# UNEXPECTED REACTION BETWEEN DCC AND CEPHALOSPORANIC ACID SULPHONES

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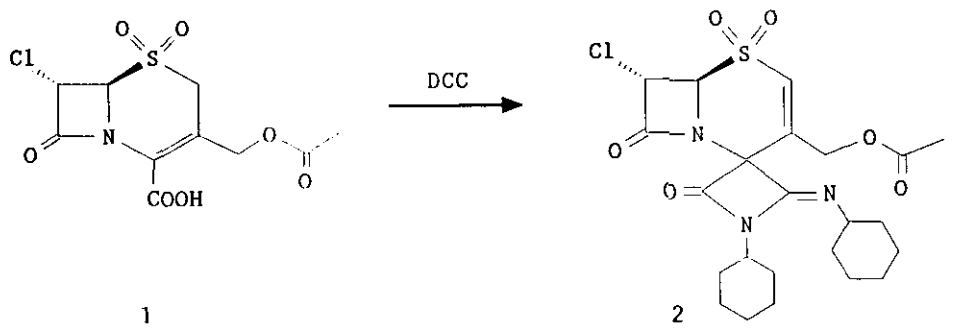
Abstract — Cephalosporanic acid sulphones react with DCC to give a novel 4-spiroazetidinyl-$\Delta^2$-cephem structure with high stereoselectivity. The reaction, though general for cephem-4-carboxylic acids, is deeply affected by oxidation at sulphur; an external base was required for the 1-sulphide 3, and stereoselectivity was lost with the 1α-sulphoxide 9.

Only few scientific works[1-3] deal with the chemistry and biological properties of cephem sulphones. Cefotaxime 1,1-dioxide was shown to possess interesting antimicrobial properties,[1] but no marketable product ensued from this finding. Compound 11, the cephalosporanic counterpart of sulbactam, did not show any β-lactamase inhibitory activity.[2] Recently, renewed interest in this area arose from the announcement by the Merck group that modified cephalosporins, in particular esters of 7α-chlorocephalosporanic acid 1,1-dioxide (1), are potent inhibitors of human leukocyte elastase.[3] We wish here to report on a singular reaction that we observed while preparing esters of cephalosporanic acid sulphones by the dicyclohexylcarbodiimide (DCC) method.

Reaction of 1 with a variety of alcohols in the presence of DCC afforded, beside the desired esters, a small amount of a less polar compound which incorporated DCC into the cephem skeleton. The new product was isolated in almost quantitative yield when the reaction was run with 2 mol equiv. of DCC and in the absence of the alcohol partner. Its molecular ion at m/z 511 in FD-ms was enough to discard the hypothesis of a trivial *N*-acylureido derivative. The peculiar spiroazetidinylcephem structure 2 was assigned on the basis of spectral data and confirmed by the synthesis of analogs differing for the sulphur oxidation level (6-8) and/or for the configuration of the new chiral center (9, 10).




1 2

The $^1$H nmr spectrum ($CDCl_3$)of 2, featuring resonances for the β-lactam (4.84 and 5.34 ppm, each d, $J$= 1.6 Hz) and acetoxy protons (2.11 ppm) characteristic of the starting material, but differing at $C_2$ for the presence of a methine (6.65 ppm, $J$= 1.5 Hz) coupled with the 3'-methylene, suggested a $\Delta^2$-cephem skeleton possessing a quaternary carbon at position 4. Five quaternary carbon atoms (162.06,158.39, 140.10, 139.63, and 73.74 ppm), apart from that of the easily recognizable acetoxy group (169.20 ppm), were detected by APT in the $^{13}$C nmr spectrum. Of these, the first two are carbonyl carbons. Their multiplicity in the all-coupled C-H spectrum (d and dd, respectively) and the results of selective decouplings (irradiation of the cyclohexyl methine at 3.66 ppm and of cephem 6-H, 7-H) enabled their assignment as *N*-cyclohexylamide and β-lactam carbonyl carbons, respectively. The methine hydrogen atoms of both cyclohexyl rings, being coupled with the carbon at 140.10 ppm (dd), identified the latter as an imine carbon arising from the central DCC atom. Finally, the quaternary carbon at 73.74 ppm, coupled with 6-H, 2-H, 3'-$H_2$, confirmed the $C_4$-disubstituted $\Delta^2$-cephem structure.

Evidences from EI-ms included accurate mass measurement of the molecular ion and the "criss-cross" fragmentation pattern[4] of monocyclic β-lactams (Figure 1). In the ir spectrum, a very strong absorption at 1700 $cm^{-1}$ was consistent with the C=N stretching anticipated for 4-iminoazetidinones,[5] while a single and less pronounced band at 1810 $cm^{-1}$ had to account for both β-lactam (iminoazetidinone and cephem) carbonyl absorptions.[6]

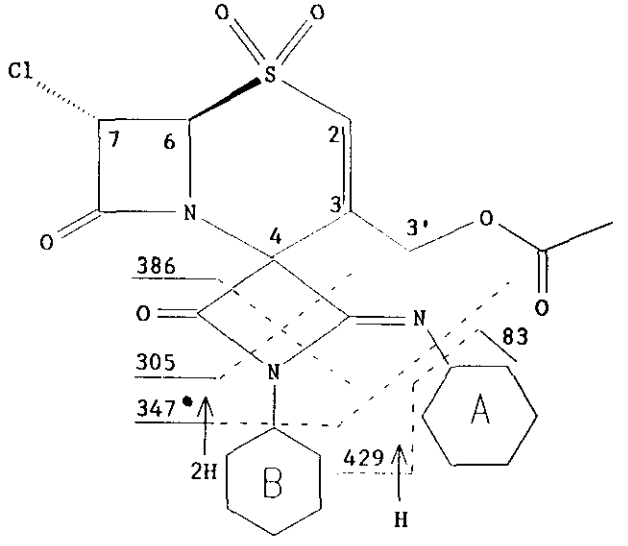
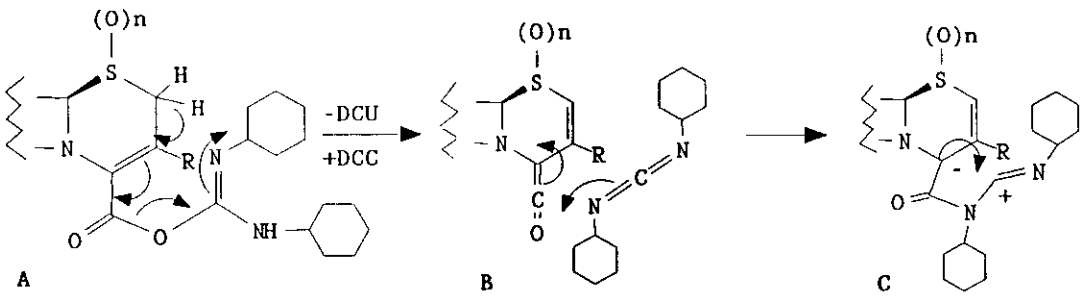



**Figure 1**

*Significant ions characterizing the spiroazetidinone moiety in the EI mass spectrum of 2.*

Most reasonably, the reaction pathway leading to 2 involves formation of the acyl-isourea A, followed by collapse to ketene B, which undergoes cycloaddition with a second molecule of DCC through the formal intermediacy[7] of zwitterion C (Scheme 1). Indeed, the ketene-imine cycloaddition is well documented, and DCC has been used previously as the imine partner.[5] However, the present reaction has peculiarities. First, we could not find precedents for ketene formation from *O*-acylisoureas.[8] Second, compared with reported cycloadditions of this type, usually requiring heating or long reaction times, the immediate formation of 2 (≤ 5 min at room temperature) is noteworthy. In turn, this reinforces the question why similar products have never been reported in cephem chemistry, in spite of the large use of DCC as a condensing agent. Finally, the stereoselectivity observed is of interest.

Scheme 1




A B C

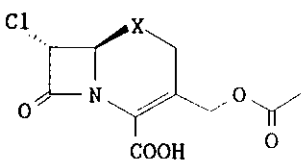
To account for these peculiarities, possible factors easening the formation and cycloaddition of ketene B were sought, as well as proofs for its actual involvement. Conversion of the carboxylic acid 1 into its chloride and treatment with $Et_3N$ in the presence of a single mol equiv. of DCC, albeit successful in producing 2 (66%), gave no conclusive evidence for ketene intermediacy. In fact, when the base was omitted, 2 was again obtained, though on longer reaction times. Carbodiimides are known to react with acyl chlorides to give the corresponding chloroformamidines;[8] in the present case the propensity of $Cl^-$ in the *N*-acyliminium intermediate to abstract a proton at $C_2$ instead of adding to the $^+N$=C double bond is not unreasonable.[9] This would offer an alternate pathway to zwitterion C when acyl chlorides are used as the starting material.

Investigations on the influence of the sulphone moiety in promoting the formation of cephem $C_4$-spiroazetidinones proved more meaningful. Generation of ketene B from acylisourea A according to Scheme 1 is expected to depend on the acidity of the cephem $C_2$ protons and thence on the oxidation state of the dihydrothiazine sulphur. Indeed, sulphide 3 failed to give the corresponding $C_4$-spiroazetidinone 6 when treated with DCC (the symmetrical anhydride being the main identified product), but 6 was obtained (13%) when triethylamine was added. By contrast, the β-sulphoxide 4 smoothly reacted with DCC in the absence of an external base affording a compound, 7, whose oxidation with 3-chloroperoxybenzoic acid (MCPBA) provided a sulphone identical in all respects with 2. Reaction of the α-oxide 5 with DCC proved more sluggish (still incomplete after ter 1 h) and non sterereoselective, epimers 8 and 9 being isolated after flash chromatography in comparable amounts (10-15%). The former compound when exposed to MCPBA oxidation gave sulphone 2, while 9 provided the $C_4$-epimer 10. The configurational relationships among compounds 1-10 were completed by the observation that 7, accompanied by a trace amount of 8, was obtained by treatment of 6 with 1 mol equiv. of MCPBA. Further, the scope of the reaction in cephem chemistry was assessed by subjecting to DCC treatment sulphones 11[2] and 12[10] as examples of 3-deacetoxycephalosporanic acid derivatives unsubstituted at $C_7$ or substituted by a β-oriented, classical acylamino side chain. By allowing a reaction time of 15 min, 13 and 14 were stereoselectively isolated in 52 and 20% yields.

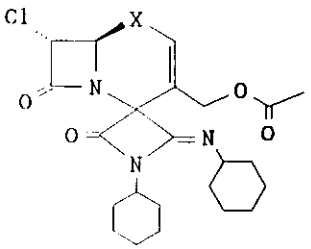
Stereochemistry at the spiro carbon atom (being established that 2 and 6-8 possess the same configuration, opposite of that of 9 and 10) could not be assigned unambiguosly. The physical state of the compounds synthesized precluded X-ray diffraction analysis, and no relevant indication came from NOE-nmr experiments. Mechanistic considerations are controversial. One would expect[11] the $C_4$ carbanion electron pair of species C, if really involved in the cycloaddition, to prefer an antiperiplanar orientation relative to the $N_5$ lone pair, which would favor β-attack of oncoming electrophiles[12], and afford 4*S*-spiro products as the major isomers. In this case, however, a further enhancement of

stereoselectivity would be expected for the α-sulphoxides,[13] which is the opposite of what experimentally found with compound 5. Inspection of Dreiding models suggests that approach of the imine carbenium from the α-face in the cycloaddition transition state is sterically favored. In compound 5 this facial bias is minimized, so that the observed loss of stereoselectivity can be accomodated by an alternate interpretation, where the reaction is governed[14] by the faster cyclization rate of the less stable zwitterion. According to this reasoning, compounds 2, 6-8, 13 and 14 should be assigned the 4*R* configuration.

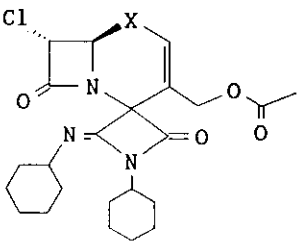
As far as reactivity is concerned, the differences observed are consistent with a reaction pathway kinetically controlled by abstraction of a $C_2$ proton from O-acylisourea A. In particular, the highest kinetic acidity among the $C_2$ protons of sulphoxide epimers 4 and 5 should be proper of the α-hydrogen of the β-oxide, due to the antiperiplanarity[15] existing in this compound between the $C_2$—$H_\alpha$ and $S_1$—$O_\beta$ bonds (within the reasonable[16] assumption of the pseudo-half chair conformation for the cephem dihydrothiazine ring). In our interpretation, this effect accounts for the higher reactivity of 4 (faster formation of ketene B).



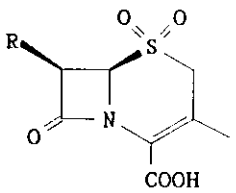
1 X = $SO_2$
3 X = S
4 X = S—O
5 X = S⋯O



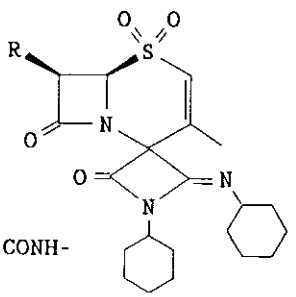
2 X = $SO_2$
6 X = S
7 X = S—O
8 X = S⋯O



9 X = S⋯O
10 X = $SO_2$



11 R = H
12 R = $PhOCH_2CONH$-



13 R = H
14 R = $PhOCH_2CONH$-

## EXPERIMENTAL

Melting points were determined in a Büchi apparatus and are uncorrected. Spectral data were recorded on the following spectrometers: Ir — Perkin Elmer 1420; FT-ir — Nicolet 20 SX; $^1H$ nmr — Varian EM390 and VXR-200; $^{13}C$ nmr — Varian VXR-400S. FD mass spectra were recorded on a Varian Mat 311/A instrument equipped with a combined EI/FI/FD ion source, using benzonitrile activated emitters.

The total voltage difference between the field emitter anode and the cathode was 9 kV. The emitter heating current was in the range 10-20 mA and the source temperature was 150 °C. EI mass spectra were obtained on a Finnigan 1020 spectrometer at an electron energy of 70 eV; the temperature of the direct inlet probe was in the range 150-230 °C and the source temperature was 250 °C. Exact mass measurement was performed on a VG Analytical 70-70 EQ spectrometer at a resolution of 10,000 (10% valley) using peak matching methods and perfluorokerosene as reference compound. Elemental analyses were performed on a Carlo Erba Erba NA 1005 analyzer. Flash chromatography was done with silica gel Merck 60 (230-400 mesh) and elution was carried out with hexane:ethyl acetate mixtures (ratio between brackets). Prior concentration under reduced pressure, all organic extracts were dried over anhydrous sodium sulphate.

3-Acetoxymethyl-7α-chloro-3-cephem-4-carboxylic acid 1,1-dioxide (1). 7-Aminocephalosporanic acid (27.2 g, 0.1 mol) was dissolved in 70% aqueous acetone (850 ml) and treated at 0-5 °C with 37% HCl (100 ml). Sodium nitrite (10.4 g) was added, the cooling bath removed and the reaction mixture was stirred for 90 min at 21 °C. Dilution with water (500 ml), extraction with $CH_2Cl_2$ (2x300 ml) and evaporation left crude 3 as a foam. Without purification, this material was dissolved in a mixture of $CH_2Cl_2$ (225 ml) and EtOH (75 ml), and treated at -20 °C with 70% MCPBA (33 g). After standing overnight at -5 °C, $Et_2O$ (250 ml) was added. Extraction with water (3 x 1 l), salting (NaCl) and back-extraction with EtOAc (3 x 200 ml) afforded technical grade 1 as a white powder (7.5 g, 24% overall), without the need for chromatographic purification (extensive 3,4-lactonization occurring otherwise); mp 55-57 °C ($Et_2O$). Ir (KBr) $\upsilon_{max}$ 1800, 1740, 1715 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 2.08 (3H, s, $COCH_3$), 3.90 (2H, ABq, $J$= 18.0 Hz, 2-$H_2$), 4.90 (1H, d, $J$= 2 Hz, 6-H), 4.92 (2H, ABq, $J$= 13.5 Hz, 3'-$H_2$), 5.28 (1H, d, $J$= 2 Hz, 7H), 9.73 ppm (1H, br s, exch. $D_2O$).

3-Acetoxymethyl-7α-chloro-4-spiro-[3'-(1'-cyclohexyl-4'-cyclohexylimino-2'-oxoazetidinyl)]-2-cephem 1,1-dioxide (2).

*a)From carboxylic acid 1* - A solution of 1 (0.323 g, 1 mmol) in ethanol-free $CH_2Cl_2$ (6 ml) was treated with DCC (0.432 g, 2.1 mmol). Separation of dicyclohexylurea took place immediately. After 5 min, water (2 drops) was added, and the mixture was vigorously stirred for 10 min. After filtration and separation from water, the solution was evaporated and the residue was purified by flash chromatography (4:1). Compound 2 (515 mg, quantitative) was obtained as a white foam, tenaceously retaining traces of solvent (elemental analysis). Ir ($CHCl_3$) $\nu_{max}$ 1810, 1755, 1700 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 1.1-1.7 (4H, m, 2-$H_2$ and 6-$H_2$ of ring B), 1.1-1.9 (12H, m, 3-$H_2$, 4-$H_2$, and 5-$H_2$ of rings A and B), 1.7-2.0 (4H, m, 2-$H_2$ and 6-$H_2$ of ring A), 2.11 (3H, s, $COCH_3$), 3.26 (1H, m, 1-H of ring A), 3.66 (1H, m, 1-H of ring B), 4.66 (2H, d, $J$= 1.5 Hz, 3'-$H_2$), 4.84 (1H, d, $J$= 1.6 Hz, 6-H), 5.34 (1H, d, $J$= 1.6 Hz, 7-H), 6.65 ppm (1H, t, $J$= 1.5 Hz, 2-H); $^{13}C$ nmr ($CDCl_3$) δ 20.49 ($CO\underline{C}H_3$), 23.85, 24.23, 24.83, 24.91, 24.96, and 25.40 ($C_3$, $C_4$, and $C_5$ of rings A and B), 29.55 and 29.71 ($C_2$ and $C_6$ of ring A), 33.86 and 34.36 ($C_2$ and $C_6$ of ring B), 53.42 ($C_1$ of ring B), 58.87 ($C_1$ of ring A), 60.51 (3-$\underline{C}H_2O$), 70.16 ($C_6$), 73.74 ($C_4$), 128.89 ($C_2$), 139.63 ($C_3$), 140.10 (spiroazetidinone $\underline{C}$=N), 158.39 (cephem $\underline{C}$=O), 162.06 (spiroazetidinone $\underline{C}$=O), 169.20 ($\underline{C}OCH_3$); FD-ms (EHC= 17 mA), m/z 511 ($M^{+\cdot}$, 100%), 386 [(M-$C_6H_{11}NCO)^{+\cdot}$, 3%]; EI-ms (see Figure 1), m/z 511 (2%), 429 (2%), 386 (4%), 347 (5%), 305 (15%), 263 (6%), 287 (4%), 136 (10%), 125 (10%), 83 (100%). Exact mass measurement of $M^+$, m/z 511.1538 ($C_{23}H_{30}ClN_3O_6S$ requires 511.1544). Anal Calcd for $C_{23}H_{30}ClN_3O_6S$: C, 54.06; H, 5.92; N, 8.22; S, 6.27; Cl, 6.94. Found: C, 55.56; H, 6.30; N, 7.91; S, 5.64; Cl, 6.64.

*b)From the carboxylic chloride of 1* - Carboxylic acid 1 (0.646 g, 1 mmol) in ethanol-free $CH_2Cl_2$ (8 ml) was treated at 0 °C with oxalyl chloride (0.21 ml) and dimethylformamide (0.01 ml). After standing for 2 h at room temperature, *the mixture was evaporated in vacuo* (2 x; dry benzene added). Crude carboxyl chloride of 1 was obtained as a white foam. This compound (two separate preparations) was immediately allowed to react with DCC (215 mg, 1.04 mmol) in $CH_2Cl_2$ (8 ml) for 30 min at room temperature either in the presence or in the absence of triethylamine (0.14 ml, 1 mmol). After aqueous workup, the organic extracts were evaporated and purified by flash chromatography (4:1). Compound 2 (tlc, nmr, ms) was isolated from the two reactions in comparable amounts (0.34 and 0.31 g, 66 and 61%, respectively).

*c)From oxidation of sulphoxides 7 and 8* - Compounds 7 and 8 (50 mg, 0.1 mmol) *in $CH_2Cl_2$ (5 ml) were separately treated with 70% MCPBA (50 mg) for 2 h at room* temperature. Workup with 4% aqueous $NaHSO_3$ and flash chromatography afforded 2 (tlc, nmr, ms) in comparable amounts (approx. 18 mg, 35%).

3-Acetoxymethyl-7α-chloro-3-cephem-4-carboxylic acid 1β-oxide (4). 7-Aminocephalosporanic acid (27.2 g, 0.1 mol) was nitrosated with $NaNO_2$ (10.4 g) in 3.5N HCl (350 ml) and acetone (600 ml) as described for the preparation of 1. The obtained compound was purified by flash chromatography (1:9), to yield 3-acetoxymethyl-7α-chloro-3-cephem-4-carboxylic acid 3 (14.5 g, 50%) as a syrup. This compound was converted to the corresponding benzhydryl ester (20.8 g, 91%) by reaction with diphenyldiazomethane (11.7 g, 0.06 mol) in dichloromethane (300 ml) for 1 h at room temperature, followed by evaporation and flash chromatography (7:3). A solution of this intermediate (10 g, 21.8 mmol) in dichloromethane (120 ml) was treated at -40 °C with 70% MCPBA (5.4 g, 21.8 mmol). The reaction mixture was stirred for 30 min at -20 °C and then sequentially washed with 4% aq. $NaHSO_3$ and $NaHCO_3$ solutions. Evaporation and flash chromatography (1:1) afforded two sulphoxide epimers (ir, nmr) as white foams, here indicated as faster-running compound A (1.4 g, 14%) and slower-running compound B (5.1 g, 49%). The major sulphoxide B (4.74 g) in $CH_2Cl_2$ (25 ml) and anisole (1.2 ml) was treated at 0 °C with trifluoroacetic acid (7 ml). The cooling bath was removed. After 30 min at 20 °C, the reaction mixture was diluted with $CH_2Cl_2$ and extracted with saturated aq. $NaHCO_3$. The solution was made acidic with 8% HCl and back-extracted with EtOAc. Evaporation left a slurry which crystallized by addition of ethyl ether (1.54 g, 50%); mp 163-165 °C ($Et_2O$). Ir (KBr) $\nu_{max}$ 1797, 1750, 1717, 1640 $cm^{-1}$; $^1H$ nmr ($CDCl_3$ + DMSO-$d_6$) δ 2.01 (3H, s, $COCH_3$), 3.40 (1H, dd, $J$= 18.0 and 1.0 Hz, 2-$H_\alpha$), 3.75 (1H, d, $J$= 18.0 Hz, 2-$H_\beta$), 4.72 (1H, m, 6-H), 4.88 (2H, ABq, $J$= 13.5 Hz, 3'-$H_2$), 5.07 (1H, d, $J$= 2.0 Hz, 7-H), 10.0 ppm (1H, br s, exch. $D_2O$); FD-ms (EHC= 18 mA), m/z 308 ($MH^+$, 100%), 263 [$(M-CO_2)^{+\cdot}$, 43%], 248 [$(M-CH_3COO\cdot)^+$, 15%]. The long-range coupling ($J$= 1.0 Hz) observed in this compound between 6-H and 2-$H_\alpha$ confirmed the β-oxide configuration.[15]

3-Acetoxymethyl-7α-chloro-3-cephem-4-carboxylic acid 1α-oxide (5). Similarly, trifluoroacetic acid treatment of the minor sulphoxide epimer A (900 mg) afforded the acid 5 (300 mg, 54%); mp 78-80 °C ($CH_2Cl_2$ — $i$-$Pr_2O$). Ir (KBr) $\nu_{max}$ 1800, 1730 br, 1640 $cm^{-1}$; $^1H$ nmr ($CDCl_3$ + DMSO-$d_6$) δ 2.10 (3H, s, $COCH_3$), 3.85 (2H, ABq, $J$= 16.0 Hz, 2-$H_2$), 4.60 (1H, d, $J$= 2.0 Hz, 6-H), 4.98 (2H, ABq, $J$= 13.0 Hz, 3'-$H_2$), 5.28 (1H, d, $J$= 2.0 Hz, 7-H), 9.30 ppm (1H, br s, exch. $D_2O$); FD-ms (EHC= 17 mA), m/z 308 ($MH^+$, 100%), 263 [$(M-CO_2)^{+\cdot}$, 9%], 248 [$(M-CH_3COO\cdot)^+$, 13%]. The geminal coupling between the $C_2$-protons ($J$= 16.0 Hz), smaller than that observed in its epimer 4 ($J$= 18.0 Hz), confirmed the α-oxide configuration.[15]

3-Acetoxymethyl-7α-chloro-4-spiro-[3'-(1'-cyclohexyl-4'-cyclohexylimino-2'-oxoazetidinyl)]-2-cephem (6). DCC (825 mg, 4 mmol) was added to a solution of acid 3 (574 mg, 2 mmol) in $CH_2Cl_2$ (10 ml). After 15 min, triethylamine (0.28 ml, 2 mmol) was added, and stirring was continued for additional 20 min. The reaction mixture was sequentially washed with 2N HCl and 4% aq. $NaHCO_3$ solutions and evaporated. Flash chromatography (4:1) afforded compound 6 as a syrup (120 mg, 13%). Ir ($CHCl_3$) $\nu_{max}$ 1820 sh, 1787, 1738, 1690 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 1.1-2.1 (20H, m, cyclohexyl $CH_2$), 2.03 (3H, s, $COCH_3$), 3.04 and 3.67 (each 1H, m, cyclohexyl CH), 4.67 (1H, d, $J$= 1.7 Hz, 6-H, and 2H, s, 3'-$H_2$), 4.83 (1H, d, $J$= 1.7 Hz, 7-H), 6.68 ppm (1H, s, 2-H); FD-ms (EHC= 16 mA), m/z 479 ($MH^{+\cdot}$, 100%). Compound 6 was not detected (tlc) in the reaction mixture before triethylamine addition.

3-Acetoxymethyl-7α-chloro-4-spiro-[3'-(1'-cyclohexyl-4'-cyclohexylimino-2'-oxoazetidinyl)]-2-cephem 1β-oxide (7).

*a)From carboxylic acid 4* - Acid 4 (100 mg, 0.33 mmol) was allowed to react with DCC (72 mg, 0.35 mmol) as described for compound 2, method a). Spiroazetidinone 7 (120 mg, 74%) was obtained as a syrup; ir ($CHCl_3$) $\nu_{max}$ 1794, 1745, 1693 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 1.1-2.1 (20H, m, cyclohexyl $CH_2$), 2.12 (3H, s, $COCH_3$), 3.5-3.8 (2H, m, cyclohexyl CH), 4.70 and 4.79 (each 1H, dd, $J$= 14.4 and 1.4 Hz, 3'-$H_2$), 4.70 (1H, d, $J$= 1.7 Hz, 6-H), 5.20 (1H, d, $J$= 1.7 Hz, 7-H), 7.00 ppm (1H, t, $J$= 1.4 Hz, 2-H).

*b)From sulphide 6* - A solution of 6 (20 mg, 0.042 mmol) in $CH_2Cl_2$ (5 ml) was treated at -40 °C with 70% MCPBA (11 mg, 0.044 mmol). The cooling bath was removed. After 1 h, the reaction mixture was sequentially washed with 4% aq. $NaHSO_3$ and $NaHCO_3$ solutions. Evaporation and flash chromatography (3:2) afforded, in this order, the 1α-oxide 8 (1-2 mg), identical (tlc, nmr) with an authentic sample (see below), and the 1β-oxide 7 (12 mg, 58%), identical (tlc, nmr) with the material described above.

3-Acetoxymethyl-7α-chloro-4-spiro-[3'-(1'-cyclohexyl-4'-cyclohexylimino-2'-oxoazetidinyl)]-2-cephem 1α-oxides (8 and 9). Chlorocephalosporanic acid 1α-oxide 5 (400 mg, 1.3 mmol) was suspended in $CH_2Cl_2$ (10 ml) and treated with DCC (536 mg, 2.6 mmol). The resulting clear solution was stirred for 1 h at room temperature, during which time dicyclohexylurea began to precipitate. The reaction mixture was filtered, washed with 4% aq. $NaHCO_3$ in order to remove some unreacted starting material, dried and evaporated. Flash chromatography (3:1) afforded, in this order, the 4-spiro(azetidinyl)cephem 1α-oxides 9 (96 mg, 15%) and 8 (64 mg, 10%) *as syrups. 4-Epimer 8: Ir* ($CHCl_3$) $\nu_{max}$ 1820, sh, 1790, 1740, 1685 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 1.1-2.1 (20H, m, cyclohexyl $CH_2$), 2.09 (3H, s, $COCH_3$), 2.90 and 3.68 (each 1H, m, cyclohexyl CH), 4.18 (1H, d, $J$= 1.5 Hz, 7-H), 4.46 and 4.82 (each 1H, dd, $J$= 14.1 and 1.2 Hz, 3'-$H_2$), 5.17 (1H, d, $J$= 1.5 Hz, 7-H), 6.67 ppm (1H, t, $J$= 1.2 Hz, 2-H). 4-Epimer 9: Ir ($CHCl_3$) $\nu_{max}$ 1820 sh, 1790, 1735, 1690 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 1.1-2.1 (20H, m, cyclohexyl $CH_2$), 2.10 (3H, s, $COCH_3$), 2.81 and 3.67 (each 1H, m, cyclohexyl CH), 4.57 (1H, d, $J$= 1.5 Hz, 6-H), 4.72 (2H, d, $J$= 1.2 Hz, 3'-$H_2$), 5.10 (1H, d, $J$= 1.5 Hz, 7-H), 6.66 ppm (1H, t, $J$= 1.2 Hz, 2-H).

3-Acetoxymethyl-7α-chloro-4-spiro-[3'-(1'-cyclohexyl-4'-cyclohexylimino-2'-oxoazetidinyl)]-2-cephem 1,1-dioxide (10). Oxidation of compound 9 (10 mg) was carried out as described for its 4-epimer 8. Sulphone 10, the 4-epimer of 2, was obtained as a syrup (4 mg, 39%). Ir ($CHCl_3$) $\nu_{max}$ 1810, 1755, 1700 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 1.1-2.1 (20H, m, cyclohexyl $CH_2$), 2.10 (3H, s, $COCH_3$), 3.6-3.8 (2H, m, cyclohexyl CH), 4.60 and 4.75 (each 1H, dd, $J$= 14.4 and 1.3 Hz, 3'-$H_2$), 4.79 (1H, d, $J$= 1.5 Hz, 6-H), 5.40 (1H, d, $J$= 1.5 Hz, 7-H), 6.78 ppm (1H, t, $J$= 1.3 Hz, 2-H); FD-ms, m/z 511 ($M^{+\cdot}$, 100%).

3-Methyl-4-spiro-[3'-(1'-cyclohexyl-4'-cyclohexylimino-2'-oxoazetidinyl)]-2-cephem 1,1-dioxide (13). Acid 11 (171 mg, 0.74 mmol) and DCC (160 mg, 0.78 mmol) were combined as desribed for the preparation of 2, method a), but a reaction time of 15 min was allowed prior isolation. Compound 13 (160 mg, 52%) was isolated as a white foam; FT-ir (DRS) $\nu_{max}$ 1827 sh, 1797.6, 1712.9 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 1.1-2.1 (20H, m, cyclohexyl $CH_2$), 1.98 (3H, d, $J$= 1.5 Hz, 3'-$H_3$), 3.33 and 3.70 (each 1H, m, cyclohexyl CH), 3.39 (1H, dd, $J$= 15.6 and 4.6 Hz, 7-$H_\alpha$), 3.67 (1H, dd, $J$= 15.6 and 1.9 Hz, 7-$H_\beta$), 4.76 (1H, dd, $J$= 4.6 and 1.9 Hz, 6-H), 6.37 ppm (1H, q, $J$= 1.5 Hz, 2-H); FD-ms (EHC= 14 mA), m/z 419 ($M^{+\cdot}$, 100%), 294 [$(M-C_6H_{11}NCO)^{+\cdot}$, 25%].

3-Methyl-7β-phenoxyacetamido-4-spiro-[3'-(1'-cyclohexyl-4'-cyclohexylimino-2'-oxoazetidinyl)]-2-cephem 1,1-dioxide (14). Similarly, from acid 12 (380 mg) compound 14 (110 mg, 20%) was obtained as a colorless syrup; ir ($CHCl_3$) $\nu_{max}$ 1795, 1700, 1690 $cm^{-1}$; $^1H$ nmr ($CDCl_3$) δ 1.1-2.0 (20H, m, cyclohexyl $CH_2$), 1.97 (3H, d, $J$= 1.5 Hz, 3'-$H_3$), 3.40 and 3.68 (each 1H, m, cyclohexyl CH), 4.52 (2H, s, $OCH_2CO$), 4.89 (1H, d, $J$= 4.6 Hz, 6-H), 6.15 (1H, dd, $J$= 10.8 and 4.6 Hz, 7-H), 6.36 (1H, q, $J$= 1.5 Hz, 2-H), 6.8-7.4 (5H, m, Ph), 8.13 ppm (1H, d, $J$= 10.8 Hz, exch. $D_2O$, CONH); FD-ms (EHC= 20 mA), m/z 568 ($M^{+\cdot}$, 100%), 443 [$(M-C_6H_5NCO)^{+\cdot}$, 5%].

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