Polynucleotide Analogues. 2.[1] Synthesis, Characterization, and Physicochemical Properties of Alternating Copolymers of Dihydropyran Containing Nucleic Acid Bases and Maleic Anhydride

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ABSTRACT: Two monomers, 2-(thymin-1-ylmethyl)-3,4-dihydro-2*H*-pyran and 2-(adenin-9-ylmethyl)-3,4-dihydro-2*H*-pyran, have been synthesized by reacting 2-[(tosyloxy)methyl]-3,4-dihydro-2*H*-pyran with thymine and adenine, respectively. After acetylation of amino groups, these monomers were copolymerized with maleic anhydride in the presence of radical initiators. These copolymerizations have produced poly[{2-(thymin-1-ylmethyl)-3,4-dihydro-2*H*-pyran}-*alt*-{maleic anhydride}] and poly[{2-(adenin-9-ylmethyl)-3,4-dihydro-2*H*-pyran}-*alt*-{maleic anhydride}]. Hydrolysis of these copolymers resulted in poly[{2-(thymin-1-ylmethyl)tetrahydropyran-5,6-diyl}{1,2-dicarboxyethylene}] (**7**) and poly[{2-(adenin-9-ylmethyl)tetrahydropyran-5,6-diyl}{1,2-dicarboxyethylene}] (**9**). These products, **7** and **9**, are the analogues of poly(thymidylic acid) and poly(adenylic acid), respectively. The alternating sequences of the copolymers have been confirmed by titrations of anhydride and carboxyl groups in the copolymers. Number-average molecular weights of the polymers are 1700 for **7** and 2000 for **9**. These values correspond to six repeating units per polymer chain. UV absorption spectra of polymers **7** and **9** in water show hypochromicities of 28.6 (271 nm) and 43.7% (260 nm) in comparison to their respective monomers. These hydrochromicities are due to the base stacking. Sodium salts of these copolymers have shown polyelectrolyte behavior in water due to the carboxylate pendant groups on the polymer chain. Electrophoresis of copolymer **9** on a cellulose sheet immersed in buffer solution (pH 7.4) shows only one band, indicating that the polymer consists exclusively of hexamers, whereas copolymers **7** shows two bands.

Introduction

Considerable research has been carried out on synthetic methods of polynucleotide analogues (PNA). Their physicochemical and biological properties have been studied to gain a better understanding of the structure of natural polynucleotides and to utilize their biological activities in polymeric drugs. There are several recently developed PNA synthetic methods, which include a method of attaching the nucleic acid bases (NAB) to vinyl monomers and polymerization of these monomers resulting in PNAs,[2-8] a synthetic method of reacting NABs with the functional groups of the selected polymers,[9-20] and polycondensation of ω-hydroxy carboxylic acid or α-amino acids which contain the NABs.[21-23]

Attempts to synthesize PNAs with structures and physical properties closely resembling their natural counterparts have not been successful. Most of the synthesized PNAs exhibit neither good solubilities in water, due to the lack of hydrophilic groups, nor optical activities, due to the absence of sugar moieties on the polymer chain. The alternating sequences between nucleosides and phosphate, observed in the natural polynucleotides, have rarely been realized in synthetic PNAs.

Recently we have reported the synthesis of analogous poly(thymidylic acids) in which the methylene phosphate groups are substituted by dicarboxyethylene or dihydroxyethylene groups.[1] These PNAs are soluble in water and optically active. They contain alternating sequences along the polymer chain. It is of interest to synthesize PNAs that contain pyranose sugar moieties instead of furanose rings.

In this paper we report the synthesis, characterization, and physicochemical properties of poly[{2-(thymin-1-ylmethyl)tetrahydropyran-5,6-diyl}{1,2-dicarboxyethylene}] (**7**) and poly[{2-(adenin-1-ylmethyl)tetrahydropyran-5,6-diyl}{1,2-dicarboxyethylene}] (**9**). These alternating copolymers are analogous of poly(thymidylic acid) or poly(adenylic acid) in which the furanosyl sugar and phosphate groups are substituted by fructopyranosyl sugar moieties and 1,2-dicarboxyethylene groups, respectively.

Experimental Section

Adenine (Aldrich, 99%) and *N,N*-dimethylformamide dineopentyl acetal (Janssen, 99%) were used as received. Maleic anhydride and AIBN were crystallized from benzene and methanol, respectively. Dimethylformamide was dried over anhydrous $MgSO_4$ and distilled.

2-Formyl-3,4-dihydro-2*H*-pyran was synthesized by Diels-Alder[24] reaction of acrolein. 2-[(Tosyloxy)methyl]-3,4-dihydro-2H-pyran (**1**) was obtained by the reaction of tosyl chloride with 2-(hydroxymethyl)-3,4-dihydro-2*H*-pyran,[25] which was synthesized by reduction of 2-formyl-3,4-dihydro-2*H*-pyran with the aid of $NaBH_4$.[25]

2-(Thymin-1-ylmethyl)-3,4-dihydro-2*H*-pyran (2). Thymine (5 g, 3.97×10^{-2} mol) and sodium hydride (1.0 g, 95%, 3.97×10^{-2} mol) were charged in 500 mL of DMF and stirred for 5 h at room temperature. To this suspension was added 2-[(tosyloxy)methyl]-3,4-dihydro-2*H*-pyran (12.75 g, 4.76×10^{-2} mol), and the mixture was stirred for 3 h at room temperature and then for 12 h at 90 °C. After solvent evaporation, the residue was dissolved in 200 mL of chloroform and the salt was removed by filtration. After evaporation of the solvent, the residue was crystallized from ethanol-ethyl ether mixture (v/v, 1:9) to yield 3.2 g of **2**: mp 139-140 °C; yield 36%.

^{1}H NMR (500 MHz, $CDCl_3$): for 2-methyl dihydropyran δ 3.65 (q, 1 H, J = 14, 8 Hz, NCH_{2a}), 4.06 (m, 1 H, NCH_{2b}), 4.07-4.11 (m, 1 H, H_2), 1.53-1.65 (m, 1 H, H_{3a}), 1.91-2.01 (m, 1 H, H_{3b}), 1.91-2.15 (m, 2 H, H_4), 4.72 (m, 1 H, H_5), 6.34 (d, 1 H, J = 6 Hz, H_6); for thymine δ 1.91 (d, 3 H, J = 1.5 Hz, CH_3), 10.32 (s, 1 H, NH), 7.12 (d, 1 H, J = 1.5 Hz, H_6).

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^{13}C NMR (125.8 MHz, $CDCl_3$): for thymine δ 151.6 (C_2), 164.8 (C_4), 109.9 (C_5), 141.8 (C_6), 12.3 (CH_3); for 2-methyldihydropyran δ 73.1 (C_2), 25.0 (C_3), 19.1 (C_4), 101.1 (C_5), 142.9 (C_6), 51.7 (NCH_2). IR (KBr): 3470, 3030 (Br), 1700 (Br), 1465, 1430, 1370, 1230 (Br), 1305, 845 cm^{-1}.

Elemental anal. calcd for $C_{11}H_{14}N_2O_3$: C, 59.46; H, 6.31; N, 12.61. Found: C, 59.13; H, 6.32; N, 12.09.

2-(Adenin-9-ylmethyl)-3,4-dihydro-2*H*-pyran (3). Adenine (10 g, 7.4×10^{-2} mol) and sodium hydride (1.97 g, 95%, 7.4×10^{-2} mol) were charged in 700 mL of DMF and stirred for 5 h at room temperature without moisture. To this suspension was added 2-[(tosyloxy)methyl]-3,4-dihydro-2*H*-pyran (23.8 g, 8.8×10^{-2} mol), and the mixture was stirred for 2 h at room temperature and for 12 h at 100 °C. After evaporation of the solvent, the residue was stirred in 200 mL of H_2O at room temperature, filtered, and washed with diethyl ether twice. The residue was crystallized from methanol to yield 11.4 g of **3**: mp 205–206 °C; yield 67%.

^1H NMR (300 MHz, DMSO-d_6): for 2-methyldihydropyran δ 1.5–2.1 (m, 4 H, H_3, H_4), 4.2–4.3 (m, 1 H, H_2), 4.35 (d, J = 14 Hz, $=NCH_2$), 4.66–4.72 (m, 1 H, H_5), 6.3 (d, 1 H, J = 6 Hz, H_6); for adenine 8.06 (s, 1 H, H_8), 8.15 (s, 1 H, H_2), 7.25 (s, 2 H, NH_2). IR (KBr): 3360, 3200 (Br), 2980, 1690 (Br), 1630, 1490, 1330, 1310, 1230, 1040 cm^{-1}.

Elemental anal. calcd for $C_{11}H_{13}N_5O$: C, 57.14; H, 5.63; N, 30.30. Found: C, 57.27; H, 5.67; N, 30.41.

2-(*N*-Acetyladenin-9-ylmethyl)-3,4-dihydro-2*H*-pyran (4). Acetic anhydride (2.2 g, 2.15×10^{-2} mol) and 1 g of **3** (4.33×10^{-3} mol) were dissolved in 25 mL of chloroform and refluxed for 5 h. After evaporation of the solvent, the residue was crystallized from THF–ethyl ether (v/v, 2:8) to produce 0.97 g of **4**: mp 85 °C; yield 82%.

^1H NMR (500 MHz, $CDCl_3$): for 2-methyldihydropyran δ 4.50 (q, 1 H, J = 14.5, 3.0 Hz, NCH_{2a}), 4.37 (q, 1 H, J = 14.5, 3.7 Hz, NCH_{2b}), 4.19 (m, 1 H, H_2), 1.55 (m, 1 H, H_{3a}), 1.99 (m, 2 H, H_{3b}, H_{4a}), 2.11 (m, 1 H, H_{4b}), 4.70–4.73 (m, 1 H, H_5), 6.32 (d, 1 H, J = 6 Hz, H_6); for *N*-acetyladenine 8.30 (s, 1 H, H_8), 8.72 (s, 1 H, H_2), 10.20 (s, 1 H, =NH), 2.64 (s, 3 H, CH_3CO).

^{13}C NMR (125.8 MHz, $CDCl_3$): for *N*-acetyladenine δ 172.0 (CO), 152.2 (C_2), 149.4 (C_4), 121.6 (C_5), 151.8 (C_6), 144.6 (C_8), 25.8 (CH_3); for 2-methyldihydropyran 47.4 ($=NCH_2$), 72.8 (C_2), 25.0 (C_3), 19.1 (C_4), 101.0 (C_5), 142.9 (C_6). IR (KBr): 3200 (Br) 1735, 1610, 1460, 1370, 1345, 1305, 1220, 1035, 1015 cm^{-1}.

Elemental anal. calcd for $C_{13}H_{15}N_5O_2$: C, 57.14; H, 5.49; N, 25.64. Found: C, 57.99; H, 5.56; N, 25.94.

2-[*N*-[(Dimethylamino)methenyl]adenin-9-ylmethyl]-3,4-dihydro-2*H*-pyran (5). *N*,*N*-Dimethylformamide dineopentyl acetal (8.9 g, 3.72×10^{-2} mol) and **3** (4.3 g, 1.86×10^{-2} mol) were dissolved in 70 mL of DMF and stirred for 10 h at room temperature under exclusion of moisture. After evaporation of the solvent, the residue was crystallized from ethanol–ethyl ether (v/v, 1:9) to give 4.5 g of **5**: mp 119–120 °C; yield 84.5%.

^1H NMR (400 MHz, $CDCl_3$): for 2-methyldihydropyran δ 4.33–4.46 (m, 2 H, $=NCH_2$), 4.17 (br s, 1 H, H_2), 4.68 (br s, 1 H, H_5), 6.31 (br s, 1 H, H_6), 1.4–2.1 (m, 4 H, H_3, H_4); for *N*-[(dimethylamino)methylene]adenine 3.19, 3.23 (d, 6 H, $N(CH_3)_2$), 8.0 (s, 1 H, H_8), 8.57 (s, 1 H, H_2), 9.0 (s, 1 H, N=CH). IR (KBr): 3500 (Br) 3100 (Br), 2940 (Br), 1650, 1575 (Br), 1410 (Br), 1320, 1220 (Br), 1090, 1030, 970 cm^{-1}.

Elemental anal. calcd for $C_{14}H_{18}N_6O$: C, 58.74; H, 6.29; N, 29.37. Found: C, 58.75; H, 6.29; N, 29.58.

Copolymerization. Calculated amounts of monomers and initiators were charged with or without the solvent into polymerization tubes (Table I). These tubes were immersed into a Dewar flask containing dry ice and acetone. Following conventional freeze–thaw treatments under N_2, the tubes were sealed and placed in an oil bath at a fixed temperature for a definite time interval. Then DMF was added, the copolymers were precipitated in ethyl acetate several times, and the product was dried in vacuo over P_2O_5 at 50 °C in a drying pistol.

Hydrolysis of the Polymers. (a) Poly[{2-(thymin-1-ylmethyl)tetrahydropyran-5,6-diyl}{1,2-dicarboxyethylene}] (7). Copolymer **6** (0.5 g) was refluxed in H_2O for 3 h. The solution was filtered, and the filtrate was evaporated to give 0.45 g of polymer **7**: yield 85%.

Table I
Copolymerization of 2 or 4 with Maleic Anhydride (MA) in Solution and in Bulk

monomers, mol/L		initiator (mol/L)	solvent	temp, °C	time, h	yield, %	polymer		
							M_n[a]	$[\eta]$[b]	MA,[c] %
2	MA								
4.5	4.5	AIBN (0.02)	DMF	70	48	10.3			
4.5	4.5	AIBN (0.09)	DMF	70	48	28.0			
1.8	3.6	AIBN (0.02)	DMF	80	48	32.0			
4.5	9.0	AIBN (0.045)	DMF	80	72	42.0	1700	0.053	48
4	MA								
0.73	0.73	AIBN (0.01)	DMF	70	48	28.4			
1.83	3.66	AIBN (0.03)	DMF	70	72	42.0			
3.66	3.66	BPO (0.05)	DMF	25[d]	72	10.7			
(mole	ratio)	(mol %)[e]							
1	1	BPO (1.0)	none	60	24	40.5			
1	2	AIBN (0.36)	none	70	24	48.0			
1	2	AIBN (0.36)	none	80	48	61.0	2000	0.12	48

[a] Number-average molecular weight measured by vapor-pressure osmometer. [b] Intrinsic viscosity in DMF. [c] Mole percent of maleic anhydride in the copolymer measured by titration. [d] Equivalent mole of *N*,*N*-dimethylaniline to initiator was added as a promotor. [e] Mole percent of initiator to the total amount of monomers.

(b) Poly[{2-(adenin-9-ylmethyl)tetrahydropyran-5,6-diyl}{1,2-dicarboxyethylene}] (9). Copolymer **8** (0.4 g) was stirred in 1 M aqueous NaOH at 90 °C for 3 h. The solution was acidified to pH 3 with 0.1 M aqueous HCl, and the solvent was evaporated to dryness under reduced pressure. The residue was dissolved in DMF and filtered. The product was obtained by precipitation of the filtrate in benzene to give 0.33 g of **9**: yield 88%.

Titration. Analysis of anhydride groups in copolymers **6** and **8** was performed by dissolving them in DMF and titrating the solution with sodium methoxide (0.1 N) in DMF–methanol by a potentiometric method.[27] Analysis of carboxyl groups in copolymers **7** and **9** was performed by back titration with 0.1 N HCl after the anhydride groups in polymer **5** were hydrolyzed with 0.1 N aqueous NaOH.

Hypochromicity (*h* %). UV spectra were recorded using a Hitachi 200-20 spectrophotometer. Solution concentrations were approximately 10^{-4} M of base residues. The percent hypochromicity (h, %) was calculated from

$$h\,(\%) = [|\epsilon_m - \epsilon_p|/\epsilon_m] \times 100 \quad (1)$$

where ϵ_m and ϵ_p denote the molar extinction coefficients of monomer and of base residues of polymer, respectively.

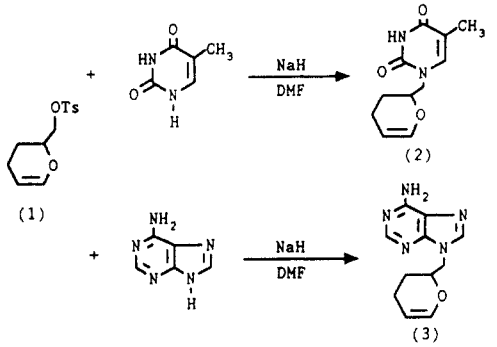
Measurements. ^1H and ^{13}C NMR spectra were recorded on a Varian T-60 or a Bruker AMX-500 spectrometer. IR spectra were obtained with a Perkin-Elmer Model 283B spectrophotometer, and UV spectra were recorded with a Hitachi Model 200-20 spectrometer. Measurements of molecular weight were carried out in DMF at 90 °C using a vapor-pressure osmometer (Knauer Co.). Elemental analysis was performed at KRICT.

Electrophoresis. Thin-layer electrophoresis was carried out on a cellulose sheet (25 cm × 2 cm, Toyo Roshi Kaisha, sheet 51A), which was immersed in a buffer solution of pH 7.4 at a constant 250 V for 3 h. The separated bands were identified in an I_2 chamber.

Results and Discussion

Synthesis of Monomers. 2-[(Tosyloxy)methyl]-3,4-dihydro-2*H*-pyran (**1**) was prepared by the reaction of tosyl chloride with 2-(hydroxymethyl)-3,4-dihydro-2*H*-pyran,[25] which was obtained by reduction of 2-formyl-3,4-dihydro-2*H*-pyran, Diels–Alder product of acrolein,[24] with the aid of $NaBH_4$.

Syntheses of 2-(thymin-1-ylmethyl)-3,4-dihydro-2*H*-pyran (**2**) and 2-(adenin-9-ylmethyl)-3,4-dihydro-2*H*-pyran (**3**) were achieved by treatment of the sodium salts of the corresponding nucleic acid bases with **1** in dimethylformamide.

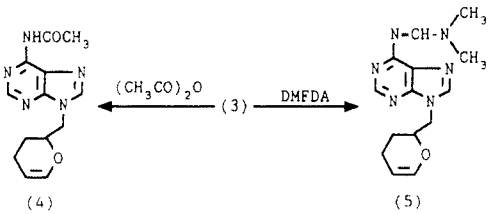


Each of the nucleic acid bases has several sites susceptible to alkylation. The alkylation of thymine may give rise to 1- or 3-substitution or their mixture. Similarly, the alkylation of adenine may produce 7- or 9-substitution or their mixture. Careful examination of the reaction products revealed for each reaction the presence of only one major derivative which had a sharp melting point and exhibited only one spot on TLC.

The ^{1}H NMR spectrum of **2** shows no evidence of the presence of a peak at 10.6 ppm due to NH^1 of the original thymine. The alkylation of thymine under similar conditions occurred exclusively at the N^1-position.[28] These facts led to the definite assignment to the N^1-position in **2**. The N^3-substitution by such a bulky group as 2,3-dihydro-2*H*-pyran-2-methyl must be sterically unfavorable because of the steric hindrance of the two adjacent carbonyl groups.

The alkylation of adenine in aprotic media with base catalyst gives N^9-substitution.[29,30] The H_8 proton of adeninyl groups in **3** gives one sharp signal in the ^{1}H NMR spectrum, indicating the N^7-substitution is excluded. Additionally, it is well-known that 9-methyladenine in ethanol absorbs at 262 nm, whereas 7-methyladenine possesses a peak at 272 nm in the UV spectrum.[31] Compound **3** showed a peak at 260 nm, which confirms N^9-substitution.

To facilitate its copolymerization with maleic anhydride, the amino groups of **3** were blocked by acetyl or dimethylaminomethylene groups, as shown in the scheme



DMFDA ; Dimethylformamide dineopentyl acetal

The ^{13}C–^{1}H COSY spectrum of monomer **4** is shown in Figure 1. Each of the two protons of methylene groups in the dihydropyran ring gives different chemical shifts: H_{3a} (δ 1.55, m), H_{3b} (δ 1.99, m), H_{4a} (δ 1.99, m), and H_{4b} (δ 2.11, m).

The rotations of C–CH_2–N bonds are sterically hindered at room temperature, so that the two protons of the methylene group also give different chemical shifts, δ 4.50 and 4.37. The detailed assignments of ^{1}H and ^{13}C are also given under Experimental Section.

Copolymerization. The radical copolymerization of 3,4-dihydro-2*H*-pyran derivatives with maleic anhydride (MA) is known to give alternating copolymers by forming charge-transfer complexes of the monomer pairs during the copolymerization.[24,25,32] Since the electron-donating character of the vinyl ether groups of **2** and **4** is negligibly influenced by the substitutions on the C_2-position of the

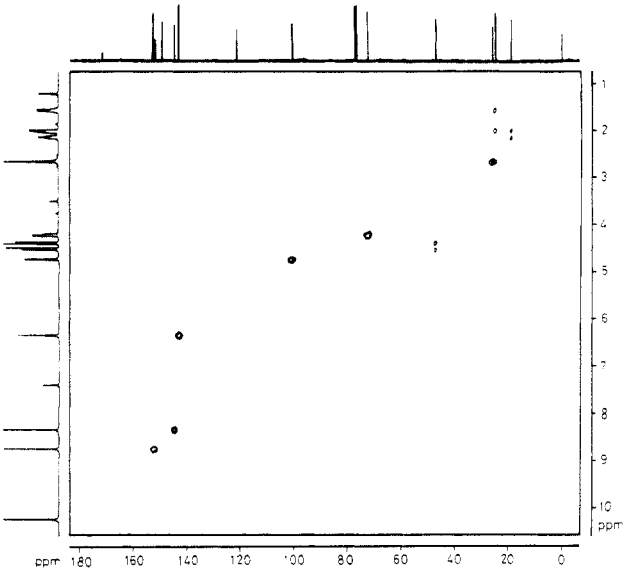
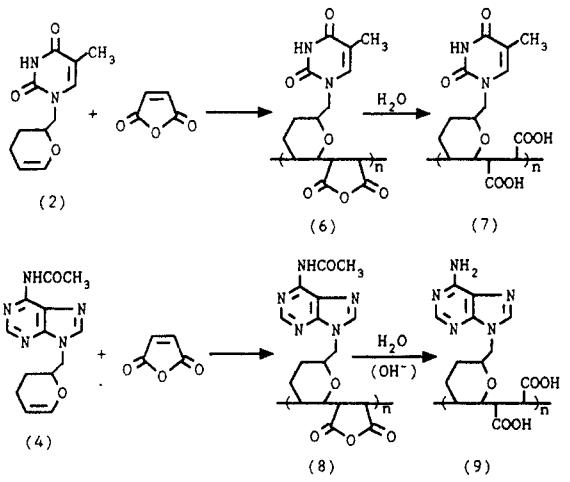


Figure 1. ^{13}C–^{1}H COSY spectrum of 2-(*N*-acetyladenin-9-ylmethyl)-3,4-dihydro-2*H*-pyran.

dihydropyran rings, copolymers, poly[{2-(thymin-1-ylmethyl)-3,4-dihydro-2*H*-pyran}-*alt*-{maleic anhydride}] (**6**) and poly[{2-(*N*-acetyladenin-9-ylmethyl)-3,4-dihydro-2*H*-pyran}-*alt*-{maleic anhydride}] (**8**), are expected to have

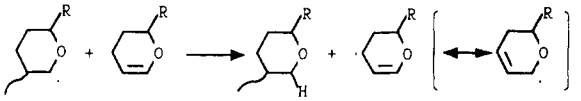


alternating sequences. Neither the dihydropyran derivatives, **2** and **4**, nor maleic anhydride is homopolymerized under the same condition; hence, the resulting polymer also must have the alternating sequences.

The copolymerizations of **2**, **4**, or **5** with maleic anhydride have been carried out either in DMF or in bulk in the presence of radical initiators (AIBN or BPO), and the copolymerization data are given in Table I. Monomer **5** did not yield a copolymer under the same conditions but formed unidentified adducts of the monomers. This conclusion is deduced from the ^{1}H NMR spectrum of the product that shows absorption peaks corresponding to the double bonds from the dihydropyran rings.

The yields of polymers (Table I) are increased with the increasing monomer concentration at the start of copolymerization, temperature, and polymerization time. Since the copolymerization of **2** with maleic anhydride in solution gave poor yield as well as low molecular weight, the copolymerization of **2** was attempted in bulk to obtain high yields.

The low molecular weight (Table I) of copolymers **6** and **8** seems to be attributable to the transfer reaction shown. The allyl and/or allyloxy radicals, formed by hydrogen



transfer from the monomer to the active center, are very stable due to formation of resonance hybrids. These stable free radicals can start the copolymerization anew. This point is confirmed by the presence of the 6.34 ppm absorption peak of dihydropyran in the ^{1}H NMR spectra of copolymers **6** and **8**, corresponding to the presence of a trace amount of vinyl protons. In an attempt to suppress the transfer reactions, the copolymerization has been carried out at a low temperature (25 °C) with *N,N*-dimethylaniline as a promoter (Table I). However, no detectable polymer was found in this case.

Acetylation of pyrimidine-2,4(1*H*,3*H*)-dione derivatives by acyl chloride occurs at the N^3- as well as the N^1-position in the presence of tertiary amines as catalysts.[26] Although it was suspected that maleoyl groups could be substituted on the N^3 of thyminyl groups of polymer **6** during copolymerization, this substitution is excluded on the basis of the following points: the proton signal of HN^3 has been found intact at δ 11.0, whereas those of the double bond at 6.2 pm in the maleoyl groups have not been found in the ^{1}H NMR spectrum of polymer **6** (Figure 2). The carboxyl group concentration of polymer **7** has been found to be equivalent to 48 mol % of maleic anhydride incorporated into polymer **6**. The substitution of maleoyl groups on N^3 of thymine would cause cross-linking and would decrease the solubility of the polymer. However, polymers **6** and **7** are completely soluble in the polar solvents.

Characterization of the Copolymers. ^{1}H NMR spectra of copolymers **6** and **8** are shown in Figure 2. The disappearance of proton signals of maleic anhydride at δ 7.2 and of vinyl protons at δ 4.7–4.8 and 6.1–6.35 in dihydropyran rings confirms the formation of copolymers. Copolymers **6** and **8** are soluble only in highly polar solvents, DMF and DMSO, and insoluble in solvents such as acetone, THF, and ethyl acetate. Number-average molecular weights of copolymers **6** and **8**, measured by vapor-pressure osmometer, are found to be 1700 and 2000, respectively. These values correspond to six repeating units per polymer chain. To confirm the alternating sequences of copolymers **6** and **8**, the anydride groups incorporated into the copolymers were titrated with standardized sodium methoxide solution[27] and the mole percent of the anhydride groups was found to be 48% for both copolymers.

Hydrolysis of the Copolymers. Hydrolysis of copolymer **6** was simply accomplished by stirring in water to give poly[{2-(thymin-1-ylmethyl)tetrahydropyran-5,6-diyl}{1,2-dicarboxyethylene}] (**7**). Deblocking of acetyl and hydrolysis of anhydride groups of copolymer **8** were performed with the aid of hydroxide catalyst to obtain poly[{2-(adenin-9-ylmethyl)tetrahydropyran-5,6-diyl}{1,2-dicarboxyethylene}] (**9**). The completion of the reaction was confirmed by the disappearance of acetyl proton signal at δ 2.3 in the ^{1}H NMR spectra. Hydrolysis of anhydride groups was monitored by IR spectra in which the cyclic anhydride peak at 1825 cm^{-1} disappeared while carboxyl groups at 1730 cm^{-1} emerged. The ^{1}H NMR spectrum of polymer **9** is shown in Figure 2. The amino groups of polymer **9** give a signal at δ 7.4. The polymers **7** and **9** are soluble in water and insoluble in organic solvents. The carboxyl groups have been titrated and found to be equivalent to 48 mol % of maleic anhydride in these polymers. The results of these titrations for anhydride and carboxyl groups confirm, within experimental error, the equimolarity of comonomers incorporated into the polymer as required for an alternating copolymer.

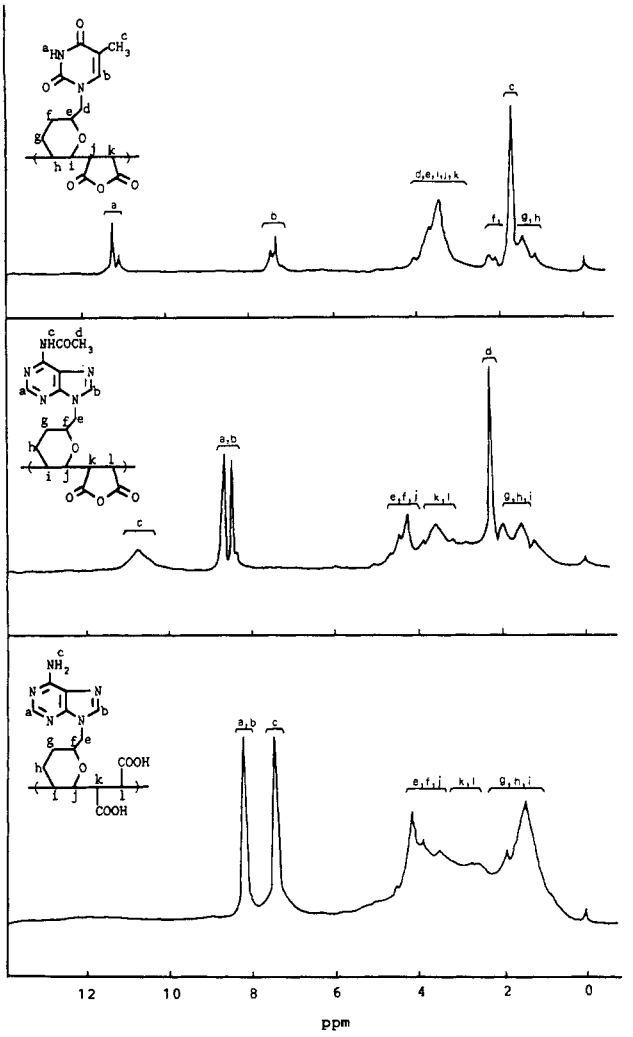


Figure 2. ^{1}H NMR spectra of copolymers **6**, **8**, and **9** in DMSO-d_6.

Base Stacking. According to the Tinoco[33] and Rhodes[34] theory, induced dipole–dipole interactions in the chromophores of nucleic acid bases can result in either hypochroism or hyperchroism, depending on the relative geometry of the stacked chromophores. Hypochroism is common to systems that are stacked with the chromophores one upon another like a deck of cards, while systems in which the chromophores are in an end-to-end aggregate are generally predicted to be hyperchromic.[35] UV spectra of monomers and copolymer are shown in Figure 3 and their data are summarized in Table II. Copolymers **7** and **9** show hypochromicities of 28.6 (λ = 271 nm) and 43.7% (λ = 260 nm), respectively, when compared with monomers **2** and **3**.

The carboxylate groups of copolymer **7** at pH 7 and of copolymer **9** at pH 12 in aqueous solution protrude outward, interacting with the hydrophilic environment. Consequently, the thymine or adenine bases are stacked one upon another, resulting in high hypochromicities. This hypochromicity is related to the degree of stacking. Both of these copolymers have the same polymer backbone with different pendant groups, thymine and adenine. Since the percentage hypochromicity (43.7%) of copolymer **9** is higher than that (28.6%) of copolymer **7**, adenine has a higher stacking ability than thymine. This can be attributed to the increased intermolecular interactions of the aromatic purine rings compared to the interactions of

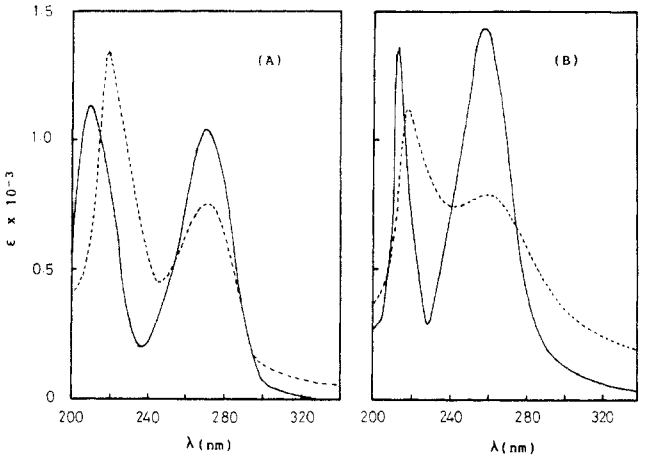


Figure 3. UV spectra of monomer and copolymers: (A) monomer **2** in MeOH–H_2O (solid line) and copolymer **7** in H_2O at pH 7 (dotted line); (B) monomer **3** in MeOH–H_2O (solid line) and copolymer **9** in H_2O at pH 12 (dotted line).

Table II
UV Data of Monomers and Copolymers at a Concentration of 1.0×10^{-4} mol/L (or Residue mol/L)

compd	solvent	pH	λ, nm	ε, L mol^{-1} cm^{-1}	hypochromicity, %
3	MeOH–H_2O		270	10.5	
7	H_2O	7	271	7.5	28.6
5	MeOH–H_2O		260	14.4	
9	H_2O	12	260	8.1	43.7

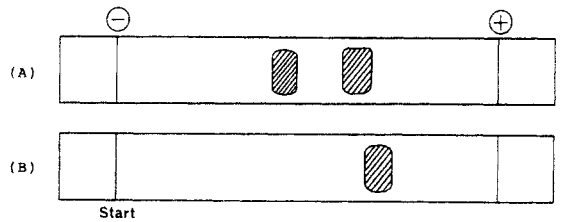


Figure 4. Thin-layer electrophoresis diagrams of sodium salts of copolymers **7** and **9** at constant 250 V for 3 h in a buffer solution (pH 7.4): (A) polymer **7**; (B) polymer **9**.

pyrimidine rings in an aqueous environment.

Polyelectrolyte Behavior. Sodium salts of polymers **7** and **9** are polyelectrolytes, and the polyanions migrate to the anode in an electrical field. The mobility (V) of charged polymers is proportional to the net charge (Z) and is inversely proportional to two-thirds power of molecular weight (M)[36]

$$V = KZM^{-2/3} \quad (2)$$

where K is a constant. Thin-layer electrophoresis diagrams of sodium salts of **7** and **9**, developed on electrophoresis celluose sheets immersed in a buffer solution (pH 7.4), are shown in Figure 4. Polymer **7** shows only one band while polymer **9** shows two bands.

The increase of one repeating unit in polymers **7** and **9** is accompanied by the increase of two net charges. According to the relationship of eq 2, the effect of net charge on mobility is higher by one-third power than that of molecular weight. The polymer band at the farther distance from the starting line, therefore, has a higher degree of polymerization than the next band. However, the polymer initiated and terminated by maleic anhydride will have more carboxylate groups than the polymer that is initiated and terminated by dihydropyran derivatives. The former will migrate further than the latter when polymers have very similar molecular weights.

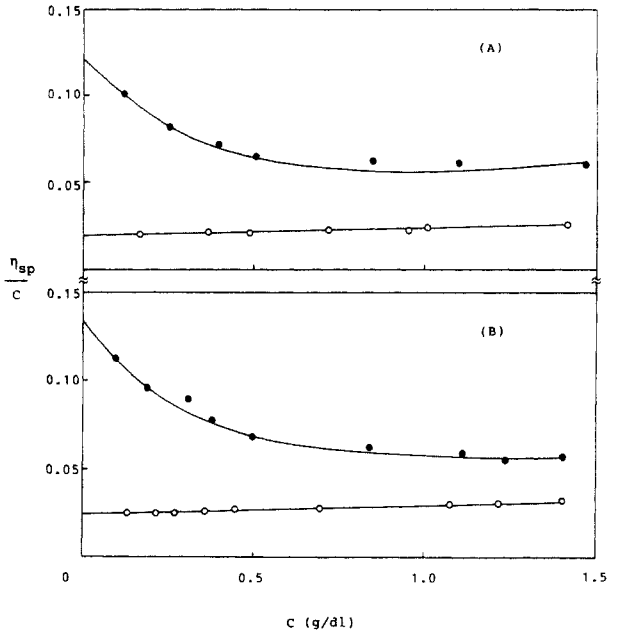


Figure 5. Reduced viscosities of sodium salts of concentrations in H_2O and aqueous NaCl solution (5%): (A) polymer **7**; (B) polymer **9**.

Polymer **7** shows only one band. It consists exclusively of hexamers, as indicated by the result of vapor-pressure osmometry. Polymer **9** shows two bands, which are mixtures of either hepta- and hexamer or hexa- and pentamer. The sharp molecular weight distribution is attributable to the transfer reactions mentioned above, which cut off high molecular weight, and to the precipitation processes after copolymerization and hydrolysis of the polymers, which result in dissolving the low molecular weight portion.

Reduced viscosities of sodium salts of copolymers **7** and **9** as a function of concentration in H_2O are shown in Figure 5. They exhibit typical polyelectrolyte behavior of the reduced viscosity decreasing at the beginning and thereafter increasing steeply with continuous dilution. By addition of neutral salts they retain normal behavior.[37] The intrinsic viscosities of copolymers **7** and **9** in aqueous NaCl solution (5%) are rather low compared with those of **6** and **8** as illustrated in Table I. This point is attributable to the chain flexibility caused by cleavage of anhydride groups in polymers **6** and **8**.

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Registry No. **2**, 140853-22-1; **3**, 140853-23-2; **4**, 140853-24-3; **5**, 140853-25-4; **6** (alternating copolymer), 140853-26-5; **8** (alternating copolymer), 140872-67-9; adenine, 73-24-5; thymine, 65-71-4; 2-[(tosyloxy)methyl]-3,4-dihydro-2*H*-pyran, 7007-32-1.