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Advancements in structure-property correlation studies of cross-linked citric acid-based elastomers in the perspective of biological application

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1. Prepolymer characterization

1.1. Synthesis protocol

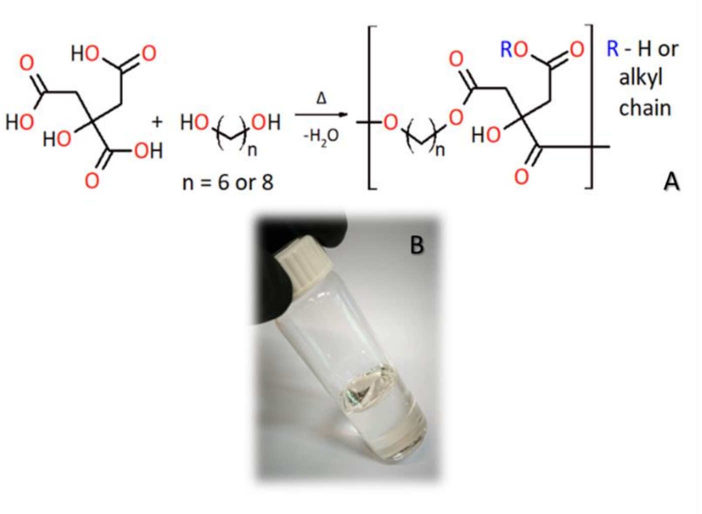

Figure S1. Synthesis scheme of PAC prepolymers fabrication (**A**) and the appearance of the final product (**B**).

1.1.1. Monitoring condensation progress

For the elaboration of the most efficient conditions for PAC prepolymers syntheses, it was crucial to choose a simple reaction parameter that could be correlated directly with its progress. Thus, we considered PAC prepolymer formation as a polycondensation reaction that involves the release of one molecule of water per one covalent bond formulated between the CA carboxyl group and the hydroxyl group in diol. The above fact implies that the reaction progress could be roughly determined through measurement of the mass of water that was expelled from the reaction mixture. For that purpose water collectors are frequently used; however, these would be impracticable in the case of synthetic protocols described in this report due to a large number of small vessels used in the syntheses. Therefore, assuming that water vapours are the only gaseous products being produced during the synthesis of PAC prepolymers, we decided to use reaction mass loss as an indication of the quantity of water being expelled. Consequently, the estimated degree of conversion (α) of substrates into particular prepolymer could be simply calculated through weighing of vials filled with reactants before and after reaction, using a formula:

$$\boldsymbol{\alpha = \left(\frac{(m_{before} - m_{after})}{M_{H_2O}}\right) * \frac{1}{m_{total}} * 100}$$

- α – degree of conversion;
- m_{before} – mass of vial with substrates before reaction;
- m_{after} – mass of vial with prepolymer after reaction;
- M_{H2O} – water molar mass;
- m_{total} – total mass of substrates – usually 20 g;
- 100 – percentage multiplier.

1.1.2. Reaction vessel and stirring process

In the literature, the PAC prepolymers are fabricated by heating reactants in round bottom flasks using a mineral oil bath or in an Erlenmeyer flask using a hot plate. Considering high viscosity accompanied by a relatively large volume of the reaction mixture, both local overheating and underheating effects cannot be neglected. Therefore, in the present studies, we decided to use 4 smaller tubular reaction vessels (25 ml each) placed in a dedicated heating block to obtain one batch of PAC prepolymers instead (Figure S1). Thus, by decreasing the reaction volume and providing more homogeneous heat distribution it was possible to minimalize local temperature fluctuations and unwanted effects associated with insufficient mixing of reactants. Furthermore, a clear effect of mixing efficiency on the degree of overall reaction yields was observed; for reactions conducted using stirring bars with different dimensions the variations in final reaction yields ranged up to 40% (cylindrical stirring bar 2.0 x 0.6 cm ~`20%, cylindrical stirring bar 2.5 x 0.8 cm ~`60%). Therefore to be able to investigate other reaction parameters, all syntheses were conducted with identical stirring bars at the same rotational speed (IKA HS-10, 100 rpm). In rare cases, where the mass loss of a particular reaction differed significantly from the group, it was possible to eliminate it from the group, not affecting the overall quality of the final batch of synthesized PAC prepolymer. Finally, after a comparison of the estimated conversion of reagents in each of the reaction vessels, it was possible to combine them and purify them as a single batch.

1.1.3. Reaction temperature

Elimination of the mixing problem allowed to determine the effect of reaction temperature on the process efficiency. In this study, only one reaction temperature (140 °C) was used for the synthesis of PAC prepolymers which is in contradiction to two temperatures described in the literature (160 °C for the first minutes of the reaction, followed by cooling to 140 °C). This suggests that the temperatures used in the literature descriptions do not improve the efficiency of the reaction and make the accurate control of the process more difficult. It was found that the higher temperature described in the literature may cause a decrease in reaction efficiency due to the reaction conducted in conditions close to the CA decomposition temperature (175 °C). Considering, frequent local overheating occurring in large vessels above fact may cause side reactions which leads to the release of decarboxylated derivatives of CA, such as itaconic, citraconic, aconitic, mesaconic acids [35]. Formulation of the above compounds during PAC synthesis, on one hand, may be an origin of unwanted radical processes and on the other hand, can lead to a decrease in a total number of ester bonds formation resulting in a reduction of reaction yield.

1.2. Mass spectra

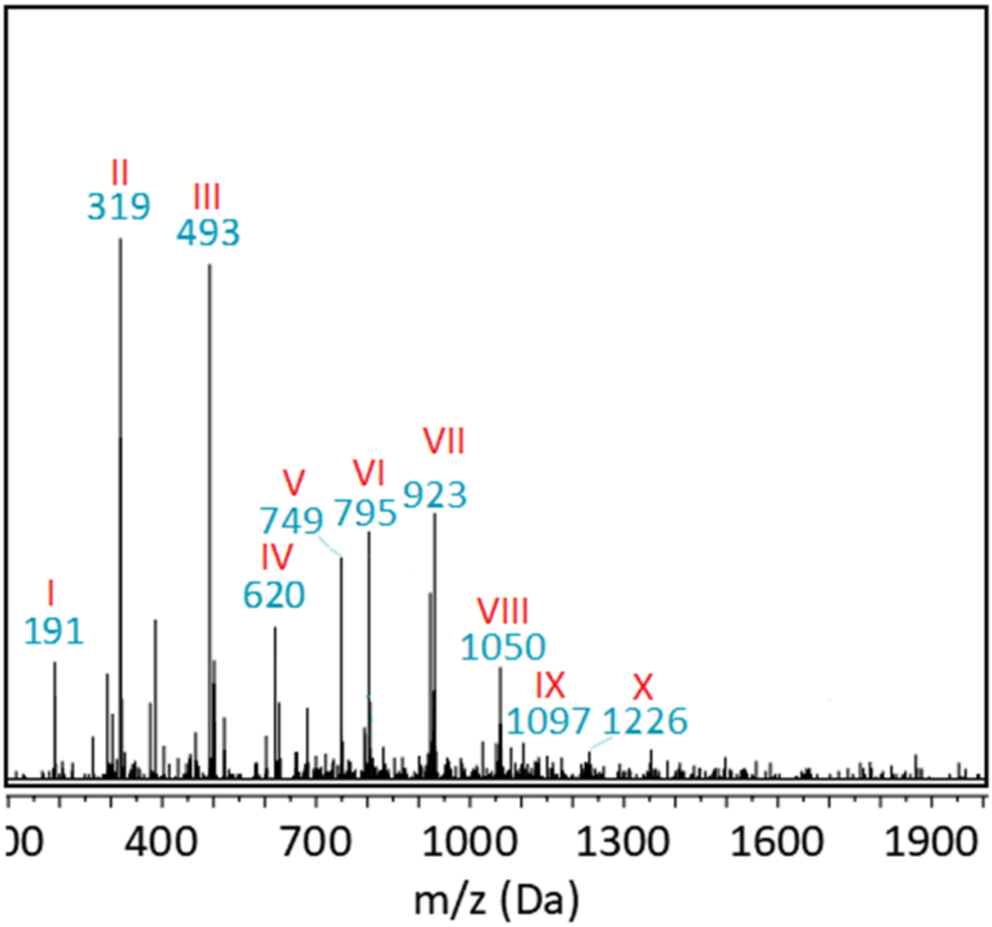

Figure S2. Mass spectrum of POC_1:1 prepolymer acquired via FIA-MS analysis (ESI$^-$). Ion labels are referred in the table below.

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m/z	Ion label	Real mass [Da]	Presumed structures
191	I	192	**C**
319	II	320	**CD**
493	III	494	**CDC**
620	IV	622	**a) CDCD** b) CD DC
749	V	750	**a) DCDCD** b) CDCD D
794	VI	796	**a) CDCDC** b) CDCD C
923	VII	924	**a) CDCDCD** b) CDCDC D
1050	VIII	1051	**a) DCDCDCD** b) CDCDCD c) CDCDCD D D
1097	IX	1098	**a) CDCDCDC** b) CDCDC D C
1226	X	1226	**a) CDCDCDCD** b) CDCDC c) CDCDCDC D D C D

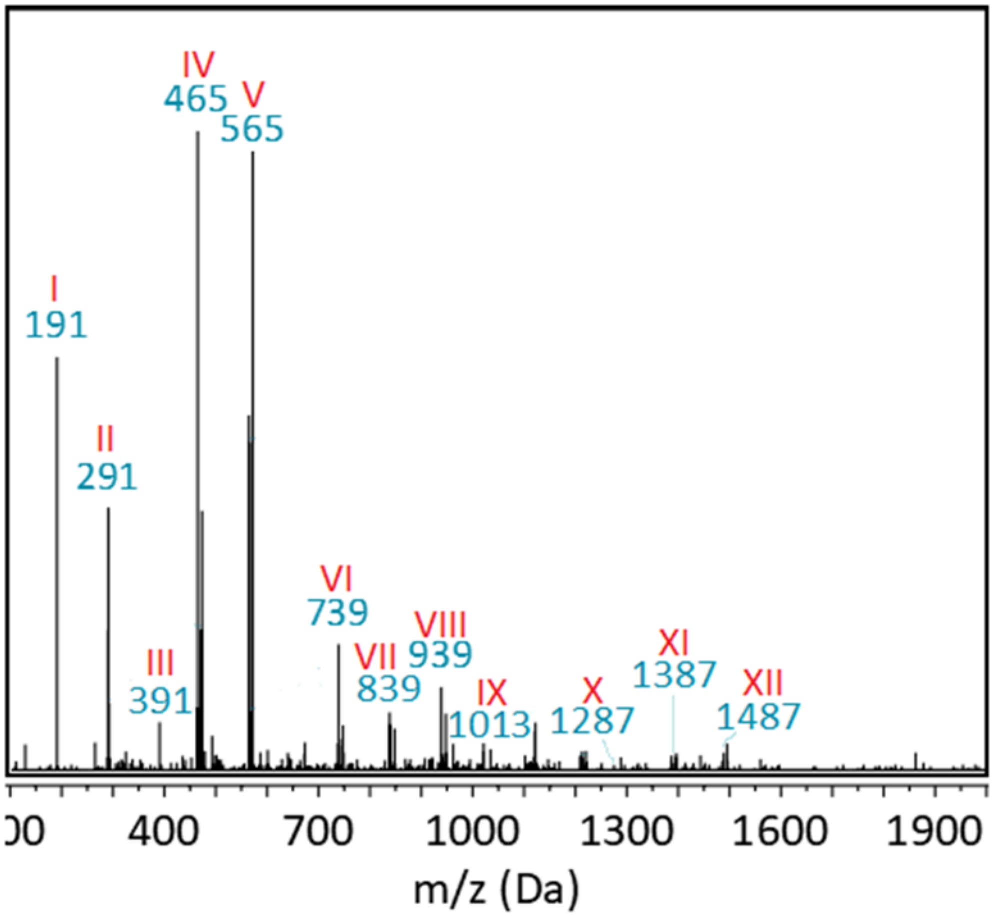

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Table S2. Identification of molecular ions found in FIA mass spectra of PHC_1:1 prepolymer and their assignments to possible chemical structures of specific oligomers (ion labels from I to XII on Figure S3). The singular citrate unit was labeled with C while the letter D denotes an alkylene group. Most probable structures of particular oligomers, according to NMR analyses, were labeled in bold.

m/z	Ion label	Real mass [Da]	Presumed structures
191	I	192	**C**
291	II	292	**CD**
391	III	392	**DCD**
465	IV	466	**CDC**
565	V	566	**a) CDCD** b) CD DC
739	VI	740	**a) CDCDC** b) CDCD C
839	VII	840	**a) CDCDCD** b) CDCDC D
939	VIII	940	**a) DCDCDCD** b) CDCDCD c) CDCDCD D D
1013	IX	1014	**a) CDCDCDC** b) CDCDC D C
1287	X	1288	**a) CDCDCDCDC** b) CDCDCDC D C
1387	XI	1388	**a) CDCDCDCDCD** b) CDCDCDC D D C
1487	XII	1488	**a) DCDCDCDCDCD** b) CDCDCDCD c) CDCDCDCDC D D D D C C D

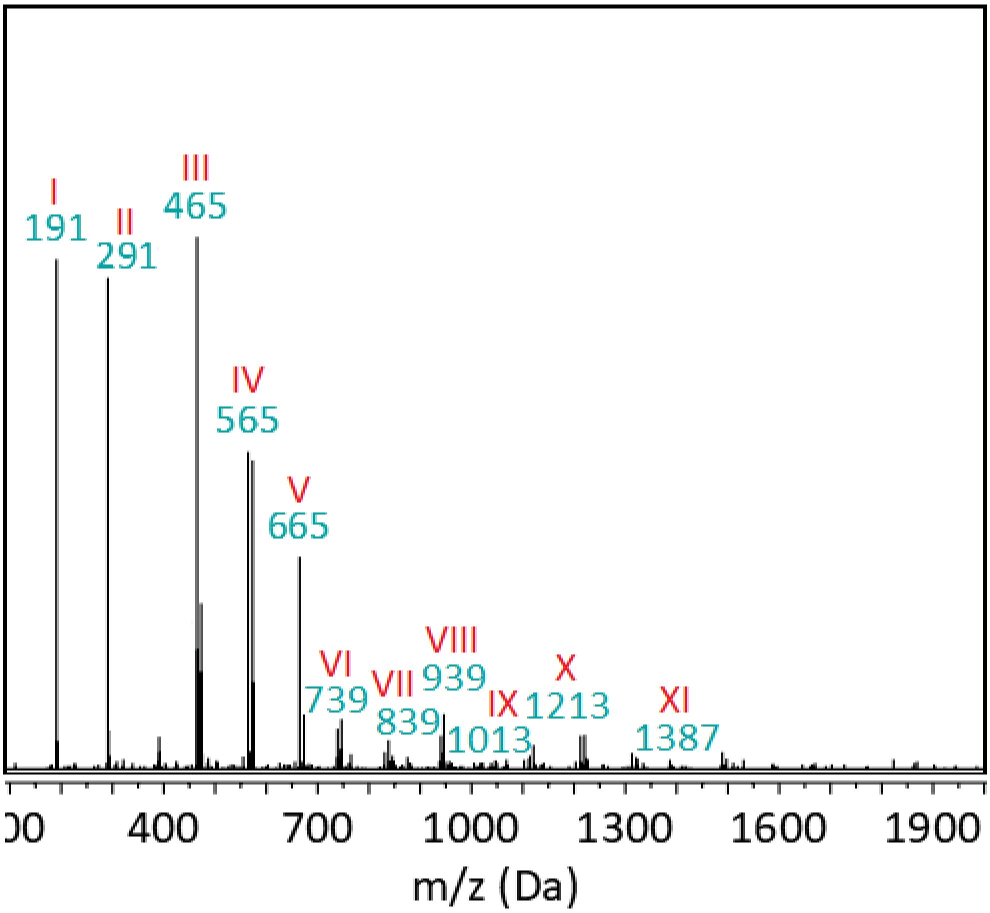

Figure S4. Mass spectrum of PHC_2:3 prepolymer acquired via FIA-MS analysis (ESI$^-$). Ion labels are referred in the table below.

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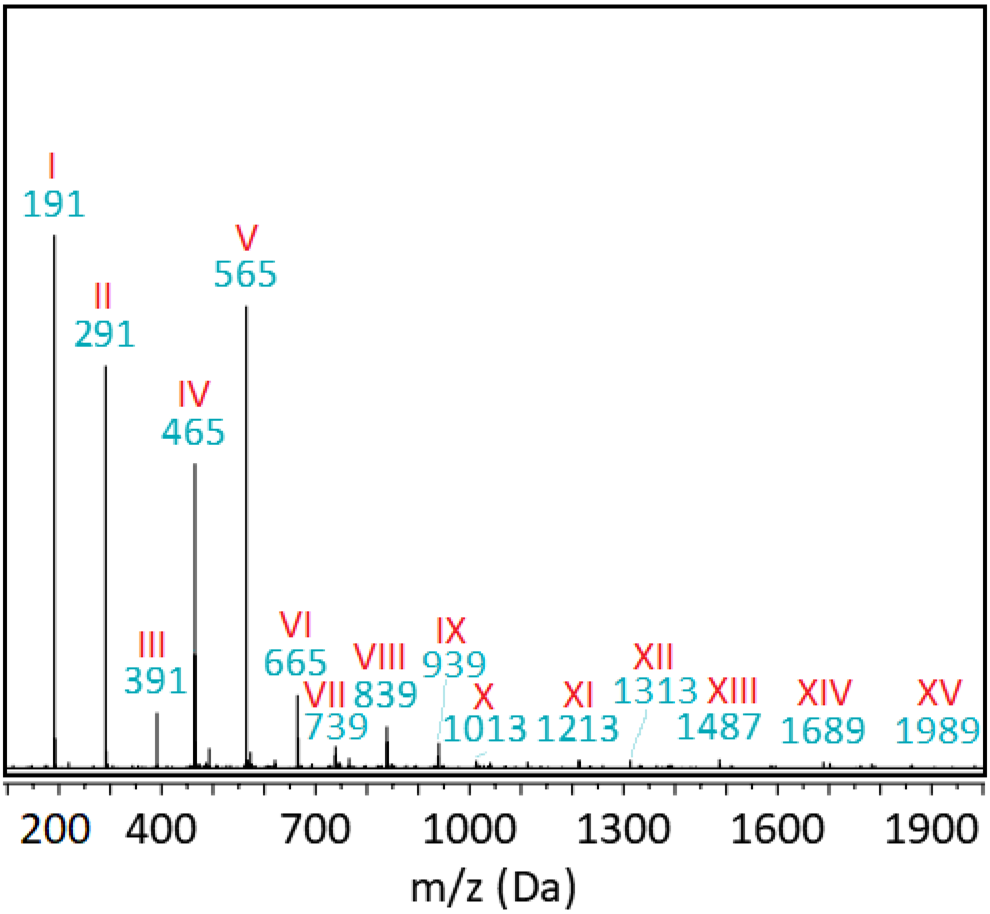
m/z	Ion label	Real mass [Da]	Presumed structures
191	I	192	**C**
291	II	292	**CD**
465	III	466	**CDC**
565	IV	566	**a) CDCD** b) CD DC
665	V	666	**a) DCDCD** b) CDCD D
739	VI	740	**a) CDCDC** b) CDCD C
839	VII	840	**a) CDCDCD** b) CDCDC D
939	VIII	940	**a) DCDCDCD** b) CDCDCD c) CDCDCD D D
1013	IX	1014	**a) CDCDCDC** b) CDCDC D C
1213	X	1214	**a) DCDCDCDCD** b) DCDCDCD c) DCDCDCD D D D D
1387	XI	1388	**a) CDCDCDCDCD** b) CDCDCDC D D C



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191	I	192	**C**
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391	III	392	**DCD**
465	IV	466	**CDC**
565	V	566	**a) CDCD** b) CD DC
665	VI	666	**a) DCDCD** b) CDCD D
739	VII	740	**a) CDCDC** b) CDCD C
839	VIII	840	**a) CDCDCD** b) CDCDC D
939	IX	940	**a) DCDCDCD** b) CDCDCD c) CDCDCD D D
1013	X	1014	**a) CDCDCDC** b) CDCDC D C
1213	XI	1214	**a) DCDCDCDCD** b) DCDCDCD c) DCDCDCD D D D D
1313	XII	1314	**a) DCDCDCDCD** b) DCDCDCD **D** D D D
1487	XIII	1488	**a) DCDCDCDCDCD** b) CDCDCDCD c) CDCDCDCDC D D D D C C D
1689	XIV	1688	**a) DCDCDCDCDCD** b) CDCDCDCD c) CDCDCDCDC **D** **D** D D D D D D DC C D D
1989	XV	1988	**a) DCDCDCDCDCD** b) DCDCDCDCD c) DCDCDCDCD **D D D D D** D D D D D D D D DC DC D D

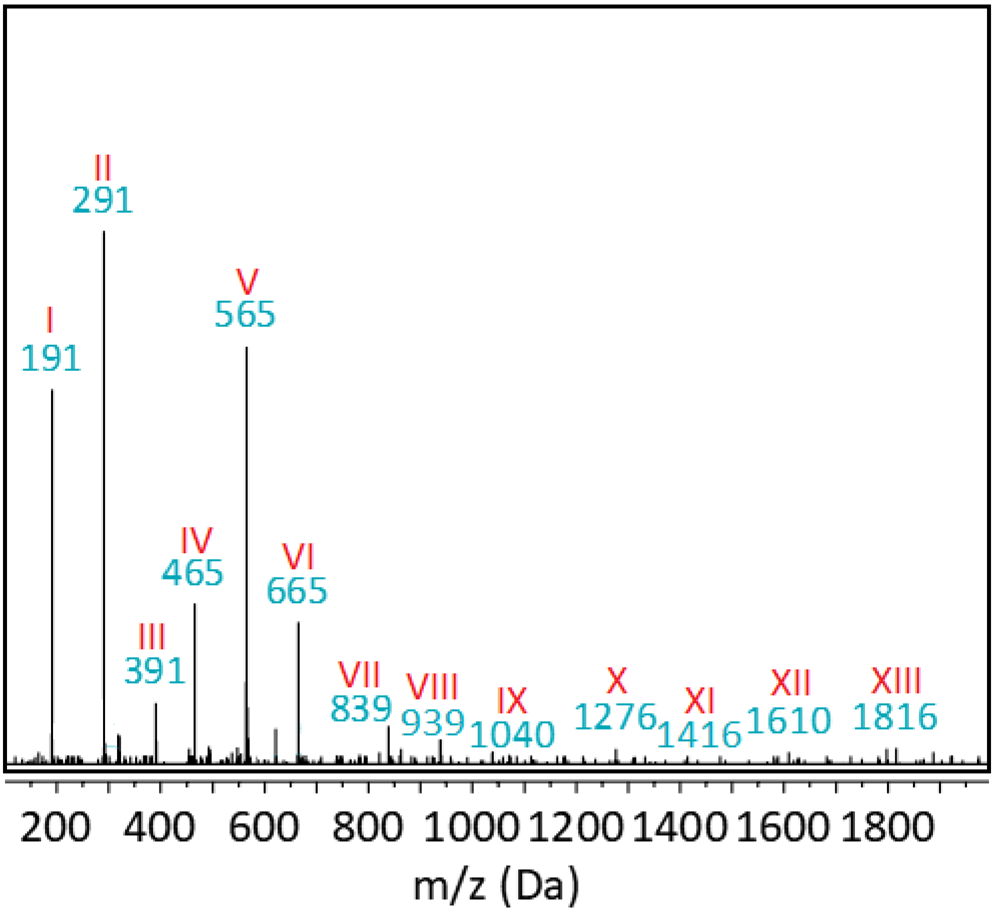

Figure S6. Mass spectrum of PHC_2:3 prepolymer obtained after 90 min of reaction, acquired via FIA-MS analysis (ESI$^-$). Ion labels are referred in the table below.

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m/z	Ion label	Real mass [Da]	Presumed structures
191	I	192	**C**
291	II	292	**CD**
391	III	392	**DCD**
465	IV	466	**CDC**
565	V	566	**a) CDCD** b) CD DC
665	VI	666	**a) DCDCD** b) CDCD D
839	VII	840	**a) CDCDCD** b) CDCDC D
939	VIII	940	**a) DCDCDCD** b) CDCDCD c) CDCDCD D D
1040	IX	1040	**a) DCDCDCD** b) DCDCDCD **D** D
1276	X	1278	**a) CDCDCD** **DC DCD** **D**
1416	XI	1416	3D cyclic structure - too complicated to present using following format
1610	XII	1608	3D cyclic structure - too complicated to present using following format
1816	XIII	1816	3D cyclic structure - too complicated to present using following format

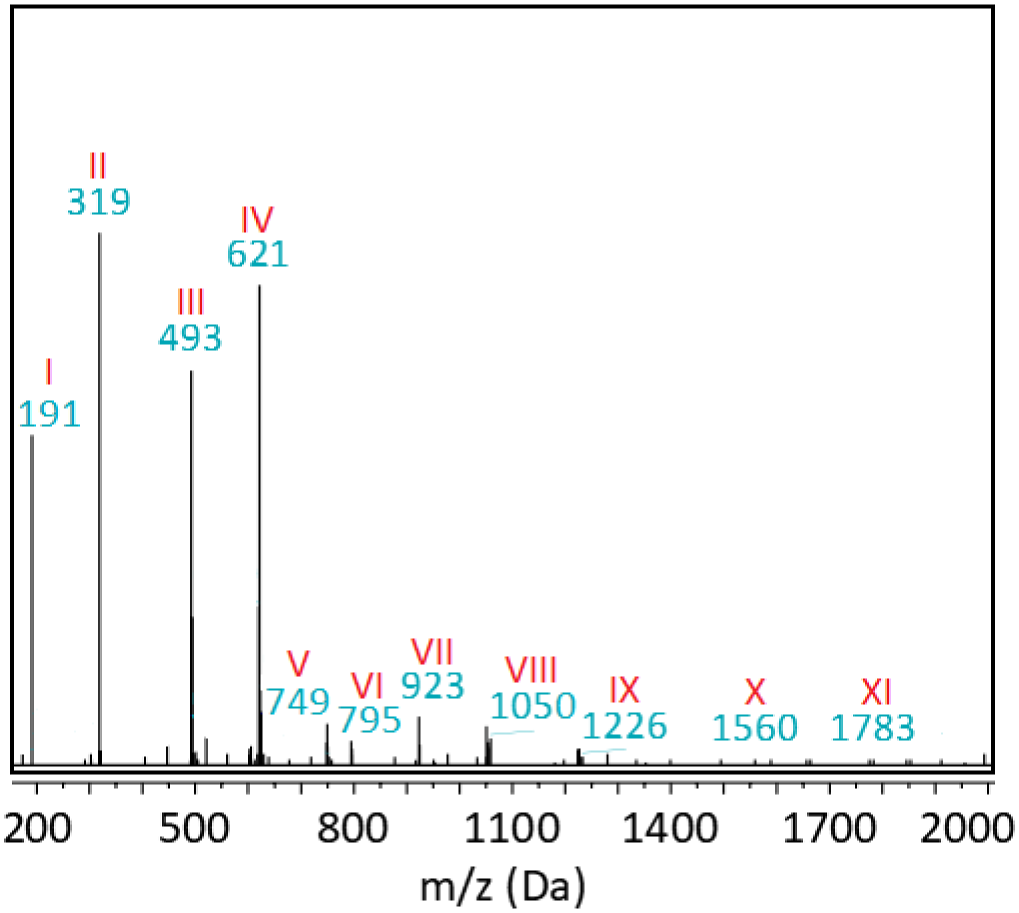
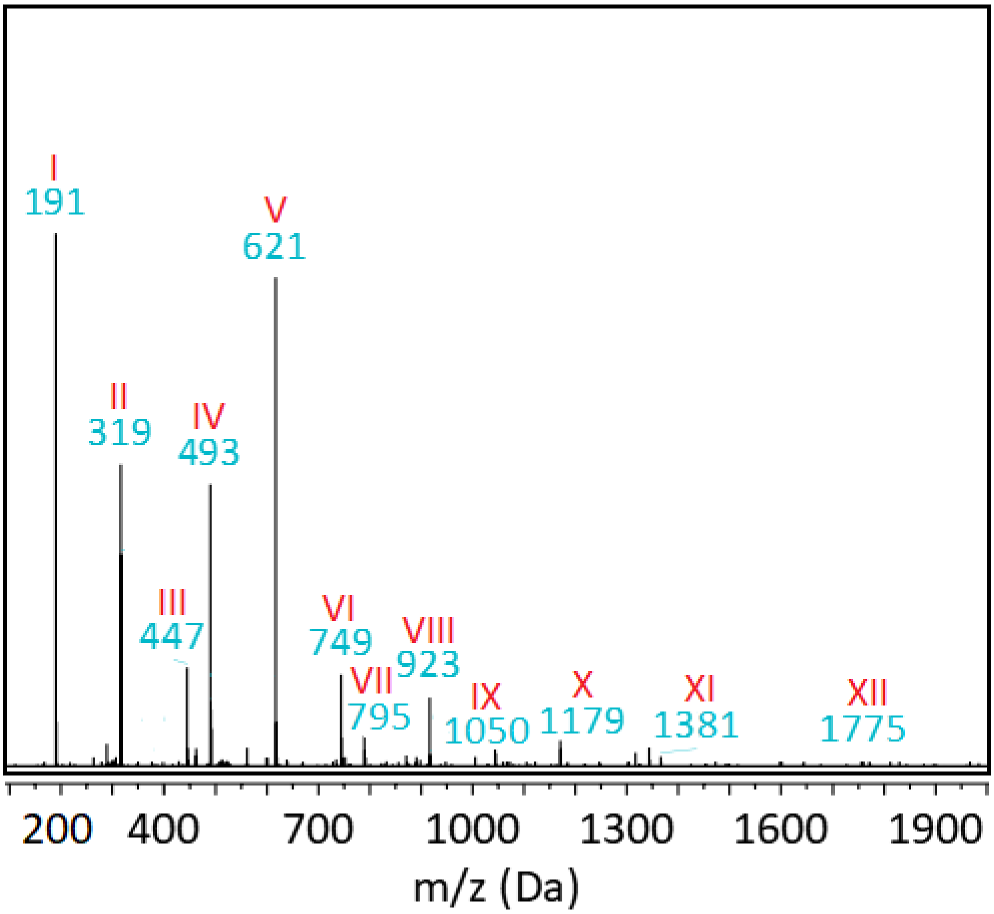


Figure S7. Mass spectrum of POC_2:3 prepolymer obtained after 60 min of reaction, acquired via FIA-MS analysis (ESI$^-$). Ion labels are referred in the table below.

Table S6. Identification of molecular ions found in FIA mass spectra of POC_2:3 prepolymer obtained after 60 min of reaction and their assignments to possible chemical structures of specific oligomers (ion labels from I to XI on Figure S7). The singular citrate unit was labeled with C while the letter D denotes an alkylene group. Most probable structures of particular oligomers, according to NMR analyses, were labeled in bold. Ion labels of which only branched or cyclic structures are possible and placed in the red frames.

m/z	Ion label	Real mass [Da]	Presumed structures
191	I	192	**C**
319	II	320	**CD**
493	III	494	**CDC**
620	IV	622	**a) CDCD** b) CD DC
749	V	750	**a) DCDCD** b) CDCD D
794	VI	796	**a) CDCDC** b) CDCD C
923	VII	924	**a) CDCDCD** b) CDCDC D
1050	VIII	1051	**a) DCDCDCD** b) CDCDCD c) CDCDCD D D
1226	IX	1226	**a) CDCDCDCD** b) CDCDC c) CDCDCDC D D C D
1560	X	1563	3D cyclic structure - too complicated to present using following format
1783	XI	1784	**a) DCDCDCDCDCD** b) DCDCDCDCDC c) CDCDCDCDC **D** D D D D D



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m/z	Ion label	Real mass [Da]	Presumed structures
191	I	192	**C**
319	II	320	**CD**
447	III	448	**DCD**
493	IV	494	**CDC**
621	V	622	**a) CDCD** b) CD DC
749	VI	750	**a) DCDCD** b) CDCD D
795	VII	796	**a) CDCDC** b) CDCD C
923	VIII	924	**a) CDCDCD** b) CDCDC D
1050	IX	1051	**a) DCDCDCD** b) CDCDCD c) CDCDCD D D
1179	X	1179	**a) DCDCDCD** b) DCDCDCD **D** D
1381	XI	1382	3D cyclic structure - too complicated to present using following format
1775	XII	1776	3D cyclic structure - too complicated to present using following format

1.3. NMR analyses

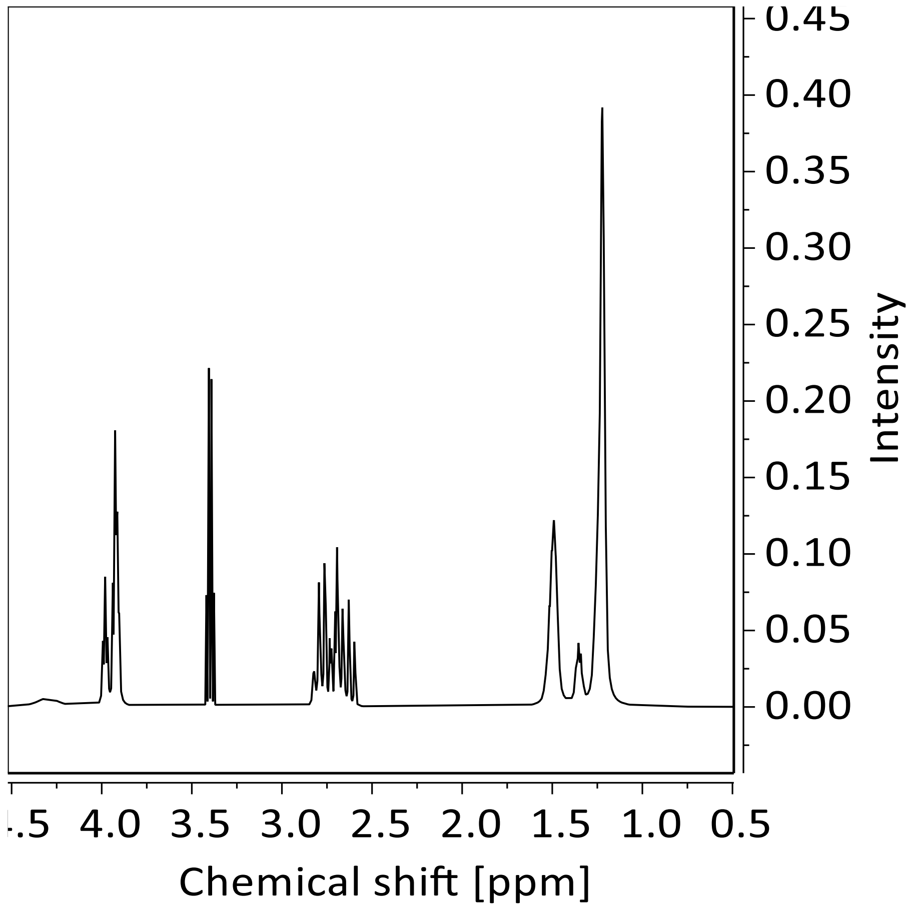

Figure S9. ^{1}H NMR spectrum for POC_1:1 prepolymer.

The ^{1}H NMR spectrum of POC_1:1 prepolymer (Figure S9) shows signals derived from aliphatic groups of octamethylene chain (three multiplets, δ 1.27 ppm, δ 1.41 ppm, δ 1.56 ppm). Multiplet at δ 2.79 ppm originates from methylene protons of citrate units. Two triplets at δ 3.95 and δ 4.05 ppm derive from the protons of -CH_2 groups of alkylene units adjacent to ester bonds. connected to the hydroxyl group of the alkylene, which has been esterified. Slightly visible multiplet δ 3.37 ppm from the protons of the extreme -CH_2 group from alkylene chain in closure to the -OH group unsubstituted with CA. A blurred signal δ 4.33 ppm belongs to hydroxyl proton from octamethylene segments.

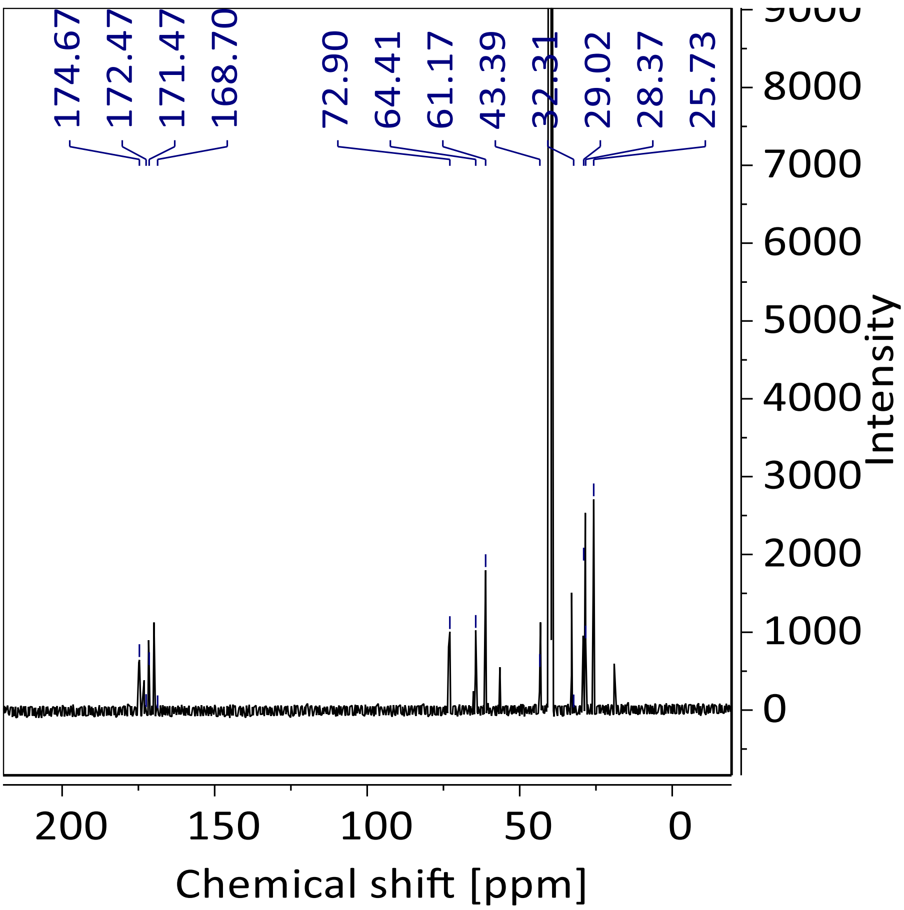

Figure S10. ^{13}C spectrum for POC_1:1 prepolymer.

The ^{13}C spectrum (Figure S10) contains signals δ 25.73, δ 28.37, δ 29.02, δ 32.31 ppm derived from carbons in the octamethylene chain. The δ 61.17 and δ 64.41 signals are derived from the outermost carbons of the -CH_2 alkylene groups, respectively, in the vicinity of the unsubstituted and substituted hydroxyl group. The δ 43.39 and δ 72.90 ppm signals are derived from citrate carbons (citrate aliphatic groups and middle quaternary carbon, respectively). Signals δ 168.70, δ 171.47, δ 172.47 and δ 174.67 ppm are derived from the carbons of citrate carboxyl groups. Signals δ 168.70 and δ 171.47 ppm come from extreme groups, respectively substituted and unsubstituted with alkylene, while signals δ 172.47 and δ 174.67 come from substituted and unsubstituted citrate central carboxyl group.

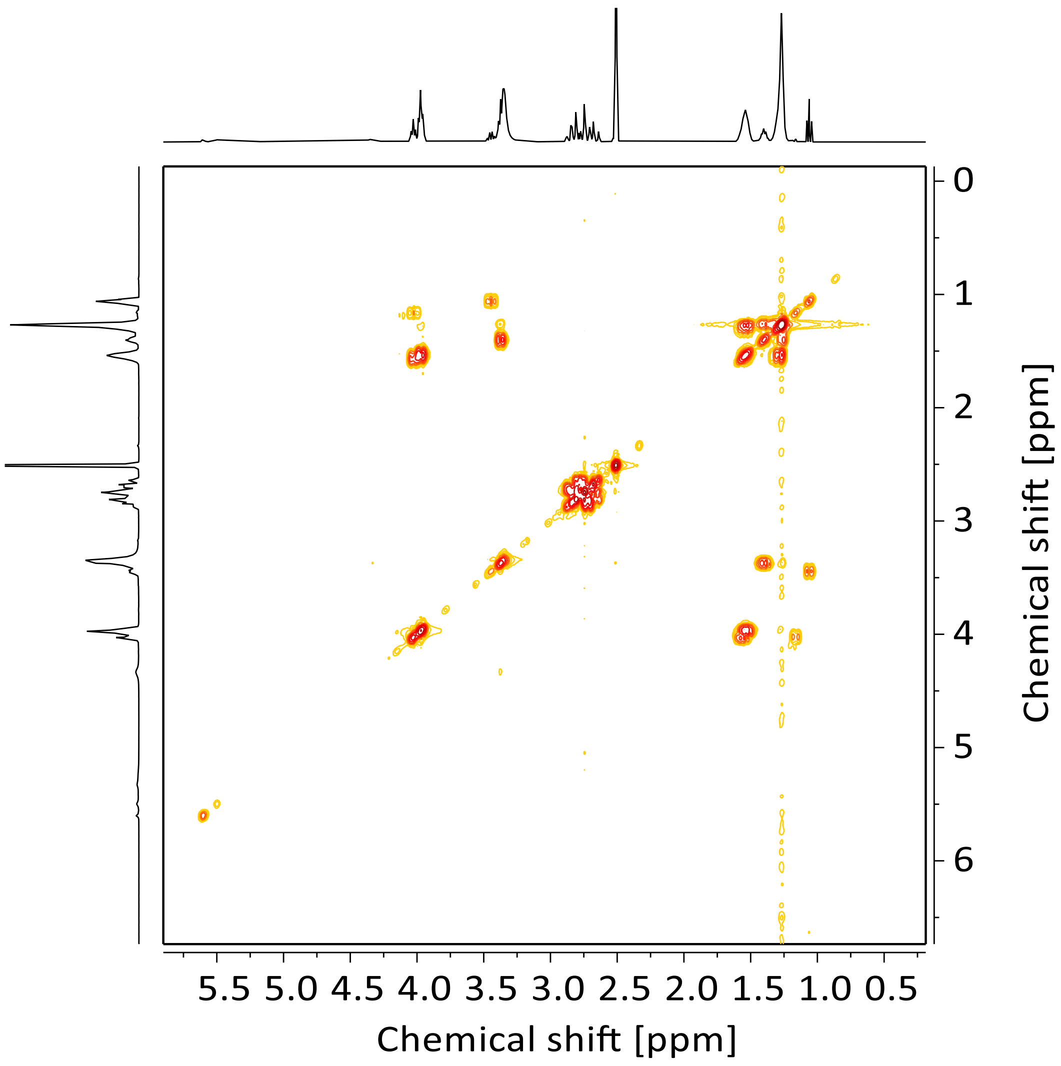

Figure S11. ^{1}H-^{1}H COSY spectrum for POC_1:1 prepolymer.

The correlation spectroscopy (COSY) spectrum (Figure S11) shows the relationship between alkylene and citrate protons. The following correlations were found: 1.25-1.41; 1.52-1.27; 1.32-1.41; 3.36-1.41; 4.05-1.57; 3.95-1.54 ppm.

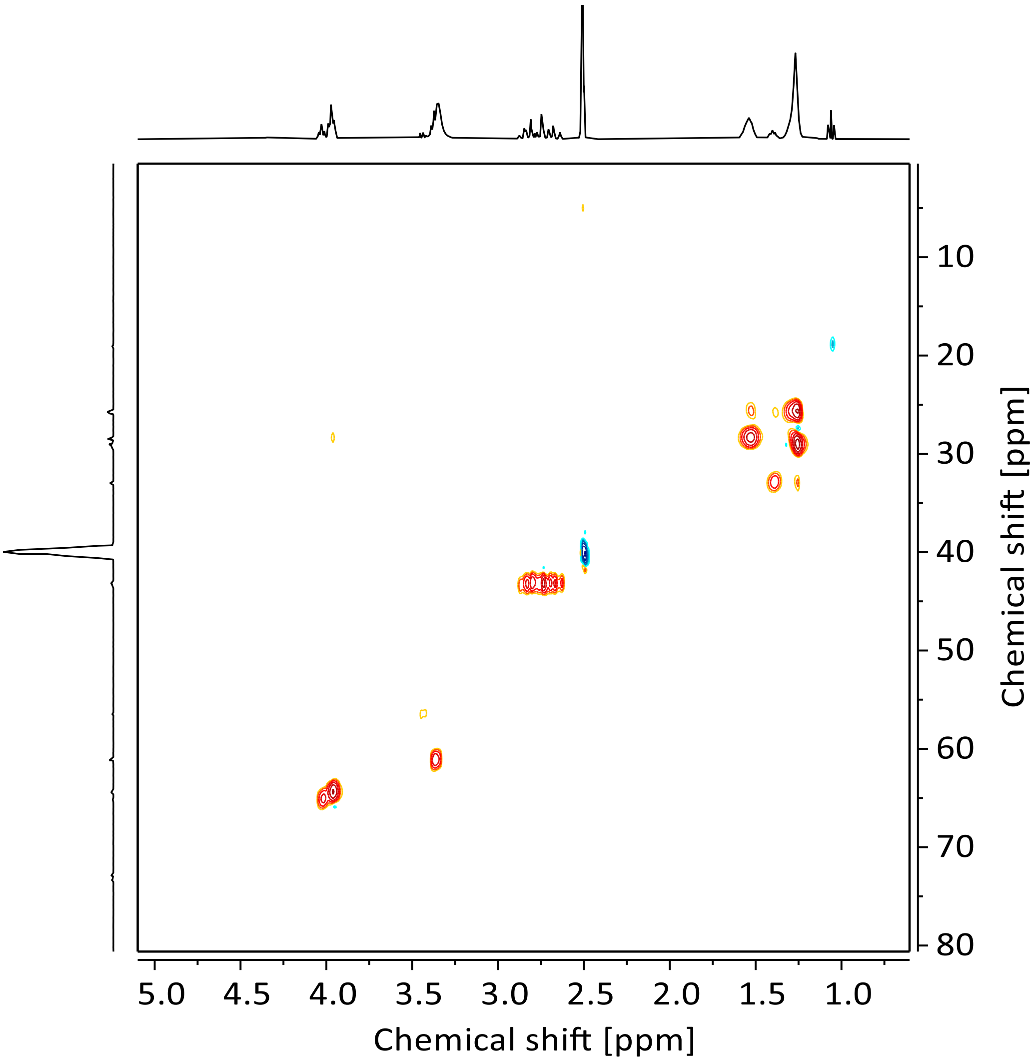

Figure S12. ^{1}H-^{13}C HSQC spectrum for POC_1:1 prepolymer.

In the HSQC spectrum (Figure S12), close-range correlations between protons and carbons were recorded: 1.49-25.52; 1.27-25.77; 1.54-25.77. 1.27-29.13; 1.40-32.99; 3.94-28.40; 2.75-43.30; 3.45-56.64; 3.38-61.26; 4.03-65.19; 3.97-64.51 ppm.

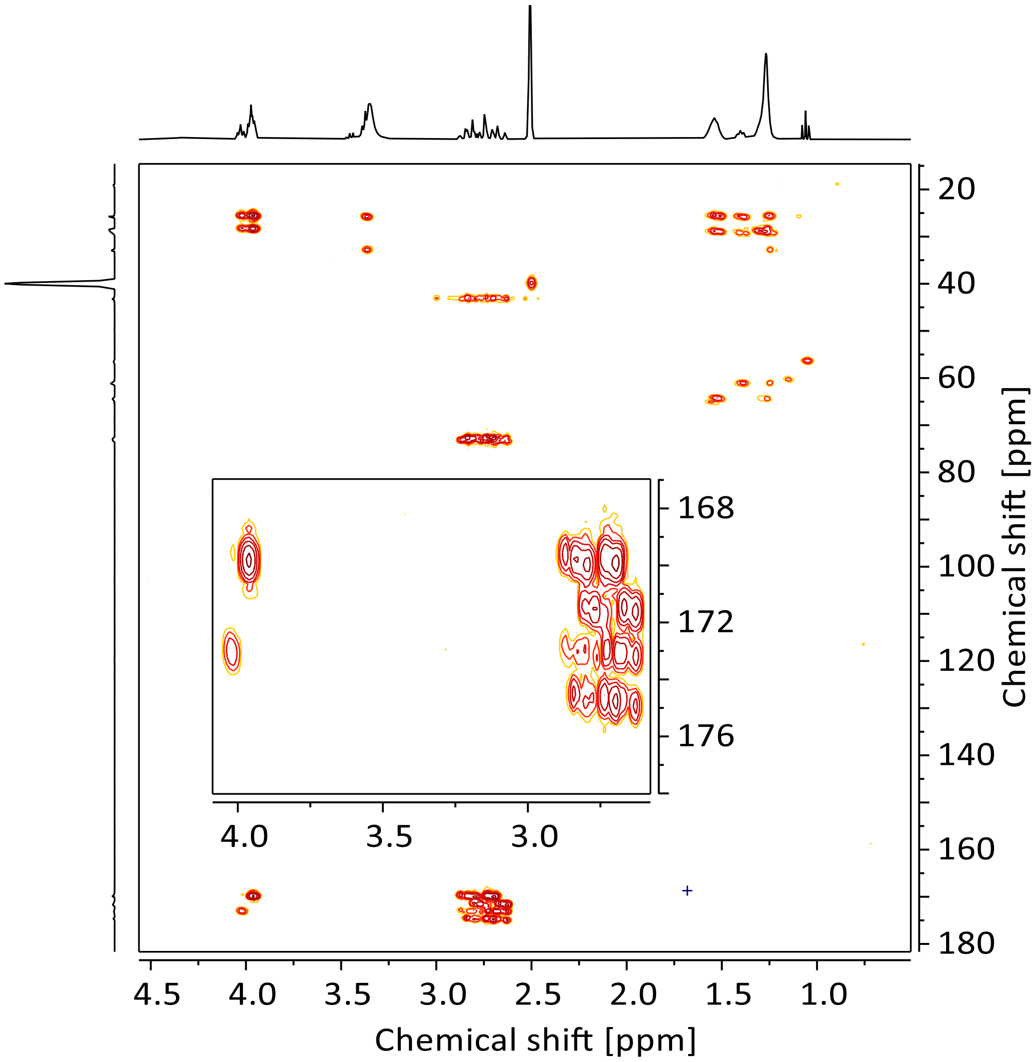

Figure S13. ^{1}H-^{13}C HMBC spectrum for POC_1:1 prepolymer. Signals from correlations in carboxyl region were enlarged.

The HMBC spectrum (Figure S13) allowed to observe the following long-range correlations: 1.26-25.76; 1.29-29.04; 1.55-25.73; 1.28-29.13; 1.40-61.20; 3.97-25.75; 3.38-25.91; 4.03-25.73; 2.64-43.30; 2.64-72.74; 2.85-72.80; 2.75-72.66; 3.96-170.00; 2.64-171.66; 2.64-174.96 ppm.

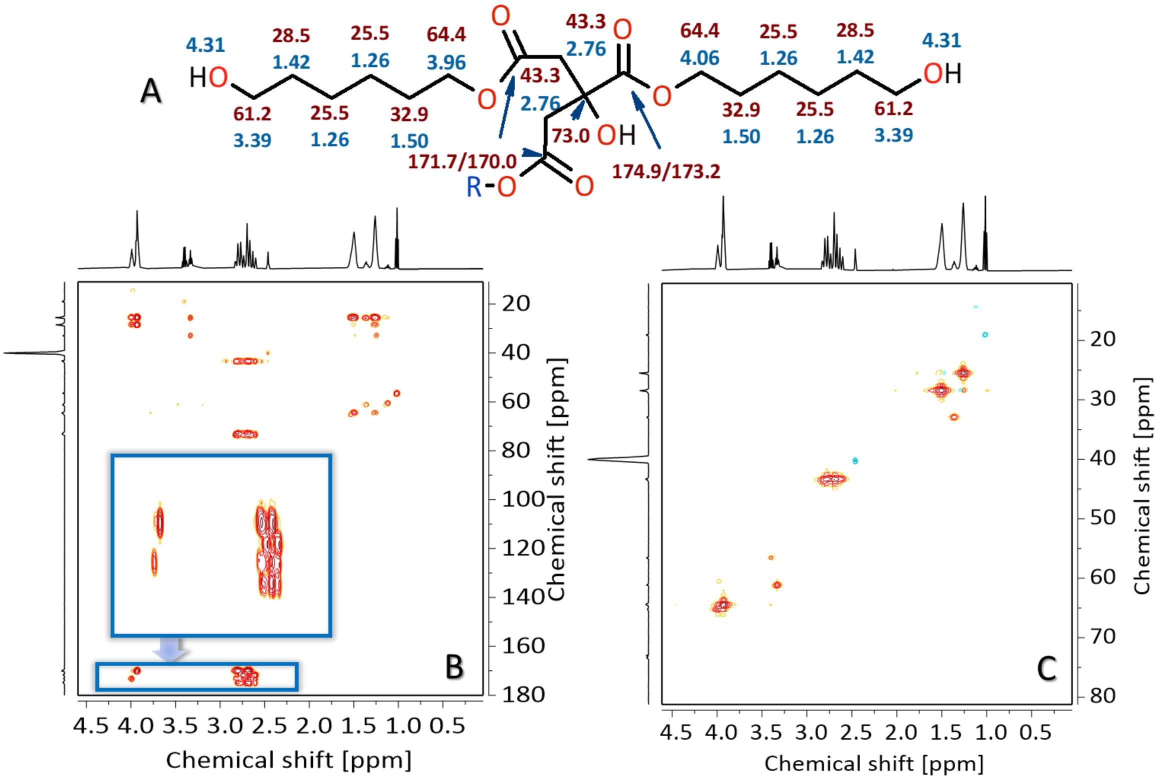

Figure S14. (**A**) Fragment of PHC prepolymer structure along with assignments of chemical shifts (crimson-carbons, blue-protons) based on NMR analysis. (**B**) HMBC spectra of PHC_1:1 prepolymer with expanded proton-carbon correlation in carboxyl region. (**C**) HSQC spectra of PHC_1:1 prepolymer.

In-depth analysis of spectra allowed for unambiguous and precise matching of assignments to the respective atoms, while confirming the proposed structure of prepolymers (Figure 3 and S14). Detailed analyses were carried out for PHC_1:1 and POC_1:1, for PHC_2:3 and POC_2:3 (both fabricated in regular and extended reaction time), due to the identical structure only ^{1}H analyses were performed (Figures S15 – S20). Differences between PHC_1:1 and POC_1:1 ^{1}H spectra are presented in the table (Table S8).

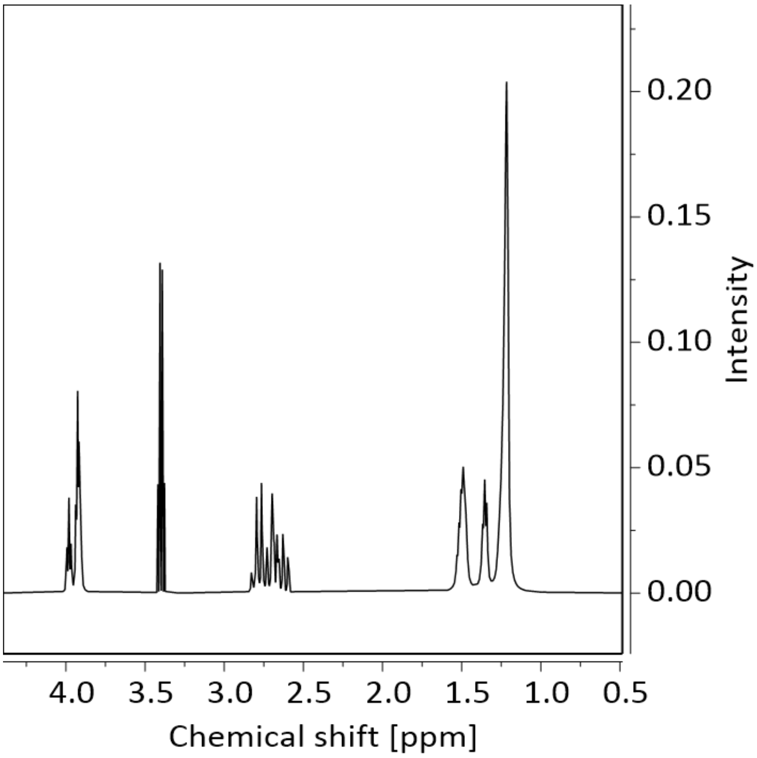

Figure S15. ^{1}H NMR Spectrum for POC_2:3 prepolymer.

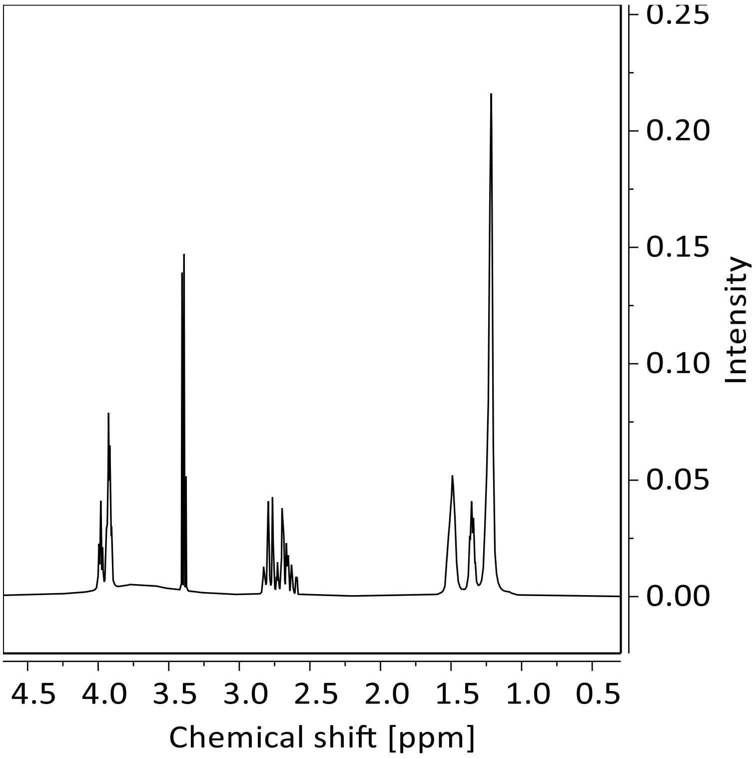

Figure S16. ^{1}H NMR Spectrum for POC_2:3 prepolymer obtained after 60 min of polycondensation.

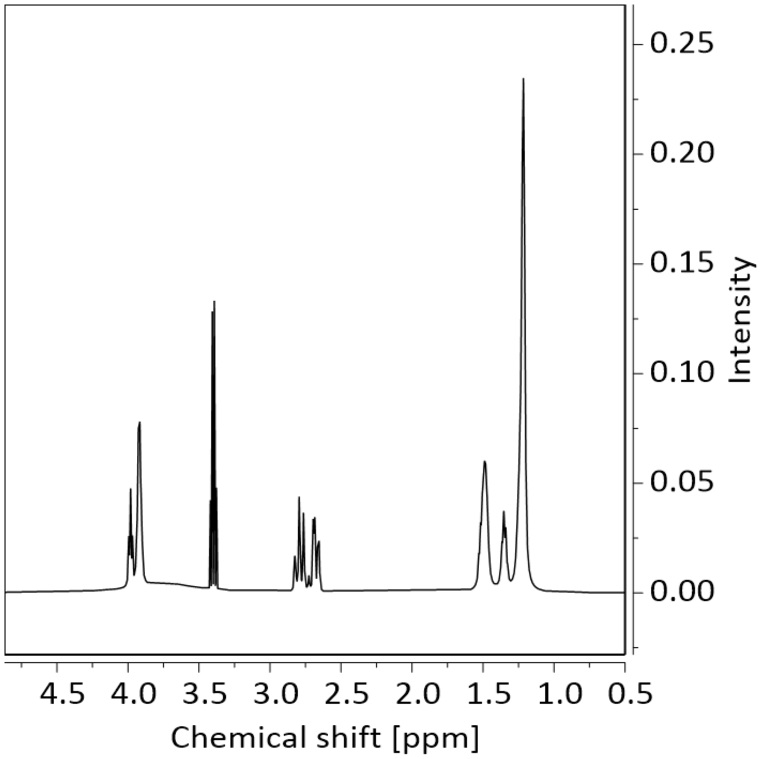

Figure S17. ^{1}H NMR Spectrum for POC_2:3 prepolymer obtained after 90 min of polycondensation.

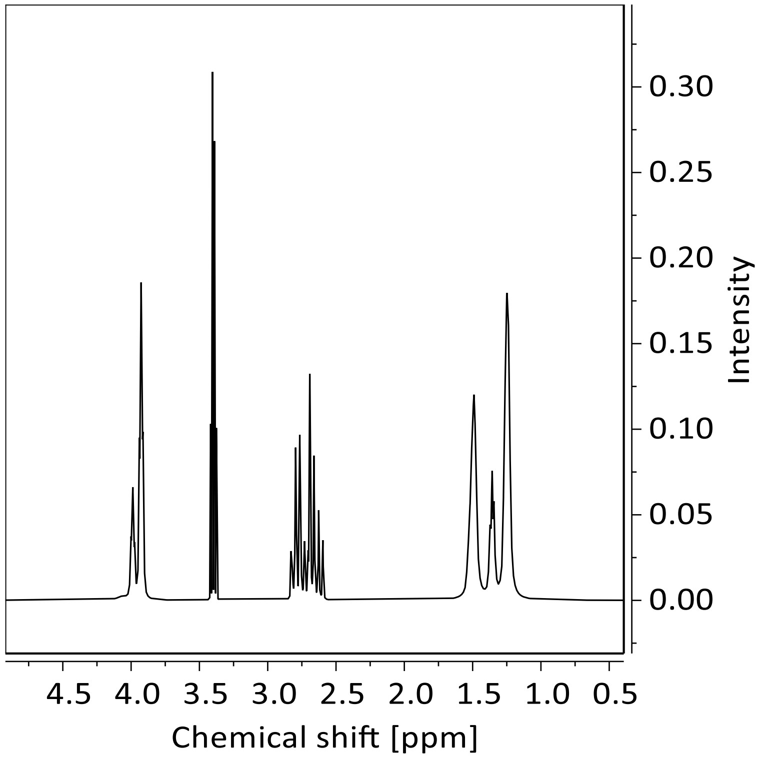

Figure S18. ^{1}H NMR Spectrum for PHC_2:3 prepolymer.

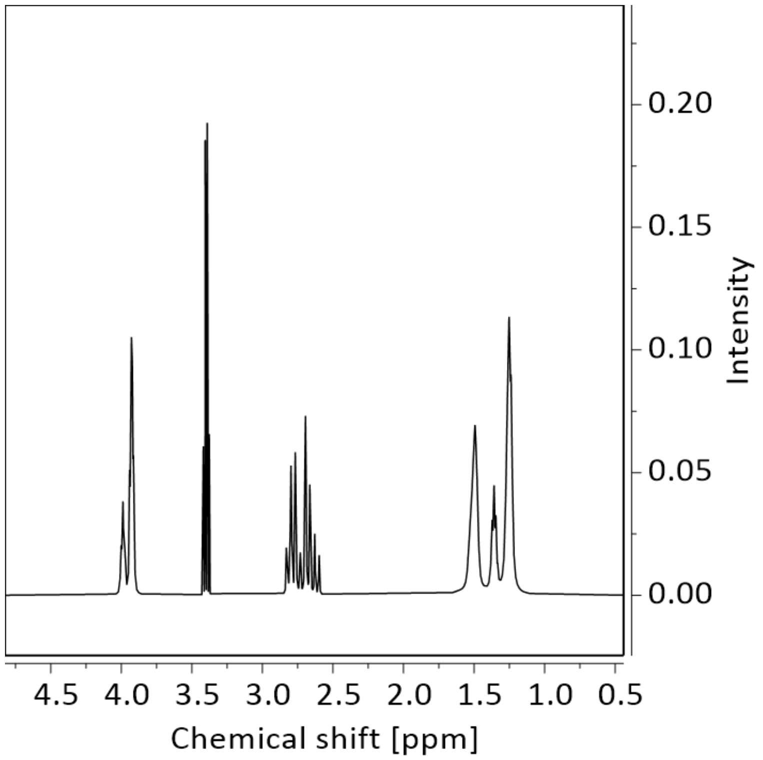

Figure S19. ^{1}H NMR Spectrum for PHC_2:3 prepolymer obtained after 60 min of polycondensation.

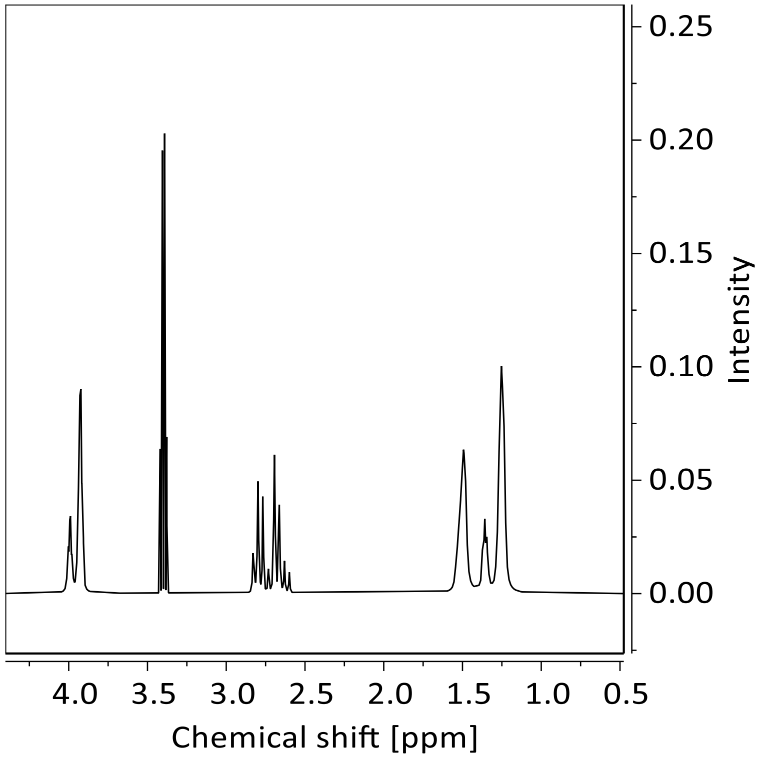

Figure S20. ^{1}H NMR Spectrum for PHC_2:3 prepolymer obtained after 90 min of polycondensation.

Table S8. ^{1}H NMR data from PHC_1:1 and POC_1:1 analysis with shifts assignment to specific groups. Protons, from which signal is derived are in bold.

PHC			POC		
δ [ppm]	**CA**	**HEX**	**δ [ppm]**	**CA**	**OCT**
1,26	---	CA-O-$(CH_2)_2$-$\mathbf{CH_2}$-$\mathbf{CH_2}$-$(CH_2)_2$-OH	1,27	---	CA-O-$(CH_2)_2$-$\mathbf{CH_2}$-$\mathbf{CH_2}$-$\mathbf{CH_2}$-$\mathbf{CH_2}$-$(CH_2)_2$-OH
1,50	---	CA-O-CH_2-$\mathbf{CH_2}$-$(CH_2)_2$-$\mathbf{CH_2}$-CH_2-OH	1,41	---	CA-O-$(CH_2)_6$-$\mathbf{CH_2}$-CH_2-OH
2,76	COOR_1-$\mathbf{CH_2}$-C(OH)COOH	---	1,56	---	CA-O-CH_2-$\mathbf{CH_2}$-$(CH_2)_6$-OH
3,39	---	CA-O-$(CH_2)_5$-$\mathbf{CH_2}$-OH	2,79	COOR_1-$\mathbf{CH_2}$-C(OH)COOH	---
3,96/4,06	---	CA-O-$\mathbf{CH_2}$-$(CH_2)_5$-OH	3,31	---	CA-O-$(CH_2)_7$-$\mathbf{CH_2}$-OH
4,31	---	CA-O-$(CH_2)_5$-**OH**	3,95/4,04	---	CA-O-$\mathbf{CH_2}$-$(CH_2)_7$-OH
			4,33	---	CA-O-$(CH_2)_7$-**OH**

2. Characteristic of cross-linked materials

2.1. Mechanical properties

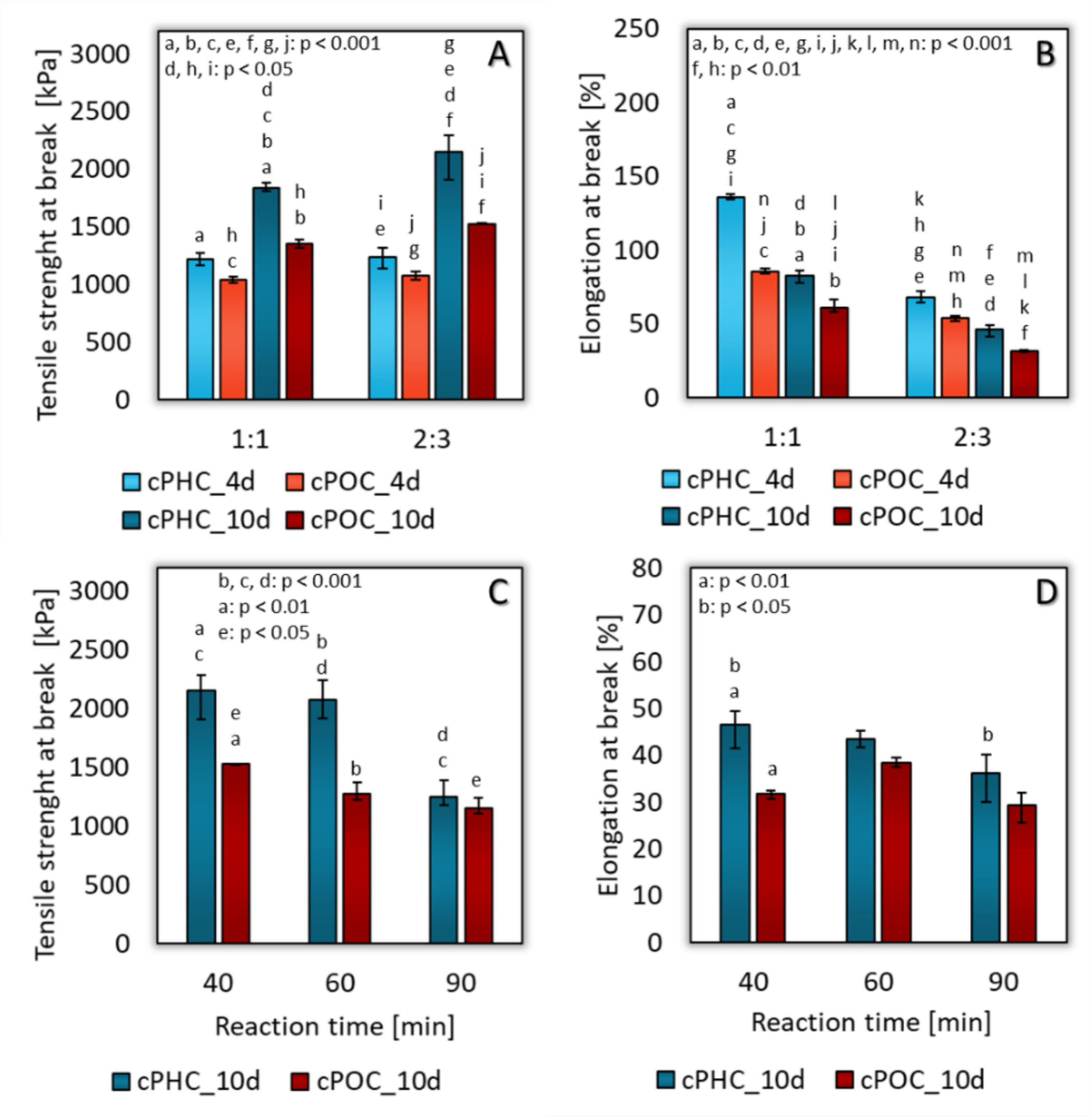

Figure S21. Graphs of mechanical properties at break point for PAC materials. **A** - tensile strength at break, **B** - relative elongation at break and their respective values (**C** and **D**) for PAC materials obtained from prepolymers synthesized in elongated reaction times. Statistical significance of measured values according to one-way ANOVA at $p < 0.001$, $p < 0.01$ and $p < 0.05$. Presented results were compared to each other for the same type of material at the various molar ratios and at the materials in one molar ratio separately. Columns paired with the same letter are significantly different.

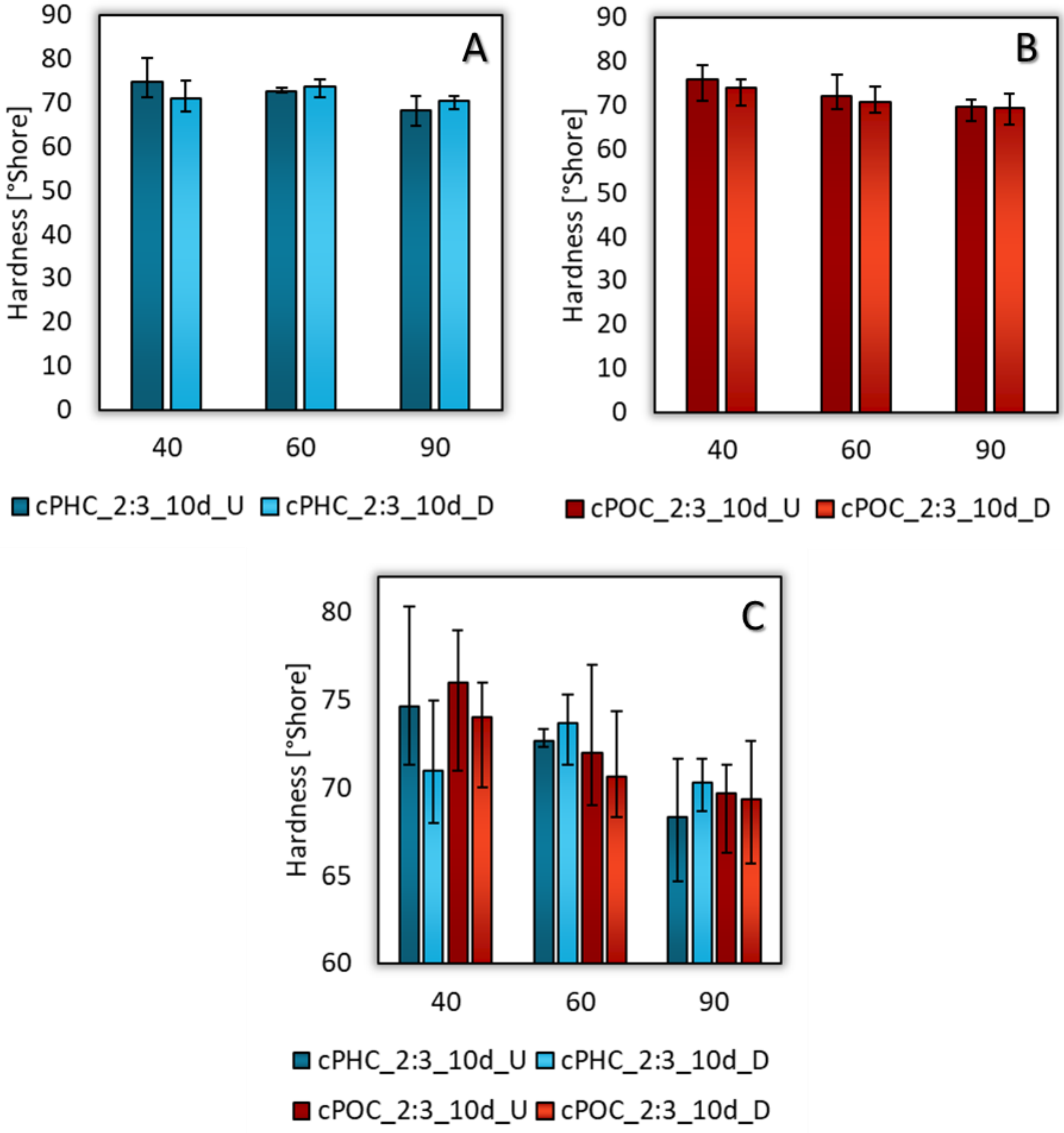

Figure S22. Graphs of hardness of cPAC_2:3 materials obtained in various reaction times, **A** – cPHC materials, **B** – cPOC materials, and closeup of their comparison.