Nylon-3 Polymers with Selective Antifungal Activity

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Materials and Methods

Dulbecco's modified eagle medium (DMEM) and cell culture supplies were obtained from Invitrogen (Carlsbad, CA); NIH 3T3 fibroblast cells were obtained from the American Type Tissue Collection (ATCC, Manassas, VA); RPMI 1640 medium (11875119) was obtained from Life Technologies (Grand Island, NY); LB medium (244610) was obtained from BD (Franklin Lakes, NJ); agar (BP1423500) was obtained from Fisher Scientific (Pittsburgh, PA); amphotericin B (46006), α-poly-L-lysine at 1-5 KDa (P0879), and α-poly-L-lysine at 4-15 KDa (P6516) were obtained from Aldrich (St. Louis, MO); CytoTox-ONE assay kits (G7892) were obtained from Promega (Madison, WI); Easivial polymethyl methacrylate (PMMA) standard for GPC column calibration (PL2020-0200) was obtained from Polymer Varian (Palo Alto, CA). All other chemicals were purchased from Sigma-Aldrich and used without further purification. ^{1}H and ^{13}C NMR spectra were collected on a Varian MercuryPlus 300 spectrometer at 300 MHz and 75 MHz, respectively, using $CDCl_3$ or D_2O as the solvent. ^{1}H NMR chemical shifts were referenced to the resonance for residual protonated solvent (δ 7.26 for $CDCl_3$ and 4.79 for D_2O). ^{13}C NMR chemical shifts were referenced to the solvent (δ 77.16 for $CDCl_3$). Mass spectra were acquired using either a Waters (Micromass) LCT mass spectrometer or a

Waters (Micromass) AutoSpec mass spectrometer. IR spectra were acquired on a Bruker Tensor 27 instrument with an ATR attachment (Pike Technologies).

Synthesis of β-lactam NM

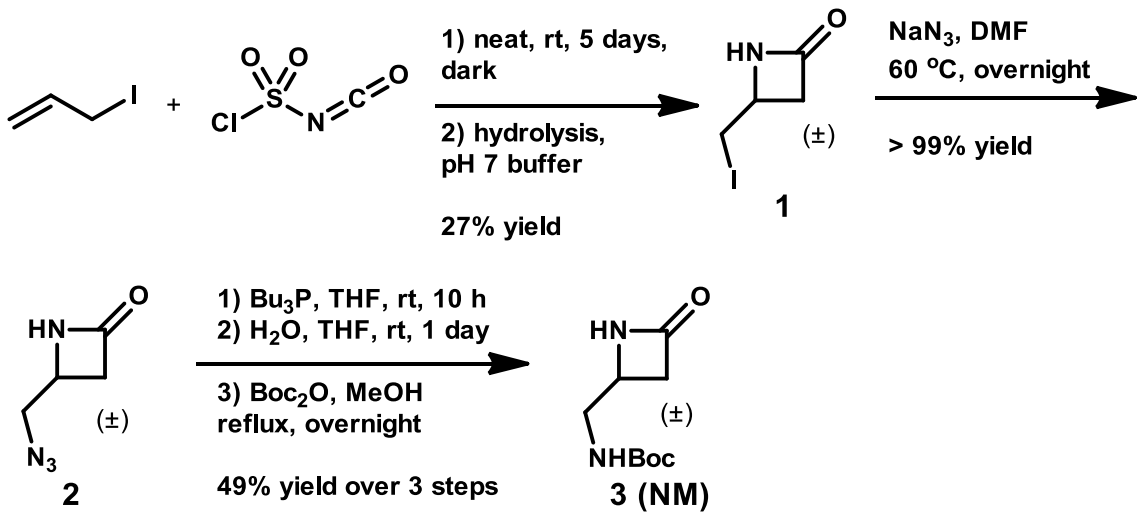


Figure S1. The synthesis of β-lactam **NM**.

4-Iodomethylazetidin-2-one (1). Compound **1** was prepared by a modification of reported methods.[1] Chlorosulfonyl isocyanate (70.4 mL, 810 mmol) in a 500 mL round bottom flask was combined with allyl iodide (200 g, 1.19 mol), and the mixture was stirred at rt for 5 days with protection from light. The reaction mixture was then diluted with CH_2Cl_2 (200 mL), and the resulting solution was poured into an ice-cold buffer solution containing sodium sulfite (306 g, 2.43 mol) and dibasic sodium phosphate (346 g, 2.43 mol). The mixture was stirred at rt overnight. The crude product was extracted with CH_2Cl_2 (3 × 500 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel chromatography (1:1 hexane:EtOAc) to afford iodo-β-lactam **1** as a light yellow to brown solid (46.2 g, 27%). Recrystallization from EtOAc provided a white solid, m.p. 106.7-107.2 °C (m.p. 106-106.5 °C in ref. 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.72 (br, 1H), 3.81–3.88 (m, 1H), 3.30 (d, J = 6.3 Hz, 2H), 3.07 (ddd, J

= 15.3, 5.1, 2.4 Hz, 1H), 2.66 (ddd, *J* = 15.3, 2.4, 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.63, 48.94, 44.45, 8.79; ESI-HRMS: *m/z* calcd for $C_4H_{10}IN_2O$ $[M+NH_4]^+$: 228.9833; found: 228.9824.

4-Azidomethylazetidin-2-one (2). A solution of iodo-β-lactam **1** (2.80 g, 13.3 mmol) in DMF (58 mL) was treated with NaN_3 (2.6 g, 39.8 mmol), and the mixture was heated to 60 °C and stirred overnight. The reaction mixture was concentrated *in vacuo,* and water (70 mL) was added. The resulting slurry was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel chromatography (1:1 hexane:EtOAc) to afford azido-β-lactam **2** as a colorless oil (1.68 g, > 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.98 (br, 1H), 3.68–3.74 (m, 1H), 3.49 (dd, *J* = 12.6, 6.3 Hz, 1H), 3.31 (dd, *J* = 12.6, 6.3 Hz, 1H), 2.96 (ddd, *J* = 14.7, 5.1, 2.1 Hz, 1H), 2.63 (ddd, *J* = 14.7, 2.4, 1.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.62, 54.20, 48.45, 41.00; IR (NaCl): 3250, 2928, 2014, 1742 cm^{-1}; HREI-MS: *m/z* calcd for $C_4H_6N_4O$ $[M]^+$: 126.0542; found: 126.0537.

4-(*N*-*tert*-Butoxycarbonyl)methylazetidin-2-one (3). A solution of azido-β-lactam **2** (1.67 g, 13.3 mmol) in THF (54 mL) was treated with Bu_3P (3.6 mL, 14.6 mmol), and the mixture was stirred at rt for 10 hr. Water (9 mL) was then added, and the resulting mixture was stirred at rt for 1 day. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in MeOH (66 mL). To this solution was added di-*tert*-butyl dicarbonate (6.1 mL, 26.5 mmol), and the mixture was heated to reflux overnight. After cooling, the reaction mixture was concentrated *in vacuo,* and water (70 mL) was added. The resulting slurry was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated. The crude product was purified by silica gel chromatography (1:1 hexane:EtOAc) to afford Boc-protected amino-β-lactam (**NM**) **3** as a white powder (1.30 g, 49% over 3 steps), which was recrystallized from EtOAc, m. p. 128.5-129.3 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.13 (br, 1H), 4.90 (br, 1H), 3.78 (br, 1H), 3.24–3.43 (m, 2H), 3.01 (ddd, *J* = 15.5, 2.1, 1.5 Hz, 1H), 2.67 (d, *J* = 15.0 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.72, 156.38, 80.13, 47.57, 43.87, 41.08, 28.46; HRESI-MS: *m/z* calcd for $C_9H_{16}N_2NaO_3$ $[M+Na]^+$: 223.1059; found: 223.1054.

Polymer synthesis and characterization

Polymerization reactions were carried out in a glove box (dry N_2 atmosphere). The reaction mixture was removed from the glove box for quenching and product isolation.

Representative synthesis and purification of 60:40 CH:NM random copolymer (target length = 20-mer). To a solution of β-lactams **CH** (37.5 mg, 0.3 mmol) and **NM** (40.0 mg, 0.2 mmol) in dimethylacetamide (DMAc, 2.5 mL) were added a solution of co-initiator *t*BuBzCl in DMAc (1.0 mL, 0.025 mmol) and then a solution of lithium bis(trimethylsilyl)amide (LiHMDS) in DMAc (0.5 mL, 0.063 mmol). The mixture was stirred at rt overnight, and the reaction was then terminated by addition of a few drops of MeOH. The resulting solution was poured into pentane (35 mL), which caused a light yellow oil to separate from the solution. The oil was collected after centrifugation and dissolved in THF (2 mL), and then pentane (40 mL) was added to induce polymer precipitation. After five or six dissolution/precipitation cycles, the side-chain-protected polymer was obtained as a white solid. This material was subjected to gel permeation chromatography (GPC) characterization. GPC analysis was limited to the side chain-protected forms of these polymers. GPC analysis of 60:40 **CH:NM**, **MM** homopolymer, and **DM** homopolymer was conducted with THF as the mobile phase, and GPC analysis of all **CH**:**NM** copolymers and **NM** homopolymer was conducted with DMAc as the mobile phase. Polymer 60:40 **CH:NM** was analyzed in both solvents, and comparable results were obtained in these two solvents.

Boc groups were removed by treating a side-chain-protected polymer with neat trifluoroacetic acid (TFA, 2 mL) at rt for 2 hr with shaking. Addition of Et_2O (40 mL) caused the deprotected polymer to precipitate as a white fluffy solid. The solid was collected after centrifugation, dried under N_2, dissolved in MeOH (2 mL), and precipitated again by addition of Et_2O (40 mL). After three dissolution/precipitation cycles, the TFA salt form of the deprotected polymer was isolated as a white powder.

Polymer characterization by gel permeation chromatography (GPC) using DMAc as the mobile phase. Side-chain-protected polymers, at a concentration of about 2 mg/mL, were dissolved in *N,N*-dimethylacetamide (DMAc) supplemented with 10 μM LiBr. The solution was filtered through a 0.2 μm polytetrafluoroethylene (PTFE) filter before GPC analysis. The GPC analysis involved two Waters Styragel HR 4E columns (particle size 5 μm) linked in series on a Waters GPC instrument equipped with a refractive index detector (Waters 2410); DMAc containing 10 μM LiBr was used as the mobile phase at a flow rate of 1 mL/min at 80 °C. Number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) were calculated using the Empower software and calibration curves obtained from at least nine PMMA standards with peak average molecular weight (Mp) ranging from 690 to 1944000. The degree of polymerization (DP) for a particular polymer was calculated based on the deduced M_n value, the initial ratio of β-lactam monomers used for the reaction, and the molecular weight of the β-lactam monomers, as described previously.[2]

Polymer characterization by GPC using THF as the mobile phase. Polymers were dissolved in tetrahydrofuran (THF) and filtered through a 0.2 μm polytetrafluoroethylene (PTFE) filter before GPC analysis. Polymers at the side chain-protected stage were analyzed using two Waters columns (Styragel HR 4E, particle size 5 μm) linked in series on a Shimadzu gel-permeation chromatography (GPC) instrument equipped with a multiangle light scattering detector (Wyatt miniDAWN, 690 nm, 30 mW) and a refractive index detector (Wyatt Optilab-rEX, 690 nm). M_n, M_w and PDI were measured using THF as mobile phase at a flow rate of 1 mL/min at 40 °C and calculated using ASTRA 5.3.4.20 software with a dn/dc value of 0.1 mL/g for all polymers. DP for a particular polymer was calculated based on the deduced M_n value, the initial ratio of β-lactam monomers in the reaction, and the molecular weight of the β-lactam monomers, as described previously.[2]

Antifungal activity assay

The minimum inhibitory concentration (MIC) assay for *Candida albicans* was conducted according to a previously described protocol.[3] The clinically isolated K1 strain of *C. albicans* was used.[4] Cells were inoculated and cultured on a Sabouraund dextrose agar plate at 37 °C for 36 hr and then suspended in 0.145 M saline at 2.5×10^6 cells/mL to generate the stock suspension. The working suspension was prepared from a 1:1000 dilution of the stock suspension using adjusted RPMI 1640 medium (containing L-glutamine but not sodium bicarbonate) buffered with 0.145 M 3-(N-morpholino) propanesulfonic acid (MOPS). Two-fold serial dilution series for nylon-3 polymers or amphotericin B were prepared in a 96-well plate using adjusted RPMI medium; each well contained 100 μL compound solution at concentrations from 200 to 1.56 μg/mL. A 100 μL aliquot of the cell working suspension was added to each well (except the cell-free blank control), followed by gentle shaking of the plate for 10 sec. The plate was then incubated at 37 °C for 48 hr, and fungal cell growth was evaluated visually. RPMI medium only (cell-free blank, negative control) and cells in RPMI without any polymer additive (positive control) were included on the same plate. The antifungal MIC was the lowest concentration of a polymer or amphotericin B that completely inhibited *C. albicans* growth, that is, for which no cell colony was visible in the well. Each experiment was performed in duplicate on a given day, and experiments were repeated on at least two different days.

In selected cases a minimum fungicidal concentration (MFC) assay was conducted after the MIC assay. Solutions from wells containing a polymer at a concentration from one dilution below the MIC to the highest polymer concentration were individually mixed with a pipette. Aliquots of 10 μL from each of these wells, as well as aliquots from the positive and negative control wells, were plated on yeast extract-peptone-dextrose (YPD) agar. The plates were incubated at 37 °C for 48 hr and then inspected visually for *C. albicans* colony formation. The MFC was defined as the lowest polymer concentration to result in zero *C. albicans* colonies.

Antibacterial activity assay

The minimum inhibitory concentration (MIC) assay for bacteria was conducted by following a protocol similar to that previously described.[5] Four bacteria were used in this study: *Escherichia coli* JM 109,[6] *Bacillus subtilis* BR151,[7] *Enterococcus faecium* A634 (vancomycin-resistant),[8] and *Staphylococcus aureus* 1206 (methicillin-resistant).[9] Bacterial cells were inoculated and cultured at 37 °C overnight on LB agar plates and then suspended in LB medium at 2×10^6 cells/mL to generate the working suspension. Two-fold serial dilution series for nylon-3 polymers were prepared in a 96-well plate using LB medium; each well contained 50 μL compound solution at concentrations ranging from 400 to 3.13 μg/mL. A 50 μL aliquot of the cell working suspension was added to each well, followed by gentle shaking of the plate for 10 sec. The plate was incubated at 37 °C for 6 hr, and the optical density (OD) of each well was then measured at 650 nm using a Molecular Devices Emax precision microplate reader. Wells containing LB medium only (blank) and wells containing cells in LB without polymer (positive control) were included on the same plate. Measurements were performed in duplicate, and measurements were repeated on at least two different days. The percentage of cell growth in each well was calculated from $\left(\%\,\text{cell growth} = \frac{A_{650}^{\text{polymer}} - A_{650}^{\text{blank}}}{A_{650}^{\text{control}} - A_{650}^{\text{blank}}} \times 100 \right)$, and plotted against polymer concentration to give the dose-response curves of antibacterial activity for these polymers. The MIC value is the minimum concentration of a given polymer to inhibit bacterial growth.

Hemolysis assay

Hemolysis assays were conducted as previously described using human red blood cells (hRBC).[3,10] hRBCs were obtained from freshly drawn human blood, washed three times with TRIS-buffered saline (TBS; 10 mM TRIS, 150 mM NaCl, pH 7.2), and diluted 1:50 in TBS to obtain a working suspension of 2% RBC relative to total RBC in whole blood. Two-fold serial dilution series for nylon-3 polymers were prepared in a 96-well plate in TBS; each well contained 100 μL compound solution at concentrations ranging from 800 to 6.25 μg/mL. An aliquot of 100 μL working suspension

was added to each well, followed by gentle shaking of the plate for 10 sec. Wells containing TBS without polymer (blank) and wells containing Triton X-100 (positive control, 3.2 μg/mL in TBS) were included on the same plate. The plate was incubated at 37 °C for 1 hr, and then centrifuged at 3700 RPM for 5 min to precipitate the RBCs. An aliquot of 80 μl of the supernatant from each well was transferred to the corresponding well in a new 96 well plate using a multichannel pipettor, and the optical density (OD) at 405 nm was measured using a Molecular Devices Emax precision microplate reader. Measurements were performed in duplicate, and each measurement was repeated on at least two different days. The percentage of hemolysis in each well was calculated from ($\%\,\text{hemolysis} = \frac{A_{405}^{\text{polymer}} - A_{405}^{\text{blank}}}{A_{405}^{\text{control}} - A_{405}^{\text{blank}}} \times 100$), and plotted against polymer concentration to give the dose-response curves of hemolysis for these polymers. The HC_{10} value for each polymer was the polymer concentration required to cause 10% lysis of RBCs.

Fibroblast cell toxicity assay

The 3T3 fibroblast cell toxicity assay was conducted by following the CytoTox-ONE protocol provided by Promega to measure the release of lactate dehydrogenase (LDH) from cells with damaged membranes.[11] 3T3 fibroblasts were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine at 37 °C in a 5% CO_2 environment. Upon reaching 80–90% confluence, cells were detached with 0.05% trypsin and 0.02% EDTA, centrifuged and resuspended in DMEM to a final concentration of 1.5×10^5 cells/mL. A 100 μl aliquot of of this cell suspension was added to each well of a 96-well plate, and the plate was incubated for 24 hr at 37 °C. Wells were then supplemented with 45 μL fresh DMEM (phenol red- and pyruvate-free) and incubated for 2 hr at 37 °C. During this period, two-fold serial dilution series of nylon-3 polymers were prepared in DMEM (phenol red- and pyruvate-free) with concentrations ranging from 800 to 6.25 μg/mL. An aliquot of 45 μL each polymer solution was added to each well of the 96-well plate containing the 3T3 fibroblasts, and the plate was incubated for 12 hr at 37 °C. Wells containing no

polymer were also included on the same plate as a blank (polymer-free) and a positive control for toxicity (to generate 100% release of LDH with addition of lysate solution later). The plate was removed from the incubator and cooled to rt over 20 min, at which point a 10 μL aliquot of 1X lysate solution (1.8% (m/v) TX-100 in DI water) was added to the positive control wells. The plate was gently shaken for 10 sec. An aliquot of 100 μL assay working solution (provided as part of the CytoTox-ONE kit) was added to each well (90 μL for the positive control wells) with protection from light, and the plate was shaken gently for 30 sec, and then incubated at rt for 10 min. At this point 50 μL stop solution was added to each well, and the plate was shaken for 10 sec. The fluorescence intensity in each well was monitored at 590 nm (excitation at 560 nm) on a Tecan Infinite M1000 microplate reader. Measurements were performed in triplicate, and each experiment was repeated on at least two different days. The percentage of cell death in each well was calculated from ($\%\,\text{death} = \frac{F^{\text{polymer}} - F^{\text{blank}}}{F^{\text{control}} - F^{\text{blank}}} \times 100$), and plotted against polymer concentration to give the dose-response curves for 3T3 fibroblast toxicity for these polymers. The IC_{10} value was the polymer concentration required to produce 10% death among 3T3 cells.

Table S1. GPC characterization of nylon-3 polymers[a]

polymer composition	PDI	M_n	M_w	DP
60:40 **CH**:**NM**	1.29 (1.20)[b]	3763 (3831)[b]	4870 (4601)[b]	23 (24)[b]
50:50 **CH**:**NM**	1.29	3832	4941	23
40:60 **CH**:**NM**	1.29	3718	4807	21
30:70 **CH**:**NM**	1.26	3792	4774	20
20:80 **CH**:**NM**	1.33	4273	5696	22
10:90 **CH**:**NM**	1.24	3502	4334	17
NM	1.13	4199	4757	20
MM	1.03[c]	4934[c]	5079[c]	22[c]
DM	1.13[c]	4240[c]	4778[c]	18[c]

[a]All polymers were characterized with side chain amino groups protected with Boc groups. Unless otherwise indicated, characterization data were obtained on a GPC using DMAc as the running solvent and PMMA standards for column calibration. [b]The data in parentheses for 60:40 **CH**:**NM** were obtained for samples dissolved in 5:95 MeOH:THF on a GPC using THF as the running solvent. [c]Polymers were dissolved in THF and characterized on a GPC using THF as the running solvent.

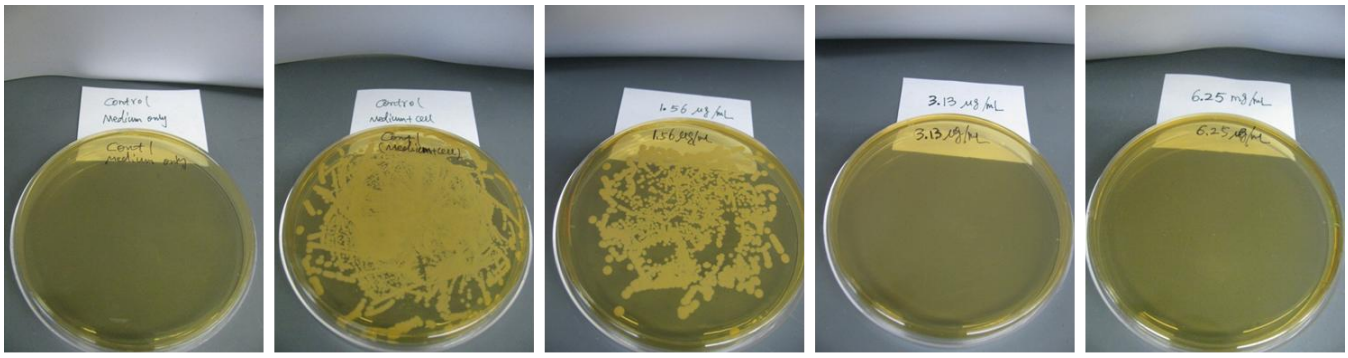


Figure S2. Monitoring of *C. albicans* growth (colony forming units) on yeast extract-peptone-dextrose (YPD) agar to determine the minimal fungicidal concentration (MFC) for the **NM** homopolymer. *C. albicans* in liquid culture was incubated with **NM** homopolymer at various concentrations. Aliquots (10 µL for each polymer concentration) were then plated on individual agar plates, which were incubated at 37 °C for 48 hr. The results indicate that MFC = MIC = 3.1 µg/mL for the **NM** homopolymer.

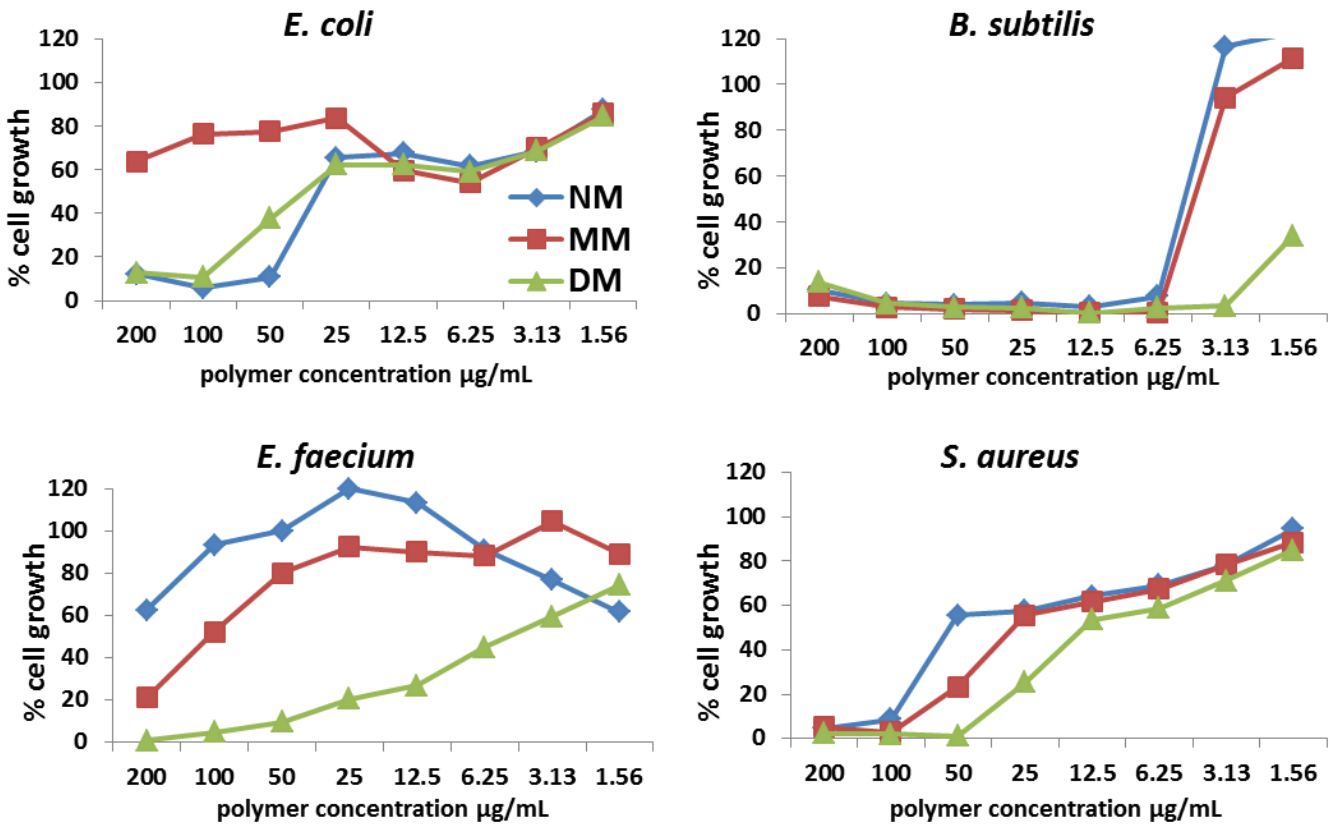


Figure S3. Bacterial growth inhibition dose-response data for homopolymers derived from **NM**, **MM**, or **DM**.

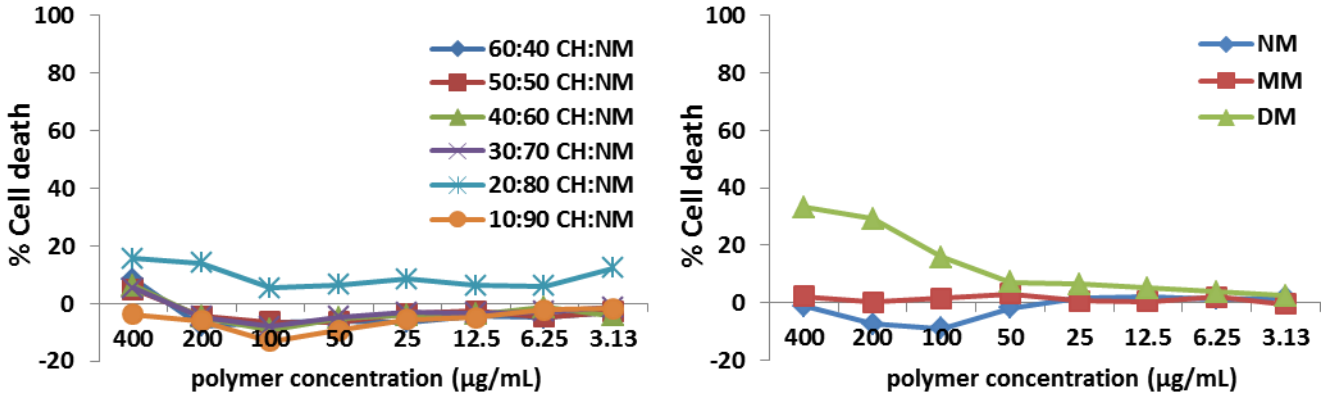


Figure S4. 3T3 fibroblast toxicity data for **CH:NM** co-polymers and **NM**, **MM**, and **DM** homopolymers.

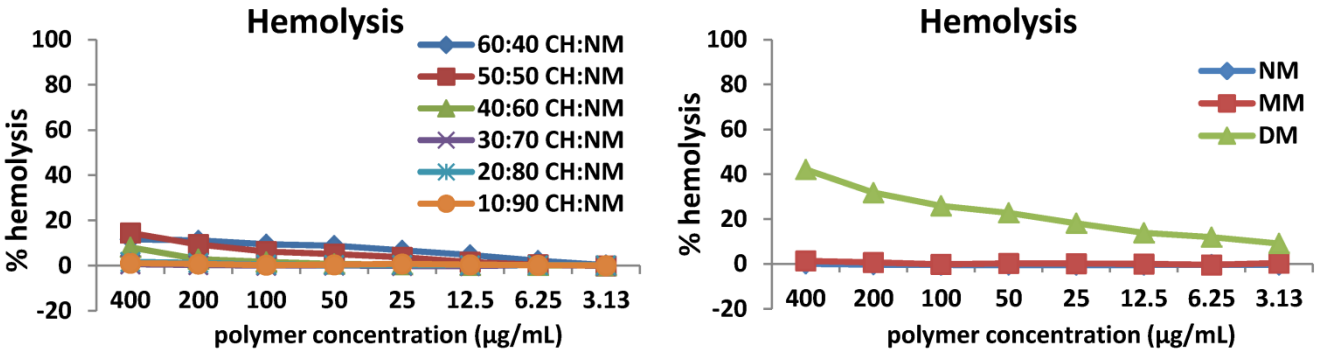


Figure S5. RBC hemolysis data for **CH:NM** copolymers and **NM**, **MM**, and **DM** homopolymers.

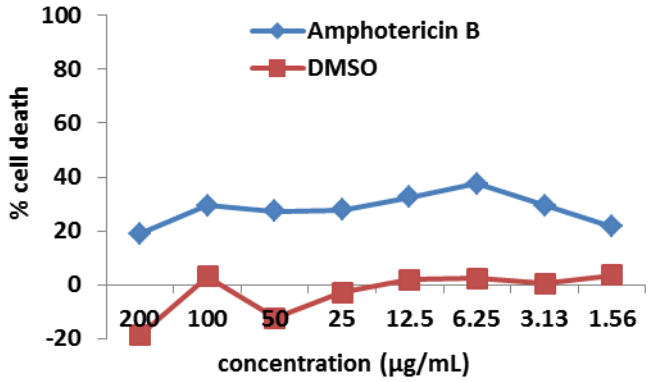


Figure S6. 3T3 fibroblast toxicity data for amphotericin B. Amphotericin B was dissolved in 1:1 DMSO:water as the stock solution for 3T3 toxicity assay. Blank sample containing DMSO in the same concentration as that in the amphotericin B sample was used as the DMSO control.

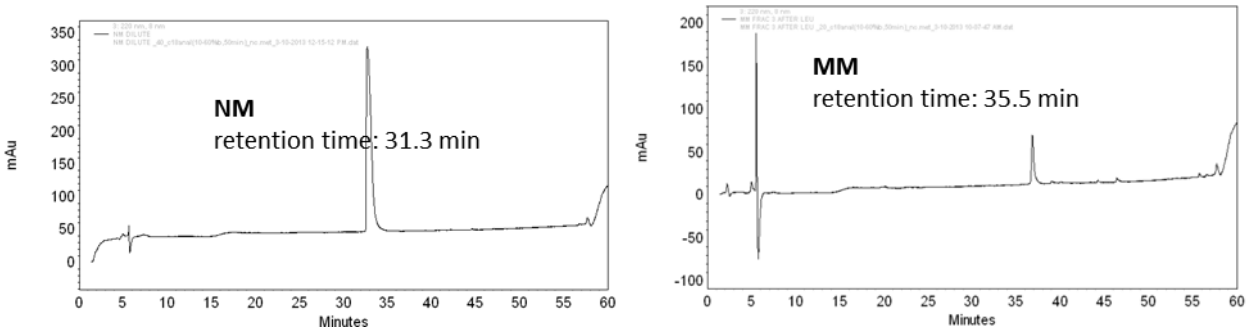
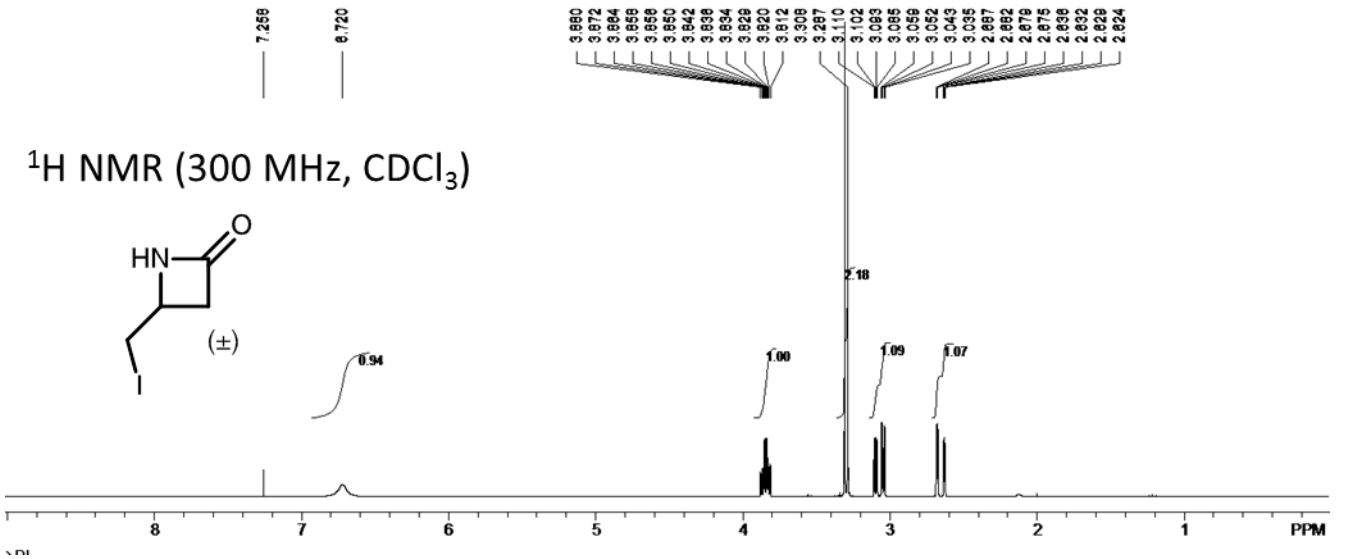


Figure S7. **NM** and **MM** β-lactam lipophilicity analysis using reverse phase HPLC. The data indicate that **NM** is less lipophilic than **MM**, since **NM** has a shorter retention time. Both β-lactams were dissolved in 30:70 CH_3CN:H_2O and analyzed on a Supelco C18 RP column (2500 × 2.6 mm ID, 5 µm particles) using a gradient eluent changing from 10% to 60% CH_3CN in water (containing 0.1% TFA) at a flow rate of 1 mL/min.

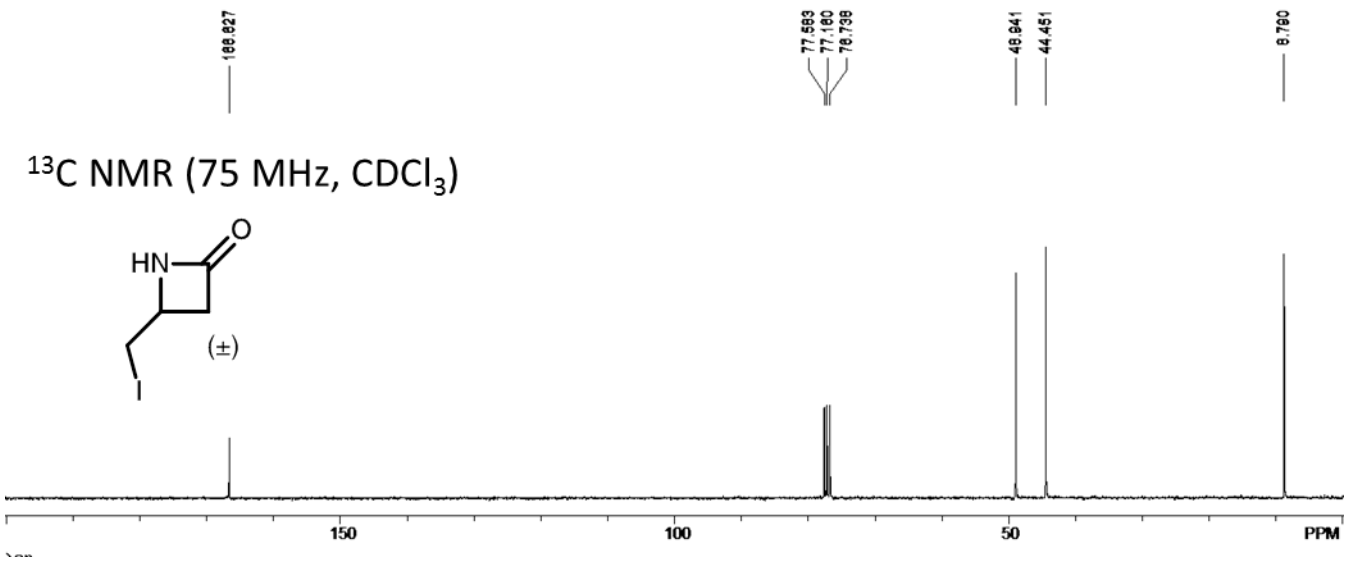
Table S2. Antifungal activity of **NM** homopolymer samples from different synthetic batches and of commercially obtained poly-lysine [a]

polymer composition	batch #	MIC, μg/mL
NM	1	3.1
NM	2	6.3
NM	3	3.1
NM	4	3.1
α-poly-L-lysine (1-5 KDa)	NA	> 200
α-poly -L-lysine (4-15 KDa)	NA	> 200

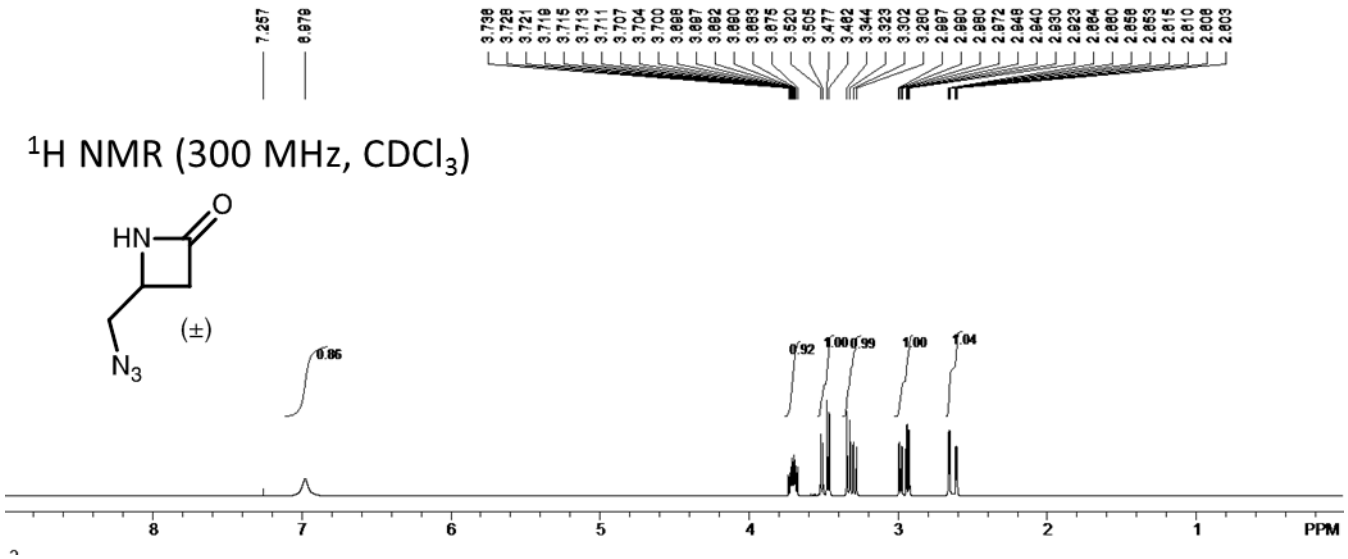
[a] **NM** homopolymer batches were synthesized on different days. The *α*-poly-L-lysine was purchased in the HBr salt form with nominal molecular weight ranges of 1-5 KDa or 4-15 KDa. NA means not applicable.



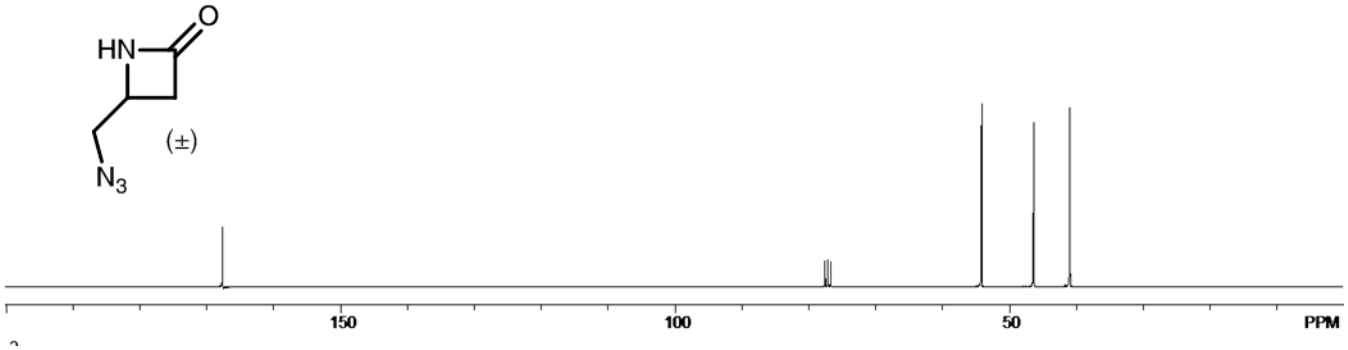
^{1}H NMR (300 MHz, $CDCl_3$)



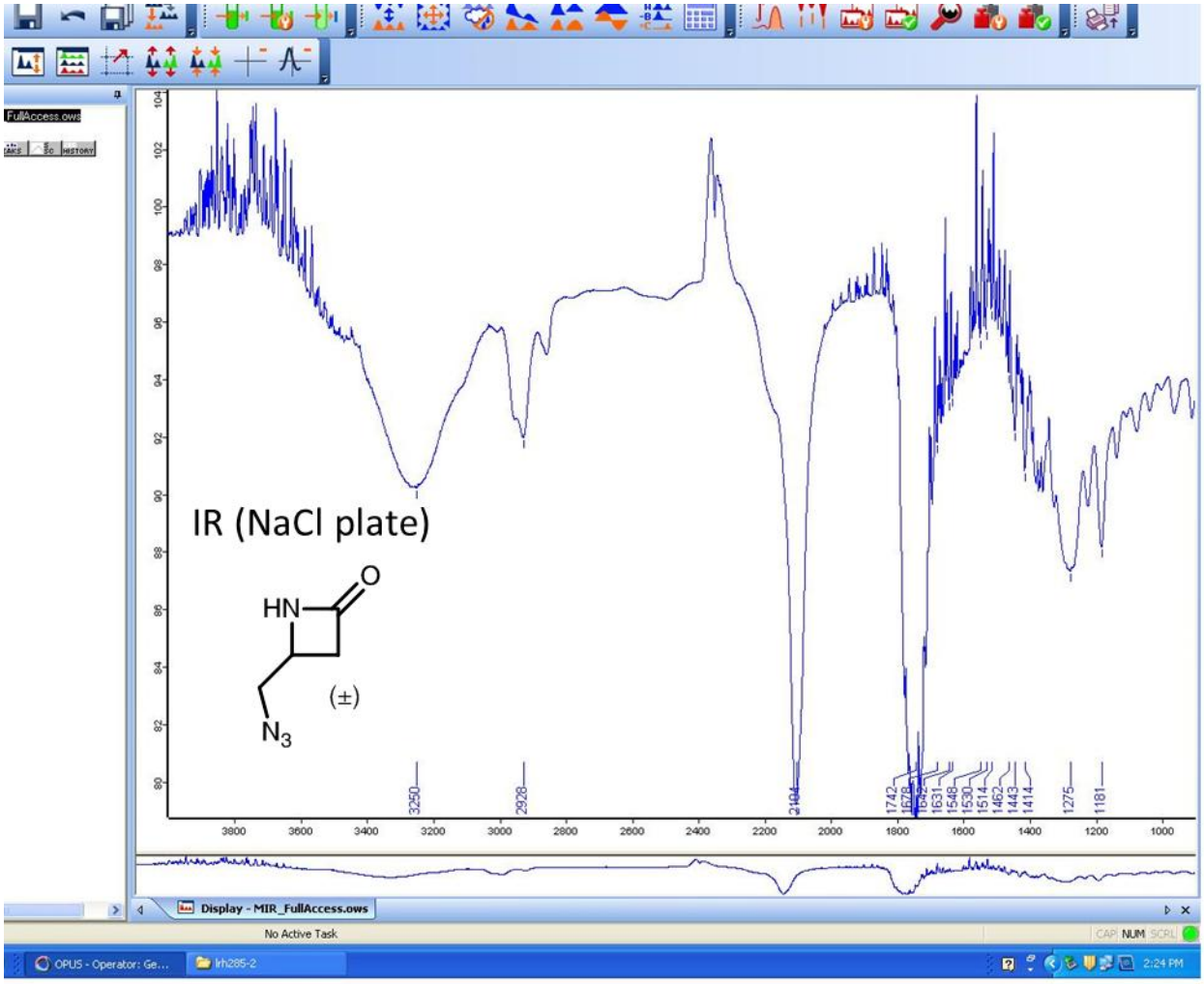
^{13}C NMR (75 MHz, $CDCl_3$)



^{1}H NMR (300 MHz, $CDCl_3$)



^{13}C NMR (75 MHz, $CDCl_3$)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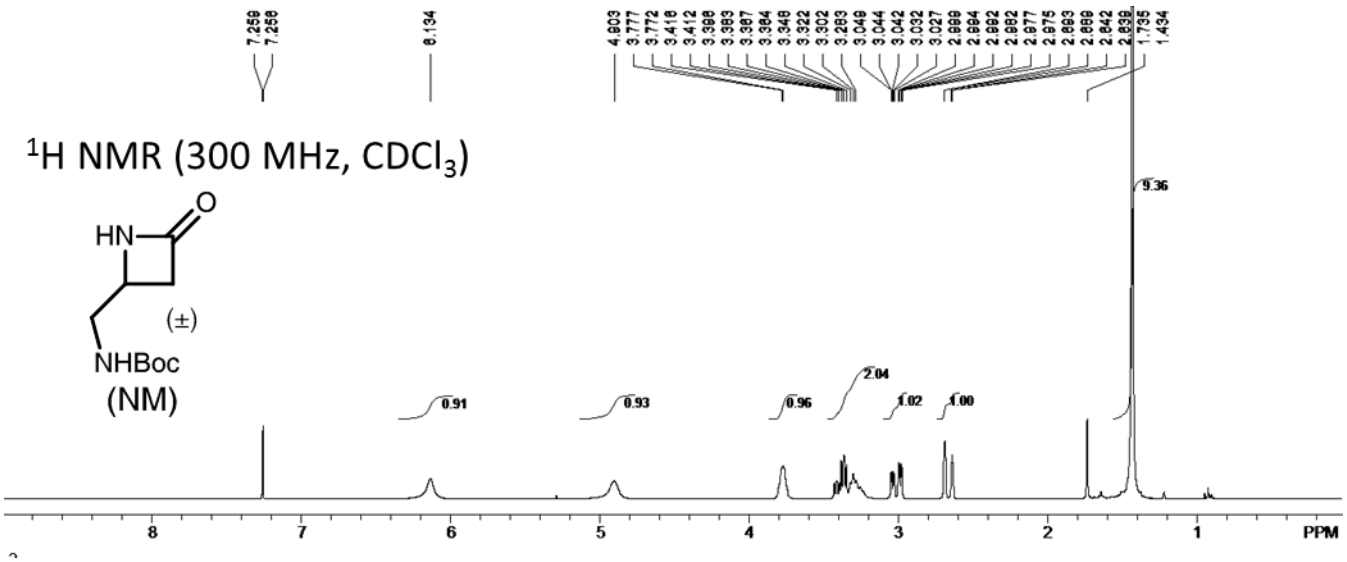
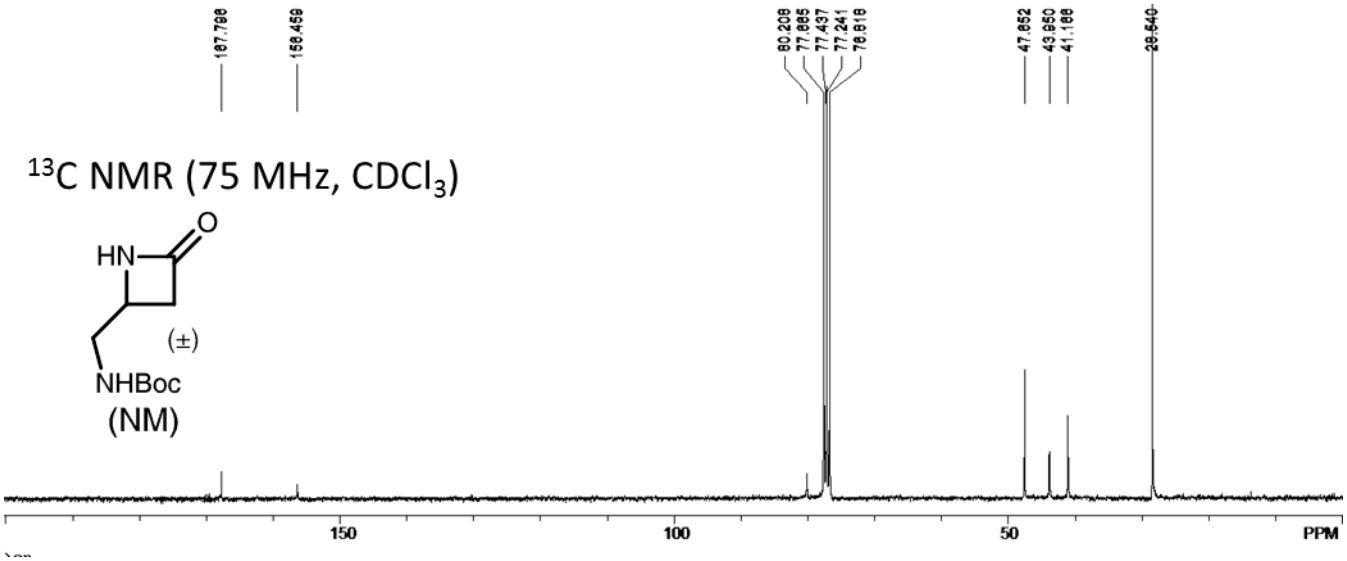
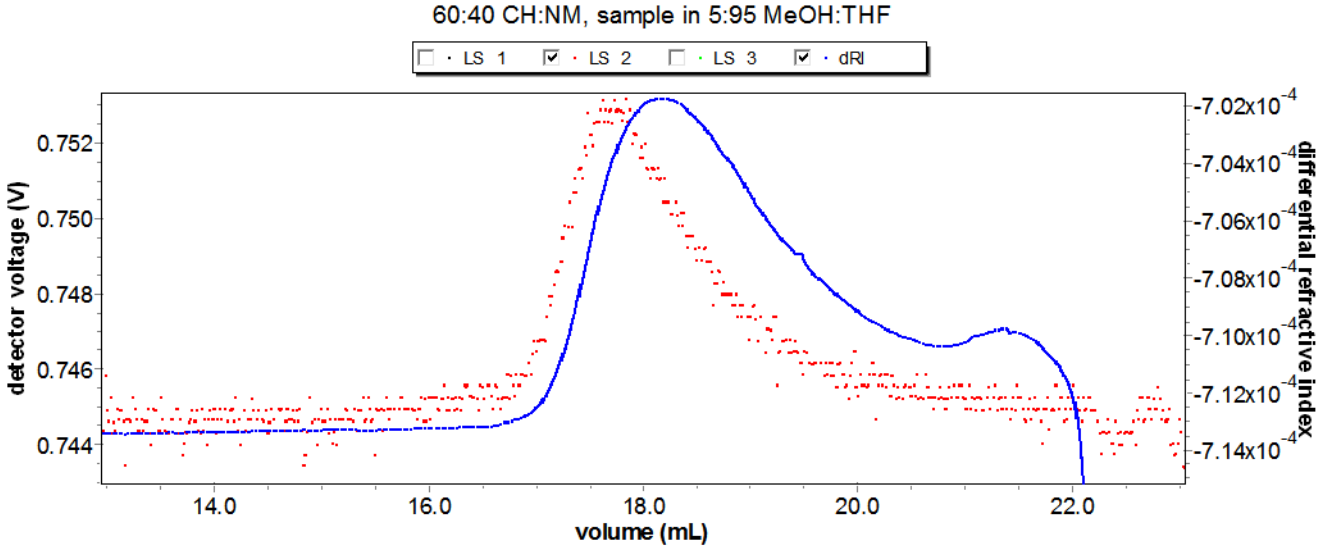


^{1}H NMR (300 MHz, $CDCl_3$)

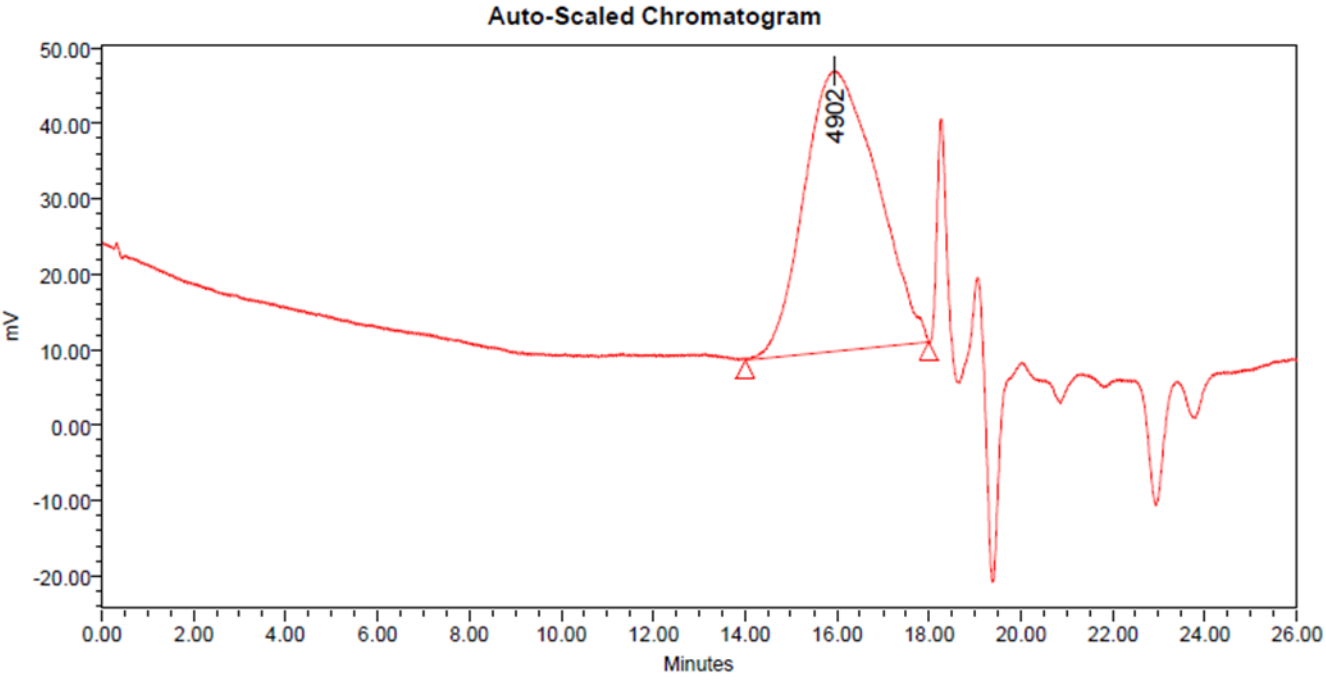


^{13}C NMR (75 MHz, $CDCl_3$)

GPC Chromatogram (60:40 **CH:NM** copolymer), mobile phase: THF



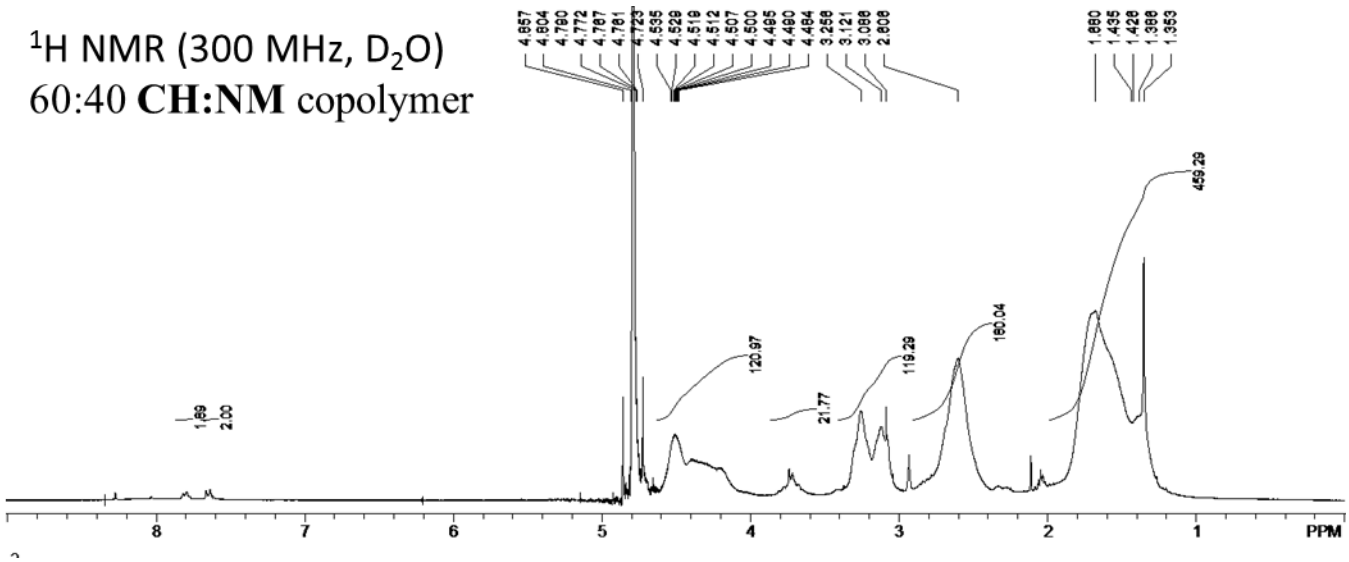
GPC Chromatogram (60:40 **CH:NM** copolymer), mobile phase: DMAc



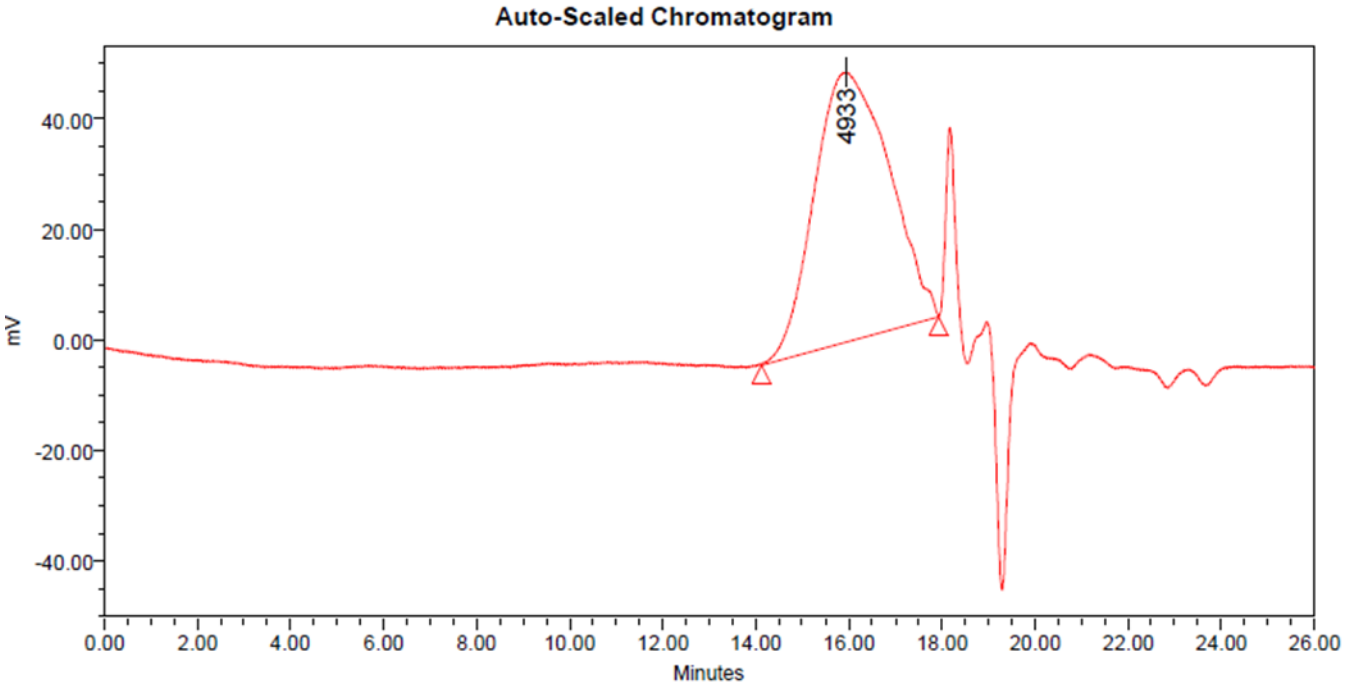
GPC Results

	Dist Name	Mn	Mw	Mv	MP	Mz	Mz+1	Polydispersity	K	alpha
1		3763	4870		4902	6133	7470	1.294324		

^{1}H NMR (300 MHz, D_2O)
60:40 **CH:NM** copolymer



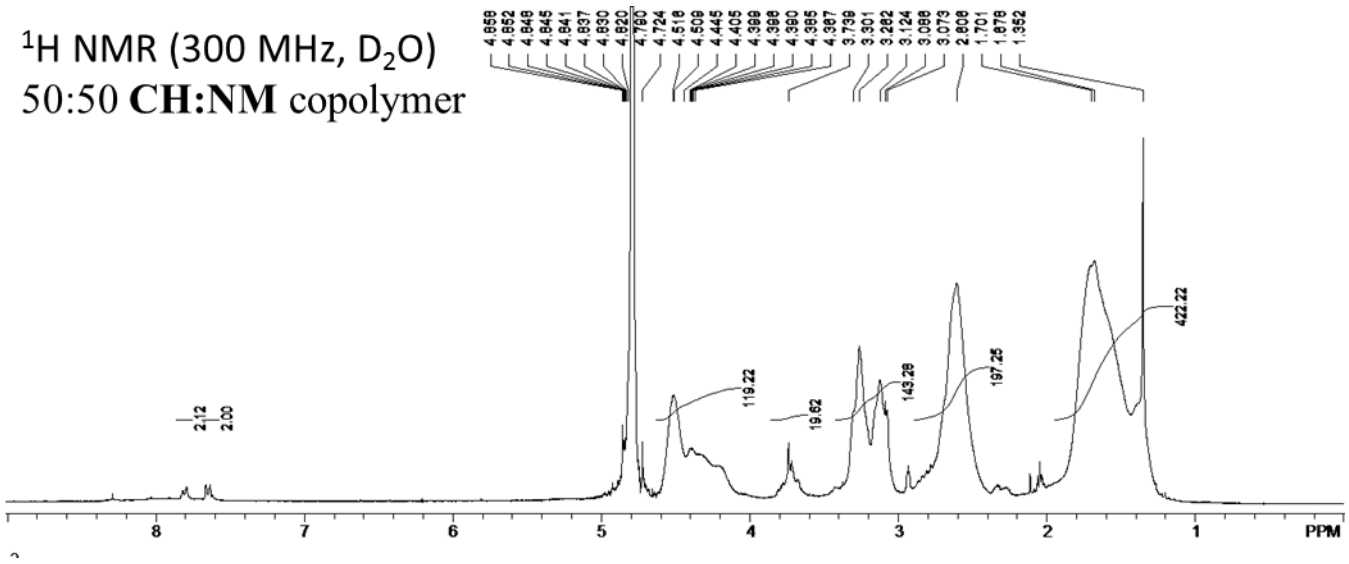
GPC Chromatogram (50:50 **CH:NM** copolymer), mobile phase: DMAc



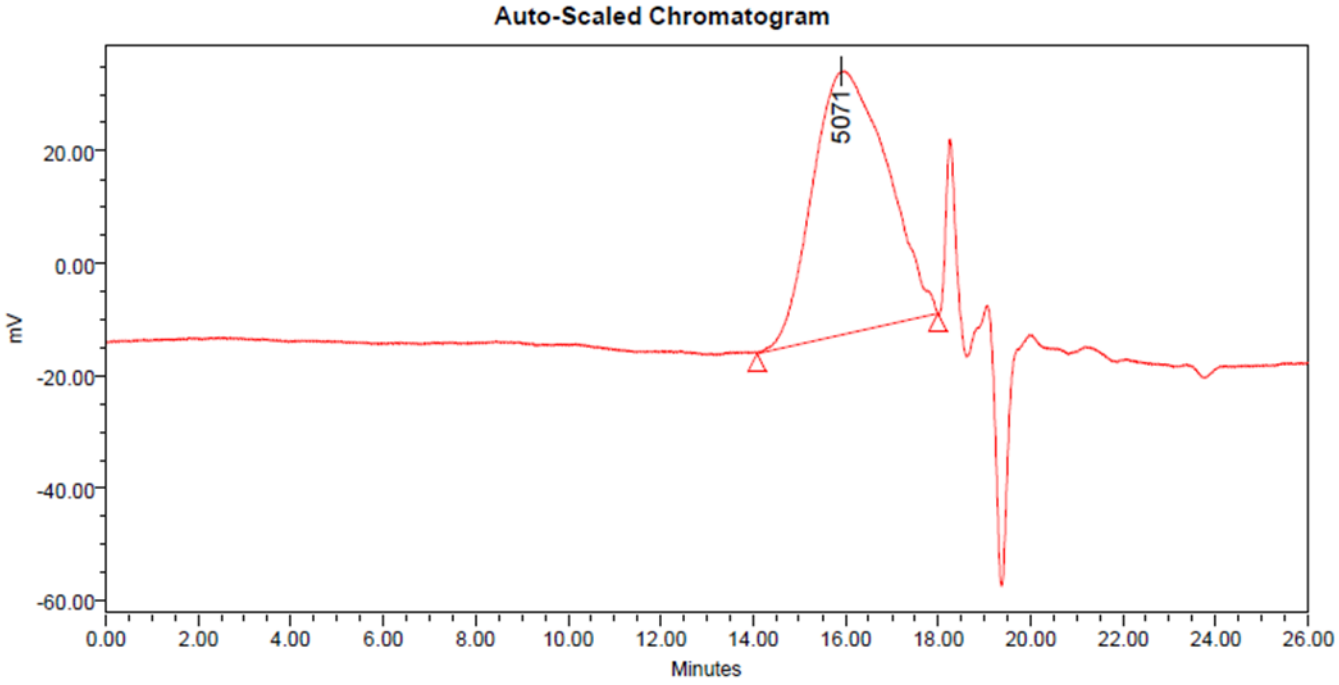
GPC Results

	Dist Name	Mn	Mw	Mv	MP	Mz	Mz+1	Polydispersity	K	alpha
1		3832	4941		4933	6193	7473	1.289620		

^{1}H NMR (300 MHz, D_2O)
50:50 **CH:NM** copolymer



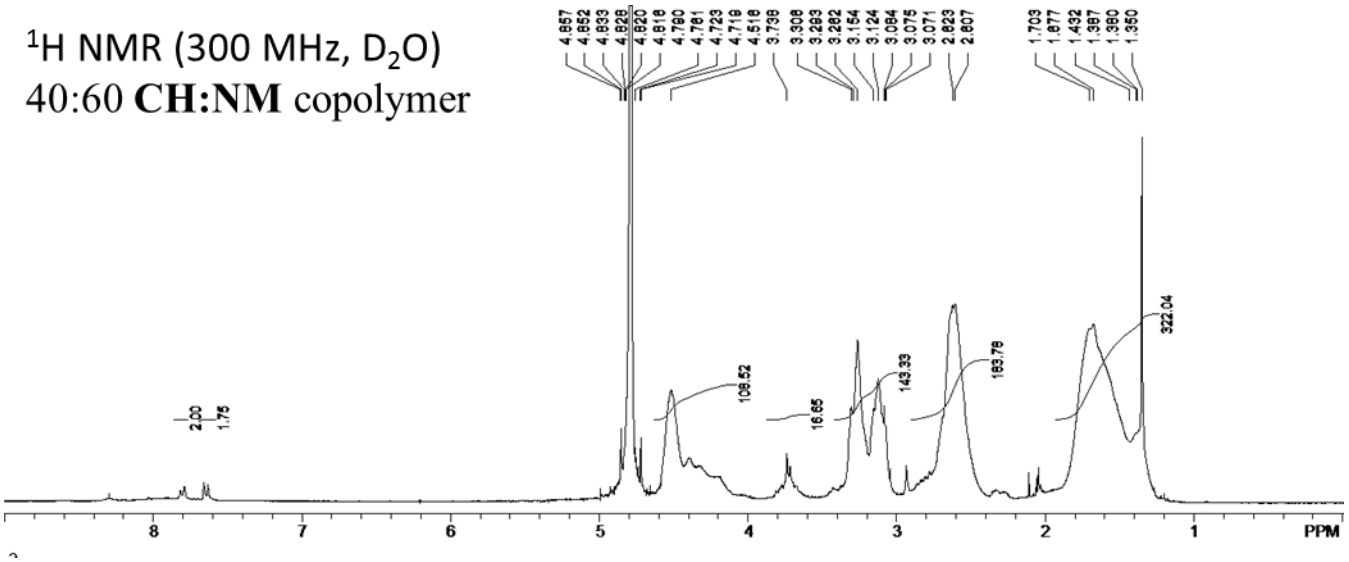
GPC Chromatogram (40:60 **CH:NM** copolymer), mobile phase: DMAc



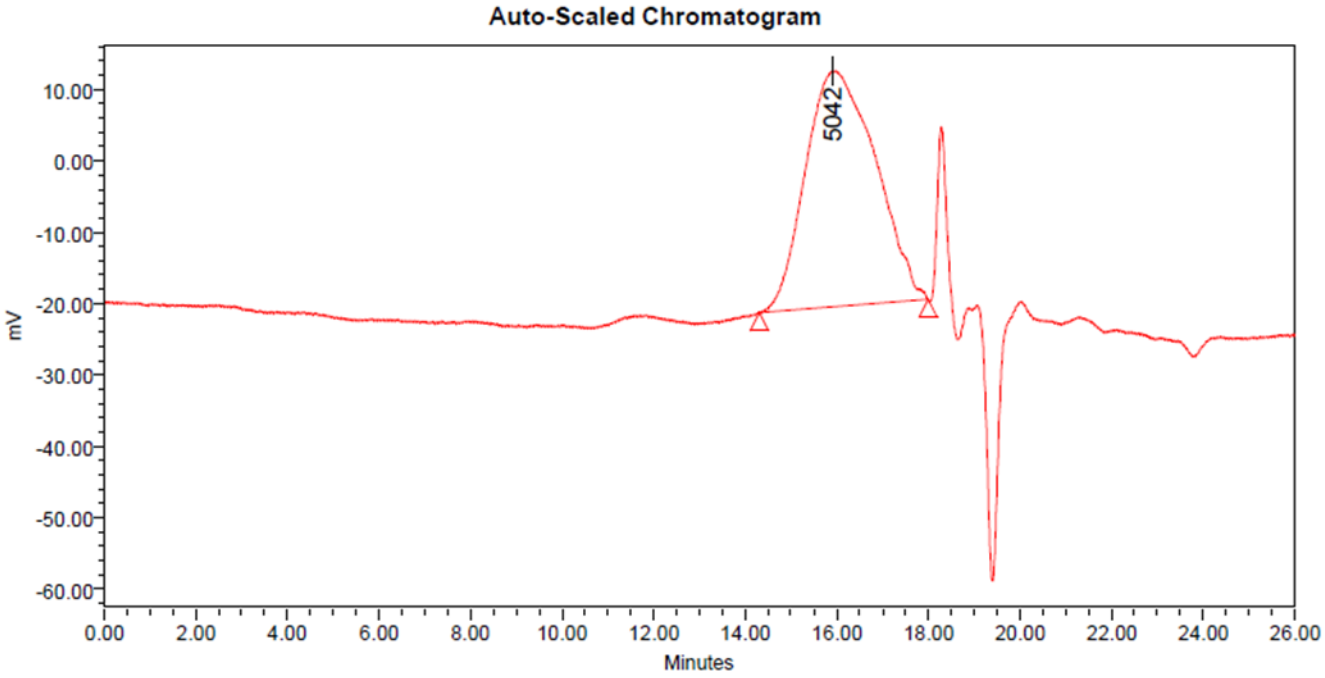
GPC Results

	Dist Name	Mn	Mw	Mv	MP	Mz	Mz+1	Polydispersity	K	alpha
1		3718	4807		5071	6024	7260	1.293081		

^{1}H NMR (300 MHz, D_2O)
40:60 **CH:NM** copolymer

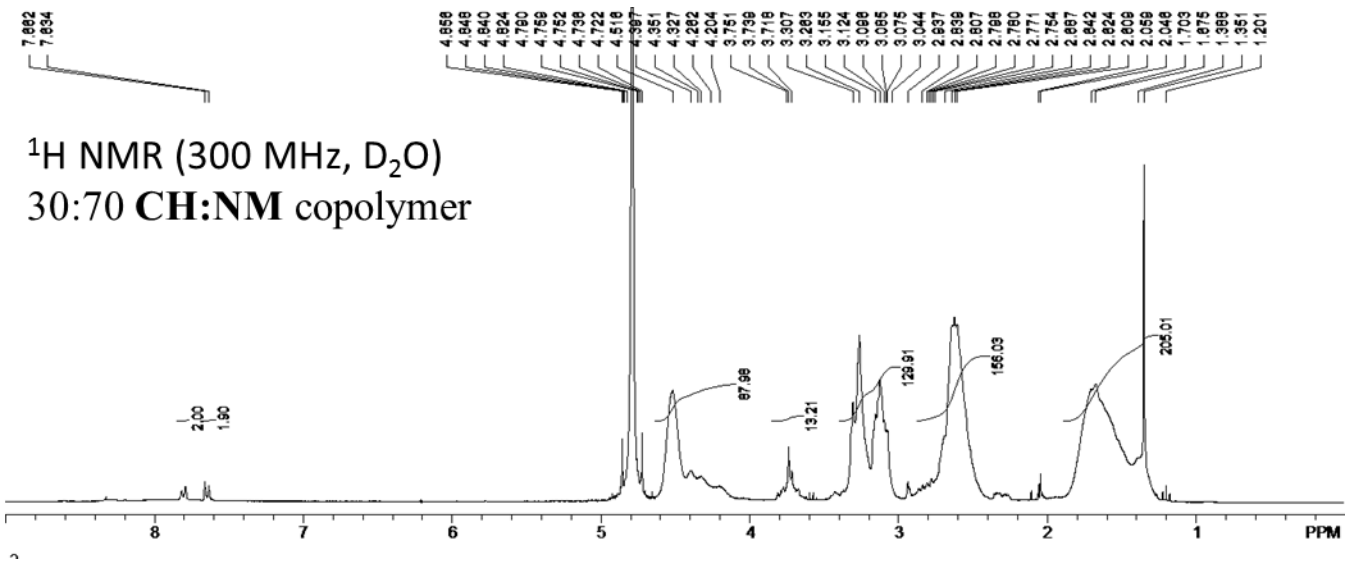


GPC Chromatogram (30:70 **CH:NM** copolymer), mobile phase: DMAc

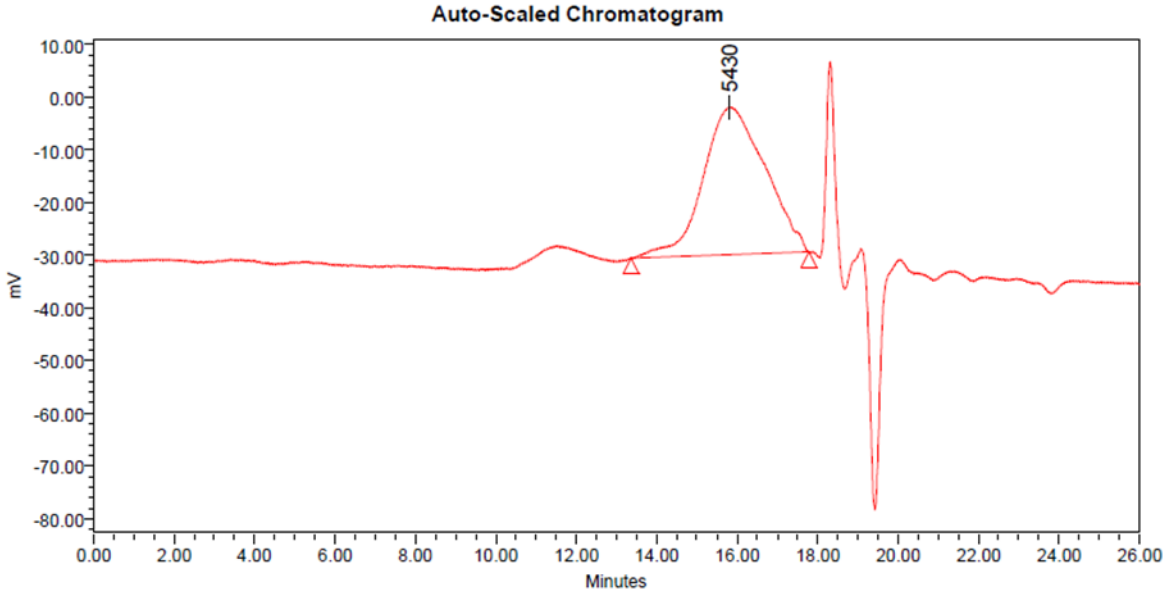


GPC Results

	Dist Name	Mn	Mw	Mv	MP	Mz	Mz+1	Polydispersity	K	alpha
1		3792	4774		5042	5835	6871	1.258871		

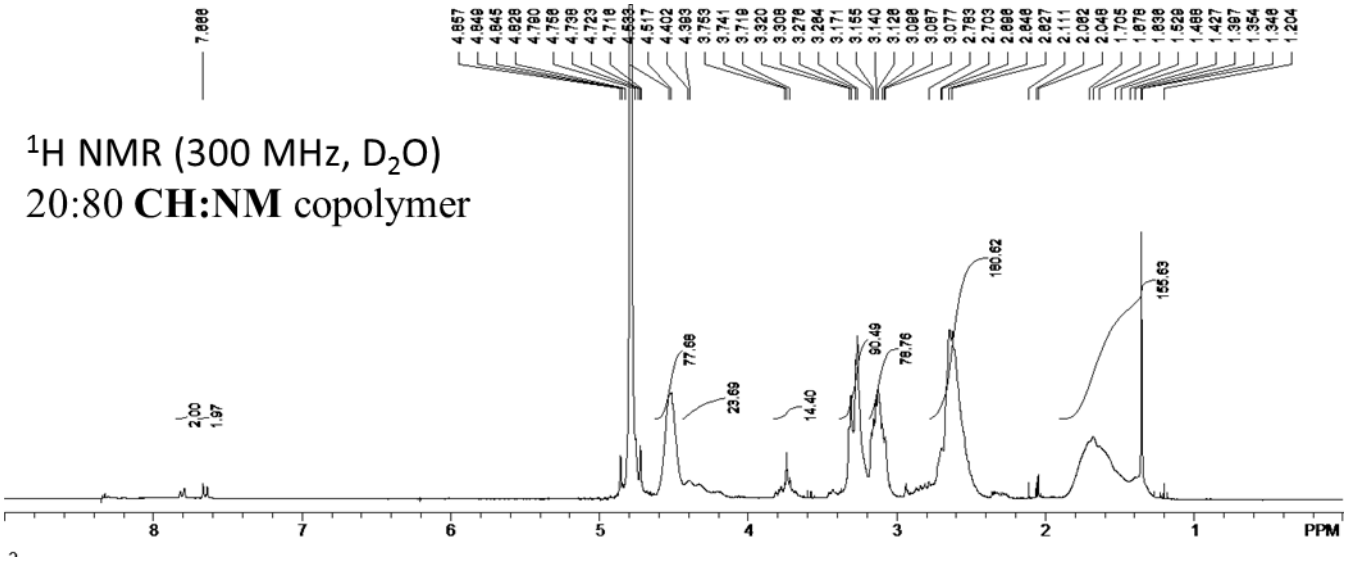


GPC Chromatogram (20:80 **CH:NM** copolymer), mobile phase: DMAc

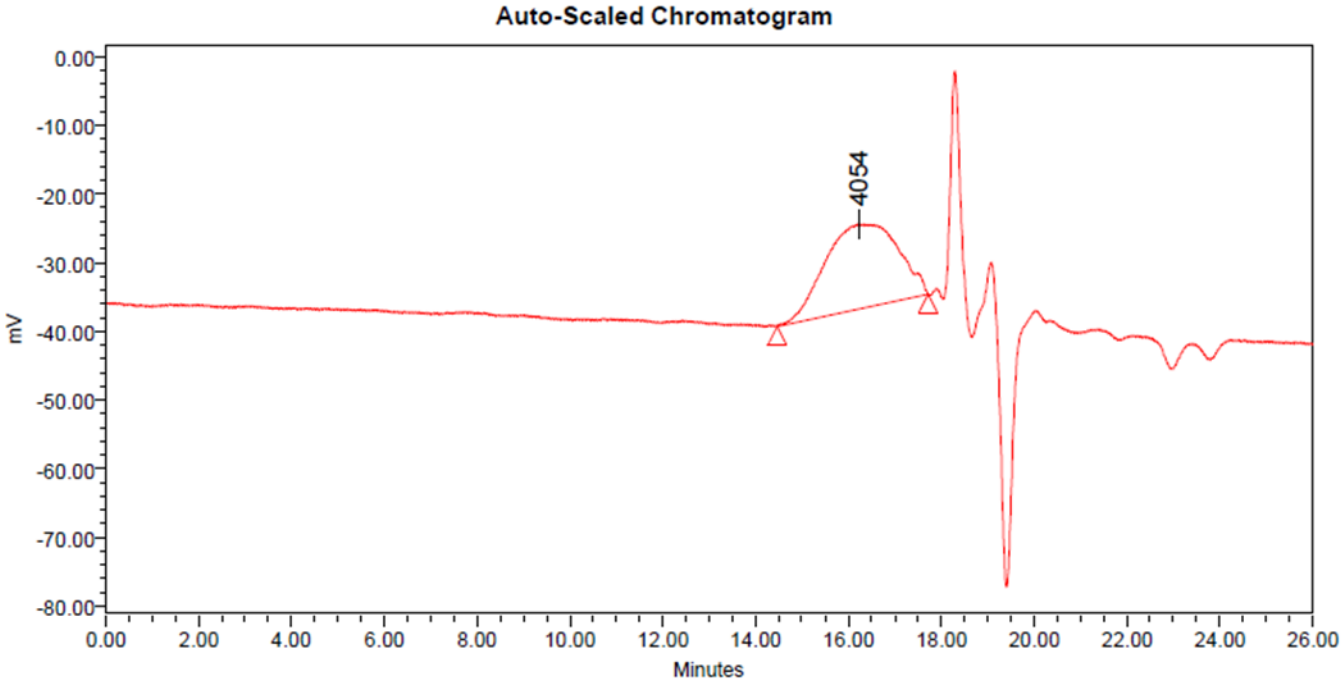


GPC Results

	Dist Name	Mn	Mw	Mv	MP	Mz	Mz+1	Polydispersity	K	alpha
1		4273	5696		5430	7878	11230	1.333021		

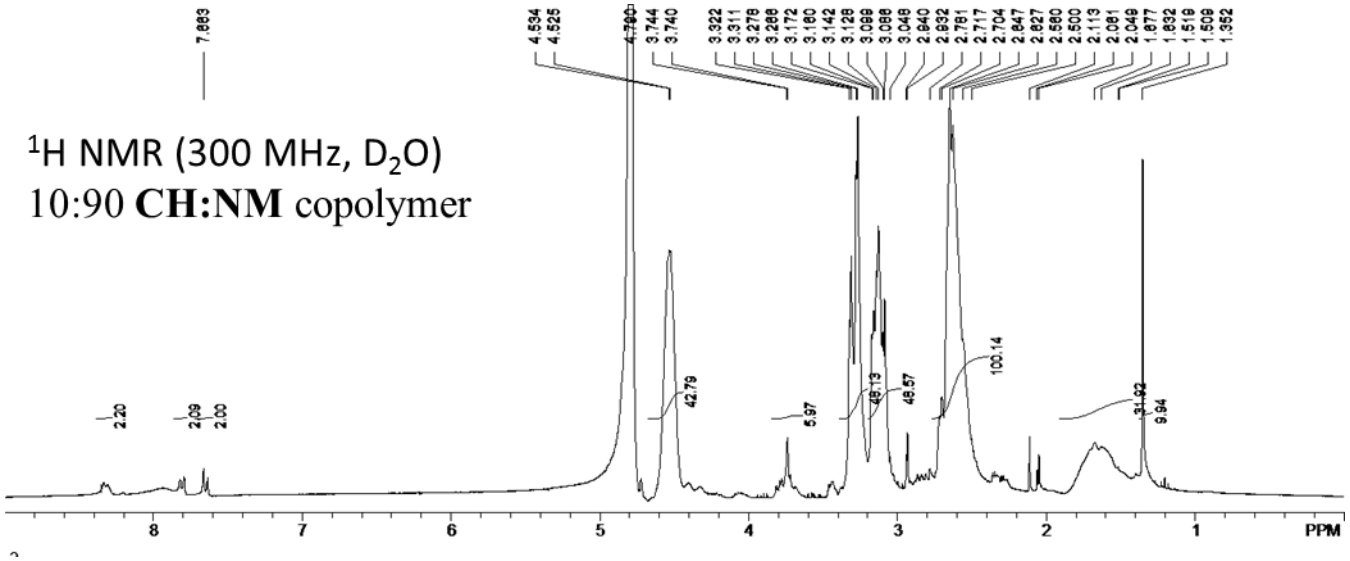


GPC Chromatogram (10:90 **CH:NM** copolymer), mobile phase: DMAc

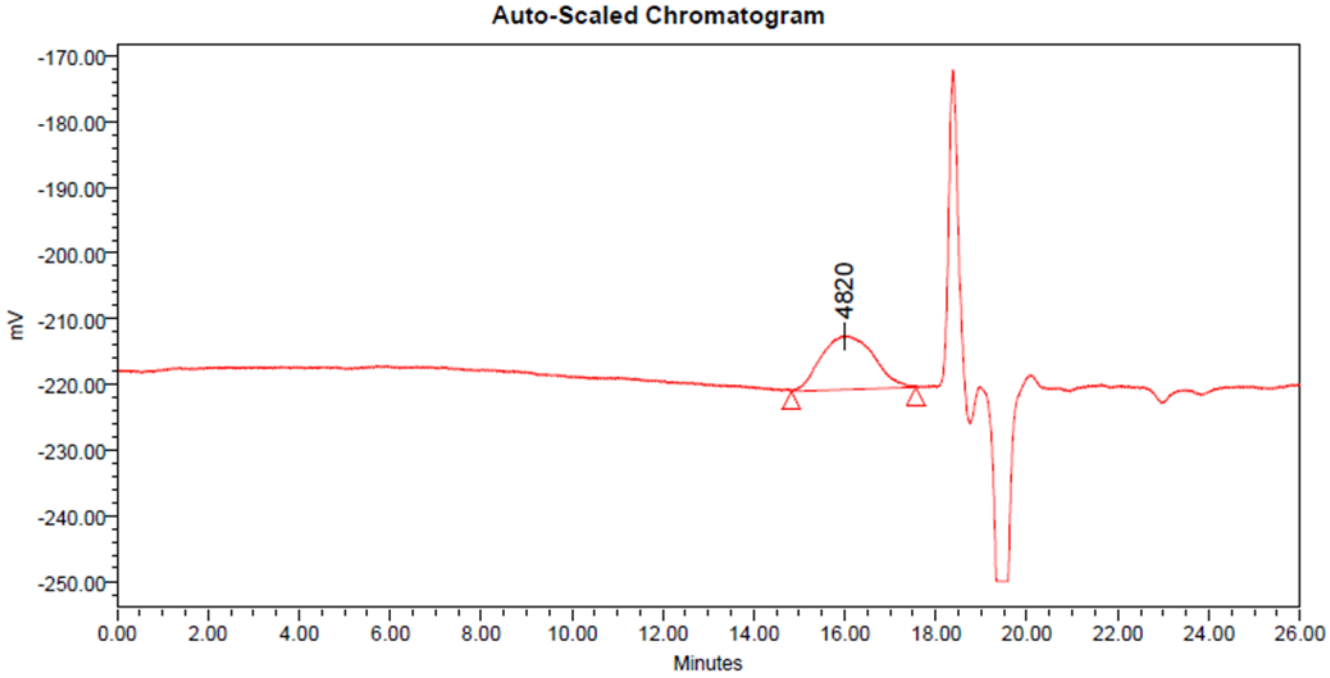


GPC Results

	Dist Name	Mn	Mw	Mv	MP	Mz	Mz+1	Polydispersity	K	alpha
1		3502	4334		4054	5302	6306	1.237488		



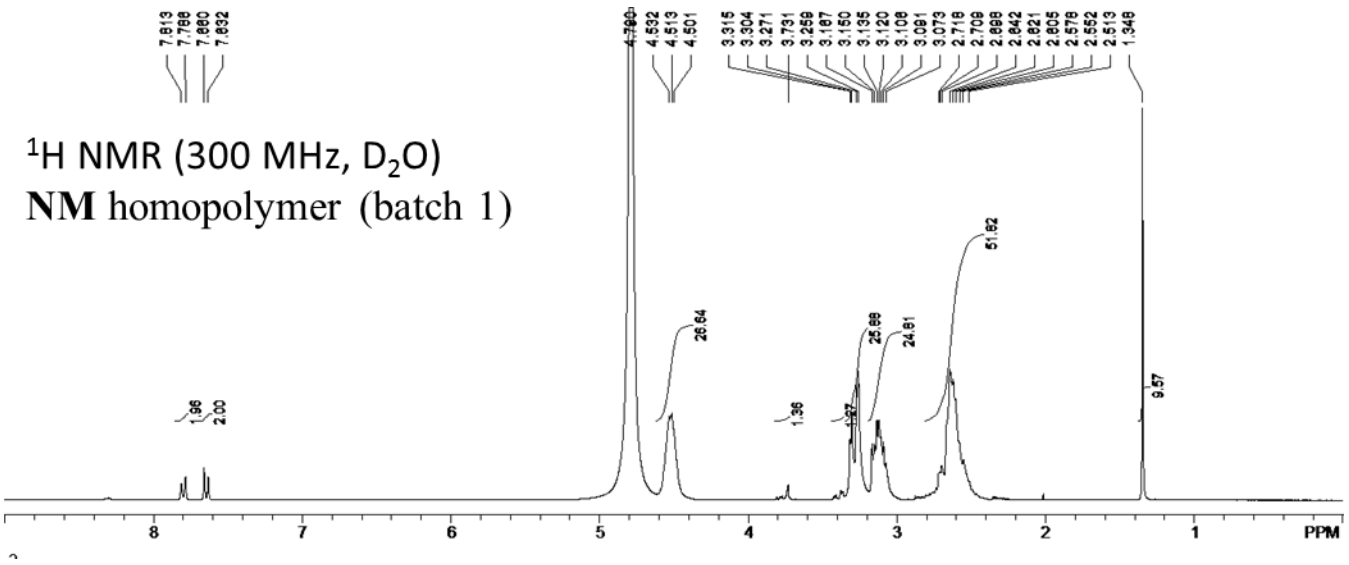
GPC Chromatogram (**NM** homopolymer), mobile phase: DMAc

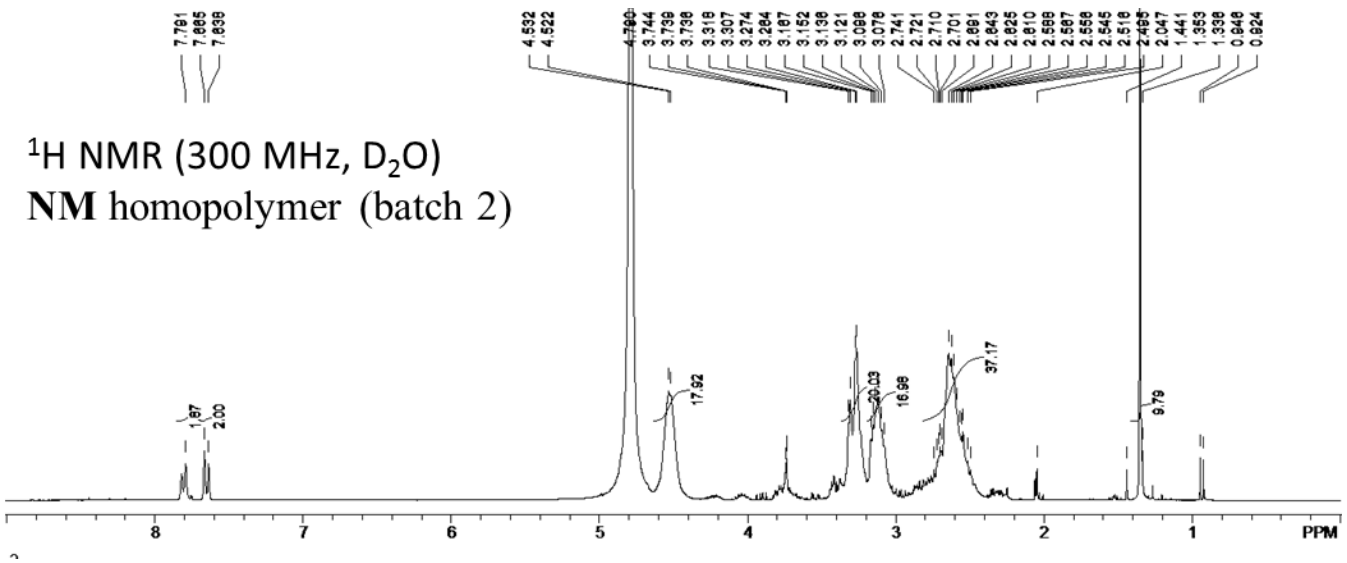


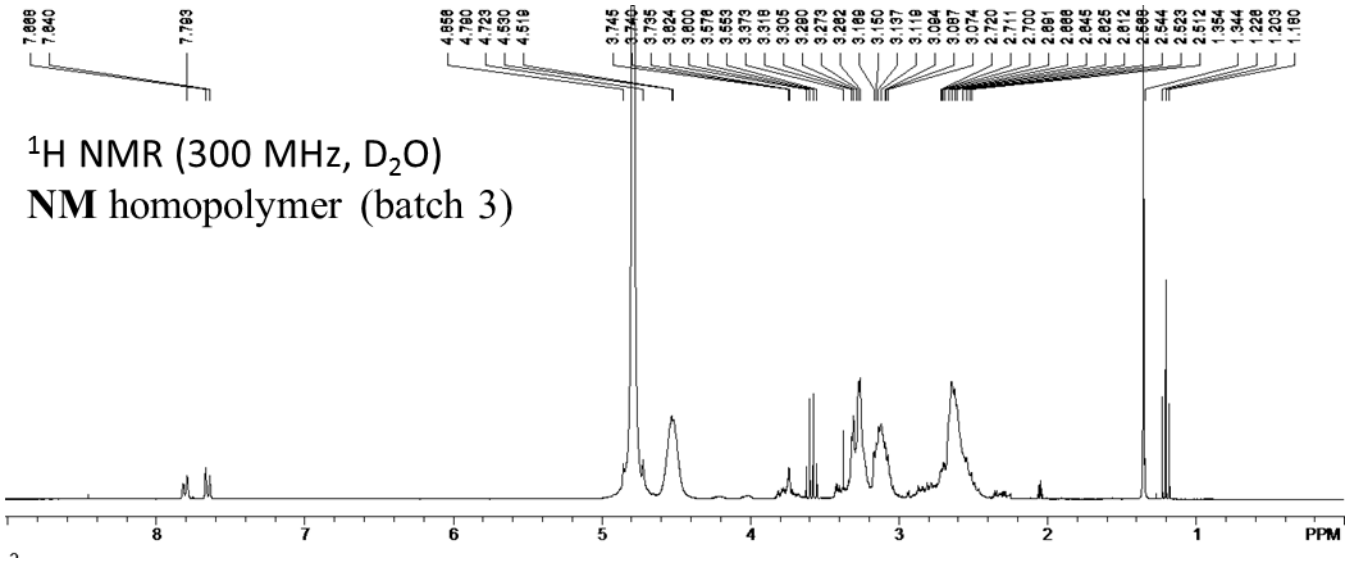
GPC Results

	Dist Name	Mn	Mw	Mv	MP	Mz	Mz+1	Polydispersity	K	alpha
1		4199	4757		4820	5336	5901	1.132934		

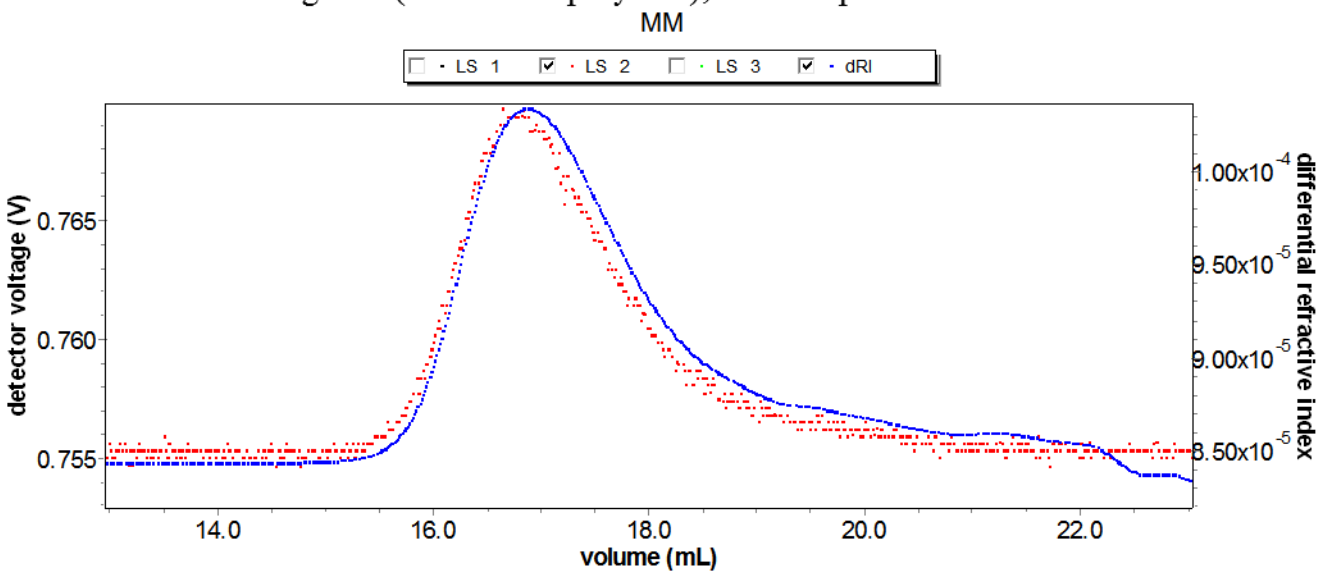
^{1}H NMR (300 MHz, D_2O)
NM homopolymer (batch 1)

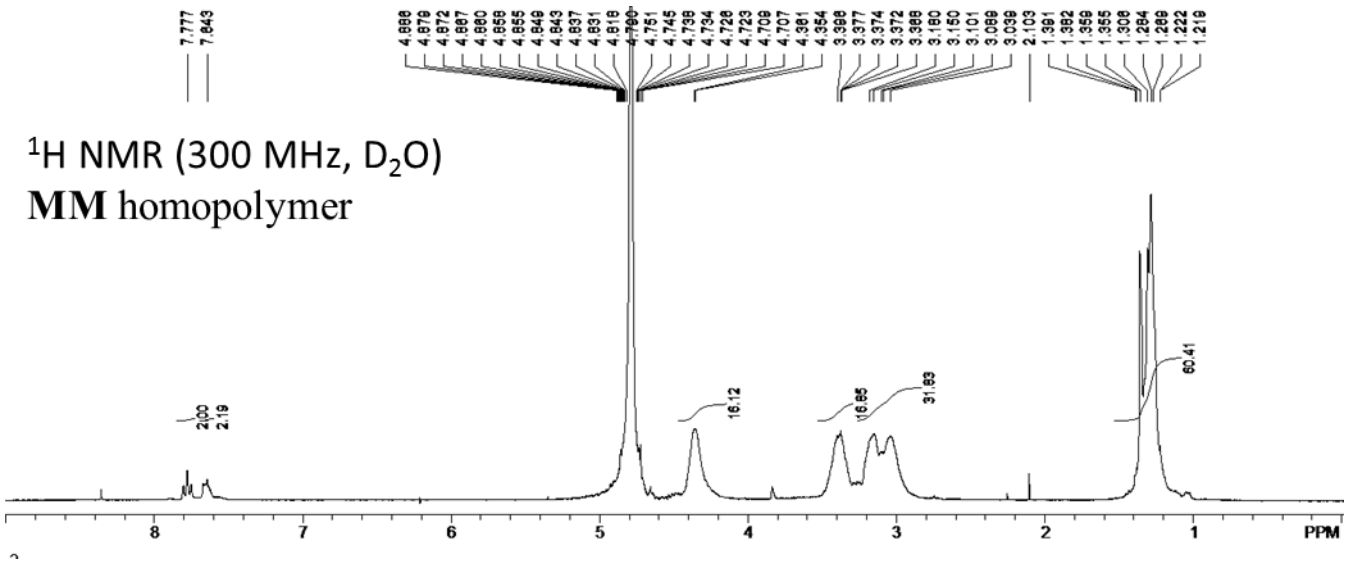



^{1}H NMR (300 MHz, D_2O)
NM homopolymer (batch 2)
PPM

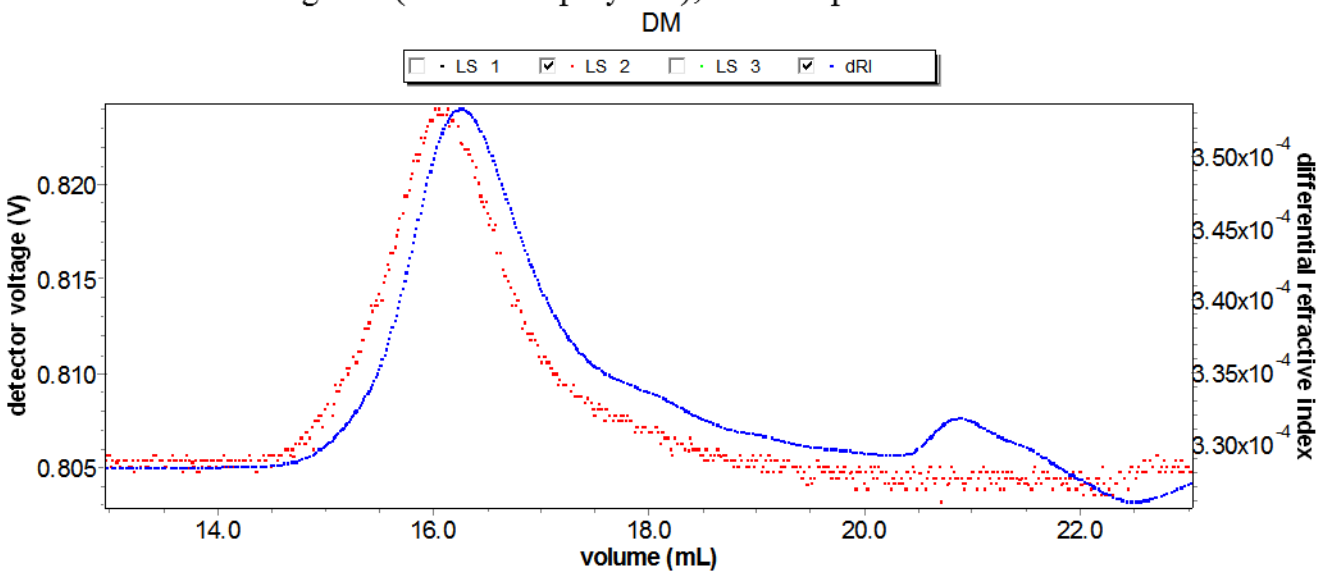

^{1}H NMR (300 MHz, D_2O)
NM homopolymer (batch 3)
PPM

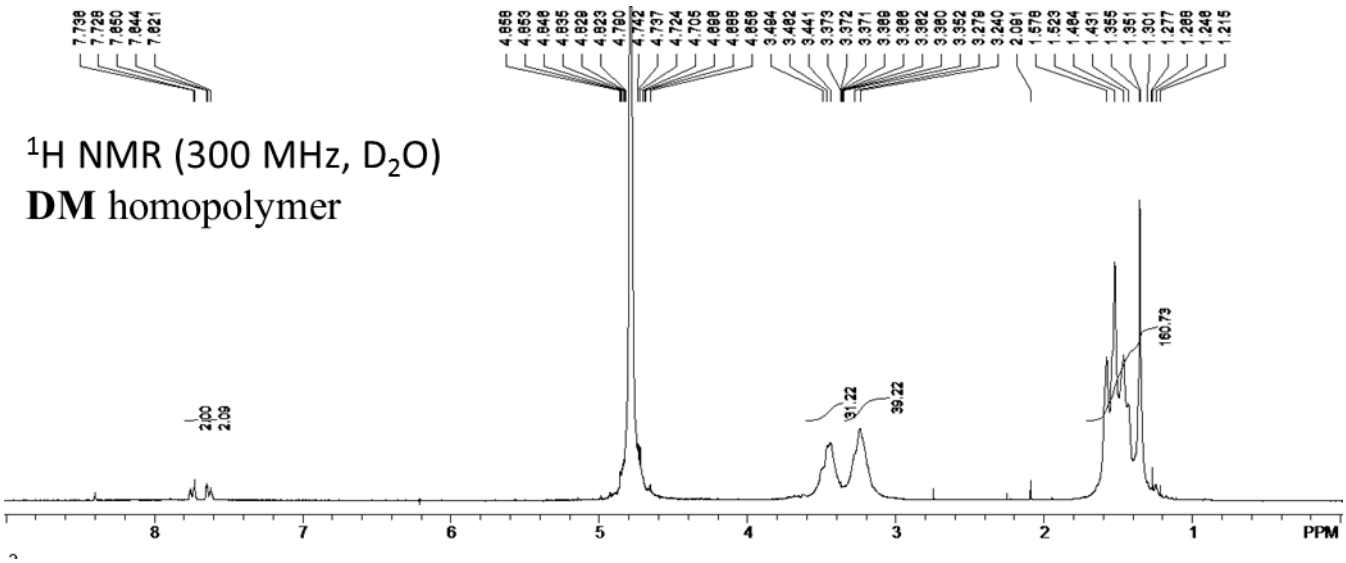
GPC Chromatogram (**MM** homopolymer), mobile phase: THF





GPC Chromatogram (**DM** homopolymer), mobile phase: THF





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