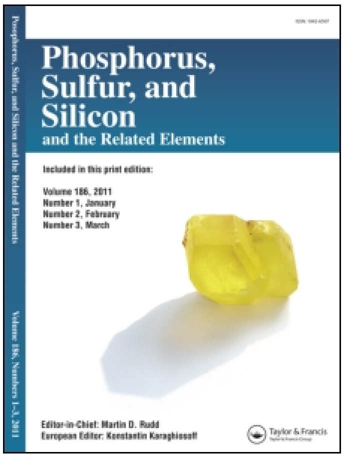

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Phosphoryl Transfer Enzymes and Hypervalent Phosphorus Chemistry: A Keynote Lecture of the 7th International Conference on Heteroatom Chemistry

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Phosphoryl Transfer Enzymes and Hypervalent Phosphorus Chemistry: A Keynote Lecture of the 7th International Conference on Heteroatom Chemistry

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The coordination tendencies of phosphorus to form a hexacoordinated state from a pentacoordinated state which might assist in describing mechanistic action of phosphoryl transfer enzymes are delineated. The factors discussed include substrate and transition or intermediate state anionicity; hydrogen bonding; packing effects, i.e., van der Waals forces; the ease of formation of hexacoordinate phosphorus from lower coordinate states; and the pseudorotation problem common to nonrigid pentacoordinate phosphorus. In view of the work reported in this account 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.

Keywords Donor coordination; hexacoordinate; hypervalent phosphorus; pentacoordinate; phosphoryl transfer enzymes

INTRODUCTION

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

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The reference numbers cited in this article correspond to those listed in R. R. Holmes, *Acc. Chem. Res.*, *37*, 746–753, 2004. Work not cited therein is individually referenced in the text of this article.

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atom.[1–3] 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 pentacoordinate phosphorus species is formulated as a trigonal bipyramid with the entering and departing group doing so at axial sites, that is, an inline process.[1,2,4–12] The coordination tendencies of phosphorus which might assist in describing the mechanistic action of phosphoryl transfer enzymes and cAMP has not been utilized 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.[1,6,13] It is the purpose of this account to delineate the dominant features of hypervalent phosphorus chemistry that may have applicability in formulating active site mechanisms of phosphoryl transfer enzymes and cAMP. 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.[1]

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

A principal tenet that will guide our discussion is the likelihood that active sites of phosphoryl transfer enzymes and cAMP will incorporate donor coordination at the phosphorus center in phosphate substrates and active site complexes. As a corollary, such coordination will distort the phosphate substrate from tetracoordinate toward pentacoordinate and alter the proposed mechanistic activated complex from pentacoordinate toward hexacoordinate. Accompanying these geometrical changes induced by coordination of donor atoms that are prevalent at active sites, consideration will be given to the effects brought about by associated changes in energy and concomitant activation energies for nucleophilic displacement reactions. What will not be treated is a digression about transition states or activated intermediates and whether *bond* making or *bond* breaking is a guiding feature of displacement reactions.[14]

As pointed out in my ACS Monograph,[2] although the term transition state refers to a crest in a potential energy surface and intermediate refers to a trough, many authors use these terms somewhat interchangeably, even though in most cases, the intermediate is not

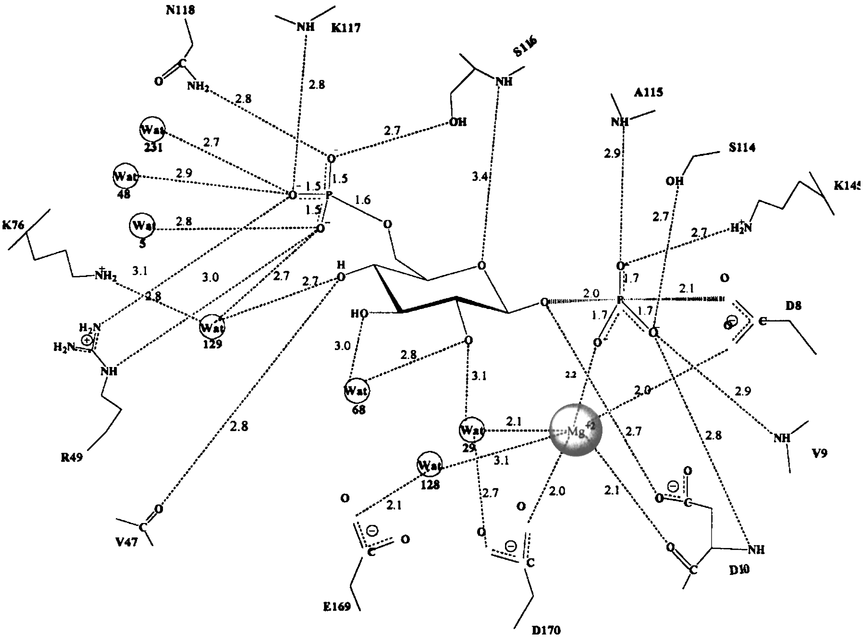


FIGURE 1 Schematic of the active site of β-phosphoglucomutase from *Lactococcus lactis*. Reprinted from reference 15 with permission. Copyright 2003 AAAS.

isolated. In a recent X-ray study, Dunaway-Mariano, Allen and coworkers (Science **2003**, *299*, p. 2067)[15] obtained the first structural evidence of a pentacoordinated phosphorane *intermediate* stabilized by an enzyme-catalyzed phosphoryl transfer reaction, the isomerization of β-glucose 1-phosphate to β-glucose 6-phosphate catalyzed by β-phosphoglucomutase from *Lactococcus lactis*. A trigonal bipyramidal oxyphosphorane intermediate formed during the phosphoryl group transfer from the C(1) oxygen atom of glucose 1,6-(bis)phosphate to the nucleophilic oxygen atom of Asp8 carboxylate. A schematic of the active site geometry is reproduced here (Figure 1).

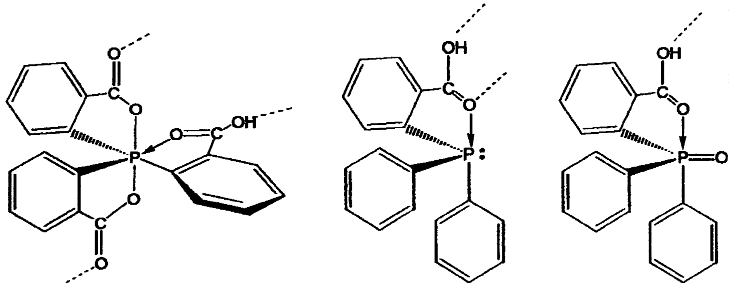
HYPERVALENT PHOSPHORUS-FACTORS TO CONSIDER

- Donor action at phosphorus
- Hydrogen bonding
- Anionicity
- Packing effects (van der Waals)
- Phosphorane reactivity

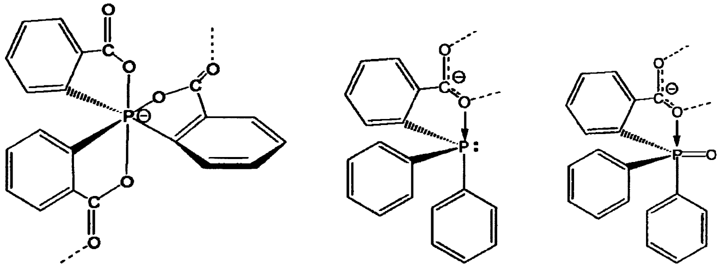
Anionic Phosphorus and Hydrogen Bonding[16,17]

Studies were conducted on a series of phosphorus compounds **I–III** containing carboxyl groups[16] and their anionic counterparts **IV–VI**[17] obtained by treatment of the precursor acid forms with amines which also served to introduce hydrogen bonding interactions. The structures, a hexacoordinated anionic phoshoranate **IVA** and **IVB**, a trigonal bipyramidal anionic phosphine **V**, and a trigonal bipyramidal phosphine oxide **VI**, revealed the presence of P–O donor coordination, which was stronger for all members than that which existed in the precursor acid forms **I** to **III**. The extent of the P–O bond distance shortening is expressed by the Δ values listed at the bottom of Chart 1.

Chart I Neutral phosphorus compounds (I–III) and their Amionic counterparts (IV–VI)



^{31}P, ppm	−56.9		−4.8	38.3
P—O, Å	2.984(2) Ave.		2.806(1)	3.075(2)
	I	**II**	**III**	



	$Et_2NH_2^+$	Et_3NH^+	$Et_2NH_2^+$	$Et_2NH_2^+$
^{31}P, ppm	−135.5	−124.9	−5.7	30.3
P—O, Å	1.901(1)	1.898(1)	2.696(2)	2.863(2)
	IVA	**IVB**	**V**	**VI**
Δ,Å	1.083	1.086	0.110	0.212

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Packing Effects (van der Waals Forces)

The study leads to the conclusion that van der Waals forces that are discussed in terms of conformational distortions in enzyme chemistry,[43] termed lattice or packing effects here, are important in controlling P—O donor interactions. The variation in P—O donor distance influenced by packing effects is found to extend over a range of nearly 0.2 Å. This is comparable to the effect of phosphorus anionicity in supporting donor action.

X-Ray vs. Solution Structures

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."[49]

Reactivity of Pentaoxyphosphoranes with Catechol[22,28]

A displacement reaction conducted with catechol for the above series of oxyphosphoranes shows an increase in reaction rate that parallels the increase in displacement from a trigonal bipyramid toward an octahedron (Figure 2). Second-order kinetics are followed implying an associative reaction.[22,40] One may conclude that there will be a tendency toward hexacoordinate formation for a proposed trigonal bipyramid engaged at an enzyme active site in donor action with a nearby residue.

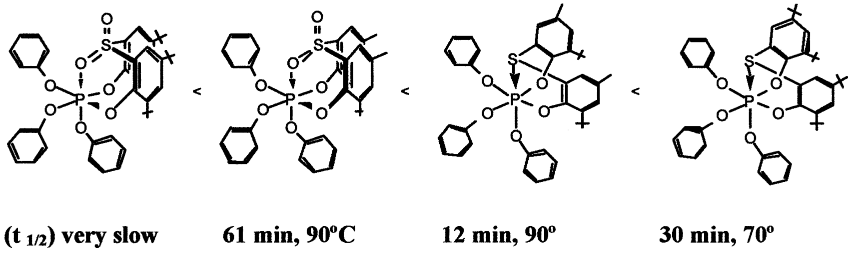
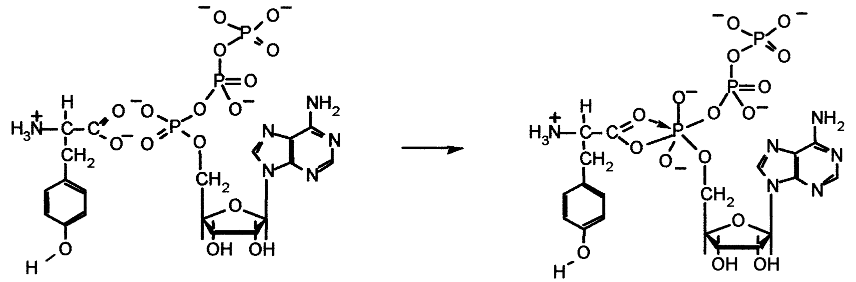


FIGURE 2 An increasing order of reactivity of a series of oxyphosphoranes with catechol on going from left to right that parallels an increase in displacement toward an octahedron from a trigonal bipyramid.[40]

GROUND-STATE COMPLEX AND PROPOSED TRANSITION-STATE COMPLEX IN THE ACTIVATION OF TYROSINE BY THE TYROSYL-tRNA SYNTHETASE

In the tyrosyl-tRNA synthetase enzyme operating on ATP, it has been proposed that the carboxylate oxygen atom of tyrosine binds to phosphorus in the transition state giving a pentacoordinate phosphorus atom (Figure 3). Consistent with our work is the likelihood that the carbonyl oxygen atom of the tyrosyl carboxylate groups also interacts with phosphorus.[1,24] This would result in a nucleophilic assisted nucleophilic-attack in cleaving ATP in an in-line mechanism.



E. Tyr. ATP Ground State[a] **E[Tyr-ATP] Transition State**[b]

[a] Fersht, AR; Knill-Jones, IW.; Bedouelle, H.; Winter, G. *Biochemistry*, **1988**, *27*, 1581.
[b] Timosheva, N.V; Chandrasekaran, A; Day, RO.; Holmes, RR *Inorg. Chem.*, **1998**, *37*, 3862–3867.

FIGURE 3 Ground state complex and proposed transition-state complex in the activation of the tyrosyl-tRNA synthetase.

PHOSPHORYL TRANSFER ENZYMES

Active Site Considerations

1. Inversion and retention
2. Pseudorotation
3. Enzyme promiscuity
4. Moonlighting enzymes
5. Substrate-transition state activity

Inversion Process

If an enzymatic-chiral reaction is involved, most of these reactions occur with inversion of configuration.

Adjacent Attack. In the more rare event of a retention process, biochemists are reluctant to propose an adjacent attack at the phosphate substrate, as this 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.[2,44]

Pseudorotation

Retention Process. In apical attack by the nucleophile relative to equatorial location of the leaving group, pseudorotation must occur to bring the leaving group to the preferred apical site for departure (Figure 4).

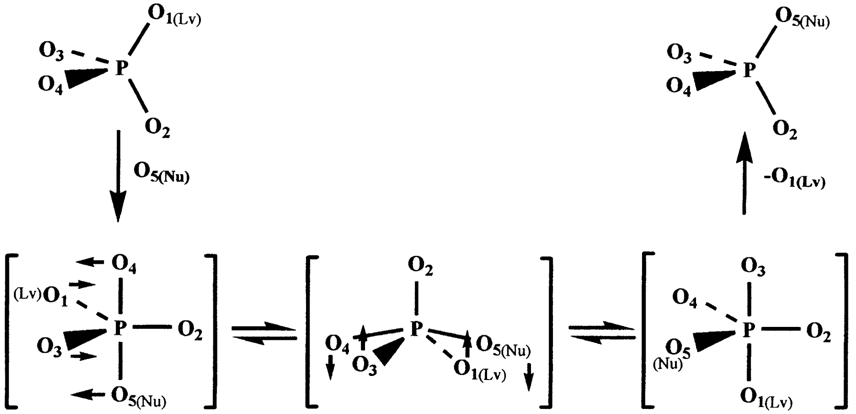


FIGURE 4 Pseudorotational process (simple Berry process) for intramolecular exchange leading to a retention mechanism in PO_4^- derivatives undergoing nucleophilic attack by OH^-. Bracketed isomers represent activated states.[45] Structural displacements of pentacoordiante main group elements including phosphoranes, closely follow the coordinate connecting a trigonal bipyramid with a square pyramid and show no evidence for the trajectory followed by the turnstile coordinate: Holmes, R. R.; Deiters, J. A. *J. Am. Chem. Soc.* **1977** *99*, 3318–3326; Holmes. R. R. "Five-Coordinated Structures," in *Progress in Inorganic Chemistry*, Vol. 32, Lippard, S. J Ed., John Wiley and Sons, New York, 1984, pp. 119–235; Holmes, R. R. *Acc. Chem. Res.* **1979**, *12*, 257–265; Holmes, R. R. "Pentacoordinated Phosphorus—Reaction Mechanisms," Volume II, ACS Monograph 176, American Chemical Society, Washington, D.C., 1980, 237 pp. Reprinted with permission from *Acc. Chem. Res. 37(10)*, 746–753, **2004**. Copyright 2004 American Chemical Society.

Enzyme Promiscuity[5,3,56]

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. An example of a promiscuous enzyme is found in the active site of chymotrypsin. It is able to catalyze both amidase and phophotriesterase reactions (Figure 5).[53]

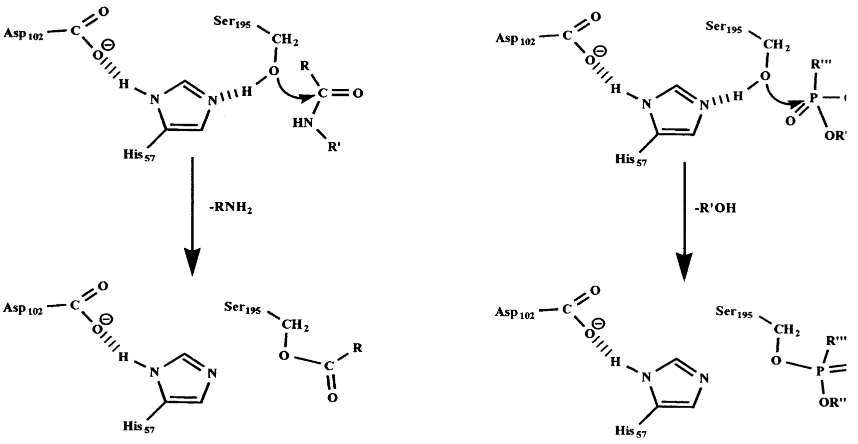


FIGURE 5 Chymotrypsin acts as a promiscuous enzyme in catalyzing the hydrolysis of both amidase substrates (left) and phosphotriesterase substrates (right).[53]

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. 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.

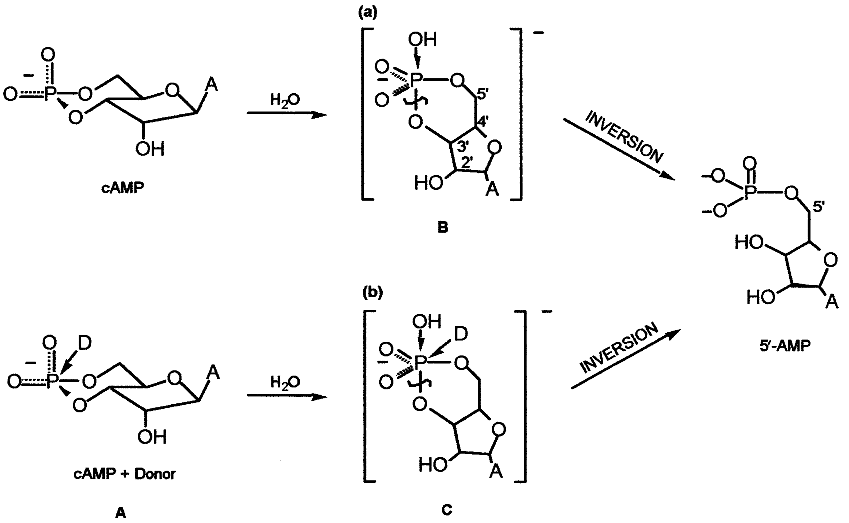
Enzymatic Hydrolysis of cAMP

(a) Formation of 5′-AMP with inversion favors in-line attack opposite the 3′-oxygen atom with phosphodiesterases.[1]

(b) Formation of the hexacoordinate activated state **c** is possible via donor coordination as illustrated in Scheme 1.

Moonlighting Enzymes[58–60]

Moonlighting enzymes support other structures and functions in addition to their original catalytic activity, some of which may involve more than one active site of the protein.



SCHEME 1

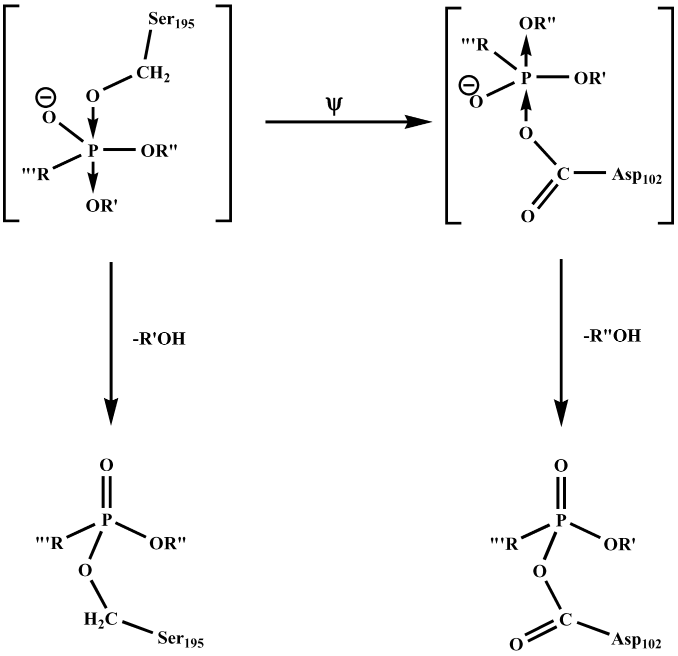


FIGURE 6 Pseudorotation (Ψ) causing attack by a different aminoacid residue. Timosheva, N.V.; Chandrasekaran, A.; Holmes, R.R., *Z. Anorg. Allg. Chem.*, **2005** in press.

An Enzymatic Rate Increase Would Parallel Increasing Hexacoordination. Due to donor coordination caused by an amino acid residue at an enzyme-pentcoordinated-activated state leading to hexacoordinated phosphorus, a loosening of all bonds to phosphorus will occur and allow the leaving group to depart more readily.

Enzyme Model for Substrate-Transition State Activity

Synthesis of a phosphate substrate and an analogous transition state model based on the preceding work was accomplished and done so in a similar encapsulated environment. The transition state model was synthesized similarly to the phosphate synthesis shown in Figure 7.

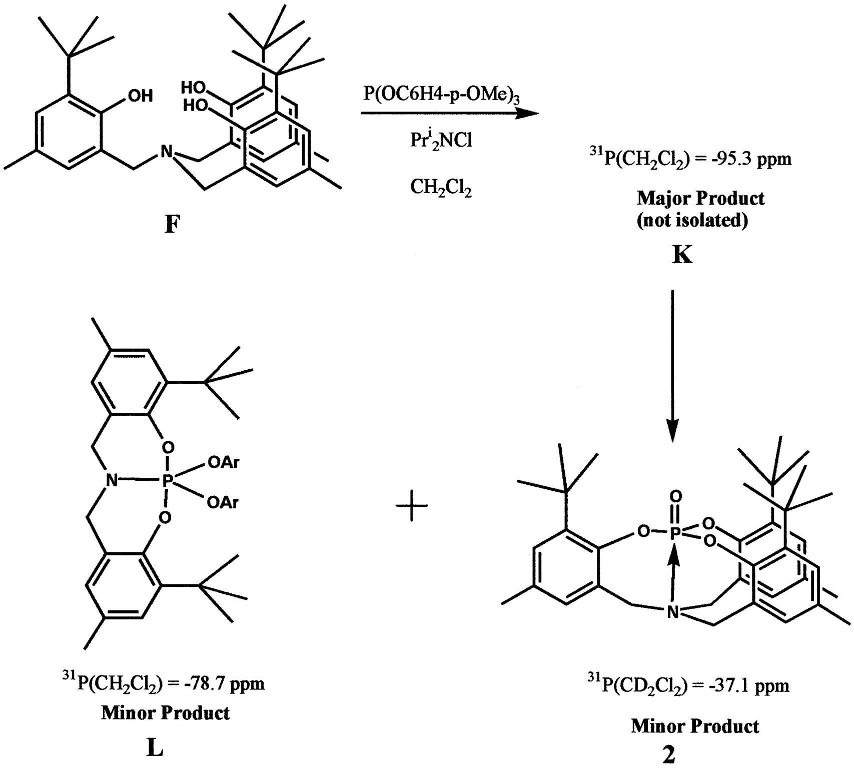


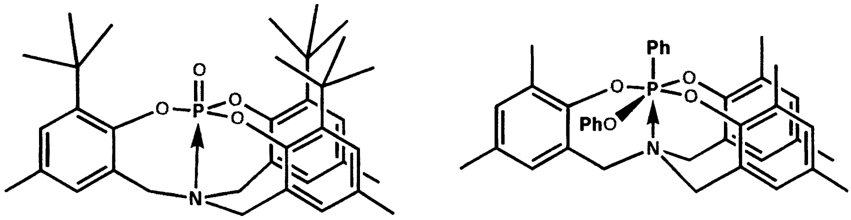
FIGURE 7

Formation of the Phosphate-Atrane

Timosheva, N. V.; Chandrasekaran, A; Holmes, R. R. Inorg. Chem. 2004, *43*, 7403-7411.

Phosphate Substrate to Activated Enzyme-Complex Analogy

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 **5** shows strong P-N coordination that is representative of an activated enzyme complex formed by an attacking nucleophile based on our work (Figure 8).



2 -37.1 ppm
P-N = 2.980 (1) Å

5 -122.2 ppm
P-N = 2.161(2) Å

FIGURE 8 Schematic diagram of phosphate **2** showing weak P—N coordination compared to oxyphosphorane-atrane **5** showing strong P—N donor coordination.

SUMMARY

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 (van der Waals terms), 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 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. A phosphoryl transfer rate-enhancement effect is envisioned by a nucleophilic-assisted nucleophilic attack.

CONCLUSIONS

The prevalence of S, O, and N donor atoms to cause an increase in coordination geometry at phosphorus centers extending from phosphines to phosphates to phosphoranes suggests that this may be an important consideration in detailing mechanisms of nucleophilic displacement reactions. In particular, 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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