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Protonation of the Pyrimidine Ring at the C(5) Position: Formation of a Stable Cationic σ-Complex

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Abstract: NMR studies showed that, in addition to the expected N(1) protonation, 2,4,6-pyrimidinetriamine, *N*,*N*,*N*′,*N*′,*N*″,*N*″-hexamethyl- (**1**) could also be protonated at the C(5) position in water, leading to an equilibrium between the C(5) and N(1) protonated forms. Analysis of the NMR titration data gives 6.87 and 6.89 for the pK_a of the C(5) and N(1) protonation equilibria. Moreover, the reaction of **1** with chloroacetyl chloride leads to a novel 1,1-bis(pyrimidin-5-yl)-2-chloroethene type derivative (**4**) that is, peculiarly, fully monoprotonated at the C(5) position in either of the pyrimidine rings, forming a stable cationic σ-complex.

Introduction

The pyrimidine system is an essential structural motif in several natural products of biological importance.[1] There is a large class of pharmacologically important, substituted 2,4-diaminopyrimidines which act as dihydrofolate reductase (DHFR) inhibitors (e.g., pyrimethamine, thrimethoprim).[2,3] Furthermore, hexaalkyl-2,4,6-triaminopyrimidines constitute important subunits of 21-aminosteroids (e.g., tirilazad) and 2-(aminomethyl)-chromans that inhibit iron-dependent lipid peroxidation and protect against central nervous system (CNS) trauma.[4,5] Protonation studies might provide basic insight into the mechanism of action as clearly demonstrated for DHFR inhibitors.[6] The site of protonation has been extensively investigated in solution and gas phase for simple aminopyrimidines; those studies indicated that protonation occurs at the pyrimidine ring nitrogen [N(1) or N(3)] or at the exocyclic amino group.[7−13] However, such investigations for hexaalkyl-2,4,6-triaminopyrimidines have not been established.

In this paper, we show that, in addition to the anticipated N(1) protonation, hexamethyl-2,4,6-triaminopyrimidine (**1**) could be C(5) protonated in water, leading to an equilibrium between the C(5) and N(1) protonated forms. More interestingly, we demonstrate that in a novel 1,1-bis(pyrimidin-5-yl)-2-chloroethene type derivative (**4**) either of the pyrimidine rings is, peculiarly, fully monoprotonated at the C(5) position and forms a stable cationic σ-complex.

Results and Discussion

Formation of the Stable σ-Complex (4). The current study was initiated by attempts to synthesize 5-(2-haloacyl)triaminopyrimidines for coupling with 21-aminosteroids and 2-(aminomethyl)chromans to find new drug candidates. However, in the reaction of 2,4,6-pyrimidinetriamine, *N*,*N*,*N*′,*N*′,*N*″,*N*″- hexamethyl-[14] (**1**) and chloroacetyl chloride (**2**), we obtained the HCl salt of the novel 2,4,6-pyrimidinetriamine, *N*,*N*,*N*′,*N*′,*N*″,*N*″-hexamethyl-5-[1-(2,4,6-tris(dimethylamino)pyrimidin-5-yl)-2-chlorovinyl]- (**4**) instead of the expected derivative **3** (Scheme 1). NMR showed that the isolated bis-pyrimidine compound **4** is, peculiarly, monoprotonated at the C(5) position in either of the pyrimidine rings, forming in solution a slowly interconverting mixture of **4**(*E*) and **4**(*Z*) isomers (see below).

Negative FAB-MS and classical methods proved that the anion is chloride. The respective conjugate base (**5**) could also be obtained by treating **4** with a strong base (NaOEt). Addition of one equivalent of aqueous HCl to the base **5** gave back the salt **4**. The structure of **4** has two novelties: First, to the best of our knowledge, the observed C-protonation is unique among (amino)pyrimidines. Second, in **4** the protonated pyrimidine ring corresponds to a stable cationic σ-complex. Up until now, only anionic σ-complexes of pyrimidines have been isolated in a stable form.[15−17]

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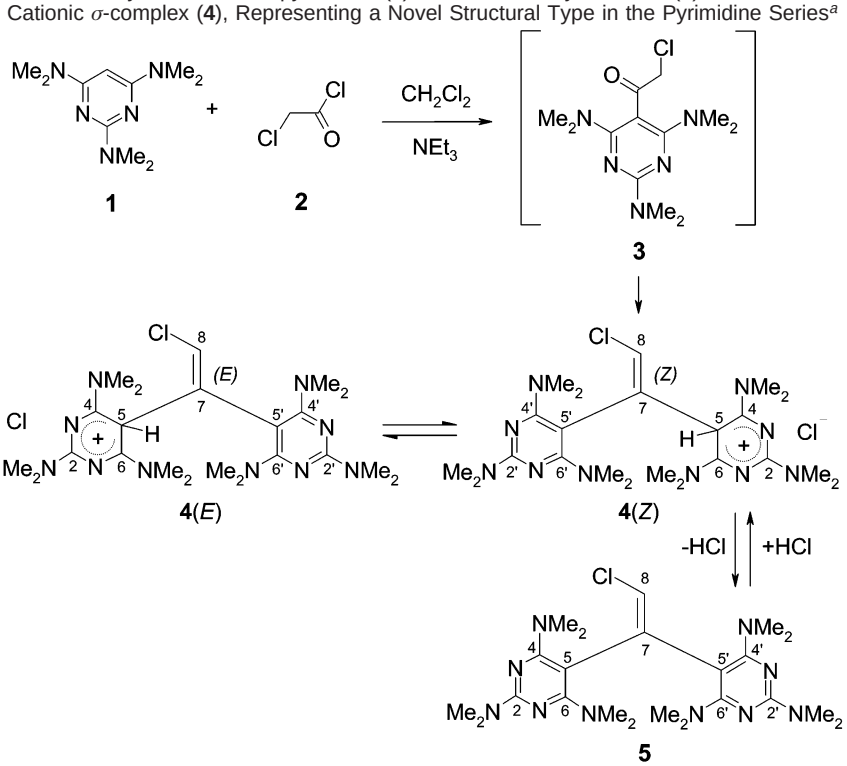
(1) For a comprehensive review of pyrimidines, see (a) Brown, D. J.; Evans, R. F.; Cowden, W. B.; Fenn, M. D. *The Pyrimidines*; Wiley-Interscience: New York, 1994. (b) *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Scriven, E. F., Eds.; Pergamon: Oxford, 1996; Vol. 6, pp 93−233. (c) *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; Vol. 3, pp 57−157.
(2) Hitchings, G. H. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 879−885.
(3) Hitchings, G. H. *Rev. Port. Farm.* **1991**, *41*, 21−29.
(4) Jacobsen, E. J.; VanDoornik, F. J.; Ayer, D. E.; Belonga, K. L.; Braughler, J. M.; Hall, E. D.; Houser, D. J. *J. Med. Chem.* **1992**, *35*, 4464−4472.
(5) Jacobsen, E. J.; McCall, J. M.; Ayer, D. E.; VanDoornik, F. J.; Palmer, J. R.; Belonga, K. L.; Braughler, J. M.; Hall, E. D.; Houser, D. J.; Krook, M. A.; Runger, T. A. *J. Med. Chem.* **1990**, *33*, 1145−1151.
(6) Feeney, K. *Angew. Chem., Int. Ed.* **2000**, *39*, 290−312.
(7) Threadgill, M. D.; Griffin, R. J.; Stevens, M. F. G.; Wong, S. K. *J. Chem. Soc., Perkin Trans. I* **1987**, 2229−2234.
(8) Städeli, W.; Philipsborn, W.; Wick, A.; Kompis, I. *Helv. Chim. Acta* **1980**, *63*, 504−522.
(9) Riand, J.; Chenon, M. Th.; Lumbroso-Bader, N. *J. Am. Chem. Soc.* **1977**, *99*, 6838−6845.
(10) Wagner, R.; von Philipsborn, W. *Helv. Chim. Acta* **1970**, *53*, 299−320.
(11) Nguyen, V. Q.; Tureček, F. *J. Am. Chem. Soc.* **1997**, *119*, 2280−2290.
(12) Riand, J.; Coupry, C.; Chenon, M.-T. *J. Chem. Soc., Perkin Trans. 2* **1981**, 783−788.
(13) Griffiths, D. V.; Swetnam, S. P. *J. Chem. Soc. Chem. Commun.* **1981**, 1224−1225.
(14) Neunhoeffer, H.; Lehman, B. *Liebigs Ann. Chem.* **1975**, 1113−1119.
(15) Cherkasov, V. M.; Remennikov, G. Ya.; Kisilenko, A. A.; Romanenko, E. A. *Chem. Heterocycl. Compd. (Engl. Transl.)* **1982**, *18*, 526−529.

Scheme 1. The Reaction of Hexamethyl-2,4,6-triaminopyrimidine (**1**) with Chloroacetyl Chloride (**2**) in the Presence of Et_3N in CH_2Cl_2 at 0−5 °C Leads to a Stable Cationic σ-complex (**4**), Representing a Novel Structural Type in the Pyrimidine Series[a]



[a] The formation of **4** can be rationalized by nucleophilic addition of **1** to the carbonyl carbon of intermediate **3**, followed by water elimination.

NMR Spectroscopic Proof of Structure 4. The following spectroscopic pieces of evidence support our structural conclusions. The ^1H and ^{13}C NMR spectra of **4** measured in DMSO-d_6 consist of a major and a minor signal set, with an intensity ratio 2.3:1, because of the **4**(*Z*) and **4**(*E*) isomers, respectively. The $^1H-^{13}C$ HSQC spectrum exhibits correlations between the ^1H minor and major signals at δ 7.31 and δ 7.87 and the ^{13}C signals at δ 129.3 and δ 131.3, respectively; therefore, these signals are assigned to H−C(8). The observed one-bond correlations between the ^1H signals at δ 5.12 and δ 5.34 and the aliphatic ^{13}C signals at δ 41.8 and δ 37.5 are assigned to the H−C(5) unit. The minor and major ^{13}C signals at δ 129.5 and δ 132.9 as well as at δ 88.8 and δ 85.5 are due to the olefinic C(7) and the pyrimidine C(5′) carbons, respectively. The observed $^1H-^{13}C$ HMBC correlations [H(5)↔C(4,6),-C(5′),C(7),C(8), as well as H(8)↔C(5),C(5′),C(7),C(4′,6′)*,-C(4,6)*, where * denotes weak but reproducible correlations through four bonds] unambiguously verify the geminal arrangement of the pyrimidine units for both the minor and major species. Assignment of the minor and major signals to the **4**(*Z*) and **4**(*E*) isomers is based on the measured vicinal $^1H-^{13}C$ heteronuclear couplings (**4**(*E*): $^3J_{H(8),C(5)} = 7.6$ Hz, $^3J_{H(8),C(5')} = 2.8$ Hz and **4**(*Z*): $^3J_{H(8),C(5)} = 4.1$ Hz and $^3J_{H(8),C(5')} = 7.4$ Hz).[18] Furthermore, the observed strong **4**(*Z*) and the weak **4**(*E*) H(5)−H(8) NOEs confirm this assignment. The inversion transfer between the minor and major signals proves that **4**(*E*) and **4**(*Z*) interconvert at a rate which is, under the applied conditions, slow on the chemical shift time scale but moderately fast on the relaxation time scale.

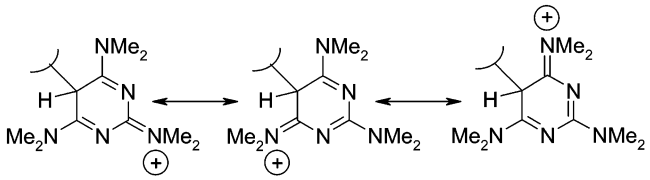


Figure 1. Mesomeric structures illustrate effective charge delocalization in the pyrimidine ring.

The observed C(5) protonation can be intuitively explained by means of steric and electronic factors: In the olefinic bond, the geminal arrangement of the pyrimidine rings, bearing relatively bulky dimethylamino groups at C(4,6), results in a strongly crowded structure in the conjugate base **5**. This congestion is appreciably relieved in **4** by protonation at C(5) because of the $sp^2 \rightarrow sp^3$ hybridization change of C(5). The positive charge is stabilized by the electron-donating resonance effect of the 2,4,6-dimethylamino groups, leading to charge delocalization as illustrated by the mesomeric structures in Figure 1. In light of the fact that 1,3,5-triaminobenzenes could form σ-complexes on protonation,[19−25] the corresponding

(16) Cherkasov, V. M.; Remennikov, G. Ya.; Kisilenko, A. A.; Romanenko, E. A. *Chem. Heterocycl. Compd. (Engl. Transl.)* **1980**, *16*, 179−182.
(17) Cherkasov, V. M.; Remennikov, G. Ya.; Romanenko, E. A. *Khim. Geterotsikl. Soedin.* **1978**, *10*, 1389−1391.
(18) Marshall, J. L. Carbon-Carbon and Carbon-Proton NMR Couplings. In *Methods in Stereochemical Analysis*; Marchand, A. P., Ed.; Verlag Chemie International: Deerfield Beach, FL, 1983; Vol. 2, pp 33−38.
(19) Glatzhofer, D. T.; Allen, D.; Taylor, R. W.; *J. Org. Chem.* **1990**, *55*, 6229−6231.
(20) Effenberger, F. *Acc. Chem. Res.* **1989**, *22*, 27−35.
(21) Effenberger, F.; Niess, R. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 1067.
(22) Yamaoka, T.; Hosoya, H.; Nagakura, S. *Tetrahedron* **1968**, *24*, 6203−6213.
(23) Yamaoka, T.; Hosoya, H.; Nagakura, S. *Tetrahedron* **1970**, *26*, 4125−4130.
(24) Knoche, W.; Schoeller, W.; Schomaecker, R.; Vogel, S. *J. Am. Chem. Soc.* **1988**, *110*, 7484−7489.
(25) Sachs, W.; Knoche, W.; Herrmann, C. *J. Chem. Soc., Perkin. Trans. 2* **1991**, 701−710.

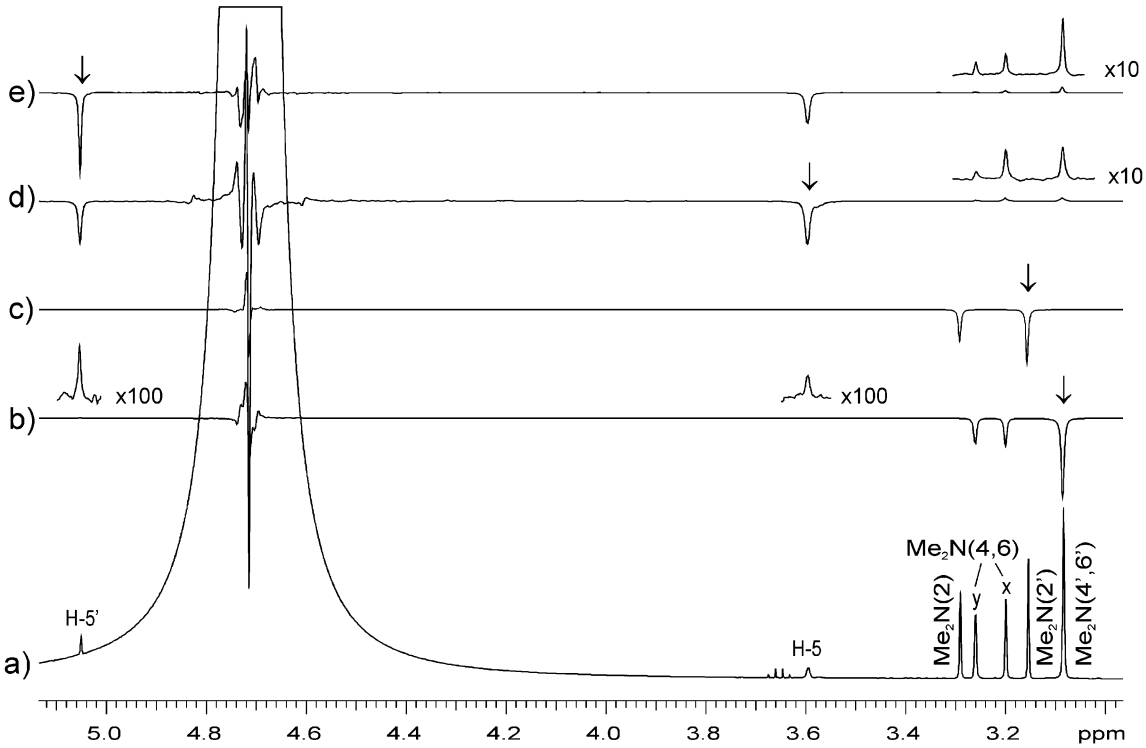
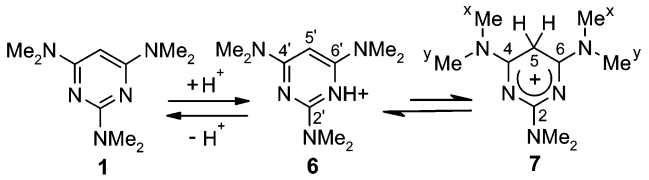


Figure 2. (a) ^{1}H NMR spectrum of **1** as measured in H_2O:D_2O (9:1) at pH 4.6 at 30 °C. (b)−(e) DPFGSE-NOE spectra indicate inversion transfer between the N(1) and C(5) protonated species. The arrows denote the site of selective excitation.

Scheme 2. Protonation of **1** Leads to an Equilibrium between the N(1) and C(5) Protonated Species, **6** and **7**, Respectively.



behavior of **4** is not unexpected. However, the analogous behavior of the pyrimidine ring was not demonstrated before.

Protonation of the Monomeric Compound 1. It is an essential control experiment to show whether the parent compound **1** could be C(5) protonated. Figure 2. shows the ^{1}H NMR spectrum of **1** and selective 1D DPFGSE-NOE experiments as recorded in H_2O:D_2O (9:1) solution at pH 4.6 at 30 °C. In the ^{1}H spectrum (Figure 2, trace a) two signal sets could be distinguished, with an intensity ratio ∼1.2:1, because of the N(1) and C(5) protonated species, **6** and **7**, respectively—see Scheme 2.

The direct ^{1}H−^{13}C heteronuclear coupling constant determined from the ^{13}C satellite of the H-5 signal at δ 3.60 ($^1J_{H(5),C(5)}$ = 131.9 Hz) indicates sp^3 hybridization as opposed to the H-5′ signal at δ 5.05 ($^1J_{H(5'),C(5)}$ = 168.4 Hz) which points to sp^2 hybridization at C(5).[26] In the phase-sensitive 2D PFG-HSQC spectrum, the correlation (in anti-phase to the correlations due to the methyl and methin groups) between the ^{1}H signal at δ 3.60 and the ^{13}C signal at δ 25.4 provides evidence for the C(5) protonation. Inversion transfer between the two signal sets observed in selective DPFGSE-NOE experiments proves that the rate of interconversion between **6** and **7** is slow on the chemical shift time scale but moderately fast on the relaxation time scale. The positive signals visible upon vertical expansion indicate that the system resides in the positive NOE regime, so the massive negative signals can be attributed to inversion transfer due to chemical exchange. The shaped pulses were carefully calibrated and selectivity was checked by control experiments to eliminate possible spillover effects. The presence of exchange between the two forms was also proved by nonselective 2D ROESY experiments (see Supporting Information).

In **7**, the Me_2N groups at the C(4) and C(6) positions give two different signals as opposed to **6**, in which only one signal is observed. The rationale for this observation is the following: If the exchange rate between the N(1) and the formally distinguished N(3) protonated species as well as the rotation around the C(4)−NMe_2 and C(6)−NMe_2 bonds were slow on the NMR chemical shift time scale, we would expect four singlet signals because of the chemically nonequivalent methyl groups in **6**. The presence of only one signal indicates that both processes should be fast on the NMR chemical shift time scale. In contrast, the C(5) protonated species possesses C_{2v} symmetry; thus, the Me_2N(4) and Me_2N(6) groups are chemically equivalent in **7**. However, in this case the rotation of the NMe_2 groups in the 4 and 6 positions is slow which results in two signals. The increased rotational activation energy indicates more effective charge delocalization in the symmetric cation **7** relative to **6**. The C(5) protonation destroys the aromatic character of the pyrimidine ring [sp^2→sp^3 hybridization change at C(5)] as compared to the N(1) protonation where the π sextet is preserved during the process. This explains the observed increased activation energy of the C(5) protonation relative to N(1), that is, the C(5) process is slower (slow on the δ time scale) than the N(1) (fast on the δ time scale).

(26) Kalinowski, H.-O.; Berger, S.; Siegmar, B. *Carbon-13 NMR spectroscopy*; John Wiley & Sons: Chichester, U.K., 1991; Ch. 4, pp 495−496.

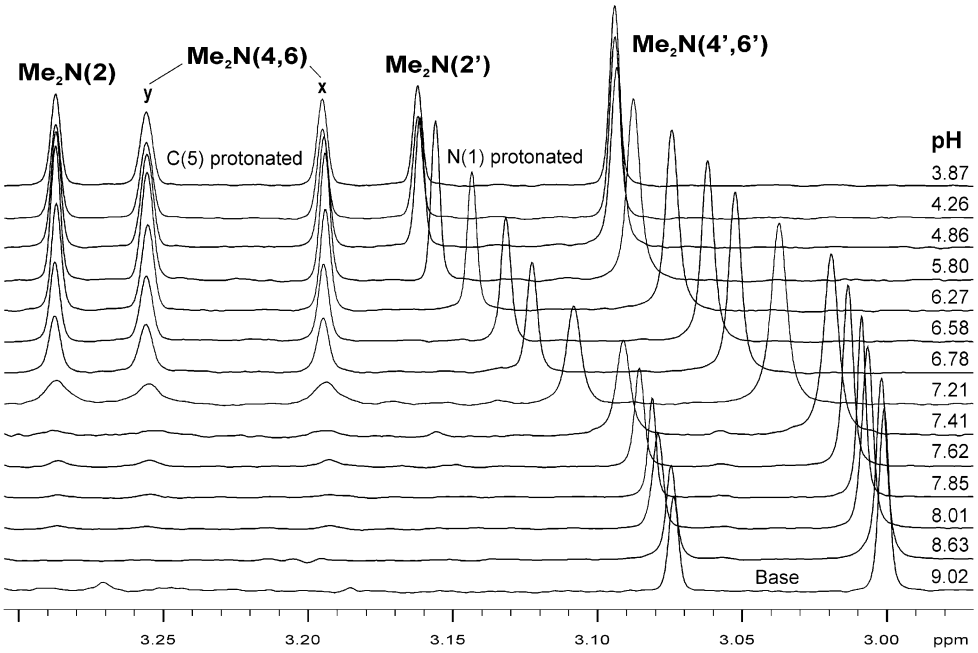


Figure 3. Section of the ^{1}H NMR spectrum of **1** as a function of pH at 25 °C in H_2O:D_2O (9:1) solution.

Determination of the pK_a of the N(1) and C(5) Protonation Equilibrium of 1. It is of particular interest to determine the pK_a of the N(1) and C(5) protonation equilibria in **1**, which could be accomplished by NMR titration. Figure 3. shows the methyl section of the ^{1}H NMR spectrum of **1** as a function of pH at 25 °C in a H_2O:D_2O (9:1) solution.

At basic conditions (pH = 9.02), only one signal set is observed because of the base form of **1**. With decreasing pH, two distinct processes could be identified. First, the methyl groups show gradual downfield shift according to a titration curve that reaches a chemical shift limit characteristic of the N(1) protonated form (**6**). Second, because of the C(5) protonated form, a new signal set appears with an intensity increasing to the equilibrium value. The chemical shift of this downfield signal set remains constant during titration. To determine the pK_a of the N(1) and C(5) protonation, we need to consider the following equilibria [eqs 1 and 2]:

$$B_CH^+ \rightleftharpoons H^+ + B \quad (1)$$

$$B_NH^+ \rightleftharpoons H^+ + B \quad (2)$$

where B and H^+ denote the base form and the proton while B_CH^+ and B_NH^+ correspond to the C(5) and N(1) protonated forms. The equilibrium constants (K_C and K_N) of the respective protonation equilibria are expressed by eqs 3 and 4.

$$K_C = \frac{[H^+]c_B}{c_C} \quad (3)$$

$$K_N = \frac{[H^+]c_B}{c_N} \quad (4)$$

where $[H^+]$ is the proton concentration, c_B, c_C, and c_N are the concentration of the base, the C(5), and the N(1) protonated forms, so that

$$c_B + c_C + c_N = c_{total} \quad (5)$$

Since on the NMR chemical shift time scale the C(5) and the N(1) protonation rates are slow and fast, respectively, the measured intensity of the downfield signal set (I_C) directly reflects the concentration of the C(5) protonated species [eq 6], while the measured intensity of the upfield signal set (I_{NB}) is proportional to the sum concentration of the N(1) protonated species and the base form [eq 7]. The above argument is valid at every pH but strictly so when the system is at thermal equilibrium.

$$I_C \cong c_C \quad (6)$$

$$I_{NB} \cong c_N + c_B \quad (7)$$

Combining eqs 3 and 4 with eqs 6 and 7 leads to a linear relationship for the measured intensity ratio $R = I_{NB}/I_C$ with the inverse of the proton concentration [eq 8] (for details of the derivation of [eq 8] see Supporting Information).

$$R = \frac{I_{NB}}{I_C} = \frac{K_C}{[H^+]} + \frac{K_C}{K_N} \quad (8)$$

A two-parameter linear least-squares fit of R versus $1/[H^+]$ gives both K_N and K_C as it is shown in Figure 4. The respective equilibrium constants obtained from NMR titration data are $K_N = 1.30 \times 10^{-7} \pm 2 \times 10^{-9}$ mol/dm^3 and $K_C = 1.35 \times 10^{-7} \pm 3 \times 10^{-9}$ mol/dm^3.[27] Accordingly, the pK_a of the N(1) and C(5) protonation equilibria is 6.89 ± 0.01 and 6.87 ± 0.01.

Another way the value of K_N becomes accessible is through the chemical shift changes observed during titration. The observed chemical shift of the upfield signal set (δ_{obs}) because of the N(1) protonation will be the population-weighted average of the characteristic chemical shift of the base (δ_B) and the N(1)

(27) Microcal Origin 6.0 (Microcal Software, Inc., One Roundhouse Plaza, Northampton, MA 01060) was used for the linear least-squares fit.

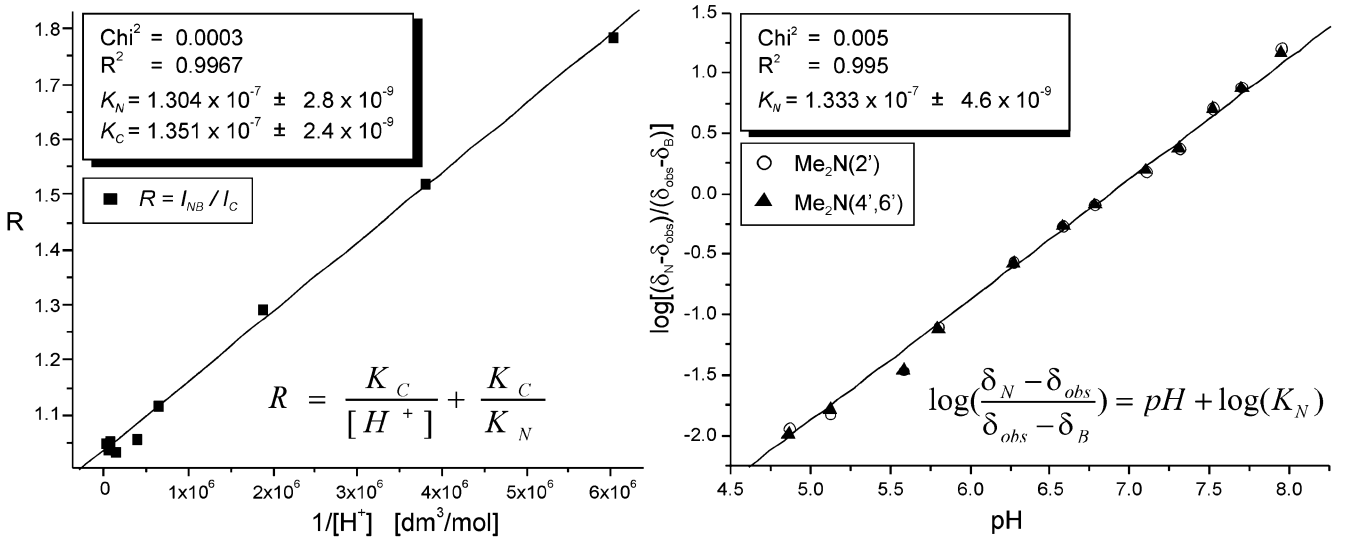


Figure 4. Left: Linear fit to the measured intensity ratio $R = I_{NB}/I_C$ vs the reciprocal proton concentration. Right: Linear fit to the observed chemical shift changes vs solution pH.

protonated form (δ_N) [eq 9]. The contribution of the C(5) protonated species to δ_{obs} can be neglected since the C(5) protonation is slow on the NMR chemical shift time scale.

$$\delta_{obs} = \delta_B p_B + \delta_N p_N \tag{9}$$

where the respective populations are defined as $p_B = c_B/(c_B + c_N)$ and $p_N = c_N/(c_B + c_N)$, so that $p_B + p_N = 1$.

Equation 9 can be expressed as the well-known Henderson−Hasselbalch equation [eq 10] (for details of the derivation of eq 10 see Supporting Information).[28]

$$\log\left(\frac{\delta_N - \delta_{obs}}{\delta_{obs} - \delta_B}\right) = \mathrm{pH} + \log(K_N) \tag{10}$$

By plotting the logarithmic term at the left side of eq 10 as a function of pH, the value of K_N can be determined by a linear least-squares fit (see Figure 4). Accordingly, simultaneous fit of the measured chemical shifts due to the $Me_2N(2')$ and $Me_2N(4',6')$ signals versus solution pH gives $1.33 \times 10^{-7} \pm 5 \times 10^{-9}$ mol/dm³ for K_N.[27] The $pK_a = 6.88 \pm 0.02$ obtained this way for the N(1) protonation is equal within experimental error to that obtained from eq 8.

We also tried to measure the pK_a value of the C-protonation in the σ-complex **4** in water. However, the solubility of conjugate base **5** turned out to be too small to allow precise evaluation. Similarly to the monomeric **1**, in the case of the σ-complex **4** we would expect that the base and the C-protonated forms are in slow exchange on the NMR chemical shift time scale. With increasing pH, a gradual disappearance of the signals because of the C-protonated form (**4**) as well as a gradual growth of a new signal set characteristic of the base form (**5**) is expected. In this case, determination of the pK_a would require simultaneous measurement of the C-protonated and base populations, that is, the signal intensity of **4** and **5** as a function of pH. We could not detect signals that could be assigned to the base form when **4** was titrated with NaOH solution up to pH = 13. Even in dilute solutions above pH = 13, precipitation of the solute was observed indicating formation of the base that is insoluble in water. On the basis of these facts, we estimate that the pK_a of the C-protonation in **4** should be larger than 13.

Although one would not, in general, expect any protonated form to be permeable across the blood-brain barrier, a compound penetrating in the base form might exert a pharmacological effect in the protonated form. Therefore, the question of the amount of protonated species present at physiological pH receives special importance regarding the pharmacological relevance of our finding. On the basis of the pK_a values obtained for compound **1**, the acid/base ratio is ca. 0.59 as calculated for pH = 7.4, that is, the population of the C(5) and N(1) protonated species is 18% and 19%, respectively. This suggests that both the C(5) and N(1) protonated forms should be equally considered in structure−activity studies of pharmaceutically active compounds containing the hexaalkil-2,4,6-triaminopyrimidine moiety.

Conclusions

We presented a novel bis-triaminopyrimidine derivative that forms a stable cationic σ-complex by C(5) protonation and we provided spectroscopic evidence of its structure. We also showed that, in analogy to 1,3,5-aminobenzenes, the monomeric hexamethyl-2,4,6-triaminopyrimidine (**1**) could also be C(5) protonated in equilibrium with the N(1) protonated form. For the monomeric **1,** the pK_a of the C(5) and N(1) protonation was determined from NMR titration data, which values suggest that considerable amounts of C(5) and N(1) protonated species are present at physiological pH. The observed C-protonation feature might be of interest in connection with structure−activity relationship studies of pharmaceutically active compounds containing a hexaalkyl-2,4,6-triaminopyrimidine moiety.

Experimental Section

General Information. The NMR spectra were recorded on a Varian *INOVA* spectrometer operating at 500 MHz (^{1}H) by using a Varian 5-mm ^{1}H{^{13}C/^{15}N} PFG indirect·nmr probe. ^{1}H chemical shifts are given relative to TMS ($\delta_{TMS} = 0.00$ ppm) as measured in DMSO-d_6/$CDCl_3$ or relative to DSS ($\delta_{DSS} = 0.00$ ppm) as measured in water at 30 °C. ^{1}H assignments were straightforward by a concerted use of

(28) Günther, H. *NMR Spectroscopy*, 2nd ed.; John Wiley & Sons: Chichester, U.K., 1996; Ch. 4, pp 76−78.

standard high-field one- and two-dimensional (2D) NMR methods: 1D DPFGSE-NOE (selective excitation by I-Burp2 shaped pulses, typically 80−100 ms excitation time) and 2D $^1H-^1H$ shift correlations (PFG-HSQC, PFG-HMBC). The obtained scalar and NOE connectivities provided abundant information to ensure unambiguous spectral assignments. $^1H-^{13}C$ heteronuclear coupling constants were measured by the 2D EXSIDE method[29] or by recording the proton-coupled ^{13}C NMR spectrum.

Preparation and Characterization of 1, 4, and 5. 2,4,6-Pyrimidinetriamine, *N,N,N′,N′,N″,N″*-hexamethyl- (1). Preparation of **1** was carried out according to the method of Neunhoeffer and Lehman.[14] Yield 87%; mp published 143 °C; mp found 142 °C; ^1H NMR (300 MHz, $CDCl_3$, 30 °C): δ 3.01 (s, 12H, $Me_2N(4,6)$), 3.11 (s, 6H, Me_2N-(2)), 4.87 (s, 1H, H-5).

5H-Pyrimidinium, 2,4,6-tris(dimethylamino)-5-[(*Z*)-1-(2,4,6-tris-(dimethylamino)pyrimidin-5-yl)-2-chlorovinyl]-, chloride and 5*H*−Pyrimidinium, 2,4,6-tris(dimethylamino)-5-[(*E*)-1-(2,4,6-tris-(dimethylamino)pyrimidin-5-yl)-2-chlorovinyl]-, chloride [4(*Z*) and 4(*E*)]. Solution of **2** (1.5 mmol in 10 mL CH_2Cl_2) was added dropwise to a mixture of **1** (1 mmol) and NEt_3 (1.5 mmol) in CH_2Cl_2 (40 mL) at 0−5 °C. The reaction mixture was stirred at 0−5 °C for 8 h and then it was allowed to warm to ambient temperature and was stirred for 3 h. After evaporation of the solvent, the residue was stirred with diethyl ether and the precipitate was collected by filtration. The precipitate was dissolved in CH_2Cl_2, washed with water, dried in vacuo to give **4**: yield 47%; mp 207−208 °C (after recrystallization from acetone); ^1H NMR (500 MHz, DMSO-d_6, 30 °C): **4**(*Z*) δ 2.79 (s, 12H, $Me_2N(4',6')$), 2.99 (s, 6H, $Me_2N(2')$), 3.08 (s, 6H, $Me_2N(2)$), 3.15 (s, 6H, $^yMe_2N(4,6)$), 3.40 (s, 6H, $^xMe_2N(4,6)$), 5.12 (s, 1H, H-5), 7.87 (s, 1H, H-8); **4**(*E*) δ 2.78 (s, 12H, $Me_2N(4',6')$), 2.99 (s, 6H, $Me_2N(2')$), 3.09 (s, 6H, $Me_2N(2)$), 3.16 (s, 6H, $^yMe_2N(4,6)$), 3.38 (s, 6H, $^xMe_2N$-(4,6)), 5.34 (s, 1H, H-5), 7.31 (s, 1H, H-8); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): **4**(*Z*) δ 35.8 $Me_2N(2)$, 36.7 $Me_2N(2')$, 38.3 $^yMe_2N(4,6)$, 38.4 $^xMe_2N(4,6)$, 41.1 $Me_2N(4',6')$, 41.8 C(5), 85.5 C(5′), 131.3 C(8), 132.9 C(7), 158.9 C(2), 161.8 C(2′), 165.2 C(4,6), 167.7 C(4′,6′); **4**(*E*) δ 35.9 $Me_2N(2)$, 36.7 $Me_2N(2')$, 38.7 $^yMe_2N(4,6)$, 38.8 $^xMe_2N(4,6)$, 37.5 C(5), 42.3 $Me_2N(4',6')$, 88.8 C(5′), 129.3 C(8), 129.5 C(7), 158.8 C(2), 161.5 C(2′), 165.3 C(4,6), 168.5 C(4′,6′); HR-MS (FAB): m/z [M^+] found: 477.2950, calcd for $C_{22}H_{38}N_{10}Cl$: 477.2969; anal. calcd for $C_{22}H_{38}Cl_2N_{10}$: C 51.46, H 7.46, Cl 13.81, N 27.28; found: C 51.15, H 7.56, Cl 14.08, N 27.07; Cl anion calcd: 6.92; found: 6.87.

2,4,6-Pyrimidinetriamine, *N,N,N′,N′,N″,N″*-hexamethyl-5-[1-(2,4,6-tris(dimethylamino)pyrimidin-5-yl)-2-chlorovinyl]- (5). 4 (1 mmol) was dissolved in abs EtOH followed by the addition of two equivalents of NaOEt. The precipitate was separated by filtration and then stirred with CH_2Cl_2. The solution was filtered and evaporated to give **5**: yield 39%; mp 166−167 °C; ^1H NMR (500 MHz, $CDCl_3$, 30 °C): δ 2.77 (s, 12H, $Me_2N(4',6')$), 2.79 (s, 12H, $Me_2N(4,6)$), 3.11 (s, 6H, $Me_2N(2)$), 3.12 (s, 6H, $Me_2N(2')$), 6.25 (s, 1H, H-8); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 36.1 $Me_2N(2)$, 36.5 $Me_2N(2')$, 40.8 $Me_2N(4,6)$, 41.1 $Me_2N(4',6')$, 89.7 C(5′), 94.3 C(5), 110.7 C(8), 136.2 C(7), 158.5 C(2′), 159.7 C(2), 164.7 C(4,6), 166.7 C(4′,6′); $^1H-^{13}C$ couplings: $^3J_{H(8),C(5)} = 7.4$ Hz, $^3J_{H(8),C(5')} = 3.1$ Hz; HR-MS (FAB): *m/z* [M^+-H] found: 477.2896, calcd for $C_{22}H_{38}N_{10}Cl$: 477.2969.

NMR Spectral Characterization of the Equilibrium Mixture of 6 and 7. 15 mg of **1** was dissolved in 0.8 mL of H_2O:D_2O = 9:1 solution and the pH was adjusted with aqueous HCl (pH = 4.6).

(6). ^1H NMR (500 MHz, H_2O:D_2O = 9:1, pH = 4.6, 30 °C): δ 3.10 (s, 12H, $Me_2N(4',6')$), 3.18 (s, 6H, $Me_2N(2')$) 5.05 (s, 1H, H-5′); ^{13}C NMR (125 MHz, H_2O:D_2O = 9:1, pH = 4.6, 30 °C): δ 39.6 Me_2N-(2′), 40.3 $Me_2N(4',6')$, 73.8 C(5′), 155.2 C(2′), 158.8* (measured at 50 °C; this signal is broadened to the extent that it escapes detection at 30 °C) C(4′,6′).

(7). ^1H NMR (500 MHz, H_2O:D_2O = 9:1, pH = 4.6, 30 °C): δ 3.22 (s, 6H, $^xMe_2N(4,6)$), 3.27 (s, 6H, $^yMe_2N(4,6)$), 3.30 (s, 6H, Me_2N-(2)) 3.60 (s, 2H, H_2-5); ^{13}C NMR (125 MHz, H_2O:D_2O = 9:1, pH = 4.6, 30 °C): δ 39.7 $Me_2N(2)$, 40.1 $Me_2N(4,6)$, 27.8 C(5), 165.9 C(2), 168.8 C(4,6).

Method of Titration. The NMR titration of **1** was carried out at 25 °C. A gentle steam of argon gas was passed through deionized water and D_2O for 1 h to remove dissolved gases. These degassed waters were used to produce a H_2O:D_2O = 9:1 solution containing 1.2 mM of **1** and 100 mM NaCl (99.9% purity). The pH was adjusted to 9.02 with NaOH solution and 0.8 mL of said solution was titrated with 20 mM HCl stock solution. After each addition of the titrant, the NMR tube was shaken thoroughly to ensure constant pH across the sample. The pH of the solution was measured with a Mettler-Toledo glass electrode (ELEC 6030-M3/180/IM/ BNC) and recorded with a Radelkis Laboratory Digital pH Meter (OP-211/1) before and after the NMR measurements to check for possible errors. The glass electrode was calibrated with IUPAC pH standards (potassium hydrogen phthalate, 50 mmol/dm^3, pH 4.005 ± 0.010, 25 °C and disodium hydrogen phosphate, 27.5 mmol/dm^3, pH 7.000 ± 0.010, 25 °C). Under the applied conditions, the small amount of **1** or the small volume of titrant do not contribute much to the ionic strength, and as such the data were not corrected to zero ionic strength and activity corrections were not applied.

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Supporting Information Available: ROESY spectra of **1**, derivation of eqs 8 and 10 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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