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Short Research Article

New approaches for the synthesis of isotopically labelled guanidine-derived amino acids and noradrenaline reuptake inhibitors†

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Abstract: A new approach for the stereoselective synthesis of guanidine-derived amino acids, L-arginine and (+)-blastidic acid, has been devised which allows the selective incorporation of isotopic labels in both the side chain of the amino acid as well as the guanidine unit. A new asymmetric synthesis of the (*S*,*R*)-diastereomer of reboxetine, an antidepressant, has also been completed which allows the specific incorporation of radiolabelled iodine for SPECT imaging. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: asymmetric synthesis; guanylation; isotope labelling; amino acids and reboxetine

Introduction

The efficient synthesis of selectively labelled biologically active compounds is important for a range of studies including metabolic investigations as well as the elucidation of protein structure by NMR spectroscopy.[1] Ideally, incorporation of the isotope should occur at a late stage of the synthesis thereby maximizing the utility of the relatively expensive isotopically labelled reagent. Herein we describe our work on new routes for the stereoselective synthesis of guanidine-containing amino acids for use in biosynthetic studies. These new routes allow the selective incorporation of stable isotopes at various positions during the later stages of the synthesis. We also describe our initial investigations in identifying a molecular imaging agent for the noradrenaline reuptake transporter (NAT) with the first synthesis of an iodinated (S,*R*)-diastereomer of reboxetine, a known inhibitor of NAT.[2]

Results and discussion

Our first objective was to develop a short and efficient synthesis of L-arginine which would also allow the selective incorporation of stable isotopes. This was achieved by initially synthesizing a suitably protected ornithine derivative and coupling this with a commercially available, 1-guanyl-pyrazole reagent to achieve the key step. The ornithine derivative was prepared as shown in Scheme 1. L-Aspartic acid **1** was converted in two steps to *N*,*N*-di-*tert*-butoxycarbonyl L-aspartic acid dimethyl ester **2**. Regioselective reduction of the β-ester, first with DIBAL-H and then with sodium borohydride gave primary alcohol **3** in good yield. The alcohol functional group was activated in two steps to give iodide **4**. Incorporation of a stable isotope was achieved by nucleophilic displacement of the iodide with potassium [^{13}C]cyanide in DMF. To prevent racemization of the stereogenic centre or any cyclization reactions from taking place, one of the Boc-protecting groups was removed using TFA and the ester functional group was hydrolysed using lithium hydroxide. Finally, hydrogenation of nitrile **5** using platinum oxide as a catalyst gave the desired ornithine derivative **6** in excellent yield.

The key step was carried out involving the reaction of ornithine derivative **6** with commercially available *N*,*N*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine

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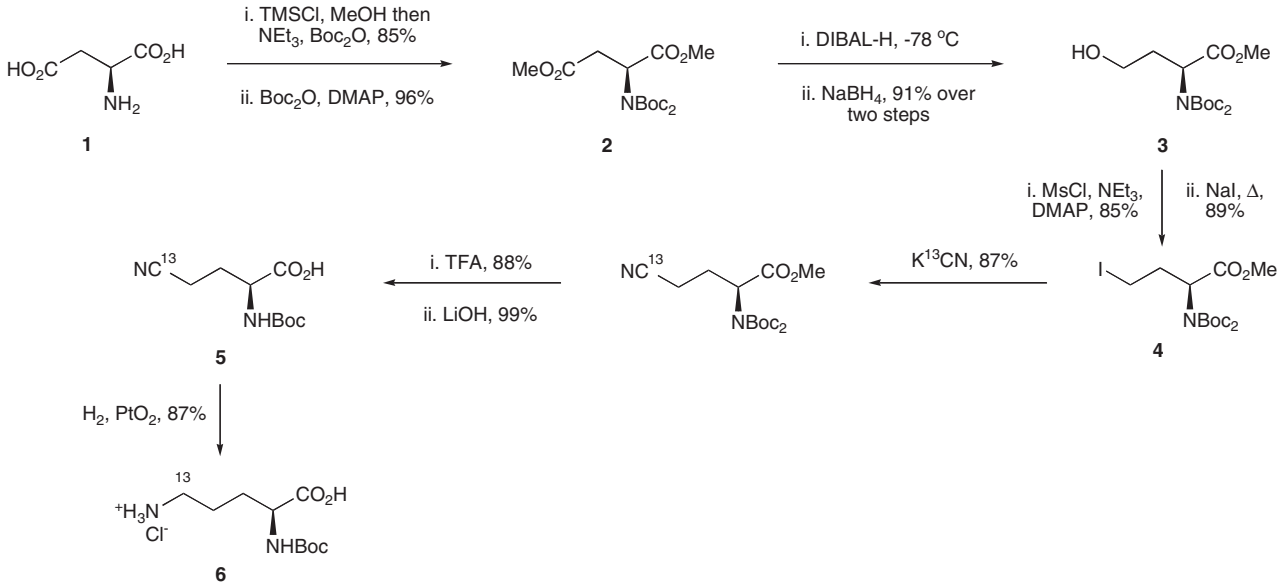
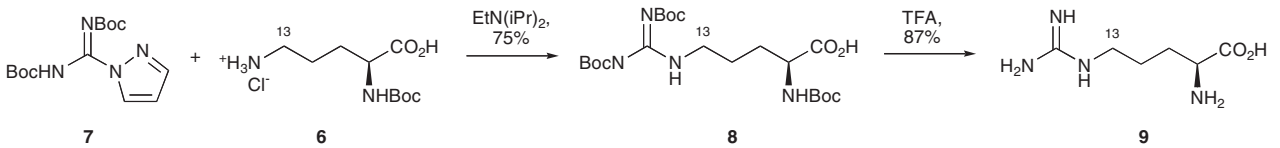


Scheme 1

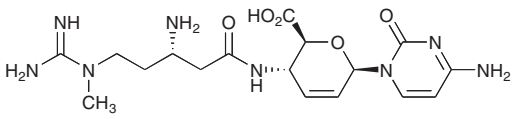


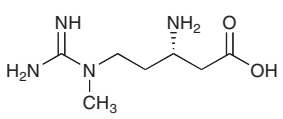
Scheme 2

7 in the presence of *N*-ethyldiisopropylamine which gave the tri-Boc protected arginine analogue **8** in 75% yield (Scheme 2). Finally, cleavage of the three Boc-protecting groups using TFA followed by ion exchange chromatography gave [5-^{13}C]-L-arginine **9** in 12-steps and in 24% overall yield.[3] We utilized this approach for the synthesis of [5-^{13}C]-L-arginine only. However, the use of other stable isotopes of potassium cyanide or isotopically labelled *N*,*N*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine **7** (prepared from commercially available isotopically labelled cyanamide)[4] would allow the preparation of further analogues of labelled L-arginine.

L-Arginine has been used to investigate the biosynthetic pathway of blasticidin S **10**, a peptidyl-nucleoside antibiotic first isolated from *Streptomyces griseochromogenes*.[5] Gould and co-workers demonstrated that the β-amino acid component of blasticidin S, blastidic acid **11**, is biosynthesized from arginine via an intramolecular migration of the α-nitrogen to the β-position.[6]

Having achieved a simple and efficient synthesis of isotopically labelled L-arginine we proposed that a similar approach involving the coupling of an ornithine derivative with *N*,*N*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine should lead to an improved synthesis of blastidic acid **11** and also allow the selective incorporation of stable isotopes for further biosynthetic studies. The ornithine derivative was prepared as outlined in Scheme 3. A one-pot reaction for the protection of β-alanine **12** followed by *N*-methylation gave **13** in excellent yield. Reduction of the ester **13** followed by a one-pot Swern/Wadsworth–Emmons reaction gave exclusively the *E*-alkene **14**. The β-amino functionality was then introduced using an asymmetric conjugate addition,[7] which gave major diastereomer **15** in 85% yield. After exchange of the benzyl groups for the Cbz-protecting group, the *tert*-butyl ester and Boc-protecting groups were cleaved using TFA to give the β-ornithine derivative **17**.





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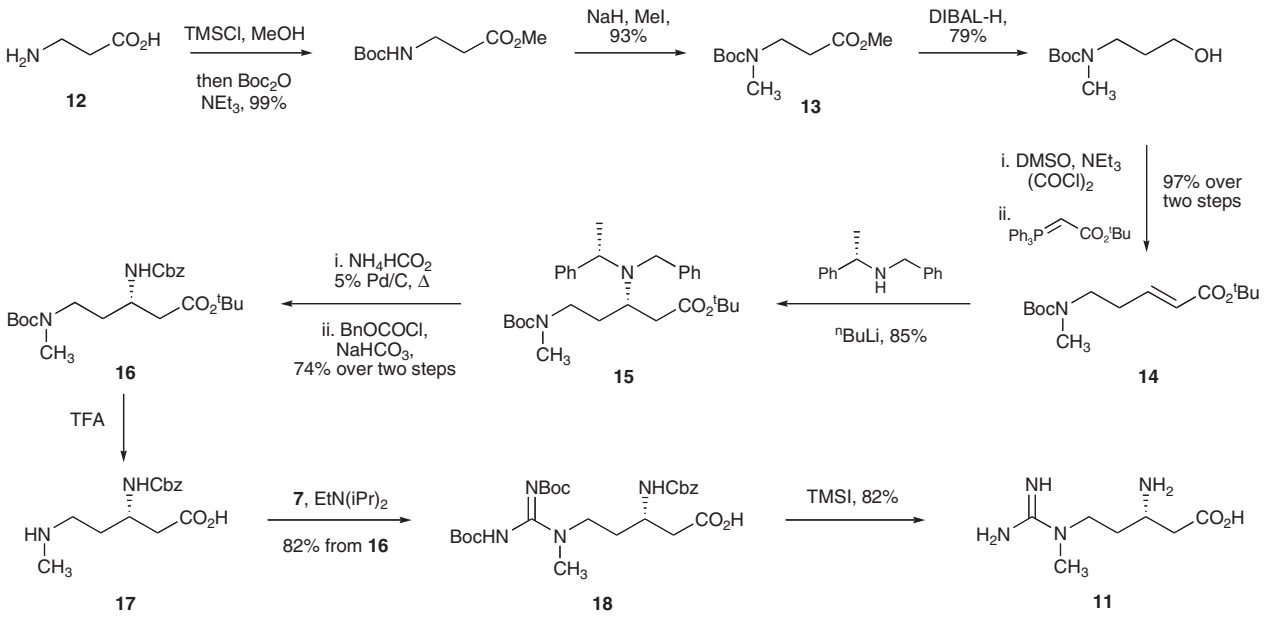
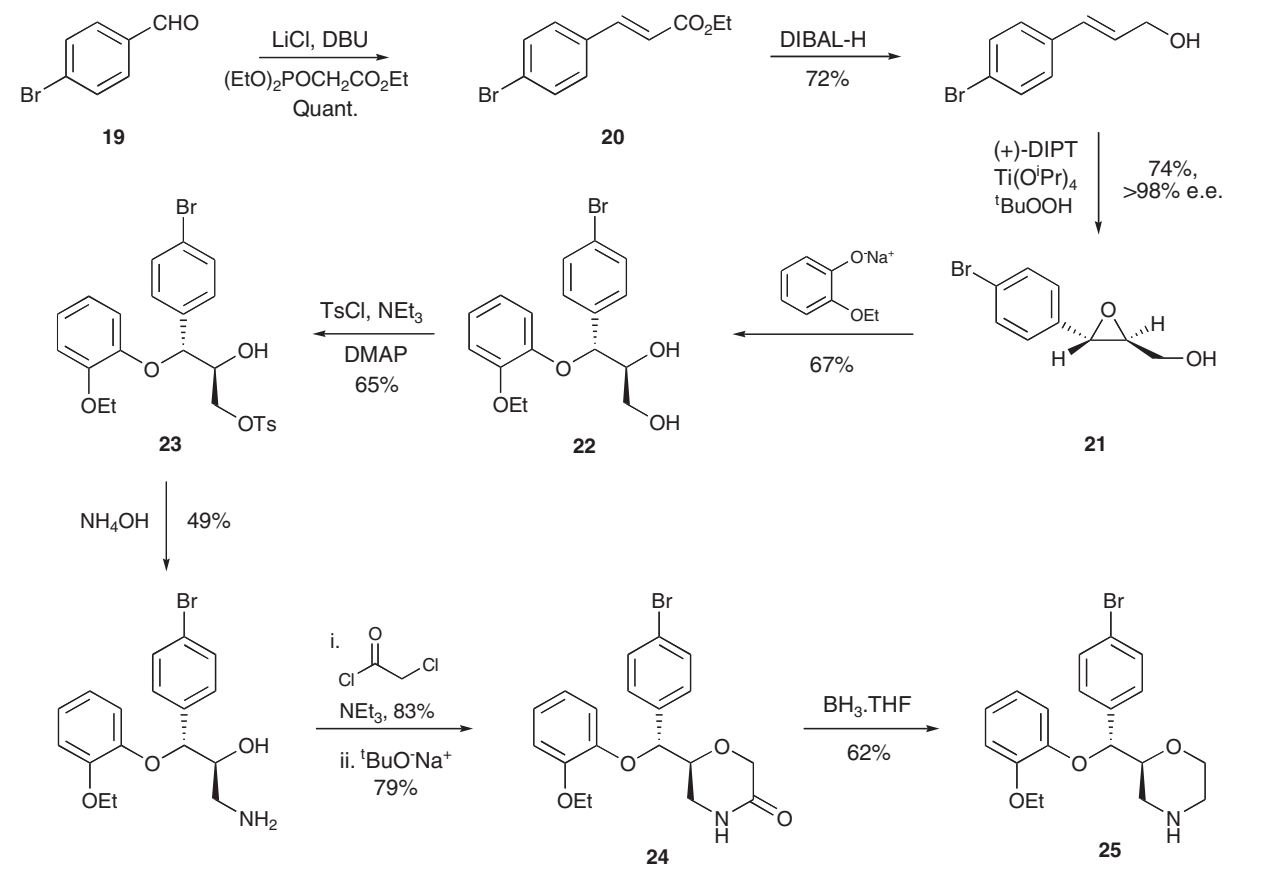


Scheme 3



Scheme 4

As described for the synthesis of L-arginine, the guanidine unit was introduced by coupling of **17** with **7** in the presence of *N*-ethyldiisopropylamine which gave **18** in excellent yield. Finally, deprotection of **18** with trimethylsilyl iodide gave blastidic acid **11** in 11 steps and 30% overall yield.[8] Again, the use of isotopically

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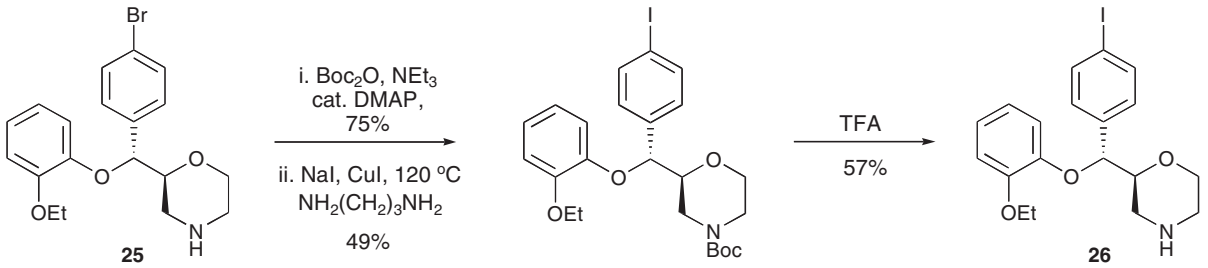

Scheme 5

labelled *N,N*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine would allow the selective incorporation of stable isotopes at a late stage of this synthesis of blastidic acid.

More recently our research programme for the synthesis of isotopically labelled, biologically active compounds has focused on identifying a new imaging tracer for the NAT. Our aim has been to synthesize radioiodinated analogues of reboxetine, a known inhibitor of NAT which could be used for SPECT imaging.

The synthesis of an iodinated (*S,R*)-diastereomer of reboxetine has been completed using a Wadsworth–Emmons reaction and a Sharpless asymmetric epoxidation to effect the key steps (Scheme 4). 4-Bromobenzaldehyde **19** was converted to the *E*-α, β-unsaturated ester **20** using Masumune and Roush conditions of the Wadsworth–Emmons reaction.[9] Reduction of the ester followed by Sharpless asymmetric epoxidation of the resulting allylic alcohol gave epoxide **21** in 74% yield.[10] Regioselective ring opening of the epoxide **21** with 2-ethoxyphenol gave diol **22** which was converted to the mono-tosylate **23** in 65% yield. Displacement of the tosyl group with ammonium hydroxide and subsequent reaction with chloroacetyl chloride and ring closure gave amide **24**. The synthesis of the morpholine ring was completed by treatment of **24** with borane-THF which gave **25** in good yield.

The synthesis of the iodinated (*S,R*)-diastereomer of reboxetine was completed by Boc-protection of amine **25** and conversion of the resulting aryl bromide to the corresponding iodide using a reaction developed by Klapars and Buchwald (Scheme 5).[11] Finally, Boc-deprotection using TFA gave the target compound **26**. Once the 'cold' compound has been analysed for binding with NAT, it will be converted to a radioiodinated analogue using organotin methodology.[12]

Conclusion

We have demonstrated a simple and efficient approach for the synthesis of guanylated amino acids which allows the incorporation of stable isotopes at the late stages of the syntheses. We have also established the first synthesis of an (*S,R*)-diastereomer of reboxetine which after binding studies will be radioiodinated for SPECT imaging.

Acknowledgement

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