Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information for:

Synthetic Mimics of Cyclic Antimicrobial Peptides via Templated Ring-Opening Metathesis (TROM)

Zhe Zhou, Cansu Ergene, and Edmund F. Palermo*

Materials Science and Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180

*Email: palere@rpi.edu

General Experimental:

Reactants and reagents including 3-thiophene ethanol, 3-methyl thiophene, 3-hexyl thiophene, N-bromo succinimide (NBS), tert-butyldimethylsilyl chloride, imidazole, diisopropylamine, tetrakis triphenylphosphine palladium, cyclooctadiene, exo-3 norbornyl carboxylic acid, diisopropyl carbodiimide, and 4-dimethyl aminopyridine were purchased from Sigma-Aldrich as ACS Reagent Plus grade and used without further purification, except where noted otherwise. Tetra-n-butylammonium fluoride (TBAF) (1.0 M solution in THF), tributyltin chloride and n-butyllithium (2.5M in hexanes) were purchased from Sigma-Aldrich and stored in a glove box under N_2 atmosphere. Organic solvents: tetrahydrofuran (THF), dichloromethane (DCM), N,N-dimethylformamide (DMF), ethyl acetate, hexane, methanol (MeOH), and Toluene were obtained from Sigma-Aldrich (USA). Bio Beads SX-1 was purchesd from Bio-Rad (USA). Deionized water was purified using EDM Millipore purification system. Flash-column chromatography was employed using silica gel (60 Å pore size, 40-63 μm technical grade, Sigma-Aldrich). Thin-layer chromatography was performed on IB2-F J.T. Baker silica gel TLC (Germany). All reactions were carried out under inert gas atmosphere under strictly anhydrous conditions, except where noted otherwise. Standard Schlenk line techniques using an inert gas/vacuum double manifold were employed for all reagent manipulations. Sheep red blood cells were purchased from MP Biomedicals. MH broth was purchased from VWR. The LIVE/DEAD confocal staining kit was purchased from ThermoFisher.

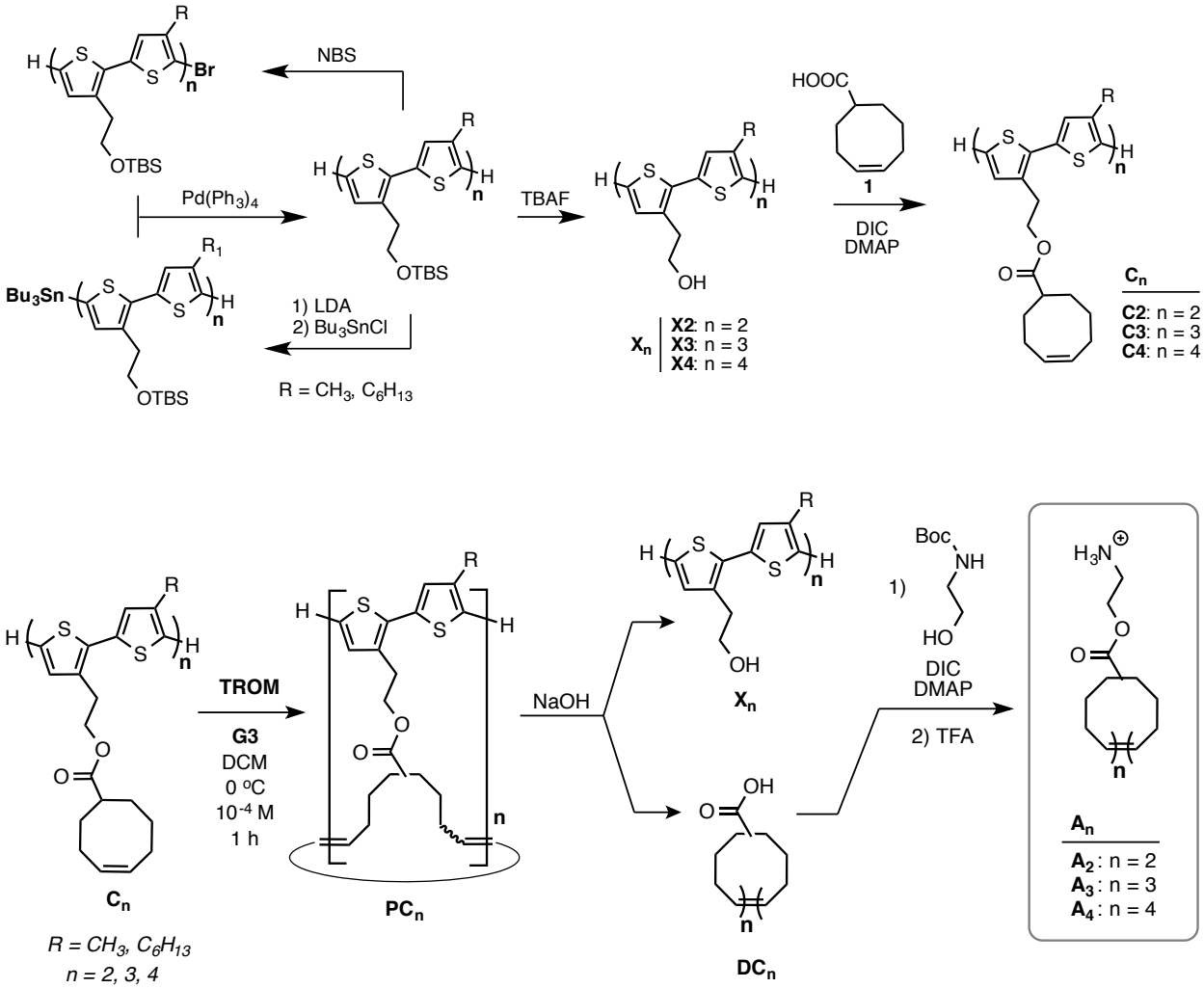
Instrumentation:

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using 500 MHz Agilent NMR spectrometer at 25 °C. NMR chemical shifts were reported in parts per million (ppm, δ) and referenced to tetramethylsilane ((CH3)4Si, 0.00 ppm) or to residual solvent signals (CDCl3 (δ 7.26)), (DMSO-d6 (δ 2.50)). Data are expressed as chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances) and integration. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded using 600 MHz Agilent NMR spectrometer at 25 °C. NMR chemical shifts were reported in parts per million (ppm, δ) and referenced to residual solvent signals (CDCl3 (δ 77.0), (DMSO-d6 (δ 39.5).

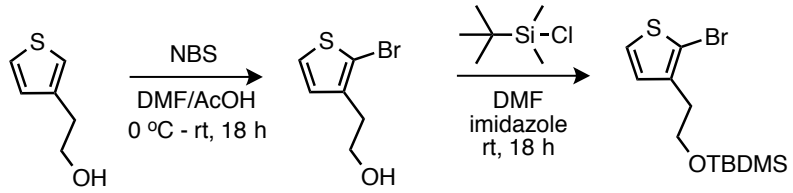
Mass spectrometric measurements were performed by the mass spectrometry service of the laboratory in the Center for Biotechnology and Interdisciplinary Studies (CBIS) on a Bruker Ultraflex III MALDI-TOF equipped with a nitrogen smart beam laser (λ = 337 nm, 150 µJ, 3 ns) at a pulse rate of 50 to 100 Hz using α-cyano-4-hydroxycinnamic acid (CHCA) as matrix. Ions were accelerated with pulsed ion extraction (PIE) by a voltage of 25 kV. The analyzer was operated in reflection mode and the ions were detected using a micro channel plate detector.

High-resolution mass measurements were performed on Thermo LTQ Orbitrap XL instrument at resolution 30,000 (at m/z 400) and mass accuracy better than 3 ppm. Samples were injected in the ESI source using Agilent 1200 HPLC system in methanol as a mobile phase at flow rate 50 ul/min.

Synthetic Overview[1]

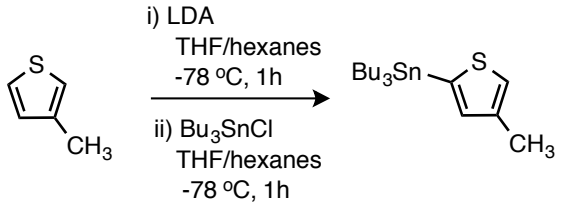


Detailed Synthetic Procedures



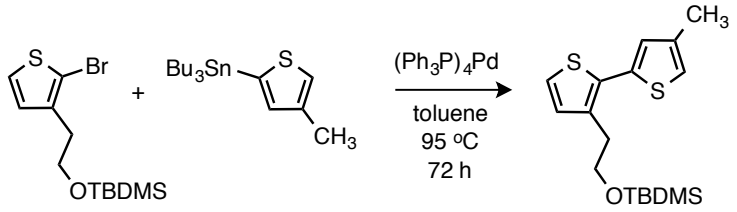
2-(2-bromothiophen-3-yl)ethan-1-ol. At 0 °C, recrystallized NBS(1.57g, 8.9mmol) was added to a solution of 2-(thiophen-3-yl)ethan-1-ol (1ml, 8.9mmol) in dry THF (20ml) under nitrogen atmosphere. The solution was allowed to warm to room temperature overnight. THF was removed under reduced pressure and mixture was dissolved in EtOAc (20ml), followed by washing with DI water (3×20ml) brine (3×20ml), drying over anhydrous sodium sulfate. After filtration, solvent was removed under high vacuum, the crude oil was purified by silica gel chromatography (Hex: EtOAc, 9:1) to give the product (1.18g, 64%). ^{1}H NMR (500 MHz, CDCl3): δ = 7.24 (d, 1H), 6.87 (d, 1H), 3.84 (t, 2H), 2.86 (t, 2H)

(2-(2-bromothiophen-3-yl)ethoxy)(tert-butyl)dimethylsilane (1). Imidazole (0.86g, 12.6 mmol) and tert-butyldimethyl silyl chloride (0.92g, 6.08mmol) were dissolved in dry DMF and 2-(2-bromothiophen-3-yl)ethan-1-ol (1.05g, 5.07mmol) was added in a single portion. The reaction was stirred at 35 °C overnight. The mixture was quenched with saturated aq. Sodium carbonate and extracted with EtOAc (3×20ml) followed by washing with DI water(3 ×20 ml) and brine(3 ×20 ml), drying over sodium sulfate. After filtration, solvent was removed under high vacuum, the crude oil was purified by silica gel chromatography (Hex) to give the product (0.97g, 60%). ^{1}H NMR (500 MHz, CDCl3): δ = 7.18 (d, 1H), 6.85 (d, 1H), 3.77 (t, 2H), 2.80 (t, 2H), 0.87(s, 9H), 0.00(s, 6H).

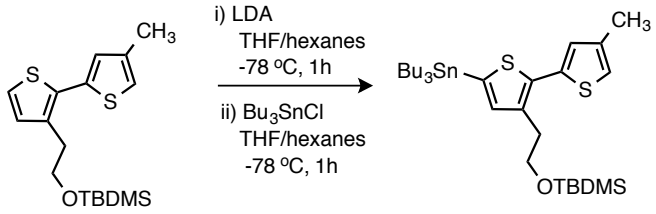


tributyl(4-methyl thiophen-2-yl) stannane (2). Lithium diisopropyl amide (LDA) was prepared by first mixing diisopropyl amine (7.15 mL, 51 mmol) with hexane (50 mL) and THF (50 mL) in a 250 mL oven-dried Schlenk flask, cooling to -78 °C, and injecting *n*-BuLi (2.5M solution, 20 mL, 50 mmol). Then, 3-methylthiophene (5 mL, 51 mmol) in THF (100 mL) was added dropwise via cannula transfer. After 30 min, tributyltin chloride (13.8 mL, 51 mmol) was injected via syringe and the reaction mixture was stirred for 1 h at -78 °C. Upon warming to rt overnight, the reaction was quenched with saturated aq. sodium carbonate, and the crude reaction mixture was extracted with 3×50mL EtOAc,

followed by washing with DI water and brine, drying over magnesium sulfate, and evaporation under reduced pressure. The crude brown oil was purified by vacuum distillation (0.1 torr, 73 °C) to yield the pure product as a clear, colorless oil (15.3 g, 78 %).

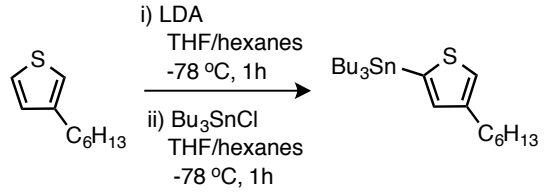


Stille Coupling, bithiophene parent "monomer" (3). In a 200 mL storage flask with a Teflon valve seal, **1** (1 g, 3.1 mmol) and **2** (1.33 g, 3.4 mmol) were dissolved in toluene (50 mL) and subjected to 3× freeze/pump/thaw/cycles. $Pd(Ph_3P)_4$ (36 mg, 0.031 mmol) as a slurry in toluene (1mL) was added and subjected to another 3× freeze/pump/thaw/cycles. The reaction vessel was sealed under vacuum with a Teflon valve, and then heated to 95 °C for 24-72 h, or until the appearance of a black precipitate. The reaction mixture was cooled to rt, diluted with 50mL THF, and stirred with 1M NaOH aq (100 mL) for 1 hr. The organic layer was washed with water and brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (hexane:toluene, 9:1, R_f = 0.35) gave the title cmpd as a colorless oil (477 mg, 45%).

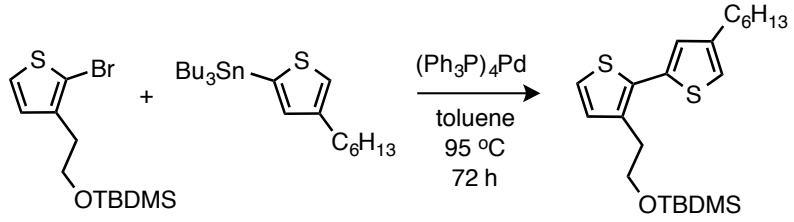


Stannylation of bithiophene parent "monomer" (4). Same procedure as for **2**. Amounts: diisopropyl amine (277 µL, 2 mmol), *n*-BuLi (784 µL, 1.95 mmol), bithiophene **3** (670 mg, 2 mmol), Bu_3SnCl (537 µL, 2 mmol). No purification required. Yield 1.03 g, 83%.

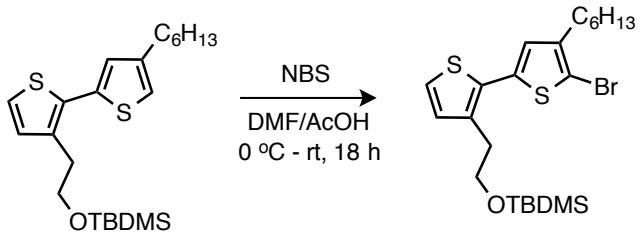
For R_1 = hexyl side chain:



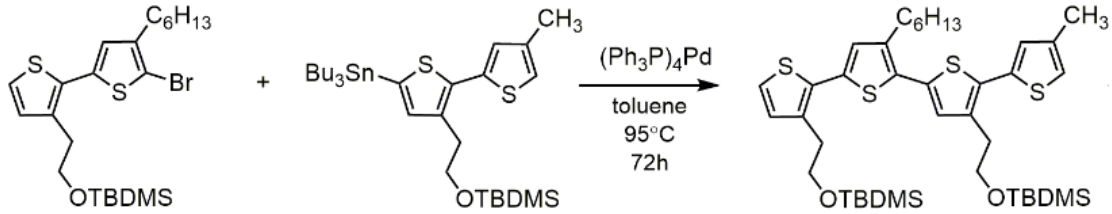
tributyl(4-hexylthiophen-2-yl)stannane. Lithium diisopropyl amide (LDA) was prepared by mixing diisopropyl amine (0.78ml, 5.56mmol) with hexane (10ml) and THF (10ml) in a 100ml oven-dried Schlenk flask, cooling to -78 °C, and injecting n-BuLi (2.5M solution, 2.2ml, 5.50mmol). Then, 3-hexylthiophene (1ml, 5.56mmol) in THF (20ml) was added dropwise via cannula transfer. After 30 minutes, tributyltin chloride (1.66ml,6.11mmol) was injected via syringe and the reaction mixture was stirred for 1 hour at -78 °C. Upon warming up to room temperature overnight, the reaction was quenched with saturated aq. Sodium carbonate, and the crude reaction mixture was extracted with EtOAc (3×20ml), followed by washing with DI water(3 ×20 ml) and brine(3 ×20 ml), drying over sodium sulfate. After filtration, solvent was removed under high vacuum, the crude oil was purified by vacuum distillation (0.16mmHg, 120°C) to yield pure pdt (2.03g, 77%).^{1}H NMR (500 MHz, CDCl3): δ = 7.19 (d, 1H), 6.96 (s, 1H), 2.65 (t, 2H), 1.63-1.51(m, 8H),1.34-1.28(m, 12H), 1.11-1.05(m, 6H), 0.91-0.86(m, 12H).



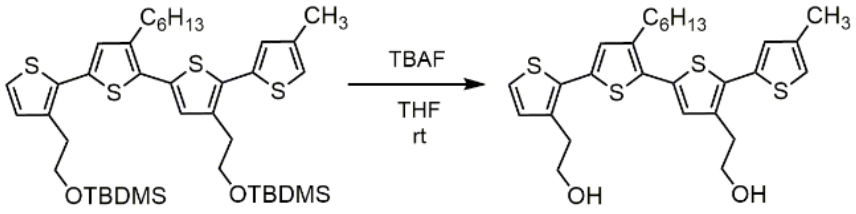
$(3HT)_2$-1OTBDMS. In a 200ml storage flask with a Teflon valve seal, (2-(2-bromothiophen-3-yl)ethoxy)(tert-butyl)dimethylsilane (1.4 g, 4.3 mmol) and tributyl(4-hexylthiophen-2-yl)stannane (2.18 g, 4.76 mmol) were dissolved in dry toluene (70ml) and subjected to 3× freeze/pump/thaw/cycles. $Pd(Ph_3P)_4$ (50.4mg, 0.043mmol) as a slurry in toluene (2ml) was added and subjected to another 3×freeze/pump/thaw/ cycles. The reaction vessel was sealed under vacuum with a Teflon valve, and then heated to 90 °C for 72 hours, or until the appearance of a black precipitate. The reaction mixture was cooled to room temperature. The organic layer was washed with water (3×50ml) and brine (3×50ml), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (Hex: Toluene, 9:1) gave pure product (1.19mg, 82%). ^{1}H NMR (500 MHz, CDCl3): δ = 7.15 (d, 1H), 6.98 (d, 1H), 6.95 (s, 1H), 6.88(s, 1H), 3.83(t, 2H), 2.98(t, 2H), 2.60(t, 2H), 1.60(m,2H) 1.33-1.31(m, 6H), 0.91-0.86(m, 12H), 0.00(s, 6H)



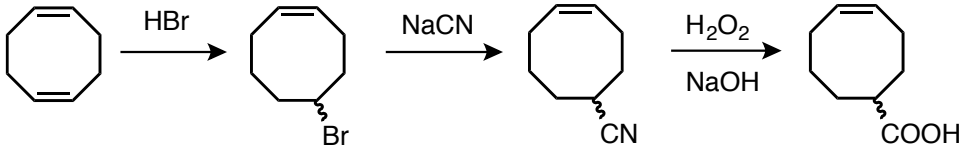
$(3HT)_2$-Br-1OTBDMS. (5) At 0 °C, recrystallized NBS(252mg, 1.40mmol) was added to a solution of $(3HT)_2$-1OTBDMS (585mg, 1.42mmol) in dry THF (7ml) under nitrogen atmosphere. The solution was allowed to warm to room temperature overnight. THF was removed under reduced pressure and mixture was dissolved in EtOAc (20 ml), followed by washing with DI water (3×20ml) brine (3×20ml), drying over anhydrous sodium sulfate. After filtration, solvent was removed under high vacuum, the crude oil was purified by silica gel chromatography (Hex: Toluene, 4:1) to give the product (484mg, 89%). ^{1}H NMR (500 MHz, CDCl3): δ = 7.14 (d, 1H), 6.93 (d, 1H), 6.81(s, 1H), 3.80(t, 2H), 2.91(t, 2H), 2.52(t, 2H), 1.57(m, 2H) 1.31-1.29(m, 6H), 0.87-0.83(m, 12H), 0.00(s, 6H)



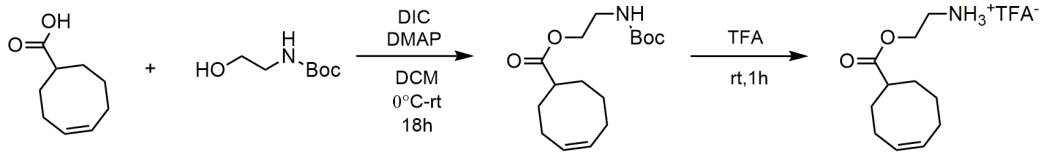
Stille Coupling, thiophene tetramer H/H (6). Same general procedure as for **3**. Amounts: **5** (50mg, 0.10mmol) and **4** (70mg, 0.11 mmol) in toluene (5 mL), $Pd(Ph_3P)_4$ (13 mg, 0.011 mmol). Purification on silica gel (hexane:toluene, 9:1) to give the pure product as a bright yellow oil (61.5mg, 81 %).^{1}H NMR (500 MHz, CDCl3): δ = 7.17 (d, 1H), 7.00-6.97 (m, 4H), 6.89 (s, 1H), 3.88-3.84(m, 4H), 3.04-2.97(m, 4H), 2.78-2.75(t, 2H), 2.62(t,2 H), 2.28(s, 3H), 1.70-1.65(m, 4H), 1.34-1.31(m, 2H), 0.91-0.86(m,21H), 0.00(m,12H)



Deprotection of Side Chains. The protected template tetrathiophene (55 mg, 0.07 mmol) was dissolved in tetrabutylammonium fluoride (TBAF, 1M in THF, 2.5 mL 2.44 mmol) and stirred overnight at room temperature. Purified by silica gel column chromatography. ^{1}H NMR (500 MHz, CDCl3): δ = 7.15 (d, 1H), 7.01-6.98 (m, 4H), 6.89 (s, 1H), 3.88-3.85(m, 4H), 3.05-3.01(m, 4H), 2.75(t, 2H), 2.60(t,2 H), 2.21(s, 3H), 1.67-1.63(m, 4H), 1.40-1.37(m, 5H).

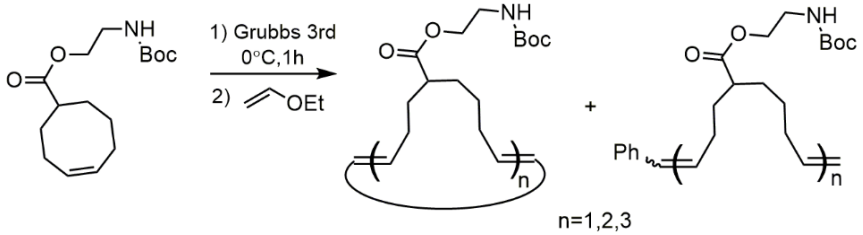


Z*-cyclooct-4-ene carboxylic acid.** Cyclooctadiene (COD, 25 mL, 0.2 mol) and 33% HBr(aq) (37 mL) were mixed in a r.b. flask at room temperature overnight. The mixture was extracted with EtOAc and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a dark brown, ***malodorous oil. The crude was distilled (40 °C, 0.4 torr) to give an odourless and colorless oil (27.5 g). Then, the brominated intermediate was dissolved in DMSO (100mL), sodium cyanide was added, and the mixture was heated at 110 °C for 2 h in a r.b. flask equipped with a reflux condenser. The product was extracted with EtOAc (3×500 mL) washed with DI water (3×500 mL) and brine (500 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a dark brown oil, that was distilled (50 °C, 0.1 torr) to give a colorless oil. This intermediate (~10g) was mixed with 33 mL of aq. KOH (30% w/v) and 2.1 mL of H_2O_2 and heated to 120 °C for 48 h. Organics were extracted with EtOAc (3×200 mL) and the organic layer was set aside. The aqueous phase (containing the conjugate base of the product) was acidified with 40% phosphoric acid to pH 4 and extracted with EtOAc (3×200 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a brown oil. Purification by distillation (120 °C, 0.15 torr) gave the pure product as a clear, colorless oil that slowly solidifies to a waxy consistency upon standing at 4 °C for a few days. Yield 12.7 g (41 %).

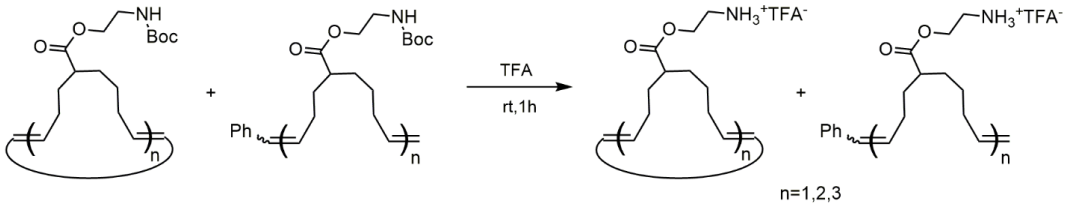


DC1-$NH_3^+TFA^-$ (A1). In a 10 mL oven-dried Schlenk flask, the cyclooctene monomer with carboxylic acid group (30 mg, 0.19 mmol) and N-(tert-Butoxycarbonyl)ethanolamine (47mg, 0.29 mmol) were dissolved in anhydrous DCM (4 mL) under nitrogen and DMAP (3 mg, 0.02 mmol) was added in one portion. The solution was cooled to 0 °C and stirred for 10 min before injection of neat DIC (60 µL, 0.38 mmol). The reaction mixture was allowed to warm to rt and stirred overnight. The organic layer was concentrated under reduced pressure. The crude product was purified by silica gel column (Hexane: Ethyl acetate= 4:1) to yield the pure product (55mg, 84%). 0.2ml TFA solution was added to the product and stirred for 1 hour. Then TFA was evaporated and the residue was washed with diethyl ether (3×5ml) to yield pure product (30mg, 91%).^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.97 (m, 3H), 5.64-5.58 (m, 2H), 4.12 (t, 2H), 3.06(t, 2H), 2.11-

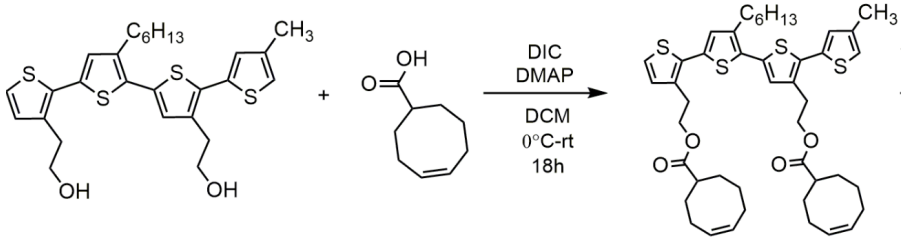
1.06(m, 11H). ^{13}C-NMR (600MHz, DMSO-d_6): δ = 176.90, 130.74, 129.98, 61.07, 42.82, 38.30, 31.49, 29.22, 27.76, 25.81, 24.04. HRMS (ESI, m/z): [M= $C_{11}H_{20}NO_2^+$] Calc. 198.1489, Found 198.1494.



Disperse poly(Boc-A1): NBoc-cyclooctene (12mg, 0.040mmol) was dissolved in anhydrous DCM (260ml) under a nitrogen flow in an oven-dried 500ml Schlenk flask, and cooled to 0°C on ice water bath. Then, the Grubbs 3rd generation catalyst (36mg, 0.022mmol) dissolved in DCM (6ml) was injected via syringe, to give an initial template concentration of 1.5×10^{-4} M. After 60 minutes, ethyl vinyl ether (0.1ml) was injected via syringe and the reaction was stirred for an additional 30 minutes before warming to room temperature. The solvent was evaporated under reduced pressure and the catalyst was removed by passing silica gel column (Hexene: Ethyl acetate 4:1) to yield pure product.

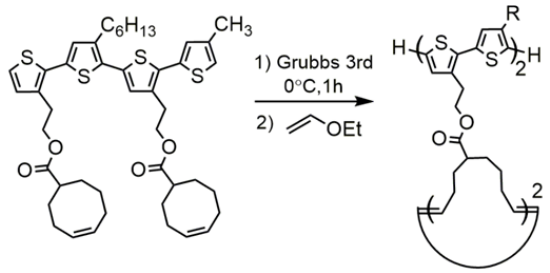


Disperse poly(A1) : 0.2ml TFA solution was added to the product and stirred for 1 hour. Then TFA was evaporated and the residue was washed with diethyl ether (3×5ml) to yield pure product.

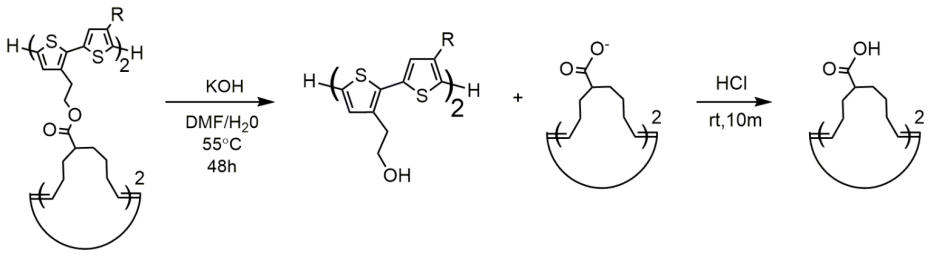


$(3HT)_4$-2cyclooctene: In a 4ml oven-dried vial, the Z-cyclooct-4-ene carboxylic acid (24mg, 0.15mmol) and $(3HT)_4$-2OH (34mg,0.066mmol) were dissolved in dry THF (2ml) under nitrogen and DMAP (1mg, 0.0084mmol) was added in one portion. The solution was cooled to 0°C and stirred 30 minutes before injection of neat DIC (0.018ml, 0.1mmol). The reaction was allowed to warm to room temperature and stirred overnight.

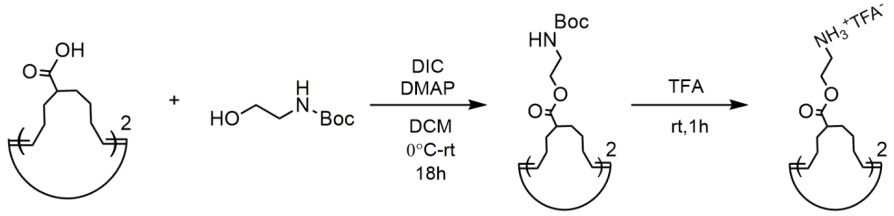
The solution was diluted with EtOAc (10ml) and washed with DI water (3×10ml) and brine (3×10ml). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by SX1 biobeads column (Toluene) to yield the pure product (39mg, 74%). ^{1}H NMR (500 MHz, CDCl3): δ = 7.21 (d, 1H), 6.99-6.92 (m, 5H), 5.69-5.63(m, 4H), 4.31-4.28(m, 4H), 3.12-3.09(m, 4H), 2.77(t, 2H), 2.86-2.79(m, 4H), 2.29(s, 3H), 2.15-2.12(m, 6H), 1.92-1.86(m, 4H), 1.69-1.56(m, 6H), 1.41-1.31(m, 8H), 0.92-0.87(m,5H).



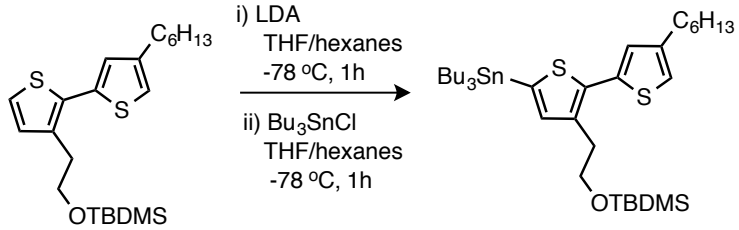
Optimized procedure for PC2 by T-ROM: $(3HT)_4$-2cyclooctene (18mg, 0.022mmol) was dissolved in anhydrous DCM (66ml) under a nitrogen flow in an oven-dried 200ml Schlenk flask, and cooled to 0 °C on ice water bath. Then, the Grubbs 3rd generation catalyst (20mg, 0.022mmol) dissolved in DCM (8ml) was injected via syringe, to give an initial template concentration of 3×10^{-4} M. After 60 minutes, ethyl vinyl ether (0.1ml) was injected via syringe and the reaction was stirred for an additional 30 minutes before warming to room temperature. The solvent was evaporated under reduced pressure and the residue was extracted with 1ml of Toluene. The catalyst was removed by passing SX1 biobeads column. NMR shows around 95% conversion and MALDI shows single peak (MW=789.024).



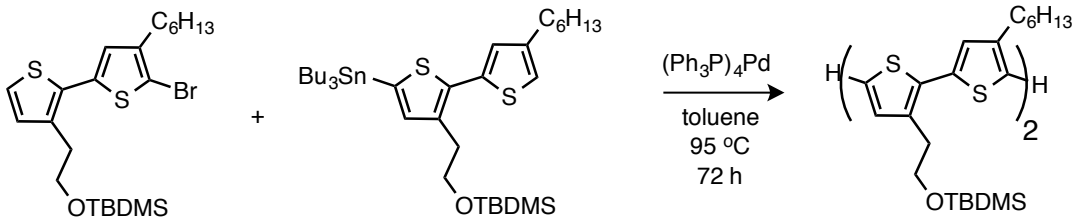
Hydrolysis of PC2 in basic solution: Potassium hydroxide (1M in H_2O, 0.5ml) was added into PC_2 (17mg, 0.021mmol) DMF solution (3.5ml) and stirred at 55 °C for 48 hours. The solvent was removed by high vacuum and the residue was subjected to 10ml 1M KOH and 10ml ethyl acetate. Collect the water phase and 1M HCl solution was added until PH>7, keep the solution for 10 minutes to fully protonate daughter oligomer (DC2). Then, the solution was diluted with ethyl acetate (10 mL) and washed with water (3x10mL). Organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The product was obtained (4.2mg, 63 %) and used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.38 (m, 4H), 2.40-2.35(m, 4H), 2.15-2.13(m, 18H).



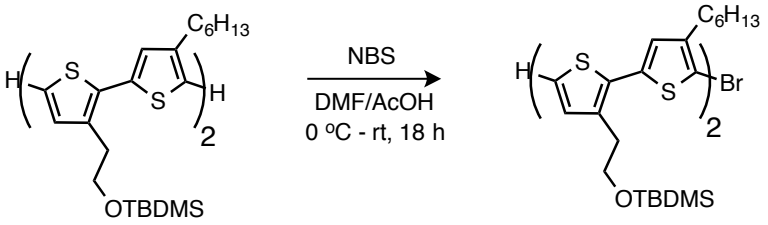
DC2-2NH$_3^+$TFA$^-$ (A2). In a 10 mL oven-dried Schlenk flask, the cyclic cyclooctene dimer with two carboxylic acid group (4.2 mg, 0.013 mmol) and N-(tert-Butoxycarbonyl)ethanolamine (8.7mg, 0.054 mmol) were dissolved in anhydrous DCM (1 mL) under nitrogen and DMAP (1.5 mg, 0.01 mmol) was added in one portion. The solution was cooled to 0 °C and stirred for 10 min before injection of neat DIC (12 µL, 0.1 mmol). The reaction mixture was allowed to warm to rt and stirred overnight. The organic layer was concentrated under reduced pressure. The crude product was purified by SX1 biobeads column (Toluene) to yield the pure product (5.3mg, 66%). 0.2ml TFA solution was added to the product and stirred for 1 hour. Then TFA was evaporated and the residue was washed with diethyl ether (3×5ml) to yield pure product (3.3mg, 72.7%).^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.88 (m, 6H), 5.26 (m, 4H), 4.08 (m, 4H), 3.01(m, 4H), 1.85(m, 4H), 1.52-1.04(m, 18H). ^{13}C-NMR (600MHz, DMSO-d_6): δ = 177.65, 130.65, 129.50, 63.46, 43.26, 31.65, 29.45, 28.39, 27.84, 25.92, 24.07. HRMS (ESI, m/z): [M−H$^+$ = $C_{22}H_{39}N_2O_4^+$] Calc. 395.2904, Found 395.2903



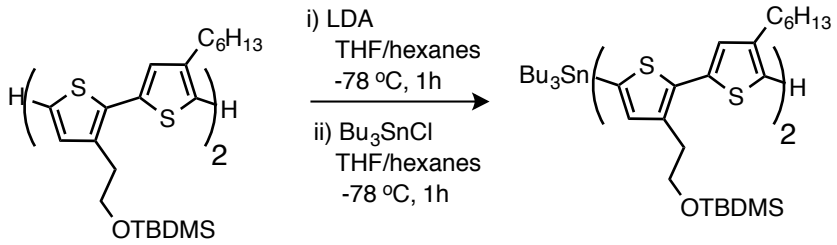
(3HT)$_2$-Bu$_3$Sn-1OTBDMS. Lithium diisopropyl amide (LDA) was prepared by mixing diisopropyl amine (0.25ml, 1.78mmol) with hexane (10ml) and THF (10ml) in a 100ml oven-dried Schlenk flask, cooling to -78 °C, and injecting n-BuLi (2.5M solution, 0.71ml, 1.78mmol). Then, (3HT)$_2$-1OTBDMS (728mg, 1.78mmol) in THF (10ml) was added dropwise via cannula transfer. After 1 hour, tributyltin chloride (0.483ml,1.78mmol) was injected via syringe and the reaction mixture was stirred for 1 hour at -78 °C. Upon warming up to room temperature overnight, the reaction was quenched with saturated aq. Sodium carbonate, and the crude reaction mixture was extracted with EtOAc (3×20ml), followed by washing with DI water(3 ×20 ml) and brine(3 ×20 ml), drying over sodium sulfate. Solvent was removed over high vacuum. The product was obtained (822.5mg, 70 %) and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.96 (s, 1H), 6.93 (s, 1H), 6.82 (s, 1H), 3.82(t, 2H), 2.98(t, 2H), 2.55(t, 2H), 1.59-1.51(m, 8H), 1.33-1.28(m, 12H), 1.09-1.04(m, 6H), 0.90-0.83(m,18H), 0.00(s,6H)



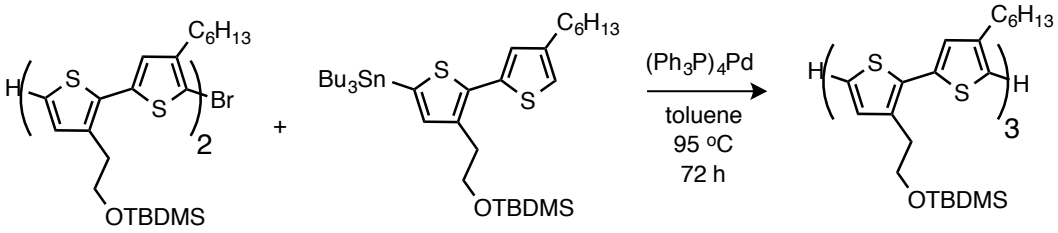
(3HT)$_4$-2OTBDMS: In a 200ml storage flask with a Teflon valve seal, (3HT)2-Br-1OTBDMS (522mg, 1.07mmol) and (3HT)$_2$-Bu3Sn-1OTBDMS (822mg, 1.17mmol) were dissolved in dry toluene (15ml) and subjected to 3 × freeze/pump/thaw/cycles. $Pd(Ph_3P)_4$ (124mg, 0.107mmol) as a slurry in toluene (1ml) was added and subjected to another 3×freeze/pump/thaw/ cycles. The reaction vessel was sealed under vacuum with a Teflon valve, and then heated to 90 ^oC for 72 hours, or until the appearance of a black precipitate. The reaction mixture was cooled to room temperature. The organic layer was washed with water (3×30ml) and brine (3×30ml), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (Hex: Toluene, 4:1) gave pure product (820mg, 94%). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.16 (d, 1H), 7.02-6.98 (m, 4H), 6.90 (s, 1H), 3.89-3.85(m, 4H), 3.05-2.99(m, 4H), 2.77(t, 2H), 2.62(t,2 H), 1.67-1.63(m, 4H), 1.37-1.33(m, 12H), 0.91-0.86(m,24H), 0.00(m,12H)



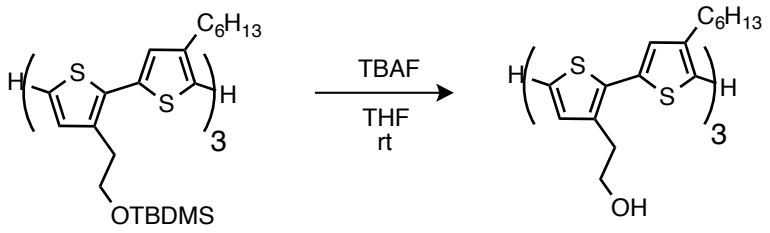
(3HT)$_4$-Br-2OTBDMS: At 0 ^oC, recrystallized NBS(140mg, 0.78mmol) was added to a solution of (3HT)2-1OTBDMS (325mg, 0.79mmol) in dry THF (4ml) under nitrogen atmosphere. The solution was allowed to warm to room temperature overnight. THF was removed under reduced pressure and mixture was dissolved in EtOAc (20ml), followed by washing with DI water (3×20ml) brine (3×20ml), drying over anhydrous sodium sulfate. After filtration, solvent was removed under high vacuum, the crude oil was purified by silica gel chromatography (Hex: Toluene, 4:1) to give the product (272mg, 70%). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.16 (d, 1H), 7.02-6.98 (t, 3H), 6.88 (s, 1H), 3.88-3.85(m, 4H), 3.02(t, 2H), 2.75(t, 2H), 2.56(t,2 H), 1.72-1.58(m, 4H), 1.43-1.25(m, 12H), 0.91-0.87(m,24H), 0.00(m,12H)



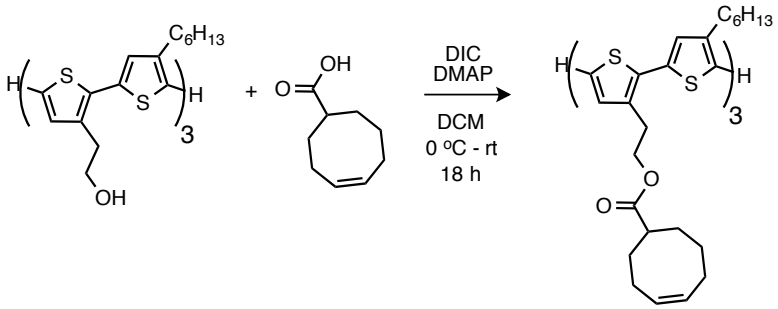
$(3HT)_4$-Bu_3Sn-2OTBDMS: Lithium diisopropyl amide (LDA) was prepared by mixing diisopropyl amine (0.074ml, 0.529mmol) with hexane (10ml) and THF (10ml) in a 100ml oven-dried Schlenk flask, cooling to -78 °C, and injecting n-BuLi (2.5M solution, 0.21ml, 0.529mmol). Then, $(3HT)_4$-2OTBDMS (432mg, 0.529mmol) in THF (10ml) was added dropwise via cannula transfer. After 1 hour, tributyltin chloride (0.143ml,0.529mmol) was injected via syringe and the reaction mixture was stirred for 1 hour at -78 °C. Upon warming up to room temperature overnight, the reaction was quenched with saturated aq. Sodium carbonate, and the crude reaction mixture was extracted with EtOAc (3×20ml), followed by washing with DI water(3 ×20 ml) and brine(3 ×20 ml), drying over sodium sulfate. Solvent was removed over high vacuum. The product was obtained (313mg, 55%) and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ =7.01-6.97 (t, 4H), 6.87 (s, 1H), 3.88-3.82(m, 4H), 3.08(t, 2H), 2.87(t, 2H), 2.76(t,2H), 2.60(t,2H) 1.70-1.57(m, 6H), 1.36-1.25(m, 25H), 1.18(m, 6H) 0.91-0.87(m,30H), 0.00(m,12H)



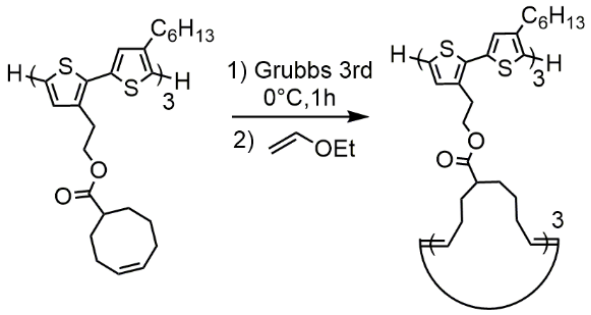
$(3HT)_6$-3OTBDMS: In a 50ml storage flask with a Teflon valve seal, $(3HT)_4$-Br-2OTBDMS (412mg, 0.46mmol) and $(3HT)_2$-Bu3Sn-1OTBDMS (378mg, 0.54mmol) were dissolved in dry toluene (15ml) and subjected to 3× freeze/pump/thaw/cycles. Pd(Ph3P)4 (53mg, 0.046mmol) as a slurry in toluene (1ml) was added and subjected to another 3×freeze/pump/thaw/ cycles. The reaction vessel was sealed under vacuum with a Teflon valve, and then heated to 90 °C for 72 hours, or until the appearance of a black precipitate. The reaction mixture was cooled to room temperature. The organic layer was washed with water (3×30ml) and brine (3×30ml), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (Hex: Toluene, 4:1) gave pure product (384mg, 68%).^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.15 (d, 1H), 7.02-6.99 (m, 5H), 6.88 (s, 1H), 3.91-3.84(m, 6H), 3.05-3.01(m, 6H), 2.82-2.77(m, 4H), 2.61(t,2 H), 1.69-1.63(m, 6H), 1.40-1.31(m, 21H), 0.90-0.88(m,33H), 0.03-0.00(m,18H)



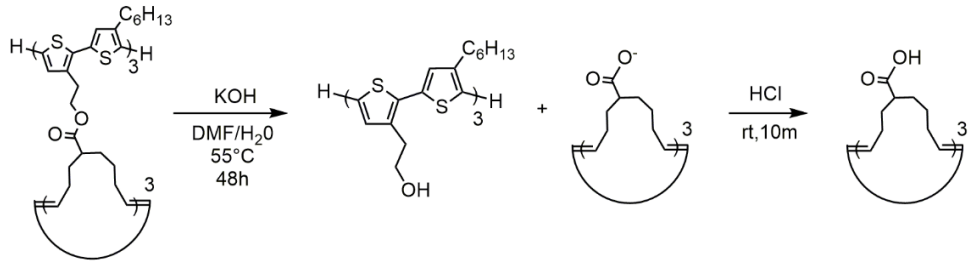
$(3HT)_6$-3OH: Tetreabutylammonium floride (1M in THF,0.4ml,0.39mmol) was added into $(3HT)_6$-3OTBDMS (96mg, 0.076mmol) THF solution (2ml) and stirred at room temperature overnight. The mixture was purified by SX1 biobeads column (THF) to yield pure product (62mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.15 (d, 1H), 7.00-6.97 (m, 6H), 6.88 (s, 1H), 3.98-3.87(m, 6H), 3.05-2.99(m, 6H), 2.79-2.76(m, 4H), 2.60(t,2 H), 1.68-1.64(m, 6H), 1.41-1.34(m, 21H), 0.88-0.83(m,6H)



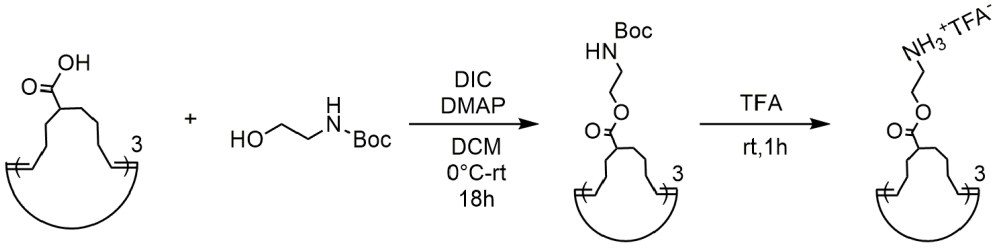
$(3HT)_6$-3cyclooctene: In a 4ml oven-dried vial, the Z-cyclooct-4-ene carboxylic acid (18.4mg, 0.12mmol) and $(3HT)_6$-3OH (30mg, 0.033mmol) were dissolved in dry THF (2ml) under nitrogen and DMAP (1mg, 0.0084mmol) was added in one portion. The solution was cooled to 0 °C and stirred 30 minutes before injection of neat DIC (0.022ml, 0.12mmol). The reaction was allowed to warm to room temperature and stirred overnight. The solution was diluted with EtOAc (20ml) and washed with DI water (3×20ml) and brine (3×20ml). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by SX1 biobeads column (THF) to yield the pure product (39mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.21 (d, 1H), 7.01-6.99 (m, 6H), 6.93 (s, 1H), 5.69-5.62(m,6H) 4.35-4.30(m, 6H), 3.15-3.11(m, 6H),2.81-2.78(m,4H), 2.64(t,2H),2.47(m, 3H), 2.37(m,3H), 2.15-2.11(m, 9H), 2.02-1.99(m,3H) 1.85-1.82(m, 3H), 1.72-1.55(m, 18H), 1.44-1.34(m,24H), 0.92-0.89(m,9H).



Optimized procedure for PC3 by T-ROM: $(3HT)_6$-3cyclooctene (28mg, 0.021mmol) was dissolved in anhydrous DCM (132ml) under a nitrogen flow in an oven-dried 250ml Schlenk flask, and cooled to 0 °C on ice water bath. Then, the Grubbs 3rd generation catalyst (19.2mg, 0.021mmol) dissolved in DCM (8ml) was injected via syringe, to give an initial template concentration of 1.5×10^{-4} M. After 60 minutes, ethyl vinyl ether (0.1ml) was injected via syringe and the reaction was stirred for an additional 30 minutes before warming to room temperature. The solvent was evaporated under reduced pressure and the residue was extracted with 1ml of toluene. The catalyst was removed by passing SX1 biobeads column. NMR shows around 92% conversion and MALDI shows single peak (MW=1287.075).

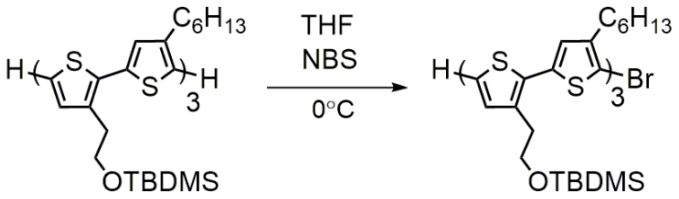


Hydrolysis of PC3 in basic solution: Potassium hydroxide (1M in H_2O, 0.5ml) was added into PC3 (25mg, 0.019mmol) DMF solution (4.5ml) and stirred at 55 °C for 48 hours. The solvent was removed by high vacuum and the residue was subjected to 10ml 1M KOH and 10ml ethyl acetate. Collect the water phase and 1M HCl solution was added until pH >7, keep the solution for 10 minutes to fully protonate daughter oligomer (DC3). Then, the solution was diluted with ethyl acetate (10 mL) and washed with water (3x10mL). The Organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The product was obtained (5.2mg, 58 %) and used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.35 (m, 6H), 2.41-2.35(m, 6H), 2.14-2.11(m, 27H).

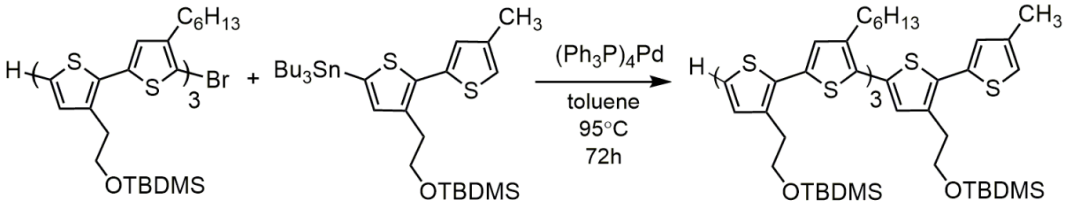


DC3-3$NH_3^+TFA^-$ (A3). In a 10 mL oven-dried Schlenk flask, the cyclic cyclooctene trimer with three carboxylic acid group (5.2 mg, 0.011 mmol) and N-(tert-Butoxycarbonyl)ethanolamine (11.2mg, 0.07 mmol) were dissolved in anhydrous DCM (1 mL) under nitrogen and DMAP (1.5 mg, 0.01 mmol) was added in one portion. The solution was cooled to 0 °C and stirred for 10 min before injection of neat DIC (12 μL, 0.1 mmol). The reaction mixture was allowed to warm to rt and stirred overnight. The organic layer was concentrated under reduced pressure. The crude product was purified by SX1 biobeads column (Toluene) to yield the pure product (7.2mg, 72%).

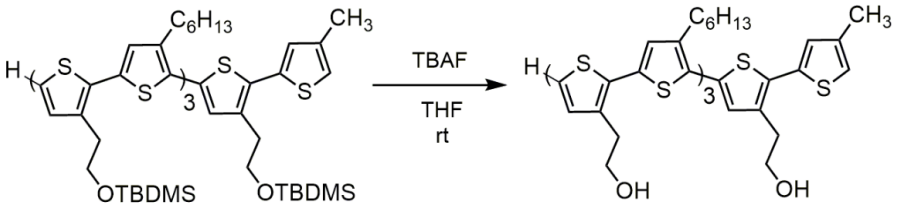
0.2ml TFA solution was added to the product and stirred for 1 hour. Then TFA was evaporated and the residue was washed with diethyl ether (3×5ml) to yield pure product (6.9mg, 66%).^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.95 (m, 9H), 5.27 (m, 6H), 4.09 (m, 6H), 3.01(m, 6H), 1.86(m, 6H), 1.52-1.04(m, 27H). ^{13}C-NMR (600MHz, DMSO-d_6): δ = 176.97, 130.37, 130.29, 61.15, 43.98, 43.43, 31.79, 30.36, 30.09, 30.01, 29.71, 26.63, 25.70, 24.33. HRMS (ESI, m/z): [M−2H$^+$ = $C_{33}H_{58}N_3O_6^+$] Calc. 592.4320, Found 592.4291



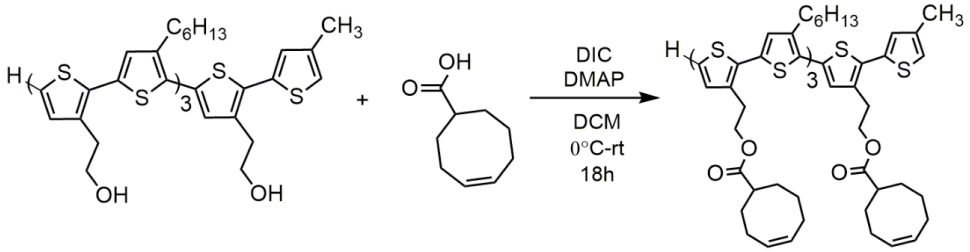
(3HT)$_6$-Br-3OTBDMS: At 0 ^{o}C, recrystallized NBS (40mg, 0.22mmol) was added to a solution of (3HT)$_6$-3OTBDMS (275mg, 0.22mmol) in dry THF (4ml) under nitrogen atmosphere. The solution was allowed to warm to room temperature overnight. THF was removed under reduced pressure and mixture was dissolved in EtOAc (20ml), followed by washing with DI water (3×20ml) brine (3×20ml), drying over anhydrous sodium sulfate. After filtration, solvent was removed under high vacuum, the product was used without any further purification (270mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.15 (d, 1H), 7.02-6.98 (m, 4H), 6.88 (s, 1H), 3.91-3.84(m, 6H), 3.05-3.01(m, 6H), 2.82-2.77(m, 4H), 2.61(t,2 H), 1.69-1.63(m, 6H), 1.40-1.31(m, 21H), 0.90-0.88(m,33H), 0.03-0.00(m,18H)



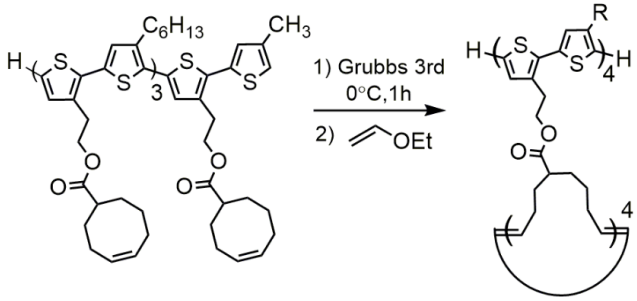
(3HT)$_8$-4OTBDMS: In a 200ml storage flask with a Teflon valve seal, (3HT)$_6$-Br-3OTBDMS (98mg, 0.075mmol) and (3HT)$_2$-Bu$_3$Sn-1OTBDMS (52mg, 0.082mmol) were dissolved in dry toluene (15ml) and subjected to 3× freeze/pump/thaw/cycles. Pd(Ph$_3$P)$_4$ (124mg, 0.107mmol) as a slurry in toluene (4ml) was added and subjected to another 3×freeze/pump/thaw/ cycles. The reaction vessel was sealed under vacuum with a Teflon valve, and then heated to 90 ^{o}C for 72 hours, or until the appearance of a black precipitate. The reaction mixture was cooled to room temperature. The organic layer was washed with water (3×30ml) and brine (3×30ml), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (Hex: Toluene, 2:1) gave pure product (83mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.16 (d, 1H), 7.01-6.98 (m, 8H), 6.89 (s, 1H), 3.90-3.84(m, 8H), 3.05-2.97(m, 8H), 2.79-2.76(t, 6H), 2.28(s,3 H), 1.71-1.66(m, 4H), 1.44-1.42(m, 12H), 1.34-1.31(m, 17H), 0.87-0.81(m,36H), 0.00(m,24H)



$(3HT)_8$-4OH: Tetreabutylammonium floride (1M in THF,0.4ml,0.4mmol) was added into $(3HT)_8$-4OTBDMS (80mg, 0.051mmol) THF solution (4ml) and stirred at room temperature overnight. The mixture was purified by SX1 biobeads column (THF) to yield pure product (46.5mg, 88%). ^{1}H NMR (500 MHz, CDCl3): δ = 7.16 (d, 1H), 7.01-6.98 (m, 8H), 6.89 (s, 1H), 3.87-3.82(m, 8H), 2.03-2.98(m, 8H), 2.80-2.77(t, 6H), 2.58(t,2H), 2.28(s,3 H), 1.71-1.66(m, 6H), 1.44-1.42(m, 21H), 0.87-0.81(m, 6H).

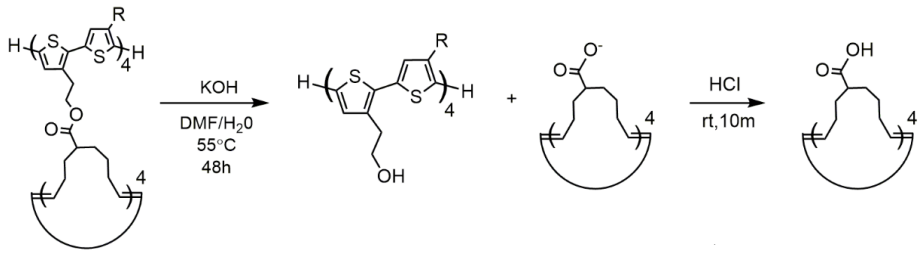


$(3HT)_8$-4cyclooctene: In a 4ml oven-dried vial, the *Z*-cyclooct-4-ene carboxylic acid (41.2mg, 0.26mmol) and $(3HT)_8$-4OH (46mg, 0.044mmol) were dissolved in dry THF (3ml) under nitrogen and DMAP (1mg, 0.0084mmol) was added in one portion. The solution was cooled to 0 ^{o}C and stirred 30 minutes before injection of neat DIC (0.054ml, 0.3mmol). The reaction was allowed to warm to room temperature and stirred overnight. The solution was diluted with EtOAc (20ml) and washed with DI water (3×20ml) and brine (3×20ml). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by SX1 biobeads column (Toluene) to yield the pure product (55.8mg, 76%). ^{1}H NMR (500 MHz, CDCl3): δ = 7.23 (d, 1H), 7.00-6.98 (m, 8H), 6.89 (s, 1H), 5.63-5.59(m, 8H) 4.30-4.27(m, 8H), 3.13-3.09(m, 8H), 2.78-2.73(m, 8H), 2.62(t, 2H), 2.43-2.32 (m, 6H), 2.10(m,3H), 1.98-1.94(m, 6H), 1.81(m,3H) 1.65-1.52(m, 20H), 1.39-1.32 (m, 26H), 0.89-0.85(m,12H).

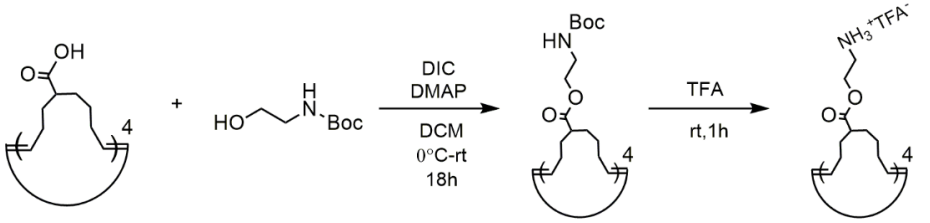


Optimized procedure for PC4 by T-ROM: $(3HT)_8$-4cyclooctene (17mg, 0.01mmol) was dissolved in anhydrous DCM (60ml) under a nitrogen flow in an oven-dried 10ml Schlenk flask, and cooled to 0°C on ice water bath. Then, the Grubbs 3rd generation

catalyst (9.1mg, 0.01mmol) dissolved in DCM (8ml) was injected via syringe, to give an initial template concentration of 1.5×10^{-4} M. After 60 minutes, ethyl vinyl ether (0.1ml) was injected via syringe and the reaction was stirred for an additional 30 minutes before warming to room temperature. The solvent was evaporated under reduced pressure and the residue was extracted with 1ml of Toluene. The catalyst was removed by passing SX1 biobeads column (Toluene). NMR shows 100% conversion and MALDI shows single peak (MW=1646.706).



Hydrolysis of PC4 in basic solution: Potassium hydroxide (1M in H_2O, 0.5ml) was added into PC4 (17mg, 0.010mmol) DMF solution (4.5ml) and stirred at 55 ^oC for 48 hours. The solvent was removed by high vacuum and the residue was subjected to 10ml 1M KOH and 10ml ethyl acetate. Collect the water phase and 1M HCl solution was added until pH >7, keep the solution for 10 minutes to fully protonate daughter oligomer (DC4). Then, the solution was diluted with ethyl acetate (10 mL) and washed with water (3x10mL). The Organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The product was obtained (3.4mg, 54 %) and used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.37 (m, 8H), 2.41-2.35(m, 10H), 2.16-2.12(m, 34H).



DC4-4$NH_3^+TFA^-$ (A_4). In a 10 mL oven-dried Schlenk flask, the cyclic cyclooctene tetramer with four carboxylic acid group (3.4 mg, 0.005 mmol) and N-(tert-Butoxycarbonyl)ethanolamine (6.4mg, 0.04 mmol) were dissolved in anhydrous DCM (1 mL) under nitrogen and DMAP (1.5 mg, 0.01 mmol) was added in one portion. The solution was cooled to 0 °C and stirred for 10 min before injection of neat DIC (5 μL, 0.04 mmol). The reaction mixture was allowed to warm to rt and stirred overnight. The organic layer was concentrated under reduced pressure. The crude product was purified by SX1 biobeads column (Toluene) to yield the pure product (5.2mg, 81%). 0.2ml TFA solution was added to the product and stirred for 1 hour. Then TFA was evaporated and the residue was washed with diethyl ether (3×5ml) to yield pure product (3.2mg, 54%).^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.96 (m, 12H), 5.31 (m, 8H), 4.13 (m, 8H), 3.04(m, 8H), 1.89(m, 10H), 1.52-1.04(m, 34H). ^{13}C-NMR (600MHz, DMSO-d_6): δ =

175.48, 131.49, 130.27, 129.76, 129.43, 61.03, 38.24, 31.82, 31.31, 30.48, 29.93, 29.47, 26.74, 22.16, 20.85. HRMS (ESI, m/z): [M−3H$^+$ = $C_{44}H_{77}N_4O_8^+$] Calc. 789.5736, Found. 789.4104.

NMR and Mass Spectra:

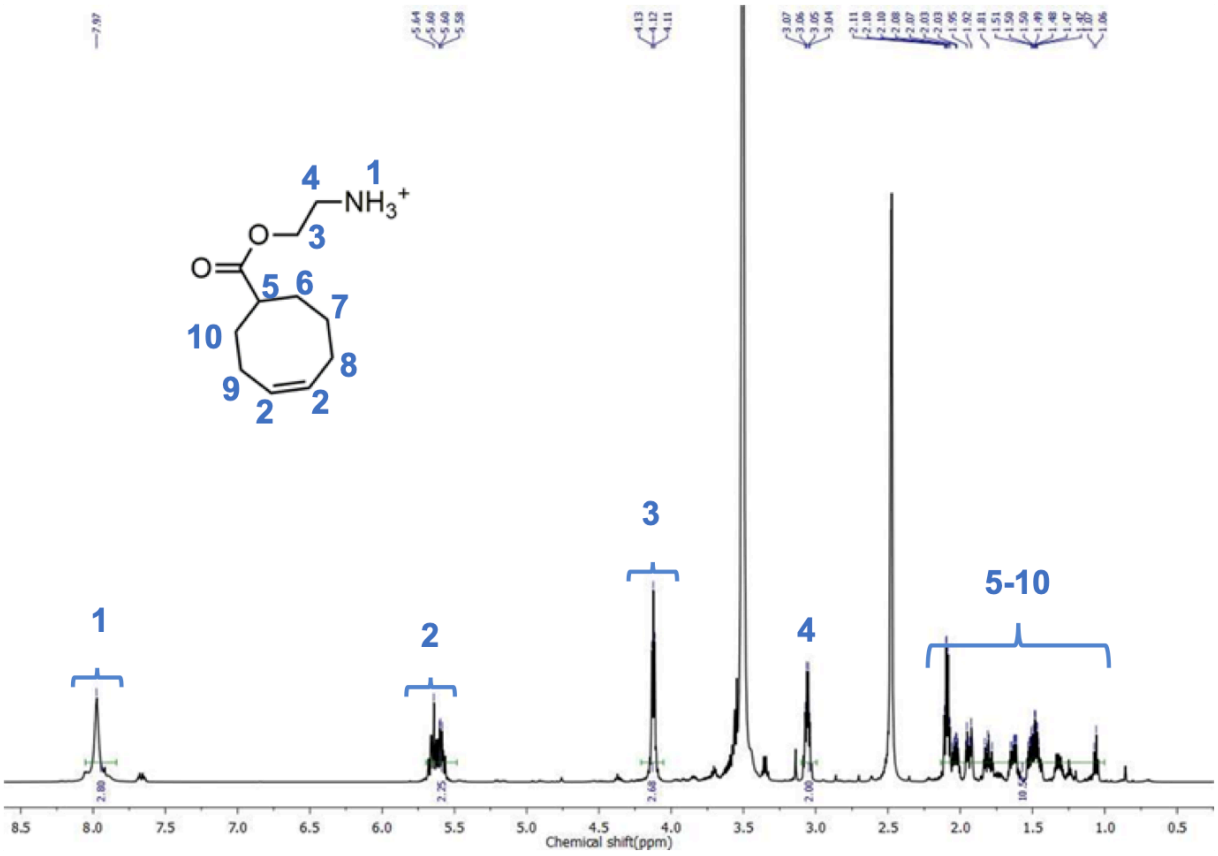


Figure S1. ^{1}HNMR spectra for **A1**

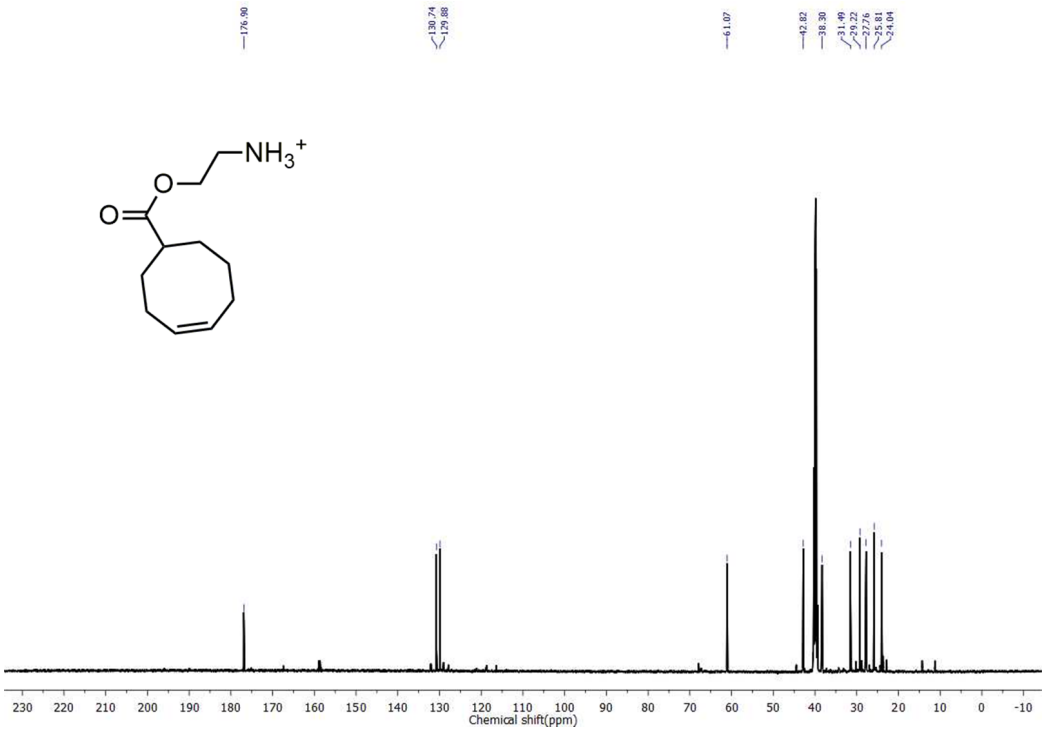


Figure S2. ^{13}CNMR spectra for **A1**

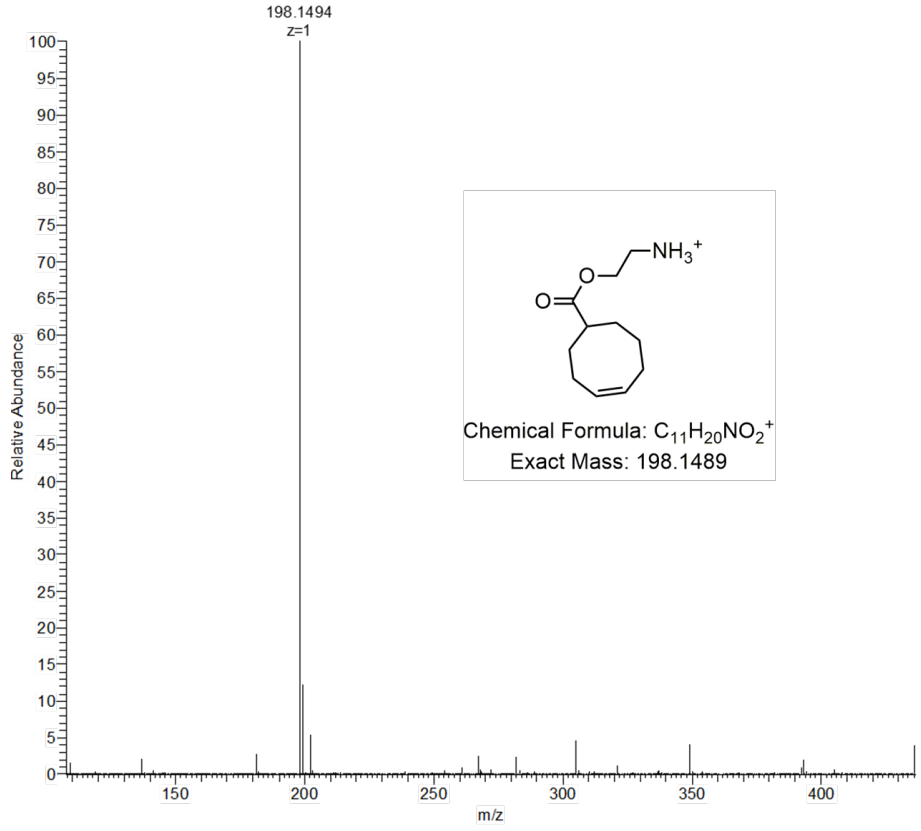


Figure S3. ESI mass spectra of **A1**

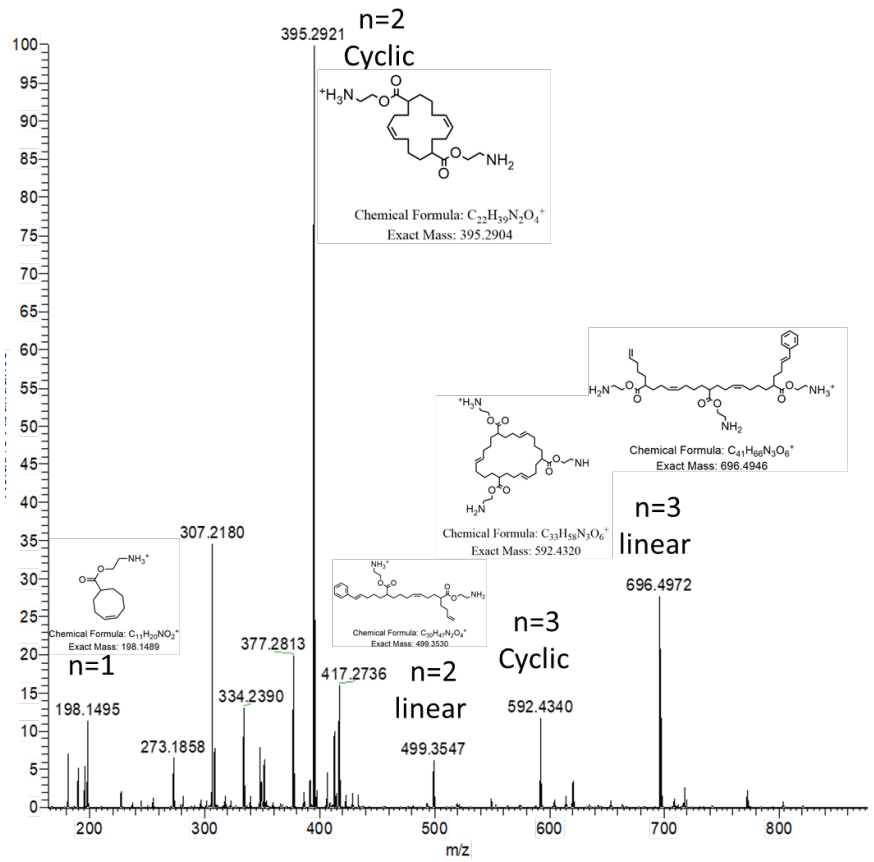


Figure S4. ESI mass spectra of **poly(A1)**

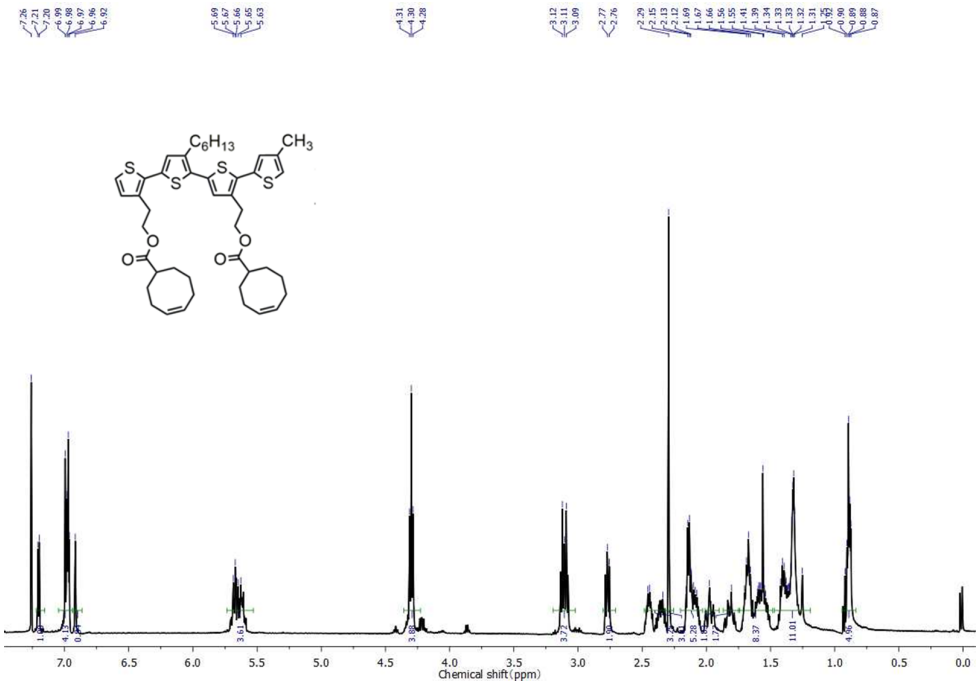


Figure S5. ^{1}HNMR spectra for **C2**

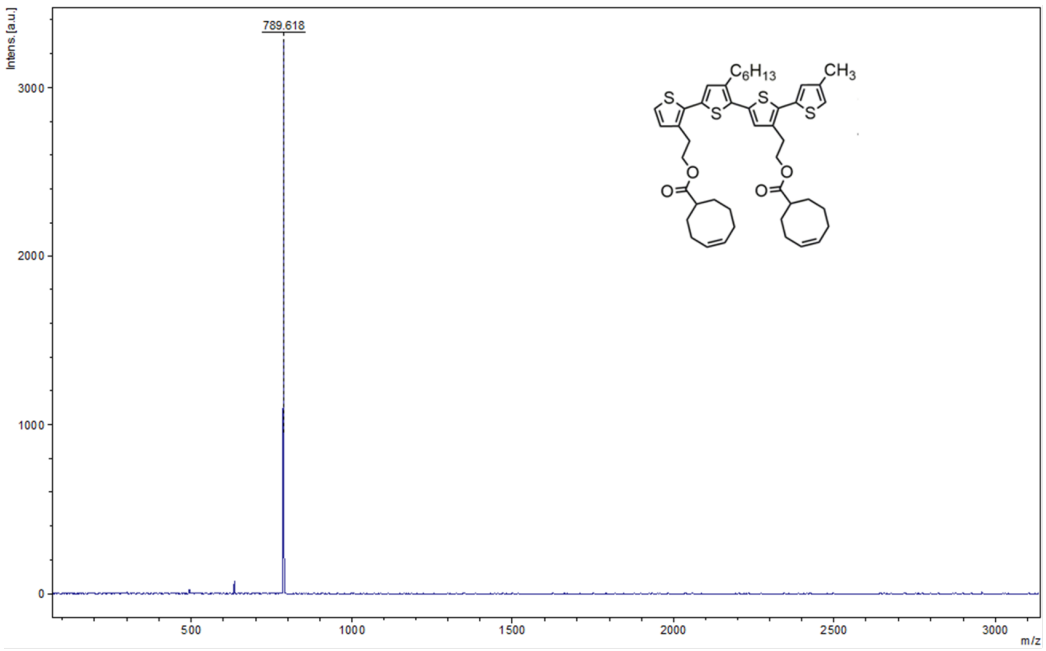


Figure S6. MALDI spectra for **C2**

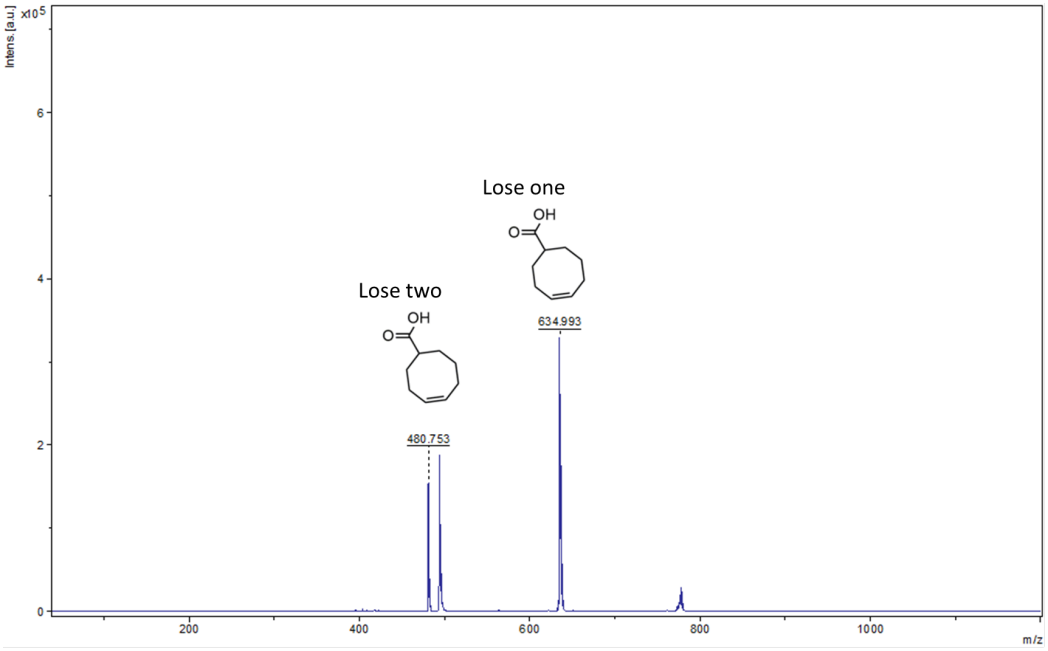


Figure S7. MALDI ms/ms spectra for **C2**

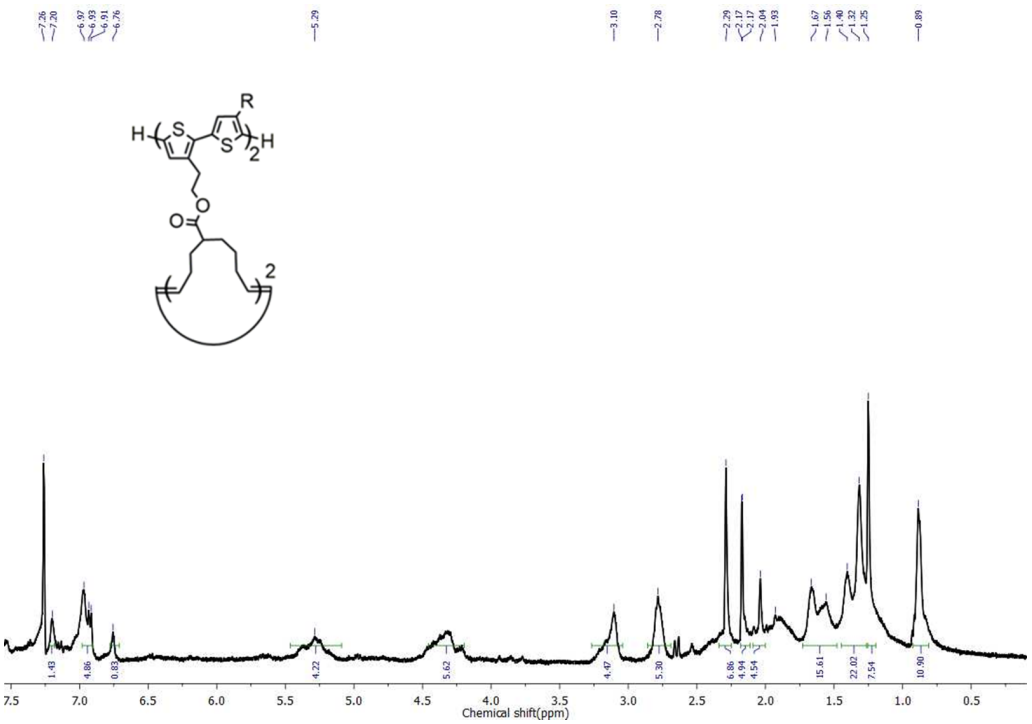


Figure S8. [1]HNMR spectra for **PC2**

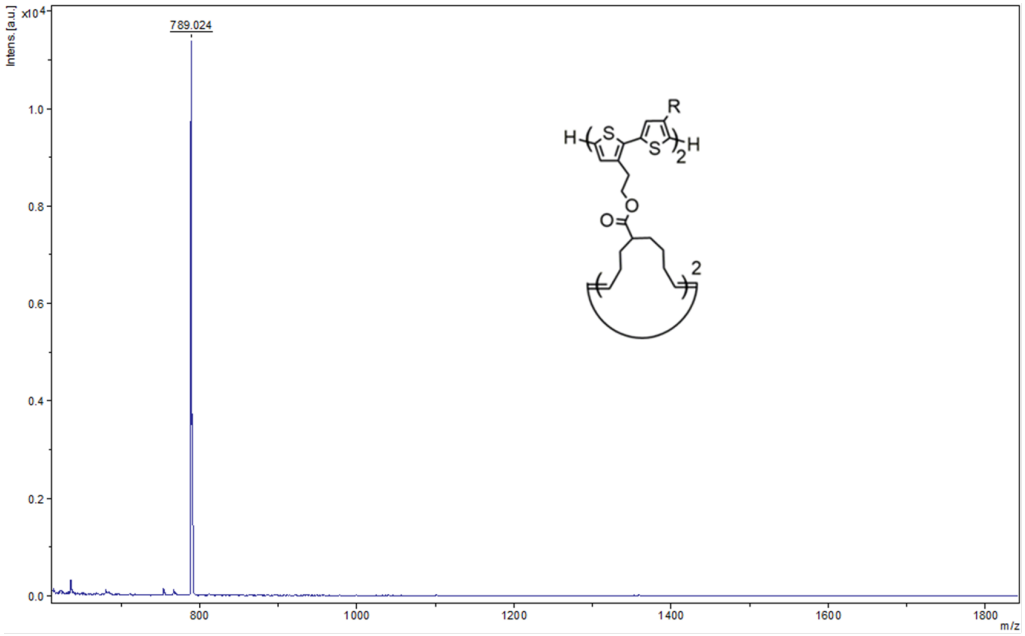


Figure S9. MALDI spectra for **PC2**

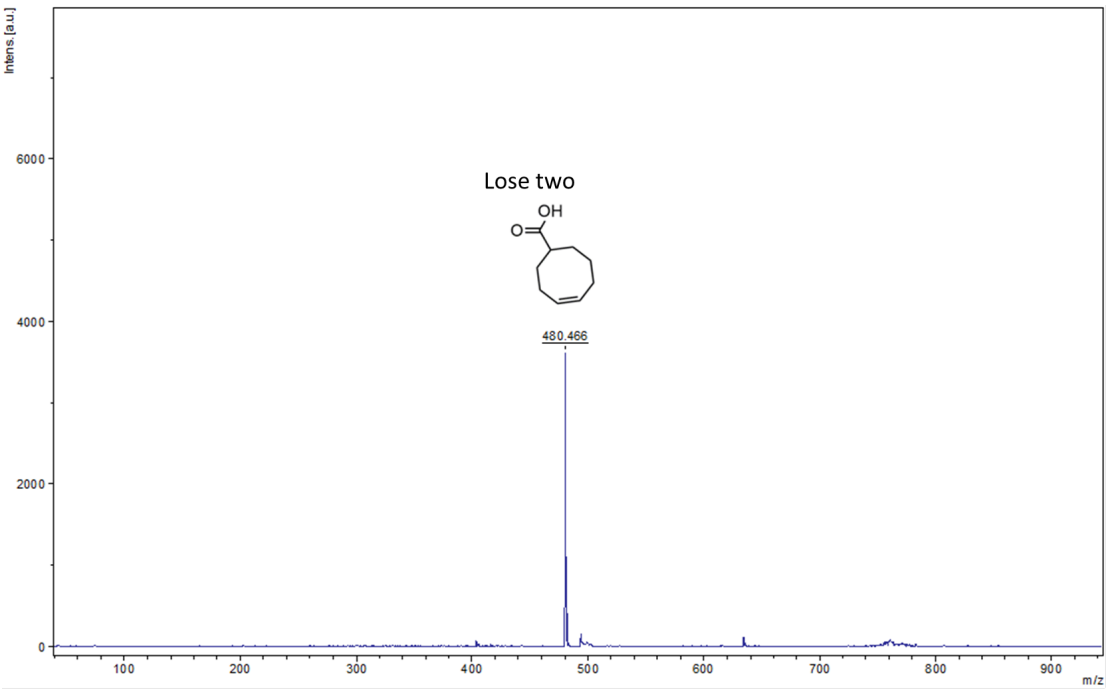


Figure S10. MALDI ms/ms spectra for **PC2**

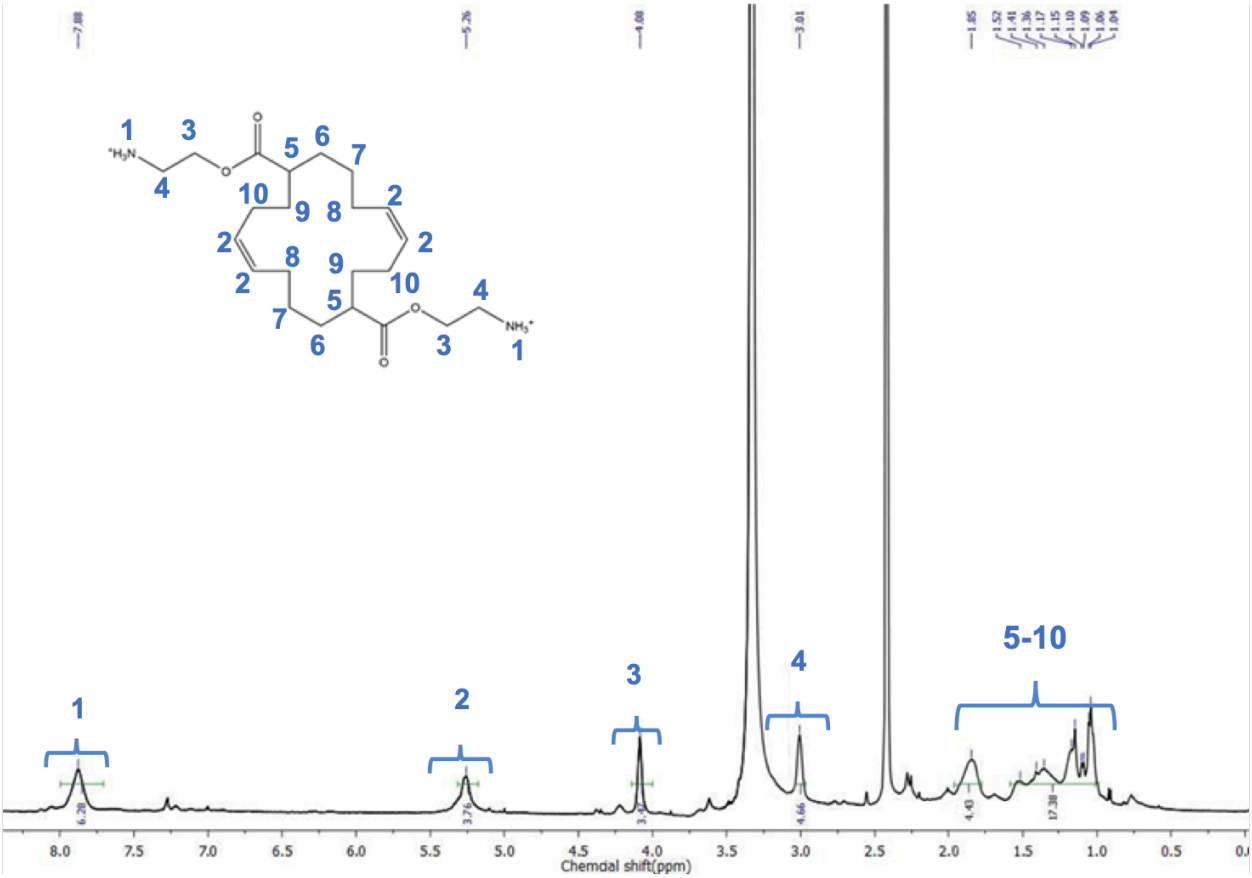


Figure S11. [1]HNMR spectra for **A2**

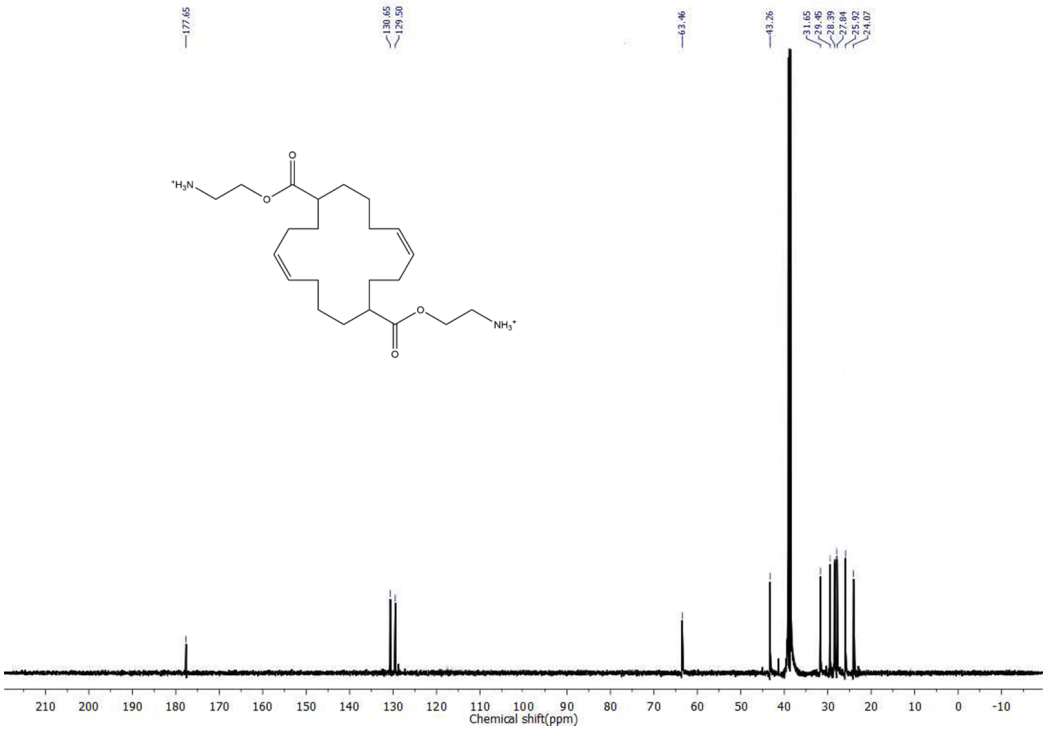


Figure S12. ^{13}C NMR spectra for **A2**

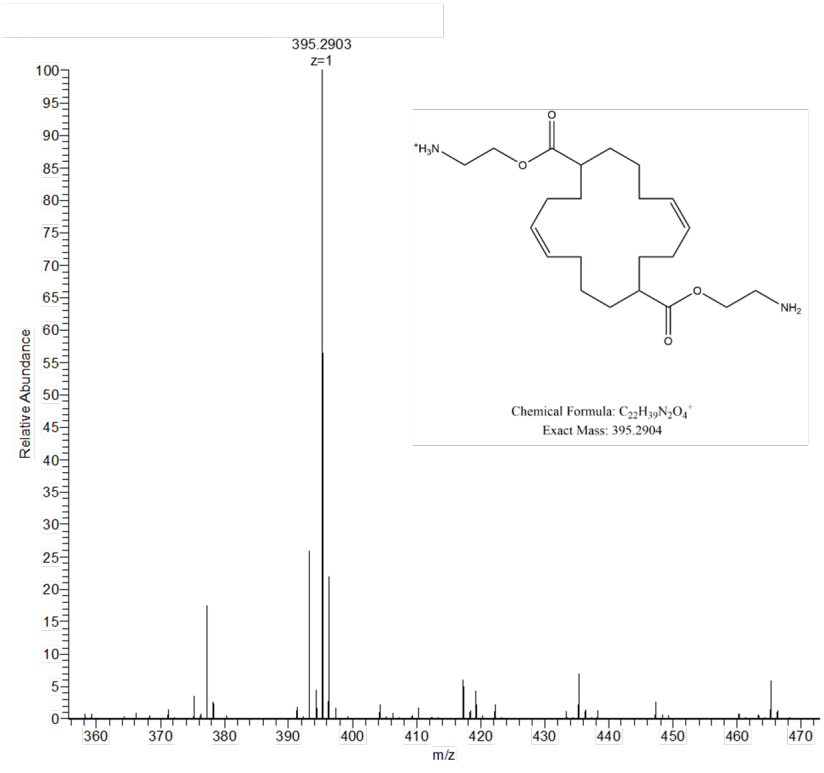


Figure S13. ESI mass spectra of **A2**

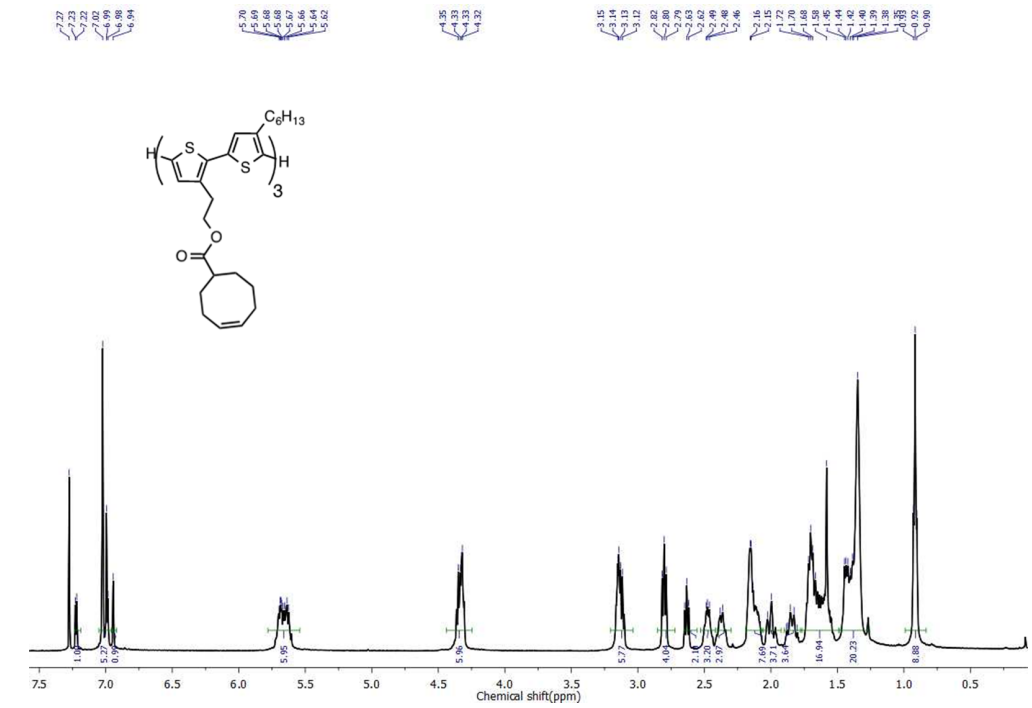


Figure S14. ^{1}HNMR spectra for **C3**

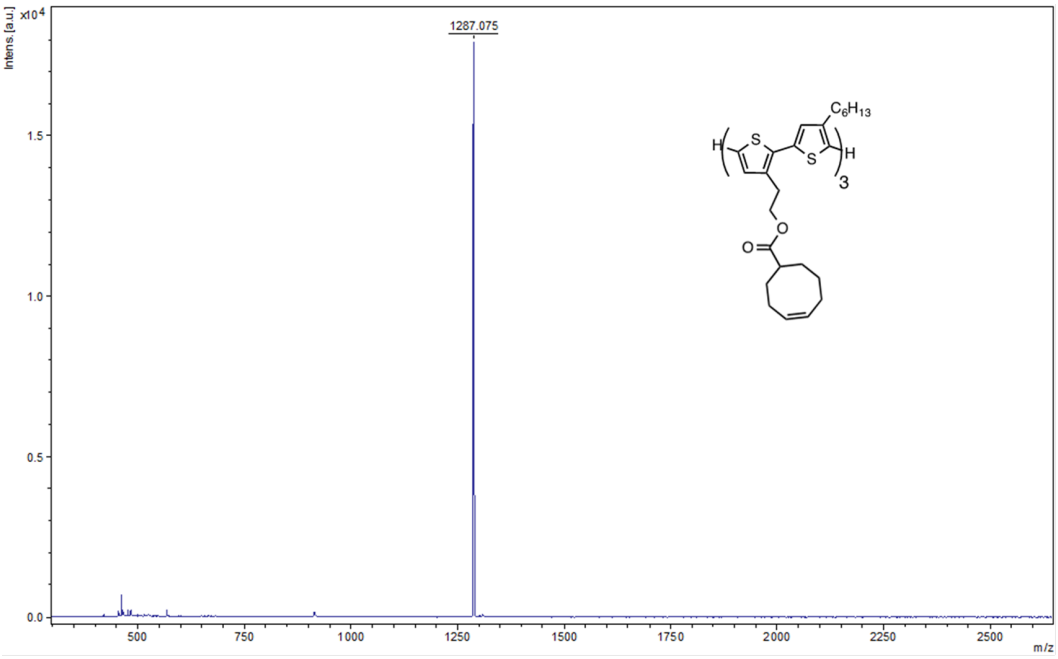


Figure S15. MALDI spectra for **C3**

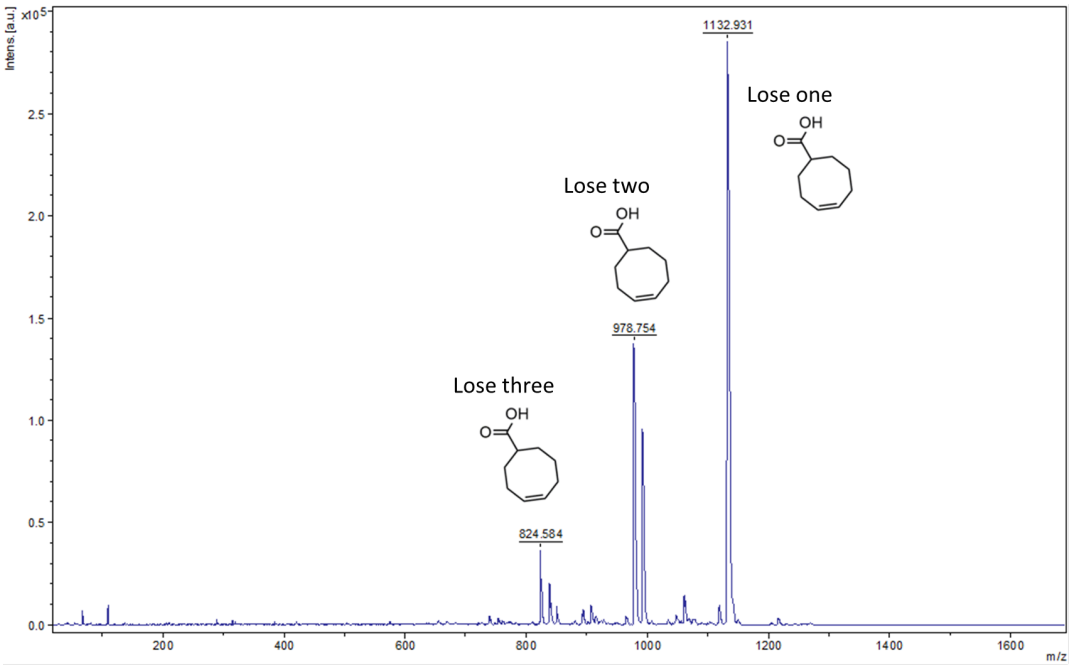


Figure S16. MALDI ms/ms spectra for **C3**

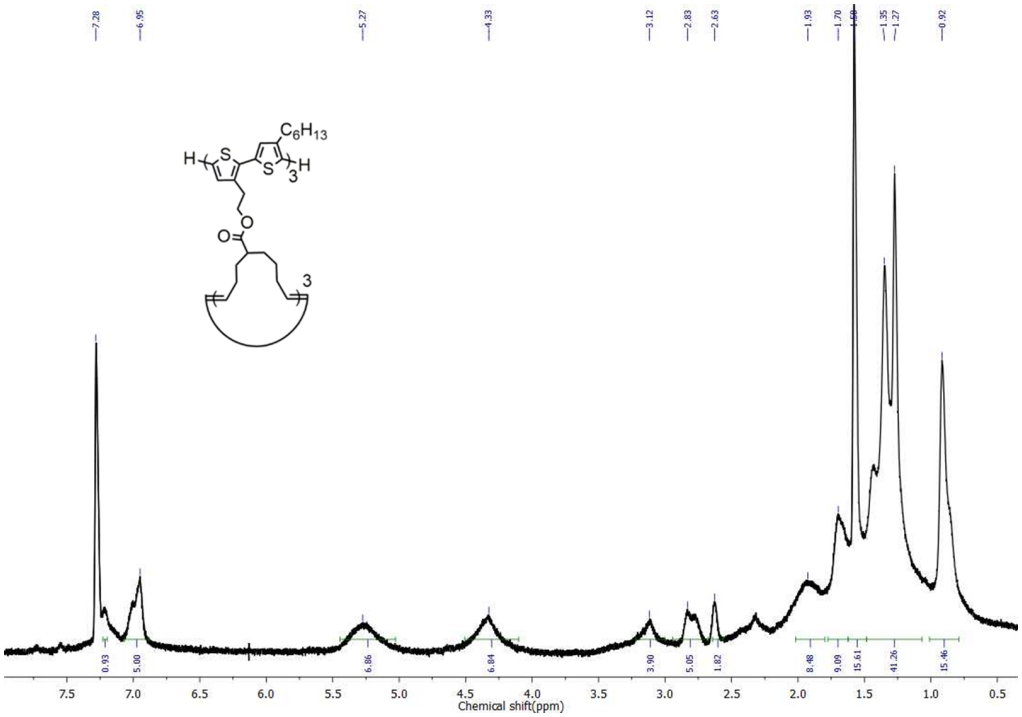


Figure S17. ^{1}HNMR spectra for **PC3**

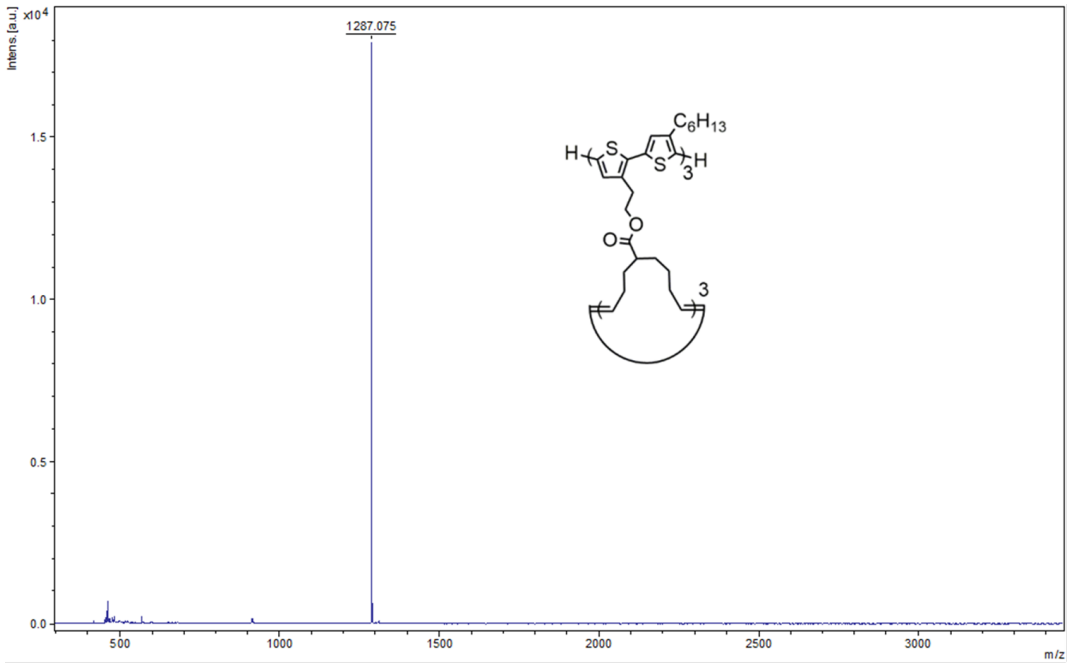


Figure S18. MALDI spectra for **PC3**

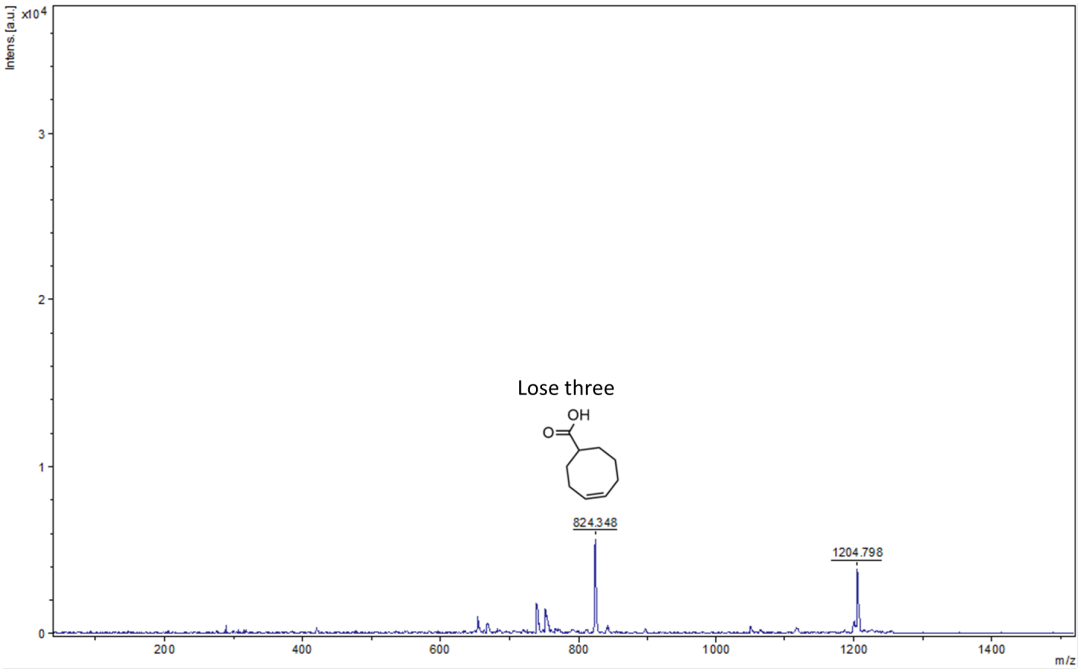


Figure S19. MALDI ms/ms spectra for **PC3**

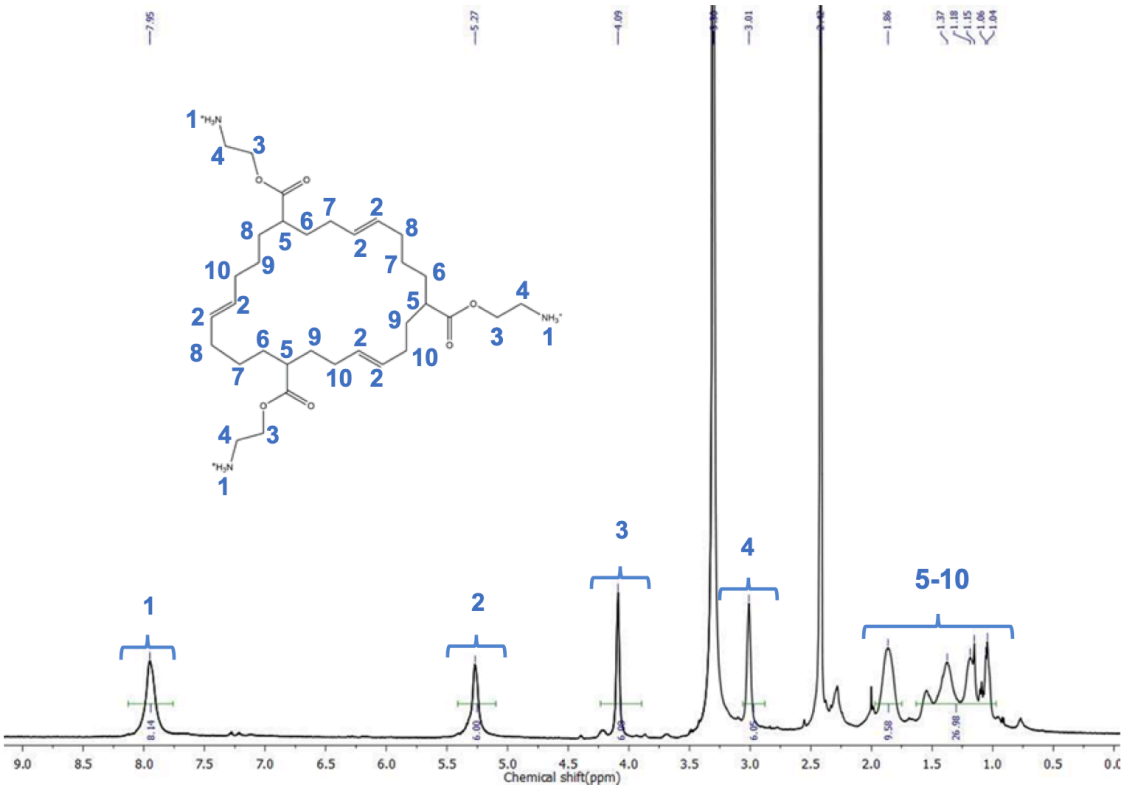


Figure S20. ^{1}HNMR spectra for **A3**

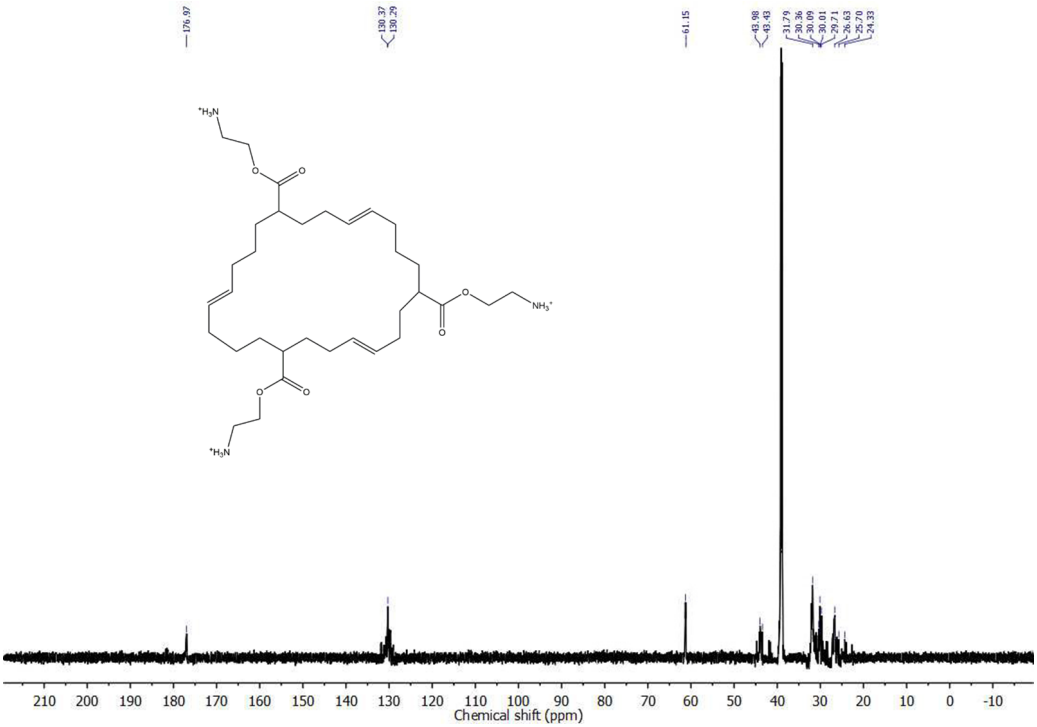


Figure S21. ^{13}CNMR spectra for **A3**

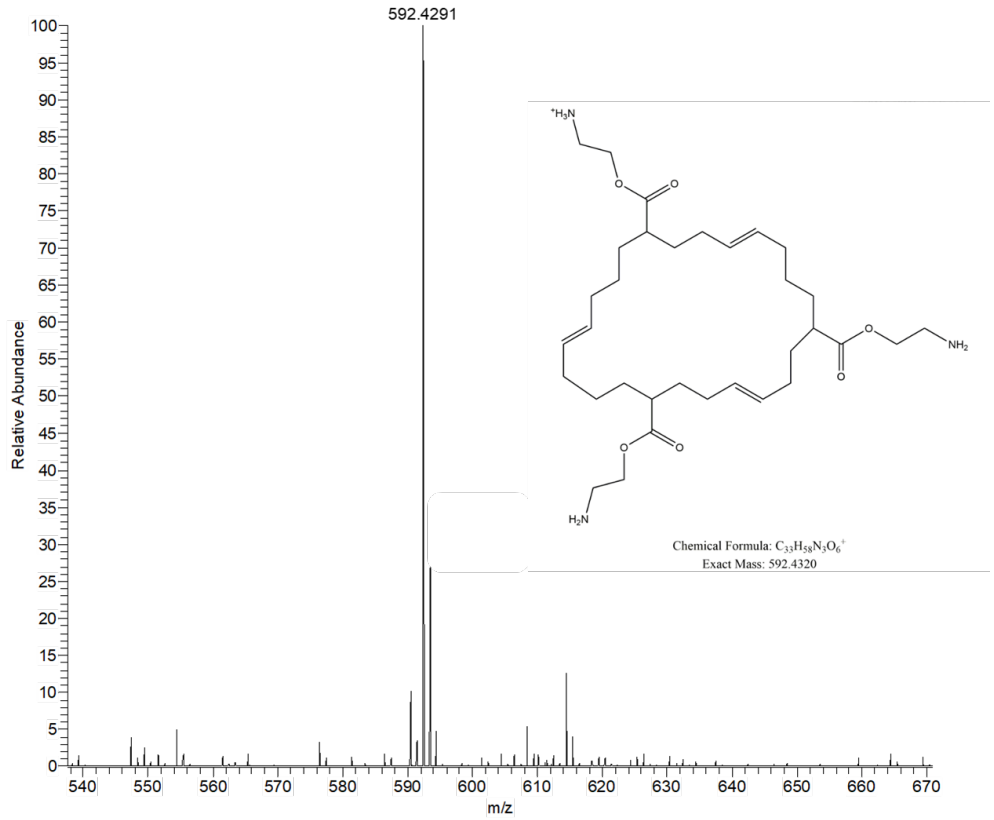


Figure S22. ESI mass spectra of **A3**

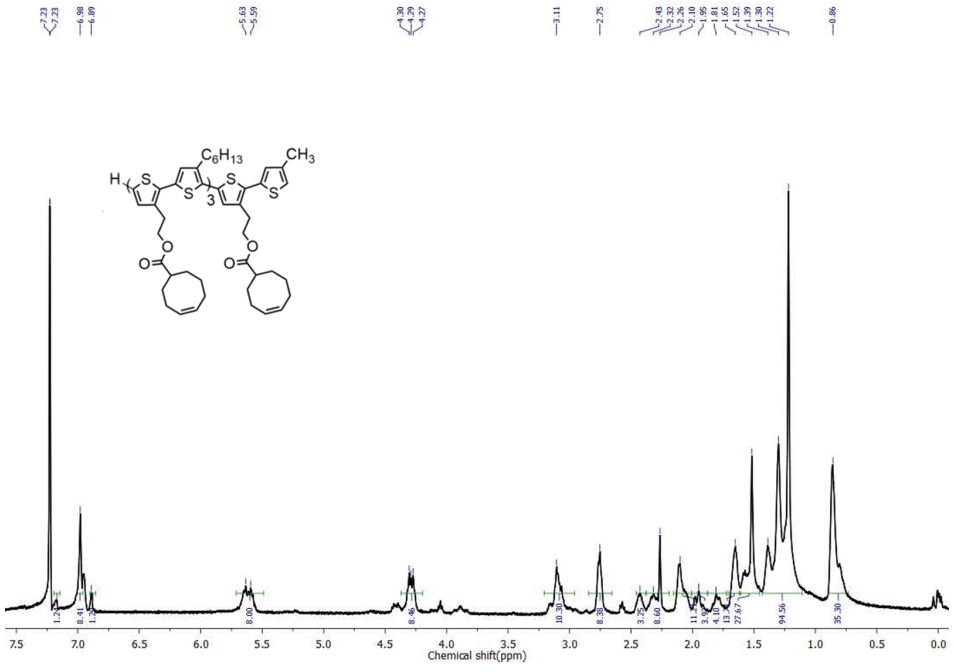


Figure S23. ^{1}HNMR spectra for **C4**

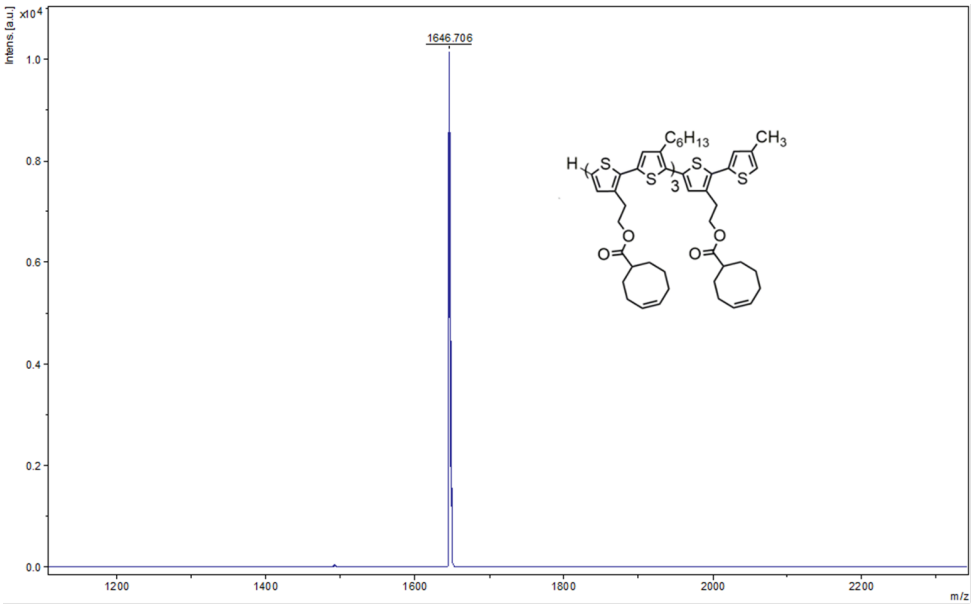


Figure S24. MALDI spectra for **C4**

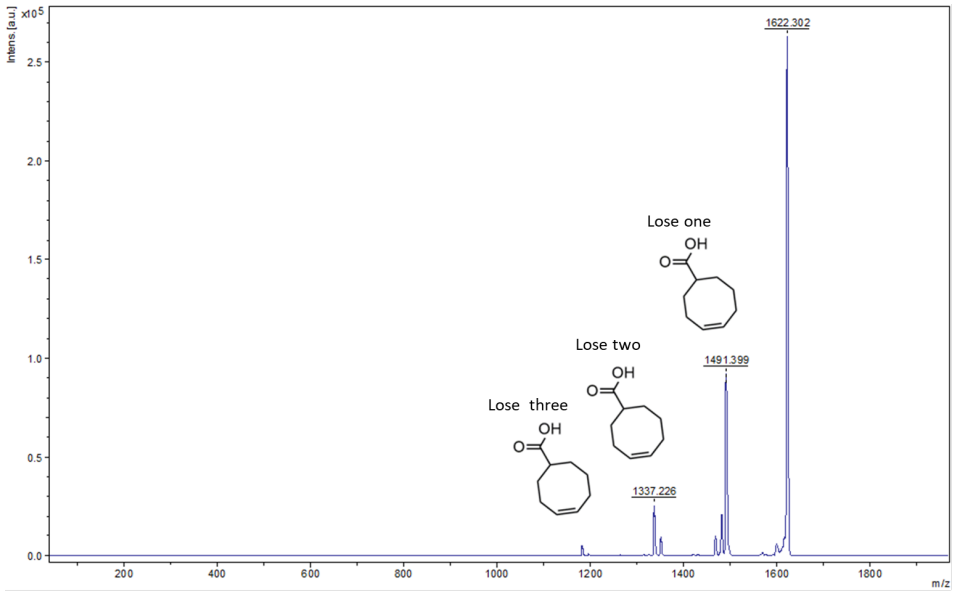


Figure S25. MALDI ms/ms spectra for **C4**

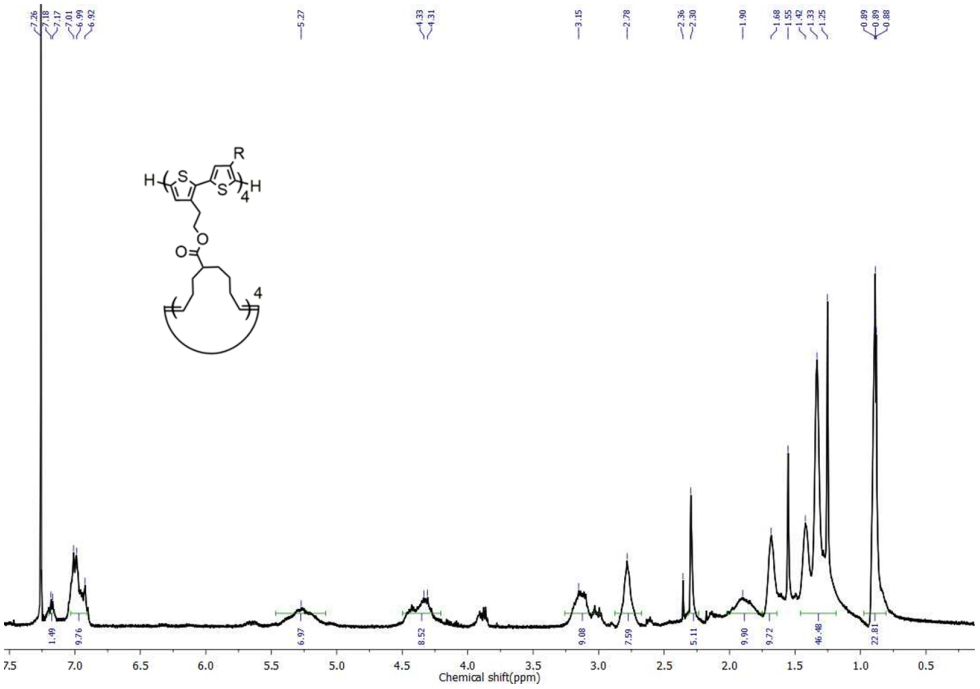


Figure S26. ^{1}HNMR spectra for **PC4**

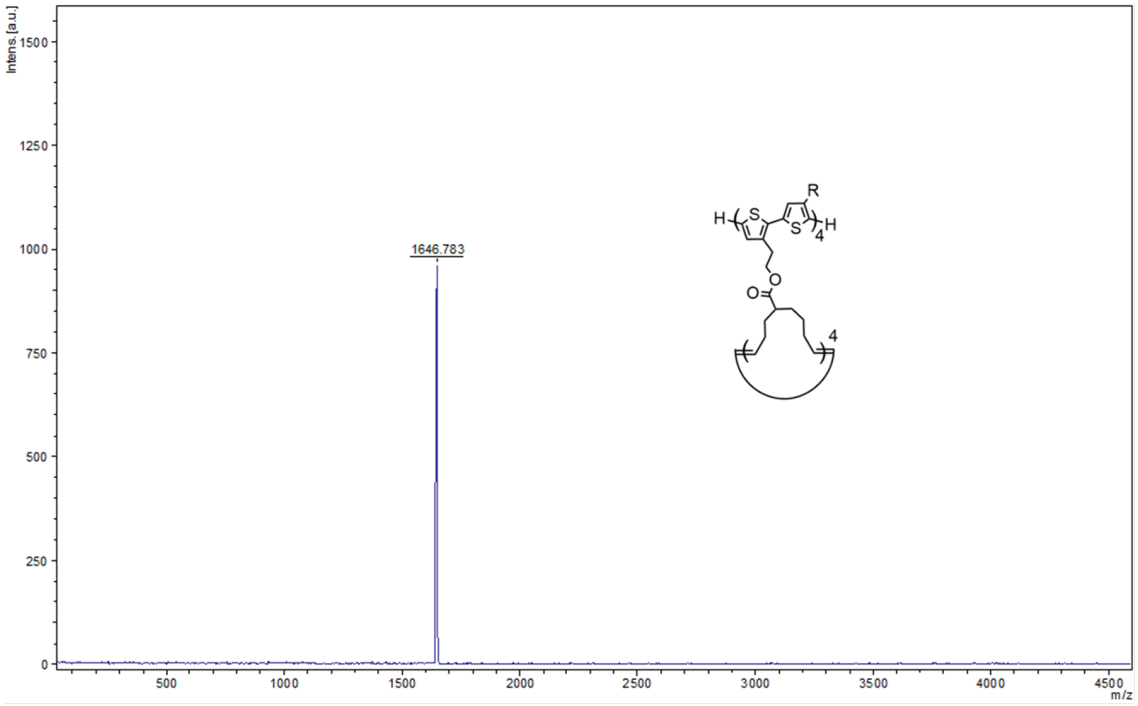


Figure S27. MALDI spectra for **PC4**

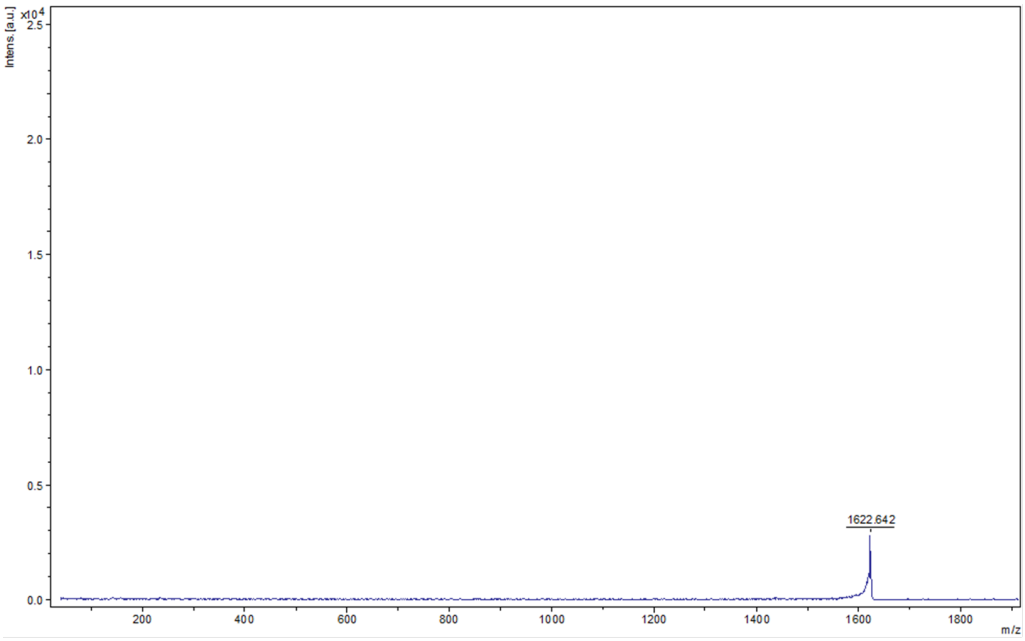


Figure S28. MALDI ms/ms spectra for **PC4**

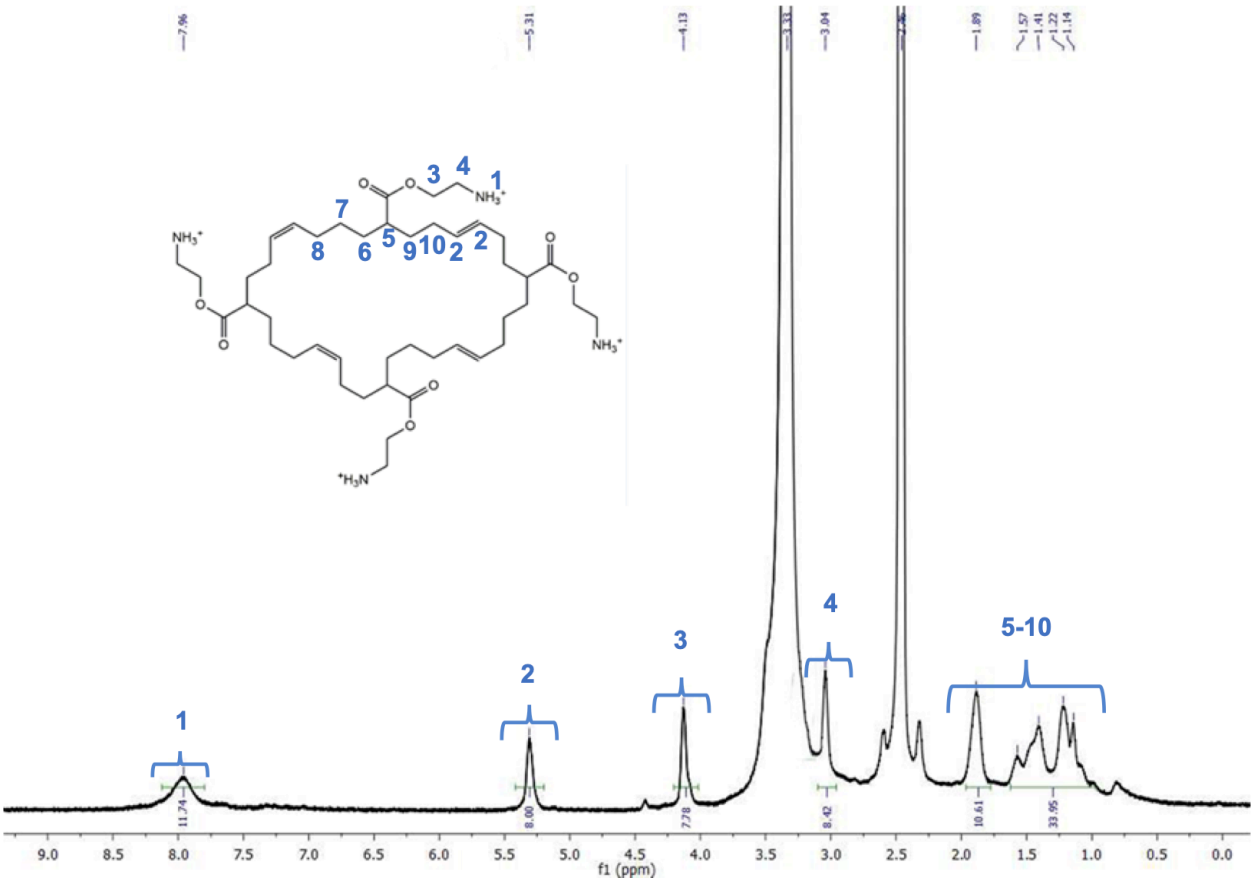


Figure S29. [1]HNMR spectra for **A4**

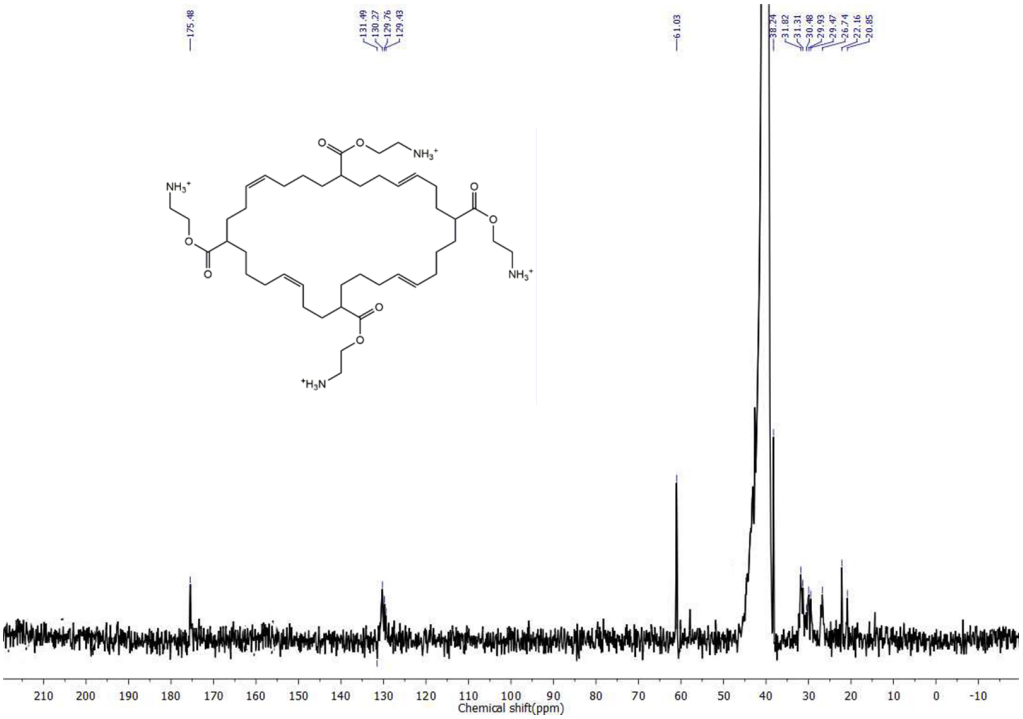


Figure S30. ^{13}CNMR spectra for **A4**

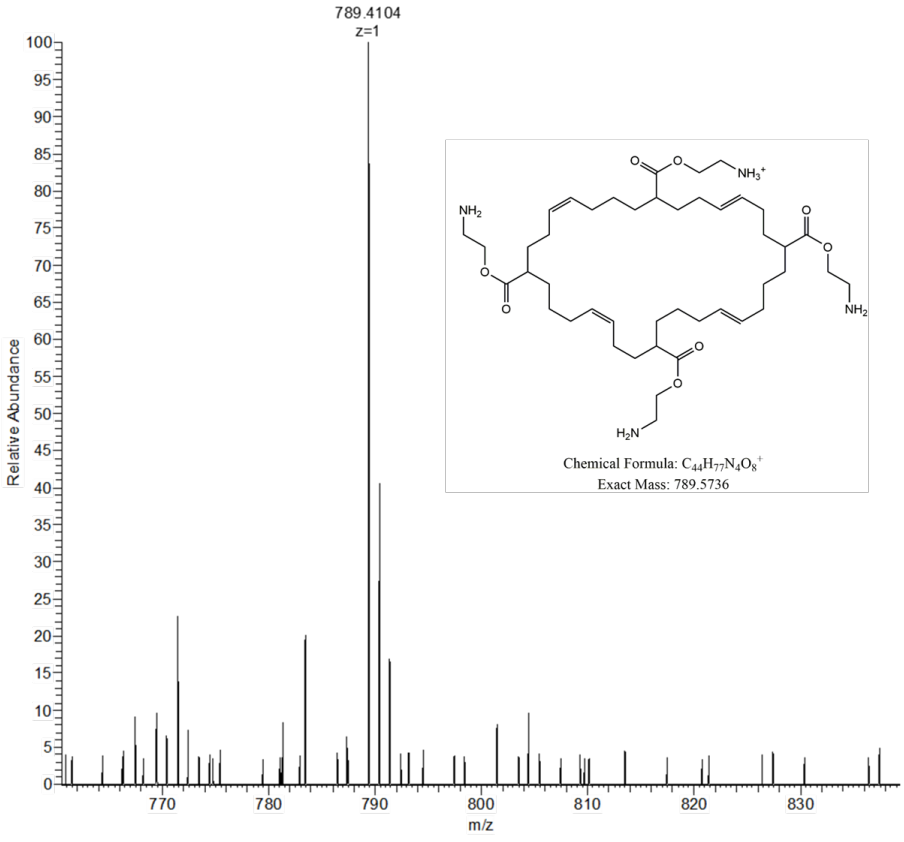


Figure S31. ESI mass spectra of **A4**

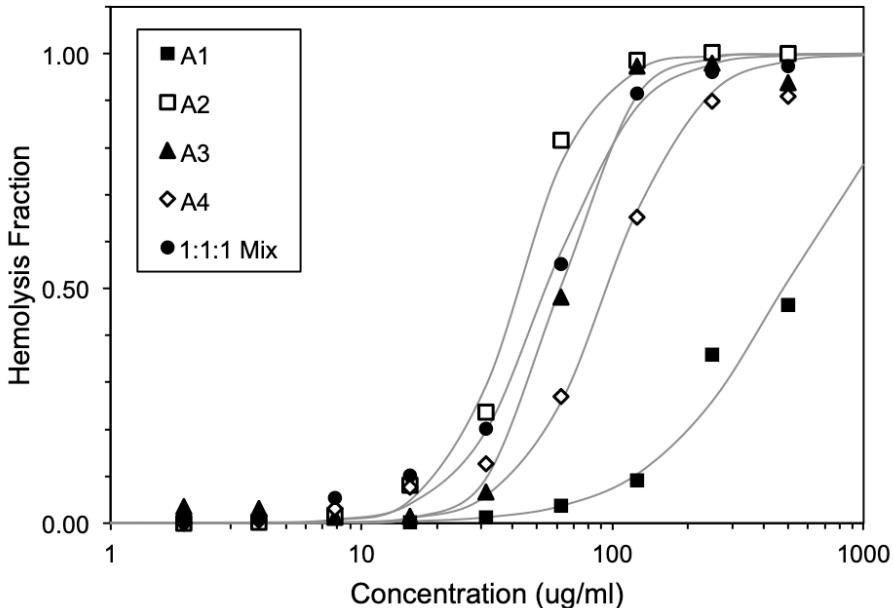


Figure S32. Hemolysis fraction of **A1** (monomer), **A2**, **A3**, **A4** and **1:1:1 molar mixture** of **A2**, **A3** and **A4**

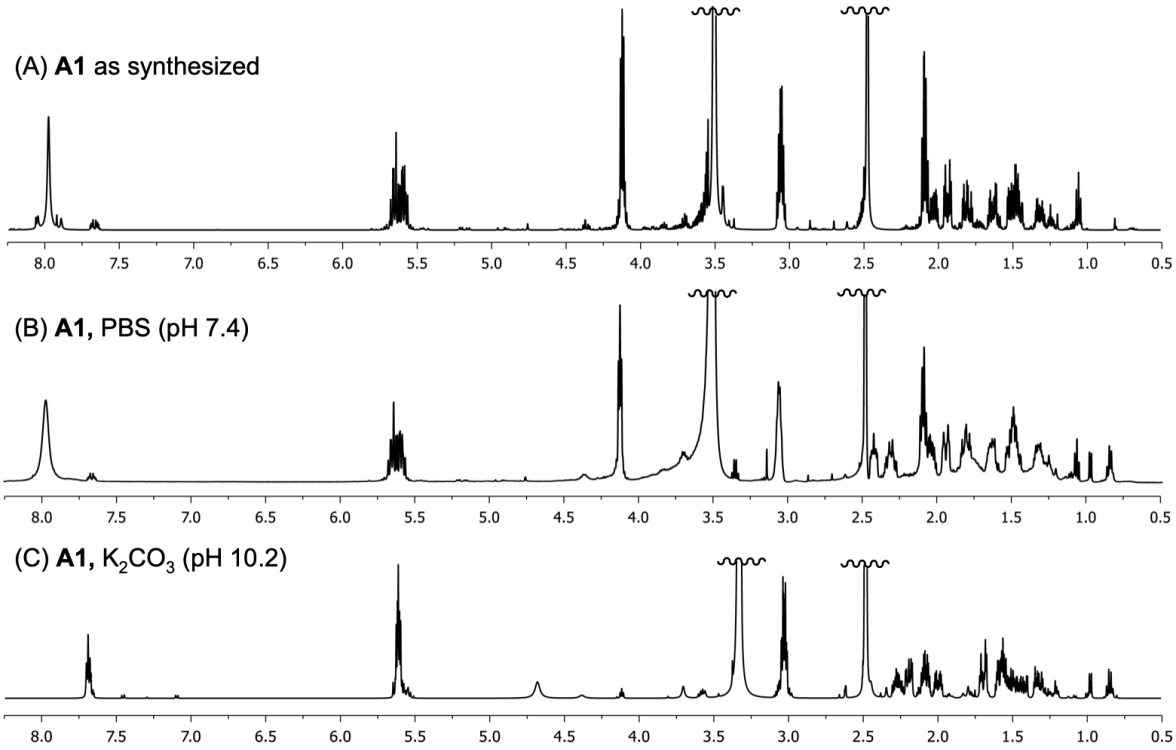


Figure S33. ^{1}H NMR spectra of **A1** (A) as made and after incubation for 24 hr at 37 °C in (B) PBS buffer, pH 7.4 and (B) K_2CO_3 buffer, pH 10.2. The monomer is stable in neutral conditions but isomerizes in basic pH.

References:

1. Zhou, Z.; Palermo, E. F., Templated Ring-Opening Metathesis (TROM) of Cyclic Olefins Tethered to Unimolecular Oligo(thiophene)s. *Macromolecules* **2018**, 51 (15), 6127–6137.