Supporting information

Investigation of antimicrobial PEG-poly(amino acid)s

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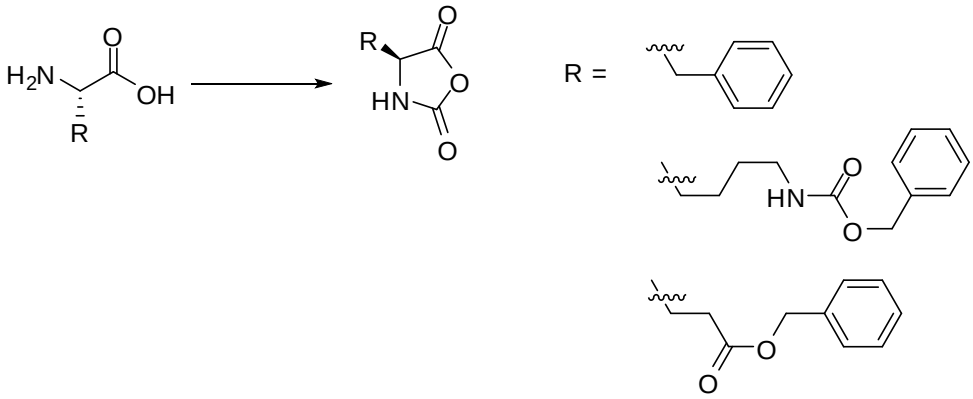
Experimental

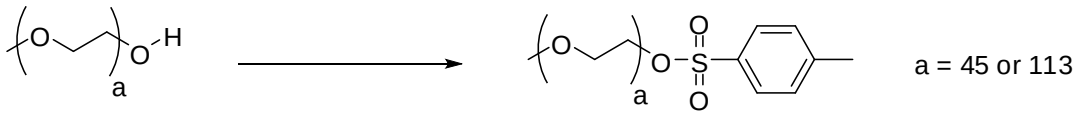
1. Synthesis of PEG-poly(amino acid)s

Synthesis of N-carboxyanhydrides (NCAs):[1]



Typically, to a round bottom flask, 5 g of the amino acid was suspended in approximately 130 mL of anhydrous THF and heated to 60 ℃. Separately, 0.34 equivalents of triphosgene was dissolved in 20 mL of anhydrous THF and added dropwise to the solution under an active N_2 atmosphere (final concentration ~0.15 M). After the solution turned clear in ~4 h, any insoluble precipitates were filtered off. The solution was concentrated under vacuum and crystallized from THF/hexane. Three to four subsequent recrystallizations were preformed until pure white compounds were obtained.

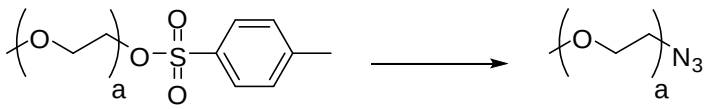
Synthesis of PEG-OTs:



a = 45 or 113

Typically, to a solution of 10 g of mPEG (mw = 2000 or 5000) in 50 mL of DCM, 0.416 ml of TEA was added and stirred for 10 min. Then 3.8 g of p-toluensulfonyl chloride (10 eq.) dissolved in DCM was added and stirred for 20 h at room temperature. The solution was washed with citric acid to neutralize TEA and then dried over $NaSO_4$. The solvent was removed and product was precipitated into ether.

Synthesis of PEG-N_3:

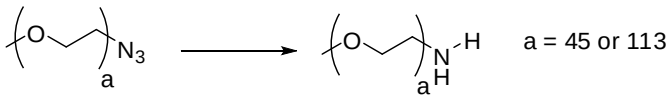


a = 45 or 113

5 g of mPEG-OTs was dissolved in 40 ml of DMF and 1.89 g (30 eq.) of NaN_3 was added. The reaction was heated to 90 ℃ for 20 h. Then 200 ml of brine was added and extracted into DCM.

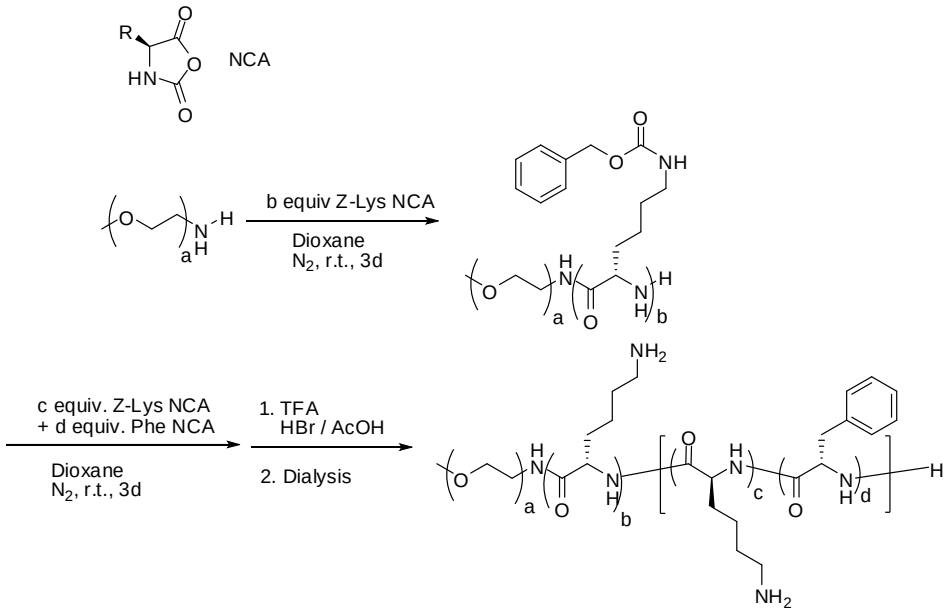
Excess water/brine was used to move all salts into the water layer along with DMF. The DCM extracts were dried over $NaSO_4$, concentrated under reduced pressure and precipitated into ether.

Synthesis PEG-NH_2:



2 g of PEG-N_3 was dissolved in 50 ml of THF (minimal) and 200 mg of LAH (2 eq.) was added at 0 ℃. The reaction was allowed to reach room temperature and was stirred overnight. Then 5 eq of water was added from a 4M NaOH solution and stirred until all the LiOH and $Al(OH)_3$ salts have precipitated out as a white solid. The salts were filtered off, and the solvent was removed to be re-dissolved in DCM. It was then washed with water/brine to remove any excess salts. DCM layers were collected, dried over $NaSO_4$, and precipitated into ether.

Polymerization procedure (except for **P1**)



Synthesis of polymer P3 (The other polymers were prepared by a similar method). 0.4 g of PEG-NH_2 was dissolved in 10 ml of anhydrous dioxane and purged with N_2. Separately 0.49 g (20 eq) of Z-lysine NCA was dissolved in 5 ml anhydrous dioxane, passed through a 2 micron filter to remove any decomposed NCA, thoroughly purged with N_2 and then added via syringe. The reaction was then allowed to proceed for 3 days under an active N_2 atmosphere. Then 10 equivalents of Z-Lysine NCA and 15 equivalents of Phenylalanine NCA were dissolved in 5 ml anhydrous dioxane, passed through a 2 micron filter, purged, and added via syringe. Polymerization was allowed to continue for another 3 days. The clear solution was then precipitated into ether and the product was collected via filtration.

Removal of Z protecting groups:

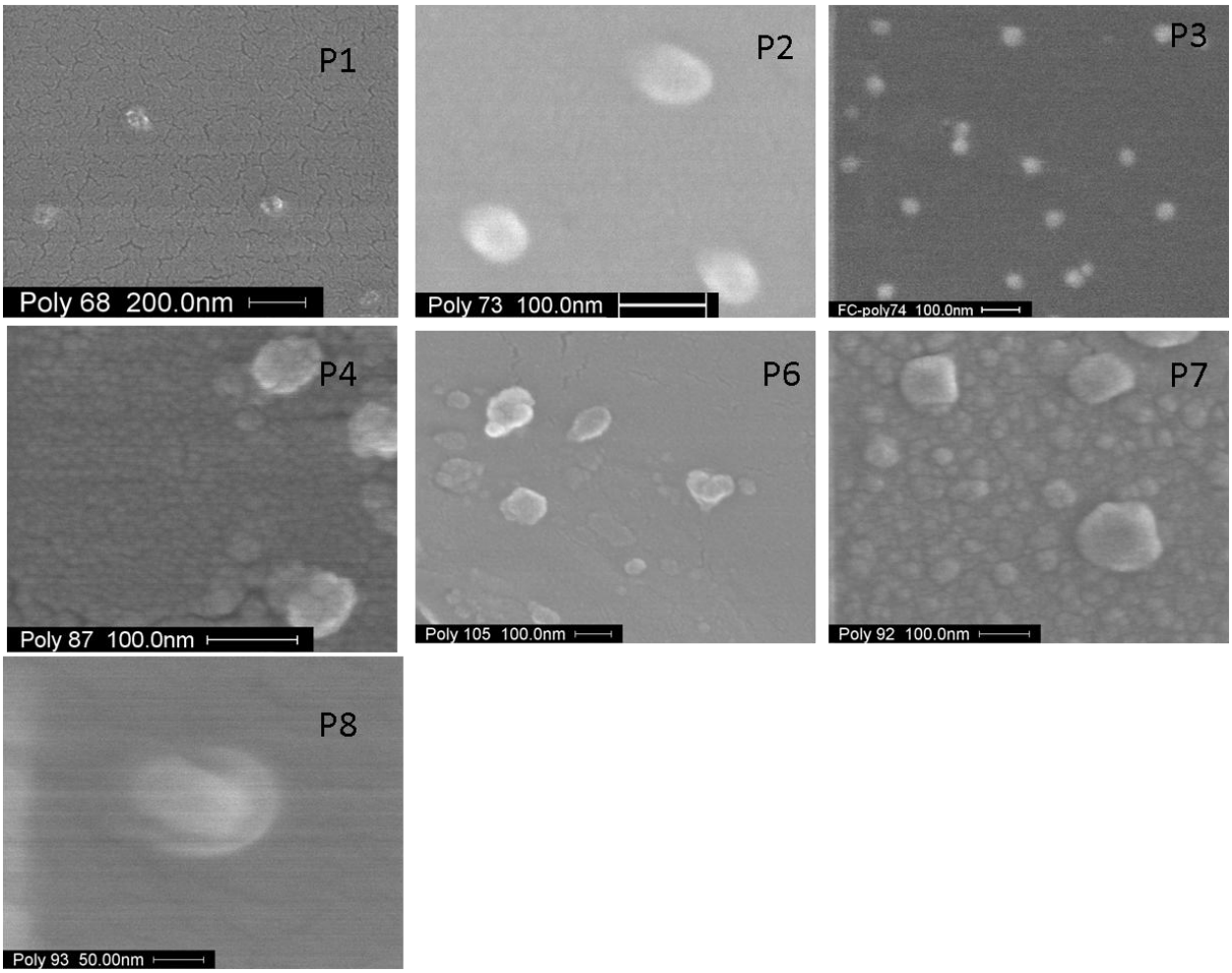
Polymers were dissolved in 10 ml of TFA and to this solution, 10 equivalents of HBr in AcOH (33% v/v conc.) was added and stirred for 4 hours. Product was then precipitated into ether and collected via filtration.

Dialysis:

The polymers were dissolved in minimal DMSO and added to dialysis tubing (MWCO = 3,500) followed by immersion in water. Dialysis was carried out for 3 days replacing the water daily. Any precipitate was then filtered out, and the clear filtrates were lyophilized to afford the final products.

Polymer #	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
MW (calculated)	10880	9030	11045	7750	4750	5485	7500	8780	4560	3920

2. The morphology of PEG-poly(amino acid)s in water.



3. Antimicrobial activity

The bacterial strains used for testing the efficacy of polymers were multi-drug resistant *S. epidermidis* (RP62A), *B. subtilis* (BR151), *K. pnuemoniae* (ATCC 13383) and multi-drug resistant *P. aeruginosa* (ATCC 27853). The antimicrobial activities of the polymers developed were determined in sterile 96-well plates by serial dilution method. Bacterial cells were grown overnight at 37 °C in 5 mL medium after which a bacterial suspension of approximately 10^6 CFU/mL in Luria broth or trypticase soy was prepared ensuring that the bacterial cells were in the mid-logarithmic phase. Aliquots of 50 µL bacterial suspension were added to 50 µL of medium containing different concentrations of polymers for a total volume of 100 µL in each well. The 96-well plates were incubated at 37 ^{0}C for about 20 h. The Biotek microplate reader was used to measure the optical density (OD) at a wavelength of 600 nm after about 20 h. The experiments were carried out as three independent biological replicates, each in duplicate. The lowest concentration at which complete inhibition of bacterial growth is observed is defined as the minimum inhibitory concentration (MIC).

4. Hemolytic activity

Freshly drawn human red blood cells (hRBC's) were used for the assay. The blood sample was washed with PBS buffer several times and centrifuged at 700g for 10 min until a clear supernatant was observed. The hRBC's were re-suspended in 1X PBS to get a 5% v/v suspension which was used to perform the assay. 50 µL of different polymers solutions were added to sterile 96-well plates. Then 50 µL of 5% v/v hRBC solution was added to make up a total volume of 100 µL in each well. The 0% hemolysis point and 100% hemolysis point were determined in 1 X PBS and 0.2% Triton-X-100 respectively. The 96 well plate was incubated at 37 °C for 1h and centrifuged at 3500 rpm for 10 min. The supernatant (30 µL) was then diluted with 100 µL of 1XPBS and hemoglobin was detected by measuring the optical density at 360nm by Biotek microtiter plate reader (Type: Synergy HT).

$$\% \text{ hemolysis} = (Abs_{sample} - Abs_{PBS}) / (Abs_{Triton} - Abs_{PBS}) \times 100$$

5. Fluorescence microscopy

A double staining method with DAPI (4',6-Diamidino-2-phenylindole dihydrochloride,Sigma,>98%) and PI (Propidium iodide, Sigma) as fluorophores was used to visualize and differentiate the viable from the dead *B. subtilis* cells. DAPI being a double stranded DNA binding dye, stains all bacterial cells irrespective of their viability. Ethidium derivatives such as propidium iodide (PI) is capable of passing through only damaged cell membranes and intercalates with the nucleic acids of injured and dead cells to form a bright red fluorescent complex. The bacterial cells were first stained with PI and then with DAPI. The bacterial cells were grown until they reached mid-logarithmic phase and then ~ $2x10^3$ cells were incubated with 100 µg/mL polymer P5 for 4 h. Then the cells were pelleted by centrifugation at 3000 g for 15 min in an Eppendorf microcentrifuge. The supernatant was decanted and the cells were washed with 1X PBS several times and then incubated with PI (5 µg/mL) in the dark for 15 min at 0 ^{o}C .The excessive PI was removed by washing the cells several times with 1X PBS several times. Lastly, the cells were incubated with DAPI (10 µg/mL in water) for 15 min in the dark at 0^{o}C. Then finally the excessive DAPI solution was removed by washing it with 1X PBS.

The controls were performed following the exact same procedure for bacteria without **P5**. The bacteria were then examined by using the Zeiss Axio Imager Z1optical microscope with an oil-immersion objective (100X). [2, 3]

6. SEM of bacteria after treatment of polymer P5.

MRSE and *B.subtilis* were grown to an exponential phase and approximately about 2 x 10^6 cells were incubated with polymer **P5** for about 20 h. The cells were then harvested by centrifugation (3000 g) for 15 min. After pelleting the cells, the cells were washed three times with DI water. The cells were then fixed with 2.5% (w/v) glutaraldeyhyde in nanopure water for about 30 min, followed by extensive wash with DI water to get rid of any excess glutaralhedyde. Graded ethanol series (30%, 50%, 70%, 95% and 100%, 5 min each) was then used to dehydrate the cells. Following the dehydration of cells hexamethyldisilazane was added for about 2 min. Then about 2 µL of sample was added to a silicon wafer followed by Gold/Pd coating, and then the samples were observed at 25KV with a HITACHI S-800 scanning electron microscope.

References

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2. Y. Niu, S. Padhee, H. Wu, G. Bai, L. Harrington, W. N. Burda, L. N. Shaw, C. Cao and J. Cai, *Chemical communications (Cambridge, England)*, 2011, **47**, 12197-12199.
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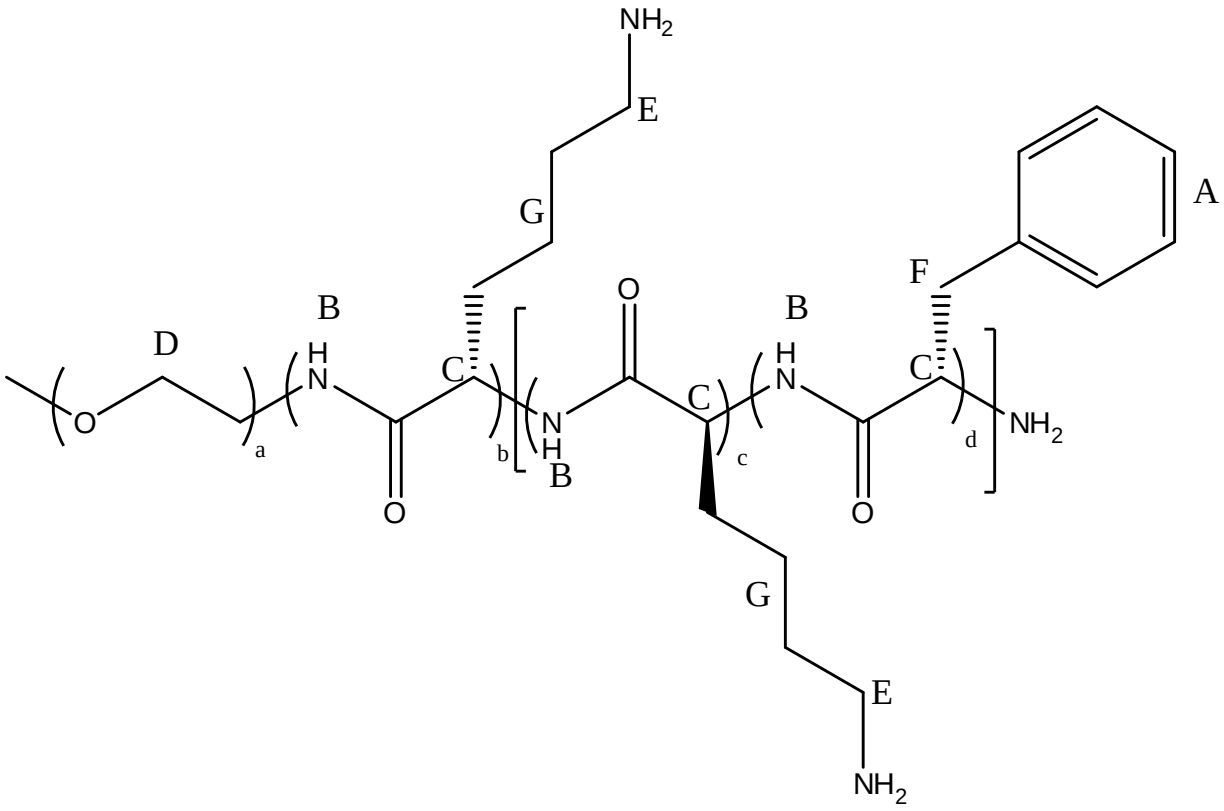
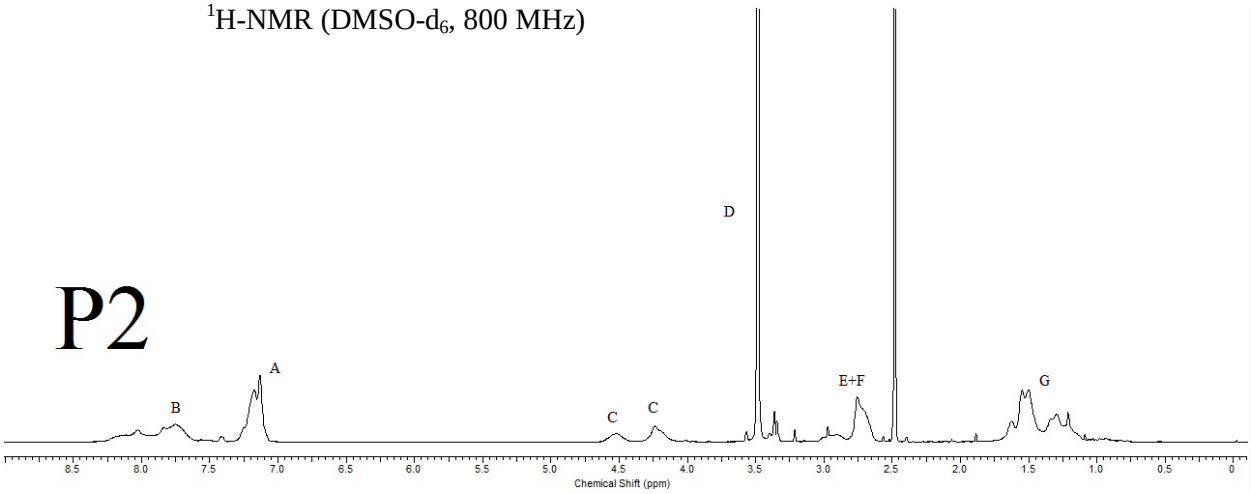
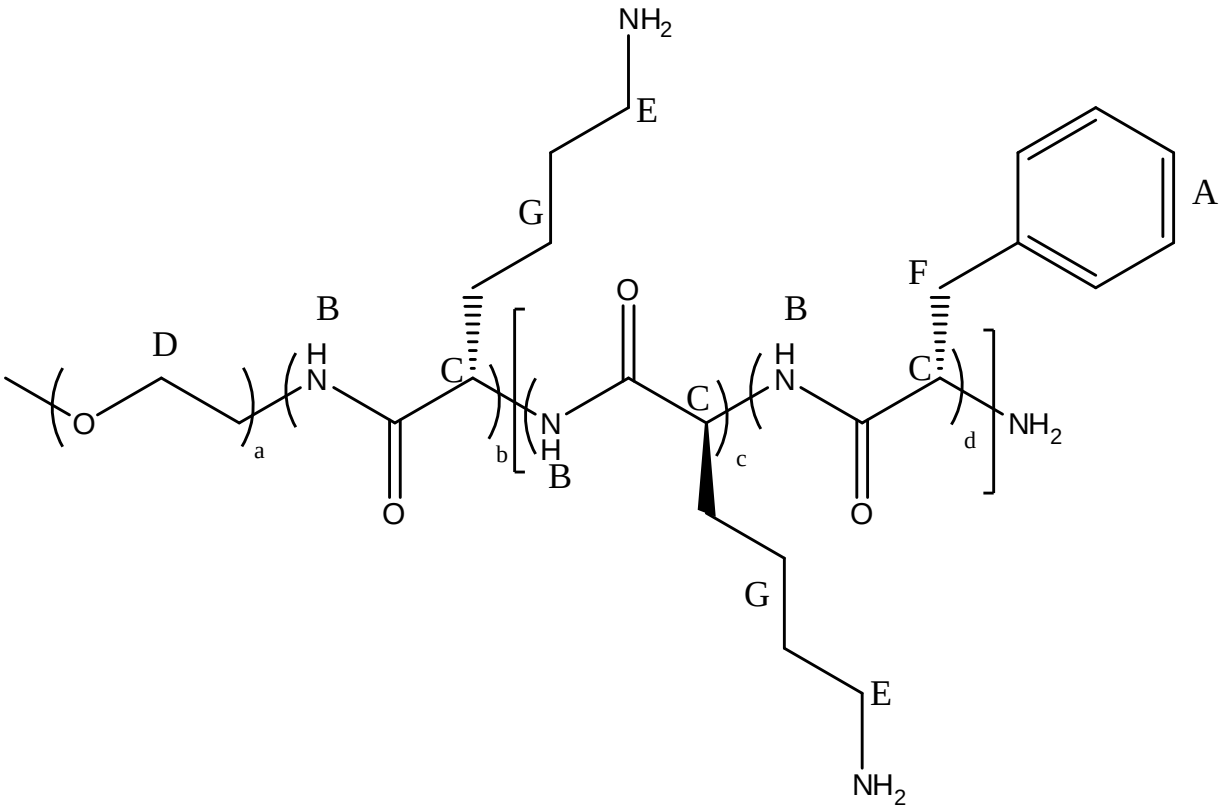
3. Y. Niu, S. Padhee, H. Wu, G. Bai, Q. Qiao, Y. Hu, L. Harrington, W. N. Burda, L. N. Shaw, C. Cao and J. Cai, *Journal of medicinal chemistry*, 2012, **55**, 4003-4009.







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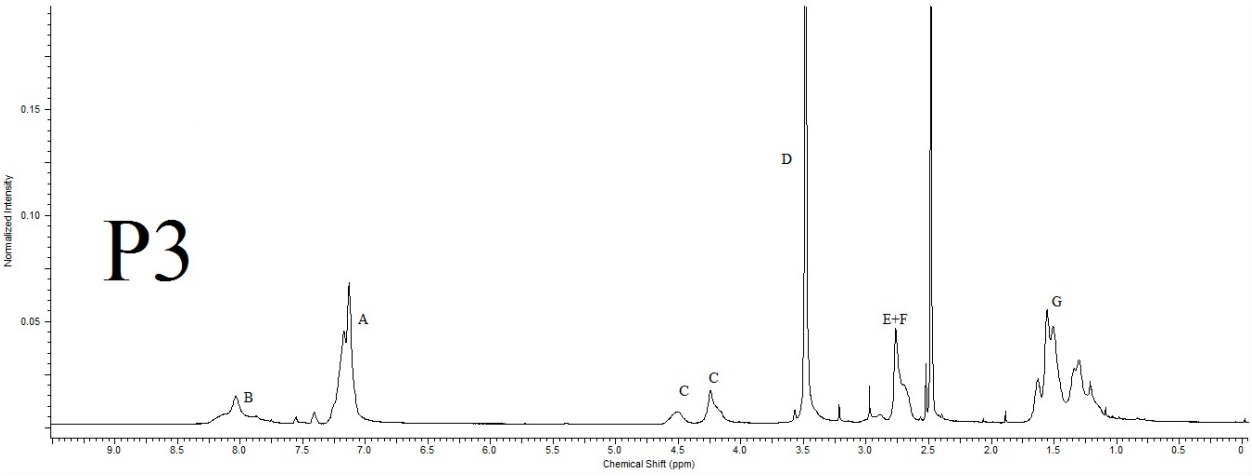

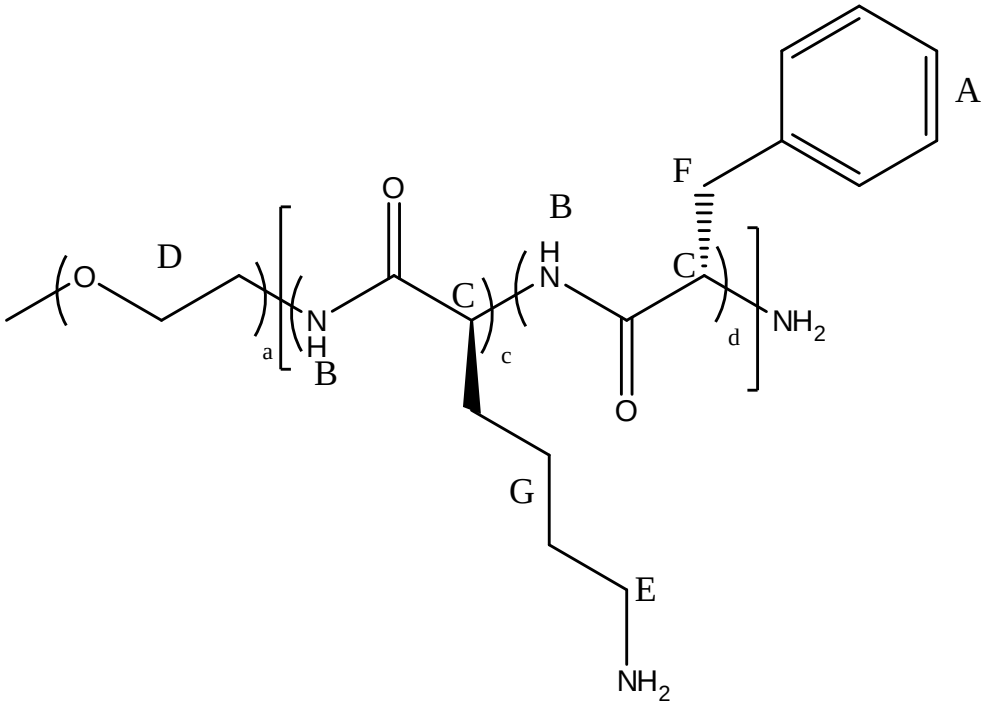

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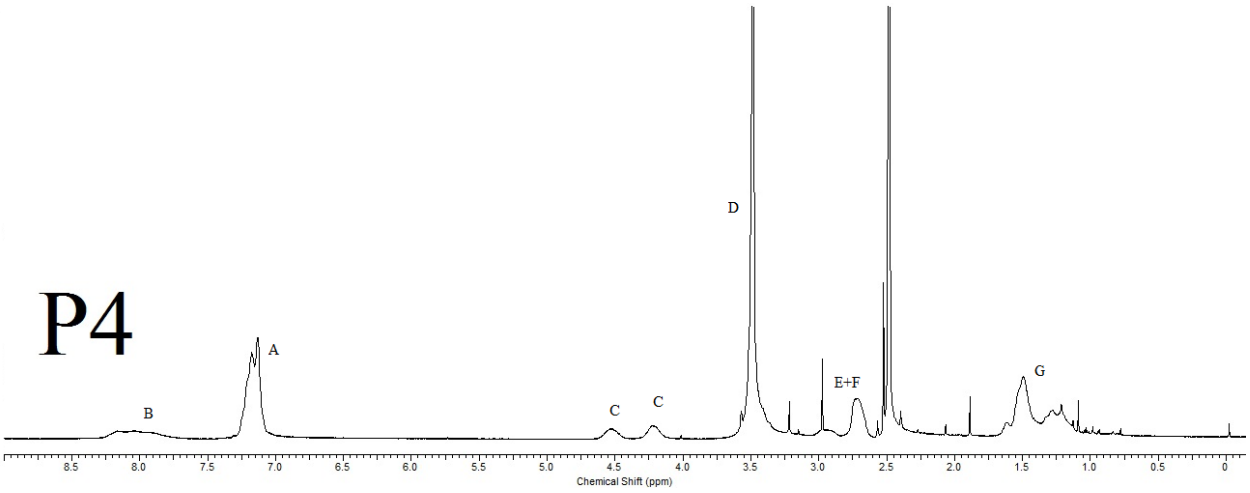

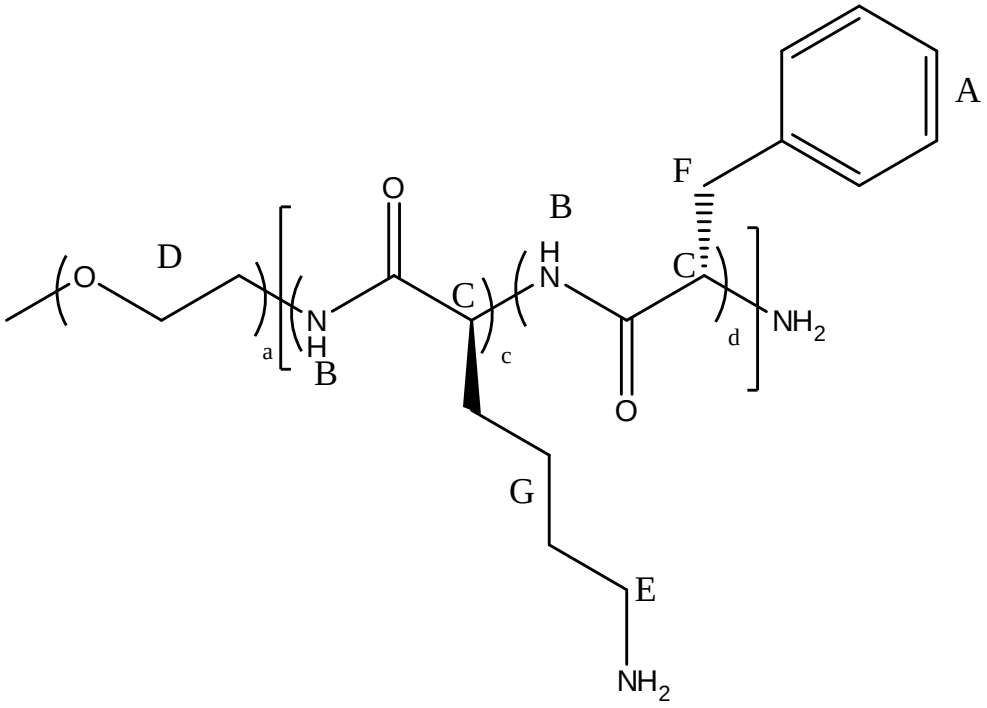

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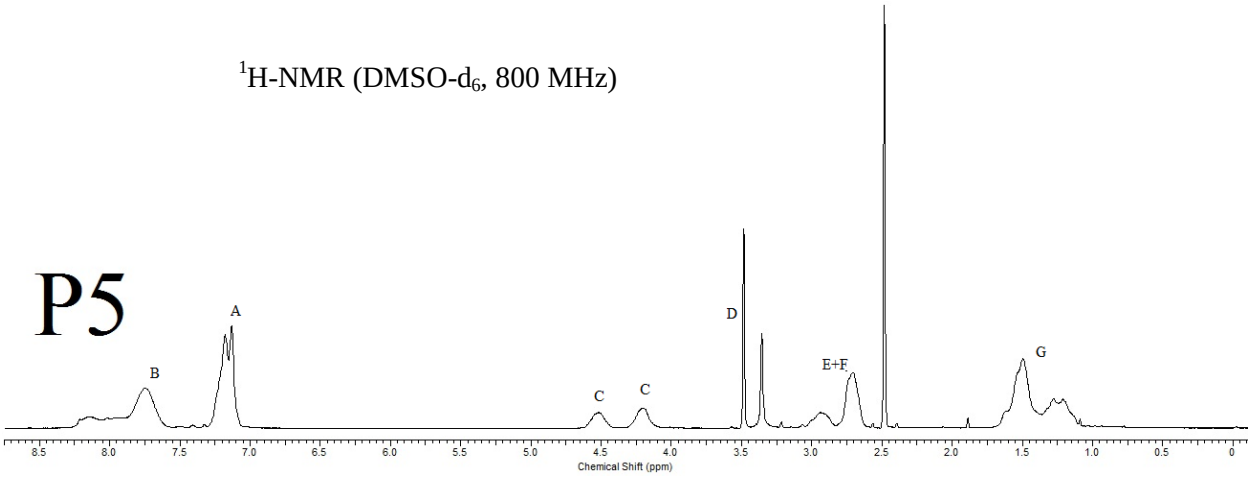

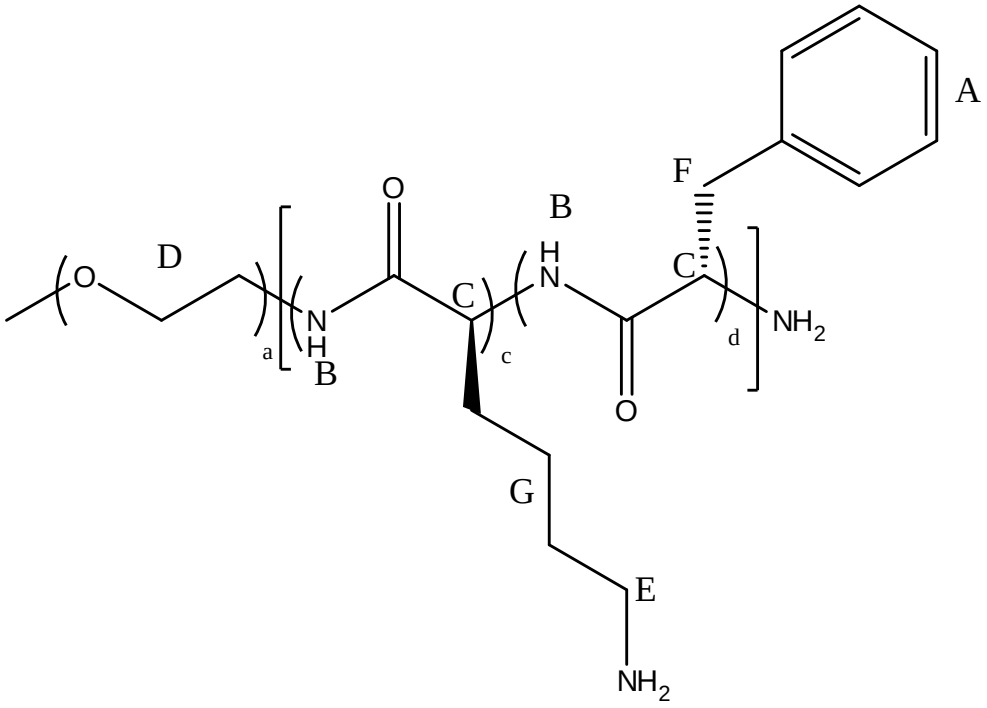

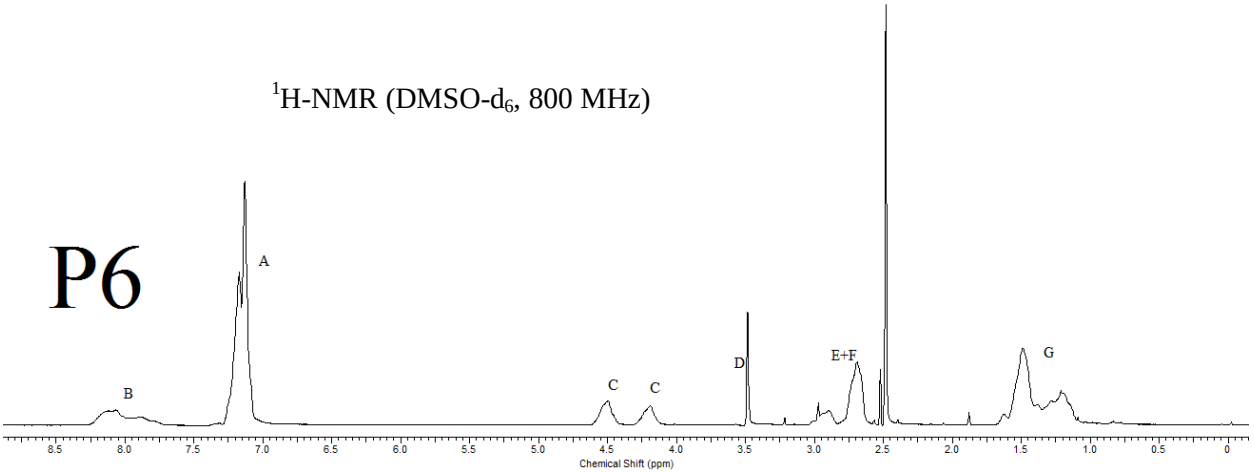

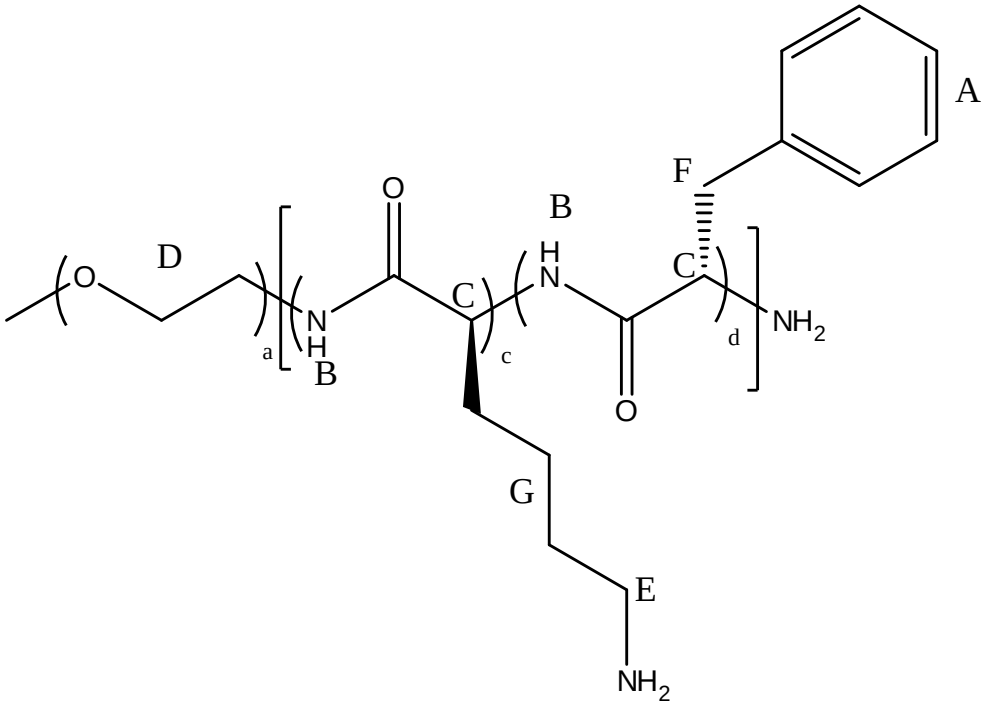

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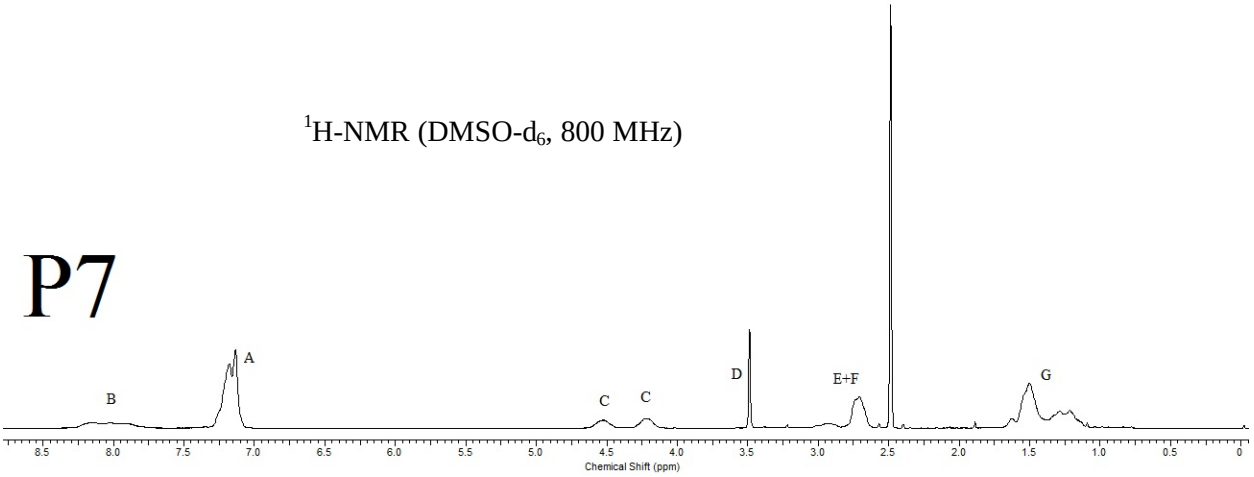

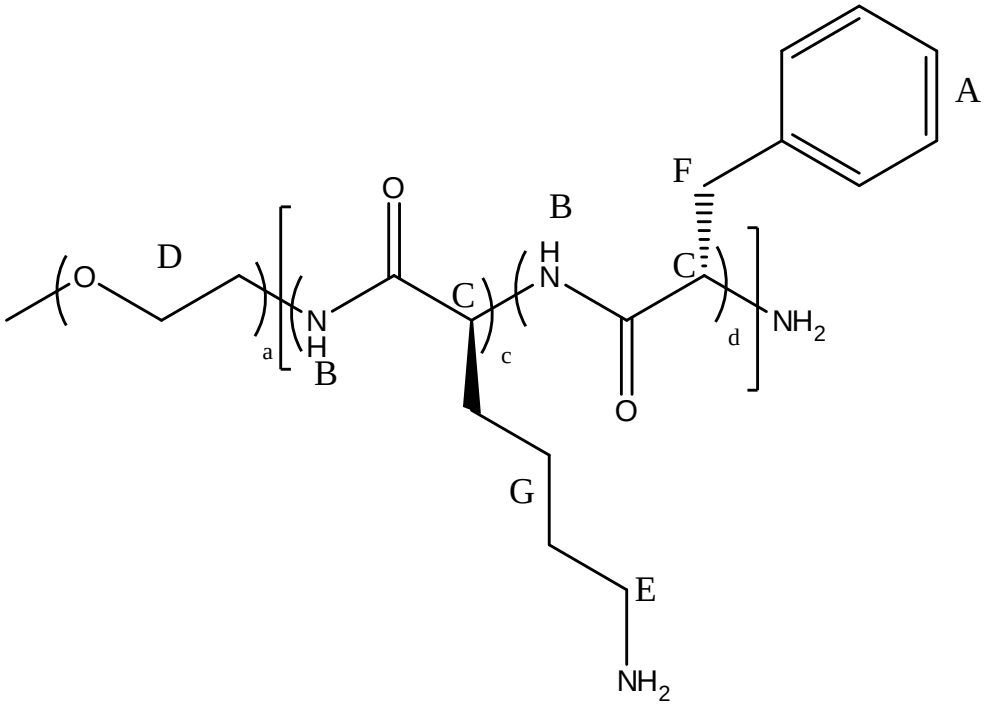
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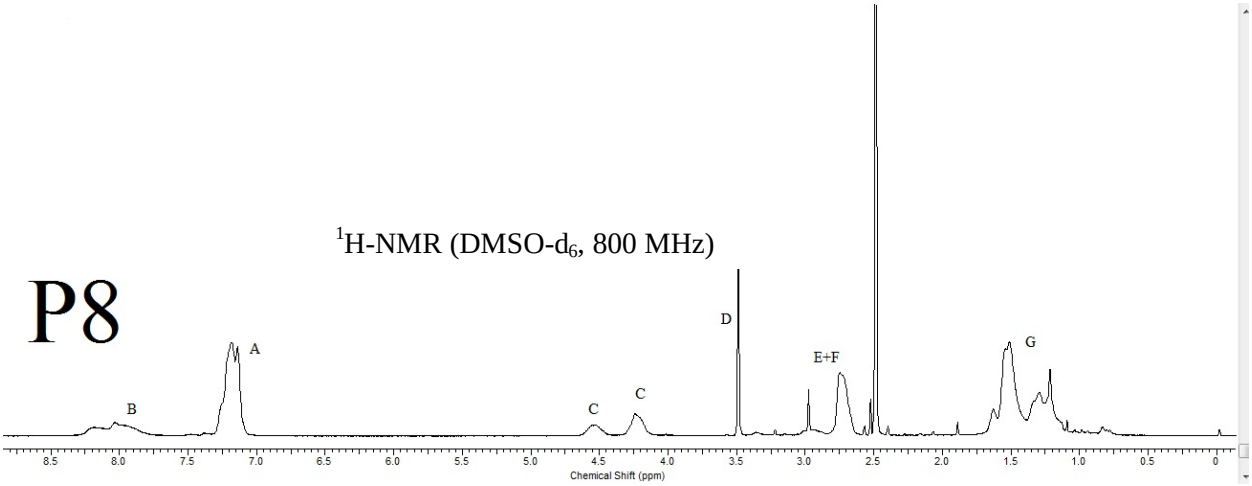
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D
E+F
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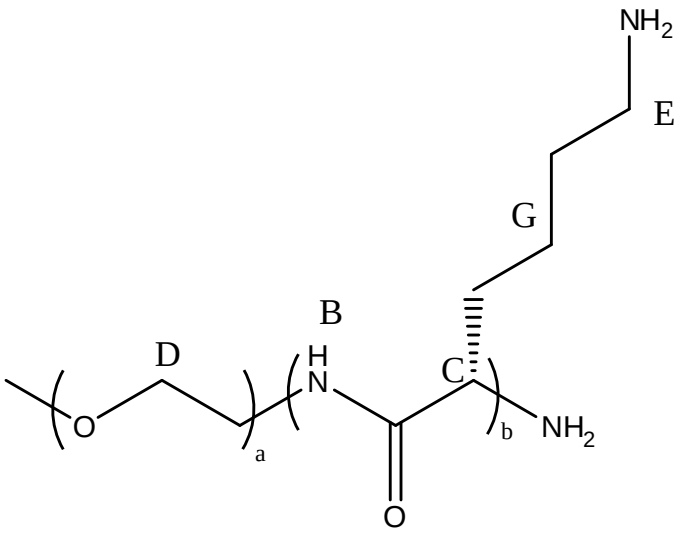
Chemical Shift (ppm)



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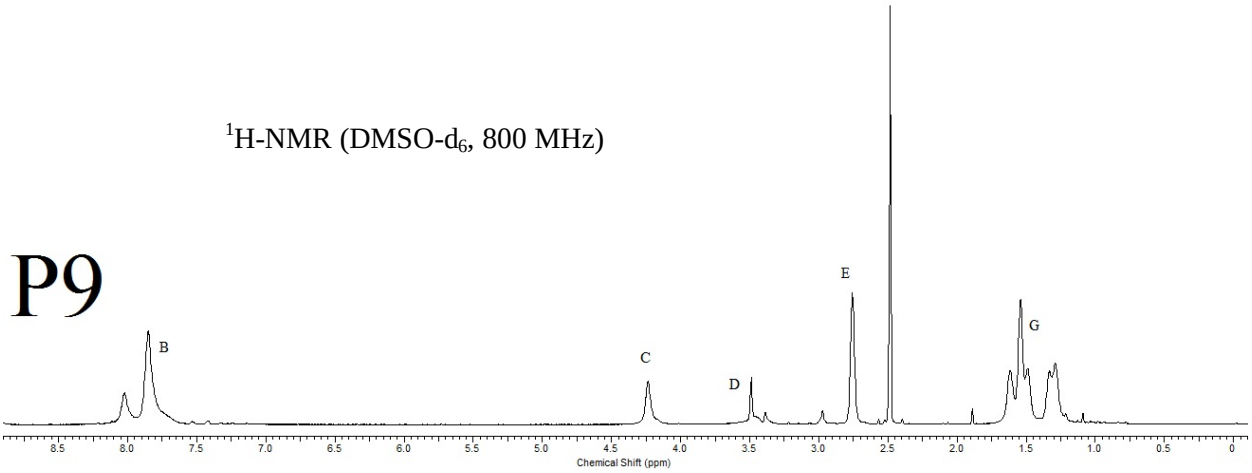

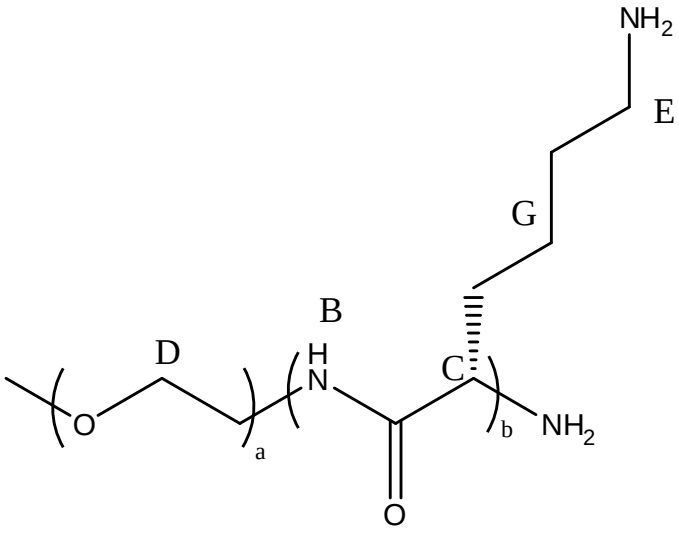
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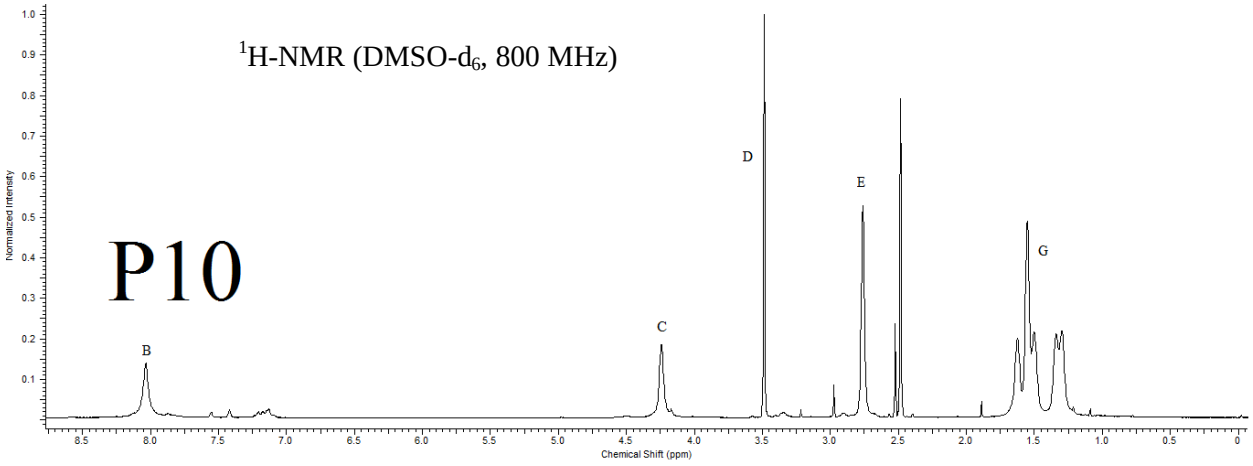
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