Efficient Synthesis of Methylenebis(phosphonate) Analogues of P^1,P^2-Disubstituted Pyrophosphates of Biological Interest. A Novel Plausible Mechanism

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Abstract: Synthesis of novel nucleoside bicyclic trisanhydrides **7** in the reaction of nucleoside-5′-methylenebis(phosphonate)s (**4**) with DCC is described. They were obtained by P^1,P^3- and P^2,P^4-dehydration of initially formed P^1,P^2,P^3,P^4-bismethylenetetraphosphonate **6**. Reaction of **7** (N = 2′,3′-*O*-isopropylideneadenosin-5′-yl) with 2′,3′-*O*-isopropylidenetiazofurin gave, after hydrolysis and deisopropylidenation, β-methylene-TAD (**10a**), the known potent inhibitor of inosine monophosphate dehydrogenase (IMPDH). Similar reaction of **7** with benzyl 2,3-*O*-isopropylidene-β-D-riboside followed by hydrolysis and deprotection afforded a new methylenebis(phosphonate) analogue of ADP-ribose **10b.** Upon reaction of **7** with riboflavin, the corresponding β-methylene-FAD (**10c**) was obtained. Bicyclic trisanhydride **7** prepared from (2′,3′-*O*-isopropylidene-N^4-acetylcytidin-5′-yl)methylenebis(phosphonate) was used in the synthesis of the methylenebis(phosphonate) analogues of CDP-ethanolamine **10d** and CDP-dipalmitoylglycerol **10e**.

Introduction

Formation of the cyclic anhydride adenosine 5′-trimetaphosphate (**1**; Figure 1) from ATP was first postulated by Michelson and Todd.[1] Glonek *et al*.[2] detected **1** using ^{31}P NMR when they treated ATP with DCC in pyridine. Orgel[3] observed efficient production of AMP from adenosine tetraphosphate (ATeP) upon treatment with water-soluble carbodiimide, postulated adenosine isotetrametaphosphate (**2**) being the intermediate. The first intermediate in hydrolysis of P_2O_5 was suggested to be P^2,P^3-anhydrotetrametaphosphate (**3**)[4] which was also formed as the end product of dehydration of orthophosphoric acid with DCC.[5] The evidence of its existence was based on ^{31}P NMR analysis of the reaction mixture which shows a surprisingly narrow-peaked spectrum containing, among others, two triplets at δ −25 to −31 and δ −35 to −40 with equal intensity. Recently we treated several P^1,P^2:P^3,P^4-bismethylene analogues of dinucleoside tetraphosphates (Np_4N) with DCC in pyridine and observed a similar formation of the corresponding bismethylene analogues of **3**. These compounds are useful intermediates in the synthesis of a variety of P^1,P^2-disubstituted methylenebis(phosphonate) analogues of biologically important pyrophosphates.

It has been generally accepted that DCC catalyzes direct coupling of nucleotides and nucleosides through formation of amidine intermediates such as **5** (Scheme 1). It was reported that reaction of **4** (A = adenosine protected as the 2′,3′-*O*-ethoxymethylene acetal) with **8** (B = 2′,3′-*O*-isopropylidenetiazofurin-5′-yl) in pyridine in the presence of DCC (4.5 equiv) afforded the methylenebis(phosphonate) analogue **10a** (β-methylene-TAD, Chart 1) of thiazole-4-carboxamide adenine dinucleotide (TAD) in 36% yield.[6] TAD and β-methylene-TAD are known to be potent inhibitors of inosine monophosphate dehydrogenase[7] which has been suggested as an important target in cancer chemotheraphy.[8] In our hands, hovewer, a similar coupling of **4** (A = 2′,3′-*O*- ispropylideneadenosin-5′-yl; Scheme 1) with nucleoside **8** did not afford β-methylene-TAD in more than 23% yield.

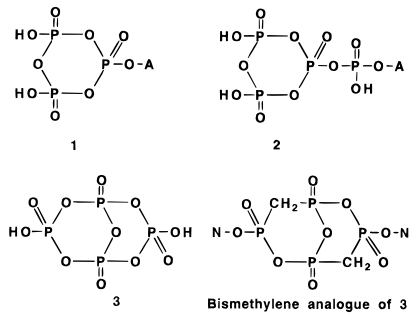

Figure 1. Structures of cyclic anhydrides. A = adenosin-5′-yl; N = nucleosid-5′-yl.

Results and Discussion

We examined the coupling of **4** and **8** and found that the early product of this reaction was the P^1,P^2:P^3,P^4- bismethylene analogue of P^1,P^4-diadenosine tetraphosphate (**6**, Ap_4A).[9] Compound **6** could be isolated in good yield after 1−2 h of reaction of **4** with DCC. We assumed that the Ap_4A analogue **6** may be involved in the final formation of β-methylene-TAD **10**. However, isolated **6** did not react with **8**. Therefore, we

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(1) Michelson, A. M.; Todd, A. R. *J. Chem. Soc.* **1949**, 2487−2490.
(2) Glonek, T.; Kleps, R. A.; Myers, T. C. *Science.* **1974**, *185*, 352−354.
(3) Ng, K. E.; Orgel, L. E. *Nucleic Acid Res.* **1987***15*, 3573−3580.
(4) Van Wazer, J. R.; Norval, S. *J. Am. Chem. Soc.* **1966**, *88*, 4415.
(5) Glonek, T.; Myers, T. C.; Han, P. Z.; Van Wazer, J. R. *J. Am. Chem. Soc.* **1970**, *92*, 7214−7216.
(6) Marquez, V. E.; Tseng, C. K. H.; Gebeyehu, G.; Cooney, D. A.; Ahluwalia, G. S.; Kelley, J. A.; Dalal, M.; Fuller, R. W.; Wilson, Y. A.; Johns, D. G. *J. Med. Chem.* **1986**, *29*, 1726−1731.
(7) Gebeyehu, G.; Marquez, V. E.; Kelly, J. A.; Cooney, D. A.; Jayaran, H. N.; Johns, D. G. *J. Med. Chem.* **1983**, *26*, 922−925.
(8) Jackson, R. C.; Weber, G.; Morris, H. P. *Nature* **1975**, *256*, 331−333.
(9) Blackburn, G. M.; Guo, M-J.; McLennan, A. G. In *Ap4A and Other Dinucleoside Polyphosphates;* McLennan, A. G., Ed.; CRC Press, Inc.: Boca Raton, FL, 1994; Chapter 11, pp 313−314.

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Scheme 1

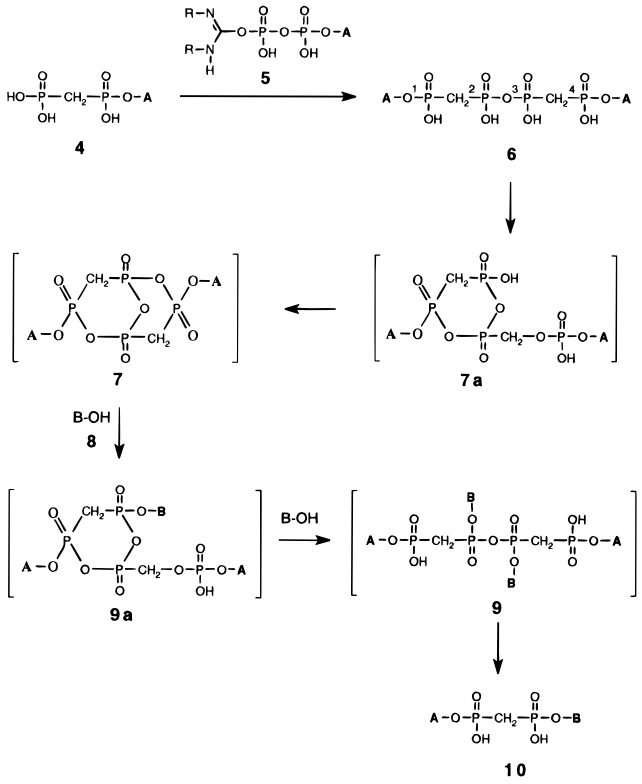
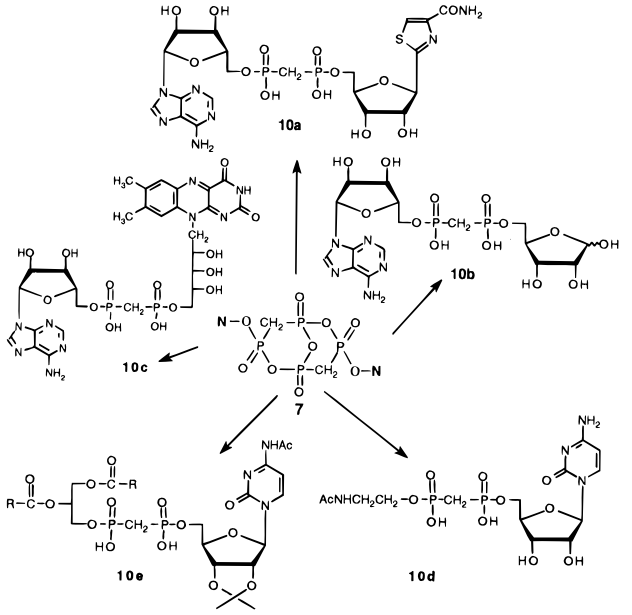


Chart 1



considered that **6** may be further converted into another intermediate which then reacts to give **10**. Indeed, treatment of **6** with DCC (2−3 equiv) for 2−3 h afforded a mixture which showed multisignal resonances in ^{31}P NMR as shown in Figure 2. Addition of tiazofurin derivative **8** (B-OH) at this stage of reaction caused gradual simplification of ^{31}P NMR signals which, after 1−2 h of heating at 60 °C, collapsed into two narrow multiplets at δ 8−9 and δ 18−21. Finally, addition of water to the reaction mixture resulted in the ^{31}P NMR showing almost exclusively an AB system of the desired product **10**. A similar course of reaction was observed when methylenebis(phosphonate) **4** was treated with DCC. After 5−6 h, multisignal resonances, such as shown in Figure 3, were detected in the ^{31}P NMR spectrum of the reaction mixture, and compound

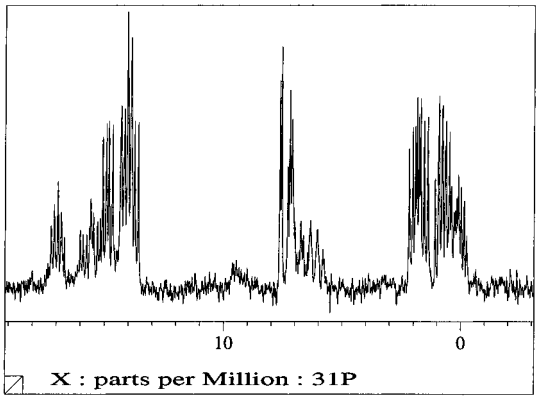


Figure 2. ^{31}P NMR spectrum of the reaction mixture of **6** (A = 2′,3′-*O*-isopropylideadenosin-5′-yl) with DCC in pyridine.

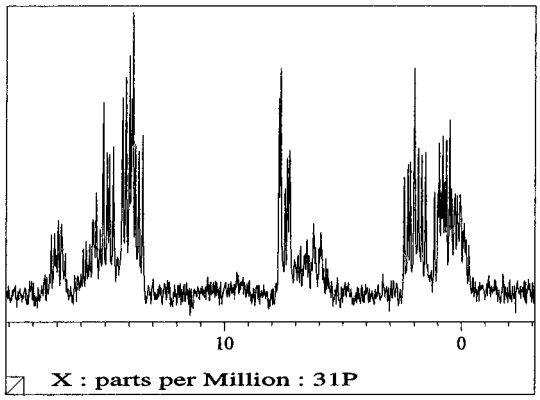


Figure 3. ^{31}P NMR spectrum of the reaction mixture of **4** (A = 2′,3′-*O*-isopropylideadenosin-5′-yl) with DCC in pyridine.

8 was added. HPLC purification of **10** (obtained directly from **4** or from isolated **6**) followed by deisopropylidenation afforded β-methylene-TAD (**10a**; Chart 1) in 92% overall yield. This procedure is amenable to gram-scale synthesis of β-methylene TAD.

Other nucleoside 5′-methylenebis(phosphonate)[10] derivatives **4** (Scheme 1, A = 2′,3′-*O*-isopropylideneuridin-5′-yl or A = 2′,3′-*O*-isopropylidene-N^4-acetylcytidin-5′-yl) reacted with DCC in pyridine, also forming the corresponding intermediate(s) **7** (A = U and C^{Ac}), which exhibit similar patterns of ^{31}P NMR features as depicted in Figures 4 and Figure 5, respectively.

The plausible mechanism for such reactions would be as follows: P^1,P^3-dehydration of **6** occurred, forming cyclic anhydride **7a** in a manner similar to dehydration of ATeP which afforded **2**. Bismethylene analogue **7a**, however, is expected to be more stable than **2** since the elimination of trimetaphosphate derivative from **7a** is not possible. Thus, further intramolecular P^2,P^4-dehydration of **7a** could lead to the formation of bicyclic trisanhydride **7**. Such an intermediate has not been reported earlier. This compound could be prepared from isolated Ap_4A analogue **6** or by treatment of **4** with DCC, diisopropylcarbodiimide (DIC), and probably other dehydrating agents. While reaction with DCC caused vigorous precipitation of dicyclohexylurea, with DIC no precipitation of the diisopropylurea occurred due to its solubility in pyridine. The bicyclic trisanhydride **7** could not be purified or isolated due to its

(10) Methylenebis(phosphonate)s of 2′,3′-*O*-isopropylidene-protected uridine and *N*-acetylcytidine were prepared from the corresponding 2′,3′-*O*-isopropylidene-5′-mesyluridine and -*N*-acetylcytidine derivatives by displacement of the 5′-mesyl group with the tris(tetrabutylammonium) salt of methylenebis(phosphonic acid) in DMSO.[11]

(11) Zatorski, A.; Lipka, P.; Mollowa, N.; Schram, K. H.; Goldstein, B. M.; Watanabe, K. A.; Pankiewicz, K. W. *Carbohydr. Res.* **1993**, *249*, 95−108.

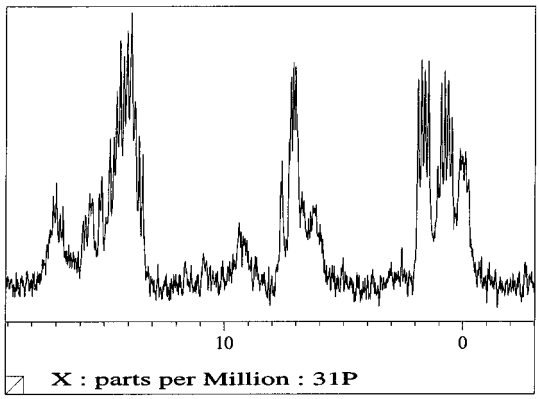


Figure 4. ^{31}P NMR spectrum of the reaction mixture of **4** (A = 2′,3′-*O*-isopropylide-*N*^{4}-cytidin-5′-yl) with DCC in pyridine.

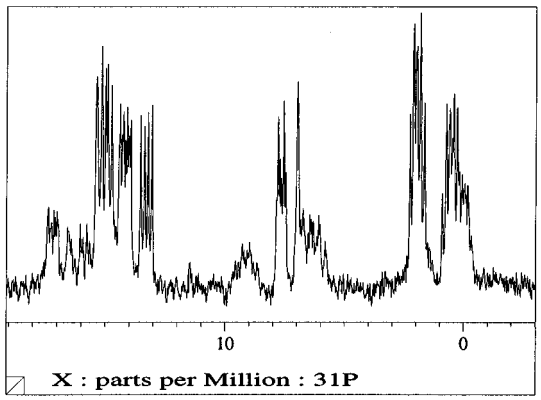


Figure 5. ^{31}P NMR spectrum of the reaction mixture of **4** (A = 2′,3′-*O*-isopropylideneuridin-5′-yl) with DCC in pyridine.

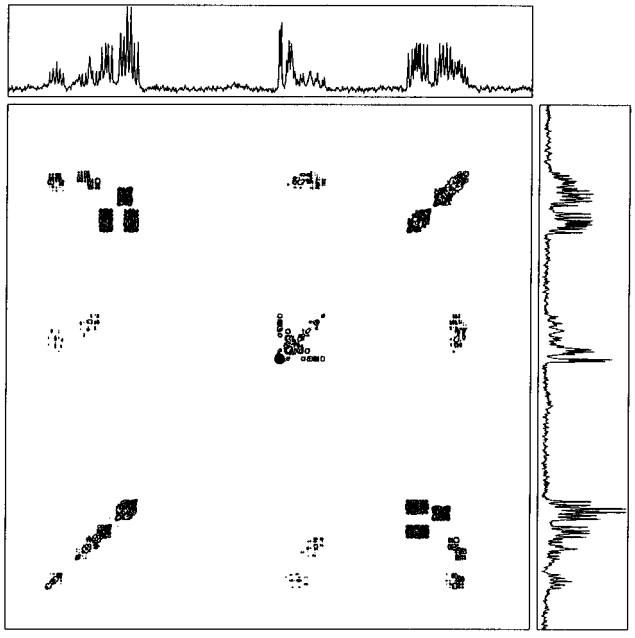


Figure 6. ^{31}P NMR (P,P)-COSY with proton decoupling of the reaction mixture of **6** (A = 2′,3′-*O*-isopropylideneadenosin-5′-yl) with DCC in pyridine.

susceptibility to hydrolysis. However, its presence could be detected by ^{31}P NMR showing multisignal resonances in two regions of δ −0.6 to −1.0 (*P*^{2},*P*^{3}) and δ 12.4−14.0 (P^1,P^4, Figure 2). The group of signals at δ 4.5−6.8, which are not coupled [as is evident from the ^{31}P NMR (P,P)-COSY spectrum of **7** (Figure 6)] with the above two groups of signals, is probably due to the presence of unreacted **7a** (approximately 25%). Although all four phosphorus atoms in the structure of **7** are chiral and up to 16 diastereomers can exist, the number of stereoisomers is reduced due to conformational restrictions. In the rigid structure of **7** the bridgehead phosphorus atoms, P^2 and P^3, can adopt *RR* or *SS* configuration, but they do not fit in this position having *SR* or *RS* geometry. Therefore, only 8 of 16 stereoisomers would be expected. If substituents at P^1 and P^4 are not chiral, these eight stereoisomers would form four diastereomers (two enatiomeric pairs each), but due to the chiral nature of adenosine moieties, there is no enatiomeric relationship between them. On the other hand, the probability of formation of some of these eight diastereomers is rather low. For example, although the chair−chair conformation of **7** with equatorially oriented *P*^{1},*P*^{4}-adenosines is expected to be most favored thermodynamically, the formation of the other chair−chair conformers is highly unlikely due to steric congestion. Moreover, equatorial preference of the bulky adenosine moieties should also influence the stereochemistry of preferred conformers. Thus, the pattern of phosphorus resonances in the spectrum of **7** would be much simpler than that expected from 16 compounds. Actually, the observed ^{31}P NMR feature was consistent with the above considerations.

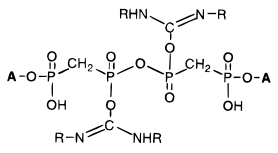
The assignment of the structure of the bicyclic trisanhydride **7** was further confirmed by its hydrolysis with $H_2{}^{18}O$ to the corresponding Ap$_4$A analogue **6a** which resulted in incorporation of two $H_2{}^{18}O$ molecules. Incorporation of only one ^{18}O isotope was also detected probably due to the presence of monocyclic derivative **7a**.

The chemical reactivity of anhydride **7** also confirmed its structural assignment. The ring opening reaction with nucleoside **8** (B-OH) occurred smoothly (due to the uncharged nature of bicyclic trisanhydride **7**) to give the corresponding tetraester **9** as a single product detected by ^{31}P NMR. Such reactivity can be explained by an assumption of a greater vulnerability of phosphorus atoms *P*^{2} and *P*^{3} than *P*^{1} and *P*^{4} of the bicyclic structure of **7** for nucleophilic attack due to the steric hindrance of bulky adenosine moieties. Thus, substitution of phosphorus *P*^{2} (alternative substitution of *P*^{3} is not discussed for clarity reasons) should result in the formation of intermediate **9a** by breaking the P^2−O−P^4 bond rather than the P^2−O−P^3 linkage. The pyrophosphate bond P^2−O−P^3 in **9a** is left intact to allow the second attack of **8** on still uncharged phosphorus P^3 of **9a** to give derivative **9**. Alternatively, the concerted attack on P^2 and P^3 atoms would also give derivative **9**. After hydrolysis with water 2 equiv of **10** was obtained from one molecule of **9** in almost quantitative yield.

Unreacted monocyclic derivative **7a** was detected in the reaction mixture of the active intermediate **7** by ^{31}P NMR and MS analysis. Excess DCC, prolonged reaction time, or elevated temperature did not eliminate **7a** whose ^{31}P NMR spectrum contains resonance signals at δ 4.5−6.8. A similar reaction of **7a** with **8** followed by hydrolysis should afford equal amounts of the desired product **10** and starting material **4**. However, **4** was not found in the final product. This is probably because the intermediate of **7a** reacted (at P^3) with nucleophile **8**, with breaking of the P^1−O−P^3 pyrophosphate bond. Then a subsequent P^2,P^4 DCC dehydration followed by the second attack of **8** on P^2 results in formation of **9**.

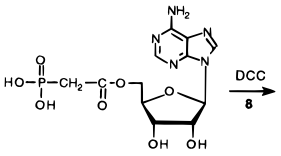
In order to explain the course of formation of P^1,P^2-disubstituted methylenebis(phosphonate)s **10** from the corresponding tetraphosphonate analogues **6**, alternative mechanisms such as (1) formation of an Ap$_4$A intermediate containing two dicyclohexylamidine groups at P^2 and P^3 or (2) formation of cyclic dimers, larger macrocyclic molecules, and/or linear polymers were also considered. As for mechanism (1) one

Chart 2



Ap_4A with two amidine groups

Scheme 2



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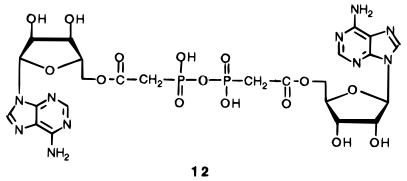

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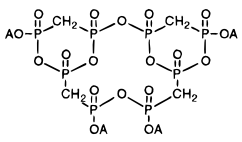
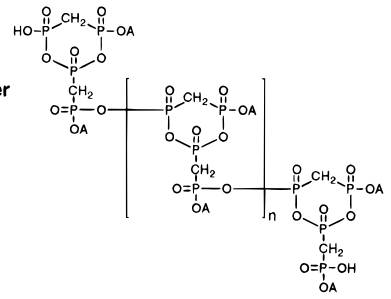
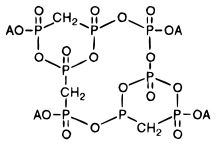
Chart 3



"Head to head" dimer





"Head to tail" dimer

"Head to tail" polymer

might argue that **6** containing two dicyclohexylamidine groups attached to P^2 and P^3 (Chart 2) may be the intermediate for the synthesis of various products described herein. These two leaving groups (cyclohexylamidine groups) could then be displaced with **8** to give tetraester **9**. However, this is rather unlikely because the ^{31}P NMR spectrum of such an intermediate would be similar to that of its product **9.** In contrast, however, the spectrum of the intermediate **7** is much more complicated than that of **9**. Moreover, we found that similar treatment of adenosine 5′-phosphonoacetate (**11**; Scheme 2) with DCC and tiazofurin **8** did not lead to the formation of the expected phosphonoacetate analogue of TAD.[12] Although adenosine phosphonoacetate anhydride (**12**) was obtained in a good yield, it neither could be dehydrated in a manner similar to that of **6** nor activated via cyclohexylamidine group(s) for efficient reaction with **8**.

Intermolecular dehydration of **7a** (mechanism 2) is also possible, leading to the formation of cyclic dimers, larger macrocyclic molecules, and/or linear polymers (Chart 3). Fortunately, further reaction of these species with **8** would result in equally efficient formation of the desired P^1,P^2-disubstituted methylenebis(phosphonate)s **10**, providing that P^2 and P^3 were preferentially attacked due to steric hindrance at P^1 and P^4. However, intermolecular dehydration of **7a** would produce an even more complicated mixture of polymeric intermediates whose ^{31}P NMR spectra could not match the ^{31}P spectra of the intermediates **7** and **7a** obtained during the course of intramolecular reaction.

P^1,P^2-Disubstituted pyrophospates play an important role in a variety of biochemical transformations. Nicotinamide adenine dinucleotide and flavin adenine dinucleotide (FAD) serve as the major electron carriers in biological dehydrogenations whereas coenzyme A (CoA) is a universal carrier of acyl groups. Cytidine diphosphodiacylglycerol (CDP-DAG), cytidine diphosphocholine (CDP-choline), and cytidine diphosphoethanolamine (CDP-ethanolamine) are activated intermediates in the *de novo* synthesis of phospholipids. UDP-glucose, UDP-galactose, GDP-mannose serve as cofactors in many sugar transfer processes. Finally, mono- and poly(ADP-ribose) derivatives which modulate function of proteins also contain the pyrophosphate (P−O−P) moiety.

We took advantage of bicyclic trisanhydrides **7** as intermediates in the synthesis of isosteric methylenebis(phosphonate) analogues of biologically important P^1,P^2-disubstituted pyrophosphates. Replacement of the pyrophosphate oxygen by a methylene group preserves the shape and size of the natural counterpart significantly, but provides modified biochemical properties, as $P^1-CH_2-P^2$ cannot be hydrolyzed by the enzymes that cleave the P−O−P bond.[13]

Thus, the reaction of intermediate **7** (N = A, Chart 1) with benzyl 2,3-*O*-isopropylidene-*β*-D-riboside (**11**) followed by hydrolysis and deprotection afforded novel ADP-ribose analogue **10b** in 72% overall yield**.** In a similar manner treatment of **7** (N = A) with unprotected riboflavin (**12**) afforded, after workup, the FAD analogue **10c** in 12.5% yield. Treatment of **7** (N = C^{Ac}) with *N*-acetylethanolamine (**13**) or 1,2-dipalmitoyl-*sn*-glycerol (**14**) gave methylenebis(phosphonate) analogues of CDP-ethanolamine and CDP-DAG (**10d** and **10e**, respectively), in high yield.

The potential application of anhydrides **7** for preparation of other methylenebis(phosphonate)s of biological importance is now under vigorous investigation.

Experimental Section

General Methods. HPLC was performed on a Dynamax-60A C18-83-221-C column with a flow rate of 5 mL/min or Dynamax-300A C18-83-243-C column with a flow rate of 20 mL/min of 0.1 M $Et_3N{\cdot}H_2CO_3$ (TEAB) followed by a linear gradient of 0.1 M TEAB−aqueous MeCN (70%). Nuclear magnetic resonance spectra were recorded on a Jeol Eclipse 270 with Me_4Si or DDS as the internal standard for ^1H and external H_3PO_4 for ^{31}P. Chemical shifts are reported in parts per million (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), and dd (double doublet). Values given for coupling constants are first order. Negative ion mass spectra were recorded using a Finnigan TSQ-700 instrument equipped with an electronspray ion source. Samples were dissolved in $CH_3CN-H_2O-Et_3N$ (90:9:1) at a concentration of 20 pM/μL and were introduced by infusion at a flow rate of 3 μL/min.

Synthesis of P^1,P^2:P^3,P^4-Bismethylene-P^1,P^4-diadenosine Tetraphosphonate. Adenosine 5′-methylenebis(phosphonate) (**4**; 0.06 mmol, 42 mg as the bis(triethylammonium) salt) was dissolved in dry pyridine (1 mL) containing DCC (37 mg, 0.12 mmol) and stirred for 2 h at room temperature. Addition of water followed by concentration *in vacuo* afforded the residue which was purified on HPLC (Dynamax-300A C18-83-243-C) to give **6** (30 mg, 72% as the tetrakis(triethylammonium) salt). ^1H NMR (D_2O): δ 1.45 (3H, s, Me-isopropylidene), 1.67 (3H, s, Me-isopropylidene), 2.36 (2H, t, P-CH_2-P, J_{P-H} = 20.2 Hz), 4.04−4.17 (2H, m, H5′, 5″), 4.57−4.60 (1H, m, H4′), 5.23 (1H, dd, H3′, $J_{2',3'}$ = 6.1 Hz, $J_{3',4'}$ = 1.9 Hz), 5.36 (1H, dd, $J_{1',2'}$ = 3.7 Hz),

(12) Zatorski, A.; Lipka, P.; Pankiewicz, K. W. *Collect. Czech. Chem. Commun.* **1993**, *58*, 122−126.

(13) Engel, R. *Chem. Rev.* **1977**, *77*, 350.

8.10, 8.41 (two 1H singlets, H2, H8). ^{31}P NMR (pyridine): δ 7.31 (br s), 15.43 (br s).

Synthesis of P^1,P^4-Disubstituted Bicyclic Trisanhydrides (BTAs) 7. Nucleoside 5′-methylenebis(phosphonate) (**4**; 0.1 mmol, prepared by reaction of 2′,3′-*O*-isopropylidene-5′-*O*-mesyladenosine, -uridine, and -N^4-acetylcytidine with the tris(tetrabutylammonium) salt of methylenebis(phosphonic acid) in DMSO[10]) was dissolved or suspended in dry pyridine (1 mL) containing DCC (103 mg, 0.33 mmol) and stirred (2−5 h) until ^{31}P NMR of the reaction mixture showed multisignal resonances characteristic for BTAs **7**. Treatment of **6** with DCC afforded an identical pattern of phosphorus resonances. See Figure 3 (adenosine-BTA obtained from **4**) , Figure 2 (adenosine-BTA obtained from **6**), Figure 4 (N^4-acetylcytidine-BTA), Figure 5 (uridine-BTA), and Figure 6 (adenosine-BTA, ^{31}P NMR (P,P)-COSY spectrum). Treatment of **4** or **6** with DIC afforded a mixture showing the same pattern of ^{31}P resonances.

Synthesis of P^1,P^2-Disubstituted Methylenebis(phosphonate)s. At the time the formation of bicyclic anhydride **7** was completed, compound **8**, **11**, **12**, **13**, or **14** (0.15 mmol) was added and the mixture was heated at 65 °C for from 2 h to 2 days until the ^{31}P NMR spectrum of the reaction showed the formation of intermediate **9** (two multiplets at δ 8−9 and δ 18−21). Then reaction was quenched by addition of water, and the mixture was stirred at room temperature for 1−3 h, filtered, and concentrated *in vacuo*. The residue was chromatographed on an HPLC column to give the following compounds.

(1) P^1-(2′,3′-*O*-Isopropylidenetiazofurin-5′yl)-P^2-(2′,3′-*O*-isopropylideneadenosin-5′-yl)methylenebis(phosphonate) (10). Yield: 95%. ^{1}H NMR (D_2O): δ 1.26 (18H, t, Et_3NH^+), 1.38, 1.45, 1.60, and 1.67 (3H each, Me-isopropylidene of tiazofurin and Ado), 2.07 (2H, t, P-CH_2-P, J_{P-H} = 20.1 Hz), 3.19 (12H, q, Et_3NH^+), 3.88−3.98 (2H, m, H5′,5″ of tiazofurin), 4.07−4.12 (2H, H5′,5″ of Ado), 4.35−4.38 (1H, m, H4′ of tiazofurin), 4.59−4.62 (1H, m, H4′ of Ado), 4.89−4.95 (2H, m, H2′, 3′ of tiazofurin), 5.09 (1H, d, H1′ of tiazofurin, $J_{1'2'}$ = 3.4 Hz), 5.22 (1H, dd, H3′, $J_{2',3'}$ = 6.2 Hz, $J_{3',4'}$ = 2.2 Hz), 5.36 (1H, dd, H2′, $J_{1',2'}$ = 3.5 Hz), 6.18 (1H, d, H1′), 8.06, 8.14, and 8.42 (three 1H singlets, H2, H8 of Ado and H5 of tiazofurin). ^{31}P NMR (D_2O): δ 17.80 (AB system, $J_{P,P}$ = 10.0 Hz). This compound was deprotected on Dowex 50/H^+ to give **10a** (97%). The ^{1}H NMR spectrum was identical with that of an authentic sample.

(2) P^1-(Benzyl-2,3-*O*-isopropylidene-β-D-ribos-5-yl)-P^2-(2′,3′-*O*-isopropylideneadenosin-5′-yl)methylenebis(phosphonate). Yield: 78%. ^{1}H NMR (D_2O) δ: 1.21 and 3.12 (Et_3NH^+), 1.23, 1.37, 1.40, and 1.62 (3H each, Me-isopropylidene) 2.09 (2H, t, P-CH_2-P, J_{P-H} = 20.0 Hz), 3.8 (2H, m, H5,5′, ribose), 4.05 (2H, t, H5′, H5″, Ado, $J_{4',5'}$ = $J_{4',5''}$ = 4.6 Hz,), 4.27 (1H, t, H4, ribose, $J_{4,5}$ = $J_{4,5'}$ = 7.7 Hz), 4.30 and 4.50 (2H, two d, $PhCH_2$, J = 11.4 Hz), 4.55 (1H, m, H4′, Ado), 4.56 (1H, d, H3, ribose, $J_{2,3}$ = 6.0 Hz), 4.78 (1H, H2, ribose), 5.06 (1H, s, H1, ribose), 5.12 (1H, dd, H3′, Ado, $J_{3',4'}$ = 2.2 Hz), 5.25 (1H, dd, H2″, Ado, $J_{2',3'}$ = 6.2 Hz), 6.09 (1H, d, H1′, Ado $J_{1',2'}$ = 3.5 Hz), 7.22 (5H, m, phenyl), 8.06, 8.37 (two 1H singlets, H2, H8, Ado). ^{31}P NMR (D_2O): δ 17.47 (d, J_{P-C-P} = 10.8 Hz), 17.73 (d, J_{P-C-P} = 10.8 Hz). Deprotection on Dowex 50/H^+ afforded **10b** as a 1:2 mixture of α,β-anomers. ^{1}H NMR (D_2O): δ 2.18 (0.7 H, t, P-CH_2-P, J_{P-H} = 19.9 Hz, α-anomer), 2.20 (1.3H, t, P-CH_2-P, J_{P-H} = 19.9 Hz, β-anomer), 3.8−4.8 (10H, all H atoms of sugars except H1 of ribose and H1′ of Ado), 5.18 (0.66H, d H1 of β-ribose, $J_{1,2}$ = 1.6 Hz), 5.30 (0.33H, d, H1 of α-ribose, $J_{1,2}$ = 4.0 Hz), 6.00 (0.33H, d, H1′ of α-anomer of Ado $J_{1',2'}$ = 4.1 Hz), 6.05 (0.66, d, H1′ of β-anomer of Ado, $J_{1',2'}$ = 5.1 Hz), 8.16, 8.50 (two 1H doublets, H2, H8, Ado). ^{31}P NMR (D_2O): δ 18.0 (brs).

(3) P^1-(Riboflavin-5′-yl)-P^2-(2′,3′-*O*-isopropylideneadenosin-5′-yl)-methylenebis(phosphonate). Yield: 12.5%. ^{1}H NMR (D_2O): δ 1.40 (3H, s, Me-isopropylidene), 1.63 (3H, s, Me-isopropylidene), 2.21 (2H, t, P-CH_2-P, J_{P-H} = 19.8 Hz), 2.32 (3H, s, Me-flavin), 2.39 (3H, s, Me-flavin), 3.89−4.06 (3H, m, H4′, H5′, H5″). Deisopropylidenation on Dowex 50/H^+ gave FAD analogue **10c**. ^{1}H NMR (D_2O): δ 2.30 (2H, t, P-CH_2-P, J_{P-H} = 19.9 Hz), 2.35 (3H, s, Me-flavin), 2.41 (3H, s, Me-flavin), 3.80−5.00 (12H, m, ribitol and ribose protons), 5.81 (1H, d, H1′, Ado, $J_{1',2'}$ = 5.1 Hz), 7.56 and 7.67 (two 1H singlets, flavin), 7.82 and 8.39 (two 1H singlets, H2, H8, Ado). ^{31}P NMR (D_2O): δ 18.17 and 18.77 (AB system, $J_{A,B}$ = 12.3 Hz).

(4) P^1-[(*N*-Acetylamino)ethyl]-P^2-(2′,3′-*O*-isopropylidene-N^4-acetylcytidin-5′-yl)methylenebis(phosphonate). Yield: 83%. ^{1}H NMR (D_2O): δ 1.23 and 3.15 (Et_3NH^+), 1.37 and 1.57 (3H each, isopropylidene), 1.89 (3H, s, *N*-Ac), 2.05 (2H, t , P-CH_2-P, J_{P-H} = 19.8 Hz), 2.19 (3H, s, N^4-Ac), 3.31 (2H, m, CH_2-N, ethanolamine), 3.89 (3H, m, CH_2O-P, ethanolamine), 4.03−4.19 (2H, m , H5′, H5), 4.69 (1H, m, H4′), 4.95 (1H, dd, H2′, $J_{2',3'}$ = 6.1 Hz), 5.01 (1H, dd, H3′, $J_{3',4'}$ = 1.4 Hz), 5.85 (1H, d, H1′, $J_{1',2'}$ = 2.2 Hz), 7.31 and 8.29 (1H each, d H5, H6, $J_{5,6}$ = 7.6 Hz). ^{31}P NMR (D_2O): δ 17.73 (br s).

Treatment with Dowex 50/H^+ gave **10d**. ^{1}H NMR (D_2O): δ 1.97 (3H, s, *N*-Ac), 2.18 (2H, t, P-CH_2-P, J_{P-H} = 19.8 Hz), 3.38 (2H, t, CH_2-N, J = 5.4 Hz), 3.95 (3H, m, CH_2-OP), 4.10−4.36 (5H, m, H2′,3′,4′,5′,5″), 5.96 (1H, d, H1′, $J_{1',2'}$ = 3.6 Hz), 6.10 (1H, d, H6, $J_{5,6}$ = 7.6 Hz), 8.00 (1H, d, H5, $J_{5,6}$ = 7.6 Hz). ^{31}P NMR (D_2O): δ 18.01, 18.05 (AB system, $J_{A,B}$ = 10.8 Hz).

(5) P^1-(1,2-Dipalmitoyl-*sn*-glycer-3-yl)-P^2-(2′,3′-*O*-isopropylidene-N^4-acetylcytidin-5′-yl)methylenebis(phosphonate) 10e. Yield 19%. ^{1}H NMR (CD_3OD): δ 0.89 (6H, t, CH_3-palmitoyl, J = 6.6 Hz), 1.28 (76H, m, CH_2-palmitoyl), 1.34 and 1. 55 (3H each, s, CH_3-isopropylidene), 1.5 (4H, m, CH_2-CH_2COOH), 2.15 (2H, t, P-CH_2-P, J_{P-H} = 19.8 Hz), 2.17 (3H, s, acetyl), 2.28 and 2.30 (2H each, t, CH_2COOH, J = 7.3 and J = 7.6 Hz), 3.18 (12H, q, CH_2-N, J = 7.3 Hz), 4.07 (2H, m, CH_2-3-glycerol), 4.19 (3H, m, H5′, H5″, CH_2-1-glycerol), 4.48 (2H, m, H4′, CH_2-1-glycerol), 4.89 (1H, dd, H3′, $J_{2',3'}$ = 6.1 Hz, $J_{3',4'}$ = 2.5 Hz), 5.01 (1H, dd, H2′, $J_{1',2'}$ = 2.7 Hz), 5.24 (1H, m, CH-2-glycerol), 6.01 (1H, d, H1′, $J_{1',2'}$ = 2.7 Hz), 7.45 (1H, d, H5, $J_{5,6}$ = 7.5 Hz), 8.41 (1H, d, H6). ^{31}P NMR (CD_3OD): δ 16.56 and 16.58 (AB system, $J_{P,P}$ = 3.5 Hz).

Adenosine Phosphonoacetate Anhydride. Adenosine phosphonoacetate[12] (16 mg, 0.03 mmol) was dissolved in pyridine-d_5 (0.5 mL) containing DCC (6.7 mg, 0.032 mmol) and kept at room temperature for 2 h until disappearance of the resonance signal of the starting material at δ 11.08 and formation of new peak of the product at δ 5.38 in ^{31}P NMR. The reaction mixture was concentrated *in vacuo*, and the residue was purified on a HPLC column to give the adenosine phosphonoacetate anhydride (26 mg, 78%) as the bis(triethylammonium) salt. ^{1}H NMR (D_2O): δ 1.19 (t, 18H, Et_3N), 2.96 (q, 12H, Et_3N), 3.08−3.18 (t, 2H, P-CH_2-CO), 4.41−4.43 (m, 3H, H4′, 5′, 5″), 4.52−4.55 (m, 1H, H3′), 4.90 (pseudo-t, 1H, H2′), 6.03 (d, 1H, H1′, $J_{1',2'}$ = 5.9 Hz), 8.09, 8.35 (two 1H singlets, H2, H8). ^{31}P NMR (D_2O): δ 4.91 (s). MS (ES): *m/z* 759 $(M - H)^-$.

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