Amide Transacylation in Penicillin and Cephalosporin Derivatives

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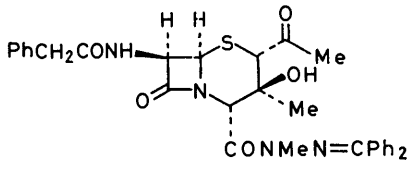
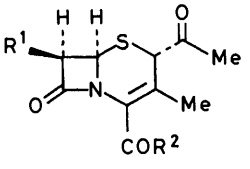
The phenylacetamido-side-chain of penicillin and desacetoxy-cephalosporin esters was exchanged by a trifluoroacetamido-group in high yield on reaction with trifluoroacetic anhydride and subsequently 1,5-diazabicyclo[4.3.0]non-5-ene. Methyl 7-trifluoroacetamidodesacetoxycephalosporanate gave the acetamido-analogue with acetyl chloride and the Δ^2-amino-acid on alkaline hydrolysis.

RECENTLY the synthesis of a novel 2α-acetyldesacetoxycephalosporin (2d) has been described.[1] A late stage in the synthesis involved the dehydration of the 3β-alcohol (1) by reaction with trifluoroacetic anhydride (TFAA) and subsequently with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). However, prolonged reaction with TFAA prior to addition of DBN gave the trifluoroacetamide analogue (2a) as the major product. Formulation as the amide (2a) was in full agreement with spectral and analytical data. Hydrolysis and oxidation [1] gave the free carboxylic acid (2c). Since this constituted a new method of acyl exchange, the reaction of TFAA and DBN with other cephem and penam derivatives was examined.

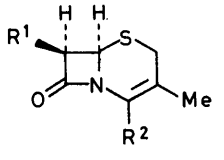


(1)



(2)

a; R^1 = CF_3CONH, R^2 = $NMeN{=}CPh_2$
b; R^1 = $PhCH_2CONH$, R^2 = $NMeN{=}CPh_2$
c; R^1 = CF_3CONH, R^2 = OH
d; R^1 = $PhCH_2CONH$, R^2 = OH



(3)

a; R^1 = $PhCH_2CONH$, R^2 = CO_2Me
b; R^1 = $(PhCH_2CO)CF_3CON$, R^2 = CO_2Me
c; R^1 = $PhCH_2COND$, R^2 = CO_2Me
d; R^1 = CF_3CONH, R^2 = CO_2Me
e; R^1 = $PhCH_2CONH$, R^2 = CO_2H
f; R^1 = $PhCH_2CONH$, R^2 = CO_2COCF_3
g; R^1 = $(PhCH_2CO)CF_3CON$, R^2 = CO_2COCF_3
h; R^1 = $(CH_3CO)CF_3CON$, R^2 = CO_2Me
i; R^1 = CH_3CONH, R^2 = CO_2Me
j; R^1 = $PhCH_2CONH$, R^2 = $CO_2CH_2CCl_3$
k; R^1 = CF_3CONH, R^2 = $CO_2CH_2CCl_3$
l; R^1 = CF_3CONH, R^2 = CO_2H
m; R^1 = NH_3^+, R^2 = CO_2^-

Reaction of the ceph-3-em ester (3a) with TFAA gave the derived *N*-acylamide (3b) (n.m.r.) which on reaction with D_2O gave exclusively deuteriated starting material (3c). Reaction, however, with DBN gave the trifluoroacetamide derivative (3d) in excellent yield. These results suggested the acyl exchange proceeded *via* phenylketen elimination (Scheme).

Penicillin and cephalosporin derivatives have been transacylated by acylation and subsequent hydrolysis.[2] In general yields were low, however, presumably due to both amides being formed in a ratio depending on the relative electrophilicities of the two carbonyl groups in the intermediate *N*-acylamide.

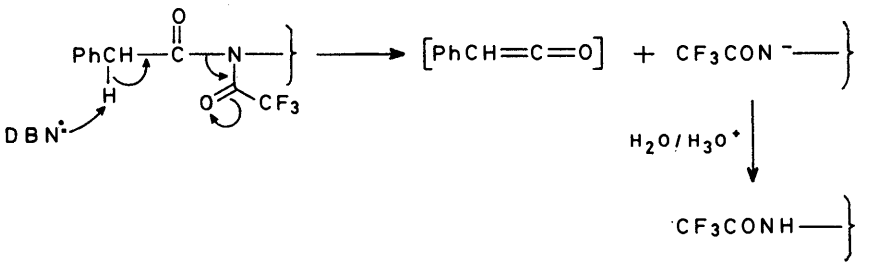
The ceph-3-em acid (3e) gave initially the mixed anhydride (3f) and subsequently the *N*-acylamide (3g) (n.m.r.) on reaction with TFAA. The second acylation was slow and was both cleaner and faster in the presence of pyridine. Addition of DBN resulted only in extensive decomposition; clearly carboxyl protection was required.

Both penam sulphide (4a) and sulphoxide (5a) were decomposed on reaction with TFAA. However, the addition of triethylamine resulted in rapid formation of the *N*-acylamides (4b) and (5b) respectively (n.m.r.). Prolonged reaction with the sulphoxide (5a) resulted in β-lactam destruction. Reaction of the acylamide (5b) with pyridine and methanol at −75 °C gave only starting material (5a). Addition of DBN, however, gave the trifluoroacetamide (5c) in 49% yield. In this case the yield was reduced because of sulphoxide nucleophilicity. Unlike the vigorous reaction of TFAA and dimethyl sulphoxide,[3] the sulphoxide function in penam (5a) reacted sufficiently slowly with TFAA to permit acyl exchange. This is consistent with the β-concave face of the molecule being sterically congested.

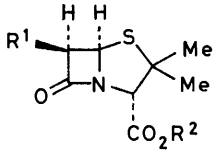
The acylpenam (4b) gave in quantitative yield the derived trifluoroacetamide (4c) with DBN. Attempts to transacylate the penicillin (4d) without carboxyl protection resulted in β-lactam destruction on DBN addition.

If a trifluoroacetamide is acylated, subsequent

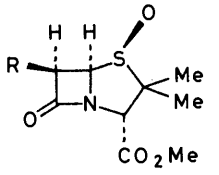
hydrolysis should provide trifluoroacetic acid and a new amide. Consistent with this hypothesis, cephem trifluoroacetamide (3d) gave the acetamide (3i) on reaction with acetyl chloride and triethylamine. Presumably the *N*-acetylamide (3h) is the intermediate. Acetic anhydride failed in the acetylation.



Trifluoroacetamides are hydrolysed under mildly alkaline conditions.[4] The cephem trifluoroacetamide (3d) on reaction with sodium hydroxide in aqueous THF rapidly gave the expected strongly dextrorotatory ceph-2-em carboxylic acid (6a) in high yield. Formulation as the Δ^2 isomer (6a) followed from spectral data especially the u.v. [$\lambda_{max.}$ 222 (ε 5 800) and 249 (sh) nm (4 200)] and elemental analysis. Prolonged reaction



(4) a; R^1 = $PhCH_2CONH$, R^2 = Me
b; R^1 = $(PhCH_2CO)CF_3CON$, R^2 = Me
c; R^1 = CF_3CONH, R^2 = Me
d; R^1 = $PhCH_2CONH$, R^2 = H

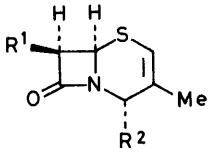


(5) a; R = $PhCH_2CONH$
b; R = $(PhCH_2CO)CF_3CON$
c; R = CF_3CONH

with aqueous sodium hydroxide resulted in trifluoroacetamide hydrolysis giving the Δ^2-amino-acid (6b) readily isolated at pH 4. Spectral data [$\nu_{max.}$ 3 300—2 200, 1 770, and 1 640, 1 610 cm^{-1}; and $\lambda_{max.}$ 226 nm (ε 4 900)] and elemental analysis supported the structural assignment.

Since alkaline hydrolysis of ceph-3-em-4-carboxylic esters is always accompanied by double-bond isomerisation, alternative carboxyl protection was investigated. The 2,2,2-trichloroethyl ester[5] (3j) on reaction with TFAA and 1,5-diazabicyclo[5.4.0]undec-5-ene in sequence gave the trifluoroacetamide analogue (3k). Zinc dust–acetic acid reduction[5] in THF gave the expected ceph-3-em-4-carboxylic acid (3l). In contrast to the Δ^2-isomer, the product absorbed in the u.v. spectrum at $\lambda_{max.}$ 259 nm (ε 7 300) and had a lower optical rotation.

Prolonged reaction of the ceph-3-em carboxylic acid (3l) with aqueous alkali in THF gave only a poor yield of impure 7-aminodesacetoxycephalosporanic acid (3m). Presumably this resulted from the β-lactam being more electrophilic in the Δ^3-isomer (3l) than in the Δ^2-isomer (6a).



(6) a; R^1 = CF_3CONH, R^2 = CO_2H
b; R^1 = NH_3^+, R^2 = CO_2^-

The transacylation herein described provides an alternative procedure to amide hydrolysis *via* reaction with phosphorus pentachloride and subsequently with an alcohol. The reaction should be applicable to an amide with α-hydrogens and acylating reagent without.

EXPERIMENTAL

Unless stated to the contrary, n.m.r. and u.v. spectra were recorded on deuteriochloroform and ethanol solutions respectively. P.l.c. was carried out on Merck GF_{254} Kieselgel. Column chromatography was carried out on Merck Kieselgel-60. The following purified solvents were used: deuteriochloroform (dried over anhydrous potassium carbonate); carbon tetrachloride and dichloromethane (freshly distilled from P_4O_{10}); diazabicyclo[4.3.0]non-5-ene (DBN), diazabicyclo[5.4.0]undec-5-ene (DBU), triethylamine, and di-isopropylethylamine (freshly distilled from molten potassium or sodium); methanol (freshly distilled from magnesium–iodine); pyridine (freshly distilled from 4A molecular sieve); and acetyl chloride (HCl-free, redistilled). Petroleum and light petroleum refer respectively to the redistilled fractions with b.p. 60—80 and 40—60 °C. All reactions were carried out in dry apparatus (150 °C overnight, assembled hot) under dry argon. All transfers were by means of syringe or double-tipped needle. Organic extracts were dried over anhydrous sodium sulphate.

Preparation of (2S,6R,7R)-2-*Acetyl*-4-(N′-*diphenylmethylene*-N-*methylcarbazoyl*)-3-*methyl*-7-*trifluoroacetamido*-

ceph-3-*em* (2a).—A solution of cepham (1) [1] (30 mg) in trifluoroacetic anhydride (1.5 ml) and dichloromethane (0.5 ml) was stirred for 14 h. The solvents were removed *in vacuo* and the residue dissolved in dichloromethane (2 ml) at 0 °C. DBN (100 μl) in dichloromethane (0.5 ml) was added dropwise. After stirring for 45 min at 0 °C, ethyl ethyl acetate (20 ml) was added and the solution washed with 5% aqueous w/v orthophosphoric acid (2 × 25 ml), and water (2 × 25 ml), dried, and evaporated. P.l.c. [EtOAc–light petroleum (1 : 1)] gave (in order of increasing polarity) minor components (2 mg); the *trifluoroacetamide* (2a) (14 mg, 50%) as an amorphous solid (from EtOAc–petroleum), $\nu_{max.}$(CH_2Cl_2) 3 390m, 1 780s, 1 738s, 1 715s, 1 660s, 1 535m, 1 360m, 1 210m, 1 170s, 1 100m, 1 020m, and 960m cm^{-1}; $\lambda_{max.}$ 241 (ε 22 000), 258 (sh) (19 000), and 305 nm (8 300); τ 1.80br (1 H, d, *J* 9 Hz, N–H), 2.63 (10 H, m, aryl-H), 4.5 (1 H, dd, *J* 9 and 5 Hz, 7-H), 5.2 (1 H, d, *J* 5 Hz, 6-H), 5.8 (1 H, s, 2β-H), 7.12 (3 H, s, N–Me), 7.63 (3 H, s, COMe), and 8.18 (3 H, s, 3-Me); *m/e* 544 (M^+), 526, 513, 502, 472, 469, 348, 210, 180, and 165 (Found: C, 57.35; H, 4.45; N, 10.15; S, 6.05. $C_{26}H_{23}F_3N_4O_4S$ requires C, 57.35; H, 4.25; N, 10.3; S, 5.9%); and cephem (2b) (2.1 mg, 3%).

Preparation of (2S,6R,7R)-2-*Acetyl*-3-*methyl*-7-*trifluoroacetamidoceph*-3-*em*-4-*carboxylic Acid* (2c).—Hydrazone (2a) (86 mg) and toluene-4-sulphonic acid monohydrate (0.25 g) in THF (1 ml) and water (0.25 ml) were stirred at room temperature for 50 min. The solution was added *drop by drop* with THF (2 ml, 1 ml) to sodium periodiate (37 mg, 1.1 equiv.) in THF (12 ml) and water (3 ml) whilst stirring vigorously. After 30 min sodium hydrogen carbonate (0.15 g) and sodium thiosulphate (to remove iodine) were added. Normal work-up [1] and chromatography (eluant ethyl acetate) gave the *cephem acid* (2c) (32 mg, 56%) as an amorphous solid (from CH_2Cl_2–petroleum); $[\alpha]_D^{20}$ −6.5° (*c* 1.14, $CHCl_3$); $\nu_{max.}$(CH_2Cl_2) 3 380m, 3 200—2 700m,br, 1 790s, 1 740s, 1 720s, 1 535m, 1 360m, 1 210s, and 1 165s cm^{-1}; $\lambda_{max.}$ 263 nm (ε 6 000); τ 1.82 (1 H, d, *J* 9 Hz, N–H), 3.55 (2 H, m, H_2O + CO_2H), 4.34 (1 H, m, 7-H), 5.16 (1 H, d, *J* 4 Hz, 6-H), 5.76 (1 H, s, 2β-H), 7.60 (3 H, s, 2α-COMe), and 7.94 (3 H, s, 3-Me); *m/e* 308, 265, 237, 210, 156, 140, and 102. Prolonged drying *in vacuo* gave an analytical sample (Found: C, 40.85; H, 3.45; N, 7.7. $C_{12}H_{11}F_3N_2O_5S$ requires C, 40.9; H, 3.15; N, 7.95%).

Reaction of Cephem (3a) *and Trifluoroacetic Anhydride*.—A solution of the cephem (3a) (62 mg) and trifluoroacetic anhydride (50 μl) in $CDCl_3$ (0.55 ml) was set aside at room temperature. After 4.5 h, n.m.r. indicated formation of the trifluoroacetylamide (3b); τ 2.65 (5 H, s, aryl–H), 4.6 (1 H, d, *J* 4 Hz, 7-H), 5.26 (1 H, d, *J* 4 Hz, 6-H), 5.8 (2 H, s, aryl–CH_2), 6.17 (3 H, s, OMe), 6.5, 6.74, 7.08, and 7.33 (2 H, AB quartet, *J* 15 Hz, 2-H), and 7.72 (3 H, s, 3-Me). D_2O (5 drops) was added and the reaction mixture stirred overnight to give exclusively the deuteriocephem (3c) [n.m.r. spectrum as the cephem (3a) with the exception of no amide N–H, and τ 4.4 (1 H, d, *J* 4 Hz, 7-H)].

Preparation of Methyl (6R,7R)-3-*Methyl*-7-*trifluoroacetamidoceph*-3-*em*-4-*carboxylate* (3d).—A solution of the cephem (3a) (0.50 g) in trifluoroacetic anhydride (3 ml) and dichloromethane (6 ml) was stirred at room temperature for 6 h. Solvents were removed *in vacuo* and the residue dissolved in CH_2Cl_2 (6 ml) at 0 °C. DBN (0.30 ml) was added dropwise during 5 min. After 45 min stirring, work-up as before and chromatography (eluant dichloromethane) gave a minor yellow pigment and [eluant ethyl acetate–dichloromethane (2 : 3)] gave the *trifluoroacetamide* (3d) (0.49 g, *ca.* 100%). Recrystallisation with charcoal treatment gave the trifluoroacetamide (3d) (388 mg, 83%) as white needles, m.p. 152.5—154.5°; $[\alpha]_D$ +168° (*c* 0.294, $CHCl_3$); $\nu_{max.}$ (Nujol) 3 270s, 1 775s, 1 730s, 1 703s, 1 630m, 1 550m, 1 440m, 1 380m, 1 312m, 1 260m, 1 220m, 1 195m, 1 155m, 1 110m, 1 070, 1 050, 990m, 920m, 820m, 770m, 750m, and 682m cm^{-1}; $\lambda_{max.}$ 257 nm (ε 5 200); τ 1.67 (1 H, br, d, *J* 8 Hz, N–H), 4.47 (1 H, dd, *J* 8 and 4 Hz, 7-H), 5.07 (1 H, d, *J* 4 Hz, 6-H), 6.24 (3 H, s, O-Me), 6.68 (2 H, s, 2-H), and 7.87 (3 H, s, 3-Me); *m/e* 324 (M^+), 296, 265, 237, 172 (100%), 154, and 140 (Found: C, 40.75; H, 3.5; N, 8.65; S, 10.05. $C_{11}H_{11}F_3N_2O_4S$ requires C, 40.7; H, 3.4; N, 8.65; S, 9.9%).

Reaction of the Cephem (3e) *and Trifluoroacetic Anhydride*.—A suspension of the acid (3e) (92 mg) and trifluoroacetic anhydride (0.15 ml) in deuteriochloroform (0.5 ml) was set aside at room temperature. The acid rapidly dissolved presumably giving the mixed anhydride (3f). N.m.r. indicated *ca.* 40% conversion into the trifluoroacetylamide (3g) during 9 h; τ 2.77 (s, aryl-H), 4.65 (d, *J* 3.5 Hz, 7-H), 5.3 (d, *J* 3.5 Hz, 6-H), 5.85 (s, aryl–CH_2), 6.47, 6.73, 7.0, and 7.24 (AB quartet, *J* 14 Hz, 2-H), and 7.64 (s, 3-Me). After 24 h reaction, n.m.r. indicated the presence of unreacted starting material (3f), trifluoroacetylamide (3g), and a new product with signals at τ 6.06 (d, *J* 4 Hz) and 7.53 (s, 3-Me). The reaction in the presence of pyridine (2 equiv.) was faster and cleaner. Subsequent reaction with DBN resulted in extensive decomposition.

Trifluoroacetylation of the Penam (4a).—Triethylamine (110 μl, 1.1 equiv.) and trifluoroacetic anhydride (0.20 ml) were added in sequence to the penam (4a) (244 mg) in $CDCl_3$ (2 ml) at 0 °C. The solution was stirred at room temperature for 2 h to give a solution of the acylamide (4b); τ 2.57, 2.66 (5 H, 2s, aryl–H), 4.5 and 4.7 (2 H, AB quartet, *J* 4 Hz, 5-H and 6-H), 5.48 (1 H, s, 3β-H), 5.84 (2 H, d, aryl–CH_2), 6.2 (3 H, s, O-Me), and 8.38 and 8.53 (6 H, 2 s, 2-Me_2). Omission of triethylamine resulted in β-lactam destruction.

Trifluoroacetylation of the Sulphoxide (5a).—Triethylamine (38.6 μl) and trifluoroacetic anhydride were added in sequence to sulphoxide (5a) (101 mg) in $CDCl_3$ (0.5 ml). After 20—30 min the n.m.r. spectrum indicated a high conversion into the acylamide (5b); τ 2.68 (5 H, s, aryl–H), 4.57 (br), 4.75 (2 H, AB quartet, *J* 4 Hz, 5-H, and 6-H), 5.33 (1 H, s, 3β-H), 5.8 (2 H, AB quartet, *J* 16 Hz, aryl–CH_2), 6.17 (3 H, s, OMe), and 8.32 and 8.77 (6 H, 2 s, 2-Me_2). Prolonged reaction resulted in β-lactam destruction.

Preparation of Methyl (1S,3S,5R,6R)-2,2-*Dimethyl*-6-*trifluoroacetamidopenam*-3-*carboxylate* 1-*Oxide* (5c).—Triethylamine (146 μl, 1.05 equiv.) and then trifluoroacetic anhydride (426 μl, 3 equiv.) were added to the sulphoxide (5a) (364 mg) in dichloromethane (4 ml) at 0 °C. The solution was stirred at room temperature for 30 min and cooled to −75 °C. Pyridine (242 μl, 3 equiv.) and then methanol (405 μl, 10 equiv.) were added. After warming to room temperature, normal work-up gave recovered starting material (5a) (364 mg). Alternatively, instead of quenching with pyridine and methanol, the solvents were removed *in vacuo* at 0 °C after 3—4 h. DBN (0.13 ml) was added dropwise to the residue in dichloromethane (2 ml) at 0 °C. After 15 min, 1% aqueous orthophosphoric acid (10 ml) and ethyl acetate (20 ml) were added. Normal work-up and p.l.c. [ethyl acetate–light petroleum (1 : 1)] gave the *trifluoroacetamide* (5c) (166 mg, 49%, 77% based on recovered starting material) as white hexagonal plates, m.p.

151—153 °C (from EtOAc–petroleum); $[\alpha]_D^{20}$ +207° (*c* 1.179, $CHCl_3$); $\nu_{max.}$(CH_2Cl_2) 3 340s, 1 800s, 1 755s, 1 730s, 1 530m, 1 370m, 1 210m, 1 170s,br, 1 130m, 1 070m, 1 040m, 982m, 900m, and 830m, cm^{-1}; τ 1.75 (1 H, d, *J* 10 Hz, N-H), 4.13 (1 H, dd, *J* 10 and 4.5 Hz, 6-H), 4.93 (1 H, d, *J* 4.5 Hz, 5-H), 5.32 (1 H, s, 3β-H), 6.18 (3 H, s, O-Me), and 8.24 and 8.76 (6 H, 2 s, 2-Me_2); *m/e* 342 (M^+), 325, 252, 207, 165, and 128 (Found: C, 38.75; H, 3.8; N, 8.35; S, 9.6. $C_{11}H_{13}F_3N_2O_5S$ requires C, 38.6; H, 3.85; N, 8.2; S, 9.35%); and the more polar starting material (135 mg).

Preparation of Methyl (3S,5R,6R)-2,2-*Dimethyl*-6-*trifluoroacetamidopenam*-3-*carboxylate* (4c).—Triethylamine (149 μl) and then trifluoroacetic anhydride (160 μl) were added to the penam (4a) (354 mg) in dichloromethane (2 ml) at 0 °C. After stirring for 3 h at room temperature, the solution was cooled to 0 °C and DBN (150 μl) added dropwise. After 45 min work-up in the usual way and chromatography [eluant dichloromethane–ethyl acetate (17 : 3)] gave the trifluoroacetamide (4c) (337 mg, *ca.* 100%) as an oil; $[\alpha]_D$ +190° (*c* 0.166, $CHCl_3$); $\nu_{max.}$(CH_2Cl_2) 3 380s, 1 790s, 1 745 (sh), 1 730s, 1 525m, 1 205m, and 1 160 (br) cm^{-1}; τ 2.78 (1 H, br d, *J* 8 Hz, N-H), 4.38 (2 H, m, 5-H and 6-H), 5.5 (1 H, s, 3β-H), 6.17 (3 H, s, OMe), and 8.32 and 8.48 (6 H, 2 s, 2-Me_2); *m/e* 326 (M^+), 212, 174, 114 [Found: *m/e* 326.0538 (M^+). $C_{11}H_{13}F_3N_2O_4S$ requires *M*, 326.0548].

Preparation of Methyl (6R,7R)-7-*Acetamido*-3-*methylceph*-3-*em*-4-*carboxylate* (3i).—Triethylamine (48 μl, 48 μl, 48μl, and 10 μl) was added in portions (0, 50, 60, and 75 min) to acetyl chloride (66 μl, 3 equiv.) and trifluoroacetamide (3d) (100 mg) in CH_2Cl_2 (1 ml). Work-up after 80 min from the first addition of triethylamine gave acetamide (3i) (44 mg, 53%) as white needles, m.p. 214—216 °C (from dichloromethane–petroleum, 2 crops); $[\alpha]_D^{18}$ +181° (*c* 0.138, $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 430m, 1 780s, 1 725s, 1 688s, 1 500m, and 1 370m cm^{-1}; $\lambda_{max.}$ 261 nm (ε 7 500); τ 3.44 (1 H, br d, *J* 9 Hz, N-H), 4.23 (1 H, dd, *J* 9 and 4.5 Hz, 7-H), 5.03 (1 H, d, *J* 4.5 Hz, 6-H), 6.15 (3 H, s, OMe), 6.33, 6.57, 6.68, and 6.99 (2 H, AB quartet, *J* 16 Hz, 2-H), 7.86 (3 H, s, 3-Me), and 7.92 (3 H, s, NCOMe); *m/e* 270 (M^+), 239, 172, and 140 (Found: C, 48.65; H, 5.3; N, 10.3. $C_{11}H_{14}N_2O_4S$ requires C, 48.85; H, 5.2; N, 10.35%). Four or possibly more by-products were formed in the reaction. Acetyl chloride and di-isopropylethylamine gave similar results.

Hydrolysis of the Cephem (3d).—(*a*) Aqueous sodium hydroxide (1.13M; 370 μl) was added to a solution of the cephem (3d) (67 mg) in THF (2.0 ml) at 0 °C. After 40 min acetic acid (11.8 μl) was added, and the mixture was evaporated, and partitioned between ethyl acetate (2 × 10 ml) and 5% aqueous orthophosphoric acid (4 ml). The organic phase was washed with saturated aqueous sodium sulphate (2 × 2 ml), dried, and evaporated to give (4R,6R,-7R)-3-*methyl*-7-*trifluoroacetamidoceph*-2-*em*-4-*carboxylic acid* (6a) (54 mg, 84%) as a white solid. Recrystallisation from THF gave white needles, m.p. 191—192.5 °C; $[\alpha]_D^{16}$ +409° (*c* 0.588, THF); $\nu_{max.}$(THF) 1 780s, and 1 730s cm^{-1}; $\lambda_{max.}$(THF) 222 (ε 5 800) and 249 (sh) nm (4 200); τ[$(CD_3)_2$CO] 1.93 (1 H, m, N-H), 3.96 (1 H, s, 2-H), 4.52 (1 H, m, 7-H), 4.78 (1 H, d, *J* 4 Hz, 6-H), 5.28 (1 H, s, 4β-H), and 8.05 (3 H, s, 3-Me); *m/e* 310 (M^+), 158 (100%), 112, and 69 (Found: C, 38.6; H, 2.9; N, 8.95. $C_{10}H_9F_3N_2O_4S$ requires C, 38.7; H, 2.95; N, 9.05%). (*b*) Aqueous sodium hydroxide (2.82M; 116 μl) was added to the cephem (3d) (52 mg) in THF (0.50 ml). After stirring at room temperature for 4 d toluene-4-sulphonic acid monohydrate was added to pH 4.0 ± 0.1 and the solution cooled to 0 °C. The crystalline (4R,6R,7R)-7-*amino*-3-*methylceph*-2-*em*-4-*carboxylic acid* (6b) (19 mg, 56%) was filtered off, washed with acetone (0.2 ml), and dried *in vacuo*; $[\alpha]_D^{18}$ +569° (*c* 0.157, DMSO); $\nu_{max.}$ (Nujol) 3 300—2 200 (br), 1 770s, 1 640—1 610m, 1 210w, 1 182w, 1 025w, 925w, 895w, 870w, 840m, and 760m cm^{-1}; $\lambda_{max.}$(H_2O) 226 nm (ε 4 900); τ (D_2O with 1 equiv. $NaHCO_3$) 4.1 (1 H, s, 2-H), 4.86 (2 H, m, 6-H and 7-H), 5.56 (1 H, s, 4β-H), and 8.18 (3 H, s, 3-Me). A sample was purified by dissolving in water containing triethylamine (1 equiv.) and adding toluene-4-sulphonic acid monohydrate (1 equiv.). The white plates were filtered off, washed with acetone–water (2 : 3) and dichloromethane, and dried *in vacuo*, m.p. 229—232 °C (decomp.) (Found: C, 45.0; H, 4.75; N, 13.1; S, 14.85. $C_8H_{10}N_2O_3S$ requires C, 44.85; H, 4.7; N, 13.1; S, 14.95%).

Preparation of 2,2,2-*Trichloroethyl* (6R,7R)-3-*Methyl*-7-*trifluoroacetamidoceph*-3-*em*-4-*carboxylate* (3k).—Reaction of the phenylacetamidocephem (3j) (926 mg) with trifluoroacetic anhydride and subsequently DBU gave, on chromatography on Kieselgel H [eluant toluene–ethyl acetate (9 : 1)] the *trifluoroacetamidocephem* (3k) (626 mg, 71%) as rosettes of white needles, m.p. 122.5—125 °C (from dichloromethane–petroleum); $[\alpha]_D^{21}$ +112° (*c* 0.235, $CHCl_3$); $\nu_{max.}$ (Nujol) 3 300m, 1 790s, 1 735—1 700s,br, 1 630m, 1 540m, 1 295m, 1 220m, 1 160m,br, 1 030m, 990m, 940m, 920m, 850m, 825m, 790m, 760m, 745m, and 702m cm^{-1}; $\lambda_{max.}$ 262 nm (ε 5 600); τ 1.97br (1 H, d, *J* 9 Hz, NH), 4.33 (1 H, dd, *J* 9 and 5 Hz, 7-H), 4.93 (1 H, d, *J* 5 Hz, 6-H), 5.15 (2 H, AB quartet, *J* 12 Hz, CH_2CCl_3), 6.6 (2 H, br s, 2-H), and 7.73 (3 H, s, 3-Me); *m/e* 443, 441, 439 (M^+), 290, 288, 140, and 112 (Found: C, 32.75; H, 2.3; N, 6.3. $C_{12}H_{10}Cl_3F_3N_2O_4S$ requires C, 32.6; H, 2.3; N, 6.35%).

Preparation of (6R,7R)-3-*Methyl*-7-*trifluoroacetamidoceph*-3-*em*-4-*carboxylic Acid* (3l).—A mixture of the cephem ester (3k) (595 mg), zinc dust (5 g), acetic acid (5 ml), and THF (10 ml) was stirred vigorously at room temperature for 260 min. The solution was filtered through Celite and the pad washed with THF and ethyl acetate. After evaporation the filtrate was dissolved in ethyl acetate (50 ml) and washed with 4% aqueous orthophosphoric acid (5 ml), water (5 ml), and saturated aqueous sodium sulphate (2 × 5 ml), dried, and evaporated. The residue was precipitated from ethyl acetate with toluene to give the *cephem acid* (3l) (418 mg, 100%) as a white solid. A sample was crystallised from ethyl acetate to give white needles, m.p. 199—203 °C (decomp.); $[\alpha]_D$ +164° (*c* 0.173, THF); $\nu_{max.}$ (dioxan) 3 500m, 3 000—2 650m, 1 785s, 1 735—1 715s, 1 640m, and 1 550m; $\lambda_{max.}$ 259 nm (ε 7 300); τ[$(CD_3)_2$CO] 0.60 (1 H, br d, *J* 9 Hz, N-H), 4.28 (1 H, m, 7-H), 4.77 (1 H, d, *J* 7 Hz, 6-H), 6.48 (2 H, s, 2-H), and 7.80 (3 H, s, 3-Me); *m/e* 310 (M^+), 308, 249, 237, 180, 158 (100%), and 140 (Found: C, 38.4; H, 3.0; N, 8.8. $C_{10}H_9F_3N_2O_4S$ requires C, 38.7; H, 2.95; N, 9.05%).

Hydrolysis of (6R,7R)-3-*Methyl*-7-*trifluoroacetamidoceph*-3-*em*-4-*carboxylic Acid* (3l).—Aqueous sodium hydroxide (2.94M, 0.29 ml) was added to the cephem (3l) (125 mg) in THF (0.50 ml). The mixture was stirred at room temperature for 4 d. Toluene-4-sulphonic acid monohydrate was added to pH 3.8 ± 0.1 and the mixture cooled to 0 °C. The brown amorphous solid (32 mg) was centrifuged off, washed with acetone and water, and dried *in vacuo*; $\lambda_{max.}$(H_2O) 261 nm (ε 3 700). The product was dissolved in water (0.20 ml) containing di-isopropylethylamine (26 μl) and toluene-4-sulphonic acid monohydrate (29 mg) added

to give crude 7-amino-3-methylceph-3-em-4-carboxylic acid (3 m) (11 mg, 13%), m.p. >240 °C (decomp.); $[\alpha]_D^{18}$ +111° (*c* 0.175 DMSO); $\lambda_{max.}$ (H_2O containing di-isopropylethylamine 1 equiv.) 263 nm (ε 4 100) [lit.,[6] m.p. 241—242 °C; $\lambda_{max.}$ 263 nm (ε 7 800)]. T.l.c. [silica, acetic acid–acetone (1 : 19) or cellulose acetone–water (17 : 3)] indicated the product to contain a slightly less polar impurity. Attempted hydrolysis of the cephem acid (3l) with benzylamine or n-butylamine (2.1 equiv.) in THF resulted in β-lactam destruction (i.r.)

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