Unsaturated α-aminopimelic acids as potent inhibitors of *meso*-diaminopimelic acid (DAP) D-dehydrogenase

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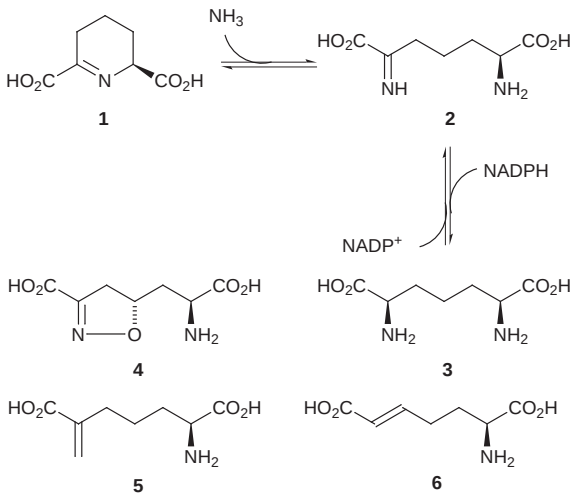
Received (in Corvallis, OR, USA) 8th January 1999, Accepted 4th February 1999

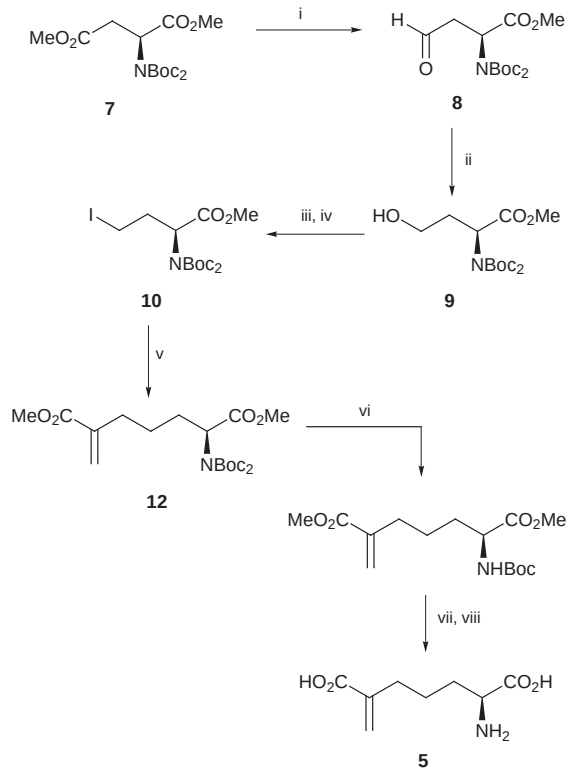
Two nonproteinogenic amino acids 5 and 6 have been efficiently prepared using an S_H2' allylstannane coupling reaction and a Wittig reaction, respectively, to effect the key steps; kinetic studies show these compounds to be reversible inhibitors of DAP dehydrogenase with K_i values of 5.3 (competitive) and 44 μM (noncompetitive), respectively.

Due to the increasing problems associated with antibiotic resistance, alternative strategies to disrupt microbial cell wall synthesis have become highly desirable.[1] The key crosslinking amino acid in the peptidoglycan cell wall layer of Gram negative bacteria is *meso*-diaminopimelic acid (DAP), the biosynthetic precursor of L-lysine, which is used as the crosslinker by many Gram positive organisms.[2] The biochemical pathway to this molecule is not present in animals, which require L-lysine in their diet, and therefore specific inhibitors of the DAP enzymes are likely to be antimicrobial agents with low mammalian toxicity. One of the enzymes used by certain bacteria[2] is *meso*-DAP D-dehydrogenase (EC 1.4.1.16), which converts L-tetrahydrodipicolinate (THDP) **1** to *meso*-DAP **3**, presumably *via* an imine **2** which is reduced stereospecifically by NADPH to generate the D-amino acid center (Scheme 1).[3] We previously demonstrated that a conformationally restricted isoxazoline analogue **4** having the DAP skeleton and a planar α-carbon attached to a basic nitrogen is a potent reversible inhibitor of DAP dehydrogenase and has antimicrobial activity.[4] However, recent X-ray crystallographic studies show that it binds in an unexpected fashion in the active site which places the L-amino acid center rather than the imine bond near the NADP moiety.[5] We now report the syntheses of two new inhibitors **5** and **6** of DAP dehydrogenase which contain a planar α-carbon but lack the characteristic basic nitrogen at the presumed reactive end of the molecule.

The synthesis of unsaturated amino acid **5** is outlined in Scheme 2. The aspartic acid derivative **7** was selectively reduced to the γ-aldehyde **8** in good yield using DIBAL-H.[6]



Scheme 1

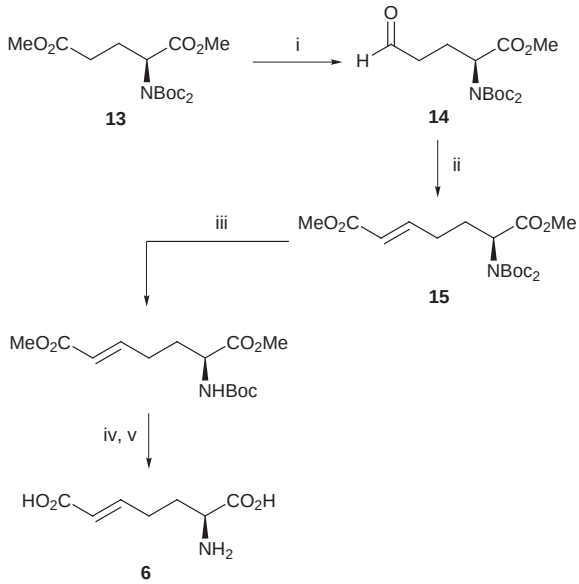


Scheme 2 *Reagents and conditions*: i, DIBAL-H (1.2 equiv.), Et_2O, −78 °C, 87%; ii, $NaBH_4$, THF, 82%; iii, MsCl, NEt_3, DMAP, CH_2Cl_2, 85%; iv, NaI, acetone, Δ 89%; v, AIBN, $MeO_2CC(=CH_2)CH_2SnPh_3$ **11**, C_6H_6, Δ, 60%; vi, TFA (1.2 equiv.), CH_2Cl_2, 76%; vii, LiOH, MeOH, H_2O, 84%; viii, TFA, CH_2Cl_2, 95%.

Further reduction gave the primary alcohol **9** which was converted to the iodide **10** using standard procedures.[7] Radical coupling of iodide **10** with stannane **11**[8] was initiated thermally using AIBN to give the protected α-aminopimelic acid analogue **12** in 60% yield. Selective removal of one of the Boc groups, followed by ester hydrolysis and finally cleavage of the second Boc group gave the parent amino acid **5** in excellent yield (20% overall for eight steps from **7**).

Reduction of the protected glutamate **13** with DIBAL-H similarly gave aldehyde **14** in good yield (Scheme 3). Wittig coupling with the stabilized ylide, methyl (triphenylphosphoranylidene)acetate, gave solely the *trans*-alkene **15**[9] which was deprotected to **6** in a similar manner as above.

With compounds **5** and **6** available, DAP D-dehydrogenase was isolated and purified from *Bacillus sphaericus* IFO 3525 as previously reported.[3a,4] Inhibition studies with this enzyme were done at pH 7.8 using the reverse reaction (oxidative deamination by $NADP^+$ of the D-amino acid center of **3** to give **1**). This produces NADPH and thereby allows continous spectrophotometric assay at 340 nm. The unsaturated amino acids **5** and **6** were first individually tested as possible substrates for DAP D-dehydrogenase in the absence of *meso*-DAP **3**, and



Scheme 3 *Reagents and conditions*: i, DIBAL-H, Et_2O, −78 °C, 86%; ii, $Ph_3P{=}CHCO_2Me$, THF, 85%; iii, TFA (1.2 equiv.), CH_2Cl_2, 82%; iv, LiOH, MeOH, H_2O, 98%; v, TFA, CH_2Cl_2, 87%.

as expected, showed no conversion. Subsequent inhibition studies showed both compounds to be very good but rapidly reversible inhibitors (not time-dependent) of DAP D-dehydrogenase. More detailed kinetic analyses demonstrated that **5** is a competitive inhibitor with an inhibition constant (K_i) of 5.3 μM. In contrast, a Michaelis–Menten plot for **6**, which has the double bond in the backbone, showed noncompetitive inhibition with a K_i value of 44 μM, indicating different binding modes for the two unsaturated analogues.

DAP D-dehydrogenase from *Corynebacterium glutamicum* exists as a homodimer with each monomer unit (*M ca*. 35200) possessing a similar fold but somewhat different overall conformation based on crystallographic studies.[5,10] Although there may be structural differences between the *B. sphaericus* and *C. glutamicum* enzymes,[11] their overall mechanism and inhibition by **4** are similar. Since isoxazoline **4** is a competitive inhibitor with respect to L-tetrahydrodipicolinate **1** (K_i = 4.2 μM) but a noncompetitive inhibitor with respect to *meso*-DAP (K_i = 23 μM),[4] it seems likely that the nearly equipotent noncompetitive inhibitor **6** may bind in a similar fashion. This indicates that interactions of the isoxazoline nitrogen and oxygen with active site water molecules seen in crystallographic studies may play a minor role in binding.[5] However, **5** is directly competitive with *meso*-DAP **3** and is one of the most potent substrate analogue inhibitors for this enzyme found thus far. This suggests that **5** may be a true mimic of the imine intermediate **2**, and that normal catalysis by DAP D-dehydrogenase can be blocked by conformational changes induced in the protein by binding of **4** or **6** in the adjacent subunit at a site normally occupied by THDP **1** (Fig. 1). Additional crystallographic studies with the unsaturated α-aminopimelic acid derivatives **5** and **6** may help to clarify the subunit interactions and the detailed mechanism of this enzyme. Further studies on the synthesis of DAP analogues[12] and their interactions with other DAP enzymes are in progress.

The authors thank Dr Jonathan Parrish for assistance with molecular modeling. Financial support from the Alberta Heritage Foundation for Medical Research (Fellowship to A. S.) and the Natural Sciences and Engineering Research Council of Canada (Scholarship to J. F. C. and Operating Grant) is gratefully acknowledged.

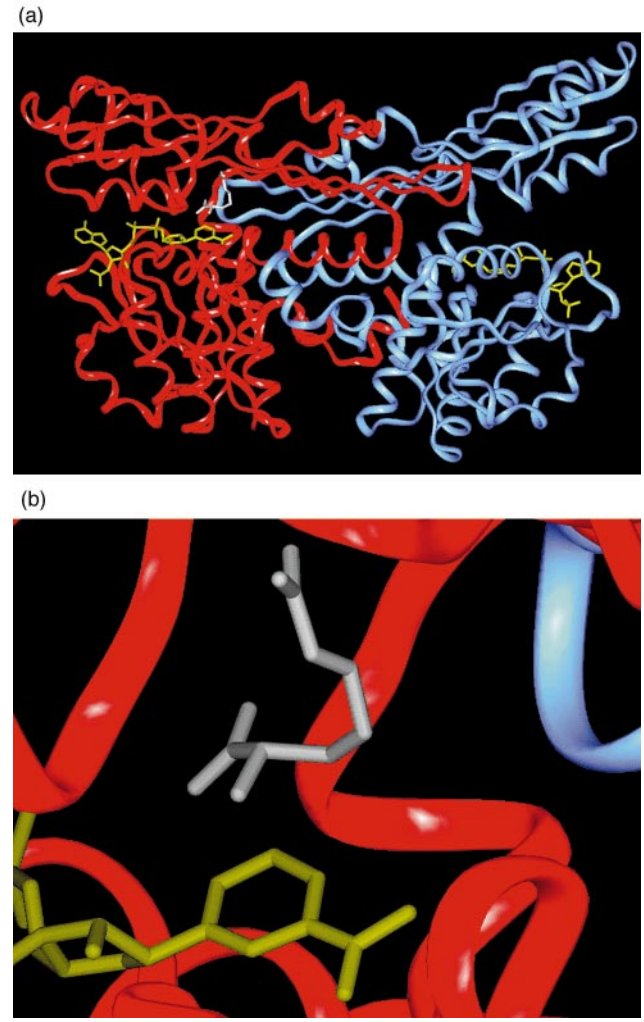


Fig. 1 (a) Model of **6** (white) docked into homodimeric DAP D-dehydrogenase (red and blue subunits) from *C. glutamicum* generated by best-fit replacement of the isoxazoline **4** in the corresponding enzyme–inhibitor crystal structure (ref. 5). These inhibitors (**4** and **6**) have their L-amino acid center proximal to the NADP moiety (yellow) and therefore cannot be oxidized by the cofactor. (b) Expansion to show the inhibitor conformation in the closed active site.

Notes and references

1 T. D. H. Bugg and C. T. Walsh, *Nat. Prod. Rep*., 1992, **9**, 199; J.-M. Ghuysen, *Int. J. Antimicrob. Agents*, 1997, **8**, 45.
2 R. J. Cox, *Nat. Prod. Rep*., 1996, **13**, 29; G. Scapin and J. S. Blanchard, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 1998, **72**, 279.
3 H. Misono and K. Soda, *J. Biol. Chem*., 1980, **255**, 10599; S. J. Maniscalco, S. K. Saha and H. F. Fisher, *Biochemistry*, 1998, **37**, 14585.
4 S. D. Abbott, P. Lane-Bell, K. P. S. Sidhu and J. C. Vederas, *J. Am. Chem. Soc*., 1994, **116**, 6513.
5 G. Scapin, M. Cirilli, S. G. Reddy, Y. Gao, J. C. Vederas and J. S. Blanchard, *Biochemistry*, 1998, **37**, 3278.
6 G. Kokotos, J. M. Padron, T. Martin, W. A. Gibbons and V. S. Martin, *J. Org. Chem*., 1998, **63**, 3741.
7 M. A. Walker, K. P. Kaplita, T. Chen and H. D. King, *Synlett*, 1997, 169.
8 J. E. Baldwin, R. M. Adlington, M. B. Mitchell and J. Robertson, *Tetrahedron*, 1991, **47**, 5901.
9 The preparation of **15** by essentially the same route has been reported very recently, after our synthesis was complete: J. M. Padron, G. Kokotos, T. Martin, T. Markidis, W. A. Gibbons and V. S. Martin, *Tetrahedron: Asymmetry*, 1998, **9**, 3381.
10 G. Scapin, S. G. Reddy and J. S. Blanchard, *Biochemistry*, 1996, **35**, 13 540.
11 H. Misono, M. Ogasawara and S. Nagasaki, *Agric. Biol. Chem.*, 1986, **50**, 2729; F. Wang, G. Scapin, J. S. Blanchard and R. H. Angeletti, *Protein Sci*. 1998, **7**, 293.
12 Y. Gao, P. Lane-Bell and J. C. Vederas, *J. Org. Chem.*, 1998, **63**, 2133.

Communication 9/00297I