Supplementary Information for:

Harnessing the Surface Chemistry of Methyl Ester Functionalized Polydicyclopentadiene and Exploring Surface Bioactivity

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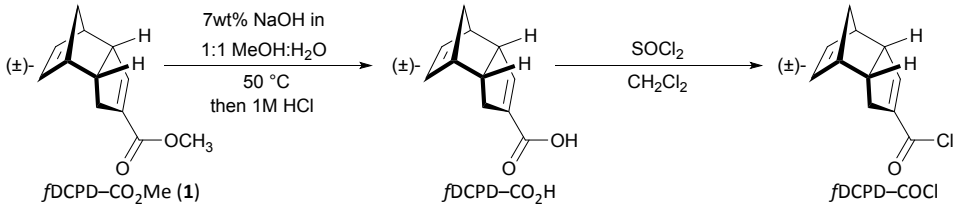
Index:

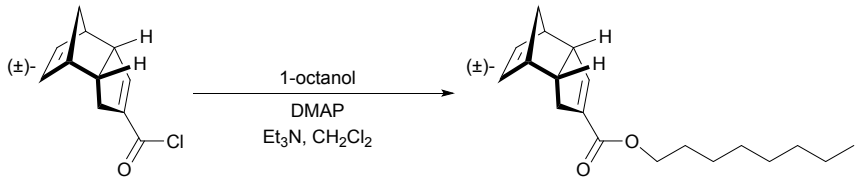
1. Synthesis of functionalized monomers.

Refer to reference 20 for a detailed discussion of the scalable synthesis of methyl ester-functionalized dicyclopentadiene monomer, **1**.



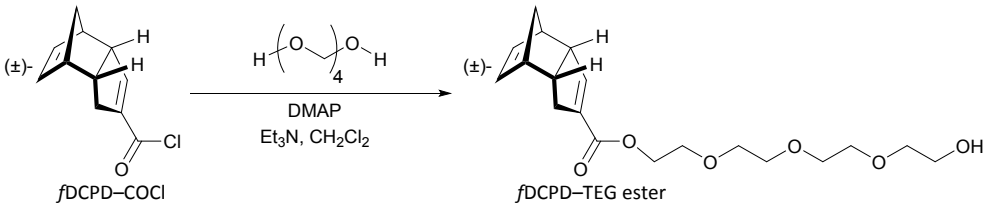
*f*DCPD–CO_2Me (**1**) *f*DCPD–CO_2H *f*DCPD–COCl

116 mg of purified *f*DCPD–CO_2Me monomer (**1**; 0.61 mmol) was added to 1.6 mL of a 7 wt% solution of sodium hydroxide (NaOH) in 1:1 MeOH : distilled water. The solution was heated to 50 °C and left to react overnight. After cooling to room temperature, 1M HCl was used to precipitate the desired carboxylic acid intermediate. After partial concentration *in vacuo* to remove methanol, the product was extracted with ethyl acetate to afford 102 mg (0.58 mmol; 95%) of crude *f*DCPD–CO_2H as a white powder. The acid intermediate was then immediately dissolved in 1.5 mL of dichloromethane, to which 0.42 mL of thionyl chloride (5.8 mmol; 10 equiv) was added under argon. The solution was left to react overnight at room temperature with stirring, after which the solvent was removed *in vacuo* to afford 100 mg (0.51 mmol; 89%) of crude *f*DCPD–COCl as a clear, yellow-brown oil.



*f*DCPD–COCl *f*DCPD–octyl ester

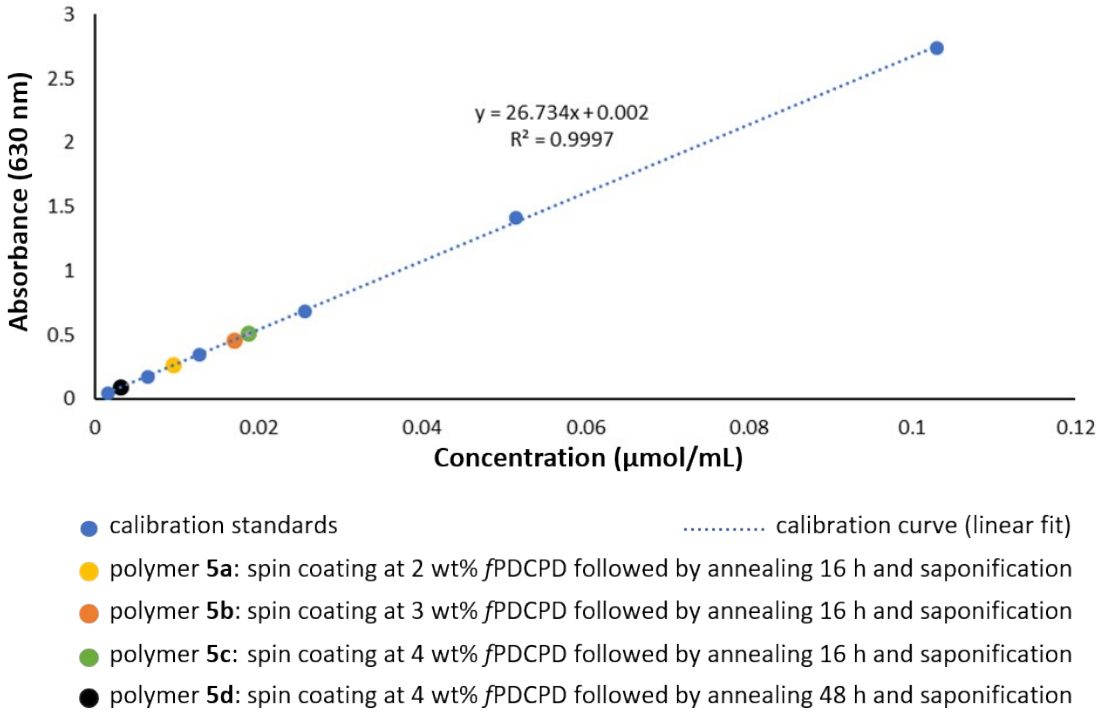
Crude *f*DCPD–COCl (195 mg; 1.00 mmol) was dissolved in 2 mL of CH_2Cl_2. *N*,*N*-Dimethylaminopyridine (12.3 mg; 0.1 mml; 0.1 equiv) was added and the mixture was cooled to 0 °C. 1-Octanol (1.6 mL; 10 mmol; 10 equiv) and triethylamine (1.4 mL; 10 mmol; 10 equiv) was added to the stirring solution. After 5 min, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature over 12 h. The reaction mixture was washed with 1 M HCl and the aqueous layer was back-extracted twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and then evaporated to afford the crude *f*DCPD–octyl ester product. This was subjected to flash-column chromatography over silica gel, using 2.5:1 hexanes:CH_2Cl_2 as the eluent. The desired product was obtained as a clear yellow oil in a yield of 50%. ^{1}H NMR (300MHz, $CDCl_3$) δ 6.54-6.52 (m, 1H), 6.03 (dd, *J* = 5.6, 2.9 Hz, 1H), 5.94 (dd, *J* = 5.6, 2.9 Hz, 1H), 4.06 (t, *J* = 6.6 Hz, 2H), 3.36-3.30 (m, 1H), 2.94-2,90 (m, 2H), 2.86-2.80 (m, 1H), 2.42 (ddt, *J* = 17.2, 10.3, 1.9 Hz, 1H), 1.94-1.86 (m, 1H), 1.62 (t, *J* = 6.6 Hz, 2H), 1.50 (dt, *J* = 8.2, 1.8 Hz, 1H), 1.43-1.14 (m, 11H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 144.1, 137.5, 135.7, 133.0, 64.2, 55.0, 50.3, 46.4, 45.6, 41.3, 33.6, 31.8, 29.7, 29.2, 28.6, 26.0, 22.6, 14.1; IR (cm^{-1}, film) 3065, 2959, 2924, 2854, 1716, 1638, 1461, 1383, 1343, 1280, 1232, 1132, 1095, 1017, 917, 755, 733, 707, 518, 470; HRMS: $[M+H]^+$ calculated for $C_{19}H_{29}O_2$ 289.2162; found 289.2161.



Crude *f*DCPD–COCl (195 mg; 1.00 mmol) was dissolved in 2 mL of CH_2Cl_2. *N*,*N*-Dimethylaminopyridine (12.3 mg; 0.1 mml; 0.1 equiv) was added and the mixture was cooled to 0 °C. Tetraethylene glycol (1.7 mL; 10 mmol; 10 equiv) and triethylamine (1.4 mL; 10 mmol; 10 equiv) was added to the stirring solution. After 5 min, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature overnight. A crude NMR spectrum was collected to confirm that the reaction had gone to completion, after which the reaction mixture was washed with 1M HCl and saturated aqueous sodium bicarbonate. Following each wash, the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and then evaporated to afford the crude *f*DCPD–TEG ester product. This was subjected to flash-column chromatography over silica gel, using 100% CH_2Cl_2 followed by 2:1 CH_2Cl_2:EtOAc and finally 30:1 CH_2Cl_2:MeOH as the eluent. The desired product was obtained as a clear, colorless oil. ^{1}H NMR (500MHz, $CDCl_3$) δ 6.61-6.55 (m, 1H), 6.02 (dd, *J* = 5.6, 2.8 Hz, 1H), 5.93 (dd, *J* = 5.6, 2.8 Hz, 1H), 4.28-4.19 (m, 2H), 3.75-3.59 (m, 14H), 3.38-3.31 (m, 1H), 3.00-2.88 (m, 2H), 2.87-2.77 (m, 1H), 2.42 (ddt, *J* = 17.3, 10.2, 1.9 Hz, 1H), 2.00-1.78 (m, 2H), 1.50 (dt, *J* = 8.2, 1.7 Hz, 1H), 1.29 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (125MHz, $CDCl_3$) δ 165.1, 144.9, 137.0, 135.7, 133.0, 72.5, 70.7, 70.6, 70.6, 70.4, 69.2, 63.3, 61.8, 55.1, 50.3, 46.3, 45.6, 41.2, 33.6; IR (cm^{-1}, film) 3461, 3060, 2933, 2872, 1712, 1635, 1454, 1343, 1287, 1230, 1129, 1083, 915, 815, 758, 731, 651, 517, 470; HRMS: $[M+Na]^+$ calculated for $C_{19}H_{28}NaO_6$ 375.1778; found 375.1775.

Quantification of accessible *f*DCPD–CO_2H groups in polymer **5**

A stock solution of toluidine blue oxide (TBO) in 50% acetic acid at a concentration of 1.03 µmol/mL was freshly prepared. Two-fold serial dilutions were carried out to prepare a series of standards, with concentrations from 0.103 µmol/mL to 0.0016 µmol/mL. The absorbance at 630 nm was measured to create a calibration curve (R^2 = 0.9997) as shown below:



Samples of polymer **5** were prepared on 15 mm micro glass cover slides (surface area = 1.76 cm^2) as described in the Experimental section, using a variety of different concentrations of linear *f*PDCPD **2** (2 wt%, 3 wt% or 4 wt%) in 1:1 toluene:CH_2Cl_2, and crosslinking for two different times (16 h or 48 h) prior to hydrolysis (NaOH in 1:1 H_2O:MeOH, as described in the Experimental section).

The prepared samples were first incubated in a TBO solution (84 mg/mL; 6.4 µmol/mL) for 1 minute, then immediately washed (dipped with slight swirling) in a 10 wt% NaOH solution three times, and then air dried. Dried samples were then incubated in 3 mL of 50% acetic acid for 10 minutes with occasional stirring. The solution was then diluted 10x, and the absorbance was recorded at 630 nm, using a 50 wt% acetic acid solution as a blank. A non-saponified surface (i.e. **4**) was used as a control to determine non-specific TBO absorption on the surface of the films and was subtracted from the saponified surfaces' absorption reading.

Table S1 summarizes the accessible concentration of COOH groups on the surface of each saponified sample. The data show that the amount of accessible carboxylate groups increases with increasing loading (i.e. a greater amount of *f*PDCPD **2** used in the spin-coating step), and decreases with crosslinking time. This is most probably due to a lack of swelling for the more-crosslinked samples. As we showed previously

(see reference 19), crosslinking density can be controlled by thermal annealing time. As annealing time (and therefore crosslinking density) is increased, the amount of solvent (and therefore TBO) that can penetrate into the polymer matrix is reduced. As a result, the amount of solvent-accessible groups naturally decreases.

Table S1: Accessible COOH Groups on the Surface of *f*PDCPD **5**

Sample	Control (**4**) 3 wt% loading; 16 h annealing	*f*PCPD-CO_2H **5a** 2 wt% loading; 1 6h annealing	*f*PCPD-CO_2H **5b** 3 wt% loading; 16 h annealing	*f*PCPD-CO_2H **5c** 4 wt% loading; 16 h annealing	*f*PCPD-CO_2H **5d** 4 wt% loading; 48 h annealing
µmol TBO per surface	0.126 ± 0.006	0.288 ± 0.049	0.511 ± 0.175	0.564 ± 0.221	0.097 ± 0.035
µmol TBO/cm^2	0.071 ± 0.003	0.164 ± 0.028	0.290 ± 0.099	0.320 ± 0.125	0.055 ± 0.020
calculated* µmol COOH/cm^2		0.092 ± 0.028	0.219 ± 0.100	0.249 ± 0.125	

*assuming 1:1 TBO:COOH binding

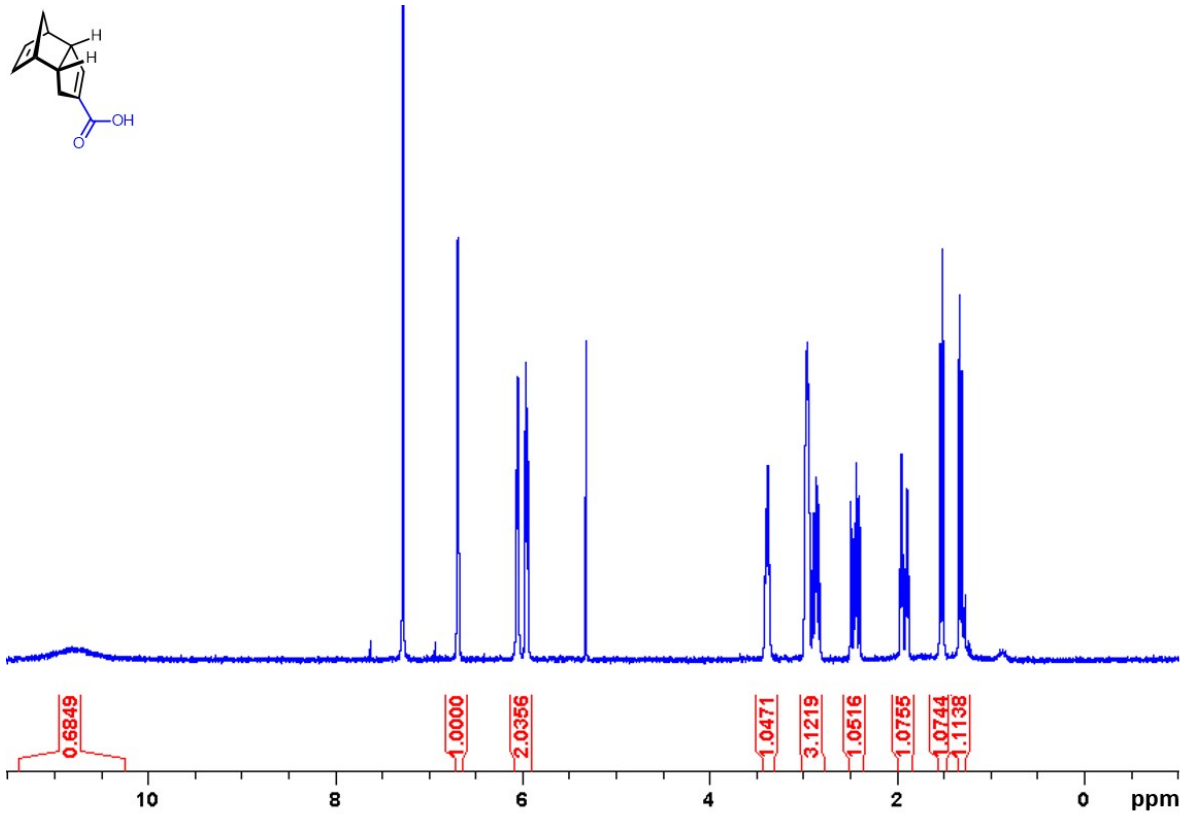


Figure S1. ^{1}H NMR spectrum for crude *f*DCPD–CO_2H in $CDCl_3$, recorded at 300 MHz.

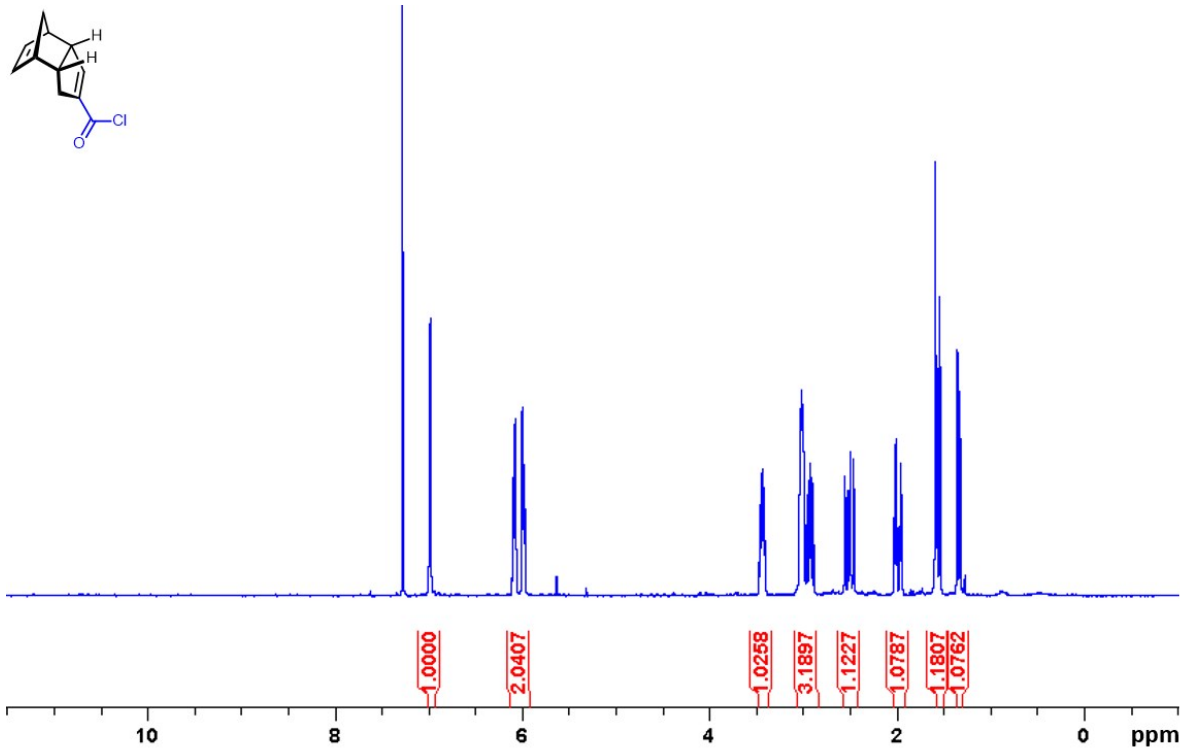


Figure S2. ^{1}H NMR spectrum for crude *f*DCPD–COCl in $CDCl_3$, recorded at 300 MHz.

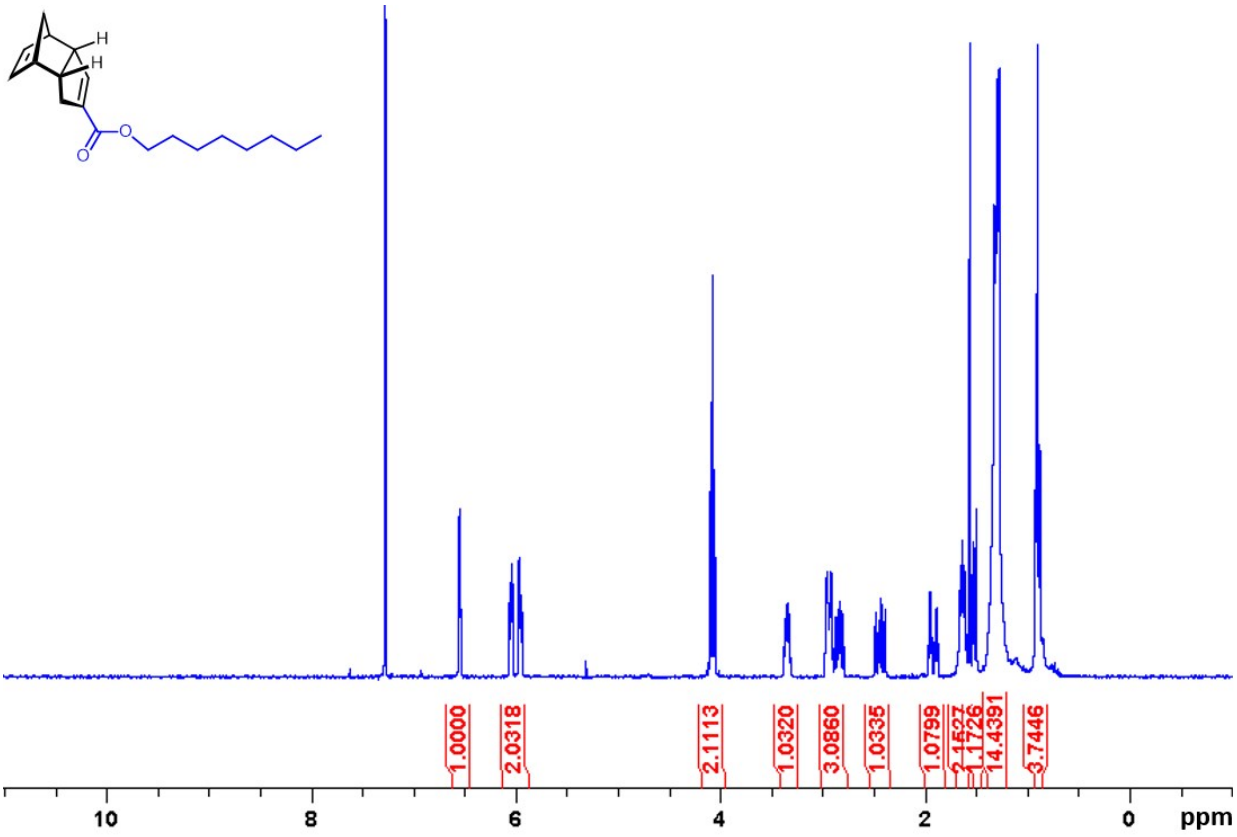


Figure S3. ^{1}H NMR spectrum for *f*DCPD–octyl ester in $CDCl_3$, recorded at 300 MHz.

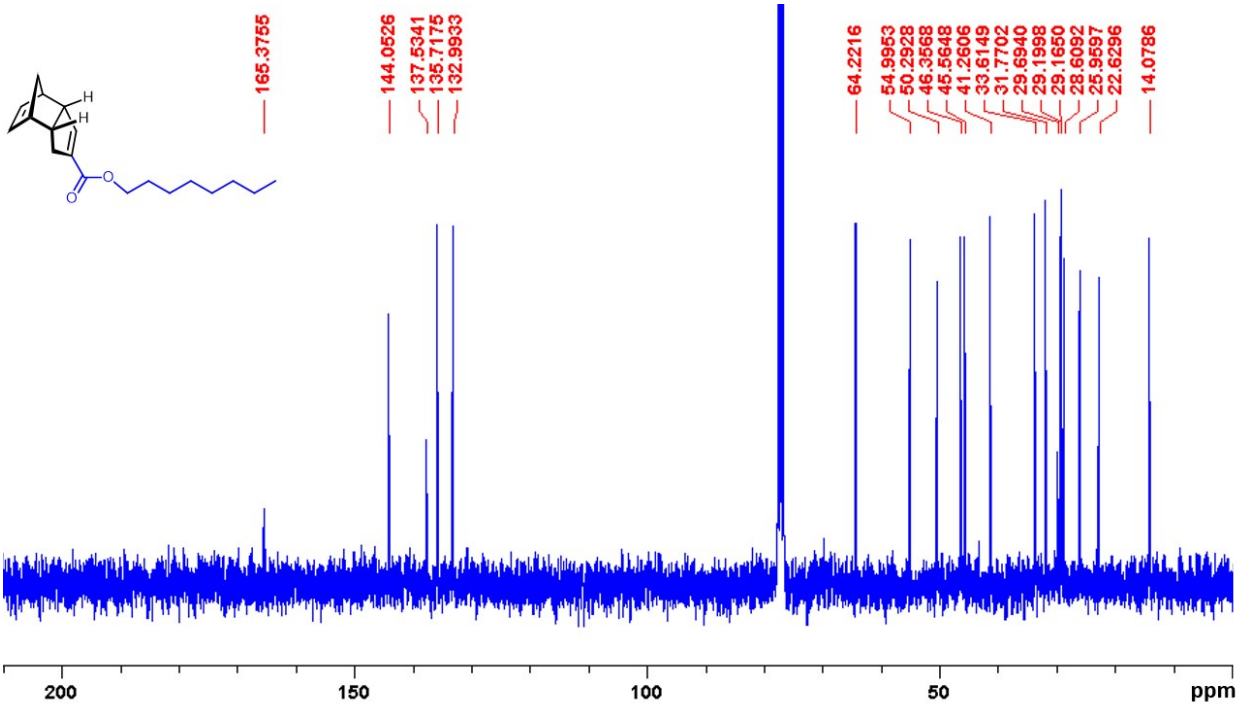


Figure S4. ^{13}C NMR spectrum for *f*DCPD–octyl ester in $CDCl_3$, recorded at 75 MHz.

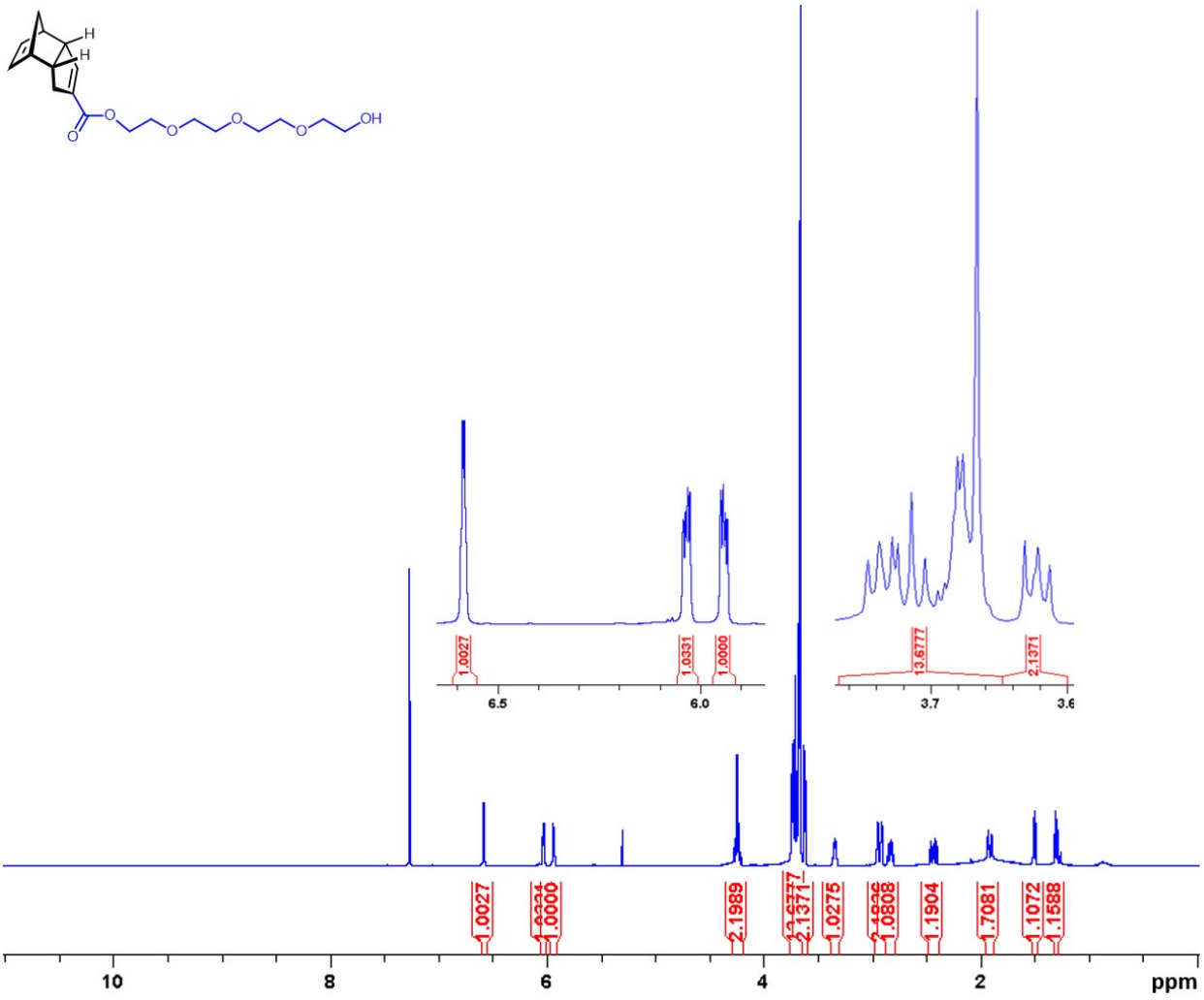


Figure S5. ^{1}H NMR spectrum for *f*DCPD–TEG ester in $CDCl_3$, recorded at 500 MHz. The inset spectra show expansions of key regions of the spectrum.

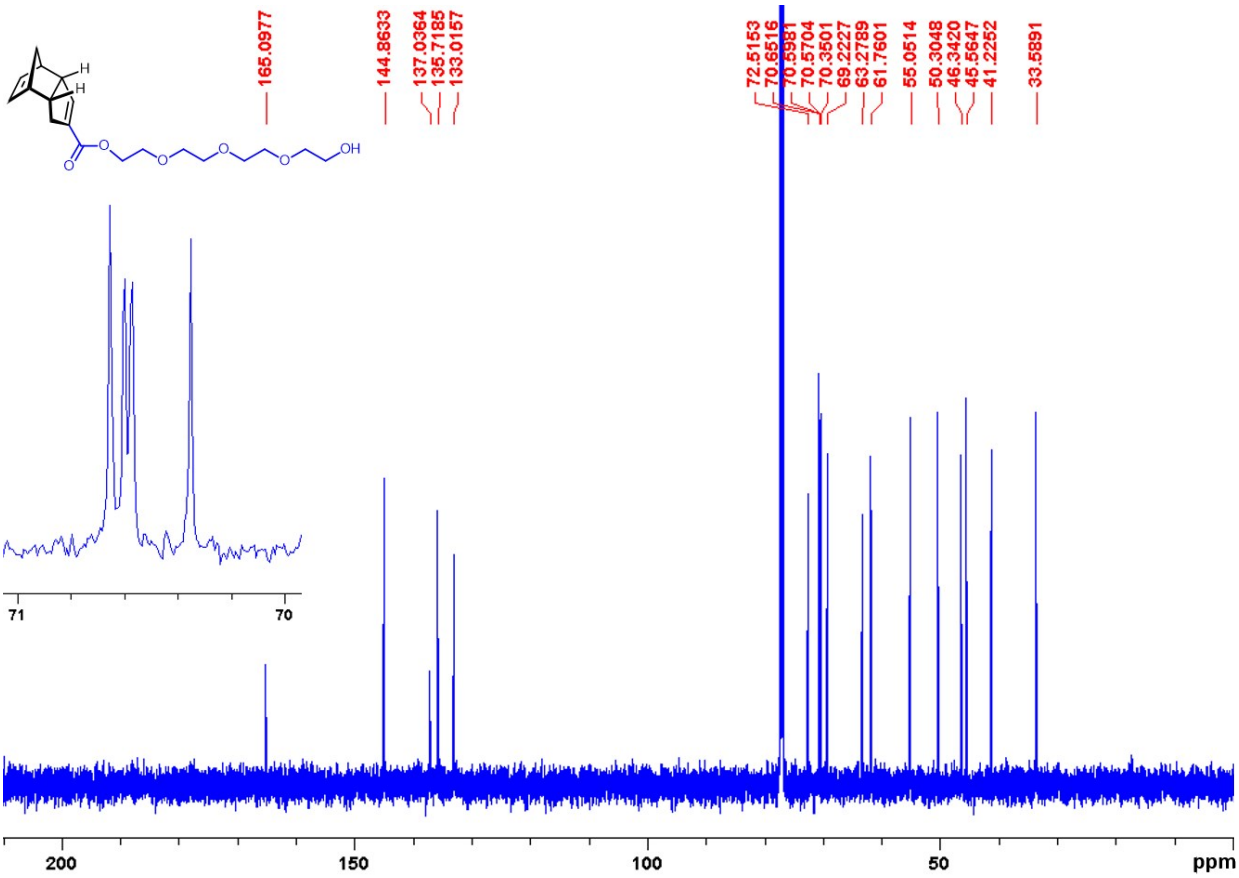


Figure S6. ^{13}C NMR spectrum for *f*DCPD–TEG ester in $CDCl_3$, recorded at 125 MHz. The inset spectrum shows an expansion in the region between 70–71 ppm, to highlight the distinct resonances visible in this region.

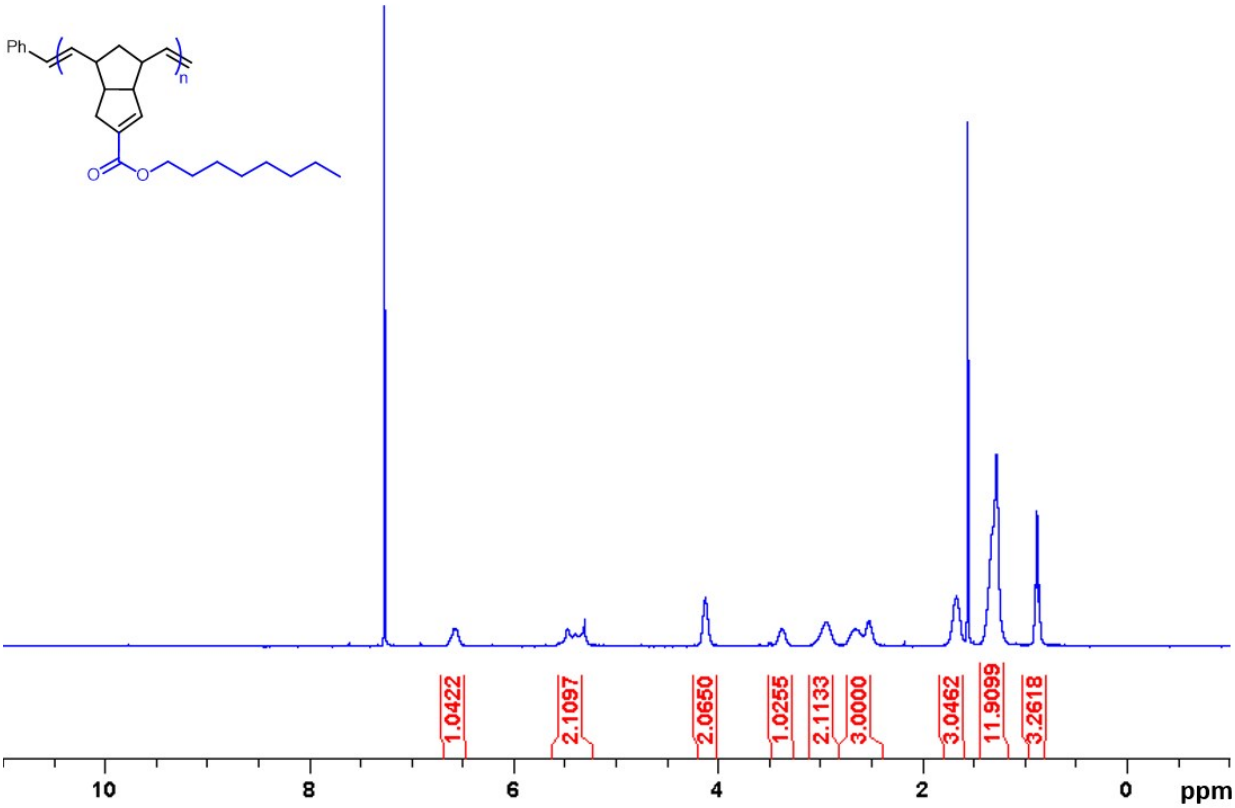


Figure S7. ^{1}H NMR spectrum for *f*PDCPD-octyl ester (linear precursor to crosslinked polymer **8**) in $CDCl_3$, recorded at 300 MHz.

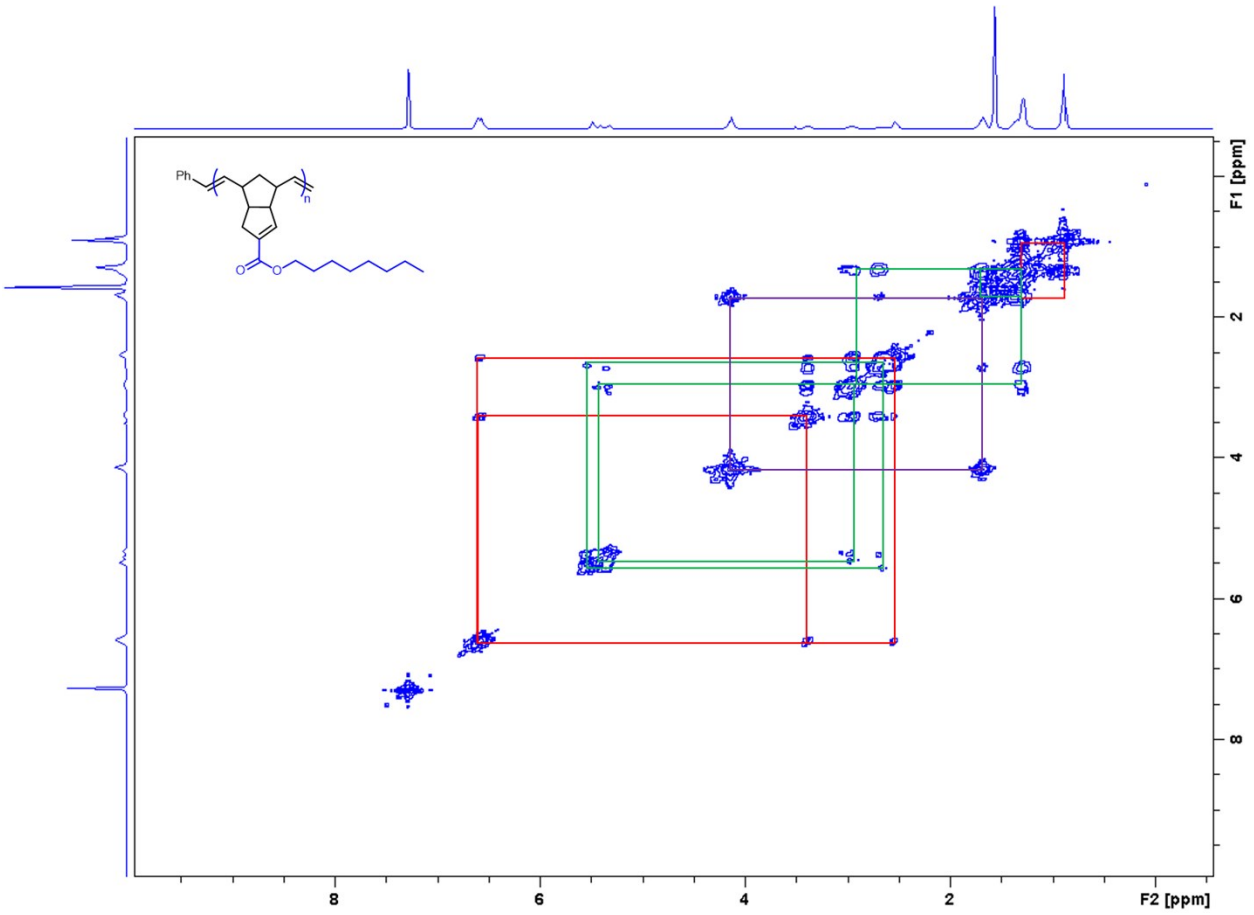


Figure S8. 500 MHz COSY NMR spectrum for *f*PDCPD-octyl ester (linear precursor to crosslinked polymer **8**) in $CDCl_3$. Colored boxes indicate correlations between ^1H signals. Refer to reference 20 for NMR data for the linear *f*PDCPD-methyl ester polymer **2**, and for assignments of the linear *f*PDCPD backbone.

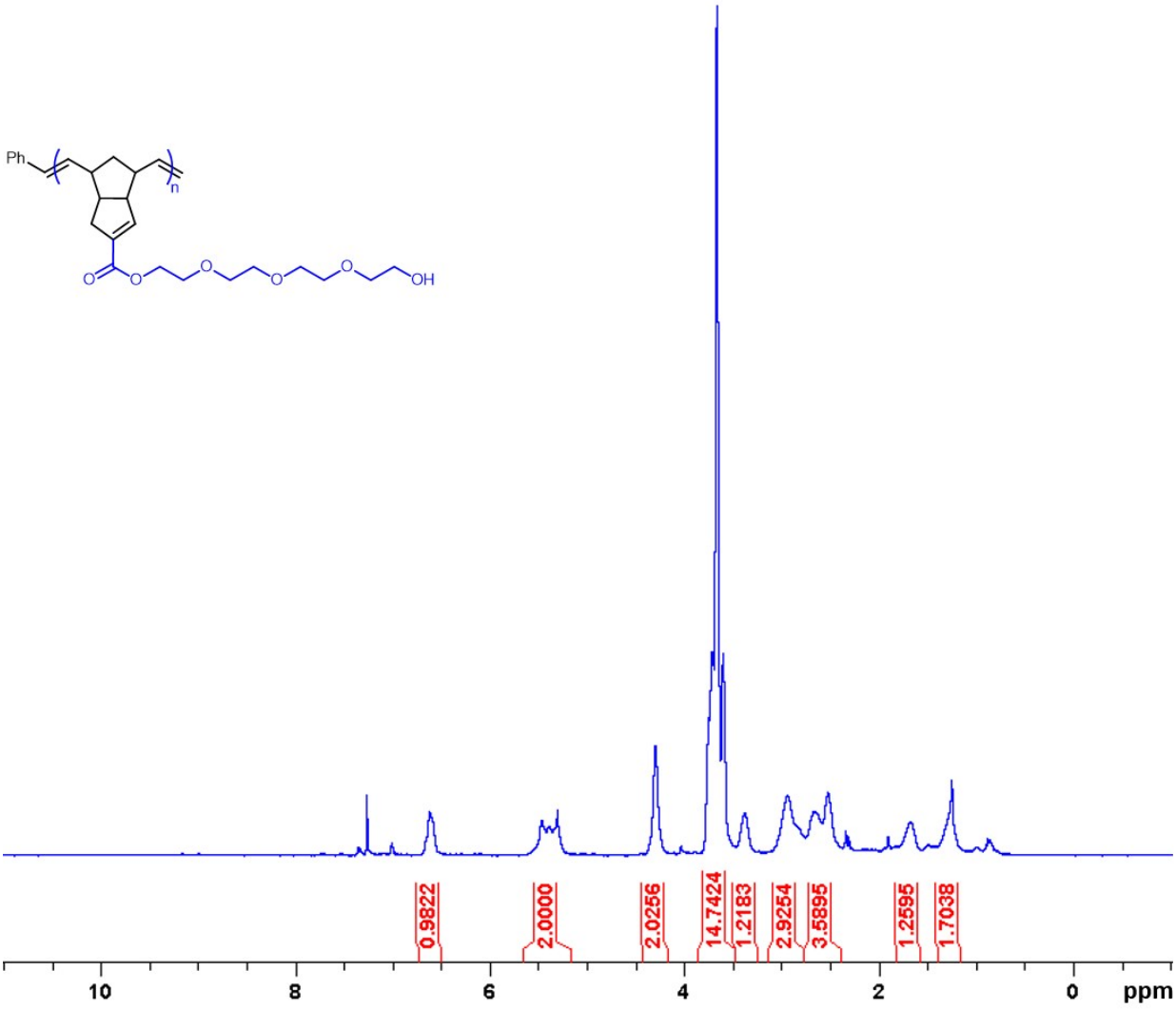


Figure S9. ^{1}H NMR spectrum for *f*PDCPD-TEG ester (linear precursor to crosslinked polymer **9**) in $CDCl_3$, recorded at 300 MHz.

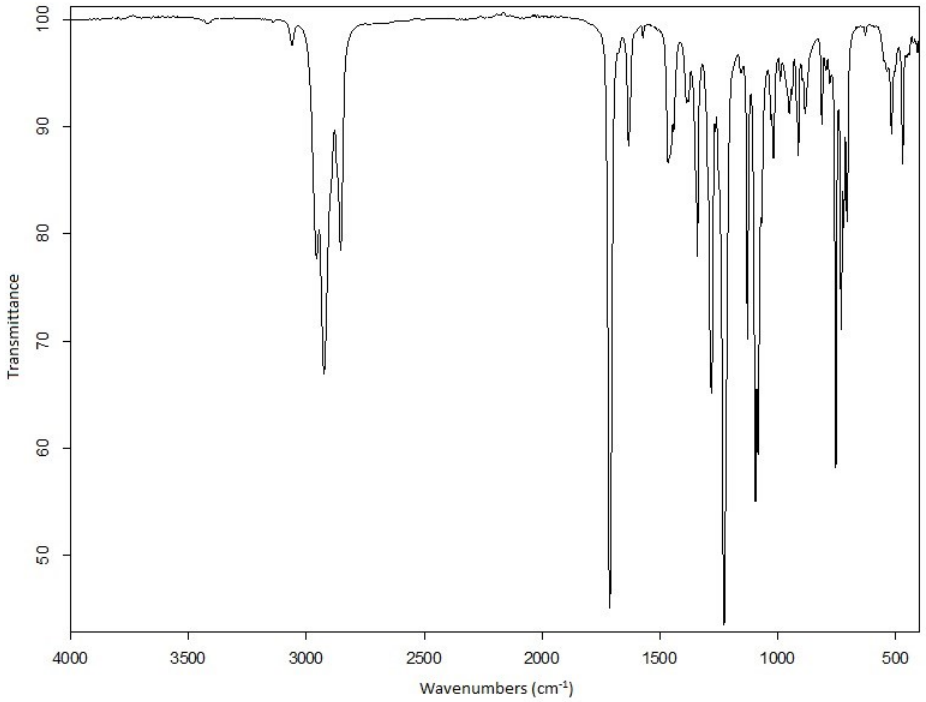


Figure S10. IR spectrum for octyl-ester functionalized dicyclopentadiene.

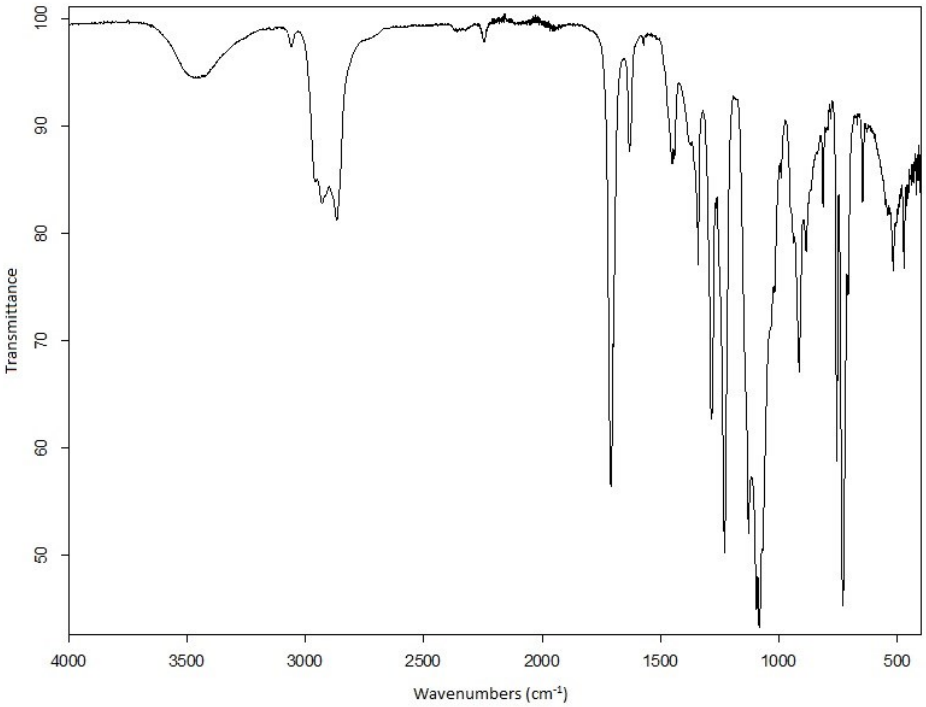


Figure S11. IR spectrum for TEG-ester functionalized dicyclopentadiene.

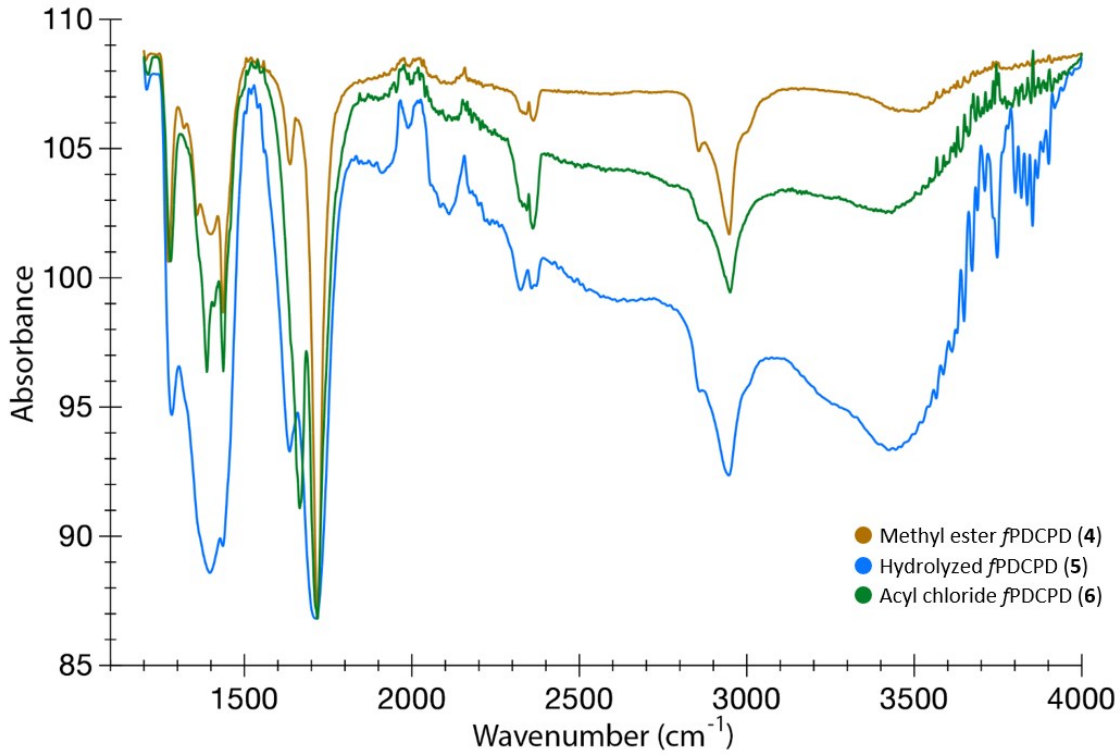


Figure S12. ATR-FTIR spectra for methyl ester *f*PDCPD (**4**), partially hydrolyzed *f*PDCPD (**5**) and the putative acyl-chloride-containing *f*PDCPD (**6**) on glass cover slides. The characteristic acyl chloride carbonyl stretch is not visible in this sample, either due to decomposition during analysis or else due to constraints of the surface-bound sample. The corresponding small-molecule analogue, *f*DCPD–COCl, formed under identical conditions, was confirmed by a downfield shift of the vinyl proton in the ^{1}H NMR spectrum (Fig. S2).

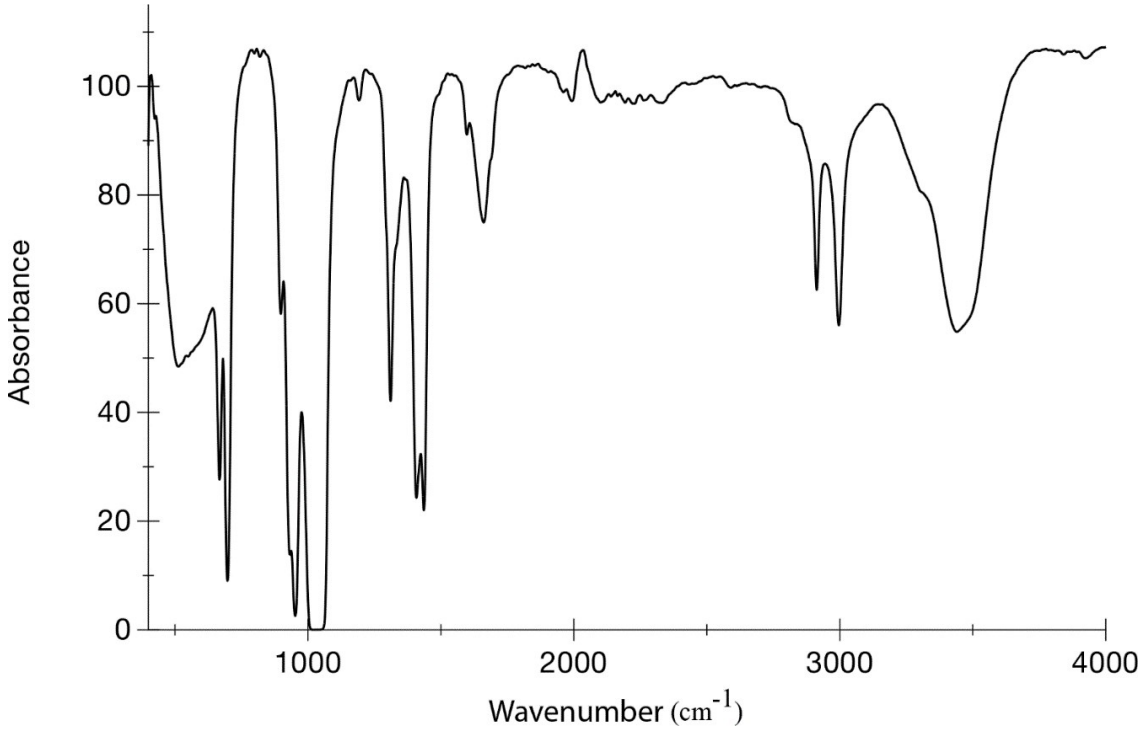


Figure S13. ATR-FTIR spectrum for 5-TAMRA-PEO3-amine in DMSO.

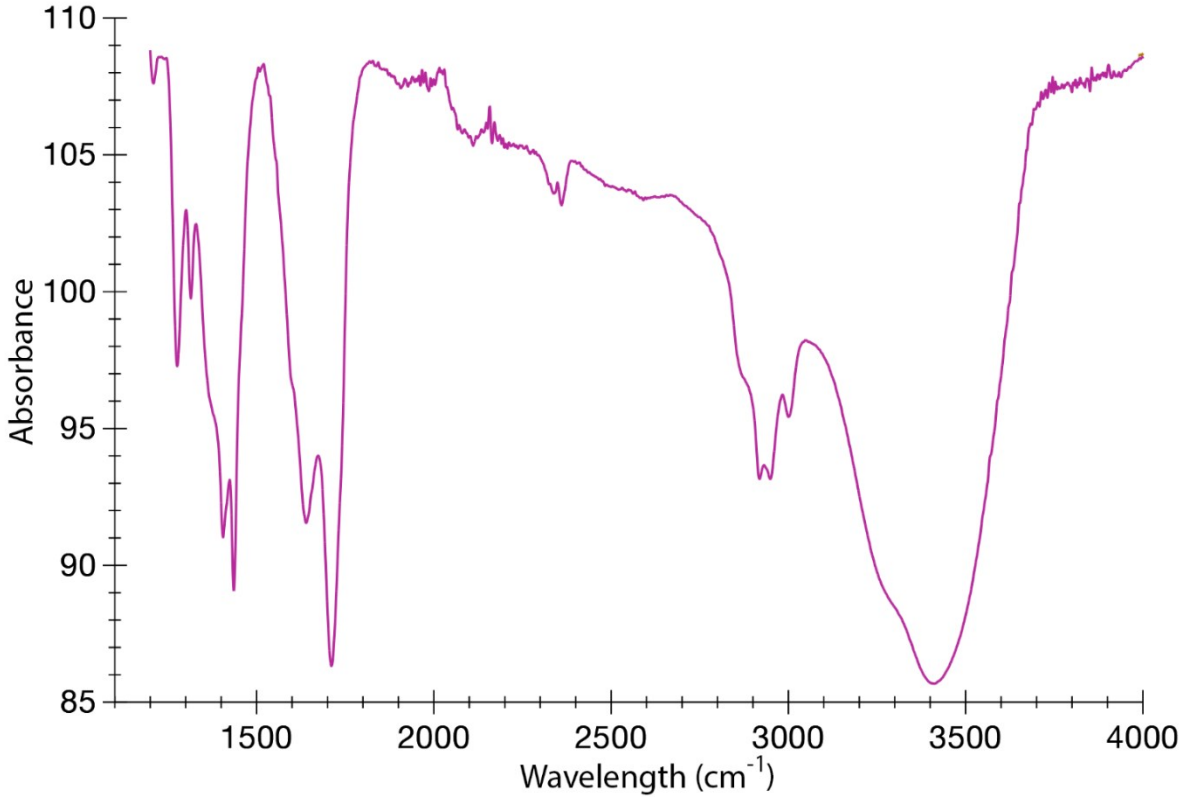


Figure S14. ATR-FTIR spectrum for *f*PDCPD spin coated cover slides functionalized with TAMRA.

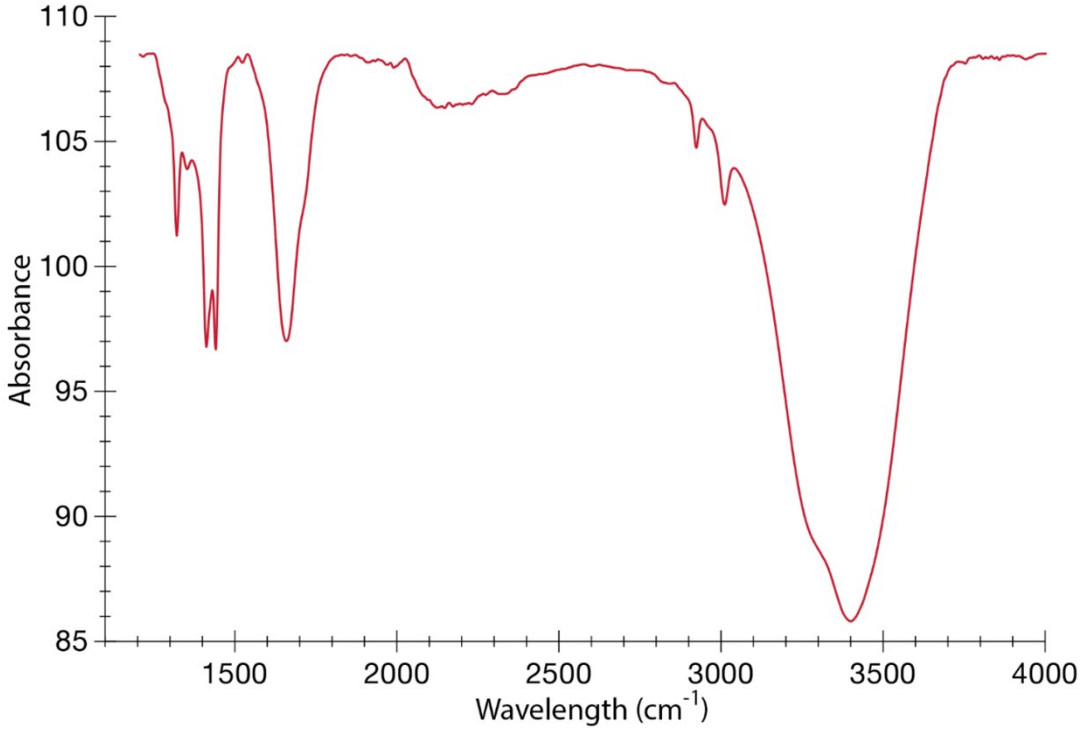


Figure S15. ATR-FTIR spectrum for *f*PDCPD spin coated cover slides functionalized with chloramphenicol.

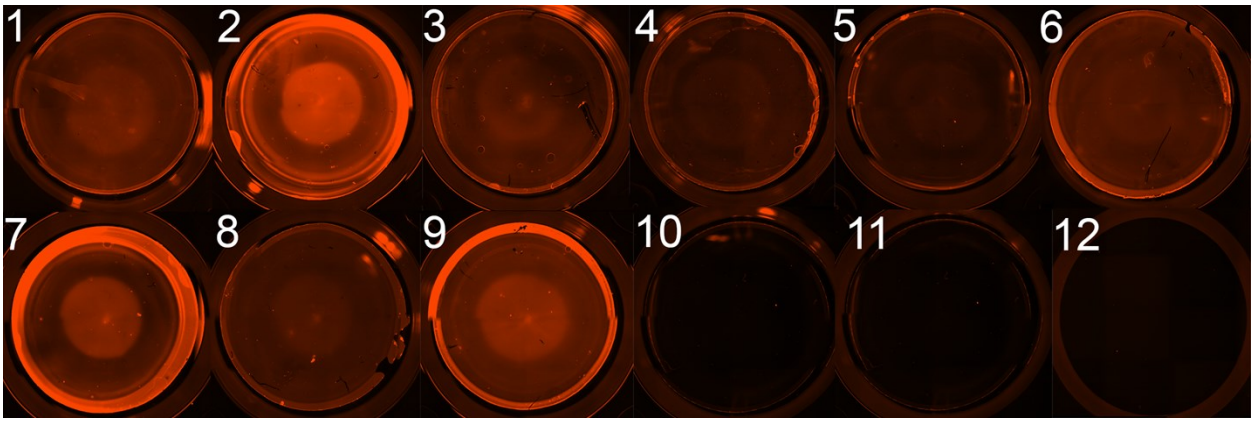


Figure S16. Images of TAMRA functionalized surfaces with varying spin speeds, acceleration and polymer volume. Also shown are the methyl ester *f*PDCPD (**4**), partially hydrolyzed *f*PDCPD (**5**) and non-polymer-coated methacrylated glass. Images were collected using a Cytation 5 multichannel plate reader using the Texas Red Filter Cube and a 1.25x objective. The instrument's Gen 3.05 software was used, and an automated experiment was conducted to ensure identical imaging parameters for each surface. Numerical labels refer to the samples identified in Table S2.

Table S2. Spin-coating parameters for the *f*PDCPD coated glass cover slides seen in Figure S16. Various volumes of 4 wt% linear *f*PDCPD were spin-coated onto methacrylated glass slides with varying maximum speed and acceleration.

Image #	***Spin Time (s)***	***Max Speed (RPM)***	***Acceleration (RPM/s)***	***Volume Spin-Coated (µL)***
1	30	3000	750	30
2	30	1000	750	50
3	30	3000	1000	25
4	30	3000	500	50
5	30	3000	1000	30
6	30	2000	750	50
7	30	1000	500	50
8	30	2000	750	25
9	30	2000	1000	50
10 (Methyl Ester Negative Control)	30	3000	1000	50
11 (Carboxylic Acid Negative Control)	30	3000	1000	50
12 (Methacrylated Glass Negative Control)	--	--	--	--

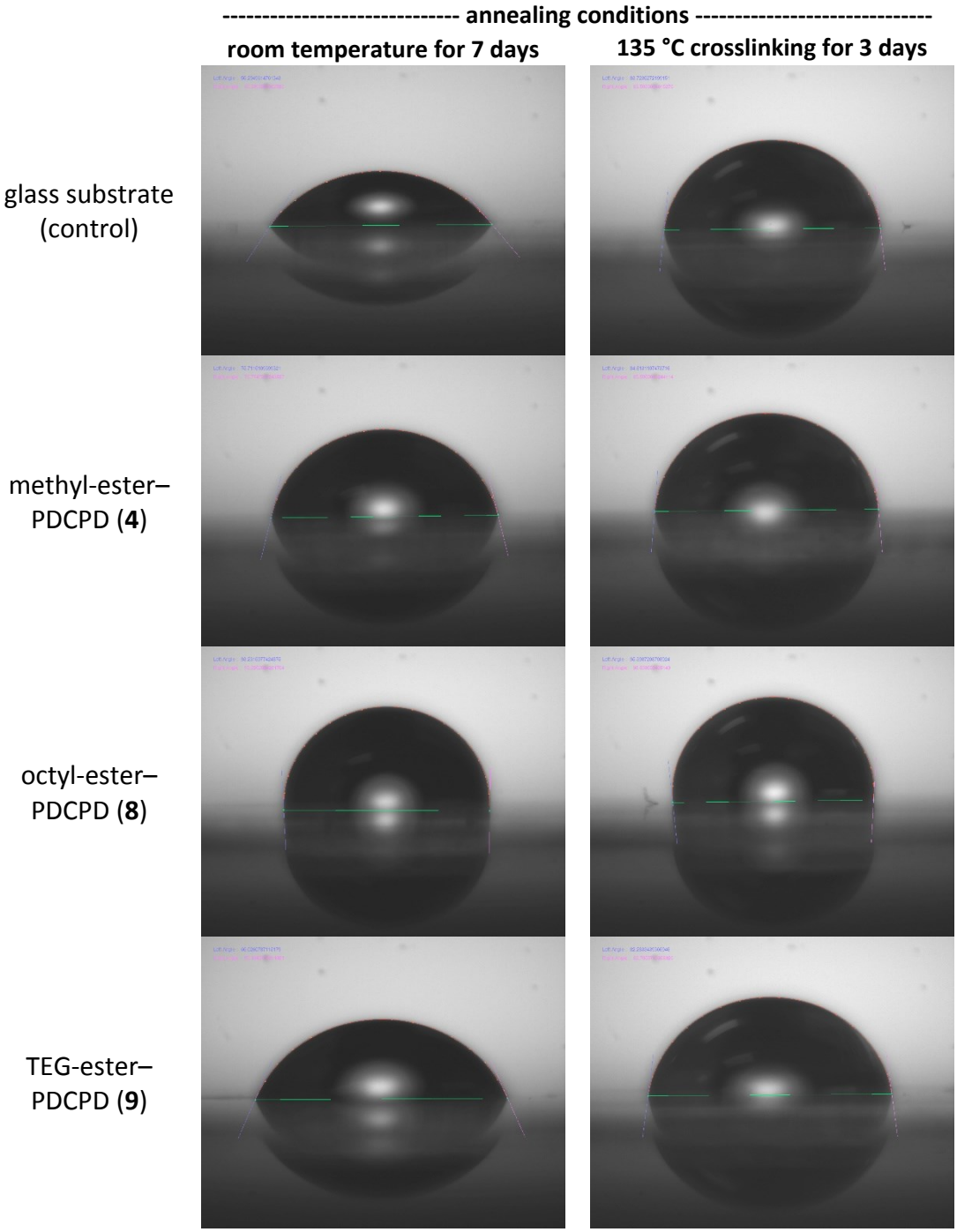


Figure S17. Representative contact angle photos.