Stereospecific Synthesis of Phosphonate Analogues of Diaminopimelic Acid (DAP), Their Interaction with DAP Enzymes, and Antibacterial Activity of Peptide Derivatives

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Analogues of diaminopimelic acid (DAP) in which the carboxyl groups are replaced with phosphonic acid moieties were synthesized as pure stereoisomers, examined as inhibitors of three DAP enzymes, and tested as antibacterial agents. Condensation of the enolate of (*S*)-1-benzoyl-2-*tert*-butyl-3-methyl-4-imidazolidinone (**1**) with 1,3-dibromopropane stereoselectively gave the expected monobromide **3** which was used to alkylate the (−)-camphor imine **7** of diethyl (aminomethyl)phosphonate to yield a 4:1 mixture of 1*R* and 1*S* diastereomers **8** and **9**, respectively. Separation and hydrolytic deprotection gave stereochemically pure (1*R*,5*S*)-(1,5-diamino-5-carboxypentyl)phosphonic acid (P-DAP) (**10**) and its (1*S*,5*S*)-isomer **11**. An analogous approach employing (+)-camphor imine **17** and monobromide **3** also allowed synthesis of **10** and **11**, but in a reversed ratio (ca. 2:3). The pure (1*R*,5*R*)-P-DAP (**14**) and (1*S*,5*R*)-P-DAP (**15**) could be made by a similar procedure using (*R*)-1-benzoyl-2-*tert*-butyl-3-methyl-4-imidazolidinone (**2**), 1,3-dibromopropane, and **7**. A DAP bisphosphonate analogue **22**, in which both carboxyl groups are replaced, was synthesized as a mixture of all possible isomers by condensation of 2 equiv of the enolate of imine **7** or **17** with 1,3-dibromopropane followed by hydrolysis. A series of di- and tripeptides of individual P-DAP isomers with L-alanine were synthesized to enhance transport into bacterial cells for antimicrobial tests. Condensation of L-alanine *N*-carboxyanhydride (**23**) with individual P-DAP isomers **10**, **11**, **14**, and **15** in aqueous Na_2CO_3/DMF gave acylation only on the amino group adjacent to the carboxyl to generate dipeptides **24-27**. Acylation of P-DAP isomers **10** or **11** with Boc-L-Ala-L-Ala proceeded similarly to give, after deprotection, tripeptides **30** and **32**. The P-DAP isomers were generally weak competitive inhibitors of purified DAP decarboxylase from wheat germ (*Triticum vulgaris*), DAP dehydrogenase from *Bacillus sphaericus*, and DAP epimerase from *Escherichia coli*. P-DAP **11** (a *meso*-DAP analogue) has the strongest effect on the decarboxylase and epimerase, and its enantiomer **14** is the strongest inhibitor of the dehydrogenase. Antibacterial tests show that the P-DAP isomers display negligible activity except against *Salmonella typhimurium* LT-2. Compound **11** is the most active isomer and its inhibition is reversed by DAP. Among the peptide derivatives, the antibacterial spectrum of **30** (the tripeptide containing **10**) includes several strains of *E. coli* and *Citrobacter freundii*.

Introduction

Analogues of α-amino acids wherein a phosphonic acid moiety replaces the carboxyl group can inhibit key enzymes or interact with receptors which normally bind the corresponding natural compounds.[1] The antibiotic alafosfolin represents a classical example; active transport into bacterial cells by a specific permease precedes cleavage to release (*R*)-(1-aminoethyl)phosphonic acid (P-Ala), which then acts as a potent slow-binding inhibitor of alanine racemase in Gram-positive bacteria (Figure 1).[2] The utility of α-aminophosphonates and their peptidic derivatives[3] as biological tools has stimulated continuing efforts for their synthesis, both in racemic[4] and enantiomerically pure[5] form. Interest in the mechanisms of enzymes involved in diaminopimelic acid (DAP) metabolism (Figure 2) and the potential of DAP-based inhibitors to act as antibiotics[6] suggest that phosphonic acid analogues of diaminopimelate (P-DAPs) and peptides derived from them could be useful biochemical probes and potent antibacterial agents. Bacteria and higher plants use the DAP pathway to manufacture L-lysine, but

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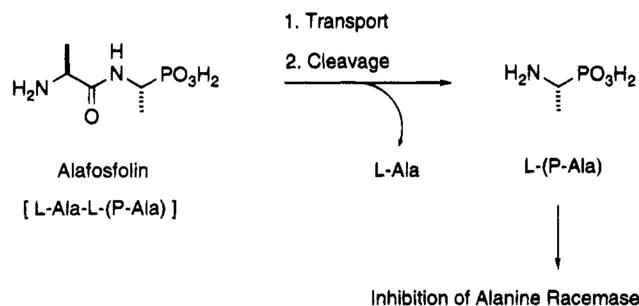

Figure 1. Mechanism of alafosfolin inhibition.

mammals lack this metabolic route and require L-lysine in their diet.[7] In addition, Gram-negative bacteria employ *meso*-DAP as a cross-linking amino acid in the peptidoglycan cell wall layer, whereas many Gram-positive microorganisms utilize L-lysine for this purpose.[8] With the possible exception of DAP-adding enzyme,[9] every protein which operates on diaminopimelic acid examined thus far has quite stringent requirements for chain length, for the presence of all functionalities (i.e. two amino and two carboxyl groups), and for correct stereochemistry at both ends.[6d,10] Recently the synthesis of P-DAP as a mixture of all four possible isomers and the failure of this mixture to significantly inhibit DAP-adding enzyme were reported.[11] Production of a stereoisomeric mixture of phosphinic acid (one oxygen less on phosphorus) analogues of DAP has also been published, but no enzymatic or biological studies were included.[12] In the present work, we describe the stereospecific syntheses of pure P-DAP isomers, their interaction with three enzymes (DAP epimerase, dehydrogenase, and decarboxylase), the specific formation of L-alanine-containing di- and tripeptide derivatives of P-DAP, and studies on the antibacterial activity of these compounds.

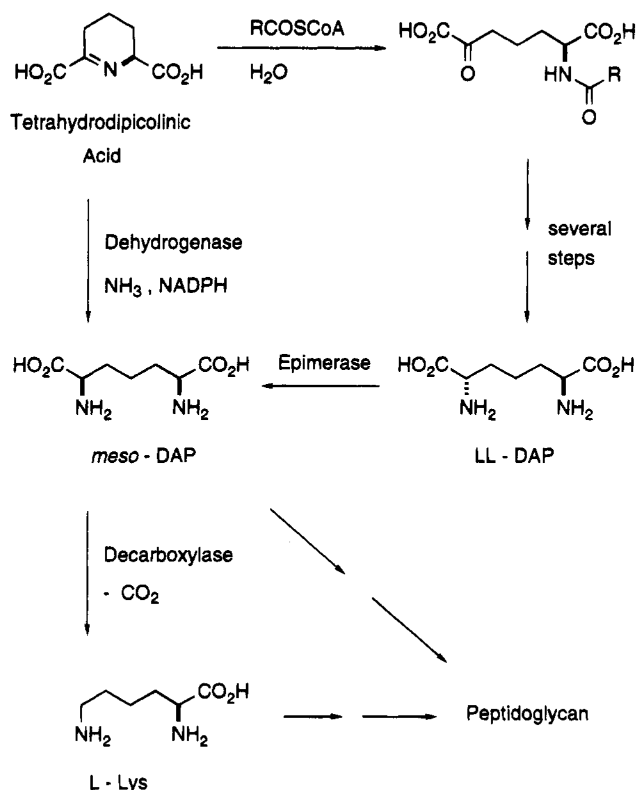

Figure 2. Portion of the DAP pathway in bacteria and higher plants showing the transformations catalyzed by DAP dehydrogenase, epimerase, and decarboxylase.

Results and Discussion

Syntheses of P-DAP Stereoisomers and Their Peptide Derivatives. The strategy for stereocontrolled generation of P-DAP employed alkylation of chiral Seebach imidazolidinones[13] with 1,3-dibromopropane followed by a second condensation, originally developed by Schöllkopf and Schütze,[14,15] with a chiral camphor imine of diethyl (aminomethyl)phosphonate (**5**)[16] (Figure 3). Thus reaction of the lithium enolates of imidazolidinone **1** and its enantiomer **2** with 1,3-dibromopropane generates the corresponding monoalkylated products **3** and **4**

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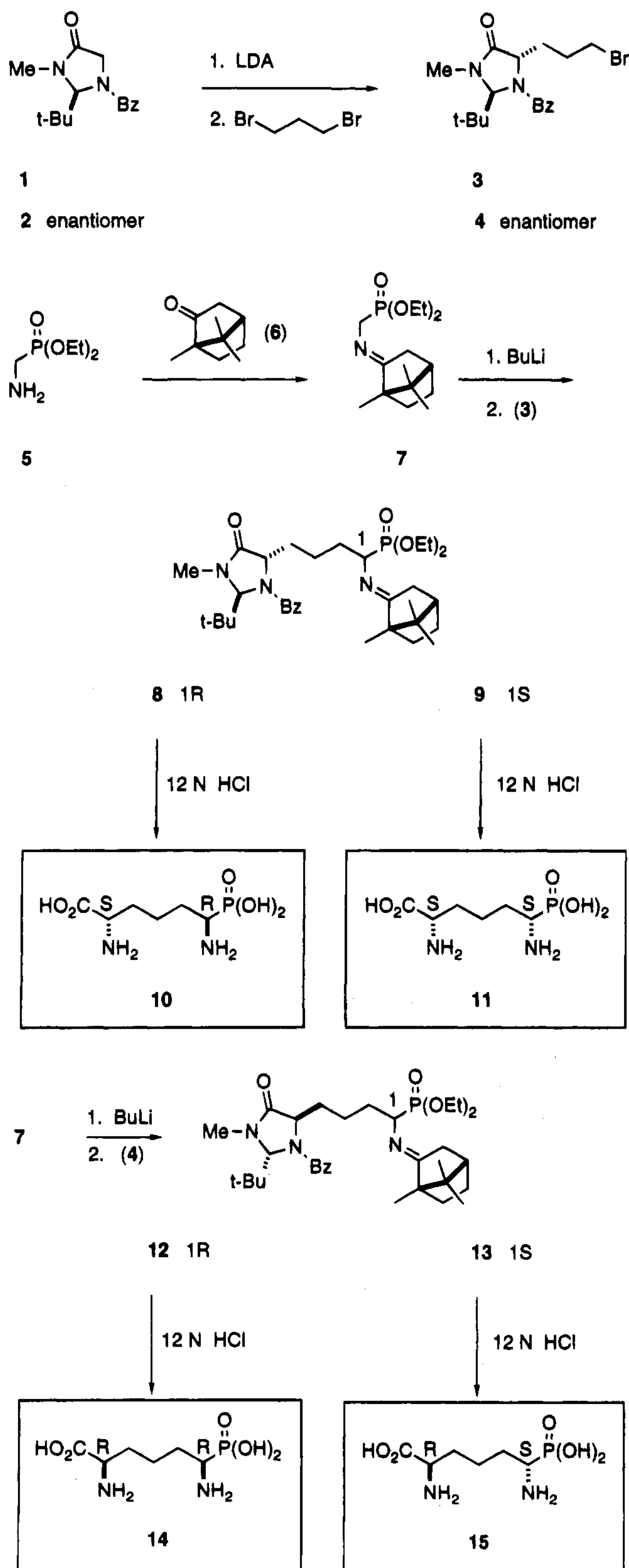

Figure 3. Synthetic routes to P-DAP isomers **10**, **11**, **14**, and **15** using (−)-camphor imine **7**.

as pure isomers (by ^{1}H NMR) in reasonable yield (57–66%). Displacement of bromide from **3** by the enolate of the (−)-camphor imine **7**[14] gives two diastereomers **8** and **9** in a ratio of 4:1 (71% yield), which can be chromatographically separated. Direct deprotection of **8** in refluxing concentrated HCl is surprisingly problematic, but a two-stage procedure, wherein room temperature hydrolysis for 12 h with this reagent precedes heating to

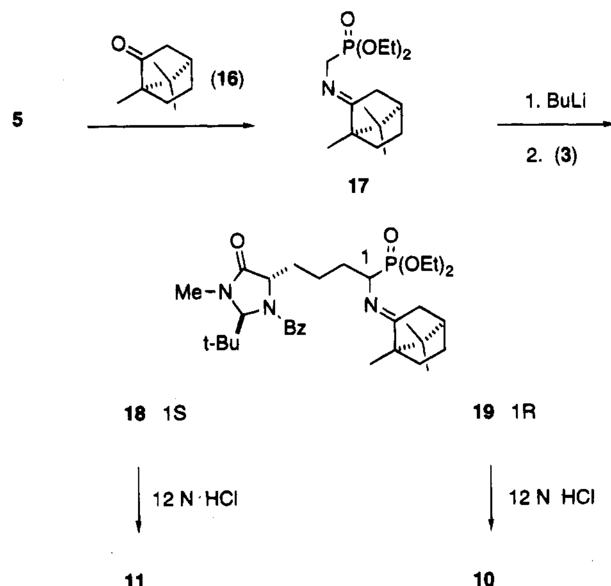

Figure 4. Synthetic route to P-DAP isomers **11** and **10** using (+)-camphor imine **17**.

reflux, readily converts **8** and **9** to the corresponding optically pure P-DAP isomers **10** and **11** in 74–88% yield after cellulose chromatography. Analogous alkylation of the same imine **7** with the enantiomeric bromide **4** generates two diastereomers **12** and **13** in a 3:2 ratio (86% yield), which after separation and hydrolysis give the pure P-DAP isomers **14** and **15**. As expected, the corresponding "mirror image" reaction of the (+)-camphor imine **17** with bromide **3** produces the same 3:2 ratio of two diastereomers **18** and **19**, which are enantiomers of **12** and **13**, respectively (Figure 4). Hydrolysis of **18** and **19** after separation gives pure P-DAP isomers **11** and **10**. The stereochemical assignments of all these compounds are based on the well-established preferences of both the Seebach[13] and Schöllkopf[14] methodologies. The optical purity of the P-DAP isomers is confirmed by generation of single diastereomers (by ^{1}H NMR analysis) upon formation of dipeptide and tripeptide derivatives (see below).

In order to briefly examine possible replacement of both carboxyls of DAP with phosphonic acid groups, the bisphosphonate analogue **22** was also synthesized as a stereoisomeric mixture (DD, LL, and *meso*) (Figure 5). Separate alkylations of the enolates of (−)-camphor imine **7** and of (+)-camphor imine **17** with 0.5 equiv of 1,3-dibromopropane generate in each case a nonstatistical mixture of all possible stereoisomers **20** and **21** (62–65% yield), respectively, because of the incomplete stereoselectivity of the process. The two mixtures are presumably enantiomeric with respect to each other. Hydrolysis with concentrated HCl gives the corresponding bis-phosphonate **22** as a mixture of LL, DD, and *meso* compounds (63% yield). Possible separation of these stereoisomers was not pursued because of the weak inhibition of DAP enzymes and the lack of antibiotic activity displayed by **22** (see below).

As exemplified by alafosfolin,[2] peptide derivatives of antibiotics often have much higher activity against bacteria because specific microbial transport systems can assist their importation into the cell where they are hydrolyzed by peptidases to the active parent

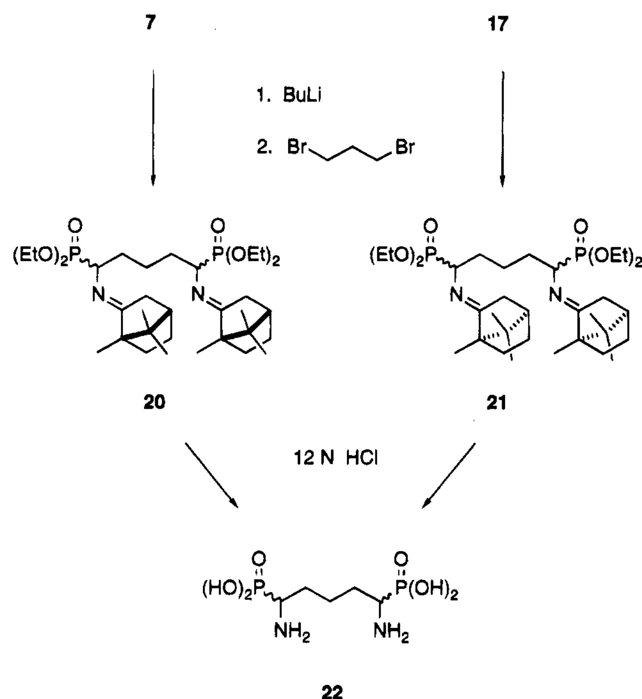

Figure 5. Syntheses of bis-phosphonate analogue 22 of DAP using enantiomeric camphor imines 7 and 17.

compound.[2b,17] Since free phosphates are known to be especially resistant to unassisted crossing of cell membranes,[18] transformation of the P-DAP isomers to di- and tripeptide derivatives with L-alanine could allow recognition by bacterial active transport systems and enhance importation. Direct reaction of the (1*R*,5*S*)-P-DAP (**10**), an analogue of LL-DAP,[19] with the L-alanine *N*-carboxyanhydride **23**[20] in sodium carbonate buffer (pH 10, 1 N) containing DMF results in N-acylation only on the amino group adjacent to the carboxylate (Figure 6). The isolated yield of **24** is low (19–47%), but formation of a single product (in addition to recovered starting material) and lack of protection–deprotection procedures affords rapid access to the desired material. The origin of the regiospecificity is not known, but it may have to do with the extent of ionization because lowering of the pH to about 8 (e.g. $NaHCO_3$ buffer) reduces the selectivity for the two amino groups to 4:1 for formation of the dipeptide **24** (major regioisomer). Similar reactions of the *N*-carboxyanhydride **23** with the other P-DAP isomers **11**, **14**, and **15** convert them to the L-alanine-containing dipeptides **25**, **26**, and **27**, respectively. The fact that single diastereomers are formed in each case, which can be distinguished in their ^{1}H NMR spectra, confirms the optical purity of the starting P-DAP isomers. To prepare the tripeptide derivatives, N-*tert*-(butoxycarbonyl)-L-alanyl-L-alanine (**28**) is first activated with *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) and is then coupled

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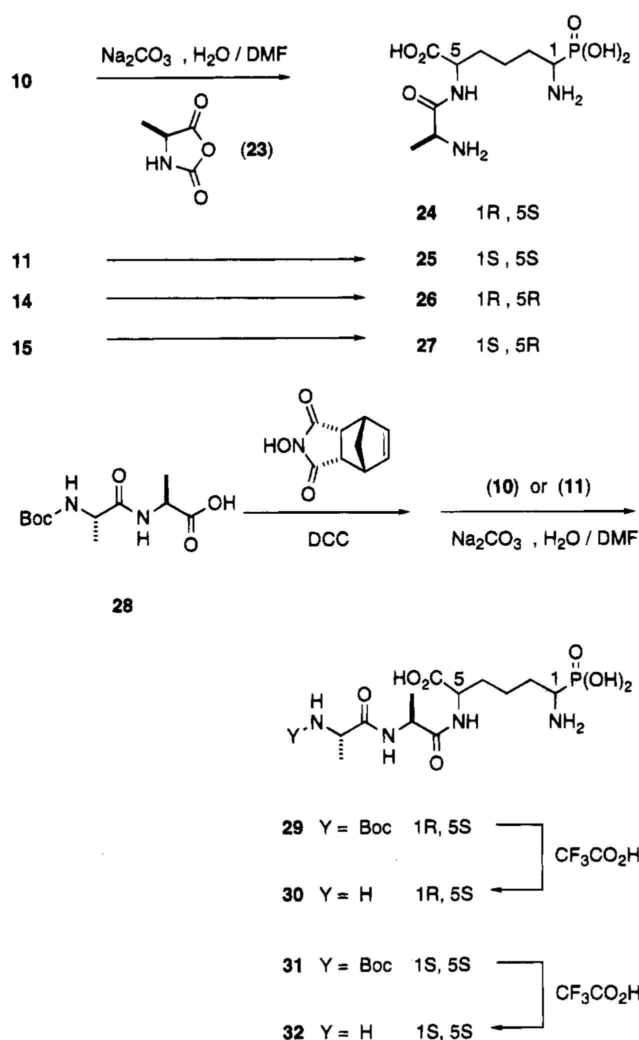

Figure 6. Formation of di- and tripeptide derivatives of P-DAP isomers.

directly to unprotected (1*R*,5*S*)-P-DAP (**10**) in aqueous sodium carbonate/DMF. As with the dipeptide formation, reaction occurs at the amino group closest to the carboxylate to give the protected tripeptide derivative **29** in 60% yield. Standard removal of the Boc group with trifluoroacetic acid quantitatively produces the target tripeptide **30** containing the (1*R*,5*S*)-P-DAP residue. A similar sequence in which **28** is coupled to (1*S*,5*S*)-P-DAP (**11**) generates the diastereomeric tripeptide **32** which bears the (1*S*,5*S*)-P-DAP moiety.

Interaction of P-DAP Isomers with DAP Decarboxylase, Dehydrogenase, and Epimerase. *meso*-Diaminopimelate decarboxylase (EC 4.1.1.20) is an unusual pyridoxal phosphate (PLP)-dependent enzyme because it catalyzes the loss of CO_2 from a center having D stereochemistry and operates with inversion of configuration (Figure 7), in contrast to all other α-amino acid decarboxylases.[21] The structural requirements for substrate recognition are quite strict, and although the enzyme will decarboxylate *meso*-lanthionine (in which the central methylene of DAP is replaced with sulfur) at about 5% of the rate for *meso*-DAP, other DAP isomers or analogues are neither substrates nor effective inhibi-

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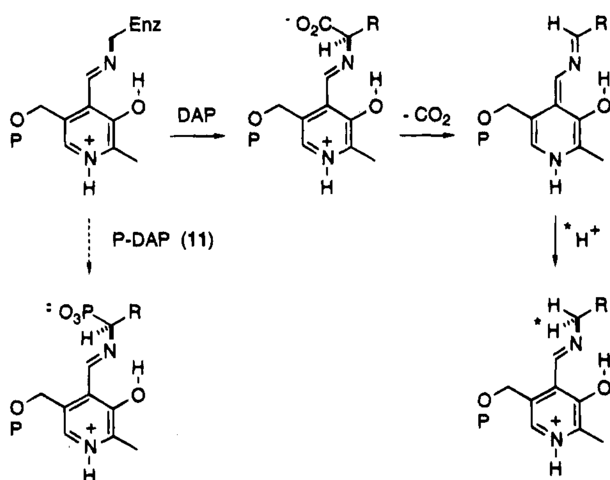

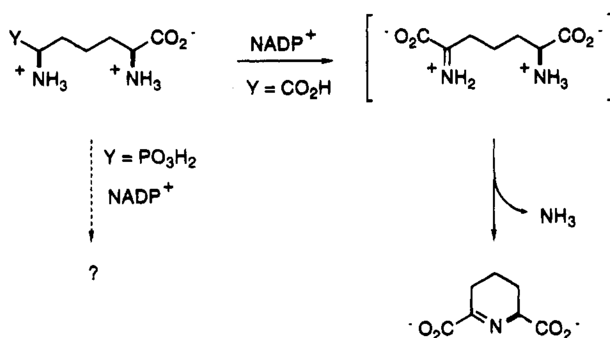

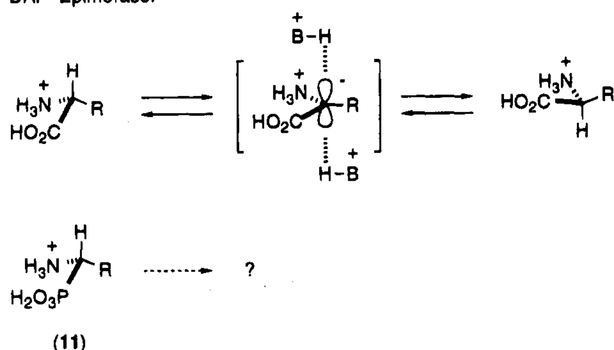

Figure 7. Proposed mechanisms of DAP decarboxylase, dehydrogenase, and epimerase indicating possible interference by (1*S*,5*S*)-P-DAP (**11**), an analogue of *meso*-DAP.

tors.[22] The sequences of this decarboxylase from five different bacterial species are known with molecular weights which range from 45462 to 48876 for the monomer unit in a dimeric, or in some cases tetrameric, enzyme.[23] The present work employs the enzyme purified from wheat germ, *Triticum vulgaris*; it has similar properties[21b,22] to the bacterial DAP decarboxylases, but its sequence has not yet been reported. It would be expected that replacement of a carboxyl group at the D-center of *meso*-DAP with a phosphonic acid moiety could lead to inhibition of this decarboxylase because PLP-assisted cleavage of the carbon–phosphorus bond is unlikely. The decarboxylase assay employs capture of radioactive $^{14}CO_2$ released from [1,7-^{14}C]diaminopimelate (statistical mixture of isomers) and gives a K_m of 59 μM for *meso*-DAP, which is much lower than the K_m values for the bacterial DAP decarboxylases (1700 to 170 μM).[23e] Among the potential inhibitors, (1*S*,5*S*)-P-DAP (**11**) is the analogue whose stereochemistry would direct binding in the decarboxylase active site such that the phosphonate is located at the position for bond cleavage. Although it is the most potent of the four monophosphonates tested (Table 1), it still shows only 50% competitive inhibition of the wheat germ enzyme at 320 μM. Surprisingly, isomer **14**, which would bind with correct α-amino acid functionality at the active site and would place the stereochemically correct polar phosphonate group at the distal recognition site, is an even weaker competitive inhibitor and does not detectably act as a substrate. No imine formation or transformation could be detected with any of the P-DAP isomers. Apparently the requirements for substrate recognition by this enzyme are so strict that the increased length of the carbon–phosphorus bond or the presence of an extra oxygen prevent effective binding to this plant DAP decarboxylase.

Although many bacteria convert tetrahydrodipicolinic acid (THDP) to LL-DAP and then employ DAP epimerase to form *meso*-DAP, certain species use an alternative pathway in which DAP dehydrogenase (EC 1.4.1.16) transforms THDP directly to *meso*-DAP using ammonia and NADPH (cf. Figures 2 and 7).[24] This enzyme probably reduces a primary imine, which results from cleavage of the THDP ring by ammonia and ultimately generates a D amino acid center. The mechanism may be similar to that of L-glutamate dehydrogenase, which has been extensively studied.[25] To examine the interaction with P-DAP analogues, DAP dehydrogenase was purified from *Bacillus sphaericus* IFO 3525 by literature procedures.[24a] This dehydrogenase is known to be a dimer of two identical subunits (each of *ca.* 40000 Da), with pH optima of about 7.5 for the forward reaction (formation of *meso*-DAP by reductive amination) and 10.5 for the reverse direction (oxidative deamination to THDP).[24a] However, the oxidative deamination reaction will proceed at pH 7.8 if ammonia is absent and the enzyme is provided with $NADP^+$ and *meso*-DAP instead of THDP.[26] The conversion of $NADP^+$ allows continuous spectrophotometric assay at 340 nm. The three-dimensional structure of DAP dehydrogenase from *B. sphaeri-*

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(26) The K_m^{app} of $NADP^+$ at pH 7.8 was determined[6d] as 8.2 μM, since only a value at pH 10.5 (83 μM) is available in the literature (see ref 24a and also: Misono, H.; Togawa, H.; Yamamoto, T.; Soda, K. *J. Bacteriol.* **1979**, *137*, 22–27). Using the literature assay procedure,[24a] the K_m^{app} for THDP was determined as 0.12 mM, in reasonable agreement with the literature value 0.24 mM. The literature values of Michaelis constants for *meso*-DAP, NADPH, and NH_3 are 2.5 mM, 0.2 mM, and 12.5 mM, respectively.[24a]

Table 1. Inhibition of DAP Enzymes by Phosphonate Analogues of DAP[a]

analogue	DAP decarboxylase, % activity remaining at 320 μM inhibitor	DAP dehydrogenase		DAP epimerase	
		K_i (mM)	type of inhibition	K_i (mM)	type of inhibition
10	89	12[b]	noncompetitive	6.2	competitive
11	50	7.4	competitive	3.9	competitive
14	93	4.3	competitive	–	none[c]
15	91	26	competitive	7.2	competitive
22	84	ND[d]		14[b]	mixed

[a] See Experimental Section for assay conditions. [b] Apparent inhibition constant, K_i'. [c] No significant inhibition could be detected at 15 mM concentration of **14**. [d] Not determined; 11% inhibition at 3 mM was observed.

cus has not been reported, but the corresponding enzyme from *Corynebacterium glutamicum* has been cloned and sequenced.[27] We have recently synthesized a heterocyclic DAP analogue which acts as a potent allosteric inhibitor of the dehydrogenase from *B. sphaericus* and inhibits bacteria which depend on this enzyme for production of *meso*-DAP.[6d] However, as with other DAP enzymes, the substrate specificity is quite high, and other stereoisomers of DAP or its derivatives are poorly bound and are not transformed by the dehydrogenase.[24a,28]

It would be expected that substitution of a phosphonate for a carboxylate group would alter the propensity for oxidation of the adjacent amino group by DAP dehydrogenase. Alternatively, if binding to the enzyme occurs such that the phosphonate is at the distal site, the tendency of the adjacent amino functionality to cyclize onto an electrophilic imine bond and form a six-membered ring should also be altered. Exposure of the P-DAPs **10**, **11**, **14**, and **15** as well as the bis-phosphonate analogue **22** to DAP dehydrogenase shows that all of these compounds are very weak inhibitors of the enzyme (Table 1). In contrast to results with DAP decarboxylase, the best competitive inhibitor is (1*R*,5*R*)-P-DAP (**14**) (K_i = 4.3 mM), the analogue of *meso*-DAP whose stereochemistry would place the phosphonate group at the distal position, away from the potential site of oxidation. However, neither this isomer nor any of the others are detectable substrates or display any time-dependent inhibition of the enzyme upon preincubation at concentrations of 2.25 mM for up to 2 h. Clearly all of the P-DAP isomers bind relatively weakly to the dehydrogenase, presumably because its requirements for substrate recognition are quite strict, as is the case with DAP decarboxylase.

DAP epimerase (EC 5.1.1.7) from *Escherichia coli* interconverts LL-DAP and *meso*-DAP (but not DD-DAP) without the aid of cofactors, metal ions, or reducible imine or keto functionalities (cf. Figure 2).[10] This enzyme is a member of a growing class of α-amino acid epimerases[29–32] which may function by an unusual mechanism in which one basic group on the protein removes the α-hydrogen and a second protonated base delivers its proton from the opposite side (Figure 7). Studies with β-fluoro DAP isomers suggest possible formation of an intermediate anion at the α-carbon during catalysis by this epimerase.[10b] As with DAP decarboxylase and dehydrogenase, the distal (nonreacting) α-amino acid site must have L-configuration, and both carboxyl and both amino groups are necessary for substrate recognition and transformation (i.e. α-hydrogen exchange) by DAP epimerase from *E. coli*.[6d]

The substitution of carboxyl in DAP by a phosphonic acid group should alter the pK_a of the adjacent α-hydrogen considerably. To examine the effect of P-DAP isomers, the epimerase was first purified from an overproducing strain of *E. coli* using an improved procedure.[6d] The enzyme was assayed by following the release of tritium from the α-carbon of uniformly tritiated DAP (statistical mixture of all possible isomers) which was diluted with unlabeled DAP, rather than by the more convenient spectrophotometric coupled assay with DAP dehydrogenase,[10] to avoid possible complications due to inhibition of the latter enzyme by P-DAP isomers. None of the P-DAP isomers **10**, **11**, **14**, and **15** or the bis-phosphonate analogue **22** show any time-dependent inhibition or are substrates of DAP epimerase based on the lack of α-hydrogen exchange with deuterated media. The compounds are weak competitive inhibitors, with (1*S*,5*S*)-P-DAP (**11**) (K_i = 3.9 mM) showing the strongest effect (Table 1). It is interesting that for both the DAP decarboxylase and the epimerase, **11** is bound more strongly in the active site than its enantiomer **14**, which is also a *meso*-DAP analogue. This indicates that the distal (nonreacting) recognition sites in DAP decarboxylase and epimerase prefer to bind the natural α-amino acid moiety and demand greater structural fidelity than the protein groups which bind the reacting terminus of the DAP molecule. The situation is reversed with DAP dehydrogenase, where **14** is a better inhibitor. Surprisingly, the phosphonate for carboxylate replacement which is effective for other enzymes that metabolize α-amino acids leads only to weak competitive inhibition with the highly specific DAP enzymes. This observation is in accord with the results previously obtained with DAP-adding enzyme using a mixture of all possible P-DAP isomers.[11]

Antibacterial Activity of P-DAP Isomers and Their Peptides. In preliminary experiments, P-DAP isomers **10**, **11**, **14**, **15** and the bis-phosphonate **22** showed little or no growth inhibition when tested in defined liquid medium against six bacterial strains by the turbidimetric method. Consistent with these results, studies employing the agar dilution method on defined minimal agar medium against 32 bacterial strains also demonstrated a general lack of activity. However, growth of one strain, *Salmonella typhimurium* LT-2, was inhibited by several of the P-DAP isomers. The most active analogue, **11**, showed a minimum inhibitory concentra-

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tion (MIC) of about 1 μg/mL. The MIC was dependent on the inoculum level; i.e. on Davis–Mingioli minimal agar, the MIC values were 0.25, 1, 2, and 8 μg/mL for 100, 10^3, 10^4, and 10^5 bacteria per spot applied to the agar plate, respectively. Inhibition was competitively reversed by LL-DAP, *meso*-DAP, and L-cysteine, but not L-lysine. These results suggest uptake by the DAP/cysteine transport system. It is interesting to note that peptidoglycan biosynthesis studies have indicated that *S. typhimurium* LT-2 has a very low intracellular DAP pool.[8c] Accordingly, interference with DAP metabolism may more easily affect growth in this strain, as compared with strains having larger DAP pools.

As described above, peptide derivatives of P-DAP isomers could be expected to enhance bacterial uptake via the well-known peptide transport systems. Compounds **24**, **26**, **30**, and **32** were tested by the agar dilution method. Although none display potent, broad-spectrum activity, the L-Ala-L-Ala-(1*R*,5*S*)-P-DAP (**30**) does show activity against several strains of *E. coli* and *Citrobacter freundii*. MIC values range from 4 to 32 μg/mL. Counter diffusion tests with *E. coli* ATCC 27856 demonstrate that this inhibition is reversed by LL-DAP and *meso*-DAP, thereby indicating that DAP metabolism is the target for the antibacterial action of tripeptide **30**. Two peptides, L-Ala-L-Ala and L-Ala-L-Ala-L-Ala, also reverse growth inhibition by **30**, but as expected, the parent amino acid, L-alanine, does not show this effect.

Conclusions

This work describes the synthesis of four stereochemically pure diaminopimelic acid analogues wherein a carboxyl group is replaced with a phosphonic acid moiety. Acylation of such P-DAP isomers in aqueous base to L-alanine-containing di- and tripeptides occurs regiospecifically on the amino group adjacent to the carboxyl. Although many phosphonate analogues of α-amino acids are potent inhibitors of enzymes involved in metabolism of the parent compounds, the P-DAP isomers display only weak competitive inhibition of DAP decarboxylase, dehydrogenase, and epimerase. None are substrates. The inability of these enzymes to accommodate replacement of a carboxyl group at either the active site or the distal (nonreacting) recognition site demonstrates that proteins involved in DAP metabolism have evolved very high specificity and allow little variation in substrate or inhibitor structure. Although the parent P-DAP isomers display negligible antibacterial activity, the tripeptide **30** containing (1*R*,5*S*)-P-DAP (an LL-DAP analogue) shows growth inhibition of certain bacterial strains. Reversal of this effect by *meso*-DAP or LL-DAP, as well as by L-alanine-containing peptides, indicates that transport into bacterial cells is assisted by such derivatization and that DAP metabolism is a target for antimicrobial action. Further studies on interaction of P-DAP derivatives with other enzymes in the DAP pathway as well as on other DAP analogues are currently underway.

Experimental Section

General Methods. Most general experimental procedures and instrumentation have been described previously.[6d] All reagents were purchased from Sigma or Aldrich and were used without further purification unless otherwise stated. All solvents were dried and distilled prior to use according to standard procedures.[33] Deionized water was purified with a Milli-Q apparatus (Millipore, Piscataway, NJ). Hydroxyapatite (Biogel HTP) and ion-exchange resins AG50W-X8 (200–400 mesh) and AG1-X8 (100–200 mesh) were purchased from BioRad (Mississauga, ON). Spectropor 2 and 4 dialysis tubing, Scintiverse E scintillation cocktail, and Tris-HCl were obtained from Fisher Chemicals (Nepean, ON). Matrex Green A resin was purchased from Amicon (W.R Grace Inc., Beverly, MA). Radiochemicals such as [G-^{3}H]diaminopimelate (statistical mixture of isomers, 1.09 Ci/mmol) and [1,7-^{14}C]-D,L-diaminopimelate (117 mCi/mmol) were obtained from Amersham (Oakville, ON). Radioactivity was determined on a Beckman LS5000TD scintillation counter using Scintiverse E scintillation cocktail. Pure stereoisomers of DAP were separated as previously described.[6d,28] Protein concentrations were determined using bovine serum albumin as a standard with a Biorad Bradford protein assay kit following manufacturer's instructions. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal standard for ^{1}H NMR analysis with aqueous (D_2O) samples, and H_3PO_4 was used as standard for ^{31}P NMR spectra.

(2*S*,5*S*)-1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-5-(3-bromopropyl)-4-imidazolidinone (3). To a solution of (2*S*)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**1**)[13] (1.50 g, 5.77 mmol) in anhydrous THF (90 mL) at −78 °C, was added freshly prepared lithium diisopropylamide (LDA) solution (1.15 equiv). The mixture was stirred at −78 °C for 25 min before the addition of 1,3-dibromopropane (1.29 g, 6.39 mmol) in THF (10 mL). After being stirred at −78 °C for 1 h, the mixture was warmed to 20 °C over 6 h and then stirred at 20 °C overnight. It was then poured into half-saturated aqueous NH_4Cl solution (40 mL). The aqueous phase was extracted with EtOAc (3 × 80 mL). The combined organic phases were dried and concentrated *in vacuo* to give an oil, which was purified by flash chromatography using EtOAc/hexane (50/50) to give **3** as an amorphous solid in 57% yield (1.25 g): IR (CH_2Cl_2 cast) 2960, 1707, 1640 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70–7.40 (m, 5 H), 5.65 (s, 1 H), 4.38 (br d, 1 H, J = 4 Hz), 3.10 (s, 3 H), 3.15–2.92 (m, 2 H), 1.92–0.92 (m, 4 H), 1.08 (s, 9 H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 171.3, 137.1, 132.2, 129.3, 128.0, 80.3, 60.8, 41.4, 32.2, 32.0, 30.0, 26.5, 26.2; exact mass 323.0392 ($M^+ - C(CH_3)_3$) (323.0396 calcd for $C_{14}H_{16}N_2O_2Br$). Anal. Calcd for $C_{18}H_{25}N_2O_2Br$: C, 56.70; H, 6.61; N, 7.34; O, 8.39. Found: C, 56.80; H, 6.65; N, 7.19; O, 8.57.

(2*R*,5*R*)-1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-5-(3-bromopropyl)-4-imidazolidinone (4). The alkylation of (2*R*)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**2**)[13] (4.50 g, 17.3 mmol) with 1,3-dibromopropane (3.87 g, 14.2 mmol) by the procedure analogous to the preparation of **3**, produced **4** in 66% yield (4.37 g): IR (CH_2Cl_2 cast) 2970, 1707, 1646 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70–7.40 (m, 5 H), 5.65 (s, 1 H), 4.38 (br d, 1 H, J = 4 Hz), 3.09 (s, 3 H), 3.15–2.92 (m, 2 H), 1.93–0.91 (m, 4 H), 1.06 (s, 9 H); MS (CI) 381 (MH^+). Anal. Calcd for $C_{18}H_{25}N_2O_2Br$: C, 56.70; H, 6.61; N, 7.34. Found: C, 57.12; H, 6.65; N, 7.26.

Diethyl [(2*S*,5*S*,1′*S*,4′*S*,1″*R*)-4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-oxoimidazolidin-5-yl]-1-[(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)amino]butyl]phosphonate (8) and Diethyl [(2*S*,5*S*,1′*S*,4′*S*,1″*S*)-[4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-oxoimidazolidin-5-yl]-1-(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-amino-butyl]]phosphonate (9). To a solution of the (−)-camphor imine **7**[14] (0.530 g, 1.76 mmol) in THF (10 mL) at −78 °C was added dropwise a *n*-BuLi solution (1.20 M, 1.69 mL, 2.03 mmol). The resulting solution was stirred at −78 °C for 25 min before addition of bromide **3** (0.671 g, 1.76 mmol) in THF (5 mL). The solution was stirred at −78 °C for 1 h, warmed to 20 °C gradually over 6 h, and stirred at 20 °C overnight. The reaction mixture was poured into half-saturated aqueous NH_4Cl solution (20 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic phases were dried and concentrated *in vacuo* to afford an oil, which was purified by flash chromatography using

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EtOAc/MeOH (95/5) to give a mixture of **8** and **9** (0.752 g, 71% yield total) in a 4:1 ratio. Compound **8** (0.529 g, 50% yield) was separated from **9** (0.110 g, 10% yield) on an MPLC column (Merck PF_{254} silica gel, 2.5 × 30 cm) using EtOAc as eluent. For **8**: IR (CH_2Cl_2 cast) 2958, 1709, 1680, 1650 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65−7.38 (m, 5 H), 5.58 (s, 1 H), 4.31 (br d, 1 H, J = 4 Hz) 4.08 (m, 4 H), 3.46 (ddd, 1 H, J = 4, 9, 12 Hz), 3.05 (s, 3 H), 2.56−2.40 (m, 1 H), 2.00−1.16 (m, 12 H), 1.26 (dt, 6 H, J_{HH} = 7.2 Hz, J_{HP} = 4.8 Hz), 1.05 (s, 9 H), 1.00 (s, 3 H), 0.94 (s, 3 H), 0.85 (s, 3 H); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^1H decoupled) δ 24.97 (s); MS (CI) 602 (MH^+). Anal. Calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.73; H, 8.86; N, 6.95.

For **9**: IR (CH_2Cl_2 cast) 2960, 1710, 1680, 1649 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65−7.38 (m, 5 H), 5.54 (s, 1 H), 4.31 (br s, 1 H), 4.24−3.92 (m, 4 H), 3.48 (ddd, 1 H, J = 4, 9.5, 14 Hz), 3.00 (s, 3 H), 2.28−1.14 (m, 13 H), 1.29 (dt, 6H, J_{HH} = 7 Hz, J_{HP} = 2.5 Hz), 1.04 (s, 9 H), 0.93 (s, 3 H), 0.90 (s, 3 H), 0.71 (s, 3 H); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^1H decoupled) δ 24.43 (s); MS (CI) 602 (MH^+). Anal. Calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.59; H, 8.69; N, 6.92.

Hydrolysis of 8 or 19 to (1*R*,5*S*)-(1,5-Diamino-5-carboxypentyl)phosphonic Acid (10). Compound **8** (0.220 g, 0.366 mmol) was dissolved in 12 N aqueous HCl (10 mL) and stirred at 20 °C for 24 h. This solution was then heated at reflux for 3 days. The solvent was removed *in vacuo*, and the residue was applied to a cellulose (Merck) column (2.5 × 22 cm) and eluted with n-BuOH/H_2O/HOAc (4/2/1) to give 95.0 mg of P-DAP **10** as the dihydrochloride salt (87% yield): IR (MeOH cast) 3600−2400, 1728, 1595, 1504, 1300−850 cm^{-1}; ^1H NMR (200 MHz, D_2O, DSS) δ 4.14 (t, 1 H, J = 6 Hz), 3.35 (dt, 1 H, J_{HH} = 6 Hz, J_{HP} = 13 Hz), 2.15−1.54 (m, 6 H); ^{13}C NMR (50.3 MHz, D_2O) δ 171.3, 52.1, 48.1 (d, J_{PC} = 144 Hz), 29.0, 27.6, 21.0 (d, J_{PC} = 9 Hz); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) (^1H decoupled) δ 14.03 (s); FAB-MS 227 (MH^+). The same procedure was used to convert **19** to **10** with identical spectral and chromatographic properties.

Hydrolysis of 9 or 18 to (1*S*,5*S*)-(1,5-Diamino-5-carboxypentyl)phosphonic Acid (11). Compound **9** (80 mg, 0.133 mmol) was hydrolyzed in 12 N aqueous HCl as described above for preparation of **10** to afford **11** as its dihydrochloride salt (35 mg, 88% yield): IR (MeOH cast) 3600−2400, 1730, 1590, 1510, 1300−850 cm^{-1}; ^1H NMR (200 MHz, D_2O, DSS) δ 4.14 (t, 1 H, J = 6.4 Hz), 3.34 (dt, 1 H, J_{HH} = 6 Hz, J_{HP} = 13 Hz), 2.15−1.54 (m, 6 H); ^{13}C NMR (50.3 MHz, D_2O) δ 171.3, 52.0, 48.1 (d, J_{PC} = 144 Hz), 28.8, 27.4, 20.9 (d, J_{PC} = 7 Hz); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) (^1H decoupled) δ 14.21 (s); FAB-MS 227 (MH^+). The same procedure was used to convert **18** to **11** with identical spectral and chromatographic properties.

Diethyl [(2*R*,5*R*,1′*S*,4′*S*,1″*R*)-[4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-oxoimidazolidin-5-yl]-1-[(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)amino]butyl]phosphonate (12) and Diethyl [(2*R*,5*R*,1′*S*,4′*S*,1″*S*)-[4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-oxoimidazolidin-5-yl]-1-[(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-amino]butyl]phosphonate (13). The procedure employed above to prepare **8** and **9** was used to alkylate (−)-camphor imine **7**[14] (2.00 g, 6.64 mmol) with the enantiomeric imidazolidinone bromide **4** (2.53 g, 6.64 mmol), thereby giving a mixture of compounds **12** and **13** in 86% yield (3.43 g) in a ratio of 3:2. Compound **12** (1.25 g, 31% yield) was separated from **13** (1.03 g, 26%) on an MPLC column (silica gel, 2.5 × 30 cm) using EtOAc as eluent. For **12**: IR (CH_2Cl_2 cast) 2958, 1709, 1680, 1647 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65−7.37 (m, 5 H), 5.57 (s, 1 H), 4.30 (br s, 1 H), 4.22−3.93 (m, 4 H), 3.48 (ddd, 1 H, J = 4.8, 8.8, 12.8 Hz), 3.04 (s, 3 H), 2.54−2.38 (m, 1 H), 1.99−1.11 (m, 12 H), 1.26 (dt, 6 H, J_{HH} = 8 Hz, J_{HP} = 4 .4 Hz), 1.04 (s, 9 H), 0.90 (s, 6 H), 0.83 (s, 3 H); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^1H decoupled) δ 24.79 (s); MS (CI) 602 (MH^+). Anal. Calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.91; H, 8.82; N, 6.89.

For **13**: IR (CH_2Cl_2 cast) 2958, 1711, 1680, 1649 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65−7.37 (m, 5 H), 5.49 (s, 1 H), 4.30 (br s, 1 H), 4.22−3.95 (m, 4 H), 3.45 (ddd, 1 H, J = 2.8, 11.2, 16 Hz), 3.01 (s, 3 H), 2.54−1.11 (m, 13 H), 1.29 (dt, 6 H, J_{HH} = 8 Hz, J_{HP} = 5.2 Hz), 1.09 (s, 3 H), 1.05 (s, 9 H), 0.98 (s, 3 H), 0.80 (s, 3 H); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^1H decoupled) δ 24.47 (s); MS (CI) 602 (MH^+). Anal. Calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.70; H, 8.79; N, 6.96.

Hydrolysis of 12 to (1*R*,5*R*)-(1,5-Diamino-5-carboxypentyl)phosphonic Acid (14). Compound **12** (0.225 g, 0.374 mmol) was hydrolyzed in 12 N aqueous HCl as described above for preparation of **10** to afford **14** as its dihydrochloride salt (82.5 mg, 74% yield): IR (MeOH cast) 3600−2400, 1730, 1590 cm^{-1}; ^1H NMR (200 MHz, D_2O, DSS) δ 4.14 (t, 1 H, J = 6.4 Hz), 3.34 (dt, 1 H, J_{HH} = 6 Hz, J_{HP} = 14 Hz), 2.15−1.56 (m, 6 H); ^{13}C NMR (50.3 MHz, D_2O) δ 171.8, 52.5, 48.5 (d, J_{PC} = 145 Hz), 29.4, 27.9, 21.4 (d, J = 8 Hz); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) (^1H decoupled) δ 14.21 (s); FAB-MS 227 (MH^+).

Hydrolysis of 13 to (1*S*,5*R*)-(1,5-Diamino-5-carboxypentyl)phosphonic Acid (15). Compound **13** (0.155 g, 0.258 mmol) was hydrolyzed in 12 N aqueous HCl as described above for preparation of **10** to afford **15** as its dihydrochloride salt (54.0 mg, 70% yield): IR (KBr) 3700−2400, 1730, 1610 cm^{-1}; ^1H NMR (200 MHz, D_2O, DSS) δ 4.14 (t, 1 H, J = 6.4 Hz), 3.34 (dt, 1 H, J_{HH} = 6 Hz, J_{HP} = 14 Hz), 2.15−1.55 (m, 6 H); ^{13}C NMR (50.3 MHz, D_2O) δ 171.9, 52.6, 48.5 (d, J_{PC} = 144 Hz), 29.4, 28.0, 21.4 (d, J = 8 Hz); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) (^1H decoupled) δ 13.88 (s); FAB-MS 227 (MH^+).

Diethyl [(2*S*,5*S*,1′*R*,4′*R*,1″*R*)-[4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-oxoimidazolidin-5-yl]-1-[(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)amino]butyl]phosphonate (18) and Diethyl [(2*S*,5*S*,1′*R*,4′*R*,1″*S*)-[4-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-oxoimidazolidin-5-yl]-1-[(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)-amino]butyl]phosphonate (19). The procedure employed above to prepare **8** and **9** was used to alkylate (+)-camphor imine **17**[14] (2.00 g, 6.64 mmol) with the imidazolidinone bromide **3** (2.53 g, 6.64 mmol), thereby giving a mixture of compounds **18** and **19** in 88% yield (3.52 g) in a ratio of 3:2. Compound **18** (1.31 g, 33% yield) was separated from **19** (0.906 g, 23% yield) on an MPLC column (silica gel, 2.5 × 30 cm) using EtOAc as eluent. For **18**: IR ($CHCl_3$ cast) 2958, 1710, 1647 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65−7.37 (m, 5 H), 5.57 (s, 1 H), 4.30 (br s, 1 H), 4.22−3.93 (m, 4 H), 3.48 (ddd, 1 H, J = 4.8, 8.8, 12.8 Hz), 3.04 (s, 3 H), 2.54−2.38 (m, 1 H), 1.99−1.11 (m, 12 H), 1.26 (dt, 6 H, J_{HH} = 8 Hz, J_{HP} = 4.4 Hz), 1.04 (s, 9 H), 0.90 (s, 6 H), 0.83 (s, 3 H); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^1H decoupled) δ 24.79 (s); MS (CI) 602 (MH^+). Anal. Calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.57; H, 8.75; N, 6.97.

For **19**: IR ($CHCl_3$ cast) 2960, 1709, 1679, 1648 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.65−7.37 (m, 5 H), 5.49 (s, 1 H), 4.30 (br d, 1 H, J = 4 Hz), 4.22−3.95 (m, 4 H), 3.45 (ddd, 1 H, J = 2.8, 11.2, 16 Hz), 3.01 (s, 3 H), 2.54−1.11 (m, 13 H), 1.29 (dt, 6 H, J_{HH} = 8 Hz, J_{HP} = 5.2 Hz), 1.09 (s, 3 H), 1.05 (s, 9 H), 0.98 (s, 3 H), 0.80 (s, 3 H); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) (^1H decoupled) δ 24.49 (s); MS (CI) 602 (MH^+). Anal. Calcd for $C_{33}H_{52}N_3O_5P$: C, 65.87; H, 8.71; N, 6.98. Found: C, 65.92; H, 8.60; N, 6.83.

Tetraethyl (1*S*,4*S*,1′*S*,4′*S*)-[1,5-Bis[(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)amino]pentane-1,5-diyl]-diphosphonate (20). To a solution of the (−)-camphor imine **7**[14] (0.709 g, 2.36 mmol) in THF (15 mL) at −78 °C was added dropwise a *n*-BuLi solution (2.59 mmol). Stirring was continued at −78 °C for 20 min, and 1,3-dibromopropane (0.240 g, 1.19 mmol) in THF (4 mL) was added. The mixture was stirred at −78 °C for 3 h, warmed to 20 °C over 2 h, and stirred overnight. It was poured into half-saturated aqueous NH_4Cl (15 mL). The product was extracted with EtOAc (3 × 60 mL). The combined organic phases were dried and concentrated *in vacuo* to give an oil, which was purified by flash chromatography using EtOAc/MeOH (95/5) to afford **20** as a mixture of all possible isomers at the α-centers in 62% yield (0.472 g): IR (CH_2Cl_2 cast) 2958, 1680 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.26−3.98 (m, 8 H), 3.72−3.50 (m, 2 H), 2.64−1.00 (m, 20 H), 1.40−1.22 (m, 12 H), 0.96 (s, 6 H), 0.92 (s, 6 H), 0.86 (s, 3 H), 0.72 (s, 3 H); ^{31}P NMR (81.0 MHz, $CDCl_3$, H_3PO_4) δ 25.25 (s) and 25.01 (s) in 7:3 ratio; exact mass 642.3930 (642.3926 calcd for $C_{33}H_{60}N_2O_6P_2$). Anal. Calcd for $C_{33}H_{60}N_2O_6P_2$: C, 61.66; H, 9.41; N, 4.36. Found: C, 61.40; H, 9.25; N, 4.20.

Tetraethyl (1*R*,4*R*,1′*R*,4′*R*)-[1,5-Bis[(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)amino]pentane-1,5-diyl]diphosphonate (21). The procedure described above for the preparation of **20** was used to convert (+)-camphor imine **17**[14] (0.683 g, 2.27 mmol) and 1,3-dibromopropane (0.230 g, 1.14 mmol) to **21** (0.474 g, 65% yield) as a mixture of all possible isomers at the α-centers. The spectroscopic properties of **21** are indistinguishable from those of **20**.

Hydrolysis of 20 or 21 to [1,5-Diaminopentane-1,5-diyl]diphosphonic Acid (22). Compound **20** (0.201 g, 0.312 mmol) was hydrolyzed in 12 N aqueous HCl as described above for preparation of **10** to afford **22** as a dihydrochloride salt (a mixture of isomers) in 63% yield (65.4 mg): IR (MeOH cast) 3700–2000, 1615, 1520, 1170, 1060, 940 cm^{-1}; ^{1}H NMR (200 MHz, D_2O, DSS) δ 3.38 (dt, 2 H, J_{HH} = 6.5 Hz, J_{HP} = 14 Hz), 2.15–1.57 (m, 6 H); ^{13}C NMR (50.3 MHz, D_2O) δ 48.41 (d, J = 145.4 Hz), 48.28 (d, J = 145.0 Hz), 27.98, 27.88, 22.44 (t, J = 8.6 Hz), 22.29 (t, J = 7.4 Hz); ^{31}P NMR (81.0 MHz, D_2O, H_3PO_4) δ 14.57 (s); FAB-MS 263 (MH$^+$). The spectral properties of **22** prepared from **21** in an analogous fashion are indistinguishable from this sample and are in good agreement with those reported in the literature for a mixture of isomers prepared by a different procedure.[11]

(1*R*,5*S*)-(N_5-(L-Alanyl)-1,5-diamino-5-carboxypentyl)phosphonic Acid (24). To a stirred solution of P-DAP **10** (26 mg, 0.115 mmol) in 1 N aqueous Na_2CO_3 (0.5 mL) at 20 °C was added simultaneously and dropwise a solution of L-alanine *N*-carboxyanhydride **23**[20] (26 mg, 0.226 mmol) in dry THF (2 mL) and 1 N aqueous NaOH (0.5 mL). The mixture was then stirred at 20 °C for 10 min. The solvent was removed *in vacuo*, and the residue was applied to a cellulose column (2.5 × 27 cm). Elution with *n*-BuOH/H_2O/HOAc (4/2/1) gave **24** (16 mg, 47% yield): IR (MeOH cast) 3600–2400, 1670, 1580 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 4.17 (t, 1 H, J = 8.8 Hz), 4.10 (q, 1 H, J = 7.2 Hz), 3.21 (m, 1 H), 2.04–1.30 (m, 6 H), 1.54 (d, 3 H, J = 7.2 Hz); ^{13}C NMR (50.3 MHz, D_2O) δ 178.40, 170.15, 55.38, 49.30 (d, J = 135.9 Hz), 49.10, 31.08, 28.40, 22.36 (d, J = 4.0 Hz), 16.45; FAB-MS 298 (MH$^+$).

(1*S*,5*S*)-(N_5-(L-Alanyl)-1,5-diamino-5-carboxypentyl)phosphonic Acid (25). The P-DAP isomer **11** (47.0 mg, 0.208 mmol) was acylated with **23**[20] (51.0 mg, 0.443 mmol) by the procedure employed above for formation of **24** to give dipeptide **25** in 23% yield (14.0 mg): IR (MeOH cast) 3600–2400, 1671, 1580–1540 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 4.22 (dd, 1 H, J = 5.2, 8 Hz), 4.14 (q, 1 H, J = 7.2 Hz), 3.27 (m, 1 H), 2.10–1.30 (m, 6 H), 1.60 (d, 3 H, J = 7.2 Hz); ^{13}C NMR (50.3 MHz, D_2O) δ 178.35, 170.16, 55.07, 49.09 (d, J = 142 Hz), 49.09, 30.90, 28.18, 22.21 (d, J = 8 Hz), 16.42; FAB-MS 298 (MH$^+$).

(1*R*,5*R*)-(N_5-(L-Alanyl)-1,5-diamino-5-carboxypentyl)phosphonic Acid (26). The P-DAP isomer **14** (40.0 mg, 0.177 mmol) was acylated with **23**[20] (41.0 mg, 0.356 mmol) by the procedure employed above for formation of **24** to give dipeptide **26** in 19% yield (10.0 mg): IR (MeOH cast) 3600–2400, 1670, 1580 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 4.21 (dd, 1 H, J = 5.5, 8 Hz), 4.14 (q, 1 H, J = 7.5 Hz), 3.25 (m, 1 H), 2.10–1.30 (m, 6 H), 1.57 (d, 3 H, J = 7.5 Hz); ^{13}C NMR (50.3 MHz, D_2O) δ 178.56, 170.37, 55.09, 49.12 (d, J = 145 Hz), 49.19, 30.69, 28.22, 22.31 (d, J = 9 Hz), 16.63; FAB-MS 298 (MH$^+$).

(1*S*,5*R*)-(N_5-(L-Alanyl)-1,5-diamino-5-carboxypentyl)phosphonic Acid (27). The P-DAP isomer **15** (54.0 mg, 0.239 mmol) was acylated with **23**[20] (57.0 mg, 0.496 mmol) by the procedure employed above for formation of **24** to give dipeptide **27** in 19% yield (14.2 mg): IR (MeOH cast) 3600–2400, 1671, 1585, 1540 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 4.22 (dd, 1 H, J = 5.2, 8 Hz), 4.15 (q, 1 H, J = 7.2 Hz), 3.27 (m, 1 H), 2.10–1.30 (m, 6 H), 1.62 (d, 3 H, J = 7.2 Hz); ^{13}C NMR (50.3 MHz, D_2O) δ 178.51, 170.41, 55.28, 49.15 (d, J = 141 Hz), 49.24, 31.08, 28.32, 22.50 (d, J = 10 Hz), 16.68; FAB-MS 298 (MH$^+$).

(1*R*,5*S*)-(N_5-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl-L-alanyl]-1,5-diamino-5-carboxypentyl]phosphonic Acid (29). L-Alanyl-L-alanine (300 mg, 1.87 mmol) dissolved in water (1.8 mL) was treated with dioxane (3.75 mL) and cooled to 0 °C. To the resulting suspension was added aqueous 1 N NaOH (1.8 mL) followed by di-*tert*-butyl pyrocarbonate (Boc_2O) (600 mg). The temperature was kept at 0 °C and the pH was adjusted to 10 with 1 N NaOH after 10, 45, and 100 min. After 150 min, a further portion of Boc_2O (200 mg) was added. The mixture was stirred for 17 h, concentrated to ca. 5 mL, and adjusted to pH 11.5. The solution was extracted with EtOAc (3 × 5 mL), acidified to pH 2 with 10% $KHSO_4$, and extracted with EtOAc (5 × 10 mL). The organic phase from the acidic extraction was dried and evaporated to give Boc-L-alanyl-L-alanine (**28**) (459 mg, 94% yield): mp 108–111 °C; $[\alpha]_D$ = −10.4 (c = 0.67, EtOAc); IR (KBr) 3440, 3352, 2980, 1685, 1670, cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.48 (s, 1 H), 6.12 (s, 1 H), 4.44 (m, 1 H), 4.16 (m, 1H), 1.40 (s, 9 H), 1.38 (d, 3 H, J = 6.5 Hz), 1.30 (d, 3 H, J = 6.5 Hz); ^{13}C NMR (100 MHz, acetone-d_6) δ 174.1, 173.3, 156.1, 79.3, 50.7, 48.5, 28.5, 18.7, 18.1; FAB-MS (Cleland) 283 (11 , MNa$^+$), 261 (43 , MH$^+$).

To make an active ester for peptide coupling, the Boc-L-Ala-L-Ala (**28**) (200 mg, 0.768 mmol) and *N*-hydroxy-5-norbornene-*endo*-2,3-dicarboximide (HONB) (138 mg, 0.770 mmol) were dissolved in anhydrous EtOAc (2.0 mL) and THF (2.0 mL). The solution was cooled to 0 °C, dicyclohexylcarbodiimide (DCC) (159 mg, 0.771 mmol) was added, and the resulting mixture was stirred at 0 °C for 30 min and then at 20 °C for 16 h. The precipitate was removed by centrifugation and washed with EtOAc (3 × 4 mL). The combined supernatants were concentrated *in vacuo* to give a white solid which was washed with pentane (2 × 5 mL) to remove unreacted DCC and then dried. The resulting Boc-L-Ala-L-Ala-ONB (317 mg) was used directly for coupling to the P-DAP isomers.

Solid (1*R*,5*S*)-P-DAP (**10**) (15 mg, 0.057 mmol) was dissolved in aqueous 0.5 M Na_2CO_3 (0.3 mL). A solution of Boc-L-Ala-L-Ala-ONB (43 mg) in DMF (0.30 mL) was added with stirring over 20 min. After 2 h, the mixture was evaporated and the residue was purified on a Merck Lobar RP-18 column (H_2O) and on a Sephadex G-10 Column (H_2O) to give 18.5 mg of the Boc-protected tripeptide **29** as its trisodium salt (60% yield): IR (KBr) 3360, 2995, 2960, 2920, 1685, 1530 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 4.33 (q, 1 H, J = 7.3 Hz), 4.12 (m, 1 H), 4.06 (q, 1 H, J = 7.3 Hz), 3.0 (m, 1 H); 2.0–1.26 (m, 12 H), 1.41 (s, 9 H); FAB-MS (Cleland) 491 (10, MNa$^+$), 469 (4, MH$^+$).

(1*R*,5*S*)-[N_5-(L-alanyl-L-alanyl)-1,5-diamino-5-carboxypentyl]phosphonic Acid (30). Protected tripeptide **29** (15 mg (dihydrate), 0.028 mmol) was dissolved in trifluoroacetic acid (1.0 mL) and kept at 20 °C for 3.5 h. The solvent was evaporated, and the residue was separated on a cellulose (Merck) column (2.5 × 22 cm) which was eluted with *n*-BuOH/H_2O/HOAc (4/2/1) to give **30** (10.8 mg, quantitative): IR (KBr) 3600–2500, 1653 cm^{-1}; ^{1}H-NMR (400 MHz, D_2O) δ 4.34 (q, 1 H, J = 7.2 Hz), 4.17 (dd, 1 H, J = 5.3, 8.0 Hz), 4.08 (q, 1 H, J = 7.1 Hz), 3.23 (m, 1 H), 1.97–1.45 (m, 6 H), 1.55 (d, 3 H, J = 7.2 Hz), 1.45 (d, 3 H, J = 7.3 Hz); ^{31}P NMR (162 MHz, D_2O) (^{1}H decoupled) δ 13.4 (s); FAB-MS (Cleland) 391 (0.4, MNa$^+$), 369 (0.9, MH$^+$).

(1*S*,5*S*)-[N_5-[*N*-(*tert*-Butoxycarbonyl)-L-alanyl-L-alanyl]-1,5-diamino-5-carboxypentyl]phosphonic Acid (31). The procedure employed above to prepare **29** was used to couple Boc-L-Ala-L-Ala-ONB (20 mg) to (1*S*,5*S*)-P-DAP (**11**) (15 mg, 0.057 mmol) to give 10 mg of protected tripeptide **31** as its trisodium salt (33% yield): IR (KBr) 3435, 2980, 2830, 1650, 1540 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 4.32 (q, 1 H, J = 7.0 Hz), 4.18 (dd, 1 H, J = 5.3, 7.8 Hz), 4.06 (q, 1 H, J = 7.1 Hz), 3.23 (m, 1 H), 2.0–1.3 (m, 12H), 1.14 (s, 9 H); FAB-MS (HCOOH–glycerol) 491 (3.0, MNa$^+$), 469 (9.9, MH$^+$).

(1*S*,5*S*)-[N_5-(L-Alanyl-L-alanyl)-1,5-diamino-5-carboxypentyl]phosphonic Acid (32). The procedure used above to deprotect **29** to **30** was employed to convert protected tripeptide **31** (9.0 mg, 0.017 mmol) to **32** (7.5 mg, quantitative): IR (KBr) 3600–2500, 1656, 1650, 1579 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 4.35 (q, 1 H, J = 7.2 Hz), 4.16 (dd, 1 H, J = 4.9, 7.6 Hz), 4.08 (q, 1 H, J = 7.2 Hz), 3.09 (m, 1 H), 2.0-1.38 (m, 6 H), 1.54 (d, 3 H, J = 7.1 Hz), 1.40 (d, 3 H, J = 7.2 Hz); ^{31}P NMR (162 MHz, D_2O) (^{1}H decoupled) δ 12.2 (s); FAB-MS (Cleland) 391 (1.2, MNa$^+$), 369 (1.5, MH$^+$).

Inhibition Studies with DAP Decarboxylase. *meso*-Diaminopimelate decarboxylase was isolated from 500 g of wheat germ (*Triticum vulgaris*, Sigma) by modification of a previous method.[22] After protamine sulfate fractionation, a 40% ammonium sulfate fractionation was performed. The pellet obtained was resuspended in buffer A (10 mM potassium phosphate buffer containing 0.025% dithiothreitol, 50 μM pyridoxal-5′-phosphate, and 20% ethylene glycol, pH 6.9) and

was dialyzed against the same buffer before loading onto a DE52 column (4.2 × 16 cm). The column was washed with buffer A and then with buffer A containing 0.1 M NaCl. The decarboxylase was eluted with buffer A containing 0.15 and 0.25 M NaCl. Active fractions eluting at 0.25 M NaCl were pooled, concentrated by ultrafiltration, and dialyzed against buffer B (5 mM potassium phosphate pH 6.9, 50 μM pyridoxal-5′-phosphate, and 0.025% dithiothreitol) before loading onto a hydroxyapatite column (2.5 × 32 cm). This column was washed with buffer B and then eluted with a gradient of 5−40 mM potassium phosphate in buffer B. The enzyme was eluted as a broad peak from 10 to 35 mM potassium phosphate. Active fractions were pooled and concentrated to give a final specific activity of 0.02 units/mg protein, where 1 unit is defined as decarboxylation of 1 μmol DAP/min.

Diaminopimelate decarboxylase was assayed as previously described by measuring the amount of $^{14}CO_2$ released from [1,7-^{14}C]diaminopimelate over 20 min at 37 °C.[21b] Potential inhibitors (320 and 560 μM) were incubated with 1 mU DAP decarboxylase with 45 μM *meso*-DAP for 20 min at 37 °C. For kinetic analyses, reaction rates over a range of substrate concentrations at inhibitor concentrations of 0, 200, 320, and 560 μM were measured, and the data were analyzed by the statistical method of Wilkinson,[34] to yield K_m = 59 μM for *meso*-diaminopimelate and K_i = 210 μM for **11**.

Inhibition Studies with DAP Dehydrogenase. DAP dehydrogenase was isolated and purified by literature procedure[24a] from *Bacillus sphaericus* IFO 3525 with modifications as previously described.[6d] Spectrophotometric assays were performed on a Hewlett Packard (HP) 8452A diode array spectrophotometer equipped with a HP Vectra ES computer loaded with kinetics and quantitation programs as part of the HP89530A MS DOS-UV/VIS operating software.

The enzyme was assayed at 25 °C in 0.1 M Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, and 0.3 mM NADP at pH 7.8, unless otherwise noted, with varying *meso*-DAP concentrations as noted below in a total volume of 1 mL. The increase in absorbance at 340 nm, using a 1 cm light path, was then followed. For examination of the inhibitors as substrates, *meso*-DAP was omitted from the assay mixture and 1.8 mU of enzyme was present. *meso*-DAP concentration was varied from 0.0105 to 0.175 mM for inhibition curves with a constant inhibitor concentration of 2.25 mM for P-DAP analogues and compared against a control performed simultaneously. The K_m for *meso*-DAP under these conditions was found to be 0.092 mM (lit. K_m 0.11mM).[10a] Possible time-dependent inhibition was tested by incubating the enzyme (1.3 mU) and the inhibitor at 25 °C and removing aliquots at 0, 30, 60, 90 and 120 min and assaying under standard conditions with 0.175 mM *meso*-DAP. These results were compared against a control performed simultaneously, where Tris-HCl buffer replaced the inhibitor.

Inhibition Studies with DAP Epimerase. DAP epimerase was isolated from a strain of *E. coli* JM109 containing plasmid pDF6 which overexpresses DAP epimerase (obtained from Dr. William Higgins, Merrell-Dow Research Institute, Strasbourg, France) using our recent modification[6b] of the literature procedure.[10] For isolation procedures and inhibition studies with the diphosphonate DAP analogue **22**, the literature coupled assay with DAP dehydrogenase was employed.[10a] LL-DAP concentration varied from 0.05 to 2 mM in the presence and absence varying concentrations of **22** (total assay volume 1 mL). Possible time-dependent inhibition was tested by incubating 23 mU of enzyme in the presence of 3 mM inhibitor at 25 °C and removing aliquots at 0, 30, 60, 90 and 120 min and assaying under standard assay conditions with 0.36 mM LL-DAP. Results were compared against a control run simultaneously.

For inhibition studies with P-DAP analogues, DAP epimerase was assayed using a slightly modified literature procedure[10a] which follows the release of tritium from [G-^{3}H]-diaminopimelate (mixture of isomers) into water. Typically 200 μL of a reaction mixture containing 6 mU of epimerase in 0.1 M Tris-HCl (pH 7.8), 1 mM EDTA, and 1 mM dithiothreitol with LL-DAP concentrations of 0.05, 0.10, 0.20, 0.40, 1.0, and 2.0 mM was incubated in the presence and absence of 3.0 mM inhibitor for 40 min. The solution was acidified with 500 μL of 10% trichloroacetic acid and applied to a column (0.5 mL) of Biorad AG 50W-X8 ion-exchange resin (H^+) form. The column was washed three times with 500 μL of water and the eluates combined and counted for radioactivity. Kinetic constants K_m and V_{max} were obtained from the nonlinear regression program Enzfitter, using Michaelis Menten kinetics and explicit weighting. Possible time-dependent inhibition was tested by incubation of 12 mU of DAP epimerase in the presence and absence of 3 mM inhibitor at 25 °C. Aliquots were removed at 0, 30, 60, and 120 min and assayed under standard assay conditions using 0.30 mM LL-DAP.

Compounds were tested as substrates for the epimerase by incubating 0.7 U of of active or heat-inactivated epimerase in the presence of 3.0 mM inhibitor in deuterated 0.1 M Tris-HCl, 1 mM EDTA, and 1 mM dithiothreitol (pD 7.8) (total volume 3.0 mL) at 25 °C for 6 h. The reactions were quenched by rapid freezing in a −78 °C bath and the thawed mixtures were immediately loaded onto columns (0.5 mL) of Biorad AG1-X8 ion-exchange resin (OH^- form) and washed with water (2 × 1 mL). Compounds were eluted with 1 M HCl (2.5 mL) and lyophilized. ^{1}H NMR spectra were obtained in 99.9% D_2O, and integrals of the α-hydrogens were compared to those of other resonances, both for analogues incubated with active enzyme and exposed to heat-inactivated enzyme.

Antibacterial Testing. Turbidimetric growth studies were done in defined liquid medium (using ammonium sulfate as a nitrogen source) as described previously.[22] Six wild-type strains were employed: *Bacillus subtilis* ATCC 6051; *B. megaterium* ATCC 15374; *E. coli* ATCC 9637; *Pseudomonas aeruginosa* ATCC 10145; *Salmonella typhimurium*; and *Serratia marcescens*. Optical density readings of growth at 6 h and 24 h showed that, within experimental error (10%), only negligible or low antibiotic activities could be detected for P-DAP isomers **10**, **11**, **14**, **15**, and bis-phosphonate **22**.

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method[35] in Davis−Mingoli minimal agar[36] with and without L-lysine supplementation at 40 mg/L. Thirty-two strains were employed: *Escherichia coli* strains 257, ATCC 25922B, 2721B, 2722B; *Salmonella typhimurium* strains LT-2, ATCC 13311; *Citrobacter freundii* strains EL-1408, BS-16; *Klebsiella pneumoniae* 369; *K. oxytoca* 1082EN; *Enterobacter cloacae* strains 4192-2, 5699, P99; *Bacillus subtilis* 558; *Serratia marcescens* strains SM, 1071, MA; *Providencia rettgeri* ATCC 9250; *Acinetobacter calcoaceticus* PCI-3; *Pseudomonas aeruginosa* strains ATCC 27853, 5712, 8780, 765, 18SH, K799/WT, K799/61, 56, 56M, 56-1B; *Pseudomonas cepacia* 1973B. Metabolite reversal tests were run in minimal agar using the counter diffusion technique.[37]

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Supplementary Material Available: ^{1}H NMR spectra for compounds **10**, **11**, **14**, **15**, **22**, **24**−**32** and HPLC data for **30** and **32** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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