Nitroxide-Mediated Polymerization of *N*-Isopropylacrylamide: Electrospray Ionization Mass Spectrometry, Matrix-Assisted Laser Desorption Ionization Mass Spectrometry, and Multiple-Angle Laser Light Scattering Studies on Nitroxide-Terminated Poly-*N*-isopropylacrylamides

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ABSTRACT: Nitroxide-mediated controlled living free radical polymerization of *N*-isopropylacrylamide using highly sterically hindered 2,2,6,6-tetraethylpiperidin-4-on-*N*-oxyl **1** is described. In addition, an improved synthesis for nitroxide **1** is presented. Poly-*N*-isopropylacrylamides (PNIPAMs) prepared are analyzed by multiple-angle laser light scattering. Moreover, the nitroxide-terminated PNIPAMs are characterized using electrospray ionization mass spectrometry, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and Fourier transform ion cyclotron MALDI-MS. Careful MS analysis reveals that chain-end degradation of nitroxide-terminated PNIPAMs occurs during MALDI analysis. A mechanism for chain end degradation is presented.

Introduction

Poly-*N*-isopropylacrylamide (PNIPAM) is a highly interesting polymer that in aqueous solution undergoes phase separation upon temperature increase.[1,2] The transition from a hydrophilic hydrated to a hydrophobic phase-separated state occurs at the lower critical solution temperature (LCST). PNIPAM is currently one of the most popular polymers investigated. Numerous publications on PNIPAM appeared during the last 5 years.[3] Many applications using PNIPAM for the construction of thermoresponsive smart materials have been published.[4] PNIPAM is generally prepared via free radical polymerization of *N*-isopropylacrylamide (NIPAM). However, classical radical polymerization techniques do not allow the control of the molecular weight of PNIPAM. Moreover, broad polydispersities are obtained. For sophisticated PNIPAM-containing materials, however, defined molecular weight and narrow polydispersities of PNIPAM are highly desirable.

Controlled free radical polymerization techniques have been intensively investigated during the past few years. Nitroxide-mediated polymerizations (NMP),[5] atom transfer radical polymerizations (ATRP),[6] and reversible addition fragmentation chain transfer (RAFT) polymerizations[7] among others are radical polymerization techniques that allow the preparation of polymers with defined molecular weight and narrow polydispersities. Whereas for ATRP[8] and RAFT[9,10] several papers on the controlled radical polymerization of PNIPAM were published, only a single example on a successful nitroxide-mediated radical polymerization of NIPAM has appeared in the literature to date.[11] Systematic studies on NMP of NIPAM are unknown. Herein we present results on successful controlled radical polymerization of NIPAM using our recently introduced alkoxyamine **2**, which is readily prepared from the highly sterically hindered nitroxide **1** (Figure 1).[12] Moreover, we present an improved synthesis for nitroxide **1**. Careful electrospray ionization mass spectrometry (ESI-MS), matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and Fourier transform ion cyclotron MALDI-MS (FTICR-MALDI-MS) analyses have been used for the characterization of nitroxide-terminated PNIPAMs.[13] In addition, we will show that multiple-angle laser light scattering (MALLS) is superior to classical gel permeation chromatography (GPC) analysis to determine the molecular weight of PNIPAM.

Experimental Section

Materials. 2-Ethylbutene (Aldrich, 95%), chlorosulfonyl isocyanate (Aldrich, 98%), *N*,*N*-(dimethylamino)pyridine (Fluka, ≥99%), di-*tert*-butyl dicarbonate (Acros, 99%), methylmagnesium bromide (Aldrich, 3 M in Et_2O), trifluoroacetic acid (Merck, ≥ 99%), and diethyl ketone (Aldrich, 99+%) were used as purchased. *N*-Isopropyl acrylamide (Aldrich, 99+%) was recrystallized twice from *n*-hexane to remove the stabilizer. Et_2O and THF were distilled over K/Na, benzene was distilled over sodium, and CH_2Cl_2 was distilled over CaH_2 before use. All other chemicals were used as received.

General. ^{1}H NMR (500 MHz, 300 MHz, 200 MHz) and ^{13}C NMR (125 MHz, 75 MHz, 50 MHz) spectra were recorded on an AMX 500 (Bruker), ARX 300 (Bruker), or ARX 200 (Bruker). Chemical shifts δ in ppm are relative to $SiMe_4$ as an internal standard. Thin-layer chromatography: silica gel 60 F_{254} plates (Merck); detection with UV or dipping into a solution of $KMnO_4$ (1.5 g in 333 mL of 1 M NaOH) or a solution of $Ce(SO_4)_2 \cdot H_2O$ (10 g), phosphormolybdic acid hydrate (25 g), concentrated H_2SO_4 (60 mL), and H_2O (940 mL), followed by heating. Flash chromatography (FC): silica gel 60 (40−63 μm, Merck or Fluka), at ca. 0.4 bar. Melting points: 510 apparatus (Büchi); uncorrected. IR spectra were recorded on an IR 750

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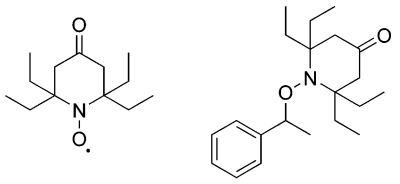


1 2

Figure 1. Nitroxide **1** and alkoxyamine **2** used in the present study.

(Nicolet Magna) or a IFS-200 (Bruker). Size-exclusion chromatography (SEC) was carried out with tetrahydrofuran (THF) or *N*,*N*-dimethylformamide (DMF) as eluents at a flow rate of 1.0 mL/minute at room temperature on a system consisting of a L-6200A Intelligent Pump (*Merck Hitachi*), a set of two PLgel 5-μm MIXED-C columns (300 × 7.5 mm, Polymer Laboratories, linear range of molecular weight: 200−2 × 10^6 g/mol), and a RI-101 detector (Shodex, halogen lamp) or a Knauer differential refractometer ($\lambda = 950 \pm 30$ nm) detector. Data were acquired through a PL Datastream unit (Polymer Laboratories) and analyzed with Cirrus GPC software (Polymer Laboratories) or with PSS WinGPC compact V 7.20 software based upon calibration curves built upon polystyrene standards (Polymer Laboratories Polystyrene Medium MW Calibration Kit S-M-10) with peak molecular weights ranging from 500 to 3 × 10^6 g/mol.

For MALDI-MS, 2,5-dihydroxybenzoic acid (DHB) (Aldrich) was used as the matrix and $NaBF_4$ (Aldrich) was added to improve ionization. The samples were prepared by mixing THF solutions of the polymer and matrix (50 mg/mL) with saturated methanolic $NaBF_4$ in a typical ratio of 1:50:2 (w/w/w, polymer/matrix/salt).

Mass spectra were measured with: Quattro LCZ (Waters-Micromass) electrospray mass spectrometer with nanospray inlet; Reflex IV (Bruker) MALDI-TOF mass spectrometer; APEX III (Bruker) FT-ICR mass spectrometer used for exact mass determination with MALDI. Scans (250) were added, each scan contained the ions of 3 laser shots which were collected within the hexapole and transferred to the ICR cell after cooling with Ar atoms. The data aquisition used 256k datapoints/scan.

For ESI measurements, the samples were diluted in methanol (approximately 0.05 mg/mL) and sprayed with a nanospray needle with internal contact. The capillary and cone voltage was adjusted for maximum signal intensity (typical values 1.3 kV, respectively, 35 V). MALDI samples where prepared by mixing equal volumes of solutions of 1 mg/mL polymer in THF and 10 mg/mL of DHB containing 10 μL of a saturated methanolic solution of $NaBF_4$. The mixture (1 μL) was applied to the target and gently evaporated. Spectra (100−200) were taken on different locations of the sample spot and added. Laser intensity was adjusted as low as possible except for the decomposition experiment.

MALLS was performed with a PSS SLD 7000 (laser: P = 30 mW, $\lambda = 660$ nm, angles = 35, 50, 75, 105, 130, 145°) at a cell temperature of about 23 °C. The light-scattering detector was used in combination with the GPC apparatus described above, and the concentration detector was thus a Knauer differential refractometer ($\lambda = 950 \pm 30$ nm). The PNIPAM samples were dissolved in THF at a concentration of 0.8−1.7 mmol/L (referring to $M_{n,theor.}$). The refractive index increment used (dn/dc = 0.0942 ± 0.00041 mL/g) was determined by Polymer Standards Service (PSS) in Mainz (Germany) using a PSS dn/dc 2010 Ablenkrefraktometer. The samples were dissolved in THF (1−8 g/L), and the measurements were performed at a temperature of 30 °C using a laser with a wavelength of 620 nm.

4,4-Diethylazetidin-2-one (3). 2-Ethylbutene (10 mL, 82 mmol) was dropwise added at room temperature (RT) (syringe pump) over a period of 1 h to a solution of chlorosulfonyl isocyanate (7.1 mL, 82 mmol) in ether (40 mL). After it was stirred for an additional hour at RT, a saturated solution of Na_2SO_3 (80 mL) was added and the mixture was stirred for 2 h at RT. Solid $NaHCO_3$ was added to adjust the pH approximately to a value of 7. The heterogenic mixture was stirred for another 30 min. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 times). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure. Drying in vacuo afforded **3** (7.80 g, 75%) as a pale-yellow viscous oil. IR (film): 3240w (NH), 2967s, 2939m, 2880m (CH), 1751w (C=O), 1460m, 1415m, 1374m, 1116m, 948m, 665m cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 6.75 (bs, 1 H, NH), 2.63 (s, 2 H, CH_2CO), 1.70 (q, J = 7.5 Hz, 4 H, CH_2CH_3), 0.93 (t, J = 7.5 Hz, 6 H, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.3 (CO), 148.3 (C), 43.7 (CH_2CO), 29.2 (CH_2CH_3), 28.2 (CH_2CH_3), 8.4 (CH_2CH_3). MS (EI): 128 (2, $[M + H]^+$), 98 (20), 84 (67), 69 (45), 56 (100), 55 (23), 43 (16), 42 (13), 41 (23), 28 (22). HRMS (EI) calcd for $C_7H_{13}NO$ (M^+): 127.0997. Found: 127.1002.

***N*-(*tert*-Butoxycarbonyl)-4,4-diethylazetidin-2-one (4).** The β-lactam **3** (7.66 g, 60.2 mmol) was dissolved in CH_2Cl_2 (55 mL), and NEt_3 (10.2 mL, 72.3 mmol) and DMAP (736 mg, 6.02 mmol) were added. A solution of di-*tert*-butyl dicarbonate (15.8 g, 72.3 mmol) in CH_2Cl_2 (20 mL) was added, and the reaction mixture was stirred at RT overnight. The mixture was washed with saturated NH_4Cl, H_2O, and brine. The organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure. Purification by flash chromatography (FC) (pentane/methyl-*tert*-butyl ether (MTBE), 8:2) afforded **4** (13.42 g, 98%) as a pale-yellow viscous oil. IR (film): 2971s, 2939m (CH), 1807s (C=O, BOC), 1718s (C=O, lactam), 1332w, 1254m, 1162s, 1094m, 1054m (C−O), 870m, 776m cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 2.72 (*s*, 2 H, CH_2CO), 1.89 (*q*, J = 7.5 Hz, 4 H, CH_2CH_3), 1.51 (*s*, 9 H, $C(CH_3)_3$), 0.95 (*t*, J = 7.5 Hz, 6 H, CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.6 (CO), 155.2 (CO), 85.9 (C), 58.3 (C), 46.5 (CH_2CO), 29.9 ($C(CH_3)_3$), 29.2 (CH_2CH_3), 9.0 (CH_2CH_3). MS (ESI): 250 (82, $[M + Na]^+$), 226 (20), 194 (100), 182 (28), 150 (52). HRMS (ESI) calcd for $C_{12}H_{21}NO_3$ (M^+): 227.1521. Found: 227.1523.

1,1-Dimethylethyl ester-(1,1-diethyl-3-oxobutyl)-carbamic acid (5). β-Lactam **4** (16.35 g, 71.93 mmol) was dissolved under argon in THF (250 mL), and the solution was cooled to −40 °C. A solution of methylmagnesium bromide (3 M in Et_2O, 31.20 mL, 93.51 mmol) was slowly added, and the reaction mixture was stirred for 2 h at −40 °C. Saturated NH_4Cl was added, and after warming to RT, the mixture was extracted with CH_2Cl_2 (2 times). The combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification by FC (pentane/MTBE 19:1) afforded **5** (14.89 g, 85%) as a colorless oil. IR (film): 3365w (NH), 2973s, 2939m, 2882m (CH), 1716s (C=O), 1506s, 1457m, 1366m, 1250m, 1170s, 1088m (C−O), 784m cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 4.66 (bs, 1 H, NH), 2.86 (s, 2 H, CH_2CO), 2.15 (s, 3 H, $C(O)CH_3$), 1.91−1.52 (m, 4 H, 2 × CH_2CH_3), 1.42 (s, 9H, $C(CH_3)_3$), 0.81 (t, J = 7.5 Hz, 6 H, 2 × CH_2CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 210.2 ($C(O)CH_3$), 156.4 ($C(O)C(CH_3)_3$), 80.6 (C), 59.0 (C), 48.8 (CH_2CO), 33.8 ($C(O)CH_3$), 30.2 ($C(CH_3)_3$), 29.6 (CH_2CH_3), 9.4 (CH_2CH_3). MS (ESI): 266 (60, $[M + Na]^+$), 250 (22), 210 (100), 194 (20), 166 (93), 140 (30), 108 (48). HRMS (ESI) calcd for $C_{13}H_{25}NO_3$ (M^+): 243.1834. Found: 243.1832.

2,2,6,6-Tetraethyl-1,2,5,6-tetrahydro-4-methylpyrimidine (6). The BOC-protected β-aminoketone **5** (5.61 g, 23.1 mmol) was dissolved in CH_2Cl_2 (11.4 mL). Trifluoroacetic acid (11.4 mL, 148 mmol) was added, and the reaction mixture was stirred for 2 h at RT. The liquids were removed in vacuo at 60 °C to afford the deprotected β-aminoketone as the trifluoroacetate salt as a viscous oil. The salt was dissolved in MeOH (11.0 mL), and diethyl ketone (12.2 mL, 115 mmol) was added. The solution was cooled to −78 °C, liquid ammonia (~30 mL) was added, and the reaction mixture was stirred at RT overnight in a sealed glass tube. The sealed glass tube was opened at −78 °C, and the mixture was allowed to warm to RT with stirring in order to remove ammonia. The residue was dissolved in CH_2Cl_2, and the solution was washed with saturated $NaHCO_3$ (2 times). The organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure. Purification by FC (CH_2Cl_2/acetone, 1:1) afforded **6** (3.86 g,

80%) as a pale-yellow oil. IR (film): 2965s, 2937m, 2877m (CH), 1672s (C=N), 1460m, 1370m, 1332m, 1167m, 1057m, 933m, 526m cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.99$ (s, 3 H, CH_3), 1.83 (s, 2 H, CH_2), 1.66−1.49 (m, 4 H, 2 × CH_2CH_3), 1.47−1.33 (m, 2 H, CH_2CH_3), 1.30−1.18 (m, 2H, CH_2CH_3), 0.88−0.79 (m, 12 H, 4 × CH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 164.0$ (C=N), 74.1 (NCN), 51.9 (C), 39.2 (CH_2C=N), 33.5 (CH_2CH_3), 31.4 (CH_2CH_3), 28.4 (CH_3C=N), 8.3 (CH_2CH_3), 7.5 (CH_2CH_3). MS (ESI): 210 (84, $[M]^+$), 195 (36), 192 (18), 154 (100), 140 (30), 126 (19), 86 (38). HRMS (ESI) calcd for $C_{13}H_{26}N_2$ (M^+): 210.2096. Found: 210.2094.

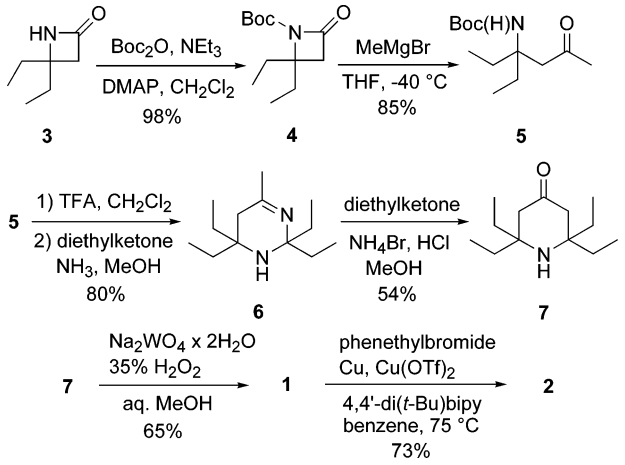
2,2,6,6-Tetraethyl-4-piperidinone (7). Tetrahydropyrimidine **6** (2.59 g, 12.3 mmol), NH_4Br (48 mg, 0.49 mmol), and diethyl ketone (6.50 mL, 61.6 mmol) were dissolved in MeOH (25 mL). Concentrated HCl (1.0 mL) was added dropwise at 0 °C, and the solution was stirred overnight at RT. Aqueous HCl (18%, 3.0 mL) was added, and the mixture was stirred for 3 days at 60 °C. After addition of aqueous K_2CO_3 (40%, until solution turned basic), MeOH was evaporated under reduced pressure. The remaining aqueous solution was extracted with CH_2Cl_2 (3 times), and the combined organic phases were dried over K_2CO_3. The solvent was removed under reduced pressure and the crude product was purified by FC (pentane/diethyl ether, 5:1) to afford **7** (1.41 g, 54%) as a yellow oil. The analytical data are in agreement with those reported in the literature.[12] The experimental procedure for synthesis of alkoxyamine **2** from **7** via **1** is described in ref 12.

Typical Procedure for the Polymerization of NIPAM. In a Schlenk tube, the alkoxyamine initiator **2** (6 mg, 0.02 mmol) and NIPAM (201 mg, 1.78 mmol) were dissolved in benzene (1.0 mL). The solution was deoxygenated by argon bubbling for several minutes. The polymerization was carried out under argon at 125 °C for 16 h. The resulting mixture was cooled to RT, dissolved in a small amount of acetone, and precipitated from Et_2O. The polymer was filtered and dried on air. Conversion was evaluated by ^1H NMR spectroscopy comparing the vinylic monomer resonance signal at $\delta = 5.31$ (dd, 1 H, $^2J = 10.1$ Hz, $^3J_{cis} = 2.2$ Hz, CH=C$H_{cis}H_{trans}$) ppm as well as the *iso*-propylic monomer signal at $\delta = 4.26-4.09$ (m, 1 H, $CH(CH_3)_2$) with the *iso*-propylic resonance of the polymer at $\delta = 4.32-4.01$ (brm, 1 H, $CH(CH_3)_2$) ppm, additionally the resonance of the methyl groups of the monomer ($\delta = 1.01$ (d, 6 H, $^3J = 6.6$ Hz, $CH(CH_3)_2$) ppm) and the polymer ($\delta = 1.45-1.02$ (brm, 6 H, $CH(CH_3)_2$) ppm) were set into relation; molecular weight was determined by MALDI-MS and MALLS. Conversion = 77%. MALDI: $M_n = 8100$ g/mol, MALLS: $M_n = 9700$ g/mol calculated from $M_w = 11\,300$ g/mol with polydispersity index (PDI) = 1.17.

Results and Discussion

Improved Synthesis of Nitroxide 1. β-Lactam **3** was readily prepared from commercially available 2-ethylbutene and chlorosulfonyl isocyanate in 75% yield according to literature procedures (Scheme 1).[14] Boc protection with Boc-anhydride in CH_2Cl_2 using *N*,*N*-(dimethylamino)pyridine (DMAP) as a catalyst afforded **4** in excellent yield. Ring opening with MeMgBr at −40 °C provided β-aminoketone **5**. *N*-Deprotection was readily accomplished using trifluoroacetic acid (TFA) to give the corresponding TFA salt in quantitative yield. Treatment of this salt with diethyl ketone and NH_3 in MeOH afforded tetrahydropyrimidine **6** in 80% yield.[15] Rearrangement/hydrolysis of **6** was performed in MeOH(aq) HCl in the presence of diethyl ketone and a catalytic amount of NH_4Br to give **7**.[16] Oxidation[17] and alkoxyamine synthesis[18] according to known procedures eventually afforded **2**. In contrast to our previous procedure where only small amounts of alkoxyamine have been obtained in each run,[12] gram quantities of **2** can readily be prepared using the novel synthetic route.

Scheme 1. Synthesis of Alkoxyamine 2[a]



[a] Boc_2O = di-*tert*-butyl dicarbonate, DMAP = *N*,*N*-(dimethylamino)pyridine, THF = tetrahydrofuran, TFA = trifluoroacetic acid, $Cu(OTf)_2$ = copper(II)triflate, 4,4′-di(*t*-Bu)bipy = 4,4′-di-*tert*-butyl bipyridine.

Polymerization of NIPAM using 2 and Characterization of Nitroxide-Terminated PNIPAMs. Polymerizations of NIPAM were conducted in perdeuterated benzene (1.78 M) using 1, 0.6, and 0.4 mol % of alkoxyamine **2** as initiator/regulator at 125 °C (oil bath temperature) in sealed tubes. Reaction time was varied from 4 to 36 h. After the mixture reached the desired reaction time, the mixture was allowed to cool to room temperature and conversion was determined by ^1H NMR spectroscopy. The reaction mixture was redissolved in acetone and precipitation of PNIPAM was achieved upon addition to Et_2O. The polymer was then dried on air and was analyzed using GPC using THF or DMF as the mobile phase. Polymerizations in *t*-BuOH were not successful. It is known that PNIPAM forms insoluble aggregates in this and other solvents.[19] Results are presented in Table 1.

As expected for a living polymerization, conversion is increasing upon increasing the reaction time from 4 to 20 h (runs 1−5). The theoretical molecular weight was calculated after determining the conversion gravimetrically from the known amount of added alkoxyamine regulator. Narrow PDIs were obtained for PNIPAM; however, the experimentally determined molecular weight (GPC) did not follow the rules of a living process. In fact, molecular weight leveled at about 2000 g/mol if THF was used as a mobile phase. For higher molecular weight PNIPAM, DMF was also tested as solvent for GPC analysis. However, determined molecular weight was far higher than expected (runs 5−7). We assumed that aggregation of PNIPAM during chromatography may be the reason for these results. Indeed, it has been reported in the literature that analysis of PNIPAM using GPC is difficult.[9] We tested several literature protocols for sample preparation such as a Li salt addition; however, GPC results were not reproducible.

We therefore decided to analyze PNIPAM by MALDI-MS. Results obtained by MALDI-MS measurements are included in Table 1. In addition, the MALDI-MS spectra of the samples obtained in the polymerizations stopped after 4, 8, 12, and 16 h are depicted in Figure 2. All of the polymers exhibited differences between the molecular weight peaks in MALDI spectra that were equal to the monomer weight of the NIPAM monomer unit.

Table 1. Polymerization of NIPAM Using Alkoxyamine 2 (in C_6D_6 Using 1 Mol % 2 at 125 °C)

entry	time (h)	conversion (%)	$M_{n,th}$ (g/mol)	$M_{n,exp}$(GPC) (g/mol)	PDI (GPC)	$M_{n,exp}$ (MALDI) (g/mol)	PDI (MALDI)	M_w (MALLS) (g/mol)
1	4	40	4 500	2 300[a]	1.16[a]	4 700	1.21	n.d.
2	8	54	6 100	2 100[a]	1.16[a]	6 100	n.d.	n.d.
3	12	73	8 300	2 000[a]	1.16[a]	6 800	n.d.	n.d.
4	16	77	8 700	1 900[a]	1.17[a]	8 100	n.d.	11 300
5	20	87	9 700	1 900[a]	1.17[a]	7 700	n.d.	12 100
				22 400[b]	1.17[b]			
6[c]	36	73	13 800	28 800[b]	1.18[b]			16 200
7[d]	36	78	22 100	39 200[b]	1.26[b]			24 500

[a] GPC in THF and polystyrene as standard. [b] GPC in DMF and polystyrene as standard. [c] Initiator **2** (0.6 mol %) was used. [d] Initiator **2** (0.4 mol %) was used. n.d. = not determined.

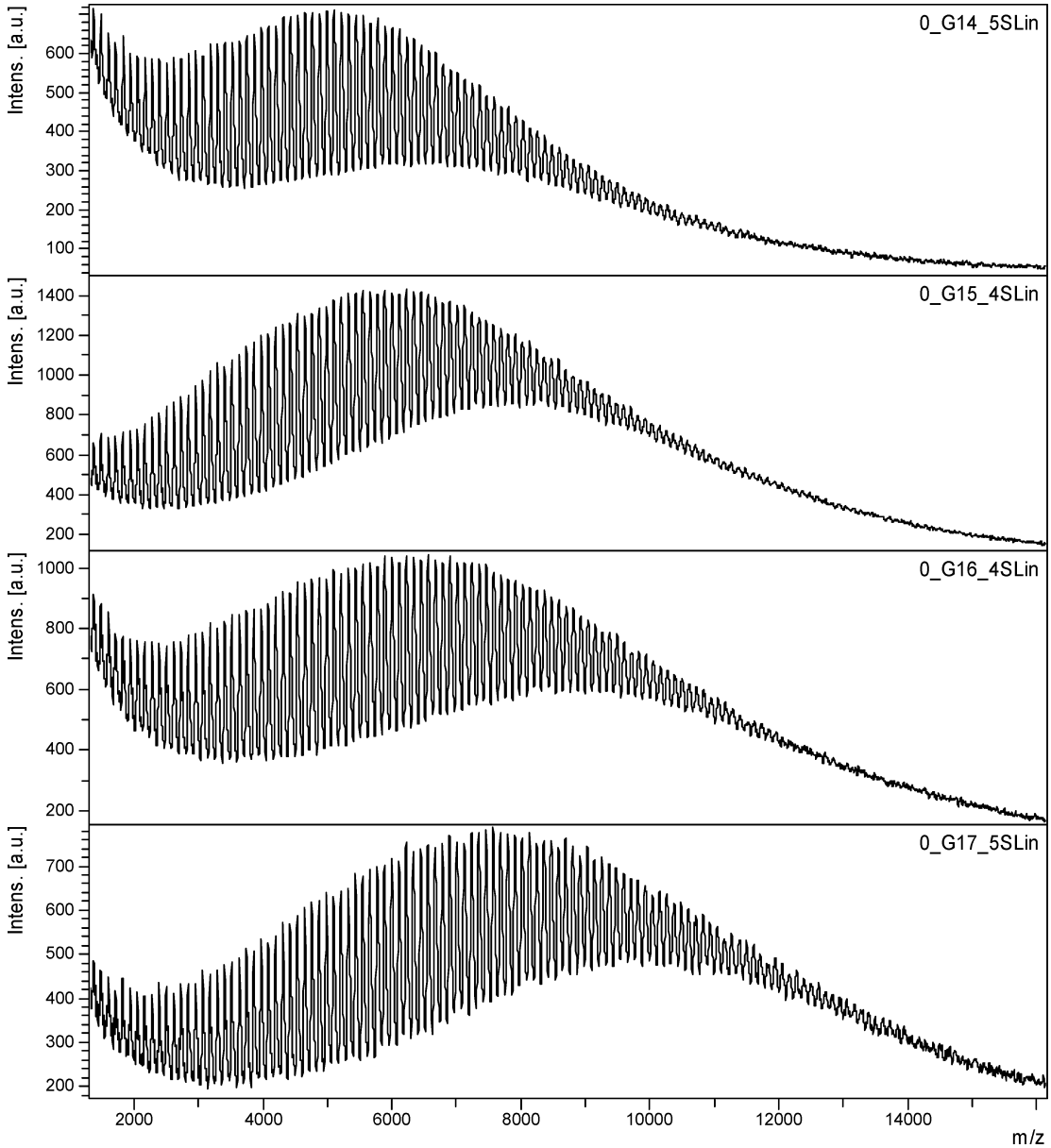


Figure 2. MALDI-MS of PNIMPAM obtained for polymerizations stopped after 4, 8, 12, and 16 h, respectively (from the top). MALDI conditions: the samples were prepared by dissolving the polymer, DHB, and $NaBF_4$ in THF. About 1 μL of the solution was applied to the MALDI target and evaporated. To gain representative information, the spot was probed at several locations and 100−200 spectra were accumulated.

In contrast to the data obtained by GPC analysis, MS-determined molecular weight is increasing from 4700 to 8100 g/mol upon increasing the reaction time from 4 to 16 h. Polymerization for 20 h delivered PNIPAM with a slightly lower molecular weight (run 5). Probably, the high laser energy used to desorb high molecular weight PNIPAM leads to chain degradation. Indeed, also the higher molecular weight PNIPAM showed a mass of about 8000 g/mol by MS analysis. Therefore, analysis of PNIPAM under our MS conditions is only possible to a molecular weight of up to 8000 g/mol. PDI was determined by careful reflectron analysis on a low

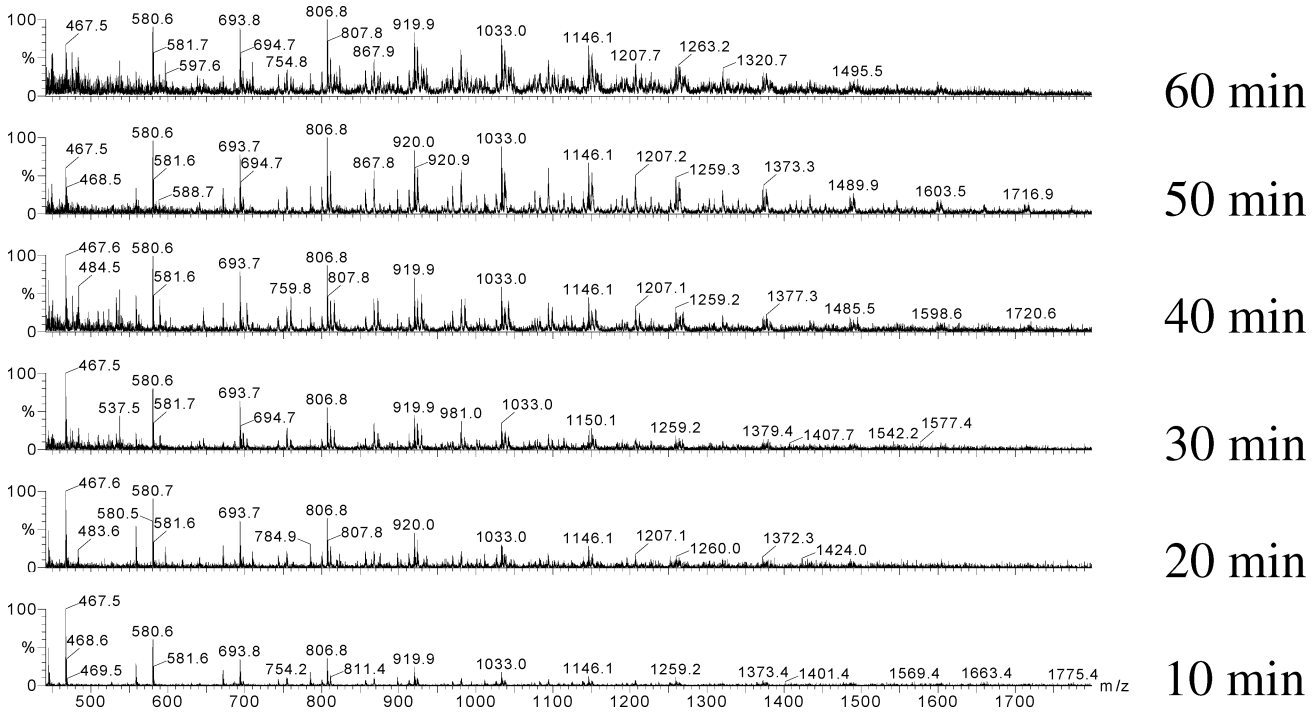


Figure 3. ESI-MS analysis of the early stage of a **2**-mediated NIPAM polymerization.

molecular weight sample (run 1, $M_{n,exp}$ = 4700 g/mol). As with GPC, a narrow PDI (1.21) was extracted from this experiment.

To determine the molecular weight of PNIPAM with a mass of >8000 g/mol, we decided to use MALLS analysis. Studies were performed in THF as solvent.[20] The MALDI-determined molecular weight could be well reproduced by MALLS analysis (entry 4: MALDI, M_n = 8100 g/mol; MALLS, M_n = 9700 g/mol calculated from M_w = 11 300 g/mol with PDI = 1.17). The higher molecular weight PNIPAM-delivered values for M_w which reasonably agree with a controlled living NIPAM polymerization (runs 5−7). Hence we can state that analysis of PNIPAM with M_n < 8000 g/mol can be perfectly performed using MALDI-MS techniques, whereas for PNIPAM with M_n > 8000 g/mol MALLS is the method of choice.

To better understand the **1**-mediated NIPAM polymerization (livingness, end-group determination), we analyzed the early stage of the polymerization process using the ESI-MS technique. The linear mode of the TOF analyzer is superior for the investigation of higher MWs, but in this mode and by the relatively high laser intensity the resolution is not sufficient at >3000 Da for an unambiguous end-group analysis.[21] For the ESI-MS studies, NIPAM polymerization was repeated under the above-described conditions (1% alkoxyamine, benzene (1.78 M) at 125 °C) and samples were taken every 10 min during the first 60 min of the reaction. These samples were directly subjected to ESI-MS analysis. The spectra are presented in Figure 3. One can readily see the progress of the polymerization in the first 60 min. The mean molecular weight is steadily increasing. As in the MALDI spectra, the mayor peaks are separated by 113 mass units (NIPAM monomer).

As example, part of the mass spectrum obtained from the sample taken after 50 min is discussed in more detail in Figure 4. Importantly, the peaks were unambiguously assigned. We found 113 series for singly, doubly, and triply charged PNIPAMs. We were very pleased to observe that all the peaks identified in the spectrum belong to styryl radical initiated nitroxide-terminated PNIPAMs. No other peaks were identified. This demonstrates the absence of any detectable amount of termination or other side reactions and is therefore an experimental proof that at least in the early stage of the polymerization nitroxide **1** is able to perfectly mediate the polymerization of NIPAM.

To get a better resolution of higher molecular weight PNIPAM (up to 3000 g/mol), the FTICR-MALDI-MS technique was also included in the present study. The sample preparation was identical to the Reflex IV measurements described above. In Figure 5, part of a spectrum of a PNIPAM sample is depicted. As with the MALDI-TOF experiments described above, the 113 series were clearly identified. However, determination of the exact mass of the individual peaks revealed that the peaks do not correspond to styryl radical initiated nitroxide-terminated PNIPAM species (simulated exact masses are also included in Figure 5). Presumably chain-end degradation occurred during mass analysis. Indeed, it has previously been reported that MALDI-MS analysis on polystyrene and poly-*n*-butyl acrylate prepared by NMP leads to chain end degradation.[13,22,23] Surprisingly, the peaks do not correspond to the expected chain-end degradation products **8** derived from alkoxyamine C−O-bond homolysis with subsequent H-transfer to nitroxide (Scheme 2). The peaks clearly correspond to protonated methylene-terminated PNIPAMs **11**.[22] Simulated and experimental determined data fit perfectly (see Figure 5). We believe that N−O-bond homolysis to generate the polymeric alkoxyl radical **9** occurs under laser irradiation. β-Fragmentation will then afford radical **10**, which upon H-loss leads to the observed polymers **11**. To the best of our knowledge, such a degradation mechanism has not been described before. We believe that this degradation mechanism may be general for nitroxide-terminated poly-

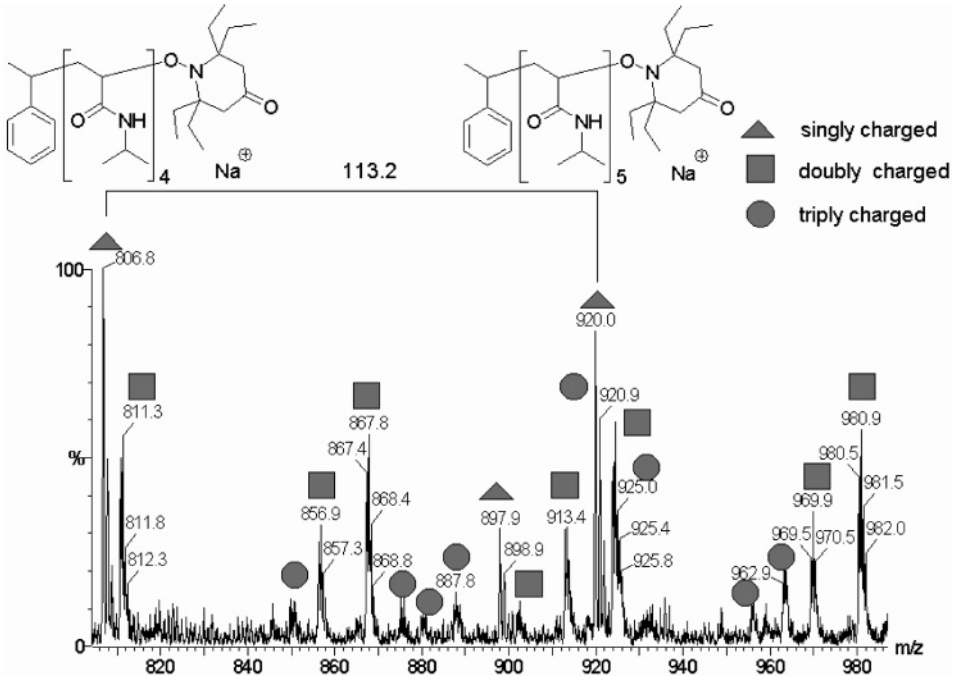


Figure 4. Part of the ESI mass spectrum of the sample taken after 50 min.

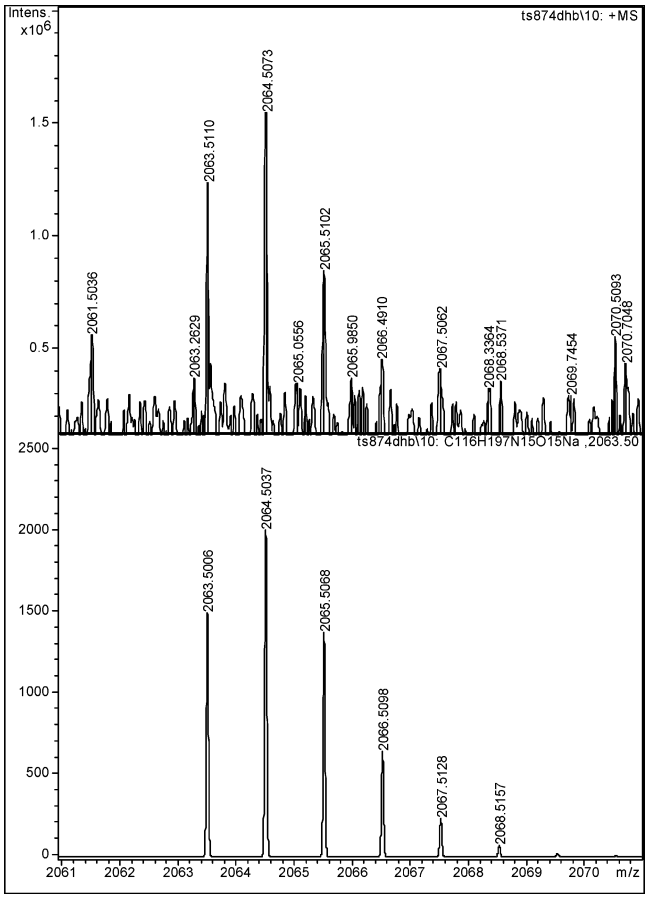
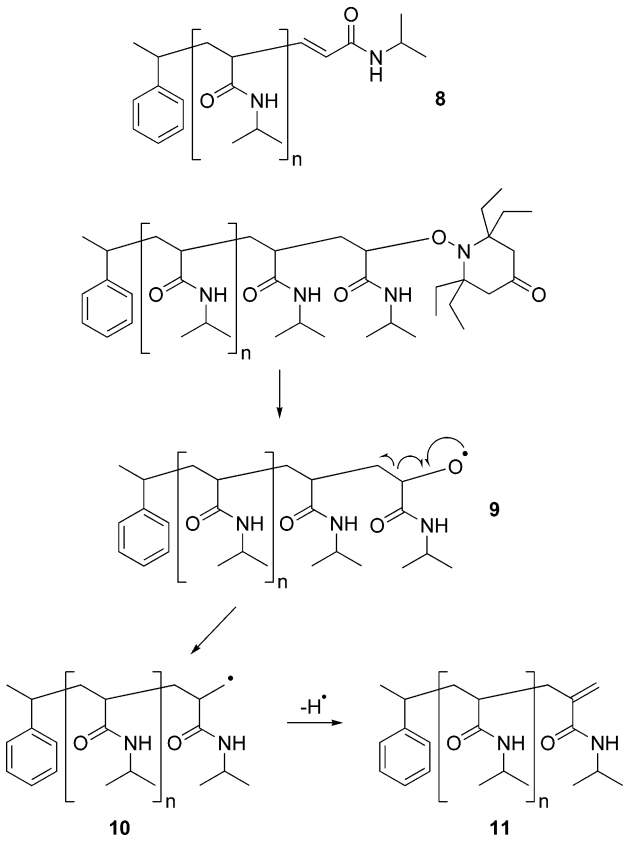


Figure 5. Characteristic region of a high resolution FT-ICR mass spectrum and the corresponding simulated spectrum.

Scheme 2. Suggested Mechanism for the Chain-End Degradation during MS Analysis



mers. In fact, degradation products identified during MALDI-TOF-MS analysis on TEMPO-terminated poly-*n*-butyl acrylates can also be explained by this mechanism.[22]

To prove whether the fragmentation suggested in Scheme 2 is induced by the laser desorption/ionization and not during the polymerization process, we ran MALDI experiments on low molecular weight polymers. With these low MW samples, the onset of ion formation is at low laser intensity (~0.5 MW/cm^2). A characteristic region of the spectra is presented in Figure 6. The first experiment was conducted using ~0.5 MW/cm^2 laser intensity. No chain-end degradation was obtained under these conditions. The major peak at 784.6 (*m/z*) corre-

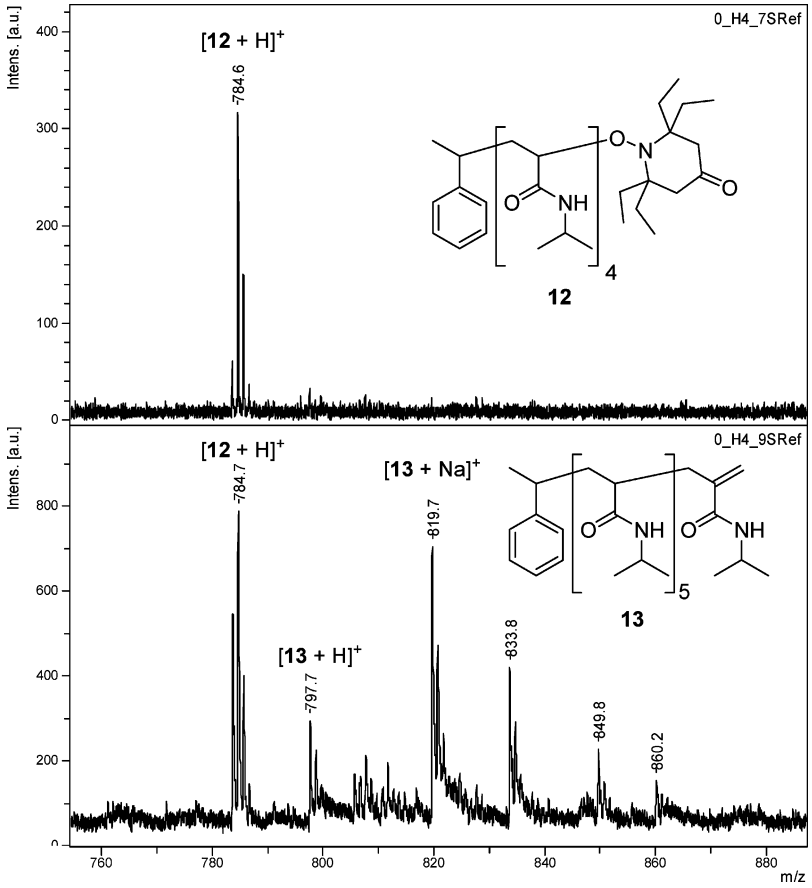


Figure 6. MALDI measurements on a low molecular weight PNIPAM sample using different laser intensities (above, ~0.5 MW/cm^2; below, ~4.0 MW/cm^2).

sponds to protonated oligomer **12** containing alkoxyamine **2** and 4 NIPAM units. Upon increasing the laser intensity to ~4.0 MW/cm^2, degradation products were clearly identified in the mass spectrum. We were pleased to observe that methylene-terminated PNIPAMs of type **11** appeared as major decomposition products among others. The peak at 797.7 corresponds to the protonated chain end degraded **13**, and the 819.7 peak belongs to the corresponding Na^+-complexed oligomer **13**. These results unambiguously show that chain-end degradation occurs during MALDI mass analysis if high laser intensity is used.

Conclusion

We showed that alkoxyamine **2** can be used as an initiator/regulator for the controlled nitroxide-mediated polymerization of NIPAM. The analysis of the molecular weight of PNIPAM using GPC is difficult. For low molecular weight PNIPAM, ESI-mass spectrometry is well suited to analyze the polymers. Moreover, we showed by ESI-MS that the **2**-mediated polymerization of NIPAM is perfectly living at least during the early stages of the polymerization. For higher molecular weight PNIPAM (up to 8000 g/mol), MALDI-MS can be used as an analytical tool.[13,22,23] For higher molecular weight PNIPAM (>8000 g/mol) MALLS analysis delivers accurate results.

In accordance to literature reports,[22,23] chain-end degradation of the nitroxide-terminated polymers occurs during MALDI analysis. The mechanism for the chain-end degradation of our nitroxide-terminated PNIPAMs was carefully studied. This mechanism may also be operative for the degradation of other nitroxide-terminated polymers during MALDI-analysis. Future experiments will show the generality. Chain-end degradation can be suppressed during MALDI-analysis using low laser intensities.

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