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Bifunctional Synthetic Enzymes via Alternating Copolymerization. I. Copolymers Containing Alternating Imidazole and Hydroxamic Functions

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ABSTRACT

The synthesis, characterization, and evaluation of bifunctional synthetic enzymes via alternating copolymerization was carried out. It was desired to obtain copolymers containing alternating placements of complementary functional groups to determine whether "cooperativity" between the groups (in the hydrolysis of ester substrates) would be greater than in the corresponding random copolymers containing the same functional groups. The bifunctional alternating copolymer, N-(β-vinyloxyethyl)imidazole-N-hydroxymaleimide, was synthesized and evaluated as a catalyst in the hydrolysis of p-nitrophenyl acetate. The effectiveness of this copolymer as an esterolysis catalyst was compared with imidazole and the homopolymer of N-hydroxymaleimide. Monomer syntheses, polymerization, and copolymer properties are also reported.

*Dedicated to Professor Georg Manecke on the occasion of his 70th birthday, June 20, 1986.

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INTRODUCTION

A great deal of attention has been given to the understanding of the catalytic properties of enzymes [1]. Enzymes are globular proteins which catalyze most of the chemical reactions in living organisms. Recently, the mechanisms by which enzymes catalyze organic reactions have been interpreted in terms of the transition state theory. As knowledge of enzyme mechanisms has grown, efforts to synthesize artificial enzymes have increased. Synthetic enzyme models have shown considerable utility as probes of enzyme kinetics [2]. These models should emulate the desirable characteristics of natural enzymes, i.e., show high selectivity and high efficiency (rate enhancement) toward the substrate molecule. To date, both characteristics have been incorporated into synthetic polymers. This paper deals with the synthesis and evaluation of alternating copolymer and its evaluation as a synthetic enzyme, including comparison of its activity with imidazole and the homopolymer of one of the monomers of the comonomer pair.

Enzymes are capable of catalyzing a great variety of chemical reactions; one of the most studied is the esterolysis (ester hydrolysis) reaction. The accepted kinetic scheme for this hydrolysis has been published [2b].

Effective binding between catalyst and substrate prior to the acylation step plays a key role in providing high catalytic activity. This pre-association step greatly increases the esterolysis rate by increasing the concentration of substrate at the active site of the catalyst. Furthermore, acylation can take place via an intramolecular reaction rather than by the much slower intermolecular pathway. At least four types of binding forces have been identified in the pre-association process: coulombic interactions, hydrophobic interactions, hydrogen bond formation, and charge-transfer interactions. The most important factor in determining the effectiveness of the catalyst, however, is that the binding take place at a site which is favorable for the subsequent acylation reaction to occur.

In order to observe a significant rate enhancement in esterolysis reactions, the substrate must first be bound to the polymer at or near the active site. Only after complexation has occurred do the actual hydrolysis steps take place. Esterolysis by synthetic enzymes has been observed to proceed both with and without a complexation step. Studies with achymotrypsin (a serine proteinase consisting of 245 amino acid residues) have shown that a serine O^- anion is responsible for catalytic acylation of substrate [1].

The serine hydroxyl group is activated for the acylation reaction, referred to as a "charge-relay system," in which an imidazole moiety plays an integral role in lowering the activation energy for catalysis. A variety of cooperative effects among the functional groups responsible for catalytic action is common in natural enzymes. Synthetic chemists have also sought to take advantage of cooperativity in order

to produce more efficient enzymes. A good example of bifunctional cooperation utilizing a molecular relay system has been demonstrated [3]. It was found that the rate of hydrolysis of p-nitrophenyl acetate (PNPA) was 1000 times faster with a terpolymer of acrylamide, 4(5)-vinylimidazide, and N-phenyl-N-hydroxyacrylamide (1) than with a copolymer of only acrylamide with the latter monomer (2).

The acylation step was demonstrated to occur primarily via the hydroxamate anion, which is known to be a highly nucleophilic species. It is also known that decomposition of an acylhydroxamate is a slow process; the fact that 1 is a much better catalyst than 2 implies that imidazole is catalyzing deacylation of the acylhydroxamate intermediate either by acting as a nucleophile or as a general base. Other examples of bifunctional catalysts exhibiting cooperativity have been published [4].

Coulombic interactions [5] as the mode of polymer-substrate binding, as well as favorable binding by hydrophobic interactions [6a], have been demonstrated. The most effective synthetic enzyme studied to date appears to be a dodecylated poly(ethyleneimine) containing imidazole residues. This material was found to approach α-chymotrypsin in catalytic activity [6b].

Catalytic properties of polymers are influenced to a large extent by the configuration (conformation) of the molecule in solution. Vinyl polymers are rather flexible as compared with enzymes, i.e., they usually lack specific secondary and unique tertiary structure. As a result, synthetic polymers lack the specific binding pocket which is typical of enzymes. Therefore, the catalytic efficiency of synthetic enzymes will depend to a large extent on the pH, ionic strength, and composition of the medium, distance of the catalytic group from the polymer backbone, degree of dissociation of catalytic groups, and many other factors. Combinations of cooperative and/or complementary functional groups are necessary to achieve high catalytic efficiency. Catalysis by hydrolytic enzymes is of the nucleophilic and acid-base type.

As previously stated, synthetic vinyl macromolecules containing bi- or multifunctionalities have been studied in other laboratories and have shown enzyme-like catalytic activity. These studies have shown a cooperativity between the functionalities leading to a rate enhancement for esterolysis reactions. However, up to this time, functionalities have been introduced into copolymers in random fashion. This implies a degree of cooperation between the functionalities which is dependent on the degree of alternation in the copolymer (3) or upon the conformation of the copolymer chain (4).

It was the purpose of this investigation to determine whether the degree of cooperativity between functional groups could be maximized by preparing regular alternating copolymers. This structure would assure that each functional group of a given type would be flanked on either side by a functional group of the complementary type.

Selection of monomer pairs which undergo regular alternating

copolymerization under free-radical conditions was fundamental to this study. Alternating copolymerization is generally believed to proceed via polymerization of 1:1 donor-acceptor complexes between electron-rich (donor) and electron-deficient (acceptor) monomer pairs. Evidence has recently been presented in support of control of both stereochemistry [7a] and monomer orientation [7b] in the copolymer via donor-acceptor complex participation.

Bifunctional synthetic enzymes via alternating copolymerization could be approached by at least two methods. The first method, which should result in fully functionalized copolymer, involves direct polymerization of monomer pairs containing the desired functional groups (or the desired functional groups in masked or protected forms). The second method involves derivatization of a preexisting alternating copolymer, a method which suffers from the fact that it is difficult to functionalize polymers completely.

This paper deals with efforts directed toward copolymerization of appropriately substituted monomer pairs. Monomer syntheses are also described. A subsequent paper describes the second method, derivatization of preexisting alternating copolymers as well as monomer syntheses, and evaluation of the copolymers as synthetic enzymes.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus or a Fisher-Johns melting point apparatus and are given in degrees celsius (uncorrected). Pressures are expressed in torrs (millimeters of mercury). Elemental analyses were performed by Atlantic Microlabs, Atlanta, Georgia, or Schwarzkopf Microanalytical Laboratories, Woodside, New York.

Proton nuclear magnetic resonance (NMR) spectra (60 MHz) were recorded on Varian A-60A or Jeol JNM-PMX-60 instruments. Carbon-13 NMR (25 MHz) and 100 MHz proton NMR spectra were recorded on a Jeol-JNM-FX-100 spectrometer. Chemical shifts are expressed in parts per million (ppm) on the δ scale downfield from tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) unless otherwise indicated. The solvent used and calibration information are given in parentheses for each spectrum reported. Multiplicities of proton and off-resonance decoupled carbon resonances are designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), or broad (br).

Infrared (IR) spectra were recorded on a Perkin-Elmer 281 spectrophotometer. Absorbances are expressed in wavenumbers (cm^{-1}) using polystyrene (1601 cm^{-1}) calibration. Solid samples were run as KBr pellets; liquid samples were analyzed neat as thin films between NaCl plates. Absorption bands are assigned the classifications weak (w), medium (m), strong (s), very strong (vs), broad (br), and shoulder (sh).

Low-resolution mass spectra (LRMS) and high-resolution mass spectra (HRMS) were recorded on an Associated Electronic Industries (AEI) Model MS-30 spectrometer.

Number-average molecular weights ($\overline{M}_n$) of polymers were determined by vapor pressure osmometry (VPO) on a Wescan 233 molecular weight apparatus. Benzil was used as a calibration standard. Intrinsic viscosities [η] were measured with an Ubbelohde viscometer (dilution viscometer). Gel permeation chromatography (GPC) of polymers was carried out on a Waters Associates liquid chromatograph using glycerated porous glass columns and both ultraviolet (UV) and differential refractometer detectors.

Compound headings appear with the common name(s) listed first, followed by the systematic name as found in Chemical Abstracts (CA). CA registry numbers of known compounds are given in brackets.

Deuterated NMR solvents were obtained from Aldrich Chemical Co. or Merck and Co. All solvents used for general applications were of reagent grade or ACS grade quality. For special applications, solvents were distilled according to accepted scientific practices.

Starting materials and reagents were obtained from Aldrich Chemical Co., Fisher Scientific Co., Mallinckrodt, Eastman Kodak Co., or Polysciences.

Synthesis of Monomers, Intermediates, and Model Compounds

3,6-Endoxo-1,2,3,6-tetrahydrophthalic Anhydride/3a,4,7,7a-Tetrahydro-4,7-4,7-epoxyisobenzofuran-1,3-dione [5426-0905] (5)

This compound was synthesized by a modification of the published procedure [8] to yield 235.9 g (88.5%) of white crystalline product (5); mp 115-116°C (dec) (literature mp 118°C (dec) [9]). IR, ^{1}H-NMR, and ^{13}C-NMR spectral data were in agreement with the proposed structure.

N-Hydroxy-3,6-epoxy-1,2,3,6-tetrahydrophthalimide/3a,4,7,7a-Tetrahydro-2-hydroxy-4,7-epoxy-1H-isoindole-1,3(2H)-dione [5596-17-8] (6)

This compound was synthesized by the published procedure [8] to yield 128.6 g (65.5%) of white solid (6); mp 189-195°C (dec) (literature mp 187-188°C (dec) [8]). IR, ^{1}H-NMR, and ^{13}C-NMR spectral data confirmed the expected structure.

N-Acetoxy-3,6-epoxy-1,2,3,6-tetrahydrophthalimide/2-(Acetyloxy)-3a,4,7,7a-tetrahydro-4,7-epoxy-1H-isoindole-1,3(2H)-dione [32463-66-4] (7)

This compound was synthesized from 6 according to the published procedure [8] to yield 34.14 g of white crystals. An additional 10.17 g

fraction was obtained from concentration of the benzene mother liquor (75.4%); mp 139-143°C (dec) (literature mp 137-138°C (dec) [8]). IR ^{1}H-NMR, and ^{13}C-NMR spectral data were in agreement with the assigned structure.

N-Acetoxymaleimide/1-(Acetyloxy)-1H-pyrrole-2,5-dione (8)

This compound was synthesized from 7 according to the published procedure [8] to yield 28.36 g (82.5%) of white crystalline product (8); mp 70.5-71.5°C (literature mp 70.5-71.5°C [8]). IR, ^{1}H-NMR, and ^{13}C-NMR spectral data and elemental analysis confirmed the structure.

Phenyl N-(3,6-Epoxy-1,2,3,6-tetrahydrophthalimidyl) Carbonate/3a,4,7,7a-Tetrahydro-2-[(phenoxycarbonyl)oxy]-4,7-epoxy-1H-isoindole-1,3(2H)-dione [60361-88-8] (9)

This compound was synthesized from 6 according to a slight modification of the published procedure [10] to yield 71.4 g (56.3%) of white needles; mp 137-139°C (literature mp 135-136°C [10]). IR, ^{1}H-NMR, and ^{13}C-NMR spectral data were in agreement with the expected structure.

Phenyl N-Maleimidyl Carbonate/1-[(Phenoxycarbonyl)oxy]-1H-pyrrole-2,5-dione [60361-89-9] (10)

This compound was synthesized from 9 according to the published procedure [10] to yield 24.51 g (81.8%) of maleimide (10) as a pale-yellow solid; mp 99-102.5°C (literature mp 98-99°C [10]). A quantity of 10 was sublimed at 1 mm (100°C) affording white crystals; mp 101-104°C. IR, ^{1}H-NMR, and ^{13}C-NMR spectral data confirmed the proposed structure.

N-Hydroxymaleimide/1-Hydroxy-1H-pyrrole-2,5-dione [4814-74-8] (11)

This compound was prepared by the published procedure [10] to yield 1.55 g (43.4%) of an off-white crystalline solid (11); mp 126-130°C (literature mp 125-126°C [10]). Spectral properties were in agreement with those of an authentic sample kindly supplied by M. Akiyama [10].

N-(4-Carbethoxyphenyl)maleanilic Acid/(Z)-4-[(3-Carboxy-1-oxo-2-propenyl)-amino]benzoic acid, 1-ethyl ester [53616-17-4] (12)

To a 500-mL Erlenmeyer flask was added 24.4 g (0.148 mol) of ethyl p-aminobenzoate and 250 mL of chloroform. The stirred solution was cooled in an ice bath, and 14.5 g (0.148 mol) of maleic anhydride was added portionwise. After 1 h, the mixture was warmed to room temperature and stirred overnight. The white precipitate was filtered, washed with $CHCl_3$, and dried in vacuo, affording 38.1 g

(98%) of maleanilic acid (12). A portion of the product was recrystallized from CH_3CN; mp 190–192° C.

^{1}H-NMR (DMSO-d_6, 2.49): 1.28 (t, 3H), 4.25 (q, 2H), 6.41 (AB q, 2H), 7.84 (AB q, 4H), 10.62 (s, 1H).

^{13}C-NMR (DMSO-d_6, 39.5): 14.20, 60.51, 118.89, 124.74, 130.30, 131.71, 143.07, 163.78, 165.34, 166.95.

IR (KBr): 3300 (m), 3205 (m), 3110 (m), 2975 (w), 1710 (s), 1635 (m), 1610 (m), 1580 (s), 1540 (s), 1470 (m), 1415 (w), 1405 (m), 1365 (m), 1330 (m), 1310 (m), 1270 (s), 1225 (w), 1175 (m), 1120 (m), 1150 (m), 1025 (m), 1010 (w), 970 (m), 900 (w), 865 (m), 850 (m), 770 (m), 695 (w), 680 (w), 610 (m).

N-(4-Carbethoxyphenyl)maleimide/[4-(2,5-Dihydro-2,5-dioxo-1H-pyrrol-1-yl)benzoic acid, ethyl ester] [14794-06-1] (13)

To a 500-mL one-necked round-bottomed flask was added 38.1 g (0.145 mol) of 12, 1.2 g (0.015 mol) of anhydrous sodium acetate, and 100 mL of acetic anhydride. A magnetic stir bar was added, and a reflux condenser was fitted. The stirring mixture was brought to 90°C over a 1.0-h period and then allowed to cool to room temperature. The resulting solution was precipitated into 1.5 L of ice water and allowed to stir overnight. The yellow solid was collected by filtration, recrystallized from ethanol-water, and dried in vacuo, giving 29.57 g (83.4%) of yellow plates; mp 112-113°C (literature mp 113°C [11]). IR, ^{1}H-NMR, and ^{13}C-NMR spectral data confirmed the structure.

N-Hydroxymaleamic Acid [4296-73-5] (14)

N-Hydroxymaleamic acid (14) was prepared from the addition of hydroxylamine (from 61.5 g (0.885 mol) of hydroxylamine hydrochloride neutralized with one equivalent of sodium methoxide in methanol) to a solution of 86.8 g (0.885 mol) of maleic anhydride in distilled dioxane at 0°C. After warming to room temperature and stirring for 1 h, the product was filtered and dried in vacuo, affording 69.3 g (60%) of 14; mp 126-129°C (dec) (literature mp 122-128°C (dec) [12]). IR, ^{1}H-NMR, and ^{13}C-NMR spectral data confirmed the structure.

N-Carbethoxymaleimide/2,5-Dihydro-2,5-dioxo-1H-pyrrole-1-carboxylic acid, ethyl ester [55750-49-7] (15)

This maleimide was prepared by the published method [13] in 44% yield; mp 55-57°C (literature mp 58-59°C [13]). IR and ^{1}H-NMR spectral evidence confirmed the expected structure.

N-[2-(4-Imidazolyl)ethyl]maleamic Acid/(Z)-4-([2-(1H-Imidazol-4-yl)ethyl]-amino)-4-oxo-2-butenoic Acid (16)

In an Erlenmeyer flask was combined 0.437 g (3.93 mmol) of histamine (17), 0.367 g (3.74 mmol) of maleic anhydride, and 20 mL of

chloroform (ethanol-free). The mixture was stirred for 20 h. The solid was filtered and dried in vacuo, affording 0.672 g (86%) of 16, which slowly decomposed above 120°C.

^{1}H-NMR (D_2O, DSS): 2.95 (m, 2H), 3.52 (m, 2H), 6.13 (AB q, 2H), 7.26 (s, 1H), 8.53 (s, 1H).

IR (KBr): 3600-2400 (br, m), 3230 (w), 3135 (w), 3060 (w), 1655 (m), 1625 (s), 1570 (br, s), 1450 (w), 1430 (w), 1398 (w), 1365 (w), 1313 (w), 1270 (m), 1208 (w), 1185 (m), 1100 (br, m), 1065 (w), 975 (m), 902 (w), 855 (br, m), 815 (m), 730 (w), 715 (m), 638 (m), 610 (m).

Histamine/1H-Imidazole-4-ethanamine [51-45-6] (17)

The free base (17) was prepared from histamine dehydrochloride by three methods [14-16]. The method of Ref. 16 proved to be superior, providing 80% yield of pure 17. The method of Ref. 14 gave poor yields, whereas the method of Ref. 15 gave only 47% yield of 17. The product of the first method [14] yielded a product of bp 134-135°C (0.075 torr) literature bp 209-210°C (18 torr) [15]). The product of the second method [15] yielded a product of bp 140-143°C (1.0 torr). Following the procedure of the latter method [16], the product was distilled in a Kugelrohr apparatus (0.050 torr, ~160°C), affording 3.245 g (80%) of a colorless oil which crystallized on standing; mp 85-88°C (literature mp 83-84°C [15]). Spectral data were in agreement with the expected structure.

IR (KBr): 3090 (w), 3000-2200 (br, m), 1655 (m), 1618 (m), 1565 (br, s), 1435 (m), 1398 (m), 1322 (m), 1270 (s), 1205 (m), 1172 (m), 1060 (m), 905 (m), 850 (m), 775 (m), 725 (m), 705 (m), 650 (m), 620 (m).

N-(4-Carboxyphenyl)maleanilic Acid/4-[(3-Carboxy-1-oxo-2-propenyl)amino]benzoic Acid [36847-92-4] (18)

This compound was previously synthesized in these laboratories [17] from p-aminobenzoic acid and maleic anhydride; mp 234°C (dec). IR and ^{1}H-NMR spectral data confirmed the expected structure.

4-[(3-Carboxy-1-oxo-2-propenyl)amino]benzeneacetic Acid (19)

This compound was previously synthesized in these laboratories [17] from p-aminophenylacetic acid and maleic anhydride, and was used without further purification. Spectral data confirmed the proposed structure.

N-[2-(4-Imidazolyl)ethyl]-3,6-endoxo-1,2,3,6-tetrahydrophthalic Acid (20)

To a 50-mL Erlenmeyer flask was added 2.505 g (0.0136 mol) of histamine dihydrochloride and 15 mL of water. To the stirred solution, 2.286 g (0.0272 mol) of $NaHCO_3$ was carefully added. Into an-

other flask was placed 2.263 g (0.0136 mol) of 5 and 22 mL of acetone. The solution of free base (17) in water was slowly added to the acetone solution with rapid stirring. Addition of more acetone (200 mL) was necessary to make the flask contents homogeneous. After stirring for 1 h, the liquid phase was decanted off, and the remaining oily precipitate was stirred over fresh acetone. The resulting fine white solid was collected and dried in vacuo to give 4.898 g of 20, apparently contaminated by NaCl. The solid gradually decomposed upon heating to 135°C.

^{1}H-NMR (D_2O, DSS): 2.73 (s, 2H), 2.67-3.63 (m, 4H), 5.07 (d, 2H), 6.43 (m, 2H), 7.12 (m, 1H), 8.48 (d, 1H).

IR (KBr): 3660-2730 (m, br), 3240 (m), 3120 (m), 1715 (w), 1650 (s), 1625 (s), 1555 (s), 1430 (m), 1395 (s), 1310 (w), 1270 (m), 1245 (w), 1218 (m), 1183 (w), 1167 (w), 1092 (w), 1060 (w), 1028 (w), 1000 (w), 982 (w), 972 (w), 930 (w), 900 (m), 838 (m), 820 (m), 808 (w), 752 (w), 730 (m), 702 (m), 627 (m).

N-Acetoxysuccinimide [14464-29-0] (21)

To a dry 250-mL three-necked round-bottomed flask fitted with a mechanical stirrer, N_2 inlet tube, and septum cap was added 3.0 g (0.026 mol) of N-hydroxysuccinimide, 100 mL of anhydrous ether, and 20 mL of distilled THF. Dry pyridine (2.06 g, 0.026 mol) was added under N_2, and the flask was cooled to 0-5°C. To the stirred solution, 1.5 mL (0.0265 mol) of acetyl chloride was added dropwise by syringe. The mixture was stirred for 0.5 h at 0°C and then at room temperature for 1 h. The flask contents were transferred to a separatory funnel and extracted with 1 N HCl. The organic layer was dried over anhydrous $MgSO_4$, the solvent removed in vacuo, and the residue triturated with hexanes to give a white solid. The solid was recrystallized from benzene-hexanes, filtered, and dried in vacuo to afford 1.496 g (36%) of needles (21); mp 131-133.5°C (literature mp 132-133°C [18]). IR, ^{1}H-NMR, and ^{13}C-NMR spectral data were in agreement with the expected structure.

N-(β-Vinyloxyethyl)imidazole/1-[2-(Ethenyloxy)ethyl]-1H-imidazole (22)

The general procedure for N-alkylation of imidazole described in the literature [19] was used to synthesize this new monomer. Short-path distillation gave 21.86 g (46.7%) of pure 22 as a colorless oil; bp 90-92°C (0.5 torr). The product was stored over CaH_2 at room temperature.

^{1}H-NMR ($CDCl_3$, TMS): 3.74-4.40 (m, 6H), 6.38 (X of ABX, 1H), 6.98 (m, 2H), 7.47 (s, 1H).

(DMSO-d_6, TMS): 3.83-4.47 (m, 4H), 6.46 (X of ABX, 1H), 6.93 (t, 1H), 7.15 (t, 1H), 7.60 (s, 1H).

^{13}C-NMR ($CDCl_3$, 77.0): 44.93, 65.96, 86.48, 118.35, 1208.08, 136.36, 149.89.

IR (neat, NaCl): 3115 (m), 2940 (m), 2880 (m), 1620 (br, s), 1506 (s), 1465 (m), 1440 (m), 1365 (s), 1325 (s), 1285 (s), 1230 (s), 1195 (br, s), 1150 (sh, m), 1110 (s), 1092 (sh, s), 1078 (s), 1038 (s), 1028 (sh, m), 990 (m), 963 (sh, s), 952 (s), 915 (m), 906 (s), 820 (br, s), 740 (br, s), 662 (s), 622 (s).

LRMS (m/e, relative intensity): 138 (M+, 19.2), 137 (11.9), 109 (19.4), 108 (96.4), 95 (14.0), 94 (10.0), 86 (19.7), 84 (32.7), 82 (20.0), 81 (100).

HRMS: m/e 138.07744 (calculated for $C_7H_{10}N_2O$ = 138.07931).

Elemental analysis: Calculated for $C_7H_{10}N_2O$: C, 60.85; H, 7.29; N, 20.27. Found: C, 60.85; H, 7.32; N, 20.28.

N-(β-Vinyloxyethyl)piperidine/1-[2-(Ethenyloxy)ethyl]-piperidine [702-06-7] (23)

This vinyl ether was synthesized by the published method [20] to yield 35.2 g (87.0%) of colorless oil (25); bp 75-76.5°C (10 torr) (literature bp 72.3-73°C (7.5 torr) [20]). IR, ^{1}H-NMR, and ^{13}C-NMR spectral data were in agreement with the expected structure.

β-Vinyloxyethyl(imidazol-4ylmethyl)piperidinium Chloride (24)

The method reported by Tonellato [21] was employed. To a 50-mL one-necked round-bottomed flask containing a stir bar was added 1.338 g (8.74 mmol) of 4-(chloromethyl)imidazole hydrochloride (25) and 10 mL of anhydrous methanol. To the stirred solution at room temperature was added 2.771 g (17.8 mmol) of 23 in one portion, and the solution was stirred for 0.5 h. Approximately 1 g of Na_2CO_3 was added, the mixture stirred for 5 min, and filtered. The filtrate was reduced in volume on a rotary evaporator and suction filtered again. Slow addition of the filtrate into ether gave an oily precipitate, which was taken up in 10 mL of anhydrous methanol, again stirred over ~1 g of Na_2SO_3, filtered, and the filtrate reduced in vacuo. The viscous oil was triturated with acetonitrile, suction filtered, and reduced in volume. Precipitation into ether gave an oily residue. This process (treatment with Na_2CO_3, etc.) was repeated two additional times to ensure complete removal of 23 in its free base. Final drying of the oily precipitate in vacuo overnight afforded 1.998 g (84%, crude) of hygroscopic solid (24).

^{1}H-NMR (D_2O, DSS): 1.50-2.17 (m, 6H), 3.25-3.69 (m, 6H), 4.17-4.53 (m, 4H), 4.63 (s, 2H), 6.60 (X of ABX, 1H), 7.52 (s, 1H), 7.86 (s, 1H).

^{13}C-NMR (D_2O, DSS): 22.17, 23.15, 58.87, 59.95, 61.80, 63.94, 91.33, 124.57, 129.11, 140.02, 153.18.

IR (KBr): 3600-2500 (br, s), 1625 (s), 1558 (w), 1495 (sh, m), 1465 (m), 1435 (sh, m), 1370 (m), 1325 (m), 1295 (w), 1195 (s), 1090 (m), 1028 (m), 977 (m), 942 (w), 897 (m), 865 (m), 830 (m), 796 (m), 663 (m), 626 (s).

4-(Chloromethyl)imidazole Hydrochloride/4-(Chloromethyl)-1H-imidazole Hydrochloride [31036-72-3] (25)

The published procedure [22] was employed for the synthesis of 25 to yield 11.50 g (71.0%) of off-white crystals; mp 141-144°C (literature mp 144°C [23]). IR and ^{13}C-NMR spectral data confirmed the proposed structure.

4-(Hydroxymethyl)imidazole Hydrochloride/1H-Imidazole-4-methanol [32673-41-9] (26)

This material was prepared by the published method [16] to give 15.45 g (69.3%) of yellow crystals; mp 105-109°C (literature mp 107-109°C [16]). A second recrystallization from absolute ethanol gave 14.25 g of pale-yellow crystals; mp 107-110°C. IR, ^{1}H-NMR, and ^{13}C-NMR spectral data were in agreement with the expected structure.

Dichlorobis(1-[2-(ethenyloxy)ethyl]-1H-imidazole-N^3)zinc (27)

The published method [24] was employed for synthesis of 27 to give 0.535 g (89%) of 27; mp 78.5-80°C.

IR (KBr): 3115 (m), 3040 (w), 2925 (w), 2875 (w), 1635 (sh, m), 1620 (s), 1525 (m), 1442 (w), 1397 (w), 1365 (w), 1322 (m), 1240 (m), 1232 (m), 1187 (s), 1112 (m), 1097 (s), 1033 (m), 992 (m), 952 (m), 850 (m), 830 (m), 760 (m), 668 (m), 653 (m), 630 (m).

Elemental analysis: Calculated for $C_{14}H_{20}N_4O_2 \cdot ZnCl_2$: C, 40.75; H, 4.89; N, 13.58; Cl, 17.18. Found: C, 40.72; H, 4.91; N, 13.51; Cl, 17.09.

Dichlorobis(1-methyl-1H-imidazole-N^3)zinc-(T-4) [23570-24-3] (28)

N-Methylimidazole-$ZnCl_2$ complex was prepared in an analogous manner [24] to 27 to yield 0.512 g (79%) of fine white solid (28); mp 205-208°C (literature mp 209°C [23]). IR spectral data and elemental analysis supported the expected structure.

4-Allylimidazole/4-(2-Propenyl)-1H-imidazole [50995-98-7] (29)

A 250-mL three-necked round-bottomed flask, a mechanical stirring rod, a condenser, and an addition funnel were assembled while hot, and were then cooled by flushing with N_2. To the flask was added 3.408 g (0.140 mol) of Mg turnings; the addition funnel was charged with 100 mL of freshly distilled THF and 10 mL (0.142 mol) of vinyl bromide. Reaction was initiated by addition of a solution of a drop of ethylene bromide in 10 mL of THF. The vinyl bromide solution was then added

at a rate which maintained a gentle reflux. When formation of Grignard reagent was complete, the solution was cooled to 0°C with an external ice bath. To the flask was added 4.289 g (0.0280 mol) of 25 in ~15 equal portions over a 2.5-h period. The rapidly stirred mixture was maintained at 0°C for an additional 0.5 h, then allowed to warm to room temperature, and was quenched by careful addition of 20 mL of saturated NH_4Br. Additional water was added to dissolve the precipitated salts, and the organic layer was separated. The aqueous layer was extracted with two 150-mL portions of $CHCl_3$, and the combined organic fractions were dried over anhydrous $MgSO_4$. Solvent was removed in vacuo, giving dark yellow oil. This oil was chromatographed on a column of silica gel using a mixture of $CHCl_3$:CH_3OH (95:5) as eluting solvent. The fractions were combined giving an $R_f \cong 0.30$ by TLC (silica gel, $CHCl_3$:CH_3OH (95.5)). Removal of solvent in vacuo afforded 1.372 g (45%) of a pale yellow oil (29) having identical ^{1}H-NMR properties as reported in the literature [26].

^{1}H-NMR ($CDCl_3$, TMS): 3.30-3.45 (m, 2H), 5.00-5.20 (m, 2H), 5.79-6.20 (m, 1H), 6.81 (d, 1H), 7.60 (d, 1H), 11.05 (br, 1H).

^{13}C-NMR ($CDCl_3$, TMS): 31.39, 116.14, 117.31, 134.76, 135.25, 135.78.

IR (neat, NaCl): 3500-2300 (br, s), 3080 (m), 3015 (w), 2985 (m), 2850 (br, m), 2740 (w), 2640 (w), 1640 (m), 1588 (m), 1570 (m), 1473 (by, m), 1730 (m), 1323 (w), 1298 (m), 1262 (m), 1230 (m), 1195 (w), 1160 (w), 1105 (m), 1088 (m), 990 (s), 940 (m), 915 (s), 820 (m), 750 (m), 662 (m), 625 (m).

LRMS (m/e, relative intensity): 109 (6.6), 108 (M+, 68.4), 107 (100), 82 (20.7), 81 (85.5), 80 (86.2), 54 (26.8), 53 (40.9).

HRMS: m/e 108.06875 (calculated for $C_6H_8N_2$ = 108.06875).

4-Nitroimidazole/4-Nitro-1H-imidazole [3034-38-6] (30)

This material was prepared in accord with the published method [27] in a 31% yield; mp 308-309°C (literature mp 308-310°C [27]). Spectral data confirmed the proposed structure.

Synthesis of Polymers and Copolymers

Poly(N-Acetoxymaleimide) (31)

To a heavy-walled polymerization tube was added 2.021 g (0.0130 mol) of 8, 0.0211 g (0.128 mmol) of AIBN, and 25 mL of freshly distilled CH_2Cl_2. After all the solid had dissolved, the tube was degassed (3 freeze-pump-thaw cycles) and sealed at $\sim 10^{-5}$ torr. Polymerization was carried out in a constant temperature bath (61°C) for 66

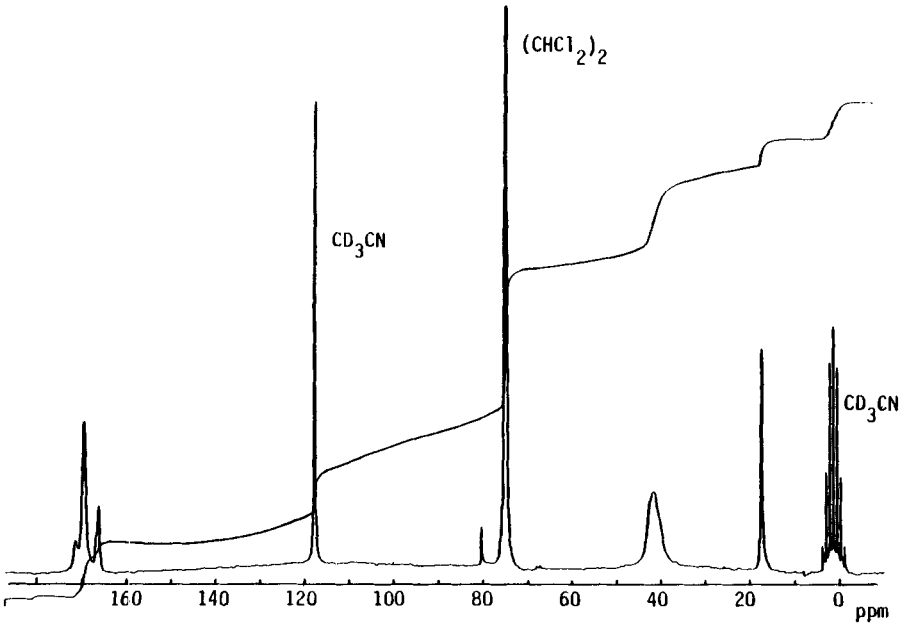


FIG. 1. Proton-decoupled ^{13}C-NMR spectrum of poly(N-acetoxymaleimide) (31) in CD_3CN–$(CHCl_2)_2$ at 60°C.

h. The tube was opened and the contents precipitated into ether. The solid was collected, redissolved in dioxane, and reprecipitated into ether. The solid was again collected and dried in a vacuum oven (100°C) overnight to afford 1.614 g (80% conversion) of pink powder (31).

^{1}H-NMR (DMSO-d_6, TMS): 2.34 (br-s, 3H), 3.45, 4.13 (br, 2H).

^{13}C-NMR (see Fig. 1).

IR (KBr): 2940 (w), 1820 (s), 1790 (s), 1730 (vs), 1625 (w), 1430 (w), 1373 (m), 1220 (s), 1160 (s), 1055 (m), 1000 (w), 820 (m), 725 (w), 640 (m).

Elemental analysis: Calculated for $C_6H_5NO_4$: C, 46.46; H, 3.25; N, 9.03. Found: C, 45.74; H, 3.38; N, 8.88.

VPO (acetone): $\overline{M}_n$ = 3850 g/mol.

Poly(Phenyl-N-maleimidyl carbonate) (32)

To a heavy-walled polymerization tube was added 3.143 g (0.01348 mol) of 10, 0.0250 g (0.152 mmol) of AIBN, and 6 mL of distilled acetone. The tube was transferred to a high-vacuum line, degassed in

the usual manner, and sealed at 10^{-5} mm. The polymerization was carried out in a constant-temperature bath (60°C) for 89 h. The tube was opened, and the solution was slowly added dropwise to a beaker of vigorously stirred ether. The precipitate was collected and dried in vacuo, giving 2.755 g (87% conversion) of pale green solid (32).

^{1}H-NMR (CD_3CN, 1.93): 3.99 (br, 2H), 7.32 (br, 5H).

^{13}C-NMR (CD_3CN, 70°C, 1.30): 42.92, 121.39, 128.50, 131.18, 150.63, 151.95, 169.49.

IR (KBr): 3060 (w), 2940 (w), 1825 (s), 1795 (s), 1735 (vs), 1600 (w), 1588 (m), 1490 (m), 1457 (m), 1375 (m), 1290 (m), 1225 (br, vs), 1160 (m), 1115 (w), 1070 (s), 1020 (m), 1005 (m), 960 (m), 905 (w), 840 (w), 775 (m), 750 (m), 682 (m), 630 (m).

Elemental analysis: Calculated for $C_{11}H_7NO_5$: C, 56.66; H, 3.03; N, 601. Found: C, 55.44; H, 3.11; N, 6.17.

Poly(N-Hydroxymaleimide) (33) from 31

To a 50-mL Erlenmeyer flask containing a stir bar was added 1.0 g (0.0144 mol) of hydroxylamine hydrochloride and 20 mL of freshly distilled methanol. To the stirred solution was added 3.9 mL (0.0144 mol) of 3.7 M sodium methoxide. After 0.5 h, the mixture was suction filtered. To the filtrate was added a solution of 31 in CD_3CN and $Cl_2CHCHCl_2$ (NMR sample ~150 mg), the transfer being aided by rinsing the tube with acetone. The resulting mixture (pink precipitate) was stirred for 48 h, and the solvents were then removed in vacuo. Trituration of the resulting solid with water gave a pink solid which was suction filtered and dried in vacuo.

^{13}C-NMR (see Fig. 2).

IR (KBr): 3640-2300 (br, m), 3470 (br, m), 2920 (w), 2800 (w), 1785 (m), 1700 (br, vs), 1620 (m), 1470 (br, m), 1385 (w), 1340 (w), 1230 (s), 1120 (m), 1070 (m), 728 (m), 645 (m).

Poly(N-Hydroxymaleimide) (33) from 32

To a 50-mL round-bottomed flask containing a stir bar was added 1.411 g (6.05 mmol of repeat units) of 32 and 20 mL of methanol. A reflux condenser was attached, and the mixture was refluxed for 20 h. The cooled solution was precipitated into 200 mL of benzene-pentane (2:1). The solid was reprecipitated from acetone into ether, filtered, and dried in vacuo, giving 0.605 g (88%) of a tan powder (33). The product decomposed above 265°C. The IR spectrum of this material was identical to that of 33 derived from 32.

Elemental analysis: Calculated for $C_4H_3NO_3$: C, 42.49; H, 2.67; N, 12.39. Found: C, 43.75; H, 3.40; N, 11.94.

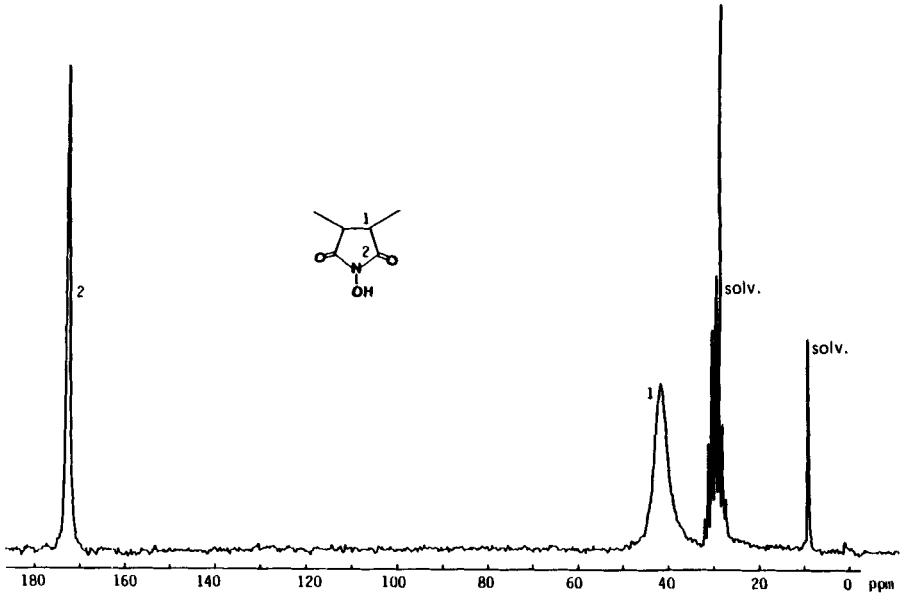


FIG. 2. Proton-decoupled ^{13}C-NMR spectrum of copolymer 33 in acetone-d_6.

Poly[N-(4-Carbethoxyphenyl)maleimide] (34)

To a heavy-walled polymerization tube was added 3.423 g (0.01396 mol) of 13, 0.0270 g (0.164 mmol) of AIBN, and 10 mL of freshly distilled DMF. When all the solid had dissolved, the tube was degassed (3 freeze-pump-thaw cycles) and sealed at $\sim 10^{-5}$ torr. Polymerization was carried out in an oil bath (75°C) for 44 h. The tube was opened, and most of the DMF was removed in vacuo. The resulting oil was dissolved in 5 mL of acetone and precipitated into ether. The solid was reprecipitated from acetone into ether, collected, and dried in vacuo to give 2.102 g (61% conversion) of a pink solid (34).

^{1}H-NMR (acetone-d_6, 50°C, TMS): 1.36 (br, 3H), 4.33, 4.40 (br, 4H), 7.47, 8.08 (br, 4H).

^{13}C-NMR (acetone-d_6, 50°C, 29.8): 14.55, 41.74, 45.64, 61.77, 127.47, 130.74, 136.44, 165.83, 175.82.

IR (KBr): 2985 (m), 2940 (w), 2910 (w), 1785 (sh, m), 1715 (vs), 1610 (m), 1510 (m), 1470 (w), 1445 (w), 1415 (sh, m), 1385 (s), 1280 (s), 1185 (s), 1110 (s), 1020 (m), 855 (m), 768 (m), 740 (m), 695 (m), 640 (m).

Elemental analysis: Calculated for $C_{13}H_{11}NO_4$: C, 63.67; H, 4.52; N, 5.71. Found: C, 63.13; H, 4.67; N, 6.05.

Poly[N-(β-Vinyloxyethyl)imidazole] (35)

All attempts to obtain homopolymer (35) of moderate molecular weight and in good yield were unsuccessful. Low yields of oligomers were generally obtained.

N-(β-Vinyloxyethyl)imidazole-N-Hydroxymaleimide Alternating Copolymer (36)

To a 100-mL round-bottomed flask was added 2.325 g (0.0168 mol) of 22 and 39.95 mL (0.0168 mol) of 0.4 N HCl. Most of the water was removed on a rotary evaporator at room temperature. The resultant viscous oil was transferred to a heavy-walled polymerization tube aided by a few milliliters of deionized water. Into a 5-mL volumetric flask was placed 2.223 g (0.0143 mol) of 8, and the flask was diluted to the mark with distilled THF. This solution was added to the polymerization tube (previously cooled to -78°C), and the volumetric flask was rinsed with 3 mL of THF. Finally, 0.0847 g (0.313 mmol) of $K_2S_2O_8$ and 0.1237 g (0.315 mmol) of Fe $(NH_4)_2(SO_4)_2 \cdot 6H_2O$ were added. The final volume of solution was 22 mL. The tube was then degassed on a high-vacuum line (3 freeze-pump-thaw cycles) and sealed at $\sim 10^{-5}$ torr. The tube was placed in a 30.0°C water bath for 91 h. The tube was opened and the contents precipitated into CH_3CN. The acetonitrile was decanted off, and the oily precipitate was taken up in 40 mL of 1 N HCl and dialyzed (2 000 MW retention) against deionized water for several days. The precipitated solid was suction filtered and dried in vacuo to afford 0.844 g (20% conversion) of light brown solid (36).

The IH-NMR spectrum is shown in Fig. 3. The ^{13}C-NMR spectra are shown in Figs. 4 and 5.

IR (KBr): 3600-3320 (br, m), 3140 (m), 2940 (w), 1780 (m), 1705 (vs), 1575 (w), 1440 (w), 1400 (w), 1355 (w), 1290 (w), 1230 (s), 1105 (s), 1080 (s), 835 (w), 760 (w), 665 (w), 625 (w).

Elemental analysis: Calculated for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.21; N, 16.73. Found: C, 49.78; H, 4.97; N, 14.68; S, 0.53.

VPO (DMSO): $\overline{M}_n$ = 488 g/mol.

Intrinsic viscosity (0.1 N HCl, 30.0°C): $[\eta]$ = 0.112 dL/g.

Dichlorobis(1-[2-(ethenyloxy)ethyl]-1H-imidazole-N^3)zinc-N-Acetoxymaleimide Alternating Copolymer (37)

To a heavy-walled polymerization tube was added a solution of 0.327 g (2.40 mmol) of $ZnCl_2$ in 6 mL of distilled THF, followed by a solution of 0.630 g (4.56 mmol) of 22 in THF (3 mL). To this

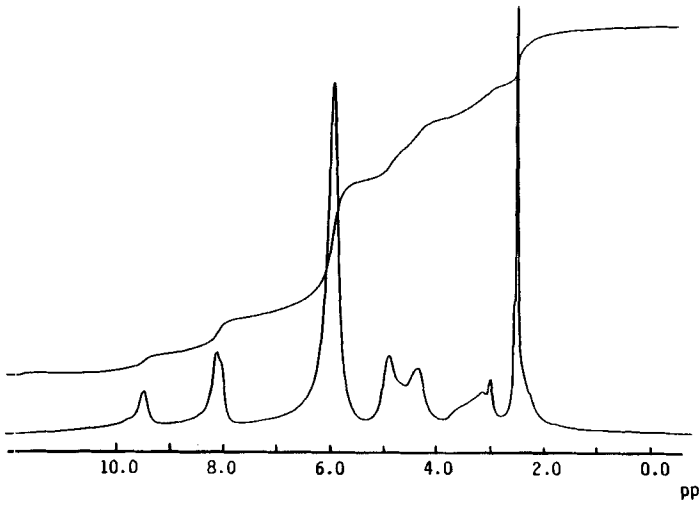


FIG. 3. ^{1}H-NMR spectrum of N-(β-vinyloxyethyl)imidazole-N-hydroxymaleimide alternating copolymer (36) in DMSO-d_6 at 120°C.

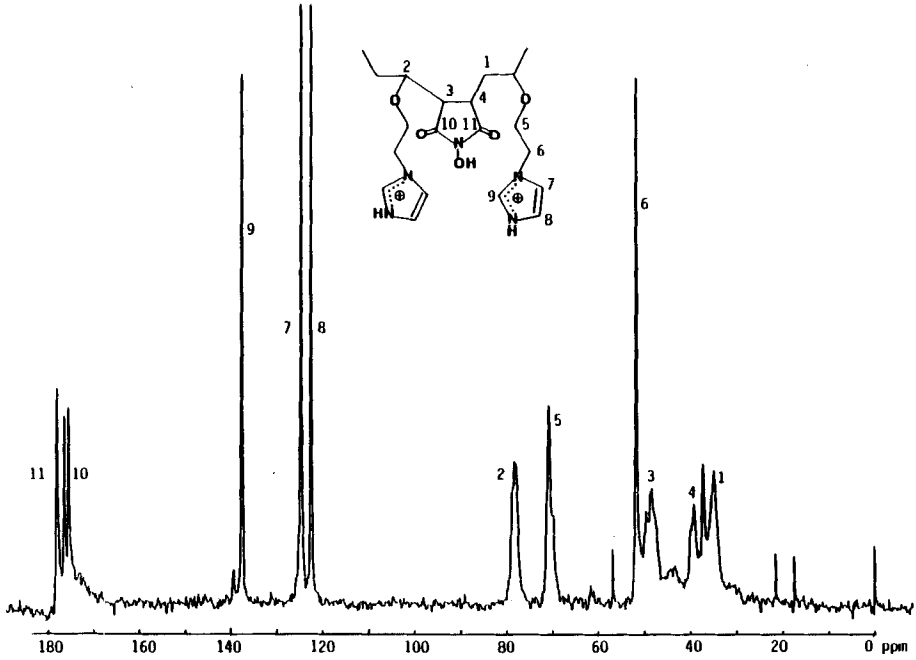


FIG. 4. Proton-decoupled ^{13}C-NMR spectrum of copolymer 36 in D_2O–HCl, 70°C.

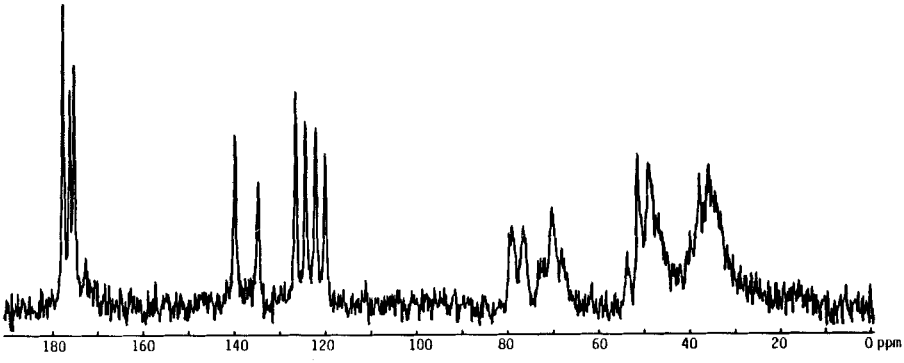


FIG. 5. Off-resonance ^{13}C-NMR spectrum of copolymer 36 in D_2O–HCl, 60°C.

solution was added 0.703 g (4.53 mmol) of 8, 0.0075 g (0.046 mmol) of AIBN, and 6 mL of THF. The solution was degassed on a vacuum line and sealed at 10^{-5} torr. Polymerization was carried out at 70°C for 3.5 h. The white precipitate was filtered, washed with THF, and dried in vacuo. The material was extracted (Soxhlet) with THF for 3 days and dried in vacuo, affording 1.110 g (67.5% conversion) of a white solid (37) which decomposed above 220°C. The ^{13}C-NMR spectrum is shown in Fig. 6.

IR (KBr): 3640-3340 (br, m), 3135 (m), 2940 (m), 2880 (w), 1818 (s), 1785 (s), 1730 (vs), 1650 (br, w), 1522 (m), 1440 (m), 1370 (m), 1290 (w), 1220 (s), 1165 (s), 1110 (s), 1095 (s), 1065 (m), 950 (m), 830 (m), 755 (m), 655 (m), 625 (w).

Elemental analysis: Calculated for $C_{26}H_{30}N_6O_{10}ZnCl_2$: C, 43.20; H, 4.18; N, 11.63; Cl, 9.81. Found: C, 42.35; H, 4.18; N, 10.98; Cl, 9.22.

Intrinsic viscosity (DMSO, 30.0°C): $[\eta]$ = 0.043 dL/g.

Attempted Copolymerization of N-Acetoxymaleimide (8) and N-(β-Vinyloxyethyl)-imidazole (25); Preparation of N-Acetoxymaleimide Cyclotrimer (38)

To a 50-mL Erlenmeyer flask was added 4.038 g (0.0260 mol) of 8 and 10 mL of CH_2Cl_2. To this colorless solution was added a solution of 0.0180 g (0.130 mmol) of 22 in 1 mL of CH_2Cl_2. A red color became immediately apparent and intensified with time. The flask was allowed to stand at room temperature for 165 h. The dark-red solution was added dropwise to a beaker of vigorously stirred ether. The

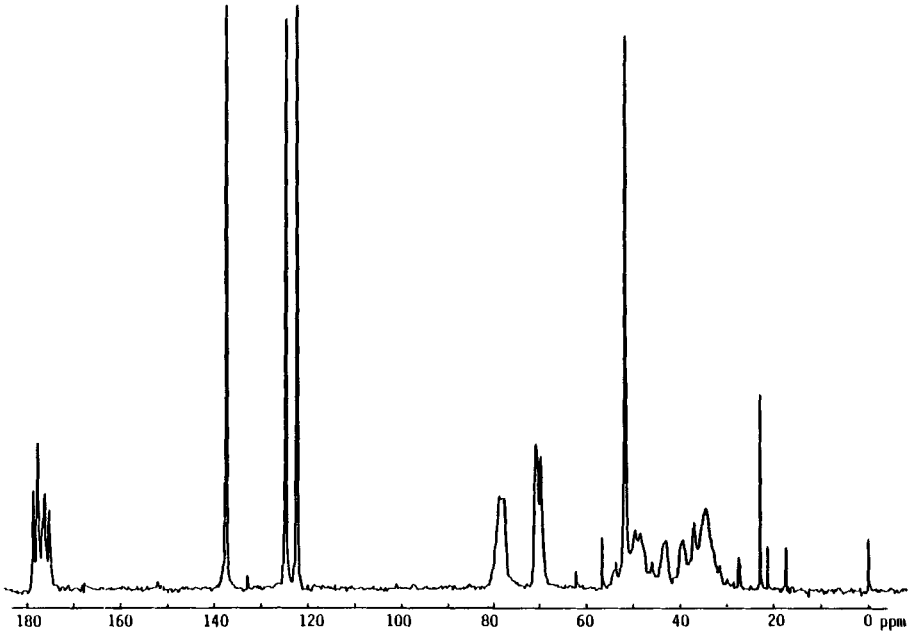


FIG. 6. Proton-decoupled ^{13}C-NMR spectrum of copolymer 37 in D_2O–HCl, 80°C.

precipitate was filtered and dried in vacuo at 100°C to afford 0.66 g (16.3%) of purple powder (38).

^{1}H-NMR (CD_3CN, TMS): 2.29 (br, 3H), 3.00-4.60 (br-m, ~2H).

The ^{13}C-NMR spectrum is shown in Fig. 7.

IR (KBr): 2945 (w), 1820 (s), 1790 (s), 1740 (vs), 1430 (w), 1375 (m), 1225 (s), 1160 (s), 1065 (m), 1005 (w), 820 (m), 670 (w), 645 (w).

Elemental analysis: Calculated for $(C_6H_5NO_4)_3$: C, 46.46; H, 3.25; N, 9.03. Found: C, 46.40; H, 3.24; N, 9.03.

LRMS (m/e, relative intensity): 465 (M+, 0.1), 423 (0.4), 113 (0.4), 60 (19.3), 45 (24.4), 44 (61.8), 43 (100).

VPO (acetone): $\overline{M}_n$ = 488 g/mol.

Reaction of Maleic Anhydride (39) and N-(β-Vinyloxyethyl)-imidazole (22)

To a 125-mL Erlenmeyer flask was added 0.967 g (7.00 mmol) of 22 and 20 mL of CH_2Cl_2. To this colorless solution was added 0.687 g

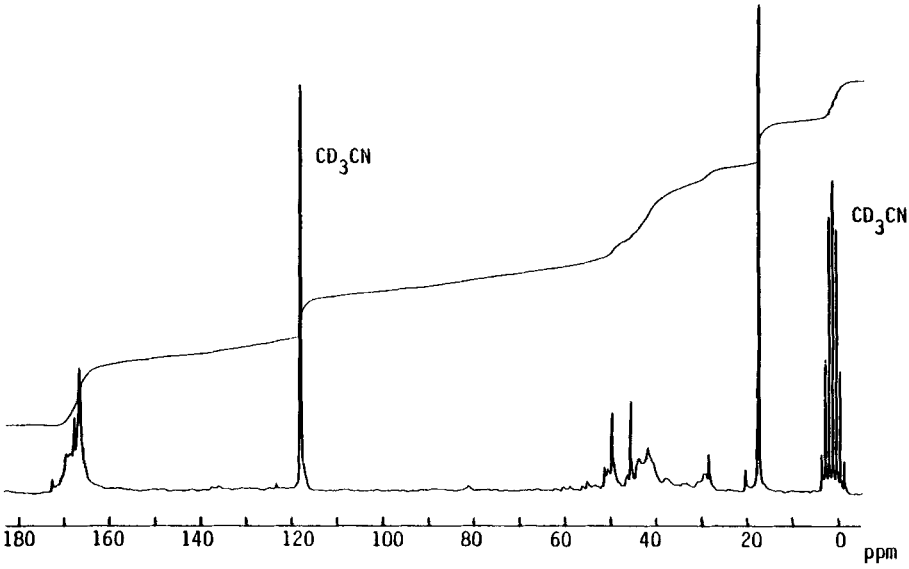


FIG. 7. Proton-decoupled ^{13}C-NMR spectrum of N-acetoxymaleimide cyclotrimer (38) in CD_3CN at 70°C.

(7.00 mmol) of 39. The solution immediately turned yellow in color and eventually became brown. The flask was allowed to stand for 11 days. The solution was decanted, leaving a black precipitate which was removed from the flask and stirred with 100 mL of acetone for 3 h. The solid was filtered and dried in vacuo, affording 0.897 g of brown powder.

IR (KBr): 3650-2320 (br, m), 3140 (m), 2950 (w), 1770 (s), 1720 (br, s), 1620 (m), 1580 (m), 1555 (m), 1445 (w), 1380 (br, m), 1220 (br, m), 1190 (m), 1135 (m), 1085 (m), 1035 (m), 935 (m), 830 (m), 750 (m), 665 (w), 625 (m).

Reaction of N-Hydroxymaleimide (11) and N-(β-Vinyloxyethyl)-imidazole (22)

To a 50-mL Erlenmeyer flask was added 0.326 g (2.88 mmol) of 11 and 10 mL of distilled acetone. To this pale yellow solution was added 0.406 g (2.94 mmol) of 22. The solution immediately assumed a darker yellow color, and a precipitate began to form. After stirring for 20 h, the precipitate was filtered, washed with acetone, and dried in vacuo, affording 0.421 g of a yellow solid which decomposed upon heating to 180°C.

IR (KBr): 3140 (w), 3100 (w), 2940 (w), 1785 (m), 1695 (br, s), 1615 (m), 1570 (w), 1555 (w), 1540 (w), 1415 (w), 1360 (w), 1320 (w), 1235 (br, m), 1190 (m), 1080 (m), 955 (w), 830 (w), 740 (w), 690 (m).

Elemental analysis: Calculated for $(C_4H_3NO_3)_3 \cdot C_7H_{10}N_2O \cdot H_2O$: C, 46.06; H, 4.27; N, 14.14. Found: C, 45.92; H, 4.21, N, 13.96.

Kinetic Measurements

Equipment and Materials

Pseudo-first-order kinetics were measured on Cary 17-D or Perkin-Elmer 330 spectrophotometers. Temperature control was provided by a Lauda K-2/R (40.0 ± 0.2°C) or a Haake A80 (25.0 ± 0.2°C) constant-temperature apparatus. A Corning-125 pH meter fitted with a Ag/AgCl pH electrode was used to measure the pH of solutions before and after the reaction with substrate. p-Nitrophenyl acetate (PNPA) was obtained from the Aldrich Chemical Co. and was recrystallized from cyclohexane before use; mp 77-78°C (literature mp 81-82°C [28]). 2,4-Dinitrophenyl benzoate (DNPB) was kindly supplied by Ann Mobley [29]. Deionized water was distilled in glass before use. DMSO and THF were purified as previously described. Tris(hydroxymethyl)aminomethane (Tris) was obtained from Fisher Chemical Co. and was used without further purification.

Kinetic measurements were carried out as follows.

Buffer Solutions

Two stock buffer solutions were prepared, 0.1 M Tris and 0.1 M Tris·HCl, both having an ionic strength (μ) of 0.1 (KCl). The first solution was prepared by adding 12.114 g (0.100 mol) of Tris and 7.455 g (0.100 mol) of KCl to a 1-L volumetric flask and diluting to the mark with distilled water. Tris·HCl was prepared by adding an ampule of 0.1 N HCl (Acculute), 12.114 g (0.100 mol) of Tris, and 7.455 g (0.100 mol) of KCl to a 1-L volumetric flask and diluting to the mark with distilled water. These two solutions were combined to give a buffer solution of the desired pH. Thus, a 2:1 volume ratio of Tris·HCl:Tris gave a pH of 7.86, and a 3:1 ratio of Tris·HCl: Tris gave a pH of 7.68 at 25°C.

Substrate Solutions

A stock solution of PNPA (2.69×10^{-3} M) in acetonitrile was used.

Catalyst Solutions

Catalyst solutions were prepared according to the concentration of functional groups. Due to difficulty in determining the exact composition of copolymers, it was assumed that the copolymers studied were strictly 1:1 alternating copolymers. The contribution to the molecular weight by end groups was also neglected in all polymer catalysts.

Kinetic Method

The following paragraph describes the kinetic method making use of the Cary 17-D spectrophotometer. The same procedure was used in conjunction with the Perkin-Elmer 330 spectrophotometer with the exception that each sample cell required a corresponding reference cell. To each of six 1-cm path-length quartz cells was added 3.0 mL of buffer solution by pipet. To four of the cuvettes was added 150 μL of catalyst solution by micropipet; to the other two cuvettes was added 150 μL of buffer solution. To one of the latter cuvettes was added 50 μL of substrate solvent (CH_3CN, DMSO, or THF), and it was placed in the reference beam of the spectrophotometer. The remaining five cuvettes were placed in the sample compartment to equilibrate thermally. The sample cuvettes were then each charged with 50 μL of substrate solution, agitated by inverting the sample holder, and replaced in the sample compartment. The release of p-nitrophenolate ion was observed at constant wavelength at constant time intervals.

The reaction was followed for at least 10 half-lives as judged by the constancy of the absorbance readings (A_∞). A plot of ln ($A_\infty - A_t$) vs time (t) was constructed, and the negative slope of the best straight line, as determined by the least-squares program of a Texas Instruments TI-55-II calculator, gave the desired rate constants (k_{meas}), which is the sum of the catalyzed (k_{obs}) and uncatalyzed (k_{blank}) rate constants. Furthermore, the second-order rate constant (k_{cat}) was calculated from the relation $k_{cat} = k_{obs}/[\text{catalyst}]$ [30]. In the case of slow reactions where A_∞ was not obtained in a reasonable time, k_{meas} was determined by a method described in the literature [31].

RESULTS AND DISCUSSION

As stated earlier, alternating copolymers containing pendant groups which would exhibit cooperative behavior in the hydrolysis of an ester substrate were sought. Substituted vinyl ether and maleimide monomer pairs were utilized in order to achieve the desired alternating of pendant functional groups, as this combination of monomers is known to give regularly alternating copolymers under free-radical initiation conditions [7]. The selection of catalytically active functional groups was made possible by earlier work [3, 32-34]. In these studies it was shown that the hydroxamic acid group is an excellent acylation catalyst for activated ester substrates. However, decomposition of an acylhydroxamate is a slow process. In order to obtain a useful catalyst, i.e., one with efficient turnover of the catalytic group, the deacylation rate must be comparable to the acylation rate. It had been found [3] that introduction of an imidazole group into the polymer would acceler-

ate the deacylation process. It was concluded that the imidazole group assists deacylation of the acylhydroxamate intermediate either by acting as a general base or as a nucleophilic catalyst.

With this information in mind, the synthesis of maleimide and vinyl ether monomers substituted with imidazole and hydroxamic acid functionalities was initiated. Initial effort was directed toward attaching an imidazole group to a maleimide; however, due to the base-sensitive nature of maleimides [35], the hydroxamic acid-maleimide combination was deemed more compatible. This logic dictated that the complementary vinyl ether monomer should contain an imidazole group. The next section describes efforts to carry out this objective.

An initial attempt to prepare an imidazole-substituted maleimide by nitration of imidazole [27], reduction, and reaction of the 4-aminoimidazole (30) with maleic anhydride (39), followed by ring closure of the intermediate maleamic acid to yield N-(4-imidazolyl)maleimide, was unsuccessful.

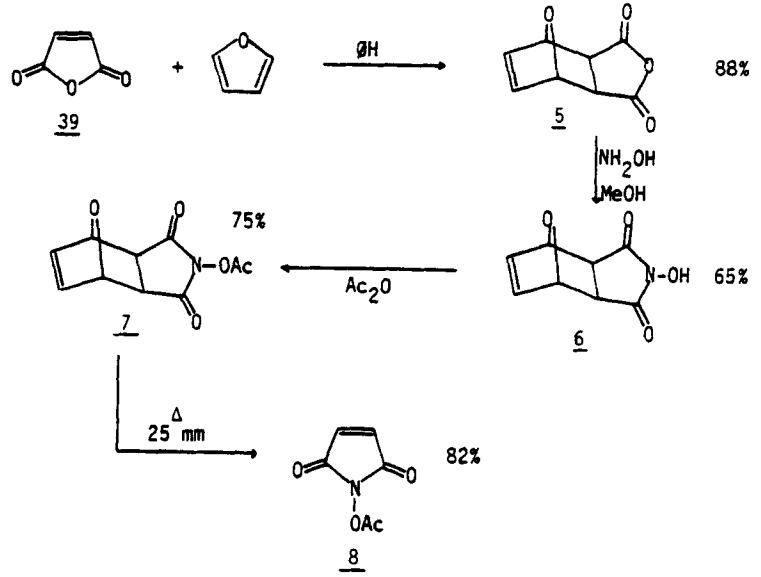
In lieu of 4-aminoimidazole, the reaction of histamine 17 with 39 was carried out. Although the maleamic acid was obtained in reasonable yield, attempts to effect dehydration to obtain N-[2-(4-imidazolyl)-ethyl]maleimide by the published method [39] were unsuccessful.

Another attempt to synthesize N-[2-(4-imidazolyl)ethyl]maleimide involved reaction of the furan-maleic anhydride adduct (5) with 17 to yield the corresponding succinamic acid (20) in good yield. However, dehydration of 20 was also unsuccessful by the Ac_2O/NaOAc [39] and N,N-dicyclohexylcarbodiimide (DCC)/DMF [40] procedures.

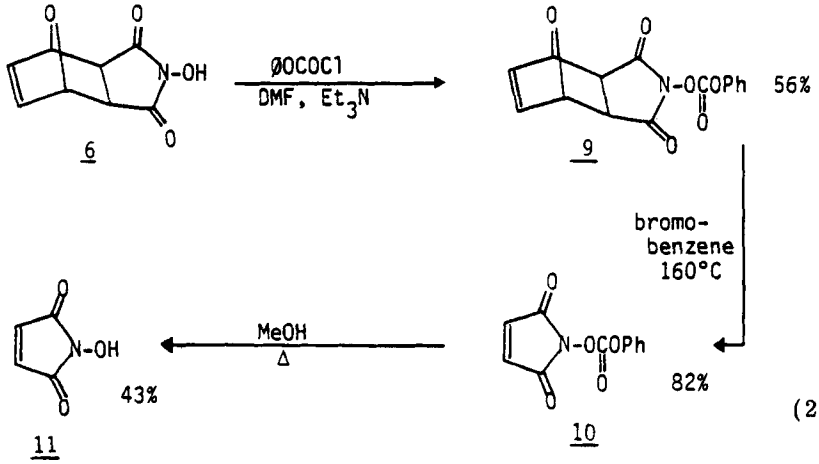
The simplest hydroxamic acid-substituted maleimide is N-hydroxymaleimide (11) (Eq. 2). The acidity of hydroxamic acids is comparable to that of carboxylic acids [41]; thus, hydroxamic acid groups incorporated into a polymer would be expected to be significantly ionized in neutral or basic media. N-Hydroxysuccinimide has a pK_a of $\sim$6.0 [42]; thus, copolymerization of 11 (Eq. 2) with a suitable imidazole-substituted vinyl ether should yield the desired copolymer. However, 11 could not be obtained by the direct reaction of 39 with hydroxylamine followed by dehydration.

The synthesis of N-acetoxymaleimide (8) has been described [8]. This monomer should be more suitable than 11, since it has been observed [33] that polymerization of monomers containing unprotected hydroxamic acid groups are efficient free-radical trapping agents. Therefore, protected maleimide (8) was synthesized via the steps outlined in Eq. (1).

The preparation of N-hydroxymaleimide (11) had been described in two earlier publications [10, 43]. This synthesis also employed the adduct 6 as intermediate for maleimide 10, the methanolysis of which gave 11. The reaction scheme, along with yields, is outlined in Eq. (2).



(1)

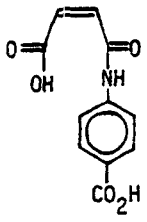


(2)

The carboxylic acid group has been incorporated into synthetic enzymes [44, 45] and also plays a key role in the "charge-relay system" of the natural enzyme chymotrypsin [46]. The synthesis of carboxylic acid containing maleimide monomers was undertaken for two reasons: evaluation of bifunctional synthetic enzymes containing the carboxylic acid residue, and conversion of the carboxylic acid group via its N-hydroxysuccinimide ester [47] to the hydroxamic acid group. Equa-

tion (11) illustrates the latter approach. A subsequent paper from this laboratory will summarize copolymer studies based on the former concept.

The direct dehydration of maleanilic acids 18 and 19 was unsuccessfully carried out using the following reagents: $Ac_2O/NaOAc$ [49], DCC/DMF [40], DCC/CH_2Cl_2 [48], and refluxing xylene [49]. On the other hand, maleanilic acid 12 was cleanly dehydrated to maleimide 13 in 83% yield by using Ac_2O/NaOAc.



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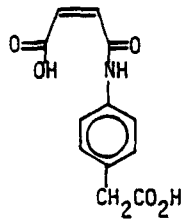

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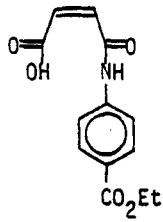

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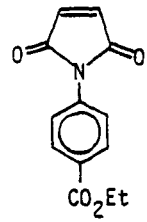
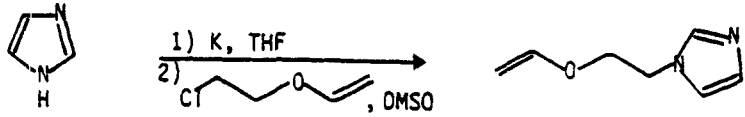


13

Clearly, the presence of the second carboxyl group in 18 and 19 is responsible for the inability to effect dehydration. Other workers [50] have reported an inability to dehydrate amino acid-maleamic acids; however, the synthesis of maleoylamino acids by reaction of maleimide with ethylchloroformate has been reported [13, 51]. Using this procedure, N-carbethoxymaleimide (15) was synthesized in 44% yield.

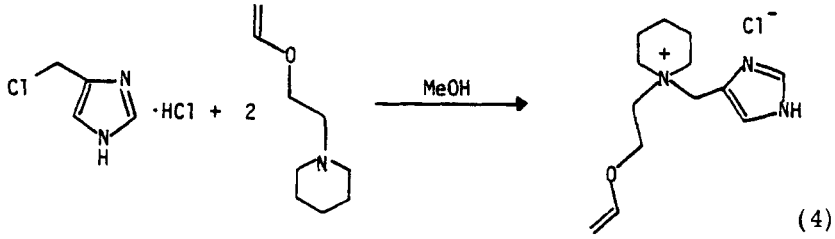
Alkylation of the potassium salt of imidazole with 2-chloroethyl vinyl ether (CEVE) in DMSO afforded the desired N-(β-vinyloxyethyl)-imidazole (22) as a colorless oil in 46% yield (Eq. 3). Vinyl ether 22 was also synthesized via the sodium salt (NaH) of imidazole, although in lower yield (25-30%). Although 22 is a tertiary (N-substituted) imidazole, there is evidence that the position of substitution would not significantly hinder this compound's ability to catalyze deacylation of the acylhydroxamate intermediate in esterolysis reactions [32].



(3)

A 4(5)-substituted imidazole-vinyl ether, β-vinyloxyethyl(imidazol-4-ylmethyl)piperidinium chloride (24), was synthesized (Eq. 4), and its ability to copolymerize with maleimide 13 was studied.

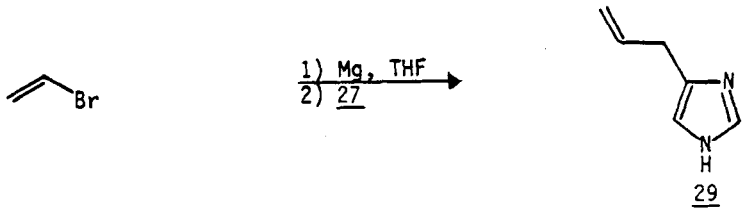
Reaction of N-(β-vinyloxyethyl)piperidine (23) and 4-(chloromethyl)-imidazole hydrochloride (25) gave 24 in yields of 70-80%. Vinyl ether 24 proved to be very difficult to purify, requiring repeated treatment



25 23 24 (4)

with Na_2CO_3 in methanol and precipitation into ethyl ether to remove excess 23 as its free base. Furthermore, 24 was isolated as a gummy hygroscopic solid, and attempts to isolate 24 as its hydrochloride salt resulted in rapid hydrolysis of the vinyl ether group. Vinyl ether 24 was consequently used for copolymerization studies in free base form. Despite prolonged drying in vacuo, a major contaminant in 24 was ethyl ether as determined from ^{1}H-NMR.

Other imidazole-containing monomers which might alternately copolymerize with N-substituted maleimides were synthesized. The reaction of vinylmagnesium bromide with 25 afforded 4-allylimidazole (29) in 45% yield (Eq. 5). This compound had been synthesized and characterized previously from the pyrolysis of N-allylimidazole, giving approximately equal amounts of 2- and 4-allylimidazole. The synthesis reported here thus represents a new and regiospecific method of obtaining 29. In view of the report that allylphenols and N-substituted maleimides copolymerize in alternate fashion [52], copolymerization studies with 29 were carried out.



29 (5)

Copolymerization of Maleimides with N-(β-Vinyloxyethyl)-imidazole (22)

Addition of 22 to a CH_2Cl_2 solution of 8 resulted in the immediate appearance of a blood red color which intensified with time. No color change occurred when CEVE was added to a solution of 8, suggesting that the imidazole group was responsible for the red coloration. After appreciable reaction times, the red solutions were precipitated into hexanes or ether, giving pale red solids in each case. Evaporation of

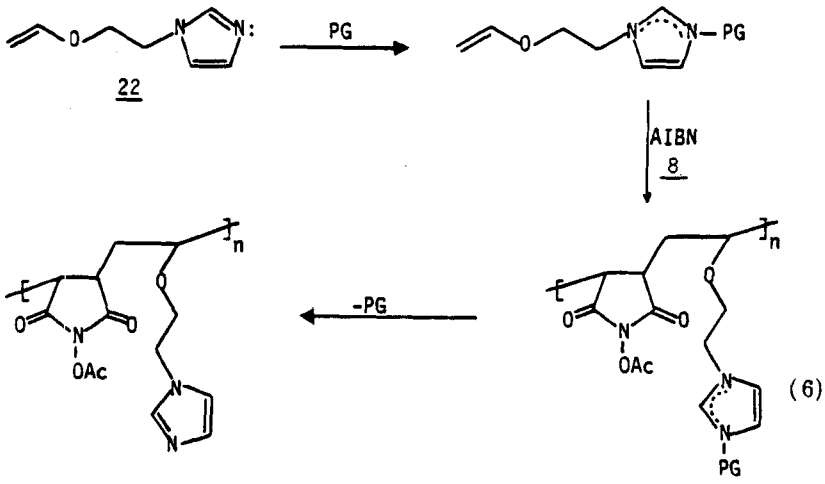
the filtrate in vacuo gave an oil, the ^{1}H-NMR spectrum of which revealed a preponderance of N-substituted imidazole over 8. The red solids appeared to be the same substance as judged from their IR spectra (1820, 1790, 1740, 1225, 1160 cm^{-1}) and appeared to be the homopolymer of 8. The postulate that N-substituted imidazoles were catalyzing homopolymerization of 8 was demonstrated by carrying out the reaction using 0.5 mol% of 22.

Further insight into the structure of 38 was gained by determining the molecular weight by VPO ($\overline{M}_n$ = 488). This information revealed that 38 was the N-acetoxymaleimide, a cyclotrimer of 8.

This reaction has been reported previously using N-alkylmaleimides, and the major product was the maleimide cyclotrimer [53]. A zwitterionic mechanism was invoked to account for the observed product [54]. Furthermore, in addition to the Michael adduct, 1-25% of maleimide cyclotrimer was formed when imidazole was reacted with N-substituted maleimides in stoichiometric amounts [53].

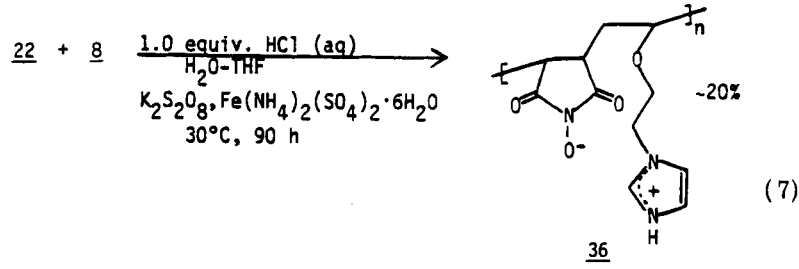
Although these results were discouraging, copolymerization of 8 and 22 using AIBN as initiator was attempted. However, only homopolymers of 8 were obtained. Direct copolymerization of maleimides 10 and 11 with 22 were also unsuccessful.

Recognizing that the cyclotrimerization reaction was responsible for the inability to obtain alternating copolymer, ways were sought to circumvent this side reaction. It was reasoned that reaction of 22 with a Lewis acid or other protecting group (PG) would effectively retard the effect of the imidazole residue to catalyze cyclotrimerization (Eq. 6). The ideal PG would coordinate strongly enough with the imidazole moiety to preclude cyclotrimerization yet be easily removed following copolymerization. The hydrochloride salt of 22 might afford adequate protection; however, when 22 was treated with anhydrous HCl in ether or THF solution, oligomerization of 22 occurred with concomitant cleavage of the vinyl ether.



(6)

Vinyl ether 22 and maleimide 8 were successfully copolymerized when 22 was pretreated with 1.0 equivalent of aqueous HCl. Maleimide 8 was added in acetone or THF solution to give a homogeneous mixture. Polymerization was initiated by redox conditions, $K_2S_2O_8$, and an Fe(II) salt (Eq. 7).



(7)

The copolymer was purified by precipitating the reaction mixture into acetone (to remove maleimide homopolymer), redissolving the oily precipitate in aqueous HCl, and dialyzing this solution against deionized water. Copolymer 36 precipitated from solution during dialysis. The IR spectrum of 36 indicated the presence of both monomers, carbonyl stretching frequencies at 1780 and 1705 cm^{-1} (indicating that the N-acetoxy group had been hydrolyzed), and C–O–C ether stretch at 1105 cm^{-1}. The carbonyl absorbances for 36 are identical to those reported for N-hydroxymaleimide-styrene copolymer [55]. Evidence for alternation can be found in the ^{13}C-NMR spectrum of 36 (Fig. 4). Three peaks appear in the carbonyl region in area ratios of ~2:1:1. This is consistent with an alternating copolymer with a homogeneous sequence distribution whose stereochemistry at the succinimide unit is exclusively cis or trans and whose carbonyls can "see" relative stereochemistry two bonds distant. Carbon 10 can be assigned as the upfield doublet reflecting random relative stereochemistry between C-2 and C-3. Carbon 11 then appears as a singlet as a result of its inability to "see" relative stereochemistry three bonds distant. The carbonyl region of 36 is analogous to the carbonyl region of N-phenylmaleimide-2-chloroethyl vinyl ether alternating copolymer as reported earlier from this laboratory [7a].

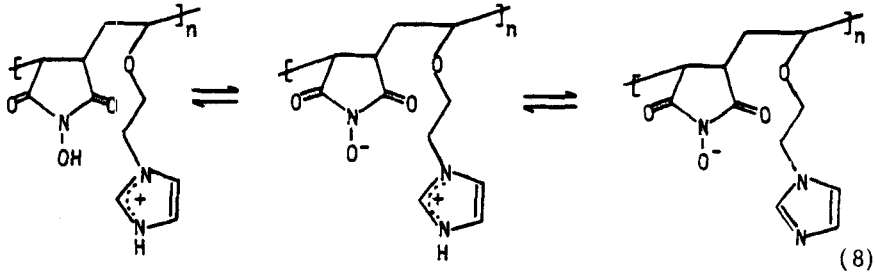
The assignment given carbonyl carbons 10 and 11 can also be rationalized by empirical chemical shift parameters reported earlier [56]. Substituents other than protons which are situated α or β to a carbon of interest cause a downfield shift (~9 ppm) relative to a similar compound without substitution. Substituents other than protons which are situated γ to a carbon of interest cause an upfield shift (~2 ppm). Examination of the copolymer structure reveals that carbons 10 and 11 have equal numbers of α and β substituents. Carbonyl 10, however, has an additional γ substituent by virtue of branching at C-2. Therefore, C-10 should appear further upfield than C-11.

The resonances between 120 and 140 ppm were assigned to the carbons of the imidazole ring. The signal appearing farthest downfield was assigned to C-9, whereas no distinction could be made between C-7 and C-8.

The resonances between 70 and 80 ppm are typical for carbon atoms α to an oxygen atom. Differentiation between C-2 and C-5 could be made after examination of an off-resonance spectrum (Fig. 5). The signal farthest upfield appeared as a triplet (C-5) and the downfield signal as a doublet (C-2). Carbon-6 also appeared as a triplet in the off-resonance ^{13}C spectrum.

Further evidence for alternation can be obtained from the chemical shifts of the succinimide backbone carbons, C-3 and C-4. In the ^{13}C-NMR spectrum of N-hydroxymaleimide homopolymer (33), the backbone carbons appear as a broad singlet centered at ~42 ppm. In copolymer 36, the succinimide backbone carbons appear as two signals at ~39 and ~48 ppm. Carbon-4 was assigned to the upfield signal on the basis of having one additional γ substituent vs C-3. In addition, C-3 has one additional β substituent than does C-4. The broad hump appearing between C-3 and C-4 at ~42 ppm is probably attributable to the methine carbons of homomaleimide sequences. The methylene backbone carbon atom (C-2) was assigned to the signal appearing farthest upfield.

Copolymer 36 possesses very interesting solubility characteristics. As isolated, 36 is virtually insoluble in all common organic solvents, although it will dissolve in DMSO at elevated temperatures. Interestingly, 36 is water soluble at pH < 3.6 and pH > 7.1 but insoluble between these limits. This solution behavior led to the belief that 36 is a polyampholyte (Eq. 8). Solubility is attained when the copolymer is protonated or deprotonated, giving rise to net positive or negative charges. Under these conditions, 36 might be expected to behave as a polyelectrolyte. At the isoelectric point, however, the attraction between oppositely charged side chains should result in tight coiling, hence a lack of solubility. In this case an increase in the ionic strength of the medium should lead to expansion of the chains and impart water solubility. Such behavior has been noted for poly(vinyl imidazolium sulfobetaine) (40).



(8)

36

TABLE 1. Solubility of 36 in Salt Solutions[a]

Cation	Anion							
	Cl^-		Br^-		I^-		BF_4^-	
Li^+	sat. 5.0 M	+ P	sat.	+				
Na^+	sat.	P	sat.	-	sat. 3.0 M	+ P		
K^+			sat.	P	sat.	-	sat.	-

[a] sat. = saturated solution
+ = soluble
- = insoluble
P = partially soluble

The solubility of 36 in various salt solutions is shown in Table 1. It can be seen that certain salts are more effective than others with respect to dissolving power. Interestingly, both saturated LiCl and saturated LiBr completely dissolve 36, whereas 5.0 M LiCl only partially dissolves 36. It is also not clear why 36 is soluble in saturated NaI, partially soluble in saturated NaCl, and apparently insoluble in saturated NaBr. It had been found earlier [57] that large cations, e.g., K^+, and large anions, e.g., ClO_4^-, were more effective solubilizing ions than were smaller ions. Minimum salt concentrations were of the order of 0.03-0.52 M for 40, whereas saturated salt solutions were necessary to impart solubility to 36.

Determination of the molecular weight of 36 proved difficult, and the results were ambiguous. VPO analysis of DMSO at 100°C gave $\overline{M}_n \cong 500$ g/mol. It is believed that this number represents a minimum value as 36 was observed to decompose under similar conditions in the NMR probe. As VPO is a colligative technique, the presence of decomposition fragments would result in a lower $\overline{M}_n$ than expected. A maximum molecular weight value was calculated from endgroup analysis (elemental analysis for S). Presumably the initiating species in the redox system employed is the sulfate radical anion [58]. Assuming the incorporation of one sulfate group per polymer chain, from calculation of an empirical formula a molecular weight of $\cong$ 6000 g/mol was derived.

The intrinsic viscosity $[\eta]$ of 36 was determined in 0.1 N HCl at 30.0°C to be 0.112 dL/g. Although this value cannot be directly related to molecular weight, it is an indication of the hydrodynamic volume of 36. Of course, the size of the polymer chains should vary

with the pH of the medium, and one would expect a change in $[\eta]$ dependent on the degree of ionization of imidazole groups.

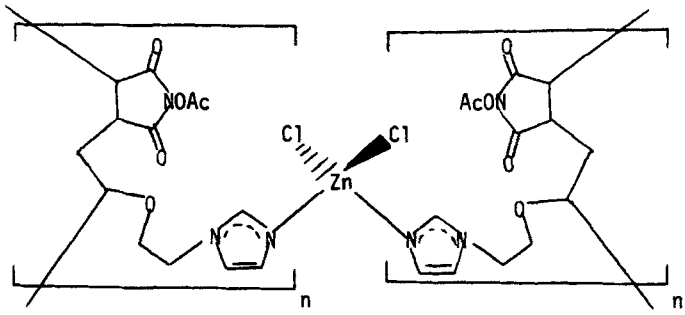
Gel permeation chromatography of 36 revealed two components in a 3:1 area ratio, the larger component having the shorter retention volume. The presence of two components precluded accurate molecular weight determination, as the individual viscosities could not be determined independently. It was suspected that the minor component was attributable to N-hydroxymaleimide homopolymer (33), which could not be separated from 36.

In another set of experiments, 22 was mixed with $ZnCl_2$ in THF before addition of AIBN and 8. In this case, a white, THF insoluble powder was obtained after only a few hours at 60°C. The IR spectrum of this material indicated that both 8 and 22 had been incorporated as evidenced by carbonyl stretching frequencies at 1818, 1785, and 1730 cm^{-1} and C–O–C ether stretch at 1110 and 1095 cm^{-1}. The incorporation of $ZnCl_2$ into this material was inferred by elemental analysis for Cl (9.22%). Elemental analysis was reasonably consistent with a structure containing two equivalents of 22 and two equivalents of 8 per equivalent of $ZnCl_2$.

Reaction of 22 with four equivalents of $ZnCl_2$ in ethanol gave, after dilution with ether, an 89% yield of 27; mp 78.5–80°C (Eq. 9). The stoichiometry of this complex was determined from elemental analysis and is consistent with N-alkylimidazole-$ZnCl_2$ complexes studied by Welleman et al. [25]. On the basis of the structure of 27 and the elemental analysis data for the $ZnCl_2$ copolymer, the latter's repeating unit was assigned structure 37.

(9)

27



37

This crosslinked structure accounts for the insolubility of 37 in common organic solvents. However, 37 dissolves in warm DMSO (~60°C), presumably giving a DMSO-$ZnCl_2$ [59] complex and free copolymer. The $[\eta]$ of a DMSO solution of 37 at 30.0°C was equal to 0.043 dL/g. Copolymer 37 is also soluble in water at pH > 13 (NaOH) and pH < 3.5 (HCl).

The proton-decoupled ^{13}C-NMR spectrum of 37 is shown in Fig. 6. Similarities to the ^{13}C-NMR spectrum of 36 (Fig. 4) are apparent, and the assignment of carbon atoms is the same as in 36. Two additional peaks (one in the carbonyl region and one at ~22 ppm) can probably be assigned to acetic acid (from hydrolysis of the N-acetoxy group). The resonance at ~42 ppm most likely indicates the presence of homomaleimide sequences.

Copolymerization of Maleimide (13) With β-Vinyloxyethyl(imidazol-4ylmethyl)piperidinium Chloride (24)

After it was established that copolymer 36 was not an efficient catalyst, new catalysts were sought. It was proposed that a better catalyst could be obtained by changing the nature of the hydroxamic acid group and by varying the position of substitution on the imidazole ring. The strategy involved copolymerizing a 4(5)-imidazole-substituted vinyl ether with an ester-maleimide and converting the ester group to a hydroxamic acid group following the copolymerization step.

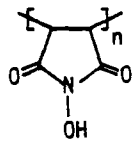
To this end, maleimide 13 and vinyl ether 24 were synthesized and their copolymerization attempted. Because of the extreme rapidity with which 24 hydrolyzed under acidic conditions, no copolymer was formed under the redox conditions by which 8 and 22 were successfully copolymerized. The hydrophobic nature of 13 necessitated the use of a water-miscible organic solvent to obtain a homogeneous reaction mixture. Addition of an organic solvent to an acidic-aqueous solution of 24 resulted in an immediate exothermic reaction probably indicative of hydrolysis of the vinyl ether group. Therefore, it was believed that complete hydrolysis took place before any copolymerization could occur. When 13 and 24 were allowed to react in organic solvents with AIBN as initiator (no acid present), only homopolymers of 13 were obtained.

Copolymerization of Maleimide (8) With 4-Allylimidazole (29)

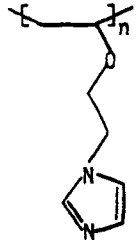
Copolymerization of 29 and 8 was attempted with and without acid protection of 29. In one case a copolymer was obtained (as evidenced by the presence of imidazole and carbonyl resonances in the ^{13}C-NMR spectrum) in low yield. It could not be determined whether the co-

polymer was random or alternating in structure. The IR spectrum, however, indicated that the N-acetoxy group had been hydrolyzed to the N-hydroxy group (1780, 1710 cm^{-1}).

In order to understand the catalytic activity exhibited by copolymer 36 more fully, the homopolymers of 11 and 22 were needed for comparison purposes.

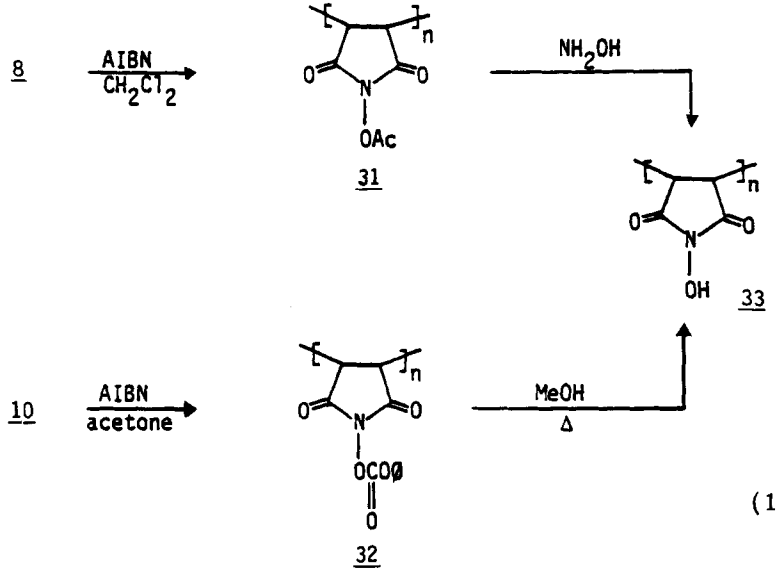


33



35

Poly(N-hydroxymaleimide) (33) was synthesized via two routes (Eq. 10). Polymerization of 8 with AIBN in CH_2Cl_2 afforded homopolymer (31) in 80% conversion [$\overline{M}_n$ (VPO) = 3850 g/mol]. The copolymer structure was confirmed by its spectral properties as well as by comparison with the spectral properties of model compound 21. Treatment of 31 with hydroxylamine gave 33. Homopolymer 33 exhibited carbonyl stretching frequencies of 1785 and 1770 cm^{-1} and broad resonances in the ^{13}C-NMR spectrum centered at 42.0 and 172.7 ppm.

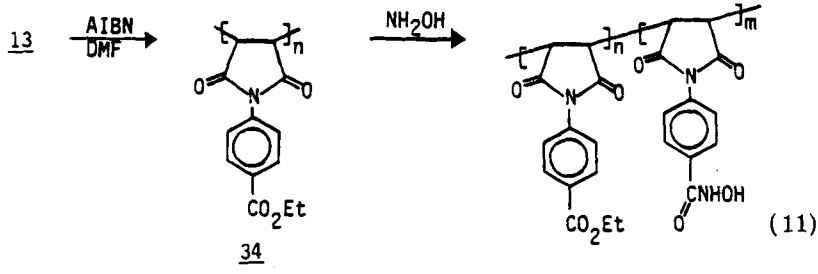


(10)

Homopolymer 33 was more conveniently prepared from the methanolysis of 32 in 88% yield. Polymerization of 10 with AIBN in acetone afforded 32 in 87% conversion. The proton decoupled ^{13}C-NMR spectrum of 33 is shown in Fig. 2. The proton decoupled ^{13}C-NMR spectrum of precursor 31 is shown in Fig. 1.

Homopolymerization of 24 was not as straightforward. Although the polymerization of vinyl ethers with cationic initiators is well documented [60-62], 35 could not be obtained in high conversion or in moderate molecular weight. The low yields obtained under cationic conditions might be due to the imidazole's ability to coordinate with the initiator, thereby rendering the latter ineffective.

N-(4-Carbethoxyphenyl)maleimide (13) was homopolymerized with AIBN in DMF to give 34 in 61% conversion. Surprisingly, insoluble polymers resulted when the polymerization was carried out with AIBN in CH_2Cl_2 or acetone. Reaction of 34 with hydroxylamine resulted in only partial conversion of ester to hydroxamic acid groups as judged by elemental analysis for nitrogen (Eq. 11).



34 (11)

Kinetic Studies With Imidazole, 33, and 36

Pseudo-first-order kinetics were measured using imidazole, 33, and 36 as catalysts as previously described. The results obtained with these catalysts are shown in Table 2. Esterolysis in the presence of homopolymer 33 was only marginally faster than esterolysis in the absence of a catalyst. This was interpreted to mean that 33 is a poor acylating agent compared to the phenyl hydroxamate ion studied earlier [33]. Catalysis by 33 is slightly enhanced at higher pH, which might indicate that catalytic activity increases with the degree of deprotonation of 33. This explanation is speculative, however, without knowledge of the pK_a of 33. In any case, the rate enhancement in the presence of 33 is small and may not be statistically important.

The catalytic activity of copolymer 36 was found to be significantly higher than 33 yet much lower than monomeric imidazole. Due to the lack of catalytic activity shown by 33, it is concluded that neutral imidazole residues are responsible for the rate enhancement exhibited by

TABLE 2. Esterolysis[a] of PNPA with Imidazole, 33, and 36

Catalyst	pH[b]	[catalyst], N	k_{obs}, min^{-1}	k_{cat}, $L \cdot mol^{-1} min^{-1}$
Imidazole	7.68	7.92×10^{-4}[c]	34.9×10^{-3}	44
33	7.68	7.77×10^{-4}[d]	0.30×10^{-3}	0.39
36	7.68	7.89×10^{-4}[e]	4.41×10^{-3}	5.6
Blank	7.68	-	4.92×10^{-3}[f]	-
Imidazole	7.86	7.75×10^{-4}[c]	35.4×10^{-3}	45.7
33	7.86	7.73×10^{-4}[d]	0.68×10^{-3}	0.88
36	7.86	7.78×10^{-4}[e]	5.03×10^{-3}	6.5
Blank	7.86	-	6.55×10^{-3}[f]	-

[a]Method A conditions: [Tris buffer] = 0.1 M, μ = 0.1 (KCl), 1.5: 98.5 $CH_3CN:H_2O$ (v/v), initial substrate concentration = 4.20×10^{-5} N, T = 40.0°C.

[b]pH at 25°C.

[c]Approximate concentration of imidazole groups.

[d]Approximate concentration of N-hydroxysuccinimide groups.

[e]Approximate concentration of imidazole and N-hydroxysuccinimide groups.

[f]k_{meas} of uncatalyzed reaction.

copolymer 36. Once again, a rate enhancement was observed at higher pH, which might be related to the degree of ionization of protonated imidazole groups. Alternatively, an increase in pH might expand the polymer chains, allowing easier access of the substrate to the reaction site.

Additional kinetic studies were not carried out on this system due to the poor catalysis exhibited by 36, the knowledge that 36 was impure, the failure to obtain homopolymer 35, and the apparent lack of cooperative behavior between hydroxamic acid and imidazole groups in 36.

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