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The Chemical Reactivity of β-Lactams, β-Sultams and β-Phospholactams

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Abstract—β-Lactam antibiotics display a range of biological activities. The origin of this diverse biological activity is discussed with reference to the chemical reactivity of the small ring system. The reactions of β-sultams and β-phospholactams with simple nucleophiles are reported and their potential as mechanism based inhibitors of bacterial and mammalian serine proteases is described.

Introduction

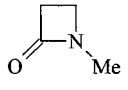
The chemical and biological activity of β-lactams

β-Lactams are active acylating agents, acylating nucleophilic residues in a diverse range of bacterial[1] and mammalian serine active enzymes.[2] They are used in a very large number of clinically important therapeutic areas: in defence against bacterial infection[3] and in the combat of pulmonary disease.[4]

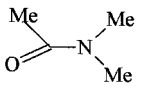
In the area of antibacterial therapy the effectiveness of β-lactam antibiotics is quickly being eroded as bacterial resistance to β-lactam antibiotics increases.[5] Bacteria resistant to all clinically used antibiotics are being identified in hospitals around the world[6] and there is now an urgent need to develop new antibiotics. To assist in the successful development of new therapeutic agents it is necessary to understand the origins of the unique biological activity of the small ring system of β-lactams. In this paper we discuss the origins of the biological activity of β-lactams and describe the work we have undertaken to determine if structural analogues of β-lactams, β-sultams and β-phospholactams, possess similar activity.

Chemical and biological reactivity of β-lactams

The significant biological activity of β-lactams is frequently attributed to the high intrinsic chemical reactivity of the four-membered cyclic amide. Comparison of the second order rate constants for the base catalysed hydrolysis of the *N*-methyl β-lactam (**1**) (6.1×10^{-6} dm^3 mol^{-1} s^{-1}) and *N*,*N*-dimethylethanamide (**2**) (2.3×10^{-6} dm^3 mol^{-1} s^{-1}) clearly indicates, however, that the difference in reactivity is, in fact, small.[7] Crystal structures of bicyclic β-lactams indicate the presence of a non-planar amide (**3**) with the nitrogen being out of the plane described by its substituents by up to 0.5 Å.



(1)



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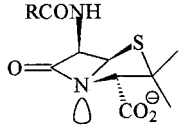
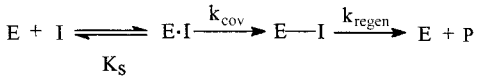


(3)

The non-planar amide of the bicyclic β-lactam systems and the presumed reduction in amide π-resonance is frequently quoted as being responsible for their increased biological activity. However, there is little evidence to support this argument. There is no direct correlation between the antibacterial activity of bicyclic ring systems and a measure of the degree of distortion of the amide bond.

β-Lactams are mechanism-based inhibitors of serine active enzymes; they act by acylating a nucleophilic residue of the enzyme.[8,9] Before considering the specific details of the inhibition by β-lactams it is appropriate to list the basic requirements for successful mechanism based inhibition. There are at least three steps involved in the successful inhibition of enzymes by compounds using the enzymes' normal catalytic machinery (mechanism-based inhibitors, Scheme 1):

- The target enzyme must be able to recognise and bind the



Scheme 1.

Keywords: β-lactamase inhibitors; β-sultams; β-lactams; β-phospholactams.
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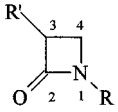
inhibitor. In binding the inhibitor favourable binding interactions compensate for changes in the order of the system and modifications of the inhibitor which activate the inhibitor leading to an increased rate of covalent attachment.

- In the second step the enzyme must be able to catalyse the redistribution of electron density associated with bond breaking and bond formation and the movement of nuclei associated with covalent attachment. This will be represented by a large rate constant for bond formation, i.e. a large k_{cov}.
- If the inhibition is to be effectively irreversible the covalent linkage between the enzyme and inhibitor, or that resulting from a subsequent modification of the covalently bound inhibitor, must be stable to a subsequent reaction leading to the regeneration of the enzyme i.e. the rate constant of turnover of the inhibitor should be small, k_{regen}.

Each of the above criteria must be met for successful in vitro inhibition. Why are β-lactams successful mechanism based inhibitors of a range of serine active enzymes?

Substrate recognition. The binding of β-lactams by serine active enzymes is a function of the amino acid residues and geometry of the active site of the individual enzymes. The bacterial proteases, transpeptidases[10] and β-lactamases,[11] have a positively charged residue, either arginine or lysine, that forms a salt bridge with a negatively charged entity that is attached to the nitrogen of the β-lactam ring (**4**, R=CH_2CO_2H or SO_3H). In penicillins (**3**) the negative charge is a carboxylate group. In sharp contrast, the mammalian proteases, human leukocyte elastase (HLE) and cathepsin G, require neutral substrates. [12] A similar variation in recognition requirements for the side chain (**4**, R′) is observed. Good antibacterial activity is associated with the presence of an aromatic acylamido group attached at 3-position of the azetidinone ring[13] (R′) whereas the most potent inhibitors of HLE have a small aliphatic group at this position.[14] The only truly common recognition motif for serine proteases is the presence of the 'oxyanion hole'.[15]

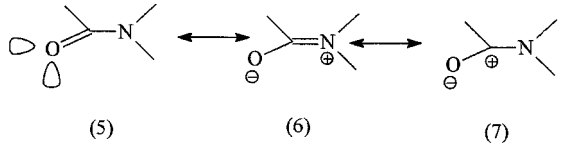


(4)

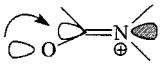
The oxyanion hole is a term used to describe the hydrogen-bonded network that surrounds the amide carbonyl oxygen of substrates. In mammalian proteases the oxyanion hole is formed by a hydrogen bond from the NH of the active site serine and a hydrogen bond from a second NH, this is normally from a backbone residue, to the carbonyl oxygen.[16] It is frequently stated that these hydrogen bonds are crucial to the catalytic process, however there is no direct evidence to indicate if this is an absolute requirement. The strength of these hydrogen bonds presumably increases in moving from the carbonyl oxygen of the ground state (pK_a −1) to the more basic oxygen anion (pK_a 9–12) of the tetrahedral intermediate. The oxyanion-hole is presumed to lower the energy of the transition structure for the enzyme reaction compared with that in water. However, using model systems, it has been demonstrated that the ability of hydrogen bonds to stabilise oxygens of differing basicity is limited.[17] Any enthalpic advantage offered to the transition state in the enzyme catalysed reaction by the oxyanion is small when compared to the enthalpy changes accompanying solvation changes for the reaction in water. The true catalytic advantage may come from entropic terms. Wladkowski et al.[18] have suggested that the primary function of the oxyanion hole is in the binding of the substrate in a correct orientation within the substrate active site.

Is there a structural feature, electronic or geometric, of the β-lactams, which enable them to interact to a greater extent, during the catalytic process, with the oxyanion hole compared with their acyclic counterparts?

The nature of bonding in amides has been the subject of recent debate.[19] A classical resonance picture describes the bond as a hybrid formed among three resonance forms (**5**, **6** and **7**).



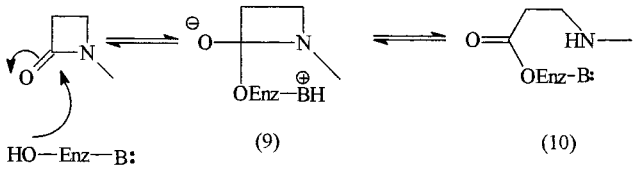
(5) (6) (7)



(8)

In addition to resonance involving the π-network it is possible for the oxygen's non-bonded orbitals to overlap with the antibonding C to N sigma bond (**8**). The consequences of amide distortions, either by pyramidalisation of nitrogen or rotation of nitrogen about an axis of the C–N bond will be to reduce the π-resonance. Reduction in amide resonance is expected to favour resonance form (**5**) and result in an increase in the length of the C–N bond and a reduction in the length of the C–O bond. Experimental evidence suggests that whilst there is an increase in the C–N bond length there is little change in the C–O bond.[20] The latter may be a consequence of the opposing effect of π-resonance and the $n(O)$-$\sigma^*(C–N)$ interaction. Molecular orbital calculations and studies of the photoelectron-spectra of distorted amides[21] suggest that there is little change in the electron density on the carbonyl oxygen compared with that in planar systems. Based on electron density, the carbonyl oxygen of distorted amides would not be expected to participate in a greater degree of hydrogen bonding in the ground state with the oxyanion hole than the equivalent acyclic counterpart.

Covalent attachment. Acylation of the active site serine residue is the second step in the inhibition process. Although there is no direct evidence, it is generally accepted that acylation of the enzyme proceeds via the formation of a tetrahedral intermediate (**9**) and this involves at least two steps, Scheme 2. Each step will have its own transition state and the extent of any catalysis is determined by the degree to which the highest energy barrier is reduced. In the base



Scheme 2.

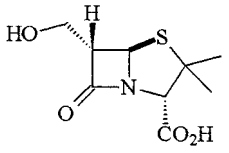
catalysed solvolyses of acyclic amides breakdown of the tetrahedral intermediate is rate limiting.[22] In the alcoholysis of bicyclic β-lactams breakdown of the tetrahedral intermediate is also rate limiting.[23] For amide hydrolysis the transition states are close in structure to the tetrahedral intermediate identified in the solvolyses reactions. It is a general belief that the rate enhancement observed in enzyme catalysis is brought about by the enzyme's ability to stabilise the transition state, the active site having a complementary structure to the transition state. Questions are being raised regarding the extent to which entropy terms contribute towards this process.[18]

The entropy difference between the enzyme catalysed reaction and that in the absence of the enzyme may favour the reaction if the reactants, and any necessary catalytic groups are preorganised. Preorganisation is energetically driven by the formation of favourable interactions between the substrate and the enzyme. What are the necessary catalytic groups in serine active enzymes?

Studies of the alcoholysis of penicillins indicate a requirement for a general acid catalyst in the breakdown of the tetrahedral intermediate (**9**). [23] In mammalian serine proteases, chymotrypsin and trypsin, histidine, [24] acts in this role. The same histidine is also used as a general base catalyst in the formation of the tetrahedral intermediate (**9**). The identity of the general base in the bacterial serine active enzymes is the subject of some debate. [25] For the bacterial β-lactamases a number of different mechanisms have been proposed for transporting the serine hydroxyl proton to the β-lactam nitrogen, these include: concerted mechanisms involving direct transfer,[26] the use of a proton shuttle[27] and transfer to and from a basic lysine residue or a glutamate carboxylate.[28]

Inhibitor turnover. The normal catalytic action of serine proteases requires the enzyme to catalyse both the acylation and deacylation steps effectively. In the inhibition of serine active enzymes by β-lactams there is a need for the covalent linked acyl-enzyme, an ester, to be stable. A modest rate of acylation can be tolerated if the rate of deacylation is negligible, the reverse is not true. Factors controlling the rates of deacylation are complex. The source of the serine active enzyme, or more specifically the geometric arrangement of catalytic groups within the active sites of the different enzymes, is important. A number of bacterial and mammalian proteases form stable acyl-enzymes with penicillins[29] and with cephalosporins derivatives.[30] In contrast, the acyl-enzymes formed between bacterial β-lactamases and penicillins are very short lived.[31] Hydrolysis of the acyl-enzyme is enzyme catalysed and frequently the catalytic machinery used for the acylation step is used in the deacylation step.

In the development of mechanism based β-lactamase inhibitors, a number of different strategies have been adopted, each of which rely on the production of stable acyl-enzymes. The different approaches have been described in detail[32] and only the most salient features will be discussed here. One of the least recognised but perhaps the most effective method of producing a stable acyl-enzyme is to prevent access of the hydrolytic water to the carbonyl-carbon of the ester. Access can be denied by obstructing the path of the hydrolytic water or, alternatively, by directly displacing the hydrolytic water from the location it occupies in the free enzyme. The latter approach was utilised by Mobashery[33] in the design of 6α-(hydroxymethyl)penicillanate (**11**), a potent inhibitor of the TEM β-lactamases. A crystal structure of the acyl-enzyme formed between (**11**) and the TEM-1 β-lactamase indicates that the hydroxymethyl group has displaced the hydrolytic water to a position where the general base Glu-166 cannot activate it.[34]



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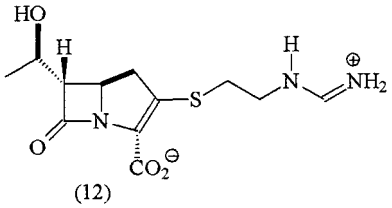
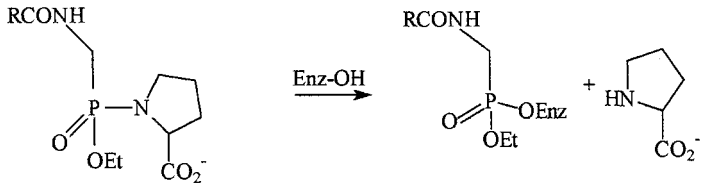


(12)

An indication of the importance of the positioning of the hydrolytic water comes from a study of the emergence of bacterial resistance to what has often been described as one of the two antibiotics of last resort, the carbapenem,[35] imipenem (**12**). New nonmetallocarbapenemases (NMC-A) are emerging which are able to hydrolyse imipenem, and crystal structure data[36] suggest that modifications in the active site of the NMC-A β-lactamase provide additional space for the hydrolytic water, allowing approach to the carbonyl of the acyl enzyme. Whilst the penicillin derivative (**11**) is as good a substrate as (**12**) for the NMC-A enzyme, replacement of the hydroxymethyl of (**11**) with the 1R-hydroxyethyl group of (**12**) provides compounds that can form a stable acyl-enzyme with NMC-A.[37] The additional methyl group of (**12**) can block the approach of the hydrolytic water.

Phosphyl transfer activity of bacterial serine active enzymes. Serine active enzymes catalyse a range of acyl transfer reactions including acyl transfer from amides, esters[38] and thioesters.[39] More recently it has been demonstrated that specific β-lactamases are capable of catalysing phosphoryl[40] and phosphonyl transfer.[41] The class C-β-lactamase from *Enterobacter cloacae* P99 is, in fact, extremely efficient in enhancing the rate of phosphonyl transfer and the pH dependence of the kinetic parameters indicate that similar catalytic machinery is used for both the acyl and phosphonyl transfer reactions. The class C-β-lactamase from *Enterobacter cloacae* P99 is inactivated by the



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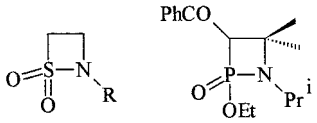
Scheme 3.

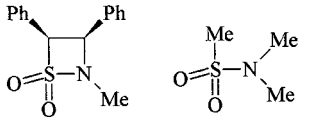
phosphonamidate (**13**) (R=$PhCH_2O$) by a phosphonylation process analogous to the enzyme catalysed acylation by penicillins (Scheme 3).

Both diastereoisomers of the phosphonamidate (**13**) (R=$PhCH_2O$) completely and irreversibly inactivate the P99 class C β-lactamase in a time dependent manner to give apparent first order rate constants, k_{obs}, for inactivation. These, in turn show a first order dependence on the concentration of the phosphonamidate to give the second-order rate constants, k_i, for inactivation. The two diastereoisomers show different rate constants for inactivation—5.10 and 0.14 $dm^3\ mol^{-1}\ s^{-1}$. But there is no discernible difference in the chemical reactivity of the two diastereoisomers towards alkaline hydrolysis. The 36-fold difference in reactivity is therefore good evidence of selectivity in the reaction of the enzyme with the two phosphonamidates and indicative of specific interactions between the inactivators and the protein. The time dependent inactivation is indicative of covalent bond formation between the enzyme and the phosphonamidate, which is confirmed by electrospray mass spectrometry (ESMS). For the most reactive diastereoisomer, inactivation of the enzyme occurs by formation of a 1:1 covalently bound enzyme-inactivator complex in which a proline residue has been displaced by a nucleophilic group on the enzyme—presumably the active site serine. Conversely, the less reactive diastereoisomer reacts with β-lactamase by displacing ethanol, which can be detected by gas chromatography. The observation that the enzyme reacts with one diastereoisomer by displacing proline and with another by displacing ethanol is again indicative of a stereoselective reaction occurring at the active site. The fact that the enzyme catalyses phosphonylation of the serine residue and that the assistance given to the phosphonylation is similar to that for acylation has been demonstrated. Consideration of the stereochemical course for acyl-transfer and phosphyl-transfer suggests that there must be a significant movement of the catalytic groups allowing the active site to catalyse these two very different processes. The products of the phosphonyl transfer reactions are phosphonyl esters that are stable and are turned over very slowly. These findings open the possibility that the heterocyclic β-lactam analogues, β-sultams and β-phospholactams, are potentially inhibitors of serine active enzymes.

β-Sultams and β-phospholactams. In an effort to develop new active inhibitors for bacterial serine active enzymes we have undertaken a detailed kinetic study of the acid and base catalysed solvolyses reactions of β-sultams (**14**) and β-phospholactams (**15**).



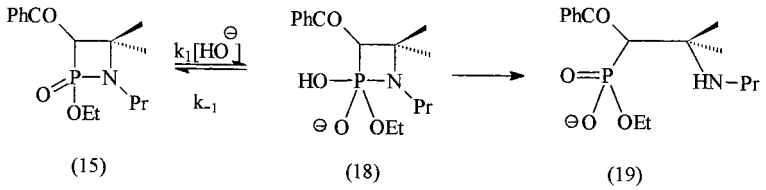
(14) (15)



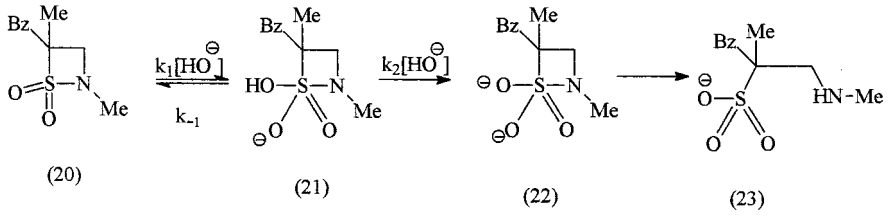
(16) (17)

A limited range of β-sultams[42] and β-phospholactams[43] has been synthesised and X-ray structures have been reported for the β-sultam (**16**)[44] and for the β-phospholactam (**15**).[43] Replacement of the carbonyl group of β-lactam with a sulphyl and phosphyl centre distorts the four membered rings, the sultam ring is buckled 14.7° whilst the phospholactam ring is buckled 8°. Whilst the CSN bond angle and CPN bond angles are very distorted, 82 and 81°, respectively, the length of the bond between the two heteroatoms is only marginally greater than that found in the related acyclic sulfonamides and phosphonamides. These bond lengths are still much greater than that of the C–N bond of amides. The P–N bond in **15** is 1.623 Å and the S–N bond in **16** is 1.643 Å, both bonds are substantially longer than the average carbon-nitrogen bond in planar β-lactams (1.35 Å) and non planar bicyclic β-lactams (1.33–1.45 Å).[45] Variations in the geometry and charge distribution in the different four membered ring systems will influence both the chemical and enzymatic reactivity of these molecules.

Base catalysed solvolyses reactions. In contrast to simple β-lactams, β-sultams and β-phospholactams react at rates that are substantially greater than that of their comparable acyclic counterparts. The second order rate constant for the hydroxide catalysed hydrolysis of the β-phospholactam **(15)** is at least 10^8 times greater than that for the phosphonamidate (**13**)[46] and the rate for the β-sultam (**14**, R=Me) is 10^7 times greater than that recorded for the acyclic sulphonamide (**17**).[47] The products of the hydrolysis reactions were the expected β-amino sulphonic acid (from the β-sultam) and the β-amino phosphonic acid (from the β-phospholactam). Attempts to observe deuterium incorporation at the α-carbon, during the sodium deuteroxide catalysed reactions, were unsuccessful suggesting that the hydrolysis proceeds via reaction at the heteroatom and does not occur by a carbanion promoted dissociative pathway (E1cB). Direct substitution at phosphonyl[48] and sulphonyl centres[49]



Scheme 4.



Scheme 5.

is known to proceed by inversion of configuration and is thought to occur with the attacking and departing groups occupying the two apical positions of a trigonal bipyramid, Schemes 4 and 5. Two possible mechanistic pathways exist for the substitution, a concerted pathway in which the attack and departure occur at the same time in a single step or a reaction in which there is an identifiable intermediate. Knowledge of the geometry of the reaction does not allow the two pathways to be separated.

Whilst there is firm evidence for the existence of trigonal bipyramidal intermediates (TBPIs) in substitutions at phosphorus[50] the same is not true for reaction at sulphonyl sulphur.[51]

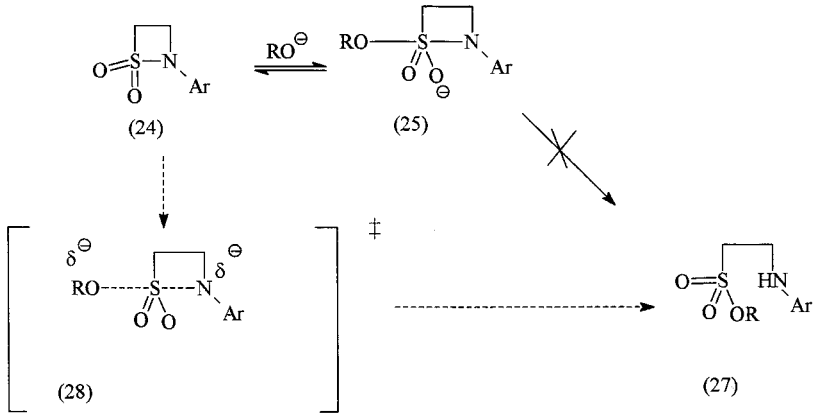
One of the main pieces of evidence for the existence of a tetrahedral intermediate on the reaction pathway for the base catalysed hydrolysis of amides comes from the observation of a second order dependence on hydroxide concentration for reaction of some anilides[52] and acetylpyrroles.[53] Rate constants measured for the hydroxide catalysed hydrolysis of the β-sultam (**20**) show a second order dependence on hydroxide and this is the dominant term in the rate law.[54] The second order term in the rate law is evidence for the formation of the TBPI (**22**) with a hypervalent sulfur. Initial but reversible attack of hydroxide ion on the β-sultam (**20**) generates a mono-anionic tetrahedral intermediate (**21**) that requires deprotonation by a second hydroxide ion before the intermediate can collapse to products.

The presence of a TBPI offers an explanation for the difference in reactivity of the β-sultams and β-phospholactams compared with that of β-lactams. The C–(S/P)–N bond angles in the four membered ring of β-sultams and β-phospholactams are close to 80° and will impart considerable angle strain to these molecules compared with the ideal tetrahedral geometry of 109°. If nucleophilic substitution occurs through the formation of a TBPI and the transition-state resembles this geometry then there will be relief of strain energy because the 5-coordinate heteroatom will be contained in a four-membered ring and the latter would be attached apical/equatorial, i.e. with an approximately normal 90° endocyclic bond angle. However, it is worth noting that the alkaline hydrolysis of five-membered cyclic sulfate and phosphate esters occurs orders of magnitude faster than that of the corresponding acyclic analogues, whether this is due to differences in strain or solvation energy remains controversial.[55]

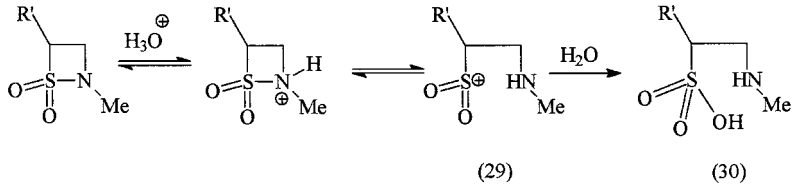
The nucleophilic substitution reaction occurring with serine acting enzymes is more directly related to alcoholysis. Studies of the alcoholysis of penicillins indicate that oxygen nucleophiles react only via their anions and this is used to indicate the requirement for general base catalysis in the enzyme catalysed reactions. With basic alkoxide anions, nucleophilic attack generates penicilloyl esters, analogous to the acyl-enzyme. In addition, penicillins react in aqueous solution with amine and thiol nucleophiles.[7] In sharp contrast, all attempts to observe alcoholysis, thiolysis and aminolysis of the *N*-aryl β-sultam (**24**) have failed.

A possible explanation for our failure to observe sulphonyl transfer to nucleophiles, other than hydroxide, is that such substitution may be inhibited by stereoelectronic factors and proton transfer steps. Alcoholysis of the β-sultam (**24**) would generate the TBPI (**25**) in which the attacking alkoxide and the leaving amino-group are located on the apical axis (Scheme 6). If the alkaline hydrolysis of *N*-aryl β-sultams requires proton removal from the OH group in the TBPI to facilitate S–N bond fission then this is not possible for alcoholysis and thiolysis. Exchange of apical and equatorial positions is also not possible in TBPI (**25**) because of the restrictions of pseudorotation due to the four-membered ring.

Acid catalysed solvolyses reactions of β-sultams. Details concerning the reactivity of small ring systems are available from studying the hydronium ion catalysed hydrolyses reactions. We have reported[47] the results of our studies of the acid catalysed hydrolysis of the β-sultams (**14**, R=Me) and (**20**). The reactions are characterised by having small negative entropies of activation (**14**, $-80\ J\ K^{-1}\ mol^{-1}$) and



Scheme 6.



Scheme 7.

a very substantial dependence of the second order rate constant for the acid catalysed hydrolysis on the inductive effects of substituents (R′) at the 4-position of the β-sultam ring. There is an enormous rate retardation by electron-withdrawing substituents at the 4-position, a Hammett ρ_I value of −10 can be estimated from the limited data available.

A mechanism consistent with the limited experimental data involves specific acid catalysed protonation of the substrate followed by a rate limiting unimolecular A1 fission of the substrate to generate the sulfonylium ion (**29**) which reacts rapidly with water to give the desired β-amino sulphonic acid (**30**) (Scheme 7). The reaction mechanism proposed for the acid catalysed hydrolysis of the β-sultam is similar to that reported for β-lactams[7] and β-phospholactams.[56]

General acid catalysis. The hydrolysis of β-sultams is buffer catalysed and the rate law for the hydrolysis of *N*-benzyl-β-sultam in carboxylate buffers is thus given by Eq. (1) demonstrating general acid catalysis by the buffer.[57]

$$k_{obs} = k_H[H^+] + k_{HA}[HA] \quad (1)$$

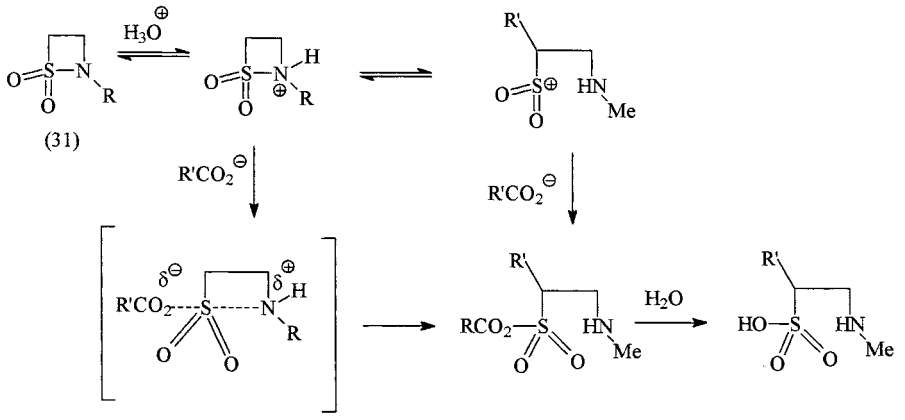
The values of the second-order rate constants k_{HA} are given in Table 1, from which it can be seen that they increase with decreasing pK_a of the carboxylic acid buffer. The observation of general acid catalysed hydrolysis is in contrast to the general base catalysis seen with the buffer catalysed hydrolysis of β-lactams of penicillins. Although catalysis by acidic species other than the protonated solvent is usually referred to as 'general acid catalysis', mechanistically the reaction may proceed via different but kinetically equivalent processes. Mechanistic general acid catalysed hydrolysis could involve nucleophilic attack by water that is concerted with the protonation of the β-sultam by the undissociated carboxylic acid. Interestingly, there is no evidence of intramolecular general acid catalysis in the hydrolysis of *N*-carboxymethyl β-sultam (**14**)(R=CH(CO_2H)Ph). Alternatively, a specific acid—general base catalysed pathway could involve pre-equilibrium protonation of the β-sultam nitrogen, followed by the general base catalysed attack by water, the nucleophilicity of which is enhanced as a result of proton abstraction by the carboxylate anion.

Table 1. Second-order rate constants ($M^{-1}s^{-1}$) for the carboxylic acid catalysed hydrolysis of *N*-benzyl-β-sultam at 30°C, I=1.0 M (KCl)

Carboxylic acid	p*K*a	k_{HA} ($M^{-1}s^{-1}$)
$ClCH_2CO_2H$	2.70	7.61×10^{-2}
$MeOCH_2CO_2H$	3.38	2.95×10^{-2}
HCO_2H	3.67	7.53×10^{-2}
CH_3CO_2H	4.57	4.14×10^{-3}

The third and probable mechanism of buffer catalysis involves specific acid–nucleophilic catalysis (Scheme 8). The β-sultam undergoes reversible protonation, probably on nitrogen, followed by direct nucleophilic attack of the carboxylate anion to form a mixed acid anhydride intermediate that is subsequently hydrolysed. In contrast to the hydroxide catalysed reaction, the concerted S_N2 type reaction would now be favoured by the expulsion of the neutral amine and the reaction could proceed without formation of a TBPI.

Nucleophilic catalysis in the carboxylate buffer hydrolysis of β-sultams was confirmed by trapping the mixed acid anhydride intermediate with aniline to give acetanilide. These observations provide conclusive evidence that the carboxylate buffer catalysed hydrolysis of β-sultams is due to specific acid—nucleophilic catalysis. There is evidence that the protonation of sulfonamide occurs on



Scheme 8.

nitrogen and the general acid-catalysed hydrolysis of the β-sultam could occur by a unimolecular A-1 type process with the carboxylate anion trapping the reversibly formed electron deficient sulfonylium ion (**31**). The evidence for such a mechanism in the hydrolysis of five-membered γ-sultams is ambiguous,[58] although most of it is consistent with a bimolecular mechanism.

The Brønsted plot (not shown) for the carboxylic acid catalysed hydrolysis of *N*-benzyl-β-sultam gives a good correlation between the values of log k_{HA} and the pK_a for 2-chloroacetic, 2-methoxyacetic and acetic acids with a slope of −0.67. This corresponds to a β_{nuc} of 0.33 for the specific acid-nucleophilic mechanism, indicative of an early transition state in which there has been a small amount of neutralisation of the negative charge on the carboxylate anion. Formic acid shows a positive deviation from this line which is again indicative of a nucleophilic pathway for catalysis.

The solvent isotope effect k_{H_2O}/k_{D_2O} of 1.57 for the chloroacetate buffer hydrolysis of *N*-benzyl-β-sultam is compatible with the specific acid-nucleophilic process, as is the observed entropy of activation of $-148\ J\ K^{-1}\ mol^{-1}$ for the chloroacetic acid catalysed hydrolysis.

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