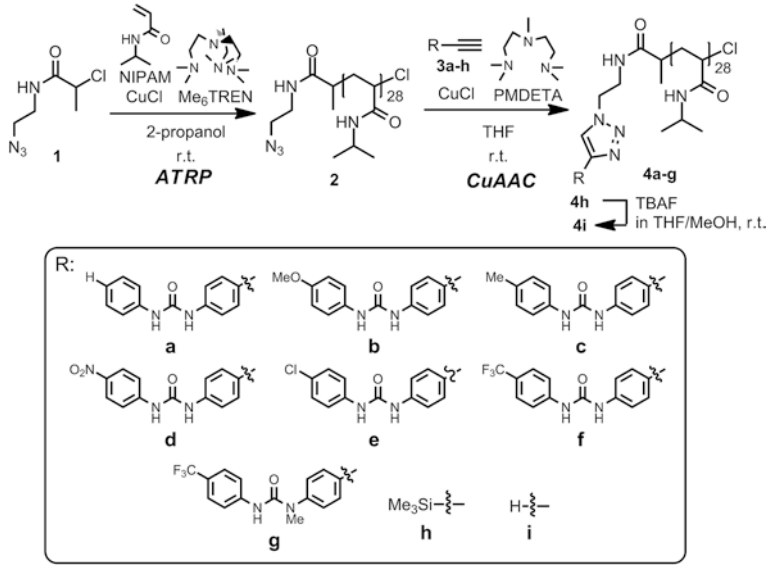
Chapter 2
Control of Thermoresponsive Properties of Urea End-Functionalized Poly(*N*-isopropylacrylamide) Based on the Hydrogen Bond Assisted Self-Assembly in Water

2.1 Introduction

The hydrogen bond has been recognized as one of the representative non-covalent interactions for realizing a three-dimensionally organized molecular architecture. In fact, biological macromolecular systems ingeniously construct specific higher-order structures that are stable in an aqueous medium, in which multiple hydrogen bonds are indispensable for stabilizing the structures. Although there are a number of artificial molecular architectures fabricated through hydrogen bonding in organic solvents, the realization of a supramolecular assembly based on hydrogen bonding in water has still remained an interesting issue because the intermolecular hydrogen bonds are easily disrupted in protic and polar solvents, such as water.

For the supramolecular assembly based on hydrogen bonding in water, the hydrophobic microenvironment approach that is inspired by a biological system have been developed for protecting the hydrogen bonding capability from disruptive solvation by water molecules, thus making it possible to construct a well-ordered self-assembly from synthetic molecules in water [1–3]. For example, Meijer et al. revealed that bifunctional ureidotriazines can self-assemble to form a helical supramolecular polymer in water through cooperative hydrogen bonds that are shielded by the hydrophobic microenvironment [4]. Kimizuka et al. achieved fabrication of a hierarchically self-assembled bilayer membrane in water through complementary hydrogen-bond pairs [5]. Sijbesma et al. further succeeded in the formation of the self-assembly from an amphiphilic triblock copolymer in water, in which urea groups that are placed at the center of a nonpolar segment contribute to the strong intermolecular hydrogen bonding [6]. Despite the definite validity of these approaches, the construction of stable intermolecular hydrogen bonding in water has still been a challenging task because an accurate and strict molecular design is required for the functional groups involved in the intermolecular hydrogen bonding, hydrophilic chains for water-solubility, and a hydrophobic microenvironment for realization of the hydrogen bonding properties. Additionally, only the construction of the self-assembly with a specific structure and morphology

K. Fuchise, *Design and Precise Synthesis of Thermoresponsive Polyacrylamides*, Springer Theses, DOI: 10.1007/978-4-431-55046-4_2, © Springer Japan 2014



Scheme 2.1 Synthesis of various urea end-functionalized PNIPAMs (**4a**–**g**) by a combination of the ATRP and the copper(I) catalyzed azide-alkyne cycloaddition (CuAAC)

has been achieved by the hydrogen bonding in water. Therefore, the control of the characteristic feature and function of the self-assembled polymer by adjusting its hydrogen bonding ability in water will be significant.

As one of the demonstrations of the macromolecular assembly based on the hydrogen bonding in water, the author now focuses on controlling the thermoresponsive properties of the urea end-functionalized poly(*N*-isopropylacrylamide) (PNIPAM) by adjusting the hydrogen bond formation between the chain end urea groups. PNIPAM is a representative thermoresponsive polymer and exhibits a coil to globule transition of the polymer structure in water at a temperature known as the lower critical solution temperature (LCST), which is generally around 32 °C [7]. Due to its versatility, many applications are expected in the diverse fields of material science including bioengineering [8–11] and nanotechnology [12–15]. Currently, it has been clarified that the cloud point of PNIPAM varies depending on its primary structure, such as molecular weight [16–18], stereoregularity [19–22], and chain end structure [17, 18, 23–26]. However, there is no report about the influence of the intermolecular hydrogen bonding of the end-functional group in PNIPAM on its cloud point.

In this chapter, the author describes the control of the cloud point of PNIPAM using the hydrogen bonding of urea groups in the polymer chain end. The well-defined PNIPAMs with urea groups as the end-functionality were prepared using a combination of the atom transfer radical polymerization (ATRP) and the copper(I) catalyzed azide-alkyne cycloaddition (CuAAC), as illustrated in Scheme 2.1. First, the azido end-functionalized PNIPAM (**2**) was synthesized

by the ATRP of NIPAM using *N*-(2-azidoethyl)-2-chloropropionamide (**1**). The CuAAC between **2** and the diphenylurea derivatives (**3a–f**) was subsequently conducted for conjugating the various urea groups, producing PNIPAMs bearing a series of chain end urea groups (**4a–f**). The urea groups to be introduced at the chain end of the PNIPAM were expected to impart a hydrogen bonding ability to the polymer, leading to its hierarchical self-assembly [6, 27]. Additionally, the diphenylurea derivatives were designed for facilitating the efficient formation of intermolecular hydrogen bonds between the urea groups in water. The relationship between the changes in the cloud point of the resulting polymers and the hydrogen bonding ability of the various urea groups was clarified by a turbidimetric analysis. In addition, in order to clarify the influence of the hydrogen bond on the cloud point, PNIPAMs bearing *N*-methylated urea (**4g**) and triazolyl groups (**4i)** were also synthesized, as shown in Schemes 2.1, and their cloud points were compared to those of **4a–f**. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) measurements were performed for the direct observation of the polymer assembly in water, providing an insight into the variation of the cloud point based on the supramolecular assembly.

2.2 Experimental Section

2.2.1 Materials

N-Isopropylacrylamide (NIPAM) was kindly supplied from the Kohjin Co., Japan, and recrystallized twice from hexane/toluene (10/1, v/v) prior to use. *N*-(2-Azidoethyl)-2-chloropropionamide (**1**) was prepared according to our previous report [18]. Tris[2-(dimethylamino)ethyl]amine (Me_6TREN) was donated by the Mitsubishi Chemical Co., Japan, and distilled over CaH_2 under reduced pressure. 2-Propanol (HPLC Grade), tetrahydrofuran (THF), phenyl isocyanate (98 %), dichloromethane, ethyl acetate, and acetone were purchased from Kanto Chemicals Co., Inc., and used as received. Acetonitrile was purchased from Kanto Chemicals, and dried over activated molecular sieves 4A for one day, and then distilled over CaH_2. 4-Ethynylaniline was purchased from Wako Pure Chemical Industries, Ltd., and used without further purification. *N*-Methyl-4-ethynylaniline was prepared according to the literature [28]. 4-(Trifluoromethyl) phenyl isocyanate (98 %), 4-methoxyphenyl isocyanate (98 %), 4-nitrophenyl isocyanate (98 %), trimethylsilylacetylene (98 %), and *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 98 %) were purchased from Tokyo Kasei Kogyo Co., Ltd., and used as received. 4-Methylphenyl isocyanate (98 %), 4-chlorophenyl isocyanate (98 %), tetra-*n*-butylammonium fluoride (TBAF) in THF (1.0 mol L^{-1}), and copper(I) chloride (CuCl, 99.999 %) were obtained from Aldrich Chemicals Co., Inc., and used as received.

2.2.2 Instruments

The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using a JEOL JNM-A400II instrument. The IR spectra were recorded using a Perkin-Elmer Paragon 1000 FT-IR instrument. Size exclusion chromatography (SEC) was performed at 40 °C using a Jasco high performance liquid chromatography (HPLC) system (PU-980 Intelligent HPLC pump, CO-965 column oven, RI-930 Intelligent RI detector, and Shodex DEGAS KT-16) equipped with a Shodex Asahipak GF-310 HQ column (linear, 7.6 mm × 300 mm; pore size, 20 nm; bead size, 5 μm; exclusion limit, 4×10^4) and a Shodex Asahipak GF-7M HQ column (linear, 7.6 mm × 300 mm; pore size, 20 nm; bead size, 9 μm; exclusion limit, 4×10^7) in *N*,*N*-dimethylformamide (DMF) containing lithium chloride (0.01 mol L^{-1}) at the flow rate of 0.4 mL min^{-1}. The number-average molecular weight ($M_{n,SEC}$) and polydispersity index (M_w/M_n) of the synthesized polymers were determined on the basis of a polystyrene calibration. The ultraviolet-visible (UV-vis) spectra were measured using a 10-mm path length cell by a Jasco V-550 spectrophotometer equipped with an EYELA NCB-1200 temperature controller and a Jasco ETC-505T temperature controller. Dynamic light scattering (DLS) was measured by an Otsuka Electronics FDLS-3000 light scattering spectrometer with an Otsuka Electronics NM-454L temperature controller. Transmission electron microscopy (TEM) was performed on a JEOL JEM-1230.

2.2.3 Synthesis of 1-(4-Ethynylphenyl)-3-phenylurea (3a)

Phenyl isocyanate (2.30 mL, 21.2 mmol) was added to a solution of 4-ethynylaniline (2.61 g, 22.3 mmol) in dry acetonitrile (47.5 mL) at room temperature under an N_2 atmosphere. The reaction mixture was refluxed for 5 h. After removal of the solvent, the residue was purified by column chromatography on silica gel with acetone/dichloromethane (1/14, v/v) to give **3a** as a solid. Yield = 4.48 g (90 %). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.04 (s, 1H, *H*C ≡ C–), 6.98 (t, J = 7.3 Hz, 1H, aromatic), 7.29 (t, J = 7.3 Hz, 2H, aromatic), 7.39 (d, J = 8.8 Hz, 2H, aromatic), 7.45 (d, J = 8.4 Hz, 2H, aromatic), 7.48 (d, J = 8.8 Hz, 2H, aromatic), 8.72 (s, 1H, –N*H*–CO–),8.87 (s, 1H, –N*H*–CO–). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 79.34, 83.74, 114.53, 117.87, 118.30, 122.01, 128.78, 132.43, 139.42, 140.38, 152.24. Anal. Calcd for $C_{15}H_{12}N_2O$ (236.27): C, 76.25; H, 5.12; N, 11.86. Found: C, 76.19; H, 5.12; N, 11.91.

2.2.4 Synthesis of 1-(4-Ethynylphenyl)-3-(4-methoxyphenyl) urea (3b)

A solution of 4-ethynylaniline (1.85 g, 15.8 mmol) in dry THF (15 mL) was added to a solution of 4-methoxyphenyl isocyanate (1.94 mL, 15.1 mmol) in dry THF (35 mL) at room temperature under an N_2 atmosphere. The reaction mixture

was stirred for 18 h. After removal of the solvent, **3b** was obtained as a solid. Yield = 2.79 g (69 %). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.72 (s, 3H, −OCH_3), 4.02 (s, 1H, HC ≡ C–), 6.86 (d, 2H, J = 8.9 Hz, aromatic), 7.35 (d, J = 8.9 Hz, 2H, aromatic), 7.38 (d, J = 8.4 Hz, 2H, aromatic), 7.47 (d, J = 8.4 Hz, 2H, aromatic), 8.52 (s, 1H, –NH–CO–), 8.79 (s, 1H, –NH–CO–). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 55.13, 79.21, 83.77, 113.97, 114.29, 117.74, 120.16, 132.38, 140.57, 152.41, 154.49. Anal. Calcd for $C_{16}H_{14}N_2O_2$ (266.29): C, 72.16; H, 5.30; N, 10.52. Found: C, 71.90; H, 5.36; N, 10.50.

2.2.5 Synthesis of 1-(4-Ethynylphenyl)-3-(4-methylphenyl) urea (3c)

A solution of 4-ethynylaniline (1.97 g, 16.8 mmol) in dry THF (23 mL) was added to a solution of 4-methylphenyl isocyanate (2.01 mL, 16.0 mmol) in dry THF (30 mL) at room temperature under an N_2 atmosphere. The reaction mixture was stirred for 18 h. After removal of the solvent, **3c** was obtained as a solid. Yield = 3.64 g (91 %). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.24 (s, 3H, −CH_3), 4.02 (s, 1H, HC ≡ C–), 7.08 (d, J = 8.4 Hz, 2H, aromatic), 7.33 (d, J = 8.4 Hz, 2H, aromatic), 7.38 (d, J = 8.9 Hz, 2H, aromatic), 7.46 (d, J = 8.9 Hz, 2H, aromatic), 8.61 (s, 1H, –NH–CO–), 8.82 (s, 1H, –NH–CO–). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 20.29, 79.25, 83.74, 114.39, 117.79, 118.39, 129.14, 130.85, 132.39, 136.82, 140.46, 152.27. Anal. Calcd for $C_{16}H_{14}N_2O$ (250.29): C, 76.78; H, 5.64; N, 11.19. Found: C, 76.83; H, 5.78; N, 11.16.

2.2.6 Synthesis of 1-(4-Ethynylphenyl)-3-(4-nitrophenyl) urea (3d)

A solution of 4-ethynylaniline (2.30 g, 19.6 mmol) in dry THF (15 mL) was added to a solution of 4-nitrophenyl isocyanate (2.92 g, 17.8 mmol) in dry THF (35 mL) at room temperature under an N_2 atmosphere. The reaction mixture was stirred for 43 h. After removal of the solvent, dichloromethane was added to the residue. The soluble part was filtered and washed with 1 mol L^{-1} HCl. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by recrystallization from acetonitrile to give **3d** as a yellow solid. Yield = 2.67 g (54 %). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.07 (s, 1H, HC ≡ C−), 7.42 (d, J = 8.8 Hz, 2H, aromatic), 7.50 (d, J = 8.8 Hz, 2H, aromatic), 7.69 (d, J = 9.3 Hz, 2H, aromatic), 8.20 (d, J = 9.3 Hz, 2H, aromatic), 9.12 (s, 1H, –NH–CO–), 9.48 (s, 1H, –NH–CO–). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 79.65, 83.58, 115.30, 117.51, 118.34, 125.13, 132.47, 139.67, 141.15, 146.12, 151.75. Anal. Calcd for $C_{15}H_{11}N_3O_3$ (281.27): C, 64.05; H, 3.94; N, 14.94. Found: C, 63.76; H, 4.01; N, 14.93.

2.2.7 Synthesis of 1-(4-Ethynylphenyl)-3-(4-chlorophenyl) urea (3e)

A solution of 4-ethynylaniline (1.82 g, 15.5 mmol) in dry THF (20 mL) was added to a solution of 4-chlorophenyl isocyanate (2.27 g, 14.8 mmol) in dry THF (35 mL) at room temperature under an N_2 atmosphere. The reaction mixture was stirred overnight. After removal of the solvent, the residue was stirred in 1 mol L^{-1} HCl, and then filtered. The filtrate was dried in vacuo to give **3e** as a solid. Yield = 3.52 g (87 %). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.02 (s, 1H, *H*C ≡ C−), 7.33 (d, J = 8.9 Hz, 2H, aromatic), 7.40 (d, J = 8.8 Hz, 2H, aromatic), 7.48 (d, J = 8.8 Hz, 2H, aromatic), 7.49 (d, J = 9.1 Hz, 2H, aromatic), 8.88 (s, 1H, –N*H*–CO–), 8.93 (s, 1H, –N*H*–CO–). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 79.32, 83.71, 114.73, 118.00, 119.83, 125.58, 128.60, 132.43, 138.45, 140.21, 152.17. Anal. Calcd for $C_{15}H_{11}N_2OCl$ (270.71): C, 66.55; H, 4.10; N, 10.35; Cl, 13.10. Found: C, 66.54; H, 4.13; N, 10.28; Cl, 13.13.

2.2.8 Synthesis of 1-(4-Ethynylphenyl)-3-[4-(trifluoromethyl) phenyl]urea (3f)

A solution of 4-ethynylaniline (1.55 g, 17.2 mmol, 1.05 eq) in dry THF (20 mL) was added to a solution of 4-(trifluoromethyl)phenyl isocyanate (2.45 mL, 16.4 mmol) in dry THF (35 mL) at room temperature under an N_2 atmosphere. The reaction mixture was stirred for 14 h. After removal of the solvent, the residue was dissolved in ethyl acetate and washed with 1 mol L^{-1} NaOH, followed by water. The organic layer was dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by recrystallization from acetonitrile to give **3f** as a solid. Yield = 3.26 g (65 %). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 4.06 (s, 1H, *H*C ≡ C−), 7.41 (d, J = 8.8 Hz, 2H, aromatic), 7.49 (d, J = 8.8 Hz, 2H, aromatic), 7.64 (d, J = 9.4 Hz, 2H, aromatic), 7.67 (d, J = 9.0 Hz, 2H, aromatic), 9.01 (s, 1H, –N*H*–CO–), 9.16 (s, 1H, –N*H*–CO–). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 79.40, 83.64, 114.99, 117.95, 118.15, 121.97 (q, J = 32 Hz), 124.49 (q, J = 270 Hz), 126.02 (q, J = 3.5 Hz), 132.43, 139.98, 143.21, 152.04. Anal. Calcd for $C_{16}H_{11}N_2OF_3$ (304.27): C, 63.16; H, 3.64; N, 9.21. Found: C, 62.96; H, 3.76; N, 9.06.

2.2.9 Synthesis of 1-(4-Ethynylphenyl)-1-methyl-3-[4-(trifluoromethyl)phenyl]urea (3g)

4-(Trifluoromethyl)phenyl isocyanate (0.69 mL, 4.91 mmol) was added to a solution of *N*-methyl-4-ethynylaniline (0.57 g, 4.35 mmol) in dry THF (20 mL) at room temperature under an N_2 atmosphere. The reaction mixture was then stirred for 5 h. After removal of the solvent, the residue was dissolved in ethyl acetate and

washed with 1 mol L^{-1} HCl, followed by water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography on silica gel with dichloromethane to provide **3g** as a solid. Yield = 0.86 g (63 %). ^{1}H NMR (DMSO-d_6, 400 MHz, δ): 3.30 (s, 3H, –N–CH_3), 4.20 (s, 1H, *H*C ≡ C-), 7.34 (d, J = 8.6 Hz, 2H, aromatic), 7.50 (d, J = 8.5 Hz, 2H, aromatic), 7.58 (d, J = 8.8 Hz, 2H, aromatic), 7.67 (d, J = 8.8 Hz, 2H, aromatic), 8.80 (s, 1H, –N*H*–CO–). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 37.25, 80.67, 83.16, 118.75, 119.29, 122.01 (q, J = 32 Hz), 124.54 (q, J = 270 Hz), 125.99 (q, J = 4.1 Hz), 132.57, 143.87, 144.18, 154.20. Anal. Calcd for $C_{17}H_{13}N_2OF_3$ (318.29): C, 64.15; H, 4.12; N, 8.80; Found: C, 64.23; H, 4.13; N, 8.84.

2.2.10 Synthesis of Azido End-Functionalized PNIPAM (2) by the ATRP

NIPAM (50.0 g, 442 mmol) and CuCl (1.75 g, 17.7 mmol) were added to a round bottom flask. The flask was capped with a septum, and purged with argon. Degassed 2-propanol (116 mL), Me_6TREN in degassed 2-propanol (6.00 mL, 17.7 mmol, 2.95 mol L^{-1}), and **1** in degassed 2-propanol (6.00 mL, 17.7 mmol, 2.95 mol L^{-1}) were then sequentially added. The reaction mixture was stirred for 4 h at room temperature. The polymerization was quenched by exposure to air. Aliquots were removed from the reaction mixture to determine the conversion of NIPAM from the ^{1}H NMR spectrum (97.1 %). The reaction mixture was diluted with THF and passed through a short column of silica gel to remove the copper complex. The obtained solution was dialyzed using a cellophane tube (Spectra/Por® 6 Membrane; MWCO: 1000) in methanol and finally reprecipitated from THF into hexane to provide the azido end-functionalized PNIPAM (**2**) as a white solid. Yield = 23.8 g (45 %). $M_{n,NMR}$ = 3.3 kg mol^{-1}, $M_{n,SEC}$ = 5.7 kg mol^{-1}, M_w/M_n = 1.19.

2.2.11 The CuAAC of 2 and Alkyne Compounds for the Synthesis of End-Functionalized PNIPAMs (4a–h)

A typical procedure for the CuAAC is as follows, a solution of CuCl (70.2 mg, 0.708 mmol) and PMDETA (296 μL, 1.42 mmol) in degassed THF (0.50 mL) was added to a solution of **2** (600 mg, 0.182 mmol) and **3f** (215 mg, 0.708 mmol) in degassed THF (7.00 mL) under an argon atmosphere. After stirring for 7 h at room temperature, the reaction mixture was diluted with THF and then passed through a short column of silica gel to remove the copper complex. The obtained solution was dialyzed using a cellophane tube (Spectra/Por® 6 Membrane; MWCO: 1000) in methanol and finally freeze-dried from water to give the 4-(trifluoromethyl) phenyl urea end-functionalized PNIPAM (**4f**) as a white solid. Yield = 322 mg

(54 %). $M_{n,SEC} = 8.9$ kg mol^{-1}, $M_w/M_n = 1.17$. The reactions using other alkynes were carried out with the same procedures.

2.2.12 Synthesis of Triazolyl End-Functionalized PNIPAM (4i) from 4h

TBAF in THF (1.50 mL, 1.50 mmol, 1.0 mol L^{-1}) was added to a solution of trimethylsilyl end-functionalized PNIPAM (**4h**) (182 mg, 55.2 μmol) in dry THF (1.25 mL) and methanol (1.00 mL) at room temperature under an N_2 atmosphere [29]. After stirring overnight, the reaction mixture was diluted with THF then passed through a short column of silica gel. The obtained solution was dialyzed using a cellophane tube (Spectra/Por® 6 Membrane; MWCO: 1000) in methanol followed by water and finally freeze-dried to give the polymer **4i** as a white solid. Yield = 51.4 mg (29 %). $M_{n,SEC} = 5.9$ kg mol^{-1}, $M_w/M_n = 1.22$.

2.2.13 Turbidimetric Analysis

A typical procedure for the turbidimetric analysis is as follows: an aqueous solution of a polymer sample (2.0 mg mL^{-1}) was prepared and cooled in an ice bath for 2 min. The resulting clear solution was then transferred to a poly(methyl methacrylate) cell with a 1-cm path length. The transmittance at 500 nm of the aqueous solution was recorded by a UV-vis spectrophotometer equipped with a temperature controller. The solution temperature was gradually increased at the heating rate of 1.0 °C min^{-1}.

2.2.14 Dynamic Light Scattering Measurement

The hydrodynamic diameter (D_h) and size distribution for the polymer aggregated in an aqueous solution were determined by dynamic light scattering (DLS) at the fixed scattering angle of 90°. The polymer sample for the measurement was dissolved in deionized water at room temperature to prepare an aqueous solution (0.20 mg mL^{-1}). All samples were first filtered through a 0.45 μm membrane filter. The measurement was carried out after the sample stood for at least 1 h at 25 °C. The D_h and particle size distribution were calculated by the CONTIN analysis.

2.2.15 Transmission Electron Microscopy Measurement

To prepare a sample for the TEM measurement, an aqueous solution of **4f** (0.20 mg mL^{-1}) was stirred for 72 h at room temperature in order to reach thermodynamic equilibrium. A drop of the polymer solution was then deposited onto a

200-mesh carbon-coated copper grid and dried overnight in a desiccator to achieve dryness. The TEM measurement was performed on the obtained polymer sample at the acceleration voltage of 100 kV.

2.3 Results and Discussion

2.3.1 Synthesis of Diphenylurea End-Functionalized PNIPAMs

In this study, the author attempted to compare the thermoresponsive properties of a series of urea end-functionalized PNIPAMs with the same number-average degree of polymerization in order to accurately evaluate the effect of the terminal urea groups on the thermoresponsive properties of PNIPAM by excluding interference from other factors. Thus, the synthesis of the urea end-functionalized PNIPAMs was carried out by following the synthetic strategy for end-functionalized PNIPAMs previously demonstrated by Kakuchi et al., which is based on (i) the synthesis of azido end-functionalized PNIPAM by the atom transfer radical polymerization (ATRP) of NIPAM using an initiator bearing an azido group, and (ii) the CuAAC reaction between the resultant azido end-functionalized PNIPAM and an alkyne compound, as shown in Scheme 2.1 [18]. In principle, a variety of end-functionalized PNIPAMs with the same number-average degree of polymerization is obtained when the same azido end-functionalized PNIPAM is used as the starting material. First, the ATRP of NIPAM was carried out using *N*-(2-azidoethyl)-2-chloropropionamide (**1**) as the initiator and CuCl/Me_6TREN as the catalyst system to afford the azido end-functionalized PNIPAM (**2**), which was a precursor polymer for further urea conjugation. The number-average molecular weight determined by a ^{1}H NMR measurement ($M_{n,NMR}$) of the resulting product was 3.3 kg mol^{-1} and in good agreement with the theoretical value of 2.9 kg mol^{-1}. The number-average degree of polymerization for the obtained **2** was determined to be 27.6 from the $M_{n,NMR}$. The polydispersity index (M_w/M_n) of the obtained **2** was estimated to be 1.19 as the result of the SEC measurement in DMF containing 0.01 mol L^{-1} of LiCl using narrow-dispersed polystyrene as the calibration standard. Subsequently, the CuAAC between **2** and various diphenylurea derivatives (**3a**–**f**) was carried out in THF at room temperature using the CuCl/PMDETA complex as the catalyst system. **3a**–**f** were synthesized from 4-ethynylaniline and various isocyanates. Completion of the reaction was confirmed by the ^{1}H NMR and IR analyses. Figures 2.1 and 2.2 show the change in the NMR and IR spectra of **2** and **3f** as an example. The signal due to the two methylene groups at the chain end of **2** at 3.49 ppm, signals *g* and *f*, was not observed in the ^{1}H NMR spectrum of **4f**, as shown in Fig. 2.1. Instead, those of the methylene group adjacent to the triazolyl group, signal h, as well as the aromatic groups, signal aromatic, were observed at 4.6 ppm and 7.4–8.2 ppm, respectively. The absorption of the azido group originally observed at 2,102 cm^{-1} in the IR

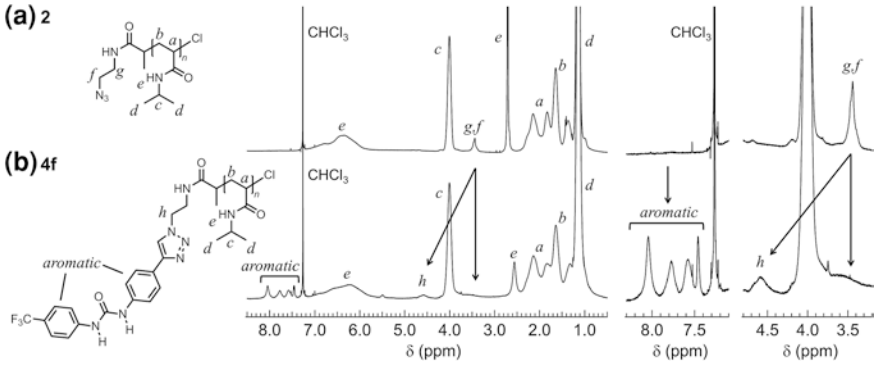


Fig. 2.1 ^{1}H NMR spectra of **a 2** and **b 4f** in $CDCl_3$

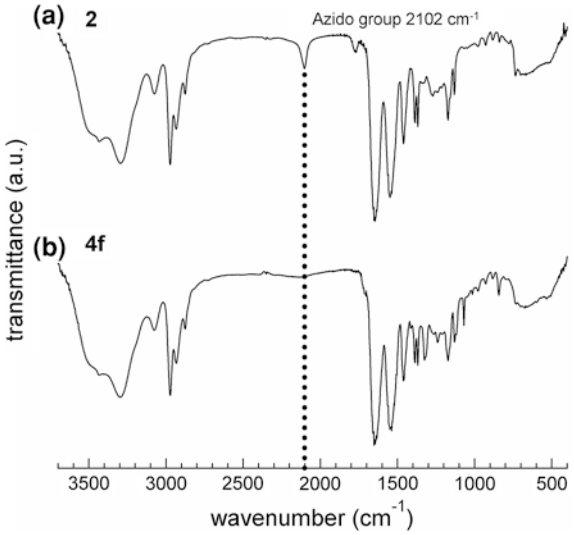


Fig. 2.2 IR spectra of **a 2** and **b 4f**

spectrum of **2** disappeared in the spectrum of **4f**, as shown in Fig. 2.2. The same change was observed for the obtained **3a**–**e**. Thus, the precise synthesis of **3a**–**f** was achieved by the combination of the ATRP and the CuAAC.

Moreover, the CuAAC of **2** and **3g**, namely, the *N*-methylated analogue of **3f**, was carried out to produce **4g** in order to elucidate the contribution of the hydrogen bonding ability of the terminal urea groups to the change in the thermoresponsive properties of PNIPAM. The hydrogen bonding ability of the terminal urea group in **4g** was expected to be weaker than that of **4f**. Completion of the reactions was confirmed by the ^{1}H NMR and IR analyses. Furthermore, the triazolyl end-functionalized PNIPAM (**4i**) as the control polymer was synthesized by the CuAAC between **2** and trimethylsilylacetylene followed by desilylation with TBAF in

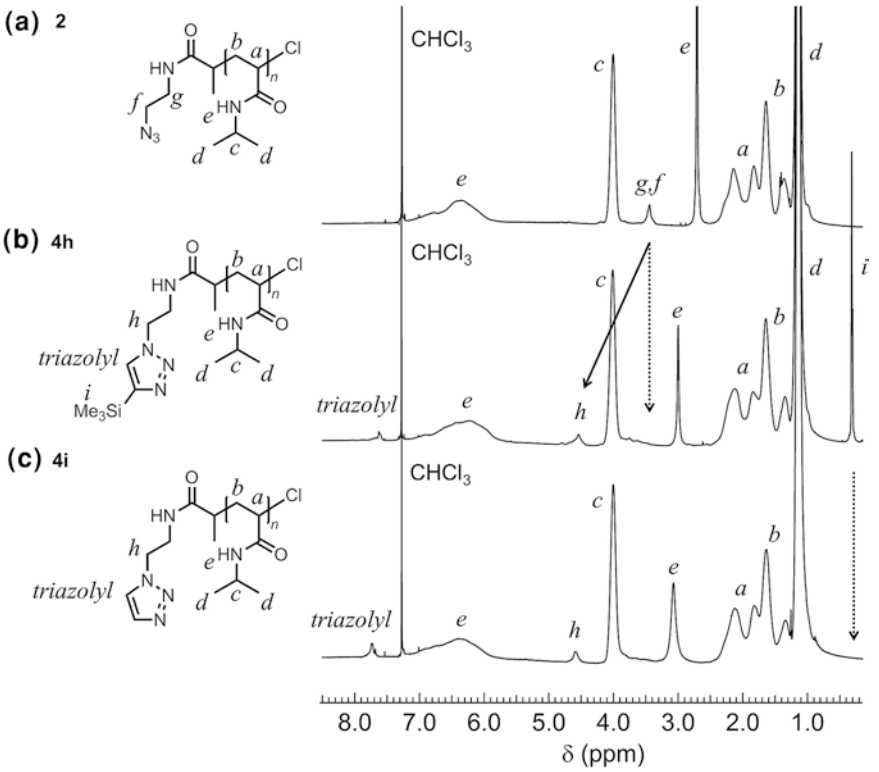


Fig. 2.3 ^{1}H NMR spectra of **2 (a)**, **4h (b)**, and **4i c** in $CDCl_3$

order to evaluate the effect of the diphenylurea moiety on the thermoresponsive properties of the urea end-functionalized PNIPAMs. Completion of the two reactions was confirmed by the ^{1}H NMR analyses, as shown in Fig. 2.3. The signal due to the two methylene groups at the chain end of **2** disappeared in the ^{1}H NMR spectrum of **4h**. Instead, the signal due to the triazolyl group, signal *triazolyl*, and the trimethylsilyl group, signal *i*, were observed at 7.6 and 0.3 ppm, respectively. The signal due to the trimethylsilyl group disappeared in the spectrum of **4i**. In summary, urea end-functionalized PNIPAMs (**4a**–**i**) with the same number-average degree of polymerization were successfully synthesized by the CuAAC between the PNIPAM precursor **2** and various urea derivatives.

2.3.2 Effect of the Terminal Urea Group on the Thermoresponsive Properties of PNIPAM

The thermoresponsive properties of a series of PNIPAMs with different terminal structures were characterized by a turbidimetric analysis, as shown in Fig. 2.4. Every end-functionalized PNIPAM showed the characteristic phase transition

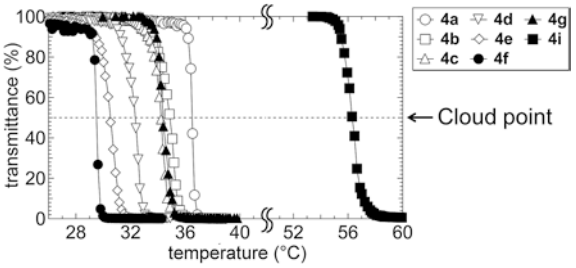
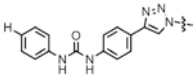
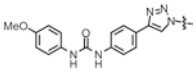
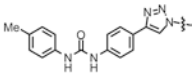
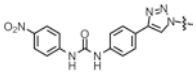
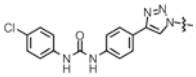
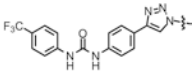
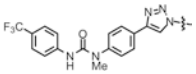


Fig. 2.4 Transmittance ($\lambda = 500$ nm) versus temperature for aqueous solutions of **4a**–**g** and **i** (2.0 mg mL^{-1})

Table 2.1 Cloud points of the obtained end-functionalized PNIPAMs (**4a**–**g** and **i**)

Polymer	Terminal structure	Cloud point (°C)[a]
4a		36.5
4b		34.8
4c		34.3
4d		32.4
4e		30.6
4f		29.5
4 g		34.4
4i		56.3

[a]Determined by turbidimetric analysis
Conditions: concentration = 2.0 mg mL^{-1}, heating rate = 1.0 °C min^{-1}, cloud point was determined by the temperature which the transmittance ($\lambda = 500$ nm) of aqueous solution reach 50 %

phenomenon at a temperature specific to each polymer sample. The cloud point was defined as the temperature at which the transmittance of the sample solution reached 50 %. Table 2.1 summarizes the cloud points of all the polymers. The cloud points of **4a**–**f** ranged from 29.5 to 36.5 °C, whereas **4i**, the control polymer, had a cloud point at 56.3 °C. Thus, the introduction of the diphenylurea group to the chain end of PNIPAM caused more than a 20 °C change in the drastic decrease of the cloud point.

A significant variation in the cloud point was observed for **4a**–**f**, though only the substituent group in the terminal diphenylurea group is the difference among the polymer structures. **4d**, **4e**, and **4f** bearing an electron withdrawing group, namely a nitro group, a chlorine atom, and a trifluoromethyl group, in the urea group showed cloud points at 32.4, 30.6, and 29.5 °C, respectively. In contrast, higher cloud points of 34.8 and 34.3 °C were observed for **4b** and **c** bearing an electron donating group, namely a methoxy group and a methyl group, in the urea group. Therefore, the variation in the cloud point was found to obviously correlate with the substituent effect, though the exceptionally high cloud point of **4a**, 36.5 °C, cannot be explained only by such an effect. Given that the substituent effect of the aromatic ring directly influences the electronic properties of the neighboring urea group, these results suggest that such variations in the cloud point should be ascribable to the hydrogen bonding ability of the terminal urea functionality.

In order to provide further insight into the relationship between the hydrogen bonding ability and the cloud point, the thermoresponsive behavior of **4g**, of which the hydrogen bonding ability was essentially inhibited, was examined. Despite the very small difference in the structure, the cloud point of **4g** was 5 °C higher than that of **4f**. The cloud point of **4g** should be lower than that of **4f** or at least unchanged if the cloud point varies only by the hydrophobicity of the polymer structure [17, 18]. Thus, this comparison well demonstrated the importance of the hydrogen bonding ability on the variation in the cloud point. Based on all the results regarding the thermoresponsive properties, the hydrogen bonds derived from the terminal urea groups can be concluded to efficiently work even in water, leading to a decrease in the cloud point.

2.3.3 Aggregation State of Urea End-Functionalized PNIPAM in Water

In the previous section, the robust and efficient hydrogen bonding was revealed to be actually present even in water for the series of urea end-functionalized PNIPAMs. Given that hydrogen bonding of the urea groups realizes a continuous and hierarchical intermolecular interaction, the polymers should self-assemble in water even at a temperature below the cloud point. Thus, the ^{1}H NMR measurements of **4f** and **g** were carried out in D_2O at 25 °C to elucidate the aggregation behavior of the polymers, as shown in Fig. 2.5. No significant difference was observed in the signals originated from the main chain protons of **4f** and **g**. Moreover, a direct investigation of the urea protons was unavailable because of the rapid exchange with the deuterium atoms of D_2O. An obvious difference was observed in the signals due to the aromatic protons at the chain end, which appeared between 6.8 and 8.0 ppm. The signals due to the aromatic protons of **4f** were broad, while those of **4g** were observed as sharp peaks. Therefore, the peak broadening was found to closely correlate with the hydrogen bonding ability of the

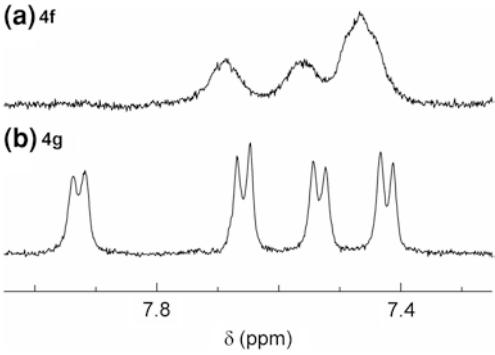


Fig. 2.5 ^{1}H NMR spectra of (**a**) **4f** and (**b**) **4g** in D_2O (2.0 mg mL^{-1}) at 25 °C

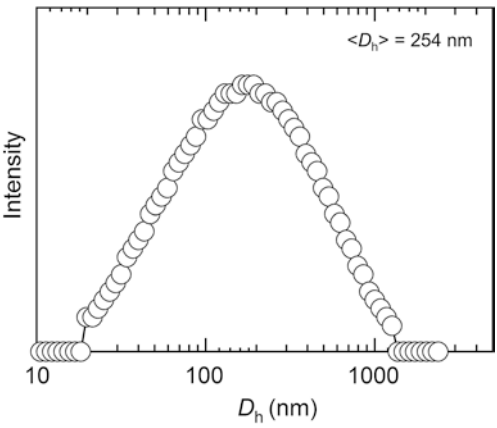


Fig. 2.6 Intensity weighted size distributions of **4f** in water (0.20 mg mL^{-1}) at 25 °C obtained by the DLS measurement using the CONTIN analysis

urea moiety. These results indicate that the mobility of the chain ends for **4f** was probably restricted due to the strong hydrogen bonding ability of terminal urea group, implying that the polymer chains assemble via intermolecular hydrogen bonding even at a temperature below the cloud point.

In order to prove the existence of the polymer assemblies in water, DLS and TEM measurements were carried out for **4f** in water, which was considered to have the highest hydrogen bonding ability in the series of urea end-functionalized PNIPAMs. The DLS measurement was conducted using aqueous solutions of **4f** (0.20 mg mL^{-1}) at 25 °C. The result was analyzed using the CONTIN method, and Fig. 2.6 depicts the obtained particle size distribution. From the DLS analysis, **4f** was found to actually form the aggregation under this condition, of which the average hydrodynamic diameter ($\langle D_h \rangle$) was determined to be 254 nm. It should be noted that the thermoresponsive behavior of PNIPAM itself is excluded from the driving force for such an aggregation process because the measurement was carried out at a temperature below the cloud point of **4f**. Thus, this result indicates that the strong hydrogen bonding ability of the terminal urea group on **4f** enabled the formation of the aggregates in water. However, the broad size distribution suggested a multiplicity of the aggregation morphology.

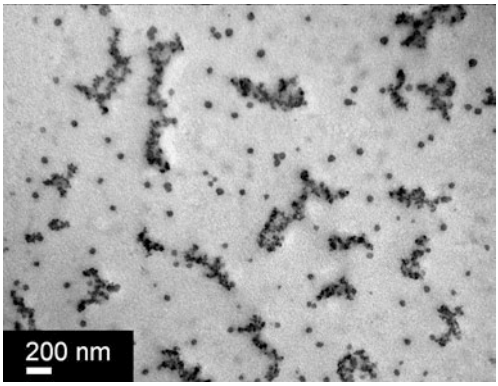


Fig. 2.7 TEM micrograph of aggregates in the aqueous solution of **4f** (0.20 mg mL^{-1}) at room temperature

For the direct observation of the aggregate of **4f** in water, the TEM measurement was carried out, in which the sample was prepared from an aqueous solution of **4f** (0.20 mg mL^{-1}) at room temperature. The resulting TEM image showed spherical nanoparticles distributed over the entire area, as shown in Fig. 2.7. Each particle was considered to be an aggregate of **4f**, of which the diameter ranged from 35 to 45 nm. Interestingly, part of the nanoparticles seemed to further assemble and form larger aggregates with diameters greater than 100 nm, which should correspond to the bigger scatterers observed in the DLS measurement. These results substantiated that **4f** supramolecularly assembled in water through the intermolecular hydrogen bonding of the chain end urea groups.

2.4 Conclusions

The author has demonstrated an adjustment of the thermoresponsive properties of PNIPAM through the hydrogen bonding in water by introducing a series of diphenylurea groups to the chain end. The combination of the ATRP and the CuAAC was employed for preparing the urea end-functionalized PNIPAMs with the same number-average degree of polymerization and different substituent groups in the urea group, which allowed evaluating the effect of the introduction of the urea group on the thermoresponsive properties of PNIPAM while excluding all other factors that influence the thermoresponsive properties. For the series of urea end-functionalized PNIPAMs, the intermolecular hydrogen bonding of the chain end urea group was revealed to work even in water, leading to the self-assembly of the polymer chains at a temperature below the cloud point. Such an antecedent aggregation made it possible to facilitate the phase transition process, resulting in the drastic decrease of the cloud point. Thus, the present study realized the control of the macromolecular function by the hydrogen bonding formation in water for the first time.

References

1. Paleos CM, Tsiourvas D (1997) Molecular recognition of organized assemblies via hydrogen bonding in aqueous media. Adv Mater 9:695–710
2. Fenniri H, Packiarajan M, Vidale KL, Sherman DM, Hallenga K, Wood KV, Stowell JG (2001) Helical rosette nanotubes: Design, self-assembly, and characterization. J Am Chem Soc 123:3854–3855
3. Jung JH, John G, Masuda M, Yoshida K, Shinkai S, Shimizu T (2001) Self-assembly of a sugar-based gelator in water: Its remarkable diversity in gelation ability and aggregate structure. Langmuir 17:7229–7232
4. Brunsveld L, Vekemans J, Hirschberg J, Sijbesma RP, Meijer EW (2002) Hierarchical formation of helical supramolecular polymers via stacking of hydrogen-bonded pairs in water. Proc Natl Acad Sci USA 99:4977–4982
5. Kawasaki T, Tokuhiro M, Kimizuka N, Kunitake T (2001) Hierarchical self-assembly of chiral complementary hydrogen-bond networks in water: Reconstitution of supramolecular membranes. J Am Chem Soc 123:6792–6800
6. Chebotareva N, Bomans PHH, Frederik PM, Sommerdijk N, Sijbesma RP (2005) Morphological control and molecular recognition by bis-urea hydrogen bonding in micelles of amphiphilic tri-block copolymers. Chem Commun 4967–4969
7. Schild HG (1992) Poly(*N*-isopropylacrylamide): experiment, theory and application. Prog Polym Sci 17:163–249
8. Coughlan DC, Quilty FP, Corrigan OI (2004) Effect of drug physicochemical properties on welling/deswelling kinetics and pulsatile drug release from thermoresponsive poly(*N*-isopropylacrylamide) hydrogelsJ Control Rel 98:97–114
9. Tsuda Y, Yamato M, Kikuchi A, Watanabe M, Chen GP, Takahashi Y, Okano T (2007) Thermoresponsive microtextured culture surfaces facilitate fabrication of capillary networks. Adv Mater 19:3633–3636
10. De P, Li M, Gondi SR, Sumerlin BS (2008) Temperature-regulated activity of responsive polymer−protein conjugates prepared by grafting-from via RAFT polymerization. J Am Chem Soc 130:11288–11289
11. Klouda L, Mikos AG (2008) Thermoresponsive hydrogels in biomedical applications - a review. Eur J Pharm Biopharm 68:34–45
12. Ito T, Hioki T, Yamaguchi T, Shinbo T, Nakao S, Kimura S (2002) Development of a molecular recognition ion gating membrane and estimation of its pore size control. J Am Chem Soc 124:7840–7846
13. Nykanen A, Nuopponen M, Laukkanen A, Hirvonen SP, Rytela M, Turunen O, Tenhu H, Mezzenga R, Ikkala O, Ruokolainen J (2007) Phase Behavior and temperature-responsive molecular filters based on self-assembly of polystyrene-*block*-poly(*N*-isopropylacrylamide)-*block*-polystyrene. Macromolecules 40:5827–5834
14. Tokuyama H, Iwama T (2007) Temperature-swing solid-phase extraction of heavy metals on a poly(*N*-isopropylacrylamide) hydrogel. Langmuir 23:13104–13108
15. Wang H, An YL, Huang N, Ma RJ, Li JB, Shi LQ (2008) Contractive polymeric complex micelles as thermo-sensitive nanopumps. Macromol Rapid Commun 29:1410–1414
16. Kujawa P, Segui F, Shaban S, Diab C, Okada Y, Tanaka F, Winnik FM (2006) Impact of end-group association and main-chain hydration on the thermosensitive properties of hydrophobically modified telechelic poly(*N*-isopropylacrylamides) in water. Macromolecules 39:341–348
17. Xia Y, Burke NAD, Stöver HDH (2006) End group effect on the thermal response of narrow-disperse poly(*N*-isopropylacrylamide) prepared by atom transfer radical polymerization. Macromolecules 39:2275–2283
18. Narumi A, Fuchise K, Kakuchi R, Toda A, Satoh T, Kawaguchi S, Sugiyama K, Hirao A, Kakuchi T (2008) A versatile method for adjusting thermoresponsivity: synthesis and ‘click’ reaction of an azido end-functionalized poly(*N*-isopropylacrylamide). Macromol Rapid Commun 29:1126–1133

19. Ray B, Isobe Y, Matsumoto K, Habaue S, Okamoto Y, Kamigaito M, Sawamoto M (2004) Macromolecules 37:1702–1710
20. Ray B, Okamoto Y, Kamigaito N, Sawamoto M, Seno K, Kanaoka S, Aoshima S (2005) Effect of tacticity of poly(*N*-isopropylacrylamide) on the phase separation temperature of its aqueous solutions. Polym J 37:234–237
21. Hirano T, Okumura Y, Kitajima H, Seno M, Sato T (2006) Dual roles of alkyl alcohols as syndiotactic-specificity inducers and accelerators in the radical polymerization of *N*-isopropylacrylamide and some properties of syndiotactic poly(*N*-isopropylacrylamide). J Polym Sci, Part A: Polym Chem 44:4450–4460
22. Katsumoto Y, Kubosaki N (2008) Tacticity effects on the phase diagram for poly(*N*-isopropylacrylamide) in water. Macromolecules 41:5955–5956
23. Duan Q, Miura Y, Narumi A, Shen X, Sato S, Satoh T, Kakuchi T (2006) Synthesis and thermoresponsive property of end-functionalized poly(*N*-isopropylacrylamide) with pyrenyl group. J Polym Sci, Part A: Polym Chem 44:1117–1124
24. Duan Q, Narumi A, Miura Y, Shen XD, Sato S-I, Satoh T, Kakuchi T (2006) Thermoresponsive property controlled by end-functionalization of poly(*N*-isopropylacrylamide) with phenyl, biphenyl, and triphenyl groups. Polym J 38:306–310
25. Furyk S, Zhang YJ, Ortiz-Acosta D, Cremer PS, Bergbreiter DE (2006) Effects of end group polarity and molecular weight on the lower critical solution temperature of poly(*N*-isopropylacrylamide). J Polym Sci, Part A: Polym Chem 44:1492–1501
26. Kujawa P, Tanaka F, Winnik FM (2006) Temperature-dependent properties of telechelic hydrophobically modified poly(*N*-isopropylacrylamides) in water: Evidence from light scattering and fluorescence spectroscopy for the formation of stable mesoglobules at elevated temperatures. Macromolecules 39:3048–3055
27. Obert E, Bellot M, Bouteiller L, Andrioletti F, Lehen-Ferrenbach C, Boué F (2007) Both water- and organo-soluble supramolecular polymer stabilized by hydrogen-bonding and hydrophobic interactions. J Am Chem Soc 129:15601–15605
28. Qu WC, Kung MP, Hou C, Oya S, Kung HF (2007) Quick assembly of 1,4-diphenyltriazoles as probes targeting beta-amyloid aggregates in Alzheimer's disease. J Med Chem 50:3380–3387
29. Chan DCM, Laughton CA, Queener SF, Stevens MFG (2002) Structural studies on bioactive compounds. part 36: design, synthesis and biological evaluation of pyrimethamine-based antifolates against *Pneumocystis carinii*. Bioorg Med Chem 10:3001–3011

http://www.springer.com/978-4-431-55045-7

Design and Precise Synthesis of Thermoresponsive Polyacrylamides

Fuchise, K.

2014, XVIII, 81 p. 60 illus., 6 illus. in color., Hardcover
ISBN: 978-4-431-55045-7