Pyridin-2-yl Guanidine Derivatives: Conformational Control induced by Intramolecular Hydrogen Bonding Interactions

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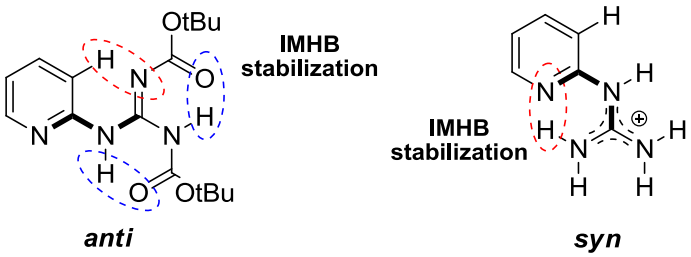

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Abstract

The synthesis and conformational analysis of a series of pyridin-2-yl guanidine derivatives using NMR, X-ray crystallography and B3LYP/6-31+G** theoretical studies are reported. A remarkable difference was observed in the ^{1}H NMR spectra of the guanidinium salts compared with their *N,N'*-di-Boc protected and neutral analogues. This difference corresponds to a 180° change in the dihedral angle between the guanidine/ium moiety and the pyridine ring in the salts compared to the Boc protected derivatives; a conclusion which was supported by theoretical studies, X-ray data and NMR analysis. Moreover, our data sustains the existence of two intramolecular hydrogen bonding systems: (i) between the pyridine N1 atom and the guanidinium protons in the salts and (ii) within the *tert*-butyl carbamate groups of the Boc protected derivatives. To verify that the observed conformational control arises from these intramolecular interactions, a new series of *N*-Boc-*N'*-propyl substituted pyridin-2-yl guanidines was also prepared and studied.

INTRODUCTION

Guanidine derivatives have a wide range of applications throughout the field of medicinal chemistry. During the last 10 years our group has been working on the synthesis[1] and biological evaluation of aromatic guanidine derivatives both as α-adrenoceptor ligands for the treatment of CNS disorders[2,3,4,5] and as DNA minor groove binders.[6,7] The biological importance of guanidines is highlighted by the prevalence of guanidine-carboxylate salt-bridges in protein structures, a feature which is often closely linked to protein function.[8] In addition, aromatic guanidines have found use in a diverse range of therapeutic and biological applications.[9] Guanidines have also proven effective as catalysts for a wide variety of organic transformations[10,11] and have been studied as ligands both in metalloproteins[12] and in synthetic metal complexes.[13]

The structure of guanidine in the solid-state was recently resolved, revealing the high tendency of this molecule to form hydrogen bond (HB) interactions.[14] Tautomerism in guanidines has been the object of a recent ^{15}N NMR study;[15] as have its vibrational properties, which have been the subject of a theoretical study.[16] In an attempt to elucidate their mode of binding to biological receptors, Kleinmaier *et al*. have investigated the conformational preferences of mono-alkylated acyl guanidines.[17] Moreover, we have recently published a theoretical study of the π-cation interactions in solution between the guanidinium and simple aromatic systems.[18] In spite of all these studies, the molecular basis for ligand-receptor interactions in aromatic guanidines remains poorly understood, and further knowledge of their conformational preferences would be of great benefit in elucidating their mode of binding to biological receptors.

Moreover, carbamates and amides are important building blocks in artificially folded molecules, in conjunction with pyridines. This topic has been thoroughly reviewed by Huc who found that aromatic oligoamide foldamers can be efficiently designed, are easy to synthesize, and permit to obtain many different stable folded states. Furthermore, he describes that conformational rearrangements may be induced by changing the conformational preference of aryl−amide bonds.[19] In addition, pH-active pyridines are considered as interesting units in molecular motion controlled by pH. Thus, Kolomiets *et al*. using NMR and circular dichroism have studied how oligopyridine carboxamide strands experience reversible folding/unfolding when protonated.[20]

In our search for new aromatic guanidine derivatives, we present here not only the preparation of a number of novel 2-guanidino pyridines (Figure 1) following our own synthetic approach,[1] but also a thorough structural study both at experimental (^{1}H NMR and X-ray spectroscopy) and computational (DFT) levels.

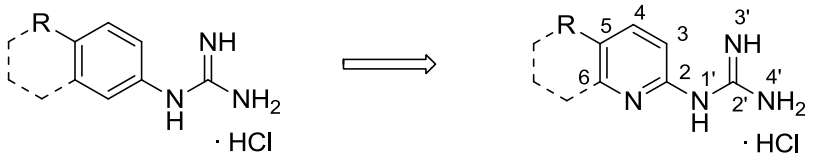


FIGURE 1. General structures of previously prepared phenyl guanidines and new pyridine-2-yl guanidine derivatives, indicating the numbering scheme used throughout this work.

To the best of our knowledge, these pyridine-2-yl guanidines have not yet been studied and only a few pyridinyl guanidine derivatives have been reported to date. These include those in which the pyridine and guanidine moieties are separated by a linker, several cobalt[21] and zinc[22] complexes that were studied for their potential in sensor technology and as lactide

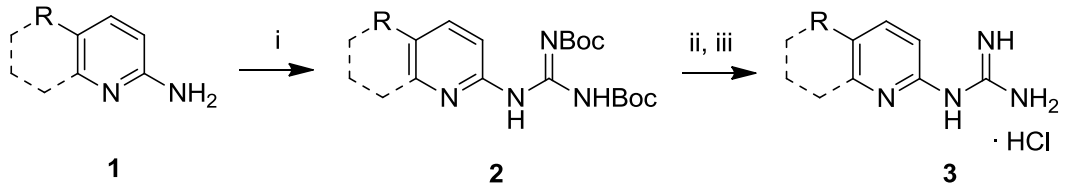
polymerisation catalysts, respectively, and a number of 2-pyridinylguanidines prepared as urokinase inhibitors.[23] Additionally, the crystal structures and conformational properties of *N,N'*-diphenylguanidine and its *N*-methylated derivatives have been previously investigated by X-ray crystallography and NMR by Kagechika and co.[24]

RESULTS AND DISCUSSION

Synthesis and characterisation of N-(pyridin-2-yl)guanidinium salts

A series of novel *N*-(pyridin-2-yl)guanidinium salts and the closely related bicyclic analogue *N*-(5,6,7,8-tetrahydroquinolin-2-yl)guanidinium were synthesised following our standard synthetic procedure for the preparation of aromatic guanidines[1] (Scheme 1).

SCHEME 1. General synthetic pathway



1 **2** **3**

(**a**) R = H; (**b**) R = Cl; (**c**) R = Br; (**d**) R= CH_3; (**e**) R = -$(CH_2)_4$-

Reagents and conditions: (i) $HgCl_2$, Et_3N, *N,N'*-bis(*tert*-butoxycarbonyl)-S-methylisothiourea, CH_2Cl_2, 0 °C to rt, 18 h; (ii) CF_3CO_2H, CH_2Cl_2, 3 h; (iii) Amberlite IRA-400 (Cl^- form), H_2O, 24 h.

Starting from commercial or synthetically prepared[25] 2-aminopyridines (**1**), guanidylation was performed in the presence of mercury (II) chloride using Boc protected 2-methyl-2-thiopseudourea to afford the corresponding *N,N'*-di-Boc protected intermediates (**2**). Removal of the Boc groups using trifluoroacetic acid followed by anion exchange with Amberlite IRA-400 resin afforded the desired guanidinium chloride salts (**3**) in good to excellent overall yields (65 - 93%).

Significantly, in the course of the characterisation of these compounds, a striking difference was observed in the ^{1}H NMR chemical shifts of hydrogen **H3** recorded for the neutral di-Boc protected compounds **2** compared to those recorded for the hydrochloride salts **3** (Figure 2).

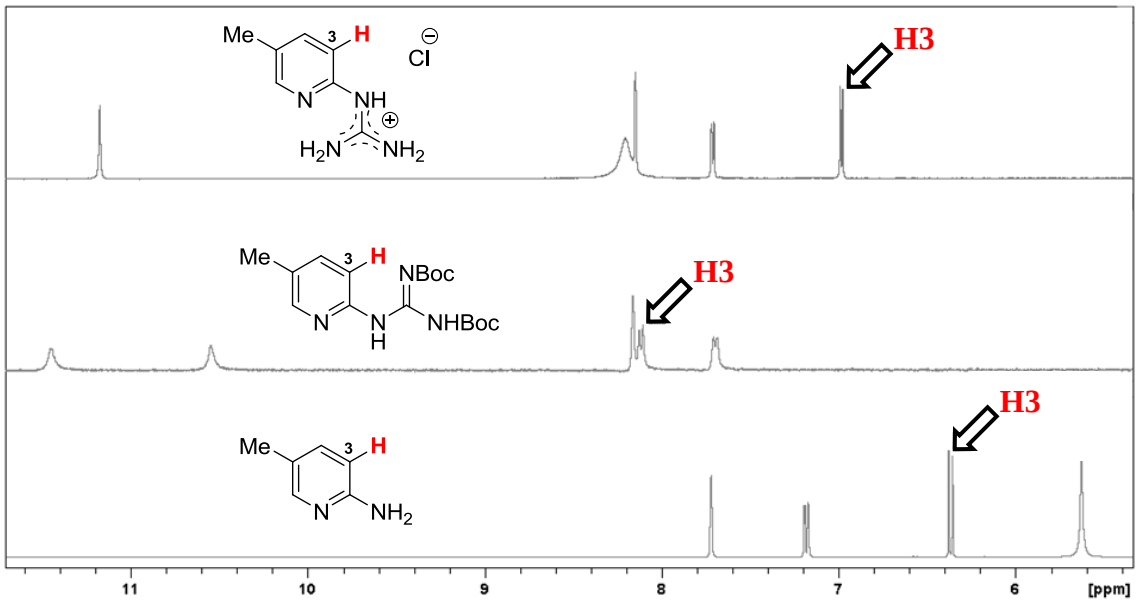


FIGURE 2. Aromatic region of the ^{1}H NMR spectra (DMSO-d_6) of 5-methyl-2-aminopyridine **1d** (*bottom*), 1-[2,3-di-(*tert*-butoxycarbonyl)guanidino]-5-methylpyridine **2d** (*middle*) and *N*-(5-methylpyridin-2-yl)guanidinium chloride **3d** (*top*).

This difference was preserved across a variety of R substituents with varying electronic properties (Cl, Br, H and CH_3), both in $CDCl_3$ and DMSO-d_6. For the sake of this discussion, only shifts acquired in DMSO-d_6 will be quoted, unless otherwise stated. Regardless of the electronic effect induced by these R substituents, the difference in the ^{1}H-NMR spectra between series **2** and **3** remained significant, thereby indicating that this phenomenon might be the result of conformational restrains. Considering the importance of conformation in the potential pharmacological application of these compounds, a complete structural study (NMR

spectroscopy, DFT calculations and X-ray crystallographic analysis) of these molecules was carried out.

Theoretical studies on conformation and tautomerism of aromatic guanidines

Considering the relative position of the guanidine/ium and pyridine moieties with respect to the **N1-C2-N1'-C2'** dihedral angle, two extreme geometries result which can be labelled as *anti* and *syn* conformations (Figure 3).

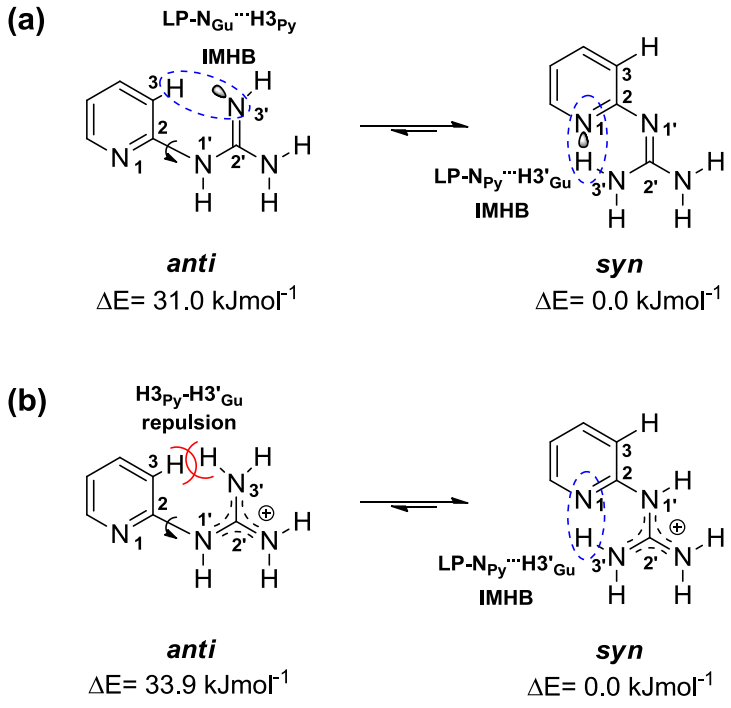


FIGURE 3. *Anti* and *syn* conformations available to (a) 2-pyridinoguanidine and (b) 2-pyridinoguanidinium [series **3**] *via* rotation around the **C2-N1'** bond.

It is known that in pyridyl guanidinium systems the *syn* conformation can be stabilized by intramolecular hydrogen-bonding interactions (IMHB) when the guanidinium carrying group

is placed in the *ortho* position.[26] Hence, a theoretical DFT study at B3LYP/6-31+G** computational level of all possible geometries of the neutral 2-pyridinoguanidine series (including *E/Z* isomers, syn/anti rotamers, and tautomers) was initially performed to approach our systems. We found that, as expected, the absolute minimum corresponds to one of the *syn* geometries (Figure 3a) which is 30 kJ mol^{-1} more stable than the best of the minima found in *anti* conformation (for the sake of clarity only the most stable *anti*/*syn* minima are shown). Thus, in 5-substituted-2-guanidine-pyridines, the stabilization induced by the IMHB through the pyridine nitrogen [LP-$N_{Py}\cdots H3'_{Gu}$] is considerably larger than that through the guanidine nitrogen [LP-$N_{Gu}\cdots H3_{Py}$], in the absence of electronic or steric secondary factors.

As mentioned before, we have experimentally observed that the guanidinium salts showed a NMR pattern very different to that observed in their Boc derivatives and that this could be due to important differences in their geometries. Hence, using B3LYP/6-31+G**, we continued with our theoretical study to confirm this hypothesis. In the case of the protonated series **3**, a quantum theoretical intrinsic reaction coordinate (IRC) study of the C2-N1' rotation process[27,28] was carried out (gas phase) resulting in two energetic minima and two transition states (Figure 4). As in the case of the neutral guanidine (Figure 3a), the global minimum of the guanidinium series corresponds to the *syn* conformation and features a co-planar arrangement of the guanidinium and pyridine moieties stabilised by the same type of IMHB interaction [LP-$N_{Py}\cdots H3'_{Gu}$]. In this case, the prevalence of the *syn* conformation is also favoured by steric constraints in the *anti* rotamer (**H3--H3'** repulsion, Figure 3b), which was also localised as a second local minimum exhibiting a 'twisted' geometry to minimise the aforementioned repulsion. Further, two stationary states were localised: **TS1** which corresponds to the transition between the *anti* and *syn* minima and **TS2** which results from the transition between the two possible degenerate *anti* minima ('twisted' above and below the

pyridine ring). Although the calculated barriers to the rotation are not high enough to completely restrict the C2-N1' rotation (36.9 and 45.7 kJ mol^{-1}), the energetic difference between the *anti* and *syn* minima (34.0 kJ mol^{-1}) suggests an almost quantitative preference for the *syn* conformation.

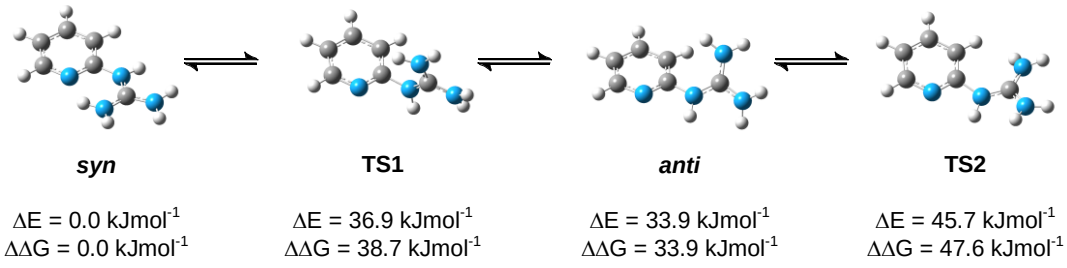


FIGURE 4. Stationary structures resulting from the IRC study at B3LYP/6-31+G** computational level of the **C2-N1'** rotation in the guanidinium derivative **3a**. ΔE refers to the difference in total energy between a given state and the global energetic minimum in gas phase while ΔΔG is the difference in free energy.

The study of conformation and rearrangement in the Boc protected series **2** is considerably more complicated. In this case, the guanidine subunit is not only triply substituted but also neutral; therefore, the localisation of the **C2'=N(3',4')** bond results in a number of tautomers. Additionally, *E/Z* isomerism in the imino group and the possible internal rotation of the adjacent secondary amines results in further geometries. Unlike guanidinium series **3**, the combination of these issues for the Boc protected series **2** results in a vast array of possible conformations/isomers/tautomers and any *a priori* prediction of geometrical preference becomes untenable. To approach this problem in a systematic way, we have carried out a complete screening (by means of a systematic search) of the less computationally demanding

di-acyl analogues of series **2**. Those structures showing total energies within a range of 30 kJ mol^{-1} from the minimum were used as templates for the di-Boc derivatives which were then fully optimised until a minimum energy geometry was obtained (see Supplementary Information).

The ^{1}H NMR spectra of the majority of the *N,N'*-di-Boc protected phenylguanidine derivatives previously synthesised in our group[3,4,5] show the CH_3 protons of the *tert*-butyl groups as two separate signals integrating for nine protons each, suggesting the chemical inequivalence of the two *N*-Boc groups. This could be explained by the formation of two IMHBs involving the Boc carbonyl groups and guanidine hydrogens **H1'** and **H4'**, which is supported by the deshielding recorded for these two protons' signals (>10 ppm). In addition, these two Boc groups are not equivalent because one is connected to an amino-N and the other one is connected to an imine-N group.

The proposed IMHB network confers rigidity to the *N,N'*-di-Boc substituted guanidine system, which could be considered as a single "pseudo bicyclic" structure similar to that previously described for β-diketones.[29] Thus, the conformational problem in these Boc derivatives is reduced to a simple rotation around the **C1-N1'** bond connecting the phenyl ring and the rigid Boc protected guanidine. Due to the symmetry of the phenyl ring this rotation results in a degenerate pair of conformations stabilised by IMHBs between the guanidine **N3'** and phenyl protons **H2** or **H6**, respectively (Figure 5a). This kind of C(Ar)-H···N IMHB has previously been described for compounds such as 2,2'-bipyridine,[30] 1,l'-bipyrazole[31] and 9-azaphenyl-9H-carbazoles,[32] and C-H···X (X = O, S, N) HBs have been widely discussed in the literature by Desiraju, among others.[33]

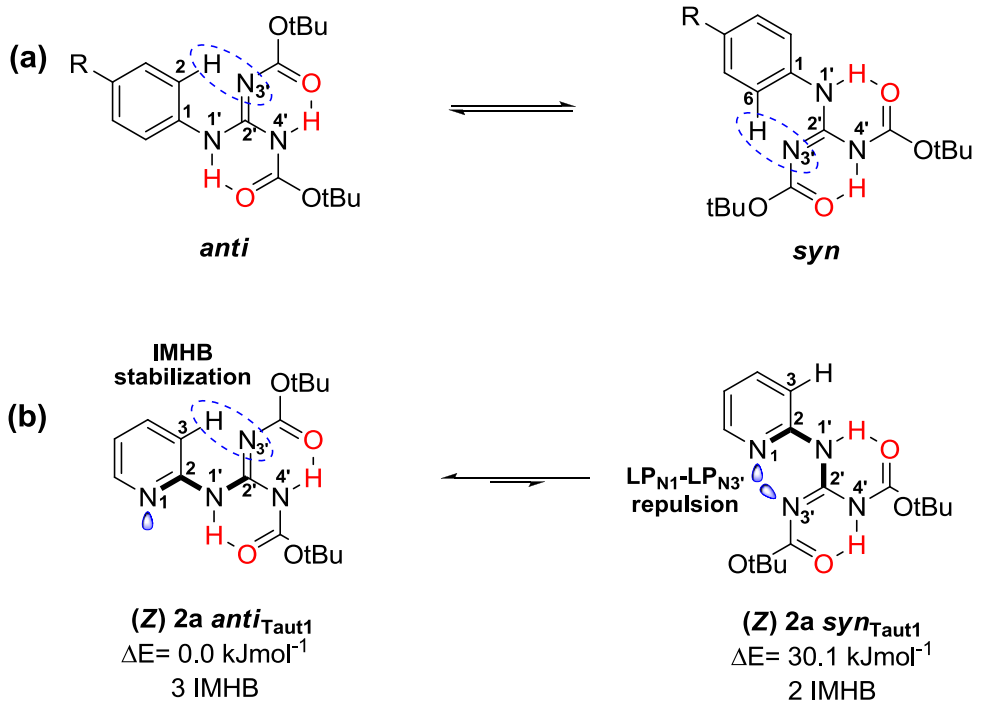


FIGURE 5. (a) Degenerate conformations for *N,N'*-di-Boc substituted phenylguanidines and (b) *anti* and *syn* conformations for Boc protected pyridin-2-ylguanidine **2a** (*Z* isomer, same tautomer 1), indicating the calculated relative energies (B3LYP/6-31+G**). Highlighted in bold the N1-C2-N1'-C2' dihedral angle and in red the NH···OC IMHB interaction.

Similarly, in the Boc protected pyridin-2-yl guanidines **2**, the conformational issue could be reduced to a rotation around the **N1-C2-N1'-C2'** dihedral angle. However, in this series **2**, the resulting *anti* and *syn* conformations are no longer degenerate (Figure 5b). In the *syn* conformation, a repulsive effect may exist between the **N1** and **N3'** lone pairs, while in the *anti* conformation a stabilising IMHB involving **N3'** and **H3** may exists [LP-N_{Gu}···$H3_{Py}$]. B3LYP/6-31+G** calculations performed for the *Z* isomer of compound **2a** (same tautomer 1) confirmed that the *anti* conformation [**(*Z*) 2a *anti*$_{Taut1}$**] was 30.1 kJ mol^{-1} more stable than the *syn* one [**(*Z*) 2a *syn*$_{Taut1}$**], which shows a twisted geometry (**N1-C2-N1'-C2'** dihedral angle = 53.0º) resulting from the **N1** and **N3'** lone pairs repulsion (Figure 5b).

As mentioned, the conformational analysis in series **2a** was systematically extended to all the possible isomers/tautomers/rotamers and, hence, several reasonably stable **2a** *syn* minimum energy geometries were found showing at least one of the previously described IMHBs, plus a second IMHB involving pyridine **N1** (Figure 6). These minima exhibited, in all cases, a co-planar arrangement of the pyridine and guanidine subunits, strongly supporting the existence of the postulated IMHBs (**N1-C2-N1'-C2'** dihedral angle ≈ 0.0º). However, their relative energies reveal that the tautomer 1 of the *Z* isomer in its *anti* conformation (compound **2a** ***anti***$_{\mathbf{Taut1}}$, Figure 5b) remains the most stable by a significant energetic margin (>15.0 kJ mol^{-1}). Therefore**,** these results strongly suggest that compounds in series **2** should preferentially adopt the *anti* conformation.

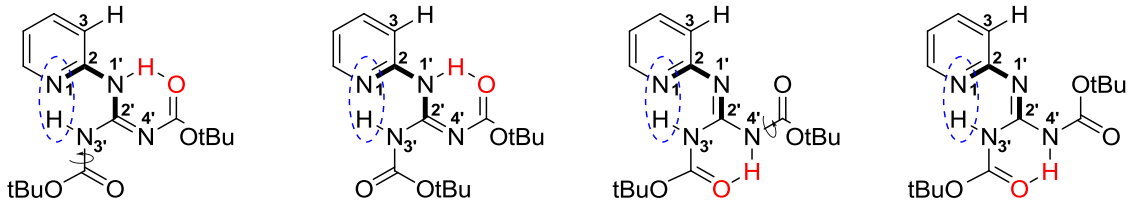


FIGURE 6. Relative energies, referred to the minimum energy structure [(*Z*) **2a** *anti*$_{\mathrm{Taut1}}$ in Figure 5b] calculated at B3LYP/6-31+G** level, for the most favourable energetic minima tautomers/rotamers selected in the *syn* geometry of compound **2a**. Highlighted in bold N1-C2-N1'-C2' dihedral angle and in red the NH···OC IMHB interaction.

Summarising, the Boc protected derivatives **2** exhibit a clear preference for the *anti* conformation, while the guanidinium salts **3** and the neutral 2-pyridinylguanidine exist

primarily in the *syn* conformation. In addition, the energetic differences computed between these two conformations seem large enough to allow predicting the predominant conformation across series **2** and **3** under experimental conditions.

X-Ray analysis and NMR studies of the new pyridin-2-yl guanidines

Confirmation of these computational predictions was achieved with the X-ray crystal structure of some of these pyridine-2-yl guanidine/ium derivatives (Figure 7). Thus, slow recrystallization from a mixture of hexane/EtOAc provided a crystal structure for the 5-chloro derivative of the di-Boc protected pyridin-2-yl guanidines (**2b**). This structure is planar, and the **N1-C2-N1'-C2'** dihedral angle is 180°, in agreement with the predicted *anti* conformation (Figure 7, **2b**). This crystal structure also reveals an extensive IMHB network involving the Boc **CO** and **N3'** lone pairs as HB acceptors and the **H1'**, **H4'** and **H3** as donors, as previously described in Figure 5b. Using the same solvent system, a second crystal structure was obtained for the di-Boc protected guanidine-4-ethyloxybenzene **4** (Figure 7), whose preparation has been described by us elsewhere[4] and which is structurally related to series **2**. The crystal structure obtained exhibited the *anti* conformation and bears similar features to those of its pyridine analogue **2b**. A summary of the most relevant distances and angles of these crystal structures is presented in Table 1. The HB distances found for the **O¨¨H1'** interaction (~1.9 Å) in both compounds are in agreement with very strong HBs while those distances found for the **N3'¨¨H3(C)** contacts (~2.3 Å) correspond to medium-weak HB interactions (see Table 1). However, the HB formed between **O¨¨H4'** is shorter for compound **4** than for compound **2b**, indicating that even though medium to very strong HBs are established in both cases the one corresponding to compound **4** is stronger.

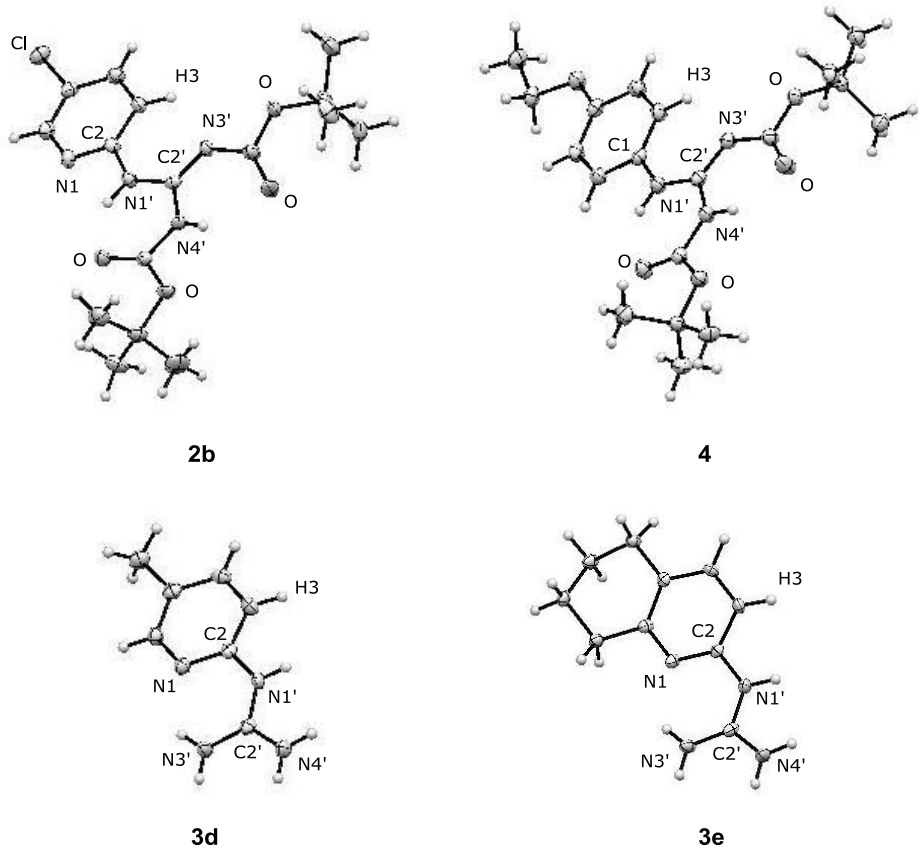


FIGURE 7. X-ray crystal structures of 2-[2,3-di-(*tert*-butoxycarbonyl)guanidino]-5-chloropyridine (**2b**), 1-[2,3-di-(*tert*-butoxycarbonyl)guanidino]-4-ethoxybenzene (**4**), *N*-(5-methylpyridin-2-yl)guanidinium chloride (**3d**) and *N*-(5,6,7,8-tetrahydroquinolin-2-yl)guanidinium chloride (**3e**).

In addition, crystals for two of the 1-(pyridin-2-yl)guanidinium chloride salts (**3d** and **3e**) were obtained, using a slow diffusion of diethyl ether in a cold methanolic solution, and resolved by X-ray crystallographic analysis (Figure 7). These structures **3d** and **3e** exhibit *syn* conformation featuring pyridine/guanidine co-planarity and an IMHB between **N1** and the **H3'/H4'** guanidine protons. In both compounds, the distances observed for the **N1¨H3'** interaction (Table 1) correspond to strong HBs.

Furthermore, looking at the three C-N bond distances within the guanidine/ium moieties, it was observed that in the neutral Boc-protected structures (**2b** and **4**) different values were observed ranging from clear single C-N bonds for **C2'-N3'** to C=N double bonds for **C2'-N4'** while **C2'-N1'** would be slightly shorter than a single bond indicating a degree of delocalisation. In the case of the guanidinium derivatives, **3d** and **3e**, the distances found for **C2'-N1'** and **C2'-N4'** are similar to the **C2'-N1'** in the Boc derivatives showing delocalisation. The **C2'-N3'** bond corresponding to the **N1···H3'** interaction was slightly shorter than the other two C-N bonds due to this IMHB. The relatively longest C-N bond corresponded, in both structures, to the **C2'-N1'** bond that connects to the pyridine system.

TABLE 1. Relevant HB distances (Å) and angles (°) found in the crystal structures of compounds **4**, **2b**, **3d** and **3e**.

	N3'···H3	**N3'···H3-C3**	**O···H1'**	**O···H1'-N1'**	**O···H4'**	**O···H4'-N4'**
4	2.314	120.9	1.950	139.7	1.872	133.8
2b	2.311	119.2	1.952	139.3	2.109	123.7
	C2'-N1'	**C2'-N3'**	**C2'-N4'**		**N1···H3'**	**N1···H3'-N3'**
4	1.339	1.385	1.315		-	-
2b	1.354	1.388	1.300		-	-
3d	1.343	1.315	1.326		2.091	129.8
3e	1.350	1.309	1.333		2.085	129.9

Curiously, in the crystal structure of *N,N'*-diphenylguanidine one of the C-N bonds connected to a phenyl ring show double bond characteristics while the other two (C-NH_2 and C-NHPh) appear as single bonds.[24] In the case of the neutral Boc derivatives **2b** and **4** it seems that the guanidine double bond is localised not over the N atom attached to the pyridine/phenyl ring but over the N atom connected to a Boc group not involved in hydrogen bonding with N1' (**C2'-N4'**). However, in the crystal structure of *N,N'*-dimethyl-*N,N'*-diphenylguanidine the C-N double bond is localized over the unsubstituted N atom,[24] similar to what is observed in our neutral guanidines. Regarding the hydrochloride salts **3d** and **3e** and the hydrobromide salt of *N,N'*-dimethyl-*N,N'*-diphenylguanidine,[24] very similar C-N distances are obtained for the three C-N bonds involved in the guanidinium group (between 1.31 and 1.38 Å)

Significantly, NMR experiments in solution supported the persistence of the conformational effects that have been theoretically predicted (DFT calculations) and experimentally observed in the solid state by X-ray crystallographic studies (Table 2).

In agreement with the *anti* conformation, the existence of an IMHB between **N3'** and **H3** in the di-Boc protected series (**2**) is strongly supported by the ^{1}H NMR data recorded for proton **H3** (Table 2). Throughout series (**2**), this signal is broadened and strongly shifted to high field (7.93 – 8.27 ppm). In contrast, the **H3** signal recorded for the guanidinium salt series (**3**) consistently appears as a sharp doublet at 6.80 – 7.13 ppm, which is much closer to that observed for the corresponding series of 2-aminopyridines (**1**) (6.20 – 6.45 ppm). As shown, this variation in the chemical shift of **H3** is significant even considering electronic effects.

TABLE 2. ^{1}H NMR shifts of series **1** – **3** obtained in DMSO-d_6. The difference in chemical shift with reference to the corresponding 2-aminopyridine is shown in brackets.

Compound	R	δH3	δH4	δH6	δH1'	δH3'	δH4'
1a	**H**	6.41	7.33	7.88	5.85		
1b	**Cl**	6.45	7.39	7.88	6.14		
1c	**Br**	6.42	7.49	7.94	6.16		
1d	**CH_3**	6.37	7.18	7.72	5.63		
1e	**-$(CH_2)_4$-**	6.20	7.03	-	5.53		
2a	**H**	8.22 (*+1.81*)	7.87 (*+0.54*)	8.32 (*+0.44*)	11.44		10.59
2b	**Cl**	8.27 (*+1.82*)	8.02 (*+0.63*)	8.38 (*+0.50*)	11.36		10.65
2c	**Br**	8.22 (*+1.80*)	8.13 (*+0.64*)	8.44 (*+0.50*)	11.36		10.64
2d	**CH_3**	8.11 (*+1.74*)	7.69 (*+0.51*)	8.15 (*+0.43*)	11.46		10.54
2e	**-$(CH_2)_4$-**	7.93 (*+1.73*)	7.53 (*+0.50*)	-	11.53		10.49
3a	**H**	7.07 (*+0.66*)	7.88 (*+0.55*)	8.32 (*+0.44*)	11.39	8.30[a]	
3b	**Cl**	7.13 (*+0.68*)	7.98 (*+0.59*)	8.35 (*+0.47*)	11.70	8.28[a]	
3c	**Br**	7.06 (*+0.64*)	8.09 (*+0.60*)	8.43 (*+0.49*)	11.53	8.23[a]	
3d	**CH_3**	6.98 (*+0.61*)	7.71 (*+0.53*)	8.15 (*+0.43*)	11.17	8.20[a]	
3e	**-$(CH_2)_4$-**	6.80 (*+0.60*)	7.55 (+0.52)	-	11.08	8.22[a]	

[a] **H3'** and **H4'** are chemically equivalent due to free rotation around the **C2'N1'**, **C2'N3'** and **C2'N4'** bonds.

The effect of the Boc-guanidine subunit upon chemical shift generally resembles that of the guanidinium cation (Figure 8). Focussing on the conformationally unaffected protons **H4** and **H6**, the average difference in chemical shift with reference to the starting 2-aminopyridine

changes little between the Boc-guanidine and guanidinium subunits (+0.57/+0.56 for **H4** and +0.47/+0.46 for **H6**, respectively). However in the case of **H3**, the average change in deshielding is considerably higher for the Boc-protected series **2** (+1.78) than for the guanidinium series **3** (+0.64); this marked discrepancy can be explained by the electronic effect of a hydrogen bonding interaction.

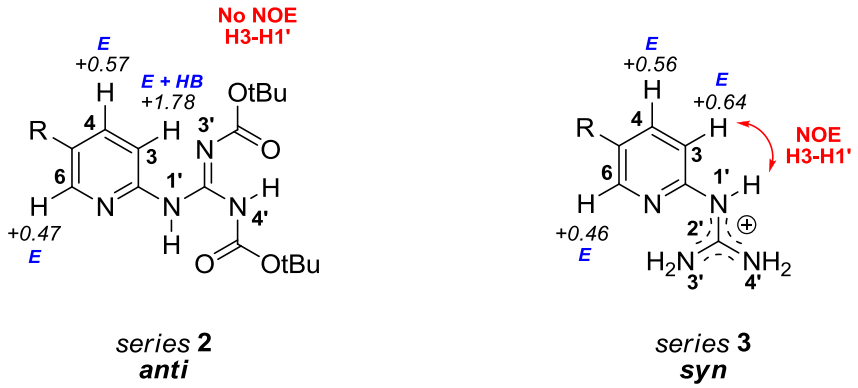


FIGURE 8. Average value of ^{1}H NMR deshielding in aromatic protons H3, H4 and H6 in series 2 and 3 with respect to the corresponding 2-aminopyridine 1. *E* and *HB* indicates *electronic* or *hydrogen bonding* effects, respectively.

The chemical inequivalence of the Boc groups in series **2** was confirmed by the occurrence of two separate signals for the *tert*-butyl groups. The CH_3 protons appeared as two singlets each integrating for nine protons at 1.43 – 1.44 and at 1.44 – 1.51 ppm, in agreement with the rigid, "pseudo bicyclic" structure suggested for the Boc-guanidine subunit. In the case of the Boc-guanidine 4-ethoxybenzene derivative **4**, for which a corroborating X-ray crystal structure was obtained, these signals appear at 1.44 and 1.50 ppm.

Further evidence of the conformational arrangement was available *via* the ^{1}H NMR signals of the guanidine **NH** protons. In the Boc protected series **2**, **H1'** and **H4'** are highly shifted

(11.36–11.53 and 10.49–10.65 ppm respectively) with respect to the starting amine, suggesting the participation of these protons in the HB network proposed for the *anti* conformation. In the *syn* conformation of the guanidinium series **3**, **H1'** is highly deshielded (11.08 – 11.70 ppm) due to its acidity induced by the adjacent pyridine ring; while **H3'** and **H4'** appear as a single signal at 8.22–8.28 ppm as they are chemically equivalent. This value is higher than the corresponding chemical shift in non-substituted guanidinium chlorides (7.07 ppm),[34] and this deshielding can be explained by the effect of the nearby pyridine nitrogen lone pair.

Nuclear Overhauser Effect (NOE) experiments further supported the postulated conformational preferences. A through-space interaction was observed between **H3** and **H1'** in the *N*-(pyridin-2-yl)guanidinium chlorides **3**, in agreement with the *syn* conformation. Conversely, the absence of an NOE signal for these same protons in the di-Boc protected series **2** suggests that they are not nearby in space, supporting the prevalence of the *anti* conformation.

Moreover, variable temperature ^{1}H NMR was carried out on compounds **2d** and **3d** in order to identify if equilibrium exists between the possible conformations in both series. Spectra were recorded in DMSO-d_6 at ten degree increments from room temperature to 80 °C and a final spectrum was recorded on re-cooling the sample to room temperature. Neither compound showed significant changes, the spectra indicating in each case that in fact a single conformer exists experimentally in solution, confirming our predictions. The characteristic downfield shift and broad nature of **H3** in compound **2d** was maintained throughout, as was the high field shift of the NH protons at 10.5 and 11.4 ppm (see Supporting Information). Some degradation of the compound was observed above 70 °C. The possibility that this was as a

result of another conformation of the same molecule was ruled out by its persistence on re-cooling the compound to room temperature. For compound **3d** no observable changes occurred on heating the solution (see Supporting Information). In particular, the high shift seen for the NH signals was maintained. No degradation of the compound was observed in this case. This leads us to conclude that the *syn* conformation for compounds **3** dominates in solution as well as in the solid state.

Based on these experiments, we have calculated the temperature dependence of the ^{1}H NMR chemical shifts of those hydrogens involved in the IHMB in compounds **2d** and **3d** (H3 and H3' respectively) as shown in Figure 9. It can be observed that compound **3d** experiences more internal hydrogen bonding than **2d** and both seem to show similar temperature dependence.

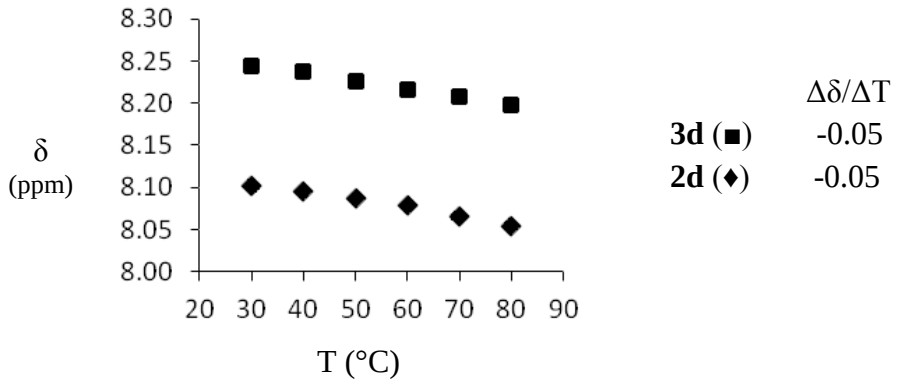


FIGURE 9. ^{1}H NMR chemical shifts of H3 (compound **2d**, ♦) and H3' (compound **3d**, ■) as a function of temperature (°C). The corresponding Δδ/ΔT coefficients are also shown

The Δδ/ΔT coefficients obtained (-0.05 for **2d** and **3d**) are larger than those reported by Gellman *et al*. for IMHBs in diamides[35] (from -0.0025 to -0.0100) suggesting that the IMHB

formed in both cases are solvent-exposed. It can be concluded that upon increasing the temperature the IMHBs have been slightly distorted since a small negative shift is observed for the corresponding signals and that this distortion is larger for compound **2d** than for **3d** in agreement with the strength of each of the IMHBs involved (C-H···N for **2d** which is weaker than N-H···N for **3d**).

Conformational control in N-Boc-N'-alkyl pyridin-2-yl guanidines

Considering the theoretical and experimental evidence so far obtained, it was hypothesised that substitution of one of the Boc protecting groups by an alkyl chain could suppress the rigid IMHB**s** network in series **2**, and might thereby induce a change from the *anti* to the *syn* conformation. Thus, a new series of *N*-Boc-*N'*-alkyl substituted pyridin-2-yl guanidines (**5**) was studied. As for the previous series, a systematic conformational study for **5a** was performed at B3LYP/6-31+G** level showing that, in agreement with our hypothesis, the *syn* conformation is >25.9 kJ mol^{-1} more stable than the best *anti* one (Figure 10). However, due to the asymmetry of this system, two different *syn*/*anti* energetic minima, **5a(i)** and **5a(ii)**, had to be analysed. In the case of the *syn* conformers these minima differ by only 5.0 kJ mol^{-1} suggesting the existence of two possible *syn* conformers under experimental conditions.

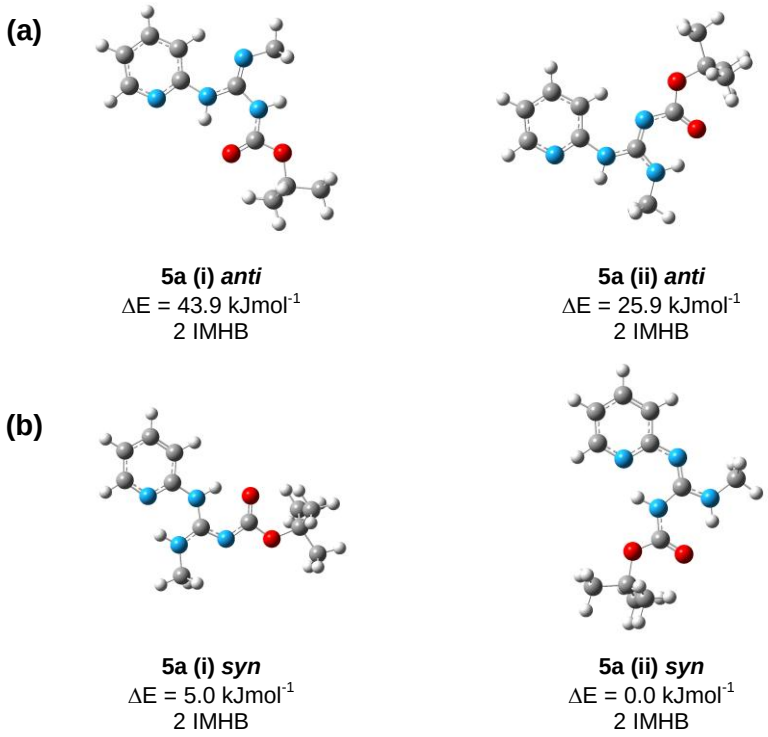


FIGURE 10. Energetic minima calculated for (a) the best *anti* conformation of *N*-Boc-*N'*-methyl-(pyridin-2-yl)guanidine **5a** and (b) the best *syn* conformation of the same molecule. To reduce the computational cost of these calculations, the propyl group present in the experimental compounds was replaced with a methyl group.

Compounds **5b** and **5d**, *N*-Boc-*N'*-propylpyridin-2-yl guanidine substituted analogues to **5a**, were experimentally prepared following a synthetic procedure recently developed within our research group.[36] The characterisation of these compounds confirmed the theoretical prediction that the molecules of series **5** can exist as a nearly equimolar mixture of two isomers (see Figure 11).

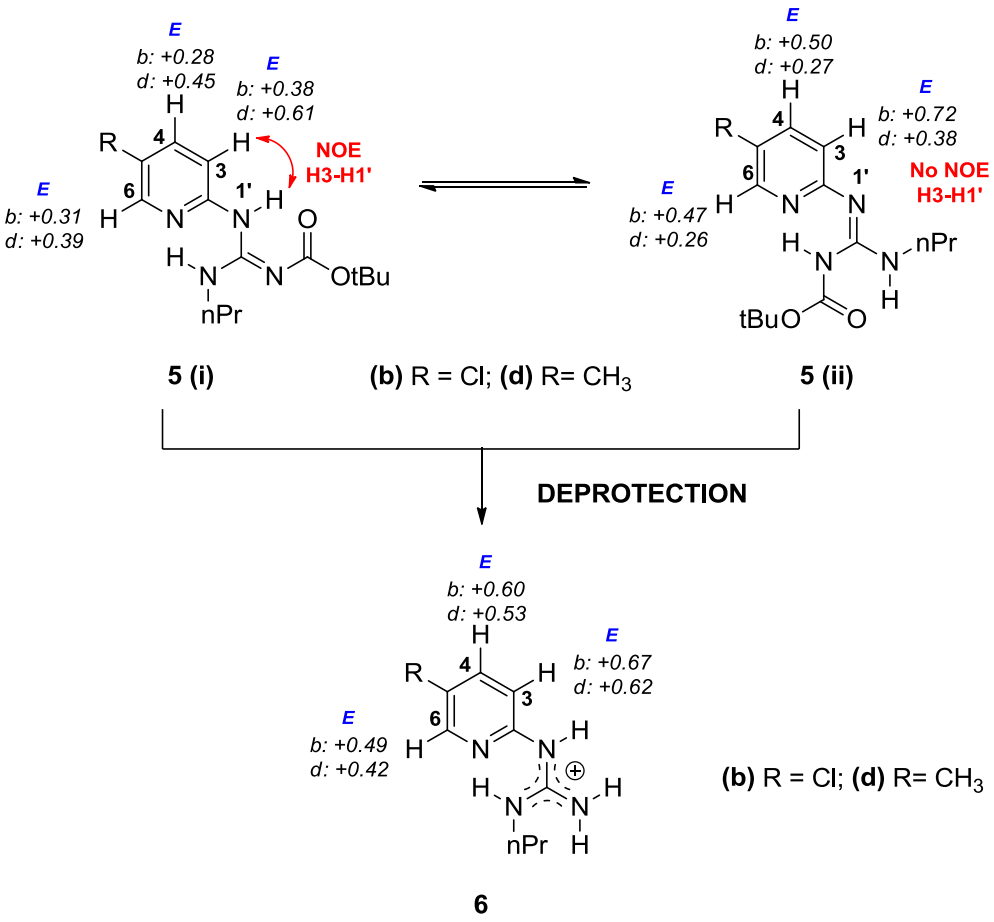


FIGURE 11. Isomers **(i)** and **(ii)** obtained for the *N*-Boc-*N'*-propylpyridin-2-yl guanidines **5b** and **5d**. Indicated are the ^{1}H NMR shifts of protons **H3**, **H4** and **H6** in compounds **5** and **6** with respect to the corresponding 2-aminopyridines **1**. *E* denotes *electronic* effects.

In the case of compound **5b** (R= Cl), a 3:4 mixture of isomers **(i)** and **(ii)** was formed, adjudged by ^{1}H NMR integration. These isomers proved to be inseparable by either column chromatography (silica gel) or by recrystallisation. For compound **5d** (R= CH_3) a 6:5 mixture of isomers **(i)** and **(ii)** was obtained, and, in this case, isolation of the **(i)** isomer was possible using silica gel column chromatography (9:1, Hexane:EtOAc). The similar isomeric ratios found in both compounds suggest little difference in their relative energies in agreement with our calculations. Temperature-dependent ^{1}H NMR experiments were carried out with the mixture of **5b** isomers in DMSO-d_6 and heating from 20 to 100 °C. It was observed that

signals corresponding to **5b(ii)** increased with temperature (up to 75 °C) until a 50:50 proportion with those of **5b(i)** indicating that the equilibrium was reached, and finally the compound decomposed at 100 °C. More importantly, the ^{1}H NMR spectra of both isomers did not show the **H3** shift characteristic of the *anti* conformation, but one closer to that of the starting 2-aminopyridines (Table 3), suggesting that it is uninvolved in IMHB interactions, in agreement with the *syn* conformation. These *syn* conformation in isomer **(i)** was further evidenced by the presence of NOE signals between **H1'** and **H3** and by the high-field shift recorded for **H1'**, as expected for an IMHB with a **CO** lone pair [**5b(i)**: 12.42 and **5d(i)**: 11.74 ppm]. These NOE signals were not observed in isomers **(ii)**, as expected.

TABLE 3. ^{1}H NMR (DMSO-d_6) shifts in compounds **5** and **6**.

Compound	Isomer	δH3	δH4	δH6	δH1'	δH3'	δH4'
5b (R= Cl)	**i**	6.83	7.67	8.19	12.42	-	7.89
	ii	7.17	7.89	8.35	-	11.89	9.74
5d (R= CH_3)	**i**	6.98	7.63	8.11	11.74	-	10.12
	ii	6.75	7.45	7.98	-	13.09	10.11
6b (R= Cl)		7.12	7.99	8.37	11.47	9.17	8.58
6d (R= CH_3)		6.99	7.71	8.14	11.34	9.43	8.59

The prevalence of the *syn* conformation in compounds **5** confirms that neither the *N*-Boc electronic effect, the unprotonated state of the molecules, nor the steric bulk around the guanidine subunit are sufficient to induce the *anti* conformation observed in the di-Boc

derivatives **2**. Thus, the conformational control in pyridin-2-yl guanidines remains firmly rooted in the IMHB interactions established between the guanidine and pyridine subunits.

As expected, cleavage of the Boc group in compounds **5**, results in the abolition of any isomeric mixture as shown the ^{1}H NMR of the corresponding *N*-propyl **6** derivatives, which exhibit the *syn* conformation (see Figure 10). It is worth noting that the introduction of an alkyl substituent in the protonated guanidinium **6** does not produce any significant variation in the chemical shift of protons **H3**, **H4** and **H6** with respect to the guanidiniums **3**.

CONCLUSIONS

During preparation of a new series of pyridin-2yl guanidine derivatives following our standard synthetic method, a striking difference between the ^{1}H-NMR spectra of the *N,N'*-di-Boc protected and guanidinium salt derivatives (series **2** and **3**, respectively) was observed. This difference remained across the series regardless of the substituent in position 5 of the pyridine ring; we therefore considered that this effect might result from conformational constraints. Hence, we have carried out a complete theoretical and experimental structural study using B3LYP/6-31+G** calculations, NMR spectroscopy and X-ray crystallographic analysis.

Considering the two possible conformational extremes (*anti* and *syn*), DFT theoretical studies yielded two main conclusions. First, the Boc protected derivatives **2** exhibit a preference for the *anti* conformation, while the 2-pyridinoguanidine and the guanidinium salts **3** exist primarily in the *syn* conformation. Second, the computations suggested the formation of two IMHB**s** involving the Boc carbonyl groups and guanidine hydrogens, which confer rigidity to

the *N,N'*-di-Boc substituted guanidine system in series **2**, and in the final guanidinium salts (series **3**) another IMHB is suggested to be formed between the pyridine nitrogen and protons **H3'** [LP-$N_{Py}\cdots H3'_{Gu}$] in the guanidinium moiety.

X-ray crystal structures obtained for two of the 1-(pyridin-2-yl)guanidinium chloride salts (**3d** and **3e**) confirm the existence of the *syn* conformation and demonstrate a co-planar arrangement of the pyridine and guanidinium subunits, and the existence of a IMHB interaction between the pyridine nitrogen and guanidine protons. Moreover, the X-ray crystal structures of the *N,N'*-di-Boc protected pyridin-2-yl guanidine **2b** (R= Cl) and its phenyl analogue **4** (R= OEt) confirm the existence of an extensive IMHB network involving the adjoining carmabate and guanidine groups.

Further, solution-phase ^{1}H NMR, variable temperature and NOE experiments confirm that the conformational preferences suggested by our computational studies and found in solid-state X-ray crystallographic studies are maintained in solution.

Finally, to verify that conformational control results from IMHB interactions and not from electronic or steric effects, a new series of *N*-Boc-*N'*-propyl substituted pyridin-2-yl guanidines **5** has been studied. B3LYP/6-31+G** studies predicted a shift from the *anti* to the *syn* geometry in these new derivatives. Preparation of the corresponding derivatives **5b** and **5d** (R= Cl and CH_3) followed by Boc deprotection to yield the propyl guanidinium salts (**6b** and **6d**) allowed us to confirm the existence of the predicted *syn* geometry by means of ^{1}H NMR studies. Hence, conformational control in pyridin-2-yl guanidines seems to be entirely dependent on the IMHB interactions within the guanidine group (in the case of the *N,N'*-di-Boc derivatives), and between the guanidine and pyridine subunits (in the guanidinium salts).

EXPERIMENTAL SECTION

Geometries were fully optimised at the B3LYP theoretical level with the 6-31+G** basis set as implemented in the Gaussian03 program.[37] Harmonic frequency calculations verified the nature of the stationary points as minima (all real frequencies) or transition states (one imaginary frequency). The scanning of the rotation was performed using the IRC type calculation implemented in the Gaussian03 program at the same level. To reduce the computational cost, the initial optimisation of all possible tautomers, isomers and rotamers of the di-Boc-protected and *N*-Boc-*N'*-alkyl pyridin-2-yl guanidine derivatives (**2a** and **5a** respectively) was performed using an *N*-acetyl group in place of the *N*-Boc group. The most significant conformers (only those structures with relative energies within 30 kJ/mol from the minimum) were then optimised using the full *N*-Boc structure. The complete data regarding these preliminary calculations are available in the Supporting Information.

A suitable crystal from each compound **2b**, **4**, **3d** and **3e** was selected and mounted using inert oil on a 0.3 mm diameter glass fiber tip and placed on the goniometer head in a 123K N2 gas stream. Non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were placed into geometrically calculated positions and refined using a riding model. Temperature was 123 K in all cases. The agreement between the data and the model (R1) for all structures was 5% or below.

Spectra were recorded on a spectrometer operating at 600.1 MHz for ^{1}H NMR and 150.9 MHz for ^{13}C NMR. NMR peak assignments were confirmed using the following COSY and ROESY experiments: Phase-sensitive gradient enhanced (ge) 2D multiplicity-edited HSQC using PEP and adiabatic pulses with gradients in back-inept (hsqcedetgpsisp2), Phase-

sensitive ge-2D HMBC using a two-fold low-pass J-filter (hmbcetgpl2nd), and selective gradient enhanced 1D ROESY (selrogp).

General procedure for the synthesis of di-Boc protected guanidine derivatives: To a solution of starting amine (1.0 eq), *N*,*N*′-*bis*-(*tert*-butoxycarbonyl)-*S*-methylisothiourea (1.1 eq), and triethylamine (3.5 eq) in CH_2Cl_2 at 0 ^{0}C was added mercuric chloride (1.1 eq). The mixture was stirred for 30 min at 0 ^{0}C, then warmed to RT and stirred until reaction was adjudged complete by TLC analysis. The reaction mixture was diluted with EtOAc and filtered through a pad of Celite to remove any mercury by-products. The filtrate was washed with brine (20 mL) and water (20 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield a residue that was purified by silica gel column chromatography, eluting with the appropriate hexane:EtOAc mixture.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]pyridine (2a): Clear crystalline solid (282 mg, 79%); mp 138-140 °C, clean melt. ^{1}H NMR (400 MHz, DMSO) δ 1.44 (s, 9H, CH_3), 1.51 (s, 9H, CH_3), 7.16 (app. t, 1H, J = 5.8, 5.6 Hz, Ar), 7.87 (app.t, 1H, J = 7.2, 7.7 Hz, Ar), 8.22 (d, 1H, J = 7.9 Hz, Ar), 8.32 (d, 1H, J = 3.8 Hz, Ar), 10.59 (broad s, 1H, NH), 11.44 (broad s, 1H, NH). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.53 (s, 18H, CH_3), 7.01 (app. t, 1H, J = 5.4 Hz), 7.70 (app. t, 1H, J = 7.3 Hz), 8.29 (d, 1H, J = 4.0 Hz), 8.37 (broad s, 1H), 10.89 (broad s, 1H, NH), 11.53 (broad s, 1H, NH). ^{13}C NMR (150 MHz, $CDCl_3$) δ 28.0 (CH_3), 28.1 (CH_3), 79.9 (quat. ^{t}Bu), 83.9 (quat. ^{t}Bu), 116.0 (CH Ar), 119.7 (quat. Ar), 138.1 (CH Ar), 148.0 (CH Ar), 150.6 (quat. Ar), 152.6 (quat. C=O), 153.1 (quat. C=O), 163.2 (quat. C=N). IR (cm^{-1}) υ 3253 (NH), 2979 (NH), 1725 (C=N, Gua), 1621 (C=O, Boc), 1586, 1570, 1479, 1398, 1368, 1332, 1302, 1293, 1256, 1229, 1147, 1121, 1089, 1030, 994, 883, 848, 809, 763, 733, 710, 669. HRMS (*m/z* ESI$^+$): 337.1876 calcd for $C_{16}H_{24}N_4O_4$ $[M + H]^+$, found 337.1882.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-5-chloropyridine (2b): White solid (433 mg, 75%); mp 124 °C, clean melt. ^{1}H NMR (400 MHz, DMSO) δ 1.44 (s, 9H, CH_3), 1.50 (s, 9H, CH_3), 8.02 (dd, 1H, J = 8.8, 2.3 Hz, Ar), 8.27 (d, 1H, J = 8.7 Hz, Ar), 8.38 (d, 1H, J = 2.3 Hz, Ar), 10.65 (broad s, 1H, NH), 11.36 (broad s, 1H, NH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 9H, CH_3), 1.54 (s, 9H, CH_3), 7.67 (dd, 1H, J = 8.9, 2.6 Hz, Ar), 8.24 (d, 1H, J = 2.6 Hz, Ar), 8.40 (d, J = 8.9 Hz, Ar), 10.92 (broad s, 1H, NH), 11.51 (broad s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.0 (CH_3), 28.1 (CH_3), 80.1 (quat. ^{t}Bu), 84.1 (quat. ^{t}Bu), 116.8 (CH Ar), 126.9 (quat. Ar), 137.7 (CH Ar), 146.6 (CH Ar), 148.9 (quat. Ar), 152.6 (quat. C=O), 152.9 (quat. C=O), 163.0 (quat. Gua). IR (cm^{-1}) υ 3249 (NH), 2981 (NH), 1741 (C=N), 1716, 1633 (C=O), 1576, 1559, 1476, 1455, 1407, 1383, 1367, 1322, 1287, 1252, 1235, 1219, 1142, 1123, 1101, 1059, 1028, 1006, 967, 917, 880, 848, 837, 801, 782, 743, 728, 685. HRMS (*m/z* ESI^{+}): 371.1486 calcd for $C_{16}H_{23}ClN_4O_4$ $[M + H]^{+}$, found 371.1489.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-5-bromopyridine (2c): White solid (383 mg, 80%); mp 126 °C, clean melt. ^{1}H NMR (400 MHz, DMSO) δ 1.44 (s, 9H, CH_3), 1.50 (s, 9H, CH_3), 8.13 (dd, 1H, J = 8.9, 1.9 Hz, Ar), 8.22 (d, 1H, J = 8.9 Hz, Ar), 8.44 (d, 1H, J = 1.9 Hz, Ar), 10.64 (broad s, 1H, NH), 11.36 (broad s, 1H, NH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 18H, CH_3), 7.75 (dd, J = 8.7, 1.3 Hz, Ar), 8.28 (d, 1H, J = 2.1 Hz, Ar), 8.31 (d, 1H, J = 8.3 Hz, Ar), 10.86 (broad s, 1H, NH), 11.49 (broad s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.8 (CH_3, Boc), 27.9 (CH_3, Boc), 79.9 (quat. ^{t}Bu), 83.9 (quat. ^{t}Bu), 114.7 (CH Ar), 117.2 (quat. Ar), 140.3 (CH Ar), 148.6 (CH Ar), 149.1 (quat. Ar), 152.5 (quat. C=O), 152.7 (quat. C=O), 162.8 (quat. Gua). IR (cm^{-1}) υ 3247 (NH), 2980 (NH), 1715 (C=N, Gua), 1632 (C=O, Boc), 1593, 1553, 1453, 1404, 1367, 1324, 1287, 1252, 1232, 1148, 1129, 1101, 1057, 1028,

1004, 878, 841, 793, 745. HRMS (*m/z* ESI^+): 415.0981 calcd for $C_{16}H_{23}BrN_4O_4$ $[M + H]^+$, found 415.0990.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-5-methylpyridine (2d): White crystalline solid (460 mg, 71%); mp 139 °C, clean melt. ^1H NMR (400 MHz, DMSO) δ 1.44 (s, 9H, CH_3), 1.51 (s, 9H, CH_3), 7.69 (d, 1H, J = 8.4 Hz, Ar), 8.11 (d, 1H, J = 8.4 Hz, Ar), 8.15 (s, 1H, Ar), 10.54 (broad s, 1H, NH), 11.46 (broad s, 1H, NH). ^1H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H, CH_3), 1.49 (s, 9H, CH_3), 2.23 (s, CH_3), 7.45 (dd, 1H, J = 8.4, 1.9 Hz, Ar), 8.07 (d, 1H, J = 1.9 Hz, Ar), 8.20 (broad s, 1H, Ar), 10.78 (broad s, 1H, NH), 11.52 (broad s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8 (CH_3), 28.0 (CH_3), 28.1 (CH_3), 79.7 (quat. ^{t}Bu), 83.7 (quat. ^{t}Bu), 115.6 (CH Ar), 129.1 (quat. Ar), 138.6 (CH Ar), 147.9 (CH Ar), 148.4 (quat. Ar), 152.7 (quat. C=O), 153.0 (quat. C=O), 163.3 (quat. C=N). IR (cm^{-1}) υ 3244 (NH), 2978 (NH), 1720 (C=N), 1632 (C=O), 1585, 1560, 1475, 1454, 1404, 1374, 1324, 1305, 1289, 1268, 1252, 1230, 1151, 1136, 1107, 1058, 1025, 882, 856, 838, 803, 758, 749, 709. HRMS (*m/z* ESI^+): 351.2032 calcd for $C_{17}H_{26}N_4O_4$ $[M + H]^+$, found 351.2036.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-5,6,7,8-tetrahydroquinoline (2e): Brown solid (302 mg, 83%); mp 164-165 °C, clean melt. ^1H NMR (400 MHz, DMSO) δ 1.43 (s, 9H, CH_3), 1.44 (s, 9H, CH_3), 1.73 (m, 2H, CH_2), 1.80 (m, 2H, CH_2), 2.71 (m, 2H, CH_2), 2.68 (m, 2H, CH_2), 7.53 (d, 1H, J = 8.7 Hz, Ar), 7.93 (d, 1H, J = 8.2 Hz, Ar), 10.49 (broad s, 1H, NH), 11.53 (broad s, 1H, NH). ^1H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 18H, CH_3), 1.78 (m, 2H, CH_2), 1.84 (m, 2H, CH_2), 2.70 (t, 2H, J = 6.3, 6.1 Hz, CH_2), 2.78 (t, 2H, J = 6.1, 6.3 Hz, CH_2), 7.35 (d, 1H, J = 8.3 Hz, Ar), 8.04 (broad s, 1H, Ar), 10.64 (broad s, 1H, NH), 11.54 (broad s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.7 (CH_2), 22.9 (CH_2), 28.00 (CH_3), 28.02 (CH_2), 28.04 (CH_3), 32.0 (CH_2), 79.6 (quat, ^{t}Bu), 83.6 (quat, ^{t}Bu), 113.6 (CH Ar), 128.3 (quat. Ar),

138.7 (CH Ar), 147.6 (quat. Ar), 152.6 (quat. C=O), 152.0 (quat. C=O), 155.4 (quat. Ar), 163.3 (quat. Gua). IR (cm^{-1}) υ 3251 (NH), 2979 (NH), 1714 (C=N, Gua), 1645 (C=O), 1629, 1588, 1565, 1449, 1395, 1369, 1354, 1325, 1313, 1279, 1247, 1231, 1151, 1112, 1058, 1029, 996, 941, 899, 874, 864, 840, 804, 755, 713. HRMS (*m/z* ESI^+): 391.2345 calcd for $C_{20}H_{30}N_4O_4$ $[M + H]^+$, found 391.2342.

1-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-4-ethoxybenzene (4): White solid (330 mg, 76%); mp 118 - 120 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, 3H, J = 7.1 Hz, CH_3), 1.51 (s, 9H, CH_3), 1.56 (s, 9H, CH_3), 4.03 (q, 2H, J = 7.1 Hz, CH_2), 6.87 (d, 2H, J = 8.7 Hz, Ar), 7.47 (d, 2H, J = 8.7 Hz, Ar) 10.20 (br s, 1H, NH), 11.67 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4 (CH_3), 27.6 (CH_3), 27.8 (CH_3), 63.1 (CH_2), 79.0 (CH_3), 83.1 (CH_3), 114.2 (CH Ar), 123.4 (CH Ar), 129.2(quat. Ar), 152.9 (quat. Ar), 153.2 (quat. C=O), 155.7 (quat. C=O), 163.2 (quat. Gua). IR (cm^{-1}) υ 3280 (NH), 3165 (NH), 2933, 1716 (C=N), 1630 (C=O), 1605, 1573, 1511 (Aryl), 1390, 1345, 1227, 1155, 1117, 1057. HRMS (*m/z* ESI^+): 380.2185 calcd for $C_{19}H_{29}N_3O_5Na$ $[M + Na]^+$, found 380.2181.

General procedure for the synthesis of *N*-Boc-*N'*-propyl guanidine derivatives: To a solution of *N-tert*-butoxycarbonyl-*N'*-propylthiourea (**7**, described below), the starting amine (1.0 eq), and triethylamine (3.5 eq) in CH_2Cl_2 at 0 ^0C was added mercuric chloride (1.2 eq). The mixture was stirred for 30 min at 0 ^0C, then warmed to RT and stirred until reaction was adjudged complete by TLC analysis. The reaction mixture was diluted with EtOAc and filtered through a pad of Celite to remove any mercury by-products. The filtrate was washed with brine (20 mL) and water (20 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield a residue that was purified by silica gel column chromatography, eluting with the appropriate hexane:EtOAc mixture.

***N*-*Tert*-butoxycarbonyl-*N'*-propylthiourea (7):** To a solution of thiourea (500 mg, 6.58 mmol) in dry THF (120 mL) at 0 °C was added NaH (60% dispersion in mineral oil, 1184 mg, 29.60 mmol), followed by di-*tert*-butyl dicarbonate (3155 mg, 14.47 mmol). After 8 h stirring at RT, the reaction was cooled again to 0 °C and a second portion of NaH (442 mg, 11.05 mmol) was added followed 1 h later by trifluoroacetic anhydride (1.41 mL, 10.13 mmol). After 45 min, 1-propylamine (0.83 mL, 10.13 mmol) was added neat the reaction was stirred at RT for 18 h. The reaction was quenched with dropwise H_2O (20 mL) followed by extraction with EtOAc (3 x 20 mL). The organic phase was dried over $MgSO_4$, solvents were removed under vacuum, and the crude product was purified by flash chromatography (4:1 Hexane:EtOAc, Rf 0.3). Recrystallisation from hexanes afforded the product as a white, crystalline solid (1019 mg, 71%); mp 58 - 60 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (t, 2H, J = 7.3 Hz, $CH_2CH_2C\underline{H}_3$), 1.52 (s, 9H, Boc), 1.71 (q, 2H, J = 7.3 Hz, $CH_2C\underline{H}_2CH_3$), 3.63 (m, 2H, $C\underline{H}_2CH_2CH_3$), 7.97 (br s, 1H, NH), 9.74 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO) δ 11.3 ($CH_2CH_2\underline{C}H_3$), 21.5 ($CH_2\underline{C}H_2CH_3$), 27.8 (CH_3 Boc), 47.1 ($\underline{C}H_2CH_2CH_3$), 83.4 (Cq Boc), 151.8 (C=O), 179.4 (C=S). IR (cm^{-1}) υ 3245, 3175 (NH), 2961, 2934, 2875, 1720 (C=O), 1523 (C=N), 1243, 1206, 1142 (C=S), 1073, 1008. HRMS (*m/z* ESI^+): 219.1167 calcd for $C_9H_{19}N_2O_2S$ $[M + H]^+$, found 219.1167.

1-[2-(*Tert*-butoxycarbonyl)-3-propylguanidino]-5-chloropyridine (5b): Clear gum (247 mg, 39%, 4:3 mixture of isomers); ^{1}H NMR (600 MHz, DMSO) δ 0.91 (m, 6H, CH_3), 1.43 (s, 9H, CH_3), 1.48 (s, 9H, CH_3), 1.56 (m, 4H, CH_2), 3.31 (m, 4H, CH_2), 6.83 (d, 1H, J = 8.8 Hz, Ar), 7.17 (d, 1H, J = 8.0 Hz, Ar), 7.67 (dd, 1H, J = 8.8, 2.6 Hz, Ar), 7.89 (dd, 1H, J = 8.8, 2.5 Hz, Ar), 7.89 (broad s, 1H, NH), 8.19 (d, 1H, J = 2.6 Hz, Ar), 8.35 (d, 1H, J = 2.5 Hz, Ar),

9.74 (broad s, 1H, NH), 11.89 (broad s, 1H, NH), 12.42 (broad s, 1H, NH). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.00 (m, 4H, CH_3), 1.53 (s, 9H, CH_3), 1.54 (s, 9H, CH_3), 1.65 (app. sex, 4H, J = 7.3 Hz, CH_2), 3.41 (broad s, 2H, CH_2), 3.49 (m, 2H, J = 7.0, 5.5 Hz, CH_2), 6.82 (d, 1H, J = 8.8 Hz, Ar), 6.88 (broad s, 1H, Ar), 7.46 (d, 1H, J = 6.9 Hz, Ar), 7.60 (dd, 1H, J = 8.8, 2.3 Hz, Ar), 7.87 (broad s, 1H, NH), 8.12 (broad s, 1H, Ar), 8.17 (d, 1H, J = 2.3 Hz, Ar), 9.77 (broad s, 1H, NH), 12.28 (broad s, 1H, NH), 12.62 (broad s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5 (CH_2), 28.2 (CH_3), 28.4 (CH_3), 42.6 (CH_2), 78.8 (quat. ^{t}Bu), 114.2 (CH Ar), 121.8 (CH Ar), 122.8 (quat. Ar), 124.9 (quat. Ar), 137.6 (CH Ar), 138.4 (CH Ar), 143.8 (CH Ar), 144.5 (CH Ar), 149.8 (quat. Ar), 151.4 (quat. Ar), 153.7 (quat. C=O), 157.2 (quat. C=O), 160.1 (quat. Gua), 164.4 (quat. Gua). IR (cm^{-1}) υ 3359 (NH), 2968 (NH), 1730 (C=N), 1645 (C=O), 1585, 1560, 1476, 1455, 1406, 1380, 1329, 1322, 1288, 1259, 1234, 1170, 1156, 1120, 1032, 980, 832, 799, 734, 689. HRMS (*m/z* ESI^{+}): 335.1251 calcd for $C_{14}H_{21}ClN_4O_2$ $[M + Na]^{+}$, found 335.1246.

1-[2-(*Tert*-butoxycarbonyl)-3-propylguanidino]-5-methylpyridine (5d): Column chromatography (5:1 Hexane:EtOAc) on the mixture of isomers 5di and 5dii, followed by recrystallisation (Hexane/EtOAc) yielded **5d** as a white powder (75.0 mg, 28%, single isomer); mp 102-104 °C, clean melt; ^{1}H NMR (400 MHz, DMSO) δ 0.91 (t, 3H, J = 7.4 Hz, CH_3), 1.42 (s, 9H, CH_3), 1.54 (m, 2H, CH_2), 2.24 (s, 3H, CH_3), 3.31 (m, 2H, CH_2), 6.98 (d, 1H, J = 8.2 Hz, Ar), 7.63 (d, 1H, J = 8.2 Hz, Ar), 8.11 (s, 1H, Ar), 10.12 (broad s, 1H, NH), 11.74 (broad s, 1H, NH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 7.4 Hz, CH_3), 1.51 (s, 9H, CH_3), 1.62 (app. sex, 2H, J = 7.6, 7.2 Hz, CH_2), 2.24 (s, 3H, CH_3), 3.46 (m, 2H, CH_2), 6.72 (d, 1H, J = 8.4 Hz, Ar), 7.42 (dd, 1H, J = 8.4, 2.1 Hz, Ar), 10.17 (broad s, 1H, NH), 11.97 (broad s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.5 (CH_3), 17.5 (CH_3), 22.5 (CH_2), 28.4 (CH_3), 42.4 (CH_2), 78.4 (quat. ^{t}Bu), 112.7 (CH Ar), 126.7 (quat. Ar), 139.2 (CH Ar),

145.3 (CH Ar), 150.9 (quat. Ar), 157.5 (quat. C=O), 164.2 (quat. Gua). IR (cm^{-1}) υ 3355 (NH), 2965 (NH), 2930, 2875, 1712 (C=N), 1638, 1597 (C=O), 1562, 1495, 1474, 1347, 1300, 1245, 1172, 1154, 1125, 1056, 1026, 961, 909, 821, 805, 774, 739, 665. HRMS (*m/z* ESI^+): 315.1797 calcd for $C_{15}H_{24}N_4O_2$ $[M + Na]^+$, found 315.1792.

General Procedure for the synthesis of guanidinium chloride salts: To starting 1-[2,3-di-Boc-guanidino]pyridine (1.0 eq) was added slowly a solution of trifluoroacetic acid (TFA) in CH_2Cl_2 (25.0 eq, 25% v/v). Stirring at RT was continued until the reaction was adjudged complete by TLC analysis (≤ 3 h). Solvent and excess TFA were removed under reduced pressure to yield the trifluoroacetate salt as an off-white solid. This was dissolved in H_2O, to which Amberlite IRA-400 resin in its chloride form (excess) was added. The mixture was stirred for 36 h at room temperature after which the Amberlite resin was removed by vacuum filtration. The aqueous layer was washed with DCM and concentrated under reduced pressure to yield an off-white solid, which was purified by small-scale reverse-phase chromatography using C-8 silica with 100% H_2O as mobile phase. Conversion to the chloride salt was checked by ^{19}F NMR and if necessary stirring in Amberlite was repeated.

***N*-(Pyridin-2-yl)guanidinium chloride (3a):** Orange/white solid (46 mg, 81%); mp 78 °C, clean melt. ^1H NMR (600 MHz, DMSO) δ 7.07 (d, 1H, J = 8.2 Hz, Ar), 7.18 (dd, 1H, J = 6.9, 5.4 Hz, Ar), 7.88 (app t, 1 H, Ar), 8.30 (broad s, 4H, NH_2), 8.32 (d, 1H, J = 4.1 Hz, Ar), 11.39 (broad s, 1H, NH). ^{13}C NMR (150 MHz, DMSO) δ 113.2 (CH Ar), 119.3 (quat. Ar), 139.5 (CH Ar), 146.7 (CH Ar), 151.9 (quat. Ar), 155.3 (quat. CN). IR (cm^{-1}) υ 3312 (NH), 3180 (NH), 3130 (NH), 1679 (C=N), 1622, 1596, 1561, 1462, 1416, 1319, 1274, 1244, 1154, 1054, 1020, 998, 874, 775. HRMS (*m/z* ESI^+): 137.0827 calcd for $C_6H_8N_4$ $[M + H]^+$, found 137.0827. Purity by HPLC: 97.2% (*t*R 16.81 min).

***N*-(5-Chloropyridin-2-yl)guanidinium chloride (3b):** Oily white solid (95 mg, 85%); mp 164 °C, clean melt. ^{1}H NMR (600 MHz, DMSO) δ 7.13 (d, 1H, J = 8.8 Hz, Ar), 7.98 (dd, 1H, J = 8.8, 2.6 Hz, Ar), 8.28 (broad s, 1H, NH), 8.35 (d, 1H, J = 2.6 Hz, Ar), 11.70 (broad s, 1H, NH). ^{13}C NMR (150 MHz, DMSO) δ 115.0 (CH Ar), 126.6 (quat. Ar), 139.2 (CH Ar), 144.7 (CH Ar), 151.0 (quat. Ar), 155.7 (quat. CN). IR (cm^{-1}) υ 3313 (NH), 3178 (NH), 2953, 1685 (C=N), 1617, 1587, 1551, 1465, 1364, 1310, 1274, 1236, 1136, 1113, 1022, 1009, 924, 876, 828, 757, 734, 718. HRMS (*m/z* ESI^{+}): 171.0437 calcd for $C_6H_7ClN_4$ $[M + H]^{+}$, found 171.0433. Purity by HPLC: 99.0% (*t*R 22.68 min).

***N*-(5-Bromopyridin-2-yl)guanidinium chloride (3c):** Oily white solid (118 mg, 74%); mp 74-81 °C. ^{1}H NMR (600 MHz, DMSO) δ 7.06 (d, 1H, J = 8.8 Hz, Ar), 8.09 (dd, 1H, J = 8.8, 2.5 Hz), 8.23 (broad s, 1H, NH), 8.43 (d, 1H, J = 2.5 Hz, Ar), 11.53 (broad s, 1H, NH). ^{13}C NMR (150 MHz, DMSO) δ 114.7 (quat. Ar), 116.1 (CH Ar), 142.9 (CH Ar), 148.1 (CH Ar), 151.7 (quat. Ar), 155.9 (quat. CN). IR (cm^{-1}) υ 3313 (NH), 3218 (NH), 3011 (NH), 1684.0 (C=N), 1618, 1582, 1551, 1465, 1360, 1308, 1275, 1234, 1137, 1094, 1006, 925, 874, 825, 732. HRMS (*m/z* ESI^{+}): 214.9932 calcd for $C_6H_7BrN_4$ $[M + H]^{+}$, found 214.9926. Purity by HPLC: 99.1% (*t*R 23.88 min).

***N*-(5-Methylpyridin-2-yl)guanidinium chloride (3d):** Clear crystalline solid (94 mg, 84%); mp 188-192 °C, clean melt. ^{1}H NMR (600 MHz, DMSO) δ 2.27 (s, 3H, CH_3), 6.98 (d, 1H, J = 8.3 Hz, Ar), 7.71 (dd, 1H, J = 8.3, 1.9 Hz, Ar), 8.15 (broad s, 1H, Ar), 8.21 (broad s, 4, NH), 11.17 (broad s, 1H, NH). ^{13}C NMR (150 MHz, DMSO) δ 17.1 (CH_3), 112.7 (CH Ar), 128.5 (quat. Ar), 140.1 (CH Ar), 146.1 (CH Ar), 149.7 (quat. Ar), 155.2 (quat. CN). IR (cm^{-1}) υ

3268 (NH), 2889 (NH), 1677 (C=N), 1621, 1601, 1563, 1489, 1376, 1315, 1285, 1242, 1086, 1035, 1023, 1002, 909, 873, 832, 798, 738, 718. HRMS (*m/z* ESI^+): 151.0984 calcd for $C_7H_{10}N_4$ $[M + H]^+$, found 151.0979. Purity by HPLC: 98.3% (*t*R 21.57 min).

***N*-(5,6,7,8-Tetrahydroquinolin-2-yl)guanidinium chloride (3e):** Brown solid (71 mg, 85%); mp 224-228 °C (decomposed). ^1H NMR (600 MHz, DMSO) δ 1.73 (m, 2H, J = 5.8, 6.0 Hz, CH_2), 1.80 (m, 2H, J = 6.1, 5.9 Hz, CH_2), 2.68 (app. t, 2H, J = 6.2, 6.0 Hz, CH_2), 2.78 (app. t, 2H, J = 6.1, 6.0 Hz, CH_2), 6.80 (d, 1H, J = 8.2 Hz, Ar), 7.55 (d, 1H, J = 8.2 Hz, Ar), 8.22 (broad s, 4H, NH), 11.08 (broad s, 1H, NH). ^{13}C NMR (150 MHz, DMSO) δ 22.1 (CH_2), 22.2 (CH_2), 27.2 (CH_2), 31.5 (CH_2), 110.5 (CH Ar), 127.3 (quat. Ar), 140.1 (CH Ar), 149.2 (quat. Ar), 154.0 (quat. Ar), 155.2 (quat. CN). IR (cm^{-1}) υ 3323 (NH), 3149 (NH), 2961 (NH), 1679 (C=N, Gua), 1634, 1597, 1566, 1465, 1031, 813. HRMS (*m/z* ESI^+): 191.1297 calcd for $C_{10}H_{14}N_4$ $[M + H]^+$, found 191.1293. Purity by HPLC: 98.1% (*t*R 27.00 min).

1-(5-Chloropyridin-2-yl)-3-propylguanidinium chloride (6b): Yellow crystalline solid (120 mg, 93%); mp 102-104 °C, clean melt. ^1H NMR (600 MHz, DMSO) δ 0.94 (t, 3H, J = 7.4 Hz, CH_3), 1.59 (app. sex, 2H, J = 7.2, 7.3 Hz, CH_2), 3.29 (m, 2H, J = 6.7 Hz, CH_2), 7.12 (s, 1H, Ar), 7.99 (dd, 1H, J = 8.8, 2.5 Hz, Ar), 8.37 (d, 1H, J = 2.5 Hz, Ar), 8.58 (broad s, 2H, NH), 9.17 (broad s, 2H, NH), 11.47 (broad s, 2H, NH).^{13}C NMR (150 MHz, DMSO) δ 11.3 (CH_3), 21.9 (CH_2), 42.9 (CH_2), 115.0 (CH Ar), 125.7 (quat. Ar), 139.7 (CH Ar), 145.1 (CH Ar), 154.1 (quat. Gua). IR (cm^{-1}) υ 3268 (NH), 3097 (NH), 3059, 2958, 2931, 2875, 1673, 1646, 1629 (C=N), 1591, 1558, 1469, 1385, 1371, 1342, 1315, 1271, 1243, 1145, 1109, 1075, 1044, 1012, 966, 903, 869, 828, 772, 737, 658. HRMS (*m/z* ESI^-): 211.0745 calcd for $C_{10}H_{14}N_4$ $[M - H]^-$, found 211.0750. Purity by HPLC: 99.3% (*t*R 26.40 min).

1-(5-Methylpyridin-2-yl)-3-propylguanidinium chloride (6d): Yellow crystalline solid (32 mg, 90%); mp 102-104 °C, clean melt. ^{1}H NMR (600 MHz, DMSO) δ 0.94 (t, 3H, J = 7.5 Hz, CH_3), 1.58 (app. sex, 2H, J = 7.5, 7.1 Hz, CH_2), 2.25 (s, 3H, CH_3), 3.29 (m, 2H, J = 6.6 Hz, CH_2), 6.99 (broad s, 1H, Ar), 7.71 (dd, 1H, J = 8.4, 1.9 Hz, Ar), 8.14 (d, 1H, J = 1.9, Ar), 8.59 (broad s, 2H, NH), 9.43 (broad s, 2H, NH), 11.34 (broad s, 2H, NH). ^{13}C NMR (150 MHz, DMSO) δ 11.0 (CH_3), 17.2 (CH_3), 21.6 (CH_2), 42.5 (CH_2), 112.6 (CH Ar), 128.3 (q Ar), 140.3 (CH Ar), 145.8 (CH Ar), 150.0 (q Ar), 154.1 (q, Gua). IR (cm^{-1}) υ 2965 (NH), 1674 (C=N), 1632, 1608, 1572, 1484, 1343, 1283, 1244, 1136, 1079, 1029, 968, 831, 743. HRMS (*m/z* ESI$^+$): 193.1453 calcd for $C_{10}H_{17}N_4$ $[M + H]^+$, found 193.1458. Purity by HPLC: 96.9% (*t*R 24.57 min).

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SUPPORTING INFORMATION

Copies of ^{1}H and ^{13}C NMR spectra of all compounds described in series **2**, **3**, **4**, **5** and **6**; characterisation data for the 2-aminopyrimidines described in series **1**; X-ray crystal structural data for compounds **2b**, **3d**, **3e** and **4**; copies of the variable temperature ^{1}H NMR spectra of compounds **2d** and **3d** and cartesian coordinates of all the structures optimised at the B3LYP/6-31+G** level are available free of charge via the Internet at http://pubs.acs.org.

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