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The Preparation of (*S*)-Aspartate Semi-Aldehyde Appropriate for Use in Biochemical Studies

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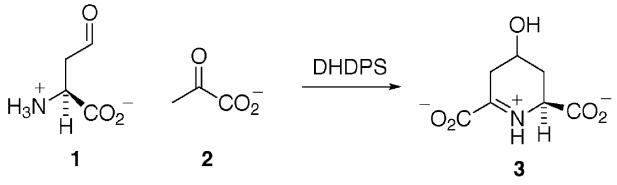
Abstract—We report two three-step syntheses of (*S*)-aspartate semi-aldehyde, an important synthetic and biosynthetic precursor, from diprotected aspartic acid. The first synthesis proceeds via a thioester, the second via a Weinreb amide. Each route yields pure (*S*)-aspartate semi-aldehyde in excellent yield. (*S*)-Aspartate semi-aldehyde prepared in this manner has proved appropriate as a substrate for detailed enzyme studies.

The lysine biosynthesis pathway is essential for plants and micro-organisms, but is not present in mammals. As such, it has remained a target for therapeutic agents for many years, although no commercial product has yet been shown to inhibit this pathway.[1,2] The enzyme that catalyses the branchpoint of the diaminopimelate pathway to lysine is dihydrodipicolinate synthase (DHDPS). Despite intense scrutiny over many years, no potent inhibitor of this pathway has yet been found. DHDPS catalyses the condensation of (*S*)-aspartate semi-aldehyde (ASA, **1**) and pyruvate (**2**) to form an unstable heterocycle, formally thought to be dihydrodipicolinate, but now believed to be 4-hydroxytetrahydrodipicolinate (**3**) (Fig. 1).[2] (*S*)-ASA is drawn here as the aldehyde, but is actually thought to exist predominantly in the hydrated form.[3,4]

For detailed biochemical analysis of this reaction, a convenient source of pure (*S*)-ASA is required. In particular, for screening of inhibitors of potential therapeutic use, the preparation of ASA employed must be free of impurities that may inhibit the enzyme and confuse the results.

Enantomerically pure ASA derivatives are also increasingly important synthetic intermediates, as the aldehyde moiety can be functionalised to yield more complex structures. The potential access to a variety of polyfunctional non-proteinogenic and unnatural amino acids using ASA and its derivatives has already been demonstrated.[1] ASA manipulation has also proved to be important in the synthesis of pharmaceuticals, aroma and flavour chemicals, pesticides and herbicides, dyes and pigments.[5]



1 **2** **3**

Figure 1. The condensation of pyruvate and (*S*)-ASA to form 4-hydroxytetrahydrodipicolinate, catalyzed by DHDPS.

ASA is difficult to synthesize and characterize since, like many amino aldehydes, it has a marked tendency to polymerize and is only stable in aqueous strong acid.[3–5] There are three methods for synthesizing (*S*)-ASA that are commonly used for biochemical studies.[3,6,7] The later methods[3,7] are derived from the original method of Black and Wright,[6] which requires the ozonolysis of (*S*)-allylglycine. Other methods have also been reported in the literature, which yield either free ASA or diprotected ASA, but they are often complex multi-step procedures and/or are extremely low yielding procedures and have not been widely adopted.[1,4,8,9]

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In the original synthesis of (*S*)-ASA, the aldehyde moiety was obtained by oxidative cleavage of the double bond of (*S*)-allylglycine by ozonolysis.[6] The reaction is carried out in a 1 M aqueous hydrochloric acid solution at 0 °C, with ozone bubbled through the solution. The reported yield of the desired product is 90–100%, as determined by an enzymatic assay following the conversion of ASA into homoserine by homoserine dehydrogenase.[6] However, (*S*)-ASA produced by this method has been found to have variable purity and no chemical characterisation of the product has ever been reported.[3,4] This method is still commonly used today, presumably due to the one step procedure from allylglycine.[10–12] However, studies in our laboratory using the DHDPS/DHDPR coupled enzymatic assay[7] have suggested that the ozonolysis product is contaminated by material that has an inhibitory effect on the DHDPS enzyme.[13]

To address the deficiencies of the Black and Wright method, Tudor and coworkers have investigated the ozonolysis of (*S*)-*N*-*tert*-butoxycarbonyl allylglycine *p*-methoxybenzyl ester.[3] Removal of the protecting groups with TFA affords the hydrate of ASA as a trifluoroacetate salt **4**, which can be stored for many months at 0 °C as a stable solid. This method has since been modified so that a Lemieux–Johnson oxidation is used in place of the ozonolysis.[7] In our hands, the overall yield of the latter method for the preparation of (*S*)-ASA was 59%, an increase on previously reported values of 42 and 43%.[3] However, the route is multistep, involves several purifications, and is particularly tedious for those focusing on biochemical studies of DHDPS.

We report herein two convenient syntheses of (*S*)-ASA from commercially available α-*tert* butyl (*S*)-*N*-*tert*-butoxycarbonyl aspartate (**5**).[14] Literature procedures were employed to activate the aspartic acid side chain, firstly, to a thio-ester[15] and secondly, a Weinreb amide.[15]

In the first synthesis, α-*tert* butyl (*S*)-*N*-*tert*-butoxycarbonyl aspartate (**5**) in dichloromethane was reacted with DCC, ethanethiol and DMAP at room temperature. Purification was achieved by flash chromatography yielding the thioester **6** as a clear oil in 94% yield. Reduction of the thioester to the aldehyde **7** was achieved in 84% yield using triethylsilane/10% Pd/C. The method of Tudor and coworkers[3] was used to deprotect the aldehyde **7** by stirring in trifluoroacetic acid in dry dichloromethane. (*S*)-ASA was isolated as the hydrated trifluoroacetate salt **4**, as a pale-yellow solid, in 96% yield.

This procedure involves only three steps and was relatively simple, cutting out the need to use osmium tetroxide. The overall reaction yield was 75% and the purity of the ASA produced was also of a very high standard, as determined by the DHDPS/DHDPR coupled enzymatic assay (99%). The only drawback of this method is that there are two purification steps required.

In a bid to cut down the number of purification steps required, the procedure of Wernic and coworkers was investigated as an alternative to obtaining (*S*)-ASA (Fig. 2).[16] α-*tert* Butyl (*S*)-*N*-*tert*-butoxycarbonyl aspartate (**5**) was converted to the corresponding Weinreb amide **8** in excellent yield (86%), using *N*,*O*-dimethylhydroxylamine hydrochloride, (benzotriazol-1-yloxy) tris(dimethylamino) phosphonium hexafluorophosphate (BOP·PF_6), and triethylamine. The resulting product **8** was reduced with diisobutyl aluminium hydride at −78 °C to give aldehyde **7** in 95% yield, which required no further purification. The method of Tudor[3] was again used to deprotect the aldehyde **7** to afford (*S*)-ASA as the hydrated trifluoroacetate salt **4** in 96% yield. This procedure was a much faster and easier method for obtaining (*S*)-ASA than the oxidation of diprotected (*R*,*S*)-allylglycine by osmium tetroxide/periodate or by ozonolysis.[6]

The overall yield of the reaction from the diprotected aldehyde **5** was also greatly increased (82%) when compared to other literature procedures.[3] The sequence has routinely been carried out on a 2 millimole scale. The above procedure is only three steps to the pure aldehyde, eliminating the need to use the very expensive and poisonous dihydroxylating agent osmium tetroxide. Another promising feature is that only one purification step is required in this scheme. The purity of the ASA generated was also of a very high standard (99%) as checked by the coupled assay of DHDPS and DHDPR. No evidence of contaminating inhibitory compounds was found.

(*S*)-ASA has been synthesised from commercially available α-*tert* butyl (*S*)-*N*-*tert*-butoxycarbonyl aspartate (**5**) in three steps via side-chain activation with an overall yield of 82%. This represents a significant advance over previously published routes to (*S*)-ASA and is the preferred synthetic route to (*S*)-ASA for future biochemical investigations.

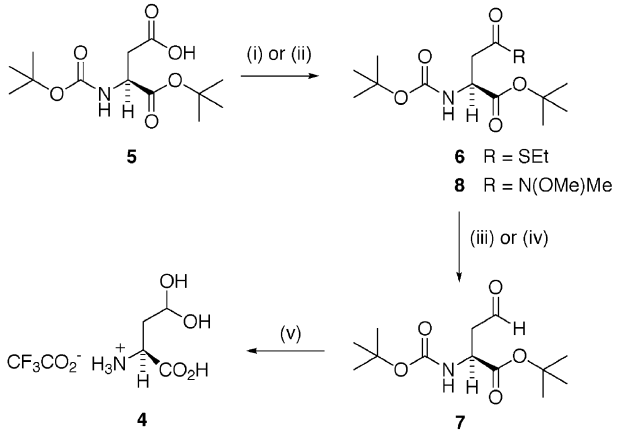

Figure 2. Three-step syntheses of (*S*)-ASA via either the thioester or Weinreb amide. (i) DCC, EtSH, DMAP, CH_2Cl_2 (94%); (ii) BOP.PF_6, Et_3N, CH_3ONHCH_3.HCl, CH_2Cl_2 (86%); (iii) Et_3SiH/10% Pd/C, CH_2Cl_2 (84%); (iv) DIBAL/THF −78 °C (95%); (v) CF_3CO_2H (96%).

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