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Macrocyclization of Di-Boc-guanidino-alkylamines Related to Guazatine Components: Discovery and Synthesis of Innovative Macrocyclic Amidinoureas

Daniele Castagnolo,[a] Francesco Raffi,[a] Gianluca Giorgi,[b] and Maurizio Botta*[a]

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The synthesis of new and innovative macrocyclic amidinoureas from linear di-Boc-guanidino-alkylamines related to guazatine was accomplished. The macrocyclization reaction proceeds under mild conditions affording 11- to 16-membered rings with a new and previously undescribed structure in good yields. Enantiomerically pure macrocyclic amidinoureas were also synthesised. The strict correlation between macrocyclic amidinoureas and the natural product guazatine makes them very actractive also from a biological point of view.

Introduction

The guanidine and urea functional groups are crucial components in many medicinally interesting molecules,[1] and therefore practical methods for straightforward syntheses of guanidine- or urea-containing molecules are of great interest in drug discovery and lead optimization.[2] Similarly, amidinoureas, molecules containing both guanidine and urea moieties, are of great interest in medicinal chemistry in treating gastrointestinal, spasmolytic, cardiovascular disorders and parasitic infestations. Furthermore, amidinourea has also been shown to be the essential structural feature for antimalarial compounds[3] and for the potent antibacterial TAN-1057A-D,[4] a naturally occurring dipeptide–amidinourea antibiotic isolated from the bacteria Flexibacter sp. PK-74 and PK-176. Few methods have been reported so far for the synthesis of linear amidinoureas, and most of them are inefficient or require numerous steps.[2a,5,6] However, no examples of cyclic or macrocyclic amidinoureas are known so far. The design and synthesis of macrocyclic structures with an appropriate ring size and predictable function is an important research topic and still remains an attractive challenge in organic synthesis. For instance, macrocyclic peptides play a significant role in biology since they can interact with a large protein surface disrupting protein–protein interaction,[7] which is a new important target in medicinal chemistry. Less common natural macrocyclic polyamine lactams and macrocyclic lactams containing the biogenetic base spermine are of great interest in view of the broad activity, which was established for the spermine-containing compounds in biological systems.[8] More recently, novel macrocylcic urea compounds were prepared showing potent Chk1 (serine/threonine protein kinase) inhibitory activity.[9] Herein we report the first design and synthesis of a novel class of macrocyclic amidinoureas with general structure **B** starting from linear precursors di-Boc-guanidino-alkylamines **A** related to guazatine components.[10] (Figure 1).

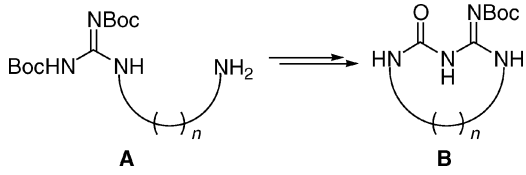

Figure 1. General structures of linear di-Boc-guanidino-alkylamines and macrocyclic amidinoureas.

The strict correlation between these macrocyclic amidinoureas and guazatine components, which in our recent studies were found to have a potent antifungal activity on all the *Candida* strains with MIC_{50} values ranging between 10 and 80 μM,[10b] makes these compounds very attractive both from a synthetic as well as a biological point of view.

Results and Discussion

During our recent studies on guazatine-analogous compounds,[10c,11] we observed that aminoguanidines such as **A**, bearing both a di-Boc-guanidine and an amine moiety, were converted by simply heating in THF into unexpected derivatives with unrecognized structure.[10c] Hence, a series of aminoguanidine precursors **2** were synthesised starting

[a] Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena,
Via A. de Gasperi 2, 53100 Siena, Italy
Fax: +39-0577-234333
E-mail: botta@unisi.it
[b] Dipartimento di Chimica, Università degli Studi di Siena,
Via A. Moro, 53100 Siena, Italy
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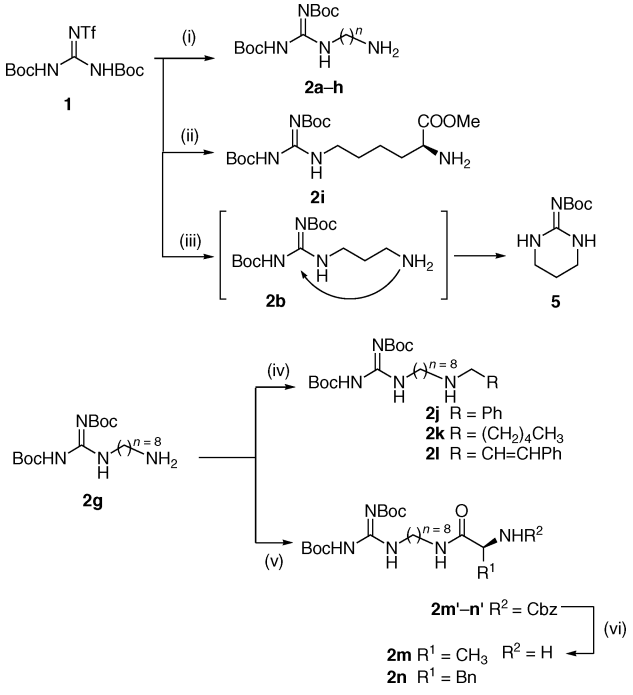
from guanidine **1** (Scheme 1) with the aim to investigate and generalize their behaviour under the same conditions and to understand the structure of the newly formed reaction products. Reaction of **1** with different diamines led to aminoguanidine precursors **2a**–**h** in high yield. Surprisingly, when **1** was treated with 1,3-diaminopropane compound **5** was isolated in 94% yield as the only product. HPLC-MS analysis revealed that in the early stages of the reaction the linear product **2b** was present in the reaction mixture together with a larger amount of **5**.



Scheme 1. Synthesis of macrocyclization precursors. Reagents and conditions: (i) alkyldiamine, Et_3N, DCM (90–98%); (ii) lysine methyl ester, Et_3N, DCM (72%); (iii)1,3-diaminopropane, Et_3N, DCM (94%); (iv) aldehyde, $NaBH_4$/*p*TSA, DCE (40–55%); (v) *N*-Cbz-alanine (for **2m′**) or *N*-Cbz-phenylalanine (for **2n′**), EDC, HOBt, DIPEA, DMF; (vi) H_2, Pd/C, EtOH (52% for **2m**, 49% for **2n**; 2 steps).

Hence, as **2b** was forming, its free amino group immediately collapsed on the quaternary electrophilic carbon atom to afford in this way the stable six-membered cyclic guanidine **5** as illustrated in Scheme 1. Amine **2i** was obtained by reaction of **1** with D-lysine methyl ester in 72% yield.[12] Secondary amines **2j**–**l** were synthesised through reductive amination reaction of **2g** with different aldehydes (namely benzaldehyde for **2j**, valeraldehyde for **2k** and cinnamaldehyde for **2l**).[13] Finally, coupling of **2g** with *N*-Cbz-alanine and *N*-Cbz-phenylalanine in the presence of DCC led to **2m′**,**n′** which were converted into precursors **2m**,**n**, respectively, after removal of the Cbz protecting group by hydrogenolysis. The di-Boc-aminoguanidine **2g**, which is the protected component GN of guazatine,[10] was then chosen to investigate the new reaction. Heating of **2g** in THF at 70 °C for 8 h afforded the macrocyclic amidinourea **3g** (48%) as the major product together with the dimer **4g** (8%) (Scheme 3). The innovative structure of macrocycle **3g** was confirmed by X-ray analysis (Figure 2). The proposed

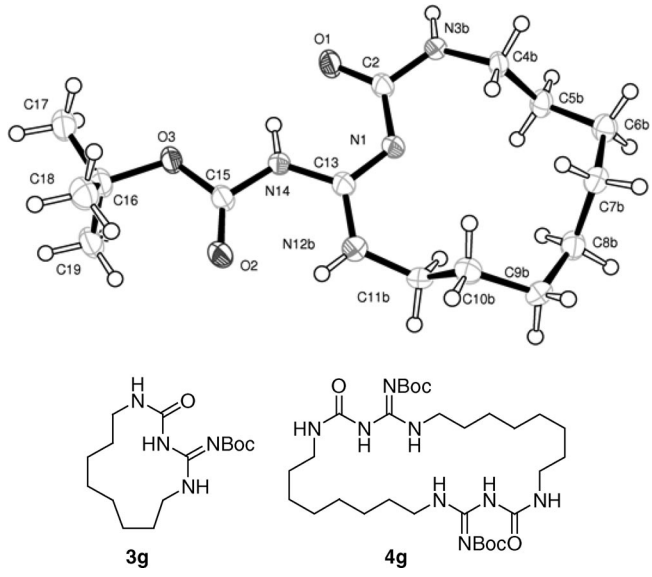
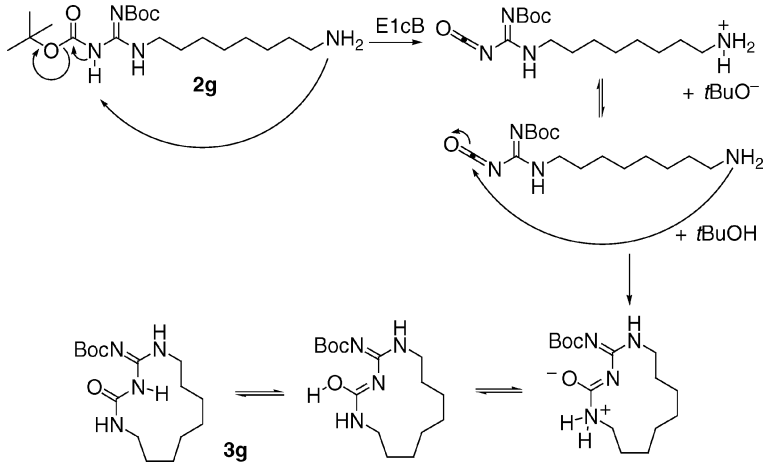


Figure 2. X-ray structure of macrocycle **3g**.

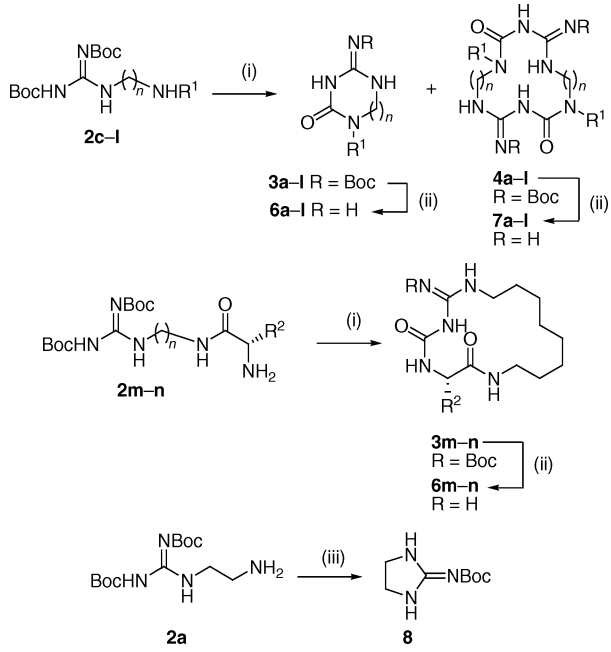


Scheme 2. Proposed mechanism for the macrocyclization.

mechanism for the macrocyclization reaction of **2g** is outlined in Scheme 2. Linear amidinoureas have been previously prepared by Rault and Miel through cross reaction of tri-Boc- or di-Boc-guanidines with different amines under mild conditions and in the presence of a base.[6] The authors also proposed that their formation could proceed via an isocyanate intermediate,[6a] formed from the Boc-protecting group by an E1cB mechanism with a conjugate base and subsequent addition of external amines as nucleophilic agents.[2b] Since in our cases no external conjugate bases are present in the reaction mixtures, we hypothesised that the amino group of aminoguanidines **2** could work both as the conjugate base in the formation of the isocyanate intermediate and as the nucleophilic agent. The formation of the dimer **3b** might be due to the cross attack of two isocyanate intermediates.

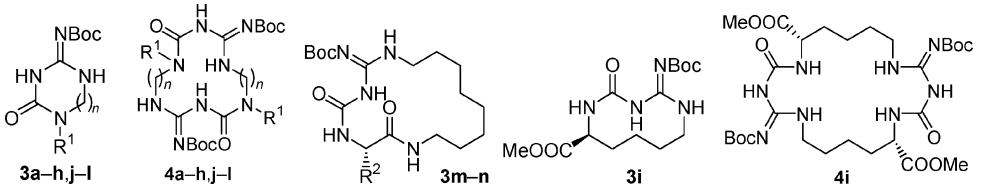
Precursors **2a**–**n** were then refluxed in THF for the required time to afford cyclic amidinoureas **3** and in some cases the corresponding dimers **4** (Scheme 3). Finally, **3** and **4** were treated with TFA to afford deprotected amidinoureas **6** and dimers **7**. The yields are reported in Table 1.

Surprisingly, when **2a** was refluxed in THF, cyclic guanidine **8** was formed after only 2 h and isolated as the only product of the reaction in 92% yield (Scheme 3). No traces of the desired amidinourea **3a** were detected (Entry 1). The formation of the more stable five-membered ring **8** was favoured over the formation of the more constrained seven-membered ring **3a**. Heating of **2c**,**d** in THF led to the formation of dimers **4c**,**d** as the only products (Entries 2, 3).[14] When **2e** was refluxed in THF, cycle **3e** was isolated in 6% yield together with 12% of dimer **4e** (Entry 4).[15] On the other hand, amines **2f**,**g** led to **3f**,**g** as major products (30–48% yield) together with smaller amounts of their dimers **4f**,**g** (Entries 5–6). Heating of **2h** led exclusively to macrocycle **3h** (Entry 7), whereas from **2i** only the dimer **4i** was formed (Entry 8). Macrocyclization of secondary amines **2j**–**l** occurred in refluxing THF to afford **3j**–**l** together with traces of the corresponding dimers **4j**–**l** (Entries 9–11). Finally, compounds **2m**,**n** afforded the macrocylcic amidinoureas **3m**,**n** as the only products with complete retention of configuration (Entries 12–13). No traces of dimeric compounds **4m**,**n** were detected. It is clear that a linear correlation exists between the length of the chain of **2** and the



Scheme 3. Macrocyclization reactions. Reagents and conditions: (i) THF, 70 °C (6–51%); (ii) TFA, DCM (quant.); (iii) THF, 70 °C (92%).

Table 1. Results and yields of the macrocyclization reactions.



Entry	Amine **2**	n	R^1	R^2	Time [h]	Cycle **3** (%)[a]	Dimer **4** (%)[a]
1	**a**	2	H		2	n.d.[b,c]	n.d.[b]
2	**c**	4	H		7	n.d.[b]	20
3	**d**	5	H		7	n.d.[b]	15
4	**e**	6	H		7	6	12
5	**f**	7	H		7	30	9
6	**g**	8	H		7	48	8
7	**h**	9	H		7	51	n.d.[b]
8	**i**	5	H		7	n.d.[b]	13
9	**j**	8	Bn		7	31	6
10	**k**	8	pentyl		7	39	4
11	**l**	8	cinnamyl		7	32	traces[d]
12	**m**	8		Bn	7	44	n.d.[b]
13	**n**	8		CH_3	7	42	n.d.[b]

[a] Isolated yield. [b] n.d. = no detected compound; compound was not formed. [c] Cyclic guanidine **8** was formed. [d] Traces of dimer **4l** were observed by HPLC-MS analysis.

formation of **3** and/or **4**. When the length of the chain of **2** is n = 4–6, dimers **4c**–**e** and **4i** were isolated as the major (or only) products (Entries 2–4 and 8). On the contrary, when the chain length is n = 9, no traces of dimeric compound **4h** were detected, and **3h** was the only product.

The amidinourea moiety being planar, the macrocyclization is conformationally hindered when the length of the chain is n = 4, 5. The amino group is too far away to reach the isocyanate moiety on the same molecule, and it prefers to cross-react with the isocyanate moiety of a second molecule to lead to the formation of dimers **4**. On the other hand, when $n > 6$, aminoguanidine **2** has a greater conformational flexibility, and the amino group can reach the isocyanate moiety to lead to macrocyles **3** bearing a planar amidinourea moiety. The geometric and stereoelectronic constraints on the transition state for the ring closure play a fundamental role in the formation of dimers **4** over cycles **3** or vice versa. This could explain why the 18-membered ring **4c** was formed instead of the nine-membered ring **3c**, whose rate of ring closure would be expected to be faster.[16]

Conclusion

A novel class of innovative macrocyclic amidinoureas **3e**–**h**,**j**–**n** was discovered and synthesised from easily accessible aminoguanidines **2** in good yields and under mild conditions. In particular, enantiomerically pure **3m**,**n** could represent interesting scaffolds in the synthesis of peptidomimetic compounds. Macrocyclic amidinoureas also represent an attractive class of new compounds with strong antifungal activity,[17] such as guazatine,[10] or potential antibacterial activity, resembling the structure of natural macrolide antibiotics. Preliminary biological results seem to be promising. The macrocyclization dimers **4c**–**g**,**i**–**k** were obtained as sideproducts in moderate yields; they also prove to be an interesting new class of macrocycles containing two amidinourea moieties, and are at the moment under biological study.

Supporting Information (see footnote on the first page of this article): Experimental data, ^{1}H and ^{13}C NMR spectra; selected crystal data and ORTEP view of **3g**.

Acknowledgments

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[12] The product derived from diguanylation of D-lysine methyl ester was isolated in 23% yield.

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[14] Compounds **4c**,**d** were obtained together with traces of a second compound, which on the basis of HPLC-MS data was hypothesised to be a trimeric derivative of **2c**,**d**.

[15] When **2e** was heated in THF at 0.3 mM instead of 3.0 mM concentration, compounds **3e** and **4e** were always isolated in a 1:2 ratio. Thus, formation of dimers **4** results to be independent of the concentration of the reaction solution and is strictly correlated to the structure of the substarte **2**.

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