Electronic Supplementary Material (ESI) for ChemComm.

PEG-conjugated pyrrole-based polymers: One-pot multicomponent synthesis and self-assembly into soft nanoparticles for drug delivery

Alexandre Moquin,[a,b] Ramez Hanna,[a] Tongyue Liang,[a] Huseyin Erguven,[a] Evan Rizzel Gran,[b] Bruce A. Arndtsen,[*a] Dusica Maysinger[*b] and Ashok Kakkar,[*a]

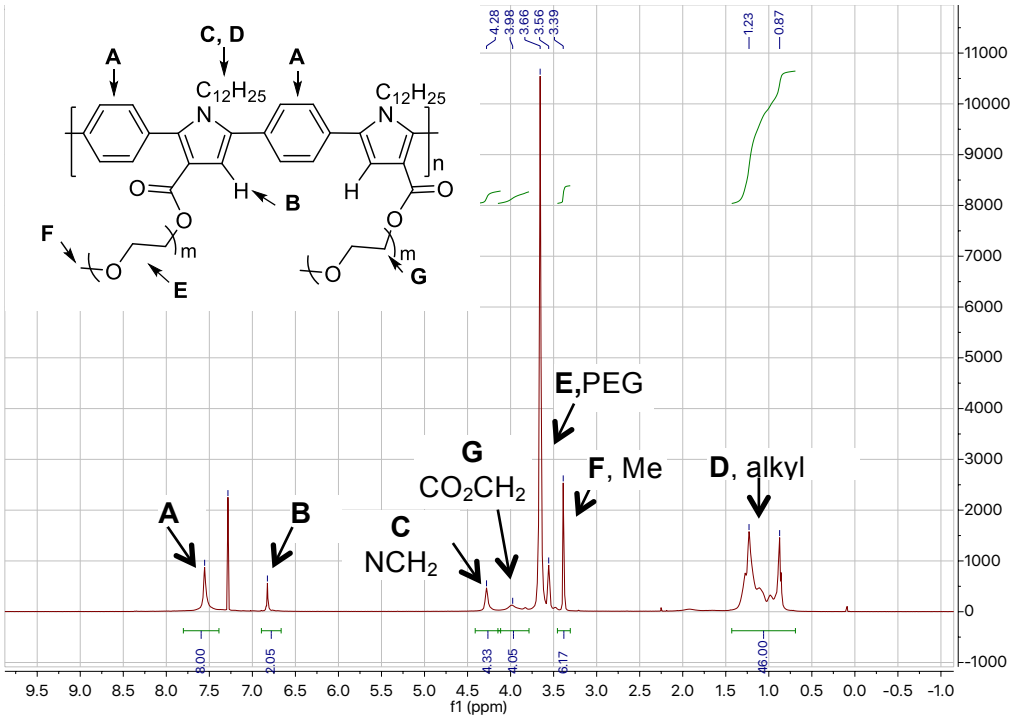
[a]Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Quebec H3A 0B8
[b]Department of Pharmacology and Therapeutics, McGill University, 3655 Prom. Sir William Osler, Montreal, Quebec H3G 1Y6

Table of Contents

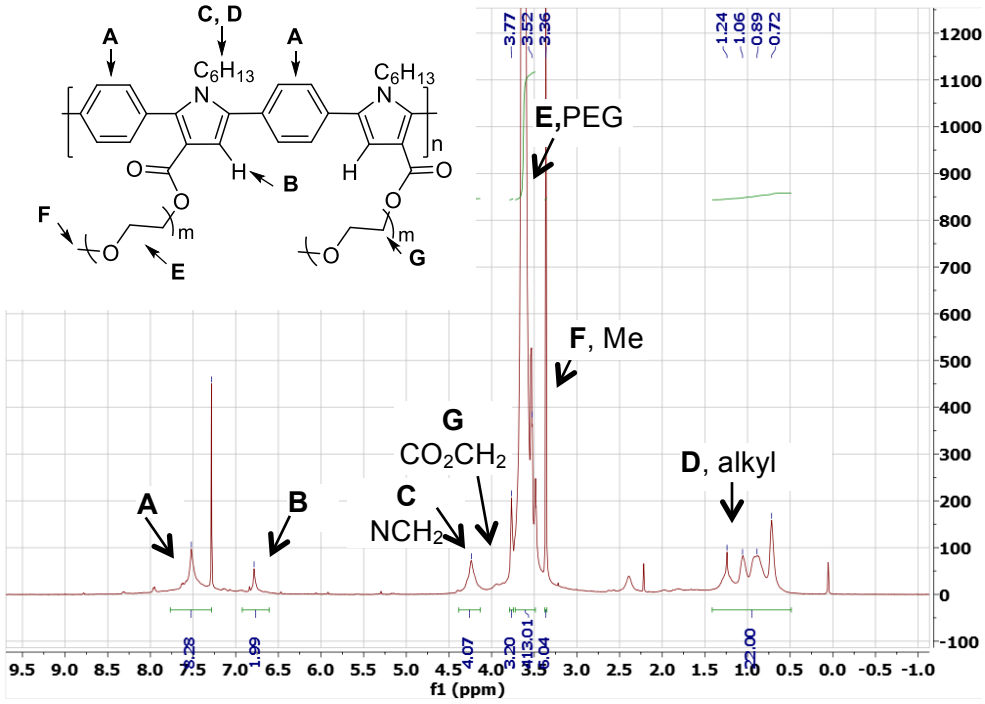
I.	**Supplementary Figures**	**S2**
II.	**General Synthesis Procedures**	**S11**
III.	**Self-Assembly and Analysis of 2b**	**S13**
IV.	**References**	**S17**
V.	**^{1}H and ^{13}C NMR Spectra**	**S19**

I. Supplemental Figures:

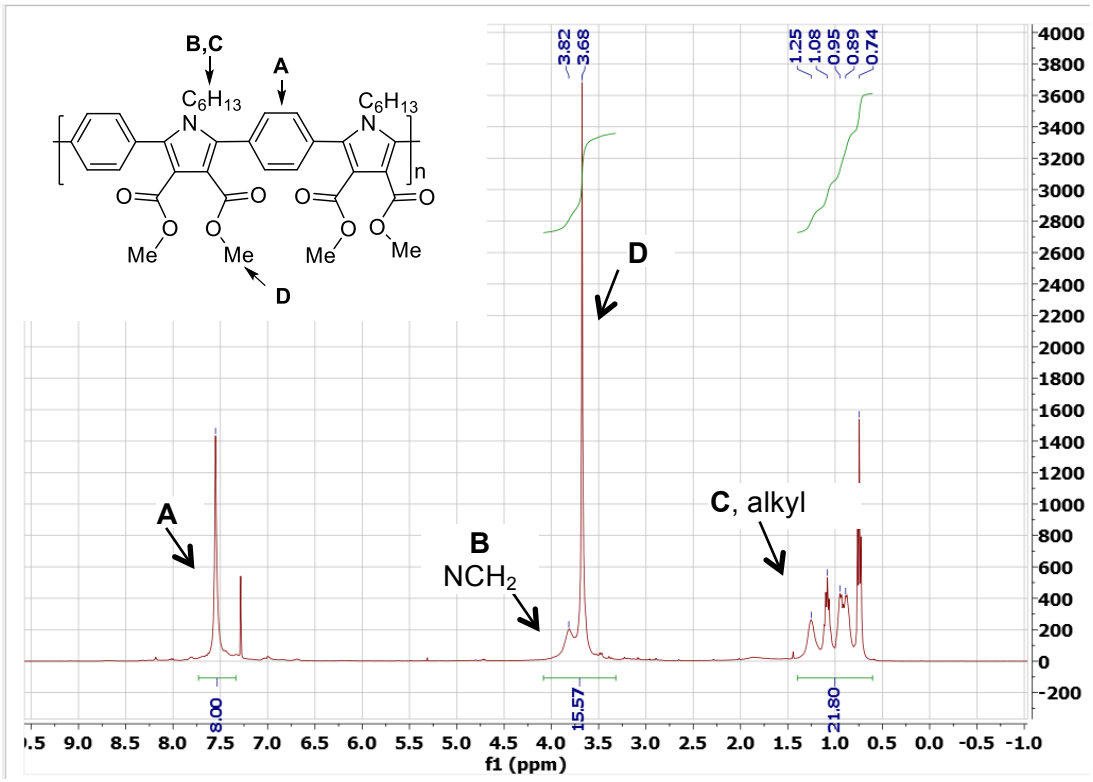
a) ^{1}H NMR ($CDCl_3$) of **2a**



b) ^{1}H NMR ($CDCl_3$) of **2b**



c) ^{1}H NMR ($CDCl_3$) of model polymer



d) ^{1}H NMR (CD_3CN) of model pyrrole

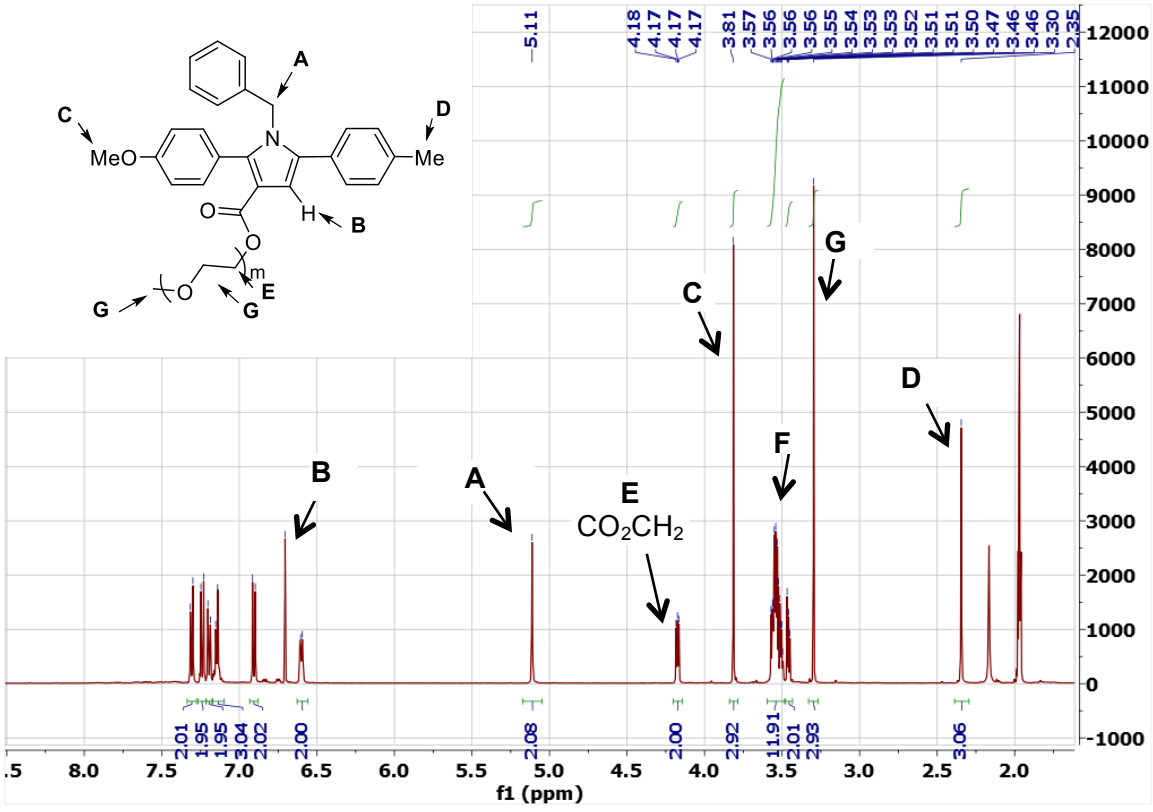


Figure S1. ^{1}H NMR spectra of: a) Polymer **2a**, b) Polymer **2b**, c) Model polymer prepared with dimethylacetylene dicarboxylate alkyne, d) Model pyrrole.

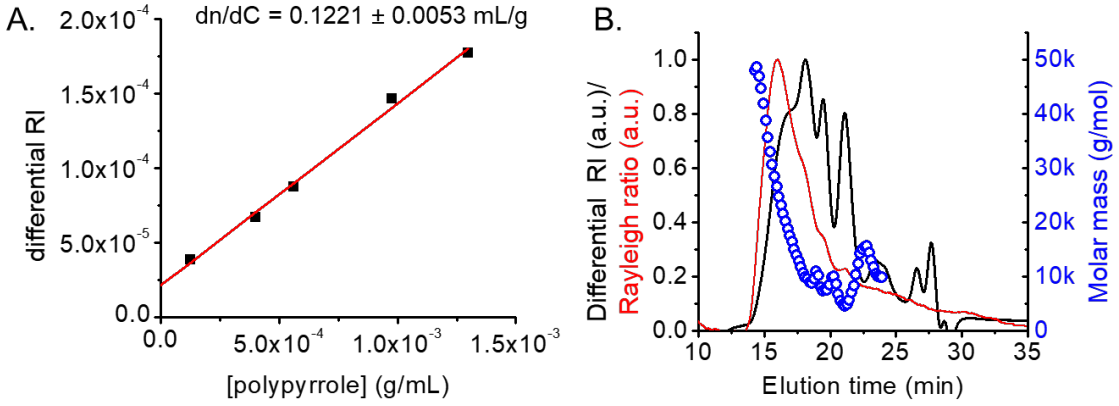


Figure S2. (A) determination of d*n*/dC for polypyrrole-C_6H_{13}-PEG_{2kDa} in THF. (B) GPC-RI-MALS measurement of polypyrrole-C_6H_{13}-PEG_{2kDa} in THF using a cross-linked Styrene Divinyl Benzene column (300 mm x 4.6 mm, 5 µm) at elution rate of 0.3 mL/min. The black line represents the refractive index change as a function of elution time. The red line represents the light scattering intensity as a function of elution time. MALS was used to plot the molar mass of the eluting sample (blue circles).

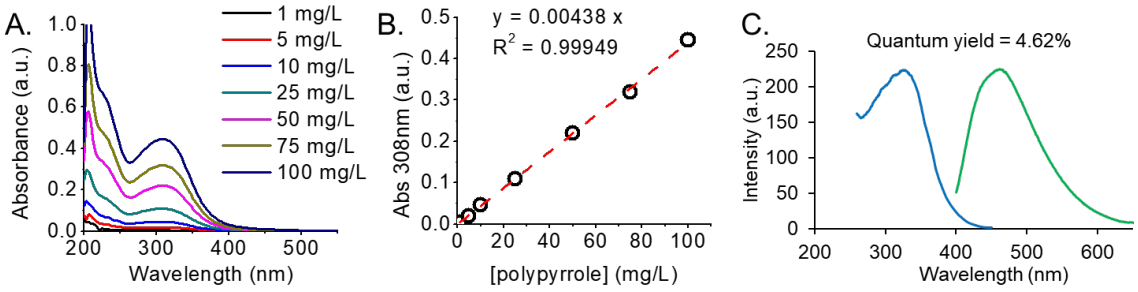


Figure S3. (A) UV-visible absorption spectra of polypyrrole dissolved in methanol, (B) calibration curve of absorption at 308 nm as a function of polypyrrole concentration, (C) Fluorescence excitation (λ_{em} = 455 nm) and emission (λ_{ex} = 330 nm) spectra of polypyrrole at 500 mg/L using Cary spectrofluorometer (slits 5 nm each, PMT = 600V).

Polypyrrole polymer is composed of a succession of aromatic rings which provides absorbance and fluorescence properties. UV-vis absorbance and fluorescence spectra were measured. The polymer absorbs in the UV range with a main absorbance peak at 308 nm (**Figure S2A**). The absorbance evolves linearly with the polymer concentration in the range of 0-100 mg/L (**Figure S2B**). Absorbance of polypyrrole can be used to

determine mass concentration of the polymer. Due to the aromatic polypyrrole rings, the polymer system demonstrates fluorescence properties with an excitation peak matching the absorbance spectra (λ_{ex} = 308 nm) and an emission peak centered at 459 nm (**Figure S2C**). The quantum yield (Q.Y.) was determined to be 4.62% using a quinine sulfate solution in sulfuric acid as a reference molecule because of its absorbance and emission spectra are similar to the ones of polypyrrole (**Figure S3**).

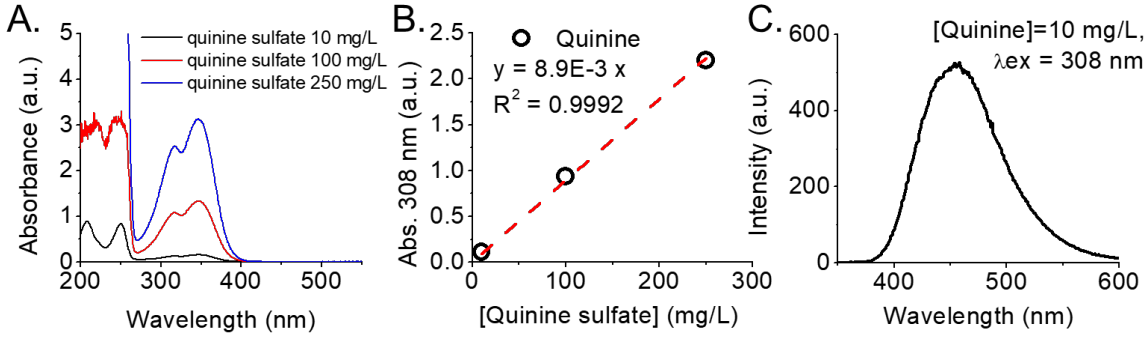


Figure S4. (A) UV-visible absorption spectra of quinine sulfate solution in sulfuric acid (0.5 M). (B) calibration curve of quinine sulfate in sulfuric acid at λ = 308 nm (at the peaks λ_{abs} = 317 nm and λ_{abs} = 346 nm, the slopes are 10.22 E-3 $L.mg^{-1}$ and 12.635 E-3 $L.mg^{-1}$, respectively). (C) Fluorescence emission spectra of quinine sulfate in sulfuric acid after excitation at 308 nm.

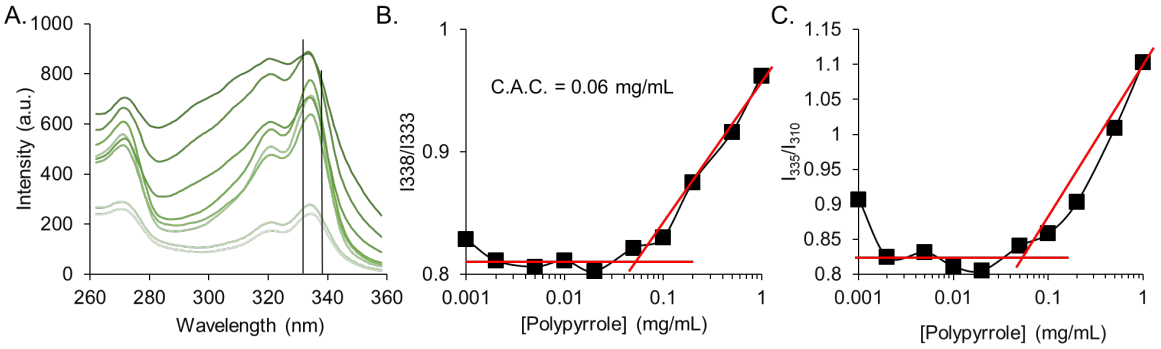


Figure S5. Determination of the critical aggregation concentration using pyrene fluorescence. (A) Pyrene excitation spectra (λ_{em} = 390 nm) as a function of increasing polypyrrole polymer concentration. Concentrations used 1, 2, 5, 10, 20, 50, 100, 200 mg/L. (B) Ratio of excitation intensity at 338 nm vs. 333 nm as a function of polypyrrole concentration. The inflexion point was measured at a polypyrrole concentration of 60 mg/L. (C) Ratio of excitation intensity at 335 nm vs. 310 nm as a function of polypyrrole concentration in the absence of pyrene.

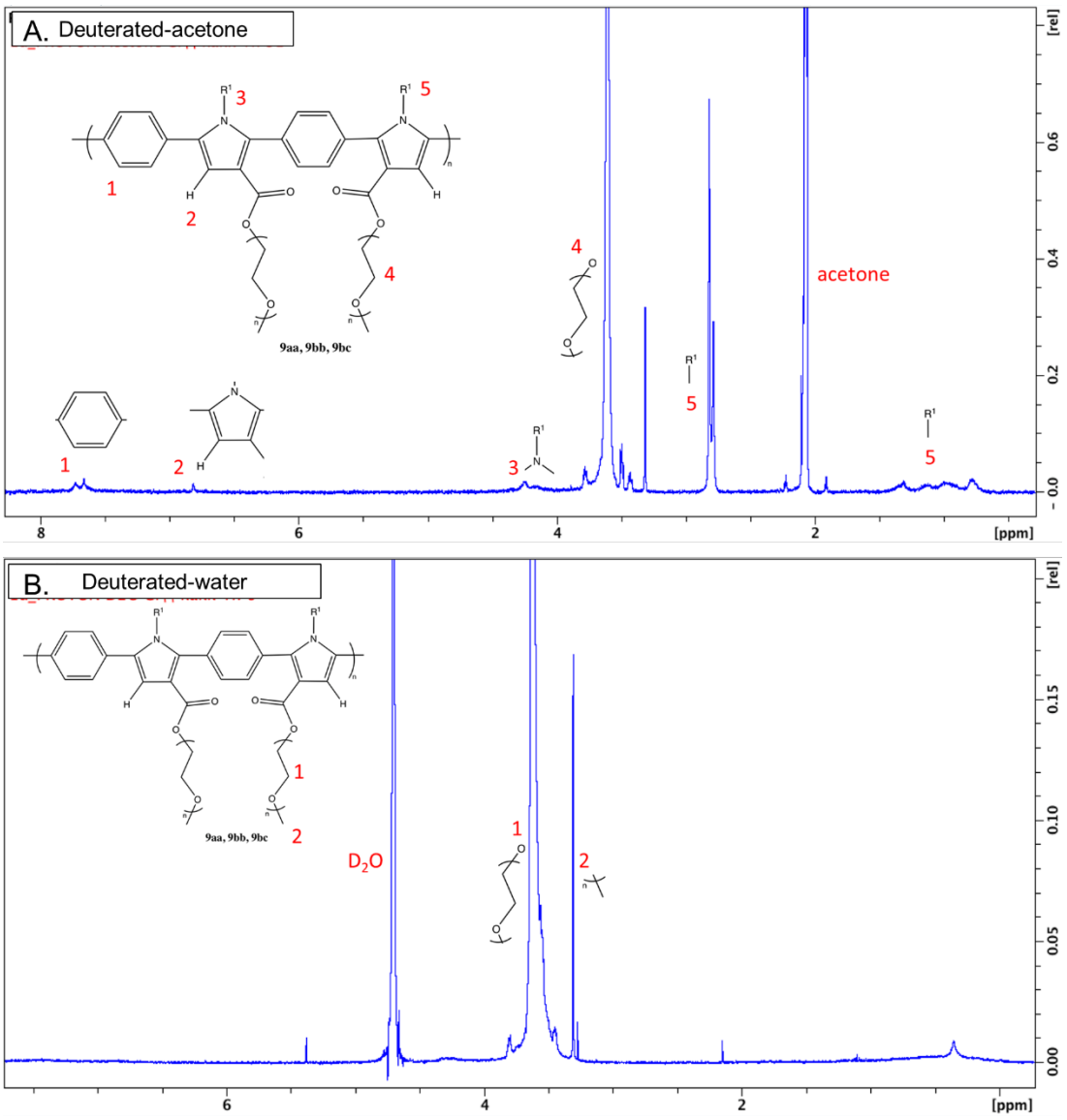


Figure S6. ^{1}H NMR spectra of polypyrrole polymer at concentration above CAC dissolved in deuterated acetone (A) and D_2O (B).

	$D_h \pm$ SEM (nm) Intensity distribution	D_h (nm) Number distribution
Unloaded NPs	220.1 ± 23.1	31.3
Cur-NPs	154.3 ± 41.1	21.5
Res-NPs	166.5 ± 12.3	21.4
Cel-NPs	283.9 ± 71.7	45.5

Table S1. Hydrodynamic diameters (intensity weighted, and number weighted)of unloaded polypyrrole-based nanoparticles and loaded with curcumin, resveratrol and celastrol.

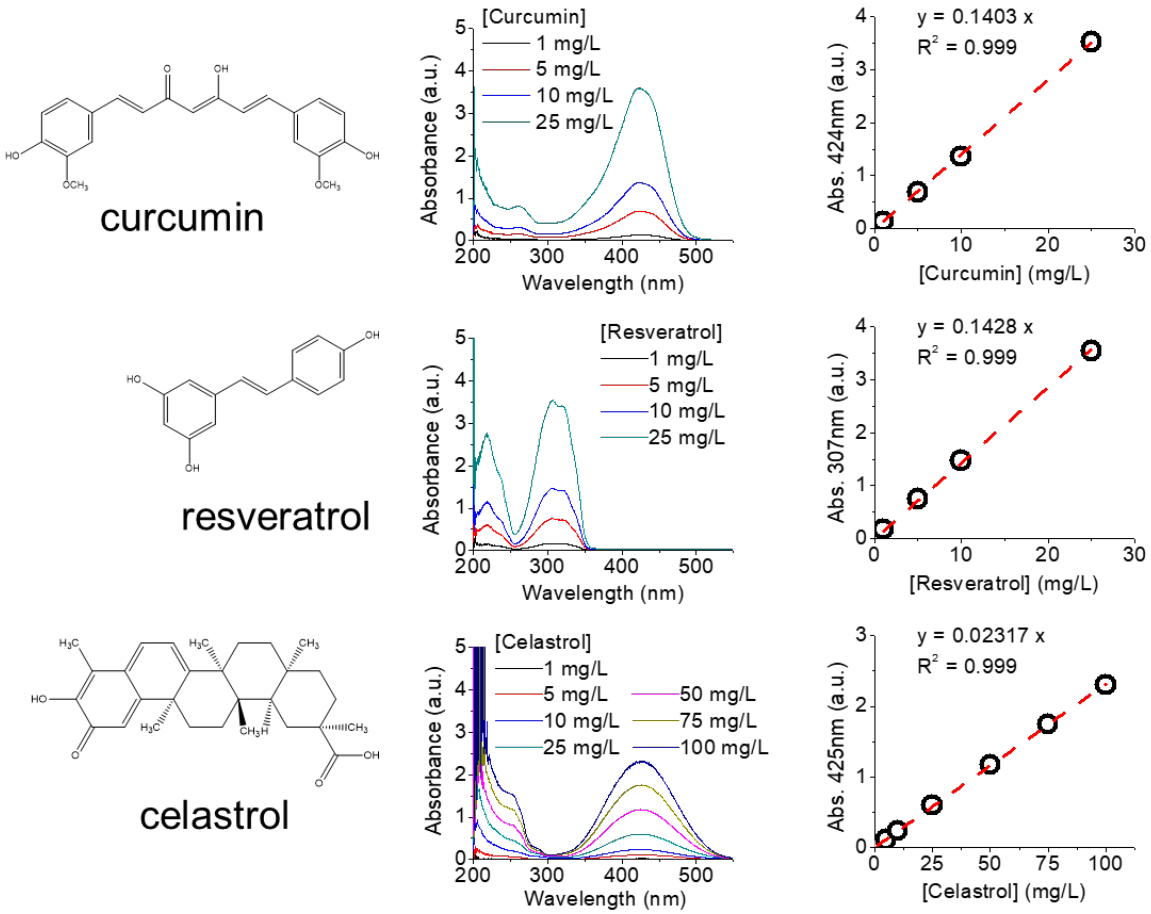


Figure S7. (left) Chemical structure of curcumin, resveratrol and celastrol. (middle) UV-visible absorption spectra of curcumin, resveratrol and celastrol in methanol at varying concentrations. (right) calibration curves of the three drugs measured at 424 nm for curcumin, 307 nm for resveratrol and 425 nm for celastrol in methanol.

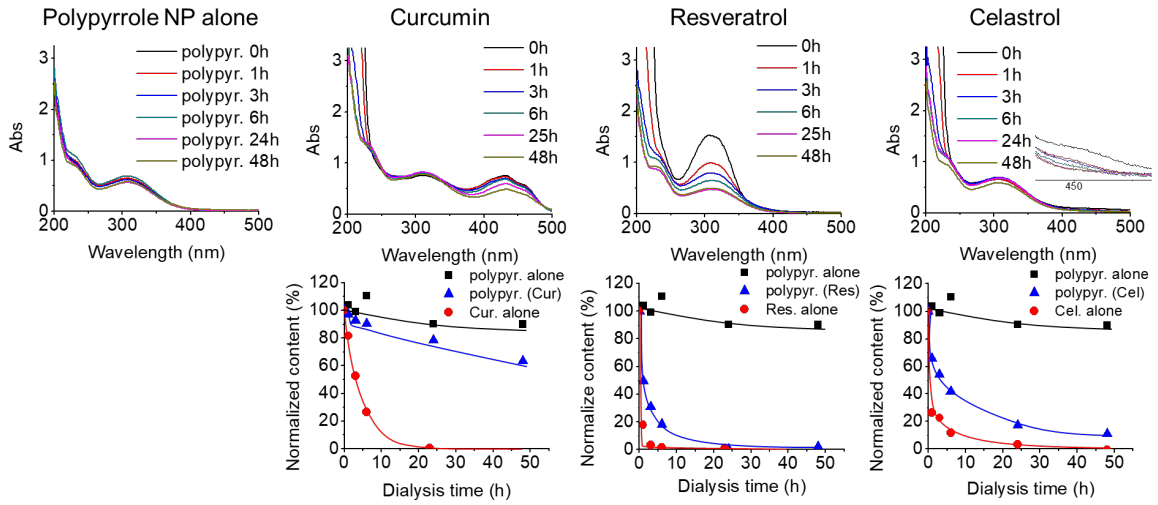


Figure S8. Drug release kinetic study using dialysis (regenerated cellulose dialysis bag, 3.5 kDa molecular weight cut-off). Normalized drug content was plotted as a function of incubation time by measuring drug absorbance from aliquots dissolved in methanol.

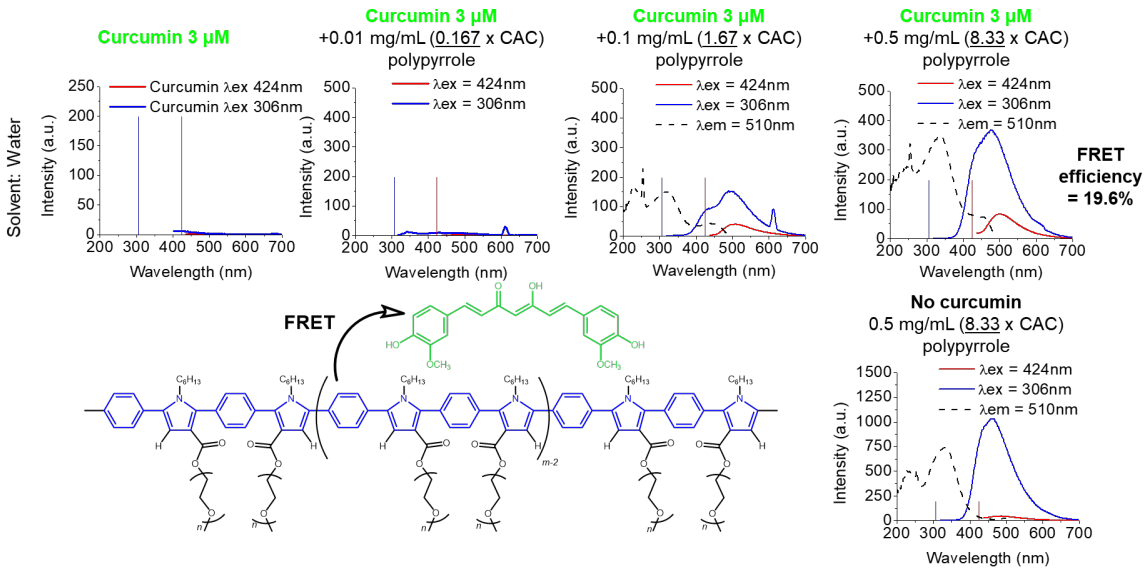


Figure S9. (Top) Fluorescence excitation and emission spectra of curcumin (3 µM) in water alone or in the presence of increasing amounts of the polypyrrole system (C<CAC, C~CAC, C>CAC). (Bottom) Schematic of fluorescence resonance energy transfer between the polypyrrole backbone and curcumin when in close proximity.

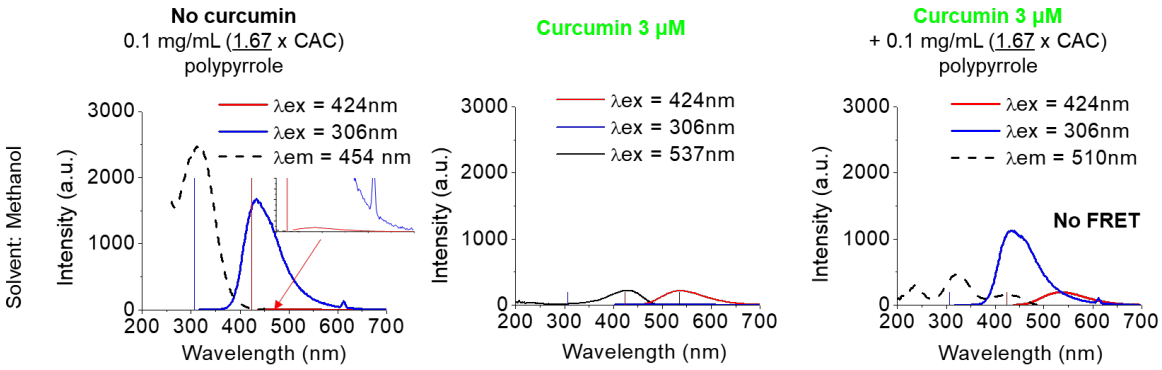
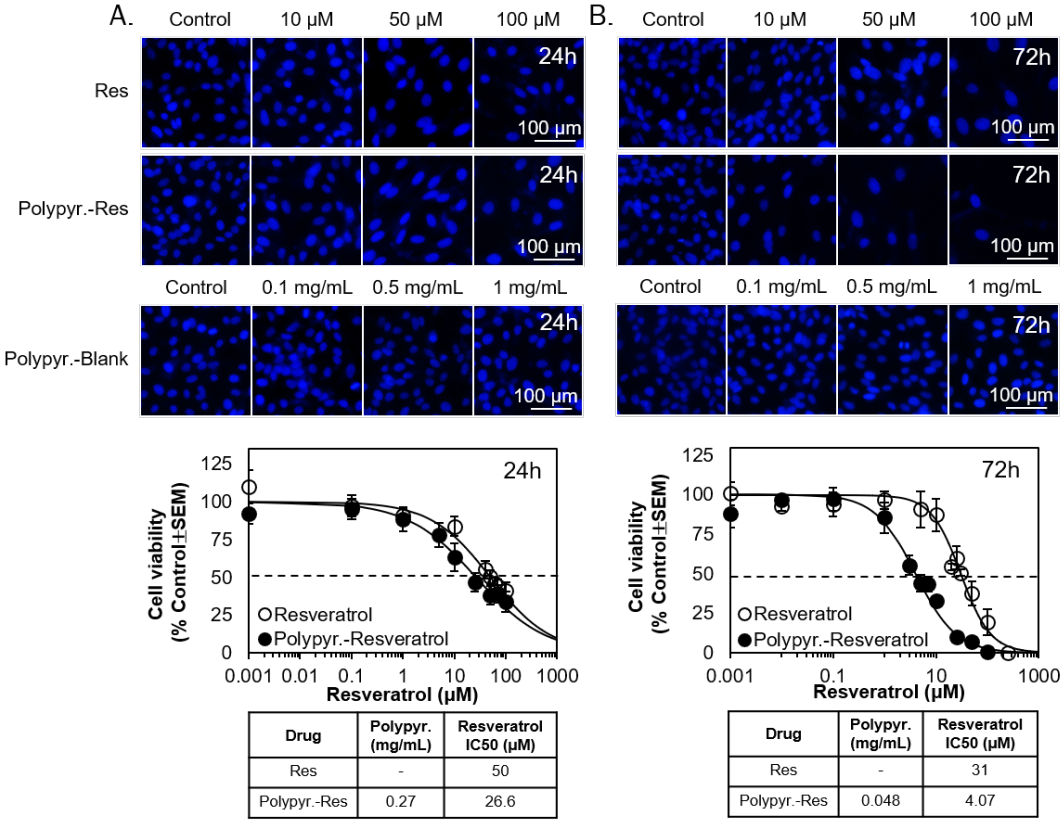


Figure S10. Fluorescence excitation and emission spectra of polypyrrole, curcumin and the mixture of polypyrrole with curcumin at 0.1 mg/mL polypyrrole concentration and 3 µM curcumin concentration in methanol.



Drug	Polypyr. (mg/mL)	Resveratrol IC50 (µM)
Res	-	50
Polypyr.-Res	0.27	26.6

Drug	Polypyr. (mg/mL)	Resveratrol IC50 (µM)
Res	-	31
Polypyr.-Res	0.048	4.07

Figure S11. (A) U251N cells were treated for 24 hours with polypyrrole loaded-resveratrol or resveratrol at a wide range of resveratrol concentrations (0-100 µM) in serum-supplemented media. IC50: 26.6±4.38 µM (polypyr.-resveratrol) vs 50.0±1.59 µM (resveratrol); p=0.0085. U251N cells were treated for 24 hours with blank polypyrrole-based nanoparticles at a wide range of polypyrrole concentrations (0-1 mg/mL) in serum-supplemented media. IC50: 4.16±0.04 mg/mL (polypyr.-blank).(B) U251N cells were treated for 72 hours with polypyrrole loaded-resveratrol or resveratrol at a wide range of resveratrol concentrations (0-250 µM) in serum-supplemented media. IC50: 4.07±0.52

μM (polypyr.-resveratrol) vs 31±1.00 μM (resveratrol); p=0.00016. U251N cells were treated for 72 hours with blank polypyrrole-based nanoparticles at a wide range of polymer concentrations (0-1 mg/mL) in serum-supplemented media. IC50: 0.95±0.05 mg/mL (polypyr.-blank). Following treatment, cells were fixed with 4% paraformaldehyde and labeled with 10 μM Hoechst 33342 nuclear stain. Average values were normalized to untreated controls and standard error of the mean (SEM) are shown from at least three independent experiments performed in quadruplicates.

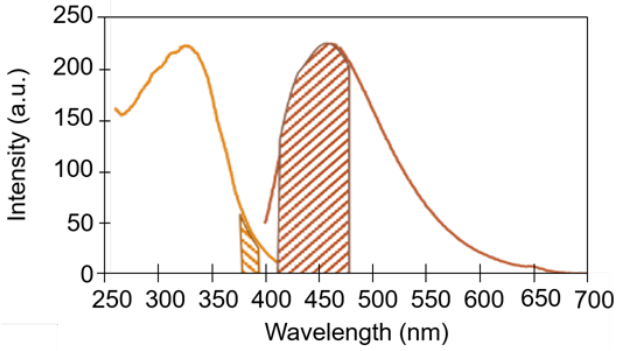


Figure S12. Comparison of the filter settings of the fluorescent microscope (hashed region) *vs.* the excitation and emission spectra of the polypyrrole polymer.

II. Synthetic Procedures

General: The chemicals and reagents employed in this study were used as received from Sigma-Aldrich. Nuclear magnetic resonance (NMR) spectra were acquired on Mercury instruments (Varian, Palo Alto, CA, USA), operated through VNMRJ 2.2D (Chempack 5) and VNMRJ 2.3A (Chempack 5) software using a 5 mm smart probe. The chemical shifts in ppm are reported relative to tetramethylsilane (TMS) as an internal standard for ^{1}H and ^{13}C in deuterated solvents. Mass spectra analyses (HRMS, ESI) were performed and analysed on an Exactive Plus Orbitrap-API (Thermo Scientific) high-resolution mass spectrometer and on MALDI Autoflex III – TOF (Brucker) instrument. GPC analysis was performed with a styrene divinyl benzene column using THF as eluent.

Synthesis of Poly(ethylene glycol) methyl ether monopropiolate ester (1a): The alkyne was prepared using a modified procedure of that reported by Qie et al.[1] In a Dean-Stark trap apparatus, $MPEG_{350}$ (4.22g, 0.012 mol), propionic acid (2.11g, 0.03 mol) and toluenesulfonic acid (0.57g, 0.003 mol) were added together with benzene (6 mL) and DCM (10 mL). The trap was half-filled with anhydrous Na_2SO_4. The reaction was heated to 70 °C for 8 hours under nitrogen. Afterwards, upon cooling the mixture to room temperature, it was extracted with 5% $NaHCO_3$ (aq, 10 mL) and brine (10 mL). Upon filtration, the solution was dried over anyhydrous Na_2SO_4. The solvent was then removed under vacuum and a dark orange liquid (1.34 g, 32%) was obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.59 (s, 1H), 4.27 (m, 2H), 3.63 (m, 2H), 3.52 (m, 21H), 3.42 (m, 2H), 3.25 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.5, 75.9, 74.4, 71.8, 70.5, 68.4, 65.1, 58.8.

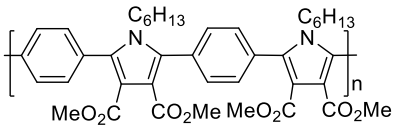
Synthesis of Poly(ethylene glycol) methyl ether monopropiolate ester (1b): The analogous procedure to that employed above was performed using $MPEG_{2000}$, affording **1b** as a pale yellow solid (16.58 g, 64%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.32 (t, 2H), 3.63 (m, 186H, PEG), 3.36 (s, 3H), 2.98 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.7, 75.5, 74.6, 72.0, 70.7, 68.6, 65.3, 59.1.

Synthesis of polymer 2a: Conjugated pyrrole-based polymers were prepared by adopting the procedure reported by the group of one of the authors.[2] In a glove box, bis-imine (R = $C_{12}H_{25}$, 75.4 mg, 0.16 mmol), terephthaloyl chloride (32.7 mg, 0.16 mmol) and (catechyl)PPh (139.1 mg, 0.64 mmol) were dissolved in 0.6 mL of dichloromethane in a sealed reaction vessel, and heated at 55 °C for 24h. The solution was then slowly added dropwise to a large vial (20 mL) containing DBU (68.5 mg, 0.48 mmol) in dichloromethane (5mL). The reaction mixture was left to stir at r.t. for 30 min before concentrating the solution under vacuum. The resulting dark solid was washed with 3x1mL of acetonitrile, dissolved/suspended in dichloromethane (10 mL), and then alkyne **1a** (180.9 mg, 0.48 mmol) was added. The reaction mixture was left to stir at r.t. for 18h in the glovebox. Subsequently, the reaction was brought out of the glovebox and quenched with water (0.1 mL). The polymer was extracted from DCM/H_2O (1:1) and filtered through an alumina plug at room temperature. The volatiles were removed in vacuo to afford polymer **2a** (101 mg, 46% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (s, 8H), 6.81 (s, 2H), 4.26 (t, 4H), 3.96 (m, 4H), 3.64 (s, 52H), 3.37 (s, 6H), 1.21-0.85 (m, 46H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.3, 139.5, 134.3, 132.34, 132.24 130.4, 129.2,

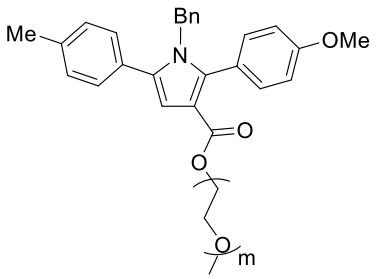
113.6, 111.5, 71.9, 70.6, 69.2, 62.6, 59.0, 31.9, 30.5, 29.7, 29.6, 29.5, 29.4, 29.3, 28.9, 26.3, 22.7, 14.1.

Synthesis of polymer 2b: In a glovebox, bis-imine (R = C_6H_{15}, 94.3 mg, 0.31 mmol), terephthaloyl chloride (63.7 mg, 0.31 mmol) and (catechyl)PPh (162.9 mg, 0.75 mmol) were dissolved with 1 mL of dichloromethane in a sealed reaction vessel, and heated at 55 °C for 24h. The solution was then slowly added dropwise to a large vial (20 mL) containing DBU (143.4 mg, 0.94 mmol) in dichloromethane (5mL). The reaction mixture was left to stir at room temperature for 30 min before concentrating the solution under vacuum. The resulting dark solid was washed with 3x1mL of acetonitrile, dissolved/suspended in dichloromethane (10 mL) and then alkyne **1b** (1.93 g, 0.94 mmol) was added. The reaction mixture was left to stir at room temperature for 60h in the glovebox. Subsequently, the reaction was brought out of the glovebox and quenched with water (0.1 mL). The polymer was extracted from DCM/iced H_2O (1:1) and filtered through an alumina plug. The volatiles were removed in vacuo to afford the desired polymer **2b** (1.41 g, 61% yield). ^{1}H NMR (500 MHz, MeOD) δ 7.63 (br, 8H), 6.80 (s, 2H), 4.23 (m, 4H), 3.77- 3.44 (m, 416H), 3.35 (s, 6H), 1.28-0.72 (m, 22H). ^{1}H NMR average molecular weight analysis (see spectra below): 47.3 kDa. MALS GPC: 10.3 kDa (PDI = 1.4).

Synthesis of model polymer. An analogous procedure to that above for **2b** was followed employing dimethylacetylene dicarboxylate (DMAD, 132.1 mg, 0.94 mmol) as the alkyne. 24h after addition of the alkyne, the reaction was brought out of the glovebox and quenched with water (0.1 mL). The polymer was precipitated and triturated with methanol (3x5 mL) then redissolved in $CHCl_3$ and filtered through an alumina plug. The volatiles were removed in vacuo to afford the polymer as a yellow solid (151 mg, 70% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (s, 8H), 3.82 (s, 4H), 3.68 (s, 12H), 1.43 – 0.72 (m, 22H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.1, 136.0, 131.5, 130.4, 115.1, 51.5, 45.4, 30.9, 30.3, 26.0, 22.3, 13.8. GPC: M_n = 7.4 kDa, PDI = 3.2.



Synthesis of model pyrrole. In order to confirm the polymer repeat unit structure, the model pyrrole shown was generated by the following procedure: In the glovebox, (4-$CH_3C_6H_4$)HC=NBn (93.5 mg, 0.45 mmol) and 4-methoxybenzoyl chloride (76.3 mg, 0.45 mmol) were combined in 1.0 mL of chloroform and allowed to stand for 30 min. PPh(catechol) (146.1 mg, 0.68 mmol), DBU (136.0 mg, 0.90 mmol) and **1a** (539.3 mg, 1.35 mmol) were added in order as chloroform solutions to obtain a total of 2.0 mL chloroform. The reaction was complete 60 minutes after all additions were made. The solution was concentrated *in vacuo* to give the crude product which was purified by column chromatography using ethyl acetate/haxanes (5%-30%) as eluent. A yellow product (151.1 mg, 86%) was obtained. ^{1}H NMR (500 MHz, CD_3CN) δ 7.31 (d, J = 8.1 Hz, 2H), 7.24 (d, J = 8.8 Hz, 2H), 7.19 (d, J = 7.9 Hz, 2H), 7.15 (m, 3H), 6.91 (d, J = 8.8



Hz, 2H), 6.70 (s, 1H), 6.60 (d, J = 5.6 Hz, 2H), 4.17 (m, 2H), 3.81 (s, 3H), 3.55 (m, 12H), 3.46 (m, 2H), 3.30 (s, 3H), 2.35 (s, 3H) ^{13}C NMR (126 MHz, CD_3CN) δ 165.1, 160.7, 140.8, 139.3, 138.7, 136.1, 133.0, 130.5, 130.1, 130.0, 129.2, 127.9, 126.5, 125.0, 115.2, 114.12, 111.3, 72.6, 71.2, 71.1, 71.0, 63.6, 58.8, 55.9, 49.0, 21.1.

III. Self-Assembly and Analysis of Polymer 2b

Drug Calibration Curves

The UV-Vis absorption spectra were taken on the Varian Agilent CARY50 Bio spectrophotometer.

Calibration curves were prepared for each drug and for the polypyrrole system by plotting the absorbance at the species absorption peaks (celastrol λ = 425 nm, curcumin λ = 424 nm, resveratrol λ = 307 nm, polypyrrole λ = 308 nm) as a function of the drug's/polymer's concentration. Methanol was used as the solvent for the drugs and polypyrrole system as it is a good solvent over the range of concentrations used and is optically clear in the UV-visible region at which the species absorb, which is not the case for acetone which has a strong absorption peak centered at 275 nm. The calibration curves were plotted between 1-25 mg/L for resveratrol and curcumin and 1-100 mg/L for celastrol and the polypyrrole.

Preparation of Blank and Drug-Loaded Nanocarriers

The co-solvent evaporation method was used to induce the self-assembly of the polypyrrole system.[3] Specific weights of the polypyrrole system and drugs were weighed and dissolved in tetrahydrofuran (THF) to a concentration of *ca.* 1 mg/mL in polymer. The solution was added dropwise (1 drop/10 s) to 2 mL of magnetically stirred deionized water. The mixture was stirred in the dark for 24 h to remove THF and trigger nanoparticle formation. The mixture was diluted to a polymer concentration of 2 g/L and filtered through a 0.45 μm PVDF filter to remove the precipitated un-encapsulated drug. Aliquots of the micellar solutions were tested by dynamic light scattering (DLS) to determine the hydrodynamic diameter (D_h) and polydispersity index (PDI) of the nanoparticles.

Physico-chemical Characterization

NMR spectra of the polymer were taken by a 400 MHz Bruker NMR Spectrometer. NMR spectra were performed in different deuterated solvents such as deuterated water, deuterated methanol (MeOD), and deuterated DMSO. The chemical shifts are relative to

tetramethylsilane (TMS) as the internal standard for the 1H NMR spectra. Molecular weight of the polymer was estimated by analysing the integrations of the 1H NMR spectra of polymer 1 in MeOD and by using GPC-MALS.

Gel permeation chromatography (GPC) measurements were carried out using a GPC system with an Agilent 1100 isocratic pump, a Dawn EOS multiangle laser light scattering detector (Wyatt Technology Corp., Santa Barbara, USA) and an Optilab DSP interferometric refractometer (Wyatt Technology Corp.) using a styrene divinyl benzene column eluted with THF at 40 °C at a flow rate of 0.3 mL/min. Solutions for analysis had a polymer concentration of 1.0 mg/mL and the injection volume was set at 100 μL. For dη/dC measurements, solutions of each polymer of concentration ranging from 0.1 to 1.25 mg/mL were prepared in THF. The refractive index change was plotted as a function of elution time. The light scattering intensity was plotted as a function of elution time at 100°. MALS was used to plot the molar mass of the eluting sample.

The dynamic light scattering (DLS) data was measured using a Brookhaven 90Plus particle size analyser. The refractive index used was that of poly(styrene) (1.587). The samples were filtered through a 0.45 μm PVDF membrane prior to measurements. This machine was used to determine the mean hydrodynamic diameter and the polydispersity index of the nanocarriers with and without the drugs. Triplicates of each solution were taken at room temperature. UV-Vis absorption data were obtained with an Agilent CARY50Bio spectrometer and an Agilent CARY5000 spectrometer.

Asymmetrical flow field-flow fractionation (AF4, Eclipse from Wyatt Technologies) with UV-vis diode (Shimadzu) and dynamic light scattering detectors (Dawn Heleos II, Wyatt Technologies) was used to monitor elution of size separated polypyrrole polymer using UV-vis absorbance to distinguish absorption specific to the drugs and to the polypyrrole. Hydrodynamic sizes of the eluting species was monitored and measured as the size separated species eluted out of the AF4 channel by the in-line DLS instrument.

Drug loading and encapsulation efficiency

Aliquots of the solution were freeze dried and a known amount was resuspended in methanol to determine the weight proportion of the drug associated to the nanoparticles and assess the encapsulation efficiency. Encapsulation efficiencies and drug loading capacities were estimated by measuring the UV-Vis absorption of the different samples and comparing them with the calibration curves of the drugs alone in methanol.

The drug loading (D.L.) and encapsulation efficiency (E.E.) were calculated for each drug separately from the following equations:

$$E.E.(\%) = \frac{Weight\ of\ the\ drug\ in\ the\ nanoparticles}{Weight\ of\ the\ drug\ used\ initially}$$

$$D.L.(weight\ \%) = \frac{Weight\ of\ the\ drug\ in\ the\ nanoparticles}{Total\ weight\ of\ the\ nanoparticles\ tested\ (polymer + drug)}$$

Electron microscopy imaging

A Philips CM200 200 kV TEM with AMT XR40B CCD camera and EDAX genesis EAS analysis system was used to take TEM micrographs to get another estimate of the sizes of the nanocarriers and to see the shapes they would form. Micrographs were taken of blank nanocarriers and resveratrol-loaded nanocarriers and the size distribution was analyzed.

Critical aggregation concentration (CAC) determination

A pyrene stock solution (180 μM) in acetone was prepared. The same amount of the acetone solution (66.7 μL) was pipetted into 11 glass vials and the acetone was evaporated under air flow. The co-solvent evaporation method was applied by adding dropwise different quantities of the polypyrrole dissolved in THF into deionized water (2 mL) under constant stirring in the presence of the dry pyrene. The mixtures were stirred in the dark overnight until all the THF evaporated. Volumes were corrected to 2 mL by adding milli-Q water when necessary. Excitation spectra of the solutions were recorded between 260 and 360 nm measuring at fixed emission λ_{em} = 390 nm.

The ratios of the pyrene excitation intensities at 338 and 333 nm (I_{338}/I_{333}) were plotted against the concentration of the polypyrrole used in the co-solvent evaporation method.[4] The critical aggregation concentration was determined as the inflexion point.

Drug release studies

Drug release kinetic studies were done by dialysis (3.5 kDa molecular weight cut-off, regenerated cellulose, SpectrumLabs). The concentration of free drugs or of nanoparticle-encapsulated drugs were monitored as a function of dialysis time. Nanoparticles encapsulating celastrol, curcumin, or resveratrol were transferred into a dialysis tube which was placed in a beaker with constant stirring. The release media was phosphate-buffer saline (PBS, 10 mM at pH 7.4, NaCl 137 mM) containing 1% Tween-80 to maintain sink conditions. The beaker was incubated at 37°C for the duration of the release study. At predetermined time points (0h, 1h, 3h, 6h, 24h, and 50h) aliquots from the dialysis tube and from the release media were taken and analysed by UV-vis spectroscopy. The aliquot from the dialysis tube was diluted several folds in methanol to break apart the nanoparticles and solubilize the drugs. Concentrations were measured using the calibration curves. Free drugs were first dissolved in DMSO and diluted in a solution of PEG_{800}/water/dimethyl acetamide (45:40:15, v/v/v) which was placed in the dialysis tubing.

Fluorescence Quantum Yield Determination

The quantum yield of the polypyrrole was calculated from the following equation:

$$Q_{polypyrrole} = Q_{ref} \frac{I_{polypyrrole}}{I_{ref}} \frac{A_{ref}}{A_{polypyrrole}} \frac{\eta_{polypyrrole}{}^2}{\eta_{ref}{}^2}$$

The equation above is used where $Q_{polypyrrole}$ is the quantum yield of the polypyrrole and Q_{ref} is the quantum yield of the reference standard. Here $I_{polypyrrole}$ and I_{ref} are the integrated fluorescence intensities of the polypyrrole and the reference after excitation at λ_{ex} = 306 nm, respectively, while $A_{polypyrrole}$ and A_{ref} are the respective absorbance values of the polypyrrole and the reference at $\lambda_{abs.}$ = 306 nm. $\eta_{polypyrrole}$ and η_{ref} are the refractive indices of the solvents used for polypyrrole and the reference, respectively. The absorbance and fluorescence of the polypyrrole were measured in water at 0.1 g/L of the polypyrrole.

The reference compound used was quinine sulfate in sulfuric acid 0.5 M (0.01 g/L, Q_{ref} = 0.546 at λ_{ex} = 306 nm).[5-7] This solution was diluted until the absorption intensity at excitation wavelength of the polymer was approximately 0.1 to avoid inner filter effects.

Relative Fluorescence Resonance Energy Transfer determination

Relative fluorescence resonance energy transfer (FRET), also known as the proximity ratio, was calculated as $E_{rel.} = \frac{I_A}{I_A + I_D}$, where I_A and I_D are the total acceptor and donor fluorescence intensities following donor excitation. As the donor and acceptor spectra are overlapping, the mixed spectra were first decomposed into the isolated donor and acceptor component spectra. As curcumin is not excited using donor excitation, the contribution from direct acceptor excitation to I_A was considered negligible. Polypyrrole and curcumin have similar quantum yields and the relative FRET efficiency was not corrected for this difference. Curcumin's quantum yield in ethanol is reported to be 6.3% with λex = 430 nm.[8]

Cell culture

Human U251N GBM cells were originally obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Life Technologies Inc. Burlington, ON, Canada) in the presence of 5% (v/v) fetal bovine serum (FBS; Gibco) and supplemented with 1% penicillin-streptomycin (P/S; Gibco). Cells were incubated at 37°C with 5% CO_2 and 95% relative humidity.

In vitro imaging of polypyrrole-based nanocarriers in U251N glioblastoma cells

U251N cells were seeded at a density of 5,000 cells per 12 mm diameter coverslip (Glaswarenfabrik Karl Hecht GmbH & Co., KG, 97647 Sondheim, Germany) and left to attach for 24 hours in serum-supplemented DMEM media (5% FBS, 1% P/S). U251N cells were treated with polypyrrole-based nanoparticles at different concentrations (0, 0.17 and 0.27 mg/mL) for 4 hours. Cells were stained with DRAQ5 nuclear dye (Pierce Biotechnology, Rockford, IL, USA) and incubated at 37°C for 20 minutes. Cells were washed with PBS prior to imaging. Cells were imaged using Leica DMI 4000B fluorescence microscope (Leica microsystems) with DAPI-1160A-LDMK-ZERO and CY5-404A-LDMK-ZERO filters (Semrock. Rochester, NY, USA). Images were analyzed using ImageJ where average fluorescence intensity per cell ± SEM (background subtracted) was normalized to cell area (μm^2) and untreated controls. Images of polypyrrole nanoparticles were converted from blue to green pseudo-coloring.

Cell Viability Assay

U251N glioblastoma cells were seeded in 96-well cell culture plates (Corning Incorporated, Corning, NY, USA) at a density of 10,000 cells/well (for 24-hour cell viability assays) or 5,000 cells/well (for 72-hour cell viability assays) in serum-supplemented medium (DMEM, 5% FBS, 1% P/S). Cells were left to attach for 24 hours and washed with PBS before being treated for the following 24 or 72 hours. Following treatment, cells were fixed with 4% paraformaldehyde in phosphate buffer saline (PBS) for 10 minutes at room temperature. Paraformaldehyde was aspirated and cells were stained with 10 μM Hoechst 33342 (Thermo Fischer scientific, USA) for 10 minutes at room temperature. The 96-well cell culture plates were imaged using Leica DMI 4000B fluorescence microscope (Leica microsystems) and analyzed using ImageJ. Quadruplicate measurements were performed in at least three independent experiments. The average percentage of cell number normalized to untreated controls ±SEM were plotted against the concentration of the drug or polypyrrole used.

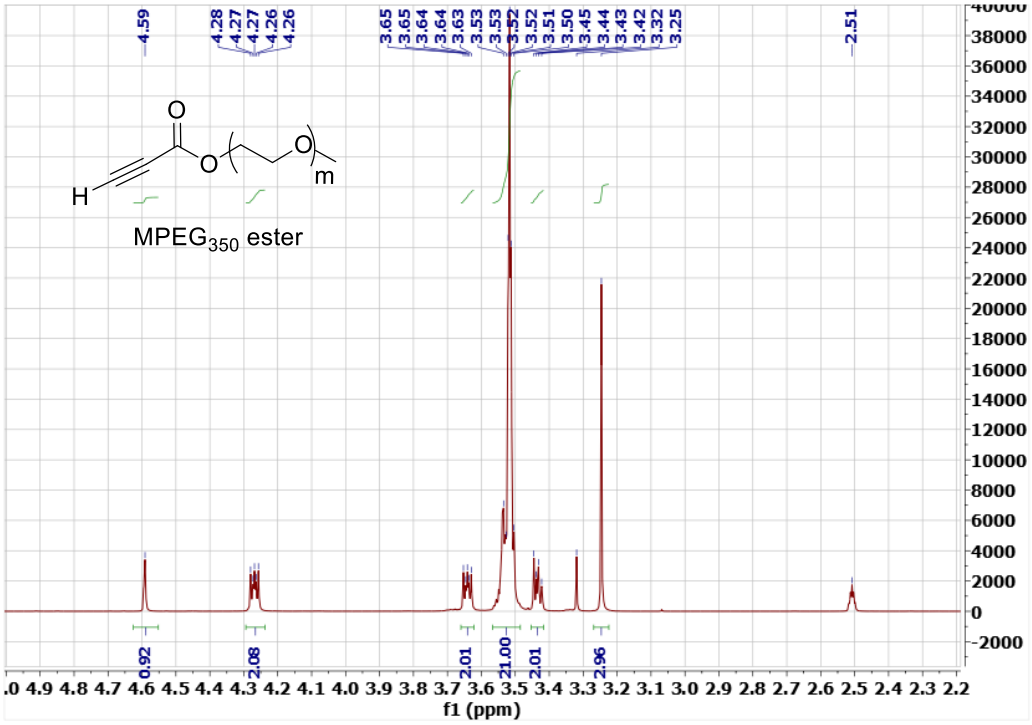
IV. References

1. Qie, J. J.; Wei, B.; Zhang, L. Q.; Yue, D. M., Synthesis and Self-Assembly of P-Chirality Helical Polymers Containing Mpeg Side Chains. *Acta Polymerica Sinica* **2012,** *10* (10), 1170-1176.
2. Kayser, L. V.; Vollmer, M.; Welnhofer, M.; Krikcziokat, H.; Meerholz, K.; Arndtsen, B. A., Metal-free, multicomponent synthesis of pyrrole-based π-conjugated polymers from imines, acid chlorides, and alkynes. *Journal of the American Chemical Society* **2016,** *138* (33), 10516-10521.
3. Soliman, G. M.; Sharma, R.; Choi, A. O.; Varshney, S. K.; Winnik, F. M.; Kakkar, A. K.; Maysinger, D., Tailoring the efficacy of nimodipine drug delivery using nanocarriers based on A2B miktoarm star polymers. *Biomaterials* **2010,** *31* (32), 8382-92.

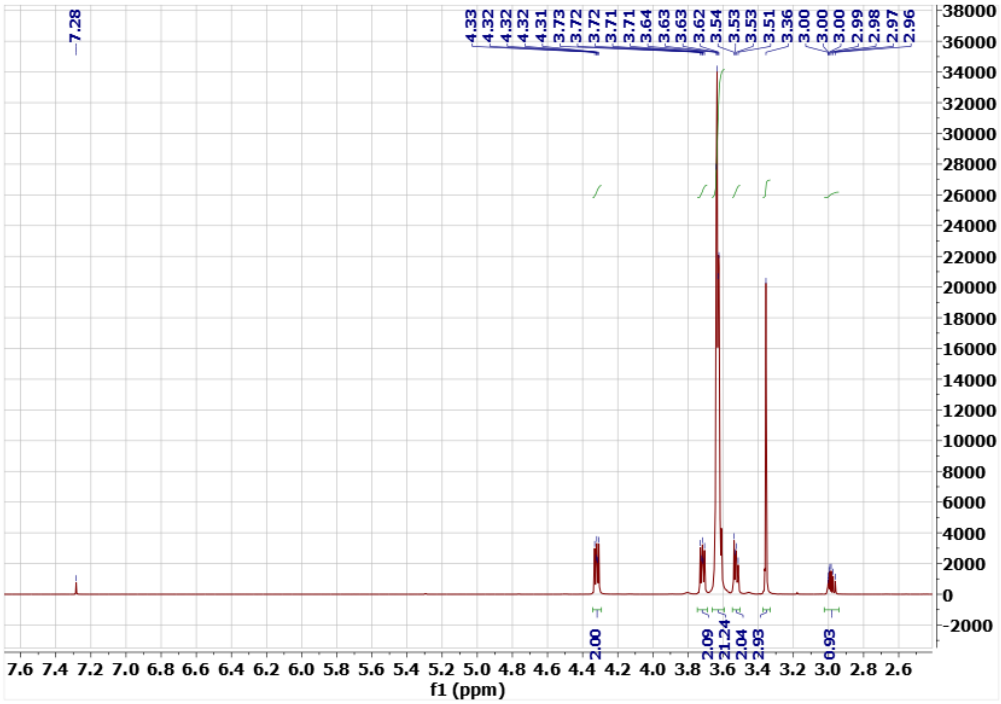
4. Soliman, G. M.; Redon, R.; Sharma, A.; Mejia, D.; Maysinger, D.; Kakkar, A., Miktoarm star polymer based multifunctional traceable nanocarriers for efficient delivery of poorly water soluble pharmacological agents. *Macromol Biosci* **2014,** *14* (9), 1312-24.
5. Brouwer, A. M., Standards for photoluminescence quantum yield measurements in solution (IUPAC Technical Report). *Pure and Applied Chemistry* **2011,** *83* (12), 2213-2228.
6. Eaton, D. F., Reference materials for fluorescence measurement. *Pure and Applied Chemistry* **1988,** *60* (7), 1107-1114.
7. Melhuish, W. H., A Standard Fluorescence Spectrum for Calibrating Spectro-Fluorophotometers. *Journal of Physical Chemistry* **1960,** *64* (6), 762-764.
8. Chignell, C. F.; Bilski, P.; Reszka, K. J.; Motten, A. G.; Sik, R. H.; Dahl, T. A., Spectral and photochemical properties of curcumin. *Photochem Photobiol* **1994,** *59* (3), 295-302.

V. ^{1}H and ^{13}C NMR Spectra

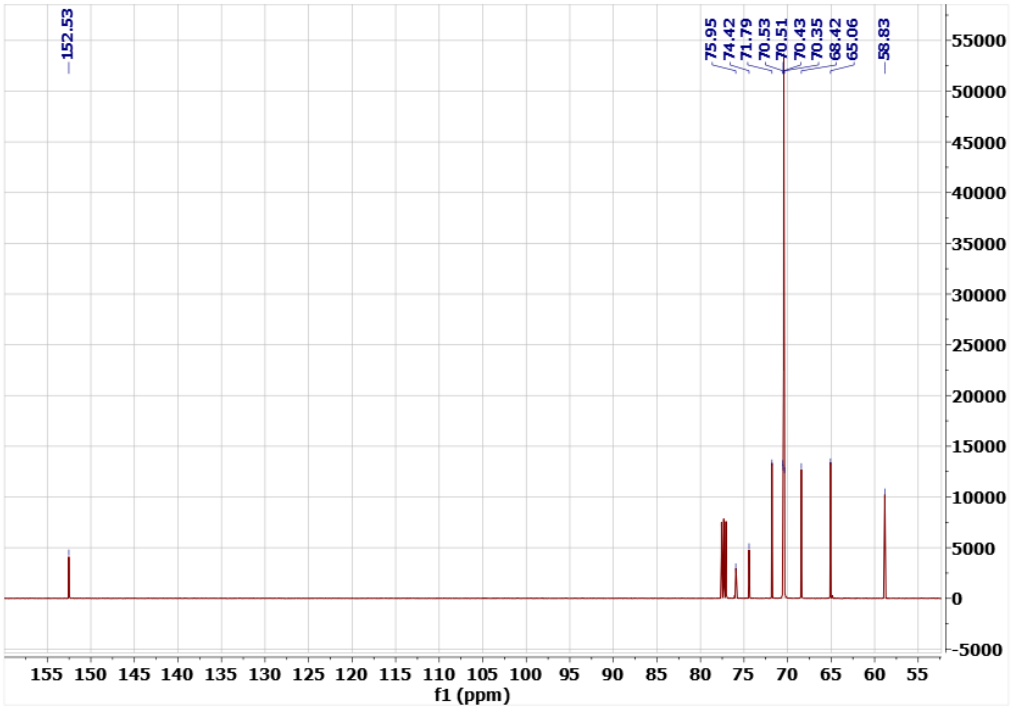
1a ^{1}H NMR (DMSO)



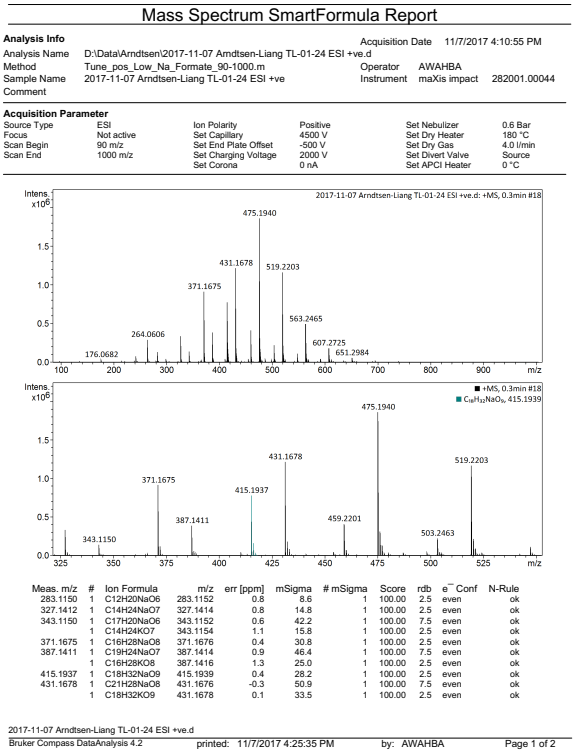
1a ^{1}H NMR ($CDCl_3$.)



1a ^{13}C NMR ($CDCl_3$).



1a Mass Spectrum report.



Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Arndtsen\2017-11-07 Arndtsen-Liang TL-01-24 ESI +ve.d	Acquisition Date	11/7/2017 4:10:55 PM
Method	Tune_pos_Low_Na_Formate_90-1000.m	Operator	AWAHBA
Sample Name	2017-11-07 Arndtsen-Liang TL-01-24 ESI +ve	Instrument	maXis impact 282001.00044
Comment			

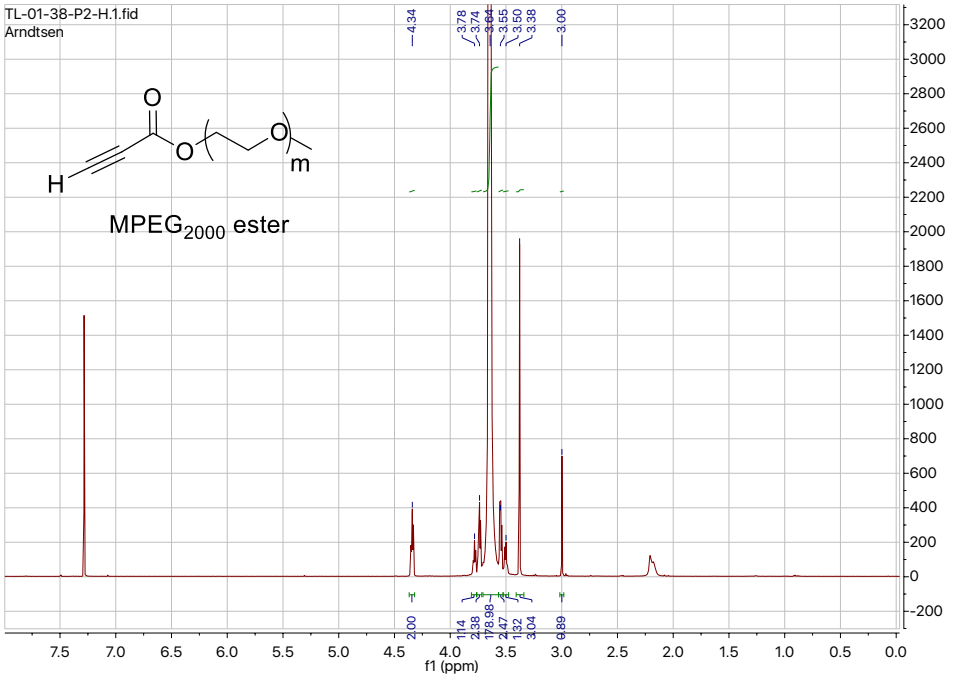
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	90 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

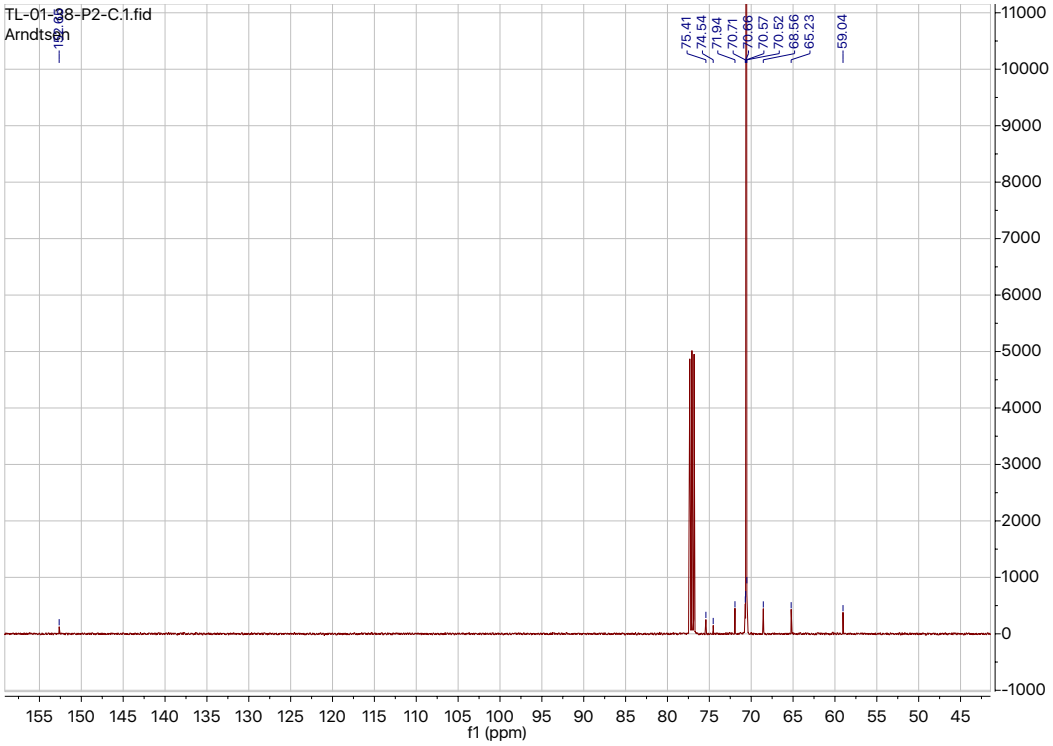
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
283.1150	1	C12H20NaO6	283.1152	0.8	8.6	1	100.00	2.5	even	ok
327.1412	1	C14H24NaO7	327.1414	0.8	14.8	1	100.00	2.5	even	ok
343.1150	1	C17H20NaO6	343.1152	0.6	42.2	1	100.00	7.5	even	ok
	1	C14H24KO7	343.1154	1.1	15.8	1	100.00	2.5	even	ok
371.1675	1	C16H28NaO8	371.1676	0.4	30.8	1	100.00	2.5	even	ok
387.1411	1	C19H24NaO7	387.1414	0.9	46.4	1	100.00	7.5	even	ok
	1	C16H28KO8	387.1416	1.3	25.0	1	100.00	2.5	even	ok
415.1937	1	C18H32NaO9	415.1939	0.4	28.2	1	100.00	2.5	even	ok
431.1678	1	C21H28NaO8	431.1676	-0.3	50.9	1	100.00	7.5	even	ok
	1	C18H32KO9	431.1678	0.1	33.5	1	100.00	2.5	even	ok

2017-11-07 Arndtsen-Liang TL-01-24 ESI +ve.d
Bruker Compass DataAnalysis 4.2 printed: 11/7/2017 4:25:35 PM by: AWAHBA Page 1 of 2

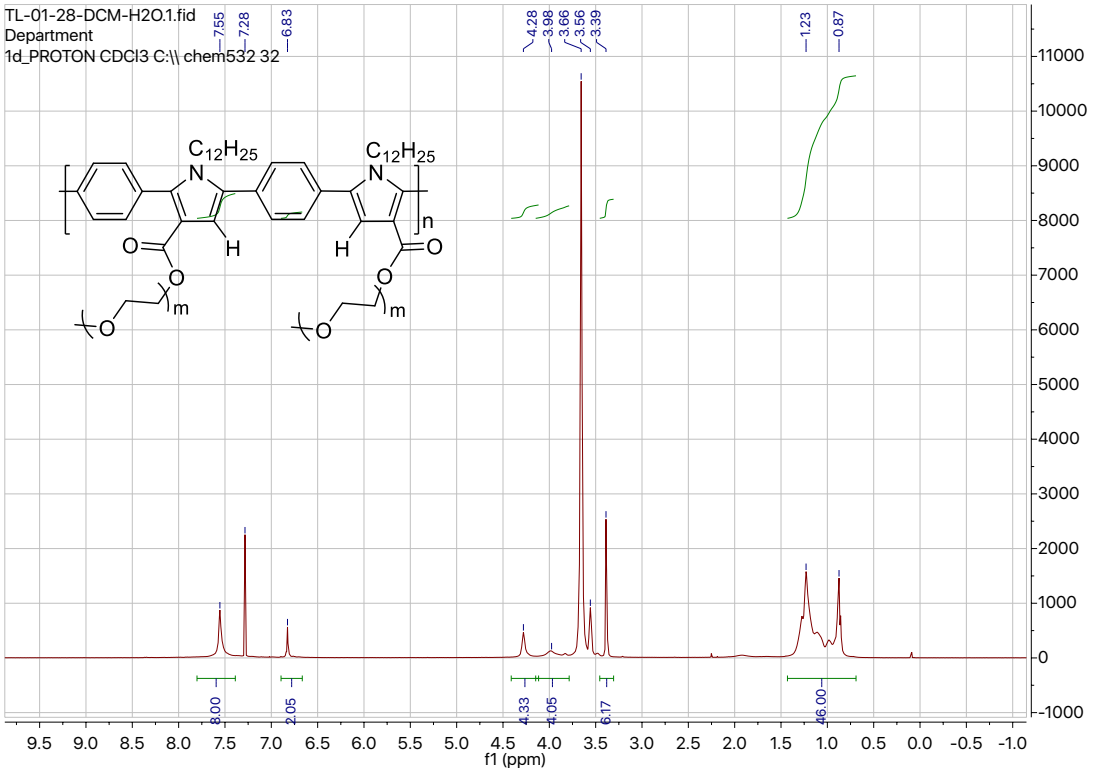
1b ^{1}H NMR ($CDCl_3$)



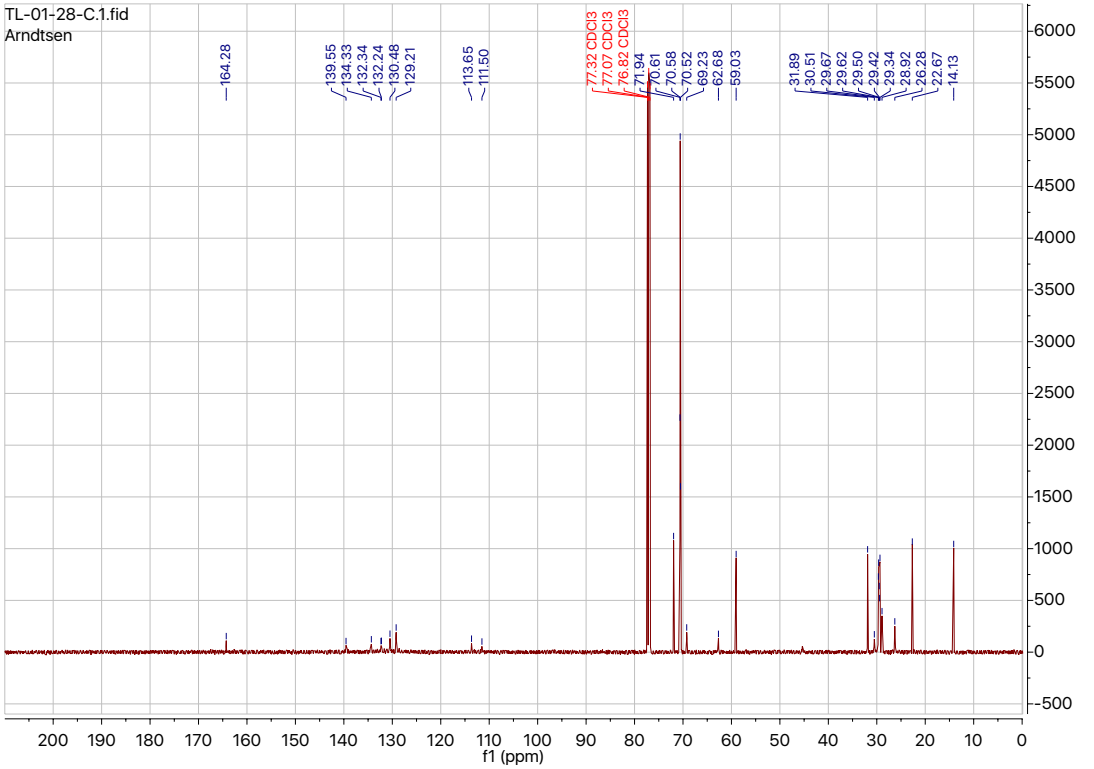
1b ^{13}C NMR ($CDCl_3$)



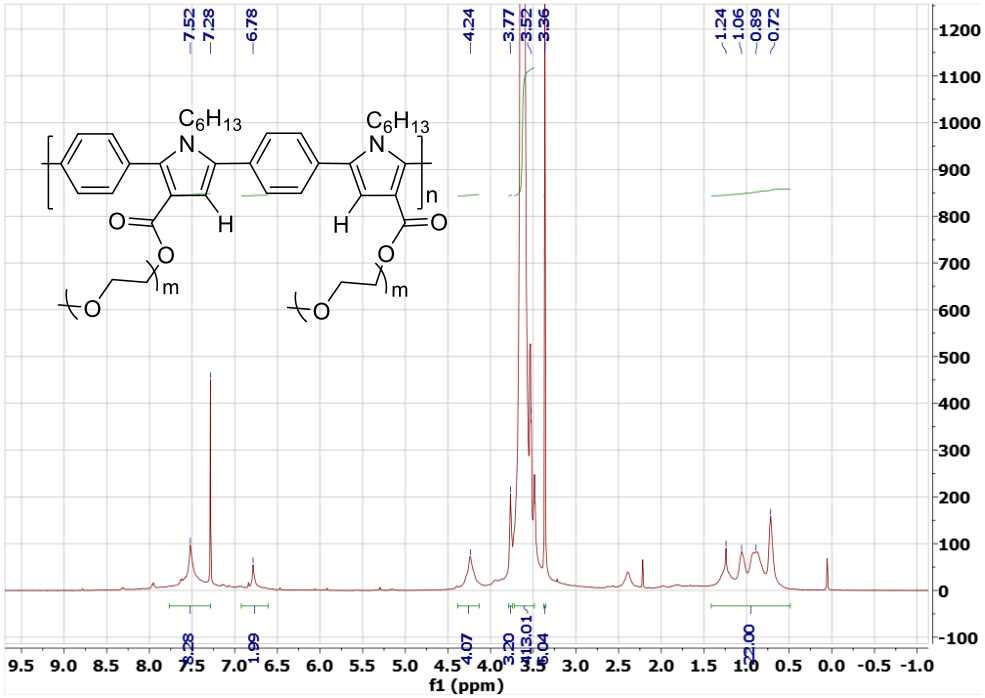
2a ^{1}H NMR ($CDCl_3$)

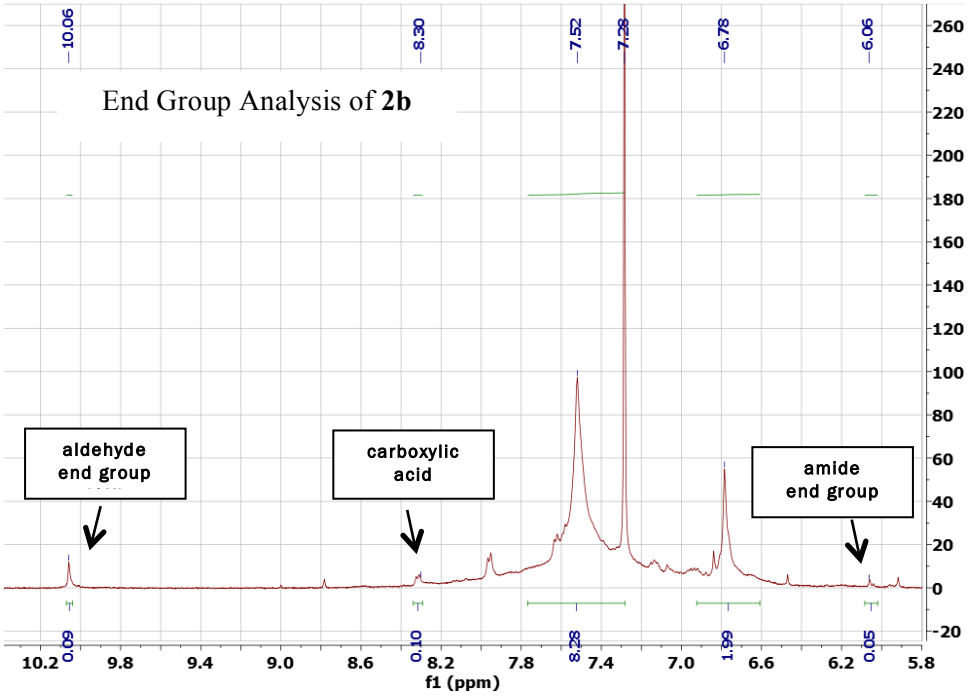


2a ^{13}C NMR ($CDCl_3$)



2b ^{1}H NMR ($CDCl_3$)

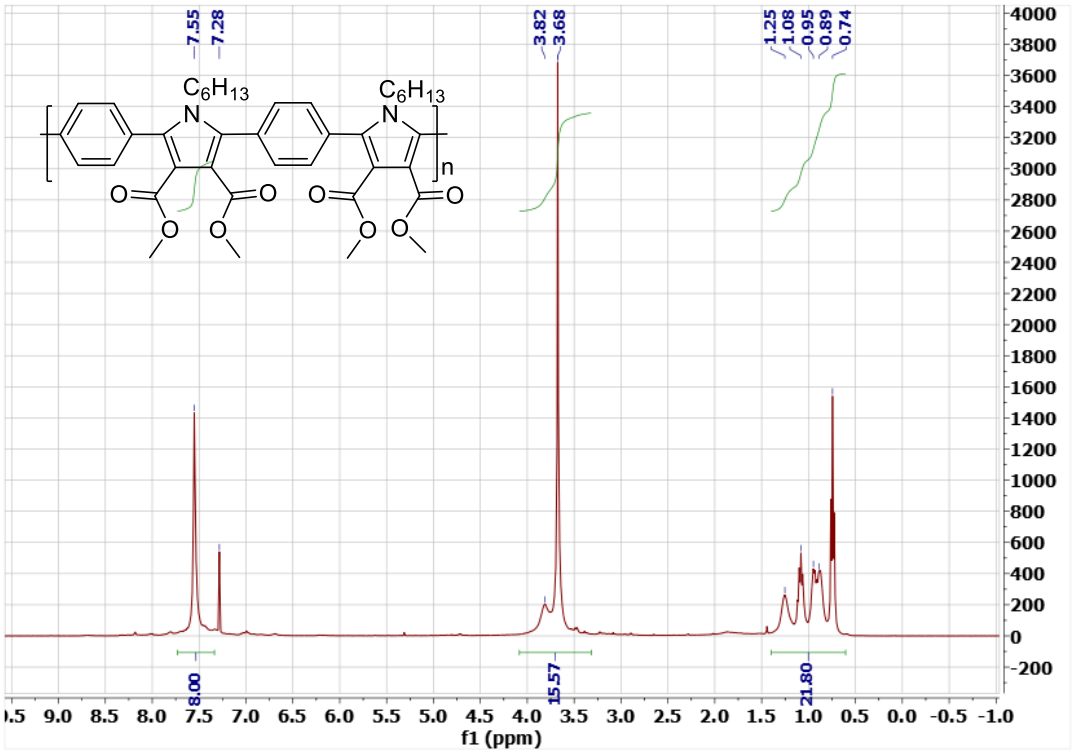




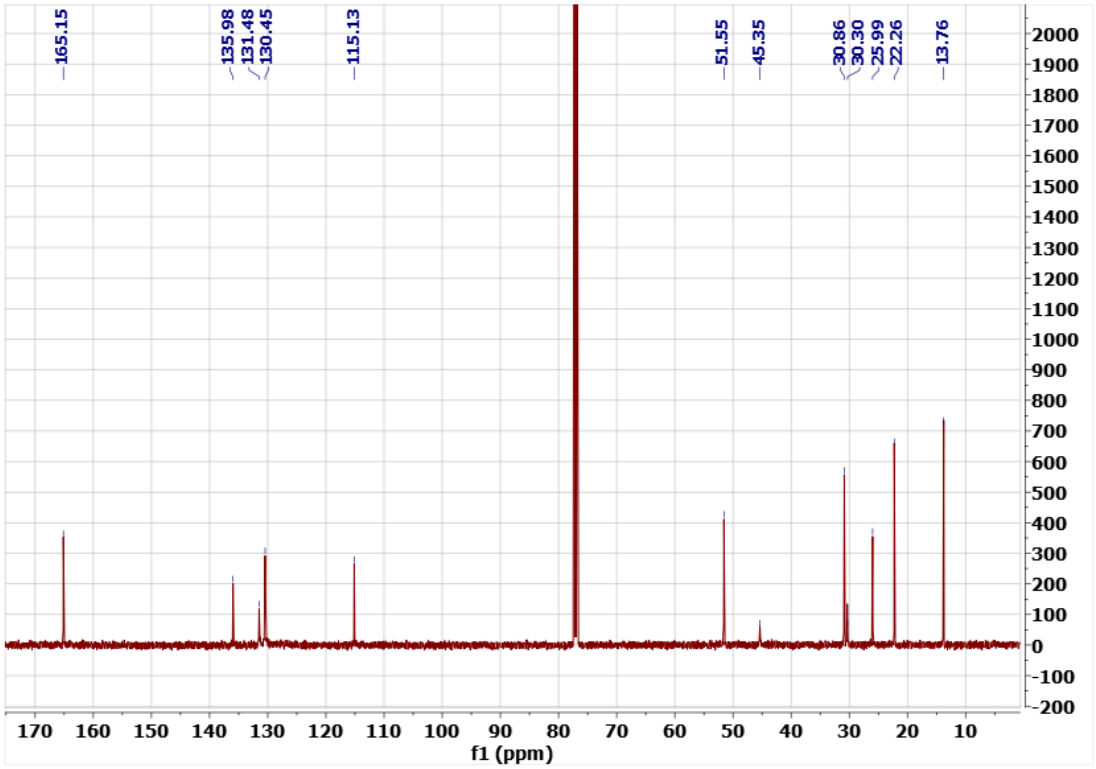
Number of repeating units: 2/(0.09+0.05+0.05)=10.5

Average Mw=4504 Da*10.5=47.3 kDa

Model polymer ^{1}H NMR ($CDCl_3$)

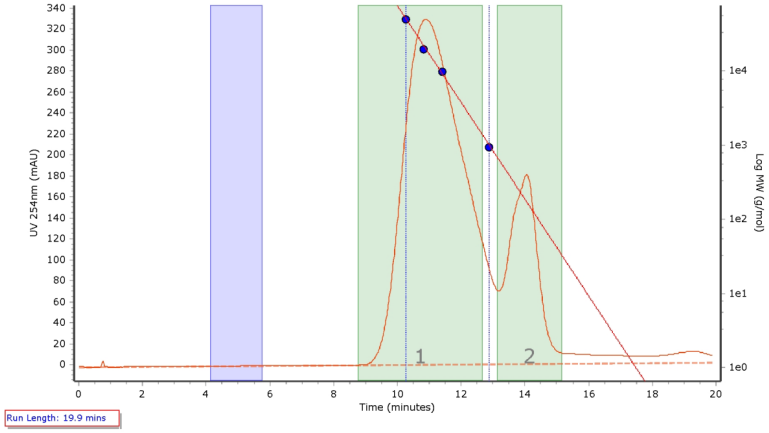


Model Polymer ^{13}C NMR ($CDCl_3$)



GPC of model polymer.

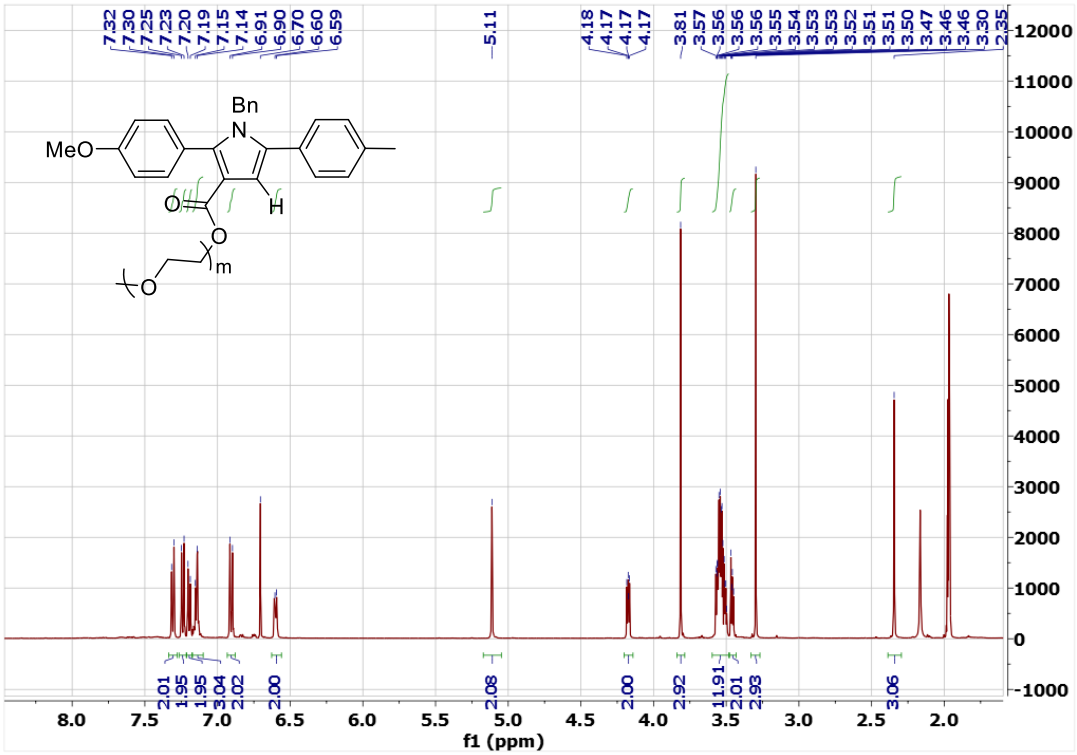
UV absorbance trace in $CHCl_3$ using a crosslinked polystyrene column (300 mm x 8.0 mm, 10 µm, target MW range 300-2000000 Da) at a flow rate of 0.8 ml/min. Sample was analyzed versus monodispersed polystyrene standards.



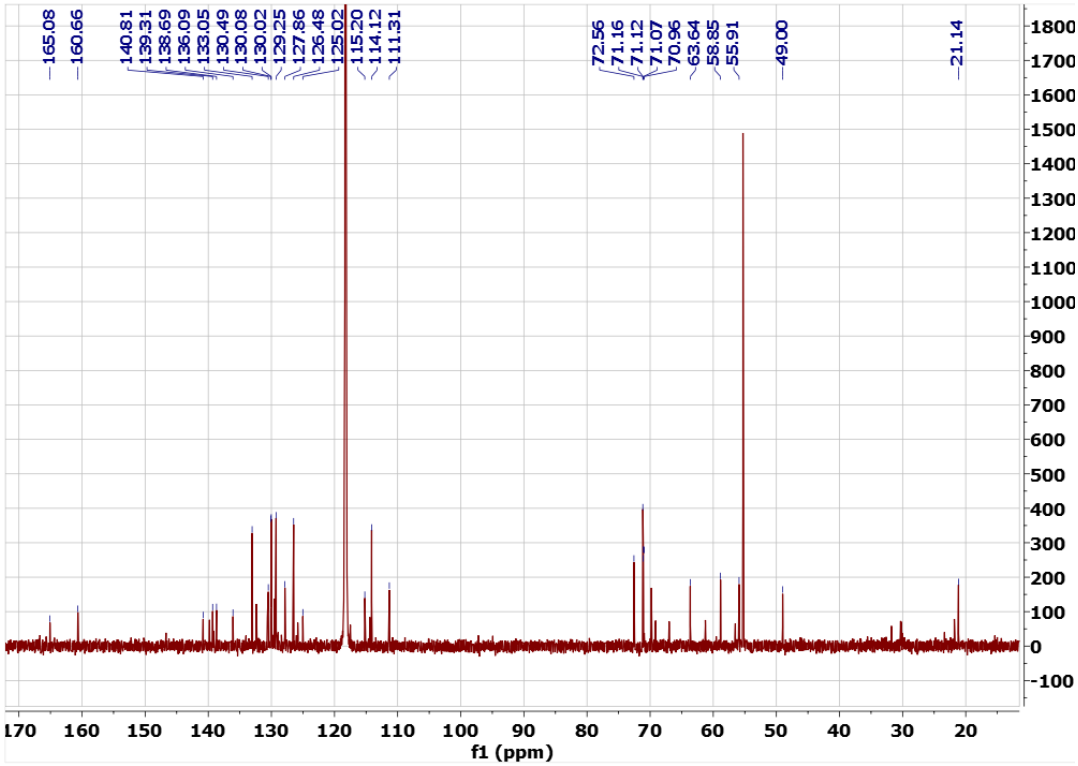
Molecular Weight Averages

Peak	Mp (g/mol)	Mn (g/mol)	Mw (g/mol)	Mz (g/mol)	Mz+1 (g/mol)	Mv (g/mol)	PD
Peak 1	19868	7441	24201	57608	98456	53390	3.252
Peak 2	170	163	237	322	401	313	1.454

Model pyrrole ^{1}H NMR (CD_3CN)



Model pyrrole ^{13}C NMR (CD_3CN)



Model pyrrole 2D NOESY (CD_3CN)