parison of ROTMS peaks with internal standard hydrocarbon peaks by glpc after work-up of samples with pentane and dilute acetic acid, thus avoiding uncontrolled reactions during glpc analysis. It was secured

that there was no measurable reaction during or after work-up by analysis of test mixtures with a known composition of ROH, ROTMS, and hydrocarbon in the absence and in the presence of excess HMDS.

Measurements with 4-tert-butylcyclohexanols (Table II) show that an OH group reacts ten times faster in the

Table II. Pseudo-First-Order Rate Constants^a for the Reaction of ROH with HMDS^b

ROH	OH position	$k_1 \times 10^5$, sec ⁻¹
4-tert-Butylcyclohexanol, trans cis	Eq Ax	$\frac{13.8 \pm 1.2}{1.4 \pm 0.1}$
Cyclohexanol 4-Methylcyclohexanol, cis 2-Norborneol, exo endo	90 % eq 90 % ax	$17.0 \pm 2.0 \\ 3.3 \pm 0.3 \\ 18.3 \pm 2.0 \\ 6.8 \pm 0.7$
Fenchol, endo		0.07 ± 0.007

^a The second-order rate constants are: $k_2 = k_1/[HMDS]$. ^b Reaction conditions: [ROH] = 0.053 M, [HMDS] = 0.46 M in pyridine at 25.0°.

equatorial than in the axial position, and the rate with cis-4-methylcyclohexanol properly reflects the equilibrium with predominantly (92%) axial OH. The rate differences between exo- and endo-2-norborneol are surprisingly smaller than in the cyclohexyl case, but still high compared to the practically equal succinate saponification rates.¹² That the HMDS reaction is very sensitive to steric hindrance by substituents is shown by the 100-fold rate decrease found for endo-fenchol. While it is clear from the cyclohexyl OTMS A value and from the cmr shifts that steric interactions are not larger for the OTMS than for the OH group in the ground state, it is evident from the kinetic measurements that the transition states for introduction of a trimethylsilyl group are quite sensitive to the steric environment of the OH group.

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Unexpected Conformational Stability of Poly(2'-azido-2'-deoxyuridylic acid)

Sir:

Although the conformational stability of polyribonucleotides and polydeoxyribonucleotides differs markedly, no satisfactory theory has been developed for this phenomenon. Intramolecular hydrogen bonding through the 2'-hydroxyl cannot be the reason for the greater stability of polyribonucleotides, because poly-

(2'-O-methyluridylic acid) (poly U_m)¹ and poly(2'-Oadenylic acid) (poly A_m)² are thermally more stable than poly U or poly A. The most recent variation, viz., poly(2'-chloro-2'-deoxyuridylic acid) (poly U_{Ci}),³ is unusual in that it has no stable secondary structure as a single strand, but forms a stable double-stranded complex with poly A. We now wish to report on poly-(2'-azido-2'-deoxyuridylic acid) (poly U_z) which, quite unexpectedly, possesses a highly ordered structure both in the single- and double-stranded forms.

Tritylation of 2'-azido-2'-deoxyuridine⁴ (I) gave II⁵ (glass; mp 89–91°; $\nu_{max} 2120 \text{ cm}^{-1}(N_3)$; pmr (CDCl₃) δ 9.60 (s, 1, NH), 7.83 (d, 1, J = 8 Hz, H-6), 7.33 (br s, 15, trityl H), 5.94 (d, 1, J = 3 Hz, H-1'), 5.37 (d, 1, J = 8 Hz, H-5), 4.50 (br m, 1, H-3'), 4.12 (br m, 2, H-2') and H-4'), 3.55 (br s, 2, H-5'), 3.08 (d, 1, J = 6 Hz, 3'-OH)). Reaction of II with acetic anhydride in dry pyridine for 12 hr at 0° gave III (85%; glass; mp 87– 89°; ν_{max} 2120 (N₃) and 1740 cm⁻¹ (OAc); pmr (CD-Cl₃) δ 9.07 (s, 1, N–H), 7.75 (d, 1, J = 8 Hz, H-6), 7.36 (br s, 15, trityl H), 6.04 (d, 1, H-1'), 5.45 (d, 1, J = 8Hz, H-5), 5.25 (br m, 1, H-3'), 4.24 (br m, 2, H-2' and H-4'), 3.56 (br s, 2, H-5'), 2.24 (s, 3, acetate- CH_3)). Hydrolysis of III in 80% HOAc gave IV (88%; prisms; mp 189–191°; ν_{max} 2120 (N₃) and 1750 cm⁻¹ (OAc); uv $\lambda_{\max}^{CH_{4}OH}$ 260 nm). Phosphorylation of IV by a modification of the cyanoethyl phosphate procedure⁶ gave 2'-azido-2'-deoxyuridine 5'-monophosphate (V; 55%; $\nu_{\rm max} 2120 \text{ cm}^{-1}$ (N₃); $R_{f(\rm UMP)} = 2.0$ (system A, isobutyric acid-1 M NH₄OH-0.2 M EDTA, 100:60: 0.8)). Bacterial alkaline phosphatase digestion of V gave I quantitatively. The phosphate V was converted, via the morpholidate,⁷ to 2'-azido-2'-deoxyuridine 5'-diphosphate (VI; 60%; ν_{max} 2120 cm⁻¹ (N₃); uv $\lambda_{\max}^{\text{H20}}$ 262 nm (ϵ_{\max} 10,000); $R_{f(\text{UDP})} = 1.75$ (system A)). Treatment of VI with alkaline phosphatase gave I quantitatively. The diphosphate VI was polymerized by polynucleotide phosphorylase⁸ (M. luteus) with Mg²⁺ as cofactor. After deproteinization with Genetron 113, the polymer was isolated in 30% overall vield by gel filtration on a Sephadex G-100 column from which the polymer was excluded in the void volume. Use of Mn^{2+} as cofactor⁹ raised the yield by 10-20%, but was not necessary for de novo synthesis. This contrasts sharply with the behavior of other nucleoside diphosphates modified in the 2' position,^{1, 3, 10} since such substrates usually require Mn²⁺ for polymerization.

Poly U_z prepared in this manner (as Dr. C. B. Klee of this Institute kindly determined) had $s_{20,w} = 8.0$ S (0.1 M NaCl, 0.01 M NaH₂PO₄, 0.001 M EDTA, pH 6.5) and gave only I upon hydrolysis with a mixture of

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alkaline phosphatase, snake venom phosphodiesterase, and micrococcal nuclease.¹¹ As required by the absence of a 2'-OH function, poly U_z was completely resistant to degradation by pancreatic ribonuclease.¹²



The thermal stability of (presumably) single-stranded poly U₂ is novel (Figure 1A), because with $T_m = 12^\circ$, it surpasses $T_m = 6^\circ$ for poly U under comparable conditions.¹³ The shape and midpoint of the phase transition did not depend on the solvent (1.0 *M* NaCl, pH 7.5, or 0.01 *M* MgCl₂, pH 7.4), but became less cooperative and markedly elevated (~10°) in the presence of 0.1 *M* MgCl₂. Poly U₂ formed a 1:1 complex with poly A as evidenced by the eutectic point at 50 mol %. This complex underwent a smooth cooperative transition with $T_m = 59^\circ$ (Figure 1B), not markedly different from poly A · poly U.¹⁴ All transitions were completely reversible.

Poly U_z is the first example of a stable secondary structure in a single-stranded polynucleotide without a 2'-oxygen function.³ When the 2'-hydroxyl of poly U is replaced by chlorine, the single-stranded poly U_{C1} is destabilized,³ whereas substitution by the azido group has the opposite effect. Since both poly U_z and poly U_{C1} form equally stable double-stranded complexes with poly A, the predominant stabilizing influence must differ in the single-stranded forms.

The availability of a model as unique as poly U_z should prove helpful in further studying the nature of the forces operative in stabilization, transcription, ¹⁵ and interferon stimulation ¹⁶ now in progress.

After this paper was submitted for publication, the syntheses and physical properties of poly (2'-fluoro-2'-deoxyuridylic acid)¹⁷ (poly U_F) and poly(2'-amino-2'-deoxyuridylic acid)¹⁸ (poly U_A) appeared. Surprisingly, both poly U_F and poly U_A are devoid of significant secondary structure at temperatures > 2°. The latter authors¹⁸ were also able to prepare poly U_z but did not characterize it further. Thus, while 2'-fluoro, 2'-chloro, 2'-amino, and 2'-deoxy substituents decrease

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Figure 1. (A) Uv absorption-temperature profile of poly U (O) and poly $U_z(\times)$ in 0.01 *M* MgCl₂, pH 7.6. A_T/A_I is the ratio of absorbance at temperature *T* over the absorbance at the initial temperature. (B) Uv absorption-temperature profile of the complex poly A·2 poly U (O) and poly A·poly $U_z(\times)$ in 0.1 *M* NaCl, 0.01 *M* NaH₂PO₄, pH 7.5. The inflection at 45° represents melting of the trajle-stranded poly A·2 poly U complex while the transition at 57° represents melting of the double-stranded poly A·poly U complex (also see ref 14).

the observed secondary structure of polyuridylic acid, the 2'-methoxy and 2'-azido substituents *alone* give rise to an increase in secondary structure.

(19) National Institutes of Health Staff Fellow, July 1969-present.

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Helix-Coil Transition of a Synthetic Polypeptide Monitored by Fourier Transform Carbon-13 Nuclear Magnetic Resonance

Sir:

We wish to report the preliminary results of the first¹ application of 13 C nmr to the study of the helix-coil transition of a synthetic homopolypeptide. We

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