Surface grafted poly(ε-caprolactone) prepared using organocatalysed ring-opening polymerisation followed by SI ATRP

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Supplementary Information

Materials

Unless otherwise stated, all chemicals were purchased from Sigma Aldrich and used as received. All transfers of compounds for ROP (including solvents) were performed under an inert nitrogen atmosphere. ε-Caprolactone (ε-CL) and benzyl alcohol were freshly distilled into sealable ampoules from CaH_2 under an inert atmosphere and stored in a glove box at ambient temperature.

Diphenyl phosphate (DPP) was powdered in a mortar and pestle and subsequently dried over P_2O_5 in a vacuum desiccator for one week, with the P_2O_5 replaced every day. The solid was stored in a glove box at ambient temperature. The white crystalline γ-BMPCL monomer, which was prepared according to a previously reported literature procedure (Mecerreyes D. *et al, Macromolecules*, 1999, 32, 5175-5182), was taken up in anhydrous dichloromethane (CH_2Cl_2), transferred by cannula onto activated 3 Å molecular sieves and left to stand for 24 h before being transferred for a second time onto fresh 3 Å sieves and left to dry for a further 24 h. The CH_2Cl_2 solution was then transferred to a dried Schlenk flask using a filter cannula and the CH_2Cl_2 removed under vacuum on a vacuum manifold. The solid was left to dry under vacuum overnight. The resulting white solid was then transferred to a dry container and stored in a glove box at ambient temperature.

Benzyl alcohol was distilled under reduced pressure, degassed and stored over 4 Å molecular sieves. Monomer and dried over 3 Å molecular sieves in dry CH_2Cl_2. Deuterated chloroform ($CDCl_3$) was purchased from Apollo Scientific Ltd was dried over 3 Å molecular sieves, flash distilled, degassed and back-filled with nitrogen, before being stored in a glove box at ambient temperature.

CH_2Cl_2 and toluene were purified over Innovative Technology SPS alumina solvent columns and degassed before use. A weak basic anion exchange resin, Amberlyst A21 (Organo Co., Ltd.) was washed with MeOH and dried in a vacuum oven overnight before use.

Cu(I)Br was purified by washing 3× with glacial acetic acid, 2× with absolute ethanol and 2× with diethyl ether followed by drying in a vacuum oven overnight.

General Considerations and Analysis

Ring-opening polymerisations were performed in a moisture and oxygen controlled glovebox (specifically <0.1ppm H_2O). The SI grafting procedure was conducted in an oxygen controlled glovebox.

^1H and ^{13}C NMR spectra were recorded on a Bruker DPX-400, AC-400, or DRX-500 spectrometer at 298 K. Chemical shifts are reported as δ in parts per million (ppm) and referenced to the chemical shift of the residual solvent resonances ($CHCl_3$: ^1H δ = 7.26 ppm; ^{13}C δ = 77.16 ppm).

Size exclusion chromatography (SEC) was conducted on a system composed of a Varian 390-LC Multi detector suite fitted with differential refractive index, light scattering, and ultraviolet detectors equipped with a guard column (Varian Polymer Laboratories PLGel 5 μM, 50 × 7.5 mm) and two mixed D columns (Varian Polymer Laboratories PLGel 5μM, 300 × 7.5 mm). The mobile phase was $CHCl_3$ (HPLC grade) with 0.5% TEA, at a flow rate of 1.0 mL min^{-1}. SEC samples were calibrated against Varian Polymer Laboratories Easi-Vials linear polystyrene standards (162 - 2.4 × 10^5 g mol^{-1}) using Cirrus v3.3 software.

X-ray Photoelectron Spectroscopy (XPS) analysis was performed using an AXIS Ultra DLD spectrometer (Kratos Analytical Inc., Manchester, UK) with a monochromated Al Kα source at a power of 150 W (15 kV × 10 mA), a hemispherical analyser operating in the fixed analyser transmission mode and the standard aperture (analysis area: 0.3 mm × 0.7 mm) The total pressure in the main vacuum chamber during analysis was typically between 10^{-9} and 10^{-8} mbar. Charging of the samples during irradiation was reduced by an internal flood gun, coupled with a magnetic immersion lens. Survey spectra were acquired at a pass energy of 160 eV, while high resolution spectra were recorded from individual peaks at 20 eV pass energy. Each specimen was analysed at an emission angle of 0° as measured from the surface normal. Under these conditions the XPS analysis depth typically ranges between 5 and 10 nm depending on the element.

Data processing for XPS was performed using CasaXPS processing software version 2.3.15 (Casa Software Ltd., Teignmouth, UK). The atomic concentrations of the detected elements were calculated using integral peak intensities and the sensitivity factors supplied by the manufacturer. Curve fitting was performed using a simplex algorithm where residuals were minimised with multiple iterations. Binding energies were referenced to the aliphatic hydrocarbon peak at 285.0 eV.

Static water contact angle testing was performed using a PG-X portable goniometer. Each sample was measured 5 times over the course of 30 seconds and averages calculated for each sample. Standard deviations were calculated from the five measurements taken.

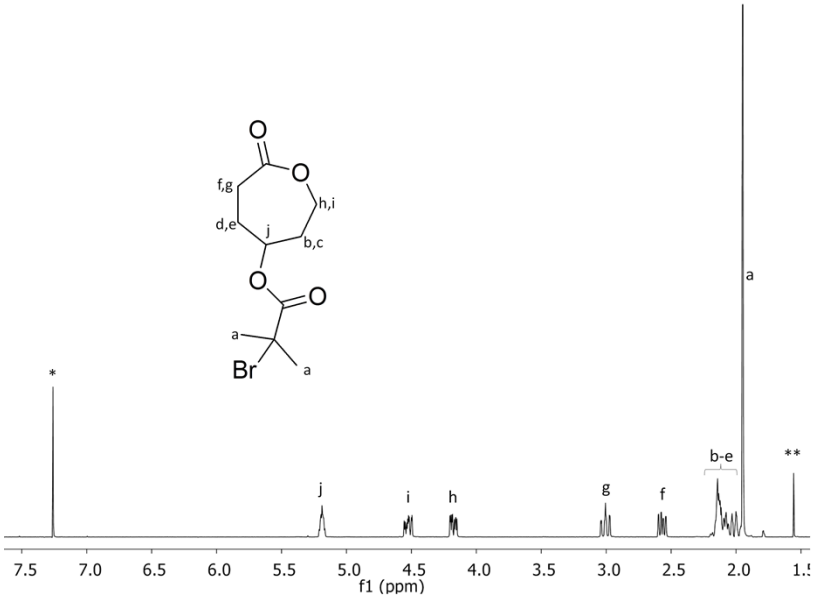
Synthesis of γ-BMPCL monomer:

(4-Hydroxycyclohexyl)-2-bromo-2-methylpropionate: 1,4-Cyclohexanediol (16.0 g, 138 mmol) and triethylamine (39.9 g, 206.6 mmol) were dissolved in dry THF (150 mL) under nitrogen. 2-Bromoisobutyryl bromide (31.7 g, 138 mmol) was then slowly added dropwise whilst cooling in ice. The mixture was left to stir under ice cooling for an hour and then left to stir at room temperature for a further 6 h. The mixture was then filtered and the precipitate washed several times with THF. The filtrate was collected and the THF was then removed by evaporation. CH_2Cl_2 (150 mL) was then added, and the solution was washed with dilute HCl (1 M) (2×), H_2O (1×) and saturated brine (1×) before being dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified using a short column of SiO_2 gel, firstly using DCM, to remove the bis-acylated by-product and then the product was eluted out using diethyl ether. Product elution was followed with thin layer chromatography (TLC). A second purification was performed to remove traces of bis-acylated by-product using SiO_2 gel column chromatography (using hexane/EtOAc gradient as eluent, 20-50% of EtOAc). The product was isolated as a clear oil after removal of solvent. Yield: 14.0 g (40%). ^{1}H NMR (mixture of isomers) ($CDCl_3$): δ 1.38-2.01 (m, 14H, 4× CH_2 and 2× CH_3), 3.65-3.83 (m, 1H, -C*H*OH-), 4.75-4.83 (m, 1H, -CHO-). ^{13}C NMR ($CDCl_3$): δ 27.29, 27.46, 30.33, 30.66, 30.70, 31.37, 56.26, 56.37, 68.15, 71.16, 73.20, 170.98, 171.11.

(4-Ketocyclohexyl)-2-bromo-2-methylpropionate: Pyridinium chlorochromate (PCC) (12.9 g, 60 mmol) was added to a solution of (4-hydroxycyclohexyl)-2-bromo-2-methylpropionate (13.2 g, 49 mmol) in CH_2Cl_2. After the mixture had been stirred for 3 h, anhydrous ethyl ether (200 mL) was added. The obtained mixture was filtered through silica gel. The yellow solution was then evaporated and purified using column chromatography (silica gel using hexane/EtOAc gradient as eluent; 10-50 % EtOAc). The product was isolated as a white crystalline powder. Yield: 8.4 g (65%). ^{1}H NMR ($CDCl_3$): δ 1.96 (s, 6H, 2× CH_3), 2.02-2.67 (m, 8H, 4× CH_2), 5.19-5.23 (m, 1H, -CHO-). ^{13}C NMR (CDCl3): δ 30.03, 30.72, 37.08, 56.09, 69.97, 170.93, 209.59.

(2-Bromo-2-methylpropionyl)-ε-caprolactone (γ-BMPCL): (4-Ketocyclohexyl)-2-bromo-2-methylpropionate (6.9 g, 26 mmol) was dissolved in 50 mL of $CHCl_3$ and added dropwise into a solution of 3-chloroperoxybenzoic acid (70%) (14.0 g, equivalent to 56.8 mmol) in $CHCl_3$ (70 mL). The mixture was stirred for 24 h and then filtered over celite. The obtained yellow solution was washed twice with $NaHCO_3$ (2 M) and brine (1×). The extracted product was then purified by column chromatography (silica gel using hexane/EtOAc gradient as eluent; 10-50 % EtOAc). The product was then passed through a basic alumina column to remove any co-eluted chloroperoxybenzoic acid. The crude monomer was recrystallized from

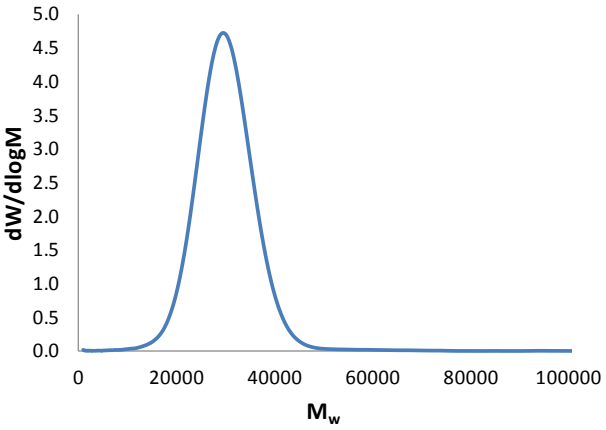
anhydrous ethyl ether to give the product as a white crystalline powder. Yield: 4.7 g (65%). ^{1}H NMR ($CDCl_3$): δ 1.95 (s, 6H, 2×CH_3), 1.98-2.20 (m, 4H, 2×CH_2-), 2.50-2.6 (m, 1H, -CH_2CO-), 2.97-3.04 (m, 1H, -CH_2CO-), 4.15-4.20 (m, 1H, -CH_2O-), 4.49-4.56 (m, 1H, -CH_2CO-), 5.17-5.21 (m, 1H, -CH-). ^{13}C NMR ($CDCl_3$): δ 27.36, 28.56, 30.83, 33.76, 56.14, 63.45, 71.47, 170.27, 175.12.



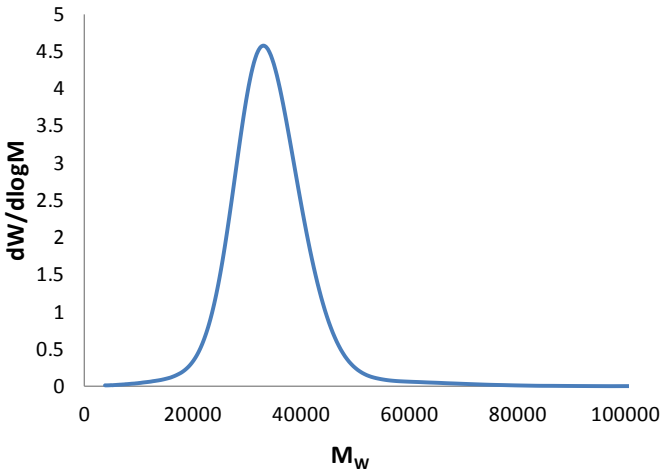
General Ring Opening Polymerization method

DPP was added to a solution of ε-CL and γ-BMPCL in dry $CDCl_3$. Benzyl alcohol was then added (from a stock solution in dry $CDCl_3$). After the desired amount of time the polymerization was quenched by the addition of basic Amberlyst A21 resin. The resin was removed by filtration and the solvent removed under reduced pressure. The residual monomer and catalyst were removed and the polymer isolated by column chromatography (silica, ethyl acetate: DCM). Polymer sample **2e** was polymerised in toluene and isolated by precipitation into MeOH multiple times.

Representative analysis of poly(γ-BMPCL) homopolymer: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.77 (br s, -$(CH_2)_n$- from end monomer unit), 1.82 - 2.01 (m, 4H × 100, backbone -CH_2- + 6H × 100, pendant -$(CH_3)_2$), 2.30 - 2.46 (m, 2H × 100, backbone -OCOCH_2-), 3.59 – 3.70 (m, 2H, -(CH_2)OH from end monomer unit), 4.02 – 4.23 (m, 2H × 100, backbone -CH_2OCO-), 5.00 - 5.09 (m, 1H × 100, backbone -CH-), 5.11 (overlapping s, 2H, -CH_2Ar end group), 7.35 (br s, 5H, ArH end group); monomer conversion: 27 %; M_n = 28,000 g mol^{-1} (^{1}H NMR estimate); M_n = 27,500 g mol^{-1}, M_w/M_n = 1.05 (SEC CH_3Cl + 0.5% TEA).



Representative analysis of p(γ-BMPCL)-*co*-p(ε-CL) copolymer: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.33 – 1.41 (m, 2H × 106, backbone -CH_2-, ε-CL), 1.59 – 1.68 (m, 4H × 106, backbone -CH_2-, ε-CL), 1.91 (br s, 6H × 19, pendant -$(CH_3)_2$, γ-BMPCL), 1.91 - 2.04 (overlapping m, 4H × 19, backbone -CH_2-, γ-BMPCL), 2.27 - 2.31 (m, 2H × 106, backbone -$OCOCH_2$-, ε-CL), 2.36 – 2.41 (m, 2H × 19, backbone -$OCOCH_2$-, γ-BMPCL), 4.03 - 4.06 (m, 2H × 106, backbone -CH_2OCO-, ε-CL), 4.10 - 4.13 (m, 2H × 19, backbone -CH_2OCO-, γ-BMPCL), 5.02 - 5.09 (m, 1H × 19, backbone -CH-, γ-BMPCL), 5.11 (overlapping br s, 2H, -CH_2Ar, end group), 7.35 (br s, 5H, ArH, end group); Monomer Conversion: 39 % ε-CL and 35 % γ-BMPCL; M_n = 17,500 g mol^{-1} (^{1}H NMR estimate); M_n = 30,400 g mol^{-1}, M_w/M_n = 1.07 (SEC CH_3Cl + 0.5% TEA).



Spin coating of 2e planar substrate for SI Grafting

Br-PCL **2e** was made up at 2% (w/v) in toluene, and spin coated onto ethanol-cleaned polystyrene film. PS film was used to prevent the layer from coming off during grafting. Substrates were cut to fit 48 well plates.

ATRP Surface-Initiated Grafting method

The procedure was adapted from the literature (S. A. Ahmad, G. J. Leggett, A. Hucknall and A. Chilkoti, Biointerphases, 2011, 6, 8-15.). MilliQ^{TM} water (MQ) and methanol were

separately deoxygenated using nitrogen sparging within a glovebox for 30 minutes. Cu(I)Br (3.4 mg, 2.4×10^{-5} mol) and 2,2′-bipryidine (bpy) (7.0 mg, 4.5×10^{-5} mol) were weighed into a glass vial, while oligo(ethylene glycol) methyl ether methacrylate, average M_n 475 g/mol (OEGMA-475) (713 mg, 1.50×10^{-3} mol) was weighed into a separate vial. These were transferred into a glovebox along with Br-PCL substrates. Deoxygenated MQ (0.47 ml) was added to the monomer and this was further sparged with nitrogen for 20 minutes. During this time, substrates were washed for 5 minutes in deoxgenated ethanol and twice in deoxygenated MQ. The OEGMA solution was split into two. To one solution was added 0.94 mL of deoxygenated methanol to form a negative control solution, while to the other was added the Cu(I)Br/bpy in the same volume of deoxygenated methanol. The final concentration of the reagents in the ATRP solution was 500 mM : 16mM : 30mM (PEGMA : CuBr : bpy) in 80:20 volume % methanol:water solution. Solutions were gently pipetted onto the substrates (0.5 mL/well) and the surface grafting reaction was allowed to proceed for 4 hours with gentle shaking. Oxygen levels within the glovebox remained at <0.01% throughout the procedure. The reaction was quenched by removing the substrates from the glovebox, where substrates were rinsed in MQ, soaked in 50 mM ethylenediamine tetraacetic acid disodium salt (Na_2EDTA) (15 minutes) followed by a further 4 MQ rinses. They were then dried under vacuum and stored in a vacuum desiccator.

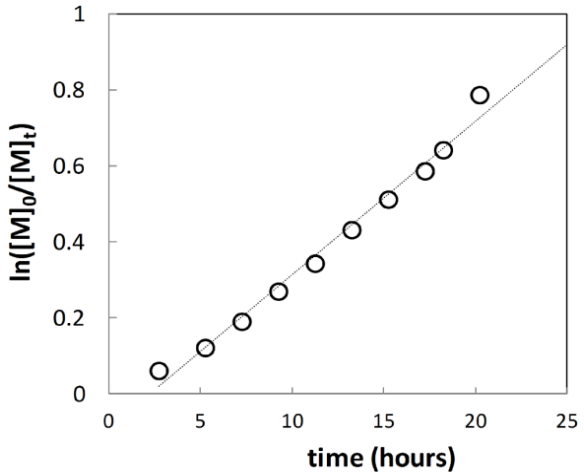


Fig. S1: Kinetic Plot for homopolymerisation of ε-CL at ambient temperature, where $[M]_0:[I]_0$=300:1, $[M]_0$ = 2.5M in $CDCl_3$, where benzyl alcohol = initiator (I); 2 mol% DPP versus monomer. Solid line represents line of best fit plotted for points 3-20 hours

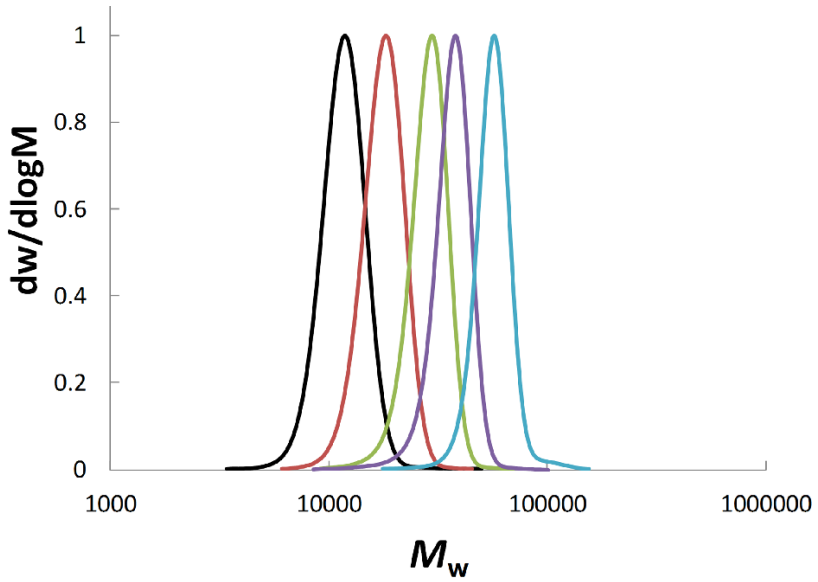
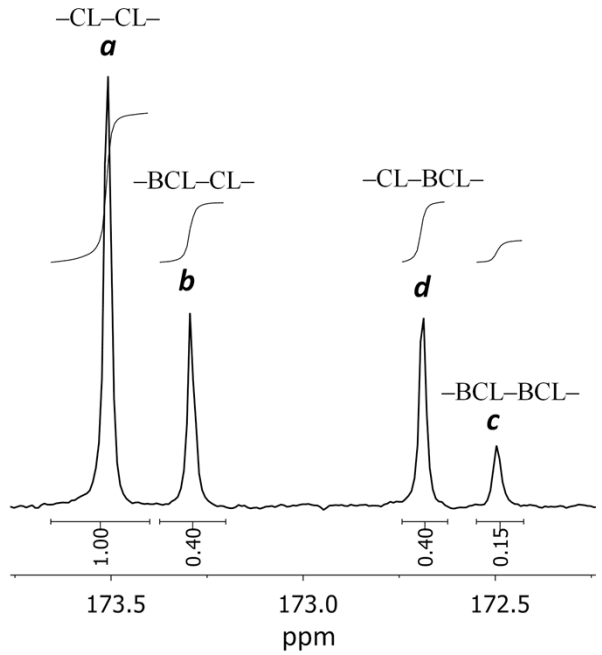


Fig. S2: Evolution of SEC traces during preparation of poly(γ-BMPCL) (polymerisation performed in $CHCl_3$ at ambient temperature, where $[M]_0$:$[I]_0$ = 300:1, $[M]_0$ = 2.5 M in $CDCl_3$, with 2 mol% DPP vs. monomer, benzyl alcohol = initiator (I). % Monomer Conversions for peaks from left to right: 11, 17, 27, 36 and 55%.



$$L_{CL} = \frac{I_{CL-CL}}{I_{BCL-CL}} + 1 = \frac{I_{CL-CL}}{I_{CL-BCL}} + 1$$

$$L_{BCL} = \frac{I_{BCL-BCL}}{I_{BCL-CL}} + 1 = \frac{I_{BCL-BCL}}{I_{CL-BCL}} + 1$$

$$MF_{BCL} = \frac{L_{BCL}}{L_{BCL} + L_{CL}} \qquad MF_{CL} = \frac{L_{CL}}{L_{BCL} + L_{CL}}$$

Fig. S3: ^{13}C spectrum (100 MHz, 298K, $CDCl_3$) showing expanded carbonyl region of copolymer **2a** with integrated signals of the four diads (**a-d**). L_{BCL}= average sequence length of γ-BMPCL; L_{CL}= average sequence length of ε-CL; MF_{BCL}= molar fraction of γ-BMPCL; MF_{CL}= molar fraction of ε-CL. BCL=γ-BMPCL; CL= ε-CL;

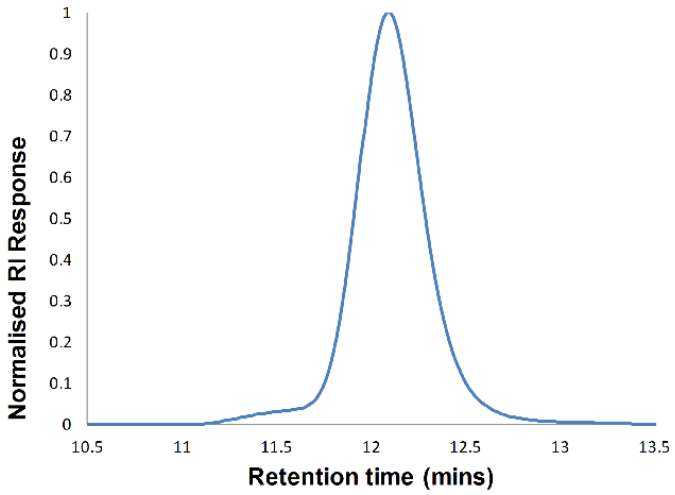


Fig. S4: SEC trace **2e** copolymer (polymerization performed in toluene at ambient temperature, where $[M]_0:[I]_0 = 300:1$, $[M]_0 = 2.5$ M, with 2 mol% DPP vs. monomer, benzyl alcohol = initiator (I).

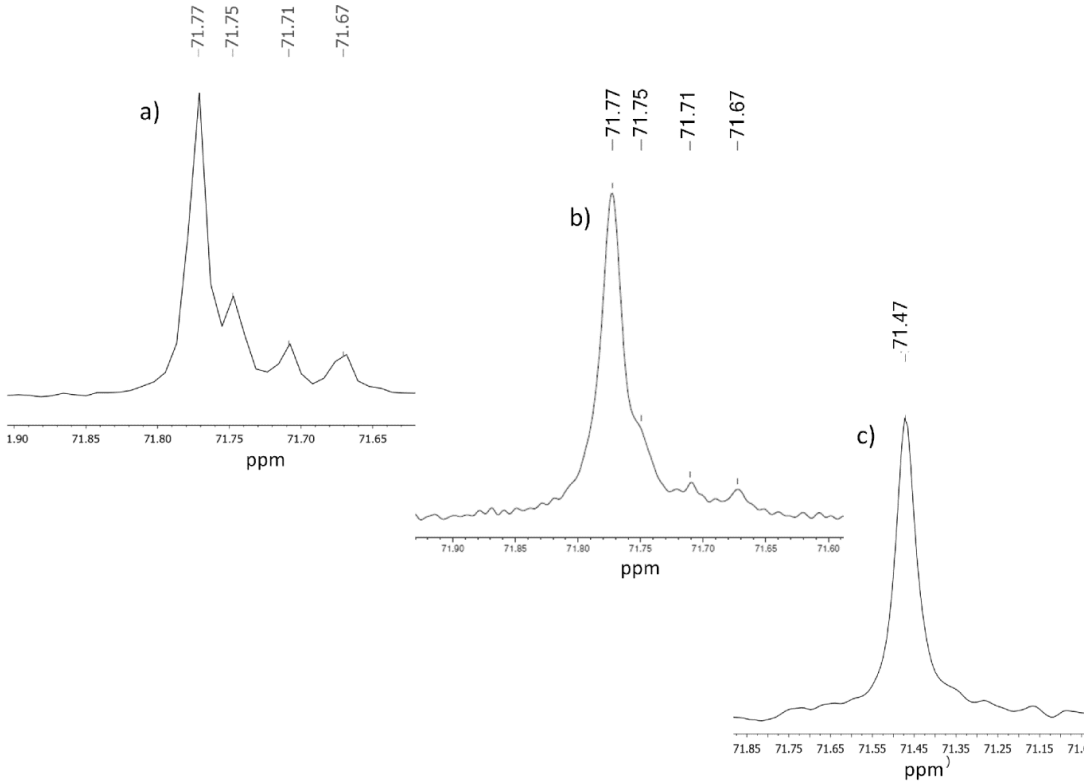


Fig. S5: ^{13}C spectra (100 MHz, 298K, $CDCl_3$) showing expansion of **2a** copolymer (a, top); **2e** copolymer (b, middle) vs. γ-BMPCL monomer (c, bottom).

Table S1: XPS measured elemental composition (%) of Br-PCL ungrafted sample vs. theoretical values.

Peak	Measured	Theoretical*
C 1s	78.9	73.2
O 1s	19.9	25.4
Br 3d	1.0	1.5
Other (Si 2p, Cl 2p)	<0.2	-

*Based on sample **2e** with 13% γ-BMPCL

Table S2: XPS Measured elemental composition of PCL-*g*-p(OEGMA) sample vs. theoretical values based on pure OEGMA composition.

Peak	Measured	Theoretical*
C 1s	70.8	67.7
O 1s	29.0	32.3
Br 3d	0	0
Si 2p	<0.2	0

*Based on OEGMA composition with an average of 8 EG units

Table S3: Measured XPS spectrum vs. theoretical for Br-PCL based on C 1s curve fitting.

Approximate peak position	Species*	Measured (%/total C)	Theoretical** (%/total C)
285	C-C & C-H	57	48
285.7	(O-C=O)-C-C & (O-C=O)-C-Br	13	17
286.6	C-C-O	14	17
287.3	Unknown	3	0
289.1	(O-C=O)-C	13	17
291	Aromatic shakeup (PS)	0.8	0

* The carbon being examined is underlined
** Based on sample **2e** with 13% γ-BMPCL

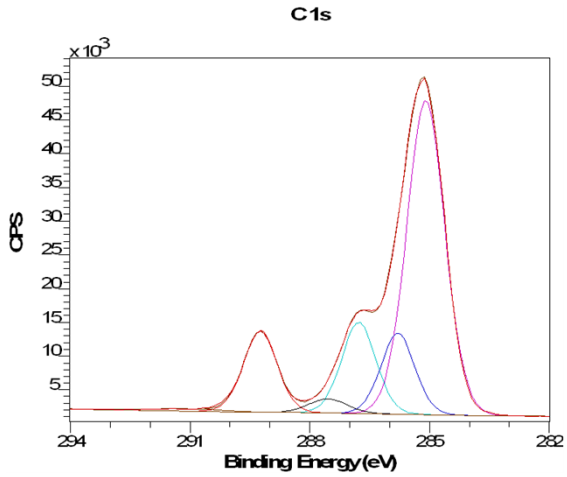


Fig. S6: XPS high intensity C 1s spectrum of Br-PCL with curve fitting

Table S4: Measured XPS spectrum vs. theoretical for PCL-*g*-p(OEGMA) based on C 1s curve fitting.

Approximate peak position	Species*	Measured (%/total C)	Theoretical** (%/total C)
285	C-C/C-H	22	10
286.6	C-C-O	65	81
285.7	(O-C=O)-C-C	7	5
289.1	(O-C=O)-C-C	7	5

* The carbon being examined is underlined
** Based on OEGMA composition with 8 EG units

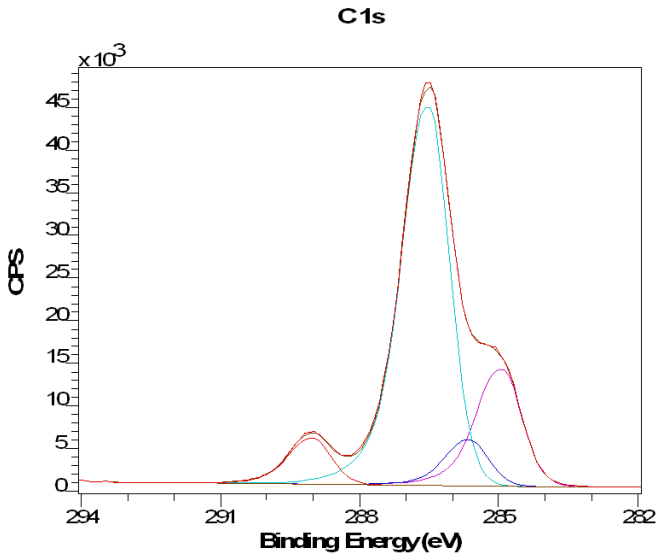


Fig. S7: XPS high intensity C 1s spectrum of PCL-*g*-p(OEGMA) with curve fitting