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A new synthesis of phosphoramidates: inhibitors of the key bacterial enzyme aspartate semi-aldehyde dehydrogenase†

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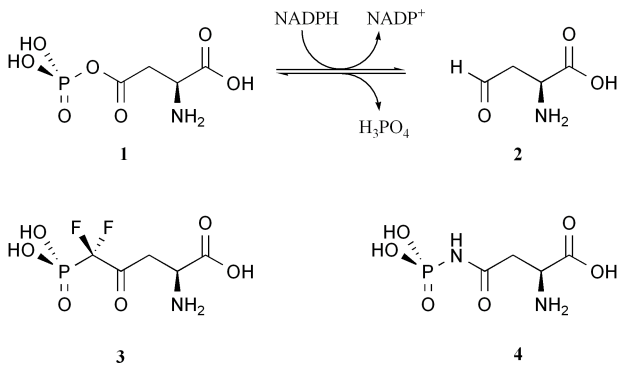
A new, mild and high yielding synthesis of phosphoramidates is described: potassium salts of carboxylic acids are treated with ethylchloroformate and the resulting activated anhydride-carbonates are then treated with LiNHP(O)(OEt)$_2$ *in situ*—the methodology is especially suited to acid sensitive systems featuring BOC, ^{t}Bu or acetal protecting groups.

Phosphoramidates (*N*-phosphoryl amides) show interesting biological properties and the occurrence of this structural motif in natural products has stimulated the development of methods for its construction. It is likely that these compounds can mimic phosphate esters and diphosphate linkages, but their increased second pK_a (*ca*. 6.4)[1] compared to acyl phosphate (*ca*. 5.0)[2] and reduced propensity for hydrolysis can produce useful enzyme inhibitory effects.

We have been studying the enzyme aspartate semi-aldehyde dehydrogenase (ASA-DH), a key bacterial enzyme involved in the biosynthesis of the aspartate family of amino acids.[3] This enzyme utilises aspartyl phosphate **1** as its substrate (Scheme 1). L-Aspartate semi-aldehyde **2** then serves as a precursor for the biosynthesis of L-lysine, L-threonine, L-methionine and L-isoleucine. We have already shown that simple analogues of **1**, such as the difluoromethylene phosphonate **3**, can cause significant inhibition of ASA-DH and such compounds are leads for the development of potential new antibacterial compounds.[4] In this case, **3** showed poor competition *vs* inorganic phosphate and hence low inhibition of ASA-DH, perhaps due to its lowered phosphate pK_a in comaprison to **1**. However, in the absence of inorganic phosphate, **3** showed likely thiohemiacetal formation with the active site cysteine-135 of ASA-DH due to the high electrophilicity of the γ-carbonyl, and hence overall good inhibition. We wished to examine the phosphoramidate **4** as a potential inhibitor of ASA-DH because its phosphate pK_a should be somewhat higher than **3** and because of the lowered electrophilicity of the γ-carbonyl.



Scheme 1 Reaction catalysed by aspartate semi-aldehyde dehydrogenase and structures of putative inhibitors.

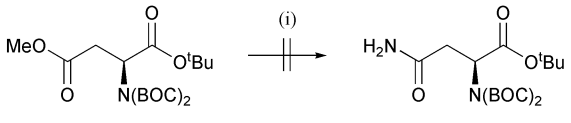
For these reasons we expected **4** to mimic acyl-phosphate **1** better than **3**, but not to form a covalent adduct at the active site of ASA-DH.

Two main routes have been described for the synthesis of phosphoramidates, relying either on P–N bond formation or N–C bond formation. The usual P–N routes are *via* either *N*-phosphorylation of deprotonated amides, using substituted phosphorchloridates,[5] or rarely by Arbuzov reaction of *N*-chloroamides with trialkylphosphites.[6] Alternatively, N–C bond formation can be achieved by coupling of phosphoramides with acyl chlorides as has recently been described.[7] We had easy access to the fully protected aspartate **5** and so we considered that formation of the corresponding primary amide **6**, followed by *N*-phosphorylation should proceed as described in the literature.

Attempts to synthesise the primary amide **6** failed (Scheme 2). Treatment of **5** with NH_3 in THF at reflux for 24 h did not result in formation of **6**. The use of neat refluxing NH_3 (−33 °C) was similarly unsuccessful and even refluxing NH_3/Na (−33 °C) failed to produce the primary amide.[8] In the absence of the amide we next considered the other known methods for phosphoramidate synthesis. However these methods rely on the formation of acyl chloride species which would be incompatible with the BOC and *tert*-butyl ester protection scheme.

Treatment of **5** with 1.0 equivalent of KOH in H_2O/CH_3CN gave a quantitative yield of the corresponding potassium salt **7** after lyophilisation (Scheme 3). This compound was conveniently soluble in CH_2Cl_2 and treatment with a slight excess of ethylchloroformate gave the mixed anhydride carbonate **8** *in situ*, with the concomitant precipitation of KCl. Addition of diethyl phosphoramidate **9** to this solution, however, did not result in the formation of the expected phosphoramidate **11**. An alternative procedure involving the pretreatment of diethyl phosphoramidate **9** with one equivalent of butyllithium to generate lithiated anion **10** before slow addition to the activated acyl species **8** was much more effective, generating the desired **11** in 49% yield after purification.[9]

In order to test the generality of this method we took a number of potassium salts and subjected them to similar procedures. In all cases the reaction proceeded smoothly and resulted in good to high yields of products (Scheme 4). The reaction sequence is compatible with protected amino acids such as **7**, **12** and **14**, and simple carboxylates such as **16**. More biologically relevent substrates such as octanoate **18** are also converted to their corresponding phosphoramidates: **19** mimics lipoyl-AMP,[10] a key intermediate in the post-translational modification of core metabolic enzymes such as pyruvate dehydrogenase. Phosphoramidate **21** is a potential intermediate



Scheme 2 Attempted synthesis of primary amide **6**. *Reagents and conditions*: (i) NH_3, THF, heat; or NH_3, heat; or NH_3/Na, heat.

† Electronic supplementary information (ESI) available: $[\alpha]_D^{20}$ measurements for compounds **4**, **5** and **11**; analytical data for **11**. See http://www.rsc.org/suppdata/cc/b2/b206199f/

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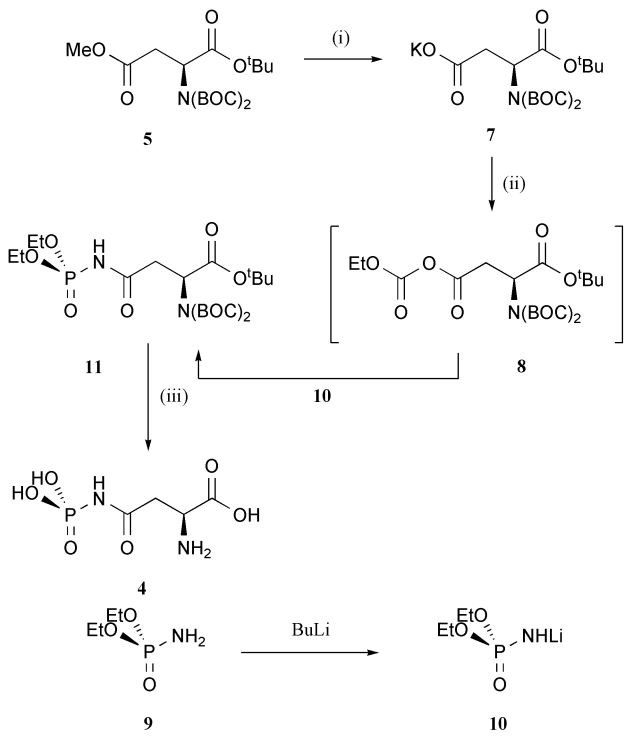
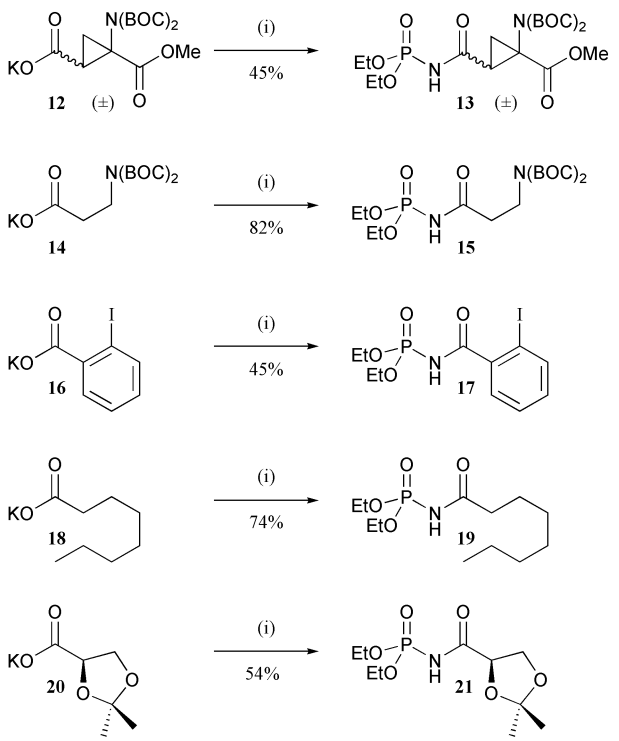


Scheme 3 *Reagents and conditions*: (i) 1.0 eq. KOH, H_2O/CH_3CN, RT, then freeze-dry, quantitative; (ii) EtOCOCl, CH_2Cl_2, RT then $LiNHP(O)(OEt)_2$, −78 to 18 °C, 49%; (iii) 5.0 eq. TMSI, 0 °C, 72%.



Scheme 4 *Reagents and conditions*: (i) EtOCOCl, CH_2Cl_2, RT then $LiNHP(O)(OEt)_2$, −78 to 18 °C, unoptimised yields as indicated.

towards the synthesis of mimics of 1,3-bisphosphoglygerate, a central intermediate in glycolysis.

The protected phosphoramidate **11** was cleanly deprotected by treatment with precisely 5.0 equivalents of trimethylsilyl iodide (TMSI) to yield **4**. In the presence of excess TMSI both aspartic acid and asparagine were observed by TLC as

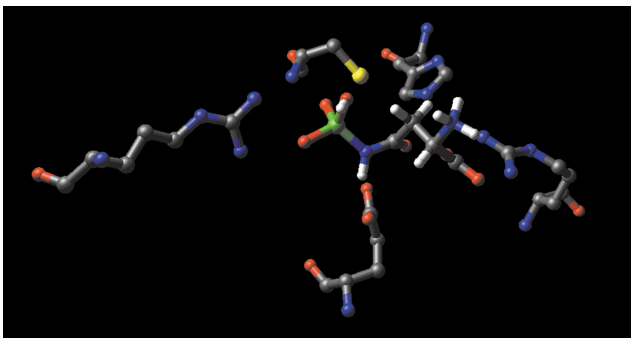


Fig. 1 Model structure created with SYBYL® (Tripos Software) showing **4** (centre) at the active site of ASADH. Residues: Arg102 (left), Arg267 (right), Cys135 (rear), His274 (rear) and Glu241 (below).

contaminating biproducts, but these were minimised with the use of pre-distilled TMSI at 0 °C.

In inhibition tests with ASA-DH, the phosphoramidate **4** showed clear competitive inhibition *vs.* both ASA (K_I = 214 ± 120 μM) and phosphate (K_I = 92 ± 40 μM). These results show that the phosphoramidate competes with phosphate more effectively than the difluoromethylene phosphonate **3** (K_I > 2 mM in similar assays),[4] perhaps due to its elevated pK_a.[1] As expected, no time dependent inhibition was observed when **4** was incubated with ASA-DH alone, even at elevated concentrations of **4** (20 mM) indicative of a lack of covalent adduct formation at the active site. This is the opposite effect shown by **3**.[4]

Model studies of **4** bound at the active site of ASA-DH (Fig. 1) support this idea. In the model, **4** is bound between Arg102 and Arg267, with the backbone near to catalytic Cys135 and His274. However, the phosphoramidate carbonyl is located 4.54 Å away from the cysteine nucleophilic sulfur—well beyond the 1.81 Å sum of covalent radii.

Overall we have demonstrated a new, straight-forward, mild and efficient synthesis of phosphoramidates compatible with acid sensitive protecting strategies. The ASA-DH inhibitor **4** shows useful competitive inhibition but, as expected, no time-dependent inhibition. This indicates that future inhibitor design for ASA-DH will have to heed phosphate mimic pK_a, while for best results, enhanced electrophilicity of the γ-carbonyl is required for active site covalent attachment.

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