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((Additional Supporting Information may be found in the online version of this article.))

ABSTRACT

2,5-diketopiperazines (DKPs) are the smallest cyclic dipeptides found in nature with various attractive properties. In this study, we have demonstrated the successful modification of proline-based DKPs using anionic ring-opening polymerization (AROP) as a direct approach. Four different proline-based DKPs with various side chains and increasing steric hindrance, were used as initiating species for the polymerization of 1,2-epoxybutane or ethoxyethyl glycidyl ether in the presence of t-BuP_4 phosphazene base. The addition of a Lewis acid, tri-isobutyl aluminum, to the reaction mixture strongly decreased the occurrence of side-reactions. Impact of the DKP side chain functionalities on molar mass control and dispersity was successfully evidenced.

Keywords: Diketopiperazine • Anionic ring-opening Polymerization • Peptidomimetics • Macromolecular engineering • Organocatalysis

INTRODUCTION

Peptides are naturally occurring structures with various functions in biology, but they are not easy to handle and have solubility and stability issues.[1] Thus, modified peptide analogues are becoming more and more attractive due to the possibility to improve the stability as well as the pharmacokinetics of their native counterparts.[2,3] 2,5-diketopiperazines (DKPs) are the smallest cyclic peptides which are found in the nature. They have a wide range of biological properties: they are for example very efficient antibacterial,[4-6] anti-cancerous,[7-9] anti-inflammatory[10] or neuroprotective[11] agents. They are attractive scaffolds for drug discoveries and their modifications have been recently the subject of intense research leading to attractive molecules for medical, catalysis or self-assembly applications.[12-13] Nevertheless, DKPs have received little attention in the field of polymer chemistry.[14] To the best of our knowledge, only 2 examples of polymer N-grafted DKP have been published. *N*-modified DKPs were used as initiator for reversible addition-fragmentation polymerization (RAFT) polymerization[15-16] and as monomer in acyclic diene metathesis (ADMET) polymerization[17] to develop polymers integrating natural and structurally constrained moieties. Amide and carbamate moieties have been shown to be deprotonated with phosphazene bases[18] to initiate the polymerization of epoxide monomers.[19-22] Based on these results, our group published the first N-modification of symmetric cyclic dipeptides through controlled anionic ring-opening polymerization[23] paving the way toward the synthesis of various peptide-based functional polymers readily applicable in the biomedical field.[24-25]

In this paper, we have extended our previous results[23] to the N-functionalization of proline-based cyclic dipeptide by AROP. In cyclic dipeptides containing proline, the 2,5-diketopiperazine ring is fused to a cyclic five-membered ring that imposes rigid constraints on the structure producing a stable boat configuration.[13] The effects of the DKP structural flexibility reduction on the NH-deprotonation by the voluminous and hindered phosphazene base and on the control of the oxiran ring-opening polymerization will be studied. Proline-based DKPs containing glycine, alanine, leucine and phenyl alanine residues (Figure 1) were chosen to additionally study the effect of increasing hydrophobicity and steric hindrance on the Nitrogen functionalization. Proline based DKPs were modified in one step reaction using their only secondary amide moiety (-CO-NH-) on the scaffold as initiating sites for AROP in the presence of phosphazene base (t-BuP_4) or in the presence of t-BuP_4 and tri-isobutyl aluminum (i-Bu_3Al). N-functionalization studies and optimizations have been first carried out with commercially available 1,2-epoxybutane. The influence of additional experimental conditions, such as phosphazene base excess, temperature decrease of monomer addition rate, were carefully studied. Eventually, this method of N-functionalization has been extended for the first time to ethoxy ethyl glycidyl ether monomer (EEGE) creating DKPs conjugated to water-soluble polymeric chains. Due to the possible occurrence of carbonyl nucleophilic attacks,[26, 27] the structural integrity of the DKP scaffolds has been verified by NMR and MALDI ToF analyses.

EXPERIMENTAL

Materials

All the DKPs, cyclo(alanine-proline) (Bachem), cyclo(glycine-proline) (Bachem), cyclo(leucine-proline) (Bachem) and cyclo(phenylalanine-proline) (Bachem), phosphazene base *t*BuP_4 solution (0.8 mol L^{-1} in hexane, Aldrich), 2-methyltetrahydrofuran (MeTHF, 98%, Aldrich), and tri-isobutyl aluminum (*i*-Bu_3Al) solution (1.0 mol L^{-1} in hexane, Aldrich) were used without further purification. 1,2-epoxybutane (99%, Aldrich) was distilled over CaH_2 two times prior to use. Tetrahydrofuran was dried with an MBRAUN MB SPS-800 solvent purification system under nitrogen.

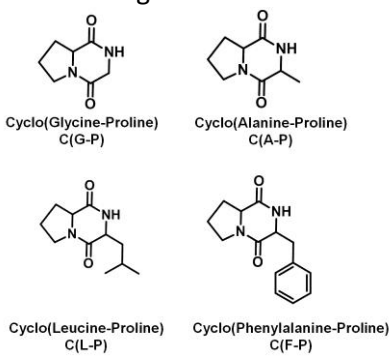

FIGURE 1 Cyclic Proline Based Dipeptide

Instrumentation

^{1}H and ^{13}C NMR analyses were performed on a Bruker Avance 300 MHz and a Bruker Avance 400 MHz spectrometers. All spectra are internally referenced to residual proton signals of the deuterated solvent: $CDCl_3$. Samples were prepared with $Cr(acac)_3$ (~25 mg/ml) for quantitative ^{13}C NMR and spectra collected with D1=10s.

Polymer molar masses were determined by size exclusion chromatography (SEC) with three PLGel Mixte C 5µm columns (7.5 × 300 mm; separation limits: 0.2 to 2000 kg.mol^{-1}) maintained at 40°C coupled with 2 modular detectors: a differential refractive index (RI) detector Viscotek 3580 and a Diode Array UV Detector Shimadzu SPD20-AV. THF was used as the mobile phase at a flow rate of 1 mL.min^{-1}, toluene was used as a flow rate marker. All polymers were injected (100 µL) at a concentration of 5 mg.mL^{-1} after filtration through a 0.45 µm pore-size membrane. OmniSEC 4.7 software was used for data acquisition and data analysis. The number-average molar masses (M_n), the weight-average molar masses (M_w), and the molar mass distributions ($Đ = M_w/M_n$) were determined by SEC with a calibration curve based on narrow poly(methyl methacrylate) (PMMA) standards

(from Polymer Standard Services), using the RI detector.

MALDI-ToF. Mass spectra were recorded by matrix-assisted laser desorption and ionization time-of-flight (MALDI-ToF) mass spectrometry (MS) using a Bruker autoflex III smartbeam mass spectrometer, equipped with the laser that produces pulses at 337 nm using dithranol as a matrix and NaI as cationizing agent. Spectra were recorded in reflectron or in linear mode at an accelerating potential of 20 kV. Samples were prepared by dissolving the polymer in THF at a concentration of 5 $mg.mL^{-1}$. A 10 µL aliquot of this solution was mixed with 20 µL of dithranol solution as matrix (at 20 $mg.mL^{-1}$ in THF) and 10 µL of NaI solution (at 10 $mg.mL^{-1}$ in THF). Standards (poly(ethylene oxide) of known structures, M_n = 1470 and 4250 $g.mol^{-1}$ purchased from Polymer Standards Service were used to calibrate the mass scale.

Polymerization conditions for Table 1.

The polymerization was carried out according to the following typical procedure. The initiator precursor DKP (0.119 mmol) was weighted into a reaction flask. The rest of the reactants was added in a glove-box. Following initiator, 1.5 mL of THF were added to the reactor. Then 0.150 mL of t-BuP_4 (0.120 mmol) were added. After complete dissolution of DKP (~5min), 0.310 mL of 1,2-epoxybutane (3.57 mmol) were added and the reactor was closed with a septum. After closure of the reaction flask, the reaction mixture was stirred at room temperature and left to react for the required period of time. A small portion of the reaction mixture was sampled through a septum at various reaction times for NMR analyses. The reaction was quenched by addition with 0.1 mL of 1% acetic acid in H_2O. The polymer was dissolved in chloroform and purified by passing through neutralized aluminum oxide, filtering, and removing the solvent under vacuum at 50 °C to give a colorless viscous liquid. Yield: ≥ 85%.

Polymerization conditions for Table 2.

The polymerization was carried out according to the following typical procedure. The initiator precursor DKP, cyclo(Alanine-proline) (20 mg, 0.119 mmol) was weighted into a reaction flask. Then, the rest of the reactant was added in a glove-box. After the initiator, solvent (MeTHF, 1.5 ml) was added to the reactor. Then the requested amount of t-BuP_4 was added. After complete dissolution of DKP (~5min), the requested amount of i-Bu_3Al was added, if not specified differently in the table. Finally, 0.310 mL of 1,2-epoxybutane (3.57 mmol) were added by a micro syringe as indicated in the table. After closure of the reaction flask, the reaction mixture was stirred at the indicated temperature and left to react for the required period of time. A small portion of the reaction mixture was sampled through a septum at various reaction times for NMR analyses. The reaction was quenched by addition with 0.1 mL of 1% acetic acid in H_2O. The polymer was dissolved in chloroform and purified by passing through neutralized aluminum oxide, filtering, and removing the solvent under vacuum at 50 °C to give a colorless viscous liquid. Yield: ≥ 85%.

TABLE 1 Polymerization of 1,2-epoxybutane initiated by cyclic proline dipeptides using t-BuP_4 as catalyst, at a monomer concentration of 2M in THF.

Run	Initiator	Monomer/ Initiator/ t-BuP_4 (mol)	T°C	Time (h)	Conv (%)[a]	Mn theo (g/mol)	Mn $_{13C\ NMR}$ (g/mol)	Mn_{SEC} (g/mol)	Mw/Mn (SEC)
P1-1	C(A-P)	30/1/1	25	24	95	2200	2500	2500	1.13
P1-2	C(G-P)	30/1/1	25	24	95	2200	n.d.	2300	1.16
P1-3	C(L-P)	30/1/1	25	24	93	2200	3400	2800	1.20
P1-4	C(F-P)	30/1/1	25	46	95	2250	3500[b]	2900	1.25

[a] Determined by 1H NMR of the reaction mixture by comparing the signal of the -CH_2CH_3 of both monomer and polymer to the CH group signal of the oxiran group; [b] Mn determined by ^{1}H NMR in Acetone-d6.

RESULTS AND DISCUSSION

Polymerization of 1,2-epoxybutane by Proline Based DKPs/ t-BuP_4 as Initiating System.

Initially, the polymerization of 1,2-epoxybutane was carried out in the presence of proline-based DKPs and t-BuP_4 as initiating system in THF at 25°C (Figure 2). Polymerizations were successfully initiated by the DKP-phosphazene base systems and polymer chains were conjugated on the Nitrogen atoms of the amide groups. For DKP with low steric hindrance (Table 1, P1-1 and P1-2), molar masses obtained by ^{13}C quantitative NMR and SEC analysis were in good agreement with the theoretical values and narrow molar mass distributions were obtained (Table 1). ^{13}C quantitative NMR was used for the calculation of M_n because of peak overlapping in the ^1H NMR. The molar mass was calculated from the ratio of -CH_3 side chain of DKP ring to the -CH_3 of polymer backbone. For the polymerizations initiated by DKP with higher steric hindrance (Table 1, P1-3 and P1-4), dispersities are slightly broader (Đ=1.2-1.25) and experimental molar masses are higher than the theoretical ones. Polymerization kinetics is slower in the case of phenylalanine functionalized DKP (Table 1 and Figure S4). In addition, low molar mass tailing on GPC-curve (Figure S3c) is suggesting a slow initiation step meaning propagation step is terminated before quantitative initiation.[28]

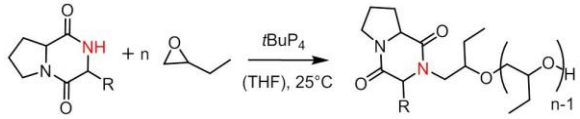

FIGURE 2 Polymerization of 1,2-butylene initiated by Cyclo(Alanine-Proline).

MALDI ToF analyses were carried out for all the polymers synthesized in Table 1 (Figure 3, S1-S3). In all cases, the major population corresponds to the expected polymer structure cationized with Na^+ and end-terminated by a DKP on one end and by a hydroxyl group on the other end. Nevertheless, MALDI-TOF analyses demonstrated additional minor populations (particularly visible in linear mode) indicating the presence of transfer or side reactions for all the polymers (Figures 3 and S1-S3). This undesired populations are probably generated by nucleophilic attack on the DKP ring carbonyl groups as previously evidenced in the case of cyclic dipeptides[23] with the apparition of ester bonds (Scheme S1). Polymerizations were conducted at lower temperatures to decrease the side reaction occurrence, however, no improvement could be evidenced.

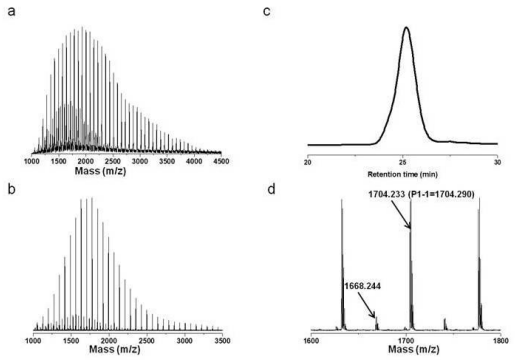

FIGURE 3 MALDI-TOF spectrum and GPC trace of P1-1. a. MALDI-TOF spectrum of P1-1 in linear mode. b. MALDI-TOF spectrum of P1-1 in reflectron mode. c. GPC trace of P1-1 using THF as eluent. d. Zoom in region of P1-1 MALDI-TOF-spectrum in reflectron mode.

Phosphazene base t-BuP_4 was proven efficient in the deprotonation of DKP amide groups and their functionalization by polyether chains having narrow dispersities. However, side-reactions have been detected which does not guarantee the presence of one DKP per macromolecule. The following part of this work is devoted to the elimination of this side reaction in order to prepare macromolecules quantitatively functionalized by DKP. C(A-P) was selected as a model DKP for these optimization studies. Indeed, in the peptide/protein chemistry, alanine residues are used to determine the role of specific residues on the function of peptides by replacing each peptide sequence with alanine, one by one which is called alanine mapping.[29] In this study, cyclic dipeptide, C(A-P) has a neutral, alanine function as well.

Polymerization of 1,2-epoxybutane by C(A-P)/t-BuP_4/i-Bu_3Al as Initiating System.

Incorporation of Lewis acid to the reaction mixture has already been used to promote activated monomer mechanism for the polymerization of oxirane monomers with various initiators.[30]

TABLE 2 Polymerization of 1,2-epoxybutane initiated by Cyclo(Ala-Pro) using t-BuP_4/ i-Bu_3Al as catalyst system, at a monomer concentration of 2M in MeTHF.

Run	Monomer /C(A-P)/ t-BuP_4/ i-Bu_3Al (mol)	T°C	Time (h)	Conv (%)[a]	$M_{n\ theo}$ (g/mol)	M_n[a] $^{13C\ NMR}$ (g/mol)	$M_{n\ SEC}$ (g/mol)	M_w/M_n (SEC)
P2-1	30/1/1/1	25	158	0	-	-	-	-
P2-2	30/1/1/2	25	3	100	2300	4100	3900	1.39
P2-3	30/1/1/3	25	3	100	2300	4600	5200	1.52
P2-4	30/1/1/2	10	17	100	2300	3800	4000	1.36
P2-5	30/1/1/2	0	3	100	2300	4100	4000	1.45
P2-6	30/1/1/2	-10	69	100	2300	3400	2300	1.53
P2-7	30/1/1/2	-20	15	100	2300	4500	4300	1.45
P2-8[b]	30/1/1/2	0	23	100	2300	5200	5300	1.31
P2-9[b]	30/1/1/2	-20	20	96	2200	4100	4200	1.47
P2-10	30/1/1.25/2	25	1	100	2300	4000	3900	1.33
P2-11	30/1/1.25/2	-20	15	94	2200	4500	3600	1.49
P2-12[c]	30/1/1.25/2	25	1	100	2300	3000	3100	1.33
P2-13[d]	30/1/1.25/2	25	1.5	100	2300	3200	3200	1.32
P2-14[e]	30/1/1.25/2	25	1.5	100	2300	2500	2800	1.35

[a] Determined by 1H NMR of the reaction mixture by comparing the signal of the -CH_2CH_3 of both monomer and polymer to the CH group signal of the oxiran group; [b]Step-wise monomer addition 25µl h^{-1}. [c]Drop-wise monomer addition at 25°C. [d]Drop-wise monomer addition at -20°C. [e]Drop-wise i-Bu_3Al addition at -20°C.

In particular, alcohol/phosphazene base initiating systems combined with i-Bu_3Al have been used to synthesize high molar mass poly(propylene oxide)[31] or to polymerize *N*-glycidylphthalimide.[32] These systems strongly increase the rate of polymerization due to the monomer activation and minimize transfer and side reactions thanks to the selective reactivity of the Lewis acid-anion-counter-ion complexes formed as initiating and propagating species. Therefore, we have investigated the effect of C(A-P)/t-BuP_4/i-Bu_3Al initiating system on the reactivity of active centers and the occurrence of side reactions during polymerization. MeTHF was chosen as a polymerization solvent instead of THF to avoid the complexation with i-Bu_3Al.[33]

Effect of C(A-P)/i-Bu_3Al ratio: Initially, the ratio of C(A-P)/t-BuP_4 was fixed to 1 and then varied (P2-1, P2-2 and P2-3, Table 2). In the first attempt, 1eq. i-Bu_3Al was used, and it did not lead to polymerization (P2-1, Table 2) because i-Bu_3Al contributed only in the formation of a complex with the deprotonated amide group which is the initiator. i-Bu_3Al complexed amidates are not nucleophilic enough to react with oxirane monomers, therefore the polymerization is inhibited which is in agreement with previous reports.[30] Consequently, we increased the i-Bu_3Al to initiator ratio to 2 (P2-2, Table 2) to promote the activation of monomer through complexation of i-Bu_3Al with oxirane ring and start the polymerization (Figure 4).

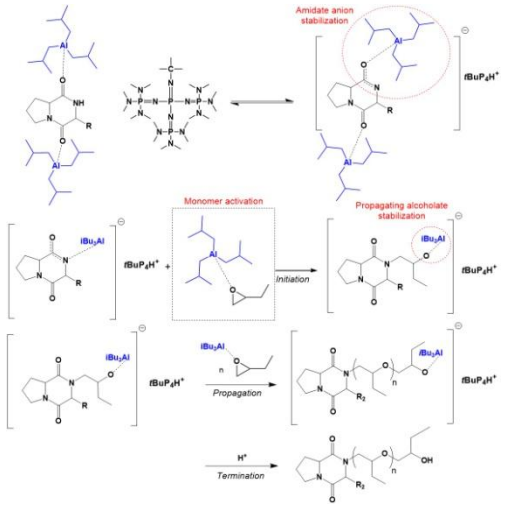

FIGURE 4 Proposed mechanism of initiation, propagation and termination steps of polymerization in the presence of i-Bu_3Al.).

In this case, the reaction yielded a high conversion in a very short time (~3h), suggesting increased monomer reactivity through oxygen coordination allowing initiation and subsequent propagation. The MALDI-TOF analyses of the resulting polymers show that all the polymer chains have the expected structure, i.e., polybutylene oxide initiated with DKP and terminated with a hydroxyl group (Figure 5). The addition of i-Bu_3Al to the reaction medium reduced the reactivity of anionic species, hence side reactions which were observed in the polymerization with t-BuP_4 alone were eliminated. However, molar masses (M_n) determined by both ^{13}C quantitative NMR (4100 g/mol) and SEC (3900 g/mol) were higher than the expected value (2300 g/mol) with a quite broad dispersity (M_w/M_n) (M_w/M_n=1.39, P2-2, Table 2). The higher molar mass values than expected and the characteristics of molar mass distribution (broad dispersity with low molar mass tailing) strongly suggest a rather slow initiation step. The i-Bu_3Al complexed amidates of phosphazenium are probably less reactive than the non-complexed amidates of phosphazenium. Meaning, the lower reactivity of initiating sites (k_i) compared to propagating species (k_p) causes an increase of the reactivity rates of propagating species to initiating centers (k_p/k_i) leading to broadening of molar mass distributions.[28] Louis Gold has shown that for living polymerization the dispersity and the percentage of reacted initiator is related to both k_p/k_i and polymerization degree.[34] For example, for k_p/k_i=100 and targeted *X*n = 28, the theoretical dispersity Đ = 1.295 and only 40 % of the initiator will initiated a polymer chain.[34] Later, we tested the addition of 3eq. of i-Bu_3Al (P2-3, Table 2), and a polymer without detectable side or transfer reactions was obtained. However, the M_n is way higher than the previous case (4600 g/mol by NMR and 5200 g/mol by SEC), and a large dispersity (M_w/M_n=1.52, P2-3, Table 2) was obtained.

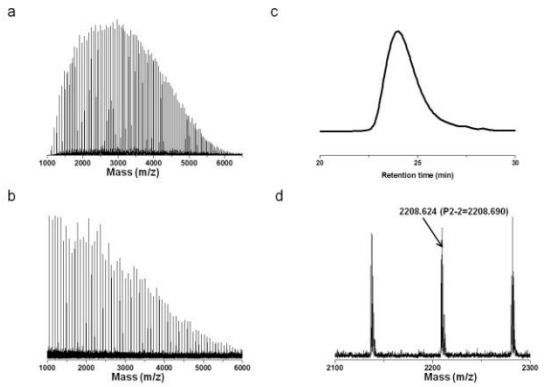

FIGURE 5 MALDI-TOF- spectrum and GPC trace of P2-2. a. MALDI-TOF- spectrum of P2-2 in linear mode. b. MALDI-TOF- spectrum of P2-2 in reflectron mode. c. GPC trace of P2-2 using THF as eluent. d. Zoom in region of P2-2 MALDI-TOF-spectrum in reflectron mode.

In summary, all the existing side reactions were completely eliminated (Figure 5) for [i-Bu_3Al]/[I] ≥ 2. Thus, our primary goal is reached: each macromolecule is functionalized with one cyclic dipeptide. The amount of added i-Bu_3Al has to be adjusted in order to enable the polymerization without being too detrimental to molar mass and dispersity. Therefore, 1/1/2 ratio of C(A-P)/t-BuP_4/i-Bu_3Al was selected for further optimization studies.

Effect of temperature: We concluded that the ratio of k_p/k_i increased in the presence of 2 eq.

i-Bu_3Al, leading to broad molar mass distributions. Hence, we performed the reactions at lower temperatures assuming a difference in activation energies of initiation and propagation steps in order to decrease the k_p/k_i ratio and gain a better control over the M_n and M_w/M_n. Reactions were conducted at 10°C, 0°C, -10°C and -20°C (P2-4, P2-5, P2-6, and P2-7, respectively, Table 2). However, lowering the temperature did not improve the results. Besides, the M_w/M_n values indicate that the polymers were obtained even with broader distributions (P2-5, P2-6 and P2-7, Table 2). Decreasing reaction temperature decreased the rate of propagation, but it further slowed down the rate of initiation which resulted in larger molar mass distributions.

Step-wise monomer addition: A recent literature by Groll and coworkers demonstrated that the dispersity of poly(glycidyl ether)s was controlled by slow monomer addition.[35] Therefore, we followed a similar approach, and the monomer was added step-wise (one addition of 25 µL monomer every hour) into the solution containing C(A-P)/t-BuP_4/i-Bu_3Al in MeTHF at both 0°C and -20°C (P2-8 and P2-9, respectively, Table 2). However, we have not seen an improvement in the case of step-wise monomer addition.

Effect of excess t-BuP_4: *t-BuP_4* was added 25% in excess in the reaction mixture to improve the equilibrium of active species in the reaction medium. By simply adding *t-BuP_4* in excess in the reaction medium, we have achieved to slightly improve molar mass distribution (P2-10, Table 2). However, the obtained M_n was still higher than the theoretical value suggesting that all the initiator precursors were not efficient during the initiation step. Next, we attempted to perform the same reaction at -20°C to see the effect of temperature (P2-11, Table 2). At the end, we obtained a lower molar mass than the previous case (3600 g/mol vs 3900 g/mol), but the molar mass distribution was much broader (1.49 vs. 1.33, P2-11 and P2-10, respectively, Table 2). A higher molar mass (Xn=100) was also targeted under the same conditions as P2-10 (Table S5, P-S5-2). The obtained polymer has a dispersity of 1.20 in spite of the presence of a small high molar mass shoulder (Figure S10).

Effect of drop-wise monomer addition: In all the polymerization conditions in the presence of i-Bu_3Al, we obtained the molar masses higher than the targeted values. The propagation step of polymerization is an exothermic process. Moreover, polymerization kinetics in the presence of i-Bu_3Al is observed to be very fast which could cause local increase of the temperature and be detrimental to the control of the polymerization.

Thus, to control the heat release, we added the monomer drop-wise (77 µL min^{-1}) at both 25°C and -20°C (P2-12 and P2-13, respectively, Table 2). In both cases, molar masses were closer to the theoretical value (P2-12 = 3100 g/mol and P2-13 = 3200 g/mol) with 1.30-1.35 dispersities. For the drop-wise monomer addition at -20°C, the presence of side reactions was observed in MALDI-ToF- analysis. On the contrary, the MALDI-ToF- spectrum of P2-12 demonstrates a unique peak distribution corresponding to the expected polymer structure cationized with a sodium ion, initiated by DKP and terminated by a hydroxyl group (Figure 6).

In addition, NMR characterization also confirms the expected polymer structure (COSY-NMR, Figure S11, HSQC-NMR, Figure S12 and HMBC-NMR, Figure S13). Further, the addition order was changed and i-Bu_3Al was added drop-wise at -20°C according to a technique applied by Carlotti and coworkers.[36] The molar mass obtained by SEC using a conventional calibration with PMMA standards is in good agreement with the theoretical one and the dispersity is equal to 1.35 (P2-14, Table 2). However MALDI-ToF- analysis demonstrated that this condition increased the occurrence of side-reactions (Figure S6).

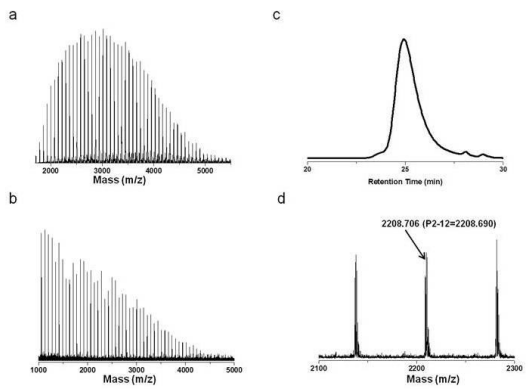

FIGURE 6 MALDI-ToF- spectrum and GPC trace of P2-12. a. MALDI-ToF- spectrum of P2-12 in linear mode. b. MALDI-ToF- spectrum of P2-12 in reflectron mode. c. GPC trace of P2-12 using THF as eluent. d. Zoom in region of P2-12 MALDI-ToF- spectrum in reflectron mode.

(2700 g/mol, P3-2, Table 3) without side reactions (Figure S8). In addition, in polymers initiated by glycine (P3-1, Table 3) and phenyl alanine (P3-3, Table 3) containing DKPs, side reactions were drastically diminished although not completely eliminated (Figure S7 and Figure S9, P3-1 and P3-3, respectively). Both polymerization reactions (P3-1 and P3-3, Table 3) yielded higher molar masses than the theoretical ones. These results show that there is a significant importance of the DKP chemical structure on the characteristics of resulting polymers. The presence of benzyl function next to the amide functionality significantly slows down the reaction kinetics. Slow rate of initiation could be explained by amide proton exchange rate of peptide backbone N-H which is directly related to backbone N-H acidity. In case of aromatic amino acid side chains, amide

TABLE 3 Polymerization of 1,2-epoxybutane and EEGE initiated by proline cyclic dipeptides using t-BuP_4/i-Bu_3Al, at a monomer concentration of 2M in MeTHF.

Run	Initiator	Monomer	Monomer/Initiator/ t-BuP_4/i-Bu_3Al (mol)	Time (h)	Conv (%)[a]	$M_{n,theo}$ (g/mol)	$M_{n,NMR}$ (g/mol)	$M_{n,SEC}$ (g/mol)	M_w/M_n (SEC)
P3-1	C(G-P)	1,2-epoxybutane	30/1/1.25/2	1	100	2300	n.d.	3900	1.37
P3-2	C(L-P)	1,2-epoxybutane	30/1/1.25/2	1	100	2400	2500[b]	2700	1.32
P3-3	C(F-P)	1,2-epoxybutane	30/1/1.25/2	1	100	2400	3000[b]	3400	1.38
P3-4	C(L-P)	EEGE	30/1/1.25/2	2	100	4300	4500[c]	4900	1.28
P3-5	C(F-P)	EEGE	30/1/1.25/2	2	100	4300	6200[c]	5200	1.28

[a] Determined by ^{1}H NMR of the reaction mixture by comparing the signal of the -CH_2CH_3 of both monomer and polymer to the CH group signal of the oxiran group; [b] M_n determined by ^{13}C quantitative NMR in $CDCl_3$. [c] M_n determined by ^{1}H NMR in CD_2Cl_2.

To sum up, the addition of i-Bu_3Al to the polymerizations of 1,2-epoxybutane initiated by C(A-P)/t-BuP_4 decreased the reactivity of ionic species suppressing the side-reactions leading to the functionalization of all polymer chains by a DKP. A drawback of the reduced amidate reactivity is the low initiation efficiency. However, an excess of t-BuP_4 and the dropwise monomer addition resulted in a better control of the polymer molar mass and narrower dispersities. The best polymerization conditions were identified as the ones used for the polymerization of in P2-12 (Table 2).

Later on, we applied the optimized polymerization condition for the modification of other proline-based DKPs (Table 3). Polymerization initiated by C(L-P) proceeded well and resulted in controlled molar mass

proton exchange rates have been reported to be slower which is also in correlation with the steric blocking effects of the aromatic rings.[37-39] Therefore, higher molar mass and broad distribution in phenylalanine functionalized proline based DKP could be explained by the poor accessibility of N-H protons which is directly related to the initiation efficiency of backbone N-H initiator precursors. The limited control in the presence of glycine residue compared to leucine or phenylalanine could be explained by the decrease of the electron donating effect and of the steric hindrance due to the absence of alkyl side chains leading to a higher carbonyl group reactivity and a higher susceptibility to nucleophilic attacks.

Eventually, C(L-P) and C(F-P) were used to initiate the polymerization of a protected

version of water soluble monomer glycidol, ethoxyethyl glycidyl ether (EEGE) to demonstrate the possible functionalization of proline based DKPs with hydrophilic, water-soluble polymer chains. In the case of C(L-P), molar masses determined by SEC and NMR are in good agreement with the theoretical one and the dispersity is narrower than in the polymerization of 1,2-epoxybutane. For C(F-P), molar masses obtained by both NMR and SEC were higher than expected values as in the case of 1,2-epoxybutane (Table 3) but with narrower dispersities. It is noteworthy that the proton signal for H_4 is observed at 4.07 ppm for C(F-P) (Figure S26 and S27) and at 2.50 ppm for the conjugate P3-5 (Figures S23 and S25). According to Ishizu et al.[40], this upfield shift is due to the epimerization from C(L-F-L-P) to cyclo(L-F-D-P) and the formation of hydrophobic interactions between H_4 of cyclodipeptide skeleton and the benzene ring.

CONCLUSIONS

We have successfully demonstrated the modification of proline-based DKPs through AROP using t-BuP_4 as polymerization promoter. We achieved to eliminate most of the side reactions in the presence of i-Bu_3Al and thus, 100% DKP-functionalized polymer chains could be synthesized. This study also demonstrated the significant impact of functional groups of the peptides on the polymerization control. We have demonstrated the possibility to modified proline-based DKPs with either hydrophobic or hydrophilic polyether chains using the polymerization of 1,2-epoxybutane or EEGE monomers, respectively. The use of Lewis acids with different strengths is currently considered to further optimize the control of the polymerization by modulating the stability of the Lewis acid-base adducts. In addition, AROP offers the possibility to modify DKPs using other various functional monomers, such as glycidyl phthalimide that yields primary amine functionalities after deprotection or allylglycidyl ether. A copolymerization approach will be considered to obtain amphiphilic structures[41] with superior outcomes and self-assembly properties.

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GRAPHICAL ABSTRACT

Özgül Tezgel, Valentin Puchelle, Haiqin Du, Nicolas Illy and Philippe Guégan

Modification of Proline-based 2,5-diketopiperazines by Anionic Ring Opening Polymerization

Proline-based 2,5-diketopiperazines (cyclic dipeptides) are used in the presence of phosphazene base as anionic ring-opening polymerization initiators of two epoxide monomers, 1,2-epoxybutane or protected glycidol (ethoxyethylglycidyl ether). Addition of Lewis Acid to the reaction mixture strongly decreases the occurrence of side reactions and allows the synthesis of 100% DKP-functionalized polymer chains.