Living Polymerization of *N*-Substituted β-Alanine *N*-Carboxyanhydrides: Kinetic Investigations and Preparation of an Amphiphilic Block Copoly-β-Peptoid

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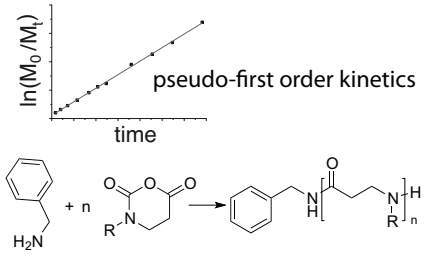
Poly(α-peptoid)s (*N*-substituted polyglycines) are interesting peptidomimetic biomaterials that have been discussed for many applications. Poly(β-peptoid)s (*N*-substituted poly-β-alanines), although equally intriguing, have received much less attention. Here we present results that suggest that while *N*-substituted β-alanine *N*-carboxyanhydrides can undergo a living nucleophilic ring-opening polymerization, the solubility of poly(β-peptoid)s can be very poor, which contributes to the limited accessibility using other synthetic approaches. The living character of the polymerization was utilized for the preparation of the first polymerized amphiphilic block copoly-β-peptoid. Our results may open a new route towards highly defined functional poly(β-peptoid)s which could represent biomaterials.



1. Introduction

β-Peptoids (β-POIs) are a group of peptidomimetic oligomers introduced by Hamper et al. who prepared them by solid-phase synthesis.[1] They consist of a β-alanine backbone, comprising a substituent at the nitrogen atom. Their flexible backbone, the missing hydrogen donating ability for hydrogen bonding,[2] and their proteolytic stability in comparison to peptides,[3,4] are properties which make them interesting for a variety of applications, in particular as a biomaterial.[5,6] Typically, the polymerization of suitable monomers can give much easier and more rapid access to oligomers and polymers. An analogue to the smaller homologues, α-peptoids, β-peptoids can be obtained by ring-opening polymerization from *N*-substituted *N*-carboxyanhydrides (NCAs).

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Only a few publications on the ring-opening polymerization (ROP) of *N*-substituted β-alanine-*N*-carboxyanhydrides (β-NNCAs) can be found in the literature. In 1954, Birkofer et al. reported the successful synthesis of *N*-*p*-tolyl-β-alanine-*N*-carboxyanhydride and its polymerization by thermal and *p*-toluidine initiation.[7] Two years later, the same group described the synthesis of *N*-phenyl-β-alanine-*N*-carboxyanhydride and its polymerization, initiated with water or by heating to the melting point.[8] Zilkha et al. studied the synthesis of poly(*N*-benzyl-β-alanine)s (poly(N-Bn-β-ala)) and published detailed results.[9] Since then, reports about poly(β-peptoid)s by ROP have been rare.

In 2002, Jia et al. developed an alternative route for the synthesis of β-peptoids; living alternating copolymerization of *N*-alkylaziridines and carbon monoxide under cobalt catalysis in dioxane.[10] Poly(*N*-methyl-β-alanine) (poly(N-Me-β-ala)) and poly(*N*-ethyl-β-alanine) (poly(N-Et-β-ala)) were prepared, end-functionalized with thiol groups and finally grafted onto gold surfaces. Interestingly, poly(N-Me-β-ala) is a very hydrophilic polymer with water contact angles in the range of 15°–20°.

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Poly(N-Et-β-ala) is less hydrophilic and the values for the contact angle were somewhat higher. These β-peptoid coated surfaces exhibit antifouling properties.[2] In addition, β-peptoids were reported as non-hemolytic materials and to show antibacterial activity.[4,11] It is important to note that by solid-phase synthesis approaches, oligomers with more than 10 repeat units have not been reported, to the best of our knowledge. Moreover, the resource and time-consuming preparation using this approach must be justified by the need of sequence-specific products. Interestingly, it has been reported that the polymers commonly precipitate during polymerization,[2,9] which may also limit solid-phase synthesis approaches of larger polymers. For example, Jia et al. observed the precipitation and crystallization of poly(N-Me-β-ala) during polymerization, therefore the degree of polymerization (DP) was limited to 20.[2]

Considering the potential of β-peptoids as (bio)materials, there is a significant lack in our understanding of their preparation and physicochemical properties.

Living nucleophilic ROP of NNCAs offers excellent synthetic control and can be performed in a wider range of solvents.[12] Thus, we hypothesized that ROP, using β-NNCAs, should also be a robust living polymerization with the possibility to prepare previously unknown block copoly-β-peptoids. As a first example, we present the preparation of poly(N-Me-β-ala) with DP > 20 and provide evidence that the polymerization has characteristics of a living polymerization. Moreover, the living character is demonstrated by the preparation of an amphiphilic block copolymer comprising poly(N-Me-β-ala) as hydrophilic and poly(N-Bn-β-ala) as a hydrophobic block.

2. Experimental Section

General materials and methods as well as monomer synthesis can be found in the Supporting Information.

2.1. Preparation of Homopoly-β-peptoids

Poly(N-Bn-β-ala)$_{25}$ (**P1**): *N*-Benzyl-β-alanine-*N*-carboxyanhydride (0.237 g, 1.16 mmol) was dissolved in 1.16 mL of dry *N*-methyl-2-pyrrolidinone (NMP) in a Schlenk tube. The reaction vessel was sealed carefully. Outside the glove box, the initiator benzylamine (4.9 μL, 0.046 mmol) was added ($[M]_0/[I]_0$ = 25). The polymerization solution was stirred at 25°C and about 40 mbar. After approximately 7 h, the reaction mixture was precipitated in cold diethyl ether, centrifuged, and dried to obtain the poly(N-Bn-β-ala) (0.221 g, 115%). The yield exceeded 100% due to residual solvent, which was found difficult to remove completely. Attempts to re-precipitate subsequently failed due to the poor product solubility in suitable solvents.

Exemplarily, the preparation of poly(N-Me-β-ala)**25** (**P2**) is described: *N*-Methyl-β-alanine-*N*-carboxyanhydride (0.088 g, 0.68 mmol) was dissolved in 1.14 mL of dry dimethyl sulfoxide (DMSO) under argon atmosphere using Schlenk methods. Subsequently, the initiator benzylamine (2.9 mg, 0.027 mmol) was added ($[M]_0/[I]_0$ = 25). The solution was stirred at 25 °C and 40 mbar. After 1 h, the mixture was precipitated in cold diethyl ether, centrifuged, dissolved in water and freeze-dried to obtain the title compound with minor residual solvent impurities (0.062 g, 100%).

Poly(N-Me-β-ala)$_{50}$ (**P3**): MALDI: $\overline{M}_n$ = 3.1 kg mol^{-1} ($Đ_{MALDI}$ = 1.04).

Poly(N-Me-β-ala)$_{100}$ (**P4**): MALDI: $\overline{M}_n$ = 4.2 kg mol^{-1} ($Đ_{MALDI}$ = 1.03).

2.2. Preparation of Poly[(*N*-Methyl-β-Alanine)$_{25}$-*block*-(*N*-Benzyl-β-Alanine)$_{10}$] (P5)

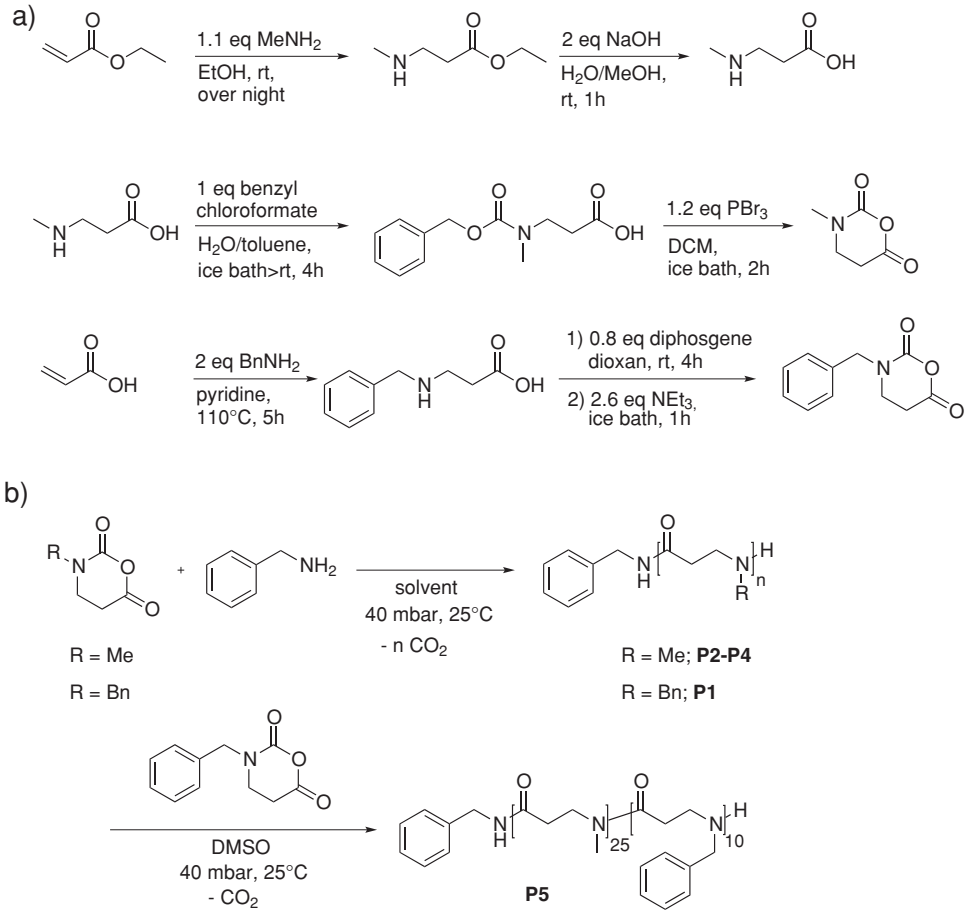
N-Methyl-β-alanine-*N*-carboxyanhydride (0.1281 g, 0.99 mmol) was dissolved in 1.64 mL of dry DMSO under argon atmosphere with the help of Schlenk methods. Subsequently, the initiator benzylamine (2.9 mg, 0.039 mmol) was added ($[M]_0/[I]_0$ = 25). The solution was stirred at 25 °C and 40 mbar. After 27 min, *N*-benzyl-β-alanine-*N*-carboxyanhydride (0.081 g, 0.039 mmol) was added (stock solution of monomer: 0.094 g mL^{-1}). After 133 min, the homogenous reaction mixture was precipitated into cold diethyl ether, centrifuged, and freeze-dried to obtain **P5**. MALDI: $\overline{M}_n$ (1st block) = 1.5 kg mol^{-1} ($Đ_{MALDI}$ = 1.07), $\overline{M}_{n\ (diblock)}$ = 2.1 kg mol^{-1}.

3. Results and Discussion

The synthesis of *N*-substituted β-alanine-NCAs is typically realized starting from acrylic acid or acrylates by the Michael-addition of primary amines and subsequent ring closure by different approaches (Scheme 1a). In our case, the overall yields were poor (16% for *N*-benzyl-β-alanine-NCA, less than 5% for *N*-methyl-β-alanine-NCA) but purity was sufficient for polymerization.

All polymerization reactions were carried out at 25 °C under reduced pressure (approx. 40 mbar) to remove the evolving carbon dioxide (Scheme 1b). To the best of our knowledge, no kinetic investigations regarding the ROP of β-NNCA have been reported to date. Therefore, we used IR measurements to monitor the relative intensity of the C=O stretching bond at 1795 cm^{-1} (see for IR and NMR spectra of monomers in the Supporting Information, Figure S1 and S2). The ln(*M*0/*M*t) vs. time plot for the polymerization of *N*-benzyl-β-alanine-NCA (**P1**) was linear to high monomer conversion of about 80%, after which the reaction was stopped (Figure 1).

This suggests a living character of the polymerization. When *N*-unsubstitued α/β-amino acid NCA are polymerized with an amine as initiator, deprotonation of the nitrogen can occur. The formed anion can rearrange to the isomeric α/β-isocyanatocarboxylate ion. The attack of an amine at the isocyanate group would lead to *N*-substituted α/β-ureidocarboxylic acid as product and thus, termination of the



Scheme 1. a) Monomer synthesis of *N*-methyl-β-alanine *N*-carboxyanhydride and *N*-benzyl-β-alanine *N*-carboxyanhydride. b) Schematic representation of the preparation of polymers **P1**–**P5**.

polymer.[13] This side reaction can be excluded in the polymerization of β-NNCAs (as with α-NNCAs). The apparent polymerization rate was calculated to be 1.65×10^{-3} L mol^{-1} s^{-1} under these experimental conditions. It is to be expected that this will vary strongly with solvent polarity as known for α-NNCAs, but solvent variation appears to be restricted by polymer solubility in the case of β-NNCAs. The analytical data for poly(N-Bn-β-ala)$_{25}$ (**P1**) are listed in Table 1. Interestingly, the $\overline{M}_n$ (4.7 kg mol^{-1}) obtained by GPC slightly exceeds the theoretical value (4.1 kg mol^{-1}) for complete monomer conversion. The $\overline{M}_n$ determined by MALDI is 4.5 kg mol^{-1} (Figure 2a, Figure S3a, Supporting Information). This is in good agreement with predicted values, even considering that the polymerization was not driven to full monomer consumption (approx. 90%). In addition, a low dispersity and essentially Poisson-like polymer chain length distribution was obtained ($Đ_{MALDI}$ = 1.03). The somewhat higher Đ, obtained from GPC (Đ = 1.08) and the higher molar mass is attributed to the use of poly(methyl methacrylate) (PMMA) calibration standards.

A major problem during the synthesis of poly(N-Me-β-ala) has been described by Jia et al. to be the solubility

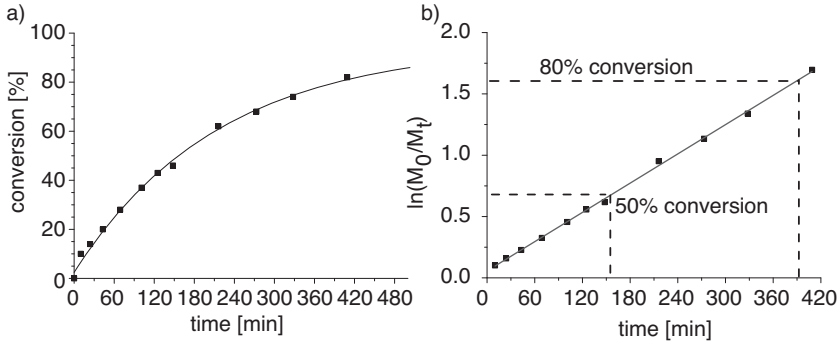


Figure 1. a) Monomer consumption vs. time of the polymerization of *N*-benzyl-β-alanine *N*-carboxyanhydride in NMP at 25 °C ($[M]_0$ = 1 M, $[M]_0/[I]_0$ = 25). b) Linear pseudo-first order kinetic plot of the same polymerization, demonstrating high fidelity of propagating species to high monomer conversion.

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Table 1. Selected analytical data of polymers **P1** – **P5**.

ID	Polymer	$\overline{M}_{n,calc}$[d] [kg mol^{-1}]	$\overline{M}_{n,GPC}$[e] [kg mol^{-1}]	$\overline{M}_{n,MALDI}$[f] [kg mol^{-1}]	$Đ^{MALDI,f)}$	$Đ_{GPC}$
P1[a]	poly(N-Bn-β-ala)$_{25}$	4.1	4.7	4.5	1.03	1.08
P2[b]	poly(N-Me-β-ala)$_{25}$	2.2	n.d.	2.2	1.04	n.d.
P3[b]	poly(N-Me-β-ala)$_{50}$	4.4	n.d.	3.0	1.04	n.d.
P4[c]	poly(N-Me-β-ala)$_{100}$	8.6	n.d.	4.2	1.03	n.d.
P5	1st block[b] poly(N-Me-β-ala)$_{25}$ (**P5a**)	2.2	n.d.	1.5	1.07	n.d.
	poly(N-Me-β-ala)$_{25}$-*b*-(N-Bn-β-ala)$_{10}$	3.8	n.d.	2.1	n.d.	n.d.

[a]1 M reaction mixture; [b]0.6 M reaction mixture; [c]0.3 M reaction mixture; [d]As calculated from $[M]_0/[I]_0$; [e]As determined by GPC using linear PMMA standards; [f]As calculated from simulated distribution functions.

of the polymer in the reaction media dioxane.[2] Thus, in first attempts, we chose *N*-methyl-2-pyrrolidinone and benzonitrile, which are known as good solvents for the polymerization of a range of *N*-substituted glycine-NCA.[12] However, we observed a white precipitate after only a few minutes into the polymerization which was not further characterized. Fukui et al. reported that poly(N-Me-β-ala) is soluble in hot water and glacial acetic acid[14] while Jia et al. reported very low water contact angles for poly(N-Me-β-ala) grafted onto gold, suggesting a highly polar polymer.[2] Therefore, we investigated other highly polar, non-protic solvents. However, the polymer also started to precipitate after approximately 30 min in acetonitrile and DMSO. In the case of DMSO, no gas formation was observed visually 20 min after initiation, suggesting monomer consumption was (nearly) complete and only subsequently the reaction mixture became more and more cloudy. Surprisingly, MALDI-ToF analysis revealed a successful synthesis of poly(N-Me-β-ala) (**P2**) with a degree of polymerization of 24 and a low dispersity of $Đ_{MALDI}$ = 1.04. Detailed investigation of the polymerization of *N*-methyl–alanine-NCA has not been performed to date, but we observed that the polymerization appears to be faster compared to *N*-methyl glycine-NCA (i.e., sarcosine NCA) under identical experimental parameters. In a control experiment, we found less than 40% of sarcosine NCA conversion after 20 min (data not shown). Two main factors are expected to contribute to the polymerization rate of β-NNCAs: ring-strain and nucleophilicity of the propagating species. The methyl-substituent at the nitrogen atom, with its +I-effect is similar in the sarcosine- and *N*-methyl-β-alanine-NCA. The amide group has electron withdrawing properties in both cases. However, in the case of β-POIs, the additional methylene unit attenuates the electron withdrawing effect. Therefore, the propagating chain end of the β-POIs is more nucleophilic than that of the POIs.[9] On the other hand, ring strain is considerable in α-NNCAs but less so in β-NNCAs, therefore, β-NNCAs should be expected to be less eager to undergo ring-opening. It will be interesting to investigate the kinetics of the copolymerization and the copolymerization parameters of α-NNCAs and β-NNCAs in the future.

Since poly(N-Me-β-ala)$_{25}$ (**P2**) was obtained with essentially the theoretical DP and low dispersity, other homopolymers with a higher DP were synthesized in the same solvent and under similar conditions. Interestingly, **P3** ($DP_{theo.}$ = 50; DP_{MALDI} = 36) and **P4** ($DP_{theo.}$ = 100; DP_{MALDI} = 50) showed considerably lower values for DP than expected. Nevertheless, both β-peptoids are obtained with low dispersities, as evidenced by MALDI-ToF-MS. It might be that the low DPs are a result of solubility problems, because again the polymers precipitated after a few minutes. Importantly, alternative polymer species, for example, water-initiated poly(N-Me-β-ala), were not detected by MALDI-ToF-MS in these cases. In summary, we report poly(N-Me-β-ala) with a DP > 20 for the first time. It should be noted, that while we were unable to identify an organic solvent able to solubilize poly(N-Me-β-ala) well, the polymer is very well soluble in water. A table with the results of a preliminary screening of the solubility of poly(N-Me-β-ala) in a variety of solvents can be found in the Supporting Information (Table S1). It is striking that the polymer, apart from in water, appears to be particularly soluble only in a mixture of lower alcohols and chloroform. Please note that the poor solubility in most solvents also did not allow us to perform GPC analysis of this polymer at this time. Similar problems have been reported by others.[2]

The two presented β-NNCA monomers are ideal candidates to form an amphiphilic block copoly-β-peptoid. If the ROP of β-NNCAs is indeed of living character, the preparation of block copoly-β-peptoids should be straightforward by subsequent polymerization of *N*-methyl-β-alanine-*N*-carboxyanhydride and *N*-benzyl-β-alanine-*N*-carboxyanhydride. To this end, we polymerized a first block of poly(N-Me-β-ala) (**P5a**), ensured full monomer consumption (by ATR-IR), and added *N*-benzyl-β-alanine-*N*-carboxyanhydride solution after retrieval of an aliquot

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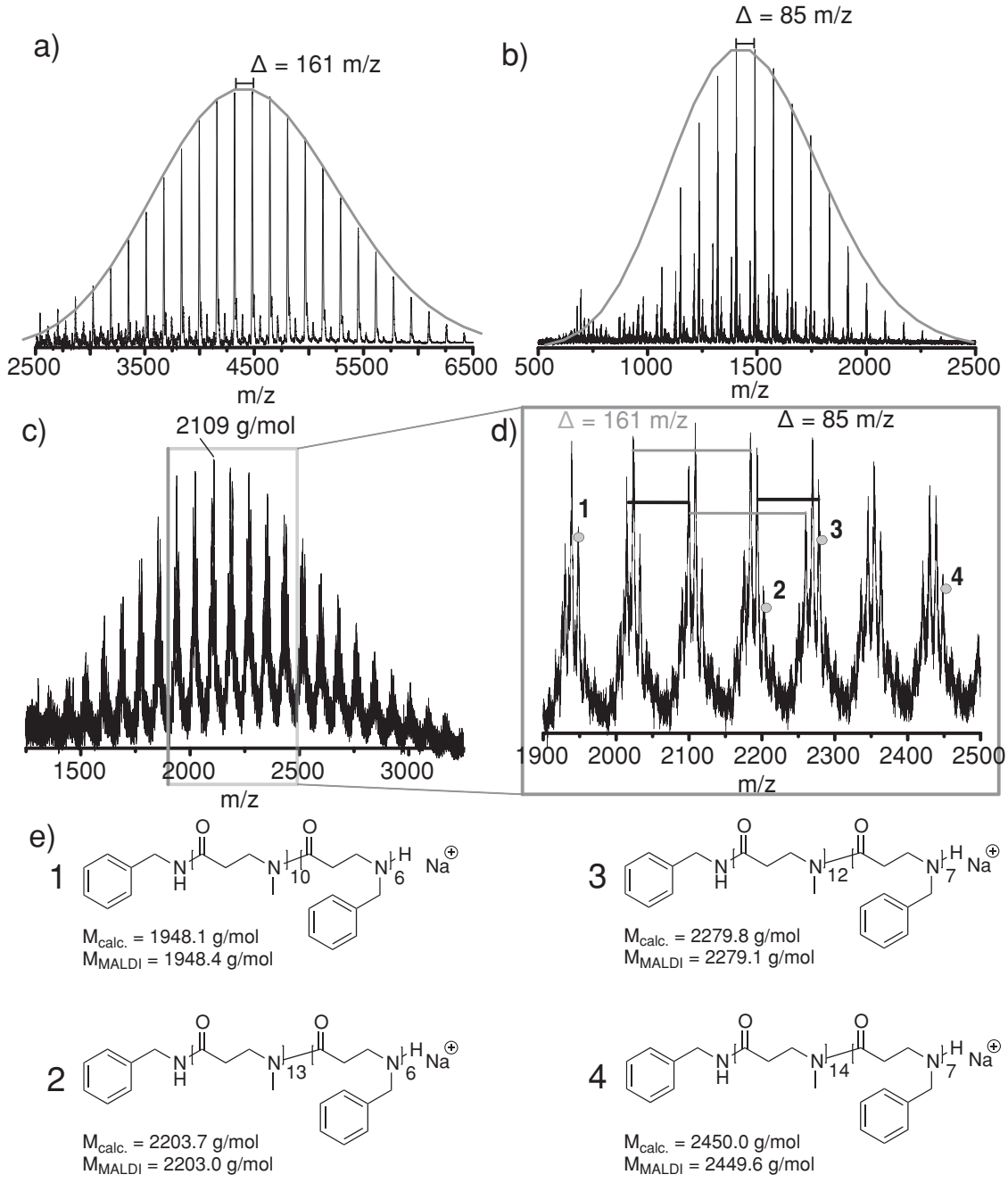

Figure 2. MALDI-ToF mass spectra (matrix: sinapinic acid) of polymers **P1** (a), and **P5** (b–d). While (b) shows the mass spectra of the first block (poly(N-Me-β-ala)), (c) depicts the mass spectra of the diblock copoly-β-peptoid. A detailed zoom into (c) is shown in (d). Gray lines represent simulated Poisson distributions, solely for a comparative purpose. e) Possible structural assignments of individual copolymer species to signals present in the mass spectra of block copolymer **P5**. Detailed signal assignment of **P1** and first block of **P5a** can be found in the Supporting Information (Figure S3).

of the reaction media for later analysis of the first block (for MALDI-ToF analysis see Figure 2b, Figure S3b). Importantly, the first block remained fully dissolved at this time, supporting our previous observation that precipitation started only after full monomer consumption. Similar to the case of **P1**, a detailed analysis of mass spectra reveals a minor fraction of water-initiated polymer (HO-**P5a**). Again, this does not interfere with the living character of the polymerization as such, but limits the predetermination of the DP. Immediately after addition of the monomer for the second block, CO_2 evolution was observed again and the polymer remained fully dissolved throughout the

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experiment. The polymer was precipitated from diethyl ether to yield a colorless solid. Redispersion in water gave a fully water-soluble product with considerable surface activity (foam formation). After freeze-drying from water, MALDI-ToF analysis was performed. Comparison of the MALDI-ToF MS spectra of the first block and the block copolymer confirmed successful preparation of the block copolymer (Figure 2c–e). However, it should be noted that the DPs were only approx. 50% of the calculated values.

4. Conclusion

We present the first evidence that ROP of *N*-substituted β-alanine-*N*-carboxyanhydrides is of living character as demonstrated by the linear pseudo-first order kinetics with respect to monomer consumption and the Poisson-type chain length distributions of the products. At this moment, we are unable to reliably predetermine molar masses of products, which will be addressed in future, more extensive work. As we were unable to identify water-initiated polymer chains by MALDI-ToF MS in most cases, we do not believe that this is due to problems associated with solvent/monomer purity, but rather is associated with the minute amount of initiator and monomers used in the current study and thus, related difficulties to exactly measure initiator volume. The successful preparation of an amphiphilic block copolymer comprising poly(N-Me-β-ala) and poly(N-Bn-β-ala) further supports the living character of the nucleophilic ROP of *N*-substituted β-alanine-*N*-carboxyanhydrides. The synthetic approach described here, resembles polymerization from C- to N-terminus, which is complementary to the previously developed method employing alternating polymerization of aziridines and CO, which resembles N- to C-terminus polymerization. Many questions regarding materials properties, and the polymerization of poly(β-peptoid)s using both approaches remain to be elucidated but the results presented suggest that highly defined and more complex poly(β-peptoid)s will be accessible in the future.

Supporting Information

Materials and methods, experimental details of monomer synthesis, IR spectra of monomers and polymers **P1** and **P4**, as well as NMR spectra of monomers are provided in the Supporting Information. In addition, a detailed view of the MALDI-ToF mass spectra of compounds **P1** and **P5a** are provided. This material is available from the Wiley Online Library or from the author.

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