Synthesis and Chemical Reactivity of C-5 Substituted 6,7-Bis-(hydroxymethyl)-1*H*-pyrrolizine Biscarbamate Tumor Inhibitors [1]

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A series of C-5 substituted pyrrolizine biscarbamate alkylating agents were synthesized and evaluated in an *in vitro* alkylation assay. Introduction of electron-withdrawing substituents at the C-5 position resulted in a dramatic decrease in chemical reactivity toward the model nucleophile 4-(4-nitrobenzyl)pyridine (NBP).

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The acylated vinylogous carbinolamine tumor inhibitors refer to a class of chemically reactive nitrogen heterocycles that have demonstrated significant and reproducible antineoplastic activity in a number of tumor models [3,4]. The pyrrolizine biscarbamate **1** is representative of this class and acts as a bifunctional alkylating agent where both carbamate moieties serve as leaving groups in an *O*-alkyl ester cleavage reaction with the incipient positive charge stabilized by electron delocalization through the π-excessive pyrrole ring. Previous structure-activity relationship studies have shown that electron-withdrawing substitutents on the C-5 phenyl ring produce analogs that retain antitumor activity while also resulting in decreased acute and continuing toxicity as evidenced by an increase in number of toxicity day survivors as well as a reduction in the degree of animal weight loss [3,5]. As part of this ongoing research we prepared a series of pyrrolizine biscarbamates where the C-5 position of the pyrrole ring was directly substituted with an electron-withdrawing substituent (Figure 1). Removal of the phenyl ring and direct substitution of the C-5 position should result in a more pronounced substituent effect and provide an effective means of controlling the chemical reactivity of this class of compounds. This report describes the synthesis and chemical reactivity toward nucleophiles of the C-5 substituted pyrrolizine biscarbamates, **2a-2f**.

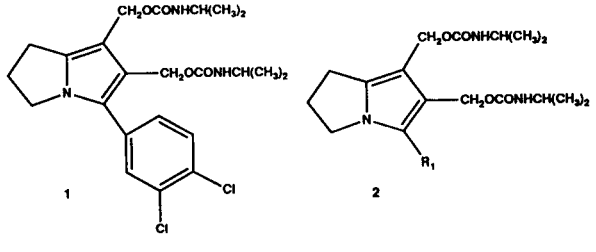
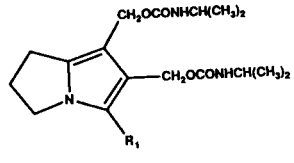


Figure 1. General structures of the analogs chosen for study.

Chemistry.

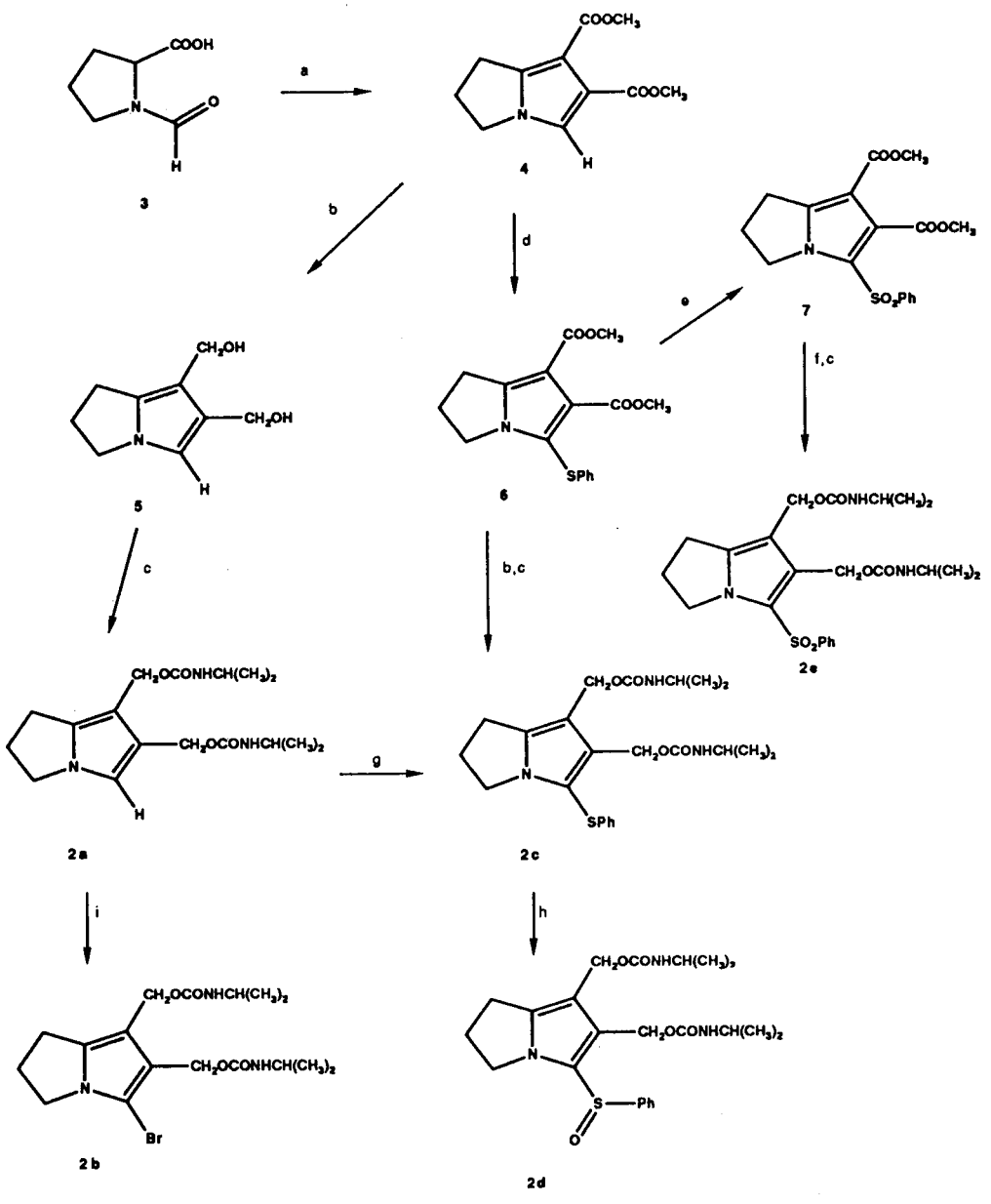
The structures of the analogs chosen for study and the physico-chemical properties of the C-5 substituent are given in Table 1. The unsubstituted dimethyl 1,3-dihydropyrrolizine-6,7-dicarboxylate, **4**, [7] was prepared *via* the 1,3-dipolar cycloaddition reaction with dimethyl acetylenedicarboxylate (DMAD) and the mesoionic oxazolone intermediate generated *in situ* by acetic anhydride mediated cyclodehydration of *N*-formylproline [8]. Reduction of **4** with lithium aluminum hydride followed by treatment of the crude diol, **5**, with 2-propyl isocyanate and di-*n*-butyltin diacetate gave the corresponding biscarbamate, **2a**. Treatment of **4** with phenylsulfenyl chloride [9] in dichloromethane gave **6** in high yield (88%). Reduction of **6** and carbamoylation as described above afforded the corresponding biscarbamate, **2c**. Treatment of **2a** with phenylsulfenyl chloride in the presence of an acid scavenger (triethylamine) also gave **2c** in moderate yield (78%). Similarly, treatment of **2a** with bromine in the presence of a slight excess of triethylamine afforded **2b** in 65% yield. Oxidation of the sulfur atom of **2c** with *m*-chloroperoxybenzoic acid in a biphasic mixture of dichloromethane and 0.5 *M* sodium bicarbonate solution gave the sulfoxide biscarba-

Table 1. Structures and Physico-chemical Parameters of Analogs **2a-2f** [6].



compound	R_1	π	σ_p	F	R
2a	H	0.00	0.00	0.00	0.00
2b	Br	0.86	0.23	0.44	-0.17
2c	SPh	2.32	0.18	-	-
2d	SOPh	-	-	-	-
2e	SO_2Ph	0.27	0.70	0.56	0.18
2f	SO_2CH_3	-1.63	0.72	0.54	0.22

SCHEME 1 [a]

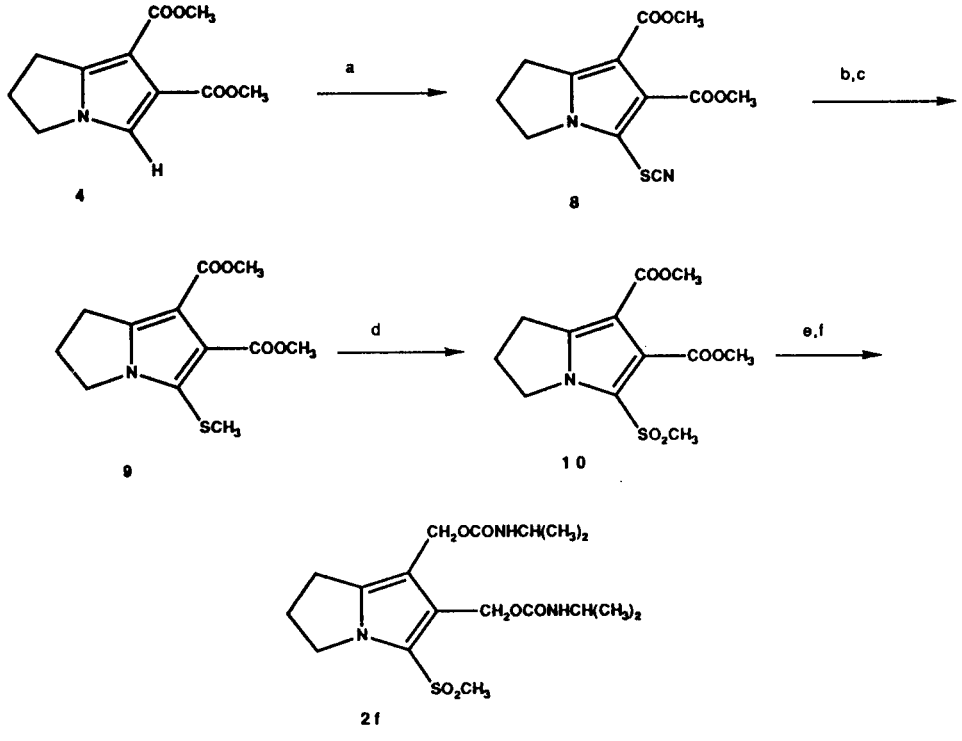


[a] Reagents: a: Ac_2O/DMAD; b: $LiAlH_4$/THF; c: $(CH_3)_2CHNCO/(n\text{-}Bu)_2Sn(OAc)_2/CH_2Cl_2$; d: $PhSCl/CH_2Cl_2$; e: CH_3CO_3H/CH_3COOH; f: AlH_3/THF; g: $PhSCl/Et_3N/CH_2Cl_2$; h: MCPBA/CH_2Cl_2; i: $Br_2/Et_3N/CH_2Cl_2$.

mate, **2d**. The phenylsulfonyl analog **2e** was prepared *via* peracetic acid oxidation of **6** to give the corresponding sulfone diester, **7**; reduction of the ester groups with aluminum hydride [10] followed by carbamoylation gave the corresponding biscarbamate in moderate yield (30% overall from **6**).

The synthesis of the methylsulfonyl biscarbamate analog is outlined in Scheme 2. Treatment of **4** with thiocyanogen chloride gave the corresponding thiocyano diester, **8**. Selective reduction of the thiocyano group of **8** with sodium borohydride in ethanol followed by alkylation of the crude thiol with iodomethane afforded the methyl-

SCHEME 2 [a]



[a] Reagents: a: ClSCN/CH_3COOH; b: $NaBH_4$/EtOH; c: KOH/CH_3I; d: CH_3CO_3H/CH_3COOH; e: AlH_3/THF; f: $(CH_3)_2CHNCO/(n\text{-}Bu)_2Sn(OAc)_2$.

thio diester, **9**. Peracetic acid oxidation of **9** followed by aluminum hydride reduction and carbamoylation of the crude diol afforded **2f** in an overall yield of 40% from **9**.

Chemical Reactivity. 4-(4-Nitrobenzyl)pyridine Alkylation Assay.

The compounds prepared in this study were evaluated for chemical reactivity toward the model nucleophile 4-(4-nitrobenzyl)pyridine (NBP) [11]. The pyrrolizine biscarbamate **1** was also synthesized for comparison in this assay. The NBP assay provides a measure of the relative reactivity of a structurally similar group of alkylating agents and gives no indication of whether the analog is reacting as a bifunctional or monofunctional electrophile. The results of the NBP assay are given in Figure 2 and Table 2. As expected, the unsubstituted analog **2a** exhibited the highest reactivity with NBP (k′ = 2.79). The 3,4-dichlorophenyl analog, **1**, was less reactive than **2a** by a factor of 6.50. Direct substitution of the C-5 position resulted in a dramatic decrease in the chemical reactivity of the pyrrolizine biscarbamates. Substitution of the C-5 position with a bromine atom (**2c**) decreased the relative rate of alkylation by a factor of 41.0 when compared with **2a** and 6.3 when compared with **1**. Since the bromine substituent is both electron withdrawing by field effects and electron donating *via* resonance effects [6], the diminished chemical reactivity of **2b** relative to **2a** indicates that field effects may dominate over resonance effects in determining the relative rate of alkylation of the C-5 substituted analogs. Similarly, the phenylthio derivative was 68.0 and 10.5 times less reactive than analogs **2a** and **1** in the NBP assay. The oxidized sulfur analogs failed to react with NBP on prolonged heating (60° for >1 hour).

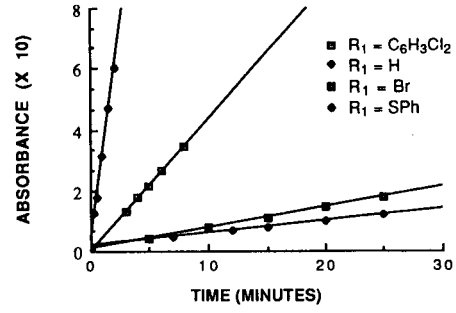


Figure 2. Results of the NBP Assay.

Table 2. Relative Reactivities of the Biscarbamates with NBP. [a]

compound	k' (E^{570}/min)	r^2	k'_H/k'_X	k'_1/k'_x
2a	2.79	0.999	1.00	0.15
1	0.430	0.998	6.50	1.00
2b	0.068	0.998	41.0	6.30
2c	0.041	0.998	68.0	10.5

[a] The value k' represents a pseudo-first-order rate constant representing relative rates of alkylation (see Experimental Section for a more detailed discussion). The value r^2 is the square of the correlation coefficient for the linear regression analysis.

Previous studies have shown the unsubstituted pyrrolizine biscarbamate **2a** to be less active and more toxic in the Murine P388 Lymphocytic Leukemia assay than the 3,4-dichlorophenyl derivative, **1** [7]. This difference in biological activity was attributed to the increased chemical reactivity of **2a** relative to **1**, a conclusion that is supported by the results of the NBP assay described above. Direct substitution of the C-5 position with either a bromine atom or a phenylthio group resulted in a marked reduction in the chemical reactivity of the pyrrolizine biscarbamate skeleton relative to both **2a** and **1**. Although a more pronounced substituent effect for the C-5 substituted analogs was expected, the total lack of chemical reactivity of analogs **2d-2f** even under forcing conditions was unexpected. Since **2b** and **2c** displayed alkylating activity in the NBP assay, both analogs are currently being evaluated for antileukemic activity. The lack of chemical reactivity of compounds **2d-2f** indicates an inability of these agents to function as alkylating agents and were not submitted for evaluation for antitumor activity.

EXPERIMENTAL

Melting points (uncorrected) were determined in an open capillary with a Thomas-Hoover Unimelt apparatus. The ir spectra were determined with either a Perkin-Elmer 727B spectrophotometer or a Nicolet FT-IR interferometer for Nujol mulls unless otherwise specified. The ^{1}H nmr spectra were determined on a Varian T-60A spectrometer. Microanalyses were performed by Atlantic Microlab, Atlanta, GA.

Dimethyl 2,3-Dihydro-5-phenylthio-1*H*-pyrrolizine-6,7-dicarboxylate (**6**).

A solution of phenylsulfenyl chloride (8.80 g, 60.8 mmoles) in dry dichloromethane (20 ml) was added dropwise to a stirred solution of dimethyl 2,3-dihydro-1*H*-pyrrolizine-6,7-dicarboxylate (**4**) (12.94 g, 57.9 mmoles) in dry dichloromethane (40 ml) and the reaction mixture was stirred at room temperature for 15 minutes. Volatile components were removed *in vacuo* to give a yellow oil that crystallized upon addition of cold ether. Recrystallization from acetone-isopropyl ether gave 16.88 g (88%) of **6** as long white needles, mp 105-106.5°; ^{1}H nmr (deuteriochloroform): δ 2.50 (m, 2H), 3.15 (t, 2H, J = 7 Hz), 3.70 to 4.0 (m, 8H), 7.15 (s, 5H); ir (potassium bromide): ν 2998 (CH), 2948, 1727 (C=O), 1706 (C=O), 1579, 1544, 1480, 1438, 1403, 1290, 1205, 1092, 979, 782, 738, 690 cm^{-1}.

Anal. Calcd. for $C_{17}H_{17}NO_4S$: C, 61.62; H, 5.17; N, 4.23. Found: C, 61.71; H, 5.20; N, 4.22.

Dimethyl 2,3-Dihydro-5-phenylsulfonyl-1*H*-pyrrolizine-6,7-dicarboxylate (**7**).

A solution of 31% hydrogen peroxide (6.62 ml, 60.4 mmoles) was added to a stirred solution of **6** (5 g, 15.1 mmoles) and concentrated sulfuric acid (0.1 ml) in glacial acetic acid (25 ml) and the reaction mixture was stirred at room temperature for 24 hours, and then heated at 50° for an additional 24 hours. The mixture was poured into water (150 ml), neutralized with solid sodium carbonate, and the aqueous solution extracted with dichloromethane (2 x 100 ml). The combined organic layers were dried (sodium sulfate) and concentrated *in vacuo* to give a white precipitate that was purified by column chromatography (silica gel-dichloromethane-ethyl acetate, 9:1) to give 2.86 g (52%) of **7**, mp 171-172°; ^{1}H nmr (deuteriochloroform): δ 2.50 (m, 2H), 3.10 (t, 2H, J = 7 Hz), 3.80 (s, 3H), 3.90 to 4.25 (m, 5H), 7.40 to 7.60 (m, 3H), 7.90 to 8.05 (m, 2H); ir (potassium bromide): ν 2948 (CH), 1748 (C=O), 1713 (C=O), 1537, 1501, 1438, 1332 (S=O), 1290, 1205, 1156, 1099, 1071, 845, 782, 718, 584, 556 cm^{-1}.

Anal. Calcd. for $C_{17}H_{17}NO_6S$: C, 56.19; H, 4.72; N, 3.86. Found: C, 56.10; H, 4.76; N, 3.78.

Dimethyl 2,3-Dihydro-5-thiocyano-1*H*-pyrrolizine-6,7-dicarboxylate (**8**).

A solution of thiocyanogen chloride was prepared by adding potassium thiocyanate (5.48 g, 56.4 mmoles) in one portion to a solution of chlorine (3.82 g, 53.9 mmoles) in acetic acid (200 ml) which had previously been dried by refluxing with acetic anhydride (5 ml) under a nitrogen atmosphere for 1 hour. The resulting solution was stirred at ambient temperature for 30 minutes. Solid **4** (10.94 g, 49 mmoles) was added in one portion and the reaction mixture was stirred at ambient temperature for 15 hours. Removal of volatile components *in vacuo* gave a yellow solid that was dissolved in dichloromethane (150 ml) and washed with 10% aqueous sodium bicarbonate (2 x 100 ml). The organic layer was dried (sodium sulfate) and concentrated *in vacuo* to give a yellow solid that was purified by column chromatography (silica gel-dichloromethane-ethyl acetate, 9:1) to afford 7.05 g (51%) of **8** as a fluffy white solid, mp 99-100.5°; ^{1}H nmr (deuteriochloroform): δ 2.60 (m, 2H), 3.15 (t, 2H, J = 7 Hz), 3.75 (s, 3H), 3.90 (s, 3H), 4.15 (t, 2H, J = 7 Hz); ir (potassium bromide): ν 2998 (CH), 2955, 2158 (SCN), 2087, 1713 (C=O), 1495, 1438, 1396, 1283, 1212, 1092, 979, 873, 795, 669 cm^{-1}.

Anal. Calcd. for $C_{12}H_{12}N_2O_4S$: C, 51.42; H, 4.32; N, 9.99. Found: C, 51.28; H, 4.27; N, 9.92.

Dimethyl 2,3-Dihydro-2-methylthio-1*H*-pyrrolizine-6,7-dicarboxylate (**9**).

Solid sodium borohydride (0.91 g, 23.4 mmoles) was added portionwise to a stirred solution of **8** (6 g, 21.3 mmoles) in absolute ethanol-tetrahydrofuran (1:1, 60 ml) and the reaction mixture was

stirred at ambient temperature for 30 minutes. The reaction mixture was cooled to 0° and a solution of potassium hydroxide (1.32 g, 23.4 mmoles) in absolute ethanol (20 ml) was added. The mixture was stirred at ambient temperature for 1 minute, iodomethane (1.59 ml, 25.6 mmoles) was added and the reaction mixture was stirred at 0° for 15 minutes, then stirred at ambient temperature for 2 hours. Volatile components were removed *in vacuo* to give a yellow precipitate that was dissolved in dichloromethane (150 ml) and washed with 10% aqueous sodium bicarbonate (2 x 100 ml). The organic layer was dried (sodium sulfate) and concentrated *in vacuo* to give a tan solid that was purified by crystallization from acetone-isopropyl ether to give 5.22 g (91%) of **9** as light tan needles, mp 79.5-81°; ^{1}H nmr (deuteriochloroform): δ 2.35 (s, 3H), 2.60 (m, 2H), 3.10 (t, 2H, J = 7 Hz), 3.80 (s, 3H), 3.90 (s, 3H), 4.0 (t, 2H, J = 7 Hz); ir (potassium bromide): ν 2998 (CH), 2948, 2927, 1720 (C=O), 1501, 1438, 1290, 1205, 1184, 1120, 1085, 979, 782 cm^{-1}.

Anal. Calcd. for $C_{12}H_{15}NO_4S$: C, 53.52; H, 5.61; N, 5.20. Found: C, 53.45; H, 5.61; N, 5.19.

Dimethyl 2,3-Dihydro-5-methylsulfonyl-1*H*-pyrrolizine-6,7-dicarboxylate (**10**).

A solution of 31% aqueous hydrogen peroxide (8.47 ml, 77.2 mmoles) was added to a stirred solution of **9** (5.22 g, 19.3 mmoles) and concentrated sulfuric acid (0.1 ml) in glacial acetic acid (40 ml) and the reaction mixture was stirred at ambient temperature for 24 hours. The reaction mixture was poured into water (150 ml) and neutralized with solid sodium carbonate, and the aqueous solution was extracted with dichloromethane (2 x 100 ml). The combined organic layers were dried (sodium sulfate) and concentrated *in vacuo* to give a white precipitate that was purified by crystallization from acetone-isopropyl ether to give 5.83 g (92%) of **10** as white prisms, mp 188.5-190°; ^{1}H nmr (deuteriochloroform): δ 2.60 (m, 2H), 3.0 to 3.30 (m, 5H), 3.75 (s, 3H), 3.90 (s, 3H), 4.25 (t, 2H, J = 7 Hz); ir (potassium bromide): ν 3062 (CH), 2998, 2955, 2920, 1734 (C=O), 1713 (C=O), 1544, 1509, 1452, 1403, 1367, 1318, 1212, 1156 (S=O), 1120, 1099, 951, 873, 803, 767, 746 cm^{-1}.

Anal. Calcd. for $C_{12}H_{15}NO_6S$: C, 47.83; H, 5.02; N, 4.65. Found: C, 47.86; H, 5.06; N, 4.63.

2,3-Dihydro-6,7-bis(hydroxymethyl)-5-bromo-1*H*-pyrrolizine Bis[*N*-(2-propyl)carbamate] (**2b**).

A solution of bromine (0.15 ml, 2.96 mmoles) in dichloromethane (5 ml) was added dropwise to a stirred solution of **2a** (1 g, 2.96 mmoles) and triethylamine (1.66 ml, 11.9 mmoles) in dry dichloromethane (20 ml) at −23° and the reaction mixture was stirred at that temperature for 5 minutes. The mixture was washed with saturated aqueous sodium bicarbonate (2 x 20 ml), the organic layer was dried (sodium sulfate) and concentrated *in vacuo* to give a white precipitate that was purified by crystallization from dichloromethane-hexanes to give 0.80 g (65%) of **2b** as fluffy white needles, mp 155-157°; ^{1}H nmr (deuteriochloroform): δ 1.20 (d, 12H, J = 7 Hz), 2.50 (m, 2H), 2.90 (t, 2H, J = 7 Hz), 3.95 (s, 4H); ir (nujol): ν 3338 (NH), 2894, 1682 (C=O), 1527, 1464, 1379, 1260, 1076, 943 cm^{-1}.

Anal. Calcd. for $C_{17}H_{26}N_3O_4Br$: C, 49.04; H, 6.30; N, 10.09. Found: C, 48.90; H, 6.35; N, 10.03.

2,3-Dihydro-6,7-bis(hydroxymethyl)-5-phenylthio-1*H*-pyrrolizine Bis[*N*-(2-propyl)carbamate] (**2c**).

Method A.

A solution of **6** (3 g, 9.05 mmoles) in anhydrous tetrahydrofuran (40 ml) was added dropwise to a stirred suspension of lithium aluminum hydride (0.90 g, 22.6 mmoles) in anhydrous tetrahydrofuran (20 ml) and the reaction mixture was stirred at ambient temperature for 2 hours. The excess reducing agent was decomposed by dropwise addition of water (2 ml) followed by 10% aqueous sodium hydroxide (2 ml). The mixture was filtered and the inorganic material washed with several portions of dichloromethane. The combined filtrate was dried (sodium sulfate) and concentrated *in vacuo* to give a colorless oil that was dissolved in dry dichloromethane (25 ml) and treated with isopropyl isocyanate (2.22 ml, 22.6 mmoles) and dibutyltin diacetate (0.1 ml). The reaction mixture was stirred at ambient temperature for 20 hours, volatile components were removed *in vacuo* to give a white precipitate that was purified by crystallization from dichloromethane-hexanes to give 2.49 g (62%) of **2c** as a fluffy white solid. This compound was identical to that obtained from method B.

Method B.

A solution of phenylsulfenyl chloride (1.29 g, 8.89 mmoles) in dry dichloromethane (10 ml) was added dropwise to a stirred solution of **2a** (3 g, 8.89 mmoles) and triethylamine (1.37 ml, 9.78 mmoles) in dry dichloromethane (20 ml) and the reaction mixture was stirred at ambient temperature for 10 minutes. The mixture was washed with saturated aqueous bicarbonate (20 ml), dried (sodium sulfate) and concentrated *in vacuo* to give a white precipitate that was purified by crystallization from dichloromethane-hexanes to give 3.10 g (78%) of **2c** as a fluffy white solid, mp 144-145.5°; ^{1}H nmr (deuteriochloroform): δ 1.10 (d, d, 12H), 2.40 (m, 2H), 2.95 (t, 2H, J = 7 Hz), 3.45 to 4.0 (m, 4H), 4.20 to 4.60 (br s, 2H), 5.05 (s, 2H), 5.15 (s, 2H), and 6.90 to 7.10 (m, 5H); ir (nujol): ν 3329 (NH), 3061 (CH), 2976, 2941, 2892, 1685 (C=O), 1586, 1537, 1438, 1367, 1332, 1254, 1087, 944, 732, 690, 648 cm^{-1}.

Anal. Calcd. for $C_{23}H_{31}N_3O_4S$: C, 62.00; H, 7.01; N, 9.43. Found: C, 61.96; H, 7.06; N, 9.42.

2,3-Dihydro-6,7-bis(hydroxymethyl)-5-phenylsulfinyl-1*H*-pyrrolizine Bis[*N*-(2-propyl)carbamate] (**2d**).

Solid *m*-chloroperoxybenzoic acid (1.90 g, 8.79 mmoles) was added portionwise to a stirred solution of **2c** (2.61 g, 5.86 mmoles) in dichloromethane (40 ml) at 0° and the reaction mixture was stirred at that temperature for 10 minutes. The mixture was washed with saturated aqueous sodium bicarbonate (30 ml), dried (sodium sulfate), and concentrated *in vacuo* to give a white precipitate that was purified by column chromatography (florisil-ethyl acetate) to give 1.1 g (41%) of **2d** as a fluffy white solid, mp 178-179°; ^{1}H nmr (deuteriochloroform): δ 1.20 (d, 12H, J = 7 Hz), 2.20 to 2.60 (m, 2H), 2.60 to 3.20 (m, 3H), 3.55 to 4.20 (m, 3H), 4.60 to 4.90 (br s, 2H), 5.0 (s, 2H), 5.30 (s, 2H), 7.30 to 7.70 (m, 5H); ir (nujol): ν 3329 (NH), 3061 (CH), 2976, 2934, 1685 (C=O), 1537, 1466, 1367, 1325, 1262, 1092, 1078, 1036 (S=O), 993, 944, 834, 746, 679 cm^{-1}.

Anal. Calcd. for $C_{23}H_{31}N_3O_5S$: C, 59.85; H, 6.77; N, 9.10. Found: C, 59.62; H, 6.81; N, 8.96.

2,3-Dihydro-6,7-bis(hydroxymethyl)-5-phenylsulfonyl-1*H*-pyrrolizine Bis[*N*-2-(propyl)carbamate] (**2e**).

Solid aluminum hydride-ether complex (1.13 g, 20.64 mmoles)

was added portionwise to a stirred solution of **7** (2.50 g, 6.88 mmoles) in dry tetrahydrofuran (40 ml) at 0° and the reaction mixture was stirred at that temperature for 1 hour. The excess reducing agent was decomposed by dropwise addition of water (1 ml) followed by 10% aqueous sodium hydroxide (1 ml). The mixture was filtered and the inorganic material washed with several portions of dichloromethane. The combined filtrate was dried (sodium sulfate) and concentrated *in vacuo* to give a white precipitate that was dissolved in dry dichloromethane (35 ml) and treated with isopropyl isocyanate (1.69 ml, 17.2 mmoles) and dibutyltin diacetate (0.1 ml) and the reaction mixture was stirred at ambient temperature for 20 hours. Volatile components were removed *in vacuo* to give a white precipitate that was purified by crystallization from dichloromethane-hexanes to give 1.90 g (58%) of **2e** as a fluffy white powder, mp 136-137.5°; ^{1}H nmr (deuteriochloroform): δ 1.20 (d, 12H, J = 7 Hz), 2.20 to 3.0 (m, 4H), 3.40 to 4.65 (m, 6H), 3.95 (s, 2H), 5.35 (s, 2H), 7.35 to 7.50 (m, 3H), 7.70 to 8.0 (m, 2H); ir (nujol): ν 3315 (NH), 3061 (CH), 2977, 2934, 1685 (C=O), 1530, 1459, 1367, 1325 (S=O), 1254, 1156, 1135, 1078, 993, 944, 718, 890 cm^{-1}.

Anal. Calcd. for $C_{23}H_{31}N_3O_6S$: C, 57.84; H, 6.54; N, 8.80. Found: C, 57.91; H, 6.59; N, 8.57.

2,3-Dihydro-6,7-bis(hydroxymethyl)-5-methylsulfonyl-1*H*-pyrrolizine Bis[*N*-2-(propyl)carbamate] (**2f**).

Solid aluminum hydride-ether complex (1.90 g, 34.7 mmoles) was added portionwise to a stirred solution of **10** (3 g, 9.92 mmoles) in dry tetrahydrofuran (40 ml) at 0° and the reaction mixture was stirred at that temperature for 2 hours. The excess reducing agent was decomposed by dropwise addition of water (2 ml) followed by 10% aqueous sodium hydroxide (2 ml). The mixture was filtered and the inorganic residue was dried (sodium sulfate) and concentrated *in vacuo* to give a white precipitate that was dissolved in dry dichloromethane (30 ml) and treated with isopropyl isocyanate (2.44 ml, 24.8 mmoles) and dibutyltin diacetate (0.1 ml). The reaction mixture was stirred at ambient temperature for 24 hours. Volatile components were removed *in vacuo* to give a white precipitate that was purified by crystallization from dichloromethane-ether to give 1.98 g (48%) of **2f** as a fluffy white solid, mp 187-189°; ^{1}H nmr (deuteriochloroform): δ 1.20 (d, 12H, J = 6 Hz), 2.10 to 3.0 (m, 4H), 3.15 (s, 3H), 3.50 to 4.40 (m, 4H), 4.40 to 4.85 (br s, 2H), 5.0 (s, 2H), 5.30 (s, 2H); ir (nujol): ν 3343 (NH), 3033 (CH), 2976, 2934, 1678 (C=O), 1530, 1459, 1382, 1311 (S=O), 1247, 1156 (S=O), 1078, 958, 782, 768 cm^{-1}.

Anal. Calcd. for $C_{16}H_{29}N_3O_6S$: C, 52.03; H, 7.04; N, 10.11. Found: C, 51.99; H, 7.06; N, 10.04.

Determination of the Relative Rates of Alkylation.

The test compounds were dissolved in 1,2-dimethoxyethane (8 μmoles/ml) added (0.5 ml aliquots) to NBP solution [10% w/v 1,2-dimethoxyethane, 1.0 ml] and aqueous acid [20:20:1, water-dimethoxyethane-acetic acid, 1 ml]. The tubes were stoppered, the contents mixed, and the tubes were placed in a water bath maintained at 60°. At different time intervals, a tube was cooled in an ice bath and base [1:1 v/v triethylamine-acetone, 1.0 ml], was added. The contents were mixed for 3 seconds with a vortex-type mixer and diluted with acetone (7.0 ml). The solution was transferred to a cuvette, and the absorbance of the reaction was read at 570 nm with a Bausch and Lomb (Spectronic 20) spectrometer. The instrument had been previously adjusted to 0% absorbance against a mixture that contained NBP solution (1.0 ml), aqueous acid solution (1.0 ml), dimethoxyethane (0.5 ml), base solution (1.0 ml), and acetone (7 ml). The color of the reaction fades with time, and the absorbance readings should be taken as quickly as possible. The slope of the line obtained by the technique of least squares when absorbances was plotted against time gave the comparative alkylating activity k', which was calculated by an unweighted linear regression analysis. The reaction obeys the integrated second-order rate equation:

$$kt = [1/(a - b)]\, In\, [b(a - x)/a(b - x)]$$

where a is the initial concentration of the alkylating agent, b is the initial concentration of NBP, and x is the amount reacted in time t. If pseudo-first-order conditions are employed ($b >> a$), and the rate is measured only at the initial phase of the reaction ($x << a$ and $bkt << 1$), then the above equation simplifies to the following expression:

$$k = CEE^{570}/abt$$

where CE is a proportionality constant that is a function of the chromophore system, k is the pseudo-first-order rate constant, and E^{570} is the absorbance reading at 570 nm. If the structures of the alkylating agents under comparison are similar, then CE will show little variation and the above equation simplifies to the following:

$$k' = \beta k = E^{570}/t$$

where β includes the standard initial concentration of the reactants and k' is the comparative alkylating activity. Therefore, the relative rates of alkylation may be determined by plotting the absorbance readings *vs* time and determining the slopes of the linear plots. Duplicate determinations were made for each compound studied.

REFERENCES AND NOTES

[1] Vinylogous Carbinolamine Tumor Inhibitors. **25**. For part **24** see reference [2].

[2] W. K. Anderson, D. C. Dean and T. Endo, *J. Med. Chem.*, in press.

[3] W. K. Anderson, *Cancer Res.*, **42** 2168 (1982).

[4] W. K. Anderson, C.-P. Chang, P. F. Corey, M. J. Halat, A. N. Jones, H. L. McPherson, Jr., J. S. New and A. C. Rick, *Cancer Treatment Rep.*, **66**, 91 (1982).

[5] W. K. Anderson and P. F. Corey, *J. Med. Chem.*, **20**, 812 (1977).

[6] C. Hansch and A. Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology", Wiley, New York, 1979, Chapter 2.

[7] W. K. Anderson and R. H. Mach, *J. Med. Chem.*, **30**, 2109 (1987).

[8] M. T. Pizzorino and S. M. Albonico, *J. Org. Chem.*, **39**, 731 (1974).

[9] D. N. Harpp, B. T. Friedlander and R. A. Smith, *Synthesis*, 181 (1979).

[10] W. K. Anderson and R. H. Mach, *Synth. Commun.*, **16**, 911 (1986).

[11] T. J. Bardos, N. Datta-Gupta, P. Hebborn and D. J. Triggle, *J. Med. Chem.*, **8**, 167 (1965).