

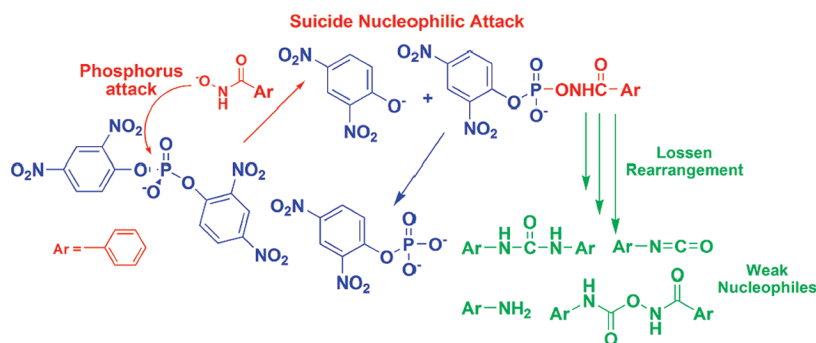
Suicide Nucleophilic Attack: Reactions of Benzohydroxamate Anion with Bis(2,4-dinitrophenyl) Phosphate

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The reaction between the benzohydroxamate anion (**BHO⁻**) and bis(2,4-dinitrophenyl)phosphate (**BDNPP**) has been examined kinetically, and the products were characterized by mass and NMR spectroscopy. The nucleophilic attack of **BHO⁻** follows two reaction paths: (i) at phosphorus, giving an unstable intermediate that undergoes a Lossen rearrangement to phenyl isocyanate, aniline, diphenylurea, and *O*-phenylcarbamyl benzohydroxamate; and (ii) on the aromatic carbon, giving an intermediate that was detected but slowly decomposes to aniline and 2,4-dinitrophenol. Thus, the benzohydroxamate anion can be considered a self-destructive molecular scissor since it reacts and loses its nucleophilic ability.

Introduction

Extensive recent studies have been reported on hydrolysis and nucleophilic substitution on phosphoesters,^{1–3} which are important because these reactions are biologically significant and are not fully understood mechanistically.⁴

In some conditions, phosphodiester are the least reactive esters of phosphoric acid; for example, the spontaneous hydrolysis of bis(2,4-dinitrophenyl)phosphate, **BDNPP**, at pH 7

proceeds with P–O bond fission forming the much more reactive dianionic 2,4-dinitrophenyl phosphate monoester (**DNPP**),⁵ which cannot be detected in solution because the spontaneous hydrolysis of the 2,4-dinitrophenyl phosphate dianion is over 10³-fold faster than that of the corresponding **BDNPP**.⁵ Conversely, at high pH, hydrolysis of **BDNPP** is faster than that of **DNPP**, which complicates development of efficient catalysts that can fully degrade phosphodiester. Phosphate monoester dianions, the initial products of phosphodiester hydrolyses, are insensitive to anionic nucleophiles (with the possible exception of fluoride) due to the electrostatic barrier.

The P–O fission of aryl phosphoesters is especially accelerated by α -effect nucleophiles, such as hydroxylamine,⁶ hydra-

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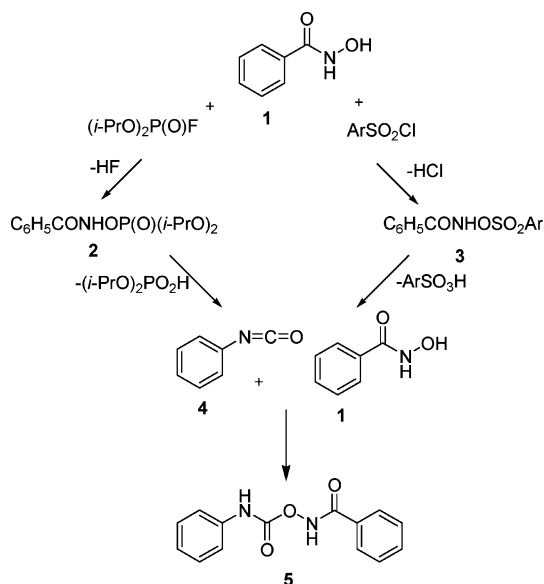
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SCHEME 1. Reaction of Benzohydroxamic Acid with Phosphoryl and Sulfonyl Halides


zine,⁷ and hydroxamic acid,^{8,9} which have unshared electron pairs on the atom adjacent to the nucleophilic center. For example, dephosphorylation of **BDNPP** anion by hydroxylamine (0.1 M) at pH 7 is over 10^3 -fold faster than by water.⁶ In this reaction, nucleophilic attack can occur on aromatic carbon and on phosphorus.⁹ Similar results were observed for reactions of α -nucleophiles with 2,4-dinitrophenyl phosphate.² Thus, these relatively fast reactions with α -nucleophiles are not completely understood mechanistically for many phosphate esters.

Hydroxamic acids have been extensively studied, in particular, due to their potential nucleophilicity,¹⁰ and some reactions of hydroxamic acids with sulfonyl and phosphoryl halides, particularly Sarin, involve Lossen rearrangements.^{11–17} For reactions of benzohydroxamic acid (**BHOH**, **1**) with benzenesulfonyl chloride and with diisopropyl phosphorofluoridate,¹³ a mechanism was proposed in which the hydroxamic acid is first sulfonated or phosphorylated to form an unstable intermediate (**2** and **3**) which breaks down by a Lossen-type rearrangement to give phenyl isocyanate **4**, which reacts with **BHOH** (**1**), yielding *O*-phenylcarbamyl benzohydroxamate (**5**) (Scheme 1).

Because the actual intermediates and products for the reactions of activated phosphate esters with hydroxamic acids have not been experimentally detected and/or isolated, we examined dephosphorylation of bis(2,4-dinitrophenyl)phosphate (**BDNPP**) by the α -nucleophilic benzohydroxamic acid

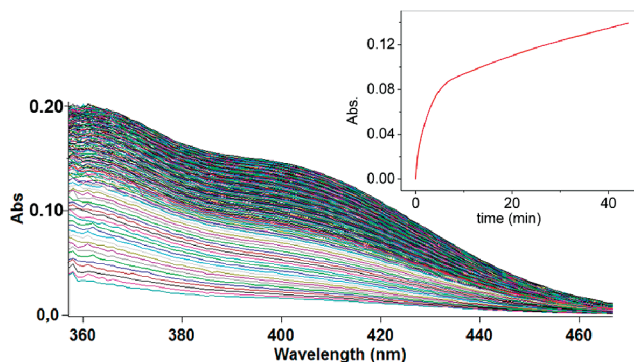
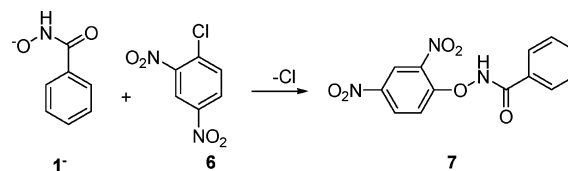


FIGURE 1. Typical kinetic behavior for the reaction of **BDNPP** with **BHO⁻** at pH 10, 25 °C. Absorbance vs time was calculated at 400 nm.

SCHEME 2. Reaction of Benzohydroxamate Anion (1⁻**) with 1-Chloro-2,4-Dinitrobenzene (**6**)**


(**BHOH**) as a function of pH and monitored intermediates and products to identify the reaction mechanism.

Results and Discussion

Reactions of **BDNPP** with **BHO⁻** were followed spectrophotometrically, as shown in Figure 1. The postulated reaction course is attack of **BHO⁻** (i) on phosphorus, liberating 2,4-dinitrophenoxide ion (**DNP**) and/or (ii) on the aromatic ring, giving more complex products. The appearance of **DNP** was observed at 400 nm, although another product absorbing at ca. 360 nm formed more rapidly in a complex two-step reaction.

The results indicate that reaction of **BDNPP** with **BHO⁻** follows parallel paths with hydroxamate attack on phosphorus and on the aromatic ring. Evidence for attack of **BHO⁻** on the aromatic ring was obtained by following the reaction of 1-chloro-2,4-dinitrobenzene (**CDNB**, **6**) with **BHO⁻** (**1⁻**) 0.05 M at pH 10 (Scheme 2), giving initially a product, **7**, which in solution slowly decomposes (*vide infra*). Although these experiments do not allow estimation of the amount of each reaction, they show that attack on the ring forms a compound with UV absorption spectra (see Supporting Information) similar to that observed for reaction of **BDNPP** with **BHO⁻** (Figure 1).

Product Characterization: Mass and ¹H NMR spectroscopy allowed elucidation of the mechanistic paths. First, ESI-MS in the negative mode monitors the course of reaction by collecting snapshots of its anionic composition. Reagents, intermediates, and products present as anions were expected to be transferred directly from the reaction solution to the gas phase and then detected by ESI-MS. Figure 2 shows a characteristic ESI-MS for the reaction solution.

In this spectrum, a series of key anions were detected and identified including the reagents **BDNPP** anion, *m/z* 429, and **BHO⁻** of *m/z* 136 and anions from the cleavage of **BDNPP**, such as the phenoxide **DNP** of *m/z* 183 and the anionic monoester **DNPP** of *m/z* 263. The hydroxamate attacks on both the aromatic ring and phosphorus were confirmed by the detection of the anionic products of *m/z* 302 and 216, respectively.

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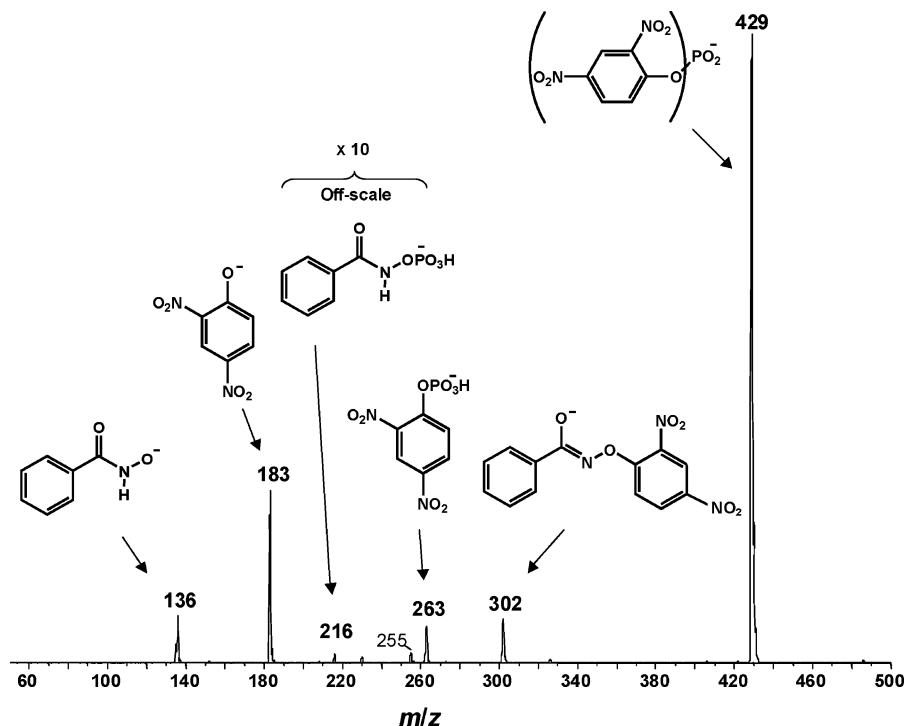
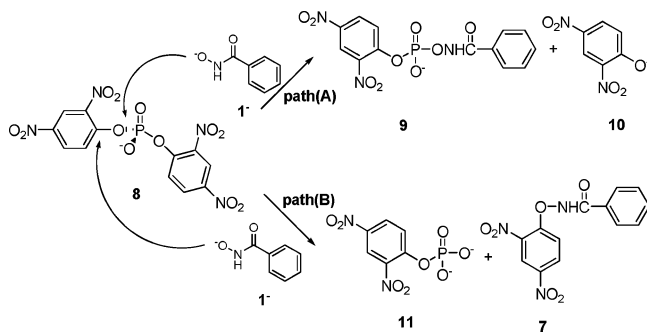


FIGURE 2. ESI-MS after 30 min for the aqueous reaction solution of BHO^- with **BDNPP** at pH 10.0 and 25 °C.

SCHEME 3. Nucleophilic Attack of BHO^- by Two Reaction Paths: Path (A) at Phosphorus and Path (B) on the Aromatic Carbon



The anion of m/z 255 is the anionic form of the carbamyl derivative, **5** (Scheme 1), from a Lossen rearrangement of the intermediate formed by hydroxamate attack on phosphorus.

ESI-MS/MS was then used to characterize some of these important species via collision-induced dissociation. The resulting tandem mass spectra supported the structural assignments: the anion of m/z 216 dissociates to the fragment ion of m/z 183, by loss of a HOO radical and to H_2PO_4^- of m/z 97 and PO_3^- of m/z 79. The anion of m/z 302 dissociated nearly exclusively to the phenoxide, **DNP** ion of m/z 183. The anion of m/z 263 also dissociated by the loss of HPO_3 to the **DNP** ion of m/z 183 and PO_3^- and to m/z 79.

Scheme 3 shows the different intermediates and products for the reaction of BHO^- with **BDNPP**. Path (A) corresponds to attack on phosphorus and path (B) to attack on the aromatic ring. For convenience, we numbered the various species where initial reactants **BHO** and **BDNPP** are **1** and **8**, respectively. Reactions occur simultaneously, forming the expected cleavage

products of **BDNPP**,¹⁸ **10**, and **11**, and form intermediates **7** and **9**, which subsequently generate **10** and other products as discussed later.

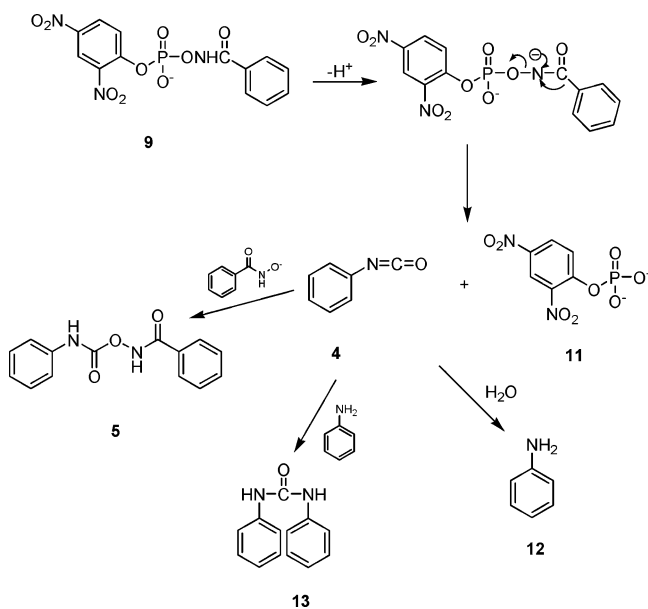
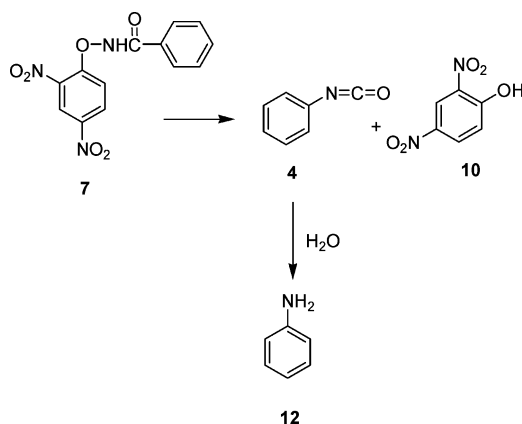
Low-resolution mass spectrometry (EI-MS) with the direct injection mode identified other important nonanionic products, undetectable by ESI-MS, such as those from the Lossen rearrangement (see Supporting Information). The products were extracted with ether from the reaction of **BDNPP** with aqueous BHO^- (1:1) at pH 10, 25 °C after 50 min, and EI-MS data were consistent with the formation of phenyl isocyanate (**4**, m/z 119), aniline (**12**, m/z 93), and diphenylurea (**13**, m/z 212). The results are consistent with Scheme 4 and indicate that the O-phosphorylated intermediate **9**, in basic media, decomposes to **DNPP** and phenyl isocyanate (**4**) in a reaction similar to the Lossen rearrangement. Subsequently, the isocyanate **4** decomposes to aniline (**12**), which reacts with phenyl isocyanate (**4**), giving diphenylurea (**13**). Scheme 4 is conceptually extremely important since it shows that the benzohydroxamate anion, which is a very reactive α -nucleophile, is in fact a self-destructive molecular scissor since it reacts effectively with **BDNPP** (which is normally used as a DNA model) and then loses its nucleophilic ability.

¹H NMR spectra were obtained over time for the reaction of **BDNPP**, **8** (6×10^{-3} M) with excess BHO^- , and **1** (0.184 M) at pH 9.0 in D_2O . The spectra (see Supporting Information) allowed identification of some of the expected products of reactions in Schemes 3 and 4, as well as reactants **BDNPP** (**8**) and BHO^- (**1**). The chemical shifts, multiplicities, and coupling constants (see Supporting Information) are fully consistent with the literature.^{6,7,19}

In order to confirm the structural assignments of product **7**, ¹H NMR spectra were recorded during the reaction of BHO^- (**1**) with 1-chloro-2,4-dinitrobenzene (**CDNB**, **6**), which leads

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SCHEME 4. Self Destruction of the Nucleophile via Lossen Rearrangement

SCHEME 5. Lossen Rearrangement of 2,4-Dinitrophenyl Benzohydroxamate


only to the attack on the aromatic ring (Scheme 2). NMR spectra (see Supporting Information) obtained as a function of time for this reaction demonstrated the formation of **7** in the first 5 min, which subsequently breaks down forming phenyl isocyanate (**4**), aniline (**12**), and **DNP** (**10**) (Scheme 5).

NMR spectroscopy allowed monitoring the changing concentrations of the most important species with time for the reaction of **BDNPP** (**8**) with **BHO**⁻ (**1**) (Figure 3). There is rapid disappearance of **BDNPP** (**8**) and appearances of **DNPP** (**11**) and intermediate **7**. Also, **DNP** (**10**) forms initially and continues to be generated during the reaction, probably by breakdown of **7** and of **DNPP** (**11**). As expected, the intermediate **9** is too unstable to be detected by this technique, but attack of **BHO**⁻ (**1**) on phosphorus is demonstrated by the initial formation of **DNP** (**10**), which must follow this path. The extents of attack of **BHO**⁻ on the aromatic ring and on the phosphorus of **BDNPP**, calculated from Figure 3, for the first 20 min of reaction were 76 and 24%, respectively.

Except for aniline (**12**), the expected products from the Lossen rearrangement of the intermediate **9**, observed mass spectrometrically in small quantities, were not identified by NMR spectroscopy. These overall results identify the complete mech-

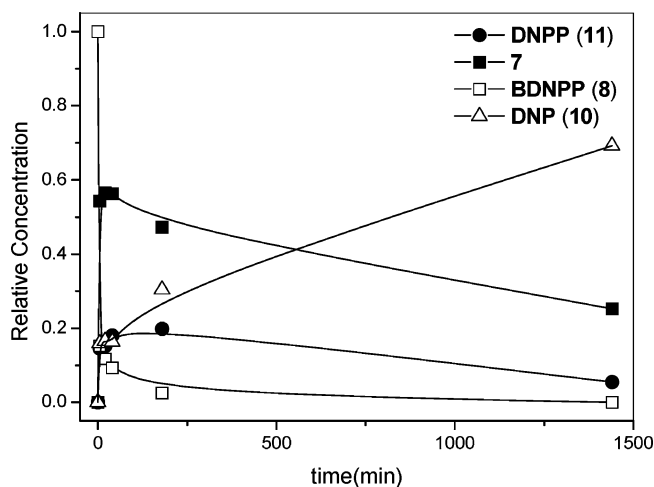


FIGURE 3. Relative concentrations of reagent and products (Schemes 3 and 4) of the reaction of **BDNPP** (6×10^{-3} M) in the presence of **BHO**⁻ (0.184 M), at pH 9.0 and 25 °C in D₂O.

anism in reactions of hydroxamic acids with phosphate esters (Scheme 3) which, although well studied,^{9,20–22} was not completely understood. Results from ESI-MS indicate nucleophilic attack of **BHO**⁻ (**1**) on the aromatic ring and on phosphorus of **BDNPP** (**8**) by detection of products **DNP** (**10**), **DNPP** (**11**), **7**, and **5**. Nonionic phenyl isocyanate (**4**), aniline (**12**), and diphenylurea (**13**) were not detected by this technique, but other mass spectrometric results confirmed the proposed reaction paths proposed, and all the Lossen rearrangement products (**4**, **5**, **12**, and **13**) were detected, as were those from attack on the aromatic ring (**7**, **10**, and **11**). NMR evidence confirms the reaction paths in Schemes 3 and 4 by detection of the **DNP** (**10**), **DNPP** (**11**), aniline (**12**), and **7**. Most importantly, this NMR analysis illustrates relative concentrations of some important species, confirming the simultaneous nucleophilic attack on the aromatic ring and on phosphorus, with the latter giving an intermediate that undergoes a Lossen rearrangement. We note that the rearrangement observed for the studied reactions was not observed with free hydroxamic acid at room temperature.¹⁴ As noted previously, benzohydroxamate anion represents a unique type of reactive α -nucleophile since it behaves as a one-cut self-destructive molecular scissor, losing its nucleophilic ability after destroying the **BDNPP** target.

Kinetic Studies. In the pH–rate profile for the first step of the reaction of **BDNPP** with **BHO**⁻ (0.05 M) (Figure 4), k_{obs} increases with pH up to a plateau, and the rate constant maximum corresponds to the pH region where the benzohydroxamate anion is the predominant species in solution (reported pK_a values for the acid dissociation constant of benzohydroxamic acid are 8.79 and 9.03 for ionic strengths 0.1 and 3, respectively).²³ Data for the minor contribution of the spontaneous hydrolysis of **BDNPP** are included for comparison, although its contribution is minor in the pH region studied.⁵

The first-order rate constant for the initial reaction of **BDNPP** with **BHO**⁻, at pH 10.0, increases linearly as a function of

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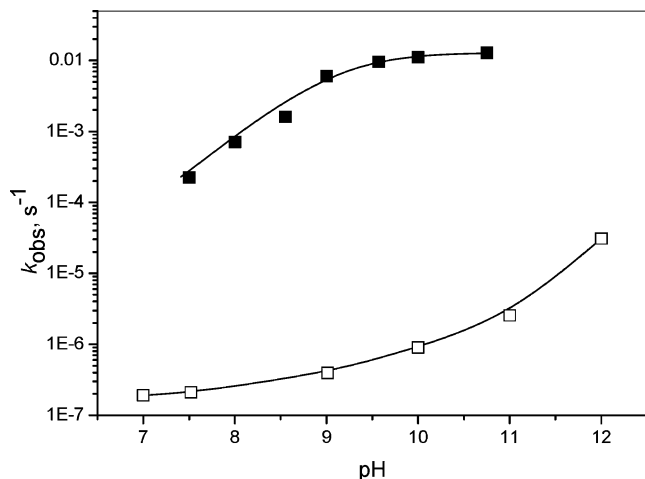


FIGURE 4. Variation of k_{obs} with pH for the reaction of **BDNPP** with (■) BHO^- (0.05 M) at 25 °C, $I = 1$ M (KCl). The solid line is theoretical (eq 2) with the parameters shown in Table 1. Data for the spontaneous hydrolysis of **BDNPP** are included for comparison, (□).⁵

TABLE 1. Rate and Dissociation Constants Used in the Kinetic Fitting for the Reaction of BHO^- with **BDNPP**

parameters	calculated value
$k_{\text{BHO}^-}^{\text{obs}}/\text{M}^{-1} \text{s}^{-1}$	$(2.61 \pm 0.03) \times 10^{-1}$
$k_{\text{BHO}^-}^{\text{C}}/\text{M}^{-1} \text{s}^{-1}$	$(1.98 \pm 0.03) \times 10^{-1}$
$k_{\text{BHO}^-}^{\text{P}}/\text{M}^{-1} \text{s}^{-1}$	$(6.21 \pm 0.03) \times 10^{-2}$
$\text{p}K_{\text{a}}$	9.16 ± 0.07

[BHO^-] (rate data in the Supporting Information), and the data are consistent with eq 1, where k_0 is the observed rate constant for the spontaneous water reaction, k_{HO^-} and k_{BHO^-} are the second-order rate constants for reaction with hydroxide ion and BHO^- , respectively.

$$k_{\text{obs}} = k_0 + k_{\text{OH}^-}[\text{OH}^-] + k_{\text{BHO}^-}[\text{BHO}^-] \quad (1)$$

Although, reaction of **BDNPP** with hydroxide ion can be neglected because the rate constant at pH 9.15, in the absence of BHO^- , is ca. $4 \times 10^{-7} \text{ s}^{-1}$, the experimental value reported in the literature was used as a fixed intercept to calculate the second-order rate constant.⁵ The calculated second-order rate constant ($k_{\text{BHO}^-} = 0.108 \text{ M}^{-1} \text{ s}^{-1}$) is much greater than that of the spontaneous water reaction of **BDNPP** ($k_0 = 1.90 \times 10^{-7} \text{ s}^{-1}$);⁵ that is, there is up to 5×10^5 -fold rate enhancement (for 1 M BHO^-), typical of α -nucleophiles. The data in Figure 4 are consistent with Scheme 6, allowing derivation of eq 2, which was used to fit the data.

$$k_{\text{obs}} = \frac{k_{\text{BHO}^-}[\text{BHOH}_T]}{1 + \frac{[\text{H}^+]}{K_{\text{a}}}} \quad (2)$$

In eq 2, BHOH_T is the total concentration of benzohydroxamic acid, k_{BHO^-} is the rate constant for the reaction with benzohydroxamate anion, and K_{a} is the acid dissociation constant

SCHEME 6

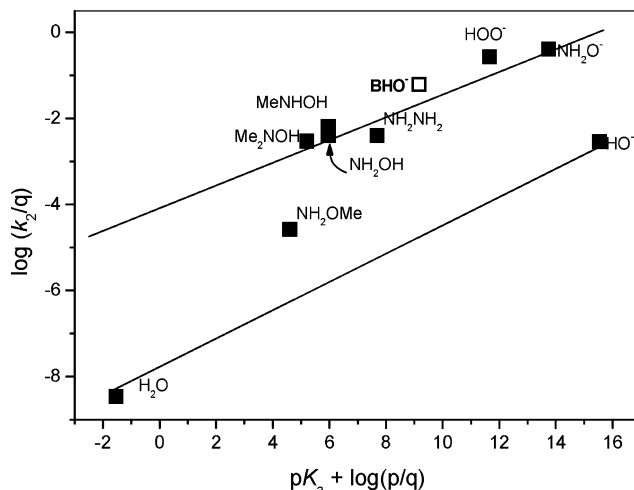
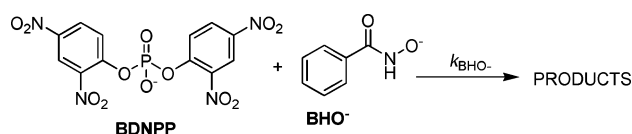


FIGURE 5. Logarithmic plot of rate constants against nucleophilic $\text{p}K_{\text{a}}$ values for reactions with **BDNPP**. Rate constants and $\text{p}K_{\text{a}}$ values are statistically corrected,²⁴ (■) nucleophiles from literature⁷ and (□) BHO^- . The line is drawn to guide the eye.

of benzohydroxamic acid forming BHO^- . Values of the individual rate and acid dissociation constants are in Table 1. Considering the difference in experimental conditions, the calculated acid dissociation constant for benzohydroxamic acid is in reasonable agreement with the value reported at high ionic strength (vide supra). On the basis of the percentage of attack on the aromatic ring and on the phosphorus atom calculated from Figure 3, the constant k_{BHO^-} was separated into two constants, $k_{\text{BHO}^-}^{\text{C}}$ and $k_{\text{BHO}^-}^{\text{P}}$, that correspond to the attack on the aromatic ring and phosphorus, respectively.

Data in Figure 4 and Table 1 show that reaction is largely of the anionic oxygen of BHO^- . The second-order rate constant $k_{\text{BHO}^-}^{\text{P}}$ for the reaction of BHO^- with **BDNPP** via attack on the phosphorus was compared with those for other nucleophiles with **BDNPP** from the literature, which fit a Brønsted plot. The data in Figure 5 show that the reactivity of BHO^- for attack on the phosphorus atom is similar to those of such nucleophiles as hydroxylamine and its methyl derivatives, which react preferentially via the oxygen atom of the zwitterionic ammonia oxide tautomers ($\text{NH}_3^+\text{-O}^-$). In fact, as expected, the OMe derivative of hydroxylamine shows very low reactivity.²

Conclusions

The reaction of **BDNPP** with BHO^- was monitored by ESI-MS, EI-MS, NMR, and UV-vis. The results indicate that BHO^- reacts simultaneously at the phosphorus atom and the aromatic carbon, and expected products were observed. The intermediate **9** from nucleophilic attack on phosphorus was not detected because it undergoes a Lossen rearrangement, leading to phenyl isocyanate (**4**), aniline (**12**), diphenylurea (**13**), and *O*-phenylcarbonyl benzohydroxamate (**5**), which were detected. Reaction via nucleophilic attack on the aromatic carbon was confirmed by appearance of the intermediate **7**, and its formation was confirmed by comparison with the reaction of 1-chloro-2,4-dinitrobenzene with BHO^- . Kinetics for the reaction of **BDNPP** with 0.05 M BHO^- showed a significant enhancement of up to 10^5 -fold over the spontaneous reaction. The benzohydroxamate anion was the most reactive species, with efficiency superior than expected on the basis of the Brønsted plot for the

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hydroxylamine derivatives, which are considered highly reactive α -nucleophiles. Benzohydroxamate anion represents a unique type of reactive α -nucleophile since it behaves as a one-cut self-destructive molecular scissor, which loses its nucleophilic ability after destroying the phosphate diester target. This type of reactive nucleophilic reagent may be of utility in the design of special scissors for gene therapy, and we are currently investigating the reactivity of other hydroxamate anions in similar systems.

Experimental Section

Materials. **BDNPP** as the pyridinium salt was prepared as described,⁵ and the pyridinium ion was exchanged for Na^+ on the cation-exchange resin in the Na^+ form. Benzohydroxamic acid (**BHOH**) was prepared following a described procedure,¹¹ with modifications summarized in the Supporting Information, resulting in the formation of white crystals: mp 125–126 °C (lit.²⁵ 124–125 °C). All other inorganic and organic reagents, such as 2,4-dinitrophenol and 1-chloro-2,4-dinitrobenzene, were of the highest purity and were used as purchased.

Kinetics. Reactions followed spectrophotometrically were started by adding 10.0 μL of a stock solution of the substrate (4×10^{-3} M) in MeCN to 3.0 mL of the aqueous reaction mixture, with a large excess of the nucleophile, assuring first-order kinetics for the initial nucleophilic attacks upon the substrate. Solutions were self-buffered from pH 8–10 with **BHOH/BHO⁻** and aqueous 0.1 M NaOH and for pH 6–8, with 0.01 M phosphate buffer.

Formation of 2,4-dinitrophenoxide ion (**DNP**) was monitored at 400 nm in the water jacketed thermostatted cell holder, at 25.0 ± 0.1 °C, of a diode-array spectrophotometer. Observed first-order rate constants (k_{obs}) were calculated from linear plots of $\ln(A_{\infty} - A_t)$ against time for at least 90% of the reaction by using an iterative least-squares program; correlation coefficients were >0.999 for all kinetic runs. Reactions of **BDNPP** with **BHOH** as a function of pH were first-order with respect to **BDNPP**, and in all cases, more than 1.5 mol of **DNP** was always formed in comparison to reactant.

Mass Spectrometry: In order to identify intermediates and reaction products of **BDNPP** with **BHO⁻**, direct infusion electrospray ionization mass spectrometry analyses were performed with a hybrid triple quadrupole linear ion-trap mass spectrometer.^{1,26,27} For typical electrospray ionization (ESI-MS) conditions, 1 mL of 1×10^{-6} M **BDNPP**, in aqueous medium at pH 10, was mixed with 100 $\mu\text{L min}^{-1}$ of 0.1 M aqueous **BHO⁻**. A microsyringe pump

delivered the reagent solution into the ESI source at a flow rate of 10 $\mu\text{L/min}$. ESI and the QqQ (linear trap) mass spectrometer was operated in the negative-ion mode. Main conditions: 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. Some of the main anionic species detected by ESI-MS were subjected to ESI-MS/MS by using collision-induced dissociation (CID) with nitrogen and collision energies ranging from 5 to 45 eV.

Other MS analyses were performed to detect nonanionic products in the reaction medium. Reaction of **BDNPP** with **BHO⁻** (1:1) was followed in aqueous solution at pH 10 and 25 °C for 50 min, and the products were extracted with diethyl ether, and after evaporation, the EI-MS spectrum was obtained in a low-resolution instrument by using the direct injection mode, with electron ionization (EI) at 70 eV. The samples were placed in a sample vial fixed onto the probe. Probe temperatures were programmed as follows: 10 °C min^{-1} up to 85 °C and held for 3 min, increasing from 85 to 300 at 20 °C min^{-1} and held for 3 min.

NMR Spectroscopy. All ¹H spectra were monitored on a spectrometer (400 MHz) at 25 °C, in D₂O, except for reactions with 1-chloro-2,4-dinitrobenzene, where 10% CD₃CN was used. Some aromatic ¹H signals were obscured by signals of the excess **BHO⁻**. The ¹H chemical shifts are referred to internal sodium 3-(trimethylsilyl) propionate (TSP), and pD was the observed pH +0.4 in D₂O at 25 °C.²⁸

Potentiometric Titration. Potentiometric titrations were carried out with a pH meter and a automatic buret, in a 150.0 mL thermostatted cell, under N₂ at 25 °C. A solution of 20.0 mL of 5.0 mM of **BHO⁻** was acidified with 1.0 mL of 0.1 M HCl and titrated with small increments of CO₂-free 0.1008 M KOH with 0.1 ionic strength (KCl). The FITEQL v.4.0 program²⁹ was used to calculate the constants.

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Supporting Information Available: Preparation of benzo-hydroxamic acid, UV absorption spectra for the reaction of 1-chloro-2,4-dinitrobenzene (**CDNB**) with benzohydroxamate anion (**BHO⁻**), low-resolution mass data for product identification, NMR spectral data for product identification, and kinetic data reaction of **BDNPP** with **BHO⁻**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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