Review Paper

Fluorinated penicillins and other β-lactams: chemistry and biological activity

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Abstract

Fluorinated penicillins and other β-lactams are reviewed.

Historical perspective

Looking back over the past 62 years, the development of the chemistry of penicillins, other β-lactam antibiotics, β-lactam inhibitors of β-lactamases, and their medical applications have evolved on a scale undreamt of by the original workers.

The historical observation of the antibacterial properties of a culture of the fungus *Penicillium notatum* in 1929 by Alexander Fleming marks the starting point of penicillin antibiotics and the antibiotic era. By 1940 a research team at Oxford University led by Abraham, Chain and Florey had isolated for the first time penicillin (later named penicillin G) in a reasonably pure form. This team discovered its clinical importance in the treatment of bacterial infections in human patients. Also, in 1940, Abraham and Chain reported that bacteria contain an enzyme capable of destroying penicillin, and called it penicillinase, since the structure of penicillin was not then known. Enzymes of this type are today generically called β-lactamases. This finding marks the discovery that bacteria produce β-lactamases to defend themselves against the action of penicillin. Currently, it is known that bacteria produce β-lactams, and so β-lactamases clearly had the natural role of destroying β-lactam antibiotics. The clinical importance of β-lactamases became clear by 1950, when staphylococcal β-lactamase, threatened the usefulness of penicillin [1a, b].

At the begining of the chemical research on penicillin controversy surrounded β-lactam and thiazolidine rings [1c]. The X-ray crystallographic

*This review is dedicated to Professors Gunther Snatzke and Alois Haas, Ruhr University, Bochum, Germany.

determination of the structure of penicillin in May 1945 by Hodgkin and Rogers-Low established the fused bicyclic β-lactam-thiazolidine nucleus and revealed the relative stereochemistry of the penicillin molecule (**1**) (Fig. 1).

The penam (**2**) and penicillanic acid (**3**) naming system were first proposed by Sheehan *et al.* in 1953 [2] (Fig. 2).

The study of the chemistry of penicillins received a great impetus with the total synthesis of Penicillin V, 6-aminopenicillanic acid (6-APA) (**4**) in 1959 and the development of 6-APA by fermentation in the Beecham Laboratories in 1960. The acylation of 6-APA has led to the explosive development of the semi-synthetic penicillins, a development which continues to the present day.

Another landmark of β-lactamase research was the discovery of clavulanic acid (**5**) an inhibitor of β-lactamase, by workers at Beecham Pharmaceuticals in 1976 [3]. This novel inhibitor is itself a β-lactam. This discovery stimulated the search for synthetic inhibitors which possess the penam nucleus and its analogs. As a result, a number of such compounds have emerged in recent years including 6β-bromopenicillanic acid (**6a**) [4], 6β-iodopenicillanic acid (**6b**) [5], penicillanic acid 1,1-dioxide (**7a**) [6] and 6α-chloropenicillanic acid 1,1-dioxide (**7b**) [7] (Fig. 3).

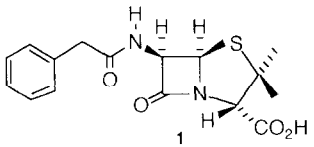

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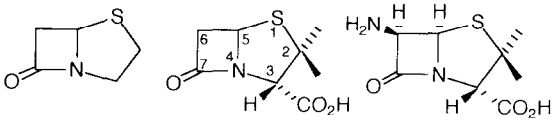
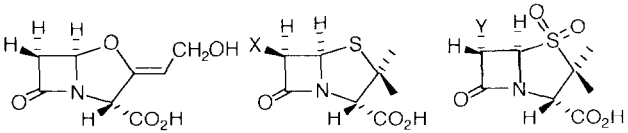
Fig. 1. The structure of benzyl penicillin (penicillin G).



Penam nucleus — Penicillanic acid — 6 β-aminopenicillanic acid

2 3 4

Fig. 2. Nomenclature systems.



Clavulanic acid

5

6 a. X = Br

6 b. X = I

7 a. Y = H

7 b. Y = Cl

Fig. 3. Representative examples of β-lactamase inhibitors: (**5**) clavulanic acid; (**6a**) 6β-bromopenicillanic acid; (**6b**) 6β-iodopenicillanic acid; (**7a**) penicillanic acid sulfone and (**7b**) 6α-chloropenicillanic acid sulfone.

Today a large family of antibiotics exists whose single common structural feature is the possession of a β-lactam ring. The β-lactam antibiotic group includes besides penicillins, cephalosporins, cephamycins, carbapenems and monobactams [8]. The β-lactam antibiotics exert their antibacterial activity because they disrupt bacterial cell wall synthesis by inhibiting one or more penicillin-binding proteins (PBPs) which act as transpeptidases that catalyze the cross-linking reactions of D-alanylpeptides on peptidoglycan strands of the growing cell wall [9]. The β-lactam ring acts as an acylating agent on transpeptidases.

Currently, many different β-lactamases are known, with differing specificity for the various types of β-lactams. Some of them are chromosomally mediated and others are plasmid coded. A great deal has been written about β-lactamases, especially during the last 15 years [10]. The story of β-lactamases showed another important landmark with the determination by Herzberg and Moult of the X-ray crystal structure of β-lactamase from *Staphylococcus aureus* PC 1 at 2.5 Å resolution [11], the Cd^{II} analog of β-lactamase II from *Bacillus cereus* at 3.5 Å resolution by Phillips *et al.* [12] and very recently a high-resolution structure of β-lactamase of *Bacillus licheniformis* 749/C at 2.0 Å by Moews *et al.* [13].

This discussion will now focus on transpeptidases, β-lactamases, β-lactam antibiotics and β-lactam inhibitors.

A β-lactam is a substrate for β-lactamases if acylation is followed by hydrolysis of the ester linkage which regenerates the active enzyme but not the β-lactam. Any compound that slows down or prevents enzyme catalysis from occurring is an enzyme inhibitor. An irreversible inhibitor, also called an inactivator, is one that prevents the return of enzyme activity for an extended period of time. Mechanistic investigation of the interaction of **5**, **6a, b** and **7a, b** with the β-lactamases has shown that they all belong to the class of mechanism-based inhibitors, sometimes referred to as suicide substrates, in the sense that they are recognized by the enzyme as potential substrates yet lead, in a diversion from the normal course of the hydrolytic reaction, to inhibition and/or inactivation of the enzyme [14, 15].

Penicillin G is a typical example of compounds which are good inhibitors of the transpeptidases, but good substrates for β-lactamases. Ceftazidime and cefoxitin, amongst other β-lactam antibiotics, are good inhibitors of the transpeptidases and poor substrates for β-lactamases.

Clavulanic acid, 6β-bromopenicillanic acid and penicillanic acid 1,1-dioxide are very poor inhibitors of the transpeptidases but irreversible 'suicide' inactivators of β-lactamases. What is conspicuous by its absence is a good inhibitor of the transpeptidases which also irreversibly inactivates β-lactamases.

A new approach to the treatment of infection caused by bacteria that produce β-lactamases is the association of a β-lactam antibiotic and a β-lactam inhibitor of β-lactamases. Currently, there are at least three commercial products of this kind on the market in Europe and the United States.

Chemistry of fluorinated penicillins and other β-lactams

The aim of this review is to deal mainly with developments that have taken place recently in the chemistry and biochemistry of fluoropenicillins and other β-lactam compounds.

First we will review the work done by other researchers on the introduction of one or two fluorine atoms at specific sites of the bicyclic nucleus of penicillins, cephalosporins, carbapenam, or the monocyclic β-lactam ring of azetidinones. Secondly, we will describe our own contributions to this field.

Synthesis of C(6)-fluoropenicillins and C(7)-fluorocephalosporins

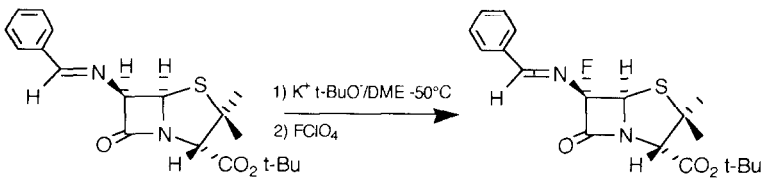
The first report on the introduction of a fluorine atom into cephalosporine in the C-7 position was the work done at the Squibb Institute (USA) in 1973 by Slusarchyk *et al.* [16]. They generated the C-6 carbanion from the benzaldehyde Schiff base (**8**), which was then monofluorinated by the addition of electrophilic fluorine from perchloryl fluoride (Scheme 1).

No data were reported on the stereochemistry of the C(6)-fluoro substituent or the antimicrobial activity.

A somewhat similar approach was used in 1974 to generate the C-6 carbanion of 6-iminochloride penicillin (**10**) [17] (Scheme 2).

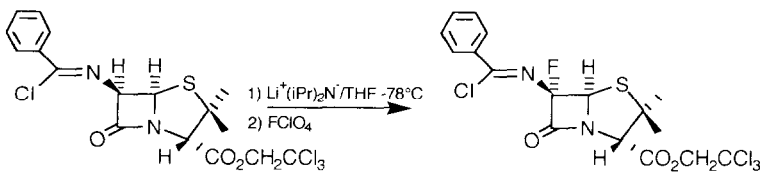
The stereochemistry of the C(6)-fluoro substituent was presumed to be 6α based on analogy with previous alkylation and acylation on the carbanion of the Schiff base of penicillins. No data appeared on the testing of biological activity.

In 1980 and 1983 two patents by the same authors [18] appeared, claiming the synthesis of several 6β-halopenicillanic acid and ester derivatives, including 6β-fluoropenicillanic acid. The value of these patents is difficult to assess. No data are given on yield, characterization, stereochemistry and biological activity of the compounds included in the patent.



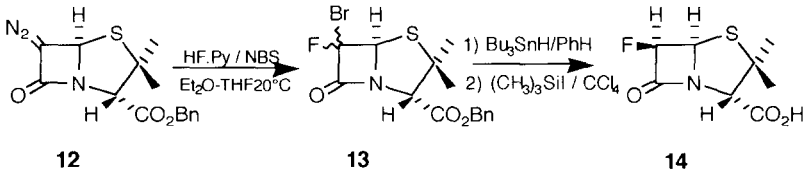
8 **9**

Scheme 1.



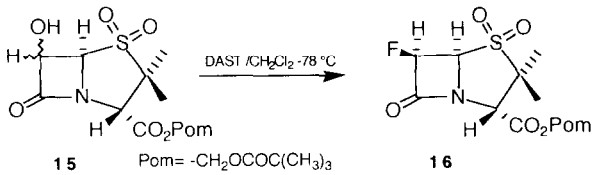
10 **11**

Scheme 2.



12 13 14

Scheme 3.



15 Pom= $-CH_2OCOC(CH_3)_3$ 16

Scheme 4.

The route used for the synthesis of 6β-fluoropenicillanic acid is outlined overleaf (Scheme 3).

Treatment of benzyl diazopenicillanate (**12**) with *N*-bromosuccinimide and pyridinium poly(hydrogen fluoride) as a source of fluoride gave the corresponding geminal 6,6-bromofluoropenicillanate (**13**). The C(6)-bromo function was then converted into the corresponding C(6α)-hydrogen derivative by treatment with tributyltin hydride in benzene. Removal of the benzyl ester was achieved with iodotrimethylsilane in carbon tetrachloride leading to 6β-fluoropenicillanic acid (**14**).

The same authors had also reported a second synthetic approach for the introduction of a fluorine atom at position 6 with β-orientation based on the conversion of hydroxy-to-fluoro groups, with diethylaminosulfur trifluoride (DAST) (Scheme 4).

No assignment of the stereochemistry of the substituents on C-5 and C-6 was given.

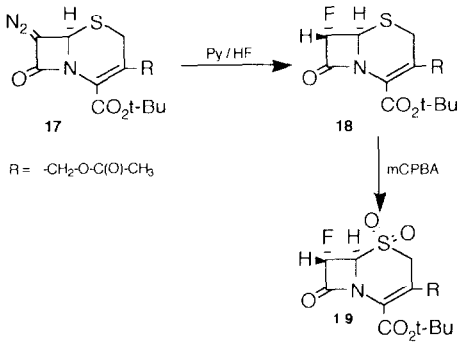
The Merck group has patented [19a] a process for the synthesis of t-butyl-7α-fluorocephalosporinate.

Hydrofluorination of t-butyl-7-diazocephalosporinate (**17**) with pyridinium poly(hydrogen fluoride) followed by oxidation with *m*-chloroperbenzoic acid (*m*CPBA) gave the corresponding 7α-fluorocephalosporinate sulfone (**19**), thus establishing the potential of this route for the introduction of a fluorine atom into the 7α orientation of cepham and the 6α of the penam nucleus (see Scheme 5).

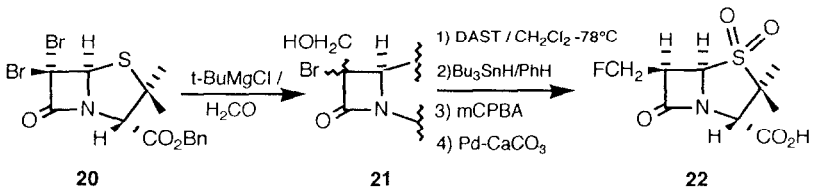
The Merck group found that the sulfones of t-butyl-7α-fluorocephalosporinate and a group of new 7α-substituted cephalosporins are potent elastase inhibitors and, therefore, useful anti-inflamatory/antidegenerative agents [19b].

Synthesis of C(6)-fluoroalkylpenicillins

Scheme 6 illustrates the route which has been followed by the Pfizer group [18] in order to convert benzyl 6,6-dibromopenicillanate (**20**), via



Scheme 5.



Scheme 6.

benzyl 6-bromo-6-hydroxymethylpenicillanate (**21**), to fluoromethylpenicillanic acid sulfone (**22**).

The principal drawback of this route is the lack of data on the stereochemistry of substituents at C-6. No epimers were reported on reactions that generated a new chiral center at C-6. The patent did not report data on biological activity.

The second functionalization of C-6 with a fluoromethyl group was reported in 1989 [20]. Scheme 7 exemplifies the transformation of the $-CH_2OH$ group into a $-CH_2F$ group with DAST.

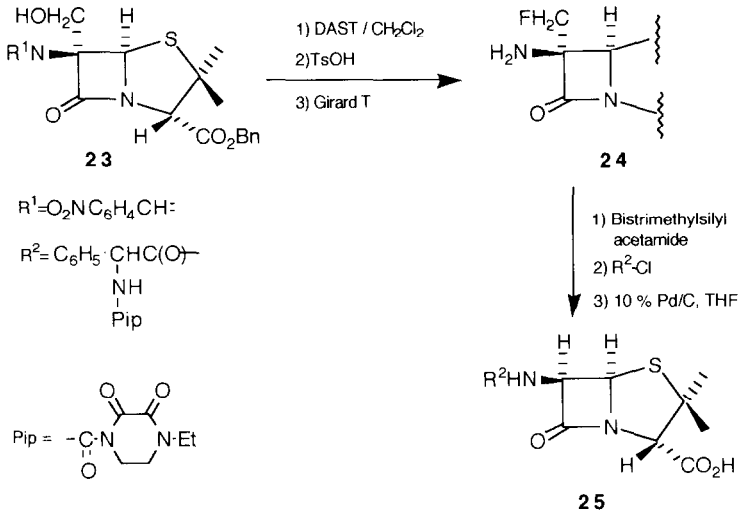
The same workers found that the 6α-formylpenicillanate (**26**) treated with DAST gave a mixture of fluorohydrins (**27**) (Scheme 8).

It was reported that both the 6α-fluoromethylpenicillanic acid (**24**) and the two fluorohydrin penicillanic acid isomers (**27**) showed weak antibacterial activity.

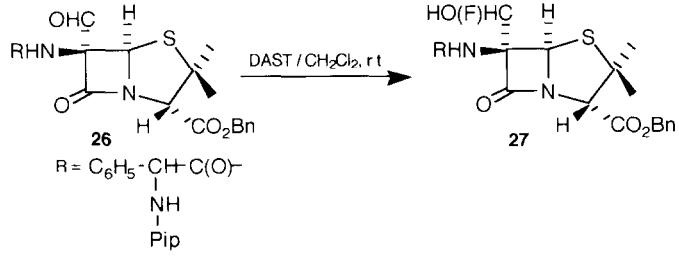
Synthesis of C(2)-fluoromethylpenicillins

The first route to the introduction of a *gem*-difluoro group at the 2β and 2α methyl groups was reported by Baldwin *et al.* [21]. It involves the transformation of the 2β-formyl group with DAST, giving the 2β-difluoromethylpenicillin (**29**) in 90% yield (Scheme 9).

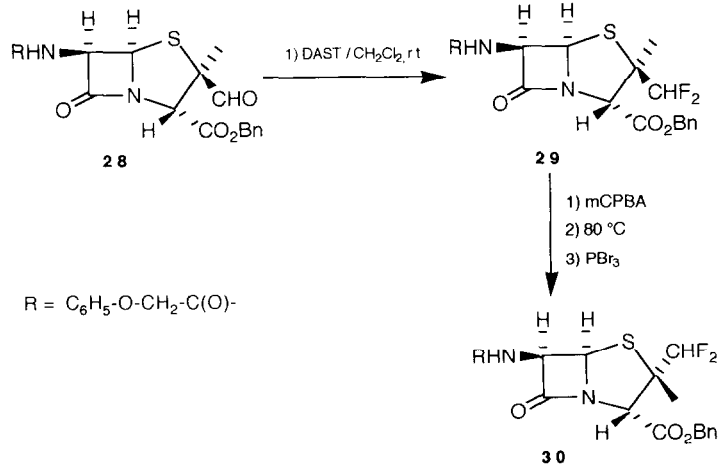
Oxidation of **29** with *m*CPBA, followed by thermal isomerization of the α-sulfoxide ester and reduction of the α-sulfoxide with PBr_3 gave the 2α-difluoromethylpenicillin (**30**).



Scheme 7.



Scheme 8.



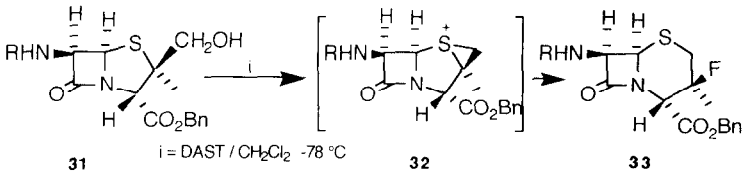
Scheme 9.

An attempt to monofluorinate the 2β-methyl group by treating 2β-hydroxymethylpenicillanate (**31**) with DAST provided the 3β-fluorocepham (**33**) in 63% yield as the only product. An episulfonium intermediate (**32**) was proposed to explain the ring expansion of the thiazolidine to cepham (**33**) (Scheme 10).

All the corresponding free acids of these fluoropenicillins showed lower antibacterial activity than the parent compound, penicillin V, against *Bacillius subtilis* (Gram-positive).

An abstract by Von Daehne and coworkers reports the formation of a mixture of 6β-bromo-2β-fluoromethylpenam (**34b**), 7β-bromo-3β-fluorocepham (**35**) and 7β-bromocepham (**36**), by nucleophilic substitution with fluorine of 6β-bromo-2β-bromomethylpenam (**34a**) (Fig. 4). No details of this reaction were provided [22].

In an extension of the above method, the azetidinones (**37**) (R and R' diverse) or the 2β-bromo- or chloro-methylpenicillanates (**38a** and **38b**) were treated with AgF giving only 3β-fluoro-3α-methylcepham (**39**) in low yield [23] (Scheme 11).



Scheme 10.

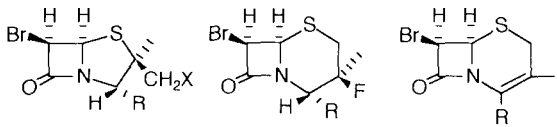
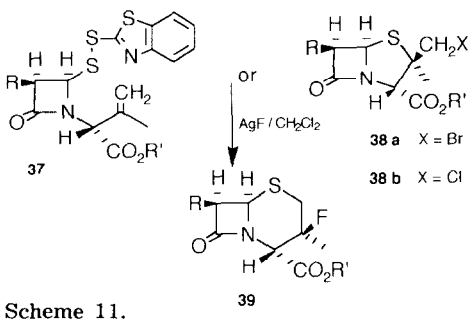


Fig. 4. Structures of 6β-bromo-2β-bromomethylpenam (**34a**), 6β-bromo-2β-fluoromethylpenam (**34b**), 7β-bromo-3β-fluorocepham (**35**) and 7β-bromocepham (**36**).



Scheme 11.

It is evident that the formation of compounds **33, 35** and **39** occurred through an episulfonium intermediate.

The known conversion of hydroxymethyl, $-CH_2OH$, and formyl, $-CHO$, groups into fluoromethyl and difluoromethyl respectively was exploited with the C(3)-cephem nucleus [24]. No details of antibacterial activity were provided (see Fig. 5).

Synthesis of C(6)-fluoroisopropylcarbapenams

Converting, with the use of DAST, a hydroxymethyl group into a fluoromethyl group with inversion of the stereochemistry at this chiral center was exploited in the reaction of the carbapenem derivative (**42**) [25] (Scheme 12).

Synthesis of 1,1-difluorocarbapenems

In 1987 Shah and Cama reported the first synthesis of potassium (5*R*, 6*R*)-1,1-difluoro-2-phenyl-6-(1*R*-hydroxyethyl)carbapen-2-en-3-carboxylate (**45**) [26].

3-(1*R*-allyloxycarbonate-ethyl)-4-(2-phenyl-2-oxo-1,1-difluoro)azetidin-2-one (**44**) was converted to the 1,1-difluorocarbapenem (**45**) using a modification of a well-established glyoxalate route [26] (Scheme 13).

The substitution at the 1-position of a carbapenem with a *gem*-difluoro group was found to give a rather unstable carbapenem. The authors of the

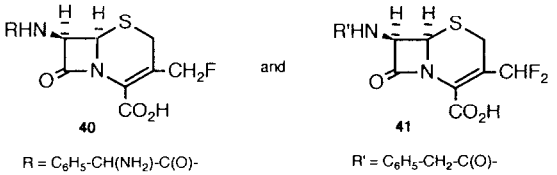
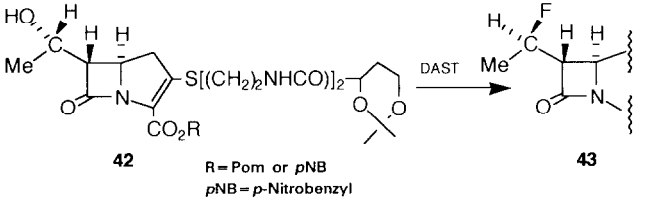
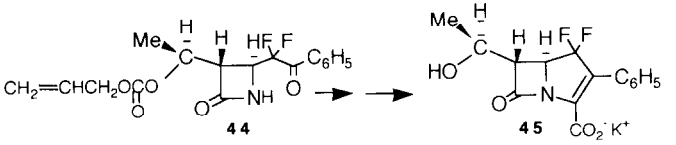


Fig. 5. Structures of 3-fluoromethyl- and difluoromethyl-cephem derivatives.



Scheme 12.



Scheme 13.

synthesis reported that this chemical instability prevented them performing a meaningful *in vitro* assay of the antibiotic activity, although, they mentioned that **45** was active against *Staphylococcus aureus*, *Streptococcus* sp and Enterobacter sp.

Synthesis of 3-(1-fluoroalkyl)-4-alkyl- and -4-sulfur-azetidin-2-ones

Transformation of 3-(1-hydroxyalkyl) into 3-(1-fluoroalkyl) groups with DAST was also applied to monocyclic β-lactams [27]. Reaction of benzyl 3-(1-hydroxyethyl) azetidin-2-one 4-acetate (**46**) with DAST afforded a mixture of products, the expected benzyl 3-[(1-*R*)-fluoro-ethyl)]azetidin-2-one 4-acetate (**47**) was obtained in 20% yield (Scheme 14).

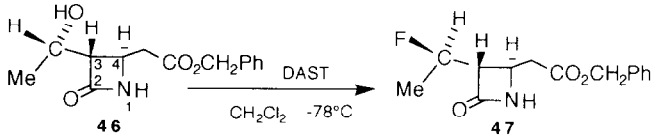
The inversion of the configuration at the chiral center was explained by direct displacement of the leaving group by fluoride (S_N2). Piperidinosulfur trifluoride was almost as effective as DAST in the fluorination reaction. Under identical reaction conditions, benzyl-3-[(1*R*)-hydroxyethyl)]azetidin-2-one 4-acetate did not afford the corresponding fluoroazetidin-2-one.

Synthesis of C(4)-fluoroazetidin-2-ones and C(4)-fluoromethylazetidin-2-ones

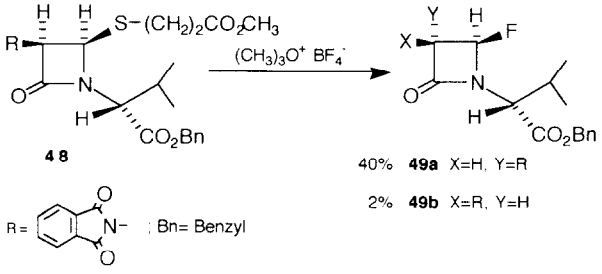
Reaction of secopenicillanate benzyl esters with trimethyloxonium tetrafluoroborate yielded 4-fluoroazetidinone derivatives (**49a**) and (**49b**), presumably via the intermediacy of secopenicillanate sulfonium tetrafluoroborate salts [28] (Scheme 15).

Synthesis of C(4)-fluoromethylazetidin-2-ones

Six 4-fluoromethylazetidin-2-ones were prepared from γ-fluorothreonine [29] (Fig. 6). No chemical data for these synthesis have been published.



Scheme 14.



Scheme 15.

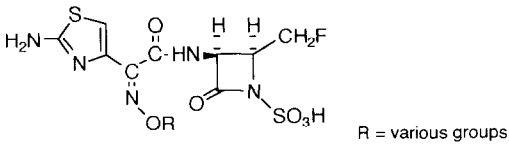
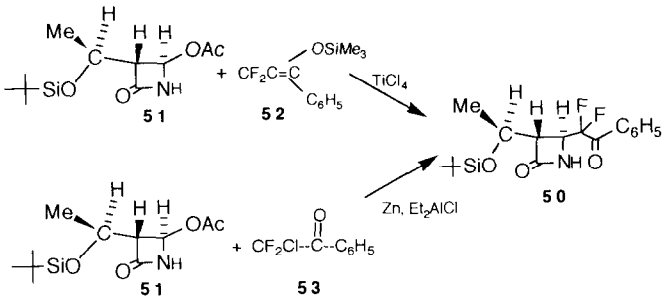
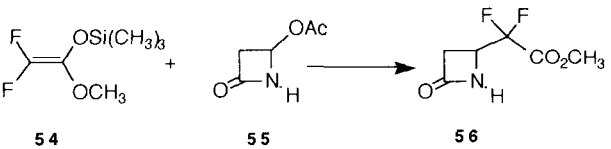


Fig. 6. Basic structure of 4-fluoromethylazetidin-2-ones.



Scheme 16.



Scheme 17.

These novel compounds showed strong antibacterial activity against Gram-negative bacteria including *Pseudomonas aeruginosa* and good stability to various β-lactamases [30].

Synthesis of 4-(1,1-difluoro)azetidin-2-ones

In 1987 Shah and Cama reported the synthesis of (3*R*, 4*R*)-3-(1*R*-t-butyldimethylsilyloxyethyl)-4-(2-phenyl-2-oxo-1,1-difluoro)azetidin-2-one (**50**) by an alkylation reaction [26].

Treatment of (3*R*, 4*R*)-3-(1*R*-t-butyldimethylsilyloxyethyl)-4-acetoxyazetidin-2-one (**51**) with difluoroenol-silyl ether (**52**) in the presence of titanium tetrachloride gave the *gem*-difluoroazeditin-2-one (**50**) in 20% yield. The same transformation was also reported by alkylation of **51** with the aluminum enolate of chlorodifluoroacetophenone (**53**) giving compound **50** in 27% yield (Scheme 16).

This 4-(difluoromethyl acetophenone)azetidin-2-one (**50**) was a key intermediate in the synthesis of the novel 1,1-difluorocarbapenem (**45**).

Introduction of the difluoroacetate moiety into the 4-position of azetidin-2-one was reported by Kobayashi *et al.* in 1988, by the reaction of the ketene silyl acetal (**54**) with 4-acetoxyazetidin-2-one (**55**) to give the methyl(4-difluoroacetate)azetidin-2-one (**56**) in 40% yield [31] (Scheme 17).

Synthesis of 3,3-difluoroazetidin-2-ones

Kobayashi *et al.* reported in 1988 the synthesis of 3,3-difluoro-2-azetidin-2-ones (**60**) via the Reformatsky reaction of iodo- or bromo-difluoroacetates with the *N*-benzylimine of (*R*)-glyceraldehyde acetonide. The reaction of **57** and **59** was also carried out with diverse imines [31].

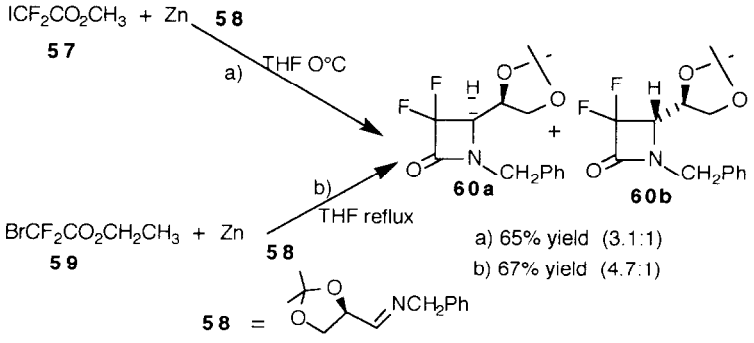
Reaction of the Zn reagent, formed by treating methyl iododifluoroacetate (**57**) with Zn powder and the *N*-benzylimine of (*R*)-glyceraldehyde acetonide (**58**) gave a diastereoisomeric mixture of the difluoroazetidin-2-ones (**60a**) and (**60b**) in 65–67% yield (see Scheme 18). To generate the equivalent reagent from ethyl bromodifluoroacetate (**59**), higher temperatures were needed (Scheme 18).

Recently Baldwin, Lynch and Schofield [32] used compound **60a** as the starting material in their synthesis of difluorohomocysteine (**61**) (see ref. 32 and Scheme 19).

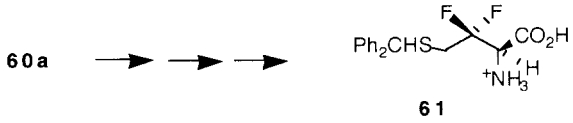
The difluorohomocysteine (**61**) was converted by a well-established procedure to the tripeptide (**62**) and then incubated with *Isopenicillin synthase* (IPNS) giving the thiocarboxylic and carboxylic compounds (**63**) and (**64**), respectively (Scheme 20).

Contributions from our laboratory at IQUIOS [33]

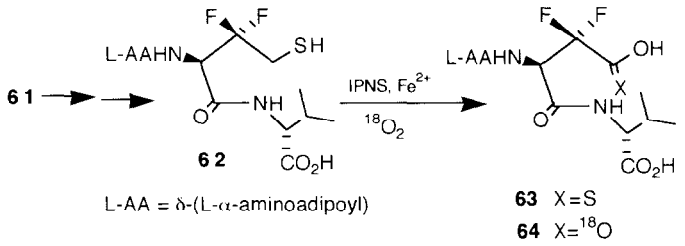
In our own search for β-lactam inhibitors, we have undertaken a broad and detailed study of regio- and stereo-specific ways of introducing a fluorine atom at position 6 of the penam nucleus with either α or β orientation.



Scheme 18.



Scheme 19.



Scheme 20.

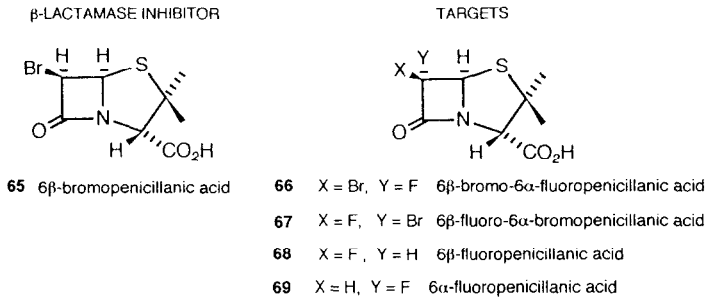


Fig. 7. Structures of 6-fluoropenicillanic acids analogues of 6β-bromopenicillanic acid.

General objective

The continuing problem of the β-lactamase-mediated resistance of clinically important bacteria to β-lactam antibiotics, generated by the β-lactamase propensity for rapid transmission of a plasmid-borne gene throughout the bacterias and the hetereogeneity of β-lactamases, has encouraged us to search for more effective inhibitors of these enzymes.

A compilation of a series of chemical structures together with their activity in eliciting some biological response forms the usual basis of perceiving effects that structural modifications have on biological activity, i.e. structure–activity relationships (SARs).

Synthetic targets

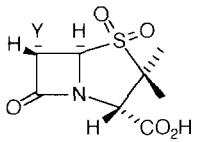
A series of analogues of β-lactamase inhibitors have been selected in which the C-6 hydrogen or bromine atoms were replaced by a fluorine atom (Figs. 7 and 8).

Rationale and outline of our research

Due to the physicochemical and spatial properties of fluorine and hydrogen atoms (electronic configuration, electron affinity, ionisation energy, bond energy and van der Waals'-radius) the bioisosteric replacement of a hydrogen or a bromine atom by a fluorine atom in organic compounds is of considerable interest [34].

Chemo-, regio- and stereo-selective fluorination is an extremely effective tool for modifying the reactivity of biological compounds [33b, 35]. Partic-

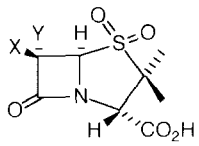
β-LACTAMASE INHIBITORS



70 Y = H; Penicillanic acid 1,1-dioxide (Sulbactam)

71 Y = Cl; 6α-chloropenicillanic acid 1,1-dioxide

TARGETS



72 X = H, Y = F; 6α-fluoropenicillanic acid 1,1-dioxide

73 X = F, Y = H; 6β-fluoropenicillanic acid 1,1-dioxide

74 X = Y = F; 6,6-difluoropenicillanic acid 1,1-dioxide

Fig. 8. Structures of 6-fluoropenicillanic acid sulfone analogues of 6α-chloro- and penicillanic acid 1,1-sulfones.

ularly, the use of selectively fluorinated inhibitors and inactivators of enzymes is of considerable interest at the present time for the study of enzyme inhibitor interactions, and to provide a useful probe for NMR studies [33b, 36]. We expect to use this approach in examining the β-lactamase-6-fluoropenicillin complex in solution. It is noteworthy that these interactions can ideally be characterized by the crystal structure of the inhibitor–enzyme complex, and the X-ray diffraction method remains the most accurate approach to describe the spatial molecular organization [34b].

Our primary interest in introducing a fluorine atom at C-6 of the penicillin molecule was due to the knowledge that replacement of hydrogen atoms by fluorine atoms will not have significant steric consequences at β-lactamase binding sites. However, fluorine with its very high electron affinity, will alter the acidity of geminal C-6 hydrogen and the acylation reactivity of the vicinal β-lactam carbonyl group. Furthermore, the *gem*-difluoro group ($-CF_2-$) has a steric profile similar to that of the methylene group ($-CH_2-$) but has a very different polarity and a drastically altered reactivity.

In order to introduce a fluorine atom selectively into our target molecules, it is important to understand (a) what factors control the regio- and stereospecific introduction of a fluorine atom into the penam nucleus, and (b) how to accomplish these selectivities.

The chemical reactivity of the bicyclic nucleus of penicillin was referred to by the late Professor Robert B. Woodward "as a diabolical concatenation of reactive groups" [37], while, on the other hand, Sheehan has characterized the β-lactam ring of penicillin as "the enchanted ring" [37].

Indeed, we agree with both points of view and through the past five years we have learned, and are still discovering, new aspects of the chemical reactivity of the penicillin molecule.

At this stage we would like to summarise schematically some structural features and the chemical reactivity of the penam nucleus (see Fig. 9).

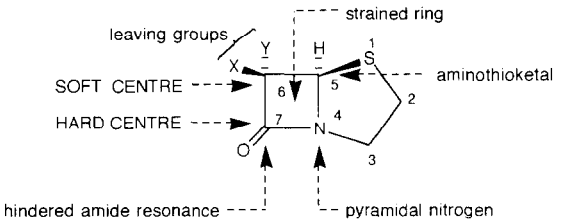
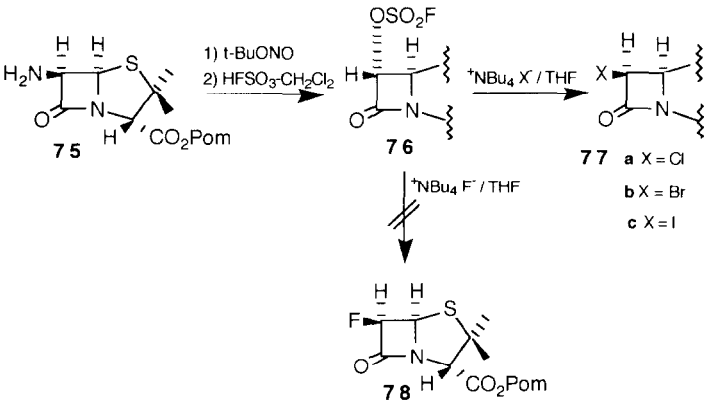


Fig. 9. A summary of some structural features and chemical reactivity of the penam nucleus.



Scheme 21.

Syntheses

We will present here several reactions that can lead to selective fluorination.

Attempts to synthesise Pom 6β-fluoropenicillanate (78) [33a]

Such a synthesis has been attempted on the basis of S_N2 reactions, starting from appropiate precursors of inverse stereochemistry.

We have found that the fluorosulfonyl group can be conveniently and stereospecifically introduced in the 6α orientation by a single-step procedure in a reasonable yield (60%) by treatment of Pom 6β-aminopenicillanate with t-butyl nitrite followed by fluorosulfonic acid in methylene chloride (Scheme 21).

Pom 6α-[(fluorosulfonyl)oxy]penicillanate (**76**) gave the 6β-halogenopenicillanates (**77a–c**) in high yield (better than 90%) upon treatment with 1.0 equiv of tetrabutylammonium halide (X = Cl, Br and I) in THF. However, attempts to convert the 6α-[(fluorosulfonyl)oxy] group into the corresponding 6β-fluoropenicillanate (**78**), employing tetrabutylammonium fluoride [TBAF] [38] under the same conditions, led simply to recovered starting material. The same results were obtained when **76** was treated with TBAF/SiO_2 in THF [39], Amberlyst A-26 (F^-) [40] in refluxing benzene for 15 h or CsF in DME at room temperature. Longer periods of heating or higher temperatures resulted in an intractable mixture of products.

We have attributed the failure of these displacement reactions to the fact that the fluoride anion is harder than iodide, bromide and chloride anions [41] and other soft nucleophiles used successfully [5a, 42] and a rather strong base. Also, the nucleophilicity of the fluoride anion is affected by solvation by water [43].

*Stereospecific synthesis of Pom 6β-bromo-6α-fluoropenicillanate (**80**)*

Electrophilic and simultaneous nucleophilic addition of formal bromine monofluoride, generated *in situ*, yields Pom 6-diazopenicillanate.

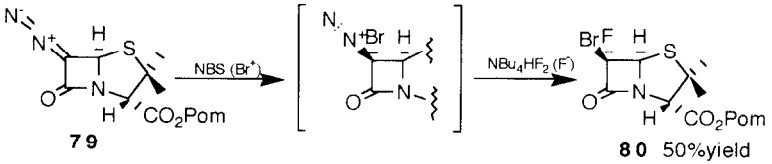
In view of the failure of attempts to prepare Pom 6β-fluoropenicillanate by displacement of a super nucleofuge group placed in the 6α orientation, we concentrated on the synthesis of the 6β-bromo-6α-fluoropenicillanate target, because we envisaged that the 6β-fluoropenicillanate (**78**) would be obtained by reductive removal of the bromine with a tin hydride reagent.

The conversion of 6-diazopenicillanates into 6-bromo-6-fluoropenicillanates using *N*-bromosuccinimide and hydrogen fluoride–pyridine has been reviewed above (see also ref. 18b).

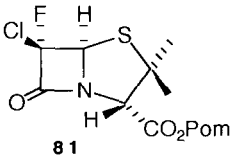
In our hands, the use of this procedure was unsatisfactory when applied to the Pom ester of 6-diazopenicillanate (**79**), giving rise to a complex mixture of products. We also investigated the use of mixtures of NBS and a range of other fluorides including tetrabutylammonium fluoride, the KF-18-crown-6 complex [44] and CsF without success. However, after considerable experimentation, the combination of tetrabutylammonium hydrogen bifluoride (TBABF) [45] and *N*-bromosuccinimide in dichloromethane was found to be more reliable than the procedure described in ref. 18 and gave the desired Pom 6β-bromo-6α-fluoropenicillanate (**80**) in 50% yield (see Scheme 22).

In our continued attempts to increase the yield of **80** we have found that tetrabutylammonium dihydrogen trifluoride (TBADTF) [46] as a source of nucleophilic fluoride, and NBS, or a combination of TBABF with 1,3-dibromo-5,5-dimethylhydantoin, as a source of electrophilic bromine, with dichloromethane as solvent, can be successfully used in the preparation of **80** and the corresponding benzyl ester [33f]. Extention of the procedure to include *N*-chlorosuccinimide was also successful and gave Pom 6β-chloro-6α-fluoropenicillanate (**81**) (see Fig. 10) in 25% yield.

These halofluorination reactions with an electrophilic bromine or chlorine and the nucleophilic anions HF_2^- or $H_2F_3^-$ proceed steroeselectively with



Scheme 22.



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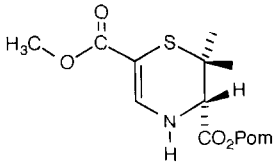


82

Fig. 10. Structure of (pivaloyloxy)methyl 6β-chloro-6α-fluoropenicillanate.

Fig. 11. Structure of (pivaloyloxy)methyl 3,4-dihydro-6-(methoxycarbonyl)-2,2-dimethyl-2*H*-1,4-thiazine-3-carboxylate.

the electrophilic atom being placed in a 6β orientation, and the nucleophilic fluoride atom in a 6α orientation, in agreement with our proposed two-step mechanism for the displacement reaction [47] (see Scheme 22).

It is interesting to consider why the HF_2^- and $H_2F_3^-$ anions displace the diazonium group while the F^- anion does not. In our explanation we consider two factors affecting the ability to transfer fluoride from these anions; one is the effect of solvation on their reactivity. It is well known that anhydrofus or 'naked' fluoride anion does not really exist [38]. The second factor comes from inspection of the reported structure of these anions [48]. Hydrogen difluoride anion is linear with $D_{\infty h}$ symmetry, whereas the dihydrogen trifluoride anion has a bond angle (FHFHF) of 130 ° (139°) and C_{2v} symmetry. It is expected than the hardness of these anions will decrease in the same order as the nucleophilicity and the basicity; $F^- \gg HF_2^- > H_2F_3^-$ [43]. If the soft–soft and hard–hard interactions of the HSAB principle are in operation in our reactions, we would expect the hard base F^- to attack the β-lactam's carbonyl group whereas the softer HF_2^- and $H_2F_3^-$ bases attack the soft C-6 of the penam nucleus.

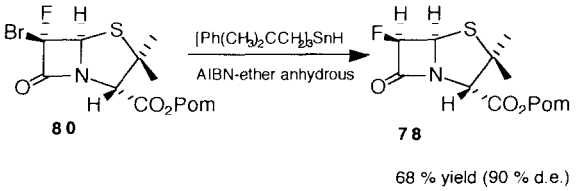
We have already demonstrated [33a] that the hard methoxide anion (CH_3O^-) attacks the carbonyl group of the penam nucleus leading, through a well-established rearrangement process [49], to the 2,3-dihydro-1,4-thiazine (**82**) (Fig. 11).

Stereoselective synthesis of Pom 6β-fluoropenicillanate (78)

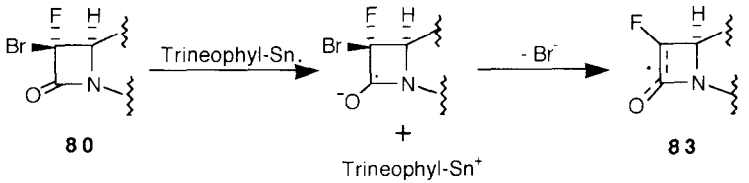
This involves the free-radical chemo- and diastereo-selective reductive dehalogenation by trineophyltin hydride [33d, e].

With the compounds **80** and **81** in hand, we have investigated their very highly chemo- and diastereo-selective reduction with tris(2-methyl 2-phenylpropyl)tin hydride to obtain the Pom 6β-fluoropenicillanate (**78**) in 68% isolated yield (Scheme 23).

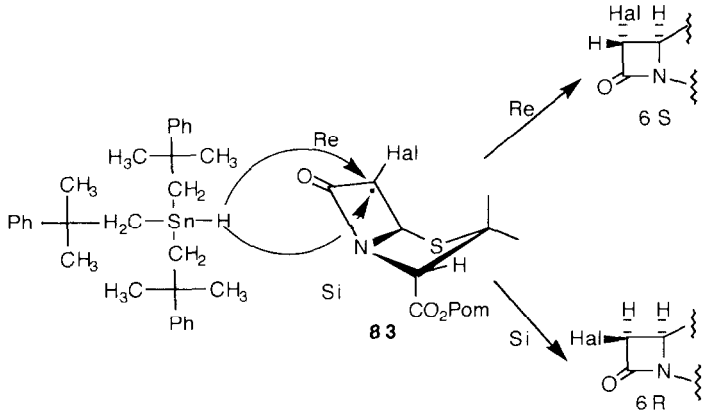
We explain the high degree of chemoselectivity according to the electron transfer-hydrogen atom-abstraction mechanism (SET) of Tanner and Singh [50], and have suggested [51] that an electron is transferred from the trineophyltin radical (Scheme 24) to the carbonyl group of the β-lactam to form a β-lactamoyl radical. In the following step the more nucleofugal of the geminal heterodihalides departs as a halide anion. It is well known that



Scheme 23.



Scheme 24.



Hal	F	Cl	Br	I
6*R*:6*S* (%)	95:5	95:5	100	100

Scheme 25.

the relative order of nucleofugality is $I^- > Br^- > Cl^- \gg F^-$ [52]. Furthermore, the departure of the bulkier halogen atom from the β face of penam nucleus, i.e. the 6β-bromo atom in compound **80**, would be sterically assisted, since it is associated with a decrease in steric crowding. Qualitatively, we have found [51] that the chemoselectivity of the process for dissimilar 6,6-dihalogenopenicillanates indicates that iodide is eliminated faster than the bromide and that, in turn, the bromide departs faster than the chloride.

To explain the high degree of diastereoselectivity we assume that the conformation in solution of the intermediate radical **83** is that depicted in Scheme 25, which implies that the β-lactam ring is planar or very nearly

so, and the conformation of the thiazolidine ring has both the 2β and 3α substituents pseudo axial with respect to the five-membered ring [53].

The better diastereoselectivity achieved with trineophyltin hydride compared with tributyltin hydride reductions, indicates a better diastereofacial discrimination of the approaching trineophyltin hydride to donate a hydrogen atom (H·) to the unencumbered diastereotopic Si face of the C-6 intermediate radical **83** [33d].

The nature of the C-6 halogen substituents also influence the degree to which Si face attack is favoured, leading to an increased amount of the 6*R* (β) product; thus, the 6*R*:6*S* diastereoselectivity increases in the case of the radical **83** from 95:5 for Hal=F or Cl to 100% for Hal=Br or I (Scheme 25). The increase seems to be caused by the steric effects of the halogen atom. A similar influence of α substituents on the stereoselectivity has been reported for the addition of alkyl mercury hydride to planar alkyl maleic anhydride [54].

*Synthesis of Pom 6β-fluoropenicillinate sulfone (**84**) [33e]*

The 6β-fluoropenicillanate (**59**) was converted by a phase-transfer catalytic oxidation using potassium permanganate [55] into the Pom 6β-fluoropenicillanate sulfone (**84**) (Fig. 12).

*First steroespecific synthesis of Pom 6α-fluoropenicillanate (**85**) and its sulfone derivative **86** [30c]*

This involves the hydrofluorination of a 6-diazopenicillanate achieved via the introduction of one fluorine atom with 6α orientation and a hydrogen atom with 6β orientation (Scheme 26). A similar procedure was previously reported for the hydrofluorination of 7-diazocephalosporinate [19].

Treatment of Pom 6-diazopenicillanate (**79**) with pyridinium poly(hydrogen fluoride) [56] (30% pyridine–70% hydrogen fluoride) in chloroform gave, after chromatographic separation, the fluorinated penicillanate **85** in 15% yield (Scheme 26).

Several attempts were made to improve the low yield of this reaction. Use of anhydrous hydrogen fluoride in ether at 0 °C gave, after 30 min, only a 10% yield of the desired fluoride **85**. Similarly unsuccessful was the reaction of Pom-6β-aminopenicillanate (**75**) with pyridinium poly(hydrogen fluoride)–tetrabutylammonium nitrite in ether at 0 °C which gave only 7% yield of **85**.

Oxidation of **85** under similar conditions to those used for 6β-fluoropenicillanate (**78**) gave in 80% yield Pom 6α-fluoropenicillanate sulfone (**86**).

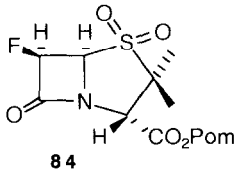


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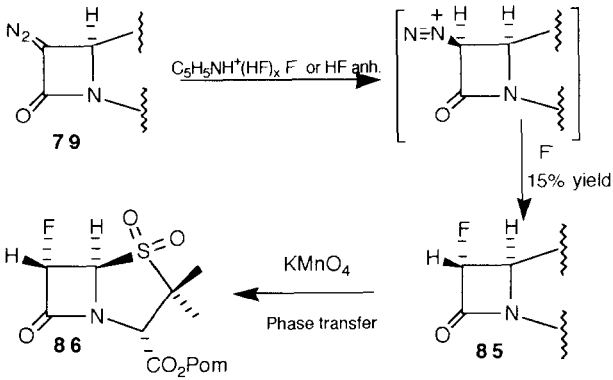
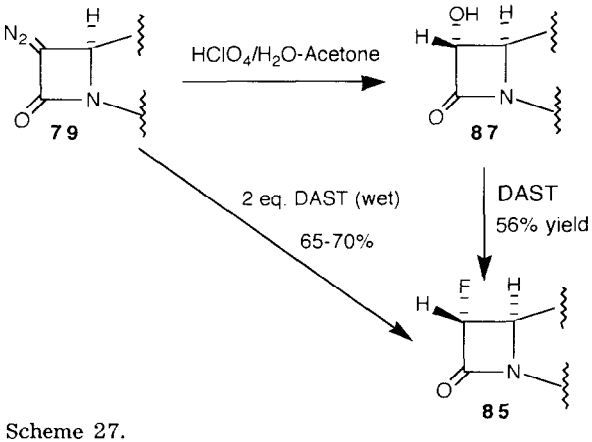
Fig. 12. Structure of (pivaloyloxy)methyl 6β-fluoropenicillanate sulfone.



Scheme 26.



Scheme 27.

Second stereospecific synthesis of Pom 6α-fluoropenicillanate (85)

This involved conversion of a 6α-hydroxy oriented group into a 6α-fluoro group with DAST.

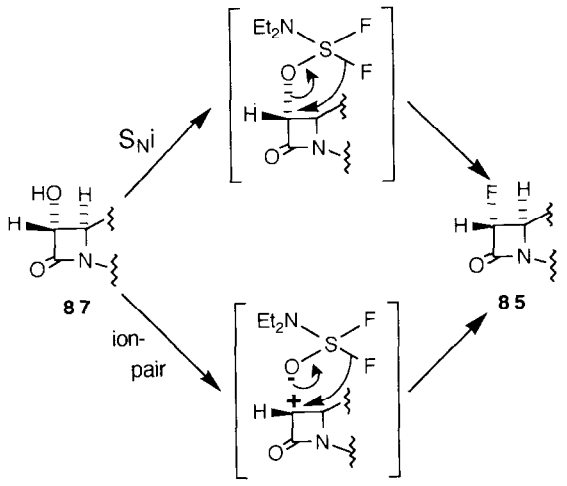
To synthesize the Pom 6α-hydroxypenicillanate (**87**), we followed the method described by Sheehan *et al.* [57].

Treatment of **87** with DAST gave **85** exclusively in 56% yield. Furthermore, reaction of wet Pom 6-diazopenicillanate (**79**) with 2 equiv of DAST gave **85** in 65% yield. In this one-pot reaction, we assume that **87** is generated *in situ* and then reacts with DAST (Scheme 27).

The stereochemistry (retention of configuration) can be explained by a S_Ni (intramolecular) mechanism or alternatively by formation of an intimate ion pair (Scheme 28).

Attempts to transform a 6β-hydroxy group into a 6β-fluoro group

As an extention of the procedure for transforming **87** into **85**, we examined the reaction of DAST with Pom 6β-hydroxypenicillanate [5a].



Scheme 28.

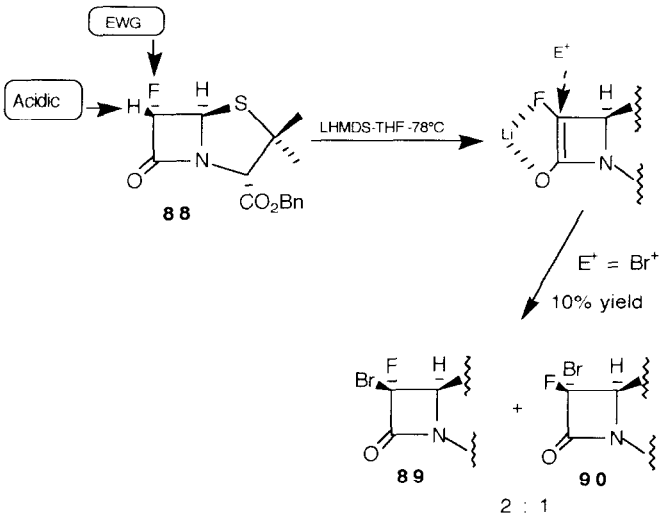
However, all attempts at this nucleophilic substitution were unsuccessful, with intractable mixtures of products being obtained. Similarly unsuccessful was the attempted displacement of the triflate group of Pom 6α-[(trifluoromethyl)sulfonyl]penicillanate with tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) [58]. Pom 6α-[(trifluoromethyl)sulfonyl]penicillanate was prepared in 70% yield by reacting 6-diazopenicillanate (**79**) with *N*-fluoropyridinium triflate [59]. The introduction of the 6α-[(trifluoromethyl)sulfonyl)] group was previously reported by reaction of trifluoroethyl sulfonyl chloride with a 6α-hydroxypenicillanate [5a].

Synthesis of benzyl 6α-bromo 6β-fluoropenicillanate (73)

Regiospecific generation of β-lactam [60] and penicillanate [61] metal enolates is a relatively well-documented process. Enolates derived from 6α-bromo- or 6α-iodo-penicillanates and 6,6-dibromopenicillanates have been generated *in situ* by a metal–halogen exchange process at -78 °C using either n-butyllithium or methyl magnesium bromide [61a]. These β-lactam and penicillanate metal enolates reacted with a variety of electrophiles, e.g. aldehydes, ketones, esters and reactive alkyl halides. However, to our knowledge, reaction with electrophilic halogen atoms has not been reported in the literature.

We describe here our preliminary results on the addition of electrophilic bromine to penicillanate metal enolates. Reaction of benzyl 6α-fluoropenicillanate (**88**) with lithium hexamethyldisilazide (LHMDS) in THF at -78 °C followed by condensation of the lithium enolate formed with electrophilic bromine from *N*-bromosuccinimide gave a mixture of the diastereoisomeric pair **89** and **90** in 10% yield in a 2:1 ratio [62] (Scheme 29).

Predominant addition to the hindered β-face of a penicillin molecule (with tetrahydrofuran as solvent) is well precedented in aldol condensations [61a, b].



Scheme 29.

The detailed mechanistic and conformational aspects of the 'wrong' stereoselectivity of addition of electrophilic bromine to a 6-fluoropenicillanate lithium enolate or carbanion are not clear to us at this moment.

Attempts to synthesise 6,6-difluoropenicillanic acid

We have made several attempts to introduce two fluorine atoms in the 6-position of penam system without success. An early attempt was made to introduce a geminal difluoro group by reaction of Pom 6-diazopenicillanate (**79**) [63] with diluted molecular fluorine (1% F_2 in N_2) under the conditions described by Rozen and Brand [64]. At −78 °C no reaction took place, while at higher temperature an intractable mixture of products was obtained. Similarly unsuccessful were the reaction of Pom 6-oxopenicillanate with DAST [65] and the attempted halogen exchange of 6,6-diiodopenicillanate with silver tetrafluoride [AgF_4] under the conditions reported by Bloodworth, Bowyer and Mitchell for conversion of *gem*-diiodides and dichlorides into the corresponding *gem*-difluoride [66].

New synthetic approaches to this target compound are currently underway in our laboratory.

Geminal versus vicinal halofluorination reactions

6-Diazopenicillanates have been found to be useful intermediates for the introduction of many different substituents at C-6 [67]. Reactions with halogens and interhalogens have provided routes to *gem*-6,6-dihalogenopenicillanates. In particular, one of the most fundamental reactions that we have used in fluoropenicillins concerns the halofluorination of 6-diazopenicillanates [33e, f].

These reactions proceed stereoselectively with the electrophilic halogen being placed in a β orientation and the nucleophilic halide atom in an α orientation, in agreement with the proposed two-step mechanism for the displacement reactions [47].

It is interesting to note that the *gem*-halofluorination of 6-diazopenicillanates parallels the *gem*-halofluorination of diazo ketones and the *vic*-halofluorination of alkenes.

The halofluorination of alkenes occurs stereospecifically to afford the *anti*-addition products, and with unsymmetrical alkenes a marked Markovnikow-type regioselectivity is observed.

Both the *gem*- and *vic*-halofluorination, via electrophilic and nucleophilic substitution reactions, are usually carried out with halonium fluoride (XF; X = Cl, Br, I) [68] or with a combined reagent system consisting of a fluoride ion source (e.g. $Bu_4N^+ HF_2^-$, [45b] $Bu_4N^+ H_2F_3^-$ [69], $C_6H_5NH^+ (HF)_x F^-$ [56], $NH_4^+ HF_2^- - AlF_3$ [70], SiF_4 [71]) and a halonium source (e.g. *N*-halosuccinimides, *N*-haloacetamides or 1,3-dibromo-5,5-dimethylhydantoin [71, 72]).

Use of pivaloyloxymethyl (Pom) esters

The use of double esters as pro-drugs in penicillins and cephalosporins is well documented [73]. The pivaloyloxymethyl group was advantageously introduced in 98% isolated yield, by treatment of amino-unprotected 6-APA with 1,8-diazabicyclo[5, 4, 0]undec-7-ene (DBU) and chloromethyl pivalate [33c]. However, it is well known that the application of conventional methods of ester cleavage to ester deprotection of penicillins presents problems [74]. We have found that replacement of the carboxy group in 6β-bromopenicillanic acid by a 3α-hydroxymethyl, fluoromethyl or cyano group results in either a drastic decrease or a complete loss of the β-lactamase inhibitory activity as compared with the parent compounds. Therefore, the presence of the carboxylic acid function in the 6-fluorinated penicillins appears to be an essential requirement for their β-lactamase inhibitory properties. The C-3 carboxylate of penicillins has been postulated to act as an electrostatic anchor with lysine-234 in the β-lactamase catalysis [11]. This interaction has been probed by site-directed mutagenesis [75].

We have recently developed a simple and effective method for the removal of the Pom ester of penicillins by the action of bis(tributyltin) oxide in non-hydroxylic solvents [76]. However, attempts to effect the cleavage of Pom 6β-bromo-6α-fluoro- and 6α-fluoro-penicillanates with this reagent brought about destruction of the β-lactam ring. This is probably due to the fact that a fluorodestannylation reaction occurs [77].

We have found a solution to this problem by preparing the benzyl esters of 6α-fluoro- and 6β-fluoro-penicillanate. Benzyl esters were deprotected by using the hydrogenolytic conditions previously reported for other benzyl penicillanates [21]. Hydrogenolytic conditions were inadequate for the cleavage of benzyl 6β-bromo- 6α-fluoro- and 6α-bromo-6β-fluoropenicillanates, since

in this series catalytic hydrogenolysis of the carbon-6-bromine bond occurred [78].

For these compounds hydrolysis with pig liver esterase (PLE) afforded the desired 6β-bromo-6α-fluoropenicillanic acid. The use of PLE with monofluorinated esters [79] an other esters [80] is well documented.

Biological activity

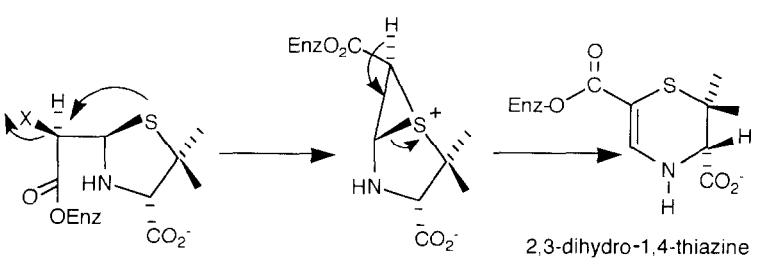
Knowledge of the mechanism of inactivation of β-lactamase by 6β-bromopenicillanic acid and penicillanic acid sulfone should permit better understanding of the relative effectiveness of the 6-fluoropenicillanic acids as β-lactamase inhibitors and perhaps lead to application with human leukocyte elastase.

The mechanism of the inactivation of *Bacillus cereus* β-lactamase I by 6β-bromopenicillanic acid (**65**) was studied by Pratt, Waley and coworkers [4b, d, 14b, c]. Acylation of the β-lactamase by 6β-bromopenicillanic acid is accompanied by rearrangement and cyclization of the inhibitor to a 2,3-dihydro-1,4-thiazine-3,6-dicarboxylic acid derivative. The acylated aminoacid residue was demonstrated to be Ser-70.

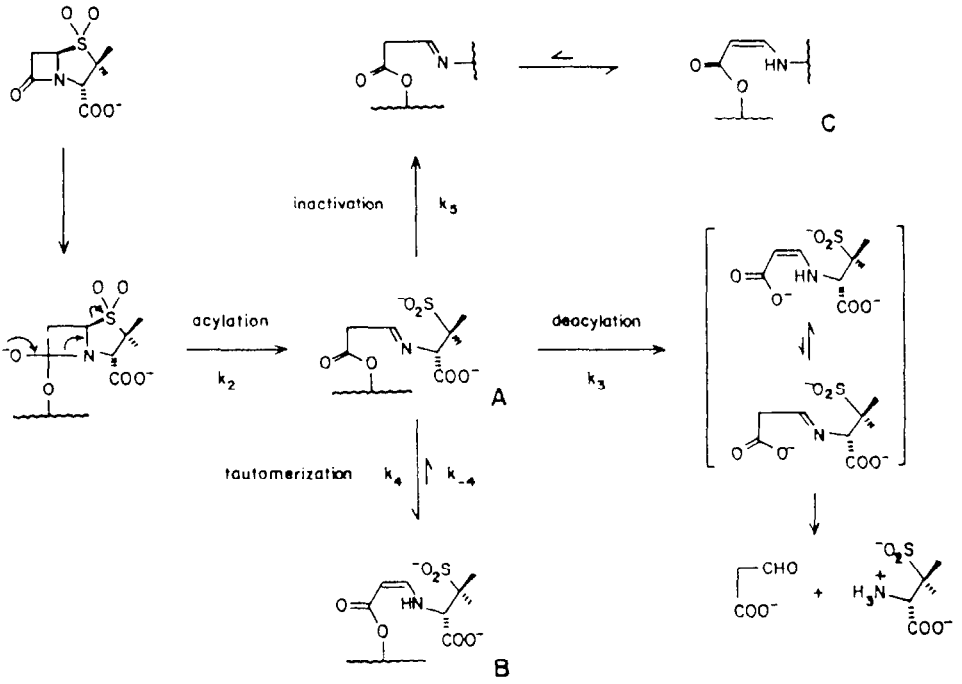
Loss of tritium from the [^{3}H]-6β-bromopenicillanic acid occurs rapidly on interaction of the latter with the enzyme [14c]. Knowles and coworkers have suggested [81] that an enzymatic base is present, which stereospecifically assists proton removal from the 6α orientation and that this is the driving force for a *trans* elimination across the C-5 and C-6 position.

The kinetics and mechanism of the rearrangement of 6-halopenicillanates to 2,3-dihydro-1,4-thiazines have been studied recently by Pratt and Cahn [49]. They conclude that the most likely mechanism involves direct intramolecular nucleophilic attack by the thiazolidine sulfur atom on the C-6 β-bromo substituent, yielding a bicyclic episulfonium ion intermediate, which then collapses to the dihydrothiazine (Scheme 30). Pratt and Cahn also presented an excellent discussion between this mechanism and several other mechanistic possibilities.

With penicillanic acid sulfone (**70**), the mechanism is slightly different. The detailed mechanisms of the interaction of **70** have been elucidated by Knowles and coworkers [15] using the RTEM β-lactamase. The pathways that account for this interaction are illustrated by the sequence shown in Scheme 31.



Scheme 30.



Scheme 31.

Following the formation of an acyl–enzyme intermediate (**A**) between the carbonyl group of the β-lactam and the hydroxy group of the active-site serine residue, three separate sequences can occur. First, the penicillanic acid sulfone acts as a substrate for the enzyme and the β-lactam ring is hydrolytically cleaved. Second, the enzyme is trapped as a transiently inhibited form (**B**). Third, the enzyme is irreversible inactivated. The acyl–enzyme intermediate **A** is converted into a β-imino ester by an elimination reaction, this event is followed by a transimination reaction by an enzyme lysine residue. Such a reaction would lead to a β-amino-acrylate crosslinked to two active site residues of an inactivated enzyme. The β-amino-acrylate fragment (**C**) is derived from C-5, C-6 and C-7 of the original β-lactam; 7000 molecules of penicillanic acid sulfone are hydrolyzed for each molecule of inactivated enzyme formed (turnover).

The 6-fluoropenicillins synthesized were tested against β-lactamase I of *Bacillus cereus* (strain 569/H)*.

In preliminary experiments, β-lactamase activity was instantaneously inhibited by the addition of 6β-fluoropenicillanic acid sulfone, 6β-fluoropenicillanic acid or 6α-fluoropenicillanic acid sulfone (Table 1). The instantaneous effect was followed by a progressive increase in the inhibition.

*We thank Dr O. A. Roveri (Rosario University) for carrying out these essays. Details on these will be presented elsewhere.

TABLE 1

Inhibitory activity of 6-fluoropenicillins against β-lactamase I of *Bacillus cereus*

β-Lactam compound	I_{50} value (10^{-3} M)	
	Without pre-incubation	With 10 min pre-incubation
6β-Bromopenicillanic acid	1.0	0.5[a]
6β-Bromo-6α-fluoropenicillanic acid	>5.0	>5.0
6α-fluoropenicillanic acid	>5.0	>5.0
6β-fluoropenicillanic acid	>2.5	0.4
Penicillanic acid sulfone	0.025	0.025
6β-fluoropenicillanic acid sulfone	1.0	0.25
6α-fluoropenicillanic acid sulfone	4.0	2.25

[a]100% inhibition.

β-Lactamase I was purified as described by Davies *et al.* [82] from *Bacillus cereus* 569/H. The enzymatic activity was measured spectrophotometrically at 482 nm in 100 mM sodium phosphate (pH 7.0) at 30 °C using 0.1 mM nitrocefin [83] as the substrate and from 0.6 to 1.2 μg β-lactamase. The I_{50} values reported were determined by addition of different concentrations of the β-lactam compound either after or 10 min before the addition of substrate. In the latter case, 100% of the activity was determined by incubation of the enzyme during 10 min in the absence of inhibitor.

Moreover, when the enzyme was preincubated with the inhibitors for 10 min before adding the substrate, the hydrolysis of nitrocefin was more strongly inhibited. On the other hand, the addition of 6α-fluoropenicillanic acid and of 6β-bromo-6α-fluoropenicillanic acid produced only a slight inhibition of the enzyme activity which did not increase with time (see Table 1).

Incubation of the β-lactam compounds under study with more enzyme was accompanied (except for 6β-fluoropenicillanic acid) by hydrolysis of the β-lactam ring, indicating that these compounds are also substrates of the enzyme. Therefore, on the basis of the inhibitory action and/or their ability to behave as enzyme substrate, it can be concluded that all the compounds under study not only bind to the enzyme but can also acylate it. Hence, they fulfill one of the conditions postulated by Knowles *et al.* for a β-lactamase inhibitor [81].

The lack of inhibitory activity observed with 6β-bromo-6α-fluoropenicillanic acid and 6α-fluoropenicillanic acid can be related to the fact that both compounds lack the 6α-hydrogen that, according to the same authors, is another requirement for inhibitory action. Thus, the β orientation of the fluorine atom and the 6α orientation of a hydrogen atom or other atom or group easily eliminated as cation, e.g. chloronium (Cl^+), must play a crucial role in the interaction with β-lactamase I. Work is in progress to ascertain the nature of this inhibition.

Examining the results listed in Table 1 obtained after 10 min pre-incubation, it is not clear to us why the 6β-fluoropenicillanic acid sulfone is 10-times less potent than penicillanic acid sulfone.

Based on the stereospecifically labelled 6α[^{2}H], 6β[H] and 6α[H], 6β[^{2}H]-penicillanic acid sulfone, Brenner and Knowles [15b] had proposed that the 6β-hydrogen is preferentially abstracted in the formation of the transiently stable intermediate. Based on our results with 6β-fluoro- and 6α-fluoro-penicillanic acid sulfone, it appears that this possibility has to be ruled out.

It seems to us that other mechanistic possibilities for the proposed β-elimination and transimination reactions are also possible. As a working hypothesis we consider that β-elimination (*trans* mode) across the 6α-hydrogen or chlorine in the case of 6α-chloropenicillanic acid sulfone and the C-5 linked sufone group will provide an acyl–enzyme–enamine intermediate directly, which then can undergo a Michael-type addition leading to the suggested β-amino-acrylate structure in the inactive protein.

The β-lactam inhibitors of Human Leukocyte Elastase (HLE) have added a new landmark to the story of β-lactam. The reported mechanism of action of elastase inhibitors and the structure–activity relationship of a group of 7α-substituted cephalosporinate sulfones including t-butyl-7α-chloro- and by extension 7α-fluoro-cephalosporinate sulfone [19] and our results with 6β-fluoro- and 6α-fluoro-penicillanic acid sulfone, clarify the operational similarities and differences between β-lactam elastase inhibitors and β-lactam-β-lactamase inhibitors. Adequately functionalised C-3, 6α-chloro- and 6α-fluoro-penicillanate sulfones remain to be tested as elastase inhibitors.

On the basis of our results [33f], the proposed β-lactamase inactivation general mechanism [82], crystallographic studies [11, 13] and site-directed mutagenesis studies [76], we postulate that the following structural features seem to be required for a penicillin compound to act as an effective β-lactamase inhibitor: (i) the C-3 carboxy group for the initial recognition of penicillins by β-lactamases: (ii) an appropiate β-lactam for acyl–enzyme formation; and (iii) an atom, such as hydrogen or chlorine, in the 6α orientation able to leave as a cation in a β-*anti*-elimination reaction.

Summary and outlook

We have developed a stereoselective synthesis of Pom 6β-bromo-6α-fluoro- and 6β-chloro-6α-fluoro-penicillanates (**80**) and (**81**) from the readily available Pom 6-diazopenicillanate (**79**). Compound **80** was a very convenient substrate for chemo- and diastereo-selective reductive dehalogenation with the hindered trineophyltin hydride to afford the expected Pom 6β-fluoro-penicillanate (**78**) in good yield. On the other hand, reaction of wet diazo (**79**) with DAST produced the 6α-fluoro isomer (**85**) exclusively in good yield, possibly via the ion-pair mechanism indicated in Scheme 28.

Our choice of methods for the introduction of a fluorine atom into chiral penicillins was made in order to obtain high stereoselectivity and versatility for exploiting the special shape of the penam nucleus for stereocontrol on the formation of a new chiral center at carbon-6. Regiospecific and stereoselective labelling of 6-fluoropenicillins with deuterium or tritium at C-6β

and C-6α is also possible [33e, 78a, b]. The unsatisfactory results for the introduction of the *gem*-difluoro group into penicillins will eventually be improved and that the gaps will be filled.

Further studies for rationalizing the β-lactamase and Human Leukocyte Elastase inhibitory activities of existing compounds and predicting the activity of new compounds are required.

Finally, we conclude this review looking back over 51 years (1991–1940). It seems to us that the battle between bacteria and man is endless. However, we are now approaching a complete understanding of the structure–activity relationships in the β-lactamase inhibitors, an issue of outstanding importance. It is hoped that progressive application of the knowledge of enzyme mechanism and enzyme interactions with inhibitors will render new useful synthetic drugs.

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References

1 (a) J. H. C. Nayler, *Trends Biochem. Sci., 16* (1991) 195, 234; (b) S. M. Roberts, *Chem. Ind. (London,* (1984) 162; (c) E. P. Abraham, *J. Chem. Soc., Nat. Prod. Rep.*, (1987) 41.

2 J. C. Sheehan, K. R. Hénery-Logan and D. A. Johnson, *J. Am. Chem. Soc., 75* (1953) 3292.

3 For recent reviews, see (a) J. R. Knowles, *Acc. Chem. Res., 18* (1985) 97; (b) S. J. Cartwright and S. G. Waley, *Med. Res. Rev., 3* (1983) 341.

4 (a) R. F. Pratt and M. J. Loosemore, *Proc. Nat. Acad. Sci. USA, 75* (1978) 4145; (b) M. J. Loosemore and R. F. Pratt, *J. Org. Chem., 43* (1978) 3611; (c) J. A. Aimetti, E. S. Hamanaka, D. A. Johnson and M. S. Kellog, *Tetrahedron Lett.*, (1979) 4631; (d) V. Knott-Hunziker, B. S. Orlek, P. G. Sammes and S. G. Waley, *Biochem. J., 177* (1979) 365; (e) V. Knott-Hunziker, S. G. Waley, B. S. Orlek and P. G. Sammes, *FEBS Lett., 99* (1979) 59.

5 (a) J. G. Kemp, M. Closier, S. Narayanaswami and M. H. Stefaniak, *Tetrahedron Lett., 21* (1980) 2991; (b) R. Wise, J. M. Andrews and N. Patel, *J. Antimicrob. Chemother., 7* (1981) 531.

6 (a) A. R. English, J. A. Retsema, A. E. Girard, J. E. Lynch and W. E. Barth, *Antimicrob. Agents Chemother., 14* (1978) 414; (b) D. G. Brenner and J. R. Knowles, *Biochemistry, 20* (1981) 3680; (c) R. A. Volkmann, R. D. Carroll, R. B. Drolet, M. L. Elliot and B. S. Moore, *J. Org. Chem., 47* (1982) 3344.

7 (a) S. J. Cartwright and A. F. W. Coulson, *Nature (London), 278* (1979) 360; (b) B. S. Moore (Pfizer Inc.), *USP at. 4 420 426* (1983) *Chem. Abstr., 100* (1984) 174 534a.

8 (a) W. Durckheimer, J. Blumbach, R. Latrell and K. H. Scheunemann, *Angew. Chem., Int. Ed. Engl., 24* (1985) 180; (b) C. E. Newall and P. D. Hallam, P. G. Sammes (ed.), *Comprehensive Medicinal Chemistry*, Pergamon Press, Oxford, 1990, p. 609; (c) A. G. Brown, M. J. Pearson and R. Southgate, *ibid.*, p. 655.

9 (a) P. M. Blumberg and J. L. Strominger, *Proc. Nat. Acad. Sci. USA, 54* (1965) 1133; (b) For a review, see D. J. Waxman, R. R. Yocum and J. L. Strominger, *Philos. Trans. R. Soc. London, Ser. B, 289* (1980) 257.

10 (a) J. M. T. Hamilton Miller and J. T. Smith (eds.), The β-Lactamases, Academic Press, New York, 1979; (b) R. P. Ambler, *Philos. Trans. R. Soc. London, Ser. B, 289* (1980) 321; (c) S. G. Waley, *Chem. Ind. (London)*, (1981) 131; (d) R. B. Sykes, *J. Infec Dis., 145* (1982) 762; (e) A. A. Medeiros, in L. Leive and D. Schlessinger (eds.), *Microbiology*, Am. Soc. Microbiol., Washington, DC, 1984, p. 385; (f) S. G. Waley, *Sci. Prog. (Oxford), 72* (1988) 579.

11 O. Herzberg and J. Moult, *Science, 236* (1987) 694.

12 B. J. Sutton, P. J. Artymiuk, A. E. Cordero Borboa, C. Little, D. C. Phillips and S. G. Waley, *Biochem. J., 248* (1987) 181.

13 P. C. Moews, J. R. Knox, O. Dideberg, P. Charlier and J. M. Frère, *Proteins, 7* (1990) 156.

14 For leading references on the mechanism of action of 6β-bromopenicillanic acid, see (a) ref. 4(d); (b) C. D. Foulds, M. Kosmirak, A. C. O Sullivan and P. G. Sammes, in S. M. Roberts (ed.), *Recent Advances in the Chemistry of β-Lactam Antibiotics, Spec. Publ. No. 52*, R. Soc. Chem., London, 1984, p. 255; (c) M. J. Loosemore, S. A. Cohen and R. F. Pratt, *Biochemistry, 19* (1980) 3990; (d) S. A. Cohen and R. F. Pratt, *Biochemistry, 19* (1980) 3996.

15 For leading references on the mechanism of action of penicillanic acid sulfone, see (a) J. Fisher, R. Charnas, S. M. Bradley and J. R. Knowles, *Biochemistry, 20* (1981) 2726; (b) D. G. Brenner and J. R. Knowles, *ibid., 20* (1981) 3680; (c) C. K. Kemal and J. R. Knowles, *ibid., 20* (1981) 3688; (d) D. A. Brenner and J. R. Knowles, *ibid., 23* (1984) 5833.

16 W. A. Slusarchyk, H. E. Applegate, P. Funke, W. Koster, M. S. Puar, M. Young and J. E. Dolfini, *J. Org. Chem., 38* (1973) 943.

17 W. A. Spitzer, T. Goodson, M. O. Chaney and N. D. Jones, *Tetrahedron Lett.*, (1974) 4311.

18 (a) M. S. Kellog and E. S. Hamanaka (Pfizer Inc.), *Ger. Offen, 3 008 316* (1980) *Chem. Abstr., 94* (1981) 84 113r. This patent was later abandoned; (b) M. S. Kellog and E. S. Hamanaka (Pfizer Inc.), *US Pat. 4 397 783* (1983) *Chem. Abstr., 99* (1983) 175 473c.

19 (a) J. B. Doherty, P. E. Finke, R. A. Fuirestone, W. K. Hagaman, S. K. Shah and K. R. Thompson (Merck Inc.), *Eur. Pat. 124 081* (1984) *Chem. Abstr., 102* (1985) 148 985v; (b) J. B. Doherty, B. M. Ashe, L. W. Argenbright, P. L. Barker, R. J. Bonney, G. O. Chandler, M. E. Dahlgren, C. P. Dorn, P. E. Finke, R. A. Firestone, D. Fletcher, W. K. Hagmann, R. Mumford, L. O'Grady, A. L. Maycock, J. M. Pisano, S. K. Shah, K. R. Thompson and M. Zimmerman, *Nature (London), 322* (1986) 192; (c) J. B. Doherty, B. M. Ashe, P. L. Barker, T. J. Blacklock, J. W. Butcher, G. O. Chandler, M. E. Dahlgren, P. Davies, C. P. Dorn, P. E. Fink, R. A. Firestone, W. K. Hagmann, T. Halgren, W. B. Knight, A. L. Maycock, N. A. Navia, L. O'Grady, J. M. Pisano, S. K. Shah, K. R. Thompson, H. Weston and M. Zimmerman, *J. Med. Chem., 33* (1990) 2513; (d) P. E. Finke, B. M. Ashe, W. B. Knight, A. L. Maycock, M. A. Navia, S. K. Shah, K. R. Thompson, D. J. Underwood, H. Weston, M. Zimmerman and J. B. Doherty, *ibid., 33* (1990) 2522; (e) S. K. Shah, K. A. Brause, G. O. Chandler,

P. E. Fink, B. M. Ashe, H. Weston, W. B. Knight, A. L. Maycock and J. B. Doherty, *ibid*, *33* (1990) 2529, and references therein.
20 A. W. Guest, P. H. Milner and R. Southgate, *Tetrahedron Lett.*, *30* (1989) 5791.
21 J. E. Baldwin, J. E. Cobb and L. N. Sheppard, *Tetrahedron*, *43* (1987) 1003.
22 W. Von Daehne, E. T. Hansen and N. Rastrup-Anderson, in A. G. Brown and S. M. Roberts (eds.), *Recent Advances in the Chemistry of β-Lactam Antibiotics*, R. Soc. Chem., London, 1985, p. 375.
23 R. G. Micetich, S. Betageri, R. Singh and P. Payne, *J. Org. Chem.*, *52* (1987) 915.
24 B. Muller, H. Peter, P. Schneider and H. Bickel, *Helv. Chim. Acta*, *58* (1975) 2469.
25 T. Yoshioka, A. Watanabe, N. Chida and Y. Fukagawa, *J. Antibiot.*, *42* (1989) 1520; see also, A. Watanabe, M. Sakamoto, Y. Fukagawa and T. Yoshioka, *Chem. Pharm. Bull.*, *39* (1991) 335.
26 N. L. Shah and L. D. Cama, *Heterocycles*, *25* (1987) 221.
27 C. P. Mak and K. Wagner, in A. G. Brown and S. M. Roberts (eds.), *Recent Advances in the Chemistry of β-Lactam Antibiotics*, R. Soc. Chem., London, 1985, p. 368; see also, C. P. Mak, K. Wagner, C. Mayerl and H. Fliri, *Heterocycles*, *19* (1982) 1399.
28 J. Brennan, F. H. S. Hussain and P. Virgili, *Tetrahedron Lett.*, *27* (1986) 3199.
29 M. Sanada, T. Mizano, S. Iwadare, J. M. Williamson, B. H. Arison and J. L. Smith, *J. Antibiot.*, *39* (1986) 259.
30 K. Matsuda, S. Nakagawa, F. Nakano, M. Inoue and S. Mitsuhashi, *J. Antimicrob. Chemother.*, *19* (1987) 753.
31 T. Taguchi, O. Kitagawa, Y. Suda, S. Ohkawa, A. Hashimoto, Y. Litaka and Y. Kobayashi, *Tetrahedron Lett.*, *29* (1988) 5291.
32 J. E. Baldwin, G. P. Lynch and C. J. Schofield, *J. Chem. Soc., Chem. Commun*, (1991) 736.
33 (a) E. L. Setti and O. A. Mascaretti, *J. Org. Chem.*, *51* (1986) 3217; (b) E. G. Mata, E. L. Setti, O. A. Mascaretti, S. B. Boggio and O. A. Roveri, *J. Chem. Soc., Perkin Trans. 1*, (1988) 1551; (c) E. L. Setti and O. A. Mascaretti, *J. Chem. Soc., Perkin Trans. 1* (1988) 2059; (d) E. G. Mata, O. A. Mascaretti, A. E. Zuñiga, A. B. Chopa and J. C. Podesta, *Tetrahedron Lett.*, *30* (1989) 3905; (e) E. G. Mata, E. L. Setti and O. A. Mascaretti, *J. Org. Chem.*, *55* (1990) 3674; (f) G. O. Danelon, M. A. Laborde, O. A. Mascaretti, S. B. Boggio and O. A. Roveri, submitted for publication.
34 (a) J. Mann, *Chem. Soc. Rev.*, *16* (1987) 381; (b) D. Seebach, *Angew. Chem., Int. Ed. Engl.*, *29* (1990) 1320, and references cited therein.
35 (a) R. Filler and Y. Kobayashi, *Biomedical Aspects of Fluorine Chemistry*, Kodansha and Elsevier Biomedical Press, Tokyo and Amsterdam, 1982; (b) J. T. Welch and S. Eswarakrishnan, *Fluorine in Bioorganic Chemistry*, Wiley, New York, 1990; (d) J. T. Welch, *Selective Fluorination in Organic and Bioorganic Chemistry, ACS Symp. Ser. No. 456*, Am. Chem. Soc., Washington, DC, 1991.
36 L. B. Dugad and T. Gerig, *Biochemistry*, *27* (1988) 4310, and references therein.
37 J. C. Sheehan, *The Enchanted Ring. The Untold Story of Penicillin*, MIT Press, Cambridge, MA, 1982.
38 TBAF is an extremely hygroscopic material; see, D. P. Cox, J. Terpinsky and W. Lawrynowicz, *J. Org. Chem.*, *49* (1984) 3216.
39 J. H. Clark, *J. Chem. Soc., Chem. Commun.*, (1978) 789.
40 (a) G. Cainelli, F. Manescalchi, *Synthesis*, (1976) 472; (b) S. Colonna, A. Re, G. Gelbard and E. Cesarotti, *J. Chem. Soc., Perkin Trans. 1* (1979) 2248.
41 (a) R. G. Pearson, *J. Org. Chem.*, *54* (1989) 1423; (b) R. G. Parr and R. G. Pearson, *J. Am. Chem. Soc.*, *105* (1983) 7512.
42 P. Mooney, S. M. Roberts, J. G. Kemp and M. D. Closier, *Synth. Commun.*, *12* (1982) 85.
43 (a) D. Landini, A. Maia and A. Rampoldi, *J. Org. Chem.*, *54* (1989) 328, and references therein; (b) A. Ahrland, *Pure Appl. Chem.*, *54* (1982) 1451.
44 C. L. Liotta and H. P. Harris, *J. Am. Chem. Soc.*, *96* (1974) 2250.
45 (a) P. Bosch, F. Camps, E. Chamorro, V. Gosel and A. Guerrero, *Tetrahedron Lett.*, *28*

(1987) 4733; (b) F. Camps, E. Chamorro, V. Gosel and A. Guerrero, *J. Org. Chem.*, *54* (1989) 4294.
46 P. Albert and J. Cousseau, *J. Chem. Soc., Chem. Commun.*, (1985) 961; (b) J. Cousseau and P. Albert, *Bull. Soc. Chim. Fr.*, (1986) 910.
47 D. U. Belinzoni, O. A. Mascaretti, P. M. Alzari, G. Punte, C. Faerman and A. Podjarny, *Can. J. Chem.*, *63* (1985) 3177.
48 K. M. Harmon and I. Gennik, *J. Mol. Struct.*, *38* (1977) 97.
49 R. F. Pratt and D. J. Cahn, *J. Am. Chem. Soc.*, *110* (1988) 5096.
50 D. D. Tanner and H. K. Singh, *J. Org. Chem.*, *51* (1986) 5182.
51 D. U. Belinzoni, E. L. Setti and O. A. Mascaretti, *J. Chem. Res. (S)*, (1988) 176; *J. Chem. Res. (M)*, (1988) 1501.
52 H. G. Kuivila, *Synthesis*, (1970) 499.
53 C. J. Salomon, O. A. Mascaretti, C. E. Strouse and G. Punte, *Can. J. Chem.*, *69* (1991) 578.
54 B. Giese and G. Kretzchmar, *Chem. Ber.*, *117* (1984) 3175.
55 G. W. Gokel, H. M. Gerdes and D. M. Dishong, *J. Org. Chem.*, *45* (1980) 3634.
56 G. A. Olah, J. T. Welch, Y. D. Vankar, N. Nojima, I. Kerekes and J. A. Olah, *J. Org. Chem.*, *44* (1979) 3872.
57 J. C. Sheehan, Y. S. Lo, J. Loliger and C. C. Podewell, *J. Org. Chem.*, *39* (1974) 1444.
58 TASF has mainly been used as a source of anhydrous fluoride anion, and was successfully used for the displacement of triflates in carbohydrates; see, B. Doboszwski, G. Hay and W. A. Szarek, *Can. J. Chem.*, *65* (1987) 412.
59 (a) T. Umemoto, K. Kawada and T. Tomita, *Tetrahedron Lett.*, *27* (1986) 4465; (b) T. Umemoto and G. Tomizawa, *J. Org. Chem.*, *54* (1989) 1726.
60 (a) T. Durst and M. J. Le Belle, Can. J. Chem., 50 (1972) 3196; (b) T. Durst, R. Van Den Elzen and R. Legault, *Can. J. Chem.*, *52* (1974) 3206; (c) I. Ojima and Y. Pei, *Tetrahedron Lett.*, *31* (1990) 977; (d) I. Ojima, T. Komata and X. Qiu, *J. Am. Chem. Soc.*, *112* (1990) 770.
61 (a) F. DiNinno, T. R. Beattie and B. G. Christensen, *J. Org. Chem.*, *42* (1977) 2960; (b) J. A. Aimetti and M. S. Kellog, *Tetrahedron Lett.*, (1979) 3805; (c) L. A. Reed, D. A. Charleson and R. A. Volkman, *Tetrahedron Lett.*, *28* (1987) 3431.
62 G. O. Danelon and O. A. Mascaretti, unpublished results.
63 T. B. Patrick, J. S. Scheibel and G. L. Cantrell, *J. Org. Chem.*, *46* (1981) 3917.
64 S. Rozen and M. Brand, *J. Org. Chem.*, *51* (1986) 3607.
65 K. Boulton and B. E. Cross, *J. Chem. Soc., Perkin Trans. 1*, (1979) 1354.
66 A. J. Bloodworth, K. J. Bowyer and J. C. Mitchell, *Tetrahedron Lett.*, *28* (1987) 5347.
67 V. J. Jephcote, I. C. Jowett, D. I. John, P. D. Edwards, K. Luk, A. M. Slawin and D. J. Williams, *J. Chem. Soc., Perkin Trans. 1*, (1986) 2187, and references therein.
68 S. Rozen and M. Brand, *J. Org. Chem.*, *50* (1985) 3342.
69 M. Kuroboshi and T. Hiyama, *Tetrahedron Lett.*, *32* (1991) 1215.
70 I. Ichichara, K. Funabiki and T. Hanafusa, *Tetrahedron Lett.*, *31* (1990) 3167.
71 M. Shimizu, Y. Nakahara and H. Yoshioka, *J. Chem. Soc., Chem. Commun.*, (1989) 1881.
72 D. Y. Chi, D. O. Kiesewetter, J. A. Katzenellenbogen, M. R. Kilbourn and M. J. Welsch, *J. Fluorine Chem.*, *31* (1986) 99.
73 For a review, see H. Ferres, *Chem. Ind. (London)*, (1980) 435.
74 S. Torii, H. Tanaka, M. Taniguchi and Y. Kamayama, *J. Org. Chem.*, *56* (1991) 3633.
75 L. M. Ellerby, W. A. Escobar, A. L. Fink, C. Mitchinson and J. A. Wells, *Biochemistry*, *29* (1990) 5797, and references therein.
76 E. G. Mata and O. A. Mascaretti, *Tetrahedron Lett.*, *29* (1988) 6893.
77 C. J. Salomon, E. G. Mata and O. A. Mascaretti, *Tetrahedron Lett.*, *32* (1991) 4239.
78 (a) E. L. Setti and O. A. Mascaretti, *J. Org. Chem.*, *54* (1989) 2233; (b) E. L. Setti, D. U. Belinzoni and O. A. Mascaretti, *J. Org. Chem.*, *54* (1989) 2235.

79 T. Katazume, T. Sato, T. Kobayashi and J. T. Lin, *J. Org. Chem.*, *51* (1986) 1003.
80 (a) G. Sabbioni and J. B. Jones, *J. Org. Chem.*, *52* (1987) 4565; (b) for a recent review, see L. M. Zhu and M. C. Tedford, *Tetrahedron*, *46* (1990) 6587.
81 J. Fisher, J. G. Belasco, R. L. Charnas, S. Khosla and J. R. Knowles, *Philos. Trans. R. Soc. London, Ser. B*, *289* (1980) 309.
82 R. B. Davies, E. P. Abraham and J. Melling, *Biochem. J.*, *143* (1974) 115.
83 C. H. O'Callaghan, A. Morris, S. M. Kirby and A. H. Shingler, *Antimicrob. Agents Chemother.*, *1* (1972) 283.

Note added in proof

A report on a stereoselective synthesis of 3-fluoro-azetidinones has been published since the submission of our review: K. Araki, J. A. Wichtowski and J. T. Welch, *Tetrahedron Lett.*, *32* (1991) 5461.