under N_2 cooled to ice-bath temperature. The reaction mixture was stirred at ice-bath temperature for 15 min and then at room temperature for 2 h. To the reaction mixture was added anhydrous hydrazine (0.96 g, 0.03 mol) followed by addition of 1 N HCl until the reaction mixture was at pH 9. The reaction mixture was stirred for an additional 2 h at ambient temperature and then poured into cold 10% aqueous NaOH (100 mL). The basic mixture was extracted twice with CH_2Cl_2. The CH_2Cl_2 solution was washed with brine, dried ($MgSO_4$), and evaporated in vacuo to provide crude free base **10** (530 mg) as an oil. A monohydrochloride was prepared by dissolving free base **10** (530 mg, 2.4 mmol) in 25 mL of MeOH and then adding 0.1 N HCl (24 mL, 2.4 mmol). The solution was concentrated in vacuo and residual solid was recrystallized from MeOH–EtOAc to give 350 mg of **10** monohydrochloride (27%): mp 290 °C dec; ^{13}C NMR (Me_2SO-d_6) δ 10.90 ($CH_2CH_2CH_3$), 15.70 ($CH_2CH_2CH_3$), 20.25 (CH_24), 27.44 (CH_29), 49.81 (CH_26), 53.40 ($CH_2C_2H_5$), 61.82 (CH4a), 62.62 (CH_27), 72.94 (CH8a), 110.54 (C3a), 129.96 (C3), 139.96 (C9a); ^1H NMR (Me_2SO-d_6, 270 MHz) δ 0.95 (t, $CH_2CH_2CH_3$), 1.74 (m, $CH_2CH_2CH_3$), 2.58/3.11 (m, CH_29), 2.88/3.30 (m, CH_24), 3.00/3.29 (m, $CH_2C_2H_5$), 3.18/3.49 (m, CH_26), 3.38 (br, H4a), 4.04 (m, CH_27), 4.15 (br, H8a), 7.77 (s, H3). Anal. ($C_{12}H_{20}ClN_3O$) C, H, Cl, N.

***trans*-(±)-4,4a,7,8,8a,9-Hexahydro-8-propyl-6*H*-thiazolo[5,4-*g*][1,4]benzoxazin-2-amine Dihydrobromide (11).** To a solution of **9** (1.97 g, 0.01 mol) in 20 mL of glacial HOAc was introduced 2.3 mL of freshly prepared 38% HBr in glacial HOAc. The reaction mixture was irradiated with a UV lamp, and a solution of Br_2 (0.4 mL) in 5 mL of glacial HOAc was added dropwise. The reaction mixture was stirred at room temperature for an additional 30 min and then concentrated in vacuo. The crude α-bromo ketone was dissolved in 50 mL of anhydrous EtOH, and thiourea (0.84 g, 0.011 mol) was added to the solution. The reaction mixture was refluxed for 16 h under N_2. The reaction mixture was cooled, and a precipitate was collected. The solid was recrystallized from MeOH to give 1.6 g (39%) of **11** dihydrobromide: mp 297 °C dec; ^{13}C NMR (Me_2SO-d_6) δ 10.85 ($CH_2CH_2CH_3$), 15.78 ($CH_2CH_2CH_3$), 22.74 (CH_29), 28.49 (CH_24), 49.96 (CH_27), 53.76 ($CH_2C_2H_5$), 60.77 (CH8a), 71.43 (CH4a), 110.13 (C9a), 130.64 (C3a), 169.24 (C2); ^1H NMR (Me_2SO-d_6, 270 MHz) δ 0.95 (t, $CH_2CH_2CH_3$), 1.75 (m, $CH_2CH_2CH_3$), 2.52/3.05 (m, CH_24), 2.93/3.45 (m, CH_29), 3.06/3.27 (m, $CH_2C_2H_5$), 3.27/3.60 (m, CH_27), 3.65 (br, CH8a), 4.08 (m, CH_26), 4.22 (br, CH4a). Anal. ($C_{12}H_{21}Br_2N_3OS$) C, H, Br, N, S.

***trans*-(±)-5a,6,7,8,9a,10-Hexahydro-6-propyl-8*H*-pyrimido[5,4-*g*][1,4]benzoxazin-2-amine Hydrochloride (12).** A solution of **9** (2 g, 0.011 mol) and tris(dimethylamino)methane (5 g, 0.034 mol) in 40 mL of toluene was refluxed under N_2 for 2 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in 80 mL of anhydrous EtOH. To the EtOH solution was added guanidine carbonate (1.98 g, 0.011 mol), and the reaction mixture was refluxed under N_2 for 16 h. The reaction mixture was cooled to ice-bath temperature to precipitate a yellow solid. The precipitate was collected, and the filter cake was washed with H_2O. The crude product was dissolved in 100 mL of $CHCl_3$. The $CHCl_3$ solution was washed with dilute NH_4OH and brine. The $CHCl_3$ solution was dried ($MgSO_4$) and evaporated in vacuo to provide 600 mg of crude **12** as free base. A monohydrochloride was prepared by dissolving **12** free base (600 mg, 2.4 mmol) in 25 mL of THF and adding 0.1 N HCl (24 mL, 2.4 mmol). The solution was concentrated in vacuo, and the residual solid was recrystallized from MeOH–EtOAc to provide 440 mg (14%) of **12** monohydrochloride: mp 290 °C dec; ^{13}C NMR (Me_2SO-d_6) δ 10.95 ($CH_2CH_2CH_3$), 15.87 ($CH_2CH_2CH_3$), 24.18 (CH_25), 36.74 (CH_210), 49.97 (CH_27), 53.40 ($CH_2C_2H_5$), 61.12 (CH5a), 62.75 (CH_28), 72.03 (CH9a), 113.14 (CH4a), 157.83 (CH4), 161.47 (C2 or C10a), 162.16 (C10a or C2); ^1H NMR (Me_2SO-d_6, 270 MHz) δ 0.94 (t, $CH_2CH_2CH_3$), 1.73 (m, $CH_2CH_2CH_3$), 2.70/2.95 (m, CH_210), 2.95/3.20 (m, $CH_2C_2H_5$), 3.2/3.5 (m, CH_27), 3.3 (br, CH5a), 3.3 (m, CH_25), 4.04 (m, CH_28), 4.14 (br, CH9a), 8.05 (s, CH4). Anal. ($C_{13}H_{21}ClN_4O$) C, H, N.

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Synthesis and Antitumor Activity of N-Terminal Proline-Containing Peptide–(Chloroethyl)nitrosoureas

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The N^{α}-(2-chloroethyl)-*N*-nitrosocarbamoyl derivatives of H-Pro-Lys(X)-Pro-Val-NH_2 (X: *tert*-butyloxycarbonyl, formyl, (2-chloroethyl)nitrosocarbamoyl) were synthesized. It was found that the bis-substitution of the urea N^3 in these derivatives does not decrease the antitumor activity influenced mainly by the nature of the carrier molecule as a whole.

One of the most effective cytostatic (2-chloroethyl)-nitrosoureas ($ClCH_2CH_2N(NO)$-CO-NHR) is the 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU).[1] This compound, although of excellent antitumor activity, is rather toxic. Numerous analogues with modified R groups have been synthesized in order to obtain less toxic and more selective compounds.

For the same reason, we have recently prepared peptide–(chloroethyl)nitrosoureas,[2] where the peptide moieties were fragments of polypeptide hormones (α-melanotropin, gastrin) with a receptor-recognizing ability. The antineoplastic activity of these peptide derivatives against L1210 leukemia in mice[3] and against human melanoma xenograft[4] proved to be significant. The Q(NO)-Pro-Val-NH_2 (Q(NO) = $ClCH_2CH_2N(NO)CO$), although inactive as a melanotropic agent, showed strikingly good

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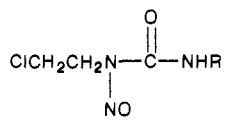
Table I. Antitumor Action of the Peptide–(Chloroethyl)nitrosoureas on L1210 Leukemia in Mice[a]

compound (Q(NO): $ClCH_2CH_2N(NO)$-CO)	dose mg/kg	dose mmol/kg	% ILS	LD_{50}, mg/kg
Q(NO)-Pro-NH_2 (XIII)	100	0.403[b]	420	140
Q(NO)-Pro-Val-NH_2 (XIV)	100	0.28[b]	cure:[c] 8/10	150
Q(NO)-Lys-Pro-Val-NH_2·HCl	130	0.25[b]	cure:[c] 6/10	175
Q(NO)-Lys(QNO)-Pro-Val-NH_2	100	0.16	380	250
Q(NO)-Pro-Lys(QNO)-Pro-Val-NH_2 (III)	30	0.042	100	
	100	0.14	cure:[c] 10/10	
	200	0.28	120	220
Q(NO)-Pro-Lys(BOC)-Pro-Val-NH_2 (VI)	30	0.045	no effect	
	100	0.15	no effect	
	200	0.30	30	300
Q(NO)-Lys(For)-Pro-Val-NH_2 (XI)	30	0.059	70	
	100	0.20	110	
	200	0.40	190	260
Q(NO)-Pro-Lys(For)-Pro-Val-NH_2 (XII)	50	0.083	40	
	100	0.16	80	
	200	0.32	80	250
H-Lys(For)-Pro-Val-NH_2·HCl	100	0.24	no effect	

[a] Control group (saline or solvent) median survival time: 10 days. Each experimental group comprised 10 mice. [b] Maximal effective dose; detailed data in ref 3, 4. [c] Cures observed on the 60th day after transplantation: numerator, number of cured mice; denominator, number of mice in the group.

cytostatic activity and low toxicity. Responsible for this is most likely its chemical structure, since, while the effective (2-chloroethyl)nitrosoureas generally have the Q(NO) group attached to a secondary nitrogen atom as in **1**, in the case of peptides with proline terminus it is coupled to a tertiary nitrogen atom as in **2**.



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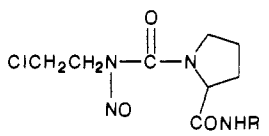

2

Suami et al.[5] published the synthesis and antitumor potency of Q(NO)-Pro-NH_2. We have also prepared this compound and found it more effective in one single dose against L1210, as compared with the Japanese administration in three doses. This discrepancy may be due to the applied lower doses in their experiments. Rodriguez et al.[6] reported on the relatively weak cytotoxic activity of Q(NO)-Pro-$NHCH_2CH_2Cl$ and explained it on the basis of the finding of Johnston et al.[7] that bis-substitution of the N^3 of the (2-chloroethyl)nitrosoureas leads to less active compounds. This is, however, in contradiction with the observed high cytostatic activity of the sarcosin derivatives[5,6] having also a tertiary nitrogen atom in the N^3 position of the urea.

The aim of our present work is to investigate how the N-terminal incorporation of a proline residue into an already tested peptide–(chloroethyl)nitrosourea alters its antitumor activity. For this purpose we chose the C-terminal Lys-Pro-Val-NH_2 sequence of α-melanotropin, because both the α-mono-[3] and the α,ϵ-disubstituted (2-chloroethyl)nitrosocarbamoyl derivatives have proved to be potent antineoplastic agents (unpublished results).

Chemistry

For the synthesis of the appropriate peptide carrier, BOC-Pro-OH (BOC: *tert*-butyloxycarbonyl) was coupled to H-Lys(BOC)-Pro-Val-NH_2[8] by using the modified mixed anhydride method[9] to obtain BOC-Pro-Lys(BOC)-Pro-Val-NH_2 (I). The BOC groups were removed with trifluoroacetic acid, and after ion-exchange chromatography, H-Pro-Lys-Pro-Val-NH_2·2HCl (II) was isolated. This compound was allowed to react with *N*-(2-chloroethyl)-*N*-nitrosocarbamic acid succinimido ester,[2,10] to form the disubstituted peptide derivative Q(NO)-Pro-Lys(Q(NO))-Pro-Val-NH_2 (III).

Attempts to synthesize a monosubstituted derivative were unsuccessful: Z-Pro-OH (Z: benzyloxycarbonyl) was coupled to H-Lys(BOC)-Pro-Val-NH_2 to give Z-Pro-Lys(BOC)-Pro-Val-NH_2 (IV), which was hydrogenolyzed to H-Pro-Lys(BOC)-Pro-Val-NH_2 (V) and then converted to Q(NO)-Pro-Lys(BOC)-Pro-Val-NH_2 (VI). For the removal of the BOC group, the same procedure was used (HCl in cold formic acid) as in the case of the previously synthesized peptide–(chloroethyl)nitrosoureas,[2] but in this case rapid denitrosation occurred. A similar observation was made by Snyder and Stock[11] during the acid-catalyzed decomposition of *N*-nitrosoureas: the compound with a tertiary nitrogen atom proved to be more susceptible to denitrosation.

To make the investigation of the monosubstituted derivative possible, we decided to use a compound protected on the ϵ-amino group of the lysine derivative. As bulky protecting groups greatly alter the character of the carrier peptide, we chose the formyl group for this purpose. Z-Lys(For)-Pro-Val-NH_2 (VII) (For: -CHO) was prepared from Z-Lys(For)-ONP (VIII) (ONP: $-OC_6H_4NO_2$) and H-Pro-Val-NH_2. Hydrogenolysis of this compound was carried out in formic acid–methanol, and, after the addition of hydrogen chloride, H-Lys(For)-Pro-Val-NH_2·HCl[12] was isolated. This compound was coupled with Z-Pro-OH to yield Z-Pro-Lys(For)-Pro-Val-NH_2 (IX). The Z group was removed in the same way as above, to give H-Pro-Lys(For)-Pro-Val-NH_2·HCl (X). These two peptides with free

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amino terminus were then converted to the corresponding *N*-(2-chloroethyl)-*N*-nitrosoureas (XI, XII) in the usual way.

The end products were purified by column chromatography on silica gel in a cold room. These compounds are best characterized by the determination of their nitroso content. For this purpose in our previous work we used spectrophotometric measurements at 400 nm and compared the molar extinction coefficients to those of pure, crystalline *N*-(2-chloroethyl)-*N*-nitrosocarbamoyl amino acids (ϵ_{400nm}^{max} 94). With these derivatives bearing a proline at the N-terminus, we always found lower values for ϵ. Therefore, we synthesized [*N*-(2-chloroethyl)-*N*-nitrosocarbamoyl]proline amide (XIII) in pure, crystalline state and found its absorption maximum shifted to 395 nm with a molar extinction coefficient of 87.

Biological Results

The compounds synthesized in the present study were evaluated for antitumor activity against L1210 in vivo in mice. In Table I, the increase of life span (ILS) and/or the percentage of surviving animals on day 60 (long survivors) and the LD_{50} values are given. For comparison, we also indicated the previously measured data[3] of the *N*-(2-chloroethyl)-*N*-nitrosocarbamoyl derivatives of proline amide, prolylvalinamide, and lysylprolylvalinamide.

Since we did not succeed in preparing Q(NO)-Pro-Lys-Pro-Val-NH_2, and as we were unable to compare the cytostatic activity of Q(NO)-Pro-Lys-Pro-Val-NH_2 with that of Q(NO)-Lys-Pro-Val-NH_2, we can only make a direct comparison between the two bis-substituted compounds Q(NO)-Lys(QNO)-Pro-Val-NH_2 and Q(NO)-Pro-Lys(QNO)-Pro-Val-NH_2. The latter compound shows higher antitumor activity and, what is more, in our test it proved to have a curative effect. In the case of the other peptide–(chloroethyl)nitrosoureas with proline terminus, there is a dramatic decrease in the antitumor activity, caused very likely by the substitution of the ϵ-amino group of lysine.

When this group was substituted with the BOC group (VI), no activity was observed, and even in the case of the formyl substitution (XI, XII), only a moderate activity was found. In this context, it is remarkable that, by the N-formylation of melphalan ester, another alkylating agent, antitumor activity decreases, without changing the chemical reactivity.[13] It is perhaps worth mentioning that the two antitumor alkaloids vincristine and vinblastine have different antitumor spectra and toxicity, the former containing a formyl group in the position of a methyl group in vinblastine.[14]

Thus it may be concluded that the bis-substitution of the N^3 in the *N*-(2-chloroethyl)-*N*-nitrosoureas per se does not cause any decrease in the antitumor activity influenced mainly by the general pharmacokinetic properties of the whole carrier molecule.

Experimental Section

Thin-layer chromatography (TLC) was carried out on Merck DC-Alufolien Kieselgel 60 and on Kieselgel 60 F254 (nitroso compounds). Column chromatography was performed on silica gel (Merck, Kieselgel 60, Art. 10832) below 5 °C. UV spectra were determined on a Specord UV–vis spectrometer (Karl Zeiss, Jena) in 96% ethanol and in 4-cm-long cuvettes at 25 °C.

The following solvent systems were used for chromatography (v/v): A, ethyl acetate–pyridine–acetic acid–water, 120:20:6:11; B, butanol–pyridine–acetic acid–water, 4:1:1:1; C, butanol–acetic acid–water, 4:1:1; D, ethyl acetate–pyridine–acetic acid–water, 60:20:6:11; E, chloroform–methanol, 9:1; F, ethyl acetate–methanol, 3:1; and G, chloroform–methanol, 8:2.

Evaporation or concentration in vacuo was carried out by using a rotation evaporator at a pressure of 1–15 mbars, so that the bath temperature never exceeded 37 °C.

BOC-Pro-Lys(BOC)-Pro-Val-NH_2 (I). To the stirred solution of 861 mg (4 mmol) of BOC-Pro-OH in 20 mL of absolute CH_2Cl_2 at −5 °C were added 0.44 mL (4 mmol) of *N*-methylmorpholine (NMM) and 0.42 mL (4.2 mmol) of ethyl chloroformate, and stirring was continued for 20 min at −5 °C. The solution was then washed successively with ice-cold 10% citric acid, water, 10% sodium bicarbonate, and water (5 mL), dried over Na_2SO_4, and filtered off. H-Lys(BOC)-Pro-Val-NH_2·CH_3COOH[8] (2 g; 4 mmol) was dissolved in 10 mL of absolute CH_2Cl_2 with 0.44 mL (4 mmol) of NMM at 0 °C and was poured into the cold solution of the mixed anhydride. Stirring was continued at 0 °C for a half-hour and at room temperature for 2 h. The reaction mixture was washed successively with ice-cold water, 10% citric acid, water, 10% $NaHCO_3$, and water (5 mL), dried over Na_2SO_4, and evaporated in vacuo. The residue was triturated with ether and filtered off (1.8 g; 70%). The crude product was dissolved in ethyl acetate and precipitated with petroleum ether (1.5 g; 59%): R_f 0.87 (A), 0.78 (B); $[\alpha]^{25}_D$ −101.21° (*c* 1.00, methanol). Anal. ($C_{31}H_{54}N_6O_8$) C, H, N.

H-Pro-Lys-Pro-Val-NH_2·2HCl (II). Compound I (1.27 g; 2 mmol) was dissolved in 10 mL of trifluoroacetic acid, and after 15 min at room temperature, ether was poured into the solution. The precipitate was filtered off, washed several times with ether, and dried in a desiccator over KOH to give 0.96 g (94%) of H-Pro-Lys-Pro-Val-NH_2·2CF_3COOH. The product was dissolved in water and applied onto a column of Amberlite IRA 400 in chloride cycle, and the effluent was collected and lyophilized to give II in quantitative yield: R_f 0.27 (B), 0.13 (C); $[\alpha]^{25}_D$ −88.72° (*c* 1, methanol). Anal. ($C_{21}H_{40}N_6O_4Cl_2$) C, H, N. Amino acid analysis (after hydrolysis with 5.7 N HCl, 105 °C, 24 h) gave Pro 2.05, Val 1.00, Lys 1.05.

Q(NO)-Pro-Lys(QNO)-Pro-Val-NH_2 (III). To the solution of 511 mg (1 mmol) of II in 6 mL of absolute dimethylformamide (DMF) were added 0.28 mL (2 mmol) of triethylamine (TEA) and 500 mg (2 mmol) of *N*-(2-chloroethyl)-*N*-nitrosocarbamic acid succinimido ester with stirring and ice cooling. After ca. 3 h, the reaction mixture was evaporated in vacuo. The residue was diluted with cold ethyl acetate and extracted twice with cold water. After drying (Na_2SO_4), the ethyl acetate was evaporated in vacuo. The crude product was purified on a silica gel column in solvent system F to give pure III in 63% yield: R_f 0.70 (F), 0.5 (E); ϵ_{395nm}^{max} 178. Anal. Calcd for $C_{27}H_{44}N_{10}O_8Cl_2$ (707.64): N, 19.79; Cl, 10.02. Found: N, 19.11; Cl, 10.07.

Z-Pro-Lys(BOC)-Pro-Val-NH_2 (IV). The compound was prepared from Z-Pro-OH and H-Lys(BOC)-Pro-Val-NH_2 by the same procedure as that used for compound I: yield, 75%; R_f 0.70 (A), 0.81 (C); $[\alpha]^{25}_D$ −95.26° (*c* 1, methanol). Anal. ($C_{34}H_{52}N_6O_8$) C, H, N.

H-Pro-Lys(BOC)-Pro-Val-NH_2 (V). Compound IV (1.34 g; 2 mmol) was hydrogenolyzed in methanol in the presence of Pd/C catalyst for 3 h. After filtration and evaporation, the residue was triturated with ether to give the desired product in quantitative yield: R_f 0.17 (D), 0.58 (B); $[\alpha]^{25}_D$ −89.24° (*c* 1, methanol). Anal. ($C_{26}H_{46}N_6O_6$) C, H, N.

Q(NO)-Pro-Lys(BOC)-Pro-Val-NH_2 (VI). The title compound was prepared with the same procedure as that used for III. The crude product was purified on a silica gel column in solvent system E to yield VI (55%): R_f 0.44 (E), 0.72 (B); ϵ_{395nm}^{max} 85.2. Anal. Calcd for $C_{29}H_{49}N_8O_8Cl$ (673.21): N, 16.64; Cl, 5.27. Found: N, 16.13; Cl, 5.74.

Z-Lys(For)-Pro-Val-NH_2 (VII). H-Pro-Val-NH_2·HCl[15] (3.5 g; 14 mmol), 1.5 mL (14 mmol) of NMM, and 6 g (14 mmol) of VIII were dissolved in 50 mL of DMF with stirring, and the solution was left overnight at room temperature. An excess of water was poured into the reaction mixture, and the precipitate was filtered off. The product was washed on the filter several

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times with the 1:2 mixture of 0.5 M K_2CO_3 and 0.5 M $KHCO_3$ solutions and finally with water. After the crude material was dried, it was boiled in 50 mL of ethanol, then cooled down, filtered off, and washed on the filter several times with ether to give 4.6 g (66%) of VII, mp 217–219 °C (lit.[11] mp 217–218 °C).

Z-Lys(For)-ONP (VIII). To the solution of 1.54 g (5 mmol) of Z-Lys(For)-OH[12] in 7 mL of absolute CH_2Cl_2 were added 0.69 g (5 mmol) of *p*-nitrophenol and 1.03 g (5 mmol) of dicyclohexylcarbodiimide with stirring and ice cooling. Stirring was continued for 2 h at 0 °C, and then the reaction mixture was left overnight at room temperature. The precipitated dicyclohexylurea was filtered off, and the solution was evaporated in vacuo. The residue was crystallized from acetone–petroleum ether to yield 1.5 g (70%) of VIII: mp 115–116 °C; $[\alpha]^{25}_D$ −30.12° (*c* 1, methanol). Anal. ($C_{21}H_{23}N_3O_7$) C, H, N, O.

Z-Pro-Lys(For)-Pro-Val-NH_2 (IX). The title compound was prepared from H-Lys(For)-Pro-Val-NH_2·HCl[12] and Z-Pro-OH by the same procedure as described for compound I. Purification was carried out on a silica gel column in solvent system A to give IX in 64% yield: R_f 0.33 (A), 0.76 (B); $[\alpha]^{25}_D$ −107.06° (*c* 1, methanol). Anal. ($C_{30}H_{44}N_6O_7$) C, H, N.

H-Pro-Lys(For)-Pro-Val-NH_2·HCl (X). Compound IX (1.2 g; 2 mmol) was suspended in 15 mL of methanol containing 10% formic acid and hydrogenolyzed in the presence of Pd/C catalyst for about 1 h. After filtration, the solution was evaporated in vacuo, the residue was dissolved in 10 mL of methanol, 2 mL of 1 N hydrochloric acid was added, and the solution was evaporated again. The residue was dissolved in water and lyophilized to give 1 g (94%) of X: R_f 0.15 (C), 0.45 (B); $[\alpha]^{25}_D$ −102.25° (*c* 1, methanol). Anal. ($C_{22}H_{39}N_6O_5Cl$) C, H, N, Cl.

Q(NO)-Lys(For)-Pro-Val-NH_2 (XI). To the solution of 1.1 g (2.7 mmol) of H-Lys(For)-Pro-Val-NH_2·HCl in 10 mL of absolute DMF were added 0.75 mL (5.4 mmol) of TEA and 675 mg (2.7 mmol) of *N*-(2-chloroethyl)-*N*-nitrosocarbamic acid succinimido ester with ice cooling and stirring. After 1 h, the reaction mixture was concentrated in vacuo. The residue was dissolved in cold ethyl acetate and washed three times with water. Sodium chloride was added to the combined water solution, which was extracted three times with ethyl acetate. The combined ethyl acetate solution was dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was chromatographed on a silica gel column in solvent system F to give 800 mg (59%) of XI: R_f 0.52 (F), 0.60 (A); $\epsilon_{400nm}{}^{max}$ 92. Anal. Calcd for $C_{20}H_{34}N_7O_6Cl$ (504.00): N, 19.45; Cl, 7.03. Found: N, 18.89; Cl, 6.73.

Q(NO)-Pro-Lys(For)-Pro-Val-NH_2 (XII). The compound was prepared from X by the same procedure as that used for XI. Purification was carried out on a silica gel column in solvent system G to give compound XII in 60% yield: R_f 0.78 (G), 0.28 (F); $\epsilon_{395nm}{}^{max}$ 86.6. Anal. Calcd for $C_{25}H_{41}N_8O_7Cl$ (601.12): N, 18.64; Cl, 5.89. Found: N, 18.10; Cl, 5.63.

Q(NO)-Pro-NH_2 (XIII). The title compound was prepared from H-Pro-NH_2 by the same procedure as that used for III. Column chromatography on silica gel was performed in solvent system E: yield, 70%; R_f 0.8 (E); $\epsilon_{395nm}{}^{max}$ 87; mp 94–95 °C (dec). Anal. ($C_8H_{13}N_4O_3Cl$) C, H, N, Cl.

Q(NO)-Pro-Val-NH_2 (XIV). H-Pro-Val-NH_2·HCl (1.25 g; 5 mmol) was suspended in 15 mL of absolute DMF; 1.4 mL (10 mmol) of TEA and 1.25 g (5 mmol) of *N*-(2-chloroethyl)-*N*-nitrosocarbamic acid succinimido ester were added to it with stirring and ice cooling. After 1 h, the reaction mixture was diluted with ice-cold water and the precipitate was filtered off and washed well on the filter with water. The product was dried over P_2O_5 to give 0.91 g (53%) of XIV: R_f 0.8 (G); mp 130 °C (dec); $\epsilon_{395nm}{}^{max}$ 87. Anal. ($C_{13}H_{22}N_5O_4Cl$) N, Cl.

Measurements on L1210 Leukemia in Mice. Six to ten week old C57B1 × DBA_2 F_1 hybrid mice of either sex, bred in our institute, were inoculated intraperitoneally (ip) with 10^6 cells of L1210 leukemia. The L1210 was maintained by weekly transplantation in DBA_2 mice. Compounds in the amount equivalent to the dose related to kilograms of body weight were dissolved in 0.2 mL of dimethyl sulfoxide and diluted to 10 mL with 0.9% NaCl. Treatment consisted of a single ip injection 1 day after transplantation in a volume of 0.1 mL/10 g of body weight. The percentage increase in life span (% ILS) of treated animals was compared with the untreated tumor bearer and was calculated as follows: % ILS = [(T − C)/C] × 100 (T and C = median survival in days for treated and control groups, respectively). The experiments were repeated three times.

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Registry No. I, 106039-75-2; II, 106039-76-3; III, 106039-77-4; IV, 106039-78-5; V, 106039-79-6; VI, 106039-80-9; VII, 106039-81-0; VIII, 14373-54-7; IX, 106039-82-1; X, 106039-83-2; XI, 106039-84-3; XII, 106039-85-4; XIII, 81965-44-8; XIV, 97209-67-1; BOC-Pro-OH, 15761-39-4; H-Lys(BOC)-Pro-Val-NH_2·AcOH, 92603-22-0; H-Pro-Lys-Pro-Val-NH_2·2CF_3COOH, 106039-87-6; Z-Pro-OH, 1148-11-4; H-Lys(BOC)-Pro-Val-NH_2, 53267-79-1; H-Pro-Val-NH_2·HCl, 51165-72-1; Z-Lys(For)-OH, 20807-05-0; H-Lys(For)-Pro-Val-NH_2·HCl, 106039-88-7; H-Pro-NH_2, 7531-52-4; Q(NO)-Lys(QNO)-Pro-Val-NH_2, 87230-62-4; Q(NO)-Lys-Pro-Val-NH_2·HCl, 87230-64-6; *N*-(2-chloroethyl)-*N*-nitrosocarbamic acid succinimido ester, 80354-49-0.

Antitumor Agents. 86.[1,2] Synthesis and Cytotoxicity of α-Methylene-γ-lactone-Bearing Purines

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α-Methylene-γ-lactones covalently linked to adenine (**3**), hypoxanthine (**4**), and guanine (**5**) were synthesized by using the convenient Reformatsky-type reaction between ethyl α-(bromomethyl)acrylate and the proper purine ketones. In vitro cytotoxicity data demonstrated that these compounds were active against L-1210 tissue culture cells with **3** being most potent (ED_{50} = 0.3 μg/mL).

Alkylating agents have played a major role in the development of antitumor agents.[3] One member of this class of chemotherapeutic agents is α-methylene-γ-lactone-containing sesquiterpenes, such as the cytotoxic antitumor helenalin.[4-6] It has been demonstrated that the O=C—C=CH_2 system which is present in helenalin may act as an alkylating center through a Michael-like reaction with

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(2) A preliminary account of this work was presented at the International Research Congress on Natural Products, Chapel Hill, NC, July 7–12, 1985; Abstr. 121.
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