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Synthesis, Base-Catalyzed Hydrolytic Reactivity, and Anticancer Evaluation of *O*-Aryl Phosphorodiamidates as a Novel Class of Pro(phosphorodiamidic acid mustards)

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Bis(2-chloroethyl)phosphoramidic dichloride [$MP(O)Cl_2$, M = $N(CH_2CH_2Cl)_2$] has been used as the starting material for the synthesis of *O*-aryl phosphorodiamidates having the general structure MP(O)(NHR)OAr: **9**, R = H, Ar = 4-$NO_2C_6H_4$; **10**, R = H, Ar = C_6F_5; **11**, R = C_6H_5, Ar = C_6F_5; **12**, R = 4-MeC_6H_4, Ar = C_6F_5; and **13**, R = 4-$EtOC_6H_4$, Ar = C_6F_5. The phosphorodiamidic chloride precursor to **13** (**14**) was also isolated. Kinetics for the base-catalyzed hydrolysis of compounds **9**-**13** were investigated by UV and NMR methods and are considered in connection with service of these compounds as pro(phosphorodiamidic acid mustards) [MP(O)(NHR)OAr → MP(O)(NHR)OH] via an E1cB mechanism involving the intermediacy of a mustard-bearing metaphosphorodiimide [MP(O)=NR]. Anticancer screening tests against L1210 lymphoid leukemia in mice indicated that **9**-**14** are inactive; similar negative results were obtained with the KB cell culture, except in the case of **14** which was marginally active.

The cytotoxicity of nornitrogen mustard [$HN(CH_2CH_2Cl)_2$] results from DNA cross-linking by a sequence of two alkylation reactions, each involving aziridinium ion formation followed by nucleophilic ring opening.[1] Bis-alkylating activity of nitrogen mustards can be predictively moderated by delocalization of the electron pair on nitrogen, and the synthesis[2] of resonance-stabilized phosphorylated mustards [$>P(O)N(CH_2CH_2Cl)_2 \leftrightarrow >P(O^-)$-$=N^+(CH_2CH_2Cl)_2$] capable of enzymatic "activation" (P-N bond hydrolysis) in tumors represents one of the first prodrug[3] approaches to selective cytotoxicity against cancer cells. The subsequent emergence of cyclophosphamide (**1**) as a phosphorylated mustard having clinical utility was followed by studies which have discounted the originally intended mechanism of action[4] and instead support enzymatic oxidations leading to the preferential generation

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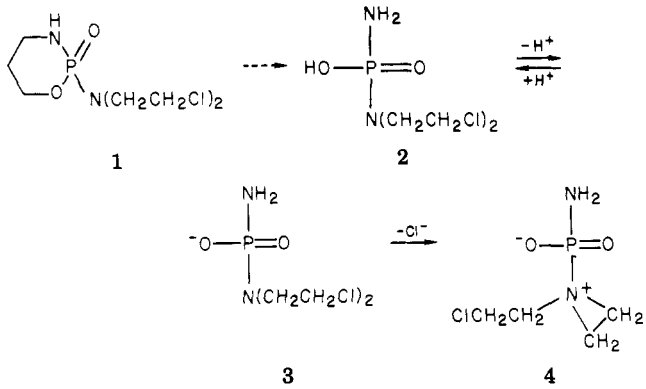
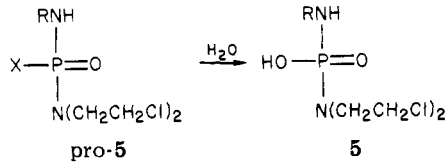
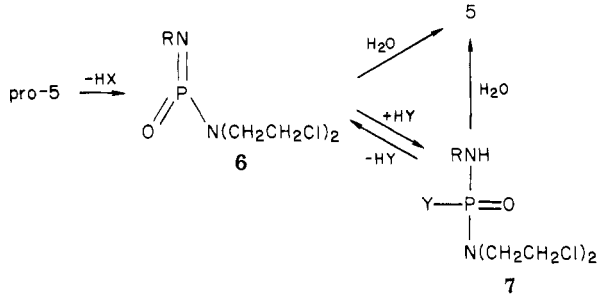


of phosphorodiamidic acid mustard **2** in cancerous vs. normal cells.[5] Potent cytotoxicity of **2** relative to its alkyl esters had been known for many years and was rationalized by Friedman[6] in terms of the negative-charge density in conjugate base **3**, which facilitates the formation of **4**. More recently, Colvin et al.[7] have convincingly demonstrated the bisalkylating capability of intact **2** and the intermediacy of **4**.

While details concerning the oncostatic selectivity of **1** are incomplete at this time, substituted analogues[8-10] and "preactivated"[11-13] versions of **1** are of current interest as alternative pro-**2** compounds which may exhibit improved anticancer activity. One of our studies in this general area has dealt with hydrolytically labile precursors to phosphorodiamidic acid mustard **5**, viz., pro-**5** systems. Pre-

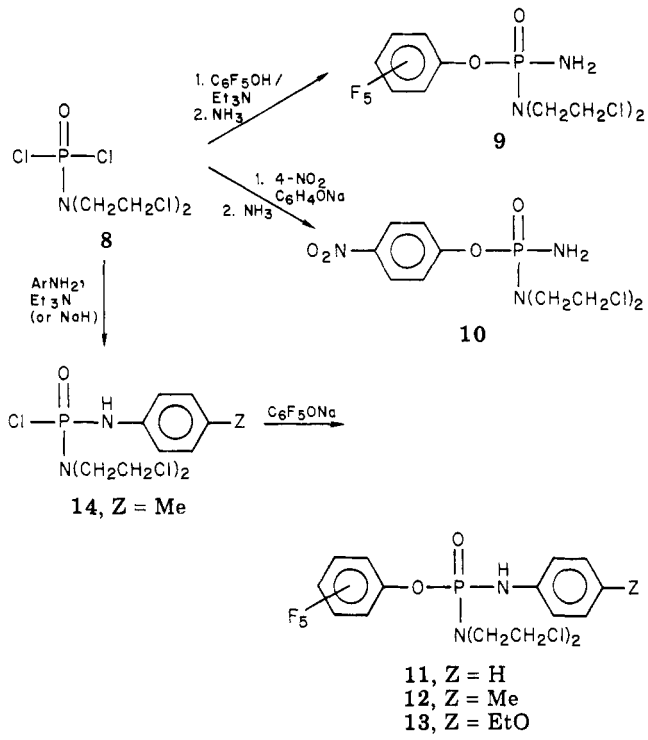


vious attempts to obtain selective hydrolysis by exploiting pH differences between normal and tumor tissue have assumed the latter to be more acidic, which led to anticancer screening of acid-sensitive promustards having diazoamino [$ArN{=}NN(CH_2CH_2F)_2$] and enamine structures [$RCH{=}CR'N(CH_2CH_2X)_2$, X = F, Cl, Ms];[14,15] however, the uniform lack of promising results suggested to us that the relatively unexplored base-catalyzed "activation" schemes with compounds like pro-**5** were reasonable options. An additional motivating factor was that basic hydrolysis of pro-**5** compounds with good leaving groups (X) is expected[16] to afford the reactive intermediate **6**, which is a mustard-bearing derivative of meta-



phosphorodiimide [$H_2NP(O){=}NH$]. Metaphosphorodiimides[17] and other cognates of metaphosphate [$HOP(O){=}O$][18] have been extensively studied with regard to their role in phosphoryl-group transfer processes, and it was of interest to evaluate the biological consequences of generating **6**. Intermediate **6** has the combined potential for reversible phosphorylation (**6** ⇄ **7**) and bisalkylation (**6** or **7** → **5**), which somewhat resembles the carbamylating

Scheme I



and alkylating capacity of nitrosourea anticancer drugs such as BCNU and CCNU.[19]

In the work reported here we have synthesized initial representatives of pro-**5** having aryloxy leaving groups (X = ArO) and either an amino (R = H) or anilino (R = Ar) functionality for hydrolytic comparisons under alkaline conditions, which served to roughly gauge structure vs. reactivity features. The results of anticancer screening tests with these new compounds are also presented.

Results and Discussion

Synthesis. A survey of hydrolysis data[16a] for various organophosphorus model systems suggested that reasonable rates for base-catalyzed conversion of pro-**5** into **5** should be obtained with X = ArO. However, earlier cytotoxicity studies[6] using the KB cell culture had shown pro-**5** with X = PhO and R = H to be at least 30-times less potent than **2** and, therefore, further suggested to us that ArO leaving groups having electron-withdrawing substituents were needed to accelerate hydrolytic formation of **5**. The use of an ArNH moiety in pro-**5** was also desired as a mechanistic probe and to control hydrolytic reactivity. Compounds **9**–**14** were thus synthesized according to Scheme I, starting from the readily available phosphoramidic dichloride **8**, and final products were isolated in 30–80% overall yield.

Hydrolytic Studies. The application of UV spectroscopy to monitor the rate of 4-nitrophenoxy ($4\text{-}NO_2\text{-}C_6H_4O^-$) displacement from organophosphorus esters akin to **9** is a convenient kinetic technique[20] that was applied to dilute solutions of **9** (ca. 0.1–0.01 mM) in unbuffered 1:1 (v/v) H_2O–EtOH containing excess NaOH (0.008–1.00 M). Linear plots for log $[(A_\infty - A)/(A_\infty - A_0)]$ vs. time were obtained with absorbance (A) values measured at 400 nm, and the derived pseudo-first-order hydrolytic rate constants (k') and half-lives ($\tau_{1/2}$) are listed in Table I.

The inductively stabilized $C_6F_5O^-$ anion was expected to significantly facilitate the hydrolysis of **10**, relative to **9**, and comparative kinetic measurements following the above procedure were next pursued. The UV spectra of $C_6F_5O^-$ vs. **10** differ primarily in the intensity of absorption

Table I. Kinetic Data for Base-Catalyzed Hydrolysis of Pro(phosphorodiamidic acid mustards)[a]

no.	concn, M	[NaOH], M	k',[b] s^{-1}	$\tau_{1/2}$, s
9	8.3×10^{-5}	8.33×10^{-3}	1.17×10^{-3}	590
	1.0×10^{-5}	2.00×10^{-2}	9.91×10^{-4}	699
	1.0×10^{-5}	4.00×10^{-2}	2.20×10^{-3}	315
	1.0×10^{-5}	1.00×10^{-1}	4.34×10^{-3}	160
	1.0×10^{-5}	5.00×10^{-1}	2.30×10^{-2}	30
	1.0×10^{-5}	7.50×10^{-1}	3.70×10^{-2}	19
	1.0×10^{-5}	1.00	4.38×10^{-2}	16
10	8.3×10^{-5}	2.08×10^{-3}	5.80×10^{-3}	120
	8.3×10^{-5}	3.33×10^{-3}	8.10×10^{-3}	80
	8.3×10^{-5}	5.00×10^{-3}	1.33×10^{-2}	52
	8.3×10^{-5}	6.66×10^{-3}	1.59×10^{-2}	43
	8.3×10^{-5}	8.33×10^{-3}	1.94×10^{-2}	36
11[c]	5.0×10^{-2}	1.00×10^{-3}	2.18×10^{-3}	318
12[c]	5.0×10^{-2}	1.00×10^{-3}	1.77×10^{-3}	392
13[c]	5.0×10^{-2}	1.00×10^{-3}	1.41×10^{-3}	490

[a] All runs refer to 1:1 (v/v) H_2O–EtOH at 26 °C, except as noted. [b] Pseudo-first-order rate constants from linear least-squares treatment of log $[(A_\infty - A)/(A_\infty - A_0)]$ vs. time at 400 nm for 9 and 290 nm for 10, except as noted. [c] 1:4 (v/v) D_2O–Me_2SO-d_2 solution containing NaOD; values of k' refer to initial (linear) portions of log $([compd]_0/[compd]_t)$ vs. time plots using relative NMR signal integrations to monitor concentration (see Figure 2 and Experimental Section).

rather than the value of λ_{max}, which contrasts with 4-$NO_2C_6H_4O^-$ vs. 9; however, the kinetics of hydrolysis could be followed at 290 nm in an acceptable manner, as judged by the linearity of the logarithmic absorbance vs. time plots leading to the k' and half-life data given in Table I.

Phosphorodiamidates bearing a labile α proton can undergo base-catalyzed hydrolysis by three distinct mechanisms: an E1cB process, which is the favored[16a,b] pathway for 9 and 10, and direct S_N2 attack at phosphorus by either hydroxide or water. A mechanistically unbiased steady-state analysis[16a] of these reaction pathways leads to the expression rate = $[ester]k^*/[1 + K_w/(K_a[OH])]$, wherein k^* is a composite parameter involving rate constants for all three mechanisms. Consequently, regardless of how a given pro-5 compound specifically partitions itself in the alkaline hydrolysis reaction manifold, the measured values of k' for pro-5 should linearly correlate with hydroxide ion concentration.[16c] Data for 9 and 10 given in Table I were plotted as $1/k'$ vs. 1/[NaOH], and the expected straight-line relationships (Figure 1) were used for extrapolation to lower hydroxide concentrations that would crudely approximate physiological pH ranges. The calculations for 9 (1×10^5 M; slope = 20.2 s/M^{-1}) indicated that this compound is relatively "inert" at pH 8–9, whereas 10 (8.3×10^{-5} M; slope = 0.41 s/M^{-1}) is estimated to have a half-life of ca. 80–8 h over this pH range.

The efficiency of α-proton removal from the RNH functionality in pro-5, which triggers formation of intermediate 6 according to the E1cB mechanism, should be responsive to the electronic nature of R. For direct conjugation of the nitrogen lone pair with a π system such as R = Ar, it was anticipated that the electron-donating 4-Me and 4-EtO substituents in 12 and 13 would disfavor pro-5 → 6 and thus decrease the overall hydrolysis rate, relative to 11. The aforementioned UV kinetic method could not be applied to compounds 11–13 due to their spectral similarity with $C_6F_5O^-$; consequently, high-resolution ^1H NMR spectroscopy was considered as a likely alternative, especially in view of its past utility in hydrolysis studies of 1[21] and related compounds.[10]

The relatively low solubility of 11–13 in the aqueous EtOH used for the kinetic study of 9 and 10 necessitated a solvent change, and 1:4 (v/v) D_2O–Me_2SO-d_6 was found

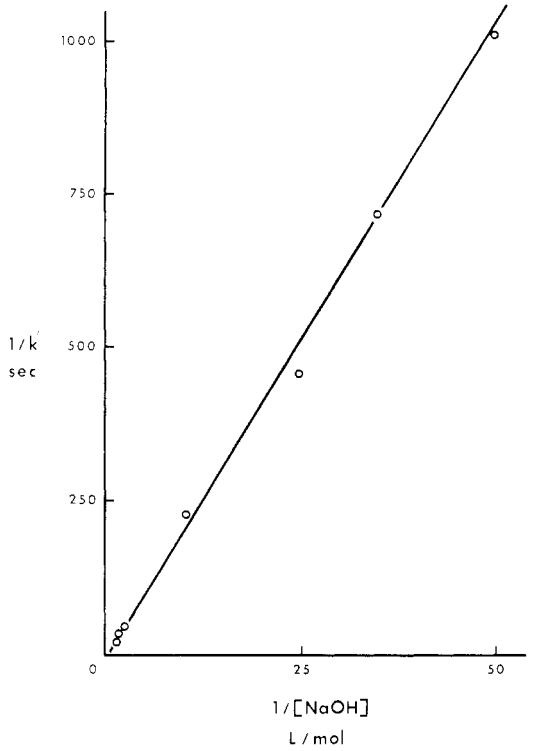


Figure 1. Hydrolysis data listed in Table I for 4-nitrophenyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (9) fitted to a linear function of hydroxide ion concentration.

to give substrate concentrations (50 mM) suitable for continuous-wave NMR measurements. Preliminary hydrolysis studies with 11–13 indicated that the mustard proton region was not amiable to quantitative analysis; however, each starting material and its respective initial hydrolysis product (5) exhibited aromatic ring proton signals that could be used to monitor the course of the reaction. Representative partial spectra obtained with tolyl compound 12 are shown in Figure 2, while less obvious spectral changes encountered with analogues 11 and 13 are summarized under the Experimental Section. Electronic signal integrations as a function of time and/or manual measurement of peak areas were used in each case to obtain standard plots of log $([ester]_0/[ester]_t)$ vs. time, t. The initial portion of each hydrolysis reaction showed acceptable linearity, while later data points led to negative curvature due to the consumption of base. Derived values of k' and $\tau_{1/2}$ are given in Table I and show the decrease in reactivity that was predicted for increased electron donation by the aryl group. Quantification of such long-range substituent electronic effects is subject to Hammett correlations of the type log $k = \rho\sigma$, where σ is an appropriate substituent parameter and ρ is the reaction constant or "sensitivity factor". The rate constants for 11–13 were analyzed in this fashion and it was found that $\rho = -0.9$. We are unaware of ρ values for suitable model reactions; however, it appears that the response of k' to aromatic ring substituents in 11–13 is approximately one-half that observed for aryloxy substituents in the E1cB hydrolysis of $(PhNH)_2P(O)OAr$ ($\rho = 1.58$).[16a]

It was desirable to compare the leaving group ability of $C_6F_5O^-$ with a more common anion, such as Cl^-; however, the relatively high solvolytic reactivity of chloro compound 14, coupled with solubility factors for this material and its initial hydrolysis product, foiled kinetic measurements by ^{31}P Fourier-transform NMR in the 20% aqueous Me_2SO solvent used for 13. The use of ^1H NMR was also investigated and it was found that no aromatic ring proton

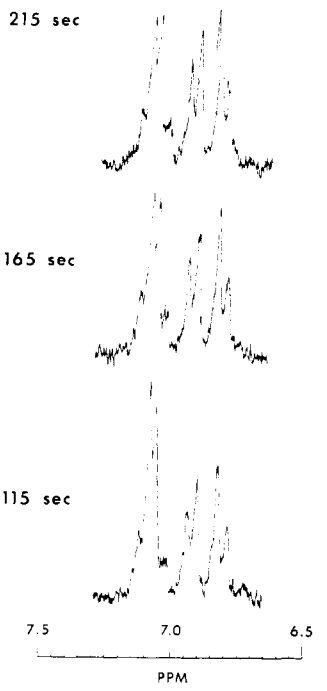


Figure 2. Continuous-wave 220-MHz ^1H NMR spectrum of the aromatic ring proton region of pentafluorophenyl *N,N*-bis(2-chloroethyl)-*N'*-(4-methylphenyl)phosphorodiamidate (**12**; initial concentration 50 mM) as a function of time at 20 °C in 1:4 (v/v) D_2O–Me_2SO-d_6 containing NaOD (initial concentration 1 mM). The two AA'BB' patterns between 7.25–7.05 and 7.05–6.80 ppm are due to **12** and hydrolysis product **5** (R = 4-MeC_6H_4). Chemical shifts are referenced to internal TSP (1%).

signals for the starting material ($[\mathbf{14}]_0$ = 25 mM) could be detected 60 s after the addition of D_2O (0.1 mL) to the Me_2SO-d_6 solution (0.7 mL), which implies a 10–20-s upper limit to the half-life of **14** under these noncatalyzed hydrolysis conditions.

Proton-catalyzed hydrolysis of **9–13**, by analogy to related compounds which have been studied,[22,23] requires substantial levels of acidity; consequently, acidic hydrolysis of these materials at physiological pH would necessitate the intervention of an enzyme. As a check for this type of hydrolytic conversion, **9** (0.3 mM) and **10** (1.5 mM) were incubated at 25 °C with orthophosphoric monoester phosphohydrolase from wheat germ in acetate buffer at pH 5.0. No P–O bond cleavage was detected by UV monitoring at 400 and 290 nm, respectively, and after 2 h in each case the addition of assay substrate (2-carboxyphenyl phosphate) was used to demonstrate that these pro-**5** compounds neither inhibited nor denatured the enzyme.

Anticancer Screening Data. The in vivo anticancer activity of compounds **9–14** was evaluated against L1210 lymphoid leukemia in either female or male mice according to the National Cancer Institute standard protocol for analogues of **1**.[24] Test samples were administered ip in an aqueous Tween-80 (polysorbate) vehicle on day 1 only using halved doses that began with 500 mg/kg and generally ended at 62.5 mg/kg of body weight. Results are evaluated on day 30, with mean survival time serving as the evaluation parameter and samples exhibiting a test/control (T/C) value equal to or greater than 125% are considered to be active in this screening system. None of the compounds in this pro-**5** series were found to be active; however, **9** and **10** appeared to be somewhat more toxic than the remainder: nontoxic doses of **9** and **10** began at 62.5 and 31.2 mg/kg, respectively, while all of the *N*-aryl samples were nontoxic even at the 500 mg/kg dose level. The in vitro test data obtained with the KB cell culture in the usual manner[24] indicated no activity for **9–13**, while the hydrolytically more reactive chloro compound **14** led to confirmed albeit low-level activity.

Conclusions. The present work has elucidated the kinetic characteristics for base-catalyzed hydrolysis of pro(phosphorodiamidic acid mustards) **9–14** and has established the degree of influence upon the reaction rate which attends remote substitution in a pendant *N*-aryl functionality. Granted that the E1cB mechanism is operative with these compounds, the resultant generation of a mustard-bearing metaphosphorodiimide intermediate (**6**) is not associated with anticancer activity in the L1210 test system. These negative results have turned our attention to other classes of potential anticancer prodrugs and such work will be reported in the future.

Experimental Section

All reactions were carried out with protection from water, and all reagents and solvents refer to anhydrous materials. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Analytical TLC utilized Analtech plates (5 × 20 cm) precoated with a 0.25-mm layer of silica gel GF, while preparative work employed 1-mm precoated plates (20 × 20 cm); component visualization was accomplished with I_2 vapor and/or UV light. ^1H NMR spectra were obtained with either a Varian A-60 or HR-220 instrument using $CDCl_3$ and Me_4Si as an internal reference, unless otherwise noted. Compounds **9–14** exhibited routine NMR spectral features which were entirely consistent with their assigned structures. ^{31}P NMR spectra were recorded at 40.25 MHz on a JEOL FX-100 spectrometer. Normal operating conditions were as follows: a 26-μs ($\pi/2$) pulse, 5-kHz spectral window and filter, 8192 data points, and exponential multiplication prior to Fourier transform so as to result in an additional 1-Hz line broadening in the frequency domain spectrum. Broad-band ^1H decoupling was continuously employed, and the resultant ambient sample temperature was estimated to be ca. 30 °C. UV spectra were obtained with a Cary 15 spectrometer using 1-cm cells. Elemental analyses were performed by Chemalytics, Inc.

Bis(2-chloroethyl)phosphoramidic dichloride (**8**) was synthesized as previously described.[9] Pentafluorophenol was used as received from Tridom/Fluka, and orthophosphoric monoester phosphohydrolyase type I from wheat germ was purchased from Sigma Chemical Co.

4-Nitrophenyl *N,N*-Bis(2-chloroethyl)phosphorodiamidate (9). A mixture of the sodium salt of 4-nitrophenol (338 mg, 2.1 mmol), **8** (520 mg, 2.0 mmol), and dibenzo-18-crown-6 (72 mg, 0.2 mmol) in Et_2O (15 mL) was stirred for 15 h at 5 °C. Solids were removed by suction filtration, and the concentrated filtrate was column chromatographed on silica gel using $CHCl_3$ solvent. Anhydrous NH_3 was bubbled for 3 h through a chilled C_6H_6 (10 mL) solution of the crude monochloro intermediate that was obtained, and filtration to remove NH_4Cl followed by solvent removal in vacuo gave **9** (70%), mp 95.9–96.5 °C, after recrystallization from C_6H_6–petroleum ether. Anal. ($C_{10}H_{14}N_3O_4PCl_2$) C, H.

Pentafluorophenyl *N,N*-Bis(2-chloroethyl)phosphorodiamidate (10). A solution of pentafluorophenol (921 mg, 5 mmol) and **8** (1.29 g, 5 mmol) in C_6H_6 (10 mL) was heated to reflux, and Et_3N (0.8 mL) was then added over a 40-min period. After 2 h of continued reflux, the Et_3N·HCl precipitate was removed by suction filtration, and anhydrous NH_3 was then bubbled through the chilled filtrate for 3 h. Removal of NH_4Cl and solvent gave **10** (65%), mp 102.5–103.5 °C (C_6H_6–petroleum ether). Anal. ($C_{10}H_{10}N_2O_2PF_5Cl_2$) C, H.

Pentafluorophenyl *N,N*-Bis(2-chloroethyl)-*N'*-phenylphosphorodiamidate (11). A suspension of PhNHNa was prepared by adding a solution of $PhNH_2$ (0.46 mL, 5 mmol) in C_6H_6 (4 mL) to magnetically stirred NaH (12 mg, 5 mmol) in chilled C_6H_6 (5 mL) over a 30-min period, and a solution of C_6F_5ONa (5 mmol) in C_6H_6 (9 mL) was then prepared in an analogous manner. The suspension of PhNHNa was added (20 min) via syringe to a chilled solution of **8** (1.30 g, 5 mmol) in C_6H_6

(5 mL), and after continued stirring for 6 h the NaCl precipitate and solvent were removed. A C_6H_6 solution (10 mL) of the residue was added to the C_6F_5ONa solution over a 30-min period and stirring was continued for 6 h at room temperature. After removal of NaCl and decolorization with charcoal (2 g), the solution was concentrated to half its volume and petroleum ether was then added to induce crystallization of **11** (30%): mp 127.5–128.5 °C; TLC R_f 0.57 (7% MeOH in C_6H_6). Anal. ($C_{16}H_{18}N_2PO_2F_5Cl_2$) C, H, N.

Pentafluorophenyl *N*,*N*-Bis(2-chloroethyl)-*N'*-(4-methylphenyl)phosphorodiamidate (12). A solution of Et_3N (0.80 mL, 5 mmol) in C_6H_6 (4 mL) was added (45 min) to a refluxing C_6H_6 (10 mL) solution of 4-methylaniline (535 mg, 5 mmol) and **8** (1.30 g, 5 mmol); reflux was continued for 2 h and the reaction was allowed to stir at room temperature for 15 h. After removal of Et_3N·HCl and solvent, the residue was diluted with C_6H_6 (10 mL), and C_6F_5ONa (5 mmol) in C_6H_6 (vide supra) was slowly added (30 min) at 5 °C. Stirring was continued at room temperature for 15 h, and removal of NaCl and solvent led to the isolation of impure crystals of **12** from C_6H_6–petroleum ether. This material was column chromatographed on silica gel using 7% MeCN in C_6H_6 as solvent (R_f 0.42), which afforded analytically pure **12** (50%), mp 114–115 °C. Anal. ($C_{17}H_{20}N_2PO_2F_5Cl_2$) C, H, N.

Repetition of the first reaction step in the above procedure gave an oily sample of **14**, ***N*,*N*-bis(2-chloroethyl)-*N'*-(4-methylphenyl)phosphorodiamidic chloride.** Careful treatment of this material with Et_2O–$CHCl_3$ led to the crystallization of sharp-melting white prisms, mp 173.5–174 °C, which, nevertheless, failed to give an acceptable elemental analysis. The identity of **14** therefore rests upon its conversion to **12**, as well as its ^1H and ^{31}P NMR spectra, the latter of which featured a single absorption at −3.47 ppm, relative to external H_3PO_4.

Pentafluorophenyl *N*,*N*-Bis(2-chloroethyl)-*N'*-(4-ethoxyphenyl)phosphorodiamidate (13). A solution of Et_3N (0.80 mL, 5 mmol) in C_6H_6 (5 mL) was added over 30 min to a refluxing solution of 4-ethoxyaniline (0.65 mL, 5 mmol) and **8** (1.32 g, 5.1 mmol) in C_6H_6 (15 mL). After additional reflux for 1 h, Et_3N·HCl and solvent were removed, and a C_6H_6 solution (15 mL) of the residue was treated at 5 °C with C_6F_5ONa (5 mmol) in C_6H_6 solvent, which had been previously prepared (vide supra). Stirring at room temperature for 18 h followed by removal of the NaCl precipitate and solvent gave crude product as crystals from C_6H_6–petroleum ether. Chromatographic purification as described above for **12** yielded **13** (80%): mp 126–127 °C; TLC R_f 0.62 (7% MeOH in C_6H_6). Anal. ($C_{18}H_{22}N_2PO_3F_5Cl_2$) C, H, N.

Kinetic Measurements. Compounds 9 and 10. The reference and sample cells of the UV spectrometer were each filled with 2.5 mL of 1:1 (v/v) H_2O–EtOH containing 1.00 M NaOH and were then allowed to thermally equilibrate (26 °C) while being irradiated with 400-nm light. A 25-μL aliquot of a stock solution of **9** (1×10^{-3} M) in the same solvent was injected into the sample cell. After rapid mixing, the absorbance (A) was continuously monitored at 400 nm until the value of A reached A_∞, which had been previously determined with the sodium salt of 4-nitrophenol under identical conditions. Variation of the concentration of NaOH and **9** together with linear least-squares analysis of $\log[(A_\infty - A)/(A_\infty - A_0)]$ vs. time plots afforded the k' values given in Table I. The same procedure was applied to **10**; however, a wavelength of 290 nm was used to monitor the progress of the reaction, and relatively small contributions due to the absorbance of starting material were neglected.

Compounds 11–13. A small amount of compound (0.05 mmol) was weighed into a glass vial and was then dissolved in Me_2SO-d_6 (0.80 mL). An aliquot (0.20 mL) of D_2O–NaOD (4.79×10^{-3} M) containing 1% TSP as internal reference was injected and the rapidly shaken sample was quickly transferred to an NMR tube, which was then inserted into the HR-220 spectrometer probe maintained at 20 °C. Continuous-wave spectra of the aromatic ring proton region were repeatedly recorded (1000-Hz sweep width, 250-s sweep time) until spectral changes were no longer discernible.

For compound **11**, the sum of the integrated signal intensity for the two meta protons (7.02 ppm, t) in **11** and that for the two ortho protons (6.63 ppm, d) in the hydrolysis product remained constant, which therefore represented a relative measure of $[11]_0$. The intensity of the meta protons in **11** was taken as a measure of $[11]_t$. Linear least-squares analysis of a $\log([11]_0/[11]_t)$ vs. time plot gave k' listed in Table I.

For compound **12**, the entire AA'BB' absorption pattern for the aromatic ring protons was anisochronous with the AA'BB' pattern of the hydrolysis product (see Figure 2). The sum of the integrated signal intensities of these multiplets remained constant and thereby allowed for a kinetic analysis like that described for **11**.

For compound **13**, the sum of the integrated signal intensity of the low-field portion (7.45 ppm, apparent d, 2 H) of the aromatic ring proton pattern for **13** and the high-field portion (7.06 ppm, apparent d, 2 H) of the aromatic ring proton pattern for the hydrolysis product remained constant. The remaining aromatic ring protons from both **13** and the hydrolysis product were accidentally isochronous (7.20 ppm, apparent d). Extraction of k' from the aforementioned nonoverlapping spectral portions was thus analogous to the case with **11**. A duplicate run indicated the precision to be ca. 5%.

Enzyme Studies. Assay for enzyme activity toward 2-carboxyphenyl phosphate gave 2.1 units/mg. An aliquot (0.5 mL) of an aqueous solution of the enzyme (0.1 mg/mL) was added to the sample cell which contained an aliquot (0.5 mL) of an aqueous solution of **9** (1.2 mM) and an aliquot of buffer (2.0 mL of 0.15 M NaOAc, pH 5.0). The reference cell was identical with the sample cell, except that it lacked enzyme. After 2 h at 26 °C, no absorbance for $4\text{-}NO_2C_6H_4O^-$ was detected at 400 nm. Addition of 2-carboxyphenyl phosphate to the sample cell at this time led to measurements which confirmed the enzyme's original level of activity.

Repetition of the above procedure with **10** (1.76 mg) required the use of acetate buffer (2.5 mL) and glycerin (0.5 mL) to achieve a sufficient concentration of substrate (1.5 mM). After 2 h no absorbance for $C_6F_5O^-$ was detected at 290 nm, and subsequent addition of 2-carboxyphenyl phosphate was again used to confirm enzyme activity.

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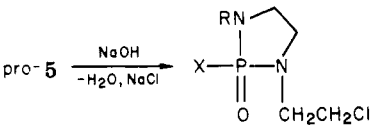
mechanisms of base-catalyzed hydrolysis, pro-5 representatives 9–13 are unique in that deprotonation of the amidic position can be followed by intramolecular cyclization via displacement of chlorine in the mustard group (pro-5 → i), which is a reaction mode having precedence in the



i

chemistry known for 1.[21] In addition, these compounds offer the possibility for direct displacement of chlorine by hydroxide. Our unpublished ^{31}P NMR kinetic studies of 2 [T. W. Engle, G. Zon, and W. Egan] have indicated a half-life of ca. 15 min (pH ≥7, 37 °C) for intramolecular chlorine displacement to aziridinium ion 4 and, consequently, monitoring the rate of chloride production during the present studies of 9–13 was not pursued as mechanistically informative data. In any event, the aforementioned considerations do not substantially affect the composite nature of k^* and the linear correlation with k'.

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Alkylating Nucleosides. 2.[1] Synthesis and Cytostatic Activity of Bromomethylpyrazole and Pyrazole Nitrogen Mustard Nucleosides

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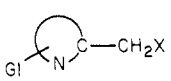
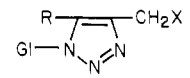
Glycosylation of ethyl 3(5)-(bromomethyl)pyrazole-5(3)-carboxylate (3) and 3(5)-(bromomethyl)pyrazole-5(3)-carboxamide (4) with poly-*O*-acetylated sugars via an acid-catalyzed fusion method afforded the corresponding ethyl 3-(bromomethyl)pyrazole-5-carboxylate and 3-(bromomethyl)pyrazole-5-carboxamide substituted nucleosides 5 and 7, respectively. In some cases, the positional isomers 6 and 8 were also obtained. Treatment of 5 and 7 with methanolic ammonia gave the deprotected 3-(aminomethyl)pyrazole-5-carboxamide nucleosides 9. Reaction of 3–5 and 7 with bis(2-chloroethyl)amine led to the corresponding pyrazole nitrogen mustards 10–13. All the bromomethylpyrazole nucleosides described showed significant cytostatic activity against HeLa cell cultures.

With the aim of obtaining new types of possible anticancer drugs, we have in our laboratories several ongoing research programs concerning the synthesis, cytostatic evaluation, and mode of action of *N*-glycosyl heterocyclic compounds in which a halomethyl group, as alkylating moiety, is attached to the heteroaromatic ring (1).



1, X = halogen
Gl = glycosyl

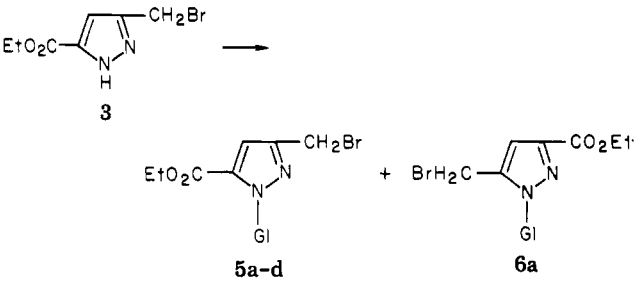


2, X = halogen
R = H or CH_2X
Gl = glycosyl

In the first paper of this series,[1] the synthesis of several *N*-glycosylhalomethyl-1,2,3-triazoles, 2, by cycloaddition of glycosyl azides to propargyl halides or by halogenation of the corresponding *N*-glycosylhydroxymethyl-1,2,3-triazoles was reported. These and other halomethyl-1,2,3-triazole nucleosides, having different sugar residues, were evaluated as cytostatic agents.[1,2] In general, all those bromo- and iodomethyl derivatives showed significant "in vitro" activities. 4-(Bromomethyl)- and 4-(iodomethyl)-1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2,3-triazole (2, R = H, Gl = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) were also effective against ECA and P388 tumor systems. A study on the mode of action of these two latter compounds has demonstrated that they really act as alkylating agents.[2]

We now report the synthesis and cytostatic activity of nucleosides of ethyl 3(5)-(bromomethyl)pyrazole-5(3)-carboxylate and 3(5)-(bromomethyl)pyrazole-5(3)-carboxamide (3 and 4) via an acid-catalyzed fusion method. We also describe the preparation of *N*-glycosyl-3-(aminomethyl)- and *N*-glycosyl-3-[[bis(2-chloroethyl)amino]-methyl]pyrazoles by transformation of the corresponding *N*-glycosyl-3-(bromomethyl) derivatives.

Scheme I



a, Gl = 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl
b, Gl = 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl
c, Gl = 2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl
d, Gl = 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl

Chemistry. The bromomethylpyrazoles 3 and 4 were prepared by treating ethyl 3(5)-(hydroxymethyl)pyrazole-5(3)-carboxylate and 3(5)-(hydroxymethyl)pyrazole-5(3)-carboxamide, respectively,[3] with phosphorus tribromide.

Fusion of 3 with penta-*O*-acetyl-β-D-glucopyranose in the presence of *p*-toluenesulfonic acid provided a mixture of

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