Articles

Nitrobenzyl-Based Photosensitive Phosphoramide Mustards: Synthesis and Photochemical Properties of Potential Prodrugs for Cancer Therapy

Robert Reinhard and Brigitte F. Schmidt*

Center for Light Microscope Imaging and Biotechnology, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213

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Several nitrobenzyl-based photosensitive phosphoramide mustards were synthesized. The nitrobenzyl moiety was structurally varied to find the most promising prodrug candidates in respect to photorelease and activity of the alkylating species. The synthesis of these compounds proved to be applicable even in regard to compounds with additional functionalization. The target molecules **13a**,**b** to **14** exhibited the expected red shift in their absorption spectra maximum compared to the parent nitrobenzyl moiety. As seen by UV and ^{31}P NMR spectroscopy, the phosphoramide mustard was quickly liberated upon irradiation with mercury arc lamps. Assaying the structurally different prodrugs on their alkylating activity showed that compounds **13b** and **14**, derived from secondary benzyl alcohols, are promising prodrug candidates. Their water solubility and the possibility of attaching macromolecules are encouraging vis-à-vis future investigations on their in vitro cytotoxicity.

Introduction

Photolabile ("caged") compounds provide an important tool in the investigation of many biological processes.[1] Most functionalities occurring in biological molecules, such as amines,[2] alcohols,[3] carboxylic acids,[4] phosphates,[5] and thiols,[6] have been caged, and the feasibility of this concept has been established by the reactivation of their biological function after irradiation with light of appropriate wavelength. A number of light-sensitive protective groups have been developed.[1a,7] The nitrobenzyl group has been shown to be among the most useful members in this group of compounds, primarily due to its ability to derivatize a number of functional groups, the reliability of its photochemical reaction in different systems, and the often straightforward synthesis of the caged moiety.[8] The mechanism of the intramolecular redox process responsible for the decaging reaction has been well studied, especially by Trentham and coworkers.[9] Initial applications involved "caged ATP"[10] and continued with the caging of a series of biologically important molecules. Applications include modern techniques such as fluorescent imaging,[11] light-directed solid-phase synthesis,[12] and combinatorial chemistry.[13]

Antiproliferative agents play a very important role in cancer chemotherapy.[14] Compounds with alkylating activity remain among the most valuable in this field, in particular the phosphoramide mustard-based agents (e.g., cyclophosphamide).[15] Most of the phosphoramide mustard-derived reagents in and of themselves are inactive "prodrugs". They are activated by different mechanisms that involve hydrolytic,[16] bioreductive,[17] and

* To whom correspondence should be addressed. E-mail: Bschmidt@andrew.cmu.edu.

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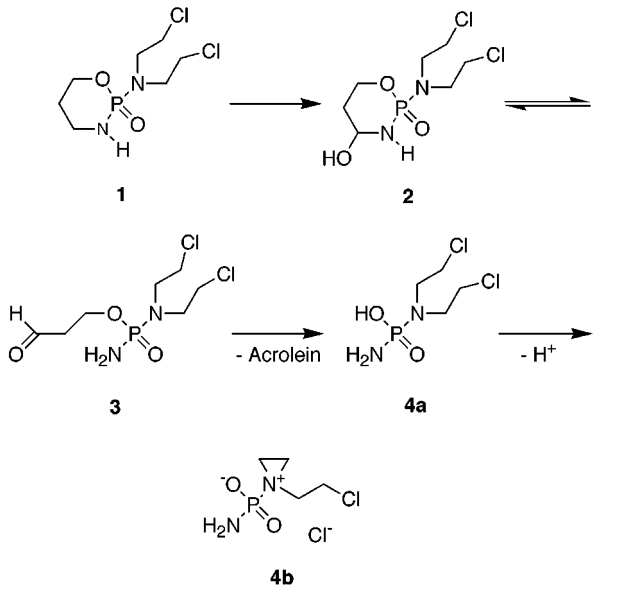
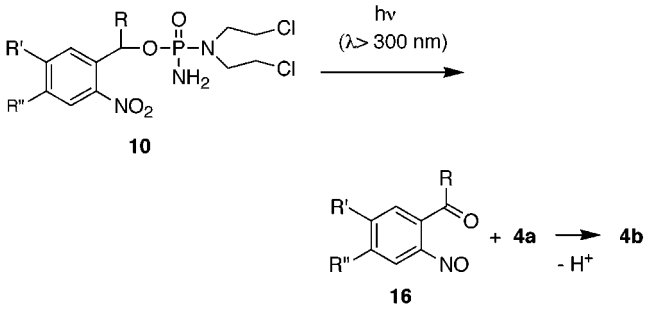
Scheme 1



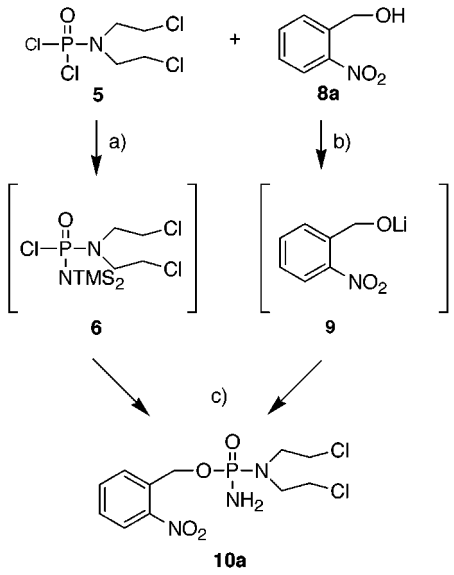
Scheme 2



biooxidative[18] pathways. Interestingly, photochemical activation of alkylating species has not been developed, despite the potential advantage of controllable release. This form of activation requires light of a particular wavelength, typically $\lambda > 340$ nm.

The metabolism of cyclophosphamide (**1**)[15] (Scheme 1) suggests a strategy of introducing the caging moiety. To get a light-sensitive prodrug candidate, we synthesized nitrobenzyl esters of the phosphoric acid derivative **4a**. Irradiation of these compounds (type **10**) should directly lead to the buildup of the aziridinium cation **4b**, which is the active alkylating species (Scheme 2). Molecule **10** also indicates the possible structural variations that will affect the conditions of the photochemical decaging reaction. Electron-donating residues R′ and R″ on the aromatic ring are known to shift the absorption maximum of the photosensitive group further to the red, thus allowing efficient irradiation with longer wavelengths. On the other hand, residues R on the benzyl position are known to affect the reaction rates and yields of the decaging reaction. With these aspects in mind, it was our goal to synthesize a number of structurally different caged phosphamides and to compare their photochemical behavior. In particular, we wanted to develop compounds where R′ is not only an electron-donating residue but also a linker that would allow attachment to other molecules. These molecules include peptides and peptidomimetics

Scheme 3[a]



[a] Reagents and conditions: (a) 2 $HNTMS_2$, THF; (b) LHMDS, THF, 0 °C; (c) THF, 0 °C then H^+.

for cell-specific uptake, dyes for signaling, or macromolecules for solid-phase chemistry in future applications.

Results and Discussion

Synthesis. We describe the synthesis of nitrobenzyl-based phosphoramide mustard prodrugs that can be activated by light. The feasibility of this approach, to temporally inactivate phosphoramide mustard and restore its activity via irradiation, was tested with the model compound 2-nitrobenzyl *N*,*N*-bis(2-chloroethyl)-phosphordiamidate (**10a**). The synthesis of this compound is outlined in Scheme 3. *N*-Bis(2-chloroethyl)-amidophosphoric acid dichloride (**5**), prepared from phosphorus oxychloride and the appropriate amine hydrochloride,[19] was converted to the bisamide **6** by the reaction with 2 equiv of hexamethyldisilazane. The 2-nitrobenzyl alcohol **8a** was deprotonated with lithium bis(trimethylsilyl)amide (LHMDS) and reacted with in situ generated **9** to yield, after final hydrolysis of the silyl groups, the nitrobenzyl-protected phosphoramide mustard **10a**. After proving the successful photochemical release of the phosphoramide mustard from our model compound **10a** (see next section), we developed a general route for the synthesis of caged phosphoramide mustards from higher functionalized 2-nitrobenzyl alcohols. We reduced the commercially available 6-nitroveratraldehyde (**7a**) with sodium borohydride to give the primary benzyl alcohol **8b** (Scheme 4). The secondary benzyl alcohol **8c** was prepared by stannous fluoride-mediated alkylation of 2-nitrobenzaldehyde (**7b**) with allyl iodide. Analogously, we synthesized the 5-hydroxy-2-nitrobenzyl alcohols **11a** and **11b** from the commercially available 5-hydroxy-2-nitrobenzaldehyde (**7c**). Compounds **11a** and **11b** opened a route to water-soluble caged phosphoramide mustards or prodrugs that could be linked to other molecules such as dyes and proteins. The primary alcohol **11a** and the secondary alcohol **11b** were alkylated at the phenolic position by reaction with methyl bro-

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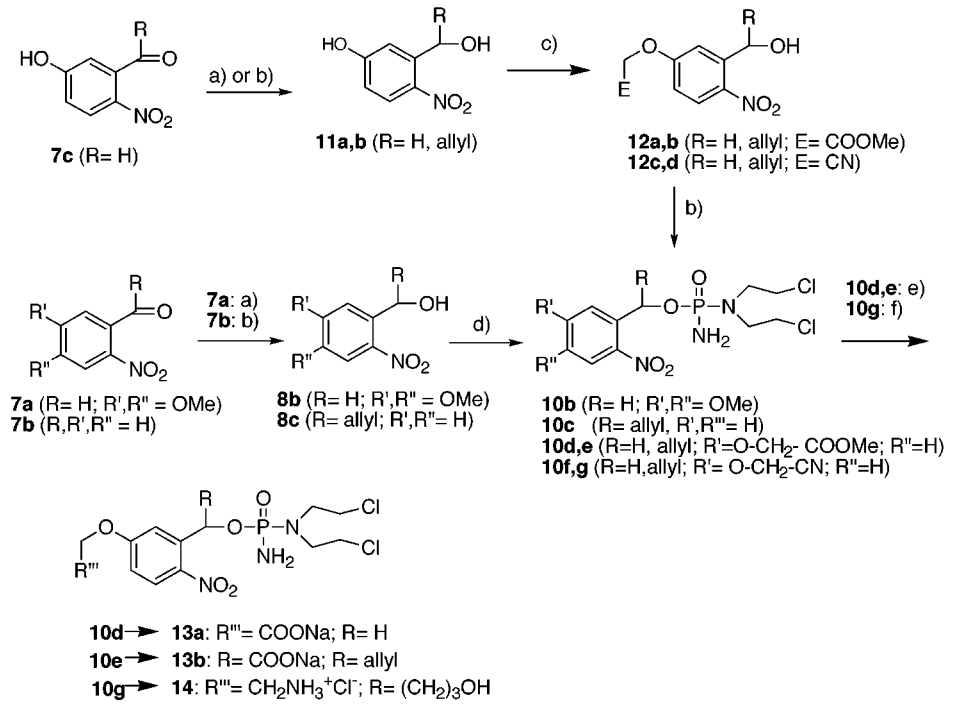
Scheme 4[a]



[a] Reagents and conditions: (a) $NaBH_4$, EtOH; (b) SnF_2, C_3H_5I, DMF; (c) NaOH, $BrCH_2E$, MeOH, reflux; (d) LHMDS, THF, 0 °C; **5**; then NH_3(g); (e) 0.1 N NaOH (1 equiv), MeOH, 0 °C; (f) $BH_3{\cdot}SMe_2$ (excess), THF, rt; $NaOH/H_2O_2$; HCl.

moacetate to give **12a** (R = H) and **12b** (R = allyl) and with bromoacetonitrile to yield **12c** (R = H) and **12d** (R = allyl). Sodium hydroxide was used as a base in both cases. In the next step, we introduced the phosphoramide mustard moiety in the substituted 2-nitrobenzyl alcohols. It turned out that the protected phosphoramide mustards of this work, with the exeption of our model compound **10a**, could be prepared in a one pot synthesis via direct reaction of phosphoric acid dichloride **5** and the substituted 2-nitrobenzyl alcohols **8b**,**c** and **12a**−**d** as outlined in Scheme 3. The alcohols **8b**,**c** and **12a**−**d** were initially deprotonated with LHMDS, reacted with the phosphoric acid dichloride **5**, and finally converted to the phosphoric acid diamidates **10b**−**g** by bubbling ammonia through the solution. Interestingly, in the case of the nonsubstituted 2-nitrobenzyl alcohol **8a**, this direct route resulted in the phosphordiester amidate **15**.

The carboxylic acid methyl ester functionalized compounds **10d** and **10e** were converted into the corresponding sodium carboxylates **13a** and **13b** by hydrolysis with 0.1 N aqueous sodium hydroxide. The nitrile **10g** was reduced to the amine by an excess of borane−dimethyl sulfide complex; as the C=C of the allyl residue is also hydroborated in this step as well, an intermediate oxidation step of the alkylborane was necessary. The final addition of hydrochloric acid yielded the ammonium chloride salt **14**.

Even though the nitrile **10f** could be reduced to its amine with borane−dimethyl sulfide complex as well, the product did not appear to be sufficiently stable toward hydrolysis at the benzyl position for isolation of a pure compound. As shown by the ^{1}H NMR spectrum of the product mixture, there was always a significant amount of the corresponding benzyl alcohol present (δ(CH_2OH) 4.82 (s) ppm (D_2O); δ(CH_2OP) 5.25 (d) ppm (D_2O)), even after chromatographic purification. We did not make further attempts to isolate this product, since an easily hydrolyzable product was not expected to be a useful prodrug with respect to their application in a biological medium.

The products were characterized by ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra. The phosphorester diamidates showed typical signals in their ^{31}P NMR spectra. While **10a**,**b**,**d**,**f** and **13a** showed one singlet, the diastereomeric mixtures of **10c**,**e**,**g** and **14** appeared as two singlets in the region between δ 22.54−21.15 ppm, indicating a mixture of diasteromers. Attempts to separate any of the diastereomers were not successful. The doubling of signals in the carbon spectra reflects the appearance of diasteromers as well. The formation of the phosphorester bond in all "caged" phosphoramide mustards (**10a**−**g** and **13**−**15**) was indicated by the ^3J(P,C) coupling to the benzylic carbon (primary, 64−66 ppm; secondary, 72−75 ppm) in the range of 2.5−5.0 Hz and a ^3J(P,C) coupling in the range of 4.15−6.16 Hz to the α-carbon (48−50.5 ppm) of the bis(chloroethyl)amine moiety. Furthermore, infrared spectra exhibited strong absorptions for the carboxylic acid methyl ester groups at ν 1760 cm^{-1} for **10d** and **10e** and weak absorptions for the cyano groups at ν 2260 and 2200 cm^{-1} for **10f** and **10g**, respectively. The phosphoramidates **13**−**14** are highly water-soluble compounds, while compounds **10a**−**g** and **15** showed only poor or no water solubility.

Photolysis of the Caged Phosphoramide Mustards. In the following section, the examination of the photolysis properties of products **10a**−**g** and **13**−**15** will be described. The photochemical reaction of compound **10a** (R, R′, R″ = H) is outlined in Scheme 2. As can be seen from Figure 1, irradiation of a solution of the nitrobenzyl phosphoramide mustard **10a** in acetonitrile (0.2 mM) for 20 s resulted in the expected changes in the absorption spectrum. The nitrobenzyl chromophore of

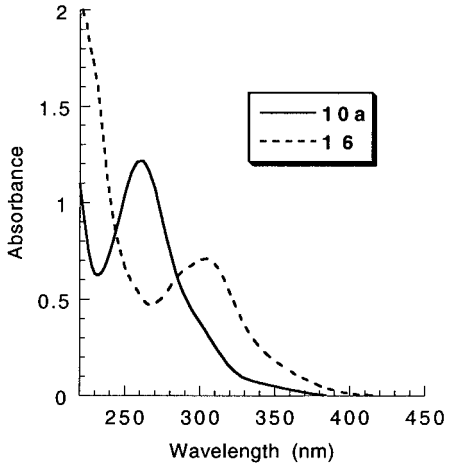
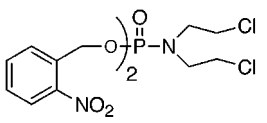


Figure 1. UV spectral recording of the photolysis of nitrobenzylphosphoramide mustard (**10a**) in acetonitrile solution (0.2 mmol). The sample was photolyzed in a quartz cuvette (1 cm path length) for 20 s to give **16** (λ_{max} = 304 nm).

10a showed an absorption maximum at λ 262 nm, the nitrosobenzaldehyde photoproduct **16** (R, R′, R″ = H) at λ 304 nm.

Since the absorption spectrum could only detect the photochemical nitrosobenzaldehyde byproduct and not the actual decaged substrate, we sought further proof of the successful cleavage reaction. In this respect, ^{31}P NMR spectroscopy was a powerful tool. The conversion of the phosphoric acid ester derivative **10a** to the free phosphoric acid derivative **4a** (see Scheme 2) was expected to result in a significant upfield shift of the observed ^{31}P NMR resonance. As predicted, the free phosphoric acid derivative **4a** resonated at δ 3.15 ppm, while the signal for the educt **10a** was observed at δ 21.17 ppm (CD_3CN/H_2O (1:1)) (Figure 2). Solutions of higher concentration lead to longer reaction times because of internal filter effects of the nitrosobenzaldehyde photoproduct. The use of a 12 mM solution required 6 min for an almost complete reaction in this experiment. Due to further reactions of **4a** in the aqueous medium (see its metabolism in Scheme 1), a whole group of signals was actually observed, which resulted in an overall broad signal.

In addition to these spectroscopic investigations of the cleavage reaction, an assay that elucidated the alkylating activity of the irradiated solutions was desirable. It was not only the goal to obtain a comparison of the overall activity of the structurally different types of caged phosphoramide mustards **10a**−**g** and **13**−**15** but also to



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determine the dependence of decaging rate on irradiation time. Solutions were assayed for alkylating activity after irradiation of the samples for 0, 2, 4, 6, and 10 min with the well-established 4-(4-nitrobenzyl)pyridine (NBP) reagent.[20] Prior to the interpretation of the resulting data shown in Figure 3, some comments are necessary.

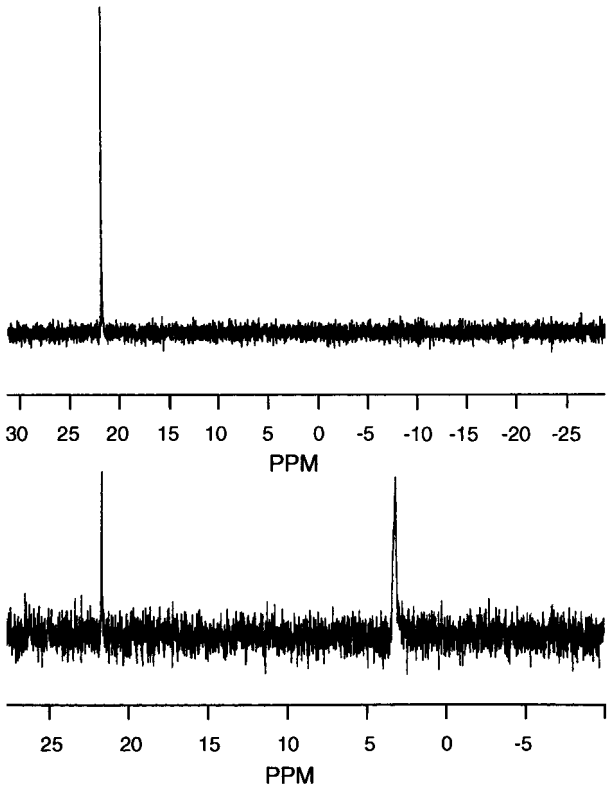

Figure 2. (a) ^{31}P NMR spectrum of **10a** in CD_3CN/H_2O solution (1:1) (12 mM); (b) ^{31}P NMR spectrum of the solution in (a) after irradiation for 3 min in a 10 mm NMR tube (see the Experimental Section for details).

The irradiation-coupled NBP assay was performed with a mercury arc lamp with a maximum emission at λ 360 nm and a wavelength range of λ 300−400 nm. The energy output of this lamp was measured to be 3.5% of the EFOS mercury arc lamp used in the spectroscopic photolysis studies (Figures 1 and 2). Since concentrations of the irradiated sample solutions were much lower for the NBP assay experiments than for the spectroscopic studies (0.2 mM compared to 12 mM), the less powerful illumination system yielded experimentally more convenient time frames (0−10 min, see Figure 3). The use of a filter system that transmitted light in the range λ 350−500 nm with a maximum at λ 402 nm resulted in very slow and incomplete reaction, even for the absorption red-shifted derivative **10b**. Therefore, all compounds were photolyzed in the wavelength range of λ 300−400 nm.

It should be mentioned that, in all cases studied, there was an absorption present at t = 0 min (before irradiation), which was slightly shifted to the blue (λ 515−520 nm) as compared to the chromophore for the alkylated (nitrobenzyl)pyridine absorbing at λ 542 nm. To define the origin of this absorption, we synthesized bis(2-nitrobenzyl) phosphate (**17**) as a control (see the Experimental Section) and performed the NBP assay with this compound (t = 0, 10 min). A weak absorption at λ 516 nm was observed and did not change during photolysis. Other experiments showed that nonphosphorylated compounds such as **12a** do not have a similar absorption. Therefore, we concluded that an adduct between the NBP and the phosphate moiety[21] is responsible for the "baseline" absorption at λ = 516 nm.

(20) We used a slightly modified procedure of the one described by: Friedman, O. M.; Boger, E. *Anal. Chem.* **1961**, *33*, 906−910 (see Experimental Section).

(21) Getz, M. E.; Watts, R. R. J. *Assoc. Off. Agric. Chem.* **1964**, *47*, 1094−1096.

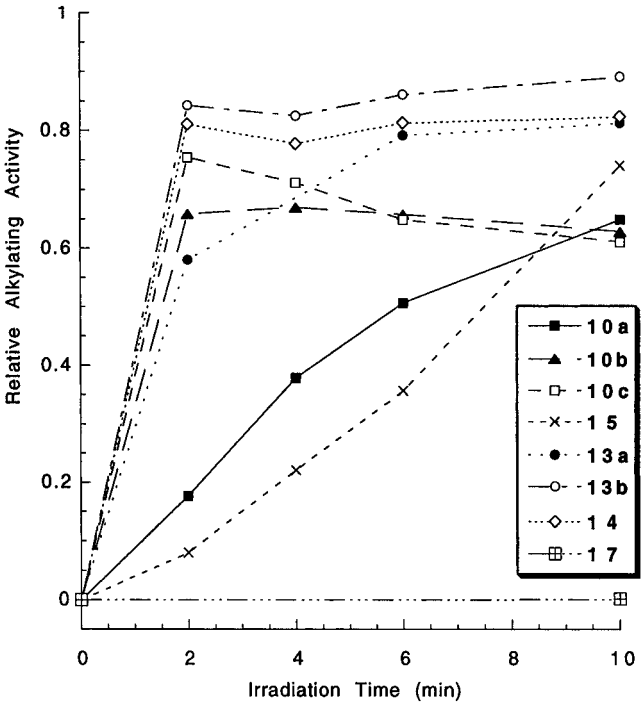

Figure 3. 4-(4-Nitrobenzyl)pyridine (NBP) assay of irradiated solutions (t = 0, 2, 4, 6, 10 min) of **10a**−**c** and **15** (in CH_3CN), **13a**,**b** and **14** (in H_2O), and **17** (in EtOH/H_2O 1:1). The relative alkylating activity was determined by measuring the absorbance of the alkylated NBP chromophore at λ = 542 nm. The numbers are relative numbers. See the text and Experimental Section for details.

In addition to the baseline absorption, there are weak absorptions present at λ 542 nm for some compounds even without irradiation. Typically, the derivatives of the primary nitrobenzyl group exhibited this phenomenon, which probably originated from hydrolysis of the compounds due to the rather harsh reaction conditions (boiling in an aqueous buffer solution, pH 4.6, for 20 min) required for the NBP assay. Bis(nitrobenzyl)phosphoramide mustard **15** showed the highest final alkylation activity. The compound's final absorption (t = 10 min) at its maximum at λ 542 nm was set to 1, and all other absorption values were normalized. The phosphordiester amidate **15** also showed the highest baseline absorption. This compound was more likely to undergo hydrolysis under NBP assay conditions than the phosphorester bis(amidates), thus showing a higher baseline value. The data presented in Figure 3 were finally corrected for their baseline absorption to take this nonillumination-based effect into account. When the irradiation was continued after the compounds exhibited their maximum activity, the curves tended to drop slightly, indicating reactions of the photolyzed reaction products. The results in Figure 3 can be summarized as follows:

Bis(nitrobenzyl)phosphoramide mustard **15** cleaved with the slowest reaction of all compounds. The illumination-mediated final alkylating activity of **15** did not significantly differ from **10d**, **13b**, and **14**, considering that its t = 0 value was higher than the other derivatives.

The derivatives **13b** and **10c** with an allyl substitutent at the benzyl position exhibited faster photochemical conversions than their nonsubstituted analogues **13a** and **10a**. The maximum activity for **13b**, **10c**, and **14** was reached within the first 2 min of irradiation, with **13b** exhibiting the highest alkylating activity. Substituents at the benzyl position have been shown to stabilize the aci-nitro intermediate of the photoreaction, thus enhancing the rate of photolysis.[10]

Comparison of the primary alcohol-derived phosphoramidates **10a**,**b** and **13a**, with respect to their absorption maxima, at (**10a**) λ_{max} 262 nm, (**10b**) λ_{max} 346 nm, (**13a**) λ_{max} 316 nm, showed that irradiation with light of a wavelength maximum at 360 nm was best suited for the compound **10b**. Although the rate of photorelease of **10a** was significantly slower, it reached the same overall activity after 10 min as the 4,5-dimethoxy-substituted 2-nitrobenzyl derivative **10b**. Compound **13a** showed the most efficient photorelease of the alkylating moiety of the phosphoramides derived from primary benzylic alcohols.

In summary, we found a convenient method to synthesize a number of nitrobenzyl-caged phosphoramide mustards. This synthesis turned out to be a general route, even if the compounds bear additional functional groups. The main target molecules, the water-soluble phosphoramide mustards **13a**,**b** and **14**, were among the most promising photosensitive alkylating agents. Prodrugs **13b** and **14**, based on secondary benzyl alcohol caging moieties, exhibited the best results.

Our ongoing research on these compounds will focus on the study of their antiproliferative effect on living cells. Phosphoramide mustard, the active metabolite of cyclophosphamide, is not stable as such. Our prodrugs are well suited to the study of phosphoramide mustards in different cell types in a time- and space-controlled fashion, independent of metabolic prodrug transformations. Functional groups at the aromatic ring of the caging moiety would allow linking these prodrugs to molecules that mediate specific cellular uptake to investigate tissue specificity.

Experimental Section

Synthesis. General Methods. All reactions were conducted under a dry argon atmosphere and under subdued light. Anhydrous THF (Aldrich Chem. Co.) and all reagents were used as received. Other solvents were reagent grade and used without further purification. ^{1}H NMR spectra were recorded at 300 MHz with the residue solvent peak used as reference relative to TMS; ^{31}P NMR spectra were run at 121 MHz with trimethyl phosphite (1% in benzene) as external standard (δ 141 ppm). Flash chromatography was performed on silica gel (J. T. Baker, 40 μm particle size). Melting points are not corrected. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA).

Safety Note. Because cyclophosphamide is a potent biological alkylating agent, it is only prudent to assume that derivatives of its active metabolite phosphoramide mustard are also potentially toxic and carcinogenic. At all times, efficient hoods and protective clothing should be used in working with these substances.

Bis(2-chloroethyl)phosphoramidic dichloride (5) was prepared as previously described.[19]

2-Nitrobenzyl *N*,*N*-Bis(2-chloroethyl)phosphordiamidate (10a). Bis(2-chloroethyl)phosphoramidic dichloride **5** (0.2 g, 0.77 mmol) was dissolved in 5 mL of THF, the solution cooled to 0 °C, and hexamethyldisilazane (0.33 mL, 1.56 mmol) was slowly added. The solution was brought to room temperature and stirred for 15 h. A white precipitate was formed. Ether (15 mL) was added, and the flask was put in a freezer (−22 °C) for 1 h. The mixture was filtered, and the solvents were removed. The resulting colorless oil was dissolved in THF (10 mL). Meanwhile, 2-nitrobenzyl alcohol (**8a**) (0.12 g, 0.78 mmol) was dissolved in 10 mL of THF and cooled to 0 °C, and a 1 M solution of LHMDS in THF (0.78 mL, 0.78 mmol) was slowly added. This solution was stirred at 0 °C for 10 min and then

added with vigorous stirring at 0 °C to the above phosphordiamide solution by means of a transfer needle. The resulting yellow solution was kept at 0 °C for 3 h. The THF was evaporated, and the resulting oil was extracted with CH_2Cl_2 (50 mL) and water (50 mL). The layers were separated, and the aqueous layer was extracted (2×) with 20 mL of CH_2Cl_2. After evaporation of the solvent, an oil resulted, which primarily consisted of **8a** and product **10a**. Using flash chromatography, **8a** was separated by elution with EtOAc. During chromatography, the silyl groups of the phosphordiamidate were rapidly hydrolyzed. Product **10a** was eluted with a 5:1 mixture of EtOAc/MeOH (R_f 0.55) and crystallized from ether to yield 0.18 g (66%) of yellow crystals: mp 76 °C; IR (KBr) 1260 (s, P═O) cm^{-1}; ^1H NMR (CD_3CN) δ 8.09 (dd, J = 8.0, 0.9 Hz, 1 H), 7.81 (d, J = 8.0 Hz, 1 H), 7.74 (dt, J = 8.0, 1.0 Hz, 1 H), 7.54 (t, J = 8.0 Hz, 1 H), 5.30 (d, J = 6.9 Hz, 2 H), 3.63 (t, J = 6.9 Hz, 4 H), 3.46−3.42 (br, 2 H), 3.46−3.22 (m, 4 H);^{13}C NMR (acetone-d_6) δ 147.8, 134.7, 134.6, 134.4, 129.3, 125.3, 63.9 (J_{CP} = 2.57 Hz), 50.1 (J_{CP} = 4.23 Hz), 43.1; ^{31}P NMR (CD_3CN) δ 22.00; UV (CH_3CN) λ_{max} 262 nm (ϵ_{max} 5520). Anal. Calcd for $C_{11}H_{16}Cl_2N_3O_4P$: C, 37.09; H, 4.53; N, 11.80. Found: C, 37.24; H, 4.56; N, 11.74.

4,5-Dimethoxy-2-nitrobenzyl Alcohol (8b). This compound was prepared by sodium borohydride reduction of 6-nitroveratraldehyde (**7a**) in ethanol solution: yield 96%; mp 142 °C; IR (KBr) 3500 (s, OH), 1520 (s, NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.59 (s, 1 H), 7.07 (s, 1 H), 4.86 (d, J = 6.5 Hz, 2 H), 3.90 (s, 3 H), 3.85 (s, 3 H), 2.64 (t, J = 6.5 Hz, 1 H); ^{13}C NMR (acetone-d_6) δ 154.9, 148.4, 139.7, 134.8, 110.4, 108.6, 61.7, 56.4.

1-(2-Nitrophenyl)-3-buten-1-ol (8c). 2-Nitrobenzaldehyde (**7b**) (2.00 g, 13.23 mmol) was dissolved in DMF (30 mL), and 1.25 mL (1 equiv) of allyl iodide was added. The solution was cooled to 0 °C, and stannous fluoride (2.07 g, 1 equiv) was added. The ice bath was removed, and the solution was stirred for 2 h. A slightly exothermic reaction occurred. Water (60 mL) was poured into the DMF solution, and the aqueous layer was extracted (3×) with 30 mL of ether. The organic layer was then washed with 2 × 50 mL of saturated NH_4Cl solution. After evaporation of the ether, a yellow oil remained; its identity as **8c** was checked by ^1H NMR, and it was used without further purification: yield 74%; IR (film) 3400 (br, m, OH), 3080 (w, ═CH), 1525 (vs, NO_2) cm^{-1}; ^1H NMR (CD_3CN) δ 7.85 (dd, J = 8.0, 1.2 Hz, 1 H), 7.79 (dd, J = 7.9, 1.2 Hz, 1 H), 7.66 (dt, J = 8.0, 1.2 Hz, 1 H), 7.43 (dt, J = 7.7, 1.5 Hz, 1 H), 5.96−5.82 (m, 1 H), 5.22−5.17 (m, 1 H), 5.10−5.02 (m, 2 H), 3.57 (d, br, J = 3.6 Hz, 1 H), 2.57−2.49/2.44−2.34 (m, 2 H); ^{13}C NMR (acetone-d_6) δ 148.5, 141.0, 135.5, 133.7, 129.0, 128.6, 124.4, 117.5, 68.9, 43.7.

4,5-Dimethoxy-2-nitrobenzyl *N*,*N*-Bis(2-chloroethyl)-phosphordiamidate (10b). Typical Procedure. Alcohol **8b** (0.60 g, 2.81 mmol) was dissolved in 20 mL of THF and cooled to 0 °C, and a solution of LHMDS in THF (1 M, 2.8 mL, 2.8 mmol) was slowly added. This mixture was stirred at 0 °C for 10 min. Meanwhile, **5** (0.72 g, 2.80 mmol) was dissolved in 20 mL of THF and cooled to 0 °C. The solution of the alkoxide was transferred into this flask by means of a cannula, and stirring was continued for 1 h at 0 °C. Ammonia was bubbled through the solution at a moderate rate for 30 min at 0 °C, and then the solution was warmed to room temperature and stirred for an additional 2 h. The same workup procedure was applied as described for compound **10a**, R_f (EtOAc/MeOH 5:1) 0.50. The product formed a pale yellow powder when ether was added: mp 121 °C; yield 60%; IR (KBr) 1280 (vs, P═O) cm^{-1}; ^1H NMR (CD_3CN) δ 7.67 (s, 1 H), 7.21 (s, 1 H), 5.42 (dd, J = 7.0, 3.9 Hz, 2 H), 3.87 (s, 6 H), 3.68 (t, J = 6.7 Hz, 4 H), 3.45 (dt, J = 11.7, 6.7 Hz, 4 H); ^{13}C NMR (acetone-d_6) δ 155.1, 149.4, 140.4, 129.7 (J_{CP} = 8.4 Hz), 111.5, 109.3, 64.3 (J_{CP} = 3.3 Hz), 56.9, 56.8, 50.5 (J_{CP} = 4.4 Hz), 43.3; ^{31}P NMR (CD_3CN) δ 21.90; UV (CH_3CN) λ_{max} 346 nm (ϵ_{max} 6875). Anal. Calcd for $C_{13}H_{20}Cl_2N_3O_6P$: C, 37.51; H, 4.84; N, 10.10. Found: C, 37.51; H, 4.85; N, 10.00.

3-Buten-1-(2-nitrophenyl) 1-*N*,*N*-bis(2-chloroethyl)-phosphordiamidate (10c) was prepared as described above for **10b**. For flash chromatography, ether was used as the first solvent (elutes mainly **8c**), followed by EtOAc (R_f 0.5), which gave **10c** as a yellow oil: yield 41%; IR (film) 1255 (s, P═O) cm^{-1}; ^1H NMR (CD_3CN) δ 7.94 (d, J = 8.7 Hz, 1 H), 7.78−7.68 (m, 2 H), 7.51 (m, 1 H), 5.93−5.81 (m, 2 H), 5.11−5.05 (m, 2 H), 3.62 (t, J = 7.2 Hz, 2 H), 3.53−3.03 (m, 6 H), 2.71−2.57 (m, 2 H); ^{13}C NMR (acetone-d_6) δ 148.4/148.2, 137.9/137.6, 134.3/132.2, 134.2, 129.6/129.5, 129.4, 125.0, 118.6, 72.7 (J_{CP} = 3.65 Hz)/72.6 (J_{CP} = 3.68 Hz), 50.2, 43.1, 42.9/42.8; ^{31}P NMR (CD_3CN) δ 21.54, 21.50 (2s); UV (CH_3CN) λ_{max} 260 nm (ϵ_{max} 4760). Anal. Calcd for $C_{14}H_{20}Cl_2N_3O_4P$: C, 42.44; H, 5.09; N, 10.61. Found: C, 42.71; H, 5.13; N, 10.50.

4-Hydroxy-2-(hydroxymethyl)-1-nitrobenzene (11a) was prepared by dissolving 5-hydroxy-2-nitrobenzaldehyde (**7c**) (8.45 g, 50 mmol) in 50 mL of 1 N sodium hydroxide solution (1 equiv) and adding 0.95 g (25 mmol) of sodium borohydride pellets. This solution was stirred for 4 h, acidified to pH 2 with 1 N HCl, and extracted with EtOAc (4 × 50 mL). Removal of the solvent yielded a pale yellow powder: mp 105 °C; yield 93%; IR (KBr): 3400 (m, OH), 3100 (s, br, OH), 1540 (s, NO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.91 (s, br, 1 H), 8.05 (d, J = 9.0 Hz, 1 H), 7.24 (d, J = 2.8 Hz, 1 H), 6.78 (dd, J = 9.0, 2.8 Hz, 1 H), 5.52 (t, J = 5.4 Hz, 1 H), 4.80 (d, J = 5.4 Hz, 2 H); ^{13}C NMR (acetone-d_6)δ 163.6, 143.3, 140.3, 128.4, 115.3, 114.8, 62.0.

4-Hydroxy-2-(1-hydroxy-3-butenyl)-1-nitrobenzene (11b). This compound was prepared analogously to **8c** from 2.00 g (12.0 mmol) of 5-hydroxy-2-nitrobenzaldehyde (**7c**), 1.10 mL (1 equiv) of allyl iodide, and 1.88 g (1 equiv) of stannous fluoride: yield 94% of a pale yellow oil; IR (KBr) 3500 (w, OH), 3280 (s, br, OH), 1510 (s, NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.97 (d, J = 8.8 Hz, 1 H), 7.20 (d, J = 2.5 Hz, 1 H), 6.97 (s, br, 1 H), 6.74 (dd, J = 8.8, 2.5 Hz, 1 H), 5.91−5.77 (m, 1 H), 5.46 (dd, J = 8.2, 3.6 Hz, 1 H), 5.08−5.01 (m, 2 H), 2.80−2.40 (s, br, 1 H), 2.70−2.62 (m, 1 H), 2.29 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 161.1, 142.6, 140.2, 133.6, 128.2, 119.1, 115.1, 114.2, 69.3, 42.3.

Methyl [3-(Hydroxymethyl)-4-nitrophenoxy]ethanoate (12a). Typical Procedure. Phenol **11a** (3.25 g, 19.0 mmol) was placed in a flask and dissolved in MeOH (50 mL). NaOH pellets (0.76 g, 1 equiv) were powdered and added to the solution. The color of the solution turned deep yellow as the NaOH gradually dissolved. The solution was kept at room temperature for 15 h. Methyl bromoacetate (1.98 mL, 1.1 equiv) was added, and the mixture was heated to reflux (oil bath temperature: 100 °C) for 15 h. After cooling, the MeOH was evaporated and the solid residue dissolved in CH_2Cl_2 (50 mL) and water (100 mL), which was brought to pH 9 by adding solid K_2CO_3. Extraction was repeated twice with 50 mL of CH_2Cl_2. The combined organic layer was washed (3×) with 30 mL of water. After evaporation of the solvent, the remaining solid was recrystallized from chloroform−ether to yield 3.26 g (71%) of **12a** as a pale yellow powder: mp 120 °C; IR (KBr) 1740 (vs, C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.12 (d, J = 9.1 Hz, 1 H), 7.20 (d, J = 3.0 Hz, 1 H), 6.83 (dd, J = 9.2, 2.9 Hz, 1 H), 4.94 (d, J = 4.4 Hz, 2 H), 4.69 (s, 2 H), 3.76 (s, 3 H); ^{13}C NMR (acetone-d_6) δ 169.7, 163.3, 142.9, 141.6, 128.1, 114.7, 113.8, 66.0, 61.9, 52.4.

Methyl [3-(1-Hydroxy-3-butenyl)-4-nitrophenyloxy]-ethanoate (12b). This compound was prepared using the procedure described for **12a** to give 65% of **12b** as yellow crystals; crystallization was achieved from ether−hexane: mp 64 °C; IR (KBr) 1770 (s, C═O) cm^{-1}; ^1H NMR (CD_3CN) δ 8.00 (d, J = 9.4 Hz, 1 H), 7.27 (d, J = 2.7 Hz, 1 H), 6.91 (dd, J = 9.4, 2.7 Hz, 1 H), 5.98−5.84 (m, 1 H), 5.33 (quintet, J = 4.1 Hz, 1 H), 5.09−5.03 (m, 2 H), 4.80 (s, 2 H), 3.74 (s, 3 H), 3.51 (d, J = 4.6 Hz, 1 H); ^{13}C NMR (acetone-d_6) δ 169.2, 162.8, 145.2, 142.2, 135.8, 127.7, 117.5, 114.7, 114.3, 69.3, 66.0, 52.4, 43.8.

[3-(Hydroxymethyl)-4-nitrophenoxy]ethanenitrile (12c). This compound was prepared analogously to **12a** using bromoacetonitrile to give 66% of **12c** as a pale brown powder that was crystallized from ether: mp 103 °C; IR (KBr): 3500 (vs, br, OH), 1510 (vs, NO_2) cm^{-1}; ^1H NMR (CD_3CN) δ 8.17 (d, J = 9.0 Hz, 1 H), 7.45 (d, J = 2.9 Hz, 1 H), 7.04 (dd, J = 9.1, 2.8 Hz, 1 H), 5.01 (s, 2 H), 4.94 (s, 2 H), 3.59 (br, 1 H); ^{13}C NMR (acetone-d_6) 161.7, 143.1, 142.5, 128.2, 116.0, 114.8, 114.1, 61.7, 54.7.

[3-(1-Hydroxy-3-butenyl)-4-nitrophenoxy]ethanenitrile (12d). This compound was prepared analogously to **12a** using bromoacetonitrile to give 84% of **12d** as a yellow oil. The oil was further purified by column chromatography on silica gel (eluent: hexane/ether 1:1) to give a yellow solid: mp 47−49 °C; IR (film) 2250 (w, CN) cm^{-1}; ^1H NMR (CD_3CN) δ 8.03 (d, J = 8.8 Hz, 1 H), 7.40 (d, J = 3.0 Hz, 1 H), 7.02 (dd, J = 8.8, 3.0 Hz, 1 H), 5.98−5.85 (m, 1 H), 5.34 (m, 1 H), 5.13−5.04 (m, 2 H), 5.00 (s, 2 H), 3.60 (d, J = 4.5 Hz, 1 H), 2.58−2.49 (m, 1 H), 2.34 (m, 1 H); ^{13}C NMR (acetone-d_6) δ 161.0, 145.1, 142.8, 135.5, 127.8, 117.6, 115.9, 114.7, 114.5, 69.1, 54.5, 43.6. Anal. Calcd for $C_{12}H_{12}N_2NO_4$: C, 58.06; H, 3.4.87; N, 11.29. Found: C, 58.21; H, 4.93; N, 11.24.

5-[(Carbomethoxy)methoxy]-2-nitrobenzyl *N,N*-Bis(2-chloroethyl)phosphordiamidate (10d). Typical Procedure. Methyl [3-(hydroxymethyl)-4-nitrophenoxy]ethanoate (**12a**) (0.2 g, 0.83 mmol) was dissolved in THF (20 mL) and cooled to 0 °C. A 1 M solution of LHMDS in THF (0.91 mL, 1.1 equiv) was slowly added with vigorous stirring, and the solution was kept at 0 °C for 10 min. Meanwhile, **5** (0.24 g, 1.1 equiv) was dissolved in 20 mL of THF and cooled to 0 °C. The solution of the alkoxide was transferred into this flask by means of a transfer needle, and stirring was continued for 1 h at 0 °C. Ammonia was bubbled through the solution at a moderate rate for 30 min at 0 °C, and stirring was continued for 30 min at that temperature. The THF was evaporated without heating the solution. The oily residue was dissolved in 50 mL of CH_2Cl_2 and 50 mL of water, and the aqueous layer was extracted twice with 25 mL of CH_2Cl_2. The resulting almost colorless oil was purified by flash chromatography. Initial elution with EtOAc separated byproducts. The product was eluted with EtOAc/MeOH (5:1) (R_f 0.4) and crystallized from CH_2Cl_2−ether to yield 0.15 g (40%) of **10d** as pale yellow crystals: mp 109 °C; IR (KBr) 1760 (vs, C═O), 1230 (vs, P═O) cm^{-1}; ^1H NMR (CD_3CN) δ 8.15 (d, J = 9.1 Hz, 1 H), 7.28 (d, J = 2.8 Hz, 1 H), 6.95 (dd, J = 9.1, 2.8 Hz, 1 H), 5.30 (dd, J = 6.5, 2.8 Hz, 2 H), 4.81 (s, 2 H), 3.74 (s, 3H), 3.64 (t, J = 7.4 Hz, 4 H), 3.58−3.34 (m, 4 H); ^{13}C NMR (CD_3CN) δ 169.6, 163.4, 141.8, 138.1 (J_{CP} = 8.0 Hz), 128.8, 115.3, 114.7, 66.5, 64.6 (J_{CP} = 3.2 Hz), 53.0, 50.2 (J_{CP} = 4.4 Hz), 43.6; ^{31}P NMR (CD_3CN) δ 22.27 (s).

3-Butenyl-1-[5-[(Carbomethoxy)methoxy]-2-nitrophenyl] 1-*N,N*-bis(2-chloroethyl)phosphorodiamidate (10e). This compound was prepared from alcohol **12b** according to the procedure described for **10d** to give 42% of **10e** as a mixture of diastereoisomers: colorless oil; R_f (EtOAc) 0.4; IR (film) 1760 (s, C═O), 1250−1200 (vs, P═O) cm^{-1}; ^1H NMR (CD_3CN) δ 8.04 (d, J = 9.4 Hz, 1 H), 7.21/ 7.18 (2d, J = 3.0 Hz, 1 H), 6.97 (dd, J = 9.4, 3.0 Hz, 1 H), 6.03−5.96 (m, 1 H), 5.95−5.81 (m, 1 H), 5.10−5.04 (m, 2 H), 4.82, 4.81 (2s, 2 H), 3.75 (s, 3 H), 3.63 (t, J = 7.4 Hz, 1 H), 3.54−3.05 (m, 7 H), 2.73−2.53 (m, 2 H); ^{13}C NMR (CD_3CN) δ 169.6/169.5, 162.9/162.8, 142.1, 141.5/141.1, 134.5/134.4, 128.4, 119.2/119.1, 115.5/115.4, 115.2/115.1, 73.1 (J_{CP} = 4.36 Hz)/73.0 (J_{CP} = 4.43 Hz), 66.5, 53.0, 50.2 (J_{CP} = 4.65 Hz)/50.1 (J_{CP} = 6.16 Hz), 43.6 (J_{CP} = 2.78)/43.4 (J_{CP} = 2.78); ^{31}P NMR (CD_3CN) δ 21.32, 21.15 (2 s).

5-(Cyanomethoxy)-2-(nitrobenzyl) *N,N*-Bis(2-chloroethyl)phosphorodiamidate (10f). This compound was prepared from alcohol **12c** according to the procedure described for **10d** to give 44% of **10f** as an orange oil: R_f (EtOAc/MeOH 5:1) 0.5; IR (film) 2260 (w, CN), 1250−1200 (s, P═O) cm^{-1}; ^1H NMR (CD_3CN) δ 8.19 (d, J = 8.9 Hz, 1 H), 7.37 (d, J = 2.8 Hz, 1 H), 7.08 (dd, J = 9.0, 2.9 Hz, 1 H), 5.34 (d, J = 6.0 Hz, 2 H), 5.03 (s, 2 H), 3.65 (t, J = 6.8 Hz, 4 H), 3.45−3.37 (m, 4 H); ^{13}C NMR (CD_3CN) δ 161.9, 142.7, 138.1 (J_{CP} = 8.55 Hz), 128.9, 116.2, 115.8, 115.1, 64.7 (J_{CP} = 3.4 Hz), 61.1, 55.4, 50.2 (J_{CP} = 4.5 Hz), 43.6; ^{31}P NMR (CD_3CN) δ 22.52 (s).

3-Butenyl-1-[5-(cyanomethoxy)-2-nitrophenyl] 1-*N,N*-Bis(2-chloroethyl)phosphordiamidate (10g). This compound was prepared from alcohol **12d** according to the procedure described for **10d** to give 52% of **10g** as a mixture of two diastereoisomers: yellow oil; R_f (EtOAc/MeOH 5:1) 0.55; IR (film) 2200 (w, CN), 1285 (s, P═O) cm^{-1}; ^1H NMR (CD_3CN) δ 8.09, 8.08 (2 d, J = 8.9 Hz, 1 H), 7.30, 7.29 (2 d, J = 3.0 Hz, 1 H), 7.11−7.06 (m, 1 H), 6.04−5.97 (m, 1 H), 5.96−5.81 (m, 1 H), 5.11−5.04 (m, 2 H), 5.03/5.01 (s, 2 H), 3.66−3.12 (m, 8 H), 2.75−2.53 (m, 2 H); ^{13}C NMR (acetone-d_6) δ 161.3, 142.9/142.8, 141.7/141.2 (J_{CP} = 3.16 Hz), 134.2/134.1, 128.2, 118.8, 116.1, 115.9/115.8, 115.4/115.2, 72.9 (J_{CP} = 4.2 Hz), 72.8 (J_{CP} = 4.2 Hz), 54.9/54.8, 50.5 (J_{CP} = 4.8 Hz)/50.4 (J_{CP} = 4.5 Hz), 43.3/43.2, 42.8 (J_{CP} = 4.7 Hz)/42.7 (J_{CP} = 5.0 Hz); ^{31}P NMR (CD_3CN) δ 21.52, 21.41 (2 s). Anal. Calcd for $C_{16}H_{21}Cl_2N_4O_5P$: C, 42.59; H, 4.69; N, 12.42. Found: C, 42.41; H, 4.78; N, 12.27.

[3-Methyl-*N,N*-bis(2-chloroethyl)phosphordiamidato-4-(nitrophenyl)oxy]acetate, Sodium Salt (13a). Typical Procedure. Methyl ester **10d** (0.14 g, 0.315 mmol) was dissolved in 10 mL of MeOH and cooled to 0 °C, and 3.15 mL of 0.1 N NaOH solution (3.15 mmol) was added dropwise. The solution was stirred at room temperature for 3 h. Most of the solvent was evaporated, and 2-propanol was added until the solution became cloudy. The flask was put in a freezer (−22 °C); the product was obtained as a pale yellow powder: mp 145 °C; yield 63%; IR (KBr) 1620 (vs, C═O), 1285 (s, P═O) cm^{-1}; ^1H NMR (D_2O) δ 7.99 (d, J = 9.3 Hz, 1 H), 7.00 (d, J = 2.8 Hz, 1 H), 6.77 (dd, J = 9.3, 2.8 Hz, 1 H), 5.16 (d, J = 6.6 Hz, 2 H), 4.41 (s, 2 H), 3.53 (t, J = 6.4 Hz, 4 H), 3.30 (dt, J = 11.1, 6.4 Hz, 4 H), 3.17 (s, 2 H); ^{13}C NMR (CD_3OD/D_2O)δ 174.5, 164.5, 141.0, 137.4 (J_{CP} = 8.7 Hz), 128.7, 115.3, 115.1, 68.4, 65.2 (J_{CP} = 3.3 Hz), 50.2 (J_{CP} = 4.5 Hz), 43.3 (J_{CP} = 1.4 Hz); ^{31}P NMR (D_2O) δ 22.41 (s); UV (H_2O) λ_{max} 316 nm (ϵ_{max} 9460). Anal. Calcd for $C_{13}H_{17}Cl_2N_3NaO_7P$: C, 34.53; H, 3.79; N, 9.29. Found: C, 34.65; H, 3.86; N, 9.17.

3-[3-Butenyl-1-(*N,N*-bis(2-chloroethyl)phosphordiamidato)]-4-(nitrophenyl)oxyacetate, Sodium Salt (13b). This compound was prepared from methyl ester **10e** using the procedure given for **13a** to give 70% of **13b** as a brown powder: mp 120 °C; IR (KBr) 1615 (vs, C═O), 1285 (s, P═O) cm^{-1}; ^1H NMR (D_2O) δ 8.00/7.99 (2 d, J = 9.4 Hz, 1 H), 7.07/7.04 (2 d, J = 2.7 Hz, 1 H), 6.84 (m, 1 H), 5.96−5.89 (m, 1 H), 5.85−5.68 (m, 1 H), 5.01−4.94 (m, 2 H), 4.45 (s, 2 H), 3.53/3.34 (2 t, J = 6.6 Hz, 4 H), 3.43−2.97 (m, 4 H), 2.55/2.50 (2 m, 2 H); ^{13}C NMR (D_2O) δ 175.2/175.0, 162.8, 140.1/139.5, 139.7/139.6, 133.2/133.0, 128.1/128.0, 119.1, 114.9/114.4, 113.7, 73.2 (J_{CP} = 3.67 Hz)/72.8 (J_{CP} = 3.67 Hz), 67.3/67.2, 48.1 (J_{CP} = 4.15 Hz)/47.9 (J_{CP} = 4.31 Hz), 42.3/42.2, 41.7/41.6; ^{31}P NMR (D_2O) δ 21.09 (s); UV (H_2O) λ_{max} 316 nm (ϵ_{max} 8170). Anal. Calcd for $C_{16}H_{21}Cl_2N_3NaO_7P$: C, 39.04; H, 4.30; N, 8.54. Found: C, 38.98; H, 4.37; N, 8.45.

[3-(Butan-4-ol-1-(*N,N*-bis(2-chloroethyl)phosphordiamidato)-4-nitrophenyl)oxy]-2-ethylammonium Chloride (14). The nitrile **10g** (0.35 g, 0.77 mmol) was dissolved in THF, and a 2 M solution of borane−dimethyl sulfide complex in THF (2.31 mL, 6 equiv) was added dropwise at room temperature. Evolution of gas occurred for about 5 min. The solution was stirred for 15 h at room temperature. The flask was placed in a water bath (20 °C), and 4.62 mL (4.62 mmol, 6 equiv) of a 1 N solution of NaOH was added, followed by 0.48 mL (4.62 mmol, 6 equiv) of a 30% solution of H_2O_2. This mixture was stirred for 2 h at room temperature; eventually, a white solid separated. After filtration and removal of the solvents, water and dilute HCl were added to bring the pH to 1. The aqueous layer was extracted twice with 30 mL of EtOAc and then brought to pH 9 by addition of a K_2CO_3 solution, and the product was extracted with EtOAc (4 × 40 mL). The solvent was evaporated, the remaining oil weighed and dissolved in MeOH (10 mL), and a slight excess of the theoretical amount of 0.1 N HCl was added. Solvents were removed, the oily residue dissolved in EtOH, and ether was added until the solution became cloudy. On standing at −22 °C, the product was obtained as a yellow powder: mp 115 °C; yield 54%; ^1H NMR (D_2O) δ 8.06 (d, J = 9.1 Hz, 1 H), 7.21 (d, J = 2.8 Hz, 1 H), 6.99 (dd, J = 9.1, 2.8 Hz, 1 H), 5.93 (m, 1 H), 4.32 (t, J = 4.9 Hz, 2 H), 3.58 (t, J = 6.6 Hz, 4 H), 3.51 (m, 2 H), 3.37 (t, br, J = 4.7 Hz, 2 H), 3.35−3.28 (m, 4 H), 1.90−1.83 (m, 2 H), 1.62−1.59 (m, 2 H); ^{13}C NMR (D_2O) δ 162.7, 140.8, 140.5, 128.6, 114.7, 114.13, 75.0 (J_{CP} = 5.1 Hz), 65.0, 61.6, 48.1 (J_{CP} = 5.0 Hz), 42.4, 41.39.2, 34.1 (J_{CP} = 6.5

Hz), 28.0; ^{31}P NMR (D_2O) δ 21.70/ 21.32 (2 s); UV (H_2O) λ_{max} 308 nm (ϵ_{max} 7350).

Bis(2-nitrobenzyl) *N*,*N*-Bis(2-chloroethyl)phosphoramidate (15). Alcohol **8a** (0.6 g, 3.92 mmol) was dissolved in THF (15 mL), and the solution was cooled to 0 °C. A 1 M solution of LHMDS in THF (3.9 mL, 1 equiv) was slowly added, and stirring was continued for 10 min at 0 °C. Bis(2-chloroethyl)phosphoramidic dichloride (**5**) (0.51 g, 1.96 mmol) was added in small portions, and the solution was kept at 0 °C for 45 min. The THF was evaporated, and the oily residue was extracted with CH_2Cl_2 (3 × 3 0 mL) and water. The resulting oil was subjected to flash chromatography. The first fraction, eluted with ether/CH_2Cl_2, contained mainly **8a**. Product **15** eluted cleanly with EtOAc as solvent (R_f 0.7) and was crystallized as colorless crystals from ether/hexane: mp 81 °C; yield 47%; IR (KBr): 1260 (s, P═O) cm^{-1}; ^{1}H NMR (CD_3CN) δ 8.09 (d, J = 8.0 Hz, 2 H), 7.73 (m, 4 H), 7.56 (m, 2 H), 5.43 (d, J = 7.0 Hz, 4 H), 3.65 (t, J = 6.8 Hz, 4 H), 3.42 (dt, J = 11.5, 6.7 Hz, 4 H); ^{13}C NMR (acetone-d_6) δ 148.4, 134.8, 133.3 (J_{CP} = 7.93 Hz), 130.0, 129.8, 125.6, 65.6 (J_{CP} = 3.99 Hz), 49.8 (J_{CP} = 4.28 Hz), 42.8; ^{31}P NMR (CD_3CN) δ 14.78 (s); UV (CH_3CN) λ_{max} 262 nm (ϵ_{max} 9330). Anal. Calcd for $C_{18}H_{20}Cl_2N_3O_7P$: C, 43.92; H, 4.10; N, 8.54. Found: C, 44.02; H, 4.06; N, 8.51.

Bis-2-nitrobenzyl Phosphate (17).[22] Methyl dichlorophosphate (0.1 mL, 0.98 mmol) and 2-nitrobenzyl alcohol (0.3 g, 1.96 mmol) were mixed in pyridine (10 mL) at 0 °C. The ice bath was removed, and the solution was stirred for 15 h. The mixture was poured into 50 mL of aqueous $NaHCO_3$ (10%) and extracted with 2 × 10 mL of ether. The aqueous layer was acidified with dilute HCl to pH 1 and the product extracted with chloroform. Most of the solvent was evaporated, and the product **17** crystallized on standing at −22 °C: mp 98 °C; yield 46%; ^{1}H NMR ($CDCl_3$) δ 12.8−11.5 (br, 1 H), 7.92 (d, J = 8.1 Hz, 2 H), 7.72 (d, J = 7.7 Hz, 2 H), 7.50 (t, J = 7.7 Hz, 2 H), 7.30 (t, J = 7.7 Hz, 2 H), 5.34 (d, J = 6.7 Hz, 4 H); ^{13}C NMR (D_2O/acetone-d_6) δ 148.0, 135.4, 134.1 (J_{CP} = 8.23 Hz), 130.1, 129.9, 125.8, 65.9 (J_{CP} = 3.47 Hz); ^{31}P NMR (CD_3CN/D_2O) δ 1.99 (s). Anal. Calcd for $C_{14}H_{13}N_2O_8P$: C, 45.66; H, 3.56; N, 7.61. Found: C, 45.62; H, 3.60; N, 7.64.

Photolysis. General Methods. Irradiations for the UV spectroscopic detection of the nitrosobenzaldehyde photoproduct and the ^{31}P NMR spectroscopic detection of released phosphoramide mustard were performed with a mercury arc lamp (EFOS Ultracure 100ss plus; output 1 W/cm^2). The irradiations in connection with the NBP assay were performed with a mercury arc lamp (Carl Zeiss, HBO 200W/4, L1; output 35 mW/cm^2). The output was passed through a glass filter that transmitted light of wavelengths λ 300−400 nm (λ_{max} 362 nm). The samples were mounted at a 2 cm distance from the optics and were cooled during photolysis by a fan.

Irradiations for Assaying Alkylating Activity. Photolysis was performed in quartz cuvettes (1 cm path length); 3 mL of 0.2 mM solutions, nonstirred, were used: **10a**−**c** and **15** in CH_3CN, **13a**,**b** and **14** in H_2O, **17** in EtOH/H_2O (1:1). Aliquots were taken after t = 0 (without irradiation), 2, 4, 6, and 10 min and used in the following procedure.

Determination of the Alkylating Activity by NBP Assay. The known procedure[20] was slightly modified. In each of five screw-capped and round-bottomed 10 mL test tubes were placed 2 mL of distilled water, 1 mL of 0.025 M NaOAc buffer (pH 4.6), and 0.5 mL of 5% NBP in acetone. Two hundred μL aliquots of the irradiated sample (t = 0, 2, 4, 6, and 10 min) were added and the test tubes placed in a boiling water bath for 20 min. The samples were then immediately placed in an ice bath. One mL of acetone and 3 mL of ethyl acetate were added to each tube. Handling one tube at a time, 1 mL of a 0.25 N NaOH solution was added and the tube immediately vortexed for 30 s; 1 min was allowed for complete phase separation, and then a sample (1.5 mL) of the upper phase was transferred into a quartz cuvette and the absorption measured at λ 542 nm. Because the baseline of the five different readings tended to shift slightly, a baseline absorption at λ 700 nm was also read and subtracted from the chromophore absorption. The reading with the overall maximum absorption (compound **10a** after t = 10 min) was set to 1.00, and all other readings were scaled in relation to this number. For the illustration of the illumination-based NBP absortion in Figure 3, the t = 0 absorption value was subtracted: 0.234 (**10a**), 0.0990 (**10b**), 0.190 (**10c**), 0.260 (**15**), 0.0790 (**13a**), 0.0500 (**13b**), 0.112 (**14**), 0.134 00 (**17**)).

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(22) This compound was synthesized by a procedure similar to one reported by: Baldwin, J. E.; McConnaughie, A. W.; Moloney, M. G.; Pratt, A. J.; Shim, S. B. *Tetrahedron* **1990**, *46*, 6879−6884.