Synthesis of 3-Fluorodiaminopimelic Acid Isomers as Inhibitors of Diaminopimelate Epimerase: Stereocontrolled Enzymatic Elimination of Hydrogen Fluoride

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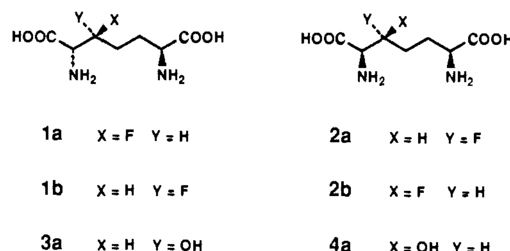
Abstract: Two hydroxylated and four fluorinated analogues of diaminopimelic acid (DAP) were synthesized in stereochemically pure form and examined for inhibition of diaminopimelate epimerase from *Escherichia coli.* The hydroxylated derivative of L,L-DAP, (2*S*,3*R*,6*S*)-2,6-diamino-3-hydroxydiaminopimelic acid (**3a**), and the corresponding analogue of *meso*-DAP, (2*R*,3*S*,6*S*)-2,6-diamino-3-hydroxydiaminopimelic acid (**4a**), were prepared by Seebach condensation of (2*S*)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**5**) and its enantiomer **8**, respectively, with (*S*)-3-(benzyloxycarbonyl)-4-(3-oxopropyl)-5-oxazolidinone (6). Compounds **3a** and **4a** proved to be very weak inhibitors of DAP epimerase (50% inhibition at 2.5 and 4.0 mM, respectively). Compound **3a** was epimerized at the nonhydroxylated end, but **4a** was not transformed. The 3-fluoro analogues of L,L-DAP, the 2*R*,3*S*,6*S* isomer **1a** and the 2*R*,3*R*,6*S* isomer **1b**, were synthesized by Schoellkopf aldol condensation of (3*R*)-3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazine (**11**) with aldehyde 6, followed by fluorination with (diethylamino)sulfur trifluoride and subsequent hydrolytic deprotection. Corresponding 3-fluoro derivatives of *meso*-DAP, the 2*S*,3*R*,6*S* isomer **2a** and the 2*S*,3*S*,6*S* isomer **2b**, were generated analogously from **12** (the enantiomer of **11**) and 6. All four fluoro DAP analogues were potent competitive inhibitors of DAP epimerase (IC_{50} 4–25 μM). Compounds **1b** and **2a**, having a 3*R* configuration at the carbon bearing fluorine, undergo rapid epimerase-catalyzed elimination of hydrogen fluoride without detectable epimerization at C-2 to eventually form tetrahydrodipicolinic acid (**19**). In contrast, the enzyme epimerizes **1a** and **2b** extensively at C-2 before slow elimination of hydrogen fluoride occurs. The results are discussed in terms of stereoelectronic requirements for elimination and possible conformational orientations of the enzyme-bound fluoro DAP analogues.

Recent studies in several laboratories demonstrate that a number of compounds which inhibit the formation or metabolism of 2,6-diaminopimelic acid (DAP) by bacteria possess antibiotic activity.[1,2] The L,L and meso isomers of this widely occurring amino acid lie on the biosynthetic path to L-lysine in bacteria[3,4] and higher plants[5] (Scheme I) and are key constituents of the peptidoglycan cell wall layer in many bacteria.[6] Since mammals lack the diaminopimelate pathway and require L-lysine in their diet,[7] specific inhibitors of the enzymes along this route are potential antimicrobial agents or herbicides with low toxicity.[1,2] One of the most interesting targets is L,L-diaminopimelate epimerase (EC 5.1.1.7), an unusual enzyme that interconverts L,L- and *meso*-DAP without the aid of detectable metals, cofactors, or intermediates that are sensitive to reagents which react with imine or keto functionalities.[8] Its mechanism has been suggested[8] to be similar to that of proline racemase;[9] an active-site thiol group may act as one of two bases in an "in-line" deprotonation–protonation sequence to accomplish the epimerization (Figure 1). Hence the α-carbon might be expected to develop anionic character that could lead to elimination of an adjacent electronegative substituent. Recently we showed that a mixture of isomers of *N*-hydroxydiaminopimelate strongly inhibits DAP epimerase.[1e] While this work was in progress, it was reported that mixtures of isomers of β-chlorodiaminopimelate[1f] and β-fluorodiaminopimelate[1g] also give potent inhibition of this enzyme, with possible involvement of elimination reactions. In the present work we describe the syntheses of pure isomers of 3-fluoro-2,6-diaminopimelic acid (**1a**, **1b**, **2a**, and **2b**) and examine the interaction of these conformationally mobile compounds with DAP epimerase from *Escherichia coli.* The results show that conformational orientations of enzyme-bound fluorodiaminopimelates control subsequent reactions and hence inhibitory properties.

Results

Synthesis of DAP Analogues. Examination of previous syntheses of β-fluorinated amino acids[10] suggested that an efficient ste-



reospecific route to β-fluorodiaminopimelates **1** and **2** might involve transformation of the corresponding β-hydroxy amino acids **3** and

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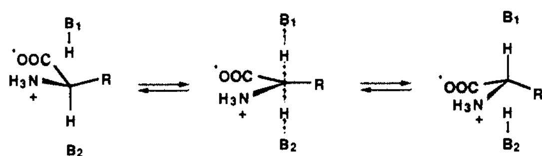
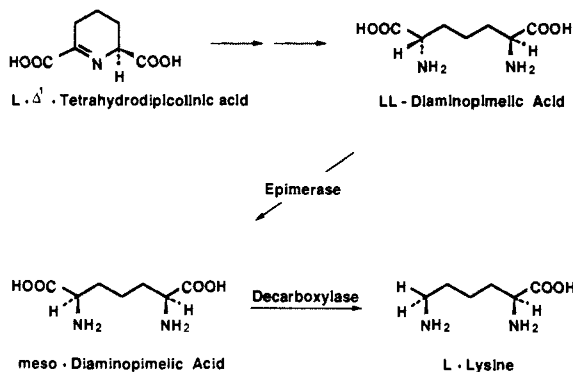
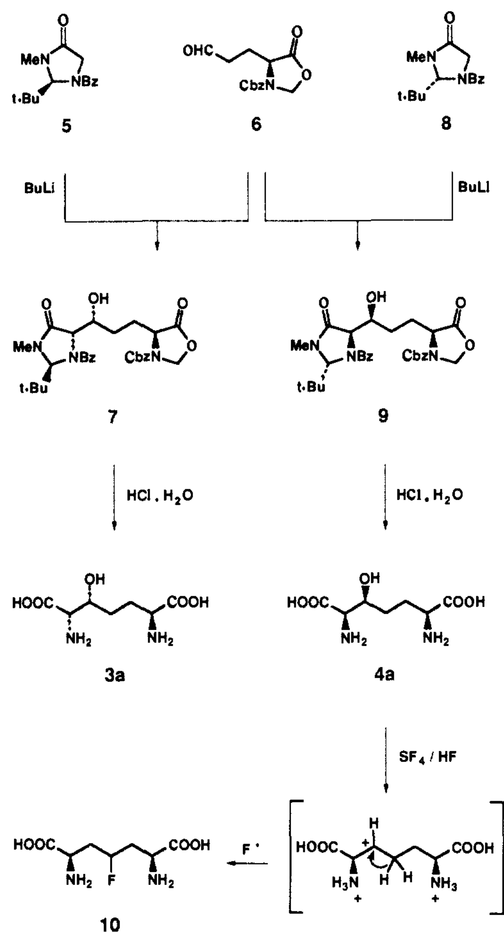


Figure 1. Two-base in-line mechanism proposed for the action of DAP epimerase on DAP. Here, B_1 and B_2 designate enzymic acids/bases of unspecified structure. At least one of these groups is thought to be a cysteine sulfhydryl moiety. See ref 8.

Scheme I. Diaminopimelate Pathway to L-Lysine in Bacteria and Plants



Scheme II



4 with sulfur tetrafluoride in liquid hydrogen fluoride.[11] Compound **3a** was obtained in 49% overall yield as shown in Scheme II by condensation of the chiral imidazolidinone **5**[12] devised by Seebach and co-workers[13] with the known aldehyde **6**[14] derived from L-glutamic acid and subsequent hydrolysis.[15] ^{1}H and ^{13}C NMR spectral analyses of **7** and of **3a** and comparison to other β-hydroxydiaminopimelate isomers (see below) indicate that the condensation is highly stereospecific (>95% de). Isomer **4a** was prepared analogously by use of the enantiomeric imidazolidinone **8**[12] to give **9** (49% yield), which was hydrolyzed as before in quantitative yield. Unfortunately, treatment of (2*R*,3*S*,6*S*)-3-hydroxy-2,6-diaminopimelate (**4a**) with sulfur tetrafluoride in hydrogen fluoride did not produce any of the desired β-fluorodiaminopimelates but generated instead the symmetrical γ-fluorodiaminopimelic acid (**10**) in 50% yield, presumably via a 1,2 hydride shift in a carbocationic intermediate.[11] This probably occurs because both nitrogens in the initially formed carbocation are protonated and hence positively charged. Attempts to transform the protected derivatives **7** and **9** with (*N*,*N*-diethylamino)sulfur trifluoride (DAST)[16] to corresponding fluoro compounds afforded complex mixtures that did not contain detectable quantities of the desired materials.

An alternative route employing condensation[17] of the Schoellkopf chiral bis(lactim ethers) **11** and **12**[18] with aldehyde **6** proved successful (Scheme III). However, in these cases the aldol reaction is much less stereoselective and yields a 55:45 ratio for **13a**/**13b** (3*R*/3*S*) and a 83:17 ratio for **14a**/**14b** (3*S*/3*R*), respectively. In each case the stereochemistry of the major isomer was confirmed to be threo by hydrolysis of the mixture and comparison to the pure β-hydroxydiaminopimelates (**3a** or **4a**) obtained previously by the Seebach procedure. Although attempts at separation of the bis(lactim ether) mixtures **13ab** and **14ab** were unsuccessful, treatment with DAST gives the expected[19] fluoro derivatives **15ab** and **16ab**, respectively, in low yields (8–15%). Separation of the isomeric mixture **15ab** affords pure **15a** (major component) and impure **15b**, which upon hydrolysis yield (2*R*,3*S*,6*S*)-3-fluoro-2,6-diaminopimelic acid (**1a**) (47% yield from **15a**) and the 2*R*,3*R*,6*S* isomer **1b** (2.3% overall yield from **13ab**), respectively.[20] Similar separation and hydrolysis of **16ab** produce the pure 2*S*,3*R*,6*S* compound **2a** (major product, 51% yield from **16a**) and its diastereomer **2b** (0.8% yield from **14ab**).

Since one aspect of the studies on enzyme inhibition involves examination of the isotope effect for removal of the α-hydrogen by the epimerase, a specifically deuterated analogue, **2c**, was required. To obtain this, the synthetic sequence was repeated with dideuterated bis(lactim ether) **17**, which was readily available by repeated deprotonation–protonation of **12**. The complete retention of α-deterium throughout each step indicates that no epimerization occurs at C-2.

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(9) (a) Cardinale, G. J.; Abeles, R. H. *Biochemistry* **1968**, *7*, 3970–3978. (b) Rudnick, G.; Abeles, R. H. *Biochemistry* **1975**, *14*, 4515–4522. (c) Albery, W. J.; Knowles, J. R. *Biochemistry* **1986**, *25*, 2572–2577 and references therein.

(10) For leading references see: Pansare, S. V.; Vederas, J. C. *J. Org. Chem.* **1987**, *52*, 4804–4810.

(11) Kollonitsch, J.; Marburg, S.; Perkins, L. M. *J. Org. Chem.* **1979**, *44*, 771–777.

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(13) For aldol condensations of **5** see: Seebach, D.; Juaristi, E.; Miller, D. D.; Schickli, C.; Weber, T. *Helv. Chim. Acta* **1987**, *70*, 237–261.

(14) Lee, B. H.; Miller, M. J. *Tetrahedron Lett.* **1984**, *25*, 927–930.

(15) (a) Racemic β-hydroxydiaminopimelates have been previously synthesized. Certain isomers support growth of DAP-requiring auxotrophs of *E. coli* and are incorporated into peptidoglycan: Sundharadas, G.; Gilvarg, C. *J. Biol. Chem.* **1966**, *241*, 3276–3282. (b) Very recently β-hydroxydiaminopimelate **4a** has been synthesized by using aldehyde **6**: Bold, G.; Duthaler, R. O.; Riedeker, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 497–498.

(16) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574–578.

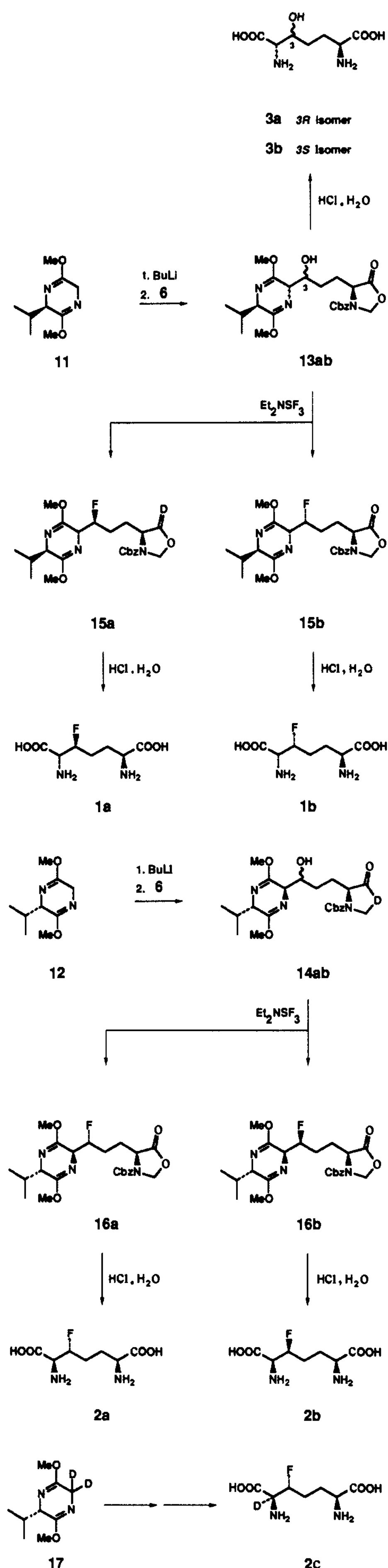
(17) Schoellkopf, U.; Groth, U.; Gull, M. R.; Nozulak, J. *Liebigs Ann. Chem.* **1983**, 1133–1151.

(18) Schoellkopf, U.; Groth, U.; Deng, C. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 798–799.

(19) Groth, U.; Schoellkopf, U. *Synthesis* **1983**, 673–675.

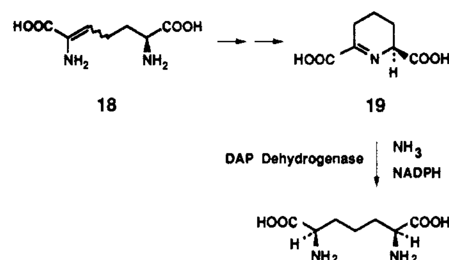
(20) The presence of fluorine at the β-position of **1** and **2** changes the priority of groups in nomenclature, and hence the "L,L isomer" has 2*R*,6*S* configuration whereas the analogue of *meso*-DAP possesses 2*S*,6*S* stereochemistry.

Scheme III



Analyses of β-fluorodiaminopimelates **1a**, **1b**, **2a**, **2b**, and **2c** as well as mixtures of these compounds by ^{1}H, ^{13}C, and ^{19}F NMR spectroscopy confirm their purity. The absolute stereochemistries at the α-carbons (C-2 and C-6) are assigned in each case from the configurations of the corresponding β-hydroxy compounds, which were available by the two independent routes (Schemes II and III) with well-precedented stereochemical outcomes.[13,17] The configuration of the newly created asymmetric center at C-3 could then be determined from the general observation that *erythro*-β-fluoro-α-amino acids have smaller coupling constants ($^{3}J_{HH}$ = 2.0–3.4 Hz) between the C-2 and C-3 hydrogens than do the corresponding threo isomers ($^{3}J_{HH}$ = 2.0–4.8 Hz).[11,21,22] Hence the 2.6-Hz coupling constant for these hydrogens seen in **1a** and **2a** in comparison to the corresponding values of 4.8 Hz for **1b** and 4.4 Hz for **2b** completes the configurational assignments. Together with the relative ratio (before separation) of fluorinated compounds **15a**/**15b** obtained from the unequal mixture of hydroxy precursors **13a**/**13b** and the corresponding ratio of **16a**/**16b** produced from **14a**/**14b**, the NMR results indicate that the DAST fluorination reaction proceeds predominantly with inversion of configuration. This is in contrast to the lack of stereoselectivity previously observed during DAST fluorodehydroxylation of other protected β-hydroxy-α-amino acid derivatives.[10]

Interaction of 3-Hydroxydiaminopimelates 3a and 4a with DAP Epimerase. Compounds **3a** and **4a** were tested as inhibitors of DAP epimerase. The epimerase was assayed by following the release of tritium into the solvent from uniformly tritiated DAP.[1e,8] The experiment was repeated in the presence of increasing amounts of **3a** and **4a**. Compounds **3a** and **4a** were found to be weak inhibitors of DAP epimerase, producing 50% inhibition at concentrations of 2.5 and 4.0 mM, respectively. Experiments were undertaken to determine whether DAP epimerase would catalyze the elimination of water from **3a** and **4a** to produce an enamine, **18**, which would spontaneously cyclize to tetrahydrodipicolinic acid (**19**). Compound **19** is an intermediate in the lysine/DAP



pathway in bacteria (Scheme I) and is also a substrate for the enzyme *meso*-DAP dehydrogenase (EC 1.4.1.16), which catalyzes the reductive amination of **19** to produce *meso*-DAP.[4] Thus, the production of **19** can be conveniently followed in the presence of DAP dehydrogenase by monitoring the decrease in absorbance at 340 nm as NADPH is consumed. Addition of either **3a** or **4a** to a mixture of DAP epimerase and DAP dehydrogenase in the presence of NADPH and ammonia did not result in a decrease in absorbance at 340 nm, indicating that these analogues were not converted to **19**.

The epimerization of **3a** and **4a** by DAP epimerase was also studied. Epimerization of compound **4a**, which is an analogue of *meso*-DAP, is forced to occur at the hydroxylated end of the molecule. This is based on the fact that the D,D isomer of DAP is neither a substrate nor a product for DAP epimerase. Apparently, the region of the enzyme that binds the nonepimerized end of DAP can only accommodate the L configuration. Compound **4a** was incubated with DAP epimerase, and the reaction mixture was injected onto an HPLC system capable of resolving

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(22) The assignments were confirmed by coupling constant changes induced upon addition of crown ether.[21a]

Table I. Interaction of 3-Substituted DAP Analogues with DAP Epimerase

compound	IC_{50}, μM[a]	k_{cat} for HF loss,[b] s^{-1}
3a	2500	0
4a	4000	0
1a	4	0.049[c]
1b	10	5.22
2a	25	2.35
2b	8	0.049[c]

[a] Determined by the radiometric assay (see Experimental Section). [b] The k_{cat} value for the DAP epimerase catalyzed epimerization of L,L-DAP to *meso*-DAP is 84 s^{-1} (see ref 8). [c] See text.

the DAP isomers. No epimerization product could be detected, even after 48 h of incubation. Compound **3a** is an analogue of L,L-DAP, and its interaction with DAP epimerase is more complicated in that epimerization could, in principle, occur at either end of the molecule. When **3a** was incubated with DAP epimerase, a new peak was seen by HPLC analysis that migrated in the region of *meso*-DAP and its hydroxy analogue **4a**. The rate of production of this compound was similar to the rate of epimerization of L,L-DAP. In order to establish which end of **3a** was being epimerized, the enzymatic reaction was done in D_2O. The protons attached to carbons 2 and 6 of **3a** were easily distinguished by high-resolution ^1H NMR, and this analysis showed that only the proton attached to C-6 became progressively deuterated (spectra not shown). This indicates that the epimerization of **3a** occurs primarily at the nonhydroxylated end of the molecule.

Interaction of 3-Fluorodiaminopimelates 1a, 1b, 2a, and 2b with DAP Epimerase. Fluorinated DAP analogues **1a**, **1b**, **2a**, and **2b** were tested as inhibitors of DAP epimerase. In contrast to the 3-hydroxy-DAP analogues, the corresponding 3-fluoro compounds were found to be potent inhibitors of DAP epimerase. By radiometric assay, **1a**, **1b**, **2a**, and **2b** produced 50% inhibition at concentrations of 4, 10, 25, and 8 μM, respectively (Table I). These IC_{50} values are well below the K_m values of 320 μM and 160 μM for *meso*-DAP and L,L-DAP, respectively. In the radiometric assay, the concentration of substrate is well below its K_m. In this case, the IC_{50} value for a competitive inhibitor is equal to the inhibitor dissociation constant (K_i).[23]

Interestingly, although *meso*-DAP is readily converted into **19** in the presence of $NADP^+$ and DAP dehydrogenase, compound **2a**, which is an analogue of *meso*-DAP, was neither a substrate nor a inhibitor of this enzyme. It was therefore possible to examine the inhibition of DAP epimerase by **2a** in a coupled enzymatic assay, in which the production of *meso*-DAP from L,L-DAP by DAP epimerase was coupled to the consumption of $NADP^+$ in the presence of DAP dehydrogenase. In this spectrophotometric assay, the initial velocity of the DAP epimerase reactions was measured as a function of the concentration of L,L-DAP substrate in the presence of three difference concentrations of **2a**. The data were analyzed by using a standard Lineweaver–Burk plot.[23] A pattern of intersecting lines was observed (data not shown). This is indicative of simple competitive inhibition.[23] From this analysis, a K_i value for **2a** of 11 μM was determined. This value agrees reasonably well with the value of 25 μM determined with the radiometric assay.

As described above for the hydroxylated DAP isomers, it is possible that DAP epimerase catalyzes the elimination of HF from the fluoro-DAP analogues to produce imine **19**. Again, DAP dehydrogenase was used to monitor the formation of **19**. When DAP epimerase, DAP dehydrogenase, compound **2a**, ammonia, and NADPH were mixed together in buffer, a rapid consumption of NADPH was observed, as judged by the loss of absorbance at 340 nm. The product of this coupled enzymatic reaction was shown to be *meso*-DAP by HPLC analysis. The epimerase-catalyzed elimination of HF from **2a** was verified by using a fluoride-selective electrode, which showed that addition of DAP epimerase to solutions of **2a** in buffer led to a steady production

(23) Segel, I. H. *Enzyme Kinetics*; Wiley: New York, 1975.

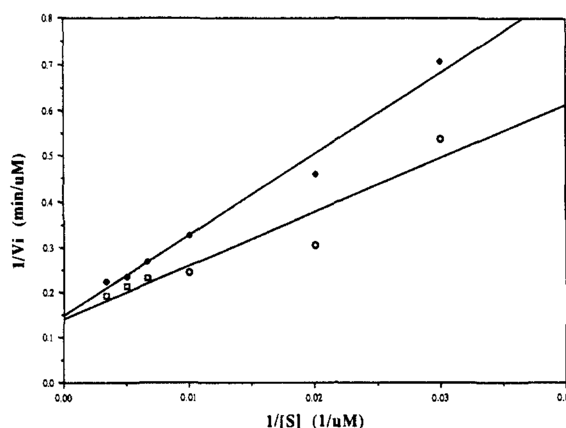


Figure 2. Lineweaver–Burk plot for the DAP epimerase catalyzed elimination of HF from **2a** (□) and from the deuterated analogue **2c** (◆).

of fluoride. DAP epimerase catalyzed HF elimination was also detected with isomer **1b** by the coupled enzymatic assay. In contrast, NADPH consumption was not detected with isomer **1a**, suggesting that either the elimination of HF was not occurring or that it was too slow to be detected with the enzymatic assay. Studies with the fluoride-selective electrode in the presence of large amounts of DAP epimerase indicated that DAP epimerase can catalyze the slow release of fluoride from **1a**. This was also verified by ^{19}F NMR studies, described below. The maximal velocities for the conversion of the fluoro DAP analogues to **19** are reported in Table I. It is clear that the fluorinated DAP analogues are better classified as slow competitive substrates for DAP epimerase rather than as simple competitive inhibitors. In this case, the apparent K_i values derived from the inhibition analyses should equal the K_m values for the elimination reaction.[23] This was examined with compound **2a** by carrying out a more detailed kinetic analysis in which the initial velocity of the elimination reaction was determined with several different concentrations of **2a**. The results shown in Figure 2 demonstrate that a normal hyperbolic, Michaelis–Menten pattern is seen. The data were fit to the Michaelis–Menten equation to give a K_m value for the turnover of **2a** of 53 μM. This value agrees reasonably well with the K_i value of 25 μM determined in the radiometric inhibition assay.

Since the fluorinated DAP analogues are actually consumed by DAP epimerase, it might be expected that the inhibitory properties of these compounds would diminish if they are preincubated with the enzyme prior to initiation of the epimerization reaction by addition of substrate. This was shown to be the case. When compound **2a** was preincubated with the epimerase, followed by the addition of substrate, the degree of inhibition continuously decreased to zero over 20 min. In contrast, the inhibition by **1a**, which is consumed much more slowly than **2a**, did not change over this same period. Thus **1a** is not only a more potent inhibitor than **2a** but it is also the more persistent inhibitor.

An analogue of **2a** deuterated at C-2, compound **2c**, was prepared (Scheme III). With this material, the isotope effects on the kinetic parameters for the DAP epimerase catalyzed elimination of HF were determined. The initial velocity versus substrate concentration plot is shown in Figure 2. Fitting the curves for both the deuterated and nondeuterated substrates to the Michaelis–Menten equation indicated an isotope effect on V_{max} of 1.1 and on K_m of 0.58.

In addition to being slow substrate inhibitors of DAP epimerase, the fluorinated DAP analogues might also undergo enzyme-catalyzed epimerization. Epimerization reactions were examined by a combination of ^{19}F NMR spectroscopy and HPLC analysis. Figure 3 shows the ^{19}F NMR spectrum of **2a** in the presence of DAP epimerase. Spectra were acquired at periodic intevals after the addition of enzyme. For isomer **2a**, the enzymatic epimerization, if any, should occur at the α-carbon adjacent to the fluorinated carbon. Epimerization at the other α-carbon position would generate a fluorinated analogue of D,D-DAP, which violates

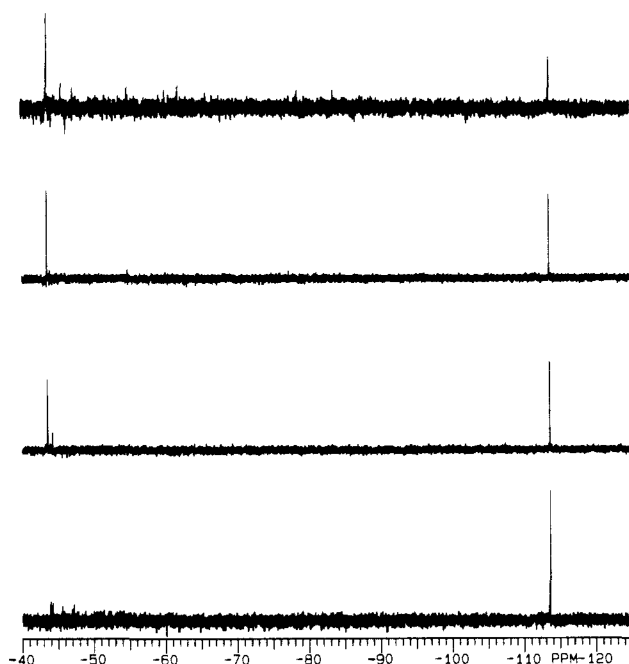


Figure 3. ^{19}F NMR analysis of the interaction of DAP epimerase with **2a**. DAP epimerase (0.2 unit) was added and spectra were recorded (bottom to top) after 0, 24, 33, and 45 min of incubation. See Experimental Section.

the substrate specificity rules of DAP epimerase. Figure 3 shows that, at zero time, a single ^{19}F NMR resonance is seen at −113.5 ppm for isomer **2a**. With time, this signal decreases in intensity and a new signal at −43.5 ppm, due to fluoride, increases in intensity. No additional NMR resonances were seen, suggesting that other fluorinated DAP isomers are not formed during the DAP epimerase catalyzed elimination reaction. Epimerization of **2a** at the fluorinated end would have generated isomer **1b**, which would have a different ^{19}F NMR chemical shift (see below). HPLC analysis of the reaction mixture confirmed the results obtained by NMR in that **1b** was not seen during the elimination reaction. It may be argued that enzymatic epimerization of **2a** is occurring but that the product **1b** is never seen because it rapidly undergoes an elimination reaction. This is not the case, since it has already been shown that isomers **1b** and **2a** are converted to imine **19** at similar rates (Table I). It is conceivable that epimerization of **2a** does occur and that the product is never released from the enzyme but only goes back to enzyme-bound **2a**, which then proceeds through the elimination pathway. It is difficult to rule out this latter possibility. If epimerization of **2a** is occurring, it might be possible to incorporate deuterium from the solvent into remaining **2a** during the elimination reaction. This would happen if the enzymic base that abstracts the proton from the substrate exchanges its proton with the medium prior to the regeneration of the enzyme-bound **2a**. To examine this, the enzymatic elimination of HF from **2a** was done in buffered D_2O, followed by isolation of remaining **2a** after approximately 50% of the material had undergone elimination. Mass spectral analysis of the remaining **2a** as its bis(*N*-trifluoroacetyl)dimethyl ester derivative indicated that deuterium was not incorporated. In summary, all of the above results suggest that DAP epimerase catalyzes the elimination of HF from **2a** without a competing epimerization reaction.

Figure 4 shows the ^{19}F NMR spectra of isomer **1b** in the presence of DAP epimerase. In principle, epimerization at either end of the molecule is possible. At zero time, a single resonance is seen at −116.1 ppm. With time, a resonance from free fluoride at −44 ppm develops, along with a small amount of a new resonance at −116.5 ppm. If epimerization were to occur at the fluorinated end, the product would be compound **2a**. This is not the case, because the addition of a small amount of **2a** to the NMR tube generates a third resonance, confirming that the chemical shifts of **2a** and the epimerization product are different (spectrum

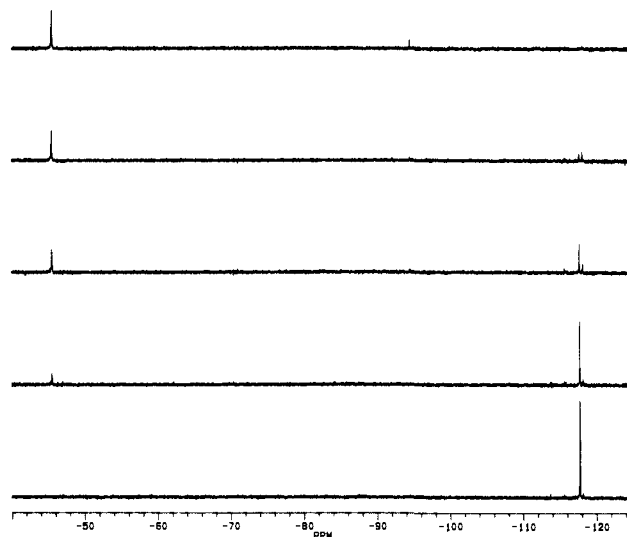


Figure 4. ^{19}F NMR analysis of the interaction of DAP epimerase with **1b**. DAP epimerase (0.11 unit) was added and spectra were recorded (bottom to top) after 0, 33, and 160 min of incubation. An additional portion of enzyme (0.22 unit) was added and spectra were recorded after 206 and 1000 min of incubation. See Experimental Section.

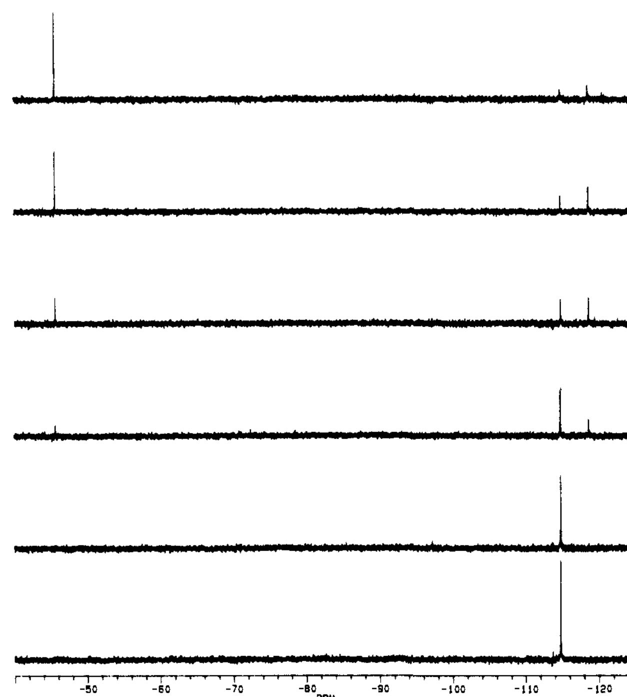


Figure 5. ^{19}F NMR analysis of the interaction of DAP epimerase with **1a**. DAP epimerase (0.88 unit) was added and spectra were recorded (bottom to top) after 0, 9, and 30 min of incubation. An additional portion of enzyme (6 units) was added and the incubation was continued for 90, 109, and 160 min. See Experimental Section.

not shown). The possibility that free **2a** is formed but cannot be observed by ^{19}F NMR spectroscopy because of rapid conversion to **19** is unlikely, because **1b** and **2a** undergo elimination reactions at similar rates (see above). Thus it is concluded that this new product is the result of epimerization at the nonfluorinated end of **1b**. This result is consistent with the epimerization of the hydroxy DAP analogue **3a**, which also occurs on the unsubstituted end. Presumably, the product of epimerization of **1b** cannot undergo an elimination reaction, since this would require interaction of the D center with the distal (nonepimerizing) binding site of the enzyme, which would violate the substrate specificity rules of the epimerase. These results indicate that DAP epimerase catalyzes the elimination of HF from **1b** without competing epimerization at the fluorinated end and that a small amount of epimerization occurs at the nonfluorinated end.

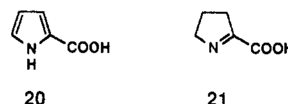
Figure 5 shows the ^{19}F NMR spectrum of isomer **1a** in the presence of DAP epimerase. At zero time, there is a single resonance at −113.3 ppm. With time, a resonance from free fluoride at −44 ppm appears. In addition, a new peak at −117.1 ppm develops, and both this peak and the original peak from **1a** eventually decrease in intensity as the free fluoride peak continues to grow. These observations suggest that a fluorinated analogue of *meso*-DAP is forming concomitantly with the elimination reaction. These NMR results were confirmed by HPLC analysis. In principle, the epimerization of **1a** can occur at either end of the molecule. Epimerization at the fluorinated end of **1a** would generate isomer **2b**, whereas inversion at the opposite end would generate the 2*R*,3*S*,6*R* isomer. This latter isomer is the enantiomer of **2a**, and both compounds would have identical chemical shift values. Since the chemical shifts of the epimerization product (−117.1 ppm) and **2a** (−113.5 ppm) are significantly different, the peak at −117.1 ppm is assigned to isomer **2b** and it is concluded that the epimerization of **1a** occurs only on the fluorinated end of the molecule. Surprisingly, epimerization at the nonfluorinated end of **1a** does not occur. The substrate specificity rules[1e,8] of the epimerase exclude the possibility that epimerization proceeds at the nonfluorinated end (C-6), with the product of this reaction not being seen because of rapid consumption by enzyme-catalyzed elimination. The latter reaction of this sequence would require unfavorable interaction of the epimerization product with its D center at the distal "L-specific" binding site of the epimerase. It might be argued that the epimerization at the fluorinated end is favored by an electronic effect of the adjacent fluorine that increases the acidity of the α-hydrogen. However, this cannot explain the lack of epimerization at the opposite end of the molecule, since reaction at that site would still be expected to proceed at a rate comparable to the rate of epimerization of nonfluorinated DAP. Rather, this result is probably due to a repulsive effect of the fluorine that prevents the binding of **1a** with the nonfluorinated end in a catalytically productive complex with the epimerase. This idea is not inconsistent with the results described above, in which the epimerizations of **1b** and of the hydroxy analogue **3a** occur on the unsubstituted end. For isomer **1a**, the fluorine lies on the opposite side of the DAP skeleton compared to the substituent in analogues **1b** and **3a**. This degree of substrate specificity expressed by DAP epimerase is remarkable in that the replacement of a single hydrogen by fluorine at C-3 is sufficient to prevent the epimerization three carbons away at C-6.

The results in Table I demonstrate that the elimination of hydrogen fluoride from **1a** and **2b** is much slower than from isomers **2a** and **1b**. The spectra in Figure 5 show that the interconversion of **1a** and **2b** by enzyme-catalyzed epimerization is much faster than the elimination reaction from either of these two isomers, and hence they appear to be in rapid equilibrium as the loss of hydrogen fluoride occurs. Although it is not clear which enzyme-bound species, **1a**, **2b**, or both, undergo elimination, removal of HF from the **1a**/**2b** mixture occurs with an overall V_{max} that is 0.058% of the V_{max} for epimerization of L,L-DAP to *meso*-DAP (Table I).

Preliminary Tests for Antibacterial Activity of Fluorinated Diaminopimelates. Compounds **1a** and **2a** were individually tested for antimicrobial activity in defined liquid media (with ammonium nitrate as nitrogen source) with wild-type organisms: *Bacillus subtilis* ATCC 6051, *Bacillus megaterium* ATCC 15374, *E. coli* ATCC 9637, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhimurium*, and *Serratia marcescens*. Optical density readings of overnight growth showed that within experimental error (10%) no antibiotic activity could be detected except against *B. megaterium*, which is inhibited by **2a** to the extent of about 14% at a concentration of 5 μg/mL, 20% at 20 μg/mL, and 50% at 100 μg/mL. The inhibition is relieved by addition of DAP as a mixture of isomers (100 μg/mL). Compound **1a** showed no inhibition. These results were initially surprising in view of the previously reported antibiotic activity of mixtures of isomers of β-fluorodiaminopimelates and β-chlorodiaminopimelates.[1f,g] However, some of the earlier studies have employed *E. coli* mutants that were DAP auxotrophs. In addition, it is likely that transport of DAP analogues into the bacterial cell is variable and may be quite poor in many cases.[1a] This problem could potentially be overcome by formation of di- or tripeptides, which could exploit the peptide transport systems and subsequently be cleaved within the bacterial cell by peptidases.[1a]

Discussion

The results described in this work indicate that the fluorinated DAP analogues **1a**, **1b**, **2a**, and **2b** are potent inhibitors of DAP epimerase from *E. coli*. The original rationale for this study was that enzyme-catalyzed proton abstraction from the α-carbon of the DAP analogue would be accompanied by elimination of an electronegative substituent from the adjacent β-carbon to generate the enzyme-bound enamine **18**. For enamine **18**, the α- and β-carbons as well as the attached α-amino and α-carboxyl groups are confined to a plane. This enzyme-bound intermediate might therefore be a structural mimic of the planar transition state that is formed in the enzyme-catalyzed epimerization of the DAP substrate (Figure 1). On the basis of the concept of transition-state stabilization by the enzyme,[24] it might be expected that **18** would be tightly bound to the active site of DAP epimerase and released very slowly into solution. This view is analogous to the inhibition of proline racemase by analogues of proline (**20** and **21**) that have a trigonal arrangement of atoms at the site of racemization.[9a,25]



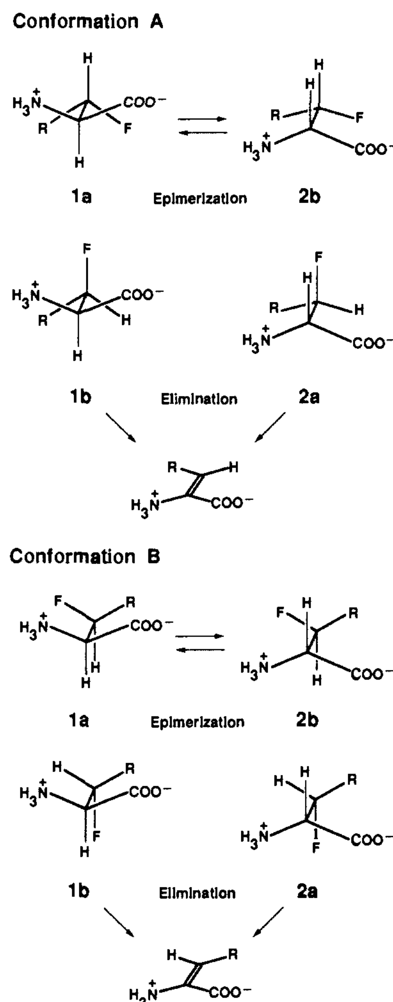
After release from the enzyme, **18** could tautomerize to an imine, which would then cyclize to give tetrahydrodipicolinate (**19**). The elimination of hydrogen fluoride was observed with all of the fluorinated DAP isomers in the presence of DAP epimerase, and production of **19** was confirmed for **2a** by its conversion to *meso*-DAP with DAP dehydrogenase. It is also possible that the predominant epimerase-bound intermediate is an imine tautomer that has the same planar geometry as enamine **18**. Such a species could potentially suffer attack by a nucleophilic functionality on the enzyme, such as the active-site thiol,[8] to form a covalently bound enzyme–inhibitor adduct. Since none of the fluoro DAP analogues were irreversible inhibitors of the epimerase, the formation of such an adduct would have to be a reversible process.

Enzyme-catalyzed elimination was not seen with the hydroxy DAP analogues **3a** and **4a**. Although this might initially be attributed to the poorer leaving-group potential of the hydroxyl group compared to the fluoro group, a more significant feature is that addition of a hydroxyl group to the DAP skeleton can prevent binding of the analogue to the enzyme in a catalytically productive manner. For example, the analogue of *meso*-DAP, compound **4a**, which in principle could be epimerized on the hydroxylated end, was not a substrate for DAP epimerase. In addition, the corresponding derivative of L,L-DAP, **3a**, is epimerized exclusively at the nonhydroxylated end even though epimerization at the hydroxylated end is conceivable. The hydroxylated analogues **3a** and **4a** were very weak inhibitors of the epimerase compared to the fluorinated compounds.

The results in Table I indicate that the maximal velocities for the enzymatic elimination of HF from the fluoro DAP analogues fall into two groups. Compounds **1b** and **2a** undergo elimination much more rapidly than compounds **1a** and **2b**. An attempt to explain these results is illustrated in Scheme IV. Since the elimination reaction is fast for isomer **1b**, it is reasonable to propose that its conformation when bound to the epimerase is such that the α-hydrogen and β-fluorine atoms are oriented in either an anti coplanar (Scheme IV, conformation A) or a syn coplanar (Scheme IV, conformation B) fashion. According to stereoelectronic theory,[26] these two geometries are predicted to lead to facile

(24) Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman: New York, 1985; pp 190–214.
(25) Keenan, M. V.; Alworth, W. L. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 500–504.

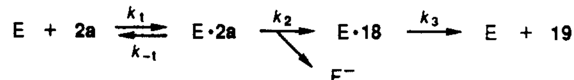
Scheme IV. Possible Conformations of Fluoro DAP Analogues Bound to DAP Epimerase



elimination of HF. Wiseman and Nichols have suggested that the epimerization of DAP occurs by a two-base mechanism in which one enzymic base removes a proton from the α-carbon and an enzymic acid delivers a proton to the opposite face of the substrate (Figure 1).[8] In addition, there must be a binding pocket on the enzyme for the distal amino and carboxyl groups of the substrate. This is based on the rigid stereochemical requirements of the epimerase, which only accepts isomers of DAP with the L configuration at the distal end.[1e,8] If **1b** is bound with the eliminating groups in an anti coplanar fashion (conformation A), then the remaining portion of the substrate (designated R in Scheme IV) would be positioned on the same side as the 2-amino group. Alternatively, a syn coplanar arrangement of the eliminating groups of **1b** (conformation B) would place the R group on the opposite side of the 2-amino group (Scheme IV). Since the structure of the bound inhibitor is not known, these possibilities cannot be distinguished at the present time. However, it is likely that all of the fluorinated DAP isomers bind in a manner that maintains the same relative position of the charged functional groups on the enzyme surface. On the basis of these arguments, it is possible to predict the relative ease of HF elimination for the other three fluorinated DAP isomers examined in this study. Facile elimination is predicted for isomer **2a** and this was found to be the case. In contrast, binding of both **1a** and **2b** to the enzyme would leave the eliminating groups in a gauche conformation (Scheme IV), which is not favorable for elimination. Again, this is consistent with the results in Table I.

Interestingly, when the elimination of HF is a facile process (isomers **1b** and **2a**), no competing epimerization at the carbon adjacent to fluorine is seen. In contrast, when hydrogen fluoride loss is disfavored (isomers **1a** and **2b**), epimerization at the α-carbon adjacent to the fluorinated carbon occurs readily. As discussed under Results, it is difficult to rule out the possibility that epimerization of **1b** and **2a** at the fluorinated end happens on the surface of the enzyme but that the product of the epimerization is never released into solution. Since deuterium from solvent was not incorporated into **2a** during the enzymatic conversion to **18**, it is likely that epimerization does not accompany the elimination reaction.

In light of the above results, a kinetic scheme for the interaction of **2a** with the epimerase is



The enzyme E binds **2a** to produce the Michaelis complex (E·**2a**), which undergoes an irreversible loss of fluoride to give the enzyme–enamine complex (E·**18**). Product dissociation regenerates the free enzyme and eventually **18** cyclizes to form **19**. It is not clear from the present results whether the transformation of **18** to **19** occurs on the enzyme or in solution after release from the enzyme. If **18** is converted to **19** prior to release into solution, it might be possible to obtain stereospecific incorporation of deuterium from solvent into the 3-position of **19** as **18** tautomerizes on the enzyme. Unfortunately, this could not be observed because the protons attached to C-3 of **19** were found to exchange rapidly with solvent in the absence of enzyme. As discussed above, E·**2a** apparently is not epimerized by the enzyme and such a step has therefore not been included in the above scheme. The K_m (also the K_i in this case) for the above kinetic scheme is given by the expression[24]

$$K_m = \left(\frac{k_{-1} + k_2}{k_1}\right)\left(\frac{k_3}{k_2 + k_3}\right)$$

If enamine **18** is released very slowly from the enzyme compared to the rate of the elimination step (i.e., $k_3 \ll k_2$), then the second term in the expression for K_m will be much less than unity and this will lower the value of K_m. This is the case, since the isotope effect on the maximal velocity of the HF elimination is close to unity (1.1). The expression for the isotope effect on V_{max} is

$$\frac{^{H}V_{max}}{^{D}V_{max}} = \left(\frac{^{H}k_2}{^{D}k_2}\right)\left(\frac{^{D}k_2 + k_3}{^{H}k_2 + k_3}\right)$$

It can be seen that the isotope effect on V_{max} will approach unity under the condition $k_3 \ll k_2$. Thus the potent inhibition of DAP epimerase by **2a** seems to be explained by a slow step that occurs after the loss of fluoride. This step is likely to be the release of enamine **18** from the enzyme.

Of the four fluoro DAP isomers studied, compound **1a** was found to be the most potent inhibitor of DAP epimerase. Enzyme-catalyzed elimination of HF from enzyme-bound **1a** would generate the identical enzyme–enamine complex that forms from isomer **2a**, and the rate of release from the enzyme (k_3) would necessarily be identical in both cases. For isomer **2a**, the V_{max} for the elimination reaction is limited by a step that occurs after the loss of fluoride. Yet isomer **1a** undergoes an elimination reaction that is some 50-fold slower than for isomer **2a**. Clearly the elimination of HF from **1a** and **2a** cannot share the same rate-determining step. Therefore, it appears that the enzymatic turnover rate for **1a** is not limited by a step that occurs after the elimination of hydrogen fluoride. The small K_m value (or K_i value) for isomer **1a** is not the result of a slow release of enamine **18** from the enzyme. Instead the intact fluoro DAP, rather than a chemically modified form, seems to bind tightly to the epimerase. Similar arguments apply to isomer **2b**. From the present results, it is difficult to explain the potent inhibition of DAP epimerase by **1a** and **2b**. Such an explanation must await a high-resolution structure analysis of the enzyme–inhibitor complex. The present study indicates that DAP epimerase binds diaminopimelic acid

(26) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon: New York, 1985; pp 319–323.

and its analogues fairly rigidly on the enzyme surface and that the resulting conformational restriction, together with stereoelectronic requirements for elimination, controls capture of anionic character at the α-carbon through loss of a β-substituent. Further studies on the mechanism of this unusual enzyme are in progress.

Experimental Section

General Methods. Most general procedure, instrumentation, and biochemical methods have been previously described.[1e,2,27] The *R* and *S* isomers of 3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazine were prepared by literature procedures[18] or purchased from Theodor Schuchardt Co. (8011 Hohenbrunn, Federal Republic of Germany). [G-^{3}H]DAP (630 Ci/mol) was obtained from Amersham as a mixture of stereoisomers in statistical proportion. Pure DAP isomers (L,L, D,D, and meso) were obtained by enzymatic resolution as previously described.[1e] DAP epimerase was purified as described[1e] from a strain of *E. coli* harboring the plasmid PDF6/JM109, which contains the epimerase gene (obtained as a generous gift from Dr. William Higgins, Merrell-Dow Research Institute, Strasbourg, France). The final specific activity was 30–35 units/mg. Protein concentrations were determined as previously reported.[1e] DAP dehydrogenase was purified from *Bacillus sphaericus* as described.[1e] Unless otherwise stated, ^{19}F NMR spectra were obtained on a Bruker AM400 spectrometer at 376.5 MHz with broad-band ^{1}H decoupling and with solvent as reference relative to $CFCl_3$ set to $\delta = 0$.

Antibacterial testing employed liquid cultures of the following bacteria from the American Type Culture Collection (Rockville, MD): *B. subtilis* 6051, *B. megaterium* 15374, *E. coli* 9637, *P. aeruginosa* 10145, *S. typhimurium*, and *S. marcescens*. Conditions under which these organisms were grown as well as experimental details for growth inhibition studies have been previously reported.[1c] Diaminopimelate analogues were added at concentrations of 0, 5, 20, and 100 μg/mL. The latter experiment was also done with addition of 100 μg/mL diaminopimelic acid to test for relief of growth inhibition.

Assay of DAP Epimerase. Radiometric assays of DAP epimerase were done in 100 μL of buffer A (100 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, pH 7.8) containing 0.5 μCi of [G-^{3}H]DAP. Enzyme was added (typically 3 milliunits), and the mixture was incubated at 25 °C for 10 min and then quenched by the addition of 100 μL of 10% trichloroacetic acid. The mixture was applied to 1 mL of Dowex 50X8-200 resin (H^+ form) in a Pasteur pipet column. The tritiated water released by the epimerase was eluted from the column with 2 mL of water, and a 0.4-mL portion was mixed with scintillation fluid and counted in a Beckmann LS 7500 scintillation counter.

The activity of the epimerase was also measured in a coupled assay with DAP dehydrogenase.[8] The assay mixture contained 0.3 mM $NADP^+$, 0.2 unit of DAP dehydrogenase, and a variable amount of L,L-DAP in a total volume of 0.4 mL of buffer A. The solution was placed in a cuvette and the reaction was followed by the addition of DAP epimerase (typically 4 milliunits). The increase in absorbance at 340 nm was monitored in a Perkin-Elmer Lambda 3A spectrophotometer at 25 °C.

Assay of Elimination Reactions Catalyzed by DAP Epimerase. The elimination of HF from the fluoro DAP analogues was monitored in 400 μL of buffer A containing 0.1 mM NADPH, 20 mM NH_4Cl, 0.2 unit of DAP dehydrogenase, and 4 milliunits of DAP epimerase. The reaction was started by the addition of variable amounts of the fluoro DAP analogues. The consumption of NADPH was monitored as the loss of absorbance at 340 nm in a spectrophotometer at 25 °C. The generation of fluoride was also monitored with a fluoride-selective electrode (K401 fluoroselectrode, Radiometer Corp.) in buffer A.

HPLC Analysis of the DAP Isomers. The L,L, D,D, and meso isomers of DAP were separated on a reverse-phase HPLC column with a chiral additive in the mobile phase.[8] Epimerase reaction mixtures (10 μL) were quenched with an equal volume of 10% trichloroacetic acid. After neutralization with 7 μL of 2 N NaOH, the solution was injected onto a reverse-phase column (4.6 mm × 250 mm, Vydac 218TP, 10 μm). The column was developed with a mixture of 8 mM *N,N*-di-*n*-propyl-L-alanine and 4 mM cupric acetate in 3% acetonitrile in water. The DAP isomers were detected by postcolumn derivatization with *o*-phthaldehyde[8] in a helium-driven reagent pump (Lazar Inc.). The derivatization reagent consisted of *o*-phthaldehyde (0.14 g), 2-mercaptoethanol (1.12 mL), Brij 35 (1.68 mL, 30% solution, Pierce Chemical Co.), methanol (2.8 mL), and borate buffer (350 mL) (0.4 mol of boric acid and 0.38 mol of KOH in 1 L of water). The reagent was stored for up to 4 days. The column eluent and derivatization reagent were mixed in a stainless steel T-joint, and the outflow was passed through knotted Teflon tubing (to ensure proper mixing) into a fluorescence detector (excitation at 340 nm, emission at 420 nm). The Teflon tubing was heated in a water bath at 65 °C. The DAP isomers eluted in the following order: D,D, meso, and L,L.

^{19}F NMR Analyses of Reactions Catalyzed by DAP Epimerase. The action of DAP epimerase on the fluoro DAP analogues was monitored by ^{19}F NMR by adding the fluoro compound (1 mg) to 0.4 mL of buffer A containing about 10% D_2O for spectrometer locking. The reactions were started by adding DAP epimerase. Spectra were collected on an IBM NR/300 spectrometer operating at 282.2 MHz with sodium trifluoroacetate in water as an external reference standard (assigned to 0 ppm). All spectra were obtained with broad-band ^{1}H decoupling.

DAP Epimerase Catalyzed Elimination of 2a in D_2O and Mass Spectral Analysis. Compound **2a** (0.75 mg) was dissolved in 300 μL of 100 mM potassium phosphate, 1 mM DTT, and 1 mM EDTA in D_2O, pD 7.4. DAP epimerase (0.3 unit) was added, and the reaction was allowed to proceed at 25 °C until approximately 50% of the fluorinated DAP was consumed (as judged by HPLC analysis). The reaction mixture was frozen and lyophilized. Acetonitrile (200 μL) and trifluoroacetic anhydride (100 μL) were added and the mixture was left at room temperature for 10 min. The liquid was removed with a stream of N_2. The residue was dissolved in water (200 μL), acidified to pH 1 with 6 N HCl, and extracted twice with an equal volume of ethyl acetate. The organic extracts were combined and concentrated with a stream of N_2. The residue was dissolved in ether (200 μL), and excess diazomethane in ether was added. The liquid was removed with a stream of N_2, and the residue was submitted for mass spectral analysis in a VG 70SEQ (VG Analytical) spectrometer operating in the positive-ion FAB mode.

(*S*)-3-(Benzyloxycarbonyl)-4-(3-oxopropyl)-5-oxazolidinone (6). To a solution of (*S*)-3-[3-(benzyloxycarbonyl)-5-oxooxazolidin-4-yl]-propanoyl chloride[14,28] (10.0 g, 32.1 mmol) in dry THF (60 mL) at −78 °C was added $LiAl(O\text{-}t\text{-}Bu)_3H$ (6.53 g, 25.7 mmol) in dry THF (80 mL) over a period of 2.5 h. The reaction mixture was then stirred at −78 °C for another 3 h. Water (20 mL) was added and the solvent was removed in vacuo. The residue was dissolved in dichloromethane, the mixture was filtered, and the filtrate was extracted with water and 5% aqueous $NaHCO_3$ solution. The organic phase was dried (Na_2SO_4) and concentrated in vacuo to give the known[14,15] aldehyde **6** (6.52 g, 73% yield), which could be used without further purification: IR ($CHCl_3$ cast) 2920 (m), 1800 (s), 1720 (s), 1410 (s), 1360 (m), 1040 (m), 760 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.68 (s, 1 H, C*H*O), 7.36 (s, 5 H, Ph), 5.55 (m, 1 H, OCH*H*N), 5.22 (m, 3 H, OC*H*HN and C*H*$_2$Ph), 4.38 (t, 1 H, *J* = 6 Hz, NC*H*CO), 2.70–2.10 (m, 4 H, C*H*$_2$C*H*$_2$); exact mass 277.0947 (277.0950 calcd for $C_{14}H_{15}NO_5$). Anal. Calcd for $C_{14}H_{15}NO_5$: C, 60.65; H, 5.45; N, 5.05. Found: C, 60.71; H, 5.31; N, 5.03.

(4*S*,3′*R*,2″*S*,5″*S*)-4-[3-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-oxoimidazolidin-5-yl]-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (7). The procedure described below to convert **8** to **9** was employed for condensation of (2*S*)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**5**)[12] (1.53 g, 5.88 mmol) with **6** (1.64 g, 5.92 mmol) to afford a 75:25 ratio of **7** and a minor isomer in which the benzoyl group has migrated to the hydroxyl oxygen[13] (1.60 g, 50% yield): IR ($CHCl_3$ cast) 3400 (m), 2970 (m), 1800 (s), 1720 (s), 1690 (s), 1650 (s), 1410 (s), 1360 (s), 1260 (m), 760 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ (major isomer 75%) 7.75–7.28 (m, 10 H, 2Ph), 5.65 (s, 1 H, NC*H*NBz), 5.51 (br s, 1 H, CbzNC*H*HO), 5.22–5.06 (m, 3 H, CbzNCH*H*O and C*H*$_2$Ph), 4.45 (d, 1 H, *J* = 4 Hz, OCC*H*NBz), 4.39 (d, 1 H, *J* = 12 Hz, O*H*), 4.22 (t, 1 H, *J* = 6 Hz, OCC*H*NCbz), 3.05 (s, 3 H, CH_3N), 2.20–1.90 (m, 2 H, CH_2CH_2), 1.50–1.20 (m, 2 H, CH_2CH_2), 1.04 (s, 9 H, *t*-Bu); ^{1}H NMR (200 MHz, $CDCl_3$) δ (minor isomer 25%) 8.06–7.99 (m, 2 H, ArH), 7.68–7.58 (m, 8 H, ArH), 5.51 (br s, 1 H, CbzNC*H*HO), 5.22–5.06 (m, 3 H, CbzNCH*H*O and C*H*$_2$Ph), 4.22 (t, 1 H, *J* = 6 Hz, OCC*H*NCbz), 4.20 (s, 1 H, *t*-BuC*H*), 2.89 (s, 3 H, CH_3N), 2.20–1.90 (m, 2 H, CH_2CH_2), 1.50–1.20 (m, 2 H, CH_2CH_2), 0.98 (s, 9 H, *t*-Bu); exact mass 537.2465 (537.2474 calcd for $C_{29}H_{35}N_3O_7$); CI (NH_3) MS 538 (MH^+). Anal. Calcd for $C_{29}H_{35}N_3O_7$: C, 64.80; H, 6.56; N, 7.82. Found: C, 64.65; H, 6.42; N, 7.62.

(4*S*,3′*S*,2″*R*,5″*R*)-4-[3-[1-Benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-oxoimidazolidin-5-yl]-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (9). A solution of lithium diisopropylamide (LDA) (1.56 mmol, 1.8 mL, prepared from diisopropylamine and *n*-BuLi) was slowly added to a stirred solution of (2*R*)-1-benzoyl-2-(1,1-dimethylethyl)-3-methyl-4-imidazolidinone (**8**)[12] (0.367 g, 1.41 mmol) in dry THF (10 mL) at −78 °C. The mixture was stirred at −78 °C for 40 min to afford a deep red solution. Aldehyde **6** (0.381 g, 1.37 mmol) in dry THF (8 mL) was added dropwise at −78 °C, and stirring was continued an additional 1.5 h. The mixture was treated with half-saturated NH_4Cl (20 mL) and

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dichloromethane (20 mL) at −78 °C. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to give an oil, which was purified by flash chromatography (ethyl acetate/hexane, 60:40) to afford an amorphous solid (0.361 g, 49% yield). This material consists of an 88:12 mixture of **9** and a minor isomer in which the *N*-benzoyl group has migrated to the hydroxyl oxygen:[13] IR ($CHCl_3$ cast) 3420 (s), 2960 (m), 1800 (s), 1720 (s), 1690 (s), 1650 (m), 1410 (m), 1360 (m), 1260 (m), 700 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (major isomer 88%) 7.66–7.30 (m, 10 H, 2Ph), 5.67 (s, 1 H, NCHNBz), 5.52 (br s, 1 H, CbzNCHHO), 5.26–5.14 (m, 3 H, CbzNCHHO and CH_2Ph), 4.46 (d, 1 H, *J* = 4.0 Hz, OCCHNBz), 4.41 (d, 1 H, *J* = 12 Hz, OH), 4.24 (t, 1 H, *J* = 6 Hz, OCCHNCbz), 3.74 (m, 1 H, CHOH), 3.04 (s, 3 H, CH_3N), 2.12–1.90 (m, 2 H, CH_2CH_2), 1.55–1.20 (m, 2 H, CH_2CH_2), 1.05 (s, 9 H, *t*-Bu); ^1H NMR (200 MHz, $CDCl_3$) δ (minor isomer 12%) 8.18–8.00 (m, 8 H, ArH), 7.96–7.88 (m, 2 H, ArH), 5.57 (br s, 1 H, OCHHNCbz), 5.33–5.11 (m, 3 H, OCHHNCbz and CH_2Ph), 4.40 (t, 1 H, *J* = 6 Hz, OCCHNCbz), 4.19 (br s, 1 H, *t*-BuCH), 3.77 (br s, 1 H, OCCHNBz), 2.90 (s, 3 H, CH_3N), 2.36–1.80 (m, 5 H, NH and CH_2CH_2), 0.98 (s, 9 H, *t*-Bu); exact mass 537.2440 (537.2474 calcd for $C_{29}H_{35}N_3O_7$). Anal. Calcd for $C_{29}H_{35}N_3O_7$: C, 64.80; H, 6.56; N, 7.82. Found: C, 65.10; H, 6.42; N, 7.48.

(2*S*,3*R*,6*S*)-3-Hydroxy-2,6-diaminopimelic Acid (3a). Hydrolysis of **7** following the procedure described below for production of **4a** from **9** gave a quantitative yield of **3a**: IR (KBr) 3680–2400 (br s), 1630 (br s), 1507 (s), 1404 (m), 1350 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.16–4.04 (m, 1 H, H-3), 3.82 (t, 1 H, *J* = 6 Hz, H-6), 3.70 (d, 1 H, *J* = 5.2 Hz, H-2), 2.25–1.55 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.5 MHz, D_2O) δ 175.2, 173.4, 70.1, 60.0, 55.3, 30.1, 27.9; FAB MS (glycerol) 207 (MH^+). Anal. Calcd for $C_7H_{14}N_2O_5{\cdot}2HCl$: C, 30.11; H, 5.73; N, 10.03. Found: C, 29.90; H, 5.53; H, 9.95.

(2*R*,3*S*,6*S*)-3-Hydroxy-2,6-diaminopimelic Acid (4a). A mixture of **9** (0.709 g, 1.32 mmol) and 6 N HCl (50 mL) was heated to reflux for 12 h. The solution was cooled and extracted with dichloromethane (2 × 50 mL), and the aqueous phase was concentrated in vacuo. The residue was purified by ion-exchange chromatography (AG 50X8, H^+ form, 68 equiv bed volume) using water (100 mL) and then 1 N aqueous ammonia (300 mL). The ammonia fractions were concentrated in vacuo to afford pure **4a** (0.272 g, 99%). The compound could be recrystallized from water/ethanol: mp 228–230 °C dec; IR (KBr) 3640–2400 (br s), 1640 (s), 1610 (s), 1500 (s), 1410 (m), 1345 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 4.13–4.01 (m, 1 H, H-3), 3.79 (t, 1 H, *J* = 6 Hz, H-6), 3.65 (d, 1 H, *J* = 5.2 Hz, H-2), 2.20–1.54 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.5 Hz, D_2O) δ 175.2, 173.5, 70.0, 60.1, 55.2, 30.1, 27.9; FAB MS (glycerol) 207 (MH^+).

(2R,6S)-4-Fluoro-2,6-diaminopimelic Acid (10). Since hydrogen fluoride is highly toxic, the reaction was done in an efficient hood and full protective clothing (face shield, rubber gloves, etc.) was used. (2*R*,3*S*,6*S*)-3-Hydroxy-2,6-diaminopimelic acid (**4a**) (500 mg, 2.43 mmol) was dissolved in anhydrous liquid hydrogen fluoride (10 mL) at −78 °C in a polyethylene vessel equipped with a drying tube. Sulfur tetrafluoride (0.70 mL, 12.3 mmol) was then introduced into the solution and stirring was continued at −78 °C for 10 h. The excess HF and SF_4 were removed by passing a stream of argon gas over the solution, which was gradually warmed to room temperature. The toxic effluent gases were absorbed in cold 3 N NaOH solution. The residue was taken up in concentrated HCl and evaporated in vacuo to dryness. This process was repeated three times to give a solid compound (680 mg), a part of which (39.0 mg) was purified by HPLC[29] to afford pure **10** (14.4 mg, 50% yield): IR (KBr) 3600–2500 (s), 1631 (s), 1507 (m), 1407 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 5.17 (br d, 1 H, $^2J_{HF}$ = 50 Hz, CHF), 3.91 (t, 2 H, *J* = 6.5 Hz, 2OCCHN), 2.50–2.02 (m, 4 H, $2CH_2$); ^{13}C NMR (75.5 MHz, D_2O) δ 174.55 (s, CO), 92.60 (d, $^1J_{CF}$ = 165.3 Hz, CF), 52.57 (d, $^3J_{CF}$ = 6.8 Hz, CHN), 36.71 (d, $^2J_{CF}$ = 18.9 Hz, CH_2); ^{19}F NMR (376.5 MHz, D_2O) δ −182.57 (s); FAB MS (glycerol) 209 (MH^+).

(4*S*,3′*RS*,3″*R*,6″*S*)-4-[3-[3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl]-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (13ab). This compound was made by following the procedure described below for **14ab** with (3*R*)-3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrzine (**11**)[18] (0.361 g, 1.96 mmol) and **6** (0.544 g, 1.96 mmol). The purified product was an inseparable mixture of **13a** (3′*R* isomer) and **13b** (3′*S* isomer) (55:45 ratio) (total 0.318 g, 35% yield): IR ($CHCl_3$ cast) 3350 (br), 2960 (m), 1802 (s), 1700 (s), 1416 (s), 1240 (s), 765 (m), 700 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ (major diastereomer 55%) 7.30 (m, 5 H, Ph), 5.50 (m, 1 H, CbzNCHHO), 5.18 (m, 1 H, CbzNCHHO), 5.15 (s, 2 H, CH_2Ph), 4.34 (t, 1 H, *J* = 7 Hz, OCCHNCbz), 3.96 (m, 1 H, *i*-PrCHN), 3.93 (br, 1 H, HCOH), 3.86 (m, 1 H, NCHCOMe), 3.69 (s, 3 H, OCH_3), 3.64 (s, 3 H, OCH_3), 2.22 (m, 2 H, CH_2CH_2), 1.02 (d, 3 H, *J* = 8 Hz, CH_3), 0.70 (d, 3 H, *J* = 8 Hz, CH_3); ^1H NMR (200 MHz, $CDCl_3$) δ (minor diastereomer 45%) 7.30 (m, 5 H, Ph), 5.50 (m, 1 H, CbzNCHHO), 5.18 (m, 1 H, CbzNCHHO), 5.15 (s, 2 H, CH_2Ph), 4.39 (t, 1 H, *J* = 6 Hz, OCCHNCbz), 3.96 (m, 1 H, *i*-PrCHN), 3.93 (br, 1 H, HCOH), 3.86 (m, 1 H, NCHCOMe), 3.66 (s, 3 H, OCH_3), 3.64 (s, 3 H, OCH_3), 2.22 (m, 1 H, MeCHMe), 2.33–1.86 (m, 3 H, OH and CH_2CH_2), 1.86–1.46 (m, 2 H, CH_2CH_2), 1.01 (d, 3 H, *J* = 8 Hz, CH_3), 0.70 (d, 3 H, *J* = 8 Hz, CH_3); exact mass 461.2153 (461.2162 calcd for $C_{23}H_{31}N_3O_7$). Anal. Calcd for $C_{23}H_{31}N_3O_7$: C, 59.86; H, 6.77; N, 9.11. Found: C, 59.83; H, 6.86; N, 8.75.

(4*S*,3′*RS*,3″*S*,6″*R*)-4-[3-[3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl]-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (14ab). To a solution of (3*S*)-3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazine (**12**)[18] (0.287 g, 1.56 mmol) in dry THF (8 mL) at −78 °C was added dropwise a solution of *n*-BuLi in hexane (1.23 N, 1.27 mL, 1.56 mmol). The mixture was stirred at −78 °C for 35 min. Aldehyde **6** (0.432 g, 1.56 mmol) in dry THF (12 mL) was added dropwise to the yellow basic solution, which turned colorless. The mixture was stirred at −78 °C for another 7 h, quenched at −78 °C with HOAc (0.19 g, 3.17 mmol) in THF (3 mL), and warmed to 20 °C. The solvent was removed in vacuo, the residue was partitioned between water and dichloromethane, and the aqueous phase was extracted with dichloromethane (2 × 30 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give an oil, which was purified by flash chromatography (ethyl acetate/petroleum ether, 50:50) to afford an inseparable mixture of **14a** (3′*S* isomer) and **14b** (3′*R* isomer) (83:17 ratio) (total 0.376 g, 52% yield): IR ($CHCl_3$ cast) 3460 (m), 2940 (m), 1800 (m), 1700 (br s), 1415 (m), 1238 (m), 765 (m), 700 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ (major isomer 83%) 7.35 (m, 5 H, Ph), 5.53 and 5.24 (AB, 2 H, *J* = 6 Hz, $CbzNCH_2O$), 5.22 and 5.20 (AB, 2 H, CH_2Ph), 4.40 (t, 1 H, *J* = 6 Hz, OCCHNCbz), 3.99 (t, 1 H, *J* = 4 Hz, *i*-PrCHN), 3.96 (br, 1 H, HCOH), 3.90 (t, 1 H, *J* = 4 Hz, NCHCOMe), 3.74 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 2.25 (m, 1 H, $CHMe_2$), 2.03 (br d, 1 H, *J* = 7 Hz, OH), 2.25–2.05 (m, 2 H, CH_2CH_2), 1.85–1.65 (m, 2 H, CH_2CH_2), 1.05 (d, 3 H, *J* = 8 Hz, CH_3), 0.74 (d, 3 H, *J* = 8 Hz, CH_3); ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ (minor isomer 17%) 7.35 (m, 5 H, Ph), 5.51 and 5.16 (AB, 2 H, $CbzNCH_2O$), 5.22 and 5.20 (AB, 2 H, CH_2Ph), 4.33 (t, 1 H, *J* = 7 Hz, OCCHNCbz), 4.12 (t, 1 H, *J* = 4.8 Hz, *i*-PrCHN), 3.96 (br, 1 H, HCOH), 3.70 (s, 3 H, OCH_3), 3.68 (s, 3 H, OCH_3), 2.81 (d, 1 H, OH), 2.25 (m, 1 H, $CHMe_2$), 2.25–2.05 (m, 2 H, CH_2CH_2), 1.85–1.65 (m, 2 H, CH_2CH_2), 1.04 (d, 3 H, *J* = 8 Hz, CH_3), 0.74 (d, 3 H, *J* = 8 Hz, CH_3); ^{13}C NMR (75.5 MHz, D_2O) δ 172.3, 166.0, 161.7, 151.51, 135.5, 128.7, 128.6, 128.3, 78.2, 72.0, 68.0, 61.1, 60.4, 59.4, 54.9, 52.7, 32.1, 28.6, 19.0, 16.9; exact mass 461.2166 (461.2162 calcd for $C_{23}H_{31}N_3O_7$). Anal. Calcd for $C_{23}H_{31}N_3O_7$: C, 59.86; H, 6.77; N, 9.11. Found: C, 59.75; H, 6.89; N, 8.95.

Hydrolysis of 13ab to (2*S*,3*RS*,6*S*)-3-Hydroxy-2,6-diaminopimelic Acid (3ab). Deprotection of **13ab** (32.0 mg, 69.4 μmol) in 6 N HCl (10 mL) by a procedure analogous to that described below for hydrolysis of **16a** to **2a** produced **3ab** (18.2 mg, 94% yield) as its hydrochloride salt. The sample showed chromatographic and spectral properties identical with those of **3a** except for the following: ^1H NMR (300 MHz, D_2O, DSS) δ (major isomer 55%, peaks enhanced if pure **3a** added) 4.25 (m, 1 H, CHOH), 4.11 (t, 1 H, *J* = 6 Hz, CH_2CHN), 4.02 (d, 1 H, *J* = 5.2 Hz, NCHCHOH), 2.26–1.60 (m, 4 H, CH_2CH_2); ^1H NMR (300 MHz, D_2O, DSS) δ (minor isomer 45%) 4.25 (m, 1 H, CHOH), 4.14 (d, 1 H, *J* = 3.5 Hz, NCHCHOH), 4.11 (t, 1 H, *J* = 6 Hz, CH_2CHN), 2.26–1.60 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.5 MHz, D_2O) δ (major isomer 55%, peaks enhanced if pure **3a** added) 172.75, 171.28, 69.23, 58.61, 53.44, 29.64, 27.30; ^{13}C NMR (75.5 MHz, D_2O) δ (minor isomer 45%) 172.75, 170.26, 69.88, 58.24, 53.60, 28.88, 27.58.

Hydrolysis of 14ab to (2*R*,3*RS*,6*S*)-3-Hydroxy-2,6-diaminopimelic Acid (4ab). Deprotection of **14ab** (23.8 mg, 51.6 μmol) in 6 N HCl (10 mL) by a procedure analogous to that described below for hydrolysis of **16a** to **2a** produced **4ab** as a free amino acid in quantitative yield. The sample showed chromatographic and spectral properties identical with those of **4a** except for the following: ^1H NMR (300 MHz, D_2O, DSS) δ (major isomer 83%, peaks enhanced if pure **4a** added) 4.13–4.01 (m, 1 H, CHOH), 3.79 (t, 1 H, *J* = 6 Hz, CH_2CHN), 3.65 (d, 1 H, *J* = 5.2 Hz, NCHCHOH), 2.20–1.54 (m, 4 H, CH_2CH_2); ^1H NMR (300 MHz, D_2O, DSS) δ (minor isomer 17%) 4.13–4.01 (m, 1 H, CHOH), 3.79 (t, 1 H, *J* = 6 Hz, CH_2CHN), 3.66 (d, 1 H, *J* = 4.8 Hz, NCHCHOH), 2.20–1.54 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.5 Hz D_2O) δ (major isomer 83%, peaks enhanced if pure **4a** added) 175.14, 173.42, 69.93, 60.06, 55.17, 30.06, 27.85; ^{13}C NMR (75.5 MHz, D_2O) δ (minor isomer 17%)

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175.14, 173.42, 70.08, 60.01, 55.17, 30.01, 27.85.

(4*S*,3′*S*,3″*R*,6″*R*)-4-[3-[3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl]-3-fluoropropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (15a). Compound **15a** was prepared by the procedure described below for **16a** with **13ab** (217 mg, 0.470 mmol) and (diethylamino)sulfur trifluoride (146 mg, 0.910 mmol). Repeated chromatographic separation afforded pure **15a** (18.0 mg, 8.3% yield) (R_f = 0.23; ethyl acetate/hexane, 25:75) and impure **15b** (R_f = 0.28; ethyl acetate/hexane, 25:75), which was hydrolyzed directly to **1b** (see below). **15a**: IR ($CHCl_3$ cast) 2960 (m), 1802 (s), 1717 (s), 1700 (s), 1415 (m), 1241 (s), 765 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.36 (s, 5 H, Ph), 5.54 (br s, 1 H, CbzNCH*H*O), 5.24–5.16 (m, 3 H, CbzNCH*H*O and C*H*$_2$Ph), 4.79 (br d, 1 H, $^2J_{HF}$ = 48 Hz, C*H*F), 4.33 (t, 1 H, *J* = 6 Hz, CbzNC*H*CO), 4.25 (d of t, 1 H, $^3J_{HH}$ = 2.8 Hz, $^3J_{HF}$ = 22 Hz, NC*H*COMe), 3.92 (t, 1 H, *J* = 3 Hz, *i*-PrC*H*N), 3.71 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 2.28 (m, 1 H, MeC*H*Me), 2.21–1.63 (m, 4 H, CH_2CH_2), 1.08 (d, 3 H, *J* = 8 Hz, CH_3), 0.71 (d, 3 H, *J* = 8 Hz, CH_3); ^{19}F NMR (376.5 MHz, $CDCl_3$) two lines with equal intensities, δ −190.99, −191.09; exact mass 463.2126 (463.2118 calcd for $C_{23}H_{30}N_3O_6F$). Anal. Calcd for $C_{23}H_{30}N_3O_6F$: C, 59.60; H, 6.53; N, 9.07. Found: C, 60.06; H, 6.66; N, 8.68.

(4*S*,3′*R*,3″*S*,6″*S*)-4-[3-[3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl]-3-fluoropropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (16a). A solution of **14ab** (0.280 g, 0.61 mmol) in dry dichloromethane (5 mL) was added dropwise to a stirred solution of (diethylamino)sulfur trifluoride (DAST) (0.16 mL, 1.21 mmol) in dry dichloromethane (5 mL) at −78 °C, and the mixture was stirred at −78 °C for an additional 2 h. Aqueous $NaHCO_3$ solution (0.20 g/20 mL of water) was added, the mixture was warmed to 20 °C, and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 20 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to afford an oil (0.280 g), which was separated by MPLC (silica gel column, 2.3 cm × 30 cm; ethyl acetate/hexane, 25:75). The fractions were monitored by TLC (ethyl acetate/hexane, 25:75; for **16a**, R_f = 0.23; for **16b**, R_f = 0.28) with detection by phosphomolybdic acid/cerium sulfate spray. Repeated chromatography was necessary to obtain pure **16a** (42 mg, 15% yield) and impure **16b**, which was hydrolyzed directly to **2b** (see below). **16a**: IR ($CHCl_3$ cast) 2960 (br), 1803 (s), 1719 (s), 1705 (s), 1668 (s), 1649 (m), 1416 (br), 1360 (s), 1242 (s), 1051 (m), 765 (m) 699 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.36 (s, 5 H, Ph), 5.56 and 5.22 (AB, 2 H, CbzNC*H*$_2$O), 5.19 (s, 2 H, C*H*$_2$Ph), 4.82 (br d, 1 H, $^2J_{HF}$ = 48 Hz, C*H*F), 4.34 (t, 1 H, CbzNC*H*CO), 4.27 (d of t, 1 H, $^3J_{HF}$ = 20 Hz, *J* = 4 Hz, NC*H*COMe), 3.94 (t, 1 H, *J* = 4 Hz, *i*-PrC*H*N), 3.72 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 2.30 (m, 1 H, MeC*H*Me), 2.25–1.86 (m, 4 H, CH_2CH_2), 1.06 (d, 3 H, *J* = 7.2 Hz, CH_3), 0.71 (d, 3 H, *J* = 7.2 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.86, 165.79, 160.09, 153.00, 135.38, 128.79, 128.70, 128.33, 93.49 ($^1J_{CF}$ = 178.3 Hz), 68.12, 60.76, 58.84, 58.56, 54.52, 52.79 ($^2J_{CF}$ = 9.3 Hz), 31.51, 25.87, 19.16, 16.65; ^{19}F NMR (376.5 MHz, $CDCl_3$) two lines with equal itensities, δ −190.48, −190.63; exact mass 463.2132 (463.2118 calcd for $C_{23}H_{30}N_3O_6F$). Anal. Calcd for $C_{23}H_{30}N_3O_6F$: C, 59.60; H, 6.53; N, 9.07. Found: C, 59.93; H, 6.54; N, 8.60.

(3*S*)-[6,6-$^2H_2$]-3,6-Dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazine (17). To a solution of (3*S*)-3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyazine (**12**)[18] (1.55 g, 8.43 mmol) in dry THF (20 mL) at −78 °C was added dropwise a solution of *n*-BuLi (2.40 M, 3.86 mL, 1.1 equiv). Stirring was continued at −78 °C for 40 min, at which point the yellow solution was quenched with CD_3OD (0.334 g, 1.1 equiv). The sequence of anion formation and quenching was then repeated twice with 1.2 and 1.3 equiv of *n*-BuLi, respectively. After an additional 40 min at −78 °C, the reaction was further quenched with D_2O (2 mL). The solution was partitioned between dichloromethane (30 mL) and H_2O (30 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated in vacuo. The resulting liquid was distilled under reduced pressure (70 °C/2 Torr) to afford **17** as a colorless liquid (1.21 g, 77% yield): IR ($CHCl_3$ cast) 2980 (s), 1695 (s), 1436 (m), 1302 (s), 1237 (s), 1139 (s), 1031 (m), 745 (s) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.00 (d, 1 H, *J* = 4 Hz, *i*-PrC*H*N), 3.74 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 2.24 (m, 1 H, MeC*H*Me), 1.03 (d, 3 H, *J* = 7 Hz, CH_3C), 0.76 (d, 3 H, *J* = 7 Hz, CH_3C); exact mass 186.1334 (186.1335 calcd for $C_9H_{14}D_2N_2O_2$), no peak for $C_9H_{15}DN_2O_2$. Anal. Calcd for $C_9H_{14}D_2N_2O_2$: C, 58.06; H, 8.72; N, 15.05. Found: C, 58.27; H, 8.95; N, 14.97.

(2*R*,3*S*,6*S*)-3-Fluoro-2,6-diaminopimelic Acid (1a). The protecting groups were removed from **15a** (32.0 mg, 69.1 μmol) by a hydrolysis procedure analogous to that described below for production of compound **2a** to give (after chromatography on cellulose) pure **1a** (9.2 mg, 47% yield) (TLC, silica gel, $MeOH/CHCl_3$/concentrated NH_3, 50:35:15, R_f = 0.23): IR (MeOH cast) 3420 (br m), 2920 (br s), 1666 (s), 1580 (m), 1487 (s) cm^{-1}; ^1H NMR (200 MHz, D_2O, DSS) δ 4.96 (br d, 1 H, $^2J_{HF}$ = 48 Hz, C*H*F), 4.12 (d of d, 1 H, $^3J_{HF}$ = 24.6 Hz, $^3J_{HH}$ = 2.6 Hz, CFC*H*N), 3.95 (t, 1 H, *J* = 6 Hz, CH_2C*H*N), 2.20–1.58 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.5 MHz, D_2O) δ 174.74 (s, *C*O), 170.64 (s, *C*O), 92.40 (d, $^1J_{CF}$ = 175.2 Hz, CF), 58.0 (d, $^2J_{CF}$ = 26.4 Hz, N*C*HCF), 54.90 (s, CH_2*C*HN), 27.35 (s, *C*H_2CHN), 26.65 (d, $^2J_{CF}$ = 21.71 Hz, CHF*C*H_2); ^{19}F NMR (376.5 MHz, D_2O) δ −190.88; FAB MS (glycerol) 209.14 (MH^+).

(2*R*,3*R*,6*S*)-3-Fluoro-2,6-diaminopimelic Acid (1b). The fraction (60.0 mg) from MPLC purification containing crude **15b** was hydrolyzed by a procedure analogous to that described below for production of **2a** from **16a**. Repeated purification by ion-exchange chromatography on AG50 (H^+ form, 100 mesh, 6 N HCl) and cellulose chromatography (as described for **2a**) afforded pure **1b** (6.8 mg, 2.3% overall yield for DAST fluorination and hydrolysis) (TLC, silica gel, $MeOH/CHCl_3$/concentrated NH_3, 50:35:15, R_f = 0.23): IR (MeOH cast) 3600–2600 (br s), 1734 (m), 1653 (s), 1580 (m), 1496 (m), 1405 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O, DSS) δ 5.02 (br d, 1 H, $^2J_{HF}$ = 47.2 Hz, C*H*F), 4.03 (d of d, 1 H, $^3J_{HF}$ = 24.0 Hz, $^3J_{HH}$ = 4.8 Hz, CFC*H*N), 3.97 (t, 1 H, *J* = 6Hz, CH_2C*H*N), 2.20–1.70 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.5 MHz, D_2O) δ 173.47 (s, *C*O), 170.83 (s, *C*O), 92.15 (d, $^1J_{CF}$ = 175.2 Hz, *C*F), 57.80 (d, $^2J_{CF}$ = 22.6 Hz, N*C*HCHF), 53.92 (s, CH_2*C*HN), 28.10 (d, $^2J_{CF}$ = 20.6 Hz, CHF*C*H_2), 26.70 (s, *C*H_2CHN); ^{19}F NMR (376.5 MHz, D_2O) δ −192.22; FAB MS (glycerol) 209 (MH^+).

(2*S*,3*R*,6*S*)-3-Fluoro-2,6-diaminopimelic Acid (2a). The protecting groups were removed by aqueous hydrolysis. Compound **16a** (36.0 mg, 77.8 μmol) was heated to reflux in 6 N HCl (10 mL) for 6 h. The aqueous solution was then washed with ethyl ether (2 × 20 mL) and concentrated in vacuo to give a solid compound, which was purified by column chromatography on cellulose powder (2.5 cm × 26 cm, solvent system *n*-BuOH/H_2O/CH_3CO_2H, 4:2:1) with slight pressure of argon or nitrogen. Since β-fluorodiaminopimelates are unstable under basic conditions, compound **2a** was obtained as its dihydrochloride by lyophilization from dilute hydrochloric acid to give 11.2 mg (51% yield): IR (KBr) 3600–2600 (br s), 1735 (m), 1630 (m), 1505 (m), 1400 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O, DSS) δ 4.95 (br d, 1 H, $^2J_{HF}$ = 46.4 Hz, C*H*F), 4.12 (d of d, 1 H, $^3J_{HF}$ = 24.6 Hz, $^3J_{HH}$ = 2.6 Hz, CFC*H*N), 3.94 (t, 1 H, *J* = 6 Hz, CH_2C*H*N), 2.20–1.60 (m, 4 H, CH_2CH_2); ^{13}C NMR (75.5 MHz, D_2O) δ 172.90 (s, *C*O), 169.63 (s, *C*O), 92.12 (d, $^1J_{CF}$ = 175.8 Hz, *C*F), 57.63 (br s, CF*C*HN), 53.60 (br s, CH_2*C*HN), 26.94 (s, *C*H_2CHN), 26.67 (d, $^2J_{CF}$ = 18.9 Hz, CHF*C*H_2); ^{19}F NMR (376.5 MHz, D_2O), δ −191.38; FAB MS (glycerol) 209.13 (MH^+).

(2*S*,3*S*,6*S*)-3-Fluoro-2,6-diaminopimelic Acid (2b). The fraction (93 mg) from MPLC purification containing crude **16b** was hydrolyzed by a procedure analogous to that described for production of **2a** from **16a**. Repeated purification by chromatography on cellulose and ion-exchange chromatography on AG 50 (H^+ form, 1-mL bed volume, 6 N HCl) afforded **2b** (2.4 mg, 0.8% overall yield for DAST fluorination and hydrolysis): IR (KBr) 3600–2600 (br s), 1718 (m), 1657 (s), 1593 (m), 1490 (s), 1399 (m), 1150 (m) cm^{-1}; ^1H NMR (200 MHz, D_2O, DSS) δ 4.92 (br d, 1 H, $^2J_{HF}$ = 48.0 Hz, C*H*F), 3.79 (d of d, 1 H, $^3J_{HF}$ = 24.3 Hz, $^3J_{HH}$ = 4.4 Hz, CFC*H*N), 3.69 (t, 1 H, *J* = 6 Hz, CH_2C*H*N), 2.10–1.65 (m, 4 H, CH_2CH_2); ^{19}F NMR (376.5 MHz, D_2O), δ −191.83; FAB MS (glycerol) 209.11 (MH^+).

(2*S*,3*R*,6*S*)-[2-^{2}H]-3-Fluoro-2,6-diaminopimelic Acid (2c). The aldol condensation between the deuterated bis(lactim ether) **17** (1.09 g, 5.85 mmol) and aldehyde **6** (1.62 g, 5.85 mmol) was accomplished by a procedure analogous to that described for generation of **14ab**. After purification as before, 0.948 g (35% yield) of (4*S*,3′*RS*,3″*S*,6″*R*)-[6″-^{2}H]-4-[3-[3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl]-3-hydroxypropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (**14c**) was obtained: IR ($CHCl_3$ cast) 3480 (br m), 2959 (s), 1803 (s), 1699 (s), 1419 (s), 1240 (s), 1128 (m), 1010 (m), 765 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ (major diastereomer, 88%) 7.35 (m, 5 H, Ph), 5.53 and 5.24 (AB, 2 H, *J* = 6 Hz, CbzNC*H*$_2$O), 5.22 and 5.20 (AB, 2 H, C*H*$_2$Ph), 4.40 (t, 1 H, *J* = 6 Hz, OCC*H*NCbz), 4.00 (d, 1 H, *J* = 4 Hz, *i*-PrC*H*N), 3.74 (s, 3 H, OC*H*$_3$), 3.69 (s, 3 H, OC*H*$_3$), 2.25 (m, 1 H, MeC*H*Me), 2.25–2.05 (m, 2 H, C*H*$_2$CH$_2$), 1.85–1.65 (m, 2 H, CH_2C*H*$_2$), 1.76 (br d, 1 H, O*H*), 1.05 (s, 3 H, *J* = 8 Hz, C*H*$_3$C), 0.74 (s, 3 H, *J* = 8 Hz, C*H*$_3$C); ^1H NMR (400 MHz, $CDCl_3$, 50 °C) δ (minor diastereomer, 12%) 7.35 (m, 5 H, Ph), 5.51 and 5.16 (AB, 2 H, *J* = 3 Hz, CbzNC*H*$_2$O), 5.22 and 5.20 (AB, 2 H, C*H*$_2$Ph), 4.33 (t, 1 H, *J* = 7 Hz, OCC*H*NCbz), 4.12 (d, 1 H, *J* = 4 Hz, *i*-PrC*H*N), 3.70 (s, 3 H, OC*H*$_3$), 3.68 (s, 3 H, OC*H*$_3$), 2.90 (d, 1 H, O*H*), 2.25 (m, 1 H, MeC*H*Me), 2.25–2.05 (m, 2 H, C*H*$_2$CH$_2$), 1.85–1.65 (m, 2 H, CH_2C*H*$_2$), 1.04 (d, 3 H, *J* = 8 Hz, C*H*$_3$), 0.74 (d, 3 H, *J* = 8 Hz, C*H*$_3$); CI MS 463 (MH^+). Anal. Calcd for $C_{23}H_{30}DN_3O_7$: C, 59.80; H, 6.76; N, 9.09. Found: C, 59.99; H, 6.75; H, 8.87.

The fluorination of **14c** (0.0892 g, 1.93 mmol) with DAST (0.51 mL, 3.9 mmol) employed a procedure analogous to that described for generation of **16a** to produce 90.2 mg (10% yield) of (4*S*,3′*R*,3″*S*,6″*S*)-[6-

^{2}H]-4-[3-[3,6-dihydro-2,5-dimethoxy-3-(1-methylethyl)pyrazin-6-yl]-3-fluoropropyl]-3-(benzyloxycarbonyl)-5-oxazolidinone (**16c**): IR ($CHCl_3$ cast) 2960 (s), 1803 (s), 1718 (s), 1668 (s), 1647 (m), 1416 (s), 1242 (s), 1052 (m), 765 (m) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33 (m, 5 H, Ph), 5.53–5.22 (AB, 2 H, CbzNC*H*$_2$O), 5.21 and 5.20 (AB, 2 H, C*H*$_2$Ph), 4.77 (br d, 1 H, $^{2}J_{HF}$ = 44 Hz, C*H*F), 4.38 (t, 1 H, *J* = 8 Hz, CbzNC*H*CO), 3.93 (d, 1 H, *J* = 3.2 Hz, *i*-PrC*H*N), 3.70 (s, 3 H, OC*H*$_3$), 3.68 (s, 3 H, OC*H*$_3$), 2.28 (m, 1 H, MeC*H*Me), 2.23–1.85 (m, 4 H, C*H*$_2$C*H*$_2$), 1.02 (d, 3 H, *J* = 7 Hz, C*H*$_3$), 0.67 (d, 3 H, *J* = 7 Hz, C*H*$_3$); CI MS (NH_3) 465 (MH^+). Anal. Calcd for $C_{23}H_{29}DN_3O_6F$: C, 59.54; H, 6.52; N, 9.05. Found: C, 59.94; H, 6.61; N, 8.64.

The protected β-fluoroamino acid **16c** (28.1 mg, 60.6 μmol) was hydrolyzed by a procedure analogous to that described above for production of **2a** to afford 7.7 mg (45% yield) of (2*S*,3*R*,6*S*)-[2-^{2}H]-3-fluoro-2,6-diaminopimelic acid (**2c**): IR (MeOH cast) 3600–2500 (br s), 1725 (m), 1660 (m), 1580 (m), 1498 (s), 1400 (m) cm^{-1}; ^{1}H NMR (400 MHz, D_2O, DSS) δ 4.88 (d of d, 1 H, $^{2}J_{HF}$ = 48 Hz, *J* = 10.4 Hz, C*H*F), 3.85 (t, 1 H, *J* = 6 Hz, CH_2C*H*N), 2.10–1.58 (m, 4 H, CH_2CH_2); ^{19}F NMR (376.5 MHz, D_2O) δ −191.69; FAB MS (glycerol) 210 (MH^+).

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Sequential Biocatalytic Kinetic Resolutions

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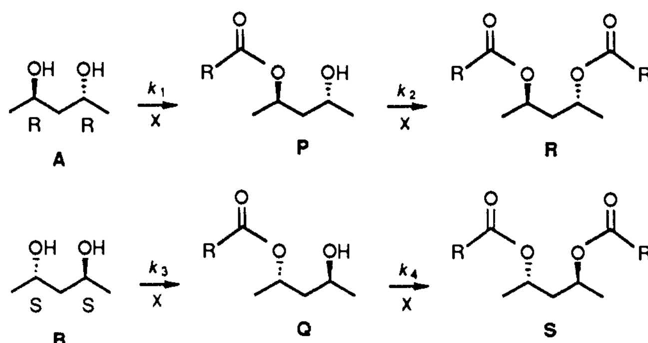
Abstract: Quantitative expressions that govern sequential kinetic resolutions have been developed for calculation of the relative kinetic constants to allow optimization of chemical and optical yields. Enantiomerically pure (2*R*,4*R*)- and (2*S*,4*S*)-2,4-pentanediols have been prepared by biocatalyzed sequential enantioselective esterification in anhydrous isooctane.

The synergistic coupling of an enantioselective reaction with a subsequent kinetic resolution in an asymmetric synthesis leads to a marked enhancement in the enantiomeric purity of the product. In a series of enzymatic studies, it was shown that the initial enantiotopically selective hydrolysis of an achiral diester (asymmetric synthesis) generates optically active monoesters; the same enzyme in turn catalyzes the preferential hydrolysis of the minor antipodal monoester (kinetic resolution). This kinetic pattern is manifested by an increase in the enantiomeric purity of the monoester fraction as the reaction progresses toward completion. This concept has been successfully employed in several enzymic and nonenzymic systems.[1] Similarly, in sequential kinetic resolutions, the second step could likewise improve the enantioselectivity of the first. We had earlier applied this strategy to the preparation of optically active binaphthols.[2] In this paper, we disclose in detail the derivation of the quantitative expressions that govern sequential kinetic resolutions. These equations allow one to define the underlying kinetic parameters that determine the enantiomeric excess (ee) of any chiral species for a given conversion.

Theory

During the enzymatic enantioselective esterification of racemic axially disymmetric diols, two esterification reactions are operating sequentially



where **A** is the fast-reacting enantiomer, (2*R*,4*R*)-(−)-pentanediol, and **B** is the slow-reacting enantiomer, (2*S*,4*S*)-(+)-pentanediol; **P** and **Q** are the corresponding enantiomeric monoesters; **R** and **S** are the corresponding enantiomeric diesters; **X** is the acyl donor, hexanoic acid; and k_1, k_2, k_3, and k_4 are the four relative second-order rate constants. When the reactions are virtually irreversible and product inhibition is absent in the initial stages, we may write the following:

$$-d\mathbf{A}/dt = v_a = k_1[\mathbf{A}][\text{acyl-ENZ}] \quad (1)$$

$$-d\mathbf{B}/dt = v_b = k_3[\mathbf{B}][\text{acyl-ENZ}] \quad (2)$$

$$d\mathbf{R}/dt = v_P = k_2[\mathbf{P}][\text{acyl-ENZ}] \quad (3)$$

$$d\mathbf{S}/dt = v_Q = k_4[\mathbf{Q}][\text{acyl-ENZ}] \quad (4)$$

$$d\mathbf{P}/dt = v_a - v_P \quad (5)$$

$$d\mathbf{Q}/dt = v_b - v_Q \quad (6)$$

Divide (1) by (2) and one obtains

$$d\mathbf{A}/d\mathbf{B} = k_1[\mathbf{A}]/k_3[\mathbf{B}] \quad (7)$$

Let

$$E_1 = k_1/k_3 \quad (8)$$

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