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Carbon acidity of the α-pyridinium carbon of a pyridoxamine analog

Juan Crugeiras,[a] Ana Rios,[a] Tina L. Amyes[b] and John P. Richard*[b]

[a] *Departamento de Química Física, Facultad de Química, Universidad de Santiago, 15706 Santiago de Compostela, Spain*

[b] *Department of Chemistry, University at Buffalo, SUNY, Buffalo, NY 14260, USA. E-mail: jrichard@chem.buffalo.edu*

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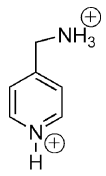
We report second-order rate constants of $k_{DO} = 120$ dm^3 mol^{-1} s^{-1} and $k_B = 6.4 \times 10^{-4}$ dm^3 mol^{-1} s^{-1} for exchange for deuterium of the first α-methylene proton of the 4-(aminomethyl)pyridine dication in D_2O at 25 °C and $I = 1.0$ (KCl). These data are consistent with a carbon acid pK_a between 17 and 19 for ionization of this simple carbon acid and they show that the effect of an α-pyridinium substituent on carbon acidity is similar to that of an α-ester substituent.

Introduction

We are interested in characterizing the effects of strongly electron-withdrawing substituents on carbon acidity and have reported new protocols for determination of the kinetic and thermodynamic acidities of a wide variety of weak carbon acids in water.[1–10] We have now applied these methods to characterization of the kinetic and thermodynamic acidity of the simple α-pyridinium-substituted carbon acid **1-H$_2$**.



1-H$_2$

These determinations have relevance in both chemistry and biology.

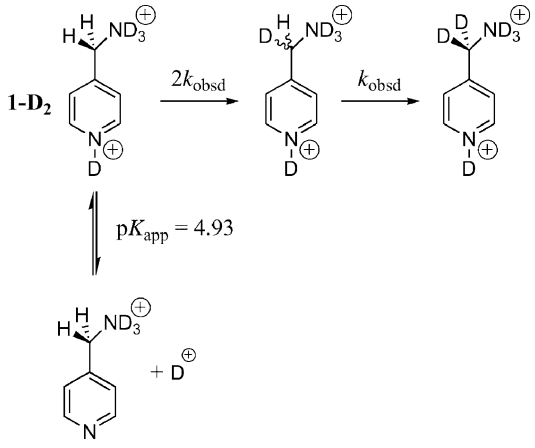
(1) The ketimines that form by addition of 5′-pyridoxamine phosphate to α-ketoacids are intermediates of enzyme-catalyzed transamination reactions that occur by a 1,3-hydrogen shift (Scheme 1).[11] The α-pyridinium proton of the ketimine intermediate is no doubt strongly activated for proton transfer by the pyridinium ring. However, the extent of this activation has not been ascertained through the direct determination of carbon acid pK_as for ionization of the ketimine or aldimine intermediates of pyridoxal/pyridoxamine phosphate-catalyzed transamination, nor can they readily be estimated from the results of the rather limited studies on related model carbon acids.[12–16]

(2) Determination of the carbon acidity of model compounds such as **1-H$_2$** will define the effect of an α-pyridinium substituent on carbanion stability relative to other strongly electron-withdrawing groups, such as the α-carbonyl group of ketones,[17] oxygen esters,[2] thiolesters[1] and amides,[8] and the α-cyano group of nitriles.[5]

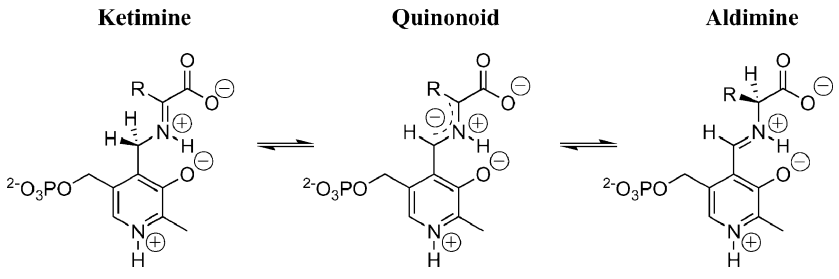
We report here a kinetic study of deprotonation of the 4-(aminomethyl)pyridine dication in D_2O at neutral pD by deuteroxide ion and by phosphate dianion. The data define the kinetic acidity of **1-H$_2$** and provide strong evidence that the effect of a pyridinium substituent on the stability of a neighboring carbanion is similar to that of an ester group.

Results

The exchange for deuterium of the *first* α-methylene proton of 4-(aminomethyl)pyridine in buffered D_2O (pD 6.4–8.4) at 25 °C and $I = 1.0$ (KCl) was followed by ^{1}H NMR spectroscopy at 500 MHz. Deuterium exchange results in the disappearance of the singlet at 4.269 ppm due to the α-CH_2 group of the substrate and the appearance of a poorly resolved upfield-shifted triplet at 4.255 ppm due to the α-CHD group of the first-formed monodeuteriated product (Scheme 2). The deuterium isotope effect on the ^{1}H chemical shift and the H–D coupling constant are similar to those observed in our previous work.[1–5,8,9,18,19]



Scheme 2



Scheme 1

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Table 1 Rate constants for exchange for deuterium of the first α-methylene proton of 4-(aminomethyl)pyridine in buffered D_2O[a]

Buffer system	[Buffer]/mol dm^{-3}	pD	f_B [b]	k_{ex}/s^{-1} [c]	$(k_{ex})_o$/s^{-1} [d]	$(k_B)_{obsd}$/dm^3 mol^{-1} s^{-1} [e]
Phosphate	0.01	6.40	0.2	4.96×10^{-8}	$(1.4 \pm 0.8) \times 10^{-8}$	2.23×10^{-5}
	0.02	6.40		1.08×10^{-7}	$(k_{ex})_o/f_{N^+} =$	$(k_B)_{obsd}/f_{N^+} =$
	0.05	6.38		2.36×10^{-7}	4.3×10^{-7} s^{-1}	6.8×10^{-4} dm^3 mol^{-1} s^{-1}
	0.10	6.37		4.74×10^{-7}		
	0.15	6.36		6.76×10^{-7}		
Phosphate	0.01	6.97	0.5	4.40×10^{-8}	$(1.4 \pm 0.5) \times 10^{-8}$	5.82×10^{-6}
	0.02	6.99		7.26×10^{-8}	$(k_{ex})_o/f_{N^+} =$	$(k_B)_{obsd}/f_{N^+} =$
	0.05	6.99		1.63×10^{-7}	1.6×10^{-6} s^{-1}	6.5×10^{-4} dm^3 mol^{-1} s^{-1}
	0.10	7.00		2.96×10^{-7}		
	0.15	7.02		4.56×10^{-7}		
Phosphate	0.02	7.53	0.8	4.06×10^{-8}	$(1.9 \pm 0.4) \times 10^{-8}$	1.45×10^{-6}
	0.05	7.55		8.14×10^{-8}	$(k_{ex})_o/f_{N^+} =$	$(k_B)_{obsd}/f_{N^+} =$
	0.10	7.59		1.32×10^{-7}	7.8×10^{-6} s^{-1}	5.9×10^{-4} dm^3 mol^{-1} s^{-1}
	0.15	7.62		1.94×10^{-7}		
Pyrophosphate	0.01	8.40	0.5	3.28×10^{-8}	$(1.50 \pm 0.4) \times 10^{-8}$	3.86×10^{-6}
	0.02	8.42		5.60×10^{-8}	$(k_{ex})_o/f_{N^+} =$	$(k_B)_{obsd}/f_{N^+} =$
	0.04	8.46		9.14×10^{-8}	5.2×10^{-5} s^{-1}	1.3×10^{-2} dm^3 mol^{-1} s^{-1}

[a] For reactions at 25 °C and $I = 1.0$ (KCl). [b] Fraction of the buffer in the reactive basic form. [c] Observed first-order rate constant for exchange for deuterium of the first α-methylene proton of 4-(aminomethyl)pyridine. The values of k_{ex} are reproducible to ±10%. [d] Observed first-order rate constant for solvent-catalyzed deuterium exchange at the pD of the experiment. [e] Apparent second-order rate constant for general-base-catalyzed deuterium exchange.

First-order rate constants k_{obsd} (s^{-1}) for exchange for deuterium of a *single* proton of the α-methylene group of the substrate were determined as the slopes of semilogarithmic plots of reaction progress R [eqn. (6), derived for Scheme 2] against time according to eqn. (7) (not shown). The reaction of the α-CH_2 group of the substrate to give the product containing an α-CHD group occurs twice as fast as the exchange of a *single* proton of the α-CH_2 group, so that $k_{ex} = 2k_{obsd}$ (Scheme 2), where k_{ex} (s^{-1}) is the first-order rate constant for exchange for deuterium of the first α-methylene proton of the substrate.[4,5,9,20]

The values of k_{ex} (s^{-1}) for the deuterium exchange reactions of 4-(aminomethyl)pyridine at pD 6.4–8.4 maintained with phosphate or pyrophosphate buffer are reported in Table 1. Fig. 1 shows the dependence of k_{ex} (s^{-1}) on the total concentration of phosphate buffer at pD 6.4–7.6. These data show a good fit to eqn. (1), where $(k_{ex})_o$ is the observed first-order rate constant for solvent-catalyzed deuterium exchange (Table 1), and k_{Buff} is the observed second-order rate constant for buffer-catalyzed exchange.

$$k_{ex} = (k_{ex})_o + k_{Buff}[\text{Buffer}] \quad (1)$$

The plot (not shown) of $(k_{ex})_o/f_{N^+}$ against [DO^-] according to eqn. (2) is linear, where f_{N^+} is the fraction of the substrate present in the reactive pyridinium form that was calculated from the solution pD and $pK_{app} = 4.93$ for ionization of **1-D$_2$** at the pyridine nitrogen in D_2O at 25 °C and $I = 1.0$ (KCl) (Scheme 2).

$$\frac{(k_{ex})_o}{f_{N^+}} = k_{DO}\left[DO^-\right] \quad (2)$$

The slope gives $k_{DO} = 120$ dm^3 mol^{-1} s^{-1} as the second-order rate constant for exchange of the first α-methylene proton of **1-D$_2$** catalyzed by DO^-. Fig. 2 (●) shows the pD–rate profile for the DO^--catalyzed exchange reaction of **1-D$_2$**. The solid line through the data was calculated from the value of $k_{DO} = 120$ dm^3 mol^{-1} s^{-1} using the logarithmic form of eqn. (2) in which [DO^-] is related to pD through eqn. (5).

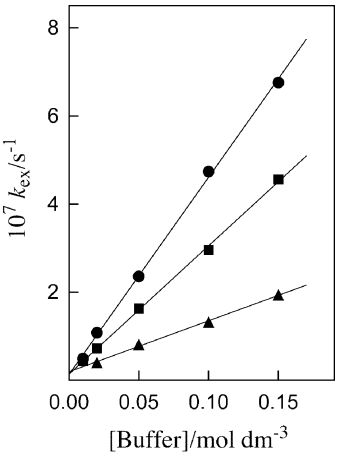


Fig. 1 Dependence of k_{ex} (s^{-1}) for exchange for deuterium of the first α-methylene proton of 4-(aminomethyl)pyridine on the total concentration of phosphate buffer in D_2O at 25 °C and $I = 1.0$ (KCl). The slopes of these correlations give the observed second-order rate constants k_{Buff} for buffer-catalyzed deuterium exchange, and the intercepts give the observed first-order rate constants $(k_{ex})_o$ for solvent-catalyzed deuterium exchange [eqn. (1)]. (●) 20% free base buffer at pD 6.4; (■) 50% free base buffer at pD 7.0; (▲) 80% free base buffer at pD 7.6.

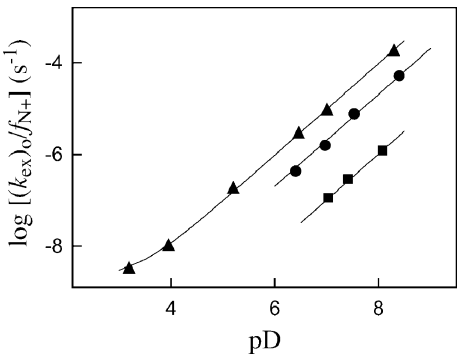
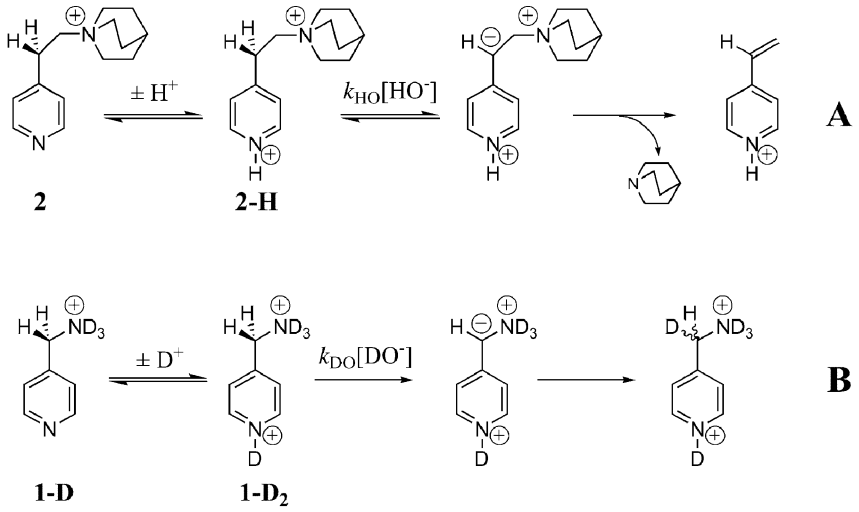


Fig. 2 pD–rate profiles of $(k_{ex})_o/f_{N^+}$ for solvent-catalyzed exchange for deuterium of the first α-methylene proton of the 4-(aminomethyl)pyridine dication (●, this work), *N*-protonated glycine methyl ester **3** (■, data from ref. 4) and betaine methyl ester **4** (▲, data from ref. 4) in D_2O at 25 °C and $I = 1.0$ (KCl).

Table 1 reports the *apparent* second-order rate constants $(k_B)_{obsd} = k_{Buff}/f_B$ for deuterium exchange catalyzed by phosphate dianion, where f_B is the fraction of phosphate buffer present in the reactive dianionic form. True second-order rate



Scheme 3

constants k_B for the deuterium exchange reaction of the dication **1-D$_2$** catalyzed by phosphate dianion were calculated from the values of $(k_B)_{obsd}$ according to eqn. (3) (Table 1). The average of the values of k_B determined for the exchange reactions of **1-D$_2$** at pD 6.4, 7.0 and 7.6 is $k_B = (6.4 \pm 0.5) \times 10^{-4}$ dm^3 mol^{-1} s^{-1}.

$$\frac{(k_B)_{obsd}}{f_{N^+}} = k_B \qquad (3)$$

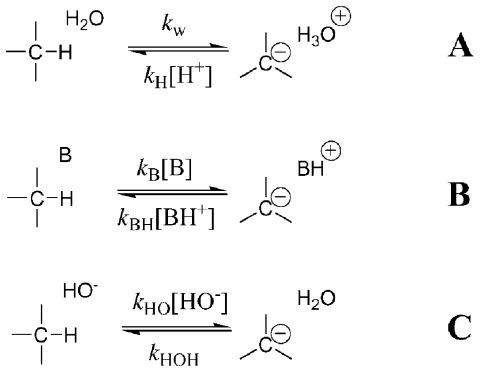
Discussion

The base-catalyzed elimination reaction of the *N*-[2-(4-pyridyl)ethyl]quinuclininium ion (**2**) has been shown to proceed by a stepwise mechanism through an α-pyridinium-stabilized carbanion intermediate (Scheme 3A).[12,14] This provides good precedent for the formation of a related α-pyridinium-stabilized carbanion as an intermediate of the base-catalyzed deuterium exchange reactions of **1-D$_2$** in D_2O. The arguments that deuterium exchange into simple carbon acids usually proceeds through free, solvent-equilibrated, carbanion intermediates (Scheme 3B) have been summarized in earlier work.[2,5,21]

The observed first-order rate constants $(k_{ex})_o$ for solvent-catalyzed deprotonation of 4-(aminomethyl)pyridine remain constant (±20%) as the pD is increased from 6.4 to 8.4, which corresponds to a 100-fold increase in [DO$^-$] (Table 1). This observation is consistent either with deprotonation of the more abundant monocation form of the substrate **1-D** by D_2O, or with the kinetically equivalent deprotonation of the more reactive dication form of the substrate **1-D$_2$** by the more reactive base DO$^-$ (Scheme 3B). Protonation of the pyridine nitrogen of **2** to give **2-H** results in a 53 000-fold increase in k_{HO} for deprotonation of the α-pyridyl carbon by hydroxide ion.[12] A similar activation is expected upon conversion of **1-D** to **1-D$_2$**, so that at pD $\leqslant$ 4 units above pK_{app} = 4.93 for **1-D$_2$** in D_2O (see Fig. 2), the observed deuterium exchange reaction will be due largely to the deprotonation of **1-D$_2$** by DO$^-$ (Scheme 3B). The deuterium exchange reaction catalyzed by phosphate buffer is likewise expected to proceed by deprotonation of **1-D$_2$** by the Brønsted base phosphate dianion. The value of $k_{DO} = 120$ dm^3 mol^{-1} s^{-1} for deprotonation of **1-D$_2$** by DO$^-$ in D_2O at 25 °C reported here is similar to $k_{HO} = 180$ dm^3 mol^{-1} s^{-1} for deprotonation of **2-H** by HO$^-$ in H_2O at the higher temperature of 50 °C.[12]

Carbon acidity of 4-(aminomethyl)pyridine

In water, simple carbon acids may undergo deprotonation by the solvent (Scheme 4A), by a buffer base (Scheme 4B) or by the conjugate base of the solvent, hydroxide ion (Scheme 4C). **1-D$_2$** is too weakly acidic to undergo detectable deprotonation



Scheme 4

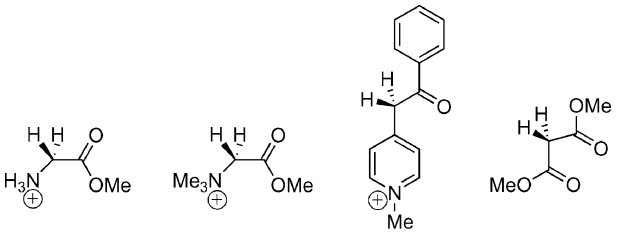
by solvent water but its deprotonation by buffer bases and deuteroxide ion in D_2O is observed (Table 1).

Deprotonation of 1-D$_2$ by DPO$_4^{2-}$ in D$_2$O. The carbon acid pK_a for ionization of the α-pyridinium carbon of **1-D$_2$** can be obtained from the rate constants for its deprotonation by a buffer base to give the free carbanion (k_B) and for the reverse protonation of the carbanion by the conjugate acid of this base (k_{BH}) according to eqn. (4), derived for Scheme 4B, where pK_{BH} is the pK_a of the buffer base.

$$pK_a = pK_{BH} + \log\left(\frac{k_{BH}}{k_B}\right) \qquad (4)$$

Our data give $k_B = 6.4 \times 10^{-4}$ dm^3 mol^{-1} s^{-1} for deprotonation of **1-D$_2$** by DPO$_4^{2-}$ in D_2O, and the value for deprotonation of **1-H$_2$** by HPO$_4^{2-}$ in H_2O should be very similar to this because the solvent deuterium isotope effect on carbon deprotonation by Brønsted bases is close to unity.[2,22,23] The value of $k_B = 6.4 \times 10^{-4}$ dm^3 mol^{-1} s^{-1} can then be combined with the upper limit of $k_{BH} \approx 10^9$ dm^3 mol^{-1} s^{-1} for the reverse diffusion-limited protonation of the carbanion by H$_2$PO$_4^-$,[24] along with pK_{BH} = 6.5 for H$_2$PO$_4^-$ in H_2O at $I = 1.0$,[25] to give p$K_a \leqslant 18.7$ for carbon ionization of **1-H$_2$** in H_2O [eqn. (4)].

The value of $k_B = 6.4 \times 10^{-4}$ dm^3 mol^{-1} s^{-1} for deprotonation of **1-D$_2$** by DPO$_4^{2-}$ is *ca.* 1000-fold larger than $k_B = 5.6 \times 10^{-7}$ dm^3 mol^{-1} s^{-1} for carbon deprotonation of *N*-protonated glycine methyl ester (**3**), for which the carbon acid pK_a in water is 21.0, by the same base.[3,4] This is consistent with a more than 3 unit lower pK_a for deprotonation of **1-H$_2$** in water (pK_a < 18.0), provided there are similar Marcus intrinsic barriers for the two proton transfer reactions (see below).



3 **4** **5** **6**

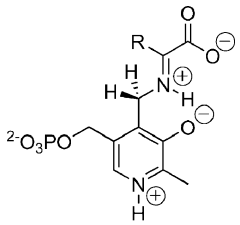
Deprotonation of 1-D_2 by DO^- in D_2O. Fig. 2 shows the pD–rate profiles for carbon deprotonation of the cationic amino acid esters **3** (■), **4** (▲), and of **1-D_2** (●) in D_2O at 25 °C. The second-order rate constant $k_{DO} = 120$ dm^3 mol^{-1} s^{-1} for deprotonation of **1-D_2** by deuteroxide ion lies between the values of $k_{DO} = 6.0$ dm^3 mol^{-1} s^{-1} and $k_{DO} = 570$ dm^3 mol^{-1} s^{-1} for deprotonation of **3** and **4**, respectively.[4] This implies that the kinetic acidity for carbon ionization of **1-H_2** in water lies closer to that of the cationic ester **4** ($pK_a = 18.0$ in water) than to that of **3** ($pK_a = 21.0$ in water), again provided that there are similar Marcus intrinsic barriers for deprotonation of these carbon acids.[26,27] This assumption is supported by the similar Marcus intrinsic barriers of $\Lambda = 13.8$ kcal/mol for deprotonation of the α-pyridinium-substituted carbon acid **5** by benzylmethylamine[16] and $\Lambda = 13.9$ kcal mol^{-1} for deprotonation of dimethyl malonate **6** by dialkylamines.[28] These data provide direct evidence that the α-pyridinium group at **5** and the α-carbonyl group at **6** have similar effects on the Marcus intrinsic barrier for proton transfer at carbon.

In summary, the kinetic data for deprotonation of **1-D_2** by phosphate dianion are consistent with $pK_a < 18$ for **1-H_2** in water, while the data for deprotonation of **1-D_2** by deuteroxide ion are consistent with a pK_a slightly greater than 18. A compromise reflecting the uncertainty in these two analyses is that the carbon acid pK_a of **1-H_2** in water lies between 17 and 19.

The results reported here show that α-pyridinium and α-ester substituents have similar effects on the acidity of simple carbon acids in water. By comparison, the addition of an α-pyridinium group to acetophenone ($pK_a = 18.3$)[17] to give **5** ($pK_a = 7.7$)[15] results in a 10.6 unit decrease in the carbon acid pK_a, which is similar to the 8.7 unit lower carbon acidity of acetoacetate methyl ester ($pK_a = 10.6$)[29] than of acetone ($pK_a = 19.3$).[17]

Biological relevance

The ketimine intermediate **7** of pyridoxal-dependent enzyme-catalyzed transamination reactions should be a relatively strong carbon acid as a combined result of the strongly electron-withdrawing α-pyridinium and α-iminium ion groups.



7

A high carbon acidity is typically observed for carbon that is activated by two strongly electron-withdrawing groups. For example, pK_as of 11.2 and 13.3 have been reported for malonitrile[30] and dimethyl malonate,[31] respectively. We are not aware of literature data that would allow us to estimate the effect of the second resonance electron-withdrawing iminium ion group on the carbon acidity of **7**. However, the pK_a of 7.7 reported for **5**[15] shows that the combined presence of an α-pyridinium and an α-carbonyl substituent results in a carbon acid that readily ionizes at neutral pH. This suggests that there may in fact be little thermodynamic barrier to deprotonation of the ketimine **7** at physiological pH.

Much or all of the kinetic barrier to the non-enzymatic deprotonation of the α-amino carbon of amino acids results from the large thermodynamic barrier for carbon deprotonation.[32] We propose that pyridoxal-dependent enzymes solve this problem entirely through the formation of a covalent adduct to the amino acid. This still leaves a myriad of roles for the protein in catalysis, including recognition of the pyridoxal cofactor and amino acid substrates, and in providing stereoelectronic control over the position of bond cleavage at the enzyme-bound aldimine intermediate.[11]

Experimental

Materials

4-(Aminomethyl)pyridine, deuterium chloride (37 wt%, 99.5% D), potassium deuteroxide (40 wt%, 98+ % D), and deuterium oxide (99.9% D) were from Aldrich. All other organic and inorganic chemicals were reagent grade and were used without further purification.

General methods

The acidic protons of K_2HPO_4 and KH_2PO_4 were exchanged for deuterium before use, as described previously.[1] Phosphate buffers were prepared by mixing stock solutions of K_2DPO_4 and KD_2PO_4 in D_2O at $I = 1.0$ (KCl) to give the desired acid/base ratio. Pyrophosphate buffers were prepared by dissolving the basic form and KCl in D_2O followed by the addition of DCl to give the desired acid/base ratio at $I = 1.0$ (KCl).

Solution pH was determined at 25 °C using a Radiometer PHM82 pH meter equipped with a Radiometer pHC4006-9 electrode. Values of pD were obtained by adding 0.40 to the observed reading of the pH meter.[33] The concentration of deuteroxide ion at any pD was calculated using eqn. (5), where $K_w = 10^{-14.87}$ is the ion product of D_2O at 25 °C,[34] and $\gamma_{OL} = 0.78$ is the apparent activity coefficient of lyoxide ion for the particular electrode under our experimental conditions, determined as described previously.[2]

$$[DO^-] = \frac{10^{pD - pK_w}}{\gamma_{OL}} \quad (5)$$

Apparent pK_as of 4.93 and 9.17 for ionization of the 4-(aminomethyl)pyridine dication at the pyridine and amino nitrogens, respectively, in D_2O at 25 °C and $I = 1.0$ (KCl) were determined by potentiometric titration of an 8 mM solution of the substrate with KOD.

^{1}H NMR spectroscopy

^{1}H NMR spectra at 500 MHz were recorded in D_2O at 25 °C using a Bruker AMX500 NMR spectrometer. Spectra were recorded with a sweep width of 2600 Hz, a 90° pulse angle, an acquisition time of 6 s and zero filling of the data to 64 K. Relaxation times T_1 were determined using a 10 mM solution of substrate at $I = 1.0$ (KCl). In all cases the relaxation delay between pulses was at least 10-fold longer than the longest T_1 for the protons of interest. Chemical shifts are reported relative to HOD at 4.67 ppm. Baselines were subjected to a first-order drift correction before determination of integrated peak areas.

Kinetic measurements

All reactions were carried out in D_2O at 25 °C and $I = 1.0$ (KCl). The deuterium exchange reactions of 4-(aminomethyl)pyridine were initiated by mixing solutions of the substrate and buffer in D_2O at an identical ionic strength of 1.0 (KCl) to give a final substrate concentration of 10 mM. The pD of the reaction

mixture was closely monitored and was found to be constant to within 0.03 pD units during the time the isotope exchange reaction was followed. At timed intervals an aliquot (700 μL) of the reaction mixture was transferred to an NMR tube for ^{1}H NMR analysis at 25 °C. For reactions at pD > 8, aliquots of the reaction mixture were adjusted to pD 7 with concentrated DCl prior to ^{1}H NMR analysis.

The exchange for deuterium of the first α-methylene proton of 4-(aminomethyl)pyridine in D_2O buffered at pD 6.4–8.4 was followed by monitoring the disappearance of the singlet at 4.269 ppm due to the α-CH_2 group of the substrate and the appearance of a poorly resolved triplet at 4.255 ppm due to the α-CHD group of the product by ^{1}H NMR spectroscopy. Reactions were monitored during the exchange for deuterium of 25–80% of the first α-methylene proton of the substrate. Values of R, which is a measure of the progress of the deuterium exchange reaction,[5,20] were calculated according to eqn. (6), where A_{CH_2} and A_{CHD} are the integrated areas of the singlet due to the α-CH_2 group of the substrate and the triplet due to the α-CHD group of the product, respectively. Observed first-order rate constants, k_{obsd} (s^{-1}), for exchange for deuterium of a *single* α-methylene proton of the substrate were determined from the slopes of linear semilogarithmic plots of reaction progress against time [eqn. (7)].[4,5,20] The values of k_{obsd} have been shown in our earlier work to be reproducible to ±10%.[3,4]

$$R = \frac{A_{CH_2}}{A_{CH_2} + A_{CHD}} \quad (6)$$

$$\ln R = -k_{obsd}\, t \quad (7)$$

Acknowledgements

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