Development of Novel Quinone Phosphorodiamidate Prodrugs Targeted to DT-Diaphorase

Carolee Flader, Jiwen Liu, and Richard F. Borch*

Departments of Chemistry and Pharmacology, University of Rochester, Rochester, New York 14642, and Department of Medicinal Chemistry and Molecular Pharmacology and the Cancer Center, Purdue University, West Lafayette, Indiana 47907

Received April 24, 2000

A series of naphthoquinone and benzimidazolequinone phosphorodiamidates has been synthesized and studied as potential cytotoxic prodrugs activated by DT-diaphorase. Reduction of the quinone moiety in the target compounds was expected to provide a pathway for expulsion of the phosphoramide mustard alkylating agent. All of the compounds synthesized were excellent substrates for purified human DT-diaphorase ($k_{cat}/K_m = 3 \times 10^7 - 3 \times 10^8$ M^{-1} s^{-1}). The naphthoquinones were toxic to both HT-29 and BE human colon cancer cell lines in a clonogenic assay; however, cytotoxicity did not correlate with DT-diaphorase activity in these cell lines. The benzimidazolequinone analogues were 1−2 orders of magnitude less cytotoxic than the naphthoquinone analogues. Chemical reduction of the naphthoquinone led to rapid expulsion of the phosphorodiamidate anion; in contrast, the benzimidazole reduction product was stable. Michael addition of glutathione and other sulfur nucleophiles provides an alternate mechanism for activation of the naphthoquinone phosphorodiamidates, and this mechanism may contribute to the cytotoxicity of these compounds.

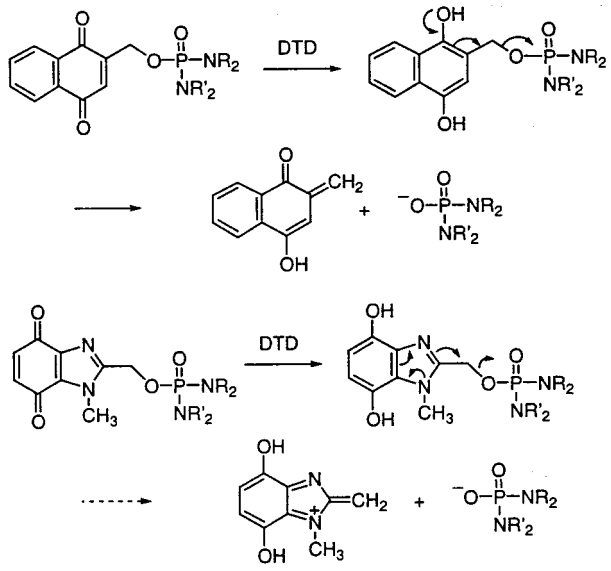
Introduction

DT-diaphorase (E.C. 1.6.99.2, NAD(P)H:quinone-acceptor oxidoreductase) is a cytosolic FAD-containing enzyme that catalyzes an obligatory two-electron reduction of a variety of quinones using NAD(P)H as cofactor.[1] Although the physiological role of this enzyme is not known, it is believed to involve the detoxification of quinones by inhibiting formation of highly reactive radical anion intermediates.[2] Substantial evidence exists that DT-diaphorase is overexpressed in a number of solid tumors,[3] and the enzyme has become an attractive target for the design of bioreductively activated prodrugs.[4−6] The strategy underlying the selectivity of these prodrugs is that two-electron reduction of the quinone moiety markedly increases the electrophilicity of appended functional groups, thereby converting the prodrugs into DNA-reactive species. We have an ongoing interest in the design of prodrugs that utilize phosphoramidate release to deliver cytotoxins to tumor cells.[7] In this approach, intracellular reduction of the prodrug generates an intermediate that should spontaneously release a cytotoxic phosphoramide mustard that can cross-link DNA. We describe herein the synthesis, activation mechanisms, and cytotoxicity of a series of unique quinone phosphorodiamidates that are excellent substrates for human DT-diaphorase and exhibit significant cytotoxicity in vitro.

Results and Discussion

Chemistry. The mechanistic rationale for the proposed activation of these compounds is outlined in Scheme 1. Two-electron reduction of the quinone generates the corresponding hydroquinone in each case. The naphthoquinone reduction product can expel the phosphorodiamidate anion directly. In the case of the benzimidazolequinone, reduction will increase electron density on the imidazole nitrogen and thereby facilitate expulsion of the phosphorodiamidate. A series of naphthoquinone and benzimidazolequinone prodrugs containing either *N*,*N*-bis(haloethyl)- or *N*,*N*′-haloethylphosphorodiamidates were prepared (Chart 1). In addition, compounds containing a novel bis[*N*-methyl-*N*-(2-haloethyl)]phosphorodiamidate (**6a**−**c**) were synthesized. It was hypothesized that this bis-alkylating moiety might be more cytotoxic than compounds containing an *N*,*N*-bis(2-haloethyl)amine substituent because of the presumed faster rate of aziridinium ion formation resulting

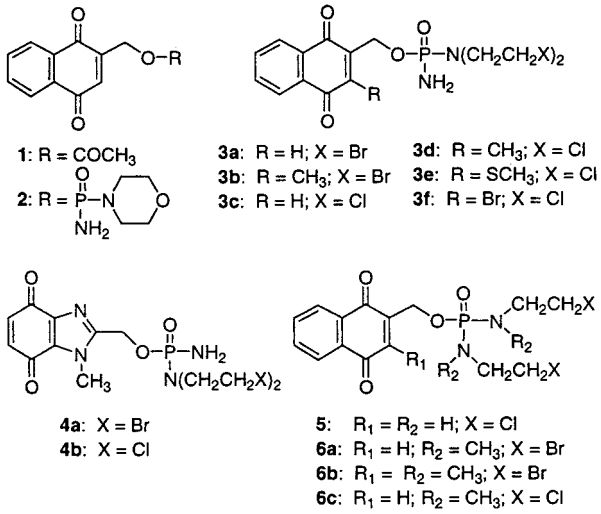
Scheme 1



* Address for correspondence: Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907. Tel: (765) 494-1403. Fax: (765) 494-1414. E-mail: rickb@pharmacy.purdue.edu.

10.1021/jm000179o CCC: $19.00
Published on Web 07/18/2000

Chart 1



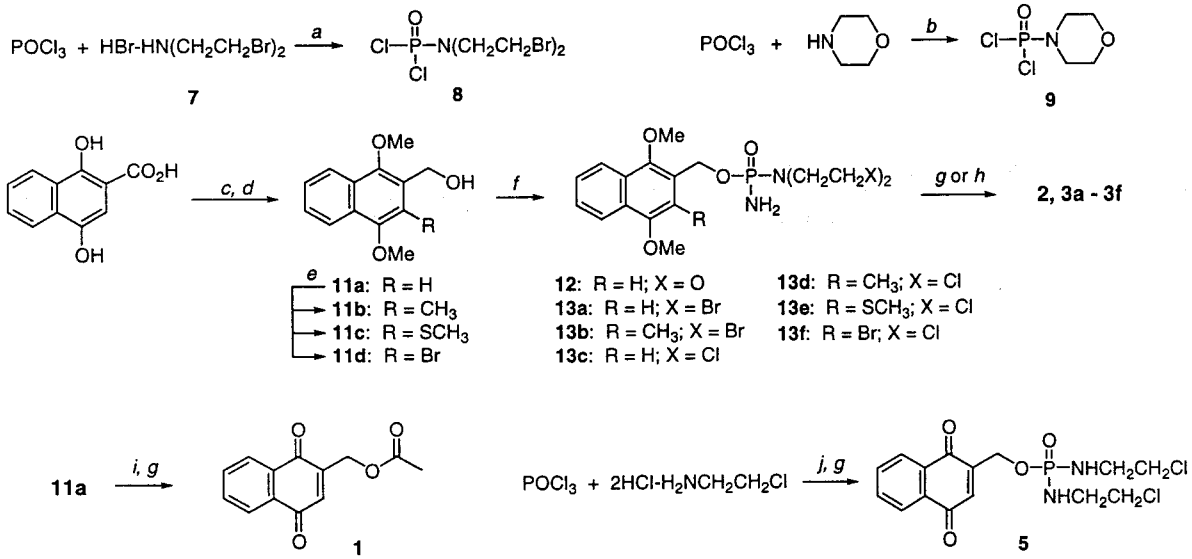
from the inductive effect of the *N*-methyl substituent. Finally, two compounds (acetate **1** and morpholine phosphorodiamidate **2**) that do not contain an alkylating moiety were prepared. They were used to assess the substituent effect on enzyme substrate activity and the contribution of the quinone methide product to the cytotoxicity of the prodrugs.

The synthesis of the target naphthoquinone and benzimidazolequinone phosphorodiamidates was carried out as shown in Schemes 2−4. Naphthoquinones **1**, **2**, **3a**−**f**, **5,** and **6a**−**c** were synthesized from 1,4-dimethoxy-2-(hydroxymethyl)naphthalene (**11a**), which was prepared by complete methylation[8] of 1,4-dihydroxy-2-naphthoic acid followed by reduction of the ester to the alcohol (Scheme 2). Substituted alcohols **11b**−**d** were prepared by deprotonation of **11a** with *n*-butyllithium followed by reaction with electrophiles methyl iodide, dimethyl disulfide, and 1,2-dibromotetrafluoroethane,[9] respectively. Phosphorylating agent **9** was prepared according to the procedure used to phosphorylate *N*-methyl-*N*-(2-bromoethyl)amine hydrobromide.[10] Bis-(2-bromoethyl)amine hydrobromide (**7**)[11] was phosphorylated in a similar manner, except that the reaction temperature was maintained below 0 °C. Surprisingly, at higher temperatures a side product [*N*-(2-chloroethyl)-*N*-(2-bromoethyl)phosphoramidic dichloride] was formed that could not be separated from the desired product. The side product presumably arises from the displacement by chloride released in the phosphorylation reaction. Compounds **12** and **13a**−**f** were generated by reaction of alcohols **11a**−**d** with the respective phosphoramidic dichloride followed by reaction with ammonia. 1,4-Dimethoxynaphthalenes **12** and **13a**−**e** were oxidized to their respective quinones **2** and **3a**−**e** using ceric ammonium nitrate,[12] and quinone **3f** was generated from **13f** using silver(II) oxide as oxidant.[13] The acetate **1**[6b,14] was prepared by acetylation of alcohol **11a** followed by oxidation with ceric ammonium nitrate. Isophosphoramide mustard prodrug **5** was synthesized in two steps from alcohol **11a**, phosphorus oxychloride, and *N*-(2-chloroethyl)amine hydrochloride.

Compounds **4a**,**b** were synthesized by nitration of 1,4-dimethoxybenzene to give a 8:1 ratio of 2,3- and 2,5-dinitro-1,4-dimethoxybenzenes (Scheme 3).[15] The mixture was reduced to the diamines[16] with stannous chloride and reacted with glycolic acid[17] to give benzimidazole **17**. Compound **17** was methylated and the product (**18**) phosphorylated as described above to give **19a,b**, which were converted to the benzimidazolequinones **4a,b** by silver(II) oxide oxidation.

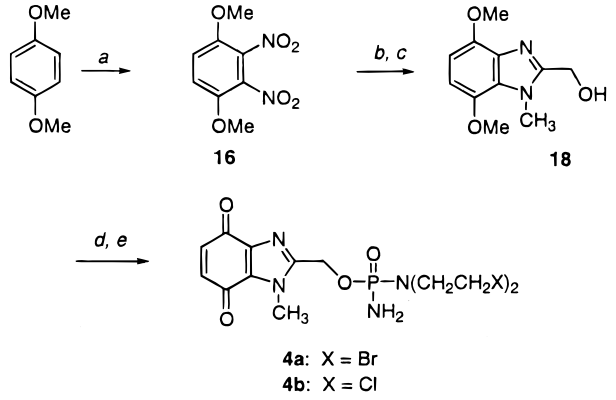
Naphthoquinone prodrugs containing a bis[*N*-methyl-*N*-(2-bromoethyl)]- (**6a**,**b**) or bis[*N*-methyl-*N*-(2-chloroethyl)]phosphorodiamidate (**6c**) were prepared using phosphorus(III) chemistry as outlined in Scheme 4. Alcohol **11a** or **11b** was reacted with phosphorus trichloride in the presence of base at −70 °C. Two equivalents of the *N*-methyl-*N*-(2-haloethyl)amine hydrohalide[10] were then added, and without isolation, the intermediate was oxidized with *tert*-butyl hydroperox-

Scheme 2[a]



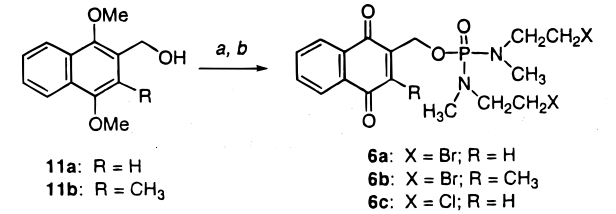
[a] Reagents: (a) NEt_3, CH_2Cl_2, −40 to 0 °C; (b) NEt_3, CH_2Cl_2, 0 °C to rt; (c) CH_3I, K_2CO_3, acetone, reflux; (d) $LiAlH_4$, ether, reflux; (e) nBuLi, THF, −78 °C to rt, then CH_3I, CH_3SSCH_3 or $F_2BrCCBrF_2$; (f) $LiN(TMS)_2$, THF, −78 °C, then **8**, **9**, or $Cl_2P(O)N(CH_2CH_2Cl)_2$, −78 °C, then NH_3, −20 °C; (g) $Ce(NH_4)_2(NO_3)_6$, CH_3CN/H_2O; (h) AgO, THF, 6 N HNO_3; (i) CH_3COCl, NEt_3, CH_2Cl_2; (j) iPr_2NEt, CH_2Cl_2, −78 °C to rt, then **11a** + nBuLi, −78 °C to rt.

Scheme 3[a]



[a] Reagents: (a) HNO_3, 90 °C; (b) $SnCl_2$, HCl, 100 °C, then glycolic acid, H_2O, reflux; (c) $LiN(TMS)_2$, 0 °C to rt, then CH_3I; (d) $LiN(TMS)_2$, THF, −78 °C, then **8** or $Cl_2P(O)N(CH_2CH_2Cl)_2$, −78 °C, then NH_3, −20 °C; (e) AgO, THF, 6 N HNO_3.

Scheme 4[a]



[a] Reagents: (a) PCl_3, iPr_2NEt, CH_2Cl_2/CH_3CN, −70 °C, then 2 equiv $HX\text{-}HN(CH_3)CH_2CH_2X$, iPr_2NEt, −60 to −50 °C, then tBuOOH, −40 to −20 °C; (b) $Ce(NH_4)_2(NO_3)_6$, CH_3CN/H_2O.

ide. The resulting dimethoxynaphthalene phosphorodiamidates **20a**−**c** were oxidized to their respective naphthoquinones **6a**−**c** using ceric ammonium nitrate.

Enzyme Kinetics. Each of the quinones was assayed spectrophotometrically at 25 °C with purified human DT-diaphorase to determine its enzyme kinetic parameters. A standard coupled assay monitoring the absorbance change of cytochrome *c* was used.[18] All of the compounds exhibited some degree of product and substrate inhibition at substrate concentrations near K_M, so a Hanes analysis was used to determine K_M and V_{max}. Reaction velocities were obtained from the initial, linear portion of the reaction progress curve. The results of the kinetic analyses are shown in Table 1; menadione is included for comparison. All of the haloethylphosphorodiamidates are excellent substrates for the enzyme ($k_{cat}/K_M > 3 \times 10^7$ M^{-1} s^{-1}), and the differences in K_M and V_{max} among the haloethylphosphorodiamidates are modest. Introduction of a 3-methyl substituent in the naphthoquinone results in a 3−4-fold increase in K_M (**3a** vs **3b**, **3c** vs **3d**, **6a** vs **6b**). Bis(methyl haloethyl) compounds **6a**−**c** had somewhat higher K_M and V_{max} values than the other haloethylphosphorodiamidates, and the benzimidazolequinones had slightly lower turnover numbers than the other compounds.

In Vitro Cytotoxicity. The cytotoxicity of these prodrugs was assessed using HT-29 and BE human colon carcinoma cell lines. HT-29 cells have a high level of DT-diaphorase activity (1410 nmol cytochrome *c* reduced/min/mg protein). In contrast, the BE cell line has a C-to-T point mutation in the gene that encodes for DT-diaphorase, so this cell line produces a mutant that has no measurable enzymatic activity.[19] The results

Table 1. Kinetic Analysis of Naphthoquinones and Benzimidazolequinones[a]

compound	K_m (μM)	V_{max} (μM/s)	V_{max}/K_m	k_{cat} (1/s)	k_{cat}/K_m (M^{-1} s^{-1})
menadione[b]	1.2	0.60[b]	0.50[b]	700	5.8×10^8
1	1.7	2.4	1.4	280	1.6×10^8
2	0.98	0.81	0.83	15	1.6×10^7
3a	0.21	0.41	2.0	46	2.2×10^8
3b	0.69	0.56	0.81	63	9.1×10^7
3c	0.26	0.46	1.8	51	2.0×10^8
3d	0.91	0.69	0.76	78	8.6×10^7
3e	0.59	0.64	1.1	72	1.2×10^8
3f	0.32	0.86	2.7	97	3.0×10^8
4a	2.1	0.59	0.28	66	3.1×10^7
4b	0.91	0.37	0.40	41	4.5×10^7
5	0.38	0.78	2.1	88	2.3×10^8
6a	0.89	1.0	1.1	115	1.3×10^8
6b	3.6	1.6	0.44	180	5.0×10^7
6c	1.4	1.7	1.2	190	1.4×10^8

[a] See Experimental Section for details of enzyme kinetics assays. [b] Enzyme assays with menadione were conducted using 1/10 of the enzyme concentration used for the other assays.

Table 2. Clonogenic Survival of HT-29 and BE Cells[a]

	LC_{99}[b] (μM)			LC_{99}[b] (μM)	
compound	HT-29	BE	compound	HT-29	BE
1	64	22	**3f**	4.1	3.3
2	89	72	**4a**	130	120
3a	7.8	4.6	**4b**	220	300
3b	1.7	1.2	**5**	14	7.8
3c	11	13	**6a**	4.5	1.2
3d	2.1	1.0	**6b**	1.5	0.44
3e	4.2	5.9	**6c**	4.5	2.8

[a] 2-h drug treatment; see Experimental Section for details of the clonogenic assay. [b] Concentration that reduces clonogenic survival by 2 log units (99%).

of the 2-h drug treatments are summarized in Table 2. Several important structure−activity relationships are apparent from these data. First, the presence of an incipient alkylating group markedly enhances cytotoxicity; the cytotoxicity of the nonalkylating morpholine phosphorodiamidate **2** is approximately 1 order of magnitude less than that of the alkylating phosphorodiamidates. Second, introduction of the 3-methyl substituent enhances the cytotoxicity of the naphthoquinones in both cell lines. Third, cytotoxicity is not strongly dependent on the nature of the alkylating group; while the bis(methyl bromoethyl)phosporodiamidate compounds are somewhat more cytotoxic, the isophosphoramide prodrug **5** is comparable in cytotoxicity to the bis(haloethyl) compounds.

Two unexpected results emerged from the cytotoxicity experiments. First, the benzimidazolequinone analogues **4a**,**b** are essentially inactive in this assay. The enzyme kinetics experiments suggest that these compounds are excellent substrates for the enzyme, indicating that there may be a problem with the phosphorodiamidate expulsion step. Second, cytotoxicity is comparable in the HT-29 and BE cell lines. It was expected that the quinone phosphorodiamidates would be significantly more toxic to the HT-29 cells because of the high level of enzyme activity, but this was not the case. Compound **3a** was evaluated in the NCI human tumor cell line in vitro screen. The compound showed modest cytotoxicity across all cell lines but showed significantly greater cytotoxicity against melanoma (ca. 1 order of magnitude more potent than the mean for 5/8 cell lines). Examination of the GI_{50} values for **3a** and cellular levels of DT-

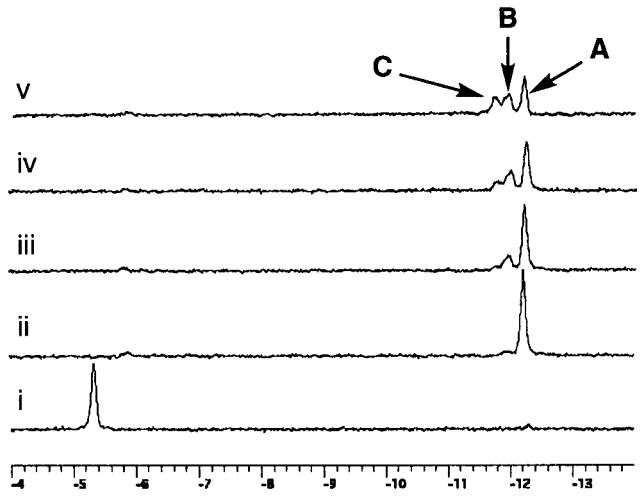


Figure 1. Reduction of quinone phosphorodiamidate **3c** with sodium dithionite (3 equiv, 3:4 CH_3CN:0.4 M cacodylate buffer, pH ~ 7.4, 37 °C): **A**, phosphoramide mustard; **B**, monosubstituted product; **C**, disubstituted product; (i) 0, (ii) 5, (iii) 13, (iv) 21, and (v) 29 min. Chemical shifts are reported relative to the TPPO reference. See Results and Discussion for details.

diaphorase[20] showed that a weak correlation exists (Pearson correlation coefficient = 0.31) between growth inhibition and DT-diaphorase activity. Presumably activation mechanisms other than quinone reduction must be contributing to the cytotoxicity of these compounds.

Chemical Activation of Phosphorodiamidates. The cytotoxicity of the quinone phosphorodiamidates presumably relies on the facile release of the phosphorodiamidate anion following reduction to the hydroquinone. If the phosphorodiamidate anion is released too slowly, the hydroquinone can diffuse out of the cell in which it was activated and the selective delivery of the active drug will be decreased. Reoxidation of the hydroquinone by oxygen can also occur if the release step is too slow. It is therefore important to ascertain whether these prodrugs deliver the alkylating moiety quickly and efficiently. To this end, the chemical reduction of the naphthoquinone prodrug **3c** using sodium dithionite (3 equiv, 3:4 CH_3CN:0.4 M cacodylate buffer, pH ~ 7.4, 37 °C) was followed using ^{31}P NMR; the results are shown in Figure 1. The resonance for quinone **3c** at −5.3 ppm (spectrum i) had disappeared within 5 min and was replaced by the resonance for phosphoramide mustard **A** at −12.2 ppm (spectrum ii). Similar results were obtained for compounds **3a**, **5,** and **6a**. The subsequent alkykation chemistry previously reported by Ludeman et al.[21] was also observed, except that in this case the mono- (**B**) and bis- (**C**) bisulfite substitution products (−12.0 and −11.8 ppm, respectively; spectra iii and iv) were formed instead of glutathione products. Bisulfite, a product of the dithionite reduction and a contaminant in commercial sodium dithionite, is a stronger nucleophile than water so that attack of bisulfite on the aziridinium ion intermediate predominates over alkylation and hydrolysis reactions.[22]

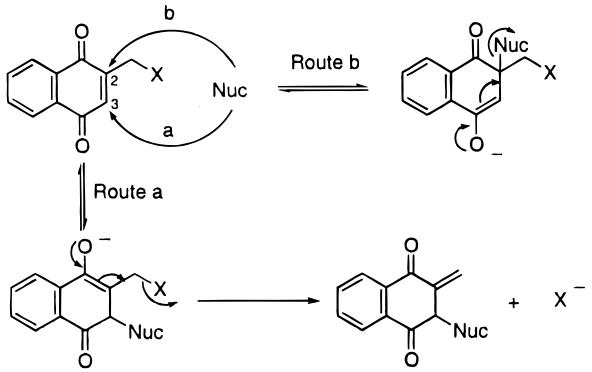
The chemical reductions of naphthoquinones **3a** and **6a**,**c** were also monitored by ^{31}P NMR in order to estimate the half-lives for the cyclization of the resulting phosphorodiamidate anions. The phosphoramidate obtained from reduction of **6a** was the most reactive; 80% of the phosphorodiamidate anion was converted to solvolysis products within 3 min at room temperature. The half-lives of the phosphorodiamidates resulting from reduction of **3a** ($t_{1/2}$ = 12 min at room temperature) and **6c** ($t_{1/2}$ = 2.9 min at 37 °C) were determined by linear regression analysis of the disappearance of the phosphorodiamidate resonance. Two *N*-methyl-*N*-(2-bromoethyl)amine substituents on phosphorus (phosphorodiamidate from **6a**, $t_{1/2}$ < 1 min) increase the rate of aziridinium ion formation by at least 1 order of magnitude when compared to the *N*,*N*-bis(2-bromoethyl)phosphorodiamidate from **3a**. Similarly, cyclization of the bis[*N*-methyl-*N*-(2-chloroethyl)]phosphorodiamidate (from **6c**) at 37 °C is a factor of 8 faster than the *N*,*N*-bis(2-chloroethyl) analogue (from **3c**, $t_{1/2}$ = 23 min) and a factor of 22 faster than the *N*,*N*′-bis(2-chloroethyl) analogue (from **5**, $t_{1/2}$ = 64 min). These differences presumably result from the electron-donating effects of the *N*-methyl group compared to the electron-withdrawing effects of a second haloethyl group.

The chemical reduction of benzimidazolequinone phosphorodiamidate **4a** was also carried out using sodium dithionite as described above. In contrast to the naphthoquinones, however, the resulting hydroquinone was stable for >12 h and did not expel the phosphorodiamidate anion. This result provides an explanation for the absence of cytotoxicity for **4a**,**b**; although the prodrugs are good substrates for DT-diaphorase, the phosphorodiamidate anion is not appreciably released from the resulting hydroquinone.

Other Cellular Mechanisms of Quinone Activation. The absence of a correlation between DT-diaphorase activity and in vitro cytotoxicity of the quinone phosphorodiamidates suggests that the cells are using other mechanisms to activate the compounds, and an attempt was made to determine what those mechanisms might be. Other enzymes that might be capable of activating the compounds in HT-29 and BE cells were considered; the levels of cytochrome b_5 reductase and cytochrome P-450 reductase in the cell lines[20] were investigated. The activities of these enzymes (40−65 nmol cytochrome *c* reduced/min/mg protein) were nearly equivalent for the two cell lines and were much lower than the DT-diaphorase levels found in HT-29 cells. Furthermore, no correlation was observed between the GI_{50} values of **3a** and the levels of these enzymes in the NCI panel.

The Michael addition of sulfur nucleophiles to naphthoquinones is well-known,[23] and addition to the 3-position might provide a pathway for phosphorodiamidate anion release. Two possible products could be predicted from the Michael addition of a nucleophile to the naphthoquinone (Scheme 5): addition at the 2-position (route b) leads to reversible formation of the kinetic product;[23] addition at the 3-position (route a) would give an intermediate that could expel the nucleophile in the reversible reaction or could expel the phosphorodiamidate anion in an irreversible step. Preliminary experiments were carried out using sodium dimethyldithiocarbamate (DDTC), which contains a highly nucleophilic sulfur that is anionic at physiologic pH. Activation of **3a** with DDTC (3 equiv, 3:4 THF:0.4 M cacodylate buffer, pH ~ 7.3, room temperature) was monitored using ^{31}P NMR. Three equivalents of nucleophile were used, assuming that one equivalent would be consumed

Scheme 5



by the Michael addition and the other two would be consumed by reaction with the resulting phosphorodiamidate anion. The resonance for quinone **3a** (−5.7 ppm) had disappeared and was replaced by the resonance for the phosphorodiamidate anion at −13.1 ppm within 5 min after addition of DDTC, confirming that nucleophilic activation of the naphthoquinone is rapid and complete. Subsequent phosphorodiamidate anion chemistry was also observed, generating primarily the mono- and bis-DDTC substitution products at −12.9 and −12.6 ppm, respectively. Reaction of **3a** with one equivalent of DDTC resulted in complete conversion to the phosphorodiamidate anion within 3.5 min after addition of DDTC, suggesting that nucleophilic activation of **3a** is complete before cyclization of the phosphorodiamidate anion to the aziridinium ion takes place.

The experiments described above show that compound **3a** is activated by a potent sulfur nucleophile. It was of interest to know whether glutathione might react in the same way. Glutathione, the primary nonprotein intracellular thiol, is responsible for maintaining the cellular redox environment and removing potential electrophilic cytotoxins. It is overproduced (1−10 mM intracellular concentrations) in many cancer cell lines, particularly those that have acquired resistance to anticancer drugs.[24,25] Thus the reaction of **3c** with glutathione (3 equiv, 1:1.3 CH_3CN:0.4 M cacodylate buffer, pH ∼ 7.5, room temperature) was monitored by ^{31}P NMR. The reaction with glutathione was essentially identical to that of DDTC; the resonance for quinone **3c** had disappeared and was replaced by the resonance for phosphorodiamidate anion within 4 min of glutathione addition. The result was essentially identical when only one equivalent of glutathione was used, again suggesting that activation of this compound is complete before cyclization of the phosphorodiamidate anion can occur.

A study of the activation of the 3-substituted naphthoquinones by glutathione was then undertaken. It was hypothesized that both the steric and electronic effects of the substituents might influence the rate of glutathione activation. The reactions of the naphthoquinone phosphorodiamidates substituted with a methyl (**3d**), 3-methylthio (**3e**), or bromo (**3f**) group were carried out using three equivalents of glutathione. In each case the quinone was completely converted to its respective phosphorodiamidate anion within 4 min at room temperature, suggesting that substituent effects are minimal in this reaction.

To assess whether the intramolecular glutathione reaction might be affecting the activity of **3a** in vitro, the NCI data were examined to determine whether growth inhibition correlates with glutathione *S*-transferase activity (GST-P or GST-M) or with cellular glutathione levels.[25] No correlation was observed with GST-P or GST-M activities; however, a correlation was observed between GI_{50} and glutathione levels (Pearson correlation coefficient = 0.43). This result suggests that intracellular glutathione may be contributing to the intracellular activation of the naphthoquinones.

In Vivo Cytotoxicity. The antitumor activity of **3a** was evaluated in a xenograft model. Nude mice were injected subcutaneously with human renal carcinoma cells ($A498_2LM$), and the tumors were allowed to grow until a measurable mass was present (22 days). The $A498_2LM$ cell line is a highly metastatic subline of A498 with high levels of DT-diaphorase (1010 nmol cytochrome *c* reduced/min/mg protein). A single dose of **3a** (25 mg/kg) was administered intraperitoneally, and the tumor size was monitored for 7 weeks. Compound **3a** modestly retarded tumor growth, exhibiting a 40% reduction in tumor size at all time points.

Conclusion

A series of naphthoquinone and benzimidazolequinone phosphorodiamidates has been prepared in the expectation that they would function as mechanism-based anticancer drugs. All of the compounds are excellent substrates for DT-diaphorase. The naphthoquinones undergo facile activation and expulsion of the cytotoxic phosphorodiamidate either via reduction or reaction with glutathione. The naphthoquinone analogues are potent cytotoxic agents, but they do not exhibit selectivity in vitro for cells with high levels of DT-diaphorase, presumably because of the glutathione-catalyzed activation in cells with high glutathione levels. Phosphorodiamidate anions containing the bis(*N*-methyl-*N*-haloethyl) moiety cyclize to the aziridinium ion approximately 1 order of magnitude faster than those containing the bis(haloethyl)phosphorodiamidate, but this increase in reactivity results in only a modest increase in cytotoxicity. The benzimidazolequinone phosphorodiamidates were not cytotoxic in vitro, because the hydroquinone produced by enzymatic activation does not eliminate the cytotoxic phosphorodiamidate anion.

Experimental Section

General Methods. All ^1H NMR spectra were measured on a 250-MHz Bruker NMR system equipped with a multinuclear (^1H, ^{13}C, ^{19}F and ^{31}P) 5-mm probe. The NMR data acquisition/processing program MacNMR was used with the Tecmag data acquisition system. ^1H chemical shifts are reported in parts per million from tetramethylsilane. All ^{31}P NMR spectra were obtained on the same instrument using broadband gated decoupling and a pulse width of 10 μs. Chemical shifts are reported in parts per million from a coaxial insert containing 5% phosphoric acid in H_2O (structure characterization) or 1% triphenylphosphine oxide (TPPO) in benzene-d_6 (^{31}P kinetics); the chemical shift difference between these references is 24.7 ppm. All variable temperature experiments were conducted using a Bruker variable temperature unit. Chromatographic purifications were carried out by flash chromatography using silica gel grade 60. Melting points were determined on a Mel-Temp II apparatus and are uncorrected. Elemental analyses were performed by the Purdue University Microanalysis Lab, West Lafayette, IN. Mass spectral data were obtained from

the Purdue University Mass Spectrometry Service, West Lafayette, IN, using fast atom bombardment with a 3-nitrobenzyl alcohol matrix. A glass calomel electrode on either a radiometer pH meter or an Orion PerpHect LogR meter, model 330, was used for acidity measurements. IR spectra were recorded on a Nicolet Magna IR 550 spectrometer using either a thin film or Nujol suspension, as noted, between NaCl plates. All anhydrous reactions were carried out in either a flame-dried or oven-dried flask under argon. All organic solvents were distilled prior to use.

Bis(2-bromoethyl)amine Hydrobromide (7). According to a modification of the procedure described by Cortese,[11] hydrobromic acid (75 mL, 0.45 mol, 48% by wt) was added slowly, with stirring, to diethanolamine (11.0 g, 0.10 mol) at 0 °C. The reaction mixture was heated to reflux and then water was removed by distillation through a vigreaux column. The distillate temperature was 100 °C for the first 15 mL collected and 125 °C for the remainder. After a total of 40 mL of distillate was collected, additional 48% HBr (50 mL, 0.30 mol) was added and 50 mL of distillate was collected. Hydrobromic acid (25 mL, 48%) was added and the reaction mixture was refluxed overnight. The reaction mixture was then distilled (~30 mL collected) and the still pot residue was poured into acetone at −78 °C. The white solid that precipitated (10.3 g, 22% diethanolamine hydrobromide and 78% product **7** as determined by ^{1}H NMR) was collected by filtration. This impure product can either be used directly in the subsequent phosphorylation reaction or can be purified by repeated recrystallization from acetone. This recrystallization procedure actually crystallizes out diethanolamine hydrobromide, so that purer product **7** is recovered from the filtrate upon successive recrystallizations: ^{1}H NMR (D_2O) δ 3.55 (t, 4H), 3.45 (t, 4H); ^{1}H NMR of diethanolamine hydrobromide (D_2O) δ 3.78 (t, 4H), 3.12 (t, 4H).

Bis(2-bromoethyl)phosphoramidic Dichloride (8). Phosphorus oxychloride (0.30 mL, 3.2 mmol) was added slowly to a suspension of **7** (1.00 g, 3.2 mmol) in CH_2Cl_2 (22 mL) at −40 °C under argon. Triethylamine (1.20 mL, 8.6 mmol) was added dropwise via syringe over 5 min with vigorous stirring to avoid local heating. The cloudy white reaction mixture was warmed to 0 °C over 2.5 h and stirred at 0 °C for 4 h. Saturated ammonium chloride (8 mL) was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (20:80 EtOAc:hexanes) afforded **8** (0.76 g, 68%) as a white solid: R_f 0.56 (20:80 EtOAc:hexanes); mp 43−44 °C; ^{1}H NMR ($CDCl_3$) δ 3.73 (dt, 4H), 3.55 (t, 4H); ^{31}P NMR ($CDCl_3$) δ 16.48.

Morpholinophosphoramidic Dichloride (9). Morpholine (2.6 mL, 30 mmol) was added dropwise over 10 min to a vigorously stirred solution of phosphorus oxychloride (2.8 mL, 30 mmol) in CH_2Cl_2 (25 mL) at 0 °C under argon. Triethylamine (4.6 mL, 33 mmol) was added dropwise via syringe and the resulting thick suspension was warmed to room temperature and stirred overnight. The mixture was poured over ice and extracted with CH_2Cl_2 (3×). The combined organic layers were washed with saturated NaCl, dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (25:75 EtOAc:hexanes) afforded **9** (4.27 g, 70%) as a pale yellow liquid: R_f 0.41 (25:75 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 3.75 (t, 4H), 3.38 (dt, 4H); ^{31}P NMR ($CDCl_3$) δ 14.70.

Methyl 1,4-Dimethoxy-2-naphthoate (10). According to a modification of the procedure of Brimble et al.,[8] potassium carbonate (30.50 g, 0.22 mol) and methyl iodide (27.5 mL, 0.44 mol) were added to a solution of 1,4-dihydroxy-2-naphthoic acid (6.00 g, 0.029 mol) in acetone (120 mL) under argon. The mixture was refluxed for 48 h. Water (50 mL) was added and the mixture was extracted with CH_2Cl_2 (5×). The combined organic layers were washed with H_2O (2×), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (15:85 EtOAc:hexanes) afforded **10** (7.05 g, 97%) as a solid: R_f 0.44 (15:85 EtOAc:hexanes); mp 48−50 °C; ^{1}H NMR ($CDCl_3$) δ 8.24 (m, 2H), 7.59 (m, 2H), 7.16 (s, 1H), 4.02 (s, 3H), 4.01 (s, 3H), 4.00 (s, 3H).

1,4-Dimethoxy-2-hydroxymethylnaphthalene (11a). A solution of lithium aluminum hydride (29.2 mL, 29.2 mmol, 1 M in ether) in ether (30 mL) was heated to reflux under argon. A solution of **10** (7.18 g, 29.2 mmol) in ether (30 mL) and THF (8 mL) was added dropwise over 35 min. The resulting mixture was refluxed for 3 h and then cooled to 0 °C. Methanol (10 mL) was added dropwise and the resulting clear yellow solution was stirred for 1 h. Saturated NH_4Cl (20 mL) and aqueous HCl (5 mL, 10%) were added and the mixture was extracted with ether (6×). The combined organic layers were washed with saturated Na_2CO_3 (2×) and H_2O (2×), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (35:65 EtOAc:hexanes) afforded **11a** (5.82 g, 92%) as a pink solid: R_f 0.48 (35:65 EtOAc:hexanes); mp 69−70 °C; ^{1}H NMR ($CDCl_3$) δ 8.23 (dd, 1H), 8.03 (dd, 1H), 7.52 (m, 2H), 6.82 (s, 1H), 4.89 (s, 2H), 3.99 (s, 3H), 3.92 (s, 3H), 1.95 (bs, 1H).

1,4-Dimethoxy-3-methyl-2-hydroxymethylnaphthalene (11b). *n*-Butyllithium (7.3 mL, 18.3 mmol, 2.5 M in hexanes) was added dropwise via syringe to a solution of **11a** (1.00 g, 4.58 mmol) in THF (30 mL) at −78 °C under argon. The solution was slowly warmed to room temperature and stirred for 1 h. Methyl iodide (0.34 mL, 5.50 mmol) was added dropwise and the solution was stirred for 20 min. Water (8 mL) was added and the mixture was extracted with EtOAc (4×). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (45:55 EtOAc:hexanes) afforded **11b** (0.61 g, 57%) as a yellow solid: R_f 0.59 (45:55 EtOAc:hexanes); mp 116−117 °C; ^{1}H NMR ($CDCl_3$) δ 8.07 (dd, 2H), 7.51 (m, 2H), 4.94 (s, 2H), 3.98 (s, 3H), 3.88 (s, 3H), 2.52 (s, 3H), 1.73 (bs, 1H).

1,4-Dimethoxy-3-thiomethyl-2-hydroxymethylnaphthalene (11c). *n*-Butyllithium (0.73 mL, 1.83 mmol, 2.5 M in hexanes) was added dropwise to a solution of **11a** (100 mg, 0.458 mmol) in THF (6 mL) at −78 °C under argon. The solution was slowly warmed to room temperature and stirred for 1 h. Dimethyl disulfide (0.050 mL, 0.55 mmol) was added dropwise and the resulting mixture was stirred for 30 min. Water (1 mL) was added and the mixture was extracted with EtOAc (3×). The combined organic layers were washed with saturated NaCl, dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (20:80 EtOAc:hexanes) afforded **11c** (71.2 mg, 59%, 82% based on recovered **11a**) as an oil: R_f 0.26 (20:80 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.55 (m, 2H), 5.07 (s, 2H), 4.03 (s, 3H), 3.99 (s, 3H), 2.53 (s, 3H), 1.60 (bs, 1H).

1,4-Dimethoxy-3-bromo-2-hydroxymethylnaphthalene (11d). *n*-Butyllithium (2.9 mL, 7.33 mmol, 2.5 M in hexanes) was added dropwise to a solution of **11a** (400 mg, 1.83 mmol) in THF (25 mL) at −78 °C under argon. The reaction mixture was slowly warmed to room temperature and stirred for 1 h. 1,2-dibromotetrafluoroethane (0.26 mL, 2.20 mmol) was added dropwise and the solution was stirred for 1 h. Water (10 mL) was added and the mixture was extracted with EtOAc (3×). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (35:65 EtOAc:hexanes) afforded **11d** (273 mg, 50%, 63% based on recovered **11a**) as a yellow solid: R_f 0.62 (35:65 EtOAc:hexanes); mp 115−117 °C; ^{1}H NMR ($CDCl_3$) δ 8.11 (m, 2H), 7.57 (m, 2H), 5.03 (s, 2H), 4.02 (s, 3H), 3.99 (s, 3H), 2.36 (bs, 1H).

2-(1,4-Dimethoxynaphthyl)methyl Morpholinophosphorodiamidate (12). Lithium bis(trimethylsilyl)amide (3.8 mL, 3.8 mmol, 1 M in THF) was added dropwise to a solution of **11a** (750 mg, 3.44 mmol) in THF (10 mL) at −78 °C under argon. The resulting solution was stirred for 5 min and added dropwise via syringe to a solution of **9** (840 mg, 4.13 mmol) in THF (20 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 1.5 h and then warmed to −20 °C. Gaseous ammonia was passed through the reaction mixture for 15 min. Hydrochloric acid (2%, 40 mL) was added and the mixture was extracted with EtOAc (6×). The combined organic layers were washed with saturated NaCl (2×), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (5:

95 MeOH:$CHCl_3$) afforded **12** (960 mg, 76%) as a pale yellow foam: R_f 0.13 (5:95 MeOH:$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.24 (dd, 1H), 8.06 (dd, 1H), 7.54 (m, 2H), 6.85 (s, 1H), 5.23 (dd, 2H, $J_{P,H}$ = 7.8 Hz), 4.00 (s, 3H), 3.94 (s, 3H), 3.63 (t, 4H), 3.19 (dt, 4H), 2.64 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 15.10.

2-(1,4-Naphthoquinonyl)methyl Morpholinophosphorodiamidate (2). Ceric ammonium nitrate (1.03 g, 1.88 mmol) in H_2O (6 mL) was added in portions over 15 min to a solution of **12** (300 mg, 0.82 mmol) in CH_3CN (15 mL) at −8 °C. The solution was stirred at −8 °C for 40 min and then was warmed to 0 °C and stirred for 50 min. The solution was extracted with $CHCl_3$ (3×), and the combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (8:92 MeOH:$CHCl_3$) afforded **2** (54 mg, 20%) as a brown solid; R_f 0.40 (8:92 MeOH:$CHCl_3$); mp 104−106 °C; IR (Nujol) 1661, 1632, 1594 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.08 (m, 2H), 7.78 (m, 2H), 7.03 (t, 1H), 5.01 (m, 2H, $J_{P,H}$ = 7.3 Hz), 3.68 (t, 4H), 3.23 (dt, 4H), 2.89 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 15.00; FAB MS ($C_{15}H_{17}N_2O_5P$) calcd 337.0953, obsd 337.0957.

2-(1,4-Dimethoxynaphthyl)methyl *N,N*-Bis(2-bromoethyl)phosphorodiamidate (13a). Lithium bis(trimethylsilyl)amide (6.05 mL, 6.05 mmol, 1 M in THF) was added dropwise via syringe to a solution of **11a** (1.20 g, 5.50 mmol) in THF (20 mL) at −78 °C under argon. The resulting solution was stirred for 5 min and then added dropwise to a solution of **8** (2.29 g, 6.60 mmol) in THF (50 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 1.5 h and then warmed to −20 °C. Gaseous ammonia was passed through the reaction mixture for 10 min. The mixture was stirred for 10 min, aqueous HCl (2%, 70 mL) was added and the mixture was extracted with EtOAc (3×). The combined organic layers were washed with saturated NaCl (2×), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (70:30 EtOAc:hexanes) afforded **13a** (1.20 g, 43%) as a white solid: R_f 0.33 (70:30 EtOAc:hexanes); mp 112−113 °C; ^{1}H NMR ($CDCl_3$) δ 8.23 (dd, 1H), 8.06 (dd, 1H), 7.54 (m, 2H), 6.84 (s, 1H), 5.26 (m, 2H, $J_{P,H}$ = 8.1 Hz), 4.01 (s, 3H), 3.94 (s, 3H), 3.50 (m, 8H), 2.78 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 15.67.

2-(3-Methyl-1,4-dimethoxynaphthyl)methyl *N,N*-bis(2-bromoethyl)phosphorodiamidate (13b) was prepared from **11b** as described for the synthesis of **13a** (2.07-mmol scale). Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **13b** (480 mg, 44%) as a viscous yellow oil: R_f 0.63 (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.09 (d, 2H), 7.41 (m, 2H), 5.31 (m, 2H, $J_{P,H}$ = 7.1 Hz), 3.98 (s, 3H), 3.89 (s, 3H), 3.48 (m, 8H), 2.77 (bs, 2H), 2.53 (s, 3H); ^{31}P NMR ($CDCl_3$) δ 15.32.

2-(1,4-Naphthoquinonyl)methyl *N,N*-Bis(2-bromoethyl)phosphorodiamidate (3a). Ceric ammonium nitrate (1.83 g, 3.33 mmol) in H_2O (15 mL) was added in portions over 15 min to a solution of **13a** (680 mg, 1.33 mmol) in CH_3CN (60 mL). The reaction was stirred at room temperature for 1 h and extracted with $CHCl_3$ (3×). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **3a** (550 mg, 86%) as a yellow solid: R_f 0.51 (2:98 MeOH:EtOAc); mp 118−119 °C; IR (Nujol) 1664, 1627, 1591 cm^{-1}; $_1$H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H), 5.05 (m, 2H, $J_{P,H}$ = 7.0 Hz), 3.55 (m, 8H), 2.98 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 16.18. Anal. ($C_{15}H_{17}Br_2N_2O_4P$) C, H, N.

2-(3-Methyl-1,4-naphthoquinonyl)methyl *N,N*-bis(2-bromoethyl)phosphorodiamidate (3b) was prepared from **13b** as described for the synthesis of **3a** (0.90-mmol scale). Column chromatography of the crude product (6:94 MeOH:$CHCl_3$) afforded **3b** (230 mg, 52%) as a yellow solid: R_f 0.28 (6:94 MeOH:$CHCl_3$); mp 117−119 °C; IR (Nujol) 1662, 1626, 1594, 1568 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.12 (m, 2H), 7.76 (m, 2H), 5.06 (m, 2H, $J_{P,H}$ = 7.6 Hz), 3.50 (m, 8H), 3.02 (bs, 2H), 2.34 (s, 3H); ^{31}P NMR ($CDCl_3$) δ 15.72; FAB MS ($C_{16}H_{19}Br_2N_2O_4P$) calcd 492.9527, obsd 492.9542.

2-(1,4-Dimethoxynaphthyl)methyl *N,N*-Bis(2-chloroethyl)phosphorodiamidate (13c). Lithium bis(trimethylsilyl)amide (2.50 mL, 2.50 mmol, 1.0 M in THF) was added dropwise via syringe to a solution of **11a** (500 mg, 2.29 mmol) in THF (10 mL) at −78 °C under argon. The resulting solution was stirred for 5 min and added dropwise via syringe to a solution of bis(2-chloroethyl)phosphoramidic dichloride (710 mg, 2.75 mmol) in THF (20 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 1.5 h and then was warmed to −20 °C. Gaseous ammonia was passed through the reaction mixture for 10 min. The mixture was stirred for an additional 10 min, aqueous HCl (2%, 30 mL) was added and the mixture was extracted with EtOAc (4×). The combined organic layers were washed with saturated NaCl (2×), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **13c** (480 mg, 50%) as a yellow oil: R_f 0.59 (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.25 (dd, 1H), 8.06 (dd, 1H), 7.54 (m, 2H), 6.85 (s, 1H), 5.33 (m, 2H, $J_{P,H}$ = 7.8 Hz), 4.01 (s, 3H), 3.94 (s, 3H), 3.65 (t, 4H), 3.48 (m, 4H), 2.77 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 15.72.

2-(3-Methyl-1,4-dimethoxynaphthyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (13d) was prepared from **11b** as described for the synthesis of **13c** (2.15-mmol scale). Column chromatography of the crude product (1:99 MeOH:EtOAc) afforded **13d** (440 mg, 47%) as a pale yellow foam: R_f 0.44 (1:99 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.08 (m, 2H), 7.53 (m, 2H), 5.31 (m, 2H, $J_{P,H}$ = 6.8 Hz), 3.98 (s, 3H), 3.89 (s, 3H), 3.64 (t, 4H), 3.45 (dt, 4H), 2.77 (bs, 2H), 2.52 (s, 3H); ^{31}P NMR ($CDCl_3$) δ 15.65.

2-(3-Thiomethyl-1,4-dimethoxynaphthyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (13e) was prepared from **11c** as described for the synthesis of **13c** (0.27-mmol scale). Column chromatography of the crude product (EtOAc) afforded **13e** (77 mg, 61%) as a yellow oil: R_f 0.22 (EtOAc); ^{1}H NMR ($CDCl_3$) d 8.12 (m, 2H), 7.57 (m, 2H), 5.48 (d, 2H, $J_{P,H}$ = 4.9 Hz), 4.04 (s, 3H), 4.00 (s, 3H), 3.64 (t, 4H), 3.49 (dt, 4H), 2.73 (bs, 2H), 2.51 (s, 3H); ^{31}P NMR ($CDCl_3$) δ 14.62.

2-(3-Bromo-1,4-dimethoxynaphthyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (13f) was prepared from **11d** as described for the synthesis of **13c** (0.91-mmol scale). Column chromatography of the crude product (EtOAc) afforded **13f** (353 mg, 78%) as a white solid: R_f 0.17 (EtOAc); mp 112−114 °C; ^{1}H NMR ($CDCl_3$) δ 8.12 (m, 2H), 7.60 (m, 2H), 5.40 (m, 2H, $J_{P,H}$ = 5.9 Hz), 4.02 (s, 3H), 4.00 (s, 3H), 3.66 (t, 4H), 3.48 (dt, 4H), 2.82 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 14.38.

2-(1,4-Naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (3c) was prepared from **13c** as described for the synthesis of **3a** (1.14-mmol scale). Column chromatography of the crude product (6:94 MeOH:$CHCl_3$) afforded **3c** (360 mg, 81%) as a yellow solid: R_f 0.45 (6:94 MeOH:$CHCl_3$); mp 105−107 °C; IR (Nujol) 1664, 1630, 1589 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.09 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H), 5.05 (m, 2H, $J_{P,H}$ = 7.1 Hz), 3.68 (t, 4H), 3.54 (m, 4H), 3.01 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 16.42. Anal. ($C_{15}H_{17}Cl_2N_2O_4P$) C, H, N.

2-(3-Methyl-1,4-naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (3d) was prepared from **13d** as described for the synthesis of **3a** (1.01-mmol scale). Column chromatography of the crude product (3:97 MeOH:$CHCl_3$) afforded **3d** (289 mg, 71%) as a yellow solid: R_f 0.16 (3:97 MeOH:$CHCl_3$); mp 129−130 °C; IR (Nujol) 1665, 1660, 1628, 1594 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.11 (m, 2H), 7.76 (m, 2H), 5.06 (m, 2H, $J_{P,H}$ = 7.3 Hz), 3.66 (t, 4H), 3.47 (dt, 4H), 3.02 (bs, 2H), 2.34 (s, 3H); ^{31}P NMR ($CDCl_3$) δ 14.27. Anal. ($C_{16}H_{19}Cl_2N_2O_4P$) C, N, H.

2-(3-Thiomethyl-1,4-naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (3e) was prepared from **13e** as described for the synthesis of **3a** (0.287-mmol scale). Column chromatography of the crude product (2:98 MeOH:$CHCl_3$) afforded **3e** (72 mg, 57%) as a viscous orange oil: R_f 0.29 (2:98 MeOH:$CHCl_3$); IR (Nujol) 1666, 1643, 1588, 1542 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.75 (m, 2H), 5.21 (m, 2H, $J_{P,H}$ = 6.1 Hz), 3.66 (t, 4H), 3.46 (dt, 4H), 2.97 (bs, 2H), 2.74 (s, 3H); ^{31}P NMR ($CDCl_3$) δ 15.25. Anal. ($C_{16}H_{19}Cl_2N_2O_4PS$) C, H, N.

2-(3-Bromo-1,4-naphthoquinonyl)methyl *N,N*-Bis(2-chloroethyl)phosphorodiamidate (3f). Silver(II) oxide (300

mg, 2.4 mmol) was added in one portion to a solution of **13f** (200 mg, 0.40 mmol) in THF (15 mL) at room temperature. Nitric acid (6 N, 2 mL) was added to this suspension. The mixture was stirred for 20 min at room temperature, H_2O (5 mL) was added and the mixture was extracted with $CHCl_3$ (3×). The combined organic layers were washed with saturated NaCl and $NaHCO_3$ (1 M), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (2:98 MeOH:$CHCl_3$ to remove impurities, then 6:94 MeOH:$CHCl_3$) afforded **3f** (156 mg, 83%) as a yellow solid: R_f 0.36 (6:94 MeOH:$CHCl_3$); mp 134−135 °C; IR (Nujol) 1680, 1675, 1659, 1605, 1589 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.18 (m, 2H), 7.80 (m, 2H), 5.21 (m, 2H, $J_{P,H}$ = 6.5 Hz), 3.66 (t, 4H), 3.48 (dt, 4H), 2.97 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 15.32. Anal. ($C_{15}H_{16}BrCl_2N_2O_4P$) C, H, N.

2-Acetoxymethyl-1,4-dimethoxynaphthalene (14). Triethylamine (0.35 mL, 2.52 mmol) was added with stirring to a solution of **11a** (500 mg, 2.29 mmol) in CH_2Cl_2 (25 mL) in a room-temperature water bath under argon. Acetyl chloride (0.18 mL, 2.52 mmol) was added dropwise, and the solution was stirred at room temperature for 3 h and concentrated under reduced pressure. The residue was resuspended in EtOAc and the salts were separated. Column chromatography of the crude product (1:9 EtOAc:hexanes) afforded **14** (540 mg, 91%) as a colorless oil: R_f 0.40 (1:9 EtOAc:hexanes); ^1H NMR ($CDCl_3$) δ 8.23 (dd, 1H), 8.07 (dd, 1H), 7.54 (m, 2H), 6.76 (s, 1H), 5.34 (s, 2H), 3.99 (s, 3H), 3.94 (s, 3H), 2.13 (s, 3H).

2-Acetoxymethyl-1,4-naphthoquinone (1). Ceric ammonium nitrate (2.84 g, 5.19 mmol) in H_2O (25 mL) was added in portions over 15 min to a solution of **14** (540 mg, 2.07 mmol) in CH_3CN (30 mL) at room temperature. The mixture was stirred at room temperature for 30 min and extracted with $CHCl_3$ (4×). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (3:7 EtOAc:hexanes) afforded **1** (420 mg, 88%) as a yellow solid: R_f 0.66 (3:7 EtOAc:hexanes); mp 107−109 °C (lit.[15] mp 109−110 °C); IR (Nujol) 1739, 1659, 1630, 1593 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.77 (m, 2H), 6.91 (t, 1H, J = 2 Hz), 5.15 (d, 2H, J = 2 Hz), 2.20 (s, 3H).

2-(1,4-Dimethoxynaphthyl)methyl Bis[*N*-(2-chloroethyl)]phosphorodiamidate (15). Phosphorus oxychloride (0.043 mL, 0.46 mmol) was added to a suspension of *N*-(2-chloroethyl)amine hydrochloride (112 mg, 0.96 mmol) in CH_2Cl_2 (10 mL) at −78 °C under argon. Diisopropylethylamine (0.34 mL, 1.92 mmol) was added dropwise and the reaction mixture was warmed to room temperature over 2 h and stirred for 7 h. *n*-Butyllithium (0.18 mL, 0.46 mmol, 2.5 M in hexanes) was added to a solution of a **11a** (100 mg, 0.46 mmol) in CH_2Cl_2 (5 mL) in a second flask at −78 °C under argon. The resulting solution was stirred for 15 min. The phosphoryl chloride solution was added via syringe to the alkoxide solution with vigorous stirring at −78 °C. The reaction mixture was slowly warmed to room temperature, stirred overnight, and then concentrated under reduced pressure until the volume was reduced to one-third. The salts were separated and the crude product was purified by column chromatography (78:22 $CHCl_3$:acetone to remove impurities, then 67:33 $CHCl_3$:acetone) to afford **15** (62.0 mg, 32%, 87% based on recovered **11a**) as a pale yellow oil: R_f 0.31 (78:22 $CHCl_3$:acetone); ^1H NMR ($CDCl_3$) δ 8.24 (dd, 1H), 8.06 (dd, 1H), 7.55 (m, 2H), 6.84 (s, 1H), 5.25 (d, 2H, $J_{P,H}$ = 7.9 Hz), 4.01 (s, 3H), 3.94 (s, 3H), 3.59 (t, 4H), 3.28 (dt, 4H), 3.04 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 14.72.

2-(1,4-Naphthoquinonyl)methyl bis[*N*-(2-chloroethyl)]phosphorodiamidate (5) was prepared from **15** as described for the synthesis of **3a** (0.37-mmol scale). Column chromatography of the crude product (78:22 $CHCl_3$:acetone to remove impurities, then 67:33 $CHCl_3$:acetone) afforded **5** (42.4 mg, 74%) as a yellow solid: R_f 0.24 (67:33 $CHCl_3$:acetone); mp 109−110 °C; IR (Nujol) 1662, 1633, 1594 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H, J = 1.9 Hz), 5.04 (dd, 2H, J = 1.9 and 6.8 Hz), 3.65 (t, 4H), 3.36 (dt, 4H), 3.20 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 14.83. Anal. ($C_{15}H_{17}Cl_2N_2O_4P$) C, H, N.

2,3-Dinitro-1,4-dimethoxybenzene (16) was prepared as described.[15] 1,4-Dimethoxybenzene (3.00 g, 2.17 mmol) was added slowly with vigorous stirring to concentrated HNO_3 (27 mL) at 0 °C. The thick, yellow suspension was warmed to room temperature and stirred for 1 h. The mixture was then heated to 90 °C and stirred at 90 °C for 1 h, cooled to room temperature and poured into ice water (125 mL). The product was collected by filtration, washed with water, and dried to give 4.39 g (89%) of a bright yellow solid. The product was determined to be an 8:1 mixture of **16** and 2,5-dinitro-1,4-dimethoxybenzene by ^1H NMR. The crude product was used without further purification in the subsequent reaction: R_f 0.20 (50:50 EtOAc:hexanes); ^1H NMR ($CDCl_3$) δ 7.20 (s, 2H), 3.94 (s, 6H).

4,7-Dimethoxy-2-hydroxymethylbenzimidazole (17). Stannous chloride (22.4 g, 0.118 mol) was added in portions to a suspension of **16** (3.00 g, 0.013 mol) in concentrated HCl (33 mL) at 0 °C. The ice bath was removed and the reaction mixture became warm. The temperature was maintained below 100 °C by occasional application of an ice bath. When the temperature of the mixture fell to 70 °C, the mixture was heated to 90−100 °C for 2 h. The pale yellow mixture was cooled to room temperature, and water (100 mL) and glycolic acid (2.60 g, 0.034 mol) were added. The mixture was refluxed overnight. The clear, pale green solution was cooled to room temperature, slowly neutralized with saturated Na_2CO_3, and extracted with EtOAc (6×). The combined organic layers were washed with saturated NaCl (2×), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (10:90 MeOH:EtOAc) afforded **17** (2.11 g, 78%) as an amorphous gray solid: R_f 0.41 (10:90 MeOH:EtOAc); ^1H NMR (DMSO-d_6) δ 6.56 (s, 2H), 4.57 (s, 2H), 3.85 (s, 6H).

4,7-Dimethoxy-2-hydroxymethyl-1-methylbenzimidazole (18). Lithium bis(trimethylsilyl)amide (10.1 mL, 10.1 mmol, 1 M in THF) was added dropwise via syringe to a solution of **17** (1.00 g, 4.80 mmol) in THF (50 mL) at 0 °C under argon. The mixture was warmed to room temperature and stirred for 20 min. A solution of methyl iodide (0.33 mL, 5.3 mmol) in THF (20 mL) was added dropwise via syringe. The solution was stirred for 7 h, H_2O (40 mL) was added and the mixture was extracted with EtOAc (4×). The combined organic layers were washed with saturated NaCl (3×), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (10:90 MeOH:EtOAc) afforded **18** (0.98 g, 92%) as a brown solid: R_f 0.50 (10:90 MeOH:EtOAc); mp 153−155 °C; ^1H NMR ($CDCl_3$) δ 6.57 (d, 1H), 6.50 (d, 1H), 4.86 (s, 2H), 4.02 (s, 3H), 3.96 (s, 3H), 3.89 (s, 3H).

2-(4,7-Dimethoxy-1-methylbenzimidazolyl)methyl *N,N*-Bis(2-bromoethyl)phosphorodiamidate (19a). Lithium bis(trimethylsilyl)amide (2.80 mL, 2.80 mmol, 1 M in THF) was added dropwise via syringe to a solution of **18** (560 mg, 2.52 mmol) in CH_2Cl_2 (10 mL) and THF (4 mL) at −78 °C under argon. The resulting solution was stirred for 5 min and then added via syringe to a solution of **8** (1.05 g, 3.02 mmol) in THF (28 mL). The mixture was stirred at −78 °C for 1.5 h and then warmed to −20 °C. Gaseous ammonia was passed through the solution for 7 min and the cloudy mixture was stirred for 8 min. Hydrochloric acid (2%, 35 mL) was added and the mixture was extracted with EtOAc (4×). The combined organic layers were washed with saturated Na_2CO_3 (2×) and saturated NaCl (2×), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (68:29:3 $CHCl_3$:acetone:MeOH) afforded **19a** (610 mg, 47%) as an amorphous tan solid: R_f 0.24 (68:29:3 $CHCl_3$:acetone:MeOH); ^1H NMR ($CDCl_3$) δ 6.60 (d, 1H), 6.52 (d, 1H), 5.26 (m, 2H, $J_{P,H}$ = 8.2 Hz), 4.10 (s, 3H), 3.96 (s, 3H), 3.90 (s, 3H), 3.47 (m, 8H), 3.13 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 16.27.

2-(4,7-Dimethoxy-1-methylbenzimidazolyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (19b) was prepared from **18** and bis(2-chloroethyl)phosphoramidic dichloride as described for the synthesis of **19a** (1.80 mmol). Column chromatography of the crude product (68:29:3 $CHCl_3$:acetone:MeOH) afforded **19b** (390 mg, 51%, 62% based on recovered **18**, 70 mg) as a tan foam: R_f 0.30 (68:29:3 $CHCl_3$:acetone:

MeOH); ^{1}H NMR ($CDCl_3$) δ 6.59 (d, 1H), 6.52 (d, 1H), 5.26 (m, 2H, $J_{P,H}$ = 8.2 Hz), 4.10 (s, 3H), 3.96 (s, 3H), 3.90 (s, 3H), 3.64 (t, 4H), 3.46 (dt, 4H), 3.05 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 16.43.

2-(1-Methyl-4,7-benzimidazolequinonyl)methyl *N*,*N*-bis(2-bromoethyl)phosphorodiamidate (4a) was prepared as described for **3f** using **19a** (0.194-mmol scale). Column chromatography of the crude product (68:29:3 $CHCl_3$:acetone:MeOH to remove impurities, then 53:42:5 $CHCl_3$:acetone:MeOH) afforded **4a** (62.7 mg, 67%) as a yellow foam: R_f 0.18 (68:29:3 $CHCl_3$:acetone:MeOH); IR (Nujol) 1665, 1521 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.72 (d, 1H), 6.65 (d, 1H), 5.24 (m, 2H, $J_{P,H}$ = 8.5 Hz), 4.07 (s, 3H), 3.50 (m, 8H), 3.16 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 16.53; FAB MS ($C_{13}H_{17}Br_2N_4O_4P$) calcd 482.9433, obsd 482.9432.

2-(1-Methyl-4,7-benzimidazolequinonyl)methyl *N*,*N*-bis(2-chloroethyl)phosphorodiamidate (4b) was prepared from **19b** as described for the synthesis of **3f** (0.47-mmol scale). Column chromatography of the crude product (68:29:3 $CHCl_3$:acetone:MeOH) afforded **4b** (146 mg, 79%) as a yellow foam: R_f 0.18 (68:29:3 $CHCl_3$:acetone:MeOH); IR (neat) 1665, 1591, 1518 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.72 (d, 1H), 6.65 (d, 1H), 5.22 (m, 2H, $J_{P,H}$ = 8.3 Hz), 4.07 (s, 3H), 3.66 (t, 4H), 3.49 (dt, 4H), 3.09 (bs, 2H); ^{31}P NMR ($CDCl_3$) δ 16.74; FAB MS ($C_{13}H_{17}Cl_2N_4O_4P$) calcd 395.0445, obsd 395.0461.

2-(1,4-Dimethoxynaphthyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)]phosphorodiamidate (20a). A solution of **11a** (100 mg, 0.46 mmol) in CH_2Cl_2 (2 mL) and CH_3CN (2 mL) was cooled to −70 °C and stirred under argon. Phosphorus trichloride (0.23 mL, 0.46 mmol, 2 M in CH_2Cl_2) was added dropwise followed by the dropwise addition of diisopropylethylamine (0.08 mL, 0.46 mmol). The reaction mixture was stirred for 25 min at −70 °C. A solution of *N*-methyl-*N*-(2-bromoethyl)-amine hydrobromide[10] (200 mg, 0.92 mmol) in CH_3CN (2.5 mL) was added slowly via syringe, followed by additional diisopropylethylamine (0.32 mL, 1.84 mmol). The temperature was raised to −60 to −50 °C and the reaction was stirred for 1.25 h. *tert*-Butyl hydroperoxide (1.1 mL, 5−6 M in decane) was added, and the reaction mixture was warmed to −40 to −20 °C and stirred for 30 min. Water (3 mL) was added, and the mixture was warmed to room temperature and extracted with CH_2Cl_2 (4×). The combined organic layers were washed with H_2O and saturated NaCl, dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **20a** (190 mg, 77%) as a pale yellow oil: R_f 0.33 (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.24 (dd, 1H), 8.06 (dd, 1H), 7.54 (m, 2H), 6.87 (s, 1H), 5.24 (d, 2H, J = 7.4 Hz), 4.01 (s, 3H), 3.93 (s, 3H), 3.43 (m, 8H), 2.71 (d, 6H, J = 9.7 Hz); ^{31}P NMR ($CDCl_3$) δ 17.60.

2-(3-Methyl-1,4-dimethoxynaphthyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)]phosphorodiamidate (20b) was prepared from **11b** and *N*-methyl-*N*-(2-bromoethyl)amine hydrobromide[10] as described for the synthesis of **20a** (0.43 mmol). Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **20b** (135 mg, 57%) as a yellow oil: R_f 0.37 (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.09 (dd, 2H), 7.52 (m, 2H), 5.28 (d, 2H, J = 5.8 Hz), 3.97 (s, 3H), 3.89 (s, 3H), 3.41 (m, 8H), 2.67 (d, 6H, J = 9.7 Hz), 2.53 (s, 3H); ^{31}P NMR ($CDCl_3$) δ 17.28.

2-(1,4-Dimethoxynaphthyl)methyl bis[*N*-methyl-*N*-(2-chloroethyl)]phosphorodiamidate (20c) was prepared from **11a** and *N*-methyl-*N*-(2-chloroethyl)amine hydrochloride[10] as described for the synthesis of **20a** (0.458 mmol). Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **20c** (132 mg, 64%) as a pale yellow oil: R_f 0.33 (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.24 (dd, 1H), 8.06 (dd, 1H), 7.54 (m, 2H), 6.88 (s, 1H), 5.24 (d, 2H, J = 7.3 Hz), 4.00 (s, 3H), 3.93 (s, 3H), 3.63 (t, 4H), 3.37 (dt, 4H), 2.72 (d, 6H, J = 9.6 Hz); ^{31}P NMR ($CDCl_3$) δ 17.91.

2-(1,4-Naphthoquinonyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)]phosphorodiamidate (6a) was prepared from **20a** as described for the synthesis of **3a** (0.372-mmol scale). Column chromatography of the crude product (2:98 MeOH:CH_2Cl_2) afforded **6a** (164 mg, 87%) as a viscous brown oil: R_f 0.16 (2:98 MeOH:CH_2Cl_2); IR (neat) 1664, 1632, 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H, J = 2.0 Hz), 5.04 (dd, 2H, J = 2.0 and 6.4 Hz), 3.49 (m, 8H), 2.78 (d, 6H, J = 9.7 Hz); ^{31}P NMR ($CDCl_3$) δ 18.00. Anal. ($C_{17}H_{21}Br_2N_2O_4P$) C, H, N.

2-(3-Methyl-1,4-naphthoquinonyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)]phosphorodiamidate (6b) was prepared from **20b** as described for the synthesis of **3a** (0.235-mmol scale). Column chromatography of the crude product (3:97 MeOH:CH_2Cl_2) afforded **6b** (99 mg, 81%) as a yellow oil: R_f 0.20 (3:97 MeOH:CH_2Cl_2); IR (neat) 1663, 1626, 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.12 (m, 2H), 7.75 (m, 2H), 5.04 (d, 2H, J = 6.4 Hz), 3.43 (m, 8H), 2.71 (d, 6H, J = 9.7 Hz), 2.35 (s, 3H); ^{31}P NMR ($CDCl_3$) δ 17.77. Anal. ($C_{18}H_{23}Br_2N_2O_4P$) C, H, N.

2-(1,4-Naphthoquinonyl)methyl bis[*N*-methyl-*N*-(2-chloroethyl)]phosphorodiamidate (6c) was prepared from **20c** as described for the synthesis of **3a** (0.29-mmol scale). Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **6c** (105 mg, 87%) as a brown oil: R_f 0.55 (2:98 MeOH:EtOAc); IR (neat) 1664, 1633, 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H, J = 2.0), 5.03 (dd, 2H, J = 2.0 and 6.4 Hz), 3.66 (t, 4H), 3.42 (m, 4H), 2.78 (d, 6H, J = 9.9 Hz); ^{31}P NMR ($CDCl_3$) δ 18.21 ppm. Anal. ($C_{17}H_{21}Cl_2N_2O_4P$) C, H, N.

Enzyme Kinetics. The DT-diaphorase activity of the synthesized quinone compounds was determined using a standard coupled assay according to a modification of the procedure described by Ernster.[18] The reactions were monitored using Kinetics software by Varian, version 1.0, and a CARY 3 UV−visible spectrophotometer. Reaction mixtures, totaling 1 mL in volume, contained Tris buffer (0.05 M, pH 7.6) and dithiothreitol (0.01 mM), NADH (1 mM), purified human enzyme (4.8×10^{-4} mg), cytochrome *c* (55 μM) and a suitable concentration of the quinone in DMSO (10 μL). The reactions were run in triplicate at 25 °C and started by the addition of the quinone. They were monitored by recording an increase in absorbance at 550 nm due to the reduction of cytochrome *c*, and appropriate controls were performed without the addition of enzyme to determine the background rates for the reactions. Rates of reduction were measured from the initial linear portion of the reaction curve and the kinetic parameters were determined using a Hanes plot. An extinction coefficient of 1.85×10^4 M^{-1} cm^{-1} for cytochrome *c* was used in the calculations.

Measurement of DT-Diaphorase, NADPH:Cytochrome P-450 Reductase, and NADH:Cytochrome b_5 Reductase Activity in HT-29 and BE Cells. S9 supernatants were prepared from HT-29 and BE cell lines using a procedure outlined by Fitzsimmons et al.,[20] except that the cell pellets were resuspended immediately. Protein concentration was determined using a standard Bradford assay.[26] Enzyme activities were measured using a modification of the procedure described by Fitzsimmons et al.[20] The same reaction mixtures were used as described above, except that menadione (2 μM) was used as the substrate and the assays were run at 37 °C. Using NADH as cofactor, the enzyme activity present when dicumarol (10 μM) was added as an inhibitor of DT-diaphorase was attributed to cytochrome b_5 reductase, and the activity that was inhibited by dicumarol was attributed to DT-diaphorase. Using NADPH as cofactor, the enzyme activity present when dicumarol was added as an inhibitor of DT-diaphorase was attributed to cytochrome P-450 reductase.[20] Enzyme activity is expressed as nmol cytochrome *c* reduced/min/mg protein.

^{31}P NMR Kinetics. The quinone prodrug was dissolved in CH_3CN (0.3 mL, THF for **3a**) and the activating agent (glutathione, DDTC or sodium dithionite) was dissolved in cacodylate buffer (0.4 mL, 0.4 M, pH 7.7). The buffer solution was added to the organic solution and the pH of the mixture was adjusted to ∼7.4. The reaction mixture was transferred to a 5-mm NMR tube, and the data acquisition was started (pulse delay 30 μs). Spectra were taken every 2.5 min for 0.5 h, then every 5 min for 0.5 h, then every 10 min for 1 h, and time points for each spectrum were assigned from the initiation of the reaction. Chemical shifts are reported relative to the

TPPO reference. The temperature of the probe was maintained at 37 °C, if necessary, using the Bruker variable temperature unit. The relative concentrations of the intermediates were determined by measuring the peak areas.

Cytotoxicity Studies in Vitro: HT-29 and BE Cells. A modification of the procedure described by Miribelli et al.[27] was used to determine the clonogenic survival of the cells. HT-29 and BE cells in exponential growth were suspended in unsupplemented Eagles MEM medium (10 mL) at a final density of $1.7-2 \times 10^5$ cells/mL. Unsupplemented medium contains MEM (Gibco) and HEPES (0.02 M). The drug stock solutions (0.1−40 mM) were prepared using either ethanol or dimethyl sulfoxide as solvent. The maximum amount of DMSO or ethanol used in the drug treatments was 1% of the final volume. Appropriate volumes (6.5−100 μL) of the drug stock solution were added to five vials of the cell suspensions, to give five different final drug concentrations, and 100 μL of solvent was added to a sixth vial for a control. The treated cells were incubated for 2 h (37 °C, 5% CO_2). The cells were spun down and rinsed three times with supplemented medium (3 mL) and then diluted in 5 mL of supplemented medium. Supplemented medium was prepared by adding fetal bovine serum (10%), gentamicin (0.05 mg/mL), l-glutamine (0.03 mg/mL), and sodium pyruvate (0.1 mM) to unsupplemented medium. The cells were counted using a Coulter Counter, plated at 2−3 different densities for each drug concentration, and incubated for 10 days. The colonies were stained with 0.5% crystal violet in 95% ethanol, and those colonies comprised of 50 or more cells were counted using a dissecting microscope and pen-style counter. The LC_{99} of each compound was determined by plotting the log surviving fraction vs drug concentration.

Acknowledgment. We thank Dr. Shiuan Chen (City of Hope Medical Center, CA) for providing purified human DT-diaphorase and Dr. David Ross (University of Colorado) for providing the BE cell line. The assistance of the Purdue Cancer Center Drug Evaluation Facility in carrying out the xenograft experiments is appreciated. Financial support from Grants R01 CA34619 and T32 GM08298 is also gratefully acknowledged.

References

(1) Ernster, L. DT-diaphorase, a historical review. *Chem. Scripta* **1987**, *27A*, 1−13.

(2) (a) Lind, C.; Hochstein, P.; Ernster, L. DT-diaphorase as a quinone reductase: A cellular control device against semiquinone and superoxide radical formation. *Arch. Biochem. Biophys.* **1992**, *216*, 178−185. (b) Radjendirane, V.; Joseph, P.; Lee, Y. H.; Kimura, S.; KleinSzanto, A. J. P.; Gonzalez, F. J.; Jaiswal, A. K. Disruption of the DT-diaphorase (NQO1) gene in mice leads to increased menadione toxicity. *J. Biol. Chem.* **1998**, *273*, 7382−7389.

(3) (a) Schlager, J. J.; Powis, G. Cytosolic NAD(P)H: (quinone-acceptor) oxidoreductase in human normal and tumor tissue: effects of cigarette smoking and alcohol. *Int. J. Cancer* **1990**, *45*, 403−409. (b) Malkinson, A. M.; Siegel, D.; Forrest, G. L.; Gazdar, A. F.; Oie, H. K.; Chan, D. C.; Bunn, P. A.; Mabry, M.; Dykes, D. J.; Harrison, S. D.; Ross, D. Elevated DT-diaphorase activity and messenger RNA content in human nonsmall cell lung carcinoma − relationship to the response of lung tumour xenografts to mitomycin C. *Cancer Res.* **1994**, *52*, 4752−4757.

(4) Workman, P. Enzyme-directed bioreductive drug development revisited: A commentary on recent progress and future prospects with emphasis on quinone anticancer agents and quinone metabolizing enzymes, particularly DT-diaphorase. *Oncol. Res.* **1994**, *6*, 461−475.

(5) Riley, R. J.; Workman, P. Commentary: DT-diaphorase and cancer chemotherapy. *Biochem. Pharmacol.* **1992**, *43*, 1657−1669.

(6) (a) Lin, A. J.; Pardini, R. S.; Cosby, L. A.; Lillis, B. J.; Shansky, C. W.; Sartorelli, A. C. Potential bioreductive alkylating agents. 2. Antitumor effect and biochemical studies of naphthoquinone derivatives. *J. Med. Chem.* **1973**, *16*, 1268−1271. (b) Antonini, I.; Lin, T.-S.; Cosby, L. A.; Dai, Y.-R.; Sartorelli, A. C. 2- and 6-Methyl-1,4-naphthoquinone derivatives as potential bioreductive alkylating agents. *J. Med. Chem.* **1982**, *25*, 730−735. (c) Skibo, E. B.; Gordon, S.; Bess, L.; Boruah, R.; Heileman, M. J. Studies of pyrrolo[1,2-*a*]benzimidazolequinone DT-diaphorase substrate activity, topoisomerase II inhibition activity, and DNA reductive alkylation. *J. Med. Chem.* **1997**, *40*, 1327−1339. (d) Skibo, E. B.; Schulz, W. G. Pyrrolo[1,2-*a*]benzimidazole-based aziridinyl quinones. A new class of DNA cleaving agent exhibiting G and A base specificity. *J. Med. Chem.* **1993**, *36*, 3050−3055. (e) Beall, H. D.; Winski, S.; Swann, E.; Hudnott, A. R.; Cotterill, A. S.; O'Sullivan, N.; Green, S. J.; Bien, R.; Siegel, D.; Ross, D.; Moody, C. J. Indolequinone antitumor agents: Correlation between quinone structure, rate of metabolism by recombinant human NAD(P)H:quinone oxidoreductase, and in vitro cytotoxicity. *J. Med. Chem.* **1998**, *41*, 4755−4766. (f) Beall, H. D.; Hudnott, A. R.; Winski, S.; Siegel, D.; Swann, E.; Ross, D.; Moody, C. J. Indolequinone antitumor agents: Relationship between quinone structure and rate of metabolism by recombinant human NQO1. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 545−548.

(7) (a) Borch, R. F.; Valente, R. R. Synthesis, activation, and cytotoxicity of aldophosphamide analogues. *J. Med. Chem.* **1991**, *34*, 3052−3058. (b) Firestone, A.; Mulcahy, R. T.; Borch, R. F. Nitroheterocycle reduction as a paradigm for intramolecular catalysis of drug delivery to hypoxic cells. *J. Med. Chem.* **1991**, *34*, 2933−2935. (c) Mulcahy, R. T.; Gipp, J. J.; Schmidt, J. P.; Joswig, C.; Borch, R. F. Nitrobenzyl phosphorodiamidates as potential hypoxia-selective alkylating agents. *J. Med. Chem.* **1994**, *37*, 1610−1615. (d) Morgan, A. S.; Sanderson, P. E.; Borch, R. F.; Tew, K. D.; Niitsu, Y.; Takayama, T.; Von Hoff, D. D.; Izbicka, E.; Mangold, G.; Paul, C.; Broberg, U.; Mannervik, B.; Henner, W. D.; Kauvar, L. M. Tumor efficacy and bone marrow-sparing properties of TER286, a cytotoxin activated by glutathione S-transferase. *Cancer Res.* **1998**, *58*, 2568−2575. (e) Borch, R. F.; Liu, J.; Schmidt, J. P.; Marakovits, J. T.; Joswig, C.; Gipp, J. J.; Mulcahy, R. T. Synthesis and evaluation of nitroheterocyclic phosphoramidates as hypoxia-selective alkylating agents. *J. Med. Chem.* **2000**, *43*, 2258−2265.

(8) Brimble, M. A.; McEwan, J. F.; Turner, P. Asymmetric diels-alder addition of cyclopentadiene to chiral naphthoquinones. *Tetrahedron: Asymmetry* **1998**, *9*, 1239−1255.

(9) Moorlag, H.; Meyers, A. I. An asymmetric synthesis of a C_2 symmetric tetrasubstituted biaryl: 2,2′-dihydroxy-6,6′-dimethyl-1,1′-diphenyl, a stable chiral system. *Tetrahedron Lett.* **1993**, *34*, 6993−6996.

(10) Fries, K. M.; Joswig, C.; Borch, R. F. Synthesis and biological evaluation of 5-fluoro-2′-deoxyuridine phosphoramidate analogues. *J. Med. Chem.* **1995**, *38*, 2672−2680.

(11) Cortese, F. β-Bromoethylamine hydrobromide. *Org. Synth.* **1938**, *18*, 13−14.

(12) Jacob, P., III; Callery, P. S.; Shulgin, A. T.; Castagnoli, N., Jr. A convenient synthesis of quinones from hydroquinone dimethyl ethers. Oxidative demethylation with cerric ammonium nitrate. *J. Org. Chem.* **1976**, *41*, 3627−3629.

(13) Snyder, C. D.; Rapoport, H. Oxidative cleavage of hydroquinone ethers with argentic oxide. *J. Am. Chem. Soc.* **1972**, *94*, 227−231.

(14) Tarbell, D. S.; Fukushima, D. K.; Dam, H. The synthesis of some derivatives of 2-methyl-1,4-naphthoquinone. *J. Am. Chem. Soc.* **1945**, *67*, 197−199.

(15) Fisher, G. H.; Moreno, H. R.; Oatis, J. E., Jr.; Schultz, H. P. Quinoxaline studies. 23. Potential antimalarials. Substituted 5,8-dimethoxyquinoxalines. *J. Med. Chem.* **1975**, *18*, 746−752.

(16) Woodward, R. B. *m*-Hydroxybenzaldehyde. *Organic Syntheses;* Wiley: New York, 1955; Coll. Vol. 3, pp 453−455.

(17) Phillips, M. A. The formation of 2-substituted benzimidazoles. *J. Chem. Soc.* **1928**, 2393−2399.

(18) Ernster, L. DT-diaphorase. *Methods Enzymol.* **1967**, *10*, 309−317.

(19) Traver, R. D.; Horikoshi, T.; Danenberg, K. D.; Stadlbauer, T. H. W.; Danenberg, P. V.; Ross, D.; Gibson, N. W. NAD(P)H: Quinone oxidoreductase gene expression in human colon carcinoma cells: Characterization of a mutation which modulates DT-diaphorase activity and mitomycin sensitivity. *Cancer Res.* **1992**, *52*, 797−802.

(20) Fitzsimmons, S. A.; Workman, P.; Grever, M.; Paull, K.; Camalier, R.; Lewis, A. D. Reductase enzyme expression across the National Cancer Institute tumor cell line panel: Correlation with sensitivity to mitomycin C and E09. *J. Natl. Cancer Inst.* **1996**, *88*, 259−269.

(21) Shulman-Roskes, E. M.; Noe, D. A.; Gamcsik, M. P.; Marlow, A. L.; Hilton, J.; Hausheer, F. H.; Colvin, O. M.; Ludeman, S. M. The partitioning of phosphoramide mustard and its aziridinium ions among alkylation and P−N bond hydrolysis reactions. *J. Med. Chem.* **1998**, *41*, 515−529.

(22) Tomasz, M.; Lipman, R. Reductive Metabolism and Alkylating Activity of Mitomycin C Induced by Rat Liver Microsomes. *Biochemistry* **1981**, *20*, 5056−5061.

(23) Menotti, A. R. Water-soluble derivatives of menadione. *J. Am. Chem. Soc.* **1943**, *65*, 1209−1211.

(24) Tew, K. D. Glutathione-associated enzymes in anticancer drug resistance. *Cancer Res.* **1994**, *54*, 4313−4320.

(25) Tew, K. D.; Monks, A.; Barone, L.; Rosser, D.; Akerman, G.; Montali, J. A.; Wheatley, J. B.; Schmidt, D. E., Jr. Glutathione-associated enzymes in the human cell lines of the National Cancer Institute drug screening program. *Mol. Pharmacol*. **1996**, *50*, 149−159.
(26) Bollag, D. M.; Rozycki, M. D.; Edelstein, S. J. *Protein Methods*, 2nd ed.; John Wiley and Sons: New York, 1996; pp 62−67.
(27) Mirabelli, C. K.; Johnson, R. K.; Sung, C. M.; Faucette, L.; Muirhead, K.; Crooke, S. T. Evaluation of the in vivo antitumor activity and in vitro cytotoxic properties of auranofin, a coordinated gold compound, in murine tumor models. *Cancer Res*. **1985**, *45*, 32−39.

JM000179O