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Polypeptoids from *N*-Substituted Glycine *N*-Carboxyanhydrides: Hydrophilic, Hydrophobic, and Amphiphilic Polymers with Poisson Distribution

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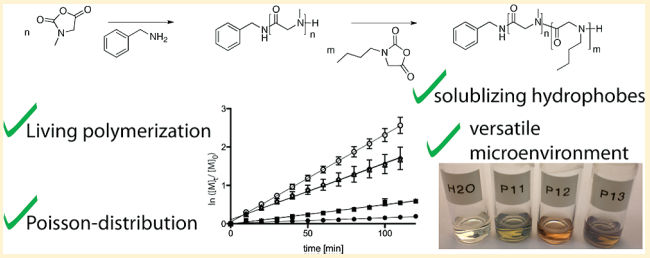
Supporting Information

ABSTRACT: Preparation of defined and functional polymers has been one of the hottest topics in polymer science and drug delivery in the recent decade. Also, research on (bio)degradable polymers gains more and more interest, in particular at the interface of these two disciplines. However, in the majority of cases, combination of definition, functionality and degradability, is problematic. Here we present the preparation and characterization (MALDI−ToF MS, NMR, GPC) of nonionic hydrophilic, hydrophobic, and amphiphilic N-substituted polyglycines (polypeptoids), which are expected to be main-chain degradable and are able to disperse a hydrophobic model compound in aqueous media. Polymerization kinetics suggest that the polymerization is well controlled with strictly linear pseudo first-order kinetic plots to high monomer consumption. Moreover, molar mass distributions of products are Poisson-type and molar mass can be controlled by the monomer to initiator ratio. The presented polymer platform is nonionic, backbone degradable, and synthetically highly flexible and may therefore be valuable for a broad range of applications, in particular as a biomaterial.



INTRODUCTION

Synthetic polymers are a proverbial part of modern life, as they appear to be intrinsically tied to biomedical applications and health care products. When hydrophilicity is a desired feature, poly(ethylene glycol) (PEG) is ubiquitous and arguably among the most important biomaterials. While many researchers maintain that PEG is safe,[1,2] other groups are actively engaged in finding alternatives to PEG.[3,4]

Among others, the following points may to be considered when designing and developing a biomaterial platform technology that might rival the broad applicability of PEG while not suffering of PEGs limitations:

- (bio)degradability/good shelf life
- synthetic versatility and definition
- scalability and availability
- good solubility in water and organic media.

As water-insoluble polymer, biodegradable polyesters (e.g., poly(lactic acid)) play a pivotal role. Only recently Möller and coworkers introduced amphiphilic block copolyesters. However, yields, reproducibility and definition were mediocre and the resulting polyelectrolyte character is suboptimal for many applications.[5]

There are advantages and disadvantages in the use of synthetic polypeptides as biomaterials. From the wide range of natural and non-natural amino acids an endless variety of different structures are available. In addition, polypeptides are degradable into, typically nontoxic, fragments. Hydrogen bonding between different monomer units can lead to distinct secondary structures (e.g., α-helices, β-sheets) which are often desired features of synthetic polypeptides.[6] However, α-helices and β-sheets also pose problems for the polymer chemist, as they are often insoluble in many solvents, including aqueous media. Poly(L-lysine), poly(L-glutamic acid), and other charged polypeptides are the only available water-soluble homopolypeptides. These are, however, polyelectrolytes with all the associated problems, especially for biological context.[7,8] A further disadvantage is the potential immunogenicity of polypeptides, especially copolypeptides and polypeptide conjugates.[9−11] Interestingly, H-bond-formation is also important for an immunogenic reaction and polymers of *N*-alkylamino acids possess limited immunogenic properties.[9,12] Several groups have reported side chain modified poly(L-lysine) and poly(L-glutamic acid) based polymers. This way, nonionic, water-soluble polypeptides are accessible.[13−15] However, monomer synthesis and in particular, purification is not trivial, rendering scale up difficult.

Only two nonionic homopolypeptides show good water and organic solubility, poly(hydroxyproline) and poly(sarcosine) (PSar), both representing in fact poly(imino acid)s, also referred to as polypeptoids (POI). Interestingly, the former comprises

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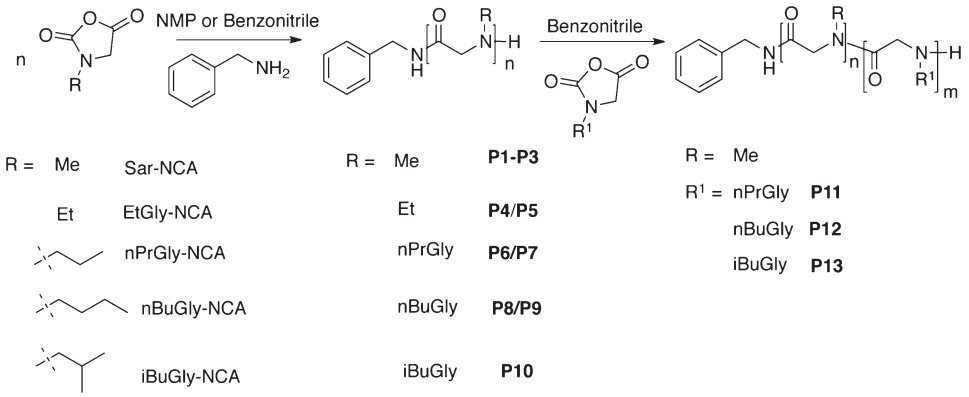

Figure 1. Schematic representation of synthesis of homo and block copolypeptoids prepared in this work.

stable helices while the latter forms random coils in water.[9] The tertiary amides in POI do not carry any H-donor, only two acceptors. Intra- and intermolecular interactions between polymer chains are impeded significantly but interaction with water (or other solvents), is favorable.

By and large, two different methods exist for the preparation of synthetic polypeptides: solid-phase peptide synthesis and the ring-opening polymerization of α-amino acid-*N*-carboxyanhydrides (NCA). The former is only of limited use for the preparation of large polypeptides due to synthetic limitations.[16] The ring-opening polymerization (ROP) NCAs is the most common route to access polypeptides.[9] It is well understood in the literature that NCA monomers can be deprotonated at the nitrogen, which leads to a different polymerization mechanism and eventually loss of control over the polymerization. Overall, a plethora of different polymerization mechanisms are confirmed or discussed (Figure S1a, Supporting Information).[17] Only in recent years have novel synthetic approaches allowed the preparation of well-defined polypeptides, block copolymers, and hybrid structures.[18−27] Most approaches have in common that the so-called activated monomer mechanism should be prevented, significantly reducing the possible reactions and thus, products (Figure S1a, Supporting Information).

In the case of *N*-substituted NCAs (NNCAs) the activated monomer mechanism cannot occur (Figure S1b, Supporting Information). Additionally, only the desired C5 is available for a nucleophilic attack.[28] However, an early report by Wessely et al. suggested that NNCAs are exceedingly sensitive to hydrolysis.[29] This may explain why NNCAs have been widely ignored over the last 6 decades. Only *N*-methylglycine *N*-carboxyanhydride (sarcosine *N*-carboxyanhydride, Sar−NCA) has received some attention.

Kimura and co-workers synthesized microcapsules, peptosomes and nanotubes comprising PSar as hydrophilic block[30−35] and peptide or ester based hydrophobic segments and reported stealth-like properties of the resulting materials (i.e., long circulation, limited nonspecific organ uptake). We hypothesized that PSar is biodegradable and biocompatible, but while the latter has been demonstrated,[34,35] the former remains to be elucidated. The reported biodegradation of poly-L-proline suggests that PSar may behave similarly.[36] Apparently, only one publication describes poly(*N*-ethylglycine) (P(EtGly)) and poly(*N*-*n*-propylglycine) (P(nPrGly)), respectively, without giving synthetic details nor physicochemical properties.[37,38]

On the other hand, POI obtained by solid-phase synthesis are well-known materials with interesting properties[39] useful for nonfouling surfaces,[40−42] self-assembling 2D and 3D structures[43,44] and glycopolypeptoids.[45] However, this synthetic approach is associated with high costs and large-scale production is not feasible.

Guo and Zhang gave a first proof of principle regarding the synthesis of block copolypeptoid.[46] They report on the preparation of cyclic POI of *N*-*n*-butylglycine and sarcosine under the catalysis of *N*-heterocylic carbenes. For comparison, a linear analog was prepared and analyzed by viscosimetry. Unfortunately, dispersity and physicochemical properties of the linear analog was not provided.

Here, we report on the synthesis, characterization of a series of POI bearing short aliphatic side chains (C1−C4) and some of their physicochemical properties. We emphasized on the polymerization kinetics to verify the livingness of this polymerization and prepared amphiphilic block copolymers with potential as drug delivery vehicles (Figure 1).

Our results suggest that POI are a very interesting platform technology for next generation (bio)materials as they combine excellent synthetic versatility and definition with main chain degradability.

EXPERIMENTAL SECTION

Materials and Methods. All substances for the preparation of monomers and polymers were purchased from Aldrich or Acros and were used as received unless otherwise stated. Benzonitrile (BN) and *N*-methyl-2-pyrrolidinone (NMP) were dried by refluxing over P_2O_5, benzylamine over BaO and petrolether over CaH_2 under dry argon atmosphere and subsequent distillation prior to use. Water levels were determined using a C20 compact coulometer (Mettler-Toledo, Giessen, Germany). In general, solvents were used at water levels of <30 ppm. Sensitive samples (monomers) were handled preferably in a glovebox (UNIlab, MBraun, Garching, Germany).

NMR spectra were recorded on a Bruker DRX 500 at room temperature (295 K). The spectra were calibrated using the solvent signals ($CDCl_3$ 7.26 ppm, D_2O 4.79 ppm, DMSO-d_6 2.50 ppm, MeOD-d_3 3.31 ppm).

Attenuated total reflectance Fourier transform infrared (ATR−FTIR) spectroscopy was performed on a Nicolet 5700 (Thermo) with an ATR sampling accessory (GladiATR, PIKE Technologies) and a MCT detector operated under OMNIC software.

Gel permeation chromatography (GPC) was performed on a PL-GPC-120 (Polymer Laboratories) running under WinGPC software (PSS, Mainz, Germany) with two consecutive Gram columns (100 and 1000 Å) with *N*,*N*-dimethylacetamide (DMAc) (5 g/L LiBr, 70 °C,

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1 mL/min) as eluent and calibrated against PMMA standards (PSS, Mainz, Germany).

Matrix assisted laser desorption/ionization time-of-flight (MALDI−ToF) mass spectra were recorded on a Bruker Biflex IV (Bruker Daltonics, Bremen, Germany) using a N_2 laser (λ = 337 nm). All spectra were recorded in positive reflector mode. The ions were accelerated by a potential of 19 kV and reflected using a voltage of 20 kV. Detection was typically set from 1000 m/z to 8000 m/z with a matrix suppression of typically 450−750 m/z. After parameter optimization for each measurement, the instrument was calibrated with Peptide Calibration standard II and/or Protein Calibration standard I (Bruker), depending on the m/z range of the individual sample. Samples were prepared with either dithranol (1,8,9-anthracenetriol, DA) or sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid, SA) as matrices using the dried-droplet spotting technique (0.5−1.5 μL). Exemplarily, samples (1 g/L) were dissolved in $CHCl_3$ (supplemented with 0.1% v/v trifluoroacetic acid (TFA)). The solution was mixed 1:1 (v/v) with saturated solution of DA in $CHCl_3$/0.1% TFA. No salt addition was necessary. Alternatively, samples (1 g/L) were dissolved in MeOH/1% TFA (v/v) and mixed 1:1 (v/v) with 10 g/L SA MeOH/1% TFA. Laser power was set slightly above the threshold, typically at 50%. Poisson distributions were calculated using eq 1, where DP_{max} is the degree of polymerization obtained from the signal with highest intensity (M_P) and k are non-negative integers. The obtained probabilities $P(DP_{max})$ are plotted against the calculated m/z at the respective DP and overlaid with the experimental MALDI−ToF mass spectra.

$$P(\mathrm{DP})_{max}(X = k) = \frac{\mathrm{DP}_{max}^{k}}{k!}\mathrm{e}^{(-\mathrm{DP}_{max})} \quad (1)$$

Gas chromatography was performed on a Varian 430-GC equipped with a Varian VF-5 ms column (30 m, 0.25 mm, 0.25 μm), N_2 carrier gas and FID detector running under Galaxy Software (Varian, Darmstadt, Germany) supplemented with a CombiPal robot arm (CTC Analytics, Zwingen, Switzerland). Customized equipment allowed Argon-flow during automated sampling while reduced pressure was applied otherwise.

Melting points were determined using a Büchi B-545 melting point apparatus.

Synthetic Procedures. *Monomer Synthesis. Sarcosine−NCA.* First 1.668 g of freshly ground sarcosine (19 mmol) and 1.99 g of triphosgene (6.7 mmol) were dried separately under 0.05 mbar for 1 h. Then, 4.01 mL (25 mmol) of (+)-limonene and 40 mL of dry THF were added to sarcosine under a steady flow of argon. Triphosgene was dissolved in 10 mL of dry THF and added to the sarcosine suspension. The steady flow of argon was turned off and the reaction mixture was heated to 65 °C and stirred for 2 h, yielding a clear solution. The solvent was evaporated under reduced pressure, yielding a brownish oil as crude reaction product. The oil was heated to 75 °C and dried under reduced pressure to obtain a solid. The crude product was dissolved in 35 mL THF and precipitated with 15 mL petrolether. This mixture stands overnight at −18 °C to crystallize. The solid was filtered under argon atmosphere, dried under reduced pressure and subsequently sublimated *in vacuo* (1.6596 g, 76%). Melting point (mp): 104.4 °C (lit. 102−105 °C).[17,47]

^{1}H NMR (500 MHz; DMSO-d_6): δ = 2.86 (3 H, s, CH_3−), 4.22 ppm (2 H, s, −CH_2−CO−).

^{13}C{^{1}H} NMR (125 MHz; DMSO-d_6): δ = 29.82 (C^4), 51.14 (CH_3), 152.62 (C^2), 176.40 ppm (C^5).

N-Ethylglycin−NCA. a. N-Ethylglycine Hydrochloride. Glyoxylic acid (15.128 g, 204.4 mmol) and ethylamine (3.838 g, 85.14 mmol) were added to CH_2Cl_2 (400 mL) and stirred at room temperature for 24 h. The solvent was evaporated, and 1 M HCl aqueous solution (400 mL) was added. The reaction mixture was heated under reflux for 24 h. The solvent was evaporated to yield a yellow solid. Recrystallization in methanol/diethyl ether (1/5, v/v) afforded the final product as white crystals (6.716 g, 57%). Mp: 179−180 °C (lit. 179−180 °C).[48]

^{1}H NMR (500 MHz; DMSO-d_6): δ = 1.19 (3 H, t, $^3J_{H,H}$ = 7.3 Hz, CH_3−CH_2−), 2.95 (2 H, q, $^3J_{H,H}$ = 6.6 Hz, CH_3−CH_2−NH), 3.84 (2 H, s, NH−CH_2−COOH), 9.13 (2 H, br, NH·HCl), 13.76 ppm (1 H, br, COOH).

b. N-Benzyloxycarbonyl-N-ethylglycine. *N*-Ethylglycine hydrochloride (4.050 g, 29.01 mmol) was suspended in 40 mL toluene and cooled to 0 °C. Sodium hydroxide (3.554 g, 88.85 mmol) was dissolved in 40 mL water and added to the cooled suspension. After slowly adding benzyl chloroformate (4.882 g, 28.62 mmol) the solution was stirred for about 4 h and allowed to phase separate subsequently. The aqueous layer was returned to the reactor and the pH value was controlled in the range from 1 to 2 with conc. HCl. The mixture was then extracted with 80 mL of ethyl acetate. After phase separation, the organic solvent was dried over magnesium sulfate and removed under reduced pressure to obtain yellowish oil (5.856 g, 86%).

^{1}H NMR (500 MHz; $CDCl_3$): δ = 1.14 (3 H, q, $^3J_{H,H}$ = 6.9 Hz, CH_3−CH_2−), 3.40 (2 H, m, CH_3−CH_2−), 4.03 (2 H, d, N−CH_2−COOH), 5.15 (2 H, d, C_6H_5−CH_2−O−), 7.33 ppm (5 H, br, C_6H_5).

c. N-Ethylglycine−NCA. To 6.413 g of *N*-benzyloxycarbonyl-*N*-ethylglycine (27.03 mmol) were added 4.322 g of acetyl chloride (55.18 mmol), and 5.537 g of acetic anhydride (54.23 mmol) under dry argon atmosphere. The mixture was heated under reflux for 6 h at 70 °C. The excess of acetyl chloride and anhydride was removed under reduced pressure, yielding a brownish oil as crude reaction product. The crude product was distilled under reduced pressure (0.04 mbar) at 120 °C (bath temperature), yielding colorless oil (2.517 g, 72%). Bp: 85−95 °C (0.03−0.04 mbar).

^{1}H NMR (500 MHz; $CDCl_3$): δ = 1.18 (3 H, t, $^3J_{H,H}$ = 7.3 Hz, CH_3−CH_2), 3.41 (2 H, q, $^3J_{H,H}$ = 7.3 Hz, CH_3−CH_2−), 4.06 ppm (2 H, s, N−CH_2−CO−).

^{13}C{^{1}H} NMR (125 MHz; $CDCl_3$): δ = 12.28 (CH_3−), 38.32 (CH_3−CH_2−), 48.24 (C^4), 151.72 (C^2), 165.80 ppm (C^5).

The other monomers were obtained accordingly.

N-n-Propylglycine−NCA. a. N-n-Propylglycine Hydrochloride. This was obtained as a colorless solid, 57%, mp. 195−197 °C.

^{1}H NMR (500 MHz; DMSO-d_6): δ = 0.89 (3 H, t, $^3J_{H,H}$ = 7.5 Hz, CH_3−C_2H_4−), 1.61 (2 H, m, CH_3−CH_2−CH_2−), 2.86 (2 H, t, $^3J_{H,H}$ = 7.5 Hz, CH_3−CH_2−CH_2−), 3.87 (2 H, s, NH−CH_2−COOH), 8.90 (2 H, br, NH·HCl), 13.78 ppm (1 H, br, COOH).

b. N-Benzyloxycarbonyl-N-n-propylglycine. This was obtained as a yellowish oil, 84%.

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.81 (3 H, m, CH_3−C_2H_4), 1.49 (2 H, m, CH_3−CH_2−CH_2−) 3.24 (2 H, q, $^3J_{H,H}$ = 8.2 Hz, CH_3−CH_2−CH_2−), 3.96 (2 H, d, N−CH_2−COOH), 5.07 (2 H, d, C_6H_5−CH_2−O−), 7.24 ppm (5 H, br, C_6H_5).

c. N-n-Propylglycine−NCA. This was obtained as a colorless oil, 79%, bp 70−90 °C (0.015−0.03 mbar).

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.90 (3 H, t, $^3J_{H,H}$ = 7.4 Hz, −CH_3), 1.57 (2 H, m, CH_3−CH_2−CH_2−), 3.31 (2 H, t, $^3J_{H,H}$ = 7.4 Hz, CH_3−CH_2−CH_2−), 4.05 ppm (2 H, s, −CH_2−CO−).

^{13}C{^{1}H} NMR (125 MHz; $CDCl_3$) 11.32 (CH_3−CH_2−), 20.98 (CH_3−CH_2−), 49.86 (−CH_2−N), 66.27 (C^4), 163.46 (C^2), 169.28 (C^5).

N-n-Butylglycine−NCA. a. N-n-Butylglycine Hydrochloride. This was obtained in 58%, mp 207 °C.

^{1}H NMR (500 MHz; DMSO-d_6): δ = 0.87 (3 H, t, $^3J_{H,H}$ = 7.4 Hz, −CH_3), 1.31 (2 H, m, CH_3−CH_2−), 1.61 (2 H, m, C_2H_5−CH_2−), 2.88 (2 H, t, $^3J_{H,H}$ = 7.7 Hz, −CH_2−NH−), 3.83 (2 H, s, −NH−CH_2−COOH), 9.21 (2 H, br, NH·HCl), 13.71 ppm (1 H, br, COOH).

b. N-Benzyloxycarbonyl-N-n-butylglycine. This was obtained as a colorless oil, 66%.

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.85 (3 H, m, CH_3−), 1.25 (2 H, m, CH_3−CH_2−), 1.47 (2 H, m, C_2H_5−CH_2−), 3.30 (2 H, m, −CH_2−N), 3.95 (2 H, m, N−CH_2−COOH), 5.10 (2 H, d, C_6H_5−CH_2−O−), 7.31 ppm (5 H, m, C_6H_5).

c. N-n-Butylglycine−NCA. This was obtained as a colorless oil, 70%, bp 110−120 °C (0.30−0.35 mbar).

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.95 (3 H, d, $^3J_{H,H}$ = 7.4 Hz, $CH_3-C_3H_6-$), 1.36 (2 H, m, $CH_3-CH_2-C_2H_4-$), 1.57 (2 H, m, $C_2H_5-CH_2-CH_2-$), 3.40 (2 H, t, $^3J_{H,H}$ = 7.4 Hz, $C_3H_7-CH_2-$), 4.08 ppm (2 H, s, $N-CH_2-CO-$).

^{13}C{^{1}H}-NMR (125 MHz; $CDCl_3$): δ = 13.50 ($CH_3-C_3H_6-$), 19.68 ($CH_3-CH_2-C_2H_4-$), 29.20 ($C_2H_5-CH_2-CH_2-$), 43.35 (C^4), 48.84 ($C_3H_7-CH_2-$), 152.02 (C^2), 165.52 ppm (C^5).

N-Isobutylglycine−NCA. a. N-i-Butylglycine Hydrochloride. This was obtained in 57%, mp 225 °C.

^{1}H NMR (500 MHz; DMSO-d_6): δ = 0.93 (6 H, d, $^3J_{H,H}$ = 6.7 Hz, $(CH_3)_2-CH-CH_2-$), 2.02 (1 H, m, $(CH_3)_2-CH-CH_2-$), 2.77 (2 H, d, $^3J_{H,H}$ = 7.0 Hz, $(CH_3)_2-CH-CH_2-$), 3.82 (2 H, s, $NH-CH_2-COOH$), 9.16 (2 H, br, $NH\cdot HCl$), 13.74 ppm (1 H, br, COOH).

b. N-Benzyloxycarbonyl-N-isobutylglycine. This was obtained as a colorless oil, 87%.

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.88 (6 H, m, $(CH_3)_2-CH-CH_2-$), 1.84 (1 H, m, $(CH_3)_2-CH-CH_2-$), 3.16 (2 H, m, $(CH_3)_2-CH-CH_2-$), 4.03 (2 H, m, $N-CH_2-COOH$), 5.15 (2 H, m, $C_6H_5-CH_2-O-$), 7.31 ppm (5 H, m, C_6H_5).

c). N-Isobutylglycine−NCA. This was obtained as a colorless solid, 70%, mp.: 39 °C

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.96 (6 H, d, $^3J_{H,H}$ = 6.7 Hz, $(CH_3)_2-CH-CH_2-$), 1.92 (1 H, m, $(CH_3)_2-CH-CH_2-$), 3.20 (2 H, d, $^3J_{H,H}$ = 7.5 Hz, $(CH_3)_2-CH-CH_2-$), 4.09 ppm (2 H, s, $N-CH_2-CO-$).

^{13}C{^{1}H} NMR (125 MHz; $CDCl_3$): δ = 19.80 ($(CH_3)_2-CH-CH_2-$), 26.96 ($(CH_3)_2-CH-CH_2-$), 49.54 (C^4), 51.21 ($(CH_3)_2-CH-CH_2-$), 152.37 (C^2), 165.45 ppm (C^5).

Preparation of Homopolypeptoids. For the kinetic measurements with IR spectroscopy and gas chromatography the polymerization mixture was prepared and sealed in a glovebox under inert and dry atmosphere.

Exemplarily, the preparation of Poly(Sar)$_{25}$ was performed as follows.

Poly(sarcosine)$_{25}$, ***P1***. Sar−NCA (0.2647 g, 2.3 mmol) was weighed into reaction vessel dissolved in 2.3 mL of dry benzonitrile and 0.228 g (2.3 mmol) of NMP. After complete dissolution the reaction vessel was closed with a septum. Outside of the glovebox the initiator benzylamine (10 μL, 0.092 mmol) was added via a syringe ($[M]_0/[I]_0$ = 25). The reaction mixture was stirred at room temperature under constant pressure (20 mbar). After several hours the reaction mixture was precipitated into diethyl ether and isolated POI was dried under reduced pressure. The product was dissolved (or suspended for water insoluble polymers) in water and subsequently freeze-dried.

GPC (DMAc): M_n = 1.3 kg/mol ($Đ_M = M_w/M_n$ = 1.31).[49]

^{1}H NMR (500 MHz; D_2O): δ = 2.89 (77 H, br, CH_3-), 4.20 (55 H, br, $-CH_2-CO-$), 7.28 ppm (5 H, br, C_6H_5-).

Poly(N-ethylglycine)$_{25}$, ***P4***. GPC (DMAc): M_n = 1.7 kg/mol ($Đ_M$ = 1.25).

^{1}H NMR (500 MHz; $CDCl_3$): δ = 1.10 (73 H, br, CH_3-), 3.40 (47 H, br, CH_3-CH_2-), 4.17 (47 H, br, $-CH_2-CO-$), 7.25 ppm (5 H, br, C_6H_5-).

Poly(N-n-propylglycine)$_{25}$, ***P6***. GPC (DMAc): M_n = 2.8 kg/mol ($Đ_M$ = 1.20).

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.83 (79 H, br, CH_3-), 1.62 (53 H, br, CH_3-CH_2-), 3.18 (50 H, br, $CH_3-CH_2-CH_2-$), 4.11 (50 H, br, $-CH_2-CO-$), 7.20 ppm (5 H, br, C_6H_5-).

Poly(N-n-butylglycine)$_{25}$, ***P8***. GPC (DMAc): M_n = 2.3 kg/mol ($Đ_M$ = 1.16).

^{1}H NMR (500 MHz; TFA (DMSO-d_6)): δ = 0.84 (65 H, br, CH_3-), 1.29 (43 H, br, CH_3-CH_2-), 1.62 (38 H, br, $CH_3-CH_2-CH_2-$), 3.33 (43 H, br, $CH_3-C_2H_4-CH_2-$), 4.39 (41 H, br, $-CH_2-CO-$), 7.19 ppm (5 H, br, C_6H_5-).

Poly(N-iso-butylglycine)$_{25}$, ***P10***. GPC (DMAc): M_n = 1.4 kg/mol ($Đ_M$ = 1.20).

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.78 (80 H, br, $(CH_3)_2-CH-CH_2-$), 1.81 (24 H, br, $(CH_3)_2-CH-CH_2-$), 3.23 (22 H, br, $(CH_3)_2-CH-CH_2-$), 4.08 (25 H, br, $-CH_2-CO-$), 7.32 ppm (5 H, br, C_6H_5-).

Preparation of Block Copolypeptoids. Poly[(Sar)$_{50}$-b-(N-nPrGly)$_{25}$], ***P11***. In a glovebox, 0.203 g (1.77 mmol) of sarcosine−NCA was weighed into reaction vessel and 2.3 mL of dry benzonitrile was added. After complete dissolution 3.9 μL of benzylamine (36 μmol) was added. The reaction mixture was stirred at room temperature and under constant pressure (20 mbar) for 3$^1/_2$ h. For analytical investigations of the first block, 40 μL were removed from the reaction mixture. Then 0.1242 g (0.87 mmol) of *N-n*-propylglycine−NCA was weighed out and dissolved in 0.87 mL benzonitrile. The solution was added to the reaction mixture of the first block. Additional 5 h the reaction mixture was stirred under constant pressure (20 mbar). The reaction mixture was precipitated into diethyl ether and isolated block copolypeptoide was dried under reduced pressure, dissolved in water and subsequently freeze-dried.

GPC (DMAc): M_n = 5.7 kg/mol ($Đ_M$ = 1.13).

^{1}H NMR (500 MHz; D_2O): δ = 0.89 (51 H, br, $CH_3-C_2H_4-$), 1.50 (35 H, br, $CH_3-CH_2-CH_2-$), 2.98 (135 H, br, CH_3-), 3.29 (30 H, br, $CH_3-CH_2-CH_2-$), 4.26 (121 H, br, $(CH_3-C_2H_4-)N-CH_2-CO-$, $(CH_3-)N-CH_2-CO-$ and $NH-CH_2-C_6H_5$), 7.33 ppm (5 H, br, C_6H_5-).

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.88 (50 H, br, $CH_3-C_2H_4-$), 1.41 (33 H, br, $CH_3-CH_2-CH_2-$), 2.96 (129 H, br, CH_3-), 3.21 (32 H, br, $CH_3-CH_2-CH_2-$), 4.27 (117 H, br, $(CH_3-C_2H_4-)N-CH_2-CO-$, $(CH_3-)N-CH_2-CO-$ and $NH-CH_2-C_6H_5$), 7.28 ppm (5 H, br, C_6H_5-).

Poly[(Sar)$_{50}$-b-(N-nBuGly)$_{25}$], ***P12***. GPC (DMAc): M_n = 6.8 kg/mol ($Đ_M$ = 1.16).

^{1}H NMR (500 MHz; $CDCl_3$): δ = 0.90 (38 H, br, $CH_3-C_3H_6-$), 1.30 (27 H, br, $CH_3-CH_2-C_2H_4-$), 1.47 (25 H, br, $CH_3-CH_2-CH_2-CH_2-$), (16 H, br, $C_3H_7-CH_2-$), 3.03 (125 H, br, CH_3-), 3.35 (23 H, br, $CH_3-C_2H_4-CH_2-$), 4.23 (105 H, br, ($C_4H_9-N-CH_2-CO-$ and $CH_3-NH-CH_2-C_6H_5$), 7.29 ppm (5 H, br, C_5H_6-).

^{1}H NMR (500 MHz; D_2O): δ = 2.96 (122 H, br, CH_3-), 4.28 (84 H, br, (CH_3-N-CH_2-CO-), 7.20 ppm (5 H, br, C_6H_5-).

Poly[(Sar)$_{50}$-b-(N-iBuGly)$_{25}$], ***P13***. GPC (DMAc): M_n = 5.6 kg/mol ($Đ_M$ = 1.09).

^{1}H NMR (500 MHz; TFA (DMSO-d_6)): δ = 1.02 (63 H, br, $(CH_3)_2-CH-CH_2-$), 2.07 (11 H, br, $(CH_3)_2-CH-CH_2-$), 3.22 (160 H, br, $(CH_3)_2-CH-CH_2-$) and CH_3-), 4.55 (109 H, br, $((CH_3)_2-CH-CH_2-)N-CH_2-CO-$, $(CH_3-)N-CH_2-CO-$ and $NH-CH_2-C_6H_5$), 7.29 ppm (5 H, br, C_6H_5-).

Investigation of Polymerization Kinetics. For the kinetic investigations using IR spectroscopy or gas chromatography the monomer solutions were prepared and sealed in a glovebox under inert and dry atmosphere (<0.1 ppm of H_2O) in custom-made Schlenk-tubes. The initiator was added outside the glovebox after sampling $[M]_0$ and was added against dry nitrogen or argon flow via a septum. The polymerization kinetics were determined following the decrease of the monomer concentration.

IR Spectroscopy. Polymerization kinetics were investigated by ATR−FTIR spectroscopy by following the decrease of the intensity of the C=O stretching band (≈1776 cm^{-1}) which responds linear with the concentration in the investigated concentration range. Samples (approximately 5 μL) were taken manually and during sampling inert gas was blown over the reaction mixtures.

Gas Chromatography. Online gas chromatographic measurement of the monomer conversion was possible for all monomers but Sar−NCA. Polymerization mixtures were sampled at regular intervals automatically. Monomer consumption was followed by the change of the ratio of the integrals of the monomer and the internal standard over time.

Statistical Analysis. Statistical analysis of the kinetic investigations was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego CA, www.graphpad.com.

Reichardt's Dye Solubilization. Solutions of polymer and Reichardt's dye in MeOH (10 g/L each) were combined in a ratio of 10/1 (v/v), the solvent was removed in a stream of nitrogen. The dry film was redissolved in phosphate buffered saline (pH 7.4) to give concentrations of 10 g/L (polymer) and 1 g/L (dye), respectively. To remove insoluble fractions, the solutions were centrifuged for 5 min at 10 000*g*. For absorbance measurements (400−700 nm), the solutions were diluted with DI water (1/1) and filtered through a 0.22 μm PVDF syringe filter.

RESULTS AND DISCUSSION

Monomer Synthesis. Monomers used in this account were obtained by direct phosgenation (Sar−NCA) or three-step synthesis from primary amines and glyoxylic acid using modified literature procedures (Figure S2, Supporting Information).[29,46,50] Overall yields of polymerization grade purified monomers were good (about 75%, Sar−NCA) to mediocre (about 30% for EtGly−NCA, nPrGly−NCA, nBuGly−NCA, and iBuGly−NCA). In contrast to nonsubstituted NCAs, all these polymers could be purified by sublimation (Sar−NCA) or distillation (EtGly−NCA, nPrGly−NCA, nBuGly−NCA, and iBuGly−NCA), which is an advantage of this monomer type over nonsubstituted NCAs (purified typically by multiple recrystallization). In modification of literature procedures we used benzyl group in the intermediate step and acetyl chloride in the cyclization step. As a consequence, benzyl acetate results as a side product, which we found problematic to remove completely in one distillation step (monomer purity >98% as judged by NMR, Figure S3, Supporting Information). ATR−FTIR spectra of all monomers can be found in Supporting Information (Figure S4). However, compared to HCl, the most common impurity in NCA synthesis, benzyl acetate as a potential impurity in the monomers should be much less problematic during storage and polymerization as it is less likely to inflict unwanted reactions.

Kinetic Investigations of Polymerization of NNCAs. It is well understood in the literature that Sar−NCA can be polymerized in a very defined manner. Many researchers have investigated the polymerization kinetics of NCAs and NNCAs in the last decades but there is little consensus on various factors such as temperature, CO_2 partial pressure and addition of acids. In particular monomer purity appears to be blamed for erratic results. More recently, it is discussed that application of so-called high-vacuum is beneficial of the polymerization of NCAs.[20,23,25,27] However, it should be kept in mind that, when performed in solution, the minimal accessible pressure is the vapor pressure of the solvent. Common solvents in NCA polymerization reactions are tetrahydrofuran (THF) and dimethylformamide (DMF) with vapor pressures of 173 mbar and 3.7 mbar (both at 293 K), respectively. Reactions under high-vacuum conditions ($<10^{-3}$ mbar) are therefore not possible using such solvents. Moreover, vacuum is reportedly applied either only at the beginning of the reaction[20] or intermittently without further specification.[23,51] Therefore, CO_2 partial pressure will be not constant during the polymerization. To the best of our knowledge, the NCA polymerization has not been investigated in dependence of a constant pressure.[52] In order to allow polymerization over a broad pressure-range, we chose the *N*-methyl-2-pyrrolidinone (boiling point (bp), 203 °C; vapor pressure, 0.3 mbar) and benzonitrile (bp, 191 °C; vapor pressure, 0.7 mbar) as solvent. High vacuum still is not accessible

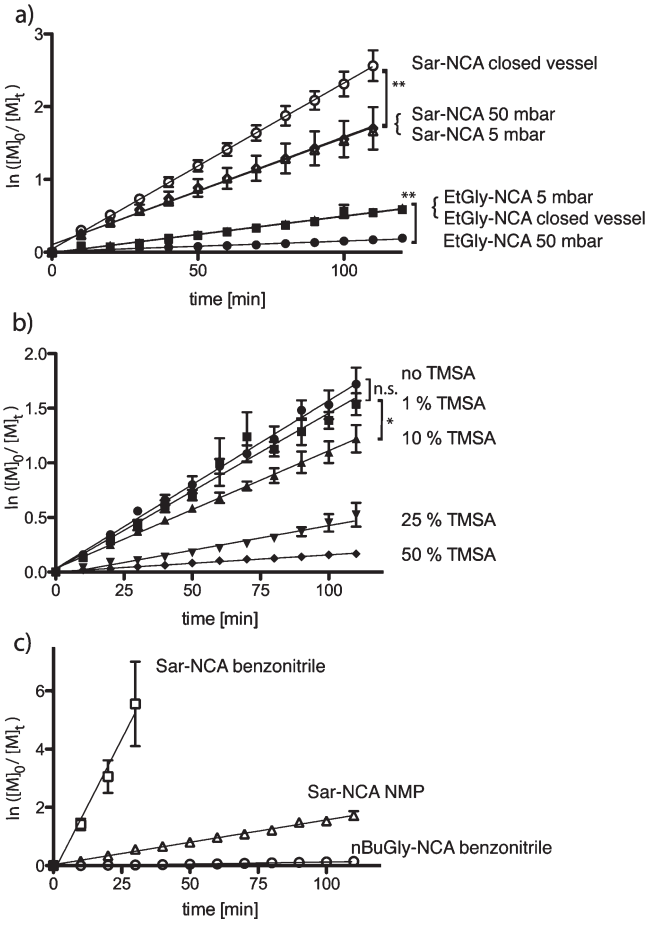

Figure 2. Linear pseudofirst order kinetic plots of the polymerization of Sar−NCA and EtGly−NCA under different conditions. (a) Dependency of the polymerization Sar−NCA in *N*-methyl-2-pyrrolidinone (NMP) and EtGly−NCA in benzonitrile on the pressure (5, 50 and closed vessel) at 20 °C (0.5 M, $[M]_0/[I]_0 = 50$). (b) Influence of the concentration of added trifluoromethanesulfonic acid (TMSA, represented as mol % of initiator) on the polymerization of Sar−NCA at room temperature in *N*-methyl-2-pyrrolidinone (0.5 M, $[M]_0/[I]_0 = 50$, all $p < 0.0001$ unless mentioned otherwise). (c) Comparison of polymerization of Sar-NCA in NMP ($[M]_0/[I]_0$ =50, 0.5 mbar, room temperature) and benzonitrile ($[M]_0/[I]_0$ = 50, 0.5 mbar, room temperature), nBuGly−NCA ($[M]_0/[I]_0$ = 50, 50 mbar, room temperature). Experiments were performed duplicate or triplicate and data are presented as means ± SEM. Pairs of linear regressions differ statistically significant (* $p < 0.001$, all ** $p < 0.0001$) unless otherwise marked (n.s.).

with such solvents, but the pressure range that can be studied is somewhat larger. Both represent polar but relatively inert solvents, which are excellently suited for NCA polymerization and can be dried to water levels below 30 ppm without undue efforts. It should be noted that all polymerization mixture remained homogeneous through the entire course of the experiment unless otherwise mentioned.

Polymerization Kinetics of Sarcosine−NCA. Polymerization kinetics were measured in duplicate at 5 and 50 mbar and in a closed vessel using NMP as a solvent and Sar−NCA as monomer. Vacuum was applied continuously, except during sampling, when inert gas was blown over the reaction mixtures. Change in monomer concentration was followed by IR spectroscopy monitoring the C═O stretching band at 1776 cm^{-1}. For the other

Table 1. Experimental Data of Kinetic Experiments of the Polymerization of Various *N*-Substituted Amino Acid *N*-Carboxyanhydrides under Different Experimental Conditions ($[M]_0$ = 0.5 M)

run ID	monomer	$[M]_0/[I]_0$	k_p^{app} [10^{-3} L/(mol·s)]	pressure [mbar]	% acid	solvent
1	Sar	50	23.11	0.5	0	NMP
2	Sar	50	305	0.5	0	BN
3	Sar	50	19.87	5	0	NMP
4	Sar	50	20.93	50	0	NMP
5	Sar	50	28.78	closed vessel	0	NMP
6	EtGly	50	7.41	5	0	BN
7	EtGly	50	2.34	50	0	BN
8	EtGly	50	8.54	closed vessel	0	BN
9	Sar	50	21.51	0.5	1	NMP
10	Sar	50	17.07	0.5	10	NMP
11	Sar	50	7.90	0.5	25	NMP
12	Sar	50	2.36	0.5	50	NMP
13	EtGly	25	6.28[a]	40	0	BN
14	EtGly	50	6.30[a]	40	0	BN
15	PrGly	25	4.60[a]	40	0	BN
16	PrGly	50	5.50[a]	40	0	BN
17	nBuGly	25	4.08[a]	40	0	BN
18	nBuGly	50	4.46[a]	40	0	BN
29	iBuGly	25	2.71[a]	40	0	BN

[a] $[M]_0$ = 1 M, BN = benzonitrile, NMP = *N*-methyl-2-pyrrolidinone.

monomers concentration could be also followed by gas chromatography. All $\ln(M_0/M_t)$ vs time plots appear linear up to high monomer conversions (>80−90%), i.e., show pseudofirst order kinetics with regard to the initiator (Figure 2a). This suggests that the concentration of propagating species remains constant and supports the living character of the polymerization. Analytical data and kinetic investigations from earlier work have often pointed toward a living polymerization of Sar−NCA.[53,54] It has been reported that the polymerization rate is not constant during Sar−NCA polymerization although this was attributed to heterogeneity of the reaction mixture,[55] while other authors claimed that Sar−NCA kinetics give strictly linear pseudofirst order kinetic plots.[56] Recently, several reports discussed the benefit of reduced pressure on the NCA polymerization[20,23,25,27] but the influence of the pressure on the polymerization kinetics remained somewhat unclear. We were unable to find a clear correlation between the polymerization rate and the pressure (Figure 2a). In the case of Sar−NCA the polymerization at 5 and 50 mbar is slower than when performed in a closed vessel. While the difference appears statistically significant ($p < 0.001$) we believe that it will be necessary to perform this experiment more often to consolidate this finding.

Although carbamates can be quite stable at room temperature,[17] the decarboxylation is reportedly not the rate-determining step in the NCA polymerization.[17,57−59] Therefore, no or only a limited effect of the pressure on the polymerization should be expected. At this stage, we tend to believe that the differences that we and others observed are due to experimental errors and will not prove significant when the experiments are replicated in sufficient number. Also the influence of acids during the polymerization of NCAs is somewhat unclear. On the one hand, small amounts (substoichiometric and stoichiometric with respect to initiator/propagating species) of weak acids reportedly increase the polymerization rate.[60] On the other hand, stoichiometric amounts of strong acids slows down polymerization considerably by protonating the propagating species.[61]

We were interested which way substoichiometric amounts of strong acids might influence the NNCA polymerization. Since the counterion of an acid represents a (weak) nucleophile, which in turn could interfere with the polymerization, we used trifluoromethanesulfonic acid (TMSA) as the triflate anion shows no considerable nucleophilicity. We added 1, 10, 25, and 50% of TMSA with respect to the initiator (benzylamine) and followed monomer conversion ($[M]_0/[I]_0$ = 50) (Figure 2b). Clearly, the addition of TMSA slows down the polymerization in all cases, although it should be mentioned that the difference between the experiments without TMSA and 1% TMSA is not statistically significant ($p > 0.05$). As expected, the apparent polymerization rate constants are practically invariant for different $[M]_0/[I]_0$ ratios (Table 1).

It is well-known that the polymerization kinetics of NCAs and NNCA is highly solvent dependent and is described to be faster in less polar solvents, suggesting a noncharged species involved in the rate-determining step.[17] Contrarily, we observed a much faster polymerization in benzonitrile as compared to NMP in the case of Sar−NCA contradicting the solvent polarity influence (about 10-fold increase, Figure 2c). It should be noted that the polymerization rates in NMP observed by us are in good agreement with rates observed by others in DMF.[54] However, also other authors found very rapid polymerization in polar solvents such as nitrobenzene and acetophenone for Sar−NCA with apparent polymerization constants very similar to our result.[56] It appears that the solvent influence on the polymerization rate of NCAs and NNCAs is more complex. However, for NCAs, limited solubility often prevents such studies in a broader range of solvents and NNCAs may be a valuable alternative. We mentioned earlier that we sometimes observe small amounts of benzyl acetate as an impurity in the monomers. One could expect that this could eventually lead to a chain transfer with acetamido terminated and benzyl alcohol initiated polymers. However, we are unable to find evidence of such polymers in the MALDI−ToF mass spectra. While this does not allow to rule out the possibility of such transfer reaction, we think it to be highly unlikely to happen to any considerable extent. All relevant data for the polymerization kinetics and apparent polymerization rate constants are listed in Table 1.

Next, we investigated the development of M_P (as determined by MALDI−ToF) vs conversion (Figure 3). The linearity (absence of transfer reactions) and the linear pseudofirst order (absence of termination reactions) to high monomer conversions (>75%) again points toward a living polymerization of Sar−NCA. However, while the first sample shows good agreement between calculated and observed M_P, the subsequent data show an increasingly marked deviation from the calculated values. Kricheldorf et al. made a very similar observation in comparable experiments. While high yields and high apparent DPs were obtained by ^{1}H NMR end-group analysis, the DPs as obtained by MALDI−ToF were much lower and in excellent agreement with our experimental data.[62] We are unable to provide a mechanism for a potential transfer reaction during the polymerization of Sar−NCA. Although the samples were taken against argon flow, minute amounts of water, e.g., absorbed at the pipet tips or cannulas, might have been introduced into the polymerization reaction. It is well-known that NNCAs are

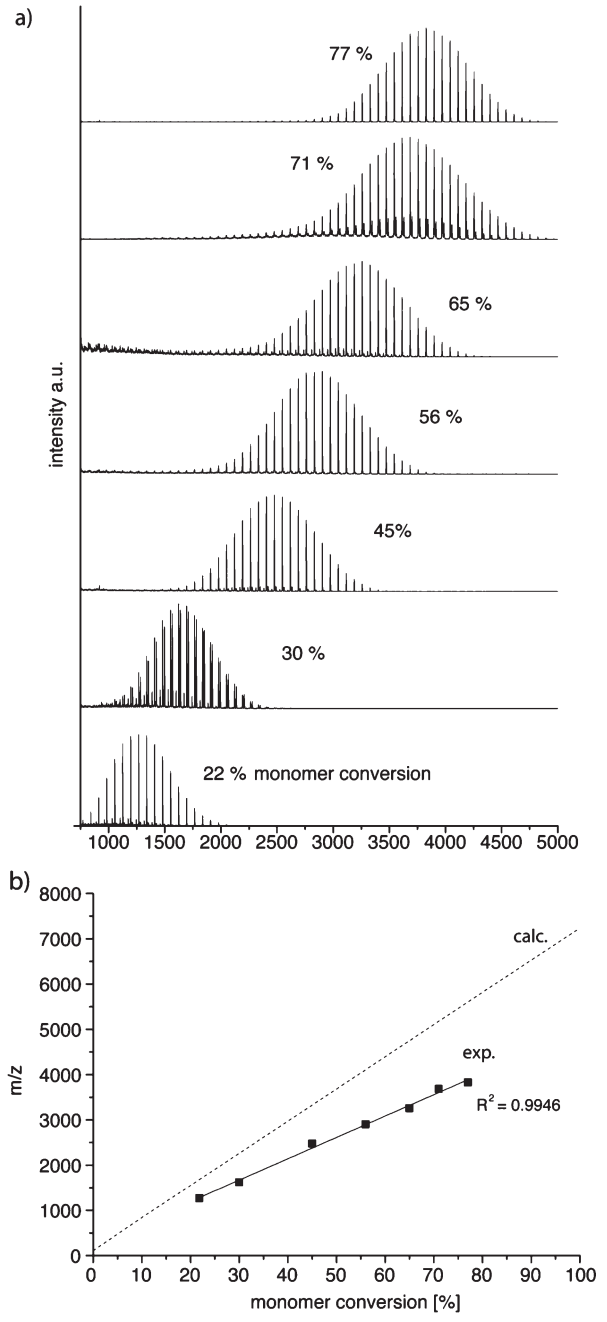

Figure 3. Development of M_p (obtained by MALDI−ToF MS, H^+ doping, matrix SA) vs monomer consumption (obtained by IR spectroscopy) of the polymerization of sarcosine−NCA initiated by benzylamine in *N*-methyl-2-pyrrolidinone (0.6 M) at room temperature and 50 mbar at a monomer to initiator ratio $[M]_0/[I]_0 = 100$. (a) The narrow distributions (Poisson-type) confirm the good control over the polymerization. (b) The linear trend suggests a high degree of control over the polymerization with very limited termination and chain transfer reactions and high initiator efficiency as well as a rapid initiation versus polymerization.

exceedingly sensitive to hydrolysis.[29] However, a marked ingress of water during sampling, leading to monomer hydrolysis and/or additional initiation of polymerization should show up in the kinetic plots (positive curvature). We would also expect to detect low molar mass polymer in MALDI−ToF if significant amount of initiator would be introduced during sampling. Moreover, in a control experiment we were unable to detect monomer consumption for 2 h without addition of initiator (data not shown) ruling out the possibility of residual water in the reagents being responsible for this observation. We are currently investigating the nature of this discrepancy in more detail. The Poisson-type distributions of the polymers are not surprising, considering the nature of the propagating species and results of an earlier report.[54] In this work by Sisido, however, considerable deviation from Poisson-type distribution was found at degrees of polymerization larger than 15. The narrow distribution even at low monomer conversion indicates that the initiation is fast enough in comparison to the polymerization as has been described before.[17] Typically, the reasons for "non-livingness" of NCA polymerizations and resulting broad product dispersities are secondary structure formation, propagation via mechanisms other than the amine mechanism or combinations thereof. At elevated temperatures, also nonideal solvents such as DMF may lead to termination reactions. In our case, all these modalities can be ruled out. However, one might expect side reactions at significantly larger degrees of polymerization (DP > 1000), but end-group analysis at such high masses will be challenging.

Polymerization of Higher Substituted NNCAs. In contrast to Sar−NCA polymerization,[54,62,63] no information regarding the living character of NNCAs with longer *N*-substituents is available. Also for NNCAs with ethyl, propyl, and butyl substituents, the polymerization can proceed to high monomer conversion with linear pseudofirst order kinetics with respect to monomer concentrations, albeit much slower (Figure 2a,c). However, we have to note that poly(*N*-(isobutyl)glycine) P(iBuGly) starts to become gel-like at degrees of polymerizations of approximately 12 (50% monomer conversion). Thus, polymerization kinetics of this monomer was not followed to higher monomer conversions and degrees of polymerizations. The apparent polymerization rates of the different monomers were in the order Sar ≫ EtGly > PrGly > nBuGly > iBuGly (Table 1). This order reflects earlier results that investigated hydrolysis vs polymerization of NNCAs in aqueous solution.[29] Regarding pressure dependency, the polymerization rates of EtGly−NCA were in the order 5 mbar ≈ closed vessel > 50 mbar, and in all cases, much smaller rates were observed as compared to Sar−NCA (Table 1).

Matrix assisted laser desorption/ionization time-of-flight mass spectrometry. Homopolymers of Sar, EtGly, PrGly, and nBuGly were accessible to MALDI−ToF mass spectrometry (Figure 4 and 5). The molar mass distributions appear narrow and in all cases the mass differences ($\Delta m/z$) between individual signal distributions reflect the mass of the polymer repeat unit (Figure 4b and Figure 5b). Furthermore, the main distribution (α) reflects a molar mass distribution, which corresponds to polymers initiated with benzylamine, bearing an amine terminus and sodium doping. Major alternative subdistributions (β, γ) are found to correspond to the same polymers bearing a proton and potassium ions instead of sodium. In most cases, the degree of polymerization (DP) as obtained by the signal with highest intensity (M_P) is somewhat smaller than expected from $[M]_0/[I]_0$. This can be explained by incomplete monomer conversion. Alternatively, significant errors in the amount of added initiator may be responsible as these were rather small (<10 μL). However, unintentional monomer hydrolysis may also be responsible for this observation, as discussed above. Nevertheless, the signal distributions observed are in excellent agreement with calculated Poisson-type distributions (Figures 4a, 5a) in all cases.

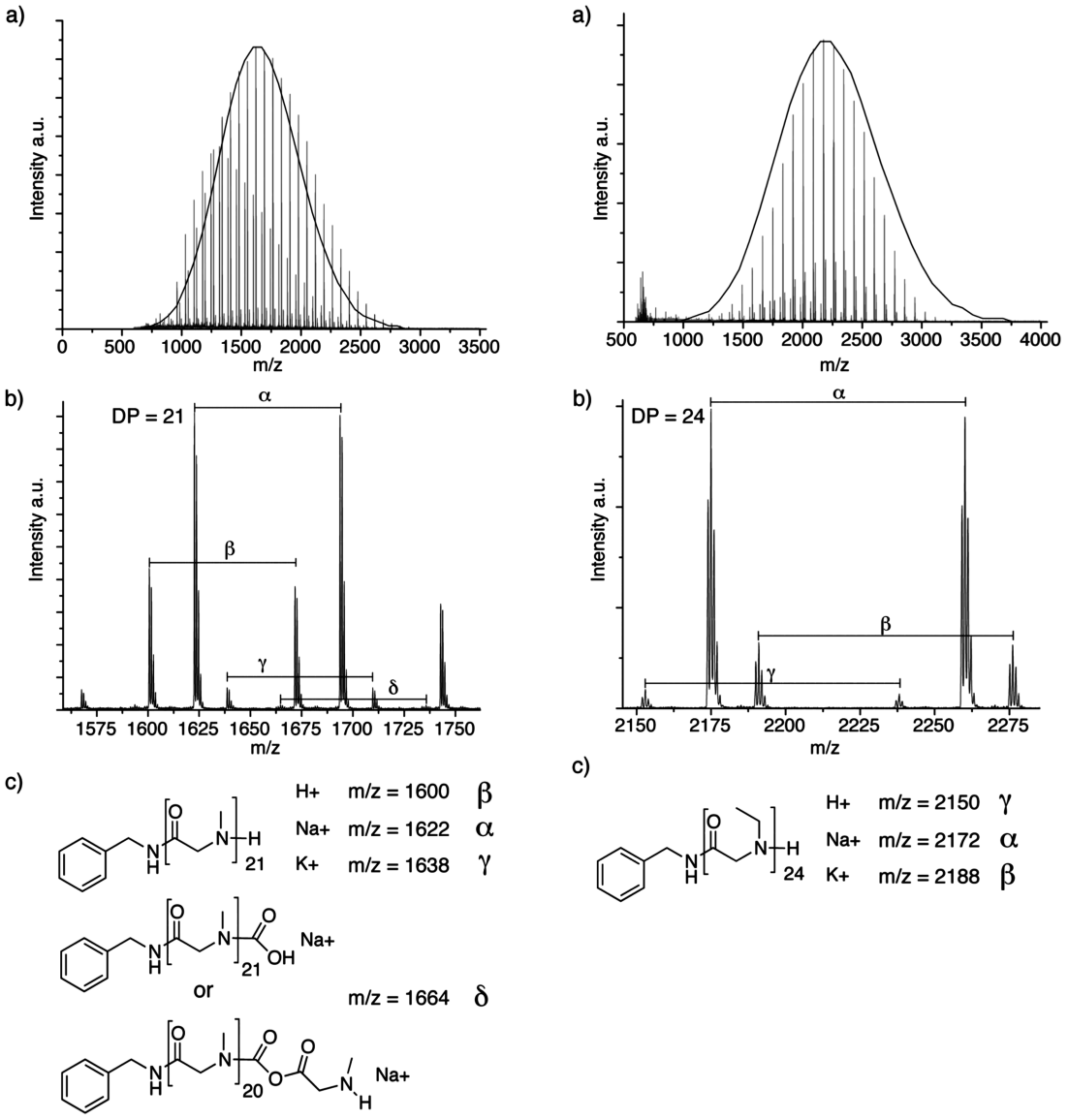

Figure 4. MALDI−ToF mass spectra (H^+ doping, matrix DA) of (a) **P1** (left column) and **P4** (right column), with an overlay of the respective Poisson distributions (black curve) along with (b) a blown up view of section comprising most intensive signals. (c) Structures of polymers that can be assigned to the different populations.

At a DP of 25, a Poisson distribution corresponds to a dispersity of $Đ_M = 1.04$. We also calculated an experimental $Đ_{M,MALDI}$ for all samples (Table 2) by summing up the intensities at all data points obtained from the MALDI−ToF experiment. The obtained values are between 1.04 and 1.08 and correspond well with the expected values. Besides the three major distributions that all can be attributed to polymers of the desired structure, we found only signals of minor intensity that correspond to different polymer structures. Distribution δ in the mass spectrum of **P1** can be attributed to polymers that did not undergo decarboxylation (i.e., carbamate mechanism) (Figure 4c). Similarly, the δ distributions in the mass spectra of **P8** fits to polymer chains which were initiated by water and bear an additional CO_2 and Na^+ doping (Figure 5b,c, right column). Signals corresponding to the same polymer but doped by protons can be seen at very low intensities (not shown). Contrarily, in the case of **P6**, it appears that this distribution (m/z e.g. 2591.1) neither derives from polymers initiated by water, nor can it be attributed to cyclic polymers, which have been reported for POI.[64] Also structures resulting from the carbamate mechanism or from a potential termination or initiation reaction with benzyl acetate do not fit this distribution.

NMR Studies on Homopolymers. The use of benzylamine as initiator allows the determination of M_n of the polymers by end-group analysis (Table 2). It is apparent from the comparison of ^1H NMR spectra of **P1**, **P4**, **P6**, and **P8** that all major signals can be attributed to the polymer structures (Figure S5, Supporting Information). For end-group analysis, the signal intensity of aromatic protons of the initiator benzylamine were related to the backbone or side-chain protons. Typically both ratios are very similar, if not, they were averaged to obtain the molar mass from NMR given in Table 2.

Infrared Spectroscopic Studies. Only limited information can be found regarding the infrared spectroscopic characteristics of *N*-substituted POI in the literature. All five types of homopolymers

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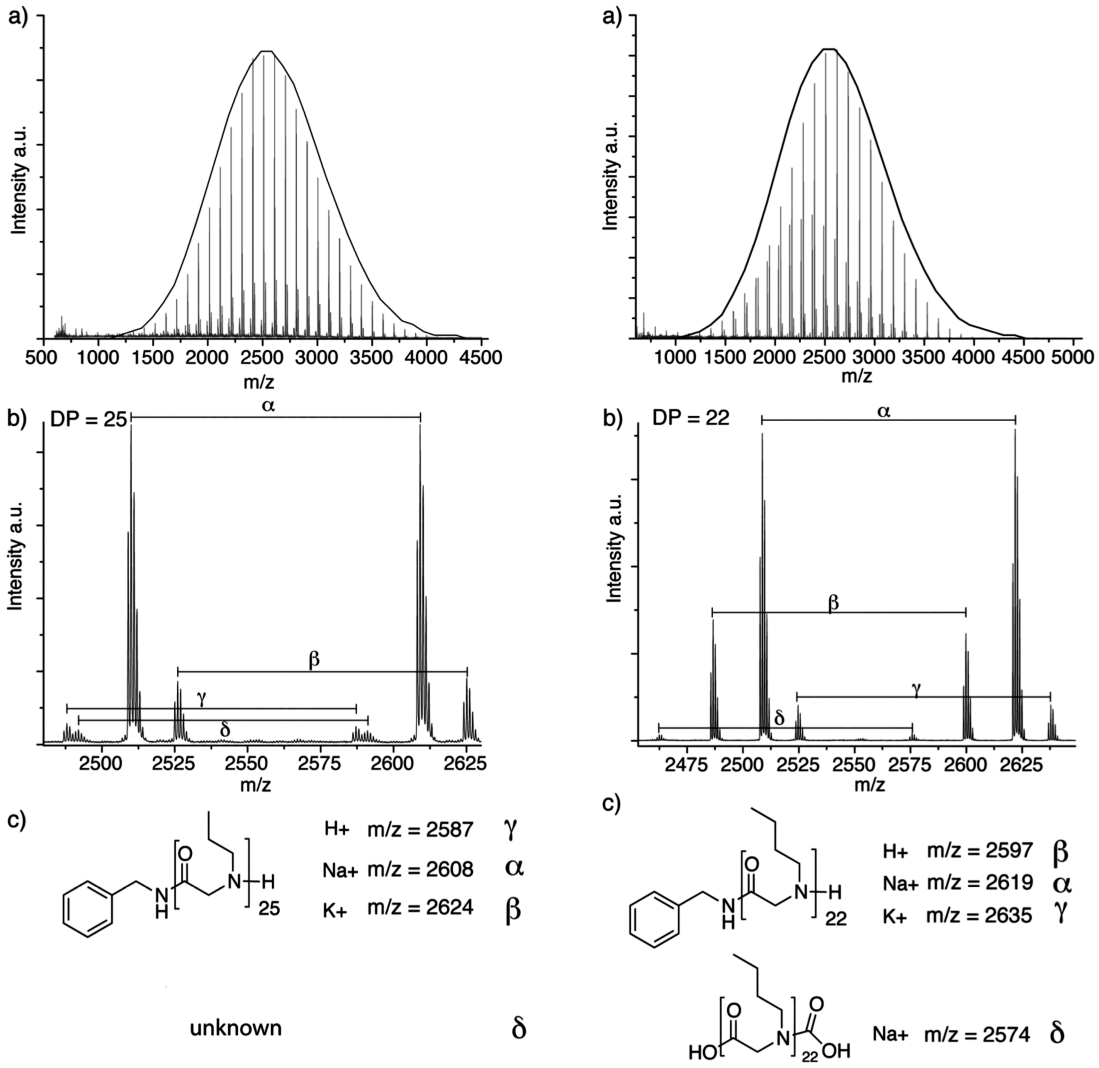

Figure 5. MALDI−ToF mass spectra (H^+ doping, matrix DA) of (a) **P6** (left column) and **P8** (right column) with an overlay of the respective Poisson distributions (black curve) along with (b) a blown-up view of section comprising most intensive signals. (c) Structures of polymers that can be assigned to the different populations.

were analyzed by ATR−FTIR spectroscopy from powder samples after freeze-drying from water (Figure 6, Table 3). It is apparent that the signal intensity in the region of C−H stretching (2973 - 2870 cm^{-1}) increases with increasing side chain length. In all spectra, the broad signal is split up in three peaks, two of which only resemble small shoulders in the case of **P1** while they are particularly well distinguishable in the case of **P8**. The amide I band (C═O stretching) at 1646 - 1642 cm^{-1} is the most prominent signal in all samples and clearly originates from the carbonyl group within the tertiary amide in the polymer backbone.[65] The next signal group is assigned to symmetric and asymmetric deformation bands of methyl and methylene groups in the polymer (1500 - 1400 cm^{-1}). Another group of signals is found around 1220 and 1110 cm^{-1}, respectively. In this range, the C−N stretching mode is expected and may be assigned to these two signal groups.[66] The prominent signals at 842 cm^{-1} may be attributed to C−C stretching while we assign the peaks around 750 cm^{-1} to a CH_2 rocking mode.

Interestingly, the broad signal at approximately 3500 cm^{-1} is only observed in the case of the two water-soluble polymers **P1** and **P4**.

Solubility of Homopolypeptoids. Solubility in water and organic solvents is a major issue for the most homopolypeptides. In contrast, the synthesized homopolypeptoids were soluble in many organic solvents, which can be attributed to the lack of intra- and intermolecular H-bonding.

Water-solubility of polymers is important for biomedical and pharmaceutical applications. The excellent water-solubility of polysarcosine is known already and we were unable to determine a limit of its water-solubility, as it appears to be miscible with water in all ratios (for DP ≤ 100). In contrast, little to no information on the water- and organosolubility of its higher analogues can be found in the literature. The few reports describing P(EtGly) and P(nPrGly) mention that both polymers as well as PSar are soluble in nitrobenzene.[37,38] We found moderate solubility in water for P(EtGly) (5 g/L) and very limited water-solubility for P(nPrGly) (0.5 g/L) while P(nBuGly) (**P9**) and P(iBuGly) (**P10**) are practically insoluble in water. Moreover, we performed preliminary solubility tests of the prepared homopolymers by addition of 10 μL of polymerization reaction mixture (in benzonitrile, containing approximately 0.5−1 mg polymer) to 1 mL of a variety of

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Table 2. Selected Analytical Data of Homopolypeptoids P1−P10

	polymer[a]	M_n[b] [kg/mol]	M_n[c] [kg/mol]	M_n[d] [kg/mol]	$Đ_M$[c]	$Đ_M$[d]	yield [%]
P1	poly(Sar)$_{25}$	2.0[e]	1.3	1.6	1.31	1.08	83
P2	poly(Sar)$_{50}$	3.8[e]	1.6	3.5	1.24	1.02	97
P3	poly(Sar)$_{100}$	5.4[e]	6.5	6.0	1.14	-	99
P4	poly(*N*-EtGly)$_{25}$	1.9[f]/2.1[g]	1.7	2.1	1.25	1.04	86
P5	poly(*N*-EtGly)$_{50}$	3.9[e]	3.3	3.3	1.25	1.06	92
P6	poly(*N*-^{n}PrGly)$_{25}$	2.3[g]/2.6[f]	2.8	2.4	1.20	1.08	(127)[i]
P7	poly(*N*-^{n}PrGly)$_{50}$	4.1[g]/4.8[f]	5.0	4.2	1.22	1.04	83
P8	poly(*N*-^{n}BuGly)$_{25}$	2.4[h]	2.3	2.5	1.16	1.04	73
P9	poly(*N*-^{n}BuGly)$_{50}$	4.5[h]	4.1	3.6	1.19	1.08	46
P10	poly(*N*-^{i}BuGly)$_{25}$	1.6[g]	1.4	-	1.20	-	49

[a] As calculated from $[M]_0/[I]_0$. [b] As determined by end-group analysis from ^{1}H NMR spectroscopy (signal intensity of aromatic protons of benzylamine-initiator vs main-chain and side-chain signal intensity). [c] As determined by gel permeation chromatography. [d] As determined by MALDI−ToF mass spectrometry. [e] Determined in D_2O. [f] Determined in CD_3Cl. [g] Determined in CD_3OD. [h] Determined in TFA-d_1 with DMSO-d_6 as external lock. [i] Sample contained considerable amount of solvent after single precipitation.

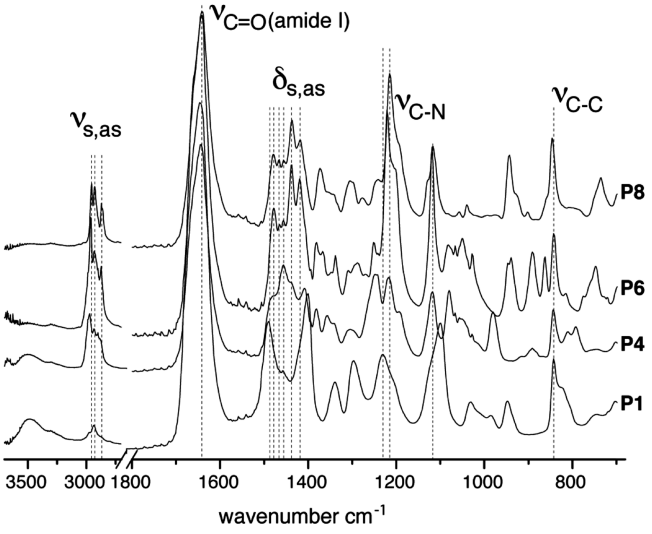

Figure 6. Comparison of IR spectra of PSar (**P1**), P(EtGly) (**P4**), P(nPrGly) (**P6**), and P(nBuGly) (**P8**). The increased intensity of the water band at around 3400 cm^{-1} reflects the increased hydrophilicity and hygroscopic character of **P1** and **P4** as compared to **P6** and **P8**.

Table 3. Assignment of IR Band Maxima of Four Different Types of Homopolypeptoids

assignment[a]	P1	P4	P6	P8
$\nu_{s,as}$(CH) of	2973 sh	2973 s	2962 s	2955 s
ν_{as}(CH$_3$)	2936 s	2933 sh	2933 sh	2933 sh
ν_s(CH$_3$)	2875 sh	2878 sh	2873 s	2870 s
ν(CO) amide I	1646 s, br	1644 s, br	1642 s, br	1642 s, br
δ_{as}(CH$_3$)	1491 s	1482 sh	1479 s	1478 sh
δ(CH$_2$−CO)	1457 w	1456 s	1456 m	1455 w
δ(CH$_2$)	-	1437 m	1437 s	1437 s
δ_s(CH$_3$)	1400 s	1408 s	1419 s	1416 m
ν(C−N)	1230 s/1100 s	1217 s/1118 s	1221 s/1119 s	1215s/1115s
ν(C−C)	842 s	842 s	842 s	845 s
r(CH$_2$)	750 w	749 w	747 m	737 m

[a] Key: s, strong; m, medium; w, weak; sh, shoulder; br, broad.

solvents (Table 4). Within the range of solvents we tested, P(EtGly) shows the most promiscuous behavior being insoluble only in cyclohexane and diethyl ether. In contrast, P(iBuGly) is insoluble in all but one solvent (chloroform). The latter solvent is also the only one that is a good solvent for all investigated products, while all polymers appear insoluble in cyclohexane.

Preparation of Block Copolymers. The living character of POI synthesis should allow access to block copolymers.[46] In fact, during many studies that investigated the so-called chain effect,[37,38,67−69] block copolymers may have been prepared, at least to some extend. In most cases, however, POI were used as macroinitiators for the polymerization of NCAs. Since the secondary amine terminus of POI must be expected to initiate the NCA polymerization also via the activated monomer mechanism, effective preparation of block copolymers is not expected using this route. Here, to obtain amphiphilic block copolypeptoids, nPrGly−NCA (**P11**), nBuGly−NCA (**P12**), and iBuGly−NCA (**P13**), respectively, were added to the reaction mixture of the first block (PSar) (Table 5). GPC traces suggest complete consumption of the first block (Figure 7). It is apparent that the GPC traces of the first block appear rather broad and with a particular pronounced shoulder at higher elution times. We attribute this, at least in part, to nonoptimized GPC conditions, as we did not find irregular MALDI−ToF mass spectra. We are currently trying to find conditions more suitable for the GPC analysis of such POI. MALDI−ToF analysis of **P11** revealed a M_P of m/z = 4730, which can be attributed to a polymer with the structure P(Sar$_{41}$-nPrGly$_{17}$) and Na$^+$ doping (Figure 8a). However, several other structures have very similar masses (e.g., P(Sar$_{38}$-nPrGly$_{19}$), K$^+$ doping, m/z = 4731 or P(Sar$_{48}$-nPrGly$_{12}$), Na$^+$ doping, m/z = 4731); therefore, unambiguous assignment is not possible as it must be expected that these species coexist in the product. Nevertheless, it is clear that a block copolymer has been successfully synthesized, as from each peak, $\Delta m/z$ representing the respective monomer units (Sar $\Delta m/z$ = 71, nPrGly $\Delta m/z$ 99) are identifiable (Figure 8b). It should be noted, that at lower m/z values we observed signals that can be attributed to nPrGly homopolymer (initiated by water, H$^+$ doping) while no signals that can be attributed to Sar homopolymer are apparent. We assume that, similar to the kinetic experiments, traces of water that were introduced with

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the addition of the second block lead to the formation of the homopolymer. However, as homopolymer are generally much easier to detect by MALDI−ToF mass spectrometry, we assume that the homopolymer is actually present in minute amounts. As mentioned, the number of possible monomer combinations in a copolymer makes a reasonable peak assignment impossible. This is visualized in Figure 8c where we plotted a collection of 46 Poisson distributions, which constitute the most intense peaks as calculated for a monomer composition of 41 units of Sar and 17 units of nPrGly. Unfortunately, we were unable to obtain mass spectra of polymer **P12** and **P13** despite several efforts.

Table 4. Solubility of Depicted As-Synthesized Homopolymers (+, Soluble; −, Insoluble)

solvent	P(Sar) (**P2**)	P(EtGly) (**P5**)	P(nPrGly) (**P7**)	P(nBuGly) (**P9**)	P(iBuGly) (**P10**)
acetone	-	+	-	-	-
acetonitrile	+	+	-	n.d.	n.d.
chloroform	+	+	+	+	+
cyclohexane	-	-	-	-	-
methylene chloride	+	+	+	n.d.	n.d.
diethyl ether	-	-	-	-	-
N,*N*-dimethyl-acetamide	+	+	+	+	-
dimetylsulfoxide	+	+	-	n.d.	n.d.
1,4-dioxane	-	+	-	n.d.	n.d.
ethanol	+	+	+	+	-
ethyl acetate	-	+	+	n.d.	n.d.
methanol	+	+	+	+	-
tetrahydrofuran	-	+	+	+	-
0.1 M NaOH	+	+	-	-	-
0.1 M HCl	+	+	-	-	-
DI water	+	+	-	-	-

Table 5. Analytical Data of Synthesized Block Copolymers

polymer id	polymer composition[a]	M_n[a] [kg/mol]	M_p[d] [kg/mol]	M_n[b] [kg/mol]	M_n[c] [kg/mol]	$Đ_M$[c]
P11	$PSar_{50}$-$nPrGly_{25}$	6.1	4.7	5.1[e]	5.7	1.13
P12	$PSar_{50}$-$nBuGly_{25}$	6.5	n.d.	4.6[e]	6.8	1.16
P13	$PSar_{50}$-$iBuGly_{25}$	6.5	n.d.	4.5[f]	5.6	1.09

[a] As calculated from $[M]_0/[I]_0$. [b] As determined by end-group analysis from ^{1}H NMR spectroscopy (signal intensity of aromatic protons of benzylamin-initiator vs main-chain and side-chain signal intensity). [c] As determined by gel permeation chromatography. [d] As determined by MALDI−ToF mass spectrometry. [e] determined in CD_3Cl. [f] determined in TFA-d_1 with DMSO-d_6 as external lock.

A simple method to have a first idea whether block copolymers compartmentalize in different solvents is ^{1}H NMR (Figure S6a−c, Supporting Information). In $CDCl_3$, both blocks are soluble and therefore well detected. As aggregates form from amphiphilic block copolymers in water, the solvent is excluded from the hydrophobic core and spin−lattice relaxation of nuclei is strongly reduced and signal intensities are diminished. The obtained analytical data for the prepared block copolymers are summarized in Table 5. In the case of **P11** this effect is not observed as P(nPrGly) is still somewhat water-soluble, and the hydrophobic character will be further augmented by the attached hydrophilic block (Figure S6a, Supporting Information).[70] In contrast, in the case of **P12**, the hydrophobic core is virtually undetectable in D_2O while it is well detectable in $CDCl_3$ (Figure S6b, Supporting Information). Interestingly, this result stands in contrast to our recent data on structurally very similar block copolymers comprising poly(2-methyl-2-oxazoline) and poly(2-butyl-2-oxazoline) as hydrophilic and hydrophobic blocks, respectively.[71] In this case the hydrophobic 2-butyl-2-oxazoline block was detectable in aqueous media, albeit with reduced intensity. Concomitantly, we observed an extraordinary high loading capacity for extremely hydrophobic drugs such as paclitaxel with the poly(2-oxazoline)s. Whether the present amphiphilic POI are able to do this remains to be seen. The prepared amphiphilic POI should be able to act as nonionic surfactant and solubilize hydrophobic components in aqueous media. As a hydrophobic model compound, we chose Reichardt's dye, a basically water-insoluble (at neutral pH) and solvatochromic dye which can be used to estimate the polarity of the microenvironment by which the dye is surrounded.[72−74] All three amphiphilic block copolymers were able to disperse the dye in the aqueous phase (Figure S6d and Figure S7, Supporting Information). Moreover, as distinct colors are observed, the dye is sensing the different natures of the hydrophobic core of the different block copolymers. Only the formulation comprising **P12** was clear prior to centrifugation, where **P11** and **P13**

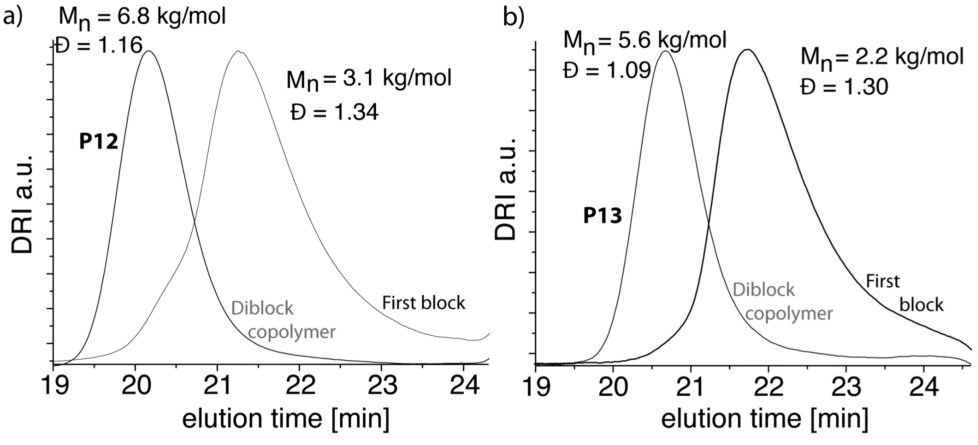

Figure 7. Gel permeation chromatography of the first block and final product of (a) **P12** and (b) **P13**. The complete consumption of the first block indicates the successful preparation of amphiphilic block copolypeptoide of Sar and nBuGly.

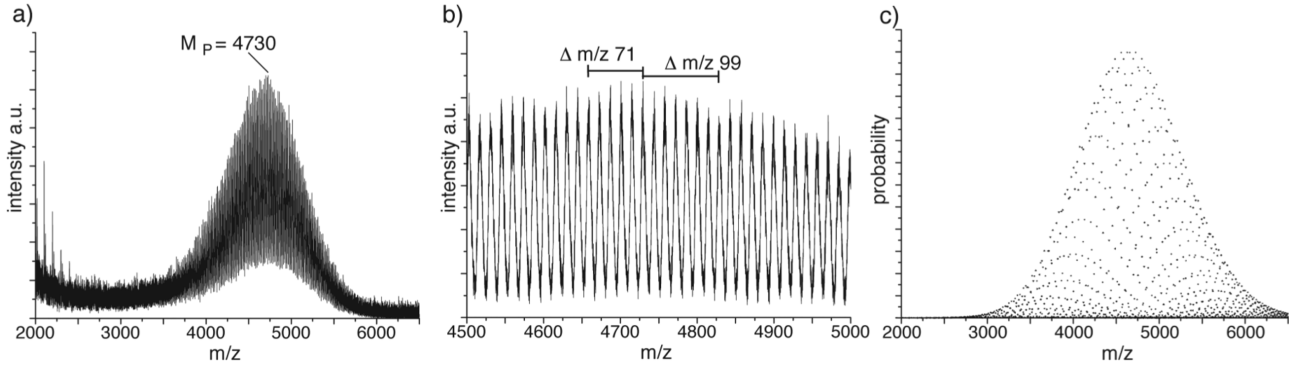

Figure 8. (a) MALDI−ToF mass spectrum (H^+ doping, DA matrix) of amphiphilic block copolymer **P11** and (b) blown-up view showing the respective $\Delta m/z$ of both monomer units (Sar $\Delta m/z$ = 71, nPrGly $\Delta m/z$ 99). (c) Collection of a selection of calculated Poisson-distributions for a copolymer with the target composition of P(Sar_{41}-nPrGly_{17}) + Na^+ for comparison with the experimentally observed MALDI−ToF mass spectrum using the same scale as in part a.

formulations appear turbid and pellets were observed after centrifugation, suggesting that **P12** has a better capacity for hydrophobic components. This, however, will be studied in future work. Surface tension measurements show that rather low critical micelle concentrations (16 mg/L for **P12**) can be achieved with even rather short hydrophobic blocks (Figure S8, Supporting Information), which would be beneficial for many applications.

CONCLUSION

We have presented evidence that POI from N-substituted NCAs (NNCAs) can be prepared in a highly defined manner yielding polymers with Poisson-distributions. We were unable to find indications for substantial termination or chain transfer during polymerization. MALDI−ToF MS data suggests that excellent end-group fidelity can be achieved which is important to allow for quantitative terminal modification with functional moieties such as fluorescent dyes, radiolabels or targeting moieties in future work. Furthermore, using the hydrophobic solvatochromic Reichardt's dye and ^{1}H NMR spectroscopy, we could show that POI based amphiphiles may serve as potentially (bio)degradable drug delivery vehicles for hydrophobic compounds. Since the monomers can be prepared without the need for phosgene or its derivatives in acceptable yields and purified by distillation or sublimation, this platform may be interesting for many researchers and applications. Synthetic versatility and definition as well as good solubility in a variety of solvents can be addressed using POI. Regarding availability and scalability we should mention that the phosgene-free synthesis with acceptable yields from readily available and inexpensive precursors is promising but certainly has significant optimization potential. The shelf life and (bio)degradability of POI are the remaining important issues that were, however, outside the scope of this paper and will be published in due time.

ASSOCIATED CONTENT

Supporting Information. Figures S1−S8, showing mechanisms, reaction schemes, ^{1}H NMR spectra, ATR−FTIR spectra, visible absorbance, and plots of surface tension polymer concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

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