Catalytic Antibody Mediated Hydrolysis of Paraoxon

Brian J. Lavey and Kim D. Janda*

Departments of Chemistry and Molecular Biology, The Scripps Research Institute and the Skaggs Institute for Chemical Biology, 10550 North Torrey Pines Road, La Jolla, California 92037

Received April 4, 1996

Phosphate ester insecticides have become the dominant form of insect control on the world market. First synthesized in the 1930s and later developed in the 1940s and 1950s, tetrasubstituted phosphorus(V) compounds such as paraoxon, parathion, and diazinon (Figure 1) became the commercial insecticides of choice after the problems associated with the persistence in the environment of aromatic halocarbons such as DDT became understood. As materials which will decompose slowly in water,[1,2] phosphotriesters such as paraoxon are believed to be less environmentally hazardous than other categories of insecticides. Unfortunately, phosphate insecticides are also most often responsible for the poisoning of agricultural workers.[3,4]

The harmful effects of phosphotriester insecticides are related to their inhibition of mammalian acetylcholinesterase, the enzyme responsible for regulating the *in vivo* concentration of the neurotransmitter acetylcholine. Early reports suggested that parathion was unreactive toward mammalian acetylcholinesterase *in vitro* and was presumably less toxic than paraoxon.[5] It was rapidly discovered, however, that parathion is converted into paraoxon *in vivo*,[6,7] by microsomal P_{450} enzymes *via* oxidative desulfuration of phosphorus.[8−10] Given the broad substrate specificity of P_{450} enzymes, it is likely that desulfuration occurs with other phosphorothioate insecticides as well.

Antibodies have a long history of being used therapeutically to treat poisoning by snake venoms and other natural toxins by binding the foreign substances and isolating them from susceptible tissues.[11] We believe that antibodies also have potential as a means of alleviating the effects of overexposure to phosphate insecticides and other phosphonate poisons, including nerve agents such as sarin and tabun. This potential may be clinically exploited further than has been done previously in affinity based antibody therapies by using an antibody to catalytically decompose the toxin of interest rather than simply immobilize it. As part of a program of applying antibody catalysis to phosphate hydrolysis,[12] we chose to study the antibody mediated hydrolysis of paraoxon.

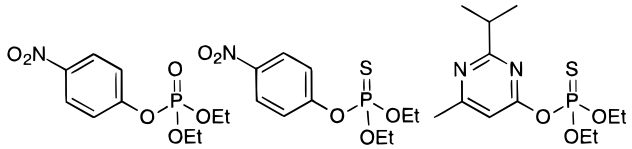


Figure 1. Some representative phosphate insecticides.

In designing haptens that will induce antibody catalysis, two strategies may be followed. In the first, a compound which mimics the geometry and charge distribution of the transition state as closely as possible is used as the hapten. In the second strategy, which is referred to as "bait and switch", a compound which is not necessarily a transition-state analog is synthesized with the intent of inducing specific complementary amino acid residues capable of mechanistic interaction with the substrate.[13−17] Previous demonstrations of this principle have focused on the placement of a general base in the active site of an antibody binding pocket. Each strategy has advantages and disadvantages with regard to a particular reaction and its mechanism. Unfortunately, in the case of phosphotriesters, enzymatic systems do not provide a useful guide for hapten design. There is one known type of phosphotriesterase[18,19] which is a highly efficient catalyst but requires two zinc ions which are held in place by four active site histidines for catalytic activity. Previous work with antibodies has successfully made use of metal cofactors to bring about catalysis.[20−23] However, designing a hapten that will induce an antibody which is capable of binding two metal cations in a precise alignment with a substrate remains problematic at this time, so other strategies were pursued.

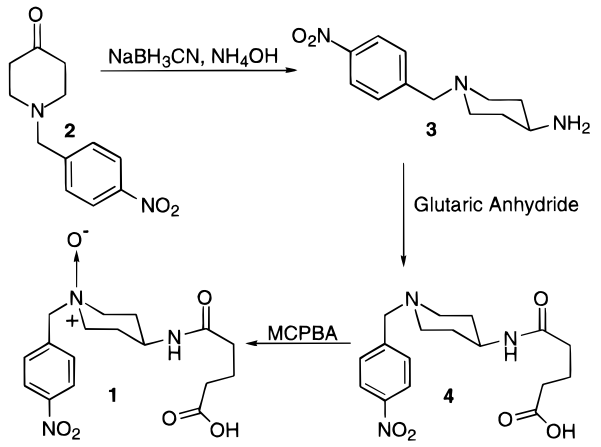
The hydrolysis of phosphotriesters is first order in hydroxide ion and is believed to proceed *via* an in-line displacement mechanism through a trigonal bipyramidal transition state with the nucleophile and leaving group in the apical positions.[24−27] This implies that the hy-

* Author to whom correspondence should be addressed. Phone: International code + (619)-554-4318. Fax: International code + (619)-554-6068. E-mail: kdjanda@scripps.edu.

(1) Coates, H. *Ann. Appl. Biol.* **1949**, *36*, 156.
(2) Ginjaar, L.; Vel, S. *Rec. Trans. Chim. Pays−Bas* **1958**, *77*, 956.
(3) Ecobichon, D. J. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 4th ed.; Amdur, M. O., Doull, J., Klaasen, C. D., Eds.; McGraw-Hill: New York, 1991; pp 565.
(4) Murphy, S. D. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 3rd ed.; Amdur, M. O., Doull, J., Klaasen, C. D., Eds.; McGraw-Hill: New York, 1986; pp 519.
(5) Aldridge, W. N.; Davison, A. N. *Biochem. J.* **1952**, *52*, 663.
(6) Dubois, K. P. ; Doull, J.; Coon, J. M. *Fed. Proc.* **1948**, *7*, 216.
(7) Diggle, W. M.; Gage, J. C. *Nature* **1951**, *168*, 998.
(8) Neal, R. A. *Biochem. J.* **1967**, *103*, 183.
(9) Nakatsugawa, T.; Dahm, P. A. *Biochem. Pharmacol.* **1967**, *16*, 25.
(10) Norman, B. J.; Poore, R. E.; Neal, R. A. *Biochem. Pharmacol.* **1974**, *23*, 1733.
(11) Butler, V. P., Jr. *Pharmacol. Rev.* **1982**, *34*, 109.
(12) Rosenblum, J. S.; Lo, L.-C.; Li, T. ; Janda, K. D.; Lerner, R. A. *Angew. Chem.* **1995**, *107*, 2448; *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2275. For other work on antibody-mediated hydrolysis of phosphates and phosphonates, see: Scanlan, T. S.; Prudent, J. R.; Schultz, P. G. *J. Am. Chem. Soc.* **1991**, *113*, 9397. Brimfield, A. A.; Lenz, D. E; Maxwell, D. M.; Broomfield, C. A. *Chem. Biol. Interact.* **1993**, *87*, 95. For recent surveys of the catalytic antibody literature, see: Lavey, B. J.; Janda, K. D. In *Antibody Expression and Engineering*; Wang, H. Y., Imanaka, T., Eds; ACS Symposium Series 604; American Chemical Society: Washington, DC, 1995; Chapter 10. Schultz, P. G.; Lerner, R. A. *Science* **1995**, *269*, 1835.
(13) Janda, K. D. *Abstracts of Papers*, 198th National Meeting of the American Chemical Society; American Chemical Society: Washington, DC, 1987; ORGN 196.
(14) Janda, K. D.; Weinhouse, M. I.; Schloeder, D. M.; Lerner, R. A.; Benkovic, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 1274.
(15) Shokat, K. M.; Leumann, C. J.; Sugasawara, R.; Schultz, P. G. *Nature* **1989**, *338*, 269.
(16) Janda, K. D. *Biotechnol. Prog.* **1990**, *6*, 178.
(17) Janda, K. D.; Weinhouse, M. I.; Dannon, T.; Pacelli, K. A.; Schloeder, D. M. *J. Am. Chem. Soc.* **1991**, *113*, 5427.
(18) Omburo, G. A.; Kuo, J. M.; Mullins, L. S.; Raushel, F. M. *J. Biol. Chem.* **1992**, *267*, 13278.
(19) Lewis, V. E.; Donarski, W. J.; Wilk, J. R.; Raushel, F. M. *Biochemistry* **1988**, *27*, 1591.
(20) Iverson, B. L.; Lerner, R. A. *Science* **1989**, *243*, 1184.
(21) Wade, W. S.; Ashley, J. A.; Jahangiri, G. K.; McElhaney, G.; Janda, K. D.; Lerner, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 4906.
(22) Cochran, A. G.; Schultz, P. G. *Science* **1990**, *249*, 781.
(23) Crowder, M. W.; Stewart, J. D.; Roberts, V. A.; et al, *J. Am. Chem. Soc.* **1995**, *117*, 5627.

S0022-3263(96)00629-9 CCC: $12.00

Scheme 1. Synthesis of *N*-Oxide Hapten 1



drolysis of paraoxon should be susceptible to specific base catalysis. Paraoxon hydrolysis also might be accelerated by either the protonation of the leaving-group alkoxide or the stabilization of developing negative charge on the P═O bond.

We believed that both of these catalytic processes might be accessible using a bait and switch approach with *N*-oxide **1** as the hapten. The partial positive charge on the tetrasubstituted nitrogen could induce a general base that would assist in the formation of hydroxide in the appropriate location to attack the phosphorus atom. The partial negative charge on the *N*-oxide oxygen can induce either a residue capable of protonating the leaving group according to a bait and switch strategy or a residue that would stabilize the developing negative charge on the phosphoryl oxygen as the transition state is approached. We hoped to define whether antibodies raised against **1** would act to hydrolyze paraoxon primarily by stabilizing transient charges in the transition state or by general acid and general base mechanisms. Hapten **1** was synthesized by treating piperidone with nitrobenzyl bromide and then forming amine **3** *via* reductive amination (Scheme 1). Attachment of the linker by treatment with glutaric anhydride followed by oxidation with *m*CPBA gave **1**.

Hapten **1** retains the nitrophenyl group and general shape of paraoxon. Previous work has shown that antibodies raised against the *p*-amino analog of paraoxon would bind to the insecticide.[28,29] However, the use of a phosphate triester as a hapten would make the incorporation of useful charged functionality difficult. The retention of a nitroaromatic moiety in the hapten was also believed to be desirable for induction of a strong immune response. As a result, the linker was attached on the cyclohexyl side of the molecule rather than the aromatic portion.

Of 25 monoclonal antibodies raised against **1**, one catalyzed the hydrolysis of paraoxon. Antibody 3H5 exhibited Michaelis−Menten kinetics and displayed turnover. At a pH of 8.25, hapten **1** is a competitive, tight-binding inhibitor of antibody 3H5 with a K_i of 0.98 μM. The k_{cat} values of antibody 3H5 ranged from 3.8×10^{-4} min^{-1} to 1.9×10^{-3} min^{-1} and increased linearly with

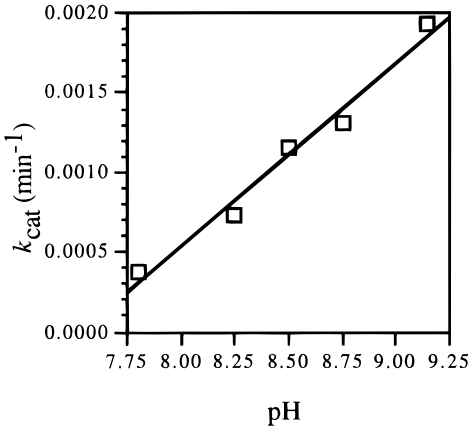


Figure 2. Change in k_{cat} with pH. The slope of 1.1 suggests that the rate of hydrolysis is proportional to hydroxide ion concentration.

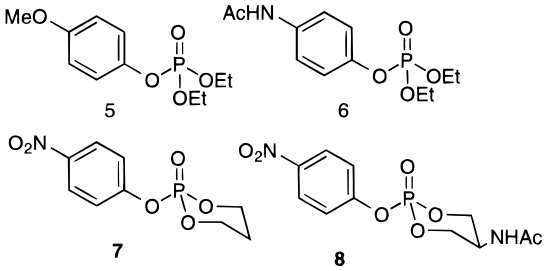


Figure 3. Compounds examined for hydrolytic activity by antibody 3H5.

Table 1. Kinetic Parameters for the Hydrolysis of Paraoxon between pH 7.8 and 9.1

pH	k_{uncat} (min^{-1})	k_{cat} (min^{-1})	K_M (mM)
7.80	7.97×10^{-7}	3.99×10^{-4}	1.60
8.25	1.80×10^{-6}	7.00×10^{-4}	1.92
8.51	2.23×10^{-6}	1.15×10^{-3}	3.73
8.75	2.95×10^{-6}	1.30×10^{-3}	4.40
9.15	5.64×10^{-6}	1.95×10^{-3}	5.05

hydroxide ion concentration in the pH range 7.8−9.1 (Figure 2, Table 1). The linear dependence of the rate of catalysis on hydroxide ion concentration implies that antibody 3H5 mediates the reaction primarily through transition-state stabilization rather than general acid or base catalysis. It is unclear whether this effect is due to the inability of an *N*-oxide to induce a general base in the antibody combining site, or whether it is simply that any side chains capable of acting as a general base are incorrectly positioned for the necessary in-line displacement by hydroxide. Unfortunately, kinetic measurements alone will not distinguish between these two posssibilities.

In order to determine the substrate specificity of antibody 3H5, four additional compounds were prepared (Figure 3). Compound **5** was prepared by treating diethyl phosphorochloridate with *p*-methoxyphenol in the presence of base. Compound **6** was synthesized from paraoxon by hydrogenolysis over platinum on carbon followed immediately by acetylation with acetic anhydride and a catalytic amount of DMAP. Compounds **7** and **8** were prepared by reacting *p*-nitrophenyl phosphorodichloridate with 1,3-propanediol and *N*-acetylserinol, respectively. Antibody 3H5 did not appreciably accelerate the rate of hydrolysis of any of these compounds. This suggests that the aromatic nitro group is one of the primary recognition sites for the antibody. The fact that

(24) Knowles, J. R. *Annu. Rev. Biochem.* **1980**, *49*, 877.
(25) Khan, S. A.; Kirby, A. J. *J. Chem. Soc. B* **1970**, 1172.
(26) Cox, J. R.; Ramsay, O. B. *Chem. Rev.* **1964**, *64*, 317.
(27) Caldwell, S. R.; Raushel, F. M.; Weiss, P. M.; Cleland W. W. *Biochemisty* **1991**, *30*, 7444.
(28) Sternberger, L. A.; et al. *US Army Sci. Conf. Proc.* **1972**, *3*, 429.
(29) Hunter, K. W., Jr.; Lenz, D. E. *Life Sci.* **1982**, *30*, 355.

the hydrolysis of compounds **7** and **8** was not accelerated suggests that the cyclohexyl and *N*-acyl portions of hapten **1**, which are closest to the KLH protein, are not recognized by the antibody.

The ability of antibody 3H5 to hydrolyze paraoxon suggests that catalytic antibodies may be an appropriate means of decomposing toxins such as insecticides *in vivo*. The advantage of using a catalytic antibody rather than an antibody which only binds to the toxin is that hydrolysis of the toxin and the subsequent release of relatively innocuous decomposition products leaves the antibody free for further substrate binding. To be fully useful in clinical therapy, higher rates of catalysis and lower K_M values than observed with 3H5 will be needed. We believe, however, that this work makes a significant first step toward developing antibodies for use in treatment of organophosphate insecticide poisoning. In the future, this principle may be applied to other materials.

Experimental Section

General. ^{13}C NMR spectra were obtained at 125 MHz. Melting points were determined on open slides and are uncorrected. Flash and sinter funnel[30] chromatography were carried out with Mallinckrodt silica gel 60 (230−400 mesh). Analytical TLC was performed on Merck glass plates coated with 0.25 mm silica. Preparative TLC was performed with Merck glass plates coated with 1 mm silica. Chromatographic and reagent solvents were reagent grade and were used as received. All reagents were obtained from commercial sources and were used without further purification. Brine refers to a saturated solution of sodium chloride. Elemental analyses were performed by Galbraith Laboratories Inc.

Antibody Preparation. Hapten **1** was coupled to keyhole limpet hemocyanin (KLH) and bovine serum albumin using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) as previously reported.[14] Mice were immunized with the KLH conjugate of **1**, and monoclonal antibodies were obtained *via* standard procedures.[31,32] Antibody 3H5 was purified *via* DEAE anion exchange chromatography, Protein G affinity chromatography, and Mono Q anion exchange chromatography and was demonstrated to be >98% homogeneous by SDS acrylamide gel electrophoresis.

Assays. Paraoxon (90%) was purchased from Aldrich and then purified *via* flash column chromatography using EtOAc/CH_2Cl_2/hexanes as the mobile phase. Assays for the hydrolysis of **6** and **7** were carried out on a Hitachi HPLC using a reverse phase column and UV detection at λ = 286 nm. All kinetic assays for the release of *p*-nitrophenol were performed at 25 ± 0.1 °C on a Molecular Devices Thermomax ELISA plate reader using the absorbance of light at 405 nm to observe the formation of *p*-nitrophenolate. Calibration curves for the absorbance of *p*-nitrophenol were obtained for each pH.

All paraoxon hydrolysis reactions were performed in 96-well microtiter plates containing 235 μL of 50 mM bicine with 20 μM antibody and 5% DMSO. For each pH, kinetic parameters were obtained from the average of two runs using substrate concentrations between 250 μM and 5 mM of paraoxon. Initial Dixon plot experiments showed the hapten to be a tight-binding inhibitor of antibody 3H5. As a result, the K_i was determined using the Copeland[33] variation of the Henderson plot.

4-Piperidinone, 1-[(4-Nitrophenyl)methyl]- (2). *p*-Nitrobenzyl bromide (7.35 g, 3.40 mmol) and 4-piperidone (5.00 g, 3.25 mmol) were suspended in 13 mL of DMF and 8 mL of Et_3N. The mixture was stirred at rt for 17 h, then filtered through a celite pad, concentrated, and chromatographed using an IPA/CH_2Cl_2 gradient to give 5.07 g (66%) of **2** as a white solid, mp 116−118. ^1H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, J = 8 Hz, 2 H), 7.53 (d, J = 8 Hz, 2 H), 3.68 (s, 2 H), 2.72 (t, J = 6 Hz, 4 H), 2.43 (t, J = 6 Hz, 4 H). ^{13}C NMR: δ 208.4, 147.2, 146.1, 129.2, 123.6, 61.1, 52.9, 41.1. HRMS (ESI+): calcd 235.1083, found 235.1076. Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.52; H, 6.02; N, 11.96. Found: C, 61.51; H, 6.13; N, 11.85.

4-Piperidinamine, 1-[(4-Nitrophenyl) methyl]- (3). (Nitrobenzyl)piperidone **2** (0.852 g, 3.64 mmol) was dissolved in 20 mL of MeOH, and 3 Å molecular sieves (1.5 g), NH_4OAc (2.845 g, 37.0 mmol), and $NaBH_3CN$ (0.127 g, 2.02 mmol) were added. The reaction mixture was stirred at rt for 16 h and then concentrated. H_2O (10 mL) was added, followed by 1 M HCl to pH 2. After gas evolution had ceased, the flask was placed in an ice bath and KOH (s) was added until the pH was 7. The solvent was evaporated to give 6.36 g of solid, which was subjected to sinter funnel chromagraphy using MeOH/NH_4OH/CH_2Cl_2 as the mobile phase to give 711 mg (83%) of yellow oil as the product. HPLC (λ = 254 nm) indicated that this material was 96% pure. It was used in the next step without further purification. ^1H NMR (300 MHz, CD_3OD): δ 8.14 (d, J = 8 Hz, 2 H), 7.53 (d, J = 8 Hz, 2 H), 3.57 (s, 2 H), 2.82 (d, J = 12 Hz, 2 H), 2.70−2.65 (m, 1 H), 2.55−2.35 (m, 1 H), 2.13 (t, J = 12 Hz, 2 H), 1.82 (d, J = 12 Hz, 2 H), 1.43−1.33 (m, 2 H). ^{13}C NMR: δ 146.9, 146.7, 129.3, 123.3, 61.9, 52.4, 48.4, 35.6. HRMS (ESI+): calcd 236.1399, found 236.1392.

Pentanoic Acid, 5-[[1-[(4-Nitrophenyl)methyl]-4-piperidinyl]amino]-5-oxo- (4). Amine **3** (521 mg, 2.21 mmol) and glutaric anhydride (0.386 g, 3.38 mmol) were dissolved in 4 mL of CH_2Cl_2 and then stirred at rt for 6.5 h. Evaporation of the solvent followed by flash chromatography (NH_4OH/MeOH/CH_2Cl_2) afforded 633 mg (82% yield) of yellow solid. ^1H NMR (300 MHz, CD_3OD): δ 8.14 (d, J = 9 Hz, 2 H), 7.53 (d, J = 9 Hz, 2 H), 4.86 (s, 1 H), 3.85 (s, 1 H), 3.85−3.70 (m, 1 H), 3.0 (d, J = 12 Hz, 2 H), 2.05 (t, J = 12 Hz, 2 H), 1.89 (d, J = 12 Hz, 2 H), 1.64−1.39 (m, 2 H). ^{13}C NMR: δ 178.4, 175.4, 175.0, 148.8, 145.7, 131.6, 124.5, 62.5, 53.3, 52.0, 47.4, 36.4, 35.4, 34.0, 32.0, 22.9, 21.8. HRMS (FAB): calcd 350.1716, found 350.1719. Calcd for $C_{17}H_{23}N_3O_5$: C, 58.43; H, 6.63; N, 12.03. Found: C, 58.53; H, 6.86; N, 11.82.

Pentanoic Acid, 5-[[1-[(4-Nitrophenyl)methyl]-4-piperidinyl]amino]-5-oxo- (1). Amino acid **4** (43 mg, 0.123 mmol) was dissolved in 5 mL of MeOH, and *m*CPBA (Aldrich 50−60%, 60 mg) was added. The reaction mixture was stirred at rt for 1 h. Purification by preparative TLC using 2% AcOH/10% MeOH/88% CH_2Cl_2 as the mobile phase gave 38 mg (84%) of white solid as product. HPLC (λ = 254 nm) indicated that this material was 99% pure. ^1H NMR (300 MHz, CD_3OD): δ 8.13 (d, J = 9 Hz, 2 H), 7.80 (d, J = 9 Hz, 2 H), 4.55 (s, 2 H), 3.75−3.9 (m, 1 H), 3.65−3.50 (t, J = 12 Hz, 2 H), 3.47−3.35 (d, J = 12 Hz, 2 H), 2.26 (t, J = 7 Hz, 2 H), 2.19 (t, J = 7 Hz, 2 H), 2.15−2.00 (m, 2 H) 1.95−1.75 (m, 4 H). HRMS (FAB): calcd 366.1665, found 366.1656.

Phosphoric Acid, Diethyl 4-Methoxyphenyl Ester- (5). Hunig's base (1mL) and *p*-methoxyphenol (255 mg, 2.06 mmol) were dissolved in 3 mL of CH_3CN, and diethyl phosphorochloridate was added (290 μL, 2.01 mmol). The reaction mixture was stirred at rt for 15 h and then concentrated and redissolved in 50 mL of EtOAc. After washing with citric acid (3 × 5 mL) and brine (2 × 5 mL), the solution was evaporated and subjected to flash chromatography using EtOAc/hexanes as the mobile phase to give 345 mg of the known[34] **5** as a clear oil (66%). ^1H NMR (500 MHz, $CDCl_3$): δ 7.12 (d, J = 10 Hz, 2 H), 6.82 (d, J = 10 Hz, 2 H), 4.22−4.16 (m, 4 H), 3.76 (s, 3 H), 1.33 (t, 7 Hz, 6 H). ^{13}C NMR: δ 156.6, 144.2, 120.8, 114.5, 64.4, 55.5, 16.0. HRMS (FAB): calcd 261.0892, found 261.0900.

Phosphoric Acid, 4-(Acetylamino)phenyl Diethyl Ester- (6). A Parr hydrogenation bottle was placed under an Ar blanket, and 5% Pt on C (20 mg) was added, followed by MeOH (10 mL) and a solution of paraoxon (365 mg, 1.33 mmol) in 5 mL of MeOH. The flask was placed under 25 psi of H_2 and shaken at rt for 4 h. The resulting mixture was filtered, and the solvent was evaporated to give 320 mg of brown oil. This material was redissolved in 20 mL of CH_2Cl_2, and Ac_2O (195 μL, 2.07 mmol), Hunig's base (340 μL, 1.95 mmol), and DMAP (12 mg, 0.098 mmol) were added. The reaction mixture was stirred at rt for 2 h, then diluted with CH_2Cl_2, washed with citric acid, $NaHCO_3$, and brine, then dried with Na_2SO_4, and evaporated to give 350 mg of a brown oil. Flash chromatography using

(30) Harwood, L. M. *Aldrichimica Acta* **1985**, *18*, 25.
(31) Köhler, G.; Milstein, C. *Nature* **1975**, *256*, 495.
(32) Engvall, E. *Methods Enzymol.* **1980**, *70*, 419.
(33) Copeland, R. A.; Lombardo, D.; Giannaras, J.; Dediddo, C. P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1947.
(34) van Hooidonk, C.; Ginjaar, L. *Recl. Trans. Chim. Pays-Bas* **1967**, *86*, 449.

1% AcOH, 5% absolute EtOH, and 94% CH_2Cl_2 as the mobile phase afforded 234 mg of **6** (61%) which HPLC (λ = 283 nm) indicated was >99% pure. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.44 (s, 1 H), 7.40 (d, J = 9 Hz, 2 H), 7.05 (d, J = 9 Hz, 2 H), 4.20 (pseudoquintet, 4 H), 2.12 (s, 3 H), 1.35 (t, J = 7 Hz, 6 H). ^{13}C NMR: δ 168.9, 146.4, 135.4, 121.3, 120.2, 64.7, 24.1, 16.1. HRMS (FAB): calcd 288.1001, found 288.0995.

1,3,2-Dioxaphosphorinane, 2-(4-Nitrophenoxy)-, 2-Oxide- (7). A 100 mL flask equipped with a stir bar was flame dried, cooled under N_2, and placed in a rt water bath. *p*-Nitrophenyl phosphorodichloridate (2.060 g, 8.048 mmol) was added, followed by 20 mL of dry THF and 2 mL of dry pyridine. A solution of 1,3-propanediol (0.615 g, 8.082 mmol) dissolved in 4 mL of THF was added, and the reaction mixture was stirred at rt for 14 h. The resulting mixture was diluted with Et_2O and filtered through a pad of Celite. The resulting solution was concentrated and subjected to flash chromatography using an EtOH/CH_2Cl_2 gradient as the mobile phase to give 0.898 mg of white solid as product (43% yield). HPLC (λ = 254 nm) indicated that this material was >99% pure. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (d, J = 9 Hz, 2 H), 7.43 (d, J = 9 Hz, 2 H), 4.65−4.45 (m, 4 H), 2.55−2.35 (m, 1 H), 1.87 (d of q, 14.9 Hz, 4.9 Hz, 2.4 Hz). HRMS (FAB): calcd 260.0324, found 260.0321.

Acetamide, *N*-[2-(4-Nitrophenoxy)-2-oxido-1,3,2-dioxaphosphorinan-5-yl]- (8). A 100 mL flask was prepared as for **8**, and *N*-acetylserinol (0.543 g, 4.078 mmol) was added, followed by 10 mL of pyridine and *p*-nitrophenyl phosphorodichloridate (1.054 g, 4.12 mmol). The reaction mixture was stirred at rt for 11 h. The pyridine was evaporated, and EtOAc (45 mL) and citric acid (10 mL) were added. The layers were separated, and the organic layer was washed with 3 × 6 mL of citric acid and 6 mL of brine. Evaporation of the solvent afforded 352 mg of white solid. After flash chromatography using a gradient of IPA/CH_2Cl_2 as the mobile phase, 95 mg of white solid was obtained as product (7% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.28 (d, J = 9 Hz, 2 H), 7.55 (d, J = 8 Hz, 1 H), 7.42 (d, J = 9 Hz, 2 H), 4.71 (d, J = 12 Hz, 2 H), 4.99 (d of d, J = 12 Hz, 2 H), 4.35 (d, J = 8 Hz, 1 H), 2.09 (s, 3 H). HRMS: calcd M + Cs^+ 449.9515, found 449.9523.

Acknowledgment. We gratefully acknowledge financial support from the Alfred P. Sloan Foundation and the NIH (GM48351). We thank Julie Coakley for technical support in raising antibodies.

Supporting Information Available: Eadie Hofstee plots of the paraoxon hydrolysis reactions (1 page). 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.

JO960629D