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Electronic Supplemental Information

for

Modular Molecular Design of Polymerized Pro-Estrogen Materials Enables Controlled Astrocyte Response

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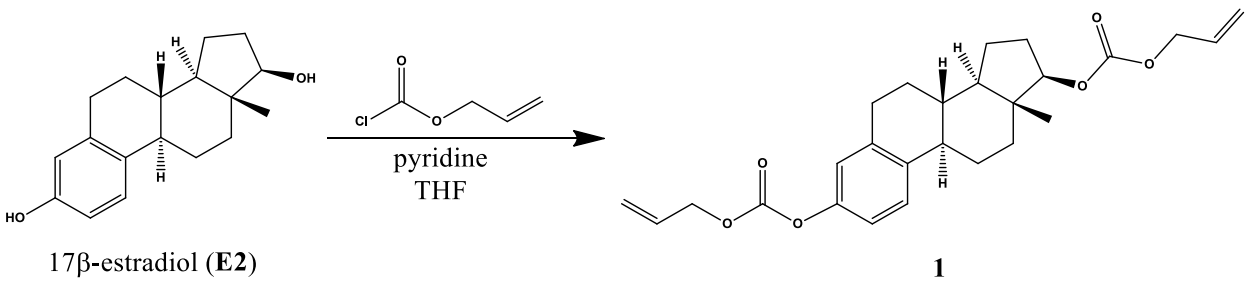
Synthetic Procedures:

Difunctional prodrug of E2 as the bisallyl carbonate (**1**) was prepared according to a as previously described. Briefly, in a nitrogen-filled glovebox, 17β-estradiol (5.00 g, 18.35 mmol, 1 eq.) was dissolved in anhydrous THF (50 mL) in a 250 mL Schlenk flask with a magnetic stir bar, and anhydrous pyridine (14.8 mL, 0.184 mol, 10 eq.) was added. Allyl chloroformate (11.7 mL, 0.110 mol, 6 eq.) was dissolved in anhydrous THF (12 mL) in a 50 mL addition funnel that was secured to the Schlenk flask and capped with a rubber septum. The apparatus was removed to an inert gas Schlenk line and the flask was cooled to 0°C over an ice bath with stirring for ~ 15 min. Under N_2, the chloroformate solution was added dropwise to the cooled solution in the flask. After complete addition, the flask was removed from the ice bath and allowed to warm to rt overnight with stirring. The resulting mixture was diluted with ethyl acetate (~75 mL) in a 250 mL separatory funnel, extracted sequentially with 0.1 M HCl aq. (100 mL), deionized water (100 mL), 0.1 M NaOH aq. (100 mL), deionized water (100 mL), and brine (100 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude oil was then purified by silica gel column chromatography (ethyl acetate: hexanes = 2:3 v/v, $R_f = 0.7$) to give a viscous yellow oil that was dried *in vacuo* overnight (3.3524 g, 41.45 % yield).



17β-estradiol (**E2**) **1**

Figure S-1. Synthesis of the diallylcarbonate of 17β estradiol (**1**).

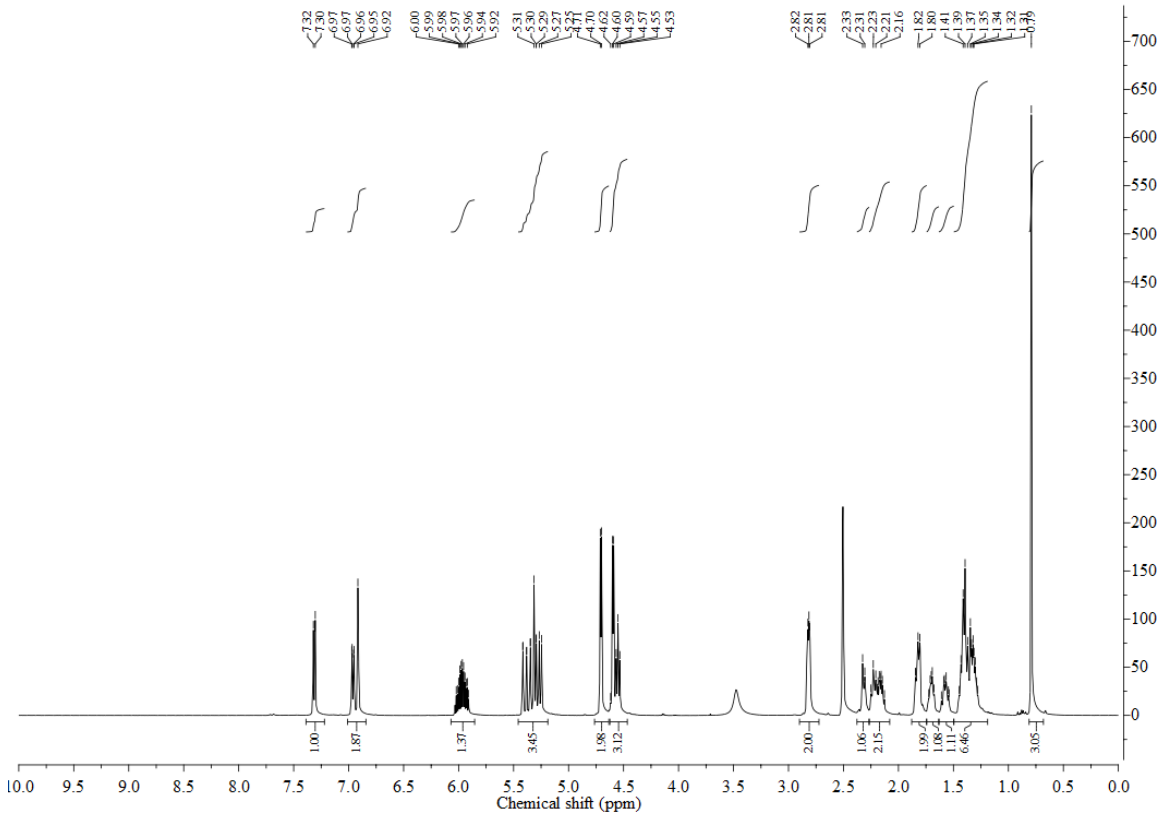


Figure S-2. ^{1}H NMR spectrum of **1** in DMSO-d6.

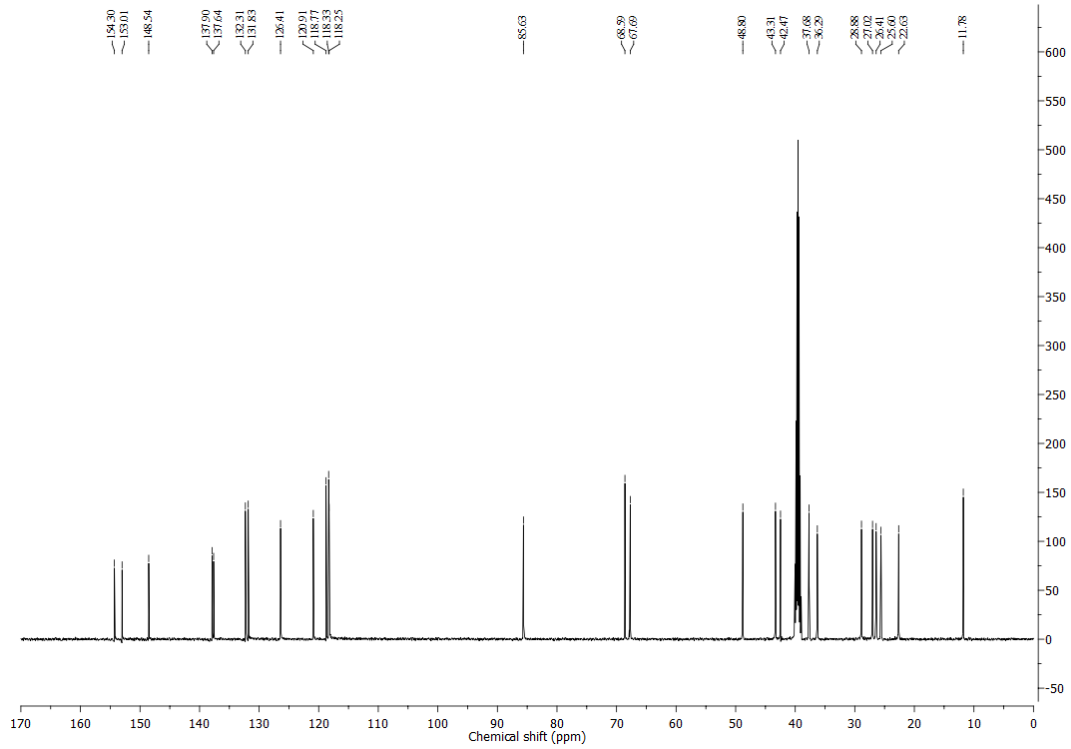
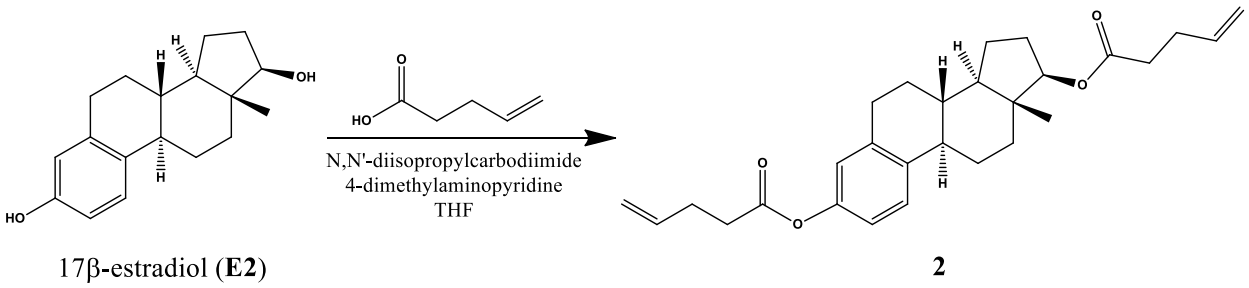


Figure S-3. ^{13}C NMR spectrum of **1** in DMSO-d6.

Difunctional prodrug of E2 as the bispentenoate ester (**2**) was prepared as follows: in a nitrogen-filled glovebox, 17β-estradiol (5.00 g, 18.35 mmol, 1 eq.) was dissolved in anhydrous THF (50 mL) in a 250 mL Schlenk flask with a magnetic stir bar. Then, 4-pentenoic acid (11.25 mL, 0.110 mol, 6 eq.) and 4-dimethylaminopyridine (DMAP, 1.8 g, 14.68 mmol, 0.8 eq) were added. The flask was capped with a rubber septum, then removed to an inert gas Schlenk line and the flask was cooled to 0°C over an ice bath with stirring for ~ 15 min. After purging a syringe with inert gas, the syringe was filled with N,N'-diisopropylcarbodiimide (DIC, 8.55 mL, 55.05 mmol, 3 eq). Under N_2, the DIC was added dropwise to the cooled solution in the flask. After complete addition, the flask was removed from the ice bath and allowed to warm to rt overnight with stirring. The resulting mixture was diluted with ethyl acetate (~75 mL) in a 250 mL separatory funnel, extracted sequentially with 0.1 M HCl aq. (100 mL), deionized water (100 mL), 0.1 M NaOH aq. (100 mL), deionized water (100 mL), and brine (100 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude oil was then purified by silica gel column chromatography (ethyl acetate: hexanes = 1:1 v/v, $R_f = 0.7$) to give a viscous yellow oil that was dried *in vacuo* overnight (3.276 g, 40.89 % yield).



17β-estradiol (**E2**) **2**

Figure S-4. Synthesis of the bispentenoic acid ester of of 17β estradiol (**2**).

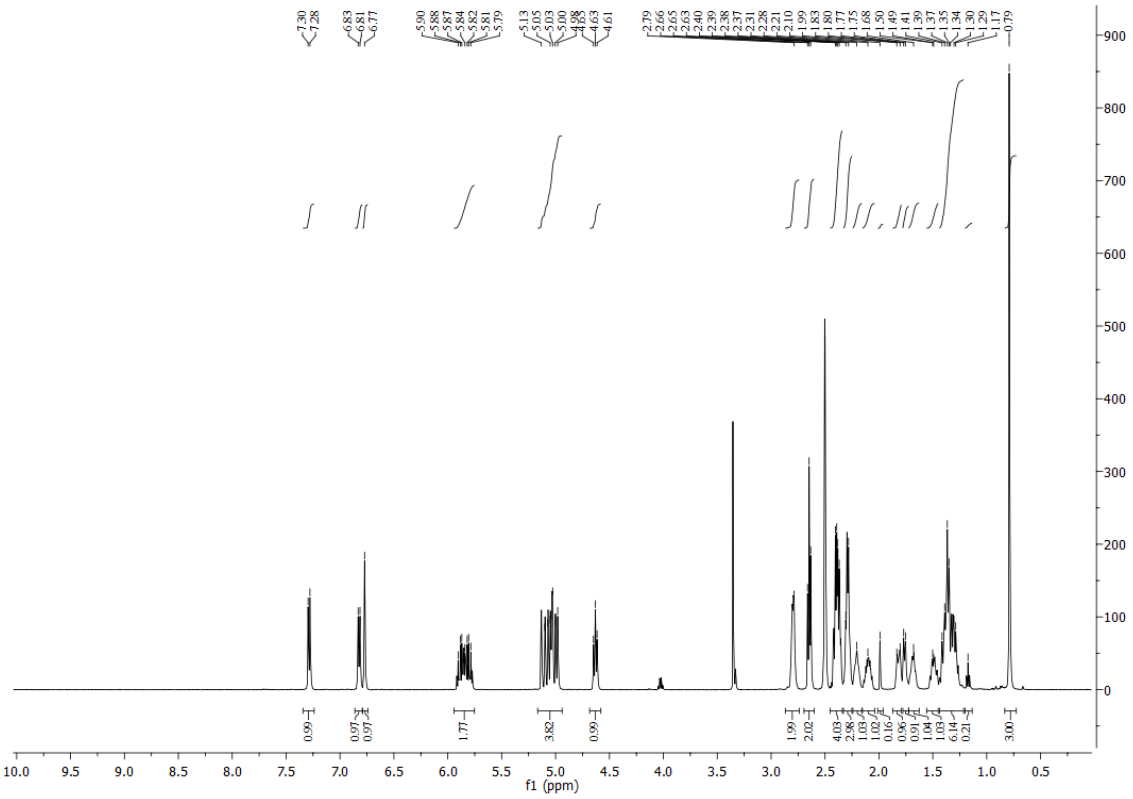


Figure S-5. [1]H NMR spectrum of **2** in DMSO-d6.

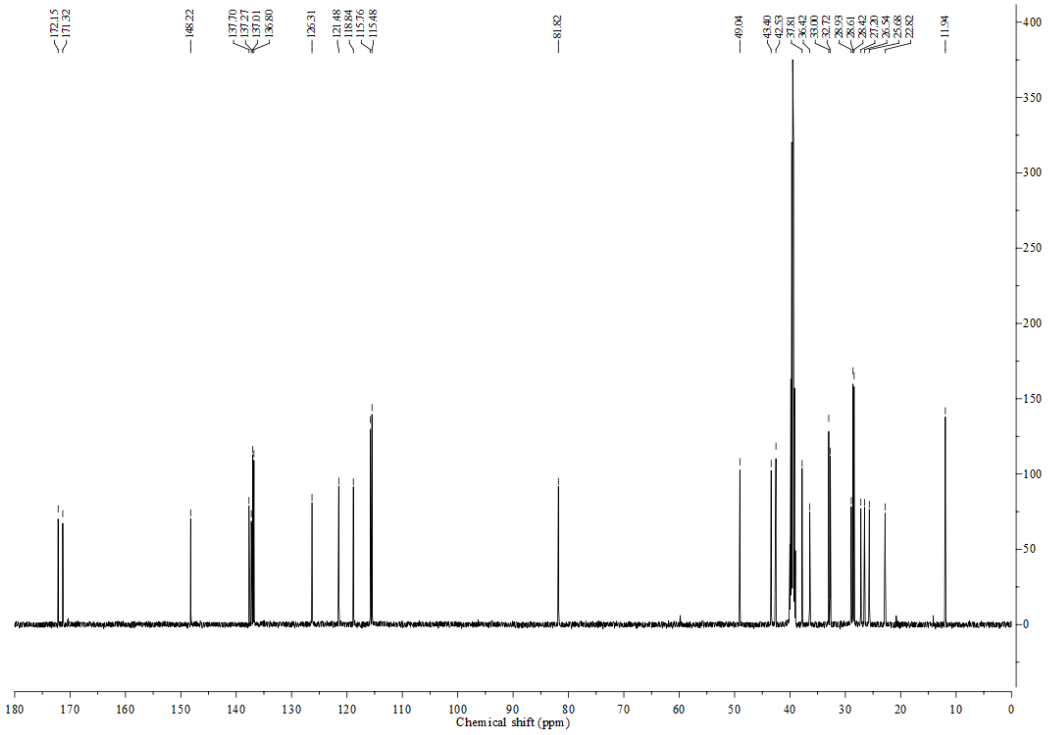


Figure S-6. [13]C NMR spectrum of **2** in DMSO-d6

PC-EG i.e. poly(E2-**1**-*alt*-**3**), was prepared by co-polymerizing E2-**1** with 2,2'-(ethylenedioxy)diethanethiol (**3**). The dicarbonate pro-E2 monomer **1** (0.277 g, 0.629 mmol, 1.00 eq) and 99.1 % pure 2,2'-(ethylenedioxy)diethanethiol (0.116 g, 0.629 mmol, 1.00 eq) were added to a 20 mL glass scintillation vial containing a magnetic stir bar. The UV photoinitiator 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 0.002 g, 0.063 mmol, 0.01 eq) was dissolved in THF (5 mL) and added to the monomer solution via pipette. The solution was concentrated under reduced pressure to remove all solvent, deoxygenated by bubbling with N_2 for 10 min, and stirred overnight at rt under a 4-W handheld TLC lamp at a distance of ~ 2 cm. Then, the resulting solidified mixture was dissolved in DCM and precipitated into cold MeOH. The ppt was concentrated by centrifugation (4500 rpm, 15 min), the supernatant liquid was decanted, and the pellet was dried *in vacuo* overnight to obtain an off-white foam (0.3174 g, 90.1 % yield).

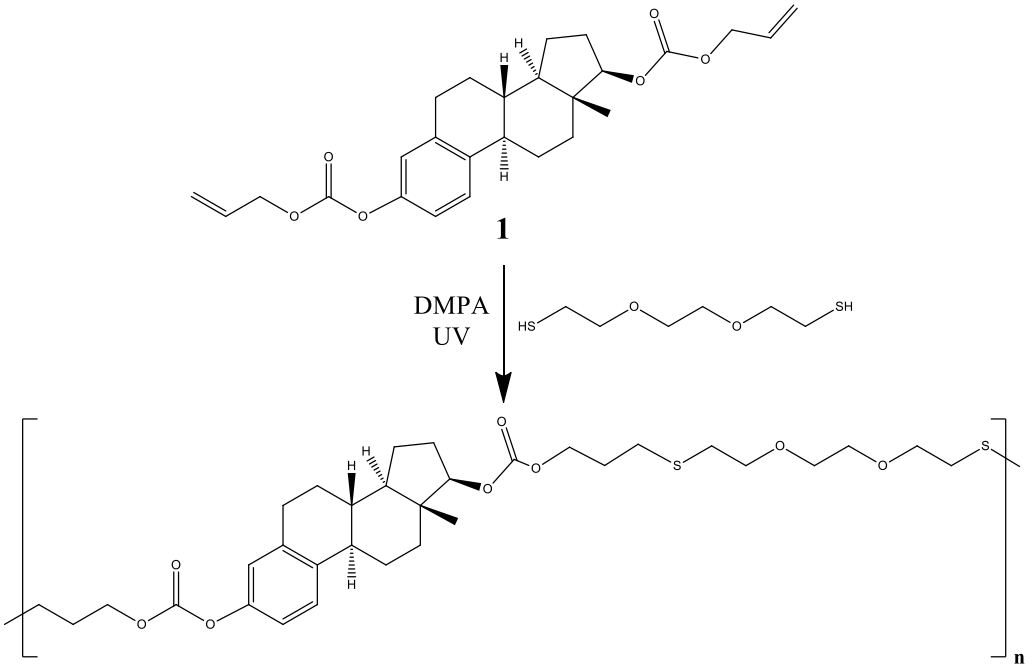


Figure S-7. Polymerization of **1** and EG dithiol (**3**) to yield PC-EG.

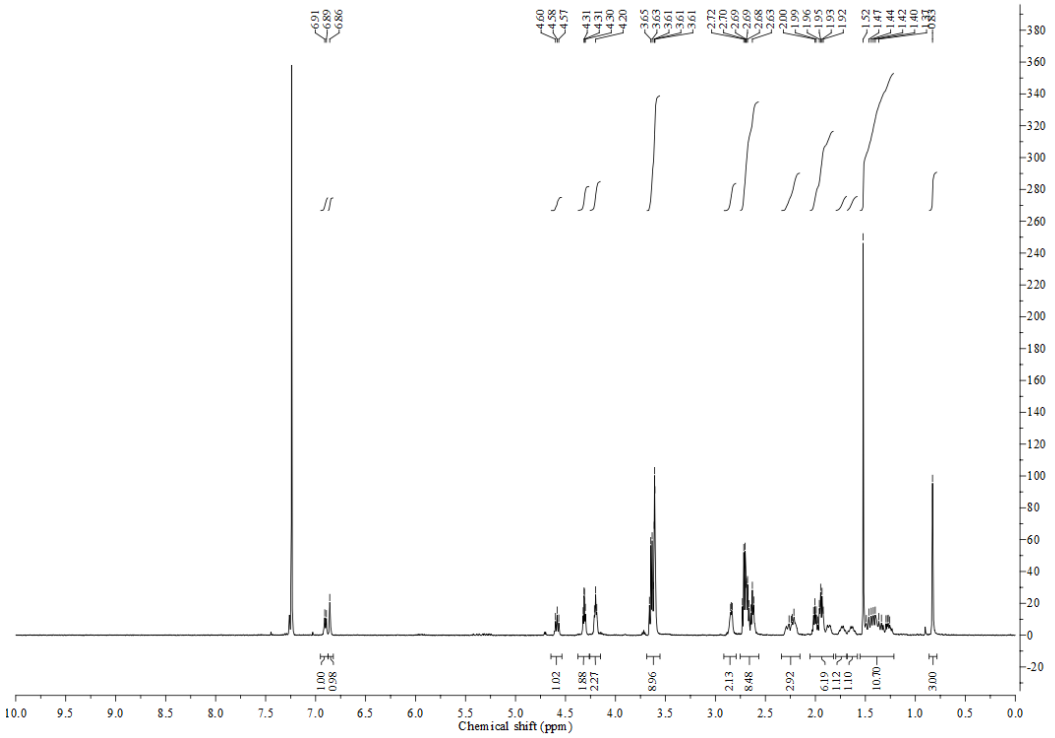


Figure S-8. ^{1}H NMR spectrum of PC-EG in $CDCl_3$.

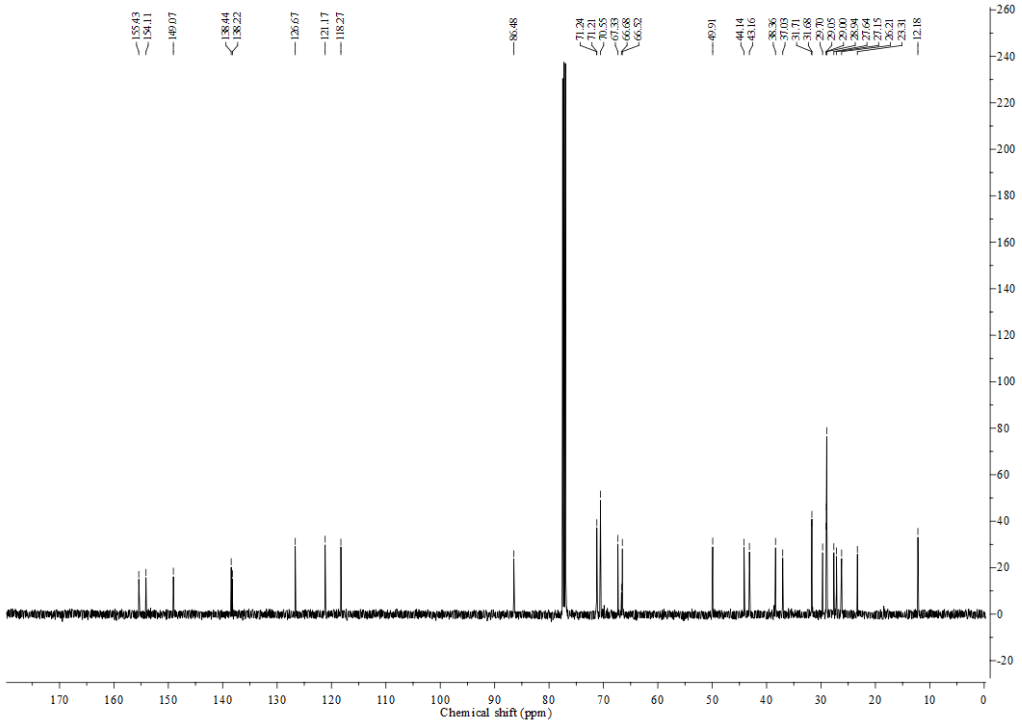


Figure S-9. ^{13}C NMR spectrum of PC-EG in $CDCl_3$.

PC-Hex i.e. poly(E2-**1**-*alt*-**4**), was prepared by co-polymerizing E2-**1** with 1,6-hexylenedithiol (**4**). The dicarbonate pro-E2 monomer **1** (0.274 g, 0.621 mmol, 1.00 eq) and 98.6% pure 1,6-hexanedithiol (0.095 g, 0.621 mmol, 1.00 eq) were added to a 20 mL glass scintillation vial containing a magnetic stir bar. The UV photoinitiator 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 0.002 g, 0.063 mmol, 0.01 eq) was dissolved in THF (5 mL) and added to the monomer solution via pipette. The solution was concentrated under reduced pressure to remove all solvent, deoxygenated by bubbling with N_2 for 10 min, and stirred overnight at rt under a 4-W handheld TLC lamp at a distance of ~ 2 cm. Then, the resulting solidified mixture was dissolved in DCM and precipitated into cold MeOH. The ppt was concentrated by centrifugation (4500 rpm, 15 min), the supernatant liquid was decanted, and the pellet was dried *in vacuo* overnight to obtain an off-white foam (0.3143 g, 94.4 % yield).

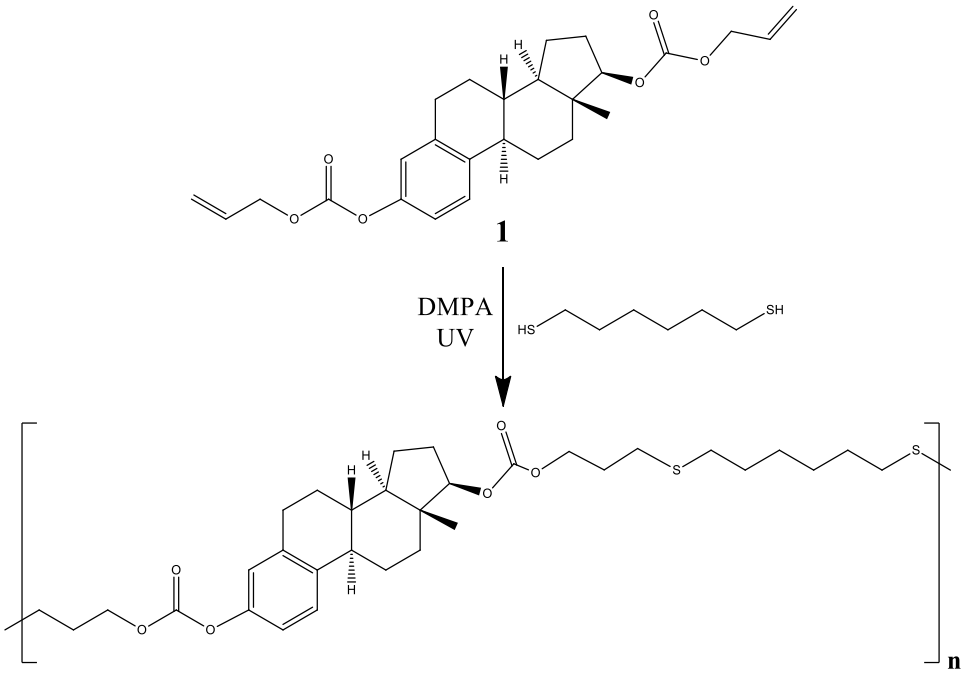


Figure S-10. Polymerization of **1** and hexane dithiol (**4**) to yield PC-Hex**.**

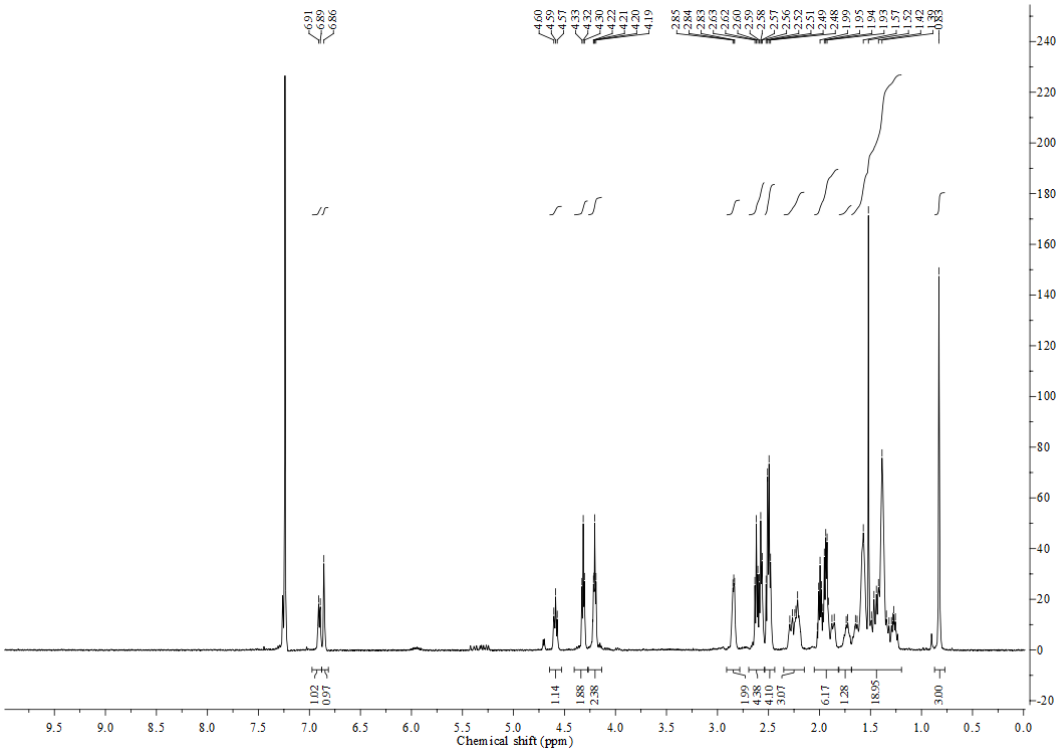


Figure S-11. ^{1}H NMR spectrum of PC-Hex in $CDCl_3$.

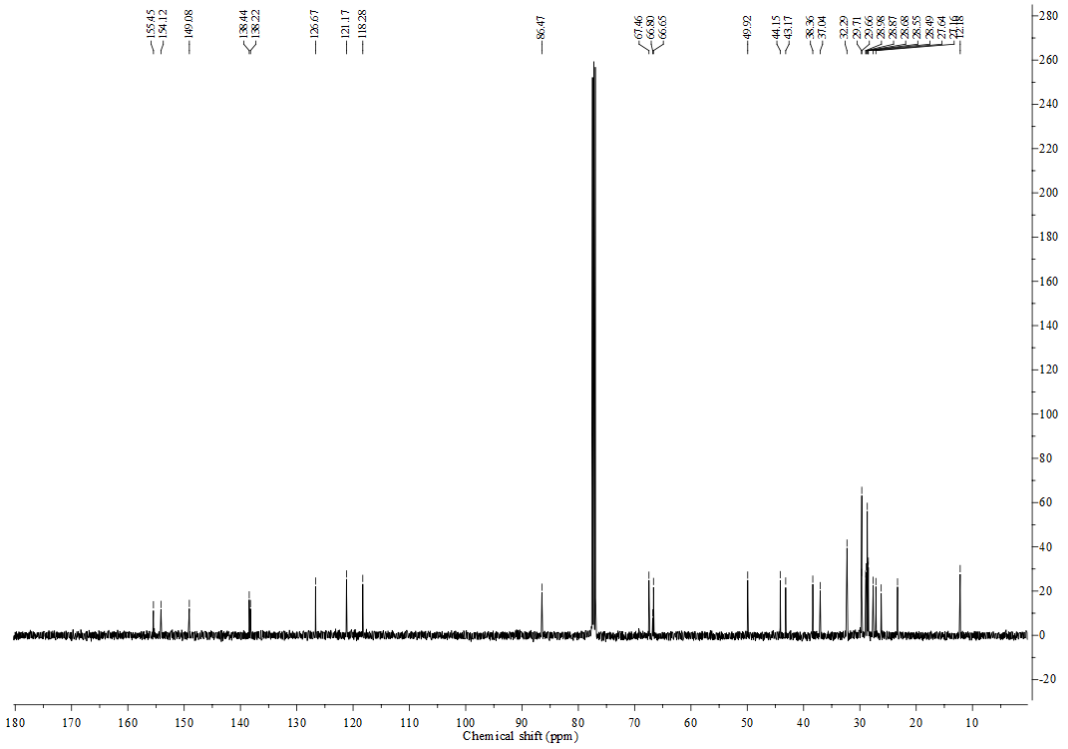


Figure S-12. ^{13}C NMR spectrum of PC-Hex in $CDCl_3$.

PE-EG, poly(**2**-*alt*-**3**), was prepared by copolymerizing **2** with 2,2'-(ethylenedioxy)diethanethiol (**3**). The diester pro-E2 monomer **2** (0.6164 g, 1.41 mmol, 1.00 eq) and 99.1% pure 1,6-hexanedithiol (0.2597 g, 1.41 mmol, 1.00 eq) were added to a 20 mL glass scintillation vial containing a magnetic stir bar. The UV photoinitiator 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 0.080 g, 0.141 mmol, 0.01 eq) was dissolved in THF (10 mL) and added to the monomer solution via pipette. The solution was concentrated under reduced pressure to remove all solvent, deoxygenated by bubbling with N_2 for 10 min, and stirred overnight at rt under a 4-W handheld TLC lamp at a distance of ~ 2 cm. Then, the resulting solidified mixture was dissolved in DCM and precipitated into cold MeOH. The ppt was concentrated by centrifugation (4500 rpm, 15 min), the supernatant liquid was decanted, and the pellet was dried *in vacuo* overnight to obtain an off-white foam (0.8252 g, 94.2 % yield).

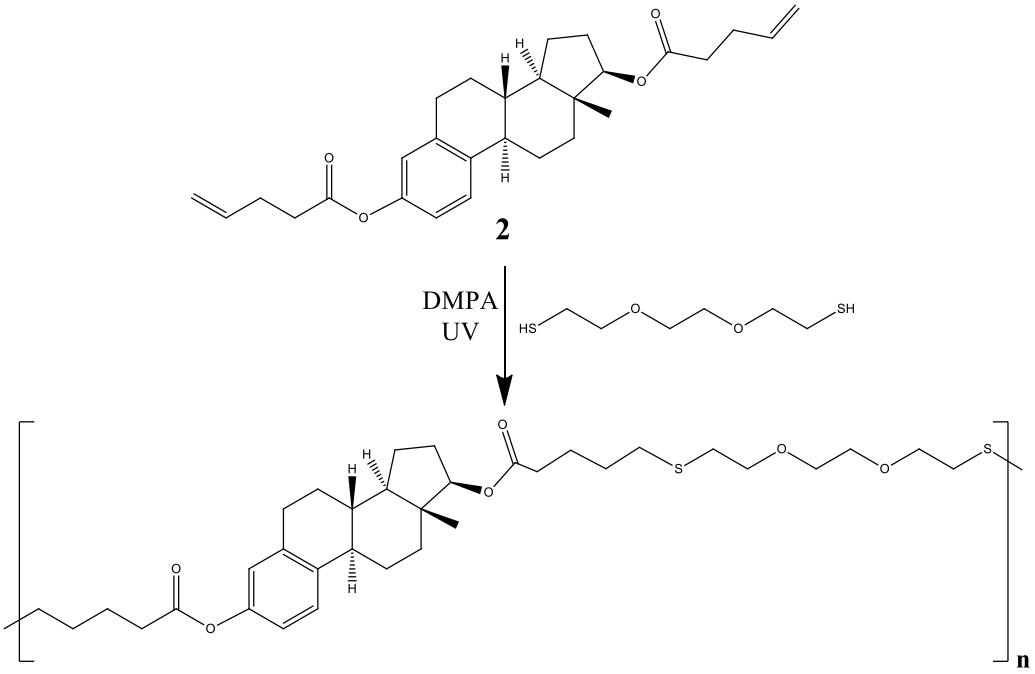


Figure S-13. Polymerization of **2** and EG dithiol to yield PE-EG**.**

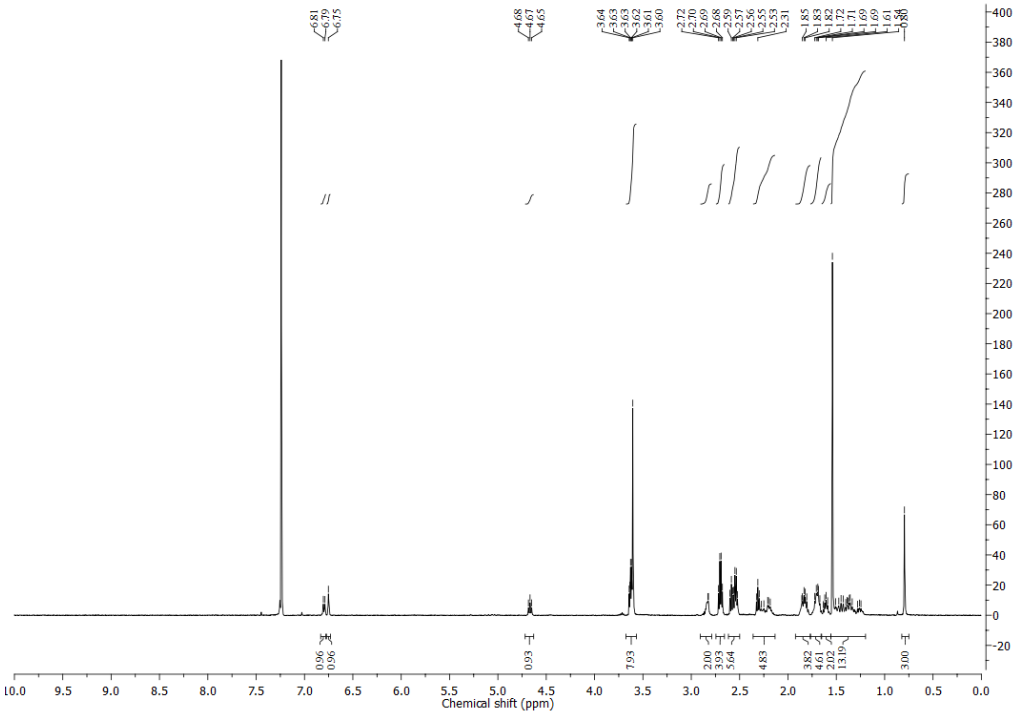


Figure S-14. ^{1}H NMR spectrum of PE-EG in $CDCl_3$.

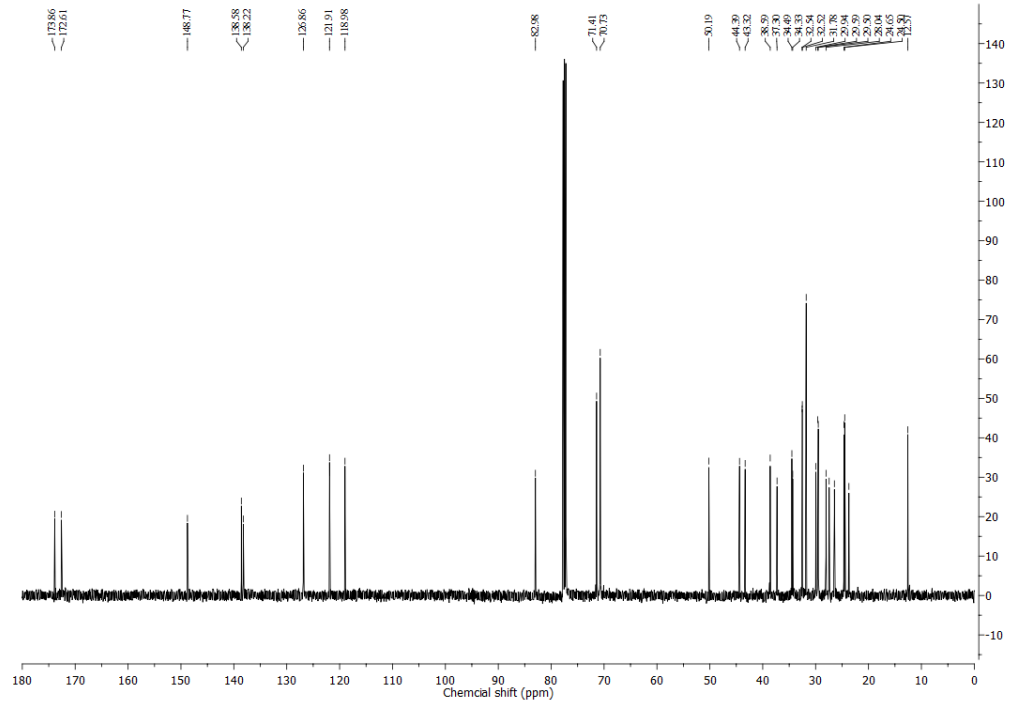


Figure S-15. ^{13}C NMR spectrum of PE-EG in $CDCl_3$.

PE-Hex, poly(**2**-*alt*-**4**), was prepared by copolymerizing **2** with 1,6-hexylenedithiol (**4**). The diester pro-E2 monomer **2** (0.271 g, 0.621 mmol, 1.00 eq) and 98.6% pure 1,6-hexanedithiol (0.095 g, 0.621 mmol, 1.00 eq) were added to a 20 mL glass scintillation vial containing a magnetic stir bar. The UV photoinitiator 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 0.002 g, 0.063 mmol, 0.01 eq) was dissolved in THF (10 mL) and added to the monomer solution via pipette. The solution was concentrated under reduced pressure to remove all solvent, deoxygenated by bubbling with N_2 for 10 min, and stirred overnight at rt under a 4-W handheld TLC lamp at a distance of ~ 2 cm. Then, the resulting solidified mixture was dissolved in DCM and precipitated into cold MeOH. The ppt was concentrated by centrifugation (4500 rpm, 15 min), the supernatant liquid was decanted, and the pellet was dried *in vacuo* overnight to obtain an off-white foam (0.2738 g, 74.8 % yield).

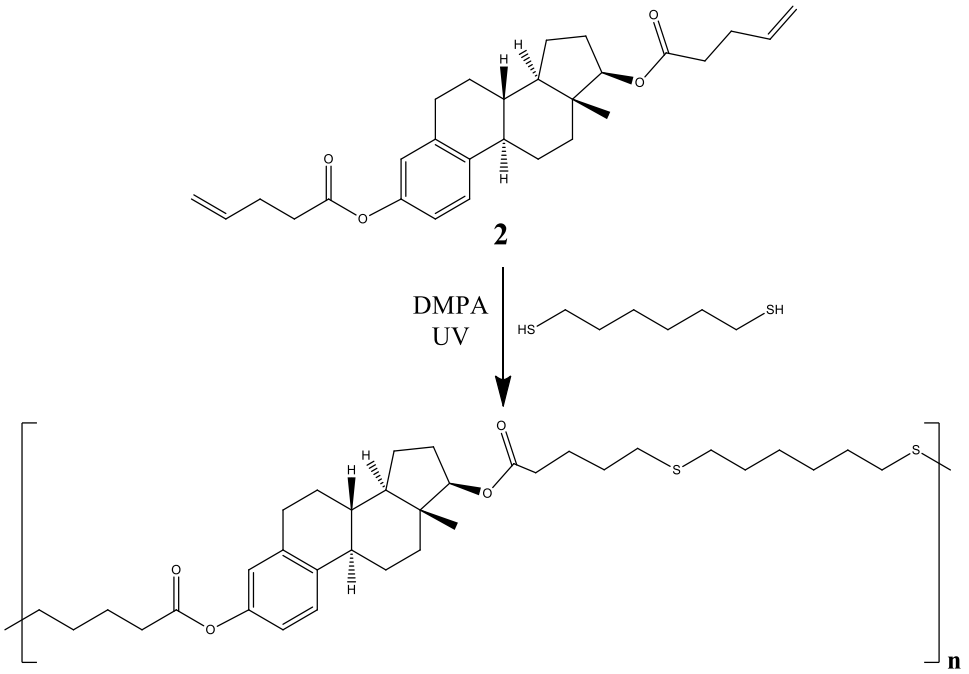


Figure S-16. Polymerization of **2** and hexane dithiol (**4**) to yield PE-Hex**.**

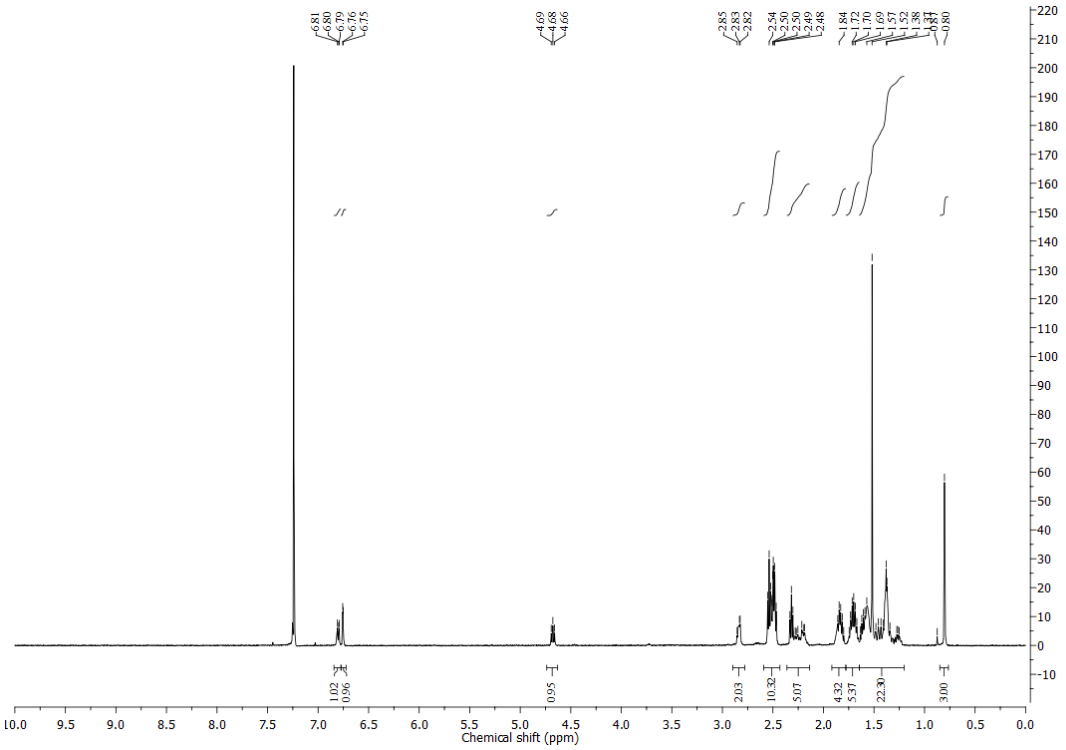


Figure S-17. ^{1}H NMR spectrum of PE-Hex in $CDCl_3$.

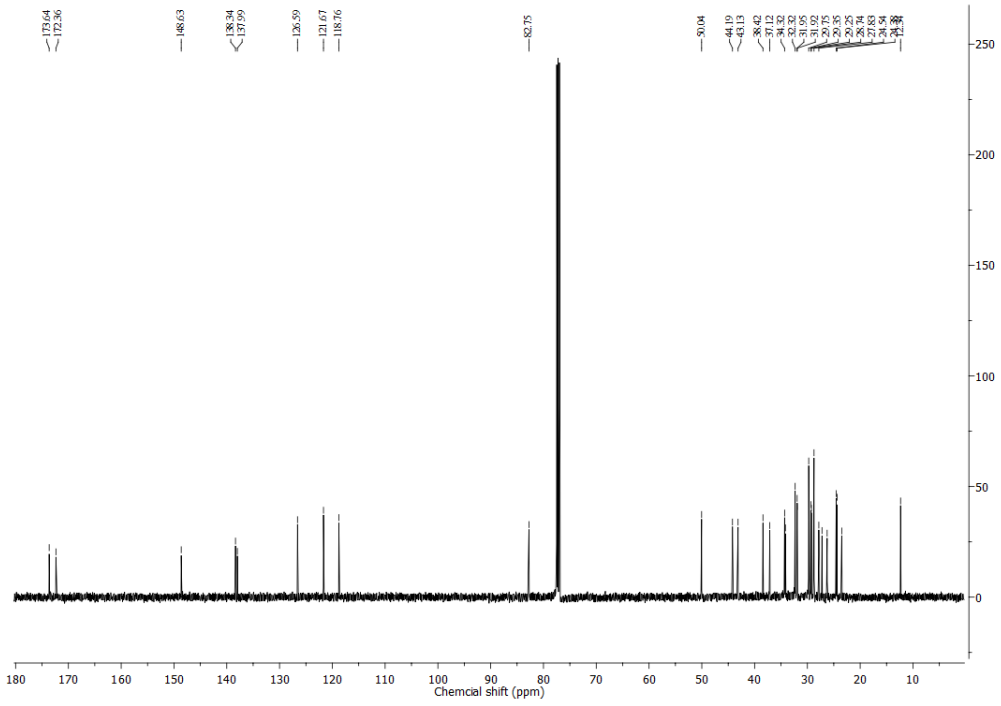
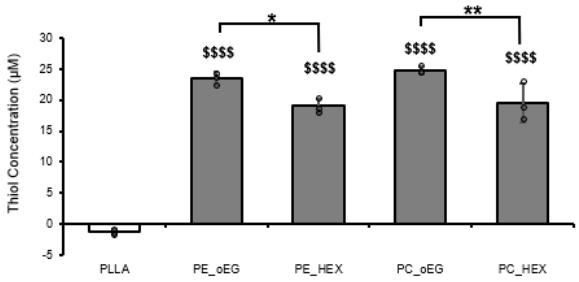


Figure S-18. ^{13}C NMR spectrum of PE-Hex in $CDCl_3$.



$-significant difference of PLLA + Exog E2 compared to all groups
*- significant difference of PE PEG **vs** PE HEX **or** PC PEG **vs** PC HEX

$ or * ≤ 0.05; $$ or ** ≤ 0.01; $$$ or *** ≤ 0.001, $$$$ or **** ≤ 0.0001

Thiol concentration based on Ellman's reagent
$F[6,14] = 206.22$, $p = 0.000$ (one-way ANOVA)

Figure S-19. All poly(pro-E2) polymers have a greater concentration of free thiol groups compared to the PLLA control. Summary graph of the free thiol concentration determined via an Ellman's test. All data are represented by the mean thiol concentration ± the standard deviation and overlayed with dots showing each individual data point for all groups.

Table S-1. Molecular weight analysis of methanol insoluble fractions over time for poly(pro-E2) variants.

PE-EG (weeks)	**M_n**	**M_w**	**Đ**	**$M_w/M_{w,0}$ (%)**
0	16.5	40.4	2.45	100
1	15.2	37.3	2.45	92.33
2	14.7	34.3	2.33	84.9
3	13 .7	33 .5	2.45	82.92
PE-Hex (weeks)				
0	17.1	30	1.75	100
1	11.9	20.7	1.74	69
2	5.49	22.9	4.17	76.33
3	6.03	14 .9	2.47	49.67
PC-EG (weeks)				
0	14.5	43.6	3.01	100
1	14.5	42.4	2.92	97.25
2	14.6	44.7	3.06	102.52
3	16 .8	61 .7	3.67	141 .51
PC-Hex (weeks)				
0	11.9	30.1	2.53	100
1	9.58	27.5	2.87	91.36
2	9.3	26.3	2.83	87.38
3	8.28	29.4	3.55	97.67

Table S-3. Comparison of astrocyte adhesion on different substrates

Adhesion	PE_oEG		PE_HEX		PC_oEG		PC_HEX	
	1 day	7 day	1 day	7 day	1 day	7 day	1 day	7 day
PLLA + Exo. E2	$F_{7,47.10} = 32.83$; $p \leq 0.0001$	$F_{7,51.46} = 20.08$; $p \leq 0.0001$	$F_{7,47.10} = 32.83$; $p \leq 0.001$	$F_{7,51.46} = 20.08$; $p \leq 0.01$	$F_{7,47.10} = 32.83$; $p \leq 0.0001$	$F_{7,51.46} = 20.08$; $p \leq 0.001$	$F_{7,47.10} = 32.83$; $p = 0.7750$	$F_{7,51.46} = 20.08$; $p = 0.0637$
PE_EG			$F_{7,47.10} = 32.83$; $p \leq 0.001$	$F_{7,51.46} = 20.08$; $p \leq 0.0001$				
PC_EG							$F_{7,47.10} = 32.83$; $p \leq 0.0001$	$F_{7,51.46} = 20.08$; $p \leq 0.001$

Key for Table # & #:	The color refers to the group indicated in the top row
	significant increase
	significant decrease
	no significant difference

Table S-4. Comparison of astrocyte spreading on different substrates

Spreading	PE_oEG		PE_HEX		PC_oEG		PC_HEX	
	1 day	7 day	1 day	7 day	1 day	7 day	1 day	7 day
PLLA + Exo. E2	$F_{7,43.53} = 45.24$; $p \leq 0.0001$	$F_{7,50.55} = 14.28$; $p = 0.2222$	$F_{7,43.53} = 45.24$; $p \leq 0.0001$	$F_{7,50.55} = 14.28$; $p \leq 0.0001$	$F_{7,43.53} = 45.24$; $p \leq 0.0001$	$F_{7,50.55} = 14.28$; $p = 0.5180$	$F_{7,43.53} = 45.24$; $p = 0.6719$	$F_{7,50.55} = 14.28$; $p \leq 0.01$
PE_EG			$F_{7,43.53} = 45.24$; $p \leq 0.01$	$F_{7,50.55} = 14.28$; $p \leq 0.0001$				
PC_EG							$F_{7,43.53} = 45.24$; $p \leq 0.0001$	$F_{7,50.55} = 14.28$; $p \leq 0.001$

Key for Table # & #:	The color refers to the group indicated in the top row
	significant increase
	significant decrease
	no significant difference

Non-Equilibrium Contact Angle Measurement

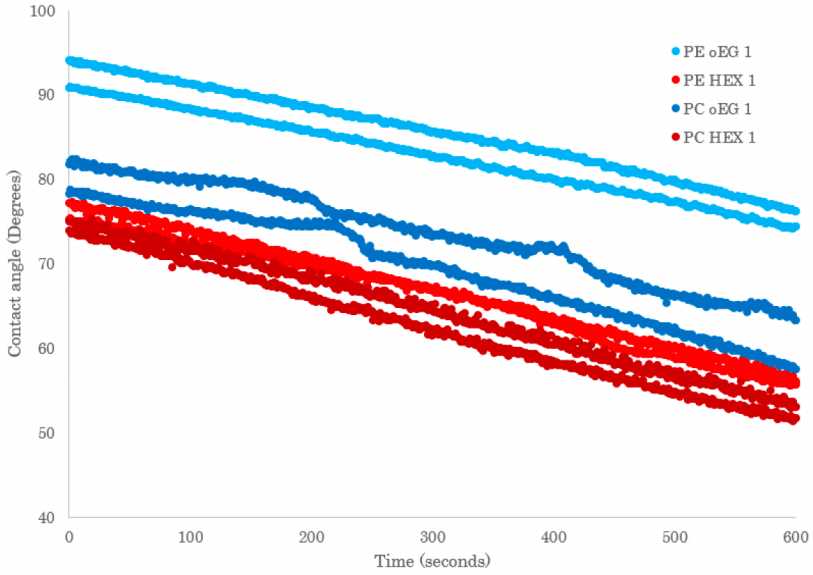


Figure S-20. Water contact angle as a function of time for the 4 polymers in this work. Equilibrium was not reached within the accessible timescale of the experiment, but it seems clear that hexylene-linked polymers are surprisingly *less* hydrophobic than their EG-linked counterparts.

Film Turbidity in the Wet vs Dry States:

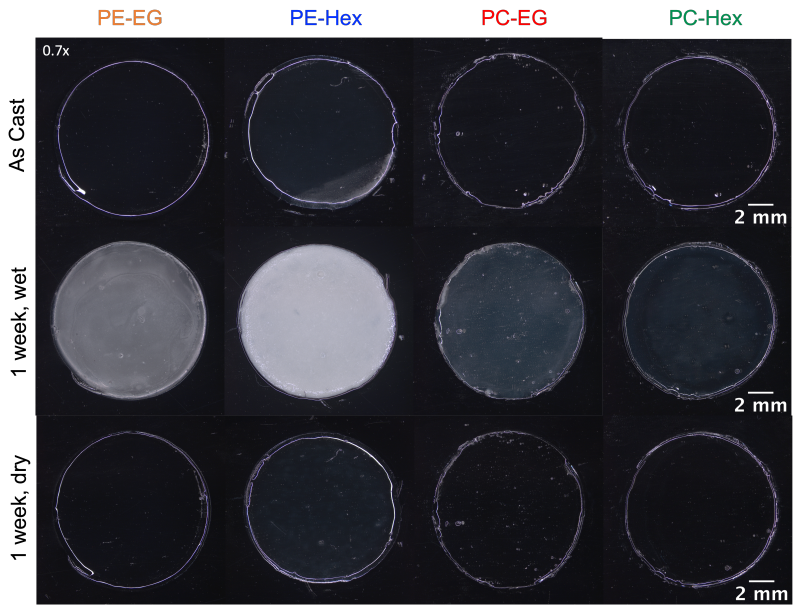


Figure S-21. Optical images of polymer films demonstrate that the materials only turbid in the *wet state* but revert to transparent upon drying.