Macromolecules **1994,** *27,* 2896–2898

Communications to the Editor

Polyribonucleotide Analogues:[1] Synthesis and Physicochemical Properties

Man Jung Han,* Ki Soo Kim, Tae Joon Cho, Ki Ho Kim, and Ji Young Chang

Department of Applied Chemistry, Ajou University, Suwon 441-749, Republic of Korea

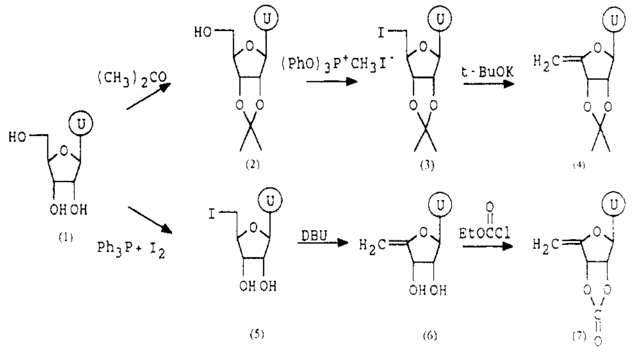
Received September 23, 1993
Revised Manuscript Received March 14, 1994

Considerable attention has been devoted to the synthesis of polynucleotide analogues as model compounds for natural ones over the last decade in an effort to elucidate functions of nucleic acids in biological systems. More recently analogues themselves were found to show biological activities, arousing hopes for their utilization in chemotherapy as polymeric drugs.[2,3] Despite numerous attempts, it had not previously been possible to synthesize polynucleotide analogues whose structures and physicochemical properties had a close resemblance to those of natural polymers.[4–16] Recently we have reported the synthesis of polynucleotide analogues, which contained nucleoside analogues and 1,2-dicarboxyethylene units in an alternating pattern.[17–19] They have shown physicochemical properties such as high solubility in water, hypochromicity, and optical properties, which resembled closely those of natural polymers.

All of the polynucleotide analogues that have been reported so far are the analogues to polydeoxyribonucleotide. In the present work, we prepared polyribonucleotide (RNA) analogues **9** and **12** containing hydroxyl groups on C-2′ of furanose rings (Scheme 2). As model compounds for RNA, synthesis and physicochemical properties of these polymers are of much interest, since RNA was recently found to show another important function, i.e., enzymatic activity (ribozyme) in biological systems.[20–30] It has been believed that hydroxyl groups on furanose rings of RNA play important roles for its activity.

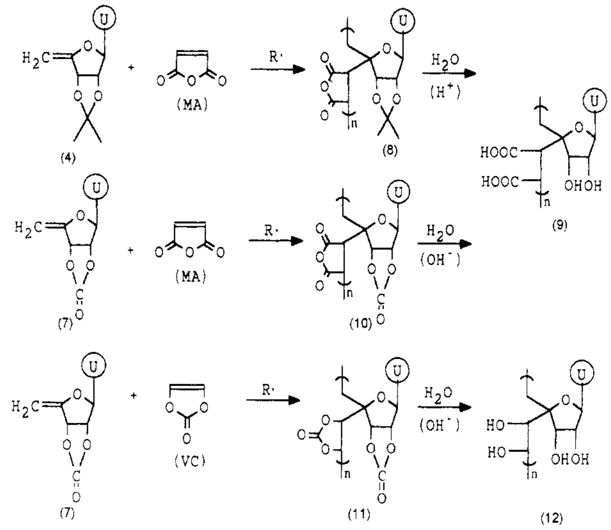
Two monomers **4** and **7** having different blocking groups of the 2′,3′-dihydroxyl group of the furanose ring were synthesized by modification of the methods in the literature[31–33] as shown in Scheme 1.

Scheme 1



DBU: 1,8-Diazabicyclo[5,4,0]undec-7-ene

Scheme 2



Uridine (**1**) was treated with acetone to give 2′,3′-*O*-isopropylideneuridine (**2**), whose 5′-hydroxyl group was transformed to a 5′-iodo group with the aid of methyltriphenoxyphosphonium iodide. The elimination reaction of compound **3** with the aid of sodium *tert*-butoxide resulted in monomer **4**, 1′-β-uracil-1-yl-5′-deoxy-2′,3′-*O*-isopropylidene-D-*erythro*-pent-4′-enofuranose. For the synthesis of monomer **7**, 1′-β-uracil-1-yl-5′-deoxy-2′,3′-*O*-carbonyl-D-*erythro*-pent-4′-enofuranose, uridine (**1**) was first transformed to 5′-deoxy-5′-iodouridine (**5**) and elimination of HI with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) was followed. The dihydroxyl group of the furanose ring was protected by carbonylation with ethyl chloroformate before polymerization.

Radical copolymerization of vinyl ethers with either maleic anhydride or vinylene carbonate is known to give alternating copolymers by forming charge-transfer complexes of the monomer pairs during copolymerization.[18,34,35] As the electron-donating character of the vinyl ether groups of monomers **4** and **7** is little influenced by either the 1′-uracilyl, 2′,3′-*O*-diether, or 2′,3′-carbonate groups, the copolymers of the monomers and maleic anhydride or vinylene carbonate are expected to have alternating sequences.

The copolymerizations of maleic anhydride (MA) with **4** or **7** and of vinylene carbonate (VC) with **7** were carried out in bulk in the presence of radical initiator (AIBN) to give poly[(1′-β-uracil-1-yl-5′-deoxy-2′,3′-*O*-isopropylidene-D-*erythro*-pent-4′-enofuranose)-*alt*-(maleic anhydride)] (**8**), poly[(1′-β-uracil-1-yl-5′-deoxy-2′,3′-*O*-carbonyl-D-*erythro*-pent-4′-enofuranose)-*alt*-(maleic anhydride)] (**10**), and poly[(1′-β-uracil-1-yl-5′-deoxy-2′,3′-*O*-carbonyl-D-*erythro*-pent-4′-enofuranose)-*alt*-(vinylene carbonate)] (**11**), respectively (Scheme 2). The copolymerization data are given in Table 1.

None of monomers **4**, **7**, and MA homopolymerized under the same conditions. Equimolar amounts of the comonomer pairs in polymers **8** and **9** were confirmed by titration of anhydride and carboxyl groups incorporated into copolymers **8** and **9**, respectively.[36] As VC can homopolymerize, the alternating copolymer **11** was obtained by controlling the feeding mole ratios of the monomers. In

0024-9297/94/2227-2896$04.50/0

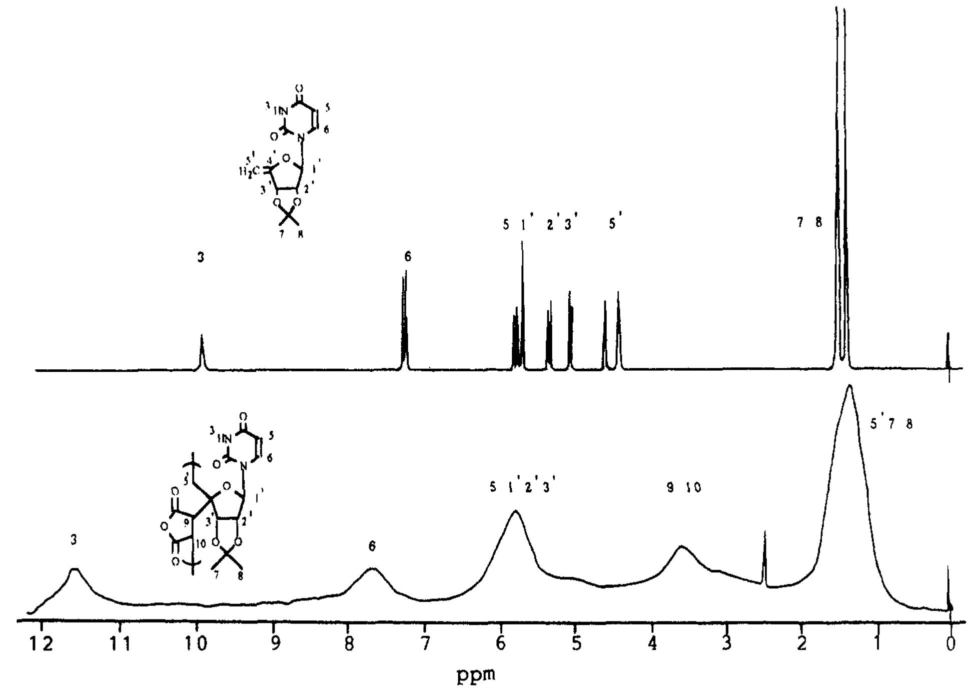


Figure 1. ¹H-NMR spectra of monomer 4 in $CDCl_3$ (200 MHz) and polymer 8 in DMSO-d_6 (500 MHz).

Table 1. Copolymerization Data in Bulk with AIBN[a]

copolymer	mole ratios of monomer	polym temp (°C)	polym time (h)	yield (%)	M_n[b]
9	4:MA = 1:2	80	24	75	10 000
9	7:MA = 1:2	95	1.5	46	4 600
12	7:VC = 1.5:1	95	24	30	

[a] Initiator (AIBN) concentration: 1 mol % of the total monomers. [b] Measured in a 0.1 N $NaNO_3$ aqueous solution by GPC.

the ¹H-NMR spectrum of polymer 8 (Figure 1), signals at δ 4.4–4.6 of vinyl protons on the 5′ position of monomer 4 disappeared and new peaks for methylene protons resulted from polymerization of double bonds overlapped with methyl proton peaks of isopropylidene groups around δ 1.3.

Hydrolysis of polymers 10 and 11 was accomplished with the aid of a hydroxide catalyst in an aqueous solution (0.1 N NaOH) to give poly[{2(*R*)-uracil-1-yl-3(*R*),4(*S*)-dihydroxytetrahydrofuran-5,5-diyl}{1,2-dicarboxypropylene}] (**9**) and poly[{2(*R*)-uracil-1-yl-3(*R*),4(*S*)-dihydroxytetrahydrofuran-5,5-diyl}{1,2-dihydroxypropylene}] (**12**), respectively. *N*-Glycosidic bonds of the nucleosides were stable under the reaction conditions, whereas they were susceptible to hydrolysis in the presence of protonic acid.[37] Although the hydrolysis of polymer 8 was attempted under weak acidic conditions (50% formic acid, 50 °C and 2 days) to remove isopropylidene groups, it was found by investigation of the ¹H-NMR spectrum of polymer 9 that about 25% of the uracil groups were eliminated from the polymer chain.

The hydrophilic groups, such as hydroxyl and/or carboxyl, on the polymer chain made polymers 9 and 12 water-soluble. The chiral atoms of C-1′, C-2′, and C-3′ in uridine were intact during synthesis of the monomers. Since C-2′ and C-3′ were bridgehead atoms, no racemization would occur at the C-2′ and C-3′ positions during copolymerization and thus most chiralities of the monomer would be retained to render polymers 9 and 12 optically active.[38] High solubility in water, optical activity, and alternating sequences of the polymer had been hardly realized in the synthesis of polynucleotide analogues except a few cases.[17-19] Since the main chains of polymers 9 and 12 were composed of C–C bonds instead of phosphate linkages, the polymers were hydrolytically stable. Susceptibility to hydrolysis of polynucleotides and the analogues often deteriorates their biological activities.[2]

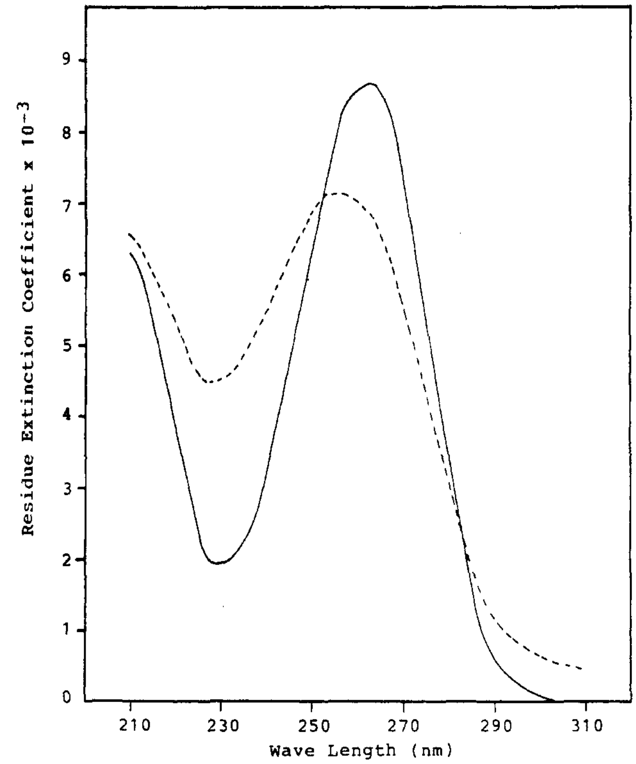


Figure 2. UV spectra of uridine (1, solid line) and polymer 9 in H_2O (dotted line) at pH 7.0.

Polymer 9 is a polyelectrolyte. It is known that the hydrodynamic volume of the polyelectrolyte increases greatly in the dilute solution. The polyelectrolyte expansion effect is strongly dependent on the ionic strength of the solution. The effect was suppressed successfully in 0.1 N $NaNO_3$ aqueous solution, which was confirmed by the universal calibration method.[39,40] Polymer 9 showed typical polyelectrolyte behavior. The molecular weight of polymer 9 was measured by gel permeation chromatography at room temperature. The number-average molecular weight of polymer 9 (Table 1) obtained from monomer **7** and MA was found to be 4600 in aqueous 0.1 N $NaNO_3$, whereas it was 113 000 in water. A similar phenomenon was also observed in the measurement of the reduced viscosity of polymer **9** in an aqueous solution, which decreased at the beginning and thereafter increased steeply with continuous dilution and retained normal behavior by the addition of $NaNO_3$.

UV spectra of uridine (**1**) and polymer **9** in water are shown in Figure 2. Polymer **9** shows a hypochromicity of 18% ($\lambda = 258$, $\epsilon = 7120$), compared with UV absorption of uridine (**1**; $\lambda = 263$, $\epsilon = 8690$) at pH 7. According to the Tinoco[41] and Rhodes[42] 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 occurs on the systems in which the chromophores are stacked one upon another like a deck of cards. The carboxylate groups of polymer **9** at pH 7 in an aqueous solution likely protruded outward, interacting with the hydrophilic environment, and the uracil bases seemed to be so stacked one upon another as to result in hypochroism.

In the preliminary experiment it was found that the hydrolysis rate of deodecyl *p*-nitrophenylphosphate in a buffer solution (pH 7) was increased in the presence of polymer **9**. Catalytic activity of the polymer like that of ribozyme seemed to be responsible for the results. A detailed analysis of catalytic, optical, and physicochemical properties as well as a more extensive discussion will be published elsewhere. Syntheses and copolymerizations of **4** and **7** substituted with other nucleic acid bases, such as adenine, guanine, cytosine, and hypoxanthine, are in progress.

Acknowledgment. This work was supported by a grant from the Korea Science and Engineering Foundation.

References and Notes

(1) The 5th paper on Polynucleotide Analogues. Paper 4: Han, M. J.; Chang, Y. S.; Park, J. Y.; Kim, K. H. *Macromolecules* **1992**, *25*, 6574.
(2) Levy, H. B.; Riley, F. L. *Polymers in Medicine*; Chiellini, E., Giusti, P., Eds.; Plenum Press: New York, 1983; p 33.
(3) Pitha, J.; Pitha, P. M. *Science* 1971, *172*, 1146.
(4) Kaye, H. *Polym. Lett.* **1969**, *7*, 1.
(5) Kondo, K.; Iwsaski, H.; Nakatani, K.; Ueda, N.; Takemoto, K.; Imoto, M. *Makromol. Chem.* **1969**, *125*, 42.
(6) Kaye, H. *J. Polym. Sci.* **1969**, *B7*, 1.
(7) Kondo, K.; Hisaoka, Y.; Takemoto, K. *Chem. Lett.* **1973**, 125.
(8) Kondo, K.; Takemoto, K. *Macromol. Chem., Rapid. Commun.* **1980**, *2*, 303.
(9) Ishikawa, T.; Inaki, K.; Takemoto, K. *Polym. Bull.* **1978**, *1*, 85.
(10) Anand, N.; Murthy, N. S. R. K.; Naider, F.; Goodman, M.; *Macromolecules* 1971, *4*, 564.
(11) Overberger, C. G.; Lu, C. X. *J. Polym. Sci., Polym. Chem. Ed.* **1987**, *25*, 1523.
(12) Overberger, C. G.; Inaki, Y. *J. Polym. Sci., Polym. Chem. Ed.* **1979**, *17*, 1739.
(13) Overberger, C. G.; Lu, C. X. *J. Polym. Sci., Polym. Chem. Ed.* **1986**, *24*, 243.
(14) Overberger, C. G.; Chang, J. Y.; Gunn, V. E. *J. Polym. Sci., Polym. Chem. Ed.* **1989**, *27*, 99.
(15) Overberger, C. G.; Chang, J. Y. *J. Polym. Sci., Polym. Chem. Ed.* **1989**, *27*, 4013.
(16) Ishirawa, T.; Inaki, Y.; Takemoto, K. *Polym. Bull.* **1978**, *1*, 215.
(17) Han, M. J.; Park, S. M. *Macromolecules* **1990**, *23*, 5295.
(18) Han, M. J.; Park, S. M.; Park, J. Y.; Yoon, S. H. *Macromolecules* **1992**, *25*, 3534.
(19) Han, M. J.; Chang, Y. S.; Park, J. Y.; Kim, K. H. *Macromolecules* **1992**, *25*, 6574.
(20) Wang, J.-F.; Downs, W. D.; Cech, T. R. *Science* **1993**, *260*, 504.
(21) Robertson, D. L.; Joyce, G. F. *Nature* **1990**, *34*, 467.
(22) Herochlag, D.; Cech, T. R. *Nature* **1990**, *34*, 405.
(23) Perreault, J.-P.; Wu, T.; Cousineau, B.; Ogilvie, K. K.; Cedergren, R. *Nature* **1990**, *29*, 759.
(24) Cech, T. R. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 759.
(25) Altman, S. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 749.
(26) Marsh, T. L.; Pace, N. R. *Science* **1985**, *229*, 80.
(27) Maniatis, T.; Robin, R. *Nature* **1987**, *325*, 673.
(28) Guerrier-Takada, C.; Haydock, K.; Allen, L.; Altman, S. *Biochemistry* **1986**, *25*, 1509.
(29) Perrota, A. T.; Been, M. D. *Biochemistry* **1992**, *31*, 16.
(30) Heidenreich, O.; Eckstein, F. *J. Biol. Chem.* **1992**, *267*, 1904.
(31) Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1970**, *35*, 2319.
(32) Verheyden, J. P. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1975**, *97*, 4386.
(33) Hampton, A. *J. Am. Chem. Soc.* **1961**, *83*, 3640.
(34) Han, M. J.; Kim, K. H.; Cho, T. J.; Choi, K. B. *J. Polym. Sci., Polym. Chem. Ed.* **1990**, *28*, 2719.
(35) Han, M. J.; Kang, S. D.; Lee, W. Y. *Bull. Korean Chem. Soc.* **1990**, *11*, 154.
(36) Fritz, J. S.; Lisicki, N. M. *Anal. Chem.* **1958**, *23*, 462.
(37) Blackburn, G. M. *Nucleic Acids in Chemistry and Biology*; Blackburn, G. M., Gait, M. J., Eds.; Oxford University Press: Oxford, U.K., 1990; p 261.
(38) The CD curve of polymer **9** showed a positive Cotton effect at 240 nm ($\theta = 2000$) and a negative one at 265 nm ($\theta = 1500$).
(39) Grubisic, Z.; Rempp, P.; Benoit, H. *J. Polym. Sci., Part C* **1967**, *5*, 753.
(40) Hamielec, A.; Styring, M. *Pure Appl. Chem.* **1985**, *57*, 955.
(41) Tinoco, I., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 5047.
(42) Rhodes, W. *J. Am. Chem. Soc.* **1961**, *83*, 3609.