Bioorganic & Medicinal Chemistry Vol. 1, No. 6, pp. 447–455, 1993
Printed in Great Britain

0968-0896/93 $6.00 + .00

Synthesis and β-Lactamase Inhibitory Activity of 6-Fluoropenicillanic Acids

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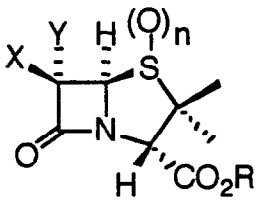
(*Received* 26 *July* 1993; *accepted* 1 *November* 1993)

Abstract—The benzyl 6-fluoro-penicillanate sulfides **4a, 6a, 7a**; and sulfones **6c, 7d** were synthesized. The conversion to their free acids **4b, 6b, 6d, 7b, 7e** and potassium salts **7c, 7f** are described. These acids and salt **7c** were evaluated as β-lactamase inhibitors using β-lactamase I from *Bacillus cereus*. The data indicate that substitution of the 6α-hydrogen by a 6α-fluorine atom on 6β-bromopenicillanic acid (**1**), leads to loss of β-lactamase inhibitory activity. In the case of the isomers 6β- and 6α-fluoropenicillanic acids the 6β-enantiomer proved to be considerably more potent. Potassium salts of 6β-fluoropenicillanate sulfide and sulfone were unstable in solid state and in water solution. The fragmentation of the sulfone in two parts in water solution is consistent with the hydrolytic behavior ot the penicillanic acid sulfone (**2**) with 0.5 N NaOH.

In the course of the development of new drugs, the need frequently arises to determine what effects structural modifications have on biological activity, i.e., to establish structure–activity relationships (SARs).

In recent years much attention has been focused on the inhibition of β-lactamases[1–4] as a means of controlling the increasing threat posed by bacteria which have developed resistance to β-lactam antibiotics. Such resistance is the consequence of the production of β-lactamases that catalyze the hydrolysis of the β-lactam ring. The previously reported inhibitors include, among others, 6β-bromopenicillanic acid (**1**)[5] and penicillanic acid sulfone (sulbactam) (**2**).[6,7] Several studies have been carried out in which some aspects of the mechanism of action of these inhibitors have been elucidated.[8–11]



1 X=Br, Y=H, n=0, R=H
2 X=H, Y=H, n=2, R=H

† Dedicated to Professor Heinz G. Floss (Seattle), on the occasion of his 60th birthday.

One of the simplest structural modifications altering reactivity is the replacement of a hydrogen atom by a fluorine atom (isosteric replacement).[12] Herein we report the synthesis and some preliminary results of a systematic study of the structure–activity relationship of 6-fluoropenicillanic acids **4b, 6b, 6d, 7b, 7e** and potassium salt **7c**, analogs of β-lactamase inhibitors **1** and **2**, with β-lactamase I of *Bacillus cereus* 569/H.

Results and Discussion

Chemistry

6-Fluoropenicillanates are known in the patent literature.[13] The formation of the fluorine-substituted β-lactam nucleus of the target 6-fluoropenicillins described herein was accomplished by our previously published methods.[14] The novel parent acids or salts were prepared by chemical deprotection of its benzyl esters.

Synthesis of benzyl esters and carboxylic acids. Treatment of benzyl 6-diazopenicillanate (**3**)[15] with *N*-bromosuccinimide (NBS) and tetrabutyl-ammonium hydrogen bifluoride (TBABF)[16] or alternatively with NBS and tetrabutylammonium dihydrogen trifluoride (TBATF)[17] in dichloromethane at -40 °C gave two products. The major product was **4a**, while the minor product was **5a** at a ratio of 30:7 as determined by ^{1}H NMR spectroscopy. The stereochemistry of the fluorine substituent in **4a**, which could not be separated from **5a**, was ascertained on the basis of the coupling constants indicating a *cis* H(5)–F(6) relationship.

Cleavage of the benzyl ester was effected by treatment with aluminum trichloride[18] to give carboxylic acids **4b** and **5b** in approximately 96% isolated yield, in a ratio 30:7 (^{1}H NMR).

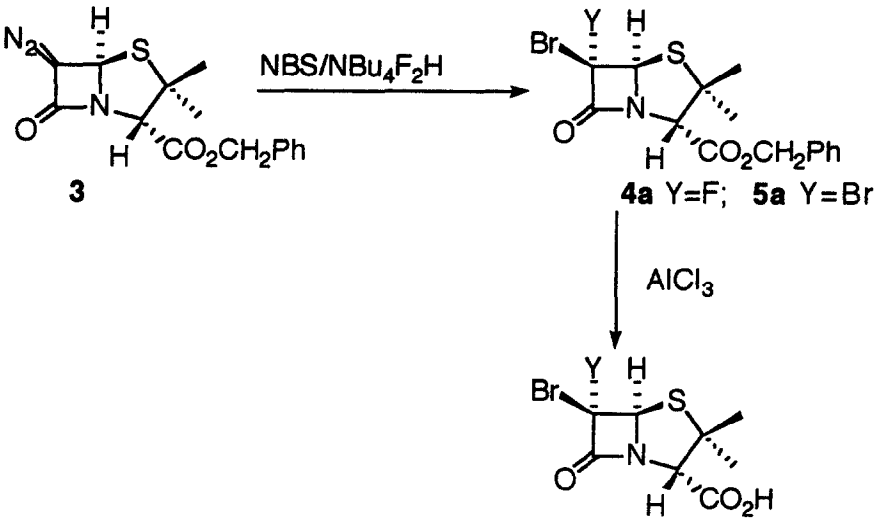
It was clear from our previous studies[14] that radical-induced reductive debromination with tris[2-methyl-2-phenyl-propyl-(neophyl)]tin hydride[14] is a very convenient method for the reductive debromination of **4a**. By using this reagent we were able to obtain the benzyl 6β-fluoro-penicillanate **6a** in 60% isolated yield. The stereochemistry at C-6 was ascertained from the *cis* coupling constant of the protons on C-5 and C-6. A detailed mechanistic rationale for this chemo- and diastereo-selective reduction has been given elsewhere.[19]

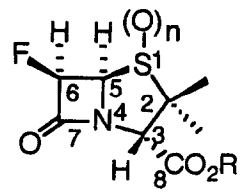
Compound **6a** was converted into the free acid **6b** in 90% isolated yield using hydrogenolytic conditions previously reported for other benzylpenicillanates.[20]

The benzyl 6α-fluoropenicillanate (**7a**) was prepared in 48% isolated yield by reaction of wet benzyl 6-diazo-penicillanate (**3**) with 2 equiv. of DAST. The structure assigned to **7a** is supported by extensive ^1H and ^{19}F NMR chemical shift and coupling analyses. The benzyl ester group of compound **7a** was cleaved by treatment with aluminum trichloride to give 6α-fluoropenicillanic acid **7b** in quantitative yield.

Since the sulfone derivatives of 6β- (**6d**) and 6α-fluoropenicillanic acid (**7e**) were also target compounds in this work, we prepared them by oxidation of benzyl fluoropenicillanates **6a** and **7a** using potassium permanganate under phase-transfer conditions[21] to obtain compounds **6c** and **7d**, both in 80% isolated yield. The benzyl protecting groups of compounds **6c** and **7d** were cleaved by hydrogenolysis to give 6-fluoropenicillanic acids sulfone **6d** and **7e** respectively, both in 90% isolated yield.

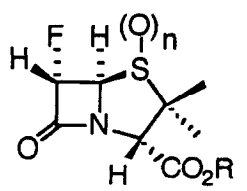
Synthesis of potassium salts. Acids **7b** and **7e** were converted into their potassium salts **7c** and **7f** by exchange with potassium 2-ethyl hexanoate.[22] However, attempts to convert the acids **6b** and **6d** into the corresponding potassium salts were unsuccesful. The first evidence of this failure was the disappearance of the β-lactam carbonyl stretching frequency at around 1800 cm^{-1}. Analysis by ^1H NMR (D_2O) of the expected potassium 6β-fluoropenicillanate showed it to be a mixture of products. This mixture was acidified to pH 3.5 and extracted with ethyl acetate. The organic phase showed by silica gel TLC the presence of a minor amount of the acid **6b** in a complex mixture of products. In the case of the attempted preparation of the potassium salt of 6β-fluoropenicillanate sulfone, data from ^1H and ^{13}C NMR indicated the structure





6a-d

a n=0, R=CH_2Ph
b n=0, R=H
c n=2, R=CH_2Ph
d n=2, R=H



7a-f

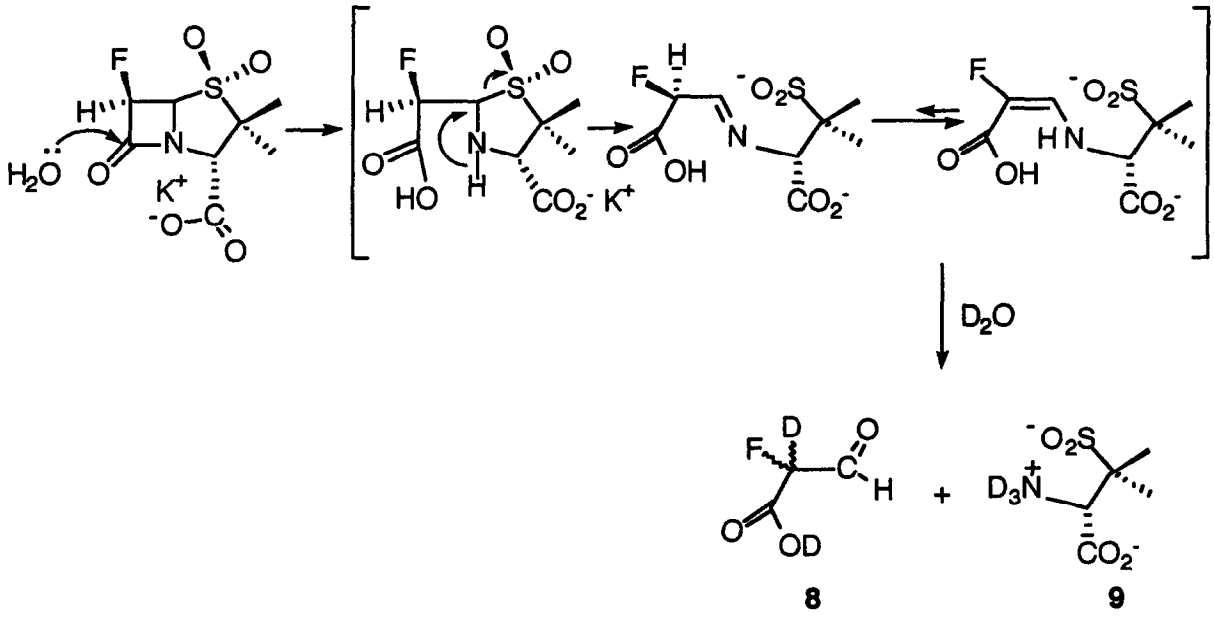
a n=0, R=CH_2Ph
b n=0, R=H
c n=0, R=K
d n=2, R=CH_2Ph
e n=2, R=H
f n=2, R=K

to be that of the sulfinate of penicillamine (**9**). This finding is well precedented[8a,b] and suggests the sequence of reactions: β-lactam hydrolysis by adventitious water, followed by or synchronous with the opening of the thiazolidine ring in the solid state, hydrolysis of the resulting imine in D_2O solution to give fluoromalonic semialdehyde (**8**)[23] and compound **9**.

We propose that these observations can be explained as follows. The different behavior of the potassium penicillanate with a fluorine atom in the 6β-orientation relative to that shown by the 6α-fluoro-oriented diastereoisomer could be due to a through-space repulsion[24] involving overlap of lone pair orbitals from the 6β-fluoro- and 1β-oxygen atom in potassium penicillanate sulfone. As a consequence of these non-bonded interactions, a conformational change was induced and the potassium ion coordinates between the carboxylate group and the β-lactam nitrogen.[25] This is not the case with potassium 6α-fluoropenicillanate sulfone indicating that one of the conformations of the penicillin would be more suitable for ion coordination. Evidently this metal coordination is lacking in the case of 6β-fluoropenicillanic acid sulfone **6d**, where we assume the predominant effect might be intermolecular hydrogen bonding.

Enzyme inhibition

The 6-fluoropenicillanic acids **4b**, **6b**, **6d**, **7b**, **7e** and potassium salt **7c** have been prepared in an attempt to study structure–activity relationships systematically within the 6-monofluoropenicillin series. The table shows the IC_{50} values of these compounds compared with those of the previously known β-lactamase inhibitors **1** and **2**.



8 **9**

Table 1. Inhibitory activity of 6-fluoropenicillins against β-lactamase I of *Bacillus cereus*

Compound	IC_{50} (mM) Without Preincubation	With Preincubation
1	(*)	(*)
2	0.025	0.025
4b	>5	>5
6b	2.5	0.4
6d	1	0.25
7b	>5	3
7c	>5	>3
7e	4	2.5

(*) Compound **1** reacts rapidly with the enzyme in a 1:1 molar ratio. The addition of **1** (0.001 mM) to the reaction medium produced an instantaneous inhibition of 50% of the enzymatic activity, inhibition that progressed with time. The incubation of the enzyme during 10 min with **1** (0.0005 mM) completely inhibited the enzyme.

It has been postulated by Knowles *et al.*[8a] that penicillanic acid sulfone **2** interacts with β-lactamases with high affinity yielding a long-lived acyl–enzyme complex. Such a complex partitions into three different pathways: (a) hydrolysis and regeneration of free enzyme; (b) formation of a transiently inhibited enzyme form; and (c) slow irreversible inactivation of the enzyme. Therefore the effectiveness of related compounds as inhibitors would depend on the relative affinity of the compounds for the enzyme, their ability to form a long-lived acyl–enzyme complex and the partitioning among the three different reaction pathways. The replacement in **2** of the 6β-hydrogen atom by a fluorine atom, as in compound **6d**, should produce an increase in the acidity of the 6α-hydrogen which, according to Fisher *et al.*,[26] would be accompanied by an increase in the β-lactamase inhibitory activity. However, such replacement rendered a compound ten times less active. Other predictable consequences of the above mentioned replacement would be an increase in the rates of acylation and deacylation of the enzyme and a decrease in the rate of thiazolidine ring opening. In addition, the IC_{50} value obtained without preincubation is 40 times higher than that obtained with the parent compound **2**, clearly suggesting that the affinity of the former for the enzyme is strongly diminished with respect to the affinity of the latter. The lower affinity and other effects produced by the presence of an electron-withdrawing substituent at C-6 can explain the lower inhibitory activity of **6d** when it is compared with **2**. The replacement of the 6α-hydrogen as in compound **7e** resulted in a greater decrease in the inhibitory activity. Nevertheless, compound **7e** was still capable of inhibiting the enzyme in spite of the fact that it lacks a 6α-hydrogen at C-6.[26] The presence of a fluorine atom could facilitate epimerization at C-6,[5,27] hence explaining the activity of the 6α-isomer. However, we have not been able to obtain evidence for such epimerization. Further kinetic studies are being carried out in order to fully determine the mechanism and the kinetic constants for the interaction of 6β-fluoro-penicillanic acid sulfone with β-lactamase I.

Compound **1** inhibits irreversibly β-lactamase I from *Bacillus cereus* 569/H. Neither hydrolysis nor formation of a transiently inhibited enzyme form has been reported to occur.[9d] Several reaction mechanisms have been postulated: (i) Cohen and Pratt[9d] suggested that the formation of the acyl–enzyme complex, is followed immediately by ring opening of the thiazolidine with formation of a thiolate–immonium zwitterion followed by rearrangement and cyclization of the inhibitor to a 2,3-dihydro-1,4-thiazine-3,6-dicarboxylic acid derivative. The last step involves a fast isomerization of an imine to an enamine which is facilitated by the acidity of the hydrogen atom on carbon-6 of the 6β-bromopenicillanic acid, (ii) a slightly different reaction scheme has been proposed by Fisher *et al.*[26] whose main differences are that previous to the five membered ring opening, an enzyme base stereospecifically assists proton removal from the carbon-6α position and that this is the driving force for an *anti* elimination across the C-5 and C-6 positions resulting in the direct formation of an enamine and ring opening of the thiazolidine, and (iii) recently Pratt and Cahn[27] have postulated an alternative mechanism: The thiazolidine ring does not open upon acyl–enzyme complex formation, but a direct intramolecular nucleophilic attack by the thiazolidine sulfur atom on the alkyl halide occurs, yielding a bicyclic episulfonium ion intermediate, which collapses to the imine form of dihydrothiazine through intramolecular participation of the lone pair on the thiazolidine nitrogen atom, followed by the isomerization of the imine double bond to an enamine form of a 2,3-dihydro-1,4-thiazine derivative. For mechanisms (i) and (iii) the more important structural feature is the presence of a good 6β-leaving group, whereas for mechanism (ii) also the presence of an acidic 6α-hydrogen is essential for the rearrangement reactions leading to the dihydrothiazine formation.

The replacement of the 6α-hydrogen in compound **1** by a fluorine atom, such as in **4b**, renders a compound capable of binding and acylating the enzyme, since it was hydrolyzed by the β-lactamase.[28] On the other hand, it only slightly inhibited β-lactamase activity and the inhibition did not increase significantly by preincubation of the enzyme with compound **4b**. Preliminary kinetic results have shown that it behaved as a poor substrate with a K_m value higher than 10 mM, in agreement with its high IC_{50} value. Such a replacement results in a compound that lacks the 6α-hydrogen. The presence of the electronegative fluorine atom would also retard the elimination of the bromide ion. However, since the hydrolysis product has not yet been identified, it is not possible to draw definite conclusions on the hydrolytic mechanism or on the role of the replaced acidic 6α-hydrogen in the inactivation mechanism of β-lactamase I.

Conversely, the 6β-fluoropenicillanic acid **6b** showed good inhibitory activity. The inhibition was a progressive one, but did not follow simple pseudo-first order kinetics. The inhibition depends largely on the inhibitor/enzyme ratio. Therefore, the formation of a transiently inhibited enzyme form is suspected in addition to the irreversible inactivation. Hence, the reaction mechanism of compound **6b** must be a branched-type mechanism similar to that reported for penicillanic acid sulfone and for other 6β-halopenicillanic acids with β-lactamases.[29] The replacement in **1** of the bromine atom by a fluorine one would produce an increase in the acidity of the 6α-hydrogen. On the other hand, fluoride is poorer than bromide as leaving group. Since compound **6b** has lower activity than its parent compound **1** it is possible to suggest that, for 6-halopenicillanic acids, the leaving capacity of the 6β-substituent is more important than the acidity of the 6α-hydrogen in terms of β-lactamase inhibition.[27] Further studies in order to determine the mechanism of interaction of compound **6b** with β-lactamase I are currently in progress.

On the other hand, 6α-fluoropenicillanic acid **7b** and its potassium salt **7c**[3] behaved similarly to compound **4b**: viz it was hydrolyzed by and it only slightly inhibited the enzyme. This result is in agreement with the mechanisms discussed above since either the thiazolidine ring opening or the formation of an episulfonium ion intermediate are easier reaction paths for 6β-substituted penicillanic acids.

Conclusion

The goals of the present investigation were to develop the synthesis of 6-fluoropenicillanic acids and their salts, and their evaluation as inhibitors of β-lactamases. Our choice of methods for the introduction of a fluorine atom into the β-lactam nucleus was made such as to obtain high stereoselectivity and versatility for exploiting the special shape of the penam nucleus for stereocontrol in the formation of a new chiral center at carbon 6. Such methods are of interest for application to other β-lactam compounds such as cephalosporins, penems, carbapenems and monobactams.

We have demonstrated that the replacement of the 6α-hydrogen atom in compound **1** by a fluorine atom results in loss of β-lactamase inhibitory activity.

For the first time some instability was noted for the 6β-fluoro stereoisomer, which points to a potentially interesting stereoelectronic effect.

These findings represent a significant step toward the design of active 6-halopenicillanic acid and salts, particularly since besides compounds **1** and **2** also 6β-iodopenicillanic acid[31] and 6α-chloropenicillanic acid sulfone[32] were found to be good inhibitors of β-lactamases.

Additional tests using these 6-fluoropenicillins against various strains of cephalosporinase, penicillinase and broad-spectrum β-lactamase-producing bacteria are in progress.

Experimental Section

Infrared spectra were recorded on either a Beckman Acculab 8 spectrometer or a Bruker IFS 25 FT-IR spectrometer with polystyrene as the reference. The ^1H, ^{13}C and ^{19}F NMR spectra were taken on a Bruker WP 80 SY and Bruker AC 200 E at 80.13, 20.15, 75.39, 200 and 50 MHz, respectively. Chemical shifts are reported for $CDCl_3$ solution unless indicated otherwise, in ppm positive downfield from internal TMS for ^1H and ^{13}C NMR and from internal $CFCl_3$ for ^{19}F NMR. Mass spectra were measured at 70 eV for electron impact (EI) and with methane or ammonia for chemical ionization (CI). Fragment ions m/z in % of the most abundant peak. The low resolution mass spectra (EI) of benzyl 6β-bromo-6α-fluoro- (**4a**), 6β-fluoro- (**6a**) and 6α-fluoropenicillanates (**7a**) followed the known fragmentation pattern described for methyl[33] and (pivaloyloxy)methyl penicillanates. The low resolution mass spectra (CI) of benzyl 6β-fluoro- (**6c**) and 6α-fluoropenicillanate sulfone (**7d**) gave very few fragment ions and contained easily discernible molecular ions.[34] Satisfactory mass spectra (EI, CI or FAB) could not be obtained[35] for penicillanic acid sulfide and sulfone **4b**, **6b**, **6d**, **7b** and **7e**. Column chromatography was performed on silica gel 60 A (100–200 mesh). Thin-layer chromatography (TLC) was done on silica gel GF_{254} (type 60 Merck). Benzyl 6-diazopenicillanate (**3**)[15] was prepared according to a literature procedure. All new compounds have been characterized from their spectral data. The purity of all title compounds was judged to be >95% by TLC, ^1H NMR, ^{13}C NMR determinations.

Preparation of benzyl (2S,5R,6R) 6-bromo-6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (4a)

To a solution of **3** (187 mg, 0.59 mmol) in anhydrous dichloromethane (3 mL) at -40 °C was added dropwise and simultaneously 114 mg (0.65 mmol) of *N*-bromosuccinimide in a mixture of chloroform–acetonitrile (5 mL, 4:1) and 186 mg (0.65 mmol) of tetrabutylammonium bifluoride in anhydrous dichloromethane (2 mL). The cold bath was then allowed to warm slowly to room temperature and after 1.5 h the reaction was quenched by the addition of water (6 mL). The layers were separated and the organic layer was dried (Na_2SO_4). The solvent was evaporated and the crude residue was subjected to column chromatography (elution with hexane–ethyl acetate, 8.5:1.5) to afford 95 mg of a mixture of two products in a ratio of 30:7 (^1H NMR), which were inseparable by chromatography. The major product, a pale yellow oil was identified as **4a** (≈30%): IR (film) 1800 (β-lactam), 1750 (ester) cm^{-1}; ^1H NMR δ 1.38 (s, 3H), 1.56 (s, 3H), 4.56 (s, 1H, C–3H), 5.20 (s, 2H, C–9H), 5.60 (d, 1H, $J_{5,F}$ = 5.6 Hz, C–5H), 7.37 (s, 5H, Ph–H); ^{13}C NMR δ 166.29 (C-8), 162.3 (d, $^2J_{C,F}$ = 23.6 Hz, C-7), 134.4 (Ph–C), 128.55 (Ph–C), 107.9 (d, $^1J_{C,F}$ = 307.3 Hz, C-6), 77.1 (d, $^2J_{C,F}$ = 26.8 Hz, C-5) 69.0 (C-3), 67.53 (C-9), 64.09 (C-2), 33.34 (C–βMe), 25.45 (C–αMe); ^{19}F NMR δ -114.8 (d, $^3J_{F,H}$ = 5.2 Hz, C–6F); LRMS (EI) 387 (M^+, 36.0) 190 (5), 114 (12), 91 (100); HRMS calcd for $C_{15}H_{15}BrFNO_3S$ 386.9934, found 386.9941. The minor product was identified as benzyl 6,6-dibromopenicillanate (**5a**).[36]

Preparation of (2S,5R,6R) 6-bromo-6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (4b)

A solution of $AlCl_3$ (80 mg, 0.6 mmol) in anhydrous nitromethane (1 mL) was added to a solution of the previous mixture of **4a** and **5a** (40 mg) in anhydrous dichloromethane (4 mL) under ice cooling, and the mixture was stirred for 10 min at 0 °C. The reaction solution was diluted with ethyl acetate (4 mL), washed with hydrochloric acid 0.5 N (2 mL) and the aqueous phase extracted with ethyl acetate (3 x 1 mL). The organic phase was dried and evaporated to give an inseparable mixture of **4b** and **5b** (29.5 mg ~ 96%) at a ratio of 30:7 (^1H NMR) as a yellow oil. **4b** (23.9 mg): IR (film) 1794 (β-lactam), 1734 (carboxylic acid) cm^{-1}; ^1H NMR δ 1.57 (s, 3H), 1.62 (s, 3H), 4.56 (s, 1H, C–3H), 5.61 (d, 1H, $J_{5,F}$ = 5.6 Hz, C–5H); ^{19}F NMR δ -115.0 (d, $^3J_{F,H}$ = 6.9 Hz, C–6F). **5b** (5.6 mg): IR and ^1H NMR are consistent with those reported in Ref. 6b.

Preparation of benzyl (2S,5R,6R) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (6a)

A similar procedure as previously described[14] was used. The mixture of compounds **4a** and **5a** was the starting material. Pure compound **6a** was obtained after purification by flash chromatography (elution with hexane–ethyl acetate, 8.5:1.5). Yield 60% as a pale yellow oil: IR (film) 1800 (β-lactam), 1750 (ester) cm^{-1}; ^1H NMR δ 1.43 (s, 3H), 1.64 (s, 3H), 4.54 (s, 1H, C–3H), 5.19 (s, 2H, C–9H), 5.50 (dd, 1H, $^3J_{5,F}$ = 4.0 Hz, $J_{5,6}$ = 4.0 Hz, C–5H), 5.72 (dd, 1H, $^2J_{6,F}$ = 55.0 Hz, $J_{5,6}$ = 4.0 Hz, C–6H), 7.37 (s, 5H, Ph–H); ^{13}C NMR δ 169.15 (d, $^2J_{C,F}$ = 22.1 Hz, C-7), 167.10 (C-8), 134.5 (Ph–C), 128.4 (Ph–C), 91.67 (d, $^1J_{C,F}$ = 225.0 Hz, C-6), 70.60 (C-3), 67.30 (C-9), 66.45 (d, $^2J_{C,F}$ = 22.1 Hz, C-5), 64.10 (C-2), 31.6 (C–βMe), 26.04 (C–αMe); ^{19}F NMR δ -156.62 (dd, $^2J_{F,H}$ = 55.3 Hz, $^3J_{F,H}$ = 3.4 Hz, C–6F); LRMS (EI) 309 (M^+, 8.4), 250 (4.8), 91 (100), (CI, ammonia) 327 (M^+ + 18, 31.1), 264 (45.3), 250 (60.6), 208 (39.5), 108 (88.9), 91 (40.6), 58 (100), HRMS calcd for $C_{15}H_{16}O_3NFS$ 309.0834, found 309.0821.

Preparation of (2S,5R,6R) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6b)

To a solution of **6a** (100 mg, 0.32 mmol) and 1.0 equivalent of $NaHCO_3$ in 16 mL aqueous THF (1:1) was added 100 mg of 10% Pd/C. The resulting suspension was stirred under 1 atmosphere of H_2 for 5 h and then filtered through a Celite pad and washed with aqueous THF (2 x 5 mL). The combined filtrates were acidified to pH 3.0 with hydrochloric acid and extracted with ethyl acetate (4 x 6 mL). The organic layer, after drying, was evaporated to give 64 mg (90%) of pure **6b** as a pale yellow oil: IR (film) 1784 (β-lactam), 1760 (carboxylic acid) cm^{-1}; ^1H NMR δ 1.60 (s, 3H), 1.70 (s, 3H), 4.54 (s, 1H, C–3H), 5.50 (dd, 1H, $J_{5,F}$ = 4.0 Hz, $J_{5,6}$ = 4.0 Hz, C–5H), 5.74 (dd, 1H, $J_{6,F}$ = 54.5 Hz, $J_{5,6}$ = 4.0 Hz, C–6H); ^{19}F NMR δ -156.7 (dd, $^2J_{F,H}$ = 54.0 Hz, $^3J_{F,H}$ = 5.3 Hz, C–6F).

Preparation of benzyl (2S,5R,6R) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4,4-dioxide (6c)

For the oxidation of penicillanate sulfide to penicillanate sulfone, we used a similar, previously described procedure.[14] Compound **6a** was the starting material. Yield of pure **6c** 80% as a colorless oil: IR (film) 1820 (β-lactam), 1760 (ester), 1340, 1130 (sulfone) cm^{-1}; ^1H NMR δ 1.25 (s, 3H), 1.55 (s, 3H), 4.63 (s, 1H, C–3H), 4.75 (dd, 1H, $^3J_{5,F}$ = 4.0 Hz, $J_{5,6}$ = 4.0 Hz, C–5H), 5.22 (qAB, 2H, J = 12.0 Hz, C–9H), 5.85 (dd, 1H, $^2J_{6,F}$ = 55.0 Hz, $J_{5,6}$ = 4.0 Hz, C–6H), 7.37 (s, 5H, Ph–H); ^{13}C NMR δ 168.89 (d, $^2J_{C,F}$ = 23.6 Hz, C-7), 166.13 (C-8), 134.8 (Ph–C), 128.66 (Ph–C), 89.15 (d, $^1J_{C,F}$ = 239.0 Hz, C-6) 68.13 (C-9), 67.92 (C-2), 65.42 (C-3), 64.1 (d, $^2J_{C,F}$ = 7.5 Hz, C-5), 19.72 (C–βMe), 17.64 (C–αMe); ^{19}F NMR δ -149.43 (d, 1F, $^2J_{F,H}$ = 52.3 Hz, $^3J_{F,H}$ = 3.5 Hz, C–6F); LRMS (CI, ammonia) 359 (M^+ + 18, 100); (CI, methane) 342 (M^+ + 1, 67), 185 (100), HRMS calcd for $C_{15}H_{20}O_5N_2FS$ 359.1075, found 359.1074.

Preparation of (2S,5R,6R) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid-4,4-dioxide (6d)

To a solution of **6c** (100 mg, 0.29 mmol) in ethyl acetate (10 mL) was added a suspension of 10% Pd/C in 8.0 mL of ethyl acetate. The resulting suspension was stirred under 1 atmosphere of H_2 for 1 h and then filtered through a Celite pad washed with EtAcO (2 x 3 mL) and dried to give 66 mg (90%) of pure **6d** as a colorless oil: IR (film) 1815 (β-lactam), 1735 (carboxylic acid), 1340 and 1130 (sulfone) cm^{-1}; ^1H NMR ($CDCl_3$–CD_3OD) δ 1.26 (s, 3H), 1.41 (s, 3H), 4.35 (s, 1H, C–3H), 4.75 (dd, 1H, $J_{5,F}$ = 4.0 Hz, $J_{5,6}$ = 4.0 Hz, C–5H), 5.75 (dd, 1H, $J_{6,F}$ = 52.0 Hz, $J_{5,6}$ = 4.0 Hz, C–6H); ^{19}F NMR δ - 147.15 (dd, $^2J_{F,H}$ = 52.3 Hz, $^3J_{F,H}$ = 1.7 Hz, C–6F).

Preparation of benzyl (2S,5R,6S) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (7a)

A similar, previously described procedure[14] was used. Compound **3** was the starting material, yield 48% as a yellow oil: IR (film) 1800 (β-lactam), 1750 (ester) cm^{-1}; ^1H NMR δ 1.39 (s, 3H), 1.53 (s, 3H), 4.54 (s, 1H, C–3H), 5.20 (s, 2H, C–9H), 5.33 (dd, 1H, $J_{6,F}$ = 53.0 Hz, $J_{5,6}$ = 1.6 Hz, C–6H), 5.46 (dd, 1H, $J_{5,F}$ = 4.8 Hz, $J_{5,6}$ = 1.6 Hz, C–5H), 7.37 (s, 5H, Ph–H); ^{13}C NMR δ 166.55 (C-8), 165.3 (d, $^2J_{C,F}$ = 20.1 Hz, C-7), 134.45 (Ph–C), 128.5 (Ph–C), 98.3 (d, $^1J_{C,F}$ = 229.7 Hz, C-6), 68.71 (d, $^2J_{C,F}$ = 25.6 Hz, C-5), 68.08 (C-3), 67.33 (C-9), 64.04 (C-2), 33.59 (C–βMe), 25.19 (C–αMe); ^{19}F NMR δ -170.18 (dd, $^2J_{F,H}$ = 48.8 Hz, $^3J_{F,H}$ = 5.3 Hz, C–6F); LRMS (EI) 309 (M^+, 10.7), 250 (31.3), 190 (7.0), 119 (15.3), 114 (14.6), 91 (100); (CI, ammonia) 327 (M^+ + 18, 100), 282 (49.6), 250 (31.3), 108 (27.3), 91 (50.0). HRMS calcd for $C_{15}H_{16}O_3NFS$ 309.0834, found 309.0842.

Preparation of (2S,5R,6S) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (7b)

This compound was prepared in a manner similar to that described for the synthesis of **4b**; yield 100% as a pale yellow oil: IR (film) 1800 (β-lactam), 1770 (carboxylic acid) cm^{-1}; ^1H NMR δ 1.57 (s, 3H), 1.59 (s, 3H), 4.55 (s, 1H, C–3H), 5.35 (dd, 1H, $J_{6,F}$ = 53.6 Hz, $J_{5,6}$ = 1.6 Hz, C–6H), 5.46 (dd, 1H, $J_{5,F}$ = 4.8 Hz, $J_{5,6}$ = 1.6 Hz, C–5H); ^{13}C NMR (CD_3OD) δ 170.22 (C-8), 167.65 (d, $^2J_{C,F}$ = 20.1 Hz, C-7), 99.38 (d, $^1J_{C,F}$ = 233.0 Hz, C–6H), 70.57 (C-3), 69.91 (d, $^2J_{C,F}$ = 26.6 Hz, C-5), 64.99 (C-2), 33.53 (C–βMe), 26.04 (C–αMe); ^{19}F NMR (CD_3OD) δ -170.33 (dd, $^2J_{F,H}$ = 52.3 Hz, $^3J_{F,H}$ = 5.2 Hz, C–6F).

Preparation of potassium (2S,5R,6S) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (7c)

To a solution of **7b** (19.7 mg, 0.09 mmol) in 2 mL of ethyl acetate, was added 5 mL of ethyl acetate solution

containing potassium 2-ethyl hexanoate (16 mg, 0.09 mmol) under a N_2 atmosphere, and the mixture was stirred; a precipitate formed immediately. The mixture was stirred for an additional 30 min, the solids were centrifuged, washed with ethyl acetate (2 x 3 mL) and dried to give a white solid (19.7 mg, 85%) IR (KBr) 1800 (β-lactam), 1610 and 1420 (carboxylate salt) cm^{-1}; ^1H NMR (CD_3OD) δ 1.54 (s, 6H, α and β Me), 4.27 (s, 1H, C–3H), 5.34 (dd, 1H, $J_{6,F}$ = 53.7 Hz, $J_{5,6}$ = 1.6 Hz, C–6H), 5.47 (dd, 1H, $J_{5,F}$ = 4.8 Hz, $J_{5,6}$ = 1.6 Hz, C–5H); ^{19}F NMR (CD_3OD) δ -169.97 (dd, $^2J_{F,H}$ = 52.3 Hz, $^3J_{F,H}$ = 5.8 Hz, C–6F).

Preparation of benzyl (2S,5R,6S) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4,4-dioxide (7d)

Yield 80% as a colorless oil: IR (film) 1800 (β-lactam), 1760 (ester), 1315, 1120 (sulfone) cm^{-1}; ^1H NMR δ 1.27 (s, 3H), 1.53 (s, 3H), 4.44 (s, 1H, C–3H), 4.75 (dd, 1H, $J_{5,F}$ = 4.8 Hz, $J_{5,6}$ = 1.6 Hz, C–5H), 5.25 (qAB, 2H, J = 12 Hz, C–9H), 5.75 (dd, 1H, $J_{5,F}$ = 53.0 Hz, $J_{5,6}$ = 1.6 Hz, C–6H), 7.38 (s, 5H, Ph–H); ^{13}C NMR δ 165.76 (C-8), 164.71 (d, $^2J_{C,F}$ = 20.3 Hz, C-7), 134.08 (Ph–C), 128.66 (Ph–C), 91.22 (d, $^1J_{C,F}$ = 239.0 Hz, C-6), 68.18 (C-9), 67.38 (d, $^2J_{C,F}$ = 25.8 Hz, C-5), 63.29 (C-2), 62.65 (C-3), 19.55 (C–βMe), 18.17 (C–αMe); ^{19}F NMR δ - 155.9 (dd, $^2J_{F,H}$ = 52.4 Hz, $^3J_{F,H}$ = 5.2 Hz, C–6F); LRMS (CI, ammonia) 359 (M^+ + 18, 100), HRMS calcd for $C_{15}H_{20}O_5N_2FS$ 359.1075, found 359.1072.

Preparation of (2S,5R,6S) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid-4,4-dioxide (7e)

Hydrogenolysis and sulfide oxidation for the preparation of compound **7e** were carried out under the same conditions as described for the synthesis of **6d**. Yield 90% as a colorless oil: IR (film) 1800 (β-lactam), 1720 (carboxylic acid), 1320, 1110 (sulfone) cm^{-1}; ^1H NMR δ 1.51 (s, 3H), 1.62 (s, 3H), 4.45 (s, 1H, C–3H), 4.82 (dd, 1H, $J_{5,F}$ = 4.8 Hz, $J_{5,6}$ = 1.6 Hz, C–5H), 5.78 (dd, 1H, $J_{6,F}$ = 52.0 Hz, $J_{5,6}$ = 1.6 Hz, C–6H); ^{19}F NMR δ -155.83 (dd, 1F, $^2J_{F,H}$ = 52.3 Hz, $^3J_{F,H}$ = 5.2 Hz, C–6F).

Preparation of potassium (2S,5R,6S) 6-fluoro-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4,4-dioxide (7f)

Yield 60% as a white amorphous solid: IR (KBr) 1800 (β-lactam), 1620 and 1400 (carboxylate salt), 1330, 1140 (sulfone) cm^{-1}; ^1H NMR (D_2O) δ 1.44 (s, 3H), 1.55 (s, 3H), 4.33 (s, 1H, C–3H), 5.27 (dd, 1H, $J_{5,F}$ = 4.8 Hz, $J_{5,6}$ = 1.6 Hz), 5.87 (dd, 1H, $J_{6,F}$ = 51.3 Hz, $J_{5,6}$ = 1.6 Hz); (DMSO) δ 1.35 (s, 3H), 1.43 (s, 3H), 3.82 (s, 1H, C–3H), 5.33 (dd, 1H, $J_{5,F}$ = 1.6 Hz), 5.82 (dd, 1H, $J_{6,F}$ = 52.0 Hz, $J_{5,6}$ = 1.6 Hz).

Attempted preparation of potassium 6β-fluoropenicillanate

The method described above for the preparation of the potassium salt of **7c** and **7f** was applied here to give a pale yellow amorphous solid. IR (KBr) 1652; 1616 and 1398 (carboxylate salt) cm^{-1}.

Attempted preparation of potassium 6β-fluoropenicillanate sulfone

A yellow amorphous solid was obtained: IR (KBr) 1656; 1636 and 1398 (carboxylate salt), 1016 and 958 (sulfinate) cm^{-1}; ^1H NMR (D_2O) δ 1.10 (s, 3H), 1.17 (s, 3H), 3.99 (s, 1H), ^{13}C NMR[37] (D_2O) δ 174.51 (C-8), 59.33 (C-3), 58.84 (C-2), 21.50 and 16.10 (C–β and αMe).

Enzymatic methods

For enzymatic studies, β-lactamase I was purified as described by Davies *et al.*[38] from *Bacillus cereus* 569/H. β-Lactamase assays were conducted at 30 °C in 100 mM sodium phosphate buffer, pH 7.0 using 0.1 mM nitrocefin[39] as substrate and 0.6 to 1.2 μg of β-lactamase. Various concentrations of inhibitor were assayed to determine the concentrations required for 50% inhibition (IC_{50}) of nitrocefin hydrolysis, which was measured at 482 nm. Reactions were initiated by addition of substrate without preincubation or after 10 min preincubation of enzyme and inhibitor.

Acknowledgements

We thank CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) for financial support, Professor Eric J. Thomas (University of Manchester, U.K.), Professor M. G. Sierra and Dr J. A. Bacigaluppo (IQUIOS, Rosario, Argentina) for obtaining mass spectral data and NMR assistance, respectively. We also thanks Professor Julio Podesta, Universidad Nacional del Sur, Bahía Blanca, Argentina and Glaxo Pharmaceutical Ltd, Greenford, Middlesex, U.K., for generous gifts of tris (2-methyl-2-phenylpropyl)tin hydride, and nitrocefin, respectively. G.O.D. acknowledges CIUNR (Consejo de Investigaciones Universidad Nacional de Rosario) for a fellowship.

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