(R_f ca. 0.5) when the chromatogram was developed with 5:1 chloroform–methanol. Similarly, reverse-phase HPLC (μBondapak C18 column; solvent 0.01 M aqueous ammonium dihydrogen phosphate–methanol (90:10), isocratic) of the two specimens monitored at 254 nm showed that their retention times (15.46 min and 15.45 min) were the same. The IR spectrum of the specimen obtained via **12** was identical with the IR spectrum of the specimen of *C*-EDU obtained via **6**. Thus, the melting temperatures, TLC, HPLC, and IR spectra show that the two specimens obtained by the routes outlined in Schemes I and II are identical.

Evidently, the commercial Pd–$CaCO_3$–lead catalyst was sufficiently active to reduce the ethynyl group to the ethyl group. The reduction of crude **12** (100 mg) in ethanol (10 mL) was also effected on 5% Pd–C at approximately atmospheric pressure. After 1 h in the hydrogen atmosphere, the mixture was filtered to remove the catalyst, and the filtrate (including ethanol washings of the catalyst) was concentrated to a residue that crystallized when it was triturated with ether. The white crystals were collected by filtration, washed with ether, and dried in vacuo at 56 °C: yield, 90% (92 mg). The IR and ^1H NMR spectra of this material showed that it was crude *C*-EDU.

(±)-1-[(1α,3β,4α)-3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-5-[2-(trimethylsilyl)ethyl]-2,4(1*H*,3*H*)-pyrimidinedione (13b). A mixture of 173 mg (0.43 mmol) of the 5-(trimethylsilylethynyl)pyrimidine (**11**), ethanol (20 mL), and 5% palladium-on-charcoal (100 mg) was stirred for 45 min in an atmosphere of hydrogen at approximately atmospheric pressure. The mixture was filtered to remove the catalyst, and the filtrate (including ethanol washings of the catalyst) was concentrated in vacuo to a colorless syrup: yield, 173 mg (99%). The mass spectrum (FAB) of this material showed that it was **13a**: *m/e* 411 (M + 1), 395 (M – CH_3), 351 (395 – CH_3CO – H), 213 (P + 2H), 197.

A solution of **13a** (170 mg) in ammonia–methanol (17 mL, 10% ammonia) was stirred at room temperature overnight. The reaction solution was concentrated under reduced pressure to a syrupy residue. The desired product was isolated by chromatography on silica gel with 5:1 chloroform–methanol as the developing and eluting solvent. The collection of fractions was monitored by TLC; fractions containing the desired product were combined and concentrated under reduced pressure. Further concentrated in vacuo with a vacuum pump left a crystalline residue that was triturated with 1:1 ethyl acetate–cyclohexane (3 mL). The crystalline product was collected by filtration, washed with the same solvent, and dried in vacuo at 78 °C: yield, 68 mg (50%); mp 154–156 °C (capillary inserted at 100 °C, 3 °C/min); TLC, 1 spot (5:1 chloroform–methanol); MS (FAB), *m/e* 327 (M + 1), 311 (M – CH_3), 213 (P + 2H), 197. Anal. ($C_{15}H_{26}N_2O_4Si$) C, H, N.

Antiviral Evaluations in Vitro. The compounds listed in Table I were tested for inhibition of the cytopathogenic effects produced by strain 377 (TK^+) of HSV-1 or strain MS of HSV-2 replicating in Vero cells. The data summarized in Table I were acquired by methods and procedures described previously for the evaluation of compounds for antiviral activity in vitro.[41] The general assay method was described by Ehrlich et al.,[37] but some modifications were incorporated.

Acknowledgment. This investigation was supported by Grant P01-CA34200 from the National Institutes of Health, Public Health Service. We are indebted to Dr. William C. Coburn, Jr., Marion C. Kirk, and Martha C. Thorpe for spectroscopic determinations and elemental analyses and to Carol Eldridge for technical assistance with the antiviral evalulations.

Registry No. **2**, 61849-27-2; **3a**, 98736-89-1; **5**, 98736-90-4; **6**, 98736-91-5; **7**, 98736-92-6; **8**, 98736-93-7; **9a**, 62102-28-7; **9c**, 95313-01-2; **9d**, 83967-19-5; **11**, 98736-94-8; **12**, 98736-95-9; **13a**, 98736-97-1; **13b**, 98736-96-0; lithium dimethylcuprate, 15681-48-8; methyl lithium, 917-54-4; (trimethylsilyl)acetylene, 1066-54-2.

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(*o*- and *p*-Nitrobenzyloxycarbonyl)-5-fluorouracil Derivatives as Potential Conjugated Bioreductive Alkylating Agents[1]

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A series of (*o*- and *p*-nitrobenzyloxycarbonyl)-5-fluorouracil derivatives were synthesized by reacting *o*- or *p*-nitrobenzyl chloroformate with 5-fluorouracil in the presence of triethylamine in DMF or Me_2SO. The reductive activation of these agents was hypothesized to generate a reactive methide and 5-fluorouracil, two components that are capable of synergistic interaction through complementary inhibition. Measurement of the surviving fractions of EMT6 tumor cells treated with these agents in culture under conditions of hypoxia and aerobiosis resulted in equal cell kill regardless of the state of oxygenation. One of the synthesized agents, 3-(*p*-nitrobenzyloxycarbonyl)-5-fluorouracil (**4**), appeared to be superior to 5-fluorouracil in prolonging the survival time of mice bearing intraperitoneal implants of the P388 leukemia and Sarcoma 180.

The malignant cell subpopulations of solid tumors are markedly heterogeneous with respect to a number of properties, including the degree of oxygenation and the rate of proliferation. Oxygen deficiency occurs in these neoplasms as a result of an insufficient blood supply, a phenomenon that leads to hypoxic tumor cells with significant resistance to both X-irradiation and many of the drugs used in therapy. We have hypothesized that the environment of hypoxic neoplastic stem cells is more conducive to reductive reactions than that of well-oxygenated normal cells. Such a metabolic differential is theoretically exploitable through the use of prodrugs requiring reductive activation to form reactive electrophiles; we have called this class of drugs bioreductive alkylating agents. Our laboratory has demonstrated that the quinone antibiotic mitomycin C, which may be considered a prototype bioreductive alkylating agent, is (a) preferentially

(1) This paper has been presented in part; see: Lin, T. S.; Wang, L.; Cosby, L. A.; Kirkpatrick, D. L.; Shiba, D. A.; Sartorelli, A. C. In "Abstracts of Papers"; 188th National Meeting of the American Chemical Society, Philadelphia, PA, Aug 26–31, 1984; American Chemical Society: Washington, DC, 1984; MEDI 61.

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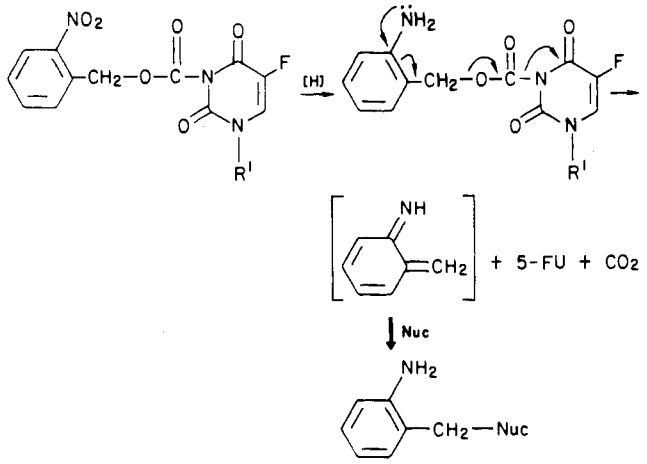
Scheme I

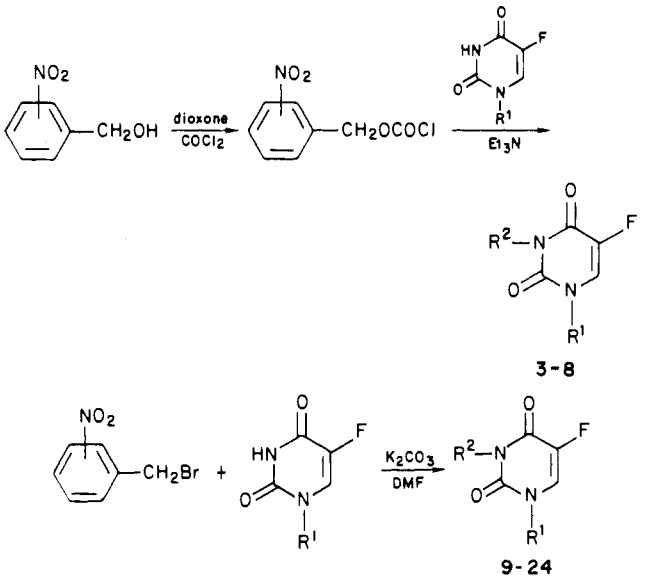


cytotoxic to hypoxic tumor cells in vitro[4] and (b) preferentially activated by metabolic reduction to a reactive species by tumor cell enzyme systems under anaerobic conditions.[5] On the basis of these concepts, we have synthesized a variety of quinone derivatives with the capacity to be activated by cellular reductive enzymes and have demonstrated their potential as bioreductive alkylating agents.[6-14]

In an effort to create an agent of this class in which the leaving group participates in a major way in the cytotoxicity that is produced, we have synthesized a series of (*o*- and *p*-nitrobenzyloxycarbonyl)- and (*o*- and *p*-nitrobenzyl)-5-fluorouracil derivatives with the potential to be activated preferentially by hypoxic cells to produce highly reactive quinone methides and 5-fluorouracil, a clinically useful antimetabolite. The reductive activation process for compounds of this class is hypothesized to occur as shown in Scheme I. The resulting products are visualized to have the potential to act in a synergistic manner to produce cytotoxicity by complimentary inhibition,[15] with the quinone methide causing damage to DNA and thereby interfering with its function as a template in replication, and the 5-fluorouracil inhibiting thymidylate synthetase after its conversion to a deoxyribonucleotide, an action which serves to minimize repair of DNA lesions.

Chemistry. Treatment of *p*-nitrobenzyl chloroformate[16] with a molar equivalent of 5-fluorouracil (1) in the presence of triethylamine in DMF at room temperature gave the N-1-substituted isomer **3**. Conversely, under similar reaction conditions, except for a change in the reaction solvent to a mixture of Me_2SO and dioxane, the N-3-substituted isomer **4** was formed. The structures of these two isomers were demonstrated by NMR spectroscopy. The chemical shifts of the methylene protons in the *p*-nitrobenzyloxycarbonyl moiety of **3** and **4** were quite different ($\Delta\delta$ = 0.57 ppm), with the methylene protons resonating at higher field (δ 4.96) being assigned to the N-1-substituted isomer **3**, and the methylene signal at lower field (δ 5.53) to the N-3-substituted isomer **4**. Since the methylene protons in **4** are situated between two carbonyl groups in the 5-fluorouracil moiety, they are more deshielded than their counterparts in the N-1-substituted isomers. The 1,3-bis(*p*-nitrobenzyloxycarbonyl)-5-fluorouracil derivative **5** was obtained by condensation of 5-fluorouracil (1) with 2 molar equiv of *p*-nitrobenzyl chloroformate in the presence of triethylamine in Me_2SO. The *o*-nitrobenzyloxycarbonyl and other related derivatives of 5-fluorouracil (**6–8**) were synthesized by similar methodology. The *p*- and *o*-nitrobenzyl derivatives of 5-fluorouracil (**9–24**) were fabricated by treatment of 5-fluorouracil (**1**) with the appropriate nitrobenzyl bromide and K_2CO_3 in DMF. The general chemical reactions for these syntheses are shown in Scheme II.

Scheme II



The chemical decomposition of the *p*-nitrobenzyloxycarbonyl derivatives of 5-fluorouracil **3** and **4** was measured in Waymouth's tissue culture medium containing penicillin/streptomycin and 15% fetal bovine serum at 37 °C by reverse-phase HPLC. The half-life for the decomposition of each compound was determined by least-squares linear regression analysis of the kinetic data. Compounds **3** and **4** had half-lives of approximately 75 and 39 min, respectively. The data for these determinations are listed in Table I.

Biological Activity. Measurement of the surviving fractions of EMT6 tumor cells treated with agents of this class in culture under conditions of hypoxia and aeration demonstrated equivalent degrees of cell kill regardless of the state of oxygenation (data not shown). These results are consistent with the findings shown in Table I, that these agents are unstable and spontaneously breakdown to generate 5-fluorouracil. Although these compounds did not produce differential kill of hypoxic cells, it appeared reasonable to test these derivatives for activity in a

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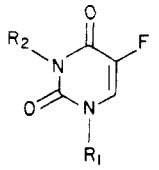

1, $R_1 = R_2 = H$ (5-FU)
2, R_1 = tetrahydrofuran-2-yl; $R_2 = H$ (ftorafur)
3, $R_1 = OCOCH_2C_6H_4NO_2$-*p*; $R_2 = H$
4, $R_1 = H$; $R_2 = OCOCH_2C_6H_4NO_2$-*p*
5, $R_1 = R_2 = OCOCH_2C_6H_4NO_2$-*p*
6, $R_1 = H$; $R_2 = OCOCH_2C_6H_4NO_2$-*o*
7, R_1 = tetrahydrofuran-2-yl; $R_2 = OCOCH_2C_6H_4NO_2$-*p*
8, R_1 = tetrahydrofuran-2-yl; $R_2 = OCOCH_2C_6H_4NO_2$-*o*
9, $R_1 = R_2 = CH_2C_6H_4NO_2$-*o*
10, $R_1 = R_2 = CH_2C_6H_4NO_2$-*m*
11, $R_1 = R_2 = CH_2C_6H_4NO_2$-*p*
12, $R_1 = CH_2C_6H_4NO_2$-*o*; $R_2 = H$
13, $R_1 = CH_2C_6H_4NO_2$-*m*; $R_2 = H$
14, $R_1 = CH_2C_6H_4NO_2$-*p*; $R_2 = H$
15, $R_1 = H$; $R_2 = CH_2C_6H_4NO_2$-*o*
16, $R_1 = H$; $R_2 = CH_2C_6H_4NO_2$-*m*
17, $R_1 = H$; $R_2 = CH_2C_6H_4NO_2$-*p*
18, $R_1 = CH_2C_6H_4NO_2$-*o*; R_2 = Ac
19, R_1 = tetrahydrofuran-2-yl; $R_2 = CH_2C_6H_4NO_2$-o
20, R_1 = tetrahydrofuran-2-yl; $R_2 = CH_2C_6H_4NO_2$-*m*
21, R_1 = tetrahydrofuran-2-yl; $R_2 = CH_2C_6H_4NO_2$-*p*
22, R_1 = 2'-deoxyribofuranosyl; $R_2 = CH_2C_6H_4NO_2$-*o*
23, R_1 = 2'-deoxyribofuranosyl; $R_2 = CH_2C_6H_4NO_2$-*m*
24, R_1 = 2'-deoxyribofuranosyl; $R_2 = CH_2C_6H_4NO_2$-*p*

standard test system. For this reason, representative compounds of the nitrobenzyloxycarbonyl and nitrobenzyl derivatives of 5-fluorouracil were evaluated for antineoplastic activity in mice bearing the P388 leukemia; the results of these tests are shown in Table II. Several compounds of the nitrobenzyloxycarbonyl series showed significant anticancer activity in this system, with compounds **4** and **6** possessing the best anticancer activity, yielding maximum T/C × 100 values of 212 and 215 at daily dosage levels of 80 and 60 mg/kg, respectively. These effects were slightly superior to those produced by an optimal daily dosage (20 mg/kg) of 5-fluorouracil, which produced a T/C × 100 value of 185. None of the compounds (**9–24**) in the nitrobenzyl series exhibited anticancer activity. Thus, it appeared that antineoplastic activity was dependent on the leaving group, even though there is no evidence that the leaving group directly contributed to this activity. Since the nitrobenzyloxycarbonyl moiety is a much better leaving group than the corresponding nitrobenzyl substituent, compound **4** was selected for further evaluation against Sarcoma 180 ascites cells in vivo. At a daily dose of 40 mg/kg, **4** produced an optimal T/C × 100 value of 332. The results of these experiments are summarized in Table III.

Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. ^1H NMR spectra were recorded at 60 MHz on a Varian T-60 spectrometer or at 500 MHz on a Brucker HX spectrometer, with Me_4Si as the internal reference standard. IR spectra were obtained with a Perkin-Elmer 15 spectrometer. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

1-(*p*-Nitrobenzyloxycarbonyl)-5-fluorouracil (3). A solution of 1.66 g (7.7 mmol) of *p*-nitrobenzyl chloroformate which was prepared by the methodology of Carpenter et al.,[16] in 10 mL of Me_2SO was added dropwise over a period of 30 min to a solution of 5-fluorouracil (1; 1.0 g, 7.7 mmol) dissolved in 10 mL of DMF containing 0.8 g (7.7 mmol) of triethylamine at 0–5 °C (ice–water bath) with stirring. The reaction mixture was stirred overnight at room temperature, the solvent was removed in vacuo below 30 °C, and the residue was dissolved in 100 mL of $CHCl_3$. The $CHCl_3$ solution was washed with 100 mL of 0.5 N HCl. During

Table I. Rate of Chemical Decomposition of 1-(*p*-Nitrobenzyloxycarbonyl)-5-fluorouracil (3) and 3-(*p*-Nitrobenzyloxycarbonyl)-5-fluorouracil (4)

time, min	peak area ratio (5-FU/3)	time, min	peak area ratio (5-FU/3)
5	0.35	75	0.70
30	0.54	125	0.66
time, min	**peak area ratio (5-FU/4)**	**time, min**	**peak area ratio (5-FU/4)**
10	0.48	60	1.24
30	0.92	120	1.29

Table II. Effects of (Nitrobenzyloxycarbonyl)- and (Nitrobenzyl)-5-fluorouracil Derivatives on the Survival Time of Mice Bearing the P388 Leukemia

compd	daily dosage,[a] mg/kg	av Δ wt, %	T/C × 100[b]
1 (5-FU)	20	−12.5	185
2 (ftorafur)	60	+12.2	100
4	20	+5.6	165
	40	+0.0	190
	60	−2.9	196
	80	−5.8	212
	100	−11.6	158
	200	−10.7	64
5	40	−2.7	136
	60	−5.5	149
	80	−7.4	158
6	20	+4.9	171
	40	+3.9	178
	60	−8.3	215
	80	−11.3	177
	100	−10.6	65
8	60	+15.1	72
	100	+8.4	103
	120	+6.6	101
9	25	+17.8	90
	50	+15.0	88
	100	+22.4	86
12	25	+13.3	90
	50	+10.0	92
	100	+20.8	88
19	25	+10.3	90
	50	+7.8	96
	100	+13.7	96

[a] Drugs were administered by intraperitoneal injection, beginning 24 h after tumor implantation, once daily for 6 consecutive days. [b] T/C × 100 represents the ratio of the survival time of treated to control animals × 100. The average survival time of the untreated tumor-bearing control animals was 11.8 ± 2.9 days. Each value represents the results from 5 to 15 mice. The results for 5-fluorouracil were those obtained at the optimum therapeutic dosage; ftorafur was found to be inactive at a daily dosage level of 60 mg/kg.

Table III. Effects of 3-(*p*-Nitrobenzyloxycarbonyl)-5-fluorouracil (4) on the Survival Time of Mice Bearing Sarcoma 180 Ascites Cells

compd	daily dosage,[a] mg/kg	av Δ wt, %	T/C × 100[b]
1 (5-FU)	20	−3.4	277
4	10	+9.6	159
	20	+2.7	124
	40	−7.6	332

[a] The dosage schedule employed was identical to that described in Table II. [b] The average survival time of untreated tumor-bearing control animals was 11.8 days.

the process a white solid precipitated, which was collected by filtration, washed with H_2O, and dried to give 0.03 g. The $CHCl_3$ layer was concentrated and washed with H_2O, and more white solid precipitated; this was also collected, washed with H_2O, and dried. The combined product weighed 0.05 g: mp 235–237 °C; TLC, R_f 0.34 ($CHCl_3$–EtOAc, 2:1.5, v/v); NMR (Me_2SO-d_6) δ 4.96 (s, 2 H, Ar CH_2O), 7.58 (d, 2 H, 2′- and 6′-H, aromatic ring, J_{AB} = 8.70 Hz), 8.20 (d, 2 H, 3′- and 5′-H, aromatic ring, J_{AB} = 8.70 Hz), 8.23 (d, 1 H, 6-H, J_{H-F} = 6.70 Hz), 11.88 (s, 1 H, 3-NH, D_2O exchangeable); IR (KBr) 2.96 (N–H), 5.80 (carbamate carbonyl), 5.90 and 6.02 (pyrimidine ring carbonyl) μm. Anal. ($C_{12}H_8FN_3O_6$) C, H, N.

3-(p-Nitrobenzyloxycarbonyl)-5-fluorouracil (4). p-Nitrobenzyl chloroformate (0.71 g, 3.33 mmol) in 3 mL of dioxane was added dropwise to a solution of 5-fluorouracil (1; 0.42 g, 3.33 mmol) and 0.33 g (3.30 mmol) of triethylamine in 25 mL of Me_2SO. The reaction mixture was stirred at room temperature for 5 h, the Et_3N·HCl salt which formed during this period was removed by filtration, and the Me_2SO solution was then poured into 100 mL of ice–water. The resulting white solid was collected, washed with ice-cold water, and dried to yield 0.92 g of crude product, which was washed thoroughly by stirring with 25 mL of $CHCl_3$ for 1 h to remove impurities. This purification process was repeated once more, and the product was then collected by filtration and dried, yielding 0.53 g (45%): mp 218–220 °C dec; TLC, R_f 0.39 ($CHCl_3$–EtOAc, 2:1.5, v/v); NMR (Me_2SO-d_6) δ 5.53 (s, 2 H, $ArCH_2O$), 7.77 (d, 2 H, 2′- and 6′-H, aromatic ring, J_{AB} = 8.57 Hz), 8.25 (d, 2 H, 6-H, J_{H-F} = 7.20 Hz), 8.26 (d, 2 H, 3′- and 5′-H, aromatic ring, J_{AB} = 8.60 Hz), 12.05 (s, 1 H, 1-NH, D_2O exchangeable); IR (KBr) 2.92 (NH), 5.73 (carbamate carbonyl), 5.82 and 5.97 (pyrimidine ring carbonyl) μm. Anal. ($C_{12}H_8FN_3O_6$) C, H, N.

1,3-Bis(p-nitrobenzyloxycarbonyl)-5-fluorouracil (5). A solution of 1.66 g (7.6 mmol) of p-nitrobenzyl chloroformate in 2 mL of dioxane was added dropwise to a solution of 5-fluorouracil (1; 0.5 g, 3.8 mmol) and triethylamine (0.8 g, 7.6 mmol) in 12 mL of Me_2SO with stirring. The reaction mixture was maintained at room temperature for 1 h and then at 43 °C (inner temperature) for 20 additional hours with continued stirring. The Et_3N·HCl salt that formed during the reaction was removed by filtration, the Me_2SO filtrate was poured onto 150 mL of ice–water, and the white solid which precipitated was collected by filtration, washed with water, and dried. The product was then extracted with $CHCl_3$ (3 × 20 mL) to remove any impurities and dried to give 0.44 g (23%): mp 213–215 °C; TLC, R_f 0.39 ($CHCl_3$–EtOAc, 2:1.5, v/v); NMR (Me_2SO-d_6) δ 5.03 (s, 2 H, 1-NCO_2CH_2), 5.50 (s, 2 H, 3-NCO_2CH_2), 7.36–8.30 (m, 8 H, 6-H and $O_2NC_6H_4$); IR (KBr) 5.75 (carbamate carbonyl), 5.82 and 5.97 (sh) (pyrimidine carbonyl) μm. Anal. ($C_{20}H_{13}FN_4O_{10}$) C, H, N.

3-(o-Nitrobenzyloxycarbonyl)-5-fluorouracil (6). A solution of o-nitrobenzyl chloroformate[17] in 3 mL of dioxane was added slowly over a period of 10 min to a solution of 5-fluorouracil (1; 0.5 g, 3.8 mmol) and triethylamine (0.4 g, 3.8 mmol) in 8 mL of Me_2SO. The reaction mixture was stirred for 5 h at room temperature, the insoluble solid (Et_3N·HCl) was removed by filtration, and the filtrate was poured onto 150 mL of ice–water. The resulting white solid was collected by filtration, washed with water, dried, and then suspended in 10 mL of $CHCl_3$. The suspension was stirred at room temperature for 1 h, filtered, and dried to yield 0.8 g (67%) of a white solid product: mp 192–194 °C; TLC, R_f 0.44 ($CHCl_3$–EtOAc, 2:1.5, v/v); NMR (Me_2SO-d_6) δ 5.69 (s, 2 H, Ar CH_2O), 7.66 (t, 1 H, 5′-H, aromatic ring, J = 7.78 Hz), 7.84 (t, 1 H, 4′-H, aromatic ring, J = 7.60 Hz), 7.97 (d, 1 H, 3′-H, aromatic ring, J = 7.72), 8.19 (d, 1 H, 6′-H, aromatic ring, J = 8.18 Hz), 8.25 (d, 1 H, 6-H, J_{H-F} = 7.16 Hz), 11.13 (s, 1 H, 3-NH, D_2O exchangeable); IR (KBr) 2.95 (NH), 5.72 (carbamate carbonyl), 5.78 and 5.95 (pyrimidine carbonyl) μm. Anal. ($C_{12}H_8FN_3O_6$) C, H, N.

1-(Tetrahydrofuran-2-yl)-3-(p-nitrobenzyloxycarbonyl)-5-fluorouracil (7). p-Nitrobenzyl chloroformate (0.16 g, 0.75 mmol) in 5 mL of dioxane was added dropwise over 10 min to a stirred solution of ftorafur (2; 0.1 g, 0.5 mmol) and triethylamine in 5 mL of Me_2SO; stirring was continued at room temperature for 24 h. The solvent was evaporated to dryness in vacuo to afford a yellow solid residue. The crude product was then purified by silica gel column chromatography ($CHCl_3$–EtOAc, 1:1, v/v) to give 0.15 g (8%) of a white powder: 127–129 °C dec; TLC, R_f 0.43 ($CHCl_3$–EtOAc, 1:1, v/v); NMR (Me_2SO-d_6) δ 1.93 (m, 2 H, 3′-H), 2.04 (m, 1 H, 2′-H_A), 2.23 (m, 1 H, 2′-H_B), 3.81 (q, 1 H, 4′-H_A), 4.26 (q, 1 H, 4′-H_B), 5.07 (d, 1 H, $ArCH_A$, J_{AB} = 15.32 Hz), 5.11 (d, 1 H, $ArCH_B$, J_{AB} = 15.32 Hz), 5.94 (d, 1 H, 1′-H), 7.55 (d, 2 H, 2″- and 6″-H, aromatic ring, J_{AB} = 8.63 Hz), 8.05 (d, 1 H, 6-H, J_{H-F} = 6.63 Hz), 8.66 (d, 2 H, 3″- and 5″-H, aromatic ring, J = 8.63 Hz); IR (KBr) 5.83 (carbamate carbonyl), 5.98 and 6.02 (pyrimidine carbonyl) μm. Anal. ($C_{16}H_{14}FN_3O_7$) C, H, N.

1-(Tetrahydrofuran-2-yl)-3-(o-nitrobenzyloxycarbonyl)-5-fluorouracil (8). A solution of o-nitrobenzyl chloroformate (1.1 g, 5.1 mmol) in 2 mL of CH_2Cl_2 was added dropwise over a period of 10 min to a stirred solution of ftorafur (2; 0.5 g, 2.5 mmol) dissolved in 5 mL of CH_2Cl_2 containing 0.51 g (5.1 mmol) of triethylamine. The reaction mixture was stirred at room temperature for 48 h and then refluxed for an additional 48 h. The insoluble white solid of Et_3N·HCl was removed by filtration and the filtrate was washed with H_2O (2 × 2 mL). The CH_2Cl_2 layer was collected, dried (Na_2SO_4), and concentrated in vacuo to afford a yellow syrup which was triturated with anhydrous Et_2O at −30 °C. The resulting solid was filtered and dried, yielding 0.52 g of product (55%); mp 100–104 °C. The crude product was then washed thoroughly with Et_2O (3 × 10 mL), filtered, and dried to give 0.25 g of analytically pure 8: mp 103–105 °C; TLC, R_f 0.45 ($CHCl_3$–EtOAc, 2:1.5, v/v); NMR (Me_2SO-d_6) δ 1.42 (m, 2 H, 3′-H), 2.05 (m, 1 H, 2′-H_A), 2.23 (m, 1 H, 2′-H_B), 3.81 (q, 1 H, 4′-H_A), 4.27 (q, 1 H, 4′-H_B), 5.28 (d, 1 H, Ar CH_A, J_{AB} = 16.73 Hz), 5.24 (d, 1 H, Ar CH_B, J_{AB} = 16.73 Hz), 5.92 (m, 1 H, 1′-H), 7.32 (d, 1 H, 6″-H, aromatic ring, J = 7.54 Hz), 7.54 (t, 1 H, 5″-H, aromatic ring, J = 7.55 Hz), 7.67 (t, 1 H, 4″-H, aromatic ring, J = 7.62 Hz), 8.03 (d, 1 H, 6-H, J_{H-F} = 6.66 Hz), 8.26 (d, 1 H, 3″-H, aromatic ring, J = 8.09 Hz); IR (KBr) 5.83 (carbamate carbonyl), 5.99 (sh) and 6.05 (pyrimidine carbonyl) μm. Anal. ($C_{16}H_{14}FN_3O_7$) C, H, N.

1,3-Bis(o-nitrobenzyl)-5-fluorouracil (9) and 1-(o-Nitrobenzyl)-5-fluorouracil (12). o-Nitrobenzyl bromide (0.83 g, 3.8 mmol) in 10 mL of DMF was added dropwise to a stirred mixture of 5-fluorouracil (1; 0.5 g, 3.8 mmol) and K_2CO_3 (1.2 g, 8.7 mmol) in 15 mL of DMF. The reaction mixture was stirred at room temperature for 2 h and then filtered. The filtrate was evaporated to dryness in vacuo, and the resulting residue was dissolved in a minimum amount of EtOAc and chromatographed on a silica gel column (C_6H_6–EtOAc, 3:2, v/v). The first fraction collected afforded 0.35 g (23%) of 9: mp 132–133 °C; TLC, R_f 0.46 (C_6H_6–EtOAc, 4:1, v/v); NMR (Me_2SO-d_6) δ 5.31 (br s, 4 H, 1- and 3-CH_2), 7.20–8.20 (m, 8 H, aromatic ring), 8.29 (d, 1 H, H-6). Anal. ($C_{18}H_{13}FN_4O_6$) C, H, N. The second fraction yielded 0.40 g (39%) of 12: mp 240–241 °C; TLC, R_f 0.47 (C_6H_6–EtOAc, 1:1, v/v); NMR (Me_2SO-d_6) 5.20 (s, 2 H, N_1- CH_2), 7.18–7.90 (m, 4 H, aromatic ring), 8.13 (d, 1 H, H-6), 11.6 (br s, 3-NH, D_2O exchangeable). Anal. ($C_{11}H_8FN_3O_4$) C, H, N.

Compounds **10**, **13**, **11**, and **14** were also synthesized by the methodology described above.

1,3-Bis(m-nitrobenzyl)-5-fluorouracil (10) and 1-(m-Nitrobenzyl)-5-fluorouracil (13). **Compound 10**: yield, 1.2 g (78%); mp 171–172 °C; TLC, R_f 0.52 ($CHCl_3$–EtOAc, 7:3, v/v); NMR (Me_2SO-d_6) δ 5.09 (br d, 4 H, 1- and 3-CH_2), 7.43–8.37 (m, 8 H, aromatic ring), 8.50 (d, 1 H, H-6). Anal. ($C_{18}H_{13}FN_4O_6$) C, H, N.

Compound 13: yield, 0.37 g (18%); mp 206–208 °C dec; TLC, R_f 0.28 ($CHCl_3$–EtOAc, 1:1, v/v); NMR (Me_2SO-d_6) δ 5.01 (s, 2 H, 1-CH_2), 7.60–8.42 (m, 5 H, aromatic ring, and H-6), 11.89 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{11}H_8FN_3O_4$) C, H, N.

1,3-Bis(p-nitrobenzyl)-5-fluorouracil (11) and 1-(p-Nitrobenzyl)-5-fluorouracil (14). **Compound 11**: yield, 1.3 g (80%); mp 161–162 °C; TLC, R_f 0.59 (C_6H_6–EtOAc, 2:3, v/v); NMR (Me_2SO-d_6) δ 5.15 (br d, 4 H, 1- and 3-CH_2), 7.50 (d, 2 H, H_A, 1-aromatic ring), 7.73 (d, 2 H, H_B, 1-aromatic ring), 8.10 (d, 2 H, H_A, 3-aromatic ring), 8.23 (d, 2 H, H_B, 3-aromatic ring), 8.42 (d, 1 H, H-6). Anal. ($C_{18}H_{13}FN_4O_6$) C, H, N.

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Compound 14: yield, 0.35 g (16%); mp 236–238 °C; TLC, R_f 0.37 (C_6H_6–EtOAc, 2:3, v/v); NMR (Me_2SO-d_6) δ 4.99 (s, 2 H, 1-CH_2), 7.58 (d, 2 H, H_A, 1-aromatic ring), 8.10–8.35 (m, 3 H, H_B, 1-aromatic ring, and H-6), 11.92 (br s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{11}H_8FN_3O_4$) C, H, N.

3-(*o*-Nitrobenzyl)-5-fluorouracil (15). A solution of 5-fluorouracil (1; 1.0 g, 7.6 mmol) and 0.2 mL of pyridine in 20 mL of acetic anhydride was refluxed with stirring for 30 min. The solvent was evaporated in vacuo to dryness and the remaining residue was dissolved in 20 mL of dioxane. *o*-Nitrobenzyl bromide (1.7 g, 7.8 mmol) and triethylamine (0.87 g, 8.6 mmol) were added to the above solution, and the resulting mixture was stirred overnight at room temperature. The insoluble Et_3N·HBr that formed during the reaction was removed by filtration. Evaporation of the filtrate yielded a residue which gave two major fractions when chromatographed on a silica gel column (C_6H_6–EtOAc, 4:1, v/v). The first fraction consisted of 0.4 g (26%) of **9** and the second 0.8 g (39%) of the desired product **15**: mp 193–194 °C; TLC, R_f 0.27 (C_6H_6–EtOAc, 2:3, v/v); NMR (Me_2SO-d_6) δ 5.28 (s, 2 H, 3-CH_2), 7.20–8.22 (m, 5 H, 3-aromatic ring, and H-6), 11.10 (br s, 1 H, 1-NH, D_2O exchangeable). Anal. ($C_{11}H_8FN_3O_4$) C, H, N.

3-(*m*-Nitrobenzyl)-5-fluorouracil (**16**) and 3-(*p*-nitrobenzyl)-5-fluorouracil (**17**) were fabricated by a similar procedure; in these preparations, however, only the desired products, **16** and **17**, were isolated.

Compound 16: yield, 0.65 g (32%); mp 211–212 °C; TLC, R_f 0.31 (C_6H_6–EtOAc, 1:1, v/v); NMR (Me_2SO-d_6) δ 5.12 (s, 2 H, 3-CH_2), 7.60–8.31 (m, 5 H, 3-aromatic ring, and H-6), 11.22 (br s, 1 H, 1-NH, D_2O exchangeable). Anal. ($C_{11}H_8FN_3O_4$) C, H, N.

Compound 17: yield, 0.4 g (39%); mp 204–207 °C; TLC, R_f 0.32 (C_6H_6–EtOAc, 2:3, v/v); NMR (Me_2SO-d_6) δ 5.11 (s, 2 H, 3-CH_2), 7.52 (d, 2 H, H_A, 3-aromatic ring), 7.90 (d, 1 H, H-6), 8.21 (d, 2 H, H_B, 3-aromatic ring), 11.32 (br s, 1 H, 1-NH, D_2O exchangeable). Anal. ($C_{11}H_8FN_3O_4$) C, H, N.

1-(*o*-Nitrobenzyl)-3-acetyl-5-fluorouracil (18). Acetyl chloride (0.48 g, 6.0 mmol) was added over a period of 10 min to a stirred mixture of 1-(*o*-nitrobenzyl)-5-fluorouracil (**12**; 0.55 g, 2.0 mmol) and triethylamine (0.4 g, 4.0 mmol) in 10 mL of dioxane. The reaction mixture was stirred at room temperature for 1 h, and the solvent and excess acetyl chloride were then removed by evaporation under reduced pressure. The resulting residue was recrystallized from Et_2O–$CHCl_3$ at −20 °C overnight to afford 0.5 g (78%) of **18**: mp 135–137 °C dec; TLC, R_f 0.49 ($CHCl_3$–EtOAc, 7:3, v/v); NMR (Me_2SO-d_6) δ 2.54 (s, 3 H, $COCH_3$), 5.28 (s, 2 H, 1-CH_2), 7.30–8.40 (m, 5 H, 1-aromatic ring, and H-6). Anal. ($C_{13}H_{10}FN_3O_5$) C, H, N.

1-(Tetrahydrofuran-2-yl)-3-(*o*-nitrobenzyl)-5-fluorouracil (19). A mixture of ftorafur (**2**; 0.2 g, 1.0 mmol), *o*-nitrobenzyl bromide (0.32 g, 1.5 mmol), and K_2CO_3 (0.5 g, 3.6 mmol) in 10 mL of DMF was stirred at room temperature for 1 h. The solution was filtered to remove any insoluble material, and the filtrate was evaporated in vacuo to give a residue which was dissolved in a small amount of EtOAc and chromatographed on a silica gel column ($CHCl_3$–EtOAc, 3:2, v/v). The fractions containing compound **19** were combined and evaporated to dryness under reduced pressure to yield 0.3 g (90%) of pure syrup which solidified upon trituration with Et_2O: mp 114–115 °C; TLC, R_f 0.53 ($CHCl_3$–EtOAc, 3:2, v/v); NMR (Me_2SO-d_6) δ 1.67–2.24 (m, 4 H, 2′- and 3′-H), 3.62–4.44 (m, 2 H, 4′-H), 5.28 (s, 2 H, 3-CH_2), 5.80–6.08 (m, 1 H, 1′-H), 7.17–8.32 (m, 5 H, 3-aromatic ring, and H-6). Anal. ($C_{15}H_{14}FN_3O_5$) C, H, N.

1-(Tetrahydrofuran-2-yl)-3-(*m*-nitrobenzyl)-5-fluorouracil (**20**) and 1-(tetrahydrofuran-2-yl)-3-(*p*-nitrobenzyl)-5-fluorouracil (**21**) were prepared by the methodology described above. These compounds were isolated and purified by silica gel column chromatography ($CHCl_3$–EtOAc, 7:3, v/v).

Compound 20: yield, 0.8 g (95%); mp 137–138 °C; TLC, R_f 0.51 ($CHCl_3$–EtOAc, 7:3, v/v); NMR (Me_2SO-d_6) δ 1.72–2.28 (m, 4 H, 2′- and 3′-H), 3.56–4.45 (m, 2 H, 4′-H), 5.06 (s, 2 H, 3-CH_2), 5.80–6.03 (m, 1 H, 1′-H), 7.46–8.18 (m, 5 H, 3-aromatic ring, and H-6). Anal. ($C_{15}H_{14}FN_3O_5$) C, H, N.

Compound 21: yield, 0.75 g (90%); TLC, R_f 0.49 ($CHCl_3$–EtOAc, 7:3, v/v); NMR (Me_2SO-d_6) δ 1.70–2.30 (m, 4 H, 2′- and 3′-H), 3.65–4.49 (m, 2 H, 4′-H), 5.12 (s, 2 H, 3-CH_2), 5.85–6.13 (m, 1 H, 1′-H), 7.60 (d, 2 H, H_A, 3-aromatic ring), 8.03 (d, 1 H, H-6), 8.22 (d, 2 H, H_B, 3-aromatic ring). Anal. ($C_{15}H_{14}FN_3O_5$) C, H, N.

3-(*o*-Nitrobenzyl)-2′-deoxy-5-fluorouridine (22). A mixture of 2′-deoxy-5-fluorouridine (0.5 g, 2 mmol), *o*-nitrobenzyl bromide (0.5 g, 2.3 mmol), and K_2CO_3 (0.5 g, 3.6 mmol) in 25 mL of DMF was stirred at room temperature for 2 h. The solution was filtered, and the filtrate was evaporated to dryness in vacuo. The remaining residue was dissolved in a minimum amount of EtOAc and chromatographed on a silica gel column (EtOAc). The eluate containing **22** was evaporated under reduced pressure to yield 0.75 g (97%) of a syrup which was solidified from Et_2O: mp 172.5–173.5 °C; TLC, R_f 0.34 (EtOAc); NMR (Me_2SO-d_6) δ 2.00–2.32 (m, 2 H, 2′-H), 3.20–4.50 (m, 5 H, 3′-, 4′-, and 5′-H, and 5′-OH, D_2O exchangeable), 5.03–5.43 (m, 3 H, 3-CH_2, and 3′-OH, D_2O exchangeable), 5.98–6.32 (m, 1 H, 1′-H), 7.14–8.60 (m, 4 H, 3-aromatic ring), 8.80 (d, 1 H, H-6). Anal. ($C_{16}H_{16}FN_3O_7$) C, H, N.

3-(*m*-Nitrobenzyl)-2′-deoxy-5-fluorouridine (**23**) and 3-(*p*-nitrobenzyl)-2′-deoxy-5-fluorouridine (**24**) were also obtained by the procedure described for the synthesis of **22**.

Compound 23: yield, 0.7 g (90%); mp 60–62 °C; TLC, R_f 0.47 (EtOAc); NMR (Me_2SO-d_6) δ 1.90–2.32 (m, 2 H, 2′-H), 3.34–4.41 (m, 5 H, 3′-, 4′-, and 5′-H, and 5′-OH, D_2O exchangeable), 4.88–5.38 (m, 3 H, 3-CH_2, and 3′-OH, D_2O exchangeable), 6.08–6.40 (m, 1 H, 1′-H), 7.45–8.53 (m, 5 H, 3-aromatic ring, and H-6). Anal. ($C_{16}H_{16}FN_3O_7$) C, H, N.

Compound 24: yield, 0.65 g (84%); mp 99–100 °C; TLC, R_f 0.39 (EtOAc); NMR (Me_2SO-d_6) δ 1.92–2.32 (m, 2 H, 2′-H), 3.33–4.40 (m, 5 H, 3′-, 4′-, and 5′-H, and 5′-OH, D_2O exchangeable), 4.92–5.28 (m, 3 H, 3-CH_2, and 3′-OH, D_2O exchangeable), 5.95–6.26 (m, 1 H, 1′-H), 7.53 (d, 2 H, H_A, 3-aromatic ring), 8.20 (d, 2 H, H_B, 3-aromatic ring), 8.40 (d, 1 H, H-6). Anal. ($C_{16}H_{16}FN_3O_7$) C, H, N.

Methodology for Evaluation of the Rate of Chemical Decomposition. The chemical decomposition of 4-nitrobenzyloxycarbonyl derivatives of 5-fluorouracil was quantitated with use of reverse-phase HPLC on a Varian Model 5000 liquid chromatograph equipped with a Varian Vari-Chrom UV–vis variable wavelength detector operated at 254 nm and a Varian CDS 111 chromatography data system. A 25-cm Whatman Partisil PXS 10/25 octadecylsilane reverse-phase column was used at ambient temperature. The sample was eluted with 35% methanol (Burdick & Jackson, Muskegon, MI) and triple distilled water.

The *p*-nitrobenzyloxycarbonyl compounds **3** and **4** (1 mg) were dissolved in 100 μL of DMF and 2 mL of Waymouth's medium containing penicillin and streptomycin and supplemented with 15% fetal bovine serum at 37 °C. At various times, 200-μL aliquots were removed and combined with 100 μL of a 0.3 μg/uL stock solution of the internal standard *p*-nitrobenzyl chloride (Aldrich Chemical Co., Milwaukee, WI), and 50 μL was injected onto the HPLC column. The quantity of 5-fluorouracil released during the chemical decomposition was determined from the ratio of the 5-fluorouracil peak area to the peak area for the internal standard. The reported half-lives were determined by least-squares linear regression analysis.

Biological Test Procedures. Transplantation of P388 murine leukemia and Sarcoma 180 ascites cells was carried out by withdrawing peritoneal fluid from donor CDF_1 mice bearing 7-day growths. The suspension was centrifuged for 2 min (1600*g*), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was determined with a Coulter particle counter and the cell population was adjusted to 10^7 cells/mL. The resulting cell suspension (0.1 mL; ca. 10^6 cells) was injected intraperitoneally into each animal. Drugs were administered by intraperitoneal injection, beginning 24 h after tumor implantation, once daily for 6 consecutive days. The test compounds were injected as fine suspensions following homogenization in two to three drops of 20% aqueous Tween 80 and then made up to volume with isotonic saline. All drugs were administered intraperitoneally in a volume of 0.5 mL. For any one experiment, animals were distributed into groups of five mice of comparable weight and maintained throughout the course of the experiment on Purina Laboratory Chow pellets and water ad libitum. Control tumor-bearing animals given injections of comparable volumes of vehicle were included in each experiment. Mice were weighed during the course of the experiments, and the percent change in body weight from onset to termination of

therapy was used as an indication of drug toxicity. Determination of the sensitivity of ascitic neoplasms to these agents was based on the prolongation of survival time afforded by the drug treatments. Each experiment was repeated at least one time.

Acknowledgment. This research was supported in part by Grant CH-211A from the American Cancer Society.

Registry No. 1, 51-21-8; 2, 17902-23-7; 3, 98652-97-2; 4, 98652-98-3; 5, 98652-99-4; 6, 98653-00-0; 7, 98653-01-1; 8, 98653-02-2; 9, 98653-03-3; 10, 98653-04-4; 11, 98653-05-5; 12, 98653-06-6; 13, 98653-07-7; 14, 98653-08-8; 15, 98653-09-9; 16, 98653-10-2; 17, 98653-11-3; 18, 98653-12-4; 19, 98653-13-5; 20, 98653-14-6; 21, 98653-15-7; 22, 98653-16-8; 23, 98653-17-9; 24, 98653-18-0; 2'-deoxy-5-fluorouridine, 50-91-9.

Peptides of 2-Aminopimelic Acid: Antibacterial Agents That Inhibit Diaminopimelic Acid Biosynthesis

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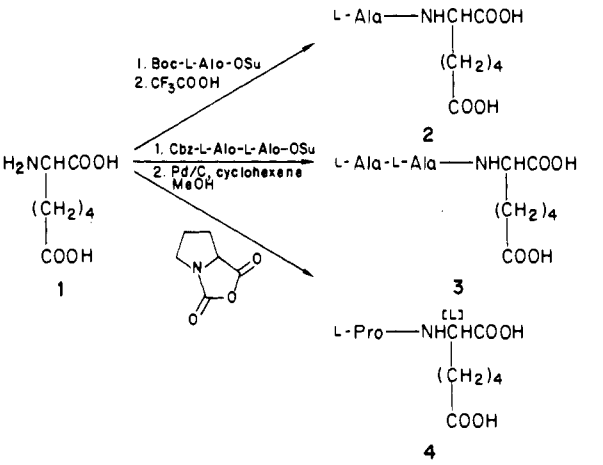
Succinyl-CoA:tetrahydrodipicolinate-*N*-succinyltransferase is a key enzyme in the biosynthesis of diaminopimelic acid (DAP), a component of the cell wall peptidoglycan of nearly all bacteria. This enzyme converts the cyclic precursor tetrahydrodipicolinic acid (THDPA) to a succinylated acyclic product. L-2-Aminopimelic acid (L-1), an acyclic analogue of THDPA, was found to be a good substrate for this enzyme and was shown to cause a buildup of THDPA in a cell-free enzyme system but was devoid of antibacterial activity. Incorporation of 1 into a di- or tripeptide yielded derivatives that exhibited antibacterial activity against a range of Gram-negative organisms. Of the five peptide derivatives tested, (L-2-aminopimelyl)-L-alanine (6) was the most potent. These peptides were shown to inhibit DAP production in intact resting cells. High levels (30 mM) of 2-aminopimelic acid were achieved in the cytoplasm of bacteria as a result of efficient uptake of the peptide derivatives through specific peptide transport systems followed, presumably, by cleavage by intracellular peptidases. Finally, the antibacterial activity of these peptides could be reversed by DAP or a DAP-containing peptide. These results demonstrate that the peptides containing L-2-aminopimelic acid exert their antibacterial action by inhibition of diaminopimelic acid biosynthesis.

Ever since its discovery in *Corynebacterium diphtheriae* by Work,[1,2] diaminopimelic acid (DAP) has been recognized as a prime site for the design of rationally based antibiotics. This conviction derives from the finding that the compound is widely distributed in prokaryotes[3] where it serves as a component of the cell wall peptidoglycan of nearly all bacteria (Gram-positive cocci excepted)[4,5] and as a precursor of lysine.[6,7] Additionally, since DAP is not found as a constituent of animal tissues and its biosynthetic pathway is absent in animals, there is a reasonable expectation that an antibiotic acting on DAP biosynthesis is unlikely to be toxic to humans. Furthermore, interference with the DAP pathway has been shown to be a lethal event; growth studies on a strain of *Escherichia coli* auxotropic for DAP showed that the organism lysed when exogenously supplied DAP had been depleted.[8]

Given the above biological rationale, it is surprising that the literature records few attempts at interdicting the biosynthesis of diaminopimelate[9] or interfering with its incorporation into peptidoglycan precursors. This is all the more remarkable since all of the intermediates in the biosynthetic pathway are known and the steps leading to DAP and lysine in bacteria have been fully elucidated (Figure 1).[10] Of the eight enzymes necessary for the conversion of pyruvate and aspartate to *meso*-DAP, the isomer usually present in the peptidoglycan of Gram-negative bacteria, four have been purified to homogeneity:

Scheme I



the aldolase (dihydrodipicolinate synthase, EC 4.2.1.52),[11] the reductase (dihydrodipicolinate reductase, EC

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