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Asymmetric Syntheses of (2S,3S,6S)-, (2S,3S,6R)-, and (2R,3R,6S)-2,3-Methano-2,6-diaminopimelic Acids. Studies Directed to the Design of Novel Substrate-based Inhibitors of L,L-Diaminopimelate Epimerase

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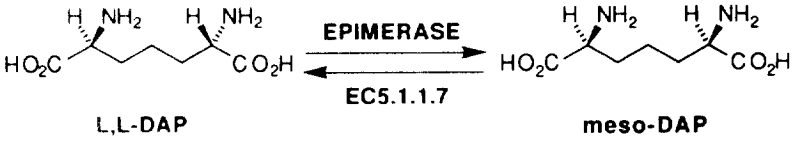
Abstract. The asymmetric syntheses of (2S,3S,6S)-, (2S,3S,6R)-, and (2R,3R,6S)-2,3-methano-2,6-diaminopimelic acids (**7a-c**) is described. The synthesis features phosphonate coupling of **13** and **14** to form the corresponding E-olefins which are subsequently cyclopropanated and deprotected under dissolving metal conditions.

INTRODUCTION

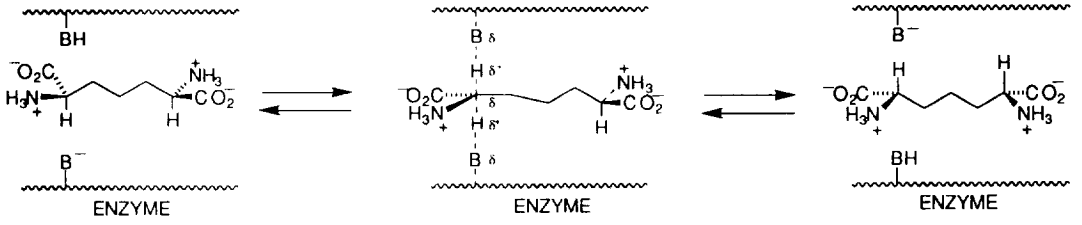
Currently there is growing interest in the design and synthesis of substrate-based inhibitors of any one of the approximately nine enzymes involved in the biosynthetic conversion of pyruvate and aspartate to *meso*-2,6-diaminopimelic acid (*meso*-DAP), an immediate biosynthetic precursor to L-lysine; a process necessary for the growth of bacteria and plants. Since mammals lack this pathway and require dietary intake of L-lysine, it is anticipated that specific inhibitors of this biochemical pathway should potentially display antibiotic activity with low host toxicity and might also serve as mechanism-based herbicides.

One key enzyme which has commanded much attention in recent years is L,L-diaminopimelate epimerase (EC 5.1.1.7),[2] an unusual enzyme responsible for the interconversion of (L,L)-2,6-diaminopimelic acid to *meso*-2,6-diaminopimelic acid without the aid of any cofactors such as pyridoxal phosphate. This epimerase enzyme was first discovered in 1957 by Antia and coworkers,[3] and was first purified from *Escherichia coli* by Wiseman and Nichols in 1984.[2] The enzyme exists as a monomer having a molecular weight of 34,000 Daltons and is believed to function via a two base in-line deprotonation-protonation mechanism (Scheme 1), much like that of proline racemase.[4] One of the basic residues is believed to be a thiol group based on an experiment in which the enzyme was alkylated and inhibited with iodoacetamide.



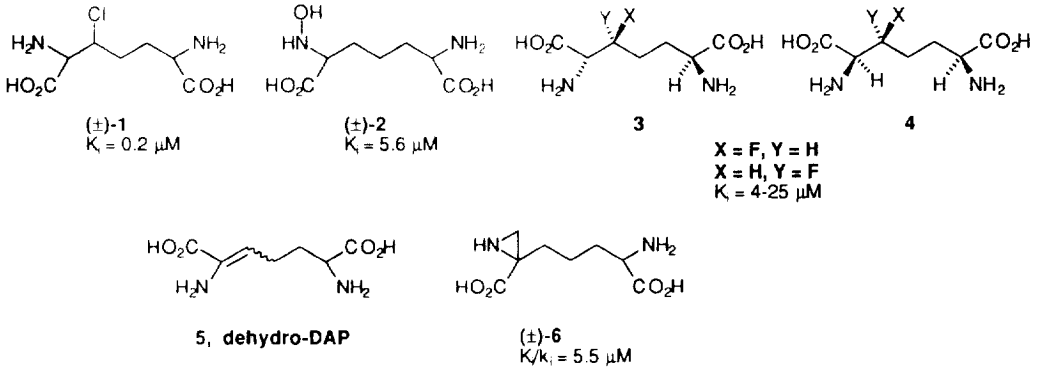
The inhibition of meso-DAP metabolism is now an active area of intense research, since this amino acid is a constituent of the cell wall peptidoglycan of virtually all Gram-negative and some Gram-positive bacteria. Peptidoglycan consists of linear sugar chains substituted with short peptide strands which are cross-linked at a D-alanine residue of one strand and *meso*-diaminopimelic acid of another via a peptide bond. The sugar chains are composed invariably of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid residues. The peptide strands consist of D- and L-configured amino acids and are generally conserved except for the third residue (e.g. L,L-DAP, *meso*-DAP, or L-lysine), which varies from species to species. Thus reduction or elimination of *meso*-DAP (or L,L-DAP or L-lysine) from bacteria should result in weakening of the cell wall, subsequent lysis, and finally cell death.

Scheme 1



Several DAP analogues have been synthesized [5] and studied for epimerase inhibition activity (Figure 1). Most of these analogues (**1**, **2**, **3**, and **4**) [5a,b,d] take advantage of the fact that some anionic character develops in the transition state on the α-carbon of (L,L)-DAP during the epimerase-catalyzed conversion to *meso*-DAP (see Scheme 1). Thus, appropriate leaving group functionalization of either the amino group or the β-carbon (e.g. hydroxyl- or halosubstitution, respectively) should result in formation of a planar transition state mimic. Therefore, enzyme-catalyzed elimination of HCl (from **1**), HF (from **3** and **4**), or H_2O (from **2**) leads to the common, unstable intermediate, dehydro-DAP (**5**).

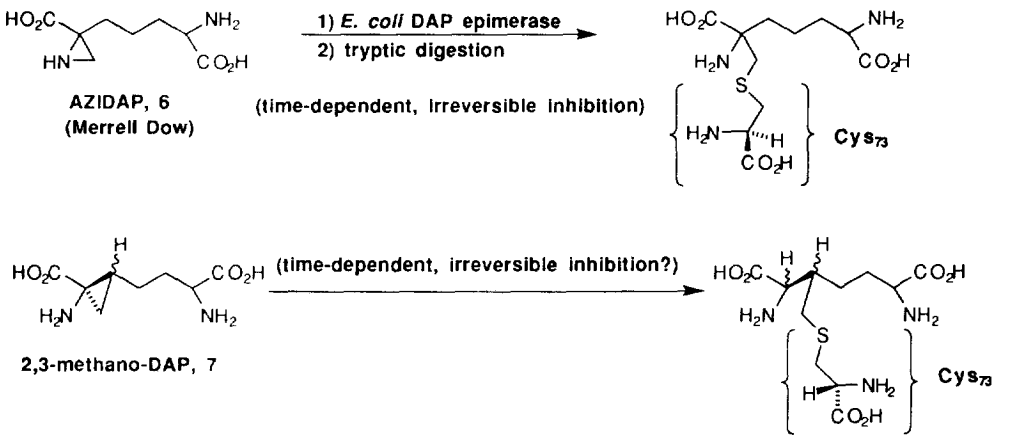
Figure 1



In a completely unique approach, stereo-random, racemic aziridino-DAP **6** was prepared and incubated with DAP epimerase and potent irreversible inhibition of the enzyme was observed.[5c] Subsequent tryptic digestion and peptide mapping of the covalently inactivated enzyme showed that the Cys^{73} residue was labeled [5d] thus supporting the original proposal of the thiol base mechanism (Scheme 2).[2] Compound **6** also displayed significant antimicrobial activity. The stereochemistry of the most active component of the stereorandom mixture of **6** has not been reported.

Based on the studies involving aziridino-DAP (**6**) as an electrophilic inhibitor of DAP epimerase, a study was initiated to explore the possibility of using 2,3-methano-2,6-diaminopimelic acid (**7**) (Scheme 2) as a potential substrate analog and electrophilic inhibitor of the same enzyme. Thus, it was envisioned that attack of the epimerase cysteine thiol on the cyclopropane group of **7** with concomitant ring opening [6] and release of strain energy would provide the necessary means for possible suicide inhibition of the epimerase.

Scheme 2

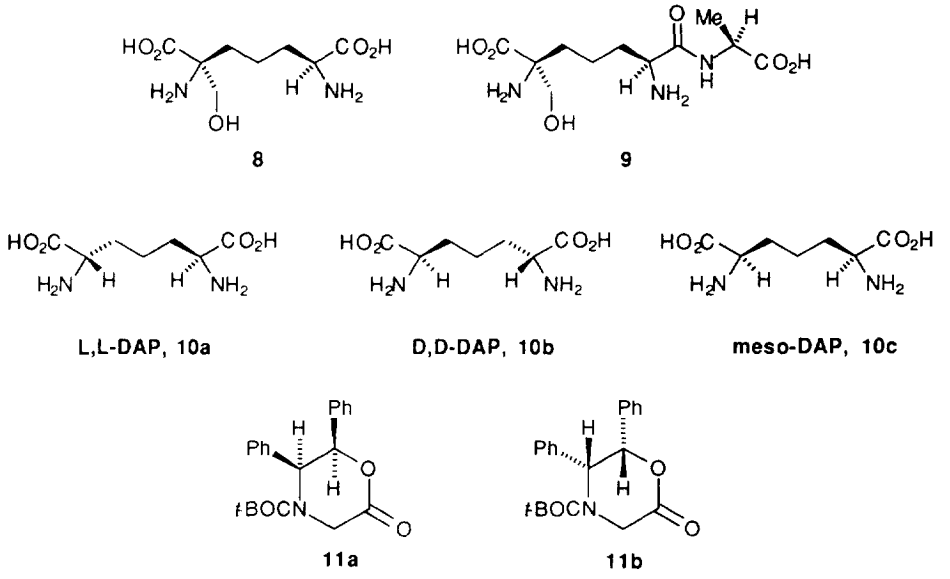


RESULTS AND DISCUSSION

During the past few years, we have been intensively interested in the design and synthesis of diaminopimelate pathway metabolites and inhibitors (Figure 2). Recently, our group[7] and an Oxford group[8] have reported the asymmetric synthesis of 2,6-diamino-6-hydroxymethylpimelic acid (**8**) which is a constituent of the natural antibiotic dipeptide N-(2,6-diamino-6-hydroxymethylpimelyl)-L-alanine (**9**).[9] In addition, we have successfully accomplished asymmetric syntheses of (R,R)-, (S,S)-, and (S,R)-2,6-diaminopimelic acids (**10a-c**) [10] from the 4-(*tert*-butoxycarbonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-ones **11a** and **11b**, which serve as versatile templates from which a variety of carbon-carbon bond-forming strategies [11,12] can be employed to access a wide structural array of non-proteinogenic α-amino acids. Herein, we report the asymmetric syntheses of (2S,3S,6S)-, (2S,3S,6R)-, and (2R,3R,6S)-2,3-methano-2,6-diaminopimelic acids (**7a**, **7b** and **7c**, respectively) from the glycinates **11a** and **11b**; the biological activities of these novel DAP analogs has been examined and is described. This work is based largely on the synthetic methodology previously

described from this laboratory for the asymmetric syntheses of 1-aminocyclopropane-1-carboxylic acid derivatives. [13]

Figure 2

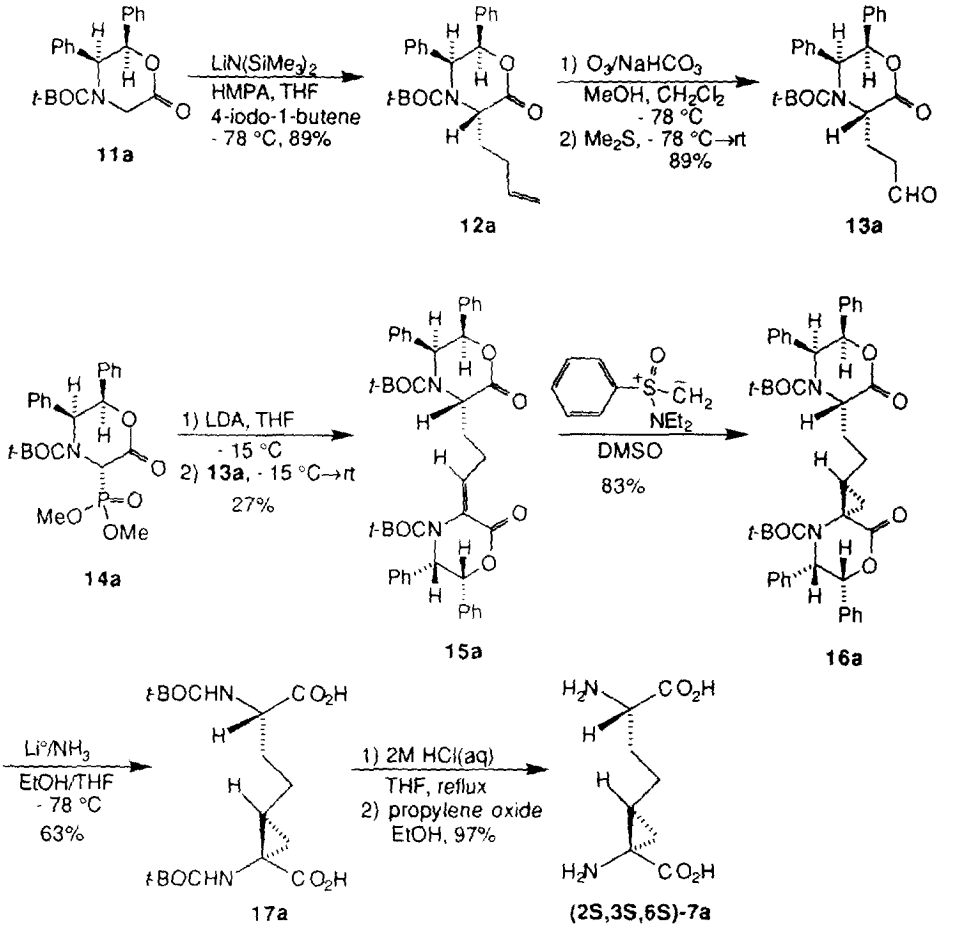


In order to successfully complete asymmetric syntheses of the individual diastereomeric forms of 2,3-methano-2,6-diaminopimelic acid (**7a-c**), two key problems had to be addressed. First, a method was required which would enable the coupling of two optically pure glycinates by a three carbon tether while simultaneously installing α,β-unsaturation to one glycinate terminus in geometrically pure form. Second, a cyclopropanating reagent was needed which could add in a highly stereoselective fashion to the carbon-carbon double bond of the coupled bis-glycinate adduct. Once these two critical problems have been solved, the only remaining tasks to finish the synthesis of 2,3-methano-2,6-diaminopimelic acids would simply involve the removal of the chiral auxiliary and deprotection of the nitrogen atoms. The issues of controlling olefin geometry and subsequent facial selectivity in the cyclopropanation reaction have been adequately addressed in our laboratory for the asymmetric syntheses of 2-alkyl-1-aminocyclopropane-1-carboxylic acids and has been reported on in full.[13]

As shown in Scheme 3, the synthesis of (2S,3S,6S)-2,3-methano-2,6-diaminopimelic acid **7a** began with the preparation of aldehyde **13a** [10] and phosphonate ester **14a**, [13] the two fragments necessary for constructing the parent backbone. Aldehyde **13a** was synthesized in 78% overall yield by first alkylating the lithium enolate of **11a** with 4-iodo-1-butene followed by ozonolysis of the resulting alkene **12a**. Emmons-Horner-Wadsworth olefination of **13a** with **14a** using LDA as base, provided exclusively (E)-alkene **15a** in 27% yield (38% yield based on recovered **14a**) as a single diastereomer. Comparison of the crude ^{1}H NMR of **15a** with that of α,β-dehydrolactones described previously[13] provided convincing evidence for the assigned olefin geometry. The

best yield of **15a** was obtained when a one mole excess of **14a** was used in the condensation reaction. The excess and unconsumed portions of **14a** could be routinely recovered during flash silica gel chromatography, whereas in experiments involving excess **13a**, attempts to recycle this reagent failed. As expected, aldehyde **13a** was unstable to the basic reaction conditions employed in the condensation process.

Scheme 3

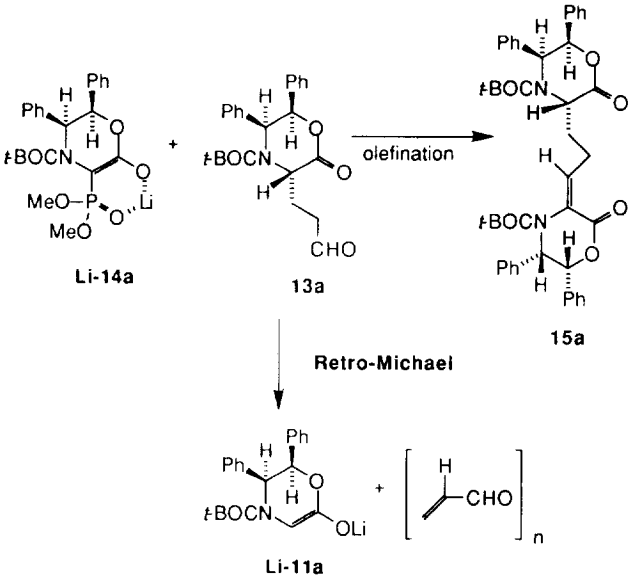


There is compelling evidence (^{1}H NMR) to suggest that the resulting low yield for this key coupling process is due to competitive retro-Michael degradation of aldehyde **13a** (Scheme 4). Despite the modest yield obtained for the olefination process, efforts to synthesize compound **7a** were pursued.

When alkene **15a** was treated with 1.5 equivalents of racemic [(diethylamino)phenyl]oxosulfonium methylide [14,15] in DMSO, cyclopropane **16a** was isolated as a single diastereomer [16] in 83% yield. Pure cyclopropane **16a** was obtained from the crude material via crystallization from absolute ethanol. Removal of

the chiral auxiliary from **16a** was accomplished by dissolving metal reduction (Li°/NH_3) to afford the bis-*t*-BOC-protected 2,3-methano-2,6-diaminopimelic acid **17a** in 62.5% yield. Deblocking of the *t*-BOC groups using HCl(aq) followed by conversion of the corresponding HCl salt to free (2S,3S,6S)-2,3-methano-2,6-diaminopimelic acid **7a** with propylene oxide was accomplished in 97% yield.

Scheme 4

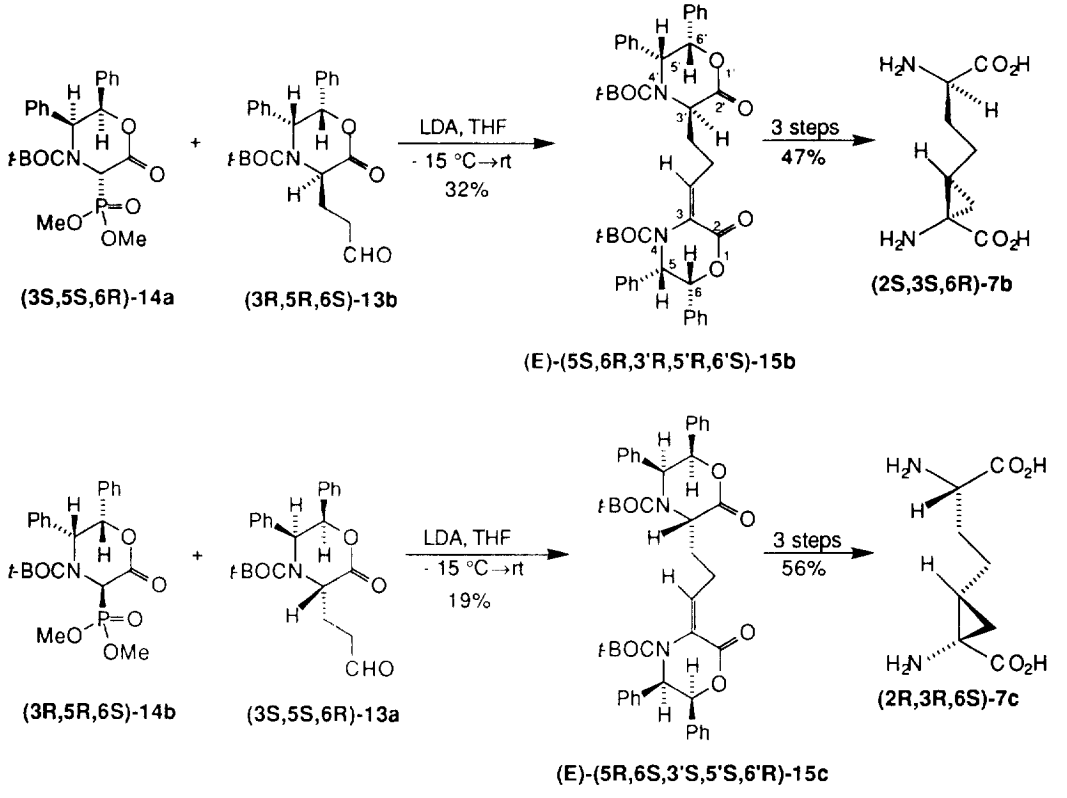


The synthesis of (2S,3S,6S)-2,3-methano-2,6-diaminopimelic acid **7a** was achieved entirely from lactone **11a**. The opportunity now existed to apply the same methodology to the synthesis of the (2S,3S,6R)- and (2R,3R,6S)-diastereomers of 2,3-methano-2,6-diaminopimelic acid **7b** and **7c**, respectively (isosteric analogs of D,L- and L,D-DAP, respectively) using a combination of both antipodes **11a** and **11b**. The purpose of this exercise was to ascertain any possible binding specificity of the DAP epimerase active site towards these DAP analogs. The (2R,3R,6R)-diastereomer of **7** was not considered in this preliminary investigation, since it represented an unnaturally-configured D,D- 2,6-diaminopimelic acid and was not expected to be processed by the enzyme.

The synthesis of (2S,3S,6R)- and (2R,3R,6S)-2,3-methano-2,6-diaminopimelic acids **7b** and **7c**, first required the synthesis of (3R,5R,6S)-**13b** and (3R,5R,6S)-**14b** from lactone **11b** respectively. As anticipated, the preparation of these intermediates occurred uneventfully and the spectroscopic data matched identically with the enantiomorphs in every respect, except for the sign of their respective optical rotation values. The remaining task simply involved the couplings of **14a** with **13b** and **14b** with **13a** to afford the desired "D,L- and L,D"-diastereomers of 2,3-methano-2,6-diaminopimelic acid according to the protocol described above. Again, the Emmons-Horner-Wadsworth reaction involving enantiomeric forms of phosphonate esters **14** and aldehydes **13** resulted in low yields of the corresponding alkenes **15**. Fortunately, both olefin diastereomers

were obtained in geometrically pure form as illustrated in the first example (see Scheme 3). A summary of the syntheses of (2S,3S,6R)- and (2R,3R,6S)-2,3-methano-2,6-diaminopimelic acids **7b** and **7c** is shown in Scheme 5.

Scheme 5



(3S,5S,6R)-14a **(3R,5R,6S)-13b** **(E)-(5S,6R,3'R,5'R,6'S)-15b** **(2S,3S,6R)-7b**

(3R,5R,6S)-14b **(3S,5S,6R)-13a** **(E)-(5R,6S,3'S,5'S,6'R)-15c** **(2R,3R,6S)-7c**

Having achieved syntheses of three diastereomers of 2,3-methano-2,6-diaminopimelic acids **7a-c**, three bioassay systems were employed to evaluate these substances for antibacterial activity. First, the three diastereomers of **7** were individually tested in Mueller Hinton agar against Gram (+) bacteria (*Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus*), Gram (-) bacteria (*Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Pseudomonas aeruginosa*), and Yeast (*Candida albicans, Saccharomyces cerevisiae*) using the agar-diffusion paper-disc technique. All microorganisms showed complete resistance to the 2,3-methano-2,6-diaminopimelic acid derivatives (**7a-c**) at concentrations up to 10 mg/mL. Since amino acids present in Mueller Hinton medium could have competed with **7** for uptake, the three diaseteriomers of **7** were individually tested for antimicrobial activity by the agar-dilution method in minimal agar in the presence and absence of L-lysine. Lysine supplementation is known to enhance the activity of compounds that block DAP synthesis.[17a] Unfortunately, none of the compounds inhibited cell growth in either medium up to 64 μg/ml.

These results, although disappointing, do not completely rule out the potential inhibition properties of **7** towards the epimerase enzyme, since the efficiency of drug transport across the cell envelope cannot be ascertained from these strictly qualitative experiments. A more rigorous set of experiments would require incubation of **7** with purified DAP epimerase to unambiguously determine activity profiles.

The third antibacterial assay directly measured peptidoglycan biosynthesis in ether-treated *E. coli* K12 JE5707. The procedure involved incubation of the microorganism with uridine diphospho-N-acetylmuramyl-L-Ala-D-Glu, *meso*- or L,L-DAP, D-Ala-D-Ala, and 2,3-methano-2,6-diaminopimelic acids (**7**). The extent of subsequent uridine diphospho-N-acetylmuramyl pentapeptide (a monomeric unit of peptidoglycan) incorporation into cell wall was analyzed with the aid of radiolabeled substrates, *meso*-2,6-diamino-(3,4,5-^{3}H)-pimelic acid or uridine diphospho[^{14}C]-*N*-acetylglucosamine. When *meso*-2,6-diamino-(3,4,5-^{3}H)-pimelic acid was employed, both (2S,3S,6S)-2,3-methano-2,6-diaminopimelic acid **7a** and (2R,3R,6S)-2,3-methano-2,6-diaminopimelic acid **7c** appeared to inhibit peptidoglycan biosynthesis to some extent (IC_{50}s of about 10 mM and 0.2 mM, respectively; Table 1).

Table 1. Peptidoglycan synthesis assay using (*meso*)-2,6-diamino-3,3,4,4,5,5-hexatritio-pimelic acid as radiolabeled substrate.

compound	concentration	% inhibition
(2S,3S,6S)-**7a**	10 mM	53
(2S,3S,6S)-**7a**	1 mM	12
(2S,3S,6S)-**7a**	0.1 mM	0
(2R,3R,6S)-**7c**	10 mM	100
(2R,3R,6S)-**7c**	1 mM	79
(2R,3R,6S)-**7c**	0.1 mM	43

Since (2S,3S,6S)-**7a** is isosteric to L,L-DAP, it was reincubated using L,L-DAP or *meso*-DAP with UDP[^{14}C]-*N*-acetylglucosamine as the radiolabeled substrate. (2S,3S,6S)-2,3-methano-2,6-diaminopimelic acid **7a** *stimulated* peptidoglycan synthesis when 50 μM L,L-DAP was used as the substrate (168% of control at 10mM), but had essentially no effect when 50 μM *meso*-DAP was used (104% of control at 10mM) (Table 2). These results appear to contradict the previous observation that (2S,3S,6S)-2,3-methano-2,6-diaminopimelic acid (**7a**) at 10 mM, slightly inhibited peptidoglycan biosynthesis. But this inconsistency can be rationalized if one considers that, (2S,3S,6S)-2,3-methano-2,6-diaminopimelic acid (**7a**) behaves as a substrate for the DAP-ligating enzyme. As a substrate, (2S,3S,6S)-**7a** competes with the radiolabeled *meso*-DAP, and therefore the reaction should produce less radioactively labeled peptidoglycan. The fact that there is no net increase in peptidoglycan biosynthesis when (2S,3S,6S)-**7a** is combined with *meso*-DAP and UDP[^{14}C]-*N*-acetylglucosamine, suggests that *meso*-DAP is a better substrate for the DAP-ligating enzyme than is (2S,3S,6S)-2,3-methano-2,6-diaminopimelic acid (**7a**). The hypothesis that (2S,3S,6S)-**7a** behaves as a substrate for the DAP-ligating enzyme was confirmed by testing the compound in the peptidoglycan biosynthesis assay in the absence of DAP (Table 3). Also, it does not appear that (2S,3S,6S)-2,3-methano-2,6-diaminopimelic acid **7a** is

an inhibitor of DAP epimerase, since peptidoglycan biosynthesis is stimulated, not inhibited, when L,L-DAP is present in the reaction medium. In similar fashion, (2R,3R,6S)-2,3-methano-2,6-diaminopimelic acid **7c**, which is isosteric to *meso*-DAP, was also found to be a substrate for the DAP-adding enzyme and appeared to only slightly inhibit peptidoglycan biosynthesis when incubated with radiolabeled *meso*-DAP (Table 2) while slightly increasing peptidoglycan biosynthesis when incubated with radiolabeled UDP-*N*-acetylglucosamine (Table 3). (2S,3S,6R)-2,3-methano-2,6-diaminopimelic acid (**7b**) was not tested in the permeabilized *E. coli*-mediated peptidoglycan biosynthesis assay system.

Table 2. Peptidoglycan synthesis assay using UDP[^{14}C]-*N*-acetylglucosamine as radio-labeled substrate.

compound	concentration	% of control
with 50 μM L,L-DAP:		
(2S,3S,6S)-**7a**	10 mM	168
(2S,3S,6S)-**7a**	1 mM	123
(2S,3S,6S)-**7a**	0.1 mM	108
with 50 μM D,L-DAP:		
(2S,3S,6S)-**7a**	10 mM	104
(2S,3S,6S)-**7a**	1 mM	107
(2S,3S,6S)-**7a**	0.1 mM	102
with 50 μM D,L-DAP:		
(2R,3R,6S)-**7c**	10 mM	71
(2R,3R,6S)-**7c**	1 mM	117
(2R,3R,6S)-**7c**	0.1 mM	110

Table 3. Peptidoglycan synthesis assay using UDP[^{14}C]-*N*-acetylglucosamine as radio-labeled substrate without addition of *meso*- or L,L-DAP.

compound	concentration	% of control	pmole incorporation
(2S,3S,6S)-**7a**	10 mM	101	59
(2S,3S,6S)-**7a**	1 mM	59	35
(2S,3S,6S)-**7a**	0.1 mM	11	7
(2R,3R,6S)-**7c**	2 mM	76	33
(2R,3R,6S)-**7c**	0.5 mM	71	31
(2R,3R,6S)-**7c**	0.1 mM	54	24
(2R,3R,6S)-**7c**	0.025 mM	29	13

The control reaction mixture contained 50 μM *meso*-DAP.

In summary, stereodefined syntheses of 2,3-methano-2,6-diaminopimelic acids (**7a-c**) have been achieved. Although these compounds did not exhibit antibacterial properties, the peptidoglycan biosynthesis assay data suggests that these compounds mimic DAP and can act as substrates for the DAP-adding enzyme as they appear to be incorporated into the cell wall. Efforts are underway to synthesize the corresponding Z-olefin isomers of **15** and the corresponding (2S,3R,6S)-, (2R,3R,6S)-, and (2S,3R,6R)-2,3-methano-2,6-diaminopimelic acids as well as a series of structures lacking one of the methylene units in the connecting chain.

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Experimental Section

General Information ^{1}H NMR spectra were obtained on a Bruker AC 300 MHz spectrometer and chemical shifts are reported in parts per million downfield from the internal standard. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR and are reported as λmax in cm^{-1}. Melting points were determined in open-ended capillary tubes on a Mel-Temp apparatus and are uncorrected. Optical rotations were obtained on a Rudolph Research Autopol III automatic polarimeter at a wavelength of 589 nm (sodium "D" line) with a 1.0-dm cell with a volume of 1 mL. Specific rotations, $[\alpha]_D$, are reported in degrees per decimeter at the specified temperature and the concentration (c) given in grams per 100 mL in the specified solvent. Elemental analyses are accurate to within ± 0.4% of the calculated values. High resolution mass spectraare accurate to within ± 3 millimass units. Column chromatography was performed with Merck silica gel grade 60, 230-400 mesh, 60 Å. Reagents and solvents were dried or purified as required. Purification of the free amino acids was accomplished by one or both of two methods: elution of an aqueous sample with H_2O on a Sep-Pak® C_{18} (reverse phase) cartridge manufactured by Waters, a Division of Millipore Corporation; or elution of the hydrochloride salt with 0.5 - 1.0 N NH_4OH on a column packed with Dowex® 50x8-100 cation-exchange resin.

Biological Assays

Antimicrobial susceptibility in Mueller Hinton agar was determined as described by Bauer, et. al. [17b] Antibacterial susceptibility assays in minimal agar medium were carried out as described by Song et al.[17c] Peptidoglycan biosynthesis was measured by following UDP[^{14}C]GlcNAc incorporation into SDS-insoluble material using *E. coli* K12 JE5707, a DAP, lys, thi mutant. Cells were starved for meso-DAP and ether-treated according to the method of Mirelman et al. (1976).[17d] Ether-treated cells were stored at -20°C at a

concentration of approximately 10 mg protein/ml until use. UDP-MurNAc-D-ala-D-glu was accumulated in *Staphylococcus aureus* ATCC 29213 grown in the absence of lysine, and isolated according to the method of Moore et al. (1979).[17e] Standard assay mixtures contained in 200 µl: [250 mM Tris pH 8.7, 25 mM $MgCl_2$, 10 mM ATP, 50 mM meso- or LL-DAP, 500 mM 2-mercaptoethanol, 100 mM UDP-MurNAc-dipeptide, 5 mM UDP-GlcNAc (75nCi), 100 mM D-ala-D-ala, 200 mg protein ether-treated *E. coli* K12 JE5707. Reactions were initiated by the addition of ether treated bacteria and incubated for 40 min at 30°C in a shaking water bath. Reactions were terminated by the addition of 1 ml of 4% SDS and boiling for 30 min. SDS-insoluble material was collected on 0.22 mm membrane filters (Millipore Corp. GSWP 025 00), washed twice with 2% SDS, and three times with deionized water. Filters were dried and placed in glass vials. Ten ml Filtron X (Dupont NEN) was added and the vials were counted in a Beckman LS 5000 TD scintillation counter.

(3S,5S,6R)-4-(*tert*-butoxycarbonyl)-5,6-diphenyl-3-(3'-butenyl)2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one (12a).

To a -78 °C mixture of lactone **11a** (1.0 g, 2.83 mmol, 1.0 equiv), 4-iodo-1-butene (1.54 g, 8.49 mmol, 3.0 equiv), HMPA (492.3 mL, 2.83 mmol, 1.0 equiv), and THF (10 mL) was added 1.0 M lithium bis(trimethylsilyl)amide in THF (3.11mL, 3.11 mmol, 1.1 equiv). The reaction was slowly warmed to room temperature and stirred overnight. The mixture was quenched with saturated NaCl (aq) and extracted with 3 x 5 mL EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. Flash silica chromatography (87 g silica, 1:10 EtOAc/hexanes) provided 1.03 g (89 %) **12a** as a clear glassy solid.

^1H NMR (300 MHz)(DMSO-d_6/K)δTMS: 1.18 (9H, s), 2.14-2.34 (4H, m), 4.81 (1H, apparent t, J = 6.9 Hz), 5.02-5.18 (3H, m), 5.85-5.98 (1H, m), 6.17 (1H, d, J = 3.2 Hz), 6.55-6.58 (2H, m), 7.04-7.27 (8H, m); IR (KBr) υ: 3072, 3031, 3007, 2981, 2932, 1752, 1699, 1638, 1397, 1272, 1176, 1056 cm^{-1}; $[\alpha]^{25}_D$ = - 54.6 ° (c = 1.0, CH_2Cl_2); mp = 152-153 °C; Anal. (recrystallized from EtOH) Calc'd for $C_{25}H_{29}NO_4$: C, 73.69; H, 7.17; N, 3.44. Found: C, 73.43; H, 7.13; N, 3.22.

(3R,5R,6S)-12b.

Yield (from **11b**) = 68 %; See spectroscopic data for the enantiomorph **12a.** $[\alpha]^{25}_D$ = + 57.2° (c = 1.0, CH_2Cl_2); mp = 149 -151°C (recrystallized from EtOH).

(3S,5S,6R)-4-(*tert*-butoxycarbonyl)-5,6-diphenyl-3-(2'-carbonylethyl)-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one (13a).

To a -78 °C slurry of **12a** (614 mg, 1.51 mmol, 1.0 equiv), $NaHCO_3$ (1.27 g, 15.07 mmol, 10.0 equiv), and 5:1 CH_2Cl_2/MeOH (60 mL) was bubbled excess O_3 until a faint blue color persisted. Excess O_3 was removed by bubbling O_2 through the slurry and the reaction quenched with excess Me_2S at - 78 °C. The mixture was warmed to room temperature and stirred overnight, filtered, and concentrated. Flash silica chromatography (31 g silica, 1:5 EtOAc/hexanes) provided 548.6 mg (89 %) **13a** as a white powder.

^1H NMR (300 MHz) (DMSO-d_6/373 K)δTMS: 1.17 (9H, s), 2.29-2.43 (2H, m), 2.66-2.75 (2H, m), 2.81-2.91 (2H, m), 4.82 (1H, apparent t, J = 7.7 Hz), 5.14 (1H, d, J = 3.0 Hz), 6.20 (1H, d, J = 3.2 Hz), 6.55-6.57 (2H, m), 7.03-7.25 (8H, m), 9.74 (1H, apparent t, J = 1.3 Hz); IR (KBr) υ: 3069, 3032, 2977, 2936, 2819, 2722, 1750, 1726, 1695, 1454, 1393, 1272, 1168, 1051 cm^{-1}; $[\alpha]^{25}_D$ = -51.3 ° (c = 1.0, CH_2Cl_2); mp = 160-162 °C; Anal. Calc'd for $C_{24}H_{27}NO_5$: C, 70.40; H, 6.65; N, 3.42. Found: C, 70.38; H, 6.71; N, 3.43.

(3R,5R,6S)-13b.

Yield (from **(3R,5R,6S)-12b**) = 84 %; See spectroscopic data for the enantiomorph **13a**. $[\alpha]^{25}_D$ = +51.6° (c = 1.0, CH_2Cl_2); mp = 164-166 °C.

(E)-(5S,6R,3'S,5'S,6'R)-alkene (15a).

To a -15 °C solution of phosphonate ester **14a** (1.35 g, mmol, 2.0 equiv) in THF (5 mL) was added 7.3 mL of 0.4 M LDA in THF (2.93 mmol, 2.0 equiv) via cannula. The mixture was stirred for 1h and **13a** (599.0 mg, 1.46 mmol, 1.0 equiv) was added in a single heap. The reaction was slowly warmed to room temperature, stirred for 1 day, and quenched with saturated NaCl (aq). The product was extracted with 3 x 5 mL EtOAc, dried over $MgSO_4$, filtered, and concentrated. Flash silica chromatography (45 g silica, 1:5 EtOAc/hexanes) followed by washing with hot EtOH provided 298.2 mg (27%, 38% based on recovered **14a**) **15a** as a white powder.

^{1}H NMR (300 MHz) (DMSO-d_6/373 K) δTMS: 1.18 (18H, s), 2.34-2.41 (2H, m), 2.69-2.80 (2H, m), 4.90 (1H, apparent t, J = 7.8 Hz), 5.20 (1H, d, J = 2.9 Hz), 5.33 (1H, d, J = 2.9 Hz), 6.00 (1H, d, J = 2.9 Hz), 6.23 (1H, d, J = 3.2 Hz), 6.58 (2H, d, J = 6.7 Hz), 6.67 (2H, d, J = 6.7 Hz), 6.72 (1H, apparent t, J = 7.8 Hz), 7.04-7.24 (16H, m); IR (KBr) υ: 3067, 3033, 2977, 2929, 1751, 1702, 1655, 1389, 1369, 1354, 1313, 1165 cm^{-1}; $[\alpha]^{25}_D$ = -73.2° (c = 1.0, CH_2Cl_2); mp = 233-235 °C (dec); Anal. (recrystallized from EtOH/THF) Calc'd for $C_{45}H_{48}N_2O_8$: C, 72.56; H, 6.49; N, 3.76. Found: C, 72.36; H, 6.59; N, 3.67.

(E)-(5R,6S,3'S,5'S,6'R)-15c. Yield (from **(3R,5R,6S)-14b** and **(3S,5S,6R)-13a)** = 19%:

See spectroscopic data for the enantiomorph, **15b**. $[\alpha]^{25}_D$ = + 46.0 ° (c = 1.0, CH_2Cl_2); mp = 229-231 °C (dec.); Anal. (recrystallized from EtOAc/hexanes). Calcd for $C_{45}H_{48}N_2O_8$: C, 72.56; H, 6.49; N, 3.76. Found: C, 72.70; H, 6.50; N, 3.77.

(E)-(5S,6R,3'R,5'R,6'S)-15b. Yield (from **(3S,5S,6R)-14a** and **(3R,5R,6S)-13b)** = 32%:

^{1}H NMR (300MHz) (DMSO-d_6/373 K) δTMS: 1.16-1.25 (20H, m), 2.28-2.38 (2H, m), 4.86-4.91 (1H, m), 5.17 (1H, d, J = 3.0Hz), 5.33 (1H, d, J = 3.2 Hz), 6.01 (1H, d, J = 2.8 Hz), 6.21 (1H, d, J = 3.1 Hz); IR (KBr)υ: 3090, 3064, 3032, 2976, 2932, 1747, 1708, 1702, 1655, 1456, 1386, 1355, 1281, 1259, 1167, 1142, 1115, 1082, 1058 cm^{-1}; $[\alpha]^{25}_D$ = - 45.0 ° (c = 1.0, CH_2Cl_2); mp = 229-231 °C (dec.).

(E)-(1S,3S,5S,6R,3'S,5'S,6'R)-Cyclopropane (16a).

To a room temperature mixture of (±)-[[(diethylamino)methyl]phenyl] oxosulfonium tetrafluoroborate (301.2 mg, 1.01 mmol, 1.5 equiv) and degreased NaH (24.2 mg, 1.01 mmol, 1.5 equiv) was added DMSO (4 mL). After stirring for 0.5 h, the freshly prepared ylide solution was added to a room temperature slurry of **15a** (500.0 mg, 0.67 mmol, 1.0 equiv) in DMSO (4 ml). The resulting reaction mixture was stirred for 5 days at room temperature. At this time the reaction was quenched with H_2O (10 mL) and EtOAc (20 mL) was added. The organic layer was thoroughly washed with several portions of H_2O, dried over MgSO4, filtered, and concentrated. Recrystallization of the crude material from absolute EtOH provided 423.3 mg (83%) **16a** as a white amorphous solid.

^{1}H NMR (300 MHz) (DMSO-d_6/373 K) δTMS: 1.11 (9H, s), 1.16 (9H, s), 1.67 (1H, dd, J_{gem} = 8.5 Hz, J_{vic} = 2.6 Hz), 1.92 (1H, m), 2.07 (1H, m), 2.23 (2H, m), 2.65 (1H, dd, J_{gem} = 9.7 Hz, J_{vic} = 4.0 Hz), 4.85 (1H, m), 5.14 (1H, d, J = 3.4 Hz), 5.33 (1H, d, J = 3.4 Hz), 6.13-6.16 (2H, m), 6.56 (2H, J = 6.7 Hz), 6.75 (2H, d, J = 7.7 Hz), 7.00-7.23 (16H, m); IR (KBr) υ: 3065, 3035, 2976, 2930, 2863, 1754, 1704, 1455, 1387, 1367, 1283, 1164, 1063 cm^{-1}; $[\alpha]^{25}_D$ = -5.0 ° (c = 1.0, CH_2Cl_2); mp = 225-226 °C (dec); Anal. (recrystallized from EtOH/THF) Calc'd for $C_{46}H_{50}N_2O_8$: C, 72.80; H, 6.64; N, 3.69. Found: C, 72.62; H, 6.70; N, 3.61.

(1S,3S,5S,6R,3'R,5'R,6'S)-16b.

Yield (from **(E)-(5S,6R,3'R,5'R,6'S)-15b** and (±)-(diethylamino)- phenyloxosulfonium methylide) = 88%:

See nmr data for the enatiomorph, **16c**. IR (KBr) υ: 3067, 3033, 2978, 2933, 1752, 1702, 1389, 1364, 1356, 1255, 1162, 1094, 1082, 1062 cm^{-1}; $[\alpha]^{25}_D$ = + 54.4 °(c = 1.0, CH_2Cl_2); mp = 209-211 °C (dec.); Anal. (recrystallized from EtOH/THF). Calcd for $C_{46}H_{50}N_2O_8$: C, 72.80; H, 6.64; N, 3.69. Found: C, 73.06; H, 6.75; N, 3.63.

(1R,3R,5R,6S,3'S,5'S,6'R)-16c.

Yield (from **(E)-(5R,6S,3'S,5'S,6'R)-15c** and (±)-(diethylamino)phenyloxosulfonium methylide) = 94%.

^{1}H NMR (300 MHz) (DMSO-d_6/373 K) δTMS: 1.05-1.41 (20H, m), 1.68 (1H, dd, J_{gem} = 8.5 Hz, J_{vic} = 2.4 Hz), 1.85-2.00 (1H, m), 2.20-2.29 (2H, m), 2.66 (1H, dd, J_{gem} = 9.5 Hz, J_{vic} = 3.4 Hz), 4.84 (1H, m), 5.13 (1H, apparent s), 5.32 (1H, d, J = 3.2 Hz), 6.14-6.17 (2H, m), 6.54-6.57 (2H, m), 6.72-6.74 (2H, m), 7.00-7.24 (20H, m); see ir data for the enantiomorph **16b**. $[\alpha]^{25}_D$ = - 52.4 ° (c = 1.0, CH_2Cl_2); mp = 208-210 °C (dec.).

(2S,3S,6S)-2,3-methano-2,6-di(N-(*tert*-Butoxycarbonyl)amino)pimelic acid (17a).

To a -78 °C slurry containing **16a** (423.0 mg, 0.56 mmol, 1.0 equiv), anhydrous EtOH (327.1 uL, 5.57 mmol, 10.0 equiv), THF(20 mL), and NH_3(45 mL) was added Li° pieces (~76 mg) until a persistent blue color was obtained. The resulting mixture was stirred an additional 10 min and then quenched with solid NH_4Cl. The ammonia was completely evaporated and the resulting white residue was dissolved in H_20 (~15 mL) and washed with Et_2O (3 x 5 mL). The aqueous layer was acidified to pH ~ 2.0 and extracted with EtOAc (3 x 5 mL). The organic extracts were combined, dried over Na_2SO_4, filtered, and concentrated. Purification of the crude product via preparative TLC (95:4:1, CH_2Cl_2:MeOH:CH_3CO_2H) provided 140.2 mg (63 %) **17a** as a clear oil.

^{1}H NMR (300 MHz) (DMSO-d_6) δTMS: 0.97-1.00 (1H, m); 1.15-1.80 (24H, m); 3.82-3.92 (1H, m); 6.94-7.10 (m) and 7.43 (s)(2H); 12.29 (2H, broad s); IR (NaCl, neat) υ: 3322, 2979, 2934, 2591, 1703(br), 1517, 1395, 1368, 1251, 1165, 1051 cm^{-1}; $[\alpha]^{25}_D$ = +17.80 ° (c = 1.03, CH_2Cl_2); Anal. Calc'd for $C_{18}H_{30}N_2O_8$: C, 53.72; H, 7.51; N, 6.96. Found: C, 53.67; H, 7.62; N, 6.95.

(2S,3S,6R)-17b.

Yield from **(1S,3S,5S,6R,3'R,5'R,6'S)-16b** = 64%:

^{1}H NMR (300 MHz) ($Cl_2CDCDCl_2$/373K)δ TMS: 1.27-1.37 (1H,m); 1.44-1.49 (18H); 1.71-2.05 (6H,m); 3.92 (apparent t, J = 6.2 Hz) ; 4.22 (1H, m); 5.13-5.52 (2H,m); 7.36 (2H, broad s). IR (NaCl/neat)υ: 3328, 2980, 2935, 2593, 1704, 1515, 1395, 1368, 1253, 1166, 1055 cm^{-1}; $[\alpha]^{25}_D$ = 0.00 ° (c = 1.0, CH_2Cl_2).

(2R,3R,6S)-17c.

Yield from **(1R,3R,5R,6S,3'S,5'S,6'R)-16c** = 60%:

$[\alpha]^{25}_D$ = 0.00° (c = 1.0, CH_2Cl_2); HRMS (FAB) calcd for $C_{18}H_{30}N_2O_8$ ($[M+H]^+$) 403.2080; obsd ($[M+H]^+$) 403.2078.

(2S,3S,6S)-2,3-Methano-2,6-diaminopimelic acid (7a).

To a room temperature solution of **17a** (201.0 mg, 0.50 mmol, 1.0 equiv) in THF (15mL) was added 2M HCl (aq) (15 mL). The resulting mixture was refluxed gently for 45 min, cooled to room temperature, and concentrated to dryness. The crude HCl salt was eluted on a Sep-pak C_{18} cartridge with H_2O and concentrated. The HCl salt was then dissolved in absolute EtOH (10 mL) and propylene oxide (~ 5 mL) was added at room temperature. Immediately, the free amino acid precipitated and was collected via Buchner filtration. This was further purified by dissolving the amino acid in H_2O (2 mL) and stirring for 1.5 h with 10 mg decolorizing charcoal. Removal of the charcoal via filtration on Celite and evaporation of solvent provided 98.0 mg (97 %) **7a** as a clear glassy solid.

^{1}H NMR (300 MHz) (D_2O) δHOD: 1.27-2.00 (7H, m), 3.68 (1H, apparent t, J = 5.5 Hz); IR (KBr) υ: 3439, 2989 (br), 1627 (br), 1509, 1407, 1346 cm^{-1}; $[\alpha]^{25}_D$ = + 10.45° (c = 0.67, H_2O); mp = 210-212 °C (dec).

(2R,3R,6S)-7c.

Yield from **(2R,3R,6S)-17c** = 99%:

^{1}H NMR (300 MHz) (D_2O) δHOD: 1.14-1.89 (7H, m), 3.56-3.62 (1H, m); IR (KBr) υ: 3673-2104 (broad), 1616, 1409, 1349, 1326, 1309, 1236, 1206 cm^{-1}; $[\alpha]^{25}_D$ = - 18.2 ° (c = 0.5, H_2O); mp = 215-217 °C (dec.); Anal. Calcd for $C_8H_{14}N_2O_4$: C, 47.52; H, 6.98; N, 13.85. Found: C, 47.33; H, 7.15; N, 13.78.

(2S,3S,6R)-7b.

Yield from **(2S,3S,6R)-17b** = 84%:

See nmr data for the enantiomorph, **7c**. $[\alpha]^{25}_D$ = + 18.8 ° (c = 0.5, H_2O); mp = 216-218 °C (dec.).

References and Footnotes

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1) Taken in part from the Ph.D. Thesis of G.J.F., Colorado State University, 1994.

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

3) Antia, M.; Hoare, D. S.; Work, E. *Biochem. J.* **1957**, *65*, 448.

4) a) Fischer, L. M.; Albery, W. J.; Knowles, J. R. *Biochemistry* **1986**, *25*, 2538. b) Fisher, L. M.; Belasco, J. G.; Bruice, T. W.; Albery, W. J.; Knowles, J. R. *Biochemistry* **1986**, *25*, 2543. c) Belasco, J. G.; Albery, W. J.; Knowles, J. R. *Biochemistry* 1986, 25, 2552. d) Belasco, J. G.; Bruice, T. W.; Albery, W. J.; Knowles, J. R. *Biochemistry* **1986**,

25, 2558. e) Belasco, J. G.; Bruice, T. W.; Fisher, L. M.; Albery, W. J.; Knowles, J. R. *Biochemistry* **1986**, *25*, 2564. f) Albery, W. J.; Knowles, J. R. *Biochemistry* **1986**, *25*, 2572.

5) Compounds **1** - **4,6** are the most potent DAP epimerase inhibitors known to date: a) Baumann, R. J.; Bohme, E. H.; Wiseman, J. S.; Vaal, M.; Nichols, J. S. *Antimicrob. Agents Chemother.* **1988**, *32*, 1119. b) Lam, L. K. P.; Arnold, L. D.; Kalantar, T. H.; Kelland, J. G.; Lane-Bell, P. M.; Palcic, M. M.; Pickard, M. A.; Vederas, J. C. *J. Biol. Chem.* **1988**, *263*, 11814. c) Gerhart, F.; Higgins, W.; Tardif, C.; Ducep, J-B. *J. Med. Chem.* **1990**, *33*, 2157 d) Gelb, M. H.; Lin, Y.; Pickard, M. A.; Song, Y.; Vederas, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 4932.

6) There is some precedence for ring opening of activated cyclopropanes by thiolate nucleophiles. See: a) Kondo, K.; Umemoto, T.; Takahatake, Y.; Tunemoto, D. *Tetrahedron Lett.* **1977**, 113. b) Zutterman, E.; DeWilde, H.; Mingheer, R.; De Clercq, P.; Vandevalle, H. *Tetrahedron Lett.* **1979**, *35*, 2389. c) Isobe, M.; Iio, H.; Kawai, T.; Goto, T. *J. Am. Chem. Soc.* **1978**, *100*, 1940. d) Iio, H.; Isobe, M.; Kawai, T.; Goto, T. *J. Am. Chem. Soc.* **1979**, *101*, 6076. d) Iio, H.; Isobe, M.; Kawai, T.; Goto, T. *Tetrahedron* **1979**, *35*, 941. For enzyme-mediated nucleophilic ring opening reactions of activated cyclopropanes, see: e) Walsh, C. T.; Pascal, R. A., Jr.; Johnston, M.; Raines, R.; Dikshit, D.; Krantz, A.; Honma, M. *Biochemistry*, **1981**, *20*, 7509. f) Hill, R. K.; Prakash, S. R.; Wiesendanger, R.; Angst, W.; Martinoni, B.; Arigoni, D.; Walsh, C. T.; Liu, H-W. *J. Am. Chem. Soc.* **1984**, *106*, 795. g) Liu, H-W.; Auchus, R.; Walsh, C. T. *J. Am. Chem. Soc.* **1984**, *106*, 5335.

7) Williams, R. M.; Im, M-N.; Cao, J. *J. Am. Chem. Soc.* **1991**, *113*, 6976.

8) Baldwin, J. E.; Lee, V.; Schofield, C. J. *Syn. Lett.* **1992**, 249.

9) Shoji, J.; Hinoo, H.; Kato, T.; Nakauchi, K.; Matsuura, S.; Mayama, M.; Yasuda, Y.; Kawamura, Y. *J. Antibiot.* **1981**, *34*, 374.

10) Williams, R. M.; Yuan, C. *J. Org. Chem.* **1992**, *57*, 6519.

11) a) Sinclair, P. J.; Zhai, D.; Riebenspies, J.; Williams, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 1103. b) Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. *J. Am. Chem. Soc.* **1988**, *110*, 1547. c) Sinclair, P. J. Ph.D. Thesis, Colorado State University, 1987; d) Williams, R. M.; Zhai, D.; Sincair, P. J. *J. Org. Chem.* **1986**, *51*, 5021; e) Williams, R. M.; Sinclair, P. J.; Zhai, W. *J. Am. Chem. Soc.* **1988**, *110*, 482; f) Williams, R. M.; Zhai, W. *Tetrahedron* **1988**, *44*, 5425. g) Zhai, D.; Zhai, W.; Williams, R. M. *J. Am. Chem. Soc.* **1988**, *110*, 2501; h) Williams, R. M.; Hendrix, J. A. *J. Org. Chem.* **1990**, *55*, 3723.

12) a) Williams, R. M.; Im, M-N. *Tetrahedron Lett.* **1989**, *29*, 6075 b) Williams, R. M.; Im, M-N. *J. Am. Chem. Soc.* **1991**, *113*, 9276.

13) Williams, R. M.; Fegley, G. J. *J. Am. Chem. Soc.* **1991**, *113*, 8796.

14) a) Johnson, C. R.; Janiga, E. R.; Haake, M. *J. Am. Chem. Soc.* **1968**, *90*, 3890. b) Johnson, C. R.; Schroeck, C. W. *J. Am. Chem. Soc.* **1968**, *90*, 6852. c) Johnson, C. R.; Haake, M.; Schroeck, C. W. *J. Am. Chem. Soc.* **1970**, *92*, 6594. d) Johnson, C. R.; Schroeck, C. W. *J. Am. Chem. Soc.* **1973**, *95*, 7418. e) Johnson, C. R.; Schroeck, C. W.; Shanklin, J. R. *J. Am. Chem. Soc.* **1973**, *95*, 7424. f) Johnson, C. R.; Janiga, E. R. *J. Am. Chem. Soc.* **1973**, *95*, 7692. g) Johnson, C. R.; Rogers, P. E. *J. Org. Chem.* **1973**, *38*, 1793. h) Johnson, C. R.; Rogers, P. E. *J. Org. Chem.* **1973**, *38*, 1798.

15) For a comprehensive review on the synthetic applications of sulfoximines, see: Johnson, C. R. *Aldrichimica Acta* **1985**, *18*, 3.

16) A comparison of the ^{1}H NMR spectra of **16** and cyclopropyllactones from our initial work[13] shows convincing evidence for analogous stereochemical outcomes in the cyclopropanation reaction.

17) a)Berges,D.A.; DeWolf,Jr., W. E.; Dunn, G. L.; Grappel, S. F.; Newman, D. J.; Taggart, J. J.; Gilvarg, C. *J. Med. Chem.* **1986**, *29*, 89. b)Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Turck, M., Am.J.Clin. Pathol. **1966**, *45*, 493; c)Song, Y.; Neiderer, D.; Lane-Bell, P. M.; Lam, L. K. P.; Crawley, S.; Palcic, M. M.; Pickard, M. A. ; Pruess, D. L.; Vederas, J. C. *J. Org. Chem.* **1994**, *59*, 5784. d)Mirelman, D. Y.; Yashouv-Gan, Y.; Schwarz, U. *Biochemistry* **1976**, *15*, 1781. e)Moore, B. A.; Jevons, S.; Brammer, K. W. *Antimicrob. Agents Chemothe.* **1979**, *15*, 513.

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