Supplementary Information for

Cyclohexyl-substituted poly(phosphonate)-copolymers with adjustable glass transition temperatures

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Materials

Solvents and chemicals were purchased from Acros Organics, Sigma Aldrich or Fluka and used as received, unless otherwise stated. All chemicals were purchased in highest purities, dry and stored over molecular sieve (4Å), if possible. 2-(Benzyloxy)ethanol was purchased from ABCR, distilled from calcium hydride and stored over molecular sieve (4Å) and under argon prior to use. TBD was purchased from Sigma Aldrich and stored under argon. Deuterated solvents were purchased from Deutero GmbH (Kastellaun, Germany) and used as received. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine albumin (FBS penicillin and streptomycin were purchased from Invitrogen, Germany.

Instrumentation and Characterization Techniques

Size exclusion chromatography (SEC) measurements were performed in DMF (1 g L^{-1} LiBr added) at 60°C and a flow rate of 1 mL min^{-1} with an PSS SECcurity as an integrated instrument, including a PSS GRAM 100-1000 column and a refractive index (RI) detector. Calibration was carried out using poly(ethylene glycol) standards provided by Polymer Standards Service. The ^{1}H, $^{13}C\{H\}$, and $^{31}P\{H\}$ NMR experiments were acquired on a Bruker 500 AMX system. The temperature was kept at 298.3 K and calibrated with a standard ^{1}H

methanol NMR sample using the topspin 3.0 software (Bruker). $^{13}C\{H\}$ NMR spectra were referenced internally to solvent signals. $^{31}P\{H\}$ NMR spectra were referenced externally to phosphoric acid. The $^{13}C\{H\}$ NMR (125 MHz) and $^{31}P\{H\}$ NMR (201 MHz) measurements were obtained with a 1H powergate decoupling method using 30 ° degree flip angle. 2D NMR experiments (1H DOSY (Diffusion ordered spectroscopy)) were measured on a Bruker 500 AMX NMR spectrometer under the same conditions as mentioned above. All spectra were processed with the MestReNova 9.0.1-13254 software. Differential Scanning Calorimetry (DSC) measurements were performed using a Mettler-Toledo DSC823 thermal analysis system in the temperature range from -80 to 50 °C under nitrogen with a heating rate of 10 °C min^{-1}. Cloud points were determined in dulbecco's phosphate buffered saline (no Ca^{2+}, no Mg^{2+}) from ThermoFischer scientific and detected by optical transmittance of a light beam (λ = 500 nm) through a 1 cm sample cell. The measurements were performed in a Jasco V-630 photo spectrometer with a Jasco ETC-717 Peltier element. The intensity of the transmitted light was recorded versus the temperature of the sample cell. The heating / cooling rate was 1 °C min^{-1} and values were recorded every 0.1 °C.

Murine macrophage-like cells (RAW 264.7) were cultivated in DMEM supplemented with 10% FBS, 100 units of penicillin, and 100 mg mL^{-1} streptomycin. Cells were grown in humidified incubator at 37°C and 5% CO_2. The effect of $\mathbf{P(1_x\text{-}\textit{co}\text{-}2_y)_n}$ on cell viability of RAW 264.7 cells was measured by CellTiter-Glo Luminescent Cell Viability Assay (Promega) according to manufacturer. Luminescent signals were measured with a Tecan infinite M1000. RAW 264.7 cells were seeded at a density of 15 000 cell per cm^2 in 96-well plates (100 µL per well). The polymers were dissolved in DMEM (stock concentration: 1 mg/mL) and further diluted to the indicated concentrations. After 24 h of incubation, the cell culture medium was replaced by the polymer supplemented medium and cells were incubated for 24 h.

Experimental procedure

Cyclohexyl phosphonic acid dichloride: The dichloride was synthesized according to literature.[1] A flame-dried 1 L three-necked round-bottom flask was equipped with a dropping funnel, a thermometer and a mechanical stirrer. Freshly ground aluminium trichloride (133 g, 1.0 mol) was provided and phosphorus trichloride (137 g, 1.0 mol) was added. The suspension was cooled to 5 °C and stirred vigorously. Cyclohexyl bromide (81 g, 0.5 mol) was added drop wise so that the temperature did not exceed 10 °C. The mixture was stirred for another 20 minutes at 5 °C and for further 30 min at 25 °C. The formed solid was dissolved in dichloromethane, the solution cooled to -20 °C by addition of $CO_{2(s)}$ and hydrolyzed with 185 mL water while keeping the temperature below 0 °C.

The precipitated solid was filtered off and the organic phase was evaporated *in vacuo*. Fractionated distillation yielded the desired product as a colorless powder (71 g, 0.36 mol, yield: 67%, b.p. 75 °C / 5x10^{-2} mbar). ^{1}H NMR (500MHz, $CDCl_3$-*d*, ppm): δ 2.43-2.33 (m, 1H), 2.24-2.16 (m, 2H), 1.95-1.86 (m, 2H), 1.77-1.69 (m, 1H), 1.55-1.43 (m, 2H), 1.37-1.17 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$-*d*, ppm): δ 51.00 (d, 1C, P-**C**H-, $^{1}J_{CP}$ = 91.3 Hz), 25.90 (d, 2C, P-CH-**C**H_2, $^{2}J_{CP}$ = 5.0 Hz), 25.58 (d, 2C, P-CH-CH_2-**C**H_2- $^{3}J_{CP}$ = 20.0 Hz), 25.18 (d, 1C, P-CH-CH_2-CH_2-**C**H_2- $^{4}J_{CP}$ = 2.5 Hz) . ^{31}P NMR (201 MHz $CDCl_3$-*d*, ppm): δ 58.28.

2-cyclohexyl-2-oxo-1,3,2-dioxaphospholane (*cy*HexPPn, (1)): The ring-closing reaction was performed according to a slightly altered literature protocol.[2] A flame-dried three-necked round-bottom flask, equipped with a magnetic stirring bar and two dropping funnels, was charged with 250 mL dry THF and cooled to -21°C. Cyclohexyl phoshonic acid dichloride (30 g, 0.15 mol) was dissolved in dry THF (250 mL) and transferred into one dropping funnel via a flame-dried stainless steel capillary. A solution of dry ethylene glycol (9 g, 0.15 mol) and dry pyridine (23 g, 0.30 mol) in THF (250 mL) was transferred into the second dropping

funnel via a flame-dried stainless steel capillary. Dropping speed was adjusted to be approximately equal for both mixtures. After complete addition the solution was stirred for 3 hours and kept over-night at -28°C to facilitate the precipitation of the pyridinium hydrochloride byproduct. The precipitate was removed by filtration via a flame-dried Schlenk funnel and the solvent was evaporated at reduced pressure. The obtained solid was dissolved in dichloromethane and washed three times with brine. The organic phase was dried with sodium sulfate and evaporated in vacuo. Fractionated distillation yielded the desired product as colorless solid (9 g, 0.05 mol yield: 33%, b.p. 115°C / 9x10-3 mbar). ^{1}H NMR (500MHz, CDCl3-d, ppm): δ 4.51-4.40 (m, 2H, P-O-CH2), 4.28-4.15 (m, 2H, P-O-CH2), 2.07-1.90 (m, 3H) 1.88-1.74 (m, 2H), 1.74-1.62 (m, 1H), 1.50-1.33 (m, 2H), 1.33-1.14 (m, 3H). ^{13}C NMR (125 MHz, CDCl3-d, ppm): δ 66.39 (d, 2C, P-O-CH2-CH2-, 2JCP = 2.5 Hz), 31.22 (d, 1C, P-CH-, 1JCP = 130.0 Hz), 21.36 (d, 2C, P-CH-CH2, 2JCP = 3.8 Hz), 21.18 (d, 2C, P-CH-CH2-CH2- 3JCP = 16.3 Hz), 20.84 (d, 1C, P-CH-CH2-CH2-CH2- 4JCP = 1.3 Hz). ^{31}P NMR (201 MHz CDCl3-d, ppm): δ 47.50.

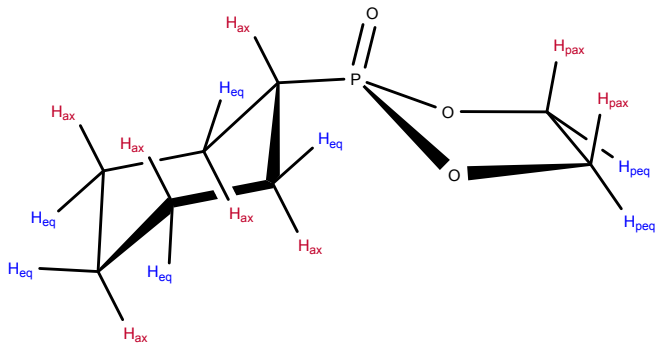
Representative procedure for the ROP of (1) and (2): The respective monomer(s) was/were placed in a flame-dried Schlenk-tube, dissolved in dry benzene and dried by repeated lyophilization. The monomer was dissolved in dry dichloromethane at a concentration of 4 mol L^{-1}. A stock solution of initiator 2-(benzyloxy)ethanol in dry dichloromethane was prepared with a concentration of 0.2 mol L^{-1} and the calculated volume was added to the monomer solution via gas tight syringe (Hamilton®). A stock solution of twice lyophilized TBD in dry dichloromethane was prepared with a concentration of 0.2 mol L^{-1}. The monomer- and catalyst solutions were cooled to 0°C. The polymerization was initiated by addition of the calculated amount of the catalyst solution (1 equivalent of TBD in respect to the initiator). The polymerization was terminated after 2h by the rapid addition of an excess of formic acid dissolved in dichloromethane with a concentration of

20 mg mL^{-1}. The colorless, amorphous polymers were purified by precipitation in cold diethyl ether and dried under reduced pressure.

Representative NMR data for P(1): ^{1}H NMR (500MHz, DMSO-d_6, ppm): δ 7.37-7.26 (m, aromatic protons), 4.22-4.02 (m, polymer backbone), 3.61 (m, 2H, terminal), 4.50 (m,benzylic protons) 1.92-1.02 (m, side-chain). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 128.19 und 127.45 (aromatic protons), 64.32 (polymer backbone), 34.50 (d, side-chain), $^{1}J_{CP}$ = 141.3 Hz), 25.50-25.27 (m, side-chain)). ^{31}P NMR (201 MHz DMSO-d_6, ppm): δ 33.11 (polymer backbone), 32.98 (terminal group).

Representative NMR data for P($\mathbf{1_x}$-*co*-$\mathbf{2_y}$)$_\mathbf{n}$: ^{1}H NMR (500MHz, DMSO-d_6, ppm): δ 7.38-7.27 (m, aromatic protons), 4.22-4.07 (m, polymer backbone), 3.65-3.61 (m, terminal group), 4.52 (m, benzylic protons) 2.08-1.95 (m, isopropyl side-chain), 1.92-1.02 (m, cyclohexyl side-chain), 1.14-1.05 (m, isopropyl side-chain). ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 128.19 und 127.45 (aromatic protons), 64.32 (polymer backbone), 34.50 (d, cyclohexyl side-chain, $^{1}J_{CP}$ = 141.3 Hz), 25.50-25.27 (m, cyclohexyl side-chain), 25.22 (d, $^{1}J_{CP}$ = 141.4 Hz, isopropyl side-chain), 16.12 (d, $^{2}J_{CP}$ = 4.9 Hz, isopropyl side-chain). ^{31}P NMR (201 MHz DMSO-d_6, ppm): δ 36.12 (isopropyl backbone), 35.90 (isopropyl terminal groups), 33.11 (cyclohexyl backbone), 32.98 (cyclohexyl terminal groups).

Additional data



Scheme S1: Assumed conformation of **(1)**. The equatorial (H_{eq}), axial (H_{ax}) protons of the six-membered ring as well as the pseudo-equatorial (H_{peq}) and the pseudo-axial (H_{pax}) protons of the five-membered ring are marked.

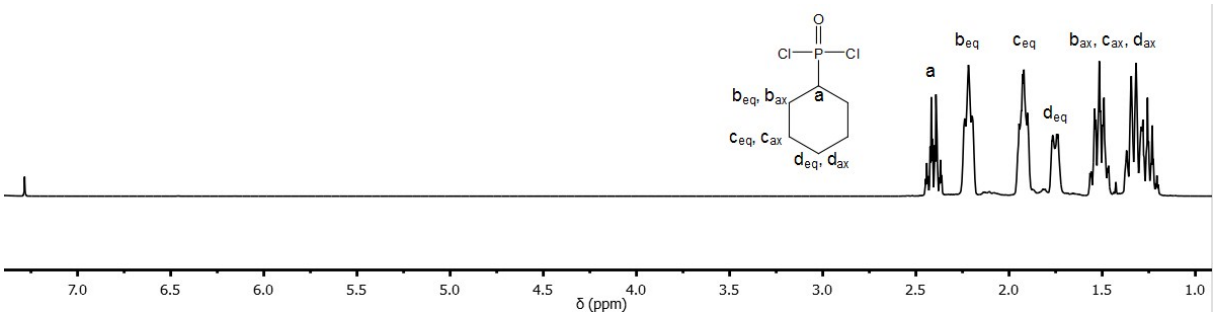


Figure S1: ^{1}H (500 MHz) NMR spectrum of cyclohexyl phosphonic acid dichloride in $CDCl_3$ at 298K.

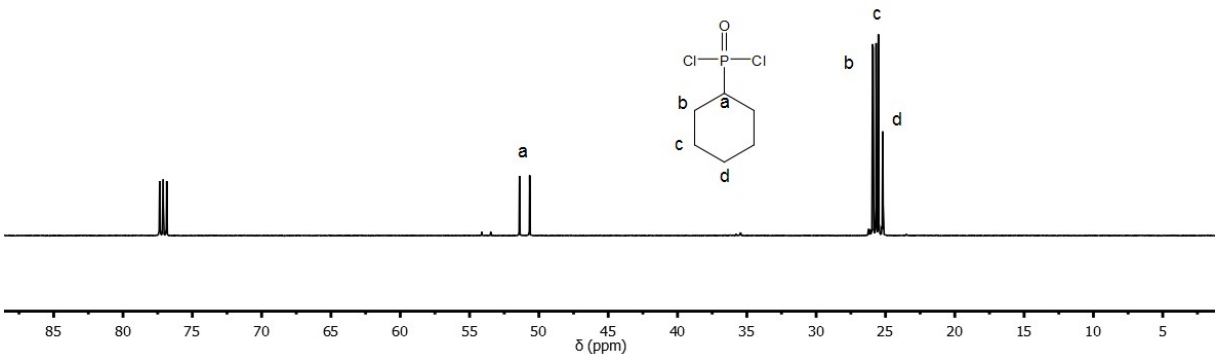


Figure S2: ^{13}C (125 MHz) NMR spectrum of cyclohexyl phosphonic acid dichloride in $CDCl_3$ at 298K.

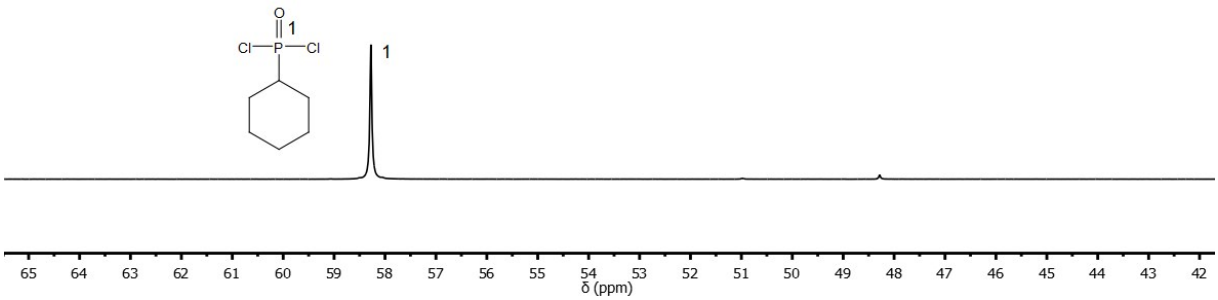


Figure S3: ^{31}P (202 MHz) NMR spectrum of cyclohexyl phosphonic acid dichloride in $CDCl_3$ at 298K.

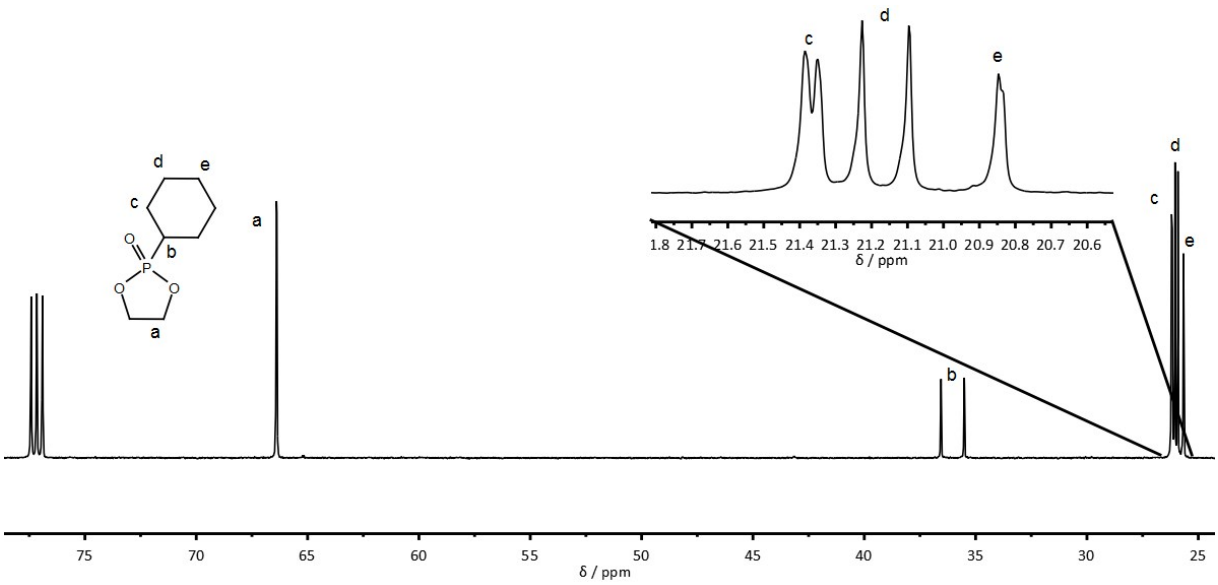


Figure S4: ^{13}C (125 MHz) NMR spectrum of **(1)** in $CDCl_3$ at 298K.

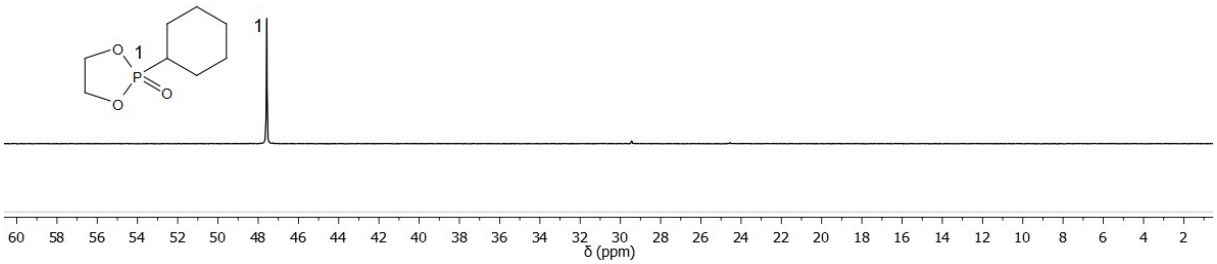


Figure S5: ^{31}P (202 MHz) NMR spectrum of **(1)** in $CDCl_3$ at 298K.

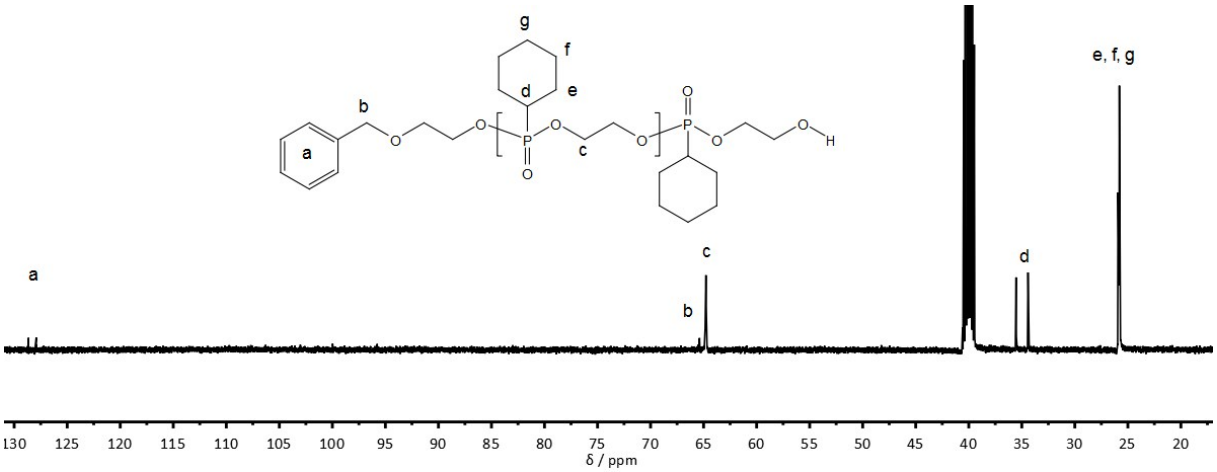


Figure S6: ^{13}C (125 MHz) NMR spectrum of **P(1)** in DMSO-d_6 at 298K.

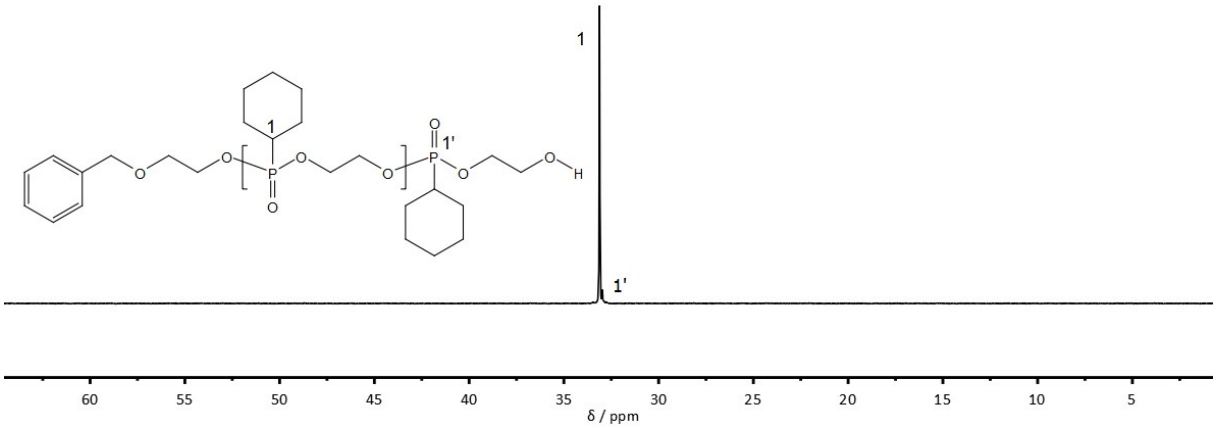


Figure S7: ^{31}P (202 MHz) NMR spectrum of **P(1)** in DMSO-d_6 at 298K.

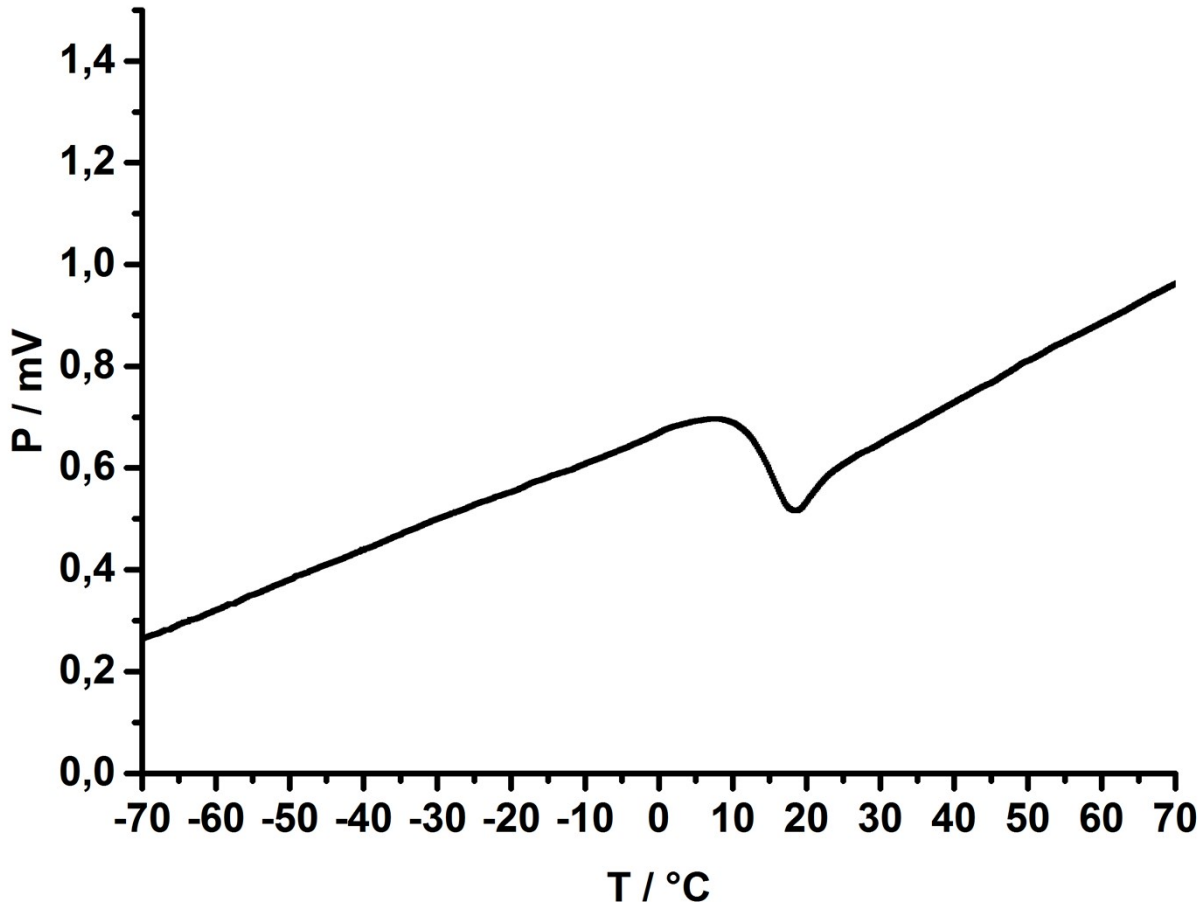


Figure S8: DSC thermogram of **P(1)** at a heating rate of 10 °C min-1 in the temperature range from -70 °C to 70 °C.

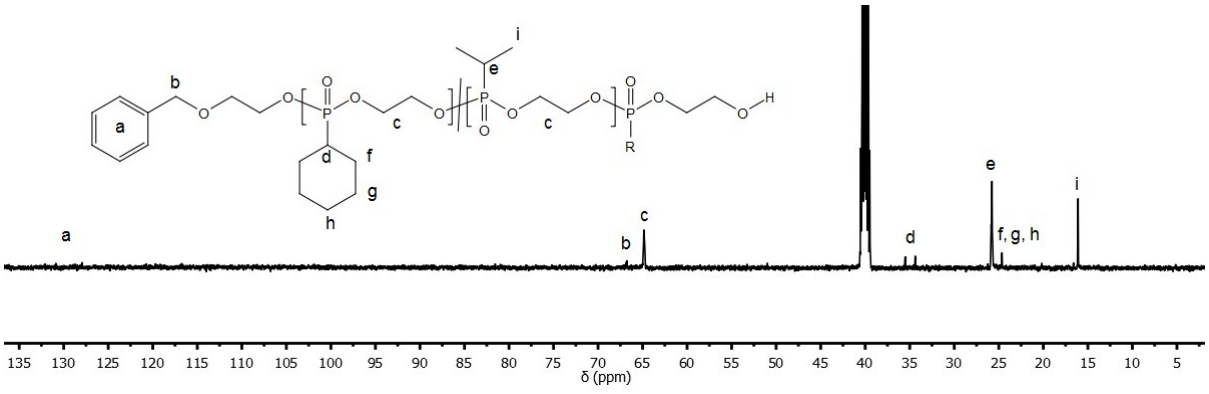


Figure S9: ^{13}C (125 MHz) NMR spectrum of **P($\mathbf{1_x}$-*co*-$\mathbf{2_y}$)$_\mathbf{n}$** in DMSO-d_6 at 298K.

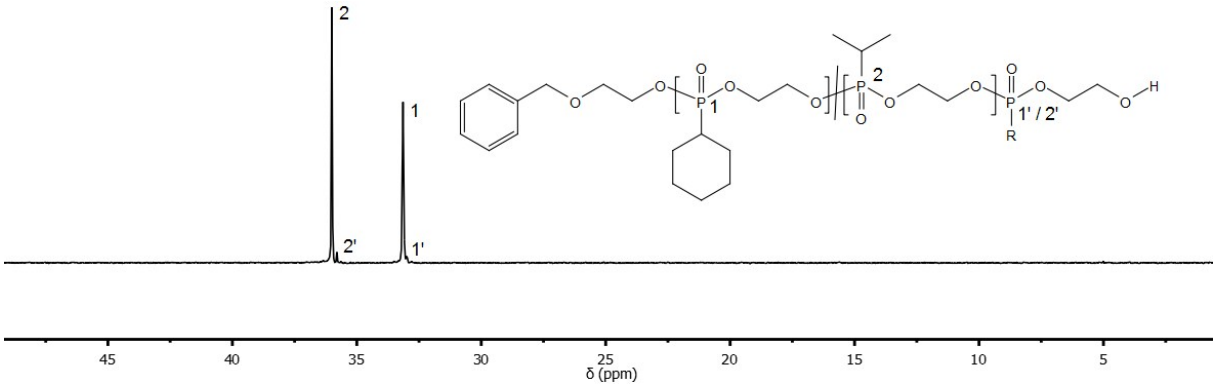


Figure S10: ^{31}P (202 MHz) NMR spectrum of **P(1$_x$-*co*-2$_y$)$_n$** in DMSO-d_6 at 298K.

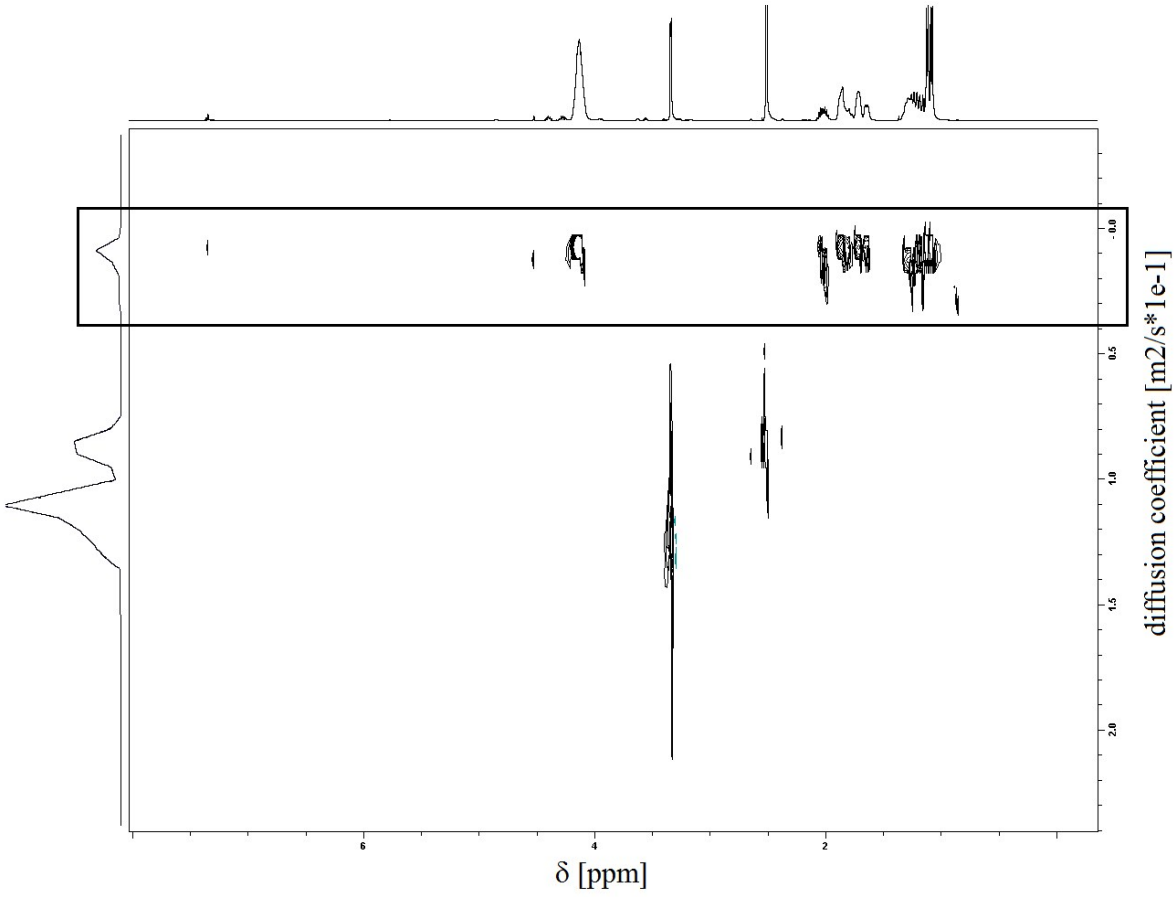


Figure S11: ^{1}H DOSY (500 MHz) NMR spectrum of **P(1$_x$-*co*-2$_y$)$_n$** in DMSO-d_6 at 298K. All copolymersignals of backbone and side-chains show a diffusion coefficient of 0.15 $m^2\ s^{-1}$ (black box) whereas the solvent DMSO-d_6 and H_2O show a significantly higher diffusion coefficient.

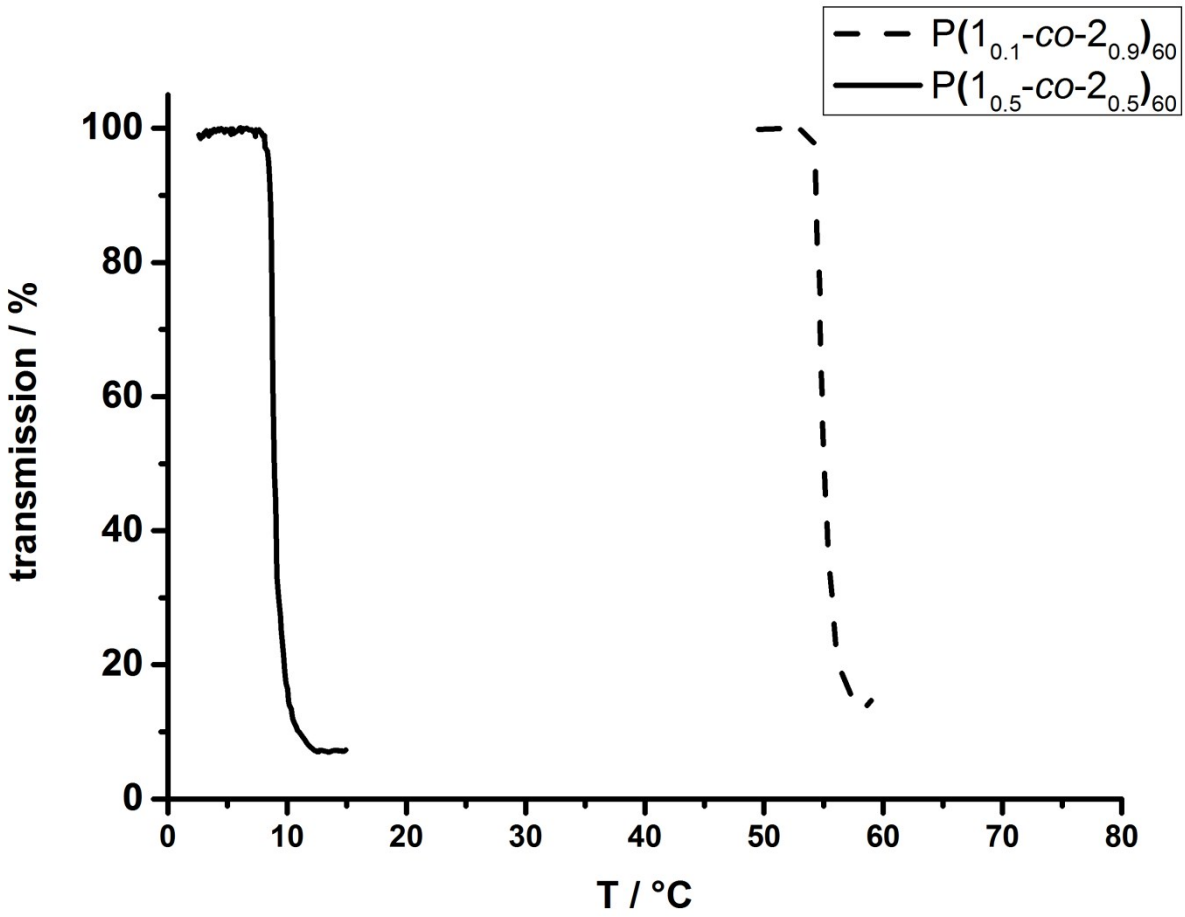


Figure S12: Turbidity measurements (heating curves) of $\mathbf{P(1_{0.1}\text{-}co\text{-}2_{0.9})_{60}}$ (dashed curve) and $\mathbf{P(1_{0.5}\text{-}co\text{-}2_{0.5})_{60}}$ (black line) in PBS at a concentration of 1 g L^{-1} and a heating rate of 1 °C min^{-1}. Transmission was measured at 500 nm. Significant increase of cloud point temperature observed upon increasing the amount of **(2)** in the polymer chain.

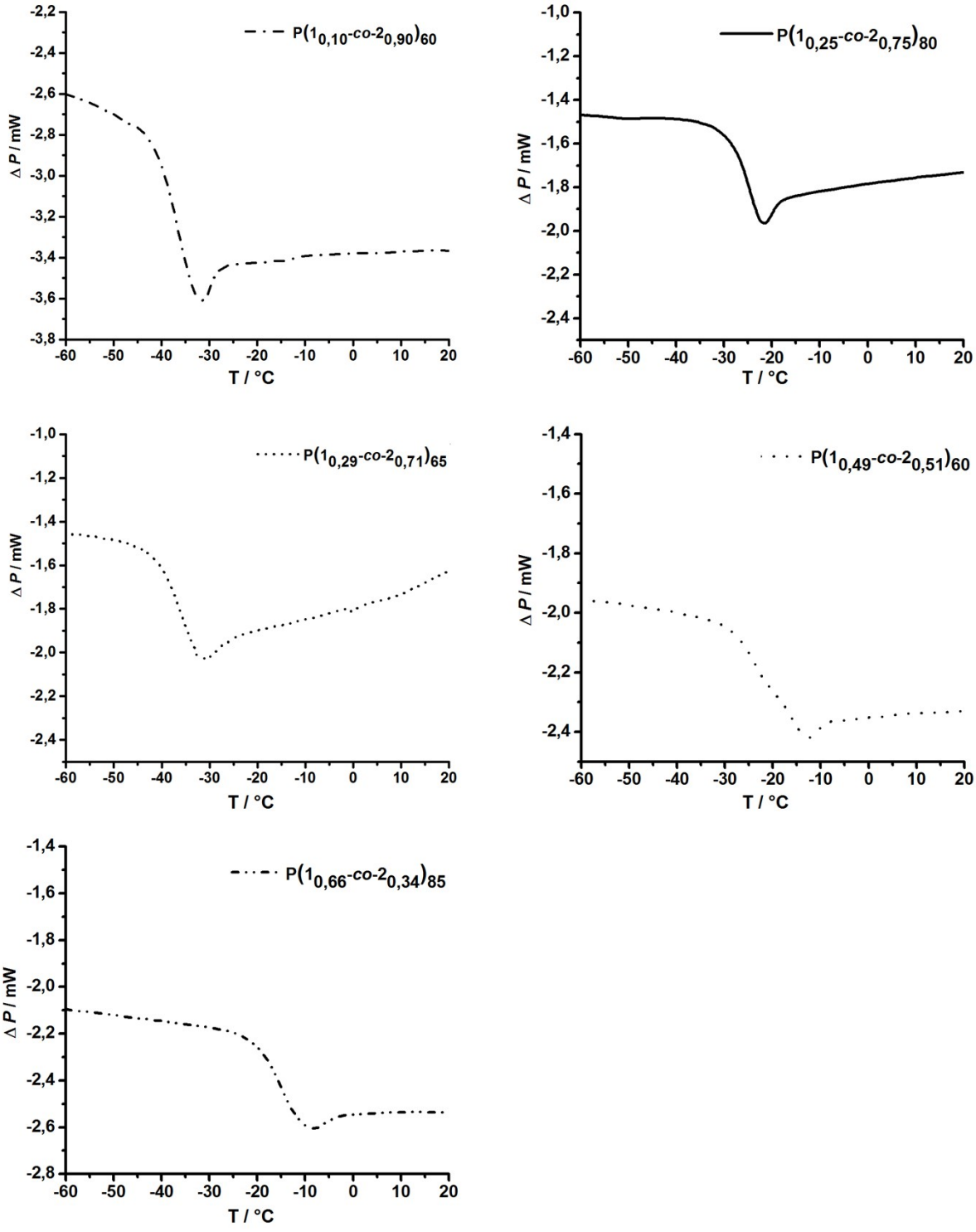


Figure S13: DSC thermograms of $\mathbf{P(1_x\text{-}co\text{-}2_y)_n}$ with different copolymer compositions at a heating range of 10 °C in the range from -60 °C to 20 °C. Increase of T_g with the incorporation of **(1)** visible.

References:

1. Clay, J. P., A New Method for the Preparation of Alkane Phosphonyl Dichlorides. *J. Org. Chem.* **1951,** *16* (6), 892-894.
2. Steinbach, T.; Ritz, S.; Wurm, F. R., Water-Soluble Poly(phosphonate)s via Living Ring-Opening Polymerization. *ACS Macro Lett.* **2014,** *3* (3), 244-248.