of 0.16 M in DEAE-cellulose chromatography using the same condition described in Figure 1. The yield was 640 OD$_{270}$ units, 30%, assuming $\epsilon_{(P)}$ = 10,400 at 280 nm. The unprotected trinucleotide pTpApG (ca. 3 OD$_{260}$ units) was degraded with purified

snake venom phosphodiesterase to give pT (0.066 μmol), d-pA (0.068 μmol), and d-pG (0.064 μmol) in paper chromatography (solvent C). The spectral properties and R_f values of the trinucleotide derivatives are given in Table I.

Communications to the Editor

## Synthesis of Isosteric Phosphonate Analogs of Some Biologically Important Phosphodiesters

Sir:

Recently we have developed syntheses of isosteric phosphonate analogs of both nucleoside 5'-phosphates (1)[1] and nucleoside 3'-phosphates (2)[2] in which the ester oxygen is replaced by a methylene group. Conversion of these compounds into analogs of natural phosphodiesters containing specific chemically and enzymatically stable bonds provides powerful tools for studying the mechanism of enzyme and hormone action. Such syntheses are described in this communication.

The intramolecular, high-dilution cyclization[3] of 1a using dicyclohexylcarbodiimide (DCC) in hot pyridine readily gave the 3',6'-cyclic phosphonate 3 as the crystalline free acid in 90% yield without need for chromatography: mp >220° dec; λ_{max} 259 mμ (ϵ 14,900).[4] The cyclization of 1a to 3 was much more facile than that of adenosine 5'-phosphate and could be carried out in concentrated solution, and even in aqueous pyridine. Thus, reaction of the tributylammonium salt of 1a with 4 equiv of DCC in refluxing pyridine–water (95:5) gave 89% of crystalline 3.

1a, B = adenine
b, B = uracil

2a, B = adenine
b, B = uracil

3
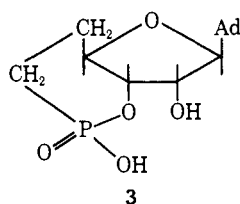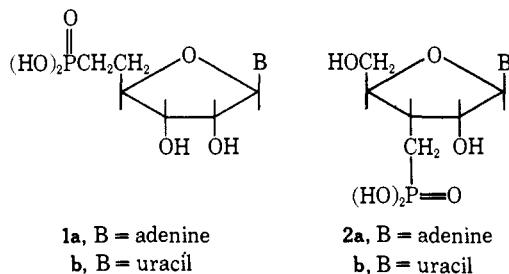
Cyclization of 2a to the branched-chain cyclic phosphonate 5 requires prior protection of the 2'-hydroxyl group. To this end 2a was converted in 70% yield into its 5'-O-monomethoxytrityl derivative (4a) as described

for adenosine 3'-phosphate.[5] Subsequent reaction of 4a with acetic anhydride in the presence of 30 molar equiv of tetraethylammonium acetate in pyridine gave the desired 2'-O-acetate 4b as the major product together with variable amounts of the 2'-cyclic ester[6]

4a, R$_1$ = MeOTr; R$_2$ = H
b, R$_1$ = MeOTr; R$_2$ = Ac

5

6a, B = Ad; R = H
b, B = Ad; R = MeOTr
c, B = Ur; R = H
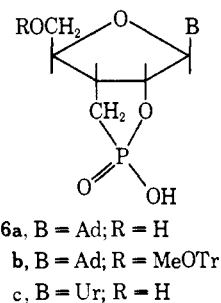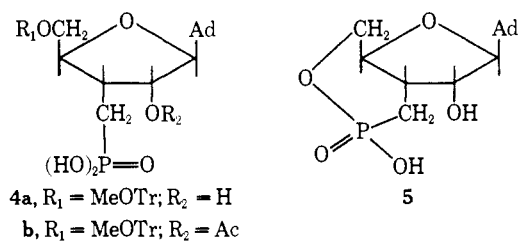
6b. The mixture was sequentially treated with 80% acetic acid to remove the methoxytrityl group, with DCC in pyridine to effect intramolecular cyclization to the 5'-hydroxyl group, and then deacetylated with ammonium hydroxide giving a mixture of the 3',5'-cyclic phosphonate 5 and the 2',3'-cyclic phosphonate 6a. Completely selective hydrolysis of the five-membered cyclic compound 6a[7] was achieved with 0.5 N hydrochloric acid at 22° for 2 hr and pure 5 was isolated by ion-exchange chromatography. Subsequent acidification gave 5 as the crystalline free acid in 10% overall yield from 2a: mp >220° dec; λ_{max} 258 mμ (ϵ 14,400). Biological studies on 3 and 5 which are phosphonate analogs of adenosine 3',5'-cyclic phosphate[8] will be described separately.

In order to obtain substrates with which to study the mechanism of enzyme action (*e.g.*, RNase) we have also prepared nucleoside 2',3'-cyclic phosphonates (6a, 6c) and the isomeric phosphonate analogs (7 and 8) of

(1) G. H. Jones and J. G. Moffatt, *J. Amer. Chem. Soc.*, 90, 5337 (1968).

(2) H. P. Albrecht, G. H. Jones, and J. G. Moffatt, *ibid.*, 92, 5511 (1970).

(3) M. Smith, G. I. Drummond, and H. G. Khorana, *ibid.*, 83, 698 (1961).

(4) All purified products gave satisfactory analytical values and 100-MHz nmr spectra.

(5) Y. Lapidot and H. G. Khorana, *J. Amer. Chem. Soc.*, 85, 3857 (1963).

(6) Using 10 equiv of tetraethylammonium acetate, which gives quantitative 2'-O-acetylation of the corresponding phosphate derivative,[5] the predominant product was the cyclic phosphonate 6b.

(7) Cf. the relative stabilities of five- and six-membered cyclic phosphates: H. G. Khorana, G. M. Tener, R. S. Wright, and J. G. Moffatt, *J. Amer. Chem. Soc.*, 79, 430 (1957).
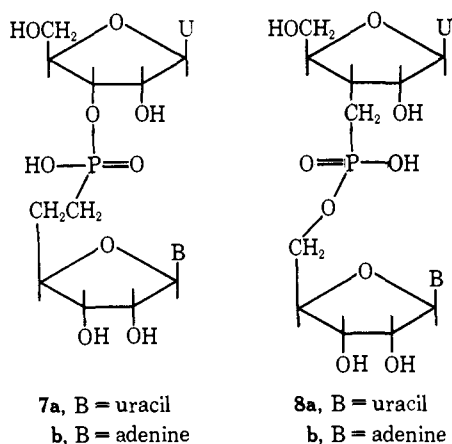
(8) R. W. Butcher, G. A. Robison, J. G. Hardman, and E. W. Sutherland, *Advan. Enzyme Regul.*, 6, 357 (1968).

7a, B = uracil
b, B = adenine

8a, B = uracil
b, B = adenine

dinucleoside phosphates. Acetylation of **1b** with acetic anhydride in the presence of excess tetraethylammonium acetate[9] gave the 2′,3′-di-O-acetyl derivative in 82% yield and the latter was condensed with 2′,5′-di-O-(4-methoxytetrahydropyran-4-yl)uridine[10] in the presence of DCC. Treatment of the product with ammonium hydroxide followed by 80% acetic acid removed the protecting groups and gave the dinucleoside phosphonate $UpCH_2U$[11] (**7a**) as its sodium salt in 78% yield following ion-exchange chromatography: $\lambda_{max}^{H_2O}$ 261 mμ ($\epsilon_{(p)}$ 21,200). Similarly, by condensation of the acetyl derivative of **1b** with 2′,5′-di-O-(4-methoxytetrahydropyran-4-yl)uridine, $UpCH_2A$ (**7b**) was obtained in 50% yield with λ_{max} 259 mμ ($\epsilon_{(p)}$ 24,200). Since **7a** and **7b** cannot be cleaved by spleen phosphodiesterase, the purity of the 3′,6′-phosphono ester was confirmed by nmr spectroscopy. Thus, the spectrum of **7b** in D_2O showed C_6H of the uracil ring as a sharp doublet ($J_{5,6}$ = 8 Hz) at 7.78 ppm while mixtures of the 2′,6′- and 3′,6′-phosphono esters prepared as above from 5′-O-p-nitrobenzoyluridine showed C_6H as a pair of doublets at 7.74 and 7.77 ppm, respectively. The nmr spectrum of **7a** showed the two C_6 protons as sharp doublets at 7.65 and 7.85 ppm. By careful time averaging[12] the purity of the 3′,6′ ester bonds in **7a** and **7b** was shown to be at least 98%.

The reactions of the triethylammonium salts of **2a** and **2b** with DCC in tert-butyl alcohol–DMF mixtures at 80° for 1 hr led to quantitative formation of the 2′,3′-cyclic phosphonates (**6a** and **6c**) which were isolated as their calcium salts. **6a** had a comparable stability to adenosine 2′,3′-cyclic phosphate being 50% hydrolyzed by 0.1 M hydrochloric acid in 35 min at 23°.[13]

Treatment of **2b** with dihydropyran in dioxane–DMF in the presence of trifluoroacetic acid gave the 2′,5′-bistetrahydropyranyl derivative which was isolated as its calcium salt in 73% yield. Condensation of the latter as its pyridinium salt with 2′,3′-O-anisylidene-uridine using DCC in pyridine followed by removal of the protecting groups with 80% acetic acid at 23° for

(9) In the absence of excess acetate ion roughly 15% of the 2′-O-acetyl 3′,6′-cyclic phosphonate was formed.

(10) D. P. L. Green, T. Ravindranathan, C. B. Reese, and R. Saffhill, Tetrahedron, 26, 1031 (1970).

(11) We shall use standard abbreviations for oligonucleotides except that the ester oxygen replaced by a methylene group is so indicated.

(12) The capable assistance of Dr. M. Maddox is gratefully acknowledged.

(13) For further kinetic data see M. R. Harris, D. A. Usher, H. P. Albrecht, G. H. Jones, and J. G. Moffatt, Proc. Natl. Acad. Sci. U. S., 63, 246 (1969).

24 hr gave UCH_2pU (**8a**) in 56% yield as its calcium salt following chromatography on DEAE–Sephadex: $\lambda_{max}^{H_2O}$ 262 mμ ($\epsilon_{(p)}$ 18,500). Hydrolysis of **8a** with 1 N sodium hydroxide at 23° for 15 hr gave equal amounts of **2b** and uridine. Similarly, condensation of the tetrahydropyranyl derivative of **2b** with 2′,3′-O-anisylidene-N^6-benzoyladenosine[14] gave UCH_2pA (**8b**) in 54% yield: $\lambda_{max}^{H_2O}$ 260 mμ ($\epsilon_{(p)}$ 23,400).

Synthesis of further derivatives of **1** and **2** and enzymatic studies on these compounds will be described shortly.

(14) S. Chladek and J. Smrt, Collect. Czech. Chem. Commun., 29, 214 (1964).

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3′-Deoxy-3′-(dihydroxyphosphinylmethyl)nucleosides. Isosteric Phosphonate Analogs of Nucleoside 3′-Phosphates

Sir:

Previous work from these laboratories has led to syntheses of isosteric[1] and nonisosteric[2] phosphonate analogs of nucleoside 5′-phosphates. We now describe a synthetic route to 3′-deoxy-3′-(dihydroxyphosphinylmethyl)nucleosides (**9**) which are isosteric analogs of nucleoside 3′-phosphates. In view of the instability of 3′-ketonucleosides[3] under basic conditions and the lack of reactivity of suitable, less basic reagents such as diphenyl triphenylphosphoranylidenemethylphosphonate[4] toward ketones, we preferred a route to the title compounds via the common, versatile carbohydrate intermediate **6**. Accordingly, 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose (**1**)[5] was condensed in tetrahydrofuran with tetraethyl methylenediphosphonate[6] in the presence of 1 equiv of n-butyllithium giving the vinyl phosphonate **2**[7] in 81% yield

1

2
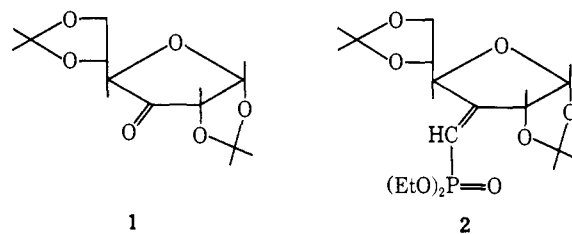
with bp 136–140° (10^{-3}mm); $[\alpha]^{22}D$ +127.6° (c 0.88, MeOH). The nmr spectrum (pyridine-d_5) indicated

(1) G. H. Jones and J. G. Moffatt, J. Amer. Chem. Soc., 90, 5337 (1968).

(2) (a) L. Yengoyan and D. H. Rammler, Biochemistry, 5, 3629 (1966); (b) D. H. Rammler, L. Yengoyan, A. V. Paul, and P. C. Bax, ibid., 6, 1828 (1967).

(3) (a) A. F. Cook and J. G. Moffatt, J. Amer. Chem. Soc., 89, 2697 (1967); (b) U. Brodbeck and J. G. Moffatt, J. Org. Chem., in press.

(4) G. H. Jones, E. K. Hamamura, and J. G. Moffatt, Tetrahedron Lett., 5731 (1968).

(5) W. A. Szarek, J. S. Jewell, I. Szczerek, and J. K. N. Jones, Can. J. Chem., 47, 4473 (1969).

(6) W. S. Wadsworth and W. D. Emmons, J. Amer. Chem. Soc., 83, 1733 (1961).

(7) All new compounds gave satisfactory elemental analyses and 100-MHz nmr spectra.