An Optimised Synthesis of 2-[2,3-Bis(*tert*-butoxycarbonyl)guanidino]ethylamine

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Abstract: This short report describes an improved, reliable, and high-yielding (>90%) synthesis of 2-[2,3-bis(*tert*-butoxycarbonyl)guanidino]ethylamine. The method is scalable (>5 g), and the product obtained directly from the reaction mixture requires no further purification. In addition, this methodology can be successfully applied to other diamine substrates (1,3-propyl and 1,4-butyl; 70% and 61% yield, respectively).

Key words: guanidine, synthesis, optimisation, cyclisation, protecting groups, NMR spectroscopy

A number of research groups have used 2-[2,3-bis(*tert*-butoxycarbonyl)guanidino]ethylamine (**1**, Figure 1) as a simple way to install a guanidine moiety with an ethyl spacer. Indeed, aminoethylguanidine **1** has been incorporated in compounds that block tumour growth (**2** and **7**),[1,2] engage in peptide recognition (**3** and **4**),[3,4] act as antibacterial agents (**5**),[5] and treat hypertension (**6**).[6]

Despite the commercial availability of aminoethylguanidine **1**, its cost prohibits routine use.[7] As a consequence a number of 'in house' synthetic methods have become available – each with limitations.

A guanylating agent is required to synthesise aminoethylguanidine **1** and typically, one of the three reagents shown in Figure 2 is used to perform this task: *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea (**8**),[8–10] *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-trifluoromethanesulfonylguanidine (**9**),[11,12] and *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (**10**).[13]

The synthesis of thiourea **8** requires low temperatures (0 °C) and the use of NaH,[8] which can become troublesome on a larger scale. Additional reagents such as $HgCl_2$[9] and *N*-iodosuccinimide (NIS)[10] are often required to activate agent **8**.

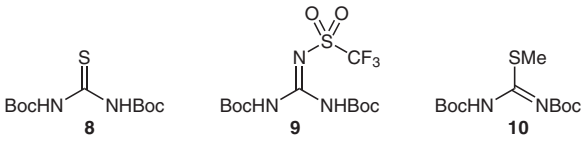

Figure 2 Guanylating agents

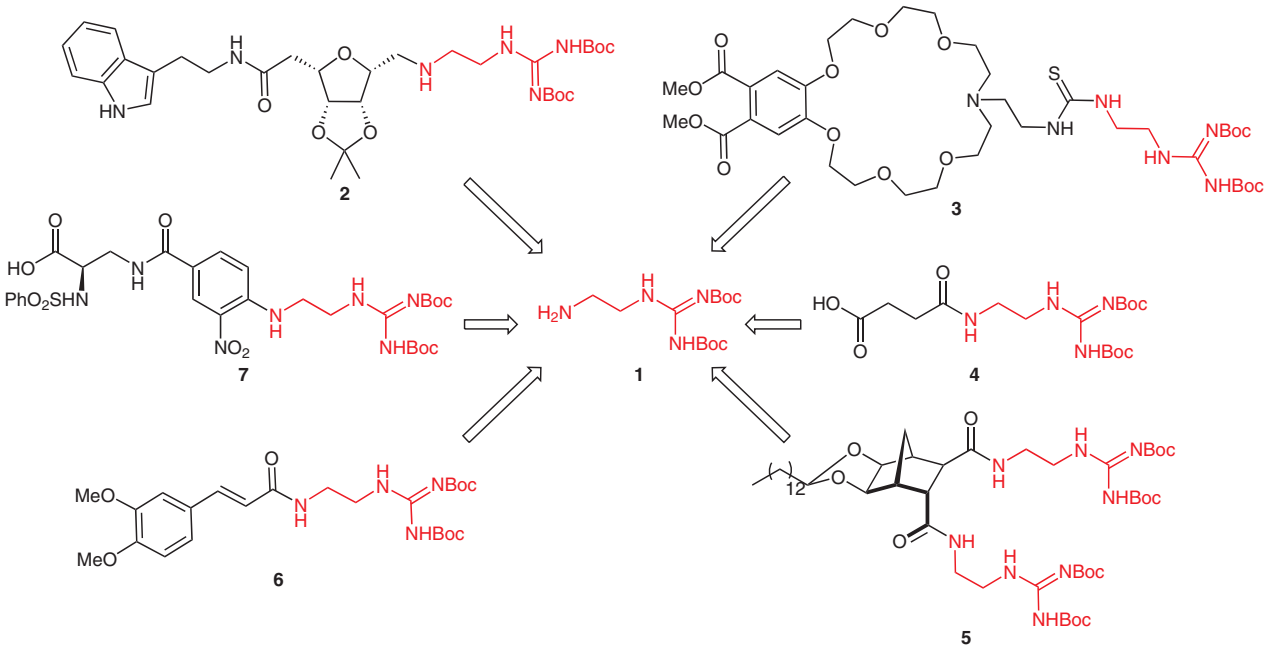

Figure 1 Examples where aminoethylguanidine **1** has been used

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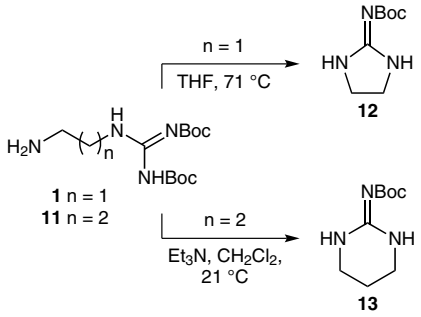
Although guanidine **9** has been shown to give excellent yields in guanidinylation reactions, it is expensive to purchase[14] or can be synthesised using a somewhat tedious procedure [again, this approach requires low temperatures (–78 °C) and multiple purification steps].[11,12] These factors make this agent less attractive if a straightforward procedure is desired.

As such, isothiourea **10** appears a cheaper and simpler alternative to the other guanylating agents (**8** and **9**). Scheme 1 depicts an approach that has been shown to be a robust method for the synthesis of isothiourea **10** and is amenable to gram-scale synthesis.[13] In addition, the method is performed at ambient temperature; an advantage for larger-scale preparations.

Even with an efficient guanylating agent other complications exist in the current methods for the synthesis of the target **1**. For example, current methods require the slow addition of isothiourea **10** to 1,2-ethylenediamine (EDA) in dilute conditions (presumably to prevent the formation of a doubly guanidinylated product). Such a slow addition rate inevitably increases reaction time. It is also common to perform this reaction at elevated temperatures.[4–6] However, it has been shown by Castagnolo et al. that an elevation in reaction temperature facilitates the intramolecular cyclisation of aminoethylguanidine **1** to form **12** (Scheme 1).[13] As a result a purification step (chromatography) is required.[15] Alternatively, crude samples of **1** have been used directly when the next step is high yielding and the product is easily isolated from a complex reaction mixture.[5]

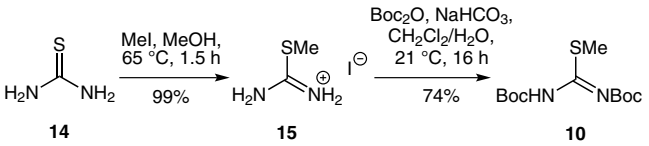


Scheme 1 Reported cyclisation of aminoalkylguanidine substrates[13]

An orthogonal protecting-group strategy (such as that used by Wakimoto in the synthesis of hordatine and aperidine[16]) would avoid intramolecular cyclisation and ultimately afford highly pure samples of aminoethylguanidine **1**, however, the multistep nature of such an approach is not ideal.

In order to pursue analogues of antibacterial agent **5**,[5] an optimised method for the synthesis of aminoethylguanidine **1** was sought. Herein, a practical, reliable, scalable, and operationally simple synthesis to access gram-scale quantities of **1** using cheap and readily accessible starting materials is described.

Synthesis of the required guanylating agent *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (**10**) was carried out as shown in Scheme 2.[17]



Scheme 2 Synthesis of *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (**10**)[17]

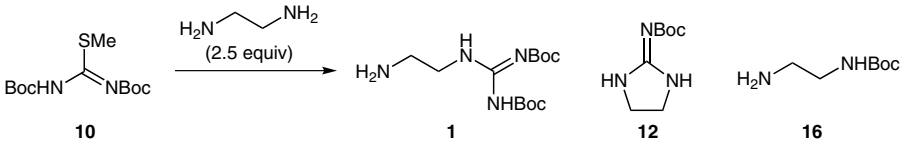
With guanylating agent **10** in hand, the procedure developed by Carmignani et al. for the synthesis of target **1** was tested.[6] In brief, a THF solution of guanylating agent **10** was added dropwise over 90 minutes to a solution of 2.5 equivalents of EDA in THF at 50 °C (Table 1, entry 1) then heating was continued for four hours. In the ^{1}H NMR spectrum of the crude product obtained from this reaction a singlet at $\delta = 3.62$ ppm was observed. This singlet was assigned to cyclic guanidine **12** which is formed from intramolecular reaction of **1** as shown in Scheme 1. Formation of this side product has been described by Castagnolo[13] and in this study its identity was confirmed after subsequent comparison with published ^{1}H NMR spectroscopic data.[18]

As aminoethylguanidine **1** has been shown to cyclise (to **12**) at elevated temperature,[13] the reaction was repeated at 21 °C using CH_2Cl_2 as the solvent (Table 1, entry 2) such that the reaction mix could be easily concentrated without heating. Gratifyingly, the reaction proceeded smoothly, and after aqueous workup the product contained only a trace of the cyclised product (as evidenced in the ^{1}H NMR spectra), decreasing from 17% to 3% (Table 1, entries 1 and 2).

The reaction was repeated on a larger scale (Table 1, entry 3), and an increase in yield was noted (87%). In an attempt to achieve faster reaction times the effect of concentration was investigated (increased from 0.02 M to 0.07 M, Table 1, entries 3–5) and in less than six hours a 74% yield of target **1** was realised (with only a trace of **12** detected by ^{1}H NMR spectroscopy; Table 1, entry 5).

Using these more concentrated conditions the reaction was again scaled up (Table 1, entry 6), however, a new side product was noted which was subsequently isolated using column chromatography and identified as *tert*-butyl *N*-(2-aminoethyl)carbamate (BocEDA, **16**).[19] Although the formation of this side product was minimal (<10% by ^{1}H NMR spectroscopy), purification would be required to remove BocEDA as it has a free amine that would compete with aminoethylguanidine **1** in ensuing reactions. Suspecting that the long reaction time was responsible for the additional side product and given that an excess of EDA (2.5 equiv) was present the reaction was repeated with the guanylating agent added in a single portion (Table 1, entry 7). Careful monitoring of the reaction mix by TLC indicated that the reaction was complete in only 3.5

Table 1 Optimisation of the Synthesis of Aminoethylguanidine **1**



10 **1** **12** **16**

Entry	Scale (g)	Conc. (M)	Temp (°C)	Addition time (min)	Reaction time	Ratio (**1**/**12**/**16**)[b]	Yield (%)[d]
1[a]	0.2	0.02	50	90	4 h	83:17:0	69
2	0.2	0.02	21	90	24 h	97:3:0	64
3	0.6	0.02	21	90	24 h	95:5:0	87
4	0.6	0.03	21	90	23 h	97:3:0	85
5	0.6	0.07	21	60	5.5 h	99:1:0	74
6	1.8	0.07	21	90	20 h	86:5:9	78
7	1.8	0.14	21	–	3.5 h	98:2:0	76
8	1.8	0.27	21	–	80 min	98:2:0	82
9	1.8	0.67	21	–	50 min	92:3:5[c]	55
10	5.8	0.27	21	–	70 min	98:2:0	90

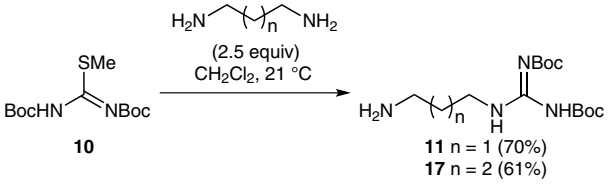
[a] This reaction was performed in THF. All other reactions were performed in CH_2Cl_2.
[b] Ratio determined from integration of the NMR spectra (see Supporting Information for example).
[c] An additional unidentified side product was formed.
[d] Isolated yield after aqueous workup (see Supporting Information for full experimental).

hours and upon workup no trace of BocEDA could be detected in the product. Indeed, formation of both side products **12** and **16** could be minimised (<2% by ^{1}H NMR spectroscopy) if the more concentrated reaction was used with the guanylating agent **10** added in one portion (Table 1, entry 7).

It was subsequently confirmed through reactions of various durations and concentration (Table 1, entries 7–9) that BocEDA only formed when longer reaction times and/or a concentration greater than 0.27 M was used.

The optimised reaction conditions were shown to be amendable for multigram-scale reactions, which was highlighted by a yield of 90% on a 5.8 gram scale (Table 1, entry 10).[20] In addition, no evidence of a diguanidinylated product was observed throughout this study, even at higher reagent concentrations. It is also worth noting that we found it necessary to store this compound at less than –20 °C as slow cyclisation was observed even at 4 °C.

To investigate the scope of the methodology the synthesis of two related aminoalkylguanidines was pursued (Scheme 3). Castagnolo et al.[13] previously reported that the propyl analogue 2-[2,3-bis(*tert*-butoxycarbonyl)guanidino]propylamine (**11**) would immediately cyclise to form the six-membered ring (**13**, Scheme 1). However, by employing the methodology developed in this study – which does not require heating – only a minimal amount of cyclised product was observed (<5% by ^{1}H NMR spectroscopy). Using column chromatography the cyclised side product was isolated as a white solid, whilst the desired aminopropylguanidine **11** was a colourless oil. The observed morphology of the products was in contrast to that previously reported by Nnanabu and Burgess who claimed that the white solid formed by recrystallisation of the crude compound was the desired compound.[21] Our findings (including the comparison of ^{1}H NMR spectra) conclude that the compound reported by Nnanabu and Burgess to be **11** was indeed the cyclised side product **13**.[21]



10
11 n = 1 (70%)
17 n = 2 (61%)

Scheme 3 Synthesis of analogues **11** and **17**

The synthesis of 2-[2,3-bis(*tert*-butoxycarbonyl)guanidino]butylamine (**17**), a precursor to the neurotransmitter agmatine,[22] was also achieved in good yield (61%) and high purity (>90%) using this methodology.

A cheap, scalable (90% yield on >5 g scale), and operationally simple synthesis of 2-[2,3-bis(*tert*-butoxycarbonyl)guanidino]ethylamine (**1**) has been developed. Compared to existing methods, reductions in reaction duration, solvent volume, and purification steps have been achieved. The product is of a high purity (>98%) following a simple extractive workup. The scope of the method-

ology was successfully expanded to the synthesis of related aminoalkylguanidines **11** and **17**. Given the convenience of the compound **1** as a means to install a guanidine group with an ethyl spacer and the current cost to purchase this reagent, the method described herein should be of practical use to researchers in the fields of organic and medicinal chemistry.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(20) **2-[2,3-Bis(*tert*-butoxycarbonyl)guanidino]ethylamine (1)** Boc-protected methylisothiourea **10** (5.81 g, 20.0 mmol) in CH_2Cl_2 (30 mL) was added in one portion to a stirred solution of 1,2-ethylenediamine (3.3 mL, 50.0 mmol) in CH_2Cl_2 (44 mL). The reaction was allowed to stir at 21 °C for 70 min. The reaction mixture was washed with H_2O (3 × 25 mL), brine (30 mL), then dried ($MgSO_4$) and filtered. Solvent was removed in vacuo at ambient temperature to afford **1** (5.37 g, 90%) as a white powder; mp 96.2–100.1 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 1.50 (9 H, br s, *t*-Bu), 1.51 (9 H, br s, *t*-Bu), 2.90 (2 H, t, *J* = 6.2 Hz, CH_2), 3.49 (2 H, app q, J_{app} = 5.5 Hz, CH_2), 8.67 (1 H, br s, NH), 11.51 (1 H, br s, NH). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 28.2, 28.4, 41.1, 43.5, 79.4, 83.2, 153.3, 156.5, 163.7. ESI-HRMS: *m/z* calcd for $C_{13}H_{26}N_4O_4$ $[M + H]^+$: 303.2027; found: 303.2032.
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