Versatile Synthesis of End-Functionalized Thermosensitive Poly(2-isopropyl-2-oxazolines)

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ABSTRACT: The synthesis of several end-functionalized poly(2-isopropyl-2-oxazolines) (P*i*PrOx) has been achieved via cationic ring-opening polymerization of 2-isopropyl-2-oxazoline. Poly(2-isopropyl-2-oxazolines) bearing primary amino groups at one chain end (Me-P*i*PrOx-NH_2, with M_n ranging from 3600 to 9700) were obtained by conversion of hydroxyl-terminated poly(2-isopropyl-2-oxazolines) (Me-P*i*PrOx-OH) via phthalimide activation of the hydroxyl groups and subsequent hydrazine treatment. Heterotelechelic P*i*PrOx carrying an α-acetal and an *ω*-hydroxyl group (acetal-P*i*PrOx-OH) were prepared via cationic ring-opening polymerization of 2-isopropyl-2-oxazoline initiated with 3,3-diethoxy-1-propyl tosylate. The polymerizations carried out under mild conditions (40−45 °C) for extended periods of time yielded polymers of well-controlled molecular weight (MW) and narrow molecular weight distribution (MWD). Analysis of the polymers by ^{1}H and ^{13}C NMR spectroscopy, ion-exchange HPLC, and MALDI−TOF mass measurements indicated that nearly quantitative end-functionalization was achieved in all cases. Aqueous P*i*PrOx solutions (10 mM PBS (pH 7.4) containing 150 mM NaCl) possess a cloud point temperature near 37 °C, as determined by turbidity. Thermosensitive telechelic P*i*PrOx offer promising applications as smart materials including bioconjugates, hydrogels, and drug carriers.

Introduction

Poly(oxazolines) (POx) have emerged recently as materials of importance in surface chemistry and biomaterials science, where they may act as nonionic surfactants, protein modifiers, hydrogels, and drug carriers.[1,2] Under appropriate conditions, the cationic ring-opening polymerization of oxazolines (Ox) is known to proceed by a living polymerization process to afford poly(*N*-acylethylenimines), often viewed as "pseudopeptides".[3] A variety of POx can be prepared by changing either the alkyl substituent of the starting oxazoline or the end group. POx possessing a short alkyl substituent, e.g., methyl or ethyl group, in the side 2-position are water-soluble. However, the hydrophilicity of POx decreases as the length of the alkyl substituent increases, resulting in insolubility in water at all temperatures or in certain temperature ranges. Of particular interest are poly(2-isopropyl-2-oxazolines) (P*i*PrOx) which possess an isopropyl group in the side 2-position. These polymers are soluble in cold water, but their aqueous solutions have a cloud point near physiological conditions,[4] like poly(*N*-isopropylacrylamide) (PNIPAM), the typical representative of thermosensitive polymers with numerous applications.[5−7] The main advantage of P*i*PrOx, which is a POx homologue, is that it is highly expected to be a biocompatible thermosensitive polymer and, as such, to be extremely useful in biomedical applications. It should be noted that POx, as a rule, are nontoxic and that several POx carry US Food and Drug Administration (FDA) approval. Zalipsky et al. reported that poly(2-ethyl-2-oxazoline)-modified liposomes exhibit enhanced biocompatibility and prolonged blood circulation times, comparable to those of conventional poly(ethylene glycol) (PEG) lipopolymers.[8,9]

Our group has established synthetic strategies, via an anionic ring-opening polymerization catalyzed by functionalized initiators, toward quantitative and selective syntheses of heterotelechelic PEG, leading to functionalized telechelic polymers with high potential utility in the biomedical field.[10−13] Applying a similar approach toward the cationic polymerization of Ox derivatives, it should be possible to prepare a variety of heterotelechelic P*i*PrOx derivatives. Moreover, end-functionalized block copolymers can be prepared by using heterotelechelics as macroinitiators, as in the case of PEG.[14] Thus, the objective of this work was to design end-functionalized P*i*PrOx homologues, which can be widely used as intelligent biomaterials bearing thermosensitivity near the body temperature of human beings.[15]

On the basis of this synthetic strategy, we devised two synthesis routes toward end-functionalized P*i*PrOx, as depicted in Scheme 1 and Scheme 2. One process leads to semitelechelic P*i*PrOx possessing primary amino groups at one chain end (Scheme 1). This amine-terminated P*i*PrOx may be used for bioconjugation or as a macroinitiator to incorporate a second monomer and create a diblock copolymer, opening up the facile generation of new biofunctional block copolymers. Furthermore, heterotelechelic P*i*PrOx of greater versatility, having α-acetal and *ω*-hydroxyl groups, can be obtained by the route depicted in Scheme 2. The acetal group is easily converted to an aldehyde group under mild acidic conditions. This group is readily amenable to conjugation with proteins, as it is stable in water and reacts rapidly with primary amines. The synthesis of these two new families of polymers is reported here, together with the polymer characterization by ^{1}H and ^{13}C NMR

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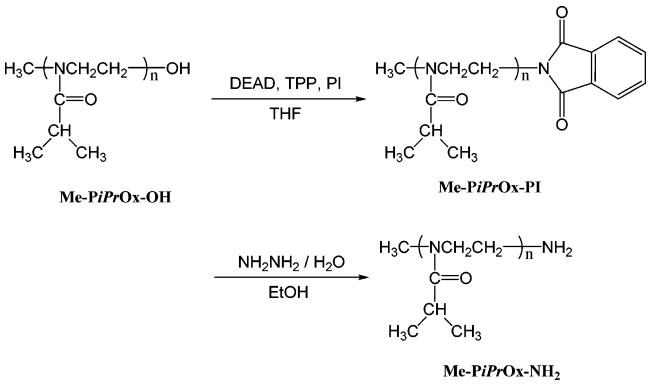
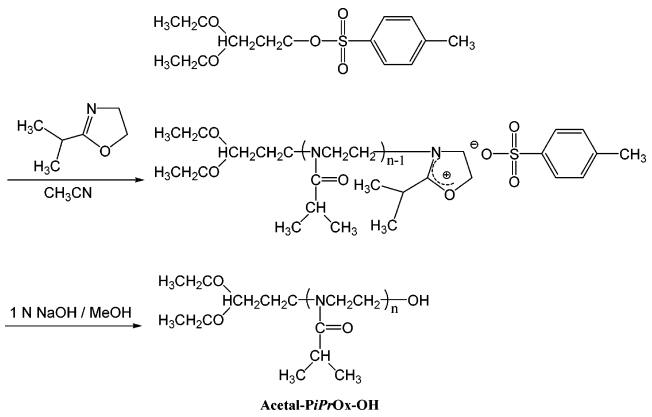
Scheme 1. Synthesis of Me-P*i*PrOx-NH_2 by Functionalization of ω-Hydroxyl Terminals



Scheme 2. Synthesis of Heterotelechelic Acetal-P*i*PrOx-OH



spectroscopy, MALDI−TOF mass spectrometry, and gel permeation chromatography. Moreover, the temperature sensitivity of P*i*PrOx aqueous solutions was examined via turbidity measurements.

Experimental Section

Materials. 2-Isopropyl-2-oxaxoline was synthesized from isobutyric acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and 2-aminoethanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as described previously (see Supporting Information, Figures S1 and S2).[16] Methyl *p*-tosylate (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) was distilled from calcium hydride under reduced pressure. 3,3-Diethoxy-1-propanol (Aldrich Chemical Co. Ltd., Milwaukee, WI) and *p*-toluenesulfonyl chloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used as received. Acetonitrile (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was distilled from calcium hydride. Tetrahydrofuran (THF) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and chloroform (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were purified by distillation following conventional procedures.[17] Other chemicals, such as 1 N NaOH aqueous solution, methanol, and ethanol, were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan, and used without further purification. Triphenylphosphine (TPP) (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan), phthalimide (PI) (Wako Pure Chemical Industries, Ltd., Osaka, Japan), diethyl azodicarboxylate (DEAD) (40 wt % in toluene; Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan), and hydrazine monohydrate (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used as received.

Techniques. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL EX 300 spectrometer (JEOL, Tokyo, Japan) at 400 MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Molecular weights and molecular weight distributions were determined using a GPC (TOSOH HLC-8220) system equipped with two TSK gel columns (G4000H_{HR} and G3000H_{HR}) and an internal refractive index (RI) detector. Columns were eluted with DMF containing lithium bromide (10 mM) and triethylamine (30 mM) with a flow rate of 0.8 mL/min and at a temperature of 40 °C. Molecular weights were calibrated with poly(ethylene glycol) standards (Polymer Laboratories, Ltd., UK). Mass measurements were performed on a MALDI−TOF mass spectrometer (Bruker REFLEX III), operating at an acceleration voltage of 23 kV in the reflection mode. UV−vis spectra were obtained with a V-550 UV/vis JASCO spectrophotometer. The functionality of amino group was determined by using an ion-exchange HPLC system equipped with TSK gel column (TOSOH SP-5PW) and an internal refractive index (RI) detector. The column was eluted with phosphate-buffered solution (2 mM PBS (pH 6.5)) at a flow rate of 0.5 mL/min and at a temperature of 30 °C.

Synthesis of 3,3-Diethoxy-1-propyl Tosylate. A solution of *p*-toluenesulfonyl chloride (9.61 g, 50.4 mmol, 1.5-fold molar excess vs OH) in chloroform (50 mL) was added to a stirred solution of 3,3-diethoxy-1-propanol (4.98 g, 33.60 mmol) and triethylamine (23.5 mL, 168 mmol, 5-fold molar excess vs OH) in anhydrous chloroform (150 mL) cooled to 0 °C and kept under an argon atmosphere. At the end of the addition, the reaction mixture was brought to room temperature and stirred for 24 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on a silica gel column eluted with chloroform. Fractions containing the desired product were dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The product was stored at −20 °C under an argon atmosphere (Figure S4).

^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 1.10 (t, J = 9.6 Hz, 3H, CH_3CH$_2$O), 2.80 (q, J = 9.6 Hz, 2H, CH$_3$CH_2O), 4.50 (t, J = 9.6 Hz, 1H, CHCH$_2$CH$_2$), 1.80 (q, J = 9.6 Hz, 2H, CHCH_2CH$_2$), 4.0 (t, J = 9.6 Hz, 2H, CHCH$_2$CH_2), 7.50 (d, J = 8.5 Hz, 2H, C$_6H_4$CH$_3$), 7.80 (d, J = 8.5 Hz, 2H, C$_6H_4$CH$_3$), 2.45 (s, J = 8.5 Hz, 3H, C$_6$H$_4$CH_3).

Synthesis of Poly(2-isopropyl-2-oxazoline) Having an Hydroxyl Group at the ω-Terminal End (Me-P*i*PrOx-OH). 2-Isopropyl-2-oxazoline (10 g, 88.4 mmol) was added via a syringe to a solution of methyl *p*-tosylate (0.186 g, 1.0 mmol) in acetonitrile (30 mL). The polymerization mixture was stirred at 42 °C for ca. 506 h under an argon atmosphere. The mixture was cooled to room temperature and treated with methanolic NaOH (1 M) to quench the poly(2-isopropyl-2-oxazoline) oxazolinium living end groups. The solution was dialyzed for 2 days against distilled water to introduce an hydroxyl group at one of the chain ends. Freeze-drying of the dialyzed solution yielded a white colorless powder. In addition, eight samples were collected in the course of the polymerization. They were subjected to the same treatment and analyzed by GPC in order to determine the conversion yield (total yields: 5.1 g, 51%).

Conversion of Terminal Hydroxyl Groups into Primary Amino Groups via the Mitsunobu Reaction (Me-P*i*PrOx-NH_2) (Scheme 1).[18] Me-P*i*PrOx-OH (100 mg, 0.022 mmol) was added to a stirred solution of triphenylphosphine (TPP) (57.7 mg, 0.22 mmol) and phthalimide (PI) (32.37 mg, 0.22 mmol) in THF (1 mL). Then, diethyl azodicarboxylate (DEAD) (38.32 mg, 0.22 mmol) was added dropwise to the mixture kept under an argon atmosphere. After being kept at room temperature for 24 h, the mixture was dialyzed first against ethanol and then against distilled water using a Spectrapor dialysis membrane with a 1000 M_r molecular weight cutoff value. The solution lyophilized for 2 days yielded the phthalimide-activated polymer "Me-P*i*PrOx-PI". The yield was 90 mg and the conversion efficiency was ca. 70%, confirmed by comparing integral ratio obtained from ^{1}H NMR analysis. This material (50 mg, 0.011 mmol) was then dissolved in ethanol (5 mL) and treated with hydrazine monohydrate (5 mL). The mixture was kept at room temperature for 24 h. After ethanol was evaporated, an aqueous sodium hydroxide was added up to pH 9−10. The polymer was extracted with methylene chloride and then purified by dialysis against ethanol and distilled water using a Spectrapor dialysis mem-

brane with a 1000 M_r cutoff value. Lyophilization of the dialyzed solution gave the amine-terminated polymer "Me-P*i*PrOx-NH_2". The yield was 30 mg and the conversion efficiency, viz., functionality of amino group, was ca. 89%, determined by the analysis of ion-exchange HPLC.

Synthesis of Heterotelechelic Poly(2-isopropyl-2-oxazoline) Containing Acetal and Hydroxyl Terminals (Acetal-P*i*PrOx-OH) (Scheme 2). 2-Isopropyl-2-oxazoline (9.74 g, 86 mmol) was added to a solution of 3,3-diethoxy-1-propyl tosylate (0.30 g, 1.0 mmol) in acetonitrile (30 mL). The resulting mixture was stirred at 45 °C for 240 h under an argon atmosphere. An aliquot taken from the reaction mixture (1 mL) was cooled to room temperature. It was added to methanolic NaOH (3 mL, 1 M) and subsequently dialyzed for 2 days against distilled water to yield heterotelechelic P*i*PrOx with hydroxyl groups at the ω-end (acetal-P*i*PrOx-OH). The solution was then evaporated, and the residue was dissolved in methanol (20 mL) and dialyzed for 2 days against distilled water. The polymer was recovered as a white colorless powder by freeze-drying of the dialyzed solution.

MALDI−TOF Mass Spectrometry. An external calibration was performed by using poly(ethylene glycol) standards (MeO-PEG-OH; MW = 5000, NOF Corp.). Ions were generated by laser desorption at 337 nm (N_2 laser, 3 ns pulse width, 10^6−10^7 W/cm^2). For each spectrum, approximately 400 transients were accumulated, and all spectra were recorded in the reflection mode. Data evaluation was performed with the Bruker XMASS program, using the reflection spectra only to achieve better signal-to-noise ratio. 1,8,9-Trihydroxyanthracene (Aldrich, Milwaukee, WI) was selected as a suitable matrix.[19] To prepare the matrix, a solution of 1,8,9-trihydroxyanthracene (20 μL, 25 mg mL^{-1}) was mixed with a solution of the polymer in THF (20 μL, 6.5 mg/mL). Finally, a solution of lithium trifluoroacetate in THF (2 μL, 2 mg/mL) was added. The resulting mixture was shaken for few seconds. An aliquot of the mixture (1 μL) was placed on the target plate and applied to the probe. The lithium salt was added to the matrix in order to promote selective and quantitative ionization of the sample.

Turbidity Measurements by Using UV−Vis Spectrophotometer. Cloud points were determined by spectrophotometric detection of the changes in transmittance (λ = 500 nm) of aqueous polymer solutions (0.07−1.0 wt %) heated at a constant rate (0.2 °C min^{-1}). The samples were thermostated with a temperature-controlled circulating water bath. Values for the cloud point of polymer solutions were determined as the temperature corresponding to 1.0% decrease in optical transmittance.

Results and Discussion

Synthesis of Me-P*i*PrOx-OH and Me-P*i*PrOx-NH_2. Cationic ring-opening polymerization of *i*PrOx, initiated with methyl *p*-tosylate, led to poly(2-isopropyl-2-oxazoline) carrying a hydroxyl group at one end (Me-P*i*PrOx-OH). For the polymerization to proceed in good yield and without undesired side reactions, mild conditions needed to be applied. We found that the polymerization of Me-P*i*PrOx-OH proceeded best, if done in acetonitrile, at a temperature (42 °C) lower than the temperature conditions (usually >70 °C) reported for the polymerization of conventional poly(2-alkyl-2-oxazolines) (POx).[3,20] Under these conditions, the polymerization had to be left to proceed for lengths of time up to 506 h, but no noticeable side reactions occurred. From the GPC trace shown in Figure 1, it was ascertained that the number-average molecular weight (M_n) and the molecular weight distribution (MWD) were consistent with a living polymerization process: the polydispersity index was low (1.02), and the experimental M_n value was close to the value predicted from the initial monomer/initiator ratio ($M_{n,calc}$ = 10 000).

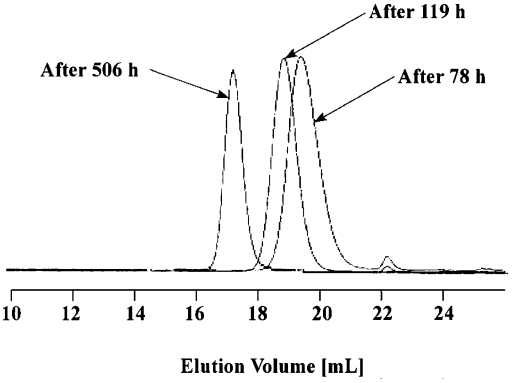


Figure 1. Gel permeation chromatograms of three Me-P*i*PrOx-OH samples having different molecular weights (PEG standard; eluent: DMF (containing 10 mM LiCl and 30 mM TEA); temperature: 40 °C; RI detection).

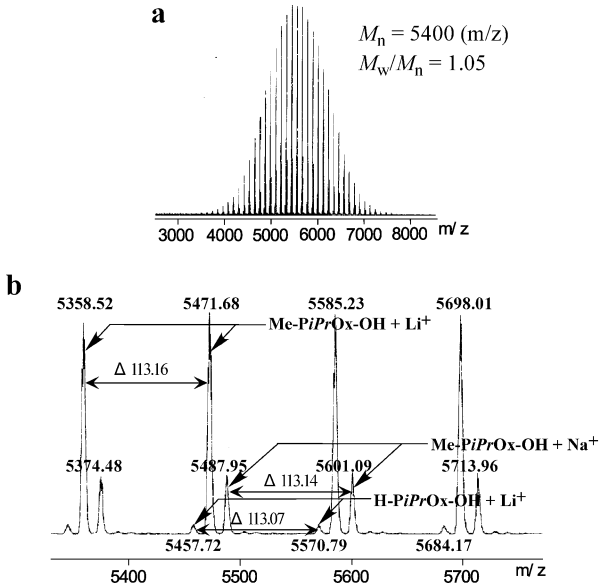


Figure 2. MALDI−TOF mass spectrum of (a) Me-P*i*PrOx-OH (after 119 h) and (b) its expanded spectrum in the region of 5300−5800.

The ^{1}H NMR spectrum of Me-P*i*PrOx-OH in $CDCl_3$ (Figure S3 in Supporting Information) presents a broad singlet at 3.4 ppm attributed to the methylene protons of polymer backbone, two broad singlets at 2.6−2.8 ppm ascribed to the side-chain isopropyl methine proton, and a strong broad singlet due to the resonance of the side-chain methyl protons (1.1 ppm). The polymer structure is supported also by the ^{13}C NMR spectrum of the polymer in $CDCl_3$, shown in Figure S5a in the Supporting Information. End-group analysis of the polymer was performed from the MALDI−TOF mass spectra recorded for a Me-P*i*PrOx-OH (polymerization time: 119 h, Figure 2). Assuming that only parent ions are detected by the MALDI−TOF mass analysis,[21] and given the molecular mass of the *i*PrOx monomer, the molar mass of the end groups, and the metal ions present, the MW of the Me-P*i*PrOx-OH samples can be determined. These values were then compared to M_n and M_w derived from GPC analysis of the same polymers. The data are summarized in Table 1, where M_p is the mass at the peak maximum of the strongest signal in the MALDI−TOF mass of each sample, except in the case of the polymer obtained after 119 h (sample 3), for

Table 1. Analytical Data As Derived from the Respective MALDI−TOF-MS along with the Results from the GPC Measurements for Comparison (MWD = M_w/M_n; Molecular Weight Distribution)

	MALDI-TOF-MS									GPC		
polymer samples[a]	ΔM_{theor}[b] [g/mol]	ΔM_{exp}[b] [g/mol]	RM_{theor}[c] [g/mol]	RM_{exp}[c] [g/mol]	M_{theor} [g/mol]	M_n [g/mol]	M_w [g/mol]	M_p [g/mol]	MWD	M_n [g/mol]	M_w [g/mol]	MWD
1 (78 h)	113.158	113.19	55.032	53.912[d]	4015.562	3800	4000	4016.95	1.04	3600	3800	1.03
2 (95 h)	113.158	113.19	38.983	39.07[e]	4678.461	4400	4600	4679.86	1.05	4300	4400	1.03
		113.07	24.957	29.181[f]	5456.541			5457.72				
3 (119 h)	113.158	113.16	38.983	38.887[e]	5470.567	5400	5700	5471.68	1.05	5100	5200	1.03
		113.14	55.032	57.23[d]	5486.616			5487.95				
4 (135 h)	113.158	113.15	38.983	39.423[e]	6262.673	6200	6400		1.03	5900	6000	1.03
5 (506 h)										9700	9900	1.02

[a] Taken at different times during the process of polymerization. [b] ΔM = mass of monomer unit. [c] RM = residual mass, M_{theor} = calculated mass with a degree of polymerization nearest to the measured value ($M_{theor} = \Delta M_{theor} n + RM_{theor}$ for the species given in footnotes *d*−*f*. [d] Me-[*i*PrOx]$_n$-OH + Na^+. [e] Me-[*i*PrOx]$_n$-OH + Li^+. [f] H-[*i*PrOx]$_n$-OH + Li^+, n = DP).

which the second and third strongest signals were included as well. The mass of sample 3 was about $M_{n,MS}$ = 5400 (m/z) ($M_{w,MS}/M_{n,MS}$ = 1.05), a value in close agreement with the GPC results. This coincidence of GPC and MS derived mass values was observed for the other polymers as well. The mass difference in the series of signals registered by MALDI−TOF mass of sample 3 (m/z 113.158, Figure 2b) agrees well with the mass of the polymer repeating unit, confirming that the ions form a homologue series originating from end-functionalized polymers. The theoretical mass of each Me-P*i*PrOx-OH is expressed by the following equation:

$$M_{theor} = \Delta M_{theor} n + RM_{theor}$$

where M_{theor} is the calculated mass of a polymer of degree of polymerization nearest to the measured value, ΔM_{theor} is the mass of the monomer unit, and RM_{theor} represents the calculated residual mass.

The most intense signal can be assigned to the lithium adduct of Me-P*i*PrOx-OH, while the second most intense signal is due to the sodium adduct of Me-P*i*PrOx-OH. The occurrence of weak signals to the left side of the lithium adduct signal in the spectrum of the Me-P*i*PrOx-OH (H-[*i*PrOx]$_n$-OH + Li^+) reflects the presence of a small fraction of polymer chain initiated by *p*-toluenesulfonic acid (H^+), formed by inadvertent hydrolysis of the methyl *p*-tosylate initiator.

We set out next to convert the terminal hydroxyl groups of Me-P*i*PrOx-OH into primary amine groups since these groups are more amenable to further conjugation with bioactive agents. This transformation was performed in good yields via the Mitsunobu reaction, which proceeds in two steps: (1) conversion of the terminal hydroxyl into a phthalimide and (2) treatment of the phthalimide-terminated polymer with hydrazine to generate the free amine (Scheme 1). The success of the reaction was assessed by ^{1}H NMR analysis comparing the integral ratio of signals and MALDI−TOF mass analysis, comparing the molar mass of the strongest signals in the spectra of the hydroxyl-terminated polymer, Me-P*i*PrOx-OH, to that of the phthalimide terminated polymer, Me-P*i*PrOx-PI (step 1). A similar comparison was also applied to the polymer obtained after hydrazine treatment, Me-P*i*PrOx-NH_2 (step 2) (Figure 3). Thus, the signals (n = 43−46) in the two spectra (b and c in Figure 3) can be assigned to homologues of the sodium adducts of Me-P*i*PrOx-PI and Me-P*i*PrOx-NH_2, respectively (Table 2). We note that the difference of mass value between M_{exp} in Me-P*i*PrOx-PI and M_{exp} in Me-P*i*PrOx-NH_2 is, within experimental error, equal to the mass value corresponding to the loss of the phthalimide groups at ω-terminals calculated to $\Delta m/z$ = −130.096 (Table 2). Differences between the theoretical and experimental mass ($M_{theor} - M_{exp}$) are also very small (<2.0 amu, Table 2).

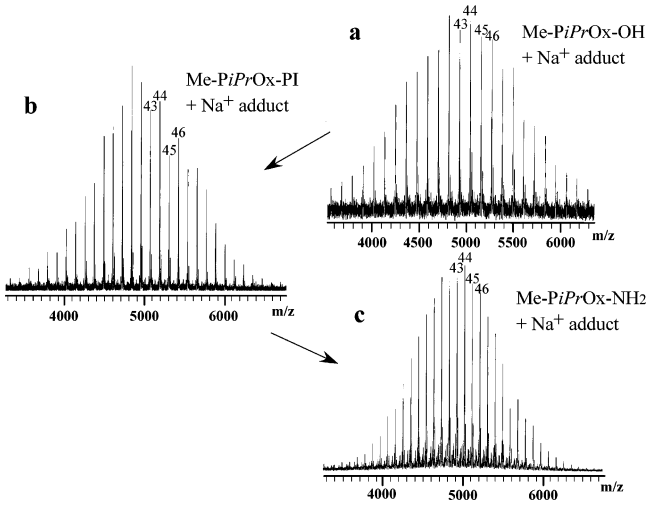


Figure 3. MALDI−TOF mass spectra of poly(2-isopropyl-2-oxazoline) having (a) hydroxyl groups (Me-P*i*PrOx-OH), (b) phthalimide groups (Me-P*i*PrOx-PI), and (c) primary amino groups (Me-P*i*PrOx-NH_2) at ω-terminal ends.

There has been an argument that the coexistence of ester−amines with hydroxyl terminals is possible unless the hydrolysis of an oxazolinium salt is conducted under the relatively high temperature condition even for a long time.[3] However, we have selected the mild conditions for avoiding the inadvertent side reaction of the dimerization of two oxazolinium living ends as well as the formation of undesirable proton initiated polymers, inclined to occur easily under the high-temperature conditions (>60 °C) just in the case of the polymerization of *i*PrOx. Note that neither the ^{1}H NMR nor the ^{13}C NMR spectrum of Me-P*i*PrOx-OH (Figures S3 and S5a in the Supporting Information) showed any undesirable signals derived from ester−amines in our system which terminated with methanolic NaOH (1 M) at room temperature. Moreover, the high conversion efficiency (ca. 70%) into phthalimide terminals and functionality of amino groups (ca. 89%) also supported the reliability about more favorable formation of hydroxyl terminals than ester−amines.

Synthesis of Acetal-P*i*PrOx-OH. The preparation of heterotelechelic P*i*PrOx was based on the cationic ring-opening polymerization of 2-isopropyl-2-oxazoline as well, but to attach a functional group at each end of the polymer, it was necessary to initiate the polymeri-

Table 2. Assignments of the Chemical Composition for Most Intensive Signals in MALDI−TOF Mass Spectra of Me-P*i*PrOx-OH, Me-P*i*PrOx-PI, and Me-P*i*PrOx-NH_2

	(a) Me-P*i*PrOx-OH + Na^+ adduct			(b) Me-P*i*PrOx-PI + Na^+ adduct			(c) Me-P*i*PrOx-NH_2 + Na^+ adduct						
n (DP)	M_{theor}[a] [g/mol]	M_{exp} [g/mol]	$M_{theor} - M_{exp}$	M_{theor}[a] [g/mol]	M_{exp} [g/mol]	$M_{theor} - M_{exp}$	M_{theor}[a] [g/mol]	M_{exp} [g/mol]	$M_{theor} - M_{exp}$	$\Delta[M_{theor}(b) - M_{theor}(a)]$	$\Delta[M_{exp}(b) - M_{exp}(a)]$	$\Delta[M_{theor}(c) - M_{theor}(b)]$	$\Delta[M_{exp}(c) - M_{exp}(b)]$
43	4920.826	4922.38	−1.55	5049.940	5050.33	−0.39	4919.844	4921.77	−1.93	+129.114	+127.95	−130.096	−128.56
44	5033.984	5035.56	−1.58	5163.098	5164.37	−1.27	5033.002	5034.97	−1.97	+129.114	+128.81	−130.096	−129.4
45	5147.142	5149.80	−2.66	5276.256	5276.65	−0.39	5146.160	5148.15	−1.99	+129.114	+126.85	−130.096	−128.5
46	5260.300	5262.88	−2.58	5389.414	5390.67	−1.26	5259.318	5261.36	−2.04	+129.114	+127.79	−130.096	−129.31

[a] M_{theor} = calculated mass with a degree of polymerization nearest to the measured value ($M_{theor} = \Delta M_{theor} n + RM_{theor}$ for the following species: Me-[*i*PrOx]$_n$-OH + Na^+, Me-[*i*PrOx]$_n$-PI + Na^+, Me-[*i*PrOx]$_n$-NH_2 + Na^+, RM = residual mass).

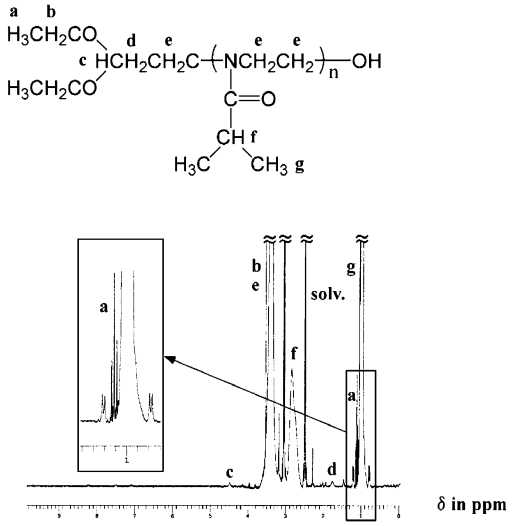


Figure 4. ^{1}H NMR spectrum of acetal-P*i*PrOx-OH in DMSO-d_6 at 80 °C.

zation with a different initiator, 3,3-diethoxy-1-propyl tosylate (Scheme 2). This initiator was synthesized by reaction of 3,3-diethoxy-1-propanol and *p*-toluenesulfonyl chloride. This material proved to be an effective cationic ring-opening polymerization initiator, as determined from the GPC trace of a polymer obtained after a polymerization time of 240 h (Figure S6 in the Supporting Information). The GPC trace for this polymer presents no shoulder, and the experimental M_n value (9600) is close to the expected value based on the initial monomer/initiator ratio ($M_{n,calc}$ = 10 000). Moreover, the molecular weight distribution (MWD) (1.1) is consistent with the proposed cationic polymerization mechanism. The structure of the polymer was confirmed by analysis of the ^{1}H NMR spectrum of acetal-P*i*PrOx-OH (Figure 4), isolated after a polymerization time of 240 h, using spectral data gathered by analyzing spectra of P*i*PrOx and 3,3-diethoxy-1-propyl tosylate used as references. In this spectrum, the signals at 4.6 and 1.2 ppm can be ascribed to the methine and methyl protons of the acetal group. Conversion of the *ω*-end group into a hydroxyl group was confirmed by the absence, in the ^{1}H NMR spectrum of acetal-P*i*PrOx-OH, of signals characteristic of the tosylate phenyl protons (7.5−7.8 ppm). Further support to the assigned structure was given by analysis of the ^{13}C NMR spectrum of acetal-P*i*PrOx-OH (Figure S5b in the Supporting Information). A MALDI−TOF mass analysis was carried out using an acetal-P*i*PrOx-OH sample obtained after a 160 h polymerization ($M_{n,GPC}$ = 5200, $M_{w,GPC}/M_{n,GPC}$ = 1.25). The difference in mass of the signals in the major mass agrees well with the expected value series (*m*/*z* 113.158, Figure 5). The experimental value of acetal-P*i*PrOx-OH (Li^+ adduct) is in agreement with the theoretical values, defined as $M_{theor} = \Delta M_{theor}(113.158)n + RM_{theor}(131.197 + 17.008 + 6.941)$, indicating near quantitative incorporation of the acetal and hydroxyl groups on the P*i*PrOx chain ends. The occurrence of small signals with a mass difference of *m*/*z* = 130.01 may reflect the presence of a small fraction of polymer chains, initiated by toluenesulfonic acid (H^+) formed inadvertently by hydrolysis of the acetal initiator.

Determination of the Cloud Points of Me-P*i*PrOx-OH and Acetal-P*i*PrOx-OH in Aqueous Solution. We determined by turbidimetry the cloud points of several Me-P*i*PrOx-OH samples of various molecular weights (M_n = 4300, M_w/M_n = 1.03; M_n = 7800, M_w/M_n = 1.02; M_n = 9700, M_w/M_n = 1.02), dissolved in phosphate-buffered saline (10 mM PBS containing 150 mM NaCl (pH 7.4)). They exhibit a remarkable depen-

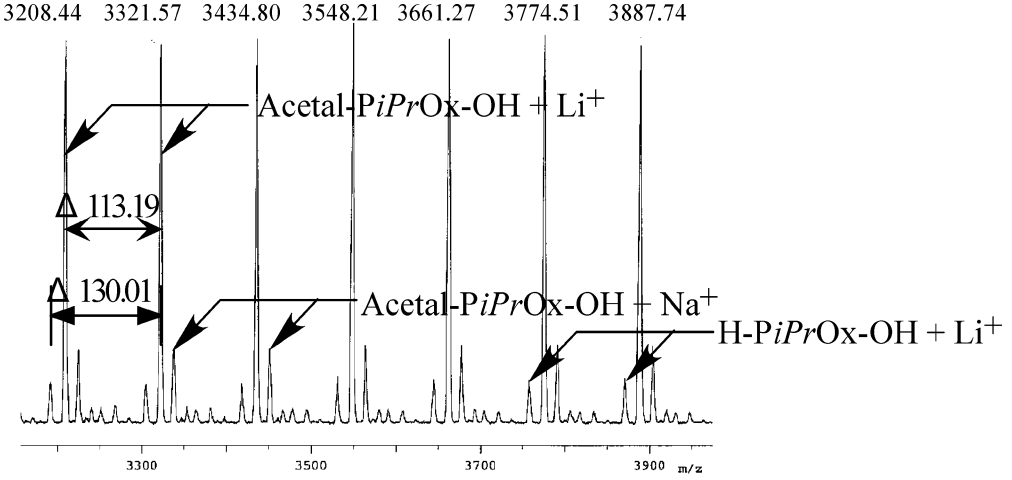


Figure 5. Enlarged detail of the MALDI−TOF mass spectrum of acetal-P*i*PrOx-OH sample obtained after 160 h.

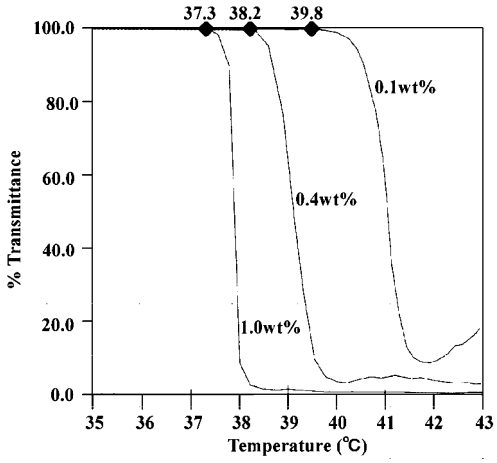


Figure 6. Transmittance changes of aqueous solutions as a function of temperature under several polymer concentrations (Me-P*i*PrOx-OH, M_n = 9700, M_w/M_n = 1.02; concentration = 0.1, 0.4, and 1.0 wt %; 10 mM PBS (pH = 7.4) containing 150 mM NaCl (rate: 0.2 deg/min; wavelength: 500 nm)).

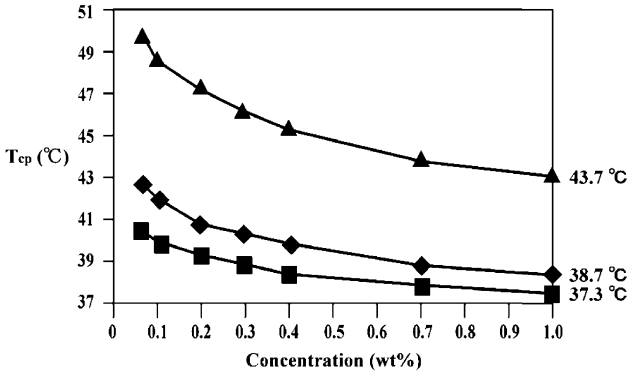


Figure 7. Relationship between cloud point (T_{cp}) and polymer concentration for three Me-P*i*PrOx-OH samples having different molecular weights (M_n = 4300 (▲), M_w/M_n = 1.03; M_n = 7800 (◆), M_w/M_n = 1.02; M_n = 9700 (■), M_w/M_n = 1.02; 10 mM PBS (pH = 7.4) containing 150 mM NaCl (rate: 0.2 deg/min; wavelength: 500 nm)).

dence on the solution concentration. For example, in the case of the polymer of M_n = 9700 and M_w/M_n = 1.02, the cloud point of its aqueous solution decreases from 39.8 to 37.3 °C, as the concentration increases from 0.1 to 1.0 wt %. The generality of this trend was confirmed by recording the concentration dependence of several poly(oxazolines) (Figure 6). For all polymer solutions, the cloud point tends to decrease with increasing concentration in the low concentration domain (0.1 to ~0.5 wt %) and to level off for solutions of higher concentration (Figure 7). This tendency becomes less pronounced as the molecular weight of the polymer increases. It is interesting to note that, in all cases, the cloud points of the poly(oxazolines) dissolved in phosphate-buffered saline remain slightly higher than the physiological temperature, opening up the possibility of controlling the temperature response of polyoxazoline aqueous solutions around physiological conditions via subtle changes in polymer molecular weight. Note that the cloud point (37.5 °C) of a 1.0 wt % solution of acetal-P*i*PrOx-OH (M_n = 9600, M_w/M_n = 1.15) is nearly identical to the cloud point of a Me-P*i*PrOx-OH (M_n = 9700, M_w/M_n = 1.02) solution measured under identical conditions (Figure 6), confirming that molecular weight is the determinant factor dictating the thermal response of poly(oxazolines).

Conclusions

The synthesis of novel thermosensitive telechelic poly(2-isopropyl-2-oxazolines) (P*i*PrOx) bearing different terminal ends (Me-P*i*PrOx-OH, Me-P*i*PrOx-NH_2, acetal-P*i*PrOx-OH) was successfully accomplished under optimum temperature conditions. The molecular weights of all Me-P*i*PrOx-OH samples were close to the theoretical value derived from the monomer/initiator ratio, with appreciably narrow molecular weight distributions, suggesting that the polymerization proceeded in a rather controlled manner without any noticeable side reactions. Furthermore, the successful conversion of Me-P*i*PrOx-OH into Me-P*i*PrOx-NH_2 opens up paths toward a variety of applications, such as the preparation of thermosensitive bioconjugates and the synthesis of biofunctional block copolymers, as they can serve to initiate the polymerization of a second monomer. The heterotelechelic acetal-P*i*PrOx-OH may also act as utility as hetero-cross-linkers in the preparation of thermosensitive conjugates of biomacromolecules, including proteins and nucleic acid derivatives.

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Supporting Information Available: Experimental procedure for the synthesis of 2-isopropyl-2-oxazoline and its characterization via ^{1}H, ^{13}C NMR spectra,^{1}H NMR spectra of Me-P*i*PrOx-OH and 3,3-diethoxy-1-propyl tosylate, ^{13}C NMR spectra of Me-P*i*PrOx-OH and acetal-P*i*PrOx-OH, and gel permeation chromatogram of acetal-P*i*PrOx-OH. This material is available free of charge via the Internet at http://pubs.acs.org.

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