Supporting Information

Primary Ammonium/Tertiary Amine-Mediated Controlled Ring Opening Polymerisation of Amino Acid *N*-Carboxyanhydrides

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CONTENTS

EQUIPMENT AND METHODS

Nuclear Magnetic Resonance (NMR)

For the polymer and benzylamine hydrochloride analyses: ^{1}H-NMR, ^{13}C-NMR and HSQC spectra were recorded on a Bruker Avance III 600 MHz Spectrometer in deuterated trifluoroacetic acid (TFA-d). Unless mentioned otherwise the number of scans was at least 32.

For all other analyses: ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance 300 MHz Spectrometer. Unless mentioned otherwise the number of sans was of 128 for ^{1}H-NMR and of 1024 for ^{13}C-NMR.

Size Exclusion Chromatography (SEC)

SEC with simultaneous UV and RI detection was performed in a solution of *N*-methyl-2-pyrrolidone (NMP) with 0.5 wt% LiBr at a flow rate of 0.8 mL·min^{-1} at 70 °C, on PSS-GRAM columns (7 µm particle size, 100 and 1000 Å porosity). Polymer solutions were of 0 to 3 mg·mL^{-1}; PMMA standards (PSS, Mainz, Germany; M_p ranged from 505 to 898,000 g·mol^{-1}) were used.

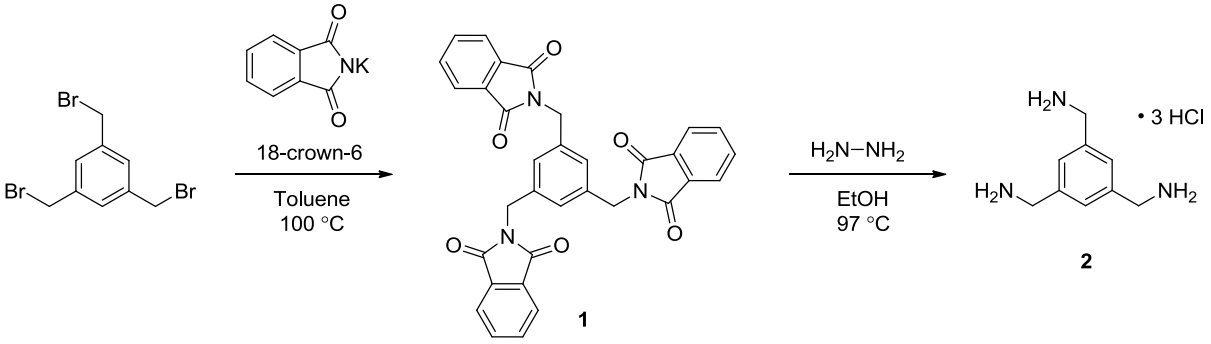
Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was performed on a Bruker Vertex 70 fitted with a PLATINUM ATR. Liquid samples were placed directly on the ATR diamond under a mixture of dry air and nitrogen flow. The spectra were acquired and processed with OPUS. The number of scans was 32, the built-in atmospheric correction function was turned on, and the background was automatically subtracted; in the case of liquid samples, the background was generated using the very same solvent as the one used for the sample.

The spectra were they processed as follows: the 1758-1815 cm^{-1} portion of the spectra were isolated ('cut' function), baseline corrected ('concave rubberband correction', 1 iteration), and fitted with a Gaussian function ('curve fit' function); the calculated area under the curve was then extracted.

SYNTHESES

Synthesis of 1,2,3-Tris(aminomethyl)benzene Trihydrochloride (TAB·3HCl)



All chemicals were purchased from Sigma-Aldrich.

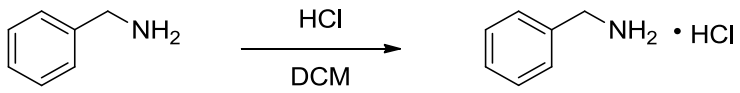
I. Synthesis of intermediate product (**1**)

1,2,3-Tris(bromomethyl)benzene (3.50 g, 1 equiv.), phtalamide potassium (6.54 g, 3.6 equiv.) and 18-crown-6 (0.78 g, 0.3 equiv.) were suspended in 45 mL of toluene in a flame-dried and nitrogen-purged round bottom flask. The mixture was refluxed for 24 h at 100 °C. 40 mL of distilled water was added and the mixture was allowed to phase separate. The aqueous layer was pipetted out and extracted 3 times with dichloromethane (DCM). The organic layers were collected and dried over $MgSO_4$, filtered, and evaporated to dryness under reduced pressure. The residue was re-dissolved in a minimum of DCM under moderate heat and purified by column chromatography using a DCM/acetone (20:1) eluent. R_f(**1**) = 0.6. Yield: 75 %. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.78 (s, 6H), 7.35 (s, 3H), 7.70-7.82 (m, 12H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 41.34, 123.52, 127.95, 132.22, 134.06, 137.44, 167.99.

II. Synthesis of TAB·3HCl (**2**)

The purest fractions containing **1** were collected and evaporated to dryness under reduced pressure. **1** (1.16 g, 1 equiv.) was then slurried with 70 mL of dry ethanol into a flame-dried and nitrogen-purged flask. Under vigorous stirring, 0.8 mL of hydrazine (6 equiv.) was added dropwise. The mixture was refluxed for 16 h at 97 °C. 3.5 mL distilled water was then added. The mixture was then acidified with fuming HCl (12.1 N) down to pH 3. The precipitate was then filtered, washed with ethanol/water (95:5, pH 3), and the filtrates were collected and evaporated to dryness under reduced pressure. The residue was slurried in acidic water, filtered, washed with ethanol/water (95:5, pH 3), and the filtrate was evaporated again. The residue was recrystallised from dioxane/water (20:1). Yield: 38 %. ^{1}H-NMR (300 MHz, D_2O): δ 4.28 (s, 6H), 7.58 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 42.88, 130.39, 134.97.

Synthesis of Benzylamine Hydrochloride (BnA·HCl)



Benzylamine (BnA) was purchased from Sigma-Aldrich.

3 mL of BnA (1 equiv.) was diluted in 7 mL of dichloromethane (DCM). Under vigorous stirring, 3 mL of fuming HCl (12.1 N) (1.1 equiv.) was added dropwise. The precipitate was filtered, washed with DCM and recrystallised from a minimum of dry ethanol to yield pearly-white needles. The latter were dried under high vacuum for 24 h. Yield: 85%. ^{1}H-NMR (600 MHz, DMSO-d6): δ 3.99 (s, 2H), 7.37-7.53 (m, 5H), 8.64 (s, 3H). ^{13}C-NMR (151 MHz, DMSO-d6): δ 42.25, 128.52, 128.69, 129.16, 134.31.

γ-Benzyl-L-glutamate NCA (BLG-NCA)

L-Glutamic acid γ-benzyl ester (≥99%) and anhydrous tetrahydrofuran (THF) (≥99%) were purchased from Sigma-Aldrich.

Typically, 7.5 g (1 equiv.) of L-glutamic acid γ-benzyl ester was placed in a flame-dried and nitrogen-purged round bottom flask. It was dried under high vacuum for 12 hours in 150 mL dry THF. Under vigorous stirring, triphosgene (3.75 g, 0.4 equiv.) was added and the mixture heated to 50 °C for 3 h, or at least 1 h after the mixture has become completely translucent (yellowish). The mixture was reduced to about 20 mL under reduced pressure and precipitated in 200 mL re-distilled heptanes under inert atmosphere. The precipitate was filtered, washed with heptanes, and dried under high vacuum for 1 h. It was then re-dissolved in 20mL of dry THF, precipitated, filtered, washed and dried for at least 12 h. The NCAs were then stored under inert atmosphere at -25 °C. Melting point = 93-94 °C. Yield = 94%. See NMR section for NMR spectra.

L-Leucine NCA (LLEU-NCA)

L-Leucine (≥99%) was purchased from Sigma-Aldrich.

The procedure used to synthesise LLEU-NCA was identical to that used for BLG-NCA (above). Typically, all other things being equal, 7.5 g (1 equiv.) of L-leucine was used for 8.55 g (0.5 equiv.) of triphosgene. Melting point = 76-78 °C. Yield = 79%. See NMR section for NMR spectra.

L-Phenylalanine NCA (LPHE-NCA)

L-Phenylalanine (≥99%) was purchased from Sigma-Aldrich.

The procedure used to synthesise LPHE-NCA was identical to that used for BLG-NCA (above). Typically, all other things being equal, 5 g (1 equiv.) of L-Phenylalanine was used for 4.5 g (0.5 equiv.) of triphosgene. Melting point = 90-91 °C. Yield = 83%. See NMR section for NMR spectra.

Polymerisations

All polymerisations were performed according to formerly reported procedures.[1]

The NCA concentration was 60 $g \cdot L^{-1}$ for the polymerisations of BLG-NCA (150 equiv.) in DMF initiated by TAB·3HCl (1 equiv.), TAB·3HCl/TEA (1:0.5 equiv.), and TEA (0.5 equiv.).

The NCA concentration was 100 $g \cdot L^{-1}$ for the polymerisations of BLG-NCA (150 equiv.) in DMF initiated by PyA·HCl (1 equiv.), PyA·HCl/TEA (x:y equiv. with x = 1 and y = 0.2, 0.5, 0.7, 0.9, 1.1, and 1.5).

The NCA concentration was 100 $g \cdot L^{-1}$ for the polymerisations of BLG-NCA, LLEU-NCA, and LPHE-NCA (100 equiv.) in DMF initiated by BnA (1 equiv.), BnA·HCl (1 equiv.), BnA·HCl/TEA (1:0.5 equiv.), and TEA (0.5 equiv.).

MONITORING MONOMER CONVERSION

Monomer conversion followed by GPC

By using an internal standard, the monomer conversion was indirectly calculated from the ratio of the area under the polymer peak to that of the internal standard in SEC elugramms, which was translated in a polymer concentration (wt %) using a calibration curve. For this method to be reliable, it is necessary that the growing polymer and internal standard peaks do not overlap; in other words, the internal standard ought to be either a small molecule or a high molar mass polymer.

In order to avoid of the difficulties associated with the integration of peaks at high elution volumes (e.g., presence of impurities, solvent or monomer peaks), we chose a polystyrene standard of Mw = 1,815,000 $g{\cdot}mol^{-1}$ (PS2M). Since such polymer is unlikely to take part in NCA polymerisations, it could be added (i) either directly to our polymerisation medium as it was the case for our PyA·HCl/TEA (x:y with x = 0 to 1 equiv. and y = 0 to 1.5 equiv.) initiated polymerisations, or (ii) in controlled amounts to know volumes sampled from our reaction media as it was the case for our TAB·3HCl/TEA (x:y with x = 0 to 1 equiv. and y = 0 to 0.5 equiv.) initiated polymerisations.

The calibration curve was prepared using a series of solutions composed of $PBLG_{51}$/PS2M (x:y) in 2.5 mL DMF, with x = 15 mg for all solutions, and y = 1, 3, 5, 10, 15, 30, and 60 mg (Fig. S1), as well as a control solution composed of 15 mg of PS2M only.

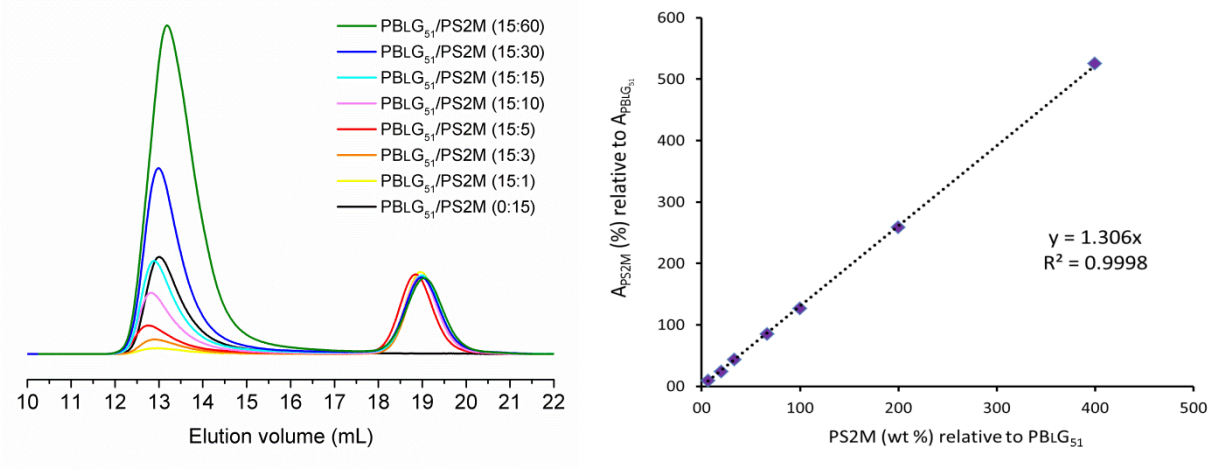


Fig. S1 (left) SEC traces of the $PBLG_{51}$/PS2M calibration series; (right) calibration curve of the $PBLG_{51}$/PS2M calibration series where the PS2M/$PBLG_{51}$ ratio of the areas under the elution peaks is plotted as a function of the PS2M/$PBLG_{51}$ weight ratios

Monomer conversion followed by FTIR

The absorbance peaks (1850-1855 cm^{-1} and 1785-1790 cm^{-1}) of the carbonyl bonds (C=O stretch) of the tested NCAs (BLG, LLEU and LPHE) do not overlap with any other absorbance peak of the corresponding polymers (Fig. S2). The 1850-1855 cm^{-1} and the 1785-1790 cm^{-1} peak were assigned by Kricheldorf to the (C-)C=O and the (N-)C=O groups, respectively. This assignment was mostly based on the fact that 2-thioxooxazolidine-5-ones (TOOs), which possess only one carbonyl group, (C-)C=O, only exhibit one carbonyl band at 1850 cm^{-1}.[2]

The peak at 1785-1790 cm^{-1} is larger, and as such likely to incur less error than the smaller 1850-1855 cm^{-1} peak; it was, therefore used to monitor the monomer conversion. The calibration series was composed of NCA solutions in DMF of concentration ranging from 0 to 100 $g.L^{-1}$ (Fig. S3). In order to ensure that the presence of polypeptide did not affect the calibration curve in any way, a control calibration series of BLG-NCA/$PBLG_{51}$ (x:y with x and y ranging from 0 to 10 and x+y=1) solutions in DMF of total concentration ranging from 0 to 100 $g{\cdot}L^{-1}$ was measured and yielded an almost identical calibration curve to that of the calibration series using BLG-NCA alone.

No internal standards were used: the area under the fitted 1850-1855 cm^{-1} peak was directly related to the NCA concentration in DMF. The consistency of the method was confirmed by a coefficient of determination (R-squared) close to 1 and almost identical linear regression equations obtained for 2 repeats. This consistency relied on a systematic processing of the spectra as described in the EQUIPMENT section.

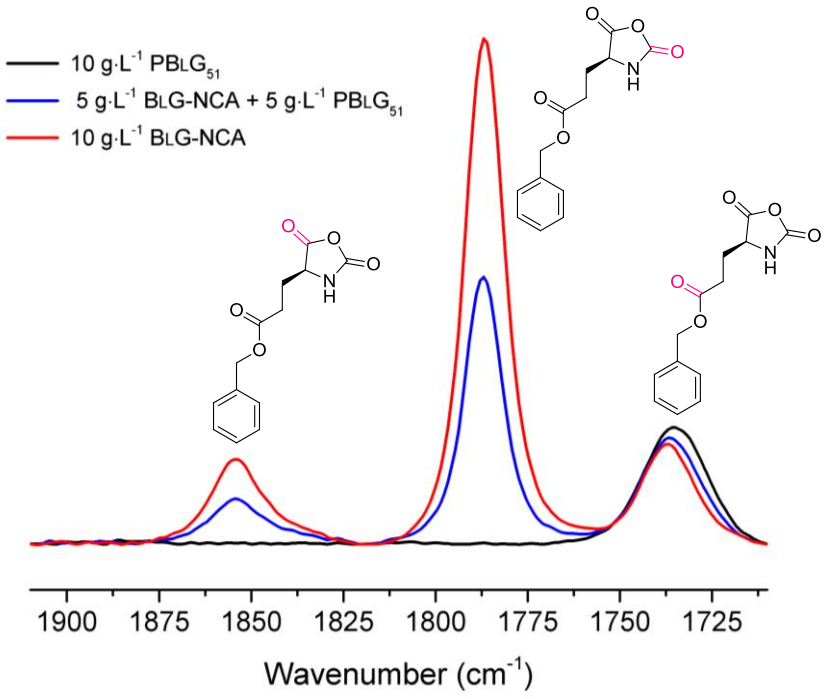


Fig. S2 FTIR absorbance peaks (C=O stretch) in the 1710-1910 cm^{-1} region of solutions of BLG-NCA and $PBLG_{51}$ in DMF (DMF background subtracted), and corresponding carbonyl groups (in pink)

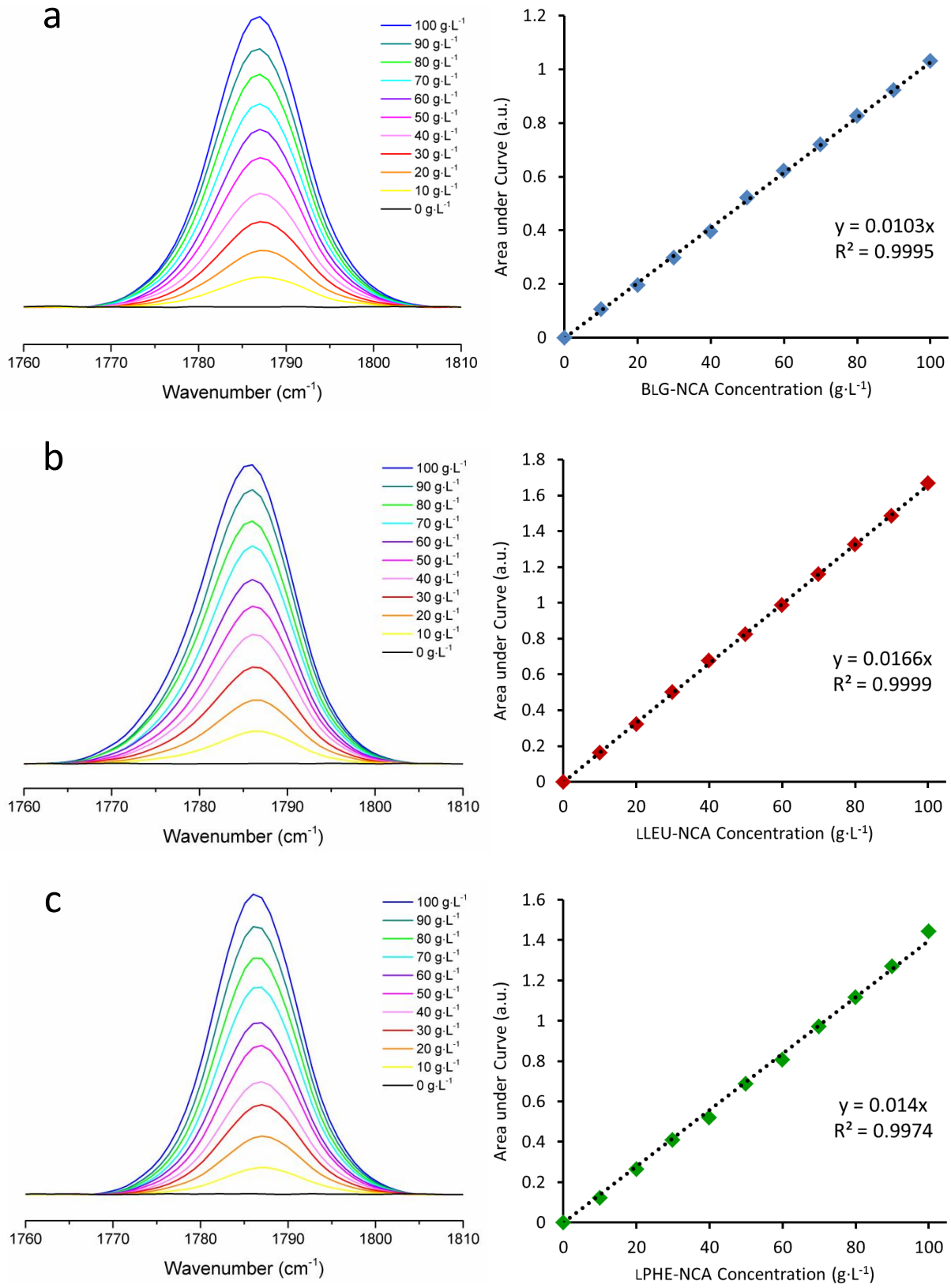


Fig. S3 (left) FTIR absorbance peaks of NCA C=O stretch from the NCA calibration series; (right) calibration curve of the NCA calibration series where the area under the 1785-1790 cm^{-1} fitted peaks is plotted as a function of the NCA concentration; for (a) BLG-NCA, (b) LLEU-NCA, and (c) LPHE-NCA

NMR SPECTRA

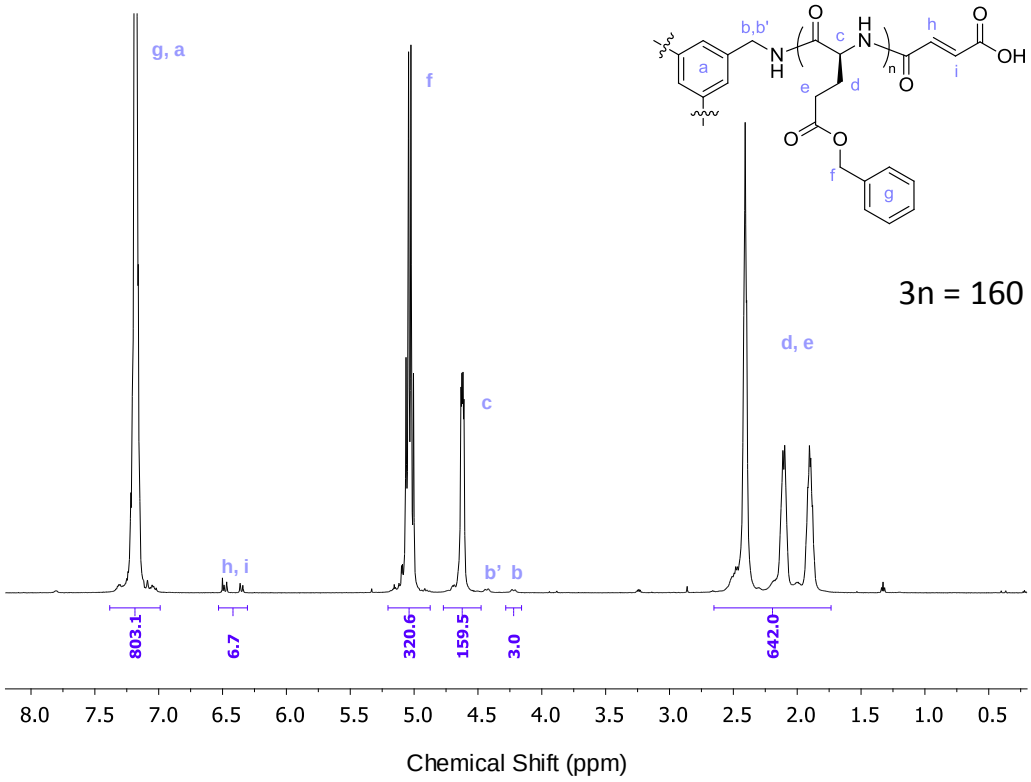


Fig. S4 ^{1}H-NMR spectrum of star $PBLG_{160}$ polymerised (DMF, r.t.) by TAB·3HCl/TEA (1:0.5 equiv. for 150 BLG-NCA equiv.) and terminated by maleic anhydride after 7 days

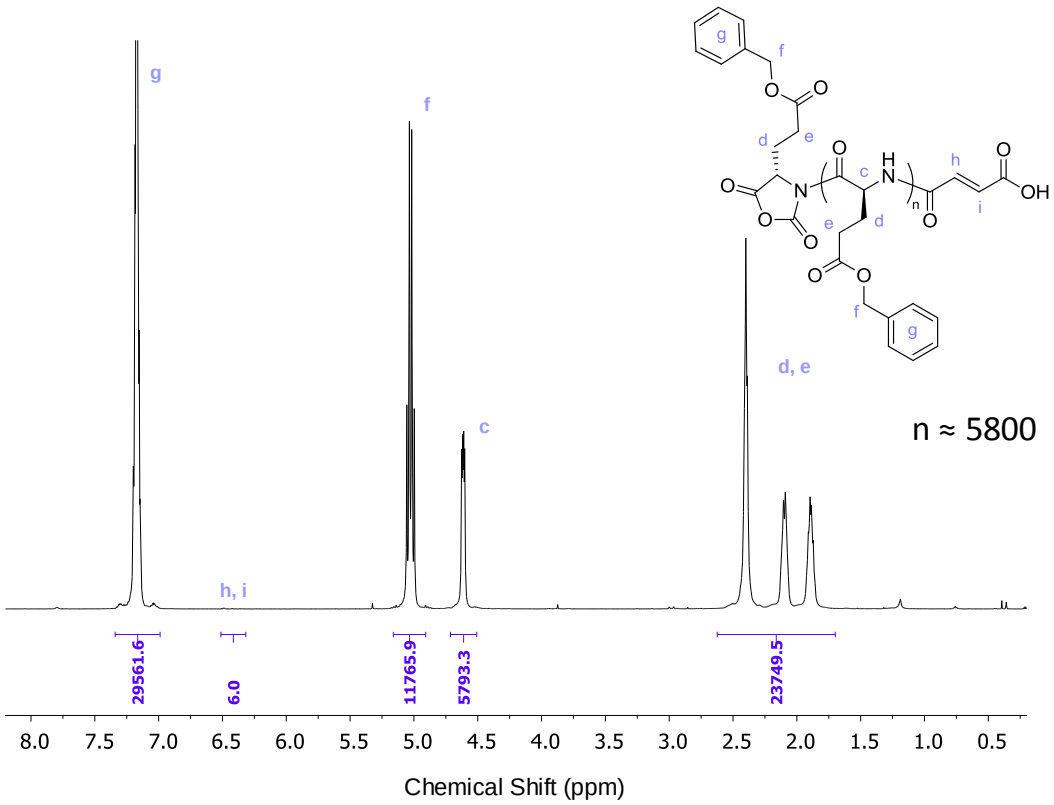


Fig. S5 ^{1}H-NMR spectrum of linear $PBLG_{5800}$ polymerised (DMF, r.t.) by TEA (0.5 equiv. for 150 BLG-NCA equiv.) and terminated by maleic anhydride after 7 days

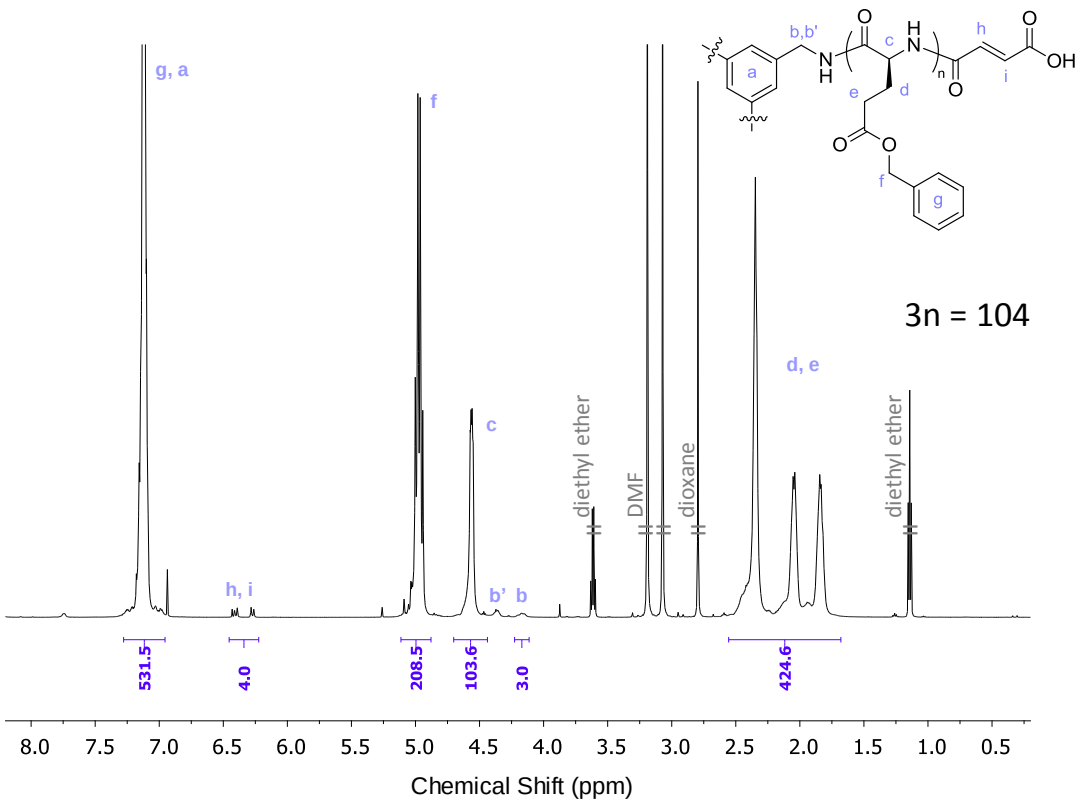


Fig. S6 ^{1}H-NMR spectrum of star $PBLG_{160}$ polymerised (DMF, 50 °C) by TAB·3HCl (1:0.5 equiv. for 150 BLG-NCA equiv.) and terminated by maleic anhydride after 7 days

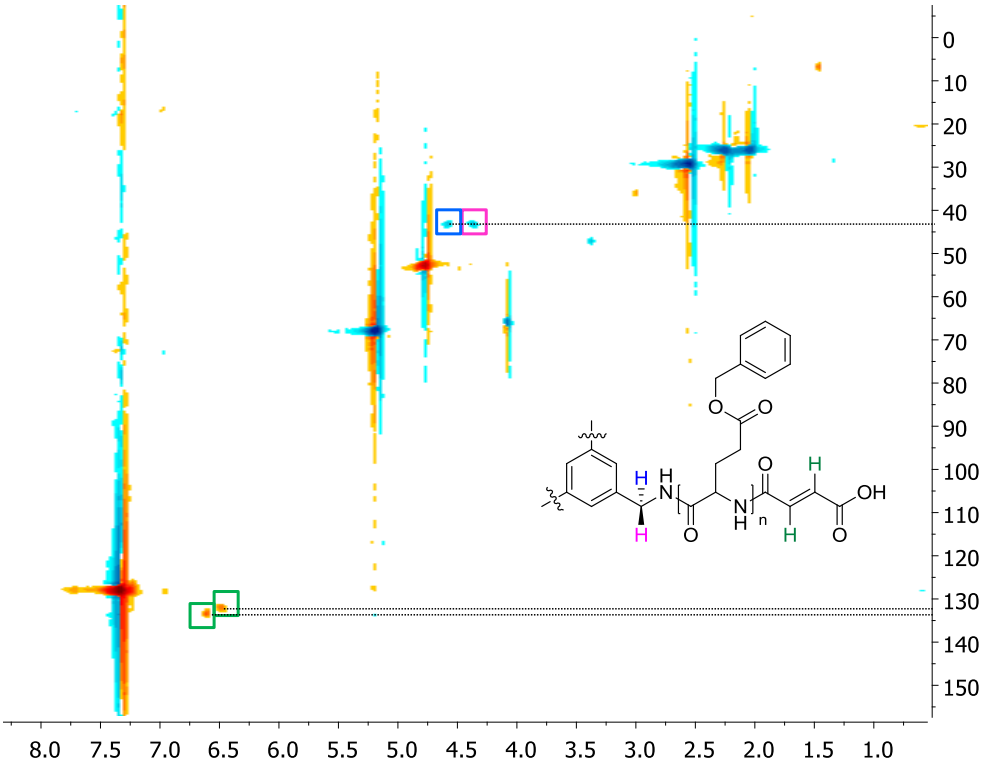


Fig. S7 Typical HSQC-NMR (x axis: ^{1}H chemical shift (ppm), y axis: ^{13}C chemical shift (ppm)) spectrum of star $PBLG_n$ polymerised by TAB·3HCl and terminated by maleic anhydride; the circled spots correspond to the colour-coded protons of the displayed polymer molecule

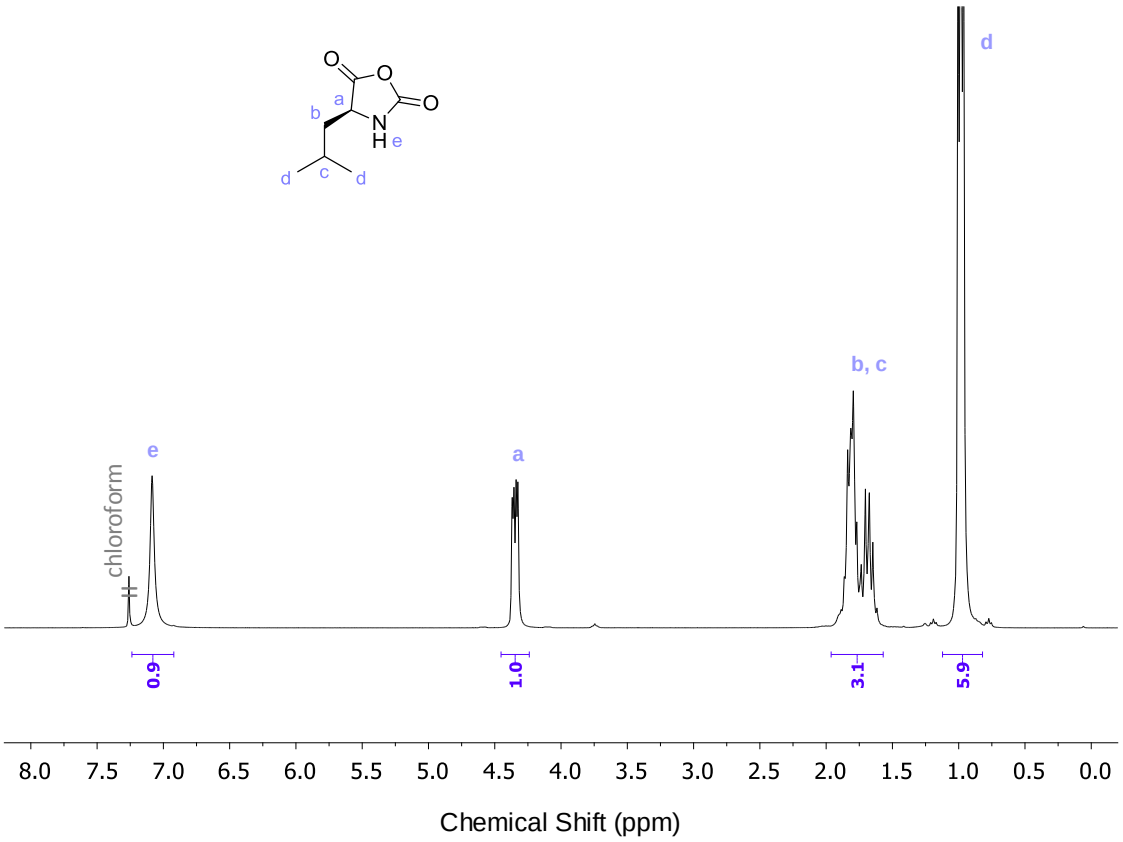


Fig. S8 ^{1}H-NMR spectrum of LLEU-NCA in $CDCl_3$

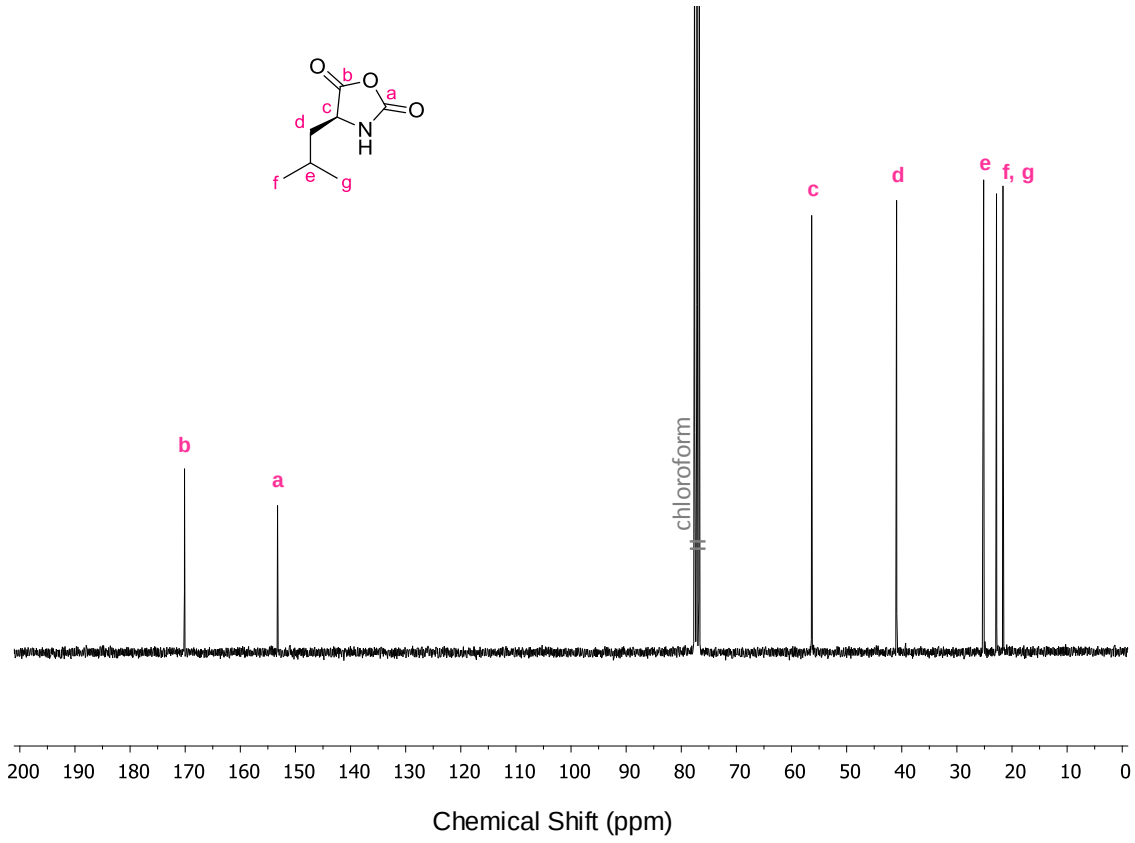


Fig. S9 ^{13}C-NMR spectrum of LLEU-NCA in $CDCl_3$

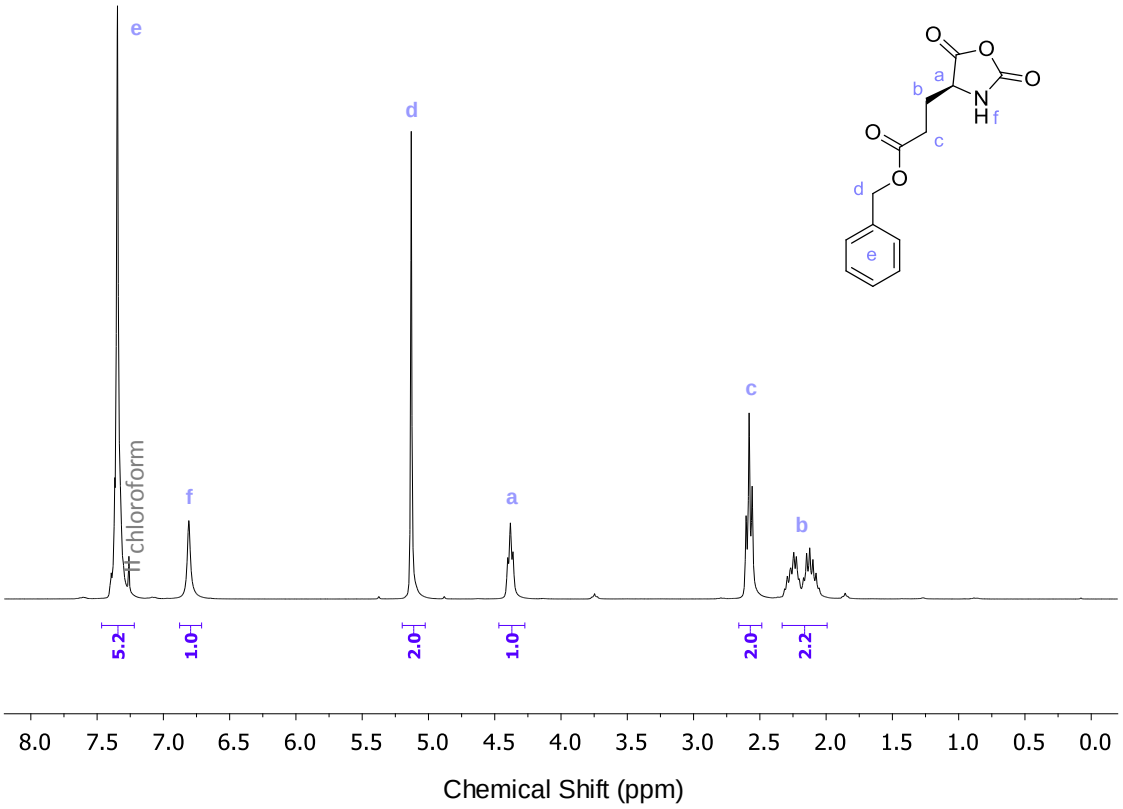


Fig. S10 ^{1}H-NMR spectrum of BLG-NCA in $CDCl_3$

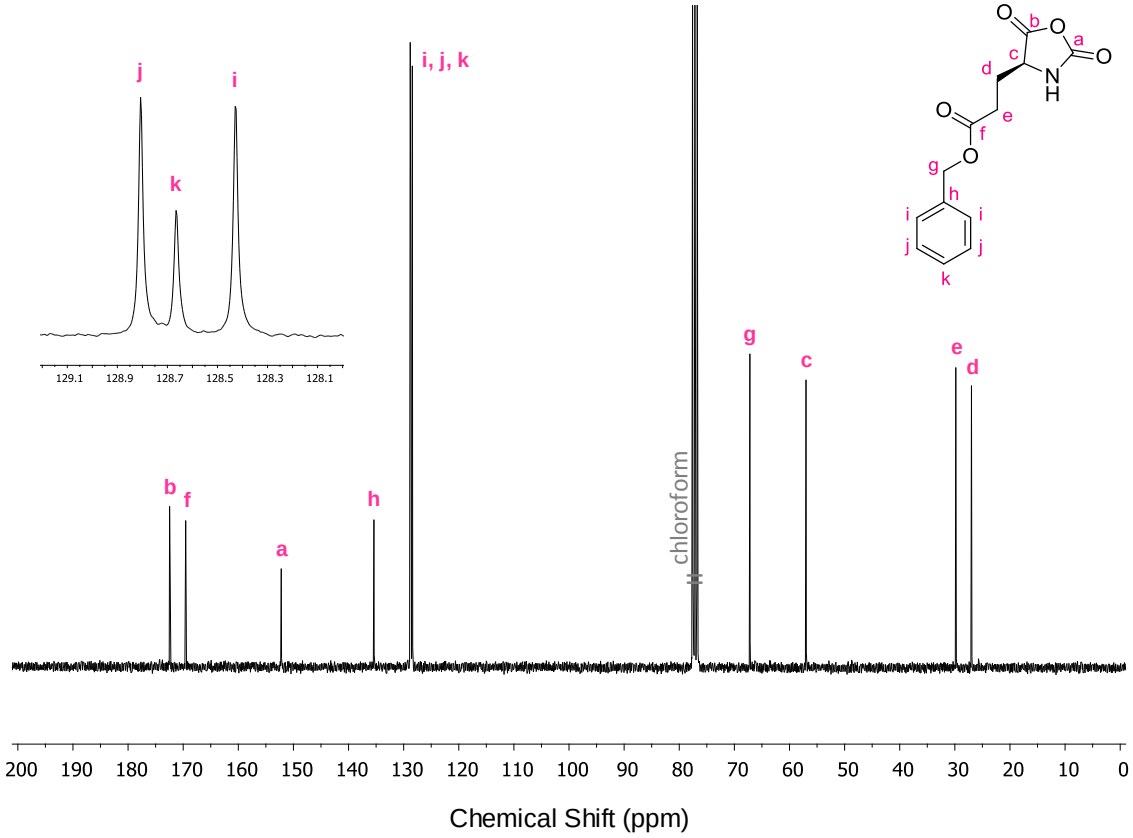


Fig. S11 ^{13}C-NMR spectrum of BLG-NCA in $CDCl_3$

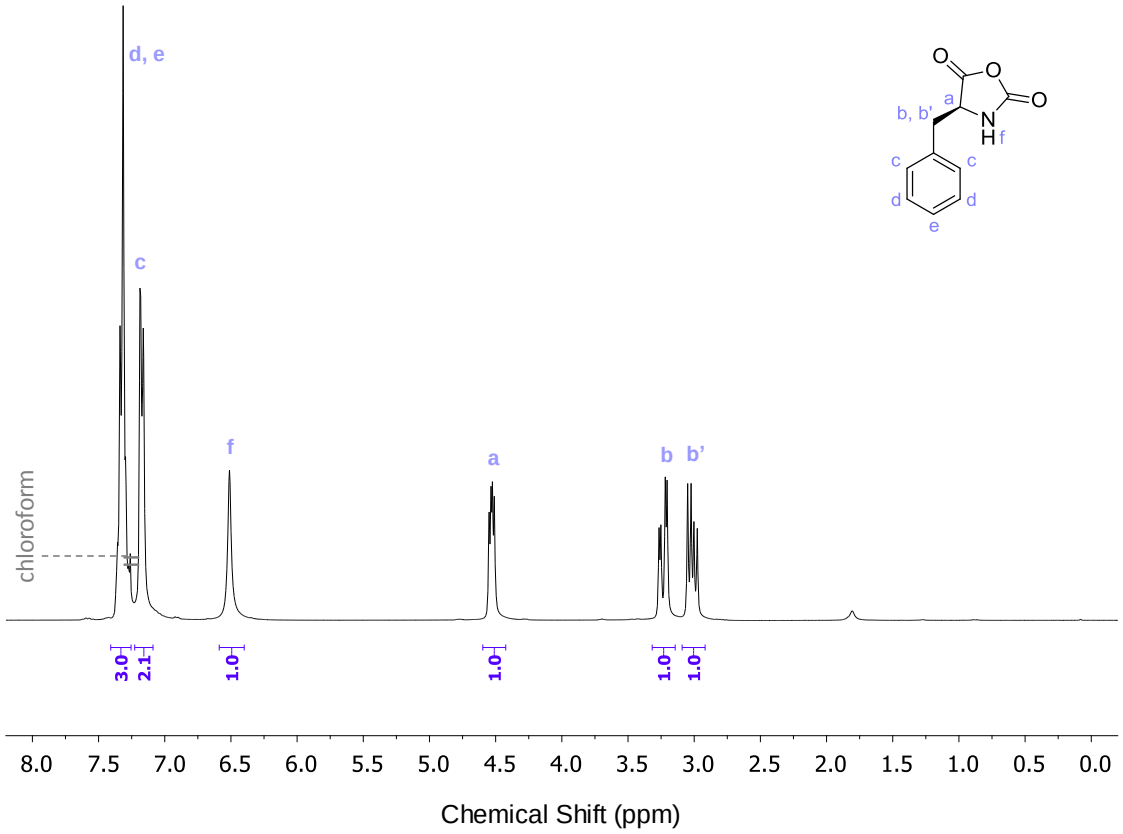


Fig. S12 ^{1}H-NMR spectrum of LPHE-NCA in $CDCl_3$

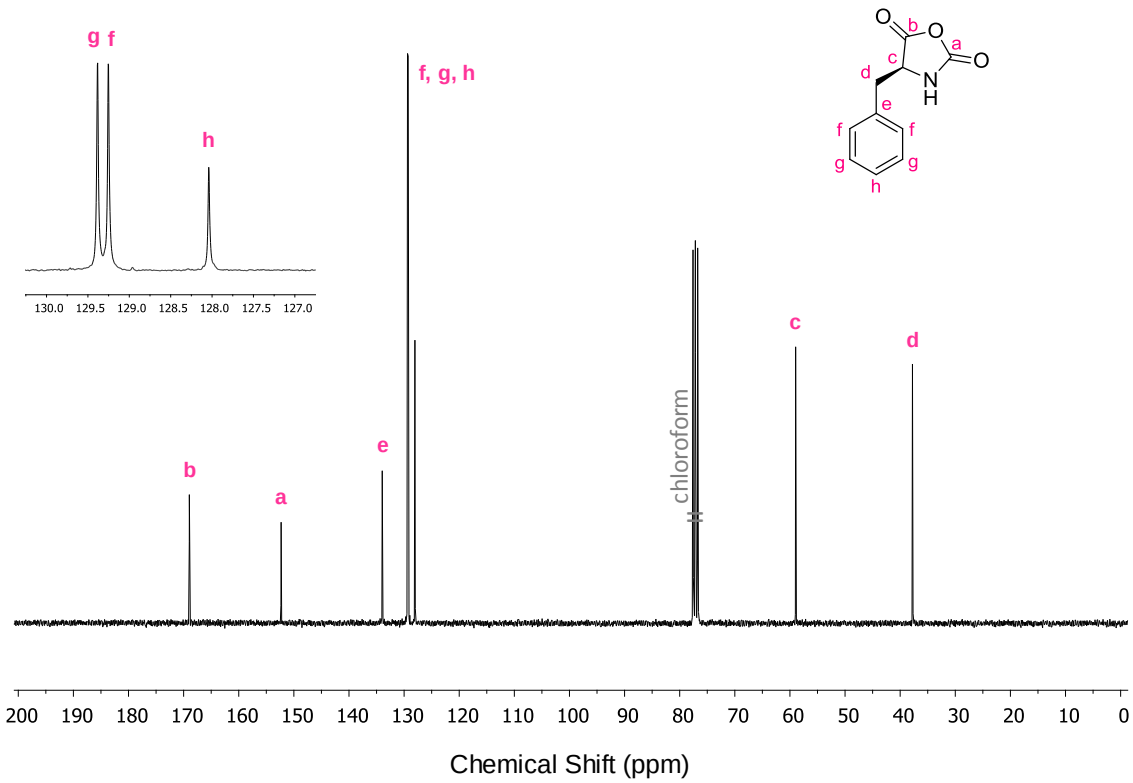


Fig. S13 ^{13}C-NMR spectrum of LPHE-NCA in $CDCl_3$

LPHE-NCA POLYMERISATION

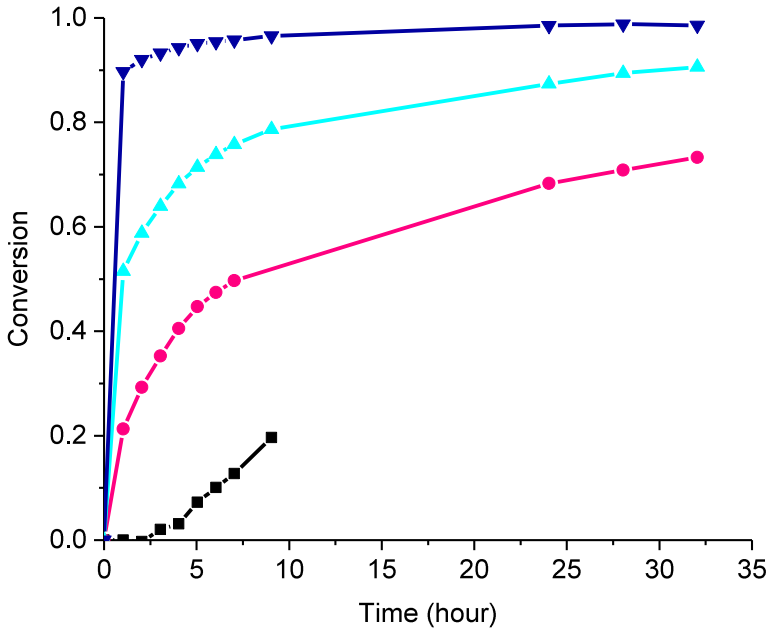


Fig. S14 Polymerisation of LPHE-NCA (100 equiv.) in DMF initiated by ■ BnA·HCl (1 equiv.), ● BnA·HCl/TEA (1:0.5 equiv.), ▲ BnA (1 equiv.), and ▼ TEA (0.5 equiv.)

COMPARISON OF PyA·HCl /TEA AND PyA·HCl /DIPEA FOR THE POLYMERISATION OF BLG-NCA

Table S1 Results of the polymerisations of BLG-NCA at room temperature initiated by PyA·HCl/TEA and PyA·HCl/DIPEA (molar ratio 1:0.5)

Tertiary Amine	TEA	DIPEA
24 h - conversion	50%	63%
- dispersity	1.07	1.08
120 h - conversion	86%	96%
- dispersity	1.08	1.09

REFERENCES

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2 H. R. Kricheldorf, *Alpha-Aminoacid-N-Carboxy-Anhydrides and Related Heterocycles*, Springer Berlin Heidelberg, 1987.