Synthesis and Physicochemical Properties of Polynucleotide Analogues Containing Pyrimidine Bases

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Polynucleotide analogues containing pyrimidine (uracil and thymine) bases, poly[(1'-β-uracil-1-yl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose)-*alt*-(maleic acid)] (**12**) and poly[(1'-β-thymin-1-yl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose)-*alt*-(maleic acid)] (**15**), were synthesized by the alternating copolymerization of relevant nucleoside derivatives and maleic anhydride, and the subsequent hydrolysis. The polymers had quite similar structures to the natural polymers and were soluble in water. They showed high hypochromicities up to 49% and excimer fluorescence due to the base stacking, and polyelectrolyte behavior. Since the polymers had compact structures, depyrimidinations, the release of pyrimidine bases from the polymer backbone, occurred in aqueous solutions with higher rates compared with those of the natural polymers.

Introduction

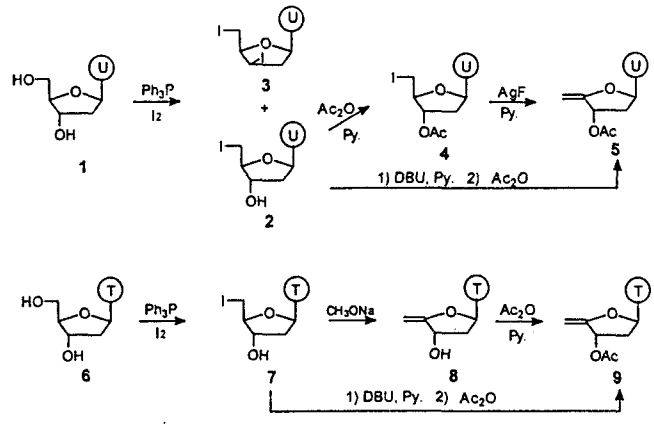
Considerable attention has been devoted to the synthesis of polynucleotide analogues (PNA) as model compounds for natural ones over the last decade in an effort to elucidate the functions of nucleic acids in biological systems. Recently the analogues themselves were found to show biological activities, arousing hopes for their utilization in chemotherapy as polymeric drugs.[1,2] We reported several polynucleotide analogues,[3-8] which had alternating sequences between nucleoside derivatives and dicarboxyalkylenes. Their structures were quite similar to those of natural polynucleotides, *i.e.*, the alternating copolymers of nucleoside and methylene phosphate groups. They also showed similar physicochemical properties to those of natural polymers such as good solubility in water, large hypochromicities due to base-stacking, double-helix formation with the natural polymers by base-pairing between the complementary bases.

In line with the effort to obtain new PNAs resembling natural polymers and study their physicochemical properties, we have synthesized two new monomers (**5** and **9**) and copolymerized them with maleic anhydride to obtain the alternating copolymers (**10** and **13**) as shown in Scheme 1 and 2, respectively. These products were hydrolyzed to give polymers **12** and **15**, which had the alternating structures of nucleoside derivatives and 1,2-dicarboxytrimethylene, replacing the methylene phosphate groups in the natural polynucleotides. Polymers **12** and **15** showed good solubility in water due to the carboxylate groups on the polymer chains, and high hypochromicity and excimer formation due to the base-stacking. These polymers underwent depyrimidination with the release of the pyrimidine bases from the nucleic acid by hydrolysis of the N-glycosidic bond. This process plays very important roles in the biological systems. Here we report on the syntheses of the polymers, their physicochemical properties, and their depyrimidination.

Results and Discussion

Monomer Synthesis. Iodination of 2'-deoxyuridine (**1**) with triphenyl phosphine and iodine gave 2',5'-dideoxy-5'-iodouridine (**2**) and 2',3',5'-trideoxy-3',5'-diiodouridine (**3**). Compound **3** was identified to be in *threo* form by ^{1}H NMR spectroscopy. The proton signals of $H_{1'}$ were a quartet and the difference in the chemical shifts of $H_{2'a}$ and $H_{2'b}$ proton was 0.4 ppm, which is similar to that for *threo* 3',5'-dideoxy-3',5'-diiodothymidine.[9] Acetylation of **2** with acetic anhydride resulted in 3'-O-acetyl-2',5'-dideoxy-5'-iodouridine (**4**). 1'-β-Uracil-1-yl-3'-O-acetyl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose (**5**) was obtained by elimination of HI from **4** with the aid of silver fluoride. Compound **5** was also obtained by elimination of HI from **2** with a base, 1,8-diazabicyclo-[5,4,0]-undec-7-ene (DBU), and subsequent acetylation *in situ*. The latter method gave a higher yield of **5**.

Thymidine was iodinated to give 5'-deoxy-5'-iodothymidine (**7**), which was transformed to 1'-β-thymin-1-yl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose (**8**) with the aid of



Scheme 1

sodium methoxide. 1'-β-Thymin-1-yl-3'-O-acetyl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose (**9**) was obtained by acetylation of **8**. Compound **9** could be obtained with a higher yield by elimination of **7** with DBU and subsequent acetylation *in situ*. Since the chiral atoms $C_{1'}$ and $C_{3'}$ in 2'-deoxyuridine (**1**) and thymidine (**6**) remained intact during the reactions, their chiralities were retained to render monomers **5** and **9** optically active.

Copolymerization. Radical copolymerization of cyclic vinyl ethers with maleic anhydride (MA) is known to give alternating copolymers through the formation of charge transfer complexes of the monomer pairs during copolymerization.[10,11] As the electron-donating character of the vinyl ether group of the monomers **5** and **9** is little influenced by either 1'-uracilyl, 1-thyminyl, or 3'-*O*-acetyl groups, the copolymers of monomers **5** and **9** with MA are expected to have alternating sequences. In previous studies[4,6-8] it was also confirmed that alternating copolymers were obtained by the copolymerization of MA with the cyclic vinyl ethers having different substituents on C_1-position on the furanose rings.

Copolymerizations of monomers **5** or **9** with MA were carried out in bulk at different temperatures in the presence of a radical initiator (AIBN). The coploymerization data are given in the Table 1. Neither monomers **5** and **9** nor MA were homopolymerized under the same conditions. As the copolymerization of cyclic vinyl ethers with MA in bulk gave a higher yield than in solution and the monomers were soluble in the molten MA (mp; 52.8 °C), the copolymerizations were carried out above 80 °C in an excess of MA. Polymer yields were found to be higher with higher temperatures (Table 1). Polymers **10** and **13** were pale brown powders, which were soluble in DMSO and DMF and insoluble in less polar solvents such as ethyl acetate, chloroform, ether, and methylene chloride. In the ^{1}H-NMR spectra of polymers **10** and **13** were found the relevant signals for the protons of monomers **5** and **9** except those for the vinyl protons at δ 4.46 and 4.85 ppm for **5** and at 4.46 and 4.85 ppm for **9**. The cyclic anhydride peaks at 1825 cm^{-1} were found in the IR-spectra of polymers **10** and **13**. The succinic anhydride contents in polymers **10** and **13** were titrated[12] to be 50.2 and 51.3 mol %, respectively, which was consistent with the alternating structures of the polymers.

Table 1. Copolymerization of **5** or **9** with maleic anhydride (MA) in bulk with AIBN[a] for 12 h at different temperatures

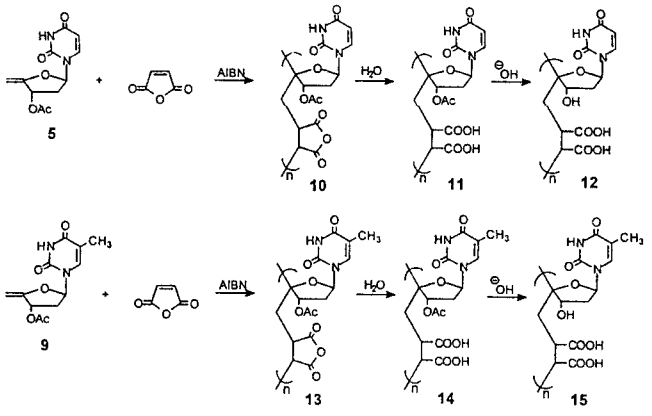
Polymer	Monomer (mole ratio)	Polym. Temp. (°C)	Yield (%)	Polymer Mn[b]	[η][c] (dl/g)	MA (%)[d]
10	**5** : MA (1:2)	80	52			
		95	63	10300	0.12	50.2
13	**9** : MA (1:2)	80	30			
		90	33	8400	0.07	51.3

[a]Initiator concentration: 1 mole % to the total amounts of monomers. [b]Number-average molecular weight of polymers **12** and **15** measured by GPC in 0.1 N aqueous $NaNO_3$ with poly(ethylene oxide) standards. [c]Intrinsic viscosity of polymers **12** and **15** in 0.1 N aqueous $NaNO_3$. [d]Mole % of maleic anhydride incorporated into polymers **10** and **13**.

Hydrolysis of the Polymers. Hydrolysis of polymers **10** and **13** in water at room temperature gave polymers **11** and **14**, whereas the process in 0.1N aqueous NaOH at 50 °C resulted in **12** and **15**, respectively. In the IR spectra of polymers **10** and **13**, the cyclic anhydride peaks at 1825 cm^{-1} disappeared while peaks for the carboxylate groups at 1730 cm^{-1} emerged. The deblocking of acetyl groups was confirmed by the disappearance of the acetyl proton signals at δ = 2.3 ppm in the ^{1}H-nmr spectra of the polymers. The sodium salts of polymers **11, 12, 14,** and **15** were soluble in H_2O and insoluble in common organic solvents. The pyrimidine bases were eliminated during hydrolysis at 50 °C, 78% uracilyl and 81% thyminyl groups remaining on polymer **12** and **15**, respectively.

^{1}H-nmr spectra for polymers **11** and **14** are shown in Figure 1. The proton signals of H_5, H_6, $H_{1'}$, and $H_{3'}$ for polymer **11** and of H_6, $H_{1'}$ and $H_{3'}$ for polymer **14** appeared between 5 and 8.5 ppm whereas the other backbone proton signals for the both polymers appeared between 1 and 4 ppm. The ratios of the integration values between them were found to be about 4 to 9 for polymer **11** and 3 to 12 for polymer **14**, which coincided with the alternating structures of both polymers.

Base-Stacking. According to the Tinoco[13] and Rhodes,[14] induced dipole-dipole interactions in the chromophores of



Scheme 2

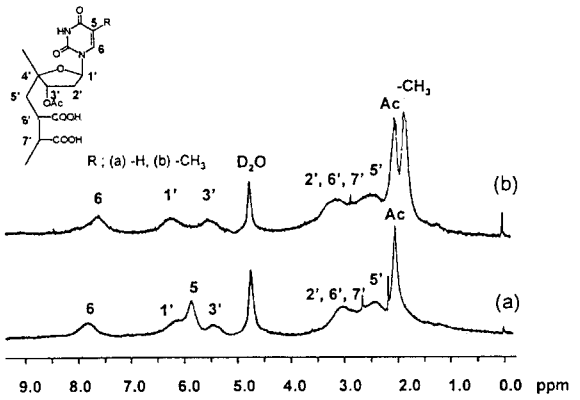


Figure 1. ^{1}H-nmr spectra in D_2O: (a) polymer **11** and (b) polymer **14**.

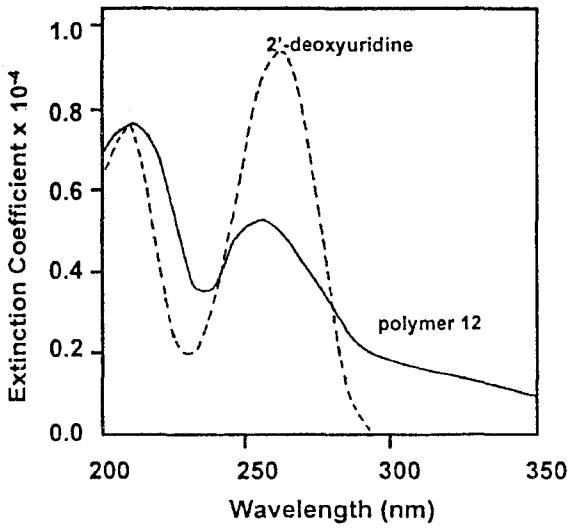


Figure 2. UV-spectra of 2'-deoxyuridine and polymer **15**. [2'-deoxyuridine] = [uracil residue in polymer**12**] = 1×10^{-4} mol/L.

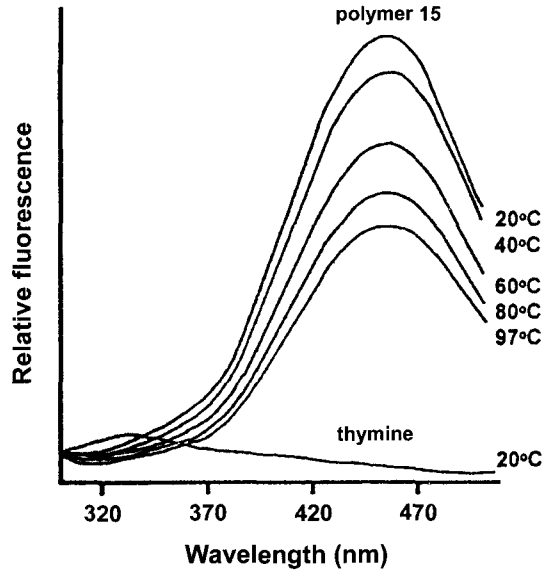


Figure 3. Fluorescence emission spectra of thymine and polyme **15** in H_2O at different temperatures after excitation at 250 nm.

nucleic acids 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 orientation are generally predicted to be hyperchromic.

UV spectra of thymidine (**6**) and polymer **15** measured in H_2O at pH 7 are shown in Figure 2. 2'-Deoxyuridine (**1**) and polymer **12** showed similar UV-curves under the same conditions. Polymers **12** and **15** showed hypochromicities of 43 (λ=262 nm) and 49% (λ=267 nm), respectively, when compared with 2-deoxyuridine (**1**) and thymidine (**6**), respectively. The carboxylate groups of the polymers in aqueous solutions protrude outward, interacting with the hydrophilic environment. Consequently, the uracil or thymine bases are stacked one upon another, resulting in high hypochromicities.

Hypochromicity is related to the extent of base stacking. The hypochromicities of polymers **12** and **15** were found to be very high when compared with those of natural polynucleotides, which showed generally less than 20% hypochromicities. Simple molecular modeling showed that the fully extended distance between adjacent ribose rings (from C4' to C4") of polymers **12** and **15** was 4.96 Å,[15] which was much shorter than that (6.19 Å) of the corresponding nucleic acid. The higher intramolecular interactions between bases due to the shorter distance would be responsible for higher hypochromicities

Excimer Fluorescence. Bichromophoric molecules, where the two aromatic chromophores are separated by a three-atom linkage, can give rise to intramolecular excimer formation.[16-18] Excimer formation in these systems requires rotational motion about the bonds in the linkage to allow the two chromophores to reach, within the lifetime of the excited state, a conformation suitable for complex formation in which the two aromatic rings overlap in a sandwich-like arrangement. When these geometrical requirements are satisfied for the pendent chromophores on the polymer chain, the polymer shows an excimer fluorescence. This has been observed in numerous poly(vinyl aromatics)s[17,19-22] and in polymers containing pendent chromophoric groups.[23] Therefore excimer fluorescence can provide further evidence for base-stacking within the polynucleotide analogues.

Fluorescence emission spectra of thymidine (**6**) and of polymer **15**, after excitation at 250 nm, were measured at the same concentrations of base groups (1×10^{-5} residue mol/L) in H_2O at different temperatures (Figure 3). 2-Deoxyuridine (**1**) and polymer **12** showed similar fluorescence emission curves under the same conditions. 2-Deoxyuridine and thymidine showed no fluorescence emissions, whereas polymers **12** and **15** exhibited a typical excimer fluorescence, giving strong broad bands with maximum intensities around 450 nm and devoid of vibrational structures. The intensities of excimer fluorescence were diminished with increasing temperature (Figure 3), probably due to the hindrance of excimer formation by thermal motions of the chromophores at elevated temperatures.

Polyelectrolyte Behaviour. Polymers **12** and **15** are polyelectrolytes. The hydrodynamic volumes of these polyelectrolytes increase greatly in dilute aqueous solutions. The polyelectrolyte expansion effect was reported to be strongly dependent on the ionic strength of the solution and successfully suppressed in 0.1 N $NaNO_3$ aqueous solution, which was confirmed by the universal calibration method.[24,25] The reduced viscosity of polymer **12** was measured in water, which steeply increased with continuous dilution and retained normal behavior by the addition of $NaNO_3$ (Figure 4). Similar results were observed for polymer **15** under the same conditions. The molecular weights for polymers **12** and **15** were measured by gel permiation chromatography at room temperature. The number-average molecular weights of polymers **12** and **15** were found to be 10,300 and 8,400 in aqueous 0.1 N $NaNO_3$, respectively.

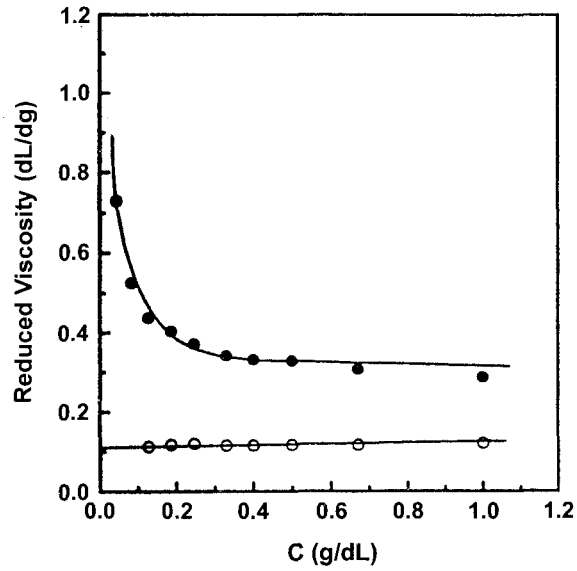


Figure 4. Reduced viscosities of the sodium salt of polymer **12** in H_2O (upper curve) and aqueous 0.1 N $NaNO_3$ solution (lower curve) at room temperature.

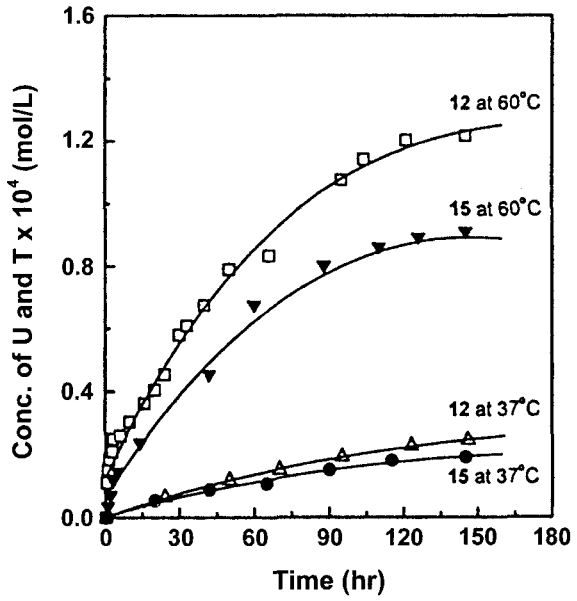
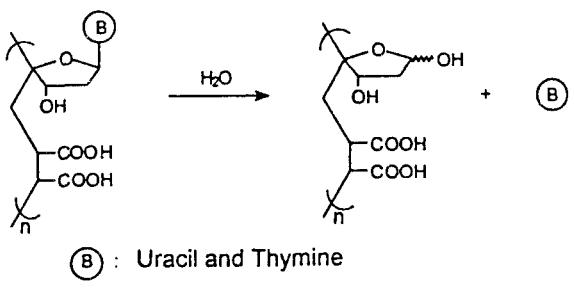


Figure 5. Released uracil (U) and thymine (T) from polymers **12** and **15** as a function of time at pH 7.4 and different temperatures.

Depyrimidination. The spontaneous depyrimidination of nucleic acids, the release of pyrimidine (cytosine, thymine, and uracil) bases from nucleic acids by hydrolysis of the N-glycosidic bond, gives rise to alterations of the cell genome.[26,27] The depyrimidination of denatured DNA *in vitro*[28] at 80 °C and pH 7.4 was found to be 1.2×10^{-9} sec^{-1}. The apyrimidinic sites resulting from depyrimidination are quite stable;[29] and cells have evolved mechanisms to repair these lesions.[30] Unrepaired apyrimidinic sites have been shown to have biological consequences, including lethality[31,32] and base-substitution errors.[31]

Recently we have reported on the depurination of a synthetic poly(inosinic acid) analogue.[33] Its rate constant was 10^5-fold higher than that for the depurination of DNA occurring in biological systems, which was attributable to the high potential energy of the polymer caused by the crowded environment around its bases. As the structures of polymers **12** and **15** are quite similar to that of the polymer reported earlier, it was of interest to investigate whether depyrimidination occurs in the former polymers in a similar trend under the same conditions.

When polymers **12** and **15** were dissolved in buffer solution (pH 7.4) above 30 °C, the N-glycosidic bonds of the polymer were spontaneously hydrolyzed to liberate uracil or thymine from the polymer backbone (Scheme 3), which was measured by HPLC. The rates of depyrimidination at 37 and 60 °C are shown in Figure 5. The reaction at the beginning was very fast at the higher temperature and slower at the lower temperature. When 50% of uracil in polymer **12** and



Scheme 3

Table 2. Depyrimidination rate constants at different temperatures

Polymer	Temperature (°C)	Rate constants$\times10^8$ (sec^{-1})
12	37	8.6
	60	58.6
15	37	5.0
	60	36.4
DNA	80	0.12

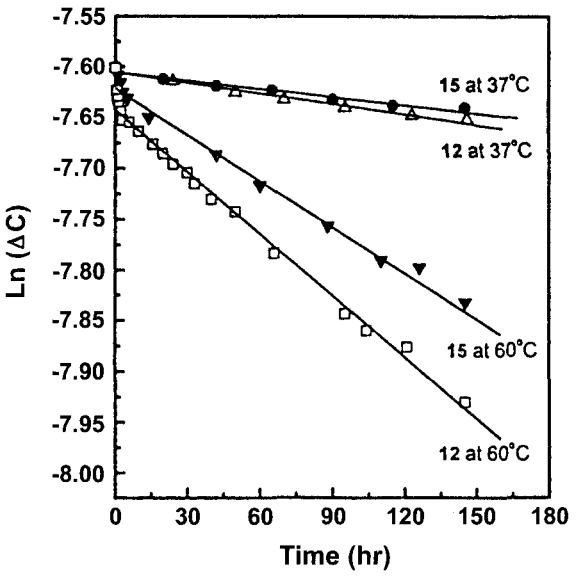


Figure 6. Logarithmic concentrations of released uracil and thymine *vs.* time at pH 7.4 and different temperatures.

47% of thymine in polymer **15** were eliminated, no more depyrimidination occurred.

To determine the rate constants, the logarithmic concentrations of uracil or thymine released from the polymer chain were plotted against time (Figure 6), which obeyed first-order kinetics up to 150 hours of reaction time. The initial rate constants at different temperatures are given in Table 2. The rate constant at 60 °C is 10^2-fold higher than that for the heat-induced depyrimidination of DNA at 80 °C,[15] which was attributable to the higher potential energy within the polymer caused by the crowded environment around the bases as observed earlier for poly(inosinic acid).[32]

Conclusion

We prepared novel polynucleotide analogues **12** and **15** containing uracil and thymine bases. These PNAs were soluble in water and contained alternating sequences betweenthe nucleoside analogues and dicarboxytrimethylene groups along the polymer chains. They showed physicochemical properties which were quite similar to those for the natural polymers, such as polyelectrolyte behavior and hyprochromicities greater than 40%. The polymers in an aqueous solution showed broad excimer fluorescence around 450 nm and underwent depyrimidination. Rate constants were about 10^2 faster than those for the heat-induced depyrimidination of DNA.

Experimental Section

Materials. Deoxyuridine, thymidine, triphenylphosphine, iodine, AgF, and 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU) were used as received. Maleic anhydride and AIBN were crystallized from benzene and methanol, respectively. Dimethyl formamide was dried over anhydrous $MgSO_4$ and distilled under reduced pressure. Pyridine was refluxed over KOH and distilled.

2',5'-Dideoxy-5'-iodouridine (2) and 2',3',5'-Trideoxy-3',5'-diiodouridine (3). Triphenylphosphine (11.39 g, 43.6 mmol) and iodine (11 g, 43.3 mmol) were added to a suspension of 2'-deoxyuridine (5 g, 21.9 mmol) in dioxane (150 mL) containing pyridine (5 mL, 61.8 mmol). After stirring the mixture for 15 h at room temperature, methanol (5 mL) was added, and then the solvents were evaporated under reduced pressure. The solution of residue in ethyl acetate (250 mL) was washed successively with 5% aqueous sodium thiosulfate and water. After drying the solution with anhydrous $MgSO_4$, the solvent was evaporated under reduced pressure to dryness. The residue was crystallized from ethanol to give 2.18 g of **3** (yield: 22%, mp: 180-182 °C). The solid substance, obtained from the filtrate by evaporating the solvent under reduced pressure, was purified by column chromatography on silica gel (CCl_4 : acetone, 2 : 1) and crystallized from acetone/CCl_4 to give 2.0 g of **2** (yield: 27%, mp: 161-162 °C).[34]

Compound **2**: ^{1}H-nmr (DMSO-d_6): δ (ppm) 2.15-2.34 (m, 2H, H_2), 3.33-3.54 (m, 2H, H_5), 3.78-3.82 (m, 1H, H_4), 4.18-4.24 (m, 1H, H_3), 4.00-4.80 (bs, 1H, OH), 5.80 (d, 1H, H_5, J = 8 Hz), 6.29 (t, 1H, H_1, J = 7 Hz), 7.62 (d, 1H, H_6, J = 8 Hz), 11.19 (bs, 1H, NH). ^{13}C-nmr (DMSO-d_6); δ (ppm) 38.5 (C_5), 72.6 (C_2), 83.5 (C_3), 83.8 (C_4), 101.5 (C_1), 138.9 (C_5), 149.4 (C_6), 162.4 (C_2 and C_4). Anal. Calcd for $C_9H_{11}IN_2O_4$: C, 31.97, H, 3.28, N, 8.28. Found: C, 31.65, H, 3.12, N, 8.05.

Compound **3**: ^{1}H-nmr ($CDCl_3$): δ (ppm) 2.80 (qq, 1H, $H_{2'a}$), 3.19 (q, 1H, $H_{2'b}$), 3.34-3.45 (m, 1H, $H_{4'}$), 3.49-3.57 (m, 2H, $H_{5'}$), 4.58-4.64 (m, 1H, $H_{3'}$), 5.81 (q, 1H, H_5, J = 5 Hz, 2 Hz), 6.55 (q, 1H, $H_{1'}$), 7.82 (d, 1H, H_6, J = 8 Hz). ^{13}C-nmr ($CDCl_3$): δ (ppm) 7.4 ($C_{5'}$), 26.2 ($C_{3'}$), 44.7 ($C_{2'}$), 82.8 ($C_{4'}$), 85.9 ($C_{1'}$), 101.9 (C_5), 140.2 (C_6), 150.0 (C_2), 162.8 (C_4). Anal. Calcd for $C_9H_{10}I_2N_2O_3$: C, 24.12; H, 2.23; N, 6.25. Found: C, 24.98; H, 2.26; N, 6.29.

3'-*O*-Acetyl-2',5'-dideoxy-5'-iodouridine (4). Acetic anhydride (30 mL) was added to a DMF solution (50 mL) containing compound **2** (3 g, 8.90 mmol) and pyridine (35 mL). The solution was stirred for 2 h at 70 °C and concentrated under reduced pressure to a syrup which was taken up in ethyl acetate (300 mL). After washing with H_2O and drying with $MgSO_4$, the solution was evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (CCl_4/acetone; 7 : 3) and crystallized from ethyl acetate to give 2.64 g of **4** (yield: 85%, mp: 138-140 °C). ^{1}H-nmr ($CDCl_3$): δ (ppm) 2.12 (s, 3H, CH_3CO-), 2.26-2.42 (m, 2H, $H_{2'}$), 3.47-3.65 (m, 2H, $H_{5'}$), 3.90-3.95 (m, 1H, $H_{4'}$), 5.07 (m, 1H, $H_{3'}$), 5.82 (q, 1H, H_5, J = 8 Hz, 2Hz), 6.31 (q, 1H, $H_{1'}$), 7.77 (d, 1H, H_6, J = 8 Hz). ^{13}C-nmr ($CDCl_3$): δ (ppm) 7.4 ($C_{5'}$), 20.9 (CH_3 of thymine), 37.5 ($C_{2'}$), 77.4 ($C_{3'}$), 82.2 ($C_{4'}$), 84.4 ($C_{1'}$), 103.2 (C_5), 139.7 (C_6), 150.1 (C_2), 162.7 (C_4), 170.5 (C=O of acetyl). Anal. Calcd. for $C_{11}H_{13}IN_2O_5$: C, 34.75; H, 3.42; N, 7.37. Found: C, 35.56; H, 3.49; N, 7.46.

1'-β-Uracil-1-yl-3'-*O*-acetyl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose (5). From compound **4**: Silver fluoride (1.66 g, 13.1 mmol) was suspended in a pyridine solution (100 mL) containing compound **4** (2 g, 5.28 mmol) and the suspension was stirred for 4 days at room temperature. The reaction mixture was passed a silica gel column and the eluent was concentrated to a syrup which was taken up in ethyl acetate (200 mL) and washed with H_2O. After evaporation of the solvent under reduced pressure, the residue was purified by a column chromatography on silica gel (CCl_4/acetone; 2 : 1) and crystallized from CCl_4/acetone to give 0.24 g of **5** (yield: 18%, mp: 147-149 °C).

From compound **2**: Compound **2** (5 g, 14.8 mmol) and DBU (4.52 g, 30.2 mmol) were dissolved in pyridine (150 mL) and stirred for 20 h at room temperature. After addition of acetic anhydride (1.81 g, 17.7 mmol), the solution was stirred for 7 h at room temperature and mixed with ethyl acetate (300 mL). The solution was washed with aqueous dilute HCl and H_2O twice. After drying ($MgSO_4$), the solvent was evaporated to dryness under the reduced pressure. The residue was purified as mentioned above to give 1.64 g of **5** (yield: 44%, mp: 148-150 °C). ^{1}H-nmr ($CDCl_3$): δ (ppm) 2.11 (s, 3H, CH_3CO-), 2.22-2.36 (m, 1H, $H_{2'a}$), 2.54-2.66 (m, 1H, $H_{2'b}$), 4.46 (d, 1H, $H_{5'a}$, *J*gem=2Hz), 4.65 (d, 1H, $H_{5'b}$, *J*gem=2Hz), 5.74 (q, 1H, $H_{3'}$), 5.82 (d, 1H, H_5, J = 8 Hz),

6.54 (t, 1H, $H_{1'}$), 7.23 (d, 1H, H_6, $J_{5,6}$ = 8 Hz), 9.59 (bs, 1H, -NH-). ^{13}C-nmr ($CDCl_3$): δ (ppm) 21.0 (CH_3 of acetyl), 37.7 ($C_{2'}$), 71.4 ($C_{3'}$), 85.9 ($C_{1'}$), 88.5 ($C_{5'}$), 103.6 (C_5), 138.6 (C_6), 150.1 (C_2), 158.4 ($C_{4'}$), 162.9 (C_4), 170.0 (C=O of acetyl). Anal. Calcd. for $C_{11}H_{12}N_2O_5$: C, 52.38; H, 4.76; N, 11.11. Found: C, 53.03; H, 4.97; N, 11.14.

5'-Deoxy-5'-iodothymidine (7) and 1'-β-Thymin-1-yl-2',5'-dideoxy-D-*glycero*-pent-4-enofuranose (8) were synthesized according to the literature.[34]

1'-β-Thymin-1-yl-3'-O-acetyl-2',5'-dideoxy-D-*glycero*-pent-4-enofuranose (9). Compound **7** (4.5 g, 12.7 mmol) and DBU (3.87 g, 25.4 mmol) were dissolved in pyridine (50 mL) and stirred for 16 h at room temperature. After addition of acetic anhydride (1.55 g, 15.2 mmol), the solution was stirred for 5 h at room temperature and mixed with ethyl acetate (300 mL). The solution was washed with aqueous dilute HCl and H_2O twice. After drying ($MgSO_4$), the solution was evaporated to dryness, which was purified by a column chromatography on silica gel (CCl_4/acetone: 2 : 1) to give 1.49 g of **9** (yield: 44%, white homogeneous form.[35] ^{1}H-nmr ($CDCl_3$): δ (ppm) 1.95 (s, 3H, CH_3-), 2.11 (s, 3H, CH_3CO-), 2.21-2.36 (m, 1H, $H_{2a'}$), 2.50-2.62 (m, 1H, $H_{2b'}$), 4.46 (d, 1H, $H_{5'a}$, *J*gem=2 Hz), 4.85 (d, 1H, $H_{5'b}$, *J*gem = 2 Hz), 5.75 (q, 1H, $H_{3'}$), 6.58 (q, 1H, $H_{1'}$), 7.00 (s, 1H, H_6), 9.12 (bs, 1H, -NH-). ^{13}C-nmr ($CDCl_3$): δ (ppm) 12.6 (CH_3 of thymine), 21.0 (CH_3 of acetyl), 37.4 ($C_{2'}$), 71.5 ($C_{3'}$), 85.6 ($C_{1'}$), 88.3 ($C_{5'}$), 112.2 (C_5), 134.0 (C_6), 150.1 (C_2), 158.4 ($C_{4'}$), 163.2 (C_4), 170.0 (C=O of acetyl). Anal. Calcd. for $C_{12}H_{14}N_2O_5 \cdot 1/2H_2O$: C, 52.36; H, 5.45; N, 10.18. Found: C, 52.94; H, 5.40; N, 9.98.

Copolymerization.

Poly[{1'-uracil-1-yl-3'-O-acetyl-2',5'-dideoxy-D-*glycero*-pent-4-enofuranose}-*alt*-{maleic anhydride}] (10) and Poly-[1'-thymin-1-yl-3'-O-acetyl-2',5'-dideoxy-D-*glycero*-pent-4-enofuranose}-*alt*-{maleic anhydride}] (13): Calculated amounts of monomers and initiators were charged into polymerization tubes (Table 1). These tubes were immersed into a Dewar flask containing dryice and acetone. Following conventional freeze-thaw treatments under N_2, the tubes were sealed and placed on an oil bath at a fixed temperature for a definite time interval. The polymers were dissolved in DMF, precipitated in ethyl acetate, filtered, and dried over P_2O_5.

Hydrolysis of the Polymers.

Poly[(1'-b-uracil-1-yl-3'-*O*-acetyl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose)-*alt*-(maleic acid)] (11) and poly-[(1'-b-thymin-1-yl-3'-O-acetyl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose)-*alt*-(maleic acid)] (14): Polymers **10** and **13** (0.3 g) were dissolved in H_2O (20 mL) and these solutions were stirred for 4 h at room temperature. The polymer solutions were dialyzed with a cellulose membrane (Spectrum Medical Ind. Inc. MWCO-1,000) using a constant flow of distilled water for 48 h at 0 °C. The retentates were freeze-dried to give polymer **11** (yield: 75%) and polymer **14** (yield: 68%).

Poly[(1'-b-uracil-1-yl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose)-*alt*-(maleic acid)] (12) and poly[(1'-b-thymin-1-yl-2',5'-dideoxy-D-*glycero*-pent-4'-enofuranose)-*alt*-(maleic acid)] (15): Polymers **10** and **13** (0.36 g) were dissolved in 40 mL of 0.1 N NaOH and these solutions were stirred for 12 h at 50 °C. The polymer solutions were dialyzed and freeze-dried as decribed above to give polymer **12** (yield: 65 %) and polymer **15** (yield: 55%). The uracilyl and thyminyl groups on the polymer chains were found to be liberated during these hydrolyses. To measure the contents of pyrimidine bases remaining on the polymer chains, the hydrolyzed solutions in prior to dialysis was subjected to analysis by HPLC. 78% of uracil and 81% of thymine were remained on the polymers **12** and **15**, respectively, which was used for the further investigation.

Hyper- or Hypochromicity. UV-spectra were recorded using a JASCO V-550 spectrophotometer. The solution concentrations were approximately 10^{-4} mol/l of base residue. The percent hyper- or hypochromicity (h %) was calculated from equation (1) where ε_p and ε_m denote the molar extinction coefficients of the base residue of the polymers and the relevant monomers at the wavelength of the absorption maxima of the monomers. The positive value of h is hyperchromicity whereas a negative value means hypochromicity.

$$h\,(\%) = 100[(\varepsilon_p - \varepsilon_m)/\varepsilon_m] \quad (1)$$

Measurements of Depyrimidination. The polymers (2.4 mg) were dissolved in Tris-buffer (pH: 7.4) solution (20 mL) at ionic strength of 0.02 (KCl), which were kept at the definite temperatures (±0.1) controlled by a thermostat. The reaction mixture was analyzed by HPLC on ultrahydrogel column in different reaction times.

^{1}H and ^{13}C NMR spectra were recorded on Varian Gemini 200 spectrometer. IR spectra were obtained with Nicolet Magna-IRTM 550 spectrophotometer. Fluorescence spectra were recorded on Kontron Instrument SFM25 fluorescence spectrophotometer. Measurement of molecular weight were carried out by gel permeation chromatography, Waters 150-CV with RI detector under the following conditions: waters ultrahydrogel 250 column with water or 0.1N $NaNO_3$ aqueous solution eluent at the flow rate of 0.8 mL/min. Elemental analysis was performed at KRICT.

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