TABLE VII

CHEMICAL SHIFT VALUES[a] FOR PURINE PROTONS IN MONOMER,
DIMER, AND TRIMER MAGNETIC ENVIRONMENTS[b]

Proton	δ_1	δ_2	δ_3
H-6	−104.0	−28.5	−5.6
H-2	−94.0	−25.8	−5.6
H-8	−72.7	−28.3	−11.9

[a] In c.p.s. from chloroform. [b] All the values are good to ±0.5 c.p.s.

in Table VII. Using the standard formula,[29] a distance of 3–4 Å. is obtained.

In conclusion, the n.m.r. data obtained for purine and 6-methylpurine in aqueous solution clearly support a model of vertical stacking with average partial

ring overlap for the mode of association of these solutes in preference to one of horizontal hydrogen bonding. Furthermore, a numerical correlation between the n.m.r. data and the osmotic data has been successful in the sense that they reinforce and support the interpretations of each other.

It is noteworthy to point out that guanosine 5'-phosphate in water was shown to stack in a vertical, partially overlapping manner when forming a helix.[30] Desoxycholate in water has also been shown to form helical polymers by Blow and Rich.[31] It is tempting to suggest that such a situation may exist for the solutes mentioned here when their chain length attains a certain value.

(29) J. A. Pople, W. G. Schneider, and H. J. Berstein, "High-Resolution Nuclear Magnetic Resonance," McGraw–Hill Book Company, Inc., New York, N. Y., 1959, p. 177–182.

(30) M. Gellert, M. N. Lipsett, and D. R. Davies, Proc. Natl. Acad. Sci. U. S., 48, 2013 (1962).

(31) D. B. Blow and A. Rich, J. Am. Chem. Soc., 82, 3572 (1959).

Studies on Polynucleotides. XXXIV.[1] The Specific Synthesis of C3'–C5'-Linked Ribooligonucleotides.[2] New Protected Derivatives of Ribonucleosides and Ribonucleoside 3'-Phosphates. Further Syntheses of Diribonucleoside Phosphates[3]

BY R. LOHRMANN AND H. G. KHORANA

Methods for the preparation of O⁵'-monomethoxytrityladenosine, O⁵'-monomethoxytritylcytidine, and O⁵'-monomethoxytritylguanosine are described. Using these derivatives and the previously known O⁵'-trityluridine, the following acylated derivatives of ribonucleosides bearing free 5'-hydroxyl groups were prepared: N,N',O²',O³'-tetrabenzoyladenosine, N,O²',O³'-tribenzoylcytidine, O²',O³'-diacetylguanosine, N,O²',O³'-triacetylguanosine, O²',O³'-dibenzoyluridine, and N,O²',O³'-tribenzoyluridine. Methods for the large-scale preparation of cytidine 3'-phosphate and guanosine 3'-phosphate are described. Condensations of the N,O²',O⁵'-triacetyl derivatives of these ribonucleotides gave generally satisfactory yields of the following compounds: cytidylyl-(3'→5')-adenosine, cytidylyl-(3'→5')-cytidine, cytidylyl-(3'→5')-guanosine, cytidylyl-(3'→5')-uridine, guanylyl-(3'→5')-adenosine, guanylyl-(3'→5')-cytidine, guanylyl-(3'→5')-guanosine, and guanylyl-(3'→5')-uridine.

The most satisfactory method so far developed for the synthesis of the C3'–C5' interribonucleotidic linkage is that which involves, as the key step, the protection of 2'-hydroxyl groups in ribonucleoside 3'-phosphates by an acyl (acetyl, benzoyl) group.[2c,e] The use of the protected ribonucleoside 3'-phosphates, in turn, requires suitably protected ribonucleosides bearing free 5'-hydroxyl groups. Our previous studies in the field of ribooligonucleotides using this approach have been limited to uridine 3'- and adenosine 3'-phosphates and to the use of a few derivatives of the ribonucleosides.[2] In initiating a comprehensive attack on the stepwise synthesis of ribopolynucleotides containing all of the four commonly occurring ribonucleoside units in predetermined sequences, we have devoted attention to (a) the preparation of useful amounts of the protected derivatives of ribonucleosides and nucleotides and (b) a generalized study of the

synthesis of the interribonucleotidic linkage using these derivatives. This first phase of the study is reported in the present paper. The work parallels the systematic study recently reported of the corresponding problems in the deoxyribonucleotide series.[4] A brief report of this work has previously been made.[5] Recently, Chladek and Smrt[6] have also reported on the synthesis of several of the dinucleoside phosphates reported in this paper using the tetrahydropyranyl group[2a,b] to protect the 2'-hydroxyl group in ribonucleoside 3'-phosphates.

Protected Ribonucleosides.—The classical approach to the synthesis of protected ribonucleosides bearing free 5'-hydroxyl groups has involved the direct tritylation of the nucleosides, acetylation of the trityl derivatives, and subsequent detritylation under acidic conditions. In this way, several ribonucleosides bearing acetyl groups on the 2'- and 3'-hydroxyl groups have been available. With the realization of the need for the additional protection of the amino groups in the heterocyclic rings, methods for the preparation of N,N',O²',O³'-tetrabenzoyladenosine and N,O²',O³'-tribenzoylcytidine[2a,b] recently were described. In the present work, the selective formation of the O⁵'-

(1) Paper XXXIII: A. Stuart and H. G. Khorana, J. Biol. Chem., in press.

(2) Previous papers which deal directly with this topic: (a) M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, J. Am. Chem. Soc., 84, 430 (1962); (b) D. H. Rammler and H. G. Khorana, ibid., 84, 3112 (1962); (c) D. H. Rammler, Y. Lapidot, and H. G. Khorana, ibid., 85, 1989 (1963); (d) Y. Lapidot and H. G. Khorana, ibid., 85, 3852 (1963); (e) Y. Lapidot and H. G. Khorana, ibid., 85, 3857 (1963); (f) C. Coutsogeorgopoulos and H. G. Khorana, ibid., 86, 2926 (1964).

(3) This work has been supported by grants from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, the National Science Foundation, and the Life Insurance Medical Research Fund, Rosemont, Pa.

(4) H. Schaller and H. G. Khorana, J. Am. Chem. Soc., 85, 3828 (1963).

(5) R. Lohrmann and H. G. Khorana, Abstracts, 145th National Meeting of the American Chemical Society, New York, N. Y., 1963, p. 37C.

(6) S. Chladek and J. Smrt, Collection Czech. Chem. Commun., 29, 216 (1964).

monomethoxytrityl derivatives of cytidine, adenosine, and guanosine was studied and the first accomplishment of this work has been the preparation of these derivatives in useful amounts by new procedures. Using these derivatives, the desired $N,N',O^{2'},O^{3'}$-tetrabenzoyladenosine and $N,O^{2'},O^{3'}$-tribenzoylcytidine were obtained in satisfactory yields.

Because the N-benzoylated derivative of guanosine was expected to be much too stable[7] to alkaline hydrolysis, the N-protection of guanosine was accomplished by acetylation. When $O^{5'}$-monomethoxytritylguanosine was treated with acetic anhydride in pyridine at room temperature, acetylation occurred only at the sugar moiety. After acidic treatment of the acetylated product to remove the monomethoxytrityl group, $O^{2'},O^{3'}$-diacetylguanosine was obtained in good yield. For the preparation of N^2-$O^{2'},O^{3'}$-triacetylguanosine (I), it was necessary to carry out acetylation of $O^{5'}$-monomethoxytritylguano-


I   II
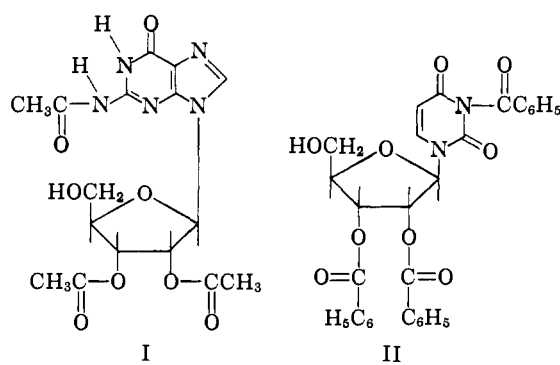
sine in anhydrous pyridine in the presence of tetraethylammonium hydroxide.[8] The N-acetyl group was stable, as expected from the previous work in the deoxyribonucleoside series,[9] to the subsequent acidic treatment necessary for the removal of the methoxytrityl group. It should be added that the use of $N^2,O^{2'},O^{3'}$-triacetylguanosine, in place of the $O^{2'}$,-$O^{3'}$-diacetyl derivative, in the synthesis of interribonucleotidic linkage not only affords complete protection against N-phosphorylation but also offers the distinct advantage of complete solubility in the medium of the condensation reactions.

The preparation of a benzoylated derivative of uridine has been recorded previously.[2b] It should be mentioned that there was lack of good agreement in the values of the elemental analysis with the values expected for a dibenzoylated derivative. In several repetitions of this work, a variation in the melting point behavior of the product obtained was also observed. Further work has provided clarification of the above results.[10] The use of strictly stoichiometric quantities of benzoyl chloride for the benzoylation of $O^{5'}$-trityluridine gives a dibenzoylated derivative from which, after acidic treatment, pure $O^{2'},O^{3'}$-dibenzoyluridine can be readily obtained in crystalline state. However, when an excess of benzoyl chloride is used, a tribenzoyl derivative is formed. The third

benzoyl group is concluded to be on the uracil ring, because of the alteration of the ultraviolet absorption spectrum. Tentatively, the ring benzoylation is concluded to occur at the N^1-position (II). $N,O^{2'},O^{3'}$-Tribenzoyluridine and $O^{2'},O^{3'}$-dibenzoyluridine both give elemental analyses in excellent agreement with those expected and are clearly separable by chromatography. Both derivatives are equally useful for most synthetic purposes.

Protected Ribonucleoside 3'-Phosphates.—The currently projected program of ribopolynucleotide synthesis requires large quantities of pure ribonucleoside 3'-phosphates. Pure adenosine 3'-phosphoric acid is a commercially available product. Disodium uridine 3'-phosphate can be satisfactorily prepared by fractional crystallization of the commercially available crystalline sodium salt of the 2'- and 3'-mixed isomers.[2f] In the present work, a large-scale procedure for the selective crystallization of disodium guanosine 3'-phosphate from the commercially available isomeric mixture has been developed. For the preparation of cytidine 3'-phosphate, initially, a large-scale adaptation of the anion exchange procedures developed by Cohn[11] was first used. However, working on a gram scale necessitated prolonged exposure of the chromatographed 3'-isomer to acidic conditions and, despite care, detectable isomerization to the 2'-isomer could not be avoided. The procedure which is completely satisfactory involves the pancreatic ribonuclease catalyzed ring opening of cytidine 2',3'-cyclic phosphate at neutral pH values. A similar procedure has been used by Smrt and Sorm[12] for the purpose. Cytidine 2',3'-cyclic phosphate was prepared in quantitative yield as the dicyclohexylguanidinium salt from the commercially available cytidine 2'(3')-phosphoric acid by an adaptation of the method developed previously.[13]

Pyridinium $N,O^{2'},O^{5'}$-triacetylcytidine 3'-phosphate (V, R = N^6-acetylcytosine) was prepared by acetylation of the parent nucleotide (III, R = cytosine) with an excess of acetic anhydride in the presence of tetraethylammonium acetate. The initial product (IV, R = N^6-acetylcytosine) was treated with aqueous

III   IV

V
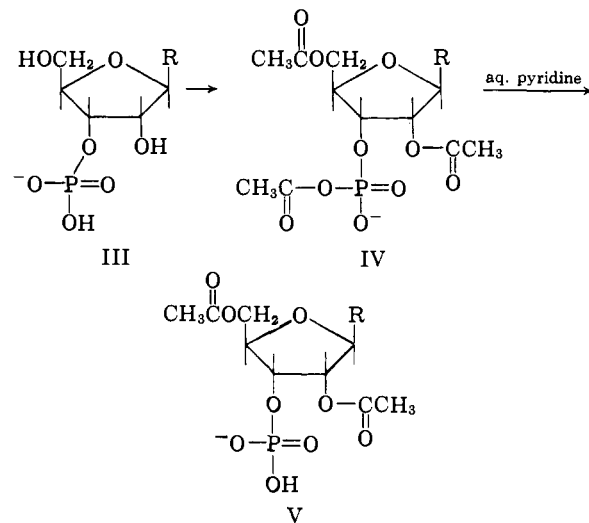
(7) M. Smith, G. I. Drummond, and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 698 (1961).

(8) H. Schaller and H. G. Khorana, *ibid.*, **85**, 3835 (1963).

(9) R. K. Ralph, W. J. Connors, H. Schaller, and H. G. Khorana, *ibid.*, **85**, 1983 (1963).

(10) Communication with Dr. D. H. Rammler, who independently arrived at the conclusions drawn in the present work, greatly facilitated the resolution of the situation.

(11) W. E. Cohn and J. X. Khym in "Biochemical Preparations," Vol. 5, D. Shemin, Ed., John Wiley and Sons, Inc., New York, N. Y., 1957, p. 40.

(12) J. Smrt and F. Sorm, *Collection Czech. Chem. Commun.*, **28**, 61 (1963).

(13) M. Smith, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6204 (1958).

pyridine under controlled conditions so as to cause the hydrolysis of the acetyl–phosphate linkage without causing any removal of the sensitive $O^{2'}$-acetyl group. The complete acetylation of the 2'-hydroxyl group in the product (V) was confirmed by the inertness of the latter toward dicyclohexylcarbodiimide in 10% aqueous pyridine, conditions which bring about quantitative formation of the 2',3'-cyclic phosphate from mononucleotides bearing an adjacent hydroxyl group. Completion of the N-acetylation in V was checked by the mobility of the latter during paper electrophoresis at an acidic pH, conditions under which the parent nucleotide shows zero mobility.

The corresponding reaction with guanosine 3'-phosphate gave $N^2,O^{2'},O^{5'}$-triacetylguanosine 3'-phosphate (V, R = N^2-acetylguanine). However, for the N-acetylation to proceed to completion, it was essential to remove the last traces of the pyridine from the mixture of anhydrous tetraethylammonium acetate and guanosine 3'-phosphate before the addition of acetic anhydride.[14]

The Synthesis of the $C^{3'}–C^{5'}$ Interribonucleotidic Linkage.—The condensation of the $N,O^{2'},O^{5'}$-triacetyl derivatives of cytidine 3'- and guanosine 3'-phosphates with the various protected ribonucleosides described above was carried out in the presence of dicyclohexylcarbodiimide in anhydrous pyridine for 4–5 days at room temperature. Two equivalents of the nucleosidic component were used, pyridinium Dowex-50 ion-exchange resin being added to remove traces of any strong inhibitory base in the reaction mixture.[15] The work-up involved an ammoniacal treatment to remove the protecting groups, the duration of this treatment being determined by the groups concerned. The N-acetyl group on the guanine ring had the slowest rate of ammonolysis, requiring 45 hr. at room temperature in 7.5 N ammonium hydroxide for complete removal. Because of the concomittant appreciable hydrolysis of the internucleotidic linkage under these conditions, alternative conditions for the ammonolysis reaction were sought. Anhydrous methanolic ammonia (15 M) was more effective, the N-deacetylation of the guanine ring being complete in 12 hr. at room temperature.[16]

The yields of the desired products (VI) were 65% to near theoretical in most cases. In two syntheses, those of guanylyl-(3'→5')-cytidine and guanylyl-(3'→5')-guanosine, the yields were surprisingly low, 27 and 31%, respectively. These low yields cannot be ascribed to the bulk of the nucleotide component[4,17] since using the same derivative the yields of guanylyl-(3'→5')-adenosine and guanylyl-(3'→5')-uridine were in the expected range (65% and 78%, respectively). It is possible that the low yields were due to some inhibitory contaminant present in the actual sample of the derivative used.[18]

(14) In the presence of traces of pyridine, even prolonged reaction periods (one week or longer at room temperature) failed to give complete N-acetylation. In such cases, complete N-acetylation could nevertheless be obtained by the addition of a strong base such as triethylamine to the acetylation mixture.

(15) See, for example, T. M. Jacob and H. G. Khorana, *J. Am. Chem. Soc.*, **86**, 1630 (1964).

(16) The use of anhydrous methanolic ammonia is now preferred to the use of aqueous ammonium hydroxide. Not only is the rate of ammonolysis of the N-acyl groups increased but the rate of internucleotidic bond cleavage is much reduced.

(17) G. Weimann and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 4329 (1962).

(18) The yields of the two products, guanylylcytidine and guanylyl-
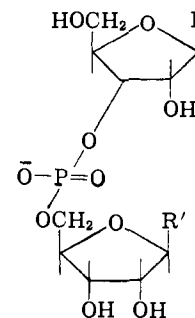
VI, R = guanine, or cytosine
R' = adenine, cytosine, uracil, or guanine

That the protected nucleotidic derivatives used gave exclusively the $C^{3'}–C^{5'}$-linked products was confirmed by appropriate enzymatic degradation of members drawn from the two series of syntheses. Thus, cytidylyl-(3'→5')-cytidine was completely degraded by pancreatic ribonuclease to cytidine 3'-phosphate and cytidine, the amount of the synthetic substrate used for this analysis being adequate to detect any enzyme-resistant material in the amount of 1% of the starting material. Similarly, the synthetic guanylyl-(3'→5')-uridine was completely susceptible to the action of the *L. acidophilus* phosphodiesterase.[19] While these tests established the fact that the products were free from the 2'→5'-linked isomers, degradation with the venom phosphodiesterase was carried out to show further that the linkage involved exclusively the 5'-hydroxyl group of the protected nucleosides. Thus, degradation of cytidylyl-(3'→5')-cytidine, cytidylyl-(3'→5')-adenosine, cytidylyl-(3'→5')-uridine, and cytidylyl-(3'→5')-guanosine with this enzyme preparation again proceeded to completion to give the 5'-phosphates of the different nucleoside components originally used in the syntheses. The result provides final proof for the structures of the protected nucleosides described in this paper.

The present work completes the basic survey of the approach to the specific $C^{3'}–C^{5'}$-interribonucleotidic linkage which has emerged as the most attractive. With the availability of the protected nucleoside and nucleotide derivatives reported now and earlier, the way is clear toward the stepwise synthesis of relatively long ribopolynucleotide chains of defined sequence. Work along these lines will be reported in forthcoming papers.

Experimental[20]

General Methods.—Reagent grade pyridine was dried over calcium hydride for at least 2 weeks prior to use in coevaporations. Pyridine for use in condensation reactions was further purified so as to remove small traces of strongly basic amines. For this purpose, chlorosulfonic acid (60 ml. for 4 l. of pyridine) was added cautiously and then distillation of pyridine was carried out over a short fractionating column. The distillate was redistilled over potassium hydroxide pellets under exclusion of moisture. The distilled product was kept over molecular sieve beads[21] (type 4xa) in the dark.

Paper chromatography was performed by descending technique using mostly Whatman No. 1 paper. The solvent systems used for paper chromatography were: solvent A, 2-propanol–concen-

guanosine, are high, being comparable with those obtained in the other syntheses, using arylsulfonyl chloride as the reagent (unpublished work).

(19) W. Fiers and H. G. Khorana, *J. Biol. Chem.*, **238**, 2780, 2789 (1963).

(20) The elemental analyses were performed by Mr. A. Bernhardt, Mülheim, Germany. All melting points are uncorrected and were obtained on a Thomas-Hoover capillary melting point apparatus.

(21) Linde Co., Division of Union Carbide.

trated ammonium hydroxide–water (7:1:2, v./v.); solvent B, 1-butanol–30% acetic acid (2:1, v./v.); solvent C, ethanol–1 M ammonium acetate (pH 7.5) (7:3, v./v.); solvent D, isobutyric acid–concentrated ammonia–water (57:4:39, v./v.); solvent E, saturated ammonium sulfate–0.1 M sodium acetate (pH 6.5)-2-propanol (79:19:2, v./v.). The R_f values of different compounds are listed in Table I.

TABLE I

PAPER CHROMATOGRAPHY OF DIFFERENT COMPOUNDS

	Solvent A	Solvent B	Solvent C	Solvent D
Cytidine	0.49	0.22	0.63	
Guanosine	0.31	0.20	0.57	
$O^{5'}$-Monomethoxytritylguanosine	0.82		0.82	
$N^2,O^{2'},O^{3'}$-Triacetylguanosine		0.72	0.81	
$O^{2'},O^{3'}$-Diacetylguanosine		0.60	0.72	
Cytidine 3'-phosphate	0.10	0.051	0.26	0.61
Guanosine 3'-phosphate	0.057	0.033	0.17	0.43
$N,O^{2'},O^{5'}$-Triacetylcytidine 3'-phosphate		0.20	0.56	
$N^2,O^{2'},O^{5'}$-Triacetylguanosine		0.21	0.53	
$O^{2'},O^{5'}$-Diacetylguanosine 3'-phosphate			0.40	
Cytidylyl-(3'→5')-adenosine	0.21	0.023	0.40	0.73
Cytidylyl-(3'→5')-cytidine	0.18	0.019	0.41	0.65
Cytidylyl-(3'→5')-guanosine	0.087	0.019	0.34	0.51
Cytidylyl-(3'→5')-uridine	0.17	0.05	0.42	0.52
Guanylyl-(3'→5')-adenosine	0.10	0.021	0.33	0.56
Guanylyl-(3'→5')-cytidine	0.10	0.03	0.29	0.51
Guanylyl-(3'→5')-guanosine	0.040	0.020	0.24	0.34
Guanylyl-(3'→5')-uridine	0.10	0.03	0.36	0.39

The protected nucleosides were checked for purity by thin layer chromatography on silica gel HF$_{254}$,[22] using chloroform–methanol mixtures as solvents. An alternative technique for separation of the protected nucleosides involved descending paper chromatography on Whatman No. 1 paper impregnated with dimethyl sulfoxide[23] using carbon tetrachloride alone or carbon tetrachloride–ethyl acetate (3:1, v./v.) as solvents. While the technique was very useful for separation of the protected nucleosides, the R_f values were greatly influenced by the relative humidity in the chromatographic tank. An atmosphere of low relative humidity (20-30%) gave the best results. The chromatographic paper was prepared by dipping Whatman No. 1 paper in 20% (v./v.) solution of dimethyl sulfoxide in benzene. The paper was air-dried at room temperature and the procedure repeated once. Samples were applied on the paper after drying in an air stream at room temperature.

The nucleotidic compounds were detected by viewing under an ultraviolet lamp. A fluorescent screen was used to increase the sensitivity of detection of certain of the compounds. The trityl- and monomethoxytrityl-containing compounds were made visible on paper chromatograms after spraying the paper with 10% perchloric acid.

Paper electrophoresis was carried out using Whatman 3 MM paper in a high voltage (4000 v.) apparatus. The paper was immersed in a water-cooled high-boiling petroleum fraction (Varsol). The buffer used was 0.03 M potassium phosphate (pH 7.1). The electrophoretic mobilities are given in Table II. Preparative electrophoresis was performed in the cold on Whatman 3 MM paper (20 cm. wide strips) immersed in carbon tetrachloride, using 0.1 M triethylammonium bicarbonate buffer (pH 7.5). The potential applied across the paper was about 15 v./cm. Enzymatic degradations of the synthetic diribonucleoside phosphates with the pancreatic ribonuclease,[2a] the *L. acidophilus* phosphodiesterase,[19] and the venom phosphodiesterase[24] were performed as described previously.

$O^{5'}$-Monomethoxytrityladenosine.—Dried adenosine (5 g.) and monomethoxytritylchloride (7 g.) in dry dimethylformamide (100 ml.) were shaken until a clear solution was obtained. After standing for three days in the dark the reaction mixture was poured with stirring into a dilute aqueous ammonia (2%)–ice mixture. The white precipitate was collected by filtration,

TABLE II

PAPER ELECTROPHORETIC MOBILITIES OF DIFFERENT COMPOUNDS[a]

Cytidine 3'-phosphate	1.0
Guanosine 3'-phosphate	0.95
$N,O^{2'},O^{5'}$-Triacetylcytidine 3'-phosphate	0.82
$N^2,O^{2'},O^{5'}$-Triacetylguanosine 3'-phosphate	0.86
Cytidine 2',3'-cyclic phosphate	0.63
Guanosine 2',3'-cyclic phosphate	0.60
Cytidylyl-(3'→5')-adenosine	0.37
Cytidylyl-(3'→5')-cytidine	0.35
Cytidylyl-(3'→5')-guanosine	0.38
Cytidylyl-(3'→5')-uridine	0.36
Guanylyl-(3'→5')-adenosine	0.34
Guanylyl-(3'→5')-cytidine	0.33
Guanylyl-(3'→5')-guanosine	0.29
Guanylyl-(3'→5')-uridine	0.32

[a] Values recorded are relative to the mobility of cytidine 3'-phosphate.

washed well with water, dried over phosphorus pentoxide *in vacuo*, and crystallized from ethyl acetate. After 20 hr., the crystalline precipitate was collected by filtration, washed with cold ethyl acetate and then with cyclohexane and pentane. This product was chromatographically pure, the yield being 5 g. (50%). The sample for analysis was further recrystallized from ethyl acetate–cyclohexane, m.p. 189–191° dec. The ultraviolet absorption characteristics in methanol were λ_{max} 260 and 233 mμ.

Anal. Calcd. for $C_{30}H_{29}O_5N_5$ (539.57): C, 66.78; H, 5.42; N, 12.98. Found: C, 66.68; H, 5.56; N, 12.75.

N,N',$O^{2'}$,$O^{3'}$-Tetrabenzoyladenosine.[2a]—To a solution of $O^{5'}$-monomethoxytrityladenosine (12 g.) in anhydrous pyridine (180 ml.) benzoyl chloride (48 ml.) was added and the solution was shaken for 24 hr. in the dark at room temperature. The solution was then poured into an excess of ice–water and the mixture was extracted with chloroform, the extract dried over sodium sulfate, and evaporated to dryness. To the pyridine-free residue 86% acetic acid (100 ml.) was added and the solution was shaken for 4 hr. at room temperature. The solvent was removed under reduced pressure (below 30°) until a solid residue was left. To remove the benzoic acid, chloroform was added and the mixture was shaken with dilute sodium bicarbonate solution until the evolution of carbon dioxide ceased. The chloroform extract was dried over sodium sulfate, concentrated to a small volume, and then passed onto a silicic acid[25] column (48 × 4 cm.). It was eluted by a linear chloroform–methanol gradient with chloroform (2 l.) in the mixing vessel and chloroform–5% methanol (2 l.) in the reservoir. The desired compound was eluted at a concentration of about 3.5% methanol. After removal of the solvent and addition of hot benzene to the residue, a white crystalline precipitate separated and was collected by filtration and washed with cyclohexane and pentane. The yield of this crop was 10.6 g. (m.p. 187–188°; lit.[2a] m.p. 185°). From the mother liquor a further amount (1.18 g.) of less pure material was recovered. The total yield of the desired product was 11.78 g. (77%).

$O^{5'}$-Monomethoxytritylcytidine.—A sealed mixture of dry cytidine (15 g.) and monomethoxytrityl chloride (21 g.) in anhydrous dimethylformamide (400 ml.) was shaken for 5 days in the dark. After pouring it into 2 l. of ice–water containing a small amount of ammonia (5 ml. of 15 N), the white precipitate was collected by filtration, carefully washed with water, and dried over phosphorus pentoxide *in vacuo*. The compound was recrystallized from ethyl acetate (150 ml.). On standing for 20 hr., the solution deposited a voluminous precipitate which was collected by filtration. After washing with cold ethyl acetate, cyclohexane, and finally pentane, the white powder was dried in air. The yield was 19.73 g. (62%), m.p. 151–153° dec. The ultraviolet absorption spectrum in methanol showed λ_{max} 274 and 232 mμ.

Anal. Calcd. for $C_{29}H_{29}O_6N_3$ (515.55): C, 67.56; H, 5.67; N, 8.15. Found: C, 66.38; H, 5.71; N, 8.43.

N,$O^{2'}$,$O^{3'}$-Tribenzoylcytidine.[2b]—A solution of $O^{5'}$-monomethoxytritylcytidine (12 g.) in anhydrous pyridine (150 ml.) and benzoyl chloride (50 ml.) was shaken for 3 hr. at room temperature. After pouring the solution onto ice, the mixture was extracted with chloroform (500 ml.) and the extract was dried over sodium sulfate and evaporated *in vacuo* to dryness. The pyri-

(22) E. Merck, A.G., Darmstadt, Germany.

(23) B. Wickberg, *Acta Chem. Scand.*, **12**, 615 (1958).

(24) H. G. Khorana and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 675 (1961).

(25) Mallinckrodt, A.R., 100 mesh.

dine-free gum was dissolved in 30 ml. of glacial acetic acid, then 70 ml. of 80% acetic acid was added. The solution was shaken for 4.5 hr. at room temperature and then evaporated *in vacuo*. The residual gum was dissolved in chloroform and the solution was extracted with dilute aqueous sodium bicarbonate solution until the carbon dioxide evolution stopped. The dry chloroform layer was evaporated and the concentrate chromatographed on a silicic acid column (48 × 4 cm. diameter). The column was eluted with four bed volumes of chloroform and then with chloroform containing 5% methanol. The fraction containing the desired product was evaporated to a small volume. On standing for 36 hr. the concentrate deposited the compound which was crystallized from ethyl acetate–pentane. The yield of the pure product was 7.4 g. (57%); m.p. 185° (lit.[2b] m.p. 180–181°).

O[5]′-Monomethoxytritylguanosine.—To a solution of recrystallized guanosine (7.5 g., dried over phosphorus pentoxide *in vacuo*) in warm anhydrous dimethyl sulfoxide (50 ml.) anhydrous pyridine (40 ml.) was added and the solution was rapidly cooled to room temperature. Monomethoxytrityl chloride (8.75 g.) was added immediately before the nucleoside started to crystallize. The mixture was shaken for 30 hr. at room temperature. The reaction mixture was poured into ice-water under vigorous stirring and the precipitate was collected by filtration. The dry cake of the product was thoroughly washed with ether. The crude product (14 g.) was purified by pouring its saturated solution in dimethylformamide into warm ethyl acetate and collecting the precipitate after cooling. The yield at this stage was 8.2 g. (56%). This product was again dissolved in a small amount of dimethylformamide and the solution was poured into boiling water (1 l.) containing some ammonia. On cooling, the compound which separated was pure. It was collected by filtration and dried over phosphorus pentoxide *in vacuo*, m.p. 189–191° dec. The ultraviolet absorption spectrum taken in methanol showed λ_{max} 235 mμ, and shoulders at 270 and 250 mμ.

Anal. Calcd. for $C_{30}H_{29}O_6N_5$ (555.57): C, 64.85; H, 5.26; N, 12.61. Found: C, 64.84; H, 5.32; N, 12.34.

O[2]′,O[3]′-Diacetylguanosine.—Dry O[5]′-monomethoxytritylguanosine (1.15 g.) was added to a mixture of acetic anhydride-pyridine (2.2 ml.:25 ml.). On shaking of the sealed mixture a clear solution resulted. After 20 hr. at room temperature the solution was poured into ice-water, and the white precipitate was collected by filtration, dissolved in 80% acetic acid (20 ml.), and shaken for 3 hr. at room temperature. The acid was then removed *in vacuo* with repeated addition of benzene. Finally, a clear concentrated methanolic solution of the residual gum was added dropwise into three volumes of anhydrous ether. After chilling of the mixture the diacetylguanosine had separated as a white crystalline precipitate which was collected by filtration and washed with cyclohexane and pentane. The yield was 650 mg. (77%), m.p. 213–216°. The ultraviolet absorption spectrum in water showed λ_{max} 252 mμ and a shoulder at 270 mμ.

Anal. Calcd. for $C_{14}H_{17}O_7N_5$ (367.32): C, 45.77; H, 4.67; N, 19.07. Found: C, 44.76; H, 4.94; N, 19.05.

N[2],O[2]′,O[3]′-Triacetylguanosine.—A solution of O[5]′-monomethoxytritylguanosine (1.15 g.) in aqueous 10% tetraethylammonium hydroxide (3.5 ml.) was rendered anhydrous by repeated addition and evaporation of pyridine. The residue was suspended in anhydrous pyridine (35 ml.) and acetic anhydride (10 ml.) was added. The sealed solution was kept at room temperature for 20 hr. and then poured on ice. Extraction with chloroform and evaporation of the extract to dryness (under reduced pressure) gave a pyridine-free gum which was dissolved in 80% acetic acid (30 ml.), and the mixture was shaken for 3 hr. at room temperature. After removal of the acid by repeated coevaporation with benzene *in vacuo*, the residual gum was taken up in a small volume of ethyl acetate and decolorized with charcoal. The concentrated solution was added dropwise into hot cyclohexane. After cooling, the white precipitate was collected by centrifugation. The yield was 720 mg. The compound was further purified by chromatography on a silicic acid column (45 × 2.5 cm. diameter) using a linear gradient of methanol in chloroform (1.5 l. of chloroform in the mixing vessel, 1.5 l. of chloroform containing 20% methanol in the reservoir). The elution of the products was followed by spotting each tube on paper and (1) looking for ultraviolet-absorbing material using a fluorescent screen and (2) spraying the paper with 10% perchloric acid and looking for the characteristic orange color of the *p*-methoxytrityl cation. After elution of monomethoxytrityl alcohol the desired compound appeared at about 6% methanol concentration. Evaporation of the pooled fractions containing the triacetylguanosine gave a gum which was dissolved in a small

volume of ethyl acetate, and the solution was added dropwise into warm cyclohexane. After cooling, the resulting white precipitate was collected by centrifugation, washed with pentane, and dried *in vacuo;* yield 510 mg. (61%). The compound had no sharp melting point, but showed sintering above 123°. The ultraviolet absorption spectrum in water showed λ_{max} 256 mμ and a shoulder at 273 mμ.

Anal. Calcd. for $C_{16}H_{19}O_8N_5$ (409.35): C, 46.94; H, 4.68; N, 17.11. Found: C, 47.03; H, 4.89; N, 16.98.

O[5]′-Trityluridine.[26]—From a solution of uridine (10 g.) in anhydrous pyridine (200 ml.) 90 ml. of the solvent was removed by distillation. After addition of trityl chloride (12 g.) to the cooled solution, the mixture was refluxed for 2 hr. and then poured into ice–water (1500 ml.). The precipitate was collected by filtration, washed with petroleum ether (200 ml., b.p. 35–36°) and dissolved in chloroform (500 ml.). The chloroform solution was extracted with concentrated salt solution (2 × 200 ml.), dried over sodium sulfate, and evaporated under reduced pressure. The pyridine-free sirup was then dissolved in dry benzene (200 ml.) and the solution kept for several hours in the cold. The resulting crystalline precipitate was collected by filtration, washed with benzene and pentane, and dried *in vacuo*. The yield of the O[5]′-trityluridine–benzene adduct was 16.4 g. Chromatography on dimethyl sulfoxide-impregnated paper using carbon tetrachloride–ethyl acetate (2:1) as the solvent showed that the compound was free of bistrityluridine, whereas the mother liquor contained both bis- and monotrityluridine.

O[2]′,O[3]′-Dibenzoyluridine.—To an anhydrous pyridine solution (25 ml.) of O[5]′-trityluridine (9.75 g.) was added a mixture of benzoyl chloride (4.7 ml.) in pyridine (15 ml.). The solution was kept at room temperature for 3.5 hr. and then poured into ice-water (500 ml.). The mixture was extracted with chloroform and the chloroform extract (after drying over sodium sulfate) was evaporated to dryness. The pyridine-free residue was dissolved in 80% acetic acid (90 ml.) and the solution was heated under gentle reflux for 20 min. The mixture was then evaporated to dryness *in vacuo*, and the acid-free residue was crystallized from absolute ethanol (100 ml.). After being kept overnight in the cold, the solution deposited white crystals which were collected by filtration and washed with cold ethanol and ether. The yield of the pure compound was 2.9 g. By reducing the mother liquors in volume (50 ml.) and addition of hot cyclohexane another crop of crystals (2.2 g.) of almost pure compound was obtained. The total yield was 5.1 g. (65% based on the O[5]′-trityluridine–benzene adduct); m.p. 195–197°. The purity of the compound was checked by chromatography on dimethyl sulfoxide treated paper with carbon tetrachloride as solvent. The R_f of this product relative to that of N,O[2]′,O[3]′-tribenzoyluridine was 0.37. The ultraviolet absorption characteristics of this compound in methanol showed λ_{max} 257 and 229 mμ, λ_{min} 251 mμ.

Anal. Calcd. for $C_{23}H_{20}O_8N_2$ (452.41): C, 61.06; H, 4.46; N, 6.19. Found: C, 61.35; H, 4.46; N, 6.48.

N,O[2]′,O[3]′-Tribenzoyluridine.—To a solution of O[5]′-trityluridine (5 g.) in anhydrous pyridine (14 ml.) benzoyl chloride (4.8 ml.) was added under cooling. After shaking at room temperature for 12 hr., the mixture was poured into ice-water. The mixture was extracted with chloroform and the extract was dried over sodium sulfate and evaporated to dryness. The pyridine-free residue was dissolved in 80% acetic acid (75 ml.), the mixture was heated under gentle reflux for 15 min., and then evaporated to dryness *in vacuo*. The acid-free residue was crystallized from a mixture of absolute ethanol–cyclohexane (3.4 g., 69%). Recrystallization from ethanol–water (3:1) afforded pure tribenzoyluridine as fine white crystals, m.p. 191–193°. The ultraviolet absorption characteristics of this compound in methanol were λ_{max} 231 mμ and a shoulder at 250 mμ.

Anal. Calcd. for $C_{30}H_{24}O_9N_2$ (556.51): C, 64.74; H, 4.35; N, 5.03. Found: C, 64.23; H, 4.06; N, 5.31.

Cytidine 2′,3′-Cyclic Phosphate.—To a solution of cytidine 2′(3′)-phosphate (3.2 g.) in 2 N ammonium hydroxide (25 ml.) and dimethylformamide (50 ml.) was added a solution of dicyclohexylcarbodiimide (10.6 g.) in *t*-butyl alcohol (26 ml.). The mixture was heated under reflux for 2 hr. (glycerol bath, temperature 120°). The completion of the cyclization reaction was ascertained by high voltage electrophoresis (pH 7.1). The *t*-butyl alcohol was then evaporated under reduced pressure and the remaining mixture was diluted with water (200 ml.), extracted (three times) with 300-ml. portions of ether, and filtered from the insoluble

(26) H. Bredereck, *Ber.*, **66**, 198 (1933).

material. After addition of an equal volume of pyridine the solution was evaporated to dryness. The residue was redissolved in pyridine and the solution was evaporated. Finally, the solution of the product in anhydrous pyridine (20 ml.) was slowly added to anhydrous ether (1 l.) under vigorous stirring. Dicyclohexylguanidinium cytidine 2′,3′-cyclic phosphate, which appeared as a fine white precipitate, was collected by centrifugation and washed several times with ether. The compound was dried *in vacuo* over phosphorus pentoxide. The yield was 3.96 g. The product was completely free from cytidine 2′(3′)-phosphate as tested by paper electrophoresis at a level of 60 optical density units (270 mμ) per application.

Cytidine 3′-Phosphate.—To a solution (pH 7) of dicyclohexylguanidinium cytidine 2′,3′-cyclic phosphate (2 g.) in water (25 ml.) was added pancreatic RNase (13 mg.) and the mixture was stirred magnetically at 37°. By the automatic addition of 9 N ammonium hydroxide from a microsyringe the pH was kept in the range of 6.8 to 7.2 The completion of the reaction (6–8 hr.) was indicated by the constancy of pH and was further confirmed by high-voltage paper electrophoresis (pH 7.1) of an aliquot (30 optical density units or higher per spot). The solution was then slowly passed through a column (40 cm. × 2.6 cm.) of pyridinium Dowex-50 (2% crosslinked, 200–400 mesh). The product was eluted with 1% aqueous pyridine (adjusted to pH 6.5 with acetic acid.) Lyophilization of the effluent gave a white product. The yield was 1.28 g. (79% based on cytidine 2′(3′)-phosphate). The compound proved to be pure 3′-isomer when a sample (6.25 mg.) was chromatographed on a Dowex-1 (8%)(formate) ion-exchange resin column (15 cm. × 1 cm.).[11]

Pyridinium N,O$^{2'}$,O$^{5'}$-Triacetylcytidine 3′-Phosphate.—Lyophilized cytidine 3′-phosphate (161 mg., 0.5 mmole) and tetraethylammonium acetate (5 mmoles) were rendered anhydrous by repeated evaporation of pyridine *in vacuo*. The last evaporation was continued until a viscous sirup remained. Acetic anhydride (1.6 ml.) was added and the sealed reaction mixture kept for 15 hr. at room temperature. Then, 5 ml. of methanol–pyridine (4:1) mixture was added and the clear solution was kept for 30 min. at room temperature. After evaporation of the solvent *in vacuo*, the residue was dissolved in cold 10% aqueous pyridine (30 ml.) and the solution was passed through a column (2.5 × 30 cm.) of Dowex-50 (8%)(pyridinium) ion-exchange resin. The column was eluted with 10% aqueous pyridine and the total effluent was kept for 2.5 hr. at room temperature. Subsequent high voltage electrophoresis at pH 7.1 indicated complete hydrolysis of the acetyl phosphate derivative. The solution was concentrated to a small volume under reduced pressure (1 mm. mercury, bath temperature 10°) and finally rendered anhydrous by repeated evaporation of pyridine. The residual gum was dissolved in anhydrous pyridine (10 ml.) and then slowly added under vigorous stirring to an excess of anhydrous ether. The pyridinium salt of N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3′-phosphate precipitated as a white powder. After centrifugation and washing with ether the compound was dried over phosphorus pentoxide. The yield of the dry powder was 210 mg. (83%). The ultraviolet absorption spectrum of the pyridine-free compound taken in water showed λ_{max} 298 and 247 mμ.

Pyridinium Guanosine 3′-Phosphate.—To a clear solution of disodium guanosine 2′(3′)-phosphate[27] (14.85 g.) in hot water (200 ml.) warm acetone (240 ml.) was added and the mixture was kept at room temperature for 12 hr. The crystalline crop was collected by centrifugation and washed with acetone–water (6:5). The crystallization procedure was repeated using 180 ml. of water and 200 ml. of acetone. The product was now washed with acetone–water (6:4). The yield was 9.63 g. (65%) of the pure 3′-isomer. An additional fraction (1.25 g.) of fairly pure compound could be isolated from the mother liquors. For conversion to the pyridinium salt, the disodium salt (2 g.) obtained above was dissolved in warm water (50 ml.) and the solution was slowly passed through a Dowex-50 (8%) (pyridinium) ion-exchange column (25 × 2.5 cm.). The column was washed with 5% aqueous pyridine (300 ml.), the eluate was lyophilized, and the product was dried over phosphorus pentoxide. The yield was 1.93 g. (89%) of the monopyridinium salt. This pyridinium salt can be kept for many months in dry state without isomerization. R_f values in solvent E were 0.54 (2′-isomer) and 0.47 (3′-isomer).

(27) Pabst Laboratories, Milwaukee, Wis. This commercial preparation contained, as determined by paper chromatography in solvent E, about 10% of the 2′- isomer, the remainder being guanosine 3′-phosphate.

Pyridinium N^2,O$^{2'}$,O$^{5'}$-Triacetylguanosine 3′-Phosphate.—A solution of the pyridinium salt of guanosine 3′-phosphate (440 mg.) in 0.5 M tetraethylammonium acetate (20 ml.) was rendered anhydrous by repeated evaporation of pyridine *in vacuo* (10^{-1} mm. pressure). The final evaporation was continued until a viscous sirup remained.[14] After addition of acetic anhydride (3 ml.) the mixture was shaken until a clear solution resulted. The solution was then kept for 3 days in the dark at room temperature. The subsequent work-up was carried out as described under N,O$^{2'}$, O$^{5'}$-triacetylcytidine 3′-phosphate. The yield of the product, a fine white powder, was 560 mg. (99%). The ultraviolet absorption spectrum taken in water showed λ_{max} 258 mμ and a shoulder at 272 mμ.

Internucleotide Bond Synthesis. General Method.—A mixture of the protected nucleotide (0.05 mmole), the protected nucleoside (0.1 mmole), and dry pyridinium Dowex-50W (8% crosslinked; 50–100 mesh) ion-exchange resin (500 mg.) was rendered anhydrous by repeated evaporation of dry pyridine. Anhydrous pyridine (0.5 ml.) and dicyclohexylcarbodiimide (115 mg.) were added to the residue and the mixture was shaken for 4 to 5 days in the dark. Then water (0.5 ml.) was added and the mixture extracted with pentane (three times). After standing for 12 hr., the mixture was diluted with aqueous pyridine (50%) and filtered from the insoluble material. The clear aqueous pyridine solution was extracted with carbon tetrachloride to remove unreacted protected nucleoside,[28] the carbon tetrachloride layer being extracted back with aqueous pyridine. The aqueous pyridine layers were combined and evaporated *in vacuo* to dryness. Cold 15 M methanolic ammonia (25 ml.) was added to the residue and the mixture was kept well stoppered at room temperature for an appropriate period. The solution was then evaporated and the products separated by preparative paper electrophoresis in triethylammonium bicarbonate buffer (0.1 M) for 6–8 hr. The band corresponding to the desired product was eluted with dilute aqueous ammonia (0.5%). The solution was then lyophilized or evaporated *in vacuo* in the presence of pyridine. In the latter case, the residual anhydrous gum was finally dissolved in anhydrous dimethylformamide (5 ml.) and the solution added dropwise into an excess of dry ether containing anhydrous ammonia. The desired compounds precipitated as white powders in the form of their ammonium salts. They were collected by centrifugation, washed with ether, and kept over magnesium perchlorate in the cold.

Cytidylyl-(3′→5′)-adenosine.—Pyridinium N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3-′phosphate and N,N′,O$^{2'}$,O$^{3'}$-tetrabenzoyladenosine were condensed in the presence of dicyclohexylcarbodiimide under the standard conditions. The deacylation was carried out with 15 M methanolic ammonia for 12 hr. at room temperature. The yield as estimated spectrophotometrically using an extinction of 16,600 at 271 mμ (pH 6) was 84% (based on the nucleotide).

Cytidylyl-(3′→5′)-cytidine.—Pyridinium N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3′-phosphate and N,O$^{2'}$,O$^{3'}$-tribenzoylcytidine were condensed as described in the general procedure. The deacylation was carried out with 15 M methanolic ammonia for 20 hr. The yield of the dinucleoside phosphate was 91%. Degradation of the compound (9.1 optical density units at 280 mμ, pH 2) with the pancreatic ribonuclease showed that after 4 hr. of incubation the starting material had completely disappeared, the only products being cytidine and cytidine 3′-phosphate (chromatography in solvent C).

Cytidylyl-(3′→5′)-guanosine.—The condensation of pyridinium N,O$^{2'}$,O$^{'5}$-triacetylcytidine 3′-phosphate and N^2,O$^{2'}$,O$^{3'}$-triacetylguanosine was performed in the usual way. The treatment with 15 M methanolic ammonia lasted for 20 hr. The yield of the product was 95% using an extinction of 16,900 at 271 mμ (pH 6).

Cytidylyl-(3′→5′)-uridine.—Pyridinium N,O$^{2'}$,O$^{5'}$-triacetylcytidine 3′-phosphate and O$^{2'}$,O$^{3'}$-dibenzoyluridine was condensed by the dicyclohexylcarbodiimide procedure. After removal of the protecting groups in aqueous 7.5 N ammonium hydroxide (7 hr.), the compound was purified by paper chromatography in solvent C. The eluate of the dinucleoside phosphate band was passed through a column of pyridinium Dowex-50 ion-exchange resin and then worked up in the usual manner. The yield was essentially 100%, no unreacted mononucleotide being detected.

Guanylyl-(3′→5′)-adenosine.—Pyridinium N^2,O$^{2'}$,O$^{5'}$-triacetylguanosine 3′-phosphate and N,N′,O$^{2'}$,O$^{3'}$-tetrabenzoyl-

(28) This extraction was omitted in case of guanosine.

adenosine were condensed according to the general procedure. The deacylation was carried out in 15 M methanolic ammonia for 20 hr. and the desired product was obtained in 65% yield using an extinction of 27,100 at 252 mμ (pH 6).

Guanylyl-(3'→5')-cytidine.—Pyridinium N^2,O$^{2'}$,O$^{5'}$-triacetylguanosine 3'-phosphate and N,O$^{2'}$,O$^{3'}$-tribenzoylcytidine were treated under the standard conditions. After giving the methanolic ammonia treatment for 20 hr. the dinucleoside phosphate was obtained in 26% yield using an extinction of 16,900 at 271 mμ (pH 6).

Guanylyl-(3'→5')-guanosine.—Pyridinium N^2,O$^{2'}$,O$^{5'}$-triacetylguanosine 3'-phosphate and N^2,O$^{2'}$,O$^{3'}$-triacetylguanosine were condensed by the dicyclohexylcarbodiimide procedure. The deacetylation was carried out in aqueous 7.5 N ammonium hydroxide (25 ml.) for 50 hr. at room temperature. The yield was 31%, assuming no hypochromicity for the product.

Guanylyl-(3'→5')-uridine.—Pyridinium N^2,O$^{2'}$,O$^{5'}$-triacetylguanosine 3'-phosphate and O$^{2'}$,O$^{3'}$-dibenzoyluridine were condensed under the standard conditions. The deacylation was performed with a mixture of aqueous concentrated ammonium hydroxide–pyridine (1:1; 25 ml.) for 35 hr. The desired product

was obtained in 78% yield using an extinction of 20,800 at 262 mμ (pH 7). Degradation by the *Lactobacillus acidophilus* R-26 phosphodiesterase[19] was carried out using 10.7 optical density units (at 252 mμ, pH 7) of the dinucleoside phosphate. Paper chromatography of the total incubation mixture in solvent C showed that the starting material was completely degraded to uridine and guanosine 3'-phosphate.

Degradations with the Snake Venom Phosphodiesterase.—Cytidylyl-(3'→5')-cytidine (0.5 μmole), cytidylyl-(3'→5')-uridine (0.6 μmole), cytidylyl-(3'→5')-adenosine (0.3 μmole), and cytidylyl-(3'→5')-guanosine (0.6 μmole) were each incubated with the purified venom phosphodiesterase preparation[29] using amounts previously standardized.[24] Degradation to the expected nucleosides and nucleoside 5'-phosphates was complete as followed by paper chromatography in the solvent 2-propanol–0.1 M boric acid–concentrated ammonia (7:2:1, v./v.). The identity of the nucleoside 5'-phosphates was further confirmed by elution of their spots and subsequent paper electrophoresis at pH 7.1.

(29) W. E. Razzell and H. G. Khorana, *J. Biol. Chem.*, **235**, 2105 (1959).

COMMUNICATIONS TO THE EDITOR

Bridgehead Adamantane Carbonium Ion Reactivities[1,2]

Sir:

Previous investigations have established the unusual bridgehead positions of bridged ring systems toward carbonium ion processes.[2-6] The considerable variation—10^{13}—in solvolysis rates between bridgehead derivatives in different ring systems[2-6] has been attributed to changes in conformational strain factors in proceeding from the ground state to the transition state.[2a,5] Estimates of angle strain provide a satisfactory quantitative explanation for the 10^3 reactivity difference between *t*-butyl bromide and 1-adamantyl bromide (I), but do not account for the further 10^3 difference between I and 1-bicyclo[2.2.2]octyl bromide (II).[5] The geometry around the reaction sites of both I and II is the same, and both would be expected to have nearly the same solvolytic reactivity, on the basis of angle strain considerations.[2-5]
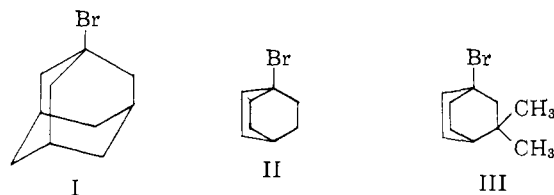

A possible explanation for the rate spread between I and II is based on electronic differences in the corre-

(1) Presented at the 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April, 1964, Abstracts, p. 22N.

(2) For background details, see (a) R. C. Fort, Jr., and P. von R. Schleyer, *Chem. Rev.*, **64**, 277 (1964); (b) P. von R. Schleyer, R. C. Fort, Jr., W. E. Watts, M. B. Comisarow, and G. A. Olah, *J. Am. Chem. Soc.*, **86**, 4195 (1964).

(3) Reviews: D. E. Applequist and J. D. Roberts, *Chem. Rev.*, **54**, 1065 (1954); U. Schöllkopf, *Angew. Chem.*, **72**, 147 (1960).

(4) W. von E. Doering, M. Levitz, A. Sayigh, M. Sprecher, and W. P. Whelan, Jr., *J. Am. Chem. Soc.*, **75**, 1008 (1953); see M. Finkelstein, Ph.D. Thesis, Yale University, 1955, and ref. 10.

(5) P. von R. Schleyer and R. D. Nicholas, *J. Am. Chem. Soc.*, **83**, 2700 (1961).

(6) H. Stetter, J. Mayer, M. Schwarz, and K. Wulff, *Ber.*, **93**, 226 (1960); H. Stetter and P. Goebel, *ibid.*, **96**, 550 (1963).

sponding bridgehead carbonium ions IV and V. The geometry of ions IV and V is ideal for C–C hyperconjugation,[7] which, conceivably, might be of greater importance for IVa (all second degree contributing hyperconjugative forms) than for Va (all first degree contributing hyperconjugative forms). Such hyperconjugation might thus account for the greater reactivity of I over II. Doering and co-workers[4] found that solvolysis of 1-bromo-3,3-dimethylbicyclo[2.2.2]octane (III) was about two times more rapid than that of II; C–C hyperconjugation (compare Va and Vb) might be responsible for the accelerative effect of methyl substituents, since in Vb one of the contributing forms is tertiary.
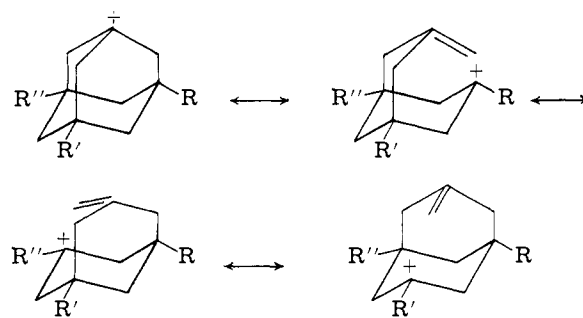
IVa, R = R' = R'' = H
b, R = CH$_3$, R' = R'' = H
c, R = R' = CH$_3$, R'' = H
d, R = R' = R'' = CH$_3$

Va, R = H
b, R = CH$_3$
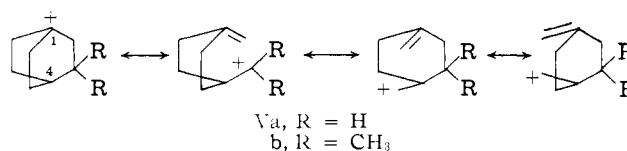
It has been argued earlier that hyperconjugative differences of this type do not contribute significantly

(7) M. J. S. Dewar, "Hyperconjugation," Ronald Press, New York, N. Y., 1962.