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# A flexible approach for the synthesis of selectively labelled L-arginine

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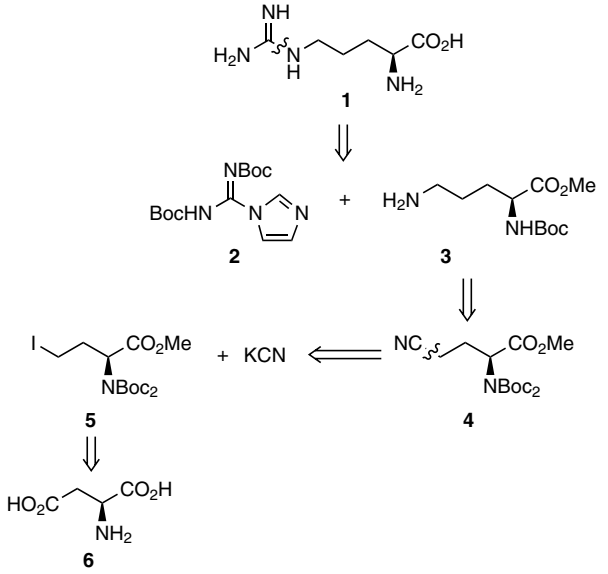
**Abstract**—A simple and efficient synthesis of L-arginine has been achieved in 12 steps and 24% overall yield. Regioselective reduction and functional group manipulation of the β-side chain of aspartic acid allowed the preparation of an ornithine derivative, which was then guanylated with a bis-protected 1-guanyl-pyrazole and deprotected to give L-arginine. This approach allows the flexible incorporation of stable isotopes and this is demonstrated using potassium $^{13}$C-cyanide, which has resulted in the preparation of 5-$^{13}$C-L-arginine.


L-Arginine **1** plays a key role in the function and structure of many proteins.[1] In particular, this amino acid is an important structural motif found conserved in type II polyketide synthase acyl carrier proteins (ACP), which are essential for the production of a specific polyketide product.[2] NMR studies of several type II ACPs have provided the solution structure of these proteins revealing a common topology consisting of a distorted four α-helical bundle.[2–7]

Elucidation of protein structures using NMR spectroscopy has been facilitated by the incorporation of amino acids selectively labelled with stable isotopes.[3,8] The importance of these biological studies has led to the development of many methods for the synthesis of isotopically labelled amino acids.[9] To further elucidate the structure and mechanisms of polyketide ACPs we were interested in developing a flexible synthesis of L-arginine, which would allow the selective incorporation of stable isotopes leading to a series of labelled arginine analogues. Several methods for the synthesis of labelled D,L-arginine have been reported.[10] However, these are limited by both the lack of stereocontrol and the introduction of the stable isotope early in the synthetic pathway. We now report a 12 step, stereospecific synthesis of L-arginine and demonstrate the utility of this approach for the preparation of selectively labelled analogues by the incorporation of a $^{13}$C-label at the C-5 position.

Our retrosynthesis of L-arginine is outlined in Scheme 1. It was proposed that **1** could be prepared by coupling commercially available *N*,*N*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine **2** and a suitably protected




**Scheme 1.**

*Keywords*: L-Arginine; Stable isotopes; Stereospecific synthesis; Guanylation.

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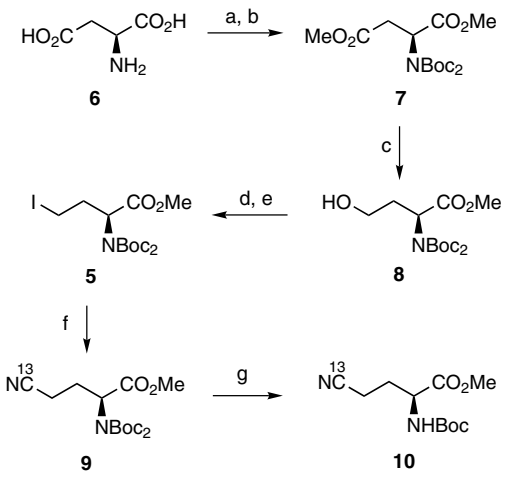
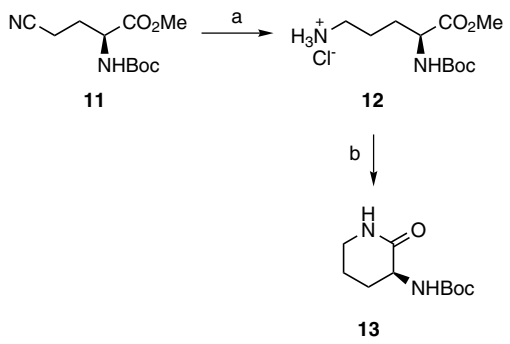
ornithine derivative **3**. We intended to synthesise **3** from L-aspartic acid **6** by regioselective reduction of the β-carboxylate group followed by functional group manipulation to give the required ornithine derivative **3** via intermediates **5** and **4**.

The first stage of the synthesis involved the preparation of nitrile **10** (Scheme 2). L-Aspartic acid **6** was converted in two steps to *N*,*N*-di-*tert*-butoxycarbonyl L-aspartic acid dimethyl ester **7**.[11] As reported by Martín and coworkers, bis-protection of the amino functional group allows regioselective reduction of the β-methyl ester using DIBAL-H to give the corresponding aldehyde.[11] The aldehyde was then further reduced using sodium borohydride to afford, after column chromatography, alcohol **8** in 91% yield over the two steps. Direct synthesis of alcohol **8** from **7** using DIBAL-H was attempted but this led to mixtures of starting material, regiomeric alcohols and also significant amounts of the diol. Alcohol **8** was then activated as the mesylate and subsequently reacted with sodium iodide to give intermediate **5** in high yield. Nucleophilic displacement of the iodide with potassium $^{13}$C-cyanide in DMF gave the isotopically labelled nitrile **9** in 87% yield. Introduction of the nitrile functional group could have been attempted using the mesylate resulting in a slightly shorter synthesis.[12] However, it was felt that displacement of the iodide leaving group rather than the mesylate with the labelled cyanide would permit more efficient utilisation of the isotopic label. Finally, selective removal of one of the Boc-protecting groups was achieved using trifluoroacetic acid to give **10** in 88% yield.[13]

The next stage required the synthesis of the ornithine analogue and this was initially investigated using unlabelled material. Hydrogenation of **11** in the presence of chloroform using platinum oxide as the catalyst gave the amine hydrochloride **12** in good yield (Scheme 3).[14]



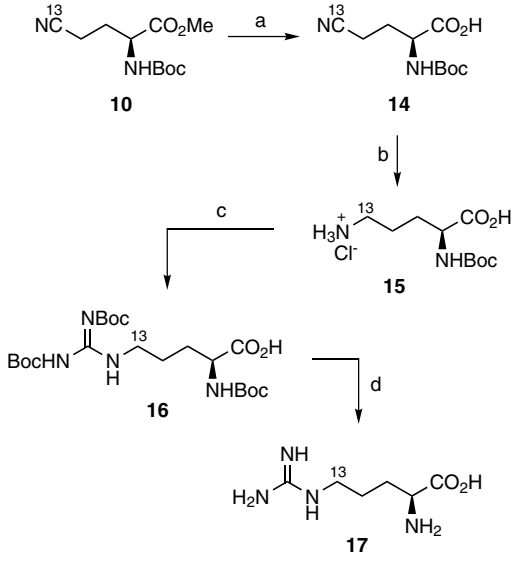

**Scheme 2.** Reagents and conditions: (a) i. TMSCl, MeOH, ii. $NEt_3$, $Boc_2O$, 85% over two steps; (b) $Boc_2O$, DMAP, MeCN, 96%; (c) i. DIBAL-H, $Et_2O$, −78 °C; ii. $NaBH_4$, THF, $H_2O$, 91% over two steps; (d) MsCl, $NEt_3$, DMAP, $CH_2Cl_2$, 85%; (e) NaI, acetone, Δ, 89%; (f) K$^{13}$CN, DMF, 87%; (g) TFA, $CH_2Cl_2$, 88%.




**Scheme 3.** Reagents and conditions: (a) $H_2$, $PtO_2$, $CHCl_3$, MeOH, 85%; (b) $NaHCO_3$, EtOAc, 75%.

The next step required deprotonation of the amine hydrochloride to release the amine for subsequent coupling with urethane protected 1-guanylpyrazole **2**. Treatment of **12** with sodium hydrogen carbonate however, resulted in the formation of the cyclic amide **13**. This was surprising as a similar ornithine derivative has been shown to be stable under such conditions.[15]

To prevent cyclisation occurring, α-methyl ester **10** was hydrolysed to the corresponding carboxylic acid **14** using lithium hydroxide in essentially quantitative yield (Scheme 4). Hydrogenation, again using platinum oxide as the catalyst gave the amine hydrochloride **15** in good yield. Direct treatment of **15** with *N*,*N*-di-isopropylethylamine (2 equiv) and *N*,*N*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine **2** gave the tri-Boc protected arginine analogue **16** cleanly and in 75% yield.[15,16] Finally cleavage of the three Boc-protecting groups in trifluoroacetic acid followed by purification by ion exchange chromatography gave 5-$^{13}$C-L-arginine **17** in 87% yield.[17]




**Scheme 4.** Reagents and conditions: (a) LiOH, MeOH, $H_2O$, 99%; (b) $H_2$, $PtO_2$, $CHCl_3$, MeOH, 87%; (c) **2**, DIPEA, MeOH, 75%; (d) TFA, 87%.

In conclusion, a simple and efficient, 12-step synthesis of L-arginine has been achieved giving the target molecule in 24% overall yield. This route has been developed to allow the selective incorporation of stable isotopes during the later stages of the synthesis thereby maximising efficacy of the isotopic label. We have demonstrated this, using the relatively inexpensive potassium $^{13}$C-cyanide for the preparation of 5-$^{13}$C-L-arginine. Similarly, use of other stable isotopes of cyanide (e.g., $KC^{15}N$ or $K^{13}C^{15}N$) or isotopically labelled *N*,*N*-bis-(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (synthesised in two steps from commercially available isotopically labelled cyanamide)[16,18] would allow the preparation of further analogues of labelled L-arginine using this approach. Further studies towards the synthesis of these compounds are currently in progress.

## Acknowledgements

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