JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2006; **19:** 446–451
Published online 30 January 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/poc.1016

Review Commentary

Comparison of the mechanisms of reactions of β-lactams and β-sultams, including their reactions with some serine enzymes†

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Received 13 September 2005; revised 14 November 2005; accepted 16 November 2005

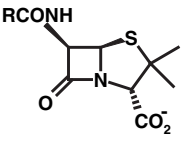
ABSTRACT: The strain energy of the four-membered ring of β-lactams is not released in the transition state to lower the activation energy of reactions involving ring-opening. The alkaline hydrolysis of *N*-aroyl β-lactams occurs with competitive exo- and endocyclic C—N ring fission, the ratio of which varies with the aryl substituent. β-Sultams are four-membered cyclic sulfonamides and are about 10^3 fold more reactive than analogous β-lactams. Nucleophiles usually attack *N*-acylsulfonamides at the carbonyl centre resulting in C—N bond fission, but the hydrolysis of *N*-acyl β-sultams occurs with S—N fission and opening of the four-membered ring. The 3-oxo-β-sultams are a unique combination of both β-lactams and β-sultams and therefore are susceptible to nucleophilic attack at either the acyl or the sulfonyl centre, but they hydrolyse with exclusive S—N bond fission. β-Sultams are novel inhibitors of the serine enzymes elastase, transpeptidase and β-lactamase due to sulfonylation of the active-site serine residue. Structure–activity relationships are used to identify differences in transition-state structures between β-sultams as inhibitors and β-lactams as substrates. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: mechanism; β-lactams; β-sultams; serine enzymes; reactivity; C—N vs S—N bond fission

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] A simple method to determine the effect of ring strain on the reactivity of β-lactams is to compare the rates of reactions of monocyclic β-lactams and their acyclic analogues. Contrary to expectations, the rate of alkaline hydrolysis of β-lactams is less than 100-fold greater than that of an analogous acyclic amide. For example, the second-order rate constant for the hydroxide-ion-catalysed hydrolysis of *N*-methyl β-lactam is only three-fold greater than that for *N*,*N*-dimethyl acetamide.[2]

β-Sultams (**2**) are the sulfonyl analogues of β-lactams (**3**) and are potential sulfonylating agents of a variety of nucleophiles by displacement of the amine leaving group. In contrast to acyl transfer reactions, the mechanisms of sulfonyl transfer reactions are much less well studied.[3] We have been interested in β-sultams both as possible inhibitors of proteolytic enzymes and as reactive sulfonyl derivatives capable of yielding useful mechanistic information for comparison with the more extensively studied β-lactams.[1] β-Lactams are well known inhibitors of DD-



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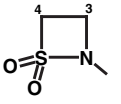

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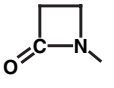

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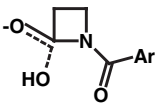

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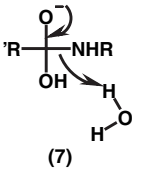

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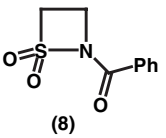

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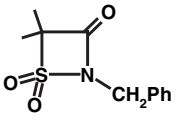

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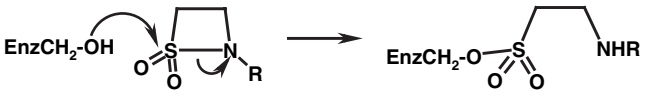
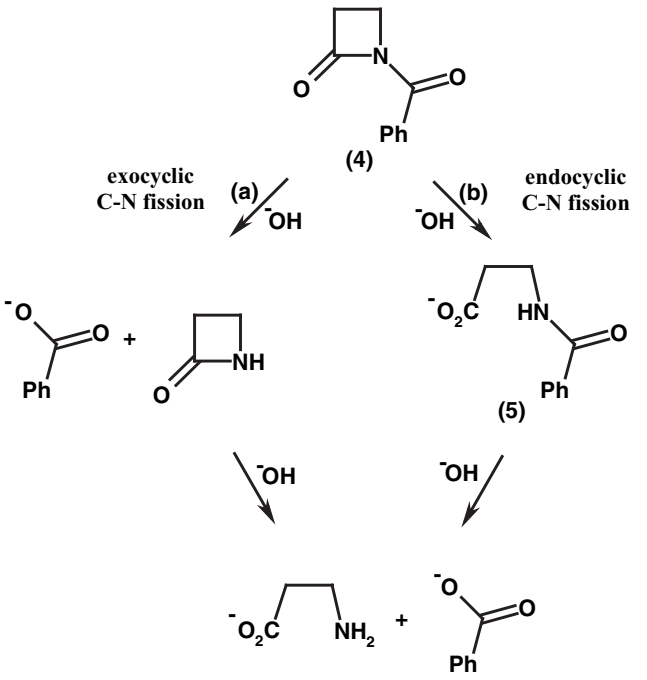
†Selected paper presented at the 10th European Symposium on Organic Reactivity, 25–30 July 2005, Rome, Italy.



Scheme 1

transpeptidase, ß-lactamase, elastase and other serine proteases by acting as acylating agents of the active site serine.[4] It is of interest to compare this activity with β-sultams, which could act as sulfonylating inhibitors of serine proteases (Scheme 1). The mechanisms of acyl transfer reactions commonly involve separate stepwise processes for nucleophilic addition and expulsion of the leaving group and rarely appear to be concerted.[3] Conversely, the mechanisms of sulfonyl transfer are usually discussed in terms of a concerted displacement and evidence for a stepwise process is often ambiguous.[5] The stereochemical and geometrical requirements for substitution at four-coordinate sulfonyl centres are also inherently different from those at three-coordinate acyl centres. Any sulfonyl transfer reaction catalysed by proteases may therefore give interesting insights about the flexibility of enzymes and any requirements for the precise alignments of atoms undergoing bond-making and -breaking.



Scheme 2

HYDROLYSIS OF *N*-AROYL β-LACTAMS

N-Benzoyl β-lactam (**4**) is an imide that contains both an endocyclic and an exocyclic acyl centre and so its alkaline hydrolysis could occur by either hydroxide ion attack at the exocyclic carbonyl to generate benzoic acid and the intact β-lactam (Scheme 2, route a) or at the endocyclic β-lactam carbonyl to give a ring-opened product (**5**) (Scheme 2, route b). The rate of the alkaline hydrolysis of *N*-benzoyl β-lactam (**4**) has a first-order dependence on hydroxide ion concentration and its second-order rate constant is $k_{OH} = 11.2\ M^{-1}\ s^{-1}$ which is in the range commonly observed for the alkaline hydrolysis of simple imides[6] and is indicative of no enhanced reactivity of the β-lactam. Surprisingly, one of the hydrolysis products is benzoic acid, which must be formed from the alkaline hydrolysis of the exocyclic amide (Scheme 2, route a). Competing exo- and endocyclic C—N bond fission was also confirmed by studying the hydrolysis reaction by ^{1}H NMR and two-dimensional ^{1}H,^{13}C HMBC (Heteronuclear Multiple Bond Correlation). The fractions of the unsubstituted azetidin-2-one and the β-amido acid (**5**) in H_2O are 0.19 and 0.81, respectively, so the second-order rate constant for the alkaline hydrolysis of the β-lactam of *N*-benzoyl β-lactam (**4**), $k^{endo}{}_{OH}$, can be calculated to be $9.07\ M^{-1}\ s^{-1}$ and that for the alkaline hydrolysis of the exocyclic amide, $k^{exo}{}_{OH}$, to be $2.13\ M^{-1}\ s^{-1}$. This is yet another example of the reluctance of four-membered rings to undergo facile opening.[1,7]

The rates of alkaline hydrolysis of some aryl-substituted *N*-aroyl β-lactams, and the product ratios and hence the relative rates of endo- and exocyclic C—N bond fission, depend on the nature of the *para*-substituents in the benzamide residue. Electron-withdrawing substituents favour attack at the exocyclic amide, presumably reflecting the relative importance of the electrophilicity of the carbonyl centre compared with the nucleofugality of the leaving group. The more electron-withdrawing the substituent, the higher is the ratio of exocyclic to endocyclic C—N bond fission and that of the simple azetidin-2-one to the β-amido acid (**5**) as products. Exocyclic C—N bond fission is the dominant reaction in the alkaline hydrolysis of *N*-*p*-nitrobenzoyl β-lactam in preference to β-lactam hydrolysis. The observation that electron-withdrawing substituents enhance the rate of reaction is compatible with either rate-limiting formation of the tetrahedral intermediate or its breakdown with amide anion expulsion occurring in the transition state. The difficulty of correlating the reaction rate with the ionization of the leaving group is the lack of available ionization constants of substituted benzamides. The ionization constants of substituted benzoic acids (analogues of benzamides) may however be used for the correlation.

The Bronsted-type plot of the rate constants for the alkaline hydrolysis of the β-lactam ring of *N*-aroyl β-lactams against the pK_a of the corresponding benzoic acids is shown in Fig. 1. The Bronsted β_{lg} value given by the slope of the line in Fig. 1 for the alkaline hydrolysis of *N*-aroyl β-lactams is −0.55. The effective positive charge on the nitrogen of imides is expected to be between 1.1 and 1.4. The observed Bronsted value of −0.55 for the alkaline hydrolysis of the β-lactam ring of *N*-aroyl β-lactams is compatible with a late transition state for the

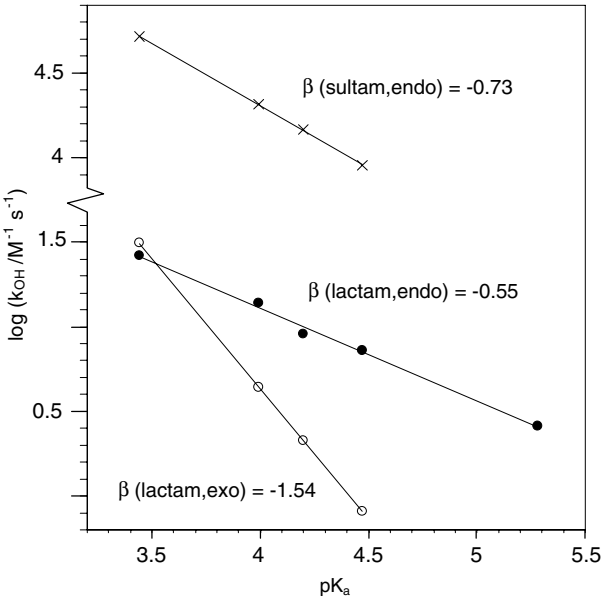


Figure 1. Bronsted plot for the second-order rate constant, k_{OH}, for the alkaline hydrolysis at the endocyclic (●) and exocyclic (○) carbonyl centre of *N*-aroyl β-lactams and *N*-aroyl β-sultam (×) against the pK_a of the corresponding benzoic acid in <5% acetonitrile–water (v/v) at 30 °C and $I = 1.0$ M (KCl)

formation of the tetrahedral intermediate (**6**). For comparison, the Bronsted β_{lg} value for the alkaline hydrolysis of *N*-substituted amides is −0.07,[8] indicative of general acid-catalysed breakdown of the tetrahedral intermediate due to partial proton transfer from water (**7**). The rates of alkaline hydrolysis of *N*-aryl β-lactams generate a Bronsted β_{lg} of −0.44, which was taken as evidence of rate-limiting formation of the tetrahedral intermediate.[1,9]

The Bronsted-type plot for the alkaline hydrolysis of the exocyclic amide of *N*-aroyl β-lactams against the pK_a of the corresponding benzoic acids is also shown in Fig. 1. The plot is reasonably linear but the dependence of the rate on the pK_a is much greater, with an apparent Bronsted β of −1.54 indicating that the effective charge at the reaction centre in the transition state for the amide hydrolysis is more negative than that in the reactant imide. This suggests that there is a relatively greater negative charge density in the transition state for the hydrolysis reaction than that in the carboxylate anion. This may indicate a rate-limiting formation of the tetrahedral intermediate with a late transition state or an early one in a rate-limiting breakdown, because in the tetrahedral intermediate the negative charge is more localized. The localized negative charge on the oxygen anion in the tetrahedral intermediate is more dependent on the stabilizing effect of electron-withdrawing groups than the more stable delocalized negative charge in the carboxylate anion.

The alkaline hydrolysis of *N*-aroyl β-lactams occurs at similar rates at the β-lactam and the exocyclic amide centres. Despite the strain energy of the four-membered ring, it is not released in the transition state to lower the activation energy and favour exclusive endocyclic C—N ring fission. The reluctance of four-membered rings to open rapidly has been noted previously.[1] The competitive rates of hydrolysis at the endocyclic and exocyclic carbonyl centres provide an opportunity to investigate the relationship between the specificity and reactivity of the acyl centres towards enzymes.

HYDROLYSIS OF β-SULTAMS

β-Sultams are formally 1,2-thiazetidine 1,1-dioxides (**2**) and are non-planar or planar quadrilaterals depending on substituents. The nitrogen in *N*-alkyl β-sultams is generally pyramidal and the nitrogen is 0.4–0.7 Å out of the plane defined by $S_1C_3C_4$.[10] In β-lactams the nitrogen ranges from being essentially in the plane of its three substituents in monocyclic β-lactams to being 0.6 Å out of the plane in bicyclic systems such as penicillins and carbapenems.[1] Exocyclic *N*-acylation of β-sultams converts the ring nitrogen to an amide and consequently the nitrogen becomes less pyramidal or even coplanar with the ring atoms. In general, the internal bond angle around sulfur is $79 \pm 1°$ compared with 113 ° in acyclic sulfonamides whilst that around nitrogen is $95 \pm 1°$ irrespective of whether the nitrogen substituent is alkyl or acyl. Finally, the S—N bond length of 1.70 Å compared with that of 1.52 Å for C_3—C_4 and the longer S—C_4 and the shorter C_3—N give the β-sultam an interesting overall geometry. There is thus considerable ring strain in β-sultams as a result of bond angle and torsional strain.

The hydrolysis of β-sultams normally occurs with exclusive S—N fission to give the corresponding β-aminosulfonic acid. Compared with sulfonamides, β-sultams are enormously reactive towards acid and base hydrolysis.[11] This is in sharp contrast to the almost identical rate of alkaline hydrolysis of β-lactams compared with that of their acylic amide analogues.[1] As demonstrated in the previous section, the strain energy inherent in the four-membered ring of β-lactams is not even partially released in the transition state to lower the activation energy for reaction. Furthermore, β-sultams are 10^2–10^3-fold *more* reactive than corresponding β-lactams, compared with the 10^4-fold *slower* rate of alkaline hydrolysis of acyclic sulfonamides compared with analogous amides.[12] This is the first example of the rate of sulfonyl transfer being greater than that of the corresponding acyl reaction.

In sharp contrast to the β-lactams,[1,13] neither *N*-alkyl nor *N*-aryl β-sultams show any reaction with amines or thiols or even oxygen nucleophiles in aqueous solutions. Nucleophiles N, S and O are not able to compete with HO^- in attacking *N*-aryl β-sultams, which would be expected to be good sulfonating agents on the basis of their reactivity. In general, sulfonyl centres are much less reactive than analogous acyl centres towards nucleophiles, with the exception of the sulfonyl halides, which readily undergo aminolysis in water.[12,14] However, *N*-

benzoyl β-sultam (**8**) is an extremely reactive β-sultam and *does* undergo reaction with nucleophiles in water, although only readily with O-nucleophiles, such as alcohols and carboxylate anions.[15] The reactivity order for *N*-benzoyl β-sultam (**8**) towards nucleophiles is $HO^- > RO^- > F^- > RCO_2^- > RNH_2 > H_2O$. In contrast to acylating agents, sulfonylation by *N*-acyl β-sultams shows a larger degree of selectivity the more reactive the compound, consistent with an inverse selectivity–reactivity relationship.[12,14,15] The more reactive *N*-acyl β-sultam is apparently more selective.

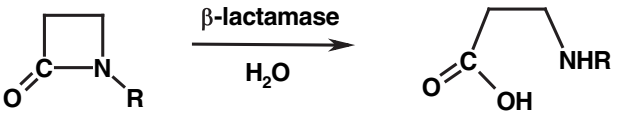
N-Acylsulfonamides normally react with nucleophiles to give N-acyl fission as a result of nucleophilic attack on the carbonyl group followed by C–N fission and displacement of the sulfonamide anion. By contrast, the alkaline hydrolysis of *N*-benzoyl β-sultam (**8**) occurs exclusively by S—N fission as a result of attack on sulfur and displacement of the carboxamide, and occurs 10^4 faster than an analogous acyclic *N*-acylsulfonamide occurring by C—N fission.[16]

The incorporation of the acyl centre within the four-membered ring gives a structure (**9**) that is both a β-sultam and a β-lactam.[16] These 3-oxo β-sultams undergo hydrolysis with preferential attack on the sulfonyl centre, leading to S—N fission. Despite the enormous strain in 3-oxo β-sultams, structure (**9**) is only 10-fold more reactive towards alkaline hydrolysis than the β-sultam with an exocyclic acyl centre.

Electron-withdrawing substituents increase the rates of alkaline hydrolysis of aryl-substituted *N*-aroyl β-sultams (**8**) and their second-order rate constants (k_{OH}) generate a Bronsted-type plot to give a β_{lg} value of −0.73 (Fig. 1). This is similar to the value of −0.55 found for the analogous *N*-aroyl β-lactams. For both series, N-protonation of the leaving group amide is thermodynamically unfavourable and amide anion expulsion could occur. If a trigonal bipyramidal intermediate (TBPI) is formed in the alkaline hydrolysis of *N*-aroyl β-sultams (**8**), and if the rate of S—N bond fission and ring-opening to expel the amide anion is greater than the rate of expulsion of hydroxide ion from the intermediate, then formation of the intermediate will be rate-limiting. Conversely, if the rate of amide anion expulsion is comparable or even slower than that for hydroxide ion, then breakdown of the intermediate may become rate-limiting. The observed β_{lg} value of −0.73 is compatible with either a late transition state for rate-limiting formation of a TBPI or with its rate-limiting breakdown with little or no S—N fission.

REACTIONS OF β-LACTAMS AND β-SULTAMS WITH SERINE ENZYMES

There are three classes of serine enzymes that recognize and react with β-lactams: β-lactamases, transpeptidases and elastase.



Scheme 3

The susceptibility of β-lactam antibiotics to the hydrolytic activity of β-lactamase enzymes is the most common and growing form of bacterial resistance to the normally lethal action of these antibacterial agents.[17] β-Lactamases catalyse the hydrolysis of the β-lactam to give the ring opened and bacterially inert β-amino acid. The mechanism uses the active-site serine residue to form an acyl-enzyme intermediate.[17] Class C β-lactamase P99 catalyses the hydrolysis of *N*-acyl monocyclic β-lactams (**4**) to give the ring-opened β-amidocarboxylic acid (Scheme 3). Despite the low level of molecular recognition, the pH-rate profile for the β-lactamase-catalysed hydrolysis of *N*-benzoyl β-lactam is sigmoidal and shows a dependence of enzyme activity on the ionization of a group of pK_a 6.0, similar to that for the 'normal' substrates such as benzylpenicillin. However, there is little effect of substituents in the *N*-acyl residue on the second-order rate constants, k_{cat}/K_m, for the enzyme-catalysed hydrolysis. A Bronsted-type plot of the logarithms of k_{cat}/K_m against the pK_a of the analogous carboxylic acid (Fig. 2) gives a Bronsted β_{lg} value of −0.06. The corresponding Bronsted β_{lg} for the alkaline hydrolysis of the same *N*-acyl β-lactams (**4**) is −0.55. Hence, relative to the hydroxide-ion-catalysed hydrolysis, there is much less removal of the positive charge on the nitrogen on going from the reactant to the

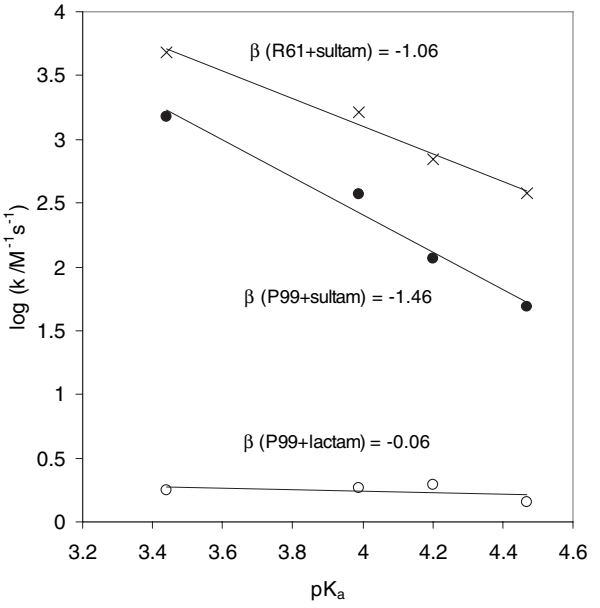


Figure 2. Bronsted plot for the second-order rate constant, k_i, for the sulfonylation of P99 β-lactamase (●) and R61 transpeptidase (×) by *N*-aroyl β-sultams and for k_{cat}/K_m for the acylation of P99 β-lactamase by *N*-aroyl β-lactams (○) against the pK_a of the corresponding benzoic acid at pH 7.0 in 1% acetonitrile–water (v/v) at 30 °C and $I = 1.0$ M (KCl)

transition state in the enzyme-catalysed reaction. This is compatible with an early transition state for the formation of the tetrahedral intermediate (TI) and β-lactamase appears to cause a move to a transition state *earlier* along the reaction coordinate.

The sulfonylation of serine enzymes (Scheme 1) offers an interesting but largely unexplored strategy for inhibition because sulfonyl derivatives are usually much less reactive than their acyl counterparts.[18] However, the reactive β-sultams have given us an opportunity to explore the mechanism of sulfonylation and inhibition of serine enzymes. *N*-Benzoyl β-sultam (**8**) is a time-dependent inhibitor of P99 β-lactamase and enzyme activity decreases with time in an exponential manner, from which can be derived the second-order rate constant for inactivation, k_i.[19] The rates of inactivation of P99 β-lactamase by the β-sultam **8** show a similar sigmoidal dependence on pH to that for the hydrolysis of β-lactams as substrate. Both rates depend on a catalytic group in the enzyme that ionizes with a pK_a of 6.3, which catalyses sulfonylation for the inhibition reaction and acylation for the hydrolysis reaction. Electrospray ionization mass spectrometry (ESIMS) of solutions of P99 β-lactamase incubated with the β-sultam **8** reveals the enzyme bound to one equivalent of β-sultam consistent with the sulfonylation of the active-site serine residue Ser-64 to form an inactive sulfonyl enzyme.[19] A Bronsted-type plot for the second-order rate constants of inactivation, k_i, gives a β_{lg} value of -1.46 (Fig. 2), indicative of a large development of negative charge on the nitrogen-leaving group in the transition state, suggesting that the leaving group is expelled as the amide anion and that there is significant S—N bond fission in the transition state. The corresponding Bronsted-type plot for the hydroxide-ion-catalysed hydrolysis of the same series of *N*-aroyl β-sultams generated a β_{lg} value of -0.73 (Fig. 1). So in contrast to the reaction with β-lactams, β-lactamase appears to cause a move of the transition state for the reaction with β-sultams to much *later* along the reaction coordinate, compared with alkaline hydrolysis. Although the enzyme appears to be using some of its catalytic machinery to facilitate the sulfonylation reaction, the geometry of the displacement reaction is not ideal and the host of favourable non-covalent interactions used by the enzyme to stabilize the transition state for the acylation of the 'natural' substrate are not fully available to lower the activation energy for the β-sultams by the maximum amount. In terms of the Hammond Postulate,[3] this would be expected to lead to a later transition state, as observed.

The DD-peptidases are the inhibition targets of β-lactam antibiotics, such as penicillin and cephalosporins. These enzymes have an active-site serine residue that is acylated by the D-Ala D-Ala natural peptide substrate as an intermediate stage of the acyl transfer reaction. *N*-Acyl β-sultams (**8**) are also time-dependent, irreversible active-site-directed inhibitors of *Streptomyces* R61 DD-peptidase.[20] The rate of inactivation is first order with respect to β-sultam concentration, and the second-order rate constants show a similar dependence on pH to that for the hydrolysis of a substrate. Inactivation is due to the formation of a stable 1:1 enzyme–inhibitor complex as a result of the active-site serine being sulfonylated by the β-sultam, as shown by ESIMS analysis and X-ray crystallography. [20] The effect of changing the basicity of the leaving group in β-sultams on the rate of inactivation of R61 was investigated by different aryl substituents in *N*-aroyl β-sultams (**8**). Electron-withdrawing substituents in the *N*-aroyl residue increase the rate of inactivation. The second-order rate constants for inactivation, k_i, for a series of substituted derivatives generate a Bronsted-type plot to give a β_{lg} value of –1.06 (Fig. 2). This value is indicative of a development of a significant negative charge on the amide nitrogen-leaving group and is compatible with the leaving group being expelled as the amide anion and that S—N bond fission is significant in the transition state.

The corresponding Bronsted β_{lg} for the alkaline hydrolysis of the same series of *N*-aroyl β-sultams is -0.73 (Fig. 1), which is indicative of much less change in charge on the leaving nitrogen in the transition state. Similar to the action of β-lactamase, the R61 transpeptidase enzyme appears to cause a move to a transition state later along the reaction coordinate for sulfonyl transfer compared with the hydroxide-ion-catalysed hydrolysis of *N*-aroyl β-sultams. The different Bronsted β_{lg} values indicate more S—N bond fission in the transition state for the enzyme-catalysed reaction.

Human neutrophil elastase (HNE) is also a serine enzyme that is one of the most destructive proteases and is able to catalyse the hydrolysis of components of connective tissue. It has been implicated in the development of diseases such as emphysema, cystic fibrosis and rheumatoid arthritis and there have been numerous studies attempting to find small molecule inhibitors of HNE.[21] β-Lactams have also been shown to be mechanism-based inhibitors of elastase when used as neutral derivatives; the first chemical step of inhibition is an acylation process in which the four-membered β-lactam ring is opened.[21] N-Acyl β-sultams are also time-dependent inhibitors of elastase and enzyme activity decreases irreversibly in a first-order rate process, giving rate constants dependent on inhibitor concentration.[22] The corresponding second-order rate constants for inactivation, k_i, vary with pH in a similar manner to that for the hydrolysis of an anilide substrate catalysed by elastase. This indicates that the rate of inactivation of elastase by β-sultams is controlled by the same catalytic groups in the active site that are used for substrate hydrolysis, i.e. active-site-directed inhibition is occurring. Covalent modification of the enzyme by sulfonylation is demonstrated by ESIMS and X-ray crystallography of the inactivated enzyme, which shows ring-opening of the β-sultam and formation of a sulfonate ester of Ser-195. One of the sulfonate oxygen atoms is located in the

Table1. A summary of the Bronsted β_{lg} values for the alkaline hydrolysis of *N*-aroyl β-lactams and *N*-aroyl β-sultams compared with those for their reactions with some serine enzymes

	N-aroyl β-lactams	*N*-aroyl β-sultams
Hydroxide ion hydrolysis	−0.55[a]	−0.73
P99 β-Lactamase	−0.06	−1.46
R61 Transpeptidase		−1.06
Elastase	− 0.5	−0.5

[a] The Bronsted β value for the exocyclic C—N bond fission of this reaction is −1.54.

oxyanion hole whereas the other occupies the upper part of the S_1 pocket.[22] Elastase also catalyses the hydrolysis of *N*-acyl monocyclic β-lactams (**4**) but, unlike the case with β-lactamase and transpeptidase, neither the rates of sulfonylation by β-sultams nor acylation by β-lactams show good correlations with the basicity of the leaving group. Nonetheless, the Bronsted β_{lg} for both reactions are similar and about −0.5.

A summary of the various Bronsted β_{lg} values for the alkaline hydrolysis of the *N*-aroyl β-lactams and *N*-aroyl β-sultams and those for the enzyme-catalysed hydrolysis of N-aroyl β-lactams and inhibition by sulfonylation with *N*-aroyl β-sultams is given in the Table 1. It is often assumed, but with little actual supporting evidence, that enzymes catalyse reactions by an exquisite positioning of the catalytic groups involved in bond-making and bond-breaking. If rigid positioning of catalytic groups within the active site was crucial then it is doubtful if an enzyme with a primary function as a catalyst for acyl transfer could be an effective catalyst for sulfonyl transfer because of the geometrical differences in the displacement mechanisms. However, this clearly is not the case, although it does appear that enzymes that have evolved to catalyse acyl transfer confer a different transition state structure on these reactions to those for sulfonyl transfer.

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