Reactivity and the mechanisms of reactions of β-sultams with nucleophiles

J. Matthew Wood, Paul S. Hinchliffe, Andrew P. Laws and Michael I. Page *

Department of Chemical and Biological Sciences, University of Huddersfield, Queensgate, Huddersfield, UK HD1 3DH

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Ethane-1,2-sultam has a pK_a of 12.12 ± 0.06 at 30 °C and its rate of alkaline hydrolysis shows a pH-dependence reflecting this so that the observed pseudo first-order rate constant at pHs above the pK_a are pH independent. There is no evidence of neighbouring group participation in the hydrolysis of either *N*-α-carboxybenzylethane-1,2-sultam or *N*-(hydroxyaminocarbonylmethyl)-2-benzylethane-1,2-sultam. Oxyanions, but not amines or thiols, react with *N*-benzoylethane-1,2-sultam in water by a nucleophilic ring opening reaction confirmed by product analysis and kinetic solvent isotope effects. A Brønsted plot for this reaction has two distinct correlations with β_{nuc} = 0.52 and 0.65 for weak and strong bases, respectively, although a statistically corrected plot may indicate a single correlation.

Introduction

Sulfonyl transfer reactions are of biological interest because of the potential use of sulfonyl compounds as sulfonating agents of serine proteases[1] and the use of sulfonamides as peptide mimics.[2] They are also of mechanistic interest for comparison with the analogous acyl transfer processes. Sulfonamides themselves are extremely resistant to alkaline and acid hydrolysis[3] and, in general, sulfonyl transfer reactions are 10^2 to 10^4-fold slower than the corresponding acyl transfer process.[4,5] The NH acidity of sulfonamides is greater than that of carboxylic acid amides and the pK_as of sulfonamides are typically around 9 to 10 so that they are fully ionised in alkaline solution.[6] However, formation of the anion is not the sole reason for the lack of reactivity because sulfonamides of secondary amines are also unreactive.[3]

There have been several studies on nucleophilic substitution at sulfonyl centres using reactive derivatives such as sulfonyl halides and aryl esters of sulfonic acids.[7,8] The modification of acyclic compounds to cyclic derivatives often changes their properties and reactivities. For example, cyclic ethylene sulfate is more than 10^7-fold more susceptible to alkaline hydrolysis than the corresponding acyclic diethyl sulfate[9] but whether this is due to strain energy or solvation effects remains controversial.[10] We have previously reported on the enhanced reactivity of the cyclic sulfonamide derivatives, the β-sultams,[11–15] *e.g.* **1**, which are the sulfonyl equivalents of the more thoroughly studied β-lactams.[16] The β-sultams are 10^2 to 10^3-fold *more* reactive than their corresponding acyl analogues, the β-lactams, whereas acyclic sulfonamides are much *less* reactive than corresponding amides. β-Sultams show extraordinary rate enhancements of 10^9 and 10^7, respectively, compared with the acid and base catalysed hydrolysis of the corresponding acyclic sulfonamides.[15]

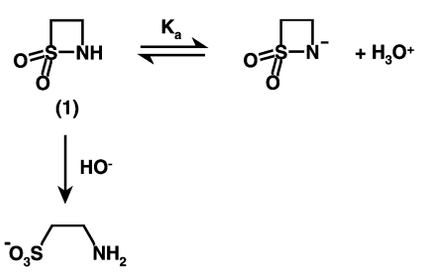
In principle, the sulfonation of serine proteases offers an interesting but largely unexplored strategy for inhibition as an alternative to the traditional mechanism-based acylation process.[17] β-Sultams are excellent candidates for exploring the mechanism of sulfonation and possible inhibition of serine protease enzymes. Ring opening of the β-sultam by the serine protease gives the sulfonate ester,[1] similar to the acyl–enzyme intermediate formed during the hydrolysis of normal substrates.[17]

Herein we report more kinetic and mechanistic studies of the reactions of β-sultams, including their alcoholysis, which is an analogous chemical process to that observed in their inactivation of serine proteases.[1]

Results and discussion

(i) Reactivity of *N*-unsubstituted β-sultam

The pK_a of ethane-1,2-sultam (**1**) was found to be 12.12 ± 0.06 by means of a reversible chromophoric change at 230 nm. In aqueous sodium hydroxide (NaOH) solutions a slow exponential decay of the chromophore of ethane-1,2-sultam (**1**) was observed at 300 nm and 30 °C. The absorbance change had a very slow rate which was found to be independent of hydroxide-ion concentration and gave a first-order rate constant of 1.00×10^{-5} s^{-1}. The observed pH independent reaction and absorbance changes are consistent with the hydrolysis pathway shown in Scheme 1.



Scheme 1

β-Sultams undergo attack by hydroxide ion at the sulfonyl centre resulting in ring opening *via* cleavage of the S–N bond and formation of a β-amino sulfonic acid. Presumably only the neutral β-sultam (**1**) can undergo this reaction and the deprotonated form is unreactive because if the ring nitrogen is ionised then the electrophilicity of the sulfonyl centre is reduced and S–N fission will not occur unless the amino-N is protonated. As a result, in solutions of pH greater than the pK_a of ethane-β-sultam (**1**) the rate of hydrolysis becomes pH independent as it depends both on the concentration of the minor species, the neutral β-sultam, which decreases as the pH is increased, and

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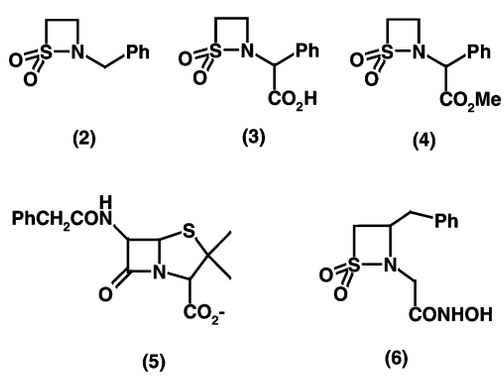
on the concentration of the hydroxide ion. The observed first-order rate constant, k_{obs}, is given by eqn. (1) and is proportional to the second-order rate constant for hydroxide ion hydrolysis, k_{OH}, the hydroxide ion concentration and the concentration of the neutral β-sultam, given by the fraction of the free 'acid'.

$$k_{obs} = k_{OH}[HO^-]([H^+]/(K_a + [H^+])) \quad (1)$$

If the solution pH is greater than the pK_a of the β-sultam, $K_a \gg [H^+]$ then this equation simplifies to eqn. (2) where $K_w = [HO^-][H^+]$, the ionic product of water, and the observed rate is pH independent.

$$k_{obs} = k_{OH}(K_w/K_a) \quad (2)$$

Using pK_w = 13.83 at 30 °C,[18] the second-order rate constant, k_{OH}, for the alkaline hydrolysis of β-sultam **1** is calculated to be 5.16×10^{-4} M^{-1} s^{-1}. This value is 20-fold lower than the second-order rate constants for the hydroxide ion hydrolysis, k_{OH}, of simple *N*-alkyl β-sultams *e.g.* *N*-methylethane-1,2-sultam (1.41×10^{-2} M^{-1} s^{-1}) and *N*-benzyl β-sultam **2** (1.07×10^{-2} M^{-1} s^{-1}).[11] For comparison, the k_{OH} values of the analogous unsubstituted β-lactam and *N*-methyl β-lactam are 2×10^{-4} and 2×10^{-5} M^{-1} s^{-1}, respectively, at 25 °C in 85% aqueous ethanol.[19,20] With these carboxylic acid amide derivatives it is the NH (unsubstituted) compound that exhibits the highest reactivity. *N*-Alkyl β-lactams may be stabilised relative to the unsubstituted compound by increased resonance of the amide bond enhanced by the electron donating alkyl substituent. Such resonance stabilisation may not occur in the β-sultam compound where the sulfonyl centre stabilises adjacent lone pairs by an inductive effect and shows little evidence of conjugation.[21]



(2) (3) (4) (5) (6)

(ii) Reactivity of the *N*-carboxybenzyl β-sultam

We are interested in exploring the possibility of neighbouring group participation by the carboxy group in the hydrolysis of β-sultams. *N*-(α-Carboxybenzyl) β-sultam **3** possesses a carboxy group β- to the sulfonyl centre. There is the potential for this group to participate in intramolecular catalysis as *N*-benzyl β-sultam **2** is known to experience specific-acid nucleophilic catalysis in carboxylic acid buffers.[13] Carboxylate anions attack the sulfonyl centre of the *N*-protonated conjugate acid of the β-sultam to produce a mixed anhydride intermediate after ring opening. However, intramolecular specific-acid nucleophilic catalysis involving the carboxylate anion in **3** is an unlikely mechanism for steric reasons.[22] Alternatively, an enhanced rate of hydrolysis of *N*-(α-carboxybenzyl) β-sultam **3** could result from intramolecular general acid or general base catalysis by the carboxylic acid or carboxylate group, respectively.

The rates of hydrolysis of *N*-(α-carboxybenzyl) β-sultam **3** were determined spectrophotometrically in a wide range of aqueous solution pHs. The observed pseudo-first-order rate constants, k_{obs} values, are plotted against pH as shown in Fig. 1. A very small inflexion is observed in the acidic limb of the pH–rate profile at *ca.* pH 3, giving two separate slopes of unit

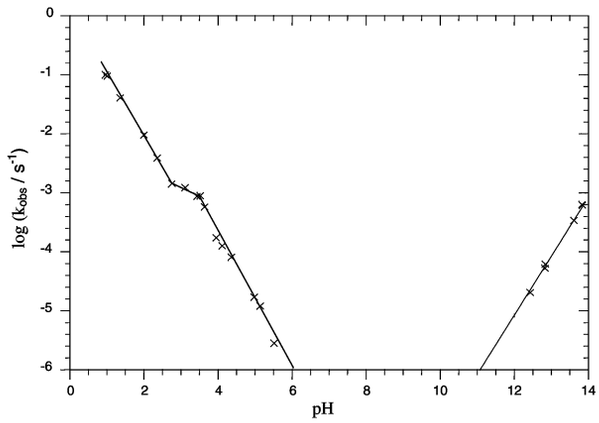


Fig. 1 pH–rate profile for the hydrolysis of *N*-(α-carboxybenzyl) β-sultam **3** in aqueous solution, 30 °C, 1% acetonitrile v/v and I = 1.0 M (KCl).

gradient which is indicative of the presence of two separate reaction pathways in the hydrolysis of *N*-(α-carboxybenzyl) β-sultam **3**. The most likely pathways in this pH region are the hydrogen ion catalysed hydrolyses of the undissociated β-sultam (RCO_2H) and the dissociated β-sultam (RCO_2^-). The rate law for the hydrolysis reaction can therefore be derived based on the existence of two different forms of β-sultam **3**, with undissociated (RCO_2H) or dissociated (RCO_2^-) carboxylic acid groups, eqn. (3);

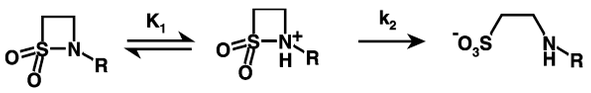
$$\begin{aligned} \text{Rate} &= k_H^{RCO_2^-}[RCO_2^-][H^+] + k_H^{RCO_2H}[RCO_2H][H^+] + k_{OH}[RCO_2^-][HO^-] \\ &= (k_0^{RCO_2H} + k_H^{RCO_2H}[H^+])[RCO_2H] + k_{OH}[RCO_2^-][HO^-] \\ k_{obs} &= (k_0^{RCO_2H} + k_H^{RCO_2H}[H^+])([H^+]/(K_a + [H^+])) + k_{OH}(K_a/(K_a + [H^+]))[HO^-] \end{aligned} \quad (3)$$

where K_a is the dissociation constant of the carboxylic acid of **3** and is equal to 2.39×10^{-3} M (pK_a = 2.62), k_H (RCO_2^-) and k_H (RCO_2H) are the second order rate constants for the acid catalysed hydrolysis of **3** with a dissociated and an undissociated carboxylic acid substituent, respectively, and k_{OH} is the second order rate constant for alkaline hydrolysis of **3** with an ionised carboxylate group. Modelling eqn. (3) to the experimental data gives the following calculated values: k_H (RCO_2H) = 0.94 M^{-1} s^{-1}, k_H (RCO_2^-) = 1.56 M^{-1} s^{-1}, and $k_{OH} = 5.75 \times 10^{-4}$ M^{-1} s^{-1}. The line in Fig. 1 represents data calculated using these values from eqn. (3). The term k_H (RCO_2^-)(H^+) is kinetically equivalent to a term, k_0 (RCO_2H), corresponding to the apparent pH independent reaction of **3** with an undissociated carboxylic acid subsituent which may be calculated from $k_0 = k_H K_a = 3.73 \times 10^{-3}$ s^{-1}. There is no term in the rate law corresponding to the pH independent hydrolysis of **3** with a carboxylate anion substituent. The less than 2-fold rate difference in the second-order rate constants for the acid catalysed hydrolysis, k_H (RCO_2H) and k_H (RCO_2^-), of the two forms of *N*-(α-carboxylbenzyl) β-sultam **3** is far too low to be attributable to intramolecular catalysis or any form of neighbouring group participation. Significantly, k_H for the methyl ester analogue, *N*-(α-methoxycarbonylbenzyl) β-sultam **4**, is 1.02 M^{-1} s^{-1}, almost identical to that of the undissociated form of **3** and consistent with an inductive effect of the carboxylic acid group rather than to any intramolecular reaction.[23] It is perhaps surprising that there is only a small difference between the values of k_H (RCO_2^-) and k_H (RCO_2H). The mechanism[11] for the acid catalysed hydrolysis of β-sultams involves *N*-protonation followed by rate limiting S–N fission, Scheme 2. The electronic demands for *N*-protonation and S–N cleavage are opposite whereas the carboxylate anion favours *N*-protonation; the rate of S–N cleavage is fastest with the more electron withdrawing undissociated carboxylic

Table 1 Second-order rate constants for the acid catalysed hydrolysis (k_H) and hydroxide ion hydrolysis (k_{OH}) of β-sultams in aqueous solution at 30 °C and I = 1.0 M (KCl)

N-substituent	k_H/M^{-1} s^{-1}	k_{OH}/M^{-1} s^{-1}
$CH(Ph)CO_2H$ (**3**)	0.94	
$CH(Ph)CO_2^-$ (**3**)	1.56	5.75×10^{-4}
$CH(Ph)CO_2Me$ (**4**)	1.02	2.24
$CH_2C(O)NHOH$ (**6**)	4.18×10^{-2}	5.58×10^{-3}
H		5.16×10^{-4}
Ph[a]	5.63×10^{-2}	6.67
m-ClC_6H_5	2.27×10^{-2}	46.0
CH_2Ph (**2**)[a]	1.52	1.07×10^{-2}
CH_3[a]	2.64	1.41×10^{-2}

[a] Ref. 11.



Scheme 2

acid. These opposing factors presumably cancel to give approximately equal second-order rate constants, k_H (RCO_2^-) and k_H (RCO_2H).

The second-order rate constant for the hydroxide ion hydrolysis of *N*-(α-carboxybenzyl) β-sultam **3** is 5.75×10^{-4} M^{-1} s^{-1}. This is 20-fold lower than that for *N*-benzyl β-sultam **2**. Similar relative rates of hydroxide ion hydrolysis have been observed for benzyl penicillin (**5**) and its methyl ester.[24] Ionised carboxy groups are known to reduce rates of hydroxide ion hydrolysis which is usually attributed to electrostatic repulsion of the incoming hydroxide ion. Interestingly, the alkaline hydrolysis of the methyl ester derivative, **4**, proceeds by a novel elimination mechanism involving carbanion formation at the exocyclic *N*-α-carbon.[25]

The lack of intramolecular catalysis in the hydrolysis of *N*-(α-carboxybenzyl) β-sultam **3** and a reduced rate of hydroxide ion hydrolysis are also observed in the hydrolysis of *N*-(hydroxyaminocarbonylmethyl) 2-benzyl β-sultam **6**. The pK_a of **6** for the deprotonation of the hydroxamate group is 8.59 ± 0.05, as expected from literature pK_as.[26] The second-order rate constants for the hydrogen ion catalysed hydrolysis and hydroxide ion hydrolysis of *N*-(hydroxyaminocarbonylmethyl) 2-benzyl β-sultam **6** are 4.18×10^{-2} and 5.58×10^{-3} M^{-1} s^{-1}, respectively. Both of these rate constants appear lower than expected for an *N*-alkyl β-sultam (Table 1).

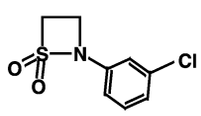
The second-order rate constant for the hydrogen ion catalysed hydrolysis, k_H, for *N*-(hydroxyaminocarbonylmethyl) 2-benzyl β-sultam **6** is about 20-fold less than that for other *N*-alkyl β-sultams including the undissociated form of *N*-(α-carboxybenzyl) β-sultam **3**, (RCO_2H) (Table 1). It is possible that the carbonyl oxygen of the hydroxamic acid moiety is more basic than the β-sultam nitrogen. Preferential carbonyl *O*-protonation would make sulfonamide *N*-protonation less favourable and reduce the effective concentration of RSO_2NH^+R in the hydroxamic acid **6** relative to the carboxylic acid **3**. As it is the *N*-protonated species that is required to facilitate ring opening the efficiency of hydrogen ion catalysis in **6** is therefore reduced.

In alkaline solution, both the hydroxamate form of compound **6** and the carboxylate compound **3** possess a negative charge on their exocyclic nitrogen substituent. They are therefore less reactive towards hydroxide ion than neutral β-sultams of equivalent leaving group pK_a due to electrostatic repulsion. The 10-fold higher second-order rate constant of hydroxide ion hydrolysis for the hydroxamate form of **6** relative to the carboxylate **3** may reflect the fact that the oxyanion is one atom further away from the sulfonyl centre.

(iii) Reactions of *N*-alkyl and *N*-aryl β-sultams with nucleophiles

Nucleophilic substitution reactions at sulfonyl centres are not well studied kinetically because of the general low reactivity of common sulfonylated derivatives.[3,8] However, sulfonyl chlorides, reactive sulfonate esters and sulfonyl imidazoles react with amines and alcohols in aqueous solution.[27–29] As we are developing the β-sultams as potential sulfonylating agents of enzymes,[1] their relative reactivities with O, S and N nucleophiles is of interest; in particular their ability to compete with hydrolysis in aqueous solution.

The synthesis of *N*-alkyl β-sultams involves intramolecular ring closure of β-amino sulfonyl chlorides *via* nucleophilic attack of the amine nitrogen at the sulfonyl centre. However there is no increase in the rate of hydrolysis of *N*-benzyl β-sultam **2** in aqueous solutions of sodium hydroxide in the presence of *n*-propylamine or hydrazine or in aqueous buffers of these amines. This is in sharp contrast to the β-lactams in penicillins and cephalosporins where the aminolysis reaction occurs readily in competition with hydrolysis.[30] The rate of alkaline hydrolysis of *N*-benzyl β-sultam is about 10-fold less than that of benzyl penicillin. Consequently, we explored potential nucleophilic reactions with more reactive β-sultams, but there is also no significant change in the observed rate of hydrolysis of *N*-(*m*-chlorophenyl) β-sultam **7** with increasing concentrations of hexafluoroisopropyl alcohol (HFIP), *n*-propylamine (PA), 2-mercaptoethanol (2-ME), hydrogen sulfide or fluoride ion in buffered solutions of these potential nucleophiles at constant pH. HFIP, PA and 2-ME were used as convenient examples of O, N and S nucleophiles, respectively. Fluoride ion (F^-) was selected as a potential nucleophile because sulfonyl chlorides are readily converted to sulfonyl fluorides in aqueous solutions of F^-.[31]



(7)

The low reactivity of *N*-(*m*-chlorophenyl) β-sultam **7** towards nucleophiles is further evidenced by the very small amount of hydrazinolysis observed in hydrazine buffers. An estimate of the maximum second-order rate constant for the reaction with the α-effect nucleophile hydrazine, k (N_2H_4), is 6.0×10^{-4} M^{-1} s^{-1}.

As there is no nucleophilic reaction or catalysis of the hydrolysis of the *N*-(*m*-chlorophenyl) compound **7** by these nucleophiles, it may be asked why do N, S and O nucleophiles not compete with HO^- in attacking *N*-aryl β-sultams? *N*-(*m*-Chlorophenyl) β-sultam **7** would appear to be a good sulfonating agent as it is a reactive compound with a k_{OH} of 46.0 M^{-1} s^{-1} and less reactive acyl systems such as β-lactams react with a variety of nucleophiles in water, showing both alcoholysis and aminolysis.[32] In general, sulfonyl centres are much less reactive than analogous acyl centres towards nucleophiles, with the exception of the sulfonyl halides.[4,5,28] However, β-sultams show greater reactivity than their acyl analogues.[11–15] For example, *N*-phenyl and *N*-methyl β-sultams are 10^3 times more reactive towards hydroxide than their corresponding β-lactams. The β-sultams are unusual sulfonyl compounds as other sulfonyl derivatives such as benzenesulfonyl chloride (k_{OH} = 40.4 M^{-1} s^{-1})[33] and *N*-tosylimidazole (k_{OH} = 3.16 M^{-1} s^{-1})[29] undergo aminolysis in water. It does appear unusual that nucleophiles cannot compete with hydroxide ion reacting with β-sultams and the apparent lack of reactivity of the *N*-(*m*-chlorophenyl) compound **7** with other nucleophiles may not simply be due to the intrinsic reactivity of the sulfonyl centre but may be the result of some peculiar aspect of the mechanism of ring opening.[34] Initial thoughts about the possible differences between hydroxide ion and other nucleophiles centred around the requirement

Table 2 Second-order rate constants k_B for the reaction of nucleophiles with *N*-benzoyl β-sultam **8** in aqueous solution, 30 °C, I = 1.0 M (KCl) and 5% acetonitrile v/v

Base	Conjugate acid pK_a	k_B/M^{-1} s^{-1}
Water	−1.74	4.56×10^{-6}
Chloroacetate	2.86	8.97×10^{-5}
Fluoride	3.17	0.30
Formate	3.56	6.85×10^{-4}
Acetate	4.72	2.41×10^{-3}
Hexafluoroacetone hydrate anion	6.53	1.07×10^{-2}
Hydrogen phosphate dianion	6.52	0.136
Hydrogen arsenate dianion	6.56	0.891
Cacodylate[a]	6.22	1.63
Hydrazine	8.11	3.43
Hexafluoroisopropyloxyl anion	9.30	246
Heptafluorobutyloxyl anion	11.40	1.15×10^{3}
2,2,2-Trifluoroethyloxyl anion	12.43	1.54×10^{4}
Propargyloxyl anion[a]	13.55	6.99×10^{4}
Hydroxide	15.63	1.46×10^{4}

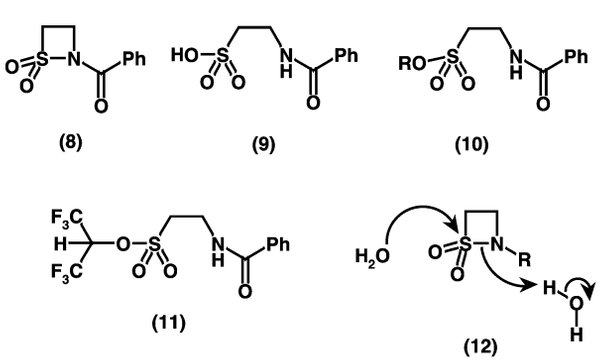
[a] The IUPAC names for cacodylate and propargyl are dimethylarsinate and prop-2-ynyl.

for there to be a negative charge on the atom apical to the amine leaving group as observed in the hydrolysis of 1-benzoyl-1-methyl-*N*-methylethane-1,2-sultam.[14] Hydrogen sulfide (HS^-) also possesses an ionisable proton which could be removed to generate a dianionic type intermediate or transition state. However, no reaction of *N*-(*m*-chlorophenyl) β-sultam **7** with HS^- was observed and although this may be due to the soft nucleophilicity of the sulfur anion, it probably also invalidates the assumption about the dianion mechanism.

It is possible that the reactions of *N*-aryl β-sultams with nucleophiles are not prevented by an unusual mechanistic requirement but are simply too slow to be observed above a relatively rapid hydroxide ion hydrolysis. Sulfonate esters are known to react rather slowly with nucleophiles other than hydroxide ion.[35] There appears to be a preference of sulfonyl compounds towards oxygen nucleophiles. In nucleophilic substitution reactions of the same sulfonate ester it has been shown that phenolate anions are 10^2-fold more reactive towards the sulfonyl centre than amines of similar basicity and the former experience a lower sensitivity to the attacking nucleophile pK_a.[36]

(iv) Reactions of *N*-benzoyl β-sultam with nucleophiles

N-Benzoyl β-sultam **8** is an extremely reactive β-sultam and undergoes alkaline hydrolysis with S–N fission and expulsion of an amide leaving group.[1] It shows a second order rate constant for alkaline hydrolysis, k_{OH}, of 1.46×10^4 M^{-1} s^{-1} (Table 2). In contrast to the *N*-aryl β-sultams just described, the more reactive *N*-benzoyl β-sultam **8** *does* react with nucleophiles in aqueous solution, although only readily with O-nucleophiles.



The reaction of *N*-benzoyl β-sultam **8** in aqueous solution was studied with a range of oxygen nucleophiles. The latter were generally used as reactants and buffers except as noted below. The observed values of the pseudo-first-order rate constants, k_{obs}, were found to increase linearly with buffer concentration. The contributing terms in the rate law were analysed by measuring the rates of reaction in a series of buffer concentrations at fixed pHs, which could be varied, constant temperature and constant ionic strength. At a fixed pH, the slopes of k_{obs} against buffer concentration give apparent second-order rate constants, k_{cat} values (Fig. 2). The values of k_{cat} vary with pH as a result of changes in the fraction of free-base, a, in the buffer solution. The particular buffer species contributing to the rate can be determined from a plot of k_{cat} against a (Fig. 3).

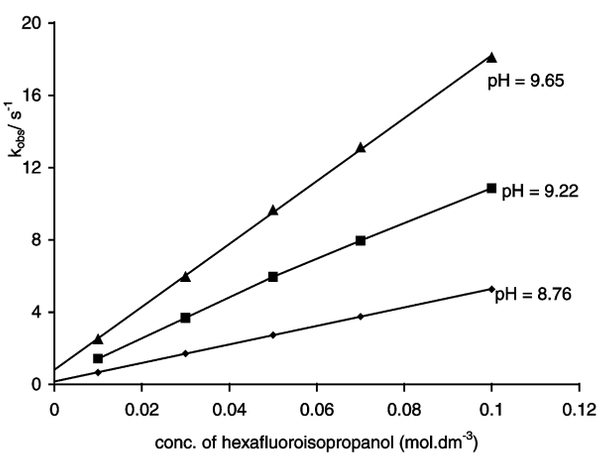


Fig. 2 Observed pseudo first-order rate constants for the reaction of *N*-benzoyl β-sultam **8** in hexafluoroisopropanol buffers, 5% acetonitrile v/v, 30 °C and I = 1.0 M (KCl).

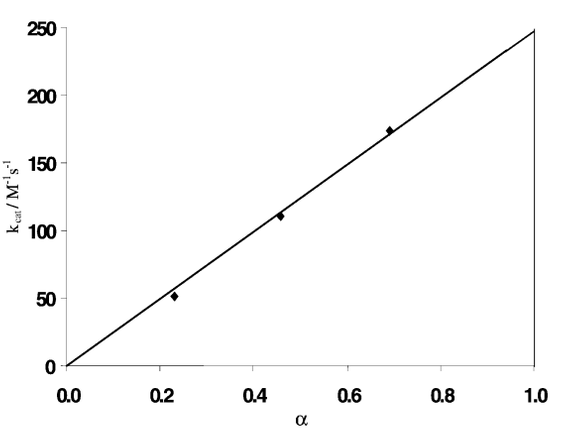
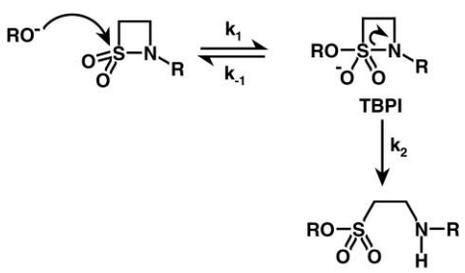


Fig. 3 Plot of k_{cat} against the fraction of free base, a, for the reaction of *N*-benzoyl β-sultam **8** in hexafluoroisopropanol buffers, 5% acetonitrile v/v, 30 °C and I = 1.0 M (KCl).

The intercept at $\alpha = 0$ corresponds to any reaction of the conjugate acid form of the buffering species and gives the second-order rate constant k_{BH}. The intercept at $\alpha = 1$ corresponds to any reaction of the basic form of the buffering species and gives the second-order rate constant k_B. The rate constants for the reaction of various buffers or bases with *N*-benzoyl β-sultam **8** are given in Table 2.

In all cases it was only the basic form of the buffer which showed a reaction. There was no contribution to the observed rate by the conjugate acid form of the buffering species. Some experiments were limited by the high reactivity of the *N*-benzoyl compound **8** which prevented the study of reactions above pH 12.5 due to the minimum stopped flow reaction time of 20 ms. Trifluoroethanol (TFE) and propargyl alcohol (PAL) were therefore used at fractions of free base less than 0.05 in solutions of non-nucleophilic buffers.

The reaction between oxyanions and the β-sultam could involve one of several mechanisms. For example, nucleophilic substitution by the oxyanion at the sulfonyl centre could occur by a stepwise (Scheme 3) or concerted process. In nucleophilic



Scheme 3

catalysed hydrolysis the intermediate sulfonate ester would rapidly hydrolyse to the sulfonic acid (**9**) whilst in alcoholysis the sulfonate ester (**10**) would be the relatively stable product. Another possible mechanism could be general base catalysed hydrolysis by the oxyanions.

A Brønsted plot showing the dependence of the logarithm of the second order rate constants, k_B, upon the pK_as of the conjugate acids of the oxyanions is shown in Fig. 4. Although

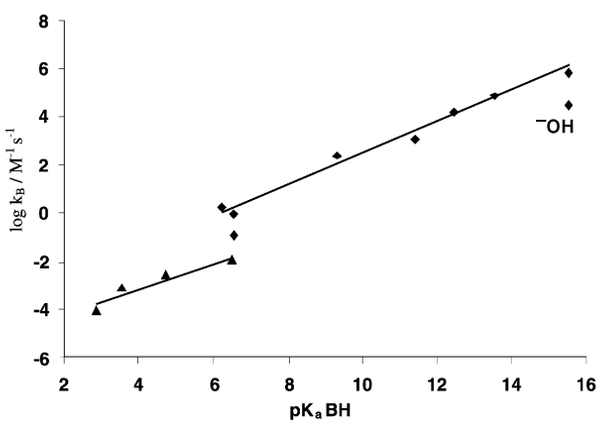


Fig. 4 Brønsted plot for the second order rate constants of the reaction of oxyanions with *N*-benzoyl β-sultam **8** against the pK_a of the conjugate acid, 30 °C, $I = 1.0$ M (KCl) and 5% acetonitrile v/v.

no reaction due to methoxide ion (pK_a 15.54) could be detected, an *upper limit* for k_{MeO^-} was made based upon an estimate of the maximum error in the observed rate constants. This estimate correlated well with the k_{RO^-} values obtained for PAL, TFE and hexafluoroisopropyl alcohol (HFIP). The slight negative deviation of heptafluorobutan-1-ol (HFIB, pK_a 11.40) may be due to solubility problems encountered in its use which led to a degree of uncertainty in the measurement. Attempts to use chloroethanol (pK_a 14.31) as an attacking nucleophile in pH 11 CAPS buffer, pH 12 phosphate buffer and 0.01 M NaOH solutions failed because of pH instability, the extent of which was dependent on the chloroethanol concentration. Even at low concentrations of the oxyanion decomposition, presumably to ethylene oxide and HCl, occurred.

There is an unusual 'step' or discontinuity which is evident in the Brønsted plot at an approximate pK_a of 6.50 (Fig. 4) and it appears to show two separate correlations, one for the carboxylate-type anions of slope +0.52 and one for the alkoxide-type anions of slope +0.65. However, before this and the magnitude of the Brønsted slopes, β, are discussed, it is relevant to describe evidence for a nucleophilic reaction.

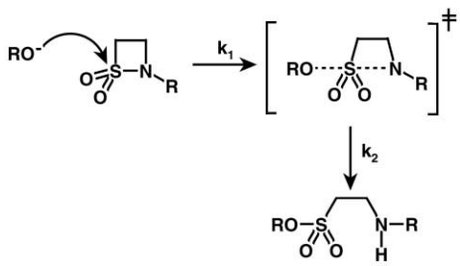
The reaction of *N*-benzoyl β-sultam **8** was followed by means of ^{1}H-NMR and ESIMS in 0.05 M deuterated hexafluoroisopropyl alcohol (HFIP) buffer pD 9.74 in 50 : 50 D_2O : CD_3CN v/v. After the reaction, there appeared to be three species present in solution, as evidenced by separate sets of CH_2 resonances: 4.38 and 3.85 ppm from intact *N*-benzoyl β-sultam **8**, 3.67 and 3.02 ppm from the ring opened sulfonic acid hydrolysis product (**9**) and 3.82 and 3.76 ppm from a new species, possibly the sulfonate ester (**11**). These signals were present in relative intensities of 10 : 2 : 6, respectively. The aromatic region was complex, containing several discrete signals, consistent with there being multiple products. The most striking piece of evidence for the existence of the sulfonate ester (**11**) was the HFIP CH resonance at 5.89 ppm. The HFIP CH signal of the free alcohol occurs at 4.67 ppm and the 1.22 ppm downfield shift from this resonance in the presence of *N*-benzoyl β-sultam **8**, under basic conditions, is consistent with the deshielding of the HFIP proton by the γ-sulfonyl moiety in the sulfonate ester (**11**). ESIMS confirmed the presence of the sulfonate ester in a mixture of products—in positive mode the ester (**11**) was observed at 381 $[M(\mathbf{11}) + D]^+$ *m*/*z* and in negative mode ESIMS revealed a single ion at 228 $[M(\mathbf{9})]^-$ *m*/*z* due to the sulfonate anion.

This evidence is consistent with the alcoholysis of *N*-benzoyl β-sultam **8** occurring in parallel with the hydroxide ion catalysed hydrolysis reaction giving both the ring opened sulfonic acid (**9**) and the sulfonate ester (**11**) as reaction products. Because the concentration of the buffer was less than that of the *N*-benzoyl compound **8** in the NMR tube, all the HFIP was consumed, the pH decreased and the reaction stopped, leaving some intact compound present. The sulfonate ester may not be stable under more basic conditions and may only have been observed in this case because of the decrease in pH.

When the experiment was repeated with a 0.1 M HFIP deuterated buffer with 0.04 M β-sultam, NMR showed there to be no intact *N*-benzoyl β-sultam **8** present. Again resonances were observed at 3.66 and 3.02 ppm from the ring opened sulfonic acid (**9**) and at 3.81 and 3.74 ppm from the sulfonate ester (**11**). The aromatic region was correspondingly clearer and this time two CH resonances due to HFIP were observed: a high intensity peak at 4.48 ppm due to the buffer solution and a low intensity peak at 5.89 ppm due to the deshielded CH in the sulfonate ester (**11**). The approximate integration ratio of CH_2 resonances for the sulfonic acid (**9**) to those of the sulfonate ester (**11**) was 1 : 10. This is similar to the product ratio of 1 : 18 predicted from the kinetic studies for a reaction at pD 9.8 with $k_{OD} = 1.93 \times 10^4$ M^{-1} s^{-1} and with 0.20 M HFIP, $\alpha = 0.75$ and k_{HFIP^-} (D_2O) = 284 M^{-1} s^{-1}. In this experiment the dominant ion in the positive mode ESIMS was due to di-deuterated sulfonate ester **11**. The sulfonate ester product is thus stable under the experimental conditions, although it presumably undergoes H–D exchange with the solvent, because of the acidity of the CH and the CH_2 (α to SO_2) protons.

No sulfonate ester could be detected in the products of HFAH catalysed hydrolysis of the *N*-benzoyl compound **8**. This is possibly due to the lability of such an ester with a leaving group pK_a of 6.5 or to a change in mechanism to general base catalysed hydrolysis.

The slopes, β, of the Brønsted plots (Fig. 4) of +0.52 and +0.65, are too low to be attributed to a stepwise process with rate limiting ring opening of the β-sultam. They are however consistent with either stepwise rate limiting formation of the trigonal bipyramidal intermediate (TBPI) (Scheme 3) or a concerted mechanism of ring opening (Scheme 4). Values of β_{nuc}



Scheme 4

similar to those observed for our reaction of nucleophiles with *N*-benzoyl β-sultam **8** have been observed for the reaction of pyridines with substituted benzenesulfonyl chlorides (0.41 for *p*-MeO, 0.56 for *p*-NO_2),[28] oxyanions with aryl benzenesulfonates (0.64 for *p*-nitrophenyl *p*-nitrobenzenesulfonate)[37] and amines with tosylimidazoles (0.48 for 3-methylimidazolium, 0.64 for 2,3-dimethylimidazolium).[29] These β_{nuc} values, however, all show single correlations and are consistent with rate limiting nucleophilic attack or concerted mechanisms of sulfonyl transfer. It is also evident that increases in the electron withdrawing character of the substituents attached to the sulfonyl centre lead to increases in the β_{nuc} value.

It appears that our Brønsted plot is best represented by two separate correlations rather than a single relationship, although this is discussed again later. It is unlikely that there are two different mechanisms occurring, with a change around pK_a 6.50. In the case of the corresponding Brønsted β_{nuc} plot for the reaction of oxygen nucleophiles with benzyl penicillin[38] a slope of 0.38 was observed for nucleophile pK_as less than 9 and a slope of 0.97 was observed for nucleophile pK_as greater than 9. This change of slope signals an upward 'bend' in a continuous plot and was attributed to a change in mechanism from general base catalysed hydrolysis by the weaker oxygen bases and a nucleophilic reaction, with rate limiting ring opening, by the stronger oxygen bases.[38]

In the region of the discontinuity of the Brønsted plot (Fig. 4) the data for hexafluoroacetone hydrate anion (HFAH), hydrogen phosphate dianion, hydrogen arsenate dianion and cacodylate (CAC) are quite remarkable. The values of the second-order rate constants, k_{RO^-}, for these four bases which all have similar pK_as *ca.* 6.5 are: $k_{HFAH^-} = 1.07 \times 10^{-2}$ M^{-1} s^{-1}, $k_{HPO_4^{2-}} = 1.36 \times 10^{-1}$ M^{-1} s^{-1}, $k_{HAsO_4^{2-}} = 0.891$ M^{-1} s^{-1} and $k_{CAC} = 1.63$ M^{-1} s^{-1}. There is a 100-fold difference between hexafluoroacetone hydrate anion and cacodylate despite their almost identical oxygen basicities.

The kinetic solvent isotope effects were determined for the reactions of some of these bases with *N*-benzoyl β-sultam **8**. A full pH–rate profile was obtained for the hydrolysis of **8** in D_2O and modelled using the Scientist software package to obtain the rate constants for the acid and base catalysed hydrolysis and the pH independent reaction. The second-order rate constants for the reaction of oxyanions are given in Table 3 with the observed solvent kinetic isotope effects (SKIEs) which vary between 1.44 for formate and 0.76 for deuteroxide. These values are consistent with those expected for a nucleophilic reaction although the higher values could be indicative of a general base catalysed hydrolysis.[39] There is no obvious distinction between the SKIE for HFAH on the lower correlation in the Brønsted plot (Fig. 4) and that for cacodylate on the upper correlation and it is concluded that both involve a nucleophilic reaction. There is an apparent trend of increasing SKIE as the basicity of the nucleophile decreases which is roughly paralleled by the decreasing difference in pK_a of the corresponding acids in H_2O and D_2O.

A SKIE of 0.76 is observed for the second-order rate constant of hydroxide ion hydrolysis, k_{OH}, of *N*-benzoyl β-sultam **8** which is consistent with rate limiting formation or breakdown of the TBPI (Schemes 3 and 4). The SKIE for the acid catalysed hydrolysis of *N*-benzoyl β-sultam **8** is 0.50 and is consistent with rate limiting ring opening of the β-sultam conjugate acid or rate limiting attack of water on the ring opened sulfonylium ion.[11,15] A large SKIE k (H_2O)/k (D_2O) of 4.4 is observed for the pH independent hydrolytic reaction, k_0. This is not consistent with water acting solely as a nucleophile with rate limiting attack at the sulfonyl centre but is indicative of rate limiting proton transfer perhaps involving general acid catalysis by water (**12**). Another alternative mechanism to account for the large SKIE is the general base catalysed addition of water.

Returning to the Brønsted plot (Fig. 4) which appears to show two separate correlations, one for the carboxylate-type anions of slope +0.52 and one for the alkoxide-type anions of slope +0.65, the oxyanions surrounding the apparent break point are cacodylate, hydrogen arsenate dianion, hydrogen phosphate dianion and hexafluoroacetone hydrate anion. These all have similar basicities as indicated by the pK_a of their conjugate acids and yet there is a 100-fold difference in their reactivities (Table 2). These differences cannot be due to bifunctional catalysis, a known feature of phosphate chemistry, because of the higher reactivity of cacodylate which lacks a proton donating group. Cacodylate and hydrogen arsenate dianion appear to lie on the correlation line for alkoxide ions whereas hexafluoroacetone hydrate anion appears to fit the line generated by carboxylate anions. Phosphate does not appear to lie on either correlation and is 6-fold less reactive than arsenate. This increased reactivity of arsenic compounds over phosphorous compounds is also observed in acyl transfer but only a 2-fold difference is observed.[40] It is difficult to envisage a significant steric difference between these tetrahedral anions, other than that due to the different As–O, P–O and C–O bond lengths,[41] which could be directly related to the ease of nucleophilic attack.

The data used for the Brønsted plot can also be treated statistically, eqn. (4), where p is the number of acidic sites and q is the number of basic sites[42] to yield the correlation shown in Fig. (5).

$$\log (k_{nuc}/q) = \beta[pK_a + \log (p/q)] + C \qquad (4)$$

The Brønsted β_{nuc} plot can now be more easily viewed as a single correlation of slope +0.81, which is still consistent with either rate limiting nucleophilic attack or a concerted mechanism of ring opening. This would indicate, in contrast to the β-lactam of benzyl penicillin, that all oxygen bases act as nucleophiles over the whole pK_a range, so that weak bases such as carboxylate anions can displace the much more basic amide

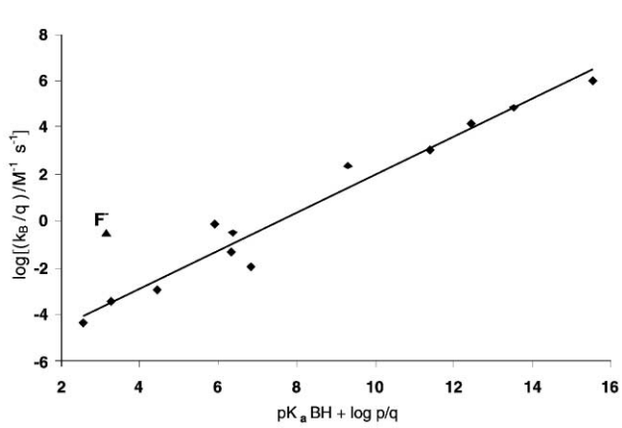


Fig. 5 Statistically corrected Brønsted plot for the reaction of nucleophiles with *N*-benzoyl β-sultam **8**, 5% acetonitrile v/v, 30 °C and I = 1.0 M (KCl).

Table 3 Rate constants and solvent kinetic isotope effects k (H_2O)/k (D_2O) for the reactions of *N*-benzoyl β-sultam **8** with nucleophiles in H_2O and D_2O at 30 °C and I = 1.0 M (KCl) and the change in pK_a for buffering species on going from H_2O to D_2O

Buffer/reactant	k_{RO^-} (H_2O)/M^{-1} s^{-1}	k_{RO^-} (D_2O)/M^{-1} s^{-1}	k (H_2O)/k (D_2O)	ΔpK_a (D_2O − H_2O)
Formate	6.85×10^{-4}	4.75×10^{-4}	1.44	0.55
HFAH	1.07×10^{-2}	8.46×10^{-3}	1.26	0.61
Phosphate	0.136	0.140	0.97	0.56
Cacodylate	1.63	1.26	1.29	0.53
HFIP	246	284	0.87	0.70
HO^-	1.46×10^{4}	1.93×10^{4}	0.76	1.07
	k_H (H_2O)/M^{-1} s^{-1}	k_D (D_2O)/M^{-1} s^{-1}	k (H_2O)/k (D_2O)	
H^+	5.57×10^{-5}	1.12×10^{-4}	0.50	
	k_O (H_2O)/s^{-1}	k_O (D_2O)/s^{-1}		
H_2O	4.56×10^{-6}	1.05×10^{-6}	4.43	

from the sulfonyl centre in the β-sultam **8**. However, there are still deviations from this correlation. The "corrected" Brønsted plot (Fig. 5) also shows the large positive deviation for fluoride ion which is *ca.* 10^3-fold more reactive than predicted from oxygen nucleophiles of similar basicity. It is clear that a number of factors are contributing to the overall appearance of the Brønsted β_{nuc} plot which reflects complex structure–reactivity relationships. There is no evidence suggestive of, or contrary to, a stepwise mechanism of hydrolysis at the sulfonyl centre.

The order of nucleophilicity of nucleophiles towards sulfonyl centres has been reported as $HO^- > RNH_2 > N_3^- > F^- > AcO^- > Cl^- > H_2O > I^-$ for benzenesulfonyl chloride[33] and aryl α-disulfones.[43] This is a similar order to that for acyl centres with soft nucleophiles having lower reactivity in both cases and indicative of sulfonyl sulfur being relatively hard.[44] The reactivity order obtained for *N*-benzoyl β-sultam **8** is similar except for the low reactivity towards nitrogen nucleophiles: $HO^- > RO^- > F^- > RCO_2^- > RNH_2 > H_2O$. This low reactivity of amines is also observed in the reactions of *p*-nitrophenyl toluene-*p*-sulfonate[35] where there is a distinct preference towards oxygen nucleophiles. It has been shown that phenolate anions are 10^2-fold more reactive towards the sulfonyl centre than amines of similar basicity.[36]

Fluoride ion shows a marked degree of reactivity towards *N*-benzoyl β-sultam **8** with a second order rate constant k_F of 0.30 M^{-1} s^{-1}. Fluoride ion is a hard nucleophile and readily reacts with hard electrophilic centres. The ratio of k_B for HO^- to that for F^- for the *N*-benzoyl compound (**8**) is 5×10^4, which is similar in order of magnitude to that observed in acyl transfer reactions.[40] However, for the latter this selectivity ratio decreases 10^3-fold as the hydrolytic reactivity of the acylating agent increases by 10^4.[40] The k_{OH}/k_F ratio for *N*-benzoyl β-sultam **8** is 20-fold greater than that for carboxylic acid esters of similar hydrolytic reactivity. This implies that, in contrast to acyl esters, the *N*-acyl β-sultams show a larger degree of selectivity in the more reactive compounds. The ratio, k_{OH}/k_F, is 53 for benzenesulfonyl chloride, for which k_{OH} = 59.0 M^{-1} s^{-1}.[33] This is consistent with an inverse selectivity–reactivity relationship for sulfonyl compounds. The high reactivity of *N*-acyl β-sultams towards hydroxide ion means that at pH 5 *N*-benzoyl β-sultam **8** reacts preferentially with 1×10^{-9} M HO^- in the presence of 1×10^{-5} M H^+ and 55 M water. The k_{OH}/k_0 ratio of **8** is 4.69×10^9 M^{-1} and can be taken as a measure of the selectivity of this sulfonating agent. Sulfonyl chlorides show an apparent inverse selectivity–reactivity relationship whilst acyl esters behave as expected. The trend for the acyl esters is as expected, the most reactive compounds (highest k_{OH} values) are the least selective (lowest k_{OH}/k_0 values). For the sulfonyl halides and *N*-acyl β-sultams, however, the more reactive compounds are apparently more selective.

The reactivity order of nucleophiles towards *N*-benzoyl β-sultam **8** indicates that the sulfonyl centre can be considered a hard electrophile. A comparison of the rate constants for the sulfonation of oxyanions by *N*-benzoyl β-sultam **8**, with the acylation of the same nucleophiles by benzyl penicillin,[38] shows a good correlation between the two electrophiles (Fig. 6). The

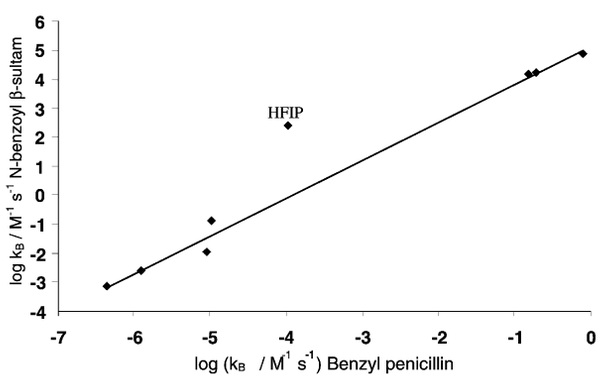


Fig. 6 Plot of log k_{RO^-} for the reaction of oxyanions with *N*-benzoyl β-sultam **8** against log k_{RO^-} for the reaction of the same oxyanions with benzyl penicillin (**5**).

slope of 1.30 indicates a higher degree of selectivity for the sulfonyl centre. The observation that ring opening of *N*-benzoyl-β-sultam **8** can be initiated by carboxylate anions is in stark contrast to *N*-benzyl β-sultam **2** where the reaction occurs solely with the undissociated form of the buffer, which is actually the kinetically equivalent mechanism of specific-acid nucleophilic catalysis.[13] The differences can be rationalised by the different reactivities of the two compounds. *N*-Benzyl β-sultam **2** has a k_H of 1.52 M^{-1} s^{-1} and a k_{OH} of 1.07×10^{-2} M^{-1} s^{-1} and ring opening by attack of the weakly basic carboxylate anions requires initial *N*-protonation to increase the nucleofugacity of the nitrogen functionality. This *N*-protonation is made favourable by the availability of the nitrogen lone pair, whereas this is not the case for *N*-benzoyl β-sultam **8** which is 3×10^4 less reactive towards acid hydrolysis. However, **8** is 10^6 more reactive towards alkaline hydrolysis with a k_{OH} of 1.46×10^4 M^{-1} s^{-1} reflecting the possible cleavage of the S–N bond by expulsion of the amide anion. *N*-Benzoyl β-sultam **8** is therefore highly reactive towards nucleophiles and extremely unreactive towards *N*-protonation, although this reactivity does not extend to aminolysis and *N*-benzoyl β-sultam **8** does not show a measurable reaction rate with *n*-propylamine.

Experimental

Kinetics

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. Stopped flow experiments used an SX.18 MV Spectrakinetic monochromator (Applied Photophysics, Leatherhead, England) equipped with an absorbance photomultiplier. The reagent syringes were thermostatted with a Grant thermostatted water circulator. pH-stat experiments were performed on a ABU 91 Autoburette (Radiometer, Copenhagen, Denmark), controlled by a VIT 90 video titrator. The SAM 90 sample station incorporated a machined aluminium E2000 sample block rotor thermostatted by a MGW Lauda M3 water circulator. pH was measured by a pHG200–8 Glass pH electrode and a REF200 'Red Rod' reference electrode (Radiometer). Temperature was monitored by a T101 temperature sensor.

pH measurements were made with either a ϕ40 (Beckman, Fullerton, USA) or 3020 (Jenway, Dunmow, England) pH meter. Electrodes were semi-micro Ag/AgCl and Calomel (Russel, Fife, Scotland and Beckman respectively). A calibration of the pH meter was carried out at 30 °C using pH 7.00 ± 0.01, pH 4.01 ± 0.02 or pH 10.00 ± 0.02 calibration buffers.

ESIMS experiments were carried out on a VG Quattro SQ II (Micromass, Altrincham, England) and NMR experiments on a 400 MHz instrument (Bruker, Germany).

AnalaR grade reagents and deionised water were used throughout. Sodium hydroxide solutions were titrated prior to use against a 1.00 M ± 0.1% hydrochloric acid volumetric reagent (D.H. Scientific, Huddersfield, England) using phenolphthalein as an indicator. For experiments carried out in D_2O (99.9+ atom%D, Sigma, Poole, England) solutions of deuterium chloride and sodium deuteroxide were prepared by diluting DCl (99+ atom%D, 20% in D_2O, Sigma) and NaOD (99+ atom%D, 40% in D_2O, Sigma) with D_2O. When not bought in fresh, organic solvents were glass distilled prior to use and stored under nitrogen. For solution pHs ≥ 3 and ≤ 11 the pH was controlled by the use of ≤0.2 M buffer solutions of formate (pK_a 3.75), ethanoate (pK_a 4.72), morpholine-4-ethanesulfonic acid (MES, pK_a 6.1), morpholine-4-propanesulfonic acid (MOPS, pK_a 7.2), 3-[tris(hydroxymethyl)methylamino]-propanesulfonic acid (TAPS, pK_a 8.4), 3-cyclohexylamino-2-hydroxypropanesulfonic acid (CAPSO, pK_a 9.6), and 3-cyclohexylaminopropanesulfonic acid (CAPS, pK_a 10.4). For general pH work, buffers were prepared by partial neutralisation of solutions of their sodium salts to the required pH. For the alcoholysis reactions, buffers were prepared by the addition of 0.25, 0.50 or 0.75 aliquots of 1 M NaOH to solutions of the alcohol. In all experiments temperatures were maintained at 30 °C and ionic strength at 1.0 M with AnalaR grade KCl unless otherwise stated. Reaction concentrations were generally within the range $\geq 2 \times 10^{-5}$ M, $\leq 2 \times 10^{-4}$ M to ensure pseudo-first-order conditions.

Hydroxide ion concentrations were calculated using pK_w (H_2O) = 13.883 at 30 °C[18] and pK_w (D_2O) = 14.699 at 30 °C[45] and the solution pD was taken as pH + 0.40.[46]

Reactions studied by UV spectrophotometry were usually commenced by injections of acetonitrile or 1,4-dioxan stock solutions of the substrate (5–50 μl) into the cells containing pre-incubated buffer (2.5 ml). Final reaction cells contained ≤5% acetonitrile or dioxan 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 were 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. If required, greater than 1% MeCN v/v was used to aid solubility. Pseudo-first-order rate constants from exponential plots of absorbance against time or gradients of initial slopes were obtained using the Enzfitter package (Elsevier Biosoft, Cambridge, England) or the CaryBio software (Varian). pH–rate profiles were modelled to theoretical equations using the Scientist program (V2.02, Micromath Software Ltd, USA).

Reactions studied by stopped flow UV spectrophotometry used stock solutions prepared at twice the standard UV concentration in 1 M KCl. Hydroxide solutions, buffer solutions or solutions of nucleophilic reagents were prepared at twice the required concentration. The substrate solution and the reaction mixture were placed in separate syringes and thermostatted at 30 °C before pneumatic injection into the reaction cell. Where applicable, the pH of solutions was measured prior to use. If greater than 1% acetonitrile v/v was required for solubility, then organic solvent concentration of all solutions used was fixed at the required reaction cell amount. The photomultplier voltage was set to a maximum on deionised water and absorbance wavelengths common to the standard UV experiments were used. Pseudo-first-order rate constants from exponential plots of absorbance against time were obtained using the supplied fitting software (Applied Photophysics).

For reactions studied by pH-stat standardised NaOH was delivered to a stirred sample solution (10 ml) held within the thermostatted sample station. All reactions were performed under nitrogen to prevent CO_2 absorption. Data was exported to a Windows PC *via* an RS232 interface and the terminal program (Microsoft Corp, USA). Conversion into an appropriate format was by means of an Excel (Microsoft Corp, USA) macro and results were fitted to first order equations *via* the Enzfitter program (Elsevier Software). The titrant used was 0.01–0.1 M NaOH standardised prior to use by means of phenolphthalein titration against 1.00 M HCl (Volumetric reagent, D.H. Scientific). Reactions were performed in 1 M KCl, 5% MeCN v/v, with a pH set point of 6–7. Concentrations of sample were in the range of 1–2 mM with expected titrant added volumes of up to 1.0 ml.

pK_a determinations

A solution of the compound was placed in a UV cell at 30 °C and typically titrated by means of additions of NaOH or HCl (≤50 μl). Absorbance values at the wavelength of maximum change and the cell solution pH were measured after each injection of 0.01, 0.05, 0.1 or 1.0 M standardised NaOH or HCl. Near the end point injections of the 0.01 M solution (5 μl) were made to ensure that sufficient pH values were covered in the proximity of the pK_a.

The results were analysed using the Enzfitter software package (Elsevier Biosoft), pK_{a1} determination equation, $A = [(A_{min} + A_{max}) \times 10^{pH - pK_a}]/(10^{pH - pK_a} + 1)$ where A = absorbance of solution at a particular pH and wavelength, A_{min} = minimum absorbance where 100% of dissociating species is in form with lowest absorption, A_{max} = maximum absorbance where 100% of dissociating species is in the form with highest absorption, pH = solution pH and pK_a = pK_a of dissociating species.

^{1}H-NMR experiments

The β-sultam (15 mg) was dissolved in 50 : 50 v/v CD_3CN : D_2O (*ca.* 3 ml) and the ^{1}H-NMR spectra acquired at 400 MHz and 300 K. One drop additions of 4% v/v NaOD in D_2O were made to the NMR tube until complete conversion of reactants had occurred with spectra being acquired after each addition. In buffer solution, the β-sultam (15 mg) was dissolved in 50 : 50 v/v CD_3CN : 0.4 M buffer solution in D_2O (*ca.* 3 ml), the pD of

which was measured before and after the experiment. The solutions were also routinely submitted for both positive and negative mode ESIMS-MS.

Materials

The synthesis of the β-sultams will be reported elsewhere.

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