Synthesis and Evaluation of Several New (2-Chloroethyl)nitrosocarbamates as Potential Anticancer Agents

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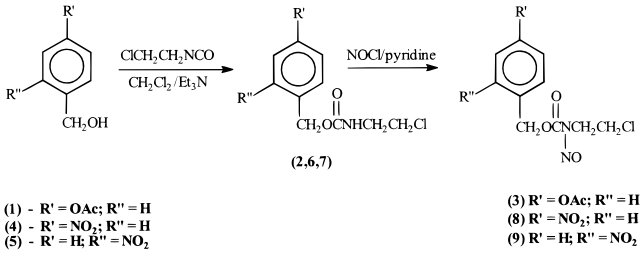
Seven new (2-chloroethyl)nitrosocarbamates have been synthesized as potential anticancer alkylating agents. These compounds were designed with carrier moieties that would either act as prodrugs or confer water solubility. All compounds were screened in an in vitro panel of five human tumor cell lines: CAKI-1 (renal), DLD-1 (colon), NCI-H23 (lung), SK-MEL-28 (melanoma), and SNB-7 (CNS). Several agents showed good activity with IC_{50} values in the range of 1−10 μg/mL against at least two of the cell lines. One compound, carbamic acid, (2-chloroethyl)nitroso-4-acetoxybenzyl ester (**3**), was selected for further study in vivo against intraperitoneally implanted P388 murine leukemia. In addition to the aforementioned compound, both carbamic acid, (2-chloroethyl)nitroso-4-nitrobenzyl ester (**9**) and carbamic acid, (2-chloroethyl)nitroso-2,3,4,6-tetra-*O*-acetyl-1-α,*β*-D-glucopyranose ester (**24**) were evaluated against subcutaneously implanted M5076 murine sarcoma in mice. None of these compounds were active in vivo.

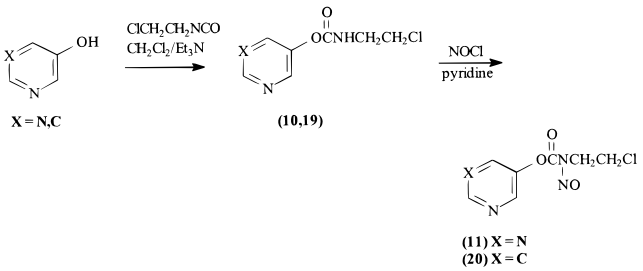
Introduction

The observed activity of ethyl (2-chloroethyl)-*N*-nitrosocarbamate,[1] and the similarity of this class of alkylating agent to the clinically used *N*-(2-chloroethyl)-*N*-nitrosourea anticancer drugs, has led us to investigate a number new of *N*-(2-chloroethyl)-*N*-nitrosocarbamates for evaluation as potential anticancer alkylating agents. Several such compounds, although only modestly water soluble, have shown significant increases in life span and/or curative benefits in mice implanted with P388 leukemia.[2] On the basis of this activity, seven new target compounds were synthesized in order to ascertain different approaches for optimizing solubility and activity of the *N*-(2-chloroethyl)-*N*-nitrosocarbamates. Among these approaches, *N*-(2-chloroethyl)-*N*-nitrosocarbamates were conjugated to small, water-soluble heterocycles (**11**, **20**), a carbohydrate (**24**), or substituted benzylic functions with the potential for in situ bioactivation (**3**, **8**, **9**, **18**) for evaluation as potential anticancer alkylating agents. Several carbohydrate-derived alkylating agents have been reported.[3] On the basis of the activity of these known agents and the potential for selective uptake of carbohydrate-alkylating agents,[4] compound **24** was synthesized and screened. The benzyl-substituted *N*-(2-chloroethyl)-*N*-nitrosocarbamates (**3**, **8**, **9**, **18**) were designed as potential prodrugs whereby deacetylation[5] (**3**, **18**) or bioreduction of the nitro function[6] (**8**, **9**) would lead to potential "self-immolative" agents as previously reported.[7] The new agents were evaluated against a panel of human tumor cell lines in vitro, and three were also evaluated in vivo against a murine leukemia and/or a murine sarcoma.

Scheme 1



Scheme 2



Chemistry

Selective acetylation of the phenolic group of 4-hydroxybenzyl alcohol (Scheme 1) by the method of Paradisi et al.[8] gave **1**. This intermediate was reacted with 2-chloroethyl isocyanate and nitrosated by standard methods[9] to give the target compound **3** in good overall yield. The commercially available nitrobenzyl alcohols (**4**, **5**) were carbamoylated and nitrosated (Scheme 1) to yield the target compounds **8** and **9**. 5-Hydroxypyrimidine[10] and commercially available 3-hydroxypyridine were likewise carbamoylated and nitrosated (Scheme 2) to yield the target heterocycle-conjugated (2-chloroethyl)-*N*-nitrosocarbamates (**11**, **20**).

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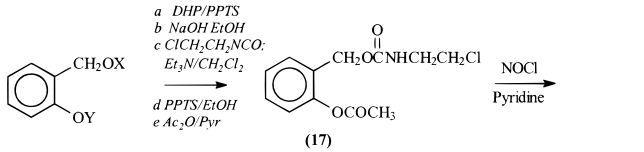
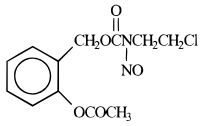
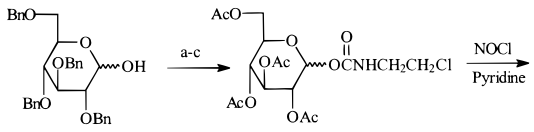
Scheme 3



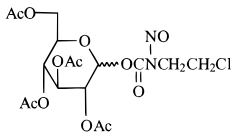
12: X = Ac, Y = H
13: X = Ac, Y = THP
14: X = H, Y = THP
15: X = $CONHCH_2CH_2Cl$, Y = THP
16: X = $CONHCH_2CH_2Cl$, Y = H



Scheme 4



a $ClCH_2CH_2NCO$; Et_3N/CH_2Cl_2
b H_2-Pd/C
c Ac_2O/Pyr



Intermediate **12** (Scheme 3) was prepared by selective acetylation[11] of the benzyl hydroxyl group of 2-hydroxybenzyl alcohol. The phenolic hydroxyl group was subsequently blocked as the THP ether, and deacetylation exposed the benzylic hydroxyl group for further elaboration. The THP derivative **14** was then carbamoylated, deprotected, acetylated, and nitrosated to yield the target **18**. Finally, commercially available 2,3,4,6-tetra-*O*-benzyl-1-α,β-D-glucopyranose was reacted with 2-chloroethyl isocyanate, deblocked by hydrogenolysis, peracetylated, and nitrosated with NOCl to give **24** (Scheme 4). All samples were characterized by FAB-MS, ^1H NMR, and IR spectroscopy and TLC and CHN analysis prior to evaluation.

Results and Discussion

Several of the new agents showed good in vitro activity with IC_{50} (see Table 1) values in the range of 1−10 μg/mL against at least two of the human tumor cell lines. Three compounds (**3**, **9**, and **24**) were selected for further study in vivo against either intraperitoneally (ip) implanted P388 murine leukemia (**3**, Table 2) or subcutaneously (sc) implanted M5076 murine sarcoma (**3**, **9**, and **24**, Table 2) in mice. Although these three compounds showed significant activity in vitro, they were not active in vivo but demonstrated notable toxicity. Target **3** was frankly toxic at a single dose of 50 mg/kg (LD_{80}) but was well-tolerated at a dosage of 25 mg/kg/dose (LD_{10} or less) and also at a dosage of 12.5 mg/kg/dose for 5 days. Compounds **9** and **24** were more toxic, yielding LD_{100} at dosages of 10 and 5 mg/kg/dose for 5 days, respectively. It has been reported that the substituted *N*-(2-chloroethyl)-*N*-nitrosocarbamates are rapidly hydrolyzed at the ester function in vivo leading to the higher toxicity and mutagenicity of this class of alkylating agent relative to the substituted *N*-(2-chloroethyl)-*N*-nitrosoureas.[12] Although these data are reported only for simple, linear, alkyl-substituted *N*-(2-chloroethyl)-*N*-nitrosocarbamates, the lack of in vivo activity and the toxicity profile of the agents reported herein suggest that these agents may also be subject to rapid hydrolysis in vivo.

Experimental Section

Chemistry. Examinations by TLC were performed on Analtech precoated (250 μm) silica gel GF plates. Column chromatographic purifications were done with silica gel (Merck, 60 A, 230−400 mesh for flash chromatography). Evaporations were performed with a rotary evaporator; higher boiling solvents (DMF, Me_2NAc, Me_2SO) were removed in vacuo (<1 mm, bath to 35 °C) and more volatile solvents with a water aspirator. Products were dried in vacuo (<1 mm) at 22−25 °C over P_2O_5 and NaOH pellets. Analytical results indicated by element symbols were within ±0.4% of the theoretical values, and where solvents are indicated in the formula, their presence was confirmed by ^1H NMR. Spectral determinations and elemental analyses were performed in the Molecular Spectroscopy Section of the Southern Research Institute under the direction of Dr. J. M. Riordan. The ^1H NMR spectra were determined with a Nicolet NMC 300 NB spectrometer using Me_4Si as internal reference. Chemical shifts (δ) listed for multiplets were measured from the approximate centers, and relative integrals of peak areas agreed with those expected for the assigned structures. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the fast-atom-bombardment (FAB) mode. Infrared spectra were recorded with a Nicolet 10 DX spectrometer using samples in KBr disks. NOCl was prepared fresh for each reaction by the method of Becham.[9] Note that all target compounds were carefully dried and stored under argon at −20 °C until used for screening.

Preparation of Carbamic Acid, (2-Chloroethyl)-4-acetoxybenzyl Ester (2). Compound **1**[8] [2.1 g (11.5 mmol)] was dissolved in 10 mL of anhydrous CH_2Cl_2. The reaction was cooled to 0 °C. Et_3N (106 μL) and then 2-chloroethyl isocyanate 1.07 mL (12.6 mmol) were added slowly. The reaction mixture was stirred for 48 h at ambient temperature and concentrated to dryness with a rotary evaporator. The resulting oil was dissolved in a minimum of CH_2Cl_2 and purified by column chromatography (silica gel 230−400 mesh) using CH_2Cl_2. The desired fractions were collected, concentrated and dried in vacuo over P_2O_5 to give an oil that solidified in the freezer: yield 3.1 g (90.3%); mp 61−63 °C; MS *m*/*z* 272 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 2.31 (S, 3H, CH_3), 3.54 (q, 2H, CH_2N), 3.61 (t, 2H, CH_2Cl), 5.1 (S, 2H, CH_2O), 5.16 (bs, 1H, NH), 7.08 (m, 2H, H-3,5), 7.37 (m, 2H, H-2,6); IR cm^{-1} 913, 1040, 1144, 1192, 1206, 1222, 1269, 1437, 1510, 1550, 1704, 1756. Anal. ($C_{12}H_{14}ClNO_4$) CHN.

Carbamic Acid, (2-Chloroethyl)nitroso-4-acetoxybenzyl Ester (3). Compound **2** [1.61 g (5.9 mmol)] was dissolved in 15 mL of anhydrous pyridine. The reaction mixture was cooled to −20 °C, and freshly prepared nitrosyl chloride (5.7 mL) was added dropwise. The resulting solution was stirred at −20 °C for 2 h, and the reaction mixture was then poured into ice-cold water. The mixture was extracted with CH_2Cl_2 (2 × 100 mL), and the extract was dried over $MgSO_4$ and concentrated to dryness. The residual yellow oil was purified by column chromatography (silica gel 230−400 mesh, elution with CH_2Cl_2). The desired fractions were combined, concentrated and dried in vacuo over P_2O_5 to give a yellow oil that solidified in the freezer: yield 1.6 g (89.8%); mp 48−50 °C; MS *m*/*z* 301 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 2.31 (S, 3H, CH_3), 3.45 (t, 2H, CH_2Cl), 4.08 (t, 2H, CH_2N), 5.5 (S, 2H, CH_2O), 7.13 (m, 2H, H-3,5), 7.5 (m, 2H, H-2,6); IR cm^{-1} 910, 1076, 1139, 1153, 1217, 1347, 1369, 1394, 1510, 1760. Anal. ($C_{12}H_{13}ClN_2O_5$) CHN.

Table 1. Cytotoxicity of Selected Drugs in the Core in Vitro Tumor Panel

			IC_{50} values in μg/mL[a] (IC_{50} molar value)				
agent	name	MW (g)	CAKI-1 (renal)	DLD-1 (colon)	NCI-H23 (lung)	SK-MEL-28 (melanoma)	SNB-7 (CNS)
3	carbamic acid, (2-chloroethyl)-*N*-nitroso-4-acetoxy-1-benzyl ester	300.69	2×10^1 (7×10^{-5})	2×10^1 (7×10^{-5})	6 (2×10^{-5})	1×10^1 (3×10^{-5})	3×10^1 (1×10^{-4})
8	carbamic acid, (2-chloroethyl)-*N*-nitroso-4-nitro-1-benzyl ester	287.65	3 (1×10^{-5})	5 (2×10^{-5})	1 (3×10^{-6})	1 (3×10^{-6})	1 (3×10^{-6})
9	carbamic acid, (2-chloroethyl)-*N*-nitroso-2-nitro-1-benzyl ester	287.65	4×10^{-1} (1×10^{-5})	6 (2×10^{-5})	3 (1×10^{-5})	4×10^{-1} (1×10^{-7})	1 (3×10^{-6})
11	carbamic acid, (2-chloroethyl)-*N*-nitroso-5-pyrimidine ester	230.60	6 (3×10^{-5})	4×10^1 (2×10^{-4})	2 (9×10^{-6})	3×10^1 (1×10^{-4})	6 (3×10^{-5})
20	carbamic acid, (2-chloroethyl)-*N*-nitroso-3-pyridine ester	229.62	$>3 \times 10^1$ ($>1 \times 10^{-4}$)	$>3 \times 10^1$ ($>1 \times 10^{-4}$)	$>3 \times 10^1$ ($>1 \times 10^{-4}$)	$>3 \times 10^1$ ($>1 \times 10^{-4}$)	3×10^1 (1×10^{-4})
24	carbamic acid, (2-chloroethyl)-*N*-nitroso-2,3,4,6-tetra-*O*-acetyl-1-D-glucopyranose ester	482.82	$>3 \times 10^1$ ($>6 \times 10^{-5}$)	2×10^1 (4×10^{-5})	3 (6×10^{-6})	1×10^1 (2×10^{-5})	1 (2×10^{-6})
18	carbamic acid, (2-chloroethyl)-*N*-nitroso-2-acetoxy-1-benzyl ester	300.69	3×10^1 (1×10^{-4})	1×10^1 (3×10^{-5})	1×10^1 (3×10^{-5})	2×10^1 (7×10^{-5})	6 (2×10^{-5})
Ara-C	cytosine arabinoside	279.68	$>5^b$	$>5^b$	1×10^{-1} [b]	1^b 2^b	1×10^{-1} [b] 9×10^{-2} [b]

[a] 72-h exposure to compound. [b] IC_{50} values are in μM.

Table 2. Summary of the in Vivo Antitumor Activity of Carbamic Acid, (2-Chloroethyl)-*N*-nitroso-4-acetoxy-1-benzyl Ester (**3**)

murine tumor[a]	optimal ip dosage (≤LD10) (mg/kg/dose)	schedule	median % ILS (dying mice only)	net log cell kill	T-C[b] (days)	tumor-free surv/total
ip P388 leukemia	25	day 1	+4	−0.3	−	0/5
sc M5076 sarcoma[c]	12.5	days 10−14	−	−	3.0	0/5

[a] $CD2F_1$ mice were implanted ip with 10^6 murine P388 leukemia cells, whereas $B6C3F_1$ mice were implanted sc with 5×10^6 murine M5076 sarcoma cells. [b] The difference in the median of times poststaging for tumors of the treated (T) groups to double in mass twice compared to the median of the control (C) group. [c] Both compounds **9** and **24** were evaluated as well and were inactive (data not shown).

Carbamic Acid, (2-Chloroethyl)-4-nitrobenzyl Ester (6). Compound **6** was prepared from 4-nitrobenzyl alcohol **(4)** (2.0 g, 13.05 mmol) over a period of 86 h using the procedure described for the preparation of **2**: yield after column chromatography ($CHCl_3$) 2.97 g (88%); mp 73−75 °C; MS *m*/*z* 259 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.58 (q, 2H, C*H*$_2$N), 3.65 (t, 2H, C*H*$_2$Cl), 5.21 (s, 2H, C*H*$_2$O), 5.26 (bs, 1H, N*H*), 7.52 (d, 2H, H-3,5), 8.23 (d, 2H, H-2,6); IR cm^{-1} 740, 847, 1016, 1075, 1157, 1259, 1273, 1312, 1350, 1373, 1458, 1510, 1548, 1605, 1690. Anal. ($C_{10}H_{11}ClN_2O_4$) CHN.

Carbamic Acid, (2-Chloroethyl)-2-nitrobenzyl Ester (7). The same procedure as described for **6** was used to prepare **7** from 2-nitrobenzyl alcohol **(5)** (2.25 g, 14.69 mmol): yield 3.42 g (90%); mp 67−69 °C; MS *m*/*z* 259 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.58 (q, 2H, C*H*$_2$N), 3.65 (t, 2H, C*H*$_2$Cl), 5.3 (bs, 1H, N*H*), 5.55 (s, 2H, C*H*$_2$O), 7.66 (m, 1H, H-3) 7.64 (m, 2H, H-4,5), 8.2 (d, 1H, H-6); IR cm^{-1} 656, 724, 749, 1003, 1073, 1153, 1197, 1263, 1273, 1318, 1348, 1436, 1465, 1520, 1545, 1693. Anal. ($C_{10}H_{11}ClN_2O_4$) CHN.

Carbamic Acid, (2-Chloroethyl)nitroso-4-nitrobenzyl Ester (8). The general procedure previously described for **3** was used to prepare **8** using **6** (2.96 g, 11.44 mmol). In this case, the reaction was stirred at −20 °C for 2 h and at −10 °C for 3 h. After column chromatography (6:4 hexanes:CH_2Cl_2), a yellow oil was obtained that solidified in the freezer: yield 3.0 g (90%); mp 27−29 °C; MS *m*/*z* 288 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.7 (t, 2H, C*H*$_2$Cl), 4.13 (t, 2H, C*H*$_2$N), 5.62 (s, 2H, C*H*$_2$O), 7.65 (d, 2H, H-3,5), 8.68 (d, 2H, H-2,6); IR cm^{-1} 767, 856, 1077, 1146, 1302, 1346, 1395, 1524, 1751. Anal. ($C_{10}H_{10}ClN_3O_5$) CHN.

Carbamic Acid, (2-Chloroethyl)nitroso-2-nitrobenzyl Ester (9). The same procedure previously described in **3** and **8** was used to prepare **9** from **7** (3.00 g, 11.59 mmol), and the reaction was stirred at −20 °C for 2 h and at −10 °C for 5 h. After column chromatography (6:4 hexanes:CH_2Cl_2) a yellow syrup was obtained: yield 3.10 g (93.09%); MS *m*/*z* 288 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.51 (t, 2H, C*H*$_2$Cl), 4.15 (t, 2H, C*H*$_2$N), 5.97 (s, 2H, C*H*$_2$O), 7.56 (m, 1H, H-4), 7.74 (m, 2H, H-3,5), 8.25 (m, 1H, H-6); IR cm^{-1} 729, 789, 1075, 1153, 1303, 1343, 1396, 1425, 1528, 1755. Anal. ($C_{10}H_{10}ClN_3O_5$) CHN.

Carbamic Acid, (2-Chloroethyl)-5-pyrimidine Ester (10). 5-Hydroxypyrimidine[10] (340 mg, 3.5 mmol) was reacted under the same conditions as those described for the preparation of **2**. The product was purified using column chromatography (99:1 CH_2Cl_2:MeOH): yield 508 mg (71.2%); mp 97−98 °C; MS *m*/*z* 202 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.7 (m, 4H, C*H*$_2$C*H*$_2$Cl), 5.61 (bs, 1H, N*H*), 8.66 (s, 2H, H-4,6), 9.7 (S, 1H, H-2); IR cm^{-1} 620, 1256, 1265, 1277, 1418, 1559, 1572, 1737, 1746. Anal. ($C_7H_8ClN_3O_2 \cdot 0.3H_2O$) CHN.

Carbamic Acid, (2-Chloroethyl)nitroso-5-pyrimidine Ester (11). Compound **11** was prepared from **10** (440 mg, 2.18 mmol) by the similar procedure as previously described for the preparation of **3**. The product was purified using column chromatography (99:1 CH_2Cl_2:MeOH), and a yellow oil was obtained that solidified in the freezer: yield 70 mg (14%); mp 78−80 °C; MS *m*/*z* 231 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.57 (t, 2H, CH_2Cl), 4.20 (t, 2H, C*H*$_2$N), 8.87 (s, 2H, H-4,6), 9.21 (s, 1H, H-2); IR cm^{-1} 719, 746, 972, 1078, 1092, 1125, 1224, 1301, 1411, 1517, 1571, 1770. Anal. Calcd for $C_7H_7ClN_4O_3$: C, 36.46; H, 3.05; N, 24.29. Found: C, 36.84; H, 3.12; N, 23.61.

Benzenemethanol, 2-[(Tetrahydro-2*H*-pyran-2-yl)oxy]-, Acetate (13). A solution of 2-acetyl salicylate[11] (7.3 g, 40.00 mmol) and dihydropyran (5.29 mL) in anhydrous CH_2Cl_2 (9 mL) containing pyridinium *p*-toluenesulfonate (PPTS; 1.094 g; 4.3 mmol) was stirred at room temperature for 4.5 h. The solution was diluted with ether and washed with brine solution to remove the catalyst. The organic layer was dried over $MgSO_4$ and concentrated to dryness. The residual product was purified by column chromatography (silica gel 230−400 mesh) and was eluted first with cyclohexane followed by 11:1 cyclohexane:EtOAc. The desired fractions were combined, concentrated, and dried in vacuo over P_2O_5 to give an oil that solidified in the freezer: yield 8.82 g (88%); mp 47−49 °C; MS *m*/*z* 251 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 1.70 (m, 3H, two H-4's, one H-3 of THP ring), 1.87 (m, 2H, H-2 of THP ring), 2.17 (m, 1H, H-3 of THP ring), 2.11 (s, 3H, C*H*$_3$), 3.61 (m, 1H, H-5 of THP ring), 3.88 (m, 1H, H-5 of THP ring), 5.21 (S, 2H, C*H*$_2$−O), 5.29 (t, 1H, H-1 of THP ring), 6.98 (dt, 1H, H-5), 7.14 (dd, 1H, H-3), 7.26−7.35 (m, 2H, H-4, H-6).

Benzenemethanol, 2-[(Tetrahydro-2*H*-pyran-2-yl)oxy]- (14). Compound **13,** 2.23 g (8.9 mmol), in 20 mL of ethanol was saponified with 19 mL of ethanolic NaOH (10 mL of 2 N NaOH in 10 mL of EtOH) for 1 h at 18 °C. The resulting solution was evaporated to dryness and the product extracted

with ether (50 mL), washed with water, dried over $MgSO_4$ and concentrated to dryness. The residue was applied to a column of silica gel (230−400 mesh) and eluted with cyclohexanes−EtOAc (6:1). Fractions containing the product **14** were combined, concentrated and dried in vacuo to give an oil: yield 1.7 g (91.9%); MS *m/z* 209 $(M + H)^+$.

Carbamic Acid, (2-Chloroethyl)-2-[(tetrahydro-2*H*-pyran-2-yl)oxy]benzyl Ester (15). The same procedure was previously described in **2** was used to prepare **15** from **14** (1.68 g, 8.0 mmol). After column chromatography (6:1 cyclohexanes−EtOAc), an oil was obtained: yield 2.48 g (98%); MS *m/z* 314 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 1.64 (m, 3H, two H-4's, one H-3 of THP ring), 1.86 (m, 2H, H-2's of THP ring), 2.00 (m, 1H, H-3 of THP ring), 3.49 (m, 5H, H-5 of THP ring and CH_2CH_2-Cl), 3.87 (dt, 1H, H-5 of THP ring), 5.15 (bs, 1H, N*H*), 5.23 (s, 2H, CH_2-O), 5.28 (bt, 1H, H-1 of THP ring), 6.98 (t, 1H, H-5), 7.15 (d, 1H, H-3), 7.24−7.35 (m, 2H, H-4, H-6); IR cm^{-1} 756, 922, 965, 1022, 1037, 1124, 1202, 1240, 1457, 1493, 1532, 1705, 1721, 2946. Anal. ($C_{15}H_{20}ClNO_4$) CHN.

Carbamic Acid, (2-Chloroethyl)-2-hydroxy-1-benzyl Ester (16). To a solution of **15** [7.25 g (23.10 mmol)] in anhydrous ethanol (30 mL) was added 611 mg (2.4 mmol) of PPTS. The reaction mixture was stirred at 50 °C for 4.5 h and concentrated to dryness. The residue was chromatographed ($CHCl_3$). The desired fractions were collected, concentrated, and dried in vacuo over P_2O_5 to give an oil that solidified upon freezing: yield 4.35 g (82%); mp 65−67 °C; MS *m/z* 330 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 2.32 (1bs, 1H, O*H*), 3.62 (m, 2H, CH_2-N), 3.69 (m, 2H, -CH_2-Cl), 4.60 (s, 2H, -CH_2-O), 5.61 (bs, 1H, N*H*), 7.11 (dd, 1H, J = 0.6 Hz, J = 8 Hz, H-3), 7.26 (dt, 1H, H-5), 7.34 (dt, 1H, H-4), 7.48 (dt, 1H, J = 0.8 Hz, J = 6 Hz, H-6); IR cm^{-1} 1050, 1182, 1226, 1249, 1271, 1487, 1540, 1711, 3319. Anal. ($C_{10}H_{12}ClNO_3$) CHN.

Carbamic Acid, (2-Chloroethyl)-2-acetoxy-1-benzyl Ester (17). Compound **16**, 4.33 g (18.85 mmol), was dissolved in anhydrous pyridine (12 mL), and the mixture was cooled to 0 °C. Acetic anhydride (1.77 mL) was added dropwise. The resulting solution was stirred overnight at ambient temperature. The mixture was poured into ice-cold water, and the product was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated to dryness, and the residual oil was purified by column chromatography ($CHCl_3$) to give an oil: yield 4.66 g (91%); MS *m/z* 272 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3H, CH_3), 3.63 (m, 2H, CH_2N), 3.69 (m, 2H, -CH_2Cl), 5.12 (s, 2H, CH_2-O), 5.55 (bs, 1H, N*H*), 7.18−7.26 (m, 2H, H-4, H-6), 7.37 (t, 1H, H-5), 7.43 (d, 1H, H-3); IR cm^{-1} 762, 952, 1028, 1041, 1122, 1184, 1222, 1246, 1310, 1363, 1381, 1438, 1455, 1490, 1532, 3327, 3332. Anal. ($C_{12}H_{14}ClNO_4 \cdot 0.1H_2O$) CHN.

Carbamic Acid, (2-Chloroethyl)nitroso-2-acetoxy-1-benzyl Ester (18). The same procedure as described for the preparation of **3** was used to prepare **18** from **17** (2.25 g, 8.28 mmol). The reaction was carried out at −20 °C for 0.5 h followed by −10 °C for an additional 2 h. The product was purified using column chromatography (CH_2Cl_2) to give a yellow oil: yield 2.39 g (95.6%); MS *m/z* 301 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3H, CH_3), 3.57 (t, 2H, CH_2Cl), 4.20 (t, 2H, CH_2N), 5.18 (s, 2H, CH_2O), 7.33−7.38 (m, 2H, H-4, H-6), 7.24−7.55 (m, 2H, H-3, H-5); IR cm^{-1} 754, 1072, 1123, 1141, 1181, 1228, 1377, 1742, 1764. Anal. ($C_{12}H_{13}ClN_2O_5$) CHN.

Carbamic Acid, (2-Chloroethyl)-3-pyridine Ester (19). Compound **19** was prepared using the procedure as described for the preparation of **2** from 3-hydroxypyridine (1.42 g, 14.9 mmol). The mixture was allowed to react for 24 h. After column chromatography ($CHCl_3$), an oil was obtained that solidified upon freezing: yield 2.53 g (84.6%); mp 47−49 °C; MS *m/z* 201 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.63−3.73 (m, 4H, -CH_2CH_2-), 5.66 (bs, 1H, N*H*), 7.33 (dd, 1H, H-5, J = 5.1 Hz, J = 8.3 Hz), 7.46 (ddd, 1H, H-4, J = 5.1 Hz, J = 8.3 Hz), 8.48 (m, 2H, H-2, H-6); IR cm^{-1} 703, 1026, 1188, 1216, 1247, 1426, 1476, 1534, 1744, 3322, 3327. Anal. ($C_8H_9ClN_2O_2$) CHN.

Carbamic Acid, (2-Chloroethyl)nitroso-3-pyridine Ester (20). The same procedure as described for the preparation of **3** was used to prepare **20** from **19** (2.5 g, 12.4 mmol). The reaction was carried out at −20 °C for 0.5 h followed by −10 °C for an additional 2 h. After column chromatography (CH_2Cl_2), a yellow oil was obtained: yield 1.62 g (56.6%); MS *m/z* 230 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.57 (t, 2H, CH_2Cl), 4.20 (t, 2H, CH_2-N), 7.44 (ddd, 1H, H-5, J = 0.6 Hz, 4.7 Hz, 8.4 Hz), 7.72 (ddd, 1H, H-4, J = 1.4 Hz, 2.8 Hz, 8.4 Hz), 8.61 (dd, 1H, H-6, J = 1.4 Hz, 4.7 Hz), 8.68 (brd, 1H, J = 2.8 Hz); IR cm^{-1} 704, 750, 795, 813, 959, 971, 1071, 1097, 1110, 1186, 1209, 1302, 1356, 1376, 1431, 1521, 1525. Anal. ($C_8H_8ClN_2O_3 \cdot 0.12H_2O$) CHN.

Carbamic Acid, (2-Chloroethyl)-2,3,4,6-tetra-*O*-benzyl-1-α,*β*-D-glucopyranose Ester (21). Compound **21** was prepared from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (2.5 g, 4.6 mmol), 2-chloroethyl isocyanate (0.42 mL) and Et_3N (125 μL) by the same procedure as described for the preparation of **2**. The mixture was allowed to react for 24 h. The product was purified by column chromatography ($CHCl_3$): yield 2.6 g (87%); mp 144−146 °C; MS *m/z* 646 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 3.48−3.96 (m's, 10H, CH_2CH_2 and H-2, H-3, H-4, H-5), 4.44−4.99 (m, 8H, four C_6H_5 CH_2's), 5.09 (t, 0.75H, NH_β), 5.26 (t, 0.25H, NH_α), 5.54 (d, 0.75H, H-1$_\beta$, J = 8.1 Hz), 6.24 (d, 0.25H, H-1$_\alpha$, J = 3.5 Hz) 7.12 (m, 2H, aromatic *H*'s), 7.25−7.35 (m, 18H, aromatic *H*'s); IR cm^{-1} 693, 716, 748, 1003, 1018, 1029, 1051, 1072, 1085, 1106, 1119, 1151, 1270, 1453, 1558, 1702, 2874, 2917, 3030, 3319. Anal. ($C_{37}H_{40}ClNO_7 \cdot 0.02CHCl_3$) CHN.

Carbamic Acid, (2-Chloroethyl)-2,3,4,6-tetra-*O*-hydroxyl-1-α,*β*-D-glucopyranose Ester (22). To a suspension of compound **21** [3.62 g (5.6 mmol)] in 150 mL of anhydrous ethanol was added 5% Pd/C (1.8 g). The suspension was hydrogenated at atmospheric pressure at 40 °C for 24 h, filtered and concentrated to dryness. The residue in a minimum of MeOH was applied to a column packed with silica gel (230−400 mesh). The column was first eluted with $CHCl_3$ followed by $CHCl_3$:MeOH (6:1). The desired fractions were combined, concentrated and dried in vacuo over P_2O_5: yield 1.36 g (85%); mp 127−129 °C; MS *m/z* 286 $(M + H)^+$; ^{1}H NMR (Me_2SO-*d*) δ 3.04−3.68 (m, H-2, H-3, H-4, H-5, H-6, CH_2CH_2), 4.53 (t, 0.3H, 6-OH$_\alpha$), 4.60 (t, 0.7H, 6-OH$_\beta$), 4.94, 4.98, 5.07, 5.10, 5.19 (five d's, 3H, 2-OH, 3-OH, 4-OH of α and β anomers), 5.26 (d, 0.7H, H-1$_\beta$, $J_{1,2}$ = 8.0 Hz), 5.83 (d, 0.3H, H-1α, $J_{1,2}$ = 3.3 Hz), 7.54 (t, 0.3H, NH_α), 7.64 (t, 0.7H, NH_β); IR cm^{-1} 1078, 1713. Anal. ($C_9H_{16}ClNO_7 \cdot 0.1C_2H_5OH$) CHN.

Carbamic Acid, (2-Chloroethyl)-2,3,4,6-tetra-*O*-acetyl-1-α,*β*-D-glucopyranose Ester (23). Compound **22**, 800 mg (2.8 mmol), was dissolved in 5 mL of anhydrous pyridine, and the resulting solution was cooled in an ice bath. Acetic anhydride (0.96 mL) was added dropwise, and the solution was stirred at ambient temperature overnight. The reaction mixture was poured into ice-cold water, the product was extracted with CH_2Cl_2 (2 × 30 mL), and the organic layer was dried over $MgSO_4$ and concentrated to dryness. The crude product was chromatographed ($CHCl_3$). The desired fractions were collected, concentrated and dried in vacuo over P_2O_5: yield 930 mg (73.2%); mp 52−54 °C; MS *m/z* 460 $(M + Li)^+$; ^{1}H NMR ($CDCl_3$) δ 2.02, 2.03, 2.04, 2.05, 2.09, 2.10 (six s's, 12H, α, β CH_3's), 3.51−3.67 (m, 4H, -CH_2CH_2-), 3.84 (ddd, 0.3H, H-5$_\beta$, J = 2.2 Hz, 4.3 Hz, 9.9 Hz), 4.07−4.14 (m, 1.7H, H-5$_\alpha$, H-6′$_\beta$, H-6′$_\alpha$), 4.28 (dd, 0.3H, H-6$_\alpha$, $J_{5,6}$ = 4.5, $J_{6,6'}$ = 12.8 Hz), 4.32 (dd, 0.7H, H-6$_\beta$, $J_{5,6'}$ = 4.4 Hz, $J_{6,6'}$ = 12.5 Hz), 5.07−5.18 (m, 2H, H-2$_\alpha$, H-2$_\beta$, H-4$_\alpha$, H-4$_\beta$), 5.26 (t, 0.7H, H-3$_\beta$, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz), 5.32 and 5.34 (two t's, 1H, N*H*), 5.47 (t, 0.3H, H-3$_\alpha$, $J_{2,3}$ = $J_{3,4}$ = 9.9 Hz), 5.67 (d, 0.7H, H-1$_\beta$, $J_{1,2}$ = 8.3 Hz), 6.25 (d, 0.3H, H-1$_\alpha$, $J_{1,2}$ = 3.7 Hz); IR cm^{-1} 1039, 1077, 1117, 1165, 1235, 1369, 1438, 1534, 1755, 3377, 3412. Anal. ($C_{17}H_{24}ClNO_{11} \cdot 0.05CHCl_3$) CHN.

Carbamic Acid, (2-Chloroethyl)nitroso-2,3,4,6-tetra-*O*-acetyl-1-α,*β*-D-glucopyranose Ester (24) Using 23 (912 mg, 2.0 mmol). The same procedure as described for the preparation of **3** was used to prepare **24**. The reaction was carried out at −20 °C for 1.5 h followed by −10 °C for an additional 1.5 h. After column chromatography (CH_2Cl_2), a pale, yellow foam was obtained upon drying in vacuo: yield 458 mg (47.2%); mp 38−40 °C; MS *m/z* 489 $(M + Li)^+$; ^{1}H NMR ($CDCl_3$) δ 2.03, 2.04, 2.05, 2.06, 2.09, 2.10 (six s's, 12H, α and β, CH_3's), 3.44−

3.55 (m, 2H, CH_2Cl), 3.94−4.36 (m, 5H, H-5$_{\alpha,\beta}$, H-6$_{\alpha,\beta}$, H-6′$_{\alpha,\beta}$, CH_2-N), 5.17−5.53 (m, 2.5H, H-2$_\alpha$, H-4$_\alpha$, H-2$_\beta$, H-3$_\beta$, H-4$_\beta$), 5.52 (t, 0.5H, H-3$_\alpha$, J = 9.6 Hz, J = 10.1 Hz), 5.99 (d, 0.5H, H-1$_\beta$, $J_{1,2}$ = 7.9 Hz), 6.63 (d, 0.5H, H-1$_\alpha$, $J_{1,2}$ = 3.6 Hz); IR cm^{-1} 1039, 1221, 1756. Anal. ($C_{17}H_{23}ClN_2O_{12}$) CHN.

Biological Evaluation. 1. In Vitro Evaluation of Cytotoxicity. Cell lines: The cell lines CAKI-1 (renal), DLD-1 (colon), NCI-H23 (lung), SK-MEL-28 (melanoma), and SNB-7 (CNS) were propagated using standard tissue culture techniques in RPMI 1640 media with 10% FBS, and 2 mmol L-glutamine at 37 °C with 5% CO_2 and humidity. For each experiment, cells were dispersed with trypsin/EDTA, suspended at 5×10^4/mL, and seeded in 96-well plates at 5×10^3 μL. Compounds were dissolved in 100% DMSO, diluted in complete media, and then added to replicate wells in 100-μL samples. For each concentration level, eight samples were prepared. Controls included mock treatment, positive control (doxorubicin, 200 μM), and vehicle control of media diluted with DMSO. The plates were incubated as described for 72 h and analyzed with either the XTT assay[13] or the neutral red dye uptake procedure.[14] The data were processed using Lotus 1-2-3 to calculate the IC_{50} values (Table 1).

2. In Vivo Evaluation of Antitumor Activity. For the in vivo evaluation of the sensitivity of transplantable murine tumors to the compound, $CD2F_1$ mice were implanted ip with 10^6 P388 leukemia cells, whereas $B6C3F_1$ mice were implanted sc with 5×10^6 M5076 sarcoma cells. Tumor implantation day was designated day 0. In each experiment, the compound(s) was administered ip at several dosage levels.

For P388 leukemia, antitumor activity (Table 2) was assessed on the basis of percent (%) median ILS (increase in life span) and net log cell kill. Calculations of net log cell kill were made using the tumor doubling time that was based on historical data. To assess tumor cell kill at the end of treatment, the survival time difference between treated and control groups was adjusted to account for regrowth of tumor cell populations that may occur between individual treatments.

For M5076 sarcoma, antitumor activity (Table 2) was assessed on the basis of delay in tumor growth (T-C). The delay in tumor growth is the difference in the median of times poststaging for tumors of the treated (T) and control (C) groups to double in mass two times. Drug deaths and any animal whose tumor failed to attain the evaluation size were excluded.

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