

Reactions of Bis(2,4-dinitrophenyl) Phosphate with Hydroxylamine

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For dephosphorylation of bis(2,4-dinitrophenyl) phosphate (BDNPP) by hydroxylamine in water, pH region 4–12, the observed first-order rate constant, k_{obs} , initially increases as a function of pH, but is pH-independent between pH 7.2 and pH 10. The initial BDNPP cleavage by nonionic NH_2OH (<0.2 M) involves attack by the OH group and follows first-order kinetics, but the overall initial reaction of BDNPP liberates ca. 1.7 mol of 2,4-dinitrophenoxide ion (DNP). This initial reaction generates a short-lived *O*-phosphorylated hydroxylamine, **2**, followed by three possible reactions: (1) reaction of **2** with hydroxylamine, generating 2,4-dinitrophenyl phosphate (DNPP, **3**), which subsequently forms DNP; (2) intramolecular displacement of the second DNP group and rapid decomposition of the cyclic intermediate to form phosphonohydroxylamine and eventually inorganic phosphate; (3) a novel rearrangement with intramolecular aromatic nucleophilic substitution involving a cyclic intermediate and migration of the 2,4-dinitrophenyl group from O to N. Values of k_{obs} increase modestly with pH > 10, the reaction is biphasic, and the yield of DNP increases. An increase in $[\text{NH}_2\text{OH}]$ also increases the yield of DNP, due largely to accelerated hydrolysis of DNPP.

Introduction

Mechanistic studies of hydrolyses and nucleophilic substitutions on phosphodiester are extensive, and such reactions are involved in metabolic processes that are still not fully understood at a molecular level. Reactions of hydroxylamine with activated acyl derivatives and aryl sulfonic and phosphate esters involve attack by the hydroxyl group, although the first-formed products may subsequently decompose.^{1–4}

Nucleophilicities toward these centers typically fit Brønsted relationships with linear plots of $\log k$ against $\text{p}K_{\text{a}}$ of the nucleophile, but the nucleophilicity of hydroxylamine in its reaction with 4-nitrophenyl acetate fits the Brønsted relationship with $\text{p}K_{\text{a}} = 6.22$, i.e., that of the amino group.^{2,3} This evidence with a simple nucleophile could indicate that NH_2 is the attacking group, but instead deprotonation of the ammonium residue generates the α -nucleophile, therefore increasing the nucleophilicity of the OH group by orders of magnitude, and the fit to the Brønsted relationship appears to be fortuitous. Hess et al.⁵ used kinetic isotope effects to show that

the reaction with 4-nitrophenyl acetate involves attack of the hydroxyl group of neutral hydroxylamine with concerted proton transfer to the amino group and interaction with putative anionoid oxygen in the transition state. This interaction probably assists reactions of the OH group of NH_2OH with other activated esters, leading to the unexpectedly high nucleophilicity of NH_2OH in terms of Brønsted relationships.

Hydroxylamine is a typical α -effect nucleophile with unshared electron pairs on oxygen and nitrogen. We were interested in the possibility that the reaction of NH_2OH with an activated bisaryl phosphate might release more than one aryloxide ion in the course of reaction. Recently the reaction of bis(4-nitrophenyl) phosphate anion with alkaline hydrogen peroxide was shown to involve simultaneous liberation of more than 1 equiv of 4-nitrophenoxide ion (NP)/mol of substrate, with no evidence for buildup of an intermediate, and it was suggested that the first-formed transient peroxophosphate ionic intermediate (Int_1) reacted with H_2O_2 , generating monoester, or intramolecularly giving the second aryloxide ion (Scheme 1).⁶

A similar reaction scheme can be envisaged for the reaction of NH_2OH with a transient intermediate, Int_2 , formed by initial attack of NH_2OH , which could decompose to the ionic monoester, or lose a second aryloxide ion (Scheme 2).

Either of the reactions of Int_2 could involve intervention by NH_2OH , and we must also consider attack of NH_2 -

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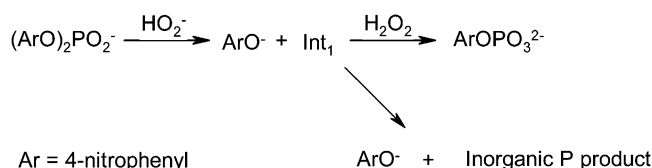
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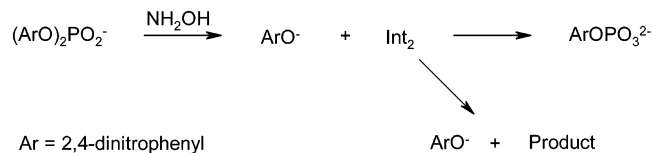
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SCHEME 1



SCHEME 2



OH on an initially formed monoester, either on phosphorus or on the aryl group, since even the less reactive 4-nitrophenyl phosphate dianion reacts with NH_2OH with a rate constant of $3.33 \times 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$.^{7a}

We chose bis(2,4-dinitrophenyl) phosphate (BDNPP) as substrate, because 2,4-dinitrophenoxide ion (DNP) is a good leaving group and there is extensive evidence of the hydrolysis of 2,4-dinitrophenyl phosphate (DNPP), including rate enhancements by tertiary amines.^{7,8} Most of our work involved NH_2OH , but we have also measured rate constants of initial reactions with NHMeOH , NMe_2OH , and MeONH_2 with the aim of distinguishing between initial phosphorylation on oxygen or nitrogen, and complete results of this work will be given elsewhere. Most of our work was in the pH range 4–12 (should this be, $10k$ increases at $\text{pH} > 10$) where nonionic hydroxylamine is dominant⁹ and the spontaneous hydrolysis of BDNPP is slow.¹⁰ At high pH NH_2O^- ($\text{p}K_a = 13.74$) is a very strong nucleophile,¹¹ but we did not investigate reaction products in detail at high pH because of the instability of the organic products in basic solutions.

Experimental Section

Materials. BDNPP as the pyridinium salt was prepared as described.¹⁰ The pyridinium ion was exchanged for sodium ion on a cation-exchange resin (Dowex 50W X8) in the Na^+ form. DNPP as the pyridinium salt was prepared by the procedure of Rawji and Milburn.¹² The hydroxylamines, as their hydrochlorides, other nucleophiles, and 2,4-dinitrophenol were of the highest purity available and were used as purchased.

Kinetics. Reactions were started by adding a 30 μL stock solution of the substrate (10^{-3} M) in water to 3 mL of the reaction mixture, which contained a large excess of the nucleophile (0.1 M), ensuring strictly first-order kinetics for the initial nucleophilic attack upon the substrate. Solutions were self-buffered by amine/amine hydrochloride at pH 4.0–7.0, prepared by addition of aqueous standard NaOH (0.1 M)

to aqueous amine hydrochloride, by borate buffer (0.005 M) from pH 8 to pH 10, and by NaOH at higher pH.

All reactions were done at 25 °C, followed by the appearance of DNP at 400 nm on a diode-array spectrophotometer with a thermostated cell holder. The pH of each reaction mixture was measured at the end of each run. Observed first-order rate constants (k_{obs}) were calculated through a nonlinear least-squares fitting of the absorbance vs time with the use of UV-vis ChemStation software. Second-order rate constants were obtained from the slopes of plots of the observed first-order rate constants against the concentration of the nucleophiles with the Origin 5.0 program.

All NMR spectra were obtained in D_2O at 25 °C, generally with a delay time of 1 s. Relaxation is slow at some positions in nitroarenes, and there were increases of 15–20% in the integrated areas of some of the signals at complete reaction when the delay time was increased to 20 s, but these long delays create problems in examining the spectra in the course of the reaction. Most of the ^{31}P NMR measurements were carried out with a delay time of 1 s, but integrated areas did not change when the delay time was increased to 3 s in measurements done at the end of the reaction. Both pyridinium and sodium salts were used in the NMR work, but ^1H signals of the pyridinium ion complicate the spectra, and the later experiments discussed here were made with the sodium salt. However, both samples gave identical signals in the spectral regions where there is no interference. The ^1H and ^{13}C chemical shifts are referred to internal sodium 3-(trimethylsilyl)propionate (TSP), and those of ^{31}P are referred to external 85% phosphoric acid. The value of pD was obtained by adding 0.4 to the observed pH of the solutions in D_2O at 25 °C.¹³

Products of the Reaction of Hydroxylamine. Most of the products and unreacted BDNPP were identified by their absorption and NMR spectra, with comparison with those of authentic material. BDNPP and DNPP (designated **1** and **3**, respectively) were prepared as described above, 2,4-dinitroaniline and 2,4-dinitrophenol were purchased and used at the appropriate pH, and *O*-phosphorylated hydroxylamine, $\text{H}_2\text{NOPO}_3^{2-}$ (designated **5**), was prepared by reaction of potassium phosphoramidate with hydroxylamine.¹⁴

***N*-(2,4-Dinitrophenyl)-*O*-phosphonohydroxylamine.** The monosodium salt of *N*-(2,4-dinitrophenyl)-*O*-phosphonohydroxylamine (designated **4**) was isolated from a reaction mixture containing BDNPP (sodium salt) (0.5 g) rapidly dissolved in ~50 mL of aqueous 0.1 M hydroxylamine, pH 8.5. The resulting solution was held at 25 °C overnight. The pH was decreased to ca. 0 by adding approximately 20 mL of 5 M HCl, the reaction mixture was cooled (ice bath), and 2,4-dinitrophenol was removed by filtration. The filtrate containing **4**, as the free acid, was concentrated (rotary evaporator, ~35 °C), NaCl was precipitated by addition of dry ether, and after filtration, the ether solution was stirred while a dilute solution of sodium ethoxide in ethanol was added dropwise, giving precipitation of a pale yellow solid. This solid was collected and washed with ether. EI-MS (70 eV): m/z (relative intensity) 197 (15), 182 (25), 181 (40), 168 (10), 167 (7), 91 (25), 75 (100), 74 (90), 63 (70). Anal. Calcd for $\text{C}_6\text{H}_5\text{N}_3\text{O}_8\text{PNa}$: C, 23.9; H, 1.66; N, 13.9. Found: C, 24.0; H, 1.77; N, 14.0. The NMR spectrum of the isolated material was identical with that observed in the reaction mixture.

The isolated sodium salt of **4** is a substrate for calf intestinal alkaline phosphatase (activity 1 unit/ μL). The reactivity of **4** was tested by using 5 μL of the phosphatase, in 50 mM Tris-HCl, pH 9.3, 1 mM MgCl_2 , 0.1 mM ZnCl_2 , and 1 mM spermidine in a final volume of 0.2 mL. The reaction mixture was kept at room temperature for 14 h, and inorganic phosphate was detected following a previously described procedure.¹⁵

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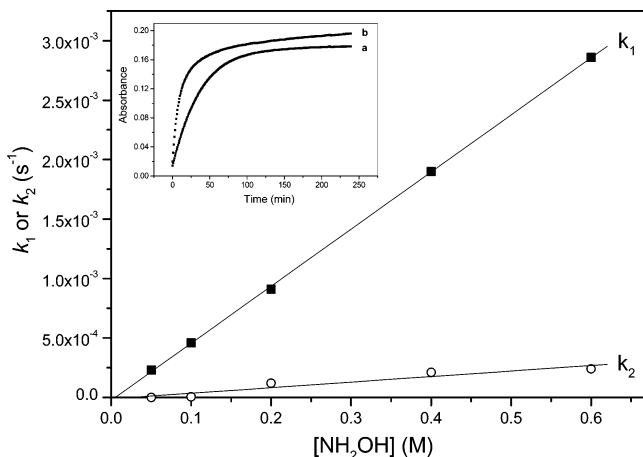


FIGURE 1. k_1 (■) and k_2 (○) as functions of $[\text{NH}_2\text{OH}]$ for nucleophilic attack on BDNPP, at pH 9 and 25 °C. Inset: kinetic plots for (a) $[\text{NH}_2\text{OH}] = 0.1 \text{ M}$ and (b) $[\text{NH}_2\text{OH}] = 0.4 \text{ M}$.

TABLE 1. Yield of DNP in the Reaction of NH_2OH with BDNPP, at pH 9 and 25 °C

| $[\text{NH}_2\text{OH}]$, M | yield of DNP, mol % | $[\text{NH}_2\text{OH}]$, M | yield of DNP, mol % | $[\text{NH}_2\text{OH}]$, M | yield of DNP, mol % |
|---------------------------------|---------------------------|---------------------------------|---------------------------|---------------------------------|---------------------------|
| 0.10 | 173 | 0.40 | 191 | 0.80 | 199 |
| 0.20 | 179 | 0.60 | 197 | | |

Products of the Reaction of *O*-Methylhydroxylamine.

An authentic sample of *N*-(2,4-dinitrophenyl)-*O*-methylhydroxylamine was prepared from the reaction of 1 mmol of 2,4-dinitrofluorobenzene with 3 mmol of *O*-methylhydroxylamine, in water at pH 7. The solvent was evaporated and a pale yellow solid collected by filtration, mp 110–112 °C. EI-MS (70 eV): m/z 213 (M^+). Anal. Calcd for $\text{C}_7\text{H}_7\text{N}_3\text{O}_5$: C, 39.4; H, 3.3; N, 19.7. Found: C, 39.5; H, 3.5; N, 19.8. The NMR spectrum of the synthetic sample was identical with that observed in the reaction mixture. The NMR spectra of the sodium salt of the *O*-methyl-*N*-(2,4-dinitrophenyl)phosphonohydroxylamine isolated from the reaction mixture was shown to be identical with that of the reaction mixture. Anal. Calcd for $\text{C}_7\text{H}_7\text{N}_3\text{O}_8\text{PNa}$: C, 35.0; H, 2.94; N, 17.5. Found: C, 34.9; H, 3.0; N, 17.6.

Geometrical Optimization. Theoretical calculations were performed by using Spartan '02 for Windows. Optimizations were carried out at the PM3 level followed by ab initio optimizations with the HF 6-31+G* basis set.¹⁶ Cartesian coordinates of the structures presented in the text are given in Table S1 of the Supporting Information. Hydration energies were calculated by the semiempirical SM 5.4 treatment of Cramer and Truhlar.¹⁷

Results and Discussion

Kinetics. Reactions of BDNPP with 0.1 M NH_2OH , monitored by the increasing absorbance at 400 nm at pH 4–12, were first-order for over 3 half-lives, up to pH 11, and in all cases more than 1.5 mol of DNP was liberated. However, the reaction is clearly biphasic in some conditions. At pH 12, or higher $[\text{NH}_2\text{OH}]$, the kinetics have the typical profile of two consecutive reactions, as shown by curve b in the inset of Figure 1, and the observed rate

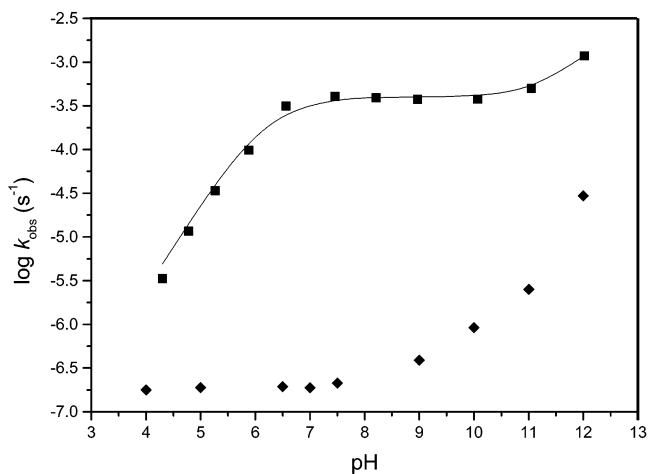


FIGURE 2. Logarithmic plots of k_{obs} for nucleophilic attack by NH_2OH on BDNPP (■) and H_2O (◆), at 25 °C.

constant (k_{obs}) could be separated into two rate constants (k_1 and k_2), by fitting the formation of DNP to eq 1. Both reactions were linearly dependent on $[\text{NH}_2\text{OH}]$, and with increasing $[\text{NH}_2\text{OH}]$ the yield of DNP increased up to approximately 200 mol % (Table 1).

$$[\text{DNP}] = [\text{BDNPP}]_0 \left\{ 1 - e^{-k_1 t} - \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \right\} + [\text{DNPP}]_0 (1 - e^{-k_2 t}) + [\text{DNP}]_0 \quad (1)$$

The data shown in Figure 2 correspond to those for the initial reaction of BDNPP with NH_2OH , and in all cases the reaction is overall second-order (Figure 1). The data for the spontaneous hydrolysis of BDNPP are included for comparison purposes and in fitting the experimental data, although contributions of the spontaneous and basic hydrolyses are minor in the pH 4–10 region of interest.¹⁰ The subsequent slow reaction, observed at high pH or higher $[\text{NH}_2\text{OH}]$, corresponds to reactions of DNPP with NH_2OH , NH_2O^- , or $\text{H}_2\text{O}/\text{OH}^-$, which eventually give the observed 200 mol % yield of DNP.

The pH rate constant profile for the initial reaction between BDNPP and hydroxylamine, shown in Figure 2, was fitted by using eq 2, where k_0 is the rate constant

$$k_{\text{obs}} = k_0 + k_{\text{OH}}[\text{OH}^-] + \frac{k_{\text{NH}_2\text{OH}}[\text{NH}_2\text{OH}]}{1 + \frac{[\text{H}^+]}{K_{a1}} + \frac{K_{a2}}{[\text{H}^+]}} + \frac{k_{\text{NH}_2\text{O}^-}[\text{NH}_2\text{OH}]}{1 + \frac{[\text{H}^+]}{K_{a2}}} \quad (2)$$

for the spontaneous reaction, k_{OH} is that for reaction with OH^- , and $k_{\text{NH}_2\text{OH}}$ and $k_{\text{NH}_2\text{O}^-}$ are rate constants for reaction with hydroxylamine and its anion. The acid dissociation constants K_{a1} and K_{a2} correspond to formation of nonionic hydroxylamine (a) and its anionic form (b), respectively (Scheme 3). Values of the individual rate and acid dissociation constants are given in Table 2.

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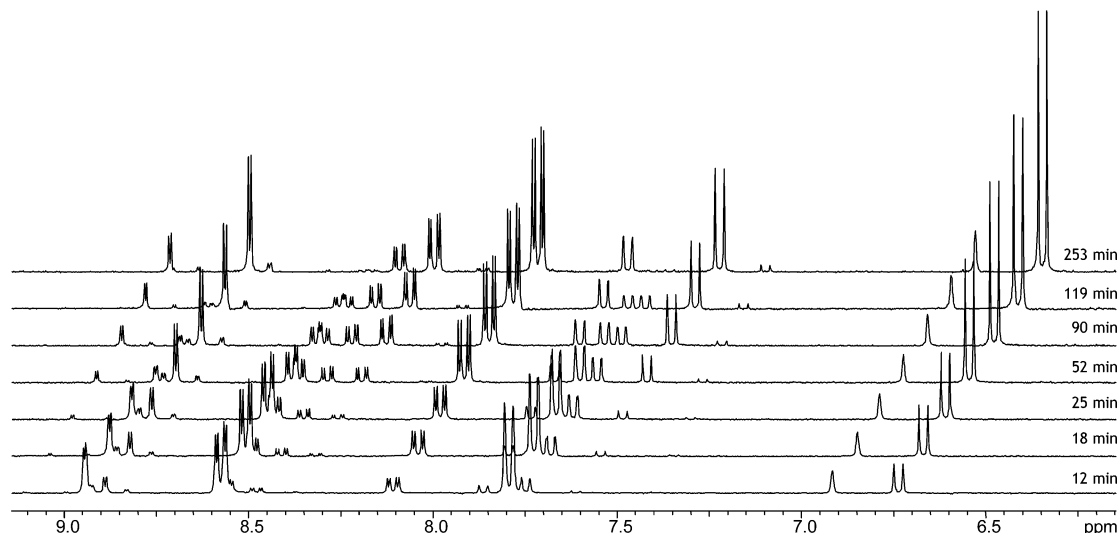


FIGURE 3. ^1H NMR spectrum of the reaction mixture of 0.01 M BDNPP with 0.1 M NH_2OH , at pH 9 and 25 °C.

SCHEME 3

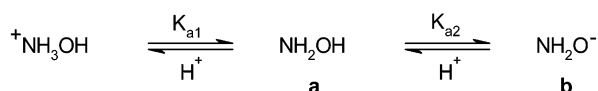


TABLE 2. Rate and Dissociation Constants^a Used in the Kinetic Fitting

| | | | | | |
|--|-----------------------|--|-----------------------|------------------|-------|
| k_0, s^{-1} | 1.90×10^{-7} | $k_{\text{NH}_2\text{OH}}, \text{M}^{-1} \text{s}^{-1}$ | 3.96×10^{-3} | $\text{p}K_{a1}$ | 6.19 |
| $k_{\text{OH}}, \text{M}^{-1} \text{s}^{-1}$ | 2.92×10^{-3} | $k_{\text{NH}_2\text{O}^-}, \text{M}^{-1} \text{s}^{-1}$ | 0.407 | $\text{p}K_{a2}$ | 13.74 |

^a Dissociation constants are from NIST Standard Reference Database 46, Version 6.0, distributed by NIST Standard Reference Data, Gaithersburg, MD 20899.

Although, the rate constants show that the anionic form of hydroxylamine reacts ca. 100 times faster than the neutral form, we did not investigate this reaction in detail due to instability of the organic products. Indeed, experiments at high pH showed, as previously reported in the absence of hydroxylamine, that 2,4-dinitrophenol is unstable in basic solutions,¹⁰ nitro groups are removed from the arene ring, and nitrite ion is detected in solution.

We have preliminary kinetic data on the reactions of BDNPP with the different nonionic methyl derivatives NHMeOH , NMe_2OH , and MeONH_2 . The results in the pH-independent region are consistent with little rate effect of methylation on nitrogen (rate constants are 1.0×10^{-3} and $2.4 \times 10^{-4} \text{ s}^{-1}$ for NHMeOH and NMe_2OH , respectively), but methylation on oxygen strongly inhibits reaction ($k_{\text{obs}} = \text{ca. } 1.4 \times 10^{-5} \text{ s}^{-1}$). Rate profiles and products of dephosphorylation by these *N*- and *O*-methyl derivatives will be discussed in detail elsewhere, but the kinetics fit our assumption that the initial reaction of NH_2OH is on oxygen.

Products. It is important to note that the yield of DNP in the first step of the reaction of BDNPP with 0.1 M NH_2OH , estimated spectrophotometrically, is approximately 1.7 equiv (Table 1); i.e., more than one molecule of DNP is formed in the first kinetically observed step of the reaction, indicating a complex reaction.

Most of the products were also identified by ^1H , ^{13}C , and ^{31}P NMR spectroscopy. The conditions, of necessity, differ from those of the kinetic work in the use of D_2O as solvent and higher substrate concentrations. However,

the difference in substrate concentration did not affect the rate constants and formation of DNP. These concentrations gave good signal-to-noise ratios for ^1H or ^{31}P , but because products were changing with time, it was difficult to use extensive accumulations, especially for the ^{31}P spectra, where weak signals could have been lost in the noise (Experimental Section). Detection of the ^1H signals was less of a problem, but some of the nuclei, especially that of H-3 of the dinitroarenes, relax very slowly, and because the reaction was progressing, we could not use delay times long enough to ensure linearity between concentrations and all signal areas (Experimental Section). Figure 3 shows ^1H NMR spectra as a function of time for the reaction mixture of 0.01 M BDNPP with 0.1 M NH_2OH , and Figure 4 shows the ^{31}P NMR spectra.

Our NMR spectra allow identification of the products, and some of the intermediates, in particular compound **2**, formed by the initial attack of NH_2OH . They show that a long-lived species is formed, probably from **2**, which does not decompose in the course of the reaction. The ^1H chemical shifts and multiplicities of the signals indicate that the 2,4-dinitrophenyl group is always present in the arene derivatives; i.e., nitro groups are not displaced by nucleophilic attack at pH < 10 (Table 3).

The NMR spectra in Figures 3 and 4 illustrate, qualitatively, the changing concentrations of products and intermediates with time, and Figure 5 gives examples of the results, among many observations. For convenience we designate the various species as **1–5**, where **1** is the starting material BDNPP. We used ^1H and ^{31}P to distinguish between species that retain dinitrophenyl and phosphoryl residues and inorganic phosphoryl derivatives. As shown in both panels, at $[\text{NH}_2\text{OH}] = 0.1$ and 0.6 M, the disappearance of starting material **1** is associated with the appearance of about 170 mol % aryloxide ion DNP, and we always detected the transient species **2**, which disappears with time. The rate of disappearance of **2** depends on $[\text{NH}_2\text{OH}]$, and this reaction generates **3** (DNPP), whose ^1H and ^{31}P NMR spectra are identical with those of authentic DNPP, as well as a stable product, **4**. It is interesting to note that as the

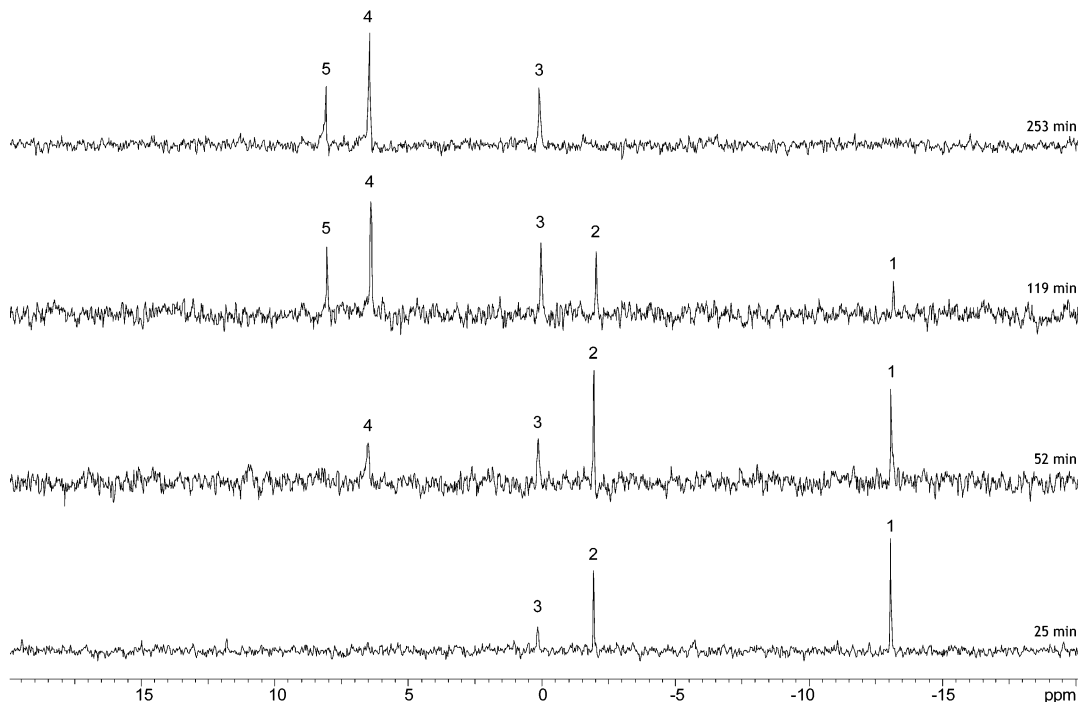
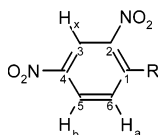


FIGURE 4. ^{31}P NMR spectrum of the reaction mixture of 0.01 M BDNPP with 0.1 M NH_2OH , at pH 9 and 25 °C.

TABLE 3. NMR Data for the Products (Scheme 4) of the Reaction of BDNPP (0.01 M) with NH_2OH (0.1 M), in D_2O , at pH 9 and 25 °C



| compd | ^1H NMR δ (ppm) | ^{31}P NMR δ (ppm) (relative integrated area, %) ^a | ^{13}C NMR δ (ppm) |
|----------|--|--|---|
| DNP | 6.74 (d, 1H, $J = 9.6$ Hz, Ar), 8.11 (dd, 1H, $J_{ab} = 9.6$ Hz and $J_{bx} = 3.0$ Hz, Ar), 8.90 (d, 1H, $J = 3.0$ Hz, Ar) | | |
| 4 | 7.62 (d, 1H, $J = 9.6$ Hz, Ar), 8.38 (dd, 1H, $J_{ab} = 9.6$ Hz and $J_{bx} = 2.7$ Hz, Ar), 9.10 (d, 1H, $J = 2.7$ Hz, Ar) | 6.43 (0) | C1, 151.2; C2, 133.3; C3, 126.5; C4, 140.0; C5, 139.5; C6, 118.5 |
| 2 | 7.75 (d, 1H, $J = 9.1$ Hz, Ar), 8.56 (dd, 1H, $J_{ab} = 9.1$ Hz and $J_{bx} = 2.7$ Hz, Ar), 8.92 (d, 1H, $J = 2.7$ Hz, Ar) | -2.04 (51) | |
| 1 | 7.80 (d, 1H, $J = 9.1$ Hz, Ar), 8.58 (dd, 1H, $J_{ab} = 9.1$ Hz and $J_{bx} = 2.9$ Hz, Ar), 8.94 (d, 1H, $J = 2.9$ Hz, Ar) | -13.17 (0) | |
| 3 | 7.86 (d, 1H, $J = 9.4$ Hz, Ar), 8.48 (dd, 1H, $J_{ab} = 9.4$ Hz and $J_{bx} = 3.0$ Hz, Ar), 8.84 (d, 1H, $J = 3.0$ Hz, Ar) | 0.04 (27) | |
| 5 | | 7.78 (22) | |

^a Relative integrated area of the signals of **1** and compounds identified at complete reaction.

initial $[\text{NH}_2\text{OH}]$ is increased the amount of **3** increases and that of **4** decreases, showing that these decompositions of **2** have different dependences on $[\text{NH}_2\text{OH}]$. With time, in a reaction that occurs after complete disappearance of starting material **1** (BDNPP), but corresponds to the slow increase in absorbance (curve b in the inset of Figure 1), and is linearly dependent on $[\text{NH}_2\text{OH}]$, **3** disappears, generating aryloxide DNP. This relatively slow decomposition of DNPP is well studied.^{7,8}

The ^{31}P NMR chemical shifts, and the relative integrated areas (Table 3), are fully consistent with the reactions shown in Scheme 4, indicating that at the end of reaction (ca. 253 min) compounds **3–5** are the main detectable phosphorus-containing products. Compound **5** was identified as *O*-phosphonohydroxylamine, by com-

parison of its NMR spectrum with that of authentic material (Experimental Section) and with the literature.^{14,18} Inorganic phosphate (Pi) is observed only after a long time of reaction (small amounts were detected at 1200 min), and it is probably formed in the spontaneous hydrolysis of DNPP.

We first consider products of the initial reaction of BDNPP with NH_2OH on the assumption that it is phosphorylation on oxygen giving DNP and the transient intermediate **2**, which breaks down in three ways.

(i) The first competing reaction involves a novel type of intramolecular rearrangement, which is somewhat

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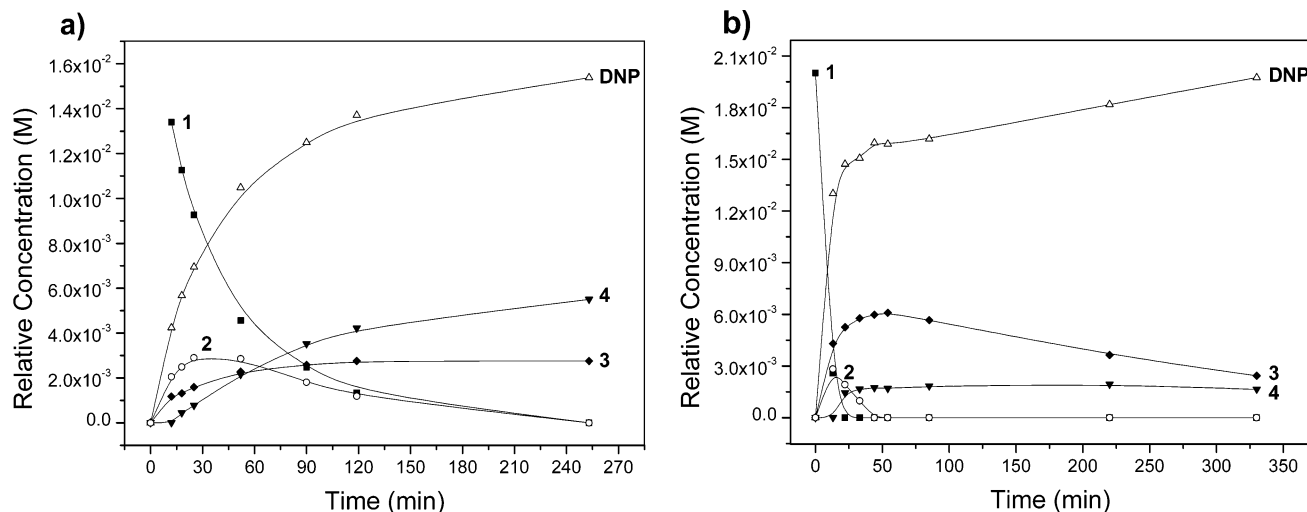
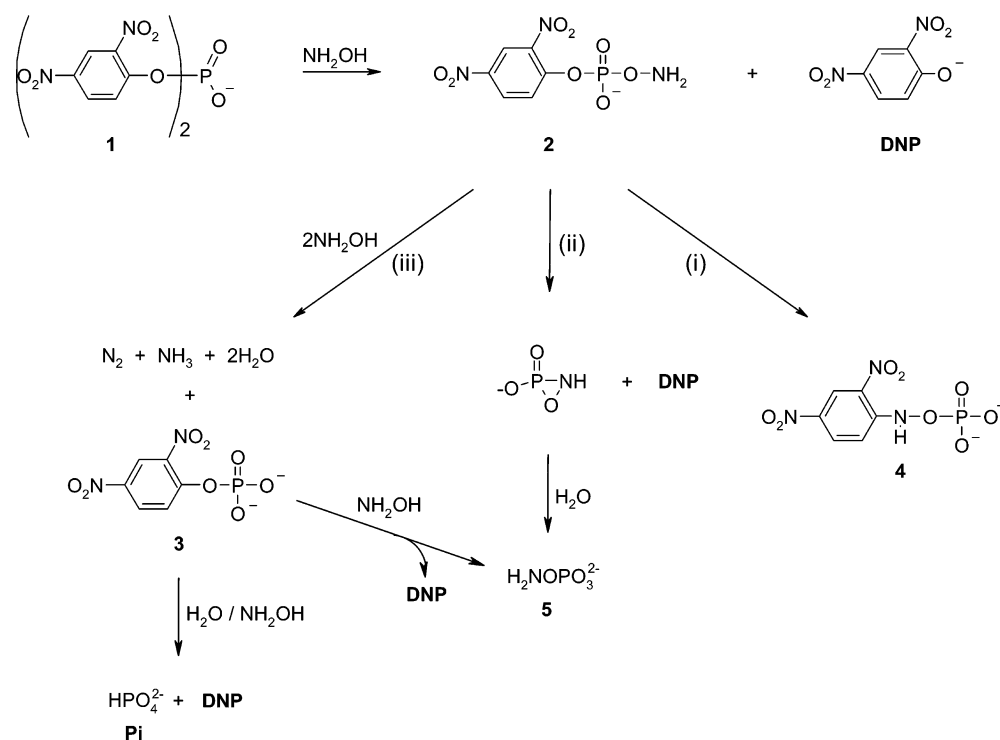


FIGURE 5. Relative concentrations of products of the reaction of BDNPP (0.01 M) with (a) $[\text{NH}_2\text{OH}] = 0.1 \text{ M}$ and (b) $[\text{NH}_2\text{OH}] = 0.6 \text{ M}$, in D_2O at pD 9 and $25 \text{ }^\circ\text{C}$: (■) **1**, (Δ) **DNP**, (\blacklozenge) **3**, (\blacktriangledown) **4**, and (\circ) **2**.

SCHEME 4



similar to both the Smiles rearrangement and the formation and breakdown of cyclic Meisenheimer complexes. The reaction involves nucleophilic attack on the arene ring and is favored by the strong electron withdrawal by the nitro groups, giving the phosphate ester **4** as one of the products (Scheme 4). However, the Smiles rearrangement has not been observed with a nitroaryl phosphoryl derivative, and the formation and breakdown of cyclic Meisenheimer complexes typically involve alkoxy or aryloxy derivatives.¹⁹ The dependence of the yield of **4** on $[\text{NH}_2\text{OH}]$ allowed us to isolate and fully characterize this product at low $[\text{NH}_2\text{OH}]$ (Experimental Section). The mass spectrum of **4** did not show the parent M^+ peak,

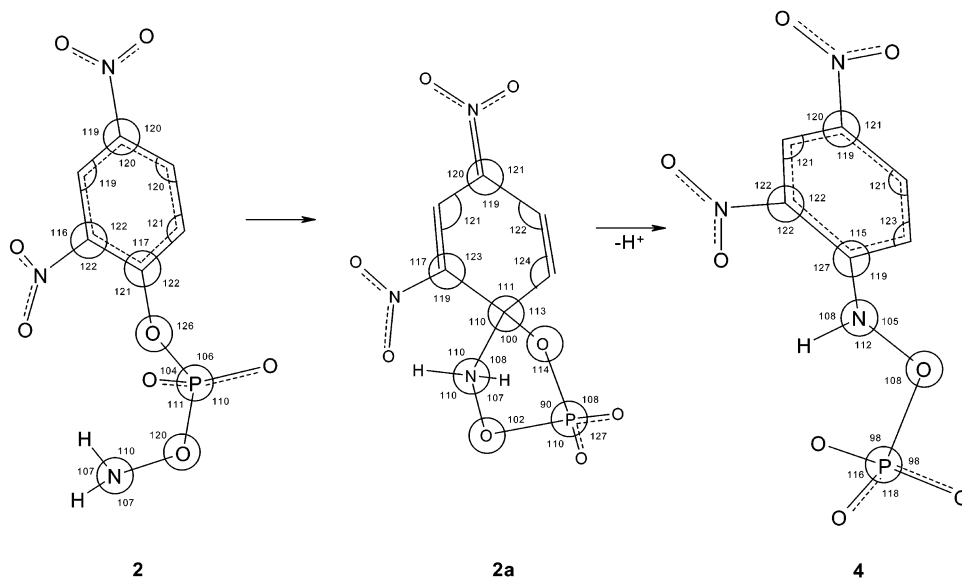
TABLE 4. ^1H NMR Chemical Shifts, ppm, of **4** as a Function of pD, in D_2O at $25 \text{ }^\circ\text{C}$

| | pD 6 | pD 12 |
|--------------|------|-------|
| H_a | 7.62 | 7.30 |
| H_b | 8.38 | 7.52 |
| H_x | 9.12 | 8.85 |

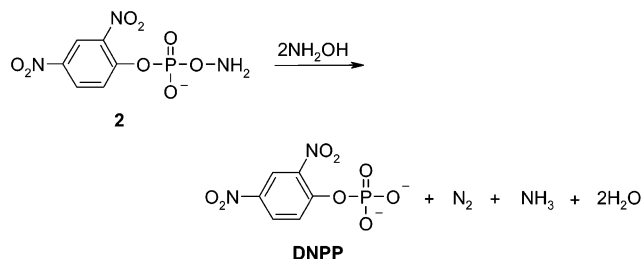
because electron impact was used, but it had all the expected fragments of the *N*-(2,4-dinitrophenyl)hydroxylamine moiety, and elemental analysis is consistent with the proposed structure. Addition of aqueous alkali solution to a solution of **4** promotes a reversible color change associated with an upfield shift of the ^1H NMR signals, indicating deprotonation at NH (Table 4). Heating the aqueous solution at $55 \text{ }^\circ\text{C}$, pD 0, for 24 h gave only the

(19) March, J.; Smith, M. B. *March's Advanced Organic Chemistry*, 5th ed.; John Wiley & Sons: New York, 2001; Chapter 13.

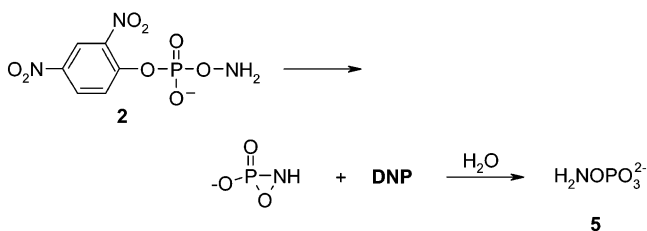
SCHEME 5



SCHEME 6



SCHEME 7



^{31}P NMR signal of H_3PO_4 , and as expected, **4** behaves as a substrate for alkaline phosphatase (Experimental Section). ^1H NMR signals of the acid hydrolyzate indicated the presence of several nitroarenes, 2,4-dinitroanilinium ion of which was identified as the major product, by comparison with authentic material, and it is probably formed by acid hydrolysis of phosphate **4** and subsequent degradation of the hydroxylamine derivative.

Our hypothesis that compound **4** is produced by the intramolecular rearrangement of the *O*-phosphorylated hydroxylamine **2** (Scheme 4), in competition with the formation of major products, e.g., **3**, formed by reaction of NH_2OH with **2**, fits all our experimental evidence. The terminal NH_2 group in **2** can readily attack the dinitrophenyl moiety to form a transient cyclic Meisenheimer complex, **2a**, higher in energy than the final product, and, on the basis of the Hammond postulate, it should rapidly ring open to give **4**. In addition, the Meisenheimer complex, **2a**, must be short-lived, because we saw no buildup of color or NMR signals typical of a Meisenheimer complex in the course of reaction. Scheme 5 shows the reaction scheme for the rearrangement, and the structures are those with optimized geometries calculated with the HF 6-31+G* basis set. The calculations indicate that the proposed intermediate has an overall stability consistent with its role in the reaction (26 kcal/mol difference between **2** and **2a**), and the angles in both rings of structure **2a** are consistent with a species with little torsional strain (Table S1, Supporting Information).

(ii) In addition to the rearrangement described above

(Scheme 5), the transient phosphorylated hydroxylamine **2** can break down by intermolecular attack by NH_2OH , giving DNPP, nitrogen, and ammonia (Scheme 6). This general reaction had been observed by Jandorf, and Harger reported decomposition of the hydroxylamine moiety of phosphonylhydroxylamine by reaction with potassium iodide.^{20,21}

This reaction is similar to the well-known decomposition of peroxy acids by reaction with H_2O_2 .^{22,23}

(iii) In competition with the above reactions **2** may generate DNP intramolecularly, or by attack of either NH_2OH or water, which is improbable because the initial yield of DNP decreases with increasing $[\text{NH}_2\text{OH}]$ (Table 1). We therefore conclude that decomposition involves intramolecular expulsion of DNP, via a hypothetical cyclic intermediate, to form the phosphorylated hydroxylamine **5**, identified by its ^{31}P NMR signal at 7.78 ppm (Scheme 7). This compound is also formed by reaction of NH_2OH with phosphoramidate, $\text{H}_2\text{NPO}_3^{2-}$.¹⁴

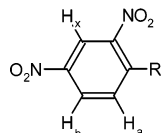
This reaction is analogous to that postulated for the more than stoichiometric production of 4-nitrophenoxide ion in the reaction of HO_2^- with bis(4-nitrophenyl) phosphate.⁶ We assume that this reaction occurs with NH_2OH and bis(2,4-dinitrophenyl) phosphate because the high leaving group ability of the 2,4-dinitrophenoxide

(20) Jandorf, B. J. *J. Am. Chem. Soc.* **1956**, *78*, 3686.

(21) Harger, M. J. P. *J. Chem. Soc., Perkin. Trans. 1* **1981**, 3284.

(22) Epstein, J.; Demek, M. M.; Rosenblatt, D. H. *J. Org. Chem.* **1956**, *21*, 796.

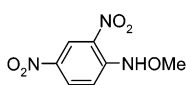
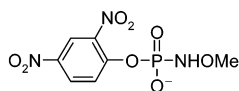
(23) Creaser, I. I.; Edwards, J. O. *Top. Phosphorus Chem.* **1972**, *7*, 379.

TABLE 5. NMR Spectral Data for Products of the Reaction of BDNPP (0.01 M) with NH₂OMe (0.1 M), in D₂O at pd 9 and 25 °C

| compd | ¹ H NMR δ (ppm) | ³¹ P NMR δ (ppm) |
|----------|---|-----------------------------|
| 6 | 3.89 (s, 3H, CH ₃), 7.52 (d, 1H, <i>J</i> = 9.6 Hz, Ar), | 0.36 |
| | 8.39 (dd, 1H, <i>J</i> _{ab} = 9.6 Hz and <i>J</i> _{bx} = 2.4 Hz, Ar), | |
| | 9.08 (d, 1H, <i>J</i> = 2.4 Hz, Ar) | |
| 7 | 3.63 (s, 3H, CH ₃), 7.74 (d, 1H, <i>J</i> = 9.1 Hz, Ar), | 0.36 |
| | 8.54 (dd, 1H, <i>J</i> _{ab} = 9.1 Hz and <i>J</i> _{bx} = 2.7 Hz, Ar), | |
| | 8.90 (d, 1H, <i>J</i> = 2.7 Hz, Ar) | |

moiety allows its expulsion by the terminal NH₂ group in compound **2**.²⁴

For comparison purposes, we are noting the products of the reaction of BDNPP with NH₂OMe, which, besides being much slower, gives less than 1 equiv of DNP. This consequence of blocking the OH group confirms that attack of the other hydroxylamine derivatives on BDNPP is preferably by oxygen rather than nitrogen. The reaction with NH₂OMe is less simple than expected for a nucleophile with one nucleophilic center, and we detected by ¹H and ³¹P NMR spectroscopy products **6** and **7** (see Table 5 and the Experimental Section), which are formed in two competitive reactions: (i) aromatic nucleophilic substitution on BDNPP, which yields DNPP and product **6**, and (ii) attack of the amino nitrogen at the phosphorus atom of BDNPP, giving a phosphorylated product (**7**) and DNP.

**6****7**

Kinetic Form and Product Formation. Reactions in dilute NH₂OH followed by the appearance of DNP follow first-order kinetics (Figures 1 and 2), but as shown in Scheme 4, some DNP is generated by reactions of the first-formed intermediate **2**, whose concentration is sufficient for its detection by NMR spectroscopy (Table 3). Adherence to a first-order kinetic form requires a single-

step reaction, an equilibrium with the intermediate in low concentration, or a stepwise reaction with an intermediate in steady state, and our reactions followed by formation of DNP should not be strictly first-order, even with dilute NH₂OH. However, several factors allow observation of reasonable first-order plots: (a) the most important is that decomposition of intermediate **2** is relatively fast and its concentration in solution is never more than ca. 10% of that of the initial substrate; (b) modest deviations from first-order kinetics are not readily detected from the kinetic form of an individual reaction. It is therefore understandable that we observed first-order rate plots with dilute NH₂OH, even though an intermediate was not strictly in steady state. The subsequent deviations from first-order kinetics, after extensive loss of BDNPP, are also understandable because here decomposition of DNPP, giving DNP, is accelerated by attack of NH₂OH and a typical profile of consecutive reactions is observed.

Conclusions

The initial reaction of NH₂OH with bis(2,4-dinitrophenyl) phosphate involves release of 1 mol of 2,4-dinitrophenoxide ion and formation of a phosphorylated hydroxylamine, **2**, which reacts readily with NH₂OH, giving the monoester **3**, which reacts relatively slowly, generating more aryloxide ion DNP. The first-formed intermediate **2** also breaks down by two other independent reactions; one involves intramolecular displacement of the aryloxide ion DNP, and the other involves migration of the 2,4-dinitrophenyl group from O to N, probably via a transient cyclic Meisenheimer complex and formation of phosphorylated 2,4-dinitrophenylhydroxylamine, **4**, which is hydrolyzed in aqueous acid but is otherwise unreactive. The products and longer-lived intermediates are identified by absorption and NMR spectroscopy.

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Supporting Information Available: Optimized geometries data (Cartesian coordinates) of structures **2**, **2a**, and **4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

(24) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 1165.