# Spirocyclic Side-chain Derivatives of Penicillin and Cephalosporin

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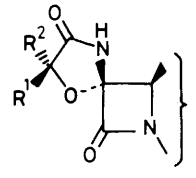
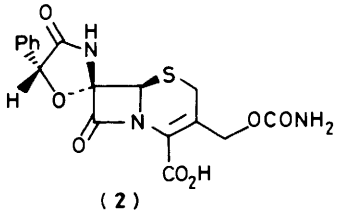
A series of two spirocyclic oxazolidinone derivatives of penicillin and four derivatives of cephalosporin have been prepared, as well as a benzoxazine conjugate of cephalosporin, with the object of freezing the conformation of the 6(7)-amide side-chain bearing a pendant aromatic ring. The antibacterial properties of these derivatives are discussed.

The nature of the side-chain acylamido groups attached to the penam and cephem nuclei of the β-lactam antibiotics is known to be extremely important in determining the range and degree of activity of these antibiotic agents.[1] It is presumed that in the binding of such agents to their receptor sites the side-chain has to be accommodated spatially, possibly in a secondary binding site. Whereas studies on preferred conformations of the 6(7)-substituents on the penam (cephem) nuclei have been reported,[2] few examples are known in which the side-chain is relatively fixed in space, for example, by introducing restrictions to free rotation about the side-chain. Herein we report some preliminary studies on the preparation of derivatives in which the side-chain is sterically inhibited from free rotation by the presence of a spirocyclic ring system (**1**).

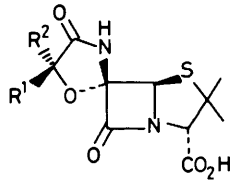
Several spirocyclic β-lactam derivatives are known [3] but one which initially attracted our attention was the cephamycin analogue (**2**), which is reported to be reasonably active as an antibiotic.[4] The analogue (**2**) was prepared from (*R*)-mandelic acid [hydroxy(phenyl)acetic acid]. Since, in this compound, the amide bond associated with the substituent is held in a *cis*-conformation, whereas in normal β-lactam derivatives the *trans*-conformer is preferred, it is possible that the aromatic phenyl group might be forced to occupy a different area of space compared with that for the acyclic derivatives and that such differences may be of importance in the shape adopted at receptor sites. A simple variant is to examine the properties of the corresponding lactam derived from (*S*)-mandelic acid in which the phenyl group is held on the opposite side of the spirocyclic ring in a completely different area of space. The properties of the cyclic isomers may be compared with those of the acyclic derivatives. Targets for this current 'spatial-mapping' study were, therefore, the penicillins (**3**) and (**4**) and the cephalosporin derivatives (**5**)—(**8**). The oxazolidinones were chosen since previous studies have highlighted the steric sensitivity of 6α(7α) substitution in the penam (cephem) systems.[5] Although the introduction of small groups, such as a methoxy group, can just be tolerated, more so in the cephem series than in the penam series, larger substituents prevent the antibiotics from reaching their target receptor sites.



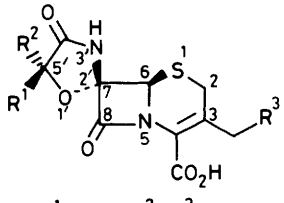
(**1**)



(**2**)



(**3**) $R^1$ = H ; $R^2$ = Ph
(**4**) $R^1$ = Ph ; $R^2$ = H

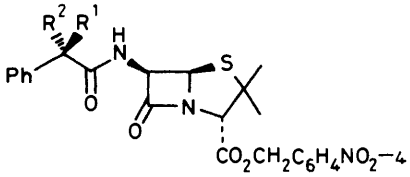


(**5**) $R^1$ = Ph ; $R^2$ = $R^3$ = H
(**6**) $R^1$ = $R^3$ = H ; $R^2$ = Ph
(**7**) $R^1$ = Ph ; $R^2$ = H ; $R^3$ = OAc
(**8**) $R^1$ = H ; $R^2$ = Ph ; $R^3$ = OAc

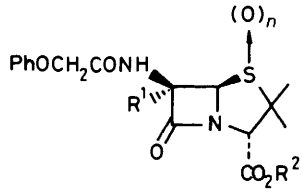
Routes to the penicillin derivatives (**3**) and (**4**) require oxidation of the related mandelamides (**9**) and (**10**) to the corresponding acylimides, followed by intramolecular cyclisation. Before engaging in this work, however, some preliminary studies on the known oxidative 6α-methoxylation of penicillin V were attempted. Use of lithium methoxide and t-butyl hypochlorite on the benzhydryl ester (**13**) according to the literature method [6] failed, whilst methoxylation of the corresponding sulphoxide (**14**), according to the method of Baldwin *et al.*[7] proved to be inefficient. More reproducible results could be achieved by preparing lithium methoxide *in situ*. Thus n-butyl-lithium was added to methanol dissolved in tetrahydrofuran to give a clear solution of the methoxide. Reaction with the *p*-nitrobenzyl ester of penicillin V (**15**), followed by addition of one equivalent of freshly prepared t-butyl hypochlorite, afforded substantially pure 6α-methoxy-penicillanate (**16**). These conditions for generating lithium methoxide in solution were used for all subsequent reactions.

Preparation of the mandelate derivatives (**9**) and (**10**) posed some problems because of the presence of the reactive hydroxy groups. Since attempts to couple mandelic acid directly with 6-aminopenicillanic acid and its esters failed, protection of the alcohol group was necessary; the formyl group proved to be suitable. (*R*)-Formyloxy(phenyl)acetic acid was converted into its acid chloride, by use of thionyl chloride, and then coupled with 6-aminopenicillanic acid, followed by protection of the free carboxy group to give the 4-nitrobenzyl ester (**11**). Removal of the formyl group was effected by stirring the ester in aqueous dimethylformamide containing sodium hydrogen carbonate as base, although some attack at the 4-nitrobenzyl ester and β-lactam groups also occurred during this step. The epimer (**10**) derived from (*S*)-mandelic acid, was similarly prepared except that deformylation of the formate (**12**) preceded esterification with *p*-nitrobenzyl bromide, a sequence which proved to be more efficient than the former one.

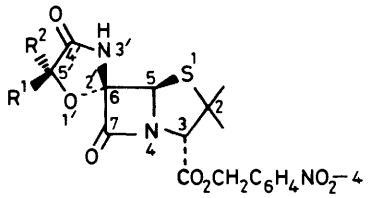
Oxidation of the alcohols (**9**) and (**10**) with lithium methoxide and t-butyl hypochlorite gave the required 6-spiro-oxazolidinones (**17**) and (**18**) respectively. None of the corresponding 6α-methoxypenicillanates were observed amongst the products.



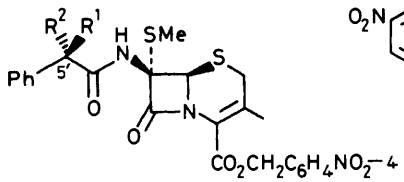
(9) $R^1$ = OH ; $R^2$ = H
(10) $R^1$ = H ; $R^2$ = OH
(11) $R^1$ = OCHO ; $R^2$ = H
(12) $R^1$ = H ; $R^2$ = OCHO



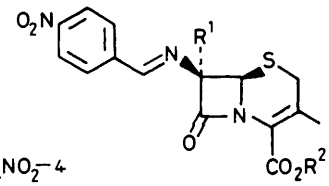
(13) $R^1$ = H ; $R^2$ = $CHPh_2$ ; $n$ = 0
(14) $R^1$ = H ; $R^2$ = $CHPh_2$ ; $n$ = 1
(15) $R^1$ = H ; $R^2$ = 4-$NO_2C_6H_4CH_2$ ; $n$ = 0
(16) $R^1$ = MeO ; $R^2$ = 4-$NO_2C_6H_4CH_2$ ; $n$ = 0



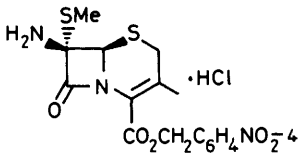
(17) $R^1$ = H ; $R^2$ = Ph
(18) $R^1$ = Ph ; $R^2$ = H



(19) $R^1$ = H ; $R^2$ = OH
(20) $R^1$ = OH ; $R^2$ = H



(21) $R^1$ = H ; $R^2$ = $SiMe_3$
(22) $R^1$ = MeS ; $R^2$ = H
(23) $R^1$ = MeS ; $R^2$ = $CH_2C_6H_4NO_2$-4



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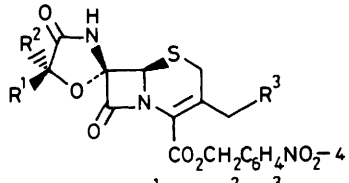
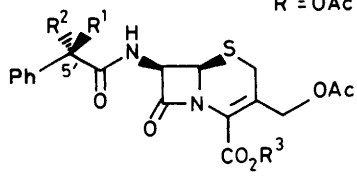


(25) $R^1$ = Ph ; $R^2$ = $R^3$ = H
(26) $R^1$ = $R^3$ = H ; $R^2$ = Ph
(27) $R^1$ = Ph ; $R^2$ = H ; $R^3$ = OAc
(28) $R^1$ = H ; $R^2$ = Ph ; $R^3$ = OAc



(29) $R^1$ = $R^3$ = H ; $R^2$ = OCHO
(30) $R^1$ = OCHO ; $R^2$ = $R^3$ = H
(31) $R^1$ = $R^3$ = H ; $R^2$ = OH
(32) $R^1$ = OH ; $R^2$ = $R^3$ = H
(33) $R^1$ = H ; $R^2$ = OH ; $R^3$ = $CH_2C_6H_4$-$NO_2$-4
(34) $R^1$ = OH ; $R^2$ = H ; $R^3$ = $CH_2C_6H_4$-$NO_2$-4

The stereochemistry at C-6 was assigned by analogy with literature results, attack on the acylimine intermediate occurring fastest from the less-hindered face. The amide bond in the spiro-compounds showed carbonyl absorption at 1 720—1 730 $cm^{-1}$, as expected for 5-membered lactams, as compared to 1 680 $cm^{-1}$ in the acyclic precursors. Deprotection of the esters in both the (*R*)- and (*S*)-series was achieved by hydrogenolysis over 10% Pd on charcoal in aqueous methanol; the spiro function was unaffected by the hydrogenation step.

Attention was next directed to preparation of the corresponding spiro-cephalosporins, (**5**)—(**8**). Initially the simpler, unsubstituted cephalosporins (**5**) and (**6**) were prepared, followed by studies on the acetoxy derivatives (**7**) and (**8**).

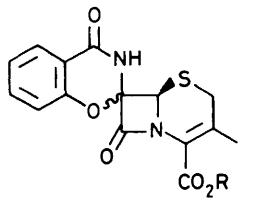
Although the methodology used in the penicillin series could be employed, difficulties were envisaged such as the known propensity for the double bond to equilibrate under basic conditions.[8] As a consequence, a different route was initially studied. This involved prior formation of the 7α-methylthio derivatives.[9] After considerable experimentation the preferred route to such systems was found to be as follows. A mixture of 7-aminodesacetoxycephalosporanic acid, *p*-nitrobenzaldehyde, and hexamethyldisilazane was heated in chloroform under reflux until all the amino acid had dissolved; removal of solvent then afforded the Schiff's base (**21**). Although the trimethylsilyl ester group could be exchanged for other groups at this point it was found to be more convenient to employ the crude trimethylsilyl ester itself in further reactions. Thus, the ester (**21**) was immediately thiomethylated by a manner analogous to reported methods,[9] using lithium di-isopropylamide as base and methyl thiotosylate as the reagent,[10] followed by careful hydrolysis of the trimethylsilyl ester, to give the acid (**22**). Esterification was achieved with 4-nitrobenzyl bromide and potassium hydrogen carbonate in dimethylformamide followed by removal of the Schiff's base (**23**) with Girard's reagent T. Treatment of the product with one equivalent of 6M-hydrochloric acid in acetone afforded the crystalline hydrochloride of the amine (**24**).[11] Overall yields of the amine hydrochloride from 7-aminodesacetoxycephalosporanic acid were in the range 12—15%. Acylation of the amine (**24**) with either (*S*)- or (*R*)-formyloxy(phenyl)acetyl chloride furnished the corresponding amides. Deformylation of these esters was achieved efficiently using potassium hydrogen carbonate in methanol–acetone mixtures and careful neutralisation of the excess of base before concentration to form the alcohols (**19**) and (**20**); failure to neutralise gave rise to double-bond isomerisation.

Formation of the spiro-ring from the alcohols (**19**) and (**20**) was effected in good yield by treatment with mercuric chloride and pyridine in dry dimethylformamide at room temperature.[9] The spiro-compounds (**25**) and (**26**) showed a characteristic shift of the amide absorption from 1 686 $cm^{-1}$ in the open-chain precursors (**19**) and (**20**) to *ca.* 1 720 $cm^{-1}$ in the spiro-cyclic lactams. The relative stereochemistry of the spiro-junction was again assigned on the known preference for nucleophilic attack on the acylimine intermediate from the α-face; no isomeric spiro-compounds could be detected in either the (*R*)- or (*S*)-series of compounds. Deprotection of the esters

(25) and (26) to the corresponding acids (5) and (6) was achieved by hydrogenolysis over 10% palladium on charcoal in a two-phase system comprised of aqueous sodium hydrogen carbonate and ethyl acetate.

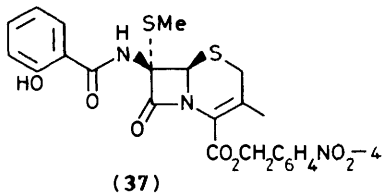
Attempts to apply the route used to prepare compounds (5) and (6) to the 7-aminocephalosporanic acid series, *i.e.* compounds (7) and (8), foundered on the difficulty in releasing the *p*-nitrobenzylidene group from the corresponding Schiff's base intermediates [*cf.* (23)]. Consequently, the lithium methoxide-t-butyl hypochlorite route was employed. Treatment of the toluene-*p*-sulphonate salt of 7-aminocephalosporanic acid with either (*S*)- or (*R*)-formyloxy(phenyl)acetyl chloride under Schotten-Baumann type conditions, *i.e.* as a solution in aqueous acetone containing sodium hydrogen carbonate as buffer, afforded the corresponding amides (29) and (30). Deprotection of the formyl group preceded attempted esterification of the carboxy group to avoid double-bond isomerisation. Esterification of the product alcohols (31) and (32) with *p*-nitrobenzyl bromide gave the required esters (33) and (34). Oxidation of the alcohols under the usual lithium methoxide t-butyl hypochlorite conditions produced the required spiro-oxazolidinones (27) and (28). These esters were deprotected under the normal hydrogenolysis conditions to liberate the acids (7) and (8), and converted, by exchange with potassium 2-ethylhexanoate, into their corresponding potassium salts.

One further derivative was prepared from the 7β-amino-7α-methylthio intermediate (24) described above. This was the related spiro-oxazine (35), in which the aromatic ring is held orthogonally to the plane of the β-lactam ring. Thus, acylation



(35) R = H

(36) R = $CH_2C_6H_4NO_2$-4



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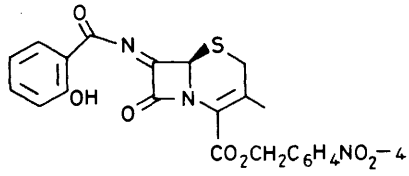

(38)

of the ester (24) with salicyloyl chloride, using propylene oxide as an acid trap, slowly produced the required amide (37). Treatment of this material was mercuric chloride and pyridine resulted in its conversion into a more polar material. Multiple elution t.l.c. showed that this material was a mixture of two related compounds, assigned as the epimeric spiro-derivatives (36) (ratio 2:1). Presumably, in this case, ring opening to the acylimine intermediate (38) is more facile than for the spiro-oxazolidinones, owing partly to the larger ring size and partly to the greater stabilisation associated with a phenolate ion as compared with an alkoxide ion. As a consequence, thermodynamic equilibration of the system can occur. The mixture (36) was deprotected by hydrogenolysis under the usual conditions to produce the corresponding acids (35).

The spiro-compounds (3)—(8) and (35) were assayed against a range of bacteria and their activities compared with those of the simple hydroxy(phenyl)acetyl derivatives (31) and (32).

**Table**

| Compound: | (7) | (8) | (31) | (32) |
|---|---|---|---|---|
| Side-chain configuration | 5′*S* | 5′*R* | 5′*S* | 5′*R* |
| *Streptomyces pyogenes* 77A | 3.12 | >200 | 0.78 | 0.07 |
| *Staph. aureus* 285 | 50 | >200 | 12.5 | 2.28 |
| *Pseud. aerug* 1771m | >200 | >200 | 200 | 9.13 |
| *Esch. coli* DC2 | 100 | >200 | 12.5 | 1.14 |

Minium inhibiting concentrations (μg/ml) of some spirocyclic and acyclic hydroxy(phenyl)acetyl derivatives. We thank Mr. John Walmsley, Hoechst Pharmaceutical Research Laboratories, Walton Manor, Walton, Milton Keynes, for these results.

Compounds (3)—(6), and (35) all showed only weak activity (minimum inhibitory concentrations of >100 μg per ml). However, the two acetoxy substituted cephalosporins (7) and (8) showed interesting properties. The 5′-(*S*)-isomer (7) showed moderate antibacterial activity whilst the 5′-(*R*)-isomer (8) was virtually inactive. For the non-spirocyclic compounds the reverse was true, the 5′-(*R*)-isomer (32)* being more active than the 5′-(*R*)-isomer (31)* (see Table). Although the results of these *in vitro* assays must be treated with caution—other effects such as differences in transport properties may be taking place—we believe these differences in activity may, in part, reflect the accommodation requirements of the pendant phenyl group at the β-lactam binding sites. The overall reduced activity of the spiro-compounds, compared with the acyclic analogues, can be explained either by the fact that the aromatic groups are held in areas not allowing full secondary binding to the receptors or by the conformational rigidity of the spirocyclic lactam function which is thus held on the edge of the range which Virudachalam and Rao predicted is necessary for resemblance between the β-lactam antiobiotics and the natural substrates, such as R-D-ala-D-ala.[2]

## Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra were recorded with a Perkin-Elmer 297 spectrophotometer on chloroform solutions, unless otherwise stated. Unless otherwise stated, $^1$H n.m.r. spectra were recorded on either a Perkin-Elmer R32 (90 MHz) instrument or a JEOL FX90Q (90 MHz) spectrometer for solutions in deuteriochloroform (tetramethylsilane as internal reference).

Thin-layer (t.l.c.) and preparative-layer (p.l.c.) chromatography were carried out on Kieselgel $GF_{254}$. Solvents were generally distilled and dried before use; light petroleum refers to the fraction of boiling range 40—60 °C and ether refers to diethyl ether throughout. Solvent ratios are described as ratios of volume before mixing.

(R)- *and* (S)-*Formyloxy(phenyl)acetic Chloride.*—The (*R*)- and (*S*)-formyloxy(phenyl)acetic acids were separately heated to reflux with redistilled thionyl chloride in the presence of one or two drops of dimethylformamide. The excess of thionyl chloride was distilled off and the residue then distilled under reduced pressure. The acid chlorides (b.p. 96/98 °C/0.5 mmHg) were obtained as colourless liquids. These were immediately used for the acylation reactions described below.

* The numbering of the side-chain in the non-spirocyclic compounds is non-systematic but has been adopted in order to allow reference to the 5′-position, *i.e.* that corresponding to the 5′-position in the spiro-compounds.

*Methoxylation of 4-Nitrobenzyl 6α-Phenoxyacetamidopenicillanate.*—Anhydrous methanol (60 mg, 1.9 mmol) was added to freshly dried tetrahydrofuran (15 ml) under nitrogen at room temperature and stirred whilst n-butyl-lithium (1.3 M solution in hexane; 1.5 ml) was added. The clear solution was stirred for 10 min and then cooled to −70 °C before a solution of the penicillin ester (**15**) (0.24 g, 0.5 mmol) in dry tetrahydrofuran (2 ml) was added to it. After 8 min a solution of t-butyl hypochlorite (54 mg, 0.5 mmol) in tetrahydrofuran (2 ml) was added to the mixture. The clear solution was stirred at −70 °C for 30 min before glacial acetic acid (0.5 ml) was added and the whole allowed to warm to room temperature. The solvent was removed under reduced pressure and the residue dissolved in dichloromethane and the solution washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated to give an amorphous yellow foam. P.l.c., using 1:1 ethyl acetate–light petroleum as eluate, afforded one major product (0.15 g, 60%), $\nu_{max.}$ 1 780, 1 750, and 1 680 $cm^{-1}$; δ 1.36 (3 H, s, Me), 1.40 (3 H, s, Me), 3.53 (3 H, s, MeO), 4.53 (1 H, s, 3-H), 4.60 (2 H, s, $ArCH_2$), 5.33 (2 H, s, $ArCH_2$), 5.65 (1 H, s, 5-H), 6.8—7.5 (7 H, m, ArH), and 8.3 (2 H, m, ArH). These data are consistent with those expected for 4-nitrobenzyl 6α-methoxy-6β-phenoxyacetamidopenicillanate (**16**).[7]

(2′R,3S,5R,6R)-6-*Formyloxy(phenyl)acetylaminopenicillanic Acid.*—6-Aminopenicillanic acid (2.61 g, 12.1 mmol) suspended in dichloromethane (30 ml) was treated with triethylamine (2.4 g, 24.2 mmol) and the mixture cooled in ice as (*R*)-formyloxy(phenyl)acetyl chloride (2.45 g, 12.3 mmol) was added dropwise over 30 min. After a further 30 min the mixture was filtered and the filtrate washed with 10% $H_3PO_4$ (×2) and water before being dried and evaporated under reduced pressure to afford, as a pale yellow foam, the title compound (4.27 g, 94%), $\nu_{max.}$(CHCl$_3$) 3 300br (OH, NH), 1 790, 1 732, and 1 690 $cm^{-1}$; δ 1.53 (3 H, s, Me), 1.60 (3 H, s, Me), 4.42 (1 H, s, 3-H), 5.4—5.7 (2 H, m, 5-H and 6-H), 6.24 (1 H, s, PhCH), 7.12 (1 H, d, *J* 9 Hz, NH), 4.70 (5 H, m, ArH), 7.79 (1 H, s, exchangeable with $D_2O$, OH), and 8.20 (1 H, s, OCHO).

(2′S,3S,5R,6R)-6-*Formyloxy(phenyl)acetylaminopenicillanic Acid.*—The above reaction was repeated using (*S*)-formyloxy-(phenyl)acetyl chloride on 6-aminopenicillanic acid (7.4 g, 34 mmol) to give the title compound (12.63 g, 97%), $\nu_{max.}$ 3 300, 1 790, 1 730, and 1 690 $cm^{-1}$; δ 1.55 (3 H, s, Me), 1.62 (3 H, s, Me), 4.47 (1 H, s, 3-H), 5.45—5.75 (2 H, m, 5- and 6-H), 6.30 (1 H, s, PhC*H*), 7.45 (6 H, m, ArH and NH), 8.20 (1 H, s, CHO), and 9.78 (1 H, s, exchangeable with $D_2O$, OH).

4-*Nitrobenzyl* (2′R,3S,5R,6R)-6-*Formyloxy(phenyl)acetylaminopenicillanate* (**11**).—(2′*R*)-6-Formyloxy(phenyl)aminopenicillanic acid (1.13 g, 3 mmol) in dimethylformamide (40 ml) at 0 °C was stirred with triethylamine (0.3 g, 3 mmol), followed by addition of 4-nitrobenzyl bromide (0.65 g, 3 mmol) in dimethylformamide (5 ml). After 1 h at 0 °C and 5 h at room temperature, dichloromethane (50 ml) was added and the solution washed repeatedly with water, dried, and then concentrated under reduced pressure to afford the *title ester* as a foam (1.03 g, 67%). A small quantity was purified by preparative-layer chromatography (3:1 benzene–ethyl acetate) and showed $\nu_{max.}$ 1 785, 1 735, and 1 690 $cm^{-1}$; δ 1.43 (3 H, s, Me), 1.61 (3 H, s, Me), 4.52 (1 H, s, 3-H), 5.28 (2 H, s, $ArCH_2$), 5.4—5.7 (2 H, m, 5- and 6-H), 6.27 (1 H, s, PhC*H*), 7.2—8.2 (9 H, m, ArH), and 8.18 (1 H, s, OCHO) (Found: C, 56.3; H, 4.6; N, 8.0. $C_{24}H_{23}N_3O_8S$ requires C, 56.1; H, 4.5; N, 8.2%).

4-*Nitrobenzyl* (2′R,3S,5R,6R)-6-*Hydroxy(phenyl)acetylaminopenicillanate* (**9**).—The ester (**11**) (10.5 g) in dimethylformamide (100 ml) and water (35 ml) was stirred with sodium hydrogen carbonate (2.5 g) at room temperature for 4 days. The product was partitioned between dichloromethane and water and the organic extract purified by preparative low-pressure chromatography through $SiO_2$ (800 g, 1:1 ethyl acetate–light petroleum, 6 atm) to give the title ester (2.31 g, 18% from 6-aminopenicillanic acid), $\nu_{max.}$ 3 390, 1 788, and 1 685 $cm^{-1}$; δ 1.40 (3 H, s, Me), 1.57 (3 H, s, Me), 3.55 (1 H, br s, exchangeable, OH), 4.47 (1 H, s, 3-H), 5.09 (1 H, s, PhC*H*), 5.25 (2 H, s, $ArCH_2$), 5.4—5.7 (2 H, m, 5- and 6-H), 7.3—8.2 (9 H, m, ArH), and 7.12 (1 H, d, *J* 9 Hz, NH).

4-*Nitrobenzyl* (2′S,3S,5R,6R)-6-*Hydroxy(phenyl)acetylaminopenicillanate* (**10**).—(2′*S*)-6-Formyloxy(phenyl)acetylaminopenicillanic acid (10 g, 26.5 mmol) was dissolved in dichloromethane (100 ml) and extracted into a solution of sodium hydrogen carbonate (10 g) in water (100 ml); the aqueous solution was stirred at room temperature for 6 h before acidification to pH 2.6 with phosphoric acid and extraction into ethyl acetate. The organic extract was washed with water, dried, and the solvent removed under reduced pressure to afford (2′*S*,3*S*,5*R*,6*R*)-6-hydroxy(phenyl)acetylaminopenicillanic acid (7.6 g, 86%). The acid (7 g, 20 mmol) in dimethylformamide (20 ml) was stirred with triethylamine (2.0 g, 20 mmol) and 4-nitrobenzyl bromide (4.3 g, 20 mmol) at room temperature for 16 h before the addition of dichloromethane (100 ml); the mixture was then washed with water (4 × 250 ml) and worked up. The crude ester was purified by chromatography through $SiO_2$ (450 g; 1:1 ethyl acetate–light petroleum) to give the *title compound* (3.95 g, 40%) as a colourless foam, $[\alpha]_D^{19}$ +129° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 360, 1 785, 1 736, and 1 672 $cm^{-1}$; $\lambda_{max.}$(EtOH) 264 nm (ε 9 600); δ 1.40 (3 H, s, Me), 1.57 (3 H, s, Me), 3.81 (1 H, s, exchangeable, OH), 4.47 (1 H, s, 3-H), 5.09 (1 H, s, PhCH), 5.24 (2 H, s, $ArCH_2$), 5.4—5.7 (2 H, m, 5- and 6-H), 7.34 (6 H, m, ArH and NH), and 8.20 and 7.51 (4 H, d, *J* 9 Hz, ArH) (Found: C, 56.8; H, 4.9; N, 8.7. $C_{23}H_{23}N_3O_7S$ requires C, 56.9; H, 4.7; N, 8.7%).

4-*Nitrobenzyl* (3S,5R,5′R,6S)-4′-*Oxo*-5′-*phenylspiro*(1′,3′-*oxazolidine*-2′,6-*penicillanate*) (**17**).*—n-Butyl-lithium (1.3M-solution in hexane; 2.03 ml) was added to methanol (0.5 ml) in tetrahydrofuran (25 ml) at 0 °C and the solution cooled to −72 °C before a solution of the ester (**9**) (0.36 g, 0.75 mmol) in tetrahydrofuran (3 ml) was added. After 5 min t-butyl hypochlorite (83 mg, 0.76 mmol) was added, followed, after a further 30 min at −70 °C, by glacial acetic acid (0.5 ml). The mixture was warmed to ambient temperature, the solvent removed under reduced pressure and the residue extracted into dichloromethane; the extract was washed with water and aqueous sodium hydrogen carbonate dried, and then evaporated. Trituration of the residue with ether afforded crystals of the *title compound* (0.145 g, 40%), m.p. 228—231 °C, $[\alpha]_D^{21}$ 65° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 410, 1 789, 1 740, and 1 720 $cm^{-1}$; δ 1.42 (3 H, s, Me), 1.55 (3 H, s, Me), 4.60 (1 H, s, 3-H), 5.28 (2 H, s, $ArCH_2$), 5.57, 5.48 (2 H, 2 s, PhCH), 7.38 (5 H, m, ArH), 7.60 (1 H, s, exchangeable, NH), and 8.25 and 7.55 (4 H, 2 × d, *J* 9 Hz, ArH) (Found: C, 57.3; H, 4.2; N, 8.9; S, 6.7. $C_{23}H_{21}N_3O_7S$ requires C, 57.1; H, 4.4; N, 8.7; S, 6.6%).

4-*Nitrobenzyl* (3S,5′S,5R,6S)-4′-*Phenylspiro*(1′,3′-*oxazolidine*-2′,6-*penicillanate*) (**18**).—4-Nitrobenzyl (3*S*,5*R*,6*R*,2′*S*)-6-formyloxy(phenyl)acetylaminopenicillanate (**10**) (2.18 g, 4.5 mmol) was oxidised under the same conditions as used for the (2′*R*) isomer. The product, which did not crystallise, was

* The rules of nomenclature for spirocyclic compounds have been modified to allow the use of unprimed numbers for the penicillanic acid nucleus.

purified by column chromatography [$SiO_2$ (100 g), 1:1 ethyl acetate–light petroleum] to give the *title compound* (1.31 g, 60%), $[\alpha]_D^{21}$ 146° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 390, 1 788, 1742, and 1 715 $cm^{-1}$; δ 1.42 (3 H, s, Me), 1.48 (3 H, s, Me), 4.60 (1 H, s, 3-H), 5.28 (2 H, s, $ArCH_2$), 5.32 (1 H, s, PhCH), 5.58 (1 H, s, 5-H), 7.2—7.6 (7 H, m, ArH), 7.78 (1 H, s, exchangeable, NH), and 8.22 (2 H, d, *J* 9 Hz, ArH) (Found: C, 57.2; H, 4.5; N, 8.7; S, 6.5. $C_{23}H_{21}N_3O_7S$ requires C, 57.1; H, 4.4; H, 8.7; S, 6.6%).

(3S,5′R,5R,6S)-4′-*Oxo*-5′-*phenylspiro*(1′,3′-*oxazolidine*-2′,6-*penicillanic Acid* (**3**).—The ester (**17**) (100 mg, 0.2 mmol) was hydrogenated in wet dioxane over 10% Pd/C (100 mg) at atmospheric pressure and temperature. After filtration and removal of solvent under reduced pressure, the product was partitioned between ethyl acetate and aqueous sodium hydrogen carbonate before re-acidification of the aqueous phase and re-extraction with ethyl acetate to give the title acid (71 mg, 98%), $\nu_{max.}$ 3 600—2 500, 1 789, and 1 730br $cm^{-1}$; δ 1.58 (6 H, s, 2 × Me), 4.56 (1 H, s, 3-H), 5.52, 5.60 (2 H, 2 × s, PhC*H*, 5-H), 5.68 (1 H, br s, exchangeable, OH), 7.38 (5 H, br s, aromatic H), and 8.08 (1 H, br s, NH).

(3S,5′S,5R,6S)-4′-*Oxo*-5′-*phenylspiro*(1′,3′-*oxazolidine*-2′,6-*penicillanic Acid* (**4**).—The 5′*S*-spiro-ester (**18**) (0.3 g, 0.6 mmol) was hydrogenated over 10% Pd/C (250 mg) in 1:9 aqueous methanol (10 ml) and the products isolated in the manner described above to afford the *title acid* (0.18 g, 84%), m.p. 204 °C (decomp.), $[\alpha]_D^{21}$ +233° (*c* 1.0, MeOH), $\nu_{max.}$ 3 400—2 500, 1 785, and 1 725br $cm^{-1}$; δ($CD_3OD$) 1.57 (3 H, s, Me), 1.62 (3 H, s, Me), 4.50 (1 H, s, 3-H), 5.38 (1 H, s, PhC*H*), 5.60 (1 H, s, 5-H), and 7.2—7.6 (5 H, m, ArH) (Found: C, 55.2; H, 4.8; N, 8.2; S, 9.2. $C_{16}H_{16}N_2O_5S$ requires C, 55.2; H, 4.6; N, 8.1; S, 9.2%).

*Trimethylsilyl* (6R,7R)-7-(4-*Nitrobenzylidene*)*amino*-3-*methylceph*-3-*em*-4-*carboxylate* (**21**).—Hexamethyldisilazane (0.32 g, 2 mmol) in dry chloroform (5 ml) was treated with 7-aminodesacetoxycephalosporanic acid (0.43 g, 2 mmol) and the mixture heated to reflux for 1 h, by which time all solids had dissolved. The solvent was removed under reduced pressure and 4-nitrobenzaldehyde (0.18 g, 1.2 mmol) added in dry chloroform (10 ml). The solution was stirred at room temperature for 1.5 h and the precipitated solid filtered off. The mother-liquor was evaporated to dryness to produce the title compound (0.42 g, 50%), m.p. 182—185 °C (decomp.), $\nu_{max.}$(Nujol), 1 747, 1 690, and 1 634 $cm^{-1}$; δ 0.41 (9 H, s, $Me_3Si$), 2.20 (3 H, s, Me), 3.26 (1 H, d, *J* 18 Hz, 2-H), 3.60 (1 H, d, *J* 18 Hz, 2-H), 5.21 (1 H, d, *J* 5 Hz, 6-H), 5.49 (1 H, br d, *J* 5 Hz, 7-H), 7.95, 8.27 (4 H, 2 d, *J* 9 Hz, ArH), and 8.76 (1 H, d, *J* 2 Hz, ArCH=N).

(6R,7S)-7-*Methylthio*-7-(4-*nitrobenzylidene*)*amino*-3-*methylceph*-3-*em*-4-*carboxylic Acid* (**22**).—Lithium di-isopropylamide [from n-butyl-lithium (0.42 ml, 1.2M-solution in hexane) and di-isopropylamine (0.55 g)] in tetrahydrofuran (10 ml) was cooled to −75 °C before addition of solution of arylidene derivative (**21**) (0.21 g, 0.5 mmol) in dimethylformamide (10 ml). A dark blue solution formed immediately, to which was added, after 15 min, a solution of methyl toluene-*p*-thiosulphonate (0.18 g, 0.9 mmol) in dimethylformamide (5 ml). The solution was left at −70 °C for 30 min before it was slowly warmed to room temperature. The yellow solution was partitioned between ethyl acetate–water and the aqueous phase adjusted to pH 3 and the mixture extracted with ethyl acetate. Work-up afforded the title compound as a gum (0.24 g), which was immediately used in subsequent transformations.

4-*Nitrobenzyl* (6R,7S)-7-*Amino*-3-*methyl*-7-*methylthioceph*-3-*em*-4-*carboxylate* (**24**).—The acid (**22**) (37.8 g) in dimethylformamide (400 ml) was esterified with 4-nitrobenzyl bromide (21.6 g, 0.1 mol) and potassium hydrogen carbonate (10 g, 0.1 mol) at room temperature overnight before the products were partitioned between ethyl acetate (500 ml) and water (400 ml). The ethyl acetate extract afforded 4-*nitrobenzyl* (6R,7S)-3-*methyl*-7-*methylthio*-7-(4-*nitrobenzylidene*)*aminoceph*-3-*em*-4-*carboxylate* as a solid foam (50.4 g, 99%). An analytical sample, isolated by t.l.c. (3:7 ethyl acetate–light petroleum) showed $[\alpha]_D^{20}$ +133° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 1 745, 1 720, and 1 634 $cm^{-1}$; δ 2.15 (3 H, s, Me), 2.25 (3 H, s, Me), 3.20 (1 H, d, *J* 18 Hz, 2-H), 3.50 (1 H, *J* 18 Hz, 2-H), 5.05 (1 H, s, 6-H), 5.30 (2 H, s, $ArCH_2$), 7.3—8.4 (8 H, m, ArH), and 8.80 (1 H, s, ArCH=N) (Found: C, 52.2; H, 3.8; N, 10.3; S, 12.1. $C_{23}H_{20}N_4O_7S_2$ requires C, 52.3; H, 3.8; N, 10.6; S, 12.1%).

To the Schiff's base (**23**) (49 g) in dimethylformamide (350 ml) was added Girard's reagent T (24 g) in water (50 ml). A further quantity of water was added until the mixture was homogeneous, followed by addition of concentrated hydrochloric acid (1 ml). After 4 h at room temperature ethyl acetate and aqueous sodium hydrogen carbonate were added and the organic layer separated, dried, and the solvent removed under reduced pressure to afford the crude amine. Chromatography through $SiO_2$ (280 g) eluting with 9:11 ethyl acetate-light petroleum afforded the title amine (13.9 g, 23% from 7-aminodeacetoxycephalosporanic acid), δ 2.23 (3 H, s, Me), 2.34 (3 H, s, Me), 2.80 (2 H, br s, exchangeable $D_2O$, $NH_2$), 3.38 (2 H, s, 2-$H_2$), 4.83 (1 H, s, 6-H), 5.38 (2 H, s, $ArCH_2$), 7.65, 8.27 (4 H, d, *J* 8 Hz, ArH). The amine was dissolved in acetone (100 ml) and 6M-hydrochloric acid (6 ml) added to produce the *amine hydrochloride*, m.p. 113—117 °C (decomp.) $[\alpha]_D^{20}$ + (*c* 1.0, MeOH), $\nu_{max.}$(Nujol) 3 380, 1 776, and 1 770 $cm^{-1}$ (Found: C, 43.0; H, 4.5; N, 9.2. $C_{16}H_{17}N_3O_3S{\cdot}HCl{\cdot}H_2O$ requires C, 42.7; H, 4.5; N, 9.3%).

4-*Nitrobenzyl* (2′S,6R,7S)-7-*Formyloxy*(*phenyl*)*acetylamino*-7-*methylthio*-3-*methylceph*-3-*em*-4-*carboxylate*.—The amine ester hydrochloride (**24**) (0.43 g, 1 mmol) in dichloromethane (50 ml) was shaken with aqueous sodium hydrogen carbonate until dissolution was complete. The organic phase was dried and the solvent evaporated to give the free amine. The amine, in dry dichloromethane (30 ml) at 0 °C was treated with propylene oxide (0.08 g, 1.4 mmol) and (*S*)-formyloxy(phenyl)acetyl chloride (0.2 g, 1.0 mmol) and the solution then stirred at room temperature overnight. Work-up in the normal manner gave the *title compound* (0.56 g, 100%) as a pale yellow foam. A sample, purified by preparative t.l.c. showed $\nu_{max.}$ 3 350, 1 779, 1 730, and 1 700 $cm^{-1}$; δ 2.20 (6 H, s, 2 × Me), 3.26 (2 H, s, 2-H), 4.93 (1 H, s, 6-H), 5.33 (2 H, s, $ArCH_2$), 6.27 (1 H, s, PhCH), 7.2—8.3 (10 H, m, ArH), and 8.21 (1 H, s, OCHO) (Found: C, 54.1; H, 4.4; N, 7.7. $C_{25}H_{23}N_3O_8S_2$ requires C, 53.9; H, 4.2; N, 7.5%).

4-*Nitrobenzyl* (2′R,6R,7S)-7-*Formyloxy*(*phenyl*)*acetylamino*-7-*methylthio*-3-*methylceph*-3-*em*-4-*carboxylate*.—This compound was prepared by the method used for the 2′*S*-isomer starting with (*R*)-formyloxy(phenyl)acetyl chloride. The *R*-isomer was slightly less polar than its epimer on t.l.c. ($SiO_2$, 9:11 ethyl acetate–light petroleum). The ester showed $\nu_{max.}$ 3 350, 1 780, 1 730, and 1 700 $cm^{-1}$; δ 2.23 (3 H, s, Me), 2.30 (3 H, s, Me), 3.22 (2 H, s, 2-H), 4.95 (1 H, s, 6-H), 5.36 (2 H, s, $ArCH_2$), 6.30 (1 H, s, PhCH), 7.3—8.4 (10 H, m, ArH), and 8.23 (1 H, s, OC*H*O).

4-*Nitrobenzyl* (5′R,6R,7S)-3-*Methyl*-4′-*oxo*-5′-*phenylspiro*-(1′,3′-*oxazolidine*-2′,7-*ceph*-3-*em*)-4-*carboxylate* (**26**).—4-Nitrobenzyl (2′*R*,6*R*,7*S*)-7-formyloxy(phenyl)acetyl-7-methylthio-3-methylceph-3-em-4-carboxylate (1.6 g, 2.9 mmol) in methanol (40 ml) and acetone (8 ml) was deformylated with potassium hydrogen carbonate (0.34 g, 3.4 mmol). After 1.25 h at room

temperature the mixture was poured into pH 7 buffer and extracted with ethyl acetate (3 × 50 ml). The extracts were washed with brine, combined, dried, and evaporated to give one major product, purified by filtration through $SiO_2$ (16 g) using 1:2 ethyl acetate–light petroleum as eluant, to produce 4-*nitrobenzyl* (2′R,6R,7S)-*hydroxy(phenyl)acetylamino-7-methylthio-3-methylceph-3-em-4-carboxylate* (**20**) (0.89 g, 59%), m.p. 87—91 °C, $[\alpha]_D^{20}$ +66° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 380, 1 774, 1 725, and 1 686 $cm^{-1}$; $\lambda_{max.}$(EtOH) 264 nm (ε 13 300); δ 2.22 (3 H, s, Me), 2.26 (3 H, s, Me), 3.01 (1 H, d, *J* 16 Hz, 2-H), 3.29 (1 H, d, *J* 16 Hz, 2-H), 3.76 (1 H, d, *J* 4 Hz, exchangeable, OH), 4.86 (1 H, s, 6-H), 5.15 (1 H, d, *J* 4 Hz, PhCH), 5.29 (2 H, s, $ArCH_2$), and 7.1—8.3 (10 H, m, ArH and NH) (Found: C, 54.4; H, 4.5; N, 7.9; S, 12.2. $C_{24}H_{23}N_3O_7S_2$ requires C, 54.4; H, 4.4; N, 7.9; S, 12.1%).

A sample of the above hydroxy(phenyl)acetyl derivative (0.5 g, 1 mmol) in dimethylformamide (10 ml) was treated with pyridine (0.16 g, 2.0 mmol) and mercuric chloride (0.23 g, 1 mmol) at room temperature, with stirring, for 3 h. The mixture was filtered and ethyl acetate and water added. The organic phase was washed copiously with water, dried, and the solvent removed under reduced pressure to produce a pale yellow gum. Chromatography through $SiO_2$ (10 g) eluting with ethyl acetate–light petroleum mixtures gave the *title compound* (0.287 g, 60%), $[\alpha]_D^{20}$ −5° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 350, 1 780, and 1 728br $cm^{-1}$; δ 2.19 (3 H, s, Me), 3.27 (1 H, d, *J* 17 Hz, 2-H), 3.43 (1 H, d, *J* 17 Hz, 2-H), 5.08 (1 H, s, 6-H), 5.36 (2 H, s, $ArCH_2$), 5.48 (1 H, s, PhCH), and 7.3—8.3 (10 H, ArH and NH). This material was used directly in the preparation of the free acid (**6**).

4-*Nitrobenzyl* (5′R,6R,7S)-3-*Methyl*-4′-*oxo*-5′-*phenylspiro*(1′,3′-*oxazolidine*-2′,7-*ceph*-3-*em*)-4-*carboxylate* (**25**).—In a similar manner to that described for the 5′*R*-epimer, 4-nitrobenzyl-(2′*S*,6*R*,7*S*)-7-formyloxy(phenyl)acetylamino-7-methylthio-3-methylceph-3-em-4-carboxylate (2.0 g) was deformylated with potassium hydrogen carbonate in 1:1 methanol–acetone. Work-up afforded the alcohol (**19**), contaminated with a little of the 2(3)-unsaturated isomer. This material was not purified but immediately processed. The crude alcohol (1.9 g, 3.6 mmol) in dry dimethylformamide (30 ml) and pyridine (0.54 g, 7 mmol) was treated with a solution of mercuric chloride (0.95 g, 3.6 mmol) in dry dimethylformamide (5 ml) at room temperature for 1 h. The precipitate was removed by filtration and the solution partitioned between ethyl acetate and brine. The crude organic extract was chromatographed through $SiO_2$ (26 g) eluting with ethyl acetate–light petroleum mixtures to give the *title compound* (0.55 g, 33%), m.p. 187—191 °C, $\nu_{max.}$ 1 787, and 1 735 $cm^{-1}$; δ 2.18 (3 H, s, Me), 3.32 (2 H, s, 2-H), 5.09, (1 H, s, 6-H), 5.34 (3 H, s, $ArCH_2$ and PhCH), and 7.3—8.3 (10 H, m, ArH and NH) (Found: C, 57.2; H, 3.9; N, 8.7; S, 6.8. $C_{23}H_{19}N_3O_7S$ requires C, 57.3; H, 3.9; N, 8.7; S, 6.7%).

(5′S,6R,7S)-3-*Methyl*-4′-*oxo*-5′-*phenylspiro*(1′,3′-*oxazolidine*-2′,7-*ceph*-3-*em*)-4-*carboxylic Acid* (**5**).—The 4-nitrobenzyl ester (**25**) (0.15 g, 0.31 mmol) in ethyl acetate (10 ml) and a solution of sodium hydrogen carbonate (27 mg, 0.32 mmol) in water (10 ml) was hydrogenated over 10% Pd/C (0.3 g) at 3 atm and 20 °C for 1 h. After filtration the aqueous phase was washed with ethyl acetate, acidified with phosphate buffer to pH 2.5, and then re-extracted with ethyl acetate (×3). The acidic organic extract was dried and the solvent removed under reduced pressure to give the title acid (0.095 g, 88%), as a colourless foam, $[\alpha]_D^{20}$ +121° (*c* 1.0, $CHCl_3$), $\nu_{max.}$(Nujol) 3 350—2 500br, 1 776, and 1 708 $cm^{-1}$; δ 2.13 (3 H, s, Me), 3.33 (2 H, s, 2-H), 5.06 (1 H, s, 6-H), 5.27 (1 H, s, PhCH), and 7.1—7.5 (5 H, m, ArH). Re-esterification of a sample of the acid, with 4-nitrobenzyl bromide, re-formed the starting ester (**25**).

(5′R,6R,7S)-3-*Methyl*-4′-*oxo*-5′-*phenylspiro*(1′,3′-*oxazolidine*-2′,7-*ceph*-3-*em*-4-*carboxylic Acid* (**6**).—The 4-nitrobenzyl ester (**26**) (0.1 g, 0.2 mmol) was hydrogenated as above using sodium hydrogen carbonate (18 mg) and 10% Pd/C (0.2 g). After work-up the *title acid* (0.065 g, 91%) showed m.p. >210 °C (decomp.), $\nu_{max.}$ 3 350—2 700br, 1 782, and 1 728 $cm^{-1}$; δ 2.19 (3 H, s, Me), 3.24 (2 H, s, 2-H), 5.07 (1 H, s, 6-H), 5.50 (1 H, s, PhCH), 7.40 (5 H br s, ArH), and 7.80 (2 H, br s, exchangeable, OH and NH) (Found: C, 55.4; H, 4.0; N, 7.9; S, 9.2. $C_{16}H_{14}N_2O_5S$ requires C, 55.5; H, 4.1; N, 8.1; S, 9.3%).

(2′R,6R,7R)-3-*Acetoxymethyl*-7-*formyloxy(phenyl)acetylaminoceph*-3-*em*-4-*carboxylic Acid* (**30**).—7-Aminocephalosporanic acid, as its toluene-*p*-sulphonate salt (2.2 g, 5 mmol) was suspended in water (10 ml) at 0 °C and stirred with sodium hydrogen carbonate (1.68 g, 20 mmol) until dissolution was complete. More water (5 ml) and acetone (10 ml) was added before the dropwise addition of (*R*)-formyloxy(phenyl)acetyl chloride (1.0 g, 5 mmol) with stirring at room temperature during 15 min. After a further 4 h, most of the acetone was removed under reduced pressure, and the solution was layered with ethyl acetate and acidified with phosphate buffer to pH 2.0. The ethyl acetate layer was dried and the solvent removed, under reduced pressure, to give the title amide as a gum (2.0 g, 95%), $\nu_{max.}$ 1 780, 1 732, 1 725, and 1 698 $cm^{-1}$; δ 2.03 (3 H, s, MeCO), 3.43 (2 H, s, 2-H), 4.7—5.3 (3 H, m, 6-H and $CH_2OAc$), 5.73 (1 H, dd, *J* 5, 8 Hz, 7-H), 6.25 (1 H, s, PhCH), 7.42 (5 H, m, H), 7.77 (1 H, d, *J* 8 Hz, NH), and 8.17 (1 H, s, OCHO).

(2′R,6R,7R)-3-*Acetoxymethyl*-7-*hydroxy(phenyl)acetylaminoceph*-3-*em*-4-*carboxylic Acid* (**32**).—The crude formate (**30**) (1.4 g) was dissolved in methanol (30 ml) and stirred with potassium hydrogen carbonate (0.5 g) for 5 h at room temperature. The mixture was then poured into ethyl acetate (40 ml) and 5% phosphoric acid (60 ml), the aqueous phase separated and re-extracted with more ethyl acetate (×2), and the combined organic extracts dried and the solvent removed under reduced pressure to give the title acid (1.2 g) as a gum. The acid was immediately used in the next stage.

4-*Nitrobenzyl* (2′R,6R,7R)-3-*Acetoxymethyl*-7-*hydroxy*-(*phenyl*)*acetylaminoceph*-3-*em*-4-*carboxylate* (**34**).—The crude acid (**32**) (1.2 g) was esterified with 4-nitrobenzyl bromide (1.1 g, 5 mmol) and potassium hydrogen carbonate (0.5 g, 5 mmol) in dimethylformamide (30 ml) in the normal manner for 5 h at room temperature. Work-up, followed by column chromatography through $SiO_2$ (40 g) using 1:1 ethyl acetate–light petroleum as eluant, gave the *title ester* (0.90 g, 33% from 7-aminocephalosporanic acid), m.p. 152—156 °C, $[\alpha]_D^{20}$ −6.5° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 660, 3 390, 1 785, 1 735, and 1 686 $cm^{-1}$; δ 2.08 (3 H, s, MeCO), 3.35 (1 H, d, *J* 3.5 Hz, exchangeable, OH), 3.42 (1 H, d, *J* 18 Hz, 2-H), 3.58 (1 H, d, *J* 18 Hz, 2-H), 5.00 (1 H, d, *J* 5 Hz, 6-H), 4.84 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.16 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.17 (1 H, d, *J* 3.5 Hz, PhC*H*), 5.37 (2 H, s, $ArCH_2$), 5.82 (1 H, dd, *J* 5, 9 Hz, 7-H), 7.05 (1 H, d, *J* 9 Hz, NH), 7.40 (5 H, br s, ArH), and 8.25 and 7.61 (4 H, m, ArH) (Found: C, 55.4; H, 4.2; N, 7.6; S, 5.9. $C_{25}H_{23}N_3O_9S$ requires C, 55.4; H, 4.2; N, 7.8; S, 5.9%).

4-*Nitrobenzyl* (5′R,6R,7S)-3-*Acetoxy*-4′-*oxo*-5′-*phenylspiro*-(1′,3′-*oxazolidine*-2′,7-*ceph*-3-*em*)-4-*carboxylate* (**28**).—Lithium methoxide [from methanol (0.5 ml) and n-butyl-lithium (1.2M; 2.9 ml)] in tetrahydrofuran (30 ml) was added to the alcohol (**34**) (0.56 g, 1 mmol) at −72 °C. After 5 min, t-butyl hypochlorite (0.11 g, 1.0 mmol) was added to the solution the colour of which changed from orange-brown to light pink. After a further 30 min at −70 °C, glacial acetic acid (1 ml) was added to the mixture which was then brought to room temperature.

Work-up in the usual manner and chromatography of the crude products through $SiO_2$ (50 g), eluting with 1:1 ethyl acetate–light petroleum gave the *title spiro-compound* (0.24 g, 44%) as a pale yellow foam, $[\alpha]_D^{20}$ −27° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 390, 1 783, and 1 735—1 720 $cm^{-1}$; δ 2.07 (3 H, s, MeCO), 3.40 (1 H, d, *J* 18 Hz, 2-H), 3.55 (1 H, d, *J* 18 Hz, 2-H), 4.82 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.14 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.38 (2 H, s, $ArCH_2$), 5.19 (1 H, s, 6-H or PhCH), 5.47 (1 H, s, 6-H or PhCH), 7.37 (5 H, br s, ArH), 7.87 (1 H, br s, NH), and 7.60 and 8.22 (4 H, m, ArH) (Found: C, 55.7; H, 4.2; N, 7.8; S, 5.9. $C_{25}H_{21}N_3O_9S$ requires C, 55.7; H, 3.9; N, 7.8; S, 5.9%).

(5′R,6R,7S)-3-*Acetoxymethyl*-4′-*oxo*-5′-*phenylspiro*(1′,3′-*oxazolidine*-2′,7-*ceph*-3-*em*)-4-*carboxylic Acid* (**8**).—The 4-nitrobenzyl ester (**28**) (68 mg, 0.13 mmol) in 1:1 methanol–ethyl acetate was hydrogenated over 10% Pd/C (80 mg) at atmospheric pressure and room temperature. When hydrogen uptake was complete the solution was filtered and extracted with aqueous sodium hydrogen carbonate. The aqueous extract was washed with ethyl acetate, before careful acidification, with phosphate buffer, to pH 2.5 and then re-extraction with ethyl acetate. The extract was dried and the solvent removed under reduced pressure to give the title acid as an amorphous solid (45 mg, 88%), $\nu_{max.}$ 1 784 and 1 735 $cm^{-1}$; δ 2.04 (3 H, s, MeCO), 3.37 (1 H, d, *J* 18 Hz, 2-H), 3.48 (1 H, d, *J* 18 Hz, 2-H), 4.86 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.11 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.07 (1 H, s, 6-H or PhCH), 5.48 (1 H, s, 6-H or PhCH), 7.35 (5 H, br s, ArH), and 7.75 (2 H, br s, exchangeable, $CO_2H$ and NH).

(6R,7R,2′S)-3-*Acetoxymethyl*-7-*hydroxy*(*phenyl*)*acetylaminoceph*-3-*em*-4-*carboxylic Acid* (**31**).—7-Aminocephalosporanic acid as its toluene-*p*-sulphonic salt (4.4 g, 10 mmol) was acylated with (*S*)-formyloxy(phenyl)acetyl chloride and the product deformylated *in situ*, with potassium hydrogen carbonate in methanol, in the manner described for the (*R*)-isomer. The product exhibited the following spectral data: $\nu_{max.}$ 1 780, 1 730, and 1 687 $cm^{-1}$; δ ([$^2H_6$]acetone) 2.08 (3 H, s, MeCO), 3.58 (1 H, d, *J* 18 Hz, 2-H), 3.73 (1 H, d, *J* 18 Hz, 2-H), 4.87 (1 H, d, *J* 13 Hz, $CH_2OAc$), 5.13 (1 H, d, *J* 13 Hz, $CH_2OAc$), 5.15 (1 H, d, *J* 5 Hz, 6-H), 5.23 (1 H, s, PhCH), 5.85 (1 H, dd, *J* 5, 9 Hz, 7-H), 6.05 (2 H, br s, $CO_2H$ and OH), 7.3—7.6 (5 H, m, ArH), and 7.98 (1 H, d, *J* 9 Hz, NH).

4-*Nitrobenzyl* (2′S,6R,7R)-3-*Acetoxymethyl*-7-*hydroxy*-(*phenyl*)*acetylaminoceph*-3-*em*-4-*carboxylate* (**33**).—The acid (**31**) (4 g, 9.8 mmol) was esterified with 4-nitrobenzyl bromide under the conditions described for the corresponding (*R*)-acid. The crude ester was subjected to column chromatography on $SiO_2$ (40 g), using 1:1 ethyl acetate–light petroleum as eluant, to produce the *title ester* (2.1 g, 40% from 7-aminocephalosporanic acid) as a pale yellow foam, $[\alpha]_D^{20}$ +83° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 380, 1 784, 1 733, and 1 687 $cm^{-1}$; δ 2.06 (3 H, s, MeCO), 3.47 (2 H br s, 2-H), 4.90 (1 H, d, *J* 5 Hz, 6-H), 4.84 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.16 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.07 (1 H, s, PhC*H*OH), 5.35 (2 H, s, $ArCH_2$), 5.76 (1 H, dd, *J* 5, 9 Hz, 7-H), 7.38 (5 H, br s, ArH), 7.71 (1 H, d, *J* 9 Hz, NH), and 7.57 and 8.20 (4 H, m, ArH) (Found: C, 55.6; H, 4.5; N, 7.7. $C_{25}H_{23}N_3O_9S$ requires C, 55.5; H, 4.3; N, 7.8%).

4-*Nitrobenzyl* (5′S,6R,7S)-3-*Acetoxymethyl*-4′-*oxo*-5′-*phenylspiro*(1′,3′-*oxazolidine*-2′,7-*ceph*-3-*em*)-4-*carboxylate* (**27**).—The alcohol (**33**) (1.08 g, 2 mmol) was cyclised using the same procedure as for the (*R*)-isomer, except that a longer time (20 min) was allowed for complete formation of the anion, thereby improving the yield. The product was purified by column chromatography through $SiO_2$ (50 g), using 1:1 ethyl acetate–light petroleum as eluant, to give the *title compound* as a crystalline solid (0.84 g, 77%), m.p. 172—174 °C, $[\alpha]_D^{20}$ +68° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 390, 1 785, and 1 733 $cm^{-1}$; δ 2.06 (3 H, s, MeCO), 3.41 (1 H, d, *J* 18 Hz, 2-H), 3.60 (1 H, d, *J* 18 Hz, 2-H), 4.82 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.12 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.09 (1 H, s, PhCH or 6-H), 5.33 (1 H, s, PhCH or 6-H), 5.37 (2 H, s, $ArCH_2$), 7.3—7.7 (5 H, m, ArH), 7.99 (1 H, s, NH), and 7.57 and 8.17 (4 H, m, ArH) (Found: C, 55.8; H, 4.2; N, 7.6; S, 5.8. $C_{25}H_{21}N_3O_9S$ requires C, 55.7; H, 3.9; N, 7.8; S, 5.9%).

(5′R,6R,7S)-3-*Acetoxymethyl*-4′-*oxo*-5′-*phenylspiro*(1′,3′-*oxazolidine*-2′,7-*ceph*-3-*em*)-4-*carboxylic Acid* (**8**).—The 4-nitrobenzyl ester (**28**) (68 mg, 0.13 mmol) in 1:1 methanol–ethyl acetate was hydrogenated under similar conditions to those used for the 5′*R*-isomer. After 1.5 h, when hydrogen uptake was complete, the mixture was worked up to afford the title compound (0.17 g, 90%), as a colourless foam, $\nu_{max.}$ 1 783 and 1 733 $cm^{-1}$; δ 2.04 (3 H, s, MeCO), 3.37 (1 H, d, *J* 18 Hz, 2-H), 3.53 (1 H, d, *J* 18 Hz, 2-H), 4.86 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.07 (1 H, d, *J* 14 Hz, $CH_2OAc$), 5.07 and 5.60 (2 H, 2 × s, PhCH and 6-H), 6.10 (1 H, br s, exchangeable, $CO_2H$), and 7.2—7.8 (6 H, m, ArH and NH).

4-*Nitrobenzyl* (6R,7S)-7-(2-*Hydroxybenzoylamino*)-3-*methyl*-7-*methylthioceph*-3-*em*-4-*carboxylate* (**37**).—The aminocephem (**24**) as its hydrochloride salt (0.86 g, 2 mmol), suspended in dichloromethane, was shaken with aqueous sodium hydrogen carbonate to liberate the free amine. The organic phase was dried and made up to a volume of 10 ml with more solvent before being cooled to 0 °C; propylene oxide (0.18 g, 2 mmol) and salicyloyl chloride (0.31 g, 2 mmol) were added and the solution stirred for 48 h at room temperature. A further quantity of salicyloyl chloride (0.1 g) was added after 24 h. The solution was washed with aqueous sodium hydrogen carbonate and then water, dried, and the solvent removed under reduced pressure. The crude product was chromatographed through $SiO_2$ (16 g), using ethyl acetate–light petroleum mixtures as eluant, to give the title compound as a colourless foam, $[\alpha]_D^{20}$ +130° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 3 430, 1 776, 1 730, and 1 646 $cm^{-1}$; $\lambda_{max.}$(EtOH) 242 nm (ε 22 200); δ 2.25 (3 H, s, Me), 2.40 (3 H, s, Me), 3.35 (2 H, s, 2-$CH_2$), 5.05 (1 H, s, 6-H), 5.31 (2 H, s, $ArCH_2$), 6.7—7.6 (6 H, m, ArH, OH, NH), and 7.52—8.11 (4 H, m, ArH).

4-*Nitrobenzyl* (6R,7RS)-3′,4′-*Dihydro*-3-*methyl*-4′-*oxospiro*-(2′H-1′,3′-*benzoxazine*-2′,7-*ceph*-3-*em*)-4-*carboxylate* (**36**).—The ester (**37**) (0.3 g, 0.6 mmol) in dry dimethylformamide (7 ml) was cooled to 0 °C before pyridine (0.11 g, 1.4 mmol) and mercuric chloride (0.16 g, 0.6 mmol) in dry dimethylformamide (3 ml) were added. After 10 min at 0 °C the mixture was allowed to warm to room temperature. After 4 h ethyl acetate was added and the mixture filtered. The filtrate was washed with water (× 5), and the organic solution dried and the solvent removed under reduced pressure. The residue was chromatographed through $SiO_2$ (10 g) eluting with ethyl acetate–light petroleum mixtures to give the *title compound* as a mixture of diastereoisomers (0.16 g, 58%). T.l.c. (7:13 ethyl acetate–light petroleum, 5 elutions) showed a slight separation into two components. The substance showed $[\alpha]_D^{20}$ +71° (*c* 1.0, $CHCl_3$), $\nu_{max.}$ 1 784, 1 782, and 1 692 $cm^{-1}$; $\lambda_{max.}$(EtOH) 240 nm (ε 16 800); δ 2.27 (3 H, s, Me), 3.29, 3.24 (2 H, 2 × s, 2-$CH_2$), 5.09, 5.15 (1 H, 2 × s, 6-H), 5.36 (2 H, s, $ArCH_2$), and 6.8—8.2 (8 H, m, ArH) (Found: C, 56.6; H, 3.9; N, 8.7; S, 6.8. $C_{22}H_{17}N_3O_7S$ requires C, 56.5; H, 3.7; N, 9.0; S, 6.9%).

(6R̄,7R̄S̄)-3′,4′-*Dihydro*-3-*methyl*-4′-*oxospiro*(2′H-1′,3′-*benzoxazine*-2′,7-*ceph*-3-*em*)-4-*carboxylic Acid* (**35**).—The 4-nitrobenzyl ester (**36**) (40 mg, 0.09 mmol) was dissolved in ethyl acetate (5 ml) before addition to a solution of sodium hydrogen carbonate (10 mg, 0.12 mmol) in water (5 ml) containing a suspension of 10% Pd/C (75 mg). The mixture was filtered, the

pH reduced to 2 with phosphate buffer, and the solution extracted with ethyl acetate; the extracts were dried and the solvent removed to give the title acid (11.6 g, 41%) as a gum. T.l.c. (50:10:1 ethyl–methanol–acetic acid) showed two closely running compounds. The mixture showed δ 2.28 (3 H, s, Me), 3.29, 3.25 (2 H, 2 × s, 2-$CH_2$), 5.00 (1 H, br s, exchangeable, $CO_2H$), 5.10, 5.14 (1 H, 2 × s, 6-H), and 6.8—8.0 (4 H, m, ArH).

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