# SYNTHESIS OF DL-[2-$^{13}$C,$^{15}$N]ASPARTIC ACID

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## SUMMARY

A synthesis of the title compound from [$^{13}$C]paraformaldehyde and [$^{15}$N]ammonium chloride *via* ethyl 2-(1,3-[2-$^{13}$C]dithianyl) acetate **3a** is described. Ethyl[3-$^{13}$C]3-oxopropanoate derived *in situ* from **3a** was converted by a stepwise Strecker procedure to DL-[2-$^{13}$C,$^{15}$N]aspartic acid **7a**.

Key words: Carbon-13, Nitrogen-15, Strecker reaction

## INTRODUCTION

α-Amino acids enriched at the 2-position with $^{13}$C and in the amino group with $^{15}$N offer significant potential as probes for biosynthetic and metabolic studies (1,2). Such molecules possess in essence a 'labelled' C-N bond the integrity of which can be detected by observation of one bond coupling in either the $^{13}$C or $^{15}$N NMR spectrum. This enables the characterisation of biochemical processes which can be conjectured to involve either conservation or cleavage of C-N bonds. In the course of a study on the biosynthesis of 3-nitropropanoic acid by the fungus *Penicillium atrovenetum* (2) we required to synthesise aspartic acid enriched with $^{13}$C and $^{15}$N in this manner.

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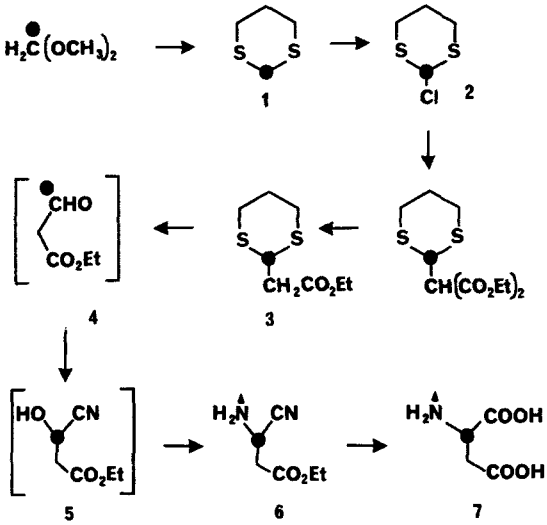
## DISCUSSION

While a variety of methods for the synthesis of racemic aspartic acid (3) and of isotopic carbon substituted aspartates (4) have been described previously none of these routes is particularly attractive for the introduction of a $^{13}C$ atom at the C-2 position. We elected therefore to evaluate a direct route *via* the unstable intermediate ethyl 3-oxopropanoate **4** the 3-$^{13}C$ isotopomer of which appeared relatively accessible by the sequence shown in the scheme below. Although esters of 3-oxopropanoic acid are known to be highly reactive we reasoned that under appropriately mild cyanolation conditions **4** might be trapped as the corresponding cyanohydrin **5** which could subsequently be aminated to give the α-aminonitrile **6** (5).

[$^{13}C$]Dimethoxymethane prepared from [$^{13}C$]paraformaldehyde by the method described by Buchler *et al* (6) was converted to 1,3-[2-$^{13}C$]dithiane **1a** by the procedure of Corey and Seebach (7). The enriched dithiane was chlorinated with $SOCl_2$ to afford **2a** which was reacted with diethyl malonate (8). The resultant




Scheme: Compounds designated **1a** to **7a** in the text were enriched with $^{13}C$ (●) and $^{15}N$ (▲)as shown above

diethyl 2-(1,3-[2-$^{13}$C]dithianyl)malonate was decarboxyethylated under basic conditions (9) to afford **3a** in an overall yield of 26% from **1a**.

In a series of trial reactions using unlabelled material it was found that while the aldehyde protecting group of **3** could be efficiently removed by reaction with N-bromosuccinimide (10) the resultant ethyl 3-oxopropanoate rapidly polymerised on standing. Not surprisingly when the product was reacted under one-pot Strecker conditions (11) none of the desired α-aminonitrile **6** could be detected. However treatment of the liberated ethyl 3-oxopropanoate with aqueous NaCN at pH 4-5 and 0°C resulted in formation of the corresponding cyanohydrin which was subsequently reacted with ammonium chloride in basic solution to afford **6** in moderate yield (50-60% from **3**).

Elaboration of the $^{13}$C,$^{15}$N-enriched aminonitrile **6a** from **3a** was carried out in a similar manner and the product subjected to acid hydrolysis without purification to afford DL-[2-$^{13}$C,$^{15}$N]aspartic acid **7a**. The $^{13}$C NMR spectrum ($D_2O$-DCl, pH 1) of **7a** exhibited a single doublet resonance for C-2 at 49.2 ppm ($^1J_{CN}$ 7.0 Hz).

## EXPERIMENTAL

1,3-[2-$^{13}$C]Dithiane **1a** (7): A suspension of [$^{13}$C]paraformaldehyde (0.5 g, 90 atom % $^{13}$C) in 10% MeOH-$H_2O$ (2 ml) was heated at reflux for 2h, diluted with 10% MeOH-$H_2O$ (1 ml) and refluxed for a further 1 h. The residue was removed by filtration, washed with MeOH (2.5 ml) and the combined filtrate and washings acidified with 6M HCl (0.1 ml) and heated at reflux over $CaCl_2$ (3 g) for 5 min. Distillation afforded [$^{13}$C]dimethoxymethane (0.84 g, b.p. 40-44°) which was converted to **1a** (1.0 g, 75% after sublimation, 0.5 mm Hg at 60°) as described (7) m. 50-52°, $\delta_C$ ($CDCl_3$, 75 MHz) 36.32 (t, C-2, $^1J_{CH}$ 150.2 Hz).

Ethyl 2-(1,3-[2-$^{13}$C]dithianyl)acetate **3a** (9): A solution of $SOCl_2$ (0.71 g, 5.3 mmol) in dry $CH_2Cl_2$ (2 ml) was added to a stirred solution of **1a** (0.6 g, 5 mmol)

in $CH_2Cl_2$ (10 ml) held at -30° under a dry argon atmosphere. After 1 h the mixture was allowed to come to 0° and the solvent evaporated under a stream of dry argon. The solid residue of 2-chloro-1,3-[2-$^{13}$C]dithiane **2a** was dissolved in dry THF and used directly for the next step. [$\delta_H$ (THF, 60 MHz) 6.28 (1H, d, $^1J_{CH}$ 142 Hz, H-2).

Diethyl malonate and **2a** were reacted as previously described (8) and the crude product [$\delta_C$ ($CDCl_3$, 75 MHz) 41.33 (d, C-3)] decarboxyethylated (9) to give **3a** as a viscous oil which was purified by distillation at reduced pressure to give a pure sample of **3a** (305 mg, b.p. 95° at 0.04 mm Hg); $\delta_C$ ($CDCl_3$, 75 MHz) 41.74 (d, C-3); $\delta_H$ ($CDCl_3$, 80 MHz) 4.34 (1H, dt, $^1J_{CH}$ 142 Hz, $^3J_{HH}$ 7 Hz, H-3).

DL-[2-$^{13}$C,$^{15}$N]Aspartic acid **7a**: A solution of **3a** (0.2 g, 1.0 mmol) in $CH_3CN$ (0.5 ml) was slowly added to a stirred solution of N-bromosuccinimide (1 g) in 80% aq $CH_3CN$ (10 ml) at -5° under argon (10). After 10 min the solution was diluted with chilled brine (10 ml) and extracted with cold diethyl ether (10 ml x 3). The ethereal extract was added to a cold solution of 0.01 M aq HCl:$CH_3CN$ (3:1, 10 ml) and the ether removed by evaporation at reduced pressure. Aqueous NaCN (0.1 g, 2.0 mmol, 1 ml) was added to the chilled solution at such a rate to maintain the pH below 6.0. After addition the pH was adjusted to 5.0 with 1 M NaOH and the solution stirred at 0° for 12 h. The reaction mixture was saturated with NaCl and extracted with ether (10 ml x 3). The ethereal layer was washed with brine (10 ml), evaporated to 0.1 ml at 0° under reduced pressure and taken up in 50% aq ethanol (1 ml). The solution was heated to 70° with stirring and a solution of $^{15}NH_4Cl$ (55 mg, 1 mmol), 99 atom % $^{15}$N) in 2 M NaOH (0.5 ml) added over 0.5 h. The cooled reaction mixture was diluted with brine (2.5 ml), extracted with $CH_2Cl_2$ (2.5 ml x 3) and the organic extract washed with brine (2 ml), concentrated to *ca* 2 ml under vacuum and extracted with 6 M HCl (2.5 ml x 2). The combined acidic extract was heated at 100° for 12 h, evaporated to a residue and the residue extracted with ethanol (5 ml). Evaporation of the ethanol extract afforded DL-[2-$^{13}$C,$^{15}$N]aspartic acid, HCl salt (60 mg).

Final purification of the product was effected by dissolving the salt in water (5 ml), adjusting to pH 4 with 2 M NaOH, and ion exchange chromatography on Biorad 50W x 2 resin ($H^+$ form, 200-400 mesh, 10 ml) eluting with a $H_2O$-1 M pyridine gradient. Lyophilisation of the ninhydrin positive fractions afforded **7a** which migrated with authentic DL-aspartic acid on paper electrophoresis.

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