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Difluoromethylene analogues of aspartyl phosphate: the first synthetic inhibitors of aspartate semi-aldehyde dehydrogenase

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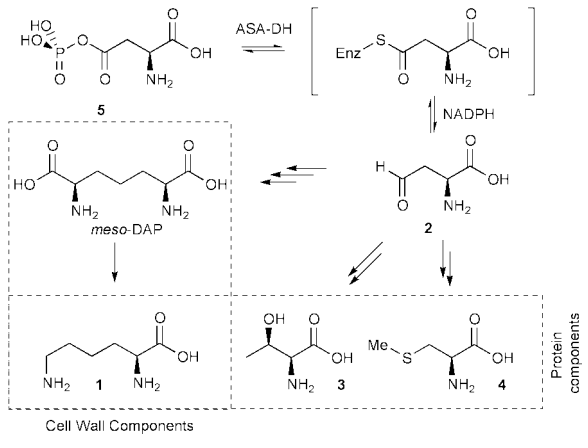
The difluoromethylene analogue of aspartyl phosphate 6 has been prepared by the fluoride catalysed coupling of diethyl trimethylsilyldifluoromethyl phosphonate with an appropriate aldehyde followed by Dess–Martin oxidation and deprotection; the deprotected compound inhibited (K_I 95 μM) aspartate semi-aldehyde dehydrogenase, a key enzyme involved in bacterial amino acid and peptidoglycan biosynthesis.

Bacterial resistance to current classes of antibacterial compounds is increasing alarmingly.[1,2] Many successful antibiotics target bacterial cell wall biosynthesis, and the efforts of our group have concentrated on attempts to design and synthesise inhibitors of enzymes from this pathway.[3] We have focussed on inhibitors of L-lysine **1** biosynthesis since L-lysine and its precursor, diaminopimelic acid (DAP), form the strength bearing cross-links of the peptidoglycan layer of bacterial cell walls (Scheme 1).[4] One of the earliest enzymes on the pathway, aspartate semi-aldehyde dehydrogenase (ASA-DH, EC 1.2.1.11) forms the key intermediate L-aspartate semi-aldehyde **2** the precursor of DAP and also L-threonine **3** and L-methionine **4**. Inhibitors of ASA-DH would therefore block dual key bacterial metabolic processes forming proteins and peptidoglycan.

The mechanism of ASA-DH is believed to involve an active site nucleophilic cysteine.[5] γ-Aspartyl phosphate **5**, is bound at the active site. Thiolester formation then occurs with concomitant loss of phosphate. NADPH then transfers its 4-*proS* hydride to reduce the thiolester, releasing the aspartate semi-aldehyde **2** and regenerating the nucleophile (Scheme 1). The 3D crystal structure of ASA-DH has recently been solved, but as no substrates were bound in the structure little information is available regarding the active site geometry and potential binding sites.[6] We decided to synthesise specific ASA-DH inhibitors. Ideally these compounds would mimic the natural substrate and contain an electrophillic centre for covalent attachment to the active site nucleophile.

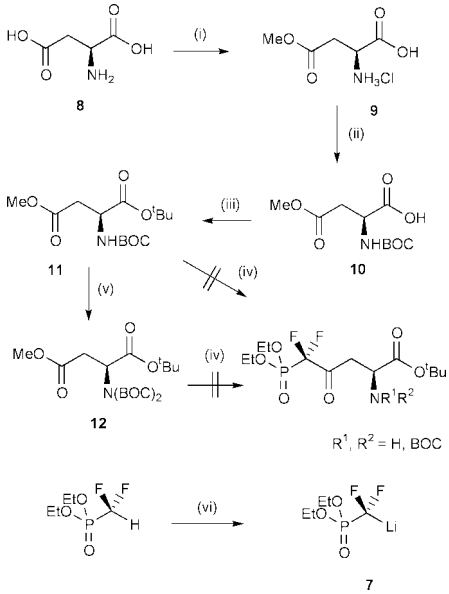


Scheme 1 Bacterial pathways to cell wall and protein components.

Difluoromethylene phosphonates have found use as phosphate mimics in numerous applications.[7] The difluoromethylene unit effectively mimics oxygen and the pK_a of difluoromethylene phosphonates closely matches that of the analogous phosphates.[8] Compounds such as **6** could form effective inhibitors of ASA-DH. Additionally the difluoromethylene α to the carbonyl would have the effect of making it an excellent electrophile and likely target for thiol attachment.

Berkowitz has reacted difluoromethylene anions such as **7** with methyl esters to prepare ketones directly.[9] We attempted to follow this methodology for the synthesis of **6** (Scheme 2). L-Aspartic acid **8** was selectively protected at the γ-ester using methanol and thionyl chloride to form the mono ester **9**. BOC protection to give **10** followed by formation of the α-*tert*-butyl ester then gave the required methyl ester **11**. However this ester was unreactive under the reaction conditions of Berkowitz[9] and we considered that the mono-protected amine could be the source of an acidic proton. Formation of the doubly BOC protected aspartate **12** was achieved, but this also could not be transformed to the required phosphonate. The bis(methyl ester) **13** was also synthesised by parallel methodology, but was similarly unreactive towards anion **7**.

We next attempted to add **7** to aldehydes (Scheme 3).[10] Treatment of **13** with DIBAL-H yielded a mixture of the corresponding alcohol **14** (22%) and aldehyde **15** (68%);[11] **14** could be converted to **15** by treatment with the Dess–Martin periodinane.[12,13] When **15** was treated with excess **7** (*ca.* 7.5 eq.) at −78 °C we were able to isolate the mixture of diastereomers **16a** and **16b** in very low yield—no products were



Scheme 2 *Reagents and conditions*: (i) $SOCl_2$, MeOH, −10 °C, 78%; (ii) BOC_2O, $NaHCO_3$–MeOH, 75%; (iii) $(CH_3)_3COH$, DMAP, EDCI, 64%; (iv) **7**, THF, −78 °C–0 °C; (v) NaH, BOC_2O, THF, Δ, 86%; (vi) LDA, −78 °C–0 °C.

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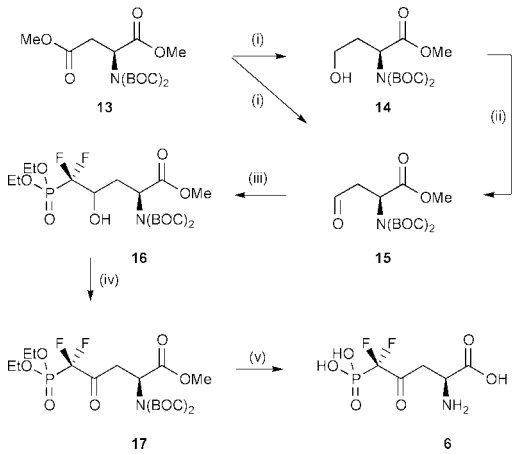
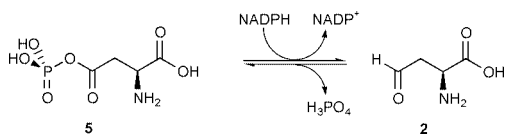


Scheme 3 *Reagents and conditions*: (i) DIBAL-H, THF, −78 °C, 68%; (ii) Dess–Martin periodinane, CH_2Cl_2, RT, 75%; (iii) $(EtO)_2P(O)CF_2SiMe_3$, THF, 10 mol% TBAF, −60 °C RT, 55%; (iv) Dess–Martin periodinane, CH_2Cl_2, RT, 69%; (v) TMSI, then aq. KOH, then Dowex AG50 WX8, 95%.

formed with lower excesses or stoichiometric amounts of **7**. Under milder conditions treatment of **15** with $(EtO)_2P(O)CF_2SiMe_3$ in THF at 0 °C in the presence of a catalytic fluoride source such as CsF, TBAF or TBAT ($Bu_4N^+SiPh_3F_2^-$) yielded the required products. The most favourable conditions required the use of 1.3 eq. of $(EtO)_2P(O)CF_2SiMe_3$, 10 mol% TBAF and one equivalent of **15**. This reaction yielded a mixture of diastereomers **16a** (2*S*,4*S*) and **16b** (2*S*,4*R*)[14] in 3 : 1 ratio in overall 55% (Scheme 3) with the remaining mass balance being unreacted aldehyde. The alcohols were smoothly converted to the ketone **17**. A two-step deprotection involving treatment of **17** with 5 eq. of TMSI (Me_3SiI) followed by KOH treatment to remove the methyl ester yielded **6** in excellent yield as a 45 : 55 mixture of keto- and hydrate forms.[15] After purification by ion exchange chromatography, **6** was used in inhibition assays using recombinant *E. coli* ASA-DH.

The ASA-DH assay (Scheme 4) requires that the enzyme be run in the 'reverse' direction as aspartyl phosphate is unavailable whereas aspartate semi-aldehyde is obtained by reductive ozonolysis of allyl glycine in 1 M aqueous HCl. In the assay, reaction is observed at 340 nm as NADPH is formed from $NADP^+$ (150 μM). In order to ensure satisfactory reaction rates the other substrates (PO_4^{3-} and ASA) must be present in large excess (15 and 0.35 mM respectively). In initial inhibition reactions under these conditions **6** showed little observable effect. However in pre-incubation reactions in which ASA-DH was incubated with **6** *prior* to addition of the other assay components clear inhibition was observable varying with time (Fig. 1A) and concentration (Fig 1B). Based on this assay a K_I of 95 μM was measured.[16] The rate of inhibition of ASA-DH by **6** was diminished in the presence of phosphate (Fig. 1B) indicating that inhibition occurs at the active site. The inhibition appears to be slowly reversible as indicated by the regeneration of activity upon dilution of the inhibited enzyme into the assay cuvette. This behaviour is consistent with that expected for a slow binding model of inhibition and is most likely caused by covalent bond formation between **6** and the active site thiol of ASA-DH.



Scheme 4 Assay of ASA-DH. In the reverse reaction phosphate and ASA are present in large excess.

Thus we have shown that a rational approach can successfully be used for the design and synthesis of ASA-DH inhibitors. The route should enable the synthesis of a range of related compounds designed to probe the mechanism and active

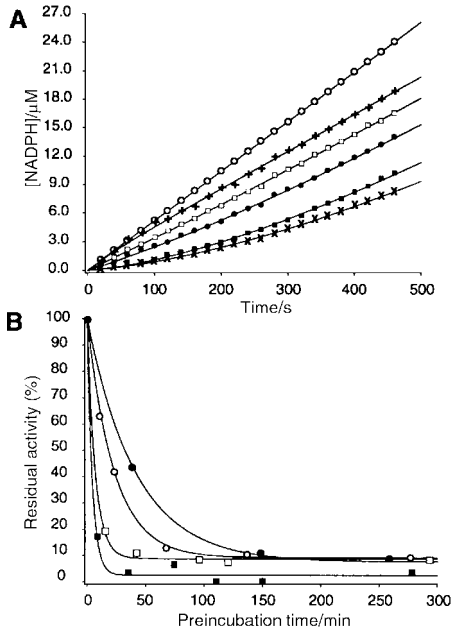


Fig. 1 A. Inhibition of ASA-DH by preincubation with **6** at 0.66mM. ○ uninhibited reaction; + preincubation with **6** for 0 min; □ preincubation with **6** for 12 min; ● preincubation with **6** for 25 min; ■ preincubation with **6** for 68 min; X preincubation with **6** for 138 min. B. Rate of inhibition of ASA-DH at varying **6** concentration (○ 0.66; □ 2.5; ■ 5.0 mM) and at ● 2.5 mM **6** in the presence of phosphate (15 mM).

site geometry of ASA-DH. The inhibited enzyme is currently undergoing crystallisation trials.

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Notes and references

1 House of Lords Select Committee on Science and Technology, Seventh Report, 1998.
2 *The Management and Control of Hospital Acquired Infections in Acute NHS Trusts in England and Wales* Report by the Comptroller and Auditor General, House of Commons, 14th February 2000.
3 R. J. Cox, A. Sutherland and J. C. Vederas, *Bioorg. Med. Chem.*, 2000, **8**, 843.
4 R. J. Cox, *Nat. Prod. Rep.*, 1996, **13**(1), 29.
5 W. E. Karsten and R. E. Viola, *Biochim. Biophys. Acta*, 1991, **1077**, 209.
6 A. Hadfield, G. Kryger, J. Ouyang, G. A. Petsko, D. Ringe and R. Viola, *J. Mol. Biol.*, 1999, **289**(4), 991.
7 M. J. Tozer and T. F. Herpin, *Tetrahedron*, 1996, **52**(26), 8619.
8 G. M. Blackburn, D. E. Kent and F. Kolkmann, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1119.
9 D. B. Berkowitz, M. Eggen, Q. Shen and R. K. Shoemaker, *J. Org. Chem.*, 1996, **61**, 4666.
10 S. F. Martin, D. W. Dean and A. S. Wagman, *Tetrahedron Lett.*, 1992, **33**, 1839.
11 A. Sutherland, J. F. Caplan and J. C. Vederas, *J. Chem. Soc., Chem. Commun.*, 1999, 555.
12 D. B. Dess and J. C. Martin, *J. Am. Chem. Soc.*, 1991, **113**, 7277.
13 R. E. Ireland and L. B. Liu, *J. Org. Chem.*, 1993, **58**(10), 2899.
14 R. J. Cox, M. Murray and M. B. Mayo-Martin, unpublished.
15 Selected data for **6. Hydrate:** δ_H(400 MHz, D_2O) 4.05 (1 H, dd, $^3J_{HH}$ 4.1, 8.05, αCH), 2.35 (1H, dd, $^2J_{HH}$ 15.4, $^3J_{HH}$ 4.4, βCH), 2.17 (1H, dd, $^2J_{HH}$ 15.4, $^3J_{HH}$ 8.2, βCH); δ_F(283 MHz, D_2O) −122.6 (1F, dd, $^2J_{FF}$ 301.8, $^2J_{FP}$ 90.6), −123.5 (1F, dd, $^2J_{FF}$ 300.0, $^2J_{FP}$ 90.3); δ_P(122 MHz, D_2O) 3.26 (dd, $^2J_{PF}$ 86.6, 89.1). **Ketone:** δ_H(400 MHz, D_2O) 4.05 (1H, dd, $^3J_{HH}$ 4.02, 8.05, αCH), 3.39 (1H, dd, $^2J_{HH}$ 19.8, $^3J_{HH}$ 4.1, βCH), 3.32 (1H, dd, $^2J_{HH}$ 20.1, $^3J_{HH}$ 7.8, βCH); δ_F(283 MHz, D_2O) −120.2 (br d, $^2J_{FP}$ 84.1); δ_P(122 MHz, D_2O) 0.67 (t, $^2J_{PF}$ 85.3); *m/z* (ESMS, CH_3CN–H_2O) 247.9 ([M]H^+, 100%), 265.9 ([M + H_2O]H^+, 75%), 270.0 ([M]Na^+, 50%), 289.9 ([M + CH_3CN]H^+, 45%), 494.8 ([2M]H^+, 20%).
16 *Fundamentals of Enzyme Kinetics*, ed. A. Cornish-Bowden, Portland Press, London, 1995.