Reaction of Uridine and Uridine 5′-Phosphate with Diiminosuccinonitrile and Cyanogen Bromide in Aqueous Solution. Direct Synthesis of the 2,2′-Anhydronucleoside Linkage at 2 °C

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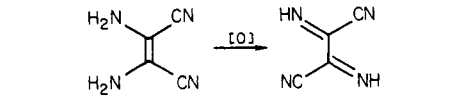
Reaction of uridine with diiminosuccinonitrile (DISN) at 2 °C in aqueous solution yields 2,2′-anhydrouridine 3-carbamate (7) (54%) along with 2,2′-anhydrouridine (8) (10%), arabinofuranosyluracil (9) (9%), uridine 2′,3′-carbonate (10) (5%), uridine 2′-carbamate (11) (3%), and uridine 3′-carbamate (12) (9%). A similar distribution of reaction products was obtained when BrCN was used in place of DISN. The same reaction products were isolated from the reaction of DISN or BrCN with uridine 5′-phosphate after the phosphate grouping was cleaved from the initial reaction product mixture with alkaline phosphatase. A reaction pathway is proposed in which the imidocarbonate derivative 6 is a common intermediate for product formation. The vicinal 2′,3′-hydroxyl groups are essential for reaction as shown by the failure to form stable reaction products with thymidine (17). Adenosine (13), which has vicinal 2′,3′-hydroxyl groups, is converted to a mixture of the 2′- and 3′-carbamates (15 and 16) via an imidocarbonate (14) intermediate with DISN or BrCN. The relevance of these studies to chemical evolution is discussed.

One focus of our studies of the origins of life has been the possible role of HCN and cyano compounds as precursors to biological molecules. HCN is present in the interstellar medium and the atmospheres of Titan and Jupiter. It is formed readily by the action of electrical discharges, shock waves, and ultraviolet light on a variety of gas mixtures which have been suggested as models for the atmosphere of the primitive earth. We conclude from these observations that HCN was present on the early earth and its reactions may have contributed to the chemical processes leading to the origins of life.[1]

In our earlier studies, in which we investigated the formation of adenine and some amino acids from HCN,[2] it was observed that all the biologically significant purines can be prepared from HCN and simple derivatives of HCN along with representative amino acids and pyrimidines.[1] A tetramer of HCN, diaminomaleonitrile (1), which forms spontaneously in dilute, pH 9 HCN solutions, is a central intermediate in these prebiotic syntheses.[1]



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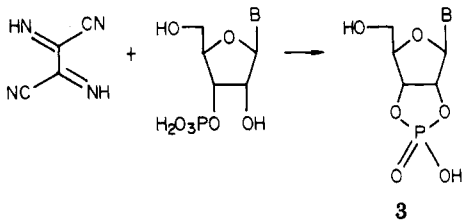

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The possible role of the oxidation product of diaminomaleonitrile (1), diiminosuccinonitrile (DISN) (2), as a prebiotic condensing agent is currently under investigation. DISN is readily prepared by the oxidation of diaminomaleonitrile with ferric iron,[3] DDQ,[4] or MnO_2,[5] and its reaction with nucleophiles has been studied in some detail.[5] We observed the cyclization of 3′-UMP and 3′-AMP to the corresponding 2′,3′-cyclic nucleotide 3 with DISN and BrCN.[6] In this paper we report a markedly different pathway in the reaction of DISN with 5′-UMP.

Results and Discussion

The successful conversion of 3′-nucleotides to the corresponding 2′,3′-cyclic nucleotides[6] prompted the investigation of the reaction of 5′-nucleotides with DISN with the goal of forming an internucleotide linkage. TLC analysis of the reaction mixture formed by heating 5′-UMP and DISN for 3 h at 60 °C indicated that 5′-UMP did not react. In addition, no 3′,5′-cyclic AMP could be detected by HPLC analysis when 5′-AMP was heated with DISN either in the absence of metal ions or in the presence of Mg^{2+}, Pb^{2+}, or Mn^{2+}.[7] However, when the reaction of 5′-UMP and DISN (2) was performed at 0–5 °C for 4 days, all the 5′-UMP was converted to new products which appeared by TLC analysis to regenerate 5′-UMP on warming the reaction solution to 60 °C. Since the latter hydrolytic conditions (60 °C, 3 h, pH 7) were too mild for the cleavage of a phosphodiester or pyrophosphate linkage, it was concluded that the observed reaction products were not due to the formation of oligonucleotide derivatives. This conclusion was supported by the observation that the HPLC retention time of the product mixture was not changed on incubation with venom or spleen phosphodiesterase or RNase T_2, enzymes which cleave the phosphodiester linkage or pyrophosphatase an enzyme that cleaves the pyrophosphate linkage.[8] Alkaline phosphatase, an enzyme that cleaves simple alkyl phosphate esters, catalyzed the hydrolysis of the phosphate group from the adduct of DISN with 5′-UMP. This finding indicated that the new adduct was not formed by reaction at the 5′-phosphate group since alkaline phosphatase cleaved the starting 5′-UMP to uridine under the same reaction conditions. The conflicting observation which suggested the presence of a derivative of 5′-phosphate grouping was the absence of phosphate cleavage with 5′-nucleotidase. This

(1) Ferris, J. P.; Hagan, W. J., Jr. *Tetrahedron* **1984**, *40*, 1093–1120.
(2) Oro, J.; Biochem. *Biophys. Res. Commun.* **1960**, *2*, 407–412. Oro, J.; Kimball, A. P. *Arch. Biochem. Biophys.* **1961**, *94*, 217–226. Oro, J.; Kimball, A. P. *Ibid.* **1961**, *94*, 293–313.
(3) Ferris, J. P.; Hagan, W. J., Jr.; Alwis, K. W.; McCrae, J. *J. Mol. Evol.* **1982**, *18*, 304–309.
(4) Webster, O. W.; Hartter, D. R.; Begland, R. W.; Sheppard, W. A.; Cairncross, A. *J. Org. Chem.* **1972**, *37*, 4133–4136.
(5) Webster, O. W. U.S. Patent 3 862 205, Jan 21, 1975.
(6) Ferris, J. P.; Yanagawa, H.; Dudgeon, P. A.; Hagan, W. J., Jr.; Mallare, T. E. *Origin Life*, in press.
(7) Unpublished work of T. Mallare in this laboratory.
(8) Zubay, G. "Biochemistry", Addison-Wesley: Reading, MA, 1983.

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enzyme cleaved 5′-UMP to uridine but it failed to catalyze the cleavage of the phosphate grouping from the DISN adduct of 5′-UMP. It is not clear why the reaction with 5′-nucleotidase was negative.

Since the main body of data indicated that observed products were not due to reaction at the phosphate grouping of 5′-UMP, the reaction of uridine (**4**) and DISN (**2**) was investigated as nucleoside derivatives are more amenable to analysis by TLC and HPLC than the corresponding nucleotides. Reaction of DISN with uridine under the same conditions as used for the reaction with 5′-UMP gave a product mixture that exhibited five spots on TLC. The major product (54%) was shown to be 2,2′-anhydrouridine 3′-carbamate (**7**) by its characteristic 2,2′-anhydrouridine UV spectrum[9] with maxima at 221 and 246.6 nm, a 3′-H signal in the ^{1}H NMR at δ 5.24 which was shifted downfield relative to that of 2,2′-anhydrouridine (**8**) at δ 4.40, and a signal at δ 158.3 in the ^{13}C NMR spectrum for the carbamate carbon.[10] The spectral assignments were confirmed by the alkaline hydrolysis of **7** to arabinofuranosyluracil (**9**) in 90% yield.[11]

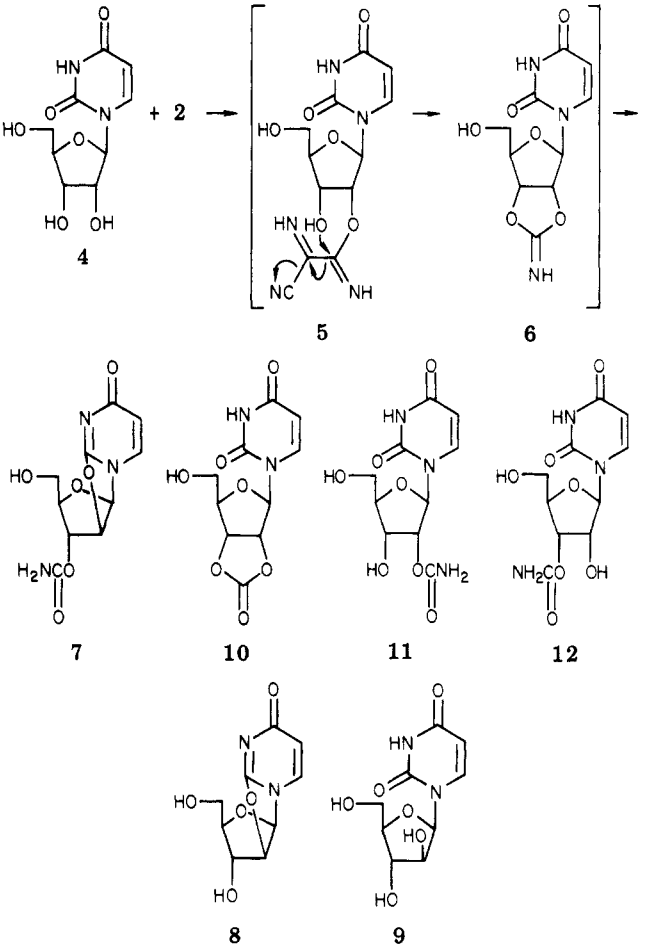
The 2′,3′-carbonate of uridine (**10**),[12] 2,2′-anhydrouridine (**8**),[9] and arabinofuranosyluracil (**9**)[9] were each formed in about 5–10% yield and were identified by direct comparison with authentic samples. The 2′- and 3′-carbamates of uridine were formed in about 10% overall yield. The structural assignments of these carbamates was made on the bases of the downfield shift of the 2′-H and 3′-H, respectively, relative to that of the signals for the corresponding protons in uridine, the signal at δ 155.44 in the ^{13}C NMR spectrum assigned to the carbonyl grouping in the more abundant 3′-carbamate, and the acidic and alkaline hydrolysis of both isomers to uridine at 60 °C.

The structure analysis of the adducts of 5′-UMP with DISN was readily performed once the analysis of the uridine–DISN reaction mixture was completed. Incubation of the product mixture resulting from the reaction of DISN and 5′-UMP with alkaline phosphatase cleaved the 5′-phosphate grouping as shown by the marked increase in the mobility of the product mixture on TLC (a change in R_f from 0 to 0.4 and greater). The TLC profile of hydrolyzed reaction mixture was identical with that observed with the product mixture resulting from the reaction of DISN and uridine. The same ensemble of reaction products was then isolated from the 5′-UMP reaction after cleavage of the phosphate grouping as was obtained from the reaction of uridine with DISN.

The absence of a product which can be attributed to the reaction of the 5′-phosphate of UMP with DISN does not mean that a reaction does not take place with the phosphate group. It is more likely that a reaction does take place but the DISN adduct formed is hydrolyzed. The corresponding reaction of DISN with 3′-UMP to give the 2′,3′-cyclic phosphate can only be understood on the basis of the formation of an activated phosphate adduct which then reacts with the proximate 2′-OH.[6] Water is the proximate nucleophile to the DISN adduct of 5′-UMP and it hydrolyzes the adduct back to 5′-UMP.

The reaction pathway in Scheme I, illustrated for the uridine reaction, was initially postulated on the basis of

Scheme I



the observed reaction products. The 2′-OH is proposed as the principal initial site of reaction because its nucleophilicity is greater than that of the 3′-OH.[13] The cyclization of **5** to imidocarbonate **6** follows from the isolation of carbamate derivatives **7**, **11**, and **12**. The proposed fragmentation of the DISN molecule in the conversion of **5** to **6** has precedence in the formation of urea in the reaction of DISN with NH_3.[14] The intramolecular nucleophilic displacement of the imidocarbonate function at the 2′-position to give the anhydronucleoside **7** is analogous to the formation of anhydronucleosides from pyrimidine 2′,3′-cyclic carbonates.[9] However, the remarkably mild conditions for the cyclization of uridine to the 2,2′-anhydronucleoside derivatives is noteworthy. This reaction proceeds at 2 °C in aqueous medium while the conversion of the 2′,3′-cyclic carbonate **10** to 2,2′-anhydrouridine is performed in dimethylformamide containing 1 equiv of water at 150 °C.[9] Acid catalysis by protonation of the imido nitrogen may accelerate the conversion of **5** to **7**. 2,2′-Anhydrouridine (**8**) and arabinofuranosyluracil (**9**) are formed by the hydrolysis of **7**. The conversion of **10** to **8** which in turn is hydrolyzed to **9** is not considered likely because **10** is hydrolyzed to uridine on heating in aqueous base.[12] Furthermore, the conversion of **7** to **9** was shown to proceed in 90% yield.

It is now apparent why TLC analysis of the reaction product formed on heating 5′-UMP with DISN at 60 °C indicated that the 5′-UMP had not been reacted. The products that formed were hydrolyzed either to 5′-UMP or arabinofuranosyluracil 5′-phosphate. These polar nu-

(9) Hampton, A.; Nichol, A. W. *Biochemistry*, **1966**, *5*, 2076–2082. Ogilvie, K. K.; Iwacha, D. *Can. J. Chem.* **1969**, *47*, 495–497. Holy, A. *Collect. Czech. Chem. Commun.* **1977**, *37*, 4071–4087.

(10) Levy, G. C.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists"; Wiley-Interscience: New York, 1972; pp 126, 132.

(11) Michelson, A. M., "The Chemistry of Nucleosides and Nucleotides", Academic Press, New York, 1963.

(12) Letsinger, R. L.; Ogilvie, K. K. *J. Org. Chem.* **1967**, *32*, 296–300.

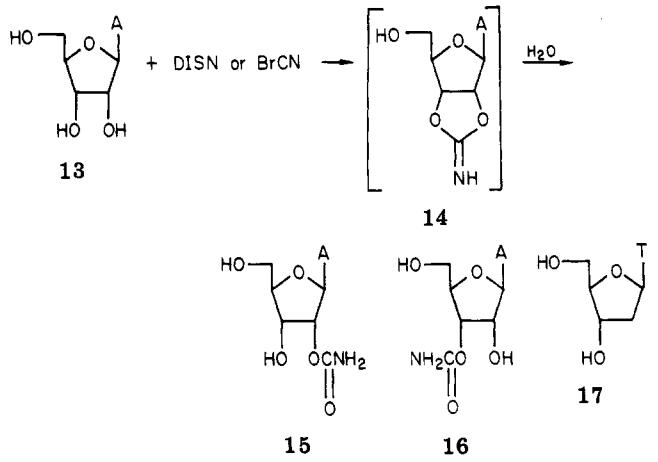
(13) Lohrmann, R.; Orgel, L. E. *Tetrahedron* **1978**, *34*, 853–855.

(14) Ferris, J. P.; Ryan, T. J. *J. Org. Chem.* **1973**, *38*, 3302–3307.

cleotides would be expected to migrate at very close to same rate on silica gel TLC and as a consequence it would appear that only 5'-UMP was present.

Evidence for the reaction pathway proposed in Scheme I was obtained by the substitution of BrCN for DISN in the reaction with uridine and 5'-UMP. Since BrCN is known to react with carbohydrates[15] and diols[16] to form imidocarbonates, the same ensemble of reaction products should be obtained by using BrCN as were observed with DISN if **6** were the reaction intermediate. The reaction of BrCN and uridine was complete in 45 min at pH 11.5 (vs. 4 days at pH 7 with DISN) to give the same products (compounds **7**, **10**, **11**, **12**, **8**, and **9**) as were obtained in the DISN reaction. This finding provides strong support for **6** as a reaction intermediate in the DISN–uridine reaction.

An investigation of the reaction of adenosine (**13**) with DISN and BrCN provided further support for the reaction pathway proposed in Scheme I. HPLC analysis of the reaction mixture, while the reaction was in progress, showed the conversion of adenosine to one new product, presumably the imidocarbonate **14**. HPLC analysis after



13 14 15 16 17

freeze-drying the reaction mixture showed the presence of two products, neither of which was identical with the product that was detected while the reaction was in progress. It was not possible to cleanly separate these two products by fractional crystallization but it was possible to assign their structures as the 2'- and 3'-carbamates of adenosine (**15** and **16**) on the basis of NMR studies. The ^1H NMR signals for each isomer could be assigned because the yield of 3'-carbamate exceeded that of the 2'-carbamate by a factor of 2–5 so its NMR signals were correspondingly more intense. The 2'- and 3'-carbamates could be differentiated on the basis of the low field shift of the 2'- and 3'-signals in the ^1H NMR. The carbamate carbon of the 2'- and 3'-isomers was detected by signals at δ 156.22 and 155.44, respectively, in the ^{13}C NMR spectra. The formation of **15** and **16** follows directly from the proposed imidocarbonate **14** intermediate.

The important role of the vicinal glycol moiety in the stabilization of the initial DISN adduct was demonstrated in its reaction with thymidine (**17**), a compound lacking the 2'-OH. When the course of the reaction was monitored by HPLC, two products were detected. All attempts to isolate these compounds resulted in their conversion to thymidine. These observations are consistent with the formation of adducts with the 3'- and 5'-hydroxyl groups, which are hydrolyzed because there is no low energy intramolecular reaction pathway available for the formation of a stable cyclic intermediate.

Relevance to Chemical Evolution

Experiments designed to model the prebiotic coupling of monoribonucleotides to oligonucleotides by using condensing agents in aqueous solution have met with limited success. Water-soluble carbodiimides do effect the formation of the internucleotide linkage in aqueous solution but it is difficult to envisage the prebiotic formation of carbodiimides.[17,18] Attempts to form the oligoribonucleotides by using cyanogen and other electrophilic nitriles, which are plausible prebiotic condensing agents, have been unsuccessful.[19] It has been possible to synthesize oligodeoxyribonucleotides in 3–4% yield from thymidine 5'-monophosphate in aqueous solution by using cyanamide as the condensing agent.[20]

Our studies indicate that the condensation of 5'-ribonucleotides to oligonucleotides does not proceed in aqueous solution because stable adducts are formed with the 2'- and 3'-hydroxyl groups. These adducts block the formation of the internucleotide linkage even though an activated adduct of the 5'-phosphate group is formed. It has been possible to form oligodeoxynucleotides by using cyanamide[20] because the adduct of the 3'-hydroxyl groups are readily hydrolyzed and, as a consequence, there will be a steady-state concentration of this 3'-hydroxyl group available for reaction with an activated 5'-phosphate grouping. The significance of the formation of oligodeoxynucleotides under prebiotic conditions is debatable since ribonucleotides, and not deoxyribonucleotides, are generally considered to have been the genetic material of the first forms of life.[21] Since the reaction of 3'-ribonucleotides with electrophilic nitriles yields the 2',3'-cyclic nucleotide, we are forced to conclude that other reagents or intermediates, in addition to the nucleotide and condensing agent, are required for such a synthesis to be successful. Examples include the polymerization of 5'-phosphorimidazolides[22] or 2',3'-cyclic nucleotides,[23] reactive intermediates formed from nucleotides.

2,2'-Anhydronucleotides have been proposed as intermediates in the formation of oligonucleotides on the primitive earth.[24] Our research provides some support for this hypothesis by demonstrating the facile formation of 2,2'-anhydronucleotide linkage under plausible prebiotic conditions.

Experimental Section

General Methods. IR spectra were measured in KBr, unless noted otherwise, on a Perkin-Elmer 298 spectrophotometer and UV spectra were determined on a Cary 219 spectrophotometer. ^1H NMR spectra were determined on a Varian XL-200 or Hitachi Perkin-Elmer R-600 and ^{13}C NMR a Varian XL-200 or FT-80 using Me_4Si as internal standard. Mass spectra were obtained with either a Hitachi Perkin-Elmer RMU-6E by electron impact at 70 eV or with a Kratos MS-25 by chemical ionization with CH_4 at 12 eV. Liquid chromatography of nucleosides was performed on a Whatman ODS-3 4.6 mm × 25 cm reverse-phase column by

(15) Lowe, C. R.; Dean, P. D. G. "Affinity Chromatography"; Wiley: New York, 1974; pp 206–211.
(16) Addor, R. W. *J. Org. Chem.* **1964**, *29*, 738–742.
(17) Naylor, R.; Gilham, P. T. *Biochemistry* **1966**, *5*, 2722–2728.
(18) Sulston, J.; Lohrmann, R.; Orgel, L. E.; Miles, H. T. *Proc. Nat. Acad. Sci. U.S.A.* **1968**, *59*, 726–733. Sulston, J.; Lohrmann, R.; Orgel, L. E.; Schneider-Bernloehr, H.; Weiman, B. J. *J. Mol. Biol.* **1964**, *40*, 227–234.
(19) Orgel, L. E.; Lohrmann, R. *Acc. Chem. Res.* **1974**, *7*, 368–377.
(20) Ibanez, J. D.; Kimball, A. P.; Oro, J. *Science (Washington, D.C.)* **1971**, *147*, 444–446.
(21) Ferris, J. P.; Usher, D. A. "Biochemistry"; Zubay, G., Ed.; Addison-Wesley: Reading, MA, 1983; p 1227.
(22) Inoue, T.; Orgel, L. E. *J. Mol. Biol.* **1982**, *162*, 201–217 and references therein.
(23) Usher, D. A.; McHale, A. H. *Science (Washington, D.C.)* **1976**, *192*, 53–54.
(24) Tapiero, C. M.; Nagyvary, J. *Nature (London)* **1971**, *231*, 42–43.

using a mobile phase of 0.01 M $NH_4H_2PO_4$ (pH 3.5):CH_3OH (9:1) and of nucleotides on a Varian 4 mm × 30 cm Micro Pak AX-10 anion exchange column by using 0.05 M KH_2PO_4 (pH 2.85) as the mobile phase.[25] Yields were determined from the HPLC peak areas by comparison with the areas measured for authentic samples chromatographed under identical conditions. Precoated silica gel sheets from Eastman Kodak were used for TLC analysis using 1-propanol–H_2O mixtures as eluants. Preparative TLC analysis was performed on Analtech 2-mm precoated plates or those prepared from Merck silica gel GF_{254}. Merck silica gel 60 (240–400 mesh) was employed for flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA.

Reaction of Uridine with DISN. A solution of uridine (0.976 g, 3.70 mmol) and DISN (2.12g, 20 mmol) in 50 mL of 0.2 M imidazole·HCl buffer (pH 7.0) was stirred for 4 days at 2 °C. The course of the reaction was monitored by TLC and HPLC. The reaction mixture was filtered, the filtrate freeze-dried, and the residue extracted with methanol. The methanol extract was stirred with Norit 211 at room temperature for 2 h and filtered. The methanol was evaporated and the residue extracted with ethanol. The residue remaining on evaporation of the ethanol was extracted with acetone and the acetone extract was concentrated to dryness and flash chromatographed on silica gel with ethyl acetate as the eluant. Uridine 2′,3′-carbonate (**10**) (5%) was eluted and purified by crystallization from ethyl acetate–chloroform: mp 105–110 °C (lit.[12] mp 120–125 °C). The product was shown to have IR, UV, mass spectrum, and ^1H and ^{13}C NMR spectra that were identical with that of an authentic sample of mp 120–125 °C.[12]

The acetone-insoluble fraction was purified by preparative TLC on silica gel by using 9:1 1-propanol:water as the eluant. 2,2′-Anhydrouridine 3′-carbamate (**7**) (54%) was eluted with methanol from a band at R_f 0.27: mp 182–184 °C dec from methanol–H_2O; ^1H NMR ($(CD_3)_2SO$) δ 3.69 (2 H, m, H-5′) 4.31 (1 H, t, $J_{4',5'}$ = 4.0 Hz, H-4′), 5.24 (1 H, s, $J_{3',4'}$ = 0 Hz, H-3′), 5.43 (1 H, d, $J_{2',3'}$ = 0 Hz, H-2′) 5.92 (1 H, d, $J_{5,6}$ = 7.2 Hz, H-5) 6.41 (1 H, d, $J_{1',2'}$ = 6.3 Hz, H-1′) 6.95 (2 H, br s, $OCONH_2$) 7.89 (1 H, d, H-6); ^{13}C NMR (D_2O) δ 61.74 (C-5′), 67.40 (C-3′), 79.28 (C-4′), 88.46 (C-2′), 91.92 (C-1′), 109.57 (C-5), 139.07 (C-6), 158.30 (carbamate), 162.01 (C-2), 173.33 (C-4); IR (KBr) 3400, 1720, 1653, 1625, 1525, 1480, 1390, 1354, 1240, 1090, 1045, 963, 830 cm^{-1}; UV_{max} (H_2O) 221 nm (ε 6.57 × 10^3), 246.6 (5.23 × 10^3), (0.01 N HCl) 222, 246.7 (0.01 N KOH), 220, 250; mass spectrum (CI), *m/e* 269 (M^+) 226, 195, 177, 167, 137, 115, 114, 113, 98, 85, 69, 57).

Anal. Calcd for $C_{10}H_{11}O_6N_3$: C, 44.62; H, 4.12; N, 15.61. Found: C, 44.57; H, 4.17; N, 15.59.

2,2′-Anhydrouridine (**8**) (10%) was eluted from the preparative TLC plate in a band of R_f 0.36 and was purified by crystallization from methanol, mp 215–217 °C dec (lit.[9] mp 238–244 °C). The product obtained had IR, UV, mass spectrum, and ^1H and ^{13}C NMR spectra that were identical with those of an authentic sample with mp 229–233 °C.[9]

A mixture of the 2′- and 3′-carbamates of uridine was eluted from the preparative TLC plate in a band of material at R_f 0.6. Additional amounts of the 3′-carbamate were eluted in a band of material at R_f 0.8. A purified sample of the 3′-carbamate **12** (9%) was obtained by crystallization of the latter band from ethanol: mp 129–132 °C; ^1H NMR ($(CD_3)_2SO$) δ 3.62 (2 H, m, H-5′), 4.01 (1 H, q, $J_{4',5'}$ = 2.7 Hz, H-4′), 4.26 (1 H, dd, $J_{2',3'}$ = 5.7 Hz, H-2′), 4.97 (1 H, dd, $J_{3',4'}$ = 2.7 Hz, H-3′), 5.74 (1 H, d, $J_{5,6}$ = 8.0 Hz, H-5), 5.89 (1 H, d, $J_{1',2'}$ = 7.4 Hz, H-1′), 6.72 (2 H, br s, $OCONH_2$), 7.93 (1 H, d, H-6); ^{13}C NMR ($(CD_3)_2SO$) δ 61.00 (C-5′), 72.37 (C-3′), 72.38 (C-2′), 83.22 (C-4′), 86.90 (C-1′), 102.16 (C-5), 140.29 (C-6), 150.81 (carbamate), 155.33 (C-2), 162.98 (C-4); IR (KBr) 3420, 1695, 1630, 1520, 1480, 1380, 1240, 1104, 1055, 824, 755 cm^{-1}; UV_{max} (H_2O) 257 nm; mass spectrum, *m/e* 288 (M + 1), 244, 226, 218, 210, 205, 186, 169, 113, 112, 69, 57, 44. It was not possible to obtain a sample of the 2′-carbamate **11** (yield ~3%) that was entirely free of the 3′-isomer as shown by HPLC. The following ^1H NMR spectrum in $(CD_3)_2SO$ is derived from analysis of the spectrum of a mixture of the 2′- and 3′-carbamates: δ 3.72 (2 H, m, H-5′), 3.91 (1 H, q, $J_{4',5'}$ = 2.7 Hz, H-4′), 4.20 (1 H, dd, $J_{3',4'}$ = 2.7 Hz, H-3′), 5.03 (1 H, dd, $J_{2',3'}$ = 6.5 Hz, H-2′), 5.74 (1 H, d, $J_{5,6}$ = 8.0 Hz, H-5), 6.07 (1 H, d, $J_{1',2'}$ = 6.0 Hz, H-1′), 7.18 (2 H, br s, $OCONH_2$), 7.97 (1 H, d, H-6).

The mixture of **11** and **12** was hydrolyzed to uridine (HPLC analysis) by heating for 17 h in H_2O (pH 5.2) or triethylamine:H_2O (75 μL:0.25 mL, pH 11.5).

Arabinofuranosyluracil (**9**) was eluted from the preparative TLC plate from a band of R_f 0.72. The eluate was crystallized from ethanol: mp 220–222 °C (lit.[26] mp 220–221 °C). The product had IR and ^1H NMR spectra identical with an authentic sample (Sigma).

Hydrolysis of 2,2′-Anhydrouridine 3′-Carbamate (7) to Arabinofuranosyluracil (9). 2,2′-Anhydrouridine 3′-carbamate (54 mg, 0.2 mmol) and triethylamine (0.6 mL, 4.3 mmol) were dissolved in 20 mL of distilled water (pH 11.5), and the mixture was stirred at 60 °C for 2 days and then freeze-dried. The solid product was extracted with chloroform to remove triethylamine salts, and the chloroform-insoluble fraction was purified by preparative TLC. The band with R_f of 0.7 was eluted with methanol (40 mg, 90%) and crystallized from ethanol to give arabinofuranosyluracil: mp 219–223 °C (lit.[26] mp 220–221 °C). The product was shown to be identical with an authentic sample (Sigma) by direct comparison of IR and ^1H and ^{13}C NMR spectra.

Reaction of Uridine 5′-Phosphate with DISN. A solution of 0.5 g (1.21 mmol) of uridine 5′-phosphate sodium salt and 0.641 g (6.05 mmol) of DISN in 25 mL of 0.2 M imidazole·HCl (pH 7.0) was stirred for 3 days at 2 °C. The reaction mixture was filtered and the filtrate freeze-dried and extracted with ethyl acetate. The ethyl acetate insoluble fraction was then extracted with methanol, the insolubles were removed by filtration, and the filtrate was concentrated to give a light brown powder. This HPLC peak of the product was difficult to resolve from that of 5′-UMP. Incubation at 37 °C for 30 min with the following enzymes did not result in a change of the HPLC profile: 1 μg of venom phosphodiesterase 1 (Sigma), pH 8.0 (TRIS·HCl); 2 μg of spleen phosphodiesterase (Sigma), pH 6.0 (imidazole·HCl) 1 μg of nucleotide pyrophosphatase (Sigma), pH 9.0 (Tris·HCl); 0.05 μg of RNase T_2, pH 5.0 (acetate), and 0.4 μg of 5′-nucleotidase (Sigma), pH 9.0 (Tris·HCl). The following enzymes cleaved the phosphate groupings from both the product and 5′-UMP: bovine intestine alkaline phosphatase (Sigma), pH 9.0 (Tris·HCl) and *E. coli* alkaline phosphatase (Sigma), pH 9.0 (Tris·HCl). In a preparative-scale experiment 100 mg of the powder obtained in the reaction of DISN with 5′-UMP was dissolved in 10 mL of distilled water and was added to 15 mL of 150 units of bovine alkaline phosphatase in 0.2 M Tris·HCl pH 8 buffer and incubated at 37 °C for 4 h. The digest was freeze-dried and the dephosphorylated products were isolated by the procedure outlined in the reaction of DISN with uridine to give the following products: uridine 2′,3′-carbonate (3%), 2,2′-anhydrouridine 3′-carbamate (32%), 2,2′-anhydrouridine (21%), arabinofuranosyluracil (6%), uridine 2′-carbamate (2%), and uridine 3′-carbamate (8%).

Reaction of Uridine with BrCN. Uridine (1.95 g, 7.99 mmol), BrCN (3.39 g, 32.0 mmol), and triethylamine (9.6 mL, 6.9 mmol) were dissolved in 100 mL of distilled water (initial pH 11.5), and the solution was stirred at 2 °C for 45 min. The reaction mixture was freeze-dried to give a yellow powder, which was extracted with chloroform to remove triethylamine hydrobromide. The chloroform-insoluble material was purified as described for the reaction of uridine with DISN, and uridine 2′,3′-carbonate (4%), 2,2′-anhydrouridine 3′-carbamate (37%), 2,2′-anhydrouridine (2%), arabinofuranosyluracil (13%), uridine 2′-carbamate (2%), and uridine 3′-carbamate (27%) were isolated as reaction products.

Reaction of Uridine 5′-Phosphate with BrCN. Uridine 5′-phosphate sodium salt (0.5 g, 1.21 mmol), BrCN (0.513 g, 4.84 mmol) and triethylamine (1.45 mL, 10.43 mmol) were dissolved in 15 mL of distilled water (initial pH 11.3) and stirred at 2 °C for 80 min. The reaction mixture was freeze-dried and the residue was extracted with chloroform to remove triethylamine hydrobromide. A portion of the chloroform-insoluble material (0.1 g) was dissolved in 10 mL of distilled water and added to 15 mL of 0.2 M Tris·HCl (pH 9.0) containing 150 units of bovine alkaline phosphatase and incubated at 37 °C for 4 h. The digest was

(25) Edelson, E. H.; Lawless, J. G., Wehr, C. T.; Abbott, S. R. *J. Chromatogr.* **1979**, *174*, 409–419.

(26) Shannahoff, D. H.; Sanchez, R. A. *J. Org. Chem.* **1973**, *38*, 593–598.

freeze-dried, and the residue was purified as described for the reaction of uridine with DISN.

Reaction of Adenosine with DISN. Adenosine (0.053 g, 0.20 mmol) and DISN (0.212 g, 2.0 mmol) were dissolved in 10 mL of 0.2 M imidazole·HCl buffer (pH 7.0), and the solution was stirred at 2 °C for 3 days. The reaction mixture was filtered and the filtrate was freeze-dried. The residue was extracted with methanol and the methanol-soluble fraction was treated with Norit 211 for 1 h at room temperature. The methanol was concentrated to give a powder which was dissolved in 0.6 M boric acid and was purified by preparative TLC on silica gel by development with 1-propanol:0.3 M boric acid (9:1). A mixture of adenosine 3′-carbamate (**16**) and adenosine 2′-carbamate (**15**) was eluted in a band of R_f 0.5. The mixture was crystallized from methanol–ethanol, and the product contained a 4.5:1 ratio of **16**(41%):**15**(9%) as shown by HPLC. Further crystallization yielded a pure sample of **16**: ^{1}H NMR ($(CD_3)_2SO$) δ 3.62 (2 H, m, H-5′), 4.11 (1 H, q, $J_{4',5'}$ = 2.4 Hz, H-4′), 4.81 (1 H, dd, $J_{2',3'}$ = 5.4 Hz, H-2′), 5.10 (1 H, dd, $J_{3',4'}$ = 2.2 Hz, H-3′), 5.91 (1 H, d, $J_{1',2'}$ = 7.1 Hz, H-1′), 6.72 (2 H, br s, $OCONH_2$), 7.43 (2 H, br s, NH_2), 8.19 (1 H, s, H-8), 8.46 (1 H, s, H-8); ^{13}C NMR ($(CD_3)_2SO$) δ 59.65 (C-5′), 72.03 (C-2′), 72.83 (C-3′), 84.03 (C-4′), 87.53 (C-1′), 119.33 (C-5), 139.63 (C-8), 149.12 (C-4), 152.37 (C-2), 155.44 (carbamate), 155.96 (C-6); IR (KBr) 3360, 1720, 1645, 1600, 1478, 1420, 1400, 1335, 1305, 1250, 1205, 1125, 1084 cm^{-1}.

The NMR spectral analysis of adenosine 2′-carbamate (**15**) was performed by comparison of the NMR spectra of the mixture of **15** and **16** with that of the purified sample of **16**. The derived spectra of **15** are as follows: ^{1}H NMR ($(CD_3)_2SO$) δ 3.62 (2 H, m, H-5), 4.41 (1 H, q, $J_{4',5'}$ = 2.4 Hz, H-4′), 4.42 (1 H, dd, $J_{3',4'}$ = 3.2 Hz, H-3′), 5.51 (1 H, dd, $J_{2',3'}$ = 4.9 Hz, H-2′), 6.11 (1 H, d, $J_{1',2'}$ = 6.7 Hz, H-1′), 6.72 (2 H, br s, $OCONH_2$), 7.43 (2 H, br s, NH_2), 8.19 (1 H, s, H-2), 8.46 (1 H, s, H-8); ^{13}C NMR ($(CD_3)_2SO$) δ 61.50 (C-5′), 69.08 (C-2′), 74.62 (C-3′), 85.30 (C-4′), 86.40 (C-1′), 119.33 (C-5), 139.63 (C-8), 149.12 (C-4), 159.39 (C-2), 156.10 (C-6), 156.22 (carbamate carbon).

Reaction of Adenosine with BrCN. Adenosine (1.50 g, 5.61 mmol), BrCN (2.38 g, 22.4 mmol), and triethylamine (6.73 mL, 48.4 mmol) were dissolved in 100 mL of distilled water (initial pH 11.0), and the mixture was stirred at 2 °C for 3 h. The reaction mixture was freeze-dried and the residue was extracted with chloroform. The chloroform-insoluble fraction was extracted with methanol and the extract was concentrated to dryness. The residue was dissolved in 0.6 M boric acid and purified by preparative TLC as outlined for the reaction of adenosine with DISN. A crystalline mixture of **16** (45%) and **15** (19%) was obtained in a 2.3:1 ratio, respectively.

Reaction of Thymidine with DISN. Thymidine (0.97 g, 4.0 mmol) and DISN (2.12 g, 20.0 mmol) were dissolved in 50 mL of 0.2 M imidazole·HCl buffer (pH 7) and the mixture was stirred at 2 °C for 3 days. The reaction mixture was filtered, and the filtrate was freeze-dried and extracted with methanol. The methanol-soluble fraction was treated with Norit 211 at 0 °C for 30 min and filtered and the filtrate concentrated in vacuo to a gummy solid. Attempted purification by preparative TLC resulted in the recovery of thymidine. HPLC analysis before workup indicated an adduct of thymidine formed which was hydrolyzed to thymidine during the concentration of the methanol extract and preparative HPLC.

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Cyclizations of ω-Allenyl Radicals[1a]

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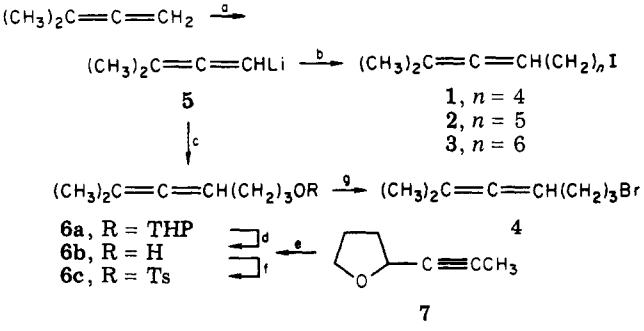
Allenyl halides of structure $(CH_3)_2C{=}C{=}CH(CH_2)_nX$ with n = 3–6 have been prepared and reacted with n-Bu_3SnH to generate the corresponding radicals for examination of the cyclization reactions of these reactive intermediates. The observed hydrocarbon products indicate that cyclization occurs for the n = 3, 4, and 5 radicals but not for the n = 6 species. The n = 3 radical isomerizes very efficiently by intramolecular addition to the sp carbon of the allene group. The n = 5 intermediate reacts relatively slowly by attack at the near sp^2 carbon. Both cyclization modes are observed for the n = 4 species. The details of these radical cyclizations are discussed.

The cyclizations of olefinic free radicals have been the subject of intense study over the past two decades, during which time a reasonable understanding of the scope of these reactions has gradually developed.[2] The analogous reactions of acetylenic radicals have also received some attention.[2,3] However, practically nothing is known concerning the intramolecular additions of free radicals to the allene function.[4,5] The unique bonding situation char-

Scheme I[a]



[a] a, RLi; b, $I(CH_2)_nI$; c, $Br(CH_2)_3OTHP$; d, TsOH, MeOH; e, $(CH_3)_2CuLi$; f, TsCl; g, $MgBr_2$.

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(2) (a) Beckwith, A. L. J. *Tetrahedron* **1981**, *37*, 3073. (b) Beckwith, A. L. J.; Ingold, K. U. "Rearrangements in Ground and Excited States"; de Mayo, P., Ed.; Academic Press; New York, 1980; Vol. I, p 161.

(3) Michaely, W. J. Ph. D. Dissertation, Indiana University, Bloomington, IN, 1971. Pradhan, S. K.; Kolhe, J. N.; Mistry, J. S. *Tetrahedron Lett.* **1982**, *23*, 4481. Stork, G.; Mook, R. *J. Am. Chem. Soc.* **1983**, *105*, 3720. Okabe, M.; Abe, M.; Tada, M. *J. Org. Chem.* **1982**, *47*, 1775. Okabe, M.; Tada, M. *Ibid.* **1982**, *47*, 5382. Choi, J-K.; Hart, D. J.; Tsai, Y-M.; *Tetrahedron Lett.* **1982**, *23*, 4765.

0022-3263/84/1949-2125$01.50/0