

Short Note

# 1-(4-Aminobutyl)guanidine

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**Abstract:** Isotope labelling of otherwise endogenous metabolites has emerged as a powerful approach to study metabolism-related biological processes, when used in conjunction with nuclear magnetic resonance or chromatography-supported mass spectrometry. Given the advantages of metabolite tracing in uncovering metabolic pathways, there is always a need to develop new methods to generate isotopically labelled compounds. In this direction, we developed a new synthetic route to access the labelled agmatine. To access labelled agmatine, we developed a two-step method that includes the treatment of labelled cyanamide with *N*-Boc-1,4-butanediamine, followed by a BOC deprotection. Structural confirmation was achieved by 1DNMR, 2DNMR and IR spectroscopy. This isotopologue of agmatine can be very helpful to study the pharmacokinetics and bio-distribution of this neurotransmitter and its metabolites in vitro and in vivo or used as an internal standard in mass spectrometry measurements.

**Keywords:** agmatine; labelled agmatine; diamino-butane; cyanamide; arginine; trifluoroacetic acid



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## 1. Introduction

Agmatine is a ubiquitous compound, discovered by Albrecht Kosse in herring sperm [1]. It is a polyamine synthesized from the decarboxylation of L-arginine catalyzed by arginine decarboxylase. Until 1994, it was believed that agmatine was found only in bacteria, plants, and invertebrates, but then subsequent studies confirmed that agmatine is also expressed in mammals [2]. Recent reports also demonstrated agmatine as a novel neurotransmitter [3]. Ever since its discovery in humans, extensive research has been done to investigate its metabolic role, and that of the ensuing metabolites. Agmatine is known for its anti-depressive, anti-nociceptive, anxiolytic, and anti-inflammatory properties, and has been found to regulate heart rate and blood pressure, which makes it a potential therapeutic agent for the treatment of hypertension, ischemia, diabetes, and other cardiovascular ailments [4–8].

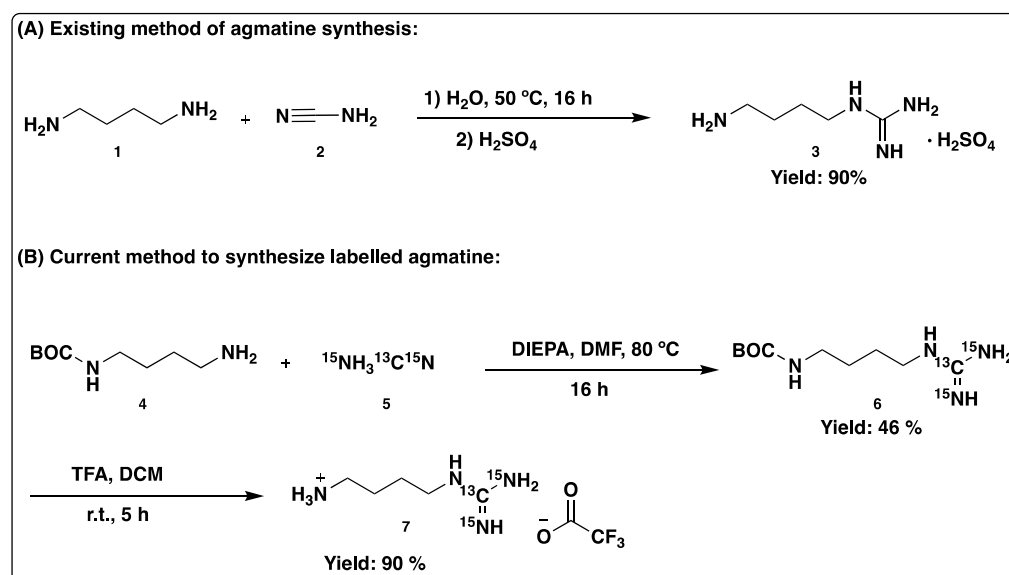
The biological importance of agmatine and its therapeutic properties highlight the importance of the simple and efficient synthesis of agmatine. There are a limited number of chemical routes for the production of agmatine, but several biotechnical techniques exist in the literature. Multiple enzymatic methods have been reported for the production of agmatine, including the use of immobilized arginine decarboxylase and substrate-specific protein engineering [8–10]. Through a whole-cell approach, Zhang et al. implemented the fermentative production of agmatine by metabolic engineering in *Escherichia coli* [11]. A similar approach was reported by Yang and co-workers, where the team overexpressed arginine decarboxylase in *Corynebacterium crenatum* [12]. Despite several biotechnical methods to synthesize agmatine, there are only a handful of chemical methods available to access agmatine. The existing chemical/industrial method of agmatine synthesis involves the treatment of 1,4-diaminobutane and cyanamide. To our knowledge, there is no reported synthetic route to produce labelled agmatine. In this work, we report a novel synthetic route to access labelled agmatine. Given the biological importance of agmatine and that of its metabolites, an efficient route to access labelled agmatine is necessary to investigate the biochemical pathways that regulate agmatine and its metabolites' production, and the

pathways that agmatine might regulate. With this facile synthetic route, we anticipate that agmatine production on a larger scale can be implemented as well.

## 2. Results and Discussion

Metabolites are continuously produced and consumed by a variety of biochemical reactions and pathways. To address this pool of biochemical information, measurements of metabolites by metabolomics using NMR and mass spectroscopy have gained much attention. Metabolomics has gained traction in a vast number of applications in the basic sciences, biology, medicine, and translational research [13].

To provide insights into the metabolism of agmatine, we developed a chemical route (Scheme 1) to synthesize agmatine (3) and labelled agmatine (7). Initial attempts to synthesize agmatine using the existing literature method were unsuccessful [9]. We found that when a mixture of 1,4-diaminobutane (1) and labelled cyanamide (5) was heated at 50 °C under the reported conditions, no product formation was observed [9]. Even changing the reaction parameters, such as increasing the temperature to 100 °C and increasing the reaction time, or the addition of a mild base such as potassium carbonate, failed to yield compound 7.

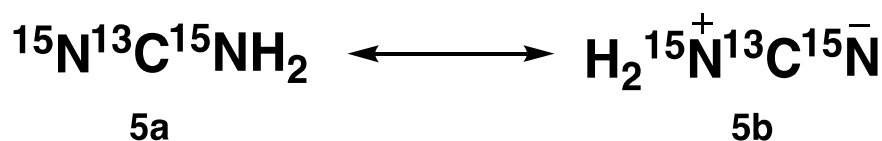


**Scheme 1.** Synthetic scheme of compounds 3 and 7.

Therefore, we sought to develop a new strategy for the facile generation of 7 that is based on a two-step approach. The first step involves the treatment of *N*-Boc-1,4-butanediamine (4) with labelled cyanamide (5) to generate *N*-Boc-protected agmatine, followed by deprotection with trifluoroacetic acid (TFA) to yield the desired compound 7 (Scheme 1). The resulting compound (compound 7) was successfully characterized by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR,  $^{19}\text{F}$  NMR, HSQC, and mass spectroscopy.

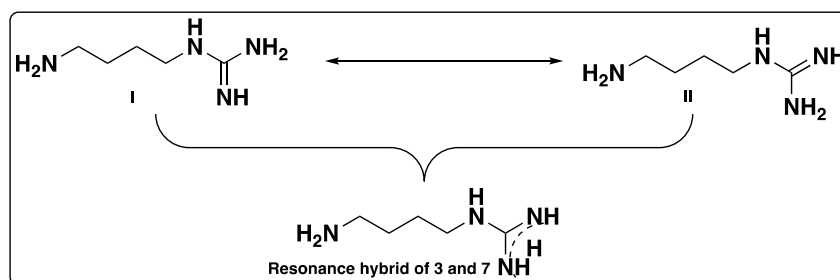
$^1\text{H}$  NMR of compound 7 (labelled agmatine) showed the desired peaks corresponding to agmatine.  $^{13}\text{C}$  NMR too supported the product formation, which was evident from the  $^{13}\text{C}$  labelled peak at 164 ppm along with other desired peaks (Supplementary Materials).

$^{13}\text{C}$  NMR (no decoupling) spectra were recorded for cyanamide (5) and labelled cyanamide (7), which revealed two distinct multiplets at 120 ppm and 163 ppm for labelled cyanamide (compound 5), while unlabelled cyanamide (compound 2) appeared as a singlet at 117 ppm. Literature reports revealed that  $^{15}\text{N}$ -labelled cyanamide (compound 5) can exist in two resonance forms (Figure 1) [14,15].



**Figure 1.** Resonance forms of compound 5 (cyanide (5a) and carbodiimide (5b)).

Therefore, the two distinct peaks observed in the  $^{13}\text{C}$  NMR spectra of compound 5 were consistent with this. Further  $^{13}\text{C}$  NMR of compounds 3 and 7 were recorded, which showed singlets for both the compounds. A difference in the compounds was evident when  $^{13}\text{C}$  spectra were recorded with no decoupling. We noticed that in the case of compound 7, guanidium (terminal) labelled carbon that appeared to be a singlet in the  $^{13}\text{C}$  NMR spectrum (with decoupling), it appeared as two distinct triplets, while no such splitting was observed in the case of the unlabelled derivative (Supplementary Materials). Due to resonance or conjugation, compounds 3 and 7 can exist as form I or form II, and the delocalization can also be shown in the form of a resonance hybrid, as shown in Figure 2. Structural identification of compound 7 was further supported by IR spectroscopy, which identified the presence of amine peaks at  $\sim 3200\text{ cm}^{-1}$ , and C=N and C-N peaks appeared at  $1674$  and  $1200\text{ cm}^{-1}$ , respectively (Supplementary Materials).



**Figure 2.** Resonance forms of 3 or 7.

Further  $^1\text{H}$ - $^{15}\text{N}$  HMBC confirmed the presence of  $^{15}\text{N}$  atoms. In addition, DEPT spectra were recorded to confirm the carbon skeleton. DEPT-135 of labelled agmatine (7) showed a singlet around 160 ppm in the positive phase, while no such peak was observed in the case of the unlabelled derivative. The appearance of  $^{13}\text{C}$  in the positive phase in the DEPT-135 spectrum can be rationalized by the difference in the chemical environment (conjugation/resonance effects) imposed by two  $^{15}\text{N}$  atoms on the  $^{13}\text{C}$  atom in 7. In contrast, DEPT135 of compound 3 did not show a similar effect, and this was in agreement with the literature reports [16].

Other 2D experiments such as COSY and HSQC helped in the structural identification of 7. Overall, the above experimental results support the unambiguous structural elucidation of labelled agmatine.

### 3. Methods and Reagents

All commercially available reagents and solvents were used without further purification. Reactions were monitored by both TLC and  $^1\text{H}$  NMR.  $^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  NMR spectra were recorded on a Bruker Avance III HD 400 spectrometer at 400.11, 100.62, and 376 MHz, respectively, using the residual proton signal ( $^1\text{H}$ ) for  $^1\text{H}$  NMR and the naturally abundant  $^{13}\text{C}$  carbon signal ( $^{13}\text{C}$ ) of a deuterated solvent (DMSO, and  $\text{CDCl}_3$ ) for  $^{13}\text{C}$  NMR as an internal standard. Chemical shifts are reported in the  $\delta$  scale relative to residual  $\text{CHCl}_3$  (7.26 ppm),  $\text{D}_2\text{O}$  (4.79), DMSO (2.5) for  $^1\text{H}$  NMR and for  $^{13}\text{C}$  NMR to the central line of  $\text{CDCl}_3$  (77.16 ppm), DMSO (39.52).  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR (including DEPT,  $^{13}\text{C}$  NMR with no decoupling), and  $^{19}\text{F}$  NMR spectra were reported for the selected compounds and are included in the Supplementary Materials. The following abbreviations have been used for the multiplicities: s = singlet, br = broad, d = doublet, and m = multiplet. Coupling

constants (J) are reported in Hertz (Hz). Analytical TLCs were performed with Merck silica gel 60 F254 plates, and visualization of TLCs was accomplished under UV light. Mass spectra were obtained on an LTQ Orbitrap XL Mass Spectrometer (HESI source, positive polarity, capillary temp 200 °C, source voltage 3.0 kV). IR spectra were recorded using an FT-IR-4100, using potassium bromide as a supporting material (ATR method). HRMS and ESI spectra, along with the IR spectra, were recorded and reported for the selected compounds and can be found in the Supplementary Materials. All solid reagents were weighed in an analytical balance without excluding moisture and air. Labelled cyanamide (cyanamide (stabilized with < 0.1% acetic acid) ( $^{13}\text{C}$ , 99%;  $^{15}\text{N}_2$ , 98%) 85%+ purity) was obtained from Cambridge Isotope Laboratories. Deionized water was used for all the aqueous reactions.

### 3.1. Synthesis of Tert-butyl N-[4-[(azanylcarbonimidoyl)amino]butyl]carbamate (6)

N-Boc-diaminobutane (4) (100 mg, 102  $\mu\text{L}$ , 0.53) was added to labelled cyanamide (5) (20 mg, 0.44 mmoles) in anhydrous DMF (1 mL) and stirred to a clear solution. To this solution, diisopropylethylamine (74 mg, 100  $\mu\text{L}$ , 0.57 mmoles) was added and the reaction mixture was heated to 80 °C for 24 h. Reaction progress was monitored by TLC. After completion, the reaction mixture was cooled to room temperature, water was added, and the resulting solution was extracted with ethyl acetate. The organic layer was separated, dried over magnesium sulfate, and, after filtration, the solvent was distilled under reduced pressure to yield a brown residue. The resulting crude compound was purified by flash column chromatography using a mixture of hexanes and ethyl acetate, and the pure intermediate (5) was isolated as a brown syrupy residue (yield: 46%).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ),  $\delta$ , ppm: 1.29 (s, 9H, BOC-Hs), 1.42–1.54 (m, 4H, 2 $\text{CH}_2$ ), 2.98–3.11 (m, 2H,  $-\text{CH}_2\text{-NHBOC}$ ), 3.17–3.26 (m, 2H,  $-\text{CH}_2\text{NH}$ ), 4.85 (br s,  $-\text{NH}$ ), 6.53 (br s,  $-\text{NH}$ );  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ ),  $\delta$ , ppm: 26.5, 27.4, 37.6, 79.1, 156.1 ( $-\text{C}=\text{O}-$  of BOC), 161.5 (guanidium carbon).

### 3.2. Synthesis of 1-(4-Aminobutyl)guanidine- $^{13}\text{C}$ -2,3- $^{15}\text{N}_2$ (7)

To a solution of compound (5) (50 mg, 0.202 mmoles) in 2 mL of dichloromethane was added trifluoroacetic acid (TFA) (500  $\mu\text{L}$ , 6.49 mmoles) at room temperature. The resulting solution was stirred at room temperature for 5 h. Once the reaction was complete, excess solvent and TFA was distilled off and co-distilled with dichloromethane twice. The resulting residue was dried under reduced pressure overnight to yield the labelled agmatine as a triflate salt (brown syrup, yield: 90%).

$^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ),  $\delta$ , ppm: 1.44–1.57 (m, 4H, 2 $\text{CH}_2$ ), 2.85–2.89 (t, 2H,  $-\text{CH}_2\text{NH}$ ,  $J = 14.2$  Hz), 3.11–3.14 (t, 2H,  $-\text{CH}_2\text{NH}$ ,  $J = 13$  Hz);  $^{13}\text{C}$  NMR (101 MHz,  $\text{D}_2\text{O}$ ),  $\delta$ , ppm: 24.0, 25.2, 37.1, 38.9, 164.2 ( $^{13}\text{C}$ -labelled carbon). (TFA peaks: 111.8–120.5 (q), 162.1–163.2 (q);  $^{19}\text{F}$  NMR (376 MHz,  $\text{D}_2\text{O}$ ),  $\delta$ , ppm:  $-75.6$ .

$^1\text{H}$  NMR (400 MHz, DMSO),  $\delta$ , ppm: 8.03 (br s, NH), 7.75 (br s,  $\text{NH}_2$ ), 3.06 (q, 2H,  $J = 6.3$  Hz), 2.76 (t, 2H,  $J = 6.5$  Hz), 1.4–1.51 (br m, 4H); IR ( $\text{D}_2\text{O}$ ) (KBr,  $\text{v}$ ,  $\text{cm}^{-1}$ ): 3281 (NH), 3063 (NH), 2947 (CH), 1674 (C=N), 1202 (C-N), 1132 (C-N); HRMS (calculated mass: (M- $\text{NH}_2$ ): 117.1649; observed mass (M- $\text{NH}_2$ ): 117.1020.

## 4. Conclusions

In conclusion, we developed an easy-to-implement method for the preparation of labelled agmatine. The structure of the resulting compound was successfully characterized by NMR and mass spectroscopy. This two-step approach is also easily applied to the production of non-labelled agmatine. We anticipate that this route can be scaled up to generate larger quantities of labelled and unlabelled agmatine. With this chemistry, the tracing of agmatine and of its analogs will be enabled and ready for use by biologists and analytical scientists who seek to characterize the metabolic profile of this class of neurotransmitters, in order to gain insights into the important biological processes that they regulate.

**Supplementary Materials:** The following are available online: Copies of NMR spectra of compounds 2, 3, 5 and 7.

**Author Contributions:** Conceptualization, J.D. and M.E.M.; methodology, J.D.; validation, J.D. and M.E.M.; formal analysis, J.D. and M.E.M.; investigation, J.D.; resources, M.E.M.; data curation, J.D.; writing—original draft preparation, J.D.; writing—review and editing, J.D. and M.E.M.; visualization, J.D.; supervision, M.E.M.; project administration, M.E.M.; funding acquisition, M.E.M. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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