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Organocatalyzed Ring-Opening Polymerization of (*S*)-3-Benzylmorpholine-2,5-Dione

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A 3-benzylmorpholine-2,5-dione monomer is synthesized from the natural amino acid L-phenylalanine and characterized by means of nuclear magnetic resonance and infrared spectroscopy, electrospray ionization mass spectrometry, and elemental analysis. Subsequent to preliminary polymerization studies, a well-defined poly(ester amide) homopolymer is synthesized via ring-opening polymerization using a binary catalyst system comprising 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and a 1-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexylthiourea (TU) cocatalyst with a feed ratio of M/I/DBU/TU = 100/1/1/10. Kinetic studies reveal high controllability of the dispersities and molar masses up to conversions of almost 80%. Analysis by mass spectrometry hints toward excellent end-group fidelity at these conditions. In consequence, utilization of hydroxyl-functionalized poly(ethylene glycol) and poly(2-ethyl-2-oxazoline) as macroinitiators results in amphiphilic block copolymers. Bulk miscibility of the building blocks is indicated by differential scanning calorimetry investigations. As more and more promising new drugs are based on hydrophobic molecules featuring aromatic moieties, the novel polyesteramides seem highly promising materials to be used as potential drug delivery vehicles.

1. Introduction

The utilization of biocompatible and biodegradable hydrophobic polymers is advantageous for the encapsulation of hydrophobic drugs.[1] Polyesters such as poly(lactic acid), poly(glycolic acid) or their copolymers (PLGA) are the most commonly used polymers exploited for this purpose.[2,3] Poly(ester amides) (PEA) derived from natural amino acids feature a similar structure, altered by the presence of more stable amide moieties while retaining the hydrolytically sensitive ester moieties in an alternating pattern.[4] Degradation of these so-called poly(depsipeptide)s results in biocompatible hydroxy acids alongside with the respective amino acid.[5] Already the diversity of proteinogenic α-amino acids opens a huge parameter space when aiming to tailor the polymer properties.

Such poly(ester amide)s are obtained by ring-opening polymerization (ROP) of morpholine-2,5-diones. Although bulk ROP catalyzed by tin-(II) octoate ($Sn(Oct)_2$)[6–8] or by enzymatic catalysis have been well established,[9–11] these polymerizations often result in PEA with broad molar mass distributions. More recent publications focused on the polymerization in solution utilizing highly active organocatalysts such as 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) which are already proven to facilitate the controllability of the ROP of lactones at room temperature.[12,13] Although TBD enables the polymerization of morpholine-2,5-diones, side reactions such as transesterification occurred at monomer conversions exceeding 50%, resulting in rather broad dispersed polymers.[14] The utilization of a binary catalytic system, using thiourea derivates as cocatalysts was demonstrated to prevent such side reactions enabling access to well-defined poly(ester amide)s.[15] The 1-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexylthiourea (TU) is to date the most commonly used thiourea cocatalyst for this purpose, as was demonstrated for the polymerization of different morpholine-2,5-dione derivates.[15,16]

Within the last decades, the number of novel drugs exhibiting high hydrophobicity increases.[1] PEA from rather hydrophobic amino acids such as phenylalanine could be advantageous to tackle this issue. Moreover, the benzylic side chain functionality could be of benefit, presumably enabling additional interactions with aromatic drugs via π–π stacking. Although the synthesis of the 3-benzylmorpholine-2,5-dione monomer was already presented in literature decades ago,[17,18] only few attempts to polymerize this monomer have been carried out. Ohya et al. obtained a triblock copolymer based on a bifunctional poly(ethylene glycol) (PEG) macroinitiator by anionic

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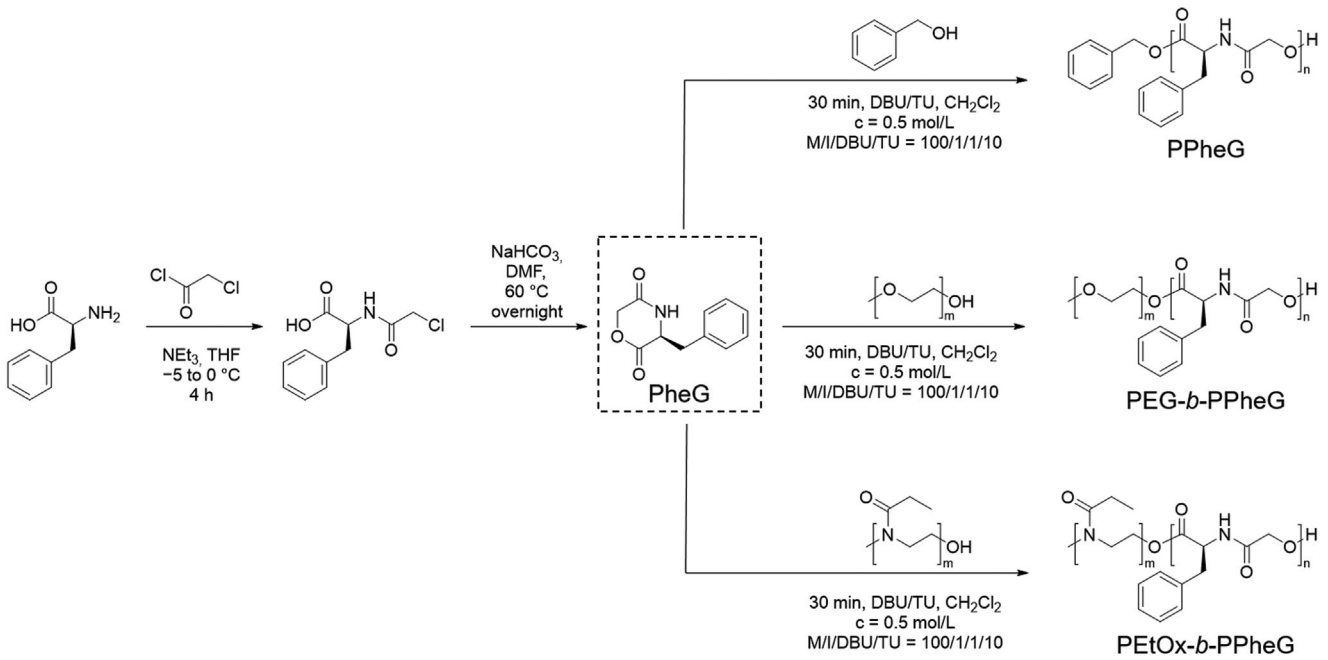

Scheme 1. Schematic representation of the synthesis route to (S)-3-benzylmorpholine-2,5-dione and its polymerization yielding a PPheG homopolymer as well as PEG-b-PPheG and PEtOx-b-PPheG block copolymers.

polymerization, comprising oligomeric 3-benzylmorpholine-2,5-dione blocks of six repeating units on each side of the PEG block.[19] A homopolymerization catalyzed by $Sn(Oct)_2$ has been only claimed in a patent by Pfizer Inc.[20] However, an organocatalyzed poly(3-benzylmorpholine-2,5-dione) has not been presented in literature so far, to the best of our knowledge.

Here we present the synthesis and characterization of the hydrophobic L-phenylalanine-based (S)-3-benzylmorpholine-2,5-dione (PheG) and its polymerization catalyzed by a binary DBU/TU catalyst system in solution at room temperature. The ROP was optimized using benzyl alcohol as initiator yielding a PPheG homopolymer. To demonstrate the possibility to obtain amphiphilic block copolymers, we selected two hydrophilic polymers known to exhibit "stealth" behavior: PEG[21–24] and poly(2-ethyl-2-oxazoline) (PEtOx).[25] The respective hydroxyl end-functionalized PEG-OH and PEtOx-OH were used as macroinitiators resulting in PEG-*b*-PPheG and PEtOx-*b*-PPheG block copolymers (**Scheme 1**).

2. Results and Discussion

The PheG monomer was synthesized via a two-step approach starting from the natural amino acid L-phenylalanine according to a procedure adapted from Ohya et al.[19] The first step was an amidation reaction of L-phenylalanine with chloroacetyl chloride resulting in a linear precursor, which underwent an intramolecular cyclization in a highly diluted DMF solution in the following step. The overall yield of 25% is in accordance with yields achieved for other morpholine-2,5-diones[14] and significantly increased compared to the yield of 12% from the original procedure.[19] The PheG monomer was characterized by means of ^1H, ^{13}C, ^{13}C-DEPT, HSQC and HMBC NMR spectroscopy, HR-ESI mass spectrometry, IR spectroscopy, and elemental analysis (see Figures S1–S6, Supporting Information). The molecular structure of PheG was additionally confirmed by X-ray crystallography (Figure S7, Supporting Information). Even though the compound crystallized as very thin needles and the collected diffraction data did not allow for adequate structure refinement, the structure could be determined unambiguously on a qualitative level.

The polymerizations of the L-phenylalanine-based monomer were performed in dichloromethane utilizing benzyl alcohol as an initiator and a catalyst system comprising DBU and a 1-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexylthiourea (TU) co-catalyst at room temperature. A suitable monomer concentration as well as cocatalyst feed was determined in initial test polymerizations (Figure S9, Supporting Information). Polymerization conditions suitable for other morpholine-2,5-diones adapted from literature (M/I/DBU/TU = 100/1/1/5, $[M]_0$ = 0.7 mol L^{-1})[14,26] resulted in a fast polymerization accompanied with broad molar mass distributions already after 30 min polymerization time. The amount of cocatalyst hence was increased whereas the initial monomer concentration $[M]_0$ was decreased to slow down the polymerization. A feed of M/I/DBU/TU = 100/1/1/10 with a $[M]_0$ of 0.5 mol L^{-1} was suitable to yield polymers with still narrow molar mass distributions at monomer conversions up to almost 80%.

At these polymerization conditions, detailed kinetic studies were performed to verify the controlled character of the polymerization. Aliquots were taken regularly, directly quenched with benzoic acid, and analyzed by means of SEC and ^1H NMR spectroscopy to determine the dispersities, molar masses, and monomer conversions, respectively (**Figure 1**).

The first-order kinetic plot revealed a linear increase within the first 45 min. However, at higher polymerization times, the polymerization rate decreased significantly, presumably due to inter- and intramolecular chain transfer reactions through

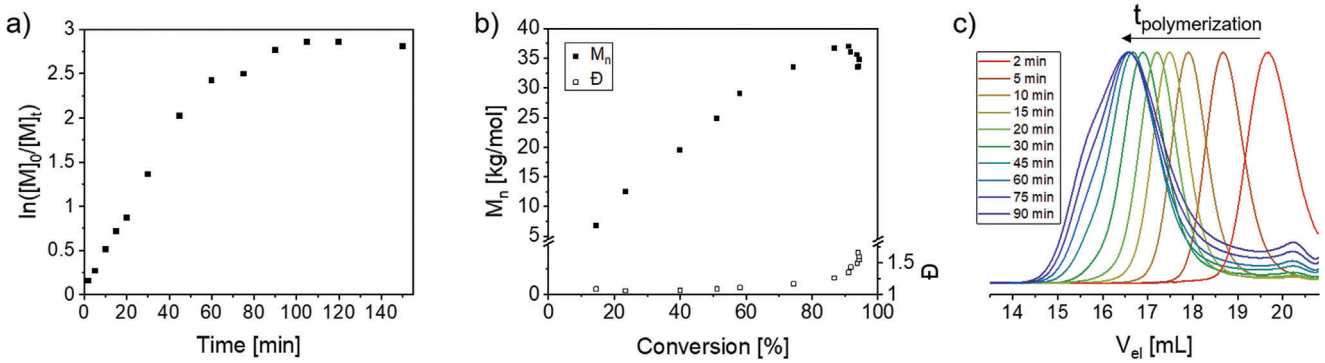

Figure 1. Kinetic studies of the organocatalyzed ring-opening polymerization of PheG. a) First-order kinetic plot. b) Development of the molar mass and the dispersity with the monomer conversion. c) Polymerization progress followed by SEC (DMAc, 0.21% LiCl) of the first 90 min of the polymerization. Elugrams of the samples with polymerization times of > 90 min can be found in the Supporting Information (Figure S10, Supporting Information).

Table 1. Selected characterization data from the PPheG homopolymer, the PEG-OH and PEtOx-OH macroinitiators as well as the PEG-*b*-PPheG and the PEtOx-*b*-PPheG block copolymers.

Polymer	Conv.[a)] [%]	$DP_{theor.}$ [b)]	$M_{n,theor.}$ [b)] [g mol^{-1}]	DP_{NMR} [c)]	$M_{n,NMR}$ [c)] [g mol^{-1}]	$M_{n,SEC}$ [d)] [g mol^{-1}]	$Đ$ [d)]	T_g [e)] [°C]	T_m [f)] [°C]
PPheG	78	- / 78	16 100	–	–	34 100	1.22	86	–
PEG-OH	–	45	2 000	45	2 000	3 200	1.06	–	52
PEG-*b*-PPheG	79	45 / 79	18 200	45 /75	17 400	26 700	1.29	48	–
PEtOx-OH	–	20	2 000	22	2200	4500	1.09	45	–
PEtOx-*b*-PPheG	77	20 / 77	17 800	22 / 75	17 600	27 100	1.22	83	–

[a)] Conversion; determined from ^{1}H NMR data; [b)] Estimated according to $M_{n,theor.} = M/I \times$ conv; data for PEG according to manufacturer's declaration; [c)] Determined from ^{1}H NMR data of the purified polymer; [d)] Determined by SEC in DMAc (0.21 wt% LiCl, PS calibration, RI detection); [e)] Glass transition temperature; determined by DSC in the third heating run at 10 K min^{-1}; inflection values are reported; [f)] Melting temperature; determined by DSC in the third heating run at 10 K min^{-1}.

transesterification dominating upon depletion of the monomer. Significant broadening of the molar mass distributions and occurrence of low molar mass macromolecules confirmed this assumption (Figure S10, Supporting Information). However, polymers with narrow molar mass distributions ($Đ < 1.3$) and molar masses up to 16 000 g mol^{-1} could be obtained by quenching the polymerization at a monomer conversion below 80%. Up to this point, the molar mass increased with conversion in a linear fashion, demonstrating the possibility to control the polymer molar masses.

Based on these findings, a polymerization time of 30 min was chosen for the synthesis of a homopolymer initiated by benzyl alcohol. The conversion of 78% determined by means of ^{1}H NMR spectroscopy and the monomodal molar mass distribution with $Đ = 1.22$ for the purified polymer (**Table 1**) point towards the robustness of the developed polymerization process as they are in accordance with the values observed during the kinetic studies.

End group and molar mass determination by means of ^{1}H NMR spectroscopy was impeded due to the overlapping signals in the aromatic region of the spectrum. To unambiguously confirm the presence of the benzyl alcohol initiator as the α-end group, MALDI TOF MS measurements were performed. Although the only m/z distribution in the high molar mass range corresponded to benzyl alcohol initiated chains, the high laser energy required for the ionization lead to fragmentation indicated by additional very broad m/z distributions occurring only at lower m/z values (Figure S11, Supporting Information). To circumvent this problem, the kinetic sample after 2 min polymerization was utilized for additional MALDI TOF MS measurements, improving the ionizability due to the shorter chain length (**Figure 2**). The most abundant species was assigned to the sodium adduct of the desired benzyl alcohol initiated polymer chains equipped with a hydroxyl ω-end group. The latter was formed by protonation of the active chain end due to the termination with benzoic acid. Less prominent distributions can be ascribed to PEA chains with a carboxylic acid α-end group and a hydroxyl ω-end group ionized with a sodium cation. These species were already observed for the high molar mass polymer. Indeed, they could be generated either by initiation of the ROP by water impurities or by hydrolysis of covalently bound DBU at the α-chain end, which was already observed in polymerizations with TBD catalysts.[27] However, the shift of the distribution to considerably lower m/z compared to the benzyl alcohol initiated polymer indicates a fragmentation of the polymer chains due to the high laser energy required during the ionization.

One advantage of the ROP is the straightforward formation of block copolymers by utilization of polymers with hydroxyl end groups as macroinitiators. Representing polymers featuring "stealth effect," a PEG-OH as well as a PEtOx-OH polymer of equal molar masses were utilized as hydrophilic macroinitiators to result in amphiphilic block copolymers. Polymerizations using the same conditions as applied for the PEA homopolymer resulted in monomer conversions of 79% for the PEG-OH initiated polymer and 77% for the PEtOx-OH initiated polymer, confirming the excellent reproducibility of the developed ROP conditions. Separated signals of both polymer blocks were observed in

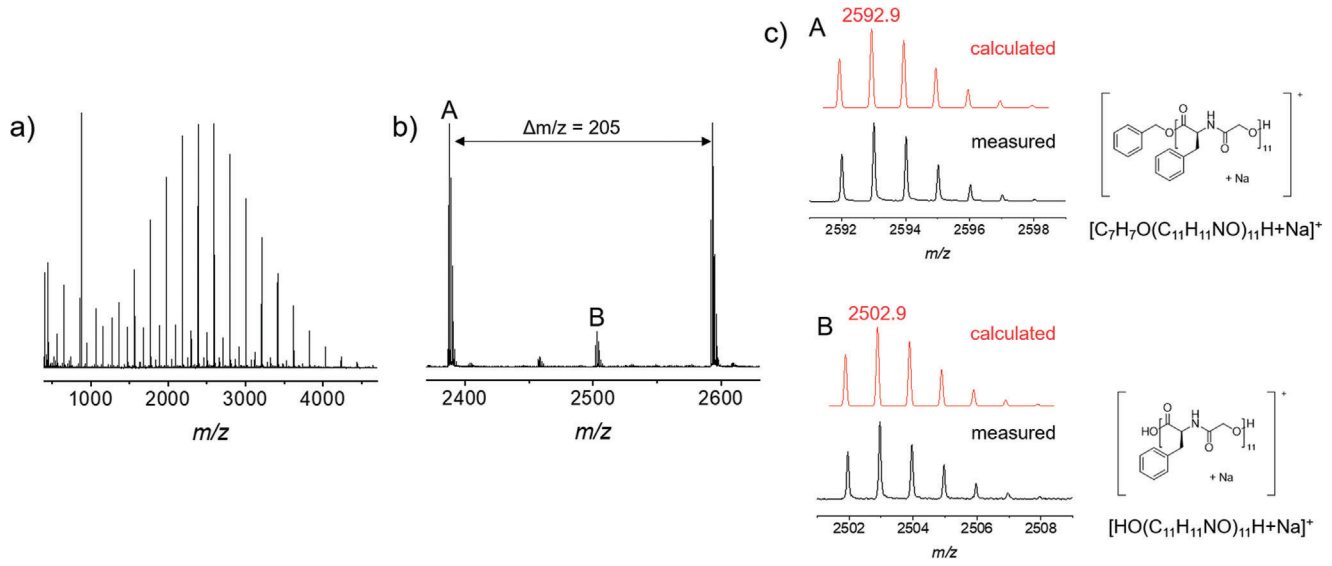

Figure 2. MALDI TOF MS analysis of the polymerization mixture after 2 min (matrix: CHCA + NaTFA). a) Full mass spectrum. b) Zoom into a *m/z* region displaying one repeating unit ($\Delta m/z = 205$) with the assignment of the two most abundant species A: $[C_7H_7O(C_{11}H_{11}NO_3)_nH + Na]^+$; B: $[HO(C_{11}H_{11}NO_3)_nH + Na]^+$). c) Overlay of the measured and the calculated isotopic pattern of the most prominent species ($[M + Na]^+$).

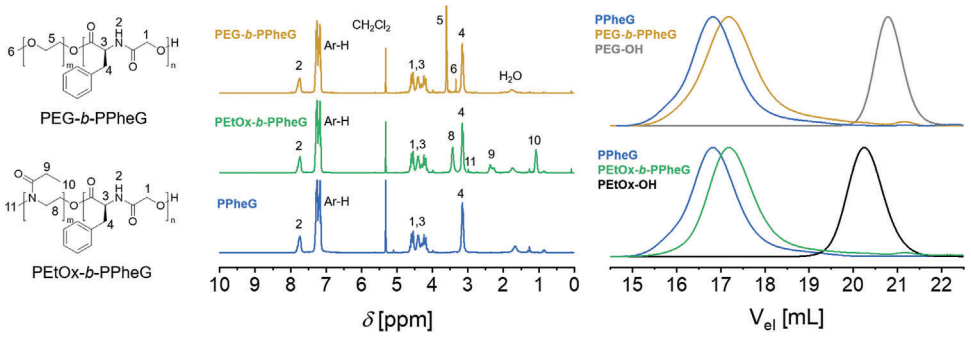

Figure 3. Characterization of the PEG-OH and PEtOx-OH macroinitiators, the PPheG homopolymer and the PEG-*b*-PPheG and PEtOx-*b*-PPheG block copolymers. a) Overlay of the ^{1}H NMR spectra (300 MHz, CD_2Cl_2) and assignment of the signals to the schematic representation of the structures b) Overlay of the SEC elugrams (DMAc, 0.21 wt% LiCl, RI detection).

the respective ^{1}H NMR spectra, enabling to determine the degree of polymerization of the PEA blocks DP_{NMR} (**Figure 3**). It was in agreement with the value expected from the M/I ratio and the conversion $DP_{theor.}$ for both block copolymers. In consequence, the respective molar mass values $M_{n,NMR}$ and $M_{n,theor.}$ matched. The molar mass determined by SEC was considerably larger due to the high hydrodynamic volume of the PheG-based polymers in comparison to the polystyrene standards used for calibration. The SEC elugrams revealed narrow, unimodal molar mass distributions ($Đ = 1.29$ for PEG-*b*-PPheG and $Đ = 1.22$ for PEtOx-*b*-PPheG) and unambiguous shifts towards lower elution volumes for the block copolymers compared to the respective macroinitiators, confirming the successful initiation with the macroinitiators and the covalent attachment of the two blocks. In summary, these findings highlight the presence of a ROP that is controlled in terms of molar mass as well as end groups.

Thermal analysis of the polymers was performed to investigate their bulk miscibility. Thermal stability was investigated by means of TGA. With a degradation just below 250 °C, the PPheG homopolymer featured a higher stability compared, e.g., to the L-valine based PEA known from literature which already degraded below 200 °C,[25] but a lower stability compared to other PEAs with aromatic substituents[15] or methionine-based PEAs,[26] which were stable up to almost 300 °C. Due to higher degradation temperatures of the PEG and PEtOx building blocks, a two-step degradation was observed for the PEG-*b*-PPheG and PEtOx-*b*-PPheG block copolymers (Figure S12, Supporting Information). DSC measurements (**Figure 4**) revealed an amorphous behavior for the PPheG homopolymer which was also observed for most of the PEAs in literature. However, with a T_g of 86 °C the PPheG homopolymer revealed a significantly higher T_g compared to the L-valine-,[25] the methionine-[26] or the L-leucine-based PEA,[16] but in a similar range than other PEAs bearing aromatic substituents.[15] The PEG-*b*-PPheG and PEtOx-*b*-PPheG block copolymers also revealed amorphous behavior. Apparently, the semicrystallinity of the PEG-OH macroinitiator was suppressed in the block copolymer as recrystallization during the cooling or cold crystallization during the heating were not

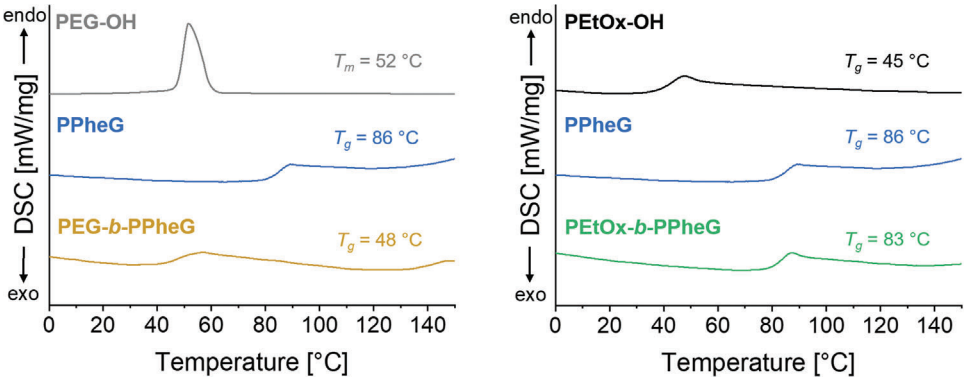

Figure 4. DSC thermograms of the PEG-OH and PEtOx-OH macroinitiator, the PPheG homopolymer and the PEG-*b*-PPheG and the PEtOx-*b*-PPheG block copolymers (third heating run, 10 K min^{-1}).

observed. The occurrence of solely one T_g indicated the miscibility of the PEA block with the respective PEG or PEtOx block. With a T_g value at 48 °C for PEG-*b*-PPheG and 83 °C for the PEtOx-*b*-PPheG the glass transitions of both block copolymers occurred between those of the individual blocks. Note that the T_g of the PEG-OH would appear significantly below its melting point.

3. Conclusion

In summary, a (*S*)-3-benzylmorpholine-2,5-dione monomer was synthesized in a two-step approach from L-phenylalanine and polymerized utilizing DBU and the thiourea derivate 1-(3,5-bis(trifluoromethyl)phenyl)-3-cyclohexylthiourea as binary catalyst system. The molar mass of the resulting poly(3-benzylmorpholine-2,5-dione) was controlled up to a monomer conversion of almost 80%, as proven by the kinetic studies. In addition, MALDI TOF MS indicated the controllability of the ROP in terms of end groups. Accordingly, the utilization of PEG-OH and PEtOx-OH as macroinitiators resulted in block copolymers. Bulk miscibility of the two building blocks was indicated by DSC measurements. Further investigations regarding the synthesis of polymers with higher molar masses as well as nanoparticle formulation including the encapsulation of specific drugs supported by computational calculations are currently under investigation in our laboratories.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords

degradable polymers, L-phenylalanine, morpholine-2,5-dione, poly(ester amide), ring-opening polymerization

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