The aminolysis of *N*-aroyl β-lactams occurs by a concerted mechanism

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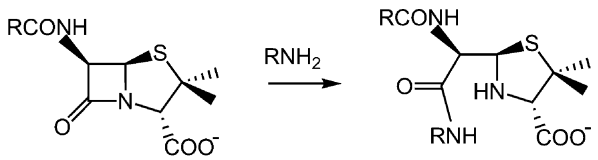
Received 9th November 2006, Accepted 30th November 2006
First published as an Advance Article on the web 21st December 2006
DOI: 10.1039/b616420j

N-Aroyl β-lactams are imides with exo- and endocyclic acyl centres which react with amines in aqueous solution to give the ring opened β-lactam aminolysis product. Unlike the strongly base catalysed aminolysis of β-lactam antiobiotics, such as penicillins and cephaloridines, the rate law for the aminolysis of *N*-aroyl β-lactams is dominated by a term with a first-order dependence on amine concentration in its free base form, indicative of an uncatalysed aminolysis reaction. The second-order rate constants for this uncatalysed aminolysis of *N*-*p*-methoxybenzoyl β-lactam with a series of substituted amines generates a Brønsted β_{nuc} value of +0.90. This is indicative of a large development of positive effective charge on the amine nucleophile in the transition state. Similarly, the rate constants for the reaction of 2-cyanoethylamine with substituted *N*-aroyl β-lactams gives a Brønsted β_{lg} value of −1.03 for different amide leaving groups and is indicative of considerable change in effective charge on the leaving group in the transition state. These observations are compatible with either a late transition state for the formation of the tetrahedral intermediate of a stepwise mechanism or a concerted mechanism with simultaneous bond formation and fission in which the amide leaving group is expelled as an anion. Amide anion expulsion is also indicated by an insignificant solvent kinetic isotope effect, $k^{H_2O}{}_{RNH_2}/k^{D_2O}{}_{RNH_2}$, of 1.01 for the aminolysis of *N*-benzoyl β-lactam with 2-methoxyethylamine. The Brønsted β_{lg} value decreases from −1.03 to −0.71 as the amine nucleophile is changed from 2-cyanoethylamine to propylamine. The Brønsted β_{nuc} value is more invariant although it changes from +0.90 to +0.85 on changing the amide leaving group from *p*-methoxy to *p*-chloro substituted. The sensitivity of the Brønsted β_{nuc} and β_{lg} values to the nucleofugality of the amide leaving group and the nucleophilicity of the amine nucleophiles, respectively, indicate coupled bond formation and bond fission processes.

Introduction

β-Lactam antibiotics such as penicillins (**1**) and cephalosporins are often considered unusually reactive compared with normal amides because most of their biologically important reactions involve the opening of the highly strained four-membered ring.[1] However, the rate of alkaline hydrolysis of β-lactams is, at most, a hundred-fold greater than that of an analogous acyclic amide and is, in fact, often very similar.[2] In water the β-lactam ring of β-lactam antibiotics undergoes attack by nucleophilic reagents such as amines and alcohols in competition with that by hydroxide-ions. Nucleophilic substitution at the carbonyl centre of β-lactams is an acyl transfer process involving covalent bond formation between the carbonyl carbon and the nucleophile as well as C–N bond fission of the β-lactam. Previous studies with nitrogen and oxygen nucleophiles have shown that the mechamism of substitution is a stepwise process, covalent bond formation to the incoming nucleophile occurs before C–N bond fission, resulting in the reversible formation of a tetrahedral intermediate.[3–5] The rate-limiting step in these reactions is normally the breakdown of the tetrahedral intermediate which may involve ring opening.[5–7] The reaction of amines with penicillins gives the corresponding penicilloyl amide and requires at least two proton transfers, proton removal from the attacking amine and proton addition to the leaving amino group.

The aminolysis of penicillins to give penicilloyl amides is strongly catalysed by bases (Scheme 1) which in itself is indicative of a stepwise process and reversible formation of a zwitterionic tetrahedral intermediate and is supported by a non-linear dependence of the rate of aminolysis upon hydroxide-ion concentration due to a change of rate-limiting step. In a stepwise process the neutral amine nucleophile becomes positively charged after forming a covalent bond with the carbonyl carbon which also significantly changes its pK_a. Proton transfer from the protonated amine in the tetrahedral intermediate to a catalytic base becomes thermodynamically favourable and general base catalysed aminolysis is the dominant reaction pathway.[8] In fact, it is experimentally difficult to determine the rate constant for the uncatalysed aminolysis[9] but the evidence suggests that the rate-limiting step is thought to be the β-lactam C–N bond fission.[7,9–10]



(1)

Scheme 1

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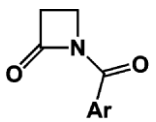
Incorporating a benzamide as a potential leaving group into a simple azetidin-2-one gives the imide *N*-benzoyl β-lactam (**2**), which contains an endocyclic and an exocyclic acyl centre, both of which are potential sites for nucleophilic attack. Contrary to expectations and despite the belief that ring strain should increase the reactivity of β-lactams, there is competition between the two sites for hydroxide-ion attack and between endo- and exocyclic C–N fission as a function of substituents in the aroyl group.[11] Herein we report our studies of the aminolysis of *N*-aroyl β-lactams (**2**) which, unlike the aminolysis of natural cephalosporins[12] and penicillins,[9] does not require general base catalysis. Linear free-energy relationships for the aminolysis of *N*-aroyl β-lactams were investigated by determining the effect on the rate of reaction of varying the basicity of the amine nucleophiles and substituents in the aryl residue of the amide leaving group.



(2)

Results and discussion

The rates of the aminolysis of *N*-aroyl β-lactams were studied by UV-spectrophotometry in 1% acetonitrile-water (v/v) at 30 °C with 1.0 M ionic strength (*I*) maintained by KCl and using the amine as both a nucleophile and a buffer. The site of nucleophilic attack was determined by a product analysis of the reaction of 1.0 M 2-cyanoethylamine at pH 8.07 (0.6 fraction of free base) with *N*-*p*-nitrobenzoyl β-lactam (100 mg, 45 mmol) in 20% acetonitrile-water (v/v). NMR and ESI-MS (electrospray ionisation mass spectrometry) analysis of the product shows that at least 90% of nucleophilic attack occurs at the β-lactam carbonyl. This is demonstrated by ^{1}H, ^{13}C HMBC (heteronuclear multiple bond correlation) NMR which shows the coupling of the α-methylene protons of 2-cyanoethylamine with a carbonyl carbon of the substrate which is, in its turn, coupled to the methylene protons of the β-lactam. ^{1}H, ^{13}C HMBC NMR also shows the two carbonyl carbons of the product were coupled to the methylene protons of the β-lactam ring indicating that there was insignificant attack at the exocyclic amide. ESI-MS (positive mode) shows an observed mass peak of 291 corresponding to the mass of the β-lactam ring opened aminolysis product (Scheme 2).

The kinetics of aminolysis were studied spectrophotometrically measuring the change in absorbance due to product formation using the amine as both buffer and reactant and, with excess amine over *N*-aroyl β-lactam, obey simple first-order kinetics. The dependence of the observed first-order rate constants, k_{obs}, for the reaction of *N*-benzoyl β-lactam in aqueous solution buffered with 2-cyanoethylamine is linearly dependent on total amine concentration (Fig. 1). Extrapolation of the reaction rate

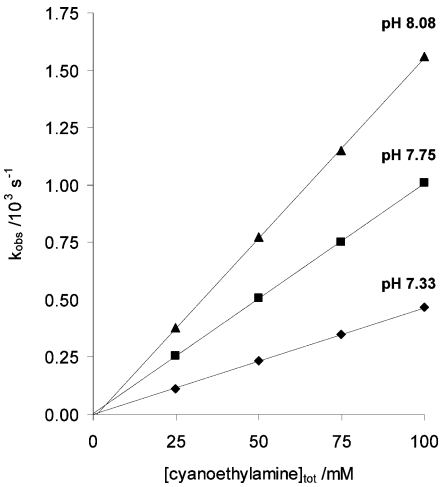


Fig. 1 Dependence of the observed pseudo-first-order rate constants, k_{obs}, for the reaction of *N*-benzoyl β-lactam with 2-cyanoethylamine against total amine concentration at various pHs in 1% acetonitrile-water (v/v) at 30 °C with 1.0 M ionic strength (KCl).

to zero buffer concentration corresponds to that calculated for the background hydrolysis but is indistinguishable from zero (Fig. 1). The slopes of these plots give the second-order rate constants, k_{cat}, for the aminolysis by both the protonated and the deprotonated form of the amine. The reaction was studied using different fractions of free base, *a*, of the amine and a plot of k_{cat} against *a* gives a straight line that passes through the origin (Fig. 2), indicating that the reactive form of the amine is the free base form and there is no significant reaction with the protonated form.

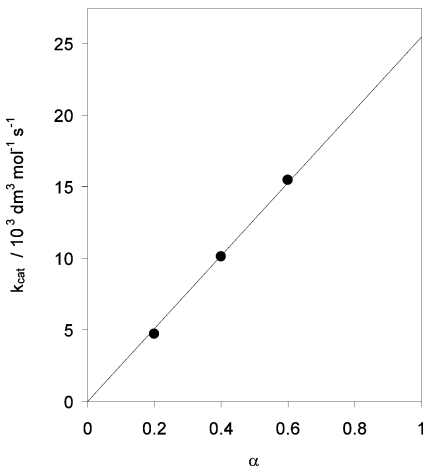
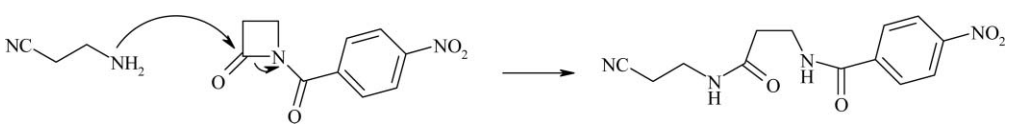


Fig. 2 Dependence of the second-order rate constant, k_{cat}, on the fraction of free base, *a*, of 2-cyanoethylamine in the aminolysis of *N*-benzoyl β-lactam in 1% acetonitrile-water (v/v) at 30 °C with 1.0 M ionic strength (KCl).



Scheme 2

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The fact that the pseudo-first-order rate constant, k_{obs}, for the aminolysis of *N*-benzoyl β-lactam is linearly dependent on the amine concentration is in sharp contrast to the aminolysis of penicillin which shows a rate law with a large term which is second-order in amine concentration. The rate law that adequately describes the reaction is thus given as eqn 1:

$$\frac{\text{Rate}}{[\beta-\text{lactam}]} = k_{obs} = k_o + k_{RNH_2} a[RNH_2]_{tot} \quad (1a)$$

$$= k_o + k_{RNH_2}[RNH_2] \quad (1b)$$

where k_o is the first-order rate constant for the background hydrolysis and k_{RNH_2} is the second-order rate constant for the aminolysis of the β-lactam.

General base catalysis is observed in the aminolysis of penicillin,[9] cephalosporin[12] and *N*-aryl β-lactams[13] and accounts for the major contribution to the observed rate for all of these three substrates. Interestingly, no such catalysis is observed with the aminolysis of *N*-benzoyl β-lactams reported here. The observation of general base catalysed aminolysis seen with other β-lactams has been taken to indicate a stepwise process and reversible formation of a tetrahedral intermediate[1–4] (Scheme 3). Formation of this unstable intermediate changes proton transfer from the amine nucleophile to the base from being thermodynamically unfavourable in the reactants to favourable in this adduct. This route, k_B, is more favourable than the uncatalysed breakdown of the intermediate, k_o (Scheme 3). Although the aminolysis of *N*-benzoyl β-lactam is not general base catalysed, the reaction could be solvent catalysed with water acting as a general base, k_{H_2O} in Scheme 3. However, it is difficult to see why this should be the exclusive pathway and more favourable than to more basic bases. The solvent kinetic isotope effect, $k^{H_2O}{}_{RNH_2}/k^{D_2O}{}_{RNH_2}$, for the reaction of *N*-benzoyl β-lactam with 2-methoxyethylamine is 1.01 (Table 1).

The insignificant isotope effect indicates that the reaction does not involve a large degree of proton transfer in the transition state of the rate-limiting step and is incompatible with a solvent catalysed mechanism. Therefore, the simplest conclusion is that the rate-limiting step is formation or breakdown of the tetrahedral intermediate or that the reaction mechanism is concerted.

The base catalysed aminolysis reaction can be expressed in terms of the rate constants, k_1, for the formation of the tetrahedral intermediate, k_{-1} for its breakdown to the unreacted β-lactam, and k_2 for the diffusion controlled encounter of the base and the tetrahedral intermediate (Scheme 4) and the corresponding overall rate equation (eqn 2).

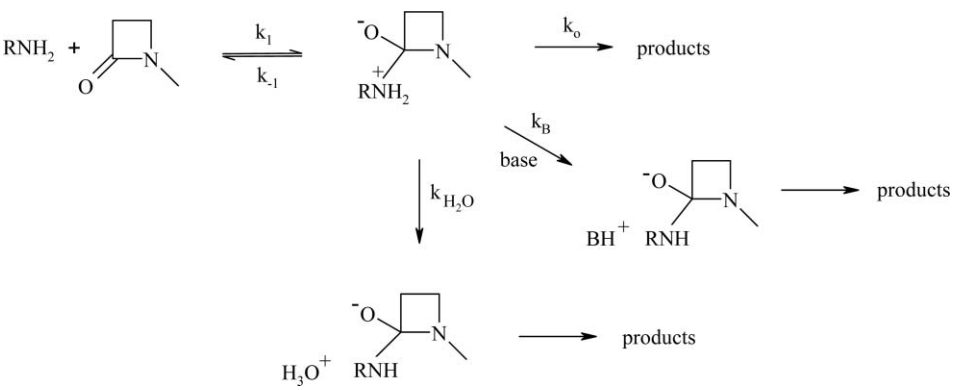
Table 1 Second-order rate constants for the aminolysis of *N*-aroyl β-lactams in water at 30 °C, $I = 1.0$ M (KCl)

	pK_a	$k^{H_2O}{}_{RNH_2}$ / M^{-1} s^{-1}
***N*-*p*-Nitrobenzoyl β-lactam**		
2-cyanoethylamine	7.91[a]	$(1.44 \pm 0.04) \times 10^{-1}$
***N*-*p*-Chlorobenzoyl β-lactam**		
2-Cyanoethylamine	7.91[a]	$(3.96 \pm 0.06) \times 10^{-2}$
2-Methoxyethylamine	9.66[b]	1.24 ± 0.01
Propylamine	10.79[b]	10.9 ± 0.1
***N*-Benzoyl β-lactam**		
Ethylenediamine monocation	7.43[a]	$(1.71 \pm 0.04) \times 10^{-2}$
2-Cyanoethylamine	7.91[a]	$(2.55 \pm 0.04) \times 10^{-2}$
2-Methoxyethylamine	9.66[b]	$(7.95 \pm 0.09) \times 10^{-1}$
Ethylenediamine	10.07[b]	4.25 ± 0.09
Propylamine	10.79[b]	8.30 ± 0.22
***N*-*p*-Methoxybenzoyl β-lactam**		
2-Cyanoethylamine	7.91[a]	$(1.23 \pm 0.01) \times 10^{-2}$
2-Methoxyethylamine	9.66[b]	$(4.21 \pm 0.06) \times 10^{-1}$
Propylamine	10.79[b]	5.02 ± 0.20

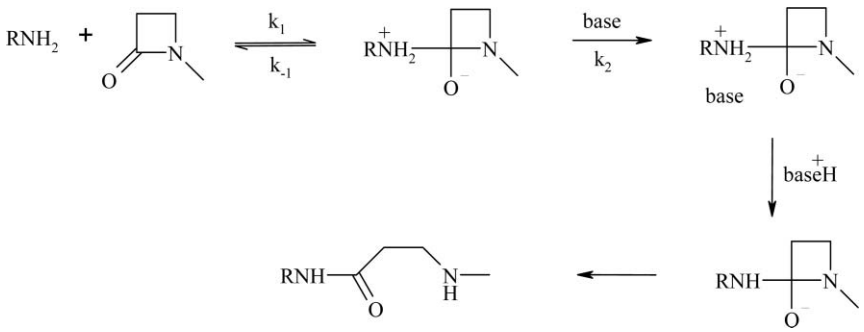
[a] Ionisation constants measured potentiometrically at 30 °C with 1.0 M ionic strength in water. [b] Ionisation constants obtained from Ref. 21.

$$\frac{\text{Rate}}{[\beta-\text{lactam}]} = k_{obs} = \frac{k_1 k_2[RNH_2][\text{base}]}{k_{-1} + k_2[\text{base}]} \quad (2)$$

The rate-limiting step is determined by the relative rates of partitioning of the tetrahedral intermediate to reactants and products, k_2[base]/k_{-1}. For the aminolysis of penicillins[9] and *N*-aryl β-lactams,[13] normally $k_{-1} \gg k_2$[base] and the reaction rate is dependent on base concentration. The rate-limiting step is the diffusion controlled encounter of the base and the tetrahedral intermediate, the k_2 step.[9] A change in rate-limiting step from the diffusion step to the formation of the tetrahedral intermediate can be observed if $k_{-1} \ll k_2$[base] due to a high concentration of a strong base.[14] Under these conditions the rate becomes independent of base concentration and simply first-order in amine concentration; the rate-limiting step then becomes the formation of the tetrahedral intermediate, k_1. This pathway is very different from the small amount of uncatalysed aminolysis observed under normal conditions which corresponds to rate-limiting proton transfer from the tetrahedral intermediate to water, k_{H_2O} (Scheme 3).



Scheme 3



Scheme 4

The observation seen here for the uncatalysed aminolysis of *N*-aroyl β-lactams step could also be due to the normal pathway shown in Scheme 4 but with proton transfer to base occurring after the rate-limiting step, *i.e.* if $k_{-1} \ll k_2$[base] arising from a faster k_2 process and/or a slower k_{-1} step. However, the rate of breakdown of the zwitterionic tetrahedral intermediates, formed from *N*-benzoyl β-lactam and other β-lactams such as *N*-aryl β-lactams, to reactants by expulsion of the attacking amine (the k_{-1} step) is unlikely to vary significantly between different types of β-lactams. Also, the rate of encounter of the base and the tetrahedral intermediate (the k_2 step) is diffusion controlled and should have a similar rate in the aminolysis of *N*-benzoyl β-lactam and other β-lactams under similar experimental conditions. Therefore, the uncatalysed aminolysis of *N*-benzoyl β-lactam must undergo a different pathway involving a different process for the breakdown of the tetrahedral intermediate in the forward direction.

To further investigate the mechanism of the uncatalysed aminolysis of *N*-aroyl β-lactams, linear free-energy relationships were studied by varying the basicity of the amine nucleophile and also by varying the amide leaving group with different substituents in the aryl residue.

The Brønsted β_{nuc} value for the attacking amine nucleophile

The second-order rate constants for the aminolysis of *N*-benzoyl β-lactam with a series of amines of varying basicity are given in Table 1. There is a 500-fold increase in the second-order rate constant as the basicity of the attacking amine is increased by 3.4 pK_a units. As with other amines, the pseudo-first-order rate constant, k_{obs}, for the aminolysis in solutions of ethylenediamine mono- and dications as buffer, is linearly dependent on the total amine concentration indicating the insignificance of the term which is second-order in amine concentration in the rate law. However, in this case the second-order rate constants, k_{cat}, exhibit a sharp upward curvature in the plot against the fraction of its monocation, basic form (Fig. 3). The second-order rate constant, k_{cat}, is indistinguishable from zero as the fraction of monocation approaches zero, showing that the ethylenediamine dication is not catalytically important. The non-linear dependence of the second-order rate constants, k_{cat}, on a is kinetically compatible with a hydroxide-ion catalysed aminolysis mechanism. The rate law can be described as eqn 3:

$$\frac{\text{Rate}}{[\beta-\text{lactam}]} = k_{obs} = k_o + k_1 a[EDAH^+]_{tot} + k'_2 a[EDAH^+]_{tot}[OH^-] \quad (3)$$

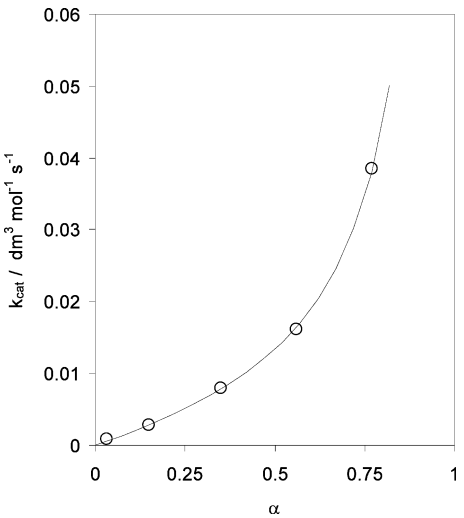


Fig. 3 Dependence of the second-order rate constant, k_{cat}, on the fraction of free base, a, of ethylenediamine monocation in the aminolysis of *N*-benzoyl β-lactam in 1% acetonitrile-water (v/v) at 30 °C with 1.0 M ionic strength (KCl), using a mixture of mono- and dication as the buffer. The theoretical line was generated by using eqn 4.

where k_1 is the second-order rate constant for the aminolysis of *N*-benzoyl β-lactam with the ethylenediamine monocation, $EDAH^+$, and k_2 is the apparent third-order rate constant for hydroxide-ion catalysed aminolysis. The apparent second-order rate constant, k_{cat}, is (eqn 4 and 5):

$$k_{cat} = \frac{k_{obs}}{[EDAH^+]_{tot}} = k_1 a + k'_2 a[OH^-] \quad (4)$$

$$\frac{k_{cat}}{a} = k_1 + k'_2[OH^-] \quad (5)$$

A plot of k_{cat}/a against the hydroxide-ion concentration gives a straight line of slope k'_{-1} (Fig. 4). Although the k'_{-1} term formally represents hydroxide-ion catalysed aminolysis by $EDAH^+$ there is no catalysis observed with other bases. This suggests that the sharp upward curvature in the plot of k_{cat} against a in the aminolysis with $EDAH^+$ is due to the aminolysis with unprotonated EDA because this term is kinetically indistinguishable from the aminolysis with neutral diamine, as shown in eqn 6a–b.

$$K_a = \frac{[EDA][H^+]}{[EDAH^+]} = \frac{[EDA]K_w}{[EDAH^+][OH^-]} \quad (6a)$$

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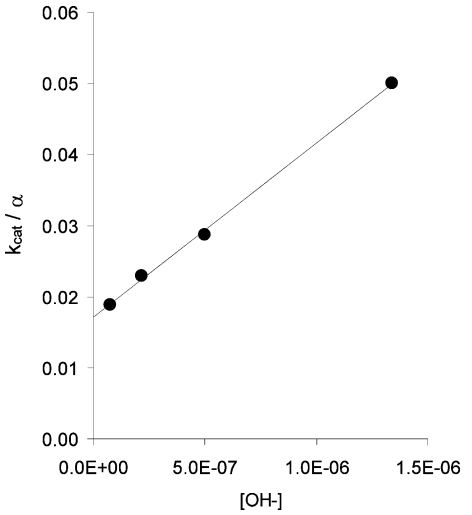


Fig. 4 Dependence of the rate constant, k_{cat}/a, on the concentration of hydroxide-ion in the aminolysis of *N*-benzoyl β-lactam. The rate constants were determined in 1% acetonitrile-water (v/v) at 30 °C with 1.0 M ionic strength (KCl).

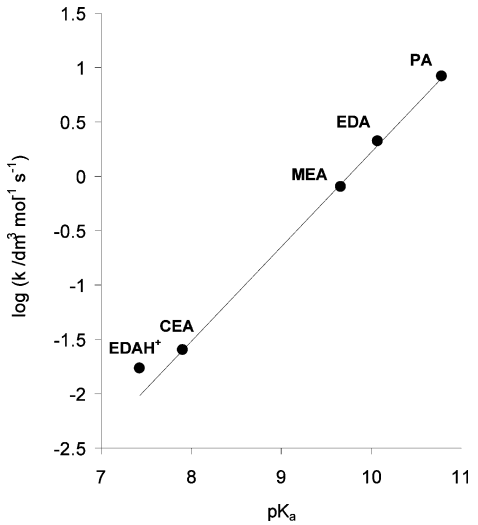


Fig. 5 Dependence of the statistically corrected second-order rate constants for the reaction of amines with *N*-benzoyl β-lactam as a function of the basicity of the amine in 1% acetonitrile-water (v/v) at 30 °C with 1.0 M ionic strength (KCl), where $EDAH^+$ = ethylenediamine monocation; CEA = 2-cyanoethylamine; MEA = methoxyethylamine; EDA = neutral ethylenediamine; PA = propylamine.

Hence,

$$[EDAH^+][OH^-] = \frac{[EDA]K_w}{K_a} \quad (6b)$$

$$k'_2[EDAH^+][OH^-] = k_2[EDA] \quad (6c)$$

where $k_2 = k'_2 \frac{K_w}{K_a}$

The second-order rate constant for the aminolysis of *N*-benzoyl β-lactam with neutral ethylenediamine, EDA, k_2 can thus be obtained even in buffers of mono- and dication even when the concentration of the unprotonated EDA is very small. The rate law for the aminolysis with ethylenediamine thus becomes (eqn 7):

$$\frac{\text{Rate}}{[\beta - \text{lactam}]} = k_{obs} = k_o + k_1[EDAH^+] + k_2[EDA] \quad (7)$$

The second-order rate constants, k_{RNH_2}, for the aminolysis of *N*-benzoyl β-lactam are linearly dependent on the pK_a of the amines, including EDA, and give a Brønsted β_{nuc} value of +0.87 (Fig. 5). This indicates that approximately an effective unit positive charge is developed on the nitrogen of the amine nucleophile in the transition state of the rate-limiting step. There are two classes of Brønsted β_{nuc} values seen for the uncatalysed aminolysis of penicillins. At high pH, where $k_2[OH^-] \gg k_{-1}$ (Scheme 4) the Brønsted β_{nuc} value is +0.3 for rate-limiting formation of the tetrahedral intermediate.[14] There is also a Brønsted β_{nuc} value of +1.0 for the uncatalysed aminolysis which corresponds to rate-limiting breakdown of the tetrahedral intermediate.[9] An unusually large sensitivity to the basicity of the attacking amine was observed in the uncatalysed aminolysis of acetylimidazole with a Brønsted β_{nuc} value of +1.6, which was interpreted as rate-limiting breakdown of the tetrahedral intermediate with complete C–N bond formation between the amine and the acyl centre in the transition state in which there was a large degree of bond fission to the leaving group.[15] The Brønsted β_{nuc} value of +0.87 seen here for the aminolysis of *N*-benzoyl β-lactam is compatible with a late transition state in the formation of the tetrahedral intermediate followed by fast expulsion of the amide leaving group, possibly rate-limiting breakdown with little bond fission to the leaving group or a concerted substitution mechanism.

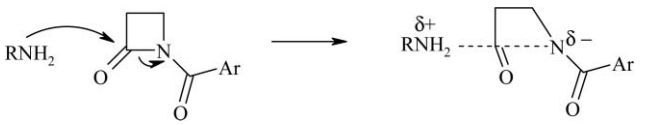
Interestingly, the second-order rate constant for the aminolysis of *N*-benzoyl β-lactam with neutral ethylenediamine shows no deviation from the Brønsted plot (Fig. 5) when statistically corrected for the two amine groups. This is in contrast to the 30-fold rate enhancement observed with the aminolysis of benzyl penicillin by ethylenediamine,[9] attributed to intramolecular general base catalysed aminolysis in which the terminal amino group of the diamine acts as a general base abstracting a proton from the amine nucleophile in the tetrahedral intermediate. Given the absence of a general base catalysed aminolysis pathway for *N*-aroyl β-lactams, it is not surprising to see that ethylenediamine falls on the Brønsted plot and acts as a simple monoamine. Similarly, although the rate constant for the reaction of ethylenediamine monocation with benzyl penicillin shows a 100-fold rate enhancement, as a result of intramolecular general acid catalysis by the terminal protonated amine facilitating the breakdown of the tetrahedral intermediate by donating a proton to the β-lactam nitrogen,[9] that with *N*-benzoyl β-lactam shows no significant deviation from the Brønsted plot. The importance of general acid catalysis in the breakdown of the tetrahedral intermediate formed during the aminolysis of penicillin and cephalosporin has been demonstrated recently with hydroxylamine which showed a 1×10^6-fold rate enhancement compared with that predicted from a Brønsted plot for other primary amines.[16] There is thus clear evidence that the aminolysis of penicillin involves a rate-limiting step that occurs after formation of the tetrahedral intermediate. The fact that the rate constants for the aminolysis of *N*-benzoyl β-lactam with ethylenediamine and its monocation are those expected for their respective basicities suggests that the reaction does not involve either general base or acid catalysis. This is compatible with the solvent kinetic isotope effect of 1.01 for the aminolysis of *N*-benzoyl β-lactam with methoxyethylamine (Table 1).

The aminolysis of *N*-benzoyl β-lactam may thus occur with an earlier rate-limiting step compared with that of other β-lactams, *i.e.* formation rather than breakdown of the tetrahedral intermediate. The leaving group of penicillins and cephalosporins is an amine and for *N*-benzoyl β-lactam is an amide. Amide anions are undoubtedly better leaving groups than amine anions and may even be better than neutral amines giving rise to an earlier rate-limiting step because $k_o \gg k_{-1}$ (Scheme 3). At first sight it may appear unusual that the Brønsted β_{nuc} of +0.87 for the aminolysis of *N*-benzoyl β-lactam is greater than that observed for rate-limiting formation of the tetrahedral intermediate with other β-lactams ($\beta_{nuc} = +0.3$).[14] This suggests nucleophilic attack by the amine occurs with a later transition state along the reaction coordinate for *N*-benzoyl β-lactams compared with *N*-alkyl β-lactams.

The Brønsted β_{lg} value for the amide leaving group

The second-order rate constants, k_{RNH_2}, for the aminolysis of *N*-*p*-chlorobenzoyl β-lactam and *N*-*p*-methoxybenzoyl β-lactam were determined with a series of amines and are given in Table 1. There is a 10-fold increase in the second-order rate constant for the aminolysis with 2-cyanoethylamine as the substituent in the arylamide leaving group is changed from *p*-methoxy to *p*-nitro. As there are no literature values of the ionisation constants for substituted benzamides, the ionisation constants for substituted benzoic acids were used for the correlation. The rate constants are linearly dependent on the pK_a of the corresponding benzoic acid and give a Brønsted β_{lg} value of −1.03 for the aminolysis of *N*-aroyl β-lactams with 2-cyanoethylamine (Fig. 6). This indicates that there is a large change in effective charge on the leaving group going from the reactant state to the transition state. The amide nitrogen, in its reactant state, is made positive by resonance and its effective charge is 0.7+.[17] As discussed elsewhere[11,18] we assume that the effective charge in imides is greater than that in amides and use a value of +1.1. In the tetrahedral intermediate the effective charge on the amide nitrogen will be +0.7. The observed Brønsted β_{lg} value of −1.03 thus indicates that the amide nitrogen has lost considerable positive charge and has an effective charge of only +0.1 in the transition state. It is therefore likely that the amide leaving group is expelled as its anion and there is significant C–N bond fission in the transition state. The rate of expulsion of the amide anion must be *faster* than that of the diffusion controlled encounter of the base and the tetrahedral intermediate so that base catalysis is not observed and the rate of aminolysis is first-order in amine concentration. If the reasonable assumption[19] is made that the diffusion-controlled step, k_2, has a value of *ca.* 1×10^{10} M^{-1} s^{-1}, with the highest amine total concentration of 0.1 M used in this study, the pseudo-first-order rate constant for this diffusion step would be *ca.* 1×10^9 s^{-1}. The rate of expulsion of the amide anion must then be at least *ca.* 1×10^{10} s^{-1} in order to be faster than the general base catalysed pathway which is not observed (Scheme 3). Such a high value is not unreasonable.[14,15]

It is interesting that the aminolysis of *N*-aroyl β-lactams has a high sensitivity to *both* the basicity of the attacking amide and that of the amine leaving group. This could be the result of a rate-limiting breakdown of the tetrahedral intermediate as discussed earlier for acetylimidazole,[15] but the β_{nuc} of +0.87 is not sufficiently large to be fully compatible with significant ring opening and reforming the carbonyl double bond. Therefore the aminolysis of *N*-aroyl β-lactams appears to occur by a concerted pathway in which bond formation and fission occurs simultaneously (Scheme 5). A high dependence on both the basicity of the attacking nucleophile and that of the leaving group was also seen in the reactions of substituted phenolate anions with phenyl formate, with a Brønsted β_{nuc} and β_{lg} value of +0.90 and −0.90, respectively, and was interpreted as a concerted reaction.[20]



Scheme 5

As a function of attacking amine basicity, the Brønsted β_{lg} value decreases from −1.03 for 2-cyanoethylamine to −0.71 for propylamine (Table 2), *i.e.* the Brønsted β_{lg} decreases with increasing basicity of the attacking amine. This indicates less charge development on the leaving group amide (less C–N bond fission) in the transition state with more basic amine nucleophiles. However, there is little variation in Brønsted β_{nuc} for the attacking amine as a function of substituents in the leaving group. Based on the limited data, it appears that there is a small decrease in β_{nuc} from +0.90 to +0.85 when the leaving groups change from

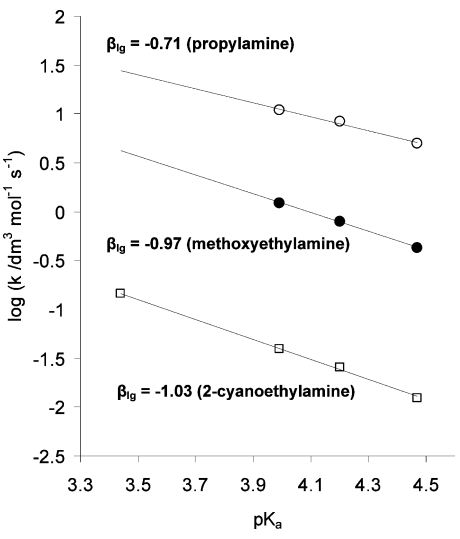


Fig. 6 Brønsted plots of the second-order rate constants, k_{RNH_2}, for the aminolysis of *N*-aroyl β-lactams against the basicity of the benzamide leaving group with different amines indicated. The rate constants were determined in 1% acetonitrile-water (v/v) at 30 °C with 1.0 M ionic strength (KCl).

Table 2 The dependence of the Brønsted β_{lg} values for the aminolysis of *N*-aroyl β-lactams on the pK_a of the amine nucleophiles

	pK_a	β_{lg}
2-Cyanoethylamine	7.91[a]	−1.03
Methoxyethylamine	9.66[b]	−0.97
Propylamine	10.79[b]	−0.71

[a] Ionisation constants measured potentiometrically at 30 °C with 1.0 M ionic strength in water. [b] Ionisation constants obtained from Ref. 21.

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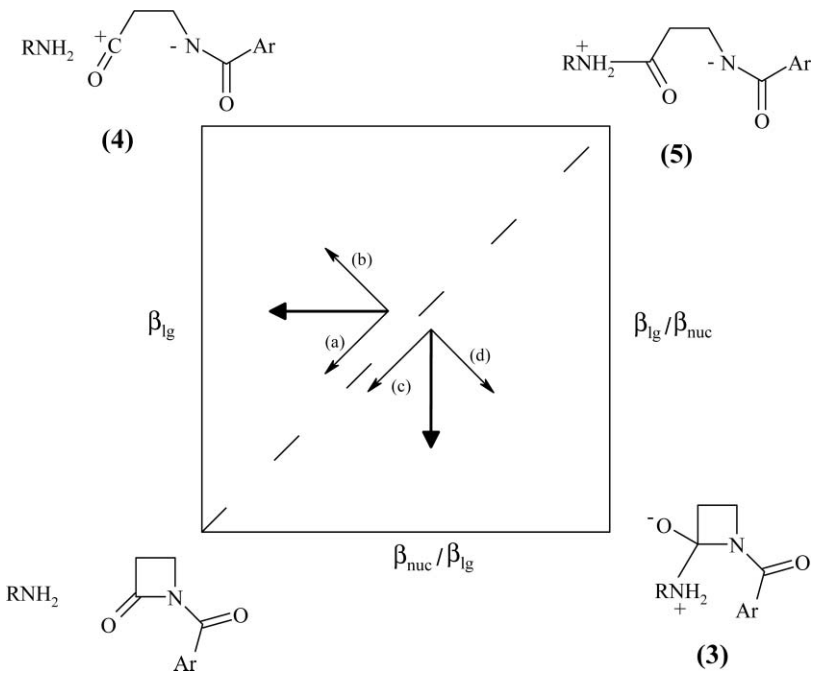
Table 3 The dependence of the Brønsted β_{nuc} values for the aminolysis of *N*-aroyl β-lactams on the basicity of the substituted benzamide leaving group. The pK_a of the carboxylic acid corresponding to exocyclic aryl carboxamide is given as an indicator of the inductive effect of the substituent in the aromatic ring. pK_a values are taken from Ref. 21

	pK_a	β_{nuc}
p-Cl	3.99	0.85
p-H	4.20	0.87
p-OMe	4.47	0.90

p-methoxy to *p*-chloro-benzamide (Table 3). This may not be meaningful, but would suggest there is *less* charge development on the attacking amine nucleophile with a better leaving group. The changes in the Brønsted β_{lg} and β_{nuc} values observed by changing the nucleophilicity of the nucleophile and the nucleofugacity of the leaving group are consistent with a concerted mechanism.

Bond making to the attacking nucleophile and bond breaking to the leaving group are coupled in the transition state of a concerted reaction. Changing either the nucleophilicity of the nucleophile or the nucleofugacity of the leaving group will affect the transition state position along the reaction coordinate of a concerted pathway. This may be monitored by changes in the Brønsted β_{nuc} and β_{lg} values of the reaction and the effect may be illustrated by the Jencks–More O'Ferrall diagram.[17] In principle, the expulsion of the amide anion could result from a stepwise associative mechanism, a stepwise dissociative mechanism, or a concerted pathway (Scheme 6). In the associative pathway, the reaction involves the formation of a tetrahedral intermediate (**3**) formed between the amine nucleophile and the β-lactam which subsequently expels the amide anion leaving group. In the dissociative pathway, an acylium centre and an amide anion would be produced in the intermediate (**4**) and the acylium centre is then trapped by the amine nucleophile. The axes of Scheme 6 are measured by the Brønsted β_{nuc} or β_{lg} values or both as indicated (Scheme 6). In the concerted mechanism, the bond making to the attacking nucleophile and the bond breaking to the leaving group takes place simultaneously, does not involve a reaction intermediate and its trajectory is indicated by the dashed diagonal line in Scheme 6. Increasing the acidity of the amide leaving group with electron withdrawing groups would stabilise the amide anion product (**5**) and the transition state will move *away* from the product corner to that of the reactants [arrow (a)], as predicted by the 'parallel' effect and the Hammond postulate.[17] Electron withdrawing groups in the amide leaving group would also stabilise the amide anion (**4**) of the dissociative pathway and destabilise the tetrahedral intermediate (**3**) in the associative pathway. In both cases, the transition state will tend to move *towards* the amide anion (**4**) corner [arrow (b)] as a Thornton or 'perpendicular' effect. The net result is predicted to be a reduced Brønsted β_{nuc} value as the acidity of the leaving group is increased, as observed. Increasing the basicity of the amine nucleophile would stabilise the amide product (**5**), which causes a movement of the transition state away from the product corner (**5**) towards the reactants [arrow (c)]. A more basic amine nucleophile would also stabilise the tetrahedral intermediate (**3**), which will move the transition state towards this corner [arrow (d)] by a 'perpendicular' effect. A reduced Brønsted β_{lg} is then predicted by increasing the basicity of the amine nucleophiles.

The aminolysis of *N*-aroyl β-lactams thus appears to occur by a concerted mechanism with significant coupling between bond formation and bond fission and involves a transition state with a large amount of charge development on the incoming nucleophile as well as on the leaving group amide anion. Acyl transfer reactions predominantly occur by a stepwise process[17] and this is true for nucleophilic substitution of β-lactams. In the case of *N*-aroyl β-lactams the concerted mechanism is presumably *enforced*[17,23] by the instability of the normal tetrahedral intermediate



Scheme 6

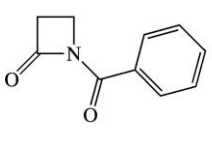
Table 4 A comparison of the second-order rate constants, k_{OH}, for the alkaline hydrolysis and, k_{RNH_2}, for the aminolysis of some β-lactams with 2-propylamine

	$k_{OH}/M^{-1}\ s^{-1}$	$k_{RNH_2}/M^{-1}\ s^{-1}$	$\frac{k_{OH}}{k_{RNH_2}}$
N-Benzoyl β-lactam (**6**)	9.07[a]	8.30[a]	1.09
N-*p*-Nitrophenyl β-lactam (**7**)	4.42×10^{-2}[b]	2.5×10^{-4}[c]	177
Benzylpenicillin (**1**)	1.54×10^{-1}[ae]	1.32×10^{-2}[d]	11

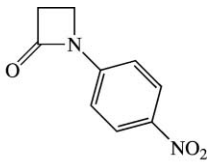
[a] At 30 °C with 1.0 M ionic strength. [b] At 25 °C with 1.0 M ionic strength, from Ref. 22. [c] At 25 °C with 0.1 M ionic strength, from Ref. 12. [d] At 30 °C with 0.25 M ionic strength. [e] From Ref. 1.

(**3**)—expulsion of the good leaving group amide anion is facilitated further by ring opening of the β-lactam and occurs with a faster rate than expulsion of the amine to regenerate the reactants.

It is of interest to compare the reactivity of *N*-aroyl β-lactams with that of other β-lactams. The second-order rate constants, k_{OH}, for the alkaline hydrolysis and, k_{RNH_2}, for the uncatalysed aminolysis of penicillin (**1**),[9] *N*-benzoyl β-lactam (**6**), and *N*-*p*-nitrophenyl β-lactam (**7**)[13] with propylamine are given in Table 4. Although the uncatalysed aminolysis of penicillin (**1**) represents the very minor pathway, a reasonable estimate of the second-order rate constant has been measured.[9] It is interesting that the rate of the uncatalysed aminolysis varies by more than 1×10^4 while the rate of alkaline hydrolysis varies more moderately by 1×10^2 between the substrates. For penicillin (**1**) the rate constant for hydrolysis is at least 10-fold greater than that of the uncatalysed aminolysis, and is 100-fold greater for *N*-*p*-nitrophenyl β-lactam (**7**) but they are similar for *N*-benzoyl β-lactam (**6**). The ratio, k_{OH}/k_{RNH_2}, indicates the relative reactivity towards hydroxide-ions and amine nucleophiles; it is much smaller for *N*-benzoyl β-lactam (**6**) than that of the other two β-lactams by 10–1 × 10^2. Since the rates of alkaline hydrolysis of the β-lactams are similar, the significantly reduced k_{OH}/k_{RNH_2} ratio for *N*-benzoyl β-lactam (**6**) may also be indicative of a different mechanism for the aminolysis of *N*-benzoyl β-lactam (**6**) compared with the other two β-lactams.



(6)



(7)

It is worth noting that although the alkaline hydrolysis of *N*-*p*-nitrobenzoyl β-lactam occurs with competitive endo- and exocyclic C–N bond fission,[11] the aminolysis reaction occurs exclusively with β-lactam ring opening. Although hydroxide-ion attack at the exocyclic carbonyl group is competitive with that at the β-lactam centre, amines prefer the β-lactam carbonyl group. The second-order rate constant for aminolysis at the endocyclic β-lactam carbonyl is at least 100-fold greater than that at the exocyclic carbonyl centre. In the aminolysis of the β-lactam of *N*-aroyl β-lactams, the zwitterionic tetrahedral intermediate appears to breakdown by expelling the benzamide leaving group as its anion. However, for the reaction of the exocyclic carbonyl centre, the leaving group is the β-lactam amide anion, which is less stable than the amide anion of *p*-nitrobenzamide. The rate of reaction with weaker nucleophiles, such as amines, compared with hydroxide-ions has a greater dependence on the basicity of the leaving group as shown by the Brønsted β_{lg} values, −0.55 for hydroxide-ion catalysed hydrolysis[11] and −0.97 for aminolysis with methoxyethylamine (Table 2) for reactions at the β-lactam centre. A similar situation exists in the inability of an intramolecular carboxylate to displace the β-lactam amide anion in competition with the alkaline hydrolysis of the β-lactam in *N*-*o*-carboxybenzoyl β-lactam. There is no evidence of anhydride formation with *N*-*o*-carboxybenzoyl β-lactam at neutral pH.[11,18]

Experimental section

(i) Synthesis

N-Aroyl β-lactams were prepared according to the general procedure: to a −78 °C stirred solution of 2-azetidinone (0.5 g, 7.03 mmol) in dry dichloromethane (DCM) (20 ml) was added 4,4-dimethylaminopyridine (0.1 g, 0.82 mmol) and a solution of aroyl chloride (1.57 g, 8.46 mmol) in dichloromethane (10 ml) dropwise over 5 minutes. Triethylamine (0.98 ml, 7.02 mmol) was added dropwise over 10 minutes forming a white precipitate. The reaction mixture was stirred at −78 °C for 1 hour and for a further 24 hours at ambient temperature. DCM (10 ml) was added to the reaction mixture and the solution washed with water (15 ml) and saturated brine (2 × 15 ml). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure by rotary evaporation at 30 °C to yield a pale yellow oil, which was purified by column chromatography.

1-(4′-Methoxybenzoyl)-1-azetidin-2-one. Yield, 0.6 g (42%); mp 125–127 °C; IR υ_{max} (cm^{-1}) ($CHCl_3$): 3020, 3009, 2975, 2912, 2842, 1784, 1668, 1606, 1325, 1259, 1195, 1108, 1028; ^{1}H NMR: δ ($CDCl_3$) 7.99 (2H, d, J 8.88), 6.92 (2H, d, J 8.97), 3.85 (3H, s, CH_3), 3.77 (2H, t, J 5.44, CH_2N), 3.02 (2H, t, J 5.45, CH_2CO); ^{13}C NMR: δ ($CDCl_3$) 165.23 (C=O), 163.92 (C=O), 132.57 (quaternary carbon), 131.92 (ArCH), 123.76 (quaternary carbon), 113.948 (ArCH), 55.21 (CH_3), 36.45 (CH_2N), 34.44 (CH_2CO); HREI-MS (high resolution electron ionisation mass spectroscopy) $[M + H]^+$ for $C_{11}H_{11}NO_3$ calculated 206.0812 measured 206.0812.

1-Benzoyl-1-azetidin-2-one. IR υ_{max} (cm^{-1}) ($CHCl_3$): 3020, 1786, 1673, 1327, 1298, 1216, 1192; ^{1}H NMR: δ ($CDCl_3$) 8.00 (2H, d, J 7.43), 7.61 (1H, t, J 7.29), 7.49 (2H, t, 7.83), 3.81 (2H, t, J 5.49), 3.14 (2H, t, J 5.49); ^{13}C NMR: δ ($CDCl_3$) 166.26 (C=O), 163.95 (C=O), 133.19 (PhCH), 131.83 (quaternary carbon), 129.69 (PhCH), 128.12 (PhCH), 36.77 (CH_2), 35.05 (CH_2).

1-(4′-Chlorobenzoyl)-1-azetidin-2-one. Yield, 0.85 g (58%); IR υ_{max} (cm^{-1}) ($CHCl_3$) 3020, 1788, 1673, 1593, 1404, 1324, 1284, 1217, 1093; ^{1}H NMR: δ ($CDCl_3$) 7.95 (2H, d, J 6.68), 7.44 (2H, d, J 8.66), 3.77 (2H, t, J 5.5, CH_2N), 3.12 (2H, t, J 5.61, CH_2CO); ^{13}C NMR: δ ($CDCl_3$) 165.01 (C=O), 163.84 (C=O), 139.51 (quaternary carbon), 131.14 (CH), 130.1 (quaternary carbon), 128.44 (ArCH), 36.73 (CH_2N), 34.99 (CH_2CO); HREI-MS $[M + H]^+$ for $C_{10}H_8NO_2Cl$ calculated 210.0316 measured 210.0316.

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1-(4′-Nitrobenzoyl)-1-azetidin-2-one. Yield, 1.23 g (80%); mp 130–131 °C; IR υ_{max} (cm^{-1}) ($CHCl_3$) 3020, 1787, 1686, 1599, 1523, 1397, 1309, 1252, 1204, 992; ^1H NMR: δ ($CDCl_3$) 8.33 (2H, d, J 8.75), 8.14 (2H, d, J 8.74), 3.85 (2H, t, J 5.58, CH_2N), 3.2 (2H, t, J 5.57, CH_2CO); ^{13}C NMR: δ ($CDCl_3$) 164.16 (C=O), 163.79 (C=O), 150.29 (quaternary carbon), 137.32 (quaternary carbon), 130.76 (CH), 123.28 (CH), 37.0 (CH_2N), 35.5 (CH_2CO).

(ii) Kinectic Procedures

Standard UV spectroscopy was carried out on a Cary 1E UV-visible spectrophotometer (Varian, Australia) equipped with a twelve compartment cell block. The instrument was used in double beam mode, allowing six reaction cells to be followed in a single run. The cell block was thermostatted using a peltier system.

pH measurements were made with a ϕ40 pH meter (Beckman, Fullerton, USA) using a semi-micro calomel electrode (Beckman). A calibration of the pH meter was carried out at 30 °C using pH 6.99 ± 0.01, pH 4.01 ± 0.02 or pH 9.95 ± 0.02 calibration buffers. For solution pHs ≥3 and ≤11 the pH was controlled by the use of ≤0.2 M buffer solutions of the amine. Buffer solutions were prepared by partial neutralisation of solutions of their sodium salts to the required pH. Hydroxide-ion concentrations were calculated using pK_w (H_2O) = 13.83 at 30 °C.[24]

In all experiments temperatures were maintained at 30 °C and ionic strength at 1.0 M with AnalaR grade KCl unless otherwise stated. AnalaR grade reagents and deionised water were used throughout. Organic solvents were glass distilled prior to use and stored under nitrogen.

Reactions studied by UV spectrophotometry were commenced by injections (20 μl) of acetonitrile stock solutions 1×10^{-2} M of the substrate into the cells containing pre-incubated buffer (2.0 ml). Final reaction cells contained ≤1% acetonitrile v/v. The pH of the reaction cells was measured before and after each kinetic run at 30 °C, kinetic runs experiencing a change >0.05 units were rejected. Reactant disappearance or product appearance was followed at absorbance change maxima for individual compounds. The solubility of compounds was ensured by working within the linear range of absorbance in corresponding Beer–Lambert plots. Reaction concentrations were generally within the range of 2×10^{-5} M to 2×10^{-4} M. Pseudo first-order rate constants from exponential plots of absorbance against time or gradients of initial slopes were obtained using the Cary Win UV kinetics application (Version 02.00(26)). pH–Rate profiles were modelled to theoretical equations using the Scientist program (V2.02, Micromath Software Ltd, USA).

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