Preparation and Properties of 7α-Formamido Cephalosporins

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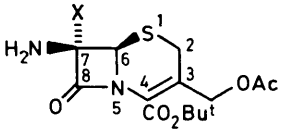
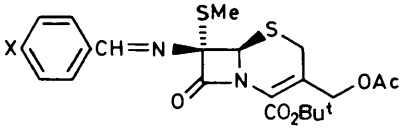
The preparation of novel antibacterially active 7α-formamido cephalosporins from the corresponding 7α-(methylthio) analogues by mercury(II)-mediated displacement with ammonia and subsequent formylation is described. The amine (**2**), a versatile intermediate, was prepared and acylated to give a wider range of 7α-formamido cephalosporins. Modifications at C-3 are discussed, in particular the preparation of the 3-(heterocyclylthiomethyl) cephalosporin (**44**) and the 3-(pyridylmethyl) derivative (**49**).

The isolation of the cephamycins[1] and subsequent identification of temocillin[2] demonstrated that the presence of a 7α(6α)-methoxy substituent conferred β-lactamase stability on cephalosporins and penicillins. Whilst considerable effort has been directed, both in these laboratories[3] and in others,[4] towards the syntheses of derivatives possessing alternative 7α(6α)-functionality, until recently none showed superiority over methoxy with respect to biological activity. However, in a preliminary communication[5] we reported that the introduction of a 7α(6α)-formamido group provided certain cephalosporins and penicillins which were found to be potent, β-lactamase-stable antibiotics. It is perhaps remarkable that, soon after our discovery, the isolation of several naturally occurring 7α-formamidocephalosporins was disclosed;[6–8] however, the Squibb compounds, SQ 28516 and SQ 28517,[6] the chitinovorins,[7] and the cephabacins,[8] all possess mediocre antibacterial activity.

The starting material for our syntheses was t-butyl 7β-amino-7α-(methylthio)cephalosporanate (**1**) which we prepared as a salt from the Schiff's base (**4**)[9] by treatment with toluene-4-sulphonic acid. Initially we had used the 4-nitrobenzylidene Schiff's base (**3**), but found, as had Sammes and Smith,[10] that there were difficulties associated with removal of the 4-nitrobenzylidene group.

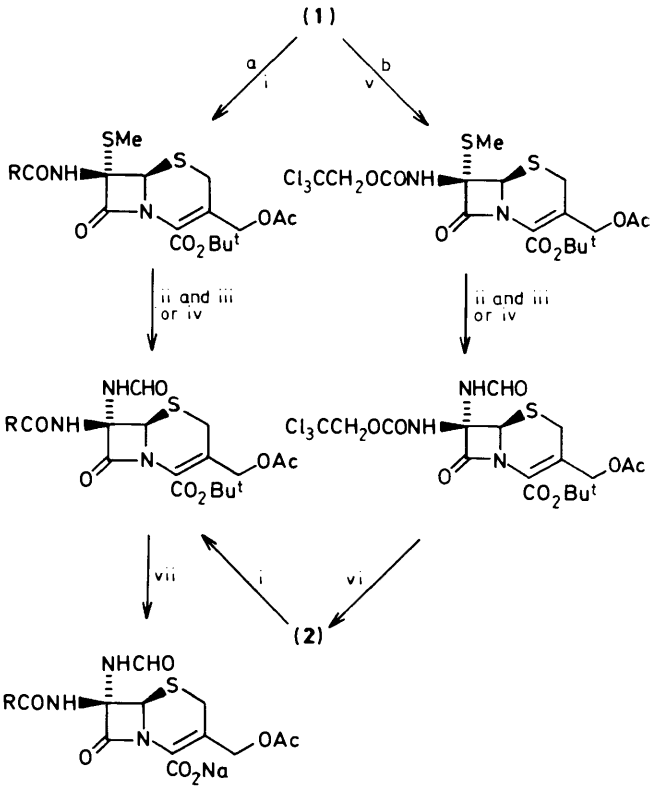


(**1**) X = SMe
(**2**) X = NHCHO



(**3**) X = NO_2
(**4**) X = H

The route to the target molecules (**21**)—(**24**) is shown in Scheme 1. Following route (a) the amine (**1**) was acylated with the appropriate acid chloride to give the cephalosporanates (**5**) and (**6**). The methylthio group was replaced by formamido in a two-step process involving a mercury(II)-mediated amination in *NN*-dimethylformamide (DMF), followed by formylation with acetic formic anhydride. The 7α-formamido derivatives (**13**) and

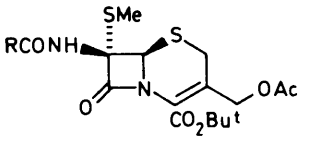


Scheme 1. *Reagents:* i, RCOCl; ii, NH_3, Hg^{II}; iii, $CH_3COOCHO$; iv, $(Me_3Si)_2NCHO$, Hg^{II}; v, Cl_3CCH_2OCOCl; vi, Zn, H^+; vii, CF_3CO_2H or HCO_2H, Na^+

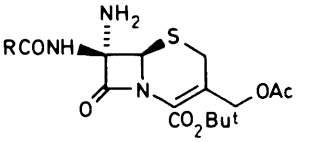
(**14**) so formed were treated with formic acid to provide the corresponding sodium salts (**21**) and (**22**).[11]

Whilst a wide variety of 7α-formamidocephalosporins were available by this route a more versatile intermediate was provided by the highly crystalline 7β-amino-7α-formamido ester (**2**). This was readily prepared as outlined in Scheme 1, route (b), *via* acylation of the 7α-(methylthio) amine (**1**) with (2,2,2-trichloroethoxy)carbonyl chloride to give the protected amine (**7**), followed by successive amination, formylation, and zinc–hydrochloric acid reduction.[12] It is also possible to introduce the 7α-formamido moiety directly by treatment of the 7α-(methylthio) compound (**7**) with *NN*-bis(trimethylsilyl)-formamide in the presence of mercury(II) acetate.[13]

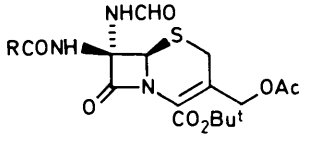
Restricted rotation about the C–N bond of the formamido group results in two rotameric forms being observed in the ^1H n.m.r. spectra of these compounds. The major, *Z*, rotamer possesses $^3J \sim 1$ Hz for N*H*–C*H*O, the corresponding coupling



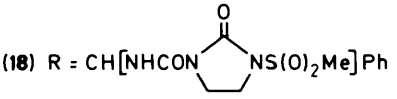
(5) R = CH_2(2-thienyl)
(6) R = CH(NHPip)Ph
(7) R = OCH_2CCl_3
(8) R = OCH_2Ph



(9) R = CH_2(2-thienyl)
(10) R = CH(NHPip)Ph
(11) R = OCH_2CCl_3
(12) R = OCH_2Ph

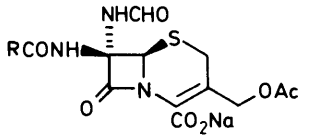


(13) R = CH_2(2-thienyl)
(14) R = CH(NHPip)Ph
(15) R = OCH_2CCl_3
(16) R = $CH(NHCO_2CH_2CCl_3)Ph$
(17) R = $CH(NH_2)Ph$

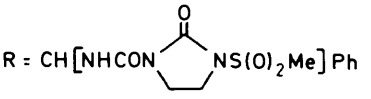


(18) R = CH[NHCON NS(O)$_2$Me]Ph

(19) R = $CH(NHPip)C_6H_3(OAc)_2$(3, 4)
(20) R = OCH_2Ph

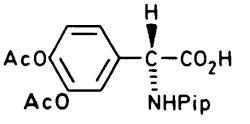


(21) R = CH_2(2-thienyl)
(22) R = CH(NHPip)Ph



(23) R = CH[NHCON NS(O)$_2$Me]Ph

(24) R = $CH(NHPip)C_6H_3(OAc)_2$(3, 4)



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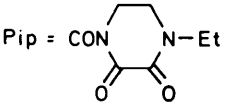


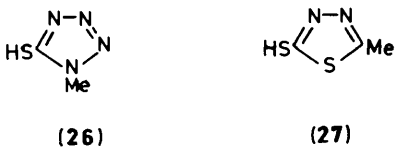
constant for the minor, *E*, rotamer ($\leq 30\%$) being $^3J \sim 11$ Hz.[14] A variable-temperature ^{1}H n.m.r. study on the 7α-formamido amine (**2**) in $(CD_3)_2SO$ solution showed that the resonance for CHO [δ 8.05 (*Z*) and 8.39 (*E*) at 30 °C] and NH_2 (δ 2.78 and 3.07 at 30 °C) coalesced at 100 °C, and those due to 6-H (δ 4.97 and 5.07 at 30 °C) coalesced at 80 °C, whilst the formamido N–H resonances (δ 8.91 and 8.96 at 30 °C) coalesced at a poorly defined but lower temperature.

Having prepared the amine (**2**) we than examined *NN*-dicyclohexylcarbodi-imide (DCC)-mediated acylation with the D-diacetoxyphenylglycine derivative (**25**). A long reaction time, *ca.* 16 h, was required to generate the cephalosporanate (**19**). This poor reactivity of the 7β-amino group was also evident in other 7α-substituted cephalosporins. For example in early attempts to prepare the 7α-formamido compound (**2**) we had sought to use benzyloxycarbonyl protection for amine (**1**), but reaction with benzyloxycarbonyl chloride did not proceed to completion even after prolonged treatment with excess of the carbonyl chloride. Chromatographic purification of the product was ineffective, but after amination and formylation of the mixture the product (**20**) was isolated in 30% *yield*. We were unable to remove the amine protecting group by hydrogenation.

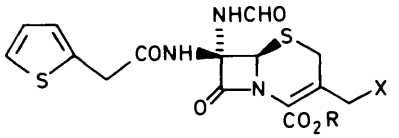
As might be expected shorter reaction times and improved yields were achieved by acylation of the amine (**2**) with acid chlorides. Thus using the appropriate amino acid derivative the fully protected cephaloglycin analogue (**16**) was formed. Partial deprotection was effected using zinc–hydrochloric acid in tetrahydrofuran (THF)–aqueous potassium dihydrogen phosphate[12] to give the amino ester (**17**). This proved a useful material for further derivatisation as exemplified by the preparation of the acylamino cephalosporanate (**18**). Subsequent deprotection of the ester (**18**) gave the sodium salt (**23**). An alternative route to compound (**14**) was also provided by this procedure.

Hepatic metabolism causes hydrolysis of the 3-(acetoxymethyl) group of simple cephalosporanates, effectively reducing the biological activity of the parent compound *in vivo*. It was therefore of interest to examine the displacement of the acetoxy group by nucleophiles such as thiols and pyridines which confer metabolic stability on the derived cephalosporins. First we investigated displacement of the acetoxy group of various 7α-formamido cephalosporins with heterocyclic thiols. When the cephalosporanic acid (**21**) was treated with the tetrazolethiol (**26**) in refluxing 1,2-dichloroethane (DCE),[15] the desired 3-tetrazole derivative (**28**) was obtained. Similarly the thiadiazolethiol (**27**) gave the cephalosporanic acid (**30**). Pure samples of (**28**) and (**30**) were conveniently obtained *via* chromatographic purification as the corresponding diphenylmethyl esters (**29**) and (**31**), and subsequent removal of the carboxyl protecting group using trifluoroacetic acid (TFA).

Applying this procedure to derivatives with more complex side-chains such as (**22**), we failed to effect displacement. Similarly, reactions carried out under aqueous conditions[16] resulted in recovery of starting material or degradation products depending upon the temperature employed. It was therefore necessary to devise a method for preparing a range of 7α-formamido cephalosporin nuclei with different 3-substituents. Our initial strategy is outlined in Scheme 2. When the carbamate (**15**) was de-esterified with TFA and the product treated with tetrazolethiol (**26**) in refluxing DCE the tetrazole derivative (**33**) was obtained. Esterification afforded the diphenylmethyl compound (**34**), but cleavage of the *N*-protecting group with zinc–hydrochloric acid[12] was accompanied by concomitant removal of the tetrazolylthio moiety to give the *exo*-methylene compound (**35**). Since removal of the



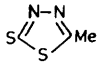
(26) (27)

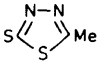


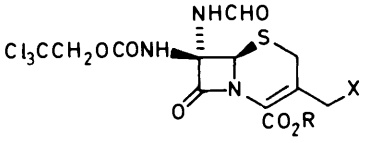
(21) X = OAc R = Na

(28) X =  R = Na

(29) X =  R = $CHPh_2$

(30) X =  R = Na

(31) X =  R = $CHPh_2$

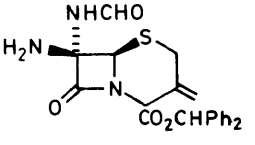


(15) X = OAc R = Bu^t

(32) X = OAc R = H

(33) X =  R = H

(34) X =  R = $CHPh_2$



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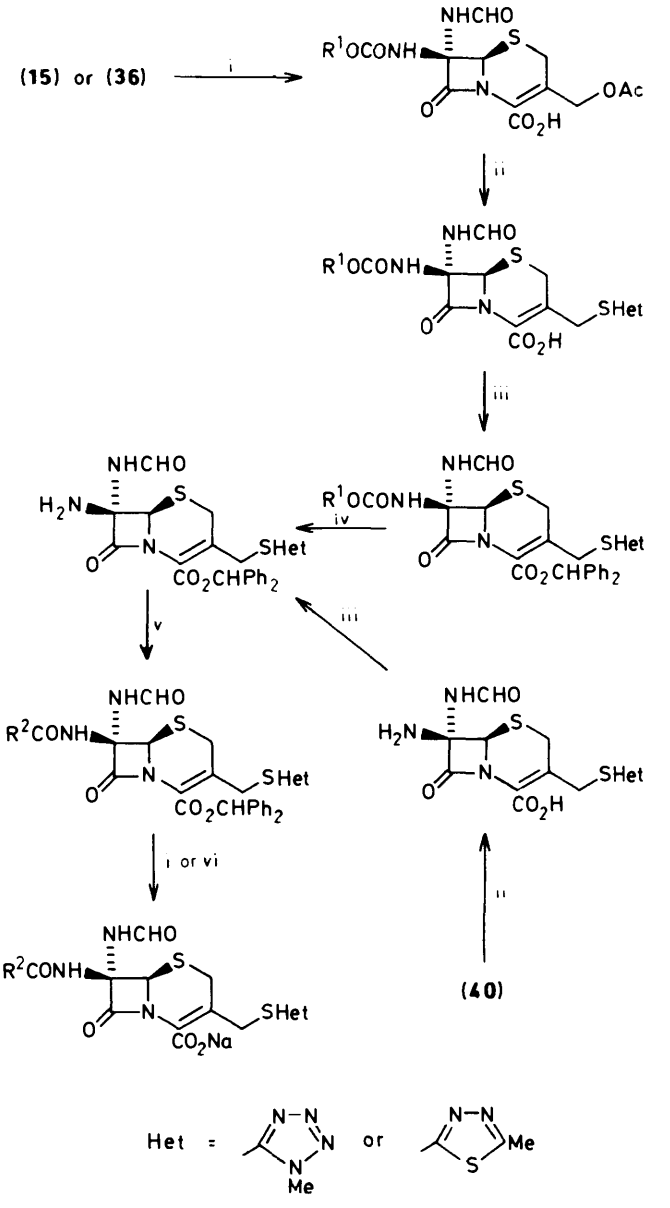
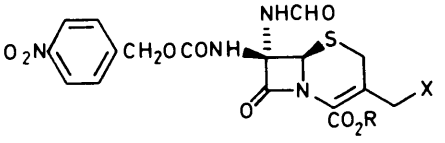


Scheme 2. *Reagents:* i, CF_3CO_2H; ii, HetSH, H^+; iii, Ph_2CN_2; iv, H_2–Pd for R^1 = 4-nitrobenzyl; v, R^2COCl; vi, HCO_2H, Na^+

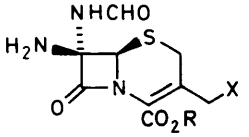
(2,2,2-trichloroethoxy)carbonyl group was not compatible with a 3-(heterocyclylthiomethyl) substituent, use of the (4-nitrobenzyloxy)carbonyl group was investigated. While the *N*-protected acid (**37**), the corresponding 3-(heterocyclylthiomethyl) derivative (**38**), and ester (**39**) were all readily prepared by previously described methodology, removal of the (4-nitrobenzyloxy)carbonyl group proceeded only in low yield (8%). This was not an entirely unexpected result in view of our previous experience with the benzyloxycarbonyl-protected compound (**20**).

Since this obviously did not provide a viable route to the desired intermediates we turned our attention to similar transformation on the amine (**40**),* generated from the ester (**2**) by treatment with TFA. Reaction of compound (**40**) with the tetrazolethiol (**26**) at 60 °C in aqueous acetone at pH 3.5—4.0 gave derivative (**41**), which was converted into the ester (**42**) by treatment with diphenyldiazomethane. Initially we used DMF as solvent for the esterification as this gave the best solubility of the crude displacement product (**41**). However, shorter reaction times and greater yields were achieved when acetonitrile[17] was used, despite the apparent insolubility of the reactants. This procedure was of general utility and allowed the synthesis of several different 3-(heterocyclylthiomethyl) derivatives, details of which have appeared in a patent.[11] Application of the standard acylation–deprotection sequence developed for compound (**2**) led to the synthesis of a range of cephalosporins with exceptional antibacterial activity. In particular the use of the diacetoxyphenylglycine derivative (**25**) provided the sodium salt (**44**), a direct analogue of BRL 36650,[18] which is a penicillin of potential clinical utility.

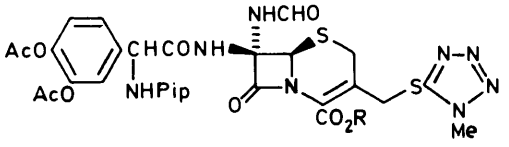
* Compound (**40**) was originally described in ref. 11 as the TFA salt of the amine, but was later found to exist as the amino acid.



(36) X = OAc R = Bu^t
(37) X = OAc R = H
(38) X =  R = H
(39) X =  R = $CHPh_2$

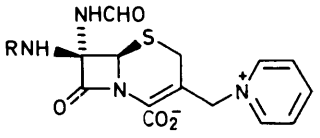


(2) X = OAc R = Bu^t
(40) X = OAc R = H
(41) X =  R = H
(42) X =  R = $CHPH_2$

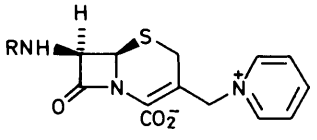


(43) R = $CHPh_2$
(44) R = Na

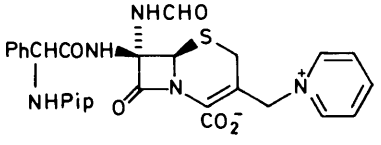
We next turned our attention to the replacement of acetoxy by pyridinium. The cephalosporin (**21**) was chosen as a suitable model compound, since displacements on analogous derivatives lacking the 7α-formamido group are extremely efficient.[19] Accordingly, compound (**21**) was treated with pyridine and sodium iodide in water at 60 °C, and purification of the crude product on Diaion HP20SS then gave the betaine (**45**). The yield was disappointing (14%), and it was therefore not totally unexpected that the process was found to be inapplicable to cephalosporins bearing more complex side-chains such as the diacetoxyphenyl piperazine derivative (**24**). Clearly the key intermediate was the 3-(pyridiniomethyl) compound (**46**). The corresponding 7α-H derivative (**47**) is available *via* cleavage of the acyl side-chain from derivatives such as cephaloridine (**48**),[20] the reaction of 7-aminocephalosporanic acid (7-ACA) with pyridine[21] being extremely inefficient. Selective cleavage of the 2-thienylacyl side-chain from 7α-formamido cephalosporin (**45**) was considered an unattractive proposition. We were therefore very gratified to discover that reaction of 7β-amino-7α-formamido cephalosporanic acid (**40**) under the standard displacement conditions afforded compound (**46**) in 47% yield. Presumably the successful outcome of this reaction is attributable to the reduced basicity of the 7β-amino group in compound (**40**) as compared with that in 7-ACA. Acylation of amine (**46**) with 2-thienylacetyl chloride, in DMF to aid solubility, followed by Diaion HP20SS chromatography gave an improved yield (44%) of the cephalosporin (**45**). However, modification of the procedure was required for acylations involving more complex acid chlorides, such as that generated from the phenylglycine derivative (**50**). In order to obtain products of good purity it was found necessary first to silylate the intermediate (**46**) using chlorotrimethylsilane and *NN*-dimethylaniline (DMA). In this way the derivative (**49**) was prepared in 45% yield.



(45) R = $COCH_2$(2-thienyl)
(46) R = H



(47) R = H
(48) R = $COCH_2$(2-thienyl)



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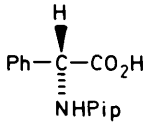

(50)

All the 7β-acylamino-7α-formamidocephalosporins described herein exhibited broad-spectrum antibacterial activity and β-lactamase stability, and a detailed discussion of these properties will be published elsewhere.

Experimental

M.p.s. were determined with a Büchi melting-point apparatus. I.r. spectra were recorded for THF and dichloromethane (DCM) solutions 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 obtained using $CDCl_3$ solutions incorporating tetramethylsilane as internal standard and D_2O solutions using either tetramethylsilane (90 MHz) or HOD (250 MHz) as internal standard on Perkin-Elmer R32 (90 MHz) or Brucker WM 250 (250 MHz) instruments. While 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 VG 7070 or a VG ZAB spectrometer operating in the electron-impact mode. Fast-atom-bombardment 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 plastic or aluminium sheets precoated with 0.2 mm thickness of silica gel 60 F_{254} (Merck 5735 and 5554), and of all sodium salts and betaines 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 using a 'flash' technique[22] on either silica gel 60 (finer than 230 mesh ASTM) (Merck 7729) with increased pressure provided by a Medcalf Hy-flo pump, or on 'Diaion HP20SS' resin (Mitsubishi Chemical Corp.). Solutions were dried using magnesium sulphate. Solvents were dried prior to use and work-up of extracts involved evaporated under reduced pressure below 30 °C on a Büchi rotary evaporator. All reactions in anhydrous solvents were carried out under an atmosphere of argon.

It should be noted that many of our products were not obtained in crystalline form, and we were unable to provide satisfactory microanalytical data in support of the structures. Furthermore, extensive attempts to obtain electron-impact mass-spectral characterisation did not furnish measurable molecular ions. However, the fast-atom-bombardment technique enabled low-resolution measurement of the molecular ions, and since the products were homogenous by either t.l.c. or h.p.l.c. we have claimed these materials as new.

The numbering scheme used for the n.m.r. data is shown in structure (**1**).

t-Butyl 7α-(*Methylthio*)-7β-[(2-*thienyl*)*acetamido*]*cephalosporanate* (**5**).—(2-Thienyl)acetic acid (0.85 g, 6.0 mmol) was heated in thionyl chloride (5 ml) at reflux for 1 h, and the solution was then evaporated under reduced pressure. The acid chloride was dissolved in DCM (10 ml) and the solution was added dropwise to a solution of the amine (**1**) (1.8 g, 4.8 mmol) and pyridine (0.57 ml, 7.0 mmol) in DCM (25 ml) at 0 °C. The reaction mixture was stirred at room temperature for 2 h, then evaporated under reduced pressure. The residue was dissolved in ethyl acetate, and the solution was washed successively with dil. hydrochloric acid, dil. aqueous sodium hydrogen carbonate, and saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–hexane (1:3) as eluant gave the *ester* (**5**) as a foam (1.75 g, 73%), $\nu_{max.}$(KBr) 3 270, 1 775, 1 735sh, 1 720, and 1 670 cm^{-1}; δ_H (90 MHz; $CDCl_3$) 1.51 (9 H, s, Bu^t), 2.04 (3 H, s, OCOMe), 2.24 (3 H, s, SMe), 3.30 and 3.42 (2 H, ABq, *J* 18 Hz, 2-H_2), 3.84 (2 H, s, ArCH_2), 4.84 and 5.05 (2 H, ABq, *J* 13.5 Hz, 3-CH_2), 4.89 (1 H, s, 6-H), 6.46 (1 H, s, NH), 6.92—7.04 (2 H, m, ArH), and 7.16—7.30 (1 H, m, ArH) (Found: M^+, 498.0908. $C_{21}H_{26}N_2O_6S_3$ requires *M*, 498.0930).

t-Butyl 7α-*Amino*-7β-[(2-*thienyl*)*acetamido*]*cephalosporanate* (**9**).—A solution of 7α-(methylthio) compound (**5**) (1.0 g, 2.0 mmol) in DMF (20 ml) at −40 °C was treated with a solution of mercury(II) acetate (0.64 g, 2.0 mmol) in DMF (5 ml) followed by a solution of ammonia (0.03 g, 2.0 mmol) in DMF (1.5 ml). The reaction mixture was allowed to warm to 0 °C during 1.5 h, and was then poured into ethyl acetate, washed well with water, dried, and evaporated to give the *amine* (**9**) (0.90 g, 96%) (Found: C, 51.5; H, 5.4; N, 8.5. $C_{20}H_{25}N_3O_6S_2$ requires C, 51.4; H, 5.4; N, 9.0%); $\nu_{max.}$ (THF) 3 260, 1 760, 1 745, 1 725, and 1 680 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.53 (9 H, s, Bu^t), 2.09 (3 H, s, OCOMe), 2.51 (2 H, br s, NH_2), 3.26 and 3.51 (2 H, ABq, *J* 18 Hz, 2-H_2), 3.85 (2 H, s, ArCH_2), 4.79 and 5.02 (2 H, ABq, *J* 13 Hz, 3-CH_2), 4.91 (1 H, s, 6-H), 6.60 (1 H, s, NH), 6.99 (2 H, m, ArH), and 7.27 (1 H, m, ArH); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol–NaOAc) MNa$^+$, 490.

t-Butyl 7α-*Formamido*-7β-[(2-*thienyl*)*acetamido*]*cephalosporanate* (**13**).—A solution of the 7α-amino derivative (**9**) (0.90 g, 1.9 mmol) in DCM (20 ml) at 0 °C was treated successively with pyridine (1.51 ml, 19 mmol) and acetic formic anhydride (0.75 ml, 9.5 mmol). The reaction mixture was stirred at 0 °C for 1 h, washed successively with dil. hydrochloric acid, dil. aqueous sodium hydrogen carbonate, and saturated brine, dried, and evaporated under reduced pressure. The *cephalosporanate* (**13**) crystallised from DCM–hexane (0.62 g, 66%), m.p. 160—164 °C (Found: C, 50.7; H, 5.1; N, 8.4. $C_{21}H_{25}N_3O_7S_2$ requires C, 50.9; H, 5.1; N, 8.5%); $\nu_{max.}$(KBr) 3 335, 1 770, 1 740, 1 720, 1 695, and 1 660 cm^{-1}; δ_H [90 MHz; $(CD_3)_2CO$] 1.54 (9 H, s, Bu^t), 2.00 (3 H, s, OCOMe), 3.33 and 3.61 (2 H, ABq, *J* 18 Hz, 2-H_2), 3.92 (2 H, s, ArCH_2), 4.73 and 4.99 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.21 (1 H, s, 6-H), 6.8—7.1 (2 H, m, ArH), 7.2—7.4 (1 H, m, ArH), and 8.19 (1 H, s, NCHO).

Sodium 7α-*Formamido*-7β-[(2-*thienyl*)*acetamido*]*cephalosporanate* (**21**).—The ester (**13**) (0.10 g, 0.2 mmol) was added to ice-cold 98% formic acid (8 ml) containing water (0.2 ml), and the mixture was stirred at room temperature. After 4 h the formic acid was evaporated off under reduced pressure. The residue was dissolved in dil. aqueous sodium hydrogen carbonate and the solution was washed with ethyl acetate. The aqueous solution was acidified to pH 1.5 and extracted with ethyl acetate. The extracts were washed with saturated brine, dried, and evaporated under reduced pressure. The residue was taken up in water, and the solution was adjusted to pH 6.5 with dil. aqueous sodium hydrogen carbonate and freeze-dried to afford the *sodium salt* (**21**) (0.054 g, 58%), $\nu_{max.}$(KBr) 3 330, 1 770, 1 735, 1 720, 1 695, and 1 660 cm^{-1}; δ_H (90 MHz; D_2O) 2.09 (3 H, s, OCOMe), 3.23 and 3.57 (2 H, ABq, *J* 17 Hz, 2-H_2), 3.92 (2 H, s, ArCH_2), 5.28 (2 H, s, 3-CH_2), 5.60 (1 H, s, 6-H), 6.9—7.2 (2 H, m, ArH), 7.3—7.5 (1 H, m, ArH), and 8.14 (1 H, s, NCHO); *m/z* (positive xenon F.A.B.; 1-thioglycerol) MH$^+$, 462; MNa$^+$, 484.

t-Butyl 7β-{D-2-[(4-*Ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]-2-*phenylacetamido*}-7α-(*methylthio*)*cephalosporanate* (**6**).—A solution of D-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]-2-phenylacetic acid (**50**) (0.48 g, 1.5 mmol) in DCM (30 ml) with oxalyl chloride (0.26 ml, 3.0 mmol) was stirred for 1 h and then the excess of oxalyl chloride was evaporated off under reduced pressure. The residual acid chloride was dissolved in DCM (20 ml) and the solution was added dropwise to a stirred mixture of the amine (**1**) (0.56 g, 1.5 mmol) and ground 4A molecular sieves (3.0 g) in DCM (25 ml) at 0 °C. After 0.5 h the mixture was allowed to warm to room temperature and was stirred for a further 2 h. The sieves were removed by filtration and the filtrate was evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate-hexane (2:1 ⟶ 1:0) as eluant gave the *cephalosporanate* (**6**) (0.61 g, 60%), $\nu_{max.}$(CH_2Cl_2) 3 280, 1 790, 1 720, and 1 695 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.22 (3 H, t, *J* 7 Hz, NCH_2Me), 1.53 (9 H, s, Bu^t), 2.08 (3 H, s, OCOMe), 2.29 (3 H, s, SMe), 3.20 and 3.39 (2 H, ABq, *J* 18 Hz, 2-H_2), 3.40—3.67 (4 H, m, NCH_2Me and NCH_2), 3.86—4.23 (2 H, m, NCH_2), 4.76 and 5.04 (2 H, ABq, *J* 13 Hz, 3-CH_2), 4.91 (1 H, s, 6-H), 5.67 (1 H, d, *J* 7 Hz, ArC*H*), 7.16 (1 H, s, NH), 7.28—7.54 (5 H, m, ArH), and 10.01 (1 H, d, *J* 7 Hz, NH); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol–NaOAc) MNa$^+$, 698.

t-Butyl 7α-*Amino*-7β-{D-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)-*carbonylamino*]-2-*phenylacetamido*}*cephalosporanate* (**10**).—A solution of the 7α-(methylthio)cephalosporanate (**6**) (0.52 g, 0.77 mmol) in DMF (15 ml) was treated with solutions of mercury(II) acetate (0.25 g, 0.77 mmol) in DMF (0.77 ml) and ammonia (0.014 g, 0.85 mmol) in DMF (1 ml) as described above. The crude product was chromatographed on silica gel with ethyl acetate as eluant to give the amine (**10**) (0.20 g, 41%), $\nu_{max.}$(CH_2Cl_2) 3 380, 3 280, 1 790, 1 725, and 1 695 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.23 (3 H, t, *J* 7 Hz, NCH_2Me), 1.54 (9 H, s, Bu^t), 2.08 (3 H, s, OCOMe), 2.95 (2 H, br s, NH_2), 3.09 and 3.37 (2 H, ABq, *J* 18 Hz, 2-H_2), 3.38—3.66 (4 H, m, NCH_2Me and NCH_2), 3.86—4.18 (2 H, m, NCH_2), 4.74 and 4.96 (2 H, ABq, *J* 12.5 Hz, 3-

CH_2), 4.87 (1 H, s, 6-H), 5.51 (1 H, d, *J* 7 Hz, ArC*H*), 7.27—7.52 (5 H, m, ArH), 7.65 (1 H, s, NH), and 9.99 (1 H, d, *J* 7 Hz, NH).

t-Butyl 7β-{D-2-[(4-*Ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]-2-*phenylacetamido*}-7α-*formamidocephalosporanate* (**14**).—*Method* (*a*). A solution of the 7α-amino derivative (**10**) (0.182 g, 0.29 mmol) in DCM (20 ml) was cooled to 0 °C and treated successively with pyridine (0.095 g, 1.2 mmol) and acetic formic anhydride (0.053 ml, 0.6 mmol). After being stirred for 0.25 h at 0—5 °C, and then for 0.75 h at room temperature, the reaction mixture was washed successively with dil. hydrochloric acid, dil. aqueous sodium hydrogen carbonate, and saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate as eluant afforded the *cephalosporanate* (**14**) as an off-white glass (0.059 g, 37%), $\nu_{max.}$(CH_2Cl_2) 3 280, 1 790, 1 725, and 1 695 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.24 (3 H, t, *J* 7 Hz, NCH_2Me), 1.55 (9 H, s, Bu^t), 2.08 (3 H, s, OCOMe), 2.94 and 3.27 (2 H, ABq, *J* 18 Hz, 2-H_2), 3.38—4.24 (6 H, m, $NCH_2CH_2NCH_2$), 4.76 and 4.99 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.17 (1 H, s, 6-H), 5.56 (1 H, d, *J* 6 Hz, ArC*H*), 7.23—7.58 (5 H, m, ArH), 7.93 (1 H, s, NH), 8.17 (1 H, s, NH), 8.18 (1 H, s, CHO), and 10.03 (1 H, d, *J* 6 Hz, NH); *m/z* (positive xenon F.A.B.; 1-thioglycerol–ammonium thiocyanate) *M*NH_4^+, 690.

Method (*b*). A solution of the amine (**17**) (0.05 g, 0.1 mmol) in DCM (5 ml) at 0 °C was treated with pyridine (0.012 ml, 0.15 mmol) followed by a solution of 4-ethyl-2,3-dioxopiperazine-1-carbonyl chloride (0.021 g, 0.1 mmol) in DCM (5 ml). The reaction mixture was stirred at between 0 and 5 °C for 0.5 h, and then for 2 h at room temperature. The solution was then washed successively with dil. hydrochloric acid, dil. aqueous sodium hydrogen carbonate, and saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–ethanol (19:1) as eluant gave the cephalosporanate (**14**) (0.015 g, 22%).

Sodium 7β-{D-2-[(4-*Ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]-2-*phenylacetamido*}-7α-*formamidocephalosporanate* (**22**).—The ester (**14**) (0.057 g, 0.085 mmol) was dissolved in ice-cold 98% formic acid (5 ml) containing water (0.2 ml) as described above to afford the *sodium salt* (**22**) as a freeze-dried solid (0.017 g, 32%), $\nu_{max.}$(KBr) 1 770, 1 710, 1 680, and 1 610 cm^{-1}; δ_H (250 MHz; D_2O) 1.20 (3 H, t, *J* 7 Hz, NCH_2Me), 2.09 (3 H, s, OCOMe), 3.05 (1 H, d, *J* 18 Hz, 2-H), 3.52 (5 H, m, NCH_2Me, NCH_2, and 2-H), 3.80—4.10 (2 H, m, NCH_2), 4.62 and 4.84 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.28 (1 H, s, 6-H), 5.52 (1 H, s, ArC*H*), 7.35—7.60 (5 H, m, ArH), and 8.15 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol–NaOAc) *M*Na^+, 639.

t-Butyl 7β-{D-2-(3,4-*Diacetoxyphenyl*)-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]*acetamido*}-7α-*formamidocephalosporanate* (**19**).—The D-diacetoxyphenylglycine derivative (**25**) (0.11 g, 0.25 mmol) was dissolved in DCM (10 ml) and the solution was added dropwise to a solution of the amine (**2**) (0.09 g, 0.24 mmol) and DCC (0.55 g, 0.24 mmol) in DCM (10 ml). After the solution had been stirred at room temperature for 3 days the solvent was evaporated off under reduced pressure. The residue was dissolved in ethyl acetate, and the solution was washed successively with dil. hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–ethanol (19:1) as eluant gave the *cephalosporanate* (**19**) as a pale yellow foam (0.067 g, 35%), $\nu_{max.}$(CH_2Cl_2) 3 270, 2 930, 1 780, 1 715, and 1 690 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.24 (3 H, t, *J* 8 Hz, NCH_2Me), 1.54 (9 H, s, Bu^t), 2.07 (3 H, s, OCOMe), 2.25 and 2.27 (6 H, 2 s, ArOCOMe), 2.87 and 3.20 (2 H, ABq, *J* 16 Hz, 2-H_2), 3.4—4.2 (6 H, m, $NCH_2CH_2NCH_2$), 4.76 and 5.03 (2 H, ABq, *J* 14 Hz, 3-CH_2), 5.11 (1 H, s, 6-H), 5.65 (1 H, d, *J* 7 Hz, ArC*H*), 7.1—7.5 (3 H, m, ArH), 7.97 (1 H, br s, NH), 8.11 (1 H, s, CHO), 8.56 (1 H, br s, NH), and 10.08 (1 H, d, *J* 7 Hz, NH); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol–NaOAc) *M*Na^+, 811.

Sodium 7β-{D-2-(3,4-*Diacetoxyphenyl*)-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]*acetamido*}-7α-*formamidocephalosporanate* (**24**).—The ester (**19**) (0.068 g, 0.086 mmol) was stirred with TFA (5 ml) for 1 h and the mixture was then evaporated under reduced pressure. The residue was triturated with diethyl ether, then dissolved in water, and the solution was carefully adjusted to pH 6.5 with dil. aqueous sodium hydrogen carbonate, then washed with ethyl acetate and freeze-dried to give the *sodium salt* (**24**) as a pale yellow solid (0.048 g, 74%), $\nu_{max.}$(KBr) 3 440, 1 765, 1 710, 1 675, and 1 610 cm^{-1}; δ_H (250 MHz; D_2O) 1.07 (3 H, t, *J* 7 Hz, NCH_2Me), 2.06 (3 H, s, OC*O*Me), 2.33 (6 H, s, ArOCOMe), 2.97 and 3.35 (2 H, ABq, *J* 18 Hz, 2-H_2), 3.46 and 3.53 (2 H, q, *J* 7 Hz, NCH_2Me), 3.68 (2 H, m, NCH_2), 3.99 (2 H, m, NCH_2), 4.48 (3-CH_2 covered by HOD signal), 5.26 (1 H, s, 6-H), 5.53 (1 H, s, ArC*H*), 7.25—7.57 (3 H, m, ArH), and 8.12 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; 1-thioglycerol) *M*H^+, 755; *M*Na^+, 777.

t-Butyl 7β-(*Benzyloxycarbonylamino*)-7α-(*methylthio*)-*cephalosporanate* (**8**).—A solution of the 7β-amino derivative (**1**) (0.25 g, 0.67 mmol) in DCM (10 ml) containing pyridine (0.11 ml, 1.34 mmol) at 0 °C was treated dropwise with a solution of benzyloxycarbonyl chloride (0.51 ml, 3.6 mmol) in DCM (2 ml). After 2 h at room temperature the mixture was diluted with ethyl acetate, washed successively with dil. hydrochloric acid and saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–hexane (1:4) as eluant gave a mixture of the product (**8**) and starting material (**1**) (0.127 g, *ca.* 4:1) and a small quantity of the pure *cephalosporanate* (**8**) (0.035 g, 10%), $\nu_{max.}$(CH_2Cl_2) 3 400, 1 785, and 1 730 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.55 (9 H, s, Bu^t), 2.09 (3 H, s, OCOMe), 2.37 (3 H, s, SMe), 3.34 and 3.50 (2 H, ABq, *J* 18 Hz, 2-H_2), 4.82 and 5.09 (2 H, ABq, *J* 13 Hz, 3-CH_2), 4.88 (1 H, s, 6-H), 5.14 and 5.23 (2 H, ABq, *J* 12 Hz, ArCH_2), 5.59 (1 H, s, NH), and 7.37 (5 H, m, ArH) (Found: M^+, 508.1338. $C_{23}H_{28}N_2O_7S_2$ requires *M*, 508.1338).

t-Butyl 7α-*Amino*-7β-(*benzyloxycarbonylamino*)*cephalosporanate* (**12**).—A solution of the 7α-(methylthio) derivative (**8**) (0.177 g, 0.35 mmol) in DMF (10 ml) at −40 °C was treated successively with mercury(II) acetate (0.111 g, 0.35 mmol) and a solution of ammonia (0.006 g, 0.35 mmol) in DMF (0.25 ml). The reaction mixture was allowed to warm to 0 °C during 1 h, then was diluted with ethyl acetate, washed exhaustively with water and then with saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–hexane (1:2 ⟶ 1:1) as eluant gave the amine (**12**) (0.074 g, 45%), $\nu_{max.}$(CH_2Cl_2) 3 400, 1 785, and 1 725 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.54 (9 H, s, Bu^t), 2.09 (3 H, s, OCOMe), 2.64 (2 H, br s, NH_2), 3.28 and 3.52 (2 H, ABq, *J* 18 Hz, 2-H_2), 4.80 and 5.03 (2 H, ABq, *J* 13 Hz, 3-CH_2), 4.90 (1 H, s, 6-H), 5.07 and 5.16 (2 H, ABq, ArCH_2), 5.78 (1 H, s, NH), and 7.36 (5 H, s, ArH).

t-Butyl 7β-(*Benzyloxycarbonylamino*)-7α-*formamidocephalosporanate* (**20**).—A solution of the 7α-amino derivative (**12**) (0.061 g, 0.13 mmol) in DCM (5 ml) at 0 °C was treated successively with pyridine (0.10 ml, 1.3 mmol) and acetic formic anhydride (0.052 ml, 0.65 mmol) in the manner described above. Chromatography on silica gel with ethyl acetate–hexane (1:1) as eluant gave the *formamido cephalosporanate* (**20**) (0.029 g,

45%), $\nu_{max.}$ (CH_2Cl_2) 3 400, 1 790, and 1 715 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.55 (9 H, s, Bu^t), 2.09 (3 H, s, OCOMe), 3.25 and 3.45 (2 H, ABq, *J* 17 Hz, 2-H_2), 4.96 and 5.07 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.16 (3 H, m, ArCH_2 and 6-H), 6.18 (1 H, s, NH), 7.35 (5 H, m, ArH), and 8.20 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol) MH^+, 508.

t-Butyl 7α-(Methylthio)-7β-[(2,2,2-trichloroethoxy)carbonylamino]cephalosporanate (**7**).—A solution of the 7β-amine (**1**) (6.92 g, 18.5 mmol) in DCM (50 ml) containing pyridine (2.2 ml, 27.2 mmol) was cooled to 0 °C and treated dropwise during 0.5 h with a solution of (2,2,2-trichloroethoxy)carbonyl chloride (2.5 ml, 18.5 mmol) in DCM (10 ml). The mixture was stirred for a further 5 min after addition was complete, then washed successively with dil. hydrochloric acid and saturated brine, dried, and evaporated under reduced pressure to give the *cephalosporanate* (**7**) as a yellow foam (9.86 g, 97%), $\nu_{max.}$(CH_2Cl_2) 3 380, 1 780, 1 740, and 1 620 cm^{-1}; δ_H (90 MHz; $CDCl_3$) 1.54 (9 H, s, Bu^t), 2.06 (3 H, s, OCOMe), 2.38 (3 H, s, SMe), 3.35 and 3.49 (2 H, ABq, *J* 18 Hz, 2-H_2), 4.6—5.2 (5 H, m, 6-H, 3-CH_2, and CH_2CCl_3), and 6.08 (1 H, s, NH) (Found: M^+, 548.0010. $C_{18}H_{23}Cl_3N_2O_7S_2$ requires *M*, 548.0010).

t-Butyl 7α-Amino-7β-[(2,2,2-trichloroethoxy)carbonylamino]-cephalosporanate (**11**).—A solution of the 7α-(methylthio) compound (**7**) (0.271 g, 0.49 mmol) in DMF (10 ml) at −40 °C was treated with a solution of mercury(II) acetate (0.156 g, 0.49 mmol) in DMF (3 ml), followed by a solution of ammonia (0.008 g, 0.49 mmol) in DMF (0.5 ml) as described above, to give the *amine* (**11**) as a pale yellow solid (0.217 g, 85%), m.p. 168 °C (Found: C, 39.7; H, 4.3; N, 8.1; S, 6.0; Cl, 20.3. $C_{17}H_{22}Cl_3N_3O_7$ requires C, 39.4; H, 4.2; N, 8.1; S, 6.2; Cl, 20.5%); $\nu_{max.}$(THF) 3 200, 1 790, 1 740, and 1 730 cm^{-1}; δ_H (90 MHz; $CDCl_3$) 1.56 (9 H, s, Bu^t), 2.10 (3 H, s, OCOMe), 2.75 (2 H, br s, NH_2), 3.32 and 3.52 (2 H, ABq, *J* 18 Hz, 2-H_2), 4.4—5.2 (5 H, m, 6-H, 3-CH_2, and CH_2CCl_3), and 6.53 (1 H, s, NH); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol–NaOAc) MNa^+, 540.

t-Butyl 7α-Formamido-7β-[(2,2,2-trichloroethoxy)carbonylamino]cephalosporanate (**15**).—A solution of the 7α-amino derivative (**11**) (0.217 g, 0.43 mmol) in DCM (10 ml) was cooled to 0 °C and treated with pyridine (0.34 ml, 4.3 mmol) and acetic formic anhydride (0.17 ml, 2.15 mmol) in the usual way. Chromatography on silica gel with ethyl acetate–hexane (1:1) as eluant gave the *formamido cephalosporanate* (**15**) (0.172 g, 75%), m.p. 167—170 °C (Found: C, 39.6; H, 4.1; N, 7.5; S, 5.9; Cl, 19.5. $C_{18}H_{22}Cl_3N_3O_8S$ requires C, 39.5; H, 4.0; N, 7.7; S, 5.9; Cl, 19.5%); $\nu_{max.}$(CH_2Cl_2) 3 380, 1 790, 1 735, and 1 700 cm^{-1}; δ_H (90 MHz; $CDCl_3$) 1.53 (9 H, s, Bu^t), 2.07 (3 H, s, OCOMe), 3.28 and 3.46 (2 H, ABq, *J* 17 Hz, 2-H_2), 4.7—5.3 (5 H, m, 6-H, 3-CH_2, and CH_2CCl_3), 6.66 (1 H, s, NH), 7.63 (1 H, br s, NH), and 8.22 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol–NaOAc) MNa^+, 568.

t-Butyl 7β-Amino-7α-formamidocephalosporanate (**2**).—A solution of the protected derivative (**15**) (8.13 g, 14.9 mmol) in THF (100 ml) and M-aqueous potassium dihydrogen phosphate (20 ml) was stirred with zinc powder (15 g) which had been freshly activated by successive washing with 5M-hydrochloric acid and water. After 6 h at pH 4—5 the reaction mixture was filtered and THF was evaporated off under reduced pressure. The residue was diluted with ethyl acetate, and the solution was washed with water, and then saturated brine, dried, and evaporated under reduced pressure to give the *amine* (**2**) (2.5 g, 45%), m.p. 166—170 °C (decomp.; from ethyl acetate–hexane) (Found: C, 48.5; H, 5.9; N, 11.4. $C_{15}H_{21}N_3O_6S$ requires C, 48.5; H, 5.7; N, 11.3%); $\nu_{max.}$(CH_2Cl_2) 3 410, 1 790, 1 740, and 1 700 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.58 (9 H, s, Bu^t), 2.10 (3 H, s, OCOMe), 2.43 (2 H, s, NH_2), 3.35 and 3.55 (2 H, ABq, *J* 18 Hz, 2-H_2), 4.81 and 4.99 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.11 (1 H, s, 6-H), 6.92 (1 H, d, *J* 1 Hz, NH), and 8.26 (1 H, d, *J* 1 Hz, CHO).

t-Butyl 7α-Formamido-7β-{D-2-phenyl-2-[(2,2,2-trichloroethoxy)carbonylamino]acetamido}cephalosporanate (**16**).—A solution of D-2-phenyl-2-[(2,2,2-trichloroethoxy)carbonylamino] acetic acid (0.163 g, 0.5 mmol) and DMF (0.05 ml) in DCM (10 ml) was treated with oxalyl dichloride (0.087 ml, 1.0 mmol). After being stirred for 1 h at room temperature, the reaction mixture was evaporated to dryness under reduced pressure. The resulting acid chloride was dissolved in DCM (5 ml) and the solution was added dropwise to a solution of the amine (**2**) (0.185 g, 0.5 mmol) and pyridine (0.061 ml, 0.75 mmol) in DCM (15 ml) at 0 °C, and the mixture was stirred at 0 °C for 0.25 h, then at room temperature for 2.5 h. The reaction mixture was washed successively with dil. hydrochloric acid, dil. aqueous sodium hydrogen carbonate, and saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–hexane (1:1) as eluant afforded the *cephalosporanate* (**16**) (0.167 g, 50%) (Found: C, 45.7; H, 4.2; N, 7.9. $C_{26}H_{29}Cl_3N_4O_9$ requires C, 45.9; H, 4.3; N, 8.2%); $\nu_{max.}$(CH_2Cl_2) 3 400, 3 290, 1 792, 1 740, 1 725sh, and 1 698 cm^{-1}; δ_H (90 MHz; $CDCl_3$) 1.50 (9 H, s, Bu^t), 2.02 (3 H, s, OCOMe), 3.02 and 3.28 (2 H, ABq, *J* 17 Hz, 2-H_2), 4.67 (2 H, s, CH_2CCl_3), 4.80 and 5.00 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.14 (1 H, s, 6-H), 5.41 (1 H, d, *J* 7 Hz, Ar*CH*), 6.47 (1 H, d, *J* 7 Hz, ArCH*NH*), 7.20—7.50 (5 H, m, ArH), 7.66 (1 H, s, CONH), 8.01 (1 H, s, N*H*CHO), and 8.11 (1 H, s, NHC*H*O); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol—NaOAc) MNa^+, 701.

t-Butyl 7β-(D-2-Amino-2-phenylacetamido)-7α-formamidocephalosporanate (**17**).—A mixture of a solution of the protected compound (**16**) (0.083 g, 0.122 mmol) in THF (6 ml) and M-aqueous potassium dihydrogen phosphate (1 ml) with freshly acid-washed zinc powder (0.50 g) was maintained at pH 4.0 for 5.5 h as described above to give the crude amine (**17**) (0.05 g) which was used without further purification.

t-Butyl 7α-Formamido-7β-(D-2-{[3-(methylsulphonyl)-2-oxoimidazolidin-1-yl]carbonylamino}-2-phenylacetamido)cephalosporanate (**18**).—A solution of 3-(methylsulphonyl)-2-oxoimidazolidine-1-carbonyl chloride (0.136 g, 0.6 mmol) in DCM (10 ml) was added dropwise to a stirred solution of the amine (**17**) (0.302 g, 0.6 mmol) and pyridine (0.071 g, 0.9 mmol) in DCM (10 ml) at 0 °C. After being stirred for 0.5 h at 0—5 °C and for 3 h at room temperature the mixture was washed successively with dil. hydrochloric acid, dil. aqueous sodium hydrogen carbonate, and saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–hexane (2:1 ⟶ 1:0) as eluant gave the *cephalosporanate* (**18**) (0.081 g, 19%), $\nu_{max.}$(CH_2Cl_2) 3 325, 1 790, 1 738, 1 695, and 1 675 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.52 (9 H, s, Bu^t), 2.06 (3 H, s, OCOMe), 2.88 and 3.25 (2 H, ABq, *J* 17 Hz, 2-H_2), 2-H_2), 3.43 (3 H, s, SO_2Me), 3.60—4.10 (4 H, m, NCH_2CH_2N), 4.75 and 5.00 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.20 (1 H, s, 6-H), 5.63 (1 H, d, *J* 7 Hz, Ar*CH*), 7.40 (5 H, br s, ArH), 8.00—8.45 (3 H, m, NHCHO and CONH), and 9.05 (1 H, d, *J* 7 Hz, ArCHN*H*); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol—NaOAc) MNa^+, 717.

Sodium 7α-Formamido-7β-(D-2-{[3-(methylsulphonyl)-2-oxoimidazolidin-1-yl]carbonylamino}-2-phenylacetamido)cephalosporanate (**23**).—The ester (**18**) (0.67 g, 0.097 mmol) was dissolved in TFA (5 ml) at 0 °C and the solution was stirred at room temperature for 0.5 h. Excess of TFA was evaporated under reduced pressure and the residue was dissolved in dil. aqueous sodium hydrogen carbonate. The solution was washed with ethyl acetate, and was then saturated with sodium chloride,

acidified to pH 1.5 with dil. hydrochloric acid, and extracted with ethyl acetate–THF (1:1). The extracts were washed with saturated brine, dried, and evaporated under reduced pressure. The *sodium salt* (**23**) was then obtained as a freeze-dried solid in the manner described above (0.54 g, 84%), $\nu_{max.}$(KBr) 1 765, 1 730, 1 675, and 1 605 cm^{-1}; $\lambda_{max.}$(water) 256 nm (ε 5 411 dm^3 mol^{-1} cm^{-1}); δ_H (90 MHz; D_2O) 1.93 (3 H, s, OCOMe), 2.85 and 3.26 (2 H, ABq, *J* 17 Hz, 2-H_2), 3.21 (3 H, s, SO_2Me), 3.50—3.85 (4 H, m, NCH_2CH_2N), 4.35—4.75 (m, 3-CH_2), 5.11 (1 H, s, 6-H), 5.35 (1 H, s, ArC*H*), 7.20—7.50 (5 H, m, ArH), and 8.00 (1 H, s, NHC*H*O); *m/z* (positive xenon F.A.B.; 1-thioglycerol) *M*H^+, 661; *M*Na^+, 683.

Diphenylmethyl 7α-Formamido-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-7β-[2-(2-thienyl)acetamido]ceph-3-em-4-carboxylate (**29**).—The cephalosporanic acid derived from the salt (**21**) (0.35 g, 0.8 mmol) was heated in DCE (15 ml) with 1-methyl-1*H*-tetrazole-5-thiol (**26**) (0.10 g, 0.86 mmol) at 80 °C for 6 h, after which the mixture was allowed to cool and was kept at room temperature for 18 h. A solution of diphenyldiazomethane (0.27 g, 1.4 mmol) in DCM (10 ml) was added and the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched with glacial acetic acid (1 ml) and the mixture was evaporated under reduced pressure. Chromatography of the residue on silica gel with ethyl acetate–hexane (1:1) as eluant gave the *tetrazolylcephem* (**29**) (0.072 g, 14%), $\nu_{max.}$(CH_2Cl_2) 3 380, 3 290, 1 790, 1 698, and 1 630 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 3.22 and 3.48 (2 H, ABq, *J* 16 Hz, 2-H_2), 3.85 (2 H, s, ArCH_2), 3.87 (3 H, s, NMe), 4.36 and 4.52 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.17 (1 H, s, 6-H), 6.86—7.04 (3 H, m, 2 × ArH and Ar_2CH), 7.2—7.6 (11 H, m, ArH), and 8.16 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol) *M*H^+, 662.

Sodium 7α-Formamido-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-7β-[2-(2-thienyl)acetamido]ceph-3-em-4-carboxylate (**28**).—A solution of the ester (**29**) (0.07 g, 0.11 mmol) in TFA (5 ml) was stirred for 0.5 h at room temperature, and was then treated as described above to afford the sodium salt (**28**) as a freeze-dried solid (0.03 g, 55%), $\nu_{max.}$(KBr) 1 764, 1 677, and 1 610 cm^{-1}; δ_H (250 MHz; D_2O) 3.34 and 3.68 (2 H, ABq, *J* 17 Hz, 2-H_2), 3.84—4.09 (6 H, m, ArCH_2, NMe, and 3-CH), 4.25 (1 H, d, *J* 13 Hz, 3-CH), 5.28 (1 H, s, 6-H), 6.95—7.15 (2 H, m, ArH), 7.30—7.45 (1 H, m, ArH), and 8.16 (1 H, s, CHO).

Diphenylmethyl 7α-Formamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-7β-[2-(2-thienyl)acetamido]ceph-3-em-4-carboxylate (**31**).—The cephalosporanic acid derived from the salt (**21**) (0.50 g, 1.14 mmol) was treated in DCE (20 ml) with 5-methyl-1,3,4-thiadiazole-2-thiol (**27**) (0.18 g, 1.36 mmol) in a similar way to the preparation of compound (**29**) described before. After treatment with a solution of diphenyldiazomethane (0.27 g, 1.4 mmol) in DCM (10 ml), chromatography of the residue on silica gel with ethyl acetate–hexane (1:1) as eluant gave the *thiadiazolylcephem* (**31**) (0.042 g, 6%), $\nu_{max.}$(CH_2Cl_2) 3 380, 1 789, 1 728, and 1 695 cm^{-1}; δ_H (90 MHz; $CDCl_3$) 2.64 (3 H, s, Me), 3.21 and 3.38 (2 H, ABq, *J* 16 Hz, 2-H_2), 3.78 (2 H, s, ArCH_2), 4.28 and 4.58 (2 H, ABq, *J* 13.5 Hz, 3-CH_2), 5.15 (1 H, s, 6-H), 6.83—7.02 (3 H, m, Ar_2CH and 2 × ArH), 7.10—7.75 (12 H, m, ArH and NH), 7.86 (1 H, s, NH), and 8.06 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; *a*, 3-nitrobenzyl alcohol; *b*, 3-nitrobenzyl alcohol–NaOAc) *a*: *M*H^+, 678; *b*: *M*Na^+, 700.

Sodium 7α-Formamido-3-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-7β-[2-(2-thienyl)acetamido]ceph-3-em-4-carboxylate (**30**).—The ester (**31**) (0.038 g, 0.06 mmol) was treated with TFA (4 ml) in the manner described previously to give after work-up, the *sodium salt* (**30**) as a freeze-dried solid (0.018 g, 63%), $\nu_{max.}$(KBr) 1 767, 1 678, and 1 574 cm^{-1}; δ_H (250 MHz; D_2O) 2.72 (3 H, s, Me), 3.24 and 3.62 (2 H, ABq, *J* 17.5 Hz, 2-H_2), 3.82—3.98 (3 H, m, ArCH_2 and 3-CH), 4.34—4.44 (1 H, d, *J* 18 Hz, 3-CH), 5.23 (1 H, s, 6-H), 6.9—7.4 (3 H, m, ArH), and 8.13 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol) *M*Na^+, 556.

Diphenylmethyl 7α-Formamido-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-7β-[(2,2,2-trichloroethoxy)carbonylamino]ceph-3-em-4-carboxylate (**34**).—A solution of the t-butyl ester (**15**) (1.44 g, 2.6 mmol) in cold TFA (15 ml) was stirred at room temperature for 0.75 h and was then evaporated under reduced pressure. The residue was diluted with toluene, the solution was evaporated to dryness, and the residue was dissolved in dil. aqueous sodium hydrogen carbonate. The solution was washed with ethyl acetate, acidified to pH 1.5 with dil. hydrochloric acid, and extracted into ethyl acetate–THF (1:1). The combined extracts were washed with brine, dried, and evaporated under reduced pressure to give the acid (**32**) (0.96 g, 74%) which was used without further purification.

The acid (**32**) (0.49 g, 1.0 mmol) and 1-methyl-1*H*-tetrazole-5-thiol (**26**) (0.13 g, 1.1 mmol) were heated in DCE (30 ml) at reflux for 7 h; the solution was cooled, and then evaporated under reduced pressure. The crude acid product (**33**) was dissolved in DCM (25 ml) and the solution was treated with a solution of diphenyldiazomethane in DCM (12 ml) (sufficient for completion of reaction as judged by t.l.c.). After being stirred at room temperature for 0.5 h, the mixture was treated with glacial acetic acid (0.15 ml) and was then stirred for a further 0.25 h. The reaction mixture was evaporated under reduced pressure and the residue was chromatographed on silica gel with ethyl acetate–hexane (1:2 ⟶ 1:1) as eluant to give the title ester (**34**) (0.121 g, 17%), $\nu_{max.}$(CH_2Cl_2) 3 390, 1 798, 1 735, and 1 705 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 3.48 and 3.62 (2 H, ABq, *J* 17 Hz, 2-H_2), 3.87 (3 H, s, NMe), 4.37 and 4.60 (2 H, ABq, *J* 13 Hz, 3-CH_2), 4.72 and 4.81 (2 H, ABq, *J* 12 Hz, CH_2CCl_3), 5.20 (1 H, s, 6-H), 6.61 (1 H, s, OCONH), 6.93 (1 H, s, Ar_2CH), 7.20—7.60 (11 H, m, ArH and N*H*CHO), and 8.21 (1 H, s, NHC*H*O).

Diphenylmethyl 7β-Amino-7α-formamido-3-methylenecepham-4-carboxylate (**35**).—A solution of the tetrazolylcephem (**34**) (0.110 g, 0.154 mmol) in THF (5 ml) and M-aqueous potassium dihydrogen phosphate (1 ml) with freshly acid-washed zinc powder (0.25 g) was maintained at pH 4.5 by addition of dil. hydrochloric acid for 4 h with further additions of acid-washed zinc (2 × 0.25 g). The reaction mixture was filtered and diluted with ethyl acetate. The solution was washed successively with water and saturated brine, dried, and evaporated under reduced pressure to give the *methylenecepham* (**35**) as a white foam (0.049 g, 75%), $\nu_{max.}$(CH_2Cl_2) 3 395, 3 300, 1 775, 1 745, and 1 695 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 2.41 (2 H, br s, NH_2), 3.23 and 3.42 (2 H, ABq, *J* 14.5 Hz, 2-H_2), 5.25 (2 H, s, 4-H and 3-CH), 5.29 (1 H, s, 3-CH), 5.39 (1 H, s, 6-H), 6.86 (1 H, s, Ar_2CH), 7.18—7.53 (11 H, m, ArH and N*H*CHO), and 8.14 (1 H, d, *J* 1 Hz, CHO); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol–NaOAc) *M*Na^+, 446.

t-Butyl 7α-Formamido-7β-[(4-nitrobenzyloxy)carbonylamino]cephalosporanate (**36**).—A solution of the amine (**2**) (1.11 g, 3.0 mmol) and pyridine (0.36 ml, 4.5 mmol) in THF (30 ml) was added dropwise to a solution of (4-nitrobenzyloxy)carbonyl chloride (0.71 g, 3.3 mmol) in THF (10 ml) at 0 °C. The reaction mixture was stirred at room temperature for 6 h with addition of another four aliquots of (4-nitrobenzyloxy)carbonyl chloride (total 2.5 g, 11.6 mmol). The solvent was evaporated off under reduced pressure and the residue was dissolved in ethyl acetate. The solution was washed successively with dil. aqueous sodium hydrogen carbonate, dil. hydrochloric acid, and saturated brine, dried, and evaporated under reduced pressure. Chromatography

on silica gel with ethyl acetate–hexane (1:2 ⟶ 3:2) as eluant gave the *cephalosporanate* (**36**) (1.06 g, 64%), $\nu_{max.}$ (CH_2Cl_2) 3 395, 3 300, 1 795, 1 740, 1 725, and 1 700 cm^{-1}; δ_H (90 MHz; $CDCl_3$) 1.51 (9 H, s, Bu^t), 2.06 (3 H, s, OCOMe), 3.21 and 3.45 (2 H, ABq, *J* 17 Hz, 2-H_2), 4.82 and 5.07 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.15 (1 H, s, 6-H), 5.21 (2 H, s, ArCH_2), 6.60 (1 H, s, OCONH), 7.49 and 8.18 (4 H, 2d, *J* 8 Hz, ArH), 7.61 (1 H, br s, N*H*CHO), and 8.20 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; *a*, 3-nitrobenzyl alcohol; *b*, 3-nitrobenzyl alcohol–NaOAc) *a*: *M*H^+, 551; *b*: *M*Na^+, 573.

7α-Formamido-7β-[(4-nitrobenzyloxy)carbonylamino]-cephalosporanic Acid (**37**).—The ester (**36**) (0.94 g, 1.7 mmol) was dissolved in TFA (10 ml) at 0 °C and the solution was stirred at room temperature for 0.75 h. TFA was evaporated off under reduced pressure and the residue was taken up in ethyl acetate (5 ml). Addition of diethyl ether precipitated the acid (**37**) (0.66 g, 78%), $\nu_{max.}$(KBr) 1 780, 1 720, and 1 680sh cm^{-1}; δ_H (90 MHz; TFA) 2.26 (3 H, s, OCOMe), 3.39 and 3.62 (2 H, ABq, *J* 17 Hz, 2-H_2), 5.20—5.60 (5 H, m, 3-CH_2, ArCH_2, and 6-H), 7.64 (2 H, d, *J* 8 Hz, ArH), 8.33 (2 H, d, *J* 8 Hz, ArH), and 8.42 (1 H, s, CHO).

Diphenylmethyl 7α-Formamido-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-7β-[(4-nitrobenzyloxy)carbonylamino]ceph-3-em-4-carboxylate (**39**).—The acid (**37**) (0.60 g, 1.21 mmol) and thiol (**26**) (0.16 g, 1.34 mmol) were heated together in DCE (50 ml) at reflux for 6.5 h to give an insoluble brown gum. The solvent was decanted from the gum which was then treated with a solution of diphenyldiazomethane (0.38 g, 1.96 mmol) in DCM (14 ml) and the mixture was stirred at room temperature for 0.5 h. The reaction was quenched with glacial acetic acid (0.05 ml) and the solvent was evaporated off under reduced pressure. Chromatography on silica gel with ethyl acetate–hexane (1:1 ⟶ 2:1) as eluant gave the *tetrazolylcephem* (**39**) (0.198 g, 23%), $\nu_{max.}$(CH_2Cl_2) 3 395, 1 795, 1 725, and 1 700 cm^{-1}; δ_H (90 MHz; $CDCl_3$) 3.47 (2 H, br s, 2-H_2), 3.79 (3 H, s, NMe), 4.27 and 4.51 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.14 (2 H, s, ArCH_2), 5.24 (1 H, s, 6-H), 6.61 (1 H, s, OCONH), 6.88 (1 H, s, Ar_2CH), 7.10—7.60 (13 H, m, 12 × ArH and N*H*CHO), 8.05—8.25 (3 H, m, 2 × ArH and CHO); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol) *M*H^+, 717.

Diphenylmethyl 7β-Amino-7α-formamido-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylate (**42**).—*Method* (*a*). A suspension of 10% palladium–charcoal (0.10 g) in a mixture of THF (10 ml) and water (1 ml) was pre-hydrogenated for 0.5 h. A solution of the cephem (**39**) (0.10 g, 0.14 mmol) in THF (5 ml) was added and hydrogenation was continued for 1.25 h with one change of catalyst. The catalyst was filtered off and washed well with ethyl acetate. The filtrate was washed with saturated brine, dried, and evaporated. Chromatography on silica gel with ethyl acetate–hexane (2:1 ⟶ 1.0) as eluant gave the *amine* (**42**) (0.006 g, 8%), $\nu_{max.}$(CH_2Cl_2) 3 395, 1 782, 1 720sh, and 1 695 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 2.43 (2 H, br s, NH_2), 3.60 and 3.67 (2 H, ABq, *J* 16 Hz, 2-H_2), 3.86 (3 H, s, NMe), 4.31 and 4.45 (2 H, ABq, *J* 13 Hz, 3-CH_2), 5.17 (1 H, s, 6-H), 6.48 (1 H, s, N*H*CHO), 6.97 (1 H, s, Ar_2CH), 7.20—7.55 (10 H, m, 2 × Ph), and 8.23 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; *a*, thioglycerol; *b*, 3-nitrobenzyl alcohol–NaOAc) *a*: *M*H^+, 538; *b*: *M*Na^+, 560.

Method (*b*). A solution of the ester (**2**) (0.10 g, 0.23 mmol) in TFA (5 ml) was stirred at room temperature for 0.5 h. The solution was evaporated to dryness and the pale yellow solid was triturated with diethyl ether, filtered, and dried to give the *acid* (**40**) (0.083 g, 98%), $\nu_{max.}$(KBr) 3 320, 1 795, 1 780, 1 725, and 1 665 cm^{-1}; δ_H (250 MHz; TFA) 2.31 (3 H, s, OCOMe), 3.77 and 3.86 (2 H, ABq, *J* 17 Hz, 2-H_2), 5.32 and 5.49 (2 H, ABq, *J* 15 Hz, 3-CH_2), 5.48 (1 H, s, 6-H), and 8.59 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; 3-nitrobenzyl alcohol–NaOAc) *M*Na^+, 338.

A solution of the acid (**40**) (0.2 g, 0.63 mmol) in a mixture of water (10 ml) and acetone (3 ml) was adjusted to pH 6.5 with dil. aqueous sodium hydrogen carbonate, and was treated with the thiol (**26**) (0.4 g, 3.4 mmol). The reaction mixture was stirred at pH 3.5 and 60 °C for 5 h, allowed to cool, acidified to pH 2.0 with dil. hydrochloric acid, and evaporated under reduced pressure. The residue was dissolved in DMF (10 ml) and the solution was treated with a solution of diphenyldiazomethane (0.14 g, 0.72 mmol) in DCM (5 ml). After 4 h at room temperature the reaction was quenched with glacial acetic acid (0.05 ml), and the mixture was diluted with ethyl acetate, washed well with water and then with saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–hexane (3;1) as eluant gave the ester (**42**) (0.075 g, 22%).

Method (*c*). The acid (**40**) (1.57 g, 4.98 mmol) was prepared as described above and dissolved in water (40 ml) by addition of dil. aqueous sodium hydrogen carbonate to pH 6.5. A solution of the thiol (**26**) (0.59 g, 5.12 mmol) in acetone (15 ml) was added. The reaction mixture was adjusted to pH 4.0 with dil. aqueous sodium hydrogen carbonate and was stirred at 60 °C for 6 h. After acidification to pH 2.0 the reaction mixture was evaporated under reduced pressure. The residue was dried, then stirred in acetonitrile (40 ml) and treated with excess of diphenyldiazomethane in DMF. The reaction mixture was stirred at room temperature for 4.5 h and quenched with glacial acetic acid (1 ml). The mixture was filtered through Kieselghur and evaporated under reduced pressure. The residue was dissolved in ethyl acetate, and the solution was washed successively with water, dil. aqueous sodium hydrogen carbonate, and saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–hexane (3:7 ⟶ 1:0) as eluant gave the amine (**42**) (1.17 g, 44%).

Diphenylmethyl 7β-{D-2-(3,4-Diacetoxyphenyl)-2-[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonylamino]acetamido}-7α-formamido-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylate (**43**).—A solution of the acid (**25**) (1.28 g, 2.94 mmol) in DCM (20 ml) containing DMF (0.05 ml) was cooled to 0 °C and treated with oxalyl dichloride (0.52 ml, 6.47 mmol). After being stirred at room temperature for 1 h the reaction mixture was evaporated under reduced pressure. The acid chloride was dissolved in DCM (10 ml) and the solution was added dropwise to a solution of the amine (**42**) (0.632 g, 1.18 mmol) and pyridine (0.1 ml, 1.25 mmol) in DCM (10 ml) at −20 °C. The reaction mixture was allowed to warm to 0 °C during 1 h, then diluted with ethyl acetate, washed successively with dil. hydrochloric acid, saturated brine, dil. aqueous sodium hydrogen carbonate, and saturated brine, dried, and evaporated under reduced pressure. Chromatography on silica gel with ethyl acetate–methanol (1:0 ⟶ 19:1) as eluant gave the *cephem* (**43**) as an off-white foam (0.80 g, 74%), $\nu_{max.}$(CH_2Cl_2) 3 280, 1 779, 1 718, and 1 692 cm^{-1}; δ_H (250 MHz; $CDCl_3$) 1.19 (3 H, t, *J* 7 Hz, NCH_2Me), 2.22 and 2.23 (6 H, 2 s, 2 × OCOMe), 2.78 and 3.04 (2 H, ABq, *J* 17.5 Hz, 2-H_2), 3.33—3.67 (4 H, m, NCH_2 and NCH_2Me), 3.84 (3 H, s, NMe), 3.85—4.06 (2 H, m, NCH_2), 4.26 and 4.56 (2 H, ABq, *J* 12.5 Hz, 3-CH_2), 5.18 (1 H, s, 6-H), 5.65 (1 H, d, *J* 7 Hz, ArCH), 6.88 (1 H, s, Ar_2CH), 7.05—7.60 (13 H, m, ArH), 8.02 (1 H, s, N*H*CHO), 8.10 (1 H, s, CHO), 8.51 (1 H, br s, CONH), and 10.10 (1 H, d, *J* 7 Hz, ArCHN*H*); *m/z* (positive xenon F.A.B.; thioglycerol) *M*H^+, 955; *M*Na^+, 977.

Sodium 7β-{D-2-(3,4-*Diacetoxyphenyl*)-2-[(4-*ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]*acetamido*}-7α-*formamido*-3-[(1-*methyl*-1H-*tetrazol*-5-*yl*)*thiomethyl*]*ceph*-3-*em*-4-*carboxylate* (**44**).—A solution of the ester (**43**) (0.05 g, 0.052 mmol) in TFA (1 ml) was stirred at room temperature for 10 min and evaporated under reduced pressure. The residue was triturated with diethyl ether, taken up in water which was adjusted to pH 6.5 with dil. aqueous sodium hydrogen carbonate, and chromatographed on HP20SS with water–acetone (1:0 ⟶ 4:1) as eluant to give the *sodium salt* (**44**) as a freeze-dried solid (0.026 g, 63%), $\nu_{max.}$(KBr) 1 770, 1 676, and 1 620 cm^{-1}; δ_H (250 MHz; D_2O) 1.19 (3 H, t, *J* 7 Hz, NCH_2Me), 2.32 (6 H, s, 2 × OCOMe), 2.97 and 3.36 (2 H, ABq, *J* 17 Hz, 2-H_2), 3.51 (2 H, q, *J* 7 Hz, NCH_2Me), 3.67 (2 H, m, NCH_2), 3.96 (6 H, m, NMe, NCH_2, and 3-CH), 4.21 (1 H, d, *J* 14 Hz, 3-CH), 5.24 (1 H, s, 6-H), 5.53 (1 H, s, ArC*H*), 7.27—7.58 (3 H, m, ArH), and 8.12 (1 H, s, CHO); *m/z* (positive xenon F.A.B.; 1-thioglycerol) MH^+, 811; MNa^+, 833.

7β-*Amino*-7α-*formamido*-3-(*pyridiniomethyl*)*ceph*-3-*em*-4-*carboxylate* (**46**).—The acid (**40**) (0.60 g, 1.9 mmol) was suspended in water (10 ml) and the mixture was treated with sodium iodide (2.0 g) and pyridine (1.0 ml). The mixture was heated at 60 °C for 4.5 h, then cooled and concentrated under reduced pressure. Chromatography on HP20SS with water–acetone (1:0 ⟶ 49:1) as eluant followed by removal of acetone under reduced pressure and freeze drying gave the *betaine* (**47**) (0.30 g, 47%), $\nu_{max.}$(KBr) 1 765, 1 670, and 1 610 cm^{-1}; δ_H (90 MHz; D_2O) 3.13 and 3.59 (2 H, ABq, *J* 18 Hz, 2-H_2), 5.21 (1 H, s, 6-H), 5.36 (2 H, AA′, 3-CH_2), 7.95—8.20 (3 H, m, ArH and CHO), 8.56 (1 H, d, *J* 8 Hz, ArH), and 8.89 (2 H, d, *J* 8 Hz, ArH); *m/z* (positive xenon F.A.B.; 2,4-di-t-pentylphenol–$CHCl_3$) MH^+, 335.

7α-*Formamido*-3-(*pyridiniomethyl*)-7β-[(2-*thienyl*)*acetamido*]*ceph*-3-*em*-4-*carboxylate* (**45**).—*Method.* (*a*) The salt (**21**) (0.30 g, 0.65 mmol) was dissolved in water (*ca.* 2 ml) containing sodium iodide (0.975 g, 0.65 mmol) and pyridine (*ca.* 0.5 ml). The mixture was adjusted to pH 6.5 with phosphoric acid, heated at 65 °C for 3 h, cooled, and diluted with water (5 ml). Chromatography on HP20SS with water–acetone (1:0 ⟶ 10:1) as eluant, as before, gave the freeze-dried *betaine* (**45**) (0.045 g, 14%), $\nu_{max.}$(KBr) 3 390, 3 240, 1 775, 1 675, and 1 615 cm^{-1}; $\lambda_{max.}$(water) 236 nm (ε 13 800 $dm^3\ mol^{-1}\ cm^{-1}$); δ_H (250 MHz; D_2O) 3.05 and 3.56 (2 H, ABq, *J* 17 Hz, 2-H_2), 3.91 (2 H, s, ArCH_2), 5.29 and 5.45 (2 H, ABq, *J* 12 Hz, 3-CH_2), 5.36 (1 H, s, 6-H), 6.97—7.10 (2 H, m, thienyl H), 7.30—7.42 (1 H, m, thienyl H), 8.00—8.16 (2 H, m, pyridyl H), 8.17 (1 H, s, CHO), 8.51—8.64 (1 H, m, pyridyl H), and 8.80—8.99 (2 H, m, pyridyl H); *m/z* (positive xenon F.A.B.; 2,4-di-t-pentylphenol–$CHCl_3$) MH^+, 459.

Method (*b*). A solution of the betaine (**46**) (0.167 g, 0.5 mmol) in DMF (5 ml) containing pyridine (0.047 ml, 0.6 mmol) was cooled to 0 °C and treated with a solution of (2-thienyl)acetyl chloride (0.096 g, 0.6 mmol) in DMF (5 ml). The mixture was stirred at *room* temperature for 1.5 h and evaporated under reduced pressure. The residue was triturated with diethyl ether and the solid thus obtained was partially dissolved in water and then chromatographed on HP20SS as before to give the betaine (**45**) (0.10 g, 44%).

7β-{D-2-[(4-*Ethyl*-2,3-*dioxopiperazin*-1-*yl*)*carbonylamino*]-2-*phenylacetamido*}-7α-*formamido*-3-(*pyridiniomethyl*)*ceph*-3-*em*-4-*carboxylate* (**49**).—A solution of the betaine (**46**) (0.167 g, 0.5 mmol) in DCM (10 ml) was treated with DMA (0.507 ml, 4.0 mmol) and chlorotrimethylsilane (0.254 ml, 2.0 mmol). The mixture was stirred vigorously and heated at reflux for 1 h, then cooled to room temperature. A solution of the acid (**50**) (0.191 g, 0.6 mmol) in DCM (10 ml) with DMF (0.05 ml) was treated with oxalyl dichloride (0.152 g, 1.2 mmol). After being stirred at room temperature for 1 h the reaction mixture was evaporated under reduced pressure. The acid chloride was dissolved in DCM (10 ml) and the solution was added to the above reaction mixture, which was then stirred for 16 h. The solution was extracted with water and the extracts were washed with DCM and concentrated under reduced pressure. Chromatographed on HP20SS with water–acetone (1:0 ⟶ 3:1) as eluant gave the *betaine* (**49**) as a freeze-dried off-white solid (0.165 g, 52%), $\nu_{max.}$(KBr) 1 780, 1 710sh, 1 670, and 1 620 cm^{-1}; δ_H (250 MHz; D_2O) 1.18 (3 H, t, *J* 7 Hz, NCH_2Me), 2.85 (1 H, ½ABq, *J* 18 Hz, 2-H), 3.35—3.57 (3 H, m, NCH_2Me and 2-H), 3.58—3.76 (2 H, m, NCH_2), 3.80—4.10 (2 H, m, NCH_2), 5.22 and 5.40 (2 H, ABq, *J* 15 Hz, 3-CH_2), 5.33 (1 H, s, 6-H), 7.30—7.58 (5 H, m, Ph), 8.00—8.12 (2 H, m, pyridyl H), 8.14 (1 H, s, CHO), 8.50—8.62 (1 H, m, pyridyl H), and 8.80—9.00 (2 H, m, pyridyl H); *m/z* (positive xenon F.A.B.; 2,4-di-t-pentylphenol–$CHCl_3$) MH^+, 636.

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