(E) Conversion of (+)-8a to (−)-2. Ten milliliters of ethanol (anhydrous) was treated with sodium hydride (30 mg, 1 mmol) and stirred for 5 min, and then **8a** (108 mg, 0.2 mmol) was added. After reflux for 1 h, the solution was poured into water, extracted with methylene chloride, and concentrated by evaporation. The residue was dissolved in 3.5 mL of a 6% ethanolic nickel chloride solution and treated dropwise with an ethanolic sodium borohydride solution at room temperature until the black coloration persisted. After rotary evaporation the residue was taken up in chloroform, washed once with concentrated ammonia solution, and concentrated by evaporation. The product was 90 mg of a viscous oil consisting of (−)-**2** which contained about 20% of the corresponding diethyl ester. The oil was reacted with (1*S*)-(−)-camphanic acid chloride (**3**), analogously to procedure A. Mp (recrystallization from ether) and HPLC showed that the product was identical to **4a**.

Acknowledgment. We thank Drs. P. Schmitt and Ch. Wünsche for the spectroscopic data, Drs. J. Lenfers and V. Muschalek for chromatographic separation, and U. Appel, S. Borgmann, and P. Hilker for preparative work. We are grateful to C. Lettner for producing the manuscript.

Supplementary Material Available: Tables of bond distances, bond angles, torsion angles, positional parameters, and general displacement parameter expressions (11 pages). Ordering information is given on any current masthead page.

Synthesis and Functional Evaluation of a Peptide Derivative of 1-β-D-Arabinofuranosylcytosine

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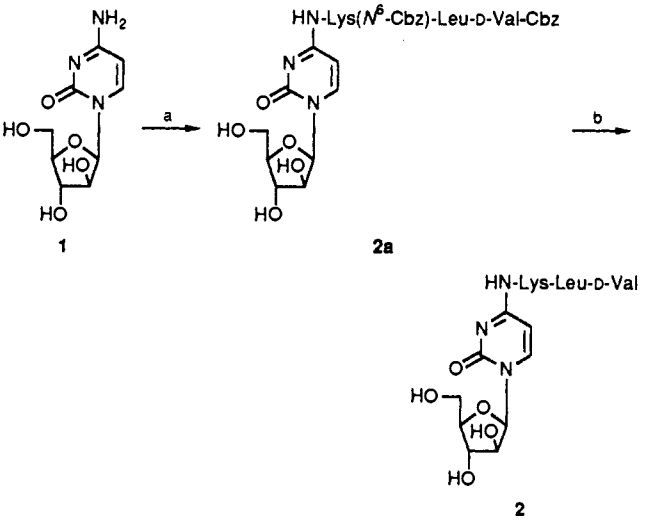
We have synthesized a peptidyl prodrug derivative of 1-β-D-arabinofuranosylcytosine (1) designed to be a selective substrate of plasmin. D-Val-Leu-Lys-*ara*-C (**2**) was obtained by coupling the protected peptide Cbz-D-Val-Leu-(N^6-Cbz)Lys-OH and *ara*-C (1) by a water-soluble carbodiimide (EDCI), followed by the removal of the Cbz groups by using catalytic hydrogenolysis over Pd/C. The kinetic constant of hydrolysis of **2** in the presence of plasmin demonstrated effective release of 1. The amino group of 1, which is sensitive to the removal by cytidine deaminase, is protected in **2** by the formation of the amide bond resulting in a prolonged half-life of **2** in biological milieu. The antiproliferative efficiency of **2** against L1210 leukemic cells was significantly higher than that of 1. The activity of **2** was abolished in the presence of serine proteinase inhibitor, (4-amidinopheny)methanesulfonyl fluoride. These data indicate that **2** is a prodrug form of 1 in systems generating plasmin.

The cytotoxic S-phase-specific antimetabolite *ara*-C[1,2] (1) is the most frequently used compound for the treatment of patients with acute myelogenous leukemias. Its application for treating patients, however, has a few disadvantages. Firstly, its specificity is low, since the compound may readily affect normal and healthy cells causing many unwanted toxic side effects. Secondly, the amino group of the cytosine in the drug is sensitive to deaminases, thus it can be easily removed leading to the inactivation of the drug by formation of an ineffective uridine derivative.[3]

A number of analogues of 1 have been prepared, most of them being less toxic than 1. An early attempt was the synthesis of the 2,2'-anhydro analogue of 1, which is resistant to cytidine deaminase and is slowly hydrolyzed releasing the active drug.[4] Other prodrugs of 1 include a large number of derivatives monosubstituted in either 5' or the 3' position of the arabinose or disubstituted in both of these positions or at the N-4 position of the cytosine ring.[5-7] These derivatives are particularly inter-

Scheme I[a]



[a] (a) DMSO, EDCI, Cbz-D-Val-Leu-Lys(N^6-Cbz); (b) Pd/C, H_2.

(1) Bhuyan, B. K.; Scheidt, L. G.; Fraser, T. J. Cell cycle phase specificity of antitumor agents. *Cancer Res.* **1972**, *32*, 398-407.
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Table I. Properties of Protected Dipeptides and Tripeptides

compd	purification[c]	yield, %	recryst solvent	mp, °C	optical rotation: $[\alpha]_D$, deg (c, solvent)	formula[d]	anal.
6[a]	B (0.88)	89	light petroleum	92–93	−38.44 (0.45, MeOH)	$C_{20}H_{30}N_2O_5$	CHN
7[b]	B (0.70)	75	EtOAc/hexane 4:1	134–136	−28.90 (0.65, MeOH)	$C_{19}H_{28}N_2O_5$	CHN
8[a]	A (0.87)	78	EtOAc	181–184	−32.00 (0.25, MeOH)	$C_{34}H_{48}N_4O_8$	CHN
9[b]	A (0.53)	68	Et_2O/light petroleum 8:1	161–163	−24.50 (0.20, MeOH)	$C_{33}H_{46}N_4O_8$	CHN

[a] Method A. [b] Method B. [c] Solvent used for column chromatography. Numbers in parentheses are R_f values detected by TLC. [d] ^1H NMR spectra of compounds were consistent with the assigned structures.

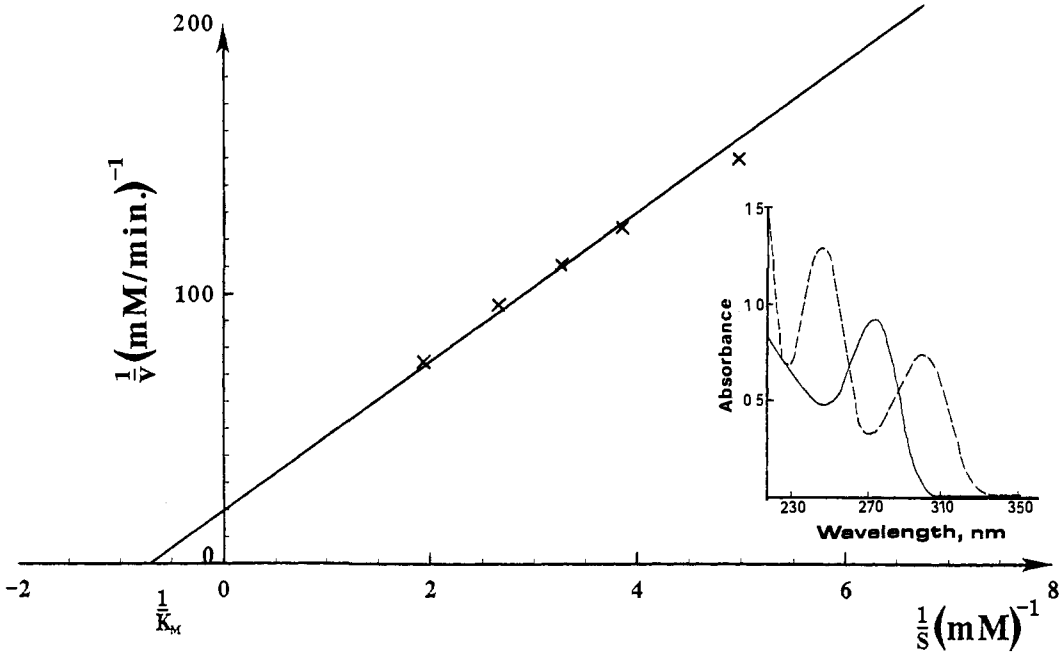

Figure 1. Determination of the K_m value of D-Val-Leu-Lys-*ara*-C (**2**) by Pli (EC 3.4.21.7). Enzymatic cleavage of amide bond between lysine and *ara*-C (1) was measured as follows: 30 μL plasmin (1 Cu/mL) was added to the thermostated buffer composed of 50 mM Tris-HCl, pH 7.4 and 110 mM NaCl followed by the addition of substrate solution of **2** (1 mM) to a final volume of 1.0 mL at 37 °C. Cleaved **2** was detected spectrophotometrically by recording the decrease in absorption of **2** at 305 nm. Initial hydrolysis rates for each substrate concentration were measured through linear least squares curves fitting of concentration vs time data for the reaction of the first 10% of substrate. K_m and V_{max} values were determined from a graphical Lineweaver–Burk plot of these initial rates. Insert: UV absorption spectra of 1 (—) and **2** (– – –) measured at 0.1 mM concentration. The molecular absorption coefficient at 305 (ε, 7.5) was used for determination of the amount of **2** in the presence of 1.

esting prodrug forms of 1 because not only are the drugs acting as slow-release forms of 1, but they are also resistant to cytidine deaminase. Therefore, as long as the prodrug is present, they will not be degraded and, consequently, the effectivity of the prodrug will be increased.

Various human cells have been described to release plasminogen activators of the urokinase-type plasminogen activator (u-PA) or tissue-type plasminogen activator (t-PA).[8,9] Apart from the involvement of t-PA in the fibrinolytic system,[10] the physiological role of these two enzymes is still not completely clear. These activators are produced by a wide variety of cells including macrophages[11] and neutrophil polymorphonuclear leucocytes.[12] PAs of both t-PA and u-PA types are expressed in various kinds of tumor cell lines and tumor tissues. A correlation exists between the expression of u-PA and the aggressiveness as well as metastatic potential[13] of the malignant cells. Leukemic cells also secrete these enzymes.[14]

Carl et al.[15] reported that the peptide prodrugs of several anticancer agents designed to be specific plasmin (Pli)

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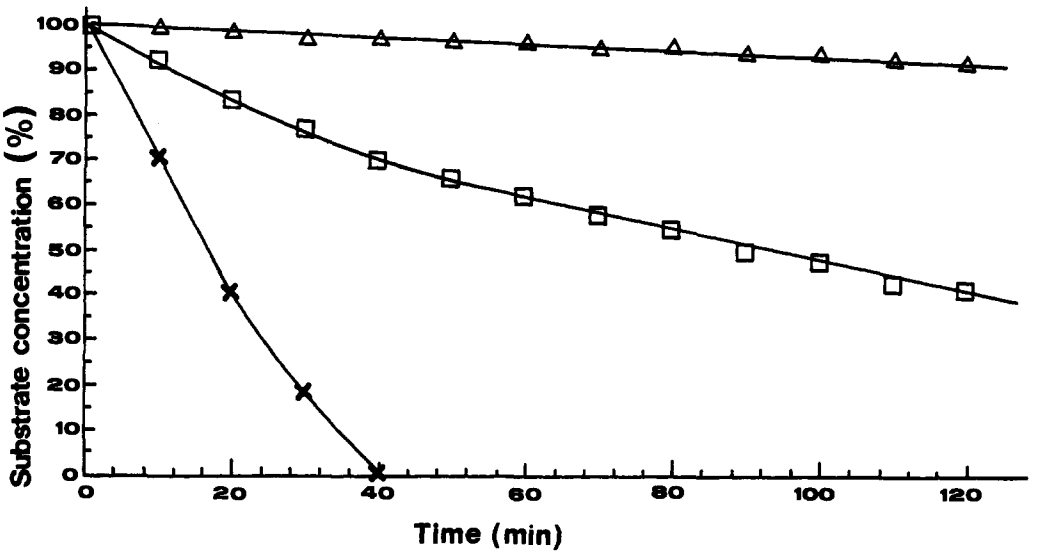

Figure 2. Hydrolysis during the incubation of 0.3 mM cytidine (×), 1 (□), and 2 (△) with 0.07 units/mL cytidine deaminase (CD) (EC 3.5.4.5) at 30 °C. One unit of CD is the amount of enzyme which hydrolyzes a standard substrate (e.g. cytidine) at the rate of 1.0 μmol/min at 27 °C. Partially purified CD was obtained from yeast, and its activity was determined by the method of Ipata.[17] A volume of 0.3 mL of substrate solutions prepared in distilled water were diluted with 0.6 mL of 0.1 M pH 7.0 Tris-Cl buffer in a quartz microcuvette with 1-cm light path and thermostated at 30 °C in a Beckman 35 spectrophotometer. Reaction was initiated by addition of 0.1 mL of CD solution. The decrease in absorbance was recorded at 286 and 305 nm, against a reference cuvette in which the substrates were replaced by water.

substrates showed selective cytotoxicity. In the present paper the preparation of a prodrug derivative of a cytostatic compound 1 is described. The drug was attached to the carboxy terminus of a tripeptide, D-valyl-leucyl-lysine. This prodrug was designed to be hydrolyzed directly by Pli formed as a result of PAs secretion from leukemic cells. In this way a slow-release drug carrier system was obtained with the following advantage that the drug was protected against metabolic degradation, and cytotoxicity was achieved at low drug concentration.

Chemistry

Scheme I outlines the procedure used in the preparation of 2. The free nucleoside 1 was coupled with *N*-Cbz-D-Val-Leu-N^6-Cbz-Lys-OH (9) using *N*-hydroxysuccinimide and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDCI) to give the protected tripeptidyl-*ara*-C derivative **2a**. Hydrogenolysis of the *N*-Cbz groups of **2a**, using palladium, was performed on carbon catalyst to give 2. The synthesis of the protected tripeptide 9 was accomplished by the following route. Cbz-D-Val-Leu-OMe (6) was obtained by coupling Cbz-Val-OH (5) with Leu-OMe (4) in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenztriazole (HOBT) to give the dipeptide methyl ester 6. The corresponding dipeptide 7 resulted from the saponification of compound 6. Compound 7 was coupled with N^6-Cbz-Lys-OMe[16] (3) by using DCC and HOBT as coupling reagents to give the protected tripeptide derivative (8). Saponification of 8 furnished Cbz-D-Val-Leu-N^6-Cbz-Lys-OH (9). Table I lists the properties of 6–9.

Biological Results

To assess the suitability of 2 as a substrate for Pli, the kinetic constants of its hydrolysis were determined. The data are shown in Figure 1. Pli degrades the prodrug effectively as indicated by the values (k_{cat} = 52 s^{-1}, K_m = 1.4 mM and k_{cat}/K_m = 37.1 mM^{-1} s^{-1}).

The water solubility of 2 was 325.0 mM at 23 °C whereas the partition coefficient of 2 between octanol and water was log P = −2.3 at 23 °C.

The stability of 2 against cytidine deaminase obtained from yeast[17] was also studied. As seen in Figure 2, less than 6% of 2 was deaminated. In the spontaneous hydrolysis of 2, liberated 1 was near 8% (not shown) by incubation for 2 h at 37 °C whereas about 60% of 1 was deaminated under the same experimental conditions.

The prodrug should be stable in plasma and be hydrolyzed by Pli near the target cells to localize the effect of 2. This should result in a continuous, relatively high concentration of the active agent around cells which can generate Pli. To check how much nonspecific enzymatic and spontaneous hydrolysis of 2 occur in plasma, the stability of 2 was examined under various conditions. It was found that the amide bond was hydrolyzed in aqueous solutions of various pHs (Figure 3). The release of 1 was rather accelerated in alkaline compared with acidic pH. The times required for the release of 50% of 1 from 2 ($t_{1/2}$) were 0.7 and 2.0 h at pH 9.0 and 8.0; on the other hand, at pH 6.0 and 4.0 the corresponding values were 69.3 and 37.5 h (last time not shown). At physiological pH the $t_{1/2}$ of 2 was 13 h. Measured in plasma, the $t_{1/2}$ of 2 was 1 h while in the presence of a potent serine protease inhibitor (4-amidinophenyl)methanesulfonyl fluoride (APMSF) it was increased to 5.5 h.

Antiproliferative activity in vitro was investigated by measuring growth inhibition of L1210 lymphoid leukemic cells in culture.[18] The concentration of the compounds which resulted in 50% inhibition (IC_{50}) of growth at 24 (Figure 4), 48, and 72 h were estimated from response

(16) Balajthy, Z. An improved preparation of N-benzyloxycarbonyl-L-lysine methyl ester hydrochloride. *Org. Prep. Proced. Int.* 1991, *23*, 375–376.

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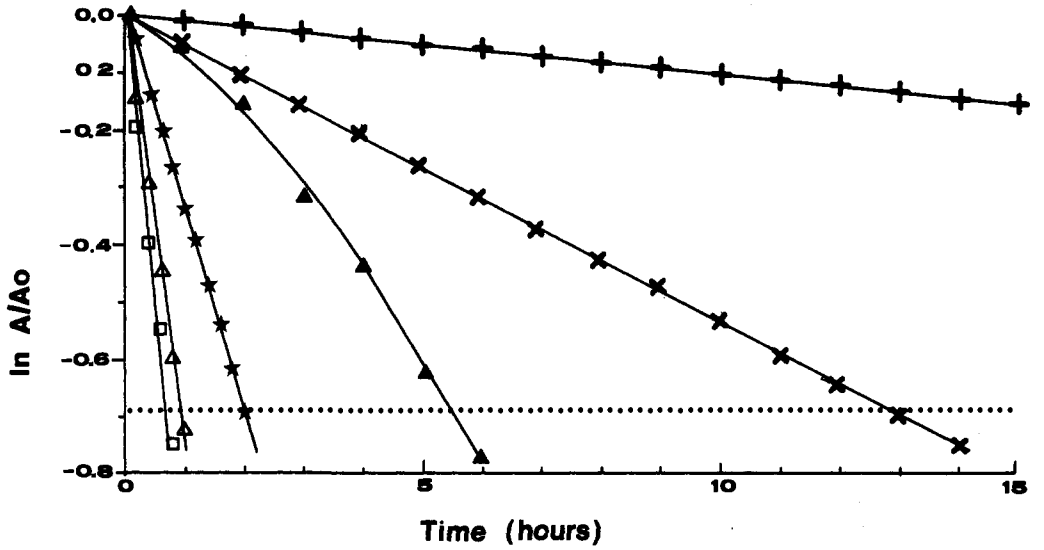

Figure 3. Plots showing the apparent first-order kinetics of spontaneous hydrolysis of 2 (0.2 mM) at pH 6.0 (+), 7.4 (×), 8.0 (*), and 9.0 (□). Decomposition of 2 was monitored by determining the change in absorption at 305 nm. Nonspecific enzymatic hydrolysis of 2 was measured in plasma (△) and in the presence 2 μg/mL APMSF in plasma (▲). 2 (2 μmol) was incubated with fresh human plasma (1 mL) against a reference where 2 was replaced by buffer at 37 °C and monitored by the change in absorption at 325 nm (ε, 1.55).

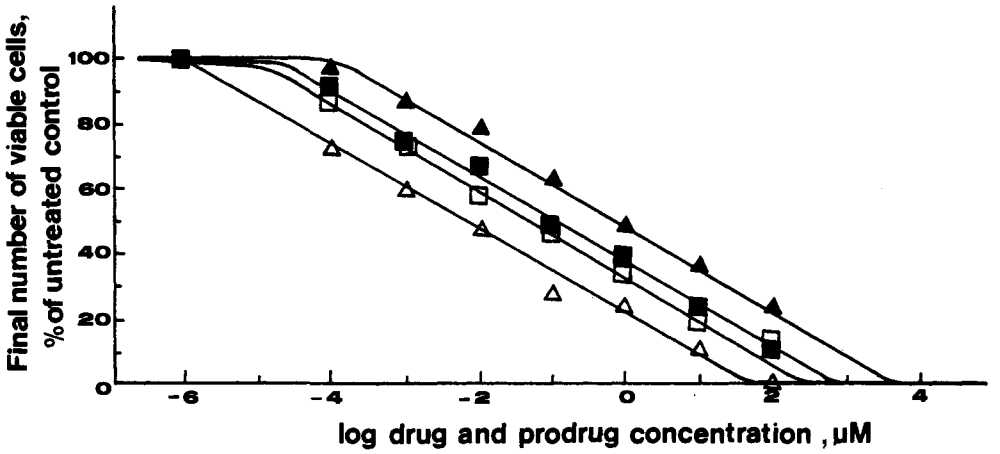

Figure 4. Demonstration of the antiproliferative activity of 2 (△) and 1 (□) at 24 h using the method of Kato et al.[18] Solid symbols: APMSF (2 μg/mL) was also added. For further details see footnote in Table II.

curves of three separate experiments as shown in Table II. Compound 2 was found to be 10 times as active as 1 at 24 h, twice as active as 1 at 48 h, and was clearly as active as 1 at 72 h. When the Pli inhibitor APMSF was added to the culture, the prodrug showed a 10-fold decrease in its activity at 24 h. That is, its effectiveness depends upon Pli generation by the tumor cells. When 2 was preincubated in culture medium for 24 h, its antiproliferative activity was not different from 1, however. Control experiments showed that the tripeptide D-Val-Leu-Lys at 10 μM had no effect on cells (data not shown).

Discussion

In an attempt to overcome the problems associated with known S-phase-specific drugs, including 1, such as hydrolytic deamination, proteolytic instability leading to short duration of action, rapid excretion, and nonselective inhibition of tumor cell growth, we sought a peptidic ligand which could be bound to 1 at the N-4 position. This was done in order to produce the new prodrug (2) which would be protected against cytidine deaminase and, with the help of the ligand's positive charges of 2, would be trapped outside on the surface of the cells. Finally the peptidic ligand would be cut off enzymatically, e.g. by Pli generated by an increased production of PA which is a characteristic feature of many malignant cells distinguishing them from their normal counterparts. We hoped that the synthesized prodrug would be a nontoxic form of the parent drug.

A rate constant in the presence of Pli could be calculated from the Michaelis–Menten equation for the purpose of making a comparison between the rate constants of en-

Table II. Effects of Compounds on the Proliferation of L1210 Lymphoid Leukemia Cells in Culture[a]

compd	length of preincub, h	IC_{50} (μM)[b] at 24 h	at 48 h	at 72 h
1	0	0.10	0.01	0.01
2	0	0.01	0.005	0.01
1 + APMES[c]	0	0.10	0.01	0.01
2 + APMES	0	1.00	0.005	0.01
2	24	0.10		

[a] Antiproliferative activity was tested in 96-well flat-bottomed microtest plates. One hundred microliters of a serially diluted compound solution in Fischer's medium supplemented with 10% horse serum and 100 μL of L1210 cell suspension (2 × 10^4/well) were incubated for 72 h. At 24, 48, and 72 h the viable cells were counted by the Trypan blue exclusion method. [b] IC_{50} was given as the concentration required for 50% inhibition of cell growth. [c] Cells were treated with the plasmin inhibitor (4-amidinophenyl)-methanesulfonyl fluoride at 2 μg/mL for 0.5 h prior to the addition of compounds.

zymatic specific, as well as nonspecific and spontaneous hydrolysis processes. The Michaelis–Menten equation: $-dS/dt = V_{max}/(K_m + S) \times S$ is simplified to $-dS/dt = V_{max}/K_m \times S$, if the substrate concentration is considerably lower than the K_m. Thus in these conditions, which are similar to those which normally prevail in vivo for prodrug hydrolysis, the enzymatic reaction becomes first-order with a rate constant equal to V_{max}/K_m. Therefore, when $1/V$ is plotted against $1/S$, a straight line is obtained with a slope of K_m/V_{max} from which the rate constant can be calculated. The rate constant of the Pli-catalyzed hydrolysis in our experiments is $k = 5.78 \times 10^{-4}$ s^{-1}.

The rate constants of spontanous and nonspecific enzymatic hydrolysis of 2 were determined under physiological pH and in human plasma at 37 °C, as well. At an initial concentration of about 2×10^{-4} M the progress of hydrolysis of 2 followed strict first-order kinetics over the half-life as illustrated in Figure 3. The pseudo-first-order rate constants for the hydrolysis were calculated from the corresponding half-lifes obtained from $k = 0.693/t_{1/2}$. Its value in aqueous solution under physiological pH is 1.48×10^{-5} s^{-1}, whereas in plasma $k = 1.92 \times 10^{-4}$ s^{-1}. Comparing the hydrolytic rate constant of 2 at physiological pH with that measured in the presence of Pli, it was found to be 40 times as high as that measured in the absence of Pli. Consequently the concentration of 1 should be elevated around cells which generate Pli.

If one compares the half-life measured under physiological pH ($t_{1/2}$ = 13 h) to that measured in plasma ($t_{1/2}$ = 1 h) or in the presence of the potent serine proteinase inhibitor APMSF in the plasma ($t_{1/2}$ = 5.5 h), it is evident, that in the plasma, additional effects, for example the hydrolyzing ability of plasmin-α_2 macroglobulin (Pli-α_2-M) complex[19,20] as a nonspecific enzymatic process, should also be taken into account in the liberation of free *ara*-C (1) from 2. This is surprising because the plasma should not contain any Pli, only the inactive plasminogen form, which is unable to hydrolyze the small molecule substrates. The 4.5-h increase in the half-life of 2 in the presence of serine proteinase inhibitor would stem from the remaining activity of Pli-α_2-M complex, which can be inhibited by APMSF. Our prodrug is a small molecule which is able to diffuse to the active site of Pli bound to the α_2-M and be hydrolyzed because α_2-M links up to the primary amino group of Pli. In this way the enzyme is bound to α_2-M, the active site of which is not blocked but is still reactive with small substrates which are not sterically hindered from reacting with the active site. Bound Pli can then be inhibited by APMSF which blocks its active site. This action may be one that we have seen in the increasing of the half-life of 2 in the presence of APMSF and means that the half-life of 2 would be increased if the nonspecific enzymatic processes could be excluded by modifying the structure of 2.

Antiproliferative activity of 1 and 2 was demonstrated in the presence and absence of a serine proteinase inhibitor (APMSF) against L1210 cells, which were proposed by NCI[21] for initial screening of synthetic agents (Table II). Although it is known, that B_{16} melanoma cells have higher PA activity in tumor-bearing mice than the L1210 cells, which show only 2–3 times higher activity over the serum background level,[22] we have selected the fast-growing L1210 cells instead of the slow-growing B_{16} cells, since 1 is an S-phase-specific drug. 2 was ten times as active as 1 at 24 h and it showed a 100-fold decrease in its activity in the presence APMSF at 24 h, suggesting that 2 is an inactive form of 1.

The difference between the IC_{50}s of 2 and 1 at 24 h might be explained by the fact that although 1 is a substrate for the facilitated diffusion system for nucleosides,[23] 2 is not. However, 2 may be absorbed on the surface of the cells more strongly than 1, by the help of 2's positive charges on the amino groups. In this way when the amide bond between *ara*-C (1) and tripeptide is hydrolyzed by the enzyme near the cells, the released 1 would be able to pass through the cell wall in a greater amount than when the initial concentration of 1 was equivalent to that of 2, but it could diffuse away. At the same time this hydrolytic process may happen farther from the cells, which is what happened during preincubation in the medium proceeding from the nonspecific activation of 2 increasing its IC_{50}. This process is supported by the fact that the half-life of 2 is 3.0 h measured in Fischer's medium with 10% horse serum at 37 °C, and 4.5 h in the presence of APMSF (data not shown). The half-life of 2 measured in plasma or medium indicates that the stability of the prodrug is still not enough to be degraded mainly in the surroundings of the cells. We are planning to investigate the characteristics of the macromolecule-bound 2, expecting that this modification will increase the stability of the prodrug, especially against the Pli-α_2-M and the other enzyme inhibitor complexes which are probably the most important hydrolyzing factors in the plasma.[20,24]

Summary

It has been shown that compound 2 is readily hydrolyzed in vitro by Pli. The kinetic constants of hydrolysis indicate an effective breakdown of 2. The amide bond, formed between the COOH group of the peptide and the NH_2 group of 1, protects 1 against deamination. Finally, 2 appears to be a more effective antiproliferative compound than 1.

Experimental Section

Melting points (uncorrected) were determined with a Boetius apparatus. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. Ultraviolet absorption spectra were recorded with a Beckman 35 spectrophotometer. ^{1}H-NMR spectra were recorded with a Burker WP 200 (200 MHz) using Me_4Si as an internal standard. Reactions were monitored by thin-layer chromatography (TLC) on Alluroller Kieselgel 60F 254 (Merck) with detection under UV light, ninhydrin, or *o*-tolidine spray. Kieselgel 60 G (Reanal) was used for column chromatography. The following solvents were used: A, ethyl acetate–pyridine–acetic acid–water (480:20:6:11); B, ethyl acetate–pyridine–acetic acid–water (240:20:6:11); C, ethyl acetate–pyridine–acetic acid–water (120:20:6:11); D, ethyl acetate–pyridine–acetic acid–water (60:20:6:11).

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Cell culture and in vitro cytotoxicity testing were performed as described.[18]

General Procedures. Method A. Coupling Reaction with Dicyclohexylcarbodiimide/1-Hydroxybenzotriazole. To a solution of amino acid ester hydrochloride (13 mmol) in dry pyridine (10 mL) were added *N*-methylmorpholine (1.64 mL, 15 mmol), Cbz-protected amino acid (13 mmol), HOBT (20 mmol), and slowly a solution of DCC (20 mmol) in dry pyridine (20 mL), at 0 °C. The reaction mixture was stirred at 0 °C for 4 h and then 36 h at room temperature. The urea formed was removed by filtration, and the filtrate was evaporated. The oily residue was dissolved in ethyl acetate (200 mL) and washed successively with 5% $NaHCO_3$, water, 0.1 M HCl, and water, dried ($MgSO_4$), and evaporated. The crude product was obtained as a solid. The final purification was achieved by silica gel column chromatography and recrystallization.

Method B. Saponification of Peptide Esters in Aqueous Dioxane. To a solution of peptide esters (10 mmol) in a mixture of dioxane (90 mL) and water (10 mL) was added 1 M NaOH (13 mmol), and the mixture was stirred at room temperature overnight. The aqueous phase was acidified with solid citric acid while being chilled. Solvent was removed under reduced pressure at 45 °C. The residue was dissolved in ethyl acetate (200 mL) and washed with 0.01 M HCl and water. The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to yield crude product. In most cases, the products obtained needed no further purification.

L-Leucine Methyl Ester Hydrochloride (4). The title compound was prepared from L-leucine (5 g, 0.03 mol) according to the method used to prepare N^6-Cbz-lysine methyl ester hydrochloride:[15] yield 11 g (93%); mp 153-154 °C; R_f (D) 0.45.

***N*-(Benzyloxycarbonyl)-D-valine (5).** D-Valine (6 g, 0.05 mol) was dissolved in a mixture of 2 M NaOH (117 mL) and ether (26 mL) and stirred at 5 °C, and benzyl chloroformate (11.7 mL, 0.08 mol) was added drop by drop. The reaction mixture was stirred for 3 h at 5 °C, and 25 °C for an additional 30 h. The aqueous phase was separated from ether. The pH was then adjusted to 2.0 with 2 M HCl, and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with water, dried over Na_2SO_4, and evaporated under reduced pressure: yield 11 g (87%); mp 58-60 °C; R_f (A) 0.67.

***N*-[*N*-(Benzyloxycarbonyl)-D-valylleucyl-N^6-(benzyloxylcarbonyl)lysyl]-1-β-D-arabinofuranosylcytosine (2a).** A solution of **9** (1.3 g, 2.1 mmol), *N*-hydroxysuccinimide (0.35 g, 3.0 mmol), and EDCI (0.4 g, 3.0 mmol) in dry DMF (50 mL) was stirred for 2 h at room temperature. To the solution was added **1** (0.5 g, 2.1 mmol). The mixture was stirred at 45 °C for 20 h. The solvent was removed in vacuum, and the oily residue was dissolved in ethyl acetate, washed successively with 0.01 M HCl and water, dried ($MgSO_4$), and concentrated under vacuum. The product was purified by column chromatography on silica gel 60 with solvent B to give 1.44 g (82%) of **2a** (after crystallization from ethyl acetate–ether): mp 136-138 °C; $[\alpha]^{20}_D$ = +16.7° (*c* = 0.7, MeOH); R_f (B) 0.33; ^{1}H NMR (DMSO-d_6) δ 0.85 (q, *J* = 5 Hz, 12 H, 2 C(CH_3)$_2$), 1.00-2.05 (m, 10 H, 4 CH_2, 2 CH), 2.97 (m, 2 H, CH_2), 3.32 (s, 1 H, NH), 3.62 (m, *J* = 5 Hz, 2 H, 5′-CH_2), 3.75-4.00 (m, 3 H, 3 OH), 4.06 (m, 1 H, CH), 4.37 (m, 2 H, 2CH), 4.90-5.15 (m, 5 H, 2 OCH_2, H-4′), 5.50 (m, 2 H, H-2′, H-3′), 6.05 (d, *J* = 4 Hz, 1 H, H-1′), 7.15 (d, *J* = 8 Hz, 1 H, CH), 7.35 (m, 11 H, 2 C_6H_5, NH), 7.90 (m, 1 H, NH), 8.05 (d, *J* = 8 Hz, 1 H, CH), 8.18 (m, 1 H, NH), 10.90 (d, 1 H, NH). Anal. ($C_{42}H_{57}N_7O_{12}$) C, H, N.

***N*-(D-Valylleucyllysyl)-1-β-D-arabinofuranosylcytosine (2).** Compound **2a** (1.27 g, 1.5 mmol) was hydrogenolyzed over 10% Pd/C (0.4 g) in dry methanol (100 mL) in the presence of 2% HCOOH. The catalyst was removed by filtration, the filtrate was evaporated in vacuum, and the residue was recrystallized from methanol–ether to afford the title compound: yield 0.7 g (87%); mp 126-130 °C; $[\alpha]^{20}_D$ = +14.6° (*c* = 0.2, MeOH); ^{1}H NMR (DMSO-d_6) δ 0.80 (q, *J* = 5 Hz, 12 H, 2 C(CH_3)$_2$), 1.07-2.05 (m, 10 H, 4 CH_2, 2 CH), 2.75 (m, 2 H, CH_2), 3.22 (d, 1 H, CH), 3.62 (d, *J* = 5 Hz, 2 H, 5′-CH_2), 3.80-4.10 (m, 3 H, 3 OH), 4.40 (m, 2 H, 2 CH), 4.50-6.00 (m, 7 H, 2 NH_2, 3 H, H-3′, H-2′, H-4′), 6.05 (d, *J* = 5 Hz, 1 H, H-1′), 7.15 (d, *J* = 8 Hz, 1 H, CH), 8.10 (d, *J* = 8 Hz, 1 H, CH), 8.35 (m, 2 H, NH). Anal. ($C_{26}H_{45}N_7O_8$) C, H, N.

cis-Bis(pyridine)platinum(II) Organoamides with Unexpected Growth Inhibition Properties and Antitumor Activity

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The platinum(II) organoamides [Pt(NRCH$_2$)$_2$L$_2$] (L = pyridine (py), R = *p*-HC_6F_4, C_6F_5, *p*-IC_6F_4, *p*-ClC_6F_4, *p*-$C_6F_5C_6F_4$; L = 4-methylpyridine, R = *p*-HC_6F_4) and [Pt(NRCH$_2$CH$_2$NR′)(py)$_2$] (R = *p*-HC_6F_4, R′ = C_6F_5, *p*-BrC_6F_4, or *p*-MeC_6F_4) inhibit the growth of murine L1210 leukemia cells in culture with ID_{50} values for continuous exposure in the range 0.6-2.7 μM. Representative complexes are also active against L1210 cells in 2-h pulse exposures, as well as against the cisplatin-resistant variant L1210/DDP and human colonic carcinoma cell lines HT 29 and BE. Three complexes [Pt(NRCH$_2$)$_2$L$_2$] (R = *p*-HC_6F_4, C_6F_5, or *p*-IC_6F_4) have good activity ($T/C \geq 180\%$) against P388 leukemia in mice, and all other compounds tested are active except when R = *p*-$C_6F_5C_6F_4$, L = py. Although the molecular basis of the biological activity of these complexes is not known, the observation of good activity for amineplatinum(II) compounds with no hydrogen substitutents on the nitrogen donor atoms introduces a new factor in the anticancer behavior of platinum(II) complexes.

Introduction

Initial studies of the antitumor effects of *cis*-[$PtCl_2(py)_2$] (py = pyridine) revealed moderate activity against Ehrlich ascites carcinoma in vivo[1] but none against the sarcoma 180 tumor model.[2] More recently, the compound has been shown to be cytostatic against murine L1210 leukemia cells in culture, though with substantially less potency than either *cis*-[$PtCl_2(NH_3)_2$] (cisplatin) or, surprisingly,

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