Phosphonates as Mimics of Phosphate Biomolecules: Ab Initio Calculations on Tetrahedral Ground States and Pentacoordinate Intermediates for Phosphoryl Transfer

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The use of phosphonates as analogs of phosphate biomolecules was explored using ab initio SCF calculations at the 3-21G(*) and 3-21+G(*) levels. Fully optimized geometries were obtained for the tetrahedral ground-state monoanions $CHF_2PO_3H^-$, $CH_2FPO_3H^-$, $CH_3PO_3H^-$, $BH_3PO_3H_2^-$, $H_2PO_3^-$, and $O^-{}_2\overline{PXCH_2CH_2O}$ (X = O, CFH) and torsional energy profiles obtained for $CH_2FPO_3H^-$ and $H_2PO_3^-$. Comparison was made of (1) structure and conformational dependence for these species and (2) electrostatic potential maps for ethylene phosphate and its monofluoromethylene phosphonate analog. The results suggest that, despite the isopolar relationship of fluoromethyl phosphonates and the parent phosphates, binding at a receptor site may be considerably perturbed for the phosphonate analogs. Fully optimized geometries were located for isomers of the pentacoordinate trigonal bipyramidal species PH_4X (X = CH_3, CF_3, CF_2H, CFH_2, BH_3^-, BF_3^-, O^-, OH) and $H_3\overline{PXCH_2CH_2O}$ (X = O, CH_2, CFH, CF_2). Torsional energy profiles were explored for PH_4X (X = CH_3, CF_3, CF_2H, CFH_2). The calculated relative apicophilicity scale in PH_4X ($CF_3 > CF_2H > CFH_2 > CH_3 > OH > O^- \gg BF_3^- > BH_3^-$) varies in the five-membered cyclic phosphoranes by reversal of CH_3 and OH. It is concluded that mono- and difluoromethylene phosphonates have similar ligand preferences to the parent phosphates in the pentacoordinate state and by consideration of the fully optimized geometry of the pentacoordinate dianion $HO_3{}^{2-}\overline{PCFHCH_2CH_2O}$ that these phosphonates are capable of forming transition-state analogs at the active site of phosphoryl transfer enzymes.

The concept of bioisosterism is of great importance in medicinal and biological chemistry.[1] This is particularly so in the field of phosphate chemistry, owing to the great importance of phosphate-containing molecules in biological processes.[2] The driving force for development of analogs of the phosphate moiety is often the unwanted lability of the ester P–O bond. The search for phosphate analogs that retain biological activity but possess diminished lability has included the use of phosphorothioates, which in some cases have demonstrated spectacular success,[3] relying on the poor turnover of phosphorothioates by many phosphoryl-transfer enzymes.[4]

Other traditional approaches have centered on the substitution of the labile phosphate ester oxygen by carbon or nitrogen to give phosphonates and phosphoramidate analogs, respectively.[5] The P–C bond is generally stable to hydrolytic cleavage except when the α-C is perhalogenated[6] or bears a carbonyl oxygen.[7] Nevertheless, the use of phosphonate analogs has been critized by Blackburn, on the evidence of pK_a values and spectroscopic parameters which demonstrate a gross alteration in the characteristics of phosphonates versus the parent phosphates.[8] Indeed, the alteration in ionization constants can be expected to perturb binding of phosphonate analogs at protein receptor sites. The suggested advantages in the use of (fluoromethylene)phosphonates as phosphate mimics,[8,9] although intellectually persuasive, have as yet not been clearly demonstrated by experiment. The further practical problem of membrane permeability of charged phosphates and their analogs has been confronted with the use of methyl and (difluoromethyl)phosphonates[10] and more recently boranylphosphonates.[11]

The rationale for the use of phosphonates as analogs of phosphate biomolecules lies in the perceived isosteric relationship of the phosphate and its mimic. Further, α-halo phosphonates are argued to be isosteric, isopolar analogs of the parent phosphates,[8] and boranylphosphonates are proposed as "structurally similar" isoelectronic analogs of phosphate diesters.[11] The term bioisostere is not rigorous. Bioisosteres are defined as groups or molecules which have chemical and physical similarities producing broadly similar biological properties.[12] Biological systems may be forgiving of gross changes in

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structure: clearly $CH_3P{=}O$ is not isosteric nor isopolar with PO_2^- and yet methyl phosphonate oligonucleotides have been reported showing biological activity presumably through mimicry of the natural nucleotide.[10a] Conversely, the small change in the use of mono and difluoro derivatives of phosphonoacetic acid successively decreases biological activity against HSV-2.[9b]

In this paper we employ ab initio calculations at the 3-21+G(*) and 3-21G(*) level to explore and compare conformational preferences and conformational energies for phosphate and its analogs, both in the tetracoordinate ground state and in the pentacoordinate state corresponding to the putative intermediates for phosphoryl transfer.[13] The influence of inclusion of phosphorus in a five-membered ring is studied along with perturbation of electrostatic interactions in phosphonate analogs. These calculations allow comparison with experiment and provide predictions on the use of phosphate analogs in particular as probes of enzymic phosphoryl transfer processes.

Methods

Standard SCF Hartree–Fock calculations were performed with the HONDO-8 program as contained in the MOTECC 90 package.[14] All equilibrium structures were fully geometry optimized with no symmetry or additional constraints, except where indicated in the text. In general, the standard 3-21+G(*) basis set was used for anionic species and 3-21G(*) for neutrals.[15] Torsional scans were performed either (1) by point calculations on fixed geometries with resulting minima tested by full geometry optimization or (2) by fixing the torsional angle with full optimization of all other parameters. No symmetry constraints were employed in torsional scans.

Results

Choice of Basis Set. It is generally understood that the use of basis sets supplemented with d-functions is essential for faithful representation of geometrical parameters for compounds containing second-row atoms.[16] The 3-21G(*) basis set recently has been used for phosphoranes[18] and 3-21+G(*) for phosphates[19] and oxyphosphoranes.[20] In cases where comparisons can be made,

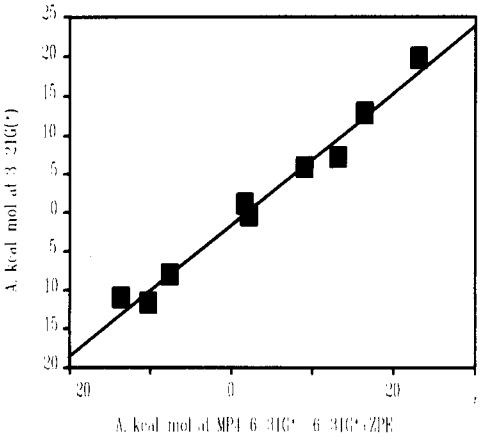

Figure 1. Correlation of apicophilicity, as defined in text for PH_4X TBP species at HF/3-21G(*)//6-31G* and MP4/6-31G*+ZPE//6-31G*; data drawn from ref 16b.

3-21G(*) qualitatively reproduces trends displayed with higher level basis sets (Figure 1).[16b,17] Despite correlations such as in Figure 1, it has been stated that it is necessary to use a basis set "at least as large" as 6-31G* for phosphoranes,[21] on the basis of Magnusson's work.[16a] However, Magnusson's work treats P(III) compounds and simply concludes that STO-3G* is unacceptable, d-functions are essential, and 6-31G* is preferable.[16a] Indeed, it is implied in this work that even for nonanionic species additional, diffuse functions, such as those contained in the 3-21+G(*) basis set, are required despite the cost in computing time. It can clearly be seen from the limited comparisons in this paper (see Table I) that diffuse functions are important for anions. Thus, 3-21+G(*) provides an acceptable basis set, which allows calculations on larger anionic molecules such as oxyphosphoranes which are of relevance to biological processes, in particular when *trends* in molecular properties are being computed.

Apicophilicity. Considerable experimental evidence suggests that nucleophilic substitution at phosphorus may proceed via a trigonal bipyramidal (TBP) pentacoordinate intermediate in which incoming and leaving groups occupy the apical positions.[13,22] Isomerization of the TBP is termed pseudorotation and allows ligand reorganization.[13] The preference of a ligand for an apical position in a TBP is termed its apicophilicity. The concept was pioneered by Muetterties et al.,[23a] quantified tentatively by Trippet as "relative apicophilicity",[23b] and placed on firmer footing by Holmes.[23c] Electronegativity, π-bonding, and steric and ring strain effects all contribute to apicophilicity. Both Trippett's and Holmes's empirical models define ligand apicophilicity as the difference in energy between two TBP pseudorotamers in which the ligand is apical in the first and equatorial in the second ($A = E_I - E_{II}$). The values of apicophilicity, derived from observations on stable phosphoranes, and discrepancies with observations on reactive intermediates, have recently been thoroughly discussed with reference to apical potentiality[24] and equatophilicity.[25]

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(17) It is of course desirable to carry out calculations with a further extended basis set and with electron correlation. However, this remains computationally nonviable for large systems. It has been pointed out by a reviewer that the correlation in Figure 1 may be a fortuitous cancellation of errors. Nevertheless, the correlation exists and given the similarity with the species and properties under study in this work and in ref 16b (and the requirement for the addition of diffuse functions for the anions in our study) one is more comfortable with the veracity of results obtained at the 3-21G(*) and 3-21+G(*) levels.
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Table I. Equilibrium Energies and Geometrical Parameters (Bond Lengths d(A-B), Å) for TBP Phosphoranes, Fully Geometry Optimized Using the Stated Basis Set. See Figure 3 for Torsional Angle Definitions

compd PH_4X	basis set	$A = E(ap) - E(eq)$[a]	d(P-X_{ap})	d(P-X_{eq})	d(P-H_{ap}) ap isomer	d(P-H_{eq}) ap isomer	d(P-H_{ap}) eq isomer	d(P-H_{eq}) eq isomer	τ(FCPHap)eq TBP (deg)
X = CF_3	3-21G(*)	-11.51	1.901	1.870	1.444	1.397	1.445	1.400	0
						1.398		1.401	
CF_2H	3-21G(*)	-8.20	1.901	1.876	1.449	1.397	1.450	1.402	28
						1.404	1.451	1.403	
	3-21G(*)	-6.86		1.866			1.443	1.391	87
							1.459	1.413	
CFH_2	3-21G(*)	-4.86	1.901	1.852	1.457	1.400	1.464	1.401	89
						1.411	1.466	1.402	
	3-21G(*)	-1.72		1.851			1.448	1.402	0
							1.475		
X = CH_3	3-21G(*)	1.71	1.904	1.842	1.467	1.406	1.467	1.407	0[e]
							1.471	1.408	
X = OH	3-21G(*)	2.42	1.678	1.634	1.454	1.397	1.422	1.401	
						1.385	1.452		
	3-21+G(*)	-4.5	1.734	1.668	1.448	1.391	1.415	1.398	
						1.381	1.440	1.407	
+ H_2O	3-21G(*)	-1.5							
X = BH^-_3	3-21G(*)	26.56	1.987	2.002	1.566	1.441	1.525	1.415	
						1.443		1.420	
	3-21+G(*)[b]	28.62	1.844	1.997	3.85		1.534	1.410	
								1.411	
BF^-_3	3-21G(*)	24.90	1.963	2.008	1.526	1.443	1.491	1.423	
						1.444		1.424	
X = O^-	3-21G(*)	14.57	1.508	1.509	1.743	1.407	1.501	1.434	
						1.408			
	3-21+G(*)[c]	7.53	1.554	1.541	1.647	1.405	1.486	1.430	
						1.406			
X = OH_2^+	3-21G(*)[d]	0.01	2.47	2.46	1.381	1.371	1.382	1.371	
NH_3^+	3-21G(*)[d]	0.02	2.47	2.52	1.387	1.371	1.387	1.371	

[a] E(apical isomer) - E(equatorial isomer), kcal/mol. [b] Apical isomer dissociates to $H_2 + H_2P = BH_2$. [c] Apical isomer dissociates to $PH_3O + H^-$. [d] All TBP isomers dissociate to $PH_4\cdots Z^+$. [e] Torsion angle refers to τ(H-C-P-H) for equatorial isomer.

Ab initio calculations have been used to derive values for apicophilicity (A) and the pseudorotational barrier ($\Delta E_{PR}^{\ddagger}$).[16b,21] $\Delta E_{PR}^{\ddagger}$ may be obtained by varying the equatorial or apical XPY angle (a) rigorously by transition-state location or (b) by imposition of C_{4v} symmetry to locate the square pyramidal (SP) pseudorotational transition state. In this way, Schleyer and Streitwieser demonstrated that $\Delta E_{PR}^{\ddagger}$ (here $\Delta E_{PR}^{\ddagger} = E(SP) - E(TBP,$ ligand apical)) correlates linearly with electronegativity, but that apicophilicity (A) shows considerable deviation for first-row π-donor and acceptor ligands.[16b] The system under examination, PH_4X, precluded significant steric effects.

Full geometry optimization of PH_4X, where X = CH_3, CF_3, CF_2H, CFH_2, BH_3^-, BF_3^-, O^-, OH, OH_2^+, NH_3^+, was performed yielding equilibrium geometries and energies (Table I). At the 3-21G(*) level these values may be appended to those of Schleyer and Streitwieser[16b] to yield an extended correlation of apicophilicity with electronegativity (Figure 2). Not unexpectedly, the increased effective electronegativity of carbon substituted with fluorine atoms leads to increased apicophilicity for these ligands. Rotation about the equatorial P-C bond of PH_4X (X = CH_3, CF_3, CF_2H, CFH_2) yielded further energy minima (fully geometry optimized) resulting in apicophilicity ranges of 3.14 and 1.34 kcal/mol for X = CFH_2 and CF_2H respectively (Table I, Figures 3 and 4).

The minimum energy structures obtained for hydronium- and ammonium-substituted phosphoranes do not represent TBP structures, but are instead partially dissociated encounter complexes of $Z^+ + PH_4^-$ with bond lengths of 2.46-2.47Å and 2.47-2.52Å for $Z^+ = OH_2^+$ and NH_3^+, respectively.

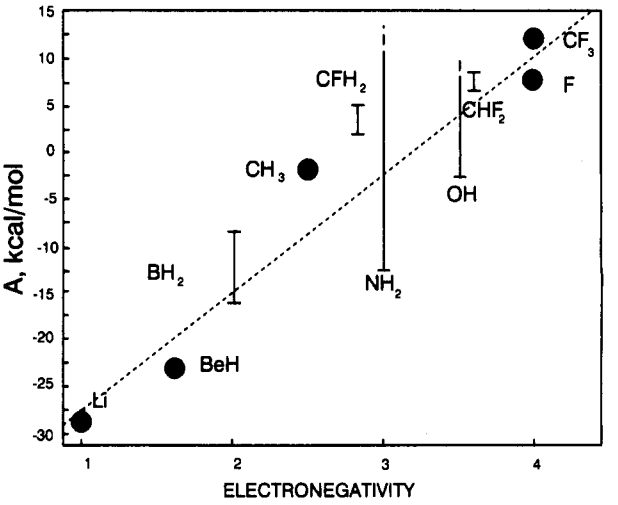


Figure 2. Correlation of apicophilicity and electronegativity; data drawn from Table I or ref 16b. Bars represent range of values resulting from torsional dependence of energy for equatorial TBP isomers, dashed lines show uncertain values owing to incomplete geometry optimization.

It can be seen from comparison of Table I and Figure 2 that the effective electronegativity of BH_3^- and BF_3^- is similar to the electronegativity of the highly electropositive ligands Li ($X = 1$) and BeH ($X = 1.5$), respectively. These ligands show the greatest preference for the equatorial position demonstrated in any study to date. A better comparison may be made with PH_4O^-. The O^- ligand, although preferring the equatorial position, has a weaker preference than BH_3^-. Streitwieser has shown PH_4O^- to be unstable in calculations on the O^- apical TBP isomer:

(25) The term *equatophilicity* was introduced to describe the preference of ligands for the equatorial position, referring in particular to methylene and π-donor ligands such as NR_2.[13] A similar term, *equatoriphilicity*, has recently been coined.[21]

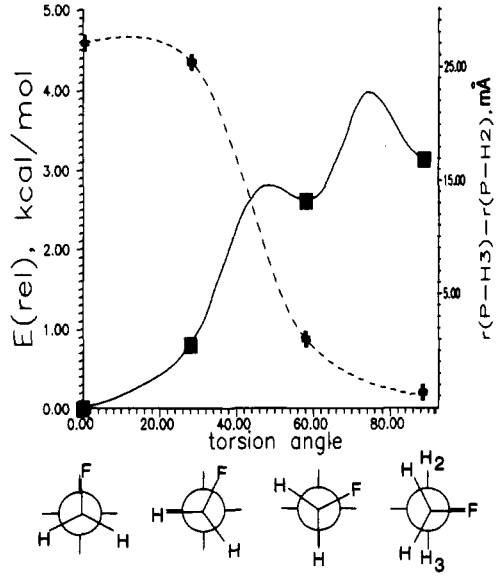


Figure 3. Plot of relative energy (■) and change in P–H(apical) bond length (+) as a function of torsion angle about P–C bond for equatorial TBP isomers of PH_4CFH_2. All energy minima shown were fully geometry optimized without constraints. The point at torsion angle = 30° was obtained by optimization of all parameters except the fixed torsion angle.

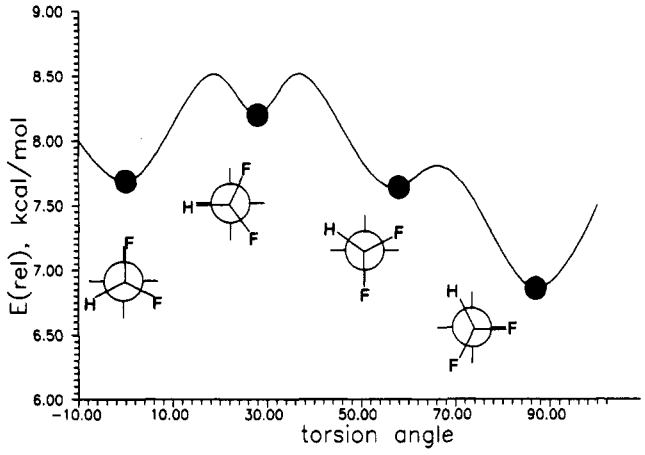


Figure 4. Plot of energy (relative to apical TBP isomer) as a function of torsion about P–C bond for equatorial TBP isomers of PH_4CHF_2. All energy minima were fully geometry optimized stationary points obtained without constraints.

the inclusion of d-functions on the apical hydrogen leads to breakdown to phosphine oxide and hydride.[16c] In our case, optimization of $PH_4BH_3^-$ at 3-21+G(*) leads to decomposition of the BH_3^- apical isomer to H_2 + $H_2P{=}BH_2^-$. At the same level of calculation, a square pyramidal species is located for $PH_4BH_3^-$ with BH_3^- at the axial position. This phosphorane lies only 1.3 kcal/mol above the equatorial TBP isomer, a difference which may disappear in more sterically crowded pentacoordinate boranyl phosphoranes.

The addition of diffuse functions in the 3-21+G(*) basis set leads to stabilization of the apical isomer relative to the equatorial isomer, yielding increased absolute values of apicophilicity (Table I). The additional functions also allow significant changes in apical bond length (for example, PH_4OH) and total structure ($PH_4BH_3^-$) (Table I), stabilizing the more localized electron density at the apical ligand.

A water molecule was included in calculations on PH_4-OH isomers (Table I). The geometry and position of the water molecule was allowed to optimize, from the same starting position relative to the OH ligand, in both apical and equatorial isomers, with the phosphorane structure frozen. The equatorial isomer yielded a bidentate hydrogen-bonded structure, whereas a monodentate structure resulted for the apical isomer.

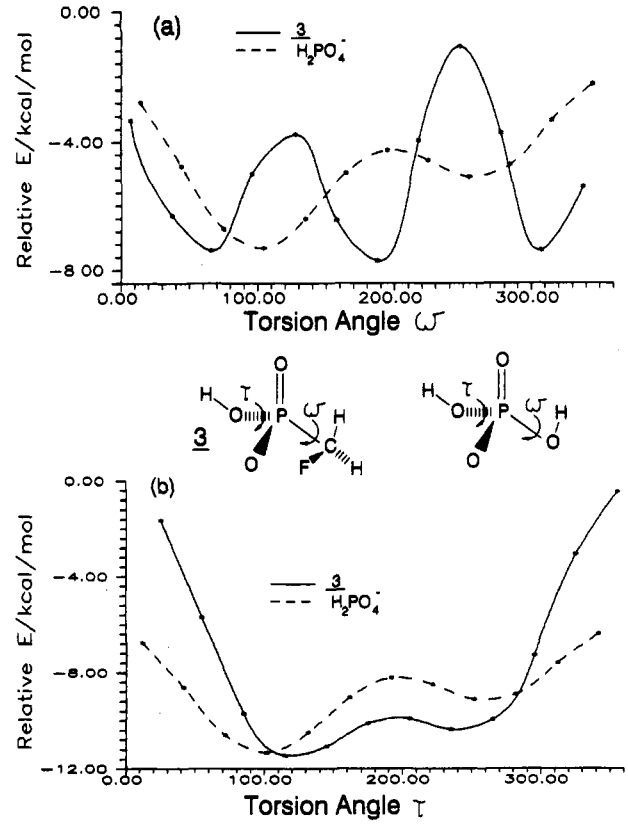
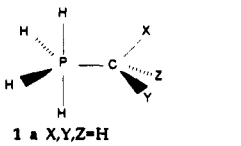
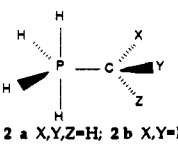


Figure 5. (a) Fixed geometry torsional scan (point calculations, no geometry optimization) of relative energy as a function of torsion angle about P–O(H) bond of $H_2PO_4^-$ ω{HOPO(H)} (dashed line) compared with torsion about P–C bond of $HO^-{}_3PCFH_2$ (**3a**) ω{HCPO(H)} (solid line). Global energy minimum structure of phosphonate used for torsional scans. (b) Fixed geometry torsional scan of relative energy as a function of torsion angle about P–O(H) bonds of $H_2PO_4^-$ τ{HOPO(H)} (dashed line) and $HO^-{}_3PCFH_2$ (**3b**) τ{HCPO(H)} (solid line). Local energy minimum structure of phosphonate used for torsional scans with ∠C–P–O(H) increased to 100° to reduce overwhelming steric effects.

Conformational Surfaces. Torsional energy profiles were studied for the pentacoordinate TBP species PH_4X ($X = CF_3, CF_2H, CFH_2$) and the tetrahedral ground states $HOPO_3H^-$ and $CF_2HPO_3H^-$. Full geometry optimization was performed at each point to obtain the profiles for PH_4X, whereas for $HOPO_3H^-$ and $CF_2HPO_3H^-$ torsional scans were obtained by point calculations on fixed geometry rotamers, accompanied by full geometry optimization of resulting energy minima (Figures 3–5; Tables I and II). Streitwieser and Schleyer identified the "staggered" equatorial conformer of PH_4CH_3 (**1a**) as the favorable TBP geometry.[16h] However, we find the conformer **2a** to be of almost identical energy (Table II). These



1 a X,Y,Z=H
b X,Y=F, Z=H
c X,Y=H, Z=F; d X=F, Y,Z=H



2 a X,Y,Z=H; **2** b X,Y=F, Z=H
c X,Z=F, Y=H
d X=F, Y,Z=H e Y=F, X,Z=H

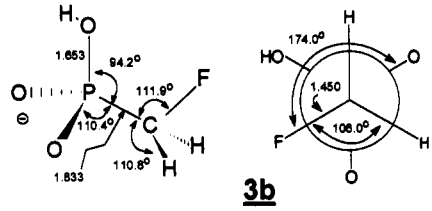
Table II. Relative Energies from Geometry Optimization of Conformers of 1 and 2, kcal/mol

structure	1a	2a	1b	2b	2c	1c	1d	1e
torsion angle[a] (deg)	0	90	58	87	28	0	58	89
energy	0.0	0.02	1.00	0.0	1.24	0.0	2.61	3.14

[a] Torsion angle defined as τ\{F-C-P-H(apical)\} or τ\{H-C-P-H(apical)\}

conformers, in which two C-substituents are staggered and one eclipsed with respect to the P-ligands, represent the minima along the more complex torsional profiles of the (mono- and difluoromethyl)phosphoranes (Figures 3 and 4). Simple torsional scans (point calculations on fixed geometries) are unable to reproduce this profile, since the geometry of the PH_4 moiety is correlated profoundly with torsion about the P–C bond. In the case of PH_4CH_2F, it can be seen that torsion about the P–C bond has profound effect on the apical P–H bond (Figure 3). A smaller effect on equatorial P–H bond lengths is observed (d(P–Heq) varies by <0.013 Å). Effects on both equatorial and apical P–H bond length are observed for PH_4CF_2H: (a) in conformer **1b** the eclipsed apical P–H bond is shorter by 0.009 Å; (b) in conformer **2b** the eclipsed equatorial P–H bond is 0.023 Å longer than the staggered equatorial P–H bond, the eclipsed C–F bond is 0.02 Å longer than the staggered C–F bond, and the P–C bond is shortened by 0.01 Å from conformer **2c**.[26]

Global and local energy minima were located for $HOPO_3H^-$ and $CFH_2PO_3H^-$ and fixed geometry, torsional scans performed on these structures at the 3-21+G(*) level. The global minimum for phosphate monoanion is symmetrical with τ\{HOPO(H)\} = 15° and −15°, although torsional distortion by ±5° incurs negligible energy cost. Two minima were located for the (monofluoromethyl)-phosphonate monoanion: the global energy minimum (**3a**) has fluorine antiperiplanar (app) to a phosphoryl oxygen, whereas in the local minimum ((**3b**) ΔE = +2.55 kcal/mol) it is app to hydroxyl. Torsional scans shows that the minima for both species are influenced by dipolar effects, but are not dominated by them.

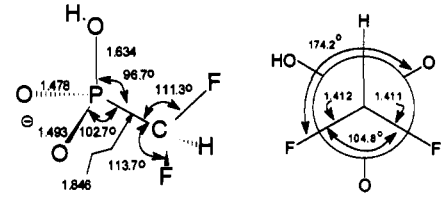


3b

Stereoelectronic effects must be invoked to account for the energy profile of phosphate monoanion, but such effects are masked in the phosphonate by steric effects. The periodicity of the phosphonate energy profile is compatible with steric effects favoring staggered conformations (Figure 5a). The global minimum is favored by dipolar effects, whereas the local minimum is disfavored. However, the presence of favorable stereoelectronic effects in the local minimum structure is indicated by the lengthening of the P–O(H) bond by 0.016 Å in the local compared to the global minimum structure.

Using the located energy minima, fixed geometry torsional scans were performed to compare the flexibility of the phosphonate to the phosphate (Figure 5a,b). Comparison of the energy profiles for rotation of the P–O(H) bonds displays similar profiles for the two species (Figure 5b). However, there is a significant barrier to rotation (12 kcal/mol) in the phosphonate, largely due to the steric interaction of the hydroxyl proton with the fluoromethyl group. A significant difference is also observed in comparison of rotation of the phosphonate P–C(H) bond with the phosphate P–O(H) bond (Figure 5a). The minimum energy conformations for the phosphate represent maxima for the phosphonate, the barrier to rotation for the latter being larger at 4–8 kcal/mol.

Tetrahedral Ground-State Structure and Geometry. A number of acyclic ground-state phosphoryl species ($CHF_2PO_3H^-$, $CH_2FPO_3H^-$, $CH_3PO_3H^-$, $BH_3PO_3H_2^-$, $H_2PO_3^-$) were geometry optimized at the 3-21+G(*) level for comparison with each other and the pentacoordinate species. In simile with the (monofluoromethylene)-phosphonate detailed above, the (difluoromethylene)-phosphonate monoanion yielded two energy minima, the global minimum ((**4b**) each fluorine app to $O^{\delta-}$), representing the minimum dipole structure, being 1.5 kcal/mol lower in energy than the local minimum ((**4a**) one fluorine ap to OH). The $CRF_2PO_3H^-$ moiety from the recent crystal



4a

structure determination of (2-amino-1,1-difluoroethyl)-phosphonic acid corresponds to the local minimum structure from the ab initio calculations.[9d] Presumably, crystal packing forces and the highly hydrogen-bonded nature of the crystal diminish the influence of dipole effects. With the exception of the C–F bond lengths, the structural parameters from crystal and calculation show good correspondence. Particularly noteworthy in comparison of phosphonates with phosphate is (1) the small CPO bond angle (96.7° in $CHF_2PO_3H^-$, 94.2° in $CH_2FPO_3H^-$ compared to 100° in $CH_3PO_3H^-$ and 100.9 for ∠OPO in phosphate) and (2) the large P–C bond lengths (1.846 Å in $CHF_2PO_3H^-$, 1.833 Å in $CH_2FPO_3H^-$, 1.852 Å in the crystal structure, 1.816 Å in $CH_3PO_3H^-$) compared to 1.65 Å for P–O in phosphate).

Similar differences in geometry are observed in the optimized geometries of ethylene phosphate (**5a**) and its monofluoromethylene phosphonate analog **5c** at the 3-21+G(*) level. (1) The P–C bond is 0.25 Å longer than the P–O bonds. (2) The CPO angle is smaller, though in both **5a** and **5c** the ring angle at phosphorus is contracted relative to the acyclic species (91.2° for the phosphate and 86.0° for the phosphonate) because of strain induced by the ring. The structures of the two cyclic compounds are superimposed in Figure 6.

The structure of the ground-state boranyl phosphonate ($BH_3PO_3H_2^-$, **8a**) was obtained at 3-21+G(*) demonstrat-

(26) The bond length changes suggest hyperconjugation between the eclipsed P–H(equatorial) and the P–C bond. Such orbital mixing interactions have been proposed in PH_4BH_2.[16b]

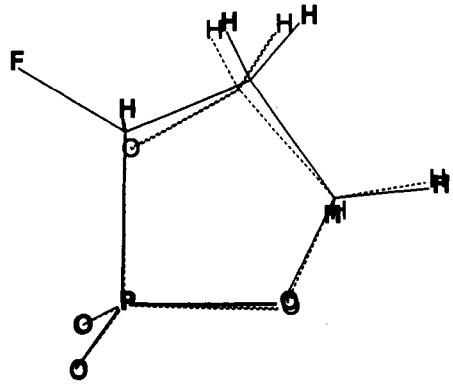
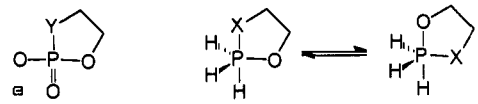


Figure 6. Structures of **5a** (dashed line) and **5c** (solid line) obtained by geometry optimization at the 3-21+G(*) level are superimposed as used for electrostatic potential mapping (Figure 8).

Table III. Apicophilicities and Bond Lengths for Cyclic Phosphoranes Calculated at 3-21G(*) Level

structure	$A(E(\text{ap}) - E(\text{eq})$	d(P–X)ap	d(P–X)eq
6a,e	+2.4	1.900	1.854
6b,f	−1.2	1.902	1.859
6c,g	−4.8	1.900	1.864
6d		1.690	1.638

ing that the long P–B bond (1.923 Å) is not peculiar to the pentacoordinate state (Table I).



5a Y=O
b Y=CH_2
c Y=CFH
d Y=CF_2

6a X=CH_2
b X=CFH
c X=CF_2
d X=O

6e
f
g

Inclusion of Phosphorus in a Five-Membered Ring. The inclusion of phosphorus in a five-membered ring has been shown to have dramatic effects on reactivity in solution.[13,22,27] Optimizations at the 3-21G(*) level allow comparison of structures and apicophilicity for the five-membered cyclic phosphoranes $H_3POCH_2CH_2X$ (X = O, CH_2, CFH,CF_2: **6a–g**). Calculations on the acyclic phosphoranes (Table I) yield the trend in apicophilicities: CF_2 > CFH > CH_2 > O, whereas the calculated ordering for the cyclic species (Table III) is CF_2 > CFH > O > CH_2. Thus, although the apicophilicity comparison is relative to H in Table II and Figure 2 and O in Table III, the trend is largely reproduced.

On the basis of calculations at the 3-21+G(*) level on the pentacoordinate TBP pentaoxyphosphorane structure **7a** it was reported that dianionic pentacoordinate intermediates derived from ethylene phosphate did not exist.[28] However, subsequently, an energy minimum corresponding to the methyl derivative of this pentaoxyphosphorane **7b** was located and reported at both the 3-21+G(*) and 3-21G(*) levels.[18,20,29] Since the phosphonate **5c** is an analog of ethylene phosphate **5a** , it was of interest to examine the structure of the dianionic pentacoordinate TBP intermediate **7c** corresponding to addition of hydroxide to this species. A structure was located corresponding to an energy minimum without addition of stabilizing methyl groups, presumably owing to the increased apicophilicity and polarizability of CFH over oxygen and ensuing stabilization of the intermediate (Figure 7).

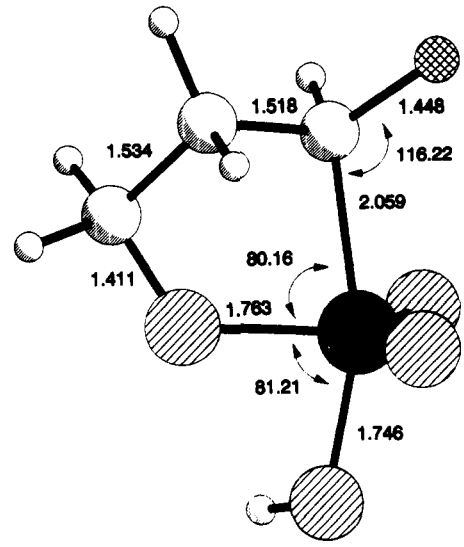
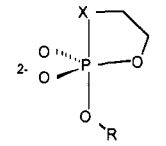


Figure 7. Structure of phosphorane **7c** obtained by geometry optimization at 3-21G(*).



7a X=O, R=H
b X=O, R=CH_3
c X=CFH, R=H

Electrostatic Potentials. The most important contribution to hydrogen bonding is electrostatic.[30] Hydrogen bonding may be the dominant force in binding of a phosphate biomolecule at an enzyme active site or protein receptor site.[31,32] Thus, a comparison was made to assess the distortion of electrostatic potential in a monofluoromethylene phosphonate versus its parent phosphate in the region of space that might be expected to be important in hydrogen bonding. The geometry-optimized structures of ethylene phosphate (**5a**) and its monofluoromethylene phosphonate analog (**5c**) were fitted, resulting in superimposition of the P–C bond of the phosphonate with an endocyclic P–O bond of ethylene phosphate (Figure 6). Electrostatic potentials were calculated in a cone shaped cross-section centered on the apical carbon atom and containing the C–F bond. The same cross-section was superimposed upon ethylene phosphate. The expected distortion of the electrostatic potential map (Figure 8) emphasizes the influence of (1) the C–F bond in displacing the center of favorable potential about 1 Å from its position

(27) Kluger, R.; Covitz, F.; Dennis, E. A.; Williams, L. D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1969**, *91*, 6066. Kluger, R.; Thatcher, G. R. J. *J. Am. Chem. Soc.* **1985**, *107*, 6006. Kluger, R.; Thatcher, G. R. J. *J. Org. Chem.* **1986**, *51*, 207. Kluger, R.; Taylor, S. D. *J. Am. Chem. Soc.* **1991**, *15*, 5714.

(28) Lim, C.; Karplus, M. *J. Am. Chem. Soc.* **1990**, *112*, 5872.

(29) It has been argued that the bulk of the methyl group is required to stabilize the negative charge in the gas phase.[18b]

(30) Hibbert, F.; Emsley, J. *Adv. Phys. Org. Chem.* **1990**, *26*, 255. Hasenein, A. A.; Hinchcliffe, A; Carbo, R., Klobukowski, M., Eds. *Studies Phys. Theo. Chem.* **1990**, *70*.

(31) For example: Luecke, H.; Quiocho, F. A. *Nature* **1990**, *347*, 402.

(32) Verlinde, L. M. J.; Noble, M. E. M.; Kalk, K. H.; Groendijk, H.; Wieranga, R. K.; Hol, W. G. J. *Eur. J. Biochem.* **1991**, *198*, 53.

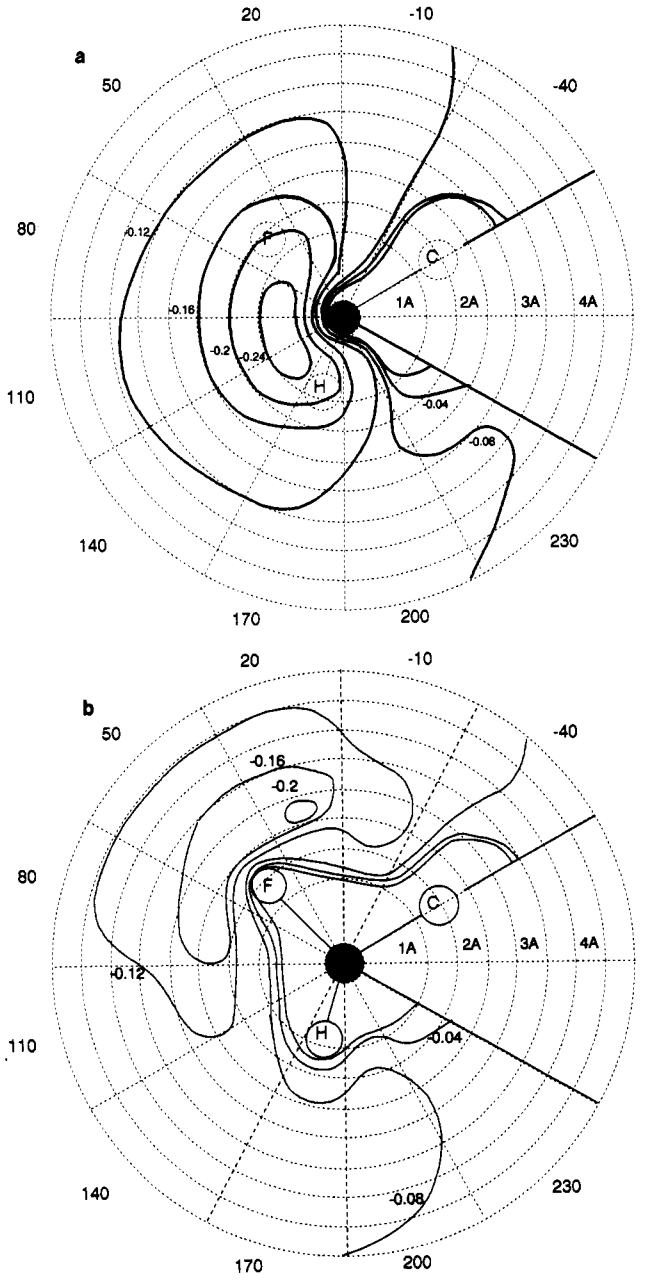


Figure 8. Electrostatic potential maps for (a) ethylene phosphate and (b) the fluoromethylene phosphonate analog **5c** as described in text. Approximate positions of C,F,H atoms attached to apical carbon of **5c** are shown in (b) and superimposed in (a) for comparison. Isopotential lines drawn every 0.04 au. Radial lines show distance from apical carbon in **5c** or apical oxygen in **5a** (angstroms).

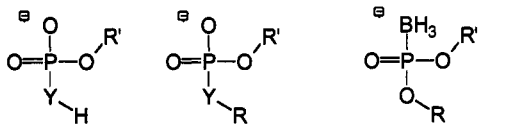
in the phosphate and (2) the C–H bond in replacing the region of favorable electrostatic potential that exists in the parent phosphate with a region of unfavorable potential on one face of the phosphonate analog.

Discussion

Bioisosterism. The optimized structures of the ground-state tetrahedral phosphonates in this study represent simple models for biological phosphates and their analogs (Scheme I: in analogs **10a–c** (R, R′ = alkyl, aryl) an ester oxygen is replaced, whereas in **9a–c** (R′ = alkyl, aryl) and **8b** a phosphoryl oxygen has been replaced). The calculated geometries for the phosphonate analogs show considerable deviation from the parent phosphate: (1) the P–X (X = C, B) bond is invariably longer than the P–O(H) bond which it replaces by a factor of 9–16%, and (2) replacement of divalent oxygen by tetracoordinate boron or carbon increases the steric requirement at this position, in particular when the carbon substituent is fluorine.[33] The

Scheme I

Biomolecule	Analog	Model
phosphate diester	8b; 10a, b, c R=R′=alkyl, aryl	3; 4; 8a; 9a, b, c R′=H
monoester monoanion	9a, b, c R′=alkyl, aryl	

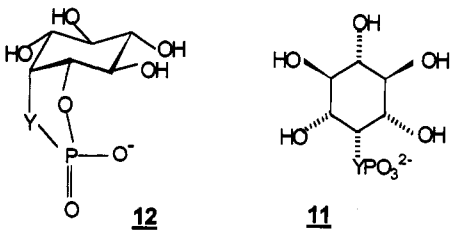


9a Y=CH_2 **10a** Y=CH_2 **8a** R=R′=H
b Y=CFH **b** Y=CFH **b** R,R′=alkyl,aryl
c Y=CF_2 **c** Y=CF_2

disruption of geometry is depicted in the superimposition of ethylene phosphate and its phosphonate analog **5c** (Figure 6).

Clearly, if binding interactions with the ester oxygen are important at a biological receptor site, the steric perturbation alone may disrupt binding. However, a further effect of the bulk of the fluoromethylene linkage is the significantly increased barrier to rotation about the P–C and P–O bond as compared to the P–O bond of the phosphate (Figures 3 and 4). The dissimilarity in the torsional profiles for phosphate and its analog suggests that the preferred conformations will be dissimilar. Coupled together these effects may present an energy cost for the phosphonate to assume the correct binding conformation, unless the phosphate is bound in a similar, but in this case, high-energy state.

The evidence for the isopolarity of α-halo phosphonates and the parent phosphates rests on pK_a determination and spectroscopic observation. Particularly outstanding is the correlation obtained by Blackburn et al. of ^{31}P NMR shift with pK_a for the series of methylphosphonic acids (XPO_3^{2-}, X = CH_3, CFH_2, CF_2H, CF_3, $CBrF_2$, CClFH).[35] Similar trends are observed in inositol phosphate analogs, both in the acyclic (**11**) and cyclic (**12**) series (Table IV).[9a,36]



12 **11**

Table IV. ^{31}P NMR Shifts for Inositol Phosphate Derivatives 11 and 12 at 162 MHz, Relative to H_3PO_4[a]

Y =	O (a)	CH_2 (b)	CHF (c)
11	4.93	29.47	12.47
12	16.72	43.03	32.52

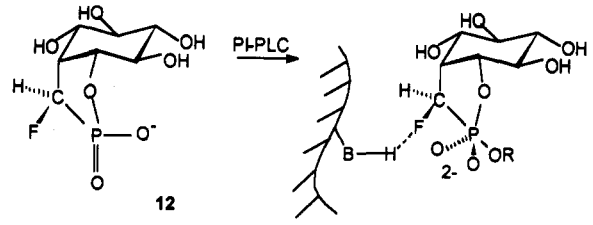
[a] Compounds **12a–c** are racemic mixtures.

(33) The C–F bonds in this study are ≈0.4 Å or 40% longer than the C–H bonds. The van der Waals volume of $-CF_3$ is twice that of $-CH_3$.[34]
(34) Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1320.

The data demonstrate that substitution of the phosphonate carbon by fluorine increases the effective electronegativity such that ^{31}P NMR shifts for (monofluoromethylene)phosphonate approach those of the parent phosphate. The proven correlation of pK_a with NMR shift allows one to assume with confidence that the electronic properties of monofluoromethylene phosphonates approach those of the phosphate. The resulting similarities between the $-PO_3^-R$ moieties of fluoromethylene phosphonate analogs and phosphate biomolecules must promote binding of the analogs at receptor sites. However, this analysis ignores the disruption of binding interactions at the ester oxygen site caused by steric perturbations. Hydrogen bonding with methylenephosphonate analogs is not possible, whereas hydrogen bonds may be formed with the fluorine atoms of (fluoromethylene)phosphonate analogs. The disruption of hydrogen bonding at a receptor site with the tetrahedral ground-state phosphate and its analog is examined by comparison of electrostatic potentials for ethylene phosphate and the phosphonate analog (**5c**) (Figures 6 and 8). Three factors are apparent: (1) the length of the P–C bond (Figure 6) displaces the region of favorable electrostatic potential in **5c**; (2) the C–H bond provides a region of unfavorable potential on one face of the molecule (Figue 8); and (3) the length of the C–F bond displaces the region of favorable potential by ≈1 Å from its position in the phosphate (Figures 6 and 8). Thus, in principle hydrogen bonding at a receptor site is possible with (monofluoromethylene)phosphonates, although displacement of the hydrogen bond donor atoms at the receptor site is inferred.

Phosphate Analogs and Phosphoryl Transfer. Design of analogs of phosphate biomolecules that bind at receptor sites in the ground state requires molecular mimicry of the tetrahedral phosphate. However, the design of inhibitors of enzymes catalyzing phosphoryl-transfer processes requires molecular mimicry of the transition state for phosphoryl transfer,[13] which is likely to be a pentacoordinate, trigonal bipyramidal species in which the nucleophile and leaving group are in-line and transapical.[37] As Blackburn has emphasized, one must consider the dynamic properties of phosphate analogs rather than the static properties discussed above.[8] The most successful phosphate transition-state analogs to date have been pentacoordinate vanadate species.[39] In order to provide a transition-state analog, phosphonate mimics must be *isodynamic* with phosphate. Application of *Westheimer's Guidelines* (see below) suggests that the major requirement will be substitution of the scissile phosphate ester linkage with a ligand possessing similar apicophilicity. Furthermore, in order for the enzyme to stabilize a transition state analog at the active site, disruption of hydrogen bonding must be minimized. This concept is illustrated for a putative transition-state analog inhibitor of phosphatidylinositol specific phospholipase C (PI-PLC) (Scheme II).

Scheme II



12

Apicophilicity. The relative apicophilicity of ligands at phosphorus in pentacoordinate TBP phosphoranes is a concept that has been in widespread usage in the prediction and explanation of both reactivity and stereochemistry in nucleophilic substitution reactions at phosphorus. Apicophilicity forms part of a series of empirical rules which have been termed *Westheimer's Guidelines for Associative Nucleophilic Substitution at Phosphorus*.[13,22] Other important rules state the following: (1) attack of nucleophile on tetrahedral phosphorus will lead to a TBP species, which may be an intermediate; (2) ligands may occupy apical or equatorial positions about TBP phosphorus, but if the TBP is an intermediate, ligand reorganization, termed pseudorotation, may occur; (3) five-membered rings must be attached apical–equatorial in TBP phosphoranes; (4) groups must enter and leave from the apical position of the TBP. Thus, apicophilicity is important in determining (1) which groups are likely to occupy the apical position of TBP intermediates and therefore represent potential leaving groups and (2) whether pseudorotation is required to place a group in the apical leaving group position. The latter factor is important in predicting and accounting for the stereochemistry of nucleophilic substitution at phosphorus.

Discrepancies have been demonstrated between experiment and prediction, in particular for nucleophilic substitution on substrates in which phosphorus is included in a five-membered ring. Notably, the apicophilicities inferred from experiment for thio, azo, and oxy ligands may show little consistency and may not conform to empirical, relative apicophilicity scales.[13,40] Carbon ligands have been shown to have the potential to occupy the apical position of TBP's when either (1) a carbon nucleophile is used or (2) the effective electronegativity of carbon is increased by electron-withdrawing substituents.[6,13,40,41]

Relative apicophilicity scales are drawn from data on stable phosphoranes. Therefore, discrepancies between this thermodynamic data and kinetic data on reaction rates and stereochemistry are not unanticipated. This discrepancy may be expected to be minimized for stepwise $A_N + D_N$ ($S_N2P(I)$) mechanisms, but maximized for concerted A_ND_N (S_N2P) mechanisms, in particular where the open, exploded transition state bears little structural resemblance to a TBP phosphorane. Such a dual-mechanism hypothesis has been discussed by DeBruin et al.[42]

The disparity between empirical relative apicophilicity scales (Trippet,[23b] Holmes[23c]) and those calculated by ab

(35) Blackburn, G. M.; Brown, D.; Martin, S. J.; Parratt, M. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 181.
(36) Campbell, A. S.; Thatcher, G. R. J., manuscript in preparation.
(37) Exceptions involve fully dissociative processes in which the nucleophile is not present in the transition state and adjacent mechanisms involving pseudorotation. There is no evidence to suggest that either alternative is of importance to enzymic processes.[13,38]
(38) Westheimer, F. H. *Org. Chem. (NY)* **1980**, *42*, 229. Knowles, J. R. *Ann. Rev. Biochem.* **1980**, *49*, 877.
(39) Campbell, A. S.; Thatcher, G. R. J. *Biomed. Chem. Lett.* **1992**, 655. Gresser, M. J.; Tracey, A. S. *J. Am. Chem. Soc.* **1986**, *108*, 1935. Gresser, M. J.; Tracey, A. S. In *Vanadium in Biological Systems*; Chasteen, N. D., Ed.; Kluwer: Dordrecht, Netherlands, 1990; p 63. Crans, D. C.; Willging, E. M.; Butler, S. R. *J. Am. Chem. Soc.* **1990**, *112*, 427. Crans, D. C.; Simone, C. M. *Biochemistry* **1991**, *30*, 6734. Lindquist, R. N.; Lynn, J. L.; Lienhard, G. E. *J. Am. Chem. Soc.* **1973**, *95*, 8762.
(40) Hall, C. R.; Inch, T. D. *Tetrahedron* **1980**, *36*, 2059. Cavell, R. G.; Poulin, D. D.; The, K. I.; Tomlinson, A. J. *Chem. Commun.* **1974**, 19.
(41) Kluger, R.; Thatcher, G. R. J.; Stallings, W. *Can. J. Chem.* **1987**, *65*, 1838.
(42) DeBruin, K. E.; Tang, C. W.; Johnson, D. M.; Wilde, R. L. *J. Am. Chem. Soc.* **1989**, *111*, 5871.

initio methods (Streitwieser,[16c] Schleyer,[16b] Thatcher (this work)) is equally striking:

Holmes:

$$F > OH > OR > Cl \approx NR_2 > Ph > H \approx O^- > CH_3$$

Trippett:

$$F > H > Cl > OR > NR_2 > Ph > CH_3$$

Streitwieser:

$$Cl > CN > F > H > CH_3 > OH > O^- > SH > NH_2$$

Schleyer:

$$Cl > F > OH \approx SH \approx CH_3 > PH_2 > NH_2 > SiH_3 > BH_2$$

Thatcher:

$$CF_3 > CF_2H > CFH_2 > OH > CH_3 > O^- \gg BF_3^- > BH_3^-$$

Streitwieser and Schleyer argue that the discrepancy in their calculated apicophilicity for CH_3 (in PH_4CH_3), which deviates 6 kcal/mol from Holme's empirical value, "may be due to the steric interactions and ring strain in more highly substituted phosphoranes".[16b] Our results lend some support, since it is observed that inclusion of carbon and oxy ligands in a five-membered ring (**6b,c**) results in a reversal in relative apicophilicity relative to the acyclic phosphoranes (Tables I and III). In addition, Holmes has tabulated steric effects to be applied in combination with his apicophilicity scale to calculate phosphorane stability.[23] In essence, steric effects cause ligands possessing α-substituents to demonstrate decreased apicophilicity. Our attention is directed at phosphate bioisosteres. In this respect, the most significant disagreement also lies in comparison of the apicophilic oxy ligand with the highly equatophilic carbon ligands; for example, CH_3 is 0.7 kcal/mol more apicophilic than OH at 3-21G(*) and 1.3 kcal/mol more apicophilic at the highest level calculated to date.[16b] However, according to Holmes's empirical values, CH_3 is 9.3 kcal/mol *less* apicophilic than OH. The quantitative disagreement in relative apicophilicity is large. The relative ordering of empirical apicophilicity, which largely mirrors relative electronegativity, is not reproduced by ab initio calculations, owing to large stereoelectronic $n \rightarrow \sigma^*$ orbital mixing effects in azo- and oxy-substituted phosphoranes. Removal of the stereoelectronic effects in PH_4X (X = OH, NH_2) restores the empirical apicophilicity ordering.[15b] The question must be asked: *are the stereoelectronic effects at pentacoordinate phosphorus, calculated as significant in the gas phase, overwhelmed so as to be insignificant in solution*? Qualitatively, it may be argued that the longer, more polar, apical P–X bond is more sensitive to stabilization by dipolar solvents than the equatorial P–X bond. Furthermore, potential hydrogen-bond acceptor ligands (e.g., oxy, azo) are liable to form stronger bonds when in the apical than equatorial position, owing to the increased negative charge density at the apical position.[43] Thus, solvation by dipolar, protic solvents is predicted to increase the apicophilicity of electronegative, hydrogen-bond acceptor ligands.

A crude measure of the effect of solvent on apicophilicity is gained by the inclusion of one water molecule in calculations on the apical and equatorial isomers of PH_4OH. The relative stabilization of the apical isomer by 4 kcal/mol results in a reversal of absolute apicophilicity for the OH ligand ($A = +2.4 \rightarrow -1.5$ kcal/mol). Calculations on a pentaoxyphosphorane + H_2O also indicate that greater stabilization results from hydrogen bonding to apical than equatorial ligands.[18b]

Although solvation effects can clearly reverse the calculated relative apicophilicity of methylene and oxy ligands, their effect on the relative apicophilicity scale, $CF_3 > CF_2H > CFH_2 > OH$, is likely to be less dramatic, since (a) the same dipolar solvation effects will apply to the highly polar P–C(F) bond and (b) hydrogen bonding is possible with the fluoromethyl moiety. Direct comparison of oxy and carbon ligands in the cyclic phosphoranes (**6b–g**) confirms (1) the higher apicophilicity of fluorocarbon ligands relative to oxygen and (2) the increasing apicophilicity of carbon ligands on sequential addition of fluorine substituents. Thus, fluorocarbon ligands can be expected to possess similar or greater apicophilicity than oxy ligands in phosphorane reaction intermediates in solution.

In contrast to (fluoromethyl)phosphoranes, calculations on boranylphosphoranes indicate that boranyl ligands strongly favor the equatorial position in TBP's. Similar steric effects to those postulated for methylphosphoranes can be expected in more highly substituted boranylphosphoranes increasing the equatorial preference still further. It is commonly understood that a high energy deficit results from placement of an oxyanion ligand in the apical position of a TBP phosphorane. In fact, this is thought to be the driving force for the pseudorotation that results in exocyclic cleavage in alkaline hydrolysis of methyl ethylene phosphate.[27] Given the considerably reduced, calculated apicophilicity of BH_3^- over O^-, it can be assumed that the BH_3^- ligand will not occupy an apical position in any phosphorane structure.

Phosphonates as Probes of Phosphoryl-Transfer Enzymes. These ab initio calculations indicate that fluoromethylene ligands have similar apicophilicity to oxygen. Furthermore, hydrogen bonding to the fluorine of these ligands may allow similar binding interactions at the active site to those with the parent phosphate. Conversely, the increased length of the P–C bond and the further displacement of any favorable hydrogen bonding region by the C–F bond are detrimental to binding of the ground-state tetrahedral phosphonates. However, these effects are beneficial in simulation of the enzyme-bound transition state by the phosphonate transition-state analog, since the long scissile P–O bond of the transition state (calculated at $\approx$2.2 Å in the dianionic transition state for nucleophilic substitution on a number of phosphate esters[45]) is superimposable with the P–C–F moiety of the transition-state analog (Figure 7). For example, the cyclization–hydrolysis reaction catalyzed by ribonuclease (and presumably PI-PLC) involves formation and cleavage of a five-membered cyclic phosphate. The essential hydrogen bond to the scissile ring oxygen of the cyclic phosphate is required for proton transfer for formation

(43) Mulliken population analysis at 3-21+G(*) indicates a charge density 200me greater at apical than equatorial OH for PH_4OH. CHELP electrostatic charges are −1.089 at the apical and −0.967 at the equatorial oxygen.[44] Natural charges confirm the increased negative charge at the apical position.[16b]

(44) Using Spartan 2.1, Hehre W. J. Wavefunction Inc., 1991.

(45) There is variation in scissile P–O bond length in the calculated transition states, depending on the position of the transition state along the reaction coordinate.[18,20,28,46]

(46) Lim, C.; Tole, P. *J. Am. Chem. Soc.* **1992**, *114*, 7245.

and breakdown of the cyclic phosphate. The (fluoromethylene)phosphorane **7c** is a model of a transition-state analog formed by the phosphonate **5c** at the active site of both ribonuclease and PI-PLC (Figure 7, Scheme I). Thus, it is predicted that fluoromethyl phosphonates will provide isodynamic analogs of phosphate biomolecules able to function as potent enzyme inhibitors through formation of transition-state analogs at the active site. Nonfluorinated phosphonates provide nonisodynamic species which are unable to form transition-state analogs, owing to the equatophilicity of CH_2 and disruption of hydrogen bonding, thereby providing a control for assessment of the mechanism of action of the fluorinated species. To date the limited studies of fluoromethyl phosphonate analogs in phosphoryl-transfer enzymes do not allow verification of these predictions.[8,9,47-49] In hand with further biological studies of the activity of (fluoromethyl)phosphonates, a rigorous study of the nonenzymic solution reactivity of cyclic and acyclic monofluoromethylene phosphonate diesters and triesters is in progress in our laboratory.

Both the high equatophilicity of the BH_3^- ligand and the tendency for boranylphosphoranes to distort to the square pyramidal structure provide some interesting opportunities for probing phosphoryl-transfer systems. If boranylphosphonates are able to bind to enzyme active sites, yet are unable to assume the trigonal bipyramidal transition-state structure, they may represent a class of nonlabile mimics of phosphate diester biomolecules. The limited study of boranylphosphonates shows these dinucleotide analogs to be resistant to phosphodiesterase activity.[11] The nonenzymic solution reactivity of these species warrants further study.

Conclusions

Several conclusions on the utility of phosphonate analogs as mimics of phosphate biomolecules may be drawn from these calculations:

1. The calculated high relative apicophilicity of mono- and difluoromethylene ligands suggests acyclic and cyclic fluoromethylene phosphonates to be *isodynamic* with the parent phosphates.

2. Consideration of the calculated molecular geometries, conformational preferences, and electrostatic potentials of mono- and difluoromethyl phosphonate monoanions suggests that ground-state receptor binding may be sufficiently perturbed to diminish the ability of the phosphonate analog to mimic the natural phosphate biomolecules.

3. Conversely, consideration of the molecular geometry for the pentacoordinate intermediate formed by a fluoromethylene phosphonate analog at an active site suggests an *isosteric, isopolar* relationship with the transition state for phosphate ester hydrolysis.

4. The isodynamic nature of (monofluoromethylene)-phosphonate analogs coupled with the isosteric and isopolar properties of the pentacoordinate intermediate state lead to a potentially potent transition-state analog inhibitor for phosphoryl-transfer enzymes.

5. It has been unclear whether modification of the properties of a phosphonate analog by use of only a single fluorine substituent is adequate for mimicry of the parent phosphate. These calculations clearly indicate that modification of methylene phosphonate analogs by inclusion of only one fluorine atom is sufficient to alter the properties of the phosphonate in the desired direction. This is particularly important, since (1) unlike the monfluoro analogs, no general route for synthesis of the difluoromethylene phosphonates exists and (2) exploitation of the chirality at the monofluoromethyl carbon may allow mapping of active site stereochemistry.

6. The differing dynamic and structural properties of methylene and fluoromethylene phosphonate analogs provide a powerful arsenal of biological probes for examination of the mechanisms and active sites of phosphoryl transfer enzymes. The interesting dynamic and structural properties of boranylphosphonates also suggests a potential role for these analogs in such studies.

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Supplementary Material Available: Cartesian coordinates for all stationary points corresponding to compounds **3a,b, 4a,b 5a,c, 6a-g, 7c, 8a,** and **9a** (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(47) The majority of studies are directed at analogs of di- and triphosphate-containing biomolecules in which perturbation of metal ion chelation in the phosphonate analog may be the dominant effect on biological activity. However, a recent paper purports to show the first clear evidence of the superiority of difluoromethylene phosphonates as enzyme inhibitors, in this case a nucleoside phosphorylase.[48a] Nevertheless, use of difluoromethyl analogs continues to yield mixed results.[48b]

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