Kinetics and Mechanism of the Cyclization of Substituted *N*-Phenyl-2-methyl-2-(2-aminophenyl)propanamides and Analogues

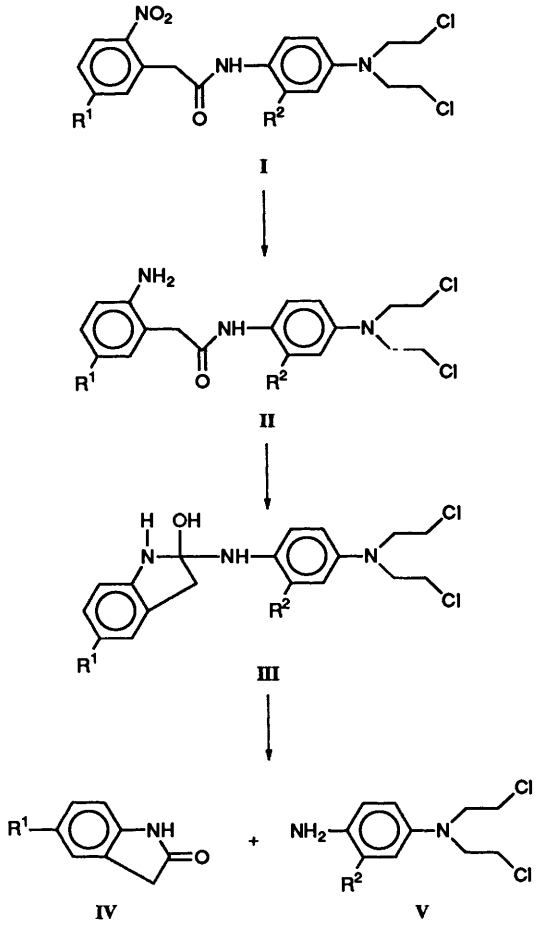
Bridget M. Sykes,[a] Graham J. Atwell,[b] William A. Denny,[b] Duncan J. McLennan[a] and Charmian J. O'Connor*,[a]

[a] *Department of Chemistry, The University of Auckland, Private Bag 92019, Auckland, New Zealand*
[b] *Cancer Research Laboratory, School of Medicine, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

The cyclization of six *N*-aryl-2-(2-aminophenyl)alkylamides has been studied at various pH values. Compounds **1a**, **2a** and **3a** had a similar leaving group (4-methoxyaniline) and varying degrees of steric bulk adjacent to the amide, while compounds **1a**–**1d** had varying substituents on the leaving amine. The cyclization of all the compounds was found to be subject to general catalysis by acidic buffer components, with the buffer-independent pH profiles obeying the equation $k_0 = k_{H_3O^+}[H_3O^+] + k_{H_2O}$. Brønsted analysis of the rate coefficients for buffer catalysis gave α values of *ca.* 0.4 for all compounds. The relative observed pseudo-first-order rate coefficients at pH 6.6 for compounds **3a**, **2a** and **1a** were 1, 9 and 800, respectively, indicating the importance of 'stereopopulation control' (Milstein and Cohen, *J. Am. Chem. Soc.*, 1972, **94**, 9158) on the rate of cyclization. However, cyclization rates were found to be independent of the electronic properties of the leaving amine. The mechanism of cyclization was considered to be rate-determining, concerted attack by the neutral amine, followed by proton transfer from a general acid to the amide oxygen.

In a recent paper,[1] we proposed the use of 2-nitroaryl-acetamides and -propanamides (**I**) as bioreductively-activated prodrugs for the hypoxia-specific release of activated amino-aromatic mustard alkylating agents. In this concept (Scheme 1), reduction of the nitro amide (**I**) to the corresponding amine (**II**) results in spontaneous cyclization to the tetrahedral intermediate (**III**). Breakdown of this results in formation of the cyclic lactam (**IV**) and concomitant release of the amine (**V**). The net conversion (**I**–**V**) expels an aromatic mustard bearing an amine substituent, which is a much more active alkylating agent than the preceding amide due to enhanced electron release.[2] In this design, the reduction potential of the nitro group and the reactivity of the mustard can be separately manipulated by the substituents R^1 and R^2. To be suitable for use as a prodrug system, it is necessary for the cyclization process to occur at a sufficiently rapid rate (with a half-life of seconds to a few minutes) under physiological conditions.

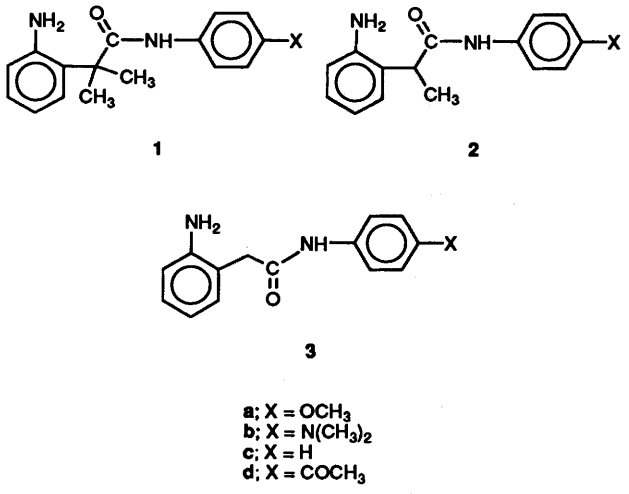
Studies of a series of model compounds showed that rates of cyclization were markedly enhanced by α-methyl substitution; at pH 6.8, the α-methyl derivative (**2a**) cyclized eight-fold faster than the unsubstituted compound (**3a**) and the α,α-dimethyl derivative (**1a**) 500-fold more rapidly than **3a**.[1] These results are consistent with earlier studies on the lactonization of 2-hydroxypropanoic acids[3,4] and 2-hydroxypropanamides.[5] However, the absolute rate of cyclization of even **1a** is probably still too slow to permit its successful use as a prodrug for amine release. As part of a programme aimed at the further development of this concept, we report here a detailed kinetic and mechanistic study of selected *N*-aryl-2-methyl-2-(2-aminophenyl)propanamides and close analogues.



Scheme 1 Reaction pathway for the cyclization-induced cleavage of 2-(2-aminophenyl)alkanamides

Experimental

Amino Amide Stock Solutions.—The 2-(2-aminophenyl)-alkanamides (**1**–**3**) were generated as required from the corresponding 2-(2-nitrophenyl)alkanamides[1] by catalytic hydrogenation of methanolic solutions (*ca.* 1 mg cm^{-3}) of the latter over Pd/C. The reduction solutions were cooled to −10 °C prior to beginning the hydrogenation, and the purity of the resultant solution of amino amide was immediately verified after hydrogenation by the HPLC assay described below. The solutions were then evaporated to dryness under reduced pressure at a temperature not exceeding 25 °C and the resulting



amines were dried in a desiccator over silica gel. Stock solutions (0.0175 mol dm^{-3}) of the amino amides were then prepared by dissolving a known weight in MeOH and stored at −18 °C. The stability of the stock solutions was determined periodically by HPLC and fresh stock solutions were prepared on detection of cyclization products.

Kinetic Measurements.—Reactions were carried out in aqueous buffer solutions prepared from commercial reagent grade chemicals, dissolved in Milli-Q water. Formate buffer was employed in the pH range 2.8–3.9, acetate in the range 3.8–5.0 and phosphate in the range 6.0–7.3. Constant ionic strength was maintained at 0.1 mol dm^{-3} by addition of NaCl. Measurements of pH were made using an Orion SA520 pH meter. Aliquots (3.5 cm^3) of buffer were thermostatted at 37 °C. The pH values of the solutions were then measured and the reactions were initiated by addition of 0.01 cm^3 of the stock solution. This gave a final amino amide concentration of 50 μmol dm^{-3} and a final solvent medium of 0.29% MeOH in water. Reactions having a half-life of less than 4 h were followed spectrophotometrically, with a suitable analytical wavelength being chosen after comparison of the spectra of the starting amino amide and the cyclization products. The spectral change was internally blanked at 450 nm, where no species absorbed. The observed pseudo-first-order rate coefficients were calculated directly from the absorbance *vs.* time data. Reactions having longer half-lives were analysed by the isocratic HPLC assay described below. In specific cases where the kinetics were studied by both methods, good agreement was seen between them. For example, the observed pseudo-first-order rate of cyclization of **1a** evaluated by UV–VIS spectrophotometry was 3.17×10^{-4} s^{-1} and by HPLC assay was 3.22×10^{-4} s^{-1} (at pH 7.23, total concentration of phosphate buffer = 0.02 mol dm^{-3}, I = 0.10 mol dm^3 and T = 37 °C).

HPLC Assay Conditions.—The loss of the starting amino amide (**II**) and the appearance of lactam (**IV**) and amine (**V**) in the reaction solutions (Scheme 1) were monitored through the use of a Hewlett Packard HPLC using an analytical wavelength of 254 nm. The isocratic assays were carried out on an Econosphere C-18 5 μm column with a mobile phase comprising 64% MeOH (analytical grade) and 36% 10 mmol dm^{-3} phosphate buffer at pH 7 (in Milli-Q water), at a flow rate of 0.7 cm^3 min^{-1}. Under these conditions all retention times were less than 14 min.

pK_a *Determinations.*—pK_a determinations were carried out spectrophotometrically using a Hewlett Packard 8452A Diode Array spectrophotometer, using solvent and temperature conditions identical to those employed for the kinetic measurements. It was not possible to determine the pK_a of any of the compounds which possessed α,α-dimethylacetamide side-chains owing to their very rapid cyclization in the requisite pH range.

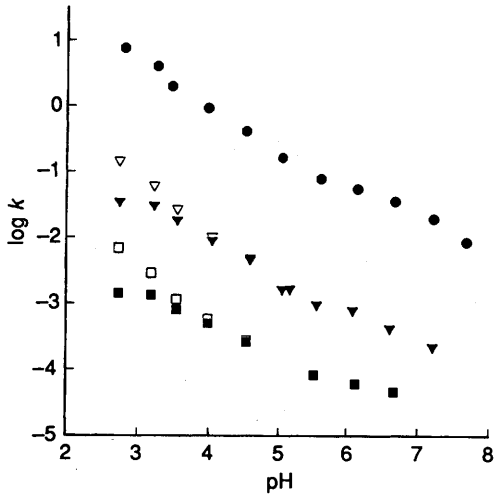


Fig. 1 pH Dependence of the observed pseudo-first-order rate coefficients of cyclization of (●) **1a**, (▼) **2a** and (■) **3a**. Filled symbols depict the rate coefficient uncorrected for amine protonation, while hollow symbols depict the corrected rate coefficient. Rate coefficients were evaluated in triplicate, with the average of the three determinations represented (total buffer concentration = 0.02 mol dm^{-3}, I = 0.1 mol dm^{-3}, T = 37 °C).

Results

Fig. 1 shows representative first-order plots for loss of **2a** at various pH values, showing that pseudo-first-order kinetics are obeyed. The pH dependence of the cyclizations of **1a** and **2a** were followed in the pH range 3.0–7.5, while (owing to the slowness of the reaction at higher pH), the cyclization of **3a** was investigated over the pH range 3.0–6.5. Both the observed first-order rate coefficient (filled symbols) and the rate coefficient corrected for amine protonation (hollow symbols) are depicted. The pK_a values of **2a** and **3a** were found to be 3.24 ± 0.08 and 3.4 ± 0.2, respectively, indicating that they will be substantially protonated at the lower end of the pH range studied. Because of the rapid cyclization of α,α-dimethyl substituted compounds in this pH range, determination of the pK_a value of **1a** was not possible and therefore no correction of the kinetics of cyclization of **1a** for amine protonation could be made. However, the pK_a of **1a** is likely to be somewhat higher than those of **2a** and **3a** (owing to increased electron donation), leading to a lesser deviation from linearity of the observed pseudo-first-order rate coefficient of **1a** at low pH (Fig. 1).

All kinetic parameters for compounds **2a** and **3a** have been derived from the values of the observed pseudo-first order rate coefficients, corrected for the effect of amine protonation according to the relationship (1). The effect of correction for

$$k_{cor} = k_{obs}\{1 + ([H_3O^+]/K_a)\} \quad (1)$$

the effect of amine protonation is significant at pH values less than 4.5. Thus k_{cor} is approximately three times greater than k_{obs} at pH 4.5.

More detailed investigation was undertaken of buffer catalysis of these cyclizations in the pH range 3.0–7.5 (**1a**), 3.0–5.0 (**2a** and **3a**) and 3.0–7.0 (**1a**, **1c** and **1d**). Compound **1b** was studied in the pH range 4.0–7.0, to avoid possible complications

Table 1 First-order, buffer-independent rate coefficients (k_0) for the loss of amino amides **1a**, **2a**, **3a**, **1b**, **1c** and **1d**

	k_0/s^{-1}		k_0/s^{-1}			k_0/s^{-1}		
pH	**1a**	pH	**2a**[a]	**3a**[a]	pH	**1b**	**1c**	**1d**
2.91	0.0595	3.04	6.97×10^{-4}	5.90×10^{-5}	3.07	—	1.16	0.113
3.36	0.0268	3.53	2.63×10^{-4}	3.70×10^{-5}	3.52	—	0.0530	0.0477
3.86	0.0105	3.98	—	6.73×10^{-6}	3.96	—	0.0187	—
3.89	9.92×10^{-3}	4.02	1.02×10^{-4}	6.87×10^{-6}	4.01	0.0235	0.0178	—
4.40	3.87×10^{-3}	4.03	1.11×10^{-4}	—	4.04	—	—	0.0188
4.94	1.12×10^{-3}	4.52	3.63×10^{-5}	2.30×10^{-6}	4.05	0.0195	—	0.0172
6.19	1.04×10^{-4}	5.03	2.02×10^{-5}	—	4.53	6.83×10^{-3}	7.07×10^{-3}	7.02×10^{-3}
6.78	2.18×10^{-5}	5.07	—	1.16×10^{-6}	5.04	2.70×10^{-3}	2.38×10^{-3}	2.48×10^{-3}
7.13	1.24×10^{-4}				6.26	3.23×10^{-4}	1.70×10^{-4}	2.13×10^{-4}
					6.78	1.80×10^{-4}	1.12×10^{-4}	1.08×10^{-4}
					7.29	1.25×10^{-4}	7.27×10^{-5}	3.82×10^{-5}

[a] Data corrected for amine protonation.

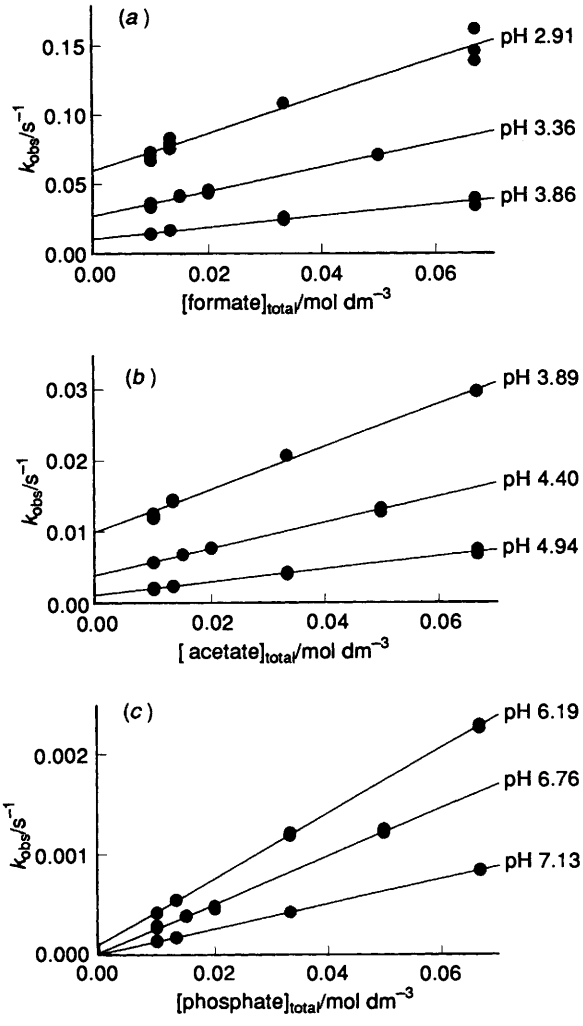


Fig. 2 Buffer dilution plots showing the dependence of the observed pseudo-first-order rate coefficient of cyclization of **1a** *vs.* total buffer concentration. Rate coefficients were determined in triplicate, with all three values depicted (I = 0.1 mol dm^{-3}, T = 37 °C).

resulting from protonation of the $N(Me)_2$ group at low pH. Figs. 2(*a*)–(*c*) show the linear dependence of the observed pseudo-first-order rate coefficient of the cyclization of **1a** on total buffer concentration. Similar relationships were observed for compounds **2a** and **3a** (pH range 3.0–5.0), **1b** and **1c** (pH range 3.0–7.5) and **1d** (pH range 4.0–7.5) (data not shown). The

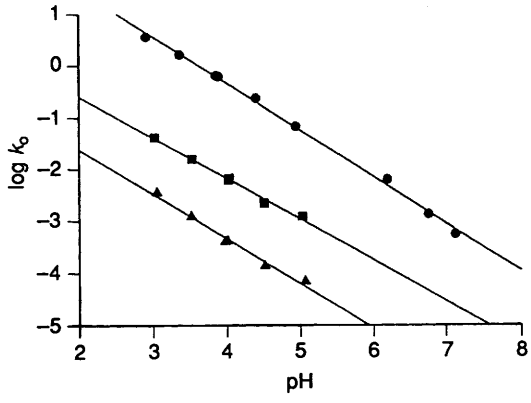


Fig. 3 Buffer independent pH–rate dependence of (●) **1a**, (■) **2a** and (▲) **3a**

data of Figs. 2(*a*)–(*c*) were found to obey eqn. (2), showing that

$$k_{obs} = k_0 + k_{cat}[B_T] \quad (2)$$

the reaction is subject to general buffer catalysis. The y-intercepts of the lines correspond to k_0, the apparent buffer-independent first-order rate coefficient (Table 1) and the slope gives values of k_{cat}, the buffer-dependent second-order rate coefficient (Table 2). The observed variations in y-intercepts given in Figs. 2(*a*)–(*c*) indicate that the buffer independent reaction is pH dependent and therefore the reaction is catalysed by hydronium and/or hydroxide ions, as well as the solvent water.

The pH dependence of the buffer-independent rate coefficients for cyclization of compounds **1a**–**3a** are represented in Fig. 3, while the buffer-independent pH profile for the series **1a**–**1d** are represented in Fig. 4. This observed buffer-independent pH dependence may be described by eqn. (3). Plots

$$k_0 = k_{H_3O^+}[H_3O^+] + k_{H_2O} \quad (3)$$

of k_{cat} against the fraction of buffer base present are shown in Figs. 5(*a*)–(*c*). Here the y-intercepts provide values for k_{HA} and the value of y corresponding to a fraction of buffer base equal to unity yields k_{A^-}. Over the pH range studied, none of the compounds showed evidence of general base catalysis and the y-value corresponding to 100% buffer base was generally less than, or very close to, zero. Table 3 gives the values of the rate coefficients for specific buffer acid catalysis. The observed pseudo-first-order rate coefficient may be described by eqn. (4).

$$k_{obs} = k_{H_2O} + k_{H_3O^+}[H_3O^+] + k_{HA}[HA] \quad (4)$$

Table 2 Second-order, buffer-dependent rate coefficients (k_{cat}) for the loss of amino amides **1a**, **2a**, **3a**, **1b**, **1c** and **1d**

	k_{cat}/dm^3 mol^{-1} s^{-1}		k_{cat}/dm^3 mol^{-1} s^{-1}			k_{cat}/dm^3 mol^{-1} s^{-1}		
pH	**1a**	pH	**2a**[a]	**3a**[a]	pH	**1b**	**1c**	**1d**
2.91	1.35	3.04	0.0283	1.18×10^{-3}	3.07	—	2.37	2.42
3.36	0.875	3.53	0.0453	5.45×10^{-4}	3.52	—	1.40	1.35
3.86	0.415	3.98	—	2.95×10^{-4}	3.96	—	0.695	—
3.89	0.298	4.02	4.40×10^{-3}	2.17×10^{-4}	4.01	0.935	0.452	—
4.40	0.183	4.03	6.05×10^{-3}	—	4.04	—	—	0.469
4.94	0.0893	4.52	2.90×10^{-3}	1.41×10^{-4}	4.04	1.08	—	0.678
6.19	0.0325	5.03	7.70×10^{-4}	—	4.53	0.450	0.285	0.308
6.78	0.0240	5.07	—	3.58×10^{-5}	5.04	0.137	0.148	0.147
7.13	0.0124				6.26	0.0372	0.0732	0.0707
					6.78	0.0192	0.0460	0.0515
					7.29	0.0104	0.0252	0.0277

[a] Data corrected for amine protonation.

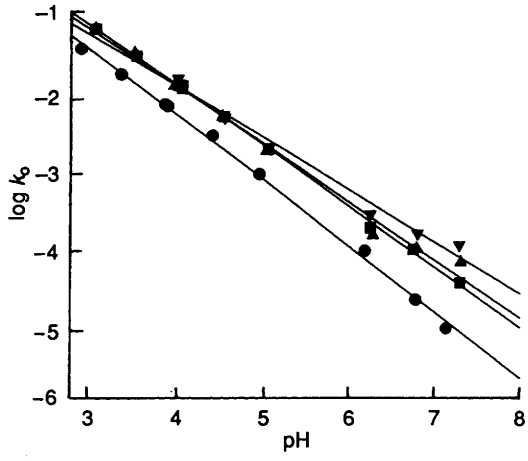


Fig. 4 Buffer independent pH–rate dependence of (●) **1a** (σ_p −0.12 ± 0.05), (▼) **1b** (σ_p −0.3 ± 0.1), (▲) **1c** (σ_p 0.00) and (■) **1d** (σ_p 0.5 ± 0.1). Values for σ_p were taken from N. B. Chapman and J. A. Shorter, A critical compilation of substituent constants, in *Correlation Analysis in Chemistry*, Plenum Press, 1978.

From Figs. 3 and 4 it appears that the water-catalysed rate coefficient, k_{H_2O}, is very small. If it is assumed that the contribution of k_{H_2O} to k_{obs} is negligible, then substituting K_a[HA]/[A$^-$] for H_3O^+ in eqn. (4), and rearranging, yields the relationship (5). Plots of k_{obs}/[HA] *vs.* 1/[A$^-$] for a given buffer

$$k_{obs}/[HA] = k_{HA} + k_{H_3O^+}K_a/[A^-] \quad (5)$$

species yield plots of slopes corresponding to k_{H_3O} and a *y*-intercept corresponding to k_{HA} (Fig. 6). Values for $k_{H_3O^+}$ were obtained from such plots for compounds **1a–1d**, **2a** and **3a**, in acetate buffers. Brønsted plots for the cyclization of **1a–3a**, where the log k_{HA} is related to the pK_a of the species responsible for the catalysis, yielded α values (-slope) of 0.41, 0.4 and 0.4, with standard deviations of 0.07, 0.2 and 0.1, respectively.

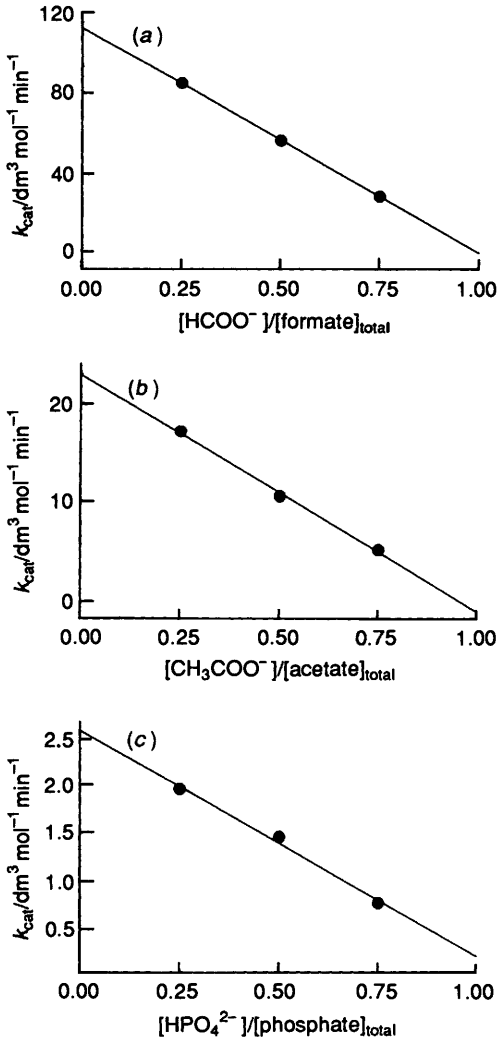


Fig. 5 Plots of k_{cat} (slopes of Fig. 4) *vs.* fraction of buffer base

Discussion

The internal cyclization of amino amides was originally developed as a means of sequencing amino acids; reduction of 2,6-dinitrophenyl derivatives of amino acids was followed by acid-catalysed internal cyclization to yield the corresponding lactam and the peptide, less one amino acid residue.[6] The facile reductive cyclization of simpler nitro amides such as **4** has also been reported.[7] In the latter case, reduction and cyclization reactions occur in the same solvent medium, with 90% release of amine observed within 30 min.

The use of the 'trimethyl lock' (a combination of α,α-dimethyl substitution in the side-chain and 6-methyl substitution on the aromatic ring) to accelerate greatly the rates of cyclization in these systems was first reported for 2-(2-hydroxyphenyl)-propanoic acids and the term 'stereopopulation control' was introduced to describe the various mechanisms contributing to the rate enhancing effect observed[3] (although subsequent work[4,8] revealed the rate enhancement to be less than was originally thought). The rates of release of amines *via* lactonization of 2-(2-hydroxyphenyl)propanamides was shown[5] to be similarly dependent on methyl substitution; thus

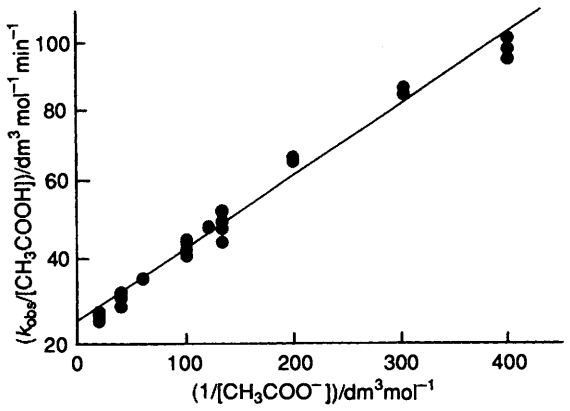


Fig. 6 Representative plot of $k_{obs}/[CH_3CO_2H]$ for **1a** *vs.* the inverse of acetate concentration. The slope of this plot yields a value for $K_a k_{H_3O^+}$, while the *y*-intercept corresponds to a value of $k_{CH_3CO_2H}$, according to eqn. (4).

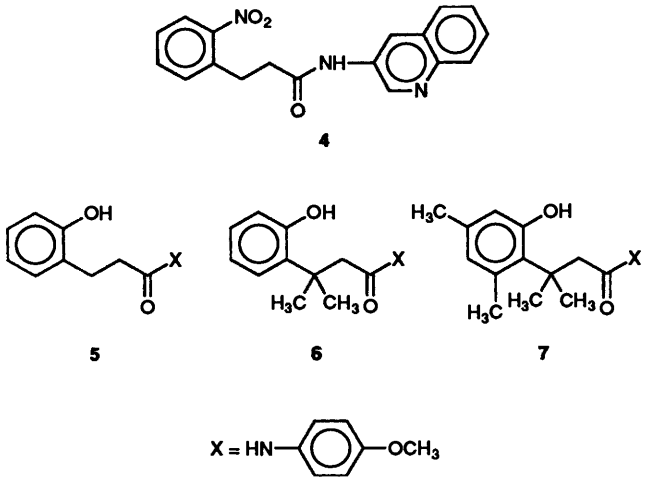


Table 3 Rate constants (s^{-1}) for the buffer acid-catalysed cyclization of amino amides **1a**, **2a**, **3a**, **1b**, **1c** and **1d**

	$k_{H_3O^+}$	k_{CHO_2H}	$k_{CH_3CO_2H}$	$k_{H_2PO_4^-}$
1a	180	1.80	0.40	0.043
2a[a]	2.0	0.038	6.3×10^{-3}	—
3a[a]	0.14	1.6×10^{-3}	3.2×10^{-4}	—
1b	450	3.7	1.5	0.048
1c	330	3.2	0.6	0.097
1d	350	3.2	0.63	0.093

[a] Data corrected for amine protonation.

the relative observed pseudo-first-order rates of cyclization of compounds **5**–**7** were 1, 44.3 and 2.57×10^4 (pH 7.5, 0.1 mol dm^{-3} phosphate buffer, $I = 0.3$ mol dm^{-3}, $T = 30$ °C).

In comparison, the relative hydronium ion catalysed rates of **3a**, **2a** and **1a** seen in the present study were 1, 14 and 1300, while the relative observed pseudo-first-order rates were 1, 9 and 800 (at pH 6.6, the highest pH studied common to all three compounds, 0.02 mol dm^{-3} phosphate buffer, $I = 0.1$ mol dm^{-3}, $T = 37$ °C). The relative enhancement of rate between **3a** and **1a** (800) is higher than that for the analogous 2-(2-hydroxyphenyl)-2-methyl-propanamides (**5**:**6**; 400). In the present series therefore, substitution in the 6-position of **1a**, to provide the full trimethyl lock, might be expected to provide a very large further rate enhancement.

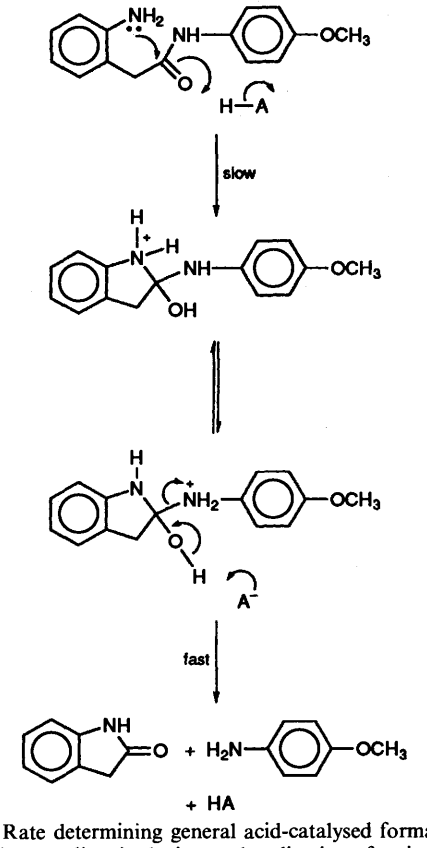
Several studies have been reported on the mechanism of cyclization of hydroxy amides, which sometimes involve quite complex pH dependence.[9–11] Breakdown of the tetrahedral intermediate is the most commonly proposed rate-determining step, although rate-determining formation of the tetrahedral intermediate has also been claimed.[10] However, only one study involving nucleophilic attack by an amine group on an amide has been reported. This was the intramolecular aminolysis of a series of 2-aminophenoxyacetamides,[12] where the effect of electronic variation in the attacking and leaving groups was investigated. The rate-determining step was proposed to be the formation of the tetrahedral intermediate because the reaction was found to be far more sensitive to the electronic environment of the attacking than of the leaving amine.

In the present study, clean isosbestic points were observed in the spectral changes accompanying the reactions, with no spectral evidence for the presence of an intermediate. Neither the buffer dilution plots, nor the buffer independent pH profiles of **1a**–**3a** (Fig. 3) and **1a**–**1d** (Fig. 4) showed deviations in kinetic behaviour over the buffer concentrations and pH ranges studied. Likewise, no changes in kinetic behaviour were observed on alteration of the compound stereochemistry (*via* methyl substitutions) or the electronic environment of the leaving amine. There is, therefore, no evidence to suggest that a change in the rate-determining step occurs with changing catalyst concentration, pH and reactant structure, over the ranges investigated.

No relationship was observed between the Hammett σ_p values (given in the caption accompanying Fig. 4) of the 4-substituents in the series **1a**–**1d** and general acid-catalysed rate constants (Table 3) and buffer independent cyclization (Fig. 4) of the amino amides. This independence of the rate of amino amide cyclization from changes in the electronic environment of the leaving amine is in contrast to the results of Kirk and Cohen,[12] who reported ρ values of 0.60 and 0.25 for the buffer acid and hydronium ion catalysed cyclization of 2-aminophenoxyacetamides, respectively. A discrepancy exists, however, between the tabulated data and resultant Hammett plots in this study and thus the influence of the leaving group is not certain. Amsberry and Borchardt[13] observed no significant leaving group effect in the lactonization of trimethyl-locked 2-(2-hydroxyphenyl)propanamides. It was proposed that the steric driving force for the reaction was so strong that any electronic effect owing to changes in the leaving amine was overwhelmed.[5] However, Carpino *et al.*[14] did observe a leaving group effect in the lactonization of similar trimethyl-locked 2-(2-hydroxyphenyl)propanamides (although the reaction conditions differed between these two studies and cyclization reactions proceeded more slowly under the conditions used by Carpino *et al.*).

It appears, therefore, that a 'steric override' of an otherwise observable leaving group may occur when internal cyclization reactions are very rapid. Such a steric override of a leaving group effect does not appear likely in the present study, as the amino amides (**1a**–**1d**) are only partially locked and the steric driving force of the reaction is expected to be considerably less in comparison to the hydroxy amides studied previously[13] (*e.g.* **1a** has $t_{\frac{1}{2}} = 14$ min) (pH 7.13, $T = 37$ °C), compared[5] with a $t_{\frac{1}{2}}$ of 65.4 s for **7** (pH 7.4, $T = 37$ °C).

The overall lack of rate dependence on the electronic environment of the leaving amine suggests that protonation of the leaving amine occurs after the rate-determining step. As a consequence, rate-determining general acid-catalysed breakdown of the tetrahedral intermediate does not appear likely. Such reaction pathways involve protonation of the amide nitrogen during, or prior to, the rate-determining step, and therefore the observed pseudo-first-order rate coefficient is expected to be influenced by the value of the pK_a of the leaving amine. A mechanism involving rate-determining concerted attack of the neutral amine and proton transfer from a general



Scheme 2 Rate determining general acid-catalysed formation of the tetrahedral intermediate in the internal cyclization of amino amides

acid to the amide oxygen is therefore proposed and is depicted in Scheme 2.

The unique pH dependence of the 2-(2-aminophenyl)-propanoic acids studied here [essentially linear over the physiological pH range (Fig. 4)] is of interest in terms of the use of this system for the generation of hypoxia-selective cytotoxins. The extracellular pH in poorly vascularized hypoxic regions of tumours, at around 6.7, is on average *ca.* 0.7 pH units lower than in normal tissue (7.4)[15] and high-resolution glass microelectrode measurements indicate values as low as 5.8.[16] Buffer independent first-order rates for the cyclization of **1a** (estimated from Fig. 3) at pH 6.7 and 7.4 suggest an enhancement of about six-fold in the rate of release between these two pH values (3×10^{-5} and 5.8×10^{-6} s^{-1}, respectively).

Acknowledgements

This work was supported by the Auckland Division of the Cancer Society of New Zealand. We thank the Research Committees of the University of Auckland and the New Zealand Vice Chancellors' Committee and Lottery Science for equipment grants.

References

1 G. J. Atwell, B. M. Sykes, C. J. O'Connor and W. A. Denny, *J. Med. Chem.*, 1994, **37**, 371.
2 W. A. Denny and W. R. Wilson, *J. Med. Chem.*, 1986, **29**, 879.
3 S. Milstein and L. A. Cohen, *J. Am. Chem. Soc.*, 1972, **94**, 9158.
4 M. Caswell and G. L. Schmir, *J. Am. Chem. Soc.*, 1980, **102**, 4815.
5 K. L. Amsberry and R. T. Borchardt, *J. Org. Chem.*, 1990, **55**, 5867.
6 R. A. Johnstone, T. J. Povall and I. D. Entwistle, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1424.
7 I. D. Entwistle, *Tetrahedron Lett.*, 1979, 555.
8 D. F. DeTar, *J. Am. Chem. Soc.*, 1982, **104**, 7205.
9 S. Milstein and L. A. Cohen, *J. Am. Chem. Soc.*, 1970, **92**, 4377.
10 R. Hershfield and G. L. Schmir, *J. Am. Chem. Soc.*, 1973, **95**, 7359.
11 T. Okuyama and G. L. Schmir, *J. Am. Chem. Soc.*, 1972, **94**, 8805.
12 K. L. Kirk and L. A. Cohen, *J. Am. Chem. Soc.*, 1972, **23**, 8142.
13 K. L. Amsberry and R. T. Borchardt, *Pharm. Res.*, 1991, **8**, 323.
14 L. A. Carpino, A. T. Salvatore and R. A. Berglund, *J. Org. Chem.*, 1989, **54**, 3303.
15 I. Tannock and D. Rotin, *Cancer Res.*, 1989, **49**, 4373.
16 P. W. Vaupel, S. Frinak and H. J. Bicher, *Cancer Res.*, 1981, **41**, 2008.

Paper 4/04266B
Received 12*th July* 1994
Accepted 21*st September* 1994