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Isotopic Exchange of Hydrogen at C-5 in Pyrimidine Derivatives: Tautomers with an sp^3-Hybridised C-5 Carbon Atom

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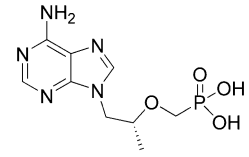
The proton-to-deuterium exchange reaction of the hydrogen atom at the 5-position of 15 pyrimidine derivatives has been studied. The exchange proceeds under both acidic and alkaline conditions. Under acidic conditions, the mechanism involves protonation at the 5-position (forming an σ complex), whereas under alkaline conditions the exchange is mainly a result of the formation of a tautomeric equilibrium, with one tautomer bearing an sp^3-hybridised carbon atom at the 5-position.

Introduction

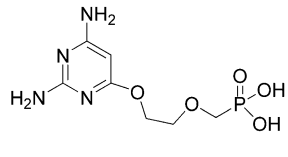
The pyrimidine system is an essential structural motif of several natural products of biological importance[1,2] and many synthetic drugs. There is a large class of pharmacologically important pyrimidine derivatives that act as dihydrofolate reductase (DHFR) inhibitors,[3,4] compounds with anti-HIV (for a review see ref.[5,6]), anti-adenovirus[7] and anti-HBV activities,[8] inhibitors of tetrahydrobiopterin synthesis,[9] regulators of pain sensitivity and persistence,[10] antidepressants[11] and inhibitors of cyclin-dependent kinase as a potential drug candidate for cancer therapy.[12–14]

Two decades ago, we discovered a novel class of biologically active compounds with an interesting antiviral activity, the acyclic nucleoside phosphonates (ANP).[15] These structurally simple compounds (e.g., Tenofovir and Viread) are characterised by the presence of a phosphonomethyl ether function at the β position of a short alkyl chain linked specifically to the N-9 position of the purine (adenine, guanine or 2,6-diaminopurine) base and show a high and stereospecific activity against DNA viruses (e.g., herpes-, pox- or adenoviruses), retroviruses (e.g., HIV-1 and -2) and the hepatitis B virus.[5,6]

In the so-called open-ring ANPs, the imidazole ring of the purine heterocyclic system is replaced by a phosphonomethoxyalkoxy substituent of the 2,4-disubstituted (amino, hydroxy) pyrimidine at the O-6 position.[16,17] These compounds have essentially the same specific properties in terms of their qualitative antiviral activity as their purine



Tenofovir
(Viread®, Truvada®, Atripla®)



Open-ring ANP

counterparts. In animal experiments as well as in metabolic studies, both of which are unavoidable parts of the preclinical phase of any potential novel drug development, it would have been very useful to have used a material labelled with radioisotope(s).

The common method for labelling pyrimidines exploits the 5-bromo derivatives that are easily accessible by direct bromination with elemental bromine or *N*-bromoacetamide.[17] The bromine/tritium exchange in the Pd/C-catalysed reaction with tritium gas in tritium oxide proceeded smoothly and yielded the expected radioisotope-containing product. However, on passing the product through a cation-exchange resin in acidic form, all the radioactivity had completely disappeared. This instability of the isotopic label at the 5-position was later supported by the finding that it had also disappeared after treatment of the product with the cell-free extract from the appropriate host cells.

Pyrimidine is a weak base in solution (pK_a = 1.3),[18] but the pK_a values of pyrimidine derivatives can vary significantly depending on the substitution pattern.[19] The site of the protonation has been investigated in solution and in the gas phase for simple aminopyrimidines; these studies indicated that protonation occurs at the pyrimidine ring nitrogen (N-1 or N-3) or at the exocyclic amino group. Protonation studies might provide a basic insight into the mechanism of action, as clearly demonstrated for DHFR inhibitors. It has recently been discovered that hexamethyl-2,4,6-

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triaminopyrimidine can be protonated at the C-5 position,[20] leading to an equilibrium between the *C*-5- and *N*-1-protonated forms. Protonation at the C-5 position was also observed for some 5-substituted 2,4,6-triaminopyrimidines.[21] The positive charge is stabilised by the resonance effect of the electron-donating substituents at the 2-, 4- and 6-positions.[20] Stable σ complexes of 1,3,5-triaminobenzenes were also observed.[22]

We thus decided to explore the pyrimidine C-5 protonation by NMR spectroscopy in a D_2O solution. If C-5 protonation takes place, the 5-H signal should disappear due to an exchange with deuterium.[23] This proton-to-deuterium exchange would serve as a suitable model for the proton-to-tritium or tritium-to-proton exchange reaction. We have chosen a series of 15 pyrimidine derivatives (Figure 1) with various substituents at the 2-, 4- and 6-positions (including cytosine **11** and uracil **13**) and studied the kinetics of the proton-to-deuterium exchange of the hydrogen at the 5-position.

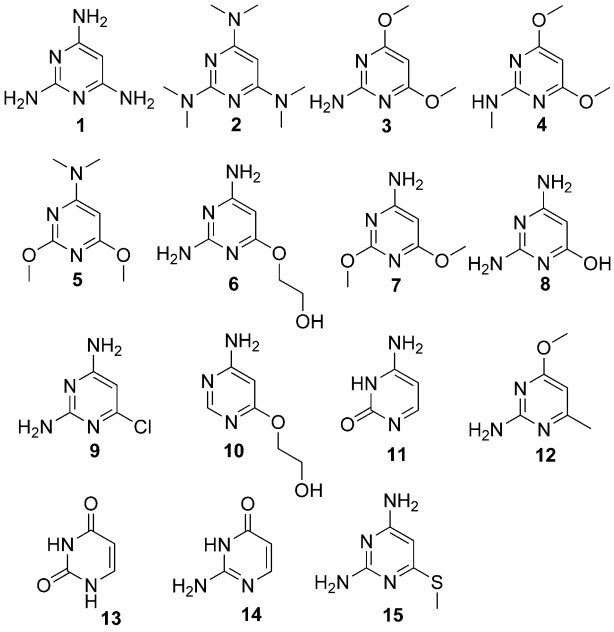
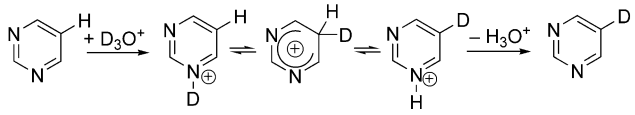


Figure 1. Selected pyrimidine derivatives **1**–**15**.

Results and Discussion

Our hypothesis concerning the mechanism of the exchange reaction is depicted in Scheme 1. We believed that the exchange reaction would take place only when protonated (deuteriated) forms of the pyrimidines (below their pK_a values) are present in the reaction mixture. For some derivatives, the pK_a values were already known, but for the other derivatives we estimated the pK_a values by UV spectroscopy using a standard method.[24] The pK_a values are given in the Supporting Information.



Scheme 1. The mechanism of 5-H isotopic exchange in acidic media.

As all of the pyrimidine derivatives studied, with the exception of uracil (**13**), were protonated at pH 1.25, we measured the 5-H exchange rates in a phosphate buffer at this pH. Compounds **1** and **2** were unstable at pH 1.25, so the exchange rates were measured at pH 4.06, which is still below the pK_a of these compounds. Uracil (**13**) is protonated at a pH value less than –2[25] and we did not attempt to observe its behaviour under such acidic conditions.

The ^{1}H NMR spectra of the samples in a D_2O buffer were measured repeatedly after specified intervals of time and the intensity of the signal of 5-H was compared with other signals in the spectra. The intensity of the 5-H signal decreased continuously because of the exchange of the proton with deuterium, as confirmed by the APT ^{13}C NMR spectra in which the signal of C-5 appeared as a singlet pointing downwards (a CH group) a few minutes after the sample's preparation. The intensity of this signal decreased with a new signal appearing after a few hours or days at the same position. This new signal pointed upwards (a carbon with no proton attached) and was split into a triplet with equal line intensity due to coupling with deuterium (see Figures S1 and S2 in the Supporting Information).

The 5-H exchange reactions of all the pyrimidine derivatives are apparently first-order reactions, and the rate constants were easily obtained from plots of the logarithm of the concentrations of the starting compound (with ^{1}H at the 5-position) versus time. The data fitted first-order kinetics very well; the correlation coefficient was usually higher than 0.99 (see the Supporting Information for an example). The experimental error in the reaction-rate determination was estimated to be less than 2%. The rate constants are reported in Table 1. The exchange rates of compounds **2** and **8** were so high they could not be measured by ^{1}H NMR spectroscopy (the hydrogen at the 5-position was fully exchanged for deuterium before the first spectrum was acquired). In contrast, the exchange reactions of cytosine (**11**) were so slow that no decrease in the starting compound was observed even after four months (the estimated integration error of 2% leads to an exchange rate of $<1.9\times10^{-9}$ s^{-1}). The exchange rate depends very much on the pyrimidine substitution pattern. As a general rule it can be stated that electron-donating substituents increase the reaction rate by stabilising the intermediate *C*-protonated species.

The reverse reaction (deuterium-to-hydrogen exchange) was also observed. The 5-deuteriated pyrimidine **3** was dissolved in a H_2O/D_2O (0.85:0.15) mixture. The ratio between the rates of the hydrogen-to-deuterium and deuterium-to-hydrogen reactions was 0.84, which corresponds (within experimental error) to the proportion of H_2O in the H_2O/D_2O mixture. The intermediate σ complex has equal probabili-

Table 1. The exchange rates of 5-H in pyrimidine derivatives **1**–**15**.

	pH	k[a] [10^{-4} s^{-1}]	$\Delta G^{\#}_{exp}$[b] [kcal mol^{-1}]	$\Delta G_{thermod}$ (calcd.) [kcal mol^{-1}]	pH	k[a] [10^{-4} s^{-1}]	$\Delta G^{\#}_{exp}$[b] [kcal mol^{-1}]	$\Delta G_{thermod}$ (calcd.) [kcal mol^{-1}]
1	4.06[c]	59.2	19.81	9.92	10	2.53	21.69	15.89
2	4.06[c]	>150	<19.25	–1.58		decomposed[d]	–	–
3	1.25	3.25	21.54	11.43	10	0.00072	26.55	21.35
4	1.25	12.2	20.75	10.46	10	0.00048	26.73	22.71
5	1.25	8.08	20.99	11.95	10	0.00054	26.73	–
6	1.25	15.6	20.60	12.13	10	0.03	24.33	18.93
7	1.25	1.11	22.18	15.11	10	0.00031	27.06	21.57
8	1.25	>150	<19.25	2.00	10	21.0	20.42	7.35
9	2.85[c]	0.047	24.06	16.24	10	<0.000019	>28.72	26.03
10	1.25	0.020	24.57	22.17	10	<0.000019	>28.72	24.14
11	1.25	<0.000019	>28.72	24.97	10	<0.000019	>28.72	21.80
12	1.25	0.000055	28.09	19.51	10	<0.000019	>28.72	24.55
13	–	–	–	–	8[e]	0.000032	28.42	18.49
14	1.25	0.00030	27.08	18.36	8[e]	0.000084	27.84	17.16
15	1.25	0.21	23.17	14.14	10	0.0039	25.55	22.07

[a] Determined with an error of less than 2%. [b] The values of $\Delta G^{\#}$ were calculated from doubled rate constants (see text). [c] Decomposition occurred at a lower pH value. [d] Decomposed at pH > 8. [e] Deprotonated at pH 10.

ties of reverting to its original *N*-deuteriated *C*-protonated form or switching to the *N*-protonated *C*-deuteriated form (Figure 2) because the energy barrier is the same for both reactions (there is no or very little difference caused by the deuterium isotopic effect). From this we can conclude that the rate constant for the reactant-to-σ complex interconversion is twice the observed rate constant for the C-5 proton/deuterium exchange. The experimental $\Delta G^{\#}$ values were obtained from the Eyring equation: $\Delta G^{\#} = RT[23.76 - \ln(k/T)]$, with the rate constant k twice the rate constant observed for 5-H exchange.

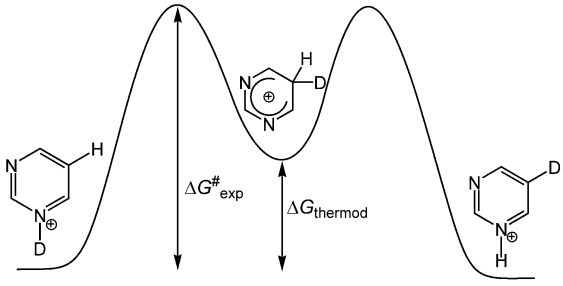


Figure 2. The mechanism and energy profile of 5-H isotopic exchange in acidic media.

We performed geometry optimisations and thermochemical analyses of the *N*- and *C*-5-protonated forms of pyrimidine derivatives **1**–**15**. The water solvent was simulated by using a polarisable continuum model (PCM). We optimised the geometries for the protonation at both possible sites of the ring nitrogen atom (N-1 and N-3) and for all the possible rotamers of the pyrimidine substituents. The lowest-energy structures are shown in the Supporting Information. The *C*-5-protonated forms are intermediates in the reaction sequence depicted in Scheme 1, not transition-state structures, which means that the $\Delta G_{thermod}$ values calculated as the difference between the *N*- and *C*-protonated forms cannot be directly compared with the $\Delta G^{\#}$ values determined experimentally: in the transition state, a water molecule or oxonium cation is probably involved. We also performed the exchange reactions of compound **3** in mixtures of DMSO and D_2O in ratios of 1:1 and 5:1. When the concentration of D_2O was lower, the exchange reaction slowed down considerably, which confirms the presence of a water molecule in the transition state of the reaction. Nevertheless, the good correlation between the calculated $\Delta G_{thermod}$ and experimental $\Delta G^{\#}$ values (Figure 3) indicates that the transition state between the *N*- and *C*-protonated forms is similar for almost all of the pyrimidine derivatives studied. One exception to this rule is compound **10**, for which the calculated free-energy difference is about 7 kcal mol^{-1} higher than anticipated. Compound **10** is the only compound in the entire series that has no nitrogen or oxygen substituent at the 2-position, and consequently its transition-state structures probably differ from the transition-state structures of the other compounds. Similar correlations of activation energies (kinetic data) with reaction energies (thermodynamic data) have been found previously (for an example see ref.[26]).

Quite surprisingly some of the pyrimidine derivatives also exchanged their 5-H in alkaline buffers, in which the compounds are in neutral, unprotonated forms. The exchange reaction was again, apparently, a first-order reaction. Table 1 reports the exchange rates of the neutral forms. There are two possible mechanisms for the exchange in alkaline media. The first mechanism that should be taken into account is the exchange of the 5-H of the cationic forms that are present in very low concentrations even in alkaline solution. This mechanism may proceed, for example, in the 5-H exchange of compound **5**, for which the exchange rate in alkaline media is clearly dependent on pH. The pH dependence of the common logarithm of the reaction rate is linear and the slope of the dependence is close to –1 (see the Supporting Information). This can be easily explained by the pH dependence of the concentration of

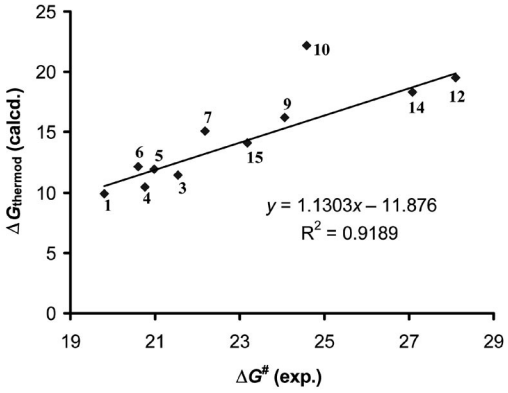
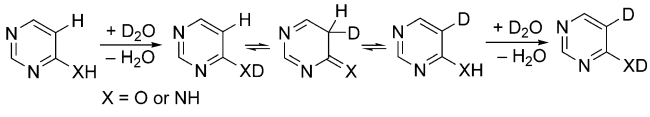


Figure 3. The correlation of the calculated free-energy differences between the *N*- and *C*-protonated forms of the pyrimidines ($\Delta G_{thermod}$) and the experimental free energy of activation measured in kcal mol^{-1}. Compound **10** is not included in the correlation (see the text).

the cationic form. When the pH is increased by 1 unit, the concentration of the cationic form of the compound **5** is 10 times lower and the 5-H exchange rate is also 10 times lower. For the pyrimidine derivatives bearing a hydroxy or amino group, this explanation is not sufficient because the 5-H exchange rate decreases only slowly when the pH is increased. This can be demonstrated, for example, by the pH dependence of the 5-H exchange rate of compound **8**. At pH 8.8, the rate constant is 20.9 h^{-1}, at pH 10 it is 7.56 h^{-1} and at pH 11.7 (at which the concentration of the protonated form is 800 times lower than at pH 8.8) the rate constant is 5.93 h^{-1}. The mechanism of this type of exchange is depicted in Scheme 2; it indicates that some pyrimidine derivatives (including uracil) exist in a water solution in an equilibrium of two (or more) forms and that one of the forms has an sp^3-hybridised carbon atom at the 5-position. The sp^3-hybridised form can be considered as another tautomer of the pyrimidine derivatives. This type of pyrimidine structure has never before been taken into consideration as a possible tautomer of nucleic acid bases.[27–29] Rare tautomers of nucleic acids may be involved in various processes, including point mutations,[30–32] the stabilisation of certain anomalous DNA structures[33] and interactions with metal ions.[34,35] We cannot totally exclude another mechanism involving deprotonated pyrimidine derivatives, but this mechanism seems to be improbable because increasing the pH always caused a decrease in the exchange rates.



Scheme 2. The mechanism of 5-H isotopic exchange in alkaline media.

We performed geometry optimisations and thermochemical analyses on a number of possible tautomers (including the sp^3-hybridised C-5) of all the pyrimidine derivatives with a hydroxy or amino substituent. The correlation between the calculated $\Delta G_{thermod}$ and experimental $\Delta G^{\#}$ values was not as good (data not shown) as was the case with the protonated forms (probably because the reaction proceeds in part by the first mechanism mentioned), and it can be seen that there is a difference between pyrimidine derivatives with an oxo substituent and those with an imino substituent at the 4- and/or 6-positions. The barrier between the sp^3- and sp^2-hybridised C-5 tautomers is higher for the oxo substituent than for the imino substituent.

Conclusions

The observed proton-to-deuterium exchange of 5-H in pyrimidines in an acidic solution is direct evidence of the presence of an σ complex (which is in fact a tautomer of a protonated pyrimidine derivative). The exchange rate (hence the stability of the σ complex) is higher when more electron-donating substituents are attached to the pyrimidine ring. The exchange reactions in alkaline water solutions were always slower and electron-donating substituents also increased the reaction rates. The isotope exchange in alkaline media can be explained by the presence of a small amount of the *C*-protonated form and/or by the presence of a rare pyrimidine tautomer with an sp^3-hybridised C-5 atom.

The results reported herein clearly explain the instability of the tritium isotopic label of the open-ring ANP with electron-donating amino or hydroxy groups. In contrast, it should be possible to use the isotopic exchange reaction for the direct ^{3}H labelling of suitably substituted pyrimidine derivatives (by a simple dissolution of a pyrimidine in ^3H_2O at an elevated temperature). This direct isotopic labelling is currently under study.

This study is also essential for the future derivatisation of the 5-position of the pyrimidine ring (for its direct derivatisation, C–H activation or lithiation). These 5-substituted pyrimidines are a new generation of acyclic nucleoside phosphonates that may have substantial potential for the treatment of a wide range of DNA virus and retrovirus infections[5,6,16,17] and, as we have discovered, 6-halouracils substituted at the 5-position by certain hydrophobic groups exhibit significant inhibitory activity against human thymidine phosphorylase.[36]

It is interesting that natural nucleic acid pyrimidine bases do not seem to have stable C-5 sp^3-hybridised tautomeric forms. The protonation of uracil is impossible under physiological conditions and the neutral form of uracil exchanges 5-H for deuterium only very slowly (the concentration of the C-5 sp^3 tautomer is very low). Cytosine does not exhibit any isotopic exchange in the cationic or neutral form (for comparison, isocytosine **14** exchanges 5-H for deuterium in both acidic and alkaline media). We might speculate that the instability of sp^3-hybridised C-5 tautomers could have played an important role in the natural selection of the pyrimidine bases of the genetic code.

Experimental Section

Synthesis: Compounds **1**, **3**, **7**–**9**, **11**–**13** and **15** were purchased from Sigma–Aldrich. Compound **14** was purchased from Lachema (Brno, Czech Republic). The preparation of **2** was carried out according to the method described in the literature.[37] The structures and purity of all the pyrimidine derivatives studied were proved by ^{1}H and ^{13}C NMR spectroscopy by using a Bruker Avance II 500 instrument (see the Supporting Information). The prepared samples were analysed by using an Agilent 6890N gas chromatograph (Santa Clara, CA, USA) coupled to a 5975B quadrupole mass spectrometer and equipped with a fused silica capillary column (HP-5ms, 30 m × 0.25 mm, 0.25 μm, Agilent). The carrier gas was helium at 1 mL min^{-1}. The injector was operated in split mode (100:1) at 230 °C. The temperature program: 60 °C (4 min), subsequently increased by 10 °C min^{-1} to 320 °C (10 min). The ESI mass spectra were measured with an LCQ Fleet spectrometer (Thermo Fisher Scientific). The standard 70 eV mass spectra were recorded in the mass range of 25–800 using a 4 min solvent delay. The temperatures of the transfer line, ion source and quadrupole were 280, 230 and 150 °C, respectively.

4,6-Dimethoxy-*N*-methylpyrimidin-2-amine (4): Compound **4** was prepared according to the procedure for the preparation of **5** with the appropriate amine (methylamine) in a 37% yield from 2,4,6-trichloropyrimidine. Colourless oil. GC–MS: t_R = 13.2 min (with no other compound being observed). ^{1}H NMR (499.8 MHz, [D$_6$]-DMSO): δ = 5.32 (s, 1 H, 5-H), 3.76 (br. s, 6 H, 2 OCH$_3$), 2.76 (s, 3 H, NCH$_3$) ppm. ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): δ = 171.39 (C-4,6), 162.15 (C-2), 76.86 (C-5), 52.93 (2 OCH$_3$), 28.00 (NCH$_3$) ppm. MS: *m*/*z* (%) = 169 (100) [M]$^+$, 154 (53). $C_7H_{11}N_3O_2$ (169.18): calcd. C 49.70, H 6.55, N 24.84; found C 49.49, H 6.70, N 24.65.

2,6-Dimethoxy-*N*,*N*-dimethylpyrimidin-4-amine (5): A solution of 2,4,6-trichloropyrimidine (1.83 g, 10 mmol) in dry acetonitrile (30 mL) was cooled to –15 °C and treated gradually with a solution of dimethylamine (0.95 g, 21 mmol) in ethanol (4 mL) and acetone (20 mL) and stirring at –15 °C was continued for 2 h. The reaction mixture was concentrated under reduced pressure without heating. The residue was separated by silica gel column chromatography (50 g; chloroform/light petroleum, 1:10 to 9:1). The evaporation and drying of the product-containing fractions afforded 187 mg of 4,6-dichloro-*N*,*N*-dimethylpyrimidin-2-amine, 209 mg of 6-chloro-*N*^{2},*N*^{2},*N*^{4},*N*^{4}-tetramethylpyrimidine-2,4-diamine and 232 mg of 2,6-dichloro-*N*,*N*-dimethylpyrimidin-4-amine. 2,4-2,6-Dichloro-*N*,*N*-dimethylpyrimidin-4-amine (150 mg, 0.78 mmol) was added to a solution of sodium methoxide in methanol (1 M, 20 mL) and this mixture was heated at reflux for 8 h. The reaction mixture was concentrated under reduced pressure and the residue in hexane (50 mL) was washed with water (2 × 20 mL). The organic layer was dried with MgSO$_4$ and evaporated to yield the desired compound (126 mg, 88%) as a colourless oil. GC–MS: t_R = 15.4 min (with no other compound being observed). ^{1}H NMR (499.8 MHz, [D$_6$]-DMSO): δ = 5.53 (s, 1 H, 5-H), 3.79 (s, 3 H, OCH$_3$), 3.78 (s, 3 H, OCH$_3$), 2.99 (s, 6 H, 2 NCH$_3$) ppm. ^{13}C NMR (125.7 MHz, [D$_6$]-DMSO): δ = 171.76 (C-4), 165.16 (C-2), 164.41 (C-4), 78.24 (C-5), 53.81 (OCH$_3$), 53.41 (OCH$_3$), 37.07 (NCH$_3$) ppm. MS: *m*/*z* (%) = 183 (84) [M]$^{+\cdot}$, 168 (79), 154 (100), 125 (40). $C_8H_{13}N_3O_2$ (183.21): calcd. C 52.45, H 7.15, N 22.94; found C 52.59, H 7.32, N 22.69.

General Procedure for the Preparation of 6 and 10: Potassium *tert*-butoxide (1.68 g, 15 mmol) was dissolved in dry ethylene glycol (20 mL) at 60 °C under an inert atmosphere. The appropriately substituted initial 6-chloropyrimidine (10 mmol) was then added at 80 °C, and the reaction mixture was heated at 120 °C for 12 h. The mixture was cooled to 0 °C and neutralised by the addition of Dowex 50 × 8(H$^+$). This mixture was subjected to Dowex 50 × 8(H$^+$) column chromatography, washed with distilled water and eluted with 5% ammonia (UV detection). The ammonia filtrate was evaporated. The crude product was crystallised from water to afford the product.

2-[(2,6-Diaminopyrimidin-4-yl)oxy]ethanol (6): Yield: 1.55 g (91%); m.p. 137 °C. ^{1}H NMR (499.8 MHz, [D$_6$]DMSO): δ = 5.04 (s, 1 H, 5-H), 4.76 (t, *J* = 5.5 Hz, 1 H, OH), 4.09 (m, 2 H, CH$_2$), 3.60 (br. q, *J* = 5.5 Hz, 2 H, CH$_2$) ppm. ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): δ = 170.25 (C-6), 166.15 (C-4), 163.08 (C-2), 76.41 (C-5), 66.64 (CH$_2$), 59.80 (CH$_2$) ppm. MS (ESI+): *m*/*z* (%) = 171 (100) [M]$^+$, 193 (82) [M + Na]$^+$. $C_6H_{10}N_4O_2$ (170.17): calcd. C 42.35, H 5.92, N 32.92; found C 42.07, H 6.02, N 32.92.

2-[(6-Aminopyrimidin-4-yl)oxy]ethanol (10): Yield: 1.33 g (86%); m.p. 131 °C. ^{1}H NMR (499.8 MHz, [D$_6$]DMSO): δ = 8.07 [d, *J*(5,2) = 1.0 Hz, 1 H, 2-H], 6.59 (br. s, 2 H, NH$_2$), 5.68 [d, *J*(5,2) = 1.0 Hz, 1 H, 5-H], 4.81 (t, *J* = 5.5 Hz, 1 H, OH), 4.18 (m, 2 H, CH$_2$), 3.64 (m, 2 H, CH$_2$) ppm. ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): δ = 169.18 (C-6), 165.50 (C-4), 157.90 (C-2), 85.78 (C-5), 67.37 (CH$_2$), 59.56 (CH$_2$) ppm. MS (ESI+): *m*/*z* (%) = 156 (33) [M]$^+$, 178 (100) [M + Na]$^+$. $C_6H_9N_3O_2$ (155.16): calcd. C 46.45, H 5.85, N 27.08; found C 46.07, H 6.07, N 27.01.

Exchange Rate Determination: Various 50 mM phosphate buffers at the required pH values were prepared, lyophilised and dissolved in D$_2$O. The pyrimidine derivatives (usually 2 mg) were dissolved in 600 μL of buffer. The ^{1}H NMR spectra of the samples were acquired periodically (with a suitable delay between them) with a Bruker Avance II 500 instrument (499.84 MHz for ^{1}H). At least 10 spectra were measured for each sample. The amount of ^{1}H at the 5-position was obtained by integration of the 5-H signal with respect to an integral intensity of another ^{1}H signal of the molecule. Derivatives without any suitable ^{1}H signal in the molecule were measured with one drop of dioxane as the internal integration standard. The rate constants were obtained from the plots of the logarithm of the 5-^{1}H concentration versus time.

Computations: All the calculations were performed by using the Gaussian 03 software package.[38] The DFT calculations were performed by using the Becke3LYP[39,40] functional with the 6-31G(d,p) basis set. The full geometry optimisations and analytical vibrational frequency analyses were carried out for all the stationary and transition-state structures. Ground-state structures were found to be minima with zero imaginary frequencies. The geometry optimisations and frequency analyses were carried out by using a polarisable continuum model[41] of water solvation.

Supporting Information (see footnote on the first page of this article): The pK_a and NMR spectroscopic data of the pyrimidine derivatives studied, the NMR spectra of compound **3**, the pH dependence of the log of the 5-H exchange rates for compound **5** in alkaline buffers, the lowest-energy geometries of *N*- and *C*-protonated compounds **1**–**15**.

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