which do polymerize (although not very well).[31] The initiation is novel but not unreasonable, and probably more reasonable than a reaction giving an alkoxy radical.

This chain, in spite of its success in accounting for the products, can hardly be taken to be more than an informed speculation, and we do not present it as more than this.

Experimental Section

Materials. The preparation of *p'*-phenylenebisdiazonium tetrafluoroborate was as described before.[5] The alcohols were commercial materials; 2-propanol-*2-d* was prepared by the lithium aluminum hydride reduction of acetone by standard procedures; it was gas chromatographically homogeneous. The deuterated allyl alcohol was made from acryloyl chloride and lithium aluminum deuteride,[32] but the product showed an impurity absorbing significantly at 254 mμ. This alcohol showed a fast reaction with the tetrazonium salt. On preparative scale gas chromatography (30% Ucon 50HB260 on 45–60 Chromosorb P) the absorbing material was removed and the rate was apparently normal. The sensitivity of the uv, the efficiency of the gas chromatography, and the rather modest rate acceleration with the impurity present lead us to believe that the reported k_D is correct, but there is a small chance that the inverse isotope effect may be due to an accelerating impurity in the deuterated species.

Kinetic Runs. A typical run describes the procedure which is straightforward except for the necessity for removing oxygen and the cautions described below. Separate solutions containing, respectively, 5.15 *M* sodium perchlorate, 5.15 *M* perchloric acid, and 6×10^{-5} *M* *p*-phenylenebisdiazonium tetrafluoroborate in 5.15 *M* perchloric acid were prepared. Prepurified nitrogen was bubbled first through water and then through each of the solutions for 4 hr. Nitrogen was also passed through 2-propanol for the same period. All solutions were kept in a water bath at 30° while the oxygen was being removed.

A 1-cm cylindrical quartz cell was fitted with a serum stopper through which two small glass tubes were passed. One was used to pass nitrogen into the cell, the other accommodated a thin Teflon tube attached to an all-glass syringe. Necessary amounts of the reagents were then withdrawn from the previously prepared solutions and added to the cell with a constant nitrogen stream flowing both during withdrawal and addition. After all the reagents were added, the glass tubes were removed from the stopper, the contents were mixed, and the cell was placed in the beam of a Cary 14 spectrophotometer in a thermostatted cell jacket. The instrument then gave a record of the absorbance at 254 nm *vs.* time.

Caution. The use of diazonium salts with perchlorates introduces a significant hazard. All diazonium perchlorates have a very limited solubility and the solids, if precipitated, are extremely sensitive, even when suspended in solution, and quite small amounts detonate with enough violence to break glassware. The amounts needed for spectrophotometric work are not hazardous, but attempts to increase the scale must be done with great caution. The solubility product of benzenediazonium perchlorate is of the order of 10^{-4} M^2, and others appear to be comparable. Thus, solid can be precipitated with even rather modest concentrations of both ions.

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A New Polymer-Support Method for the Synthesis of Ribooligonucleotide[1]

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Abstract: The ribooligonucleotide triuridine diphosphate (UpUpU) has been synthesized on a new polymer system of isotactic succinylated polystyrene. 2′-*O*-Benzoylpropionyl-3′-*O*-methoxyacetyluridine was linked to the polymer *via* a 5′ ester group and served as the first nucleoside unit. After repeated demethoxyacetylation and condensation with 2′-*O*-benzoylpropionyl-3′-*O*-methoxyacetyluridine 5′-phosphate, the trinucleotide was obtained. The yields were about 50% in each condensation step. The preparation of the suitably protected nucleosides and nucleotides used in the experiment is also described.

The synthesis of ribooligonucleotides of known sequence is important for the cytochemical study of the function of ribonucleic acid. While deoxyoligonucleotides have been prepared stepwise on a polymer support,[2-6] no successful solid-phase support procedure for the synthesis of ribooligonucleotide has so far been developed. The difficulties arise from (1) lack of suitable solid-phase support which can be used with ease and speed, and (2) the presence of the 2′-hydroxyl group in the ribofuranose moiety makes the 3′ nucleotide labile under extreme alkaline and acid conditions. We have recently found that high molecular weight isotactic polystyrene[7] can be used as a polymer support. Previously, soluble polystyrene or cross-linking polystyrene was used. The isotactic polymer possesses high crystallinity which makes it insoluble and reduces swelling in organic solvents. Therefore, this polymer should be superior for oligonucleotide synthesis since it contains less inner space when compared with the cross-linked polystyrene. In addition, the isotactic polymer can be

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(3) T. Shimixzu and R. L. Letsinger, *J. Org. Chem.*, **33**, 708 (1968).

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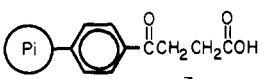
recovered completely and easily after each reaction step by a relatively simple washing procedure in such polar solvents as ethanol, methanol, or water.

Isotactic polystyrene was modified by succinylation onto the phenyl ring. The Friedel–Craft reaction between isotactic polystyrene and succinic anhydride in nitrobenzene–tetrachloroethane was carried out in the usual manner. The degree of succinylation was estimated by the infrared intensity of the carbonyl group stretching band (1680 cm^{-1}), and the extinction coefficient of β-benzoylpropionic acid was used as the reference. The degree of succinylation of **1** ranges from 11 to 16%, or 1–1.4 mmol of succinyl group per gram of polymer.



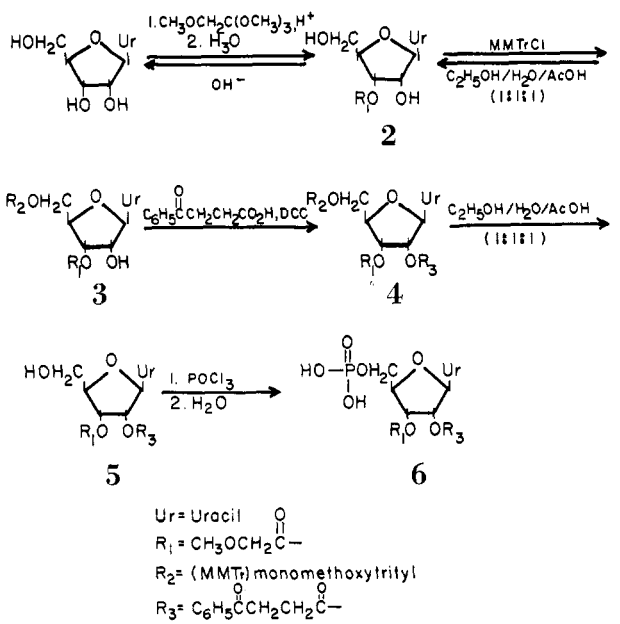
1

Pi = isotactic polystyrene backbone

The 2′- and 3′-protected ribonucleosides with a free 5′-hydroxyl group were prepared for condensation with the polymer through the succinyl group. Methoxyacetyl[8] and β-benzoylpropionyl[9] were chosen as the 3′- and 2′-protecting groups, respectively. The former protecting group has a fast basic hydrolysis rate and thus can be released, while both the polymer–nucleoside linkage and the β-benzoylpropionyl group remain attached. Further condensation with a 5′ phosphate can then be effected at the 3′ position of the polymer-supported nucleosides. Finally, the desired oligonucleotide was freed from the polymer, and blocking groups were removed by treatment with hydrazine buffer or methanolic ammonia. The cleavage of the glycosidic bond or migration of phosphodiester linkage could be avoided, therefore, since no acid was used throughout the process.

The proper protected nucleosides were prepared as shown in Chart I. When uridine was allowed to

Chart I



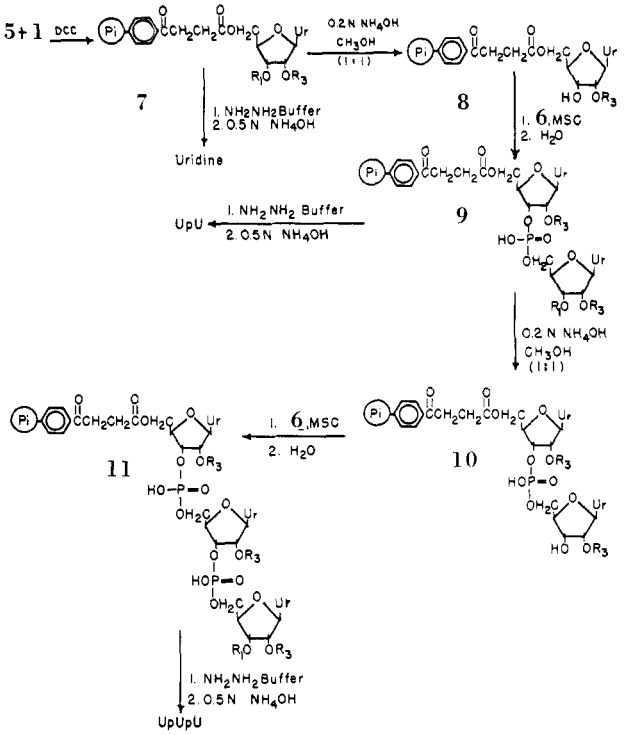
Ur = Uracil
R_1 = $CH_3OCH_2C(=O)-$
R_2 = (MMTr) monomethoxytrityl
R_3 = $C_6H_5C(=O)CH_2CH_2C(=O)-$

undergo *p*-toluenesulfonic acid catalyzed ortho ester exchange with excess trimethyl orthomethoxyacetate,[10] followed by acid treatment, the 3′-hydroxyl group was acylated selectively.[11] The 3′-*O*-methoxyacetyluridine was crystallized from absolute ethanol, and the structure was confirmed by nmr spectroscopy to be free of the 2′ isomer. The 3′-*O*-methoxyacetyl group could be completely removed by 0.2 *N* ammonium–methanol (1:1 v/v) to regenerate uridine within 30 min at room temperature. The acid-labile monomethoxytrityl group was now used to protect the 5′-hydroxyl group.

When **2** was mixed with excess monomethoxytrityl chloride in pyridine and was allowed to stand at room temperature for 2 days, 5′-monomethoxytrityl-3′-*o*-methoxyacetyluridine was obtained quantitatively. Compound **2** could also be recovered if **3** was treated with a solution of alcohol–acetic acid–water (1:1:1) for 8 hr at room temperature.[12] The 2′ blocking group was then introduced onto **3** with a fivefold excess of DCC and β-benzoylpropionic acid in pyridine.[9] The monomethoxytrityl group of **4** was then removed in a similar manner to give **5**. The percentage yield for each reaction step ranged from 93 to 45%. All products were chromatographically homogeneous and their structures were confirmed by nmr spectroscopy.

The polymer derivative **1** was now allowed to react with **5** in dry pyridine to give **7** using DCC as the coupling agent (Chart II). The amount of **5** that at-

Chart II



tached to the polymer *via* the ester linkage was estimated by the amount of uridine recovered from the

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(9) R. L. Letsinger and P. S. Miller, *J. Amer. Chem. Soc.*, **91**, 3356 (1969).

(10) Ortho ester was synthesized from methoxyacetonitrile (R. B. Wagner and H. D. Zook, "Synthetic Organic Chemistry," Wiley, New York, N. Y., 1965, Chapter 16, p 542); trimethyl orthomethoxyacetate: bp 54–55° (10 mm); nmr δ 3.35 (s, 9, $3CH_3O$–), 3.45 (s, 3, CH_3O–), 3.60 (s, 2, $-CH_2O$–).

(11) H. P. M. Fromageot, B. E. Griffin, C. B. Reese, and J. E. Sulston, *Tetrahedron*, **23**, 2315 (1967).

(12) J. Žemlička and A. Holý, *Collect. Czech. Chem. Commun.*, **32**, 3159 (1967).

hydrolysis of **7**. The hydrolysate of **7** in 0.5 *M* hydrazine in pyridine–acetic acid (4:1 v/v) followed by 0.5 *N* ammonium hydroxide was analyzed by paper chromatography, developed in solvent S_1. The uridine band was extracted, and its uv absorption at 262 mμ was measured. Hydrazine has been known to attack pyrimidines under appropriate conditions.[13,14] However, using the hydrazine buffer (*i.e.*, 0.5 *M* hydrazine in pyridine–acetic acid, 4:1 v/v) discovered by Letsinger, *et al.*,[15] uridine could be released with no degradation from the polymer **7**. At the same time, the 2′-blocking group was removed also. Later on in our work, a faster method using methanolic ammonia was also found useful for the hydrolysis of **7**. By this method, uridine was completely released from the polymer in 2.5 hr at room temperature.

The 3′-*O*-methoxyacetyl group was removed by a solution of 0.2 *N* NH_4OH–CH_3OH (1:1 v/v). No significant amount of uridine or β-benzoylpropionic acid (by uv absorption) was found in the solution.

3′-*O*-Methoxyacetyl-2′-*O*-benzoylpropionyluridine 5′-phosphate (**6**) was prepared by treating **5** with phosphorus oxychloride at 0° in trimethyl phosphate.[16] Then the product was purified on a DEAE Sephadex A-25 (HCO_3^- form) column.

The pyridinium salt of **6** was condensed with the polymer uridine derivative (270 μmol of U/g of polymer) **8** in pyridine using mesitylene sulfonyl chloride (MSC) as the condensing agent. The polymer derivative **9** was isolated by filtration. On treatment with hydrazine buffer, followed by 0.5 *N* NH_3OH, the ester linkages holding the nucleotide product to the polymer and all other protecting groups were simultaneously cleaved. Dinucleotide UpU was recovered and estimated quantitatively by paper chromatography which showed that 52% of the uridine on the polymer had reacted.

For the synthesis of trinucleotide UpUpU, the protected nucleotide **6** was again allowed to react with the polymer **10** in pyridine and MSC, using essentially the same procedure as mentioned above for the dinucleotide. A product carrying 67 μmol of UpUpU/g of polymer was obtained.

The dinucleotide and trinucleotide thus prepared were identified by their chromatographic and electrophoretic properties (Table I) and also by the hydrolysis catalyzed by venom phosphodiesterase and pancreatic ribonuclease. The dinucleotide and trinucleotide were cleaved quantitatively to uridine and uridine 5′-phosphate by snake venom phosphodiesterase. More than 95% of UpU and UpUpU were completely degraded to uridine and uridine 3′-phosphate by the action of pancreatic ribonuclease (Up/U = 0.96 for UpU and Up/U = 1.95 for UpUpU). The trinucleotide was used also in a binding study with ribosome and [^{14}C]phenylalanine.

This synthesis demonstrated that succinylated isotactic polystyrene can be used conveniently as a support for solid-phase synthesis of oligonucleotides, more than 95% of which is the 3′–5′ linked isomer. Application of this method for the synthesis of ribooligonucleotides containing unnatural bases and chromogenic or fluorogenic terminal groups is under investigation.

Table I. Chromatographic Properties of Nucleoside Derivatives and Oligonucleotides

	R_f						
	Tlc 1[a]			Tlc 2[b]	Paper C[c]		
Compd	S_1	S_2	S_3	S_3	S_1	S_3	R_m[d]
2	0.64	0.57	0.71				
3		0.60	0.80				
4		0.65	0.84				
5		0.60	0.78	0.91		0.91	
6				0.23		0.32	0.64
U	0.43	0.45	0.68	0.66	0.44	0.73	0.00
Up			0.36	0.19	0.10	0.21	1.00
UpU		0.08			0.15	0.37	0.45
UpUpU		0.12			0.03	0.25	0.65

[a] Tlc **1** was performed on Kodak Chromagram 6060 (for the solvent systems, see the Experimental Section). [b] Tlc **2** was performed on Kodak Chromagram 6065. [c] Paper chromatography was carried out on Whatman 3 MM paper. [d] For electrophoresis, see the Experimental Section.

Experimental Section

Uv absorption spectra were measured on a Beckman Model DB-G recording spectrophotometer. Ir spectra were obtained on a Perkin-Elmer Model 521 spectrophotometer. Nmr spectra were measured with a Varian spectrometer HA-100. Chemical shifts are given in parts per million on a δ scale; coupling constants are expressed in cycles per second; TMS or DSS was used as internal standards. Thin-layer chromatography was carried out by the ascending method with Eastman Chromagram Sheets 6060 (silica gel with fluorescent indicator) and 6065 (cellulose with fluorescent indicator). Paper chromatography was carried out by the descending technique on Whatman 3MM paper. For product analysis, absorbances of the blank were cut from the paper near the product and treated in the same manner as the product. Solvent systems are: S_1, 2-propanol–ammonium hydroxide–water (7:1:2); S_2, chloroform–2-propanol (2:8); S_3, ethanol–ammonium acetate (1 *M*) (7:3). Paper electrophoresis was conducted on a Savant Electrophoresis Flat Plate, using 0.03 *M* sodium phosphate buffer (pH 7) at 2000 V for 1 hr. Isotactic polystyrene (mol wt, 5.02×10^6) was ground to a fine powder with a Waring blender before use. Pyridine was dried by distillation over CaH_2 and was stored over Linde Molecular Sieves, Type 4A. The elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. For the enzyme assay, a solution containing about 1 μmol of substrate and 0.1 mg of beef pancreatic ribonuclease (Miles Laboratory) in 0.1 ml of 0.05 *M* Tris–HCl buffer (pH 8.0) was incubated at 37°. After 5 hr, the mixture was chromatographed and the uv-absorbing bands were eluted and measured spectrophotometrically. A solution containing about the same amount of substrate and 40 units of Russel's venom phosphodiesterase (Calbiochem) in 0.2 ml of 0.1 *M* Tris–HCl buffer (pH 9.4) was incubated at 37° for 5 hr.

Succinylated Isotactic Polystyrene (1). Isotactic polystyrene (1.0 g, 8.1 mmol of styrene unit) was suspended in 20 ml of warm tetrachloroethane. To the gel-like suspension, succinic anhydride (1.0 g, 10 mmol) was added, followed by aluminum chloride (2.0 g, 15 mmol), which was first dissolved in 5 ml of nitrobenzene and 5 ml of tetrachloroethane. After 10 min of stirring, more solvent (10 ml of tetrachloroethane and 5 ml of nitrobenzene) was added. The reddish mixture was stirred at 80° for 3 hr. Ice water (30 ml) was added to quench the reaction. Then the product was stirred for an additional 3 hr. More water (three 100-ml portions) was added, and the aqueous layers were decanted. Methanol (200 ml) was added to the remaining mixture. The white solid thus formed was separated by filtration and was washed thoroughly with hot methanol and hot water. A solid (1.08 g) was obtained after drying: ir a (KBr) 3300 (–OH), 1680 (C=O), 820 cm^{-1}. The degree of succinylation in the polymer was estimated by the ir of the polymer in a KBr disk. Succinylated isotactic polystyrene (2.6 mg) was mixed with 200 mg of KBr powder. The mixture was ground in a vibrator grinder for 2 min and the disk was prepared in the usual standard manner. The ir spectrum was recorded and the absorbance at 1680 cm^{-1} was measured and calibrated against β-benzoylpro-

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(14) S. Takemura and M. Miyazaki, *Bull. Chem. Soc. Jap.*, **32**, 926 (1959).
(15) R. L. Letsinger, P. S. Miller, and G. W. Grams, *Tetrahedron Lett.*, 2621 (1968).
(16) M. Yoshikawa, T. Kato, and T. Takenishi, *ibid.*, 5065 (1967).

pionyl. For instance, an adsorbance of 0.57 was found to be equivalent to 2.58 μmol of β-benzoylpropionic acid in 200 mg of KBr or 2.58/2.6 = 0.99 mmol of succinyl group per gram of polymer.

3′-*O*-Methoxyacetyluridine (2). Trimethyl orthomethoxyacetate (10 g, 67 mmol) was mixed with uridine (2.0 g, 8 mmol) and *p*-toluenesulfonic acid monohydrate (0.2 g, 1.1 mmol). The suspension was stirred at room temperature. After 1.5 hr, the solid was completely dissolved. Tlc (silica gel) revealed only one product, R_f 0.47 (S_2), and no uridine. The excess ortho ester was removed *in vacuo*. The syrup was dissolved in 20 ml of 5% acetic acid and was extracted three times with 10-ml portions of ether. The aqueous layer was separated and concentrated again to a syrup under reduced pressure. Tlc (silica gel) revealed only one spot, R_f 0.57 (S_2). The syrup was dried by repeated evaporation with absolute ethanol. Recrystallization from ethanol gave 2.0 g (80%) of **2**: mp 105–107°; uv max (H_2O) 260 mμ (ε 9300); nmr (DMSO-d_6–D_2O, 9:1, trace of CD_3COOD) δ 7.86 (d, 1, H_6), 5.82 (m, 1, J = 7.5 Hz, H_1'), 5.21 (m, 1, J = 3 Hz, H_3'), 4.33 (m, 1, J = 5 Hz, H_2'), 4.13 (s, 2, $-COCH_2O-$), 3.34 (s, 3, $-OCH_3$). *Anal.* Calcd for $C_{12}H_{16}N_2O_8$: C, 45.57; H, 5.10; N, 8.86. Found: C, 45.35; H, 5.20; N, 8.80.

3′-*O*-Methoxyacetyl-5′-*O*-mono-*p*-methoxytrityluridine (3). A solution of **2** (2.0 g, 6.4 mmol) in dry pyridine (40 ml) was treated with mono-*p*-methoxytrityl chloride (5.0 g, 16.2 mmol) for 2 days at room temperature. The product was poured with stirring into ice water (500 ml). The precipitate was collected and dissolved in chloroform. The chloroform solution was dried (Na_2SO_4) and then was added dropwise with stirring into 700 ml of petroleum ether (bp 30–60°). The white precipitate was collected by filtration and washed with petroleum ether to give 3.5 g (93%) of **3**: mp 118–120°; nmr (DMSO-d_6–D_2O, 9:1 v/v) δ 7.35 (m, 15, phenyl protons and H_6), 5.82 (m, 1, H_1'), 5.34 (m, 1, H_3'), 4.50 (m, 1, H_2'), 4.16 (s, 2, $-COCH_2O-$), 3.73 (s, 3, $PhOCH_3$), 3.36 (s, 3, $-OCH_3$); uv max (95% EtOH) 260, 232 mμ (shoulder); min 247 mμ. *Anal.* Calcd for $C_{32}H_{32}N_2O_9$: C, 65.30; H, 5.48; N, 4.76. Found: C, 65.10; H, 5.61; N, 4.76.

2′-*O*-Benzoylpropionyl-3′-*O*-methoxyacetyl-5′-*O*-mono-*p*-methoxytrityluridine (4). A solution of β-benzoylpropionic acid (4.5 g, 25 mmol) in dry pyridine (30 ml) was treated with dicyclohexylcarbodiimide (5 g, 25 mmol) for 15 min at room temperature. Compound **3** (3.0 g, 6 mmol) was then added to the mixture with stirring. After 20 hr, the product was mixed with ice water (200 ml) overnight. The solid was collected by filtration and mixed with benzene (15 ml). The insoluble white urea was removed by filtration. The benzene solution was added dropwise with stirring into petroleum ether (600 ml). The tan precipitate was collected and crystallized from ethanol–hexane to give 3.3 g (74%) of **4**: mp 146–148°; uv max (95% EtOH) 240 mμ; min 220 mμ; nmr (pyridine-d_5) δ 3.63 (s, 3, $PhOCH_3$), 3.44 (t, J = 6 Hz, 2, $PhCOCH_2-$), 3.36 (s, 3, $-OCH_3$), 3.17 (t, J = 6 Hz, 2, $-COCH_2-$). *Anal.* Calcd for $C_{42}H_{20}N_2O_{11}$: C, 67.37; H, 5.38; N, 3.74. Found: C, 67.17; H, 5.40; N, 3.62.

2′-*O*-Benzoylpropionyl-3′-*O*-methoxyacetyluridine (5). A solution of **4** (3.0 g, 4 mmol) in ethanol, water, and acetic acid (1:1:1, v/v, 90 ml) was allowed to stand at room temperature for 8 hr. Judged by tlc, essentially all of compound **4** disappeared. The solution was evaporated under reduced pressure to dryness. The residue was mixed with ether (40 ml) and ethanol (15 ml). Three 30-ml portions of water were used to extract the product. The aqueous solutions were combined, washed, and freeze dried to give 0.85 g (45%) of **5**: uv max (H_2O) 250 mμ; min 234 mμ; nmr (DMSO-d_6–D_2O, 9:1 v/v) δ 7.98, 7.66 (m, 6, phenyl proton and H_6), 6.05 (m, 1, H_1'), 5.45 (m, 2, H_2' and H_3'), 4.17 (s, 2, $-COCH_2O-$), 3.37 (s, 3, $-OCH_3$), 3.30 (t, J = 5 Hz, 2, $PhCOCH_2-$), 2.58 (t, J = 5 Hz, 2, $-COCH_2-$). *Anal.* Calcd for $C_{22}H_{24}N_2O_{10}$: C, 55.46; H, 5.08; N, 5.88. Found: C, 55.26; H, 5.19; N, 5.43.

2′-*O*-Benzoylpropionyl-3′-*O*-methoxyacetyluridine 5′-Phosphate (6). A solution of 2′-*O*-benzoylpropionyl-3′-*O*-methoxyacetyluridine (**5**) (100 mg, 0.21 mmol) in trimethyl phosphate (0.4 ml) was cooled to −60° in a Dry Ice–acetone bath. Phosphorus oxychloride ($POCl_3$) (0.6 ml, 6.5 mmol) was added, and the mixture was kept at 0° for 12 hr. Ice-water (5 ml) was then added, and after 0.5 hr the solution was evaporated to dryness. The residue was dissolved in 100 ml of water. After the pH was adjusted to 6.5, the solution (containing 2200 OD^{17} units at 260 mμ) was charged onto a DEAE Sephadex A-25 (HCO_3^- form) column preequilibrated with 0.01 M NH_4HCO_3 (pH 6.5). Elution was accomplished using a linear gradient with 0.01 M NH_4HCO_3 (pH 6.5, 2 l.) in the mixing chamber and 0.35 M NH_4HCO_3 (pH 6.5, 2 l.) in the reservoir. Fractions of 15 ml were collected, and those exhibiting the expected uv spectra were combined and freeze dried to give 40 mg of **6** (31%) as a diammonium salt: uv max (H_2O) 252 mμ; min 234 mμ. *Anal.* Calcd for $C_{22}H_{31}N_4O_{13}P$: C, 44.75; H, 5.29; N, 9.49; P, 5.25. Found: C, 44.55; H, 5.38; N, 9.25; P, 5.20.

Addition of 5 to Polymer-Support Preparation of 7. Succinylated isotactic polymer **1** (100 mg, 0.99 mmol of $COCH_2CH_2CO_2H$/g of polymer) and dicyclohexylcarbodiimide (0.55 g, 2.8 mmol) were suspended in 5 ml of dry pyridine. Compound **5** (200 mg, 0.8 mmol) was added, and the mixture was stoppered and stirred at room temperature for 24 hr. Then ice-water (20 ml) was added and the mixture was allowed to stand for an additional hour. The solid was recovered by filtration and was washed by methanol until no urea was detected from the filtrate. The product was dried under vacuum and gave 110 mg as tan solid.

Removal of Uridine from the Polymer 7. A. Compound **7** (5.0 mg) was suspended in 0.5 M hydrazine in pyridine–acetic acid (4:1 v/v, 1 ml) and was shaken for 4 hr at room temperature. Ammonium hydroxide (0.5 N, 1 ml) was added, and the mixture was extracted with three 2-ml portions of ethyl ether. Then the aqueous layer was chromatographed on Whatman 3 MM paper in the solvent system S_1 for 20 hr. The band of R_f 0.44 was eluted with water to give 13.5 OD units at 262 mμ. No other uv absorbing band was observed: (0.27 mmol of uridine/g of polymer) uv max (H_2O) 262 mμ; min 230 mμ.

B. Compound **7** (10 mg) was suspended in 1 ml of methanolic ammonia (methanol saturated with gaseous NH_3 at room temperature) and was shaken at room temperature. At different time intervals, 0.1 ml of solution was pipetted out and diluted to 5 ml with water. The solution was washed with 2 ml of ethyl ether. Concentrated HCl was used to acidify the aqueous solution which was again extracted with three 2-ml portions of ethyl ether. The aqueous solution was neutralized, and the volume was adjusted to 5 ml before the uv spectrum was taken. Under this condition, in 2.5 hr, uridine was recovered completely free from the polymer support and the blocking groups.

Removal of 3′-*O*′-Methoxyacetyl Group from the Polymer 7 (8). Polymer **7** (50 mg) was treated with methanol (2 ml) and 0.2 N ammonium hydroxide (2 ml) at room temperature for 30 min. Then the solid was separated by filtration and washed well with methanol to give 48 mg of **8** after drying. The filtrate was examined by uv, and no significant absorption was observed.

Preparation of 9. A solution of **6** (40 mg, 67 μmol) in 5 ml of pyridine–water (1:1 v/v) was passed through a Dowex-50 pyridinium 10 cm × 1.2 cm column to replace the ammonium ion. Pyridine–water (1:1 v/v) (50 ml) was used to wash the column. The eluent was combined and evaporated to dryness under reduced pressure. Then the residue was evaporated repeatedly with dry pyridine to drive off the moisture. A solution of the residue in 2 ml of pyridine was treated with mesitylenesulfonyl chloride (20 mg, 92 μmol) and polymer **8** (50 mg, 13.5 μmol of uridine). The suspension was allowed to stand at room temperature for 8 hr. Then ice-water (10 ml) was added, and after 14 hr the solid was separated by filtration. Water (100 ml) and methanol (150 ml) were used to wash the solid which then was dried under vacuum to give 49 mg of **9**.

Removal of Uridylyl-(3′–5′)-uridine from the Polymer 9. A sample of 3.0 mg of **9** was suspended in 1 ml of 0.5 M hydrazine in pyridine–acetic acid (4:1 v/v) and was shaken for 5 hr at room temperature. Ammonium hydroxide (0.5 N, 1 ml) was added, and the mixture was extracted with three 2-ml portions of ethyl ether. The aqueous layer was then chromatographed on Whatman 3 MM paper in the solvent system S_1 for 1 day. The band of the slowest mobility (R_f 0.15) was eluted with water to give 8.1 OD units at 262 mμ of the dinucleotide (51%) yield, based on the amount of uridine in the starting polymer. Unreacted uridine (4.0 OD units, R_f 0.44) was also recovered along with the dinucleotide. The dinucleotide was cleaved completely to uridine and uridine 5′-phosphate (1:0.94) by venom phosphodiesterase and underwent degradation to the extent of 96% on treatment with pancreatic ribonuclease to give uridine and uridine 3′-phosphate in the ratio 1:0.96.

Removal of 3′-*O*-Methoxyacetyl Group from the Polymer 9. Compound **9** (30 mg) was treated with methanol (2 ml) and 0.2 N ammonium hydroxide (2 ml) at room temperature for 30 min. Then the solid was separated by filtration and washed well with methanol to give 29 mg of **10** after drying. The filtrate was examined by uv, and no significant absorption was observed.

(17) OD refers to the absorbance value obtained for a 1-ml solution of a sample measured in a cuvette with a 1-cm light path.

Preparation of 11. A solution of **6** (12 mg, 20 μmol) in 5 ml of pyridine–water (1:1 v/v) was passed through a Dowex-50 pyridinium 5 cm × 1.2 cm column to replace the ammonium ion. Pyridine–water (1:1 v/v) (20 ml) was used to wash the column. The eluent was combined and evaporated to dryness under reduced pressure. The residue was then repeatedly evaporated with dry pyridine to drive off the moisture. A solution of the residue in 1 ml of pyridine was treated with mesitylenesulfonyl chloride (10 mg, 41 μmol) and polymer **10** (30 mg, 4.1 μmol of uridine dinucleotide). The suspension was allowed to stand at room temperature for 8 hr. Then ice–water (20 ml) was added, and after 14 hr the solid was separated by filtration. Water (100 ml) and methanol (150 ml) were used to wash the solid which then was dried under vacuum to give 29 mg of **11**.

Removal of Uridylyl-(3′–5′)-uridylyl-(3′–5′)-uridine from the Polymer 11. A sample of 4.6 mg of **11** was suspended in 1 ml of 0.5 *M* hydrazine in pyridine–acetic acid (4:1 v/v), and the mixture was shaken for 5 hr at room temperature. Ammonium hydroxide (0.5 *N*, 1 ml) was added, and the mixture was extracted with three 2-ml portions of ethyl ether. Then the aqueous layer was chromatographed on Whatman 3 MM paper in the solvent system S_1 for 1 day. The band of the slowest mobility (R_f 0.03) was eluted with water to give 9.2 OD units at 262 mμ of the trinucleotide, 48% yield based on the dinucleotide on the starting polymer. The trinucleotide was cleaved completely to uridine and uridine 5′-phosphate (1:1.90) by venom phosphodiesterase and underwent degradation to the extent of 95% upon pancreatic ribonuclease digestion to give uridine and uridine 3′-phosphate in the ratio 1:1.95.

The Conformation of Poly-β-alanine in Aqueous Solution from Proton Magnetic Resonance and Deuterium Exchange Studies[1]

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Abstract: Studies of poly-β-alanine (PBA) in D_2O solution were carried out on a highly branched, high molecular weight (>58,000) sample and a linear low molecular weight (18,000) sample using proton magnetic resonance (pmr) to observe the spin–spin coupling between α and β protons and deuterium exchange kinetics to test for conformational concealment of NH groups. Pmr spectra of PBA, β-alanine, *N*-acetyl-β-alanine ethyl ester (ABAEE), and *N*-acetyl-β-alanine-*N*′-methylamide (ABAMA) imply rapid internal rotation about the C^{α}–C^{β} bond with equal populations of the three staggered conformations. The pD dependence of the deuterium exchange rate of PBA is very similar to that of ABAEE and ABAMA, suggesting an absence of appreciable intramolecular hydrogen bonding in PBA, consistent with the random structure indicated by the pmr spectrum. The absence of hypochromism in the π–π^* band at 190 nm in a linear PBA (mol wt 58,000) is likewise consistent with the absence of ordered secondary structure.

The factors governing the stabilities of various chain conformations in poly-α-amino acids are gradually becoming clearer through studies of a wide variety of these materials, including both synthetic polymers and natural proteins.[2,3] The search for a full understanding of these factors is aided by studies of homologous polymers as well, since these provide additional tests of any hypothesis that may be put forward to explain conformational stability. The poly-β-amino acids are of particular interest for this purpose because of their close homology to the poly-α-amino acids.

A previous study[4] of poly-β-L-aspartic acid in aqueous solution suggested that this material in its uncharged form does take on an ordered conformation, possibly helical. Though this evidence was not conclusive, it encouraged further study of poly-β-amino acids, and the present work on poly-β-alanine, $(NHCH_2CH_2CO)_n$, seemed particularly relevant in view of its importance as the simplest representative of the family. Moreover, poly-β-alanine (PBA) is easily synthesized[5] and can be studied in aqueous solution when the sample is either highly branched or of low molecular weight.[6] The primary conformational tools used in the present study are proton magnetic resonance (pmr) spectroscopy and deuterium exchange kinetics.

Some of the properties of the PBA samples prepared by anionic polymerization of acrylamide have been reported elsewhere.[6] It was shown that chain branching often occurs due to the formation of the trifunctional β,β′-iminodipropionic acid residue in the polymer chain. Unbranched samples of high molecular weight are insoluble in water and other solvents favoring ordered conformations in poly-α-amino acids, while branching enhances water solubility enormously. The present study of aqueous solution properties is based largely on studies of a highly branched, high molecular weight sample and an unbranched, low molecular weight sample.

One of the early observations[6] suggesting an unusual polymer conformation (in a sample which later proved to be highly branched) was the strong flow birefringence of a dilute aqueous solution. This indicated a highly

(1) Adapted from the Ph.D. Thesis of J. D. Glickson, Columbia University, New York, N. Y., 1968, on work performed at Columbia and Iowa State Universities.

(2) Many aspects of this subject are reviewed in G. D. Fasman, Ed., "Poly-α-amino Acids: Protein Models for Conformational Studies," Marcel Dekker, New York, N. Y., 1967.

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