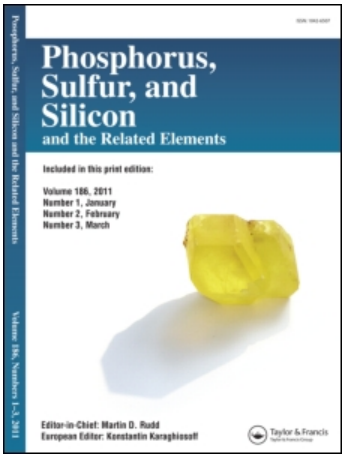

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Biologically Relevant Phosphoranes: Hypervalent Phosphorus as Applied to Phosphoryl Transfer Enzymes

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Biologically Relevant Phosphoranes: Hypervalent Phosphorus as Applied to Phosphoryl Transfer Enzymes

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The coordination tendencies of phosphorus to form a hexacoordinated state from a pentacoordinated state, which might assist in describing the mechanistic action of phosphoryl transfer enzymes, are delineated. In view of the work reported here and recent work on enzyme promiscuity and moonlighting activities, it is suggested that donor action should play a role in determining active site interactions in phosphoryl transfer enzyme mechanisms. Biochemists studying phosphoryl transfer enzymes outline mechanisms of nucleophilic attack at phosphorus that take place by way of proposed trigonal bipyramidal intermediates or transition states. However, recent work has shown the ready availability of higher coordinate forms of phosphorus, particularly the ease of formation of hexacoordinate phosphorus. Our recent work established the X-ray structure of several biorelevant phosphoranes. Included are the structures of a xylofuranose based phosphorane 1 and a thymidine based phosphorane. Dynamic equilibrium between two isomeric forms exists in solution for 1. In addition, bicyclic phosphorane 3 exists in equilibrium between pentacoordinated and hexacoordinated isomeric forms. The rapid exchange process between these two geometries reorients the nucleotidyl or carbohydrate component of the trigonal bipyramidal phosphorane. At an active site, this type of pseudorotational behavior provides a mechanism that could bring another active site residue into play and account for a means for phosphoryl transfer enzymes to express promiscuous behavior. Pseudorotation, a well-founded process in non-enzymatic phosphorus chemistry may have an application in the future of phosphoryl transfer enzyme chemistry.

I express my sincere appreciation to my students and co-workers who made this work possible, especially Dr. A. Chandrasekaran and Professor Roberta O. Day for the crystallographic work and Natalya V. Timosheva for her expertise in synthetic work.

The research reviewed here was supported by the National Science Foundation and the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the support is gratefully acknowledged.

The work summarized here formed the basis of a keynote lecture given by the author at the 17th International Conference on Phosphorus Chemistry (ICPC-17) held in Xiamen, China, April 15–19, 2007.

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Keywords Hypervalent phosphorus; nucleophilic; phosphorane; Phosphoryl transfer enzymes; pseudorotation; thymidine; xylofuranose

INTRODUCTION[1]

Enzyme active sites of phosphoryl transfer enzymes and cAMP invariably are portrayed with nearby residues involved in hydrogen bonding with the substrate but not with donor interactions at the phosphorus atom.[2–4] In general, it has been assumed that a tetracoordinate phosphate substrate will proceed to an activated complex in a nucleophilic displacement reaction where the activated complex or transition state is pentacoordinate by virtue of the incoming nucleophile.

The coordination tendencies of phosphorus that might assist in describing the mechanistic action of phosphoryl transfer enzymes and cAMP has not been used up to the present. In this regard, biochemists have persisted in referring to knowledge of hypervalent phosphorus chemistry, as it existed over a quarter of a century ago. However, the area has experienced major advances, particularly the ease of formation of hexacoordinated phosphorus and its relation to pentacoordinated phosphorus.[2,5,6] It is our contention that residues at active sites of phosphoryl transfer enzymes are capable of entering into donor interaction at the phosphorus atom and as a consequence assist in nucleophilic attack.

In what is presented here, we outline developments in higher valent phosphorus chemistry that reasonably may be used by biochemists in refining their current proposals concerning mechanistic models of nucleophilic displacements at active sites of phosphoryl transfer enzymes.

Hypervalent Phosphorus—Factors to be Discussed

The following factors are prevalent during substrate reactions at the active sites of enzyme systems in general; 1) donor action at phosphorus; 2) hydrogen bonding; 3) anionicity; 4) packing effects; and 5) phosphorane reactivity. In in-vitro studies in the absence of enzymes, we find that each one of the first three factors above approximate each other in the extent of the energy they introduce into a system as reflected in the degree of donor bond changes that occur.[7,8] This energy amount is of the order of 5 kcal/mole. This is the range of interactions encountered with the extensive array of hydrogen bond studies that are found in the literature. The energy associated with packing effects is somewhat less. One feature that is often overlooked by investigators and particularly those conducting structural studies in the solid state is that packing effects are often ignored.

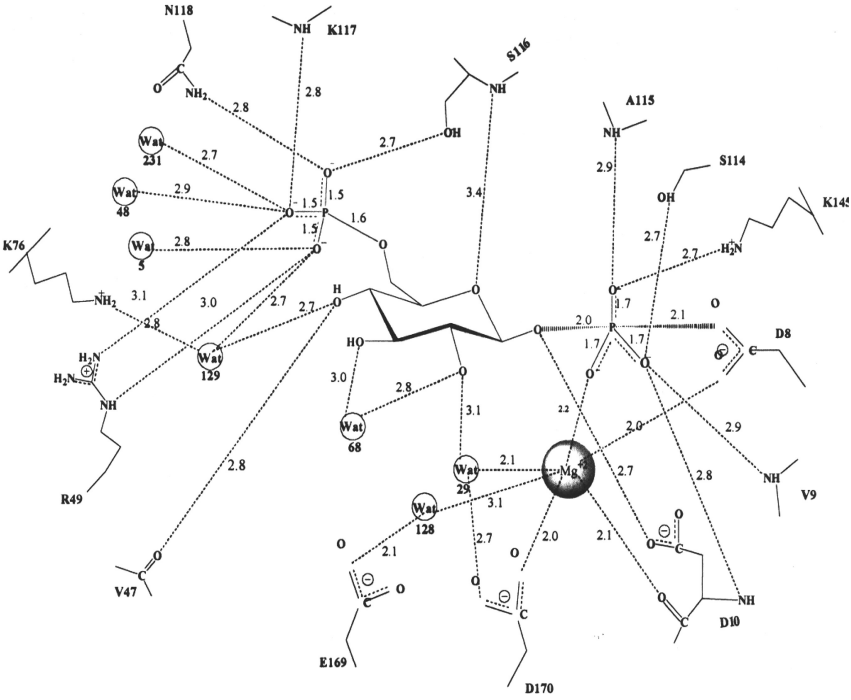


FIGURE 1 Isomerization of β = Glucose 1 = Phosphate to β = Glucose 6 = Phosphate by β = Phosphoglucomutase. Reprinted from reference 9 with permission. Copyright 2003 AAAS.

As an introduction to the area of our study, it is instructive to view the results of a recent X-ray study on a phosphate substrate (Figure 1). It is entitled, "Isomerization of β-Glucose 1-Phosphate to β-Glucose 6-Phosphate by β- Phosphoglucomutase."[9]

In this study, Dunaway-Mariano, Allen and co-workers[9] obtained the first structural evidence of a pentacoordinated phosphorane *intermediate* stabilized by an enzyme catalyzed phosphoryl transfer reaction. A trigonal bipyramidal oxyphosphorane intermediate formed during the phosphoryl group transfer from the C(l) oxygen atom of glucose 1,6-(bis)phosphate to the nucleophilic oxygen atom of Asp8 carboxylate. This particular study did not show any degree of donor action by nearby residues. However, operations of factors 2–4 above are clearly present. Static X-ray studies on enzyme environments do have limitations. The largest is that enzyme reactions are not usually conducted in the solid state. As stated by James and Tawfik, "The crystal structure of a protein might not be the only conformation adopted in solution and, owing to the influence of crystal-packing forces, might not even be the most

representative."[10] We might modify that by saying that other energy interactions are subject to change in solution as well compared to that in the solid state.

In an X-ray study[11] of the phosphonium salts **1A**, **1B**, and **2**, the anionic phosphines **3A** and **3B**, and the anionic phosphine oxide **4** exhibiting donor coordination from a nearby carbonyl group, the influence of crystal packing effects is apparent. For example, the variation in P-O donor distance for **2** extends over a range of 0.163 Å and that for **4** extends over 0.127 Å. This is comparable to the effect of phosphorus anionicity in supporting donor action, cf. the donor bond distances for the donor P-O groups in the neutral carbonyl compounds in the upper part of Chart 2 with the shorter donor bond distances for the anionic counterparts in the lower part of the chart.

Ease of Conversion of Phosphorus to Hexacoordination

The first example of a conversion of three-coordinate to six-coordinate phosphorus on going from the solid to the solution state and the existence of these two disparate geometries in equilibrium with one another in solution was made possible by the presence of a donor nitrogen atom.[12] These two processes are depicted in Schemes 1 and 2, respectively.

In a number of studies, the extent of donor coordination was obtained by comparing P-donor bond distances resulting from X-ray studies performed on 4, 5 and 6 coordinate phosphorus compounds.[2,13,14] The results are plotted in Chart 3 with the use of sulfur as the donor atom. The range of P-S distances extends from 2.8-3.2 **Å** for the lower coordinate phosphites and phosphates while much stronger coordination is apparent for the higher coordinate phosphoranes.

The feature of increased electrophilicity enjoyed by higher valent phosphorus is further manifested in its increased reactivity. For example, as displayed in Chart 4 a displacement reaction conducted with catechol for this series of oxyphosphoranes shows an increase in reaction rate that parallels the increase in displacement from a trigonal bipyramid toward an octahedron. Second-order kinetics are followed implying an associative reaction.[15–17]

Biorelevant Phosphoranes: Synthesis, Structure and Fluxional Behavior

Efforts to obtain solid-state structural information on pentacoordinate phosphorus containing nucleosides have not been successful despite studies[18] that have extended over the past 30 or so years. Most of the studies focused on solution NMR measurements. A phenylalanyl adenylate pentacoordinated phosphorane shown here (Figure 2) was recently

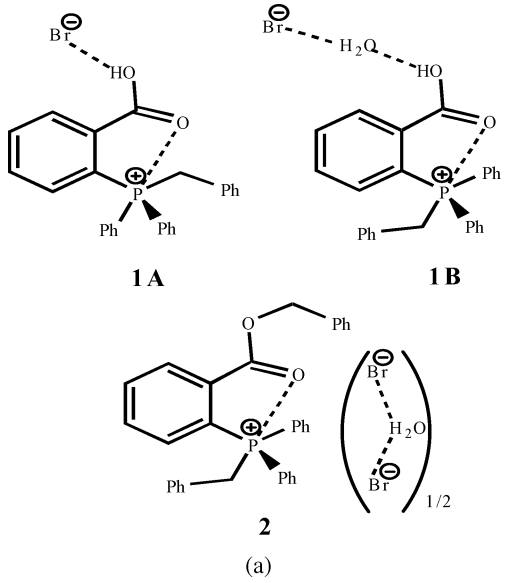


CHART 1a Packing Effects. Three independent molecules are present in each asymmetric unit in the same crystal lattice for **2**. Compound **1** crystallizes in different space groups when obtained from different solvents.

reported from Professor Zhao's laboratory.[19] This unique example contained the nucleoside component appended to one of the phosphorus oxygen atoms.

Subsequently, we reported the synthesis and first crystal structures of biorelevant nucleoside and carbohydrate-based phosphoranes,

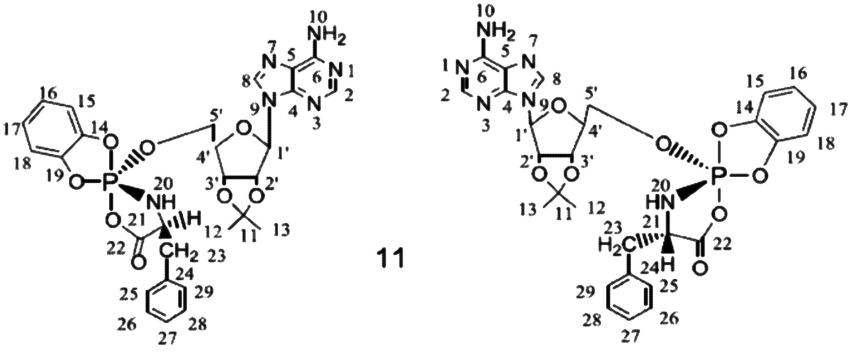
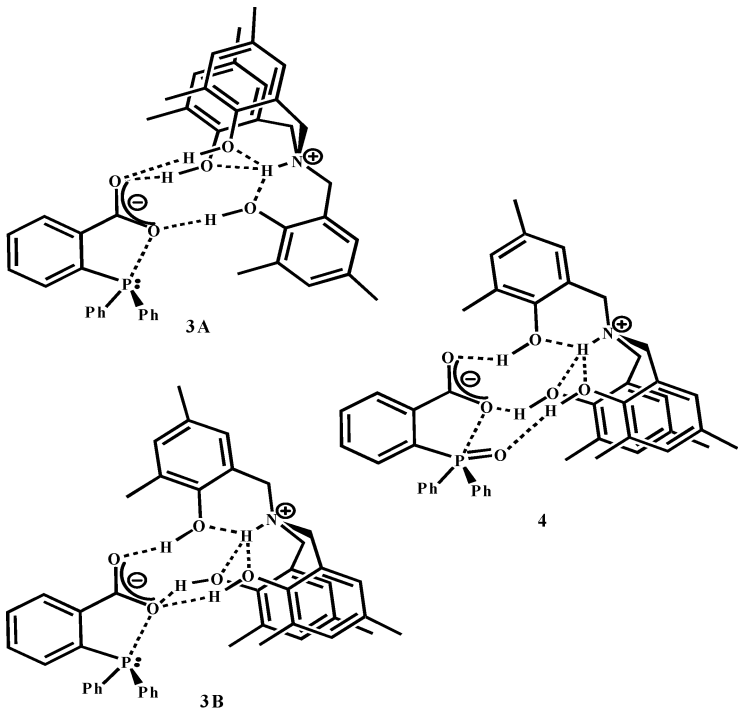


FIGURE 2 Phenylatanyl adenylate penta coordinate phosphorane that contains both an aminoacid and a nucleoside.



(b)

CHART 1b Compounds **3** and **4**. Compound **3** crystallizes in different space groups when obtained from different solvents. The P-O Distances are 2.807(4) Å to 2.970(4) Å.

1 (Scheme 3) and **2 (**Scheme **4)**, respectively.[18] NMR data showed that there is only one isomer in solution which is different from that found in the solid state. The NMR data show fluxionality.

Following a similar synthetic procedure as shown in Schemes 3 and 4, the synthesis of a xylofuranose phosphorane is outlined in Scheme 5.[20] As with several other biorelevant trigonal bipyramidal phosphoranes, NMR data suggest that there is a dynamic equilibrium in solution between two different isomers. The rapid exchange process reorients the carbohydrate component of the trigonal bipyramidal phosphorane. At an active site, this type of pseudorotational behavior provides a mechanism that could bring another active site residue into play and account for a means by which some phosphoryl transfer enzymes express promiscuous behavior.

In the course of synthesis of biorelevant phosphoranes, we discovered, after considerable trial and error, that the order of addition

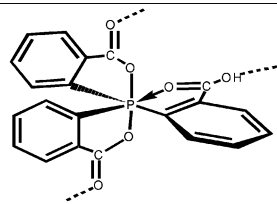
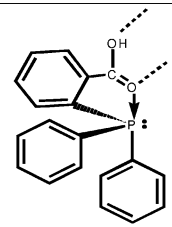
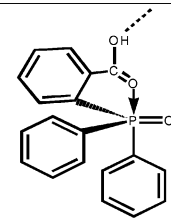
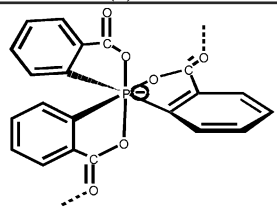
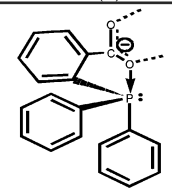
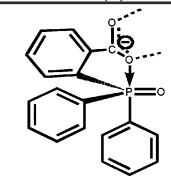
				
^{31}P, ppm P-O, Å	-56.9 2.984(2) Ave.		-4.8 2.806(1)	38.3 3.075(2)
				
^{31}P, ppm P-O, Å	$Et_2NH_2^+$ -135.5 1.901(1)	Et_3NH^+ -124.9 1.898(1)	$Et_2NH_2^+$ -5.7 2.696(2)	$Et_2NH_2^+$ 30.3 2.863(2)
Δ, Å	1.083	1.086	0.110	0.212

CHART 2 Anionic Phosphorane, Phosphine, and Phosphine Oxide Formed from Precursor Carboxylic Acids[7,8]

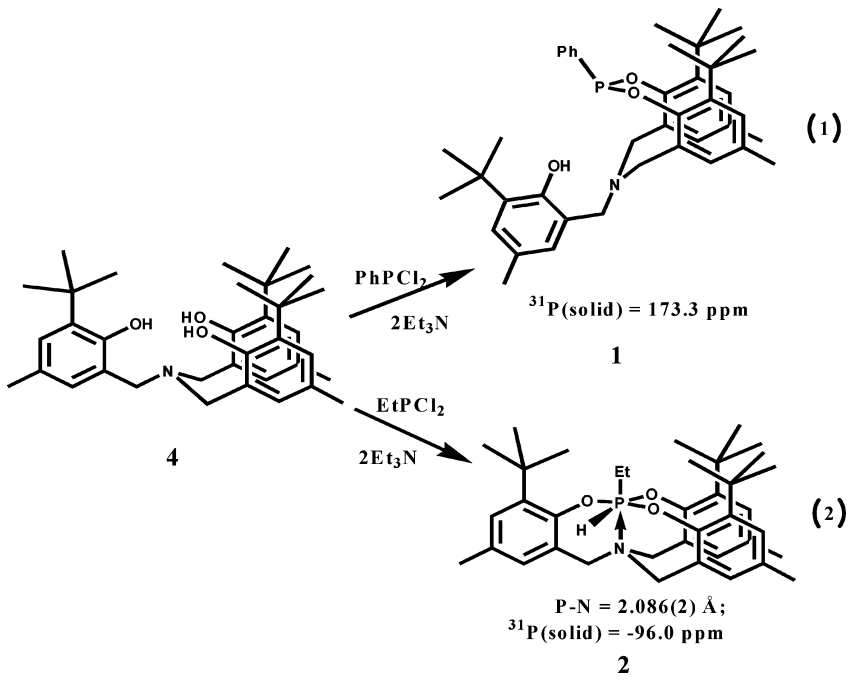
of reagents played an important role, once the problems associated with solubility, stability and crystallizability were well under control. For example, in the second step of Scheme 6, adding amine first gives **13**, whereas first adding N-Chloroamine gives phosphorane **2**.[21] However, this procedure is viable only if there is a donor atom present.

Phosphoryl Transfer Enzymes Active Site Considerations

Enzyme Model for Substrate—Transition State Activity

Synthesis of a Phosphate Substrate and an analogous Transition State model based on the preceding work and do so in a similar encapsulated environment (Scheme 7).[22]

The phosphate-atrane structure **2**, which expresses a slight degree of P-N-coordination is representative of a substrate composition in a phosphoryl transfer enzyme reaction while the first octahedrally coordinated tetraoxyphosphorane-atrane **6** based on our work[22] shows strong P-N coordination that is representative of an activated enzyme complex formed by an attacking nucleophile. The results are used to



SCHEME 1 Formation of a Tricoordinate Phosphonite and Hexacoordinate Phosphorane from an Aminotriphenol.[12]

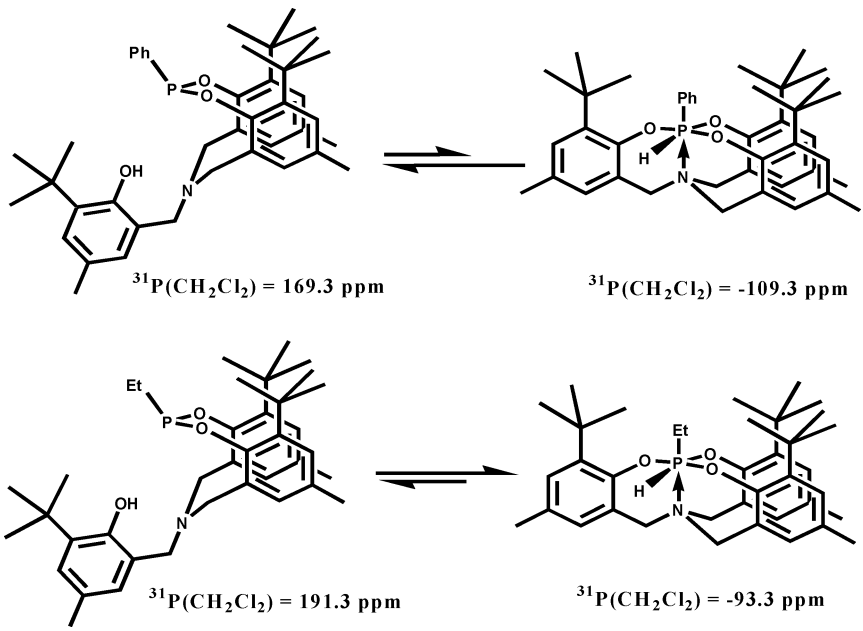
support amino acid donor action occurring at active sites of phosphoryl transfer enzymes. Due to donor coordination caused by an amino acid residue at an enzyme pentcoordinated activated state, a hexacoordinated phosphorus transition state is envisioned. As a consequence, a loosening of all bonds to phosphorus will occur and allow the leaving group to depart more readily and result in an accompanying enzymatic rate increase.

Inversion and Retention

Most biochemical processes involving chiral substrates proceed by inversion of configuration. Otherwise, the reaction involves retention that may be accomplished by multiple inversions. However, the latter is not normally substantiated by experimental procedures.

Psedorotation[3,23]

In the more rare event of a retention process, biochemists are reluctant to propose adjacent attack at the phosphate substrate, as this



SCHEME 2 Equilibria Between Tricoordinate Phosphonites and Hexacoordinate Phosphoranes in Solution.[12]

would involve pseudorotation of the trigonal bipyramidal transition state at the enzyme active site in order to bring the leaving group for departure at a preferred apical site.

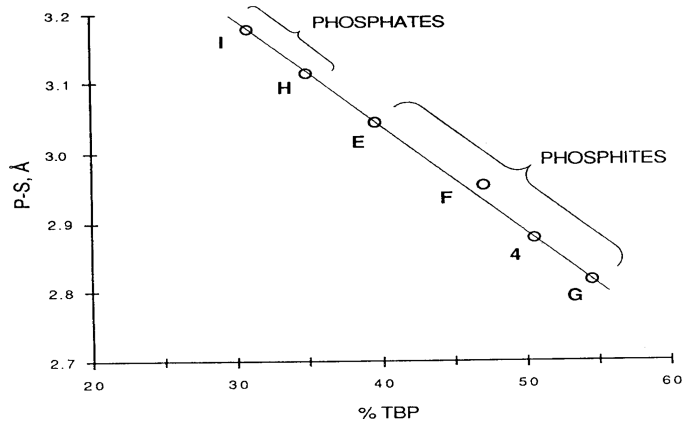


CHART 3 Phosphate and phosphite trigonal bipyramids.

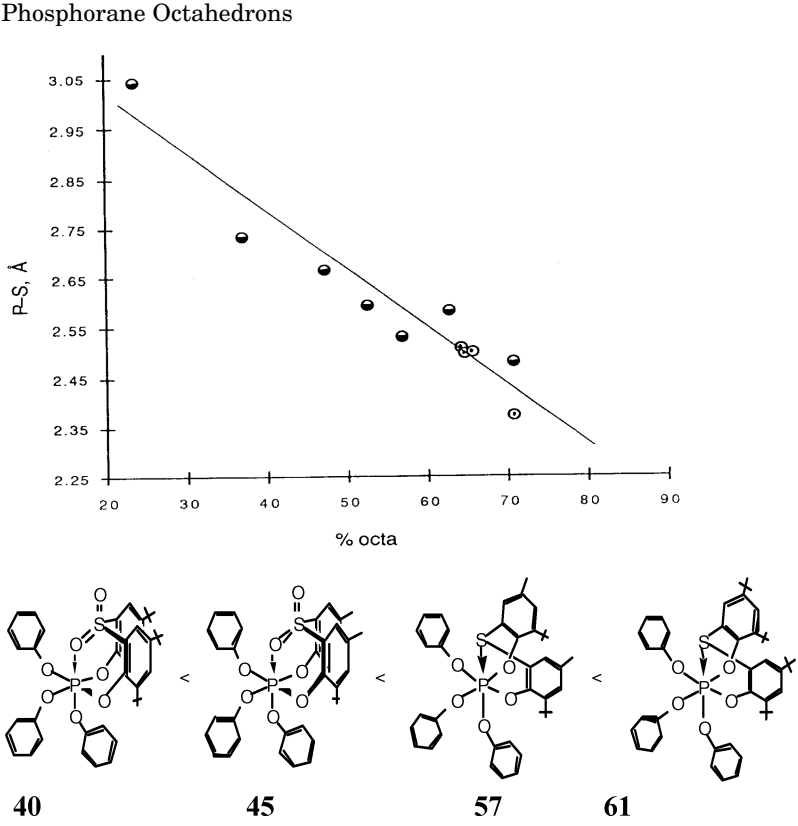


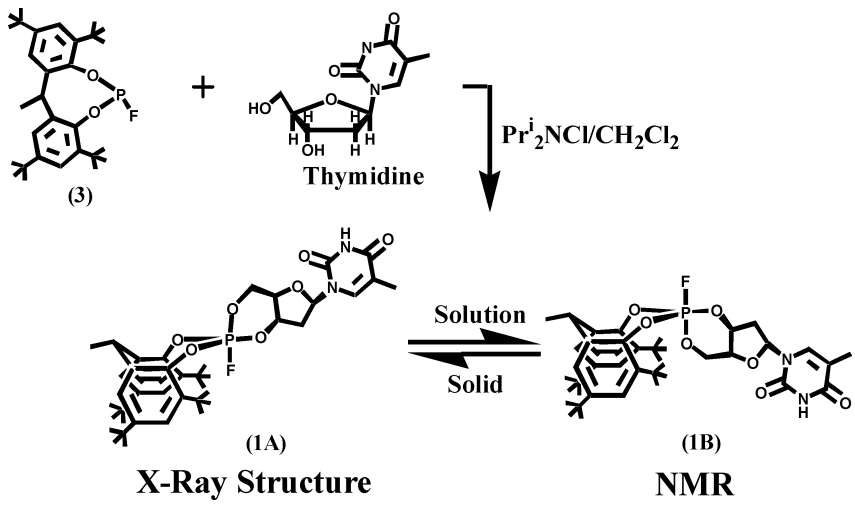
CHART 4 Reactivity of pentaoxyphosphoranes with catechol.

Enzyme Promiscuity[24–27]

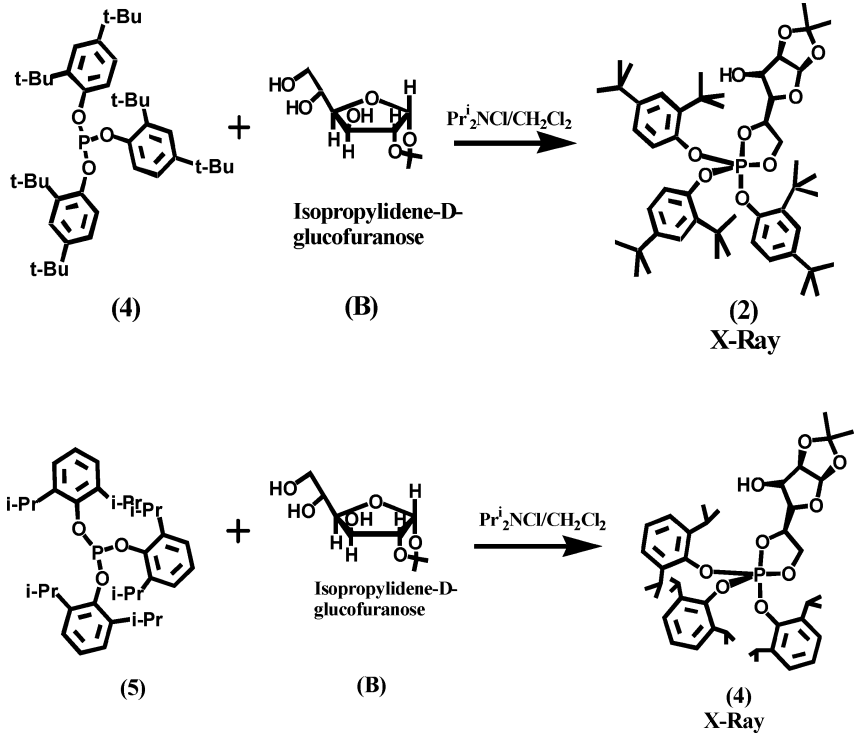
Enzyme promiscuity is defined as the ability of an enzyme to catalyze different reactions at the same active site. A number of phosphoryl transfer enzymes are known to exhibit catalytic promiscuity. Some discussions center on a switch in enzyme activity brought about by a different amino acid residue acting on the substrate at the active site.[24] Chymotrypsin is an example of a promiscuous enzyme. It is able to catalyze both amidase and phophotriesterase reactions (Scheme 8).[24]

Enzyme Diversity Supports a Likely Pseudorotation Process

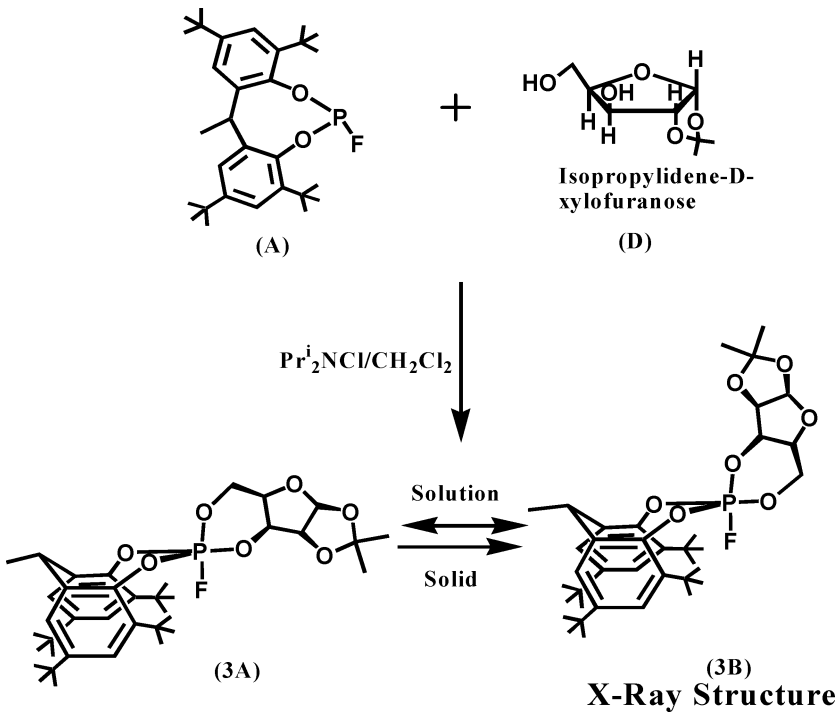
It is not unlikely that pseudorotation of a phosphorus transition state or intermediate formed at a phosphoryl transfer active site takes place.



SCHEME 3 Synthesis of a Thymidine Based Phosphorane.[18]



SCHEME 4 Structures of monocyclic glucofuranose derived phosphoranes.[18,20]

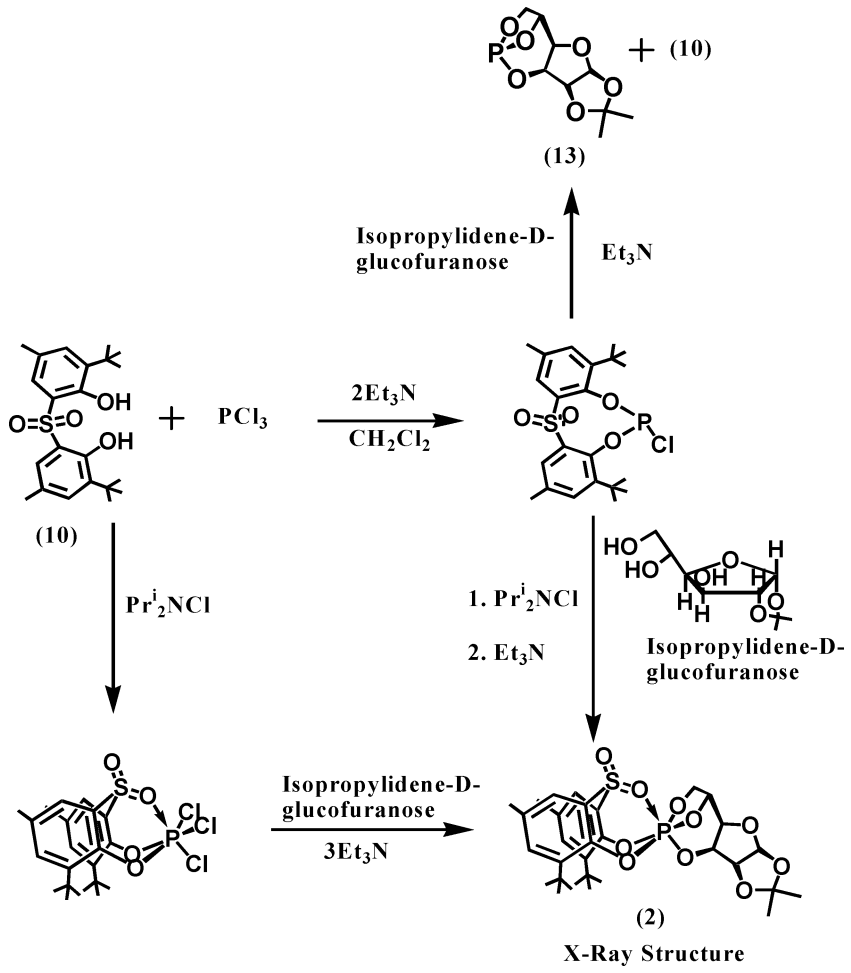


SCHEME 5 Synthesis of a Xylofuranose Based Phosphorane.[20]

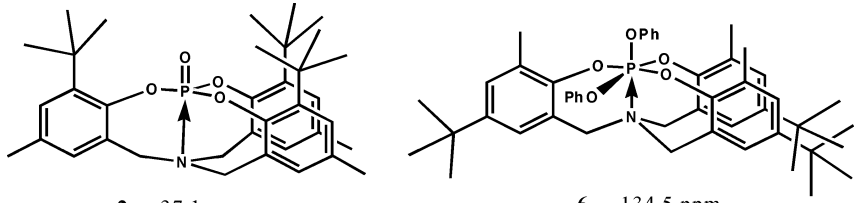
This could facilitate placement of a different nucleophile for attack or a different amino acid residue for interaction in concert with an active site reorientation resulting in a different enzymatic reaction.

CONCLUSIONS

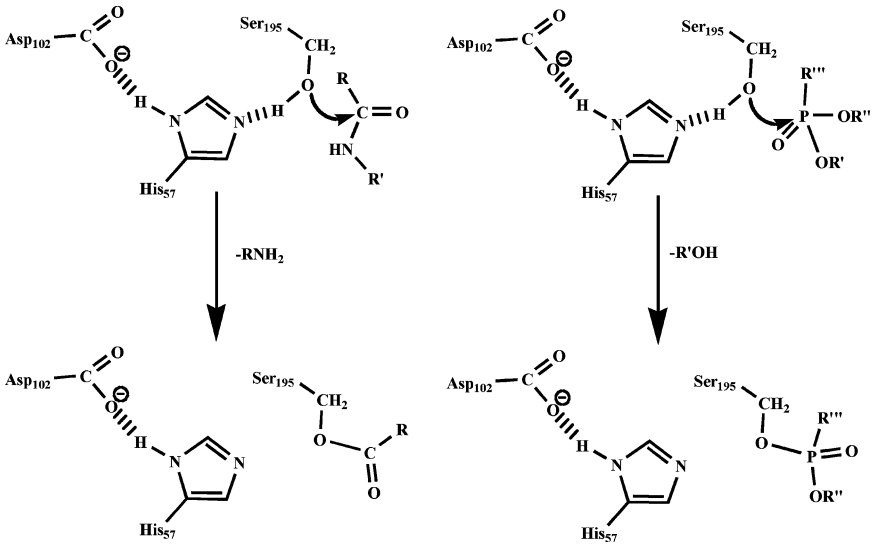
1) Donor coordination to phosphorus by O, N and S groups readily takes place.
2) Donor coordination is stronger in hexacoordinate relative to pentacoordinate phosphorus.
3) Anionicity, hydrogen bonding, packing effects and donor coordination compete with one another.
4) Pseudorotation enters into consideration in promiscuous phosphoryl transfer enzymes that proceed by retention of configuration.
5) Energies associated with the conversion of five to six coordinate phosphorus are found to be small such that the formation of a



SCHEME 6 Strategies in the synthesis of biorelevant phosphoranes.



SCHEME 7 Phosphate Substrate to Activated Enzyme Complex Analogy.[22]



SCHEME 8 Catalytic promiscuity of chymotrypsin.

hexacoordinated activated state in phosphoryl transfer reactions becomes a likely possibility.

6) The hexacoordinated state exhibits greater reactivity than pentacoordinated analogues and exhibits stronger coordination with donor atoms.
7) Nearby residues at active sites of phosphoryl transfer enzymes possessing these donor atoms may participate in altering transition state geometries from pentacoordinate to hexacoordinate as well as those of phosphate substrates from tetracoordinate to pentacoordinate and do so by providing a rate enhancement effect. As a result, a nucleophilic assisted nucleophilic displacement reaction takes place.

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[12] (a) N. V. Timosheva, A. Chandrasekaran, R. O. Day, and R. R. Holmes, *J. Am. Chem. Soc*., **124,** 7035 (2002); (b) A number of earlier studies on spirooxyphosphoranes with various donor bases have shown by ^{31}P NMR that intermolecular P(V)↔P(VI) equilibria are established in solution. For example, A. Schmidpeter,; T. von Criegern, K. Z. Blanck, *Naturforsch*., **31b,** 1058 (1976), and references therein; R. Burgada and R. Setton, *The Chemistry of Organophosphorus Compounds*, F. R. Hartley, Ed. (Wiley, New York, 1994), Vol. 3, Chap. 3 and references therein; (c) Reference 15 represents the first study establishing intramolecular exchange in solution between penta- and hexacoordinate isomers of phosphorus. The results indicate that the thermodynamic stability of these five and six coordinate isomers is approximately the same.
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