

Supporting Information

Biocompatible and bioresorbable poly(caprolactone-co-trimethylenecarbonate) urethane acrylate resins for digital light processing

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Materials

L-lysine diisocyanate ethyl ester (>96%, LDI) was obtained from Actu-All chemicals, trimethylene carbonate (99%, TMC) was provided by Huizhou Foryou Medical Devices, ϵ -caprolactone (99%, CL) and 2-hydroxyethyl acrylate (HEA) were obtained from Alfa Aesar, diphenyl (2, 4, 6-trimethylbenzoyl) phosphine oxide (TPO) and calcium hydride (CaH_2) were obtained from Sigma Aldrich, Tetrahydrofuran (anhydrous, $\geq 99\%$, THF), tin (II) 2-ethylhexanoate (92.5%, Sn(II)Oct), methanol (99.5%) and triethylene glycol ($\geq 99\%$, TEG) and toluene ($\geq 99.8\%$, anhydrous) were obtained from Merck, 2-(2-ethoxy-ethoxy) ethyl acrylate (EOEOEA) was provided by TCI Chemicals and chloroform (98%, CHCl_3) was obtained from VWR. CL was dried for 12 h over calcium hydride and distilled under reduced pressure. TMC was lyophilized for 48 h before polymerization, Sn(II)Oct was distilled under reduced pressure before use. All other chemicals were used as received.

Characterization

Nuclear magnetic resonance (^1H -NMR and ^{13}C -NMR) was recorded on a Bruker ASCEND™ 700 MHz NMR spectrometer equipped with TCI cryo probe™. Gel permeation chromatography (GPC) was conducted using *N,N*-dimethylformamide (DMF) containing 0.1 wt% LiBr as eluent and the sample concentration was fixed to 2 g L⁻¹. The GPC Shimadzu system was comprised of an autosampler, a Shodex KD-G 4A guard column (4.6 x 10 mm) with 8 μm beads, followed by two Shodex KD-802 (5 μm , 8 x 300 mm) and KD-804 (7 μm , 8 x 300 mm) columns, a refractive index detector and a photodiode array detector at 50 °C with a flow rate of 1 mL min⁻¹. The GPC system was calibrated against linear poly (methyl methacrylate) standards with molecular weights ranging from $M_p = 600$ to 265300 Da. The samples were filtered through polytetrafluoroethylene (PTFE) membranes with a pore size of 0.2 μm prior to injection. FTIR spectra were recorded on a Thermo Scientific spectrophotometer (Nicolet™ iS50 FTIR) in ATR mode.

Mechanical testing

Uniaxial tensile test was performed using ElectroForce3200 (TA instruments) equipped with a 50 N load cell at room temperature and 1% strain min⁻¹ speed. Bone shape specimens were

prepared using a cutting dye (Zwick/Roell) with a gauge section of 10 mm. All samples were measured 5 times. Values for young's modulus (E), stress at yield (σ -yield), strain at yield (ϵ -yield) and maximum strain at break (ϵ -break) and maximum stress (σ -max) are reported as averaged results.

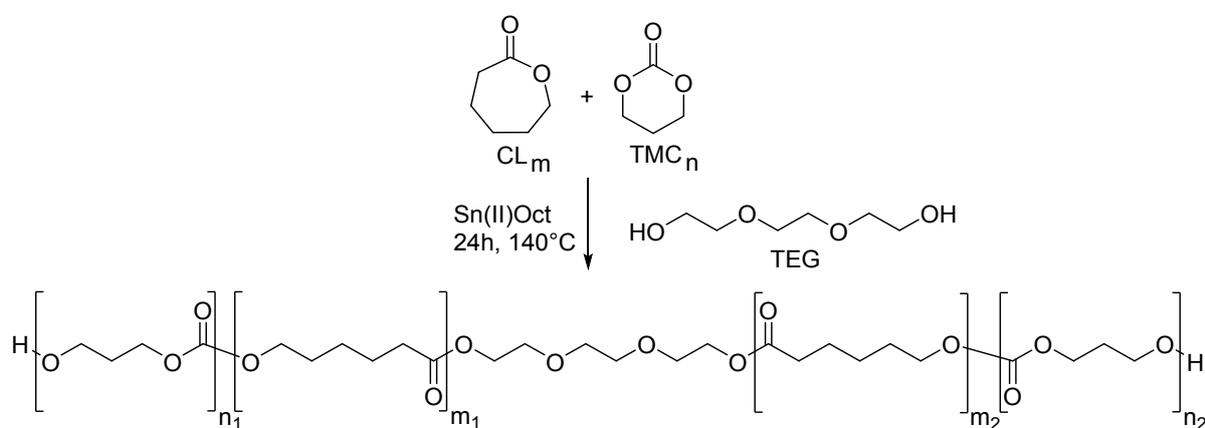
Thermal characterization

A DSC TA Q 2000 was used to analyze the thermal properties. Samples were measured in a range from -80 °C to 100 °C using heat-cool-heat cycles. The heating and cooling rate were 10 °C min⁻¹ and a nitrogen flow rate of 25 mL min⁻¹. When present, the melting point was determined at the maximum of the melting endotherm. Glass transition temperature was determined using TA Universal analysis software.

Accelerated in-vitro degradation test

Degradation was evaluated in basic conditions using 0.1 M sodium hydroxide solution. Disk shape samples (in triplicate) of all materials were prepared in the same way as the casted films but using a cylindrical mold. The samples were placed in individual glass vials, 10 mL of the solution was poured, capped, and the vials were incubated at 37 °C using a water bath. After 48 h, the samples were removed from the water bath, washed with distilled water and dried with a tissue. The wet weight was recorded and then samples were then dried for 24 h at 37 °C under reduced pressure, once dried the diameter and height of the samples was measured three times and the average was recorded. The test was prolonged for 30 days and the solution of NaOH was always refreshed after drying the samples under vacuum. In order to corroborate the fact that PD/LLA exhibit a relatively high degradation compared to PCTAc resin 75:25 (CL:DLLA) was synthesized and tested only for degradation studies.

Methods

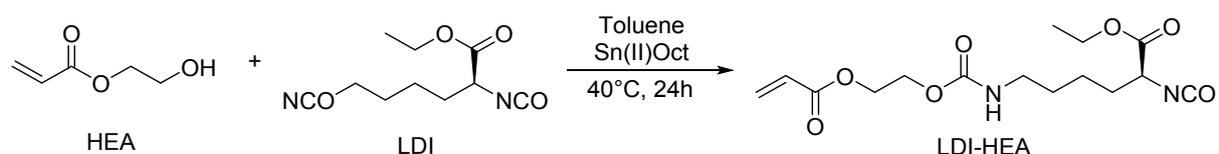


Scheme S 1. Ring opening polymerization of ϵ -caprolactone and trimethylene carbonate in presence of stannous octoate and triethylene glycol.

General procedure for Poly(CL-co-TMC) prepolymer synthesis (PCT)

Synthesis of poly(CL-co-TMC) 50:50 ratio. Typically, a 100 mL round bottom flask was equipped with a magnetic stir bar and charged with TMC (14.99 g, 147 mmol), CL (17.26 g, 147 mmol), TEG (1.16 g, 7.94 mmol) and Sn(II)Oct (57.40 mg, 0.14 mmol). The mixture was heated to 140 °C and stirred for 24 h under a nitrogen atmosphere. The final product was then dissolved in CHCl₃ and purified by precipitation in cold methanol (~0 °C). A viscous transparent solid was collected and dried under high vacuum for 48 h (20g, 62%).

LDI-HEA intermediate synthesis



Scheme S 2. Reaction scheme of HEA with LDI in presence of catalytic amounts of Sn(II)Oct.

LDI (3.0 g, 13.33 mmol) was dissolved in anhydrous toluene (5 mL, 47 mmol) then Sn(II)Oct (60 mg, 0.13 mmol) was added and stirred for 10 min under a stream of dry nitrogen. A solution of HEA (1.28 g, 13.33 mmol) in anhydrous toluene (5 mL, 47 mmol) was added dropwise under vigorous stirring. After the addition of HEA the reaction was heated to 40 °C for 12 h and kept in the dark. The reaction was monitored via IR spectroscopy by disappearance of the HEA hydroxyl band at 3500 cm⁻¹. The final product was obtained as a yellow liquid, which was used without further purification.

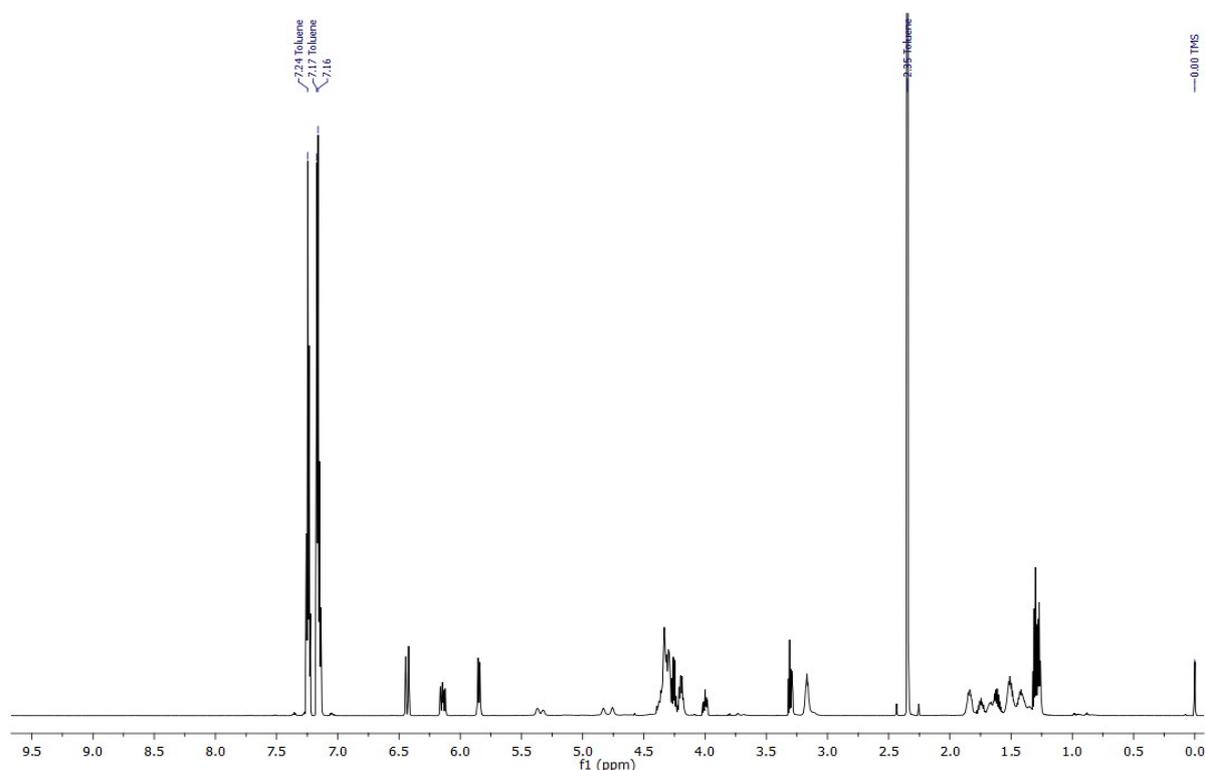


Figure S 1. ¹H-NMR in CDCl₃ of the reaction mixture LDI-HEA.

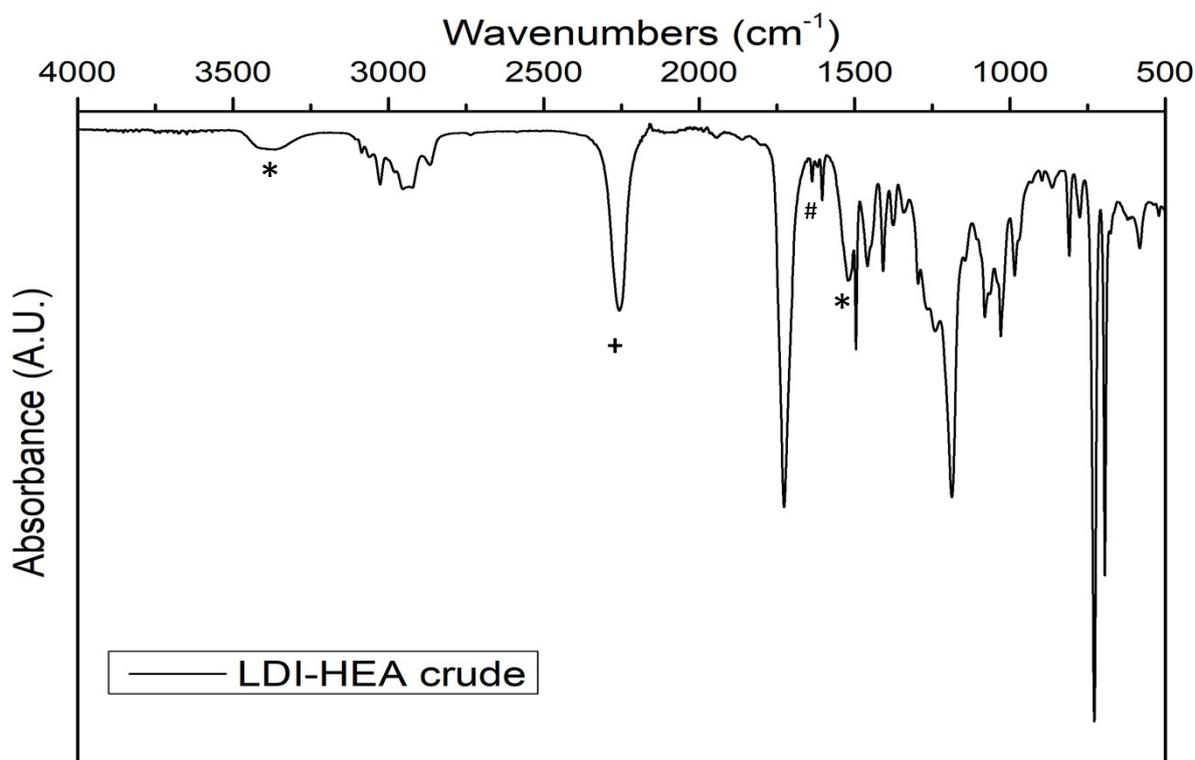


Figure S 2. IR of the crude reaction mixture of LDI-HEA showing the NH peak of the amide bond at 3300 cm^{-1} (*) and 1520 cm^{-1} (*) the double bond of the acrylate at 1600 cm^{-1} and 1630 cm^{-1} (#) and the isocyanate peak at 2270 cm^{-1} (+).

General PCTAc synthesis

Poly(CL-co-TMC) (50:50) (PCT) (M_n : 2950 g mol^{-1}) 20 g (6.7 mmol) was dissolved in chloroform (30 mL). Then, Sn(II)Oct (60 mg, 0.13 mmol) was added stirred for 10 minutes before the LDI-HEA mixture in toluene (10 ml, 13.33 mmol) was added. The mixture was heated to $40\text{ }^\circ\text{C}$ for 24 h. The final product was concentrated under reduced pressure and then purified by precipitation in cold methanol ($0\text{ }^\circ\text{C}$). A high-viscous yellow solid (14.5 g,) was obtained after drying under vacuum for 48 hours.

Preparation of photo curable resin (UV resin)

UV curable resins were prepared by mixing PCTAc with the photoinitiator (TPO, 1 wt%) and the reactive diluent (EOEOEA, 30 wt%). Then, the resin was heated to $50\text{ }^\circ\text{C}$ and mixed manually until homogenous.

AM conditions

The scaffolds were fabricated using a commercially available DLP (ARM 10, Roland, Geel, Belgium) with a customized vat and printhead. The scaffolds were printed at temperatures of around $55\text{ }^\circ\text{C}$ unless stated differently, via a heated vat. After manufacturing, the scaffolds were washed two times with acetone and acetone/ethanol (50:50) to remove all the

unreacted resin. The scaffolds were then post-cured in a UV oven at 365 nm (10 mW/cm²) for 5 min from both top and bottom sides.

Human Mesenchymal Stem Cell Culture

Human bone marrow derived Mesenchymal Stem Cells (hMSCs) (Dr. Prokop, donor 803.1), were used to assess the biocompatibility and cytocompatibility of the selected DLP fabricated films. Cells were cultured at a 1.000 cell.cm⁻² density in alpha-MEM media supplemented with Glutamax and 10% FBS at 37 °C, 5% CO₂. Cells were subcultured at 80% confluence until passage 4 and were used at passage 5 for all experiments.

Cell culture on DLP fabricated films

DLP films of 9 and 14 mm diameter were fabricated in triplicate as described previously (AM conditions) on top of glass slides and washed thoroughly in a series of 24 h steps of ethanol, acetone and ethanol, to remove any potential unreacted compound. Clean films were placed inside 24-well plates and sterilized by immersion in 70% ethanol for 15 minutes. Thereafter, the ethanol was removed and the films were rinsed 3 times with sterile phosphate buffer saline (PBS) and left overnight in sterile PBS to ensure a complete removal of ethanol. Samples were moved to new 24-well plates and hMSCs were seeded at a density of 10.000 cell.cm⁻². Controls were prepared on the same tissue culture plates (TCP) and under the same conditions. Seeding was done from a concentrated cell/media dispersion, resulting in volumes of 20-25 µL per film, that were added as a drop and incubated for 1 h (37 °C, 5% CO₂). After initial cell attachment, 1 mL of media was added to each well. Cell Attachment and Viability Studies

For cell attachment experiments, hMSC seeded DLP films were rinsed with PBS and fixed with 4 % paraformaldehyde for 15 min after only 24 h of culture. Samples were then rinsed 3 times with PBS and permeabilized for 10 min in a 0.1% Triton-X 100 solution in PBS. Samples were stained for F-Actin and DNA with Phalloidin Alexa Fluor-568 (1:100, 1 h) and Hoechst (1:1000, 15 min), respectively. For cell viability studies, after 24 h of culture cells were rinsed with PBS and a live/dead viability kit consisting on ethidium homodimer-1 and calcein (Thermofisher) was used to stain cells following manufacturer indications. After 30 min of incubation cells were imaged. All samples were under a Nikon TI-E epifluorescent microscope and analyzed using Fiji and CellProfiler free softwares.

Lactate dehydrogenase (LDH) release assay

Supernatants of hMSC cultures on 14 mm diameter DLP films (15.000 cells per film) were collected after 24 h of cell seeding and kept at -80 °C until use. As positive and negative controls, hMSCs were cultured on triplicate TCP wells. 1 h before collecting the supernatant, cells from positive controls were chemically lysed. Negative controls were used as spontaneous LDH release measures (0% release) and positive controls were used as

maximum LDH release (100%). LDH was measured with a Pierce LDH Cytotoxicity Assay Kit (Thermo Fisher Scientific) following manufacturer instructions.

DNA assay.

For DNA assays samples used for LDH measurements were harvested after 24 h of initial seeding and placed in Eppendorf tubes first frozen at $-80\text{ }^{\circ}\text{C}$ and then thawed. This process was repeated 3 times. Cells were lysed by a Proteinase K treatment. In brief, samples were incubated 16 h at $56\text{ }^{\circ}\text{C}$ with $250\text{ }\mu\text{L}$ of 50 mM Tris/ 1 mM EDTA solution containing $1\text{ mg}\cdot\text{mL}^{-1}$ Proteinase K. After sample digestion, the samples were freeze-thawed 3 times in liquid N_2 to facilitate the DNA extraction. DNA was measured with a CyQuant cell proliferation assay (ThermoFisher). In brief, cellular RNA was degraded by incubating the samples for 1 h at RT with lysis buffer containing RNase A. $100\text{ }\mu\text{L}$ of each sample (triplicates) were placed in a 96-well plate and $100\text{ }\mu\text{L}$ of 2x GR-dye solution were added and left to incubate for 15 min at RT. A standard curve was prepared with a DNA standard solution and fluorescence intensity was measured at 520 nm . Cell number was calculated assuming $6,6\text{ pg}$ of DNA per cell.

NMR Spectra of PCT prepolymers

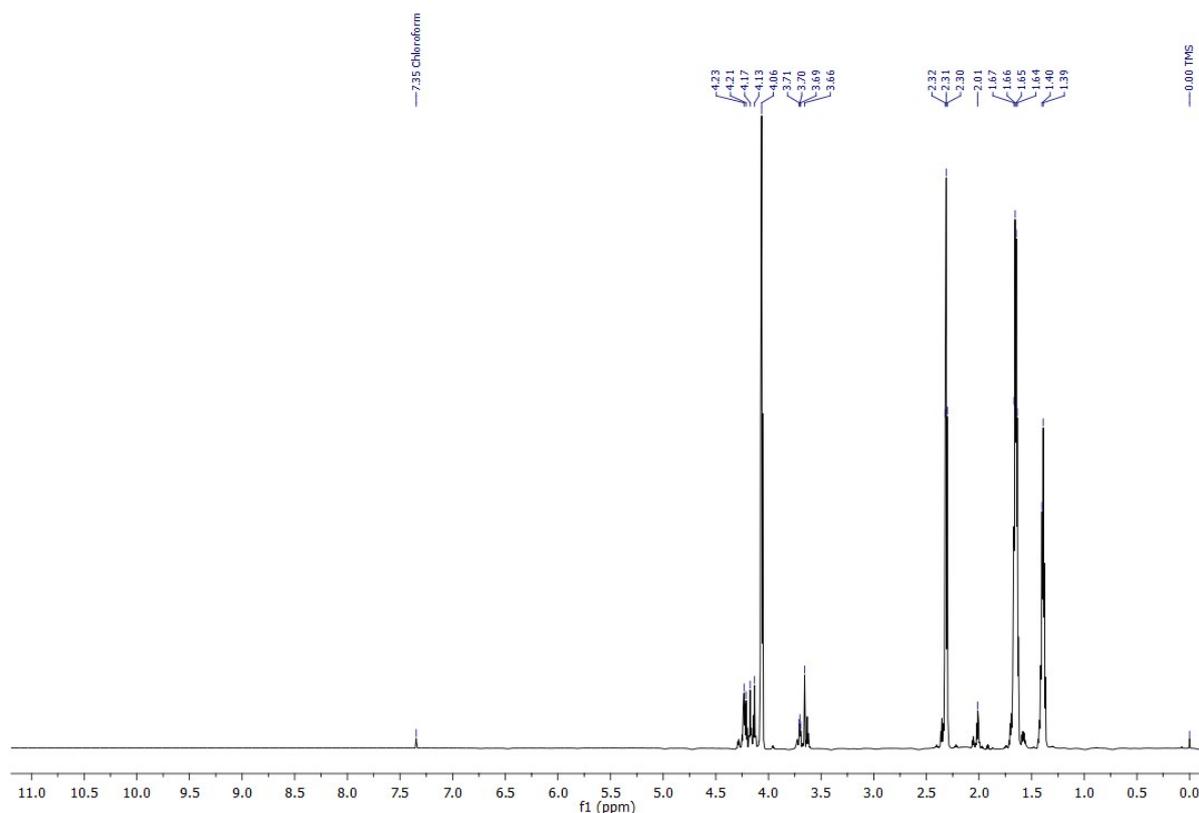


Figure S 3. $^1\text{H-NMR}$ of PCT 90:10 in CDCl_3

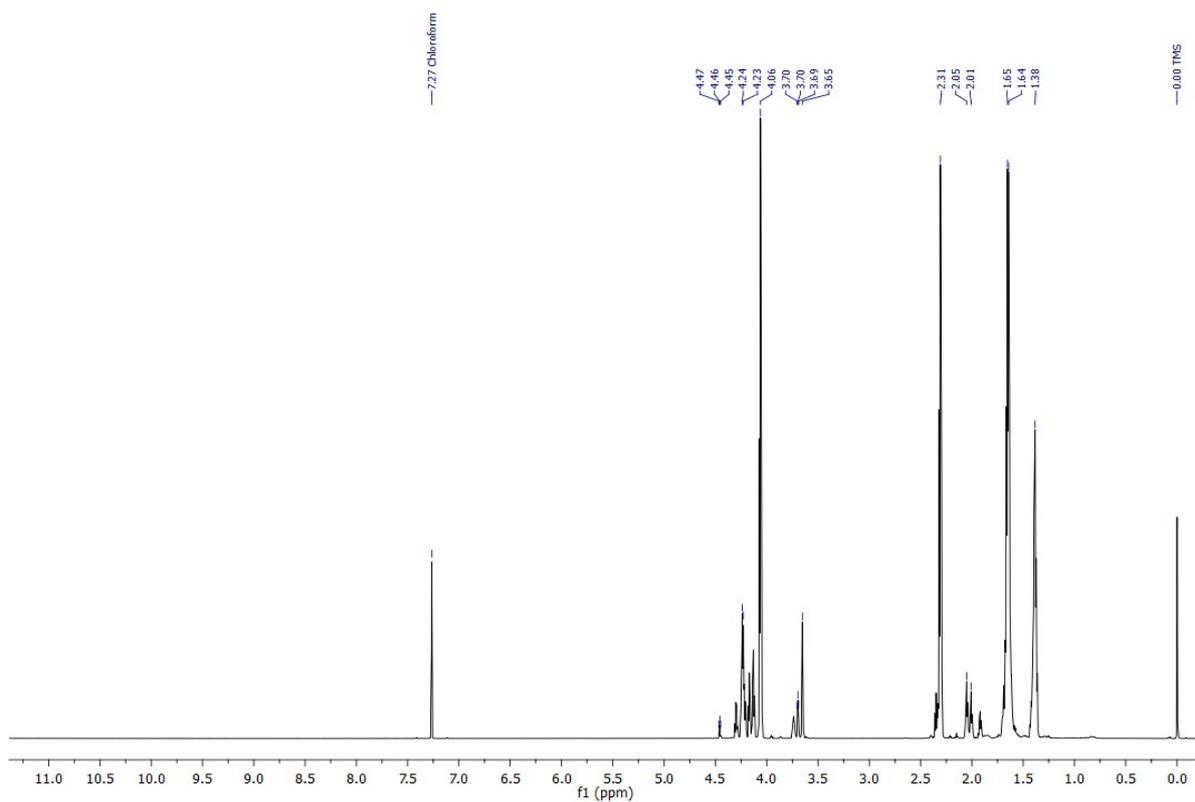


Figure S 4. $^1\text{H-NMR}$ of PCT 75:25 in CDCl_3

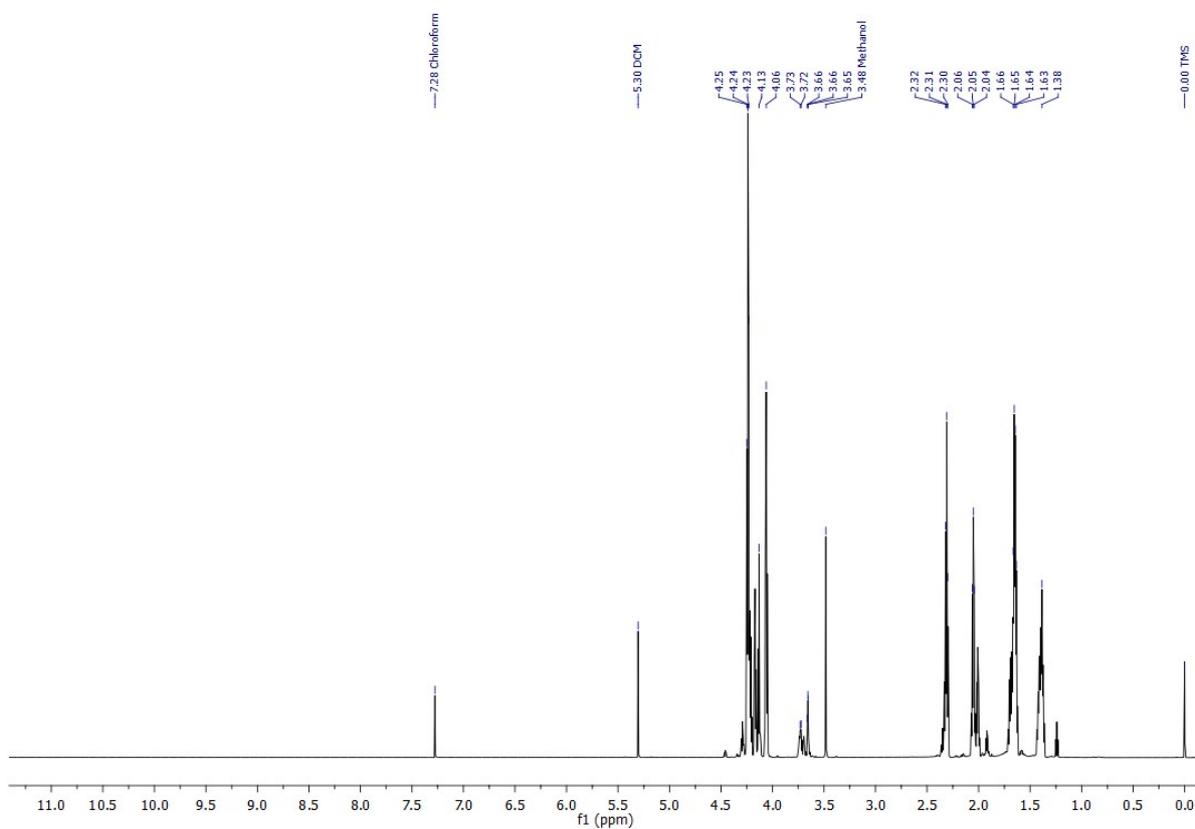


Figure S 5. $^1\text{H-NMR}$ of PCT 50:50 in CDCl_3

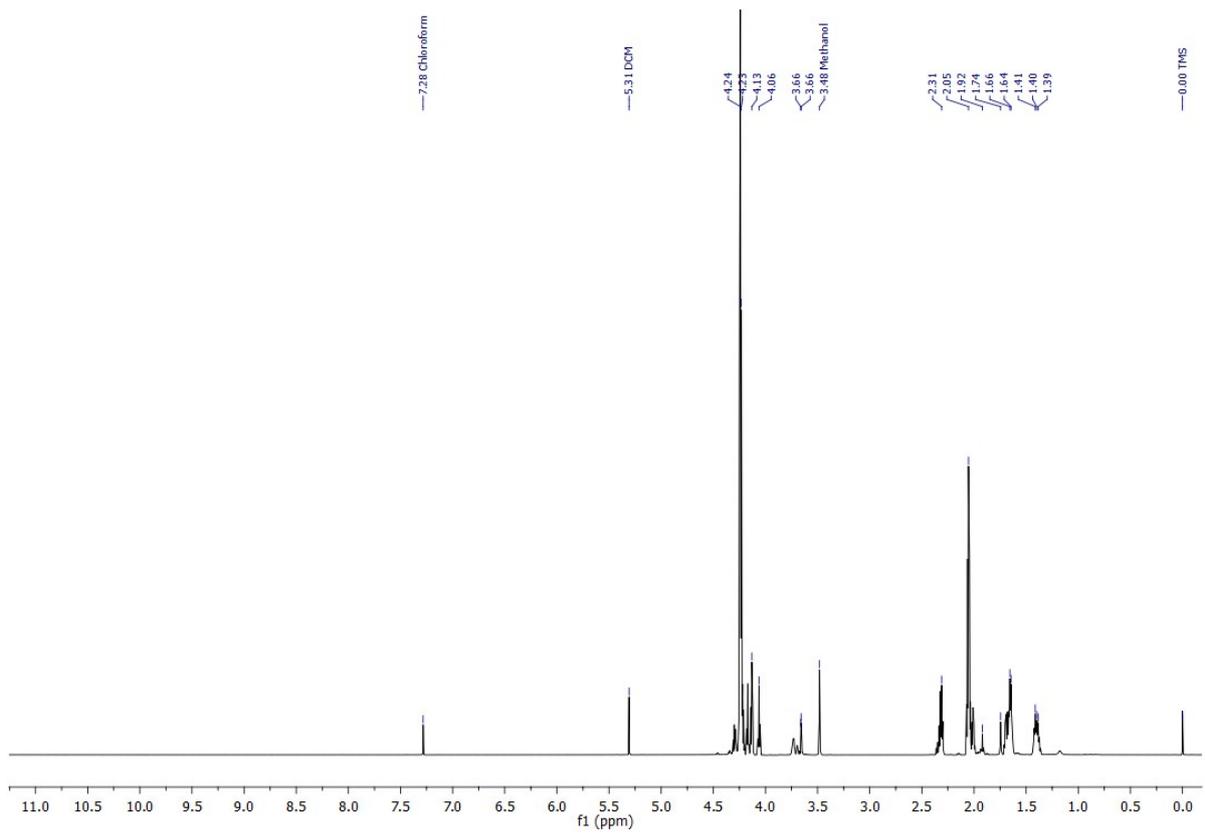


Figure S 6. $^1\text{H-NMR}$ of PCT 25:75 in CDCl_3

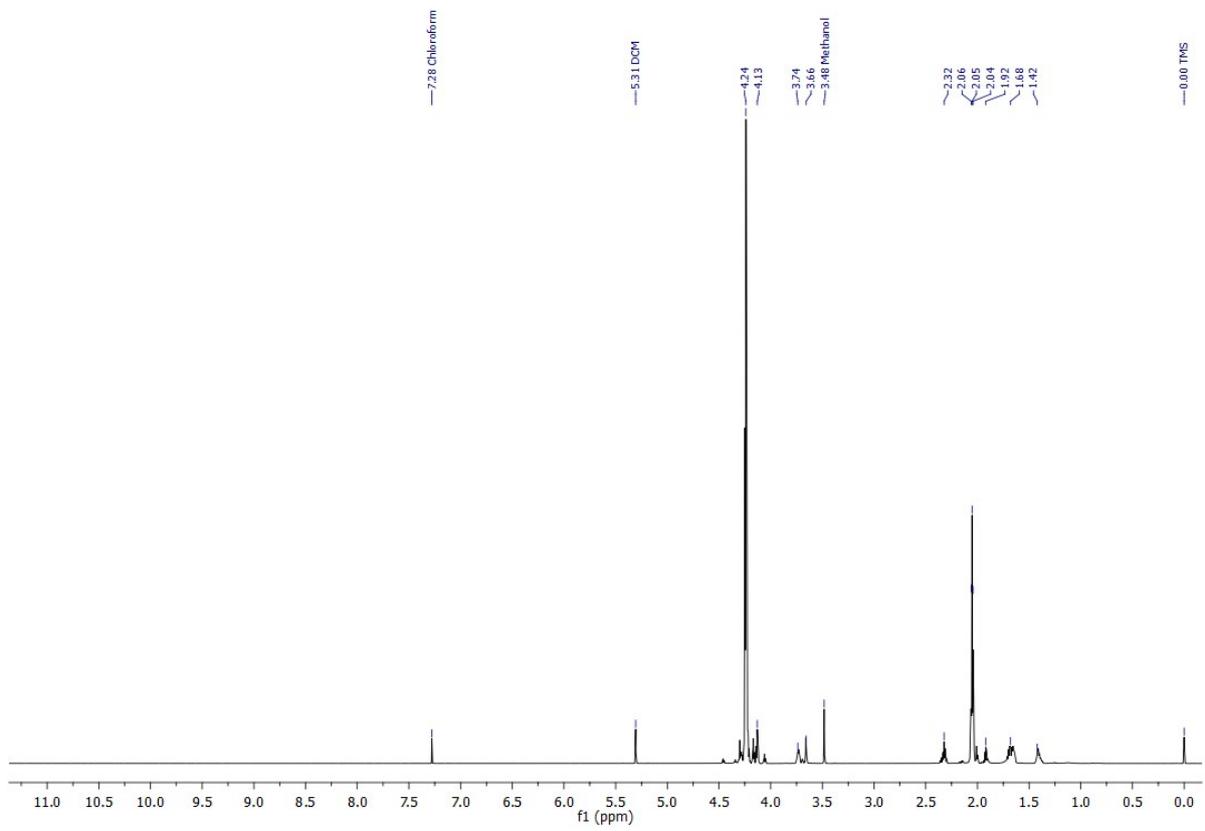


Figure S 7. $^1\text{H-NMR}$ of PCT 10:90 in CDCl_3

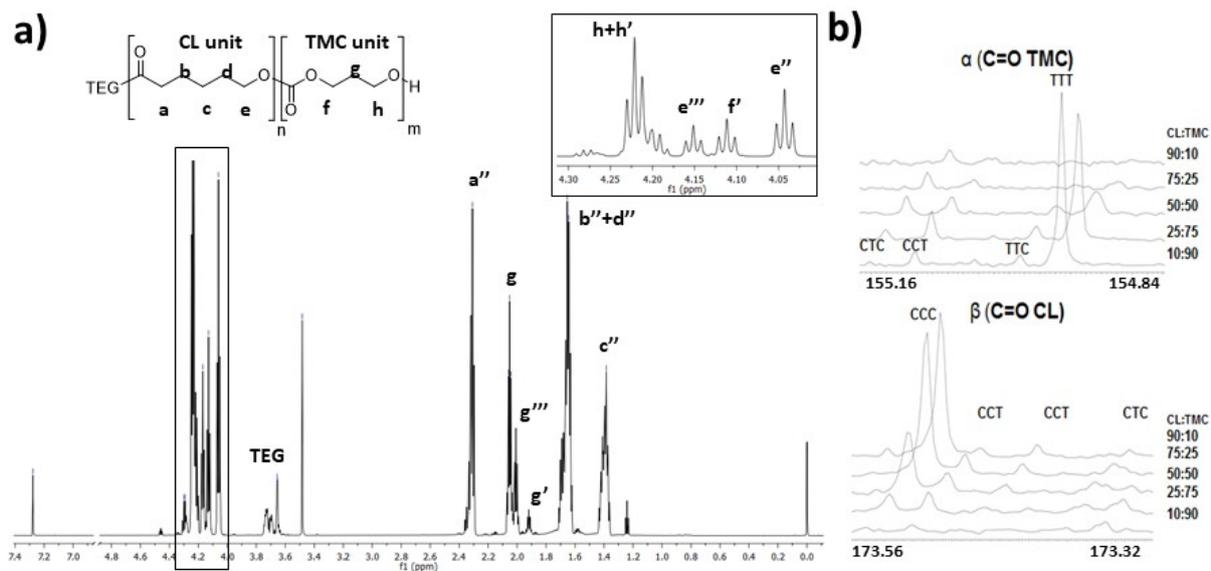


Figure S 8. NMR characterization of PCT samples. a) ¹H-NMR of PCT 50:50 with assignments based on dyad identity. The zoomed in region (4.00–4.27) shows assignments around the CH₂-O region which can be diagnostic for the polymeric microstructure. b) ¹³C-NMR of 5 different PCT samples, α corresponds to the TMC carbonyl region and β is the carbonyl region of the CL units.

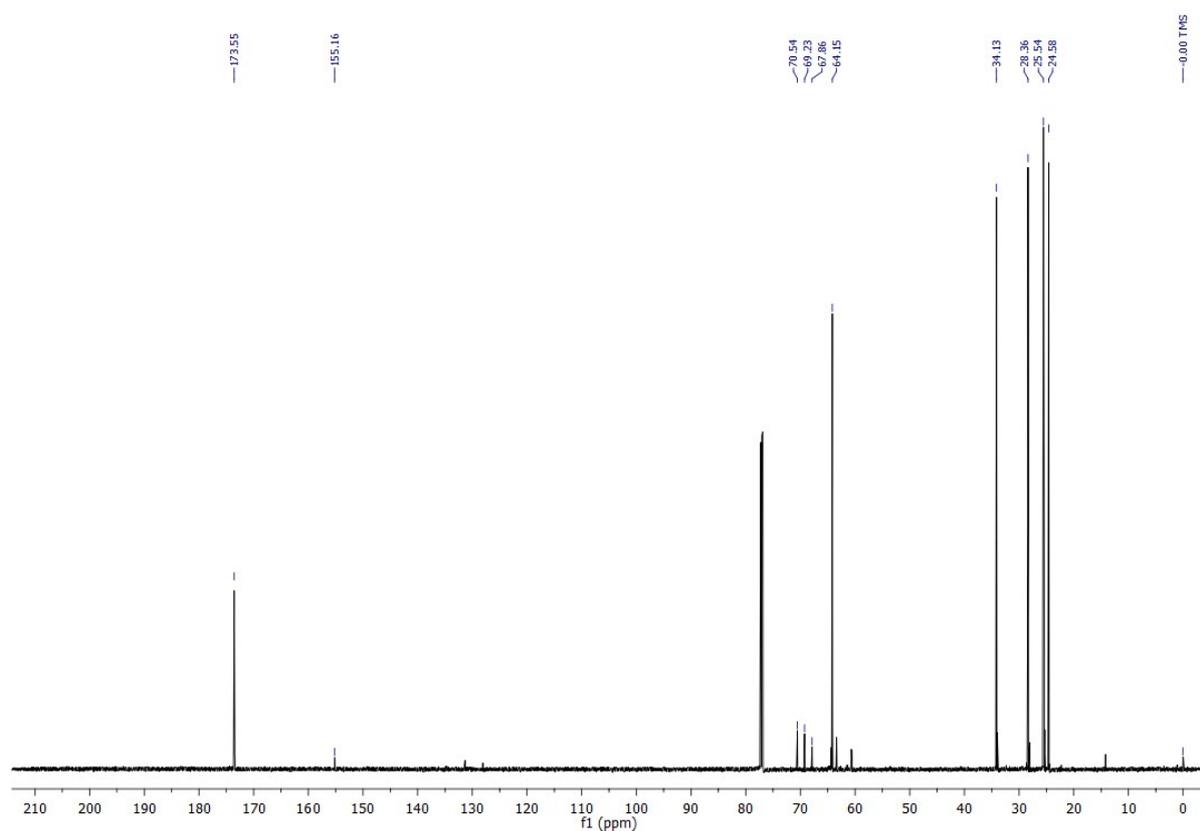


Figure S 9. ¹³C-NMR of PCT 90:10 in CDCl₃.

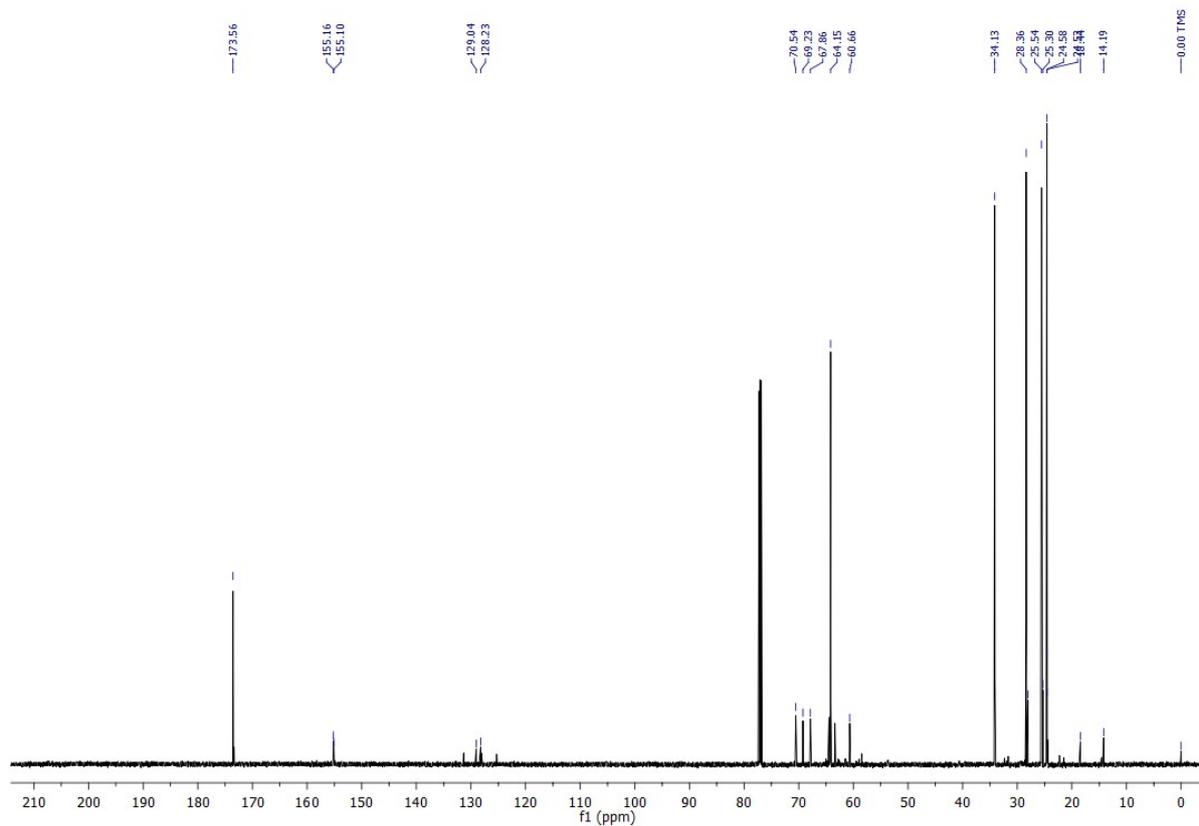


Figure S 10. ^{13}C -NMR of PCT 75:25 in CDCl_3 .

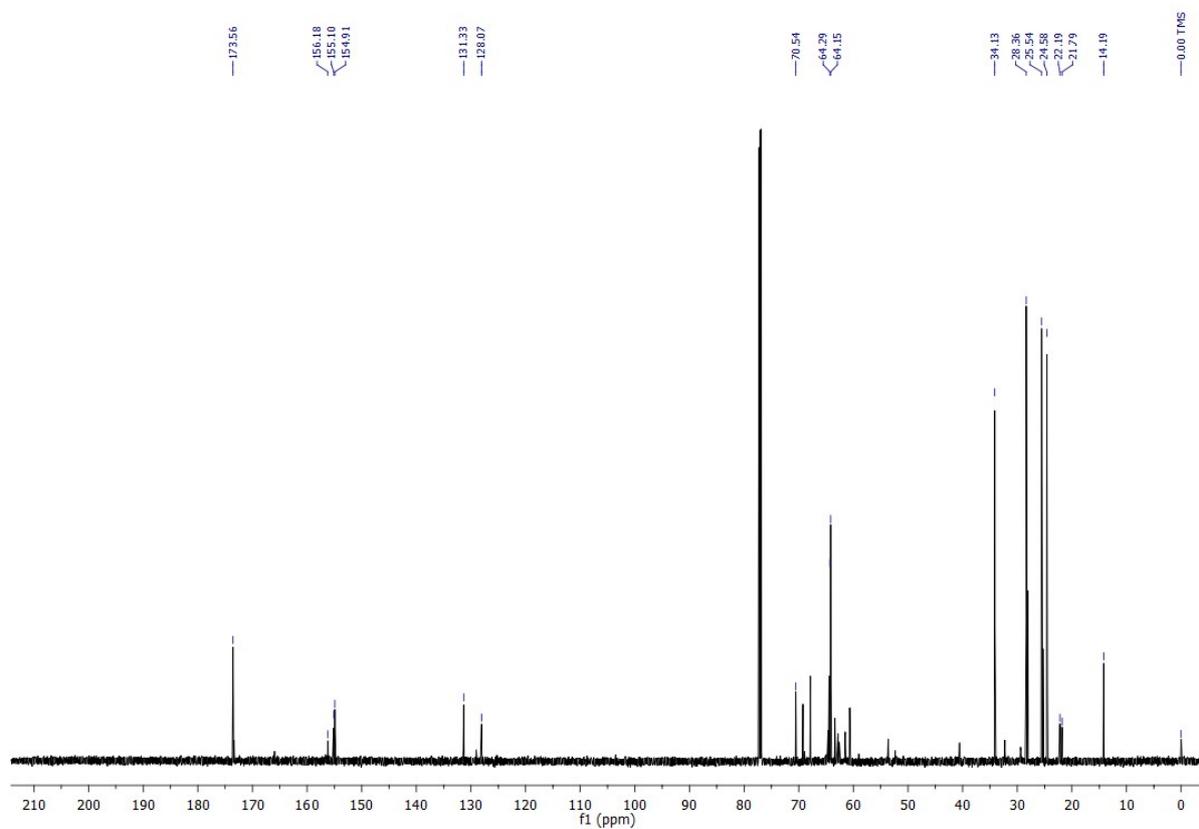


Figure S 11. ^{13}C -NMR of PCT 50:50 in CDCl_3 .

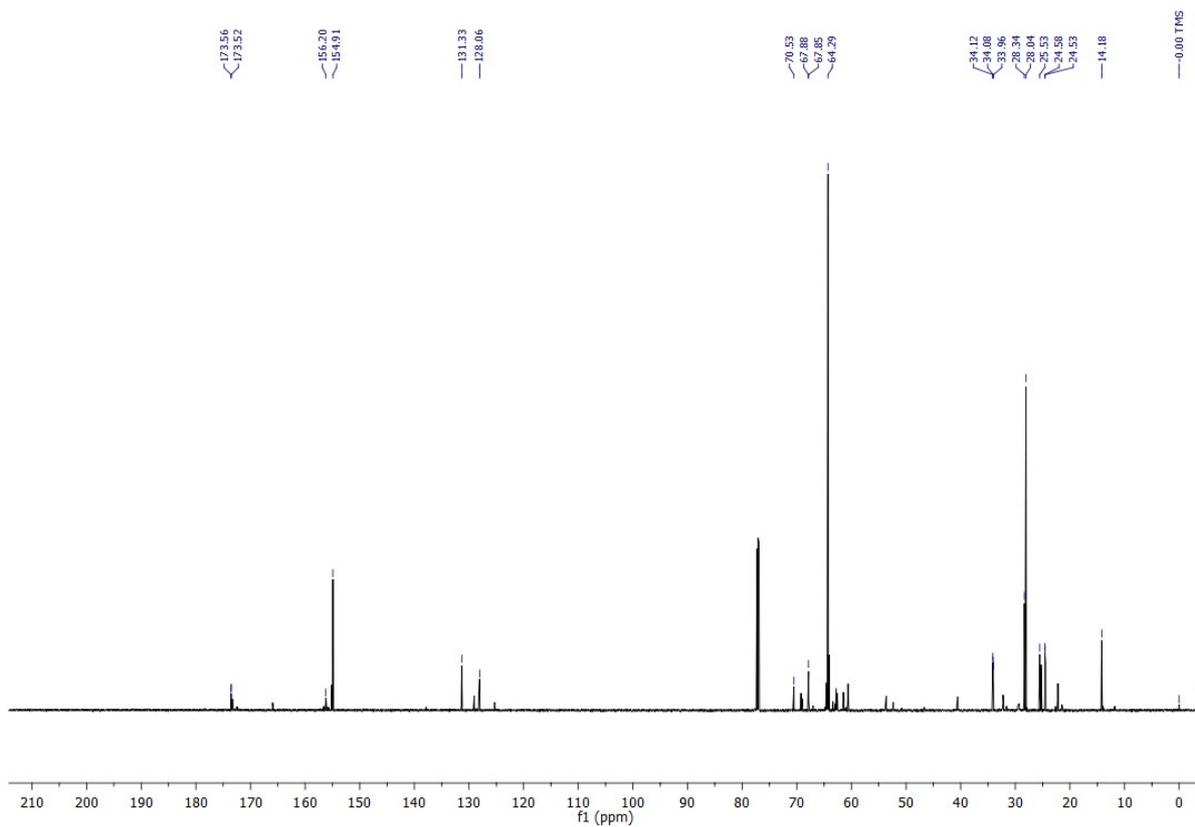


Figure S 12. ^{13}C -NMR of PCT 25:75 in CDCl_3 .

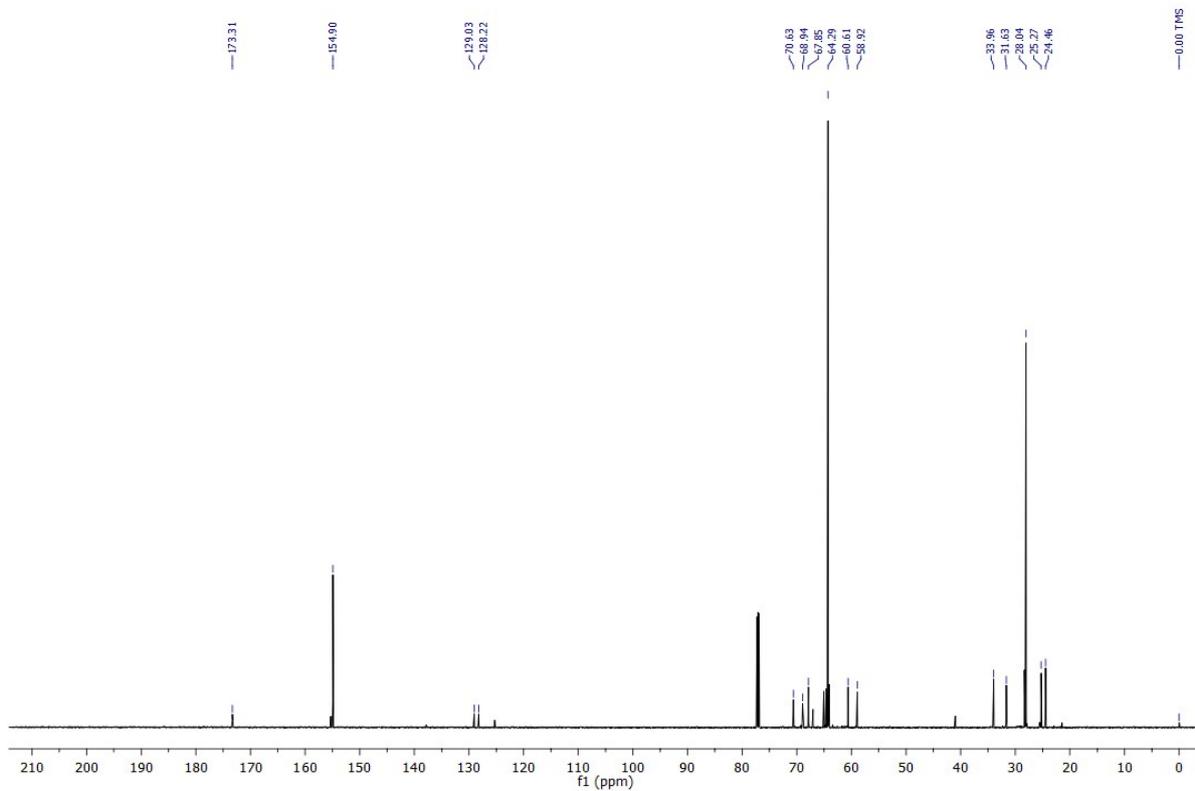


Figure S 13. ^{13}C -NMR of PCT 10:90 in CDCl_3 .

NMR Spectra of PCTAc resins

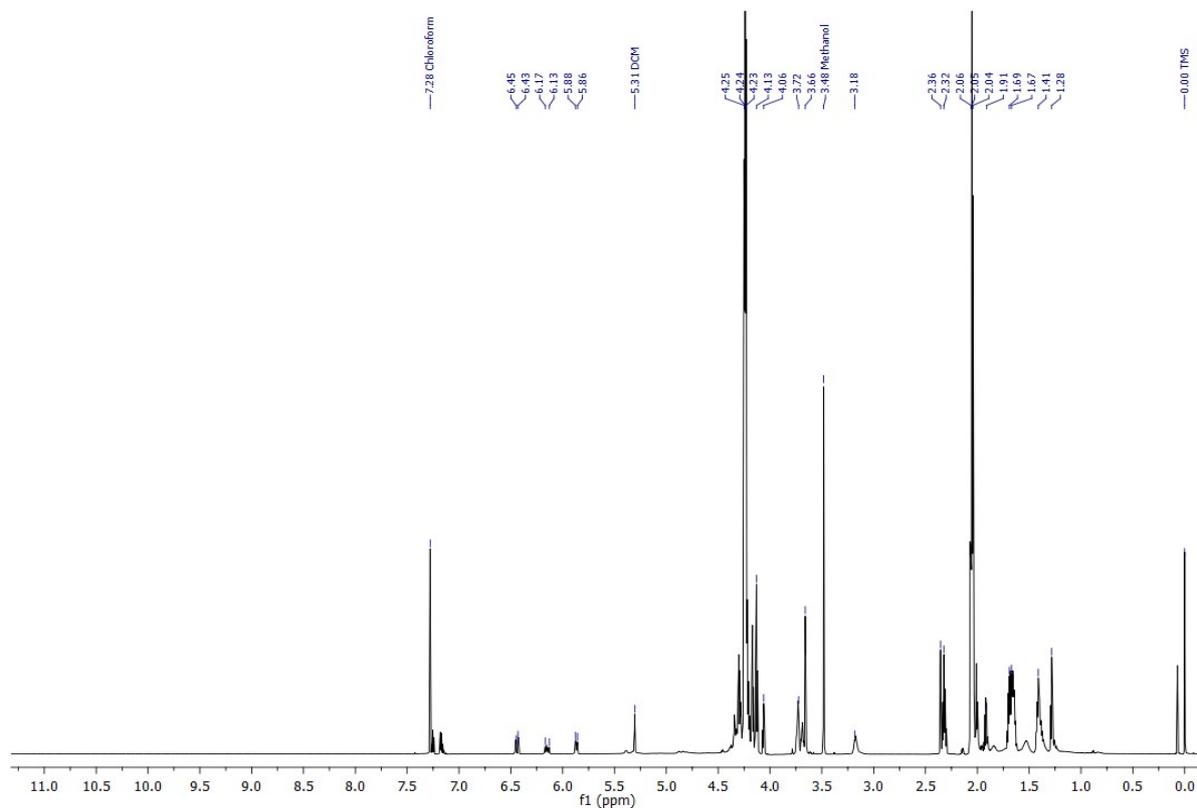


Figure S 14. $^1\text{H-NMR}$ of PCTAc 90:10 in CDCl_3 .

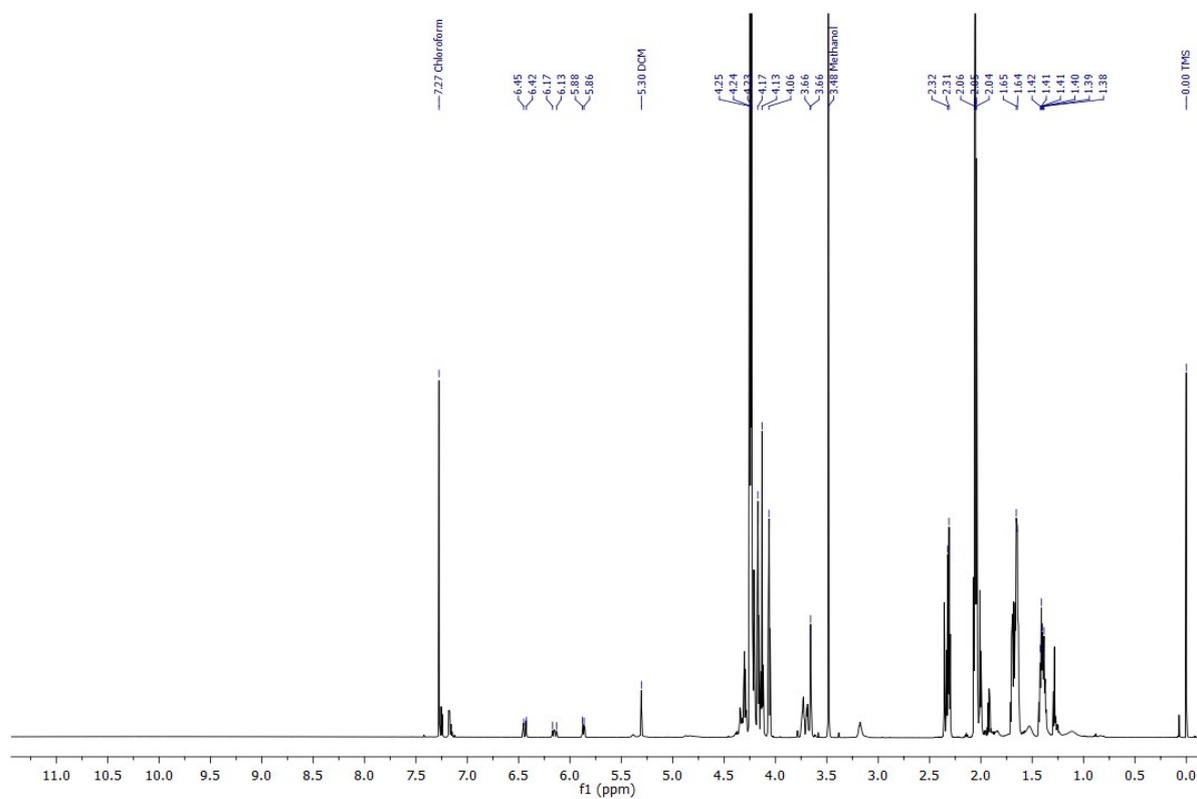


Figure S 15. $^1\text{H-NMR}$ of PCTAc 25:75 in CDCl_3 .

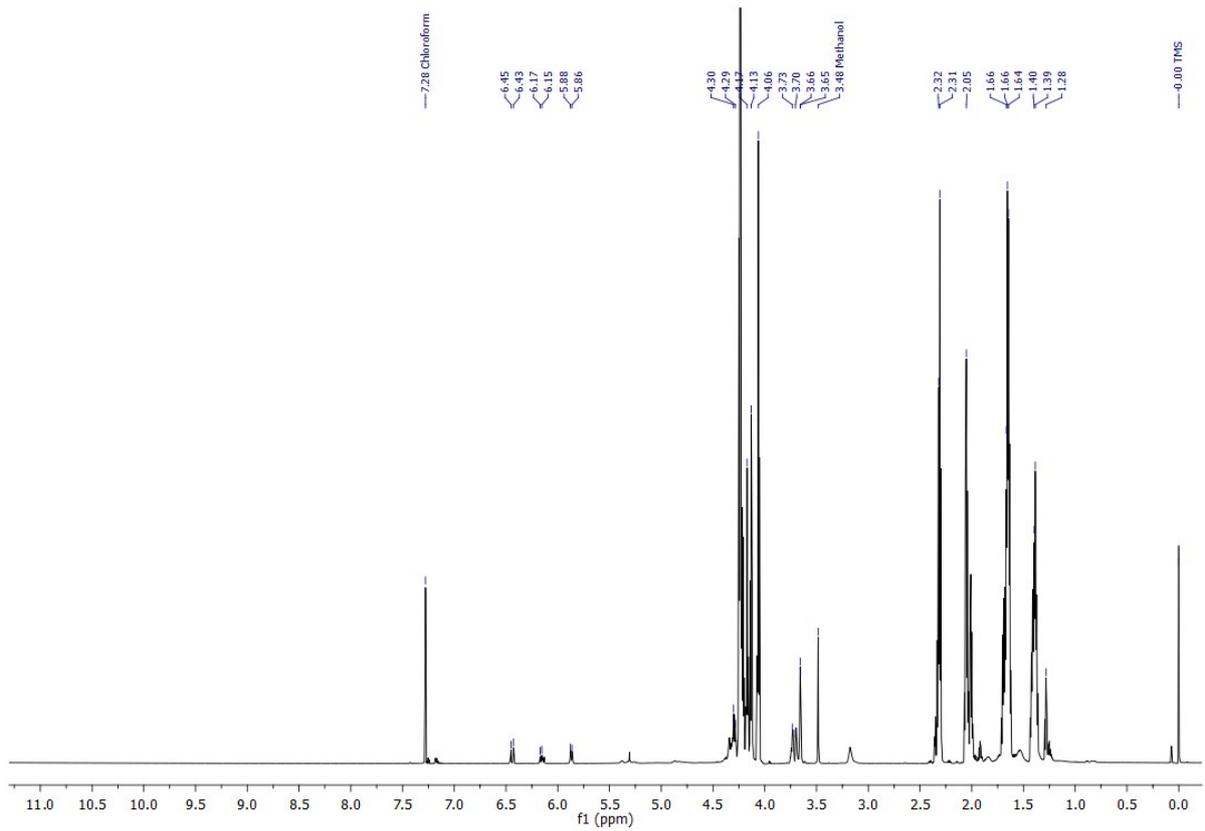


Figure S 16. $^1\text{H-NMR}$ of PCTAc 50:50 in CDCl_3 .

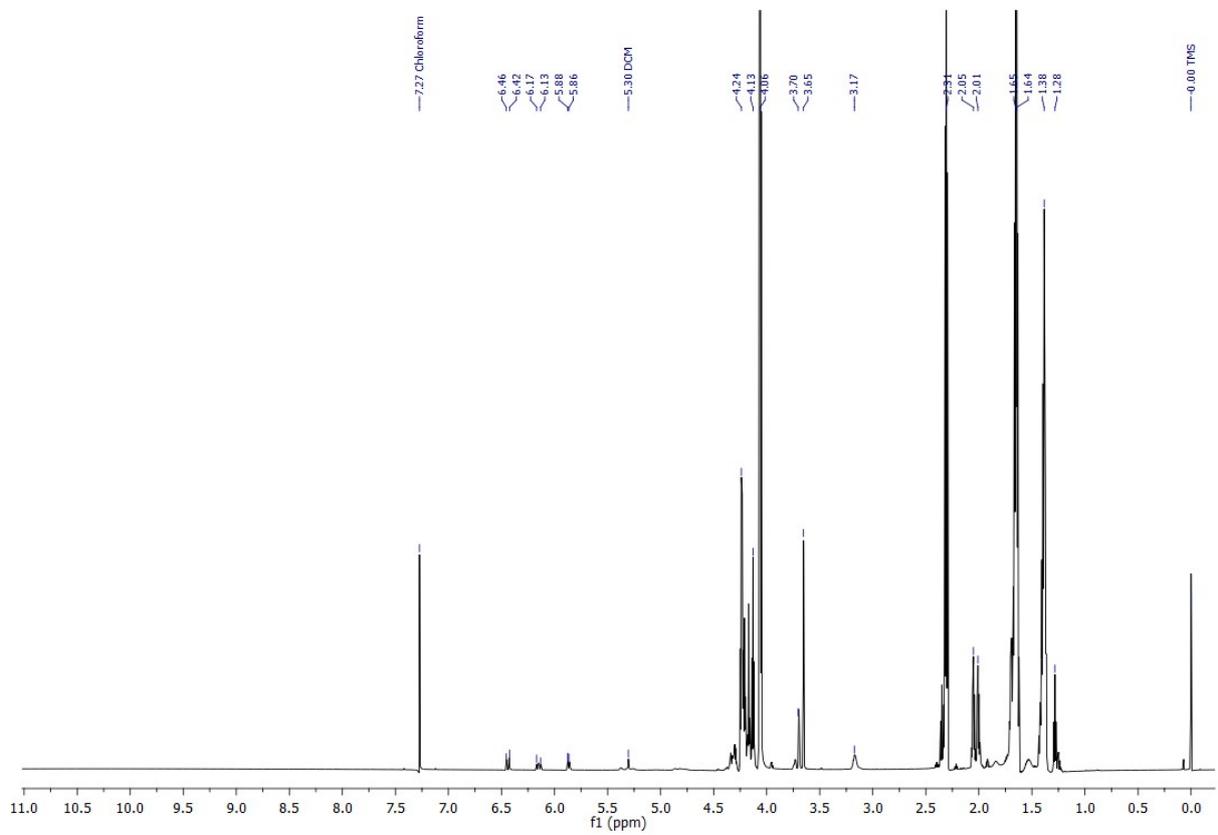


Figure S 17. $^1\text{H-NMR}$ of PCTAc 75:25 in CDCl_3 .

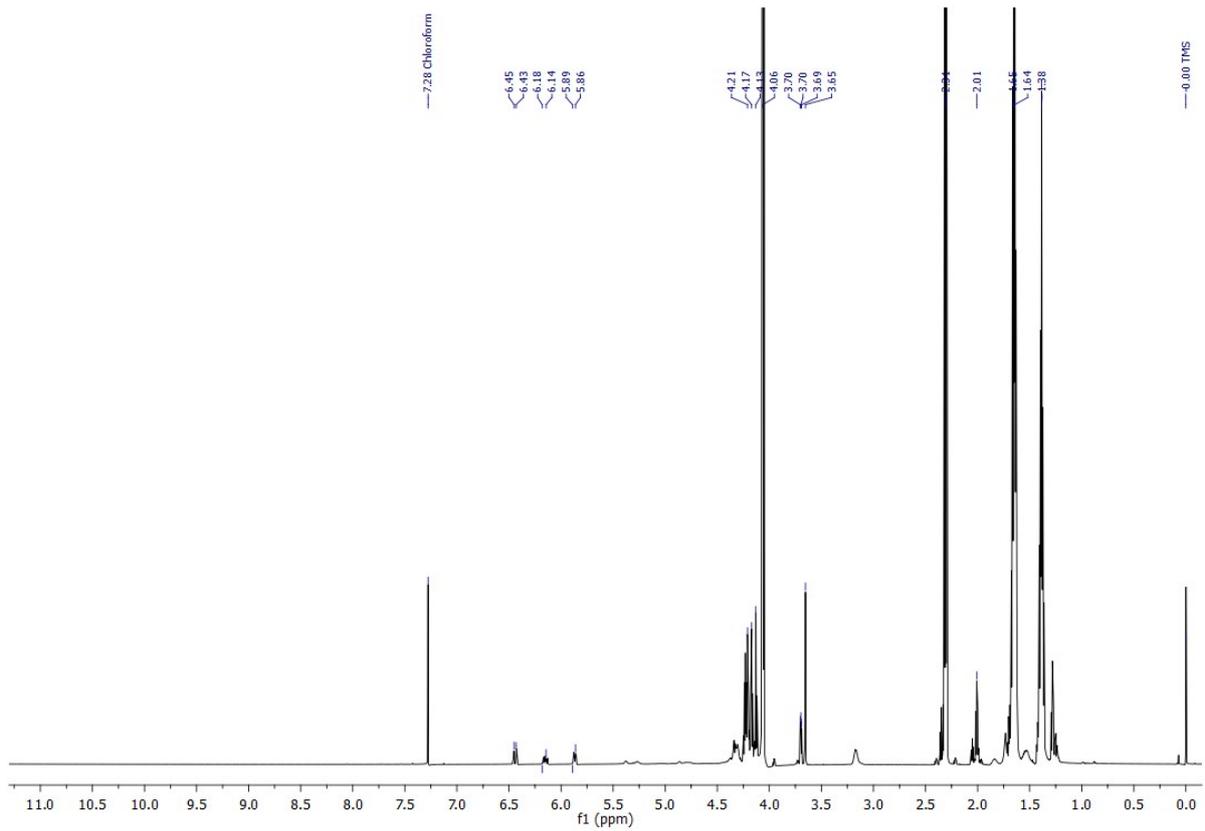


Figure S 18. ¹H-NMR of PCTAc 90:10 in CDCl₃.

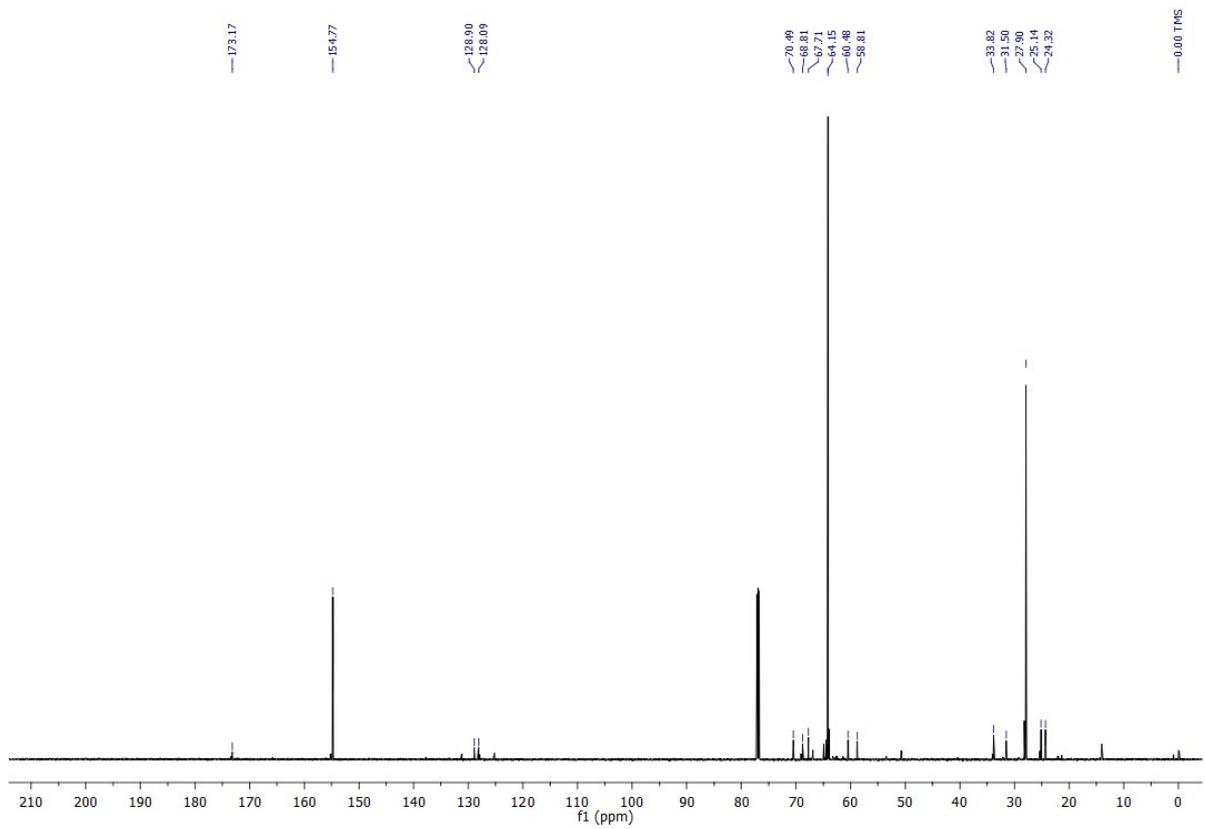


Figure S 19. ¹³C-NMR of PCTAc 10:90 in CDCl₃.

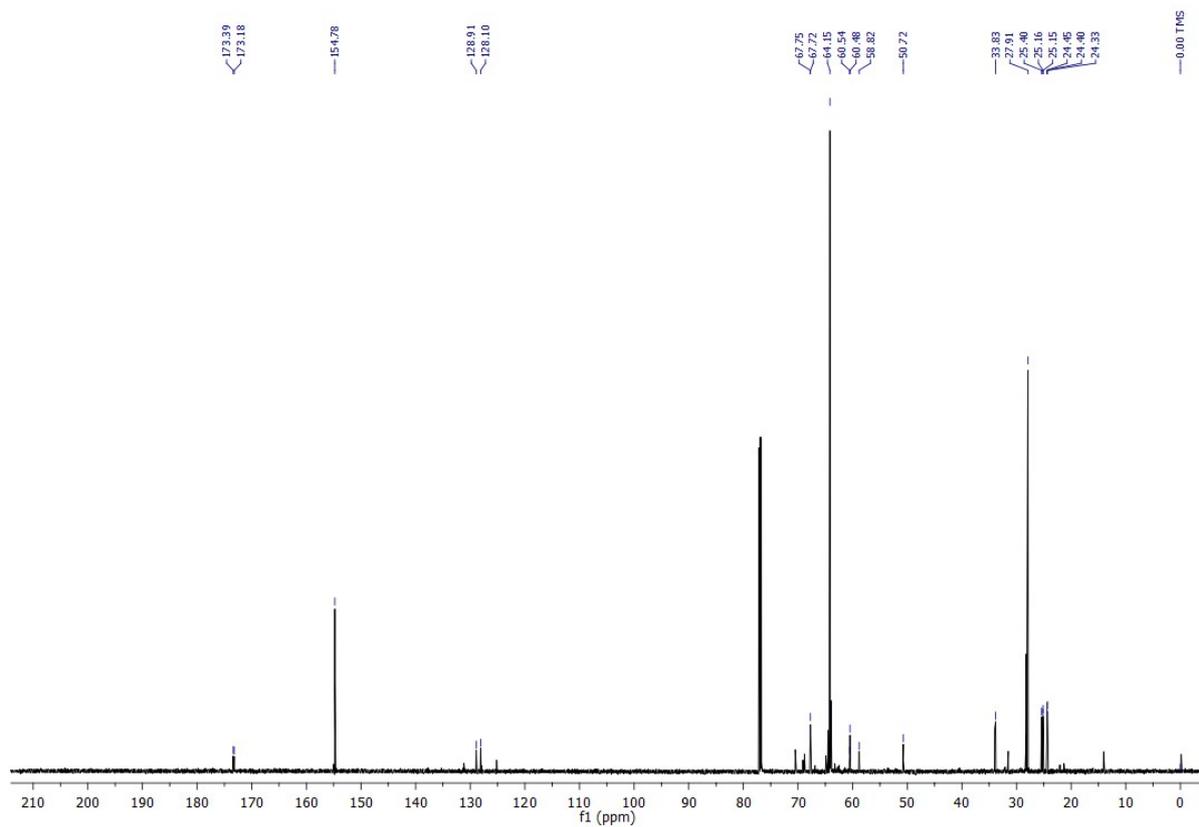


Figure S 20. ^{13}C -NMR of PCTAc 25:75 in CDCl_3 .

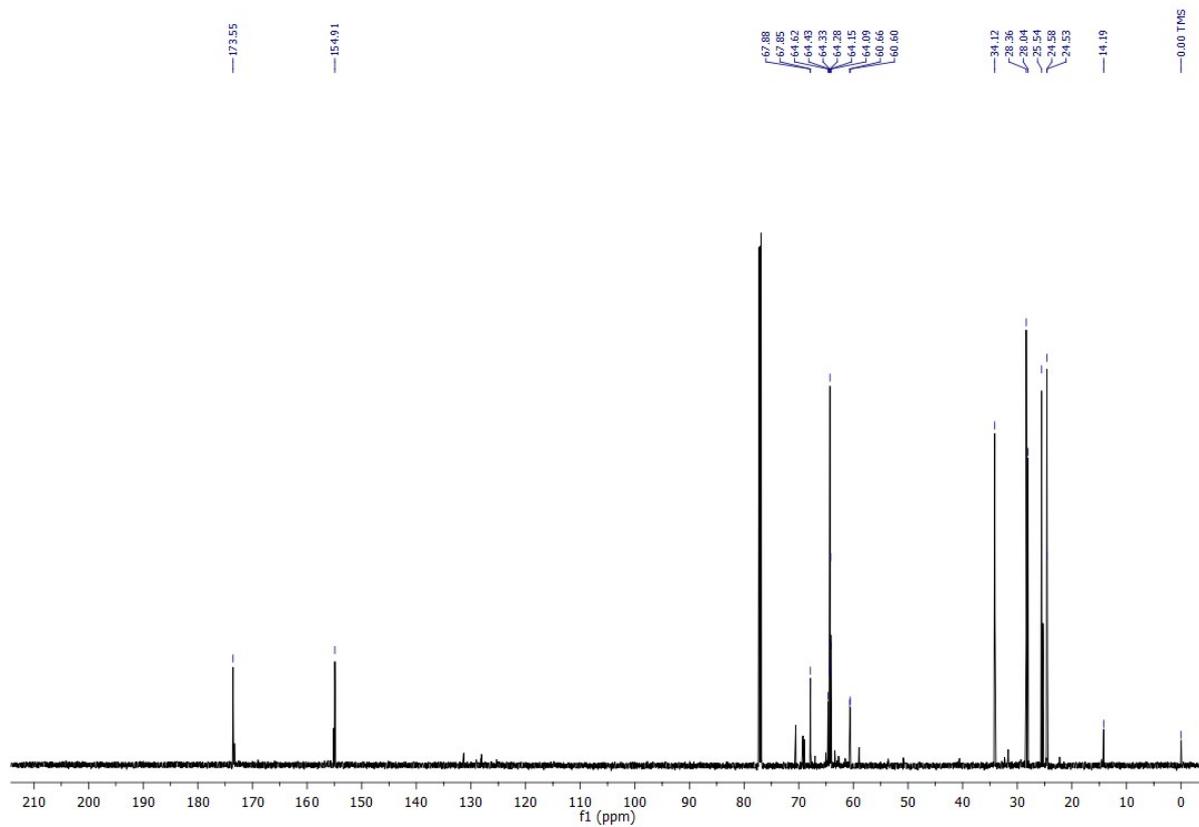


Figure S 21. ^{13}C -NMR of PCTAc 50:50 in CDCl_3 .

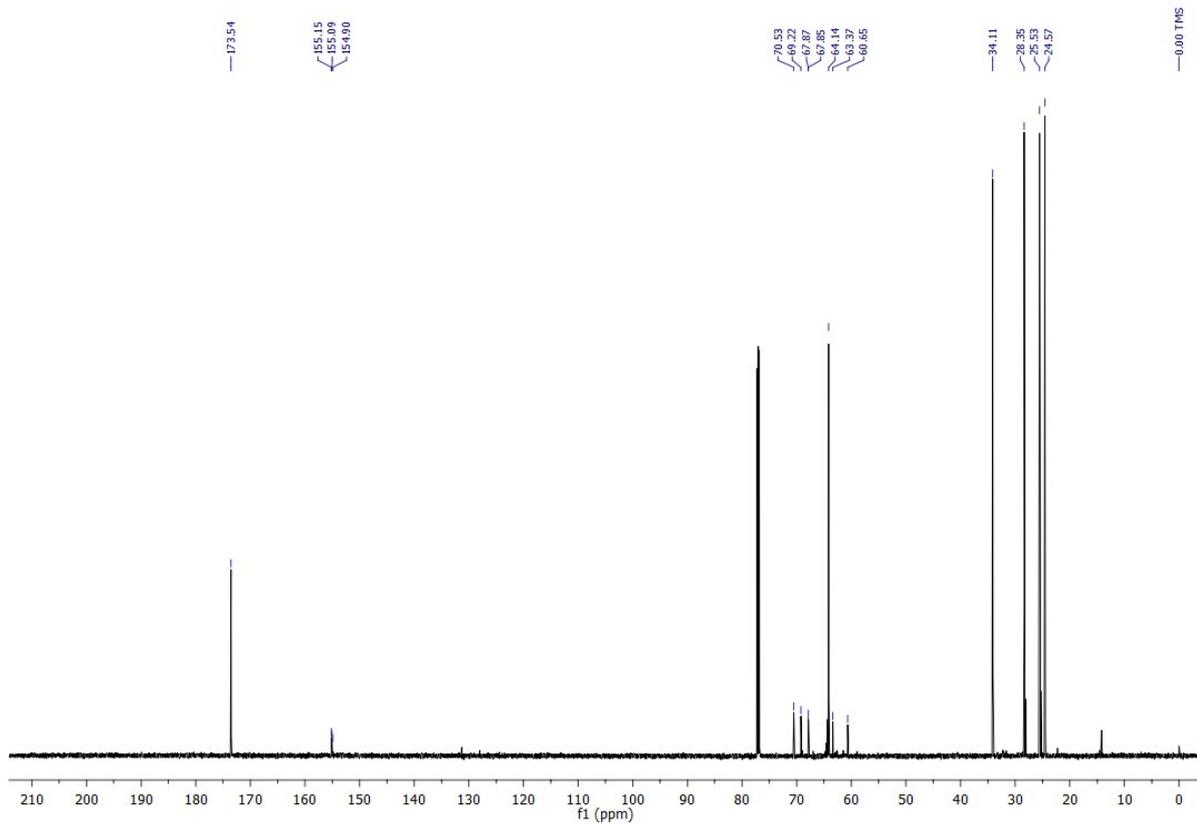


Figure S 22. ^{13}C -NMR of PCTAc 75:25 in CDCl_3 .

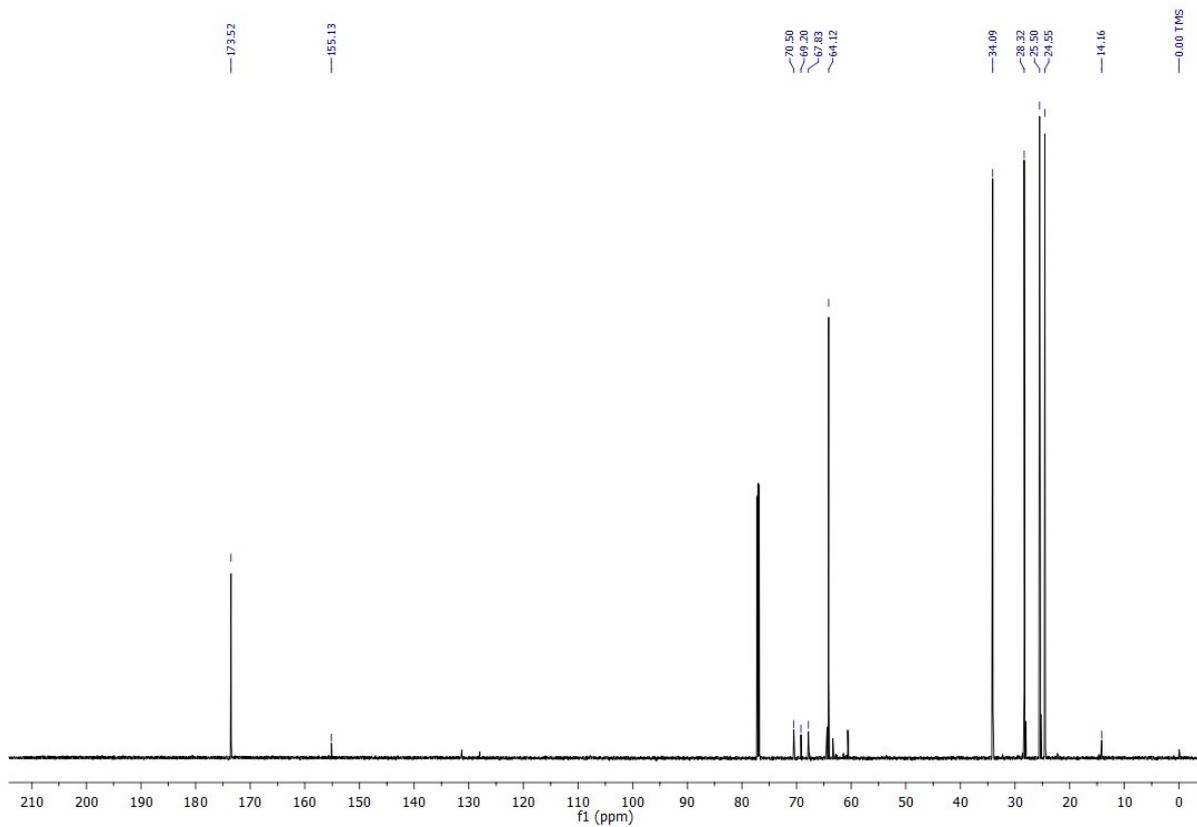


Figure S 23. ^{13}C -NMR of PCTAc 90:10 in CDCl_3 .

IR spectra of PCT and PCTAc resins

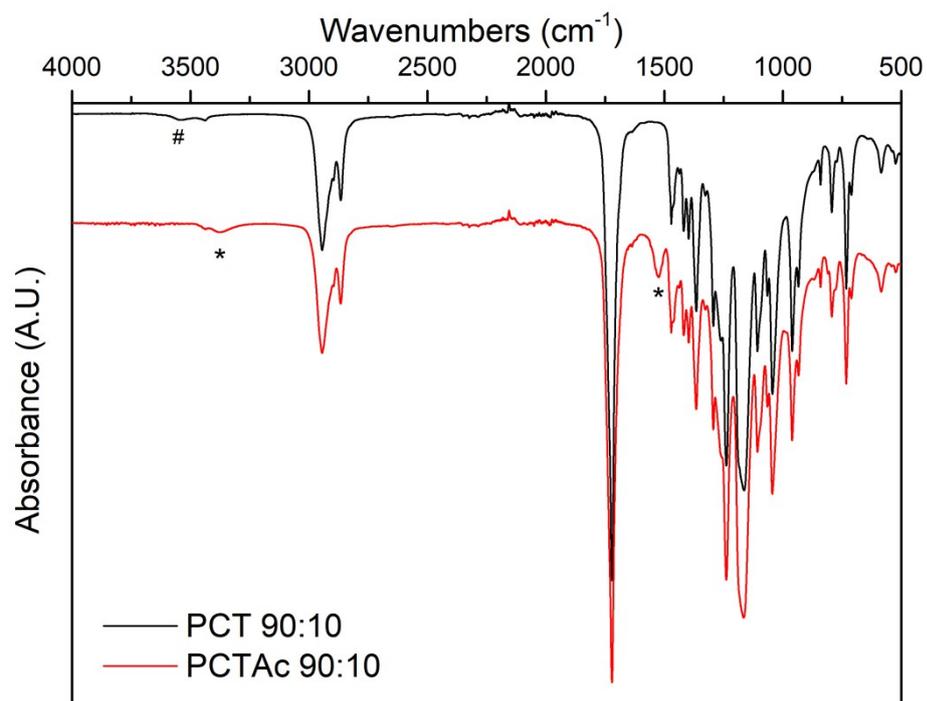


Figure S 24. IR of PCT 90:10 prepolymer and PCTAc 90:10 resin, # indicates the OH bond at 3550 cm^{-1} and * the NH bonds at 3300 cm^{-1} and 1520 cm^{-1} .

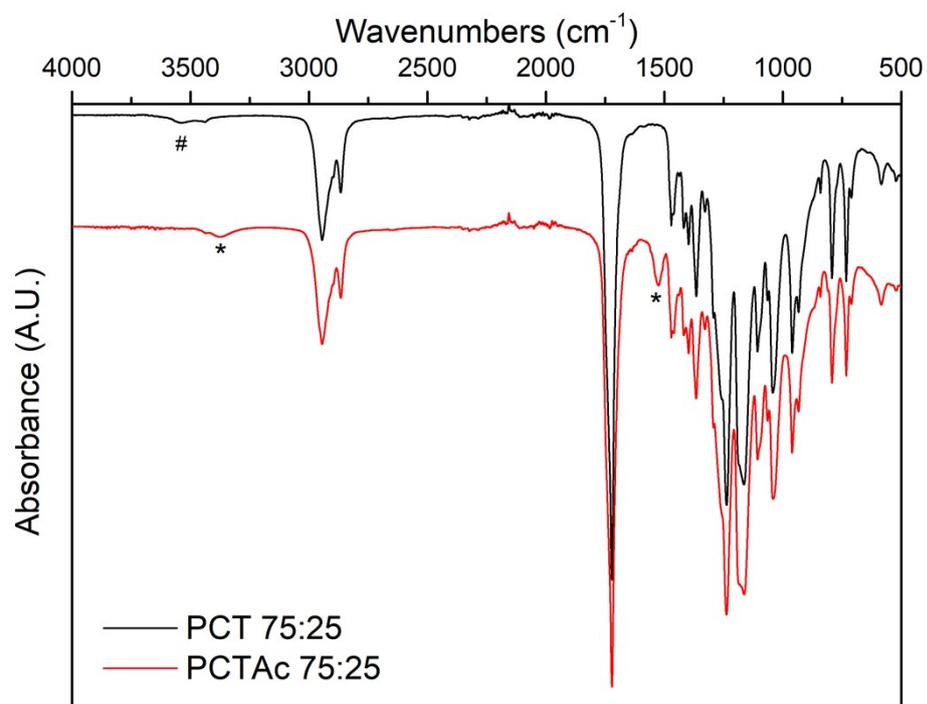


Figure S 25. IR of PCT 75:25 prepolymer and PCTAc 75:25 resin, # indicates the OH bond at 3550 cm^{-1} and * the NH bonds at 3300 cm^{-1} and 1520 cm^{-1} .

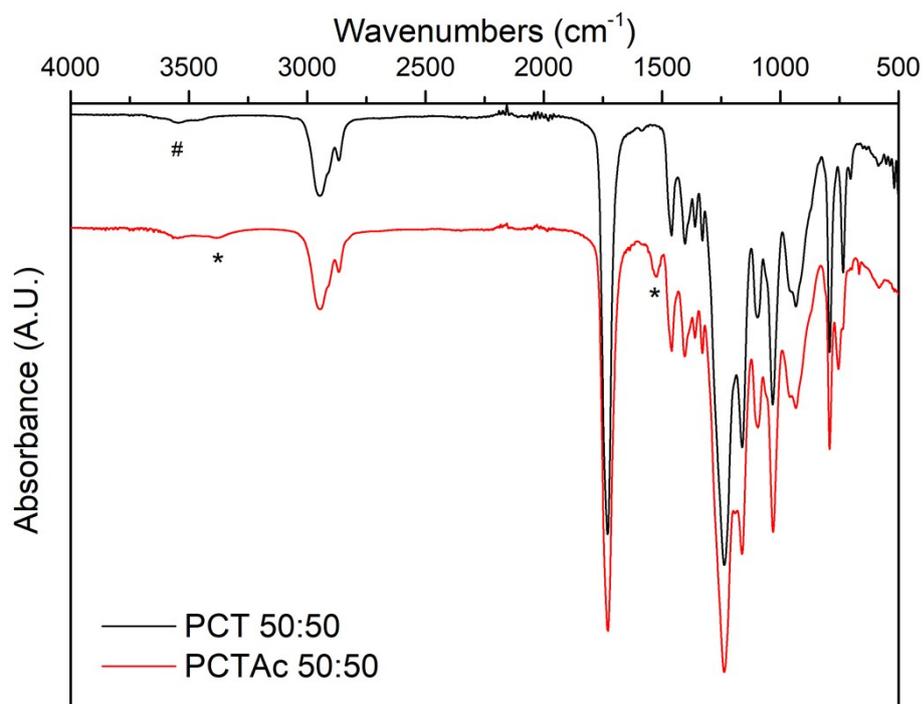


Figure S 26. IR of PCT 50:50 prepolymer and PCTAc 50:50 resin, # indicates the OH bond at 3550 cm^{-1} and * the NH bonds at 3300 cm^{-1} and 1520 cm^{-1} .

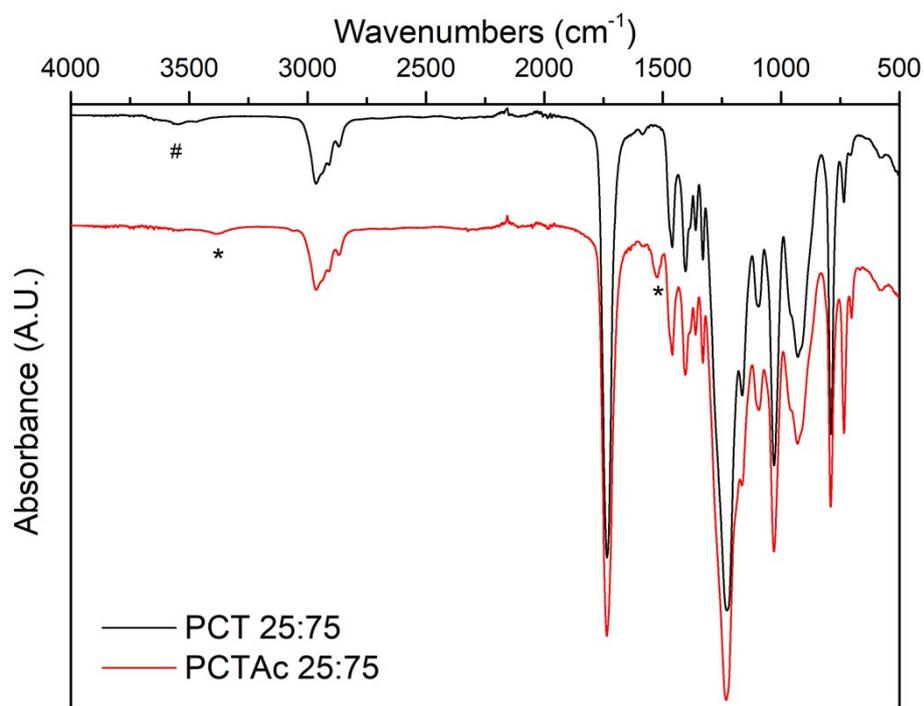


Figure S 27. IR of PCT 25:75 prepolymer and PCTAc 25:75 resin, # indicates the OH bond at 3550 cm^{-1} and * the NH bonds at 3300 cm^{-1} and 1520 cm^{-1} .

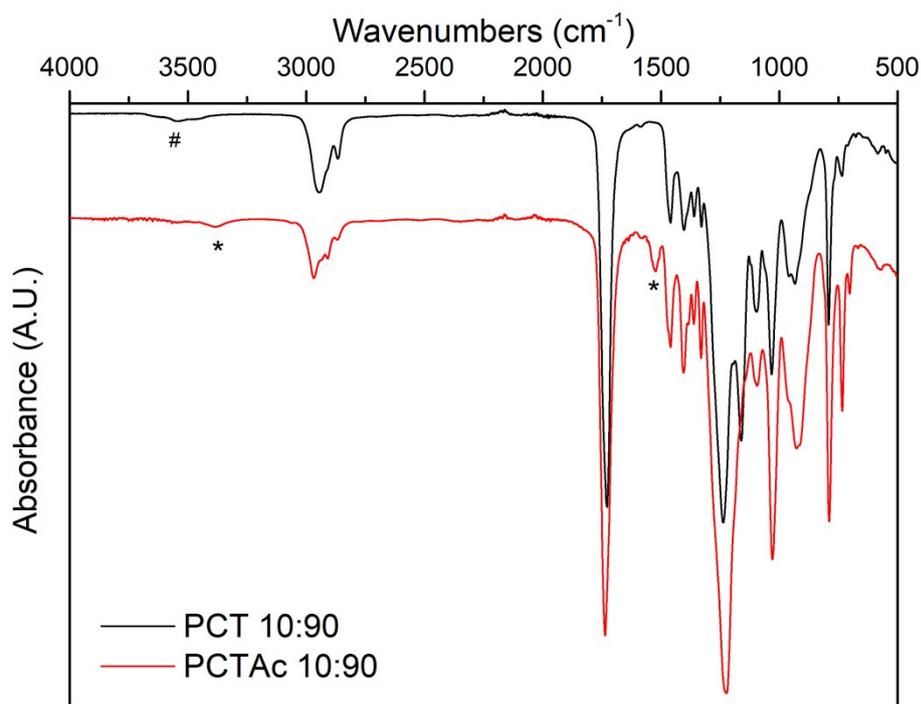


Figure S 28. IR of PCT 10:90 prepolymer and PCTAc 10:90 resin, # indicates the OH bond at 3550 cm^{-1} and * the NH bonds at 3300 cm^{-1} and 1520 cm^{-1}

GPC Traces of PCT and PCTAc

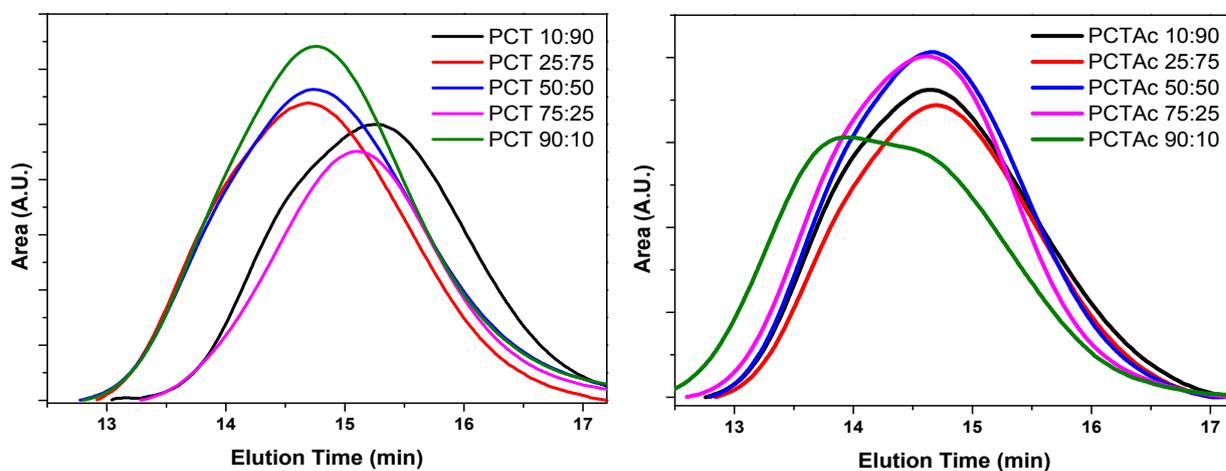


Figure S 29. GPC traces of a) PCT prepolymers and b) PCTAc showing the different weight distributions

Table S1. Determined composition, molecular weight distributions and monomer conversion for different compositions of PCT prepolymers.

Practical copolymer	Monomer
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Entry	CL:TMC	composition ^a		M_n (g·mol ⁻¹)			conversion ^b	
		CL (mol%)	TMC (mol%)	Theory	¹ H-NMR ^a	\bar{D} ^c	CL (%)	TMC (%)
1	90:10	91	9	4000	3965	1.23	99.37	88.40
2	75:25	77	23	4000	3700	1.27	99.85	91.00
3	50:50	49	51	4000	2950	1.31	99.92	84.90
4	25:75	27	73	4000	3400	1.40	99.94	95.50
5	10:90	11	89	4000	2990	1.54	99.98	95.80

a) Calculated by ¹H-NMR, b) calculated by ¹H-NMR, c) estimated via GPC (standard)

Mechanical data

Table S2. Tensile properties of photocured PCTAc resins prepared with 5 different ratios of PCTAc. Values are averaged with \pm standard deviation.

Entry	CL:TMC	E-modulus	σ_{max}	σ_{yield}	ϵ_{yield}	ϵ_{break}
		(MPa)	(MPa)	(MPa)	(%)	(%)
1	90:10	61.0 \pm 0.1	5.4 \pm 0.3	4.9 \pm 0.2	24.0 \pm 3.7	251.0 \pm 21.2
2	75:25	13.8 \pm 0.5	1.6 \pm 0.3	0.4 \pm 0.1	3.5 \pm 0.3	66.1 \pm 26.3
3	50:50	0.6 \pm 0.0(3)	0.2 \pm 0.0(2)	0.1 \pm 0.0(2)	5.1 \pm 0.2	53.0 \pm 2.4
4	25:75	1.2 \pm 0.0(2)	0.7 \pm 0.1	0.1 \pm 0.0(3)	9.1 \pm 0.7	78.2 \pm 10.4
5	10:90	1.6 \pm 0.1	0.2 \pm 0.0(1)	0.2 \pm 0.0(1)	8.4 \pm 0.1	152.3 \pm 12.0

Table S3. Tensile properties of DLP printed PCTAc resins prepared with 5 different ratios of PCTAc. Values are averaged with \pm standard deviation.

Entry	CL:TMC	E-modulus (MPa)
1	90:10	31.1 \pm 3.7

2	75:25	17.1 ± 1.6
3	50:50	0.20 ± 0.03
4	25:75	$0.06 \pm 0.00(8)$
5	10:90	0.30 ± 0.04

Accelerated degradation test

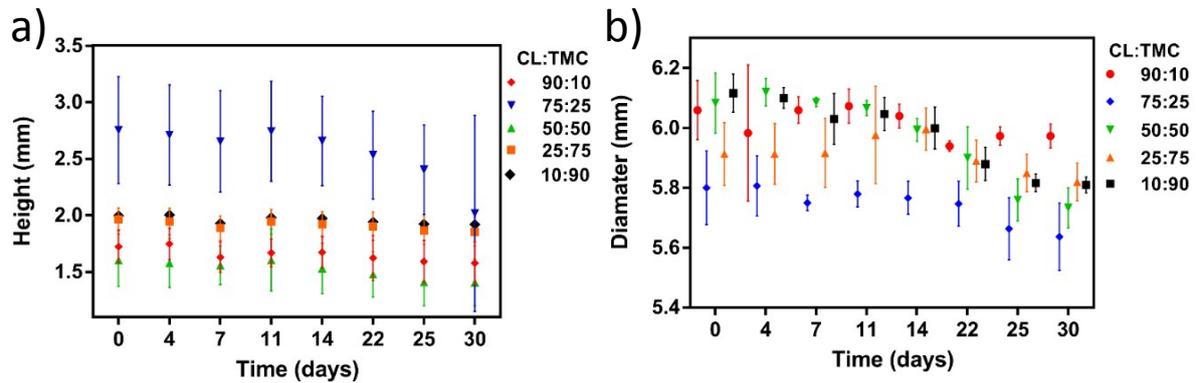


Figure S 30. Study of dimensional variation of the samples in height a) and diameter b). The graphs show no significant variation of the samples during the 30 days.

Swelling

Samples of each printed resin were immersed in 1,5 mL of PBS and kept at 37°C. After 24 h the samples were taken out dried with tissue paper and measured. Results are shown in figure S 31 and swelling % is reported as the % increase in mass.

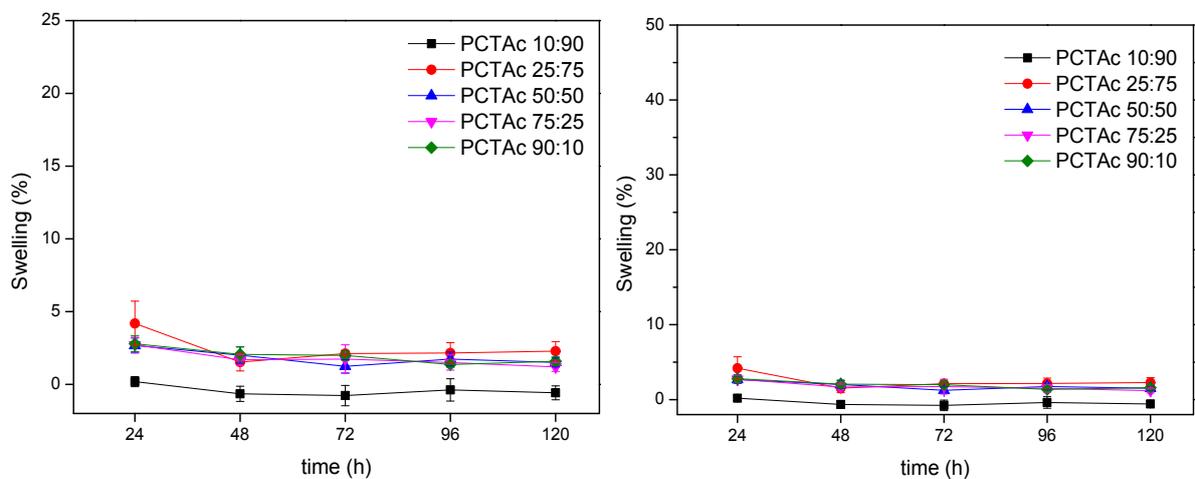


Figure S 31. Swelling over 120 h at 37°C in PBS the films show no significant swelling.