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**On the self-condensation of aminoguanidine leading to
1,1,4,10,10-pentaamino-2,3,5,6,8,9-hexaazadecan-1,3,5,7,9-pentaene
(Structure elucidation through X-ray powder diffraction)**

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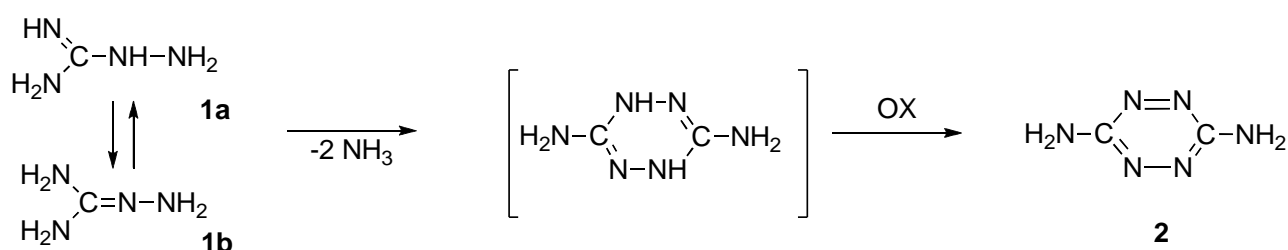
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ABSTRACT: Chemical and spectroscopic (UV; ^1H , ^{13}C , ^{15}N NMR, HRMS) studies determined the structure of 1,1,4,10,10-pentaamino-2,3,5,6,8,9-hexaazadecan-1,3,5,7,9-pentaene from the red-violet compound, which was obtained by treating aminoguanidine hydrochloride with KOH. X-ray powder diffraction and lattice-constrained PBC-DFT optimization confirmed this structure and defined a linear all-trans linear configuration.

1. Introduction

A reddish violet, basic compound was obtained by Ponzio and Gastaldi in 1913, by treating a solution of aminoguanidinium chloride with a stoichiometric amount of potassium hydroxide and leaving the solution to slowly evaporate (under a glass bell in the presence of solid KOH and concd H_2SO_4).¹ On the basis of analytical results, although not agreeing with the formula $\text{C}_2\text{H}_4\text{N}_6$, the structure 3,6-diamino-1,2,4,5-tetrazine **2** was put forward (Scheme 1).



Scheme 1. Presumed formation of diaminotetrazine from aminoguanidine

In 1954, C.-H. Lin et al.² prepared 3,6-diamino-1,2,4,5-tetrazine (**2**) by three independent and unequivocal synthetic procedures, however, the obtained compound resulted different from the one isolated by Ponzio and Gastaldi, whose structure, therefore, must be considered as undefined. Guanidine and aminoguanidine derivatives are of ever growing importance, both as synthons for novel heterocyclic compounds³ and for their potential biological activities.⁴ Indeed, the biguanide metformin is the most frequently prescribed drug for type-2 diabetes, and is also under intensive investigation for its potential applications in oncology.^{4h} In such a situation, we deemed it interesting to elucidate the structure of the above red-violet compound derived from aminoguanidine (P&G compound, **3**). For this purpose, the P&G compound and its salts with hydrochloric and nitric acids were prepared according to the described procedures.¹

2. Results and discussion

The isolated free base contains water of crystallization, whose elimination is somewhat problematic, giving rise to compounds containing variable amounts of non-removable oxygen. However, repeatable analytical results for the anhydrous base were obtained following the drying conditions described in Experimental section. Thus, the empirical formula $\text{C}_4\text{H}_{11}\text{N}_{11}$ was obtained for the anhydrous free base, which in turn allowed us to calculate the following formulas: (a) $\text{C}_4\text{H}_{11}\text{N}_{11}+2\text{H}_2\text{O}$ for the hydrated base; (b) $\text{C}_4\text{H}_{11}\text{N}_{11}+2\text{HCl}+0.5\text{H}_2\text{O}$ for the hydrochloride; (c)

$C_4H_{11}N_{11}+2HNO_3+0.5H_2O$ for the nitrate. The above molecular formula of the free base was confirmed by the HRMS (ESI LTQ Orbitrap XL) showing two main peaks, the first one corresponding to m/z (%) $[M+H]^+$ calcd for $C_4H_{12}N_{11}^+$ 214.12716, found 214.12723 (100) and the second to m/z (%) $[M+2H]^{2+}$ calcd for $C_4H_{13}N_{11}^{2+}$ =2 107.56722, found 107.56732 (73). Moreover the tandem mass spectrum of the ion at 214.12723 exhibits fragmentation peaks at m/z (%): calcd for $C_4H_9N_{10}^+$ 197.10062, found 197.10060 (8); calcd for $C_3H_8N_7^+$ 142.08357, found 142.08336 (100); calcd for $C_2H_7N_6^+$ 115.07267, found 115.07237 (12); calcd for $C_2H_6N_5^+$ 100.06177, found 100.06157 (26); calcd for $C_2H_4N_4^+$ 85.05087, found 85.05075 (24). As shown by the elemental analyses of its salts, the P&G compound is bibasic. Accordingly, it exhibits two relatively close pK_a values: pK_{a1} 5.64 ± 0.01 and pK_{a2} 7.38 ± 0.01 , respectively. The 1H NMR spectrum (DMSO- d_6) confirms the presence of 11 hydrogen atoms, 10 of which are exchangeable with D_2O . Only one 1H resonance, occurring at $\delta=8.60$, corresponds to a nonexchangeable proton, belonging to a methine group, as clearly shown by the ^{13}C DEPT NMR spectrum. The signals of the 10 exchangeable protons were grouped into 3 singlets at $\delta=5.30$, 5.90, and 6.20 ppm, which integrated, respectively, for 2, 4, and 4 equiv protons, where the resonance occurring at 5.30 ppm corresponds to the NH_2 protons on C3 and the 2 other resonances to the 4 NH_2 protons on C1 and C4 (X-ray structure numbering, Fig. 1), in agreement with the proposed X-ray structure. The ^{15}N NMR spectrum exhibited three resonances at $\delta=45.6$, 51.6, and 56.8 ppm (referred to liquid NH_3), which gave rise to three cross peaks with the above signals of the 1H NMR spectrum, indicating that the compound contains three kinds of hydrogen-bearing nitrogen atoms with a different molecular environment.

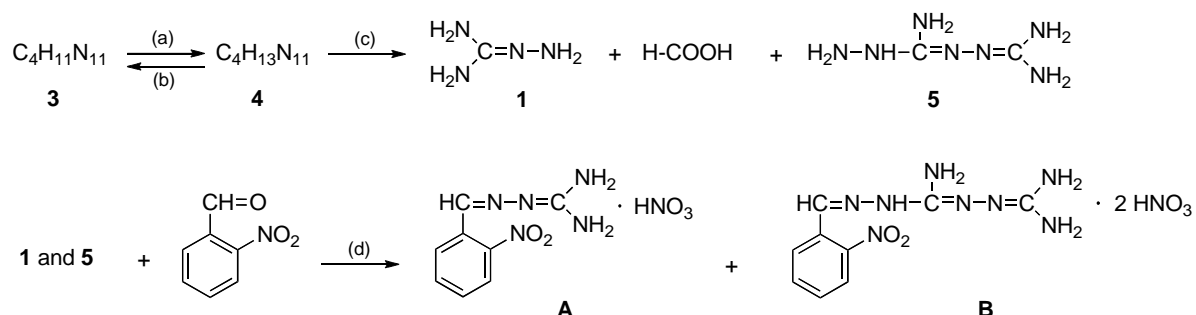
A peculiar feature of P&G compound is its very deep red-violet color, that is related to the strong absorption at 233 and 492 nm, with $\epsilon = 7015$ and 27840 respectively. The color disappears completely after reduction of P&G compound, either with hydrogen sulfide¹, or (starting from the hydrochloride) with hydrogen on Pd/C. In the latter case exactly one mole of hydrogen was absorbed by one mole of $C_4H_{11}N_{11}$. Thus, the color is related to the presence of one easily reducible double bond, as an azogroup, which, however, should be conjugated with other double bonds (not easily reducible), to give reason of the absorption at 492 nm and of the high extinction coefficient. The protonation of P&G compound produces a very strong hypsochromic shift of the higher wavelength maximum from 492 nm to 330 nm, with $\epsilon = 15000$, thus suggesting a significant modification of the assumed conjugated system of double bonds.

The reduced compound, even if easily re-oxidizable to the initial one on exposure to air, appears to be rather unstable with progressive breakdown when standing in the cold protected from re-oxidation.

The reduction of the azogroup, by interrupting the full conjugation of all double bonds, destabilizes the molecule versus hydrolysis and any other degradative reaction.

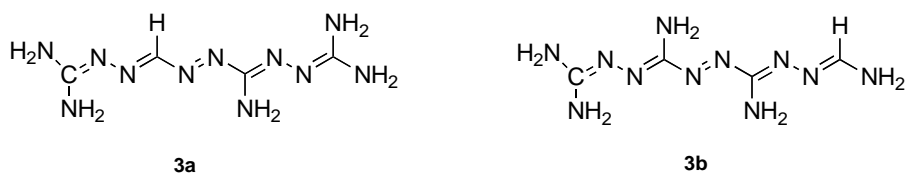
Ponzio and Gastaldi had already observed the release of formic acid and aminoguanidine, which was isolated as 2-nitrobenzaldehyde guanyldiazide nitrate (**A**) when the aldehyde and 2N HNO₃ were added to the reduction mixture. However, it has now been observed the additional formation of a second nitrate (**B**), whose elemental analysis suggests a formula C₉H₁₄N₁₀O₈, corresponding to a dinitrate of a compound of formula C₉H₁₂N₈O₂.

The latter compound may derive from the condensation of 2-nitrobenzaldehyde (C₇H₅NO₃) with a two carbon atoms fragment (C₂H₉N₇; **5**) of the reduced P&G compound, that is formally equivalent to the sum of two molecules of aminoguanidine with the elimination of one of ammonia (Scheme 2).

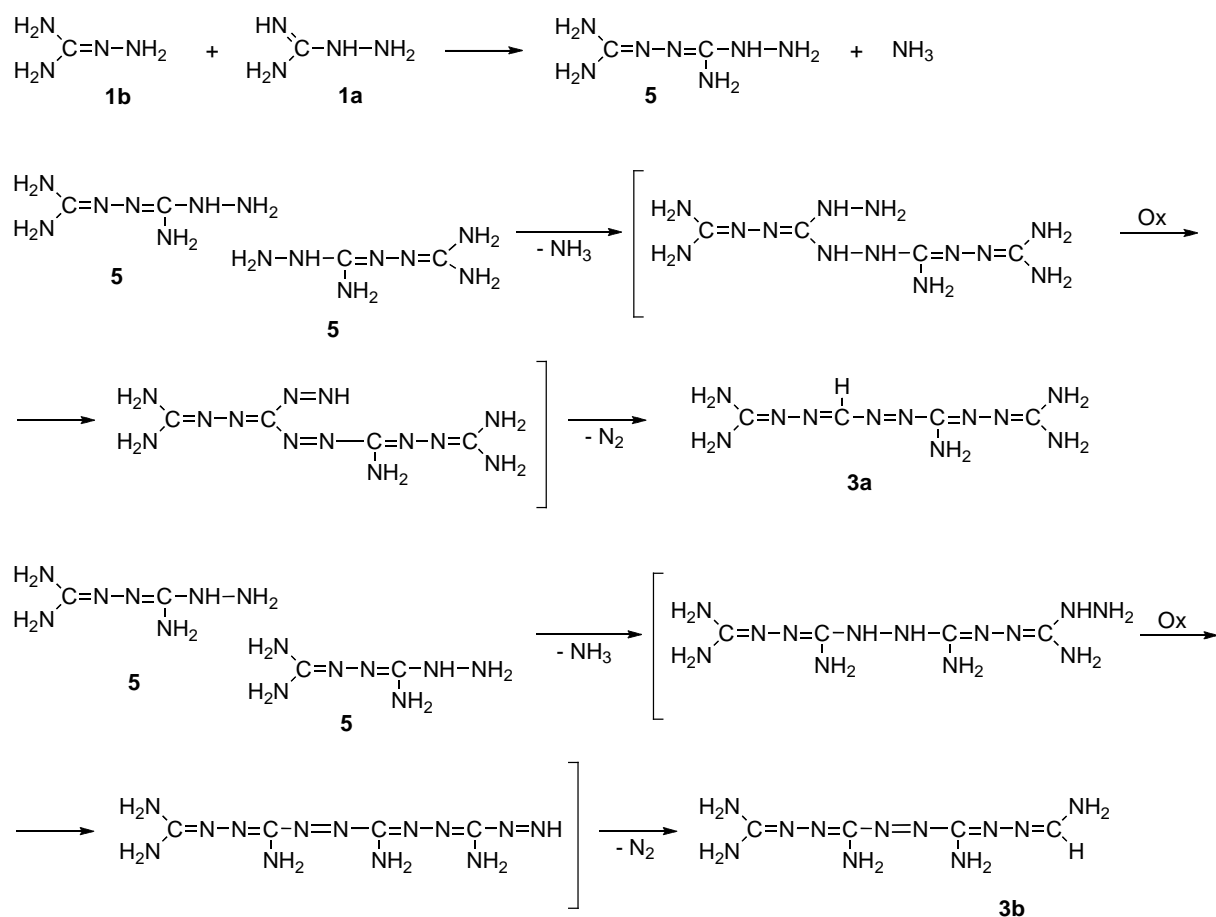


Scheme 2. Reagents and conditions: (a) H₂, Pd/C, EtOH; (b) air; (c) H₂O; (d) 2N HNO₃

The formation of formic acid, aminoguanidine and aminobiguanidine (**5**), account for the 4 carbon atoms of P&G compound, which, therefore, should contain two diaminomethylene heads and a CH group in the middle of the molecule (**3a**). However, since it cannot be excluded that the aminoguanidine might derive from a further breakdown of the aminobiguanidine, an other isomeric structure (**3b**) with only one diaminomethylene head and the CH group located at the other end of the molecule should be considered as possible.



The aminobiguanidine **5**, besides being released from the reduced P&G compound (**4**) (Scheme 2), may, indeed, be at the origin of the red-violet compound itself. It may be formed by the condensation, tail to head, of two molecule of aminoguanidine (reacting in the classic Thiele structure (**1a**) or in the symmetric one (**1b**)⁵) and by a further self condensation, tail to tail or tail to head, gives rise to P&G compound (**3a** or **3b**), as outlined in Scheme 3.



Scheme 3. Possible pathways from amino guanidine to the P&G compound.

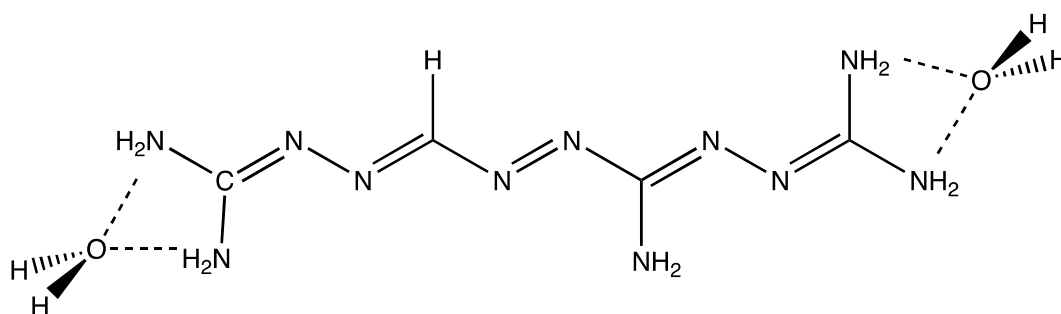
It is worth noting that self-condensation of aminoguanidine has not been described before. Even if no attempts at optimization have been made, it has been observed that the yield of the red-violet compound is rather low (20-22%), suggesting that other reactions of different assemblage and/or of

decomposition of aminoguanidine may have taken place in competition with its self-condensation leading to the P&G compound. The isolation of the latter in practically pure form must be related to its peculiar characteristics of solubility and stability in comparison to the other possible compounds. Indeed, 50 years after its preparation (by G.P. and F.S.) a sample of the dihydrated free base is still unaltered.

Even if the existence of tautomeric forms cannot be excluded, both the above fully conjugated structures **3a** (1,1,4,10,10-pentaamino-2,3,5,6,8,9-hexaazadecan-1,3,5,7,9-pentaene) and **3b** (1,1,4,7,10-pentaamino-2,3,5,6,8,9-hexaazadecan-1,3,5,7,9-pentaene) give account of all chemical and spectral data, and of the noteworthy stability of the P&G compound.

Given that no suitably grown single crystals were found, in order to check our structural hypotheses, to establish the correct connectivity pattern (**3a** or **3b**), and to ascertain if the molecule is either linear or folded (depending on the supremacy of inter- vs. intra-molecular hydrogen bonds, respectively) an *ab-initio* X-rays powder diffraction (XRPD) structural characterization of P&G compound has been carried on.

Our structural analysis clearly shows that the hydrated P&G phase, **3**•2H₂O, belongs to the Pc space group and contains 1,1,4,10,10-pentaamino-2,3,5,6,8,9-hexaazadecan-1,3,5,7,9-pentaene (**3a**) molecules, in their linear all-*trans* configuration, co-crystallized with water molecules ‘capping’ the guanidine ends. Given the almost centrosymmetric shape of the **3**•2H₂O moiety, see Scheme 4, and in spite of the non centrosymmetric space group, the overall packing is pseudo-centrosymmetric.



Scheme 4. Sketch of the **3**•2H₂O moiety present in the asymmetric unit of the hydrated P&G phase.

This diffraction-only structural model already allows to recognize the full pattern of intermolecular interactions, however, an accurate intra- and inter-molecular stereochemistry is hardly possible to achieve by a conventional Rietveld refinement, particularly when working with laboratory data.

Accordingly, in order to add otherwise missing information to our ab-initio XRPD structure determination, i.e. to better assess the extent of conjugation (size and shape of the C/N skeleton) and the stereochemistry of the NH₂ groups (their pyramidalization) which strongly depends on the actual pattern of H-bonds, we have performed a *lattice-constrained PBC-DFT optimization* (where PBC stands for periodic boundary conditions and DFT for Density Functional Theory) of our structural model. Earlier examples of a similar coupling of computational simulation and diffraction data are due to Dinnebier & al., for DFT,⁶ and to Masciocchi & al., for Molecular Mechanics.⁷

This approach eventually affords (see Experimental) a superior description of the P&G structure and of the network of H-bonds. A concise description of the network is reported in Figs. 1 and 2.

For details on the structural solution of the P&G compound, see Supplementary data.

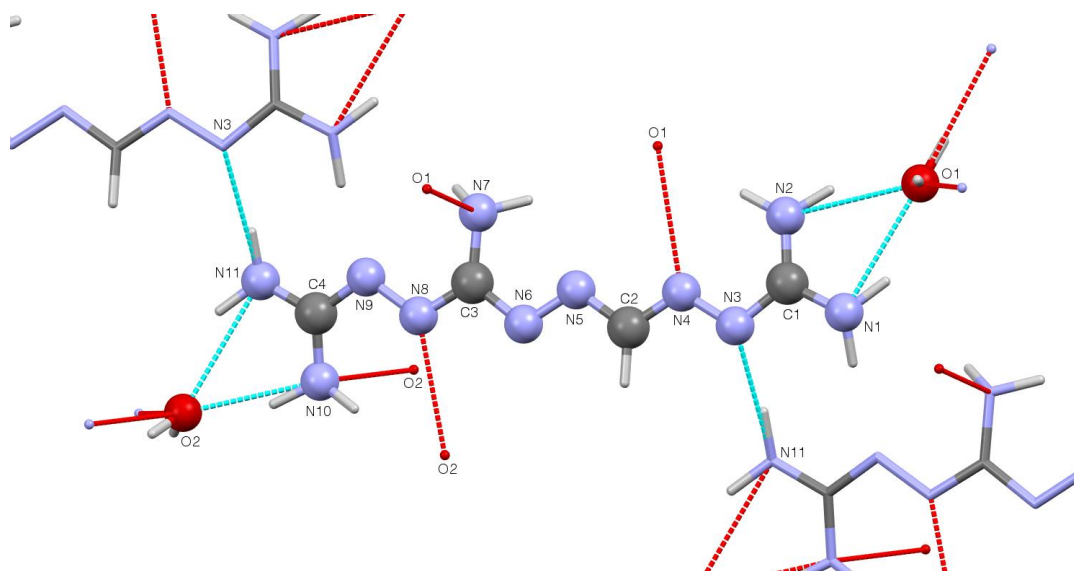


Figure 1: Ball and stick representation of the asymmetric unit of **3**•2H₂O highlighting the network of H-bonds (HB). Both water molecules are involved in four HB (O1 and O2 behaving both as HB-acceptors toward the guanidine ends, and as HB-donors toward N4, N7 and N8, N10, respectively); N1 and N2 only act as HB-donors toward O1; N10 acts both as HB-donor and -acceptor toward O2;

while N11 acts as HB-donor both toward O2 and N3. The plane of the Figure (i.e. the least square plane of the three highlighted molecules) is (-1 2 2).

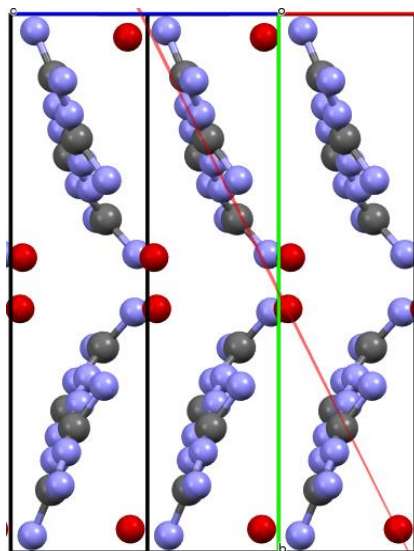
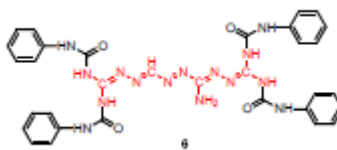


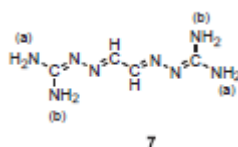
Figure 2: Packing Diagram of $3 \cdot 2\text{H}_2\text{O}$ projected down $[2, 0, 1]$, b axis vertical. P&G molecules are organized in H-bonded strips (see Figure 1) lying in the $(-1\ 2\ 2)$ plane (red line) and running along the $[2, 0, 1]$ direction. Note the herring-bone arrangement of the strips.

Due to the presence of the amino groups, the anhydrous P&G compound reacts promptly with phenyl isocyanate. The obtained brick-red compound gives poor analytical results, but still clearly suggests the condensation of only four molecules of phenyl isocyanate with one molecule of the P&G compound. This possibility is fully confirmed by the HRMS (ESI LTQ Orbitrap XL) of this derivative (m/z value for $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{32}\text{H}_{32}\text{N}_{15}\text{O}_4$ 690.27562, found in the experimental spectrum m/z 690.27628).

Even if the very low solubility of an initially formed tetraarylcarbamoyl derivative might prevent the reaction with the fifth molecule of arylisocyanate, it is possible that the observed limited reaction be related to a somewhat reduced accessibility of the amino group in the middle of the molecule, after the reaction of the arylisocyanates with the four more exposed terminal amino groups. Thus the following formulae should be preferentially attributed to the formed compound **6**.



Compound **3a**, with its full conjugated system of double bonds, ending with two diaminomethylene heads, resembles the potent antileukemic glyoxal bis-guanylhydrazone, whose structure **7** has been established both in the solid state⁸ and in solution.⁹



The ¹H NMR spectrum of **7** exhibits two singlet at $\delta = 5,64$ and $5,88$ (each integrating for 4 exchangeable protons) and a singlet at $\delta 7,83$ for the methine groups, that are consistent with those observed for **3**.

3. Conclusion

The structure of 3,6-diamino-1,2,4,5-tetrazine was assigned to the red-violet compound that was obtained by treating aminoguanidine hydrochloride with KOH by Ponzio and Gastaldi in 1913. At present, one century later, through a chemical and spectroscopic study, the structure of 1,1,4,10,10-pentaamino-2,3,5,6,8,9-hexaazadeca-1,3,5,7,9-pentaene was attributed to that compound. The X-ray powder diffraction and lattice-constrained PBC-DFT optimization confirms the above structure, defining a linear all-trans configuration. Some structural analogies between the P&G compound and the potent antileukemic glyoxal bis-guanylhydrazone suggest that the former (and/or its derivatives) could possess interesting biological activities, which indeed are now under investigation.

4. Experimental section

4.1 General Methods

Chemicals, solvents, and reagents were used as received from commercial sources (SigmaAldrich, Fluka, Lancaster). Melting points were taken in open glass capillaries on a Büchi apparatus or on a Kofler hot plate and are uncorrected. UV spectra were recorded in ethanol solution (1:100,000) with a Varian DMS80 or a Hitachi Perkin Elmer EPS-3T spectrometer. IR spectra were recorded on a Perkin Elmer 398 spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded in DMSO- d_6 solution on a Varian Gemini 200 spectrometer: chemical shifts (δ) are expressed in parts per million (ppm) from internal Me_4Si . The ^{15}N NMR spectra were recorded on a Bruker Avance 300 in DMSO- d_6 solution (from CH_3NO_2 as external reference, δ reported to liquid NH_3). 2D- ^1H , ^{15}N -HSQC spectral conditions were optimized with a gradient enhanced HSQC, 2K F2 time domain data points (^{15}N frequencies) and 512 W F1 time domain data points (^1H frequencies). Relaxation delay (D1) 1.5 s, $J_{\text{HN}}=87$ Hz.

The HRMS were obtained using a high-resolution mass spectrometer with orbital trap LTQ Orbitrap XL, using both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in positive and negative polarity. The software Qual Browser of Xcalibur and Mass Frontier were used to obtain, respectively, the molecular formulas and the theoretical fragments. The P&G compound was dissolved at a concentration of about 1 mg/mL in MeOH, while the condensation compound with phenyl isocyanate was dissolved in DMSO or in a mixture of 40% MeOH, 40% water and 20% formic acid. The suspension was sonicated at 50 °C for 30 min, centrifuged at 14,000 rpm for 3 min, and filtered through a filter with 0.4 mm cut off. The mother solution was diluted (from 1:1 to 1:50) with an acid phase formed of 70% water, 30% MeCN, and 0.1% formic acid.

Elemental analyses were performed on a Carlo Erba EA-1100 CHNS-O instrument.

The ionization constants were determined by potentiometric titration with the GLPka apparatus (Sirius Analytical Instrument Ltd, East Sussex, UK). The pKa values were obtained as means of

three titrations: aqueous solutions (ionic strength adjusted to 0.15 M with KCl) of the compound (10 mL, about 1 mM) were initially acidified to pH 1.8 with 0.5 M HCl and then titrated with standardized 0.5 M KOH to pH 12.2 at a constant temperature (25 °C) under argon atmosphere.

4.2 Synthesis

4.2.1 Synthesis of Ponzio and Gastaldi compound: 1,1,4,10,10-pentaamino-2,3,5,6,8,9-hexaazadecan-1,3,5,7,9-pentaene

A solution of aminoguanidinium chloride (11 g, 0.1 mol) in water (40 mL) was mixed, in a large Petri dish, with a solution of KOH (6 g, ~ 0.1 mol) in water (20 mL) and left for five days under a large glass bell, in the presence of KOH pellets. The initially colourless solution became progressively red-colored and red-violet threadlike needles formed on the surface. After five days the crystals were filtered and washed with ice-cold water. From the filtered solution, maintained for further days under the bell in presence of KOH, some more compound was recovered: on the whole 1.4 g of P&G compound (22%) were obtained. The compound may be recrystallized from hot, but not boiling, water (50 mL for each g) to which conc. ammonia (5 mL) was added. M.p. 204-207 °C (dec.). ¹H NMR (200 MHz, DMSO-d₆, 25°C, TMS): δ= 8.60 (s, 1H, CH), 6.23 (s, 4H, collapses with D₂O), 5.93 (broad s, 4H, collapses with D₂O), 5.31 ppm (s, 2H, collapses with D₂O). ¹³C NMR (50 MHz, DMSO-d₆, 25°C, TMS): δ= 161.8 (C), 159.0 (C), 157.8 (C), 155.60 (CH). ¹⁵N NMR (30 MHz, DMSO-d₆, 25°C, ext. ref. CH₃NO₂; δ referred to liquid NH₃): δ= 56.8, 51.6, 45.6, cross coupling, respectively, with the signals at 6.16, 5.80 and 5.29 of the proton spectrum in DMSO. UV/Vis(EtOH): λ_{max} (ε): 492 (27840), 233 nm (7015 mol⁻¹ dm³ cm⁻¹). HRMS (ESI LTQ Orbitrap XL, positive polarity) *m/z* (%): [M+H]⁺ calcd for C₄H₁₂N₁₁⁺ 214.12716, found 214.12723 (100); calcd for C₄H₉N₁₀⁺ 197.10062, found 197.10061 (8); calcd for C₃H₈N₇⁺ 142.08357, found 142.08358 (32); [M+2H]²⁺ calcd for C₄H₁₃N₁₁²⁺/2 107.56722, found 107.56732 (73). Tandem mass spectrum of ion at *m/z* 214.12723: *m/z* (%): calcd for C₄H₉N₁₀⁺ 197.10062, found 197.10060 (8); calcd for C₃H₈N₇⁺ 142.08357, found 142.08336 (100); calcd for C₂H₇N₆⁺ 115.07267, found

115.07237 (12.5); calcd for $C_2H_6N_5^+$ 100.06177, found 100.06157 (27); calcd for $C_2H_4N_4^+$ 85.05087, found 85.05075 (24).

To obtain the anhydrous product, the needles were thoroughly ground in an agate mortar and dried under high vacuum at room temperature for 3 h and then the temperature was raised up to 90 °C in 1 h and maintained at this level for 30 min. Found: C, 22.33; H, 5.29; N, 72.11. $C_4H_{11}N_{11}$ requires C, 22.53; H, 5.20; N, 72.27. When left exposed, the anhydrous compound returned rapidly to the exact dihydrated form. Found: C, 19.30; H, 5.81; N, 62.18. $C_4H_{11}N_{11}+2H_2O$ requires C, 19.28; H, 6.07; N, 61.82.

Two pKa macroconstant values were detected potentiometrically: $pK_{a1} = 5.64 \pm 0.01$ and $pK_{a2} = 7.38 \pm 0.01$.

4.2.2 Dihydrochloride

The above dihydrated base (0.5 g, 2 mmol) was triturated with 2N HCl (1.1 mL); the compound dissolved but soon afterwards the yellow salt precipitated and was filtered. The salts was dissolved in a little water and reprecipitated with acetone (0.41 g, yield 69%); m.p. ~ 200 °C (dec.). UV/Vis(EtOH): λ_{max} (ϵ): 330 nm ($15000 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$). Elemental analysis calcd (%) for $C_4H_{11}N_{11} + 2HCl + 0.5H_2O$: C 16.28, H 4.78, N 52.20, Cl 24.02; found: C 16.24, H 4.95, N 52.30, Cl 23.90.

4.2.3 Dinitrate

The dihydrated base (0.5 g, 2 mmol) was triturated with 2N HNO_3 (5 mL) until the red-violet crystals were converted to an orange powder that was filtered, washed with 1N HNO_3 and, finally, with ethanol (0.7 g, ~ quantitative yield); m.p. 180 °C (dec.). The dinitrate was also obtained by treating a water solution of the dihydrochloride (100 mg) with an excess (300 mg) of KNO_3 ; the poorly soluble dinitrate precipitates in practically quantitative yields.

Elemental analysis calcd (%) for $C_4H_{11}N_{11} + 2HNO_3 + 0.5H_2O$: C 13.80, H 4.08, N 52.29; found: C 13.65, H 4.16, N 52.13.

4.2.4 Catalytic reduction of P&G compound

a) Palladium on charcoal (5%; 100 mg) suspended in water (10 mL) was saturated with hydrogen; a solution of the dihydrochloride of P&G compound (500 mg, 1.69 mmol in 40 mL of water) was added and the hydrogenation was carried on till the hydrogen absorption stopped (41.5 mL at 18 °C and 760 Torr = 1.69 mmol).

b) After removing the catalyst, a little portion (2 mL) of the colourless solution was basified with ammonia and exposed to air: some red-violet needles were isolated after short time, indicating that the reduced compound can return to the initial one.

c) The main portion of the above colorless solution was added with 2-nitrobenzaldehyde (250 mg in 5 mL of ethanol), followed by 2N HNO_3 (3 mL) and left overnight in the cold. 2-Nitrobenzaldehyde guanylhydrazone nitrate (**A**) was recovered and recrystallized from water (300 mg, 1.11 mmol); m.p. 255-256 °C (lit.^{1,10} 251 °C). The filtered solution was extracted with ether to remove the unreacted 2-nitrobenzaldehyde and then evaporated to dryness in vacuo. The residue was taken up with boiling absolute ethanol (3 mL), from which, after cooling, a second salt (**B**, 200 mg, 0.51 mmol) was obtained; m.p. 205-206 °C.

Elemental analysis calcd (%) for $C_9H_{12}N_8O_2 + 2HNO_3$: C 27.70, H 3.62, N 35.89; found: C 27.93, H 4.25, N 36.04.

4.2.5 Reaction of Ponzio & Gastaldi compound with phenylisocyanate

a) In a pressure tube, the anhydrous compound of Ponzio and Gastaldi (200 mg, 0.94 mmol) was dissolved in anhydrous dimethylformamide (4 mL), phenylisocyanate (1 mL, 9.15 mmol) was added and the tube thoroughly closed. The mixture warmed up and a brick-red compound started to

precipitate. After 24 h the precipitate was filtered and washed with dry benzene: (270 mg, 41.7 % yield); m.p. > 320 °C (Kofler).

Elemental analysis calcd (%) for $C_{32}H_{31}N_{15}O_4 + 1.5H_2O$: C 53.63, H 4.78, N 29.31; found: C 53.79, H 5.48, N 29.21.

b) The reaction was repeated using anhydrous dioxane as solvent; the collected brick-red precipitate was washed with dry dioxane and then taken up with boiling acetonitrile to remove as much as possible the eventually present diphenylurea (220 mg, 34.0 % yield).

HRMS (ESI LTQ Orbitrap XL – positive polarity) m/z : $[M+H]^+$ calcd for $C_{32}H_{32}N_{15}O_4^+$: 690.27562, found 690.27628 (Delta mass 0.9 ppm). Elemental analysis calcd (%) for $C_{32}H_{31}N_{15}O_4$: C 55.73, H 4.53, N 30.46; found: C 55.61, H 4.81, N 28.56.

4.3 Crystallography

4.3.1 X-Ray Powder Diffraction

Gently ground powder of the P&G compound was deposited in the hollow of a holder, mm deep, equipped with a zero background plate (a quartz monocrystal). Diffraction experiments were performed on a vertical-scan diffractometer in $\theta\theta$ mode, equipped with a Ni-filtered $Cu-K\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$) and a linear Position Sensitive Detector (PSD), with the following optics: primary and secondary Soller slits, 2.3 and 2.5°, respectively; divergence slit, 0.3°; receiving slit, 8 mm. Generator setting: 40 kV, 40 mA. The nominal resolution for the present set-up is 0.08° 2θ (FWHM of the α_1 component) for the LaB6 peak at about 21.3° (2θ).

The accurate diffraction pattern at RT of P&G compound was acquired in the 5-105° 2θ range, with $\Delta 2\theta = 0.02^\circ$ and exposure time 0.5 s/step.

Indexing was performed with the aid of the single value decomposition approach,¹¹ as implemented in the TOPAS-R suite of programs,¹² leading to a monoclinic cell of approximate dimensions: $a = 4.977 \text{ \AA}$, $b = 14.271 \text{ \AA}$, $c = 8.192 \text{ \AA}$ and $\beta = 105.01^\circ$. The derived volume (563 \AA^3) addressed the

presence of two molecules in the unit cell ($Z = 2$). Systematic extinctions were consistent with a c -glide, accordingly, the Space Group (SG) of choice was Pc .

Peak shapes were described by the fundamental parameters approach.¹³ The experimental background was fit by a polynomial description. Systematic errors were modelled with sample-displacement angular shifts corrections. A common, refinable isotropic displacement parameter was assigned to Nitrogen and Carbon atoms (BM) and waters oxygen atoms (BMw). The isotropic displacement parameters of Hydrogen atoms were 1.0 \AA^2 larger than that of their leading atoms. Scattering factors, corrected for real and imaginary anomalous dispersion terms, were taken from the internal library of TOPAS.

CCDC 950267 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Crystal data for P&G compound: $C_4H_{11}N_{11} \cdot 2H_2O$, fw = 249 g mol^{-1} , monoclinic Pc (No 7), $a = 4.9788(2)$, $b = 14.2829(4)$, $c = 8.1970(2) \text{ \AA}$, $\beta = 105.014(2)^\circ$, $V = 563.00(3) \text{ \AA}^3$, $Z = 2$, $\rho_{\text{calc}} = 1.47 \text{ g cm}^{-3}$, $\mu(\text{Cu-K}\alpha) = 10.23 \text{ cm}^{-1}$. R_p and $R_{wp} = 4.81$ and 6.59 , respectively, for 5001 data collected in the $5\text{-}105^\circ$ 2θ range. $R_{\text{Bragg}} = 2.85$.

4.3.2 DFT Calculations

All the calculations were done with Gaussian 09¹⁴ using density functional calculations. The parameter-free hybrid functional PBEPBE¹⁵ was employed along with the standard valence double-polarized basis set 6-31G(d,p) for all atoms.

Supporting Information.

Copies of ^1H ^{13}C ^{15}N and HRMS (ESI) spectra for **3**; DFT calculation, details on the structural solution of the P&G compound; tables for DFT convergence path, average residual forces, and Cartesian coordinates; Rietveld refinement plot for **3**. Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.tet.2014.08.03>

References and footnotes

- (1) a) Ponzio, G.; Gastaldi, C. *Gazz. Chim. Ital.* **1913**, *43*, II, 129-137; b) G. Ponzio, G.; Gastaldi, C. *Gazz. Chim. Ital.* **1914**, *44*, I, 257-268 and 277-282.
- (2) Chao Han Lin; Lieber, E.; Horwitz, J. P. *J. Am. Chem. Soc.* **1954**, *76*, 427-430.
- (3) a) Lieber, E.; Smith, G. B. L. *Chem Rev.* **1939**, *25*, 213-271; b) Kurzer, F.; Godfrey, L. E. A. *Angew. Chem., Int. Ed.* **1963**, *2*, 459-476; c) Licandro, E.; Perdicchia, D. *Eur. J. Org. Chem.* **2004**, 665-675; d) Sugiure, M.; Kobayashi, S. *Angew. Chem. Int. Ed.* **2005**, *44*, 5176-5186.
- (4) a) Kreutzberger, A. *Progr. Drug Res.* **1968**, *11*, 356-445; b) Durant, G. J.; Roe, A. M.; Green, A. L. *Prog. Med. Chem.* **1970**, *7*, 124-213; c) Richter, P. H.; Wunderlich, J.; Schlender, M.; Keckis, A. *Pharmazie* **1993**, *48*, 83-184; d) Greenhill, J. V.; Lue, P. *Prog. Med. Chem.* **1993**, *30*, 203-326; e) Nilsson, B. O. *Inflamm. Res.* **1999**, *48*, 509-515; f) Oliver, D. W.; Dormehl, J. C.; Wikberg, J. E. S.; Dambrawa, M. *Med. Chem. Res.* **2004**, *13*, 427-438; g) Solinas, A.; Faure, H.; Roudaut, H.; Traiffort, E.; Schoenfelder, A.; Mann, A.; Manetti, F.; Taddei, M.; Ruat, M. *J. Med. Chem.* **2012**, *55*, 1559-1571.
- (5) Bharatan, P. V.; Iqbal, P.; Malde, A.; Tiwari, R. *J Phys. Chem.* **2004**, *108*, 10509-10517.
- (6) Dinnebier, R. E.; Ding, L.; Ma, K.; Neumann, M. A.; Tanpipat, N.; Leusen, F. J. J.; Stephens, P. W.; Wagner, M. *Organometallics*, **2001**, *20*, 5642-5647.
- (7) Masciocchi, N.; D'Alfonso, G.; Garavaglia, L.; Sironi, A. *Angev. Chem. Int. Ed.*, **2000**, *39*, 4478-4480.
- (8) Mutikainen, J.; Elo, H.; Tilus, P. *Z. Naturforsch.* **1993**, *48b*, 1821-1827.
- (9) Elo, H. *Spectrosc. Lett.* **1989**, *22*, 123-160.
- (10) Thiele, J.; Bihan, R. *Ann.* **1898**, *302*, 299-311.
- (11) Coelho, A. A. *J. Appl. Cryst.* **2003**, *36*, 86-95.
- (12) Topas-R: General profile and structure analysis software for powder diffraction data; Bruker AXS, Karlsruhe, Germany, 2001.
- (13) Cheary, R. W.; Coelho, A. *J. Appl. Cryst.* **1992**, *25*, 109-121.

- (14) Gaussian 09, Revision C.01, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J.; Gaussian Inc., Wallingford CT, 2009.
- (15) Perdew, J. P.; Burke, K.; Ernzerhof, M. *Phys. Rev. Lett.* **1997**, *78*, 1396.