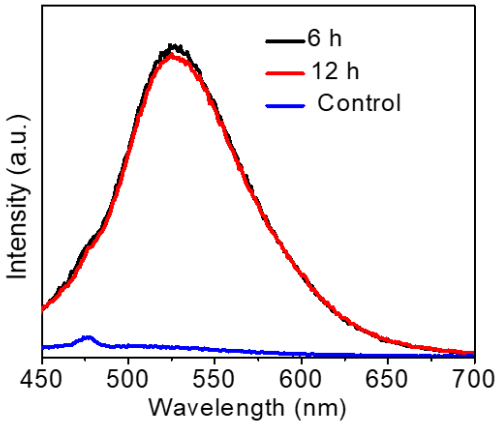


Figure S11: Fluorescence spectra (λ_{ex} = 420 nm) of cell lysates treated with P1O at different time point. Experiment was done with HeLa cells.

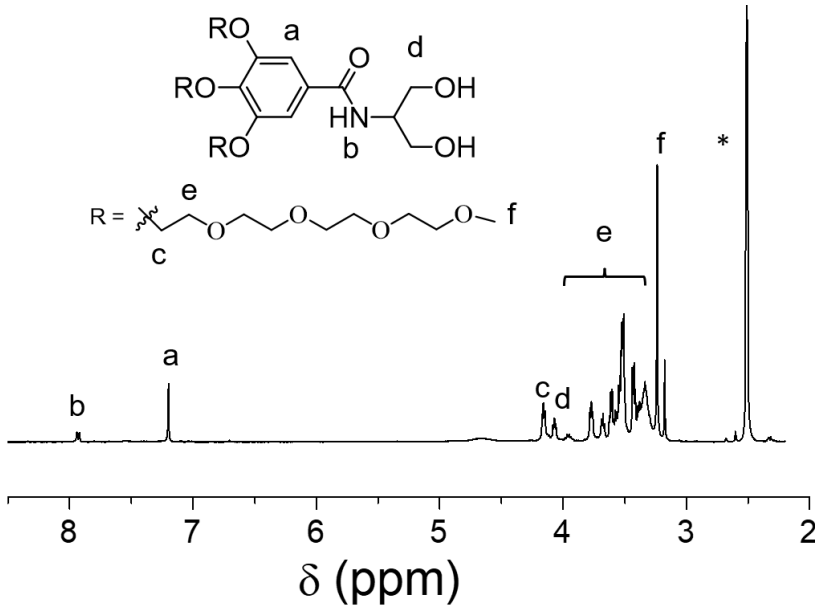


Figure S12: ^{1}H-NMR spectra of M1 in DMSO (* indicates peaks from residual solvent).

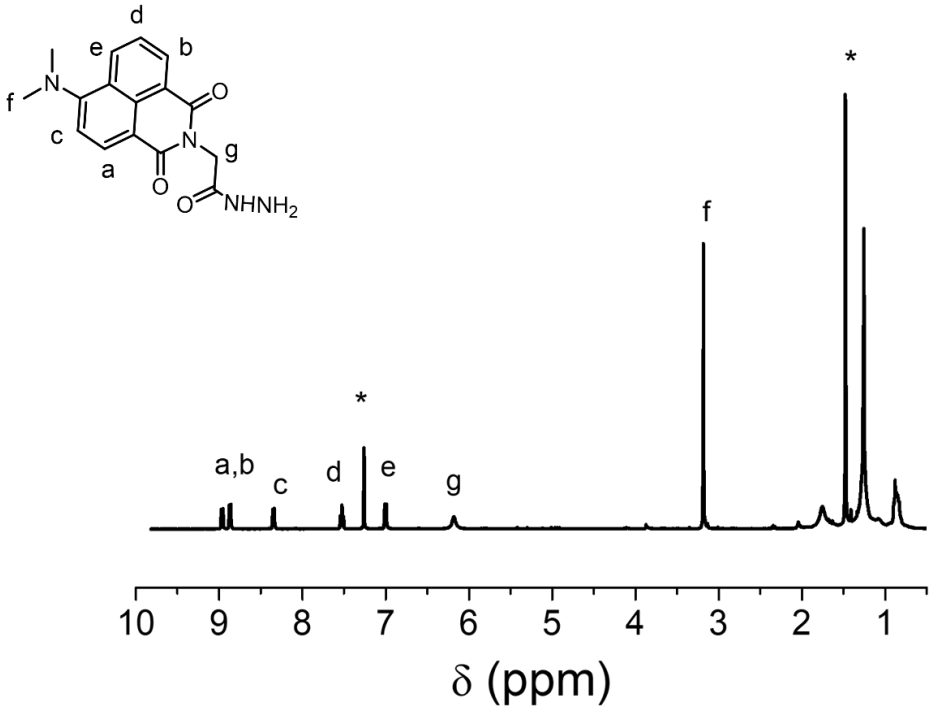


Figure S13: ^{1}H-NMR spectra of hNMIO in $CDCl_3$ (* indicates peaks from residual solvent)

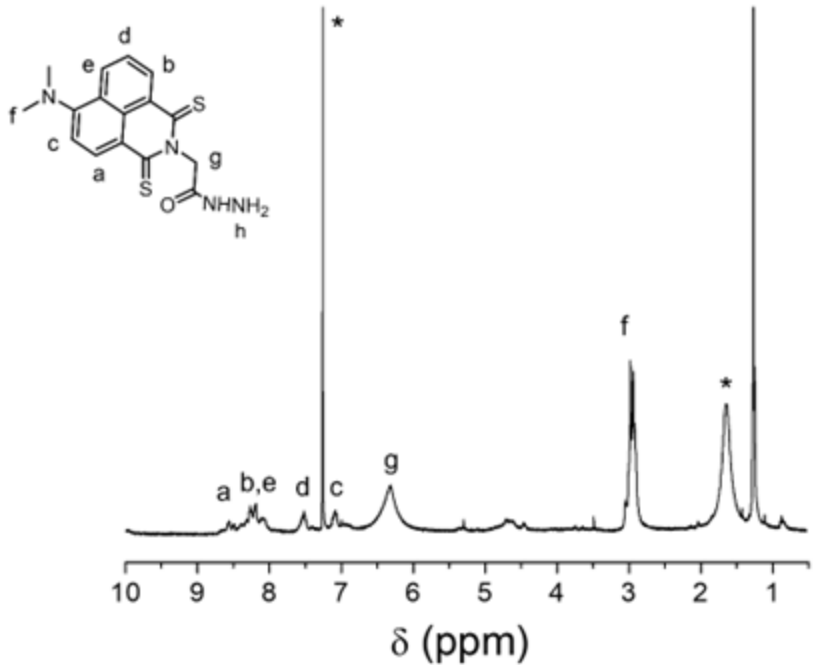


Figure S14: ^{1}H-NMR spectra of hNMIS in $CDCl_3$ (* indicates peaks from residual solvent)

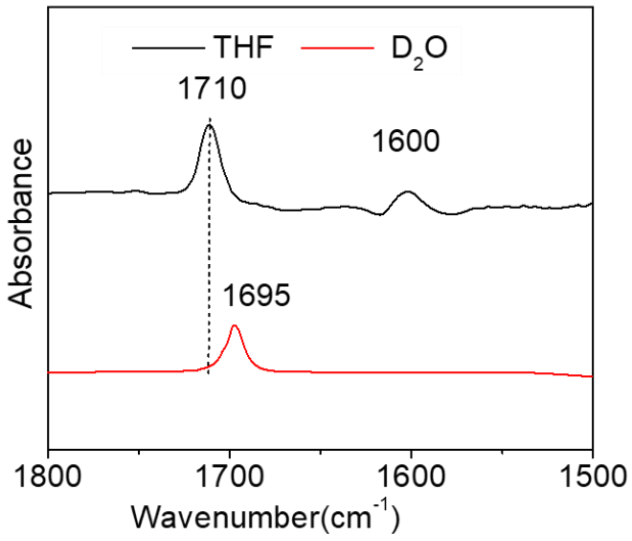


Figure S15: FTIR spectra of P1S in THF and D_2O (Concentration = 1mg/mL).

Reference

(1) Armarego, W. L. F. Purification of Laboratory Chemicals. *Purif. Lab. Chem.* **2017**, 1–1176.