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ACKNOWLEDGMENTS

The authors are grateful to the Scottish Hospital Endowments Research Trust for financial support.

Synthesis, Hydrolytic Reactivity, and Anticancer Evaluation of *N*- and *O*-Triorganosilylated Compounds as New Types of Potential Prodrugs

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Received April 24, 1981, from the *Department of Chemistry, The Catholic University of America, Washington, DC 20064*, the **Mid-Atlantic Research Institute, Bethesda, MD 20014*, and the ‡*Bureau of Biologics, Food and Drug Administration, Bethesda, MD 20205.* Accepted for publication August 6, 1981.

Abstract □ *N*- and *O*-Triorganosilylated compounds related to various anticancer agents were synthesized for evaluation as potential anticancer prodrugs. ^{1}H-NMR and UV kinetic measurements of hydrolytic desilylation were used to correlate relative rates of structural unmasking with steric bulk about the silicon reaction center. The *tert*-butyldimethylsilyl ester of chlorambucil and a number of *O*-triorganosilylated carbamate derivatives of nor-nitrogen mustard showed significant activity against P-388 lymphocytic leukemia in mice.

Keyphrases □ Prodrugs—*N*- and *O*-triorganosilylated compounds, synthesis, potential anticancer prodrug □ Anticancer agents—synthesis of *N*- and *O*-triorganosilylated compounds, potential anticancer prodrugs □ Triorganosilylated compounds—synthesis and evaluation of potential anticancer prodrugs

In an earlier investigation of potential anticancer prodrugs (1), it was found that *O*-aryl-*N*,*N*-bis(2-chloroethyl)phosphorodiamidates are resistant toward chemical activation involving P-OAr hydrolysis and failed to provide evidence for *in vivo* formation of phosphoramide mustards, which are usually cytotoxic and may exhibit anticancer activity (2). The hydrolytic lability (3) of Si—N and Si—O bonds suggested that strategically triorganosilylated derivatives of known oncostatic agents might constitute a class of compounds which are, for kinetic reasons, more suitable candidates for anticancer prodrugs. The possibility of controlling drug unmasking rates (desilylation) by manipulating the nature of the silicon reaction center represents an interesting feature of these hypothetical compounds. The expected hydrolysis byproducts, namely triorganosilanols and disiloxanes, are generally nontoxic (4). Derivatization with a triorganosilyl group increases lipophilicity; consequently, triorganosilyl prodrugs might eventually prove to be useful against central nervous system cancers, which apparently require somewhat more lipophilic chemotherapeutic agents for effective penetration of the blood–brain barrier (5).

In view of the widespread interest in the design of anticancer prodrugs (6, 7) and the development of organosilicon compounds as medicinal agents (8), the aforementioned proposal is unique in that it encompasses both of these growing research areas. This report is the first of a series of exploratory investigations of hydrolytically labile *N*- and *O*-triorganosilyl prodrugs having potential anticancer activity. These studies include the synthesis of several new classes of nitrogen mustards, measurement of hydrolytic desilylation rates by a combination of ^{1}H-NMR and UV methods, examination of the hydrolysis mechanisms by Hammett-type kinetic studies and ^{18}O-labeling, and the comparison of screening results obtained with experimental cancers in mice.

EXPERIMENTAL

NMR refers to ^{1}H-NMR at 60 MHz, except as noted; chemical shifts refer to deuterochloroform and are relative to internal tetramethylsilane, unless specified otherwise. ^{31}P-NMR spectra were recorded at 40.25 MHz using a $\pi/2$ pulse (13 μsec) and a 2-sec repetition time. All organosilicon starting materials were commercially available and were checked for purity by NMR; if necessary, further purification was achieved by either conventional distillation or recrystallization. All handling and reactions of organosilicon compounds were performed under an atmosphere of dry nitrogen; all reagents and solvents were anhydrous. Satisfactory elemental analyses were not always possible, due to hydrolytic reactivity. However, each product was reliably characterized by NMR as well as IR (9–11) and/or mass spectroscopy. In all cases, NMR signal integrations established that product purity was >90%. Electron-impact mass spectra were scanned from samples introduced *via* a solids' direct probe inlet. The probe, when loaded with a sample and properly positioned with respect to the ion source, was heated at 100°/min from ambient temperature to a final temperature of 320°. The ion source temperature was 180°, the ionizing potential was 70 eV, and the ionizing current was 50 μA. Analytical thin-layer chromatography (TLC) employed 2.5 × 10-cm plates coated with a 250-μm layer of silica gel containing a fluorescent indicator; component visualization was achieved with iodine vapor and/or a short wavelength UV lamp. Column chromatography utilized 60–200 mesh silica gel, which was dried by heating at 150° for 24 hr and then cooling to room temperature under nitrogen.

All compounds having a bis(2-chloroethyl)amino functionality are potentially toxic and/or mutagenic and should be handled with extreme care.

***O,O*-Dimethyl-*N,N*-bis(2-chloroethyl)phosphoramidate (III)** —Lithium methoxide was prepared fresh by slowly adding a benzene solution (10 ml) of methanol (6.5 ml) to a mixture of benzene (10 ml) and sliced lithium wire (15 cm, 6.11 mmoles/cm) at 25°. After an additional 3 hr of stirring, a solution of I (12) (10.4 g, 40 mmoles) in benzene (40 ml)

0022-3549/82/0500-0542$01.00/0

was added over a 30-min period. 2,3,11,12-Dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene (0.3 g) was added and stirring was continued overnight at 25°. After separation of lithium chloride by suction filtration and evaporation of solvent from the filtrate, the yellow residue was chromatographed on a column (40 × 4 cm) of dry silica gel using chloroform–methanol (24:1) as eluent; R_f = 0.60. Evaporation of solvent gave III as a pale yellow oil (75%) which was used without further purification. NMR: δ 3.82 (d, 6H, 2-CH_3) and 3.52 (m, 8H, 4-CH_2) ppm; mass spectrum: *m/z* 249 (M^+, 2-Cl).

***O,O*-Bis(trimethylsilyl)- *N,N*- bis(2-chloroethyl)phosphoramidate (IV)**—A mixture of III (0.26 g, 1 mmole), sodium iodide (0.45 g, 3 mmoles), and acetonitrile (5 ml) was treated dropwise with a solution of trimethylchlorosilane (0.35 g) in acetonitrile (3 ml) over 20 min. After 3 hr of continued stirring, followed by solvent evaporation, the residue was extracted with carbon tetrachloride (5 ml) to give IV (85%), which is extremely sensitive to atmospheric moisture and hydrolyzes to give hexamethyldisiloxane. NMR: δ 3.43 (m, 8H, 4-CH_2) and 0.28 [s, 18H, 2-Si(CH_3)$_3$] ppm.

***O,O*-Bis(*tert*-butyldimethylsilyl) -*N,N*- bis(2-chloroethyl)-phosphoramidate (V)**—Reaction of III and *tert*-butyldimethylchlorosilane in the presence of sodium iodide according to the identical procedure described for IV gave product V (56%) as a pale yellow oil. Attempts to decolorize this material by either preparative TLC or column chromatography on dry silica gel led to hydrolysis of the *tert*-butyldimethylsilyl groups, as evidenced by isolation of the corresponding triorganosilanol. NMR: δ 3.42 (m, 8H, 4-CH_2), 0.90 [s, 18H, 2-C(CH_3)$_3$], and 0.23 [s, 12H, 2-Si(CH_3)$_2$] ppm. For V (45 mg, 0.1 mmole) and tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium (156 mg, 0.13 mmole) in deuterochloroform (1 ml), the expected resonance doubling of enantiotopic groups (13) was observed and diastereotopic methyl groups in V were resolved: δ 2.37 [s, 9H, C(CH_3)$_3$], 2.09 [s, 9H, C(CH_3)$_3$], 1.97 (s, 3H, SiCH_3), 1.87 (s, 3H, SiCH_3), 1.52 (s, 3H, SiCH_3), and 1.35 (s, 3H, SiCH_3) ppm. IR (neat): 2950, 2900, and 2875 (C—H), 1366 [C(CH_3)$_3$], 1250 (P=O), and 1045 (Si—O) cm^{-1}.

***O,O*-Bis(diphenylmethylsilyl) -*N,N*- bis(2-chloroethyl)phosphoramidate (VI)**—Compound VI was synthesized (60%) from diphenylmethylchlorosilane and III by the same procedure described for IV. Purification of the crude product was achieved by rapid elution from dry silica gel using carbon tetrachloride eluent to remove 1,3-dimethyl-1,1,3,3-tetraphenyldisiloxane (R_f = 0.5, carbon tetrachloride) and then ether to collect VI (R_f = 0.85, ether) as a colorless oil. NMR: δ 7.44 (m, 20H, 4-C_6H_5), 3.15 (m, 8H, 4-CH_2), and 0.67 (s, 6H, 2-SiCH_3) ppm. For VI (0.1 *M*) and tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium (0.13 *M*) in deuterochloroform, the expected resonance doubling of enantiotopic groups was observed: δ 0.95 (s, 3H, SiCH_3) and 0.82 (s, 3H, SiCH_3) ppm. IR (neat): 3090, 3060, and 3035 (C_6H_5), 2950 (C—H), 1270 (P=O), 1050 (Si—O), and 760 and 700 (C_6H_5) cm^{-1}.

***O,O*-Bis(*tert*-butyldiphenylsilyl) -*N,N*- bis(2-chloroethyl)-phosphoramidate (VII)**—Reaction of *tert*-butyldiphenylchlorosilane and III in a manner identical to that described above for the preparation of IV was followed by column chromatography using dry silica gel, eluting first with carbon tetrachloride to remove *tert*-butyldiphenylsilanol (R_f = 0.3, carbon tetrachloride), and then with ether to remove VII (90%, R_f = 0.81, ether). NMR: δ 7.56 (m, 20H, 4-C_6H_5), 3.06 (m, 8H, 4-CH_2), and 1.14 [s, 18H, 2-C(CH_3)$_3$] ppm. For VII (0.1 *M*) and tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium (0.13 *M*) in deuterochloroform, the expected resonance doubling of enantiotopic groups was observed: δ 1.33 [s, 9H, C(CH_3)$_3$] and 0.95 [s, 9H, C(CH_3)$_3$] ppm. IR (neat): 3055 (C_6H_5), 2950 and 2850 (C—H), 1375 [C(CH_3)$_3$], 1255 (P=O), 1130 (Si—O), and 740 and 700 (C_6H_5) cm^{-1}.

***O* - Methyl -*N,N* - bis(2 - chloroethyl)phosphorodiamidate (VIII)**—A solution of methanol (1 ml, 24.7 mmoles) in benzene (5 ml) was added (20 min) to a stirred suspension of sliced lithium wire (3.6 cm, 22 mmoles) in benzene (5 ml) at 25°, and after 4 hr it appeared that all of the metal had reacted. The resultant suspension of lithium methoxide was added dropwise by syringe to a chilled mixture of I (5.2 g, 20 mmoles) and 2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene (0.1 g) in benzene (30 ml). After 5 hr of stirring at 25°, lithium chloride was removed by suction filtration and the filtrate containing intermediate II was diluted with benzene (18 ml). Dry ammonia was bubbled through the stirred filtrate at 5° until precipitation of ammonium chloride was complete. After separation of the precipitate and removal of solvent *in vacuo*, the residue was chromatographed on a dry silica gel column (40 × 4 cm) using methanol–chloroform (5:95, 250 ml). The concentrated eluate was dissolved in benzene (8 ml) and diluted with low-boiling petroleum ether until turbid. Refrigeration afforded pure VIII (51%), mp 75–78°. NMR: δ 3.84 [d ^3J(PH) = 11 Hz, 3H, CH_3], 3.80–3.35 (m, 8H, 4-CH_2), and 3.00 (broad, 2H, NH_2) ppm. IR (mull): 3403 and 3268 (NH_2), 1362 (OCH_3), and 1218 (P=O) cm^{-1}.

Anal.—Calc. for $C_5H_{13}N_2O_2PCl_2$: C, 25.55; H, 5.58; N, 11.92. Found: C, 25.49; H, 5.64; N, 11.91.

***O*-(*tert*-Butyldimethylsilyl) -*N,N*- bis(2-chloroethyl)phosphorodiamidate (X)**—A stirred mixture of VIII (0.94 g, 4 mmoles), sodium iodide (0.9 g, 6 mmoles), 2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene (0.15 g), and acetonitrile (15 ml) at 25° was treated dropwise (30 min) with a solution of *tert*-butyldimethylchlorosilane (1.1 g, 7 mmoles) in acetonitrile (10 ml). After 5 hr, solvent was removed *in vacuo* and the residue was extracted with carbon tetrachloride (15 ml). Removal of carbon tetrachloride *in vacuo* gave X as a viscous pale yellow oil (70%). Attempts to further purify X by flash chromatography on dry silica gel led to hydrolysis of the *tert*-butyldimethylsilyl group, as evidenced by isolation of the corresponding silanol. NMR: δ 3.56 (m, 8H, 4-CH_2), 3.40 (d, 2H, NH_2), 0.92 [s, 9H, C(CH_3)$_3$], and 0.25 [s, 6H, Si(CH_3)$_2$] ppm. IR (neat): 3350 (NH_2), 2980 and 2950 (C—H), 1351 [C(CH_3)$_3$], 1260 (P=O), and 1100 (Si—O) cm^{-1}.

***O*-Trimethylsilyl-*N,N*-bis(2 - chloroethyl)phosphorodiamidate (IX)**—The synthetic procedure was analogous to that described above for X, and yielded IX as a colorless oil (87%). Compound IX is extremely sensitive to atmospheric moisture and rapidly hydrolyzes to give hexamethyldisiloxane, which was identified in partially decomposed samples of IX by chemical shift comparisons with authentic material. NMR: δ 3.55 (m, 8H, 4-CH_2), 3.39 (d, 2H, NH_2), and 0.30 [s, 9H, Si(CH_3)$_3$] ppm. IR (neat): 3324 (NH_2), 2975, 2932, and 2872 (C—H), 1238 (P=O), and 1121 (Si—O) cm^{-1}.

***O*-(*tert*-Butyldiphenylsilyl) -*N,N*- bis(2-chloroethyl)phosphorodiamidate (XI)**—Reaction of VIII and *tert*-butyldiphenylchlorosilane in exactly the same manner as that described above for the synthesis of X afforded crude material, which was chromatographed on a column (40 × 2 cm) of dry silica gel using carbon tetrachloride (100 ml) to first remove *tert*-butyldiphenylsilanol (R_f = 0.3, carbon tetrachloride). Compound XI (65%) was then eluted with ether (150 ml); R_f = 0.78, ether. NMR: δ 7.57 (m, 10H, 2-C_6H_5), 3.49 (m, 8H, 4-CH_2), 3.29 (d, 2H, NH_2), and 1.12 [s, 9H, C(CH_3)$_3$] ppm. IR (neat): 3447 and 3278 (NH_2), 3094 and 3075 (C_6H_5), 2981 and 2895 (C—H), 1372 [C(CH_3)$_3$], 1230 (P=O), 1111 (Si—O), and 743 and 698 (C_6H_5) cm^{-1}.

Bis(2-chloroethyl)amine (nor-nitrogen mustard)—A stirred mixture of bis(2-chloroethyl)amine hydrochloride (35.7 g, 0.2 mole) in ice-water (100 ml) and ether (100 ml) was titrated rapidly with aqueous sodium hydroxide (1 *N*), using phenolphthalein indicator. The ether layer was separated and the aqueous layer was extracted quickly three times with ether (80 ml). The combined ether layer and washings were dried with magnesium sulfate (4 g) at 5° and then carefully concentrated on a rotary evaporator. Rapid Kugelrohr distillation of the residue gave (94%) bis(2-chloroethyl)amine[1] as a colorless oil, bp 46–50° (3 mm). NMR: δ 3.44 (t, 4H, 2-CH_2Cl), 2.77 (t, 4H, 2-NCH_2), and 1.82 (s, 1H, NH) ppm. IR (neat): 3348 (NH) cm^{-1}.

***N*-Trimethylsilyl-*N,N*-bis(2-chloroethyl)amine (XII)**—A solution of trimethylchlorosilane (30 ml, 125 mmoles) in ether (30 ml) was added dropwise (30 min) to a stirred solution of bis(2-chloroethyl)amine (7.1 g, 50 mmoles) and triethylamine (8.4 ml, 55 mmoles) in ether (150 ml) at 5°. After stirring for 15 hr at 25° and then removal of solvent *in vacuo*, triethylamine hydrochloride was separated by suction filtration. Fractional distillation of the filtrate gave (90%) XII as a colorless oil, bp 45–47° (1 mm). Compound XII decomposes relatively slowly at room temperature, but is extremely reactive toward hydrolysis of the trimethylsilyl group to give hexamethyldisiloxane. The presence of this disiloxane in partially hydrolyzed samples of XII is evidenced by chemical shift comparisons with authentic material. NMR δ 3.65 (m, 8H, 4-CH_2) and −0.06 [s, 9H, Si(CH_3)$_3$] ppm. IR (neat): 2980 and 2893 (C—H), 1262 (SiCH_3), and 980 (Si—N) cm^{-1}.

***N*-(*tert*-Butyldimethylsilyl) -*N,N*- bis(2-chloroethyl)amine (XIII)**—Bis(2-chloroethyl)amine (7.1 g, 50 mmoles), *tert*-butyldimethylchlorosilane (9.0 g, 60 mmoles), and triethylamine (9.2 ml) were refluxed in ether (150 ml) for 5 hr. After cooling, triethylamine hydrochloride was removed by suction filtration and fractional distillation of the filtrate led to isolation (83%) of XIII as a colorless oil, bp 58–60° (2 mm), which is best stored at low temperatures with complete exclusion of moisture. NMR: δ 3.95 (t, 4H, 2-CH_2Cl), 3.29 (t, 4H, 2-NCH_2), 1.20 [s, 9H, C(CH_3)$_3$], and 0.03 [s, 6H, Si(CH_3)$_2$] ppm. IR (neat): 2980, 2946, and 2863 (C—H), and 960 (Si—N) cm^{-1}.

[1] Bis(2-chloroethyl)amine undergoes rapid intramolecular alkylation in polar solvents such as water, but may be stored in ether at low temperatures for short periods of time.

Table I—Hydrolysis Kinetic Data [a]

Compound	Initial Concentration, *M*	pH[b]	Method[c]	k'[d], sec^{-1}	$\tau_{1/2}$[e], min
V	1.8×10^{-2}	7.4	NMR	3.00×10^{-3}	3.8
X	1.5×10^{-2}	7.4	NMR	4.02×10^{-4}	28.6
XV	2.3×10^{-2}	7.4	NMR	6.37×10^{-4}	18.1
	2.7×10^{-2}	6.4	UV (226 nm)	4.00×10^{-5}[f]	
	2.7×10^{-2}	7.4	UV (226 nm)	3.03×10^{-4}[f]	
	2.7×10^{-2}	8.4	UV (226 nm)	5.12×10^{-4}[f]	
XVI	2.3×10^{-2}	7.4	NMR	4.26×10^{-4}	27.0
	1.0×10^{-3}	7.4	UV (260 nm)	4.23×10^{-4}	27.2
XVII	1.0×10^{-3}	7.4	UV (260 nm)	3.37×10^{-4}	34.2
XVIII	2.1×10^{-2}	7.4	NMR	8.66×10^{-4}	13.3
	1.0×10^{-3}	7.4	UV (260 nm)	9.00×10^{-4}	12.8
XXVI	3.0×10^{-3}	6.4	NMR	—[g]	
	3.0×10^{-3}	7.4	NMR	1.07×10^{-4}	1.08
	3.0×10^{-3}	8.4	NMR	1.1×10^{-4}	1.14

[a] Compound V was studied in a dioxane–0.05 *M* lutidine buffer (pH 7.4) mixture (60:40), all other NMR studies were similarly carried out using a tromethamine buffer component. All UV studies refer to a dioxane–0.10 *M* tromethamine buffer mixture (50:50). In all cases the temperature was 27 ± 1°. [b] The indicated pH value refers to the aqueous buffer component prior to mixing with dioxane. [c] See text and Experimental section for details. [d] Pseudo first-order rate constant for desilylation, except as noted. [e] $\tau_{1/2} = \ln 2/k'$. [f] Refers to carbonate production following desilylation. [g] Not determined; the reaction was too fast to monitor by the NMR method.

***N*-Aryldimethylsilyl-*N*,*N*-bis(2-chloroethyl)amines**—The procedure described above for compound XII was applied to a series of aryldimethylchlorosilanes and afforded (80–90%) the following products, which were purified by fractional vacuum distillation and identified by NMR (CCl_4): *p*-methoxyphenyl (45–46°, 2 mm), δ 7.20 (broad s, 4H, C_6H_4), 3.60 (s, 3H, OCH_3), 3.50 (m, 8H, 4-CH_2), and 0.13 [s, 6H, $Si(CH_3)_2$]; *p*-tolyl (43–44°, 2 mm), δ 7.40 (broad s, 4H, C_6H_4), 3.60 (m, 8H, 4-CH_2), 2.50 (s, 3H, CH_3), and 0.13 [s, 6H, $Si(CH_3)_2$]; phenyl (40–42°, 2 mm), δ 7.30 (m, 5H, C_6H_5), 3.40 (m, 8H, 4-CH_2), and 0.08 [s, 6H, $Si(CH_3)_2$], *p*-fluorophenyl (41–43°, 2 mm), δ 7.30 (m, 4H, C_6H_4), 3.50 (m, 8H, 4-CH_2), and 0.07 [s, 6H, $Si(CH_3)_2$]; and *p*-chlorophenyl (45–46°, 2 mm), δ 7.40 (m, 4H, C_6H_4), 3.50 (m, 8H, 4-CH_2), and 0.06 [m, 6H, $Si(CH_3)_2$] ppm.

***O*-Trimethylsilyl-*N*,*N*-bis(2-chloroethyl)carbamate (XIV)**—A cold (−10°) solution of XII (2 g, 11 mmoles) in ether (10 ml) and pentane (35 ml) containing bis(2-chloroethyl)amine (0.16 g) as a required catalyst was reacted with anhydrous carbon dioxide gas for 4 hr. The progress of the reaction was monitored by IR with diminishing intensity of the Si—N absorption at 980 cm^{-1} and concomitant increase of the C=O absorption at 1748 cm^{-1} indicating the extent of product formation. Precipitated material was removed by suction filtration, and fractional distillation of the filtrate gave (90%) XIV as a colorless oil, bp 56–57° (1 mm). NMR: δ 3.30 (m, 8H 4-CH_2) and 0.03 [s, 9H, $Si(CH_3)_3$] ppm. IR (neat): 2970, 2933, and 2872 (C—H), 1748 (C=O), 1270 ($SiCH_3$), and 1160 (Si—O) cm^{-1}.

***O*-(*tert*-Butyldimethylsilyl)-*N*,*N*-bis(2-chloroethyl)carbamate (XV)**—Compound XV, bp 82–84° (2 mm), was prepared and isolated (65%) in a manner identical to that described above for XIV. Attempted distillation of XV at somewhat higher pressure and temperature (~100°) led to violent decomposition. NMR: δ 3.55 (broad s, 8H, 4-CH_2), 0.92 [s, 9H, $C(CH_3)_3$], and 0.22 [s, 6H, $Si(CH_3)_2$] ppm. IR (neat): 2981, 2950, and 2880 (C—H), 1750 (C=O), 1380 [$C(CH_3)_3$], 1258 ($SiCH_3$), and 1161 (Si—O) cm^{-1}.

***O*-(*tert*-Butyldiphenylsilyl)-*N*,*N*-bis(2-chloroethyl)carbamate (XVII)**—A solution of *tert*-butyldiphenylchlorosilane (27.5 g, 0.1 mole) in ether (30 ml) was added dropwise (30 min) to a cold (5°) solution of bis(2-chloroethyl)amine (12.6 g, 0.11 mole) and triethylamine (16.8 ml) in ether (270 ml). After 3 hr of stirring at 25°, the reaction mixture was cooled to −10° and (without filtration) anhydrous carbon dioxide was bubbled through it for 4 hr. Precipitated material was separated by suction filtration and ether was removed from the filtrate by fractional distillation. The residue obtained by further concentration *in vacuo* (25°, 0.1 mm, 18 hr) was extracted with benzene (25 ml), leaving undissolved material that was removed by suction filtration. Low-boiling petroleum ether was added to the filtrate until turbidity was apparent, and the solution was then stored in a freezer. Compound XVII was isolated either as an oil or a low-melting solid, mp 40–45°. NMR: δ 7.63 (m, 10H, 2-C_6H_5), 3.76 (m, 8H, 4-CH_2), and 1.22 [s, 9H, $C(CH_3)_3$] ppm. IR (neat): 3106 and 3071 (C_6H_5), 2993, 2951, and 2891 (C—H), 1715 (C=O), 1391 [$C(CH_3)_3$], 1255 ($SiCH_3$), 1121 (Si—O), and 744 and 702 (C_6H_5) cm^{-1}.

***O*-Diphenylmethylsilyl-*N*,*N*-(2-chloroethyl)carbamate(XVI)**—The same procedure described above for the synthesis of XVII gave (75%) XVI as a colorless oil, which was isolated upon cooling the benzene–petroleum ether solution of the crude product. NMR: δ 7.42 (m, 10H, 2-C_6H_5), 3.40 (t, 4H, 2-CH_2Cl), 2.70 (t, 4H, 2-NCH_2), and 0.45 (s, 3H, $SiCH_3$) ppm. IR (neat): 3092, 3075, and 3030 (C_6H_5), 1748 (C=O), 1254 ($SiCH_3$), 1122 (Si—O), and 732 and 698 (C_6H_5) cm^{-1}.

***O*-Tribenzylsilyl-*N*,*N*-bis(2-chloroethyl)carbamate XVIII**—Compound XVIII was synthesized according to essentially the same procedure as that described above for XVI, except that the reaction mixture was treated with gaseous carbon dioxide for 7 hr at −10°. Crystallization of the crude product from benzene–petroleum ether gave pale yellow crystals, mp 106–108°. NMR: δ 6.95 (m, 15H, 3-C_6H_5), 3.51 (m, 8H, 4-CH_2), and 2.00 (s, 6H, 3-$SiCH_2$) ppm. IR (mull): 3070 (C_6H_5), 1716 (C=O), 1155 (Si—O), and 722 and 685 (C_6H_5) cm^{-1}.

***O*-Triethoxysilyl-*N*,*N*-bis(2-chloroethyl)carbamate (XIX)**—The synthetic procedure described above for compound XVII yielded (75%) XIX as a colorless oil, which was isolated upon cooling the benzene–petroleum ether solution of the crude product. NMR: δ 4.06–3.55 (m, 14H, 2-NCH_2CH_2Cl and 3-OCH_2) and 1.25 (t, 9H, 3-CH_3) ppm. IR (neat): 2995 and 2896 (C—H), 1685 (C=O), and 1162 (Si—O) cm^{-1}.

***O*-Aryldimethylsilyl-*N*,*N*-bis(2-chloroethyl)carbamates**—The same procedure described above for compound XIV was applied to each member of the series of *N*-aryl-*N*,*N*-bis(2-chloroethyl)amines and afforded (50–80%) the following products, which were purified by fractional vacuum distillation and identified by NMR (CCl_4): *p*-methoxyphenyl (40–41°, 0.3 mm), δ 6.80 (m, 4H, C_6H_4), 3.80 (s, 3H, OCH_3), 3.30 (m, 8H, 4-CH_2), and 0.35 [s, 6H, $Si(CH_3)_2$]; *p*-tolyl (55–56°, 1 mm), δ 7.20 (broad s, 4H, C_6H_4), 3.20 (m, 8H, 4-CH_2), 2.30 (s, 3H, CH_3), and 0.32 [s, 6H, $Si(CH_3)_2$]; phenyl (53–54°, 1 mm), δ 7.30 (broad s, 5H, C_6H_5), 3.3 (m, 8H, 4-CH_2), and 0.30 [s, 6H, $Si(CH_3)_2$]; *p*-fluorophenyl (42–43°, 0.5 mm), δ 7.40 (m, 4H, C_6H_4), 3.2 (m, 8H, 4-CH_2), and 0.29 [s, 6H, $Si(CH_3)_2$]; *p*-chlorophenyl (50–51°, 0.5 mm), δ 7.50 (m, 4H, C_6H_4), 3.30 (m, 8H, 4-CH_2), and 0.28 [s, 6H, $Si(CH_3)_2$] ppm.

Bis-carbamoyldisiloxane (XX)—A stirred solution of bis(2-chloroethyl)amine (13.1 g, 0.12 mole) and triethylamine (17.6 ml) in ether (250 ml) at 5° was treated dropwise (1 hr) with a solution of 1,3-dichloro-1,1,3,3-tetramethyldisiloxane (10 ml, 0.052 mole) in ether (20 ml). After stirring for 2 hr at 25°, triethylamine hydrochloride was removed by suction filtration and the filtrate was reacted with gaseous anhydrous carbon dioxide for 6 hr at −10°. Further processing as described above for compound XVII led to isolation of XX as a pale yellow oil, which was isolated upon cooling the benzene–petroleum ether solution of crude product. NMR: δ 3.81 (broad s, 16H, 4-NCH_2CH_2Cl), 0.41 [broad s, 6H, 2-$Si(CH_3)_2$] and 0.23 [d, 6H, 2-$Si(CH_3)_2$] ppm. IR (neat): 2940 and 2885 (C—H), 1711 (C=O), 1269 ($SiCH_3$), and 1100 (Si—O) cm^{-1}.

***O*-(*tert*-Butyldimethylsilyl)-*N*-(2-chloroethyl)carbamate (XXI)**—A mixture of *tert*-butyldimethylsilanol (0.8 g, 6 mmoles) and 2-chloroethylisocyanate (3.6 g, 34 mmoles) was heated at 110° for 3 hr, after which time IR analysis indicated the absence of isocyanate starting material (2275 cm^{-1}) and the presence of product (1710 cm^{-1}). Volatiles were removed *in vacuo* (0.1 mm) at 25° and pentane (10 ml) soluble material was then extracted from the residue. Removal of pentane yielded (25%) crude XXI, mp 54–55°, which contained 20% isocyanate starting material and was used without further purification; compound XXI undergoes extensive fragmentation giving silanol and isocyanate when stored at 5° for 2–3 weeks. NMR: δ 5.16 (broad s, 1H, NH), 3.75–3.40 (m, 4H, NCH_2CH_2Cl), 1.03 [s, 9H, $C(CH_3)_3$], and 0.20 [s, 6H, $Si(CH_3)_2$]; integrations corrected for 20% $ClCH_2CH_2NCO$, δ 3.85 (s) ppm, mass spectrum: no M^+; base peak *m/z* 180 (M^+, −57), loss of $C(CH_3)_3$.

***O*-(*tert*-Butyldimethylsilyl)-*N*-{*p*-[*N'*,*N'*-bis(2-chloroethyl)-amino]}phenylcarbamate (XXII)**—A mixture of *p*-isocyanatophenyl mustard (1.09 g, 4.2 mmoles), *tert*-butyldimethylsilanol (0.39 g, 3

Table II—Screening Data for *In Vivo* Anticancer Activity against L-1210 Lymphoid Leukemia and P-388 Lymphocytic Leukemia in Mice

Compound	Vehicle[a]	Dose/Inj., mg/kg	Total Inj.	Treatment Schedule[b]	Screen[c]	max T/C, %[d]
V	A	750	1	1 × 1	L 1210	132
X	A	250	1	1 × 1	L 1210	138
XIII	B	18.75	3	4 × 3	P 388	171
	B	9.37	3	4 × 3	P 388	145
XIV	B	100	1	1 × 1	P 388	166
	B	100	1	1 × 1	P 388	137
XV	B	400	1	1 × 1	P 388	174
XVII	C	200	1	1 × 1	P 388	148
XVIII	D	50	3	4 × 3	P 388	214
	D	100	2	4 × 2	P 388	180
XX	C	25	9	1 × 9	P 388	165
	C	50	9	1 × 9	P 388	208
XXII	A	10	3	4 × 3	P 388	203
	A	10	3	4 × 3	P 388	158
	A	20	3	4 × 3	P 388	182
XXIII	B	200	1	1 × 1	P 388	159
XXVII	C	200	5	1 × 5	L 1210	131

[a] A = mixture of distilled water and polyoxyethylene sorbitan monooleate; B = hydroxypropylcellulose; C = mixture of saline and polyoxyethylene sorbitan monooleate; D = saline. Samples for injection were prepared immediately prior to use. [b] For example, 4 × 3 indicates a 4-day interval between treatments repeated three times; all injections were given ip. [c] For L-1210, the inoculum was 10^5 cells; for P-388, the inoculum was 10^6 cells. [d] Maximum T/C evaluation parameter which was obtained, where T/C % = (treated survivors/control survivors) × 100. A value of ≥125% indicates activity. In all cases, the max T/C % was obtained without drug-associated mortality.

mmoles), and toluene (15 ml) was refluxed until the isocyanate IR absorption (2268 cm^{-1}) was no longer detected (24 hr). Concentration *in vacuo* (0.1 mm) at 25° gave a pale yellow oil, which was extracted three times with low-boiling petroleum ether (45 ml). The combined petroleum ether extracts were concentrated to half-volume and then kept at −10° for 15 hr to afford (60%) product XXII, mp 58–60°. NMR (220 MHz): δ 7.40–6.50 (AA′BB′ q, 4H, C_6H_4), 3.64 (m, 8H, 4-CH_2), 0.85 [s, 9H, $C(CH_3)_3$], and 0.21 [s, 6H, $Si(CH_3)_2$] ppm. IR (neat melt): 3348 (NH), 3085 (C_6H_5), 2982 and 2880 (C—H), 1715 (C=O), 1260 ($SiCH_3$), 1120 (Si—O), and 837 (C_6H_4-*p*) cm^{-1}.

Anal.—Calc. for $C_{17}H_{28}N_2O_2SiCl_2$: C, 52.17; H, 7.16; N, 7.16. Found: C, 52.13; H, 7.12; N, 7.28.

***O*-(*tert*-Butyldimethylsilyl)chlorambucil (XXIII)**—A solution of chlorambucil[2] (0.92 g, 3 mmoles) in benzene (10 ml) was added dropwise (20 min) to a stirred suspension of sodium hydride (96 mg, 4 mmoles) in benzene (3 ml) at 25°. After continued stirring for 3 hr, a solution of *tert*-butyldimethylchlorosilane (0.76 g, 5 mmoles) in benzene (5 ml) was slowly added (20 min), and the mixture was refluxed for 3 hr. The reaction mixture was cooled to 25° and solid materials were removed by suction filtration. The filtrate was concentrated and further removal of volatile *in vacuo* (0.1 mm) at 25° yielded (77%) XXIII as a viscous pale yellow syrup. NMR (carbon tetrachloride): δ 6.65 (AA′BB′ q, 4H, C_6H_4), 3.56 (broad s, 8H, 2-NCH_2CH_2Cl), 2.75–1.65 (m, 6H, $CH_2CH_2CH_2$), 0.86 [s, 9H, $C(CH_3)_3$], and 0.15 [s, 6H, $Si(CH_3)_2$] ppm. IR (neat): 3030 (C_6H_4), 2975 and 2875 (C—H), 1722 (C=O), 1371 [$C(CH_3)_3$], 1200 ($SiCH_3$), 1155 (Si—O), and 832 (C_6H_4-*p*) cm^{-1}.

***O*-(*tert*-Butyldiphenylsilyl)chlorambucil (XXIV)**—Chlorambucil (0.92 g, 3 mmoles), sodium hydride (96 mg, 4 mmoles), and *tert*-butyldiphenylchlorosilane (0.83 g, 3 mmoles) were used to prepare XXIV in a manner identical to that described above for XXIII. The product was isolated (77%) as a viscous oil, which showed no evidence (NMR) of significant contamination by either the starting chlorosilane or its corresponding silanol. NMR: δ 7.90–7.15 (m, 10H, 2-C_6H_5), 7.15–6.35 (AA′BB′ q, 4H, C_6H_4), 3.55 (broad s, 8H, 2-NCH_2CH_2Cl), 2.75–1.40 (m, 6H, $CH_2CH_2CH_2$), and 1.13 [s, 9H, $C(CH_3)_3$] ppm.

***N*-(*tert*-Butyldimethylsilyl)cyclophosphamide (XXV)**—A solution of *tert*-butyldimethylchlorosilane (0.75 g, 5 mmoles) in ether (10 ml) was added slowly (30 min) to a stirred solution of anhydrous cyclophosphamide[3] (0.79 g, 3 mmoles) and triethylamine (0.5 ml) in ether (20 ml) at 5°. After continuous stirring for 24 hr at 25°, the reaction mixture was cooled to −78° and solid materials were collected at that temperature by suction filtration under a nitrogen atmosphere. The solids were immediately extracted with tetrahydrofuran, and this solvent was then removed on a rotary evaporator. The residue was chromatographed on a column (40 × 4 cm) of dry silica gel, using chloroform as the eluent, and the concentrated eluate gave XXV as low-melting crystals, mp 45–47°. NMR (220 MHz): δ 4.46–4.15 (m, 2H, OCH_2) 3.70 (t, 4H, 2-CH_2Cl), 3.45–3.20 (m, 6H, 3-NCH_2), 2.20–1.64 (m, 2H, $CH_2CH_2CH_2$), 1.09 [s, 9H, $C(CH_3)_3$], 0.37 (s, 3H, $SiCH_3$), and 0.22 (s, 3H, $SiCH_3$). The observation of resonance doubling for the $Si(CH_3)_2$ moiety is due to the chirality at phosphorus in XXV (diastereotopic CH_3 groups), and unambiguously establishes that the *tert*-butyldimethylsilyl group is bonded to the cyclophosphamide ring system. ^{31}P-NMR (deuterochloroform, 25% H_3PO_4 external reference): δ 13.82 *versus* 10.78 for cyclophosphamide ppm, mass spectrum: no M^+; *m/z* 317 (M^+, −57), loss of $C(CH_3)_3$, 2-Cl isotope cluster.

***O,O*-Bis(*tert*-butyldimethylsilyl)fluorouracil (XXVI)**—A stirred mixture of fluorouracil (1.30 g, 10 mmoles) and *tert*-butyldimethylchlorosilane (3.3 g, 22 mmoles) in benzene (20 ml) was brought to reflux, and a solution of triethylamine (3.4 ml, 22 mmoles) in benzene (8 ml) was then added dropwise over a 1-hr period. The mixture was refluxed for an additional 15 hr, and then cooled to 25° for separation of triethylamine hydrochloride and unreacted fluorouracil by suction filtration. Volatile materials were removed first on a rotary evaporator and then under high vacuum (0.1 mm) at 25° for 24 hr. The residual material was dissolved in ether (15 ml) decolorized with dry activated charcoal, and then reconcentrated to afford XXVI (67%) as a colorless oil, which crystallized upon standing at −10°, mp ~30°. Contamination of XXVI by fluorouracil was excluded by NMR and IR analyses, which showed no detectable absorptions due to the amido functionalities. Compound XXVI is extremely reactive toward moisture. NMR: δ 7.88 (d, 1H, vinylic H), 0.99 [two overlapping s, 18H, 2-$C(CH_3)_3$], 0.33 [s, 6H, $Si(CH_3)_2$], and 0.21 [s, 6H, $Si(CH_3)_2$] ppm.

***tert*-Butyldimethylsilylftorafur (XXVII)**—A stirred mixture of ftorafur (2 g, 9.8 mmoles) and *tert*-butyldimethylchlorosilane (3 g, 20 mmoles) in benzene (35 ml) was brought to reflux, and a solution of triethylamine (2 ml) in benzene (10 ml) was then added dropwise over a period of 1 hr. The mixture was further reacted and processed as described above for XXVI to afford (56%) product XXVII, mp 149–150°, which was recrystallized from carbon tetrachloride. NMR: δ 7.52 (d, $^3J_{FH}$ = 6 Hz, 1H, vinyl), 6.10 (m, 1H, methine), 4.50–3.80 (m, 2H, CH_2O), 2.70–1.85 (m, 4H, CH_2CH_2O), 0.95 [s, 9H, $SiC(CH_3)_3$], and 0.15 [s, 6H, $Si(CH_3)_2$] ppm.

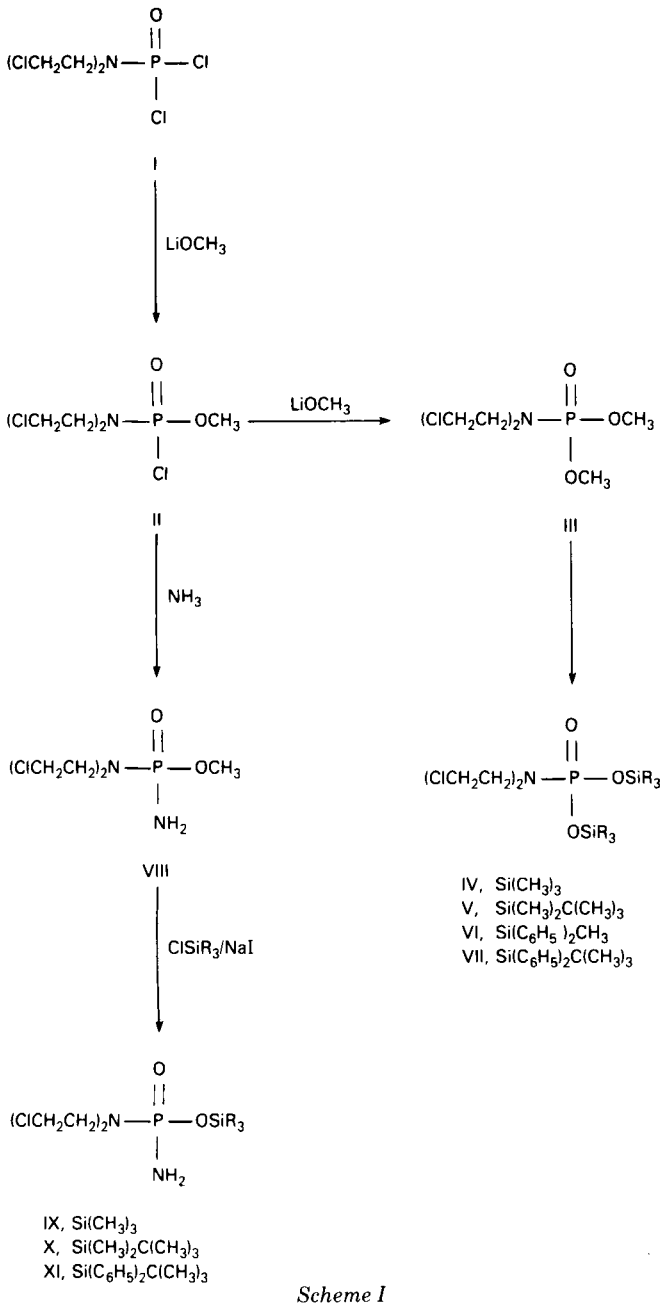
***O*-Trimethylsilylbenzohydroxamic Acid (XXVIII)**[4]—A solution of benzohydroxamic acid (1.37 g, 10 mmoles), trimethylchlorosilane (1.27 ml, 10 mmoles), and triethylamine (1.5 ml, 10 mmoles) in tetrahydrofuran (15 ml) was stirred for 3 days at 25°, and triethylamine hydrochloride was then removed from the reaction mixture by suction filtration. After initial concentration of the filtrate on a rotary evaporator, the residue was kept under high vacuum (0.1 mm) for 2 days at 25°. The crude crystalline product (~100%), mp 96–99°, was used without further purification (25% starting material) due to its hydrolytic reactivity. For XXVIII, NMR: δ 8.00 (broad s, 1H, NH), 7.80–7.42 (m, 2H, *ortho* H), 7.40–7.18 (m, 3H, *meta* and *para* H), and 0.15 [s, 9H, $Si(CH_3)_3$].

***O*-(*tert*-Butyldimethylsilyl)benzohydroxamic Acid (XXIX)**—The synthetic procedure described above for compound XXVIII was applied

[2] Chlorambucil (NSC-3088) was obtained from the Drug Synthesis and Chemistry Branch of the National Cancer Institute.

[3] Cyclophosphamide monohydrate (NSC 26271) was obtained from the Drug Synthesis and Chemistry Branch of the National Cancer Institute. Water of hydration was removed *in vacuo* over phosphorus pentoxide (0.1 mm, 25°, ≥48 hr).

[4] For the preparation of *N,O*-bis(trimethylsilyl)benzohydroxamic acid and its *para*-substituted derivatives, see Ref. 14.



Scheme I

to the reaction of benzohydroxamic acid (1.37 g, 10 mmoles) with equimolar amounts of *tert*-butyldimethylchlorosilane and triethylamine. The resultant product (~100%), mp 127–129°, was found by NMR analysis to be free of detectable contamination by its bistriorganosilylated analog. NMR: δ 8.23 (broad s, 1H, NH), 8.00–7.67 (m, 2H, *ortho* H), 7.67–7.27 (m, 3H, *meta* and *para* H), 1.00 [s, 9H, $C(CH_3)_3$], and 0.25 [s, 6H, $Si(CH_3)_2$].

Anal.—Calc. for $C_{13}H_{21}NO_2Si$: C, 62.11; H, 8.42; N, 5.57. Found: C, 62.12; H, 8.37; N, 5.81.

***N,O*-Bis(*tert*-butyldimethylsilyl)hydroxyurea (XXX)**—A solution of *tert*-butyldimethylchlorosilane (3.8 g, 25 mmoles) in ether (15 ml) was added dropwise (50 min) to a stirred solution of hydroxyurea (1.52 g, 20 mmoles) and triethylamine (3.06 ml, 20 mmoles) in ether (25 ml) at 5°, and the mixture was stirred at 25° for 18 hr. Triethylamine hydrochloride was removed by suction filtration, and the filtrate was concentrated on a rotary evaporator. The residue was extracted with ether (25 ml) and XXX was then obtained (25%) as a microcrystalline solid (mp 92.5–110°) by removal of solvent *in vacuo* (18 hr, 0.1 mm, 20°). NMR: δ 5.80 (broad s, 1H, NHO), 5.30 (broad s, 1H, NH), 0.93 [s, 18H, 2-$C(CH_3)_3$], 0.25 [s, 6H, $OSi(CH_3)_2$], and 0.17 [s, 6H, $NSi(CH_3)_2$]. Mass spectrum: *m/z* 304 (M^+), <1%; 247 (M^+, −57), 36%, loss of $C(CH_3)_3$.

Prodrug Hydrolysis Kinetics by NMR—The compound (15–23 mmoles) to be studied was placed in an NMR tube and then dissolved in dioxane (0.60 ml). Either tromethamine or lutidine buffer (0.40 ml, 0.05 *M*, pH 7.4) was added, and after rapid mixing the spectral region from δ 0.7–0 was repeatedly recorded as a function of time using a fixed-sweep rate. Total peak height for starting material and the triorganosilanol hydrolysis product remained constant and was equated with the initial concentration of starting material, C_0. The decreasing peak height for starting material was equated with the concentration of starting material at time *t*, C_t. Linear least-squares fits of $\ln(C_0/C_t)$ *versus* *t* gave the pseudo first-order rate constants (*k'*) and half-life ($\tau_{1/2}$) values listed in Table I. The NMR probe temperature (27 ± 1°) was measured using the chemical shift difference between methyl and hydroxyl protons in methanol (15).

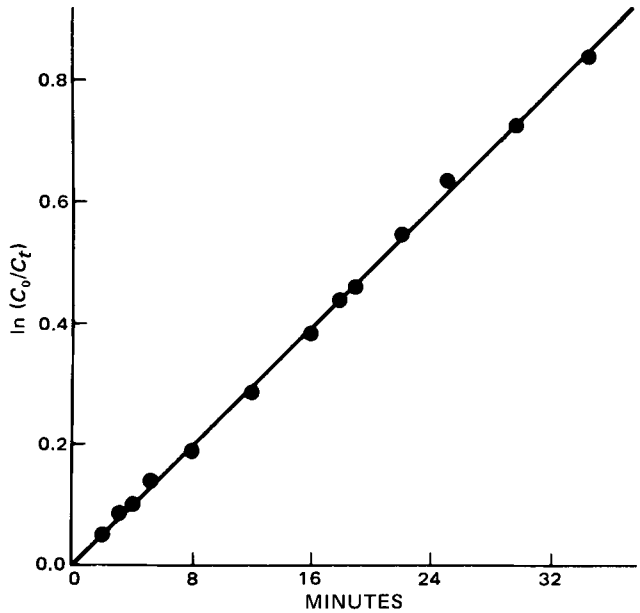


Figure 1—*Pseudo first-order kinetic plot for hydrolysis of X (1.5 × 10^{-2} M) in dioxane–0.05 M tromethamine buffer (pH 7.4) mixture (60:40) at 27°. Substrate concentration terms C_0 and C_t were determined by NMR (see text for details).*

Prodrug Hydrolysis Kinetics by UV—Stock solutions of each compound were prepared in dioxane at concentrations of 6.0 × 10^{-4}–5.4 × 10^{-2} *M*. An aliquot (1.5 ml) was rapidly mixed with an equal volume of tromethamine buffer (0.1 *M*, pH 7.4–8.4), and the reaction mixture was monitored continuously as a function of time *t*, using a fixed wave-

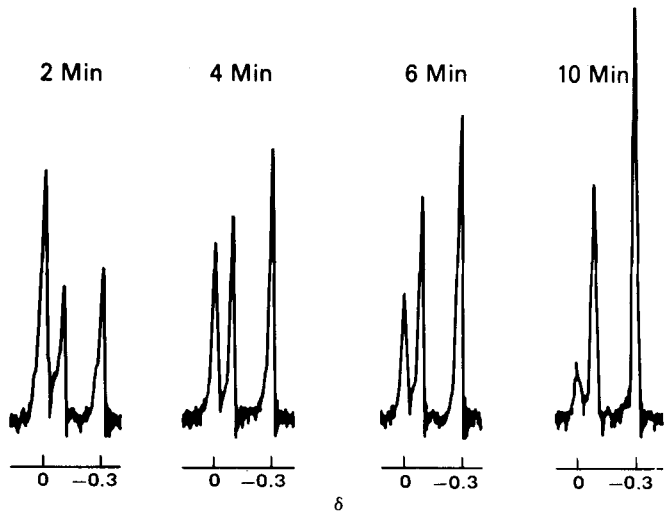
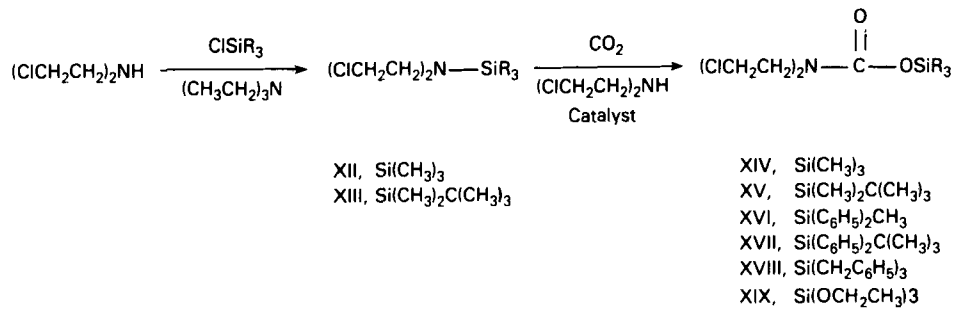
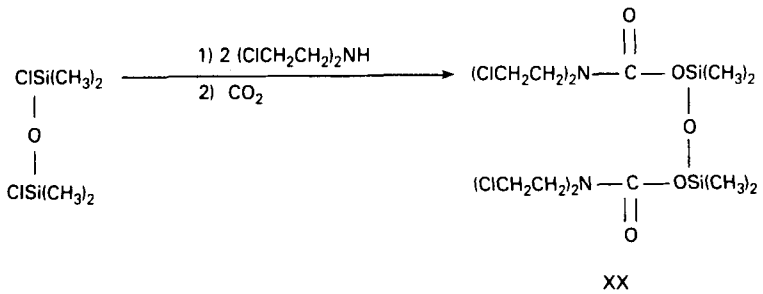
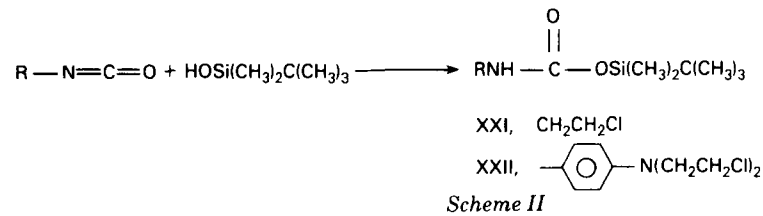


Figure 2—*Variation in the dimethylsilyl NMR (60 MHz) spectral region for V (1.8 × 10^{-2} M) as a function of time (min) in dioxane–0.05 M lutidine buffer (pH 7.4) mixture (60:40) at 27°. Chemical shifts are relative to V (0 δ); the absorption signal at −0.08 δ is assigned to the monoester derived from partial hydrolysis of V, while the* tert*-butyldimethylsilanol signal is at −0.30 δ.*





XX



Scheme II

length (Table I) and a reference sample containing the dioxane–buffer mixture (50:50). The initial absorbance was equated with A_0 and the final absorbance after 4 hr was equated with A_∞. Linear least-squares fits of $\ln [(A_\infty - A_0)/(A_\infty - A)]$ *versus* t gave the pseudo first-order rate constants (k') and half-life ($\tau_{1/2}$) values listed in Table I. The temperature (27 ± 1°) of the UV samples after equilibration in the spectrophotometer was measured with a small thermometer.

NMR Kinetic Measurements with Aryldimethylsilyl Compounds—Each of the five *N*-aryldimethylsilyl-*N*,*N*-bis(2-chloroethyl)-amines (2.5×10^{-2} mmoles) was dissolved in deuterochloroform (0.5 ml) and was then reacted with a large excess of absolute ethanol (0.5 ml) using NMR to monitor the reaction rate at a spectrometer probe temperature of 27 ± 1°. The relative concentrations of each starting material and its corresponding ethoxysilane product were determined from dimethylsilyl absorption intensities at $\delta \sim 0.15$ and ~0.08, respectively, using scale-expansion, low RF-power, and a fast sweep rate. Linear least-squares fits of $\ln(C_0/C_t)$ *versus* t had an average slope-error of ± ~5%, and gave the following pseudo first-order rate constants: p-OCH_3, 1.14×10^{-3} sec^{-1} ($\tau_{1/2}$ 10.1 min); p-CH_3, 1.43×10^{-1} sec^{-1} ($\tau_{1/2}$ 8.1 min); p-H, 2.73×10^{-3} sec^{-1} ($\tau_{1/2}$ 4.2 min); p-F, 6.86×10^{-3} sec^{-1} ($\tau_{1/2}$ 1.7 min); and p-Cl, 1.04×10^{-2} sec^{-1} ($\tau_{1/2}$ 1.1 min). The previously described NMR method for

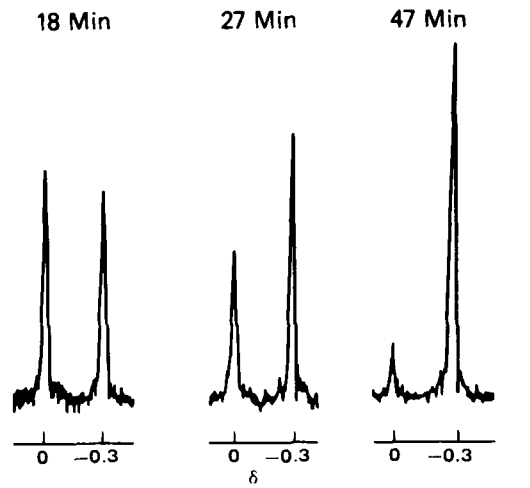


Figure 3—*Variation in the dimethylsilyl NMR (60 MHz) spectral region for XV (2.3×10^{-2} M) as a function of time (min) in dioxane–0.05 M tromethamine (pH 7.4) mixture (60:40) at 27°. The chemical shift of* tert*-butyldimethylsilanol (−0.30 δ) is relative to XV (0 δ).*

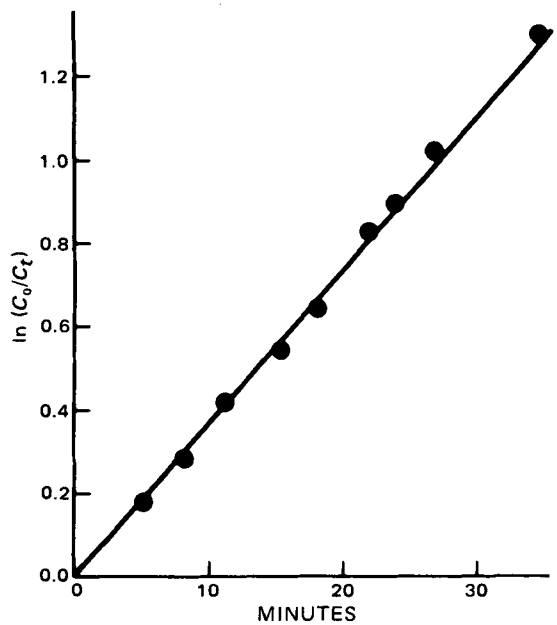
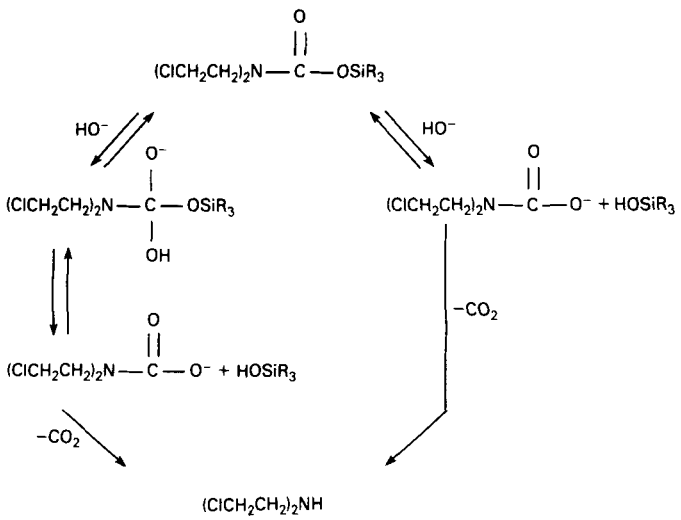


Figure 4—*Pseudo first-order kinetic plot for hydrolysis of XV (2.3×10^{-2} M) in dioxane–0.05 M tromethamine buffer (pH 7.4) mixture (60:40) at 27°. Substrate concentration terms C_0 and C_t were determined by NMR (see text and Fig. 3).*



Scheme III

measurement of prodrug hydrolysis kinetics was applied to solutions containing each of the five *O*-aryldimethylsilyl-*N*,*N*-bis(2-chloroethyl)-carbamates (2.5×10^{-2} mmoles) in a mixture of dioxane (0.5 ml) and tromethamine buffer (0.5 ml, 0.05 *M*, pH 7.4). For these reactions at 27 ± 1°, linear least-squares fits (± ~5% average slope-error) gave the following pseudo first-order rate constants: *p*-OCH_3, 1.65×10^{-4} sec^{-1} ($\tau_{1/2}$ 70.0 min); *p*-CH_3, 1.69×10^{-4} sec^{-1} ($\tau_{1/2}$ 68.3 min); *p*-H, 3.87×10^{-4} sec^{-1} ($\tau_{1/2}$ 29.8 min); *p*-F, 1.17×10^{-3} sec^{-1} ($\tau_{1/2}$ 9.9 min); and *p*-Cl, 1.58×10^{-3} sec^{-1} ($\tau_{1/2}$ 7.3 min).

^{18}O-Labeling Studies—A freshly prepared sample of silanol-free XXI (10 mg) was dissolved in a magnetically stirred mixture of dioxane (0.4 ml), tromethamine buffer (0.2 ml of 0.2 *M*, pH 7.4), and ^{18}O-enriched water (0.2 ml, >95 g-atom % ^{18}O). After 3 hr at room temperature, the reaction mixture was extracted with fractionally distilled pentane (3 × 1 ml), and the extract was then analyzed by mass spectroscopy to determine the relative proportion of ^{16}O- and ^{18}O-containing *tert*-butyldimethylsilanol. The (M^+, −57) ions observed at *m/z* 75 and 77 arise from loss of *tert*-butyl and had a normalized intensity of 99.87 and 100.00%, respectively. When unlabeled silanol (5 mg) was extracted from a simulated reaction mixture containing normal water, the ion intensities at *m/z* 75 and 77 were 100.00 and 4.78%, respectively, whereas use of 50:50 v/v mixture of water and ^{18}O-labeled water (18 hr of contact time) led to extraction of silanol having ion intensities at *m/z* 75 and 77 equal to 100.00 and 14.94%, respectively.

Anticancer Screening—Selected compounds (Table II) were evaluated against L-1210 lymphoid leukemia and P-388 lymphocytic leukemia in either male or female mice. Pertinent details and the results of these tests are given in Table II.

RESULTS AND DISCUSSION

Syntheses—The methodology for constructing *O*-triorganosilyl derivatives of phosphoramide mustard was developed by first pursuing the synthesis of diester model compounds IV–VII (Scheme I). These materials were prepared by reaction of common precursor III with the corresponding triorganochlorosilane and sodium iodide in acetonitrile solvent (16). The same demethylation–silylation procedure was used to convert common precursor VIII into target compounds IX–XI. Proton NMR spectra of these products showed two-proton amido resonance signals and were devoid of detectable methoxy signals, indicating that *N*-silylation of the amide functionality (17, 18) is not a significant side reaction.

O-Triorganosilyl carbamate derivatives of nor-nitrogen mustard (XIV–XIX, Scheme II) were obtained by Breederveld's (19) reaction sequence, namely, *N*-silylation followed by insertion of carbon dioxide into the Si—N bond, which requires excess amine as a catalyst and presumably (19) involves triorganosilyl group-transfer from an *N*-triorganosilyl-*N*,*N*-bis(2-chloroethyl)amine to the carbamic acid derivative or nor-nitrogen mustard.

The *N*-triorganosilyl-*N*,*N*-bis(2-chloroethyl)amines can be isolated (*e.g.*, XII and XIII); however, they readily decompose and were usually converted *in situ* to the final carbamate product. Biscarbamoyldisiloxane XX was similarly prepared from 1,3-dichloro-1,1,3,3-tetramethyldisiloxane, while *tert*-butyldimethylsilyl carbamate XXI was obtained by direct addition of *tert*-butyldimethylsilanol to 2-chloroethyl isocyanate. The silanol–isocyanate addition reaction has not been reported previously, and has been shown[5] to be generally applicable to variously structured triorganosilanols and alkyl/aryl isocyanates. Accordingly, it was possible to convert *p*-isocyanatophenylmustard into carbamate derivative XXII by reaction with the appropriate silanol.

Tert-butyldimethylsilyl (XXIII) and *tert*-butyldiphenylsilyl (XXIV) esters of the anticancer drug chlorambucil (20) were prepared by direct *O*-silylation using the sodium salt of this carboxylic acid. The anticancer agents cyclophosphamide (21), fluorouracil (22), ftorafur (23), benzohydroxamic acid (24), and hydroxyurea (25) were also silylated by conventional methods and gave derivatives XXV–XXX, respectively. By analogy to structurally related systems (14, 17, 26, 27), the triorganosilyl groups in compounds XXV–XXX may undergo either positional exchange (between oxygen and nitrogen bonding sites) or rotational isomerization; however, these dynamic processes are not a central issue in the present study. On the other hand, it is worthwhile to note that ^1H-NMR spectra for XXV–XXX were consistent with the presence of a single (major) isomer. The connectivities used to represent compounds XXV–XXX are the best guess structures as opposed to definite assignments of bonding.

Hydrolysis Kinetics—Hydrolytic unmasking of the candidate triorganosilyl prodrugs was first examined by ^1H-NMR monitoring of the dimethylsilyl spectral region for the phosphoramide mustard derivative X, using an organic solvent (dioxane)–aqueous buffer mixture to achieve sufficient substrate solubility for these continuous-wave measurements. Gradually decreasing intensity for the dimethylsilyl absorption of X was accompanied by increasing signal intensity for the dimethylsilyl moiety in the hydrolysis byproduct, *tert*-butyldimethylsilanol (δ −0.30, relative to X). The sum of these two resonance signals, which remained constant, provides a relative measure of the initial concentration of X, C_0, while the diminishing signal intensity for X is a relative measure of substrate concentration at time *t*, C_t. For hydrolysis of X at pH 7.4[6], 27°, a least-squares fit of $\ln(C_0/C_t)$ *versus* *t* (Fig. 1) indicated a good linear correlation and gave the pseudo first-order rate constant (k') and half-life ($\tau_{1/2}$) listed in Table I.

Spectra (Fig. 2) obtained with diester V under analogous reaction conditions showed the conversion of starting material into *tert*-butyldimethylsilanol (δ −0.30, relative to V) and a monoester intermediate (δ −0.08, relative to V), which undergoes comparatively slow hydrolysis to afford the fully unmasked phosphoramidic mustard.[7] Kinetic analysis based on the decreasing signal intensity of V afforded the monodesilylation rate data given in Table I. Multiplication of the 3.8-min half-life for V by a correction factor of 2 gives a value which is ~four times less than the 28.6-min half-life for X. This relatively small difference in half-lives is presumably due to leaving group effects, since the steric bulk about the back-side tetrahedral face of silicon is virtually the same in each compound. Pro(phosphoramide mustards) IX and XI were similarly examined by NMR in an attempt to determine the influence of the triorganosilyl group on hydrolytic stability; however, compound IX was too reactive to study by this method, and the *tert*-butyl signals from XI and its silanol byproduct were extensively overlapped at 60 MHz.

Alkaline hydrolysis of *O*-triorganosilyl carbamic acid mustards can occur by initial attack of hydroxide ion at either the carbonyl carbon, as in *O*-alkyl carbamates (28), or at silicon to give the conjugate base of carbamic acid mustards (Scheme III), which would then undergo relatively rapid decarboxylation to release nor-nitrogen mustard. While both reaction pathways lead to formation of the same bis-alkylating agent, the silicon-attack route would appear to allow for greater control over the rate of nor-nitrogen mustard release. Differentiation between the two mechanistic possibilities was first approached by studying structure–reactivity relationships. Extension of the NMR kinetic method to hydrolysis of *O*-trimethylsilyl carbamate XIV was precluded by this compound's exceedingly short lifetime in aqueous media; however, production of triorganosilanol from compounds XV, XVI, and XVIII could be conveniently monitored from changes in the methylsilyl or silyl-bearing

[5] K. C. Fichter and G. Zon, unpublished results.

[6] This and related pH values refer to the aqueous buffer component used to prepare the mixed solvent system. The true pH of such mixtures is poorly defined. Since the purpose of the present kinetic measurements was to evaluate *relative* substrate reactivities, and roughly gauge the stabilities of these potential prodrugs for possible correlation with *in vivo* biological activity, no attempt was made to devise either a more refined pH measurement or a more realistic *in vitro* kinetic system.

[7] Lutidine buffer causes a solvent-induced chemical shift separation between the dimethylsilyl signals for V and the monoester intermediate. The signals were accidentally isochronous in the dioxane–tromethamine buffer mixture used for studying X.

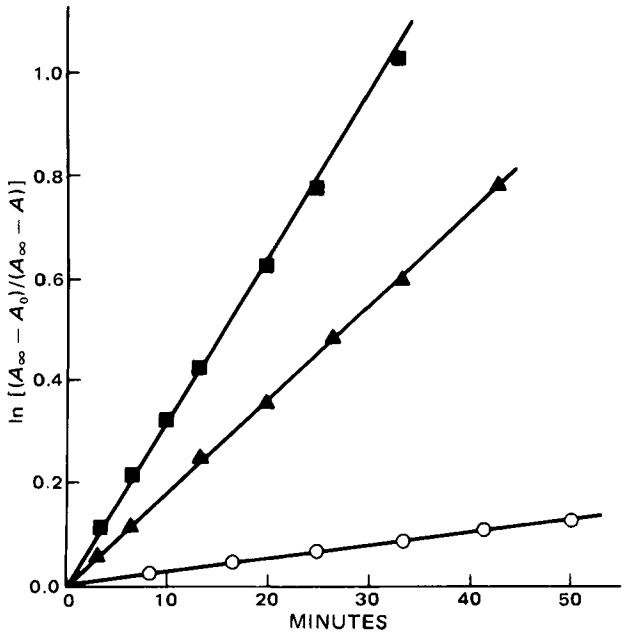


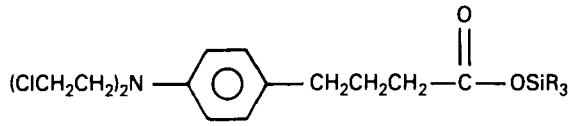
Figure 5—*Pseudo first-order kinetic plots for hydrolysis of XV (2.7 × 10^{-2} M) in 50:50 dioxane–0.10 M tromethamine buffer (pH 8.4, ■; pH 7.4, ▲; pH 6.4, ○) at 27°. The UV absorbance (A) values were measured at 226 nm (see text for details).*

methylene spectral region, as seen from representative spectra obtained with XV (Fig. 3). For each of these compounds, the signal for the triorganosilanol byproduct appeared at higher field (lower δ), relative to starting material, due to removal of the carbonyl group deshielding effect. In all cases, the formation of triorganosilanol obeyed a first-order rate law (*e.g.*, Fig. 4); however, the substrate half-life values (Table I) revealed only a twofold reactivity range for this series of SiR_3 groups: XVIII, 13.3 min; XV, 18.1 min; and XVI, 27.0 min.

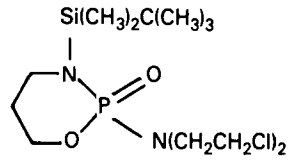
Previous studies of the alkaline hydrolysis of carbamates have employed UV spectroscopy to measure reaction kinetics (28). When this method was applied to compound XV in mixtures of dioxane and tromethamine buffer at pH 6.4, 7.4, and 8.4 (27°), the absorbance (A) at 226 nm afforded linear plots of $\ln[(A_\infty - A_0)/(A_\infty - A)]$ *versus* t (Fig. 5), and the calculated rate constants (Table I) increased with elevated concentrations of hydroxide ion. The UV-derived rate constant for hydrolysis of XV at pH 7.4 (3.03×10^{-4} sec^{-1}) is about one-half the magnitude of the rate constant (6.37×10^{-4} sec^{-1}) for production of *tert*-butyldimethylsilanol from XV measured by NMR under the same reaction conditions. This difference can be accommodated by assuming the existence of carbamic acid mustard, since the absorbance at 226 nm is most likely due to carbonate ion which is formed by decarboxylation of this metastable intermediate (28).

Compounds XVI–XVIII have phenyl substituents that obscure the 226 nm UV region. Kinetic data (Table I) for hydrolysis of these silylated carbamates at pH 7.4 were thus obtained from absorbance changes measured at 260 nm, and correspond to the rate of triorganosilanol production rather than carbonate formation. This interpretation is supported by the fact that the UV- and NMR-derived half-lives for two of these compounds are essentially equivalent: XVI, 27.2 and 27.0 min, respectively; XVIII, 12.8 and 13.3 min, respectively.

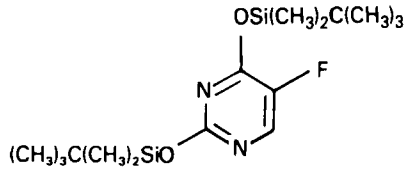
It is enlightening to compare the relative rates of desilylation in the homologous series of carbamates represented by XV–XVIII. The order of increasing substrate stability ($\tau_{1/2}$) at pH 7.4 is XVIII (13.0 min[8]) <XV (18.1 min) <XVI (27.1 min[8]) <XVII (34.2 min), which parallels the approximate order of increasing steric bulk of the triorganosilyl group in each compound. This correlation of relative reactivity with substrate structure is consistent with a hydrolysis mechanism involving hydroxide ion attack at silicon (Scheme III); however, the ratios of rate constants cover a surprisingly small range of values: XV/XVIII = 0.72, XVI/XVIII = 0.48, and XVII/XVIII = 0.38. In view of the fact that steric hindrance to S_N2 attack at silicon can lead to a more pronounced deceleration of solvolysis rates[9], and that the rate of hydroxide attack at the carbonyl



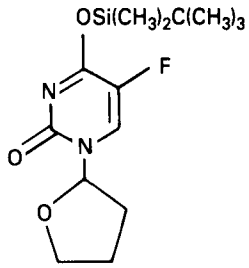
XXIII, $Si(CH_3)_2C(CH_3)_3$
XXIV, $Si(C_6H_5)_2C(CH_3)_3$



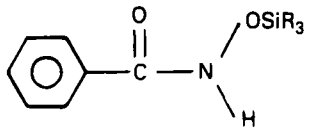
XXV



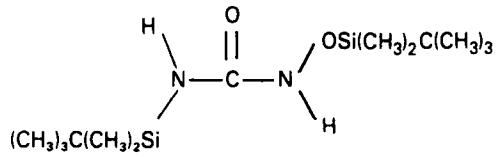
XXVI



XXVII



XXVIII, $Si(CH_3)_3$
XXIX, $Si(CH_3)_2C(CH_3)_3$



XXX

carbon in carbamates is also subject to steric retardation (28), the *O*-silyl carbamate hydrolysis mechanism was further probed by a Hammett-type kinetic study. Proton NMR measurements using a series of *para*-substituted *O*-aryldimethylsilyl carbamate derivatives of nor-nitrogen mustard afforded desilylation rate constants which correlated linearly with the σ^o substituent constant (Fig. 6) and indicated that the reaction constant, ρ, for dioxane–tromethamine buffer (pH 7.4) was equal to 2.39. The sign and magnitude of this ρ value indicate mechanistic similarity to the alkaline solvolysis of *para*-substituted aryldimethylsilanes (ρ = 1.94 for σ^o), which is known to occur by nucleophilic attack of hydroxide ion at silicon (30). Another line of evidence in support of S_N2 attack at silicon in the *O*-silyl carbamates derives from the approximately equal ρ value of 2.18 measured for ethanolysis of the corresponding series of *N*-aryldimethylsilylamines (Fig. 7), wherein silicon must be the reaction center.

The preceding mechanistic rationale can be justifiably countered by arguing that a ρ value of ~2 does not exclude the possibility of attack at

[8] Average value obtained from NMR and UV measurements.

[9] For example, triethylsilylacetate hydrolizes 38-times slower than trimethylsilyl acetate (29).

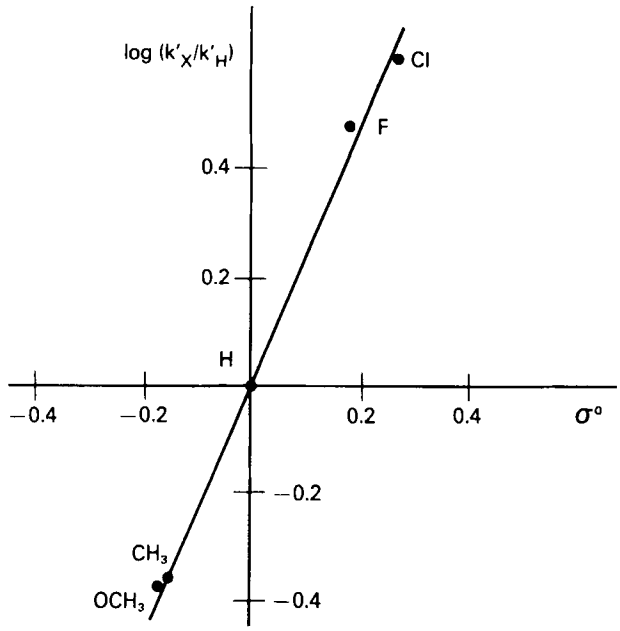


Figure 6—*NMR-derived Hammett plot (r = 0.996) for the hydrolysis of* para-*substituted* O-*aryldimethylsilyl-*N,N-*bis(2-chloroethyl)carbamates in 50:50 dioxane–0.05* M *tromethamine buffer (pH 7.4) at 27°; for details and the values of* k', *see* Experimental *section. The values used for* σ° *are as follows:* p-OCH_3, *−0.16 (30, 31);* p-CH_3, *−0.15 (30);* p-*H, 0.01;* p-*F, 0.17 (31);* p-*Cl, 0.27.*

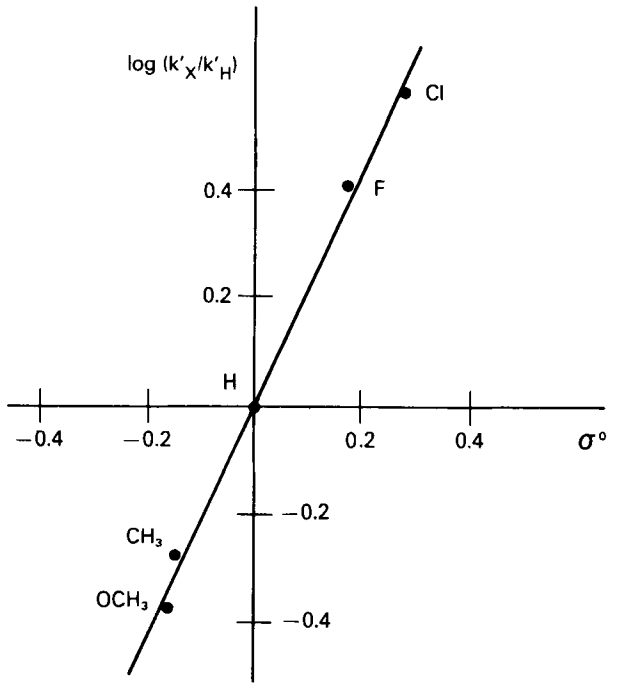


Figure 7—*NMR-derived Hammett plot (r = 0.997) for the solvolysis of* para-*substituted* N-*aryldimethylsilyl-*N,N-*bis(2-chloroethyl)amines in 50:50 ethanol–deuterochloroform at 27°; for details and the values of* k', *see* Experimental *section. The values used for* σ° *are given in the caption for Fig. 6.*

the carbamate carbonyl position, since electron-withdrawing aryl substituents would be expected to facilitate the formation of a negatively charged tetrahedral intermediate. The carbonyl-attack mechanism for hydrolysis of *O*-triorganosilyl carbamates was therefore ruled out by determining that reaction of model compound XXI in dioxane–tromethamine buffer (pH 7.4) containing a 50:50 mixture of water and ^{18}O-enriched water leads to formation of a 50:50 mixture of ^{16}O- and ^{18}O-containing silanols. Hydroxide ion attack at the carbonyl carbon in XXI cannot lead to complete incorporation of oxygen 18, as was observed, since the results of a control reaction (see Experimental) demonstrated that oxygen exchange in *tert*-butyldimethylsilanol is relatively slow (<10%) under the conditions used for hydrolysis, and a presolvolytic mechanism[10] gives an oxygen 18 content which cannot exceed ~17%. Granted that S_N2 attack at silicon also holds for analogs of XXI, the previously mentioned insensitivity to steric effects at the silicon reaction center in compounds XV–XVIII can only be speculated on. One possibility is a leveling effect which results from the fact that all of these SiR_3 moieties have relatively large R groups. An alternative explanation is that the well-known propensity for pentacoordination during nucleophilic substitution at silicon leads to either a late transition state or intermediate (32) wherein the rate of O—SiR_3 bond cleavage is primarily controlled by leaving group stability, which is a constant factor in structures XV–XVIII.

Extension of the kinetic studies to the hydrolysis of other triorganosilylated derivatives of anticancer agents focused upon evaluating the release rate of fluorouracil from compound XXVI, as there is widespread interest in developing new classes of *in vivo* precursors to fluorouracil (33–40), but apparently no available information on systems which utilize organosilicon chemistry. Proton NMR spectra obtained with compound XXVI in dioxane–tromethamine buffer (pH 7.4) revealed that the two dimethylsilyl singlets arising from the chemically nonequivalent silyl groups in XXVI decrease in intensity at the same rate, with concomitant appearance of the *tert*-butyldimethylsilanol signal. Evidently, a monosilylated intermediate is too short-lived to be detected by low-resolution NMR under these reaction conditions (Table I), which led to a half-life for XXVI of only 1.08 min. At a buffer pH of 6.4, these spectral changes were too fast to measure, while at pH 8.4 the half-life of XXVI was 1.14 min. The increase in reaction rate with decreasing pH suggests that hydrolysis in the pH range of 6.4–8.4 proceeds by hydrogen ion catalysis; *i.e.*, protonation at the N_1 and/or N_3 positions.

Anticancer Screening—Selected compounds were screened according to standard protocol (41) for either L-1210 or P-388 leukemias in either male or female mice. The hydrophobicity of these samples generally required the use of either hydroxypropylcellulose or aqueous polyoxyethylene sorbitan monooleate as the vehicle for intraperitoneal injections. For L-1210, the inoculum was 10^5 cells; for P-388, the inoculum was 10^6 cells. Mean survival time was used as the evaluation parameter: a test/control percentage (T/C%) ≥125 indicates activity. Compounds VI, VII, XI, XXV, and XXVI were inactive against the L-1210 lymphoid leukemia; compounds XVI, XXI, and XXVIII–XXX were inactive in the P-388 test system. Table II summarizes pertinent data for those compounds which exhibited activity. Since it was not possible to have the parent (nonsilylated) drugs tested in parallel with these active analogs, an evaluation of prodrug *versus* parent drug activities could only be made in a highly qualitative manner by comparisons with screening data which had been previously obtained with the parent drug against the same type of cancer using similar vehicles, dose/injection, and treatment schedules. A computer-assisted search was conducted[11], and it was found that reasonable comparisons could be made for phosphoramide mustard, nor-nitrogen mustard, and ftorafur, but not for *p*-aminophenyl mustard (XXII) and chlorambucil (XXIII). The 138% T/C obtained with X is much less than the 265% T/C value given by phosphoramide mustard at a single-dose injection of 200 mg/kg, whereas the 131% T/C determined for XXVII is essentially identical to the 130% T/C value exhibited by ftorafur under similar test conditions. Except for compound XVI, which was inactive, silylmustard XIII and *O*-silylcarbamates XIV–XVIII and XX covered a range of activities (T/C% ~150–200) roughly equal to that of the parent nor-nitrogen mustard (T/C% 210 at 37.5 mg/kg, 1 × 10), although the modality for treatment within this set of compounds was variable.

CONCLUSIONS

Kinetic measurements have demonstrated that triorganosilyl groups in *O*-silyl esters of phosphoramide mustard, *N*-silyl nor-nitrogen mustards, *O*-silylcarbamoyl nor-nitrogen mustards, and *O*-silylated fluorouracil can be used as hydrolytically labile moieties for the design of new classes of potential anticancer prodrugs. Relative hydrolysis rates, pH

[10] If it is assumed that C—OSi bond cleavage in a tetrahedral *O*-silyl carbamate ion is slow relative to proton shifts and SiR_3 migration between the three oxygen atoms in this intermediate, then a 50:50 mixture of water and ^{18}O-enriched water would scramble one ^{18}O-label among a total of six oxygens, and thus lead to ~17% incorporation at the C—OSi linkage prior to its fragmentation.

[11] NCI Automated Information Section of the Drug Evaluation Branch.

effects, Hammett studies, and ^{18}O-labeling data are all consistent with rate-limiting hydroxide ion attack at silicon in the *O*-silyl carbamates; however, increasing steric bulk about the silicon reaction center has a relatively small rate-retarding influence (<5-fold).

On the other hand, long-range electronic effects caused by *para* substituents in *O*-aryldimethylsilyl carbamates are substantial, as evidenced by a ρ value of 2.39 in dioxane–tromethamine buffer (pH 7.4).

Such electronic control may therefore be useful for fine-tuning the release of an active agent by a prodrug. Triorganosilylation of cyclophosphamide, fluorouracil, benzohydroxamic acid, and hydroxyurea obliterated the activity of these anticancer agents, whereas analogous alterations of phosphoramide mustard, nor-nitrogen mustard, *p*-aminophenylmustard, chlorambucil, and ftorafur led, in general, to retention of anticancer activity. The especially encouraging screening results obtained for *O*-tribenzylsilyl carbamate derivative XVIII have led to its recent selection by NCI for further evaluation in its tumor panel testing program. These screening results and studies of additional triorganosilylated anticancer prodrugs will be reported in the future.

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ACKNOWLEDGMENTS

Supported in part by National Institutes of Health Research Grants CA 18366 and CA 21345, PHS/DHHS, which were awarded to Gerald Zon.

All anticancer screening tests were carried out through the Drug Synthesis and Chemistry Branch (DSCB), Development Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, and with the assistance of Dr. V. L. Narayanan, Chief, DSCB.

The authors thank Dr. Philip K. G. Hodgson for carrying out some early exploratory experiments, and Mr. Nathaniel H. Greenberg (NCI, Drug Evaluation Branch) and Mr. John F. Waters (NCI, Automated Information Section) for their assistance in obtaining comparative screening data.