Synthesis and Evaluation of Antileukemic Activity of 5-Thienyl- or 5-(2-Furyl)-2,3-dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine Bis(alkylcarbamates) and Derivatives

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Treatment of *N*-(2-furoyl)proline (**1a**) or *N*-thenoylprolines (**1b**, **1c**) and *N*-(2-thenoyl)thiazolidine-4-carboxylic acid (**1d**) with acetic anhydride and dimethyl acetylenedicarboxylate gave 5-substituted derivatives of dimethyl 2,3-dihydro-1*H*-pyrrolizine-6,7-dicarboxylate (**2a**, **2b**, **2c**) and derivatives of dimethyl 5-(2-thienyl)pyrrolo[1,2-*c*]thiazole (**2d**). Reduction of **2** with lithium aluminum hydride gave the diols **3a**, **3b**, **3c**, and **3d**. These diols yielded the corresponding diacetates **4** by treatment with acetic anhydride. The bis(methylcarbamates) **5a**, **5b**, **5c**, and **5d** and bis(isopropylcarbamates) **6b** and **6c** are obtained with the appropriate isocyanates. The 1-substituted pyrrolizines were synthesized, the 1-acetoxy compounds **7b** and **7c** further transformed into 1-hydroxy (**8b**, **8c**) and 1-oxo (**9b**, **9c**) analogues. The action of hydrochloric acid on 1-acetoxy derivatives (**7b**, **7c**) gave 3*H*-pyrrolizines (**10b**, **10c**). Evaluation of antileukemic activity was investigated on the leukemia L1210 in vivo, on several bis(alkylcarbamates). The compounds **5c** and **5d** show good antileukemic activity comparable with the mitomycin.

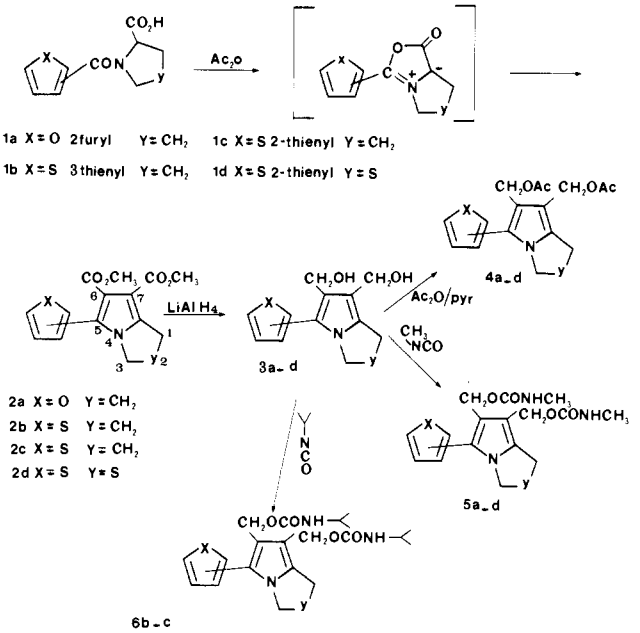
Mitomycin C is active against a broad spectrum of tumors[1] and in spite of its relatively high toxicity it is currently employed clinically for the treatment of solid tumors.[2-4] After its conversion into a mitosene by a subsequent reduction of the complexed semiquinone radical to the corresponding hydroquinone, followed by loss of methanol from the 9 and 9a positions, mitomycin C has been shown to cross-link double helical DNA.[5-8] Two electrophilic centers were unmasked at carbon 1 after aziridine ring opening and carbon 10 adjacent to the carbamate group to give bifunctional cross-linking alkylation of the genetic material DNA.[5,9,10] In theory, 1-substituted mitosenes should undergo reduction to indolohydroquinones that are able to give bifunctional alkylation.[5]

Therefore, the structure–activity relationship studies with mitomycin or mitosene derivatives were complex and also often limited by the small quantities of material available and by the relatively limited number of modifications that could be performed on these molecules. During the course of synthetic studies toward mitomycin derivatives, a concerted effort aimed at the synthesis of various analogues based on the parent 2,3-dihydro-1*H*-pyrido[1,2-*a*]indole nucleus[11-18] was made.

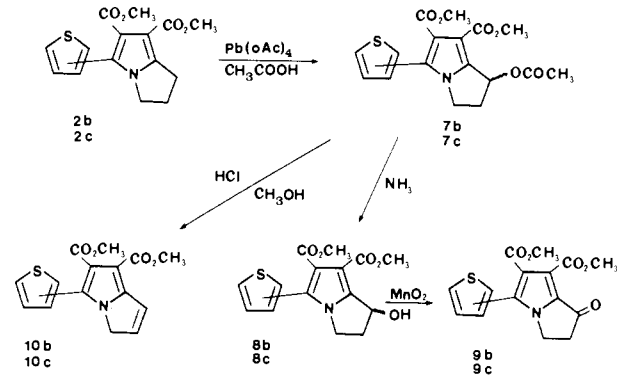
Recent reports[19-24] describe the synthesis of a series of 5-aryl-2,3-dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizines that structurally resemble reduced mitosene and that exhibit significant antitumor activity that can be related to bifunctional alkylation with DNA.

In continuation of our work on the synthetic pyrroloindoles[25-27] and with the objective of obtaining related less toxic compounds than mitomycin, we describe herein the synthesis of 5-(2-furyl)- and 5-(2- and 5-(3-thienyl)-2,3-dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine diesters and 5-(2-thienyl)-6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo-[1,2-*c*]thiazole. We chose to prepare these compounds for antitumor activity because they were simple polyfunctional molecules and, what is more, these compounds possess certain similarities to the mitosenes and some pyrrolizine alkaloids.[28] These pyrrolizine diesters were potentially bis-alkylating agents; the mechanism of the bis-alkylation was based on the potential electrophilic 6 and 7 carbons, which could be considered as allylic centers of 2,3-dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine diesters via the intermediary of an iminium ion.[19] All the compounds

Scheme I



Scheme II



obtained were stable at room temperature but only the compounds that have acylated bis(hydroxymethyl) moie-

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ties on carbon 6 and 7 have been tested for antileukemic activity. Furthermore, in order to compare these products with the mitosenes, it was necessary to investigate the introduction of substituents at C1. The introduction of substituents at C1 of dimethyl 2,3-dihydro-5-thienyl-1*H*-pyrrolizine-6,7-dicarboxylate was achieved by a linear synthesis, in the order 1-acetoxy, 1-hydroxy, and 1-oxo dimethyl 2,3-dihydro-5-thienyl-1*H*-pyrrolizine-6,7-dicarboxylate, respectively (Scheme II).

Thus Remers et al.[9a] have reported that the 1-substituted mitosenes have been achieved by a reverse sequence, 1-oxo, 1-hydroxy, 1-acetoxy pyrrolo[1,2-*a*]indole. These mitosenes only show antileukemic activity when the 1-substituent was a leaving group, for example, the 1-acetoxy group.

It was hoped that conditions could be found to reduce the carboxylate group at carbons 6 and 7 to a hydroxymethyl group, followed by carbamate formation without cleavage of the 1-acetoxy group. Although our attempts at reducing have been unsuccessful until now, we hope to find an alternative route to yield bis(hydroxymethyl)-1*H*-pyrrolizine bis(methylcarbamate) substituted at C1. The introduction of a double bond between C1 and C2 was realized to obtain dimethyl 5-thienyl-3*H*-pyrrolizine-6,7-dicarboxylates **10**. In the future these products would be potential intermediates for a trans addition synthesis of an aziridine ring.

Chemistry

The synthesis of compounds **1a**, **1b**, **1c**, and **1d** is outlined in Scheme II. The corresponding amides were prepared by condensation of the appropriate acid chlorides with proline. The reactions were conducted in aqueous sodium hydroxide solution by addition of acid chloride in acetone under Schotten–Baumann conditions. These acid products were treated with acetic anhydride to give the nonisolable mesoionic oxazolones that were heated with dimethyl acetylenedicarboxylate (DMAD) to give the diesters **2a**, **2b**, **2c**, and **2d** by 1,3-dipolar cycloaddition. The reduction of the diesters **2a–d** was accomplished in dichloromethane by addition of lithium aluminum hydride, which afforded the diols **3a**, **3b**, **3c**, and **3d**. Treatment of these 2,3-dihydrobis(hydroxymethyl)-1*H*-pyrrolizines **3** with acetic anhydride in pyridine yielded the corresponding bis(acetoxymethyl)-1*H*-pyrrolizines **4a**, **4b**, **4c**, and **4d**. The bis(hydroxymethyl)-1*H*-pyrrolizine bis(methylcarbamates) **5a**, **5b**, **5c**, and **5d** and the bis(isopropylcarbamates) **6b** and **6c** were prepared from the corresponding diol **3** by treatment with the appropriate isocyanate in dichloromethane containing a catalytic amount of triethylamine.

The introduction of a functional group at C1 (Scheme II) was achieved by using lead tetraacetate. The diester **2b** or **2c** was stirred with 1 molar equiv of lead tetraacetate in acetic acid at 40 °C to give the 1-acetoxy compound **7b** or **7c**. The crude product of this reaction was applied directly on a column of silica gel to purify the triesters **7**. The dimethyl 1-hydroxy-2,3-dihydro-5-thienyl-1*H*-pyrrolizine-6,7-dicarboxylates **8b** and **8c** were obtained by alkaline hydrolysis of the corresponding 1-acetoxy compounds **7** using ammonia in methanol. The alcohols **8b** and **8c** were oxidized to the ketone with activated manganese dioxide in methylene chloride, yielding the dimethyl 2,3-dihydro-1-oxo-5-thienyl-1*H*-pyrrolizine-6,7-dicarboxylates **9b** and **9c**. The formation of a double bond between C1 and C2 was achieved by an elimination reaction. The triesters **7** were treated by dry hydrochloric acid in methanol for 4 days to give the olefin compounds, dimethyl 5-thienyl-3*H*-pyrrolizine-6,7-dicarboxylates **10**.

Biological Results

The aim of our study was the synthesis of mitomycin analogues possessing similar chemotherapeutic activity. Table I shows the effect of seven derivatives of 5-furyl- or 5-thienyl-2,3-dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine and 5-(2-thienyl)-6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole on L1210 leukemia. These compounds were submitted to an oncostatic screening where the activity of the new analogues was compared to that of mitomycin, the reference compound. Murine leukemia was selected for this screening because it fulfilled the following three criteria: (1) It had to be sensitive to mitomycin, and consequently to its analogues, so that the risk of discarding an eventual clinically active compound (false negative) was avoided.[29] (2) It had to be sensitive to mitomycin but only mildly,[30] in order that the possible increase in the new analogues oncostatic index should be clearer. (3) It had to be mildly sensitive to some other

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Table I. Effect of Derivatives of 2,3-Dihydro-5-furyl- or -5-thienyl-6,7-bis(hydroxymethyl)-*1H*-pyrrolizine and 5-(2-Thienyl)-6,7-bis(hydroxymethyl)-*1H,3H*-pyrrolo[1,2-*c*]thiazole on L1210 Leukemia[a]

compd	acute[b] LD_{50}	dose,[c] mg/kg	$I^d = T/C$	ref $I^e = T/C$	treated $I \times 100^f$ / ref I	LD50/ED[g]
		50	117	136	92	
4c	80	30	114	140	86	
		15	100	121	83	
		100	100	125	80	
5a	>150	50	108	117	92	
		20	100	117	86	
		100	120	140	86	
5b	>150	75	120	140	86	
		40	100	125	80	
		16	100	125	80	
		100	158	124	127	1.2
		85	142 145	126 145	113 100	1.4
5c	120	65	125 118	125 146	100 81	1.8
		40	138 113	138 145	100 78	3
		35	167 133	167 162	100 82	3.4
		30	167 189	145 155	115 122	1.7
5d	50	20	125	121	103	2.5
		8	100	117	86	
		40	100	125	80	
6b	65	20	100	139	72	
		25	117	162	72	
6c	35	15	125	139	90	
		10	129	163	79	
		4	108	164	66	

[a] B6D2F1 mice were administered 10^5 L1210 leukemia cells by the ip route on day 0. [b] Mice were injected one day later with compounds by the ip route. Mortality was observed daily for 9 days. Doses are given in mg/kg. [c] Compounds were administered by the ip route 1, 5, and 9 days after L1210 grafting in 0.1- or 0.2-mL volumes of aqueous mixture containing 20%. Tween 80 per 20-g mouse body weight. Each treated group included 8 mice. Control group (12 mice) received vehicle only and reference group (8 mice) was administered mitomycin, 2 mg/kg by the ip route. Doses are given in mg/kg. [d] Mortality was observed daily and autopsies were performed to determine whether death was due to leukemia or to drug toxicity. The oncostatic index (I) = the median survival time (MST) of treated group × 100/MST of untreated groups. Underlined numbers are statistically significant ($p < 0.05$, nonparametric Wilcoxon's test[31]). When $I > 125$ and $p < 0.05$, the compound is considered as active. [e] Ref I = MST of mitocyin 2 mg/kg treated group × 100/MST of untreated control groups. [f] Results of mitomycin varied from one experiment to the other. Thus, in order to standardize the results obtained with the studied compounds, the ratio $I \times 100$/ref I is given. Underlined numbers are statistically significant ($p < 0.05$, Wilcoxon's test). [g] ED = efficient dose (mitomycin 2 mg/kg; LD50/ED = 2.5).

known clinical oncostatic drugs so that positive results should be more convincing.

L1210 fulfills the three criteria better at a time than others, such as P388 lymphocytic leukemia and was thus selected for screening. Methods are briefly described in the footnotes of Table I, where **5c** and **5d** seem to be the most interesting analogues. At their higher effective dose, they are significantly more active on L1210 leukemia than mitomycin, but the ratio LD50/ED is smaller than that of mitomycin. On the other hand, **5c** (40 and 35 mg/kg) and **5d** (20 mg/kg) are as effective as mitomycin (2 mg/kg) and their ratio LD50/ED is higher.

The fact that the same efficacy is obtained with a smaller dose in regard to LD50 is in favor of **5c** and **5d**. It would be premature to draw any conclusions on the structure–activity relationship. However, we suggest that only the compounds substituted by the 5-(2-thienyl) group possess chemotherapeutic activity.

Experimental Section

All chemical reagents are commercially available and were purchased from Aldrich Chemical Co. Melting points were determined with a Kofler Heizbank apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 257 G spectrometer and ^1H NMR spectra were obtained on a Varian EM 90 spectrometer for DMSO-d_6 solutions (unless otherwise specified). All chemical shifts are given in units (ppm) downfield from Me_4Si and the J values are given in hertz. The spectral data for all compounds were consistent with assigned structures. Satisfactory composition analyses (±0.4% for C, H, N, or S) on all products were obtained.

General Procedure for the Preparation of *N*-(2-Furoyl)-L-proline (1a), *N*-(2-Thenoyl)-L-proline (1b), *N*-(3-Thenoyl)proline (1c), and *N*-Thenoyl-2-L-thiazolidine-4-carboxylic Acid (1d). The corresponding acids were converted to the acyl chlorides by treatment with a large excess of thionyl chloride heated under reflux for 1–2 h. The thionyl chloride was vacuum distilled to give the acyl chlorides. The corresponding acyl chlorides dissolved in acetone were added dropwise to a cooled (ice bath) solution of slightly more than 1 equiv of L-proline in water containing sufficient sodium hydroxide to make the solution alkaline to litmus. Additional portions of 20% sodium hydroxide solution were added to maintain alkalinity during the addition of the acyl chlorides. After 1 h, the solutions were acidified with dilute hydrochloric acid. The reaction mixture was cooled and the *N*-furoyl- and *N*-thenoyl-L-prolines precipitated to produce white solids which were dried in air and used without purification.

***N*-(2-Furoyl)-L-proline (1a)**: white crystals, mp 185 °C, crude; IR 3110, 1720, 1570, 1470, 1420, 1210, 1180 cm^{-1}.

***N*-(3-Thenoyl)-L-proline (1b)**: white crystals, mp 140 °C; IR 3105, 1720, 1570, 1450, 1190, 750 cm^{-1}.

***N*-(2-Thenoyl)-L-proline (1c)**: mp 142 °C; IR 3080, 1710, 1590, 1510, 1430, 1260, 1240, 740 cm^{-1}.

The general procedure was employed and L-thiazolidine-4-carboxylic acid was utilized with 2-thenoyl chloride to afford a thick syrup, which was used without purification in the subsequent cycloaddition reactions.

***N*-(2-Thenoyl)-L-thiazolidine-4-carboxylic acid (1d)**: IR 3100, 1730, 1600, 1430, 1390, 1250, 740 cm^{-1}.

General Procedure for the Preparation of Dimethyl 2,3-Dihydro-5-furyl- and -5-thienyl-1*H*-pyrrolizine-6,7-dicarboxylates (2). A mixture of *N*-furoyl- or -thenoyl-L-proline 20.9 g 0.1 mol and dimethyl acetylenedicarboxylate (DMAD) 0.12 mol in acetic anhydride (200 mL) was stirred and heated to 90–110 °C (oil bath) during 2.5 h (carbon dioxide was evolved). The black solution was cooled and concentrated to dryness in vacuo. The tan residue was treated with water and $NaHCO_3$, and the mixture was stirred for 2 h and then extracted with chloroform. The

organic layer was washed with water, then dried (Na_2SO_4), treated with actived carbon, and concentrated in vacuo to yield the crude product.

Dimethyl 2,3-dihydro-5-(2-furyl)-1*H*-pyrrolizine-6,7-dicarboxylate (2a) (20.9 g, 0.1 mol of **1a**): yield (13.0 g, 45%); mp 140 °C (MeOH); IR 3140, 1705, 1690, 1440, 1230, 1150, 770 cm^{-1}; ^1H NMR 2.50 (q, C_2, J = 7 Hz), 2.96 (t, C_1, J = 7 Hz), 4.1 (t, C_3, J = 7 Hz), 3.7 (s, OCH_3), 3.75 (s, OCH_3), 6.53 (dd, H4′, $J(4'3')$ = 3.3 Hz, $J(4'5')$ = 2.1 Hz), 6.61 (dd, H3′, $J(3'4')$ = 3.3 Hz, $J(3'5')$ = 1.5 Hz), 7.66 (dd, H5′, $J(5'4')$ = 2.1 Hz, $J(5'3')$ = 1.5 Hz). Anal. ($C_{15}H_{15}NO_5$) C, H, N. A second product was obtained, insoluble in methanol. Dimethyl 5-[1-(4-acetoxy-2,3-dicarbomethoxy-7-oxabicyclo[2.2.1]-2,5-heptadienyl)]-2,3-dihydro-1*H*-pyrrolizine-6,7-dicarboxylate: mp 196 °C; IR 2950, 1760, 1710, 1450, 1295, 1200, 1080, 900 cm^{-1}; ^1H NMR 2.23 (s, $OCOCH_3$), 2.43 (m, C_2), 2.93 (m, C_1C_3), 3.46 (s, OCH_3), 3.56 (s, OCH_3), 3.66 (s, OCH_3), 3.76 (s, OCH_3), 7.5 (q, CH, J = 7, 5 Hz).

Dimethyl 2,3-dihydro-5-(3-thienyl)-1*H*-pyrrolizine-6,7-dicarboxylate (2b) (22.5 g, 0.1 mol of **1b**): yield (22.8 g, 75%); mp 138 °C (MeOH); IR 3105, 2950, 1720, 1690, 1430, 1290, 1105, 800 cm^{-1}; ^1H NMR 2.43 (q, C_2, J = 7.5 Hz), 2.96 (t, C_1, J = 7.5 Hz), 3.66 (s, OCH_3), 4.00 (t, C_3, J = 7.5 Hz), 6.45 (dd, H4′, $J(4'5')$ = 4.5 Hz, $J(4'2')$ = 1.5 Hz), 7.53 (dd, H5′, $J(5'4')$ = 4.5 Hz, $J(5'2')$ = 3.3 Hz), 7.58 (dd, H2′, $J(2'4')$ = 1.5 Hz, $J(2'5')$ = 3.3 Hz). Anal. ($C_{15}H_{15}NO_4S$) C, H, N, S.

Dimethyl 2,3-dihydro-5-(2-thienyl)-1*H*-pyrrolizine-6,7-dicarboxylate (2c) (22.5 g, 0.1 mol of **1c**): yield (24.4 g, 80%); mp 135 °C (MeOH); IR 2940, 1720, 1690, 1455, 1300, 1110, 725 cm^{-1}; ^1H NMR 2.43 (q, C_3, J = 7 Hz), 3.66 (s, OCH_3), 4 (t, C_3, J = 7 Hz), 7.03 (dd, H4′, $J(4'3')$ = 3 Hz, $J(4'5')$ = 5.1 Hz), 7.16 (dd, H3′, $J(3'5')$ = 1.2 Hz, $J(3'4')$ = 5.1 Hz), 7.53 (dd, H5′, $J(5'4')$ = 5.1 Hz, $J(5'3')$ = 1.2 Hz). Anal. ($C_{15}H_{15}NO_4S$) C, H, N, S.

Dimethyl 5-(2-thienyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole-6,7-dicarboxylate (2d) (24.3 g, 0.1 mol of **1d**): yield (26.5 g, 82%): mp 158 °C (MeOH); IR 3100, 2950, 1720, 1700, 1540, 1490, 1450, 1370, 1290, 1210, 1160, 1110, 730 cm^{-1}; ^1H NMR 3.7 (s, OCH_3), 4.3 (s, C_1), 5.16 (C_3), 7.1 (dd, H4′, $J(4'3')$ = 3.6 Hz, $J(4'5')$ = 5.1 Hz), 7.3 (dd, H3′, $J(3'4')$ = 3.6 Hz, $J(3'5')$ = 1.5 Hz), 7.63 (dd, H5′, $J(5'4')$ = 5.1 Hz, $J(5'3')$ = 1.5 Hz). Anal. ($C_{14}H_{13}O_4S_2N$) C, H, N, S.

General Procedure for the Preparation of 2,3-Dihydro-5-furyl- or -5-thienyl-6,7-bis(hydroxymethyl)-1*H*-pyrrolizines 3. A solution of **2** (0.01 mol) in dry dichloromethane (100 mL) was added dropwise over a 20-min period to a mechanically stirred mixture of lithium aluminum hydride (2.2 equiv) in anhydrous ether (50 mL). The solution was refluxed for 1 h after the addition was completed and then cooled on an ice bath. The excess hydride was carefully decomposed with a small amount of wet ether and then water until the salts were white. The mixture was filtered and the inorganic residue was washed with several portions of hot dichloromethane until the total filtrate was 800 mL; the filtrate was dried (Na_2SO_4), then filtered, and concentrated in vacuo. The crude product that was precipitated by the addition of petroleum ether (20 mL) to the oily residue was crystallized.

2,3-Dihydro-5-(2-furyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine (3a) (2.89 g of **2a**, 0.01 mol): yield (1.5 g, 64%): mp 105 °C, (Et_2O); IR 3310, 2860, 1600, 1565, 1450, 990, 970 cm^{-1}; ^1H NMR 2.4 (q, C_2, J = 7.5 Hz), 2.76 (t, C_1, J = 7.5 Hz), 4 (t, C_3, J = 7.5 Hz), 4.41 (m, CH_2OH), 6.36 (dd, H3′, $J(3'4')$ = 3.3 Hz, $J(3'5')$ = 1.2 Hz), 6.45 (dd, H4′, $J(4'3')$ = 3.3 Hz, $J(4'5')$ = 2.7 Hz), 7.55 (dd, H5′, $J(5'4')$ = 2.7 Hz, $J(5'3')$ = 1.2 Hz). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

2,3-Dihydro-5-(3-thienyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine (3b) (3.05 g of **2b**, 0.01 mol): yield (1.27 g, 51%); mp 136 °C (Et_2O); IR 3290, 2870, 1450, 1020, 790 cm^{-1}; ^1H NMR 2.4 (q, C_2, J = 6 Hz), 2.76 (t, C_1, J = 6 Hz), 3.91 (t, C_3, J = 6 Hz), 4.36 (m, CH_2OH), 4.43 (m, OH [disappeared by addition of D_2O]), 7.26 (dd, H4′, $J(4'5')$ = 4.8 Hz, $J(4'2')$ = 1.5 Hz), 7.4 (dd, H2′, $J(2'4')$ = 1.5 Hz, $J(2'5')$ = 3 Hz), 7.51 (dd, H5′, $J(5'2')$ = 3 Hz, $J(5'4')$ = 4.8 Hz). Anal. ($C_{13}H_{15}NO_2S$) C, H, N, S.

2,3-Dihydro-5-(2-thienyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine (3c) (3.05 g of **2c**, 0.01 mol): yield (1.71 g, 69%); mp 97 °C (Et_2O); IR 3280, 2840, 1450, 1430, 1010, 840, 700 cm^{-1}; ^1H NMR 2.36 (q, C_2, J = 7 Hz), 2.75 (t, C_1, J = 7 Hz), 3.9 (t, C_3, J = 7 Hz), 4.4 (m, CH_2OH [after addition of D_2O the signal was a singlet]), 7–7.06 (m, coalesc. H3′, H4′), 7.33 (dd, H5′, $J(5'4')$ = 4.2 Hz, $J(5'3')$ = 1.8 Hz). Anal. ($C_{13}H_{15}NO_2S$) C, H, N, S.

5-(2-Thienyl)-6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole (3d) (3.23 g of **2d**, 0.01 mol): yield (1.78 g, 67%); the product was a syrup; IR 3340, 2890, 1450, 1370, 1010, 710 cm^{-1}; ^1H NMR ($CDCl_3$) 4.00 (s, C_1), 4.46 (m, CH_2OH), 4.86 (s, C_3), 6.93–7 (m, coalesc. H3′, H4′), 7.23 (dd, H5′, $J(5'4')$ = 4.5 Hz, $J(5'3')$ = 1.8 Hz). Anal. ($C_{12}H_{13}NO_2S_2$) C, H, N, S.

General Procedure for the Preparation of 2,3-Dihydro-5-furyl- and -5-thienyl-6,7-bis(acetoxymethyl)-1*H*-pyrrolizines 4. A magnetically stirred solution of **3** (0.005 mol) in anhydrous pyridine (25 mL) was treated with acetic anhydride (15 mL) for 20 h at room temperature. The volatile reaction components were removed in vacuo, using a toluene azeotrope to remove traces of pyridine. The residue was crystallized in diethyl ether.

2,3-Dihydro-5-(2-furyl)-6,7-bis(acetoxymethyl)-1*H*-pyrrolizine (4a) (1.16 g of **3a**, 0.005 mol): yield (1 g, 63%); mp 90 °C (Et_2O); IR 2950, 1720, 1600, 1370, 1240, 1020 cm^{-1}; ^1H NMR 1.91 (s, $OCOCH_3$), 2.46 (m, C_2), 2.81 (t, C_1, J = 7.5 Hz), 4.03 (t, C_3, J = 7.5 Hz), 4.9 (s, CH_2OCO), 5.03 (s, CH_2OCO), 6.4 (dd, H3′, $J(3'4')$ = 3.6 Hz, $J(3'5')$ = 1.2 Hz), 6.5 (dd, H4′, $J(4'3')$ = 3.6 Hz, $J(4'5')$ = 3 Hz), 7.63 (dd, H5′, $J(5'4')$ = 3 Hz, $J(5'3')$ = 1.2 Hz). Anal. ($C_{17}H_{19}NO_5$) C, H, N.

2,3-Dihydro-5-(3-thienyl)-6,7-bis(acetoxymethyl)-1*H*-pyrrolizine (4b) (1.25 g, 0.005 mol **3b**): yield (1.16 g, 70%); mp 130 °C (Et_2O); IR 2950, 1720, 1240, 1020, 800, 785 cm^{-1}; ^1H NMR 1.95 (s, $OCOCH_3$), 2.4 (q, C_2, J = 7.5 Hz), 2.78 (t, C_1, J = 7.5 Hz), 3.93 (t, C_3, J = 7.5 Hz), 4.9 (s, CH_2OCO), 7.16 (dd, H4′, $J(4'5')$ = 4.2 Hz, $J(4'2')$ = 1.5 Hz), 7.41 (dd, H2′, $J(2'4')$ = 1.5 Hz, $J(2'5')$ = 3 Hz), 7.53 (dd, H5′, $J(5'4')$ = 4.2 Hz, $J(5'2')$ = 3 Hz). Anal. ($C_{17}H_{19}NO_4S$) C, H, N, S.

2,3-Dihydro-5-(2-thienyl)-6,7-bis(acetoxymethyl)-1*H*-pyrrolizine (4c) (1.25 g, 0.005 mol **3c**): yield (1.2 g, 72%); mp 114 °C (Et_2O); IR 2945, 1715, 1370, 1240, 1020 cm^{-1}; ^1H NMR 2 (s, $OCOCH_3$), 2.4 (q, C_2, J = 7.5 Hz), 2.85 (t, C_1, J = 7.5 Hz), 4 (t, C_3, J = 7.5 Hz), 4.96 (s, CH_2OCO), 7.11 (m, H3′, H4′), 7.5 (dd, H5′, $J(5'3')$ = 2.4 Hz, $J(5'4')$ = 3.6 Hz). Anal. ($C_{17}H_{19}NO_4S$) C, H, N, S.

General Procedure for the Preparation of 2,3-Dihydro-5-furyl- and -5-thienyl-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine Bis(methylcarbamates) 5. A solution of **3** (0.01 mol) and triethylamine (0.5 mL) in dichloromethane was cooled at 0 °C. Methyl isocyanate (3 mL) was added; then the mixture was refluxed for 2 h. The reaction mixture was concentrated to dryness in vacuo the white residue was dissolved in hot ethyl acetate, treated with charcoal, filtered, and concentrated to give a white powder.

2,3-Dihydro-5-(2-furyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine bis(methylcarbamate) (5a) (2.33 g of **3a**, 0.01 mol): yield (2.7 g, 78%); mp 177 °C; IR 3320, 2940, 1680, 1540, 1270, 1140, 970 cm^{-1}; ^1H NMR 2.46 (m, $NHCH_3$), 2.43 (m, C_2), 2.78 (t, C_1, J = 6 Hz), 4 (t, C_3, J = 6 Hz), 4.86 (s, CH_2OCO), 4.96 (s, CH_2OCO), 6.4 (dd, H3′, $J(3'5')$ = 1 Hz, $J(3'4')$ = 3.3 Hz), 6.48 (dd, H4′, $J(4'3')$ = 3.3 Hz, $J(4'5')$ = 2.1 Hz), 6.73 (m, NH), 7.61 (dd, H5′, $J(5'4')$ = 2.1 Hz, $J(5'3')$ = 1 Hz). Anal. ($C_{17}H_{21}N_3O_5$) C, H, N.

2,3-Dihydro-5-(3-thienyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine bis(methylcarbamate) (5b) (2.49 g of **3b**, 0.01 mol): yield (2.80 g, 77%); mp 174 °C; IR 3320, 1680, 1540, 1265 cm^{-1}; ^1H NMR 2.5 (m, $NHCH_3$, C_2), 2.7 (t, C_1, J = 6 Hz), 3.93 (t, C_3, J = 6 Hz), 4.84 (s, CH_2OCO), 4.88 (s, CH_2OCO), 6.73 (m, NH) [after addition of D_2O the signal disappeared], 7.23 (dd, H4′, $J(4'5')$ = 4.8 Hz, $J(4'2')$ = 1.5 Hz), 7.43 (dd, H2′, $J(2'4')$ = 1.5 Hz, $J(2'5')$ = 3 Hz), 7.56 (dd, H5′, $J(5'4')$ = 4.8 Hz, $J(5'2')$ = 3 Hz). Anal. ($C_{17}H_{21}N_3O_4S$) C, H, N, S.

2,3-Dihydro-5-(2-thienyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine bis(methylcarbamate) (5c) (2.49 g of **3c**, 0.01 mol): yield (2.4 g, 70%); mp 170 °C; IR 3320, 2945, 1675, 1550, 1280, 1155, 965, 710 cm^{-1}; ^1H NMR 2.48 (m, $NHCH_3$), 2.55 (m, $NHCH_3$), 2.51 (m, C_2), 2.78 (t, C_1, J = 6 Hz), 4.00 (t, C_3, J = 6 Hz), 4.88 (s, CH_2OCO), 6.73 (m, NH), 7.06 (m, H3′, H4′), 7.46 (dd, H5′, $J(5'4')$ = 3.9 Hz, $J(5'3')$ = 2.7 Hz). Anal. ($C_{17}H_{21}N_3O_4S$) C, H, N, S.

5-(2-Thienyl)-6,7-bis(hydroxymethyl)-1*H*,3*H*-pyrrolo[1,2-*c*]thiazole bis(methylcarbamate) (5d) (2.67 g of **3d**, 0.01

mol): yield (2.97 g, 78%); mp 203 °C; IR 3320, 2950, 1680, 1540, 1270, 1140, 970 cm^{-1}; ^1H NMR 2.5 (s, CH_3), 2.56 (s, CH_3), 4.11 (C_1), 4.86 (s, CH_2OCO), 4.9 (s, CH_2OCO), 5.06 (s, C_3), 6.76 (m, NH) [disappeared by addition of D_2O], 7.08–7.15 (m, H3′, H5′), 7.56 (dd, H5′, $J(5'4')$ = 4.8 Hz, $J(5'3')$ = 1.5 Hz). Anal. ($C_{16}H_{19}N_3O_4S_2$) C, H, N, S.

General Procedure for the Preparation of 2,3-Dihydro-5-thienyl-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine Bis(isopropylcarbamates) 6. A solution of 0.005 mol of **3** and triethylamine (1 mL) in dry dichloromethane was treated with isopropyl isocyanate (2.5 g) and refluxed for 48 h (protected from moisture by calcium chloride). Then the mixture was concentrated in vacuo and the residue was dissolved in hot ethyl acetate. The isopropylurea byproduct that precipitated from the solution was removed by filtration. The filtrate was cooled and the product was obtained by addition of petroleum ether.

2,3-Dihydro-5-(3-thienyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine bis(isopropylcarbamate) (6b) (1.25 g of **3b**, 0.005 mol): yield (1.5 g, 71%); mp 158 °C; IR 3320, 2960, 1670, 1610, 1530, 1250, 1080 cm^{-1}; ^1H NMR 1.13 (d, CH_3, J = 7 Hz), 1.16 (d, CH_3, J = 7 Hz), 2.48 (q, C_2, J = 7.8 Hz), 2.94 (q, C_1, J = 7.8 Hz), 3.85 (sept. CH, J = 7 Hz), 3.97 (t, C_3, J = 7.8 Hz), 4,54 (m, NH), 5.06 (s, CH_2), 7.17 (dd, H4′, $J(4'5')$ = 5 Hz, $J(4'2')$ = 1.4 Hz), 7.26 (dd, H2′, $J(2'5')$ = 3.5 Hz, $J(2'4')$ = 1.4 Hz), 7.36 (dd, H5′, $J(5'2')$ = 3.5 Hz, $J(5'4')$ = 5 Hz). Anal. ($C_{21}H_{29}N_3O_4S$) C, H, N, S.

2,3-Dihydro-5-(2-thienyl)-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine bis(isopropylcarbamate) (6c) (1.25 g of **3c**, 0.005 mol): yield (1.2 g, 68%); mp 144 °C; IR 3315, 2970, 1680, 1530, 1250, 1080, 935 cm^{-1}; ^1H NMR 0.96 (d, CH_3, J = 7.5 Hz), 1.05 (d, CH_3, J = 7.5 Hz), 2.46 (m, C_2), 2.81 (t, C_1, J = 7.5 Hz), 4.00 (t, C_3, J = 7.5 Hz), 3.55 (sept. CH, J = 7.5 Hz), 4.86 (s, CH_2OCO), 6.8 (m, NH) [disappeared by addition of D_2O], 7.1 (m, H3′, H4′), 7.48 (dd, H5′, $J(5'4')$ = 3.9 Hz, $J(5'3')$ = 2.7 Hz). Anal. ($C_{21}H_{29}N_3O_4S$) C, H, N, S.

Dimethyl 1-Acetoxy-2,3-dihydro-5-thienyl-1*H*-pyrrolizine-6,7-dicarboxylates 7. A mixture of 0.005 mol of **2**, lead tetraacetate (0.005 mol), and acetic acid (50 mL) was stirred for 20 h at 40 °C. The solution was concentrated in vacuo at 40 °C; then the residue was stirred with water and chloroform. The mixture was filtered to remove inorganic material. The organic layer was decanted, washed with $NaHCO_3$ solution and brine, then dried (Na_2SO_4), and concentrated. The oily residue was chromatographed on silica gel with dichloromethane/ether (4/1) as solvent and yielded **7**.

Dimethyl 1-acetoxy-2,3-dihydro-5-(3-thienyl)-1*H*-pyrrolizine-6,7-dicarboxylate (7b) (1.50 g of **2b**, 0.005 mol): yield (1.3 g, 72%); mp 174 °C (MeOH); IR 3100, 2940, 1735, 1720, 1690, 1440, 1240, 800 cm^{-1}; ^1H NMR 2 (s, $OCOCH_3$), 2.73–3.21 (m, C_2), 3.66 (s, OCH_3), 4.13 (m, C_3), 6.21 (dd, J = 6.6 Hz, J = 1.8 Hz), 7.23 (dd, H4′, $J(4'5')$ = 5.1 Hz, $J(4'2')$ = 1.5 Hz), 7.6 (dd, H5′, $J(5'4')$ = 5.1 Hz, $J(5'2')$ = 2.7 Hz), 7.7 (dd, H2′, $J(2'4')$ = 1.5 Hz, $J(2'5')$ = 2.7 Hz). Anal. ($C_{17}H_{17}NO_6S$) C, H, N, S.

Dimethyl 1-acetoxy-2,3-dihydro-5-(2-thienyl)-1*H*-pyrrolizine-6,7-dicarboxylate (7c) (1.50 g of **2c**, 0.005 mol): yield (1.12 g, 62%): mp 160 °C (MeOH); IR 3110, 3100, 2950, 1735, 1695, 1450, 1370, 1295, 1245, 1230, 1170, 1115, 720 cm^{-1}; ^1H NMR 2 (s, $OCOCH_3$), 2.96 (m, C_3), 3.66 (s, $OCH_3 \times 2$), 4.1 (m, C_3), 6.03 (dd, C_1, J = 1.8 Hz, J = 7.2 Hz), 7.06 (dd, H4′, $J(4'3')$ = 3.6 Hz, $J(4'5')$ = 4.8 Hz), 7.26 (dd, H3′, $J(3'4')$ = 3.6 Hz, $J(3'5')$ = 1.2 Hz), 7.6 (dd, H5′, $J(5'4')$ = 4.8 Hz, $J(5'3')$ = 1.2 Hz). Anal. ($C_{17}H_{17}NO_6S$) C, H, N, S.

Dimethyl 1-Hydroxy-2,3-dihydro-5-thienyl-1*H*-pyrrolizine-6,7-dicarboxylates 8. A suspension of **7** (0.005 mol) in a methanolic solution saturated with NH_3 gas was stirred for 3 h. The product was completely dissolved. The solution was neutralized with CH_3CO_2H (1/2) and then concentrated in vacuo. After the residue was dissolved in chloroform, the organic layer was washed with brine, dried (Na_2SO_4), and evaporated to afford **8**.

Dimethyl 1-hydroxy-2,3-dihydro-5-(3-thienyl)-1*H*-pyrrolizine-6,7-dicarboxylate (8b) (1.81 g of **7b**, 0.005 mol): yield (0.81 g, 51%); mp 144 °C (Et_2O); IR 3420, 2950, 1720, 1660, 1550, 1440, 1310, 1210, 1120, 800 cm^{-1}; ^1H NMR 2.06–2.9 (m, C_2), 3.66 (s, $OCH_3 \times 2$), 3.9–4.33 (m, C_3), 5.12 (t, OH) [disappeared by addition of D_2O], 5.30 (dd, C_1, J = 6 Hz), 7.2 (dd, H4′, $J(5'4')$ = 4.8 Hz, $J(4'2')$ = 1.5 Hz), 7.53 (dd, H5′, $J(5'4')$ = 4.8 Hz, $J(5'2')$ = 2.7 Hz), 7.61 (dd, H2′, $J(2'4')$ = 1.5 Hz, $J(2'5')$ = 2.7 Hz). Anal. ($C_{15}H_{15}NO_5S$) C, H, N, S.

Dimethyl 1-hydroxy-2,3-dihydro-5-(2-thienyl)-1*H*-pyrrolizine-6,7-dicarboxylate (8c) (1.81 g of **7c**, 0.005 mol): yield (0.84 g, 53%); mp 140 °C (Et_2O); IR 3430, 1720, 1660, 1560, 1445, 1310, 1220, 1120, 720 cm^{-1}; ^1H NMR 2.66 (m, C_2), 3.66 (s, $OCH_3 \times 2$), 4.06 (m, C_3), 5.1 (t, OH, J = 6 Hz) [disappeared by addition of D_2O], 5.3 (dd, C_1, J = 6 Hz), 7.06 (dd, H4′, $J(4'3')$ = 3.6 Hz, $J(4'5')$ = 5.1 Hz), 7.2 (dd, H3′, $J(3'4')$ = 3.6 Hz, $J(5'3')$ = 1.5 Hz), 7.56 (dd, H5′, $J(5'4')$ = 5.1 Hz, $J(5'3')$ = 1.5 Hz). Anal. ($C_{15}H_{15}NO_5S$) C, H, N, S.

Dimethyl 2,3-Dihydro-1-oxo-5-(3-thienyl)-1*H*-pyrrolizine-6,7-dicarboxylate (9b). A mixture of the compound **8b** (0.32 g, 0.001 mol) and activated manganese dioxide (2 g) in dichloromethane (30 mL) was stirred for 8 h, and then the inorganic material was removed by filtration and thoroughly washed with hot methylene chloride. The combined filtrate and washings were concentrated to afford a solid, which was crystallized from methanol to give **9b** (0.172 g 54%): mp 141 °C (MeOH); IR 3100, 2940, 1715, 1700, 1520, 1450, 1290, 1210, 1160, 1055, 800 cm^{-1}; ^1H NMR 3 (t, C_2, J = 5.2 Hz), 3.66 (s, OCH_3), 3.76 (s, OCH_3), 4.33 (t, C_3, J = 5.2 Hz), 7.26 (dd, H4′, $J(4'5')$ = 5.1 Hz, $J(4'2')$ = 1.5 Hz), 7.6 (dd, H5′, $J(5'4')$ = 5.1 Hz, $J(5'2')$ = 2.7 Hz), 7.86 (dd, H2′, $J(2'5')$ = 2.7 Hz, $J(2'4')$ = 1.5 Hz). Anal. ($C_{15}H_{13}NO_5S$) C, H, N, S.

Dimethyl 2,3-Dihydro-1-oxo-5-(2-thienyl)-1*H*-pyrrolizine-6,7-dicarboxylate (9c). Manganese dioxide oxidation of **8c** (0.32 g, 0.001 mol), as described for **9b**, gave **9c** (0.178 g, 58%): mp 117 °C (MeOH); IR 3100, 1710, 1700, 1520, 1450, 1290, 1220, 1135 cm^{-1}; ^1H NMR 3.03 (t, C_2, J = 6 Hz), 3.66 (s, OCH_3), 3.73 (s, OCH_3), 4.36 (t, C_3, J = 6 Hz), 7.16 (dd, H4′, $J(4'5')$ = 5.1 Hz, $J(4'3')$ = 3.6 Hz), 7.4 (dd, H3′, $J(3'4')$ = 3.6 Hz, $J(3'5')$ = 1.2 Hz), 7.73 (dd, H5′, $J(5'4')$ = 5.1 Hz, $J(5'3')$ = 1.2 Hz). Anal. ($C_{15}H_{13}NO_5S$) C, H, N, S.

Dimethyl 5-(3-Thienyl)-3*H*-pyrrolizine-6,7-dicarboxylate (10b). Dry hydrochloric acid was bubbled for 5 min in methanol (50 mL). The triester **7b** (3.63 g, 0.01 mol) was added to this solution, which was stirred for 4 days at room temperature. Then the solution was concentrated in vacuo at 30 °C. The oily residue was dissolved in chloroform, and the organic layer was washed with sodium bicarbonate solution and then water, dried (Na_2SO_4), and concentrated in vacuo to afford crystals of **10b** (1.45 g, 48%): mp 133 °C (MeOH); IR 3105, 2940, 1700, 1685, 1440, 1290, 1200, 1160, 805 cm^{-1}; ^1H NMR 3.66 (s, $OCH_3 \times 2$), 4.83 (s, C_3), 6.8 (m, C_2, C_1), 7.33 (dd, H4′, $J(4'5')$ = 4.8 Hz), $J(4'2')$ = 1.5 Hz), 7.58 (dd, H5′, $J(5'4')$ = 4.8 Hz, $J(5'2')$ = 3 Hz), 7.78 (dd, H2′, $J(2'5')$ = 3 Hz, $J(2'4')$ = 1.5 Hz). Anal. ($C_{15}H_{13}NO_4S$) C, H, N, S.

Dimethyl 5-(2-Thienyl)-3*H*-pyrrolizine-6,7-dicarboxylate (10c). Treatment of **7c** (3.63 g, 0.01 mol) with acid as described for the preparation of **10b** gave **10c** (1.4 g, 46%): mp 152 °C (MeOH); IR 3100, 2940, 1735, 1720, 1690, 1440, 1240, 800 cm^{-1}; ^1H NMR 3.66 (s, OCH_3), 3.76 (s, OCH_3), 4.83 (m, C_3), 6.83 (m, 1 H), 7.10 (m, 1 H), 7.13 (dd, H4′, $J(4'5')$ = 4.8 Hz, $J(4'3')$ = 3.6 Hz), 7.40 (dd, H3′, $J(3'4')$ = 3.6 Hz, $J(3'5')$ = 1.2 Hz), 7.56 (dd, H5′, $J(5'4')$ = 4.8 Hz, $J(5'3')$ = 1.2 Hz). Anal. ($C_{15}H_{13}NO_4S$) C, H, N, S.

Methods. Male B6D2F1/01 mice, 3 months old, were administered 10^5 L1210 leukemia cells intraperitoneally (ip) on day 0. As determined in preliminary results showing the range of toxicity of compounds, they received different dose levels by the ip route.

Mice were individually weighed and 0.1- or 0.2-mL volumes of an aqueous mixture of 20% polyoxyethylene sorbitan monooleate (Tween 80) containing the tested compounds were injected per 20 g of body weight.

Each treated group included 8 mice. A control group (12 untreated mice) received an equivalent volume of vehicle by the ip route, while a reference group (8 treated mice) was administered the optimal dose of mitomycin, i.e. 2 mg/kg in distilled water by the ip route.

Drug injections were repeated on days 5 and 9. Mortality was monitored daily and autopsies were performed to determine whether death was due to leukemia or to drug toxicity.

For each dose, the activity was expressed as an oncostatic index: $I = T/C \times 100$, where T was the median survival time of the treated group and C that of the control group.

Mortality range in the treated group was statistically compared to that in the control group according to the nonparametric Wilcoxon's test.[30] When the statistical test was significant ($p < 0.05$) and I greater than 125, the compound was considered active at the given dose. In many cases, it was necessary to do new assays, with untreated and reference control groups. For the mitomycin-treated group, the oncostatic index varied from one experiment to the other. Therefore, in order that the results from the tested compounds obtained in these experiments might be compared, the following ratio, median survival time of treated group × 100/median survival time of reference group = treated I × 100/ref I, was calculated.

Wilcoxon's test was used between reference and treated groups.

Registry No. **1a**, 117918-56-6; **1b**, 117918-57-7; **1c**, 117918-58-8; **1d**, 117918-59-9; **2a**, 117918-60-2; **2b**, 117918-61-3; **2c**, 117918-62-4; **2d**, 117918-63-5; **3a**, 117918-64-6; **3b**, 117918-65-7; **3c**, 117918-66-8; **3d**, 117918-67-9; **4a**, 117918-68-0; **4b**, 117918-69-1; **4c**, 117918-70-4; **4d**, 117918-71-5; **5a**, 117918-72-6; **5b**, 117918-73-7; **5c**, 117918-74-8; **5d**, 117918-75-9; **6b**, 117918-76-0; **6c**, 117918-77-1; **7b**, 117940-40-6; **7c**, 117918-78-2; **8b**, 117918-79-3; **8c**, 117918-80-6; **9b**, 117918-81-7; **9c**, 117918-82-8; **10b**, 117918-83-9; **10c**, 117918-84-0; DMAD, 762-42-5; mitomycin C, 50-07-7; 2-furoic acid, 88-14-2; 3-thienoic acid, 88-13-1; 2-thienoic acid, 527-72-0; L-proline, 147-85-3; L-thiazolidine-4-carboxylic acid, 34592-47-7; dimethyl 5-[1-(4-acetoxy-2,3-dicarbomethoxy-7-oxabicyclo[2.2.1]-2,5-heptadienyl)]-2,3-dihydro-1*H*-pyrrolizine-6,7-dicarboxylate, 117918-85-1.

1-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine.[1] A Highly Potent and Selective Anti-HIV Agent

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The nucleoside analogue 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine (d4T, 1) was prepared by ring opening of the 3′,5′-anhydro compound **5**. This method has been refined such that it can be used to prepare d4T (1) on a large scale. The triphosphate of d4T (8) was also synthesized from 1 in order to examine the mode of action. The in vitro inhibitory activity of d4T was found to be comparable to that of AZT in HIV-infected CEM cells. The triphosphate of d4T (8) and that of AZT inhibited the HIV reverse transcriptase with poly(rA):oligo(dT) as the template:primer with K_i values of 0.032 and 0.007 μM, respectively. The in vitro toxicity of d4T against normal human hematopoietic progenitor cells (CFU-GM) was measured in comparison to AZT. While d4T reduces colony-forming units by 50% at a concentration of 100 μM, it takes only 1 μM AZT to have a similar toxic effect. With erythrocyte burst forming units (BFU-E) the in vitro toxicities for d4T and AZT have comparable ID_{50} values of 10 and 6.7 μM, respectively.

Acquired immunodeficiency syndrome (AIDS) is the result of an infection by the human immunodeficiency virus (HIV).[3] This retrovirus shows a specific tropism for the helper/inducer T cells,[4] leading to their depletion. The resultant profound immunosuppression predisposes patients to life-threatening opportunistic infections. Although at present there is no cure for AIDS, 3′-azido-3′-deoxythymidine (zidovudine, AZT) has already proved to be an efficacious treatment in clinical trials and has been approved for use in patients with AIDS.[5] A number of other chemical agents have been reported to have biological activity against the HIV.[6-16] In addition to the aforementioned chemotherapeutic agents, a number of biological response modifiers have also been evaluated.[17]

Traditional research in the antiviral area has concentrated on nucleoside analogues for new therapies against RNA and DNA viral infections. Research for active drugs against HIV has also found nucleoside analogues to be most efficacious. One common feature among the nucleoside derivatives that have shown good in vitro activity is the lack a 3′-OH group on the sugar part of the molecule, thereby enabling these substances to act as possible chain terminators of DNA synthesis.[18] The mode of action of these compounds is believed to require the nucleoside to be metabolized to its 5′-triphosphate form by cellular

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(1) The abbreviation d4T used in this paper is derived from the non-IUPAC name 2′,3′-didehydro-2′,3′-dideoxythymidine. This compound has also been referred to as 2′,3′-dideoxythymidinene (ddeThd).
(2) J.P.S. is the recipient of a Junior Faculty Research Award from the American Cancer Society.
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