

Reaction of Bis(2,4-dinitrophenyl) Phosphate with Hydrazine and Hydrogen Peroxide. Comparison of O- and N- Phosphorylation

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Nonionic hydrazine reacts with anionic bis(2,4-dinitrophenyl) phosphate (BDNPP), giving 2,4-dinitrophenyl hydrazine and dianionic 2,4-dinitrophenyl phosphate by an $S_N2(Ar)$ reaction, and at the phosphoryl center, giving 2,4-dinitrophenoxide ion and a transient phosphorylated hydrazine that rearranges intramolecularly to *N*-(2,4-dinitrophenyl)-*N*-phosphonohydrazine. Approximately 58% of the reaction at pD = 10 occurs by N-phosphorylation, as shown by ^{31}P NMR spectroscopy. Reaction of HO_2^- is wholly at phosphorus, and the intermediate peroxophosphate reacts intramolecularly, displacing a second 2,4-dinitrophenoxide ion, or with H_2O_2 , giving 2,4-dinitrophenyl phosphate and O_2 . Rate constants of O- and N-phosphorylation in reactions at phosphorus of NH_2-NH_2 , HO_2^- , and NH_2OH and its methyl derivatives follow Brønsted relationships with similar slopes, but plots differ for oxygen and nitrogen nucleophiles. The reaction with NH_2NH_2 has been probed by using both NMR spectroscopy and electrospray ionization mass and tandem mass spectrometry, with the novel interception of key reaction intermediates in the course of reaction.

Introduction

In previous papers we discussed reactions of NH_2OH and its *N*- and *O*-methyl derivatives with bis(2,4-dinitrophenyl)phosphate monoanion (BDNPP).^{1,2} Reaction is strongly inhibited by *O*-methylation, and we concluded that reactions with NH_2OH and the *N*-methyl derivatives occur largely by attack of the OH group on phosphorus. The first-formed phosphorylated hydroxylamines can undergo subsequent reactions, which were monitored largely by visible absorption and 1H and ^{31}P NMR spectroscopy in the course of reaction and, for reaction with $NHMeOH$, by identifying ionic intermediates by electrospray ionization mass (ESI-MS) and tandem mass spectrometry (ESI-MS/MS). In this work we examine effects of amino or hydroxy-alkoxy- groups on nucleophilicities of hydroxy or amino groups for this series of α -nucleophiles and use NH_2NH_2 and HO_2^- as reagents. These two nucleophiles are conceptually simple, in that they react only via *N*- or *O*-attack, respectively. Comparison of reactions of these reagents with those of NH_2OH and its methyl derivatives provides information on the factors that control reactivities at phosphoryl and aromatic centers and the stabilities, or otherwise, of

products of the initial reactions. In general, reactions of NH_2OH and its methyl derivatives with activated carboxylic and phosphate esters involve attack of hydroxyl, rather than amino, groups at acyl or phosphoryl centers, although in some systems attack of the amino group is also observed. We also used ESI-MS and ESI-MS/MS to monitor the reaction by “fishing” ionic intermediates and products directly from solution into the gas phase. Novel interception and structural characterization of key intermediates were accomplished. This MS technique and NMR spectroscopy are complementary and powerful tools to identify, monitor, and structurally characterize reaction intermediates in solution.

Results and Discussion

Kinetics and Products: Reaction with Hydrazine. Reactions of BDNPP with 0.1 M hydrazine at pH 4–12 were followed by the increasing absorbance at 400 nm and were first order with respect to BDNPP for over 3 half-lives, but in all cases more than 1.5 mol of 2,4-dinitrophenoxide ion, DNP, was apparently liberated. However, the kinetic form depended on $[NH_2NH_2]$, as shown in Figure 1, with a deviation from linearity in a plot of k_{obs} against $[NH_2NH_2]$, which indicates that different mechanisms are simultaneously involved in the reaction with NH_2NH_2 . We show below that the overall initial reaction involves both dephosphorylation and nucleophilic aromatic substitution.

The data shown in Figure 2 are for the initial reaction of BDNPP with NH_2NH_2 , where in all cases the reaction

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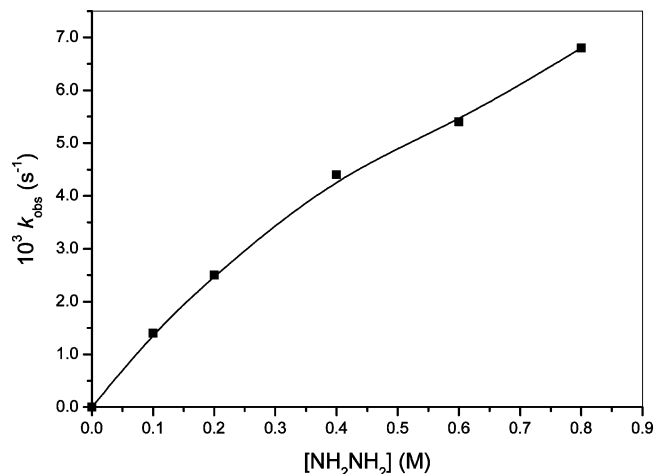


FIGURE 1. Dependence of k_{obs} (■) on $[\text{NH}_2\text{NH}_2]$ for nucleophilic attack on BDNPP in water at pH 10 and 25 °C.

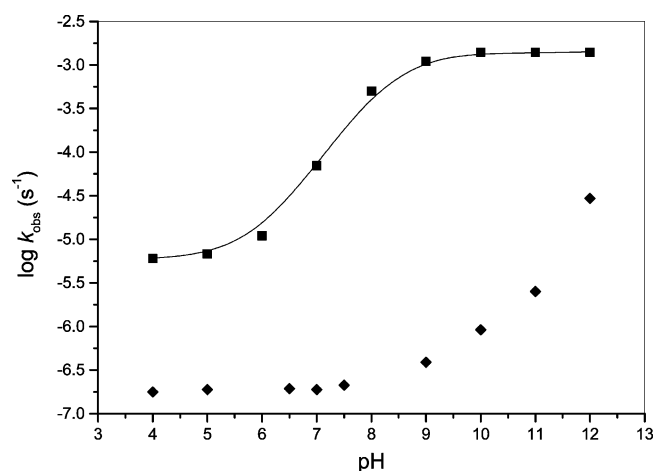


FIGURE 2. Dependence of $\log k_{\text{obs}}$ on pH for nucleophilic attack by 0.1 M NH_2NH_2 (■) and the spontaneous reaction (◆) with BDNPP, at 25 °C.

is overall second order. Data for the spontaneous hydrolysis of BDNPP are included for comparison and in fitting the kinetics, although its contribution and that of attack of HO^- are minor in the pH region of interest.^{1,3}

The pH rate constant profile for the initial reaction of BDNPP with hydrazine, shown in Figure 2, was fitted by using eq 1:

$$k_{\text{obs}} = k_0 + k_{\text{OH}}[\text{OH}^-] + \frac{k_{\text{NH}_3\text{NH}_2}[\text{NH}_2\text{NH}_2]}{1 + \frac{K_a}{[\text{H}^+]}} + \frac{k_{\text{NH}_2\text{NH}_2}[\text{NH}_2\text{NH}_2]}{1 + \frac{[\text{H}^+]}{K_a}} \quad (1)$$

where $[\text{NH}_2\text{NH}_2]$ represents the total concentration, k_0 is the rate constant for the spontaneous reaction, k_{OH} is that for reaction with OH^- , and $k_{\text{NH}_3\text{NH}_2}$ and $k_{\text{NH}_2\text{NH}_2}$ are rate constants for reaction with protonated and nonionic

SCHEME 1

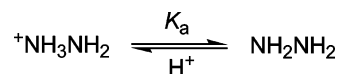


TABLE 1. Overall Rate and Dissociation Constants for Fitting the pH–Rate Profiles^a

rate constant	value
k_0 [s^{-1}]	1.90×10^{-7}
k_{OH} [$\text{M}^{-1} \text{s}^{-1}$]	2.92×10^{-3}
$k_{\text{NH}_3\text{NH}_2}$ [$\text{M}^{-1} \text{s}^{-1}$]	6.00×10^{-5}
$k_{\text{NH}_2\text{NH}_2}$ [$\text{M}^{-1} \text{s}^{-1}$]	1.38×10^{-2}
k_{HO_2} [$\text{M}^{-1} \text{s}^{-1}$]	2.95×10^{-1}
$\text{p}K_a$ (NH_2NH_3^+)	7.98
$\text{p}K_a$ (H_2O_2)	11.65

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

hydrazine, respectively. The acid dissociation constant, K_a , corresponds to formation of nonionic hydrazine (Scheme 1), and because in the pH region of interest (Figure 2) the diprotonated form of hydrazine will not be present, the kinetic dependence on pH is fitted with contributions from the neutral and monocationic species. Values of the individual rate and acid dissociation constants are in Table 1.

It is important to note that the apparent yield of DNP in the overall reaction of BDNPP with 0.1 M NH_2NH_2 , estimated spectrophotometrically, is approximately 1.5 equiv, i.e., more than one molecule of DNP appears to be formed, which indicates a complex reaction. However, as we show below, the arene hydrazine formed in an $\text{S}_{\text{N}}2$ -(Ar) reaction causes this complication because it and DNP absorb in the same spectral region and product composition cannot be estimated from the absorption spectra. Products were identified by ^1H and ^{31}P NMR and ESI-MS. The NMR spectra allowed identification of the overall products and also of a transient intermediate **2**, formed by initial attack of NH_2NH_2 on phosphorus and detected after 13 min of reaction. A long-lived species, **3**, is then formed, probably spontaneously from **2**, and does not decompose in the course of the reaction (Scheme 2). The ^1H chemical shifts and multiplicities of the signals indicate that the 2,4-dinitrophenyl moiety is always present in the arene derivatives, i.e., nitro groups are not displaced by nucleophilic attack (Table 2).

In the course of studying the products on a larger scale, a precipitate of 2,4-dinitrophenyl hydrazine, **4**, was isolated and identified by comparison of its NMR spectrum with that of authentic material. Compound **4** is formed by aromatic nucleophilic substitution by hydrazine, with displacement of the monoester DNPP, **5**, whose ^1H and ^{31}P NMR signals were identified. Therefore, the apparent initial appearance of about 150 mol % of aryloxide ion (DNP) in the disappearance of BDNPP is an overestimation due to absorbance of aryl hydrazine derivatives at 400 nm, where we initially attempted to monitor formation of DNP. The initial yield of DNP, based on the ^1H NMR signal areas, was only 58 mol % (see below), consistent with the reactions shown in Scheme 2.

The deviation from linearity in plots of k_{obs} versus $[\text{NH}_2\text{NH}_2]$, Figure 1, is kinetic evidence for the existence of an intermediate in the formation of **4** and **5**. This

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SCHEME 2

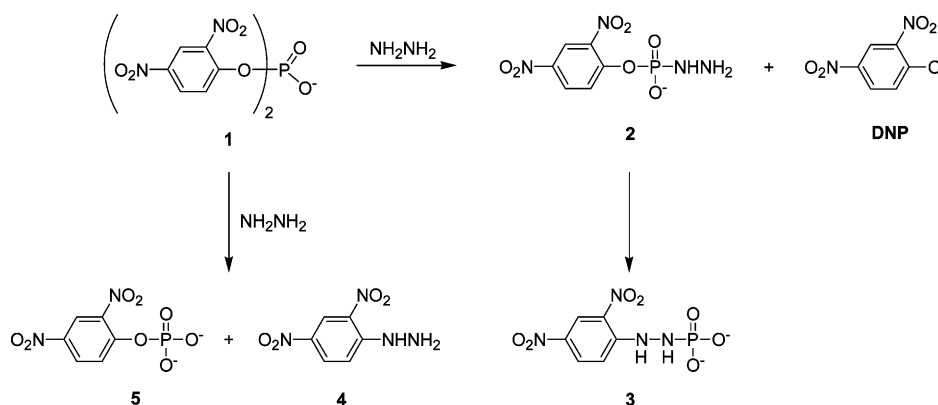


TABLE 2. NMR Spectra of Reaction Products of 0.01 M BDNPP with 0.1 M NH_2NH_2 , in D_2O , pD 10, at 25 °C^a

		³¹ P NMR δ [ppm] (rel integrated areas, %) ^b
compd	¹ H NMR δ [ppm]	
DNP	δ 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)	
1	δ 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)
2	δ 7.75 (d, 1H, J = 9.1 Hz, Ar), 8.55 (dd, 1H, J_{ab} = 8.9 Hz and J_{bx} = 2.9 Hz, Ar), 8.92 (d, 1H, J = 2.9 Hz, Ar)	4.80 (0)
5	δ 7.86 (d, 1H, J = 9.4 Hz, Ar), 8.48 (dd, 1H, J_{ab} = 9.4 Hz and J_{bx} = 2.9 Hz, Ar), 8.84 (d, 1H, J = 2.9 Hz, Ar)	0.04 (42)
4	δ 7.68 (d, 1H, J = 9.7 Hz, Ar), 8.26 (dd, 1H, J_{ab} = 9.7 Hz and J_{bx} = 2.6 Hz, Ar), 8.83 (d, 1H, J = 2.6 Hz, Ar), 10.01 (s, 1H, NH), 5.05 (s, 2H, NH_2)	
3	δ 7.86 (d, 1H, J = 9.7 Hz, Ar), 8.28 (dd, 1H, J_{ab} = 9.7 Hz and J_{bx} = 2.5 Hz, Ar), 9.08 (d, 1H, J = 2.5 Hz, Ar)	5.43 (58)

^a Compound 4 was isolated from the reaction mixture and its NMR spectrum was examined in $\text{DMSO}-d_6$. ^b Relative integrated areas of signals of 1 and products identified at complete reaction.

formation of an addition intermediate in the aromatic nucleophilic displacement of 2,4-dinitrophenyl phosphate is similar to those observed in the displacements of phosphate ion from 4-nitrophenyl phosphate⁴ and of methyl phosphate from methyl 4-nitrophenyl phosphate⁵ by piperidine and dimethylamine with attack at a carbon center. These reactions also show a complex dependence on amine concentration but change to first order in amine as its concentration is increased.

The ¹H and ³¹P NMR spectra (Table 2) allowed us to separate the contributions of carbon and phosphorus attack by hydrazine to the overall rate constant $k_{\text{NH}_2\text{NH}_2}$, eq 1, from product ratios. From yields of 42 mol % for

carbon attack and 58 mol % for phosphorus attack, we calculate values of $k_{\text{NH}_2\text{NH}_2}^{\text{S}_{\text{N}}\text{Ar}}$ and $k_{\text{NH}_2\text{NH}_2}^{\text{S}_{\text{N}}(\text{P})}$ of 5.80×10^{-3} and $8.00 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, respectively (Table 1).

The transient species **2** (Scheme 2), generates **3**, *N*-(2,4-dinitrophenyl)-*N*-phosphonohydrazine, by a rearrangement similar to those observed in reactions of BDNPP with hydroxylamine and *N*-methyl hydroxylamine, as discussed elsewhere.^{1,2} This rearrangement involves an intermediate cyclic Meisenheimer complex, **6**, but we saw no evidence for its buildup, either in the NMR spectrum or in the UV–vis region of the spectra where accumulation of such an intermediate should generate the typical color of a Meisenheimer complex. Formation of **3** occurs via a route similar to the Smiles rearrangement,⁶ except that it involves heteroatoms and is very rapid, consistent with the high nucleophilicity of the NH_2 group toward a dinitro-activated aryl group (Scheme 3).

The unreactivity of **3** toward hydrolysis is understandable, because this reaction would require protonation of a very weakly basic amino group. The rearrangement generates the major final product of the overall dephosphorylation reaction because the *N*-phosphorylated product, **2**, is a high energy intermediate, which rapidly gives **3**, with formation of an aromatic hydrazine (Scheme 2). The behavior of **2** is different from that seen for the corresponding intermediates observed in reactions of NH_2OH ¹ and MeNHOH ² for which similar rearrangements do not compete effectively with other reactions of the *O*-phosphorylated intermediates and only small amounts of rearranged products are formed.

The formation of **2** depends on $[\text{NH}_2\text{NH}_2]$ and on the reactive form of hydrazine in solution. With 0.1 M hydrazine and pH > 8 the formation of **2** is relatively fast, relative to subsequent reactions, but at low pH its formation is rate-limiting. Conversely, the *ipso* rearrangement of **2** is a slow intramolecular reaction, the monoanion is the predominant form in solution, and because we observed buildup of the first substitution product in our reaction conditions, the rearrangement cannot be very fast. Similar results were obtained in reactions of BDNPP with hydroxylamines.^{1,2} In addition, the overall reaction is followed by the increasing absorbance of dinitrophenoxide ion and the aryl hydrazine, so kinetically the *ipso* rearrangement is invisible, a fact that

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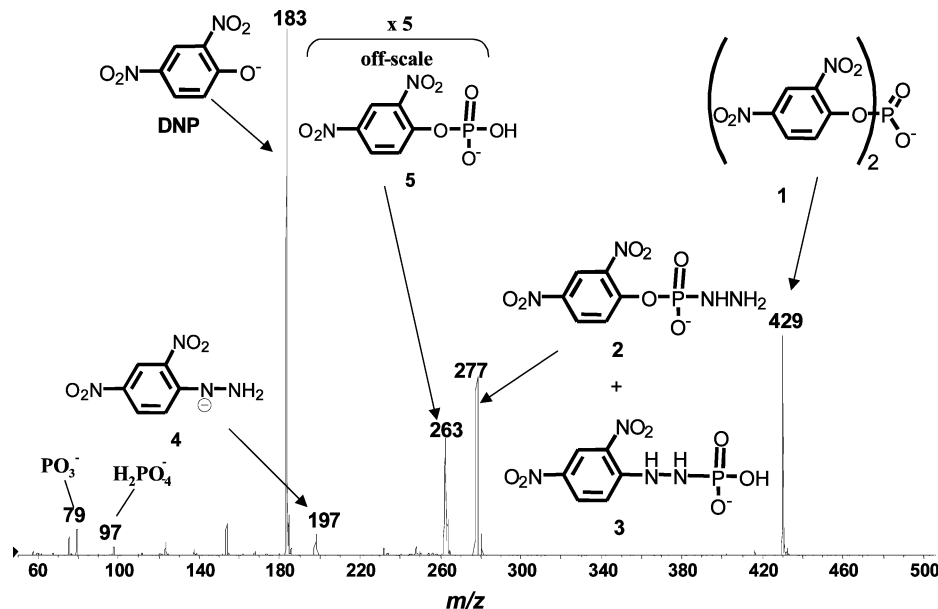
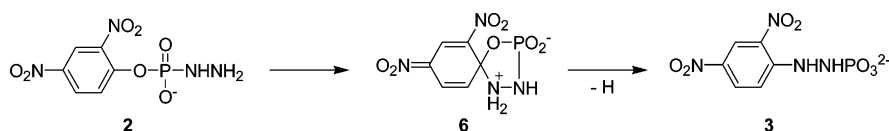


FIGURE 3. Typical ESI-MS mass spectra of the reaction mixture of 0.01 M BDNPP with 0.1 M NH₂NH₂ in aqueous methanol (50% v/v) at pH 4–7.

SCHEME 3



indicates that there is no buildup of a Meisenheimer complex and therefore it decomposes as fast as it forms.

The ¹H and ³¹P NMR chemical shifts and the relative signal areas (Table 2) are fully consistent with the reactions shown in Schemes 2 and 3, indicating that compounds **3** and **5** are the main final phosphoryl products.

Structural analysis by mass spectrometry has greatly benefited from the development of electrospray ionization (ESI),⁷ because now molecules and supramolecules⁸ of high polarity, molecular complexity, and mass can be easily ionized for MS investigation. ESI-MS is an interesting “ion-fishing” method that gently transfers pre-formed ions directly from solution into the gas phase. ESI-MS/MS is thus rapidly becoming a technique of choice for solution mechanistic studies in chemistry and biochemistry⁹ and high-throughput screening of homogeneous catalysis.¹⁰ On the basis of our background in reaction mechanism studies with MS techniques,¹¹ we used both ESI-MS and ESI-MS/MS to probe the mechanism of the hydrazine reaction as shown in Scheme 2 by transferring anions directly from solution into the gas phase, monitoring the outcome of the solution reaction

as a function of time, and characterizing structures via their collision-induced dissociation (CID) chemistry as observed by ESI-MS/MS.

Figure 3 shows the ESI-MS spectrum after 15 min of reaction of nonionic NH₂NH₂ with BDNPP in aqueous methanol (50% v/v) at pH 10, at 25 °C. Major ions clearly detected in the spectrum correspond to the reactant anion **1** (BDNPP) of *m/z* 429, isomeric intermediates **2** and **3** of *m/z* 277, DNP of *m/z* 183, **5** of *m/z* 263, and the final product **4** of *m/z* 197. The overall appearance of the spectrum and relative intensities of these ions changed little from 1 to 60 min of reaction in solution, as indicated by continuous ESI-MS monitoring. Each of these gaseous

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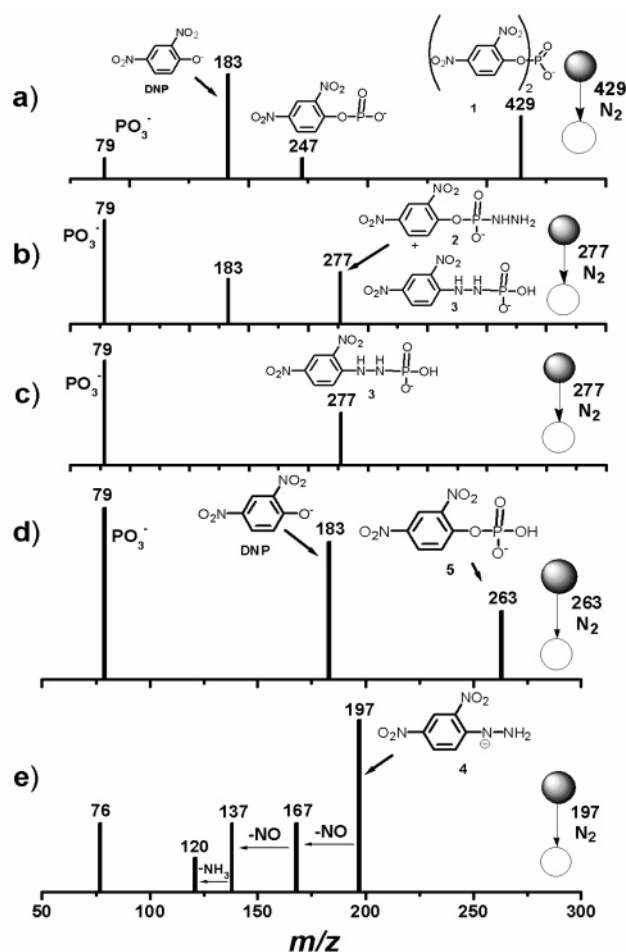
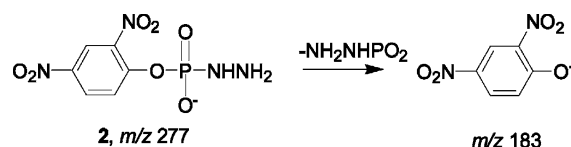


FIGURE 4. ESI-MS/MS tandem mass spectra of anionic intermediates observed in the reaction of 0.01 M BDNPP with 0.1 M NH_2NH_2 in aqueous methanol (50% v/v) at pH 10. Spectra b and c were taken at 10 and 30 min of reaction, respectively.

species was then mass-selected and structurally characterized via CID with nitrogen in ESI-MS/MS experiments. Figure 4 shows the ESI-MS/MS spectra for CID of ions of m/z 429 (**1**), 277 (**2** and/or **3**, after 10 and 30 min of reaction), m/z 263 (**5**), and m/z 197 (deprotonated **4**). These ions show dissociation behaviors that fully match proposed structures as indicated by structural assignments of fragment ions displayed in each respective mass spectrum.

Intermediates **2** and **3** in the above figure (Schemes 2 and 3) are isomers, and consistent with Scheme 2, the ESI-MS/MS spectrum of the ion of m/z 277 changes drastically with time. Spectra of samples taken after 10 min of reaction (Figure 4b) show that this ion dissociates in the gas phase mainly into two fragment ions of m/z 183 and 79. The fragment ion of m/z 183 (DNP) is formed from **2** (Scheme 4), whereas dissociation to ion of m/z 79 is characteristic of **3** (Scheme 5). Therefore **2** and **3** are probably present in the reaction mixture at an early stage of the reaction. After 30 min of reaction (Figure 4c), however, the ion of m/z 277 generates only a single fragment ion of m/z 79 (Scheme 5). This observation confirms that in a later stage of reaction intermediate **3** is a dominant species, as shown in Scheme 2.

SCHEME 4



SCHEME 5

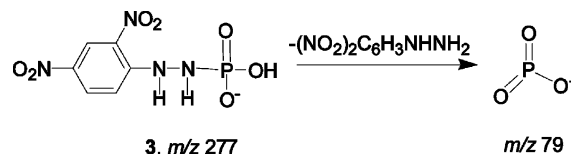
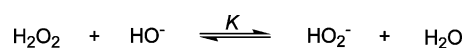


TABLE 3. First-Order Rate Constants, k_{obs} , as Function of $[\text{HO}_2^-]$ for Reaction with 0.01 M BDNPP, in Water, at 25 °C

$[\text{H}_2\text{O}_2]$, M	$[\text{HO}_2^-]^a$, M	NaOH, M	$[\text{HO}^-]^a$, M	k_{obs} , s^{-1}	pH
0.50	9.88×10^{-2}	0.10	1.2×10^{-3}	3.02×10^{-2}	11.1
0.30	9.75×10^{-2}	0.10	2.5×10^{-3}	2.93×10^{-2}	11.4
0.50	4.95×10^{-2}	0.05	5.0×10^{-4}	1.39×10^{-2}	10.7

^a Equilibrium concentrations.

SCHEME 6



Reaction with HO_2^- . Hydrogen peroxide is not very reactive toward phosphate esters, but its monoanion, HOO^- , is a very effective α -nucleophile toward activated acyl and phosphoryl derivatives.¹² In the kinetic work we used an excess of hydrogen peroxide over sodium hydroxide to limit reaction with HO^- (Table 3). The reaction was monitored by the appearance of DNP at 400 nm, and all reactions followed first-order kinetics.

The results and conditions of reactions with BDNPP are summarized in Table 3, with k_{obs} as a function of $[\text{HO}_2^-]$. The equilibrium concentrations of HO_2^- and HO^- (Scheme 6) were calculated from the stoichiometric concentrations of H_2O_2 and NaOH and the equilibrium association constant $K = (200 \pm 20) \text{ M}^{-1}$, corresponding to a pK_a of 11.65 with unit concentration of H_2O_2 .¹³

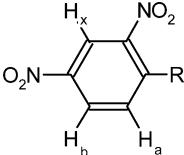
The reaction of 0.095 HO_2^- with 0.01 M BDNPP has a half-life of ca. 23 s (Table 3), and transient species are too short-lived to be identified by ^1H and ^{31}P NMR spectroscopy. Table 4 shows the NMR data for products after 12 min of reaction. DNP is a major product, but the monoester, DNPP (**5**), was also detected. It is an intermediate in the overall reaction and decomposes over time to inorganic phosphate (P_i), identified by its ^{31}P NMR spectrum, and DNP. In examining the products we used a low concentration of H_2O_2 in the false hope of increasing the lifetime of the first-formed peroxophosphate, **7** (Scheme 7).

The reaction of bis(4-nitrophenyl) phosphate anion with alkaline hydrogen peroxide involves simultaneous

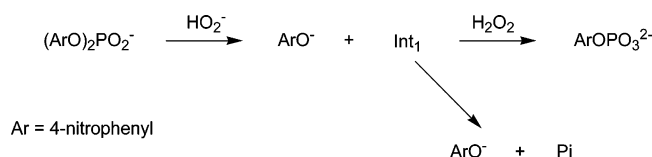
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TABLE 4. NMR Spectra of Reaction Products of 0.01 M BDNPP with 0.1 M H₂O₂, 0.3 M NaOH, in D₂O, at 25 °C

		
compd	¹ H NMR δ [ppm]	³¹ P NMR δ [ppm] (yield, mol %) ^a
DNP	δ 6.74 (d, 1H, <i>J</i> = 9.6 Hz, Ar), 8.11 (dd, 1H, <i>J</i> _{ab} = 9.6 Hz and <i>J</i> _{bx} = 3.0 Hz, Ar), 8.90 (d, 1H, <i>J</i> = 3.0 Hz, Ar)	
5	δ 7.86 (d, 1H, <i>J</i> = 9.4 Hz, Ar), 8.48 (dd, 1H, <i>J</i> _{ab} = 9.4 Hz and <i>J</i> _{bx} = 2.4 Hz, Ar), 8.83 (d, 1H, <i>J</i> = 2.4 Hz, Ar)	0.04 (58)
P _i		3.05 (42)

^a Relative signals areas of compounds identified at complete reaction.

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liberation of more than 1 equiv of 4-nitrophenoxide ion (ArO[−]) per mole of substrate as for similar reactions.¹³ There is no evidence for buildup of an intermediate, and it was suggested that the first-formed transient peroxophosphate ionic intermediate (Int₁) reacted very rapidly with H₂O₂, generating the monoester, or intramolecularly, giving the second aryloxide ion and inorganic phosphate (Scheme 7).¹³ Epstein and co-workers had observed a similar peroxo group decomposition by attack of H₂O₂ in the overall reaction of Paraoxon, (EtO)₂P(=O)O-C₆H₄NO₂, with H₂O₂/HO₂[−], where the intermediate, (EtO)₂P(=O)OOH, reacts with the excess H₂O₂, generating (EtO)₂P(=O)O[−].¹⁴

A similar reaction scheme fits the reaction of BDNPP with formation of a peroxophosphate, **7**, by initial attack of HO₂[−], followed by decomposition forming monoester, **5**, or intramolecular loss of the dinitrophenoxide ion, DNP (Scheme 8). Both the initial reaction and the subsequent intramolecular and the intermolecular reaction with HO₂[−] should be assisted by electron-withdrawing groups in the arene moiety, and the disappearance of BDNPP in our conditions is ca. 400 times faster than the subsequent hydrolysis of monoester **5**.¹⁵ The intramolecular decomposition of **7** competes effectively with the intermolecular breakdown of the peroxophosphate, which forms DNP, **5**, consistent with formation of ca. 1.5 equiv of DNP at complete reaction of BDNPP. The initially formed peroxophosphate, **7**, is a very short-lived species, and we could not detect it by NMR spectroscopy or ESI-MS.

Reaction Products. There are marked differences in the nature of products of reactions of BDNPP with NH₂–

TABLE 5. Rate Constants for Dephosphorylation in Reactions of Nucleophiles with BDNPP

nucleophile ^a	p <i>K</i> _a	<i>k</i> ₂ [M ^{−1} s ^{−1}]
water	−1.54	3.42 × 10 ^{−9}
hydrazinium	−0.24	3.48 × 10 ^{−5}
<i>O</i> -methylhydroxylamine	4.60	2.64 × 10 ^{−5}
<i>N,N</i> -dimethylhydroxylamine	5.20	3.00 × 10 ^{−3}
<i>N</i> -methylhydroxylamine	6.18	6.59 × 10 ^{−3}
hydroxylamine	5.96	3.96 × 10 ^{−3}
hydrazine	7.98	8.00 × 10 ^{−3}
hydroperoxide ion	11.65	2.95 × 10 ^{−1}
hydroxylamine monoanion	13.74	4.07 × 10 ^{−1}
hydroxide ion	15.54	2.92 × 10 ^{−3}

^a Dissociation constants are from the NIST Standard Reference Database 46, Version 6.0, distributed by NIST Standard Reference Data, Gaithersburg, MD 20899, except as specified in the text.^{1,2}

NH₂, HO₂[−], and hydroxylamine and its methyl derivatives, which depend largely upon the relative affinities of oxygen and nitrogen nucleophiles toward activated phosphoryl and aryl centers. Initial reactions of OH (or O[−]) are preferentially on phosphorus, but the adjacent amino group in initial products of phosphorylation of NH₂OH or MeNHOH can migrate intramolecularly from phosphorus to carbon,^{1,2} in a reaction similar to but less competitive than that in the phosphorylated hydrazine, **3**, Scheme 2, which generates the major product of dephosphorylation.

The initial products of phosphorylations of NH₂OH and HO₂[−] can decompose intermolecularly by attack of NH₂–OH¹⁶ or H₂O₂,^{12,14} respectively, as shown in Schemes 7 and 8 for the latter reaction. This reaction cannot occur with the methylated hydroxylamines.² Intermediate **7** can also decompose intramolecularly, with displacement of aryloxide ion at phosphorus and formation of a transient P–O bond,^{1,13} cf. Schemes 7 and 8. We saw no evidence for the corresponding reaction of the phosphorylated hydrazine, **2** (Scheme 2).

Nucleophilic Reactivities. Initial reactions can involve attack on phosphorus, preferentially by oxygen, or on the dinitroaryl group, preferentially by nitrogen. Insofar as all the nucleophiles react, at least to some extent, at phosphorus but not at the aryl group,^{1,2} we consider only rates of dephosphorylation in terms of Brönsted relationships. Table 5 shows the second-order rate constants (*k*₂^{S_N(P)}) for the initial reactions of BDNPP at phosphorus with the different nucleophiles that were used in the Brönsted plot (Figure 5). Statistical corrections were applied for hydrazine, following Bell and Evans.¹⁷ We note that the p*K*_a values used in Brönsted plots for NH₂NH₂ and HO₂[−] are for the conjugate acids at the reaction centers, whereas those for the NH₂OH and its derivatives correspond to protonation and deprotonation on nitrogen. This evidence for a simple nucleophile might erroneously indicate that NH₂ is the attacking group, but in NH₂OH and its methyl derivatives deprotonation of the ammonium residue generates the α-nucleophile, therefore increasing nucleophilicity at oxygen by orders of magnitude.

Values of log *k*₂^{S_N(P)} for phosphorylation on oxygen, for the α-nucleophiles, fit on a common line with slope β ≈

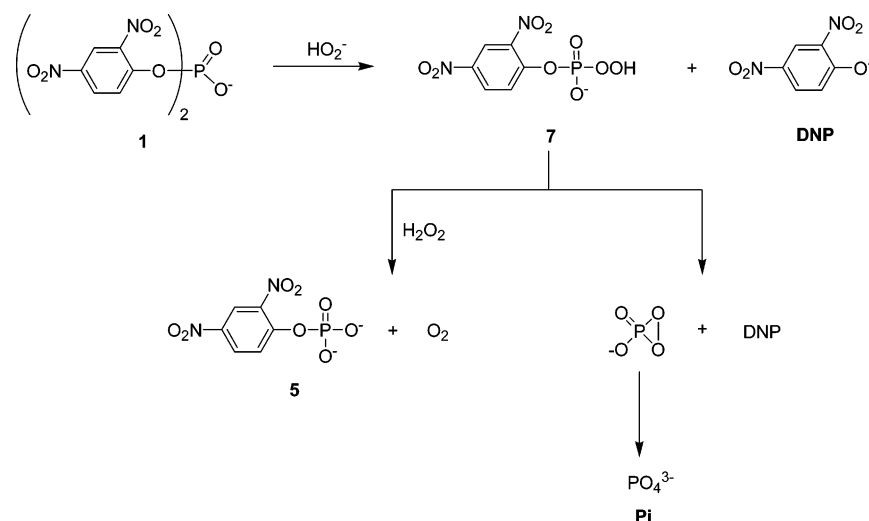
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0.3, and the limited number of points for phosphorylation on nitrogen show that the reactivity for methoxyamine is decreased by approximately 1.5 log units from the value expected for an oxygen nucleophile (but less than this for hydrazine). The slope of the Brønsted line is not very different for reactions with H_2O and HO^- , but as is general there is a decrease in reactivity of about 2000-fold in comparison with the α -nucleophiles (Figure 5).

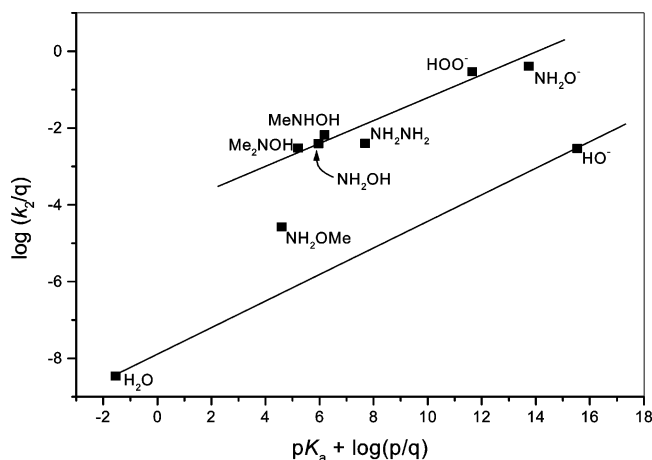


FIGURE 5. Dependence of $\log k_2^{\text{SN(P)}}$ on $\text{p}K_a$ of the nucleophiles for dephosphorylations of BDNPP. Rate constants and $\text{p}K_a$ values are statistically corrected, lines drawn to guide the eye.

There is extensive discussion of the source of the high reactivities of these α -effect nucleophiles, including the possibility of productive proton transfers that stabilize the anionoid transition states.¹⁸ However, in terms of the Avoided Crossing Model of nucleophilic substitution there is no obvious reason to expect a simple relationship between nucleophilicity and basicity.¹⁹ Such well-studied reactions as substitutions at alkyl centers and dephosphorylations by fluoride ion show no obvious relationship

between nucleophilicity and basicity, and we are agnostic as regards the general utility of Brønsted relationships in nucleophilic substitutions.

Conclusions

Reactions of NH_2NH_2 with bis(2,4-dinitrophenyl) phosphate monoanion involve nucleophilic aromatic substitution giving 2,4-dinitrophenyl hydrazine and the monoester DNPP and dephosphorylation giving an intermediate, which rapidly rearranges to a relatively stable phosphonohydrazine. Both NMR spectroscopy and ESI-MS/MS provide evidence of intermediates in the reaction of BDNPP with NH_2NH_2 and complement the kinetic data. We note that ESI-MS was used for reaction in aqueous MeOH and the other methods were applied to reactions in H_2O (D_2O). Strong signals of PO_3^- of m/z 79 were observed in the ESI-MS/MS spectra (Figure 4 and ref 2). This ion has long been postulated as an intermediate in solvolyses of anions of phosphate monoesters. Its existence in polar, nucleophilic solvents is uncertain, and it has at most a very short lifetime in these conditions.²⁰ The reaction of HO_2^- is dephosphorylation, and the initially formed peroxophosphate is rapidly decomposed by reaction with H_2O_2 or intramolecularly, generating additional dinitrophenoxide ion.

Comparison of the products of these reactions and those of NH_2OH and its methyl derivatives illustrates the generally accepted strong preference for attack of the OH group on a phosphoryl moiety and of an NH_2 group on an activated aromatic center. Rate constants of dephosphorylation follow a Brønsted relationship, but in terms of relative positions on Brønsted plots, as in Figure 5, the hydroxyl α -nucleophiles are more reactive than the amino α -nucleophiles.

Experimental Section

Materials. BDNPP as the pyridinium salt was prepared as described.³ The pyridinium ion was exchanged for sodium ion on cation-exchange resin (Dowex 50W X8) in the Na^+ form.

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DNPP as the pyridinium salt was prepared by the method of Rawji and Milburn.²¹ Hydrazine hydrochloride, 2,4-dinitrophenol, and hydrogen peroxide were of the highest purity available and were used as purchased.

Kinetics. Reactions were started by adding 30 μ L of a stock solution of the substrate (10^{-3} M) in water to 3 mL of 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.¹ Hydrazine solutions, self-buffered by the amine/amine hydrochloride at pH 4.0 to 7.0, were prepared by addition of aqueous 0.1 M NaOH to aqueous amine hydrochloride, or by borate buffer (0.005 M) from pH 8 to 10, and NaOH was used at higher pH.¹

Reactions were in H₂O (or D₂O) at 25.0 $^{\circ}$ C, followed by 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. Reactions with NH₂NH₂ and H₂O₂ gave strictly first-order kinetics over more than 5 half-lives the reaction of BDNPP, and observed first-order rate constants (k_{obs}) were calculated from the absorbance versus time profile, by using an iterative least-squares program; correlation coefficients, r , were > 0.996 for all kinetic runs.

NMR Spectroscopy. All spectra were monitored at 400 MHz for ¹H in D₂O at 25 $^{\circ}$ C, generally with a delay time of 1 s. Relaxation is slow at some nitroarene positions, and there were increases of 15–20% in areas of some of the ¹H signals when the delay time was increased to 20 s, but these long delays create problems in examining spectra in the course of reaction.¹ Most of the ³¹P NMR measurements during reaction were made with a delay time of 1 s, but the areas did not change when the delay time was increased to 3 s in measurements at the end of reaction. The ¹H chemical shifts are referred to internal sodium 3-(trimethylsilyl) propionate (TSP),

and those of ³¹P are referred to external 85% phosphoric acid. The value of pD was obtained by adding 0.4 to the observed pH of solutions in D₂O at 25 $^{\circ}$ C.²²

ESI-MS and ESI-MS/MS. All MS experiments were performed on a hybrid triple quadrupole linear ion-trap mass spectrometer generally as described in ref 2. Typical electrospray ionization (ESI) conditions were as follows: 1 mL of a 1×10^{-6} M solution of BDNPP, in aqueous methanol (50% v/v) at pH 10, was prepared, and 100 μ L of 0.1 M aqueous NH₂NH₂ was added. A microsyringe pump was used to deliver the reagent solution to the ESI source at a flow rate of 10 μ L/min. ESI and the mass spectrometer were operated in the negative-ion mode. Conditions were curtain gas nitrogen flow, 20 mL min⁻¹; ion spray voltage, -4500 eV; declustering potential, -21 eV; entrance potential, -10 eV; collision cell exit potential, -12 eV. The anionic species were subjected to collision-induced dissociation (CID) with nitrogen while ramping the collision energy from 5 to 45 eV.

Products. Most of the products were identified by their absorption, NMR, and mass spectra, with comparison with the spectra of authentic material, of which BDNPP and DNPP (designated **1** and **3**), respectively, were prepared as described above; 2,4-dinitrophenol was used at the appropriate pH. The spectra of the hydrazine products were monitored from data at pH such that reaction is wholly with NH₂NH₂.

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