Comparison of inline and non-inline associative and dissociative reaction pathways for model reactions of phosphate monoester hydrolysis

John Wilkie and David Gani

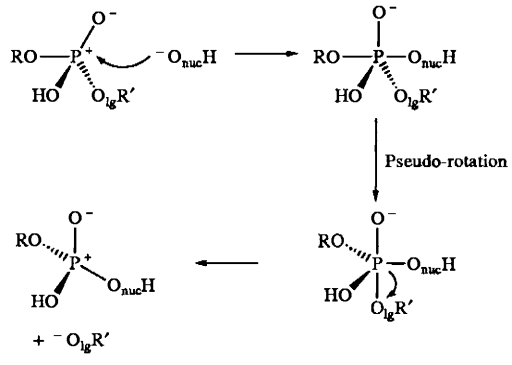
School of Chemistry and Centre for Biomolecular Science, The Purdie Building, The University, St. Andrews, Fife, UK KY16 9ST

The energies, geometries and transition state orders for several different potential phosphate ester group hydrolysis mechanisms are determined at different levels of phosphate group and nucleophile protonation to define factors which might favour one phosphoryl group transfer mechanism over another. With respect to the phosphate ester group, protonation is considered as a generic model for phosphate anion charge neutralisation by, for example, alkylation or metal ion chelation. The results indicate that the protonation state of either the phosphate group and/or the nucleophile can produce dramatic changes in the relative energies of intermediates and transition states for any particular hydrolytic mechanism and that the magnitude of the changes can be sufficiently large to cause a change in favoured mechanism. For example, the results of the calculations predict that the favoured mechanism should change from a dissociative one at low levels of phosphate group protonation, to an associative one at higher levels of phosphate group protonation. Furthermore, the results indicate that under conditions in which a highly stable pentacoordinate intermediate is formed, the favoured reaction pathway involves a non-inline displacement of the leaving group by the nucleophile rather than an inline displacement. The relevance of these findings to understanding enzyme-catalysed phosphoryl group transfer is discussed.

Introduction

The transfer of phosphoryl groups from one entity to another is of crucial importance in nature. Indeed, the regulation of virtually all cellular processes including the biosynthesis of macromolecules including proteins, RNA and DNA, energy production, signal transduction and the control of protein activity is dependent upon the phosphorylation and dephosphorylation of various different biomolecules. In most cases, the processes by which regulation is achieved are still poorly understood. Although this situation is improving with the increasing availability of biochemical and X-ray structural information,[1–3] it is clear that even the simplest phosphoryl transfer reaction, the hydrolysis of a phosphate monoester to yield an alcohol or alkoxide anion, can occur by a number of different mechanisms both in solution[4–6] and within the active site of an enzyme.[7]

Computational modelling for the binding of substrates to, and kinetic analysis of, inositol monophosphatase[8–11] has shown that the enzyme brings about phosphate hydrolysis by a direct displacement mechanism in which water, rather than the side chain of an amino acid residue, is the attacking nucleophile. There is a considerable body of evidence to suggest that the nucleophilic water molecule attacks from the same face of the phosphorus as that from which the leaving group departs, rather than from the opposite face as is more usual.[7] Such a mechanism predicts that the stereochemistry at the phosphorus centre would be retained in the product. This is because a pseudo-rotation of the pentacoordinate intermediate can occur in which the initially apical nucleophile moves to an equatorial position while the leaving group simultaneously moves to an apical position before departing, as shown in Scheme 1. The more usual direct in-line displacement mechanism, in which both the nucleophile and the leaving group occupy apical positions, proceeds with inversion of configuration at the P-atom and cannot, at this stage, be ruled out as the mechanism for the inositol monophosphatase-catalysed reaction. However, several lines of evidence and, in particular, those derived from



Scheme 1

the results of experiments designed to measure the rates of ^{18}O-label exchange from [^{18}O]-water into unlabelled inorganic phosphate[12] and structure–activity relationships for inhibitors and substrates,[13] do not fit comfortably with the inline displacement mechanism. A detailed consideration of the experimental results supporting each of the alternatives is given in ref. 11.

The pseudo-rotation mechanism is unusual and, as yet, has not been invoked for any other enzymic system.[7] However, the pseudo-rotation mechanism does appear to operate in non-enzymic 1,2-phosphoryl transfer reactions. Here Knowles and co-workers[14] have demonstrated that the transfer of a chirally labelled phosphoryl group from the 2-O-atom to the 1-O-atom of propane-1,2-diol 2-phosphate under acidic conditions occurs with retention of configuration at the migrating P-atom. In this case, the 1,2-arrangement of nucleophile and leaving group prevents an intramolecular inline displacement. Similarly we have proposed that enzyme residues in inositol monophosphatase are not suitably arranged for an inline attack of the phosphate group by a nucleophilic water.[11] Indeed, it has been proposed on the basis of kinetic experiments, theoretical calculations and X-ray diffraction data,[11,15,16] that a

magnesium ion can bind in a position ideally situated so as to activate a nucleophilic water molecule to attack *via* a non-inline direct displacement mechanism.

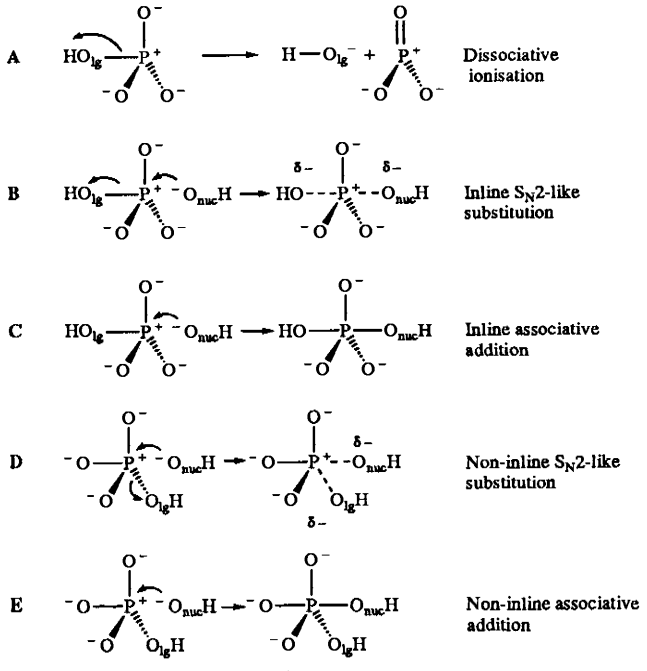
While further information on the phosphatase mechanism will come eventually from work in progress to determine the absolute stereochemical course of the hydrolysis reaction, we wished to assess the mechanistic determinants for inline and pseudo-rotation pathways at a generic level. Since no theoretical study had been reported, we set out to use computational chemistry techniques to gain insight into the nature of the reaction pathways and to examine the factors which might affect the relative energies and the importance of a number of competing pathways. Such a study is presented here for oxygen ligand exchange at the P-atom of inorganic phosphate, a simple model for phosphate ester hydrolysis.

Modelled mechanisms

In essence, the hydrolysis of a phosphate monoester is the displacement of one oxygen based ligand, typically an alcohol or alkoxide anion, by another, hydroxide or water. The simplest case is a symmetrical reaction in which the leaving group is identical to the attacking nucleophile [eqn. (1)]. This reaction

$$HPO_4^{2-} + OH^- \rightleftharpoons H_2PO_5^{3-} \rightleftharpoons HPO_4^{2-} + OH^- \quad (1)$$

can be thought of as proceeding *via* a concerted S_N2-like mechanism in which the leaving group (O_{lg}) departs as the nucleophile (O_{nuc}) approaches (B, Scheme 2), or in one of two



Scheme 2

stepwise mechanisms (A and C, Scheme 2). The first of these (A, Scheme 2) involves a metaphosphate intermediate and is characterised by a P–O_{lg} distance that is greater in the transition state than in the reactant complex, a so-called dissociative process. The second mechanism (C, Scheme 2) is described as an associative process and involves a pentavalent phosphorus intermediate and a shorter oxygen to oxygen distance in the O_{nuc}–P–O_{lg} transition state than in either the reactant or product complexes. Mechanisms B and C both proceed with inversion of configuration at phosphorus, while A gives a mixture of inversion and retention of configuration, the balance driven by the relative stability of the metaphosphate intermediate. A high stability for the intermediate leads to racemisation whereas a low stability leads to inversion at the P-atom because the leaving group can still block one face of the phosphate as the nucleophile attacks. In addition to these accepted potential mechanisms, either the S_N2-like process (B) or the associative mechanism (C) could, in principle, involve a non-inline displacement (D and E, Scheme 2). In each of these cases, the configuration at phosphorus would be retained.

In order to investigate the relative importance of these different mechanisms, and to gain insight into how changes in local pH might affect the energetic balance between them, we have calculated geometries and energies of the key turning points along the reaction path for the symmetrical oxygen exchange reaction in the gas phase [eqn. (1)]. This has been done for all five mechanisms at increasing levels of phosphate protonation using the semi-empirical PM3 Hamiltonian and parameter set[17,18] embodied in the MOPAC program[19] (see the Computational section).

Table 1 HPO_4^{2-} mechanisms[a]

Mechanism (nucleophile)	Reactant complex	Transition state	Intermediate complex	Intermediates at infinity
A (HO^-)	0	145.30		−176.44
B (HO^-)	0	826.01		
C (HO^-)	0	812.22	808.54	
D (HO^-)	0	925.28		
E (HO^-)	0	877.30	860.79	

[a] All energies in kJ mol^{-1}.

Results

There are a great many conceivable exchange mechanisms available to phosphate at each of the different protonation states considered (HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4). To simplify the description and energy comparison, the pathways have been grouped according to the phosphate species present in the reactant complex.

HPO_4^{2-}

The energies of the intermediates and transition states along the exchange pathways originating from HPO_4^{2-} are given in Table 1. Reaction pathways can be either dissociative [eqn. (2*a*)] or associative [eqn. (2*b*)]. Energies are given in kJ mol^{-1},

$$HPO_4^{2-} \rightleftharpoons PO_3^- + OH^- \quad (2a)$$

$$HPO_4^{2-} + OH^- \rightleftharpoons H_2PO_5^{3-} \quad (2b)$$

relative to the reactants (HPO_4^{2-} and hydroxide or HPO_4^{2-} alone for the dissociative path, Scheme 2, mechanism A) in either a stable complex or at infinite separation, whichever has the lower energy. Stationary points (minima and transition states) have been included for the various mechanisms, allowing comparison of barriers for the different processes. In addition to being of higher energy than the transition states for the stepwise mechanisms, the concerted S_N2-like counterparts (B and D) both show second-order transition states, with negative eigenvalues for the symmetric and anti-symmetric stretches of the forming and breaking bonds, in the case for the inline mechanism, and for independent vibrations, in the case for the non-inline mechanism. The intrinsic reaction coordinates for these second-order transition states were followed and, in addition to reactant and product complexes, structures were obtained corresponding to the intermediates for the dissociative (A) and appropriate associative (C and E) pathways.

The most favourable pathway for the reaction of HPO_4^{2-} and hydroxide, was the dissociative pathway (Scheme 2, mechanism A) with a barrier some 500 kJ mol^{-1} lower than the best associative mechanism, due principally to the greater stability of the transient intermediates and the greater separation of the two negatively charged species. For this

Table 2 $H_2PO_4^-$ mechanisms[a]

Mechanism (nucleophile)	Reactants at infinity	Reactant complex	Transition state	Intermediate complex	Intermediates at infinity
A (HO^-)	0				399.82
B (HO^-)	0		499.18		
C (HO^-)	0		385.35	274.71	
D (HO^-)	0		555.61		
E (HO^-)	0		323.36	274.71	
A (H_2O)	0	160.01[b]	164.52	116.87	166.16
B (H_2O)	0	−49.32	253.06		
C (H_2O)	0	−51.83	22.45	22.07	
D (H_2O)	0				
E (H_2O)	0	−38.46	54.84	53.30	

[a] All energies in kJ mol^{-1}. [b] As the leaving group is H_2O in this case, a proton transfer step is required to generate the appropriate reactant complex. This process is assumed to be virtually barrierless in a protic environment.

pathway there were no stable intermediate complexes, the energy fell continuously as the charges became more separated and reassociation to give products was the rate limiting factor. By comparison, the pentavalent intermediates of pathways C and E were only slightly lower in energy than the transition state (Table 1) so that the collapse of the intermediates was expected to be rapid.

A polar, or polarisable, environment would destabilise the dissociative intermediate, relative to its reactant (Scheme 2, mechanism A), as the dissociation process involves the separation of species that possess like charges which would be shielded from each other to a greater extent by a polar solvent. In the absence of any direct effect on the inherent barrier for the dissociative process, an increase in solvent polarity would serve to bring the reactant and intermediate complexes closer in energy and reduce the barrier for product formation from the intermediate structure. Similarly, a polar environment would serve to stabilise the associative intermediates (Scheme 2, mechanisms B–E), relative to their reactants as like charges combine in each case. The increase in solvent polarity also results in the reactant and intermediate complexes becoming closer together in energy with an accompanying reduction in barrier height, on this occasion, for the formation of the intermediate. Neither the transition states, nor the stable intermediates for the reaction of HPO_4^{2-} with H_2O could be found. All such structures collapsed to a reactant-like $H_2O{\cdot}HPO_4^{2-}$ complex which possessed a P–O distance of 3.73 Å.

$H_2PO_4^-$

Table 2 shows the relative energies of intermediates and transition states along the exchange pathways involving $H_2PO_4^-$ and hydroxide [eqns. (3*a*) and (3*b*)] or water [eqns. (4*a*) and (4*b*)] and follows the same protocol as for Table 1. In

$$H_2PO_4^- \rightleftharpoons HPO_3 + OH^- \quad (3a)$$

$$H_2PO_4^- + OH^- \rightleftharpoons H_3PO_5^{2-} \quad (3b)$$

$$H_2PO_4^- \rightleftharpoons PO_3^- + H_2O \quad (4a)$$

$$H_2PO_4^- + H_2O \rightleftharpoons H_4PO_4 \quad (4b)$$

this case, the nucleophile or leaving group can be either H_2O or OH^-. When the nucleophile or leaving group is H_2O rather than OH^-, there arises the possibility of, and in some cases, a requirement for, a proton transfer step. In the gas phase, this would be an intramolecular transfer out of necessity, with an associated barrier, however in solution or in the presence of other suitable proton donors, this is essentially a barrier-less process. As consideration of the proton transfer step would add considerable complexity to the system without enhancing the relevance of the model, transition states for this step have not been determined and the barrier assumed to be non-existent or at the very least, of considerably lower energy than for the oxygen exchange reaction itself.

Associative mechanisms with a hydroxide ion as the nucleophile still involve the association of like charges and show comparatively high barriers and low stability for the intermediates. As in the previous example, the concerted mechanisms (Scheme 2, B and D) involve second-order transition states and are not true reaction paths. Most surprising is that the non-inline stepwise associative mechanism (Scheme 2, E) involves the lowest energy path. No transition state for the dissociative path (Scheme 2, A) could be found because the energy increased continuously as the leaving group and metaphosphate (HPO_3) were separated. Again the energy was dominated by the electrostatic contribution even though the metaphosphate species is neutral overall, because the phosphorus atom itself possesses a significant positive charge.

H_3PO_4

Key stationary points for the mechanisms involving neutral H_3PO_4 are given in Table 3 and as before, the energies are given in kJ mol^{-1} relative to the lowest energy reactant complex. Energies are given for mechanisms involving both hydroxide [eqns. (5*a*) and (5*b*)] and water [eqns. (6*a*) and (6*b*)] as the

$$H_3PO_4 \rightleftharpoons H_2PO_3^+ + OH^- \quad (5a)$$

$$H_3PO_4 + OH^- \rightleftharpoons H_4PO_5^- \quad (5b)$$

$$H_3PO_4 \rightleftharpoons HPO_3 + H_2O \quad (6a)$$

$$H_3PO_4 + H_2O \rightleftharpoons H_5PO_5 \quad (6b)$$

nucleophile. Since there is now no requirement for the association of like charges in forming the associative intermediates, they are comparatively more stable than the less protonated associative intermediates discussed in the previous sections. No transition state corresponding to any of the concerted mechanisms could be found. With either hydroxide ion or water as the nucleophile, there was a preference for the non-inline process. By comparison, the dissociative pathway no longer had the benefit of separating like charges and was a significantly higher energy pathway than for the associative mechanisms. However as with the pathways for $H_2PO_4^-$, there was no barrier for the loss of OH^- and the energy increased continuously with increasing separation.

Discussion

The relative energies of the transition states described here are largely determined by the electrostatic component, as many of the pathways involve the separation or the coming together of charged species. For this reason, the energy differences are

Table 3 H_3PO_4 mechanisms[a]

Mechanism (nucleophile)	Reactants at infinity	Reactant complex	Transition state	Intermediate complex	Intermediates at infinity
A (HO^-)	0				1006.63
B (HO^-)	0				
C (HO^-)	0	−204.23	−124.27	−306.39	
D (HO^-)	0				
E (HO^-)	0	−204.23	−190.78	−306.39	
A (H_2O)	0	128.74[b]	178.32		164.44
B (H_2O)	0				
C (H_2O)	0	−3.55	34.53	−12.58	
D (H_2O)	0				
E (H_2O)	0		24.33	10.87	

[a] All energies in kJ mol^{-1}. [b] As the leaving group is H_2O in this case, a proton transfer step is required to generate the appropriate reactant complex. This process is assumed to be virtually barrierless in a protic environment.

expected to be much greater than for the corresponding reactions in solution, where the high relative permittivity medium would attenuate the charge interactions. Despite this, it is possible to gain an understanding of the factors affecting the likelihood of one mechanism over another.

First, a reduction in the effective negative charge on the phosphate favours associative mechanisms over the dissociative pathway. The effective charge can be reduced by protonation, insertion into a high relative permittivity medium or by association with positively charged centres such as metal ions. Second, in many cases the non-inline associative mechanism is actually of lower energy than its inline counterpart. Here the key factor in determining which pathway is followed is the stability of the pentavalent intermediate. When the phosphate is minimally protonated, the stability of the intermediate is low and the inline concerted mechanism is of similar energy to the stepwise processes. Hence it is anticipated that the collapse of the intermediate would be rapid compared with the pseudo-rotation required for the non-inline substitution, with the result that only the inline mechanism would be observed. At higher levels of phosphate protonation, the concerted mechanism is sufficiently high in energy to be insignificant, although when H_2O is the nucleophile and/or leaving group the energy difference between the inline and non-inline displacement pathways is small (< 14 kJ mol^{-1}) and the intermediate is relatively unstable. In this situation, the comparative instability of the intermediate can allow the kinetic energy of the reacting particles to dominate the choice of reaction path, again resulting in inline displacement. Non-inline displacement is favoured with hydroxide as the nucleophile and/or leaving group, when the intermediate is also at its most stable. This latter conclusion fits well with our current understanding of inositol monophosphatase, where the active site is solvated and contains many polar residues, with two α-helix dipoles arranged to minimise the effective charge of both the phosphate and the leaving group.[20] There are also two Mg^{2+} ions in close proximity to the reaction centre, and one of these is placed so as to activate a nucleophile,[11,15,16] which may well be hydroxide, for non-inline displacement. In addition, tight binding of the pentacoordinate intermediate will retard collapse of the intermediate relative to pseudo-rotation, all favouring the non-inline mechanism. The effects of Mg^{2+} ions on the solution mechanism have been described[21] and the findings show that significant catalysis is brought about by the presence of the metal ion. Fully hydrated $[Mg^{2+}(H_2O)_6]$ possesses a pK_a value of 11.5[22] and while the aspartate residue coordinated Mg^{2+} ion of inositol monophosphate may show a smaller reduction in the pK_a value for its associated nucleophilic water molecule (*i.e.* a value higher than 11.5), there will be a significant increase in the local concentration of nucleophile. The effective nucleophile concentration is increased further by pre-organisation of a reactant complex within the enzyme active site, an effect also seen to an extent in solution.[21]

In contrast to our conclusions here, Hershlag and Jencks have argued for a dissociative mechanism for the Mg^{2+} catalysed phosphate ester hydrolysis reaction in solution.[21] However, they concede that it can be difficult to distinguish experimentally between associative and dissociative pathways within the active site of an enzyme.[21,23] For inositol monophosphatase, a dissociative mechanism would still involve nucleophilic attack on the same face on account of the restricted access to a bound metaphosphate with the result that phosphorus configuration would be retained on hydrolysis. It is difficult to envisage how this could be distinguished experimentally from an associative, non-inline mechanism as the two differ only in the lengths of the P–O bonds by fractions of an Ångstrom in the transition state and intermediate. However, we believe inositol monophosphatase does not hydrolyse its substrates using a dissociative mechanism as molecules that chelate to the catalytic magnesium ion in the enzyme active site through a hydroxyethyl moiety in the inhibitor, preventing the binding of a nucleophilic water molecule, act as extremely potent *competitive* inhibitors of the enzyme.[24] If substrate hydrolysis were to occur using a dissociative mechanism, then transphosphorylation of the hydroxyethyl side-chain of the inhibitor should occur. That these Mg^{2+}-chelating inhibitors do not undergo transphosphorylation within the active site argues against a metaphosphate reaction pathway for inositol monophosphatase.[24]

In summary, it appears that the non-inline associative mechanism with pseudo-rotation (Scheme 2, E) provides a reasonable reaction pathway for phosphate ester hydrolysis and that under certain conditions the mechanism may provide the lowest energy pathway. Models described here to alter the negative charge on the phosphate anion moiety using protons more generally hold for higher alkylated phosphates (*i.e.* phosphate diesters and triesters) and apply to systems in which charge neutralisation is achieved by chelation to monovalent cations. Because divalent metal ions can chelate more than one phosphate oxygen ligand, including the leaving group and/or the nucleophile, and are most commonly involved in biological phosphate ester hydrolyses, we are currently engaged in calculations to investigate the possible effects on the mechanism of Mg^{2+} ions.

Computational

Reactant–product complexes

Geometries and energies of the reactant complexes and of isolated individual reactant species were determined by energy optimisation in internal coordinate space, using the default BFGS algorithm[25–28] and the PM3 parameters[17,18] within MOPAC,[19] terminating the optimisation when the *G*norm fell below 0.04 kJ A^{-1} (kJ rad^{-1}). The resulting complexes were identified as genuine minima following frequency determination, which yielded no negative eigenvalues. No constraints

were placed on the system during the optimisations. Starting points for the unimolecular structures could be generated by inspection, with the appropriate numbers of oxygen atoms in close contact with the central phosphorus atom, while starting points for the multimolecular complexes were generated from the final structures from the IRC calculations, see below.

Transition state structures

Geometries and energies of the transition state structures were also determined using the PM3 parameters within MOPAC by a gradient optimisation, using a non-linear least-squares algorithm (NLLSQ), again terminating with *G*norm < 0.04 kJ A^{-1} (kJ rad^{-1}). Starting points for these calculations were generated from the unimolecular intermediate or reactant structures (see above) by stretching the appropriate bonds, selected from the mechanisms in Scheme 2. The orders of identified transition states were determined by frequency calculations and the intrinsic reaction coordinate (IRC) followed in both directions and to completion, identifying reactant and product complexes for the relevant process. For higher order transition states, all negative eigenvalues were followed by means of an IRC calculation to place the turning point in context on the potential energy hypersurface.

Acknowledgements

The authors thank the EPSRC and BBSRC for grants GR/H67607 and GR/J26649 to D. G.

References

1 J. Goldberg, H.-b. Huang, Y.-g. Kwon, P. Greengard, A. C. Nairn and J. Kuriyan, *Nature*, 1995, **376**, 745.
2 E. E. Kim and H. W. Wyckoff, *J. Mol. Biol.*, 1991, **218**, 449.
3 E. G. Mueller, M. W. Crowder, B. A. Averill and J. R. Knowles, *J. Am. Chem. Soc.*, 1993, **115**, 2974.
4 D. Hershlag and W. P. Jencks, *J. Am. Chem. Soc.*, 1986, **108**, 7938.
5 D. Hershlag and W. P. Jencks, *J. Am. Chem. Soc.*, 1989, **111**, 7579.
6 D. Hershlag and W. P. Jencks, *J. Am. Chem. Soc.*, 1990, **112**, 1942.
7 D. Gani and J. Wilkie, *Chem. Rev.*, 1995, 55.
8 A. G. Cole and D. Gani, *J. Chem. Soc., Chem. Commun.*, 1994, 1139.
9 A. G. Cole and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2685.
10 A. G. Cole, J. Wilkie and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2695.
11 J. Wilkie, A. G. Cole and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1995, 2709.
12 G. R. Baker and D. Gani, *Biomed. Chem. Lett.*, 1991, **1**, 193; A. P. Leech, G. R. Baker, J. K. Shute, M. A. Cohen and D. Gani, *Eur. J. Biochem.*, 1993, **212**, 693.
13 R. Baker, C. Carrick, P. D. Leeson, I. C. Lennon and N. Liverton, *J. Chem. Soc., Chem. Commun.*, 1991, 298.
14 S. L. Buchwald, D. H. Pliura and J. R. Knowles, *J. Am. Chem. Soc.*, 1984, **106**, 4916.
15 R. Bone, L. Frank, J. P. Springer, S. J. Pollack, S. Osborne, J. R. Atack, M. R. Knowles, G. McAllister, C. I. Ragan, H. B. Broughton, R. Baker and S. R. Fletcher, *Biochemistry*, 1994, **33**, 9460.
16 R. Bone, L. Frank, J. P. Springer and J. R. Atack, *Biochemistry*, 1994, **33**, 9468.
17 J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 209.
18 J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 221.
19 J. J. P. Stewart, MOPAC v6.0, *QCPE Bull.*, 1990, **10**, 86.
20 R. Bone, J. P. Springer and J. R. Atack. *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 10 031.
21 D. Hershlag and W. P. Jencks, *Biochemistry*, 1990, **29**, 5175.
22 C. W. Childs, *Inorg. Chem.*, 1970, **9**, 2465.
23 J. R. Knowles, *Ann. Rev. Biochem.*, 1980, **49**, 877.
24 J. Schultz, J. Wilkie, P. Lightfoot, T. Rutherford and D. Gani, *J. Chem. Soc., Chem. Commun.*, 1995, 2353.
25 C. G. Broyden, *J. Inst. Math. Its Appl.*, 1970, **6**, 222.
26 R. Fletcher, *Comput. J.*, 1970, **13**, 317.
27 D. Goldfarb, *Math. Comput.*, 1970, **24**, 23.
28 D. F. Shano, *Math. Comput.*, 1970, **24**, 647.

Paper 5/07718D
Received 27*th November* 1995
Accepted 24*th January* 1996