Macromolecules
ARTICLE

Macromolecules **2010**, *43*, 1815–1822 **1815**
DOI: 10.1021/ma902405g

Ring-Opening Metathesis Block Copolymerization of Amino Acid Functionalized Norbornene Monomers. Effects of Solvent and pH on Micelle Formation

Sutthira Sutthasupa,[†] Masashi Shiotsuki,[†] Hideki Matsuoka,[†] Toshio Masuda,[‡] and Fumio Sanda*,[†]

[†]*Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura Campus, Nishikyo-ku, Kyoto 615-8510, Japan and* [‡]*Department of Environmental and Biological Chemistry, Faculty of Engineering, Fukui University of Technology, 3-6-1 Gakuen, Fukui 910-8505, Japan*

Received October 29, 2009; Revised Manuscript Received December 29, 2009

ABSTRACT: Amino acid derived norbornene monomers having unprotected carboxy (**1**) and amino groups (**2**) and 7-oxanorbornene having ester groups (**3**) were block copolymerized using the Grubbs ruthenium complexes. In the combination of **1** and **2**, block copolymers with number-average molecular weights ranging from 43 000 to 95 000 were obtained in good yields. Poly(**1**)$_{75}$-*block*-poly(**2**)$_{25}$ was soluble in 0.5 M NaOH(aq) but insoluble in 0.1 M HCl, while poly(**1**)$_{25}$-*block*-poly(**2**)$_{75}$ was soluble in 0.1 M HCl but insoluble in 0.5 M NaOH(aq). Thus, the block compositions remarkably affected the solubility of the copolymers in acidic and basic media. On the other hand, poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ was soluble in H_2O but insoluble in CH_2Cl_2 and $CHCl_3$, while poly(**2**)$_{38}$-*block*-poly(**3**)$_{62}$ was soluble in CH_2Cl_2 and $CHCl_3$ but insoluble in H_2O. In this monomer combination, the block compositions significantly affected the solubility of the copolymers in organic and aqueous media. ^{1}H NMR spectroscopic, turbidity, and dynamic light scattering measurements revealed that poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$ formed micelles with a diameter around 130 nm in 0.5 M NaOH(aq). In a similar manner, it was revealed that poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ and poly(**2**)$_{38}$-*block*-poly(**3**)$_{62}$ formed micelles with a diameter around 80 nm in H_2O and reverse micelles with a diameter around 45 nm in CH_2Cl_2, respectively.

Introduction

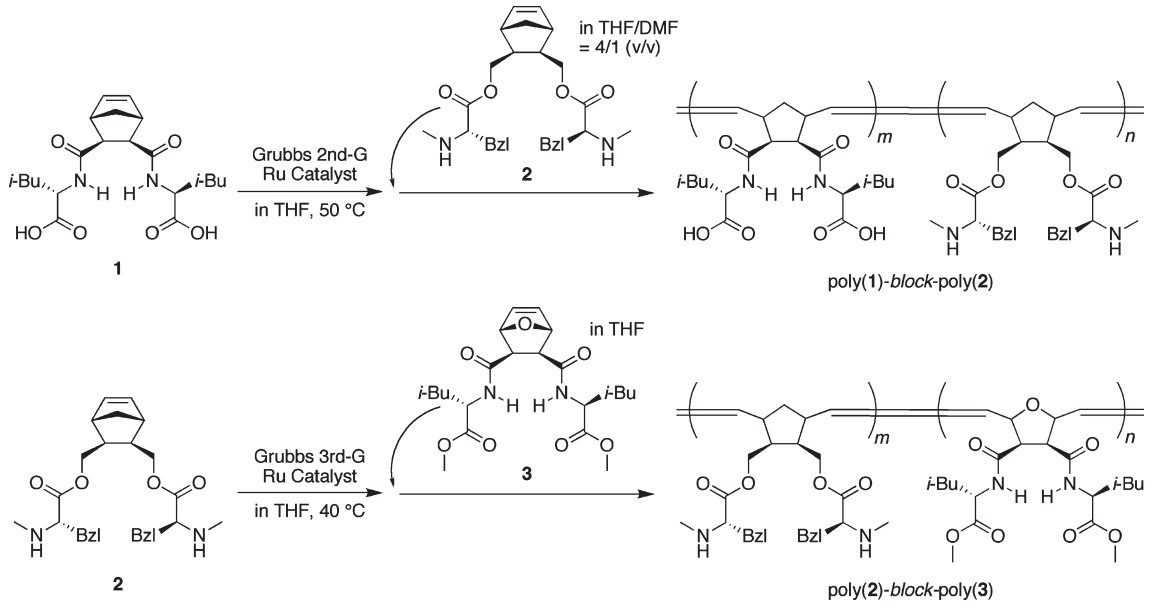
To produce materials with controlled nano- and micro-order structures,[1−3] researchers in precision polymerization chemistry continually seek ways to synthesize copolymers with controlled unit sequences. Block copolymers are also important for practical applications: for example, as thermoplastic elastomers, emulsifiers, and drug delivery materials.[4−6] The properties of block copolymers are controllable by monomer composition and block sequence. For example, amphiphilic diblock copolymers synthesized from a combination of hydrophobic and hydrophilic monomers form macromolecular assemblies in a solvent that has affinity for one block.[7−10] Block copolymers are commonly synthesized using living polymerization techniques, including anionic, cationic, atom-transfer radical polymerization, reversible addition−fragmentation chain-transfer polymerization,[5,10] and ring-opening metathesis polymerization (ROMP). Recent remarkable advances in ROMP catalysts make it possible to synthesize well-defined block copolymers with controlled molecular weights and stereostructures.[11,12] In particular, ROMP of norbornene derivatives achieves a high level of control over polydispersity, tacticity, and backbone configuration,[13−16] wherein metal carbene complexes have recently been used as catalysts. Among them, the Grubbs ruthenium (Ru) carbene complexes efficiently catalyze ROMP under ambient conditions, with high tolerance toward polar functional groups.[11] Thus, Ru-based ROMP is a promising route to prepare block copolymers with wide varies of functionalities.[17,18]

*Corresponding Author. E-mail: sanda@adv.polym.kyoto-u.ac.jp.

Meanwhile, amino acids are naturally derived optically active reagents, which can be useful as auxiliaries in asymmetric organic synthesis. Various attempts have been made to utilize amino acids, not only in peptide synthesis, but also as key components in synthetic polymers to enhance biodegradability,[19] chiral recognition,[20−22] formation of regulated chiral secondary structures,[23,24] and response to stimuli.[25−27] Gibson and co-workers have reported that amino acid derived norbornene imide monomers undergo living ROMP to produce the corresponding optically active polymers.[28] Grubbs and co-workers have synthesized polymers having peptide pendants, including glycine-arginine-glycine-aspartic acid and serine-arginine-asparagine, by ROMP of the corresponding norbornene derivatives, and they have proposed the possibility for application to cell-adhesive materials.[29] We have reported that amino acid bifunctionalized norbornene derivatives efficiently undergo ROMP to give polymers with fairly high molecular weights in good yields.[30] The polymerization proceeds in a living fashion to some extent, and the polymerizability largely depends on the substituents, stereostructure (*endo*, *exo*), solvents, and catalysts. Due to the livingness of ROMP of amino acid derived nornornenes with ester and carboxy groups, their block copolymers have been successfully synthesized. These block copolymers self-assemble to form reverse micelles in acetone.[31] Commonly, amine monomers are ROMP-inactive, because the amino group coordinates metathesis catalysts and deactivates them. We have recently achieved ROMP of an amino acid derived norbornene having unprotected amino groups,[32] wherein the amino acid spacer effectively suppresses catalyst deactivation. This remarkable finding leads us to develop alternating ROMP of amino acid derived norbornene monomers

Published on Web 01/14/2010

Scheme 1. Block Copolymerizations



carrying unprotected carboxy and amino groups. The acid−base interaction between the monomers should enhance the local monomer concentration and/or the interaction between the metal carbene propagating species and the incoming monomer.[33] Thus far, several researchers have succeeded in using anionic[34,35] and group-transfer polymerization[36,37] of methacrylates to synthesize pH-responsive block copolymers consisting of a carboxy-substituted block and an amino-substituted block. But to date, no one has synthesized copolymers consisting of carboxy and amino blocks by ROMP, presumably due to the low ROMP activity of amino group functionalized norbornene monomers. ROMP-based block copolymers having a carboxy or amino block show unique properties, including pH-response[38] and capability of traversing membranes to enter mammalian cells.[39] It is expected that ROMP-based copolymers consisting of acidic and basic blocks will exhibit interesting properties as well.

In the present article, we report the use of Grubbs Ru complexes to catalyze block copolymerizations of amino acid derived norbornene having carboxy groups (**1**) with norbornene having unprotected amino groups (**2**), as well as copolymerization of **2** with amino acid derived 7-oxanorbornene having ester groups (**3**, Scheme 1). As far as we know, the present combination (**1** and **2**) is the first example of a ROMP-based block copolymer consisting of a block having carboxy groups and a block having amino groups. The micelle formation of the block copolymers in various media was also examined to check the possibility as drug carriers.[40,41]

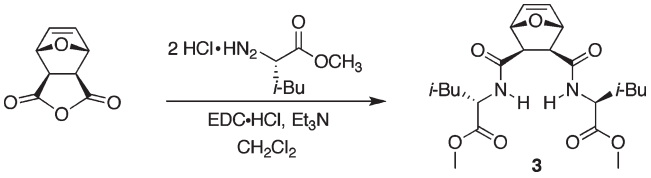
Experimental Section

Measurements. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded using tetramethylsilane (TMS) as an internal standard on a JEOL EX-400 NMR spectrometer. IR spectra were measured on a JASCO FTIR-4100 spectrophotometer. Mass spectra were measured on a JEOL JMS-HX110A mass spectrometer. Specific rotations ($[\alpha]_D$) were measured on a JASCO DIP-1000 digital polarimeter with a sodium lamp as a light source. Number- and weight-average molecular weights (M_n and M_w) of polymers were determined by gel permeation chromatography (GPC) on a JASCO Gulliver system (PU-980, CO-965, RI-930, and UV-1570) using one or more of the following polystyrene gel columns: (1) ShodexKF805L (bead size 10 μm, exclusion limit 4×10^6) using tetrahydrofuran (THF) as the eluent at a flow rate of 1.0 mL/min, calibrated by polystyrene standards at 40 °C; (2) TSK gel R-M (bead size 13 μm, exclusion limit 1×10^7) and TSK gel GMHXL (bead size 9 μm, exclusion limit 4×10^8) using a solution of LiBr (10 mM) in *N,N*-dimethylformamide (DMF) as the eluent at a flow rate of 1.0 mL/min, calibrated by polystyrene standards at 40 °C; (3) Shodex K803, K804, K805 (bead size 10 μm, exclusion limit 4×10^6) using $CHCl_3$ as the eluent at a flow rate of 1.0 mL/min, calibrated by polystyrene standards at 40 °C. Dynamic light scattering (DLS) measurements were carried out with an Otsuka Electronic SLS-7000 goniometer with a GC-1000 correlator. The time-correlation functions were analyzed by double exponential methods. The measurements were performed at four different scattering angles, and the diffusion coefficient was calculated from the slope of the straight line in the decay rate Γ vs q^2 plot, with q as the scattering vector.

Materials. Monomers **1** and **2** were synthesized according to previous reports.[31,33] *exo*-3,6-Epoxy-1,2,3,6-tetrahydrophthalic anhydride (TCI), L-leucine methyl ester hydrochloride (Watanabe Chemical Industries), *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl; Eiweiss Chemical Corp.), 4-(dimethylamino)pyridine (DMAP; Wako Pure Chemical Industries), Et_3N (Wako Pure Chemical Industries) were purchased and used without purification. 1,3-Bis-(2,4,6-trimethylphenyl)-2-(imidazolidinylidene)(dichlorophenylmethylene)(tricyclohexylphosphine)Ru (Grubbs second generation Ru catalyst) was donated by Materia, Inc., and used as received. 1,3-Bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)(dichlorophenylmethylene)(di-3-bromopyridine)Ru (Grubbs third generation Ru catalyst) was synthesized from the second generation catalyst according to a previous report.[42] THF and DMF used for polymerization were distilled over CaH_2 under nitrogen prior to use. Potassium hydrogen phthalate and indomethacin (Wako Pure Chemical Industries) were used without purification.

Synthesis of Monomer 3. *exo*-3,6-Epoxy-1,2,3,6-tetrahydrophthalic anhydride (0.93 g, 6.0 mmol) and L-leucine methyl ester hydrochloride (2.65 g, 14.0 mmol) were dissolved in CH_2Cl_2 (100 mL). EDC·HCl (2.87 g, 15.0 mmol) and Et_3N (0.37 g, 3.0 mmol) were added to the solution at 0 °C, and the resulting mixture was stirred at room temperature overnight. Then, the mixture was washed with 1 M HCl, saturated $NaHCO_3$(aq), and H_2O twice. The organic layer was separated and dried over anhydrous $MgSO_4$ and concentrated to afford **3** (Scheme 2) as a white solid. Yield 80%. Mp 115−118 °C. $[\alpha]_D = +5.4°$ ($c = 0.1$ g/dL in THF, room temperature). IR (KBr): 3324, 2956, 2870, 1749,

Scheme 2. Synthesis of Monomer 3



1683, 1654, 1535, 1437, 1368, 1330, 1310, 1263, 1199, 1157, 1038, 983, 917, 895, 828, 818, 700, 622 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.90−0.94 (m, 12H, 4 × −CHCH_3), 1.46−1.67 [m, 6H, 2 × −CH(CH_3)$_2$, 2 × −CH_2CH], 2.76−2.81 (m, 2H, 2 × −CHCO−), 3.76 (s, 6H, 2 × −COOCH_3−), 4.38−4.47 (m, 2H, 2 × −NHCH−), 5.7 (d, J = 7.3 Hz, 2H, bridge position), 6.39−6.46 (m, 3H, −CH = CH−, >CONH−), 6.76 (d, J = 7.1 Hz, 1H, >CONH−). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.25, 22.48, 22.58 (−COOCH_3), 24.63 [−CH(CH_3)$_2$], 40.85, 41.22 (>CHCH_2−), 48.23, 48.81 (−COOCH_3), 51.14 (>CHCH_2−), 52.09 (−CHCO−), 77.32, 79.98 (bridge position), 135.81, 136.76 (−HC=CH−), 170.73, 171.51 (−COO−), 172.98 (−CONH−). HRMS (FAB) $[M + Na]^+$: calcd for $C_{22}H_{34}N_2O_7Na$, 461.2264; found, 461.2242.

Block Copolymerization. Typical Procedure. Monomer **1** (86 mg, 0.21 mmol), Grubbs second generation Ru complex (3.6 mg, 4.2×10^{-3} mmol), and THF (0.5 mL) were fed into a glass tube under nitrogen, and the mixture was stirred at 50 °C for 1.5 h. A small portion was taken from the polymerization mixture of **1**, and subjected to ^1H NMR measurement to confirm the complete consumption of **1**. A solution of monomer **2** (100 mg, 0.21 mmol) in THF/DMF = 4/1 (v/v, 0.5 mL) was fed into the polymerization mixture, and the resulting mixture was further stirred for 24 h. It was poured into hexane (200 mL), and the precipitated polymer was isolated by filtration.

Spectroscopic Data for the Polymers. The data of poly(**1**) and poly(**2**) are reported in the literature.[31,33] Poly(**3**). IR (KBr): 3368 (−NH), 2958, 1742, 1676, 1560, 1542, 1438, 1207, 1155, 1019, 670 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 0.80−0.83 (broad, 12H, 4 × >CHCH_3), 1.45−1.54 [m, 6H, 2 × −CH-(CH_3)$_2$, 2 × −CH_2CH], 2.98−3.05 (broad, 2H, 2 × −CHCO−), 3.59 (s, 6H, 2 × −COOCH_3−), 4.15−4.32 (broad, 2H, 2 × −NHCH−), 4.54−4.90 (broad, 2H, bridge position), 5.47 and 5.65 (broad, 2H, −CH=CH−), 7.73 (broad, 1H, >CONH−), 8.02 (broad, 1H, >CONH−).

Results and Discussion

Monomer Synthesis. In addition to the amino acid derived norbornene monomers having carboxy (**1**) and amino groups (**2**),[31,33] we examined a novel 7-oxanorbornene monomer (**3**) as a hydrophilic monomer. Monomer **3** was synthesized by the reaction of *exo*-3,6-epoxy-1,2,3,6-tetrahydrophthalic anhydride with L-leucine methyl ester hydrochloride in 80% yield (Scheme 2). EDC·HCl was employed as a condensation agent because of easy removal of the urea derivative from the reaction mixture by washing with H_2O.[30−33] The monomer structure was determined by IR, ^1H, and ^{13}C NMR spectroscopies and high resolution mass spectrometry.

Homopolymerizations. Monomers **1**, **2**, and **3** were polymerized with the Grubbs second and third generation Ru catalysts. As listed in Tables 1 and 2, the monomers satisfactorily gave polymers with M_n's ranging from 10 900 to 26 000 in good yields.

Block Copolymerization of 1 and 2. Monomer **1** undergoes block copolymerization with the ester derivative.[31] The block copolymerization of monomer **1** with **2** was carried out with various feed ratios at 50 °C using the Grubbs second generation Ru complex. In the first stage of the polymerization of **1** in THF, **1** was quantitatively converted within 2−3 h, which was confirmed by ^1H NMR measurement of a sample portion drawn from the polymerization mixture. After that, a solution of **2** in THF/DMF was added to the polymerization mixture and then the resulting mixture was stirred for another 9−26 h, until the reaction was completed. Block copolymers with M_n's ranging from 43 000−95 000 were obtained in good yields as listed in Table 1.

Table 1. Homo and Block Copolymerizations of 1 and 2[a]

feed ratio		unit ratio[b]				
1	**2**	**1**	**2**	yield[c] (%)	M_n[d]	M_w/M_n[d]
100	0	100	0	89	26 000	1.54
75	25	77	23	87	50 000	1.80
62	38	69	31	85	54 000	1.94
50	50	53	47	97	61 700	2.02
38	62	40	60	99	43 000	1.62
25	75	29	71	99	95 000	2.68
0	100	0	100	93	10 900	1.54

[a] Solvent for the first stage (polymerization of **1**): THF, second stage (polymerization of **2**): THF/DMF = 4/1 (v/v), $[M]_{total}$ = 0.42 M, catalyst Grubbs second generation Ru complex, $[M]_{total}/[Ru]$ = 100, 50 °C, first stage 2−3 h, second stage 9−26 h. [b] Determined by ^1H NMR. [c] Hexane-insoluble part. [d] Measured by GPC (DMF, 10 mM LiBr), where the carboxy groups in the copolymers were transformed into methyl ester groups using $TMSCHN_2$.

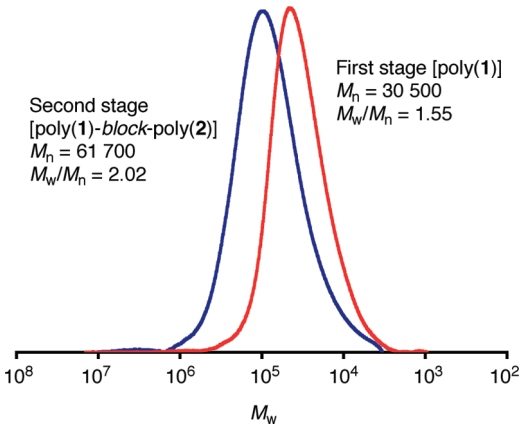


Figure 1. GPC traces of the polymers obtained by the first and second stages of the block copolymerization (feed mole ratio of **1**:**2** = 50:50). $CHCl_3$ and DMF were used as eluents for the GPC measurement (polystyrene calibration) of the polymers obtained by the first and second stages, respectively.

Figure 1 exhibits the representative GPC traces of the copolymers obtained after the first and second stages. The peak clearly shifted to a higher molecular weight region from the first to second stage, while maintaining a unimodal distribution. The order of monomer feed was important to achieve the block copolymerization of **1** and **2**. Namely, block copolymerization was unsatisfactory when monomer **2** was used as the first block. Furthermore, choice of solvent was important. When THF was used as a solvent both for the first and second stage polymerizations, a polymeric mass precipitated a couple of minutes after the addition of a solution of monomer **2** into a polymerization mixture of **1**, indicating the low solubility of the block copolymer. Employment of DMF solved the solubility issue, but the polymerization rate of **1** largely decreased, which made the copolymerization inefficient. We therefore used THF and THF/DMF in the first- and second-stage polymerizations, respectively. The copolymers were obtained in good yields by 2−3 h and 9−26 h polymerizations at the first and second stages, respectively.

As reported previously, monomers **1** and **2** undergo alternating copolymerization based on acid−base interaction

between the monomers and/or between the propagation polymer end and the incoming monomer.[33] In this study, pH-dependent self-assembly of block copolymers of **1** and **2** is expected due to their carboxy and amino groups. As listed in Table 3, poly(**1**)-*block*-poly(**2**)s were soluble in polar solvents such as DMF, DMSO, and MeOH in a manner similar to poly(**1**) and poly(**2**). Poly(**1**) was insoluble in an acidic medium (0.1 M HCl) while soluble in base (0.5 M NaOH(aq)). The solubility of poly(**2**) in acidic and basic media was opposite to that of poly(**1**) (soluble in acid and insoluble in base), as predicted from their substituent groups: carboxy in poly(**1**) and amino in poly(**2**). The solubility of poly(**1**)-*block*-poly(**2**)s in acidic and basic media depended on the composition of poly(**1**) and poly(**2**) blocks. The copolymers with at least 50% poly(**1**) block contents were insoluble in acidic medium while soluble in basic solution. Poly(**1**)$_{38}$-*block*-poly(**2**)$_{62}$ was insoluble in both acidic and basic media. Poly(**1**)$_{25}$-*block*-poly(**2**)$_{75}$ was soluble in acidic media but insoluble in base. All the copolymers were insoluble in H_2O.

Block Copolymerization of 2 and 3. 7-Oxanorbornene derivatives undergo ROMP satisfactorily to give polymers having THF skeletons. These are applicable to biological and medical materials due to their high hydrophilicity compared with their polynorbornene counterparts.[43−47] We synthesized and polymerized the oxanorbornene-derived novel monomer **3**. Poly(**3**) was more soluble in common organic solvents than poly(**1**) and poly(**2**), as listed in Table 3. Poly(**2**) and poly(**3**) obtained by the polymerization in THF showed relatively small polydispersity indices (PDIs, 1.28 and 1.18, Table 2), suggesting the possibility of block copolymerization of monomers **2** and **3**. Block copolymerization of **2** and **3** using the Grubbs second generation catalyst was attempted unsuccessfully, even though the homopolymers of these two monomers were obtained. Recently, Grubbs third generation catalyst has gained attention due to its excellent tolerance to functional groups and fast initiation rate in ROMP.[42,48] Grubbs third generation catalyst was, therefore, used in the block copolymerization of **2** and **3**. At the first stage, **2** quantitatively polymerized within 1 h, which was confirmed by ^{1}H NMR measurement of a sample portion drawn from the polymerization mixture. After that, monomer **3** was added to the mixture. The viscosity of the polymerization mixture gradually increased, suggesting that the block copolymerization had occurred. The mixture was stirred for another 24−48 h until the reaction was completed.

Table 2. Homo and Block Copolymerizations of 2 and 3[a]

feed ratio		unit ratio[b]				
2	**3**	**2**	**3**	yield[c] (%)	M_n[d] (first block)	M_w/M_n[d] (first block)
100	0	100	0	95	11000[e]	1.28[e]
75	25	80	20	93	9900	1.22
62	38	68	32	92	8800	1.20
50	50	60	40	90	7500	1.23
38	62	40	60	90	5800	1.23
25	75	35	65	85	4700	1.24
0	100	0	100	96	25500[f]	1.18[f]

[a] First stage: polymerization of **2**. Second stage: polymerization of **3**. $[M]_{total}$ = 0.42 M in THF, catalyst Grubbs third generation Ru complex, $[M]_{total}/[Ru]$ = 100, 40 °C; time, first stage 1 h and second stage 24−48 h. [b] Determined by ^{1}H NMR (DMSO-d_6). [c] Hexane-insoluble part. [d] Determined by GPC (THF). The M_n's and M_w/M_n's of the block copolymers could not be determined. [e] Poly(**2**). [f] Poly(**3**).

Table 2 summarizes the results of block copolymerization of **2** with **3**. The M_n and M_w/M_n values of the polymers [poly(**2**)] obtained after the first stage polymerization were determined by GPC eluted with THF, but no GPC peaks of the polymers obtained after the second stage polymerization were observed. The GPC of the copolymers with $CHCl_3$, DMF, H_2O, 20 mM $NaHCO_3$(aq), and potassium hydrogen phosphate buffer (pH = 4.0) eluents was also attempted, but again no GPC peaks were observed. This seems to result from the high affinity of the copolymers to the GPC column beads.

Although we could not determine the M_n value, it is considered that block copolymerization took place, judging from the solubilities of the polymers [poly(**2**)-*block*-poly(**3**)s], which are largely different from those of poly(**2**) and poly(**3**), as listed in Table 3. Poly(**3**) was soluble in CH_2Cl_2 and $CHCl_3$ and insoluble in 0.1 M HCl, and H_2O, and poly(**2**) showed the opposite solubility trend. The copolymers with at least 62% poly(**3**) block content were soluble in CH_2Cl_2 and $CHCl_3$, and copolymers with at least 50% poly(**3**) block content were insoluble in 0.1 M HCl and H_2O. If the formed polymers were not block copolymers but mixtures of poly(**2**) and poly(**3**), such solubility differences should not be observed, and there should be a GPC peak for free poly(**2**). The relatively narrow PDIs (1.20−1.24) of the first blocks indicated that living polymerization of **2** occurred. The fact that polymerization of **2** exhibited a linear relationship between the M_n values and [M]/[Ru] ratios (Supporting Information, Figure S1) also supports the livingness.

Formation of Self-Assemblies. As mentioned above, poly(**1**)-*block*-poly(**2**)s with at least 50% poly(**1**) block content

Table 3. Solubility of the Polymers[a]

	solvent											
polymer	hexane	toluene	CH_2Cl_2	$CHCl_3$	THF	acetone	DMF	DMSO	MeOH	0.1 M HCl	H_2O	0.5 M NaOH(aq)
poly(**1**)	−	−	−	−	+	−	+	+	+	−	−	+
poly(**1**)-*block*-poly(**2**)												
75:25	−	−	−	−	−	−	+	+	+	−	−	+
62:38	−	−	−	−	−	−	+	+	+	−	−	+
50:50	−	−	−	−	−	−	+	+	+	−	−	+
38:62	−	−	−	−	−	−	+	+	+	−	−	−
25:75	−	−	−	−	−	−	+	+	+	+	−	−
poly(**2**)	−	−	−	−	+	+	+	+	+	+	+	−
poly(**2**)-*block*-poly(**3**)												
75:25	−	−	−	−	+	+	+	+	+	±	+	−
62:38	−	−	−	−	+	+	+	+	+	±	+	−
50:50	−	−	−	−	+	+	+	+	+	−	−	−
38:62	−	−	+	+	+	+	+	+	+	−	−	−
25:75	−	−	+	+	+	+	+	+	+	−	−	−
poly(**3**)	−	−	+	+	+	+	+	+	+	−	−	−

[a] Symbols: (−) insoluble; (±) partly soluble; (+) soluble.

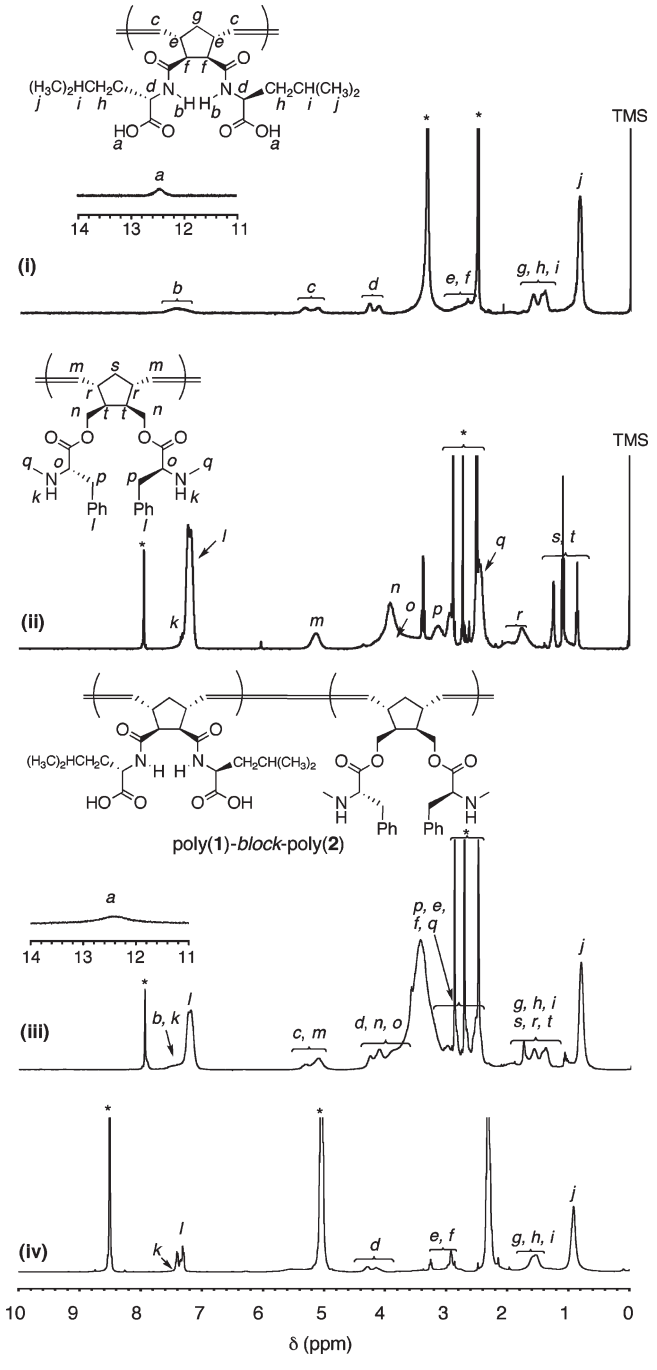


Figure 2. ^{1}H NMR spectra (400 MHz) of (i) poly(**1**) measured in DMSO-d_6, (ii) poly(**2**) in DMSO-d_6, (iii) poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$ in DMSO-d_6, and (iv) poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$ in 0.5 M NaOD/D_2O. (∗) Signal derived from solvents including residual DMF.

were soluble in basic media. The copolymer solutions were slightly turbid, suggesting that the block copolymers were self-assembled. Meanwhile, poly(**2**)-*block*-poly(**3**)s with at least 62% poly(**3**) block content were soluble in CH_2Cl_2, while those with at least 62% poly(**2**) block content were soluble in H_2O. These solutions were also turbid, suggesting self-assembly (micelle) formation, as well. Moreover, H_2O-soluble poly(**2**)-*block*-poly(**3**)s with the unit ratios of 75:25 and 62:38 became partly insoluble when the pH was lowered.

In order to clarify self-assembly of the block copolymers, ^{1}H NMR spectroscopic and DLS measurements of the polymer solutions were conducted. Figure 2 presents the ^{1}H NMR spectra of poly(**1**) and poly(**2**) in DMSO-d_6, as well as poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$ in DMSO-d_6 and in 0.5 M NaOD/D_2O. Poly(**1**) exhibits two olefinic proton signals at 5.2 and 5.4 ppm (*c* in Figure 2i), while poly(**2**) shows one olefinic proton signal at 5.1 ppm (*m* in Figure 2ii). In DMSO-d_6 [good solvent both for poly(**1**) and poly(**2**)], poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$ exhibits olefinic proton signals (*c* and *m* in Figure 2iii) as a sum of their homopolymers, together with representative carboxy (*a*) and methyl proton (*j*) signals of the isobutyl groups of poly(**1**) block and the phenyl proton (*l*) signal of poly(**2**) block. On the other hand, in NaOD/D_2O (Figure 2iv), the copolymer clearly exhibits signals based on the poly(**1**) block, especially the methyl protons (*j*) of the isobutyl groups, but signals based on the poly(**2**) block are attenuated (e.g., phenyl (*l*) protons). When block copolymers form micelles, the ^{1}H NMR signals of the core part commonly become small compared to those of the shell part due to the different mobilities.[49] The smaller and unclear proton signals of poly(**2**) block in NaOD/D_2O indicate the formation of micelles consisting of a poly(**2**) core and a poly(**1**) shell, as predicted for structures having both amino and carboxy groups. It is considered that NaOD/D_2O is the selective solvent for poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$ to form micelles.

Figure 3 shows the ^{1}H NMR spectra of poly(**2**), and poly(**3**) in DMSO-d_6, as well as poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ in DMSO-d_6, CD_2Cl_2, and D_2O. Poly(**2**) exhibits one olefinic proton signal at 5.1 ppm (*c* in Figure 3i), while poly(**3**) shows two olefinic proton signals around 5.2 and 5.4 ppm (*l* in Figure 3ii). In DMSO-d_6 [good solvent for both poly(**2**) and poly(**3**)], poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ exhibits all the signals based on both poly(**2**) and poly(**3**) blocks, including olefinic proton signals around 5.1, 5.4, and 5.6 ppm (*c* and *l* in Figure 3iii). This result indicates that the copolymer contains poly(**2**) and poly(**3**) blocks. On the other hand, in CD_2Cl_2 the copolymer exhibits clear signals based on the poly(**3**) block, including methyl protons (*o* and *s*) of the ester and isobutyl groups, but signals of phenyl protons (*b*) based on poly(**2**) are attenuated (Figure 3iv). It is considered that the copolymer forms micelles consisting of a poly(**2**) core and a poly(**3**) shell in CD_2Cl_2. Figure 3v presents the ^{1}H NMR spectrum of poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ measured in D_2O. Contrary to the case in CD_2Cl_2, the copolymer exhibits clear signals based on the poly(**2**) block, while signals from the poly(**3**) block are attenuated. These facts suggest that the copolymer forms micelles consisting of a poly(**3**) core and a poly(**2**) shell, with D_2O as the selective micelle-forming solvent.

Characterization of Micelles. The critical micelle concentration (cmc) is phenomenologically defined from sharp changes in measurable quantities. Several properties such as turbidity, solubilization, and surface tension exhibit abrupt changes at the cmc.[50] Turbidity is a simple and convenient index to detect aggregates, because it reflects changes in the sizes of particles dispersed in solution, and it is widely used to determine micelle formation, stability, and cmc.[51−56] In this work, the turbidity of the polymer solutions was evaluated as the difference in transmittance at 435 nm of the copolymer in a good solvent compared to a selective solvent ($T_{good} - T_{selective}$). Figure 4a is a plot of ($T_{good} - T_{selective}$) vs concentration of poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$ with DMF and NaOH(aq) as the good and selective solvents, respectively. The plot exhibits a turning point at *ca.* $c = 4 \times 10^{-2}$ wt%, which can be regarded as a cmc. The cmc's of poly(**2**)-*block*-poly(**3**)s were determined in a similar manner. Parts b and c of Figure 4 are plots of ($T_{good} - T_{selective}$) vs concentration of poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ and poly(**2**)$_{32}$-*block*-poly(**3**)$_{68}$ with THF as the good solvent, and H_2O and CH_2Cl_2 as selective solvents. The turning

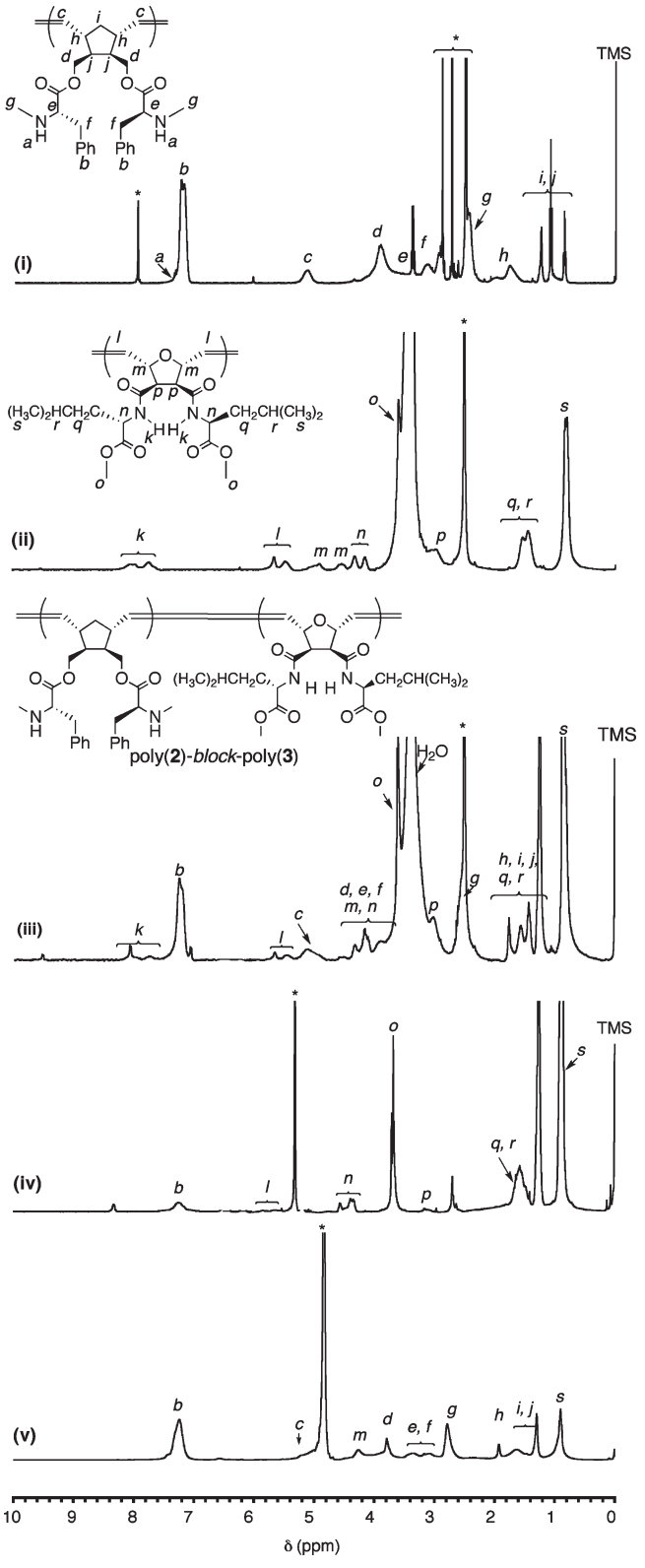


Figure 3. ^{1}H NMR spectra (400 MHz) of (i) poly(**2**) in DMSO-d_6, (ii) poly(**3**) in DMSO-d_6, (iii) poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ in DMSO-d_6, (iv) poly(**2**)$_{38}$-*block*-poly(**3**)$_{62}$ in CD_2Cl_2, and (v) poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ in D_2O. (∗) Signal derived from solvents including residual DMF.

points regarded as cmc's were $c = 2 \times 10^{-2}$ wt% (Figure 4b) and $= 1 \times 10^{-2}$ wt% (Figure 4c), respectively.

Dynamic light scattering (DLS) measurements were performed to confirm micelle formation of the block copolymers. Figure 5 shows the correlation functions of a

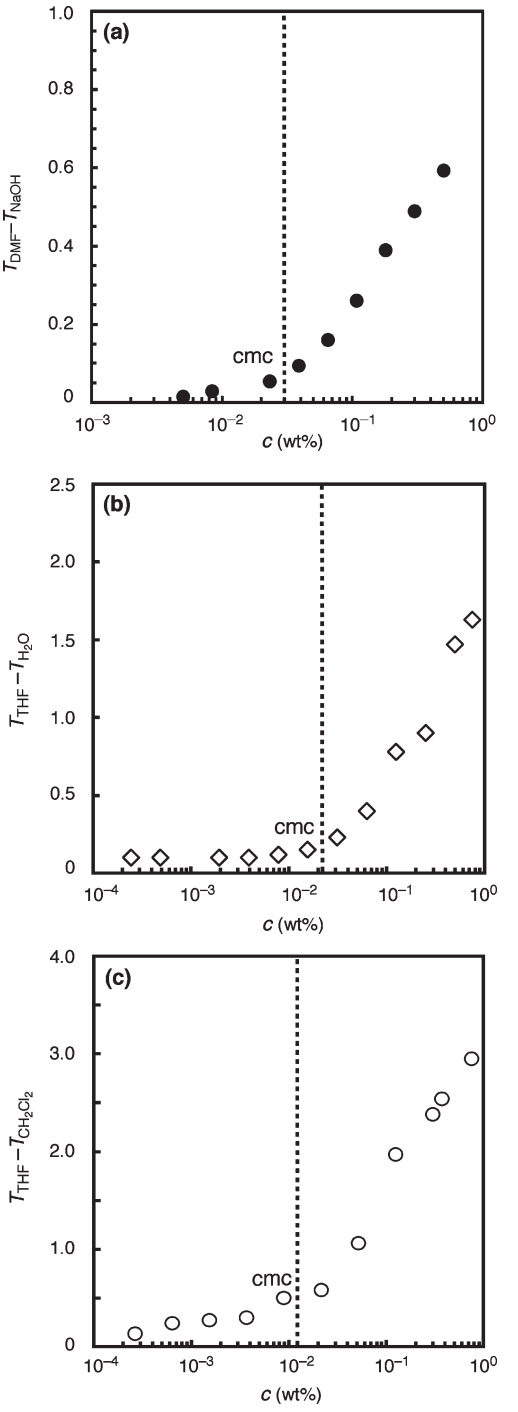


Figure 4. Plots of transmittance difference at 435 nm (T_{good} - $T_{selective}$) vs concentration of copolymers (a) DMF and 0.2 M NaOH(aq) solutions of poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$, (b) THF and H_2O solutions of poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$, and (c) THF and CH_2Cl_2 solutions of poly(**2**)$_{38}$-*block*-poly(**3**)$_{62}$.

NaOH(aq) solution of poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$, a H_2O solution of poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$, and a CH_2Cl_2 solution of poly(**2**)$_{38}$-*block*-poly(**3**)$_{62}$, all of which confirm the presence of particles. Table 4 summarizes the hydrodynamic radii (R_h and R_{large}) of the block copolymers, where R_{large} is the size of large particles considered as aggregates.

To obtain further information on the micelle sizes, molecular models of *cis*-isotactic, *cis*-syndiotactic, *trans*-isotactic, and *trans*-syndiotactic 100-mers of 2,3-*exo,exo*-dibromo-5-norbornene were constructed (Supporting Information, Figure S2).

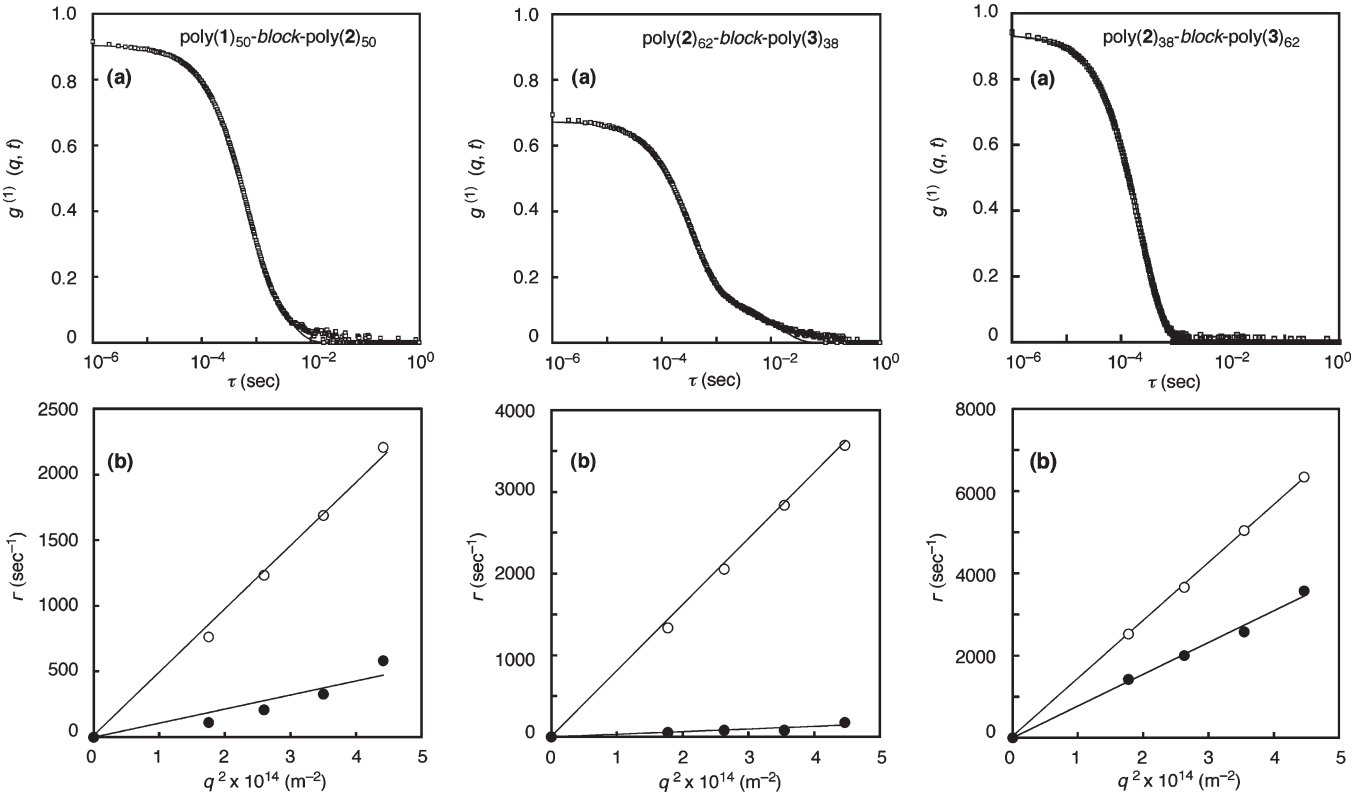


Figure 5. DLS results for solutions of poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$ 0.5 wt % in 0.2 M NaOH(aq), poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ (0.5 wt %) in H_2O, and poly(**2**)$_{38}$-*block*-poly(**3**)$_{62}$ (0.5 wt %) in CH_2Cl_2 measured at a scattering angle of 90° at 25 °C. (a) Time correlation functions for the scattered field. Solid lines are double-exponential fits. (b) Decay rate Γ vs q^2 plot for block copolymer solutions for the fast mode (○) and slow mode (●). The diffusion coefficients of the micelles were evaluated from the slopes of the straight lines for the fast mode.

Table 4. Hydrodynamic Radii (R_h) of the Micelles Determined by DLS

block copolymer	medium	R_h (nm)	R_{large} (nm)
poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$	0.2 M NaOH(aq)	132	597
poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$	H_2O	81	1964
poly(**2**)$_{38}$-*block*-poly(**3**)$_{62}$	CH_2Cl_2	45	83

The chain lengths of the 100-mers ranged from 52.1 to 55.2 nm, which moderately agree with the R_h's determined by DLS. The R_h's of block copolymers commonly depend on the surroundings as well as monomer structures.[56] The parameter measured by DLS is equivalent to the translational diffusion coefficient of spherical particles (D_0). This coefficient is then placed directly into the Stokes−Einstein equation, $R_h = kT/6\eta\pi D_0$, where k is the Boltzmann constant, T is the absolute temperature, and η is the viscosity.[57] It is likely that the carboxy groups at the shell of poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$ micelle strongly interact with NaOH(aq). The micelles seem to become extended and move slowly (small D_0), resulting in the larger R_h (132 nm). In fact, the R_h of the poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ micelle in H_2O (81 nm) is smaller than that of poly(**1**)$_{50}$-*block*-poly(**2**)$_{50}$, presumably due to the absence of such acid−base interaction. The R_h of poly(**2**)$_{38}$-*block*-poly(**3**)$_{62}$ micelle in CH_2Cl_2 is 45 nm, the smallest among the micelles listed in Table 4. This agrees with previous work showing that D_0 in an organic solvent is larger than that in aqueous media.[56]

Conclusions

In the present paper, we have demonstrated the successful block copolymerization of norbornene monomer **1** having carboxy groups, monomer **2** having amino groups, and 7-oxanorbornene monomer **3** having ester groups via ROMP. To the best of our knowledge, this is the first achievement of block copolymerization of norbornene monomers having unprotected carboxy and amino groups. The block copolymers exhibited solubilities which depended markedly on the polarity and pH of the solution, and they formed micelles in selective solvents. The micelles of poly(**2**)$_{62}$-*block*-poly(**3**)$_{38}$ loaded indomethacin, and subsequently released the drug in acidic aqueous media. We believe that the present study contributes to the progress of pH-responsive polymer synthesis by ROMP. As described in the Introduction, polymer nanoparticles prepared by ROMP have been used as polymer-drug conjugates for controlled drug release.[58−62] Micelles consisting of amphiphilic lipids and/or polymeric materials have been examined as matrices for poorly soluble compounds.[41] Micelles with hydrophobic cores can solubilize hydrophobic drugs. Amphiphilic block copolymers are promising candidates for preparation of micelles for this purpose. The drug-loading and drug-release behavior of the micelles prepared in the present study is now under investigation, and will be reported in due course.

Acknowledgment. This research was supported by a Grant-in-Aid from the Global COE Program, "International Center for Integrated Research and Advanced Education in Materials Science" from the Ministry of Education, Culture, Sports, Science and Technology of Japan. Thanks are also due to Materia, Inc. for the donation of the Grubbs second generation Ru catalyst. We are grateful to Mr. Tomoyuki Ohnishi at Kyoto University for measurement of DLS. We are indebted to Prof. Kenneth B. Wagener and Dr. Kathryn R. Williams at the University of Florida for their helpful suggestions and comments. S. S. acknowledges the help of Dr. Pensri Charoensit at Kyoto University for her suggestions about drug loading and release.

Supporting Information Available: Relationship between [M]/[Ru] and M_n of the polymers obtained by the polymerization

of **2** (Figure S1), and structures of 100-mers of 2,3-*exo*,*exo*-dibromo-5-norbornene (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hamley, W. *Nanotechnology* **2003**, *14*, 39–54.
(2) Mann, S. *Nat. Mater.* **2009**, *8*, 781–792.
(3) Vandermeulen, G. W. M.; Klok, H. A. *Macromol. Biosci.* **2004**, *4*, 383–398.
(4) Duncan, R. *Nat. Rev. Drug. Discov.* **2003**, *2*, 347–360.
(5) Lodge, T. P. *Macromol. Chem. Phys.* **2003**, *204*, 265–273.
(6) Shenhar, R.; Norsten, T. B.; Rotello, V. M. *Adv. Mater.* **2005**, *17*, 657–669.
(7) Gohy, J. F. *Adv. Polym. Sci.* **2005**, *190*, 65–136.
(8) Rodriguez-Hernandez, J.; Checot, F.; Gnanou, Y.; Lecommandoux, S. *Prog. Polym. Sci.* **2005**, *30*, 691–724.
(9) Moffitt, M.; Khougaz, K.; Eisenberg, A. *Acc. Chem. Res.* **1996**, *29*, 95–102.
(10) Hadjichristidis, N.; Pitsikalis, M.; Iatrou, H. *Adv. Polym. Sci.* **2005**, *189*, 1–124.
(11) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18–29.
(12) Schrock, R. R.; Hoveyda, A. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4592–4633.
(13) Grubbs, R. H.; Tunas, W. *Science* **1989**, *243*, 907–915.
(14) Schrock, R. R. *Acc. Chem. Res.* **1990**, *23*, 158–165.
(15) Bazan, G. C.; Schrock, R. R.; Khosravi, E.; Feast, W. J.; Gibson, V. C.; O'Regan, M. B.; Tomas, J. K.; Davis, W. M. *J. Am. Chem. Soc.* **1990**, *112*, 8378–8387.
(16) Feast, W. J.; Gibson, V. C.; Marshall, E. L. *J. Chem. Soc., Chem. Commun.* **1992**, 1157–1158.
(17) Bielawski, C. W.; Grubbs, R. H. *Prog. Polym. Sci.* **2001**, *32*, 1–29.
(18) Singh, R.; Czekelins, C.; Schrock, R. R. *Macromolecules* **2006**, *39*, 1316–1317.
(19) (a) Cohen-Arazi, N.; Katzhendler, J.; Kolitz, M.; Domb, A. J. *Macromolecules* **2008**, *41*, 7259–7263. (b) Obst, M.; Steinbuchel, A. *Biomacromolecules* **2004**, *5*, 1166–1176.
(20) Sanda, F.; Endo, T. *Macromol. Chem. Phys.* **1999**, *200*, 2651–2661.
(21) Kulkarni, R. K.; Morawetz, H. *J. Polym. Sci.* **1961**, *54*, 491–503.
(22) (a) Iwakura, Y.; Toda, F.; Suzuki, H. *J. Org. Chem.* **1967**, *32*, 440–443. (b) Iwakura, Y.; Toda, F.; Suzuki, H. *J. Polym. Sci. Part A., Polym. Chem.* **1967**, *5*, 1599–1607.
(23) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893.
(24) (a) Gao, G.; Sanda, F.; Masuda, T. *Macromolecules* **2003**, *36*, 3932–3937. (b) Gao, G.; Sanda, F.; Masuda, T. *Macromolecules* **2003**, *36*, 3938–3943. (c) Zhao, H.; Sanda, F.; Masuda, T. *Macromolecules* **2004**, *37*, 8888–8892.
(25) Deming, T. J. *Adv. Drug Deliv. Rev* **2002**, *54*, 1145–1155.
(26) Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, J. D.; Pochan, D.; Deming, T. J. *Nature* **2002**, *417*, 424–428.
(27) Checot, F.; Lecommandoux, S.; Gnanou, Y.; Klok, H. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 1339–1343.
(28) (a) Biagini, S. C. G.; Coles, M. P.; Gibson, V. C.; Giles, M. R.; Marshall, L. E.; North, M. *Polymer* **1998**, *39*, 1007–1014. (b) Biagini, S. C. G.; Davies, R. G.; Gibson, V. C.; Giles, M. R.; Marshall, E. L.; North, M.; Robson, D. A. *Chem. Commun.* **1999**, *235*, 236. (c) Biagini, S. C. G.; Davies, R. G.; Gibson, V. C.; Giles, M. R.; Marshall, E. L.; North, M.; Robson, D. A. *Polymer* **2001**, *42*, 6669–6671.
(29) (a) Maynard, H. D.; Okada, S. Y.; Grubbs, R. H. *Macromolecules* **2000**, *33*, 6239–6248. (b) Maynard, H. D.; Okada, S. Y.; Grubbs, R. H. *J. Am. Chem. Soc.* **2001**, *123*, 1275–1279.
(30) (a) Sutthasupa, S.; Terada, K.; Sanda, F.; Masuda, T. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 5337–5343. (b) Sutthasupa, S.; Terada, K.; Sanda, F.; Masuda, T. *Polymer* **2007**, *48*, 3026–3032. (c) Sutthasupa, S.; Sanda, F.; Masuda, T. *Macromol. Chem. Phys.* **2008**, *209*, 930–937.
(31) Sutthasupa, S.; Sanda, F.; Masuda, T. *Macromolecules* **2008**, *41*, 305–311.
(32) Sutthasupa, S.; Sanda, F.; Masuda, T. *Macromolecules* **2009**, *42*, 1519–1525.
(33) Sutthasupa, S.; Shiotsuki, M.; Masuda, T.; Sanda, F. *J. Am. Chem. Soc.* **2009**, *131*, 10546–10551.
(34) Gohy, J. F.; Creutz, S.; Garcia, M.; Mahltig, B.; Stamm, M.; Jerome, R. *Macromolecules* **2000**, *33*, 6378–6387.
(35) Goloub, T.; de Keizer, A.; Cohen Stuart, M. A. *Macromolecules* **1999**, *32*, 8441–8446.
(36) Patrickios, C. S.; Hertler, W. R.; Abbott, N. L.; Hatton, T. A. *Macromolecules* **1994**, *27*, 930–937.
(37) Lowe, A. B.; Billingham, N. C.; Armes, S. P. *Macromolecules* **1998**, *31*, 5991–5998.
(38) Rodriguez-Hernandez, J.; Lecommandoux, S. *J. Am. Chem. Soc.* **2005**, *127*, 2026–2027.
(39) Kolonko, E. M.; Pontrello, J. K.; Mangold, S. L.; Kiessling, L. L. *J. Am. Chem. Soc.* **2009**, *131*, 7327–7333.
(40) Bae, Y.; Kataoka, K. *Adv. Drug. Deliv. Rev.* **2009**, *61*, 768–784.
(41) Liu, J.; Lee, H.; Allen, C. *Curr. Pharm. Des.* **2006**, *12*, 4685–4701.
(42) Love, J. A.; Morgan, J. P.; Trnka, T. M.; Grubbs, R. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 4035–4037.
(43) Cazalis, C.; Herogeuz, V.; Fontanille, M. *Macromol. Chem. Phys.* **2000**, *201*, 869–876.
(44) Mortell, K. H.; Gingras, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **1994**, *116*, 12053–12054.
(45) Schueller, C. M.; Manning, D. D.; Kiessling, L. L. *Tetrahedron Lett.* **1996**, *49*, 8853–8856.
(46) Ishihara, Y.; Bazzi, H. S.; Toader, V.; Godin, F.; Sleiman, H. F. *Chem.—Eur. J.* **2007**, *13*, 4560–4570.
(47) Meier, S.; Reisinger, H.; Hang, R.; Mecking, S.; Mulhaupt, R.; Stelzer, F. *Chem. Commun.* **2001**, 855–856.
(48) Choi, T. L.; Grubbs, R. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 1743–1746.
(49) Stubenrauch, K.; Moitzi, C.; Fritz, G.; Glatter, O.; Trimmel, G.; Stelzer, F. *Macromolecules* **2006**, *39*, 5865–5874.
(50) Hamley, I. W.; In *Introduction to Soft Matter: Polymers, Colloids, Amphiphiles and Liquid Crystal*; Wiley−VCH: Weinheim, Germany, 2000; Chapter 4.6, page 217.
(51) Bakshi, M. S.; Singh, J.; Singh, K.; Kaur, G. *Colloids Surf. A: Physicochem. Eng. Asp.* **2004**, *237*, 61–71.
(52) Zhang, J. X.; Qiu, L. Y.; Jin, Y.; Zhu, K. *Colloids Surf. B: Biointerfaces* **2005**, *43*, 123–130.
(53) Huang, C. J.; Shieu, F. S. *Colloid Polym. Sci.* **2005**, *284*, 192–202.
(54) Halacheva, S.; Rangelov, S.; Tsvetanov, C. *Macromolecules* **2006**, *39*, 6845–6852.
(55) Lee, E. S.; Shin, H. J.; Na, K.; Bae, Y. H. *J. Controlled Release* **2003**, *90*, 363–374.
(56) La, S. B.; Okano, T.; Kataoka, K. J. *J. Pharm. Sci.* **1996**, *85*, 85–90.
(57) Croy, S. R.; Kwon, G. S. *Curr. Pharm. Res.* **2006**, *12*, 4669–4684.
(58) Biagini, S. C. G.; Gibson, V. C.; Giles, M. R.; Marshall, E. L.; North, M. *Chem. Commun.* **1997**, 1097–1098.
(59) Arimoto, H.; Nishimura, K.; Kinumi, T.; Hayakawa, I.; Uemura, D. *Chem. Commun.* **1999**, 1361–1362.
(60) Watson, K. J.; Anderson, D. R.; Nguyen, S. T. *Macromolecules* **2001**, *34*, 3507–3509.
(61) Bertin, P. A.; Watson, K. J.; Nguyen, S. T. *Macromolecules* **2004**, *37*, 8364–8372.
(62) Smith, D.; Clark, S. H.; Bertin, P. A.; Mirkin, B. L.; Nguyen, S. T. *J. Mater. Chem.* **2009**, *19*, 2159–2165.