

Mechanisms of Nucleophilic Substitution Reactions of Methylated Hydroxylamines with Bis(2,4-dinitrophenyl)phosphate. Mass Spectrometric Identification of Key Intermediates

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Mono- and dimethylation of hydroxylamine on nitrogen does not significantly affect rates of initial attack of NHMeOH and NMe₂OH on bis(2,4-dinitrophenyl)phosphate (BDNPP), which is largely by oxygen phosphorylation. O-Methylation, however, blocks this reaction and NH₂OMe then slowly reacts with BDNPP via N-attack at phosphorus and at the aryl group. With NHMeOH, the initial product of O-attack at phosphorus reacts further, either by reaction with a second NHMeOH or by a spontaneous shift of NHMe to the aryl group via a transient cyclic intermediate. There is a minor N-attack of NHMeOH on BDNPP in an S_N2(Ar) reaction. Reactions occurring via N-attack are blocked by N-dimethylation, and reaction of NMe₂OH with BDNPP occurs via O-attack, generating a long-lived product. Reaction mechanisms have been probed, and intermediates identified, by using both NMR and MS spectroscopy, with the novel interception of key reaction intermediates in the course of reaction by electrospray ionization mass and tandem mass spectrometry.

Introduction

As an extension of earlier experiments,¹ we have compared nucleophilic substitutions of bis(2,4-dinitrophenyl)phosphate (BDNPP) by NH₂OH and its *N*- and *O*-methyl derivatives. The initial reaction of monoanionic BDNPP, an activated bisaryl phosphate, with 0.1 M NH₂OH at pH 9 releases ca. 1.7 equiv of 2,4-dinitrophenoxide ion, DNP, and is approximately 10³ fold faster than the spontaneous hydrolysis. We showed that the initial reaction with nonionic NH₂OH generates a short-lived O-phosphorylated hydroxylamine, detected by NMR spectroscopy, and is followed by three reactions: (i) attack of hydroxylamine on this intermediate, generating 2,4-dinitrophenyl phosphate dianion (DNPP), which subsequently forms 2,4-dinitrophenoxide anion, DNP; (ii) intramolecular displacement of the second DNP anion and rapid decomposition of the cyclic intermediate to form phosphorylhydroxylamine and eventually inorganic phosphate; (iii) a novel rearrangement with intramolecular aromatic nucleophilic substitution involving a cyclic intermediate and O to N migration of the aromatic group. We found no evidence for initial N-phosphorylation or aromatic nucleophilic substitution. With NHMeOH and NMe₂OH, we saw only small rate effects of methylation on nitrogen, whereas methylation on oxygen, as expected,

strongly inhibited reaction.¹ However, reactions with Me₂NOH did not liberate more than 1 equiv of DNP and that with NH₂OMe liberated less than 1 equiv of DNP, but more than 1.5 equiv were apparently liberated in reaction with MeNHOH.¹ Other minor products were not examined, but in the present work we discuss products and kinetics in detail, particularly as regards the extent to which methylation on nitrogen affects both the initial O-attack on phosphorus and subsequent reactions of the phosphorylated species, and confirm that O-methylation blocks reactions on oxygen. Hydroxylamine and its *N*-methyl derivatives are α -effect nucleophiles that react much more readily than expected from relationships between nucleophilicity and Brønsted basicity.² We also used electrospray ionization mass and tandem mass spectrometry to monitor the reaction by “fishing” ionic intermediates and products directly from solution into the gas phase and to probe the mechanism in solution via the novel interception and structural characterization of key intermediates. This technique complements NMR spectroscopy in the identification of intermediates of reactions in solution.

Results and Discussion

Kinetics and Products. Reactions of BDNPP with 0.1 M hydroxylamine derivatives (NR₂OR', R, R' = H, Me) were followed by the increasing absorbance at 400 nm

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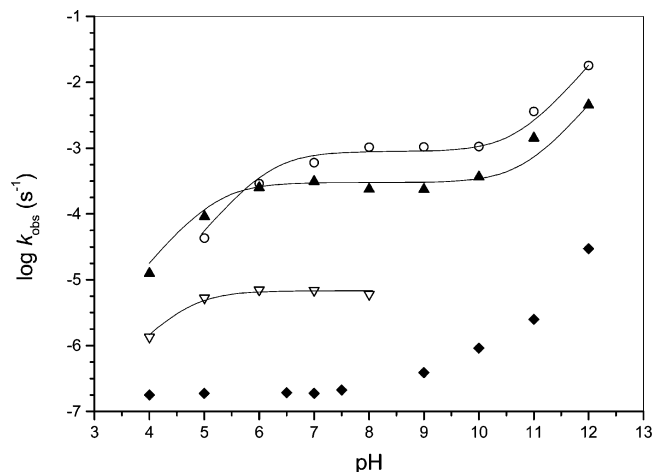
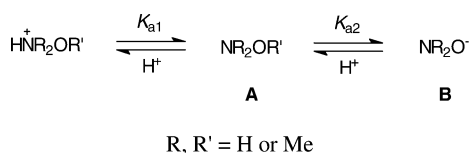


FIGURE 1. Plots of $\log k_{\text{obs}}$ versus pH for nucleophilic attack on BDNPP by MeNHOH (O), $(\text{Me})_2\text{NOH}$ (▲), NH_2OMe (▽), and H_2O (◆), at 25 °C.

SCHEME 1



in the pH range from 4 to 12. We had shown that reaction of BDNPP with NH_2OH , which releases ca. 1.7 equiv of dinitrophenoxide ion, DNP, is biphasic in some conditions. Some methyl hydroxylamine reactions show similar kinetic behavior, but with different yields of DNP. The pH–rate profiles for the initial formation of DNP are shown in Figure 1 and were fitted by using eq 1 or its modified form for NH_2OMe , where there is no reaction of a hydroxyl group or alkoxide ion.

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

In eq 1, R and R' can be H or Me, k_0 is the rate constant for the spontaneous reaction, k_{OH^-} is that for reaction with OH^- , $k_{\text{NR}_2\text{OR}'}$, $k_{\text{NR}_2\text{O}^-}$, K_{a1} , and K_{a2} are rate and equilibrium constants for the nonionic (A) and deprotonated (B) species of NR_2OH , respectively (Scheme 1); values for initial attack on phosphorus and the aromatic group are in Table 1. The spontaneous hydrolysis of BDNPP and reaction with OH^- generate monoester and dinitrophenoxide ion. These reactions are relatively unimportant at pH 9–10, where products were examined, and their contribution to product formation can be neglected.

Reaction with MeNHOH. The overall initial reaction with MeNHOH is ca. 3-fold faster than that with NH_2OH ,¹ in the pH-independent region (Figure 1), and releases ca. 1.6 equiv of DNP. This rate difference is

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

	NHMeOH	NMe ₂ OH	NH ₂ OMe
$k_{\text{S}_\text{N}}^{(\text{P})}$			
$k_{\text{HNMeOH}^+}^{\text{M}^{-1} \text{s}^{-1}}$	6.59×10^{-3}	3.00×10^{-3}	2.64×10^{-5}
yield, mol %	74	100	40
$k_{\text{HNMeOH}^+}^{\text{S}_\text{NAr}^{\text{M}^{-1} \text{s}^{-1}}$	2.31×10^{-3}		3.95×10^{-5}
yield, mol %	26		60
$k_{\text{NR}_2\text{O}^-}^{\text{M}^{-1} \text{s}^{-1}}$	9.62	2.38	
$\text{p}K_{a1}$	6.18	5.20	4.62

^a Dissociation constants are from the NIST Standard Reference Database 46, Version 6.0, NIST Standard Reference Data, Gaithersburg, MD 20899, and k_0 and $k_{\text{OH}^-} = 1.90 \times 10^{-7} \text{ s}^{-1}$ and $2.92 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, respectively.

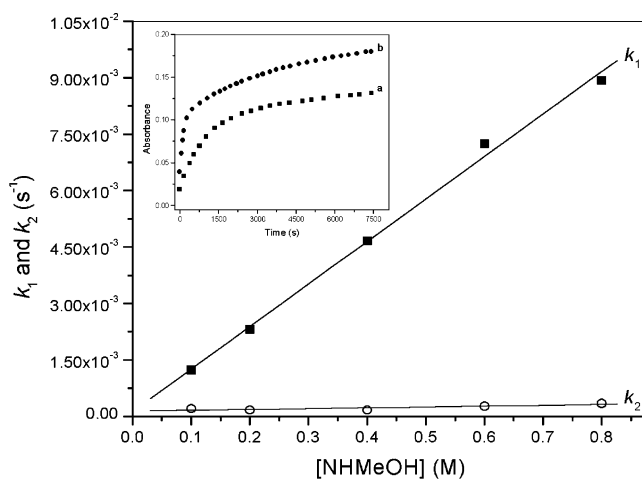


FIGURE 2. Plots of k_1 (■) and k_2 (○) as a function of $[\text{NHMeOH}]$ for nucleophilic attack on BDNPP, at pH 9 and 25 °C. Inset: kinetic plots for $[\text{NHMeOH}] =$ (a) 0.1 and (b) 0.6 M.

probably related to the increased basicity^{2,3} and is understandable in terms of the evidence of Hengge et al. regarding the role of the NH_2 group in the reaction of NH_2OH with 4-nitrophenyl acetate.⁴ Kinetics of the reaction of NH_2OH with BDNPP approximate to first order, but the reaction is biphasic.¹ The reaction kinetics with MeNHOH are typical of consecutive reactions at all pH and $[\text{MeNHOH}]$ examined (curves a and b in Figure 2). Values of the observed rate constants (k_{obs}) plotted in Figure 1 give k_1 , for the initial attack in a biphasic reaction and release of DNP, which is obtained by fitting its formation to eq 2. Figure 2 also shows the slower reaction, step k_2 , which includes hydrolysis of the initially formed monoester 2,4-dinitrophenyl phosphate (DNPP), possibly with catalysis by NHMeOH .⁵

$$[\text{DNP}] = [\text{BDNPP}]_0 \left\{ 1 - e^{-k_1 t} - \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \right\} + [\text{DNPP}]_0 (e^{-k_2 t}) + [\text{DNP}]_0 \quad (2)$$

In eq 2 k_1 and k_2 are overall first-order constants with respect to BDNPP and DNPP, respectively. The initial

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(4) Hess, R. A.; Hengge, A. C.; Cleland, W. W. *J. Am. Chem. Soc.* **1997**, *119*, 6980–6983.

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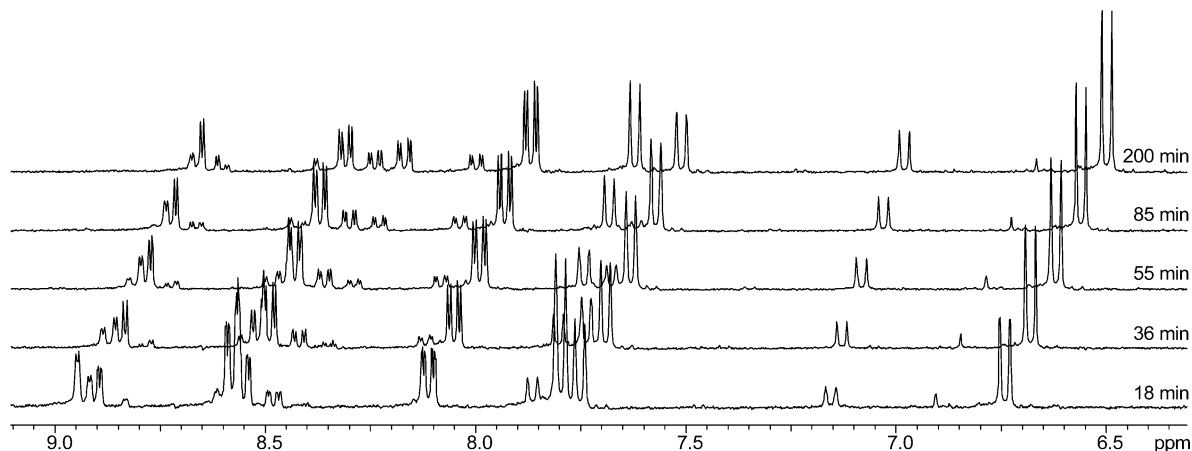
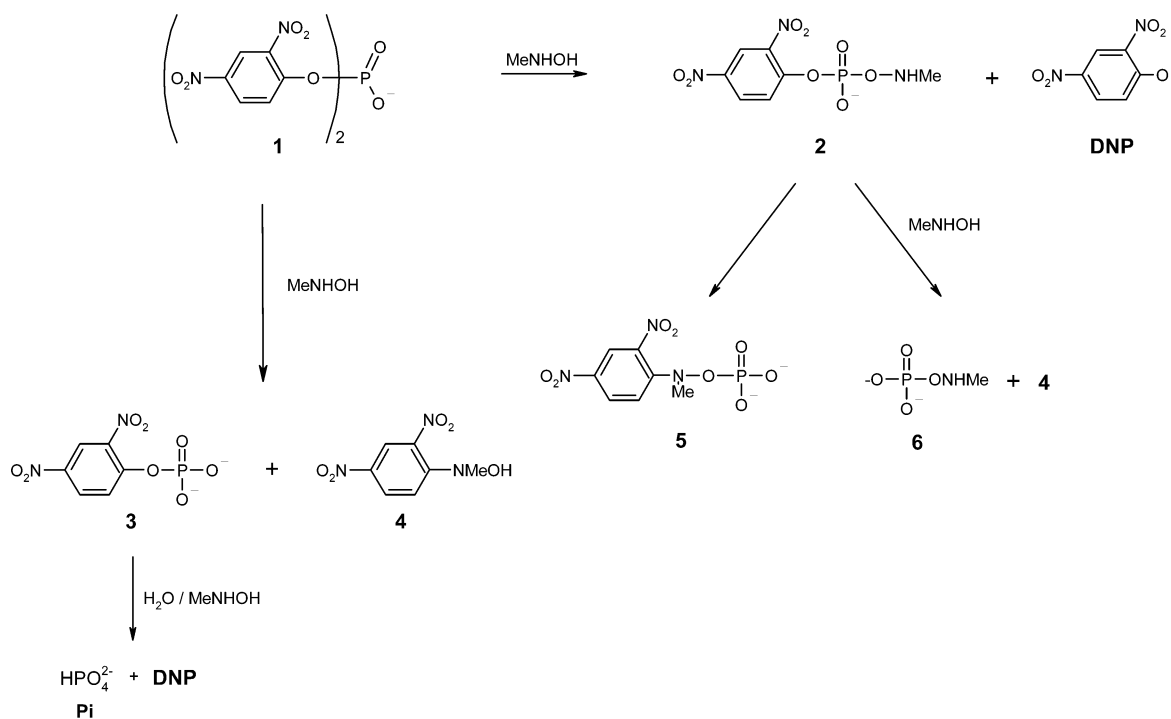


FIGURE 3. ^1H NMR spectra of the reaction mixture of 0.01 M BDNPP with 0.1 M NHMeOH, in D_2O , pD 9, and 25 °C. See Table 2 for identification of signals.

SCHEME 2



reaction of BDNPP with NH_2OH gives both stable products and intermediates, some of which are sufficiently long-lived to be identified, generally by NMR spectroscopy.¹ On the basis of this earlier evidence we postulate that a series of mechanistically reasonable reactions of MeNHOH are involved (Scheme 2), and we show that this Scheme is consistent with all of the evidence on kinetics and products. In Scheme 2, the state of ionization of species shown is that at pH 9–10, with rapid acid–base equilibration. Figures 3 and 4 show ^1H and ^{31}P NMR spectra taken at intervals, and the long-lived intermediates and products are characterized by this NMR spectral data. The NMR spectra detected four species in the course of the reaction, which were identified chemically as specified, viz., DNP, the transient species, **2**, the monoester DNPP, **3**, and a long-lived product, **5**. Intermediate **2**, which slowly decomposes, is formed in the initial reaction of MeNHOH with BDNPP

by attack of the OH group at phosphorus. Signals and their assignments are shown in Table 2 and Figures 3 and 4. Concentrations shown in Table 2 were estimated after ^1H signals of BDNPP, **1**, were no longer detected.

Figure 5 shows relative concentrations of species that form or decompose in the course of the reaction. Concentrations are based on the ^1H NMR signals in the aromatic region, and some minor products that give signals overlapping those of the major products are not shown. Nonetheless, Figure 5 shows that formation of **4**, which does not contain phosphorus (Table 2), is related to disappearance of intermediate **2** and also to the initial reaction of BDNPP, **1** (Scheme 2).

Product **4**, *N*-methyl-*N*-(2,4-dinitrophenyl) hydroxylamine, is formed by two distinct reactions, viz., aromatic nucleophilic substitution at C-1 of both BDNPP (in an initial reaction) and **2** by the amino group of MeNHOH in a subsequent reaction, and for purpose of identification

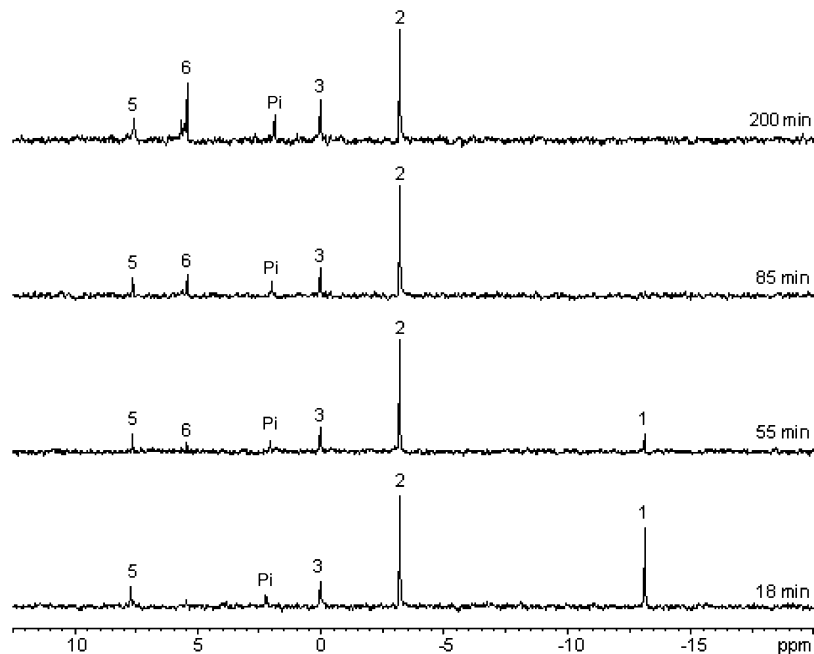
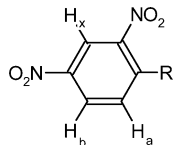


FIGURE 4. ^{31}P NMR spectra of the reaction mixture of 0.01 M BDNPP with 0.1 M NHMeOH, in D_2O , pH 9, and 25 °C. See Table 2 for identification of signals.

TABLE 2. NMR Data of Reaction Products of 0.01 M BDNPP with 0.1 M NHMeOH, in D_2O , pH 9, 25 °C



compd	^1H NMR δ (ppm)	^{31}P NMR δ (ppm)/(yield, mol %) ^a
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, $J = 9.2$ Hz, Ar), 8.55 (dd, 1H, $J_{ab}=9.2$ Hz and $J_{bx}=2.7$ Hz, Ar), 8.62 (d, 1H, $J = 2.7$ Hz, Ar)	-3.21 (36%)
3	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 (17%)
4	δ 7.22 (d, 1H, $J = 9.6$ Hz, Ar), 8.24 (dd, 1H, $J_{ab}=9.6$ Hz and $J_{bx}=2.6$ Hz, Ar), 8.62 (d, 1H, $J = 2.6$ Hz, Ar)	
5	δ 7.86 (d, 1H, $J = 9.4$ Hz, Ar), 8.48 (dd, 1H, $J_{ab}=9.4$ Hz and $J_{bx}=2.6$ Hz, Ar), 8.86 (d, 1H, $J = 2.6$ Hz, Ar)	7.56 (13%)
6		5.43 (25%)
P _i		1.86 (9%)

^a Relative areas of signals of identified compounds from ^{31}P spectra.

it was also prepared by reacting 2,4-dinitrochlorobenzene with NHMeOH. The initial $\text{S}_{\text{N}}2(\text{Ar})$ reaction of MeNHOH with BDNPP also generates DNPP, **3** (Scheme 2). Detection of the phosphorylated product, MeNHOPO_3^{2-} , **6**, at 5.43 ppm in the ^{31}P NMR spectrum, shows that there is a second aromatic nucleophilic substitution product, **4**, derived from intermediate **2**, as well as in the initial reaction of BDNPP. These results fit the reactions in Scheme 2 where, unlike for reaction with NH_2OH ,¹ there are independent competing nucleophilic substitutions on BDNPP, with attack on phosphorus and carbon.

Mass spectrometry with electrospray ionization (ESI)⁶ allows transfer of ions directly from solution into the gas phase and is characterized by the gentleness of formation of gaseous ions, and ESI-MS and ESI-MS/MS are rapidly

becoming major techniques for mechanistic studies of solution reactions involving ionic reactants, intermediates, or products.^{7,8} On the basis of our background in reaction mechanisms by using these techniques,⁸ we used both ESI-MS and ESI-MS/MS to probe the mechanism of the reactions shown in Scheme 2. We used ESI to “fish” the anionic intermediates and products directly out of solution into the gas phase, to monitor intermediates and products as a function of time and to characterize structures by CID via MS/MS. The spectrometer was

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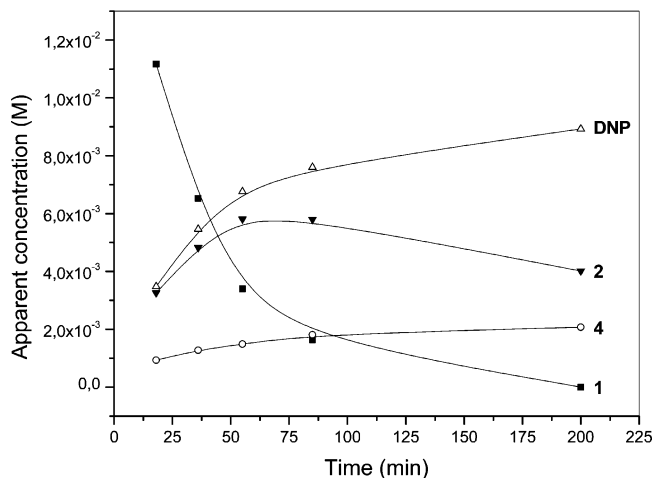


FIGURE 5. Apparent concentrations of main products of the reactions of 0.01 M BDNPP with 0.1 M NHMeOH, in D₂O, pH 9, and 25 °C: (■) **1**, (Δ) DNP, (▼) **2**, and (○) **4**.

operated in the negative-ion mode so that only anions were monitored, which simplifies treatment of reactions of BDNPP. Within the range of m/z examined only monoanions are observed (Figure 6).

Figure 6 shows the ESI-MS spectrum after 10 min of reaction of nonionic NHMeOH with BDNPP in aqueous methanol (50% v/v) at pH 10 and 25 °C. Major ions clearly detected in the mass spectrum correspond to the reactant anion **1** (BDNPP), m/z 429, intermediates and products **2** of m/z 292, DNP of m/z 183, **3** of m/z 263, and the final product **5** of m/z 292. The overall appearance of the spectrum and the relative intensities of these ions were shown, by continuous ESI-MS monitoring, to change modestly from 1 to 60 min of reaction in solution. Each of these anions was then mass-selected and structurally characterized via collision-induced dissociation (CID) with nitrogen in ESI-MS/MS measurements. Figure 7a–e show the ESI-MS/MS spectra for ions of m/z 429 (**1**), 292 (**2** and/or **5**, after 10 and 60 min of reaction, b and c,

respectively), m/z 263 (**3**), and m/z 212 (deprotonated **4**). The same ions were observed after 30 min. These ions show dissociation behaviors that fully match proposed structures of intermediates, as indicated by structural assignments of fragment ions displayed in each respective mass spectrum, and are consistent with the results from NMR spectroscopy. In Figure 7 ions shown as a solid circle are decomposed in a CID to products, shown as an open circle. As expected, the fates of the two ions with m/z of 292 depend on the time of reaction in solution (Figure 7b,c), but CID products of the other ions do not change with the time of reaction in solution.

Ionic intermediates **2** and **5** (Scheme 2) are isomers, and their relative amounts and therefore the ESI-MS/MS spectrum of the ions of m/z 292 change drastically with time. Samples taken after 10 min of reaction in solution (Figure 7b) show that these ions dissociate in the mass spectrometer by CID mainly into four fragment ions of m/z 263, 183, 97, and 79. The fragment ions, of m/z 263 and 183, are formed in the gas phase from **2** (Scheme 3), whereas dissociation to ions of m/z 97 and 79 is evidence for the presence of **5** (Scheme 4). Therefore, both **2** and **5** are present in the reaction mixture at an early stage of the reaction. After 30 or 60 min of reaction (Figure 7c), however, the ion of m/z 292 generates only the two fragment ions of m/z 97 and 79. This temporal change in product ion distribution from the CID of ion of m/z 292 confirms that in a later stage of reaction intermediate **5**, rather than **2**, become a dominant species, as shown in Scheme 2, where rearrangement of **2**, in solution, generates **5**. Although electrospray is a gentle method we did not detect **6**, m/z of 125, because it probably rapidly generates metaphosphate ion.

Based on the ESI-MS(/MS) results, NMR and kinetic data, and consistent with results on the reaction of NH₂-OH with BDNPP, we conclude that there is both aromatic substitution, generating DNP, **3**, and **4**, and initial phosphorylation of the OH group of NHMeOH by BDNPP (Scheme 2), which generates 0.7 equiv of DNP, forming intermediate **2**, which breaks down slowly by two distinct

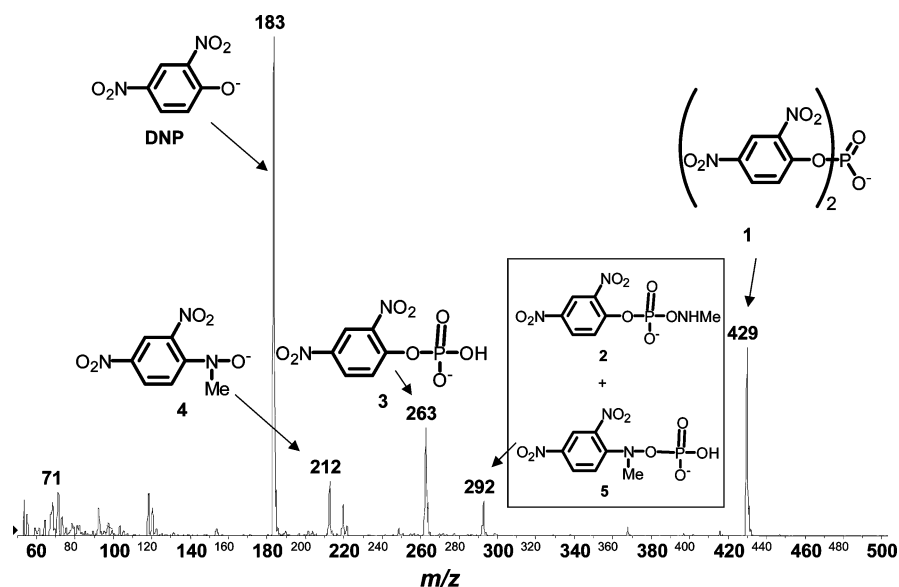


FIGURE 6. ESI-MS spectrum of the reaction mixture of 0.01 M BDNPP with 0.1 M NHMeOH, in aqueous methanol (50% v/v) at pH 10.

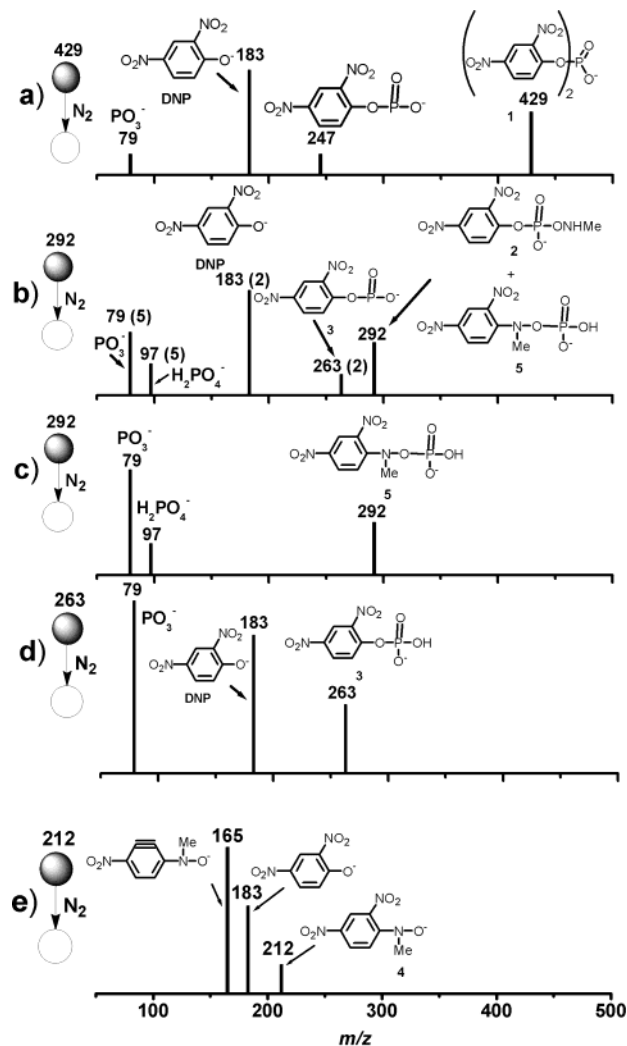


FIGURE 7. ESI-MS/MS spectra after CID of anionic intermediates observed in the reaction of 0.01 M BDNPP with 0.1 M MeNH₂OH in aqueous methanol (50% v/v) at pH 10 for ions of *m/z* 429, 292, 263, and 212.

pathways, (a) aromatic nucleophilic substitution described above, giving **4** and **6**, or (b) spontaneous rearrangement where the terminal NHMe group attacks the dinitrophenyl moiety to form a transient cyclic Meisenheimer complex, as for reaction with NH₂OH.¹ This complex rapidly ring opens, giving **5** as a long-lived product (Scheme 5). This Meisenheimer complex must be short-lived, because we saw no buildup of color or NMR signals typical of it in the course of reaction. The methyl group on nitrogen sterically hinders this intramolecular attack, and only a small amount of **5** is therefore formed. As noted, aromatic substitution on **1** also occurs, generating **4** and the monoester DNPP, **3**, although it is the minor reaction (Figure 5), and as noted, the spectral data for **4** are identical to those of an authentic sample (Table 2). The contribution of k_{HNMeOH} in eq 1 for the initial reaction of BDNPP can be separated into two rate constants according to initial product yields (³¹P NMR), $k_{\text{HNMeOH}}^{\text{S}_{\text{N}}\text{Ar}}$ (carbon attack) and $k_{\text{HNMeOH}}^{\text{S}_{\text{N}}\text{(P)}}$ (phosphorus attack), which are 2.31×10^{-3} and $6.59 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$, respectively (Table 1).

Some of these reactions in solution are similar to those identified kinetically and by NMR spectroscopy in the

overall reaction of BDNPP with NH₂OH,¹ except that there was no aromatic nucleophilic substitution on BDNPP, and with NHMeOH spontaneous conversion of the phosphorylated hydroxylamine, analogous to **2**, into DNP is not observed and is blocked by N-methylation. We note that NMR and MS spectroscopy are complementary methods leading to similar conclusions, despite differences in solvents, which were D₂O (NMR) and aqueous MeOH (MS).

Reaction with NMe₂OH. The reaction of BDNPP with NMe₂OH shows the usual first-order kinetic profile at all the pH examined and releases only 1 equiv of DNP. The first-order rate constant in the pH-independent region is lower than that with NH₂OH by about 20% (Figure 1). Dimethylation on nitrogen simplifies the reaction in that only 1 equiv of DNP is liberated by nucleophilic O-attack of Me₂NOH at phosphorus, as shown by the ¹H and ³¹P NMR spectra after approximately 3 half-lives (Table 3). Therefore, with 0.1 M NMe₂OH, the only products of reaction with BDNPP are the dinitrophenoxide ion, DNP, and the long-lived phosphorylated derivative, **7** (Scheme 6). Dimethylation on nitrogen blocks reactions of NMe₂OH similar to those of the first-formed intermediates in reactions with NH₂OH¹ and NHMeOH and also aromatic nucleophilic substitution by an amino group.

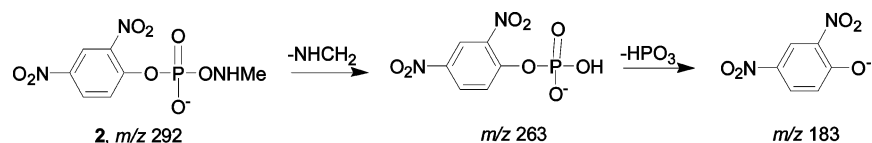
Reaction with NH₂OMe. The reaction of BDNPP with NH₂OMe is much slower than those with the N-methylated hydroxylamines. Values of k_{obs} are from initial rates, because subsequent hydrolysis of monoester DNPP, **3**, perturbs the first-order kinetics, and reaction gives less than 1 equiv of DNP (Figure 1 and Table 1). 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 subsequent decomposition of the initial products makes reaction with NH₂OMe less simple than expected for a nucleophile with only one reactive center. The half-life in the pH-independent region (Figure 1) is ca. 13.7 h, and reaction is not much faster than the spontaneous hydrolysis⁹ and is similar to that for reaction of NH₂OMe with the monoester, DNPP ($t_{1/2} = 15.5 \text{ h}$). Because these reactions are slow, other side reactions may be involved but were not identified. We detected by ¹H and ³¹P NMR spectroscopy product **9** (Table 4), from aromatic nucleophilic substitution on the substrate (Scheme 7), which is

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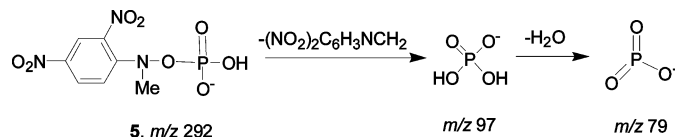
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SCHEME 3



SCHEME 4



SCHEME 5

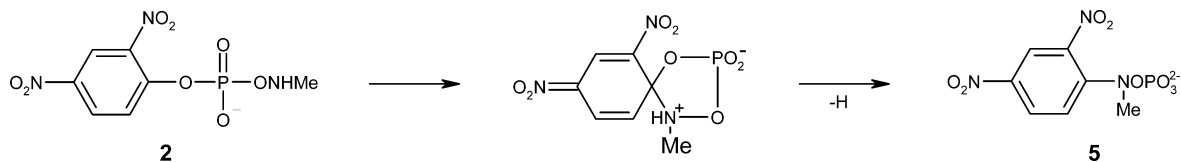
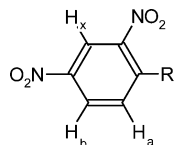


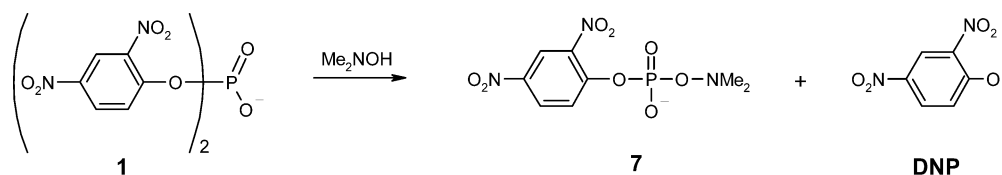
TABLE 3. NMR Spectra of Reaction Products of 0.01 M BDNPP with 0.1 M NMe₂OH, in D₂O, pD 9, 25 °C



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)	
1	δ 7.80 (d, 1H, <i>J</i> = 9.1 Hz, Ar), 8.58 (dd, 1H, <i>J</i> _{ab} = 9.1 Hz and <i>J</i> _{bx} = 2.9 Hz, Ar), 8.94 (d, 1H, <i>J</i> = 2.9 Hz, Ar)	−13.17 (12%)
7	δ 7.75 (d, 1H, <i>J</i> = 9.2 Hz, Ar), 8.55 (dd, 1H, <i>J</i> _{ab} = 9.2 Hz and <i>J</i> _{bx} = 2.7 Hz, Ar), 8.62 (d, 1H, <i>J</i> = 2.7 Hz, Ar)	−3.21 (88%)

^a Relative areas of signals of identified compounds.

SCHEME 6



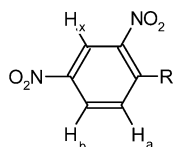
much slower than the corresponding reaction of NHMeOH. Monoester DNPP, **3**, is the other product of this S_NAr reaction with BDNPP but was only detected in low concentration because its hydrolysis, probably with amine catalysis, is not much slower than the initial attack on BDNPP. The other major product of this overall reaction is formed by attack of amino nitrogen at the phosphorus atom of BDNPP, giving the long-lived phosphorylated product, **8**, and DNP. This is the only example of nitrogen phosphorylation in solution reactions of the hydroxylamines with BDNPP. The NMR spectra were taken after approximately 48 h. Reactions shown in Scheme 7 are slow, due in part to the low basicity of this hydroxylamine.

Effects of Methylation of NH₂OH. To a first approximation mono- and dimethylation of hydroxylamine on nitrogen do not have major kinetic effects on the initial O-attack on BDNPP, but they greatly affect reactions of

the first-formed intermediates.¹ The strong inhibition of the initial reactions by O-methylation confirms the original assumption that attack on phosphorus is preferentially by the OH group,¹ although there is a slow reaction with NH₂OMe forming the *N*-phosphorylated derivative **8** and DNP (Scheme 7). Deprotonation of NHMeOH and NMe₂OH at high pH generates oxide anions, which like NH₂O[−] should be very effective dephosphorylating agents,¹⁰ and we assume that the increases in observed first-order rate constants with increasing pH involve this intervention. Values of the first p*K*_a are known for the methylated hydroxylamines (Table 1), and the second p*K*_a of NH₂OH is 13.74.¹¹ On the

(10) Simanenkov, Y. S.; Popov, A. F.; Prokop'eva, T. M.; Savyolova, V. A.; Belousova, I. A.; Zubareva, T. M. *Mendeleev Commun.* **1994**, 210–212.

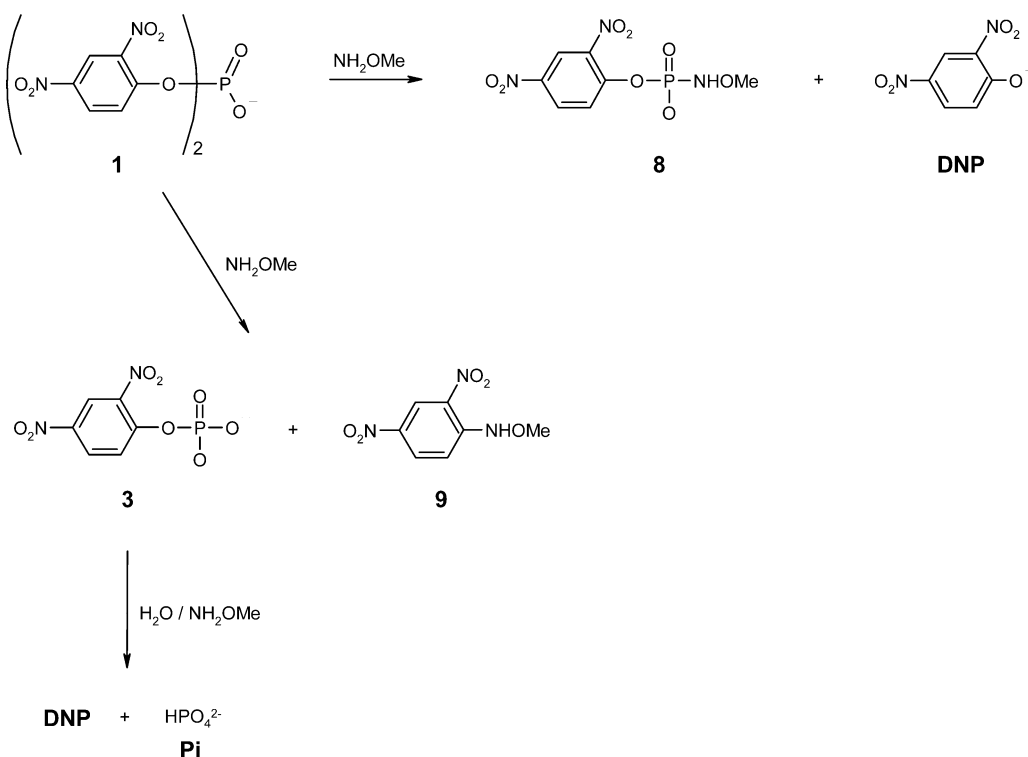
(11) NIST Standard Reference Database 46, Version 6.0; distributed by NIST Standard Reference Data, Gaithersburg, MD 20899.

TABLE 4. NMR Spectral Data for Reaction Products of 0.01 M BDNPP with 0.1 M NH₂OMe, in D₂O at pD 9, 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)	
1	δ 7.80 (d, 1H, <i>J</i> = 9.1 Hz, Ar), 8.58 (dd, 1H, <i>J</i> _{ab} =9.1 Hz and <i>J</i> _{bx} =2.9 Hz, Ar), 8.94 (d, 1H, <i>J</i> = 2.9 Hz, Ar)	-13.17 (14%)
8	δ 3.63 (s, 3H, CH ₃), 7.74 (d, 1H, <i>J</i> = 9.1 Hz, Ar), 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)	0.36 (34%)
3	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} =2.8 Hz, Ar), 8.84 (d, 1H, <i>J</i> = 2.8 Hz, Ar)	0.04 (ca. 0%)
9	δ 3.89 (s, 3H, CH ₃), 7.52 (d, 1H, <i>J</i> = 9.6 Hz, Ar), 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)	
P _i		2.17 (52%)

^a Relative areas of signals of identified compounds.

SCHEME 7



assumption that methylation has little effect on values of the first and second p*K*_a, we estimate approximate values of the second-order rate constants for attack in BDNPP by NHMeO⁻ and NMe₂O⁻ as 9.6 and 2.4 M⁻¹s⁻¹, respectively.

Dimethylation on nitrogen does not significantly affect O-attack on phosphorus (Scheme 4 and Table 1), but it blocks subsequent reactions. Aromatic substitution by an amino group is precluded by dimethylation, which also modestly hinders O-attack, probably sterically.

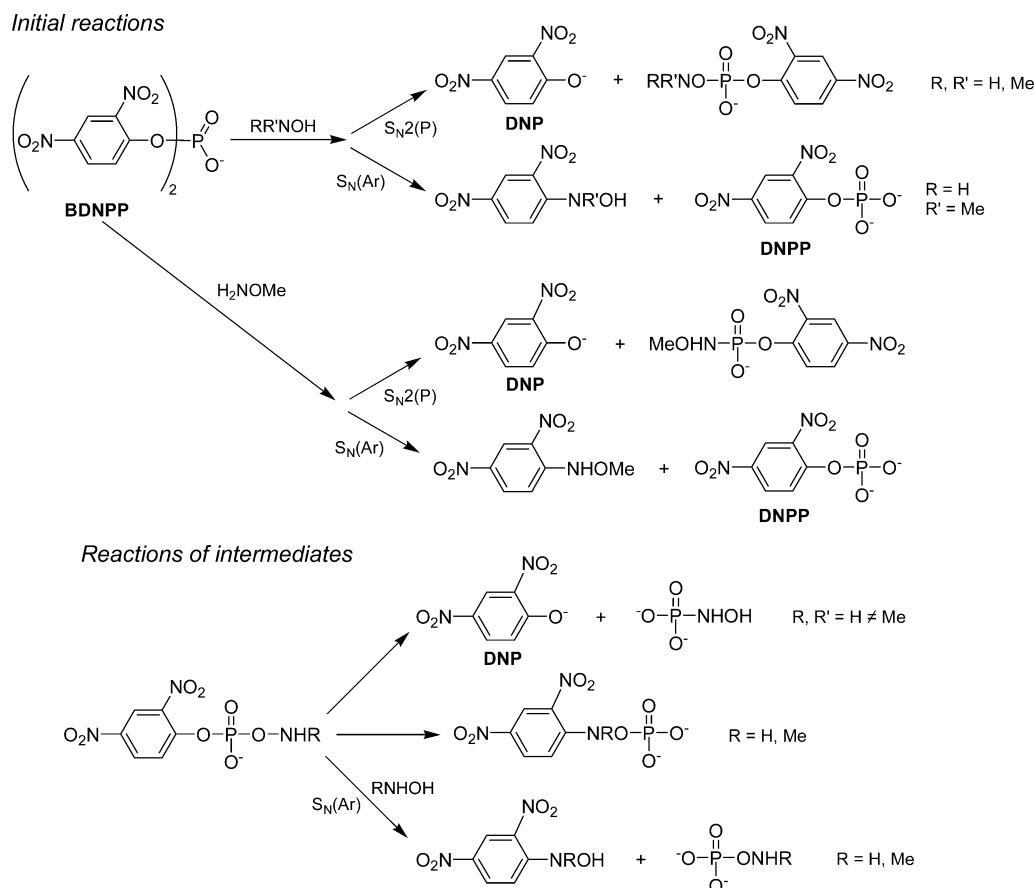
Formation of DNP in the reaction of NHMeOH with BDNPP is complex because it is formed directly by dephosphorylation and more slowly from DNPP, the product of aromatic nucleophilic substitution on BDNPP. We had seen no evidence for aromatic substitution with

NH₂OH,¹ but monomethylation in NHMeOH allows it to compete with dephosphorylation and effectively inhibits any reactions at phosphorus in the first-formed phosphorylated hydroxylamine, which would otherwise be similar to those observed earlier.¹

Conclusions

Scheme 8 summarizes our conclusions from kinetic NMR spectroscopy and ESI-MS regarding the individual reactions of BDNPP and intermediates with hydroxylamine and its methylated derivatives and the extent to which methylation controls initial reactions (top section of Scheme 8) and those of first formed intermediates (bottom section in Scheme 8). N-Monomethylation of NH₂-

SCHEME 8



OH does not significantly affect the rate of O-phosphorylation of NHMeOH by BDNPP, but it affects subsequent reactions of the first-formed intermediate. N-Dimethylation also has minor effects on the initial reaction of NMe₂OH that gives a long-lived O-phosphorylated product. O-Methylation strongly inhibits the initial attack of NH₂OMe on phosphorus and then aromatic nucleophilic substitution contributes significantly, although it is minor for the other hydroxylamines, which are much more reactive nucleophiles than NH₂OMe.

Electrospray MS and NMR spectroscopy are complementary techniques for identification of reaction intermediates and products. The former allows sampling and identification, but NMR provides structural information, although data acquisition takes time, especially for nuclei that relax slowly.

Experimental Section

Materials. BDNPP as the pyridinium salt was prepared as described.⁹ The pyridinium ion was exchanged for sodium ion on 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, and 2,4-dinitrophenol were of the highest purity available and were used as purchased.

Kinetics. Reactions followed spectrophotometrically were started by adding 30 μ L of stock solution of the substrate (1×10^{-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. Solutions were self-buffered by the amine/amine hydrochloride at pH 4.0–7.0, prepared by addition of aqueous standard NaOH (0.1 M; Merck) to aqueous amine hydrochloride, and 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. Observed first-order rate constants (k_{obs}) were calculated with a nonlinear least-squares fitting of the absorbance vs time profile.

NMR Spectroscopy. All ¹H and ³¹P NMR spectra were monitored on a spectrometer (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 at the end of reaction, but these long delays create problems in examining spectra of intermediates in the course of reaction.¹ Concentrations estimated by ¹H NMR spectroscopy were based on signals for which relaxation is relatively fast. 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 sodium salt of BDNPP was used in the NMR work, because ¹H signals of the pyridinium ion complicate the ¹H NMR spectra. Aliphatic ¹H NMR signals were not useful because they are obscured by signals of the excess methylated hydroxylamines and H–D exchange precludes examination of NH signals. The ¹H and ¹³C 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

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°C.¹³ Concentrations monitored by ¹H and ³¹P NMR spectroscopy were generally taken after the initial reaction was complete, so that compositions did not change significantly during the measurements.

ESI-MS and ESI-MS/MS. All experiments were performed on a hybrid triple quadrupole linear ion-trap mass spectrometer. For typical electrospray ionization (ESI) conditions 1 mL of 1×10^{-6} M BDNPP, in aqueous methanol (50% v/v) at pH 10, was mixed with 100 μ L of 0.1 M aqueous MeNHOH. A microsyringe pump delivered the reagent solution in to the ESI source at a flow rate of 10 μ L/min. ESI and the QqQ (linear trap) mass spectrometer was operated in the negative-ion mode. Main 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 by using collision energies ranging from 5 to 45 eV.

Products. Most reaction products and intermediates were identified by ESI-MS and ESI-MS/MS, absorption and NMR

spectroscopy, with comparisons with the spectra of authentic material of BDNPP and DNPP, which were prepared as described above; 2,4-dinitrophenol was used at the appropriate pH, and *O*-phosphorylated *N*-methylhydroxylamine, HNMeOPO₃²⁻ (designated **6**), was prepared by reaction of potassium phosphoramidate with *N*-methyl hydroxylamine.¹⁴ Spectra were monitored for reactions at pH where the nonionic nucleophile is dominant. Products were examined with the hydroxylamines in significant excess over BDNPP, and their concentrations changed little in the course of reaction.

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