residue esterified with EtOH–H_2SO_4. After the usual work-up and crystallization from benzene, **11** was obtained as bright yellow solid (25 mg, 0.08 mmol, 53%): mp 210–211°; ν max 3430, 1725, 1700, 1640 cm^{-1}; λ_{max} (95% EtOH) 420 (3040), 360 (2940), 325 (4000), 312 (3830), 239 (38,080); λ_{max} (H_2O) 415 (4770), 325 (5420), 275 (4810), 238 (49,700); nmr ($CDCl_3$–CD_3OD) 1.25 (t, 3 H, J = 7 Hz), 2.4 (s, 3 H), 2.92 (m, 2 H), 3.35 (m, 3 H), 4.18 (q, 2 H, J = 7 Hz), 7.7 (s, 1 H), 7.95 (s, 1 H), 8.45 (s, 1 H). *Anal.* ($C_{17}H_{17}NO_4$) C, H, N.

6-Carbamylmethyl-8-methyl-5,6-dihydro-7*H*-cyclopenta-[*f*]isoquinoline-3(2*H*),5-dione (12). A suspension of **11** (150 mg, 0.50 mmol) in 40 ml of EtOH was treated with 60 ml of alcoholic NH_3. A clear yellow solution was formed, which was allowed to stand at room temperature for 20 hr and then refluxed for 4 hr. The solution was evaporated and crystallized from EtOH to afford **12** as a yellow solid (15 mg, 0.06 mmol, 11%). The compound does not melt, but gradually decomposes above 300°: ν max 3450, 1700, 1680 cm^{-1}; λ_{max} (95% EtOH) 420 (3900), 360 (3660), 323 (4800), 310 (4480); λ_{max} (H_2O) 405 (5150), 317 (4870), 275 (5170). *Anal.* ($C_{15}H_{14}N_2O_3$) C, H, N.

6-Carbamylmethyl-8-methyl-7(5)*H*-cyclopenta[*f*]isoquinol-in-3(2*H*)-one (1). A mixture of **12** (65 mg, 0.24 mmol) and $NaBH_4$ (90 mg, 2.38 mmol) in 100 ml of EtOH was stirred at room temperature for 24 hr. The residue obtained after the usual work-up was refluxed with HCl in glacial HOAc (10 ml) for 1 hr. It was then evaporated to dryness and the residue was crystallized from EtOH to yield a yellow solid (10 mg, 0.04 mmol, 16%), which gradually decomposed above 250°: ν max 3440, 1645 cm^{-1}; λ_{max} (95% EtOH) 420 (1520), 347 sh (1810), 332 (2080), 263 (15,300); λ_{max} [H_2O (3% DMSO)] 418 (2500), 348 sh (1600), 333 (2000), 265 (20,000); nmr (pyridine-d_5) 2.28, 2.38 (s, 3 H, 2:1, $ArCH_3$); mass spectrum 254 (M^+). *Anal.* ($C_{15}H_{14}N_2O_2$) C, H, N.

RNA Polymerase Reaction. RNA polymerase was isolated from *E. coli* and inhibition of the RNA polymerase reaction was carried out as described by Burgess.[19] The reaction mixtures contained 10 μg of template per milliliter and 5% of dimethyl sulfoxide, the enzyme concentration being 29 μg/ml.

Thermal Transition Temperature. Thermal transition temperature was determined on a Gilford 2400-S, equipped with a variable temperature bath. Studies on calf thymus DNA and the poly(deoxyribonucleotides) were made in a phosphate–EDTA buffer (pH 7.8): PO_4^{3-} = 0.001 *M*, Na^+ = 0.002 *M*, EDTA = 10^{-4} *M*, DMSO = 0.6% by volume; concentration of DNA-P = 4×10^{-5} *M* and concentration of **1** = 2.5×10^{-5} *M*.

Difference Spectra. Difference spectra were determined[18] on a Cary 15 spectrophotometer with 0–0.1 OD scale expansion between 500 and 380 nm. Spectra were obtained using split-compartment mixing cells (Pyrocell Co., Westwood, N.J.) in which equal volumes of solutions of the compound and calf thymus DNA were placed in separate compartments of both cells. All solutions were made in phosphate buffer, pH 7.21 ± 0.01 (PO_4^{3-} = 0.001 *M*, NaCl = 0.01 *M*). The concentration of the compound after mixing was 2×10^{-4} *M* and calf thymus DNA concentration was 10 OD. All reactions were carried out at 24°.

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Chemistry of Cephalosporin Antibiotics. 30.[1] 3-Methoxy- and 3-Halo-3-cephems

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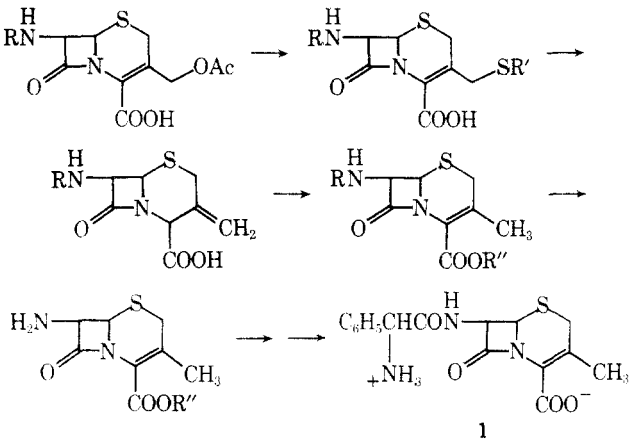
The *exo*-methylene group in esters of 7-acylamido- and 7-amino-3-methylenecephams was ozonized to give 3-hydroxy-3-cephems. Conditions are described to effect a selective N-acylation of a 3-hydroxy-3-cephem nucleus ester. Vilsmeier reagents converted 7-acylamido-3-hydroxy compounds to 3-halo-3-cephem derivatives. Diazomethane converted the 3-hydroxy compounds to 3-methoxy-3-cephem derivatives. Removal of the ester-protecting group at the C_4-carboxyl afforded a select group of cephalosporins with direct halo and methoxy substitution at C_3. A number of these compounds are potent antibiotics.

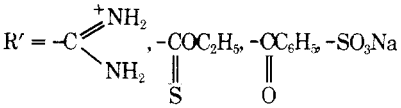
We have recently reported on the preparation of 3-methylenecephams from reductions of cephalosporanic acids in which the acetoxy group is displaced by sulfur nucleophiles[2a] (Scheme I). A reductive cleavage of the acetoxy group in cephalosporanic acids leading to 3-methylenecephams using chromium(II) salts has also been reported.[2b] As esters of the 3-methylenecephams can be readily isomerized to deacetoxycephalosporins, they were shown to be useful precursors to intermediates in published syntheses[3] of cephalexin (1).[4] This paper focuses on the oxidation of the 3-*exo*-methylene grouping in 3-methylenecephams as a step in the preparation of a new class of cephalosporins with the unique structural feature of direct heteroatom substitution at C_3.[5] These compounds possess useful antimicrobial activity.

Our initial oxidation studies were performed on 7-acylamido-3-methylenecepham esters **2** (Scheme II). Low-temperature ozonolysis of methyl 7-phenoxyacetamido-3-methylenecepham-4-carboxylate (**2a**) gave the 3-hydroxy derivative **3a**, some 1-sulfoxide of the starting cephalosporin, and decomposition products. Compound **3a** was isolated pure by preparative tlc silica gel and was shown by uv analysis (pH 7 buffer) to exist largely in the enol (3-hydroxy-3-cephem) form. Absorption in the region of 268 mμ for the

Scheme I



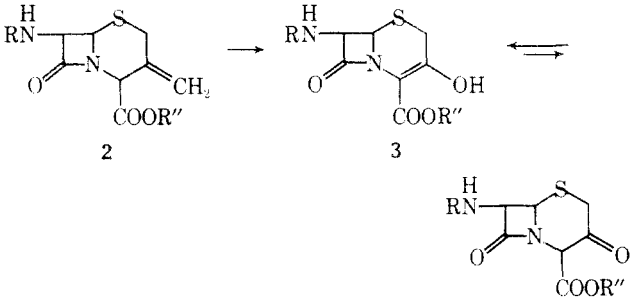
R = acyl or H

R′ = 

R″ = $-Si(CH_3)_3$, $-H_2C-C_6H_4-NO_2$, $-H_2C-C_6H_4-OCH_3$

β-lactam–Δ^3-unsaturation system had an extinction coefficient of 7,800. The nmr spectrum ($CDCl_3$) of **3a** showed no signal for an allylic proton at C_4 or for a vinyl proton at C_2 to accommodate the 3-keto or the 3-hydroxy-2-cephem structures. Similarly, ozonolysis of *p*-nitrobenzyl 7-phenylacetamido-3-methylenecepham-4-carboxylate (**2b**) gave the 3-hydroxy derivative **3b**. The acidic nature of the 3-hydroxycephalosporins was observed in titration (66% aqueous DMF, pK_a 5.6) and in their partition between organic and basic aqueous solutions. Some 3-keto character to compounds **3** is supported by the ready decarboxylation of the carboxylic acid at C_4 upon removal of ester-protecting groups.

Scheme II



2a, 3a, R = $C_6H_5OCH_2CO-$; R″ = $-CH_3$

2b, 3b, R = $C_6H_5CH_2CO-$; R″ = $-H_2C-C_6H_4-NO_2$

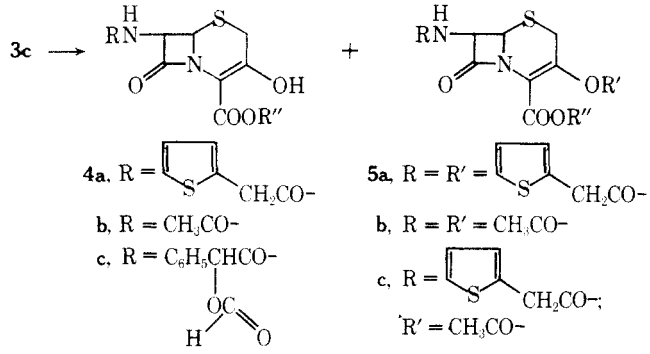
2c, 3c, R = H; R″ = $-H_2C-C_6H_4-NO_2$

An analogous ozonolysis of 3-methylenecephams was currently disclosed in the patent literature.[6]

In general, oxidation of the *exo*-methylene function in 7-acylamido derivatives was complex. The product was obtained in low yield and necessitated chromatographic purification. For example, we were not successful in isolating pure products from ozonolyses of 7-(thiophene-2-acetamido)-3-methylenecepham esters. However, the same ozonolysis of *p*-nitrobenzyl 7-amino-3-methylenecepham-4-carboxylate (**2c**) went smoothly to give high yields of **3c,** isolated directly from the reaction mixture as a crystalline hydrochloride.

Acylations of *p*-nitrobenzyl 7-amino-3-hydroxy-3-cephem-4-carboxylate (**3c**) were complicated by its zwitterion character (Scheme III). Under anhydrous conditions, ketene, acid chlorides, mixed anhydrides, and activated esters with **3c** gave intractable mixtures of N- (**4**) and N,O- (**5**) acylated products, not satisfactorily separated by chromatography over silica. On the other hand, acylations of **3c,** in the presence of water, led clearly to 3-hydroxy derivatives **4.** In a separate step, compounds **4** could be esterified in good yields to **5** under anhydrous conditions and with base catalysis. Thus, in aqueous tetrahydrofuran, **3c** reacted with thiophene-2-acetyl chloride to give pure *p*-nitrobenzyl 7-(thiophene-2-acetamido)-3-hydroxy-3-cephem-4-carboxylate (**4a**). In turn, **4a** converted to **5a** on reacting with a second mole of the acid chloride in dry acetone and in the presence of triethylamine. Similarly, **3c** reacted quantitatively with ketene in aqueous acetone. The product (**4b**) combined further with ketene in anhydrous methylene chloride containing a mole of triethylamine or with acetyl chloride or anhydride in dry dimethylformamide to give **5b.**

Scheme III



Chlorination of 7-acylamido-3-hydroxy-3-cephem esters (**4**) was accomplished in dry dimethylformamide using a number of chlorinating agents [$SOCl_2$, PCl_3, $POCl_3$, $(COCl)_2$, and $COCl_2$] (Scheme IV). At room temperature, **4a** converted to *p*-nitrobenzyl 7-(thiophene-2-acetamido)-3-chloro-3-cephem-4-carboxylate (**6a**) in good yield. Using PBr_3–DMF, the corresponding 3-bromo-3-cephem derivative **6b** was obtained.

Removal of the acyl side chain in the imino chloride cleavage reaction[7] from **6a** afforded a useful intermediate, the 3-chloro nucleus ester **7** (Scheme V). *p*-Nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate (**7**) was isolated as a hydrochloride and acylated efficiently to provide a variety of protected 3-chloro-3-cephems (**9**). A final step in the preparation of 3-halo-3-cephems for antibiotic testing was the removal of the *p*-nitrobenzyl ester protecting group. Zinc–HCl reduction or catalytic hydrogenolysis of **6a,b** and **9a,b** yielded respectively 7-(thiophene-2-acetamido-3-chloro)- (**8a**) and 3-bromo-3-cephem-4-carboxylic acid (**8b**), 7-

Scheme IV

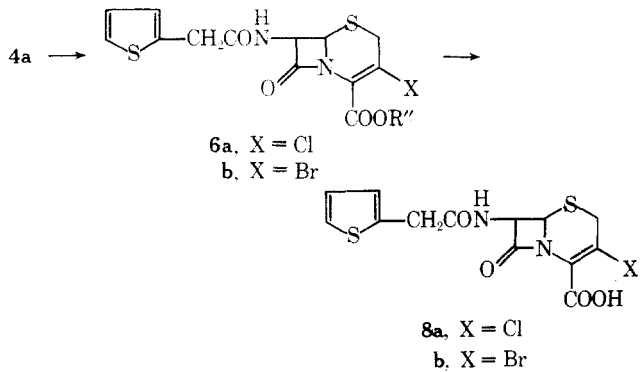
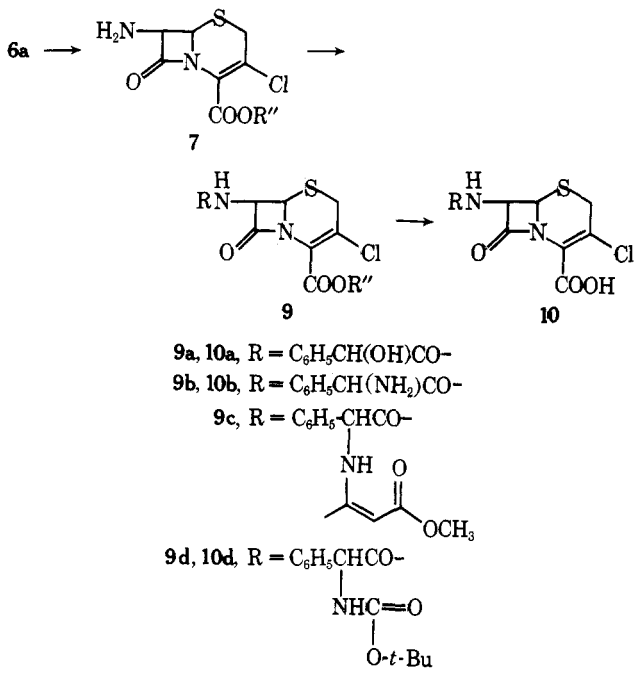


Table I. Gradient Plate Assay[a]

	Shigella sp. N9	*E. coli* N10	*K. pneumoniae* X26	*A. aerogenes* X68	*Sal. heidelberg* X514	*Ps. aeruginosa* X528	*Ser. marcescens* X99	Penicillin-resistant *Staph.* V41	Penicillin resistant *Staph.* V32	Methicillin resistant *Staph.* X400	Penicillin resistant *Staph.* V84	Non-β-lactamase producing *Staph.* X1.1
Cephalothin[b]	9.7	12.0	1.0	1.0	1.0	>200	>200	0.5	0.6	>20	<0.1	<0.1
8a	17.5	21.2	0.8	0.9	0.9	>200	>200	11.4	16.0	>20	0.5	<0.1
8b	19.8	20.8	1.0	1.0	1.0	>200	>200	12.0	15.0	>20	0.5	0.4
10a	4.0	6.3	1.0	0.7	0.6	>200	>160	5.8	8.0	>20	0.4	<0.1
10b	0.7	0.8	0.6	0.6	0.5	>200	21.5	3.0	5.0	>20	0.6	0.5
13a	48.8	57.5	11.2	9.9	9.8	>200	>200	>20	>20	>20	4.0	0.8
13b	8.0	8.6	4.5	4.3	4.8	>200	164	18.4	>20	>20	9.4	0.6
13c	4.0	3.8	3.7	3.3	3.0	>200	124	10.0	12.4	>20	3.4	0.5
Cephalexin[c]	15.0	13.5	6.9	5.2	6.3	>200	>200	6.2	7.3	>20	10.0	0.5

[a] Additional *in vivo* and *in vitro* data for these compounds will be published elsewhere. Activity expressed in minimum inhibitory concentrations (MIC). [b] Reference 8. [c] Reference 4.

Scheme V



(D-mandelamido)-3-chloro-3-cephem-4-carboxylic acid (**10a**), and 7-(D-2-amino-2-phenylacetamido)-3-chloro-3-cephem-4-carboxylic acid (**10b**).

Reactions of diazomethane with 3-hydroxy-3-cephems were another extension in our studies of the chemical properties of the 3-enolic function. While 7-acylamido-3-hydroxy-3-cephems (**4**) reacted with diazomethane to give 3-methoxy-3-cephem derivatives directly, the preferred route to compounds **12** started with a diazomethane treatment of **3c** to give the 3-methoxy-3-cephem nucleus ester **11** followed by acylation (Scheme VI). *p*-Nitrobenzyl 7-amino-3-methoxy-3-cephem-4-carboxylate (**11**) was stable as a free amino ester and acylated without complications with acid chloride, *o*-carboxy anhydride, and mixed anhydride couplings. Removal of the *p*-nitrobenzyl ester grouping in **12** afforded a select group of 3-methoxy-3-cephems for antimicrobial testing. Examples of 3-methoxy-3-cephems include 7-(thiophene-2-acetamido)-3-methoxy-3-cephem-4-carboxylic acid (**13a**), 7-(D-mandelamido)-3-methoxy-3-cephem-4-carboxylic acid (**13b**), and 7-(D-2-amino-2-phenylacetamido)-3-methoxy-3-cephem-4-carboxylic acid (**13c**).

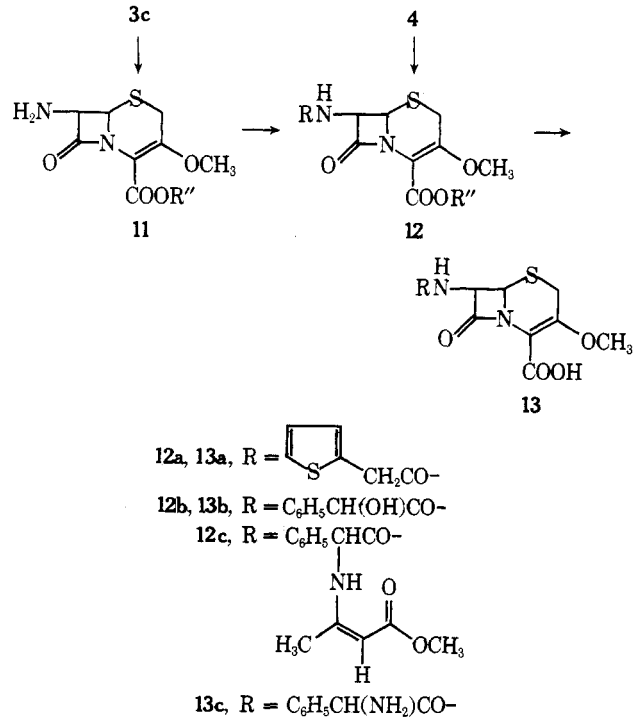
We note in ir spectroscopy a strong hydrogen bonding between the 3-enolic OH and the ester carbonyl at C_4 in compounds **4**. Ester carbonyl absorption in 3-hydroxy-3-cephems superimposes 7-amide carbonyl absorption as a broad band near 6.1 μ, while chlorination as in **9** and O-alkylation as in **12** of the 3-hydroxy function effects a shift of this band to 5.9 μ, separate from the amide π band at 6.1 μ.

Products **8a,b, 10a,b,** and **13a–c** possess both gram-positive and gram-negative activity in a gradient-plate assay as shown in Table I.

Experimental Section

Methyl 7-Phenoxyacetamido-3-hydroxy-3-cephem-4-carboxylate (3a). 2a, 1.6 g (4.4 mmol), was dissolved in 300 ml of CH_2Cl_2, cooled in acetone–Dry Ice bath, and ozonized in a Welsbach apparatus for 3 min. Sodium bisulfite, 10 g (98 mmol), was added in suspension. The mixture was sitrred vigorously at ice-

Scheme VI



water temperature. The supernatant was separated and washed with 5% HCl and then with saturated NaCl solution. The solvent was replaced with EtOAc for extraction with 5% $NaHCO_3$ solution. The aqueous phase was separated, layered with EtOAc, and acidified to pH 2.5 in the cold. The organic phase was separated, washed with water, dried (Na_2SO_4), and evaporated to dryness *in vacuo*. The residue weighed 709 mg (42% yield). An analytical sample was obtained by preparative silica gel tlc to give pure **3a:** nmr (τ, T-60 MHz, $CDCl_3$) 6.62 (s, 2 H, C_2-H_2), 6.10 (s, 3 H, ester CH_3), 5.40 (s, 2 H, α-CH_2), 4.90 (d, 1 H, C_6-H), 4.32 (q, 1 H, C_7-H), and 3.1–2.4 (m, 6 H, aromatic and C_7-NH). *Anal.* ($C_{16}H_{16}N_2O_6S \cdot H_2O$) C, H, N, S.

***p*-Nitrobenzyl 7-Phenylacetamido-3-hydroxy-3-cephem-4-carboxylate (3b). 2b** was treated as described above. **3b** crystallized (in 30% yield) from concentration, *in vacuo*, of dried EtOAc solution: mp 160–173° dec; nmr (τ, T-60 MHz, $CDCl_3$) 6.67 (AB q, 2 H, C_2-H_2), 6.38 (s, 2 H, α-CH_2), 5.03 (d, 1 H, C_6-H), 4.66 (d, 2 H, ester CH_2), 4.40 (q, 1 H, C_7-H), 2.66 (s and d, 6 H, aromatic H and C_7-NH), and 2.1 (q, 4 H, aromatic H). *Anal.* ($C_{22}H_{19}N_3O_7S$) C, H, N.

***p*-Nitrobenzyl 7-Amino-3-hydroxy-3-cephem-4-carboxylate (3c). 2c,** 3.85 g (10 mmol), was dissolved in 600 ml of CH_3OH and cooled in an acetone–Dry Ice bath for ozonolysis for 20 min. Ozonide decomposed with passing SO_2 through the reaction mixture for 2 min. The CH_3OH was replaced by 200 ml of 0.1 *N* HCl in CH_2Cl_2. Solvent and excess HCl were removed by evaporation *in vacuo*. The residue was redissolved in acetone for crystallization as a hydrochloride salt containing 0.5 mol of acetone as solute: yield 3.15 g (75%); mp 150–180° dec; nmr (τ, A-60 MHz, DMSO-d_6), 7.92 (s, 3 H, 0.5 mol of acetone), 6.22 (AB q, 2 H, C_2-H_2), 5.07 (d, 1 H, C_6-H), 4.8–4.1 (m, 3 H, ester CH_2 and C_7-H), and 1.96 (q, 4 H, aromatic H). The free amino ester was crystallized from EtOAc. *Anal.* ($C_{14}H_{13}N_3O_6S$) C, H, N.

***p*-Nitrobenzyl 7-(Thiophene-2-acetamido)-3-hydroxy-3-cephem-4-carboxylate (4a). 2c,** 3.85 g (9.2 mmol), was ozonized as described before. The crude product (**3c**) was not crystallized from acetone but was dissolved in 175 ml of THF and 50 ml of H_2O. Sodium bisulfite, 2.1 g (20 mmol), was added. While stirring at room temperature, thiophene-2-acetyl chloride, 4.8 g (30 mmol), in 200 ml of THF was added dropwise. The mixture was stirred for 2 hr. The THF was evaporated *in vacuo*. The aqueous residue was extracted with EtOAc. The EtOAc solution was washed with 5% HCl and H_2O, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The crystalline residue was triturated with ether to remove excess thiophene-2-acetic acid: yield 2.9 g (66%); mp 148–158° dec; nmr (τ, A-60 MHz, $CDCl_3$) 6.60 (s, 2 H, C_2-H_2), 6.13 (s, 2 H, α-CH_2), 4.96 (d, 1 H, C_6-H), 4.62 (d, 2 H, ester CH_2), 4.46 (q, 1 H, C_7-H), and 3.1–1.7 (m, 8 H, aromatic H and C_7-NH). *Anal.* ($C_{20}H_{17}N_3O_7S_2$) C, H, N, O, S.

***p*-Nitrobenzyl 7-Acetamido-3-hydroxy-3-cephem-4-carboxylate (4b). 3c,** 4.2 g (10 mmol), was dissolved in 325 ml of acetone and 125 ml of H_2O and cooled in an ice–water bath. Ketene was passed through the solution for 30 min. The acetone was evaporated *in vacuo*. The aqueous residue was extracted with EtOAc. The organic solution was washed with 5% HCl and saturated NaCl solution, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue crystallized in trituration with ether: yield 3.6 g (90%); mp 146–152° dec; nmr (τ, T-60 MHz, $CDCl_3$) 7.90 (s, 3 H, α-CH_3), 6.55 (s, 2 H, C_2-H_2), 4.92 (d, 1 H, C_6-H), 4.62 (d, 2 H, ester CH_2), 4.30 (q, 1 H, C_7-H), 2.81 (d, 1 H, C_7-NH), and 2.04 (q, 4 H, aromatic H). *Anal.* ($C_{16}H_{15}N_3O_7S$) C, H, N.

***p*-Nitrobenzyl 7-[*O*-(Formyl)-D-mandelamido]-3-hydroxy-3-cephem-4-carboxylate (4c). 3c,** 1.54 g (3.4 mmol), was treated with *O*-formyl-D-mandeloyl chloride,[9] 960 mg (4.9 mmol), in the manner described for **4a.** The crystalline product weighed 1.0 g (57% yield): mp 124–134° dec; nmr (τ, T-60 MHz, $CDCl_3$) 6.61 (s, 2 H, C_2-H_2), 4.95 (d, 1 H, C_6-H), 4.61 (d, 2 H, ester CH_2), 4.39 (q, 1 H, C_7-H), 3.70 (s, 1 H, α-CH_2), 2.80–1.80 (m, 11 H, C_7-NH, aromatic H and OCHO). *Anal.* ($C_{23}H_{19}N_3O_9S$) C, H, N.

***p*-Nitrobenzyl 7-(Thiophene-2-acetamido)-3-(thiophene-2-acetoxy)-3-cephem-4-carboxylate (5a). 4a,** 1.9 g (4 mmol), in 30 ml of dry acetone containing triethylamine, 405 mg (4 mmol), was treated dropwise with thiophene-2-acetyl chloride, 650 mg (4 mmol), in 20 ml of dry acetone. The mixture was stirred at room temperature for 2 hr and filtered and the solvent was evaporated *in vacuo*. The residue was redissolved in EtOAc for washes with H_2O, 5% HCl, and 5% $NaHCO_3$ solution. The organic solution was dried ($MgSO_4$) and concentrated to about 10 ml for crystallization: yield 1.1 g (45%); mp 105–126° dec; nmr (τ, A-60 MHz, $CDCl_3$) 6.55 (AB q, 2 H, C_2-H_2), 6.19 (s, 2 H, α-CH_2), 6.06 (s, 2 H, C_3-ester CH_2), 5.00 (d, 1 H, C_6-H), 4.77 (s, 2 H, C_4-ester CH_2), 4.20 (q, 1 H, C_7-H), and 3.2–1.7 (m, 11 H, aromatic H and C_7-NH). *Anal.* ($C_{26}H_{21}N_3O_8S_3$) C, H, N.

***p*-Nitrobenzyl 7-Acetamido-3-acetoxy-3-cephem-4-carboxylate (5b). 4b,** 1.9 g (4.8 mmol), was dissolved in 100 ml of cold CH_2Cl_2 containing triethylamine, 485 mg (4.8 mmol). Ketene was passed through the cold solution for 6 min. Solvent and excess ketene were removed *in vacuo*. Crystalline residue was triturated with ether: yield 1.7 g (81%); mp 198–200°; nmr (τ, T-60 MHz, $CDCl_3$–DMSO-d_6) 8.00 (s, 3 H, C_3-ester CH_3), 7.85 (s, 3 H, α-CH_3), 6.40 (AB q, 2 H, C_2-H_2), 4.85 (d, 1 H, C_6-H), 4.60 (s, 2 H, C_4-ester CH_2), 4.23 (q, 1 H, C_7-H), 1.99 (q, 4 H, aromatic H), and 1.43 (d, 1 H, C_7-NH). *Anal.* ($C_{18}H_{17}N_3O_8S$) C, H, N.

***p*-Nitrobenzyl 7-(Thiophene-2-acetamido)-3-acetoxy-3-cephem-4-carboxylate (5c). 4a** was treated with ketene as in the preparation of **5b** or with acetyl chloride, or anhydride in DMF, at room temperature. Product crystallized by trituration with ether: mp 150–178° dec; nmr (τ, T-60 MHz, $CDCl_3$) 7.86 (s, 3 H, C_3-ester CH_3), 6.55 (AB q, 2 H, C_2-H_2), 6.19 (s, 2 H, α-CH_2), 5.00 (d, 1 H, C_6-H), 4.71 (s, 2 H, C_4-ester CH_2), 4.20 (q, 1 H, C_7-H), 3.25 (d, 1 H, C_7-NH), and 3.1–1.8 (m, 7 H, aromatic H). *Anal.* ($C_{22}H_{19}N_3O_8S_2$) C, H, N.

***p*-Nitrobenzyl 7-(Thiophene-2-acetamido)-3-chloro-3-cephem-4-carboxylate (6a). 4a,** 1.9 g (4 mmol), in 10 ml of molecular-sieve-dried DMF containing freshly distilled thionyl chloride, 950 mg (8 mmol), was stored at room temperature for 6.5 hr. The reaction mixture was diluted with 100 ml of EtOAc and extracted several times with 5% HCl and saturated NaCl solution. The EtOAc solution was dried ($MgSO_4$) and filtered with talc. The solvent was removed *in vacuo* and the residue crystallized by trituration with ether: yield 1.2 g (61%); mp 164–166°. Substituting PCl_3 for thionyl chloride gave an 81% yield of the same product with less coloration: nmr (τ, T-60, MHz, $CDCl_3$) 6.39 (AB q, 2 H, C_2-H_2), 6.17 (s, 2 H, α-CH_2), 4.99 (d, 1 H, C_6-H), 4.64 (s, 2 H, ester CH_2), 4.19 (q, 1 H, C_7-H), 3.45 (d, 1 H, C_7-NH), and 3.1–1.67 (m, 7 H, aromatic H). *Anal.* ($C_{20}H_{16}ClN_3O_6S_2$) C, H, N, Cl.

***p*-Nitrobenzyl 7-(Thiophene-2-acetamido)-3-bromo-3-cephem-4-carboxylate (6b). 4a** was treated with PBr_3 as with the preparation of **6a:** nmr (τ, A-60, MHz, DMSO-d_6) 6.21 (s, 2 H, α-CH_2), 5.98 (AB q, 2 H, C_2-H_2), 4.72 (d, 1 H, C_6-H), 4.51 (s, 2 H, ester CH_2), 4.20 (q, 1 H, C_7-H), 3.04–1.74 (m, 7 H, aromatic H), and 0.66 (d, 1 H, C_7-NH). *Anal.* ($C_{20}H_{16}BrN_3O_6S_2$) C, H, N, Br.

***p*-Nitrobenzyl 7-Amino-3-chloro-3-cephem-4-carboxylate (7). 6a,** 500 mg (1 mmol), in 6 ml of CH_2Cl_2 was treated with dry pyridine, 95 mg (1.2 mmol), and with PCl_5, 237 mg (1.1 mmol). The mixture was stirred at room temperature for 1.5 hr and cooled in an ice bath for addition of 0.6 ml of isobutyl alcohol. The product crystallized as a hydrochloride out of the reaction mixture: yield 200 mg (49%); mp 168° dec; nmr (τ, T-60 MHz, DMSO-d_6) 5.97 (s, 2 H, C_2-H_2), 4.8–4.5 (m, 4 H, C_6-H, C_7-H and ester CH_2), and 2.03 (q, 4 H, aromatic H). *Anal.* ($C_{14}H_{13}Cl_2N_3O_5S$) C, H, N, Cl.

7-(Thiophene-2-acetamido)-3-chloro-3-cephem-4-carboxylic Acid (8a). 6a, 988 mg (2 mmol), in 40 ml of THF and 60 ml of CH_3OH was hydrogenolized at 50 psi, room temperature, for 1 hr using an equal weight of 5% Pd/C prereduced in 30 ml of EtOH at 50 psi, room temperature, for 30 min. The catalyst was filtered and washed successively with THF, CH_3OH, and EtOAc. Filtrate and washes were combined and evaporated to dryness *in vacuo*. Residue was redissolved in EtOAc–H_2O and adjusted to pH 7 with 1 *N* NaOH. The aqueous phase was separated, washed with EtOAc, and then back-titrated to pH 2.5 with 1 *N* HCl and in the presence of EtOAc. The organic phase was separated, washed with H_2O, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue crystallized on trituration with ether: yield 170 mg (24%); mp 114–120° dec; nmr (τ, T-60 MHz, $CDCl_3$) 6.38 (AB q, 2 H, C_2-H_2), 6.16 (s, 2 H, α-CH_2), 4.98 (d, 1 H, C_6-H), 4.20 (q, 1 H, C_7-H), and 3.1–2.5 (m, 4 H, aromatic H and C_7-NH). *Anal.* ($C_{13}H_{11}ClN_2O_4S$) C, H, N, Cl.

7-(Thiophene-2-acetamido)-3-bromo-3-cephem-4-carboxylic Acid (8b). 6b, 545 mg (1.0 mmol), was hydrogenolized as in the preparation of **8a.** Product crystallized on trituration with ether: yield 180 mg (44%); nmr (τ, 100 MHz, DMSO-d_6) 8.89 (t, ether CH_3), 6.59 (q, ether CH_2), 6.22 (s, 2 H, α-CH_2), 6.06 (AB q, 2 H, C_2-H_2), 5.76 (d, 1 H, C_6-H), 4.28 (q, 1 H, C_7-H), 3.08–2.59 (m, 3 H, aromatic H), and 0.78 (d, 1 H, C_7-NH). *Anal.* [$C_{13}H_{11}BrN_2O_4S_2 \cdot 0.5(C_2H_5)_2O$] C, H, N, Br.

***p*-Nitrobenzyl 7-(D-Mandelamido)-3-chloro-3-cephem-4-carboxylate (9a). 7,** as a hydrochloride, 812 mg (2 mmol), was suspended in 40 ml of EtOAc. Sodium bisulfite, 520 mg (5 mmol).

was dissolved in 40 ml of H_2O. The two were mixed and stirred vigorously for addition of D-mandelic acid *o*-carboxyanhydride,[10] 395 mg (2.2 mmol). The mixture was stirred at room temperature for 1.5 hr. The organic phase was separated, washed several times with H_2O, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue crystallized in trituration with ether: yield 685 mg (69%); mp 158–164° dec; nmr (τ, A-60 MHz, $CDCl_3$) 6.24 (AB q, 2 H, C_2-H_2), 5.0–4.7 (m, 2 H, C_6-H and α-CH), 4.57 (s, 2 H, ester CH_2), 6.23 (q, 1 H, C_7-H), and 2.8–1.2 (m, 10 H, aromatic H and C_7-NH). *Anal.* ($C_{22}H_{18}ClN_3O_7S$) C, H, N, Cl.

***p*-Nitrobenzyl 7-[D-2-(*tert*-Butoxyformamido)-2-phenylacetamido]-3-chloro-3-cephem-4-carboxylate (9d). 7,** 3.0 g (8.1 mmol), suspended in 200 ml of dry THF with *N*-(*tert*-butoxycarbonyl)-D-α-phenylglycine, 2.1 g (8.3 mmol), and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), 2.0 g (8.3 mmol), was stirred at room temperature overnight. The solvent was removed *in vacuo*. The residue, in EtOAc, was extracted successively with H_2O, 5% $NaHCO_3$ solution, 5% HCl, and H_2O. The EtOAc solution was dried ($MgSO_4$) and concentrated to about 50 ml for crystallization of the product. A first crop weighed 3.7 g (63%): nmr (τ, A-60 MHz, $CDCl_3$) 8.60 (s, 9 H, *t*-Boc CH_3), 6.45 (AB q, 2 H, C_2-H_2), 5.03 (d, 1 H, C_6-H), 4.67 (s, 3 H, ester CH_2 and α-CH), 4.12 (m, 2 H, C_7-H and amide NH), and 2.72–1.74 (m, 10 H, aromatic H and amide NH). *Anal.* ($C_{27}H_{27}ClN_4O_8S$) C, H, N.

***p*-Nitrobenzyl 7-(D-2-Amino-2-phenylacetamido)-3-chloro-3-cephem-4-carboxylate (9b).** The above compound (3.6 g, 6 mmol) in 60 ml of CH_3CN with *p*-toluenesulfonic acid, 2.4 g (12.6 mmol), was stirred at room temperature overnight. **9b** crystallized as a tosylate salt from the reaction mixture: yield 3.1 g (81%); nmr (τ, T-60 MHz, DMSO-d_6) 7.70 (s, 3 H, *p*-TSA CH_3), 6.61 (s, 3 H, side chain NH_3), 6.20 (AB q, 2 H, C_2-H_2), 4.94 (s, 1 H, α-CH), 4.80 (d, 1 H, C_6-H), 4.51 (s, 2 H, ester CH_2), 4.08 (q, 1 H, C_7-H), 2.95–1.62 (m, 13 H, aromatic H), and 0.32 (d, 1 H, C_7-NH). *Anal.* ($C_{29}H_{27}ClN_4O_9S_2$) C, H, N, Cl.

7-[D-2-(*tert*-Butoxyformamido)-2-phenylacetamido]-3-chloro-3-cephem-4-carboxylic Acid (10d). *p*-Nitrobenzyl 7-[D-2-*tert*-butoxyformamido)-2-phenylacetamido]-3-chloro-3-cephem-4-carboxylate, 3.0 g (5.0 mmol), was dissolved in 15 ml of molecular-sieve-dried THF and 185 ml of MeOH and hydrogenated at room temperature at 50 psi for 1 hr using 3 g of 5% palladium in carbon prereduced in 60 ml of ethanol at room temperature, 50 psi, for 30 min. The spent catalyst was filtered and washed with THF and MeOH. Filtrate and washes were combined and evaporated to dryness *in vacuo*. The residue was redissolved in water and EtOAc; the pH was adjusted to 7 with 1 *N* NaOH. The aqueous was separated, washed with EtOAc, and then back-titrated to pH 2.5 with 1 *N* HCl in the presence of EtOAc. The organic layer was separated, washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. The residue crystallized from 70 ml of ether and 20 ml of Skelly B: yield 1.75 g (75%); nmr (τ, A-60 MHz, $CDCl_3$) 8.55 (s, 9 H, *t*-Br), 6.48 (AB q, 2 H, C_2-H_2), 5.0 (d, 1 H, C_6-H), 4.63 (d, 1 H, α-CH), 4.25 (q, 1 H, C_7-H), 3.90 (d, 1 H, amide NH), and 2.59 (s, 5 H, aromatic H). *Anal.* ($C_{20}H_{22}ClN_3O_6S$) C, H, N, Cl.

***p*-Nitrobenzyl 7-[*N*-(1-Carbomethoxy-2-propenyl)-D-α-phenylglycylamido]-3-chloro-3-cephem-4-carboxylate (9c).** Methyl 3-α-carboxybenzylaminocrotonate sodium salt, 500 mg (1.85 mmol), was dissolved in 20 ml of acetonitrile containing 4 drops of dimethylbenzylamine. The mixture was stirred and cooled in a Dry Ice–carbon tetrachloride bath for slow addition of methyl chloroformate, 184 mg (1.95 mmol). After 20 min, a precooled solution made from *p*-nitrobenzyl 7-amino-3-chloro-3-cephem-4-carboxylate, 750 mg (1.85 mmol), and triethylamine, 188 mg (1.85 mmol), in 40 ml of acetone was added over a period of 3 min. The mixture was stirred in the cold for 30 min and then at room temperature for 2 hr. The mixture was filtered. The solvents from the filtrate were removed *in vacuo*. The residue was taken up in water and EtOAc; the pH was adjusted to 7 and the organic layer separated. The EtOAc solution was washed with water, dried ($MgSO_4$), and concentrated to a smaller volume. Addition of *n*-hexane precipitated the product: yield 620 mg; nmr (τ, A-60 MHz, DMSO-d_6) 8.20 (s, 3 H, enamine CH_3), 6.60 (AB q, 2 H, C_2-CH_2), 6.45 (s, 3 H, ester CH_3), 5.48 (s, 1 H, enamine CH), 4.9–4.1 (m, 5 H, C_6-H, C_7-H, α-CH, and ester CH_2), and 3.1–1.5 (m, 9 H, aromatic H). *Anal.* ($C_{27}H_{25}N_4O_8SCl$) C, H, N.

7-(D-Mandelamido)-3-chloro-3-cephem-4-carboxylic Acid (10a). 9a, 200 mg (0.4 mmol), was hydrogenolized as with **8a:** yield 7.5 mg (51%); nmr (τ, T-60, MHz, D_2O–$NaHCO_3$) 6.42 (AB q, 2 H, C_2-H_2), 4.90 (d, 1 H, C_6-H), 4.68 (s, 1 H, α-CH), 4.37 (d, 1 H, C_7-H), and 2.49 (s, 5 H, aromatic H).

7-(D-2-Amino-2-phenylacetamido)-3-chloro-3-cephem-4-carboxylic Acid (10b). Method A. 9b tosylate salt, 1.5 g (2.2 mmol), in 10 ml of dry DMF, cooled to 0°, was treated with 2 ml of concentrated HCl and zinc dust, 400 mg (6.1 mmol). The mixture was stirred in the cold for 45 min and then at room temperature for 1 hr. The mixture was filtered and adjusted to pH 6.8 with triethylamine. The product crystallized as a di-DMF solvate, weighing 800 mg (71%): nmr (τ, A-60 MHz, D_2O–DCl) 6.90 (2 s, 6 H, DMF CH_3), 6.33 (AB q, 2 H, C_2-H_2), 4.85 (d, 1 H, C_6-H), 4.64 (s, 1 H, α-CH), 4.27 (d, 1 H, C_7-H), 2.41 (s, 5 H, aromatic H), and 1.84 (s, 2 H, DMF CHO). *Anal.* ($C_{21}H_{28}ClN_5O_6S$) C, H, N, Cl.

Method B. 9c, 540 mg (0.9 mmol), was dissolved in 40 ml of acetonitrile and 20 ml of water and cooled in ice–water bath for acidification to pH 1.5 momentarily and then to pH 2.5. The solvents were evaporated *in vacuo*. The residue was redissolved in 40 ml of tetrahydrofuran and 80 ml of methanol and hydrogenolized at room temperature, 50 psi, for 1 hr using 540 mg of 5% palladium in carbon, prereduced in 20 ml of ethanol, at room temperature, 50 psi for 30 min. The catalyst was filtered and washed with THF and MeOH. Filtrate and wash were combined and evaporated to dryness *in vacuo*. The residue was redissolved in water and EtOAc for a pH adjustment to 4.5. The aqueous was separated and concentrated to a smaller volume. The product crystallized as a half hydrate. *Anal.* ($C_{15}H_{14}N_3O_4SCl \cdot 0.5H_2O$) C, H, N, Cl.

***p*-Nitrobenzyl 7-Amino-3-methoxy-3-cephem-4-carboxylate (11). 3c,** as hydrochloride, 417 mg (1 mmol), in 35 ml of THF containing 1 mol of triethylamine was treated with excess diazomethane in ether. After 30 min at room temperature, solvents and excess reagent were evaporated *in vacuo*. The residue, dissolved in EtOAc, was washed with H_2O, dried ($MgSO_4$), and crystallized by trituration with ether: yield 210 mg (58%); mp 163–164°; nmr (τ, A-60 MHz, DMSO-d_6) 7.3 (s, 2 H, C_7-NH_2), 6.27 (s, 2 H, C_2-H_2), 6.20 (s, 3 H, C_3-OCH_3), 5.30 (d, 1 H, C_6-H), 5.00 (d, 1 H, C_7-H), 4.63 (s, 2 H, ester CH_2), and 2.02 (q, 4 H, aromatic H). *Anal.* ($C_{15}H_{15}N_3O_6S$) C, H, N.

***p*-Nitrobenzyl 7-(Thiophene-2-acetamido)-3-methoxy-3-cephem-4-carboxylate (12a). Method A. 4a,** 2 g (4.2 mmol) in 50 ml of CH_2Cl_2, was treated with excess diazomethane at room temperature for 2 hr. Solvent evaporated *in vacuo*. Product crystallized from 15 ml of EtOAc: yield 750 mg (38%); mp 171–175°.

Method B. 11, 2.4 g (6.5 mmol), in 50 ml of dry acetone with $NaHCO_3$, 1.7 g (20 mmol), in suspension was treated with thiophene-2-acetyl chloride, 1.2 g (7.2 mmol), in 20 ml of acetone added dropwise. The mixture was stirred at room temperature for 15 hr after addition. Acetone was replaced by EtOAc, washed with water, dried ($MgSO_4$), and concentrated to a small volume *in vacuo*: yield 1.9 g (60%); nmr (τ, A-60 MHz, $CDCl_3$) 6.62 (s, 2 H, C_2-H_2), 6.18 (s, 3 H, C_3-OCH_3), 6.15 (s, 2 H, α-CH_2), 4.98 (d, 1 H, C_6-H), 4.71 (d, 2 H, ester CH_2), 4.42 (q, 1 H, C_7-H), 3.19 (d, 1 H, C_7-NH), and 3.1–1.65 (m, 7 H, aromatic H). *Anal.* ($C_{21}H_{19}N_3O_7S_2$) C, H, N, O, S, OCH_3.

***p*-Nitrobenzyl 7-(D-Mandelamido)-3-methoxy-3-cephem-4-carboxylate (12b). 11,** 365 mg (1 mmol), D-mandelic acid *O*-carboxyanhydride, 200 mg (1.1 mmol) in 20 ml of EtOAc, and $NaHSO_3$, 200 mg (1.9 mmol), in 20 ml of H_2O were mixed and stirred vigorously at room temperature for 30 min. The EtOAc phase was separated, washed several times with H_2O, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue crystallized on trituration with ether: yield 350 mg (70%); nmr (τ, T-60 MHz, $CDCl_3$) 6.70 (s, 2 H, C_2-H_2), 6.22 (s, 3 H, C_3-OCH_3), 5.06 (d, 1 H, C_6-H), 4.90 (s, 1 H, α-CH), 4.75 (d, 2 H, ester CH_2), 4.58 (q, 1 H, C_7-H), and 2.66–1.75 (m, 9 H, aromatic H). *Anal.* ($C_{23}H_{21}N_3O_8S$) C, H, N.

***p*-Nitrobenzyl 7-[*N*-(1-Carbomethoxy-2-propenyl)-D-α-phenylglycylamido]-3-methoxy-3-cephem-4-carboxylate (12c). 11,** 1.1 g (3 mmol), in 45 ml of acetone and cooled to −20° was added to a mixed anhydride solution prepared by mixing methyl 3-α-carboxybenzylaminocrotonate,[11] 815 mg (3 mmol), and methyl chloroformate, 303 mg (3.2 mmol), in 45 ml of CH_3CN containing 6 drops of dimethylbenzylamine. The mixture was stirred at −20° for 30 min and then at room temperature for 2 hr. The mixture was filtered. The filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in EtOAc, washed with H_2O, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The product crystallized from ethanol: yield 1.1 g (61%); mp 135–145° dec; nmr (τ, A-60 MHz, $CDCl_3$), 8.19 (s, 3 H, enamine CH_3), 6.72 (s, 2 H, C_2-H_2), 6.36 and 6.20 (2 s, 6 H, ester CH_3 and C_3-OCH_3), 5.43 (s, 1 H, enamine vinyl H), 5.05 (d, 1 H, C_6-H), 4.91 (d, 1 H, α-CH), 4.70 (s, 2 H, ester CH_2), 4.41 (q, 1 H, C_7-H), 2.63 and 2.15 (s and q, 9 H, aromatic H), and 0.56 (d, 1 H, C_7-NH). *Anal.* ($C_{28}H_{28}N_4O_9S$) C, H, N.

7-(Thiophene-2-acetamido)-3-methoxy-3-cephem-4-carboxylic Acid (13a). 12a, 490 mg (1 mmol), was hydrogenolized as in the preparation of **8a.** The product was crystallized by trituration from ether. A first crop weighed 156 mg (28%): mp 168–171°; nmr (τ, A-60 MHz, DMSO-d_6) 6.35 (s, 2 H, C_2-H_2), 6.24 (s, 5 H, C_3-OCH_3 and α-CH_2), 4.94 (d, 1 H, C_6-H), 4.55 (q, 1 H, C_7-H), 3.10–2.55 (m, 3 H, aromatic H), and 1.10 (d, 1 H, C_7-NH). *Anal.* ($C_{14}H_{14}N_2O_5S_2$) C, H, N.

7-(D-Mandelamido)-3-methoxy-3-cephem-4-carboxylic Acid (13b). 12b (1 mmol) was hydrogenolized as for **10a:** yield 115 mg (31%); mp 161–164°; nmr (τ, A-60 MHz, DMSO-d_6), 6.36 (s, 2 H, C_2-H_2), 6.23 (s, 3 H, C_3-OCH_3), 4.98–4.80 (m, 2 H, C_6-H and α-CH), 4.55 (q, 1 H, C_7-H), 3.8 (s, 1 H, α-OH), 2.78–2.38 (m, 5 H, aromatic H), and 1.45 (d, 1 H, C_7-NH). *Anal.* ($C_{16}H_{16}N_2O_6S$) C, H, N.

7-(D-2-Amino-2-phenylacetamido)-3-methoxy-3-cephem-4-carboxylic Acid (13c). 12c, 500 mg (0.83 mmol), in 30 ml of CH_3CN and 15 ml of H_2O was acidified to pH 1 momentarily and then back to 2.5. The solvents were evaporated to dryness *in vacuo*. The residue was hydrogenolized in the manner already described. After removal of solvents of hydrogenolysis, the residue was dissolved in H_2O and adjusted to pH 4.5 for extractions with EtOAc. The aqueous phase was evaporated to dryness *in vacuo*. The residue crystallized from 2 ml of H_2O and 1 ml of CH_3CN: yield 122 mg (37%); mp 148–180° dec; nmr (τ, A-60 MHz, D_2O–DCl) 6.58 (AB q, 2 H, C_2-H_2), 6.10 (s, 3 H, C_3-OCH_3), 4.87 (d, 1 H, C_6-H), 4.70 (s, 1 H, α-CH), 4.54 (d, 1 H, C_7-H), and 2.41 (s, 5 H, aromatic H). *Anal.* ($C_{16}H_{17}N_3O_5S \cdot 2H_O$) C, H,.

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Electronic Structures of Cephalosporins and Penicillins. 4. Modeling Acylation by the β-Lactam Ring†

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Molecular orbital calculations by the CNDO/2 method are used to study the molecular and electronic details involved in the initial phases of the opening of the β-lactam ring of a model cephalosporin structure, 7-amino-3-acetoxymethyl-3-cephem. The effect of a simple nucleophile, OH^-, approaching the carbonyl carbon center of the β-lactam ring is monitored by following the charge redistributions that occur in the bicyclic system and in the 3 side chain. A migration of electron density to the ester oxygen of the CH_2OAc group is observed with concomitant weakening of the CH_2–OAc bond. The results are discussed in relation to the mechanism of acylation of bacterial cell wall enzymes by β-lactam antibiotics and in relation to the hydrolysis of these molecules. The results indicate that the ability of the 3′ substituent of cephalosporins to stabilize electron density transferred to it, *i.e.*, the leavability of the 3′ moiety, can be an important factor in activating the β-lactam toward nucleophilic attack.

The mode of action of the cephalosporins and penicillins has been unraveled to a considerable degree at the molecular level.[2,3] These antibiotics are thought to inhibit the final cross-linking reaction in bacterial cell wall synthesis[4] by mimicking some part of the substrate peptidoglycan, presumably a D-alanyl-D-alanine dipeptide.[5] An irreversible chemical binding mechanism, which is believed to be an acylation by the β-lactam, results in the inactivation of at least one transpeptidase enzyme which knits the peptidoglycan into a huge macromolecule.

A number of theoretical papers based on the correctness of the above mode of action, but also providing support for its feasibility, have been addressed to (a) just how the antibiotic mimics the transpeptidase substrate in terms of the geometric and electronic similarity of the antibiotic to the natural substrate in its ground or transition state,[6] (b) the chemical mechanism of the acylation reaction leading to destruction of enzyme activity,[7] and (c) the influence of chemical modification of the antibiotic on the above chemical mechanism.[7-9] This paper is concerned to a certain extent with all three of the above details but mainly with the second. We accordingly explore relevant parts of the reaction path (or, more generally, surface) that would be traversed when a nucleophile forms a transition state complex with a representative cephalosporin. Changes calculated in electronic structure are used in making generalizations (in terms of familiar, chemical concepts) about what types of molecular modifications may be desirable in promoting biological activity of cephalosporins.

The acylation of the cell wall enzyme is believed to proceed *via* the attack at the carbonyl carbon of the antibiotic's β-lactam ring by a thiol group of the enzyme.[2,3,10-12]

† For paper 3 of this series, see ref 1.