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Synthesis of Peptide/Polymer Conjugates by Solution ATRP of Butylacrylate using an Initiator-Modified Cyclic D-*alt*-L-Peptide

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Synthesis of the cyclic peptide initiator

Details of the synthesis of the peptide-initiator were reported elsewhere.[1] In brief, the cyclic peptide is prepared using standard solid phase synthesis protocols. A linear peptide (Tentagel-L-Asp(OAll)-(D-Ala-L-Lys(Mtt))$_3$-D-Ala-Fmoc) is synthesised on the resin using conventional Fmoc chemistry. After removal of the last Fmoc and the O-allyl protecting groups the cyclization is carried out on the resin at ambient conditions. Subsequently, the Mtt protecting group is removed selectively and 2-bromo-2-methylpropionic acid is attached to the amine functions at the lysine side chains. The so-prepared peptide is cleaved off the resin and purified by recrystallization in TFA/water as well as by means of preparative HPLC (C18-RP column, 5µm, gradients of acetonitrile/water having 0.1% TFA).

Investigation of association of the cyclic peptide initiator in d^6-DMSO by ^{1}H-NMR:

Ghadiri and co-workers reported that cyclic D/L-peptides have a strong tendency to associate in non-polar solvents, depending on the concentration of the peptide.[2,3,4] So far, detailed studies have been performed in $CDCl_3$ using NMR spectroscopy. $CDCl_3$ is preferred to other solvents, as hydrogen-bond-mediated intermolecular aggregation into supra-molecular species occurs fast on the NMR time scale, thus, it can be easily monitored. Moreover, ^{1}H NMR spectra in $CDCl_3$ have been found to display significant concentration-dependent chemical shifts. In contrast to non-polar solvents, many cyclic peptides are readily dissolved as single rings in polar solvents such as DMF and DMSO [3,4].

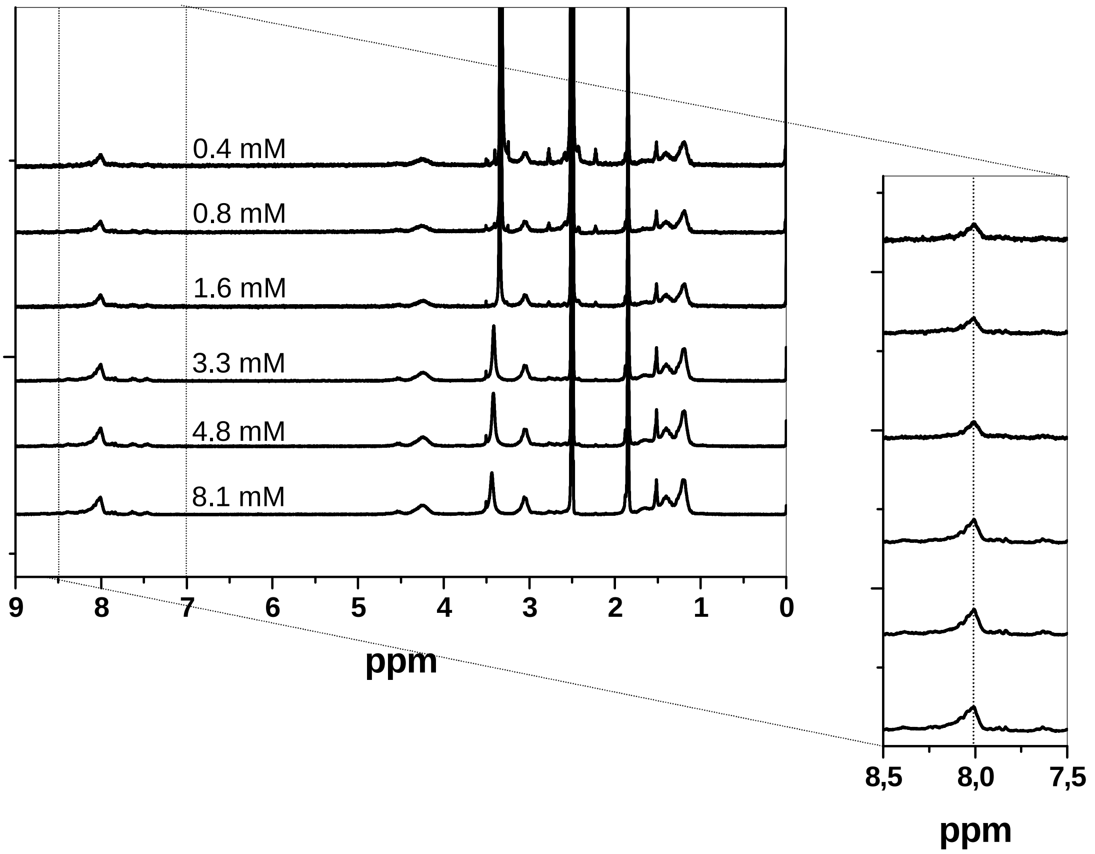


Figure S1 ^{1}H-NMR survey spectra (left) and detail spectra (right) of the cyclic peptide-initiator at different concentrations in d_6-DMSO denoted in the figure.

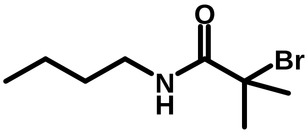
To get more information about possible aggregation of the peptide in the chosen solvent, investigations of the cyclic peptide initiator at various concentrations in d_6-DMSO were performed using ^{1}H-NMR (Figure S1). A stock solution of 8.1 mM of the cyclic peptide initiator was prepared in d_6-DMSO. Serial dilutions were carried out to produce 4.8, 3.3, 1.6, 0.8 and 0.4 mM stocks. A certain amount of each solution was transferred to separate NMR tubes and measured.

The signals at 7.5-8.00 ppm correspond to the amide N-H of the peptide ring. With increasing concentration, no visible changes to the appearance and position of the signal is observed. Thus, the NMR spectra suggest that there are no changes to the local chemical environment of the cyclic peptide initiator even at high d_6-DMSO concentrations (considerably higher than those used in the polymerizations). If cyclic peptides tend to aggregate (i.e. stack upon each other), as described above, typically a downfield shift of the amide (e.g. N-H) signals of about 0.4 ppm is observed.[5] Taken together, the investigations suggest that DMSO dissolves the peptide as single rings, even at high concentrations, which is a crucial requirement to avoid pre-aggregation during in-situ grafting of the polymer chains.

ATRP with amide-functional initiators

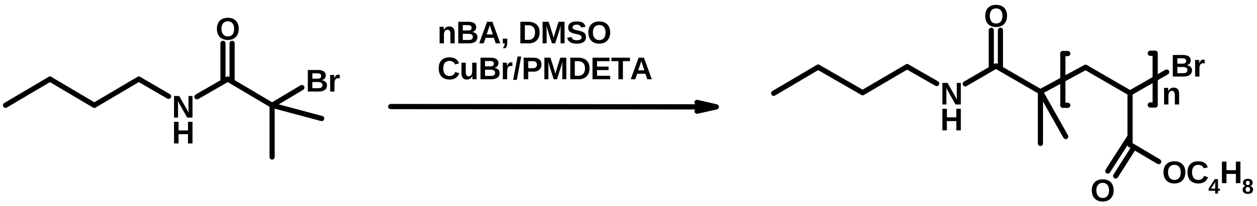
Model polymerizations were performed using an amide-based initiator, n-Butylacrylate (nBA) as monomer and Dimethylsulfoxide (DMSO) as solvent. An amide model initiator, that mimics the modified lysine side-chain of the cyclic peptide initiator, was used for the model polymerization (scheme S1). The goal of this part of the work is to investigate, and to optimize different reaction conditions to gain a maximum control over the resulting polymer molecular weight, polydispersity and end-group-functionality. Therefore, polymerizations were investigated with varying chemistry of the initiator, temperature, and added additives.

Synthesis of Poly-n-butyl acrylate in DMSO *via* ATRP



Scheme S1 Schematic description of the amide-based initiator

Scheme S1 schematically illustrates the chemical structures of the amide-functional initiator that was used in the model polymerizations. The amide-based initiator, 2-Bromo-N-butyl-2-methyl-propionamide was prepared according to Couet et al. [6]



Scheme S2 Schematic description of ATR-polymerization with nBA using amide-based initiator.

All polymerizations of nBA were carried out by dissolving the initiator in degassed DMSO, and adding a fixed amount of this initiator stock solution to a mixture of monomer, and the polymerization additives, N,N,N,N,N-pentamethyl-diethylene-triamine (PMDETA) as ligand and copper(I)bromine (CuBr) as the catalyst, and DMSO.

A schematic outline of the polymerization is shown in Scheme S2. The polymerization temperature was first kept at 55 °C. The targeted degree of polymerization was, N = 266. nBA was used as the monomer, because it yields a non-toxic hydrophobic polymer with interesting thermal properties (e.g. low Tg). The reaction conversion was calculated after precipitation of the polymer in methanol/brine solution from gravimetric analysis of the dried PnBA polymer (HPLC analysis was not performed, because of overlapping signals of monomer and solvent).

ATRP of nBA in DMSO: Influence of temperature

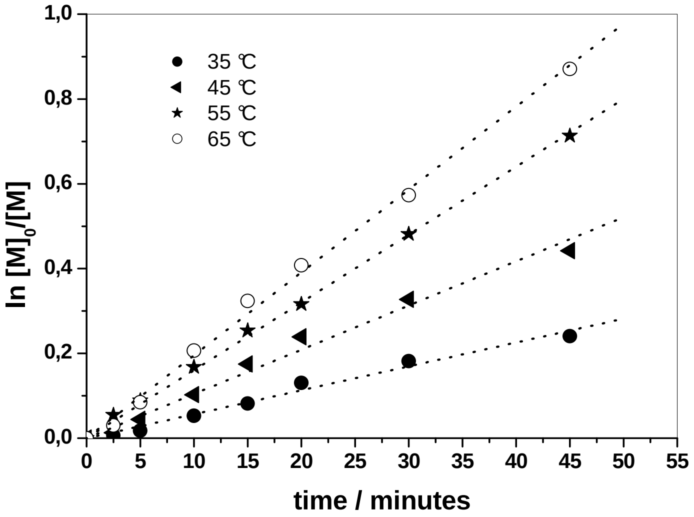


Figure S2 First-order kinetic plot of the polymerization of nBA in DMSO using an amide-functional initiator at different temperatures. Polymerization conditions: [nBA]:[amide-ini]:[CuBr]:[PMDETA] 266:1:1:1; in 65 vol% DMSO. The lines represent linear fits to the data.

We studied the temperature-dependent polymerizations, using the amide-functional initiator. The polymerizations were performed at identical monomer concentration; corresponding to a volume fraction of 65% DMSO in the polymerization feeds. Polymerizations at temperatures of 35 °C, 45 °C, 55 °C, and respectively of 65 °C were performed. Figure S2 shows the first-order kinetic plot of the polymerizations of nBA in DMSO solution at different temperatures (denoted in the Figure).

The data show a linear increase of $\ln([M_0]/[M])$ as a function of the polymerization time, up to about 45 min, which corresponds to a maximum conversion of 60 % (at T = 65 °C). With increasing temperature, the apparent propagation constant increases from 0.006 min^{-1} at 35 °C, 0.010 min^{-1} at 45 °C, to 0.016 min^{-1} at 55 °C, and to 0.020 min^{-1} at 65 °C. From the data it can be concluded that there is no significant loss in active sites and/or an increase of deactivator concentration during polymerizations.

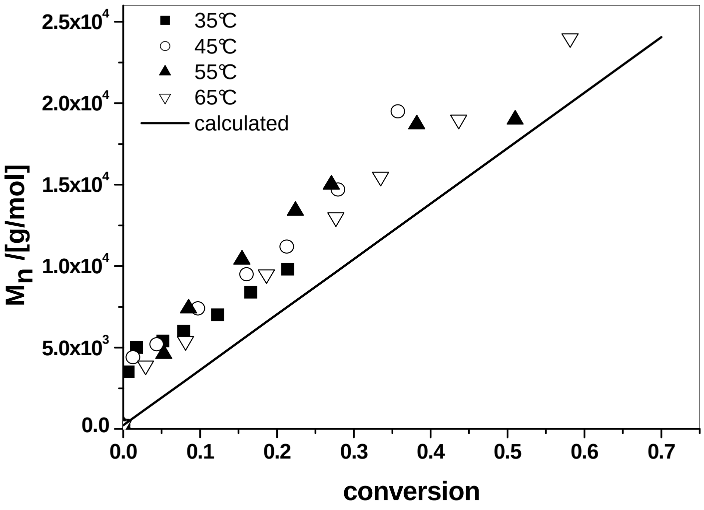


Figure S3 Number-average molecular weight of PnBA as a function of the reaction conversion at different polymerization temperatures. The solid line represents the theoretically expected mass. Polymerization conditions: [nBA]:[amide-ini]:[CuBr]:[PMDETA] 266:1:1:1; in 65 vol% DMSO.

Figure S3 shows the number average molecular weight, as a function of reaction conversion of the polymerization of nBA in DMSO at different temperatures using the amide-based initiator. The experimentally derived molecular weight increases linearly with reaction conversion, and it is slightly higher than the expected molecular weight (solid line). All superimposed experimental data fall onto one line, indicating good reproducibility of the reaction at different temperatures.

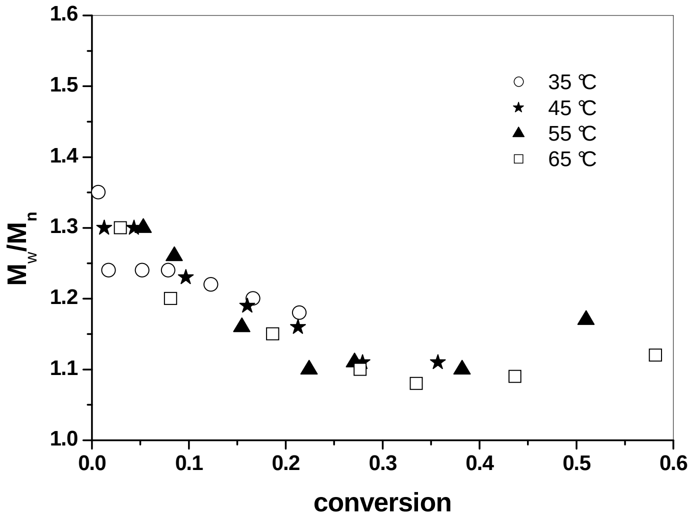


Figure S4 Polydispersity (M_w/M_n) of PnBA using amide-based initiator as a function of the reaction conversion at different polymerization temperatures.

The polydispersity (Figure S4) shows a slight decrease from about 1.3 below 10% conversion to about 1.1 above 20% conversion. It stays constant up to 60% conversion. Taken together, the temperature can be used to fine-tune molecular weight evolution with a high amount of reproducibility and control.

ATRP of nBA in DMSO: Influence of added carboxylic acid functional groups

The sensitivity of ATRP to the presence of carboxylic acids is known, as these functional groups can destroy the metal catalyst by displacing the halogen in the copper complex. Additionally a protonation of the nitrogen in the ligand system may occur, disturbing its coordination to the metal centre. [7,8]

As many peptides also carry carboxylic acid functions, including the cyclic peptide initiator that was designed in our group, it is of interest to study the effect of the presence of COOH-groups on the performed ATRP reaction (here: nBA polymerization in DMSO). In order to mimic the polymerization using the cyclic peptide initiator, we added acetic acid as model-compound to the model-polymerizations using the low molecular weight amide-initiator. To make sure that there is no interaction between the acetic acid and initiator or additives before starting the polymerizations, stock solutions of acetic acid and amide-based initiator were prepared separately. After adding a certain amount of the acetic acid stock-solution, the initiator stock-solution was added immediately to start the polymerization. Polymerizations with different acetic acid concentrations (0 equiv, 0.33 equiv and 0.66 equiv relative to the 1 equiv of amide-based initiator) were performed.

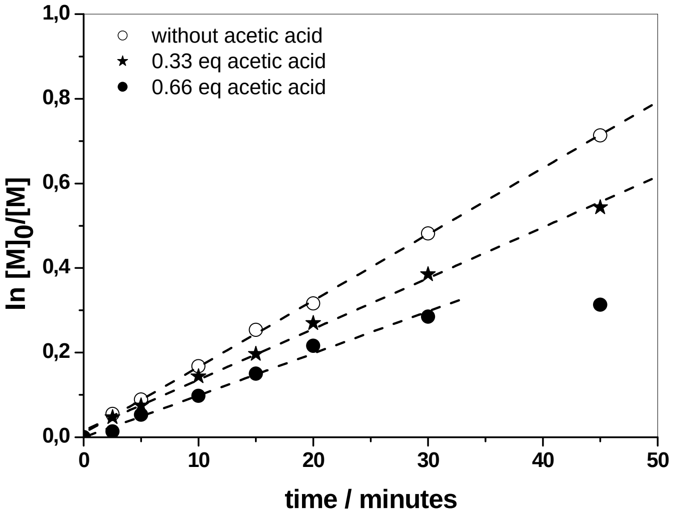


Figure S5 First-order kinetic plot of nBA in DMSO using amide-based initiator with different concentrations of acetic acid at 55°C, in 65 Vol% DMSO.

Figure S5 shows the first-order kinetic plot of the polymerizations of nBA in DMSO solution with different acetic acid concentrations. With increasing acetic acid concentration in the reaction mixture, the polymerization rate decreases without a strong effect on the linearity of the time-dependent monomer consumption. Only if 0.66 equiv acetic acid is added a slight deviation from the linear first-order kinetic can be seen at higher conversions. With increasing acetic acid concentration the apparent propagation constant decreases from 0.016 min^{-1} without acetic acid to 0.012 min^{-1} with 0.33 equiv acetic acid and 0.009 min^{-1} with 0.66 equiv acetic acid, with respect to the initiator.

A decrease in the polymerization may be explained by the interaction and thereby a possible deactivation of the catalyst system with the COOH-groups, if the concentration of the free acid is high relative to the Cu^I in the reaction.[9] Thus, carboxylic acids may directly interfere with the ATRP equilibrium at high acid concentrations, a finding that was already reported by Matyjaszewski and co-workers, when investigating the polymerization of styrene in the presence of externally added benzoic acid.[10]

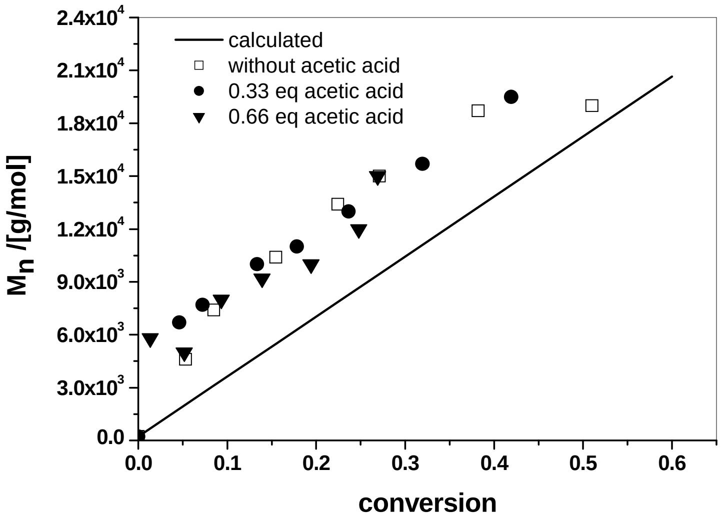


Figure S6 Number-average molecular weight of PnBA as a function of the reaction conversion at different acetic acid concentrations.

The number-average molecular weight, as a function of reaction conversion in the presence of different acetic acid concentrations is shown in Figure S6. The experimentally derived molecular weight increases linearly with reaction conversion, and it is slightly higher than the theoretical molecular weight (solid line). Possible reasons for this have been discussed above.

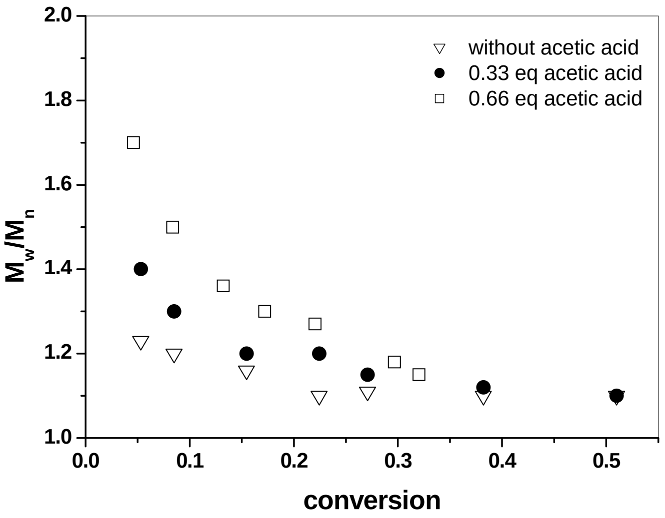


Figure S7 Polydispersity (M_w/M_n) of PnBA as a function of the reaction conversion with different acetic acid concentrations.

In Figure S7 the polydispersity is shown as a function of conversion. At low conversions, the polydispersity shows a strong increase from about 1.2 at 5% conversion to 1.7 if 0.66 equiv acetic acid is added to the reaction. At higher conversions it decreases (30% conversion) to about 1.2 for all cases and it stays (almost) constant. A possibly explanation for high polydispersities at the early stage of the polymerization may be the competition of the free carboxylic acid for the coordination of Cu^{II}, that leads to a partially deactivation of the catalyst. As the polydispersity can be correlated with the rate of constant amount of deactivators (Cu^{II}), an increase of the polydispersity occurs with a decrease of the Cu^{II} concentration.[11]

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