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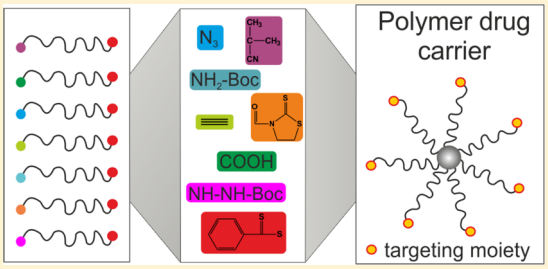
Synthesis of Well-Defined Semitelechelic Poly[*N*-(2-hydroxypropyl)methacrylamide] Polymers with Functional Group at the α-End of the Polymer Chain by RAFT Polymerization

V. Šubr,* L. Kostka, J. Strohalm, T. Etrych, and K. Ulbrich

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, v.v.i., Heyrovsky Sq. 2, 162 06 Prague 6, Czech Republic

Supporting Information

ABSTRACT: *N*-(2-Hydroxypropyl)methacrylamide polymer precursors (pHPMA) with very narrow distribution of molecular weights and polymer chain terminating in a single reactive group have big potential in the synthesis of various polymer drug and gene delivery systems. This paper shows that pHPMA can be prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization; the pHPMAs prepared by RAFT polymerization conducted to high conversions contained at the α-end of the polymer chain a single functional group originated from both, the chain transfer agent (CTA) and initiator (INI) in ratio depending on polymerization conditions. The well-defined monofunctional pHPMAs with narrow molecular weight distribution and with single reactive group situated at the α-polymer chain end can be prepared in one step by RAFT polymerization initiated by the tailor-made CTA and INI, both containing the same functional group in their structure. Synthesis of pHPMAs terminating in various reactive groups (azide, propargyl, thiazolidin-2-thione, amino, hydrazide) was also described.



INTRODUCTION

Over the last 15 years, reversible addition−fragmentation chain transfer (RAFT) radical polymerization has become an important method for the synthesis of structurally well-defined polymers with narrow molecular weight distributions.[1−4] The mechanism of RAFT polymerization based on chain transfer agents (CTAs) containing thiocarbonylthio or trithiocarbonyl groups has been described in many papers and reviews.[5−8] Because RAFT polymerization is no more than a conventional free radical polymerization conducted in the presence of a suitable thiocarbonylthio species, traditional methods for the generation of free radicals using azo compounds, such as 2,2′-azobis(2-methylpropionitrile) (AIBN) or 4,4′-azobis(4-cyanopentanoic acid) (INI-$(COOH)_2$), have been employed.[7] Progress in the field of RAFT polymerization has enabled the relatively easy synthesis of a broad range of statistical and block copolymers and α- or ω-end-functionalized polymers with well-defined architectures.[9−14]

These polymers were quickly found to have applications in polymer drug delivery systems.[15−19] For example, α-end-functionalized polymers were used in the synthesis of polymer protein/peptide conjugates.[20−23] Generally, α- or ω-end-functionalized polymers can be synthesized by RAFT polymerization via two different methods. In the first method, polymers prepared by RAFT polymerization are usually terminated at the ω-end with a thiocarbonylthio group originating from the CTA. This thiocarbonylthio group can be subsequently removed or transformed into the desired ω-end-functional group.[11,24,25] Unfortunately, not always functionality of the polymer chain ω-end is close to one, especially if reactive group has to be introduced.

In the second method, chain transfer agents with the desired functional groups were synthesized and then used in RAFT polymerization, resulting in polymers with α-end-functional groups.[20,26] In this case, the ω-end thiocarbonylthio group was removed by a method described by Perrier.[11]

Tao et al.[20] described the synthesis of a novel thiazolidine-2-thione-functionalized CTA and its use in the preparation of an α-end-functionalized pHPMA by RAFT polymerization initiated with AIBN. This α-end-functionalized pHPMA was subsequently conjugated with lysozyme. The obtained semitelechelic pHPMA was characterized by ^{1}H NMR and SEC. Unfortunately, although the exact content of the α-end thiazolidine-2-thione group can be easily measured spectrophotometrically, it was not determined.[27]

Yang et al.[28] described the synthesis of a biodegradable multiblock pHPMA polymer. The pHPMA was prepared by RAFT polymerization using a CTA containing a glycyl-phenylalanyl-leucyl-glycyl alkyne functional group initiated with AIBN. Postpolymerization modification of the ω-end thiocarbonylthio group with 4,4′-azobis(azidopropyl-4-cyanopentanoate) resulted in the formation of a heterotelechelic pHPMA containing α-terminal alkyne and ω-terminal azide groups. The biodegradable multiblock pHPMA was prepared

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from a heterotelechelic pHPMA using click chemistry. The multiblock pHPMA was separated from the unreacted mono and diblock pHPMA by size exclusion chromatography. Presence of only 38 wt % of the multiblock pHPMA in the final product indicated that the functionality of the starting heterotelechelic pHPMA was not close to one and thus the reaction was not completed. Unfortunately, the authors did not provide detailed analysis of the end-chain groups.

The impact of azo initiators on the structure of α-end-functionalized polymers in the papers mentioned above and in many other published papers studying RAFT polymerization has been neglected.[7] We found only one paper that reported on quantitative analysis of the effect of azo initiators on the structure of the α-end of the polymer chain in RAFT polymerization.[29]

Here, we focused on the synthesis of semitelechelic α-end-functionalized polymers based on *N*-(2-hydroxypropyl)-methacrylamide (pHPMAs) and the quantitative analysis of the effect of azo initiators on the structure and functionality of the α-end of the polymer chain prepared by RAFT polymerization. The study was performed with HPMA polymerized by a RAFT mechanism with 4,4′-azobis(4-cyanopentanoyl-valine or leucine methyl ester) (INI-$(Val\text{-}OMe)_2$ or INI-$(Leu\text{-}OMe)_2$) as initiators and 4-cyano-4-(thiobenzoylthio)pentanoyl-alanine methyl ester (CTA-Ala-OMe) as the chain transfer agent. Amino acids were used as labels, one amino acid in the CTA molecule and two in each INI molecule. The ratio of amino acids (Val/Ala or Leu/Ala) incorporated into the α-end of the polymer chain was determined by amino acid analysis. On the basis of these results, a series of azo initiators (INI) based on 4,4′-azobis(4-cyanopentanoic acid) and 4-cyano-4-(thiobenzoylthio)-pentanoic acid as chain transfer agents (CTA) containing different functional groups (azide, propargyl, thiazolidin-2-thione, -NH-Boc and -NHNH-Boc) were synthesized and used for the preparation of well-defined α-end-functionalized pHPMAs. The importance of such well-defined polymers is not only because of their use in the preparation of HPMA−protein or antibody conjugates[20−23] but such polymers can be used for preparation of biodegradable multiblock polymers, for polymer coating of nanoparticles and viral gene delivery vectors and also in the synthesis of water-soluble polymer drug carriers and conjugates of grafted block or star dendrimer-derived architectures.[30,31]

EXPERIMENTAL SECTION

Materials. Methacryloyl chloride (≥97%), 1-amino-propan-2-ol, 2,2′-azobis(2-methylbutyronitrile) (AIBN, ≥ 98%), 4,4′-azobis(4-cyanopentanoic acid) (INI-$(COOH)_2$, ≥ 98%), 4,5-dihydro-thiazole-2-thiol (TT), L-alanine methyl ester hydrochloride (≥99%), L-leucine methyl ester hydrochloride (≥99%), L-valine methylester hydrochloride (≥99%), 3-amino-1-propyne (≥98%), 3-aminopropyl bromide hydrobromide (≥98%), *N*-Boc-1,2-diaminoethane (≥98%), Boc-hydrazide (≥98%), bis(thiobenzoyl) disulfide (≥90%), 2-cyanopropan-2-yl benzodithioate (≥97%), 4-cyano-4-(thiobenzoylthio)-pentanoic acid (CTA-COOH, (≥97%)), 4-(dimethylamino)pyridine (DMAP, (≥99%)), *N*,*N*′-dicyclohexylcarbodiimide (DCC, (≥99%)), *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC, (≥98%)), 2,4,6-trinitrobenzenesulfonic acid (TNBSA), *N*,*N*-diisopropylethylamine (DIPEA, ≥ 99%), dimethyl sulfoxide (DMSO, ≥ 99%), *tert*-butyl alcohol (*t*-BuOH, ≥ 99%), dichloromethane (DCM) and silica gel 60 were purchased from Sigma-Aldrich, Czech Republic. All other chemicals and solvents were of analytical grade. The solvents were dried and purified by conventional procedures and distilled before use.

N-(2-Hydroxypropyl)methacrylamide (HPMA) was synthesized by a modified reaction of methacryloyl chloride with 1-aminopropan-2-ol in dichloromethane in the presence of sodium carbonate.[32]

Characterization Methods. The CTA and INI were characterized on HPLC Shimadzu system with reverse-phase column Chromolith HighResulution RP-18e, 100 × 4.6 mm (Merck, Germany) equipped with UV/vis photodiode array detector. Gradient elution with 5−95% of acetonitrile for 15 min at a flow rate of 1.0 mL/min was used. The molecular weight of CTA and INI was determined using mass spectrometer (MS LCQ Fleet, Thermo Fisher Scientific) and structure was confirmed by ^{1}H NMR (300 MHz) spectra recorded on (Bruker DPX 300) spectrometer and elemental analysis.

Number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity ($Đ$) were measured using size-exclusion chromatography (SEC) on a HPLC Shimadzu system equipped with UV, an Optilab rEX differential refractometer and multiangle light scattering DAWN 8 (Wyatt Technology, USA) detectors. For these experiments, a 20% 0.3 M acetate/80% methanol (v/v) buffer and TSKgel G3000SW or TSKgel G4000SW column were used.

The content of alanine and valine or leucine in the polymer was determined by amino acid analysis of hydrolyzed pHPMA samples (6 M HCl, 115 °C, 18 h in a sealed ampule) on a reverse-phase column Chromolith HighResulution RP-18e, 100 × 4.6 mm (Merck, Germany) using precolumn derivatization with phthalaldehyde (OPA) and 3-sulfanylpropanoic acid (excitation at 229 nm, emission at 450 nm). Gradient elution with 10−100% of solvent B for 35 min at a flow rate of 1.0 mL/min was used (solvent A, 0.05 M sodium acetate buffer, pH 6.5; solvent B, 300 mL of 0.17 M sodium acetate and 700 mL of methanol).

The content of thiazolidine-2-thione (TT) groups was determined spectrophotometrically on a Specord 205 (Jena Analytics) spectrophotometer (ε_{305} = 10 700 L.mol^{-1}.cm^{-1}; methanol)[27] and content of amino groups was determined by TNBSA method (ε_{420} = 11 550 L.mol^{-1}.cm^{-1}; borate buffer pH 9.3).[33]

Synthesis of 2-[1-Cyano-1-methyl-4-oxo-4-(2-thioxo-thiazolidin-3-yl)-butylazo]-2-methyl-5-oxo-5-(2-thioxothiazolidin-3-yl)-pentanenitrile (INI-$(TT)_2$). The INI-$(TT)_2$ was prepared by reaction of INI-$(COOH)_2$ with 4,5-dihydro-thiazole-2-thiol in the presence of DCC in tetrahydrofuran catalyzed by DMAP.[34] HPLC analysis gave two symmetrical peaks at 305 nm with a retention times of 10.24 and 10.35 min. Anal. Calcd/found: C, 44.79/44.35; H, 4.59/5.71; N, 17.41/9.45; S, 26.57/28.92. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 1.7 (s, 6H), 2.39−2.45 (m, 4H), 3.12−3.21 (m, 4H), 3.29−3.36 (m, 4H), 4.42−4.54 (m, 4H). ESI−MS: m/z = 504.83 $(M-Na)^+$.

Synthesis of 4,4′-Azobis(4-cyanopentanoylvaline methyl ester) (INI-$(Val\text{-}OMe)_2$). The INI-$(COOH)_2$ (2.0 g; 7.14 mmol), Val-OMe.HCl (2.63 g, 15.7 mmol) and DIPEA (2.82 mL, 16.4 mmol) were dissolved in dichloromethane (15 mL) and then EDC (3.61 g; 18.8 mmol) was added in several portions. Reaction mixture was stirred for 3 h and then extracted with distilled water (3 × 20 mL) and with 2 wt % $NaHCO_3$ aqueous solution. Organic layer was separated and dried with Na_2SO_4. The DCM was evaporated and the product was crystallized from mixture ethyl acetate/diethyl ether. Yield was 1.8 g (49%). HPLC gave a single peak at 220 nm with a retention time of 9.67 min. Anal. Calcd/found: C, 56.90/56.56; H, 7.56/7.64; N, 16.59/16.45. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 0.84−0.89 (m, 12H), 1.69 (s, 6H), 1.97−2.03 (m, 4H), 2.17−2.20 (m, 4H), 2.28−2.34 (m, 2H), 3.62 (s, 6H), 4.13−4.19 (m, 2H), 8.29−8.31 (d, 2H). ESI−MS: m/z = 529.00 $(M-Na)^+$.

The procedure described above for the synthesis of azo initiator INI-$(Val\text{-}OMe)_2$ was used for the synthesis of all other azo initiators containing various functional groups (leucyl methyl ester, azide, propargyl, thiazolidin-2-thione, -NH-Boc and -NHNH-Boc).

Synthesis of 4,4′-Azobis(4-cyanopentanoylleucine methyl ester) (INI-$(Leu\text{-}OMe)_2$). The INI-$(Leu\text{-}OMe)_2$ was prepared by the reaction of INI-$(COOH)_2$ (2.0 g; 7.14 mmol) with Leu-OMe.HCl (2.85 g, 15.7 mmol) in the presence of DIPEA (2.82 mL, 16.4 mmol) and EDC (3.61 g; 18.8 mmol). Yield was 1.4 g (37%). HPLC analysis gave a single peak at 220 nm with a retention time of 10.19 min. Anal: Calcd/

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found: C, 58.41/58.5; H, 7.92/8.34; N, 15.72/15.87. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 0.82−0.89 (m, 12H), 1.48−1.50 (m, 4H), 1.52−1.61 (m, 8H), 1.68 (s, 6H), 2.12−2.14 (m, 2H), 3.61 (s, 6H), 4.22−4.29 (m, 2H), 8.37−8.39 (d, 2H). ESI−MS: m/z = 556.92 $(M-Na)^+$.

Synthesis of N-(3-Azidopropyl)-4-[3-(3-azidopropylcarbamoyl)-1-cyano-1-methylpropylazo]-4-cyano-4-methylbutyramide (INI-$(N_3)_2$). 3-Azido-propylamine was synthesized by refluxing of 3-aminopropyl bromide hydrobromide with sodium azide as described earlier.[35]

The INI-$(N_3)_2$ was prepared by reaction of INI-$(COOH)_2$ (1.0 g, 3.61 mmol) with 3-azido-propylamine (0.8 g, 7.9 mmol) in the presence of EDC (1.8 g, 9.2 mmol). Yield: 1.1 g (52%). HPLC analysis: two symmetrical peaks at 220 nm with retention times of 9.1 and 9.16 min. Anal.: Calcd/found: C, 48.64/49.04; H, 6.35/5.87; N, 37.81/37.53%. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 1.64−1.68 (m,6H), 2.07−2.09 (m,4H), 2.19−2.21 (m, 4H), 2.24−2.28 (m, 4H), 3.06−3.12 (m, 4H), 3.32−3.36 (m, 4H), 8.03(t, 2H). ESI−MS: m/z = 466.67 $(M-Na)^+$.

Synthesis of 4-Cyano-4-(1-cyano-3-ethynylcarbamoyl-1-methyl-propylazo)-N-ethynyl-4-methylbutyramide (INI-(propargyl)$_2$). The INI-$(propargyl)_2$ was prepared by the reaction of INI-$(COOH)_2$ (4 g, 0.0143 mol) with 3-amino-1-propyne (1.92 mL, 0.030 mol) in the presence of EDC (1.8 g, 9.2 mmol). The final product was obtained by recrystallization from acetone/diethyl ether (1:1). Yield: 3.5 g (69%). HPLC analysis gave two symmetrical peaks at 220 nm with retention times of 8.37 and 8.46 min. Anal.: Calcd/found C, 61.00/60.96; H, 6.26/5.96; N, 23.71/23.48. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 1.68 (s, 6H), 2.29 (s, 2H), 2.48 (t, 4H), 3.09 (t, 4H), 3.85 (m, 4H), 8.42−8.46 (m, 2H). ESI−MS: m/z = 376.92 $(M-Na)^+$.

Synthesis of N′-[4-Cyano-4-(1-cyano-1-methyl-3-methylcarbamoylpropylazo)-4-methylbutyryl]hydrazinecarboxylic acid tert-Butyl Ester (INI-(NHNH-Boc)$_2$). The INI-$(NHNH\text{-}Boc)_2$ was prepared by the reaction of INI-$(COOH)_2$ (1.0 g, 3.61 mmol) with Boc-hydrazide (1.05 g, 7.9 mmol) in the presence of EDC (1.67 g, 8.7 mmol). The final product was obtained by recrystallization from ethyl acetate/diethyl ether (1:1). Yield: 1.1 g (61%). HPLC analysis gave two peaks at 220 nm with retention times of 9.12 and 9.22 min. Anal.: Calcd/found: C, 51.96/51.48; H, 7.13/7.02; N, 22.03/20.44. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 1.38 (s, 18H), 1.68 (s, 6H), 2.20−2.25 (m, 4H), 2.25−2.32 (m, 4H), 8.73 (s, 2H), 9.67 (s, 2H). ESI−MS: m/z = 531.00 $(M-Na)^+$.

Synthesis of (2-{4-[3-(2-tert-Butoxycarbonylaminoethylcarbamoyl)-1-cyano-1-methylpropylazo]-4-cyano-4-methylbutyrylamino}-ethyl)carbamic Acid tert-Butyl Ester (INI-(NH-Boc)$_2$). The INI-$(NH\text{-}Boc)_2$ was prepared by the reaction of INI-$(COOH)_2$ (0.8 g, 2.85 mmol) with *N*-Boc-1,2-diaminoethane (1.0 g, 6.2 mmol) in the presence of EDC (1.3 g, 6.85 mmol). The final product was obtained by recrystallization from ethyl acetate/diethyl ether (1:1). Yield: 1.0 g (62%). HPLC analysis gave two peaks at 220 nm with retention times of 9.26 and 9.36 min. Anal.: Calcd/found: C, 55.30/55.45; H, 7.85/7.66; N, 19.84/19.51. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 1.36 (s, 18, H), 1.67 (s, 6H), 2.17−2.19 (m, 4H), 2.24−2.28 (m, 4H), 2.95−2.99 (m, 4H), 3.02−3.05 (m, 4H), 6.76−6.80 (t, 2H), 7.96−8.00 (t, 2H). ESI−MS: m/z = 587.00 $(M-Na)^+$.

Synthesis of Dithiobenzoic Acid 1-Cyano-1-methyl-4-oxo-4-(2-thioxothiazolidin-3-yl)butyl Ester (CTA-TT). The CTA-TT was synthesized according to the literature.[36] A solution of INI-$(TT)_2$ (0.95 g, 1.96 mmol) and bis(thiobenzoyl) disulfide (0.5 g, 1.63 mmol) in ethyl acetate (250 mL) was heated at 80 °C for 6 h. After cooling, the reaction mixture was concentrated and purified by column chromatography (silica gel 60; 200 μm) using ethyl acetate/hexane (1/1, v/v) as eluent. Yield: 0.44 g (56%). HPLC analysis gave a single peak at 305 nm with a retention time of 9.96 min. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 1.84 (s, 3H), 2.45−2.51 (m, 2H), 3.30−3.31 (m, 2H), 3.42−3.44 (m, 2H), 4.42−4.44 (m, 2H), 7.41−7.44 (m, 4H), 7.60−7.62 (m, 2H), 7.81−7.83 (m, 4H). ESI−MS: m/z = 380.92 $(M-Na)^+$.

Synthesis of 2-(4-Cyano-4-methyl-4-thiobezoylsulfanylbutyrylamino)propionic Acid Methyl Ester (CTA-Ala-OMe). The Ala-OMe·HCl (0.22 g, 1.57 mmol) was dissolved in dichloromethane (10 mL) in the presence of DIPEA (0.27 mL, 1.57 mmol) and CTA-COOH (0.4 g, 1.43 mmol) was added to the solution. Then EDC (0.39 g, 2.05 mmol) was added in several portions and reaction mixture was stirred at room temperature for 3 h. Reaction mixture was extracted 3 times with distilled water (15 mL) and with 2 wt % solution of $NaHCO_3$ in water. Organic layer was dried with Na_2SO_4. Pure product was obtained after purification on Silicagel column using ethyl acetate as a mobile phase. The red colored fraction was separated and ethyl acetate was evaporated. The red oily product was obtained. Yield: 0.34 g (47%). HPLC analysis gave a single peak at 305 nm with a retention time of 9.26 min. ^{1}H NMR 300 MHz (DMSO, 296 K) ppm: 1.19−1.94 (m, 3H), 1.83 (s, 3H), 2.33−2.35 (m, 2H), 2.37−2.40 (m, 2H), 3.53 (s, 3H), 4.17−4.19 (m, 1H), 7.40−7.44 (m, 4H), 7.60−7.62 (m, 2H), 7.82−7.84 (m, 4H), 8.41 (m, 1H); ESI−MS: m/z = 387.00 $(M-Na)^+$.

The procedure described above for the synthesis of chain transfer agent CTA-Ala-OMe was used for the synthesis of all other CTAs containing various functional groups (azide, propargyl, -NH-Boc and −NHNH-Boc). The structures of synthesized azo initiators and chain transfer agents are shown in the Supporting Information (Figure 1S).

Synthesis of Dithiobenzoic Acid 3-(3-Azidopropylcarbamoyl)-1-cyano-1-methylpropyl Ester (CTA-N_3). The CTA-N_3 was prepared by the reaction of CTA-COOH (0.2 g, 0.72 mmol) with 3-azido-propylamine (0.079 g, 0.79 mmol) in the presence of EDC (0.180 g, 0.95 mmol). Pure product was obtained after purification on silica gel column using ethyl acetate as a mobile phase. The red colored fraction was separated and ethyl acetate was evaporated. The red oily product was obtained. Yield: 0.34 g (47%). HPLC analysis gave a single peak at 305 nm with a retention time of 10.12 min. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 1.54−1.59 (m, 2H), 1.83 (s, 3H), 2.30−2.32 (m, 2H), 2.34−2.40 (m, 2H), 3.02−3.05 (m, 2H), 3.21−3.26 (m, 2H), 7.40−7.44 (m, 4H), 7.60−7.62 (m, 2H), 7.79−7.84 (m, 4H), 8.00 (t, 1H). ESI−MS: m/z = 384.08 $(M-Na)^+$.

Synthesis of Dithiobenzoic Acid 1-Cyano-1-methyl-3-prop-2-ynylcarbamoylpropyl Ester (CTA-propargyl). CTA-propargyl was prepared by the reaction of CTA-COOH (0.4 g, 1.43 mmol) with 3-amino-1-propyne (0.087 g, 1.58 mmol) in the presence of EDC (0.392 g, 2.05 mmol). Pure product was obtained after crystallization from mixture ethyl acetate/hexane (1/1 v/v). Yield: 0.18 g (40%). HPLC analysis gave a single peak at 305 nm with a retention time of 11.22 min. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm:1.90 (s, 3H), 2.43−2.45 (m, 2H), 2.46−2.49 (m, 2H), 3.10−3.11 (t, 1H), 3.85−3.88 (m, 2H), 7.48−7.53 (m, 4H), 7.66−7.71 (m, 2H), 7.89−7.92 (m, 4H), 8.46−8.50 (t, 1H). ESI−MS: m/z = 339.00 $(M-Na)^+$.

Synthesis of Dithiobenzoic Acid 3-(2-tert-Butoxycarbonylaminoethylcarbamoyl)-1-cyano-1-methylpropyl Ester (CTA-NH-Boc). The CTA-NH-Boc was prepared by the reaction of CTA-COOH (0.37 g, 1.34 mmol) with *N*-Boc-1,2-diaminoethane (0.24 g, 1.47 mmol) in the presence EDC (0.31 g, 1.6 mmol). Pure product was obtained after purification on silica gel column using ethyl acetate/hexane (3/1 v/v) as a mobile phase. Yield: 0.28 g (48%). HPLC analysis gave a single peak at 305 nm with a retention time of 10.08 min. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 1.37 (s, 9H), 1.90 (s, 3H), 2.35−2.40 (m, 2H), 2.41−2.46 (m, 2H), 2.96−3.00 (m, 2H), 3.04−3.08 (m, 2H), 6.77−6.80 (t, 1H), 7.48−7.53 (m, 4H), 7.66−7.85 (m, 2H), 7.89−7.92 (m, 4H), 8.03 (t, 1H). ESI−MS: m/z = 444.00 $(M-Na)^+$.

Synthesis of Dithiobenzoic Acid 4-(N′-tert-Butoxycarbonylhydrazino)-1-cyano-1-methyl-4-oxobutyl Ester (CTA-NHNH-Boc). The CTA-NHNH-Boc was prepared by the reaction of CTA-COOH (0.4 g, 1.43 mmol) with Boc-hydrazide (0.21 g, 1.57 mmol) in the presence of EDC (0.36 g, 1.89 mmol). Pure product was obtained after purification on silica gel column using ethyl acetate/hexane (5/1 v/v) as a mobile phase. Yield: 0.31 g (55%). HPLC analysis gave a single peak at 305 nm with a retention time of 10.08 min. ^{1}H NMR 300 MHz (DMSO, 296 K), ppm: 1.39 (s, 9H), 1.90 (s, 3H), 2.38−2.41 (m, 2H), 2.43−2.44 (m, 2H), 7.48−7.53 (m, 4H), 7.66−7.71 (m, 2H), 7.88−7.94 (m, 4H), 8.76 (t, 1H), 9.71 (t, 1H). ESI−MS: m/z = 415.90 $(M-Na)^+$.

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Table 1. Characteristics of pHPMAs Synthesized by RAFT Polymerization of HPMA: Influence of the Ratio of HPMA:CTA on the Structure of the α-End Chain Group[a]

HPMA:CTA:INI, mol	Ala:Val monomer mixture, mol %	Ala:Val polymer composition, mol %	M_n Ala/Val, g/mol	M_n SEC, g/mol	$Đ$	conversion, %
100:2:1	50:50	75.6:24.4	7 900	8 300	1.11	66.1
200:2:1	50:50	73.6:26.4	13 600	15 300	1.10	68.8
300:2:1	50:50	73.1:26.9	20 600	23 200	1.07	73.8
400:2:1	50:50	70.7:29.3	27 800	29 900	1.12	57.3
500:2:1	50:50	72.0:28.0	33 600	36 600	1.04	80.7
600:2:1	50:50	70.5:29.5	42 100	47 700	1.03	73.4

[a]CTA-Ala-OMe:INI-$(Val\text{-}OMe)_2$ = 2:1, *t*-BuOH, 70 °C, 16 h.

Synthesis and Characterization of Polymers. The RAFT polymerization of HPMA was studied in *t*-BuOH at temperature range 60−80 °C for 2−16 h. The ratio of monomer (M):CTA was in a range from 100:1 to 600:1 and the ratio of CTA:INI was in the range from 2:1 to 2:0.3. Example of polymerization (ratio M:CTA:INI = 200:2:1):HPMA (0.3 g, 2.1 mmol), CTA-Ala-OMe (7.64 mg, 2.09×10^{-2} mmol) and INI-$(Val\text{-}OMe)_2$ (5.3 mg, 1.05×10^{-2} mmol) were dissolved in *t*-BuOH (2.33 mL). The solution was inserted into the ampule and bubbled with argon for 10 min. Ampule was sealed and polymerization was carried out at 70 °C for 16 h. Polymer was isolated by precipitation into the mixture acetone/diethyl ether (3:1), filtered off and dried in vacuum. Yield: 0.259 g (84.2%). The ω-end dithiobenzoate group (DTB) was removed by a method described by Perrier.[11]

Synthesis of Polymer with Thiazolidine-2-thione Group at the Chain α-End. Two HPMA polymers containing the TT reactive group at the chain α-end were prepared by RAFT polymerization. The ratio of M:CTA-TT:INI = 400:2:1 was used. To compare the influence of azo initiator, polymerizations were initiated with INI-$(TT)_2$ or INI-$(COOH)_2$.

HPMA (0.15 g, 1.05 mmol), CTA-TT (1.99 mg, 5.24×10^{-6} mmol) and INI-$(TT)_2$ (1.26 mg, 2.62×10^{-6} mmol) were dissolved in *t*-BuOH (1.16 mL). The solution was inserted into the ampule and bubbled with argon for 10 min. Ampule was sealed and polymerization was carried out at 70 °C for 16 h. Polymer was isolated by precipitation into the mixture acetone diethyl ether (3:1), filtered off and dried in vacuum. Yield: 0.043 g (29%).

HPMA (0.15 g, 1.05 mmol), CTA-TT (1.99 mg, 5.24×10^{-6} mmol) and INI-$(COOH)_2$ (0.58 mg, 2.62×10^{-6} mmol) were dissolved in *t*-BuOH (1.16 mL). The solution was inserted into the ampule and bubbled with argon for 10 min. The ampule was sealed and polymerization was carried out at 70 °C for 16 h. Polymer was isolated by precipitation into the mixture acetone/diethyl ether (3:1), filtered off and dried in vacuum. Yield: 0.023 g (15%). The DTB ω-end group was removed by the Perrier method using AIBN.[11] The polymers were characterized by molecular weight, molecular weight distribution, and content of TT groups.

Synthesis of Polymer with Amino Group at the Chain α-End. Two HPMA polymers containing the amino group at the chain α-end were prepared by the same method as described above in the presence of CTA-NH-Boc/INI-$(COOH)_2$ and CTA-NH-Boc/INI-$(NH\text{-}Boc)_2$. The Boc protecting group was removed by TFA and the content of amino group was determined by TNBSA.

RESULTS AND DISCUSSION

The synthesis of semitelechelic polymers based on HPMA with well-defined functional groups at the α-end of the chain by RAFT polymerization was studied. *t*-BuOH was selected as the polymerization solvent accommodating best the requirements for RAFT polymerization of HPMA. We focused on the effect of the structure of the azo initiator, composition of initiation system and composition of polymerization mixture on the structure and functionality of the α-end of the polymer chain of pHPMA during RAFT polymerization. The chain transfer agent 4-cyano-4-(thiobenzoylthio)pentanoylalanine methyl ester (CTA-Ala-OMe) and initiators 4,4′-azobis(4-cyanopentanoyl-valine methyl ester or leucine methyl ester) (INI-$(Val\text{-}OMe)_2$ or INI-$(Leu\text{-}OMe)_2$) were synthesized and used for RAFT polymerization of HPMA. Amino acids Val, Leu, and Ala were used as effective labels of INI a CTA residues incorporated into polymer chain at its α-end. Amino acid analysis was found to be sufficiently sensitive method for the analysis of the α-end structure and functionality of the polymer chain. The influence of the HPMA:CTA-Ala-OMe ratio on the structure of the α-end chain group was studied using a constant 2:1 ratio of CTA-Ala-OMe:INI-$(Val\text{-}OMe)_2$ which was found to be optimal to achieve high yield of polymerization and narrow molecular weight distribution. The results are summarized in Table 1, and the SEC record of polymers is presented in Figure 1. These

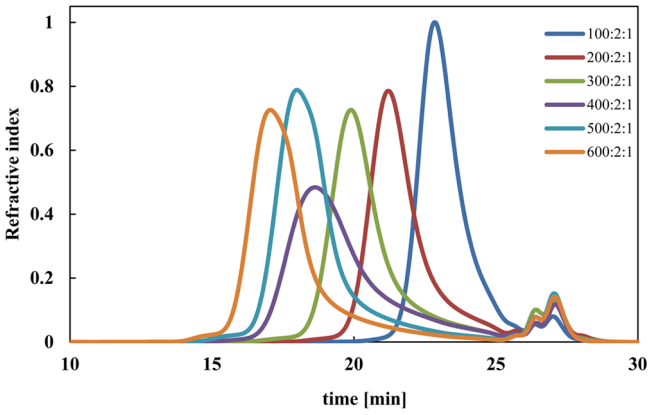

Figure 1. SEC chromatograms of pHPMAs prepared by RAFT polymerization: Influence of the ratio of HPMA:CTA on the molecular weight. CTA-Ala-OMe:INI-$(Val\text{-}OMe)_2$ = 2:1, *t*-BuOH, 70 °C, 16 h.

results show that the ratio of HPMA:CTA-Ala-OMe controls the molecular weight of the resulting pHPMA, which is in agreement with the theory of RAFT polymerization.[1,37,38] Molecular weight distributions (polydispersities, $Đ$) of all the polymers were narrow, with $Đ$ equal or below 1.22 (see Table 1 − 5). The amino acid analysis of pHPMA proved that 70 to 75% of the pHPMA chains were at the α-end terminating in a residue coming from CTA (containing Ala) and that 25 to 30% of pHPMA chains were at the α-end terminating in a residue coming from INI (containing Val).

The number-average molecular weight M_n determined by SEC is in close agreement with the M_n calculated from the content of Ala and Val in the pHPMA.

To verify whether the structure of the INI and the CTA:INI ratio influences the RAFT polymerization and composition of the α-end chain group of pHPMAs, the properties of the pHPMAs obtained by RAFT polymerization using a combina-

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Table 2. Characteristics of pHPMAs Synthesized by RAFT Polymerization of HPMA: Influence of the Ratio of CTA-Ala-OMe:INI-(Val-OMe)$_2$ on the Structure of the α-End Chain Group[a]

M:CTA:INI, mol	Ala:Val monomer mixture, mol %	Ala:Val polymer composition, mol %	M_n Ala/Val, g/mol	M_n SEC, g/mol	$Đ$	conversion, %
200:2:1	50:50	72.4:27.6	15 200	15 600	1.22	84.2
200:2:0.8	55.6:44.4	76.6:23.4	14 800	15 000	1.19	79.3
200:2:0.5	66.7:33.3	82.2:17.8	13 900	14 600	1.13	70.9
200:2:0.3	76.9:23.1	88.5:11.5	10 900	11 200	1.10	49.4

[a]HPMA:CTA-Ala-OMe = 200:2, *t*-BuOH, 70 °C, 16 h.

Table 3. Characteristics of pHPMAs Synthesized by RAFT Polymerization of HPMA: Influence of the Ratio of CTA-Ala-OMe:INI-(Leu-OMe)$_2$ on the Structure of the α-End Chain Group[a]

M:CTA:INI, mol	Ala:Leu monomer mixture, mol %	Ala:Leu polymer composition, mol %	M_n Ala/Leu, g/mol	M_n SEC, g/mol	$Đ$	conversion, %
200:2:1	50:50	67.8:32.2	16 200	17 100	1.16	87.8
200:2:0.8	55.6:44.4	72.5:27.5	14 700	16 300	1.14	85.5
200:2:0.5	66.7:33.3	78.5:21.5	14 400	16 000	1.09	74.7
200:2:0.3	76.9:23.1	86.7:13.3	13 000	13 000	1.05	54.6

[a]HPMA:CTA-Ala-OMe = 200:2, *t*-BuOH, 70 °C, 16 h.

Table 4. Characteristics of pHPMAs Prepared by RAFT Polymerization of HPMA: Effect of Temperature on the Structure of the α-End Chain Groups of pHPMAs[a]

temperature, °C	Ala:Val monomer mixture, mol %	Ala:Val polymer composition, mol %	M_n Ala/Val, g/mol	M_n SEC, g/mol	$Đ$	conversion, %
60	66.7:33.3	89.6:10.4	13 600	15 160	1.12	58.2
70	66.7:33.3	82.2:17.8	13 900	14 600	1.13	70.9
80	66.7:33.3	79.0:21.0	10 200	11 040	1.17	46.8

[a]HPMA:CTA-Ala-OMe = 200:2, CTA-Ala-OMe:INI-(Val-OMe)$_2$ = 2:0.5, *t*-BuOH, 16 h.

Table 5. Characteristics of pHPMAs Prepared by RAFT Polymerization of HPMA: Influence of the Polymerization Time on the Structure of the α-End Chain Group[a]

polymerization time, h	Ala:Val monomer mixture, mol %	Ala:Val polymer composition mol %	M_n Ala/Val, g/mol	M_n SEC, g/mol	$Đ$	conversion, %
2	66.7:33.3	95.5:4.5	4 800	4 710	1.08	7.2
3	66.7:33.3	91.7:8.3	6 100	7 040	1.04	24.1
4	66.7:33.3	88.2:11.8	8 700	9 470	1.10	39.7
6	66.7:33.3	86.5:13.5	11 300	12 940	1.10	52.3
16	66.7:33.3	82.2:17.8	13 900	14 600	1.13	70.9

[a]HPMA:CTA-Ala-OMe = 200:2, CTA-Ala-OMe:INI-(Val-OMe)$_2$ = 2:0.5, *t*-BuOH, 70 °C.

tion of CTA-Ala-OMe/INI-(Val-OMe)$_2$ or CTA-Ala-OMe/INI-(Leu-OMe)$_2$ was studied; the results are presented in Table 2 and Table 3.

The results summarized in Table 2 and Table 3 demonstrate that the use of azo initiators with different structures (INI-(Val-OMe)$_2$ or INI-(Leu-OMe)$_2$) under the same conditions of RAFT polymerization of HPMA give pHPMAs with comparable characteristics. Decreasing the ratio of CTA:INI from 2:1 to 2:0.3 decreases the percentage of pHPMA α-end chains terminated in an INI residue containing Val or Leu (decreasing from 32.2 to 11.5%) but also simultaneously decreases the conversion of polymerization. The constant ratio of HPMA:CTA-Ala-OMe controls the molecular weight of the pHPMA. The small decrease in the molecular weight of the pHPMAs can be attributed to the decrease in conversion.[2,39] The M_n determined by SEC is in good agreement with the M_n calculated from the content of the Ala and Val or Ala and Leu amino acids in the pHPMAs.

The effect of temperature on the structure of the α-end chain groups of pHPMAs at a constant ratio of HPMA:CTA-Ala-OMe and CTA-Ala-OMe:INI-(Val-OMe)$_2$ is shown in Table 4.

The percentage of the α-end chain groups terminating with INI (Val residues) increased with increasing temperature of the RAFT polymerization, rising from 10.4 (60 °C) to 21.0% (80 °C). This increase can be attributed to the higher concentration of radicals resulting from a higher rate of decomposition of the azo initiator at higher temperatures. The direct relationship between the conversion and temperature culminates at the polymerization temperature 70 °C; an increase in the polymerization temperature to 80 °C led to a decrease in the conversion. This limit may be attributable to increased rates of termination reactions or the instability of the CTA at higher temperatures. The influence of the polymerization time on conversion and the structure of the α-end chain groups of pHPMAs prepared at constant ratios of HPMA:CTA-Ala-OMe and CTA:INI-(Val-OMe)$_2$ is shown in Table 5.

The results summarized in Table 5 show a significant influence of the polymerization time and conversion on the structures of the α-end chain groups of pHPMAs. After 2 h of polymerization (conversion 7%) 95% of pHPMA chains contained a CTA residue (Ala) at the α-end, while at much higher conversion achieved after 16 h of polymerization (conversion 71%) the content of CTA residues (detected as Ala) decreased to 82.2%. The M_n increased with increasing polymerization time and conversion and when plotted against conversion, the obtained linear dependence indicated that the

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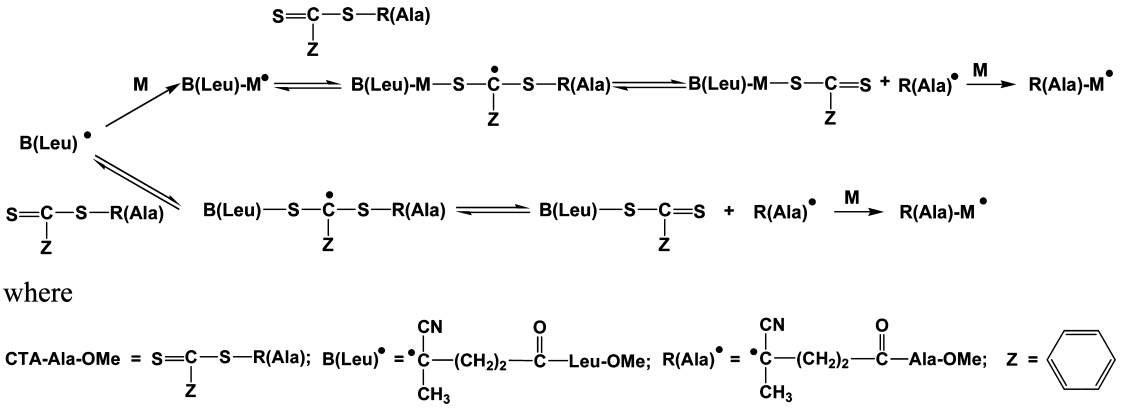
Scheme 1. Modified Scheme of CTA Activation/Initialization Process



where

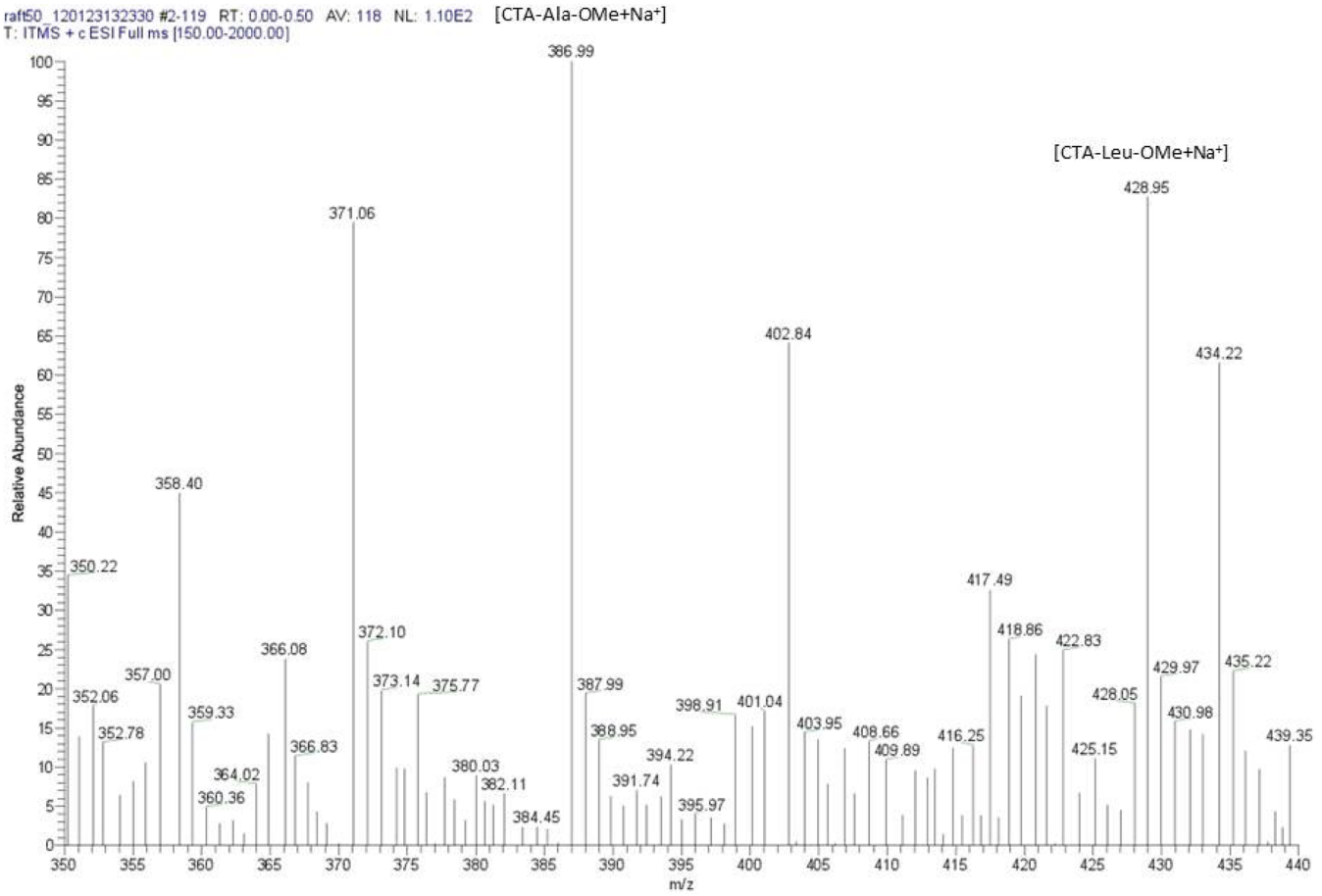


Figure 2. ESI−MS spectrum of the polymerization mixture HPMA:CTA-Ala-OMe = 100:2, CTA-Ala-OMe:INI-(Leu-OMe)$_2$ = 2:1, *t*-BuOH, polymerization time 50 min at 70 °C.

CTA provides adequate control over the polymerization process. M_n determined by SEC was again in good agreement with the M_n calculated from the content of Ala and Val in the pHPMA.

The obtained results can be explained by the CTA activation/initialization process, which was described in detail by Lowe.[7] The modified scheme of the CTA activation/initialization process adopted from Lowe's paper is shown in Scheme 1.

In the first step, the monomer (M) molecule adds to the primary radical B(Leu)• formed by decomposition of the azo initiator INI-(Leu-OMe)$_2$ to form the B(Leu)-M• adduct radical, or it may add to the RAFT agent CTA-Ala-OMe. The B(Leu)-M• radical can either add a new monomer and start to propagate or add to the RAFT agent CTA-Ala-OMe. Given the inherently high chain transfer constants of most RAFT agents, it is unlikely that more than a few monomers will add to the generated radical before adding to the RAFT agent CTA-Ala-OMe. Assuming that this forward fragmentation is favored, a new RAFT agent CTA-M-B(Leu) is formed, as well as the radical R(Ala)•. Provided that R(Ala)• is a good reinitiating species, R(Ala)• will add a new monomer and initiate the growth of the polymer chain R(Ala)-M•.

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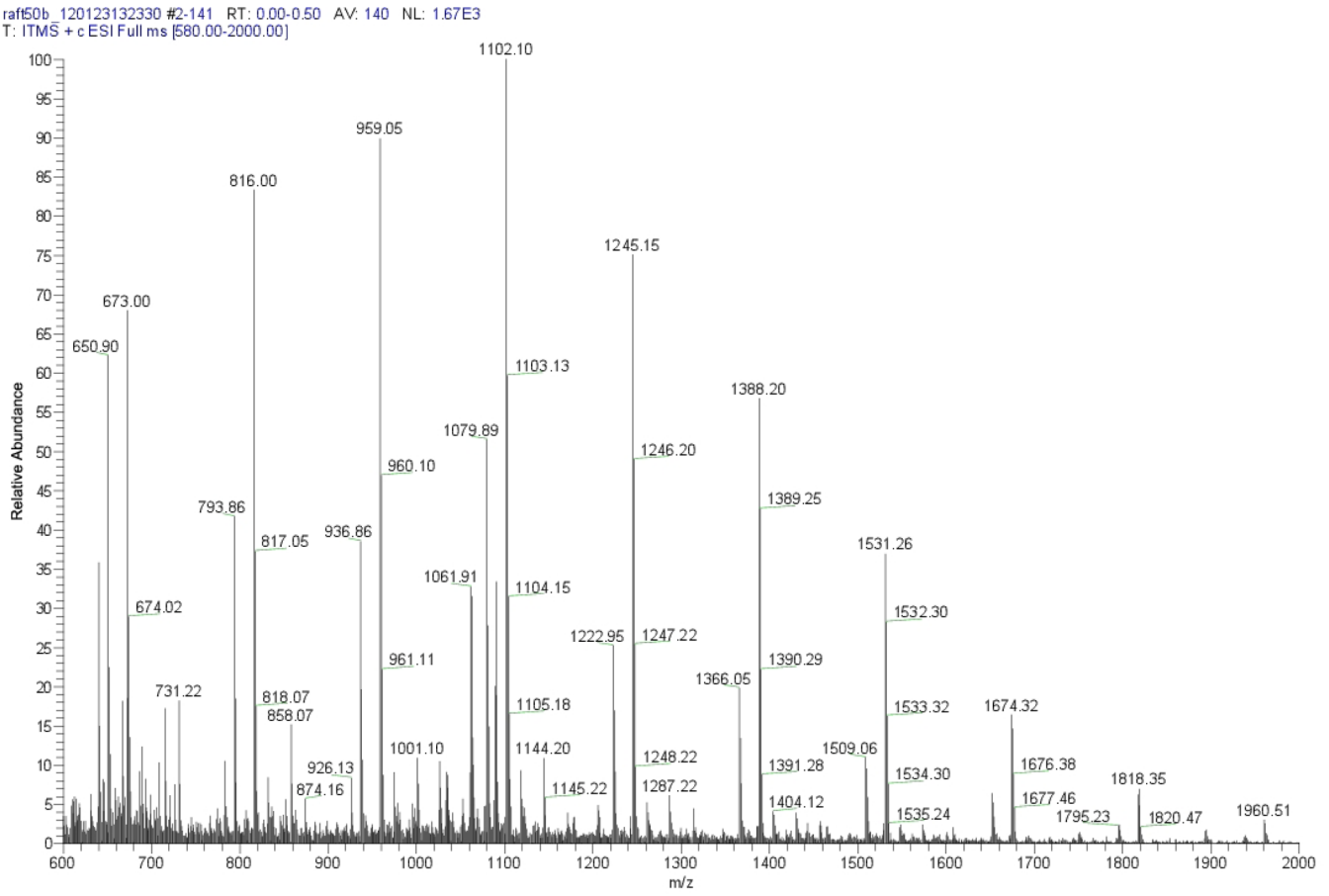

Figure 3. ESI−MS spectrum of the polymerization mixture HPMA:CTA-Ala-OMe = 100:2, CTA-Ala-OMe:INI-$(Leu\text{-}OMe)_2$ = 2:1, *t*-BuOH, after 50 min at 70 °C.

The second option for the primary radical B(Leu)• that forms from the azo initiator INI-$(Leu\text{-}OMe)_2$ is its direct addition to the RAFT agent CTA-Ala-OMe to yield the intermediate radical. This reaction is reversible, and fragmentation in the forward direction is the preferred pathway. To favor fragmentation, the formation of the new RAFT agent CTA-Leu-OMe and the radical R(Ala)• occurs, and ideally, R(Ala)• will again add the first monomer unit to initiate the growth of a new polymer chain R(Ala)-M•. This CTA activation/initialization process, with its many reversible steps, is fairly complex, and the formation of new RAFT agents CTA-M-Leu-OMe and CTA-Leu-OMe, which differ in their structures from CTA-Ala-OMe, influences the final structure of the α-end of the resulting polymer chain.

To confirm the formation of either CTA-M-Leu or CTA-Leu-OMe during the CTA activation/initiation process, we performed the RAFT polymerization of HPMA in the presence of CTA-Ala-OMe initiated with INI-$(Leu\text{-}OMe)_2$ (HPMA:CTA-Ala-OMe = 100:2, CTA-Ala-OMe:INI-$(Leu\text{-}OMe)_2$ = 2:1, *t*-BuOH, 70 °C) for 50 min and then analyzed the polymerization mixture by mass spectrometry (MS LCQ Fleet, Thermo Fisher Scientific). In Figure 2, the ESI−MS spectrum is shown, where the peak with m/z = 386.98 corresponds to $[CTA\text{-}Ala\text{-}OMe + Na]^+$ and the peak with m/z = 428.99 corresponds to the newly formed RAFT agent $[CTA\text{-}Leu\text{-}OMe + Na]^+$.

The ESI−MS spectrum presented in Figure 3 shows the peaks with m/z = 673.00, 816.00, 959.05, 1102.10, 1245.16, 1388.20, 1531.26, 1674.32, and 1818.36, which correspond to a pHPMA with the structure $[Ala\text{-}OMe\text{-}(HPMA)_n\text{-}DTB+Na]^+$ where n = 2−10, peaks with m/z = 650.90, 793.86, 936.86, 1079.89, 1222.95, 1366.05, and 1509.06, which correspond to a pHPMA with the structure $[Ala\text{-}OMe\text{-}(HPMA)_n\text{-}DTB+H]^+$ where n = 2−8, and peaks with m/z = 857.07, 1001.10, 1144.20, and 1287.23, which correspond to a pHPMA with the structure $[Leu\text{-}OMe\text{-}(HPMA)_n\text{-}DTB+Na]^+$ where n = 3−6.

On the basis of these results, it appears that the formation of RAFT agents with different structures during the polymerization can be avoided by using a CTA and an INI with the same functional group. For this purpose, a series of CTAs and azo initiators bearing various functional groups (azide, propargyl, thiazolidin-2-thione, -NH-Boc and -NHNH-Boc) were synthesized and used for the synthesis of semitelechelic α-end-functionalized pHPMAs by RAFT. The dependence of the M_n on the ratio of monomer:CTA for a series of CTA and azo initiators bearing the same functional groups was measured and is shown in Figure 4.

Figure 4 shows the dependence of the M_n of semitelechelic α-end-functionalized pHPMAs on the ratio of monomer:CTA prepared by RAFT polymerization using CTAs and INIs bearing the same functional groups. The resulting M_n of the semitelechelic pHPMA depends not only on the ratio of monomer:CTA but also on the structure of the functional group of the CTA and INI when the same functional group was used for both, INI and CTA. The combination CTA-COOH:INI-$(COOH)_2$ (i.e., both agents have COOH func-

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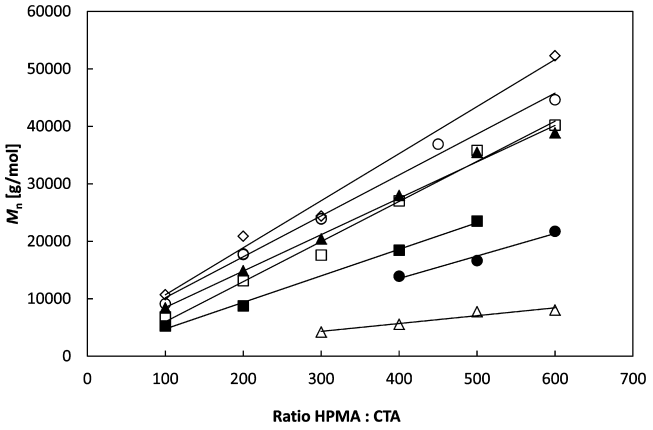

Figure 4. Dependence of the M_n on the ratio of HPMA:CTA. CTAs and INIs with the same functional groups were used. CTA:INI = 2:1, *t*-BuOH, 70 °C, 16 h. Key: (□) CTA/AIBN; (Δ) CTA-COOH/INI-$(COOH)_2$; (▲) CTA-NH-Boc/INI-$(NH\text{-}Boc)_2$; (■) CTA-TT/INI-$(TT)_2$; (●) CTA-propargyl/INI-$(propargyl)_2$; (◇) CTA-NHNH-Boc/INI-$(NHNH\text{-}Boc)_2$; (○) CTA-N_3/INI-$(N_3)_2$.

tional group) gave a semitelechelic pHPMA with a relatively low M_n. The combination of CTAs and INIs with Ala-OMe, -NH-Boc, -NHNH-Boc, -azide, and TT functional groups enables the synthesis of semitelechelic pHPMAs with well-defined structures of their α-end groups over a broad range of molecular weights that depend on the monomer:CTA ratio.

The results summarized in Table 6 show the comparison of a pHPMA prepared by RAFT polymerization in the presence of a CTA and an INI bearing the same TT functional group and a pHPMA prepared in the presence of CTA-TT and INI-$(COOH)_2$ groups, i.e., CTA and INI with diverse functional groups.

The functionality *F*, calculated as the ratio of M_n(SEC)/M_n(TT), for the pHPMA prepared by RAFT polymerization using CTA-TT/INI-$(COOH)_2$ (diverse groups) was 0.72, while the functionality of the pHPMA prepared by RAFT polymerization using CTA-TT/INI-$(TT)_2$ (for both the same TT group) was 0.96. A functionality value close to one means that all of the polymer chains contain in average one TT reactive group at the α-end. Similar results were obtained for the pHPMAs prepared by RAFT polymerization using CTA-NH-Boc/INI-$(COOH)_2$ and CTA-NH-Boc/INI-$(NH\text{-}Boc)_2$, which demonstrates the advantages of using CTAs and INIs containing the same functional groups. In this case, the absolute value of functionality was affected by error of indirect determination of NH_2 groups with TNBSA method (see characterization methods above). Important is the difference between values of CTA-NH-Boc/INI-$(COOH)_2$ and CTA-NH-Boc/INI-$(NH\text{-}Boc)_2$, which is very similar to difference of functionality values in the case of semitelechelic polymers with TT end groups.

CONCLUSION

A number of new azo initiators and RAFT chain transfer agents containing various functional groups were synthesized.

RAFT polymerization of HPMA using CTAs and INIs containing various functional groups allows for reasonable control of the molecular weights of the resulting polymers and generates polymers with narrow molecular weight distributions that terminate in functional groups that are useful for future syntheses of well-defined polymer drug carriers or for preparation of polymer-coated drug and gene delivery vectors. The temperature, composition of the initiation/chain transfer system and conversion of the reaction significantly influence the functionality of the α-end of the resulting polymer chain. The use of RAFT polymerization controlled by CTAs and INIs containing the same functional group enables the synthesis of semitelechelic polymers with very narrow molecular weight distributions and functional groups situated at the α-end of the polymer chain with functionality values very close to 1. Our recent results show that the conclusions mentioned above are fully applicable also to the synthesis of a significant number of HPMA copolymers.[30,31] This finding provides an opportunity for the design and synthesis of new well-defined HPMA copolymer carriers of various structures and architectures (linear, block and multiblock, grafted, micellar, star architectures) that are suitable for use as drug and gene delivery carriers or polymer−protein/glycoprotein conjugates.

ASSOCIATED CONTENT

Supporting Information

Structures of synthesized azo initiators and chain transfer agents (Figure 1S). This material is available free of charge via the Internet at http://pubs.acs.org/

AUTHOR INFORMATION

Corresponding Author

*E-mail: subr@imc.cas.cz. Telephone: +420 296 809 389. Fax: +420 296 809 410.

Notes

The authors declare no competing financial interest.

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Table 6. Characteristics of Polymers Prepared by RAFT Polymerization of HPMA: Influence of the Structure of CTA and INI on the Structure of the α-End Chain Group of the pHPMA[a]

CTA/INI	M_n SEC, g/mol	M_n TT, g/mol	conversion, %	*F* TT
CTA-TT/INI-$(COOH)_2$	12 260	17 100	15.0	0.72
CTA-TT/INI-$(TT)_2$	14 350	14 880	29.0	0.96
CTA/INI	M_n SEC, g/mol	M_n NH_2, g/mol	conversion, %	*F* NH_2
CTA-NH-Boc/INI-$(COOH)_2$	12 400	14 000	27.0	0.89
CTA-NH-Boc/INI-$(NH\text{-}Boc)_2$	14 400	12 300	30.0	1.18

[a]HPMA:CTA:INI = 400:2:1, *t*-BuOH, 70 °C, 16 h.

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