Selective Hydrolysis of 2,4-Diaminopyrimidine Systems: A Theoretical and Experimental Insight into an Old Rule

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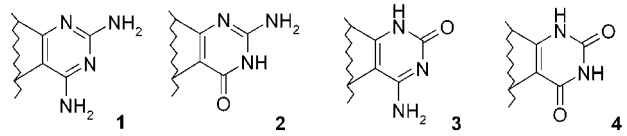
Hydrolysis of the amino groups in condensed 2,4-diaminopyrimidine systems (**1**) has been used as a common method for the synthesis of oxo-substituted pyrimidines. In particular, the treatment with 6 M HCl usually yields exclusively the 2-amino-4-oxopyrimidine isomer (**2**). During our work, we found that the hydrolysis of the amino groups present in some condensed 2,4-diaminopyrimidine systems unexpectedly afforded exclusively the 4-amino-2-oxopyrimidine isomer (**3**). In this paper, we present the experimental work and ab initio calculations carried out to understand this discrepancy. As a part of such study, eight compounds containing a 2,4-diaminopyrimidine moiety were calculated in gas phase and in aqueous solution, and some acid hydrolyses were reexamined. Results showed that the presence of an electron-donating nitrogen linked to C6 of the 2,4-diaminopyrimidine ring changes the preferred hydrolysis site to yield the 4-amino-2-oxopyrimidine isomer.

Introduction

The development of inhibitors of the enzyme dihydrofolate reductase has been a hot topic since the elucidation of the structure of folic acid, more than 50 years ago. The synthesis of the so-called "antifolates", represented by Methotrexate, has continued over these decades, promoted by successful application in the treatment of certain tumors. The presence of the 2,4-diaminopyrimidine motif (**1**) or its mono- or dioxo derivatives (**2**−**4**) is a common characteristic of these compounds (Scheme 1). Similar amino, or oxo derivatives of the pyrimidine cores are commonly found in many other biologically active molecules, such as antimalarial pyrimethamines[1] or antitumoral quinolines,[2] among others.

Selective hydrolysis of the amino groups present in a 2,4-diaminopyrimidine system (**1**) has been widely used as a method for the synthesis of oxo-substituted pyrimidines. This reaction has been extensively studied, and the general trends found are commonly used as rules of thumb in organic synthesis.[3,4] Hydrolysis products depend on the nature of the substituents in the pyrimidine ring or, alternatively, in a fused ring. In most cases, the 2-aminopyrimidin-4-ones (**2**) are formed exclusively. In other cases, the difference in reactivity between the 2-amino and the 4-amino groups is enough to stop the hydrolysis after the 2-aminopyrimidin-4-one (**2**) is formed. If the reaction is allowed to proceed further, usually both amine groups are hydrolyzed and the dioxo derivative (**4**) is formed; in some cases, mixtures of 4-oxo (**2**) and 2-oxo (**3**) products have been reported. More interestingly, selective hydrolyses affording solely 4-aminopyrimidin-2-ones (**3**) have not been described to date, to the knowledge of the authors.

Scheme 1



A number of studies can be found in the literature that deal with this hydrolysis. Taylor and Cain[5,6] studied the reactivity of a series of 2,4-diaminopteridines **5a**−**c** (**a**: $G^1 = G^2 = H$; **b**: $G^1 = G^2 = Me$; **c**: $G^1 = G^2 = Ph$) (Scheme 2) showing that the 4-amino group was selectively hydrolyzed in boiling 6 M HCl. The results also showed that, on the contrary, treatment with $NaNO_2/H_2SO_4$ caused the diazotization of the 2-amino group, yielding the corresponding 4-amino-2-oxopyrimidine system. The authors concluded that in an acidic medium, these compounds would predominantly exist as a sole tautomer in which the 4-amino group will present the imino form, easily hydrolyzable, but stable in front of nitrous acid. Thus, 6 M HCl usually affords the corresponding 2-amino-4-oxopyrimidine system (**2**).

Trattner et al. systematically explored the hydrolysis products of a series of compounds containing the 2,4-diaminopyrimidine moiety (**6**−**9**) (Scheme 2).[7] The authors found that the 4-amino group is hydrolyzed faster in all cases, and only the corresponding 4-oxopyrimidines were identified as the hydrolysis products after the first

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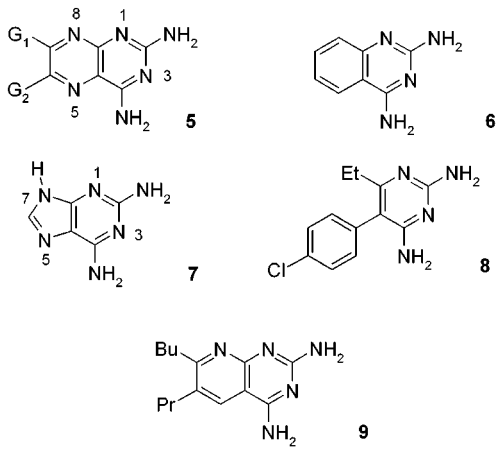
Scheme 2



hours of heating. After 24 h of reflux, the second amino group was also hydrolyzed in the case of systems **7** and **9**, but no significant secondary hydrolysis was found for compounds **6** and **8**. Trattner et al. suggested (Scheme 3) that the reactive species for the acid hydrolysis was the protonated form of the pyrimidine ring. According to the authors, protonation at N-1 is preferred over the protonation at N-3, due to the possible formation of a *p*-quinoid form that can accommodate the positive charge on the amino group linked to C-4 (note that only a less-favored *o*-quinoid form is possible if N-3 is protonated). They propose that the reaction may occur in two steps, by the addition of water to the protonated form, or in one step, by simultaneous addition of a proton and water. Among all the tetrahedral intermediates formed by the attack of water at the amino groups, the authors suggested that the attack at C-4 is again stabilized by a *p*-quinoid mesomeric form, in contrast to a less-favored *o*-quinoid form that would originate from the attack at C-2. Consequently, both the favored protonation at N-1 and the favored attack of water at C-4 are responsible for the formation of the 4-oxo product. Trattner's conclusions and results have been extensively used in the literature to rationalize the formation of 2-amino-4-oxopyrimidines by acid hydrolysis of compounds containing the 2,4-diaminopyrimidine substructure **1**.

In a later work, Griffin et al.[8,9] reexamined the hydrolysis of **8** showing that, in disagreement with Trattner's results, a mixture of the 2-oxo and 4-oxo isomers was formed in a 2:1 ratio. Griffin et al. suggested that both N-1 and N-3 are probably protonated in 6 M HCl, and that the attack at C-2 is favored by the shielding effect of the hydrophobic 4-chlorophenyl group, which hinders the nucleophilic attack by water to C-4. In the same work, various combinations of substituents on the aryl ring were tested, which afforded in all cases mixtures of the 2-oxo and 4-oxo derivatives in different ratios. It is interesting to note pyrimethamine **8** is the only monocyclic 2,4-diaminopyrimidine system considered by Trattner.

As a part of our work concerning the synthesis of folic acid analogues,[10] we needed an unequivocal method to obtain 4-amino-1,5,6,8-tetrahydropyrido[2,3-*d*]pyrimidine-4,7-diones (**11**). Taking the aforementioned results into account, we carried out the treatment of the 2,4-diaminopyrido[2,3-*d*]pyrimidine **10a** (R1 = Me, R2 = H) with 6 M HCl (Scheme 4). The reaction afforded a carbonyl compound, to which the 4-oxo-substituted structure **11a** was initially assigned. However, we proved by an independent synthesis that it was in fact the 4-amino-2-oxopyrido[2,3-*d*]pyrimidine **12a**, in other words, the hydrolysis proceeded on the theoretically less reactive amino group contrary to the prediction of Trattner. No traces of the corresponding 2-amino-4-oxo isomer were detected. We confirmed this behavior for the whole family of 2,4-diaminopyrido[2,3-*d*]pyrimidines **10a**−**d**, it being necessary to change the approach to obtain the desired compounds **11a**−**d**. In fact, **11a**−**d** were obtained by treating Michael adducts **13a**−**d** with guanidine in MeOH at reflux.

That unexpected result led us to perform a combined experimental and theoretical study to gain a better understanding of the acid hydrolysis of 2,4-diaminopyrimidines and find out which structural features predominantly affect this reaction. The results of this study follow.

Theoretical Methods and Computational Details

Following the mechanism proposed by Trattner, the first step in this study was to estimate the ease of protonation at different sites and explore the effect of the substituents and the solvent. A theoretical treatment of the kinetics of the hydrolysis reaction is out of the scope of this paper, although we accept that possibly this could be significant in some cases. It would be considered in a subsequent work if found relevant. Due to the importance of the protonation of pyrimidinic nitrogens on the hydrolysis of 2,4-diaminopyrimidines, the relative stability of different possible N-protonated species was studied by means of ab initio quantum mechanical methods.[11] Differences in basicity between various protonation sites within a given molecule can be calculated from the free energies of the various protonated terms because the neutral species is common to all of them. Actually, the relative stabilities between protonated terms give us the description of the prototropic tautomerism in the resulting cationic system. The free energy difference in solution between two protonated terms A and B (ΔG_{tot}) can be calculated as the sum of the relative free energy in gas

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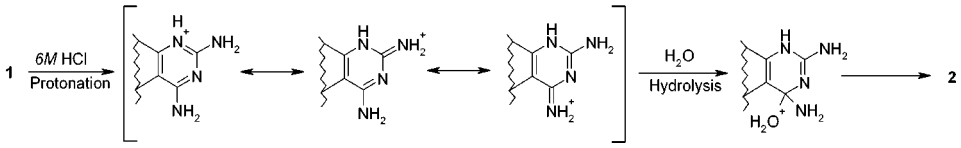
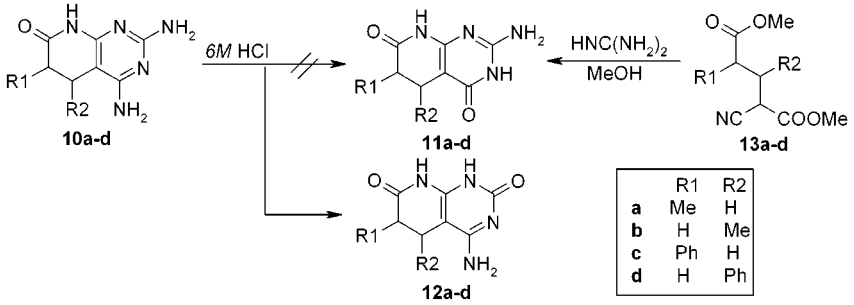
Scheme 3



Scheme 4



phase (ΔG_{gas}) and the relative free energy of solvation (ΔG_{sol}) as shown in eq 1.

$$\Delta G_{tot} = \Delta G_{gas} + \Delta G_{sol} \quad (1)$$

Gas-phase calculations were performed using the gas-phase geometries optimized at the HF/6-31G(d) level. Frequency analysis was carried out to verify the minimum-energy nature of the optimized structures. Single-point calculations were performed at the HF/6-311G(d,p) level. Correlation effects were considered from MP2 calculations performed with the same basis. Zero-point energies and thermal and entropic corrections (298 K) were evaluated from the HF/6-31G(d) frequency calculations in the rigid rotor-harmonic approximation. All gas-phase calculations were done using the Gaussian94 computer program.[11a]

Within the Polarizable Continuum Methods (PCM),[12] the free energy of solvation (ΔG_{sol}) is usually treated as a sum of steric terms (cavitation and van der Waals contributions) and nonsteric terms (electrostatic contribution, ΔG_{ele}). In this paper, only differences in free energy of solvation ($\Delta\Delta G_{sol}$) between differently charged tautomers are intended. Since the structures of the compared species were very similar (tautomers), and the electrostatic contribution is by far the leading term in charged molecules, using $\Delta\Delta G_{ele}$ to approximate $\Delta\Delta G_{sol}$ was a reasonable choice. The effect of water solvation was introduced using the PCM method developed by the Pisa group, as implemented in the Gaussian94 program. As mentioned above, only the electrostatic contribution was calculated, using a dielectric constant of 78.3 and a grid density of 200 points.

Results and Discussion

The first stage in our work was to establish the structural differences between the compounds selected by Trattner (Scheme 2) and pyrido[2,3-*d*]pyrimidines **10** that can be responsible of their unusual behavior upon hydrolysis. A simple observation of structures **5**−**9** reveals that they present, with the exception of **8** for which an anomalous behavior has been recently reported (see above), a 2,4-diaminopyrimidine ring fused to an aromatic ring through the C5−C6 bond. In some cases (**5**, **7**, and **9**) the fused ring contains a nitrogen atom that is bonded to C6 of the pyrimidine ring.

On the contrary, compounds **10** contain a tetrahydropyridone ring fused to the 2,4-diaminopyrimidine through the C5−C6 bond of this later. To determine if the abnormal behavior of compounds **10** was due to the electron-withdrawing effect of the carbonyl group on the nitrogen atom directly linked to the 2,4-diaminopyrimidine moiety, we decided to reduce such carbonyl group in **10a** and to study the acid hydrolysis of the resulting compound. Thus, **10a** was treated with borane in THF to afford 2,4-diamino-6-methyl-5,6,7,8-tetrahydropyrido-[2,3-*d*]pyrimidine (**14**) (Scheme 5). Compound **14** was later hydrolyzed in 6 M HCl, yielding a mixture of unreacted **14** and the 4-amino-2-oxo derivative **15**. This assignment was done by comparison of the ^{1}H and ^{13}C NMR spectra of the hydrolysis product and those of 4-amino-2-oxo-6-methyl-5,6,7,8-tetrahydro[2,3-*d*]pyrimidine (**15**) and 2-amino-4-oxo-6-methyl-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (**16**), unequivocally obtained from **17** and **11a**, respectively (Scheme 5). Thus, reduction of the 2-bromo substituted pyrido[2,3-*d*]pyrimidine **17** with BH_3·THF afforded **18** that was converted to **15** by hydrolysis with boiling hydrochloric acid. On the other hand, the selective reduction of the 7-oxo group present in 2-amino-6-methyl-3,5,6,8-tetrahydropyrido[2,3-*d*]pyrimidine-4,7-dione (**11a**) with BH_3·THF unequivocally yielded **16**.

The absence of a detectable hydrolysis of the 4-amino group in **10a** and **14** suggests that the amide nitrogen in **10a** and the amine nitrogen in **14** are acting as electron-donating groups respect to the 2,4-diaminopyrimidine ring, regardless of the presence of the carbonyl group in **10a**. This finding is in agreement with the presence of a relatively high-frequency C═O stretching band in the IR spectrum of **10a** (1695 cm^{-1}) and the X-ray data obtained for a quite similar 1,6-naphthyridine, which show a CO−N distance (1.362 Å) longer than normal for a simple amide.[13] All these observations point out to the electron-donating effect of the nitrogen atom bonded to C6 of the pyrimidine ring as the responsible

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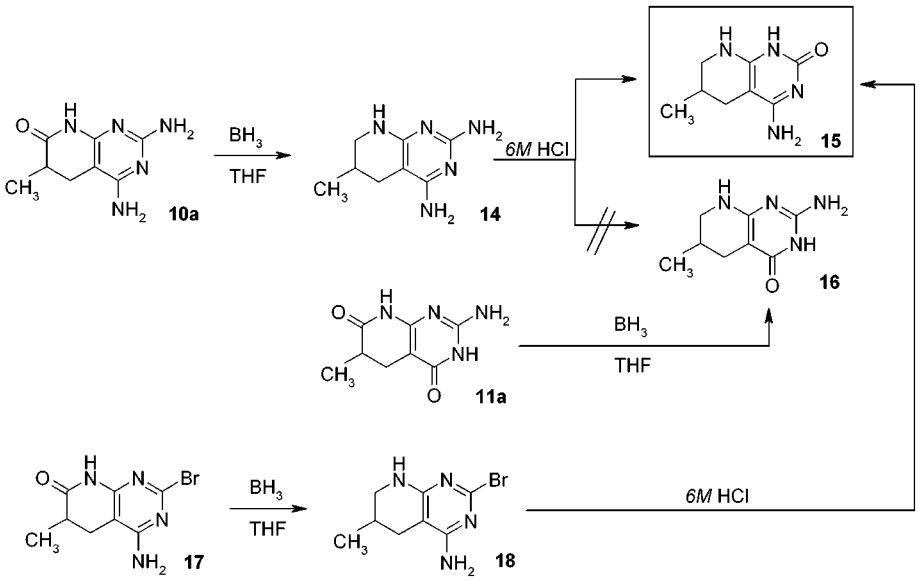
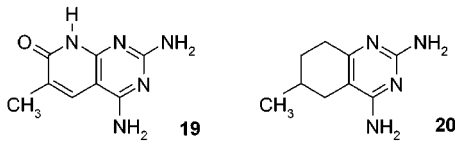
Scheme 5



Scheme 6



of the abnormal behavior of **10a** (and **14**) upon hydrolysis. The computational work that follows tried to relate this hypothesis with theoretical considerations.

Our results for the hydrolysis of **10a** and **14**, and the results described in the literature for the hydrolysis of compounds **5**−**9**, constituted the basis of our theoretical study.[14] Structures **19**, which differs from **10a** only in a double bond, and **20**, presenting a cyclohexane fused ring, were added as model compounds for calculation purposes only. They should help to determine the factors that control the unusual behavior of **10a** during the acid hydrolysis (Scheme 6).

Gas-Phase Calculations. Free energy differences in gas phase for different protonated terms of structures **5b**−**9**, **10a**, **14**, **19**, and **20** are shown in Table 1 (all results are relative to the protonation at N-1). The three levels of theory used here lead to similar conclusions, being, in general, MP2 values lower than the HF values.

Compounds **5b**, **6**, **8**, **9**, and **20** present similar characteristics upon protonation at the nitrogen atoms of the pyrimidine ring. For these molecules, protonation at N-1 is clearly preferred over all other heterocyclic nitrogens. In particular, protonation at N-3 is disfavored by more than 8 kcal/mol in all cases (MP2 values are cited if not stated otherwise). Protonation at N-8 of **5b** and **9** is predicted to be less favorable than protonation at N-1, by 5 and 2.5 kcal/mol, respectively, the slight difference between them being attributed to the ability of **9** to better delocalize the positive charge introduced by protonation at N-8. More unstable is the N-5 protonation term of **5b**, due to the interaction of the proton with the hydrogen atoms of the amine group bound to C-4. Comparison of **6** and **20** indicate that the presence of aromaticity in the fused ring does not change the preferred protonation site. A comparison of **5b** and **9** with **6** clearly shows that the presence of nitrogen atoms on the aromatic ring does not change the predilection for protonation at N-1.

In the case of purine **7**, protonation at N-1 in the N5−H tautomer[14] is the most stable one. It is interesting to see the effect of the tautomerism of the imidazole ring on the stability of the conjugated bases. Thus, in the N7−H tautomer there is almost no difference between protonation at N-3 and protonation at N-5. On the contrary, protonation at N-1 is 10 kcal/mol more stable than protonation at N-3 in the N5−H tautomer. The presence of the hydrogen atom at N-7 evens out the number of NH groups that surrounds N-1 and N-3, making them structurally more equivalent, hence, reducing the differences in basicity. On the other hand, the free energy difference for the protonation at the imidazole nitrogen is only 3 kcal/mol.

The protonation scene of systems **10a**, **14**, and **19** is quite similar. With the only exception of the hydroxypyridine form of **19**, the differences in free energy of protonated terms at N-1 and N-3 are below or equal to 1 kcal/mol. In the hydroxy form of **19**, which is in fact aromatic, the difference between the protonation at N-1 and at N-3 is close to 15 kcal/mol, in agreement with the results found for **6** (see above). Results for **19** show the preference for aromatic hydroxypyridine tautomers over pyridone tautomers; this result is commonly found when calculations are performed in gas-phase conditions. Although various conformations of the condensed ring were taken into account for **10a** and **14**, no significant variations were found between axial and equatorial positions of the methyl group. The close protonation pattern of **10** and **14** is attributable to the electron-donating character of the nitrogen linked to C6 of the 2,4-diaminopyrimidine. Such behavior is similar to the case of the N7−H

(14) To reduce the difficulties caused by the presence of flexible chains on the theoretical treatment, some simplifications have been made on the structures considered. Thus, the ethyl group present in **8** has been substituted by a methyl group. Similarly, the butyl and propyl groups present in **9** have been replaced by a hydrogen atom and a methyl group, respectively. These simplifications are not expected to affect the conclusions of this work. On the other hand, the numbering of the nitrogen atoms present in **7** (Scheme 2) was adopted for the sake of clarity and consistency within the whole series of compounds studied in this paper. Strictly speaking, **7** should be referred to as 2,6-diaminopurine and not as a 2,4-diamino-substituted compound, owing to the commonly accepted numbering for the purine skeleton.

Table 1. Gas Phase Differences[a] of Free Energies (kcal/mol) of Protonated Diaminopyrimidines. N-1, N-3, N-5, and N-8 Refer to Different Protonation Sites. Values in Parentheses Correspond to Other Tautomers Than Those Depicted in Scheme 2 (see notes below for each case)

	N-1			N-3			N-5			N-8		
method →	A	B	C	A	B	C	A	B	C	A	B	C
5b	0.0	0.0	0.0	16.1	15.5	14.7	30.3	29.8	21.5	9.0	9.1	5.0
6	0.0	0.0	0.0	14.7	14.3	11.8						
7	0.0	0.0	0.0	0.3	0.1	0.1	5.7	5.3	3.1			
	(−1.8)[b]	(−1.9)[b]	(−1.5)[b]	(11.4)[b]	(10.7)[b]	(10.9)[b]						
8	0.0	0.0	0.0	8.9	8.7	8.0						
9	0.0	0.0	0.0	20.3	19.9	18.1				3.0	2.8	2.5
10a	0.0[c]	0.0[c]	0.0[c]	0.1[c]	0.0[c]	0.0[c]						
	(1.4)[d]	(1.3)[d]	(0.3)[d]	(1.4)[d]	(1.2)[d]	(0.2)[d]						
19	0.0	0.0	0.0	0.8	0.7	1.0						
	(−8.5)[e]	(−9.7)[e]	(−10.7)[e]	(7.9)[e]	(6.2)[e]	(3.9)[e]				(−0.4)[e]	(−1.6)[e]	(−3.1)[e]
14	0.0[c]	0.0[c]	0.0[c]	0.2[c]	0.3[c]	−0.6[c]						
	(1.5)[d]	(1.6)[d]	(0.4)[d]	(1.7)[d]	(1.8)[d]	(−0.3)[d]						
20	0.0	0.0	0.0	12.3	12.0	11.1						

A: HF/6-31G(g). B: HF/6-311G(d,p). C: MP2/6-311G(d,p). [a] All values are relative to N-1 protonated forms. [b] Tautomer where N-5 bears the hydrogen atom. [c] Methyl group in equatorial position. [d] Methyl group in axial position. [e] Hydroxy form.

Table 2. Differences of Free Energies (kcal/mol) of Solvation ($\Delta\Delta G_{sol}$) and Free Energies of Protonation in Aqueous Solution ($(\Delta\Delta G_{tot})$.[a] N-1, N-3, N-5, and N-8 Refer to Different Protonation Sites. Values in Parentheses Correspond to Other Tautomers Than Those Depicted in Scheme 2 (see notes below for each case)

	N-1		N-3		N-5		N-8	
	$\Delta\Delta G_{sol}$	$\Delta\Delta G_{tot}$	$\Delta\Delta G_{sol}$	$\Delta\Delta G_{tot}$	$\Delta\Delta G_{sol}$	$\Delta\Delta G_{tot}$	$\Delta\Delta G_{sol}$	$\Delta\Delta G_{tot}$
5b	0.0	0.0	−8.8	5.9	−10.4	11.1	−0.9	4.1
6	0.0	0.0	−4.8	7.0				
7	0.0	0.0	−0.9	−0.8	−3.5	−0.4		
	(2.1)[b]	(0.6)[b]	(−7.0)[b]	(3.9)[b]				
8	0.0	0.0	−3.6	4.4				
9	0.0	0.0	−9.3	8.8			0.0	2.5
10a	0.0[c]	0.0[c]	−1.6[c]	−1.6[c]				
	(0.4)[d]	(0.7)[d]	(−1.3)[d]	(−1.1)[d]				
19	0.0	0.0	−0.4	0.6				
	(10.6)[e]	(−0.1)[e]	(1.3)[e]	(5.2)[e]			(8.7)[e]	(5.6)[e]
14	0.0[c]	0.0[c]	0.7[c]	0.1[c]				
	(0.1)[d]	(0.5)[d]	(2.3)[d]	(2.1)[d]				
20	0.0	0.0	−3.9	7.2				

[a] All values are relative to N-1 protonated forms. $\Delta\Delta G_{tot}$ derived using MP2/6-311G(d,p) results as $\Delta\Delta G_{gas}$ values (column C in Table 1). [b] Tautomer where N-5 bears the hydrogen atom. [c] Methyl group in equatorial position. [d] Methyl group in axial position. [e] Hydroxy form.

tautomer of **7** (see above). In the case of **14**, the N-3 protonated term is slightly more stable than the one resulting from protonation at N-1 (−0.6 kcal/mol). Although the presence of a carbonyl group in **10a** does not block the donating capabilities of the nitrogen atom, both protonations are estimated to be, in this case, equally favored. Finally, the presence of an extra double bond in **19** slightly hinders the donating effect of the nitrogen atom to the pyrimidine ring and, as a result, the relative stability between the N-1 and the N-3 protonated terms is reversed, being now 1 kcal/mol favorable to the protonation at N-1.

Calculations in Solution. Combination of the gas-phase results with the free energies of solvation yield the free energies in solution. Table 2 presents the differences in free energy of solvation ($\Delta\Delta G_{sol}$) and the relative free energy in aqueous solution ($\Delta\Delta G_{tot}$) for the conjugated bases studied here. $\Delta\Delta G_{sol}$ values give an idea of the relative stability gain of the various protonated terms due to the interaction with an aqueous medium. For example, a value of $\Delta\Delta G_{sol}$ close to zero means a very similar stabilization upon solvation, while a large and negative value indicates a clearly better solvation with respect to the reference compound.

In general, protonation at N-3 provides better solvated products than protonation at N-1. This is in agreement with the chemical intuition that predicts a better solvation for more polar tautomers. Note that if the protonation occurs at N-3, the new positive charge, flanked by two amino groups, generates a clear positive zone, which gives rise to a strong molecular dipole moment. This is evident in **5b**, **9**, and the N5−H tautomer of **7**, with nitrogen atoms in the fused ring, where the hydration of the N-3 protonated term is favored by more than 7 kcal/mol. The same effect can be seen for **6**, **8**, and **20** and for the protonation at N-5 of **5b** and **7**. This effect is not so clearly present in **10**, **19**, and **14**, and, therefore, the differences in free energies of solvation are not marked. Finally, the hydroxy form of **19** shows again a marked preference for the solvation of the N-3 protonated term.

Despite the large differences of free energy of solvation in favor of protonation at N-3, the values obtained in the gas phase, which actually reflects the intrinsic stability of the protonated products, are decisive in the case of systems **5b**, **6**, **8**, **9**, and **20**. These molecules are estimated to protonate preferentially at N-1 in aqueous solution (i.e., the rest of the protonated products have positive $\Delta\Delta G_{tot}$'s). In the case of purine (**7**), the solvent effect changes the preferred protonated species with respect to the gas phase. While protonation at N-1 of the N5−H tautomer was the most favored in gas phase, its poor relative solvation, together with the advantageous solvation of the N-3 protonated form of the N7−H tautomer, lead to the preference for the latter. It is

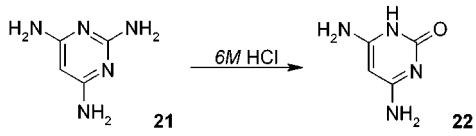
interesting to point out the effect of water solvation in the N-5 protonated form of **7**; the relative hydration effect is high enough to turn a positive 3.1 kcal/mol, with respect to protonation at N-1, into a negative −0.4 kcal/mol. This emphasizes the decisive role the solvent can play in particular cases. The solvent influence is also crucial in the protonation of the pyridopyrimidine **10a**, pointing to the protonation at N-3 as the most probable protonation site due to a better solvation. Differences for systems **19** and **20** are below 1 kcal/mol in favor of protonation at N-1. Due to the small differences found for systems **7**, **14**, and **19** (or even **10a**), caution is necessary in the estimation of preferred protonated species of these compounds. The accuracy of the theoretical estimates in solution depends on the subtle balance of the gas phase and the solvation results. The level of theory used here is expected to give a good picture of the free energy differences between different tautomers in the gas phase. On the other hand, it is known that the estimation of free energies of solvation of charged species is not as accurate as for neutral molecules. Overall, present results clearly suggest, presumably beyond the uncertainties introduced by the theoretical methods, that protonation at N-1 is preferred in aqueous solution for systems **5b**, **6**, **8**, **9**, and **20** while the tendency is not so clear in the case of **7**, **10a**, **19**, and **14**.

As we mentioned before, the available experimental results reveal different behaviors for the acid hydrolysis in 6 M HCl of 2,4-diaminopyrimidines. While pteridine **5b** and quinazoline **6** seem to hydrolyze exclusively to the 4-oxo product, pyridopyrimidine **9** hydrolyzes first to the 4-oxo product, in less than 30 min, and later to the 2,4-dioxo product. Present theoretical results predict that these compounds protonate preferentially at N-1 leading, according to the mechanism suggested by Trattner et al., to the hydrolysis of the C-4 amino group. Protonation of pyrimidine **8** is also estimated to occur at N-1, in good agreement with experimental data found in the literature,[15] suggesting a selective hydrolysis to the 4-oxo product. However, according to Griffin et al., hydrolysis of **8** yields a mixture of the 2-oxo and the 4-oxo products due to kinetic control; the attack of water at C-4 is more difficult than at C-2 due to the shielding effect of the *p*-chlorophenyl moiety. Although no experimental data were found for the hydrolysis of **20**, the calculated preference for protonation at N-1 unequivocally suggests the predominance of the 4-oxo product.

Hydrolysis of purine **7** in 6 M HCl is considerably slower than the above-described hydrolyses.[7] At the end of 6 h, a mixture of guanine (4-oxo) and xanthine (dioxo product) was detected, which turned into only xanthine at the end of 24 h. Present calculations do not indicate a clear preference for any of the protonation sites, and, in light of the above-proposed relationship, this could lead to the lack of selectivity of the hydrolysis reaction. Besides a possible kinetic control, some probable causes for this singularity are the tautomeric capabilities of the neutral base and the possible protonation at the nitrogen of the imidazole ring.

Pyridopyrimidines **10a** and **14** show a similar behavior upon hydrolysis. In both cases the 2-amino group is selectively hydrolyzed. Estimated protonation patterns show a slightly preference for protonation at N-3 in **10a**, and no preference in **14**. These results point at the presence of an electron-donating nitrogen linked to C6 of the pyrimidine ring as the reason for the similar stability of the N-1 and N-3 conjugated bases. System **19** provides a good example of this effect. While in the pyridone tautomer no significant protonation preference is evident, in the aromatic hydroxypyridine form protonation at N-1 is favored by ca. 5.3 kcal/mol. Unfortunately, no experimental hydrolysis results are available for **19**. However, according to the present prediction, **19** should afford the 4-oxo product via protonation at N-1 of the hydroxypyridine tautomer, or the 2-oxo product, via the pyridone tautomer.

Scheme 7



In the framework of the thermodynamic analysis performed here, the stability of the hydrolysis products was estimated at the HF/6-31G(d) level for selected systems **6** and **10a** (data not shown). For system **6**, the 4-oxo compound has the lowest free energy in the gas phase (by ca. 4 kcal/mol). On the contrary, the 2-oxo compound is more stable in aqueous solution by almost 5 kcal/mol. Consequently, the gas phase trend is reversed and the 2-oxo hydrolysis product is globally favored by less than 1 kcal/mol. Differences in free energy in the gas phase for hydrolysis products of quinazoline **10a** showed that the 4-oxo isomer was preferred over the 2-oxo isomer by ca. 8 kcal/mol. A better solvation of the 2-oxo product reduced the difference of free energy in aqueous solution, but did not chang the preference for the 4-oxo isomer, by c.a. 3 kcal/mol. The fact that the most favored hydrolysis product does not correspond to the experimentally detected product, neither for **6** nor for **10a**, suggests that the stability of the final product is not controlling the hydrolysis course.

Further investigations were performed to study the selectivity for the 2-oxo product in the hydrolysis of **10a** and **14**. Since the NH group in the fused ring of **10a** and **14** is equivalent to an amine group at C-6 of a pyrimidine ring, the hydrolysis of the model compound 2,4,6-triaminopyrimidine **21** was carried out (Scheme 7). Note that the two heterocyclic nitrogens of **21** are equivalent by symmetry, and therefore there are not protonation preferences. Hydrolysis of **21** in 6 M HCl selectively yielded the 4,6-diamino-2-pyrimidinone (**22**), as it was established by comparison with the commercially available 4-oxo isomer (2,6-diamino-4-pyrimidinone). This result could only be explained by a kinetic control of the hydrolysis or by the stability of the 2-oxo product **22** in aqueous solution. Free energy values calculated at the same highest level of theory used here showed that the 4-oxo isomer is the most stable compound in water solution, followed at 1.5 kcal/mol by the 2-oxo isomer and then the corresponding enolic forms. Taking into account the equal ease of protonation at N-1 and N-3, this result points at a kinetic origin for the predominance of the 2-oxo product in the hydrolysis reaction of **21**. This result suggests that the similar hydrolysis behavior of pyridopyrimidine **10a** is due to the marked aminic character of the lactam NH group. Global results suggest that, in this case, the selective formation of the 2-oxo product can be attributed to kinetic control.

(15) Philips, T.; Bryan, R. F. *Acta Crystallogr.* **1969**, *A25*, S200.

Conclusions

Combination of experimental work and theoretical calculations constitutes a powerful tool to study chemical reactivity. In this work, thermodynamic estimates from quantum mechanical calculations including the effect of the solvent provides a good picture of the stability of different protonated intermediates and some hydrolysis products. In this sense, out of the possible range of error of our calculations, present results for the hydrolysis of 2,4-diaminopyrimidines **5b**, **6**, **8**, **9**, and **20** in 6 M HCl indicate that N-1 is the preferred protonation site in aqueous solution. Excluding the amino groups attached to C-2 and C-4, these systems do not include any other electron-donor to the pyrimidine ring. Except for system **8**, Trattner's postulated mechanism is able to predict the faster or selective formation of the 4-oxo hydrolysis product from the theoretically predicted preferred protonation sites. The case of **8** could be possibly explained arguing a kinetic control, based on the nonplanarity of the chlorophenyl moiety.

On the contrary, the acid hydrolysis of 2,4-diaminopyrimidines bearing a substituent at C-6 able to donate charge density to the pyrimidine ring, such as **10a** and **21**, affords the 2-oxo product.

Rationalization of the overall results, in light of the mechanism proposed by Trattner in acidic media, allows us to confirm that those structures presenting a N-1 preferred protonation site lead to the predominant formation of the 4-oxo hydrolysis products. On the contrary, when both nitrogen atoms have similar protonation characteristics (probably due to the presence of an electron-donating group at C-6) the hydrolysis takes place preferably or exclusively at C-2 due to kinetic factors. Intermediate structures are expected to show midway behavior.

Experimental Section

All melting points were determined with a Büchi 530 capillary apparatus and are uncorrected. Infrared spectra were recorded in a Nicolet Magna 560 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were determined in a Varian Gemini-300 operating in a field strength of 300 and 75.5 MHz, respectively. Chemical shifts are reported in parts per million (δ) and coupling constants (J) in Hz, using, in the case of ^{1}H NMR, sodium 2,2,3,3-tetradeuteriotrimethylsilylpropionate as an internal standard and setting, in the case of ^{13}C NMR, the references at the signal of the solvent (163.8 ppm, CF_3COOD, TFA-*d*). Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; brs, broad singlet; br, broad signal; m, multiplet. Elemental microanalyses were obtained in a Carlo-Erba CHNS-O/EA 1108 analyzer and gave results for the elements stated with ±0.4% of the theoretical values. Tetrahydrofuran (THF) was distilled from $LiAlH_4$ and kept over 4-Å molecular sieves. Thin-layer chromatography (TLC) was performed on precoated sheets of silica 60 Polygram SIL N−HR/UV$_{254}$ (Macherey Nagel art. 804023). 2,4,6-triaminopyrimidine (**21**) and 2,6-diamino-4-pyrimidinone were obtained from Fluka (ref 90581 and ref 33050, respectively). High performance liquid chromatography (HPLC) were performed on RP-8 Chromasil columns, at room temperature, with UV detector (254 nm), a rate of 1 mL/min, and using acetonitrile/water (40/60) as the mobile phase. Compounds **10a**, **11a**, and **17** were prepared according to reported procedures.[16−19]

2,4-Diamino-6-methyl-5,6,7,8-tetrahydropyrido[2,3-*d*]-pyrimidine (14). Pyridopyrimidine **10a** (1.93 g, 10 mmol) was suspended in 20 mL of anhydrous THF under nitrogen, and 30 mL (30 mmol) of 1 M BH_3 in THF was then slowly added. During the addition, hydrogen was evolved, and the solid was completely solved. The mixture was refluxed for 6 h and then allowed to cool. The solvent was eliminated in vacuo, and the residue was treated with 50 mL of 6 M HCl for 30 min at room temperature in order to destroy the complex. The resulting solution was neutralized (pH 7−8) with 6 M NaOH. The solid obtained was filtered off, washed with THF, and dried over P_2O_5 to afford 1.74 g (97%) of **14** as a white solid: mp >300 °C; IR (KBr) 3484, 3390, 3334 (NH), 1666, 1633, 1601, 1578 cm^{-1} (C═C, C═N, *f*. N−H); ^{1}H NMR (TFA-*d*), δ 3.61−3.57 (m, 1H, H7), 3.11 (dd, ^2J = 12.9 Hz, ^3J = 9 Hz, 1H, H7), 2.60−2.56 (m, 1H, H5), 2.10 (m, 1H, H5), 2.06 (m, 1H, H6), 1.14 (d, ^3J = 6 Hz, 3H, Me); ^{13}C NMR (TFA-*d*), δ 153.2 (C4), 152.7 (C2), 151.6 (C8a), 83.1 (C4a), 49.3 (C7), 27.4 (C5), 26.9 (C6), 18.4 (Me). HPLC retention time 5.94 min. Anal. Calcd for $C_8H_{13}N_5$: C, 53.61; H, 7.31; N, 39.08. Found: C, 53.50; H, 7.35; N, 38.84.

Hydrolysis of 2,4-Diamino-6-methyl-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (14) in 6 M HCl: 4-Amino-2-oxo-6-methyl-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (15). Pyridopyrimidine **14** (1 g, 5.6 mmol) was suspended in 20 mL of 6 M HCl and heated at reflux for 48 h. The mixture was allowed to cool and was neutralized (pH 7−8) with 6 M NaOH. Half of the water was removed under reduced pressure, and the resulting solid was filtered off, washed with THF, and dried over P_2O_5 to afford 0.95 g of a mixture of **14** (45%) and **15** (55%) (evaluated by HPLC) as a white solid: IR (KBr) 3417, 3386, 3334 (NH), 1656 (C═O), 1606, 1580, 1545 cm^{-1} (C═C, C═N, *f*. N−H); ^{1}H NMR (TFA-*d*), δ 3.59 (m, 1H, H7), 3.12 (m, 1H, H7), 2.58 (m, 1H, H5), 2.13 (m, 2H, H5 and H6), 1.14 (m, 3H, Me); HPLC retention time 2.58 and 5.94 min.

4-Amino-2-bromo-6-methyl-5,6,7,8-tetrahydropyrido-[2,3-*d*]pyrimidine (18). Pyridopyrimidine **17** (2.57 g, 10 mmol) was suspended in 20 mL of anhydrous THF under nitrogen, and 30 mL (30 mmol) of 1 M BH_3 in THF was then added slowly. During the addition, hydrogen was evolved and the solid was completely solved. The mixture was refluxed for 6 h and then was allowed to cool. The solvent was eliminated in vacuo, and the residue was treated with 50 mL of 6 N HCl for 30 min at room temperature in order to destroy the complex. The resulting solution was neutralized (pH 7−8) with 6 M NaOH. The solid obtained was filtered off, washed with THF, and dried over P_2O_5 to afford 2.28 g (94%) of **18** as a white solid: mp >300 °C; IR (KBr) 3491, 3249, 3137 (NH), 1642, 1601, 1543 cm^{-1} (C═C, C═N, *f*. N−H); ^{1}H NMR (TFA-*d*), δ 3.54−3.50 (m, 1H, H7), 3.07 (dd, ^2J = 12.9 Hz, ^3J = 9 Hz, 1H, H7), 2.54−2.50 (m, 1H, H5), 2.07 (m, 1H, H5), 2.02 (m, 1H, H6), 1.13 (d, ^3J = 6 Hz, 3H, Me); ^{13}C NMR (TFA-*d*), δ 155.0 (C4), 154.3 (C2), 152.1 (C8a), 80.6 (C4a), 48.9 (C7), 27.2 (C5), 27.0 (C6), 18.2 (Me). Anal. Calcd for $C_8H_{11}N_4Br$: C, 39.51; H, 4.56; N, 23.04. Found: C, 39.42; H, 4.59; N, 22.86.

4-Amino-2-oxo-6-methyl-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (15). Pyridopyrimidine **18** (1 g, 4.1 mmol) was suspended in 20 mL of 6 M HCl and heated at reflux for 48 h. The mixture was allowed to cool and was neutralized (pH 7−8) with 6 M NaOH. Half of the water was removed under reduced pressure, and the resulting solid was filtered off, washed with THF, and dried over P_2O_5 to afford 0.67 g (91%) of **15** as a white solid: IR (KBr) 3421 (NH), 1665 (C═O), 1637, 1543 cm^{-1} (C═C, C═N, *f*. N−H); ^{1}H NMR (TFA-*d*), δ 3.54−3.50 (m, 1H, H7), 3.07 (dd, ^2J = 12.9 Hz, ^3J = 9.6 Hz, 1H, H7), 2.54−2.50 (m, 1H, H5), 2.07 (m, 1H, H5), 2.02 (m, 1H, H6), 1.13 (d, ^3J = 6 Hz, 3H, Me); ^{13}C NMR (TFA-*d*), δ 154.9 (C4), 154.2 (C2), 151.9 (C8a), 80.4 (C4a), 48.8 (C7), 27.0 (C5), 26.9 (C6), 18.1 (Me). Anal. Calcd for $C_8H_{12}N_4O$: C, 53.32; H, 6.71; N, 31.09. Found: C, 53.21; H, 6.71; N, 30.77.

2-Amino-4-oxo-6-methyl-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (16). Pyridopyrimidine **11a** (1.94 g, 10 mmol)

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(17) Victory, P.; Garriga, M. *Heterocycles* **1985**, *23*, 2853.
(18) Victory, P.; Garriga, M. *Heterocycles* **1986**, *24*, 3053.
(19) Victory, P.; Crespo, A.; Garriga, M.; Nomen, R. *J. Heterocycl. Chem*. **1988**, *25*, 245.

was suspended in 20 mL of anhydrous THF under nitrogen, and 30 mL (30 mmol) of 1 M BH_3 in THF was then slowly added. During the addition, hydrogen was evolved and the solid was completely dissolved. The mixture was refluxed for 6 h and then allowed to cool. The solvent was eliminated in vacuo, and the residue was treated with 50 mL of 6 M HCl for 30 min at room temperature in order to destroy the complex. The resulting solution was neutralized (pH 7−8) with 6 M NaOH. The solid obtained was filtered off, washed with THF, and dried over P_2O_5 to afford 1.8 g (100%) of **16** as a white solid: mp >300 °C; IR (KBr) 3473, 3318, 3264, 3170 (NH and OH), 1652, 1598, 1539 cm^{-1} (C═C, C═N, *f.* N−H); ^{1}H NMR (TFA-*d*), δ 3.03 (dd, ^2J = 16.5 Hz, ^3J = 7.2 Hz, 1H, H7), 2.86 (m, 2H, H5 and H7), 2.51 (m, 2H, H5 and H6), 1.35 (d, ^3J = 6.6 Hz, 3H, Me); ^{13}C NMR (TFA-*d*), δ 151.8 (C4), 149.0 (C2), 148.1 (C8a), 87.9 (C4a), 49.5 (C7), 27.0 (C5), 26.7 (C6), 18.2 (Me). Anal. Calcd for $C_8H_{12}N_4O$: C, 53.32; H, 6.71; N, 31.09. Found: C, 53.22; H, 6.70; N, 31.01.

4,6-Diamino-2-pyrimidinone (22). 2,4,6-Triaminopyrimidine (**21**) (1 g, 10 mmol) was suspended in 20 mL of 6 M HCl and heated at reflux for 48 h. The mixture was allowed to cool and neutralized (pH 7−8) with 6 M NaOH. Half of the water was removed under reduced pressure, and the resulting solid was filtered off, washed with THF, and dried over P_2O_5 to afford 0.99 g (79%) of **22** as a white solid: ^{1}H NMR (TFA-*d*), δ 6.09 (s, 1H, H5); ^{13}C NMR (TFA-*d*), δ 173.8 (C2), 168.2 (C4 and C6), 160.2 (C5). Anal. Calcd for $C_4H_6N_4O$: C, 38.09; H, 4.80; N, 44.42. Found: C, 37.95; H, 4.71; N, 44.06.

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