Electronic Supplementary Material (ESI) for Polymer Chemistry

Supporting Information

A Controlled and versatile NCA polymerization method for the synthesis of polypeptides.

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Experimental section

Materials

All chemicals were reagent grade, obtained from Aldrich and used without further purification, unless indicated otherwise. mPEG(2000)–NH_2 was obtained from Iris Biotech. All solvents were of analytical grade and were dried and freshly distilled.

Deuterated chloroform–*d1*, DMSO–*d6*, DMF–*d7* and D_2O were purchased from Deutero GmbH.

Preparative SEC was performed using Sephadex G–25 superfine from GE as well as PD MiniTrap G–10 ™ columns containing 2.1 mL of Sephadex™ G–10.

Ultrafiltration was performed in a Millipore ultrafiltration device fitted with a 1, 3, or 10 KDa molecular weight cut off regenerated cellulose membrane.

Characterization techniques

NMR espectroscopy. ^{1}H and ^{13}C–NMR spectra were recorded on a Bruker AC 300 at room temperature and at a frequency of 300 and 75 MHz respectively and analyzed using the MestreNova 6.2 software.

Dimethylformamide (DMF) gel permeation chromatography (GPC). For SEC measurements in DMF containing 1g L^{-1} of lithium bromide as an additive, an Agilent 1100 series system was used with a flow rate of 1 mL min^{-1} at 30 °C as an integrated instrument, including three HEMA-based columns ($10^5/10^3/10^2$ Å porosity) from MZ-Analysentechnik GmbH, a UV (275 nm) and an RI detector. Calibration was achieved with well defined PEG/DMF or poly(methyl methacrylate) (**PMMA**)/DMF standards, provided by Polymer Standards Service (PSS)/Mainz Germany.

Hexafluoroisopropanol (HFIP) gel permeation chromatography (GPC). GPC was performed with HFIP containing 3 g L^{-1} potassium trifuoroacetate as eluent at 40 °C. The columns were packed with modified silica (PFG columns particle size: 7 µm, porosity: 100 & 1000 Å. A refractive index detector (G 1362A RID) was used

to detect the polymer. Molecular weights were calculated using a calibration performed with PMMA standards (Polymer Standards Services GmbH) and toluene as internal standard.

Circular Dichroism (CD). CD Spectroscopy was performed with a J-815 CD Spectrometer (JASCO Corporation) using a Peltier thermostatted cell holder (PTC-423, JASCO Corporation) with a recirculating cooler (JULABO F250, JASCO Corporation). A nitrogen flow (~2.7 L/min) was lead through the spectrometer and controlled with a nitrogen flow monitor (Afriso Euro-Index).The samples were dissolved in HFIP for protected and water for deprotected samples and diluted to a concentration of 0.25 mg/mL. Samples were measured repeatedly (n=3) in a quartz cuvette with d= 0.1 cm at 20 °C. Obtained molar ellipticities were plotted as mean residue ellipticity.

Infrared (IR) spectroscopy analysis for polymerization monitoring. IR spectra were recorded using thermo scientific Nicolet 380 FT–IR spectrometer with a spectral range 7800 – 350 cm^{-1} , optical resolution (apodized) < 0.9 cm^{-1} resolution (standard) and peak–to–peak noise < 2.2 x 10^{-5} abs. (> 22,000:1) (1 minute scan) All samples analyzed were under solution or previously dissolved in DMF. Analysis was carried out at 25 ˚C.

Polarimetry. Chirality of the deprotected polymers was checked using the automatic polarimeter Jasco P1020 measuring 3 times x20 scans each. Solutions were all prepare in ddH_2O at 10 mg ml^{-1} concentrations. Analysis were carried out at 25 °C.

Protocols

NCA monomer synthesis. Synthesis of y–benzyl L–glutamate N–carboxyanhydride (NCA) from L–glutamic acid y–benzyl ester and diphosgene by using Limonene as HCl scavenger.

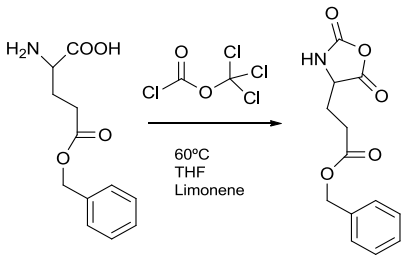


Figure 1S. y–Benzyl–L–glutamate N–carboxyanhydride synthesis scheme.

The protocol was adapted from N.M.B Smeets et al. "A Scalable synthesis of L–Leucine–N–Carboxyanhydride"[1] a variation of the *Fuchs–Farthing method*. In addition, we did some variations like the removal of remaining phosgene or HCl by nitrogen flow prior to precipitation followed by recrystallization and filtration under Schlenk conditions to avoid impurities and enhance storage stability.

Scheme of the NCA monomer synthesis is depicted in Fig. 1S. H–L–Glu(OBzl)–OH (17 g, 71.66 mmol) was added to a two–neck 250 mL round bottom flask fitted with a stirrer bar, reflux column, dropping funnel and an argon in and outlet. The apparatus was purged with Ar for 5 minutes. Afterwards tetrahydrofurane (THF) (120 mL, anhydrous) was added and the contents were heated to 60 °C. Limonene (11.6 mL, 71.66 mmol, 1 equiv) was added to the stirring suspension before diphosgene (5.2 mL, 8.5 g, 43 mmol, 0.6 equiv) dissolved in THF (10 mL, anhydrous) was added via a dropping funnel over a period of 10 minutes. The reaction was left stirring for 3 hours at 60 °C whilst purging with Ar leading to a clear solution. The reaction mixture was bubbled with Ar to aid the removal of remaining HCl for 2 hours whilst the Ar outlet was directed through an aqueous 1 M sodium hydroxide solution to neutralize the gas. The reaction solvent was reduced to a quarter of the original volume by rotary evaporation and ethyl acetate (32 mL) was added. The contents were added to ice cold hexane (200 mL) to form a white precipitate, which was isolated by vacuum filtration and washed with cold hexane.

The solid was recrystallized from toluene (50 mL, anhydrous) and THF (30 mL, anhydrous) under inert atmosphere (N_2 or Ar) by using a 250 mL two- neck round bottom flask fitted with a stirrer bar, reflux column and an argon inlet and outlet. Crystallization was induced by a dropwise addition of cold hexane (27 mL). The solution was stored for one hour at 4 °C, and then at –20 °C overnight. Finally the white crystals were filtered under Ar conditions by using Schlenk techniques, and stored at –20 °C.

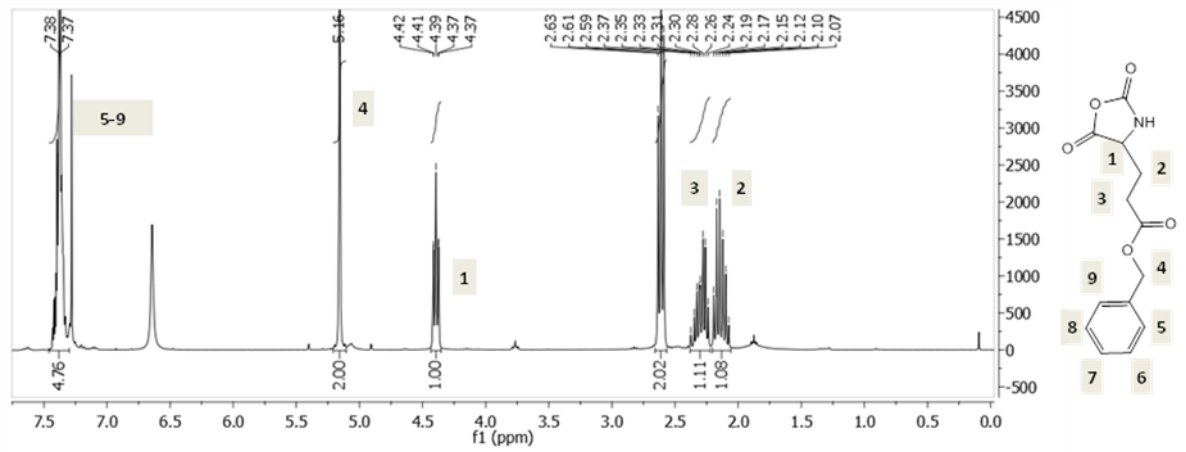
To ensure that residual HCl had been successfully remove, NCA (2–4 mg) was dissolved in THF (0.5 mL) and added to a 0.1 mmolar silver nitrate solution (1 mL) where the solution remained clear. When the Ag^+ and Cl^- ions meet they form the colorless insoluble AgCl salt, which can be easily detected.

Another test is checking the solubility in THF. The NCA is soluble in THF, if turbidity is seen in the solution, can be due to the presence of remaining hexane and should disappear by heating the solution, but if precipitation is seen is due to the presence of polymer or starting material.

Yield: 70–80 %. mp: 93.4 °C. ^{1}H–NMR: δ_H (300 MHz, $CDCl_3$): 2.00–2.30 (2H, m, CH_2), 2.52–2.60 (2H, m, CH_2), 4.30–4.34 (1H, t, CH), 5.09 (2H, s, OCH_2), 6.40 (1H, s, NH), 7.30 (5H, m, Ph).^{13}C–NMR: δ_C (75 MHz, $CDCl_3$) 27.5, 30.6, 57.6, 67.8, 129.2, 129.4, 129.5, 135.9, 152.4, 170.2, 173.3.

The proton and carbon NMR spectra in Fig. 2Sa, b show the corresponding signals of the NCA monomer.

(A)



(B)

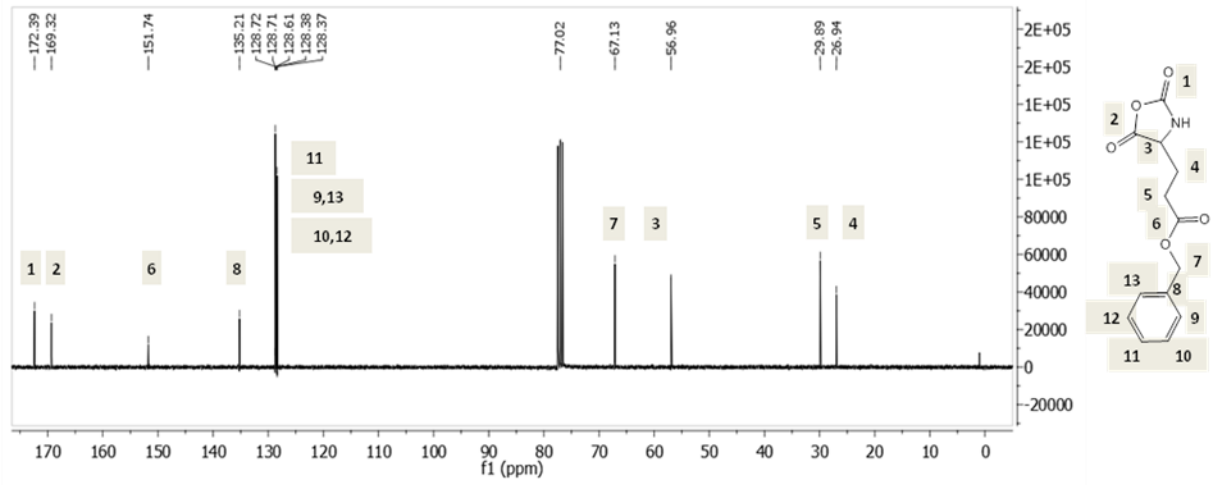


Figure 2S. ^{1}H–NMR (A) and ^{13}C–NMR (B) spectra of the γ–benzyl–L–glutamate N–carboxyanhydride (NCA–L–Glu) in $CDCl_3$.

General method for the preparation of BF_4^- salts.

These salts were easily prepared by the reaction of the corresponding amine with the HBF_4 diethyl ether complex and posterior purification by recrystallization. (Fig. 3S).

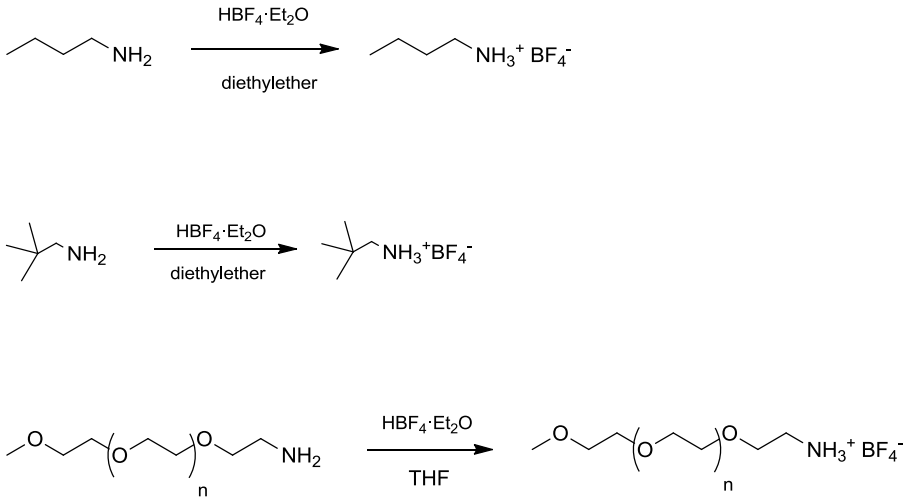
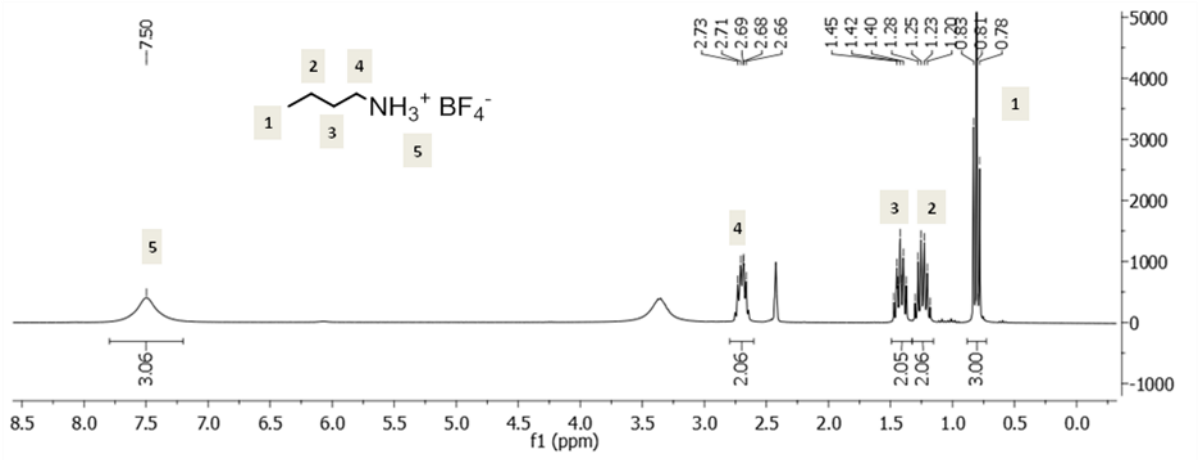


Figure 3S. Synthetic route for BF4 salts obtaining**.**

Synthesis of tetrafluoroboran n–Butyl ammonium salt.

Butyl amine (200 mg, 2.7 mmol) was dissolve in 1 mL diethyl ether, and 442 mg (2.7 mmol) of tetrafluoroboric acid diethyl ether complex, $HBF_4.Et_2O$, was added to the solution leading to the formation of a white solid salt in a quantitative yield. The product was then filtered off and recrystallized two times from ethyl acetate. The product was then dried under high vacuum and stored at –20 °C. Yield: 50% of a white solid. ^{1}H–NMR δ_H (300 MHz, DMSO–d_6) 7.58 (3H,s), 2.84 – 2.71 (2H,m), 1.56 – 1.43 (2H,m), 1.39 – 1.25 (2H, m), 0.89 (3H, t) (Fig. 4S). ^{13}C–NMR δ_C (75 MHz, DMSO–d_6) 38.64, 29.09, 19.08, 13.49. EA: C: 29.61 % (calc.: 29.85 %), H: 7.27 % (calc.: 7.51 %), N: 8.60 % (calc.: 8.70 %).

(A)



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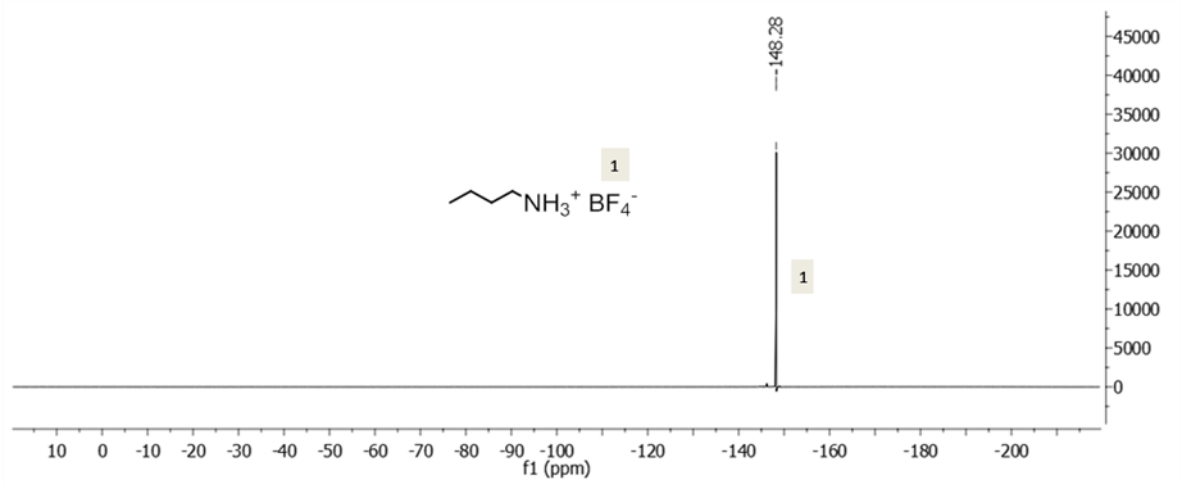
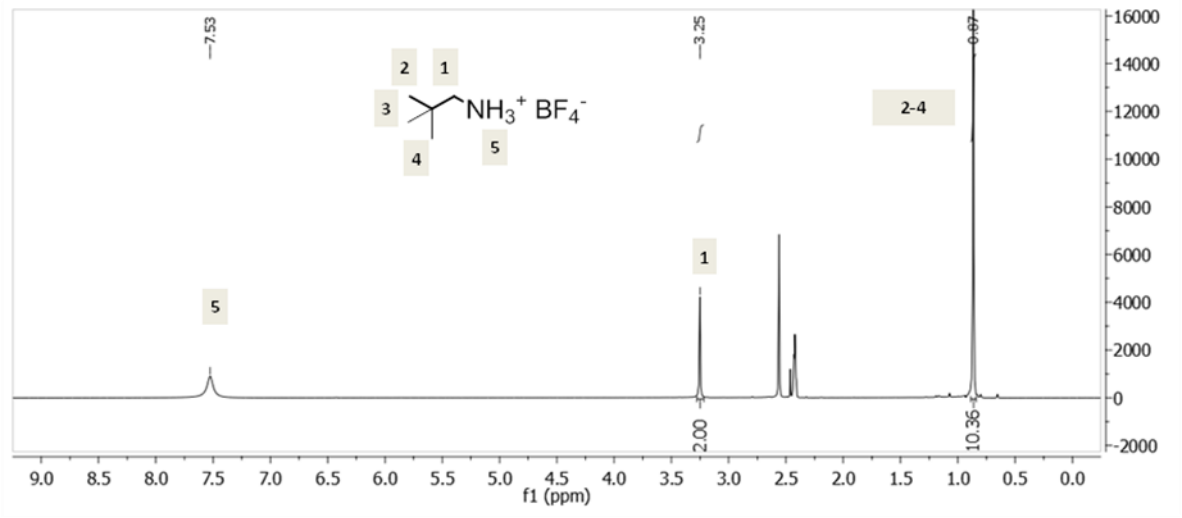


Figure 4S. NMR spectra of nBu–NH_3BF_4 (A) ^{1}H and (b) ^{19}F.

Synthesis of tetrafluoroboran neopentyl ammonium salt.

To 5 ml (5.59 g, 36.74 mmol) of tetrafluoroboric acid diethyl ether complex, $HBF_4(Et_2O)$, 4.31 mL (3.20 g, 36.74 mmol) of neopentyl amine were slowly added. The addition resulted in the precipitation of a white solid. The solvent was removed under vacuum and the solid was recrystallized twice from ethyl acetate and washed with cyclohexane.

(A)



(B)

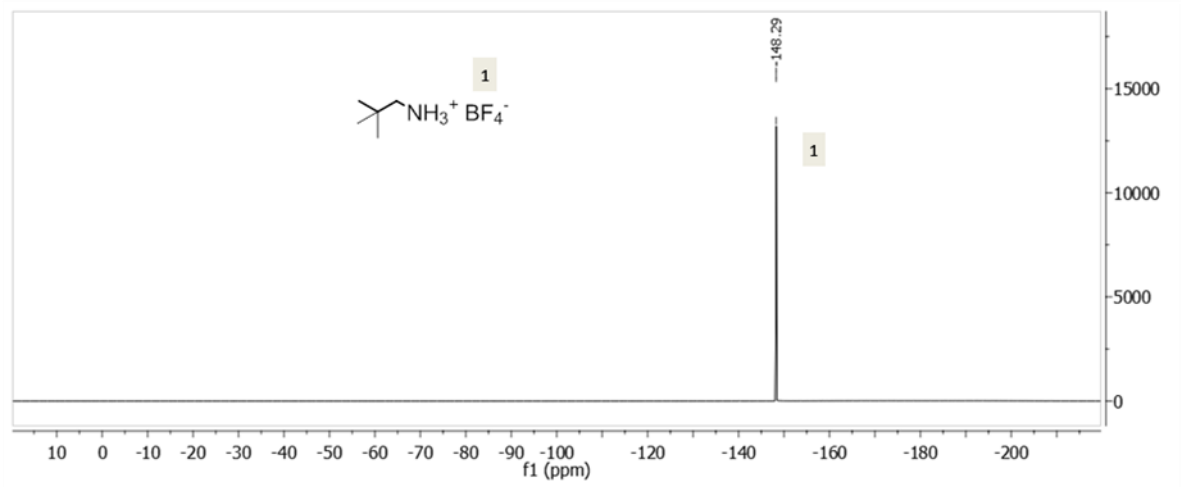


Figure 5S. NMR spectra of npt–NH_3BF_4 (A) ^{1}H and (b) ^{19}F.

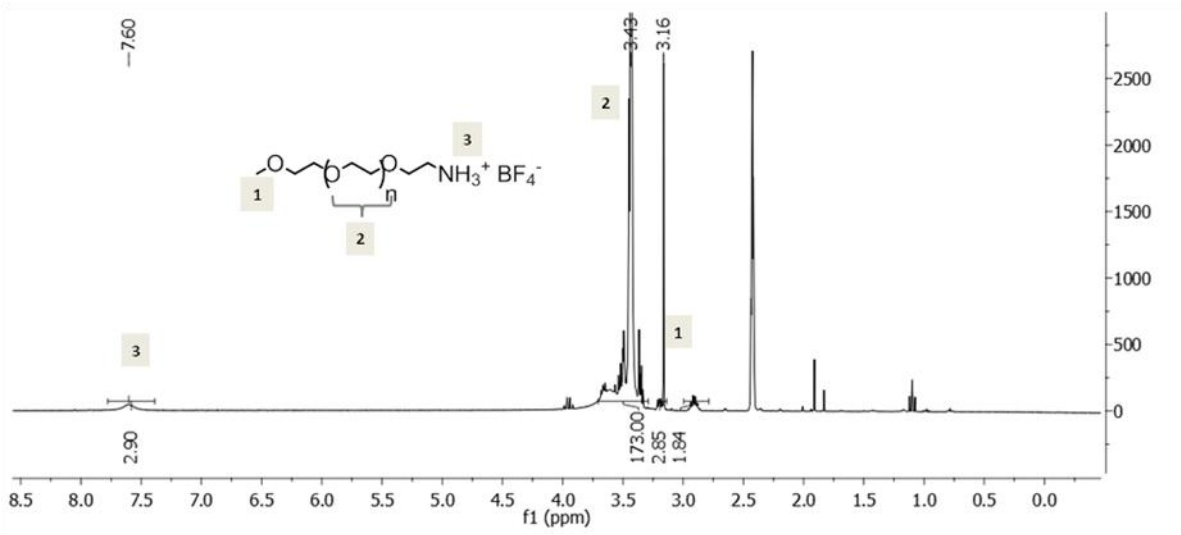
The product was dried under vacuum. Yield: 42% of a white solid. ^{1}H–NMR δ_H (300 MHz, DMSO–d_6) 7.58 (3H, s), 2.63 (2H, s), 0.93 (9H, s) (Fig. 5S). ^{13}C–NMR δ_C (75 MHz, DMSO–d_6) 49.94, 30.21, 26.78 EA: C: 34.35 % (calc.: 34.43 %), H: 7.99 % (calc.: 8.06 %), N: 8.07 % (calc.: 8.00 %).

Synthesis of tetrafluoroboran PEGammonium salt.

MeO–PEG(2000)–NH_2 (600 mg, 0.3 mmol, 1892 g mol^{-1}) was dissolve in 3 mL of THF, and 53.4 mg (0.3 mmol, 45 µL) of tetrafluoroboric acid diethyl ether complex, $HBF_4.Et_2O$, was added to the solution leading to the

formation of a faint yellow salt in a quantitative yield. The solvent was removed in a rotary evaporator. Solvent was evaporated and the solid residue was washed three times with hexane (washes were repeated until pH was not acidic). The product was then dried under high vacuum and stored at –20 °C. Yield: Quantitative.^{1}H–NMR δ_H (300 MHz, DMSO–d_6) 7.69 (3H,s), 3.78 – 3.70 (2H,m), 3.52 (139H, d), 3.47 – 3.39 (6H, m), 3.24 (3H, s), 3.06 – 2.91 (2H, m) (Fig. 6S).

(A)



(B)

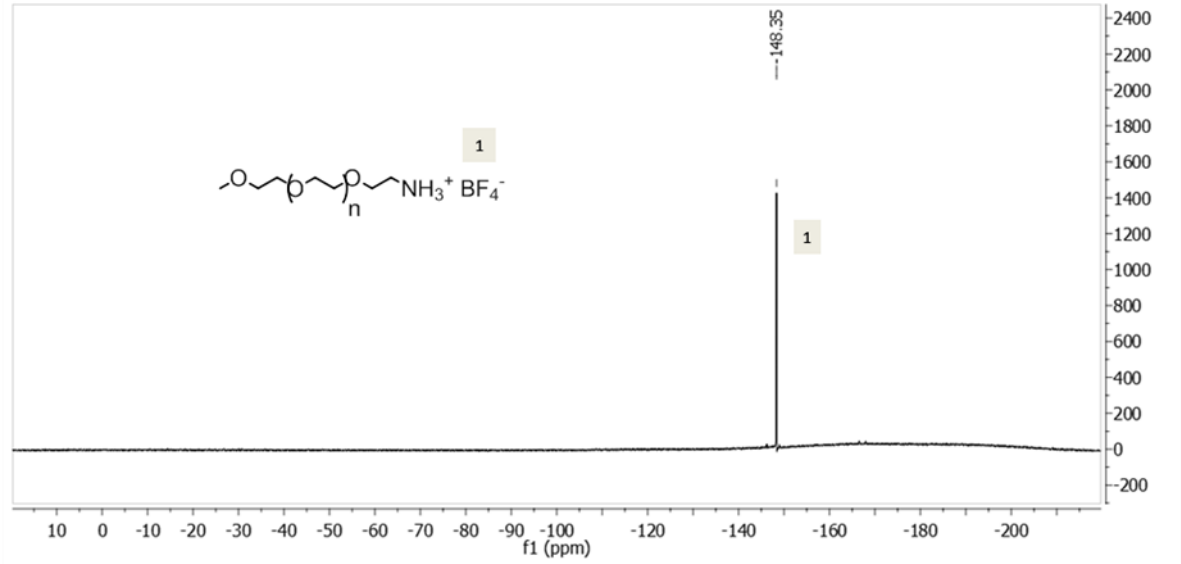


Figure 6S. NMR spectra of mPEG–NH_3BF_4 (A) ^{1}H and (b) ^{19}F.

General procedure for NCA polymerization. Synthesis of poly– y–benzyl L–glutamate (PBLG) under N_2 conditions by using Schlenk techniques.

y–Benzyl L–glutamate N–carboxyanhydride (0.5 g, 1.9 mmol, Mw= 264 g mol^{-1}) was added to a Schlenk tube fitted with a stirrer bar, a stopper and purged with 3 cycles of vacuum/Ar, under Ar flow and dissolved in 5 mL of the solvent (freshly purified). Afterwards the initiator was added and the mixture was left stirring at 40 °C in an oil bath for 3 days under Ar/N_2 atmosphere with constant pressure. After 3 days reacting the solution was poured into 40 mL of cold diethyl ether leading to a white suspension that was centrifuged at 4.000 rpm during 10 minutes. The supernatant was removed and the white solid was then suspended in milliQ water and freeze–dried. Yield: 70-90 %. ^{1}H–NMR δ_H (300 MHz, DMF) 8.58 (1H, s), 7.42 (5H, s), 5.19 (2H, s), 4.21 (1H, s), 2.81 (2H, s), 2.45 (2H, s). ^{13}C–NMR δ_C (75 MHz, DMF) 175.94 (s), 172.26 (s), 162.77 – 162.18 (m), 161.98 (s), 136.76 (s), 128.87 – 127.75 (m), 66.05 (s), 57.13 (s), 35.41 – 34.17 (m), 32.48 (s), 30.84, 30.30 – 29.04 (m), 27.28 (s), 25.99 (s).

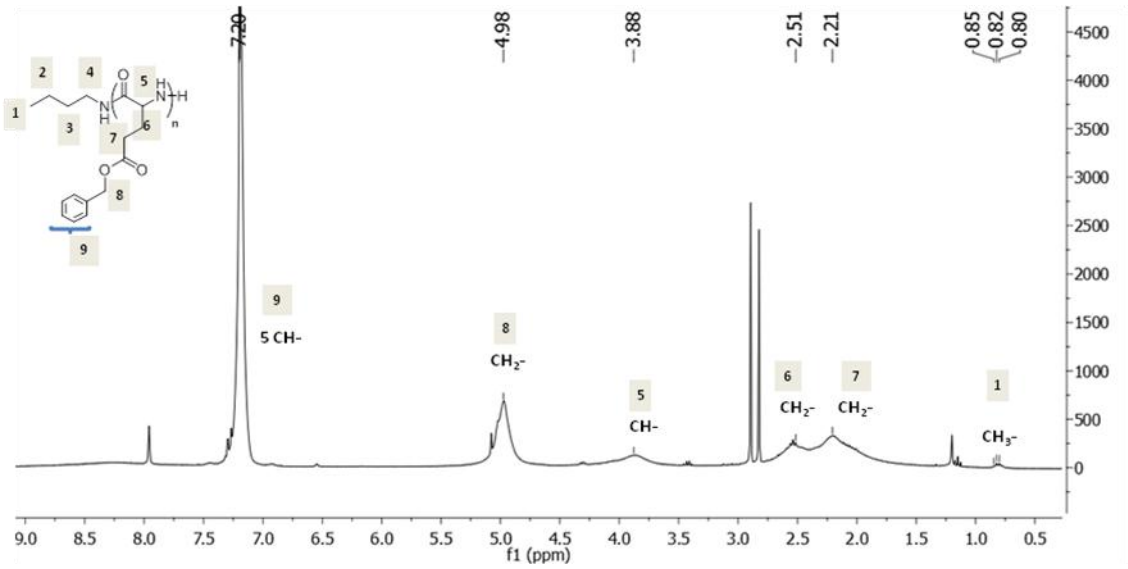


Figure 7S. ^{1}H–NMR spectra of nBu–PGA_{200} protected.

*Note: the corresponding signals of the initiator depend on the initiator used: n-Butyl ammonium; neopentyl ammonium; metoxypolyethylenglicol ammonium.

IR was used to follow the monomer conversion with the reaction time. DMF strongly absorbs in the IR region, therefore suitable peaks to follow the reaction had to be found. Figure 8S shows the IR spectra of Glu(Bzl)NCA

dissolved in DMF and PBLG in DMF. Discarding peaks obscured by DMF and those matching with the polymer, peaks corresponding only to the NCA monomer were localized at 1857, 1785 cm^{-1} and 920 cm^{-1}. Peaks at 1785 and 920 cm^{-1} were selected due to their lower signal–to–noise ratio.

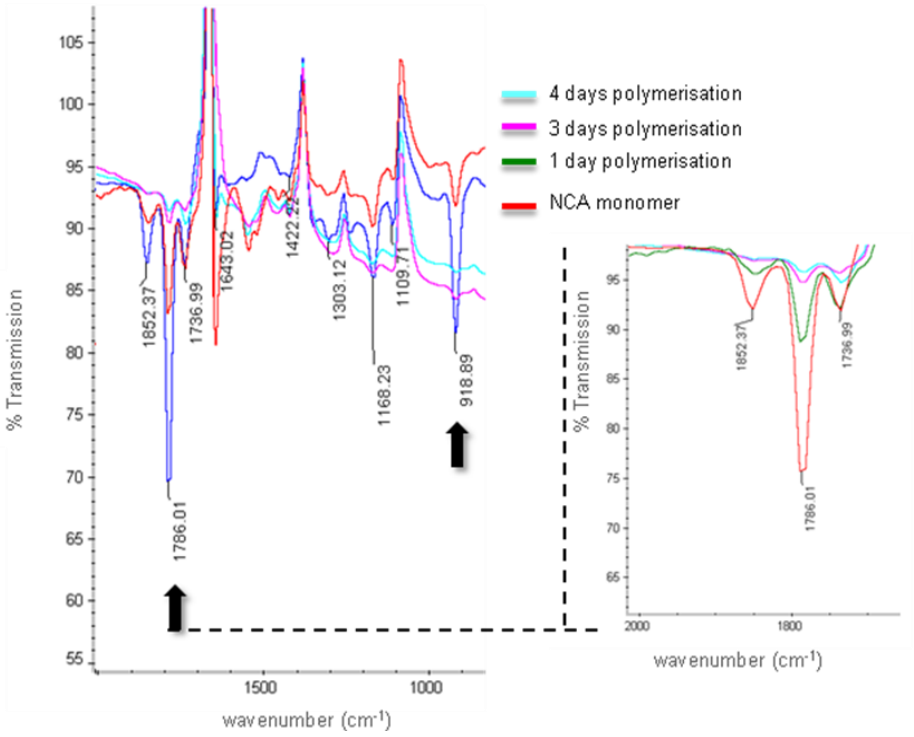


Figure 8S. Polymerization monitoring through FT-IR. Spectra of PBLG in DMF solution over time reaction. NCA monomer peaks (black arrows) are found at 1785 and 920 cm^{-1} (*red line*). They were exploited to monitor the polymerization evolution (peak at 1857 cm–1 also belongs to the NCA monomer but can be obscured by DMS absorption).

To confirm the advantages of our novel methodology, the neopentyl ammonium tetrafluoroboran salt was compared to its analogous amine form (Table 1S). NCA polymerization with the primary amine initiator was performed at 4 °C and 25 °C. However, BF4 salt initiation was carried out only at 25 °C due to the fact that the reaction is slower when temperature is diminished and therefore reaction time increased considerably. The study concluded that Mw of polymers were precisely controlled up to a degree of polymerization of 200 with NH_2 at low temperature or BF_4 at 25 °C. When higher Mw were required, only tetrafluoroborate salt initiators achieved the desired DP (Fig. 9S). The polymers had low dispersities indexes (1.05-1.20) indicating living character of the

polymerisation itself (Table 1S). Our novel initiators provided reproducibility batch-to-batch as well as enabled the scalability of the synthesis, from 100 mg to 10 g of starting NCA monomer.

Table 1S. Comparison results from NCA-ROP through different initiators (normal amine and tetrafluoroborane initiators) and temperature conditions.

Initiator	**DP_{theo}**	**T (°C)**	**Yield (%)**	**Mn^a (kDa)**	**$Đ^a$**	**DP^a**	**Mn^b (kDa)**	**$Đ^b$**	**DP^c**
Npt-NH_2	50	4	74	9.4	1.13	43	9.7	1.10	57
	100		64	27.8	1.06	127	22.2	1.11	14 4
	200		68	28.7	1.10	131	23.6	1.14	20 2
	400		90	15.7	1.14	72	29.5	1,10	19 0
Npt-NH_2	50	25	74	9.6	1.21	44	9.3	1.11	47
	100		80	11.9	1.17	54	11.0	1.14	78
	200		79	13.9	1.14	64	12.1	1.16	11 4
	400		78	16.4	1.16	75	12.5	1.16	12 1
Npt-BF_4	50	25	73	10.5	1.15	48	7.9	1.22	40
	100		70	16.4	1.10	75	15.1	1.17	73
	200		75	22.5	1.13	183	20.4	1.15	19 7
	400		85	44.2	1.08	202	35.8	1.16	40 5

a. Data obtained by SEC in N,N'-Dimethylformamide (DMF)
b Data obtained by SEC in 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP)
c. Data obtained by ^{1}H–NMR
[M]/[I]= Monomer Concentration/ Initiator Concentration, DP= degree of polymerization, Npt=neopentylamine, Đ=dispersity, Mn : apparent Mn in b

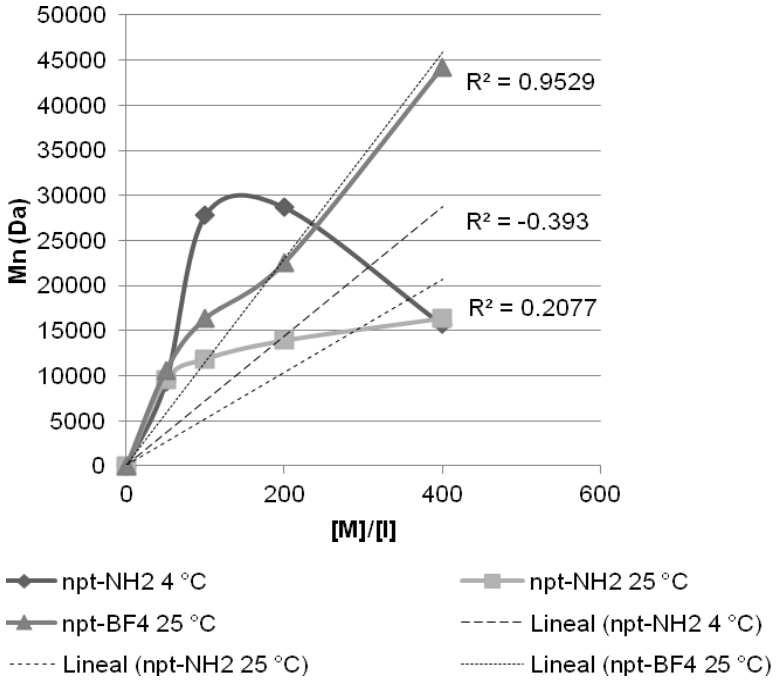
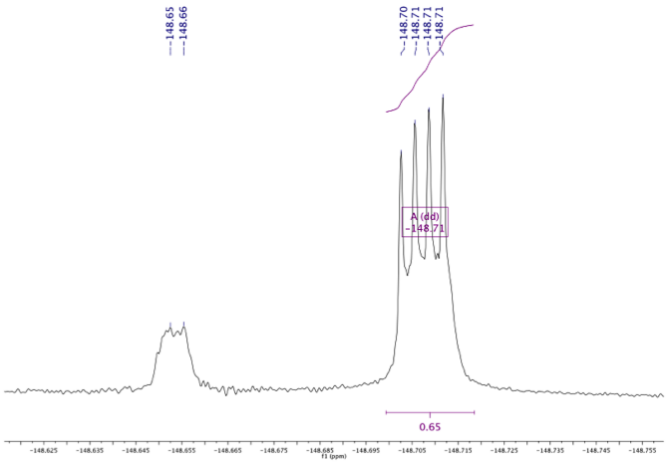


Figure 9S. Representation of the theoretical degree of polymerization versus the apparent Mn (Da) obtained in the GPC analysis (HFIP), where npt-NH_2: neopentyl amine initiator, and npt-BF_4: tetrafluoroboran neopentyl ammonium salt initiator.

Although BF_4 salts have been reported to be safe, it is important to note that, salt traces were never detected in ^{19}F–NMR after removal of the protection groups (Fig. 10S).

(A)



(B)

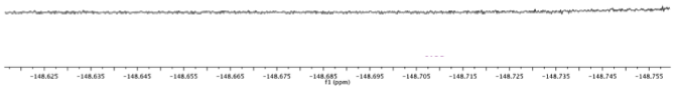


Figure 10S. A) ^{19}F–NMR of PBLG before benzyl deprotection, B)^{19}F–NMR of PGA, after benzyl deprotection. Explanation of chemical shifts: B has two isotopes with spin > ½ and because the ion is symmetric one can observed quadrupole splitting rather than quadrupole broadening. For [(^{10}B)F_4]- ^{10}B (19.58% abundance) has spin (I) 3 so (2nI +1), n =1 the resonance will be split into seven lines of equal intensity (19.58/7). On top of this (chemical shift probably but not necessarily the same) [(^{11}B)F_4] ^{11}B (80.42% abundant) has spin 3/2 so (2nI +1) the resonance will be split into 4 lines of equal intensity so about 20% (80/4) with a different B-F coupling[2]

Deprotection of poly(γ–benzyl–L–glutamate).

General reaction schemes for PBLG deprotection are pictured in Fig. 11S.

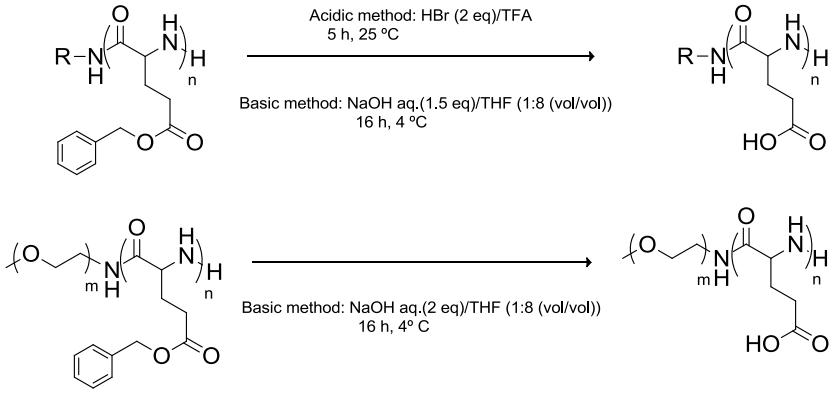


Figure 11S. Scheme conditions of acidic and basic methodologies where R represents Npt- or nBu- initiators, *n* is the number of repeating units of the glutamic block and *m* the ethylene glycol units.

Deprotection of poly(γ–benzyl–L–glutamate) with HBr in trifluoroacetic acid. Optimal conditions.

In a round bottom flask fitted with a glass stopper and a stirrer bar, 100 mg of poly(γ–benzyl–L–glutamate) (0.0035 mmol) were dissolved in 3 mL TFA. Once dissolved, 2 equiv of HBr (48% v/v, 1.49 g cm^{-3}, 81 g mol^{-1}) per carboxylic group were added drop wise, and the yellow mixture was left stirring for five hours. *Note: For big scale deprotection of PBLG (>600 mg), 16 h were needed in order to achieve full deprotection. Then, the solution was poured into a large excess of cold diethyl ether leading to a white solid that was recovered after centrifugation (2600 rpm, 4 °C, 10 minutes). The product was washed per triplicate with diethyl ether and dried over high vacuum. After that, the product was then purified by acid–base precipitation ($NaHCO_3$ /HCl 6 M). Dialysis or ultrafiltration was done leading to the sodium salt form.

Yield: 75–86%. ^{1}H–NMR δ_H (300 MHz, D_2O) 4.31–4.26 (1H, m), 2.38–2.14 (2H, m) 2.10–1.80 (2H, m) 2.10–1.80 (2H, m). (Fig. 12S).

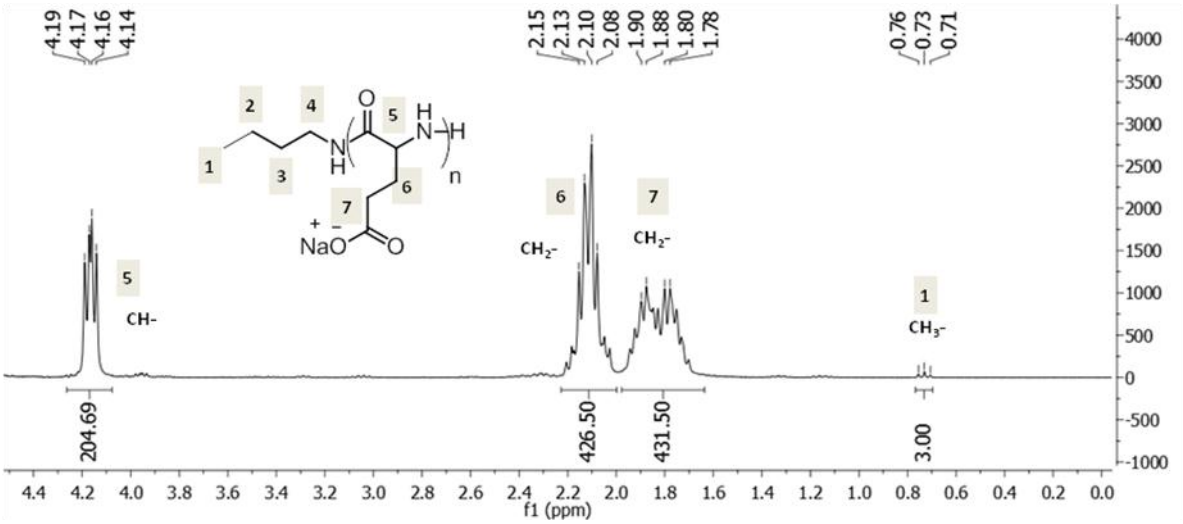


Figure 12S. ^{1}H–NMR spectra of nBu–PGA$_{200}$ deprotected.

HBr is a common reagent used for ethers cleavage and further studies confirmed that PEG was being degraded under the deprotection conditions used. PEG stability studies under acidic and basic conditions were performed obtaining the GPC profiles (Fig. 13S). This confirmed that HBr/TFA deprotection was not suitable for PEG block copolymers.

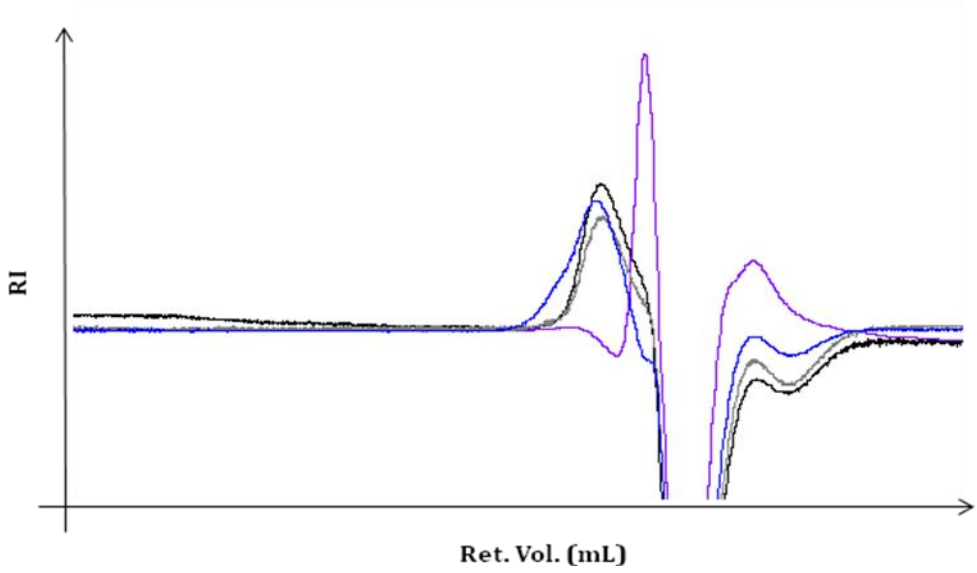


Figure 13S. PEG exposure to deprotection conditions: (violet: HBr/TFA treatment, blue: Pd/C, black: LiOH, grey: non–treated). Basic deprotection with NaOH aq./THF mixture. Optimal conditions.

HOMOPOLYMER

In a round bottom flask, PBLG (5.1 μmol, 9761 g mol^{-1}, n=50 G.A units) was dissolved in THF (16 mL) at room temperature. Then, the solution was cool down up to 4°C and kept under stirring. In a vial, 1.5 equiv of NaOH per carboxylic group (7.7 μmol, 40 g mol^{-1}) were dissolved in 2 mL of ddH_2O and then added to the main solution drop wise. Turbidity was found after NaOH addition. The solutions were left under vigorous stirring for 16 h. Afterwards, THF was removed by evaporation. The residue was diluted with ddH_2O, concentrated and purified by ultrafiltration (Vivaspin®, MWCO= 3000 Da). Upper part of the tube was freeze dried and the obtained white solid was analyzed by NMR (D_2O).

Yield: 40–60 %

PGA homopolymer ^{1}H–NMR δ_H (300 MHz, D_2O) 4.31–4.26 (1H, m), 2.38–2.14 (2H, m) 2.10–1.80 (2H, m) 2.10–1.80 (2H, m).

DIBLOCK

In a 50 mL round bottom flask, protected diblock (0.184 mmol, 12,208 g mol^{-1}, n= 50 G.A units) was dissolved in 16 mL of THF at room temperature. Then, the solution was cooled down up to 4 °C and kept under stirring. In a vial, 2 equiv of NaOH per carboxylic group of the polypeptide block (0.369 mmol, 40 g mol^{-1}) were dissolved in 2 mL of ddH_2O and then added to the main solution drop wise. Turbidity was found after NaOH addition. The solutions were left under vigorous stirring for 16 h. Afterwards, THF was removed under evaporation and the residue was diluted with ddH_2O, concentrated and purified by ultrafiltration (Vivaspin®, MWCO= 3000 Da). Upper part of the tube was freeze dried and the obtained white solid was analyzed by NMR (D_2O).

Yield: 40–70 %. ^{1}H–NMR δ_H (300 MHz, D_2O) 4.16 (1H, m), 3.45 (xH, m), 2.13 (2H, m), 1.76–1.89 (2H, m). i.e. DB_{50} (δ) 1.76–1.89 (100H, m), 2.13 (100H, m), 3.45 (172H, m), 4.16 (50H, m).

Optimization for NaOH aq/ THF deprotection study

This basic deprotection study of the polyglutamates (homopolymer and PEG–diblock) was carried out varying parameters as reaction time, NaOH equivalents and $addH_2O$/THF ratio.

In all the cases, 50 mg of the polymer were dissolved in THF at room temperature. Then, solution was cooled down and maintained under stirring at 4 °C. Finally the NaOH solution was added drop wise. Turbidity was found in all the cases once the NaOH was added. The solutions were left under vigorous stirring the desire time. All conditions exploited are detailed in Table 1S.

The organic solvent was removed under vacuum, and the aqueous phase was purified by ultracentrifugation using a Vivaspin® (MWCO= 3000 Da). The samples were concentrated, filtered (0.2 μm) and freeze–dried.

The study was monitored by polarimeter measurements to evaluate the α coefficient as a measure of the rotation angle of the polarized light. Each sample was dissolved in ddH_2O at 10 mg mL^{-1} concentration and average data was obtained after 20 measurements. The obtained products were analyzed by ^1H–NMR to prove complete deprotection.

Each deprotected polymer was evaluated to determine its specific rotation ($[\alpha]^T_\lambda$), value that was calculated according to the literature with the formula in Fig. 14S.

$$[\alpha]^T_\lambda = \frac{\alpha \cdot 100}{L \cdot c}$$

Figure 14S. Formula of the specific rotation [α] of the polypeptide chain.

where α= observed rotation, L= polarimeter cell length (dm), c= concentration (g 100 mL^{-1}) and T= temperature.

Amino acids α–carbon is chiral which confers optical activity. All natural amino acids have an L configuration, which is related to the rotation of the plane of polarized light to the left giving negative alpha values. The rotation degree of polarized light depends on the number of chiral molecules that it encounters through the polarimeter cell. For biocompatibility of our systems, L configuration is desired.

Supporting Table 2. Optimization of the THF/ H_2O ratio and concentration with 1.5 equiv, 16 h.

	equiv. GA units	equiv. NaOH	THF:$H_2$0 ratio (v/v)	t (h)	α (average)	SD	%s^2 coeff	$[\alpha]^{25}_{\lambda}$
PBLG (50 units)	1	1.5	64:1	16	*			
	1	1.5	32:1	16	*			
	1	1.5	16:1	16	–0.466	0.002	–0.330	–4.66
	1	1.5	8:1	16	–0.615	0.002	–0.2877	–6.15
	1	1.5	4:1	16	–0.237	0.001	–0.598	–2.37

*deprotection not achieved.

The next study was focused on diminishing the reaction time with 1.5 equiv of NaOH, once ratio of THF/H_2O was fixed. Results are summarized in Table 3S. Finally, 8 h were needed at least to achieve PBLG deprotection. However, a more negative alpha value was obtained for 16 h reaction.

Table 3S. Time optimizing study using 8:1 THF/H_2O (vol/vol) and 1.5 equiv. NaOH.

	equiv. GA units	equiv. NaOH	THF:$H_2$0 ratio (v/v)	t (h)	α (average)	SD	%s^2 coeff	$[\alpha]^{25}_{\lambda}$
PBLG (50 units)	1	1.5	8:1	2	*			
	1	1.5	8:1	4	*			
	1	1.5	8:1	6	*			
	1	1.5	8:1	8	–0.484	0.0027	–0.7586	–4.84
	1	**1.5**	**8:1**	**16**	**–0.615**	**0.002**	**–0.2877**	**–6.15**

*deprotection not achieved.

Therefore, this first study using the homopolymer, let us to conclude that 1.5 equiv, 16 h and THF:$H_2$0 8:1 parameters were able to deprotect the polybenzylglutamate, with the highest negative alpha value that we could obtained being around –0,6.

With the purpose of proving that the found conditions were correct regarding non–stereochemical changes along the deprotection, alpha values of comparable polymers were studied. The selected species were:

L–polyglutamates obtained by solid phase peptide synthesis (SPPS). SPPS provide a defined stereochemistry in the polypeptide chain, e.g. L. Our comparison with these polymers was centered on proving that racemization did not occur either during polymerization. The number of linked glutamic acid units obtained through this technique is limited, overall if protected side chains are necessary in the final polypeptide.

HBr deprotected polyglutamates. As named before, acid deprotection has been demonstrated not to affect the stereochemistry of the initial polymer. This procedure was carried out with our polyglutamates as well as with the SPPS commercial one.

The collected data from each species is compiled in the table below (Table 4S). [α] values of SPPS PGA (–5.12) and HBr deprotection (–5.55, –5.50) are over the same range that the ones achieved with our optimized basic deprotection products (–6.15). In addition, it was proved that strong basic conditions affect optical activity, PGA_{15} after treatment with concentrated NaOH gave positive [α] value (0.07).

Table 4S. Observed rotation for SPPS synthesized PGA in comparison with ROP-NCA synthesized PGA.

	α coefficient	SD	%s2 coeff.	$[\alpha]^{25}_{\lambda}$
PGA_{20} HBr deprotected	–0,555	0,001	–0,210	–5,55
PGA_{15} (SPPS)	–0,512	0,002	–0,357	–5,12
PGA_{15} (SPPS) treated with NaOH 2M, 16h	0,007	0,001	18,470	0,07

As a final corroboration of the basic deprotection versatility, the influence in the methodology of the polyglutamate Mw was studied. The rotation angle coefficient values obtained (Table 5S) confirm that under this conditions polypeptide is fully deprotected independently of the chain length and optical activity remains untouched.

Table 5S. Comparison of the observed rotation from PGAs with different DP after deprotection with the optimal basic protocol

$PBLG_n$	α (average)	SD	$\%s^2$ coeff.	$[\alpha]^{25}_{\lambda}$
n = 100	–0,6545	0,0022	–0,3314	–6,55
n= 200	–0,6594	0,0019	–0,2868	–6,59
n= 400	–0,6643	0,0019	–0,285	–6,64

Adaptation of the methodology for diblock copolymers deprotection

After establishing deprotection conditions with homopolymers, diblock systems were evaluated under the same protocol. However, the use of 1.5 equiv of NaOH leads to incomplete deprotections forcing to start a novel optimization process.

Using 16 h and the ratio 8:1 THF/H_2O settle parameters from homopolymer studies, the equivalents of NaOH were varied. Results proved that longer reaction times derived in less negative alpha values. (Table 6S). Therefore, following with the minimal harsh path, the lowest value was chosen: 2 equiv, even if deprotection yield had to be forfeit.

Table 6S. Optimization of the NaOH equivalents for DB deprotection using the same conditions of time and THF/H_2O ratio (16h, 8:1).

	equiv. NaOH	[NaOH]	α (average)	SD	%s2 coeff.	$[\alpha]^{25}_{\lambda}$
PEG–PGAn (n= 50 units)	2	0,02	–0,551	0,002	–0,29	–5,51
	3	0,031	–0,51	0,001	–0,292	–5,10
	4	0,041	–0,44	0,005	–0,325	–4,40

In addition when reaction time was increased, the same trend on alpha values was observed (Table 7S).

Table 7S. Optimization of the reaction time for DB deprotection.

	equiv. NaOH	t (h)	α (average)	SD	$\%s^2$ coeff.	$[\alpha]^{25}_{\lambda}$
PEG–PGA_n (n=50 units)	2	16	–0,535	0,003	–0,468	–5,35
	2	21	–0,517	0,001	–0,283	–5,17
	2	24	–0,494	0,003	–0,648	–4,94

Summarizing, 2 equivalents of NaOH per carboxyl group, 8:1 THF/H_2O ratio and 16 h handled PEG integrity whereas deprotection was successful.

To conclude, a summary of the results obtained for homo and diblock copolymers regarding basic deprotection are represented in Table 8S.

Table 8S. Summary of basic deprotection results of homo and diblock copolymer 50 units in comparison with SPPS synthesized PGA.

Polymer	Deprotection method	α (average)	SD	$\%s^2$ co-eff.	$[\alpha]^{25}_{\lambda}$
PGA_{15} (SPPS)		-0.512	0,002	-0.327	-5,12
PGA50	acidic	-0,555	0,002	-0.357	-5,45
PGA50	basic	-0,550	0,003	-0,363	-5,55
PEG-*block*-PGA50	basic	-0,551	0,002	-0,290	-5,51

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