

The Lysine Pathway as a Target for a New Genera of Synthetic Antibacterial Antibiotics?

Jean-Marc Girodeau,^{*,†,§} Constantin Agouridas,^{†,||} Maryse Masson,[†] Roland Pineau,[†] and François Le Goffic[†]

Centre de Recherche Delalande, 92500 Rueil-Malmaison, France, and Laboratoire de Bioorganique et de Biotechnologies, ENSCP, 75231 Paris Cedex 05, France. Received May 6, 1985

Unsaturated analogues of diaminopimelic acid have been synthesized. The amino acids were designed so that they would be reversible or irreversible inhibitors of both of the two last enzymes of the lysine pathway. The compounds were tested with *meso*-diaminopimelate decarboxylase. *trans*-3,4-Didehydrodiaminopimelic acid (**2**) was found to be the most potent inhibitor. The antibacterial activities did not correlate with enzyme inhibiting activities. 4-Methylenediaminopimelic acid **4** showed strong antibacterial properties. It is suggested that L,L-diaminopimelate epimerase could be the target enzyme.

Diaminopimelic acid (DAP, **1**) is a building block of the peptidoglycan of most Gram-negative as well as Gram-positive bacteria. It is introduced into this network as part of the cross-linking moiety between polysaccharide fibers.^{1,2} Of the three possible stereoisomers of DAP, the *meso* form is the most widely encountered, although the L,L isomer has been found in the peptidoglycan of some strains³ as well as L-lysine and a few other amino acids.¹⁻³ It is noteworthy that L,L-DAP, *meso*-DAP, and lysine are biosynthetically closely related as lysine is formed by decarboxylation of *meso*-DAP^{1,4} (Scheme I).

Inhibitors of peptidoglycan biosynthesis, such as β -lactams,² fosfomicine,⁵ D-cycloserine,⁶ L-alanyl-L-amino-phosphonic acid,⁷ and vancomycin,⁸ for instance have powerful antibiotic properties. Hence, the enzymes of peptidoglycan processing appear to be good targets for the rational design of a new antibacterial agents.⁵ The absence of the peptidoglycan network in mammals also implies that such an approach should provide selective toxicity against bacteria.

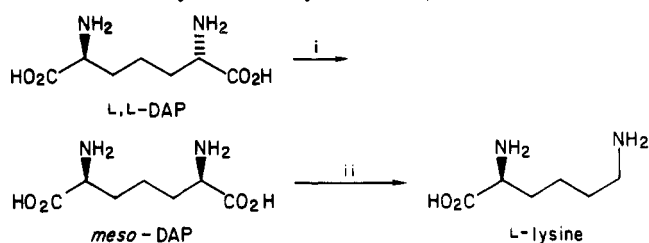
Alternatively inhibitors of amino acid biosynthesis can also provide antibacterial molecules. Propargylglycine, which shows strong antimicrobial activity,⁹ is an enzyme-activated irreversible inhibitor of cystathionine γ -synthase¹⁰ and as a consequence blocks the L-methionine pathway.

In the present approach we decided to design and synthesize didehydro analogues of DAP (Figure 1), with the expectation they will have antibacterial properties since they could be potential inhibitors of L,L-DAP epimerase (*meso*-DAP production) and/or *meso*-DAP decarboxylase (lysine production). The design of the structures of our analogues was based on two fundamental criteria.

1. Rigidity of the Molecules To Improve the Affinity for the Target Enzymes. β,γ -Didehydroornithine is, for instance, a good inhibitor of ornithine decarboxylase with a K_i 100 times lower than the K_m of its substrate.¹¹ Similar results were obtained with the didehydro analogues of α -(fluoromethyl) or α -(difluoromethyl)ornithine¹² and of α -ethynylputrescine,¹³ which are K_{cat} inhibitors of pyridoxal phosphate (PLP) dependent ornithine decarboxylase.

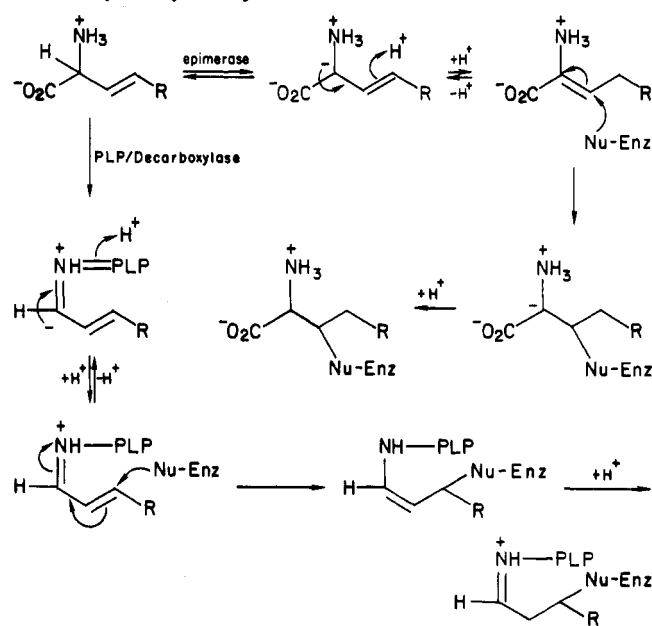
2. Inducible Chemical Reactivity of the Molecules. Decarboxylases and epimerases are known to function by related mechanisms¹⁴ that involve a carbanionic intermediate (Scheme II) and as a consequence could be inacti-

Scheme I.^a Biosynthesis of Lysine from L,L-DAP



^a Reagents: i, L,L-DAP epimerase; ii, *meso*-DAP decarboxylase.

Scheme II. Suggested Irreversible Inhibition of a PLP-Independent Epimerase by a Vinylic Amino Acid and Proposed Irreversible Inhibition¹⁴ of a PLP-Dependant Decarboxylase by a Vinylic Amino Acid



vated by amino acid derivatives possessing the same potential reactive moieties. For instance, γ -ethynyl-GABA

[†] Centre de Recherche Delalande.

[†] Laboratoire de Bioorganique et de Biotechnologies.

[§] Present address: I.C.I.-Pharma, Centre de Recherches, Zise, BP 401, 51064 Reims Cedex, France.

^{||} Present address: Centre de Recherches Roussel-Uclaf, 102 route de Noisy, 93230 Romainville, France.

- (1) Gottschalk, G. "Bacterial Metabolism"; Springer-Verlag: New York, 1979.
- (2) Ghuyssen, J. M. "Topics in Antibiotic Chemistry"; Sammes, P. G., Ed.; Ellis Horwood Ltd.: Chichester, U.K., 1980; Vol. 5.
- (3) Weiss, N. *Rev. Inst. Pasteur Lyon* 1981, 14, 3, 53.
- (4) Asada, Y.; Tanizawa, K.; Sawada, S.; Suzuki, T.; Misono, H.; Soda, K. *Biochemistry* 1981, 20, 6881.
- (5) Gale, E. F.; Cundliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. "The Molecular Basis of Antibiotic Action", 2nd ed.; Wiley: London 1981.
- (6) Lambert, M. P.; Neuhaus, F. C. *J. Bacteriol.* 1972, 110, 978.

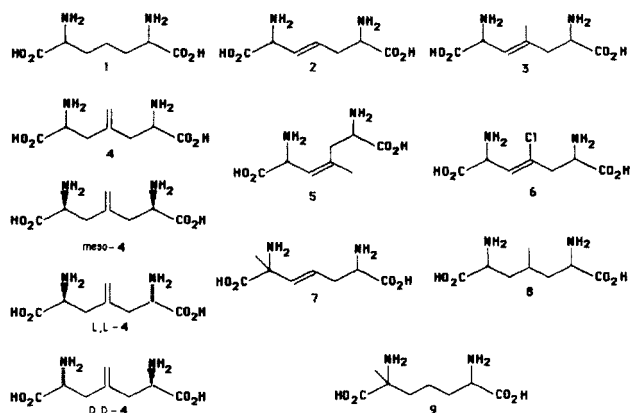
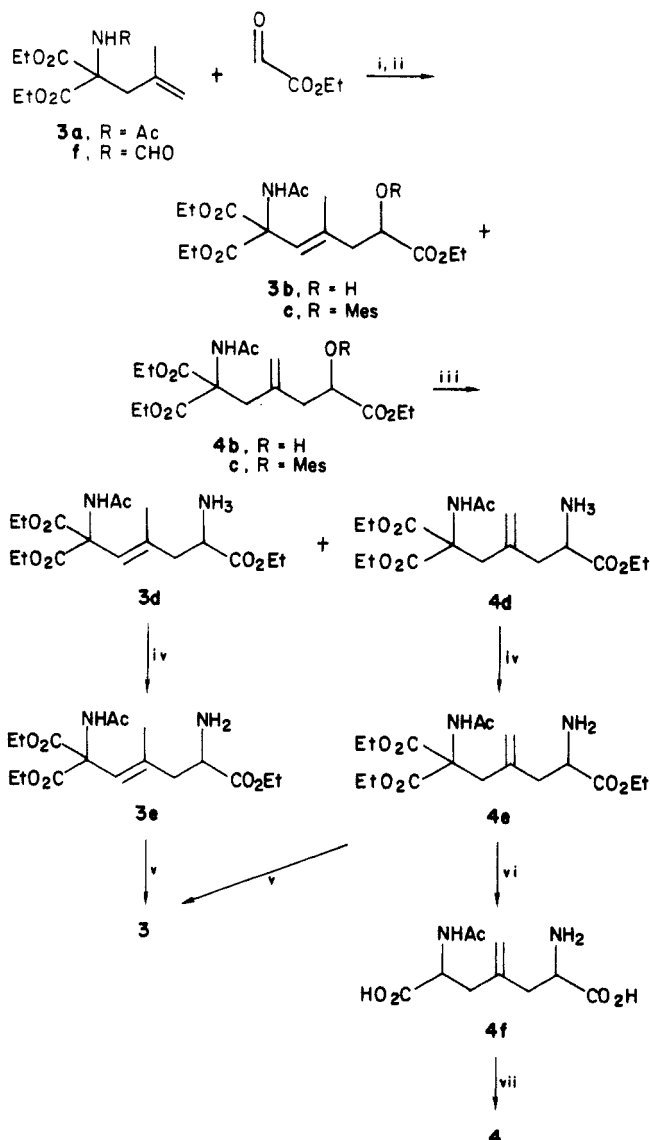
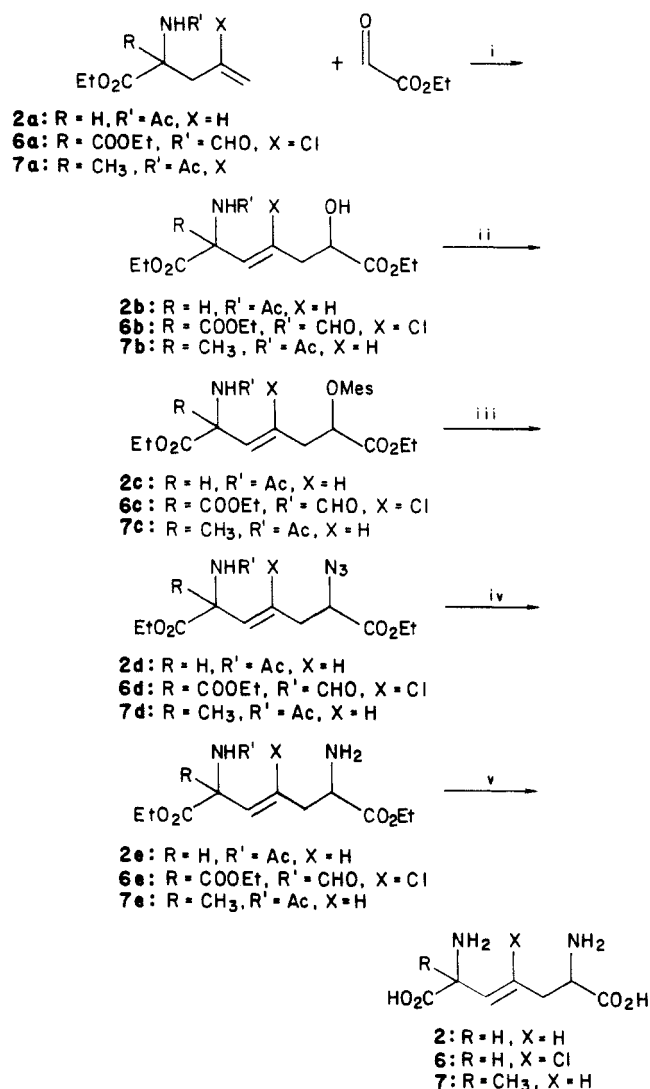


Figure 1.

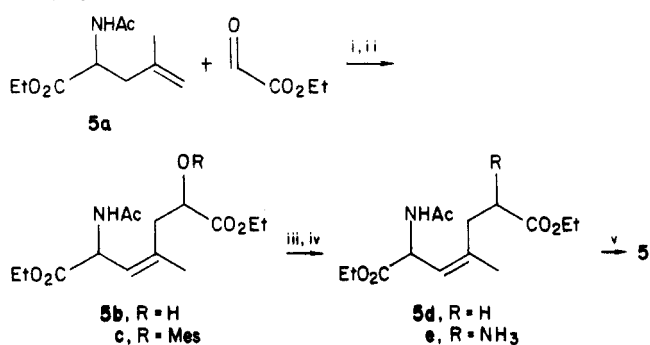
Scheme III^a

^a Reagents: i, FeCl_3 (3 equiv), CH_2Cl_2 , -10°C , 1 h; ii, MesCl , pyridine, 4°C , 16 h; iii, NaN_3 , DMF, room temperature, 16 h; iv, H_2 , Lindlar cat., EtOH , 16 h; v, 6 N HCl , reflux, 16 h; vi, (1) KOH , 80°C , 1 h, (2) $\text{IRA } 120 \text{ H}^+$, (3) 100°C , 0.5 h; vii, 2.5 N NaOH , 100°C , 20 h.

is a known suicide substrate of glutamate decarboxylase and GABA transaminase,¹⁵ two successive PLP-dependent

Scheme IV^a

^a Reagents given in Scheme III.

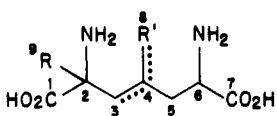
Scheme V^a

^a Reagents given in Scheme III.

enzymes in the GABA pathway. Similarly, unsaturated analogues of natural amino acids, such as 2-methyl-3,4-

(7) Allen, J. G.; Atherton, F. R.; Hall, M. J.; Hassal, C. H.; Holmes, S. W.; Lambert, R. W.; Nisbet, L. J.; Ringrose, P. S. *Antimicrob. Agent Chemother.* 1979, 15, 684.

(8) Williams, D. H.; Rajananda, V.; Williamson, M. P.; Bojen, G. "Topics in Antibiotic Chemistry"; Sammes, P. G., Ed.; Ellis Horwood Ltd.: Chichester, U.K., 1980; Vol. 5.
 (9) Johnston, M.; Jankowski, D.; Marcotte, P.; Tanaka, H.; Esaki, N.; Soda, K.; Walsh, C. *Biochemistry* 1979, 18, 4690.
 (10) Gershon, H.; Meek, J.; Fittmeier, K. *J. Am. Chem. Soc.* 1949, 71, 3573. Cheung, K. S.; Wasserman, S. A.; Dudek, E.; Lerner, S. A.; Johnston, M. *J. Med. Chem.* 1983, 26, 1733.
 (11) Relyen, N.; Rando, R. R. *Biochem. Biophys. Res. Commun.* 1975, 67, 392.

Table I. ^{13}C NMR Chemical Shifts of the DAP Analogues 1-9 (Solvent D_2O , HCl)^a


no.	C-1/C-7	C-2	C-3	C-4	C-5	C-6	C-8	C-9
1	173.1	53.8	30.2	21.1	30.2	53.8		
2	170.7	55.1	133.4	126.4	33.3	52.8		
	171.5							
3	171.5	51.9	120.7	142.0	40.9	51.9	16.7	
	172.1			142.2				
4	172.6	53.0	36.8	137.6	36.8	53.0	121.0	
							121.7	
5	173.7	53.5 ^b	122.3	140.4	34.7	53.3 ^b	22.5	
	175.1							
6	171.7	53.3 ^b	123.6	137.9	40.5	52.5 ^b		
	172.6		123.7	138.2	41.0	52.7 ^b		
7	171.5	60.6	131.9	128.9	33.4	52.9		21.9
	173.0							
8 ^c	180.2	54.4	41.3	27.4	41.3	54.4	19.0	
	181.1		42.1		42.1		20.6	
9	172.4	60.7	36.8	19.9	30.3	53.3		22.3
	174.4							
(D,D + L,L)-4 ^c	180.0	54.8	41.0	142.6	41.0	54.8	117.0	
meso-4 ^c	180.0	54.8	40.3	141.8	40.3	54.8	117.1	

^a Structural formulas given in Figure 1. ^b Close values may be exchanged. ^c Solvent D_2O + NH_4OH .

didehydroglutamic acid^{16a} or (*E*)- and (*Z*)-4,5-didehydrolysines,^{16b} are suicide substrates of their parent amino acid decarboxylase or transaminase.

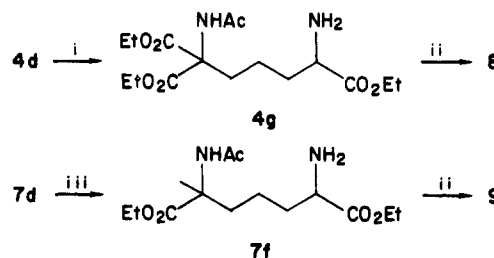
Since *meso*-DAP decarboxylase is a PLP-dependent enzyme⁴ and L,L-DAP epimerase a proline-like PLP-independent racemase,¹⁷ the structures we have designed incorporate the necessary features to be highly activated irreversible inhibitors of both enzymes, as shown in Scheme II.

In the present paper we describe the synthesis of dihydro analogues of 2,6-diaminopimelic acid, their antibacterial activities, and their influence on the enzymatic decarboxylation of *meso*-DAP.

Chemistry

The synthesis of the DAP analogues is depicted in Schemes III-V. The DAP skeleton was formed in one step concomitant with the desired unsaturation by an intermolecular ene reaction between an allylic amino acid and ethyl glyoxylate.¹⁸ The allylic alcohols **2b-7b** thus obtained were mesylated and the mesylates **2c-7c** were treated with sodium azide in DMF to give the azido derivatives **2d-7d**, which were isolated and purified. Chemospecific reduction of **3d-6d** with hydrogen in the presence of Lindlar catalyst at atmospheric pressure gave the amines **3e-6e**. Under these reaction conditions, **2d** and

Scheme VI^a



^a Reagents: i, EtOH , AcOH , H_2 , Pd/C , 10 atm, 24 h; ii, 6 N HCl , reflux, 16 h; iii, EtOH , H_2 , Pd/C , 1 atm, 16 h.

7d gave the saturated amines, so **2d** was reduced to **2e** with triphenylphosphine in THF¹⁹ and **7d** to **7e** with H_2 /Lindlar catalyst/quinoline.²⁰

Treatment of the amines **2e-3e** and **5e-7e** in refluxing 6 N HCl gave the DAP analogues **2-3** and **5-7**, respectively. The amine **4e** was treated with aqueous sodium hydroxide and then decarboxylated to give the acetamido derivative **4f**, which was deacylated by refluxing in 2.5 N aqueous sodium hydroxide. Refluxing of **4e** in 6 N HCl gave rise to the more thermodynamically stable olefin **3**.

The saturated DAP analogues **8** and **9** were obtained by reduction of the azido derivatives **4d** and **7d**, followed by treatment of the saturated amines **4g** and **7g** with refluxing 6 N HCl (Scheme VI).

The antibacterial properties of **4** justified the separation of its stereoisomers. Thus, *meso*-, L,L-, and D,D-**4** were prepared as shown in Scheme VII. The amino derivative **4i** was synthesized from diethyl methallylformamidomalonate **3f** as described in Scheme III for the synthesis of **4e** and treated with $(\text{Boc})_2\text{O}$ in methylene chloride in the presence of triethylamine. The *N*-formyl *N*-Boc derivative **4j** was deethoxycarbonylated by Krapcho's method, and the two diastereoisomers **4k** and **4l** were separated by silica gel column chromatography. Deprotection of the enantiomeric mixture **4l** provided a single com-

(12) Bey, P.; Gerhart, F.; Van Dorsselaer, V.; Danzin, C. *J. Med. Chem.* **1983**, *26*, 1551.

(13) Metcalf, B. W.; Bey, P.; Danzin, C.; Jung, M. J.; Casara, P.; Vevert, J. P. *J. Am. Chem. Soc.* **1978**, *100*, 2551. Casara, P.; Danzin, C.; Metcalf, B. W.; Jung, M. J. *J. Chem. Soc., Chem. Commun.* **1982**, 1190.

(14) Walsh, C. "Enzymatic Reaction Mechanisms"; W. H. Freeman: San Francisco, 1979.

(15) Metcalf, B.; Sjoerdsma, A. S. *Dev. Biochemistry* vol. 6. Drug Action and Design: Mechanism-Based Enzyme Inhibitors. Kalman, T. I. Ed. Elsevier/North Holland (1979).

(16) (a) Chrystal, E.; Bey, P.; Rando, R. R. *J. Neurochem.* **1979**, *32*, 1501. (b) Shannon, P.; Marcotte, P.; Coppersmith, S.; Walsh, C. *J. Am. Chem. Soc.* **1975**, *97*, 3917.

(17) Wiseman, J. S.; Nichols, J. S. *J. Biol. Chem.* **1984**, *259*, 8907.

(18) Agouridas, C.; Girodeau, J. M.; Pineau, R. *Tetrahedron Lett.* **1985**, *26*, 3115. Delalande, S. A. French Patent 8409634-20.06, 1984.

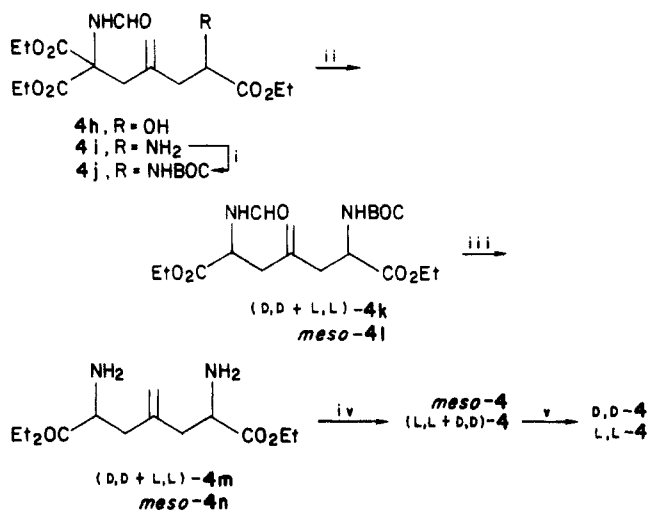
(19) Vaultier, M.; Knouzi, N.; Carrie, R. *Tetrahedron Lett.* **1983**, *24*, 763.

(20) Corey, E. J.; Nicolaou, C.; Balanson, R. D.; Machida, Y. *Synthesis* **1975**, 590.

Table II. Inhibition of Bacterial Growth and of *meso*-DAP Decarboxylase by the DAP Analogues 2-9

no.	microbiology ^a					enzymology ^b			
	1	2	5	MIC ^c	MIC ^d	1	2	5	10
2	0	0	0			65	77		
3	0	21	50				20	50	
4	90	90	95	2	4	5		15	30
<i>meso</i> -4				2	4	8		22	30
(D,D + L,L)-4				2	4	5		10	20
(-)-4				4	4				
(+)-4				6	6				
5	0	0	55			17	33	54	73
6			50	>128		18		53	
7		0	0			0	0		
8		0	0			12		43	55
9	38	74	90	512		8	18	35	

^a Percent of inhibition of bacterial growth (*E. coli* ATCC 9637) in liquid medium at 1, 2, and 5 mmol/L of the amino acids. MIC in micrograms/milliliter. ^b Percent of inhibition of DAP decarboxylase at 1, 2, 5, and 10 mmol/L of the amino acids, 1.05 mmol/L of labeled *meso*-DAP, and 32 μ mol/L of PLP. ^c *E. coli* ATCC 9637. ^d *Pseudomonas aeruginosa* A22.

Scheme VII^a

^a Reagents: i, (Boc)₂O, CH₂Cl₂, NEt₃; ii, NaCl, H₂O, Me₂SO, 160 °C, 2 h; iii, EtOH, HCl, 70 °C, 0.75 h; iv, 1 N NaOH, 20 °C, 1 h; v, cellulose.

pound (cellulose TLC): *meso*-4. Deprotection of the other enantiomeric mixture 4k gave a mixture of enantiomers, which were separated by cellulose preparative chromatography. The absolute configuration of these two enantiomers was not determined.

Results and Discussion

Enzymology. Inhibition of *meso*-DAP decarboxylase by the compounds prepared above has been studied; the results are shown in Table II. *trans*-3,4-Didehydro-DAP 2 is the most potent inhibitor of *meso*-DAP decarboxylase, and as 2 is a mixture of four stereoisomers, it is expected that one of them would be a very potent inhibitor. Interestingly it is known that *meso*-DAP decarboxylase of *Escherichia coli* has a very high stereospecificity for the *meso* isomer of DAP.^{4,21}

Small structural variations of 2 give less potent inhibitors (3 and 6) or compounds devoid of inhibitory activity (7). These results are in accordance with the suggested high structural requirements of *meso*-DAP decarboxylases of various strains.^{21,22} Although, surprisingly, the *E* and *Z* isomers 3 and 5 appear equally active.

α -Methyl amino acids are known to be generally good inhibitors of their respective parent amino acid decarboxylases and accordingly α -methyl-DAP 9 was tested

Table III. Minimum Inhibitory Concentration^a for 4 Compared to Reference Antibacterials

bacterial species	4	D-cycloserine	foscymycin	ampicilline
<i>E. coli</i> ATCC 9637	2	0.25	16	5
<i>Ps. aeruginosa</i> A22	4	128	32	512

^a Solid medium; micrograms/milliliter.

but appeared to be a poor inhibitor of *meso*-DAP decarboxylase. Similarly, γ -methyl-DAP 8 and its rigid analogue γ -methylene-DAP 4 also showed weak inhibition.

The most potent compound 2 behaves as a reversible, competitive inhibitor. Its *K_i* was determined (*K_i* = 180 \pm 10 μ M) and shows that 2 has 4 times the affinity of the substrate for the decarboxylase (*K_m* = 800 \pm 50 μ M). It was also shown that 2 is not an enzyme-activated irreversible inhibitor since incubation of *meso*-DAP decarboxylase with 2 caused no inactivation of the enzyme. In fact, none of the compounds containing a vinylic functionality in the carbon chain were irreversible inhibitors. Additionally, the lack of inhibitory activity of 7 and the low activity of 9 show that introduction of a substituent in the α position leads to compounds having very low affinity for *meso*-DAP decarboxylase. Thus, the introduction of the activatable functional group in the α position will probably not provide a suicide substrate.

These results suggest that the design of a suicide substrate of *meso*-DAP decarboxylase presents a particularly hard challenge. It is therefore suggested that the only possible remaining approach could be the design of suicide inhibitors based on the microreversibility principle.¹⁵

Bacteriology. As shown in Table II, the most powerful inhibitor of *meso*-DAP decarboxylase 2 shows no antibacterial activity. On the other hand, 4, which is a very weak inhibitor of the enzyme, shows substantial antibacterial activity against Gram-negative strains²³ (Tables II-IV). Other derivatives were inactive (7 and 8) or had weak antibacterial activity (3, 5, 6, and 9). Some analogues of DAP have already been described, and their potency as antibacterials was tested: none of them showed significant antimicrobial activity.²⁴ The isomers of the mixture 4 (*meso*; L,L; D,D) were separated and they individually showed identical antibacterial activity with that of the original mixture. When amino acids were added to the growth medium, the antibacterial activity of 4 was lowered (compare Tables III and IV, entries 8 and 9).

(21) White, P. J.; Kelly, B. J. *Biochem.* 1965, 96, 75.

(22) Lakshman, M.; Shenoy, B. C.; Raghavendra Rao, M. R. J. *Biosci.* 1981, 3, 1981.

(23) Gram-positive strains (e.g., *Staphylococcus*, *Streptococcus*) do not grow on the Davis and Mingioli's growth medium²⁹ used.

(24) Cavalleri, B.; Volpe, G.; Sartori, G.; Garniti, G.; White, R. J. *Farmaco, Ed. Sci.* 1974, 29, 257 and references therein.

Table IV. Minimum Inhibitory Concentration^a for 4 and Its Separated Diastereoisomers Compared to D-Cycloserine (DCS)

entry	bacterial species	4	meso-4	(D,D + L,L)-4	DCS
1	<i>Citrobacter freundii</i> 55.13	16	32	8	2
2	<i>Proteus rettgeri</i> 53.100	128	64	64	128
3	<i>Serratia marcescens</i> A 174	2	4	4	128
4	<i>Serratia liquefaciens</i> 52.56	2	2	4	8
5	<i>Moraxella glucidolytica</i> 52.90	16	16	8	128
6	<i>Enterobacter cloacea</i> 60.22	16	16	16	2
7	<i>Providencia alcalifaciens</i> 58.62	128	128	32	8
8	<i>Klebsiella pneumoniae</i> 53.153	32	16	16	8
9	<i>Pseudomonas aeruginosa</i> A22	16	16	32	64
10	<i>Escherichia coli</i> ATCC 9637	32	32	16	0.5
11	<i>Escherichia coli</i> UB 1005	2	2	8	32
12	<i>Escherichia coli</i> DC2	32	4	8	128

^a Solid medium supplemented with Asp, Cys, Hist, Leu, Met, Pro, Tyr, and Val (100 mg/L each);³⁰ micrograms/milliliter.

The compounds were originally designed as meso-DAP and lysine biosynthesis inhibitors; thus, the antibacterial activity of 4 was tested in the presence of these metabolites. Additionally, we wanted to know whether 4 was transported in the same way as DAP through the cystine transport system.²⁵ The results are gathered in Table V and show that L-lysine, meso-DAP, and L-cystine reverse the antibacterial activity of 4. However, although ornithine uses the same transport system as lysine,²⁶ it does not reverse the activity of 4. At this point we thought that the target was probably the L-lysine metabolic pathway and that 4 was transported through the L-cystine system.

To support the first hypothesis an *E. coli* W7 Lys, DAP mutant was grown in the presence of 4, and the results are shown in Table VI. From the table it is seen that 4 is recognized as a DAP analogue and is probably incorporated into the *E. coli* W7 peptidoglycan. Is the target enzyme present in this strain or does lysine reverse the antibacterial action of 4 in the experiment? This strain can produce neither meso-DAP nor lysine²⁷ and has probably neither of the two enzymes involved in the biosynthesis of lysine shown in Scheme I.

Conclusion

We have tried to design suicide inhibitors of the two last enzymes involved in the lysine pathway. It appears now that DAP decarboxylase was not the preferred choice for such an approach because of its high structural requirements (the epimerase was at the time the more difficult enzyme to work with). Unexpectedly, γ -methylene-DAP 4 showed potent antibacterial properties, and from this preliminary study we suggest 4 inhibits the lysine pathway and L,L-DAP epimerase could be the target enzyme. This idea is strengthened by the fact that the stereoisomers of 4 have the same antibacterial potency.

Experimental Section

Melting points were taken with a Reichert Koffler apparatus and were not corrected. The DAP analogues (zwitterions or hydrochlorides) decomposed on heating. Consequently, no reliable

melting points were observed. Infrared spectra were recorded on a Perkin-Elmer 197 spectrophotometer (KBr pellets). The optical rotations were measured with a Perkin-Elmer 141 polarimeter. Microanalyses were performed with a Perkin-Elmer 240 elemental analyzer. The NMR spectra were recorded with a Varian T60 spectrometer (¹H) and a Bruker WP80 spectrometer (¹³C). The chemical shifts are reported in ppm downfield from Me₄Si in CDCl₃ and CD₃OD or DSS in D₂O. Dioxane (67.4 ppm) was used as internal reference for ¹³C spectra in D₂O. For the carbon numbering, see Table I. Thin-layer chromatograms were made with use of Merck silica gel 60 F254 plates unless otherwise stated. Visualization was with ninhydrin or KMnO₄ solution. Preparative column chromatographies were performed with Merck silica gel 60 (230–400 mesh) unless otherwise stated. The organic extracts were dried over MgSO₄ and evaporated under reduced pressure with a rotary evaporator. Analytical samples were prepared, when necessary, by silica gel column chromatography.

Diethyl (E)-2-Acetamido-6-azido-3-heptene-1,7-dioate (2d). To a 0.25 M solution of crude alcohol 2b¹⁸ in dry pyridine was added mesyl chloride (1.2 equiv) at 0 °C. The flask was stoppered and kept at 4 °C for 15 h. The solution was poured in 4 N HCl and ice and extracted with methylene chloride. The organic extracts were washed with 2 N HCl and brine then dried. Evaporation of the solvent at room temperature yielded 2c (mp 118–121 °C). Compound 2c was dissolved in dry DMF (0.25 M solution) and sodium azide (1.2 equiv) was added at room temperature. The solution was stirred 15 h at room temperature and evaporated to dryness at 40 °C. The residue was dissolved in methylene chloride and washed with brine. After drying and evaporation of the organic layer, the residue was purified by column chromatography (70:30 heptane–ethyl acetate). Yellowish oil, yield 80% from 2a. TLC (60:40 ethyl acetate–heptane) *R*_f 0.44. IR 2100 cm⁻¹. ¹H NMR (CDCl₃) δ 1.25 (2 t, 6 H, ethoxy CH₃), 2 (s, 3 H, acetyl CH₃), 2.55 (m, 2 H, H-5), 3.9 (t, 1 H, H-6), 4.2 (2 q, 4 H, CH₂O), 5.1 (br, 1 H, H-2), 5.7 (m, 2 H, H-3 + H-4), 6.6 (br d, 1 H, NH). Anal. (C₁₃H₁₉N₄O₅·0.3H₂O) C, H, N.

Diethyl (E)-2-Acetamido-6-amino-3-heptene-1,7-dioate (2e). Compound 2d (1.25 g, 4.16 mmol) in THF (15 mL) was treated with triphenylphosphine (1.31 g, 5 mmol) at room temperature. The solution was stirred for 16 h at room temperature and H₂O (0.23 mL, 12.5 mmol) was added. The solution was stirred 24 h and evaporated to dryness. The residue was dissolved in 2 N HCl. The aqueous solution was washed with methylene chloride and evaporated to dryness to give 2e·HCl (1.2 g, 95%). The compound was used without further purification in the following reactions. TLC (ethanol), *R*_f 0.7. ¹H NMR (CD₃OD) δ 1.3 (2 t, 6 H, ethoxy CH₃), 2.05 (s, 3 H, acetyl CH₃), 2.75 (t, 2 H, H-5), 4.25 (m, 5 H, CH₂O + H-6), 4.95 (H-1 + water), 5.8 (m, 2 H, H-3 + H-4). Anal. (C₁₃H₂₂N₂O₅·HCl) C, H, N.

(E)-2,6-Diamino-3-heptene-1,7-dioic Acid (2). Compound 2e (80 mg, 0.25 mmol) was refluxed in 6 N HCl for 16 h, and the solution was evaporated to dryness. The residue was dissolved in ethanol and the resulting solution evaporated to dryness; this was repeated four times. A solution of the residue in ethanol was then treated with 2 equiv of propylene oxide. A solid was formed, which was collected and dried in vacuo. Yield 51 mg (75%). TLC (4:2:2 butanol–acetic acid–water), *R*_f 0.27. ¹H NMR (D₂O + DCl) δ 3.05 (t, 2 H, H-5), 4.55 (t, 1 H, H-6), 5.1 (H-2 + water), 6.3 (m, 2 H, H-3 + H-4). Anal. (C₇H₁₂N₂O₄·H₂O) C, H, N.

Diethyl (Z)-2-(Ethoxycarbonyl)-2-formamido-4-chloro-6-azido-3-heptene-1,7-dioate (6d). Compound 6d was synthesized from 6b¹⁸ (6.4 g, 23 mmol) by the same procedure used for the synthesis of 2d. Yield 8 g, oil (85% from 6a). TLC (60:40 ethyl acetate–heptane), *R*_f 0.45. ¹H NMR (CDCl₃) δ 1.25 (m, 9 H, ethoxy CH₃), 2.8 (m, 2 H, H-5), 4.25 (m, 7 H, CH₂O + H-6), 6.85 (s, 1 H, H-3), 7.45 (br, 1 H, NH), 8.05 (d, 1 H, CHO). Anal. (C₁₅H₂₁ClN₄O₇) C, H, N.

Diethyl (Z)-2-(Ethoxycarbonyl)-2-formamido-4-chloro-6-amino-3-heptene-1,7-dioate (6e). A solution of 6d (5.1 g, 12.7 mmol) in ethanol was hydrogenated at room temperature and atmospheric pressure in the presence of Lindlar catalyst (0.5 g). The solution was vigorously stirred for 2 h. The catalyst was removed by filtration and washed with ethanol and the solution evaporated to dryness, providing 6e, oil (4.75 g, 100%). The compound was used without further purification in the following reaction. TLC (70:30 ethyl acetate–ethanol), *R*_f 0.45. ¹H NMR

(25) Leive, L.; Davis, B. *J. Biol. Chem.* **1965**, *240*, 3685.

(26) Anraku, Y. "Microbiology Series"; Rosen, B. P., Ed.; Marcel Dekker: New York, 1978, Vol. 4, Chapter 5.

(27) Hartmann, R.; Holtje, J. V.; Schwarz, U. *Nature (London)* **1972**, *235*, 426.

Table V. Competition Experiments between 4 and *meso*-DAP, Lys, Orn, and Cystine

	4 + <i>meso</i> -DAP ^a	4 + Lys ^a	4 + Lys ^a	4 + Lys ^b	4 + Orn ^a	4 + cystine ^b
quantity	200 + 95	300 + 100	200 + 200	0.1 + 2.5	30 + 100	0.1 + 20
inhibition ^c	part. reversed	part. reversed	reversed	part. reversed	not reversed	part. reversed

^a Solid medium; values in micrograms. ^b Liquid medium; values in millimoles/liter. ^c Part. = partially.**Table VI.** Growth of *E. coli* W7 in the Presence Lys and *meso*-DAP or 4^a

	bacterial growth
liquid medium + Lys + <i>meso</i> -DAP	+
liquid medium + Lys	-
liquid medium + Lys + 4	+

^a Concentration of Lys, *meso*-DAP, and 4 = 100 μg/mL.

(CDCl₃) δ 1.25 (m, 9 H, ethoxy CH₃), 2.2 (br, 2 H, NH₂), 2.7 (m, 2 H, H-5), 3.7 (m, 1 H, H-6), 4.2 (m, 6 H, CH₂O), 6.8 (s, 1 H, H-3), 7.8 (br, 1 H, NH), 8.05 (s, 1 H, CHO). Anal. (C₁₅H₂₃ClN₂O₇) C, H, N.

(Z)-2,6-Diamino-4-chloro-3-heptene-1,7-dioic Acid (6). Compound **6e** (1.17 g, 3.09 mmol) was dissolved in 4 N HCl, Norit was added, and the solution was refluxed for 5 h. After filtration and evaporation, 6·2H₃O⁺·2Cl⁻ was obtained as a white crystalline compound (660 mg, 65%). TLC (4:2:2 butanol-acetic acid-water), *R_f* 0.22. ¹H NMR (D₂O) δ 3.15 (m, 2 H, H-5), 4.3 (t, 1 H, H-6), 4.95 (d, 1 H, H-2), 6.1 (d, 1 H, H-3). Anal. (C₇H₁₁ClN₂O₄·2H₂O) C, H, N.

Diethyl (E)-2-Acetamido-2-methyl-6-azido-3-heptene-1,7-dioate (7d). Compound **7d** was prepared from crude **7b**¹⁸ (21 g, 57 mmol) by the same procedure used for the synthesis of **2d**. Yield 14.6 g, oil (59% from **7a**). TLC (AcOEt), *R_f* 0.61. IR 2100 cm⁻¹. ¹H NMR (CDCl₃) δ 1.25 (2 t, 6 H, ethoxy CH₃), 1.6 (s, 3 H, 2-CH₃), 2 (s, 3 H, acetyl CH₃), 2.55 (m, 2 H, H-5), 3.8 (t, 1 H, H-6), 4.15 (2 q, 4 H, CH₂O), 5.65 (m, 2 H, H-3 + H-4, *J*_{H3-H4} = 16 Hz), 6.35 (br, 1 H, NH). Anal. (C₁₄H₂₂N₄O₅) C, H, N.

Diethyl (E)-2-Acetamido-2-methyl-6-amino-3-heptene-1,7-dioate (7e). Compound **7d** (1.48 g, 4.5 mmol) was dissolved in ethanol (30 mL). Lindlar catalyst (148 mg) and quinoline (1.48 mL) were added. The mixture was hydrogenated at room temperature and atmospheric pressure with vigorous stirring for 20 h. The solution was filtered and evaporated to dryness. Column chromatography (ethyl acetate, then ethanol) provided **7e**, oil (1 g, 74%). TLC (70:30 ethyl acetate-ethanol), *R_f* 0.4. ¹H NMR (CDCl₃) δ 1.25 (t, 6 H, ethoxy CH₃), 1.6 (s, 3 H, 2-CH₃), 3.2 (br, 2 H, NH₂), 3.45 (m, 2 H, H-5), 3.5 (t, 1 H, H-6), 4.2 (q, 4 H, CH₂O), 5.65 (m, 2 H, H-3 + H-4), 6.8 (br, 1 H, NH). Anal. (C₁₄H₂₄N₂O₅·0.75H₂O) C, H, N.

(E)-2,6-Diamino-2-methyl-3-heptene-1,7-dioic Acid (7). Compound **7e** (1.2 g, 39.5 mmol) was dissolved in 6 N HCl (30 mL) and the solution refluxed for 16 h. The solvent was evaporated, and the residue was dissolved in water and chromatographed on an ion-exchange column (amberlite IRA 120, H⁺, 28–35 mesh). The column was washed with water (1 L) and the product was eluted with 1 N NH₄OH. Evaporation of the fractions containing the product were collected and evaporated to dryness, providing **7**, amorphous solid (400 mg, 50%). TLC (4:2:2 butanol-acetic acid-water), *R_f* 0.23. ¹H NMR (D₂O + DCl) δ 1.55 (s, 3 H, 2-CH₃), 2.65 (m, 2 H, H-5), 3.85 (m, 1 H, H-6), 5.8 (m, 2 H, H-3 + H-4). Anal. (C₈H₁₄N₂O₄·1.25H₂O) C, H, N.

Diethyl (E)-2-(Ethoxycarbonyl)-2-acetamido-4-methyl-6-azido-3-heptene-1,7-dioate (3d) and Diethyl 2-(Ethoxycarbonyl)-2-acetamido-4-methylene-6-azido-1,7-heptanedioate (4d). The mixture of **3d** and **4d** was obtained from a mixture of **3b** and **4b**¹⁸ by the same procedure used for the synthesis of **2d**. Compounds **3d** and **4d** were separated by column chromatography (55:45 ethyl acetate-heptane). Compound **3d** (13% from **3a**): mp 64 °C. IR 2100 cm⁻¹. TLC (60:40 ethyl acetate-heptane), *R_f* 0.37. ¹H NMR (CDCl₃) δ 1.25 (m, 9 H, ethoxy CH₃), 1.65 (s, 3 H, 4-CH₃), 2 (s, 3 H, acetyl CH₃), 2.5 (d, 2 H, H-5), 3.9 (t, 1 H, H-6), 4.2 (q, 6 H, CH₂O), 6.4 (s, 1 H, H-3), 7.1 (s, 1 H, NH). Anal. (C₁₇H₂₆N₄O₇) C, H, N.

Compound **4d** (61.5% from **3a**): mp 63 °C. IR 2100 cm⁻¹. TLC (60:40 ethyl acetate-heptane), *R_f* 0.48. ¹H NMR (CDCl₃) δ 1.25 (m, 9 H, ethoxy CH₃), 2 (s, 3 H, acetyl CH₃), 2.45 (m, 2 H, H-5), 3.15 (s, 2 H, H-3), 3.9 (m, 1 H, H-6), 4.25 (q, 6 H, CH₂O), 5 (d,

2 H, 4-methylene), 6.9 (s, 1 H, NH). Anal. (C₁₇H₂₆N₄O₇) C, H, N.

Diethyl (E)-2-(Ethoxycarbonyl)-2-acetamido-4-methyl-6-amino-3-heptene-1,7-dioate (3e). Compound **3d** (4 g, 10 mmol) was dissolved in ethanol (100 mL). Lindlar catalyst (0.4 g) was added and the mixture was hydrogenated at room temperature and atmospheric pressure with vigorous stirring for 7 h. The solution was filtered and evaporated to dryness. Yield (3.7 g, 100%). TLC (90:5:5 ethyl acetate-ethanol-NH₄OH), *R_f* 0.57. ¹H NMR (D₂O + DCl) δ 1.25 (m, 9 H, ethoxy CH₃), 1.75 (s, 3 H, 4-CH₃), 2.1 (s, 3 H, acetyl CH₃), 2.7 (d, 2 H, H-5), 4.3 (q, 7 H, CH₂O + H-6), 6.2 (br s, 1 H, H-3). Anal. (C₁₇H₂₈N₂O₇·HCl) C, H, N.

(E)-2,6-Diamino-4-methyl-3-heptene-1,7-dioic Acid (3). Compound **3e** (250 mg, 0.61 mmol) was dissolved in 6 N HCl (6 mL) and the solution was refluxed for 16 h. The solution was evaporated to dryness. The residue was dissolved in water and evaporated to dryness; this was repeated three times and provided **3** as an amorphous white solid (130 mg, 78%). TLC (4:2:2 butanol-acetic acid-water), *R_f* 0.24. ¹H NMR (D₂O) δ 1.65 (s, 3 H, 4-CH₃), 2.55 (d, 2 H, H-5), 4 (t, 1 H, H-6), 4.5 (H-2 + water), 5.2 (br 1 H, H-3). Anal. (C₈H₁₄N₂O₄·2HCl·2H₂O) C, H, N.

Diethyl 2-(Ethoxycarbonyl)-2-acetamido-4-methylene-6-amino-1,7-heptanedioate (4e). Compound **4d** (46 g, 0.115 mol) was hydrogenated as described above for the synthesis of **3e**, providing **4e**, oil (33 g, 76%). TLC (90:10 ethyl acetate-ethanol), *R_f* 0.31. ¹H NMR (CDCl₃) δ 1.25 (t, 9 H, ethoxy CH₃), 2 (s, 3 H, acetyl CH₃), 2.55 (br, 4 H, H-5 + NH₂), 3.15 (s with a broad foot, 3 H, H-3 + H-6), 4.25 (q, 6 H, CH₂O), 5 (br, 2 H, 4-methylene), 6.9 (s, 1 H, NH). Anal. (C₁₇H₂₈N₂O₇) C, H, N.

2-Acetamido-4-methylene-6-amino-1,7-heptanedioic Acid (4f). Compound **4e**·HCl (1.34 g, 3.27 mmol) was dissolved in water (20 mL). KOH (1.1 g, 19.6 mmol) in water (20 mL) was added, and the solution was heated at 80 °C for 1 h. The pH of the solution was brought to ~3 at room temperature with Amberlite IRA 120 (28–35 mesh, H⁺ form), and the solution was filtered. The solution was heated at 100 °C for 0.5 h and evaporated to dryness. Compound **4f**, amorphous solid (520 mg, 70%), was obtained. TLC (4:2:2 butanol-acetic acid-water), *R_f* 0.25. ¹H NMR (D₂O) δ 2 (s, 3 H, acetyl CH₃), 1.6 (br, 4 H, H-3 + H-5), 3.95 (m, 1 H, H-6), 4.4 (m, 1 H, H-2), 5.15 (br, 2 H, 4-methylene). Anal. (C₁₂H₂₀N₂O₅) C, H, N.

2,6-Diamino-4-methylene-1,7-heptanedioic Acid (4). Compound **4f** (240 mg, 0.98 mmol) was dissolved in 2.5 N NaOH (25 mL), and the solution was refluxed for 20 h. The solution was evaporated to dryness. The residue was dissolved in the minimum amount of water, and the solution was brought to pH 5.6 with 1 N HCl. The solution was passed through a Dowex 50W X8 (70 mesh, H⁺) ion-exchange column. The column was washed with water (1 L) and eluted with 1 N NH₄OH. The fractions containing the desired compound were collected and evaporated to dryness to provide **4** (150 mg, 76%) as a white amorphous powder. TLC (4:2:2 butanol-acetic acid-water), *R_f* 0.24. ¹H NMR (D₂O + DCl) δ 2.6 (br, 4 H, H-3 + H-5), 3.85 (t, 2 H, H-2 + H-6), 5.15 (s, 2 H, 4-methylene). Anal. (C₈H₁₄N₂O₄·HCl·H₂O) C, H, N.

Diethyl (Z)-2-Acetamido-4-methyl-6-hydroxy-3-heptene-1,7-dioate (5b). Compound **5b** was prepared from **5a** (2 g) as described¹⁸ and separated from its isomers by column chromatography (85:15 methylene chloride-acetone). Yield 1 g, oil (32%). TLC (75:25 methylene chloride-acetone), *R_f* 0.52. ¹H NMR (CDCl₃) δ 1.25 (2 t, 6 H, ethoxy CH₃), 1.8 (s, 3 H, 4-CH₃), 2 (s, 3 H, acetyl CH₃), 2.7 (m, 3 H, H-5 + OH), 4.2 (m, 5 H, CH₂O + H-6), 5.15 (m, 2 H, H-2 + H-3), 6.6 (br, 1 H, NH). Anal. (C₁₄H₂₃NO₆) C, H, N.

Diethyl (Z)-2-Acetamido-4-methyl-6-azido-3-heptene-1,7-dioate (5d). Compound **5d** was obtained from **5b** (5.9 g, 19.6 mmol) by the same procedure used for the synthesis of **2d**. Yield 4.9 g (76%), mp 82 °C. TLC (80:20 ethyl acetate-heptane), *R_f* 0.55. ¹H NMR (CDCl₃) δ 1.25 (2 t, 6 H, ethoxy CH₃), 1.8 (s, 3 H, 4-CH₃), 2 (s, 3 H, acetyl CH₃), 2.75 (d, 2 H, H-5), 4.2 (m, 5 H,

$\text{CH}_2\text{O} + \text{H}-6$), 5.2 (br, 2 H, H-2 + H-3), 6.55 (br, 1 H, NH). Anal. ($\text{C}_{14}\text{H}_{22}\text{N}_4\text{O}_5$) C, H, N.

Diethyl (Z)-2-Acetamido-4-methyl-6-amino-3-heptene-1,7-dioate (5e). Compound **5d** (250 mg, 0.7 mmol) was dissolved in ethanol (5 mL) and Lindlar catalyst was added (25 mg). The mixture was hydrogenated at room temperature and atmospheric pressure for 24 h. The solution was filtered and the catalyst washed with ethanol. The solvent was evaporated to dryness, providing **5e**, oil (230 mg, 100%). TLC (80:20 ethyl acetate-ethanol), R_f 0.23. ^1H NMR (CDCl_3) δ 1.25 (2 d, 6 H, ethoxy CH_3), 1.8 (s, 3 H, 4- CH_3), 2.65 (br, 2 H, H-5), 3.05 (br, 2 H, NH_2), 3.65 (br, 1 H, H-6), 4.2 (q, 4 H, CH_2O), 5.2 (br, 2 H, H-2 + H-3), 6.8 (br, 1 H, NH). Anal. ($\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_5$) C, H, N.

(Z)-2,6-Diamino-4-methyl-3-heptene-1,7-dioic Acid (5). Compound **5e** (230 mg, 0.76 mmol) was dissolved in 6 N HCl (5 mL), and the solution was refluxed for 16 h. The solution was evaporated to dryness. The residue was dissolved in ethanol and the solution evaporated to dryness; this procedure was repeated three times. Then the ethanolic solution was treated with propylene oxide, and a solid formed, which was collected and dried. Yield 140 mg (91%). TLC (4:2:2 butanol-acetic acid-water), R_f 0.23. ^1H NMR ($\text{D}_2\text{O} + \text{DCl}$) δ 2 (s, 3 H, 4- CH_3), 2.9 (d, 2 H, H-5), 4.15 (br, 1 H, H-6), 4.9 (H-2 + water), 5.6 (br, 1 H, H-3). Anal. ($\text{C}_8\text{H}_{14}\text{N}_2\text{O}_4 \cdot 1.33\text{H}_2\text{O}$) C, H, N.

2,6-Diamino-4-methyl-1,7-heptanedioic Acid (8). Compound **4d** (1 g, 2.5 mmol) was dissolved in a 10:1 mixture of ethanol-acetic acid; Pd/C 5% (0.1 g) was added and the mixture was hydrogenated at 10 atm for 24 h. The catalyst was removed and washed with ethanol. The solvent was evaporated and the residue was dissolved in 6 N HCl. The solution was refluxed for 16 h and then evaporated to dryness. The residue was triturated in a 9:1 mixture of ethanol-water. The resulting crystalline compound was collected and dried. Yield 337 mg (66%). TLC (4:2:2 butanol-acetic acid-water), R_f 0.23. ^1H NMR (D_2O) δ 1 (d, 3 H, 4- CH_3), 1.55 (m, 5 H, H-3 + H-4 + H-5), 3.4 (t, 2 H, H-2 + H-6). Anal. ($\text{C}_8\text{H}_{16}\text{N}_2\text{O}_4 \cdot \text{H}_2\text{O}$) C, H, N.

2,6-Diamino-2-methyl-1,7-heptanedioic Acid (9). Compound **7d** (1.11 g, 3.4 mmol) and dissolved in ethanol; Pd/C 5% (0.1 g) was added and the mixture was hydrogenated at room temperature and atmospheric pressure with vigorous stirring for 16 h. The catalyst was removed and washed with ethanol. The solvent was evaporated and the residue was dissolved in 6 N HCl (20 mL). The solution was refluxed for 16 h and then evaporated to dryness. The residue was dissolved in H_2O and lyophilized to provide 9-2HCl, amorphous white solid (750 mg, 72%). TLC (4:2:2 butanol-acetic acid-water), R_f 0.35. ^1H NMR (D_2O) δ 1.65 (s with a broad foot, 5 H, 2- CH_3 + H-4), 1.9 (br, 4 H, H-3 and H-5), 4.1 (br, 1 H, H-6). Anal. ($\text{C}_8\text{H}_{16}\text{N}_2\text{O}_4 \cdot 2\text{HCl} \cdot 1.9\text{H}_2\text{O}$) C, H, N.

Diethyl 2-(Ethoxycarbonyl)-2-formamido-4-methylene-6-amino-1,7-heptanedioate (4i). Compound **4i** was synthesized from **4h**¹⁸ as described above for the synthesis of **4e** from **4b**. Overall yield from diethyl methylallylformamidomalonate (**3f**; 25.8 g, 0.1 mol) 19.6 g, oil (55%). TLC (90:10 ethyl acetate-ethanol), R_f 0.43. ^1H NMR (CDCl_3) δ 1.25 (t, 9 H, ethoxy CH_3), 2.3 (br, 4 H, H-5 + NH_2), 3.15 (s, 2 H, H-3), 3.6 (m, 1 H, H-6), 4.2 (m, 6 H, CH_2O), 4.95 (br, 2 H, 4-methylene), 7.35 (br, 1 H, NH), 8.15 (s, 1 H, CHO). Anal. ($\text{C}_{16}\text{H}_{26}\text{N}_2\text{O}_7$) C, H, N.

Diethyl 2-(Ethoxycarbonyl)-2-formamido-4-methylene-6-[(*tert*-butoxycarbonyl)amino]-1,7-heptanedioate (4j). Crude **4i** (14.3 g, 40 mmol) was dissolved in methylene chloride (200 mL). Triethylamine (0.28 mL, 2 mmol) and $(\text{Boc})_2\text{O}$ (9.6 g, 44 mmol) were added. The solution was kept at room temperature for 18 h and then evaporated to dryness, and the resulting compound was used without purification in the next reaction. TLC (10:10 ethyl acetate-ethanol), R_f 0.9. ^1H NMR (CDCl_3) δ 1.25 (m, 18 H, ethoxy and Boc CH_3), 2.35 (br, 2 H, H-5), 3.15 (s, 2 H, H-3), 4.25 (m, 7 H, CH_2O + H-6), 4.95 (br, 3 H, 4-methylene + NHBoc), 7.15 (br, 1 H, formyl NH), 8.15 (s, 1 H, CHO). Anal. ($\text{C}_{21}\text{H}_{34}\text{N}_2\text{O}_9$) C, H, N.

Diethyl 2-Formamido-4-methylene-6-[(*tert*-butoxycarbonyl)amino]-1,7-heptanedioate (4k and 4l). Crude **4j** (18.3 g, 40 mmol) was dissolved in Me_2SO (45 mL), NaCl (2.34 g, 40 mmol), and H_2O (1.44 mL, 80 mmol) were added. The solution was heated at 160 °C for 8 h. The resulting black solution was evaporated to dryness, the residue was dissolved in ethyl acetate (300 mL), and the solution was dried (Na_2SO_4). After filtration

and evaporation of the solvent, the residue was chromatographed (50:50 ethyl acetate-heptane), providing the two diastereoisomers **4k** and **4l**. Compound **4k** (2.4 g, 15.5%): TLC (70:30 ethyl acetate-heptane), R_f 0.46. ^1H NMR (CDCl_3) δ 1.25 (t, 6 H, ethoxy CH_3), 1.4 (s, 9 H, Boc CH_3), 2.05–2.65 (br, 4 H, H-3 + H-5), 4.2 (br q, 5 H, CH_2O + H-6), 4.6 (br, 1 H, H-2), 4.9 (br, 2 H, 4-methylene), 5.2 (br, NHBoc), 6.8 (br, 1 H, formyl NH), 8.1 (br, 1 H, CHO). Anal. ($\text{C}_{18}\text{H}_{30}\text{N}_2\text{O}_7$) C, H, N. Compound **4l** (2 g, 13%): TLC (70:30 ethyl acetate-heptane), R_f 0.38. ^1H NMR (CDCl_3) δ 1.25 (t, 6 H, ethoxy CH_3), 1.4 (s, 9 H, Boc CH_3), 2.5 (br t, 4 H, H-3 + H-5), 4.2 (br q, 5 H, CH_2O + H-6), 4.7 (br, 1 H, H-2), 4.95 (br s, 2 H, 4-methylene), 5.1 (br, 1 H, NHBoc), 6.5 (br, 1 H, formyl NH), 8.1 (br, 1 H, CHO). Anal. ($\text{C}_{18}\text{H}_{30}\text{N}_2\text{O}_7$) C, H, N.

Diethyl (*D,D* + *L,L*)-2,6-Diamino-4-methylene-1,7-heptanedioate (4m). Compound **4k** (2.36 g, 6.1 mmol) was dissolved in 1.9 N ethanolic HCl (45 mL) and the solution was refluxed for 0.75 h. The solvent was removed. The residue was dissolved in ethanol and the solution was evaporated to dryness; this procedure was repeated three times. Then the residue was triturated in diethyl ether containing a few drops of ethanol. The resulting crystalline compound was collected and dried, providing **4m** (1.2 g, 62%). TLC (99:1 methanol- NH_4OH), R_f 0.43. ^1H NMR (D_2O) δ 1.35 (t, 6 H, ethoxy CH_3), 2.8 (m, 4 H, H-3 + H-5), 4.4 (m, 6 H, CH_2O + H-2 + H-6), 5.4 (s, 2 H, 4-methylene). Anal. ($\text{C}_{12}\text{H}_{22}\text{N}_2\text{O}_4 \cdot 2\text{HCl}$) C, H, N.

Diethyl meso-2,6-Diamino-4-methylene-1,7-heptanedioate (4n). Compound **4n** was obtained from **4l** (1.95 g, 5 mmol) as described above for the preparation of **4m** from **4k**. Yield 1.3 g (75%). TLC (99:1 methanol- NH_4OH), R_f 0.43. ^1H NMR (D_2O) δ 1.35 (t, 6 H, ethoxy CH_3), 2.8 (d, 4 H, H-3 + H-5), 4.4 (m, 6 H, CH_2O + H-2 + H-6), 5.35 (s, 2 H, 4-methylene). Anal. ($\text{C}_{12}\text{H}_{22}\text{N}_2\text{O}_4 \cdot 2\text{HCl} \cdot 0.75\text{H}_2\text{O}$) C, H, N.

(*D,D* + *L,L*)-2,6-Diamino-4-methylene-1,7-heptanedioic Acid ((*D,D* + *L,L*)-4). Compound **4m-2HCl** (993 mg, 3 mmol) was dissolved in water (10 mL) and 10 N NaOH (1.3 mL, 13 mmol) was added. The solution was kept at room temperature for 1 h and the pH was brought to 4.5 with 1 N HCl. The solution was subjected to ion-exchange chromatography (Amberlite IR-120, H^+). The column was washed with water (400 mL) and eluted with 0.5 N NH_4OH . The fractions containing the desired compound were collected and evaporated to dryness. A solid was obtained, which was washed with hot 50:50 aqueous ethanol and dried. Yield 320 mg (51%). TLC (cellulose, Merck Art. 5552, 80:17.5:10:2.5 methanol-water-pyridine-12 N HCl),²⁸ R_f 0.27 and 0.19. 80-MHz ^1H NMR ($\text{D}_2\text{O} + \text{DCl}$) δ 2.65 (m, 4 H, H-3 + H-5), 3.87 (d of d, 2 H, H-2 + H-6), 5.2 (s, 2 H, 4-methylene). Anal. (*D,D* + *L,L*) ($\text{C}_8\text{H}_{14}\text{N}_2\text{O}_4 \cdot 0.25\text{H}_2\text{O}$) C, H, N.

(-)-2,6-Diamino-4-methylene-1,7-heptanedioic Acid ((-)-4) and (+)-2,6-Diamino-4-methylene-1,7-heptanedioic Acid ((+)-4). (*D,D* + *L,L*)-4-2HCl (38 mg) in the minimum amount of water was spotted on a cellulose preparative plate (Merck Art. 15275). The PLC plate was eluted with 80:17.5:10:2.5 methanol-water-pyridine-12 N HCl. The position of the two isomers was visualized with ninhydrin on both edges of the plate, and the middle portion of each of the two strips was recovered. Each of the two fractions of cellulose was extracted with 1 N HCl, providing crude (-)-4 and (+)-4. The two enantiomers were treated individually a second time by PLC as described above, and the cellulose extracts were chromatographed on Amberlite IR-120 (H^+). Washing with water and elution with 0.5 N NH_4OH and then evaporation of the solvent provided the two enantiomers, which were dissolved in 0.1 N HCl and lyophilized. (-)-4-2HCl (3.5 mg) and (+)-4-2HCl (3.2 mg) were obtained. TLC (cellulose, Merck Art. 5552, 80:17.5:10:2.5 methanol-water-pyridine-12 N HCl), R_f (-)-4 0.27, (+)-4 0.19.

$[\alpha]_D^{20}$ (-)-4-2HCl -18° (c 0.2, H_2O), (+)-4-2HCl +14° (c 0.17, H_2O). ^1H NMR (D_2O), same as the enantiomeric mixture.

meso-2,6-Diamino-4-methylene-1,7-heptanedioic Acid (meso-4). meso-4 was synthesized from **4n-2HCl** (993 mg, 3 mmol) as described above for the preparation of the *D,D* + *L,L* mixture. Yield 254 mg (41%). TLC (cellulose, Merck Art. 5552, 80:17.5:10:2.5 methanol-water-pyridine-12 N HCl), R_f 0.23. 80-MHz ^1H NMR ($\text{D}_2\text{O} + \text{DCl}$) δ 2.65 (m, 4 H, H-3 + H-5), 3.87 (d of d, 2 H, H-2 + H-6), 5.2 (s, 2 H, 4-methylene). Anal. ($\text{C}_8\text{H}_{14}\text{N}_2\text{O}_4 \cdot 0.25\text{H}_2\text{O}$) C, H, N.

Biological Studies. Organisms were obtained either from the American Type Culture Collection (ATCC) or from the Pasteur Institut Collection. *E. coli* W7 Lys, DAP mutant was kindly provided by Dr. J. Van Heijenoort, Institut de Biochimie, Orsay, France. The growth inhibition experiments were performed in the Davis and Mingioli's liquid medium.²⁹ The bacterial growth was followed by spectrophotometry at 660 nm, and the inhibition percentages were calculated after 6 h at 37 °C. The inoculum was 0.2 mL of 18-h liquid-medium culture of the test organism. The inoculum was added to 4.8 mL of the liquid medium, and 0.1 mL of a solution of the compound was added. The MIC values were determined on the agar Davis and Mingioli's solid medium²⁹ eventually supplemented by amino acids³⁰ and were the lowest concentration that resulted in complete inhibition of growth. Antagonism studies were conducted either by a disk diffusion method on the solid medium or by supplementing the liquid medium with the amino acid. K_m and K_i values are the arithmetic means of three determinations.

E. coli ATCC 9637 *meso*-DAP decarboxylase was isolated and purified as described by White and Kelly.²¹ Its specific activity was 3 μ m/mg. Inhibition of *meso*-DAP decarboxylase was measured following the procedure described by Beaven et al.³¹

(28) Rhuland, L. E.; Work, E.; Denman, R. F.; Hoare, D. S. *J. Am. Chem. Soc.* **1955**, *77*, 4844. Yuasa, S.; Shimada, A. *Sci. Rep. Coll. Gen. Educ.* **1982**, *31*, 13.

(29) Davis, B. D.; Mingioli, E. S. *J. Bacteriol.* **1950**, *60*, 17.

(30) Atherton, F. R.; Hall, M. J.; Hassal, C. H.; Lambert, R. W.; Ringrose, P. S. *Antimicrob. Agent Chemother.* **1979**, *15*, 677.

with [¹⁴C]-*meso*-DAP (CEA).

Acknowledgment. We are grateful to Drs. M. Langlois, Ph. Dostert, and A. R. Schoofs for valuable discussions and to A. Lalande, E. Caminade, and C. Meriaux for technical assistance.

Registry No. 2, 100910-16-5; 2a, 35047-24-6; 2b, 100910-23-4; 2c, 100910-24-5; 2d, 100910-25-6; 2e-HCl, 100910-26-7; 3-2HCl, 100910-17-6; 3a, 37944-29-9; 3b, 100910-27-8; 3c, 100910-28-9; 3d, 100910-29-0; 3e-HCl, 100910-30-3; 4-HCl, 100910-18-7; 4 (free base), 100910-60-9; (DD + LL)-4, 100910-31-4; (-)-4-2HCl, 100910-32-5; (-)-4 (free base), 100928-36-7; (+)-4-2HCl, 100910-33-6; (+)-4 (free base), 100910-61-0; *meso*-4, 100910-34-7; 4b, 100910-35-8; 4c, 100938-75-8; 4d, 100910-36-9; 4e, 100910-37-0; 4f, 100910-38-1; 4g, 100910-39-2; 4h, 100910-40-5; 4i, 100910-41-6; 4j, 100910-42-7; 4k, 100910-43-8; 4l, 100910-44-9; 4m-2HCl, 100910-45-0; 4n-2HCl, 100910-46-1; 5, 100910-19-8; 5a, 100910-47-2; 5b, 100910-48-3; 5c, 100928-35-6; 5d, 100910-49-4; 5e, 100910-50-7; 6-2HCl, 100910-20-1; 6 (free base), 100910-62-1; 6a, 100056-08-4; 6b, 100910-51-8; 6c, 100910-52-9; 6d, 100910-53-0; 6e, 100910-54-1; 7, 100910-21-2; 7a, 100910-55-2; 7b, 100910-56-3; 7c, 100910-57-4; 7d, 100910-58-5; 7e, 100910-59-6; 7f, 100910-63-2; 8, 98998-80-2; 9-2HCl, 100910-22-3; 9 (free base), 52017-67-1; MesCl, 124-63-0; (BOC)₂O, 24424-99-5; OHCCOOEt, 924-44-7; lysine, 56-87-1; *meso*-DAP decarboxylase, 9024-75-3.

(31) Beaven, M.; Wilcox, G.; Terpstra, G. *Anal. Biochem.* **1978**, *84*, 638.

Isozyme-Specific Enzyme Inhibitors. 11.¹ L-Homocysteine-ATP S-C5' Covalent Adducts as Inhibitors of Rat Methionine Adenosyltransferases

Francis Kappler,[†] Ton T. Hai,[†] Robert J. Cotter,[‡] Karen J. Hyver,[‡] and Alexander Hampton^{*†}

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, and Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University, Baltimore, Maryland 21205. Received July 15, 1985

The title compounds (**14a,b**) were 5' epimers of a derivative of a phosphonate isostere of ATP in which the CH₂OP^α system of ATP was replaced by CH(R)CH₂P^α [R = L-S(CH₂)₂CH(NH₂)CO₂H]. They resisted synthesis via attempted S-alkylation of the corresponding epimeric 5'-mercapto derivatives. A practicable route to **14a,b** commenced with Michael condensation of L-homocysteine with the diphenyl ester of the 5',6'-vinyl phosphonate analogue of 2',3'-O-isopropylideneadenosine 5'-phosphate. The resulting epimeric 5' thioethers were separated by reverse-phase HPLC. The two phenyl groups were replaced by benzyl groups, after which the α-amino acid residue was protected as an N-Boc methyl ester. Both benzyl groups were removed by hydrogenolysis, and the resulting phosphonic acid was converted into its pyrophosphoryl derivative. Blocking groups were then removed under conditions that furnished **14a** and **14b** without racemization of their L-amino acid residues. Also synthesized were the P^β-NH-P^γ imido analogue (**15a**) of **14a** and the sulfoxide derivative (**16a**) of **14a**. The structures of **14a** and **16a** were verified by FAB mass spectra, which revealed the protonated molecular ions of their sodium salts. All adducts appeared to function as dual substrate site inhibitors (competitive to ATP and to methionine) of the rat normal tissue (MAT-2) form of methionine adenosyltransferase (MAT); **14a** and **15a** [$K_M(\text{ATP})/K_i = 4$ and 9, respectively] were the most effective. Adduct **15a** was the most effective inhibitor [$K_M(\text{ATP})/K_i = 13$] of the MAT-T form from rat hepatoma tissue; the kinetic data indicated dual-site inhibition by **15a** with apparently complete coverage of the ATP site and incomplete coverage of the methionine site. The inhibition properties of the adducts indicated little preference in the order in which the two MAT forms bound ATP and methionine.

Isozyme-specific enzyme inhibitors are, as discussed previously,² of interest as potential progenitors of new antineoplastic agents. In previous work in this series, we have studied several approaches to the design of such inhibitors, using for that purpose enzymes of the most common type, i.e., ones that catalyze reactions involving two substrates and that accordingly possess two substrate binding sites per catalytic unit. These studies have shown

that two-substrate adducts linked covalently via the atoms that become bonded in the enzyme-catalyzed reaction are capable of acting as potent isozyme-selective inhibitors, either in their unsubstituted form, as was observed in the case of mammalian thymidine kinases,³ or after the at-

[†] Fox Chase Cancer Center.

[‡] Johns Hopkins University.

(1) For part 10, see: Kappler, F.; Hai, T. T.; Hampton, A. *J. Med. Chem.* **1986**, *29*, 318.

(2) Hampton, A.; Kappler, F.; Maeda, M.; Patel, A. D. *J. Med. Chem.* **1978**, *21*, 1137.

(3) Hampton, A.; Hai, T. T.; Kappler, F.; Chawla, R. R. *J. Med. Chem.* **1982**, *25*, 801.