Sequence-Defined Polypeptide−Polymer Conjugates Utilizing Reversible Addition Fragmentation Transfer Radical Polymerization

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ABSTRACT: Straightforward solid-phase-supported synthesis routes were presented to obtain novel oligopeptide-based reversible addition fragmentation transfer (RAFT) agents. These approaches include the coupling of a functional RAFT agent to a resin-bound peptide and the functionality switch of an oligopeptide ATRP macroinitiator into an oligopeptide transfer agent. The solid-phase-supported methods allowed easy purification of the transfer agents, making difficult column purification steps unnecessary. Well-defined conjugates comprising sequence-defined peptides and synthetic polymers could be accessed by applying RAFT polymerization techniques in combination with the peptide macrotransfer agents. Polymerization reactions of *n*-butyl acrylate were performed in solution, yielding peptide−polymer conjugates with controllable molecular weight and low polydispersities of around 1.1. The peptide−polymer conjugates were characterized using ^{1}H NMR spectroscopy and size exclusion chromatography (SEC), while the incorporation of the oligopeptide into the synthetic polymer and the preservation of the chirality were shown by circular dichroism (CD) spectroscopy.

Introduction

The tailored incorporation of sequence defined oligo- or polypeptides into synthetic polymers originates an interesting class of macromolecules.[1−4] These systems are of interest for the rational design of bioactive polymeric materials that have the potential to interact actively with biological systems.[5−9] To synthesize macromolecular conjugates in a well-controlled manner, existing polymerization techniques have to be adapted to obtain generally applicable access routes. Among free radical, anionic, cationic, or metal-catalyzed polymerization processes, controlled radical polymerization (CRP) seems to be the most suited method for the synthesis of these macromolecular conjugates. The main advantages of CRP methods are the control over molecular weight and molecular weight distributions as well as the high tolerance for diverse functional groups and impurities. Furthermore, a variety of well-defined polymer topologies and architectures can be realized using these methods.[10]

Recently, it has been demonstrated that sequence-defined polypeptides can be applied as macroinitiators for nitroxide-mediated radical polymerization (NMP[11]) as well as for atom transfer radical polymerization processes (ATRP[12,13]).[2,14−16] Detailed kinetic studies were reported for the solution phase ATRP of *n*-butyl acrylates.[2] This study revealed a controlled polymerization process leading to macromolecular conjugates (poly(*n*-butyl acrylate)-*block*-polypeptide) with defined molecular weight and relatively low polydispersities ($M_w/M_n \approx 1.20$).[2] Another successful approach involved the polymerization of oligopeptide macromonomers using ATRP, which allows access to interesting comb polymers that exhibit a poly(meth)acrylic backbone and multiple, functional peptide side chains.[17,18]

However, even though the ATRP process involving oligopeptide structures results in well-defined products, interactions between the copper catalyst and the peptide are evident and cannot be suppressed.[2] The polyamide backbone and some side chain functionalities of the peptide exhibit inherent ligating properties that result in a multidentate ligand for metal ions.[19] This probably causes a ligand exchange reaction with the ATRP metal complex, resulting in a partial inhibition of the catalyst, as was demonstrated recently.[2] Similar behavior has been reported for the ATRP processes of (meth)acrylamides.[20−22] Furthermore, the ligating properties of the peptides strongly depend on length and sequence, requiring an optimization of the reaction conditions based on the applied peptide. This could be a limiting factor in the use of ATRP as a general approach for the synthesis of (poly)peptide−polymer conjugates.

In our efforts to overcome these problems and to search for versatile synthesis tools to obtain well-defined macromolecular conjugates, we explored the potential of the reversible addition fragmentation transfer radical polymerization process (RAFT[23]). The RAFT process offers some advantages in its ability to tolerate functional groups, allowing the controlled polymerization of a variety of functional monomers. The RAFT technique is facile since the different components are not sensitive to air or moisture and can therefore be simply dissolved and deoxygenated prior to the polymerization. Moreover, the polymerizations are usually performed at moderate reaction temperatures (typically around 60 °C) and basic conditions are strictly avoided. This prevents racemization as well as thermal degradation of the oligopeptide segments.

Recently, Perrier et al. reported the successful application of low molecular weight amide-based transfer agents for the RAFT polymerization of diverse monomers.[24] However, the route presented requires chromatographic purification of the transfer agents, which is inconvenient for the synthesis of oligopeptide-based RAFT agents. In particular, if large and/or multifunctional peptides are targeted, a chromatographic purification is usually difficult and time-consuming, and a loss

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of material due to nonspecific adsorption to the stationary phase is often unavoidable.

With this contribution, we present straightforward, solid-phase-supported synthesis routes to obtain oligopeptide-based RAFT agents that were subsequently utilized for the polymerization of *n*-butyl acrylate (*n*BA). For the synthesis of the oligopeptide-based RAFT agents, two different strategies have been evaluated avoiding the usual chromatographic purification procedures, thereby providing a versatile route to fragile, multifunctional, or complex RAFT agents. The first approach included the coupling of a preformed carboxyl-functionalized RAFT agent to the N-terminus of a resin-bound peptide. The second synthesis route comprises a functionality switch of a solid-phase-supported oligopeptide ATRP macroinitiator into an oligopeptide transfer agent. The GDGFD peptide sequence was utilized to demonstrate the process, making the investigation directly comparable to our previous ATRP study.[2]

Experimental Section

Materials. 2-Bromopropionic acid (Aldrich, 99+%), *n*-butyl acrylate (*n*BA, Aldrich, 99%), and *N*,*N*-dimethylformamide (DMF; Aldrich, 99+%) were distilled and stored at −15 °C. THF was dried over Na/benzophenone and distilled prior to use. All other reagents were used as received from Aldrich. Fmoc−amino acid derivatives (Fmoc−Asp(*t*Bu)OH, Fmoc−GlyOH, Fmoc−PheOH), polystyrene−(2-aminoethanol-2-chlorotrityl) resin (loading: 0.3 mmol/g) and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (IRIS Biotech GmbH, Germany) were used as received. The synthesis of the oligopeptide precursors **1** and **4** was described previously.[2] 4-cyano-4-((thiobenzoyl)sulfanyl)pentane carboxylic acid (**2**) was synthesized according to literature procedures. Therefore, dithiobenzoic acid[25−27] was synthesized, oxidized to bis(thiobenzoyl) disulfide,[27,28] and subsequently reacted with 4,4′-azobis(4-cyanovaleric acid).[29]

Instrumentation. Mass spectrometry was performed on a high performance liquid chromatograph electron spray ionization mass spectrometer (LC-ESI-MS) (Shimadzu, qp8000α, Duisburg, Germany). Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker DPX-400 spectrometer at 400.1 MHz. Samples to determine the monomer conversion were taken directly from the polymerization mixture and diluted with $CDCl_3$. The conversion was determined relative to DMF as internal standard by comparing the integral intensity of the resonance of vinylic protons of the monomer with the formamide proton of the DMF. Resonances used: (δ = 6.60−5.70 ppm, 3H, $H_2C{=}CH$, monomer) and (δ = 8.01 ppm, s, DMF). GPC measurements were carried out in THF as eluent using three 5-Å MZ-SDV columns with pore sizes of 10^3, 10^5, and 10^6 Å (flow rate 1 mL/min). The detection was performed with an RI (Shodex RI-71) and a UV detector (TSP UV 1000; 260 nm). Linear PS-standards (PSS, Germany) were used for calibration. Samples were taken from the polymerization mixture, diluted with THF and used for $M_{n,app.}$ and M_w/M_n determination.

Synthesis of the oligopeptide transfer agent (Ph—C(S)—S—C(CH$_3$)(CN)—CH$_2$—CH$_2$—C(O)—Gly-Asp(*t*Bu)-Gly-Phe-Asp(*t*Bu)—NHCH$_2$CH$_2$OH) (3). 4-Cyano-4-((thiobenzoyl)sulfanyl)pentane carboxylic acid (167 mg, 0.6 mmol) was dissolved in 5 mL of dichloromethane (DCM). After the addition of DCC (62 mg, 0.3 mmol), the reaction mixture was stirred for 30 min at room temperature. The resulting anhydride solution was filtered, transferred to 2−5 mL of NMP, and added to the pre-swollen oligopeptide precursor resin **1** (0.1 mmol) in NMP, and the mixture was stirred for 4 h at room temperature under argon atmosphere (Kaiser's test results indicated that no free amine groups were left). The resin was washed thoroughly with NMP, DCM, NMP, THF, and DCM. The liberation of the macroinitiator from the support was accomplished by 5−30 min treatment with a cleavage mixture (2% (v/v) trifluoroacetic acid (TFA) in dichloromethane), followed by isolation via diethyl ether precipitation and lyophilization from acetonitrile/water (1:1). This resulted in a mixture of **3** (76%) with a thioamide side product (24%) as a pink powder. ^{1}H NMR (DMSO-d_6): δ = 8.34−8.17 (m, 3H, NH), 8.13−7.98 (m, 2H, NH), 7.91 (d, $^2J_{HH}$ = 8.2 ppm, ArH_{ortho}), 7.69 (m, ArH_{para}), 7.69 (m, ArH_{meta}), 7.3−7.1 (m, 5H, ArH), 4.7−4.4 (m, 4H, CH), 3.83−3.55 (m, 4H, CH_2), 3.37 (m, 2H, CH_2), 3.31 (m, 4H, CH_2), 3.10 (t, $^2J_{HH}$ = 5.9 Hz, CH_2), 3.02 (dd, 1H, $^2J_{HH}$ = 4.6, 14 Hz, CH_2), 2.78 (dd, 1H, $^2J_{HH}$ = 9.4, 14 Hz, CH_2), 2.66 (dd, 2H, $^2J_{HH}$ = 6.1, 16 Hz, CH_2), 2.52−2.35 (m, CH_2 + DMSO), 1.90 (s, CH_3), 1.37 (s, 18H, *t*Bu). ESI-MS: *m*/*z* (%) = 729 (4) [M2 − *t*Bu + H]$^+$, 785 (5) [M2 + H]$^+$, 791 (26) [M2 − 2*t*Bu + TFA − H_2O + Na]$^+$, 807 (9) [M2 + Na]$^+$, 948 (100) [M1 + Na]$^+$, 964 (12) [M1 + K]$^+$. M1 corresponds with the mass of **3**, and M2 corresponds with the mass of the thioamide side product.

Synthesis of the Oligopeptide Transfer Agent (Ph—C(S)—S—CH(CH$_3$)—*C*(*O*)—Gly-Asp(*t*Bu)-Gly-Phe-Asp(*t*Bu)—NHCH$_2$CH$_2$OH) (5). Under argon atmosphere, a solution of phenylmagnesium bromide, prepared from bromobenzene (1.0 mL, 9.5 mmol) and magnesium turnings (220 mg, 9.05 mmol) in THF (6 mL), was filtered into a round-bottom flask, containing anhydrous carbon disulfide (0.8 mL, 13.3 mmol) while cooling with an ice-bath. The reaction mixture was stirred for 2 h at room temperature. Then 2 mL of water was added, and the THF was removed in vacuo. Water (20 mL) and Et_2O (50 mL) were added, the aqueous layer was acidified with HCl (1 M, 15 mL), and the product from the aqueous layer was extracted twice with Et_2O (50 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated in vacuo. The dithiobenzoic acid (310 mg, 2.0 mmol) was added to the oligopeptide precursor resin **4** (0.1 mmol) pre-swollen in THF (4 mL). After the addition of pyridine (160 μL, 2.0 mmol) the reaction was stirred at 60 °C for 15 h. The reaction mixture was cooled to room temperature, and the resin was extensively washed with THF, H_2O, THF, and DCM. Liberation from the support and isolation of the final oligopeptide RAFT agent was accomplished as described above. ^{1}H NMR(DMSO-d_6): δ = 8.59 (m, 1H, NH), 8.27−8.23 (br m, 2H, NH), 8.12 (m, 1H, NH), 8.06 (d, 1H, NH), 7.92 (d, 2H, $^2J_{HH}$ = 8.4 Hz, ArH_{ortho}), 7.64 (m, 1H, ArH_{para}), 7.53 (m, 1H, NH), 7.48 (t, 2H, $^2J_{HH}$ = 7.5 Hz, ArH_{meta}), 7.3−7.1 (m, 5H, ArH), 4.73 (q, 1H, $^2J_{HH}$ = 7.0 Hz, CH), 4.63−4.42 (m, 3H, CH), 3.78 (m, 2H, CH_2), 3.66 (dd, 1H, $^2J_{HH}$ = 3.6 Hz, CH_2), 3.61 (dd, 1H, $^2J_{HH}$ = 3.6 Hz, CH_2), 3.8−3.3 (br m, H_2O), 3.37 (t, 2H, $^2J_{HH}$ = 6.2 Hz, CH_2), 3.10 (m, 2H, CH_2), 3.01 (dd, 1H, $^2J_{HH}$ = 4.5, 14 Hz, CH_2), 2.77 (m, 1H, CH_2), 2.66 (m, 2H, CH_2), 2.41 (m, 2H, CH_2), 1.54 (d, 3H, ^2J = 7.0 Hz, CH_3), 1.37 (s, 18H, *t*Bu) ppm. ESI-MS: *m*/*z* (%) = 761 (18) [M − 2*t*Bu + H]$^+$, 799 (3) [M − 2*t*Bu + K]$^+$, 817 (20) [M − *t*Bu + H]$^+$, 873 (13) [M + H]$^+$, 895 (100) [M + Na]$^+$, 911 (18) [M + K]$^+$, 967 (6) [M + TFA − H_2O + H]$^+$.

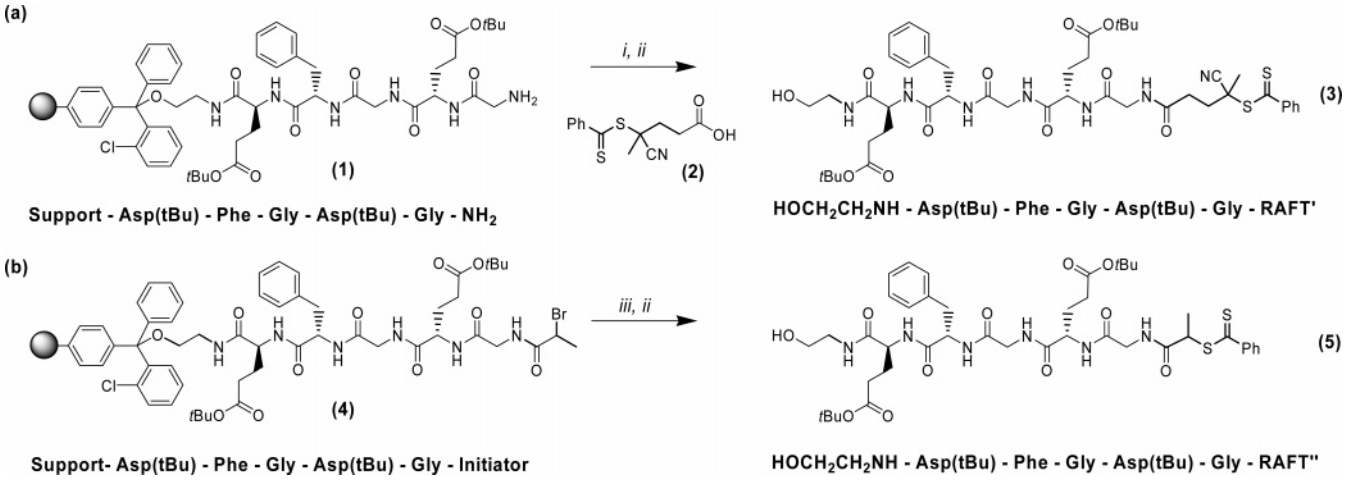
General RAFT Polymerization Procedure. The oligopeptide RAFT agent (10 mg, 10.5 μmol) was dissolved in DMF (2.12 mL). After the addition of *n*BA (11.1 mmol) and AIBN (0.091 mg, 0.55 μmol), the reaction mixture was carefully degassed and heated to 60 °C. Samples of 0.2 mL were taken for kinetic analysis (GPC, NMR).

For further characterization purposes, the polymer peptide conjugate was precipitated multiple times from DMF and THF in MeOH/H_2O (1:1) and freeze-dried from acetonitrile/benzene (1:1) to extract, eventually, the remaining peptide or peptide RAFT agent.

Results and Discussion

Combining the structural and functional control of peptides with the diversity and stability of polymers in polymer-peptide conjugates can result in biohybrid materials that have the potential to interact with biological systems. In particular, the combination of solid-phase-supported peptide synthesis and controlled radical polymerization is highly attractive to obtain such materials. Therefore, oligopeptide transfer agents were

Scheme 1. Synthesis of the Oligopeptide Transfer Agents[a]



[a] Conditions: (i) 2, DCC, NMP, 4 h, room temperature; (ii) 2% TFA/DCM; (iii) dithiobenzoic acid, THF, 15 h, 60 °C).

synthesized using solid-phase-supported peptide synthesis and subsequently RAFT polymerization techniques were performed in solution.

Synthesis and Characterization of the Oligopeptide Transfer Agent (3) and RAFT Polymerization. Oligopeptide Transfer Agent (3). The modification of an established synthetic strategy to access oligopeptide ATRP macroinitiators[2] results in the approach to couple a carboxylic acid functionalized RAFT agent to the N-terminus of a resin bound oligopeptide. This one step synthesis would allow regio-selective introduction of the transfer group either N-terminally or sequence-positioned by the modification of an ϵ-amine group of a lysine residue. Furthermore, the introduction could be performed fully automated by utilizing available peptide synthesizer protocols.

The functional RAFT agent (4-cyano-4-((thiobenzoyl)-sulfanyl)pentanoic acid, **2**) was synthesized via procedures modified from the literature. First, dithiobenzoic acid[25−27] was obtained using the Grignard reaction by starting from bromobenzene. The dithioacid could be easily oxidized into bis(thiobenzoyl) disulfide,[27,28] which was subsequently reacted with 4,4′-azobis(4-cyanovaleric acid)[29] to give **2** in good yields and high purity. As a model oligopeptide Gly-Asp-Gly-Phe-Asp (**1**) was synthesized on a polystyrene-(2-aminoethanol-2-chlorotrityl) resin following standard solid-phase-supported peptide synthesis procedures and Fmoc protocols as described previously.[2] The coupling of **2** to the peptide was facilitated by DCC in NMP yielding the oligopeptide RAFT agent **3** (Scheme 1a).

3 was cleaved from the resin using 2% (v/v) TFA in DCM, precipitated in diethyl ether and lyophilized from an acetonitrile/water mixture. ESI-MS analysis confirmed the formation of the product **3** (Figure 1) by showing the corresponding mass signals for the different ion adducts (948 *m/z* $[M + Na]^+$ and 964 $[M + K]^+$). Besides **3**, the ESI-MS analysis revealed the formation of a byproduct, caused by the nucleophilic attack of the peptide amine terminus on the dithioester. The formed substitution byproduct leads to a thioamide structure as could be confirmed by the corresponding mass signals (Figure 1). The fragmentation of the *tert*-butyl protecting groups occurred probably during the ionization process, rather than by the product liberation from the resin. This was concluded from ^{1}H NMR spectroscopy, showing no indication of a loss of *tert*-butyl protecting groups.

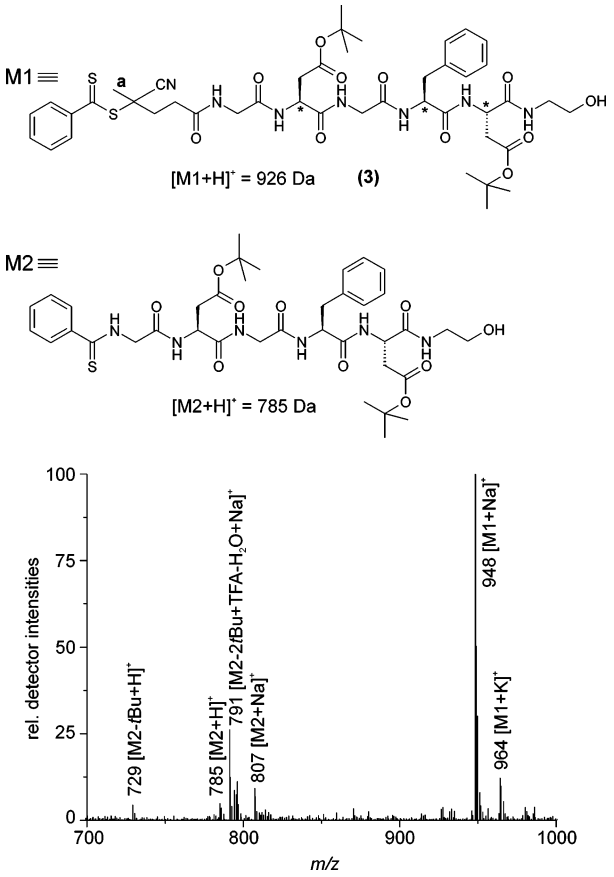


Figure 1. ESI-MS spectrum of oligopeptide transfer agent **3** (M1) and the thioamide byproduct (M2).

The formation of TFA adducts most likely occurred during the product liberation step. However, this is a common phenomenon and will not interfere with the RAFT process. It is noteworthy that no oligopeptide precursor (**1**) was detectable and that all mass signals could be assigned conclusively. This indicates that beside **3** and the thioamide side product no other products are formed.

The purity of **3** was determined to be 76% by using ^{1}H NMR spectroscopy, assuming the thioamide as the only side product. Therefore, the integral intensity of the methyl group (H_a in Figure 1) of the RAFT moiety was compared with that of the chiral α-protons (H* in

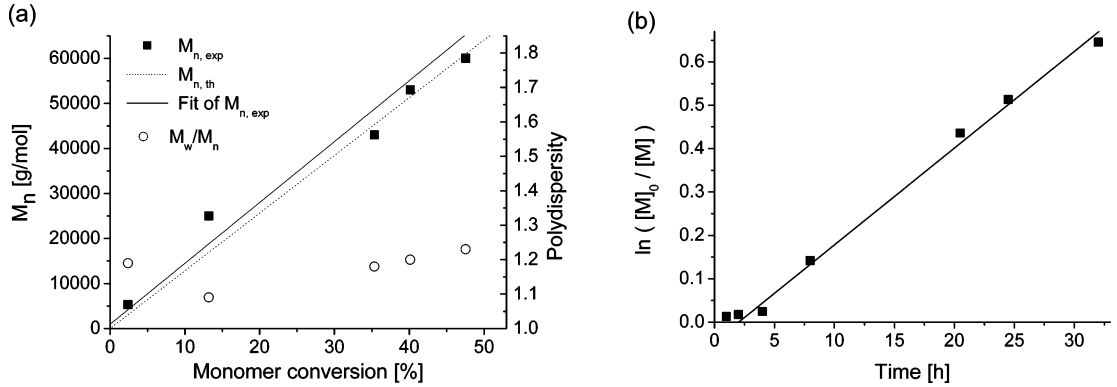


Figure 2. RAFT polymerization of *n*BA controlled by **3** at 60 °C: apparent M_n vs conversion (a) and ln($[M]_0/[M]$) vs reaction time (b) (Conditions: $[n\text{BA}]_0/[\mathbf{3}]_0/[\text{AIBN}]_0 = 1050/1/0.05$, DMF = 60 vol %.)

Figure 1) of the peptide. The optimization of the reaction conditions do not lead to a complete suppression of the formation of the thioamide side product. Nevertheless, the presented method could be applied to the coupling of a RAFT agent via hydroxyl moieties, e.g., to side chain functionalities of serine or threonine residues, where such a side reaction is not expected. The generated ester linkages between the peptide segment and the transfer group would result in a hydrolytically labile linkage between the peptide and the polymer. This will be of high importance for instance for the programmed degradation of peptide−polymer conjugates or for the liberation of the peptide segments, e.g., as biofunctional units in medical applications.

RAFT Polymerization. Even though the synthesis route for the oligopeptide macro RAFT agent **3** leads to products with 76% purity, the formed thioamide side product will not interfere with the CRP process. Because of the ease of synthesis and the potential application to ester-linked RAFT agents, **3** was utilized in a model RAFT polymerization. Therefore, the polymerization of *n*-butyl acrylate (*n*BA) was investigated, targeting oligopeptide-poly-*n*-butyl acrylate conjugates. To access well-defined peptide−polymer conjugates caution was taken in order study the polymerization within the controlled regime. Moreover, thermal stress for the oligopeptide segment as well as for the amidic solvent was reduced by limiting the time of polymerization, minimizing the risk of racemization and/or degradation of the peptide segment and solvent.

The polymerization was performed in DMF at 60 °C and 2,2′-azobis(isobutyronitrile) (AIBN) was used as radical source. DMF was chosen due to the good solvent properties for both oligopeptide and poly-*n*-butyl acrylate. Since it is not expected that the thioamide side product will interfere with the RAFT process, the polymerization of *n*-butyl acrylate with **3** was performed without further purification. However, the concentration of **3** was corrected due to the purity of the compound.

The kinetics indicate a controlled polymerization process that yields products with M_w/M_n around 1.2 (Figure 2a). The molecular weights increase linearly with the reaction time, meeting the theoretical values within the experimental error. The semilogarithmic plot of the monomer conversion vs polymerization time shows a first order kinetic after a retardation period of roughly 4 h (Figure 2b). Though retardation periods are frequently observed within RAFT processes, the causes are still discussed since the mechanism in the early stage of the polymerization remains difficult to access. It was suggested that retardation occurs due to either an intermediate radical termination[30−34] or a slow fragmentation or reinitiation within the RAFT process.[30,35]

Synthesis and Characterization of the Oligopeptide Transfer Agent (5) and RAFT Polymerization. Oligopeptide Transfer Agent (5). To avoid the formation of thioamide side product, as observed for the synthesis of **3**, a second synthesis route was developed using solid-phase-supported synthesis techniques as well. This route is outlined in Scheme 1b and comprises the functionality switch of an oligopeptide ATRP macroinitiator (**4**) into an oligopeptide transfer agent (**5**). Since within this approach the presence of a nucleophilic amine is avoided, the substitution side reaction cannot occur.

The oligopeptide ATRP macroinitiator **4** was synthesized by N-terminal coupling of 2-bromopropionic acid to the resin-bound oligopeptide **1** as was described previously.[2] The subsequent reaction of **4** with the pyridinium salt of the dithiobenzoic acid in THF yields the oligopeptide macro transfer agent **5**. Therefore, the dithiobenzoic acid[25−27] was prepared from bromobenzene using the Grignard reaction. The isolation of the dithiobenzoic acid and the in situ reaction with pyridine was proved to be a necessary step. Initial efforts to react **4** with the dithiobenzoic magnesium bromide were found to be insufficient. This was probably due to the presence of remaining colloidal magnesium that possesses a high reactivity,[36] leading to the formation of a side reaction in which the Grignard product of **4** was formed, and thereby prevents a quantitative functionality switch of **4** to **5**. In particular, in solid-phase-supported chemistry such a reaction leads to the formation of obvious amounts of side products since a large excess of reactants are required to drive the reaction toward completion.

The oligopeptide RAFT agent **5** was cleaved from the resin with 2% (v/v) TFA in DCM, precipitated in diethyl ether and lyophilized from acetonitrile/water. ^{1}H NMR spectroscopy and ESI-MS confirmed the formation of **5** (Figure 3) and HPLC analysis proved the complete consumption of the precursor (**4**). Comparison of the observed proton resonances in the ^{1}H NMR spectrum (Figure 3a) for the peptide segment of **5** with the resonances of the raft moiety allows the verification of a quantitative formation of **5**. Therefore, the integral intensities of the chiral amino acid α-protons of the peptide (H_g, H_k, H_n) were compared with the aliphatic protons of the RAFT moiety (H_d and H_e), showing the

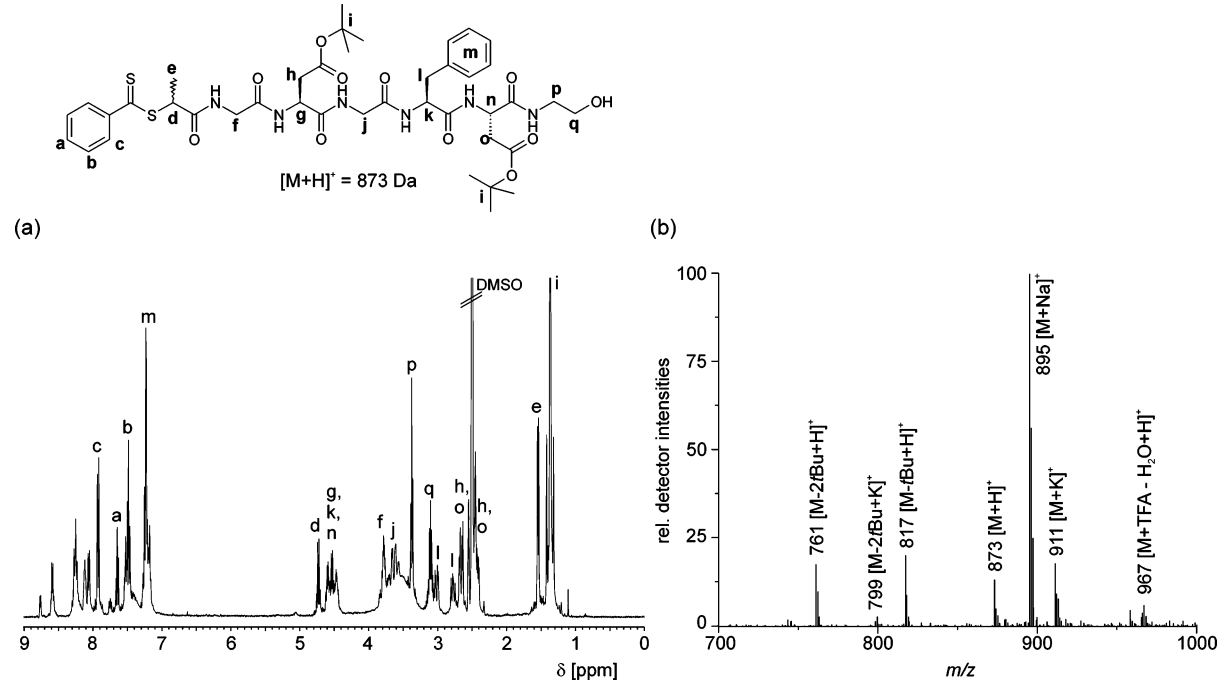


Figure 3. ^{1}H NMR spectrum (DMSO-d_6) (a) and ESI-MS spectrum of oligopeptide transfer agent **5** (b).

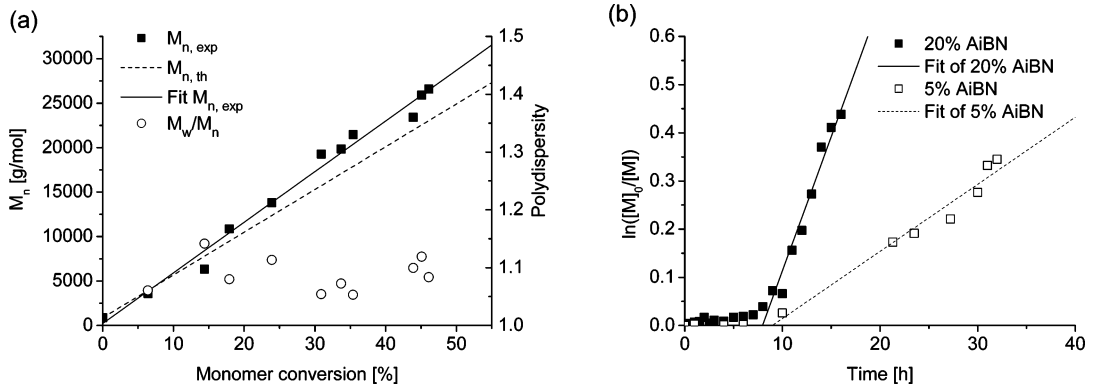


Figure 4. RAFT polymerization of *n*BA controlled by **5** at 60 °C: molecular weight ($M_{n,GPC}$) vs conversion (a) and first-order kinetic plot ln([M]$_0$/[M]) vs reaction time (b) (Conditions: [*n*BA]$_0$/[**5**]$_0$/[AIBN]$_0$ = 375/1/0.2, DMF = 60 vol %.)

complete substitution of the bromine of **4** by the dithiobenzoic acid. Moreover, the signals of the aromatic protons H_{a-c} of the dithiobenzoic acid are clearly visible (7.45, 7.65, and 7.90 ppm for H_b, H_a, and H_c, respectively). The ESI-MS spectrum revealed that besides the formation of the oligopeptide RAFT agent **5**, no side reactions occurred during the substitution reaction (Figure 3b). All observed mass signals could be assigned to different ion adducts of **5**, to a TFA adduct or to structure derivatives showing the loss of *tert*-butyl groups. The latter occurs most likely during the electrospray ionization process.

The solid-phase-supported synthesis of **5** has shown to be convenient for the synthesis of (oligo)peptide RAFT agents. The formation of **5** is quantitative, and no side reactions were observed. The products are readily purified by washing procedures prior to cleavage from the support, making chromatographic purification steps unnecessary. This is particularly advantageous if large or complex peptide sequences are desired.

RAFT Polymerization. An oligopeptide-poly-*n*-butyl acrylate conjugate (**6**) was synthesized to investigate the RAFT radical polymerization of *n*BA. Therefore, the polymerization of *n*BA was performed in DMF at 60 °C in the presence of **5** as transfer agent and 20 mol % AIBN as radical source. The kinetics indicate a controlled polymerization that yields well-defined peptide−polymer conjugates with low $M_w/M_n \approx 1.1$ (Figure 4a). The molecular weight increases linearly with monomer consumption, following the theoretical values. The semilogarithmic plot shows a first order kinetic after a retardation period of roughly 8 h (Figure 4b). Therefore, the kinetics indicates that the RAFT polymerization proceeds in a controlled manner. Also when only 5 mol % of AIBN was used a comparable retardation period of about 8 h was observed (Figure 4b). These similar retardation periods exclude the possibility that potential impurities retard the polymerization of *n*BA with **5**. Retardation periods are often observed in RAFT polymerizations but should be taken in consideration for controlled polymerizations, especially when low molecular weight compounds have to be obtained. Moreover, the structure variation of the RAFT moiety of **3** and **5** causes a different stability and reactivity of the formed primary peptide-macro radicals. This might possibly explain the different retardation times, observed for the polymerization of *n*BA using **5** compared to **3**. However, it should be emphasized that the origin for retardation

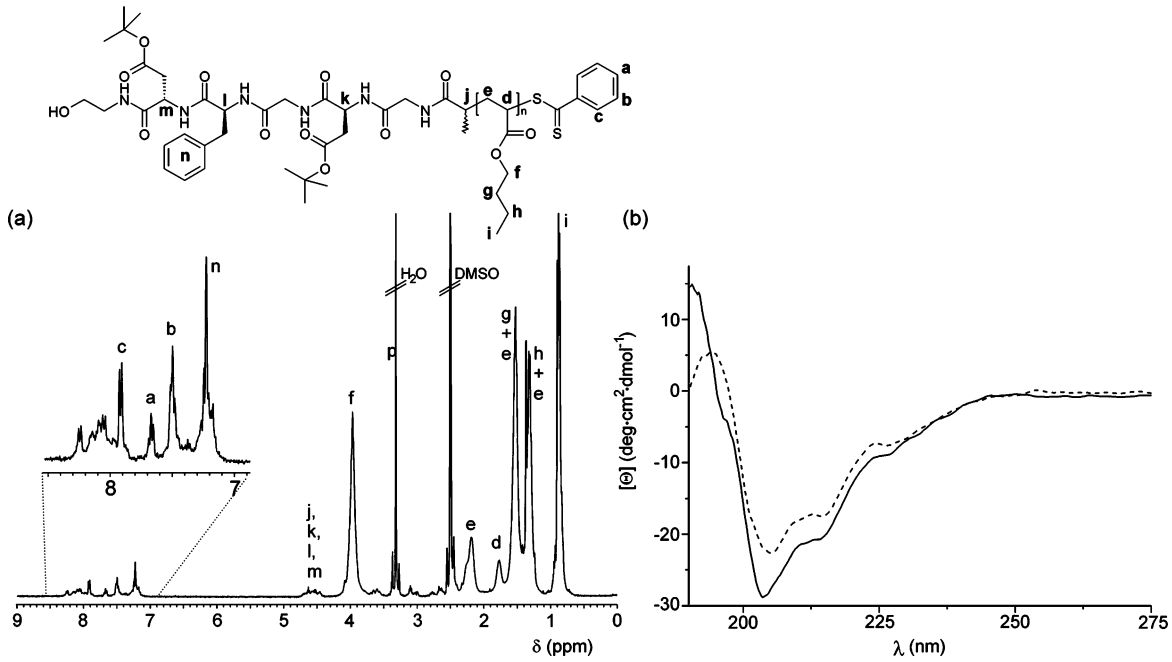


Figure 5. (a) ^{1}H NMR spectrum (DMSO-d_6) of oligopeptide-poly-*n*-butyl acrylate conjugate **6** and (b) CD spectrum of oligopeptide **5** (dashed line) and oligopeptide-poly-*n*-butyl acrylate conjugate **6** (solid line).

is not fully understood yet. The slope of the first-order kinetics plot was, as expected, 4 times lower (dashed line in Figure 4b; i.e., 0.014 and 0.056 for 5% and 20% AIBN, respectively), indicating that the overall rate of polymerization directly correlates to the amount of formed radicals.

The kinetics experiments indicate a controlled polymerization but do not prove the incorporation of the oligopeptide segment into the polymer. Therefore, a second polymerization ($[n\text{BA}]_0/[\mathbf{5}]_0/[\text{AIBN}]_0 = 120/1/0.2$, DMF = 60 vol %) was performed, targeting lower molecular weight conjugates. To show that the polypeptide segment is indeed included in the polymer the polymerization product **6** was precipitated multiple times in MeOH/H_2O. Since this is a good solvent for the oligopeptide segment, peptides that are not linked to the polymer would have been extracted. After freeze-drying from acetonitrile/benzene ^{1}H NMR spectroscopy conclusively shows that the oligopeptide-poly-*n*-butyl acrylate conjugate **6** is formed (Figure 5a). The characteristic signals were observed for the protons of the poly-*n*BA segment (H_{d-i}) and the chiral (H_{j-m}) and aromatic (H_n) protons of the oligopeptide. Moreover, the observed chemical shifts for protons of the RAFT moiety (H_{a-c}) revealed that the RAFT moiety is still present after polymerization. A number-average molecular weight $M_{n,NMR}$ = 4.6 kDa (n = 29) for **6** was determined by comparison of the integrals of the chiral protons (H_{j-m}) and the RAFT protons (H_{a-c}) with the methyl group (H_a) of the poly-*n*BA segment. The formation of dimerization products resulting from radical coupling can be excluded due to the ^{1}H NMR spectrum since both the RAFT and peptide end group functionalities were quantified in a ratio of 1:1.

These results are supported by size exclusion chromatography (SEC) showing that **6** has a monomodal and narrow molecular weight distribution with M_w/M_n = 1.18. In addition, the number-average molecular weight was determined with $M_{n,app}$ = 4.1 kDa, resembling the value calculated based on ^{1}H NMR end group analysis.

However, the structural conformation and, with this, the biological function of polypeptides are strongly influenced by the chirality of the amino acid building blocks. It is therefore essential to prove that during the synthesis of biohybrid materials the chirality of the oligopeptide segment is not altered by the polymerization process. Circular dichroism spectroscopy (CD) showed comparable spectra for **5** and **6** (Figure 5b), indicating the preservation of the chirality and structure of **6**. The comparable molar ellipticities observed for **5** and **6** support the effective incorporation of the peptide segment into the polymer and verify the absence of racemization.

Conclusion

It was shown that well-defined conjugates comprising sequence-defined oligopeptides and polymers can be accessed via RAFT polymerization methods. Therefore, oligopeptide RAFT agents were synthesized by the utilization of novel solid-phase-supported synthesis routes, making the usually required chromatographic purification step unnecessary.

A functionality switch of a resin-bound oligopeptide ATRP macroinitiator into an oligopeptide transfer agent was shown to proceed in a clean manner. The macro RAFT agent was obtained in high purity without further chromatographic purification procedures. In addition to this an alternative, straightforward synthesis route was demonstrated, involving the coupling of a carboxylic acid functionalized RAFT agent to the N-terminal amine group of a supported oligopeptide. This yielded macro transfer agents of 76% purity, showing the formation of a side product that could not be suppressed sufficiently. However, the simplicity of this method makes it potentially suitable for the fully automated coupling of RAFT agents to hydroxyl functionalities of resin-bound macromolecules since in this case a side product formation is not expected. This will be of interest for the preparation of transfer agents possessing bioorganic segments such as oligopeptides, oligosaccharides or oligonucleotides.

Kinetics investigations in which *n*-butyl acrylate (*n*BA) was polymerized in solution using the oligopeptide transfer agents revealed an efficient control of the polymerization processes. Peptide−polymer conjugates exhibiting a molecular weight distribution of $M_w/M_n \sim 1.1$ and controllable molecular weights were obtained after retardation periods of about 4−8 h. The combination of ^{1}H NMR, SEC and CD spectroscopy confirmed that the peptide segments were quantitatively incorporated into the polymer and revealed that both structure and chirality of the peptide segment have not been affected by the polymerization procedures.

The RAFT group remains at the end of the polymer chain after isolation of the conjugates, as was confirmed quantitatively by ^{1}H NMR spectroscopy. This will allow the modification of the polymer chain end as well as further block extensions that might lead to polymers with advanced architectures.

In particular, the advantages of solid-phase-supported synthesis of RAFT transfer agents will be of interest for the access to multifunctional, sensitive, or biorelated RAFT agents, since time as well as material consuming chromatographic purification procedures can be avoided. In addition to this, the on-bead RAFT polymerization is highly promising, since the RAFT process is not necessarily disturbed by a high local concentration of dormant radicals in the micro gel.

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