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Conformationally constrained diketopimelic acid analogues as inhibitors of dihydrodipicolinate synthase

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Abstract—Dihydrodipicolinate synthase (DHDPS) is a key enzyme in lysine biosynthesis and a potential antibiotic target. The enzyme catalyses the condensation of (*S*)-aspartate semi-aldehyde (ASA) and pyruvate to form dihydrodipicolinate. Constrained diketopimelic acid derivatives have been designed as mimics of the acyclic enzyme-bound condensation product of ASA and pyruvate. Several of the compounds are shown to be active, slow-binding inhibitors with improved inhibition of DHDPS.

The biosynthesis of lysine in plants and micro-organisms proceeds via the diaminopimelate (DAP) pathway, which is not present in mammals. In addition to the de novo synthesis of lysine for incorporation into proteins, lysine, and its immediate precursor, *meso*-DAP, are important constituents of the bacterial peptidoglycan cell wall. As such, enzymes in the DAP pathway have been investigated as targets for therapeutic agents.[1–4]

The enzyme that catalyses the first committed step towards lysine in the DAP pathway is dihydrodipicolinate synthase (DHDPS). DHDPS catalyses the condensation of (*S*)-aspartate semi-aldehyde (ASA, **2**) and pyruvate (**1**) to form an unstable heterocycle, 4-hydroxytetrahydrodipicolinate (HTPA, **3**), with spontaneous dehydration to give dihydrodipicolinate (DHDP, **4**) following release from the enzyme active site (Fig. 1).[5]

The DHDPS-catalysed reaction is initiated by condensation of pyruvate **1** with an active site lysine residue (lys161 in *Escherichia coli* DHDPS) forming a Schiff base. This has been confirmed by sodium borohydride trapping experiments,[6] and by X-ray crystallographic analysis.[5] Subsequent tautomerisation gives the enamine **5**. Aldol-type reaction of **5** with (*S*)-ASA **2** then gives the acyclic enzyme-bound intermediate **6** (Fig. 2). Transimination of the acyclic intermediate **6** is thought to yield the cyclic alcohol **3**, with simultaneous release of the active site lysine residue.

Many analogues of (*S*)-ASA, including glutamate semi-aldehyde, acetylaspartate semi-aldehyde and homoserine lactone, are neither substrates nor competitive inhib-

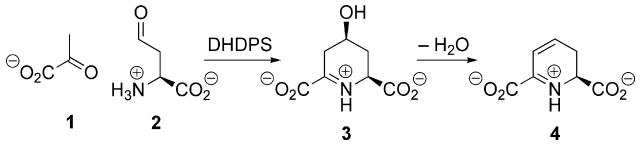

Figure 1. Condensation of pyruvate **1** and (*S*)-ASA **2** to form HTPA **3**, catalysed by DHDPS, then dehydration to give DHDP **4**.

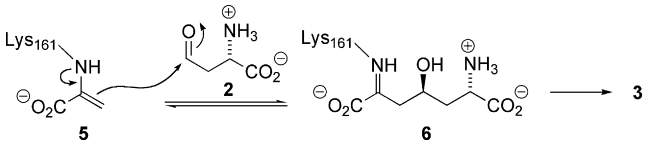

Figure 2. Condensation of pyruvate **2** and (*S*)-ASA **1** to give **3** proceeds through enamine **5** and enzyme-bound condensation product **6**.

Keywords: Dihydrodipicolinate synthase; DHDPS; Enzyme inhibitors; Lysine biosynthesis.
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itors of the enzyme.[7] Succinic semi-aldehyde—related to ASA but lacking the amino group—is a competitive inhibitor of DHDPS with respect to ASA (K_i = 0.3 mM).[5] Analogues of pyruvate are not substrates,[2] however analogues such as α-ketobutyrate, α-ketoglutarate, glyoxylate and fluoropyruvate have been shown to be competitive inhibitors of DHDPS with respect to pyruvate.[8] The majority of inhibitors reported to date have been based on DHDP **4**[7–9] or HTPA **3**,[10] but in general show only weak to moderate inhibitory activity. Dipicolinic acid and chelidamic acid show K_i = 11 mM[8] and 14 mM[10], respectively, with respect to pyruvate (Fig. 3). The reported sub-mM inhibition by related heterocyclic compounds[9] is likely due to flaws in the assay methods used.[10]

The failure of a large number of substrate and product analogues to display potent competitive inhibition led us to explore a new class of inhibitors, based on the acyclic enzyme-bound intermediate **6**. A previous report that α-ketopimelic acid is an irreversible inhibitor of DHDPS, with a K_i of 0.17 mM,[5] suggested that this might be a valid approach. The crystal structures of DHDPS with either α-ketopimelic acid or the adduct of pyruvate and succinic semi-aldehyde bound at the active site show that the pimelic acid moiety is positioned in a largely extended conformation, with torsion angles from C2 to C6 typically 141°–179°.[5] The bis(keto-acid) **8** was therefore designed as a conformationally constrained analogue of the acyclic intermediate **6**, with the ketoacid group able to condense with the active site lysine residue in the same manner as ketopimelic acid, and the aromatic ring serving to constrain the torsion angles of the central C–C bonds to 180°. Accordingly, compound **8** and various analogues **9**–**15** were synthesised and assayed for inhibition of DHDPS activity.

The parent bis(keto-acid) **8** was available in one step (98% yield) from commercially available 1,3-diacetyl benzene **7**, by oxidation with selenium dioxide (Fig. 4). Esterification in acidified methanol provided a mixture of the corresponding diester **9** in variable yield (45–97%) with the dimethyl ketal **10** being the major byproduct. The byproduct **10** could be recycled by hydrolysis to the bis(keto-acid) **8** in quantitative yield.

The bis(keto-acid) **8** was also converted to the corresponding bis-oxime **11** by treatment with hydroxylamine hydrochloride in the presence of sodium carbonate, giving the product in quantitative yield as a single isomer (Fig. 5). Similar treatment of bis(keto-ester) **9** with hydroxylamine hydrochloride and pyridine gave the bis-oxime **12** in 64% yield as a 3:2 ratio of (*Z*,*Z*)- and (*E*,*Z*)-isomers (Fig. 6).

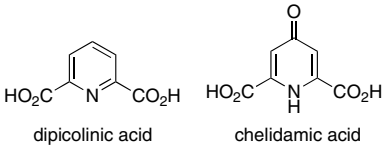

Figure 3. Heterocyclic inhibitors of DHDPS.

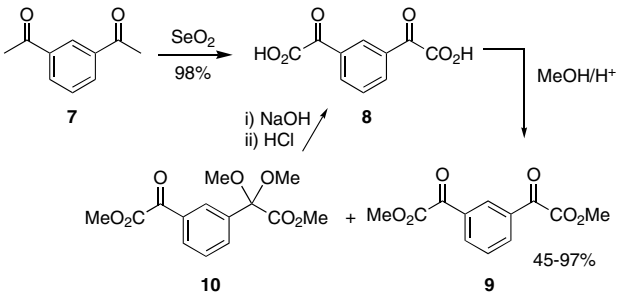

Figure 4. Synthesis of bis-ketoester **9**.

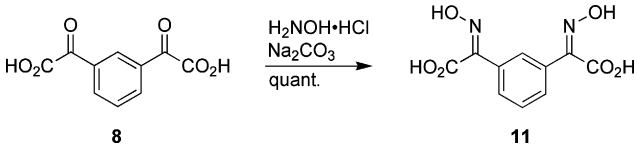

Figure 5. Synthesis of bis(oxime-acid) **11**.

The keto- and oxime-esters **9** and **12** were reduced to the corresponding diol **14** and diamine **13**. Treatment of the bis(keto-ester) **9** with sodium borohydride gave the diol **14** as a mixture of stereoisomers. Reduction of the oxime ester **12** with zinc/formic acid gave the bis-amine **13** in good yield (92%). The diol diester **14** was hydrolysed to the corresponding diacid **15** in quantitative yield by treatment with lithium hydroxide (Fig. 6).

All compounds prepared were tested for inhibition of DHDPS activity using the coupled assay, in which the NADPH-dependent reduction of DHDP **4** by the subsequent enzyme in the pathway, dihydrodipicolinate reductase (DHDPR), is followed by the absorption at 340 nm.[8,11] With the possibility of condensation of the ketone or oxime functional groups present in **8**–**12** with the active site lysine residue, these inhibitors were also tested for time-dependent inhibition. Assay conditions

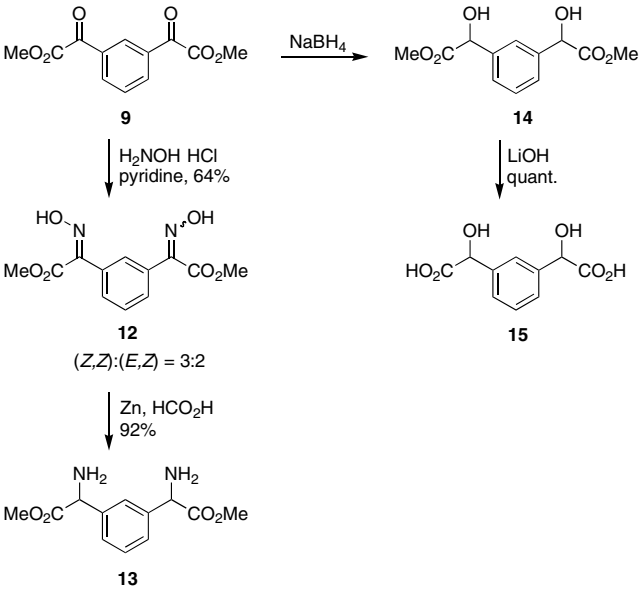

Figure 6. Synthesis of further derivatives **12**–**15**.