Direct Incorporation of a 6α(7α)-Formamido Group into Penicillin and Cephalosporin Sulphides and Sulphoxides

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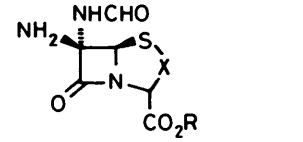
6β(7β)-[*N*-(2,2,2-Trichloroethoxycarbonyl)-*N*-trifluoromethylsulphonyl)amino]-penicillins and -cephalosporins have been converted into the corresponding 6α(7α)-formamido-6β(7β)-(2,2,2-trichloroethoxycarbonylamino) derivatives by treatment with *N,N*-bis(trimethylsilyl)formamide and triethylamine. The trifluoromethyl group could be replaced (in approximately decreasing order of effectiveness) by nonafluorobutyl, pentafluorophenyl, 2,4,5-trichlorophenyl, 2,4-dinitrophenyl, 4-nitrophenyl, *p*-tolyl, and methyl. The 6α(7α)-formamido-(2,2,2-trichloroethoxy)carbonylamino derivatives were oxidised and the structure of the derived α- and β-sulphoxides confirmed by unambiguous synthesis. The antibacterial activity of the α- and β-sulphoxide isomers of the penicillin (**45**) is presented.

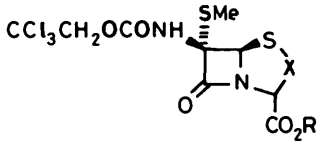
It has been known for some time that a 6α(7α)-methoxy group stabilises the penicillin and cephalosporin nuclei to attack by bacterial β-lactamases, but, this is often at the expense of antibacterial potency.[1] The search for functionalities other than methoxy to overcome this drawback generally resulted in complete loss of activity, or insufficient stability. Our recent disclosure that certain 6α(7α)-formamido-penicillins,[2,3] and -cephalosporins[4] are β-lactamase stable, highly active antibacterial agents was thus a significant step forward. Subsequent to our discovery, cephalosporins[5–10] and monocyclic β-lactams,[11,12] similarly substituted with a formamido moiety, have been isolated from various strains of bacteria.

We required a range of acylated derivatives for structure–activity studies and the amines (**1**) and (**2**) were therefore selected as the most appropriate precursors. In our initial investigations,[13] the 6α(7α)-methylthio derivatives (**3**) and (**4**) were found to be convenient substrates for the introduction of the 6α(7α)-formamido group, *via* a mercury(II) mediated displacement in the presence of *N,N*-bis(trimethylsilyl)-formamide (BSF). The carbamate protecting group was then removed by treatment with freshly activated zinc in tetrahydrofuran (THF)–potassium dihydrogen phosphate solution.[14] We report a more direct route, avoiding prior 6α(7α)-functionalisation of the β-lactam. The synthesis, structural confirmation, and biological activity of the α- and β-sulphoxides derived from oxidation of the ring sulphur of some 6α(7α)-formamido derivatives is also described.

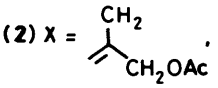
The bistrifluoromethanesulphonamide (**5**) has been shown to give the 6α-methoxytrifluoromethanesulphonamide (**9**) on reaction in methanol containing an excess of triethylamine.[15] Exclusive nucleophilic addition to the less hindered face of putative imine intermediates analogous to (**10**) has been well documented.[16] It was, therefore, surmised that if a derivative of type (**6**) could be prepared, subsequent reaction with BSF and triethylamine might afford the desired 6α-formamidopenicillin (**11**).[17] Accordingly the trifluoromethanesulphonamide (**7**)[18] was treated with 2,2,2-trichloroethoxycarbonyl chloride and triethylamine in dichloromethane (DCM). The reaction was extremely sluggish, being incomplete after 40 h at room temperature. However, the addition of 4-dimethylaminopyridine (DMAP) (0.1 equiv.) afforded the required product (**6**) in 91% yield. Treatment of (**6**) with freshly distilled BSF and triethylamine in DCM then provided the crystalline carbamate (**11**) (84%), identical in all respects to that obtained *via* the 6α-methylthio derivative (**3**).[13]



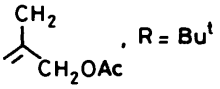
(**1**) X = Pr^i, R = CH_2Ph



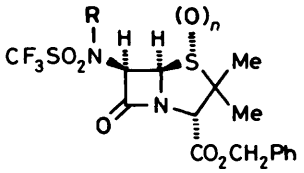
(**3**) X = Pr^i, R = CH_2Ph



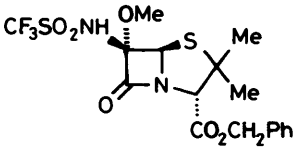
(**2**) X = , R = Bu^t



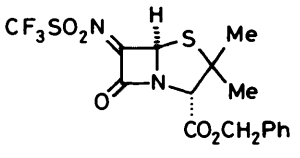
(**4**) X = , R = Bu^t



(**5**) R = SO_2CF_3, *n* = 0
(**6**) R = $CO_2CH_2CCl_3$, *n* = 0
(**7**) R = H, *n* = 0
(**8**) R = $CO_2CH_2CCl_3$, *n* = 1

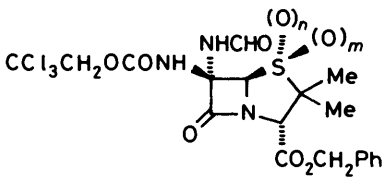


(**9**)



(**10**)

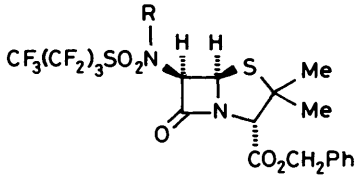
Since nonafluorobutylsulphonyl is reported[19] to be a better leaving group than trifluoromethylsulphonyl, the use of compound (**14**) was examined. The reaction of freshly prepared nonafluorobutanesulphonic anhydride[20] with the amine (**17**) proved somewhat capricious, giving mediocre, inconsistent yields of two products (**15**) (7—23%) and (**16**) (6—30%). The desired nonafluorobutanesulphonamide (**15**) was converted into the *N,N*-disubstituted derivative (**14**) using the established methodology. Reaction of (**14**) with BSF–triethylamine gave the 6α-formamidopenicillanate (**11**) (68%), but offered no advantage in rate or yield over the trifluoromethanesulphonamide route.



(11) $m = n = 0$

(12) $m = 0$, $n = 1$

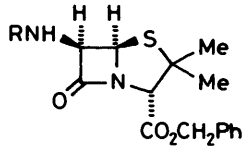
(13) $m = 1$, $n = 0$



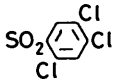
(14) R = $CO_2CH_2CCl_3$

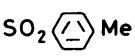
(15) R = H

(16) R = $SO_2(CF_2)_3CF_3$



(17) R = H

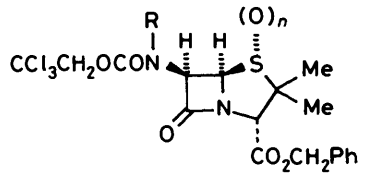
(18) R = 

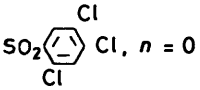
(19) R = 

The utility of groups less electron-withdrawing than trifluoromethylsulphonyl was also investigated. The arenesulphonamides (**20**) and (**21**) were prepared conventionally from the amine (**17**) *via* the respective mono derivatives (**18**) and (**19**).[21] Under the standard conditions, formamido incorporation was only observed in the case of the trichlorophenyl derivative (**20**), the toluene-*p*-sulphonamide (**21**) slowly decomposing to non-β-lactam containing products.

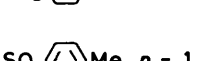
The established methodology was then extended to the cephalosporin series for an essentially similar programme of investigation. The trifluoromethanesulphonamide (**23**) was converted into the *N*,*N*-disubstituted derivative (**24**) which was treated with an excess of BSF in DCM containing triethylamine (1.1 equiv.) for 16 h at room temperature. The product was an inseparable mixture of the Δ^3 (**26**) and Δ^2 (**29**) isomers with the latter, unwanted isomer in preponderance. A slightly lower yield of a mixture containing substantially the desired isomer (**26**) was obtained when the quantity of triethylamine was increased and the reaction temperature and time reduced.

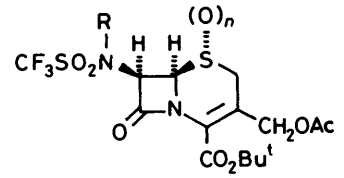
A range of leaving groups, pentafluorophenyl, 2,4-dinitrophenyl, 4-nitrophenyl, and methylsulphonyl, was then investigated. The sulphonamides (**30**)—(**33**) were prepared and converted into the corresponding *N*,*N*-disubstituted derivatives (**34**)—(**37**), which were treated with an excess of BSF–



(20) R = 

(21) R = 

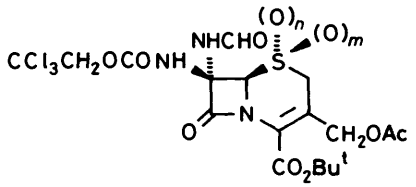
(22) R = 



(23) R = H, $n = 0$

(24) R = $CO_2CH_2CCl_3$, $n = 0$

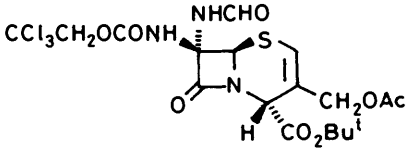
(25) R = $CO_2CH_2CCl_3$, $n = 1$



(26) $m = n = 0$

(27) $m = 0$, $n = 1$

(28) $m = 1$, $n = 0$



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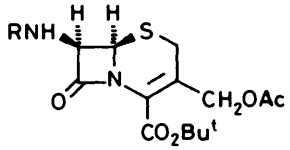
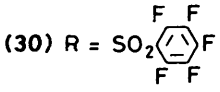
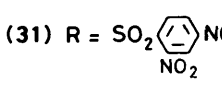
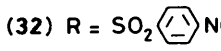
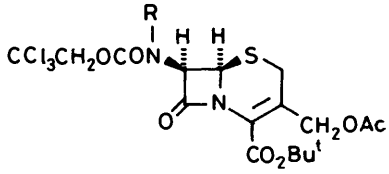


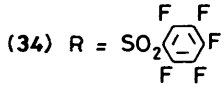
(30) R = 

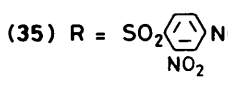
(31) R = 

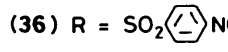
(32) R = 

(33) R = SO_2Me

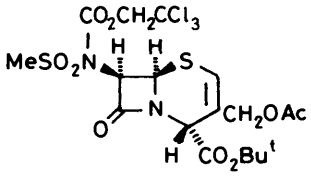


(34) R = 

(35) R = 

(36) R = 

(37) R = SO_2Me



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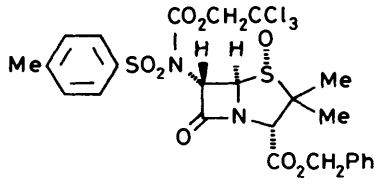


(39)

triethylamine [room temp., 7 h]. The aryl derivatives (**34**)—(**36**) gave a 2:1 mixture [3:2 in the case of (**35**)] of (**26**) and (**29**) in 62, 34, and 10% yields respectively. Further isolated products which were, in general, inseparable mixtures, were derived from double bond isomerisation and partial concomitant C-7 epimerisation. In the case of the methanesulphonamide (**37**) incomplete isomerisation to the corresponding Δ^2 isomer (**38**) was the only observed reaction.

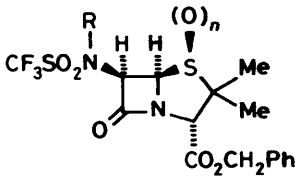
Contemporaneously with this work we had been investigating the oxidation of 6α(7α)-formamido-penicillins and -cephalosporins. In the former series oxidation of the carbamate (**11**) with *m*-chloroperbenzoic acid in 1,2-dichloroethane gave a mixture of sulphoxides in approximately a 2:1 ratio. The more polar (t.l.c.) major product could be crystallised from the mixture and the pure second component isolated by chromatography. The structures were unambiguously assigned as follows. Oxidation of the *N*,*N*-disubstituted derivative (**6**) with peracetic acid gave a single compound the α-(or *R*) sulphoxide (**8**) (82%), the approach of the oxidant being governed by steric factors as has been previously observed with phthalimidopenicillins.[22]

The sulphoxide (**8**) reacted faster with BSF–triethylamine in DCM than the corresponding sulphide (**6**) and after 40 min at −10 °C to −5 °C the authentic α-oxide (**12**) was isolated in 76% yield. The material was identical with the more polar sulphoxide derived *via* oxidation of the sulphide (**11**). The acceleration in the rate of formamido incorporation brought about by the oxidation of the thiazolidine sulphur was not unexpected since it is well documented that the 6-H is more acidic in penicillin sulphoxides than in the corresponding sulphides.[23] It was of interest therefore to ascertain whether such increased acidity would be sufficient to promote formamido incorporation in a derivative where the parent sulphide was unreactive. Accordingly the toluene-*p*-sulphonamide (**21**) was treated with peracetic acid to provide the α-oxide (**22**). Reaction of (**22**) with BSF–triethylamine did indeed afford the 6α-formamido sulphoxide (**12**) (45%), and some C(6)-epimerised material (**39**) (40%), in contrast to the behaviour of the equivalent sulphide (**21**) (*vide supra*).

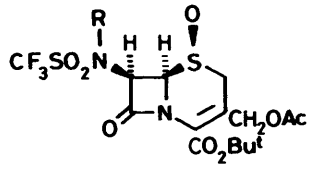
An authentic sample of the β- (or *S*-) oxide (**13**) was prepared *via* the trifluoromethanesulphonamide (**40**). Oxidation of the latter (**40**) afforded the β-oxide (**41**) as the sole product. Attack is directed from the β-face due to steric approach control, in which the side-chain NH hydrogen bonds to the oxidant. Reaction of the sulphonamide (**41**) with 2,2,2-trichloroethoxycarbonyl chloride–pyridine in DCM for 1.5 h at 0 °C then gave the *N*,*N*-disubstituted derivative (**42**) (95%). BSF–triethylamine treatment of (**42**) (DCM; −10 °C to −5 °C; 20 min) finally provided the desired β-oxide (**13**) (75%), which was identical with the less polar isomer formed by oxidation of the sulphide (**11**).

In the cephalosporin series oxidation of the sulphide (**26**) with peracetic acid gave a mixture of sulphoxides in approximately a 4:3 ratio, with the more polar (t.l.c.) predominating. The less-polar (t.l.c.) isomer was tentatively assigned the β-oxide configuration by analogy with the penicillin series. In addition, the ^{1}H n.m.r. spectrum of this isomer only, showed coupling (*ca.* 1.5 Hz) between 6-H and 2α-H. Such coupling has been reported to be unique for β-sulphoxides of 7α-H cephalosporins.[24] Nevertheless it was still considered worthwhile to examine the preparation of authentic samples of both sulphoxides (**27**) and (**28**). Accordingly, as previously described for the penicillin series, the trifluoromethanesulphonamide (**23**) was oxidised to provide the corresponding β-sulphoxide (**43**) (90%) as the sole product (6-H to 2α-H coupling present). Acylation of (**43**) with 2,2,2-trichloroethoxycarbonyl chloride (DCM–pyridine–DMAP/1 h) gave the *N*,*N*-disubstituted derivative (**44**) (63%) (6-H to 2α-H coupling present). Unfortunately, treatment of (**44**) with BSF–triethylamine or BSF–pyridine under a variety of conditions, rapidly caused cleavage to non-β-lactam material. No β-sulphoxide (**28**) was formed. However, the preparation of authentic α-sulphoxide (**27**) was more successful. Oxidation of the *N*,*N*-disubstituted amine (**24**) gave a single compound (72%), the α-sulphoxide (**25**) (no 6-H to 2α-H coupling in the n.m.r. spectrum). In a similar vein, Micetich[25] has recently reported the exclusive oxidation of 7-diacylaminocephems to their corresponding 1α-oxides. Reaction of (**25**) with BSF–triethylamine then gave the α-oxide (**27**) in low yield (25%). The material was identical with the more polar isomer derived from (**26**) thus confirming our earlier assignments.

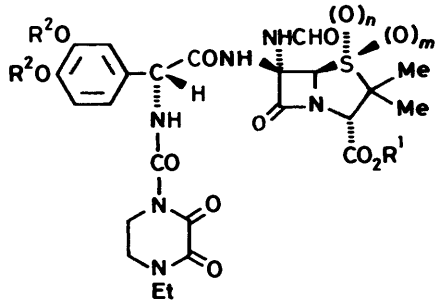
The stereochemistry of the protected derivatives (**12**), (**13**), (**27**) and (**28**) having been established, we now turned our attention to the preparation of compounds more appropriate for biological evaluation. In the sulphide series we had identified BRL 36650 (**45**)[2] as one of the most active 6α-formamido-penicillins, so it was logical to examine the preparation of the corresponding sulphoxides. The trichlorethoxycarbonyl protecting group in the α-sulphoxide (**12**) was removed with zinc in



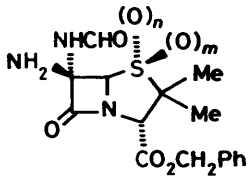
(**40**) R = H, *n* = 0
(**41**) R = H, *n* = 1
(**42**) R = $CO_2CH_2CCl_3$, *n* = 1



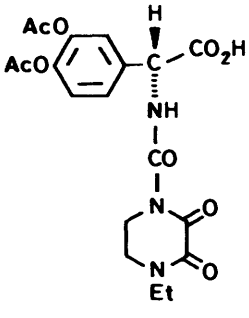
(**43**) R = H
(**44**) R = $CO_2CH_2CCl_3$



(**45**) R^1 = Na, R^2 = H, *m* = *n* = 0
(**46**) R^1 = CH_2Ph, R^2 = Ac, *m* = 0, *n* = 1
(**47**) R^1 = Na, R^2 = Ac, *m* = 0, *n* = 1
(**48**) R^1 = CH_2Ph, R^2 = Ac, *m* = *n* = 0
(**49**) R^1 = CH_2Ph, R^2 = Ac, *m* = 1, *n* = 0
(**50**) R^1 = Na, R^2 = Ac, *m* = 1, *n* = 0



(**51**) *m* = 0, *n* = 1
(**52**) *m* = 1, *n* = 0



(**53**)

Table. Antibacterial activity of 6α-formamidopenicillin sulphoxides[a]

Organism	(50)	(47)	(45)
Escherichia coli NCTC 10418	0.25	≤0.06	≤0.03
E. coli JT4[b]	0.25	≤0.06	≤0.03
E. coli JT425[c]	4	1	0.25
Enterobacter cloacae NI	8	1	0.12
Klebsiella aerogenes A	0.5	0.12	≤0.03
Serratia marcescens US32	16	1	0.25
Proteus mirabilis C977	4	0.5	0.12
Pseudomonas aeruginosa NCTC 10662	16	2	1
P. aeruginosa Dalgleish[b]	32	4	0.5

[a] MICs (μg ml^{-1}) determined by serial dilution in nutrient agar containing 5% defibrinated horse blood, inoculum 0.001 ml of an undiluted overnight broth culture. [b] Plasmid-mediated β-lactamase-producing strain. [c] Constitutive chromosomally controlled β-lactamase-producing strain.

aqueous THF–potassium dihydrogen phosphate to give an 86% yield of the nucleus (**51**) which was fairly stable to chromatography. The dicyclohexylcarbodi-imide promoted coupling of (**51**) to the D-diacetoxyphenylglycine derivative (**53**) was not so efficient as in the sulphide series[13] and (**46**) was isolated in only 22% yield. Catalytic hydrogenation readily provided the sodium salt (**47**).

The β-sulphoxide series caused more problems because deprotection of the trichloroethyl carbamate (**13**) gave a very unstable free amine (**52**), which decomposed during the reaction. Therefore, an alternative approach was adopted. Oxidation of (**48**)[13] with *m*-chloroperbenzoic acid in DCM gave a 3:1 mixture of α- and β- oxides (**46**) and (**49**). Separation of the diastereoisomers was extremely difficult and the problems were potentiated by the instability of the β-isomer (**49**) to chromatography. Eventually a small amount of β-oxide (**49**) was isolated by using ethyl acetate–dioxane mixtures as eluant. Hydrogenation then gave the sodium salt (**50**). Both sulphoxides (**47**) and (**50**) exhibited activity against Gram-negative bacteria with the α-sulphoxide (**47**) being the more potent, and only slightly less active than the parent sulphide (**45**) (see Table). The antibacterial activity of a diacetoxy derivative is generally the same as the parent catechol, since acetate esters are hydrolysed under the conditions of the *in vitro* test.

The synthesis and biological activity of some 7α-formamidoceph-3-em 1-oxides has been recently disclosed,[26] the relative potencies of the isomers essentially paralleling those observed in the penicillin series.

Experimental

M.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were recorded for chloroform solutions and Nujol mulls on a Perkin-Elmer 197 spectrophotometer and for KBr discs on Perkin-Elmer 457 or Perkin-Elmer 983 grating spectrophotometers. ^{1}H N.m.r. spectra were recorded at 90 MHz on a Perkin-Elmer R32, and at 250 MHz on a Bruker WM250 instrument, for solutions in $CDCl_3$ with tetramethylsilane as internal standard unless otherwise stated. Where two rotameric forms were observed in the ^{1}H n.m.r. spectra, only the major, *Z*, rotamer is quoted. Mass spectra were recorded on either a VG7070 or a VG ZAB spectrometer operating in the electron-impact mode. Fast-atom-bombardment (f.a.b.) spectra were recorded on a VG ZAB spectrometer and the matrix used is stated. The homogeneity of all esters was tested by t.l.c. on Schleicher and Schuell plastic pre-coated silica gel F1500/LS254 plates and of all sodium salts by analytical h.p.l.c. on a Waters μ Bondapak™ C_{18} reverse-phase column eluting with ammonium acetate–methanol–water mixtures. Preparative chromatography was carried out on columns of either Merck silica gel 60 (finer than 230 mesh) with increased pressure provided by a Medcalf Hy-flo pump, or on 'Diaion HP20SS' resin (Mitsubishi Chemical Corp.). Solutions were dried with magnesium sulphate or sodium sulphate, and solvents were removed by evaporation under reduced pressure below 30 °C on a Buchi rotary evaporator.

Benzyl 6β-[N-(2,2,2-*Trichloroethoxycarbonyl*)-N-*trifluoromethylsulphonylamino*]*penicillanate* (**6**).—The trifluoromethanesulphonamide[18] (**5**) (2.58 g) was dissolved in dry dichloromethane (DCM) (80 ml) at 0 °C and triethylamine (0.893 g) was added, followed by 4-dimethylaminopyridine (0.72 g). The cooling bath was removed. After 2 h the solution was poured into ethyl acetate and washed successively with dilute hydrochloric acid, water, dilute aqueous sodium hydrogen carbonate, and brine, dried, and evaporated. Chromatography of the residue on silica gel afforded the *penicillanate* (**6**) as an oil (3.27 g, 91%) which slowly crystallised, m.p. 89—90 °C (from EtOAc–hexane) (Found: C, 37.2; H, 3.1; Cl, 16.9; N, 4.6; S, 10.2. $C_{19}H_{18}Cl_3F_3N_2O_7S_2$ requires C, 37.2; H, 2.9; Cl, 17.3; N, 4.6; S, 10.4%); $\nu_{max.}$($CHCl_3$) 1 795, 1 785, 1 745, 1 420, and 1 130 cm^{-1}; δ_H(250 MHz) 1.42 and 1.67 (6 H, 2 s), 4.54 (1 H, s), 4.86 and 4.95 (2 H, ABq, *J* 12 Hz), 5.20 (2 H, AA′), 5.5 (1 H, d, *J* 4 Hz), 5.55 (1 H, d, *J* 4 Hz), and 7.38 (5 H, s).

Benzyl 6β-(*Nonafluorobutylsulphonylamino*)*penicillanate* (**15**).—Benzyl 6-aminopenicillanate (**17**) [ex. benzyl 6-aminopenicillanate toluene-*p*-sulphonic acid salt (466 mg)] was dissolved in anhydrous DCM (5 ml), the solution cooled to −60 °C under argon, and triethylamine (109 mg) added, followed by nonafluorobutanesulphonic anhydride (624 mg). The reaction mixture was allowed to warm to 0 °C over 1 h, diluted with ethyl acetate, and washed successively with dilute hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and brine, dried, and evaporated. Chromatography of the residue on silica gel gave two products, benzyl 6β-(*N*,*N*-bisnonafluorobutylsulphonylamino)penicillanate (**16**) (54 mg, 6%); $\nu_{max.}$($CHCl_3$) 1 795, 1 750, 1 390, and 1 140 cm^{-1}; δ_H(250 MHz) 1.44 (3 H, s), 1.63 (3 H, s), 4.52 (1 H, s), 5.20 (2 H, s), 5.26 (1 H, d, *J* 4.2 Hz), 5.58 (1 H, d, *J* 4.2 Hz), and 7.37 (5 H, s) and the *nonafluorobutanesulphonamide* (**15**) as an amorphous solid (130 mg, 23%) (Found: M^+, 588.0441. $C_{19}H_{17}F_9N_2O_5S_2$ requires *M*, 588.0436); $\nu_{max.}$($CHCl_3$) 3 345, 1 795, 1 745, 1 390, and 1 140 cm^{-1}; δ_H(250 MHz) 1.44 (3 H, s), 1.63 (3 H, s), 4.52 (1 H, s), 5.20 (2 H, s), 5.26 (1 H, d, *J* 4.2 Hz), 5.58 (1 H, d, *J* 4.2 Hz), and 7.38 (5 H, s).

Benzyl 6β-[N-(2,2,2-*Trichloroethoxycarbonyl*)-N-*nonafluorobutylsulphonylamino*]*penicillanate* (**14**).—The nonafluorobutanesulphonamide (**15**) (169 mg) was dissolved in dry DCM (5 ml) at 0 °C and 2,2,2-(trichloroethoxy)carbonyl chloride (121 mg), triethylamine (44 mg), and 4-dimethylaminopyridine (3.5 mg) added successively. The reaction mixture was removed from the cooling bath, stirred for 2 h, and poured into ethyl acetate. The organic phase was washed with dilute hydrochloric acid, dilute aqueous sodium hydrogen carbonate, and brine, dried, and evaporated. Chromatography of the residue on silica gel afforded the penicillanate (**14**) as an amorphous solid (121 mg, 55%); $\nu_{max.}$($CHCl_3$) 1 798, 1 780, 1 740, 1 350, and 1 140 cm^{-1}; δ_H(250 MHz) *inter alia* 1.43 (3 H, s), 1.69 (3 H, s), 4.57 (1 H, s), 4.85 and 4.95 (2 H, ABq, *J* 11.7 Hz), 5.19 (2 H, AA′), 5.54 (2 H, s), and 7.38 (5 H, s); *m*/*z* (c.i.; Cl_2 gas) 797, $[M + Cl]^+$.

Benzyl 6β-(2,4,5-*Trichlorophenylsulphonylamino*)*penicillanate* (**18**).—Benzyl 6-aminopenicillanate (**17**) [generated from benzyl 6-aminopenicillanate toluene-*p*-sulphonic acid salt (2.39 g)] was

dissolved in dry DCM (100 ml) and pyridine (0.87 g) added, followed by 2,4,5-trichlorobenzenesulphonyl chloride (1.54 g). After 4 days at room temperature the solution was washed successively with dilute hydrochloric acid and brine, dried, and evaporated. Chromatography of the residue on silica gel gave the *sulphonamide* (**18**) as an amorphous solid (1.78 g, 65%) (Found: M^+, 547.9807. $C_{21}H_{19}Cl_3N_2O_5S_2$ requires M, 547.9801); $\nu_{max.}$($CHCl_3$) 1 790, 1 740, 1 360, and 1 175 cm^{-1}; δ_H(90 MHz) 1.39 (3 H, s), 1.58 (3 H, s), 4.41 (1 H, s), 5.08 (1 H, dd, J 4 and 10 Hz), 5.15 (2 H, s), 5.48 (1 H, d, J 4 Hz), 5.93 (1 H, d, J 10 Hz, exch. D_2O), 7.34 (5 H, s), 7.63 (1 H, s), and 8.15 (1 H, s).

Benzyl 6β-[N-(2,2,2-*Trichloroethoxycarbonyl*)-N-(2,4,5-*trichlorophenylsulphonyl*)*amino*]*penicillanate* (**20**).—The sulphonamide (**18**) (1.099 g) dissolved in dry DCM (20 ml) at 0 °C was treated successively with 2,2,2-trichloroethoxycarbonyl chloride (0.844 g) and pyridine (0.174 g). The cooling bath was removed and after 1.5 h the solution was poured into ethyl acetate and washed with dilute hydrochloric acid and brine, dried, and evaporated. Chromatography of the residue on silica gel gave the *penicillanate* (**20**) as a white crystalline solid (1.36 g, 93%), m.p. 157—158 °C (EtOAc–hexane) (Found: C, 39.7; H, 2.8; Cl, 29.5; N, 3.8; S, 8.8. $C_{24}H_{20}Cl_6N_2O_7S_2$ requires C, 39.7; H, 2.8; Cl, 29.4; N, 3.9; S, 8.8%); $\nu_{max.}$(Nujol) 1 805, 1 790, 1 745, 1 380, and 1 160 cm^{-1}; δ_H(90 MHz) 1.43 (3 H, s), 1.67 (3 H, s), 4.55 (1 H, s), 4.62 and 4.81 (2 H, ABq, J 12 Hz), 5.19 (2 H, s), 5.58 (1 H, d, J 4 Hz), 5.92 (1 H, d, J 4 Hz), 7.35 (5 H, s), 7.63 (1 H, s), and 8.32 (1 H, s).

Benzyl 6β-(p-*Tolylsulphonylamino*)*penicillanate* (**19**).—Benzyl 6-aminopenicillanate toluene-*p*-sulphonic acid salt (2.39 g) was suspended in DCM (25 ml) at −20 °C and triethylamine (1.11 g) added, followed by toluene-*p*-sulphonyl chloride (1.05 g). The solution was allowed to warm to room temperature. After 70 h the reaction mixture was poured into ethyl acetate and washed successively with dilute hydrochloric acid, brine, aqueous sodium hydrogen carbonate, and brine, dried, and evaporated. Chromatography of the residue on silica gel gave the sulphonamide (**19**) as a white crystalline solid (1.25 g, 76%), m.p. 122—123 °C (acetone–hexane) (lit.,[21] 128.1—129.8 °C); $\nu_{max.}$($CHCl_3$) 1 790, 1 740, 1 350, and 1 160 cm^{-1}; δ_H(90 MHz) 1.32 (3 H, s), 1.52 (3 H, s), 2.39 (3 H, s), 4.38 (1 H, s) 5.02 (1 H, dd, J 4 and 11 Hz), 5.29 (1 H, d, J 4 Hz), 5.48 (1 H, d, J 1 Hz), 7.25 (2 H, d, J 8 Hz), 7.3 (5 H, s), and 7.72 (2 H, d, J 8 Hz).

Benzyl 6β-[N-(2,2,2-*Trichloroethoxycarbonyl*)-N-(p-*tolylsulphonyl*)*amino*]*penicillanate* (**21**).—The sulphonamide (**19**) (0.920 g) was converted into the *penicillanate* (**21**) as described for (**18**). The product was an amorphous solid (1.0 g, 79%) (Found: M^+, 634.0156. $C_{25}H_{25}Cl_3N_2O_7S_2$ requires M, 634.0169); $\nu_{max.}$($CHCl_3$) 1 790, 1 745, 1 380, and 1 160 cm^{-1}; δ_H(90 MHz) 1.31 (3 H, s), 1.64 (3 H, s), 2.42 (3 H, s), 4.52 (1 H, s), 4.62 and 4.78 (2 H, ABq, J 12 Hz), 5.16 (2 H, s), 5.55 (1 H, d, J 4 Hz), 5.66 (1 H, d, J 4 Hz), 7.25 (2 H, d, J 8 Hz), and 7.89 (2 H, d, J 8 Hz).

Benzyl 6α-*Formamido*-6β-(2,2,2-*trichloroethoxycarbonylamino*)*penicillanate* (**11**).—Benzyl 6β-[*N*-(2,2,2-trichloroethoxycarbonyl)-*N*-trifluoromethylsulphonylamino]penicillanate (**6**) (613 mg) in DCM (20 ml) was cooled to −50 °C and triethylamine (150 mg) and *N*,*N*-bis(trimethylsilyl)formamide (378 mg; freshly distilled) were added. The cooling bath was removed. After 1.75 h the solution was poured into ethyl acetate and washed successively with dilute hydrochloric acid and brine, dried, and evaporated. Chromatography on silica gel afforded the 6β-formamidopenicillanate (**11**) (441 mg, 84%), identical with authentic material.[13] Similarly the *N*,*N*-disubstituted derivatives (**14**) and (**20**) gave the 6α-formamidopenicillanate (**11**) (68 and 15% respectively), the reaction of (**20**) being left for 7 h at room temperature before work-up.

t-Butyl 7β-(*Trifluoromethylsulphonylamino*)*cephalosporanate* (**23**).—t-Butyl 7-aminocephalosporanate (3.28 g) in DCM (100 ml) at −65 °C was treated successively with triethylamine (1.1 g) and trifluoromethanesulphonic anhydride (3.1 g). After 30 min at −65 °C, the reaction mixture was washed with dilute hydrochloric acid, followed by brine. The solution was dried and the solvent distilled off under reduced pressure to give the *trifluoromethanesulphonamide* (**23**) as a pale yellow solid (4.5 g, 98%), m.p. 170—171 °C (decomp.) (from EtOAc–hexane) (Found: C, 39.3; H, 4.0; N, 6.1; S, 13.5. $C_{15}H_{19}F_3N_2O_7S_2$ requires C, 39.1; H, 4.1; N, 6.1, S, 13.9%); $\nu_{max.}$(Nujol) 1 820, 1 735, 1 690, and 1 640 cm^{-1}; δ_H(90 MHz) 1.52 (9 H, s), 2.07 (3 H, s), 3.45 (2 H, AA′), 4.87 and 5.05 (2 H, ABq, J 13 Hz), 4.87 (1 H, d, J 5 Hz), 5.08 (1 H, d, J 5 Hz), and 6.5—8.5 (1 H, vbr s, exch. D_2O).

t-Butyl 7β-[N-(2,2,2-*Trichloroethoxycarbonyl*)-N-*trifluoromethylsulphonylamino*]*cephalosporanate* (**24**).—The trifluoromethanesulphonamide (**23**) (920 mg) in dry DCM (30 ml) at −20 °C was treated successively with 2,2,2-trichloroethoxycarbonyl chloride (848 mg), triethylamine (303 mg), and 4-dimethylaminopyridine (25 mg). The cooling bath was removed and the solution stirred at room temperature for 3 h. It was then poured into ethyl acetate and washed successively with dilute hydrochloric acid, water, dilute aqueous sodium hydrogen carbonate, and brine. The dried organic layer was evaporated and the residue chromatographed on silica gel to give the *cephalosporanate* (**24**) as a crystalline solid (1.15 g, 90%), m.p. 96—97 °C (hexane) (Found: C, 34.0; H, 3.3; Cl, 16.7; N, 4.1; S, 10.0. $C_{18}H_{20}Cl_3F_3N_2O_9S_2$ requires C, 34.00; H, 3.1; Cl, 16.8; N, 4.4; S, 10.1%); $\nu_{max.}$($CHCl_3$) 1 795, 1 780sh, 1 735sh, 1 720, 1 420, and 1 150 cm^{-1}; δ_H(90 MHz) 1.53 (9 H, s), 2.08 (3 H, s), 3.45 (2 H, s), 4.82 and 5.13 (2 H, ABq, J 13 Hz), 4.99 (2 H, AA′ system), 5.08 (1 H, d, J 5 Hz), and 5.62 (1 H, d, J 5 Hz).

t-Butyl 7β-(*Pentafluorophenylsulphonylamino*)*cephalosporanate* (**30**).—t-Butyl 7-aminocephalosporanate (371 mg) was dissolved in dry DCM (4 ml) at −20 °C and pentafluorophenylsulphonyl chloride (305 mg) added, followed by pyridine (86 mg). After 30 min the cooling-bath was removed and the solution left at room temperature for 21 h. The solution was diluted with ethyl acetate and washed successively with dilute hydrochloric acid, aqueous sodium hydrogen carbonate, and brine, dried, and evaporated. Chromatography of the residue on silica gel eluting with ethyl acetate–DCM mixtures gave *pentafluorophenylsulphonamide* (**30**) as a crystalline solid (332 mg, 55%), m.p. 204—205 °C (EtOAc–hexane) (Found: C, 43.1; H, 3.7; N, 4.7; S, 11.3. $C_{20}H_{19}F_5N_2O_7S_2$ requires C, 43.0; H, 3.4; N, 5.0; S, 11.5%); $\nu_{max.}$(Nujol) 3 100, 1 810, 1 730, 1 700, 1 640, 1 460, 1 160, and 980 cm^{-1}; δ_H(90 MHz) 1.48 (9 H, s), 2.03 (3 H, s), 3.3 and 3.57 (2 H, ABq, J 18 Hz), 4.75 and 5.09 (2 H, ABq, J 14 Hz), 4.93 (1 H, d, J 5 Hz), 5.4 (1 H, slightly broadened, d, J 5 Hz), and 6.40 (1 H, s, exch D_2O).

t-Butyl 7β-[N-*Pentafluorophenylsulphonyl*-N-(2,2,2,-*trichloroethoxycarbonyl*)*amino*]*cephalosporanate* (**34**).—The pentafluorophenylsulphonamide (**30**) (246 mg) was dissolved in DCM (10 ml) at −20 °C and 2,2,2-trichloroethoxycarbonyl chloride (186 mg) added, followed by pyridine (52 mg). The solution was diluted with DCM, washed with dilute hydrochloric acid followed by brine, dried, and evaporated. Chromatography of the residue on silica gel gave the *cephalosporanate* (**34**) as a white solid (315 mg, 97%) after trituration with ether–hexane, m.p. 155 °C (EtOAc–hexane)

(Found: C, 38.1; H, 2.9; Cl, 14.2; N, 3.9; S, 8.9. $C_{23}H_{20}Cl_3F_5N_2O_9S_2$ requires C, 37.6; H, 2.7; Cl, 14.5; N, 3.8; S, 8.7%); $\nu_{max.}$(CHCl$_3$) 1 790, 1 730br, 1 635, 1 500, 1 160, and 990 cm^{-1}; δ_H (90 MHz) 1.53 (9 H, s), 2.04 (2 H, s), 3.42 (2 H, AA'), 4.77 (2 H, AA'), 4.77 and 5.05 (2 H, ABq, *J* 13 Hz), 5.08 (1 H, d, *J* 5 Hz), and 5.96 (1 H, d, *J* 5 Hz).

t-Butyl 7β-(2,4-*Dinitrophenylsulphonylamino*)*cephalosporanate* (**31**).—t-Butyl 7-aminocephalosporanate (742 mg) dissolved in dry DCM (15 ml) at −10 °C was treated with pyridine (173 mg) and 2,4-dinitrobenzenesulphonyl chloride (588 mg). The cooling-bath was removed and the solution stirred at room temperature for 16 h. It was then poured into ethyl acetate and washed successively with dilute hydrochloric acid, brine, dilute aqueous sodium hydrogen carbonate, and brine (×2), dried, and evaporated. Chromatography on silica gel eluting with ethyl acetate–DCM mixtures gave the *arenesulphonamide* (**31**) as a solid (588 mg, 46%), m.p. 194 °C (decomp.) (from EtOAc–hexane) (Found: C, 43.5; H, 3.9; N, 10.1; S, 11.5. $C_{20}H_{22}N_4O_{11}S_2$ requires C, 43.0; H, 3.9; N, 10.0; S, 11.5%); $\nu_{max.}$(Nujol) 3 225, 3 090, 1 795, 1 730, 1 710, 1 535, 1 450, 1 365, and 1 170 cm^{-1}; δ_H[90 MHz, $CDCl_3$ + $(CD_3)_2SO$] 1.49 (9 H, s), 2.02 (3 H, s), 3.12 and 3.48 (2 H, ABq, *J* 18 Hz), 4.72 and 5.03 (2 H, ABq, *J* 13 Hz), 4.96 (1 H, d, *J* 4.5 Hz), 5.38 (1 H, d, *J* 4.5 Hz), and 8.4—8.65 (3 H, m), SO_2NH is very broad and not clearly visible.

t-Butyl 7β-[N-(2,4-*Dinitrophenylsulphonyl*)-N-(2,2,2,-*trichloroethoxycarbonyl*)*amino*]*cephalosporanate* (**35**).—The 2,4-dinitrobenzenesulphonamide (**31**) (279 mg) was converted into the *cephalosporanate* (**35**) as described for the preparation of compound (**34**). The product (**35**) was an amorphous solid (360 mg, 96%) (Found: C, 37.5; H, 2.9; N, 7.5. $C_{23}H_{23}Cl_3N_4O_{13}S_2$ requires C, 37.6; H, 3.1; N, 7.6%); $\nu_{max.}$(CHCl$_3$) 1 790, 1 738, 1 730, 1 545, 1 345, 1 170, and 1 150 cm^{-1}; δ_H(250 MHz) 1.55 (9 H, s), 2.09 (3 H, s), 3.40 and 3.57 (2 H, ABq, *J* 17.9 Hz), 4.72 and 4.81 (2 H, ABq, *J* 11.8 Hz), 4.80 and 4.99 (2 H, ABq, *J* 13 Hz), 5.2 (1 H, d, *J* 5.1 Hz), 5.99 (1 H, d, *J* 5.1 Hz), and 8.59—8.76 (3 H, m).

t-Butyl 7β-(4-*Nitrophenylsulphonylamino*)*cephalosporanate* (**32**).—t-Butyl 7-aminocephalosporanate (1.48 g) was treated with 4-nitrobenzenesulphonyl chloride using the procedure described for the preparation of (**18**). The *4-nitrobenzenesulphonamide* (**32**) was isolated as a crystalline solid (1.6 g, 58%), m.p. 217—219 °C (decomp. from EtOAc) (Found: C, 46.8; H, 4.4; N, 8.1; S, 12.8. $C_{20}H_{23}N_3O_9S_2$ requires C, 46.8; H, 4.5; N, 8.2; S, 12.5%); $\nu_{max.}$(Nujol) 1 795, 1 740, 1 700, 1 630, 1 514, 1 470, 1 350, and 1 160 cm^{-1}; δ_H[90 MHz, $CDCl_3$–$(CD_3)_2SO$ 1:1] 1.45 (9 H, s), 1.99 (3 H, s), 3.26 and 3.53 (2 H, ABq, *J* 18 Hz), 4.67 and 4.96 (2 H, ABq, *J* 14 Hz), 4.93 (1 H, d, *J* 5 Hz), 5.33 (1 H, dd, *J* 5 Hz and 10 Hz), and 8.13 and 8.37 (4 H, ABq, *J* 9 Hz).

t-Butyl 7β-[N-(4-*Nitrophenylsulphonyl*)-N-(2,2,2-*trichloroethoxycarbonyl*)*amino*]*cephalosporanate* (**36**).—The 4-nitrobenzenesulphonamide (**32**) (513 mg) was converted into the cephalosporanate (**36**) as described for the preparation of compound (**34**). The *product* (**36**) was a crystalline solid (640 mg, 93%), m.p. 136—137 °C (EtOAc–hexane) (Found: C, 40.3; H, 3.6; Cl, 15.5; N, 6.2; S, 9.1. $C_{23}H_{24}Cl_3N_3O_{11}S_2$ requires C, 40.1; H, 3.5; Cl, 15.5; N, 6.1; S, 9.3%); $\nu_{max.}$(CHCl$_3$) 1 790, 1 742br, 1 535, and 1 170 cm^{-1}; δ_H(90 MHz) 1.54 (9 H, s), 2.05 (3 H, s), 3.47 (2 H, AA'), 4.72 (2 H, s), 4.78 and 5.08 (2 H, ABq, *J* 13 Hz), 5.1 (1 H, d, *J* 5 Hz), 5.88 (1 H, d, *J* 5 Hz), and 8.34 (4 H, AA').

t-Butyl 7β-(*Methylsulphonylamino*)*cephalosporanate* (**33**).—t-Butyl 7β-aminocephalosporanate (1.64 g) was dissolved in dry DCM (20 ml) at −20 °C and pyridine (0.45 ml) added, followed by methanesulphonyl chloride (0.38 ml). The cooling-bath was removed and after 2.5 h 4-dimethylaminopyridine (0.006 g) was added. Further pyridine (0.34 ml) and methanesulphonyl chloride (0.29 ml) were added over the next 2.5 h. After 6 h in total, the solution was diluted with ethyl acetate and washed successively with dilute hydrochloric acid, brine, saturated aqueous sodium hydrogen carbonate, and brine, dried, and evaporated. Recrystallisation afforded the *methanesulphonamide* (**33**) as a crystalline solid (1.39 g, 68%), m.p. 185—186 °C (EtOAc–hexane) (Found: C, 44.4; H, 5.5; N, 7.0; S, 15.7. $C_{15}H_{22}N_2O_7S_2$ requires C, 44.3; H, 5.4; N, 6.9; S, 15.8%); $\nu_{max.}$(Nujol) 3 200, 1 790, 1 730, 1 702, 1 335 and 1 155 cm^{-1}; δ_H(90 MHz) 1.5 (9 H, s), 2.05 (3 H, s), 3.18 (3 H, s), 3.33 and 3.61 (2 H, ABq, *J* 18 Hz), 4.78 and 5.1 (2 H, ABq, *J* 13 Hz), 4.98 (1 H, d, *J* 5 Hz) and 5.3 (1 H, dd, *J* 5 Hz), NH not clearly visible.

t-Butyl 7β-[N-*Methylsulphonyl*-N-(2,2,2-*trichloroethoxycarbonyl*)*amino*]*cephalosporanate* (**37**).—The methanesulphonamide (**33**) (406 mg) was dissolved in DCM (10 ml) at 0 °C and 4-dimethylaminopyridine (12 mg) added, followed by triethylamine (0.207 ml). To the stirred solution was added 2,2,2-trichloroethoxycarbonyl chloride (0.275 ml) in DCM (1 ml). After 15 min the solution was washed successively with dilute hydrochloric acid, brine, saturated aqueous sodium hydrogen carbonate, and brine, dried, and evaporated. Chromatography gave the cephalosporanate (**37**) as an amorphous solid (507 mg, 87%); $\nu_{max.}$(CHCl$_3$) 1 790, 1 730br, 1 380, and 1 160 cm^{-1}; δ_H(90 MHz) 1.53 (9 H, s), 2.04 (3 H, s), 3.43 (5 H, s), 4.75 and 5.72 (1 H, d, *J* 5 Hz) (Found: $[M^+-CH_3SO_2]^+$, 501.0051. $C_{17}H_{20}Cl_3N_2O_7S_2$ requires M–CH_3SO_2, 501.0057).

Reaction of the Sulphonamides (**24**) *and* (**34**)—(**36**) *with* N,N-*Bis*(*trimethylsilyl*)*formamide*.—*Method* 1. t-Butyl 7β-[*N*-(2,2,2-trichloroethoxycarbonyl)-*N*-trifluoromethylsulphonylamino]-cephalosporanate (**24**) (63 mg) in DCM (2 ml) was treated with *N,N*-bis(trimethylsilyl)formamide (72 mg) and triethylamine (11 mg). After 16 h at room temperature, the solution was poured into ethyl acetate and washed successively with dilute hydrochloric acid and brine, dried, and evaporated. Chromatography on silica gel gave the product (48 mg, 69%) which consisted of the 7α-formamidocephalosporanate (**26**) (20%) and the Δ^2 isomer (**29**) (80%). The latter gave *inter alia* δ_H(90 MHz) 1.45 (9 H, s), 2.08 (3 H, s), 4.92 (1 H, slightly br s), 5.42 (1 H, s), and 6.3 (1 H, slightly br s).

Method 2. The cephalosporanate (**24**) (63 mg) in DCM (2 ml) was cooled to −40 °C and *N,N*-bis(trimethylsilyl)formamide (72 mg) added, followed by triethylamine (15 mg). The solution was warmed to −10 °C over 30 min. After a further 30 min at 0 to −5 °C the solution was worked up as in Method 1. Chromatography on silica gel gave an amorphous solid (30 mg, 55%) consisting of the 7α-formamidocephalosporanate (**26**), containing *ca.* 5% of the Δ^2 isomer (**29**).

Method 3. The cephalosporanate (**34**) (73 mg) in DCM (3 ml) was cooled to −10 °C and *N,N*-bis(trimethylsilyl)formamide (85 mg) added followed by triethylamine (11 mg). The cooling-bath was removed and after 7 h at room temperature, the solution was worked up as in Method 1. Silica-gel chromatography gave an amorphous solid (34 mg, 62%) consisting of the 7α-formamidocephalosporanate (**26**) and the Δ^2 isomer (**29**) in a ratio of 1:2. A less polar (t.l.c.) fraction (19 mg), homogeneous by t.l.c., was a mixture (250 MHz, ^{1}H n.m.r.) which contained some cephalosporanate (**34**) (6%).

Similarly, the cephalosporanates (**35**) and (**36**) gave amorphous solids (41%) and (10%) respectively, which consisted of the 7α-formamidocephalosporanate (**26**) and the Δ^2 isomer (**29**) in a ratio of 2:3. Mixed fractions containing products derived from partial C-7 epimerisation and Δ^3 to Δ^2 isomerisation were also isolated.

Oxidation of Benzyl 6β-(2,2,2-*Trichloroethoxycarbonylamino*)-6α-*formamidopenicillanate* (**11**).—The 6α-formamido-

penicillanate (11) (5.0 g) was dissolved in 1,2-dichloroethane (75 ml) at 0 °C and *m*-chloroperbenzoic acid (1.81 g) added. After 1 h the solution was diluted with ethyl acetate (50 ml), washed successively with aqueous sodium hydrogen carbonate and brine, and dried. This solution was concentrated to a gum and redissolved in ethyl acetate (15 ml) to which hexane was added to the point of turbidity. From this crystallised the more polar (t.l.c.) isomer of benzyl 6β-(2,2,2-trichloroethoxycarbonylamino)-6α-formamidopenicillanate 1-oxide (2.45 g, 48%). The mother liquors were chromatographed on silica gel 60, eluting with ethyl acetate–hexane (6:4) to give the less polar (t.l.c.) isomer as a colourless foam (1.02 g, 20%) plus further more polar (t.l.c.) isomer (0.46 g, 9%).

Benzyl 6β-[N-(2,2,2-*Trichloroethoxycarbonyl*)-N-*trifluoromethylsulphonylamino*]*penicillanate* 1α-*Oxide* (**8**).—Benzyl 6β-[*N*-(2,2,2-trichloroethoxycarbonyl-*N*-trifluoromethylsulphonylamino]penicillanate (**6**) (613 mg) was dissolved in dry DCM (10 ml) at −10 °C and peracetic acid (5.24% solution in acetic acid; 1.5 ml) was added. After 16 h at 0 °C the solvent was evaporated under reduced pressure. After addition of toluene and repetition of the evaporation (×3), the residue was chromatographed on silica gel to give the 1α-*oxide* (**8**) as an amorphous solid (516 mg, 83%) (Found: C, 36.2; H, 3.0; Cl, 16.9; N, 4.3; S, 10.2. $C_{19}H_{18}Cl_3F_3N_2O_8S_2$ requires C, 36.2; H, 2.9; Cl, 16.9; N, 4.4; S, 10.2%); $\nu_{max.}$(CHCl$_3$) 1 810, 1 755, 1 425, and 1 130 cm^{-1}; δ_H(90 MHz) 1.18 and 1.68 (6 H, 2 s), 4.45 (1 H, s), 4.73 (1 H, d, *J* 4.5 Hz), 4.81 and 4.97 (2 H, ABq, *J* 12 Hz), 5.21 (2 H, AA′), 5.7 (1 H, d, *J* 4.5 Hz), and 7.35 (5 H, s).

Benzyl 6α-*Formamido*-6β-(2,2,2-*trichloroethoxycarbonylamino*)*penicillanate* 1α-*Oxide* (**12**).—The sulphoxide (**8**) (63 mg) was dissolved in dry DCM (2 ml) at −10 °C and *N*,*N*-bis(trimethylsilyl)formamide (40 mg) was added, followed immediately by triethylamine (10 mg). The temperature was allowed to rise to −5 °C and after 40 min the solution was poured into ethyl acetate and washed successively with dilute hydrochloric acid and brine, dried, and evaporated. Chromatography on silica gel afforded the 1α-*oxide* (**12**) as a crystalline solid (41 mg, 76%), m.p. 149—150 °C (EtOAc–hexane) (Found: C, 42.2; H, 3.7; N, 7.7; S, 5.5. $C_{19}H_{20}Cl_3N_3O_7S$ requires C, 42.2; H, 3.7; N, 7.8; S, 5.9%); $\nu_{max.}$(CHCl$_3$) 3 200br, 1 800, 1 740, 1 700, and 1 495 cm^{-1}; δ_H(90 MHz) 1.25 and 1.53 (6 H, 2 s), 4.56 (1 H, s), 4.68 and 4.89 (2 H, ABq, *J* 13 Hz), 5.16 (1 H, s), 5.2 (2 H, AA′) 7.2 (1 H, br s, exch. D_2O), 7.33 (5 H, s), 8.03 (1 H, br s, exch. D_2O), and 8.17 (1 H, s). This material was identical with the more polar (t.l.c.) isomer derived from oxidation of the 6α-formamidopenicillanate (**11**).

Benzyl 6β-(*Trifluoromethylsulphonylamino*)*penicillanate* 1β-*Oxide* (**41**).—Benzyl 6β-(trifluoromethylsulphonylamino)penicillanate (**40**) (3.12 g) was dissolved in dry DCM (50 ml) at −10 °C and peracetic acid (52.4% solution in acetic acid; 1 ml) added. After 15 min the solvent was evaporated. After addition of toluene and repetition of the evaporation (×3), the residue was chromatographed on silica gel to give the *sulphoxide* (**41**) as an amorphous solid (2.6 g, 83%) (Found: M^+, 455.0557. $C_{16}H_{17}F_3N_2O_6S_2$ requires *M*, 455.0558); $\nu_{max.}$3 250, 1 810, 1 750, 1 440, and 1 140 cm^{-1}; δ_H(90 MHz) 1.05 and 1.64 (6 H, 2 s), 4.69 (1 H, s), 4.98 (1 H, d, *J* 4.5 Hz), 5.1 and 5.28 (2 H, ABq, *J* 12 Hz), 5.31 (1 H, d, *J* 4.5 Hz), and 7.34 (5 H, s).

Benzyl 6β-[N-(2,2,2-*Trichloroethoxycarbonyl*)-N-*trifluoromethylsulphonylamino*]*penicillanate* 1β-*Oxide* (**42**).—The 1β-oxide (**41**) (988 mg) was dissolved in dry DCM (30 ml) at −10 °C and 2,2,2-trichloroethoxycarbonyl chloride (844 mg) added, followed by pyridine (240 mg). The temperature was raised to 0 °C and after 1.5 h the reaction mixture was poured into ethyl acetate–dilute hydrochloric acid. The organic layer was separated, washed with brine, dried, and evaporated. Chromatography of the residue on silica gel gave the 1β-*oxide* (**42**) as a crystalline solid (1.14 g, 84%), m.p. 133—134 °C (EtOAc–hexane) (Found: C, 36.3; H, 3.1; Cl, 17.0; N, 4.5; S, 10.1. $C_{19}H_{18}Cl_3F_3N_2O_8S_2$ requires C, 36.2; H, 2.9; Cl, 16.9; N, 4.4; S, 10.2%); $\nu_{max.}$ 1 815, 1 750, 1 420, 1 125, and 1 045 cm^{-1}; δ_H(90 MHz) 1.05 and 1.6 (6 H, 2 s), 4.7 (1 H, s), 4.88 (2 H, AA′), 5.01 (1 H, d, *J* 5 Hz), 5.13 and 5.3 (2 H, ABq, *J* 12 Hz), 5.52 (1 H, s, *J* 5 Hz), and 7.34 (5 H, s).

Benzyl 6α-*Formamido*-6β-(2,2,2-*trichloroethoxycarbonylamino*)*penicillanate* 1β-*Oxide* (**13**).—The 1β-oxide (**42**) (126 mg) was dissolved in dry DCM (3 ml) at −10 °C and *N*,*N*-bis(trimethylsilyl)formamide (80 mg) was added, followed immediately by triethylamine (20 mg). After 20 min at −10 °C to −5 °C the solution was poured into ethyl acetate, and washed with dilute hydrochloric acid and brine, dried, and evaporated. Chromatography on silica gel afforded the 1β-oxide (**13**) as an amorphous solid (81 mg, 76%); $\nu_{max.}$(CHCl$_3$) 3 400, 3 250, 1 795, 1 735br, 1 700, and 1 050 cm^{-1}; δ_H(90 MHz) 1.06 and 1.6 (6 H, 2 s), 4.68 (1 H, s), 4.72 (2 H, AA′), 5.2 (1 H, s), 5.13 and 5.32 (2 H, ABq, *J* 12 Hz), 7.27 and 7.53 (2 H, 2 br s), 7.35 (5 H, s), and 8.22 (1 H, s); *m*/*z* (positive xenon f.a.b.; dipentylphenol–CHCl$_3$) MH^+, 540. This material was identical with the less polar (t.l.c.) isomer derived from oxidation of the 6α-formamidopenicillanate (**11**).

Oxidation of t-Butyl 7α-*Formamido*-7β-2,2,2-*trichloroethoxycarbonylamino*)*cephalosporanate* (**26**).—The 7α-formamidocephalosporanate (**26**) (546 mg) was dissolved in dry DCM (10 ml) at −20 °C and peracetic acid (5.2% w/v solution in acetic acid; 1.6 ml) added. After 30 min further peracetic acid (0.36 ml) was added and the mixture stirred at 0 °C for 1 h and then evaporated under reduced pressure. After addition of toluene and repetition of the evaporation (×2), the residue was chromatographed on silica gel to give the less polar (t.l.c.) β-*isomer* (**28**) of t-butyl 7β-(2,2,2-trichloroethoxycarbonylamino)-7α-formamidocephalosporanate 1-oxide (164 mg, 29%), m.p. 180—185 °C (decomp.) (from EtOAc–hexane) (Found: C, 38.6; H, 3.9; Cl, 18.7; N, 7.3; S, 5.7. $C_{18}H_{22}Cl_3N_3O_9S$ requires C, 38.4; H, 3.9; Cl, 18.9; N, 7.5; S, 5.7%); $\nu_{max.}$(CHCl$_3$) 3 390, 3 250, 1 800, 1 720, 1 065, and 1 040 cm^{-1}; δ_H(250 MHz) *inter alia* 1.57 (9 H, s), 2.07 (3 H, s), 3.34 (1 H, d, *J* 18.5 Hz, part of AB quartet, showing fine coupling of 1.2 Hz, removed by irradiation at δ 4.87), 3.75 (1 H, d, *J* 18.5 Hz, part of ABq), 4.67 and 5.16 (2 H, ABq, *J* 13.5 Hz), 4.77 (2 H, AA′), 4.87 (1 H, d, *J* 1.2 Hz), 7.36 (1 H, s), 7.58 (1 H, slightly br s), and 8.23 (1 H, s), and the more polar (t.l.c.) α-isomer (**27**) (208 mg, 38%) (*vide infra*).

t-Butyl 7β-N-(2,2,2-*Trichloroethoxycarbonyl*-N-*trifluoromethylsulphonylamino*)*cephalosporanate* 1α-*Oxide* (**25**).—The cephalosporanate (**24**) (648 mg) was dissolved in ethyl acetate (20 ml) at −10 °C and *m*-chloroperbenzoic acid (193 mg, 90% pure) added. The solution was immediately washed with dilute aqueous sodium hydrogen carbonate and brine, dried, and evaporated. Chromatography on silica gel afforded the 1α-*oxide* (**25**) as white crystals (466 mg, 71%), m.p. 148—149 °C (Found: C, 33.4; H, 3.2; Cl, 16.1; N, 4.3; S, 9.7. $C_{18}H_{20}Cl_3F_3N_2O_{10}S_2$ requires C, 33.2; H, 3.1; Cl, 16.3; N, 4.3; S, 9.8%); $\nu_{max.}$(CHCl$_3$) 1 810, 1 750, 1 730, 1 425, 1 150, and 1 035 cm^{-1}; δ_H(250 MHz) 1.55 (9 H, s), 2.12 (3 H, s), 3.53 and 4.02 (2 H, ABq, *J* 17 Hz), 4.64 (1 H, d, *J* 5 Hz), 4.88 and 5.22 (2 H, ABq, *J* 14 Hz), 4.9 (2 H, AA′), and 5.90 (1 H, d, *J* 5 Hz).

t-Butyl 7α-*Formamido*-7β-(2,2,2-*trichloroethoxycarbonylamino*)*cephalosporanate* 1α-*Oxide* (**27**).—The 1α-oxide (**25**) (64

mg) in DCM (2 ml) was treated with *N,N*-bis(trimethylsilyl)-formamide (72 mg) and triethylamine (10 mg). After 10 min the solution was diluted with ethyl acetate, washed successively with dilute hydrochloric acid, water, dilute aqueous sodium hydrogen carbonate, and brine, dried, and evaporated. Chromatography on silica gel gave the 1α-*oxide* (**27**) as an amorphous solid (15 mg, 33%) (Found: C, 38.35; H, 4.00; Cl, 18.9; N, 7.7; S, 5.65. $C_{18}H_{22}Cl_3N_3O_9S$ requires C, 38.4; H, 3.9; Cl, 18.9; N, 7.5; S, 5.7%); $\nu_{max.}$($CHCl_3$) 3 480, 3 200br, 1805, 1 730, 1 700sh, and 1 040 cm^{-1}; δ_H(250 MHz) *inter alia* 1.5 (9 H, s), 2.11 (3 H, s), 3.58 and 4.07 (2 H, ABq, *J* 16.8 Hz), 4.7—4.9 (3 H, m), 4.91 (1 H, s), 5.11 (1 H, part of ABq, *J* 13.6 Hz), 7.44 (1 H, s), 8.16 (1 H, br s), and 8.27 (1 H, s).

t-Butyl 7β-(*Trifluoromethylsulphonylamino*)*cephalosporanate* 1β-*Oxide* (**43**).—The sulphide (**23**) (920 mg) in DCM (15 ml) was cooled to −5 °C and treated with peracetic acid (5.2% solution in acetic acid; 3.2 ml). After 2 h at 0 °C the solvent was evaporated. After addition of toluene and repetition of the evaporation (×3), the residue was chromatographed on silica gel to give the β-*oxide* (**43**) as a crystalline solid (850 mg, 90%), m.p. 155 °C (decomp.) (from EtOAc–hexane) (Found: C, 37.9; H, 4.2; N, 5.7; S, 13.2. $C_{15}H_{19}F_3N_2O_8S_2$ requires C, 37.8; H, 4.0; N, 5.9; S, 13.4%); $\nu_{max.}$($CHCl_3$) 3 450, 3 300, 1 810, 1 720br, 1 150, and 1 045 cm^{-1}; δ_H(250 MHz) 1.56 (9 H, s), 2.08 (3 H, s), 3.25 and 3.87 (2 H, ABq, *J* 19 Hz, upper field arm showing fine coupling of *J* 1.4 Hz), 4.52 (1 H, dd, *J* 1.4 and 4.8 Hz), 4.71 and 5.34 (2 H, ABq, *J* 13.9 Hz), and 5.42 (1 H, d, *J* 4.8 Hz).

t-Butyl 7β-[N-(2,2,2-*Trichloroethoxycarbonyl*)-N-*trifluoromethylsulphonylamino*]*cephalosporanate* 1β-*Oxide* (**44**).—The 1β-oxide (**43**) (476 mg) was dissolved in dry DCM (10 ml) at −10 °C and 2,2,2-trichloroethoxycarbonyl chloride (422 mg) added, followed by pyridine (114 mg). The cooling-bath was removed after 30 min and the solution left at room temperature for 1 h, and then poured into ethyl acetate–dilute hydrochloric acid. The organic layer was separated, washed successively with brine, aqueous sodium hydrogen carbonate, and brine, dried, and evaporated. Chromatography of the residue on silica gel gave the 1β-*oxide* (**44**) as a crystalline solid (409 mg, 63%) (Found: C, 33.3; H, 3.3; Cl, 16.2; N, 4.2; S, 10.0. $C_{18}H_{20}Cl_3F_3N_2O_{10}S_2$ requires C, 33.2; H, 3.1; Cl, 16.3; N, 4.3; S, 9.8%); $\nu_{max.}$($CHCl_3$) 1 815, 1 790, and 1 730 cm^{-1}; δ_H(250 MHz) 1.58 (9 H, s), 2.07 (3 H, s), 3.37 and 3.78 (2 H, ABq, *J* 18.4 Hz, higher field arm showing fine coupling of *J* 1.1 Hz), 4.44 (1 H, dd, *J* 1.1 and 4.8 Hz), 4.65 and 5.4 (2 H, ABq, *J* 13.5 Hz), 4.78 and 4.93 (2 H, ABq, *J* 11.7 Hz), and 5.77 (1 H, d, *J* 4.8 Hz).

Benzyl 6β-*Amino*-6α-*formamidopenicillanate* 1α-*Oxide* (**51**).—The carbamate (**8**) (500 mg) was vigorously stirred in a mixture of tetrahydrofuran (10 ml) and aqueous 1M potassium dihydrogen phosphate (1 ml) and treated with freshly acid-washed zinc powder (700 mg). As the reaction proceeded, the pH was maintained at 4—6 by addition of 1M hydrochloric acid and the progress was monitored by t.l.c. Further zinc (500 mg) was added after 1.5 h and deprotection was complete in a total of 3 h. The reaction product was then filtered through Celite and the filtrate concentrated until turbid. It was partitioned between ethyl acetate and water, and the organic phase was separated, washed with brine, dried (Na_2SO_4) and concentrated to afford a gum. This crude benzyl 6β-amino-6α-formamidopenicillanate 1α-oxide (**51**) (290 mg, 89%) was suitable for further reaction, but it could be chromatographed on silica gel 60 eluting with chloroform–ethanol (9:1) to give purer material (Found: M^+, 365.1041. $C_{16}H_{19}N_3O_5S$ requires *M*, 365.1045); $\nu_{max.}$($CHCl_3$) 3 380, 3 300, 2 980, 1 790, 1 740, 1 690, and 1 030 cm^{-1}; δ_H(90 MHz) 1.33 and 1.48 (6 H, 2 s), 2.67 (2 H, br s), 4.56 (1 H, s), 5.04 (1 H, s), 5.17 (2 H, s), 7.34 (5 H, s), 7.64 (1 H, s), and 8.18 (1 H, s).

Benzyl 6β-[(D)-2-(4-*Ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-(3,4-*diacetoxyphenyl*)*acetamido*]-6α-*formamidopenicillanate* 1α-*Oxide* (**46**).—The amine (**51**) (0.29 g) was stirred in dry DCM (5 ml) at 0 °C under argon. To this was added *N,N'*-dicyclohexylcarbodi-imide (0.16 g) followed by dropwise addition of (D)-2-(4-ethyl-2,3-dioxopiperazin-1-ylcarbonylamino)-2-(3,4-diacetoxyphenyl)acetic acid (0.35 g) in DCM over a period of 0.25 h. The cooling was removed and the reaction mixture stirred at room temperature for 3 h. The product was filtered and the filtrate concentrated and chromatographed on silica gel 60 eluting with ethyl acetate–ethanol (9:1) followed by further silica gel chromatography using chloroform–ethanol (9:1). This gave the 1α-oxide (**46**) as a colourless foam (0.14 g, 21%); $\nu_{max.}$($CHCl_3$) 3 300, 2 970, 1 780, 1 750sh, 1 710sh, 1 680, and 1 045 cm^{-1}; δ_H(250 MHz) 0.85 and 1.17 (6 H, 2 s), 1.22 (3 H, t, *J* 7 Hz), 2.26 (6 H, s), 3.4—4.1 (6 H, m), 4.45 (1 H, s), 5.0—5.2 (3 H, m), 5.61 (1 H, d, *J* 7 Hz), 7.1—7.4 (8 H, m), 8.09 (1 H, s), 8.26 and 9.00 (2 H, 2 br s), and 10.11 (1 H, d, *J* 7 Hz); *m/z* (positive xenon f.a.b.; dipentylphenol–$CHCl_3$) MH^+, 783.

Sodium 6β-[(D)-2-(4-*Ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-(3,4-*diacetoxyphenyl*)*acetamido*]-6α-*formamidopenicillanate* 1α-*Oxide* (**47**).—A solution of the ester (**46**) (130 mg) in dry dioxane (5 ml) was treated with 10% palladium on charcoal catalyst (130 mg) and hydrogenated at atmospheric pressure for 2.5 h. The reaction mixture was then filtered through Celite and the filtrate evaporated to *ca.* 1 ml volume. It was then treated with 1.93M sodium 2-ethylhexanoate in 4-methylpentan-2-one (0.085 ml) followed by dry diethyl ether (10 ml). The precipitate was collected, washed with acetone–diethyl ether (1:1) and dried *in vacuo* to give the salt (**47**) as a white powder (73 mg, 57%); $\nu_{max.}$(KBr) 3 436, 2 966, 1 776, 1 710, 1 678 and 1 619 cm^{-1}; δ_H(D_2O; 250 MHz) 1.01 and 1.28 (6 H, 2 s), 1.22 (3 H, t, *J* 7 Hz), 2.37 (6 H, s), 3.5—4.1 (6 H, m), 4.28 (1 H, s), 5.21 (1 H, s), 5.54 (1 H, s), 7.3—7.6 (3 H, m), and 8.19 (1 H, s); *m/z* (positive xenon f.a.b.; glycerol–thioglycerol) MH^+, 715.

Benzyl 6β-[(D)-2-(4-*Ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-(3,4-*diacetoxyphenyl*)*acetamido*]-6α-*formamidopenicillanate* 1-*Oxides* (**46**) *and* (**49**).—A solution of the sulphide (**48**) (1.0 g) in dry DCM (20 ml) was stirred at 0 °C and treated with *m*-chloroperbenzoic acid (0.25 g). After a period of 2 h the product was concentrated under reduced pressure and chromatographed on silica gel, eluting with ethyl acetate–ethanol (9:1—4:1). The product was obtained as a colourless solid (0.86 g, 85%), which was a mixture of the two diastereoisomers (**46**) and (**49**) about the sulphur atom. The mixed sulphoxide isomers were carefully rechromatographed on silica gel eluting with ethyl acetate–dioxane (2:1). This enabled the isolation of pure samples of the less polar (t.l.c.) β-oxide (**49**) and more polar (t.l.c.) α-oxide (**46**). The spectral data for the β-isomer (**46**) were $\nu_{max.}$($CHCl_3$) 3 280, 2 960, 2 850, 1 800, 1 775, 1 710, and 1 690 cm^{-1}; δ_H(250 MHz) 1.08 and 1.55 (6 H, 2 s), 1.18 (3 H, t, *J* 7 Hz), 2.26 (6 H, s), 3.4—4.1 (6 H, m), 4.63 (1 H, s), 5.15 and 5.29 (2 H, ABq, *J* 12 Hz), 5.18 (1 H, s), 5.61 (1 H, d, *J* 8 Hz), 7.1—7.4 (8 H, m), 8.03 (1 H, s), 8.08 (1 H, s), 8.48 (1 H, s), and 9.84 (1 H, d, *J* 8 Hz); *vide supra* for spectral data of 1α-oxide (**46**).

Sodium 6β-[(D)-2-(4-*Ethyl*-2,3-*dioxopiperazin*-1-*ylcarbonylamino*)-2-(3,4-*diacetoxyphenyl*)*acetamido*]-6α-*formamidopenicillanate* 1β-*Oxide* (**50**).—A solution of the ester (**49**) (8 mg), in dry dioxane (3 ml) was treated with 10% palladium on charcoal catalyst (10 mg) and hydrogenated at atmospheric pressure for 2.5 h. The resulting mixture was filtered through Celite and the filtrate concentrated under reduced pressure. The residue was partitioned between ethyl acetate and water and the pH adjusted to 6.5 with dilute aqueous sodium hydrogen carbonate. The aqueous layer was then separated and freeze-dried to give

the salt (**50**) as a white powder (6 mg, *ca.* 90%); $\nu_{max.}$(KBr micro disc) 3 430, 2 927, 1 778, 1 710, 1 677, and 1 614 cm^{-1}; $\delta_H(D_2O$; 250 MHz) 1.26 and 1.57 (6 H, 2 s), 1.20 (3 H, t, *J* 7 Hz), 2.37 (6 H, s), 3.5—4.1 (6 H, m), 4.36 (1 H, s), 5.29 (1 H, s), 5.52 (1 H, s), 7.3—7.5 (3 H, m), and 8.13 (1 H, s).

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