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Vinylogous Amide Analogues of Diaminopimelic Acid (DAP) as Inhibitors of Enzymes Involved in Bacterial Lysine Biosynthesis

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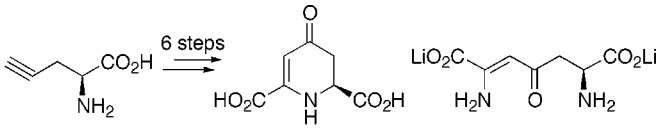
ABSTRACT



5 **6**

Vinylogous amides 5 and 6 have been synthesized from L-propargyl glycine and tested against diaminopimelate (DAP) enzymes involved in bacterial lysine biosynthesis. Both are reversible inhibitors of DAP D-dehydrogenase and DAP epimerase with IC_{50} values in the 500 μM range. Compound 5 shows competitive inhibition against the L-dihydrodipicolinate (DHDP) reductase with a K_i value of 32 μM, which is comparable to the planar dipicolinate 16 (K_i = 26 μM), the best known inhibitor of the enzyme.

The search for new broad spectrum antimicrobial agents has encouraged extensive studies on enzymes and inhibitors of the diaminopimelic acid (DAP) pathway to L-lysine (Scheme 1).[1] The meso isomer of DAP (**1**) is involved in the cross-linking of the peptidoglycan cell wall layer of Gram negative bacteria, whereas its biosynthetic product, L-lysine, has an analogous function in many Gram positive organisms.[2] Since mammals lack this pathway and require a dietary source of lysine, inhibitors of DAP enzymes may act as antibiotics with low mammalian toxicity. Several enzymes in the DAP pathway either utilize substrates having an imine bond or have transient intermediates wherein the α-carbon in the amino acid moiety becomes planar. Among these are DAP D-dehydrogenase, DAP epimerase, and dihydrodipicolinate (DHDP) reductase.[1] The X-ray crystal structures of these enzymes have been reported.[3−5] The proposed mechanism of DAP D-dehydrogenase involves generation of an acyclic imine intermediate,[5] whereas that of DAP epimerase creates anionic character at the α-center of the substrate, with one active site cysteine thiolate acting as the base and another cysteine thiol as the acid to deliver the proton from the opposite face.[6] The natural substrate of DHDP reductase is an imine. On this basis, a number of successful inhibitors of

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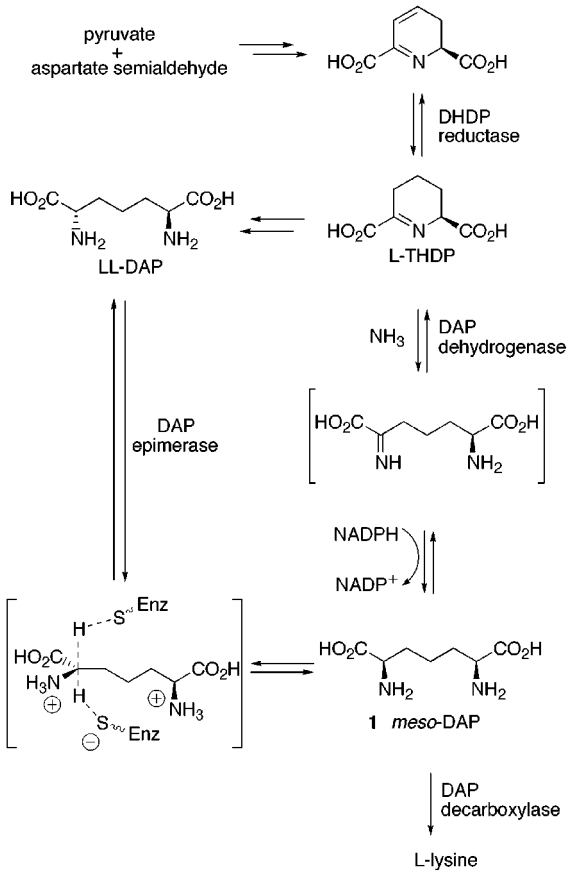
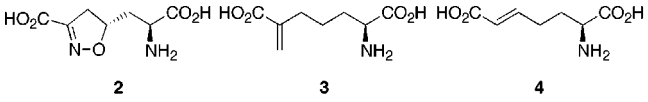
Scheme 1. Biosynthesis of L-Lysine via the DAP Pathway



DAP enzymes have been prepared which have a planar α-carbon, and some show antimicrobial activity.[1c] For example, DAP dehydrogenase is inhibited by isoxazoline **2** (K_i = 23 μM)[7] and unsaturated derivatives **3** and **4** (K_i = 5.3 μM and 44 μM, respectively).[8] Since enzymes in the

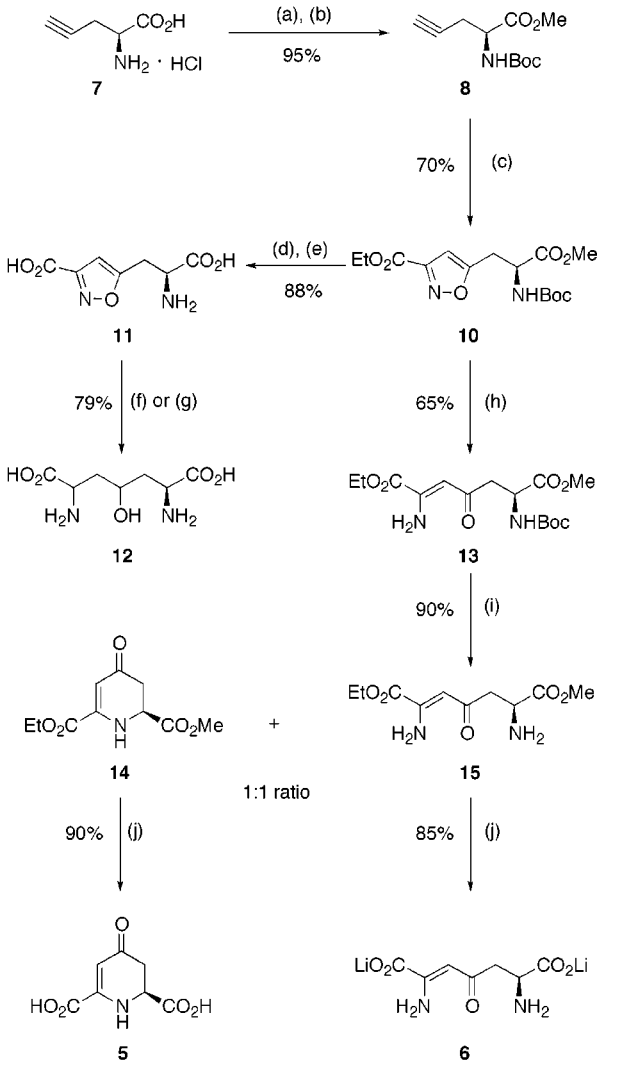


2 **3** **4**

DAP pathway tend to have relatively strict substrate specificity and excellent stereochemical recognition at the distal (nonreacting) sites, the design of potent inhibitors is often a challenging task that requires stereospecific incorporation of numerous similar functionalities into a small molecule.[1c] In the present study, we describe the preparation and testing of vinylogous amides **5** and **6** as potential inhibitors of DAP dehydrogenase, DAP epimerase, and DHDP reductase enzymes.

From the retrosynthetic perspective, it seemed that the vinylogous amide functionality could be made by reductive ring opening of an isoxazole ring.[9] Thus, protection of L-propargyl glycine (**7**) using standard protocols gives methyl *N*-(benzyloxycarbonyl)-L-propargyl glycinate (**8**)[10] in 95% yield (Scheme 2). Treatment of **8** with ethyl chlorooximidoacetate **9** under basic conditions affords the 1,3-dipolar cycloaddition product,[11] isoxazole **10**, as a single regioisomer

Scheme 2. Synthesis of Vinylogous Amide **6**[a]



[a] Reagents and conditions: (a) $SOCl_2$ in dry MeOH, 4 equiv, 10 h, 25 °C; (b) 1.2 equiv of Boc_2O, 1.2 equiv of Et_3N, MeCN, 3 h, 25 °C; (c) 3.0 equiv of $EtO_2CC(Cl)$═NOH **9**, ether, 25 °C, then 3.0 equiv of Na_2CO_3 in H_2O, via syringe pump, 3 h; (d) 2.1 equiv of LiOH, MeCN/H_2O (1:1), 17 h, 25 °C; (e) 10 equiv of TFA, CH_2Cl_2, 1 h, 25 °C; (f) H_2/Pd−C(10%), H_2O, 2 h, 25 °C; (g) 2 equiv of Na, 1 equiv of *tert*-butyl alcohol, NH_3 (liq), 1 h, − 78 °C; (h) 0.65 equiv of $Mo(CO)_6$, 1 equiv of H_2O, MeCN, 80 °C; (i) 10 equiv of TFA, CH_2Cl_2, 1 h, 25 °C; (j) 2.1 equiv of LiOH, MeCN/H_2O (1:1), 17 h, 25 °C.

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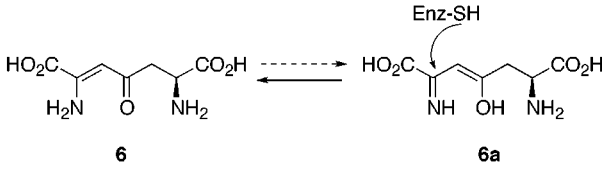
in 70% yield. Hydrolysis of the ester functionalities using lithium hydroxide, followed by deprotection of the amino moiety, generates the bis acid **11**. However, attempts to transform the isoxazole ring into the target vinylogous amide using standard conditions, such as hydrogenation or reduction with sodium in liquid ammonia,[9,12] produce exclusively the saturated alcohol **12** as a mixture of four diastereoisomers. Formation of the vinylogous amide moiety is possible by treatment of isoxazole **10** with a catalytic amount of molybdenum hexacarbonyl and 1 equiv of water in refluxing acetonitrile.[13] Subsequent exposure of **13** to excess trifluoroacetic acid (TFA) in dichloromethane for 1 h generates a 1:1 mixture of cyclic and open-chain vinylogous amides **14** and **15** in 90% yield. The yield of **15** can be improved to 80% by using 5 equiv of TFA and careful monitoring of the reaction by TLC. The cyclic derivative **14** could arise from Michael addition of the nitrogen liberated by removal of the *tert*-butoxycarbonyl moiety onto the α,β-unsaturated alkene followed by subsequent loss of ammonia. Hydrolysis of diester **14** using lithium hydroxide followed by workup gives **5** in 90% yield. Compound **15** is unstable at room temperature and is therefore best transformed immediately into the dilithium salt **6** (85% yield). Acidification of **6** leads rapidly to formation of the cyclized derivative **5**; therefore this compound was stored as its dilithium derivative. Vinylogous amide **5** is an interesting compound in terms of functional group density and structural fragments (ketone, primary amine, primary enamine) that would be incompatible or would decompose in water were it not for the stabilization afforded by the extended conjugation.

With vinylogous amides **5** and **6** available, DAP D-dehydrogenase was purified from *Bacillus spaericus* IFO 3525 as previously reported.[7a,14] In addition, DAP epimerase was isolated from an *Escherichia coli* mutant BL21(DE3) pLysS using a modified procedure.[6a,15] DAP D-dehydrogenase assay at pH 7.8 employs the reverse reaction, wherein $NADP^+$ oxidatively deaminates the D-amino acid center of *meso*-DAP (**1**) to L-tetrahydrodipicolinate (L-THDP) with generation of NADPH, thereby allowing continuous spectrophotometric assay at 340 nm.[7a,15] The stability of **6** during the enzyme assays can be monitored by TLC which reveals that cyclization begins to be detectable after 3 h in the buffer solution. Hence, each assay employed freshly dissolved inhibitor. No isomerization of the double bond geometry of **6** could be detected. Compounds **5** and **6** are not substrates for DAP D-dehydrogenase and show only poor reversible inhibition of DAP D-dehydrogenase, with IC_{50} values in the range of 400−450 μM. This is surprisingly weak binding given that these molecules possess much of the functionality present in the substrates (THDP and *meso*-DAP) and putative imine intermediate. Although the presence of an extra oxygen at the center of **6** could generate unfavorable steric interactions with the enzyme, a more likely cause for failure of effective binding to DAP dehydrogenase is the coplanarity of the vinylogous amide system. Crystallographic studies reveal that the isoxazoline **2** and the unsaturated analogue **3** bind in the active site of the DAP D-dehydrogenase with conformations which for analogues **5** and **6** could require bond rotation in the coplanar portions.[7b,8b]

Inhibition studies with DAP epimerase involve a coupled enzyme assay at pH 7.8, whereby *meso*-DAP (**1**) generated by the epimerase from LL-DAP is transformed by DAP D-dehydrogenase to produce L-THDP and NADPH, which is followed spectrophotometrically.[15] The results show that, as expected, **5** is a very poor inhibitor of DAP epimerase. Disappointingly, the acyclic vinylogous amide **6** is also a weak competitive inhibitor (IC_{50} of 500 μM) of this enzyme despite having most of its atoms in locations that might be expected to mimic the transition state or intermediate DAP-derived α-anion. Although a crystal structure of active DAP epimerase with a substrate analogue in the active site is not yet available,[16] the poor binding to DAP epimerase may be again be due to the planarity of the vinylogous amide system as well as consequent reduced basicity of the amino group on the sp^2 carbon. Initially it seemed that an enzyme-induced conformational twist around the C-3 to C-4 bond in **6** or around the C-2 to C-3 bond in its tautomer **6a** could generate reactive functionality and trigger addition of an active site thiol group, but the required tautomerization has not been observed (Scheme 3).

Scheme 3. Potential Tautomerization of **6** with Attack by DAP Epimerase



6 **6a**

Fortunately, the cyclic vinylogous amide **5**, which shows considerable structural similarity to L-DHDP proved to be a good inhibitor of L-DHDP reductase. This enzyme, which was isolated from *E. coli*, catalyzes the transfer of a hydrogen from NADPH to the γ-position of L-DHDP to give L-tetrahydrodipicolinic acid (THDP).[17] Inhibition studies demonstrate that **5** is a reversible competitive inhibitor of the DHDP reductase with respect to L-DHDP with an inhibition constant (K_i) of 32 μM. Hence **5** is comparable to the most potent competitive inhibitor of DHDP reductase reported thus far, the fully planar dipicolinic acid (**16**) (K_i 26 μM). Since

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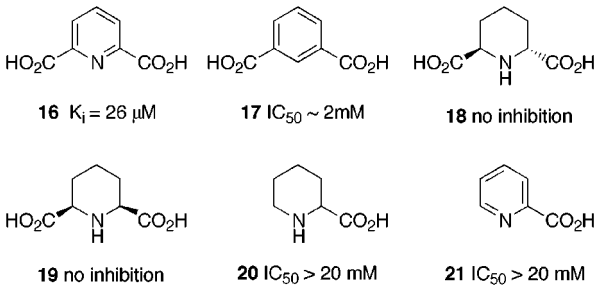
related derivatives such as isophthalic acid (**17**), a racemic mixture of *trans*-piperidine dicarboxylic acids (**18**), *cis*-piperidine dicarboxylic acid (**19**), pipecolic acid (**20**), and picolinic acid (**21**) are poor inhibitors, additional crystal-



lographic studies of DHDP reductase bound to **5** should help to clarify the substrate−enzyme interactions at the stereogenic center of DHDP. This would potentially lead to other cyclic derivatives with even greater inhibition.

In summary, we have described an efficient synthesis of highly functionalized vinylogous amides **5** and **6** in six steps starting from L-propargyl glycine. Although these compounds show only weak inhibition of DAP dehydrogenase and DAP epimerase, **5** is an excellent competitive inhibitor of DHDP reductase. Synthesis of other DAP analogues and evaluation of their interactions with DAP enzymes is currently underway in our laboratory.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **5**, **6**, **8**, and **11**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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