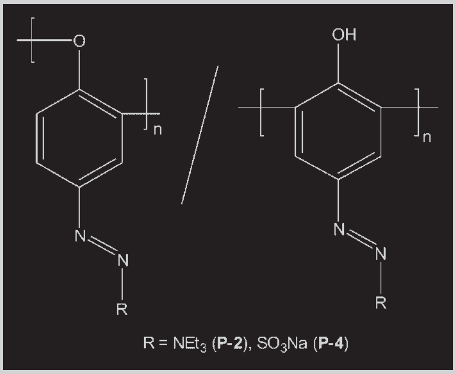
Macromol. Rapid Commun. **2003**, *24*, 185–189

Macromolecular Rapid Communications

Communication: The polymerization behavior of phenolic azosulfonates and triazene by means of enzymatic polymerization using horseradish peroxidase was studied. While for the latter one, only oligomerization was observed, sodium 4-hydroxybenzenediazosulfonate was successfully homopolymerized. The obtained polymer had an average molecular weight of $\overline{M}_n = 3\,000$ with a polydispersity index of 1.51. This is the first example of a homopolymer bearing an azosulfonate function in every monomer unit.



First Diazosulfonate Homopolymer by Enzymatic Polymerization

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Keywords: azo polymers; enzymes; horseradish peroxidase; photolabile polymer; water-soluble polymers

Introduction

Photo- and thermolabile polymers are primarily synthesized for the application in modern printing techniques and for the fabrication of microelectronic devices. In this field, many affords were made to enhance the performance of such compounds.[1,2] Their synthesis is quite challenging, because incorporated labile groups must still be sufficiently stable during the preparation and purification steps. Therefore, alternative synthetic routes allowing milder reaction conditions are needed in order to improve the yield and to broaden the structural variety of these polymers.

Potential candidates for photolabile units which can be incorporated into polymeric materials are diazosulfonates,[3] triazenes[2] and diazophosphonates.[4] They can be located within the polymer main chain[1] as well as in the side group of a monomer unit. The latter can be achieved by either polymer-analogous reaction or polymerization of functional monomers. However, both routes are not suitable for the preparation of polymers bearing functional groups in each monomer unit. Polymer-analogous reactions do not result in quantitative functionalizations under the reaction conditions restricted by the labile function.[5] Free-radical polymerization of, e.g., diazosulfonate vinyl monomers, do not result in homopolymers because of the retarding effect of the azo function. This is due to the radical addition reaction of the azo group resulting in a hydrazyl radical of low reactivity.[6] To circumvent this problem, photolabile polymeric films were prepared from statistical copolymers of methyl methacrylate or styrene with relatively low content of diazosulfonate monomers.[5]

Recently, polymerization of functional monomers employing enzymes gained increasing attention.[7] Tripathy et al. reported on the preparation of aromatic diazopolymers with phenol, aniline and sulfonic acid derivates as monomers, using horseradish peroxidase as a catalyst.[8] The enzymatic polymerization is especially intriguing for the preparation of photo- and thermolabile polymers, since the reaction conditions are mild as compared to the standard polymerization procedures. Enzymatic polymerizations are usually carried out at room temperature and in aqueous media under neutral or slightly acidic pH. This renders this polymerization technique ideal for the conversion of diazosulfonate-containing monomers.

In a first step, 4-hydroxybenzenediazosulfonate (**4**) and 4-hydroxybenzenediethyltriazene (**2**) as well as the corresponding benzene derivatives **1** and **3** were prepared

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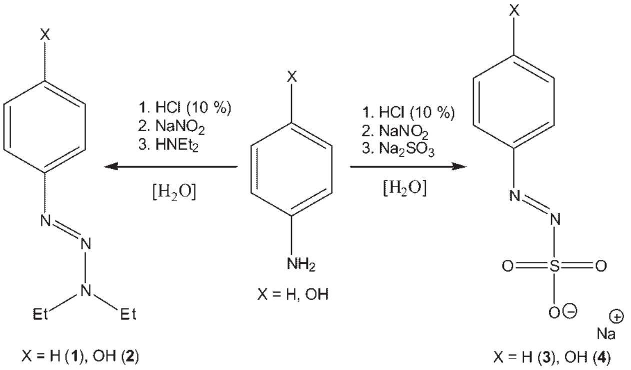


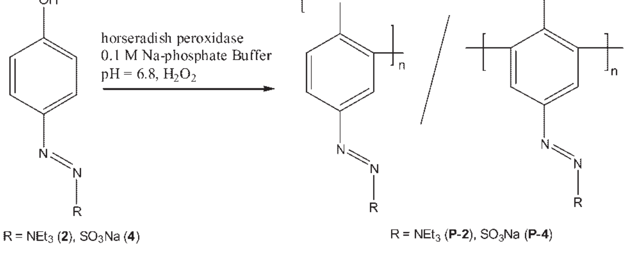
Scheme 1. Synthesis of benzenediethyltriazene (**1**, via tetrafluoroboric acid), benzenediazosulfonate (**3**), 4-hydroxybenzenediethyltriazene (**2**), and 4-hydroxybenzenediazosulfonate (**4**).

(Scheme 1) and polymerized by means of enzymatic polymerization using horseradish peroxidase (Scheme 2).

Experimental Part

Measurements

UV-vis spectra were recorded on a Hitachi U-2001 UV-vis spectrometer. FT-IR spectra were recorded using a Perkin Elmer Spectrum One FT-IR spectrometer equipped with an ATR sampling accessory (single bounce, diamond crystal) and DTGS detector. ^{1}H and ^{13}C NMR spectroscopy was performed on a Bruker 400 MHz NMR spectrometer. Thermal analysis of the polymer and monomer were studied using a Seiko Instrument TG/DTA 220 thermogravimetric analyzer (heating rate: 10 °C/min; nitrogen atmosphere). The polymer analytical values were obtained using a Tosoh gel permeation chromatography system at 60 °C (pump: Tosoh model DP-8020; detectors: RI-8020; UV-8020; oven: CO-8020; stationary phase: TSK gel; mobile phase: *N,N*-dimethylformamide (DMF) containing 1% lithium chloride). The instrument was calibrated with polystyrene standards from Wako. For irradiation experiments, a standard Hg-Xe high-pressure lamp ($\lambda_{\max} = 254$, 363 nm) was used.



Scheme 2. Enzymatic polymerization of 4-hydroxybenzenediethyltriazene (**2**) and sodium 4-hydroxybenzenediazosulfonate (**4**).

Syntheses

All chemicals and solvents (analytical grade or higher) were purchased from Wako Japan Ltd. and used as received. Synthesis and further conversion of azo-containing compounds were performed under exclusion of light. Benzenediazonium fluoroborate (**5**) was prepared according to the literature.[9]

4-Hydroxybenzenediazosulfonate (**4**)

4-Hydroxyaniline (5.45 g, 0.05 mol) was dissolved in 50 mL of aqueous HCl (10 vol.-%) at 0 °C. After 15 min, 3.45 g (0.05 mol) of sodium nitrite in 25 mL of cold water (0 °C) was added dropwise over a period of 5 min. The solution was stirred for 20 min at 0 °C and then poured into a 0 °C cold sodium sulfite solution (12.6 g, 0.1 mol, in 75 mL water). After vigorous stirring for additional 30 min at 0 °C, the final product precipitated overnight at approximately 2 °C. The yellow crystals were recrystallized from water; yield: 4.93 g (44%).

^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.89$–6.93 (m, 2 H, CH), 7.64–7.68 (m, 2 H, CH), 10.33 (br s, 1 H, OH).

^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 116.1$ (CH), 126.3 (CH), 143.2 (C_{quart}), 161.6 (C_{quart}).

IR (ATR): 3561 (s), 3500 (s), 3430 (s), 3321 (s), 3175 (s), 1653 (m), 1608 (m), 1591 (s), 1509 (m), 1472 (m), 1444 (m), 1249 (s), 1222 (s), 1198 (s), 1151 (s), 1042 (s), 900 (w), 837 (m), 757 cm^{-1} (m).

UV-vis: $\lambda_{\max}$ (H_2O) (ε): 327 (88 000), 237 nm (58 200 L $\cdot$ mol^{-1} $\cdot$ cm^{-1}).

TGA: $\tau_{\text{dec}} = 210.8$ °C, weight loss: 35.8%.

4-Hydroxybenzenediethyltriazene (**2**)

4-Hydroxyaniline (1.636 g, 15 mmol) was dissolved in 10 mL of an aqueous HCl solution (10 vol.-%) at 0 °C. After 15 min, 1.04 g (15 mmol) of sodium nitrite in 5 mL of cold water (0 °C) were added dropwise over a period of 2 min. The solution was stirred for 15 min at 0 °C. Afterwards, 15 mL of diethylamine were added to the reaction mixture, which was stirred at 0 °C for 30 min. The solution was extracted three times with ethyl acetate and the combined organic phases were dried over sodium sulfate. After filtration and evaporation of the solvent, a brown product was obtained. Further attempts of purification (flash chromatography or drying under high vacuum) only lead to decomposition of the triazene compound. Thus, the crude product was used.

^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 1.15$ (t, $^{3}J_{(\text{H,H})} = 7.0$ Hz, 6 H, CH_3), 3.64 (q, $^{3}J_{(\text{H,H})} = 7.0$ Hz, 4 H, CH_2), 6.69 (dd, $^{3}J_{(\text{H,H})} = 4.5$ Hz, $^{5}J_{(\text{H,H})} = 2.0$ Hz, 2 H, CH), 7.14 (dd, $^{3}J_{(\text{H,H})} = 4.5$ Hz, $^{5}J_{(\text{H,H})} = 2.0$ Hz, 2 H, CH), 9.21 (br s, 1 H, OH).

^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 115.5$ (CH), 121.2 (CH), 143.3 (C_{quart}), 155.3 (C_{quart}).

IR (ATR): 3300–2500 (br), 1609 (m), 1590 (s), 1501 (m), 1470 (s), 1452 (m), 1409 (s), 1376 (m), 1350 (m), 1337 (s), 1299 (w), 1265 (s), 1228 (s), 1103 (s), 1089 (m), 1072 (m), 843 (m), 815 (m), 753 cm^{-1} (m).

SEC (DMF, 1% LiCl, RI): $\overline{M}_n = 354, \overline{M}_w = 370$, PDI = 1.04.

Benzenediazosulfonate (**3**)

Aniline (9.31 g, 0.1 mol) was dissolved in 100 mL of an aqueous HCl (10 vol.-%) solution at 0 °C. After 15 min, 6.90 g (0.1 mol) of sodium nitrite in 15 mL of cold water (0 °C) were added dropwise over a period of 5 min. The solution was stirred for 15 min at 0 °C and poured into a 0 °C cold solution of 200 mL water and 26.2 g (0.2 mol) sodium sulfite, stirred vigorously for further 30 min before the solvent was removed by freeze drying. The obtained product was recrystallized (water/THF); yield: 6.45 g (31%).

^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 7.55-7.58$ (m, 3 H, CH), 7.71–7.76 (m, 2 H, CH).

IR (ATR): 3536 (w), 3060 (w), 1509 (w), 1450 (w), 1289 (s), 1228 (s), 1076 (m), 1060 (s), 757 cm^{-1} (m).

Benzenediethyltriazene (**1**)

Diethylamine (0.80 g, 11 mmol) and 1.2 g (12 mmol) of triethylamine were dissolved in 40 mL of THF. At 0 °C the benzenediazonium fluoroborate (**5**) was added in small portions and stirred for 30 min. The solution was extracted three times with hexane (in the second extraction step, brine was added to the aqueous phase). The combined organic phases were dried over sodium sulfate and the solvent was evaporated under reduced pressure to yield an orange liquid.; yield: 82% (1.45 g).

^{1}H NMR (CDCl$_3$, 400 MHz): $\delta = 1.19$ (t, $^3J_{(\text{H,H})} = 7.0$ Hz, 6 H, CH$_3$). 3.67 (q, $^3J_{(\text{H,H})} = 7.0$ Hz, 4 H, CH$_2$), 7.09 (t, $^3J_{(\text{H,H})} = 4.5$ Hz, 1 H, *p*-CH), 7.28–7.32 (dd, $^3J_{(\text{H,H})} = 7.0$ Hz, 2 H, *m*-CH), 7.42 (d, 2 H, *o*-CH).

^{13}C NMR (CDCl$_3$, 400 MHz): $\delta = 120.3$ (CH), 124.9 (CH), 128.6 (CH), 151.1 (C$_{\text{quart}}$).

IR (ATR): = 3064 (w), 3027 (w), 2974 (s), 2934 (m), 2872 (w), 1593 (m), 1482 (m), 1457 (s), 1433 (m), 1411 (s), 1377 (m), 1341 (m), 1326 (s), 1235 (s), 1195 (m), 1158 (w), 1099 (s), 1069 (s), 759 (s), 692 cm^{-1} (s).

Standard Procedure for the Enzymatic Polymerization of Phenolic Azo Compounds

After dissolving the enzyme in the buffer solution, the monomer was dissolved in either buffer or a mixture of buffer and methanol. The reaction started upon addition of hydrogen peroxide using a syringe pump over a period of 60 min. The polymerization was allowed to complete within 2 h. If the polymer was to precipitate, it was centrifuged and washed several times with water. The water-soluble polymers were purified by dialysis and freeze dried.

Poly(4-hydroxybenzenediazosulfonate) (**P-4**)

Compound **4** (1.12 g, 5 mmol) was dissolved in 30 mL of phosphate buffer (0.1 M, pH 6.8), horseradish peroxidase (5 mg), dissolved in 10 mL of phosphate buffer, and 566 µL H$_2$O$_2$ (30 vol.-%) in 1.434 mL water were added. Dialysis with water (cut-off at 2000 g · mol^{-1}); yield: 130 mg (12%).

^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.0-9.0$ (br, *o*, *m*, *p*-CH).

IR (ATR): 3645–2445 (br), 1633 (w), 1590 (m), 1496 (m), 1225 (s), 1136 (m), 1108 (m), 1046 (s), 623 cm^{-1} (w).

UV-vis: λ_{max} (H$_2$O) (ε): 318 nm (38 000 L · mol^{-1} · cm^{-1}).

SEC (DMF, 1% LiCl, RI): $\overline{M}_{\text{n}} = 3\,000$, $\overline{M}_{\text{w}} = 4\,500$, PDI = 1.51.

TGA: $\tau_{\text{dec}} = 198.9$ °C, weight loss: 13.8%.

Poly(4-hydroxybenzenediethyltriazene) (**P-2**)

Compound **2** (0.193 g, 1 mmol) in 17 mL methanol and horseradish peroxidase (15 mg), dissolved in 15 mL of phosphate buffer were mixed. 220 µL H$_2$O$_2$ (15 vol.-%) were added over 2 min. Centrifugation: 12 000 rpm, 4 °C, 15 min; yield: 126 mg (65%)

^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 0.7-1.4$ (br s, CH$_3$), 3.5–3.8 (br s, CH$_2$), 6.5–7.7 (br s, CH).

IR (ATR): 3640–2469 (br), 2974 (m), 2934 (m), 2871 (w), 2112 (w), 1660 (w), 1579 (w), 1490 (m), 1465 (m) 1451 (m), 1434 (m), 1401 (s), 1340 (s), 1234 (s), 1202 (s), 1095 (s), 1076 (s), 994 (w), 883 (w), 832 cm^{-1} (w).

SEC (DMF, 1% LiCl, RI): $\overline{M}_{\text{n}} = 880$, $\overline{M}_{\text{w}} = 1\,200$, PDI = 1.53.

Polymerizations of the benzene derivates were carried out analogously.

Results and Discussion

The enzymatic polymerization of 4-hydroxybenzenediazosulfonate (**4**) with horseradish peroxidase lead instantly to water-soluble homopolymer **P-4** with a number-average molecular weight of $\overline{M}_{\text{n}} = 3\,000$ and a polydispersity index (PDI) of 1.51. The formation of the polymer was confirmed by NMR and FT-IR spectroscopy. ^{1}H NMR spectroscopy of **P-4** showes a broadening of the phenolic proton signals (monomer signals at 6.89–6.93 and 7.64–7.68 ppm compared with 6.0–9.0 ppm in the corresponding polymer). This indicates the formation of a rigid polyphenol backbone and confirms the successful polymerization of **4**. As indicated in Scheme 2, the enzymatic polymerization of phenols yields a mixture of 3,4- and 3,5-bridged polyphenols.[10] The ratio of the two species, however, could not be determined due to the significant line broadening for polyphenols. Quantification of the phenolic proton content is not possible due to the rapid H/D exchange (D$_2$O experiments) or the poor solubility of **P-4** in dimethyl sulfoxide (DMSO).

In the FT-IR spectrum (not shown) the band broadening of all characteristic signals is also noticeable. In the polymer spectrum, the signal around 1040 cm^{-1}, which can be assigned to the S-O stretching mode of the sulfonate unit, is still present. Control experiments with the respective benzene derivatives of **1** and **3** did not show any formation of oligomeric or polymeric products. This indicates that for the polymerization as proposed in Scheme 2 the hydroxyl group is necessary for the successful conversion with horseradish peroxidase.

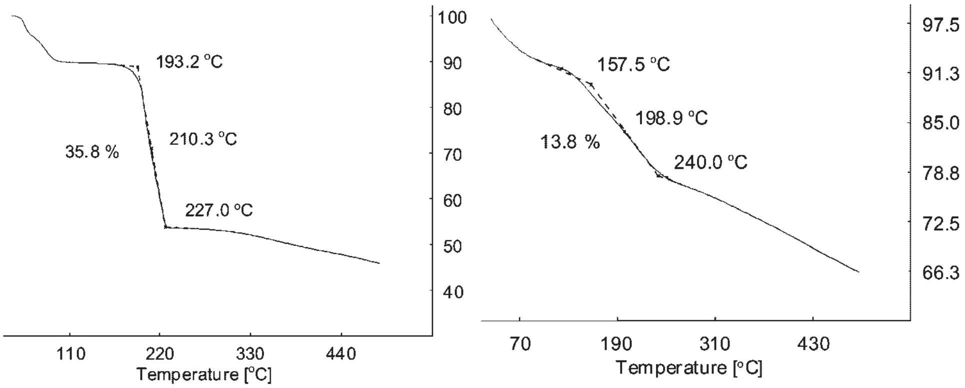


Figure 1. TGA analysis of sodium 4-hydroxybenzenediazosulfonate (**4**; left) and the corresponding polymer (**P-4**; right).

The thermal stability of the monomer and polymer **P-4** was studied by means of thermogravimetric analysis (TGA; Figure 1).

The monomer shows a maximum decomposition temperature of 210 °C whereas the polymer decomposes at lower temperature of around 199 °C. Furthermore, the temperature range for the degradation of the polymer is broader as compared with that of the monomer. While the monomer decomposes between 193 °C and 227 °C, the polymer degrades from 157 °C to 240 °C. This shift as well as the broadening of the decomposition temperature is typical for the decomposition of polymers containing diazosulfonate units. For example, 4-vinylphenylazosulfonate decomposes around 243 °C while the corresponding poly[(4-vinylphenylazosulfonate)-*co*-(methyl methacrylate)] decomposes at 225 °C. This can be explained by the amorphous structure of the corresponding polymers.

While diazosulfonate compounds show a high thermal stability in bulk, they readily decompose upon irradiation. The absorption maximum for the majority of these compounds is in the range of 300 to 370 nm. The diazosulfonate monomer and polymer synthesized have absorption maxima around 318 nm (Figure 2). Upon irradiation of an aqueous solution of **P-4** with UV light (Hg-Xe high-pressure UV lamp, $\lambda = 363$ nm) the rapid reduction of the absorption due to photolysis of the diazosulfonate group is also displayed in Figure 2.

Already after 3 s of irradiation, absorption decays by 25%, after 14 s, only 44% of the original absorption can be detected, and no further decrease was observed after about 30 s of irradiation. A slight shift of the absorption maxima from 320 nm to 308 nm can be observed. This was also noticed by Mezger et al. for diazosulfonate emulsifiers.[11]

While **4** results in the water-soluble homopolymer **P-4**, the enzymatic polymerization of phenolic triazene compound **2** yields an oligomer insoluble in water, with a degree of polymerization of 3–5 ($\overline{M}_w = 1\ 200$, $\overline{M}_n = 880$, PDI = 1.53, as measured by GPC). This anticipated a conversion of **2** to polymers of higher molar masses due to the quick precipitation. In the case of the triazene compound, multiplicity of the ethyl units is still visible.

Conclusions

For the first time, a diazosulfonate homopolymer was prepared. This was achieved by enzymatic polymerization of 4-hydroxybenzenediazosulfonate using horseradish peroxidase under mild conditions. The resulting polymer with $\overline{M}_n$ of 3 000 g · mol^{-1} and a polydispersity index of 1.51 is water-soluble, thermally stable and photosensitive. The conversion of 4-hydroxybenzenediethyltriazene only yields oligomeric products, due to its low solubility in the solvent/buffer solution. The facile reaction procedure and mild reaction conditions of enzymatic polymerization offer a unique opportunity for the preparation of photo- or thermosensitive functional polymers.

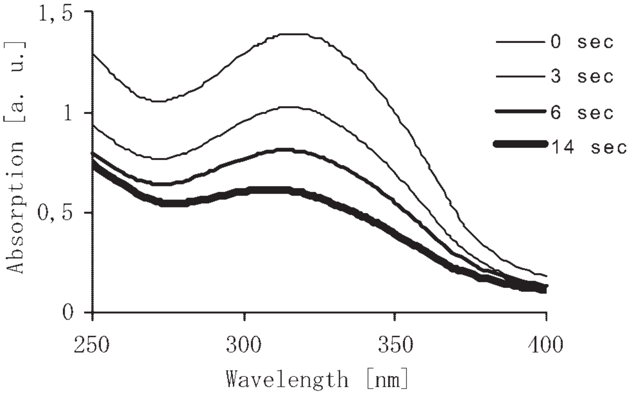


Figure 2. Irradiation of poly(4-hydroxybenzenediazosulfonate) (**P-4**) with a Hg-Xe high-pressure UV lamp ($c_{(0)} = 3.97 \times 10^{-4}$ mol L^{-1}, $A_{(0)} = 1.39$, $\varepsilon = 35\ 000$ L · cm^{-1} · mol^{-1}).

Acknowledgement: J.R. acknowledges financial support from the *DAAD (Deutscher Akademischer Austauschdienst)* for his research visit to Japan. R.J. is grateful for a Dr. Hermann-Schnell stipend provided by the *GDCh (Gesellschaft Deutscher Chemiker)*.

Received: November 12, 2002
Revised: January 3, 2003
Accepted: January 7, 2003

[1] O. Nuyken, C. Scherer, A. Baindl, A. R. Brenner, U. Dahn, R. Gärtner, S. Kaiser-Röhrich, R. Kollefrath, P. Matusche, B. Voit, *Prog. Polym. Sci.* **1997**, *22*, 93.

[2] [2a] J. Wei, N. Hoogen, T. Lippert, O. Nuyken, A. Wokaun, *J. Phys. Chem. B* **2001**, *105*, 1267; [2b] T. Lippert, J. Wei, A. Wokaun, N. Hoogen, O. Nuyken, *Macromol. Mater. Eng.* **2000**, *283*, 140; [2c] O. Nuyken, U. Dahn, N. Hoogen, D. Marquis, M. N. Nobis, C. Scherer, J. Stebani, A. Wokaun, C. Hahn, T. Kunz, T. Lippert, *Polym. News* **1999**, *24*, 257; [2d] T. Lippert, C. David, J. T. Dickinson, M. Hauer, U. Kogelschatz, S. C. Langford, O. Nuyken, C. Phipps, J. Robert, A. Wokaun, *J. Photochem. Photobiol. A* **2001**, *145*, 145.

[3] J. Dauth, O. Nuyken, P. Strohriegl, B. Voit, *Makromol. Chem.* **1992**, *193*, 723.

[4] O. Nuyken, C. Scherer, M. N. Nobis, J. Ihlemann, *Polym. Mater. Sci. Eng.* **1997**, *76*, 206.

[5] O. Nuyken, B. Voit, *Makromol. Chem.* **1989**, *190*, 1325.

[6] [6a] R. Kerber, O. Nuyken, M. Dorn, *Makromol. Chem.* **1978**, *179*, 1803; [6b] A. Stasko, O. Nuyken, B. Voit, S. Biskuic, *Tetrahedron Lett.* **1990**, *31*, 5737.

[7] [7a] S. Kobayashi, H. Uyama, *Curr. Org. Chem.* **2002**, *6*, 209, and references cited therein; [7b] S. Kobayashi, H. Uyama, M. Ohmae, *Bull. Chem. Soc. Jpn.* **2001**, *74*, 613, and references cited therein; [7c] S. Kobayashi, H. Uyama, S. Kimura, *Chem. Rev.* **2001**, *101*, 3793.

[8] [8a] K. S. Alva, T.-S. Lee, J. Kumar, S. K. Tripathy, *Chem. Mater.* **1998**, *10*, 1270; [8b] S. K. Tripathy, D. Y. Kim, L. Li, N. K. Viswanathan, S. Balasubramanian, W. Liu, P. Wu, S. Bian, L. Samuelson, J. Kumar, *Synth. Met.* **1999**, *102*, 893; [8c] S.-H. Lee, S. Balasubramanian, D. Y. Kim, N. K. Viswanathan, S. Bian, J. Kumar, S. K. Tripathy, *Macromolecules* **2000**, *33*, 6534; [8d] W. Liu, S. Bian, L. Li, L. Samuelson, J. Kumar, S. K. Tripathy, *Chem. Mater.* **2000**, *12*, 1577; [8e] D. Y. Kim, L. Li, J. Kumar, S. K. Tripathy, *Appl. Phys. Lett.* **1995**, *66*, 1166.

[9] A. Roe, "*Organic Reactions*", Wiley, New York 1957, Vol. 5, p. 193.

[10] [10a] H. Uyama, H. Kurioka, I. Kaneko, S. Kobayashi, *Chem. Lett.* **1994**, 423; [10b] H. Uyama, H. Kurioka, J. Sugihara, S. Kobayashi, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 189; [10c] H. Uyama, H. Kurioka, I. Komatsu, J. Sugihara, S. Kobayashi, *Macromol. Reports* **1995**, *A32*, 649; [10d] T. Oguchi, S. Tawaki, H. Uyama, S. Kobayashi, *Macromol. Rapid Commun.* **1999**, *20*, 401; [10e] T. Oguchi, S. Tawaki, H. Uyama, S. Kobayashi, *Bull. Chem. Soc. Jpn.* **2000**, *73*, 1389.

[11] T. Mezger, O. Nuyken, K. Meindl, A. Wokaun, *Prog. Org. Coat.* **1996**, *29*, 147.