resulting brownish syrup was dissolved in 5 mL of absolute ethanol and passed through a paper filter to remove the dark brown precipitate. The filtrate was concentrated to small volume, leading, after several hours at room temperature, to a crop of crystals (79 mg, 49%), mp 146–148 °C.[23] Concentration of the mother liquors led to a further crop of crystals (14 mg; overall yield 57%).

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Orally Active Esters of Cephalosporin Antibiotics. 3.[1] Synthesis and Biological Properties of Aminoacyloxymethyl Esters of 7-[D-(-)-Mandelamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic Acid

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The synthesis of six amino acid acyloxymethyl esters of cefamandole (**1**), a semisynthetic broad-spectrum cephalosporin antibiotic, is described. These esters were examined as potentially useful orally active antibiotic prodrugs. When tested for oral efficacy against *Streptococcus pyogenes* C203 in mouse protection tests, the esters were not notably more active than lithium cefamandole. Further studies demonstrated that significant blood and urine levels of **1** were not obtained after dosing **2a**, **2b**, and **2f** orally at 17 mg/kg in mice. A study of the stability to chemical hydrolysis and the possible relationship of hydrolysis to the lack of oral absorption of these esters is also presented.

Cefamandole (**1**), 7-(D-mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, a semisynthetic broad-spectrum cephalosporin antibiotic, is active against a wide variety of Gram-positive as well as Gram-negative organisms in vitro.[2] Particularly noteworthy is the activity of cefamandole against indole-positive *Proteus spp.* and *Enterobacter spp.*, organisms which are resistant to most cephalosporins.[3] The in vitro activity of cefamandole has been confirmed in many laboratories, and reports of its clinical efficacy are numerous.[4] Since cefamandole is not efficiently absorbed following oral administration, an investigation of the effect of esterification of the C-4 carboxyl group on oral absorption was undertaken. Acetoxymethyl and pivaloylmethyl esters of cefamandole (esters which increase the oral absorption of ampicillin[5] and cephaloglycin[6]) were only marginally active orally because they lack sufficient solubility in water for efficient absorption from the gastrointestinal tract to occur. However, when formulated with a solubilizing vehicle, solutions of these esters of

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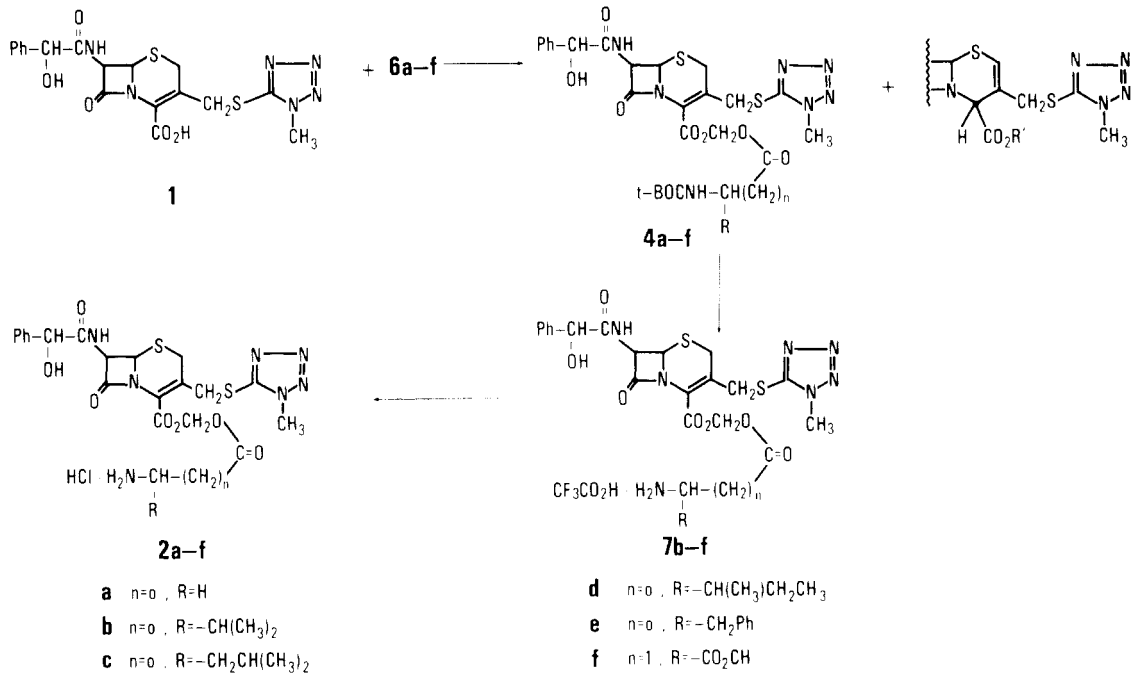
Scheme I



cefamandole achieved reasonable oral absorption, as evidenced by increased blood levels and urinary recovery.[1] To overcome the low water solubility of cefamandole esters, we prepared aminoacyloxymethyl esters, the hydrochloride salts of which might be expected to have increased water solubility. Descriptions of such esters of a variety of penicillins,[7] as well as cephalothin,[8] claiming enhanced oral absorption have appeared in the patent literature. The synthesis and biological properties of aminoacyloxymethyl esters of 1 are described herein.

Chemistry. The esters of cefamandole (**2a–f**) were prepared as outlined in Scheme I. Reaction of the sodium or triethylammonium salt of 1 with **3a** was very slow and resulted in a low yield of a mixture containing **4a** and the corresponding Δ^2-ester, as well as a preponderance of unreacted starting material 1. It was recently reported that Δ^2/Δ^3 isomerization of the double bond of cephalosporins could be prevented by alkylation with alkyl iodides, since the reaction time was very short;[9] however, treatment of 1 with **6a–f**/triethylamine in DMF for 5–10 min also resulted in a mixture of **4a–f** and the corresponding Δ^2-esters. Fortunately, crystallization (after initial purification of the crude esters by chromatography) yielded the pure Δ^3-esters.

Syntheses of the required halomethyl esters **3a–f** of the blocked amino acids are outlined in Scheme II. Each *tert*-butyloxycarbonyl-protected L-amino acid was reacted with chloroiodomethane[10] in the presence of triethylamine in DMF. In addition to the desired chloromethyl esters **3a–f**, the corresponding gem-diesters **5a–f** were also obtained. We were unable to suppress the formation of **5a–f** even by using a several-fold excess of chloroiodomethane or by changing the order of addition of the reactants. Apparently, **3a–f** were more reactive alkylating agents than chloroiodomethane. Separation of **3a–f** from **5a–f** was effected by chromatography over dry column silica gel. Conversion of **3a–f** to **6a–f** was effected by reaction with sodium iodide in acetone.

Removal of the *tert*-butyloxycarbonyl-protecting group occurred smoothly in anhydrous trifluoroacetic acid, except in the case of **4a**. Attempted conversion of the trifluoroacetate salt **7a** to the hydrochloride salt **2a** was unsuccessful. Only 1 was recovered owing to the extreme sensitivity of **2a** to base hydrolysis. Therefore, **4a** was treated with HCl/dioxane, to yield **2a** directly. The hydrochloride salts **2a–f** were all soluble in water to the extent of 50 mg/mL or greater.

Scheme II

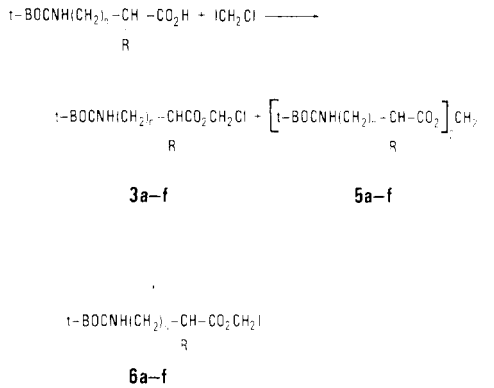


Table I. Chemical Stability of 2a–f

compd	% nonenzymat hydrolysis[a]
2a	complete hydrolysis
2b	complete hydrolysis
2c	complete hydrolysis
2d	complete hydrolysis
2e	50–60[b]
2f	20–30[b]

[a] In pH 6 phosphate buffer after 20 min. [b] These values were arrived at by comparing the visual intensities of the UV zones for the ester and 1 after paper electrophoresis of the samples removed from incubations of the ester at 37 °C and should be treated as approximations only.

Chemical Stability of 2a–f. While **2a–f** were stable in solution at 37 °C and pH 2.5, slight ester hydrolysis occurred at pH 4.5. At pH 6.0, hydrolysis of **2a–d** was complete in 20 min (Table I). The hydrolysis of **2e** was only 50–60% complete after 20 min at pH 6.0, while only 20–30% of **2f** was hydrolyzed after 20 min. The hydrolysis was followed by paper electrophoresis. Visualization of

Scheme III

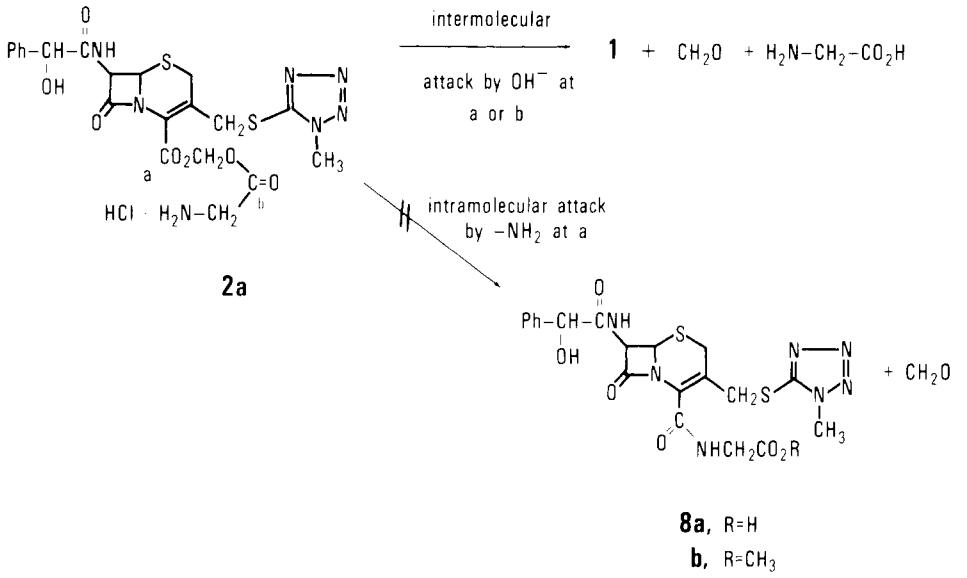


Table II. In Vivo Mouse Protection Data

compd	Oral ED_{50} vs. *S. pyogenes* C203 in mg/kg × 2[a]
2a	20.4 (12)
2b	14.3 (10.7)
2c	10.4 (17.9)
2d	8.6 (17.9)
2e	15.3 (17.9)
2f	25.2 (17.9)

[a] The oral ED_{50} of lithium cefamandole run in the same test is shown in parentheses for comparative purposes. The ED_{50} values are corrected for molecular weight differences with lithium cefamandole.

the hydrolysis products was done by UV light. Hydrolysis can conceivably occur either by attack of hydroxide ion at one of the carboxyl groups or by intermolecular or intramolecular aminolysis of the C-4 carboxyl (Scheme III). Intermolecular hydrolysis of **2a** would yield **1**, formaldehyde, and glycine, while intermolecular or intramolecular aminolysis would yield **8** and formaldehyde. The hydrolysis of **2a** at pH 6.0 was followed by TLC (*n*-BuOH/EtOH/H_2O, 8:2:2) until complete. The base-soluble fraction was isolated and esterified with diazomethane. The methyl ester obtained was indistinguishable from the methyl ester of **1** by NMR and TLC. The methyl ester of **8** was prepared by acylation of methyl glycinate with **1** in the presence of dicyclohexylcarbodiimide and was significantly different from the esterified hydrolysis product by NMR and TLC.

Biological Results. Each of the amino esters of cefamandole (**2a–f**) was tested for oral efficacy against *Streptococcus pyogenes* C203 in mouse protection tests. The results of these experiments are shown in Table II. In most cases, the oral ED_{50} for the ester was not notably better than that of lithium cefamandole; indeed, in some cases, lithium cefamandole was more effective orally than the ester. These results are disappointing in light of the efficient oral absorption of the acetoxymethyl ester of cefamandole when administered orally in solution[1] (propylene glycol/water) and the reported enhanced oral absorption (over that of penicillin G) of the valyloxymethyl and β-carbomethoxy-β-alanyloxymethyl esters of penicillin G.[7]

After oral administration of **2a**, **2b**, and **2f** at 17 mg/kg to mice, no cefamandole activity was detected in blood or urine, which further confirms lack of oral absorption. Absorption of the esters without subsequent hydrolysis cannot be the reason for the poor performance of these esters, since 75–100% of the oral dose could be isolated from the gastrointestinal tract throughout the course of the experiment. A more likely explanation for the poor absorption of these esters orally is chemical or enzymatic hydrolysis of the esters in the lumen of the small intestine back to **1** prior to absorption.

Experimental Section

Melting points are uncorrected. NMR spectra were recorded for all compounds and were recorded on either a Varian Associates HA-100 spectrometer or a Varian Associates T-60 spectrometer. UV spectra were recorded on a Cary Model 14 spectrophotometer in the solvent indicated. Elemental analyses were performed by the microanalytical group of the Lilly Research Laboratories. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4%. TLC was performed on EM Laboratories silica gel F_{254} plates.

Dry column chromatography was performed on silica gel (activity III/30 mm) from ICN Pharmaceuticals. The columns were packed without solvent. The material to be purified was adsorbed on silica gel and added to the top of the column. The material was then eluted with the solvent mixture indicated.

Each *tert*-butyloxycarbonyl-substituted amino acid was prepared by the method of Grzonka and Lammek.[11] *N*-[(*tert*-Butyloxy)carbonyl]aspartic acid α-methyl ester was prepared by the method of Schroder and Klieger.[12]

***N*-[(*tert*-Butyloxy)carbonyl]glycine Chloromethyl Ester (3a).** *N*-[(*tert*-Butyloxy)carbonyl]glycine (35 g, 0.2 mol) was dissolved in 400 mL of dimethylformamide. The stirred solution was then treated with triethylamine (48.7 mL, 0.35 mol) and chloroiodomethane (55 mL, 0.85 mol), and stirring was continued for 19 h. The DMF solution was then poured into EtOAc/H_2O and the layers were allowed to separate. The EtOAc layer was washed twice successively with water, 5% aqueous $NaHCO_3$, and saturated aqueous brine. The EtOAc layer was dried (anhydrous $MgSO_4$) and concentrated in vacuo to yield a mixture of **3a** and **5a**. The mixture was chromatographed over silica gel for dry column chromatography. The product was eluted with 3:7 EtOAc/cyclohexane, 50-mL fractions being taken. Fractions 4–14 were combined to yield 6.1 g (13.7%) of **3a** as an oil, which was used without further purification: NMR ($CDCl_3$) δ 1.42 (s, 9 H), 3.96 (d, 2 H, J = 6 Hz), 5.56 (br t, 1 H, J = 6 Hz), 5.81 (s, 2 H).

***N*-[(*tert*-Butyloxy)carbonyl]valine chloromethyl ester (3b)** was prepared by a method similar to **3a**, yielding after chromatography 7 g (20%) of a light yellow oil which was used without further purification: NMR ($CDCl_3$) δ 0.87 (d, 3 H, J = 3.5 Hz), 1.0 (d, 3 H, J = 3.5 Hz), 1.42 (s, 9 H), 2.16 (m, 1 H), 4.28 (dd, 1 H, J = 5 and 8 Hz), 5.26 (d, 1 H, J = 8 Hz), 5.7 (d, 1 H, J = 6 Hz), 5.87 (d, 1 H, J = 6 Hz).

***N*-[(*tert*-Butyloxy)carbonyl]leucine chloromethyl ester (3c)** was prepared as described for **3a**, yielding after chromatography 8.7 g (25%) of an oil: NMR ($CDCl_3$) δ 0.95 (d, 6 H, J = 5.5 Hz), 1.45 (s, 9 H), 1.6 (dd, 2 H, J = 8 and 14 Hz), 4.38 (m, 1), 4.95 (d, 1 H, J = 9 Hz), 5.92 (d, 1 H, J = 6 Hz), 6.12 (d, 1 H, J = 6 Hz).

This material was contaminated with **5c** which we were unable to remove by chromatography. An extra resonance at δ 5.9 was evident in the NMR.

***N*-[(*tert*-Butyloxy)carbonyl]isoleucine chloromethyl ester (3d)** was prepared by the procedure detailed for **3a**. The crude material was chromatographed over dry-column silica gel. Elution with 1:9 EtOAc/cyclohexane yielded 4.5 g (12%) as an oil: NMR ($CDCl_3$) δ 0.97 (d, 3 H, J = 7 Hz), 1.0 (t, 3 H, J = 10 Hz), 1.23 (m, 2 H), 1.45 (s, 9 H), 1.95 (m, 1 H), 4.35 (dd, 1 H, J = 5 and 9 Hz), 5.26 (d, 1 H, J = 9 Hz), 5.72 (d, 1 H, J = 6 Hz), 5.97 (d, 1 H, J = 6 Hz).

***N*-[(*tert*-Butyloxy)carbonyl]phenylalanine chloromethyl ester (3e)** was prepared in the manner described for **3a**. Chromatography yielded 3.3 g (8.7%) as an oil: NMR ($CDCl_3$) δ 1.45 (s, 9 H), 3.18 (d, 2 H, J = 5.5 Hz), 4.7 (m, 1 H), 5.14 (d, 1 H, J = 9 Hz), 5.73 (d, 1 H, J = 6 Hz), 5.9 (d, 1 H, J = 6 Hz), 7.36 (s, 5 H).

***N*-[(*tert*-Butyloxy)carbonyl]aspartic acid α-methyl ester, γ-chloromethyl ester (3f)**, was prepared in a manner described for **3a**. After chromatography, **3f** (1.5 g, 19%) was obtained as an oil: NMR ($CDCl_3$) δ 1.43 (s, 9 H), 3.05 (d, 1 H, J = 6 Hz), 3.80 (s, 3 H), 4.64 (m, 2 H), 5.68 (d, 1 H, J = 9 Hz), 5.76 (s, 2 H).

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic Acid, *N*-[(*tert*-Butyloxy)carbonyl]glycyloxymethyl Ester (4a). An acetone solution (50 mL) of **3a** (6.1 g, 27.2 mmol) and sodium iodide (8.2 g, 54.4 mmol) was stirred under N_2 for 16 h. The sodium chloride was removed by filtration and the filtrate was concentrated in vacuo. The residue was triturated with Et_2O and filtered. The filtrate was concentrated, redissolved in 15 mL of dimethylformamide, and added dropwise to a DMF solution (85 mL) of **1** (13.2 g, 27.2 mmol). An initial exothermic reaction subsided and stirring was continued for 10 min.

The reaction mixture was added to water and extracted with EtOAc. The EtOAc solution was washed twice with 50 mL of 3 N HCl, 10% aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The EtOAc solution was dried (anhydrous $MgSO_4$) and concentrated in vacuo to yield 10.9 g of an amorphous foam. NMR indicated the material was a mixture of **4a** and the corresponding Δ^2-ester. Crystallization from methanol yielded the pure Δ^3-ester **4a** as a white crystalline solid (6.5 g, 37%): mp 77–78 °C dec; UV λ_{max} (MeOH) 275 nm (ϵ_m 8125); NMR ($CDCl_3$) δ 1.42 (s, 9 H), 2.05 (s, 1 H, exchanges with D_2O), 3.68 (s, 2 H), 3.90 (s, 3 H), 3.98 (d, 2 H, J = 6 Hz), 4.16 (d, 2 H, J = 14 Hz), 4.54 (d, 2 H, J = 14 Hz), 4.95 (d, 1 H, J = 5 Hz), 5.14 (br s, 1 H), 5.26 (t, 1 H, J = 6 Hz), 5.76 (dd, 1 H, J = 5, 9.5 Hz), 5.86 (d, 1 H, J = 6 Hz), 5.97 (d, 1 H, J = 6 Hz), 7.35 (s, 6 H), 7.46 (d, 1 H, J = 9.5 Hz). Anal. ($C_{26}H_{31}N_7O_9S_2$) C, H, N, S.

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, *N*-[(*tert*-butyloxy)carbonyl]valyloxymethyl ester (4b), was prepared in a manner similar to that described for **4a**, producing 2.07 g (11%) of white crystals (MeOH): mp 125–127 °C dec; UV λ_{max} (MeOH) 271 nm (ϵ_m 7579); NMR ($CDCl_3$) δ 0.78 (d, 3 H, J = 7 Hz), 0.92 (d, 3 H, J = 7 Hz), 1.42 (s, 9 H), 1.85 (br s, 1 H), 2.15 (m, 1 H), 3.72 (s, 2 H), 3.95 (s, 3 H), 4.37 (m, 3 H), 4.97 (d, 1 H, J = 5 Hz), 5.3 (br s, 2 H), 5.75 (dd, 1 H, J = 5 and 9 Hz), 5.92 (s, 2 H), 7.18 (d, 1 H, J = 9 Hz), 7.37 (s, 6 H). Anal. ($C_{29}H_{37}N_7O_9S_2$) C, H, N, S.

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, *N*-[(*tert*-butyloxy)carbonyl]leucyloxymethyl ester (4c), was prepared as for **3a**, yielding, after chromatography over dry-column silica gel (eluted with 1:1 EtOAc/cyclohexane) and crystallization from 2-propanol, 2.8 g (15%) as a white crystalline solid: mp 114–117 °C dec; UV λ_{max} (MeOH) 273 nm (ϵ_m 7889); NMR ($CDCl_3$) δ 0.87 (d, 6 H, J = 6 Hz), 1.40 (s, 9 H), 1.75 (m, 3 H), 3.72 (s, 2), 3.91 (s, 3 H), 4.24 (d, 1 H, J = 14 Hz), 4.48 (d, 2 H, J = 14 Hz), 4.97 (d, 2 H, J = 5 Hz), 5.15 (br s, 1 H), 5.72 (dd, 1 H, J = 5 and 9 Hz), 5.87 (d, 1 H, J = 6 Hz), 5.90 (d, 1 H, J = 6 Hz), 7.19 (d, 1 H, J = 9 Hz), 7.35 (s, 6 H). Anal. ($C_{30}H_{39}N_7O_9S_2$) C, H, N, S.

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, *N*-[(*tert*-butyloxy)carbonyl]isoleucyloxymethyl ester (4d), was prepared as described for **4a**. Crystallization from ethanol yielded **4d** (2.02 g, 9%) as a white crystalline solid: mp 118–120 °C dec; UV λ_{max} (MeOH) 270 nm (ϵ_m 7776); NMR ($CDCl_3$) δ 0.87 (m, 6 H), 1.38 (m, 11 H), 1.87 (m, 1 H), 3.72 (s, 2 H), 3.80 (s, 1 H), 3.90 (s, 3 H), 4.28 (d, 2 H, J = 14 Hz), 4.46 (d, 1 H, J = 14 Hz), 4.91 (d, 1 H, J = 5 Hz), 5.16 (br s, 1 H), 5.75 (dd, 1 H, J = 5 and 9 Hz), 5.88 (d, 1 H, J = 6 Hz), 5.92 (d, 1 H, J = 6 Hz), 7.15 (d, 1 H, J = 9 Hz), 7.35 (s, 6 H). Anal. ($C_{30}H_{39}N_7O_9S_2$) C, H, N, S.

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, *N*-[(*tert*-butyloxy)carbonyl]phenylalanyloxymethyl ester (4e), was prepared by the procedure detailed for **4a** to yield 1.6 g (33%) after crystallization from MeOH: mp 115–118 °C dec; UV λ_{max} (MeOH) 274 nm (ϵ_m 8979); NMR ($CDCl_3$) δ 1.28 (s, 9 H), 3.1 (m, 2 H), 3.2 (br s, 1 H), 3.67 (s, 2 H), 3.82 (s, 3 H), 4.1 (d, 1 H, J = 14 Hz), 4.4 (m, 2 H), 4.95 (d, 1 H, J = 5 Hz), 5.08 (d, 1 H, J = 4 Hz), 5.85 (m, 3 H), 6.45 (d, 1 H, J = 7.5 Hz), 7.2 (m, 10 H), 8.3 (d, 1 H, J = 9 Hz). Anal. ($C_{33}H_{37}N_7O_9S_2$) C, H, N, S.

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, *N*-[(*tert*-butyloxy)carbonyl]-α-methylaspartoyloxymethyl ester (4f), was prepared in a manner similar to that described for **4a**. The crude material was chromatographed over dry-column silica gel. Elution with 1:1 EtOAc/cyclohexane and subsequent crystallization from 2-propanol yielded 1.49 g (16%) of **4f**: mp 75–77 °C dec; UV (MeOH) λ_{max} 272 nm (ϵ_m 7890); NMR ($CDCl_3$) δ 1.38 (s, 9 H), 1.81 (br s, 1 H), 2.97 (m, 2 H), 3.68 (s, 5 H), 3.90 (s, 3 H), 4.22 (d, 1 H, J = 14 Hz), 4.51 (m, 2 H), 4.95 (d, 1 H, J = 5.5 Hz), 5.18 (d, 1 H, J = 3 Hz), 5.52 (d, 1 H, J = 8 Hz), 5.75 (m, 4 H), 7.35 (s, 6 H). Anal. ($C_{29}H_{35}N_7O_{11}S_2$) C, H, N, S.

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, Glycyloxymethyl Ester, Hydrochloride Salt (2a). A mixture of **4a** (3.0 g, 4.62 mmol) was mixed with anisole (30 drops) to form a paste, and 30 mL of 0.6 N dioxane/hydrogen chloride was added. Stirring was continued for 25 min. A precipitate formed which was allowed to settle, and the solvent was decanted. The crude product was dissolved in MeOH and precipitated by the addition of Et_2O to yield 1.5 g (56%) of **2a** as a white amorphous solid: NMR (Me_2SO-d_6) δ 3.75 (m, 2 H), 3.82 (m, 2 H), 3.93 (s, 3 H), 4.21 (d, 1 H, J = 14 Hz), 4.47 (d, 1 H, J = 14 Hz), 4.98 (s, 1 H), 5.08 (d, 1 H, J = 5 Hz), 5.1 (s, 1 H), 5.71 (dd, 1 H, J = 5 and 8.5 Hz), 5.93 (s, 2 H), 7.35 (m, 6 H), 8.7 (br s, 3 H).

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, Valyloxymethyl Ester, Hydrochloride Salt (2b). A trifluoroacetic acid solution (10 mL) of **4b** (0.370 g, 0.53 mmol) was stirred at 0 °C for 15 min. The excess trifluoroacetic acid was removed in vacuo, and the residual oil was dissolved in EtOAc and washed twice successively with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. The EtOAc was filtered through anhydrous $MgSO_4$. The filtrate was treated with Et_2O, saturated with anhydrous HCl, and concentrated in vacuo to yield **2b** as a white amorphous solid: yield 0.126 g (38%); NMR (Me_2SO-d_6) δ 0.96 (d, 3 H, J = 8 Hz), 0.98 (d, 3 H, J = 8 Hz), 2.25 (m, 1 H), 3.36 (s, 1 H), 3.74 (br s, 2 H), 3.94 (s, 3 H), 4.23 (d, 2 H, J = 14 Hz), 4.46 (d, 1 H, J = 14 Hz), 5.12 (m, 2 H), 5.72 (dd, 1 H, J = 5, 9 Hz), 5.88 (d, 1 H, J = 8 Hz), 6.07 (d, 1 H, J = 8 Hz), 7.35 (m, 5 H), 8.75 (br s, 3 H).

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, leucyloxymethyl ester, hydrochloride salt (2c), was prepared in a manner similar to that described above for **2b**, yielding 0.49 g (73%) of a white amorphous solid: UV λ_{max} (EtOH) 274 nm (ϵ_m 9226); NMR (Me_2SO-d_6) δ 0.88 (d, 6 H, J = 5.5 Hz), 2.80 (m, 3 H), 3.37 (br s, 1 H), 3.75 (s, 2 H), 3.93 (s, 4 H), 4.22 (d, 1 H, J = 13.5 Hz), 4.46 (d, 1 H, J = 13.5 Hz), 5.08 (d, 1 H, J = 5.5 Hz), 5.1 (s, 1 H), 5.73 (dd, 1 H, J = 5.5 and 8.5 Hz), 5.87 (d, 1 H, J = 6 Hz), 6.05 (d, 1 H, J = 6 Hz), 7.38 (m, 5 H), 8.75 (br s, 5 H). Anal. ($C_{25}H_{32}N_7O_7S_2Cl$) C, H, N, S.

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, isoleucyloxymethyl ester, hydrochloride salt (2d), was prepared by the

procedure detailed for **2b**. The crude amorphous solid was crystallized from MeOH/Et_2O, yielding **2d** as a white crystalline solid (0.62 g, 45%): mp 102–105 °C; UV λ_{max} (MeOH) 274 nm (ϵ_m 8503); NMR δ 0.87 (t, 3 H, J = 7 Hz), 0.92 (d, 3 H, J = 7 Hz), 1.39 (q, 2 H, J = 7 Hz), 1.95 (m, 1 H), 3.35 (br s, 1 H), 3.74 (s, 2 H), 3.92 (s, 3 H), 4.0 (m, 1 H), 4.22 (d, 1 H, J = 14 Hz), 4.46 (d, 1 H, J = 14 Hz), 5.08 (d, 1 H, J = 5 Hz), 5.1 (s, 1 H), 5.7 (dd, 1 H, J = 5 and 9 Hz), 5.86 (d, 1 H, J = 6 Hz), 6.05 (d, 1 H, J = 6 Hz), 7.36 (m, 5 H), 8.75 (br s, 5 H). Anal. ($C_{25}H_{32}N_7O_7S_2Cl$) C, H, N, S.

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, phenylalanyloxymethyl ester, hydrochloride salt (2e), was prepared as described above for **2b** to yield 0.716 g (53%) of a white amorphous solid: UV λ_{max} (MeOH) 274 nm (ϵ_m 9076); NMR (Me_2SO-d_6) δ 3.32 (m, 3 H), 3.75 (s, 2 H), 3.92 (s, 3 H), 4.19 (d, 1 H, J = 13 Hz), 4.4 (m, 2 H), 5.08 (s, 1 H), 5.12 (d, 1 H, J = 5.5 Hz), 5.72 (dd, 1 H, J = 5.5 and 9 Hz), 5.87 (d, 1 H, J = 6 Hz), 5.97 (d, 1 H, J = 6 Hz), 7.37 (m, 10 H), 8.75 (m, 4 H). Anal. ($C_{28}H_{30}N_7O_7S_2Cl$) C, H, N, S.

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, α-methylaspartoyloxymethyl ester, hydrochloride salt (2f), was prepared as described for **2b**. After crystallization from MeOH/Et_2O, 0.27 g (33%) of **2f** was obtained as a white solid: mp 128–131 °C dec; UV λ_{max} (MeOH) 273 nm (ϵ_m 8777); NMR (Me_2SO-d_6) δ 3.1 (d, 2 H, J = 6 Hz), 3.37 (br s, 1 H), 3.68 (s, 5 H), 3.92 (s, 3 H), 4.19 (d, 1 H, J = 13.5 Hz), 4.32 (d, 1 H, J = 6 Hz), 4.45 (d, 1 H, J = 13.5 Hz), 5.08 (m, 2 H), 5.72 (dd, 1 H, J = 9 Hz), 5.85 (d, 1 H, J = 6 Hz), 5.92 (d, 1 H, J = 6 Hz), 7.37 (m, 5 H), 8.75 (m, 4 H). Anal. ($C_{24}H_{28}N_7O_9S_2Cl$) C, H, N, S.

Hydrolysis of 2a. An aqueous solution of **2a** (0.589 g, 1.0 mmol) was dissolved in pH 6 buffer. It was necessary to add 0.05 N NaOH to keep the pH at 6.0. The progress of the reaction was followed by TLC (*n*-BuOH/EtOH/H_2O, 8:2:2). After 2 h, the reaction was complete and the pH was raised to 8 and the aqueous mixture was washed with EtOAc. The pH was adjusted to 2 by the addition of 1 N HCl and the mixture was extracted with EtOAc. The EtOAc was dried (anhydrous $MgSO_4$) and concentrated. The residue was esterified with diazomethane. The crude product was chromatographed over dry-column silica gel (eluted with 1:1 EtOAc/cyclohexane). The product was crystallized from MeOH and was identical in all respects to the methyl ester of cefamandole (TLC, NMR, mp, and mmp).

7-(D-Mandelamido)-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-(carbomethoxymethyl)carboxamide (8b). A suspension of the sodium salt of **1** (4.84 g, 10 mmol) and glycine methyl ester hydrochloride (1.25 g, 10 mmol) in 50 mL of THF was treated dropwise with a THF solution (10 mL) of dicyclohexylcarbodiimide (2.36 g, 11 mmol). After the addition of DCC was complete, stirring was continued for 18 h at room temperature. The mixture was then filtered to remove 2.35 g of DCU. The filtrate was concentrated and the residue was dissolved in EtOAc. The EtOAc solution was washed successively with 1 N HCl, 10% aqueous $NaHCO_3$, and saturated aqueous NaCl. The EtOAc was dried ($MgSO_4$) and concentrated in vacuo. The residue was crystallized from MeOH to yield 3.5 g (66%) of **8b**: mp 177–179 °C dec; UV λ_{max} (MeOH) 267 nm (ϵ_m 9316); NMR (Me_2SO-d_6) δ 3.34 (s, 2 H), 3.65 (m, 5 H), 3.93 (s, 3 H), 4.26 (s, 2 H), 5.06 (d, 1 H, J = 5.5 Hz), 5.13 (d, 1 H, J = 5 Hz), 5.56 (dd, 1 H, J = 5.5, 9 Hz), 6.14 (d, 1 H, J = 5 Hz), 7.35 (m, 5 H), 8.76 (d, 1 H, J = 9 Hz), 8.9 (d, 1 H, J = 7 Hz). Anal. ($C_{21}H_{23}N_7O_6S_2$) C, H, N, S.

Chemical Stability of 2a–f. Each sample was dissolved in buffer (pH 2.5, 4.5, and 6.0) and stored at 37 °C. Samples were withdrawn after 0, 20, 40, and 60 min and applied to 3MM Whatman no. 1 (Type 2043A) paper strips contained in a Beckman electrophoresis cell (Durrum Type), Model R. The strips were developed at 400 V in 0.1 M pyridine–acetate buffer. After 2 h, the strips were removed and steamed lightly for 10 min to remove the pyridine. After drying, the spots were visualized by UV light. The results of these experiments are outlined in Table I.

In Vivo Studies. Groups of eight Swiss white mice (13–15 g) were injected intraperitoneally with a broth suspension of *Streptococcus pyogenes* C203 previously demonstrated to be lethal to 100% of untreated mice in 24 to 48 h. Each test consisted of five groups of infected mice to which graduated doses of a test compound were administered at 1 and 5 h after the bacterial challenge. The median effective dose (ED_{50}) was estimated by the method of Reed and Muench[13] on the basis of surviving mice at 7 days (Table II).

Cefamandole activity in mouse blood or urine was estimated using a disk-plate microbiological assay with *Micrococcus luteus* ATCC 9341 as the detecting organism. The cefamandole activity remaining in the small intestine after oral administration of **2a**, **2b**, and **2f** was determined as previously described by Wheeler et al.[14]

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