Chemical and Biological Reactivity of Sulfamidopenicillins

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A number of semisynthetic penicillins bearing a sulfamido side chain at the 6-position have been prepared and their chemical and biological reactivity examined. The compounds do not show antibiotic activity against *Escherichia coli* but do show activity against *Staphylococcus aureus*. The rates of hydroxide-ion catalysed hydrolysis correlate well with the inductive effect of the sulfamido group indicating no pronounced steric effect on the chemical reactivity of the β-lactam ring. Molecular modelling studies show that the sulfamido group does not have an effect on the 'open' *versus* 'closed' conformations of the thiazolidine ring different from that of the benzylamido group. Comparisons with some sulfonamidopenicillins are made.

The biological activity of semisynthetic penicillins is influenced by both chemical and structural parameters. The reactivity of the β-lactam ring towards cleavage by nucleophiles is important, but not a paramount factor for antibiotic activity:[1] the ability of the penicillin structure to bind at the active enzyme site is also of importance.[2] In each case the side chain at the 6-position can play an influential role as instanced by the differing stabilities of benzylpenicillin and ampicillin in aqueous acid, and their differing biological activities.[3] The general features which influence the β-lactam cleavage have been extensively studied.[4,5] The predominant effect of substituents at the 5- and 6-positions is an inductive one and in the case of hydroxide-ion catalysed hydrolysis, is correlated by a Taft relationship with a ρ_i value of 3.9, *i.e.* electron withdrawing groups activate the ring.[4] More O'Ferrall has shown that a simple dual-parameter Taft equation correlates hydrolysis and methanolysis rates over nine orders of magnitude in reactivity for monocyclic β-lactams with a ρ_i value of 5.[5] A number of studies have also been carried out to evaluate the critical conformational factors which influence biological activity.[6-8] One feature which has been examined in considerable detail is the relative conformations of the thiazolidine ring which can adopt a 'closed' or 'open' aspect with respect to the β-lactam ring (Fig. 1). A certain consensus exists which holds that penicillin structures which, in solution, cannot attain the 'open' conformation do not show antibiotic activity while those which, in solution, have a reasonably populous 'open' conformation can potentially show antibiotic activity.[7] The role of the side chain in altering the relative population of 'open' and 'closed' conformations is known and for quite a variety of side chains it has been shown that ring flipping of the thiazolidine moiety occurs quite freely in aqueous solution.[9] The direct role of the side chain on biological activity has also been considered in terms of how it might influence (a) recognition and binding at the active sites and (b) approach of a nucleophile on the β-lactam ring.[10] The side chain can also play a major role in modifying the lipophilicity of a penicillin structure.[11]

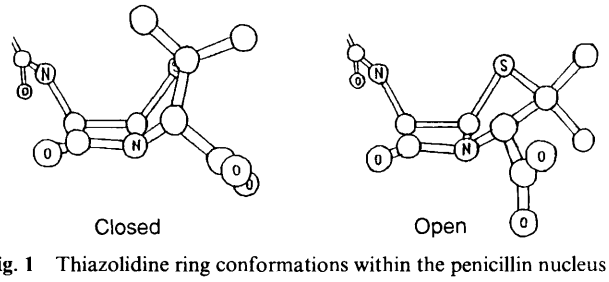
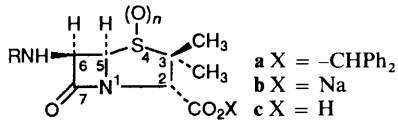


Fig. 1 Thiazolidine ring conformations within the penicillin nucleus

We have prepared a number of 6-sulfamidopenicillanic acids (Fig. 2) and examined their chemical and biological reactivities. The sulfamido group differs from the amido group in a number of ways: (a) the stereochemistry about the sulfur atom is tetrahedral[12] in contrast to the trigonal stereochemistry about the amide carbon, (b) the sulfamido group is more strongly electron withdrawing, (c) the sulfamido group is more polar and (d) the pK_a of the sulfamido group is lower by 3 to 4 units.[13]

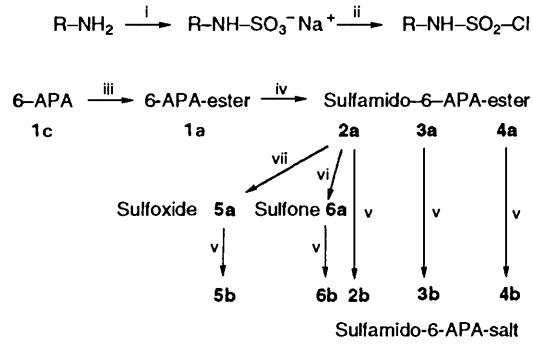
Results and Discussion

Synthesis.—The 6-sulfamidopenicillanic acid salts were prepared as outlined in Scheme 1. The sulfamoyl chlorides, prepared from the corresponding amine by the method of



1 APA R = H, n = 0
2 R = c-hex $NHSO_2$–, n = 0
3 R = $PhNHSO_2$–, n = 0
4 R = $BuNHSO_2$–, n = 0
5 R = c-hex $NHSO_2$–, n = 1
6 R = c-hex $NHSO_2$–, n = 2

Fig. 2 Structure of sulfamidopenicillins, their sulfoxides and sulfones; **a** protected as diphenylmethyl ester; **b** as sodium salts



Scheme 1 *Reagents:* $ClSO_3H$–pyridine; ii, PCl_5; iii, Ph_2CN_2; iv, $RNHSO_2Cl$; v, H_2 over Pd–C, $NaHCO_3$ extract, freeze dry; vi, *m*-CPBA (4.5 equiv.); vii, *m*-CPBA (1 equiv.)

Table 1 Antibiotic activity

Organism	Penicillin	Loading of penicillin (μg on 0.5 cm filter disk)	Diameter of halo/mm
E. coli K12	Benzylpenicillin	50	20
	2b	250	0
	3b	250	0
	4b	250	0
S. aureus FDA 209P	Benzylpenicillin	1	40
	2b	1	26
	3b	1	15
	4b	1	17

Table 2 β-Lactamase (type I *Bacillus cereus* 569H) results (20 °C)

Structure	k_{cat}/s^{-1}	$10^4 K_m$/ mol dm^{-3}	$10^{-6} k_{cat}/K_m$ dm^3 mol^{-1} s^{-1}	Relative k_{cat}/K_m
Benzylpenicillin	2044	1.15	17.7	1.00
2b	554	18.0	0.30	0.017
3b	1290	48.4	0.27	0.015
4b	511	27.2	0.19	0.010

Audrieth and Sveda,[14] were treated with the ester-protected 6-aminopenicillanic acid (6-APA; **1a**). The sulfoxide and sulfone **5** and **6** derivatives were prepared by oxidation using *m*-chloroperbenzoic acid. Hydrogenolysis of the protecting group gave the free acid which was readily converted to the sodium salt form using sodium hydrogen carbonate.

Antibiotic Activity.—The sulfamidopenicillins **2b**, **3b** and **4b** were screened for antibiotic activity, using the filter disk method, on penicillin sensitive strains of *Escherichia coli* K12 and *Staphylococcus aureus* FDA 209P. No activity was observed against the *Escherichia coli* strain but activity was observed against the *Staphylococcus aureus* strain for all three sulfamidopenicillins and this was quite significant in the case of the cyclohexylsulfamidopenicillin **2b** (see Table 1). The sulfoxide **5b** and the sulfone **6b** were screened for β-lactamase inhibitory activity against penicillin resistant strains of the above two organisms but none showed any synergistic action when used with benzylpenicillin. Compound **6b** did show some inhibitory activity when tested against a pure sample of a β-lactamase enzyme in aqueous solution (see following section).

β-*Lactamase Action.*—The sulfamido penicillins **2b**, **3b** and **4b** were found to be hydrolysed by the β-lactamase enzyme type I from *Bacillus cereus*. Values of k_{cat}, K_m and k_{cat}/K_m for these structures and for benzylpenicillin were measured and are given in Table 2. The values of k_{cat} are lowest for **2b** and **4b** but these are only four times less than that for benzylpenicillin. The difference in K_m values is somewhat greater with that for **3b** being 40 times that of benzylpenicillin. Taking K_m as an apparent dissociation constant of the penicillin from the enzyme the values for the sulfamidopenicillins suggest that these structures bind with the enzyme much more weakly than benzylpenicillin. Comparing the values of the k_{cat}/K_m term (which can be taken as an apparent second order rate constant for reaction between the enzyme and substrate) it is seen that all of the sulfamidopenicillins tested in this work are much poorer substrates for this enzyme than is benzylpenicillin.

The sulfoxide and sulfone derivatives of some penicillin structures are known to be inactivators of this enzyme *via* a 'suicide' or 'mechanism based' mode of action.[15] We examined compounds **5b** and **6b** for inhibitory activity. The rate of hydrolysis of benzylpenicillin by this enzyme was monitored by ^{1}H NMR spectroscopy before and after pre-incubation of the enzyme with **5b** or **6b**. Pre-incubation for 1.5 h at 20 °C with **6b** resulted in a 40% loss of activity by the enzyme while pre-incubation for 4 h resulted in total loss of enzyme activity. Pre-incubation with **5b** had no effect on the enzyme activity while inactivation by sulbactam was immediate. The long incubation time required with **6b** indicates that it does not have a very high association constant with the β-lactamase enzyme. This is consistent with the observation of no synergistic effect for **6b** when tested with benzylpenicillin against the penicillin resistant strains as stated in the foregoing section.

Rates of Hydrolysis.—We studied the hydrolysis of **2b**, **3b** and **4b** in carbonate buffers (0.006–0.03 mol dm^{-3}) between pH 8.5–9.0 and with unbuffered solutions at [OH^-] from 0.05–1.0 mol dm^{-3} corresponding to the pH region 13–14. At the lower pH values the sulfamido side chains are in the neutral form whereas at the higher hydroxide-ion concentrations the side chain is in the monoanion form.[13] In the pH range 13–14 the observed rates increased linearly with increasing [OH^-]; the rate data was analysed in terms of a general base mechanism. From this set of data values of $k_{OH^-(\text{side chain monoanion})}$ were determined while analysis of the buffer data (a typical set of rate data is given in Fig. 3) gave values of $k_{OH^-(\text{neutral side chain})}$. These values as well as some from the literature (entries 1, 5, 6, 7) are given in Table 3. In order to include the k_{OH^-} values for the sulfamido groups in a Taft plot it is necessary to obtain δ_i values for these groups. Experimentally determined values for these groups are not available but good estimates can be made for the neutral sulfamido group and reasonable estimates made for the sulfamido monoanions. A δ_i value of 0.38 is assigned to the neutral $-NHSO_2NH(R)/(Ar)$ group on the basis of (a) the experimental value of 0.42 available[16] for the sulfonamide group $-NHSO_2CH_3$ and, (b) the difference of 0.04 between the δ_i values of $-NHCOC_2H_5$ and $-NHCO_2NH_2$. The monoanion $-NHSO_2\bar{N}C_6H_5$ obtained by loss of the remote hydrogen, is assigned an approximate value of 0.01 by analogy with comparable structures *via.* $-CH_2SO_3^-$ and $-CH_2CO_2^-$. The remote monoanion ($-NHSO_2\bar{N}C_6H_5$) will be the form of the side chain present above pH 13 for **3b** as the pK_a for ionisation at the aryl substituted nitrogen is one unit lower than that for the alkyl substituted nitrogen.[13] The proximate monoanion $-\bar{N}SO_2NHR$, is assigned an approximate value of −0.17 by analogy with the carboxylate anion itself. The alkyl substituted sulfamide groups will also exist in the monoanion form above pH 13 and it is assumed that to a first approximation equal concentrations of the proximate- and remote-ionized stuctures ($-NHSO_2\bar{N}R/-\bar{N}SO_2NHR$) will exist to which the average δ_i value of −0.08 is assigned. These δ_i values are included in Table 3.

The Taft plot of log k_{OH^-} *vs.* δ_i for the data in Table 3 is shown in Fig. 4. The correlation has a ρ_i of 3.95 (R^2 0.99). The fact that all the sulfamido structures fit the line quite well

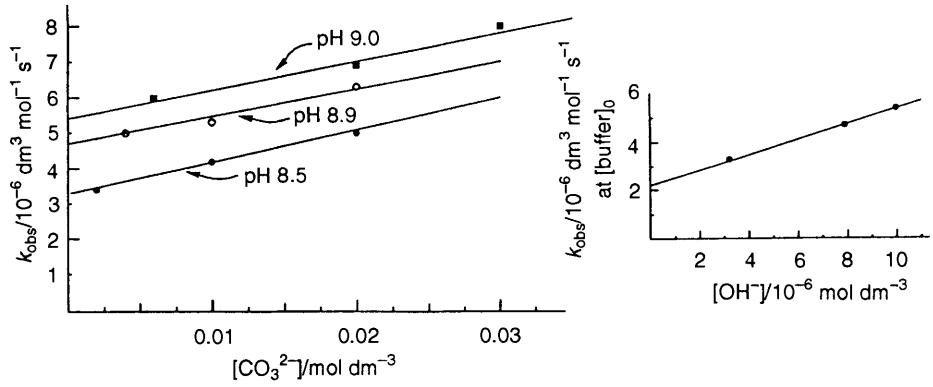


Fig. 3 Plot of k_{obs} *vs.* buffer concentration for **3b** and (insert) plot of k_{obs} at zero buffer concentration *vs.* hydroxide ion concentration to give $k_{OH^-(\text{neutral side chain})}$ for **3b**

Table 3 Rate constants (dm^3 mol^{-1} s^{-1}) for hydroxide ion catalysed hydrolysis of penicillins at 30 °C

Point	6-Substituent	$10^2 k_{OH^-}$	log k_{OH^-}	δ_i
	Neutral			
(1)	-α-Br	61.0[a]	−0.21	0.49[c]
(2)	$-NHSO_2NHC_6H_{11}$ **2b**	31.8[b]	−0.49	0.38[d]
(3)	$-NHSO_2NHC_6H_5$ **3b**	31.3[b]	−0.50	0.38[d]
(4)	$-NHSO_2NHC_4H_9$ **4b**	32.3[b]	−0.49	0.38[d]
(5)	$-NHCOCH_2Ph$	15.4[a]	−0.81	0.27[c]
(6)	-β-NH_2	6.31[a]	−1.20	0.19[c]
(7)	-H	0.74[a]	−2.13	0.00[c]
	Anionic			
(8)	$-NHSO_2\bar{N}C_6H_5$ **3b'**	0.85[b]	−2.07	0.01[d]
(9)	$-\bar{N}SO_2NHC_4H_9/-NHSO_2\bar{N}C_4H_9$ **4b'**	0.55[b]	−2.25	−0.08[d]
(10)	$-\bar{N}SO_2NHC_6H_{11}/-NHSO_2\bar{N}C_6H_{11}$ **2b'**	0.44[b]	−2.35	−0.08[d]

[a] Ref. 4. [b] This work. [c] Ref. 16. [d] Estimated as outlined in text.

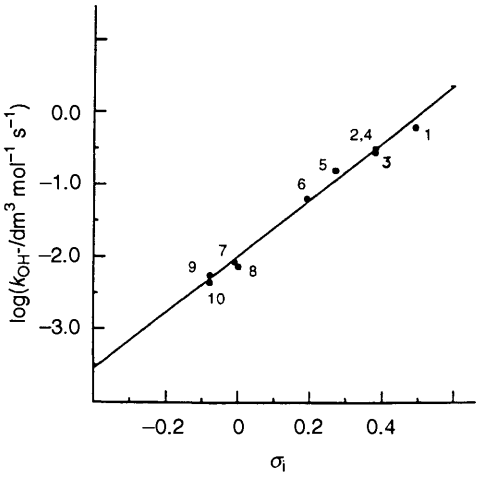


Fig. 4 Taft plot for hydroxide ion catalysed hydrolysis of 6-substituted penicillins

indicates that their predominant effect on the reactivity of the β-lactam ring is an inductive one; the tetrahedral nature of the sulfamido group does not cause any observable steric effect on the approach of the hydroxide ion to the lactam carbonyl group. The role of the inductive effect is also seen by the difference in the k_{OH^-} values between the neutral and anionic side chains of **2b** (points 2 and 10 in Table 3) with the anionic form being less reactive. The difference in k_{OH^-} values between the anionic side chain form of **3b** (point 8 in Table 3), and that of **2b** and **4b** (points 10 and 9 in Table 3) is attributable to the fact that the negative charge is more remote from the β-lactam ring in **3b'** and hence its inductive effect is lessened.

Conformational Analysis.—Molecular modelling of the thiazolidine-ring flipping between 'open' and 'closed' conformations was carried out on benzylpenicillanic acid and the free acid of **3b** using MNDO. A full structure optimisation was carried out for each structure initially: the optimised structures are shown in Fig. 5. The ring flipping was modelled by setting the dihedral angle defined by the β-CH_3 carbon to the atoms labelled 2, 1 and 5 (Fig. 5) at values from −180 to −90° in 15° intervals. The heat of formation was calculated for each structure at each of these dihedral angle settings while allowing full optimisation on all other structural parameters. The results are shown in Fig. 6. The energy profile is quite shallow over a 45° dihedral angle range (from −150 to −105°) and is essentially identical for benzylpenicillanic acid and the free acid of **3b**. The implication of this is that the phenylsulfamido group does not affect the 'open' to 'closed' thiazolidine-ring flipping in a way which is different to that of the benzylamido group. This general conclusion is supported by the ^{13}C NMR data available for **2b**. The β-CH_3 ^{13}C peak occurs at 32.03 ppm which would, according to the analysis of Dobson,[9] correspond to an equilibrium of 63% 'open' and 37% 'closed' conformers (that for the sodium salt of benzylpenicillin is at 31.7 ppm, which corresponds to 68% 'open' and 32% 'closed').[9] The ^{13}C analysis of Dobson is based on chemical shift data in the solid state and in frozen solutions for the β-CH_3 group in the 'closed' and 'open' conformers. In the solid state for the 'closed' conformer, this chemical shift is at 35.9 ppm and in the 'open' conformer is at 29.7 ppm: the solution value is taken as being the weighted average of these values.

Conclusions

The sulfamidopenicillins examined in this work appear to be quite similar to benzylpenicillin in terms of chemical reactivity

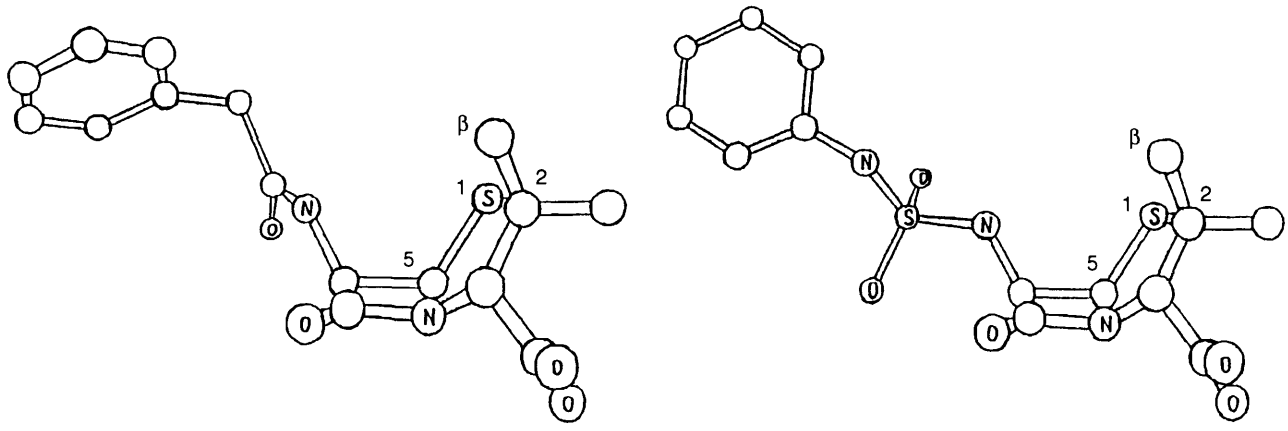


Fig. 5 Optimised (MNDO) structure of benzylpenicillanic acid and of phenylsulfamidopenicillanic acid

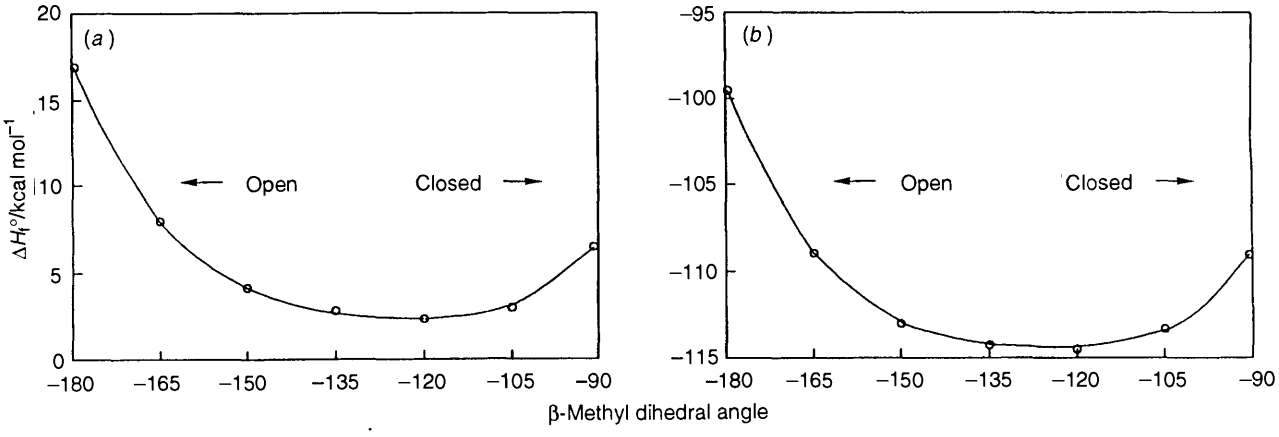
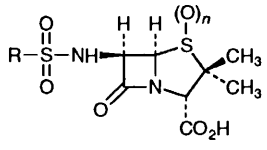


Fig. 6 Heat of formation as a function of the β-methyl dihedral in (*a*) phenylsulfamidopenicillanic acid and (*b*) benzylpenicillanic acid



	R	*n*
7	$PhCH_2$–	0
8	Ph–	2
9	*p*-tolyl–	2
10	Me–	2

and conformational flexibility of the thiazolidine ring; they differ, however, from benzylpenicillin in terms of biological activity. The poorer binding of the sulfamidopenicillins to the β-lactamase enzyme may be due to the tetrahedral stereochemistry about the sulfamido group although the increased polarity of the sulfamido group over that of the benzamido group may also be a contributing factor. This difference in polarity between the two types of side chains may be the main factor in the differing biological effects observed with *E. coli* which is a gram negative (g−) type bacterium. This type of bacterium has a membrane-like layer outside the cell wall (unlike g+ bacteria) through which the penicillin must be transported. The hydrophobicity of the penicillin side chain can be of importance in this process. The lack of any antibiotic activity of the sulfamidopenicillins on *E. coli* even at the highest concentrations used may be due to lack of transport through the outer membrane. In this respect the sulfamidopenicillins are similar to the sulfonamidopenicillin **7**. This showed essentially no antibiotic activity against *E. coli* but was active against *S. aureus* FDA 209P.[17] In contrast, it has been reported that the sulfonamide sulfones **8**, **9** and **10** were good β-lactamase inhibitors and exhibited a synergistic effect with ampicillin when tested on *E. coli* (g−), *S. aureus* (g+) and *P. mirabilis* (g−).[18] With the sulfamides, however, we found that no synergism was detectable although the sulfamide sulfone **6b** did show some inhibitory activity against a pure sample of a β-lactamase enzyme.

Experimental

Melting points (m.p.s) were determined on an electrothermal melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a JEOL FX90Q (90 MHz) instrument; *J* values are given in Hz; sodium [2,2,3,3-$^2H_4$]3-trimethylsilylpropionate is abbreviated as TSP. IR spectra were recorded on a Perkin-Elmer 577 spectrophotometer, either as a film or, for solids, in a KBr disc. UV spectra and kinetic measurements of the rates of hydrolysis were carried out on a Pye Unicam SP 1700. For kinetic measurements the temperature in the cell compartment was maintained at 30 ± 0.1 °C; the enzymatic hydrolysis measurements were carried out at 20 °C. Thin layer chromatography (TLC) was carried out on Kieselgel GF60 supported on aluminium foil, and column chromatography (flash technique)[19] through Kieselgel S (32–63 μm) with 80% chloroform–ethyl acetate as eluent. Elemental analyses were found to be satisfactory for the ester protected sulfamidopenicillins but the sodium salts generally required the inclusion of $2H_2O$. β-Lactamase type 1 *Bacillus cereus* 569H was obtained from Porton Products and was certified to contain less than 0.1% of the type II β-lactamase; the specific activity for the hydrolysis of benzylpenicillin at pH 7.0, 20 °C was 152 × 10^{-6} mol s^{-1} per mg of protein. All organic solvents were rigorously dried before use and all reactions in organic solvents were

carried out under nitrogen (with the exception of the hydrogenolysis process).

Sulfamate Salts.—Sodium cyclohexylsulfamate was the Aldrich product and was used as obtained. All other sulfamate salts were prepared from the corresponding amines by the method of Audrieth and Sveda.[14] (Scheme 1).

Sulfamoyl chlorides. The sulfamoyl chlorides were prepared from the sulfamate salts by refluxing with phosphorus pentachloride for 2 h in dry benzene. These chlorides were used as isolated from the reaction mixture without further purification; the problems associated with obtaining pure samples of these compounds have been previously reported.[20] The yields for the sulfamoyl chlorides varied from 71–74%.

Diphenylmethyl penicillanate **1a**. This compound, as the toluene-*p*-sulfonate salt, was prepared by the method of Petursson and Waley,[21] m.p. 155–156 °C (lit.,[21] 155–156 °C); for the free amine δ($CDCl_3$; Me_4Si) 1.25 (3 H, s, Me), 1.60 (3 H, s, Me), 4.43 (1 H, d, *J* 4, 6-H), 4.49 (1 H, s, 3-H), 5.45 (1 H, d, *J* 4, 5-H), 6.95 (1 H, s, Ph_2CH) and 7.3 (10 H, s, Ar).

Preparation of Sulfamidopenicillanate Esters **2a**, **3a** *and* **4a**.—The 6-APA ester **1a** (as the toluene-*p*-sulfonate salt) was added to dichloromethane and dissolved on the addition of 1 equiv. of triethylamine. The appropriate sulfamoyl chloride (1.1 equiv.) was added and the reaction mixture stirred at room temp. for 2 h. The solution was washed with water, the organic layer dried (sodium sulfate) and the solvent removed under reduced pressure. The sulfamidopenicillanate esters were all obtained as solids and were purified by recrystallization and/or by flash chromatography as indicated: yields quoted are for analytically pure compounds.

Diphenylmethyl 6-cyclohexylsulfamidopenicillanate **2a**. Recrystallised from isopropyl alcohol (3.98 g, 70%); m.p. 148–151 °C; ν_{max}/cm^{-1} 3310 (NH), 1780 (lactam carbonyl), 1730 (ester carbonyl), 1330 (SO_2 asym) and 1160 (SO_2 sym); δ_H($CDCl_3$; Me_4Si) 1.1–2.1 (16 H, br m, cyclohexyl methylene, α- and β-Me), 3.15–3.35 (1 H, m, cyclohexyl methine), 4.37 (1 H, d, *J* 10, exch. with D_2O, NH, δ_H conc. dependent), 4.55 (1 H, s, 3-H), 5.02 (1 H, dd, *J* 4, 10, 6-H; addn. of D_2O, d, *J* 4), 5.27 (1 H, d, *J* 10, exch. D_2O, NH), 5.61 (1 H, d, *J* 4, 5-H), 6.95 (1 H, s, Ph_2CH) and 7.27 (10 H, s, Ar) (Found: C, 59.4; H, 5.9; N, 7.5. $C_{27}H_{33}N_3O_5S_2$ requires C, 59.66; H, 6.11; N, 7.73%).

Diphenylmethyl 6-phenylsulfamidopenicillanate **3a**. Solid (flash chromatography; 1.22 g, 25%); m.p. 48–51 °C; ν_{max}/cm^{-1} 3245 (NH), 1785 (lactam carbonyl), 1740 (ester carbonyl), 1340 (SO_2, asym) and 1150 (SO_2, sym); δ_H($CDCl_3$; Me_4Si) 1.21 (3 H, s, Me), 1.45 (3 H, s, Me), 4.50 (1 H, s, 3-H), 5.06 (1 H, dd, *J* 4, 10, 6-H, addn. of D_2O, d, *J* 4), 5.37 (1 H, d, *J* 4, 5-H), 5.58 (1 H, d, *J* 10, exch. D_2O, NH), 6.93 (1 H, s, exch. D_2O, NH), 6.96 (1 H, s, Ph_2CH) and 7.20–7.40 (15 H, m, Ar) (Found: C, 60.2; H, 5.0; N, 7.2. $C_{27}H_{27}N_3O_5S_2$ requires C, 60.32; H, 5.1; N, 7.8%).

Diphenylmethyl 6-butylsulfamidopenicillanate **4a**. Solid (flash chromatography; 0.43 g, 26.2%); m.p. 38–40 °C; ν_{max}/cm^{-1} 3300 (NH), 1790 (lactam carbonyl), 1740 (ester carbonyl), 1335 (SO_2 asym) and 1160 (SO_2 sym); δ_H($CDCl_3$; Me_4Si) 0.9 (3 H, t, *J* 7, CH_3CH_2–), 1.15–1.7 (10 H, m, $CH_3CH_2CH_2CH_2NH$–, α- and β-Me), 2.9–3.2 (2 H, q, *J* 4, $CH_3CH_2CH_2CH_2NH$–), 4.52 (1 H, s, 3-H), 4.7–4.85 (1 H, d, *J* 10, exch. D_2O, NH, δ_H conc. dependent), 5.08 (1 H, dd, *J* 4, 10, 6-H; addn. of D_2O, d, *J* 4), 5.42 (1 H, d, *J* 10, exch. D_2O, NH, δ_H conc. dependent), 5.63 (1 H, d, *J* 4, 5-H), 6.95 (1 H, s, Ph_2CH) and 7.27 (10 H, s, Ar) (Found: C, 58.2; H, 6.05; N, 7.8. $C_{25}H_{31}N_3O_5S_2$ requires C, 58.0; H, 5.99; N, 8.1%).

Oxidation of Diphenylmethyl 6-Cyclohexylsulfamidopenicillanate **5a**.—To a chloroform (20 cm^3) solution of diphenylmethyl 6-cyclohexylsulfamidopenicillanate (1.2 g, 3.4 mmol) at 0 °C was added dropwise *m*-chloroperbenzoic acid (0.658 g, 3.81 mmol) in chloroform (10 cm^3). The reaction mixture was allowed to warm to ambient temperature and stirred for a further 2.5 h, then washed successively with aqueous sodium sulfite (1 mol dm^{-3}; 50 cm^3), 5% aqueous sodium hydrogen carbonate (50 cm^3), water (50 cm^3) and finally with saturated sodium chloride (50 cm^3). The organic layer was dried (magnesium sulfate) and the solvent removed under reduced pressure leaving a pale yellow foam (1.21 g). This was purified by flash chromatography (90% chloroform–ethyl acetate) yielding the required sulfoxide **5a** as a white foam (0.67 g, 35.25%) with m.p. 125–127 °C; ν_{max}/cm^{-1} 3300 (NH), 1800 (lactam carbonyl), 1750 (ester carbonyl), 1340 (SO_2, asym) and 1158 (SO_2, sym); δ_H($CDCl_3$; Me_4Si) 0.90–2.10 (16 H, br m, cyclohexyl methylene, α- β-Me), 3.15–3.35 (1 H, m, cyclohexyl methine), 4.50 (1 H, d, *J* 10; exch. D_2O, NH, conc. dependent), 4.76 (1 H, s, 3-H), 5.10 (1 H, dd, *J* 4, 10, 6-H; addn. of D_2O, d, *J* 4), 5.27 (1 H, d, *J* 4, 5-H), 6.17 (1 H, d, *J* 10, exch. D_2O, NH), 7.00 (1 H, s, Ph_2CH) and 7.20–7.40 (10 H, m, ArH) (Found: C, 57.6; H, 5.7; N, 7.6; O, 17.3. $C_{27}H_{33}N_3O_6S_2$ requires C, 57.9; H, 5.9; N, 7.5; O, 17.1%).

Sulfone of Diphenylmethyl 6-Cyclohexylsulfamidopenicillanate **6a**.—This was prepared according to the same procedure as the sulfoxide **5a** except that an excess of *m*-chloroperbenzoic acid (2.59 g, 15.02 mmol) was used and the reaction mixture was stirred at ambient temperature for 36 h. In the purification *via* flash chromatography a small amount of the sulfoxide was eluted ahead of the sulfone product. The sulfone **6a** was obtained as a white foam (0.65 g, 33.2%), m.p. 182–184 °C; ν_{max}/cm^{-1} 3300 (NH), 1800 (lactam carbonyl), 1750 (ester carbonyl), 1340 (SO_2, asym) and 1158 (SO_2, sym); δ_H($CDCl_3$; Me_4Si) 1.00–2.21 (16 H, br m, cyclohexyl methylene, α- and β-Me), 3.15–3.35 (1 H, m, cyclohexyl methine), 4.39 (1 H, d, *J* 10, exch. D_2O, NH, δ_H conc. dependent), 4.59 (1 H, s, 3-H), 4.78 (1 H, d, *J* 4, 5-H), 5.32 (1 H, dd, *J* 4, 10, 6-H; addn. of D_2O, d, *J* 4), 5.94 (1 H, d, *J* 10, exch. D_2O, NH), 6.99 (1 H, s, Ph_2CH) and 7.20–7.50 (10 H, m, Ar) (Found: C, 56.0; H, 5.8; N, 7.1; O, 19.1. $C_{27}H_{33}N_3O_7S_2$ requires C, 56.3; H, 5.7; N, 7.3; O, 19.4%).

Removal of Diphenylmethyl Protecting Group and Sodium Salt Formation.—To a solution of the required diphenylmethyl sulfamidopenicillanate (1.0 mmol) in ethyl acetate (20 cm^3) was added 10% Pd/C (50% H_2O content, 2 g) and this mixture was hydrogenated on a Parr apparatus at 50 psi* for 1 h at ambient temperature. The catalyst was filtered (3 times) through a Schleicher and Schuell No. 8 Glasfaser-Rund filter. The filtrate was washed with 5% sodium hydrogen carbonate (20 cm^3), acidified to pH 2.2 with HCl (1 mol dm^{-3}) and extracted with ethyl acetate (20 cm^3). The free acid of the penicillin was obtained on removal of the ethyl acetate under reduced pressure: cyclohexylsulfamidopenicillanic acid **2c** (0.27 g, 72%); phenylsulfamidopenicillanic acid **3c** (0.32 g, 86%); butylsulfamidopenicillanic acid **4c** (0.23 g, 66%); cyclohexylsulfamidopenicillanic acid 4-sulfoxide **5c** (0.34 g, 86%) and cyclohexylsulfamidopenicillanic acid 4-sulfone **6c** (0.37 g, 97%). The sodium salts were then obtained by extracting an ethyl acetate solution (20 cm^3) of the free acid (0.60 mmol) with aqueous sodium hydrogen carbonate (0.50 mmol in 10 cm^3) (*i.e.* with a deficiency of hydrogen carbonate). The resulting aqueous layer was then freeze dried to yield the salts as white crystalline solids.

Sodium 6-cyclohexylsulfamidopenicillanate **2b**. (0.18 g, 90%); ν_{max}/cm^{-1} 3300 (NH), 1770 (lactam carbonyl), 1610 (carbo-

* 1 psi ≈ 6.89 × 10^3 Pa.

xylate), 1330 (SO_2 asym) and 1160 (SO_2 sym); $\delta_H(D_2O$; TSP) 1.1–2.0 (16 H, br m, cyclohexyl methylene, α-and β-Me), 2.92–3.25 (1 H, m, cyclohexyl methine), 4.18 (1 H, s, 3-H), 5.12 (1 H, d, *J* 4, 6-H) and 5.52 (1 H, d, *J* 4, 5-H); $\delta_C(D_2O$; TSP) 26.82 (3 C, br s, cyclohexyl methylene), 28.98 (α-Me), 32.03 (β-Me), 35.29 (2 C, br s, cyclohexyl methylene), 54.72 (cyclohexyl methine), 62.01 (5-C), 66.50 (2-C), 69.14 (6-C), 75.38 (3-C), 176.16 (lactam carbonyl), 177.20 (carboxylate) (Found: C, 38.7; H, 5.9; N, 9.2. $C_{14}H_{22}N_3O_5S_2Na{\cdot}2H_2O$ requires C, 38.6; H, 5.98; N, 9.6%).

Sodium 6-phenylsulfamidopenicillanate **3b**. (0.18 g, 95%); ν_{max}/cm^{-1} 3200 (NH), 1780 (lactam carbonyl), 1605 (carboxylate), 1335 (SO_2 asym) and 1155 (SO_2 sym); $\delta_H(D_2O$; TSP) 1.45 (3 H, s, α-Me), 1.57 (3 H, s, β-Me), 4.12 (1 H, s, 3-H), 5.15 (1 H, d, *J* 4, 6-H), 5.27 (1 H, d, *J* 4, 5-H) and 7.12–7.64 (5 H, m, Ar) (Found: C, 38.2; H, 4.5; N, 8.4. $C_{14}H_{16}N_3O_5S_2Na.2H_2O$ requires C, 39.1; H, 4.6; N, 9.8%). (Although both IR and NMR spectra indicate a pure compound the elemental analysis could not be improved upon despite repeated attempts).

Sodium 6-butylsulfamidopenicillanate **4b**. (0.14 g, 75%); ν_{max}/cm^{-1} 3280 (NH), 1772 (lactam carbonyl), 1610 (carboxylate), 1325 (SO_2 asym) and 1150 (SO_2 sym); $\delta_H(D_2O$; TSP) 0.9 (3 H, t, *J* 7, CH_3CH_2), 1.15–1.70 (10 H, m, α- and β-Me, CH_3–CH_2CH_2–), 2.91–3.13 (2 H, t, *J* 4, CH_2NH), 4.2 (1 H, s, 3-H), 5.15 (1 H, d, *J* 4, 6-H) and 5.55 (1 H, d, *J* 4, 5-H) (Found: C, 35.7; H, 5.6; N, 9.7. $C_{12}H_{20}N_3O_5S_2Na{\cdot}2H_2O$ requires C, 35.2; H, 5.8; N, 10.2%).

Sodium salt of 6-cyclohexylsulfamidopenicillanate 4-sulfoxide **5b**. (0.19 g, 92.7%); ν_{max}/cm^{-1} 3320 (NH), 1780 (lactam carbonyl), 1615 (carboxylate), 1325 (SO_2 asym) and 1160 (SO_2 sym); $\delta_H(D_2O$; TSP) 1.1–2.17 (16 H, br m, cyclohexyl methylene, α- and β-methyl), 3.05–3.40 (1 H, m, cyclohexyl methine), 4.4 (1 H, s, 3-H) and 5.38 (2 H, s, 5-H and 6-H) (Found: C, 36.9; H, 5.5; N, 9.2. $C_{14}H_{22}N_3O_6S_2Na{\cdot}2H_2O$ requires C, 37.2; H, 5.7; N, 9.3%).

Sodium salt of 6-cyclohexylsulfamidopenicillanate 4-sulfone **6b**. (0.19 g, 89%); ν_{max}/cm^{-1} 3310 (NH), 1792 (lactam carbonyl), 1625 (carboxylate), 1320 (SO_2 asym) and 1155 (SO_2 sym); $\delta_H(D_2O$; TSP) 0.91–2.05 (16 H, br m, cyclohexyl methylene, α- and β-Me), 3.0–3.35 (1 H, m, cyclohexyl methine), 4.32 (1 H, s, 3-H), 5.14 (1 H, d, *J* 4, 6-H) and 5.45 (1 H, d, *J* 4, 5-H) (Found: C, 35.5; H, 5.3; N, 8.6. $C_{14}H_{22}N_3O_7S_2Na{\cdot}2H_2O$ requires C, 35.9; H, 5.5; N, 8.9%).

Biological Testing.—5 mm^3 aliquots (containing from 50–250 μg) of the sulfamidopenicillins were absorbed onto sterilised filter paper disks (0.5 cm diameter) and applied to the surface of an agar plate which had been seeded with isotonic strains of *Escherichia coli* K12* or *Staphylococcus aureus* FDA 209P* as required. The agar plates were incubated at 37 °C for 24 h after which time they were visually inspected for the formation of a halo around the paper disks. Benzylpenicillin was used as the standard reference antibiotic and sulbactam as the standard reference β-lactamase inhibitor.

Kinetics.—Determination of k_{OH^-}. The kinetics of both the alkaline and buffer hydrolysis reactions were studied in aqueous solution by monitoring the change in UV absorbance as a function of time. In the case of the alkaline hydrolysis the reactions were initiated by the addition of 25 mm^3 of a stock solution (buffered to pH 7) of the required penicillin (0.05 mol dm^{-3}) to 2.5 cm^3 of the required NaOH solution (0.1–0.5 mol dm^{-3}) at 30 °C. In the case of the buffer catalysed hydrolysis 100 mm^3 of a stock solution of the required penicillin (0.05 mol dm^{-3}) were added to 2.5 cm^3 of the aqueous hydrogen carbonate buffer (0.01–0.03 mol dm^{-3} carbonate, pH 8.5–9.0). The kinetics were thus measured using 10^{-4} mol dm^{-3} aqueous penicillin solutions. For the alkaline hydrolysis the decrease in absorbance at 270 nm was measured for **3b** and at 235 nm for **2b** and **4b**. In the case of the alkaline hydrolysis the absorbance values were treated in the usual first order manner to yield values of k_{obs} which when plotted against [OH^-] gave $k_{OH^-(\text{side chain monoanion})}$. In the case of the buffer catalysed hydrolysis the initial rate method was used as the rate of hydrolysis was quite slow. Here k_{obs} values were obtained by treating the absorbance values according to eqn. (1),[22] where Δε is the extinction coefficient difference between the penicillin and corresponding penicilloic acid, dA/dT is the slope of the linear plot of absorbance *versus* time and $[Pen]_0$ is the starting concentration of the penicillin. The following extinction coefficient differences (Δε) were obtained for the various penicillins; **2b** 464 (pH 8.5), 446 (pH 8.9) and 422 (pH 9.0); **3b** 320 (pH 8.5), 382 (pH 8.9) and 503 (pH 9.0); **4b** 411 (pH 8.5), 421 (pH 8.9) and 488 (pH 9.0). A value for $k_{OH^-(\text{side chain neutral})}$ was then determined in the usual manner by plotting the k_{obs} values against [buffer] at the three different pH values.

$$k_{obs} = -(dA/dT)/(\Delta\varepsilon[Pen]_0)^{-1} \quad (1)$$

Enzyme Kinetics.—Determination of k_{cat} *and* K_m. The β-lactamase catalysed hydrolysis was studied in aqueous solution, buffered to pH 7, by measuring the change in UV absorbance as a function of time. Reactions were initiated by adding 5 mm^3 of the enzyme solution (0.2 mg cm^{-3}) to 2.5 cm^3 of buffer containing varying concentrations (1×10^{-4}–5×10^{-4} mol dm^{-3}) of the required penicillin at 20 °C. The decrease in absorbance at 265 nm was monitored for **3b** while for **2b** and **4b** the decrease at 235 nm was measured. The slope of the linear decrease of absorbance with time gave initial rates which were then analysed in an Eadie–Hofstee plot[23] to give K_m and V_{max} values. Values of k_{cat} were then obtained from eqn. (2), where $[E]_0$ is the concentration of the enzyme which was estimated from the absorbance of the enzyme solution at 280 nm and taking a value of 2.6×10^4 dm^3 mol^{-1} for the extinction coefficient of *Bacillus cereus* β-lactamase Type I.[24]

$$k_{cat} = V_{max}/[E]_0 \quad (2)$$

β-Lactamase inhibition studies. The hydrolysis of benzylpenicillin (30 mg in 1 cm^3 of D_2O buffered to pD 7.4 in a 5 mm NMR tube) was initiated by the addition of 10 mm^3 of a β-lactamase solution (1 mg cm^{-3} in D_2O) and was monitored by recording the full spectrum at 5 min intervals. The decrease in intensity of the 3-H peak at 4.1 ppm of benzylpenicillin and the increase at 4.3 ppm of the corresponding proton in benzylpenicilloic acid was measured and used to give a reference rate for the hydrolysis. Samples of the β-lactamase enzyme (10 mm^3 of a 1 mg cm^{-3} solution) were then incubated separately with sulbactam, **5b** and **6b** (30 mg in 1 cm^3 of D_2O buffered to pD 7.4 in 5 mm NMR tubes at 20 °C). The incubation times were varied between 0–4 h. Benzylpenicillin (30 mg) was added after incubation and its rate of hydrolysis monitored as described above.

Molecular Modelling.—The calculations were carried out using MNDO as implemented in the AMPAC program.[25] Structures were obtained by full optimisation of all geometric parameters: no symmetry constraints were used. The keyword

* The penicillin sensitive strain of *E. coli* was AB1157, the penicillin resistant strain was AB1157 pUC8 (Genetic Stock Centre, Yale University); the strains of *S. aureus* were obtained from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland: the penicillin sensitive strain was NCIMB 8588 and the penicillin resistant strain was NCIMB 12864.

PRECISE was used in all calculations: this increases by one hundredfold the criteria for terminating optimisations. In the conformational analysis all the geometric parameters were allowed to optimise except the dihedral angle which was preset.

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