91444-80-3; 2-bromo-1-(4-methoxyphenyl)butan-1-one, 881-43-6; ethyl iodide, 75-03-6; propyl iodide, 107-08-4; isopropyl iodide, 75-30-9.

Supplementary Material Available: ^{1}H NMR data of methoxy-2-(methoxyphenyl)indoles (**15a–44a**), hydroxy-2-(hydroxyphenyl)indoles (**3b–45b**), and acetoxy-2-(acetoxyphenyl)-indoles (**3c–45c**) and elemental analysis of acetoxy-2-(acetoxyphenyl)indoles (18 pages). Ordering information is given on any current masthead page.

A Novel Peptide Delivery System Involving Peptidase Activated Prodrugs as Antimicrobial Agents. Synthesis and Biological Activity of Peptidyl Derivatives of 5-Fluorouracil

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As an approach to the development of antimicrobial agents, a novel peptide carrier system was designed, based on the chemical instability of α-substituted glycine analogues, with the explicit intent of actively transporting therapeutically useful compounds into microbial cells. Peptides containing 5-fluorouracil (5-FU) linked to the peptide backbone were selected to test the feasibility of this new delivery system. These peptide conjugates were designed such that they would be substrates for both the microbial peptide permeases and peptidases. After entry into cells, enzymatic hydrolysis of the peptide generates an unstable α-(5-FU)-glycine that spontaneously decomposes to release 5-FU. The 5-FU–peptide conjugates were tested for antifungal (*Candida albicans*) and antibacterial (*Escherichia coli*) activity and were found to have antimicrobial activities comparable to free 5-FU. Noninhibitory peptides antagonized the antimicrobial activities of the 5-FU–peptide conjugates but not of free 5-FU, a result consistent with peptide transport mediated entry of the peptide conjugates into cells. Further support for this conclusion was provided by the finding that biological activities were dependent upon peptide stereochemistry.

Microorganisms have been shown to possess specialized transport systems for the uptake of peptides.[1] In *Escherichia coli*, the most extensively studied bacterium, it has been found that there are separate peptide transport systems for dipeptides and for oligopeptides.[2] *Candida albicans* has also been shown to have peptide transport systems although the multiplicity in this organism has yet to be conclusively defined.[3,4] The factors that determine the recognition of peptides by microbial peptide transport systems have been the subject of numerous investigations.[1,2,5] A significant observation that has emerged from these studies, primarily in bacteria, is that peptide transport systems generally possess little demonstrable side-chain specificity. This is likely an accommodation to the diversity of side-chain combinations that occur in peptides assembled from the naturally occurring amino acids. Early indications that this process could be exploited therapeutically was demonstrated in *E. coli* where normally impermeant amino acid analogues, not recognized by the more selective amino acid transport systems, were shown to enter these cells by a peptide carrier mechanism when incorporated into the backbone of a peptide. Following transport, cytoplasmic peptidases hydrolyze the peptide to release the constituent amino acids.[2] Many natural and synthetic examples involing peptides that contain growth inhibitory amino acids have been reported[6] and considerable interest has been expressed in utilizing this approach as a means of developing novel chemotherapeutic agents.[7,8] The synthesis and development of the wide-spectrum antimicrobial agent alaphosphin[9] [L-alanyl-L-(1-aminoethyl)phosphonic acid] is a powerful example of the peptide transport concept.

In an attempt to broaden the overall scope of the peptide transport approach so that inhibitory agents other than amino acid analogues could be brought into microbial cells, we developed a method that allows the transport of sulfhydryl-containing compounds through their attachment to the cysteine residue of a peptide.[10] The results of these studies encouraged us to examine other approaches of this type.[11] In this paper we describe a more versatile peptide delivery system in which the toxophoric agent is attached to the α-carbon of a glycine residue within a peptide chain. Intracellular cleavage of the peptide by cytoplasmic peptidases results in the formation of an unstable intermediate that decomposes with release of the attached toxophoric group.

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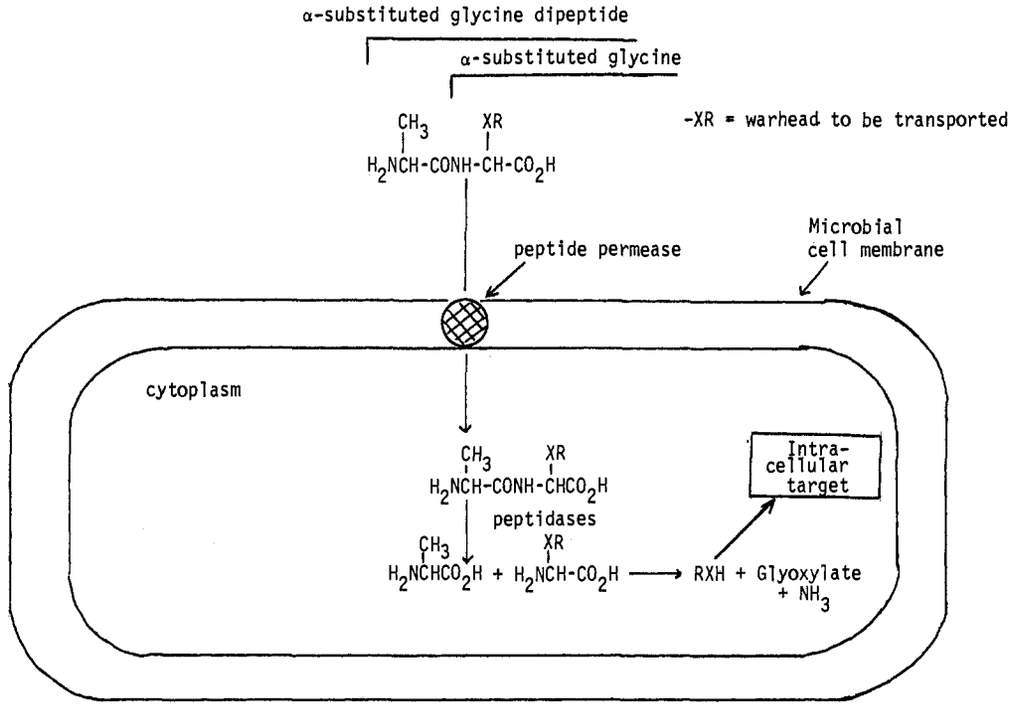
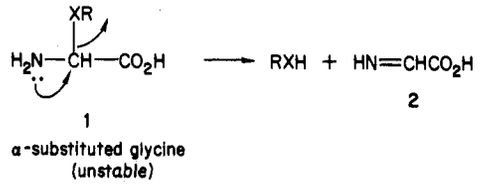
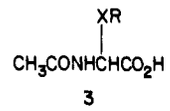
0022-2623/84/1827-1447$01.50/0

Scheme I



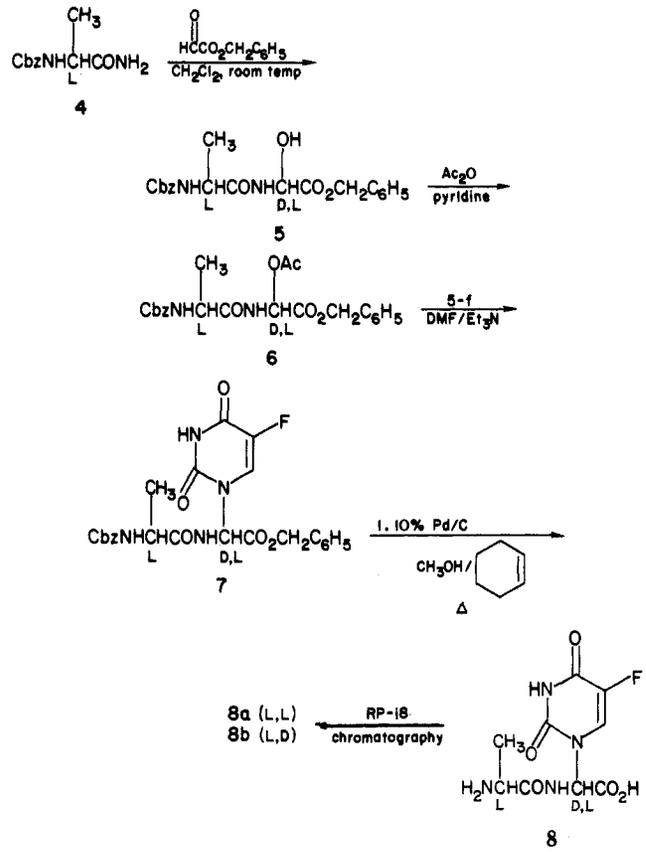
This new transport concept is based on the inherent chemical instability of α-substituted glycines, **1**, where the linked α-substituent is a good leaving group (XR = Cl, Br, OAc, SR, NR_2). Compounds of this type have been found to rapidly decompose with the expulsion of the α-substituent (i.e., **1** → **2**). However, stabilization can be achieved through acylation of the glycyl nitrogen, for example, the *N*-acyl-α-acetoxyglycine **3** is a stable molecule.[12]





Theoretically, attachment of an appropriate acyl group that would stabilize such molecules, be compatible with peptide transport, and be released readily within the cell could provide the basis for a novel form of drug delivery in which the α-substituent would be the drug delivered into microbial cells freed of the need for its own transport. However, N-terminal acyl derivatives of type **3** or peptides generated from extensions at the carboxy terminus of **3** would be poorly accepted by most microbial peptide permeases since a free terminal amino group is required for optimal transport.[1] Furthermore, peptidase activity would be unlikely to deacylate such peptides, thus preventing the formation of the unstable α-substituted glycine intermediate. On the other hand, if the stabilizing acyl group is derived from the carbonyl group of an amino acid, the resulting dipeptide should serve as a substrate for the microbial permeases as well as for the intracellular peptidases. Thus, transport of the dipeptide would be followed by hydrolysis producing the unstable α-substituted glycine unit that degrades, releasing the α-substituent within the cell (Scheme I). In order to assess the feasibility of this approach, 5-fluorouracil (5-FU), the biologically active metabolite of the clinically useful drug 5-fluorocytosine,[13]

Scheme II



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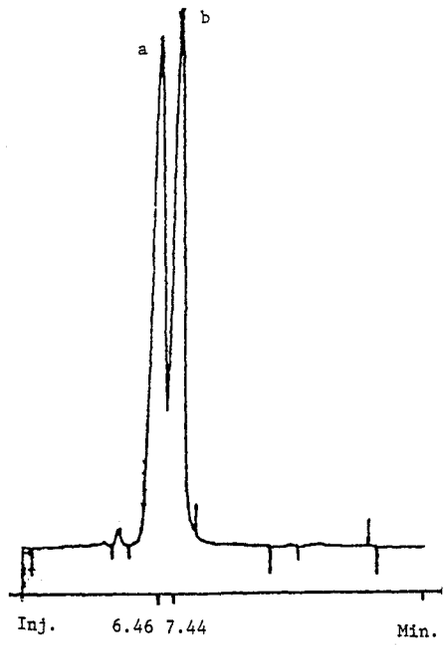

Figure 1. High-performance liquid chromatogram of diastereoisomeric mixture 8. Peaks: a = 8a; b = 8b. Column, Li-Chrosorb RP-18, 5 μm (250 × 4.6 mm); mobile phase, H_2O; flow-rate, 0.5 mL/min; absorption, 254 nm.

was selected as a pharmacologically interesting toxophoric group to study since a biological response would be guaranteed if 5-FU was brought into the cells of the test organism.

Synthesis. The key peptide required for exploring the feasibility of this transport concept, L-alanyl-2-(5-fluorouracil-1-yl)-DL-glycine, 8, was synthesized as outlined in Scheme II. Reaction of *N*-carbobenzoxy-L-alanine amide (4) with benzyl glyoxylate produced dipeptide 5. Subsequent treatment of 5 with acetic anhydride in pyridine produced the peptide acetate 6. Reaction of 6 with 5-FU gave *N*-carbobenzoxy-L-alanyl-2-(5-fluorouracil-1-yl)-DL-glycine benzyl ester (7). Removal of the blocking groups by catalytic hydrogenation gave the required peptide 8 as a mixture of diastereoisomers. These isomers were separated analytically by reverse-phase HPLC (Figure 1) and isolated preparatively by medium-pressure reverse-phase chromatography.

Stereochemical assignments of the diastereoisomeric α-substituted glycines **8a** and **8b** were based primarily on their relative antimicrobial activities (see Results and Discussion). That is, small peptides containing D-amino acids are extremely poor substrates for the transport permeases;[14,15] therefore the inactive isomer of the diastereomeric pair was assigned the L,D stereochemistry, **8b**; the active isomer was assigned the L,L stereochemistry, **8a**.[16,17]

Subsquently, it was found that compounds **8a** and **8b** had several minor differences in their NMR spectra. The alanine methyl groups of the diastereoisomeric mixture appear as a pair of doublets (δ 1.4, 1.5), which together integrate for three protons. After the stereochemical assignments were made, based on their relative biological activities, it was determined that the doublet downfield (i.e., δ 1.5) is that of the L,L dipeptide **8a**, consistent with published reports regarding chemical shifts of other peptide diastereoisomers.[18]

Table V summarizes peptide stability in aqueous solution and in the presence of various sera. Peptides 8 were found to be stable in aqueous solution where more than 90% of the peptide was intact after 10 days. However, the peptide was more rapidly degraded in serum, as measured by the appearance of free 5-FU. The rate was found to be more rapid in mouse serum than in human serum, a difference that likely correlates with a higher concentration of proteolytic enzymes in the mouse serum.

Results and Discussion

Naturally occurring antibiotic molecules exploit a variety of already existing microbial transport systems.[19] It is our contention that of the known transport systems, utilization of peptide permeases offers the greatest potential for development of novel antimicrobial agents since they are less discriminatory with regard to their substrate specificities.[1,2,5] However, a serious limitation to the exploitation of peptide transport systems has been the inability to transport potential inhibitory agents other than amino acids into microbial cells. In order to circumvent limitations of this type, we have focused on the design of peptide carriers that allow the active uptake of molecules other than amino acids. Thus, the design of appropriate peptides has been guided by the expectation that their modes of action would involve initial permease mediated transport followed by intracellular cleavage of the inhibitor from the peptide carrier portion of the molecule. The first peptide carrier to emerge as a result of this approach was the cysteine disulfide peptide carrier, which relies on the attachment of a sulfhydryl group as a disulfide to a cysteine unit in the peptide.[10] However, a serious drawback of this particular carrier system is that it is limited to the transport of inhibitors that contain a sulfhydryl group. In an attempt to obtain a more general method, the α-substituted glycine peptide carrier system was designed (Scheme I). Peptides required for evaluation of this concept were prepared as illustrated in Scheme II. In order to demonstrate the feasibility of such peptide carriers, a toxophoric group (5-FU) was selected that would elicit a strong inhibitory response once inside the cell, thus allowing biological activity to be used as a direct assay of inhibitor delivery. Since 5-FU itself is active, it is necessary to distinguish between transport of the intact peptide and extracellular breakdown of the peptide carrier followed by entry of the free 5-FU. Fortunately, it is possible to distinguish between these two possibilities by examining the ability of noninhibitory peptides to antagonize the uptake and thus the biological activity of the peptide–inhibitor conjugate. Under these conditions, no antagonism of 5-FU activity should occur since its entry is independent of peptide transport. Initial biological evaluation was performed with the diastereoisomeric mixture 8 under two separate sets of assay conditions. In one, *C. albicans* and *E. coli* were grown in a defined medium that is free of

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(16) Additional support for the stereochemical assignments is provided by the finding that only the peptide assigned the L,L stereochemistry, **8a**, is a substrate for the proteolytic enzymes leucine aminopeptidase and aminopeptidase M.

(17) Surprisingly, in subsequent separations of diastereoisomeric mixtures of this type, the L,L diastereoisomer has always eluted first from the reverse-phase column.

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Table I. Disk Diffusion Analysis of the Activity of 5-Fluorouracil and 5-Fluorouracil Peptides

compd	complex media		defined media	
	E. coli	*C. albicans*	*E. coli*	*C. albicans*
5-FU	+	+	+	+
5-FU dipeptides 8	-	-	+	+

Table II. Dialanine Antagonism of the Activity of 5-Fluorouracil and 5-Fluorouracil Peptides against *E. coli* M2626

concn, μg/disk	zone size, mm			
	5-FU	5-FU peptides (8)	5-FU + $(Ala)_2$[a]	5-FU peptides (8) + $(Ala)_2$[a]
25.0	46	31		
12.5	41	26	41	0
6.0	40	27	40	0
3.0	38	25	38	0
1.5	33	22	33	0

[a] 2 mg/disk of dialanine was codisked on 5-FU and 5-FU peptide disks.[13]

peptides, while in the other growth was followed in a complex medium known to contain peptides. The results of these tests are illustrated in Table I and imply that 8 enters the cells of the test organisms by peptide mediated transport; 5-FU is active when tested in both media, but 8 is active only in the absence of other peptides. Table II illustrates the inhibitory effects of 8 measured as a function of concentration together with the ability of dialanine to antagonize its activity. These results are in accord with the initial observations (Table I) and support peptide transport mediated entry of 8 into microbial cells.

That growth inhibition was consistent with intracellular liberation of 5-FU was supported by the fact that a mutant of *E. coli* showing reduced sensitivity to 5-FU (presumably through a defect in uracil phosphoribosyltransferase activity) also showed reduced sensitivity to the 5-FU–peptide conjugate.[11] In an analogous case, proof for the intracellular release of an attached inhibitor, thiophenol, was obtained in *E. coli*[11] as well as in *C. albicans*,[20] by using the peptide conjugate alanyl-α-(phenylthio)glycine. It was found that release of thiophenol, measured spectrophotometrically with Ellman's reagent, was rapid following entry of the peptide into the cell with the rate of release being equivalent to the rate of peptide uptake.

To our knowledge, these results represent the first definitive examples of peptide conjugates that are able to deliver pyrimidine analogues (e.g., 5-FU) into microbial cells by a peptide transport mechanism.

Since the synthesis of the 5-FU peptides produced a separable mixture of diastereoisomers, **8a** and **8b**, each diastereomer could be tested for biological activity. In view of the convincing evidence that illustrates that the peptide permeases are stereospecific, allowing only the transport of L,L dipeptides,[14,15] the L,L dipeptide should be inhibitory while the L,D dipeptide should be inactive. The biological results demonstrate that only one of the diastereomers was inhibitory (Tables III and IV), a finding that is in agreement with peptide permease mediated entry and also allows stereochemical assignments to be made for each isomer. Studies in our laboratories are presently in progress to develop methods that will permit the quantitative evaluation of relative binding differences between diastereomers, such as **8a** and **8b**, for bacterial and fungal peptide permeases. We will present a detailed description of these methods in a subsequent report.

Table III. Disk Diffusion Assay of the Activity of 5-Fluorouracil Peptides against *C. albicans* B-311

concn, μg/disk	5-FU	5-FU peptides	
		8a (L,L)	**8b** (L,D)
25.0	44	30	0
12.5	40	26	0
6.0	30	22	0
3.0	30	20	0
1.5	22	16	0

Table IV. Minimum Inhibitory Concentrations of 5-Fluorouracil and 5-Fluorouracil Dipeptides against *C. albicans*[a]

organism	MIC, μg/mL				
	5-FU	8	**8a** (L,L)	**8b** (L,D)	amphoteracin B
BC759	1.0	6.3	1.6	>100	1.25
B311	4.0	25	12.5	>100	1.25
3153A	1.0	25	12.5	>100	1.25

[a] YCB–lysine broth, ca. 10^4 cfu/mL, 37 °C overnight.

In conclusion, by overcoming the permeability barrier of the microbial cytoplasmic membrane, α-substituted glycine peptides provide unique opportunities for studying features of microbial peptide transport not previously available. In addition, this novel technique for linking potential inhibitors to a peptide backbone should prove to be of general utility, allowing the attachment and presumably the transport of a wide variety of potential microbial inhibitors. Obviously, the ideal example would be to identify a compound that inhibits a critical microbial enzyme but that has difficulty entering the cell. Intracellular delivery of substances such as these could provide therapeutically useful antiinfective agents.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra (IR) were obtained on a Perkin-Elmer 137 spectrometer; proton nuclear magnetic resonance (^1H NMR) spectra were obtained on a Varian T-60 or a JEOL FX-90 Q instrument; all values are reported in parts per million (δ) from $(CH_3)_4Si$ unless otherwise stated. Optical rotations, determined on a Perkin-Elmer 241 MC polarimeter, and elemental analyses were performed in the Analytical, Physical and Structural Chemistry Department of Smith Kline & French Laboratories.

Analytical thin-layer chromatography (TLC) was carried out with Merck silica gel 60 F-254 glass backed plates and with Whatman KC 18 F reversed-phase RP-18 glass backed plates. High-performance liquid chromatography was carried out on a Altex Model 110 A gradient liquid chromatograph fitted with a EM Lichrosorb reverse-phase C_{18} column (250 mm × 4 mm) with a UV wavelength detector set at 254 nm. Preparative column chromatography was performed on a medium-pressure liquid chromatography (MPLC) system using an Altex column (500 mm × 25 mm) packed with either Merck silica gel (40–60 μm) or Merck Lichroprep RP-18 (25–40 μm). *N*-Carbobenzoxyalanine amide was purchased from Vega Biochemicals. Dibenzyl (-)-tartrate was synthesized as described by Smith et al.[21] and benzyl glyoxylate was prepared by the method of Kelly.[22]

***N*-Carbobenzoxy-L-alanyl-D,L-2-hydroxyglycine Benzyl Ester (5).** Benzyl glyoxylate (55.3 g, 0.34 mol) was added to a stirred suspension of *N*-carbobenzoxyalanine amide (60.0 g, 0.27 mol) in methylene chloride (900 mL), and the reaction mixture was stirred at room temperature until all of the starting alanine

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Table V. Serum Stability of α-Substituted Glycine Peptide 8

	% FU								
	time, h								
condtn	0	0.5	1.5	2.5	5	8	24	50	240
H_2O	0								9
human serum	0	3	7		15	-	16	25	
mouse serum	0	12	27	36	43	-	53		

amide was reacted as indicated by TLC (silica gel; 3% CH_3OH in CH_2Cl_2). The total reaction time was 160 h and the reaction progressed from the original suspension to a clear solution back to a suspension. After this time, the reaction mixture was chilled (0–5 °C) for 5 h and the resulting solid was collected, washed with a small amount of CH_2Cl_2, and dried over P_2O_5 to give 74.3 g (71%) of a white solid: mp 117–119 °C; NMR (Me_2CO-d_6) δ 1.35 (d, 3 H, alanyl CH_3), 3.0 (br s, 3 H, NH and OH), 4.0–4.8 (br m, 1 H, alanyl CH), 5.10 (δ, 2 H, benzylic CH_2), 5.2 (s, 2 H, benzylic CH_2), 5.7 (br m, 1 H, glycyl CH), 7.2 (d, 10 H aromatic). Anal. ($C_{20}H_{22}N_2O_6$) C, H, N.

***N*-Carbobenzoxy-L-alanyl-D,L-2-acetoxyglycine Benzyl Ester (6).** A stirred suspension of **5** (20 g, 0.052 mol) in Ac_2O (250 mL) was cooled to approximately 0 °C and pyridine (180 mL) added. The solution became homogeneous and the reaction mixture was held at this temperature for 24 h and concentrated. The residue was dissolved in EtOAc and washed with H_2O (2 × 150 mL), 3 N HCl (2 × 150 mL), 5% $NaHCO_3$ (3 × 150 mL), and brine (2 × 100 mL) and dried ($MgSO_4$). Filtration, concentration of the filtrate, and trituration of the resulting residue with Et_2O gave 17.2 g (77.5%) of a white solid: mp 134–135 °C; NMR ($CDCl_3$) δ 1.35 (d, 3 H, alanyl CH_3), 2.02 (s, 3 H, acetyl CH), 4.3 (br q, 1 H, alanyl CH), 5.08 (5, 2 H, benzylic CH_2), 5.2 (s, 2 H, benzylic CH_2), 6.4 (d, 1 H, glycyl CH), 7.3 (s, 10 H, aromatics). Anal. ($C_{22}H_{24}N_2O_7$) C, H. N.

***N*-Carbobenzoxy-L-alanyl-2-(5-fluorouracil-1-yl)-D,L-glycine Benzyl Ester (7).** Acetate **6** (0.41 g, 0.96 mmol), 5-fluorouracil (0.12 g, 0.92 mmol), and triethylamine (0.92 g, 0.92 mmol) were combined in dry DMF (2 mL) and stirred for 20 h. The solvent was removed by evaporation in vacuo, and the residue was diluted with water (20 mL) and extracted with EtOAc (3 × 20 mL). The EtOAc extracts were combined and washed with water (2 × 10 mL), dried (Na_2SO_4), and evaporated in vacuo. The crude product (0.43 g) was purified by silica gel chromatography (MPLC) with 2% methanol in methylene chloride: yield 0.36 g (76%); TLC (silica gel/95:5:1 methylene chloride, methanol, formic acid) showed a single spot; NMR ($CDCl_3$) δ 1.25 (overlapping doublets, diastereomeric alanyl CH_3), 4.40 (br m, 1 H, alanyl CH), 5.00 (s, 2 H, benzylic CH_2), 5.10 (br s, 2 H, benzylic CH_2), 5.9 (br m, 2 H, glycyl CH, NH), 7.25 (s, 10 H, aromatics), 7.60 (d, 1 H, uracil 6 H).

L-Alanyl-2-(5-fluorouracil-1-yl)-D,L-glycine (8). The protected peptide **7** (0.35 g, 0.7 mmol), 10% Pd/C (0.3 g), cyclohexene (0.5 mL), and methanol (35 mL) were heated to reflux for 20 min. The reaction mixture was filtered (while hot) and the solvent was removed by evaporation in vacuo to give the desired product (0.18 g, 94%): NMR (D_2O, acetone-d_6) δ 1.45 (overlapping doublets, 3 H, diastereoisomeric alanyl CH_3), 4.15 (br m, 1 H, alanyl CH), 5.90 (s, 0.5 H, diastereoisomeric glycyl CH), 6.00 (s, 0.5 H, diastereoisomeric glycyl CH), 7.90 (d, 1 H, uracil 6 H); IR (KBr) 3600–2900 m^{-1}, 1660 cm^{-1}. Anal. ($C_9H_{11}FN_4O_5 \cdot H_2O$) C, H, N.

Separation of the L-Alanyl-2-(5-fluorouracil-1-yl)-L-glycine (8a) and L-Alanyl-2-(5-fluorouracil-1-yl)-L-glycine (8b) Diastereoisomers. A 160-mg portion of diatereomeric mixture **8** was eluted from a preparative reverse-phase column (Lichroprep RP-18) using MPLC conditions and eluting with water.

The first diastereomer eluted (**8a**) weighed 37 mg after lyophilization: $[\alpha]^{25}_D$ +131.1° (*c* 1, H_2O); NMR (D_2O, acetone) δ 1.50 (d, 3 H, alanyl CH_3), 4.15 (q, 1 H, alanyl CH), 5.90 (s, 1 H, glycyl CH), 7.90 (d, 1 H, uracil 6 H). Anal. ($C_9H_{11}FN_4O_5 \cdot 1.5H_2O$) C, H, N.

Following elution of pure **8a**, a mixed fraction containing **8a** and **8b** (40 mg) was obtained.

Elution of pure **8b** followed the mixed fraction and weighed 40 mg after lyophilization: $[\alpha]^{25}_D$ −105.9° (*c* 1, H_2O); NMR (D_2O, acetone) δ 1.40 (d, 3 H, alanyl CH_3), 4.10 (q, 1 H, alanyl CH), 6.00 (s, 1 H, glycyl CH), 7.90 (d, 1 H, uracil 6 H). Anal. ($C_9H_{11} \cdot H_2O$) C, H, N.

Stability Studies. Mouse and human whole blood was collected and the serum obtained by standard methods. A 1:1 mixture of the powdered L,L and L,D peptides **8** was added to either distilled water or undiluted serum at a concentration of 0.5 mg/mL. During the experiment the peptides in serum were held at 37 °C while the peptides in aqueous solution were held at 23 °C. The degradation of the peptides **8a** and **8b** and the formation of free 5-FU was followed by HPLC. The data in Table V are based on the increase in percent of free 5-FU. Thus, samples were taken at various times and filtered through a Gelman A-E glass fiber filter and then injected onto a Licrosorb RP-18 HPLC column (250 mm × 4.6 mm) which was fitted with a Whatman CO:PELL ODS precolumn. The peptides and 5-FU were eluted from the column with water (flow rate of 0.5 mL/min) and detected by UV at 254 nm. The retention times of the L,L peptide (**8a**), the L,D peptide (**8b**), and 5-FU were 6.7, 7.8, and 17.2 min, respectively.

Biological Testing. (A) Minimum Inhibitory Concentration (MIC). *C. albicans* and *E. coli* strains were from the SK&F collection. The MIC's of compounds were determined by twofold broth dilution tests in yeast carbon base medium (YCB, Difco) containing 200 μg/mL of lysine for *C. albicans* and in M-9 medium[23] for the *E. coli* strain. The final inoculum sizes in the tests were approximately 10^4–10^5 cfu/mL for *C. albicans* and 10^5 cfu/mL for *E. coli*. The lowest concentration that resulted in complete inhibition of growth was recorded as the MIC.

(B) Disk Diffusion Assay. For this assay, seeded plates of *C. albicans* were prepared as follows: *C. albicans* was grown in trypticase soy broth (BBL) for 7 h at 37 °C on a New Brunswick rotary shaker set at 250 rpm. One liter of yeast carbon base medium containing lysine (200 μg/mL) and agar was inoculated with 1 mL of the above inoculum. The seeded agar (15 mL) was poured into 150-mm Petri dishes. The peptides, dissolved in water, were absorbed onto 6.35-mm penicillin disks (Schleicher & Schull), which were then placed onto the seeded plates. The plates were incubated for 24 h at 37 °C, after which time zones of growth inhibition were observed. Antagonism studies were conducted by applying a second disk containing a noninhibitory di- or tripeptide over the disk containing the test peptide.

E. coli plates were prepared in a similar manner to that described above with M-9 medium.

Registry No. **5**, 89625-83-2; **6**, 89625-84-3; **7**, 91949-28-9; **8**, 91949-29-0; **8a**, 89625-90-1; **8b**, 89625-89-8; *N*-carbobenzoxyalanine amide, 13139-27-0; benzyl glyoxylate, 52709-42-9; 5-fluorouracil, 51-21-8.

(23) Anderson, E. H. *Proc. Natl. Acad. Sci. U.S.A.* **1946**, *32*, 102.