Phosphonates as Analogues of Natural Phosphates

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Contents

I. Introduction 349
II. Physical and Chemical Comparison of Phosphates and Their Phosphonic Acid Analogues 349
III. Analogues of Inorganic Pyrophosphate 350
A. Synthesis 350
B. Biological Investigations 351
IV. Analogues of Carbohydrate Phosphates 351
A. Synthesis 351
B. Biological Investigations 353
V. Analogues of Nucleotides 353
A. Synthesis 353
B. Biological Investigations 357
VI. Analogues of Phosphate Products of Glycolysis 359
A. Synthesis 359
B. Biological Investigations 360
VII. Analogues of Phospholipids 360
A. Synthesis 360
B. Biological Investigations 363
VIII. Other Phosphonic Acid Analogues of Natural Phosphates 363
A. Synthesis 363
B. Biological Investigations 364
IX. References and Notes 364

I. Introduction

During the past 15 years there has developed significant interest in the preparation and investigation of phosphonic acids and their derivatives which might be considered to be analogues of naturally occurring phosphates. This interest was generated by the recognition that phosphonic acids and their esters, used as analogues of naturally occurring phosphates, possessed intriguing possibilities for metabolic regulation or perturbation.

As it was presumed that the carbon–phosphorus bond was incapable of being hydrolyzed by the "ordinary" enzymes involved in phosphate cleavage, several mechanistic possibilities were deemed to exist for metabolic regulation by compounds bearing such a linkage in place of the usual phosphate ester linkage. (It should be noted that the presence of the carbon–phosphorus linkage does not preclude enzymatic cleavage of phosphorus–ester linkages also present.[1]) For example, given a phosphate which acts as a metabolite or metabolic regulator using enzymatic reactions at sites distant from the phosphate ester linkage, but by other extraneous routes is hydrolyzed to inorganic phosphate, the use of a phosphonic acid analogue in its place might be expected to enhance the lifetime, and thereby the integrated activity, of the metabolite or regulator. Also, as a substitute for a natural phosphate metabolite, a phosphonic acid or a phosphonate ester may be capable of inhibiting or perturbing the regular metabolism of an organism simply by nonparticipation in a normal phosphate cleavage process. Several factors leading to this nonparticipation will be discussed later. Finally, the phosphonic acid, substituting for a natural metabolite in its entrance to an organism, and possibly in several consequent reaction steps, might be capable of specific or nonspecific inhibition of one or more enzymatic processes.

Thus, the use of phosphonic acids as analogues of natural phosphates represents a more systematic approach to metabolic regulation,[2] enhancement or inhibition, than is commonly attributed to "analogue" study.

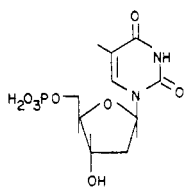
An additional factor in the development of this interest was the isolation of naturally occurring phosphonic acids from numerous organisms.[3-10] Aside from the presence of a phosphorus atom, these compounds bear other interesting structural similarities to commonly observed phosphates, and presumably the organisms involved are capable of metabolizing the carbon–phosphorus bond.[11,12] Interest here was also stimulated by the discovery that mutant strains of *Escherichia coli* could use simple phosphonic acids as a phosphorus source.[13] These results were of particular interest in light of prior efforts[14,15] indicating that the carbon–phosphorus bond could not be metabolized in rats. Still later work demonstrated that 2-aminoethylphosphonic acid could be incorporated into lipids in rat liver involving initial generation of CMP-aminoethylphosphonate.[16,17] Phosphonatase activity at a low level may be present in numerous organisms. The topic of natural phosphonic acids has been treated in detail in other places and will not be a major consideration here.

In considering analogue design, quality is usually judged by an empirical standard: is or is not the desired effect produced by the analogue. This at times might result in a narrowness of thought if one considers only a very limited range of chemical reaction types. On the contrary, the term "analogue" has more often been misused by undue expansion of the range of chemical reactions considered. Ideally, an "analogue" would contain but one structural variation from the parent substance, that, for present considerations, being the presence of a carbon–phosphorus linkage in place of either the normal phosphate ester linkage, or, a free hydroxyl on phosphorus. It will be noted that the present review also treats analogues bearing further structural variations and compounds only peripherally related to natural molecules, but containing carbon–phosphorus bonds. The value of these as analogues will be judged ultimately by the standard noted above.

II. Physical and Chemical Comparison of Phosphates and Their Phosphonic Acid Analogues

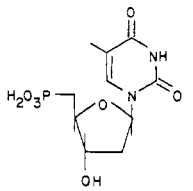
Several aspects of change must be considered upon the performance of this fundamental structural substitution, aside from rendering the phosphorus not liable to hydrolysis by normal routes.[9] First, there would be expected to result some significant decrease in acidity of the phosphorus-containing acid function upon the introduction of an electron-donating alkyl group. This could result in the existence of a different state of dissociation for the analogue compared to the natural compound at a particular (physiological) acidity associated with a biological system.

In comparison of a phosphonic acid with a simple phosphate, that is, a monoester of phosphoric acid, it is the second pK_a which is of interest, as for either system the first represents a relatively strong acid. Crofts and Kosolapoff[18] have measured the second pK_a's for a series of phosphonic acids and found them to be in the range 7.7–8.2 when a primary alkyl group is attached to phosphorus. A typical decrease was found with branching of the alkyl group. This is to be compared with second pK_a values of ca. 7.0 for the corresponding monoalkyl phosphates.[19] Slightly lower values were reported[20] for 2′-deoxythymidine 5′-phosphate (**1**) and a phosphonic acid analogue, 2′,5′-dideoxythymidine-5′-phosphonic acid (**2**).



1

pK_{a2} = 6.5



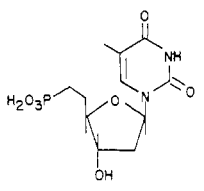
2

pK_{a2} = 7.0

If one considers analogues in which a methyl group has been substituted for a hydroxyl on phosphorus, there remains an ester linkage and a single acidic hydroxyl of low pK_a. Thus under physiological conditions this class of compounds should be considered to be completely dissociated, but of course only to a monoionic state. This point would be expected to be critical in consideration of perturbations of biological activity.

A second factor of change of greater significance is that of physical size and shape. In a comparison of structures **1** and **2**, the latter is obviously contracted in overall size, and more specifically, the distances between phosphoryl oxygen and other possible binding sites or sites of interaction (3′-hydroxyl, ring oxygen, heterocyclic base) are significantly changed unless extremely strained and highly unlikely conformations are invoked. This difference could result in great variation of biochemical or physiological activity unrelated to the simple substitution of a carbon–phosphorus linkage for that of a phosphate ester.

For this reason the "isosteric" phosphonic acid analogue of **1**, i.e., 2′,5′-dideoxy-5′-dihydroxyphosphinylmethylthymidine (**3**),[21] might be considered for some purposes as a "better"



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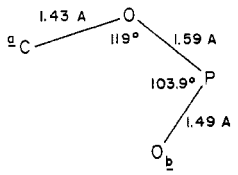
analogue than **2**. It should be noted that while the term "isosteric" strictly refers to compounds of *identical* size and shape, and **3** compared to **2** does not meet these conditions most rigorously, the bond angles and lengths involved are similar enough that the term reasonably may be applied.

Using available crystallographic data for the related compounds 2-aminoethyl phosphate[22,23] and 2-aminoethylphosphonic acid[24] (realizing all difficulties inherent in such an approach), one may predict the relative positions of phosphoryl oxygen and some other "fixed" position for a natural phosphate and its phosphonic acid "isostere"; as a generalized comparison, considering the distance between positions *a* and *b*, and *a′* and *b′*, one finds a variation of only 0.8%, the distance in the phosphonic acid being greater. The percentage variation in distances to further points (from *b* or *b′*) would be even less. With data derived from other simple phosphate esters,[25–27] similar



magnitude deviations may be calculated in both positive and negative directions. Thus the use of the term "isosteric" is clearly indicated to be reasonable for these systems.

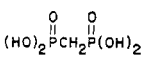
However, not for all related systems is such favorable agreement found. For inorganic pyrophosphate and its nominally isosteric phosphonic acid analogue, methylenediphosphonic acid (**4**), Larsen, Willett, and Yount[28] found signifiant angular differences (>10°) about the central atom. Thus, in an analysis of the oxygen–oxygen nonbonded internuclear distances for the five-atom-coplanar conformations of inorganic pyrophosphate and **4** (OPOPO AND OPCPO), using this and associated data,[22,23,25,27–29] that distance for the phosphonic acid is ca. 16% greater than that for the normal substance. This deviation *could* result in very serious difference in binding properties of the two functions and, as shall be noted later, is often invoked in rationalizing a lack of "predicted" properties for this phosphonic acid and analogues of nucleoside di- and triphosphates where a pyrophosphate linkage has been replaced by a methylene group.

Finally, a factor of difference should be noted which is at times separable only with difficulty from that of size and shape. That is the loss of binding function of the esterified phosphate oxygen resulting from its absence in the analogue. This is particularly a problem in those systems where a pyrophosphate linkage has been modified as there results simultaneously significant changes in geometry and binding capabilities. As noted above, such overlapping factors of variation can cause difficulties in the interpretation of experimental results.

III. Analogues of Inorganic Pyrophosphate

A. Synthesis

The introduction of phosphonic acids as analogues of inorganic pyrophosphate has been limited principally to three compounds, methylenediphosphonic acid (**4**), hydroxymethylenediphosphonic acid (**5**), and 1-hydroxyethyl-1,1-diphosphonic acid (**6**).



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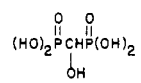

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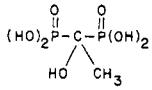

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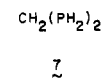

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The parent compound **4** has been synthesized by several routes, all but one involving the acidic hydrolysis of the tetraalkyl esters. The use of the Arbuzov reaction[30] on methylene dihalides has been reported[31–36] to give the desired compound in widely varying yield depending on the alkyl groups of the trialkyl phosphite and the reaction conditions used; Mastalerz has found[37] the corresponding reaction of the more reactive diethyl phenylphosphonite with methylene iodide to proceed readily. It is our experience that the most reproducible method under *ordinary laboratory* conditions is that of Kosolapoff.[31] A sequential method using an Arbuzov reaction initially, followed by a Michaelis–Becker reaction,[38] has also been reported[39,40] to give product in reasonable yield; an attempt to use the Mi-

chaelis–Becker reaction directly on methylene iodide failed.[41] An alternate approach has been to oxidize the more readily available methylenediphosphine (**7**) using nitric acid.[42]

The hydroxy compound **5** has been prepared from the tetraalkyl ester of **4** by dibromination, hydrolysis, and catalytic reduction.[43] The preparation of **6** was originally reported by von Baeyer and Hofmann[44] by a condensation reaction with phosphorous acid, the detailed nature of which was later studied by Prentice et al.[45]

B. Biological Investigations

As discussed above, sizable differences in geometry are noted for **4** compared to inorganic pyrophosphate.[28] However, it would appear that loss of the binding capabilities of the anhydride oxygen presents an even more serious source of deviation from "natural" behavior. For a number of systems, **5** is able to serve as an effective substitute for inorganic pyrophosphate (of course, without hydrolysis) whereas **4** does not interact. Moreover, the (nonphosphonate) phosphoramide analogue **8** of inorganic pyrophosphate exhibits behavior similar to **5**; it appears that an additional site bearing an unshared electron pair, and thereby some binding capabilities, often may be critical for reactivity.

$H_2O_3PNHPO_3H_2$

8

The importance of this latter consideration as compared to geometrical variations is also shown by the facile binding of analogues **4**, **5**, and **8** to divalent cations,[46,47] as is the situation with inorganic pyrophosphate. Both **4** and **5** have been found to be of use in the complexation of calcium ion and thereby in the prevention of calcium deposition in living tissues.[48–53] 1-Hydroxyethyl-1,1-diphosphonic acid (**6**) has also been investigated extensively for regulation of calcium deposition and transport.[52–64] Several mechanisms of action have been proposed including a direct effect on vitamin D metabolism.[65] The use of these reagents as drugs for the treatment of bone disorders has recently been reviewed.[66]

Zamecnik et al. have reported[67,68] the ability of **4** to become incorporated into adenosine triphosphate, presumably liberating inorganic pyrophosphate, upon reaction mediated by purified lysyl tRNA synthetase from *E. coli*. Simon and Myers have found[69] **4** to inhibit both incorporation of inorganic phosphate into nucleoside polyphosphates by polynucleotide phosphorylase, and phosphorolysis.

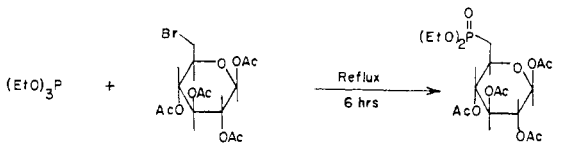
Most other uses of the isosteric analogue **4** appear to be limited to mechanistic evaluation, that is, a determination of binding requirements of inorganic pyrophosphate for enzymatic action. Kelly et al.[70,71] have studied the effect of **4** and **8** on nonspecific alkaline phosphatases of *E. coli* and bovine intestine with variable results; pyrophosphatases from yeast,[46,70,72–74] rabbit liver microsomes,[72] and bacterial sources[72] are unaffected by **4** but in many cases are strongly inhibited by **5** and **8**. This observation is indicative of a requirement for binding involving an additional site on the substrate other than the phosphoryl functions, although **6** was found to be a poor inhibitor of inorganic pyrophosphatase from pig cartilage.[75]

In further sections will be noted several other enzymatic processes in which the methylenediphosphonic acid is capable of participating.

IV. Analogues of Carbohydrate Phosphates

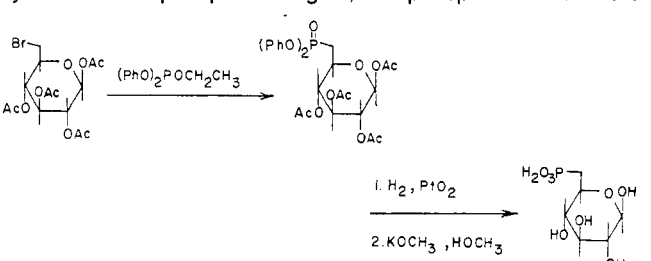
A. Synthesis

Several approaches have been made toward the preparation of nonisosteric phosphonic acid analogues related to the common carbohydrates. Griffin and Burger early reported[76] the preparation of an analogue related to glucose 6-phosphate, that is, diethyl 6-deoxyglucose 6-phosphonate (**9**); the phosphorus was introduced to a protected 6-deoxyglucose 6-bromide via an Arbuzov reaction with triethyl phosphite. The acetates could be removed from **10** by treatment with aqueous HBr yielding **9**, al-



10

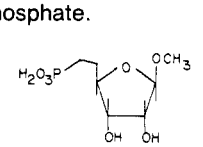
though all attempts to hydrolyze the ethyl esters resulted in decomposition. The free acid **11** was ultimately prepared via an Arbuzov reaction with diphenyl ethyl phosphite followed by hydrogenolysis and hydrolysis of the acetates. The introduction of the phosphonic diester function via the Arbuzov reaction with triethyl phosphite has also been reported by Parikh et al.[77] and Whistler and Wang[78] for the generation of nonisosteric phosphonate (diester) analogues related to ribose, galactose, and xylose terminal phosphates. Again, free phosphonic acids were



11

not obtained from the diethyl esters. Inokawa et al. have also reported[79–82] the preparation by the Arbuzov method of nonisosteric phosphonates (monoesters) related to xylose 5-phosphate. These were then converted to materials bearing the phosphorus atom in the ring system.

The conversion of the diethyl ester **10** to the free acid was later claimed in a patent[21] by the didealkylation procedure using alkali halides. This procedure, often useful where hydrolysis or hydrogenolysis is not feasible, involves heating the phosphonate diester to 150 °C with sodium iodide in dimethylformamide (DMF) followed by treatment with aqueous acetic acid. The use of this technique was claimed for a series of isosteric and nonisosteric analogues of carbohydrate phosphates (among others) although experimental details were presented for only a few systems, including methyl 5-deoxy-5-dihydroxyphosphinylmethyl-β-D-ribofuranoside (**12**), the isosteric phosphonic acid analogue related to ribose 5-phosphate, and 6-deoxy-6-dihydroxyphosphinylmethyl-α-D-galactopyranose (**13**), an analogue of galactose 6-phosphate.



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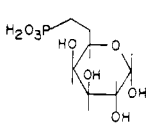

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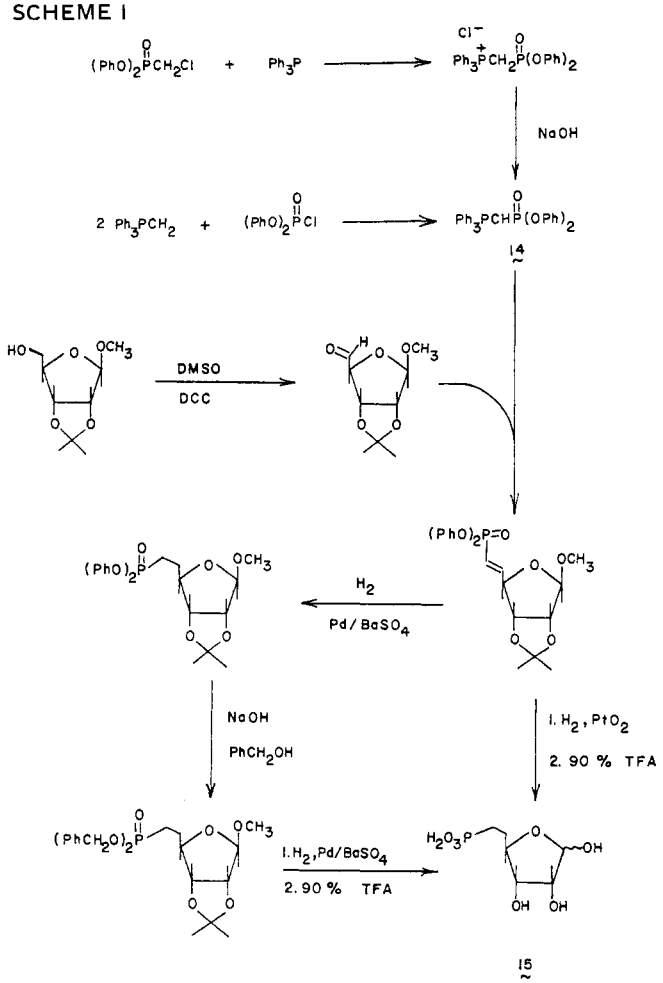
With the difficulties attendant in the use of the Arbuzov reaction for the ultimate generation of the free phosphonic acids (decomposition of sensitive functionalities upon hydrolysis of alkyl esters, lack of reactivity of triphenyl phosphite, difficulties in preparation, and use of diphenyl alkyl phosphites and tribenzyl phosphite), it was desirable that an alternate route be available for phosphorus introduction. A highly convenient one was provided through the efforts of the Syntex group[83] involving the use of a stabilized Wittig reagent. Using suitably protected carbohydrates with a 5- or 6-hydroxyl free (for pentoses and hexoses,

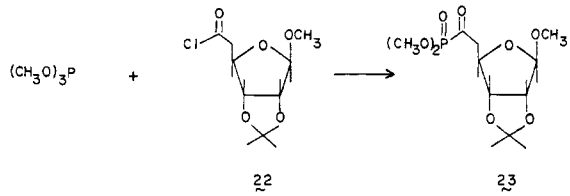
SCHEME I



respectively), oxidation to the aldehyde was performed using the previously developed dimethyl sufoxide (Me_2SO)–dicyclohexylcarbodiimide (DCC) technique[84] followed by treatment with the stable ylide **14**[85,86] to yield the vinyl phosphonate. This was then reduced, and the ester functions were removed as illustrated in Scheme I for the isosteric analogue of ribose 5-phosphate, 5-deoxy-5-dihydroxyphosphinylmethyl-D-ribose (**15**). [The nomenclature system used here and above is one used by the authors[83] which stresses the relationship to the natural molecule, ribose 5-phosphate. An alternate acceptable name for **15** which does not stress the analogue relationship would be 5,6-dideoxy-D-allofuranose-6-phosphonic acid. Both approaches are in common use and the reader would be well advised to be familiar with both.] The use of this approach is also claimed[83] for the preparation of the corresponding isosteric phosphonic acids related to D-xylose 5-phosphate (**16**), D-lyxose 5-phosphate (**17**), D-arabinose 5-phosphate (**18**), 2-deoxy-D-ribose 5-phosphate (**19**), D-glucose 6-phosphate (**20**), and D-mannose 6-phosphate (**21**), although complete experimental details for each are not provided in the patent. More recently this same approach has been described by Adams et al.[87] for the preparation of **20**.

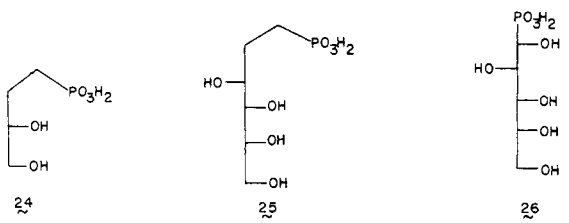
The use of this approach, along with the related Horner procedure,[88] for the introduction of the phosphonate function has also been made in an approach to several other analogues (phosphonate esters only) related to glucose 1-phosphate and arabinose 1-phosphate.[89] Final hydrogenolysis and removal of protecting groups was not performed. A nonisosteric analogue related to fructose 1-phosphate has recently been reported by Paulsen and Bartsch[90] using dimethyl methylphosphonate anion addition to an aldehyde; again, the free acid was not obtained.

Hampton et al. have reported[91] the reaction of trimethyl phosphite with the acid chloride **22** to yield the phosphonate analogue **23** related to ribose 5-phosphate. This compound, although not reported as being converted to the free acid, bears a functionality between the phosphorus and the carbohydrate ring capable of participation in binding.



Phosphonylation has also been performed by the use of phosphite addition across activated olefinic linkages under acidic[92,93] or basic[94,95] conditions; in this way various deoxy-,[93] dehydro-,[92] nitro-, and aminodeoxyphosphonocarbohydrate derivatives[94,95] have been prepared. Similar additions have been performed photochemically using dialkyl thiophosphites[96] and phosphines,[97] the product from the latter being air oxidized to a phosphorus-containing acid in a further step. Other compounds (nonisosteric) related to glucose 6-phosphate have been reported, one by the addition of hypophosphorus acid to levoglucosan yielding a phosphonite salt,[98] and a second, a carbohydrate *C*-phosphine oxide, by lithium diphenylphosphine displacement of a 6-tosylate function;[99] several others were also reported in the latter work not related directly to simple natural carbohydrate phosphates. Inokawa et al. have also reported[81,100,101] several compounds of this type (*C*-phosphine oxides) which can be considered as being indirectly related to ribose and arabinose.

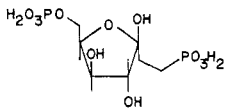
Again, preparations of protected carbohydrate derivatives related to galactose, arabinose, erythrose, and glyceraldehyde containing a phosphonic acid (esterified) linkage have been reported by Paulsen et al.[90,102] by addition to the aldehydes of dialkyl phosphites or the anion from dimethyl methylphosphonate. Zhdanov et al. have reported[103] the preparation of several of these same systems by an Arbuzov reaction on the protected 1-deoxy-1-bromo carbohydrate derivatives. Free phosphonic acids were not reported here, although those related to glyceraldehyde and arabinose, **24** and **25**, respectively, were reported[104] as prepared by a Horner reaction of the appropriately protected aldehydes[105] followed by reduction and phosphonate ester cleavage by the method of Rabinowitz.[106] It should be



noted that **24** is also an isosteric analogue of *sn*-glycerol 3-phosphate (vide infra).

In other syntheses of carbohydrate-type compounds containing the carbon–phosphorus linkage but not directly related to any naturally occurring carbohydrate phosphate, dialkyl phosphites have been added to aldehydic[107–109] and ketonic[110,111] systems under basic conditions. Paulsen et al. have used the bromomercuri salts of dialkyl phosphites in addition–displacement reactions with β-bromoacetates,[112,113] and have also reported[114,115] alkylidene protected carbohydrate derivatives with a phosphonic ester located in the "carbonyl" portion. With one system[115] a free acid (**26**) derived from arabinose was isolated. Petrov et al. have reported[116] the preparation of compounds related to galactose 6-phosphate in which the normal esteratic oxygen is present, but a methyl group is bound directly to phosphorus in place of a hydroxyl group.

In a very interesting report Stribling describes[117] the preparation of an isosteric analogue of fructose 1,6-diphosphate, 1-deoxy-1-dihydroxyphosphinylmethylfructose 6-phosphate (**27**), using a biochemical technique. The compound was isolated from the aldolase-mediated reaction of D-glycerol 3-phosphate and 4-hydroxy-3-oxobutyl-1-phosphonic acid, the isosteric phosphonic acid analogue of dihydroxyacetone phosphate (vide infra). To date this material (**27**) has not been synthesized by standard organic chemical techniques.



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B. Biological Investigations

Compared with the classes of compounds to be discussed later, relatively little biochemical investigation has been reported using phosphonic acid analogues of carbohydrate phosphates. Griffin and Burger studied[76] the glucose-related compound **11** and found that, in the absence of ATP, it inhibited acetylcholine production by choline acetylase and did not appreciably inhibit histidine decarboxylase, xanthine oxidase, or hyaluronidase.

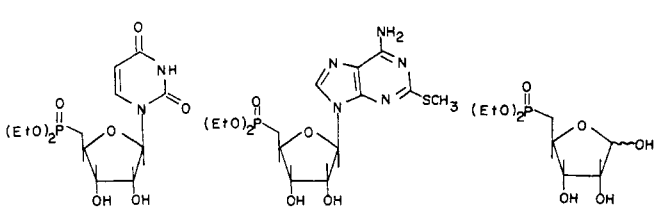
Obviously there are numerous topics available for investigation which could make use of the phosphonate analogues of carbohydrate phosphates, particularly the isosteric analogues. One might simply note that there are numerous possibilities for the use of glucose analogues in mechanistic investigations and for attempts at metabolic regulation. It is expected that many examples of such studies will be found in the next few years as these compounds become more available to biochemists.

V. Analogues of Nucleotides

A. Synthesis

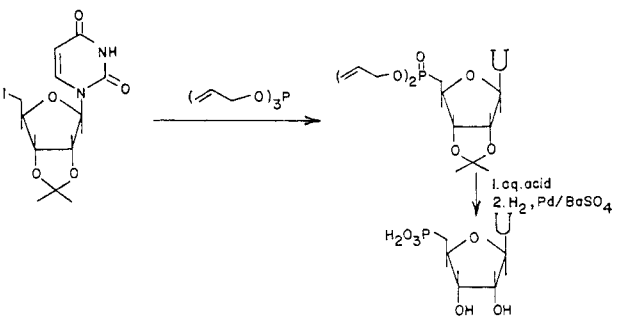
Initial efforts in regard to phosphonic acid analogues of nucleotides involved nonisosteric systems, that is, as considered above, compounds differing from the natural molecules simply by noninclusion of the normal phosphate ester oxygen. The rationale applied in the preparation and use of these was one of expediency; they were readily synthesized using only previously developed reaction systems with readily available materials. Synthesis of the isosteric analogues awaited further synthetic developments.

All efforts in this regard made use of the Arbuzov reaction for introduction of the carbon–phosphorus bond. The earliest work involved the action of triethyl phosphite on protected 6′-deoxy-6′-bromoglucopyranosyl purines.[77] Reasonable yields of phosphonate diester could be obtained although all attempts at hydrolysis caused degradation of the fundamental molecular framework, and further attempts using diphenyl ethyl phosphite (in hopes of free acid generation by hydrogenolysis[78]) yielded anomalous unidentified material. Triethyl phosphite was again used with 5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneuridine[118] yielding the diester phosphonate (**28**). However, as mentioned previously in the carbohydrate series, all straightforward attempts at hydrolysis destroyed the fundamental structure. Wolff and Burger[119] generated a nonisosteric diethyl phosphonate (**29**) related to 2-methylthioadenosine 5′-phosphate by a series of reactions adding the purine ring to the carbohydrate phosphonate (**30**) which was mentioned above.[77] Again, removal of the ester linkages could not be attained readily.



28 29 30

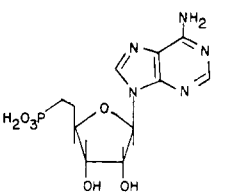
Further efforts made use of ester linkages which could be removed under milder conditions. Yengoyan and Rammler[120] successfully obtained the analogue (**31**) of uridine monophosphate in 10% overall yield from 5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneuridine using triallyl phosphite, the ester linkages being hydrogenolyzed in the final step. The corresponding analogue **32** related to 2′-deoxythymidine 5′-phosphate was later



31

reported.[20] Holy also reported[121] the synthesis of **31** in 15% overall yield using tris(2-benzyloxyethyl) phosphite as the phosphorus reagent in an Arbuzov reaction. The diester was cleaved in four steps involving hydrogenolysis, iodination, alkylation, and mild hydrolysis. Myers[122] has also considered the synthesis and (chemical) use of **31** and the corresponding compound **33** related to adenosine 5′-phosphate.

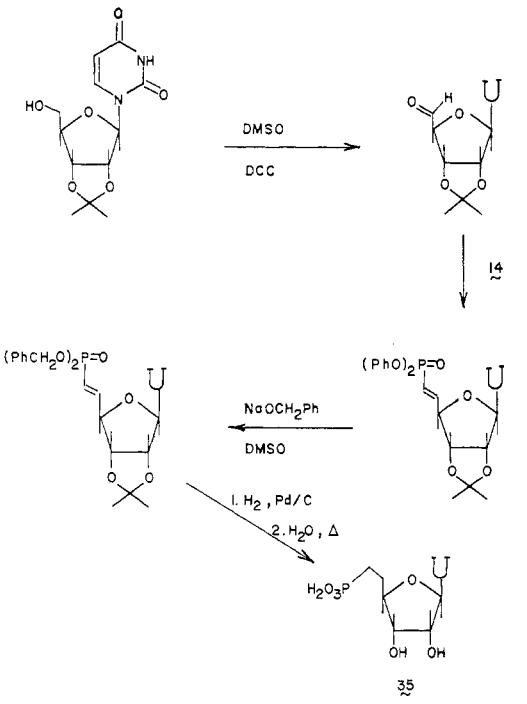
The eventual synthesis of the isosteric phosphonic acid analogues of nucleotides involved the same class of nucleoside derivatives as used in the preparation of the nonisosteric compounds mentioned above, that is, the protected 5′-deoxy-5′-halo nucleosides. These were used by Myers[122] in a displacement reaction with the anion from diphenyl methylphosphonate followed by removal of protecting groups and ester linkages. While details were given in the patent[122] only for the adenosine system **34**, the technique was claimed for other nucleosides. Several



34

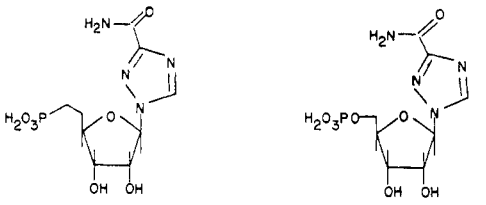
years later an alternate route to these compounds was developed by the Syntex group. The first application of the Me_2SO–DCC oxidation procedure[84] was for the preparation of the 5′-aldehydes related to protected 2′-deoxythymidine, uridine, and adenosine, with which the ylide **14** could be used for synthesis of the isosteric phosphonic acid analogues of the 5′-phosphates. For example, the analogue of UMP **35**, 5′-deoxy-5′-dihydroxyphosphinylmethyluridine, was prepared in an overall yield of 41% from 2′,3′-*O*-isopropylideneuridine[123] (Scheme II). In the original report only the preparations of the uridine **35** and adenosine **34** systems were described; other isosteric analogues of natural and derived purine and pyrimidine nucleoside monophosphates were described or claimed in several patents.[83,124,125] Modifications for improvement of yield in the ylide reaction have been suggested by Hampton.[126] Fuertes et al. have reported[127] the use of the Syntex route in the preparation of the analogue **36** related to the synthetic triazole nucleotide **37**, which

SCHEME II



35

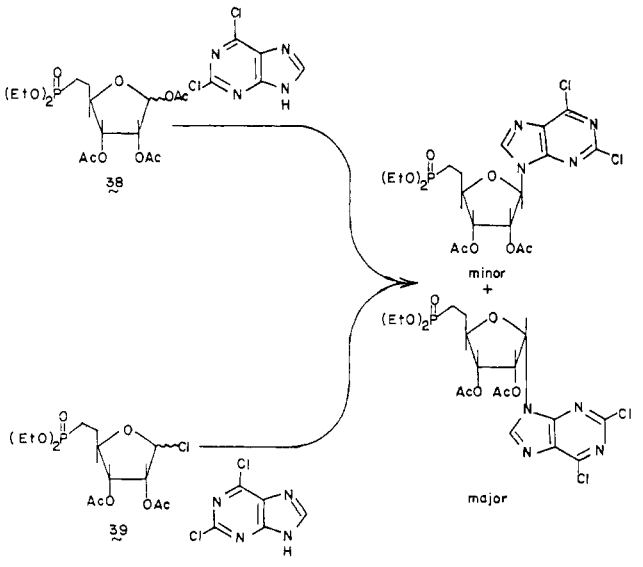
is known to have antiviral properties. This general route has also been used by Montgomery et al.[128] for the preparation of isosteric analogues of some 6-substituted purines.



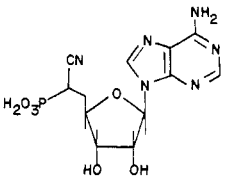
36 37

Several other approaches have been used in the preparation of the isosteric analogues of nucleotides. Montgomery and Hewson[129] attempted reactions of purines at the glycosidic carbon of protected 5-deoxy-5-diethoxyphosphinylmethylribose derivatives **38** and **39** (Scheme III); in all systems tried the major product was the α anomer.

SCHEME III

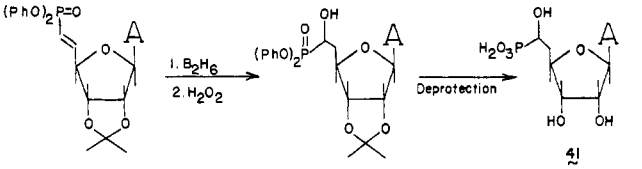


Hampton, Sasaki, and Paul[130] conducted a displacement reaction by sodio diethyl cyanomethylphosphonate on a protected derivative of 5′-deoxy-5′-iodoadenosine. This is fundamentally the same route as used by Myers[122] mentioned above. The product ultimately obtained (**40**) was not a simple isosteric



40

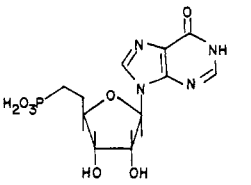
analogue, but rather that with a cyano group substituted at the 6′ position. Hampton, Perini, and Harper also prepared[131] the corresponding 6′-hydroxyl compound (**41**) by hydroboration–oxidation of the olefinic intermediate in the Syntex synthesis[123]



41

of isosteric analogues of nucleoside monophosphates. In spite of these structural variations, significant biological activity could be observed. It is possible that the introduction of a potential binding functionality at this site might be of value for numerous systems of interest.

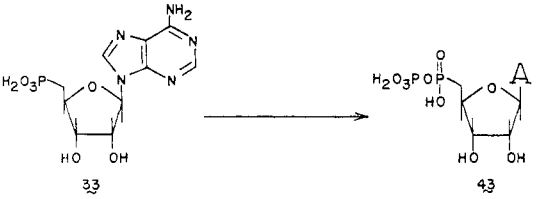
Hampton and Chu have reported[132] an interesting biochemical synthesis of the isosteric analogue **42** of IMP. This compound was isolated from the AMP deaminase reaction on compound **34**, an analogue of AMP, prepared by the Syntex route.[123]



42

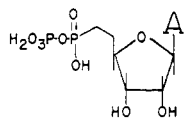
The analogues of greatest interest and use thus far for biochemical investigations have been those of nucleoside polyphosphates. Several classes of compounds may be considered here, the simplest being those wherein a single methylene group is present in place of (a) the phosphate oxygen (or the phosphate oxygen simply not included), (b) the α,β-pyrophosphate oxygen, or (c) the β,γ-pyrophosphate oxygen.

For the first of these categories Myers has reported[122] the nonisosteric analogue **43** of ADP as being prepared by coupling



33 43

of phosphoric acid with **33** mediated by DCC. In the same work were also described the preparations of two isosteric analogues of adenosine polyphosphates in this category, **44** as an analogue of ADP and **45** as an analogue of ATP. These were prepared by successive DCC couplings of phosphoric acid starting with **34.** Moffatt et al. have noted[123] such analogues of nucleoside di- and triphosphates as having been prepared from the 5′-deoxy-5′-



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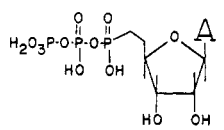
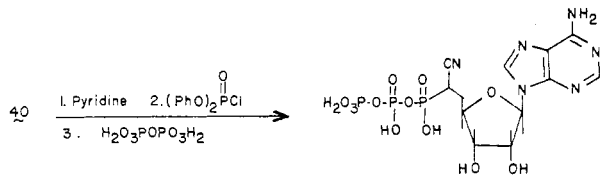


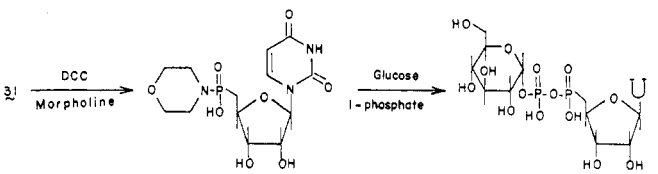
45

dihydroxyphosphinylmethyl nucleosides; no experimental details were presented although biochemical investigations have been performed using materials from this source.[133] Hampton, Sasaki, and Paul[130] have reported the preparation of the triphosphate analogue **46** derived from **40** by initial activation of the phosphonic acid with diphenyl phosphorochloridate, followed by displacement with pyrophosphate. Small amounts of the analogue of the diphosphate were also obtained.



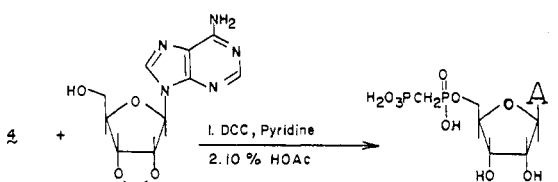
46

Rammler et al. have reported[20,120] syntheses of analogues of nucleoside di- and triphosphates beginning with the nonisosteric phosphonate analogues of the nucleoside monophosphates. For example, the UMP analogue **31** was activated by conversion to the morpholidate derivative according to the method of Moffatt and Khorana;[134] this was followed by displacement with phosphate or pyrophosphate to yield the analogues **47** and **48** of UDP and UTP, respectively.[120] For the corresponding dTDP and dTTP analogues, **49** and **50,** sequential activation by reaction with carbonyl diimidazole followed by phosphate displacement was used.[20] The preparation of a nonisosteric analogue **51** of UDP-glucose was performed in a manner similar to that mentioned above for UTP.[135] Analogous syntheses were performed with galactose and glucuronic acid derivatives.

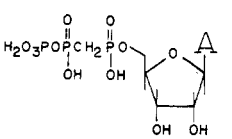


51

In the second category, Myers et al. have reported[122,136,137] the preparation of the analogue **52,** adenosine 5′-[α,β-methylene]diphosphate,[346] in which a methylene group replaces the pyrophosphate oxygen. This was obtained in 61% yield by the coupling of isopropylideneadenosine with methylenediphosphonic acid using DCC; significantly lower yields resulted if trichloroacetonitrile were used as the coupling agent. The analogue of ATP, adenosine 5′-[α,β-methylene]triphosphate (**53**), was

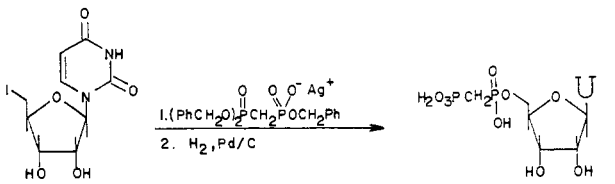


52



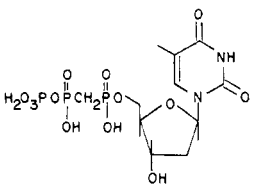
53

then prepared from **52** by DCC coupling with phosphoric acid. Myers has also described[122] the preparation of **52** and the corresponding analogue **54** of UDP by the action of silver tribenzyl methylenediphosphonate on 5′-deoxy-5′-iodo nucleosides followed by hydrogenolytic debenzylation. An analogous coupling procedure has been reported[138] for the corresponding analogue **55** of 2-Cl-ADP.



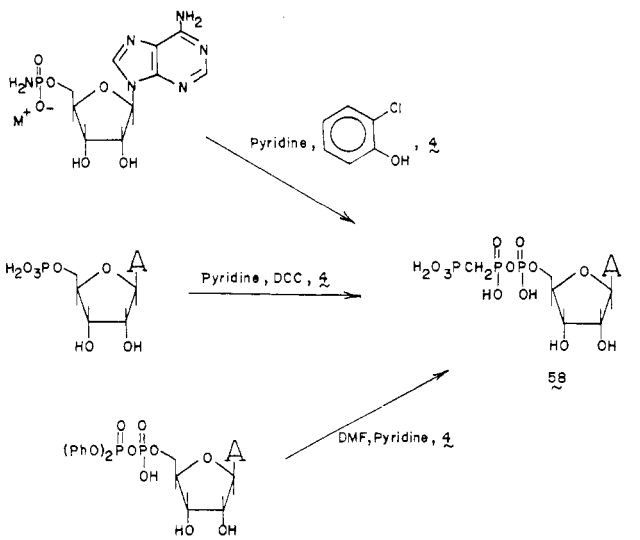
54

Englund et al. have reported[139] the preparation of 2′-deoxythymidine 5′-[α,β-methylene]diphosphate (**56**) related to dTDP and its conversion to the analogue dTTP, 2′-deoxythymidine 5′-[α,β-methylene]triphosphate (**57**). The latter preparation involved initial activation with carbonyl diimidazole followed by phosphate displacement.



57

Analogues of nucleoside triphosphates in which a methylene group is substituted for the β,γ-pyrophosphate oxygen, corresponding to the third category mentioned above, have been prepared by activation of the nucleoside monophosphate followed by displacement with methylenediphosphonic acid. Myers et al.[122,140] have reported the preparation of adenosine 5′-[β,γ-methylene]triphosphate (**58**) related to adenosine tri-

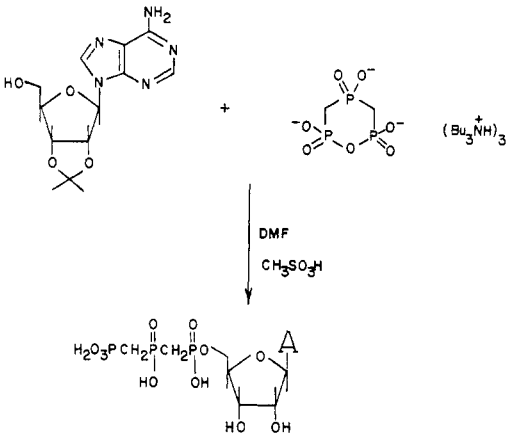


58

phosphate both by preformation of the monophosphoramide of AMP and coupling mediated by *o*-chlorophenol, and by the use of DCC in pyridine. Yount et al. have reported[141] improved yields of **58** by activation with diphenyl phosphorochloridate followed by displacement with methylenediphosphonic acid in DMF–pyridine. Hershey and Monro[142] utilized the monomorpholidate of GMP with DCC in coupling to methylenediphosphonic acid in preparing the much used analogue guanosine 5′-[β,γ-methylene]triphosphate (**59**) related to GTP. This method was also used by Gough et al.[143] in the preparation of the corresponding analogue (**60**) of 2-Cl-ATP.

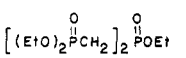
A biochemical generation of ATP analogue **58** has been reported[68] using purified lysyl tRNA synthetase from *E. coli.* With this enzyme ATP undergoes an exchange with methylenediphosphonic acid yielding **58** and inorganic pyrophosphate.

Two approaches have been used in generating analogues of ATP in which more than one "reactive" oxygen has been substituted by methylene groups. The bismethylene analogue, adenosine 5′-[α,β:β,γ-dimethylene]triphosphate (**61**), in which both pyrophosphate oxygens are replaced, has been reported by Trowbridge et al.[144,145] A modification of a common method



61

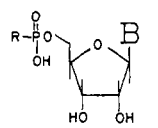
of triphosphate preparation was used in which the bismethylene analogue of trimetaphosphate (2,4,6-trihydroxy-1,2,4,6-oxatriphosphorinane 2,4,6-trioxide) was allowed to react with isopropylideneadenosine. Although only a low yield (16%) of product was obtained, this remains as the only route accomplishing the preparation of this interesting class of compounds in which only the nucleoside–phosphate linkage is capable of being cleaved. Another approach to compounds of the class of **61,** of which no attempt has been reported, would be the use of a standard coupling reagent with the protected nucleoside and the acid derived from the pentaethyl ester **62** reported by Bel'skii et al.[146]



62

In the patent of Myers[122] was reported the analogue **63** related to ATP (nonisosteric) in which the nucleoside–phosphate oxygen is missing and the β,γ-pyrophosphate oxygen is replaced by a methylene group. This was prepared by DCC mediated coupling of **33** and methylenediphosphonic acid. The analogue **64** was also claimed although no experimental details were provided.

63 **64**



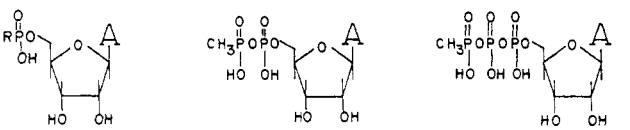
a: R = Et, B = A b: R = Et, B = U

c: R = Ph, B = A d: R = Ph, B = U

65

Significant attention has also been given to those phosphonates, as analogues of nucleoside phosphates, wherein a methyl (or other alkyl or aryl) group is substituted for a hydroxyl on phosphorus. The earliest efforts in this area were by Anand and Todd[147] who synthesized the phenyl- and ethylphosphonic acid derivatives of adenosine and uridine **65;** the route involved reaction of the protected nucleoside with the benzyl ester of the appropriate phosphonochloridate followed by hydrogenolysis and deprotection. Compound **65a** has also been prepared by Myers[137] by a coupling reaction of the nucleoside and the free phosphonic acid.

The analogues of this class closest to the natural materials (as regards bulk) are the esters of methylphosphonic acid. These have been reported for the 5′-phosphate related systems of adenosine (**66**),[136,137,148] inosine (**67**),[149] guanosine (**68**),[150] uridine (**69**),[151] azauridine (**70**),[152] and 2′-deoxy-2-bromouridine (**71**)[153] as well as the system related to 2′(3′)-AzUMP (**72**).[152] Holy et al.[151,154,155] have prepared a series of nucleoside methylphosphonates in which there are present on the methyl group substituents bearing unshared pairs of electrons and which presumably are capable of binding to electron-deficient species. In this way these compounds partially bear a reactivity lost in using the simple methylphosphonates as analogues. These include the hydroxyl derivatives related to uridine (**73**), adenosine (**74**), guanosine (**75**), cytidine (**76**), and 2′-deoxythymidine (**77**), the amino derivatives related to uridine (**78**) and adenosine (**79**), and the hydroxyethyl derivatives related to uridine (**80**). Two 3′-phosphate related compounds (mixed with 2′-substituted material) have also been reported; these are a hydroxyl derivative related to uridine (**81**)[153] and an aminoethyl derivative related to adenosine (**82**).[156] Also in this general category are compounds prepared by Gulyaev et al;[148] these include analogues **83** of acetylAMP and **84** of AMP.

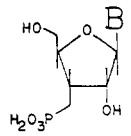


83 R = $COCH_3$ **85** **86**

Two reports are noted of nucleoside polyphosphate analogues wherein a methylphosphonic acid function is incorporated. Myers and Simon[157] prepared the ADP analogue **85** both by DCC coupling of methylphosphonic acid with AMP and by a displacement reaction of the monophosphoramide of adenosine. The ATP analogue **86** with a methyl group at the terminus was used by Tonomura et al.,[158] the compound being a gift of Moffatt; no details of its preparation have been reported.

The preparation of phosphonic acid analogues of 3′-nucleoside phosphates poses a significantly more complex problem than for the 5′-nucleoside phosphate analogue systems. Synthesis of a series of isosteric analogues has been accomplished,[159,160] in rather high overall yield, by the Syntex group utilizing a multistep route beginning with phosphonylation of 1,2:5,6-di-*O*-isopropylidene-α-*ribo*-hexofuranos-3-ulose by the Emmons modification[161] of the Horner technique.[87] The nucleoside base is interjected at the next to final step (deprotection) as the chloromercuri salt, all prior conversions being performed on the phosphonylated and protected carbohydrate derivative. It is interesting to note that unlike the similar attachment of nucleoside bases at the glycosidic carbon of phosphonate esters isosteric with carbohydrate 5-phosphate derivatives,[129] only β anomer was formed in each system here; this is probably due to a steric directing effect of the bulky phosphorus function hindering approach of the reagent from the α side of the ring system. Analogues **87** were prepared with nucleoside bases adenine, cytosine, thymine, and uracil.

Several reports have also been made regarding the preparation of analogues in which an alkyl group is substituted for a hydroxyl on phosphorus of a nucleoside 3′-phosphate. These

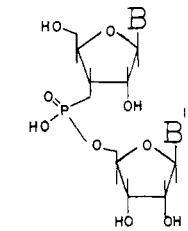


a: B = adenine b: B = cytosine c: B = thymine d: B = uracil

87

analogues include compounds wherein the alkyl group is methyl,[150,152] hydroxymethyl,[149] or aminomethyl.[156] A problem in the preparation, and use, of these compounds, which is inherent in all ribonucleosidic systems (except deoxy) where a phosphorus ester linkage is involved directly to the ring, is the ready interchange between the 2′ and 3′ positions; the compounds do not represent "pure" preparations, and difficulties in their use might be expected. This problem is obviated with the analogues (phosphonates) as prepared by the Syntex group.

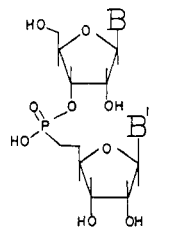
The Syntex synthesis[159,160] led directly to the preparation[162] of analogues of dinucleoside phosphates in which one of the oxygens of the normal phosphate linking the nucleosides is substituted by a methylene group. Included here are those in which the 3′ phosphate oxygen is replaced (**88**) and those in which the 5′-phosphate oxygen is replaced (**89**). These were prepared by DCC-mediated coupling of the properly protected 3′- (or 5′-) deoxy-3′- (or 5′-) dihydroxyphosphinylmethyl nucleosides with the complementary appropriately substituted nucleosides.



a: B = adenine, B′ = uracil

b: B = uracil, B′ = adenine

88



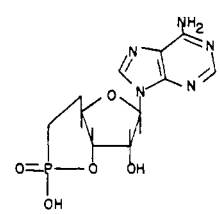
a: B = uracil, B′ = adenine

b: B = B′ = uracil

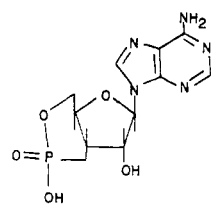
c: B = adenine, B′ = uracil

89

Accompanying these efforts, the Syntex group has also synthesized an extensive series of isosteric phosphonate analogues of cyclic nucleoside phosphates. The analogue **90** of



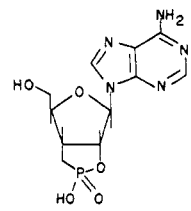
90



91

3′,5′-cyclic AMP bearing a methylene substitution for the 5′-oxygen was first noted in a patent[163] as being prepared by the DCC-mediated cyclization of **34.** This preparation and that of its isomer (**91**) in which the 3′ oxygen is substituted by a methylene group were later reported,[160,162] the latter again by a DCC-mediated intramolecular coupling involving **87a**. Once isolated, a series of compounds was prepared from it with modification in the adenine function.[164] Patent claims here include derivatives of cytosine (**92**), uracil (**93**), hypoxanthine (**94**), guanine (**95**), and thymine (**96**). Several 2′-deoxyribo systems were also noted.

Also of interest are analogues of the 2′,3′-cyclic phosphates in which the 3′ oxygen is replaced by a methylene group. These have been reported by cyclization of **87a** and **87d** with either acetic anhydride in pyridine[160] or with DCC in the mixed solvent system, DMF–*t*-BuOH[162], yielding **97** and **98,** respectively. The patent[160] includes claims for related compounds with a wide series of nucleoside bases, although experimental details were not provided. One analogue, **99,** of a 3′,5′-cyclic nucleoside phosphate has been reported[150] wherein a methyl group is substituted for the hydroxyl on phosphorus; while two ester linkages are still present, the normal acidic proton is missing.



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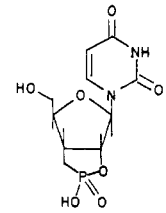

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B. Biological Investigations

Extensive and intensive biochemical and biological investigations have been performed with a variety of the compounds of which the syntheses have been discussed above. A few of these investigations have been performed in vivo in what might be termed "medicinal research", although the bulk of the efforts have been mechanistic in nature, that is, attempts to define the biochemical role of the natural phosphate by looking at interruptions or modifications of normal processes upon introduction of the phosphonic acid analogue. Although our purpose here is not to give detailed accounts of these studies, they will be enumerated and the rationale for analogue use considered.

The compound which has been used most intensively for this latter purpose, mechanistic determinations, is **59,** the analogue of GTP in which the β,γ-pyrophosphate oxygen is replaced by a methylene group. This material has been employed widely over the past 10 years for the elucidation of the mechanism of protein synthesis, a process known to require GTP. It was early discovered that the analogue **59** served as an overall inhibitor of peptide synthesis for several systems.[142,165–169]

As this topic constitutes in itself an entire area of scientific endeavor, no attempt will be made to review it here. The reader interested in an outline of this work is referred to several principal[170–172] and ancilliary reviews[173–176] and a symposium report.[177] Therein is discussed the use of **59** in the determination of the role of GTP in initial binding of an aminoacyl-tRNA to the ribosomal complex, without pyrophosphate cleavage, followed by translocation, in which pyrophosphate cleavage is involved, as it is in chain termination. For these considerations it should be kept in mind that the analogue **59** differs from GTP in more than an inability to be hydrolyzed at a specific site; accompanying the substitution of a methylene group for oxygen is a mild deviation in bond angle and thereby a slightly different spatial arrangement of possible binding functions, and the loss of reversible binding capability of the replaced oxygen atom.

The analogue **59** has found use in investigations of several other systems as well. It has been used to define the role of GTP in specific binding of glucogen with plasma membranes,[178] the stimulation of adenyl cyclase,[179–182] and RNAse V activity derived from *E. coli*[183] and has been found to be a competitive inhibitor of GTP in the action of adenylosuccinate–synthetase from *E. coli*,[184] although it interacts only weakly with tubulin from bovine brain.[185]

Because of the wide range of biochemical processes in which ATP participates, the analogue **58** in which the β,γ-pyrophosphate oxygen is substituted by a methylene group, often in

conjunction with its isomer **53,** has been employed quite extensively; these have found use in both mechanistic and medicinal type investigations.

Briefly illustrating these, **58** has been found capable of replacing ATP with RNA polymerase,[186] adenylate deaminase, both at high and low concentrations,[187] and 5-phosphoribosyl pyrophosphate synthetase.[188] Moreover, the analogue **58** was found to be an order of magnitude better than ATP in inhibition of the incorporation of inorganic phosphate into ADP by polynucleotide phosphorylase.[69] In studies of aspartate transcarbamylase from *Neurospora crassa* it was found that **58,** but not **53,** could substitute for ATP, indicating that the activity of ATP involved release of inorganic pyrophosphate.[189] For other systems, strong inhibition of activity has resulted upon replacement of ATP by **58,** indicating a necessity for binding or cleavage at the substituted site. These systems include phosphoenolpyruvate synthetase,[190] adenylate cyclase,[191,192] adenosine kinase from Ehrlich ascites-tumor cells,[193] formylglycinamide ribonucleotide amidotransferase,[194] and ATPase from carrot juice.[195] Interestingly, with ATPase from other sources, significantly different results were found with **58** indicating a difference in the mode of action of the enzyme from different sources.[196,197] Both **58** and **53** were found to be competitive inhibitors (with ATP) of ATP:L-methionine *S*-adenosyltransferase from yeast.[126] Results with yeast hexokinase indicate a variability of action of the analogue, and thereby that of ATP, with pH.[198,199] The analogue **58** has been found to be totally nonparticipatory in efforts to use it as an ATP replacement in tobacco mosaic virus[200] and for oxygen exchange between inorganic phosphate and water with rat liver mitochondria.[201,202] Analogue **53** was found to be a competitive inhibitor of ATP binding to tyrosyl-tRNA synthetase from *E. coli*,[203] and has also been found to cause relaxation in insect muscle systems.[204]

In studies of binding of ATP to macromolecules and metal ions, both **58** and **53** were found to substitute well with homogeneous rat liver fructose 1,6-diphosphatase,[205] but only **58** with clostridiol formyltetrahydrofolate synthetase,[206] indicative of specific binding with severe spatial or α,β-pyrophosphate oxygen binding requirements. EPR studies by Buttlaire and Reed[207] on this latter system indicated **58** to be a poor substitute for ATP, presumably owing to poor spatial correlation between the two species about the substituted site.

Studies using **58** and **53** with actomyosin,[140,208-210] heavy meromyosin,[211] and G-actin[212] indicate neither to be satisfactory substitutes for ATP; this is rationalized to be a problem of specific binding.[212] However, from this and other metal ion binding studies,[141,213-215] as affinity of the analogue **58** for cations mimics that of ATP, failure of **58** to participate in enzymatic processes (competitive inhibition or action as a substrate) is indicated possibly not to be due to geometry and binding of the β- and γ-phosphoryl sites. This, of course, points to an importance of reversible binding involving the β,γ-pyrophosphate oxygen. In this light, in studies using **53,** a specific interaction of the α,β-pyrophosphate oxygen is indicated in the binding of ATP to Phe-tRna synthetase from *E. coli*.[216]

In investigations using compound **52** and its 2-chloro derivative **55** as analogues of ADP, binding to creatine kinase was found to be more effective than with the natural material;[217] although it was reported that neither could replace ADP for aggregation of mammalian blood platelets,[138] further work indicates a pH dependence.[218] In the earlier work lack of binding to the ADP receptor had been invoked as a rationalization for lack of replaceability.[138] Although **52** is reported as being totally without effect as an ADP replacement with adenyl kinase, pyruvate kinase, and hexokinase, it serves as an adenine nucleotide replacement with mitochondria from several sources.[219-221] This analogue has also been used in studies of polynucleotide polymerase,[222] polynucleotide phosphorylase,[223-225] 5′-nucleotidase from several sources,[226-228] and in studies of phosphate exchange with ADP.[229]

The analogue **52** has also been investigated in the uptake and release of calcium ion in mitochondria[230,231] in which the analogue was found to deplete the ADP content of the system.

The isosteric analogue **34** of the 5′-monophosphate in the adenosine series, as mentioned above, undergoes reaction with AMP deaminase.[132] It was also noted that **34** was a competitive inhibitor of *E. coli* adenylosuccinate synthetase (as was the corresponding IMP analogue **42**), and it was concluded that this was due to changes in binding capabilities about the 5′ position. The AMP related analogue **40,** which bears a binding functionality at the carbon bound to phosphorus, exhibits significant activity;[130] it is a substrate for and competitive inhibitor of AMP deamination by rabbit muscle AMP aminohydrolase, and an inhibitor of snake venom 5′-nucleotidase. Steric requirements are exhibited in its interaction with pig muscle AMP kinase; only one epimer (at the 6′ position) serves as a substrate. A similar result is found with the related analogue **46** of ATP; one epimer only serves as phosphoryl donor in the AMP kinase reaction, that epimer being of opposite configuration to the one of **40** reacting in the pig muscle AMP kinase process. A corresponding activity could be found for the 6′-hydroxyl substituted analogue **41;**[131] while both epimers could be phosphorylated by muscle adenylate (AMP) kinase, only one of the product epimers was a substrate of muscle pyruvate kinase. Steric (bulk) problems are presumed to be involved as well in the decreased activity of **36** compared to **37**;[127] an alternate explanation for this decreased activity would be that only partial dissociation of the phosphonic acid is occurring under the conditions used.

In the 2′-deoxythymidine series, Englund et al. have shown[139] the α,β-pyrophosphate oxygen substituted analogue **57** of dTTP to bind at the same site of DNA polymerase (*E. coli*) as ordinary deoxyribotriphosphates. In an extension of this work, Geider found[232] that, for the particular systems studied, although binding to the polymerase occurs, **57** does not influence DNA synthesis, probably because binding to a replication complex is weak. Rammler et al. reported[233] that micrococcal nuclease was ineffective in cleaving and was inhibited by oligomers of the nonisosteric phosphonic acid analogue **32** of dTMP.[20] As of now, details of the preparation of these oligomers have not been provided. Kornberg et al.[133,234] have studied the isosteric phosphate oxygen and β,γ-pyrophosphate oxygen substituted analogues of dTTP (supplied by Syntex) and found that they do not support enhanced degradation of *E. coli* DNA[234] nor are they incorporated by DNA polymerase,[133] although both have the same base pairing specificity as dTTP.

In the uridine series, Holy reported[121] that, as might reasonably be expected, **31** was completely stable to crude snake venom, bovine intestinal alkaline phosphatase, and *E. coli* alkaline phosphatase. Yengoyan and Rammler found[120] the UDP analogue **47** to be incapable of polymerization with *Micrococcus lysodeikticus* polynucleotide phosphorylase, although the analogue **51** of UDP-glucose was a substrate for bovine liver UDP-glucose dehydrogenase and UDP-glucose phosphorylase.[135]

Numerous studies have also been performed using those phosphonates wherein an alkyl or substituted alkyl group replaces a hydroxyl on phosphorus. For the methylphosphonate esters related to AMP (**67**), UMP (**69**), and AzUMP (**70**), resistance to phosphatases from various sources was found,[151-153] although the corresponding 2′(3′)-AzUMP analogue **72** was significantly degraded. The UMP analogue **69** was found[235] to be without effect on thymidylate synthetase from *E. coli,* indicative of a requirement for the dibasic acid site for interaction. For the IMP analogue **67,** noncompetitive inhibition of IMP dehydrogenase was found,[149] and Wigler and Lozzio found[153] for the 2′-deoxy-2-bromouridine analogue **71** that it competed effectively with dTMP as a substrate for thymidylate kinase, could be in-

corporated into a DNA strand, and readily penetrated the cell walls of specimens from Chinese hamsters producing irreversible cytotoxicity. This latter work points up some interesting consequences of charge removal (by methyl substitution), that is, ease of membrance permeability and a polynucleotide bearing uncharged phosphorus units.

The analogue **83a** prepared by Gulyaev et al.[148] proved to be a potent inhibitor of acetylCoA synthetase. This is an interesting system in which the oxygen of a mixed anhydride linkage has been eliminated, but interaction still occurs.

Compounds bearing a hydroxy- or aminomethyl group in place of hydroxyl on phosphorus exhibit some significant differences in their biochemistry from the simple methyl systems discussed above. While all are resistant to alkaline phosphatase,[151,155,156] compounds **73–79** are cleaved by snake venom 5′-nucleotidase.[151,154,155] Compound **76** was found not to interact with *E. coli* DNA polymerase,[236] and **86** did not appear to interact with myosin B.[158] The reaction systems involved in these latter works appear to require the dianionic state for interaction.

For analogues of cyclic phosphates, Jones and Moffatt report **89,** an analogue of 3′,5′-cyclic AMP, to be equivalent to the natural material in pharmacological action, but with a longer half-life due to a lower susceptibility of hydrolysis,[163] although a later report[237] indicated it to have about one-half the activity of the natural material; etheno bridged derivatives were even less active in both the natural and phosphonate series. Compound **97,** an analogue of 2′,3′-cyclic AMP, was reported to be useful for controlling metabolism and producing metabolic deficiencies.[160] The analogue **98** related to 2′,3′-cyclic UMP was reported[238] to exhibit significantly lower susceptibility to enzymatic hydrolysis (RNAase A) than the natural material; this has been related to the comparative possibilities of pseudorotation about phosphorus in either case.

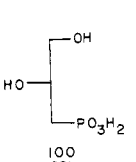
Finally, the analogues of dinucleoside phosphates **88** and **89,** as expected, were not cleaved by spleen phosphodiesterase. These compounds have been used by Richards et al.[239] in crystallographic studies of RNAase–nucleotide interactions; Griffin et al. have performed[240] studies on the same system using an NMR technique. Johnson and Schleich have reported[241] CD studies on **89** and nucleoside phosphates; it appears that the analogue in solution does not exhibit the ordinary stacking properties of the normal material, possibly due to a "solvophobic" interaction at the methylene site.

VI. Analogues of Phosphate Products of Glycolysis

A. Synthesis

Interest in phosphonic acid analogues of the natural phosphate products of glycolysis has developed rather late compared to that for the systems already discussed and the analogues of phospholipids (vide infra). However, numerous reports have appeared in the past few years with a concern for the possibilities for use of such phosphonic acids as metabolic regulators. Analogues, both isosteric and nonisosteric, for all simple phosphorus-containing products or carbohydrate degradation have been prepared and studied.

The earliest report of synthetic work in this area was by Rosenthal and Geyer[242] who prepared the 2,3-dihydroxypropyl-1-phosphonic acid (**100**) as a nonisosteric analogue of glycerol 3-phosphate by an Arbuzov reaction on allyl bromide



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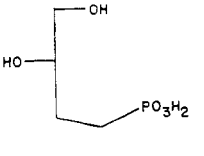
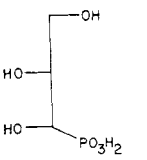


101

followed by hydroxylation and ester hydrolysis. Baer and Basu[243] later reported another synthesis of the material, only in the optically active form of the same absolute configuration as the natural *sn*-glycerol 3-phosphate. This latter route began with D-mannitol diacetonide and utilized an Arbuzov reaction on the iodide derived from it after cleavage. The racemic isosteric analogue, 3,4-dihydroxybutyl-1-phosphonic acid (**101**), was then reported[244] by a route entirely analogous to that of Rosenthal and Geyer[242] beginning with 4-bromo-1-butene. The optically active form was again reported[245] by a route beginning with D-mannitol diacetonide with phosphonylation by a Wittig reaction on the aldehyde derived from it upon cleavage. A more convenient route for this material (**101**) in optically active form has recently been developed in this laboratory beginning with *l*-malic acid.[246] Compound **101** has also been obtained with specific tritium labeling at the 3-carbon by hydride reduction of the carbonyl compound (vide infra).[247]

Two other analogues of glycerol 3-phosphate have also been reported,[245] these being the 1,2,3-trihydroxypropyl-1-phosphonic acid (**102**) and the phosphinate (**103**). Because of the relatively major structural changes (relative to the natural material) in a small molecule, neither of these would be expected to substitute as well for glycerol 3-phosphate as those mentioned previously.



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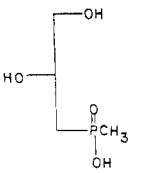
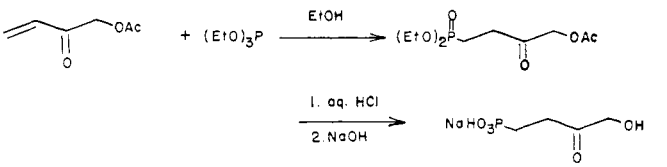


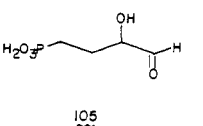
103

Two reports have appeared on the synthesis of 4-hydroxy-3-oxobutyl-1-phosphonic acid (**104**), an analogue of dihydroxyacetone phosphate. Goldstein et al.[247] first prepared the compound by hydrophosphinylation[248] of acetoxymethyl vinyl ketone followed by hydrolysis. A later report[249] described a synthesis of this material in four steps beginning with a hydrophosphinylation reaction with acrylic acid.



104

An isomer of **104,** 3-hydroxy-4-oxobutyl-1-phosphonic acid (**105**), has been prepared as an isosteric analogue of glyceraldehyde 3-phosphate;[250] the product was isolated and stored as the diethyl acetal of the dilithium salt and converted to the free aldehyde form before use by treatment with the acid form of a cation-exchange resin. The complete synthesis was accomplished in four steps beginning with a free radical addition of diethyl phosphite across the olefinic linkage of but-3-enal diethyl acetal. Earlier, Baer and Robinson[251] reported a multistep synthesis of 2-hydroxy-3-oxopropyl-1-phosphonic acid (**106**), a nonisosteric analogue, beginning with DL-glyceraldehyde dimer.



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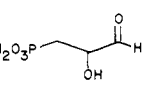

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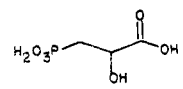
Recently there have appeared three reports on syntheses of analogues of 3-phosphoglyceric acid. Pfeiffer et al. describe[252] both the nonisosteric analogue **107** and the isosteric analogue



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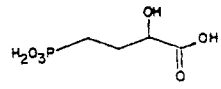
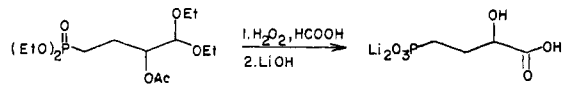


108

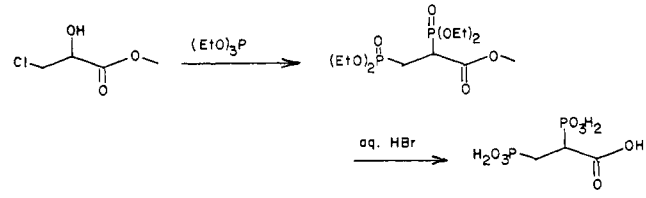
108 using syntheses involving C–P bond formation by the Arbuzov reaction and hydrolysis of the appropriate cyanohydrin. Goldstein et al. reported[250] the preparation of the trilithium salt of **108** by the oxidation of **109,** an intermediate in the preparation



109

of the analogue **105** of glyceraldehyde 3-phosphate; Dixon and Sparkes reported[249] a multistep route for the preparation of **108** from diethyl 2-bromoethylphosphonate. Baer and Robinson reported[253] a nonisosteric analogue (**110**) of 2,3-diphosphoglyceric acid by the route shown in Scheme IV. A modification of the Pfeiffer et al. technique for the preparation of **108,** wherein cyanohydrin formation is performed after phosphonylation by a hydrophosphinylation of acrolein, has been found to give increased yields of product.[246]

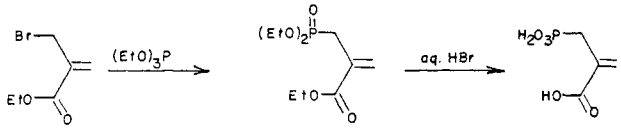
SCHEME IV



110

The last system of interest in this class is that of phosphoenolpyruvate. Stubbe and Kenyon studied[254] a number of analogues of phosphoenolypyruvate including the isosteric phosphonic acid analogue **111.** This was prepared by the route in Scheme V, phosphonylation being performed using an Arbuzov reaction.

SCHEME V



111

B. Biological Investigations

With the use of phosphonic acid analogues of glycerol 3-phosphate, significant advances toward the regulation of metabolism have been obtained for certain bacterial systems; this effort points up the potential for use of the phosphonic acids as metabolic regulators for other systems and for the treatment of higher organisms, as well as for mechanistic determinations.

The first work using the nonisosteric analogue **100** was not promising; Baer et al. found[255] it not to interact with the enzyme systems studied and concluded that the ester oxygen was necessary for activity. However, later efforts by Kabak et al.[244] and Shopsis et al.[256-258] demonstrated that the isosteric analogue **101** was capable of inhibiting the growth of mutant strains of *E. coli* at rather low concentration. The net effect was bacteriostasis and could be traced to a perturbation of the normal phospholipid production[259-261] resulting from an inability of the organism to cleave the phosphonate linkage now present in place of an ordinary phosphate. The organism is capable of being "outwitted" by **101** but not **100;** that is, the analogue substitutes for glycerol 3-phosphate for a portion of the normal metabolic process but a point of inhibition is reached as a result of the inability to release phosphate. Contrary to the original conclusion, the ester oxygen is not necessary for activity, but correspondence of size with the natural substrate is. Other work by Adams et al.[245] confirmed the ability of **101** to substitute for glycerol 3-phosphate in enzymatic processes, but the analogue **102** could not so substitute.

Whereas the analogue **101** was capable (as expected from behavior of the natural material) of penetrating the cell walls of the organism investigated, the analogue **104** of dihydroxyacetone phosphate was not. However, it did serve as a substitute for the natural material in vitro. It has been found[247,262] to serve as a substrate for L-glycerol 3-phosphate:NAD oxidoreductase and L-glycerol 3-phosphate dehydrogenase from *E. coli*; Stribling found[117] binding of **104** to the latter system to be very similar to that of the natural material, and with aldolase and glycerol 3-phosphate formed the 1-deoxy-1-phosphonomethyl isostere of fructose 1,6-diphosphate. Dixon and Sparkes[249] confirmed this work of Stribling but found **104** not to interact with triosephosphate isomerase from chicken muscle or *B. stearothermophilus.* Recently, Klein et al. have found **104** to be incorporated into the cell wall of *B. subtilis,* acting as a bacteriocidal or bacteriostatic agent depending on the strain used and the cell wall teichoic acid composition.[263]

Several studies have been performed using analogues of phosphoglyceric acid. Pfeiffer et al.[252] observed no antibacterial or enzymatic activity for either **107** or **108.** Benesch et al.[264] likewise found that **110** was incapable of replacing 2,3-diphosphoglyceric acid in oxygen release from hemoglobin. The lack of reactivity was attributed to severe deviations from the spatial characteristics of the natural material. It would be of interest to consider these processes with an isosteric analogue. Dixon and Sparkes, however, found[249] **108** capable of replacing the natural material in oxidation of NADH, and Orr and Knowles found[265] it to be a substrate for phosphoglycerate kinase with a similar k_{cat} value to that of the natural material.

Nowak et al.[266] along with Stubbe and Kenyon[254] and Lane and Hurst[267] used the analogue **111** of phosphoenolpyruvate in studies of enolase; it was found to be comparable to phosphoenolpyruvate in binding to the enzyme but much slower in overall reaction. The analogue **111** was also found to be noninhibitory of pyruvate kinase at low ratios of analogue to substrate, but Reed and Cohn[268] later found it to be inhibitory at much higher ratios. James and Cohn[269] used this analogue in proton magnetic resonance studies using a relaxation rate technique to investigate the nature of the magnesium–pyruvate kinase substrate complex.

Goldstein et al.[250] found the analogue **105** of glyceraldehyde 3-phosphate to be a substrate for rabbit muscle glyceraldehyde 3-phosphate dehydrogenase. Moreover, **105** is transported into cells of *E. coli,* as is the natural material, and completely inhibits their growth at low concentration.[270] The mode of inhibition here has not yet been elucidated.

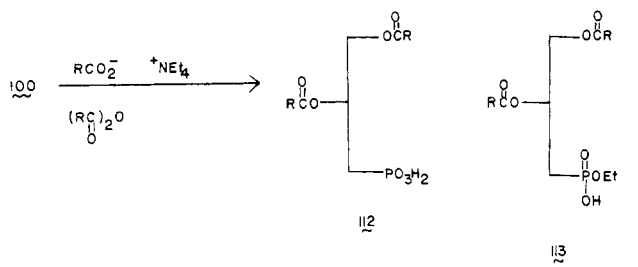
VII. Analogues of Phospholipids

A. Synthesis

There has been an exhaustive effort from several groups toward the preparation and study of phosphonic acid analogues of ordinary phospholipids. This effort was given for several stated purposes: first, the laboratory synthesis of natural phosphonolipids and compounds related to them;[9] second, the search for nonhydrolyzable lipid materials which might substitute for the natural substances; and finally, the independent synthesis of compounds which might be derived from the metabolism of other phosphonic acid analogues of natural phosphates. We shall note that included in the many compounds synthesized are those with

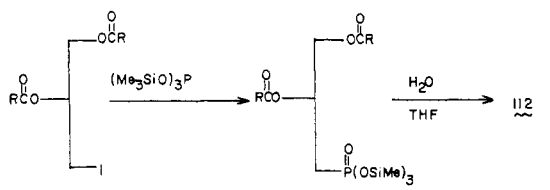
ether and hydrocarbon linkages in place of the usual fatty acid ester linkages; Paltauf has given a brief review of some of these ether lipids.[271] While these exhibit further deviations from the behavior of normal phospholipids, they remain as quite interesting substitutes for them. It should also be noted that several systems of nomenclature are in common use; here the analogues will be referred to using the generic term *phosphonolipids* as introduced by Baer.

The fundamental phosphonolipid structure is embodied in the analogues of phosphatidic acid, referred to as phosphotidic acids. The nonisosteric members of this class, **112,** bearing saturated fatty acid ester functions were reported by Baer and Basu[272] and later by Bonsen et al.[273] by acylation of **100** using a standard method. Bonsen et al. have also reported[274] a monoester **113** related to **112** by standard acylation of ethyl

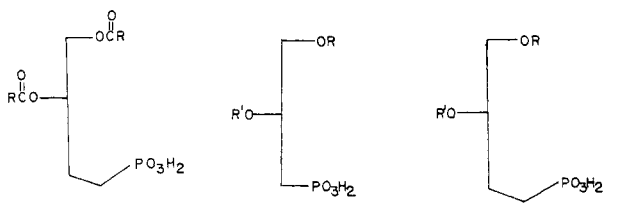


112 **113**

2,3-dihydroxypropyl-1-phosphonate. A more versatile approach has been reported by Rosenthal et al.[275,276] utilizing an Arbuzov reaction of the 2,3-diacyl-1-iodopropanes with tris(trimethylsilyl) phosphite[277,278] followed by mild hydrolysis. This technique is



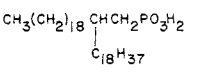
expected to be of great utility in future work by simplification of the standard Arbuzov reaction methods for generation of free phosphonic acids. The isosteric phosphotidic acids **114** bearing saturated and unsaturated fatty acid ester linkages have recently been prepared by Tang et al.[279]



114 **115** **116**

For the corresponding phosphotidic acids bearing ether linkages in place of the normal fatty acid ester functions, Rosenthal et al. have reported preparations by Arbuzov reactions using the appropriate dialkoxyalkyl iodides. Nonisosteric systems (**115**) were prepared from the 2,3-dialkoxy-1-iodopropanes;[280] included here are systems differentially substituted at the 2 and 3 positions. Isosteric (about phosphorus) analogues were later reported with phosphonylation of the 3,4-dialkoxy-1-bromobutanes.[281]

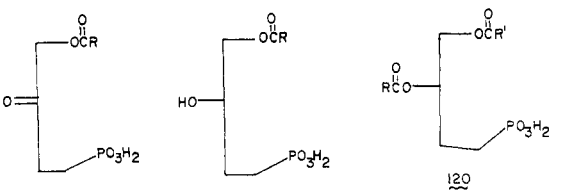
Rosenthal also reported[282] an interesting phosphonic acid (**117**) in which all nonhydrocarbon functions of the lipid backbone



117

were eliminated; this was prepared by a Becker reaction of the alkyl tosylate followed by acidic hydrolysis of the phosphonate diester.

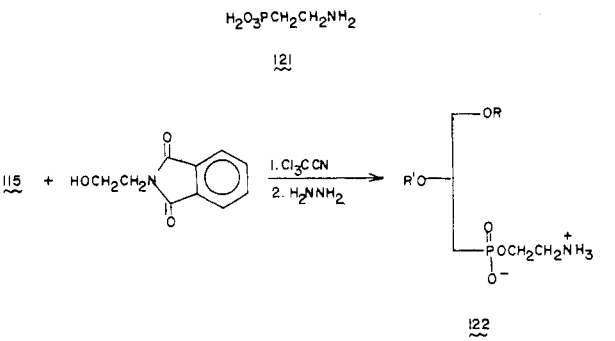
Two other interesting classes of phosphotidic acids have been prepared by Tang et al. Compound **118,** an analogue of acyldihydroxyacetone phosphate, was prepared by acylation of previously discussed **114**.[279] Upon hydride reduction there was obtained the lyso-phosphotidic acid **119**.[283,343] This material has also been acylated under standard conditions to yield the differentially substituted phosphotidic acid **120**.[283]



118 **119** **120**

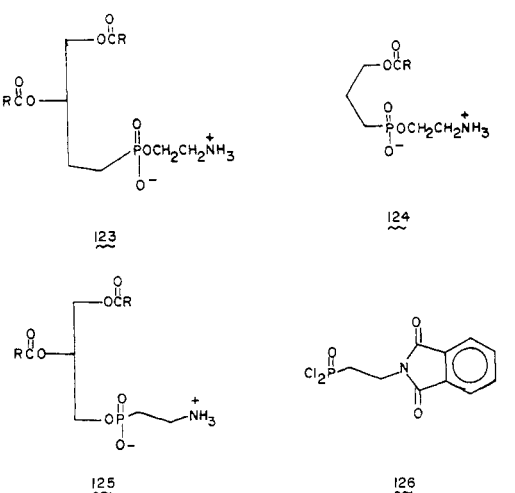
For phosphonic acids related to cephalins (phosphatidyl ethanolamines or diacylglyceryl phosphorylethanolamines), a class of derivatives of phosphatidic acid, more possibilities for structural variation are present, and accordingly a variety of approaches to their synthesis have resulted.

Most of the effort has been directed toward the laboratory preparation,of natural phosphonolipids, primarily derivatives of 2-aminoethylphosphonic acid (**121**) with less attention being



121 **122**

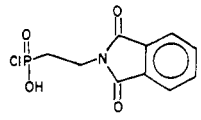
given to derivatives with a carbon–phosphorus bond in the lipid backbone. In the latter category Rosenthal has reported[282,284] the preparation of the nonisosteric diether compounds **122,** with similar and dissimilar alkoxy groups, by trichloroacetonitrile mediated coupling of the phosphotidic acid (**115**) and the phthalimide-protected ethanolamine. By similar routes the isosteric diacyl analogue **123** has been prepared,[285] and Pfeiffer et al.[286] reported the shortened acyl-bearing analogue **124** with the alkyl group being adamantyl.



123 **124** **125** **126**

Considerable attention has been given to the analogues (phosphonolipids) in which the fundamental glycerol backbone remains, but the oxygen has been eliminated from the ethanolamine portion. These have been synthesized primarily because of growing interest in natural phosphonolipids which are commonly based on 2-aminoethylphosphonic acid.

Rosenthal and Pousada first reported[287] the preparation of the dipalmitate and distearate derivatives of **125** by reaction of



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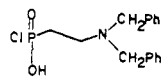

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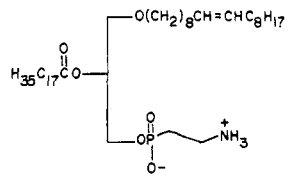
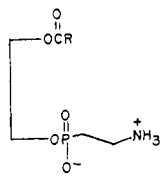


129

the α,β-diglyceride with the phosphonic dichloride (**126**), free amine being generated by hydrazinolysis. Baer et al.[288-292] synthesized a series of compounds corresponding to **125** performing ester formation with phosphonic monochlorides **127** and **128,** removing the protecting functions by hydrazinolysis and hydrogenolysis, respectively. Turner et al. later used the Baer method for the generation of **125**[293] as did Chacko and Hanahan[294] for the ether lipid **129**. Pfeiffer et al.[286] used the Rosenthal method for the synthesis of **125** where the alkyl groups were adamantyl.

Baer and Basu[295] used **128** for the synthesis of the "shortened" systems **130,** and Baer and Stanacev reported[296] the diether lipids **131** as prepared by the same route. Baer and Pa-



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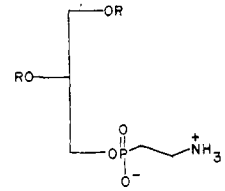
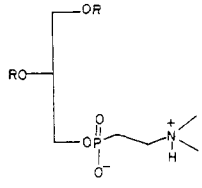


131

vanaram[297] have reported N-methyl substituted systems by a similar route, and Baer et al. have reported[298-300] the synthesis of sphinogolipid-type derivatives of aminoethylphosphonic acid. Baer and Rao have also reported[301] the preparation of the *N,N*-dimethylaminoethylphosphonate **132** bearing ether linkages on the glycerol backbone by dimethylamine reaction with the bromide **133**.



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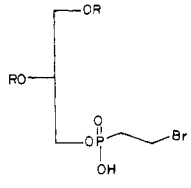
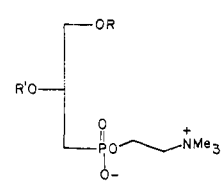


133

Lecithins (phosphatidyl cholines or diacylglyceryl phosphoryl cholines) present the same possible structural variations as seen above with the cephalins. Compounds of the types **134** and **135** with the oxygen eliminated from, or substituted by a methylene group in, the glycerol backbone have been reported by the coupling of the phosphotidic acid with choline using trichlo-



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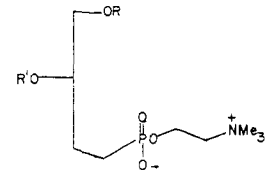
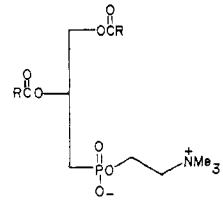


135

roacetonitrile[282] or an acid chloride;[302] by a similar method were prepared the diesters **136**[273,276] and the choline adduct **137** of **114**.[285]



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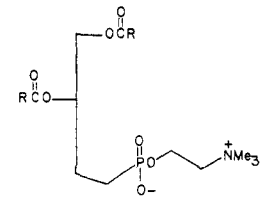
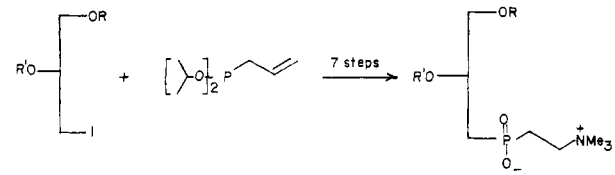


137

Rosenthal et al. have reported[303-306] phosphinate analogues **138–141** of phosphatidyl choline by an Arbuzov reaction of the appropriate alkyl iodide with diisopropyl allylphosphonite followed by functionalization of the allyl chain. Also reported was a completely nonhydrolyzable phosphinate analogue **142**.



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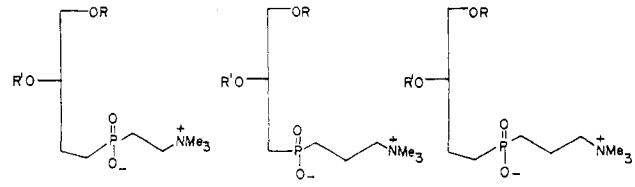
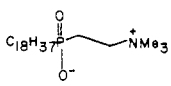


139 140 141

Another method for the preparation of these class of compounds has been developed by Rosenthal[307] using bis(trimethylsilyl)trimethylsilyloxymethylphosphonite (**143**).[308]



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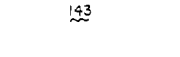

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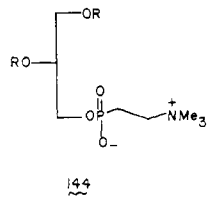
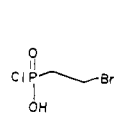


144

The phosphotidyl cholines (**144**) bearing an intact glycerol linkage but without the normal oxygen of the choline portion have been prepared by ester formation using the acid chloride **145** with the α,β-diglyceride followed by amine displacement of the



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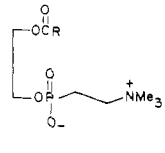
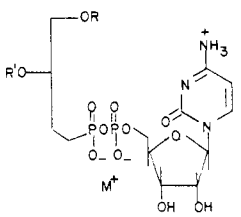


146

bromide. These have included systems where R and R′ are acyl groups[309,310] as well as those with R and R′ alkyl,[311,312] and R being alkyl with R′ being acyl.[294] Baer and Robinson have also reported[313] the preparation of the "shortened" system **146** by a similar route.

Several reports have also been made of 2-aminoethylphosphonate,[314] 2-methylaminoethylphosphonate,[315] and (2-trimethylammoniumethyl)phosphonate[316,317] esters bearing the glycerol backbone. These have been prepared by reaction of the properly substituted metaphosphonates with glycerol acetonide followed by deprotection. These compounds should be of use in the identification of natural phosphonolipids and in the study of their metabolism. In this light, Baer et al. have developed a separation scheme for identification of phosphonolipids in the presence of phospholipids.[318]

There has been a report by Tyhach et al.[319] of the preparation and study of diether lipid analogues of cytidine diphosphate diglyceride (CDP-diglyceride). Both isosteric (**147**) and nonisosteric



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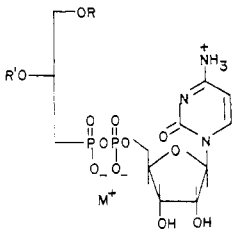
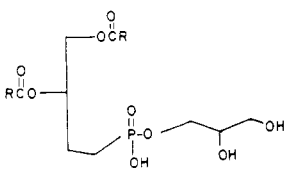


148

(**148**) analogues (as pertain to glycerol backbone) have been reported as being prepared from the phosphotidic acids **116** and **115,** respectively, using the monomorpholidate of CMP according to the technique of Raetz and Kennedy.[320] Finally, the isosteric analogue **149** of phosphatidylglycerol has been prepared[285] using a trichloroacetonitrile coupling method, as well as several analogues of phosphatidylserine.[344]



149

B. Biological Investigations

The compounds in this category have been investigated for their ability to replace natural phospholipids in various systems, being resistant to normal enzymatic cleavage routes. Rosenthal et al.[321,322] studied the analogues **115, 117,** and **116** of phosphotidic acid and found them to be inhibitors of solubilized and particulate pig kidney phosphatide phosphohydrolase. It was also found that the extent of inhibition depends on the degree of dispersion, a common problem in studies of the synthetic phosphonolipids.

Enzymatic studies with analogues of phosphatidyl choline have yielded some interesting results. For analogues of the type **144** where the glycerol backbone remains intact, phospholipase C facilitated hydrolysis occurs at a rate equal to that of the natural material,[323] although Rosenthal and Pousada found[324] the dipalmitate system to be a mild inhibitor. However, systems missing the ester linkage in the glycerol backbone, such as **134, 136,** and **135,** are inert to phospholipase C and are powerful inhibitors in a dispersed state[324-326] although the inhibition is less if the lipid is incorporated into a bilayer membrane. The phosphinates **141, 139, 138,** and **140,** with ether linkages, were, as expected, inert to and inhibitors of phospholipases A, B, C, and D.[326,327]

Inhibition of phospholipase A could be observed with several systems. These include the phosphinates,[328] several compounds of type **134,**[329] and the diacyl compound **113.** The effect resulting from the exclusion of the ammonium function in the last case is particularly interesting

Work by Bittman and Blau[330] utilized the synthetic phosphonolipids for the preparation of liposomes and the study of their interaction with sterols. Here it was found that the length of the "glycerol backbone" was quite significant; only the isosteric systems exhibited interaction with the sterols. Also of significance, it was found that the ethers were good substitutes for the esters as the C=O linkage had a negligible role in the packing of the lipids. DeKruyff et al.[331] have also reported a study of sterol interaction with phosphinate lipid analogues **140** and **142.**

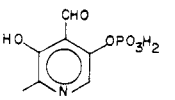
Finally, the analogues **119** of lysophosphatidic acid have been found to be substrates for lysophosphatidic acid acyl transferase.[282]

VIII. Other Phosphonic Acid Analogues of Natural Phosphates

A. Synthesis

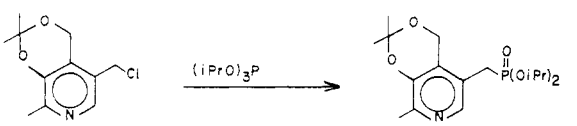
This final section is concerned with two areas of rather great biological significance, although relatively little work has been performed. We are concerned here with (a) analogues of pyridoxal phosphate and (b) analogues of phosphate intermediates in the biosynthesis of squalene and other terpenes.

For the first system, analogues in which the esteratic phosphate oxygen is removed, and those in which a hydroxyl on phosphorus is replaced, are of interest in considerations of modifications (enhancement or inhibition of activity) of transaminase reactions for which the natural phosphate (**150**) serves as coenzyme.



150

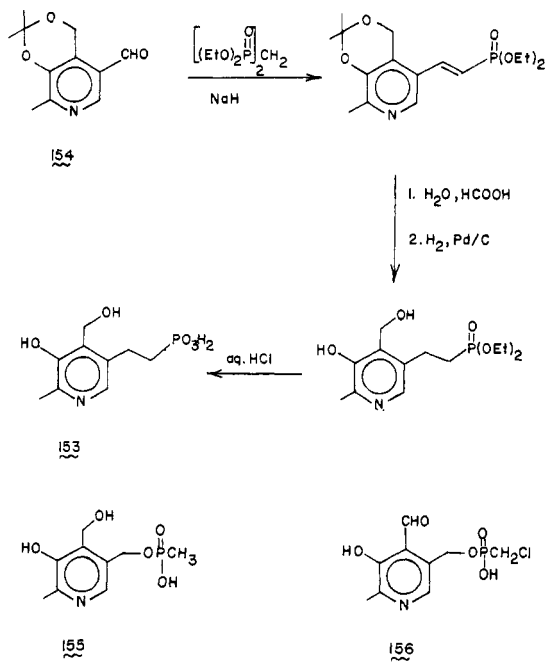
As with the systems discussed previously, the first phosphonic acid analogues prepared were nonisosteric with the natural system **150** and its derivatives. Bennett et al. first reported[332] syntheses of analogues of this type, **151,** by Arbuzov reaction involving **152.** With compound **151** in hand, modifications could be made readily (after ketal cleavage) to generate a variety of reagents for enzymatic investigations.



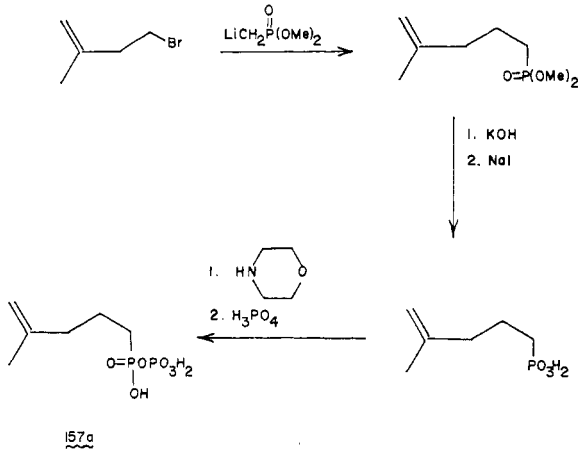
152 151

Hullar later reported[333] the preparation of the isosteric analogue 5′-deoxy-5′-dihydroxyphosphinylmethylpyridoxol (**153**), in 53% overall yield starting with the aldehyde **154.** Phosphonylation was performed by a Horner reaction, followed by reduction and hydrolysis of the ester linkages. Again, side chain modifications could readily be performed on **153.**

Two compounds have also been reported in which an alkyl group has been substituted for a hydroxyl on phosphorus of the natural material. These have been produced by coupling of the suitably protected alcohols with the phosphonic acids. Korytnyk et al.[334] reported the methyl system **155,** and several years later Gulyaev et al. reported[335] the chloromethyl compound **156** along with several other phosphate analogues.



For the second area of interest, Corey and Volante[336] have recently reported the synthesis of phosphonic acid analogues of several organic pyrophosphates known to be critical intermediates in the biosynthesis of squalene.[337] These are the isoprenoid methylphosphonophosphates (**157a–e**) related to isopentenyl, γ,γ-dimethylallyl, geranyl, farnesyl, and presqualene pyrophosphate, respectively. With the exception of the presqualene pyrophosphate analogue **157e,** these were prepared by displacement reactions on the parent bromides using dimethyl lithiomethylphosphonate followed by ester cleavage[338] and phosphorylation by the method of Cornforth and Popjak.[339]

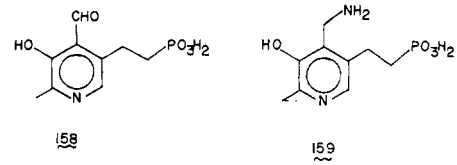


The analogue **157e** was prepared by a Wittig-type reaction[83] on the aldehyde derived from presqualene alcohol followed by ester cleavage and phosphorylation. The corresponding analogue of 5-phosphomevalonic acid, the initial phosphorylated species in this series, 5-carboxy-4-hydroxy-4-methylpentyl-1-phosphonic acid, has been reported more recently by Sarin et al.[345]

B. Biological Investigations

It appears that spatial relationships are quite critical for activity of the analogues of **150**. It was found[332] that the nonisosteric system **151** and its related derivatives were totally ineffective in replacing pyridoxal phosphate or causing inhibition of transaminase (beef heart) in vitro. Hullar found[333] that the isosteric system **153** and its derivatives, while exhibiting only minimal activity (compared to the natural material) on tyrosine decarboxylase, were similar to the natural materials in their action in the inhibition of aspartate aminotransferase.[340]

Vidgoff et al. later found[341] that by addition of the aldehyde **158,** derived from the compound **153,** rabbit muscle glycogen phosphorylase could be reconstituted to ca. 25% of its optimal activity, although at high concentration inhibition occurred. The workers concluded that for the system of interest the presence of the phosphate oxygen was not critical. Similar results were found by Feldman et al.[342] for the pyridoxamine related system **159**.



Thus far only negative activity results have been reported[334] for the phosphonate ester systems of the type **155**.

Corey and Volante[336] have studied compounds **157a–e** in cell-free systems of squalene synthetase from rat liver and kaurene synthetase from *Ricinus communis*. Varying degrees of inhibition were found for the analogues, in general that of geranyl and farnesyl pyrophosphate being the most effective. Detailed data on the effectiveness of inhibition under a variety of conditions was not noted in this preliminary communication.

Acknowledgment. The author wishes to acknowledge a NATO Senior Research Fellowship to McGill University, Montreal, during which the bulk of this review was prepared, and to the NIH and the CUNY-FRAP for grants supporting work mentioned herein but not yet published.

IX. References and Notes

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(346) Several approaches for nomenclature and abbreviated line notation are to be found in the literature for compounds in this class and those to follow. Often these approaches are misleading and at times quite incorrect. The nomenclature method used here is one of several suggested by Dr. K. L. Loening, Nomenclature Director of the Chemical Abstracts Service, which stresses the relationship of the analogue to the natural materials. In this method the position of the methylene substitution for (normal) oxygen is indicated by the notation within brackets. One may also use a numerical system wherein the phosphorus nearest the sugar ring is designated "1"; thereby **52** would be named adenosine 5'-[1,2-methylene]diphosphate. A third acceptable (and more correct in relationship to other organic nomenclature) name is adenosine 5' → O^1-(1,2-μ-methylene)diphosphate. In this article the method as already used in the text for **52** will be continued. Abbreviated notations, such as AMPCP for **52,** should be avoided as these tend to be quite ambiguous to readers.