Enzymatic Oxidative Polymerization of 2,6-Dimethylphenol

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ABSTRACT: Enzymatic oxidative polymerization of 2,6-dimethylphenol has been carried out in an aqueous organic solvent at room temperature under air. Laccase derived from *Pycnoporus coccineous* and horseradish and soybean peroxidases were active for the polymerization, yielding polymeric materials with molecular weights of several thousands. The product polymer was in all cases soluble in common organic solvents. The polymerization behavior was dependent on the enzyme type. The effects of the solvent composition have been systematically investigated with respect to the polymer yield and molecular weight. The mixing ratio between the organic solvent and buffer affected the polymer yield, and the highest yield was achieved in 60% buffer. Various water-miscible organic solvents such as acetone, methanol, and 1,4-dioxane were available as components of the mixed solvent. In using laccase catalyst, the acidic buffer afforded the polymer in high yields. NMR and matrix-assisted laser desorption/ionization time of flight mass spectroscopic analyses showed that the present polymer was exclusively composed of 2,6-dimethyl-1,4-oxyphenylene units.

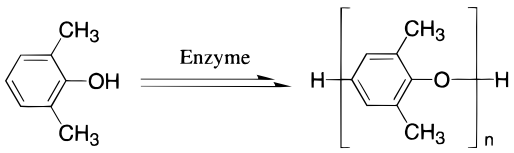
Introduction

Poly(2,6-dimethyl-1,4-oxyphenylene) (poly(phenylene oxide), PPO) is widely used in high-performance engineering plastics, since the polymer has excellent chemical and physical properties,[1] *e.g.*, a high glass transition temperature (ca. 210 °C), and is mechanically tough. PPO was first prepared from 2,6-dimethylphenol monomer using a copper/amine catalyst system.[2] Recently, a PPO derivative was synthesized by polymerization of a spiro compound involving elimination of formaldehyde.[3]

There has been much interest in polymerizations catalyzed by enzymes ("enzymatic polymerizations") as a new methodology of polymer syntheses.[4,5] Recently, enzymatic synthesis of polyaromatics has been extensively developed.[6−17] Phenol and alkylphenols were oxidatively polymerized by peroxidases in an aqueous organic solvent to produce novel polymeric materials, mainly consisting of a mixture of phenylene and oxyphenylene units.[8−11] The resulting polymers exhibited relatively high thermal stability. This process is expected to be an alternative for production of conventional phenol resins (novolak and resol resins), which involves the use of toxic formaldehyde. Bilirubin oxidase induced regioselective polymerization of 1,5-dihydroxynaphthalene, yielding a poly(phenylene) insoluble in common organic solvents.[16] *o*-Phenylenediamine was polymerized by peroxidase catalyst to give a soluble polymer having an iminophenylene unit, which is hard to synthesize by conventional oxidative polymerizations.[17]

Very recently, we have achieved enzymatic synthesis of a PPO analogue, poly(2,6-dimethoxy-1,4-oxyphenylene), by a laccase-catalyzed oxidative polymerization of 3,5-dimethoxy-4-hydroxybenzoic acid,[18] which is a new type of enzymatic polymerization involving elimination of carbon dioxide and hydrogen from the monomer. This study deals with another approach to an enzymatic PPO synthesis, by oxidative polymerization of 2,6-dimethylphenol (Scheme 1). The catalysts used in this study were laccase derived from *Pycnoporus coccineous* and horseradish and soybean peroxidases (HRP and SBP, respectively). Relevant to this study, the polymerization of this monomer catalyzed by HRP in a mixture of 1,4-dioxane and buffer (85:15 vol %) was briefly reported,[6] in which the formation of oligomeric compounds (molecular weight = 400) was observed, but the characterization of the oligomer was not well performed.

Scheme 1



Results and Discussion

Laccase-Catalyzed Polymerization. Laccase has catalytic ability not only to degrade lignin *in vivo* but also to polymerize lignin precursors.[19] In our previous communication, we reported that laccase was available as a catalyst for the enzymatic oxidative polymerization of phenol derivatives,[18] in which a mixed solvent of acetone and acetate buffer (pH 5) afforded the polymer in good yields.

In this study, the laccase-catalyzed polymerization of 2,6-dimethylphenol was first performed in a mixture of acetone and 0.1 M acetate buffer (pH 5, 40:60 vol %) at room temperature for 24 h under air. The monomer conversion was determined by HPLC to be 100%. The polymeric materials were obtained by a reprecipitation procedure (chloroform as solvent, methanol as nonsolvent) in 57% yield. The filtrate of the reprecipitation contained 3,5,3′,5′-tetramethyl-4,4′-diphenoquinone (DPQ, ca. 40% yield), which was confirmed by mass spectroscopy (m/z = 241). DPQ was produced by oxidative coupling of two molecules at the 4 position of the monomer during the polymerization. The molecular weight of the polymer, determined by gel permeation chromatography (GPC), was 2700; this value might be somewhat larger than that of the total resulting polymers due to the loss of methanol-soluble polymer parts during the purification procedure. The monomer was quantitatively recovered in the polymerization without laccase (control experiment), indicating that the present polymerization proceeded through enzymatic catalysis.

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Table 1. Laccase-Catalyzed Polymerization of 2,6-Dimethylphenol[a]

	solvent					
entry	buffer pH	organic solvent	buffer content (%)	yield[b] (%)	M_n[c]	M_w/M_n[c]
1	3.0	acetone	60	0		
2	4.0	acetone	60	53	3300	2.1
3	4.5	acetone	60	57	2900	2.0
4	5.0	acetone	20	0		
5	5.0	acetone	40	3	3400	1.6
6	5.0	acetone	50	33	3000	1.7
7	5.0	acetone	60	57	2700	2.0
8	5.0	acetone	70	40	3000	2.3
9	5.0	acetone	80	29	2800	1.9
10	5.0	acetone	90	13	3300	2.3
11	5.0	acetonitrile	60	46	3700	2.2
12	5.0	1,4-dioxane	60	61	3000	1.9
13	5.0	ethanol	60	48	3300	1.8
14	5.0	methanol	60	23	3300	2.0
15	5.5	acetone	60	0		
16	7.0	acetone	60	0		
17	12.0	acetone	60	0		

[a] Polymerization was performed at room temperature for 24 h under air. [b] Methanol-insoluble part. [c] Determined by GPC using the chloroform eluent, calibrated with polystyrene standards.

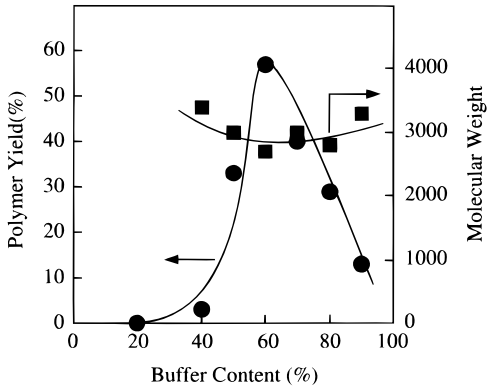


Figure 1. Effects of buffer content on the yield and molecular weight of PPO in the laccase-catalyzed polymerization of 2,6-dimethylphenol using a mixture of acetone and acetate buffer (pH 5.0) as solvent.

The polymerization was performed in the 60% buffers of different pH (Table 1). In using the buffer of pH 3, the monomer was almost recovered (the conversion was 4%). Polymer formation was observed in the range of pH from 4 to 5 (Table 1, entries 2, 3, and 7). Polymer yield and molecular weight hardly changed. The present laccase shows the highest activity in buffer of pH 4.5, which is in agreement with the pH region showing high activity for the present polymerization. In the aqueous acetone containing buffers of pH more than 5.5, however, the polymer was not obtained (Table 1, entries 15−17).

The effect of the buffer content was examined by using acetone and the acetate buffer (pH 5.0). Monomer conversion was high (>90%), except in the polymerization using 20% buffer, whereas the polymer yield strongly depended on the buffer content: there was a maximum point at 60% buffer (Figure 1). A similar behavior was observed in the peroxidase-catalyzed polymerization of phenol in a mixed solvent of 1,4-dioxane and phosphate buffer.[11] The molecular weight was almost constant.

Various water-miscible organic solvents, acetonitrile, 1,4-dioxane, ethanol, and methanol as well as acetone, were usable for the present polymerization (Table 1, entries 7 and 11−14). 1,4-Dioxane afforded the polymer in the highest yield (61%, Table 1, entry 12). The nature of the organic solvent had little effect on the molecular weight. The product polymer was in all cases soluble in common organic solvents.

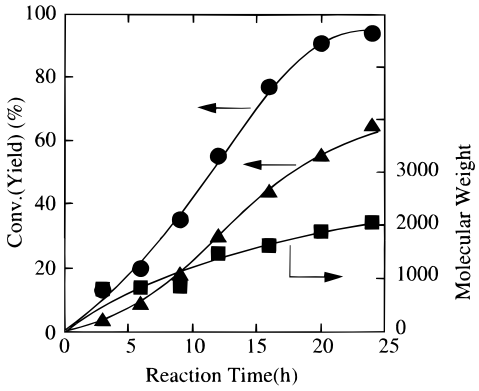


Figure 2. Polymerization time versus the monomer conversion (●), yield (▲), and molecular weight (■) of the polymer in the laccase-catalyzed oxidative polymerization of 2,6-dimethylphenol in a mixture of acetone and acetate buffer (pH 5.0, 40:60 vol %).

Table 2. Peroxidase-Catalyzed Polymerization of 2,6-Dimethylphenol[a]

	solvent						
entry	buffer pH	organic solvent	buffer content (%)	catalyst	yield[b] (%)	M_n[c]	M_w/M_n[c]
1	3.0	acetone	60	HRP	0		
2	5.0	acetone	40	HRP	2		
3	5.0	acetone	60	HRP	33	3200	1.8
4	5.0	acetone	60	SBP	38	4500	1.8
5	5.0	acetone	80	HRP	13	6900	1.9
6	5.0	acetonitrile	60	HRP	2		
7	5.0	1,4-dioxane	60	HRP	28	3500	1.5
8	5.0	ethanol	60	HRP	18	3700	1.7
9	5.0	methanol	60	HRP	25	2500	1.8
10	7.0	acetone	60	HRP	9	2900	1.3
11	8.0	acetone	60	HRP	6	2600	1.2
12	9.0	acetone	60	HRP	37	3400	1.5
13	11.0	acetone	60	HRP	20	2400	1.8

[a] Polymerization was performed using hydrogen peroxide as oxidizing agent at room temperature for 24 h under air. [b] Methanol-insoluble part. [c] Determined by GPC using the chloroform eluent calibrated with polystyrene standards.

Relationships between the monomer conversion and the yield and molecular weight of the polymer in the present polymerization are shown in Figure 2. The monomer conversion and polymer yield gradually increased as a function of polymerization time. The molecular weight (peak-top value) increased with increasing polymer yield. This polymerization behavior is different from that when using a copper/amine catalyst system: the molecular weight rapidly increased at the end of the reaction (condensation-type polymerization).[20] This may be due to the formation of the polymer precipitate during the reaction in the present laccase-catalyzed polymerization.

Peroxidase-Catalyzed Polymerization. Until now, horseradish and soybean peroxidases (HRP and SBP, respectively) have been reported to be active for the oxidative polymerization of phenol derivatives.[6−15] In this study, HRP was mainly used as catalyst. Polymerization results are summarized in Table 2.

The resulting polymer had the same structure as that obtained when using laccase catalyst. Effects of buffer pH on the polymerization have been investigated in 60% buffer. The polymer yield was more than 30% in acidic buffer (pH 5, Table 2, entry 3) as well as basic buffer

(pH 9, Table 2, entry 12). This behavior is contrasted with that of the HRP-catalyzed polymerization of phenol in aqueous 1,4-dioxane: the highest yield was achieved in the acetate buffer of pH 5, and the yield was greatly decreased in alkaline buffer (pH 9−11).[11]

The mixing ratio of acetone and the acetate buffer (pH 5) affected the polymerization behaviors (Table 2, entries 2, 3, and 5). Polymerization in 60% buffer produced the polymer in the highest yield. A similar behavior was observed in the laccase-catalyzed polymerization (Figure 1). The highest molecular weight (6900) was achieved in the 80% buffer.

The polymerization was also carried out in a mixture of various water-miscible organic solvents and the acetate buffer (pH 5, Table 2, entries 3 and 6−9). In all cases, the monomer conversion was very high (>90%), yielding not only PPO but also DPQ. Besides acetone, 1,4-dioxane, ethanol, and methanol were also effective as components of the mixed solvent (Table 2, entries 7−9); however, polymerization in the aqueous acetonitrile produced the polymer in a very low yield (Table 2, entry 6). In the polymerization catalyzed by SBP in the aqueous acetone, the monomer was quantitatively consumed. The yield and molecular weight of the polymer were larger than those obtained with HRP under similar reaction conditions (Table 2, entries 3 and 4).

The structure and function of HRP were reported to be affected by the composition of the aqueous organic solvent.[21,22] In this study, the enzyme type as well as the solvent composition greatly affected the polymerization behavior, *e.g.*, the polymerization results obtained using HRP were different from those obtained using SBP or laccase in the same solvent (Tables 1 and 2). This may be due to the difference of the enzyme activity in the aqueous organic solvent and/or of the reaction mechanism. No clear relationship between the solvent hydrophilicity and the polymerization behavior has so far been observed.

Thermal properties of the polymer were evaluated by using differential scanning calorimetry (DSC) and thermogravimetry (TG). In the DSC measurement of the polymer sample (entry 3 in Table 2) under nitrogen, the glass transition temperature (T_g) was observed at 136 °C, which was lower than the literature value (ca. 210 °C).[1] This may be because the present polymer had a low molecular weight. TG analysis under nitrogen showed that the temperature at 10 wt % loss was 360 °C, indicating that the enzymatically synthesized PPO had a relatively high thermal stability.

Structural Analysis. The polymer structure was analyzed by ^{1}H and ^{13}C NMR spectroscopy as well as matrix-assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF MS). The commercially available PPO is synthesized by oxidative polymerization of 2,6-dimethylphenol using copper/amine catalyst. This polymerization is known to involve several side reactions, resulting in Mannish base and DPQ incorporations into the polymer.[23]

In the ^{1}H NMR spectrum, two large peaks at δ 6.5 and 2.1 ascribable to protons of PPO were observed. Besides them, four small peaks were seen at δ 7.1, 6.4, 2.2, and 2.0 due to protons of the α,ω-terminal units (for detailed assignment of peaks, see Experimental Section).[24] The ^{13}C NMR spectrum exhibits five main peaks ascribed to carbons of the 2,6-dimethyl-1,4-oxyphenylene unit as well as eight small characteristic peaks, which are well assigned to the terminal units.[25]

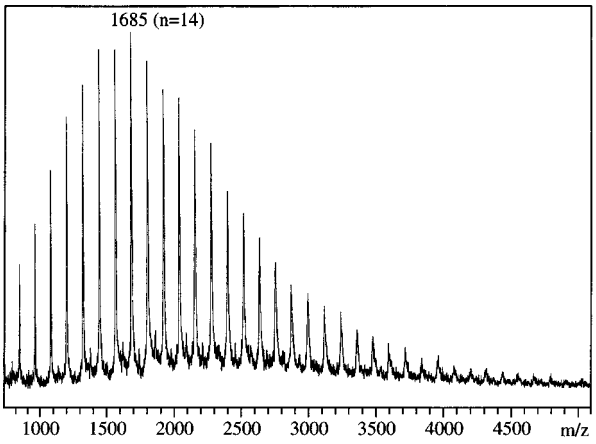


Figure 3. Positive MALDI-TOF spectrum of PPO using dithranol matrix (entry 3 in Table 2).

No additional peaks were detected in both ^{1}H and ^{13}C NMR spectra.

MALDI-TOF MS has been successfully utilized for the characterization of biomolecules.[26] Recently, this method has been applied to characterization of synthetic polymers.[27−29] In this study, we used MALDI-TOF MS for characterization of the enzymatically synthesized PPO. Figure 3 shows the mass spectrum of the PPO sample (entry 3 in Table 2). The expected molecular weight is given by $120n + 2$, where n is the degree of polymerization. The mass of the peak-top (1685) agreed with the calculated molecular weight value of cations $(M + H)^+$ ($n = 14$) and was somewhat smaller than that determined by ^{1}H NMR ($M_n = 2170$). This may be because the high molecular weight portion was partly ionized under the measurement condition. The peak-to-peak distance was 120, which is the molecular weight of the PPO repeating unit. These spectral data indicate that the present polymer was exclusively composed of 2,6-dimethyl-1,4-oxyphenylene units.

Conclusion

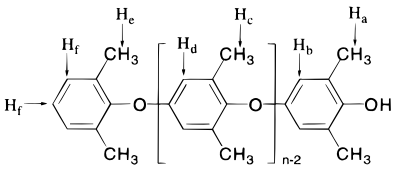
2,6-Dimethylphenol was oxidatively polymerized through enzyme catalysis in a mixture of water-miscible organic solvent and buffer to produce PPO with a molecular weight of several thousands. Although the molecular weight was not high, the product polymers are readily soluble in common organic solvents and hence will find useful applications, such as preparation of macromonomers and block copolymers containing PPO endblocks. Laccase from *Pycnoporus coccineous* and horseradish and soybean peroxidases were active for the present polymerization. The polymerization behaviors were dependent on the enzyme type as well as the solvent composition. In the laccase-catalyzed polymerization, the use of acidic buffer (pH 4−5) afforded the polymer in high yields. The resulting polymer was found to be exclusively composed of 2,6-dimethyl-1,4-oxyphenylene units by NMR and MALDI-TOF analyses. Further studies on the mechanism of the present polymerization are now under way in our laboratory.

Experimental Section

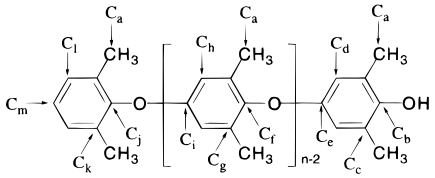
Materials. 2,6-Dimethylphenol was commercially available and used as received. Laccase, HRP, and SBP were purchased from Koken Co., Wako Pure Chemical CO., and Sigma, respectively. These enzymes were used without further purification.

Laccase-Catalyzed Polymerization. The following is a typical procedure for the polymerization (entry 7 in Table 1). 2,6-Dimethylphenol (0.31 g, 2.5 mmol) was dissolved in a mixture of acetone (10 mL) and acetate buffer (0.1 M, pH 5.0, 15 mL). The polymerization started upon the addition of laccase solution (250 μL, 2.95 mg of protein). The reaction mixture was vigorously stirred under air at room temperature for 24 h. The solvent was evaporated under reduced pressure, and the residue was washed successively with water and methanol. The residue was dissolved in a small amount of chloroform, and the solution was poured into a large amount of methanol. The preciptiates formed were collected by filtration, followed by drying in vacuo to give 0.17 g of the polymer (yield 57%).

Peroxidase-Catalyzed Polymerization. The following is a typical procedure for the polymerization (entry 3 in Table 2). Under air, 2,6-dimethylphenol (0.31 g, 2.5 mmol) and HRP (10 mg) in a mixture of 10 mL of acetone and 15 mL of 0.1 M acetate buffer (pH 5) were placed in a 50 mL flask. Hydrogen peroxide (30% aqueous solution, 28 μL, 0.25 mmol) was added to the mixture every 15 min for 10 times at room temperature. After 24 h, the solvent in the reaction mixture was evaporated under reduced pressure. The residue was washed successively with water and methanol. After the reprecipitation procedure (chloroform as solvent, methanol as nonsolvent), the polymeric material was collected and dried in vacuo (yield 0.10 g, 33%): ^{1}H NMR ($CDCl_3$) δ 2.0 (H_a), 2.1 (H_c), 2.2 (H_e), 6.4 (H_b), 6.5 (H_d), 7.1 (H_f).



^{13}C NMR ($CDCl_3$) δ 16.6−16.8 (C_a), 114.1 (C_d), 114.5 (C_h), 124.4 (C_c), 125.0 (C_m), 129.0 (C_1), 131.6 (C_k), 132.7 (C_g), 145.6 (C_f), 146.4 (C_b), 151.5 (C_j), 154.5 (C_e), 154.8 (C_i).



Measurements. GPC analysis was carried out using a Toso SC8010 apparatus with a refractive index (RI) detector under the following conditions: TSKgel, G2500H_{HR} column, and chloroform eluent at a flow rate of 1.0 mL/min. The calibration curves for GPC analysis were obtained using polystyrene standards. Monomer conversion was determined by HPLC analysis using a Hitachi 655A-12 pump and a 655A UV monitor under the following conditions: Toso ODS-120T column and aqueous methanol eluent (water:methanol = 33: 67 vol %) at a flow rate of 1.0 mL/min. Molecular weight of DPQ was measured by using a Hitachi LC/MS M-1200H mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker DPX400 spectrometer. Mass measurement of polymers was carried out using a Bruker Protein TOF mass spectrometer, equipped with a 337 nm nitrogen laser. Molecular weights were recorded using dithranol as matrix in a linear mode. Mass spectra were calibrated with substance P before measurement. DSC measurement was made at a 10 °C/min heating rate under nitrogen using a Seiko SSC/5200 differential scanning calorimeter calibrated with an indium reference standard. TG analysis was performed using a Seiko SSC/5200 apparatus for thermogravimetry/differential thermal analysis at a heating rate of 10 °C/min and a gas flow rate of 300 mL/min under nitrogen.

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References and Notes

(1) Aycock, D.; Abolins, V.; White, D. M. *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; John Wiley & Sons: New York, 1986; Vol. 13, pp 1−30.
(2) Hay, A. S.; Blanchard, H. S.; Endres, G. F.; Eustance, J. W. *J. Am. Chem. Soc.* **1959**, *81*, 6335.
(3) Kubo, M.; Itoh, Y.; Itoh, T. *Macromolecules* **1996**, *29*, 4447.
(4) Kobayashi, S.; Shoda, S.; Uyama, H. *Adv. Polym. Sci.* **1995**, *121*, 1.
(5) Ritter, H. *Trends Polym. Sci.* **1993**, *1*, 171.
(6) Dordick, J. S.; Marletta, M. A.; Klibanov, A. M. *Biotechnol. Bioeng.* **1987**, *30*, 31.
(7) Akkara, J. A.; Senecal, K. J.; Kaplan, D. K. *J. Polym. Sci., Polym. Chem. Ed.* **1991**, *29*, 1561.
(8) Uyama, H.; Kurioka, H.; Kaneko, I.; Kobayashi, S. *Chem. Lett.* **1994**, 423.
(9) Kurioka, H.; Komatsu, I.; Uyama, H.; Kobayashi, S. *Macromol. Rapid Commun.* **1994**, *15*, 507.
(10) Uyama, H.; Kurioka, H.; Sugihara, J.; Komatsu, I.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 3209.
(11) Uyama, H.; Kurioka, H.; Sugihara, J.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 189.
(12) Ayyagari, M. S.; Marx, K. A.; Tripathy, S. K.; Akkara, J. A.; Kaplan, D. L. *Macromolecules* **1995**, *28*, 5192.
(13) Bruno, F. F.; Akkara, J. A.; Kaplan, D. L.; Sekher, P.; Marx, K. A.; Tripathy, S. K. *Ind. Eng. Chem. Res.* **1995**, *34*, 4009.
(14) Uyama, H.; Kurioka, H.; Kobayashi, S. *Chem. Lett.* **1995**, 795.
(15) Kobayashi, S.; Kurioka, H.; Uyama, H. *Macromol. Rapid Commun.* **1996**, *17*, 503.
(16) Wang, L.; Kobatake, E.; Ikariyama, Y.; Aizawa, M. *J. Polym. Sci., Polym. Chem. Ed.* **1993**, *31*, 2855.
(17) Kobayashi, S.; Kaneko, I.; Uyama, H. *Chem. Lett.* **1992**, 393.
(18) Ikeda, R.; Uyama, H.; Kobayashi, S. *Macromolecules* **1996**, *29*, 3053.
(19) Hüttermann, A.; Herche, C.; Haars, A. *Holzforschung* **1980**, *34*, 64.
(20) Koch, M.; Heitz, W. *Makromol. Chem.* **1983**, *184*, 779.
(21) Ryu, K.; Dordick, J. S. *J. Am. Chem. Soc.* **1989**, *111*, 8026.
(22) Ryu, K.; Dordick, J. S. *Biochemistry* **1992**, *31*, 2588.
(23) White, D. M.; Nye, S. A. *Macromolecules* **1990**, *23*, 1318.
(24) Nava, H.; Percec, V. *J. Polym. Sci., Polym. Chem. Ed.* **1986**, *24*, 965.
(25) van Aert, H. A. M.; Venderbosch, R. W.; van Genderen, M. H. P.; Lemstra, P. J.; Meijer, E. W. *J. Macromol. Sci., Pure Appl. Chem.* **1995**, *A32*, 515.
(26) Karas, M.; Bachmann, D.; Bahr, U.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion Processes* **1987**, *78*, 53.
(27) Bahr, U.; Deppe, A.; Karas, M.; Hillenkamp, F. *Anal. Chem.* **1992**, *64*, 2866.
(28) Räder, H. J.; Spickermann, J.; Müllen, K. *Macromol. Chem. Phys.* **1995**, *196*, 3967.
(29) Chaudhary, A. K.; Critchley, G.; Diaf, A.; Beckman, E. J.; Russell, A. J. *Macromolecules* **1996**, *29*, 2213.

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