Nucleophilic and Protolytic Catalysis of Phosphonate Hydrolysis: Isotope Effects and Activation Parameters

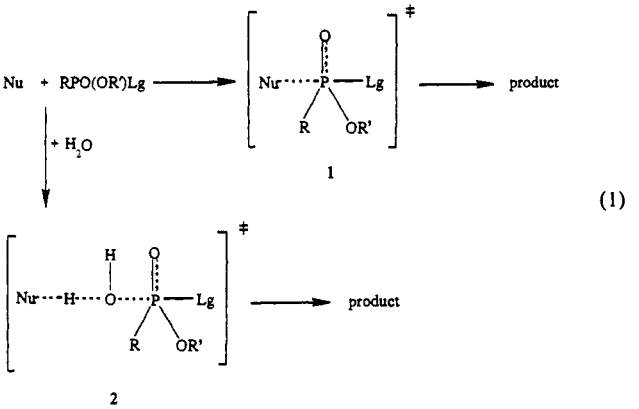
Ildiko M. Kovach,*,§ Andrew J. Bennet,† Jeffrey A. Bibbs,‡ and Qinjian Zhao§

Contribution from the Department of Chemistry, Catholic University of America, Washington, D.C. 20064, Department of Chemistry, Simon Fraser University, Burnaby, V5A 1S6, BC, Canada, and AMYLIM Inc., San Diego, California 92121

Received October 23, 1992. Revised Manuscript Received March 1, 1993

Abstract: Activation parameters for the imidazole-catalyzed hydrolysis of bis(4-nitrophenyl) methylphosphonate (NMN) are $\Delta H^{\ddagger} = 44 \pm 2$ (H_2O) and 46 ± 2 (D_2O) kJ/mol and $\Delta S^{\ddagger} = -146 \pm 5$ (H_2O) and -145 ± 5 (D_2O) J/mol K. The activation parameters for water-catalyzed hydrolysis of NMN in H_2O are $\Delta H^{\ddagger} = 28 \pm 10$ kJ/mol and $\Delta S^{\ddagger} = -270 \pm 30$ J/mol K. The solvent isotope effects for the hydrolysis of NMN at 25 °C are 2.69 ± 0.09 for imidazole catalysis and 2.91 ± 1.24 for water catalysis. The solvent isotope effect for the imidazole-catalyzed hydrolysis of 2-(3,3-dimethylbutyl) methylphosphonofluoridate (soman) is similar, 2.79 ± 0.03 at 25 °C. Nucleophilic reactions of hydroxide ion and phosphate dianion with NMN at 25 °C are associated with solvent isotope effects of 0.51 ± 0.02 and 1.54 ± 0.26, respectively, and $\Delta H^{\ddagger} = 69 \pm 4$ kJ/mol; $\Delta S^{\ddagger} = 27 \pm 12$ J/mol K for hydroxide ion. For the reactions of 4-nitrophenyl 2-propyl methylphosphonate (IMN) with imidazole, water, phosphate dianion, and hydroxide ion, the activation parameters in H_2O are $\Delta H^{\ddagger} = 56 \pm 2$ kJ/mol, $\Delta S^{\ddagger} = -150 \pm 4$ J/mol K (imidazole); $\Delta H^{\ddagger} = 34 \pm 2$ kJ/mol, $\Delta S^{\ddagger} = -143 \pm 5$ J/mol K (hydroxide ion); $\Delta H^{\ddagger} = 60 \pm 4$ kJ/mol, $\Delta S^{\ddagger} = -170 \pm 13$ J/mol K (water); and $\Delta H^{\ddagger} = 72 \pm 3$ kJ/mol, $\Delta S^{\ddagger} = -93 \pm 9$ J/mol K (phosphate dianion); the solvent isotope effects are 1.40 ± 0.07 (imidazole, 73 °C); 1.2 ± 0.2 (water, 25 °C); 1.11 - 1.15 (phosphate dianion, 55-70 °C); and 0.94 ± 0.02 (hydroxide ion, 25 °C). Secondary β-deuterium isotope effects for the reactions of hydroxide ion are 0.91 ± 0.04 for NMN and 0.936 ± 0.005 for IMN. All reactions occur with rate-determining general base-catalyzed P-O bond formation. The discrepancy observed in the character of the imidazole-catalyzed hydrolysis of NMN or soman and IMN reflects different electronic effects.

The hydrolysis of fluorophosphonates and of phosphonate esters of 4-nitrophenol exhibits nucleophilic and protolytic catalysis.[1] Based on acyl transfer reactions, rate-determining P-Nu bond formation might be the dominant mode of reaction when the conjugate acid of Lg is a reasonably stronger acid than that of Nu portrayed in eq 1. In the opposite case, protolytic catalysis of hydrolysis with a transition state structure like **2** might be the expected norm.[2a-c] Vast amounts of structure reactivity data on acyl transfer reactions[2,3a] aid in sorting out the timing of bond breaking and bond making, i.e., the existence and approximate lifetime of putative intermediates in acyl,[2,3a] phosphyl,[3-5] and sulfyl[3] transfer mechanisms. The array of phosphyl and sulfyl transfer reactions covers a greater range of electronic and steric possibilities inherent in hypervalent elements[4b,c] compared to those of acyl transfer reactions.[6] Recently, vigorous effort has been



centered on the delineation of a variety of nucleophilic reactions of phosphate esters.[3,5] The same type of systematic mechanistic analysis is lacking for phosphonates and phosphinates. The positive charge at P decreases in the order phosphate > phosphonate > phosphinate, and the pentavalent adducts formed with the latter two seldom undergo permutational isomerization.[6,7] It has been reported[8] that imidazole, a key nitrogen base in bioorganic mechanisms, reacts with phosphate esters as a nucleophile but when in an encounter with 4-nitrophenyl diphenyl-

§ Catholic University of America, correspondence should be addressed to Ildiko M. Kovach.
† Simon Fraser University.
‡ AMYLIM Inc.

(1) (a) Cox, J. R.; Ramsey, O. B. *Chem. Rev.* **1964**, *64*, 317-348. (b) Kabachnik, M. I.; Brestkin, A. P.; Godovilov, N. N.; Michelson, M. I.; Rozengart, E. V.; Rozengart, V. I *Pharmacol. Rev.* **1970**, *22*, 355-388. (c) Brass, H. J.; Bender, M. L. *J. Am. Chem. Soc.* **1972**, *94*, 7421-7428. (d) Epstein, J.; Cannon, P. L., Jr.; Sowa, J. R. *J. Am. Chem. Soc.* **1970**, *92*, 7390-7393.

(2) (a) Gold, V.; Oakenfull, D. G.; Riley, T. *J. Chem. Soc. B.* **1968**, 515. (b) Kirsch, J.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 833, 837, and references therein. (c) Bender, M. L.; Turnquest, B. W. *J. Am. Chem. Soc.* **1957**, 1652. (d) Butler, A. R.; Gold, V. *J. Chem. Soc.* **1962**, *976*, 1334, 2212. (e) Williams, I. H.; Spangler, D.; Femec, D. A.; Maggiora, G. M.; Schowen, R. L. *J. Am. Chem. Soc.* **1983**, *105*, 31. (f) Kovach, I. M.; Elrod, J. P.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 7530. For leading references to acyl transfer mechanisms, see: (g) Jencks, W. P. In *Catalysis in Chemistry and Enzymology*; McGraw-Hill Inc.: New York, 1969. (h) Lowry, T. H.; Richardson, K. S. In *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper and Row Inc.: New York, 1987; Chapter 8. (i) Jencks, W. P. *Acc. Chem. Res.* **1976**, *9*, 425. (j) Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161.

(3) (a) Williams, A. *Acc. Chem. Res.* **1989**, *22*, 387 and references therein. (b) The comprehensive term "phosphyl" is adopted from Bourne, N.; Williams, A. *J. Am. Chem. Soc.* **1984**, *106*, 7591, for all tetravalent P electrophilic groups. The comprehensive term "sulfyl" might be used for all tetravalent S electrophiles. (c) Ba-Saif, S. A.; Waring, M. A.; Williams, A. *J. Am. Chem. Soc.* **1990**, *112*, 8115.

(4) (a) Benkovic, S. J.; Schray, K. J. In *Transition States of Biochemical Processes*, Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978; Chapter 13, pp 493-521. (b) Gillespie, P.; Ramirez, F.; Ugi, I.; Marquarding, D. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 91. (c) Thatcher, G. R. J.; Kluger, R. *Adv. Phys. Org. Chem.* **1989**, *25*, 99, and references therein.

(5) (a) Herschlag, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111*, 7587. (b) Skoog, M. T.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 7597-7606 and references therein.

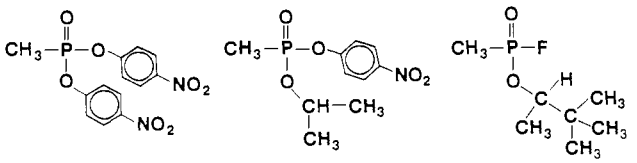
(6) (a) Kovach, I. M.; Huhta, D. *Theochem. 79* **1991**, *233*, 335. (b) Kovach, I. M.; Huhta, D.; Baptist, S. *Theochem. 72* **1991**, *226*, 99. (c) Kovach, I. M. *J. Enzyme Inhib.* **1988**, *2*, 198-208.

0002-7863/93/1515-5138$04.00/0

phosphinate, it plays the role of a general base to catalyze proton transfer from the water nucleophile. Williams and his collaborators attributed this observation to steric crowding at the electrophilic center.[8] In contrast, they noted that the mode of imidazole catalysis of acyl esters is governed predominantly by the electronic effects of the leaving groups.

Phosphonates lie between phosphates and phosphinates in structure and in complexity with respect to permutational isomerization. Imidazole-catalyzed hydrolysis of these compounds is of particular interest because it mimics some features of the inhibition of serine hydrolase enzymes by organophosphorous compounds. Recently, we reported results of solvent isotope effects and proton inventories for the inactivation of serine hydrolase enzymes.[9,10] We concluded from these studies that a single proton participates in the reaction coordinate motion which is attributed to the removal of the proton from the active-site serine hydroxyl by the imidazole of the catalytic histidine of serine proteases.[9]

Here we report results of a broader investigation of the character of transition states for nucleophilic and general base-catalyzed hydrolysis of three typical phosphonates, bis(4-nitrophenyl) methylphosphonate (NMN),[9b] 4-nitrophenyl 2-propyl methylphosphonate (IMN) and 2-(3,3-dimethylbutyl) methylphosphonofluoridate (soman), used earlier as inhibitors of serine hydrolase enzymes.[9,10]



NMN IMN Soman

We observed rate-determining P–O bond formation in spite of a broad range of reactivities in 10 reactions. Imidazole acts as a general base catalyst with all three phosphonate esters involving a large contribution of proton transfer in the transiton state of the reactions of NMN and soman and a small motion in the reaction coordinate in the reaction with IMN. This discrepancy originates from electronic effects.

Results

Rate Constants. All reactions in this study obeyed good pseudo-first-order kinetics through 6 half-lives, and the release of one stoichiometric equivalent of the leaving group was observed. Repetitive scans (240–440 nm) of the reactions of NMN and IMN with imidazole, when the other terms contributed less than 2%, showed very clear isosbestic points (300 and 320 nm, respectively). The difference spectrum of the reaction progress in 0.8 M imidazole against a reference solution of the products with added 0.8 M imidazole showed no evidence of accumulation of stoichiometrically significant intermediates. The first-order rate constants k_{obs} for the release of 4-nitrophenol from NMN and IMN in buffer solution were monitored at 400 nm and are described by eq 2

$$k_{obs} = k_{HOH} + k_B[\text{buffer}] \quad (2)$$

where [buffer] means total concentration of acidic and basic components of the buffer. For IMN hydrolysis, the dependence of k_{obs} on phosphate concentration was determined at different ratios of dianion to monoanion from which the phosphate dianion catalyzed term was calculated, while the intecept corresponding to the phosphate monoanion catalyzed term was too small to be evaluated. The same observation was reported for NMN.[1c] The dependence on temperature and phosphate concentration of the phosphate-catalyzed hydrolysis of IMN was also measured. Based on the information at 25.0 °C and with the proviso that the balance of the contribution of hydroxide ion and water attack at P does not change notably with temperature, the intercepts at pH 7.5 were used for evaluation of the temperature dependence of the water term.

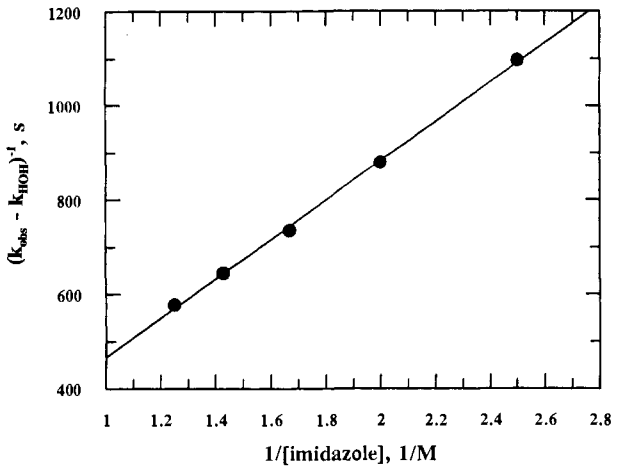


Figure 1. Double reciprocal plot for the dependence of hydrolysis of soman on imidazole base concentration; imidazole/imidazole–HCl = 9.0, 25.0 ± 0.1 °C.

In the reactions of soman with imidazole, plots of k_{obs} vs [buffer] were slightly curved, with the reaction tending toward zero order in buffer above 0.7 M imidazole base. Due to limitations inherent in the electrochemical method, the curvature was not fully characterized. Similar phenomena were earlier ascribed to saturation effects rather than the incursion of change in the rate-determining step from formation to breakdown of an intermediate.[3b,5b,11] Association between 4-nitrophenyl esters of pivalate and butyrate with imidazole at imidazole concentrations above 0.5 M were shown to be the origin of the saturation effects observed in these reactions.[11] Based on these experiences, the first-order rate constant for the hydrolysis of soman with imidazole buffer can be expressed as in eq 3

$$k_{obs} = k_{HOH} + \frac{k_{im}K[\text{Im}]}{K + [\text{Im}]} \quad (3)$$

where [Im] denotes the concentration of imidazole base, the ratio of base to conjugate acid is constant, and K is an association constant. The inverted form of eq 3

$$[k_{obs} - k_{HOH}]^{-1} = k_{im}^{-1}[\text{Im}]^{-1} + K^{-1}k_{im}^{-1} \quad (4)$$

was used for the analysis of data with soman. Figure 1 demonstrates the double reciprocal plot of $k_{obs} - k_{HOH}$ vs [Im] according to eq 4 for imidazole-catalyzed hydrolysis of soman.[11b]

(7) Morgan, W. E.; Stec, W. J.; Albridge, R. G.; Van Wazer, J. R. *Inorg. Chem.* **1971**, *5*, 926–930.

(8) (a) Williams, A.; Naylor, R. A. *J. Chem. Soc. B* **1971**, 1967. (b) Blakeley, R.; Kerst, F.; Westheimer, F. H. *J. Am. Chem. Soc.* **1966**, *88*, 112. (c) Atkinson, B.; Green, A. L. *Trans. Faraday Soc.* **1957**, *53*, 1334.

(9) (a) Kovach, I. M.; Larson, M.; Schowen, R. L. *J. Am. Chem. Soc.* **1986**, *108*, 5490–5494. (b) The system of abbreviations for the compounds is described in this paper: it is for the general formula X-R(PO)-Y, where Y is a leaving group, R a substituent attached to P, and X a second ligand. N = 4-nitrophenoxy, M = methyl, and I = isopropyl. (c) Kovach, I. M.; Schowen, R. L. In *Peptides and Proteins: Recent Advances*; Schowen, R. L., Barth, A., Eds.; Pergamon: Oxford, 1987; pp 205–212.

(10) (a) Kovach, I. M.; Huber-Ashley Harmon, J.; Schowen, R. L. *J. Am. Chem. Soc.* **1988**, *110*, 590–593. (b) Bennet, A. J.; Kovach, I. M.; Schowen, R. L. *J. Am. Chem. Soc.* **1988**, *110*, 7892–7893. (c) Bennet, A. J.; Kovach, I. M.; Bibbs, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 6424–6429. (d) Bennet, A. J.; Kovach, I. M.; Schowen, R. L. *Pestic. Biochem. Physiol.* **1989**, *32*, 78–82.

(11) (a) Gagliano, R. A., Jr.; Knowlton, R. C.; Byers, L. D. *J. Org. Chem.* **1989**, *54*, 5241–5250. (b) Under our conditions, designed for electrochemical measurements, the curvature is so slight that K is very poorly defined: it is 11 ± 12 M as calculated from the intercept.

Table I. Second-Order Rate Constants for Catalyzed Hydrolysis of Phosphonate Derivatives at 25.0 ± 0.1 °C

rate const	pK_a of base	rate const, M^{-1} s^{-1} NMN	IMN	soman
k_{HOH}	−1.7	$(6.7 \pm 2.1) \times 10^{-7}$	$(1.0 \pm 0.4) \times 10^{-7}$	1.7×10^{-6} [a]
k_{PHOS} [b]	6.7[c]	$(9.7 \pm 1.1) \times 10^{-3}$	2.65×10^{-5}	
k_{im} [d]	7.2[c]	$(6.4 \pm 0.2) \times 10^{-3}$	2×10^{-5} [e]	$(3.8 \pm 0.1) \times 10^{-3}$
k_{OH}	15.4	118 ± 3	0.27 ± 0.01	35[a]
β_{nuc}		0.48 ± 0.02	0.38 ± 0.05	0.43 ± 0.03

[a] Ref 7 for $CH_3PO[OC(CH_3)_3]F$. [b] Calculated for phosphate dianion. [c] Measured under the conditions of the kinetic experiments. [d] Calculated for imidazole free base. [e] Extrapolated from the Eyring plot to 298 K; compounded errors are estimated to be ±30%.

The reaction of hydroxide ion with NMN was measured in sodium borate solutions at different pHs (varying amounts of HCl). The rate was independent of borate concentration. The reaction of IMN with hydroxide ion was studied in aqueous sodium hydroxide solutions. The bimolecular rate constants for the reactions of hydroxide ion were determined by linear regression of four pairs of k_{obs} vs hydroxide concentration.[12]

Primary data are presented in Figures 1S–4S and Tables IS–IIIS in the supplementary material. Second-order rate constants measured at or extrapolated to 25 °C are in Table I.

Isotope Effects. In the last two columns of Table II, the isotope effects for each reaction are reported. Figures 2 and 3 illustrate the dependence of the partial solvent isotope effects k_n/k_1 (k_{im}) on the atom fraction of deuterium n in the reaction mixture for the imidazole-catalyzed hydrolysis of NMN and soman. Secondary β-deuterium isotope effects were measured for the reactions of hydroxide ion with NMN and IMN. These, along with earlier measurements[10c] for the imidazole-catalyzed reactions of the compounds, are given in the last column of Table II.

Activation Parameters. The temperature dependence of the various second-order rate constants for the hydrolysis of NMN from 25.0 to 50.0 °C and of IMN from 45.0 to 80.0 °C, in water and heavy water, were fit to the Eyring equation:

$$k_2 = kT/h(\exp -\Delta H^{\ddagger}/RT)(\exp \Delta S^{\ddagger}/R) \qquad (5)$$

Eyring plots for the imidazole base-catalyzed hydrolysis, water-catalyzed hydrolysis, and hydroxide ion attack on IMN and NMN are shown in Figures 5S–9S in the supplementary material. All activation parameters and their errors obtained by nonlinear least-squares methods are listed in Table II.

Discussion

Reaction Rates. Rate constants at 25 °C for the reactions of the three phosphonate derivatives with four different catalysts are listed in Table I. Soman and IMN are structurally similar analogs except for their leaving groups. Differences in steric hindrance to in-line attack between isopropyl and pinacolyl are small based on molecular models. Hydrogen fluoride, the conjugate acid of the leaving group in soman, is almost 10 000 times more acidic (pK 3.17)[13] than 4-nitrophenol in IMN (pK 7.2).[13] The difference between these compounds in reactivity toward each nucleophilic reagent in Table I then indicates a moderate change in the bond to the leaving group at the transition state: although comparison of the leaving tendency of fluoride to 4-nitrophenolate may seem untenable, the calcualted β_{lg} values, increasing from 0.32 for water to 0.57 for imidazole, are in the range reported for comparable phosphyl transfer reactions.[4a]

NMN is about 300 times more reactive with both imidazole base and phosphate dianion than its isopropyl analog IMN. The differences with water and hydroxide ion are 7 and 500, respectively (in the same direction). Soman is similar in reactivity to NMN with imidazole and, based on a close derivative[14] (Table I), is predicted to be one-third as reactive as NMN with hydroxide ion and similar in reactivity to NMN with water. Thus, change in the phosphonyl fragment rather than in the leaving group causes the greater change in sensitivity to a particular catalyst: the reaction of hydroxide ion shows the greatest sensitivity to the character of the phosphonyl fragment. A similar observation with acyl esters was reported.[2b] Computational studies show that there is a greater positive charge at P in NMN than in IMN in the ground state.[6a] The question is then how does the charge distribution compare between the two at the transition state? Other questions might be raised about the changes in "back-bonding" and steric effects from ground state to transition state in both NMN and IMN.[4,6a] Whatever the details are, the effect of a change in substitution of an isopropyl group (IMN) in phosphorus for 4-nitrophenyl (NMN) causes an increase in reactivity which nearly equals and therefore cancels the decline in rate observed on substitution of fluorine (soman) for 4-nitrophenol (IMN).

Catalysts. Reaction rates for each compound with the catalysts in Table I followed linear Brønsted relationships with β_{nuc} values between 0.38 and 0.48. It is curious that imidazole, the only N-nucleophile in the series, barely falls below the line for the O-nucleophiles. Although this may just be fortuitous, more likely it indicates that a P–O bond is formed in the rate-determining step in all cases, and hence the transition-state properties are similar in all reactions. This would then imply that imidazole acts as a general base in the removal of a proton from an attacking water molecule. Results of other mechanistic probes of this study actually support this contention. Brønsted β_{nuc} values of this small magnitude indicate a small sensitivity to the nature of the catalyst and are typical for protolytic catalysis of acyl ester hydrolysis, whereas nuclophilic reactions of amine nucleophiles in a series including imidazole gave β_{nuc} values around 0.8–1.0.[2b,f,g,i,j] It is true that β for the equilibrium transfer of phosphoryl was also reported to be small (1.2),[4a] which may be similar for phosphonyl transfer.[15]

Imidazole was the focus of our attention as a potential model general base for the enzymic general base-catalyzed phosphonylation by these irreversible inhibitors of serine hydrolases. Kinetics of the imidazole-catalyzed reactions showed no evidence of either the reversible formation of an intermediate or buildup of a stoichiometrically viable intermediate.[16] The rate dependence on imidazole concentration was linear with NMN and IMN and linear up to approximately 0.7 M imidazole with soman. The imidazole dependence started to fall off slightly at this concentration. Because soman was the only compound of the three that demonstrated this behavior with imidazole in the same concentration range, the origin of the interaction must be related to the

(12) Nucleophilic attack at P was evidenced by full incorporation of ^{18}O into P from $H_2{}^{18}O$ monitored by integration of the ^{31}P NMR peaks (0.033 ppm apart) for the ^{16}O and ^{18}O containing isotopomeric products of the reaction of NMN in 0.12 M NaOH. An incursion of nucleophilic aromatic substitution is even less likely in the other systems.

(13) Jencks, W. P.; Regenstein, J. In *Handbook of Biochemistry and Molecular Biology*, 3rd Ed.; CRC Press: Cleveland, OH, 1976; Vol. 1, p 314.

(14) Gubaidullin, M. G. *J. Gen. Chem. USSR* **1982**, *52*, 2182–2184.

(15) Brønsted β_{nuc} values should be interpreted with caution, because they are not unique measures of either charge development or bond order at the transition state for many reactions. Noncompliance with the Brønsted relationship includes multistep reactions that go through intermediates[15a,b] and reactions with imbalanced transition states and/or high energy barriers which are best described by curved reaction coordinates.[15c] (a) Hammond, R. B.; Williams, I. H. *J. Chem. Soc., Perkin Trans. II* **1989**, 59. (b) Pross, A.; Shaik, S. S. *J. Am. Chem. Soc.* **1982**, *104*, 1129. (c) Bernasconi, C. F. *Acc. Chem. Res.* **1987**, 301.

(16) Some spectroscopic indication of the occurrence of an *N*-(phosphonyl)imidazole intermediate, even if short lived, seems very likely. For example, release of a second 4-nitrophenol from an *N*-((4-nitrophenyl)methylphosphonyl)imidazole formed with NMN would be a real possibility. Reversible formation of an intermediate caused deviations from first-order behavior as the reaction advanced with acyl esters.[2b] The accumulation of *N*-(dibenzylphosphoryl)imidazole [8b] and other *N*-phosphorylimidazoles[8c] was also spectroscopically observable, on similar time scales to the reactions with NMN. The absence of any spectroscopic signal, then, is very likely to indicate no buildup of an N-phosphonylated intermediate.

Table II. Activation Parameters at the 1 M Standard State and Kinetic Isotope Effects for Buffer-Catalyzed Hydrolysis of Phosphonyl Derivatives

substrate buffer	concn range temp range (°C)	$\Delta H^{\ddagger}$ ± SD (kJ/mol) H_2O	 D_2O	$\Delta S^{\ddagger}$ ± SD (J/mol K) H_2O	 D_2O	k_{HOH}/k_{DOD} ± SD	k_{CH_3}/k_{CD_3} ± SD[h]
NMN, $CH_3PO)OpNP)_2$[m]	2–8 × 10^{-5} M						
imidazole[a]	25.0–55.0 ± 0.1	44 ± 2	46 ± 2	−146 ± 5	−145 ± 5	2.69 ± 0.09[h]	0.94 ± 0.02[l]
hydroxide[b]	25.0–45.0 ± 0.1	69 ± 4		27 ± 12		0.51 ± 0.02[h]	0.91 ± 0.04
water[a,b]	25.0–45.0 ± 0.1	28 ± 10		−270 ± 31		2.91 ± 1.24[h]	
phosphate[c]						1.54 ± 0.26[h]	
IMN, $CH_3PO(OiPr)(OpNP)$[m]	1–7 × 10^{-5} M						
imidazole[d]	45.0–80.0 ± 0.1	56 ± 2	57 ± 2	−150 ± 4	−152 ± 4	1.40 ± 0.07[l]	0.96 ± 0.02[l]
hydroxide[e]	25.0–46.0 ± 0.1	34 ± 2		−143 ± 5		0.94 ± 0.02[h]	0.936 ± 0.005
water[d]	45.0–80.0 ± 0.1	60 ± 4	65 ± 5	−170 ± 13	−158 ± 18	1.20 ± 0.18[l]	
phosphate[f]	55.0–75.0 ± 0.1	72 ± 3		−93 ± 9		1.11;[j] 1.15[k]	
soman, $CH_3PO(OPin)F$[m]	10–12 × 10^{-5} M						
imidazole[g]						2.79 ± 0.03[h]	0.96 ± 0.02[l]

[a] Imidazole/imidazole·HCl = 1.0, total concentration 0.1–1.0 M, pH 7.2 at 25.0 ± 0.1 °C, μ = 0.5 M (KCl), 1% v/v CH_3CN. [b] 0.025 M Sodium borate–HCl, hydroxide concentration 0.001–1 × 10^{-5} M, 0.2 M KCl, 1% v/v CH_3CN. [c] K_2HPO_4/KH_2PO_4 = 1.25, 0.05–0.20 M, pH 6.8 at 25.0 ± 0.1 °C, μ = 0.6 M (KCl). [d] Imidazole/imidazole·HCl = 9.0, total concentration 0.2–1.0 M, pH 8.0 at 25.0 ± 0.1 °C, μ = 1.0 M (KCl). [e] 0.001–0.48 M NaOH, μ = 1.0 M (KCl). [f] K_2HPO_4/KH_2PO_4 = 19, 0.05–0.20 M, pH 8.0 at 25.0 ± 0.1 °C, μ = 1.0 M (KCl). [g] Imidazole/imidazole·HNO_3 = 9.0, total concentration 0.4–0.8 M, pH 8.2 at 25.0 ± 0.1 °C, μ = 1.0 M (KNO_3), 10^{-3} M EDTA. [h] 25.00 ± 0.05 °C. [i] 73.0 ± 0.2 °C. The intercept values from the hydroxide dependence at 25.0 °C gave 1.7 ± 0.7. [j] 54.7 ± 0.2 °C. [k] 70.0 ± 0.2 °C. [l] Reference 10c. [m] OpNP = 4-nitrophenyl; OiPr = isopropyl; OPin = pinacolyl.

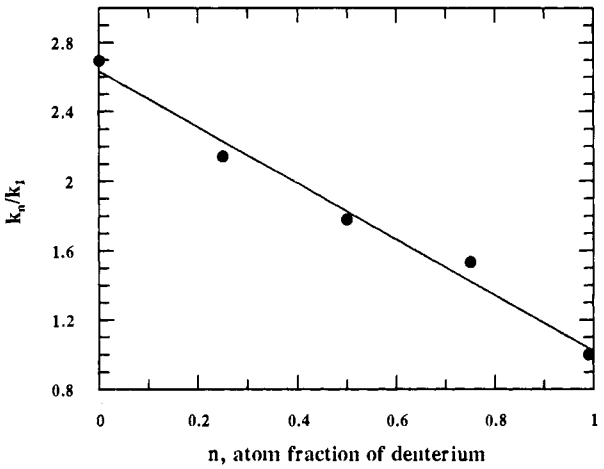


Figure 2. Proton inventory for imidazole-catalyzed hydrolysis of NMN.

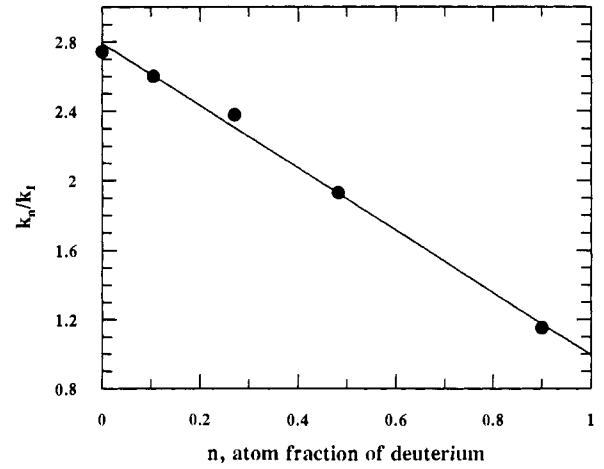


Figure 3. Proton inventory for imidazole-catalyzed hydrolysis of soman.

greater number of methyl groups in soman than in the other two esters. This judgement is based on similar phenomena observed for reactions of branched alkyl acid esters of 4-nitrophenol with imidazole and other aryl esters with methylimidazole but not with systems that have fewer hydrophobic groups.[11] van der Waals interactions between the pinacolyl side chain of soman and the approaching imidazole base can become inhibitory for this system at lower imidazole concentrations than for the other two.

Imidazole as a catalyst in the hydrolysis of these compounds is inferior to the analogous enzyme catalysts by factors of 10^5 and 10^8 when compared to the highest phosphonylation rates of chymotrypsin and acetylcholinesterase (AChE), respectively.[6c] Structural features of transition states for the imidazole-catalyzed reactions of soman, IMN, and its fluoro analog sarin were recently compared to those of their inactivation of AChE using β-deuterium isotope effect probes.[10c] The imidazole-catalyzed reactions showed looser or earlier transition states than phosphonylation of AChE. It appears that AChE with its evolutionarily highly developed catalytic machinery can compress and poise the structure in the transition state for phosphonylation, something a simple catalyst as imidazole cannot do.

Solvent Isotope Effects and Proton Inventories. The solvent isotope effects for the hydrolytic reactions of the three compounds fall into three distinct categories: large normal, small normal, and inverse. Large solvent isotope effects of 2.69 ± 0.09 and 2.79 ± 0.03 were measured for the imidazole-catalyzed hydrolyses of NMN[17] and soman, respectively. Solvent isotope effects of this magnitude are typical for protolytic reactions. Both reactions show a linear dependence on the atom fraction of deuterium in the reaction mixture for the imidazole-catalyzed attack, strongly indicating a single-proton bridge in the transition state.[18,19a] The solvent isotope effect (2.9 ± 1.2) observed for the water-catalyzed reaction with NMN, however imprecise, together with the large negative entropies, also suggests a termolecular transition state involving water-catalyzed attack of water. Thus, general base-catalyzed attack of water on these phosphonates seems to proceed with significant motion of the proton from water to catalyst and passes through early and polarized transition states.

Imidazole- and water-catalyzed reactions involving IMN show solvent isotope effects of 1.4 ± 0.07 and around 1, respectively. These are more similar to the reactions of IMN and NMN with phosphate dianion than to the imidazole reactions of the two other and more reactive phosphonate derivatives. Nonetheless, the steric hindrance, suggested by Williams and co-workers,[3a,8] to nucleophilic imidazole attack at P does not seem to be

(17) Solvent isotope effects for imidazole-catalyzed and phosphate-catalyzed hydrolysis of NMN were reported earlier to be 2.37 and 1.15 (error limits unknown).[1c]

(18) Venkatassubban, K. S.; Schowen, R. L. The Proton Inventory Technique. *Crit. Rev. Biochem.* **1984**, *17*, 1–44.

(19) (a) Alvarez, F.; Schowen, R. L. In *Isotopes in Organic Chemistry;* Buncel, E., Lee, C. C., Eds.; Elsevier: New York, 1984; pp 1–60; (b) p 42; (c) p 16. (d) Kresge, A. J.; More O'Ferrall, R. A.; Powell, M. F. In *Isotopes in Organic Chemistry;* Buncel, E., Lee, C. C., Eds.; Elsevier: New York, 1984; pp 177–276.

significantly diminished in molecular models of IMN when compared to those of soman and NMN.[20]

A rationale for the discrepancy in solvent isotope effects may be based on the consequences of general base-catalyzed water attack at a poorer electrophile in IMN. At the transition state, the loss of a smaller positive charge in IMN might be matched with a small development of negative charge at the attacking oxygen, in simplistic thinking. The reaction coordinate for such a reaction might be dominated by the movement of the heavy atoms, particularly the oxygen nucleophile, with subordinate proton transfer characterized by a low amplitude of motion at the transition state. This mode of general base catalysis could proceed with a very small solvent isotope effect, as described by Alvarez and Schowen.[19b] Certainly, the model is consistent with the arguments raised above and with the activation paramenters discussed below.

Solvent isotope effects near unity were observed for the reactions of phosphate dianion with IMN and NMN, respectively. These are probably nucleophilic reactions since the magnitude of the entropy for the phosphate-catalyzed reaction of IMN is also fitting for a bimolecular reaction. It is noteworthy though, that phosphate dianion, with a pK value 0.5 units lower than that of imidazole, reacts faster as a nucleophile than imidazole with both NMN and IMN.

The reactions of hydroxide ion with NMN and IMN are characterized by inverse solvent isotope effects. The solvent isotope effect for hydroxide attack on NMN is largely inverse, 0.51 ± 0.02, at 25 °C, which is consistent with a nearly complete loss of the contribution of the hydrogen-bonding interactions with the water hydrate shell. The overall fractionation factor for hydroxide ion calculated from exchange equilibria[19] is in the range of 0.41–0.50.[21] From the solvent isotope effect of 0.51, the transition-state fractionation factor (ϕ_T) is calculated to be between 0.85 (ϕ_{OH} = 0.434)[19d] and 0.98 (ϕ_{OH} = 0.5).[19a] This change in the fractionation factor from reactant state (0.41–0.50) to transition state (0.85–0.98) reflects the greatly diminished role of the solvent shell on covalent bond formation between oxygen and phosphorus. Consequently, the negative charge on the nucleophile is fully liberated at the transition state and stabilized by the positive charge on P. On the contrary, the solvent isotope effect for hydroxide attack on IMN is only slightly inverse, 0.94 ± 0.02 ($\phi_T \simeq 0.5$), and consistent with a small change in overall solvent reorganization from ground state to transition state. The effect may comprise a large loss of solvent from hydroxide ion compensated by a greater solvation of the phosphoryl center at the transition state. It appears that all the nucleophilic reactions we observed for IMN are characterized by little or no charge accumulation at the transition state.

Secondary β-Deuterium Isotope Effects. The effects on hydroxide ion attack at P of NMN and IMN were both inverse, 0.91 ± 0.04 and 0.936 ± 0.005, respectively, and consistently lower than the values for the imidazole reaction with the same compounds (Table II). As in the analogous acyl transfer reactions,[2f,22] alterations of the vibrational force field of the atoms in question occur in the course of the reaction.[10c] Here too, the major contribution to changes in the vibrational force constants (and inverse secondary β-deuterium isotope effects) arises from rehybridization or internal rotations[23] on going from reactants to transition states. The more inverse the effect is, the greater the departure from the sp^3 configuration in the reactant state is in the activated complex (or the greater the change is in internal rotation). Quantitation of the extent of internal restructuring from β-deuterium isotope effects is not yet possible, but the range for this effect in acyl transfer reactions, 13% (k_{3H}/k_{3D} = 0.87 maximum) per 3 D,[2f,10c] may be an acceptable guideline. Formation of a full-fledged pentavalent intermediate certainly would mean greater changes in geometry along the reaction coordinate than that in a concerted reaction. Overall, results of this probe fully support the solvent isotope effects and activation enthalpies (*vide infra*), which all point toward a less solvated transition state for NMN than for IMN with hydroxide ion. The β-deuterium isotope effect probe is consistent with greater geometric changes and charge separation for the reactions of NMN than for IMN.

Activation Parameters for NMN and IMN. The entropies of activation, shown in Table II, are similar for the imidazole-catalyzed hydrolysis of the two 4-nitrophenyl phosphonate esters; thus, the difference in reactivities must have an enthalpic origin. The entropies are large and negative and in the range expected for termolecular reactions.[2b,24] Water attack on NMN involves an even larger negative entropy. The activation parameters for water attack on IMN are similar to those for the imidazole reaction, as are the solvent isotope effects.

The entropy of activation (27 J/mol K) for hydroxide attack on NMN is much more favorable than the entropy for the other nucleophiles and most likely indicates liberation, at the transition state, of previously more tightly bound hydrate molecules around the hydroxide ion. A quite large enthalpy of activation (69 kJ/mol) and the transition-state fractionation factor also point to extensive solvent reorganization at the transition state. Rate-determining P–O bond formation in the intramolecular attack of a carbonyl hydrate anion in phosphonate ester hydrolysis occurs with a small activation enthalpy and a less negative entropy.[25] Activation parameters for hydroxide attack at IMN are much different; a sizable negative entropy and a small enthalpy are supportive[15c] of the earlier conclusions from isotope effects about a smaller change in solvent restructuring while reaching the transition state for this reaction. A less negative entropy of activation was obtained for the phosphate dianion catalyzed decomposition of IMN than that for the imidazole-catalyzed reaction, which is also consistent with a bimolecular reaction between IMN and phosphate dianion but with a termolecular reaction for the imidazole reaction. The enthalpy of activation for phosphate attack on IMN is 72 kJ/mol and may be associated with a more advanced transition state.

(20) Although N-acylation of the His residue of serine proteases has been suggested by Hubbard and Kirsch (Hubbard, C. D.; Kirsch, J. F. *Biochemistry* **1972**, *11*, 2483) for interpretation of small solvent isotope effects in the reactions of certain substrates with chymotrypsin, we find it difficult to envision nucleophilic attack at P in IMN by the imidazole of His at the active site of these enzymes.

A variation on the model may be used to explain the different behavior of imidazole toward IMN and the other two phosphonates only, but it would not account for the differences observed with water and with the serine protease enzymes: it would involve the proposition of nucleophilic attack by imidazole with formation of a pentacovalent zwitterionic transient that would need to lose the proton from the imidazole before elimination of the leaving group could take place. Proton transfer steps to solvent and other acceptors can become partly or fully rate-limiting in ionic reactions. This could be the case for the imidazole reactions with soman and NMN that may have a lower barrier for bond formation between N of imidazole and P of the phosphonate, whereas, the reaction of imidazole with IMN would be expected to involve a higher barrier for the same process. In the former case, but not in the latter, the barrier to proton transfer would be partly or fully rate-limiting.

(21) This originates from one covalently bonded hydrogen and from hydrogen bonds, probably one from each of the three water solvate molecules from the first hydration shell. The balance between the contributions of each of these is currently uncertain, except for the fact that the solvent isotope effects for methoxide attack at carbonyl compounds often are of the same magnitude (around 0.45–0.50) as with hydroxide. A covalently bonded hydrogen is not present in methoxide, only in hydroxide, but what is likely to be in common for the two nucleophiles is the composition of the solvate shell. Therefore, it might be more realistic to consider the overall fractionation factor for hydroxide ion to consist of an internal fractionation factor of unity and three fractionation factors, as in methoxide ion, of approximately 0.74 each ($\phi_{OH} = \phi_{ini}\phi_{solv}^3$ = 0.41–0.50).

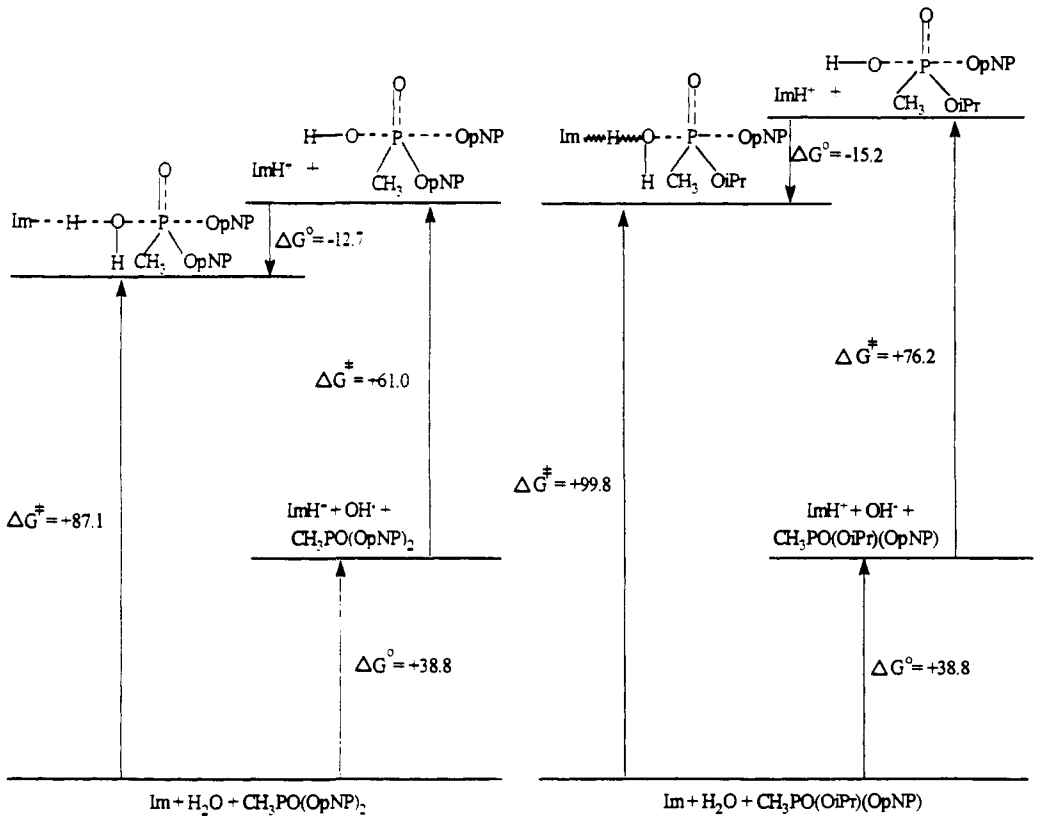
(22) (a) Hogg, J. L. See ref 4a. (b) Williams, I. *Theochem.* **1983**, *105*, 105–107. Changes in mass/moment of inertia have been found to be minor contributions to β-deuterium isotope effects.

(23) Huskey, W. P. *J. Phys. Chem.* **1992**, *96*, 1263–1265.

(24) Bunnett, J. F. In *Techniques of Chemistry*, 4th ed.; Bernasconi, C. F. Ed.; Wiley: New York, 1986; Chapter IV, pp 251–253.

(25) Kovach, I. M.; Zhao, Q.; Keane, M.; Reyes, R. *J. Am. Chem. Soc.*, submitted for publication.

Scheme I



Transition States for Reactions of the 4-Nitrophenyl Phosphonate Esters. Scheme I portrays a free energy diagram for 1 M standard states at 25.0 °C for the imidazole-catalyzed water attack and the hydroxide ion attack on NMN and IMN each. The reactant-state free energies for the two reactions are shown at the same level for easy comparison of the activation free energies for the two reactions of each compound. The standard free energy for the ionization equilibrium for the protonation of neutral imidazole by water with the generation of hydroxide ion at the 1 M standard state was calculated to be 38.8 kJ/mol from the pK.[13]

There is a 12.7 kJ/mol lower transition-state free energy for the water reaction, with the proton transferred extensively to imidazole, than that for the hydroxide ion reaction with NMN in the presence of imidazolium ion, that is, the binding of imidazolium ion, a hydrogen donor, to the transition state for nucleophilic attack liberates this free energy.[26] Both transition states are characterized by considerable accumulation of negative charge on the nucleophile to counter the positive charge at P.

The transition-state stabilization by imidazolium ion, involving a proton transferring with a low amplitude relative to that of the hydroxide ion reaction with IMN, is 15.2 kJ/mol. Although the difference in the values of the β-deuterium isotope effects between the hydroxide and imidazole reactions is the same within experimental error as that for NMN, a greater reorganization in the internal structure may be associated with the larger energy change on binding imidazole to the nucleophilic transition state in IMN than in NMN. Both of these transition states with IMN are expected to be less polarized than those for NMN.

Internal structural reorganization in the course of binding of acetic acid to the transition state for nucleophilic attack by hydroxide ion at phenyl acetate was greater than what is observed in these reactions.[2f] These changes in the geometry of transition states upon binding of a hydrogen donor, compared to essentially no changes in the geometry of stable species upon hydrogen-bonding, reflect the high degree of plasticity of transition states with respect to stable states.[2f] In the acyl-transfer case, the energy release on binding acetic acid was −15 kJ/mol, similar to that of the IMN reactions. This energy is probably smaller than the total energy of interaction between imidazole and the reorganized transition state. It might be considered to be the algebraic sum of two terms: an endergonic term for distortion, ΔG_{dis}, and a significantly exergonic term for binding, ΔG_{bin}.[27]

Summary. We find that P–O bond formation is dominant in the rate-determining process in reactions of three phosphonates with four different catalysts. Imidazole and water act as general base catalysts in the hydrolysis of NMN and soman: the transition states for these reactive phosphonates are early and have a significant component from a single proton transferring from water to imidazole. Small enthalpies and large negative entropies of activation are also in full agreement with this analysis. The imidazole-catalyzed reaction of IMN involves a small solvent isotope effect, a larger enthalpy than that of NMN, and a large negative entropy, indicative of a more advanced transition state with a small motion of the proton in the rate-limiting step. This difference in the mode of reaction between NMN or soman and IMN is a consequence of a smaller change in the charge at P between reactant and transition states for IMN than for the other two. Phosphorus in the reactant state of IMN is less positive than P in NMN and soman,[6,7] and our isotope effect data support the contention that negative charge does not accumulate significantly at P in the transition state either. Transition states for inactivation of serine proteases have similar characteristics to the imidazole-catalyzed hydrolysis of these compounds.[9]

Phosphate dianion and hydroxide ion react as nucleophiles with NMN and IMN. The hydroxide reactions involve a

(26) Assuming that the maximal β-deuterium isotope effect in phosphonyl transfer is at least as much as it is in acyl transfer, 13% per 3 D, the internal structural rearrangement accompanying the complexation is a 0.23 decrease in bond order corresponding to an increase in the P–O bond length by 0.36 Å, as calculated from the kinetic β-deuterium isotope effects.

(27) The rationale here may be reminiscent of Jencks' idea of intrinsic transition-state binding energy in enzyme catalysis: Jencks, W. P. *Adv. Enzymol. Relat. Areas Mol. Biol.*, **1975**, *43*, 219.

significant solvent reorganization at the transition state for NMN and much smaller changes in solvation at the transition state for IMN. The transition state for the latter reaction is imbalanced.[15c]

Experimental Section

Materials. Inorganic salts and buffer components were reagent grade chemicals, which were used as purchased or dried, recrystallized, or distilled as necessary. The imidazole base (Aldrich) was twice recrystallized from benzene, and the HNO_3 was titrated against NaOH standards (Fisher). Dry HCl gas was used to generate the imidazole hydrochloride. Water was distilled from a copper-bottom still, passed through a Barnstead mixed-bed ion-exchange column, boiled for 20 min, and cooled suddenly. Deuterium oxide (Norell Inc. 99.9% deuterium) was used shortly after opening the bottle. Soman was provided by the Army Medical Institute for Chemical Defense in 10^{-2} M stock solutions. These solutions were analyzed for intact soman[10d] and further diluted, for kinetic work, to 10^{-4} M. The synthesis of 4-nitrophenyl phosphonate esters and their β-CD_3 isotopomers was published earlier.[9,10c] NMN was recrystallized three times from methanol/acetone, and IMN was purified by HPLC. The fresh eluent of IMN in methanolic solution and stock solutions of NMN were hydrolyzed to 4-nitrophenol for spectrophotometric measurement at 400 nm before each experiment. The ester content of the samples was above 99%.

Rate Measurements. Automated acquisition of 200–1000 data points at 400 nm with a Perkin-Elmer Lambda-7 spectrophotometer interfaced to a Zenith Z-100 microcomputer was used for 4-nitrophenyl derivatives. First-order rate constants were calculated for 4 half-lives by least-squares fit of absorbance/time coordinates and then four to five repeats were averaged to obtain k_{obs}. The temperature was controlled with a Lauda K4/DR circulating water bath furnished with a thermistor probe attached to a digital readout. Measurements of the pH of kinetic solutions before and after reaction were performed with a Radiometer PHM 84 pH meter. Fluoride release from soman was monitored with a Radiometer PHM 84 Research pH meter that had been interfaced into a Zenith-158 computer. A Radiometer F-1052F fluoride electrode and a Radiometer K801 AgCl reference electrode were used. The temperature was controlled within ±0.1 °C of the working temperature using a Neto 01-T-623 temperature controller that provided circulation of water into the jacket of a cell holder. The temperature was monitored continually inside the reaction vessel with a thermometer. All fluoride determinations were carried out in polyethylene vials. Readings in millivolts were converted to concentrations using the Nernst equation. The time course of concentration was then analyzed for 4 half-lives using a nonlinear least-squares program. Least-squares slopes from k_{obs} vs concentration of imidazole free base gave individual rate constants according to eq 1. If curvature was observed in primary plots, then $k_{obs} - k_{HOH}$ values vs imidazole concentration were replotted according to eq 3 (see Figure 1). Linear least-squares slopes of these reciprocal plots gave satisfactory (inverse) values of k_{Im}, but the intercept values were associated with errors too large for an accurate assessment of the other parameter.

Solutions. The reactions of soman were carried out at pH 8.2, (p*D* 8.7) in imidazole buffers that contained a ratio of 9:1 free base to the HNO_3 salt in mixtures of water and heavy water. Imidazole–HCl was used in the analogous buffer system for the reactions of IMN. The reactions of NMN were carried out at pH 7.2 (p*D* 7.7) in buffers of imidazole/imidazole–HCl, 1:1. Serial dilutions of a 1 M imidazole buffer solution at μ = 1.0 were made with 1 M KNO_3 and 10^{-3} M EDTA for the phosphonofluoridates and with 1 M KCl for the 4-nitrophenolates in aqueous and D_2O solutions. Phosphate buffers were diluted from a 0.2 M total phosphate solution at pH 7.5 (p*D* 8.0) and 6.7, μ = 1.0 (KCl) with 1 M KCl in water and heavy water, respectively. Sodium borate solutions, 0.025 M and 0.2 M in KCl, were adjusted with HCl to give pH values of 8.7–9.2 in H_2O and 9.3–9.7 in D_2O. Sodium hydroxide solutions were used in 0.001–0.01 M concentrations in H_2O and in D_2O in conventional measurements and in 0.01–0.5 M concentrations in stopped-flow measurements and were 1 M in KCl. The base content was determined through titration against potassium hydrogen phthalate acid standard (Fischer Scientific Co.).

Kinetic Protocol. The 4-nitrophenyl phosphonate esters were used in $1–3 \times 10^{-3}$ M stock solutions. Stock solutions of NMN were prepared in reagent grade acetonitrile. In a typical kinetic experiment, the appropriate volume of buffer was equilibrated within 0.05 °C of the working temperature (as monitored with a thermistor probe) in a quartz cuvette in the cell compartment of the instrument. Ten microliters of the substrate solutions were introduced into 1 mL total volume to initiate the reaction. Only one 4-nitrophenol was released from NMN in the time frame of these experiments. Our experiments as well as previous work[1b,c] showed at least a 1000-fold difference in leaving tendency of the 4-nitrophenol group between NMN and its first hydrolysis product, the negatively charged phosphonate hemiester. The good linearity of the first-order plots reflects this fact.

For fluoride measurements, the fluoride electrode was calibrated each time with solutions of the serial dilution of a 0.1 M fluoride standard (Radiometer) into the appropriate imidazole buffers and 1 M KNO_3. All solutions were allowed to equilibrate for 15 min in the thermostatted titration cell in a total volume of 5 mL. The reaction was initiated by the injection of 250 μL of a stock solution of soman into the preequilibrated reaction mixture (4.75 mL).

Stopped-Flow Measurements. An Olis stopped-flow apparatus interfaced to a Zeos 386 computer and furnished with Olis software was used for these measurements. The temperature was controlled through a simple water bath with a pump to provide circulation around the cell block and syringes. IMN stock solutions were diluted with dilute HCl to a concentration of 10^{-5} M to be used in one of the drive syringes, which were then diluted 1:1 in the cell compartment.

Acknowledgment. The encouragement and most helpful suggestions of Professor R. L. Schowen are gratefully acknowledged. This work was supported in part by NSF grant DMB 9009344 and in part by Contract No. DAMD-17-83-C-3199 from the U.S. Army Medical Research and Development Command.

Registry No. Bis(4-nitrophenyl) methylphosphonate, 6395-57-9; 4-nitrophenyl 2-propyl methylphosphonate, 3735-97-5; 2-(3,3-dimethylbutyl) methylphosphonofluoridate, 96-64-0,

Supplementary Material Available: Primary kinetic data and nine figures (15 pages). Ordering information is given on any current masthead page.