Electronic Supplementary Material (ESI) for Polymer Chemistry
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Supplementary information

for

Determination of copolymerisation characteristics in the N-carboxy anhydride polymerisation of two amino acids

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1. NCA synthesis

Glu(Bzl)-NCA. 15.2 g H-Glu(Bzl)-OH were dissolved in 120 ml ethyl acetate and 30 ml α-pinene (3 equ.) were added. The suspension was heated to 90°C and stirred under reflux and N_2 for 15 min. A solution of 9.5 g triphosgene (0.5 equ.) in 60 ml ethyl acetate was added dropwise. The mixture was stirred under N_2 at 90°C under reflux for 3 h until the reaction turned into a clear, yellow solution.
The reaction was allowed to cool down, filtered and the solvent was reduced to approximately 30% of its original volume. The product was precipitated through addition of *n*-heptane. The

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recrystallisation was repeated twice. The product was dried in a vacuum oven at 50°C over night to yield 14.57 g of white crystals (87% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.05-2.35 (m, 2 H, CH_2-CH), 2.59 (t, J = 6.9 Hz, 2 H, CH_2-COO), 4.38 (t, J = 6.1 Hz, 1 H, NH-C*H*-C=O), 5.13 (s, 2 H, CH_2-O), 6.60 (bs, 1 H, N*H*), 7.30-7.42 (m, 5 H, 5 x C$H_{aromatic}$).
^{13}C NMR (400 MHz, $CDCl_3$): δ = 27.0 (1 C, *C*H_2), 30.0 (1 C, *C*H_2), 57.1 (1 C, *C*H), 67.2 (1 C, *C*H_2), 128.5 (2 C, 2 x *C*$H_{aromatic}$), 128.7 (1 C, *C*$H_{aromatic}$), 128.8 (2 C, 2 x *C*$H_{aromatic}$), 135.3 (1 C, *C*$_{aromatic}$), 151.9 (NH-*C*(=O)-O), 169.5 (CH-*C*(=O)-O), 172.5 (C_6H_5-O-*C*(=O)).

m/z (theoretical): 263.08
m/z (ESI-MS): 262.08 $[M–H]^-$

Lys(Z)-NCA. 15.1 g H-Lys(Z)-OH were dissolved in 120 ml ethyl acetate and 21 ml α-pinene (2.5 equ.) were added. The suspension was heated to 90°C and stirred under reflux and N_2 for 15 min. A solution of 10.0 g triphosgene (0.6 equ.) in 60 ml ethyl acetate was added dropwise. The mixture was stirred under N_2 at 90°C under reflux for 4 h until the reaction turned into a clear solution.
The reaction was allowed to cool down, filtered and the solvent was reduced to approximately 30% of its original volume. The product was precipitated through addition of *n*-heptane. The recrystallisation was repeated twice. The product was dried in a vacuum oven at 50°C over night to yield 13.54 g of white crystals (82% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30-1.60 (m, 4 H, 2 x CH_2), 1.70-2.05 (m, 2 H, CH_2-CH), 3.20 (p, J = 6.7 Hz, 2 H, CH_2-NH), 4.26 (t, J = 5.9 Hz, 1 H, C*H*), 4.95 (bs, 1 H, N*H*-CH_2), 5.09 (s, 2 H, CH_2-O), 6.92 (bs, 1 H, CH-N*H*-C=O), 7.27-7.40 (m, 5 H, 5 x C$H_{aromatic}$).
^{13}C NMR (400 MHz, $CDCl_3$): δ = 21.2 (1 C, *C*H_2), 29.3 (1 C, *C*H_2), 30.8 (1 C, *C*H_2), 40.0 (1 C, *C*H_2), 57.6 (1 C, *C*H), 67.1 (1 C, *C*H_2), 128.1 (1 C, *C*$H_{aromatic}$), 128.3 (1 C, *C*$H_{aromatic}$), 128.4 (1 C, *C*$H_{aromatic}$), 128.6 (1 C, *C*$H_{aromatic}$), 128.7 (1 C, *C*$H_{aromatic}$), 136.5 (1 C, *C*$_{aromatic}$), 152.3 (1 C, NH-*C*=O)-O-C(=O)), 157.1 (1 C, C_6H_5-O-*C*(=O)-NH), 169.9 (1 C, CH-*C*(=O)-O).

m/z (theoretical): 306.12
m/z (MALDI-ToF): 329.15 $[M+Na]^+$, 345.13 $[M+K]^+$

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2. HPLC peak verification

To verify that the signals observed in the HPLC chromatograms correspond to the original side chain protected amino acids, the chromatograms after NCA quenching were compared with those of the unmodified side chain protected amino acids (Figure S1). Small concentration dependent variations in the retention time were observed (see also Figure S4). LC-MS analysis of both the original amino acids and the quenched NCAs yielded the same m/z values (i.e. the m/z values of the original side-chain protected amino acids), confirming that the compounds observed in the chromatograms are identical and the NCA quenching procedure enables recovery of the unmodified amino acids.

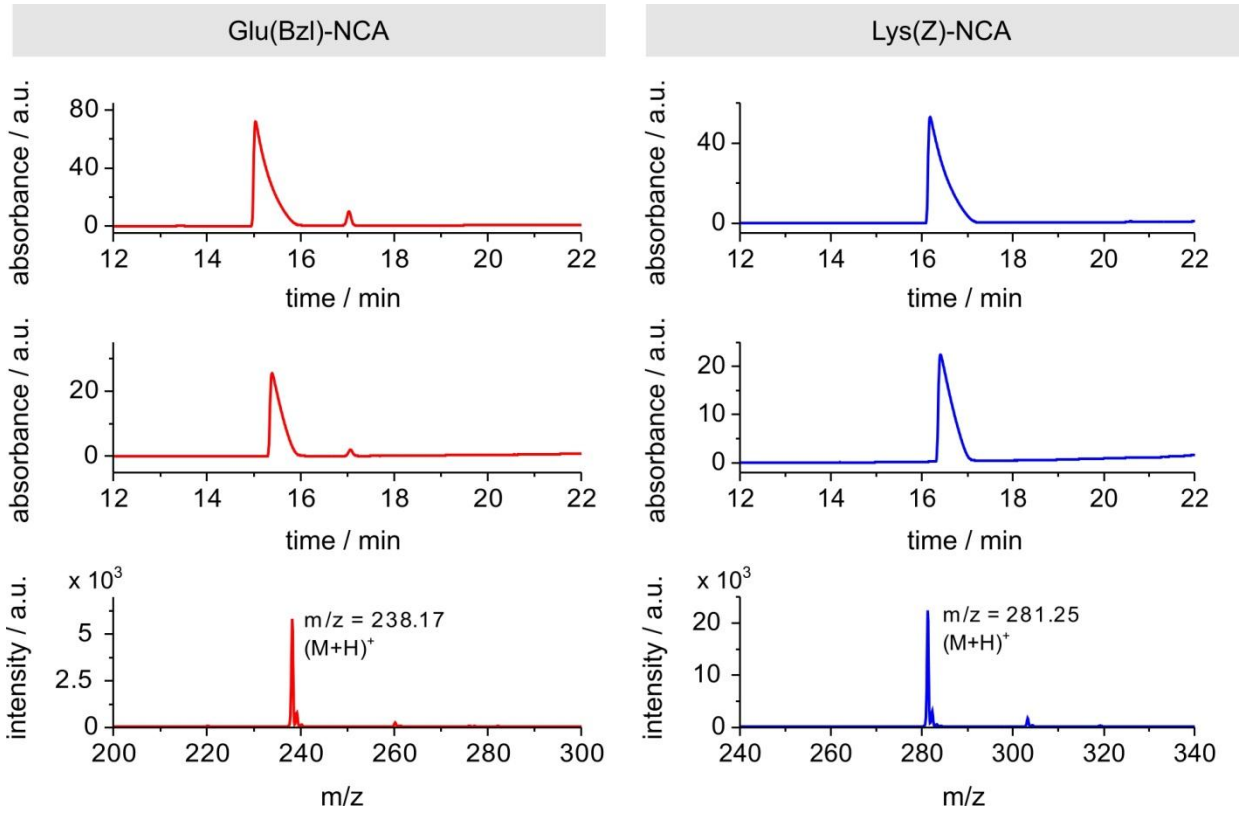

Figure S1. Chromatograms of the side chain protected amino acids at 10 mg/ml (top) and the quenched amino acid NCAs at 100 mg/ml (middle) obtained from HPLC measurements. Mass spectra of the quenched amino acid NCAs (bottom) obtained from LC-MS analysis.

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3. HPLC calibrations

Calibration curves were obtained by analysing quenched solutions of known amino acid NCA concentrations. Stock solutions of Glu(Bzl)-NCA (0.5 g/ml) and Lys(Z)-NCA (0.5 g/ml) were prepared in dry DMF and further diluted to obtain a series of samples with decreasing concentration. Following the same procedure used to quench samples from polymerisation reactions, the amino acid NCAs were converted back to the original side chain protected amino acid derivatives. The samples were subsequently filtered and analysed with the HPLC to obtain the calibration curves shown in Figure S2. The measurements were repeated 3 times and the average values were used to compute the linear regression used for the determination of the amino acid concentrations of the polymerisation samples.

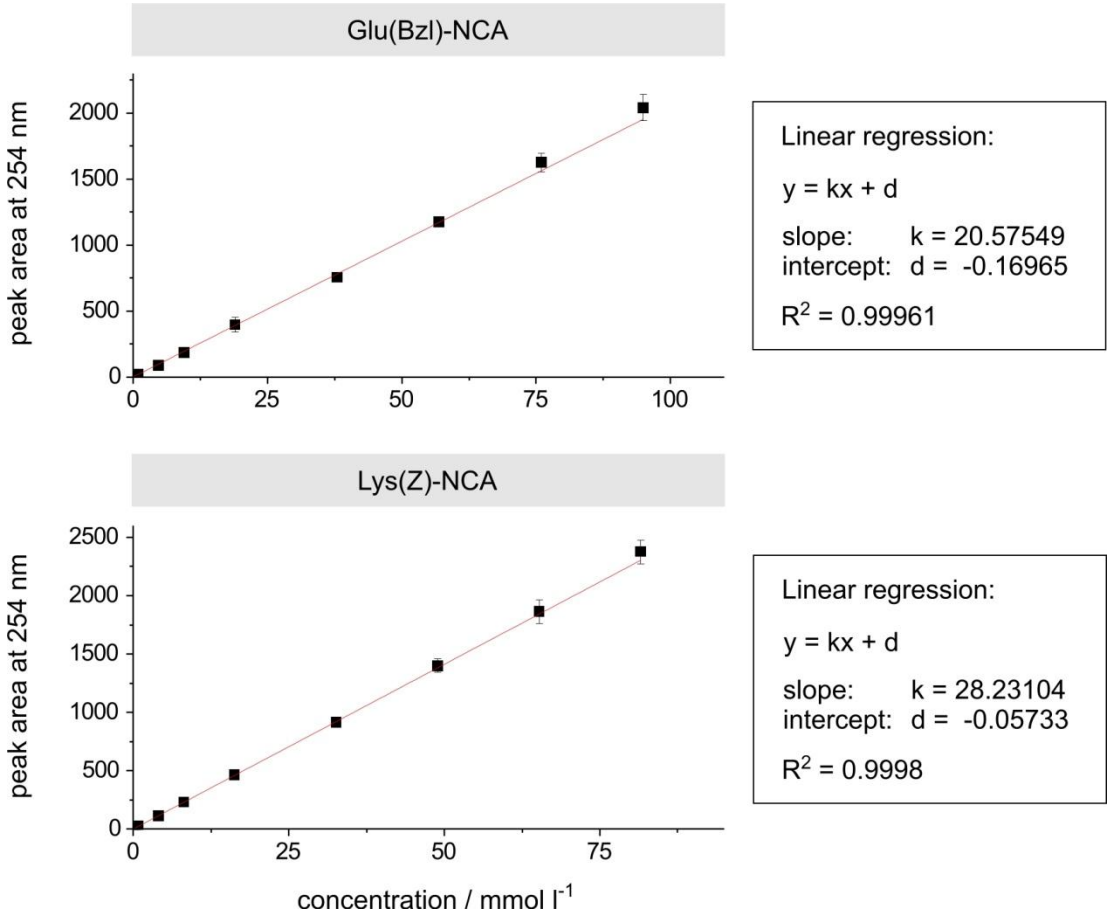

Figure S2. Calibration curves for the amino acid NCAs. The average values are shown and have been used to obtain the linear calibration lines (n = 3, error bars represent standard deviations).

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4. NCA consumption in homopolymerisations

The ability to follow the consumption of amino acid NCAs was first investigated in homopolymerisations of Glu(Bzl)-NCA and Lys(Z)-NCA. Various NCA starting concentrations (0.5, 1, 1.5 and 2 M) were tested. The results are shown in Figure S3.

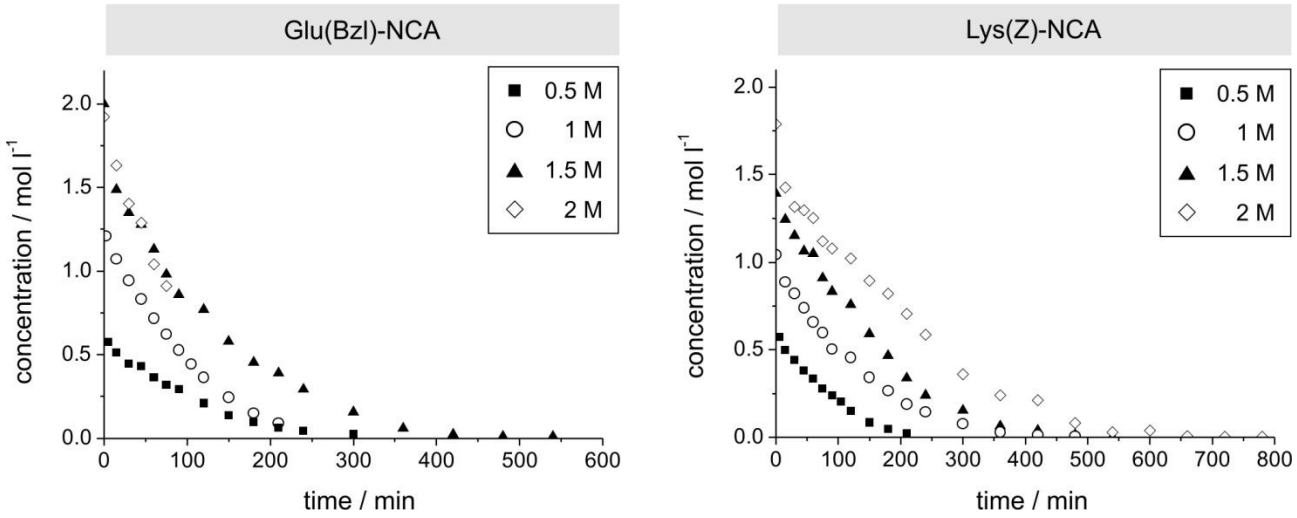

Figure S3. Monomer concentration during the polymerisation of Glu(Bzl)-NCA and Lys(Z)-NCA at various starting concentrations determined by HPLC.

5. Resolution of HPLC signals in the binary mixture

The signals of the two NCAs were resolved in the HPLC without overlap (Figure S4). The small signal just above 17 min is attributed to small amounts of the cleaved benzyl group from Glu(Bzl).

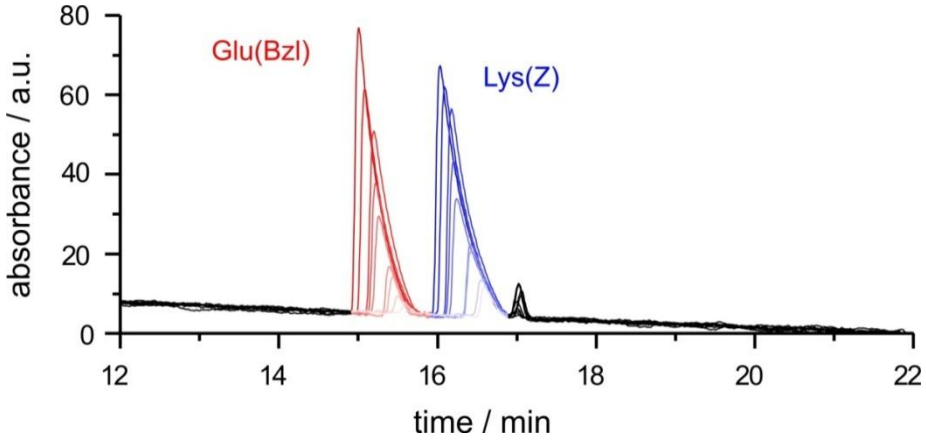

Figure S4. HPLC chromatograms for time-points at 60 min intervals during the copolymerisation of Glu(Bzl)-NCA and Lys(Z)-NCA at a monomer ratio of 1:1.

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6. Determination of copolymerisation parameters

The copolymerisation parameters of the Glu(Bzl)-NCA / Lys(Z)-NCA system were determined by copolymerising mixtures of the two amino acid NCAs at various ratios to low conversion (20 min time-points). The formed polymers were isolated and analysed by NMR (Figure S5). Peak areas of the CH regions of the polypeptide backbone were obtained by curve fits (Figure S6) and used to calculate the copolymerisation parameters r_{Glu} and r_{Lys} for Glu(Bzl)-NCA and Lys(Z)-NCA, respectively. The peak areas and calculated values are given in Table S1, the copolymerisation parameters are reported in Table 3 in the main manuscript.

Method by Fineman-Ross

$$\frac{f(F-1)}{F} = \left(\frac{f^2}{F}\right) r_{Glu} - r_{Lys} \qquad \text{(Equ 1)}$$

with

$$f = \frac{n_{Glu}}{n_{Lys}} \qquad \text{(Equ 2)}$$

and

$$F = \frac{N_{Glu}}{N_{Lys}} \qquad \text{(Equ 3)}$$

with n_{Glu} and n_{Lys} being the mole-fraction of Glu(Bzl)-NCA andLys(Z)-NCA in the feed and N_{Glu} and N_{Lys} the mole-fractions of Glu(Bzl) and Lys(Z) in the polymer.

A linear regression of the plot of $f(F-1)/F$ vs f^2/F yields r_{Glu} and r_{Lys} as the slope and the intercept, respectively (Figure S7a).

Method by Kelen-Tüdös

$$\eta = \xi\left(r_{Glu} + \frac{r_{Lys}}{\alpha}\right) - \frac{r_{Lys}}{\alpha} \qquad \text{(Equ 4)}$$

with

$$\eta = \frac{f(F-1)}{F\left(\alpha + \frac{f^2}{F}\right)} \qquad \text{(Equ 5)}$$

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$$\xi = \frac{\left(\frac{f^2}{F}\right)}{\alpha + \left(\frac{f^2}{F}\right)} \qquad \text{(Equ 6)}$$

and

$$\alpha = \left(\left(\frac{f^2}{F}\right)_{max} \times \left(\frac{f^2}{F}\right)_{min}\right)^{1/2} \qquad \text{(Equ 7)}$$

This equation equally yields a linear plot of η vs ξ for which slope and intercept can be determined (Figure S7b). r_{Lys} can be determined when ξ = 0, which allows rearrangement of Equ 4 to

$$r_{Lys} = -\eta_{(\xi=0)} \times \alpha = -d \times \alpha \qquad \text{(Equ 8)}$$

If ξ = 1, $r_{Glu} = \eta$ which can be calculated from the equation of the linear regression according to

$$r_{Glu} = \eta_{(\xi=1)} = k + d \qquad \text{(Equ 9)}$$

with *k* and *d* being the slope and the intercept of the linear regression, respectively.

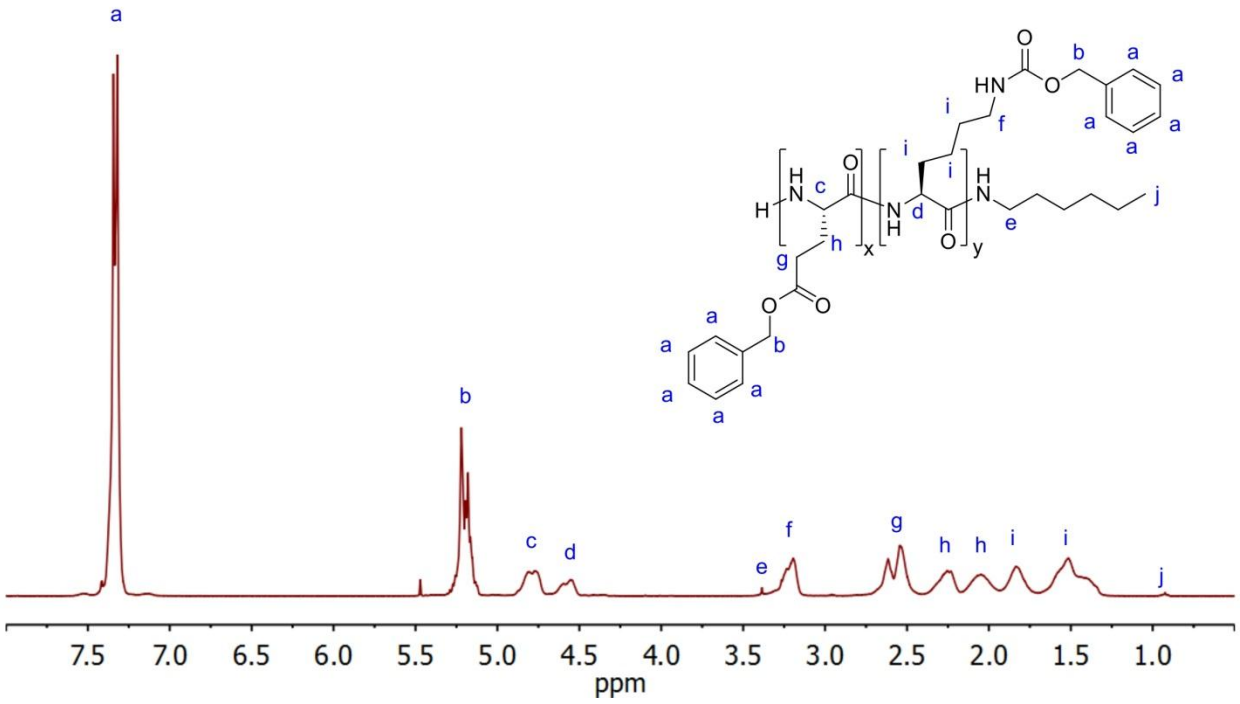

Figure S5. [1]H-NMR spectrum of the copolypeptide in TFA obtained after reaction of Glu(Bzl)-NCA and Lys(Z)-NCA for 20 min at a ratio of 1:1.

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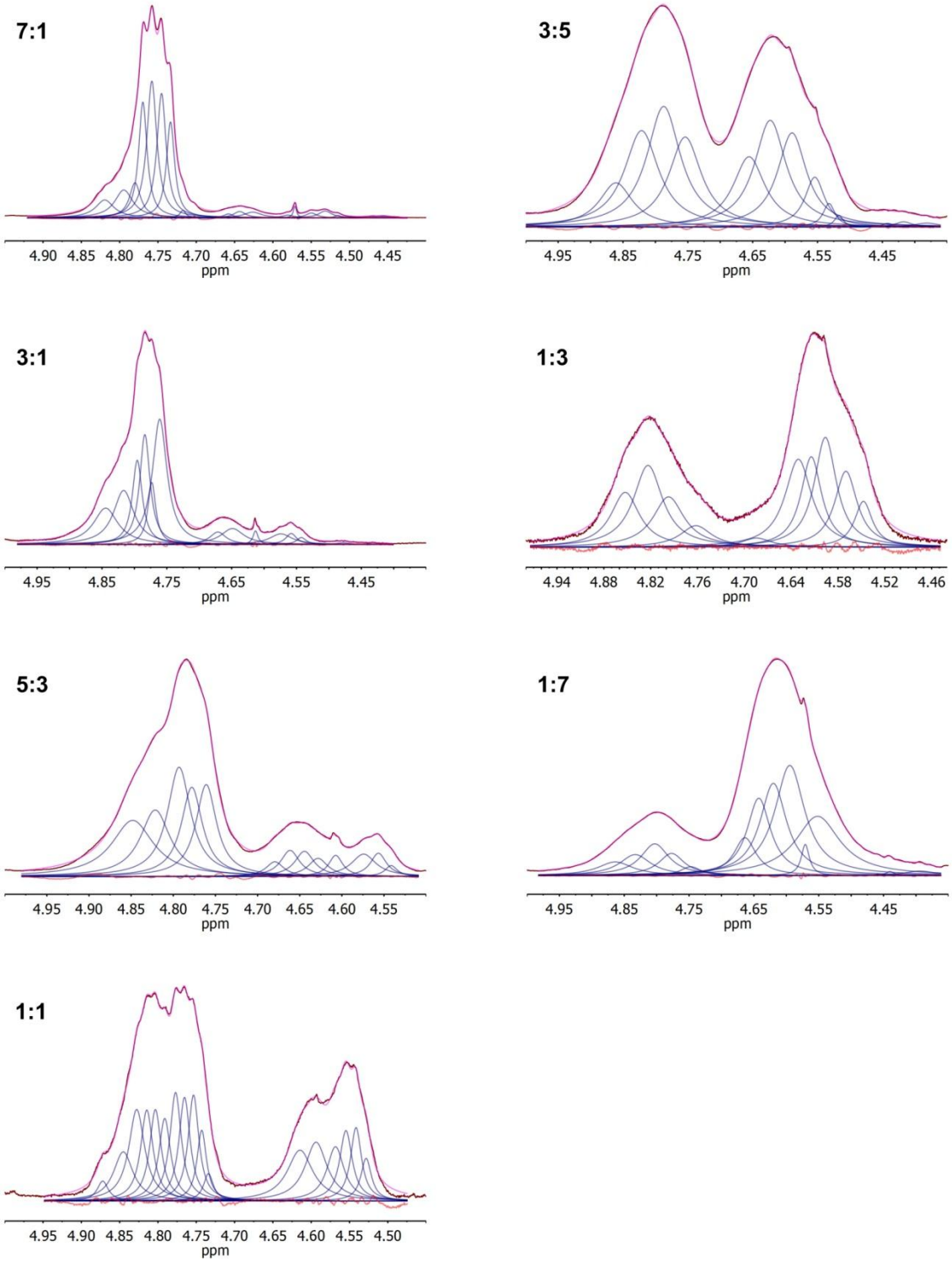

Figure S6. Peak fits to the backbone CH signals in the ^{1}H-NMR spectra of polypeptides obtained after copolymerisation of mixtures of Glu(Bzl)-NCA and Lys(Z)-NCA at various ratios (ratios are displayed as Glu(Bzl)-NCA : Lys(Z)-NCA).

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Table S1. Data used for the calculation of the copolymerisation parameters for the Glu(Bzl)-NCA / Lys(Z)-NCA system.

feed composition	feed ratio (f)	polymer composition[a]			calculated values			
		peak area Glu(Bzl)	peak area Lys(Z)	ratio (*F*)	f^2/F	f(F-1)/F	ξ	η
7:1	7.00	87202153	7239500	12.05	4,07	6,42	0,87	1,38
3:1	3.00	170925598	29591677	5.78	1,56	2,48	0,73	1,16
5:3	1.67	48529755	13434887	3.61	0,77	1,21	0,57	0,89
1:1	1.00	111453631	61616533	1.81	0,55	0,45	0,49	0,39
3:5	0.60	64713768	54642255	1.18	0,30	0,09	0,34	0,10
1:3	0.33	59628212	90606843	0.66	0,17	-0,17	0,22	-0,22
1:7	0.14	21372067	88335031	0.24	0,08	-0,45	0,13	-0,67

[a] obtained from ^{1}H-NMR spectra by fitting peaks to the backbone CH regions

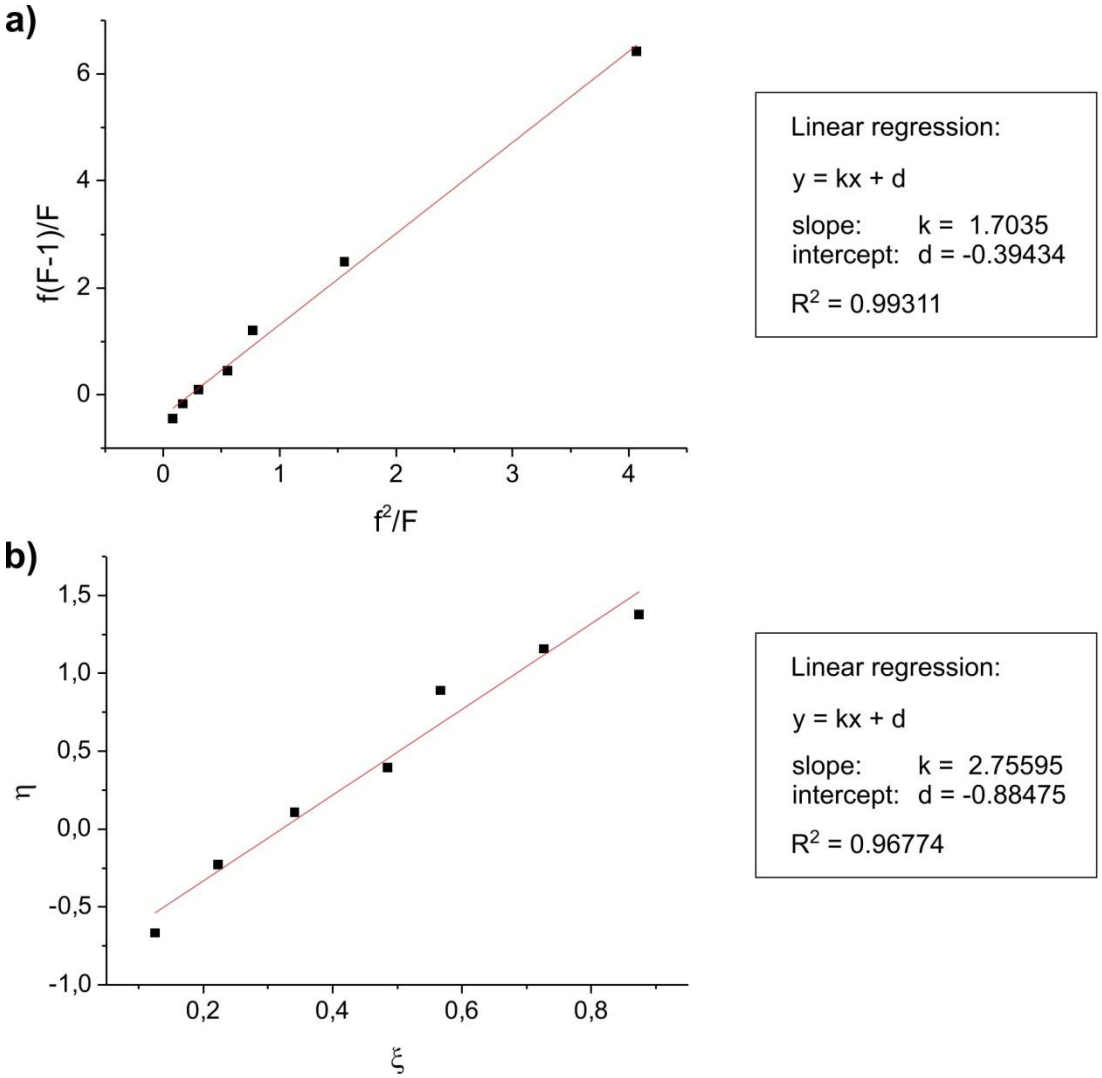

Figure S7. Determination of the copolymerisation parameters using the approach by Fineman-Ross (a) and Kelen-Tüdös (b).