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Toward Functionalization of Thermoresponsive Poly(*N*-vinyl-2-pyrrolidone)

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ABSTRACT: A series of new monomers with different substituents at the 3-position of *N*-vinyl-2-pyrrolidone (NVP) were synthesized. The substituents include simple alkyl (methyl, ethyl, propyl, and isopropyl), ether (methoxy ethyl and ethoxy ethyl), and functional groups (e.g., aldehyde, epoxy, and acetylene). These monomers were (co)polymerized radically to produce a family of (co)polymers based on poly(*N*-vinyl-2-pyrrolidone) (PVP), and the copolymer compositions could be controlled through varying comonomer feed ratio. When the monomers are substituted with ethyl-, methyl-, or ether-containing alkyl chains, their homopolymers are soluble in cold water but display sensitive and reversible phase transition upon heating to a cloud point temperature (CP). Control over CP of homopolymers was achieved by changing the hydrophilicity of the substituents. CP could also be tuned by copolymerization of different monomers or adding NaCl to the polymer aqueous solution. The mechanism of the thermoresponsive properties was studied by temperature-dependent ^{1}H NMR and microcalorimetry. The results confirmed that the phase transitions of (co)polymers bearing ether substituents were less cooperative with lower phase transition enthalpy and less dehydration even at temperatures well above the CP, and the transition is predominately liquid to liquid. In addition, aldehyde, epoxy, and acetylene groups were introduced to the (co)polymer chains as reactive groups; model reactions of these groups with other molecules were very efficient and simple. Thus, these polymers can subsequently be modified to impart additional functionality to be used as thermoresponsive polymers for bioconjugation. Finally, these polymers are demonstrated to be at least as biocompatible as PVP.

Introduction

Thermoresponsive polymers, especially those displaying lower critical solution temperatures (LCST) in water, have been extensively studied and widely used in various fields such as catalysis, intelligent drug delivery, tissue engineering, etc.[1−5] Poly(*N*-isopropylacrylamide) (PNIPAm) is one of the most documented thermoresponsive polymers, mainly because its cloud point temperature (CP) is close to the human body temperature and it is easy to tune the CP by copolymerization of NIPAm with other monomers.[6] Recently, many other types of thermoresponsive polymers have been reported, for example, poly(*N*,*N*-dimethylacrylamide),[7] poly(*N*-vinylcaprolactam) (PVCL),[8] poly(vinyl methyl ether) (PVME),[9] poly(2-oxazoline),[10,11] and poly(meth)acrylates, polystyrenics, or dendritic polymers that have oligo(ethylene glycol) chains.[12−14] Control over the CPs of thermoresponsive polymers has been achieved by manipulating either the chemical factors, such as polymer structure, composition, chain-end groups, and polymer molecular weights, or the physical factors, such as additives and organic solvent.[15−20]

Poly(*N*-vinyl-2-pyrrolidone) (PVP) is a water-soluble, neutral, and biocompatible polymer widely used in pharmaceutical industry as a drug delivery system.[21] But it lacks functional groups along the polymer chain, which limits its potential applications in biotechnology fields like bioconjugation. One of the traditional methods to solve this problem is to radically copolymerize NVP with other functional monomers.[22,23] But the inherent reactivity difference between NVP and other common vinyl monomers such as (meth)acrylates always leads to only a small incorporated amount of NVP monomer at the early stage of the polymerization. To overcome this problem, carboxyl or hydroxyl groups have been introduced to the NVP monomers.[24−27] Copolymerization of these NVP derivatives with NVP shows that they have similar reactivity ratio, leading to relatively uniform random copolymers having different functional groups along the copolymer chain. Recently, we and Ritter's group found that if an ethyl group was substituted to the 3-position of NVP (C_2-NVP), the corresponding homopolymer (C_2-PVP) displayed sensitive and reversible phase transition upon heating and cooling in water, a feature of thermoresponsive polymer with LCST. The CP of C_2-PVP is around 25 °C,[28,29] which can be tuned by copolymerization with NVP.[29a] Cai's group recently reported another type of thermoresponsive polymer based on the methacrylates of pyrrolidone derivatives.[30] Compared our thermoresponsive (co)polymers with those polymers, they exhibit the following notable features: easy synthesis of monomers, random copolymer structure with NVP, tunable thermoresponsive behavior, diverse chemical modification, and good biocompatibility.

The aim of this paper is to systematically investigate the relationship between (co)polymer structure and their thermoresponsive behavior and to impart functionality on the polymers. We have therefore synthesized a series of new monomers based on NVP (structures shown in Scheme 1). These monomers have different substituents at the 3-position of NVP; the substituents are simple alkyl chains and alkyl chains with ether or reactive groups. Free radical (co)polymerization of these monomers will

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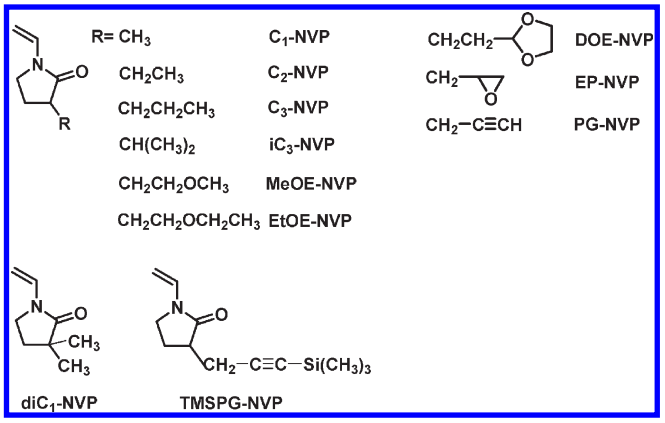
Scheme 1. Structures of New NVP Derivatives



therefore produce a family of (co)polymers. With these polymers, the influence of different substituents on the thermoresponsive behavior and phase transition mechanism can be elucidated. Moreover, the reactive groups along the polymer chains can be further modified via diverse chemistry under mild conditions to impart additional functionalities on the (co)polymers.

Experimental Section

Materials. Ethyl bromide, *n*-propyl bromide, isopropyl bromide, and methyl iodide were purchased from Beijing Chemicals Co. (China). 2-Bromoethyl methyl ether, 2-bromoethyl ethyl ether, 2-(2-bromoethyl)-1,3-dioxolane, *n*-butyllithium (*n*-BuLi, 2.5 M in hexane), benzyl mercaptan, and $NaCNBH_3$ were purchased from Alfa Aesar. Propargyl bromide (80 wt % solution in toluene, Aldrich), epibromohydrin (TCI), and 2,2-dimethoxy-2-phenylacetophenone (Acros) were also used as received. Diisopropylamine (Beijing Chemicals Co.) was refluxed with CaH_2 under argon for 6 h, followed by distillation. Tetrahydrofuran (THF, Beijing Chemicals Co.) was refluxed under argon with Na and diphenylmethanone until the solution became dark blue, and then it was distilled. *N*-Vinyl-2-pyrrolidone (NVP, Alfa Aesar, 95%) was distilled under reduced pressure prior to use. Azobis(isobutyronitrile) (AIBN) (Beijing Chemicals Co.) was purified by recrystallization from chloroform/methanol twice. All the other solvents and reagents were purchased from commercial sources and used as received.

Synthesis of Monomers. Monomer structures are shown in Scheme 1. Synthesis and polymerization of 3-ethyl-*N*-vinyl-2-pyrrolidone (C_2-NVP) were reported in previous papers.[28a,29a] All the other monomers were prepared by the reactions of methyl iodide or alkyl bromides with NVP in the presence of lithium diisopropylamide (LDA) followed by the general procedure as for the synthesis of C_2-NVP. The purification procedure was slightly different depending on the products. When alkyl bromides were used, usually two products were obtained: one is the monoalkylated NVP, and the other is the dialkylated NVP. We focused on the monoalkylated NVPs except for dC_1-NVP, so only the purification and characterization of these new NVP derivatives were described.

Synthesis of 3-Propyl-*N*-vinyl-2-pyrrolidone (C_3-NVP). In a dried reaction flask, diisopropylamine (6.4 mL, 45 mmol) and THF (60 mL) were mixed. The flask was immersed in a liquid nitrogen/acetone bath (−78 °C), and *n*-BuLi (16 mL, 2.5 M in hexane, 40 mmol) was then dropwise added into the solution within 10 min. The mixture was warmed to 0 °C under stirring for 10 min and was cooled back to −78 °C. NVP (4.4 mL, 41 mmol) was then slowly added into the solution within 5 min, and the mixture was kept at −78 °C for 1 h. Finally, *n*-propyl bromide (40 mmol) was added into the mixture, and the solution was slowly warmed to room temperature and stirred overnight. The reaction was quenched by adding deionized water (50 mL). The organic layer was separated, and the aqueous layer was extracted three times with ether (20 mL). The organic phases were combined, dried with Na_2SO_4, and concentrated by rotary evaporation. The raw product was purified using a silica column with ethyl acetate/petroleum ether (1:15 v/v) as eluent, giving 3-propyl-*N*-vinyl-2-pyrrolidone (R_f: 0.17; yield: 3.49 g, 57%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, Figure S7): 0.96 (t, 3H, J = 3.0 Hz, $-CH_3$), 1.33 (m, 3H, $-CH_2CH_2CH_3$, $-CH_2CH_2CH_3$), 1.74 (m, 1H, $-CH_2CH_2CH_3$), 1.83 (m, 1H, $-NCH_2CH_2-$), 2.30 (m, 1H, $-NCH_2CH_2-$), 2.53 (m, 1H, $-COCH-$), 3.43 (m, 1H, $-NCH_2CH_2-$), 3.51 (m, 1H, $-NCH_2CH_2-$), 4.36 (d, 1H, J = 15.6 Hz, vinyl $CHCH_2$), 4.42 (d, 1H, J = 8.4 Hz, vinyl $CHCH_2$), 7.11 (dd, 1H, J_1 = 16.2 Hz, J_2 = 9.0 Hz, vinyl $CHCH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm, Figure S8): 14.9 ($-CH_3$), 20.4 ($-CH_2CH_2CH_3$), 24.4 ($-NCH_2CH_2-$), 32.3 ($-CH_2CH_2CH_3$), 42.2 ($-NCH_2CH_2-$), 43.7 ($-COCH-$), 94.1 (vinyl $CHCH_2$), 129.6 (vinyl $CHCH_2$), 175.4 (carbonyl). ESI-MS (Figure S9): m/z 176.1 [M + Na^+].

Synthesis of 3-Methyl-*N*-vinyl-2-pyrrolidone (C_1-NVP). One equivalent of methyl iodide to NVP was used. The product was purified using a silica column with ethyl acetate/petroleum ether (1:9 v/v) as eluent. R_f: 0.22; yield: 6 g (100%). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, Figure S1): 1.23 (d, 3H, J = 7.2 Hz, $-CH_3$), 1.70 (m, 1H, $-NCH_2CH_2-$), 2.34 (m, 1H, $-NCH_2CH_2-$), 2.57 (m, 1H, $-COCH-$), 3.38 (m, 1H, $-NCH_2CH_2-$), 3.50 (m, 1H, $-NCH_2CH_2-$), 4.37 (m, 1H, J = 16.0 Hz, vinyl $CHCH_2$), 4.42 (m, 1H, J = 9.0 Hz, vinyl $CHCH_2$), 7.09 (dd, 1H, J_1 = 16.0 Hz, J_2 = 9.0 Hz, vinyl $CHCH_2$). ^{13}C NMR (100.5 MHz, $CDCl_3$, δ, ppm, Figure S2): 16.0 ($-CH_3$), 26.6 ($-NCH_2CH_2-$), 37.1 ($-NCH_2CH_2-$), 42.6 ($-COCH-$), 93.9 (vinyl $CHCH_2$), 129.6 (vinyl $CHCH_2$), 175.7 (carbonyl). ESI-MS (Figure S3): m/z 148.1 [M + Na^+].

Synthesis of 3,3-Dimethyl-*N*-vinyl-2-pyrrolidone (diC_1-NVP). Two equivalents of methyl iodide to NVP were used. The product was purified using a silica column with ethyl acetate/petroleum ether (1:9 v/v) as eluent. R_f: 0.35, yield: 1.70 g (61%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, Figure S4): 1.15 (s, 6H, $-CH_3$), 1.93 (t, 2H, J = 7.2 Hz, $-NCH_2CH_2-$), 3.43 (t, 2H, J = 6.9 Hz, $-NCH_2CH_2-$), 4.39 (m, 1H, J = 16.0 Hz, vinyl $CHCH_2$), 4.43 (m, 1H, J = 9.0 Hz, vinyl $CHCH_2$), 7.07 (dd, 1H, J_1 = 15.9 Hz, J_2 = 9.0 Hz, vinyl $CHCH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm, Figure S5): 24.6 ($-CH_3$), 33.5 ($-NCH_2CH_2-$), 41.2 ($-NCH_2CH_2-$), 43.7 ($-COC-$), 94.1 (vinyl $CHCH_2$), 129.8 (vinyl $CHCH_2$), 178.1(carbonyl). ESI-MS (Figure S6): m/z 140.1 [M + H^+].

Synthesis of 3-Isopropyl-*N*-vinyl-2-pyrrolidone (iC_3-NVP). One equivalent of isopropyl bromide was used. The product was purified using a silica column with ethyl acetate/petroleum ether (1:10 v/v) as eluent. R_f: 0.33, yield: 0.42 g (6.6%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, Figure S10): 0.79 (d, 3H, J = 6.9 Hz, $-CH_3$), 0.99 (d, 3H, J = 7.2 Hz, $-CH_3$), 1.81 (m, 1H, $-NCH_2CH_2-$), 2.04 (m, 1H, $-CH(CH_3)$), 2.11 (m, 1H, $-NCH_2CH_2-$), 2.47 (m, 1H, $-COCH-$), 3.30 (m, 2H, $-NCH_2CH_2-$), 4.35 (d, 1H, J = 15.9 Hz, vinyl $CHCH_2$), 4.42 (d, 1H, J = 9.0 Hz, vinyl $CHCH_2$), 7.07 (dd, 1H, J_1 = 15.9 Hz, J_2 = 9.0 Hz, vinyl $CHCH_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm, Figure S11): 17.7 ($-CH_3$), 19.3 ($-NCH_2CH_2-$), 20.6 ($-CH_3$), 28.5 ($-CH(CH_3)$), 43.1 ($-NCH_2CH_2-$), 48.2 ($-COCH-$), 94.1 (vinyl $CHCH_2$), 129.5 (vinyl $CHCH_2$), 169.8 (carbonyl). ESI-MS (Figure S12): m/z 176.1 [M + Na^+].

Synthesis of 3-(2-Methoxyethyl)-*N*-vinyl-2-pyrrolidone (MeOE-NVP). One equivalent of 2-bromoethyl methyl ether to NVP was used. The product was purified using a silica column with ethyl acetate/CH_2Cl_2 (1:20 v/v) as eluent. R_f: 0.18, yield: 0.88 g (13%). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, Figure S13): 1.60 (m, 1H, $-CH_2CH_2OCH_3$), 1.80 (m, 1H, $-CH_2CH_2OCH_3$), 2.18 (m, 1H, $-NCH_2CH_2-$), 2.33 (m, 1H, $-NCH_2CH_2-$), 2.64 (m, 1H, $-COCH-$), 3.49 (s, 3H, $-OCH_3$), 3.51 (m, 1H, $-NCH_2CH_2-$), 3.54 (m, 3H, $-NCH_2CH_2-$, $-CH_2CH_2OCH_3$), 4.41 (d, 1H, J = 16.0 Hz, vinyl $CHCH_2$), 4.44 (d, 1H, J = 9.0 Hz, vinyl $CHCH_2$),

7.10 (dd, 1H, $J_1 = 16.0$ Hz, $J_2 = 9.0$ Hz, vinyl C*H*CH$_2$). ^{13}C NMR (100.5 MHz, $CDCl_3$, δ, ppm, Figure S14): 24.8 (−NCH$_2$*C*H$_2$−), 30.7 (−*C*H$_2$CH$_2$OCH$_3$), 39.7 (−N*C*H$_2$CH$_2$−), 42.5 (−CO*C*H−), 58.4 (−OCH$_3$), 70.5 (−CH$_2$*C*H$_2$OCH$_3$), 93.9 (vinyl CH*C*H$_2$), 129.5 (vinyl *C*HCH$_2$), 174.8 (carbonyl). ESI-MS (Figure S15): m/z 170.1 [M + H$^+$].

Synthesis of 3-(2-Ethoxyethyl)-*N*-vinyl-2-pyrrolidone (EtOE-NVP). One equivalent of 2-bromoethyl ethyl ether to NVP was used. The product was purified using a silica column with ethyl acetate/petroleum ether (1:5 v/v) as eluent. R_f: 0.31, yield: 1.94 g (26.7%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, Figure S16): 1.11 (m, 3H, −C*H*$_3$), 1.60 (m, 1H, −C*H*$_2$CH$_2$OCH$_2$CH$_3$), 1.80 (m, 1H, −C*H*$_2$CH$_2$OCH$_2$CH$_3$), 2.15 (m, 1H, −NCH$_2$C*H*$_2$−), 2.30 (m, 1H, −NCH$_2$C*H*$_2$−), 2.64 (m, 1H, −COC*H*−), 3.33−3.53 (m, 6H, −CH$_2$C*H*$_2$OCH$_2$CH$_3$, −CH$_2$CH$_2$OC*H*$_2$CH$_3$, −NC*H*$_2$CH$_2$−), 4.41 (d, 1H, J = 16.0 Hz, vinyl CHC*H*$_2$), 4.44 (d, 1H, J = 9.0 Hz, vinyl CHC*H*$_2$), 7.10 (dd, 1H, $J_1 = 16.0$ Hz, J_2 = 9.0 Hz, vinyl C*H*CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm, Figure S17): 15.2 (−*C*H$_3$), 24.9 (−NCH$_2$*C*H$_2$−), 31.2 (−*C*H$_2$-CH$_2$OCH$_2$CH$_3$), 39.9 (−N*C*H$_2$CH$_2$−), 42.9 (−CO*C*H−), 66.1 (−CH$_2$CH$_2$O*C*H$_2$CH$_3$), 68.4 (−CH$_2$*C*H$_2$OCH$_2$CH$_3$), 94.1 (vinyl CH*C*H$_2$), 129.6 (vinyl *C*HCH$_2$), 175.1 (carbonyl). ESI-MS (Figure S18): m/z 184.1 [M + H$^+$].

Synthesis of 3-[2-(1,3-Dioxolan-2-yl)ethyl]-*N*-vinyl-2-pyrrolidone (DOE-NVP). One equivalent of 2-(2-bromoethyl)-1,3-dioxolane to NVP was used. The product was purified using a silica column with ethyl acetate/petroleum ether (1:5 v/v) as eluent. R_f: 0.21, yield: 1.93 g (23%). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, Figure S19): 1.53 (m, 1H, −C*H*$_2$CH$_2$CH (O$_2$C$_2$H$_4$)), 1.78 (m, 3H, −C*H*$_2$CH$_2$CH(O$_2$C$_2$H$_4$), −CH$_2$C*H*$_2$CH(O$_2$C$_2$H$_4$)), 2.01 (m, 1H, −NCH$_2$C*H*$_2$−), 2.32 (m, 1H, −NCH$_2$C*H*$_2$−), 2.57 (m, 1H, −COC*H*−), 3.39 (m, 1H, −N*C*H$_2$CH$_2$−), 3.49 (m, 1H, −NC*H*$_2$CH$_2$−), 3.84 (d, 2H, −CH$_2$CH$_2$CH(O$_2$C$_2$*H*$_4$)), 3.95 (d, 2H, −CH$_2$CH$_2$CH(O$_2$C$_2$*H*$_4$)), 4.39 (d, 1H, J = 16.0 Hz, vinyl CHC*H*$_2$), 4.42 (d, 1H, J = 9.0 Hz, vinyl CHC*H*$_2$), 4.88 (t, 1H, J = 4.5 Hz, −CH$_2$CH$_2$*C*H(O$_2$C$_2$H$_4$)), 7.07 (dd, 1H, J_1 = 16.0 Hz, J_2 = 9.0 Hz, vinyl C*H*CH$_2$). ^{13}C NMR (100.5 MHz, $CDCl_3$, δ, ppm, Figure S20): 24.1 (−*C*H$_2$CH$_2$CH(O$_2$C$_2$H$_4$)), 25.1 (−NC-H$_2$*C*H$_2$−), 30.8 (−CH$_2$*C*H$_2$CH(O$_2$C$_2$H$_4$)), 41.7 (−N*C*H$_2$CH$_2$−), 42.5(−CO*C*H−), 64.6 (−CH$_2$CH$_2$CH(O$_2$*C*$_2$H$_4$)), 93.8 (vinyl CH*C*H$_2$), 103.8 (−CH$_2$CH$_2$*C*H(O$_2$C$_2$H$_4$)), 129.2 (vinyl *C*HCH$_2$), 174.4 (carbonyl). ESI-MS (Figure S21): m/z 212.2 [M + H$^+$].

Synthesis of 3-(2,3-Epoxypropyl)-*N*-vinyl-2-pyrrolidone (EP-NVP). One equivalent of epibromohydrin to NVP was used. The product was purified using a silica column with ethyl acetate/CH_2Cl_2 (1:25 v/v) as eluent. R_f: 0.29, yield: 3.0 g (45%). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, Figure S22): 1.41 (m, 0.5H, −C*H*$_2$CH-(CH$_2$O)), 1.89 (m, 2H, −C*H*$_2$CH(CH$_2$O), −NCH$_2$C*H*$_2$−), 2.26 (m, 0.5H, −NCH$_2$C*H*$_2$−), 2.42 (m, 1H, −NCH$_2$C*H*$_2$−), 2.50 (m, 0.5H, −COC*H*−), 2.54 (m, 0.5H, −COC*H*−), 2.72 (m, 1H, −CH$_2$CH(C*H*$_2$O)), 2.80 (m, 1H, −CH$_2$C*H*(CH$_2$O)), 3.10 (m, 1H, −CH$_2$CH(C*H*$_2$O)), 3.42 (m, 1H, −NC*H*$_2$CH$_2$−), 3.57 (m, 1H, −NC*H*$_2$CH$_2$−), 4.45 (d, 1H, J = 16.0 Hz, vinyl CHC*H*$_2$), 4.48 (d, 1H, J = 9.0 Hz, vinyl CHC*H*$_2$), 7.10 (dd, 1H, $J_1 = 16.0$ Hz, J_2 = 9.0 Hz, vinyl C*H*CH$_2$). ^{13}C NMR (100.5 MHz, $CDCl_3$, δ, ppm, Figure S23): 24.3 (−NCH$_2$*C*H$_2$−), 24.8 (−NCH$_2$*C*H$_2$−), 33.4 (−*C*H$_2$CH(CH$_2$O)), 34.3 (−*C*H$_2$CH(CH$_2$O)), 39.8 (−N*C*H$_2$-CH$_2$−), 40.70 (−N*C*H$_2$CH$_2$−), 42.8 (−CO*C*H−), 42.8 (−CO-*C*H−), 46.7 (−CH$_2$CH(*C*H$_2$O)), 46.8 (−CH$_2$CH(*C*H$_2$O)), 49.9 (−CH$_2$*C*H(CH$_2$O)), 51.1 (−CH$_2$*C*H(CH$_2$O)), 94.4 (vinyl CH*C*-H$_2$), 129.3 (vinyl *C*HCH$_2$), 174.1 (carbonyl). ESI-MS (Figure S24): m/z 190.1 [M + Na$^+$].

Synthesis of 3-Propargyl-*N*-vinyl-2-pyrrolidone (PG-NVP). One equivalent of propargyl bromide to NVP was used. The product was purified using a silica column with ethyl acetate/petroleum ether (1:10 v/v) as eluent. R_f: 0.11, yield: 6.0 g (100%). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, Figure S25): 1.99 (m, 1H, −CC*H*), 2.04 (m, 1H, −NCH$_2$C*H*$_2$−), 2.37 (m, 1H, −NCH$_2$-C*H*$_2$−), 2.46 (m, 1H, −COC*H*−), 2,65 (m, 1H, −C*H*$_2$CCH), 2.77 (m, 1H, −C*H*$_2$CCH), 3.41 (m, 1H, −NC*H*$_2$CH$_2$−), 3.55 (m, 1H, −NC*H*$_2$CH$_2$−), 4.41 (d, 1H, J = 16.0 Hz, vinyl CHC*H*$_2$), 4.49 (d, 1H, J = 9.0 Hz, vinyl CHC*H*$_2$), 7.08 (dd, 1H, $J_1 = 16.0$ Hz, J_2 = 9.0 Hz, vinyl C*H*CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm, Figure S26): 20.2 (−NCH$_2$*C*H$_2$−), 23.4 (−*C*H$_2$CCH), 41.4 (−N*C*H$_2$CH$_2$−), 42.9 (−CO*C*H−), 70.1 (−*C*CH), 81.0 (−*C*CH), 94.8 (vinyl CH*C*H$_2$), 129.4 (vinyl *C*HCH$_2$), 173.9 (carbonyl). ESI-MS (Figure S27): m/z 172.1 [M + Na$^+$].

Synthesis of 3-(3-Trimethylsilanylpropargyl)-*N*-vinyl-2-pyrrolidone (TMSPG-NVP). In a dried reaction flask, diisopropylamine (6.4 mL, 45 mmol) and THF (60 mL) were mixed. The flask was immersed in a liquid nitrogen/acetone bath (−78 °C), and *n*-BuLi (16 mL, 2.5 M in hexane, 40 mmol) was then dropwise added into the solution within 10 min. The mixture was warmed to 0 °C under stirring for 10 min and was cooled back to −78 °C. PG-NVP (40 mmol) was then slowly added into the solution within 5 min, and the mixture was kept at −78 °C for 2 h. Finally, chlorotrimethylsilane (5 mL, 40 mmol) was added into the mixture, and the solution was slowly warmed to room temperature and stirred overnight. The reaction was quenched by adding deionized water (50 mL). The organic layer was separated, and the aqueous layer was extracted with ether (20 mL × 3). The organic phases were combined, dried with Na_2SO_4, and concentrated by rotary evaporation. The raw product was purified using a silica column with ethyl acetate/petroleum ether (1:10 v/v) as eluent, giving the target compound (R_f: 0.23, yield: 4.2 g, 47%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, Figure S28): 0.16 (s, 9H, −Si(C*H*$_3$)$_3$), 2.00 (m, 1H, −NCH$_2$C*H*$_2$−), 2.33 (m, 1H, −NCH$_2$C*H*$_2$−), 2.50 (m, 1H, −COC*H*−), 2.64 (m, 1H, −C*H*$_2$CCSi(CH$_3$)$_3$), 2.69 (m, 1H, −C*H*$_2$CCSi(CH$_3$)$_3$), 3.39 (m, 1H, −NC*H*$_2$CH$_2$−), 3.53 (m, 1H, −NC*H*$_2$CH$_2$−), 4.41 (d, 1H, J = 15.8 Hz, vinyl CHC*H*$_2$), 4.45 (d, 1H, J = 9 Hz, vinyl CHC*H*$_2$), 7.07 (dd, 1H, $J_1 = 16.2$ Hz, J_2 = 9.0 Hz, vinyl C*H*CH$_2$). ^{13}C NMR (100.5 MHz, $CDCl_3$, δ, ppm, Figure S29): −0.1 (−Si(*C*H$_3$)$_3$), 21.9 (−NCH$_2$*C*H$_2$−), 23.4 (−*C*H$_2$CCSi(CH$_3$)$_3$), 41.5 (−N*C*H$_2$CH$_2$−), 43.0 (−CO*C*H−), 86.4 (−CH$_2$*C*CSi(CH$_3$)$_3$), 94.6 (vinyl CH*C*H$_2$), 103.4 (−CH$_2$-*C*CSi(CH$_3$)$_3$), 129.4 (vinyl *C*HCH$_2$), 173.1 (carbonyl). ESI-MS (Figure S30): m/z 222.1 [M + H$^+$].

General Procedure of Radical (Co)polymerization. The solution polymerization was carried out in THF with AIBN as the initiator. In general, a polymerization tube containing a mixture of monomer (2 mmol) and AIBN (1.6 mg, 0.01 mmol) in 0.72 g of THF was degassed by three freeze/thaw cycles, sealed under vacuum, and then it was placed in a preheated oil bath set at 60 °C with stirring. After 24 h, the polymerization was stopped. Homopolymers C_1-PVP, diC_1-PVP, C_2-PVP, MeOE-PVP, EtOE-PVP, DOE-PVP, and EP-PVP were precipitated from petroleum ether, collected by filtration, and dried in vacuo for 12 h at room temperature. Homopolymers C_3-PVP, iC_3-PVP, and TMSPG-PVP should be purified via silica column chromatography. First, ethyl acetate and petroleum ether mixture (1/5, v/v) was used as eluent until no monomer was detected by TLC. Then, the eluent was changed to methanol/CH_2Cl_2 mixture (1/10, v/v). Polymers were recovered after evaporating the solvents.

Four series of copolymers were synthesized by radical copolymerization of the following monomer pairs in different feed ratios: C_2-NVP and MeOE-NVP (copolymer A), C_2-NVP and DOE-NVP (copolymer B), C_2-NVP and EP-NVP (copolymer C), NVP and TMSPG-NVP (copolymer D). These copolymers were obtained by precipitation into petroleum ether and dried in vacuo for 12 h at room temperature.

Deprotection of Copolymer B. Copolymer B (300 mg) was dissolved in 20 mL of acetone. Then 0.3 mL of water and 6 mg of pyridine/*p*-toluenesulfonic acid were added into the solution. The mixture was allowed to react at 50 °C for 1 day, then 10 mL of water was added, and the mixture was extracted by CH_2Cl_2 (15 mL × 3). The organic phase was combined, dried with Na_2SO_4, and concentrated to 5 mL by rotary evaporation. The concentrated solution was dropwise added into 100 mL of petroleum ether, and the precipitate was collected by filtration and

dried in vacuo for 12 h. The resulting copolymers having free aldehyde groups were named as copolymer B′.

Reaction of Copolymer B2′ with Aniline. The above-prepared copolymer B2′ (50 mg, about 0.065 mmol aldehyde groups, prepared from B2 shown in Table 3) was dissolved in 5 mL of methanol. Aniline (46.5 mg, 0.5 mmol) was added. The mixture was stirred at room temperature overnight, and then 1 mL of acetic acid and 100 mg of $NaCNBH_3$ were added. After being stirred for another 4 h, the solution was dialyzed (MWCO 1 kDa) against water for 3 days and lyophilized to give a white fluffy solid.

Reaction of Copolymer C with Aniline. Copolymer C1 (100 mg, about 0.071 mmol epoxy groups, shown in Table 4) was dissolved in 15 mL of ethanol. Then, aniline (0.186 g, 2 mmol) was added. After being stirred at 80 °C for 1 day, the solution was dialyzed (MWCO 1 kDa) against water for 3 days and lyophilized to get a white fluffy solid.

Deprotection of Copolymer D. Copolymer D (200 mg) was dissolved in 10 mL of methanol. Then, 100 mg of K_2CO_3 was added into the solution. After being reacted at ambient temperature overnight, the solution was dialyzed (MWCO 1 kDa) against water for 3 days and lyophilized to give a white fluffy solid. The resulting copolymers having free acetylene groups were named as copolymer D′.

"Click" Reaction of Copolymer D1′ with Benzyl Mercaptan. The above-prepared copolymer D1′ (115 mg, about 0.1 mmol alkynyl group, prepared from D1 shown in Table 5) was dissolved in 5 mL of THF. Benzyl mercaptan (0.39 mL, about 3 mmol) and a photoinitiator 2,2-dimethoxy-2-phenylacetophenone (8.13 mg) were added. After being irradiated by UV light (λ_{max} = 365 nm) for 2 h, the solution was dropwise added into 200 mL of petroleum ether with stirring, and the obtained precipitate was collected by filtration and dried in vacuo for 12 h.

Characterization. The number-average molecular weights (M_n) and polydispersity index (PDI, M_w/M_n) of all the (co)polymers were determined by gel permeation chromatography (GPC). The measurements were carried out in THF (flow rate: 1 mL/min) at 35 °C with a Waters 1525 binary HPLC pump equipped with a Waters 2414 refractive index detector and three Waters Styragel columns (1×10^4, 1×10^3, and 500 Å pore sizes). A family of narrow dispersed polystyrenes was used as the standards, and Breeze 3.30 SPA software was applied to calculate the molecular weight and PDI. For the copolymers D and D′, the M_n and PDI were determined by GPC in DMF containing 100 mM LiBr (flow rate: 1 mL/min) at 35 °C on an equipment composed of Waters 515 pump and Waters 2410 refractive index detector and two Waters Styragel columns (1×10^3 and 500 Å pore sizes). Monodisperse polystyrene standards were used for calibration, and data analysis was made on Millennium[32] software. ^{1}H NMR (400 or 300 MHz) and ^{13}C NMR (100.5 or 75 MHz) spectra were recorded on a Bruker ARX 400 MHz spectrometer or a Varian Mercury Plus 300 MHz spectrometer in $CDCl_3$, D_2O, or d_6-acetone with tetramethylsilane as the internal reference for chemical shifts. ESI-MS data were obtained on a Thermo LCQ DECA XP Plus ESI mass spectrometer under positive mode.

Transmittance Measurement. Cloud point temperatures (CPs) of the (co)polymers were determined by transmittance measurements in a 1 cm quartz cell on a Shimadzu 2101 UV−vis spectrometer equipped with a temperature controller. The polymer concentration was 1 mg/mL. The transmittance at 500 nm was monitored, and the data were collected after the solution being equilibrated for 30 min at each temperature during the heating or cooling process. CP was defined as the temperature when the transmittance was 50%.

Laser Light Scattering (LLS) Measurement. LLS measurements of the polymer aqueous solutions (1.0 mg/mL) were carried out on a commercial spectrometer (Brookhaven Inc., Holtsville, NY) equipped with a BI-200SM goniometer and a BI-TurboCorr digital correlator. A 200 mW vertically polarized solid-state laser (532 nm, CNI, Changchun, China) was used as the light source. Prior to the measurement, polymer samples were dissolved in Milli-Q water (Millipore), and the solutions were equilibrated for 1 day at 4 °C. The polymer solutions were filtered through a 0.45 μm Millipore filter into a dust-free vial prior to measurements. In each heating/cooling step, the solutions were equilibrated for 30 min at each temperature. In dynamic light scattering, the intensity−intensity time autocorrelation function was measured in the self-beating mode. The Laplace inversion program, CONTIN, was applied to obtain the average line width Γ and its distribution at varying scattering angle q. The apparent hydrodynamic radius ($R_{h,app}$) was obtained by extrapolating Γ/q^2 to zero angle, followed by the calculation based on Stokes−Einstein equation.

Calorimetric Measurement. The measurements were carried out using a differential scanning microcalorimeter (MICRO DSC III, Setaram Co., France) with an automatic system for data acquisition and processing. Polymer concentration was varied in the range of 1−5 mg/mL. About 600 mg of polymer solution was degassed and transferred into the sample cell. Polymer-free solutions of the same weight were degassed and placed in the reference cell. Measurements were performed in a temperature range of 10−80 °C at a heating rate of 0.5 °C/min.

Cytotoxicity Measurement. MTT assay was applied to evaluate the cytotoxicity of polymers in B16F10 and Chinese Hamster Ovary (CHO) cells. PVP (M_w 8 kDa) and branched polyethylenimine (M_w 25 kDa) were used as the negative and positive controls, respectively. B16F10 and CHO cells seeded in 96-well plate at a density of 5000/well were cultured at 37 °C in 5% CO_2 humidified atmosphere for 24 h. Polymer solutions of different concentrations (10 μL) were added to each well, and the cells were subjected to MTT assay after being incubated for another 24 h. The absorbance of the solution was measured on a Bio-Rad model 550 microplate reader at 570 nm. Cell viability (%) was equal to $(A_{sample}/A_{control}) \times 100$, where A_{sample} and $A_{control}$ denote absorbance of the sample well and control well (without polymer), respectively. Experiments were performed in triplicate.

Results and Discussion

Synthesis of Monomers. All the monomers were synthesized via alkylation of the α-carbon to the NVP carbonyl groups in the presence of LDA with methyl iodide or alkyl bromides. The yields and characterization of the monomers are listed in the Experimental Section and Supporting Information (Figures S1−S30). When 1 equiv of methyl iodide to NVP was used, C_1-NVP was obtained quantitatively, suggesting the high reactivity of methyl iodide; therefore, 2 equiv of methyl iodide to NVP were used to synthesize diC_1-NVP. When alkyl bromides were used to react with NVP in an equal molar ratio, two products were usually obtained: 3-alkyl-*N*-vinyl-2-pyrrolidone and 3,3-dialkyl-*N*-vinyl-2-pyrrolidone, which could be easily purified by silica column chromatography. Simple alkyl bromides ($C_nH_{2n}Br$, like ethyl bromide, *n*-propyl bromide, *n*-butyl bromide) showed high reactivities and consumed completely to get 60% C_n-NVP and 20% diC_n-NVP. The yield of monomer iC_3-NVP was quite low because isopropyl bromide preferred elimination reaction under such basic conditions. Very recently, Ritter et al. reported the synthesis of PY-NVP by a similar approach, and they obtained mixtures of mono- and double-substituted products.[29b] However, in our experiment, the yield of monomer PY-NVP was nearly 100% due to the low reaction temperature. Since the free acetylene groups may cause chain transfer reaction during radical polymerization, we transformed PY-NVP to TMSPG-NVP in 47% yield by reaction with chlorotrimethylsilane.

Compared with the simple alkyl bromides, those bromides having electron-donating ether groups, such as 2-bromoethyl

methyl ether, 2-bromoethyl ethyl ether, 2-(2-bromoethyl)-1, 3-dioxolane, and epibromohydrin, exhibited low reactivities toward nucleophilic substitution reaction. Therefore, the four monomers MeOE-NVP, EtOE-NVP, DOE-NVP, and EP-NVP were obtained in relatively lower yields.

C_1-NVP, diC_1-NVP, C_2-NVP, MeOE-NVP, EtOE-NVP, DOE-NVP, and EP-NVP are soluble in water, ethanol, DMF, dichloromethane, and chloroform. But they are insoluble in nonpolar solvents such as hexane and petroleum ether, while C_3-NVP, iC_3-NVP, PG-NVP, and TMSPG-NVP are soluble in nonpolar solvents, aprotic solvents, chlorinated solvents, and alcohols but are only slightly soluble in water.

Synthesis of Homopolymers and Copolymers by Radical (Co)polymerization. The homopolymers of these monomers were prepared via conventional free radical polymerization with AIBN as initiator in THF at 60 °C for 24 h. Characterizations of the homopolymers are summarized in Table 1. Polymer yields are usually higher than 40%, and the M_ns of polymers are in the range 4−16 kDa. Similar to their corresponding monomers, polymers C_1-PVP, diC_1-PVP, MeOE-PVP, EtOE-PVP, DOE-PVP, and EP-PVP are soluble in cold water, ethanol, DMF, and chloroform but are insoluble in nonpolar solvents. Polymers C_3-PVP, iC_3-PVP, and TMSPG-PVP are soluble in most organic solvents but only show very slight solubility in water.

Table 1. Characterization of Homopolymers[a]

homopolymer	monomer	yield (%)	M_n[b]	M_w/M_n[b]	CP (°C)[c]
C_1-PVP	C_1-NVP	56	6 300	1.25	64.0
diC_1-PVP[d]	diC_1-NVP	82	29 000	1.54	41.0
C_2-PVP[e]	C_2-NVP	86	6 700	1.75	25.0
C_3-PVP	C_3-NVP	61	14 000	1.65	
iC_3-PVP	iC_3-NVP	52	4 000	2.65	
MeOE-PVP	MeOE-NVP	86	15 000	2.43	64.0
EtOE-PVP	EtOE-NVP	47	6 100	2.66	36.0
DOE-PVP	DOE-NVP	76	11 000	1.99	47.5
EP-PVP	EP-NVP	86	9 000	1.71	soluble
TMSPG-PVP	TMSPG-NVP	40	11 000	1.73	

[a] AIBN as initiator in THF at 60 °C for 24 h. [monomer]/[AIBN] = 200:1. [b] Determined by GPC in THF with polystyrene standards. [c] Determined by transmittance change during the heating process. [d] Polymerized in ethanol. [e] Reported in ref 28b.

We also synthesized four series of copolymers by free radical copolymerization of the following monomer pairs: C_2-NVP and MeOE-NVP, C_2-NVP and DOE-NVP, C_2-NVP and EP-NVP, NVP and TMSPG-NVP. The polymerization procedure was the same as for homopolymerization, and all of the copolymerization results are summarized in Tables 2−5.

The yields and molecular weights of the copolymers were comparable to the homopolymers. By varying the feed ratio of the two monomers, copolymers with different compositions were obtained. The compositions of the copolymers were determined by ^{1}H NMR measurements. Take copolymer A1 as an example (Table 2); the ^{1}H NMR spectrum together with the assignments is presented in Figure 1. By comparing the integration at 0.93 ppm (peak g) with that from 4.00 to 2.60 ppm, we can estimate that the molar content of MeOE-NVP in the copolymer is 25.4%, very close to the feed ratio (25%). We also measured the compositions of the other three types of copolymers in a similar way (Figures S31−S33) and found that the copolymer compositions are always close to the feed ratio (Tables 3−5). These results are similar to other reports on the radical copolymerization of NVP derivatives.[24−27]

Thermoresponsive Behavior of Homopolymers and Copolymers. We and Ritter et al. have reported that C_2-PVP displays thermoresponsive behavior in water; this polymer is soluble in water below 25 °C (CP) but turns to aggregate and precipitate in water above this temperature.[28b,29a] We have also found that the CP of C_2-PVP is independent of the polymer concentration (from 0.4 to 10 mg) and molecular weights (from 2500 to 16 000 Da).[28b] CPs of copolymers from C_2-NVP and NVP have been found to increase linearly with increasing the molar contents of NVP units.[29a] Here, we investigated in detail the effects of (co)polymer structure and

Table 2. Characterization of Copolymers from C_2-NVP and MeOE-NVP[a]

	MeOE-NVP content					
copolymer	in feed (%)	in polymer (%)[c]	yield (%)	M_n[b]	M_w/M_n[b]	CP (°C)[d]
A1	25.0	25.4	76	11 000	1.53	34.5
A2	50.0	53.4	84	16 000	1.69	41.3
A3	75.0	72.9	69	13 000	1.35	51.0

[a] AIBN as initiator in THF at 60 °C for 24 h. [comonomer]/[AIBN] = 200:1. [b] Determined by GPC in THF with polystyrene standards. [c] Determined by ^{1}H NMR in $CDCl_3$. [d] Determined by transmittance change during the heating process.

Table 3. Characterization of Copolymers from C_2-NVP and DOE-NVP[a]

	DOE-NVP content					
copolymer	in feed (%)	in polymer (%)[c]	yield (%)	M_n[b]	M_w/M_n[b]	CP (°C)[d]
B1	5.0	5.7	95	15 000	1.90	27.7
B2	25.0	26.0	97	16 000	1.89	30.5
B3	50.0	50.5	87	16 000	2.07	32.4

[a] AIBN as initiator in THF at 60 °C for 24 h. [comonomer]/[AIBN] = 200:1. [b] Determined by GPC in THF with polystyrene standards. [c] Determined by ^{1}H NMR in $CDCl_3$. [d] Determined by transmittance change during the heating process.

Table 4. Characterization of Copolymers from C_2-NVP and EP-NVP[a]

	EP-NVP content					
copolymer	in feed (%)	in polymer (%)[c]	yield (%)	M_n[b]	M_w/M_n[b]	CP (°C)[d]
C1	10.0	10.7	83	12 000	1.70	32.0
C2	25.0	23.3	82	14 000	1.77	39.7
C3	50.0	46.1	69	15 000	1.70	60.6

[a] AIBN as initiator in THF at 60 °C for 24 h. [comonomer]/[AIBN] = 200:1. [b] Determined by GPC in THF with polystyrene standards. [c] Determined by ^{1}H NMR in $CDCl_3$. [d] Determined by transmittance change during the heating process.

Table 5. Characterization of Copolymers from TMSPG-NVP and NVP[a]

copolymer	TMSPG-NVP content in feed (%)	TMSPG-NVP content in polymer (%)[c]	yield (%)	M_n[b]	M_w/M_n[b]	CP (°C)[d]
D1	10.0	9.1	94	44 000	1.85	59.0
D2	25.0	22.2	92	53 000	1.98	
D3	50.0	52.0	86	49 000	2.20	

[a] AIBN as initiator in THF at 60 °C for 24 h. [monomer]/[AIBN] = 200:1. [b] Determined by GPC in DMF containing 100 mM LiBr with polystyrene standards. [c] Determined by ^{1}H NMR in $CDCl_3$. [d] Determined by transmittance change during the heating process.

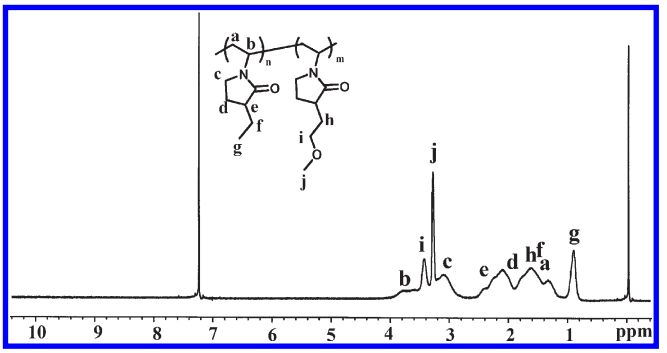


Figure 1. ^{1}H NMR spectrum of copolymer A1 in $CDCl_3$.

composition on the CP of this type of thermoresponsive polymer (see below).

We first measured the CPs of the water-soluble homopolymers (C_1-PVP, diC_1-PVP, MeOE-PVP, EtOE-PVP, and DOE-PVP) by transmittance methods (Figure S34). CP of the polymers was estimated as the temperature when the transmittance of the polymer solution was 50%, and the data are compiled in Table 2. As already known, PVP itself is soluble in water and displays no CP in a temperature range of 10−80 °C, though addition of organic acids will render PVP some hydrophobicity and result in the appearance of CP in the above temperature range.[19a] Both C_1-PVP and DiC_1PVP showed sensitive and reversible phase transitions in water upon heating and cooling as C_2-PVP. CP of polymer C_1-PVP is 64 °C, much higher than that of C_2-PVP (25 °C), which can simply be attributed to the higher hydrophilicity of C_1-PVP. Interestingly, CP of diC_1-PVP (41 °C) is still much higher than that of C_2-PVP. CPs of MeOE-PVP, DOE-PVP, and EtOE-PVP are 64, 47.5, and 36 °C, respectively. These temperatures are all higher than that of C_2-PVP, reflecting the increase of hydrophilicity due to the existence of ether groups. Moreover, these polymers showed less sensitive phase transition with a broader temperature range. This behavior can be explained by the formation of less dense and rather hydrophilic hydrated aggregates due to the more hydrophilic nature of these homopolymers.[31] To summarize, by changing the substituent groups, we can adjust the CP of the PVP derivatives in a controlled way; increasing the hydrophilicity of the polymer leads to an increase of CP.

Another general way to tune the CP of a thermoresponsive polymer is through random copolymerization of different monomers. Herein, the CPs of copolymers A and B were investigated (both heating and cooling process). They all display reversible phase transition, with a small tailing at low transmittance region and little hysteresis upon cooling. CPs of copolymer A can be varied linearly from 25 to 64 °C as presented in Figure 2, and CPs of copolymer B are in the range 25−47.5 °C (Figure S35) by simply increasing the content of DOE-NVP. For copolymer C, with increasing the EP-PVP content in the copolymer, the CP of the copolymer can be linearly increased to 60 °C when the content of EP-NVP was about 50% (Figure S36). As reported in the literature, the

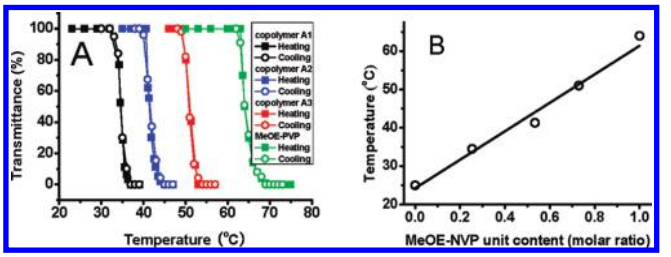


Figure 2. (A) Transmittance (500 nm) vs temperature plots of copolymer A (1 mg/mL). (B) Plots of the measured CP as a function of the experimentally determined copolymer composition.

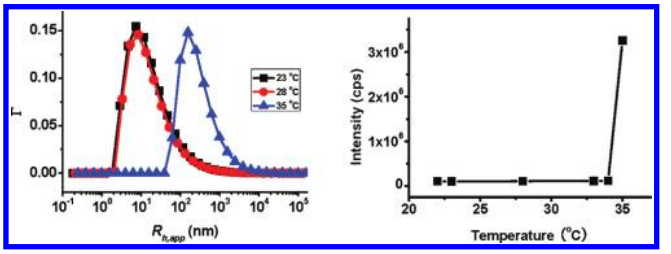


Figure 3. (A) CONTIN analysis of copolymer A1 solution at different temperatures. (B) Temperature dependence of the excess scattered intensity of the copolymer aqueous solutions upon the heating procedure. Polymer concentration: 1.0 mg/mL; detection angle: 90°.

CPs of the copolymers can thus be adjusted by varying the copolymer compositions in a linear way.

Mechanism of the Thermo-induced Phase Transition. We used LLS to study the thermo-induced phase transition behavior of the above (co)polymers in water. All of the water-soluble (co)polymers showed a single model distribution, which means that there is only one component existing in the solution below the CP. The $R_{h,app}$ of this component is smaller than 10 nm, implying that the copolymer exists as the single polymer chain and does not aggregate in aqueous solution below the CP. Figure 3 presents the temperature dependence of the excess scattered intensity and $R_{h,app}$ of copolymer A1. It can be seen that the intensity is low and remains constant below 35 °C (CP), while upon heating through this temperature a dramatic increase in intensity was observed; at the same time, the $R_{h,app}$ became larger than 100 nm, indicating formation of polymer aggregates at this temperature. Interestingly, for homopolymer C_2-NVP, the $R_{h,app}$ of which was about 8 nm at a temperature below 24 °C, at 25 °C, DLS intensity size distribution showed the coexistence of two components with different diameters, $R_{h,app}$ of the smaller one being about 6 nm and that of the larger one being about 50 nm. The smaller component disappeared at 26 °C with the increase of the $R_{h,app}$ of the larger one to more than 100 nm. We speculated that the mechanism of phase transition of C_2-PVP may be different as that of the copolymer A.

In order to get more information on the thermo-induced phase transition of these polymers, we measured the temperature-dependent ^{1}H NMR spectra of copolymer A1 as shown in Figure 4. As reported in the previous paper,[28b] for

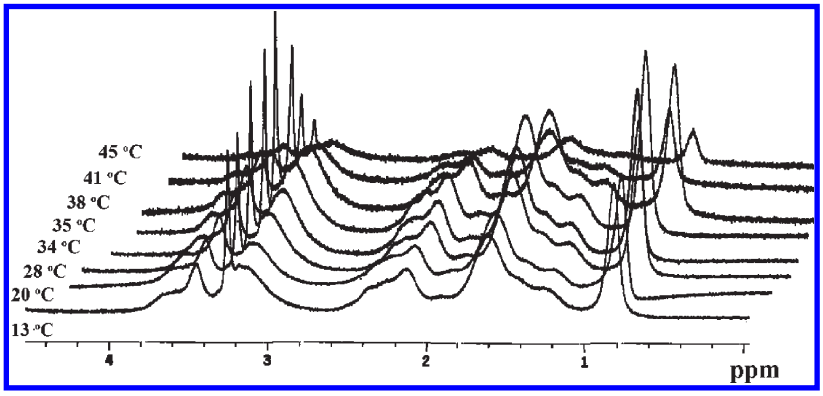


Figure 4. Temperature-dependent ^{1}H NMR spectra of copolymer A1 in D_2O (3 mg/mL).

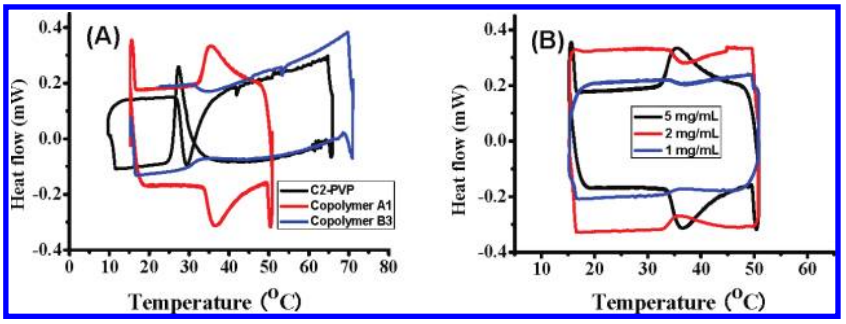


Figure 5. DSC thermograms of C_2-PVP and copolymers A1 and B3 at 5 mg/mL (A) and those of copolymer A1 at three different concentrations (B).

Table 6. Thermodynamic Parameters of the Phase Transition of (Co)polymer Aqueous Solutions

samples	C_2-PVP		copolymer A1			copolymer B3
concentration (mg/mL)	5	1	5	2	1	5
onset T (°C)[a]	26.4	27.8	33.8	34.1	34.8	31.2
peak T (°C)[a]	27.0	30.1	36.6	37.2	36.3	36.0
CP (°C)[b]	25.0	25.0	34.5	34.5	34.5	32.4
ΔH (J/g)[a]	54	41	34	34	27	17

[a] Determined by microcalorimetric measurement during the heating process. [b] Determined by transmittance change during the heating process.

C_2-PVP, the proton signals of the polymer main chain begin to drastically reduce at 24 °C and almost disappear at 35 °C, 10 °C above its CP. However, in the case of copolymer A1, all of the proton signals are still well-resolved at 35 °C (CP), though the aqueous solution begins to be turbid at this temperature. The signals become broadened at 45 °C, but each peak signal can still be clearly observed. The results confirm that the dehydration of copolymer A is incomplete and indicate that copolymers containing MeOE-NVP exhibit a typical liquid−liquid transition upon heating through the CP.[20]

The difference in the hydration states of copolymers and C_2-PVP was further demonstrated by their thermodynamic parameters determined by microcalorimetry. Figure 5 presents the DSC thermograms of the (co)polymer aqueous solutions in one heating−cooling cycle, and the corresponding data are summarized in Table 6. Upon heating, C_2-PVP and copolymers A1 and B3 showed the endothermic peaks with the maxima (T_{max}) at 27.0, 36.6, and 36.0 °C, respectively, which were about 2 °C higher than the CPs obtained by transmittance methods. In the cooling process, the exothermic peaks are observed for all the polymers with the transition temperatures of ca. 1−2 °C lower than those in the heating process.

Furthermore, there is a marked difference between the transition enthalpies (ΔH) of C_2-PVP and the copolymers. In the heating process, ΔH of 5 mg/mL C_2-PVP solution was 7.5 kJ/mol per repeating unit (54 J per gram of polymer), comparable to that of PNIPAm (ca. 5−8 kJ/mol) with a typical coil−globule phase transition.[6,32,33] However, we could not detect any transition enthalpies for both homopolymers MeOE-PVP and DOE-PVP under similar conditions, which may suggest the incomplete dehydration of these two homopolymers because of the high hydrophilic nature. More solvated homopolymer chains may still exist even at a temperature above the CP as implied by the above NMR results. The transition enthalpies of both copolymer A and copolymer B are detectable, but much smaller than that of C_2-PVP, and they decrease with increasing either MeOE-NVP or DOE-NVP unit, implying that the ΔH of copolymer A1 (34 J/g, 75 mol % of C_2-NVP) and copolymer B3 (17 J/g, 50 mol % of C_2-NVP) may mainly be contributed by the C_2-NVP repeat units in the copolymers. We are currently investigating the hydration behavior of these (co)polymers by other methods including IR[9b,18c] and UV/vis absorption spectroscopy[31] to get more insight into this behavior.

On the basis of the above results, we are quite sure that a hydration shell is formed by hydrogen bonds between water and amide groups in C_2-PVP, which makes this polymer dissolve in water at low temperature. Upon heating through CP, many hydrogen bonds break and water molecules are expelled from the hydration shell, thus leading to efficient dehydration with a larger enthalpy. However, for copolymers A and B, besides the amide groups, additional ether bonds in the polymer side chains can also form hydrogen bonds with

water. Therefore, the hydrogen bonds between amide groups and water break quickly upon heating, but those with ether bonds remain, forming coacervate droplets above CP. The phase transition is thus a typical liquid−liquid transition with a small enthalpy change because of incomplete dehydration.[20c]

Addition of NaCl to the polymer aqueous solution can adjust water structure directly and affect the hydrogen bonds indirectly,[18b] so the effects of NaCl on the CP of these (co)polymers were studied at pH = 7.4 (40 mM phosphate buffer solution) (Figure S37). Adding NaCl into the aqueous solution of C_2-PVP results in a linear decrease of the CPs with the NaCl concentration, and the smaller the polymer molecular weights, the more pronounced the effect. For example, at 1 M NaCl, CPs of three C_2-PVPs (M_n = 2500 Da, M_n = 7900 Da, M_n = 16 000 Da) decrease to 16, 17, and 19.5 °C, respectively. Similar results have also been reported for PNIPAm.[18b] The existence of NaCl leads to a partial dehydration of the polymer even below CP and consequently decrease the hydrophilicity of the polymers, leading to a decrease of CP.

In addition, compared to C_2-PVP, the effect of NaCl is more remarkable on (co)polymers having ether bonds. CPs of homopolymers MeOE-PVP and DOE-PVP are found to be about 18 °C lower in the presence of 1 M NaCl, while the CP of copolymer A1 decreases 9.5 °C. Similar large salt effects on CP have been observed in other thermoresponsive polymers when the hydrophilicity of the polymer was increased.[18d] In addition, these results may also indicate the influence of different substituents on the thermal-induced phase transition mechanism; hydrogen bonds between ether bonds and water are stronger than those between amides and water, but they seem to be easily destroyed by NaCl.

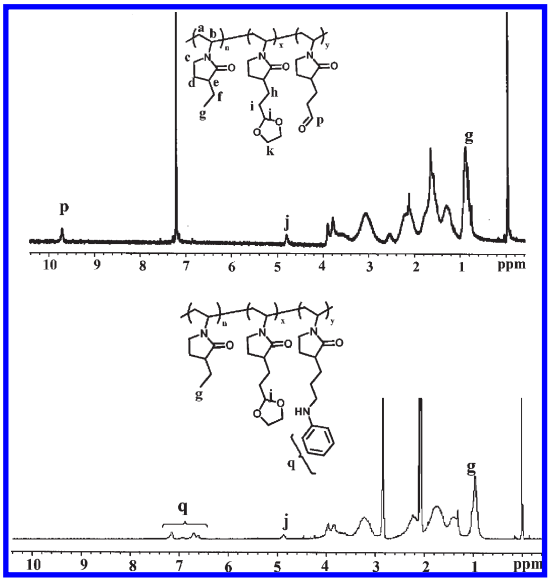


Figure 6. ^{1}H NMR spectra of copolymer B2′ in $CDCl_3$ (top) and the reaction product of this copolymer with aniline in d_6-acetone (down).

Functionalization of the Copolymers Having Aldehyde, Epoxy, and Alkynyl Groups. As we discussed above, it was easy to introduce highly reactive groups including aldehyde, epoxy, and acetylene groups along the (co)polymer backbones. Reaction of these groups with other functional molecules will be an easy way to confer the polymers with functionality.[34−36] Herein, reactions of simple small molecules with three copolymers are used as examples to demonstrate the feasibility. For easy characterization with ^{1}H NMR spectra, we select simple aromatic compounds for model reactions.

First, the aldehyde groups of copolymer B2 have been released via carbonyl exchange reaction in acetone with trace of pyridine/*p*-toluenesulfonic acid as catalyst at 50 °C.[34,37] Figure 6 presents the ^{1}H NMR spectrum of copolymer B2′; from the resonance of aldehyde protons at 9.8 ppm, it is estimated that about 70% of free aldehyde groups have been recovered. CP of copolymer B2′ is 2 °C lower than that of the parent copolymer, and the transition temperature range is broader, which implies that the aldehyde groups are less hydrophilic. Aniline was used to react with the aldehyde groups in copolymer B2′ via a one-pot reductive amination reaction. Conversion of the aldehyde groups is quantitative as determined by the ^{1}H NMR spectrum of the final product (Figure 6).

Second, the epoxy groups of copolymer C1 was allowed to react with aniline in ethanol at 80 °C for 1 day. As expected, all the epoxy groups have been consumed to produce a new copolymer with the structure and NMR spectrum shown in Figure 7.

Third, copolymer D1 was quantitatively converted to D1′ under weak basic condition at ambient temperature. The free acetylene groups of copolymer D1′ were allowed to react with an excess benzyl mercaptan irradiated with UV light in the presence of 2,2-dimethoxy-2-phenylacetophenone as a photoinitiator; conversion of acetylene groups was quantitative.[36,38] The ^{1}H NMR spectrum of the final product is presented in Figure 8; as expected, each acetylene group is connected to two benzyl groups.

Thus, we have demonstrated that the copolymers with three different reactive groups all show high reactivities toward small molecules containing amino or thiol groups, which makes it possible for future bioconjugation of biomacromolecules.

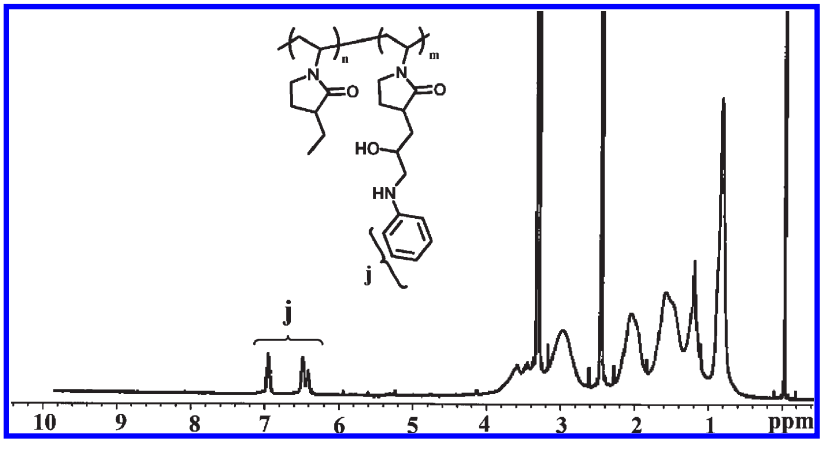


Figure 7. ^{1}H NMR spectrum of the reaction product of aniline with copolymer C1 in d_6-DMSO.

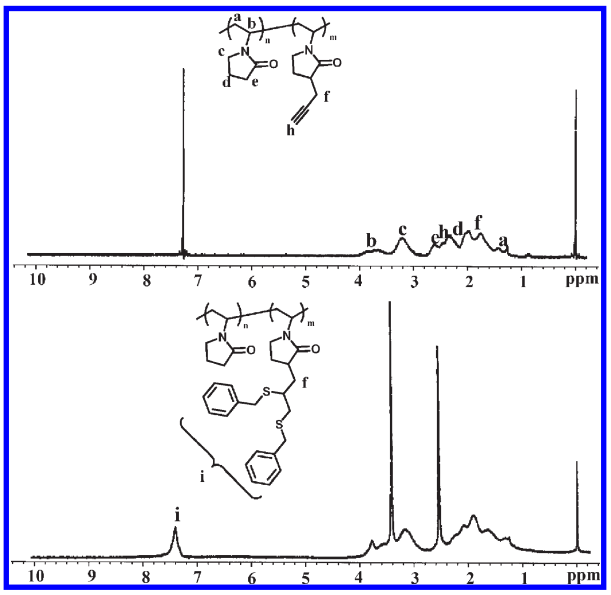


Figure 8. ^{1}H NMR spectra of copolymer D1′ in $CDCl_3$ (top) and the reaction product of this copolymer with benzyl mercaptan in d_6-DMSO (bottom).

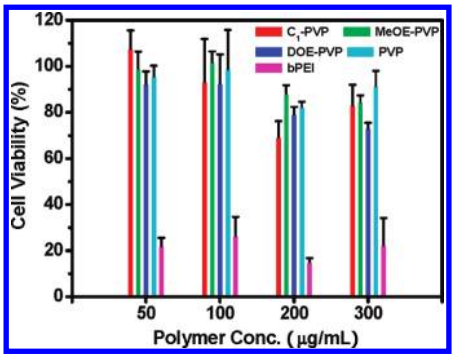


Figure 9. MTT assay in B16F10 cell lines. Results are presented as the mean ± SD in triplicate. The cells were incubated with the polymers for 4 h and cultured for another 24 h.

Cytotoxicity of Polymers. One of the most important potential applications of these copolymers is for biotechnology. Though PVP itself is of high biocompatibility, we have to evaluate the cytotoxicity of the new (co)polymers. Thus, the cytotoxicity of C_1-PVP, MeOE-PVP, and DOE-PVP with CP above 37 °C was measured in B16F10 and CHO cell lines using MTT assay. PVP and branched polyethylenimine are used as the negative and positive controls, respectively. Figure 9 presents the in vitro MTT assay results of cytotoxicity of polymers in B16F10 cell lines. All polymers based on PVP exhibited high cell viability similar as PVP, demonstrating that the alkyl or ether groups along the polymer main chain did not reduce the biocompatibility of PVP, and all the polymers had very low cytotoxicity within the tested polymer concentration range until 300 μg/mL. These polymers also exhibit high cell viabilities in CHO cell lines (Figure S38).

Conclusion

We have demonstrated that by simple modification of NVP at the 3-position we can obtain new types of thermoresponsive and functional (co)polymers based on PVP. It is easy to tune the CP of the (co)polymer in a wide temperature range by changing the substituent groups or the copolymer composition. Homopolymers C_1-PVP, C_2-PVP, and diC_1-PVP aqueous solution displays highly sensitive and reversible temperature-induced phase transitions with no hysteresis upon cooling. While (co)polymers containing ether substituents show higher CP, the phase transitions are less cooperative with lower phase transition enthalpies and less dehydration even at temperatures well above the CP. In addition, functional groups can be introduced into thermoresponsive (co)polymers by simple chemistry under mild conditions. These polymers all show low cytotoxicity comparable to that of PVP. Considering the good biocompatibility, easy functionalization, and thermoresponsive property of these (co)polymers, it is expected that they may find a wide range of applications in biotechnology like bioconjugation and drug delivery system. We are currently working on drug/gene delivery based on these (co)polymers.

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Supporting Information Available: Supporting ^{1}H NMR, ^{13}C NMR, and MS spectra of all the monomers; ^{1}H NMR spectra of some copolymers, thermoresponsive properties of the polymers, and biocompatibility experiment results. This material is available free of charge via the Internet at http://pubs.acs.org.

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