Copenhagen). ID_{50} values and relative potencies of the compounds were calculated[26,27] from the degree of maximum inhibition of total acid output. The ID_{50} is defined as the dose which caused 50% inhibition of total acid output in this series of dogs.

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Orally Active Esters of Cephalosporin Antibiotics. Synthesis and Biological Properties of Acyloxymethyl Esters of 7-(D-2-Amino-2-phenylacetamido)-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylic Acid

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The synthesis of the acetoxymethyl (AOM), pivaloyloxymethyl (POM), and phthalidyl (PHTH) esters of 7-[D-(−)-2-amino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylic acid (**1a**), a broad-spectrum semisynthetic cephalosporin antibiotic, is described. These esters were examined as potential orally active antibiotic prodrugs. The superior oral absorption of the three esters relative to the unesterified parent, **1a**, is demonstrated by differential blood levels as well as measurement of the rate at which doses of the ester leave the gastrointestinal tract and appear in the urine. A study of the decreased stability of the three esters relative to **1a** at pH 4.5, 6.5, and 7.5 is also presented.

Sodium cephalothin [sodium 7-(2-thienylacetamido)-3-acetoxymethyl-3-cephem-4-carboxylate, KEFLIN, Lilly], the first cephalosporin antibiotic available for clinical use, was marketed in the U.S. in 1964. The remarkable safety and efficacy of this parenteral antibiotic prompted a major medicinal chemistry effort to prepare analogues which were well absorbed orally in addition to having high antimicrobial potency and broad spectra. As a result, cephaloglycin [7-(D-2-amino-2-phenylacetamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid, KAFOCIN, Lilly][1] and cephalexin [7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid, cephalexin monohydrate, KEFLEX, Lilly][2] were made available as safe, effective, orally absorbed antibiotics. While cephaloglycin is more potent than cephalexin,[3] the latter is more efficiently absorbed orally.[4] Recently, however, Binderup and coworkers reported that administration of acetoxymethyl and pivaloyloxymethyl esters of cephaloglycin resulted in more efficient oral absorption of cephaloglycin in both rats and man.[5] These esters are enzymatically degraded to cephaloglycin both during the process of absorption from the gastrointestinal tract and after absorption occurs.

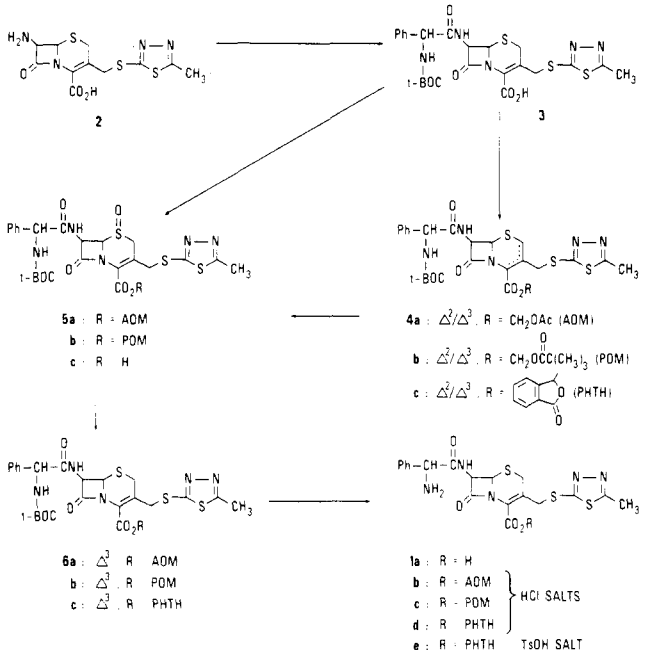
Esters of ampicillin [6-(D-2-amino-2-phenylacetamido)penicillanic acid][6] and carbenicillin [6-(2-carboxyl-2-phenylacetamido)penicillanic acid; disodium carbenicillin, Geopen, Roerig][7] are well absorbed orally giving higher blood levels than the unesterified parent compound in both laboratory animals and humans. Reports on the clinical efficacy of pivampicillin (the pivaloyloxymethyl ester of ampicillin) are numerous.[8–10] The phthalidyl ester of ampicillin is efficiently absorbed orally in both laboratory animals and man.[11,12] Bacampicillin (the ethoxycarbonyloxyethyl ester of ampicillin) is rapidly converted to ampicillin in vivo in rats, dogs, and man.[13] Oral administration of bacampicillin results in earlier and higher peak blood levels of ampicillin than when an equimolar

Table I. Biological Activity of 1a[a]

	Gram-positive[b]		Gram-negative[b]				
	Resistant *Staph. aureus*[c]	*Shigella* sp.	*E. coli*	*Klebsiella pneumoniae*	*Enterobacter aerogenes*	*Salmonella heidelberg*	*Serratia marcescens*
1a	0.7	1.0	1.0	0.6	0.8	1.0	26.5
Cephalexin[d]	4.5	9.0	8.5	7.0	6.8	5.5	140

[a] The MIC values for cephalexin are included for reference. [b] MIC in μg/mL by gradient-plate assay. [c] MIC in μg/mL by gradient-plate assay. The figure is an average value obtained using three penicillin-resistant, coagulase-positive *S. aureus* strains. [d] Reference 3.

Scheme I



dose of ampicillin itself is given orally. The indanyl ester of carbenicillin has recently been marketed in the U.S. for oral usage.[14]

The in vitro microbiological activity for 7-(D-2-amino-2-phenylacetamido)-3-[5-methyl-(1,3,4-thiadiazol-2-yl)-thiomethyl]-3-cephem-4-carboxylic acid (**1a**), a broad-spectrum semisynthetic cephalosporin,[15] is summarized in Table I. Examination of the blood and urine levels in mice following oral administration indicated that **1a** was inefficiently absorbed from the gastrointestinal tract. Accordingly we wish to report the preparation and biological evaluation for a series of esters of **1a**.

Chemistry. The key intermediate for the preparation of the esters of **1a** was the corresponding *tert*-butyloxycarbonyl-protected (*t*-BOC) derivative **3** obtained by acylation of the nucleus **2**[15] with *tert*-butyloxycarbonylphenylglycine (Scheme I). Esterification of salts of **3** with the various activated alkyl halides yielded mixtures of the corresponding Δ^2- and Δ^3-esters (the Δ^2 esters result from the base-catalyzed isomerization of the Δ^3-esters.[16] Bentley et al. have recently suggested that the unreacted carboxylate anion serves as a base to promote isomerization[17]). Although the esters **4a,b** appeared to be pure by TLC, the presence of the Δ^2-esters was obvious from the NMR spectra of **4a–c**. A significant peak at δ 6.37 ppm due to the C-2 vinyl proton of the Δ^2-ester is present with a concomitant reduction in the δ 3.6 ppm peak (C-2 methylene). Esterification of **3** with α-bromophthalide leads to a diastereomeric mixture of Δ^3-**4c** as well as the corresponding epimers of Δ^2-**4c**. The separation of the diastereomers was easily followed by TLC and by the different chemical shifts of their phthalidylmethine protons (δ 7.42 and 7.54 ppm, respectively). While diastereomerically pure **6c**[18] could be successfully separated from the mixture by fractional crystallization from MeCN, all attempts to crystallize **6a,b** from the Δ^2/Δ^3 mixture of **4a,b** failed. The epimer of **6c** with the methine resonance at δ 7.54 ppm was obtained. Concentration of the mother liquors yielded the other epimer, although impure, which resisted crystallization. No attempts to further purify this epimer were made.

Table II. Nonenzymatic Decomposition of Esters and Parent

Compd[a]	$t_{1/2}$, min		
	pH 4.5	pH 6.5	pH 7.5
1a	2820	76	54
1b	1620	60	25
1c	1920	45	25
1d		25	15

[a] Substrate concentration 50 μg/mL in 0.1 M sodium phosphate buffer at 37.5 °C.

Following the procedure of Kaiser et al.,[19] the Δ^2/Δ^3-esters **4a,b** were oxidized to the corresponding Δ^3-sulfoxide esters **5a,b** and subsequently reduced with PCl_3–DMF in $CHCl_3$ at 40 °C. After chromatography of the crude reaction mixture, **6a,b** were crystallized from Et_2O. Alternatively, sulfoxide ester **5a** could be obtained in higher yield by esterification of the sulfoxide acid **5c**.

Removal of the *t*-BOC-protecting group could then be effected by treatment of **6a–c** with either *p*-TsOH–MeCN[20] or CF_3CO_2H (TFA);[21] however, a pure crystalline tosylate salt was obtained only in the case of **1e**. The HCl salts **1b,c,d** were obtained by treatment of the free amino esters with HCl–Et_2O. All attempts to obtain an HCl salt directly from **6a–c** by treatment with anhydrous HCl in various solvents were unsuccessful. Amorphous salts were obtained for **1c,d**; however, **1b** was crystallized from MeOH–Et_2O.

Biological Results. The effect of esterification on the oral absorption of **1a** in mice was examined by comparing the esters of **1a** with respect to disappearance from the gastrointestinal (GI) tract, appearance in the urine, and blood level. Groups of mice were treated with solutions of the esters or parent compound, and, at intervals, the bioactivity was determined in blood, urine, and the GI tract.

Chemical Stability of the Esters. Esterification decreases the chemical stability of **1a** as measured by the loss of bioactivity at physiological temperature and pH. At pH 4.5 and 37.5 °C there is little loss in several hours; however, at higher pH, the rate of loss of both parent and ester increases sharply (Table II). It is assumed that the loss is the result of intramolecular attack on the β-lactam ring by the un-ionized amino group in the side chain.[22]

Disappearance from the Gastrointestinal Tract. The results in Table III show that after oral administration, the bioactivity of all three esters disappeared from the GI tract at a faster rate than the unesterified parent

Table III. Disappearance of Orally Dosed Esters and Parent Compound from the GI Tract

Compd	% of oral dose remaining in GI tract after[a]						
	5 min	20 min	40 min	60 min	80 min	100 min	120 min
1a	98.9 ± 3.0	81.2 ± 6.1	67.5 ± 3.1	60.4 ± 2.5	47.6 ± 4.5	36.0 ± 2.0	33.5 ± 7.4
1b	65.2 ± 7.4	39.1 ± 4.1	31.5 ± 3.0	27.9 ± 1.3	18.2 ± 2.2	13.2 ± 3.7	9.2 ± 3.3
1c	43.2 ± 7.2	40.5 ± 4.9	25.0 ± 1.5	20.3 ± 2.2	15.1 ± 5.3	15.8 ± 3.3	12.3 ± 5.0
1d[b]	70.4	59.0	35.0	25.0	24.0		

[a] Mean of four or five mice ± SEM; dose, 17 mg/kg. [b] Compound 1d supply limited. Average of only two mice at each time point.

Table IV. Urinary Excretion of Drug after Dosage to Mice

Compd	Dose route	Cumulative % of dose found in the urine after[a]						
		5 min	20 min	40 min	60 min	80 min	100 min	120 min
1a	sc	2.8 ± 0.4	25.0 ± 2.8	46.5 ± 5.4	50.7 ± 1.2	56.7 ± 4.2	54.1 ± 3.9	54.1 ± 4.6
1a	Oral	n.d.[b]	2.8 ± 0.9	8.4 ± 2.4	13.2 ± 0.9	17.5 ± 2.4	22.4 ± 6.2	14.1 ± 2.1
1b	Oral	Trace	20.4 ± 2.3	32.5 ± 4.8	33.1 ± 4.6	36.1 ± 3.4	38.3 ± 3.8	36.8 ± 4.1
1c	Oral	3.6 ± 1.1	20.8 ± 5.2	38.9 ± 3.2	43.2 ± 4.3	48.2 ± 4.7	41.8 ± 2.1	41.5 ± 6.3
1d[c]	Oral	n.d.	8.3	22.1	35.0	30.5	34.0	

[a] Mean of four or five mice ± SEM; dose, 17 mg/kg. [b] None detected. [c] Compound 1d supply was limited. These data represent the average of only two mice at each time point.

Table V. Relative Efficiency of Ester Oral Absorption Based on Urinary Recovery

Compd	Dose route	Urinary excretion of bioactivity after oral ester expressed as % of urinary excretion of bioactivity after sc dose of parent compound at[a]			
		60 min	80 min	100 min	120 min
1a	sc	100	100	100	100
1a	Oral	26	30.8	41.4[b]	26.1
1b	Oral	65.2	63.6	70.7	68.0
1c	Oral	85.2	85.0	77.3	76.7

[a] Average of four or five mice; dose, 17 mg/kg. [b] One value out of five appeared abnormally high.

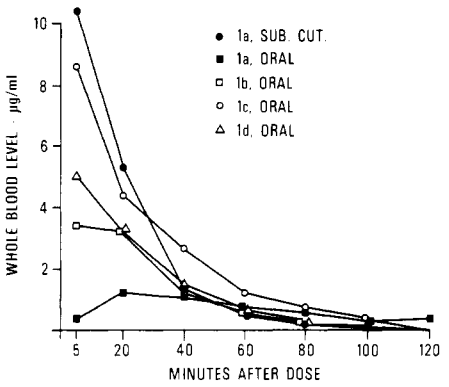


Figure 1. Blood levels of drug after dosage to mice. Comparison of oral doses of three esters of **1a**, the acetoxymethyl ester **1b**, the pivaloyloxymethyl ester **1c**, and the phthalidyl ester **1d**, with **1a** dosed either orally or subcutaneously. All doses are at 17 mg/kg and each point represents the mean value from four or five mice, except for **1d** where only two mice represent each time point due to limited supply of this compound.

drug **1a**. The blood and urine levels confirm that the disappearance is mainly due to absorption and not to decomposition.

Appearance of Bioactivity in the Urine. The results in Table IV show that after oral dosage of each of the three esters, unesterified antibiotic **1a** appears in the urine at a faster rate and to a higher cumulative level than after a comparable oral dose of the parent drug itself. Chromatography of the urine revealed only **1a** and no surviving intact ester. These data complement the disappearance data in Table III and show that a true increase in oral absorption results from esterification. An estimate of the efficiency of the oral absorption of the esters and parent drug relative to a parenteral dose of the parent can be made. Table V shows the urinary excretion of **1a** after oral doses of two of the esters (**1b,c**) expressed as a percent of urinary excretion of **1a** after a subcutaneous dose of the parent. Both esters show substantial improvement in oral absorption over the parent (**1a**).

Blood Levels. Mouse blood levels after dosage with esters or parent drug are shown in Figure 1. These results confirm the oral absorption advantages of the esters as shown previously by urinary and GI tract levels. The more prolonged blood levels seen with oral **1a** are probably the result of slower but extended absorption as contrasted with fast absorption and excretion of the esters.

Discussion and Conclusions

The superior oral absorption of the three cephalosporin esters relative to the unesterified parent compound is demonstrated by the direct measurement of the disappearance of the doses from the gastrointestinal tract, the concurrent appearance in the urine, and the increased blood levels. These results suggest that **1c**, the pivaloyloxymethyl ester, may exhibit better oral absorption than **1b**, the acetoxymethyl, or **1d**, the phthalidyl ester. The reduced chemical stability of the esters when compared with the parent in solution at neutral or alkaline pH is not a desirable property. Although the degree cannot be assessed from these data, such instability must reduce the oral efficacy of the esters by the degree to which decomposition occurs in the lumen of the intestine prior to passage through the intestinal wall. These doses were administered in unbuffered solution. The extent of decomposition in the human intestinal tract when administered as a dry dosage form cannot be inferred from these data.

Experimental Section

Chemistry. 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.

7-[D(−)-2-*tert*-Butyloxycarbonylamino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-

cephem-4-carboxylic Acid (3). A THF solution (400 mL) of monotrimethylsilylacetamide (MSA) (90 g, 0.68 mol) was stirred at room temperature and **2**[14] (78 g, 0.172 mol) was added. Stirring was continued until all of **2** dissolved (gentle warming is sometimes necessary).

A THF solution (600 mL) of isobutyl chloroformate (22.6 mL, 0.172 mol) was cooled to −10 °C and a mixture of D(−)-*tert*-butyloxycarbonylphenylglycine (43.2 g, 0.172 mol) and triethylamine (24 mL, 0.172 mol) in THF (200 mL) containing 20 drops of *N,N*-dimethylbenzylamine was added dropwise. Stirring at −10 to −20 °C was continued for 1 h after the addition was complete. The trimethylsilyated nucleus was added to the mixed anhydride all at once and stirring was continued at −10 to −20 °C for 2 h and then overnight at room temperature.

The mixture was filtered and the filtrate concentrated in vacuo. The residue was dissolved in MeOH to hydrolyze the trimethylsilyl ester and again concentrated in vacuo. The residue was dissolved in EtOAc and washed successively with 1 N HCl (twice) and saturated aqueous NaCl solution (twice). The EtOAc solution was concentrated in vacuo whereupon the material crystallized (41 g). Further concentration yielded additional material (15 g). This material was sufficiently pure by TLC [silica gel PF_{254}, Et_2O–HOAc–H_2O (15:3:1)] and NMR for subsequent work; however, the material could be further purified by recrystallization from EtOAc: mp 135–137 °C dec; UV max (EtOH) 271 nm (ϵ_M 14 815); NMR ($CDCl_3$) δ 1.25 (t, 3 H), 1.4 (s, 9 H), 2 (s, 3 H), 2.7 (s, 3 H), 3.61 (s, 2 H), 4.12 (q, 2 H), 4.25 (d, 1 H, J = 13.5 Hz), 4.55 (d, 1 H, J = 13.5 Hz), 4.93 (d, 1 H, J = 5 Hz), 5.45 (d, 1 H, J = 8 Hz), 5.80 (dd, 1 H, J = 5, 8 Hz), 6.35 (d, 1 H, J = 8 Hz), 7.4 (m, 5 H), 9.05 (d, 1 H, J = 8 Hz), 9.8 (br s, 1 H). Anal. ($C_{24}H_{27}N_5O_6S_3$) C, H, N, S.

7-[D(−)-2-*tert*-Butyloxycarbonylamino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylic Acid 1-Oxide (5c). A chloroform solution (200 mL) of **3** (10.0 g, 17.4 mmol) was treated dropwise with a $CHCl_3$ solution (50 mL) of *m*-chloroperbenzoic acid (*m*-CPBA) (3.4 g, 17.4 mmol) at 0 °C. After the addition was complete, stirring was continued for an additional 2 h. The resulting suspension was filtered and the solid was washed with Et_2O to remove any residual *m*-chlorobenzoic acid. The resulting solid (10.2 g) was recrystallized from acetone to yield **5c** as a white crystalline solid (7.3 g, 70%): mp 167–169 °C; UV max (EtOH) 271 nm (ϵ_M 15 225). Anal. ($C_{24}H_{27}N_5O_7S_3$) C, H, N.

Acetoxymethyl 7-[D(−)-2-*tert*-Butyloxycarbonylamino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylate 1-Oxide (5a). Method A. A mixture of **3** (1.5 g, 2.6 mmol), acetoxymethyl bromide (0.26 mL, 2.6 mmol), and diisopropylethylamine (DIPEA) (0.335 g, 2.6 mmol) in 50:50 MeCN–acetone (50 mL) was stirred for 16 h at room temperature. The mixture was concentrated in vacuo and the residue was redissolved in EtOAc. The HBr salt of DIPEA crystallized and the mixture was filtered. The filtrate was washed successively (twice) with H_2O, 1 N HCl, 10% aqueous $NaHCO_3$, and saturated aqueous NaCl, then dried (anhydrous Na_2SO_4), and concentrated in vacuo. An amorphous solid (1.5 g) was obtained from EtOAc–hexane. TLC (EtOAc) indicated only minor contaminants; however, the NMR indicated the presence of a significant amount of the Δ^2-ester.

A chloroform (200 mL) solution of the Δ^2/Δ^3 mixture of **4a** (4.6 g, 7.1 mmol) was oxidized by the dropwise addition of *m*-CPBA (1.4 g, 7.1 mmol) in 25 mL of $CHCl_3$ at 0 °C. After the addition was complete ($\simeq$1 h) the solution was diluted with an additional 100 mL of $CHCl_3$ and washed successively with 10% aqueous $NaHCO_3$ and saturated aqueous NaCl solution. The solution was dried (anhydrous Na_2SO_4) and concentrated in vacuo to a gum. The gum was redissolved in a small volume of $CHCl_3$ and diluted with aqueous MeOH. Upon reconcentration the sulfoxide ester **5a** crystallized (3.4 g). This material was impure by TLC (EtOAc) and was subsequently recrystallized twice from absolute MeOH to yield **5a** (1.73 g, 42%). This material was identical with that prepared by method B.

Method B. Acetoxymethyl bromide (1 mL, 10 mmol) was added to a stirred acetone (150 mL) suspension of **5c** (5.93 g, 10 mmol). An acetone solution (30 mL) of DIPEA was added dropwise. When dissolution of **5c** was complete, stirring was continued for 3 h. The mixture was filtered and the acetone was removed in vacuo. The residue was dissolved in EtOAc and washed with water (twice), followed by saturated aqueous $NaHCO_3$ and finally saturated aqueous brine. The EtOAc solution was dried (Na_2SO_4) and concentrated in vacuo to yield 5 g of the crude ester **5a**. Recrystallization from MeOH afforded 3.49 g (53%) of **5a**: mp 125–128 °C dec; UV max (EtOH) 275 nm (ϵ_M 12 645); NMR ($CDCl_3$) δ 1.39 (s, 9 H), 2.12 (s, 3 H), 2.70 (s, 3 H), 3.45 (d, 1 H, J = 18 Hz), 3.98 (d, 1 H, J = 14 Hz), 4.0 (d, 1 H, J = 18 Hz), 4.44 (d, 1 H, J = 5 Hz), 4.86 (d, 1 H, J = 14 Hz), 5.15 (d, 1 H, J = 6 Hz), 5.56 (d, 1 H, J = 6 Hz), 5.98 (m, 3 H), 7.35 (s, 6 H). Anal. ($C_{27}H_{31}N_5O_9S_3$) H, N; C: calcd, 48.71; found, 47.80.

Pivaloyloxymethyl 7-[D(−)-2-*tert*-Butyloxycarbonylamino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylate 1-Oxide (5b). Bromomethyl pivalate was prepared in situ by the reaction of sodium bromide (1.9 g, 18.5 mmol) with chloromethyl pivalate (2.6 mL, 18.5 mmol) in DMF (10 mL). After the precipitation of NaCl was complete the mixture was filtered and the filtrate was added to a mixture of **3** (5.33 g, 9.24 mmol) and dicyclohexylamine (1.8 mL, 9.24 mmol) in 140 mL of DMF. The resulting mixture was stirred for 4 h and filtered. The filtrate was poured into 750 mL of 2:1 hexane–Et_2O. The solvent was decanted and the residual oil was triturated with 750 mL of fresh solvent. After decantation the residue was partially dissolved in EtOAc and filtered. Dilution of the filtrate with hexane caused precipitation of **4b** as an amorphous solid (5.3 g). NMR and TLC (silica gel, EtOAc) indicated approximately a 50:50 mixture of Δ^2- and Δ^3-esters. A chloroform solution of **4b** (1.35 g, 1.95 mmol) was dissolved in chloroform (50 mL) and chilled to 0 °C during the dropwise addition of *m*-CPBA (0.394 g, 1.95 mmol). The mixture was stirred at 0 °C for an additional 0.5 h, then diluted with an additional 150 mL of $CHCl_3$, and washed twice successively with 5% aqueous $NaHCO_3$ solution and saturated brine. After drying (Na_2SO_4), the solvent was removed in vacuo and the residue was crystallized from MeOH to yield 0.89 g (64%) of **5b**: mp 167–169 °C; UV max (EtOH) 275 nm (ϵ_M 11 993); NMR (Me_2SO-d_6) δ 1.23 (s, 9 H), 1.40 (s, 9 H), 2.70 (s, 3 H), 3.45 (d, 1 H, J = 18 Hz), 4.04 (d, 1 H, J = 18 Hz), 4.06 (d, 1 H, J = 14 Hz), 4.42 (d, 1 H, J = 5 Hz), 4.82 (d, 1 H, J = 14 Hz), 5.18 (d, 1 H, J = 6 Hz), 5.68 (d, 1 H, J = 6 Hz), 5.87–6.1 (m, 3 H), and 7.35 (m, 6 H). Anal. ($C_{30}H_{37}N_5O_9S_3$) C, H, N.

Acetoxymethyl 7-[D(−)-2-*tert*-Butyloxycarbonylamino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)-thiomethyl]-3-cephem-4-carboxylate (6a). Phosphorus trichloride (1.78 mL, 21 mmol) was added to a mixture of **5a** (3.49 g, 5.25 mmol) and DMF (10 mL) in CH_2Cl_2 (250 mL) at −40 °C. The reaction was followed by TLC (EtOAc) and after 1.5 h the reaction was complete. The mixture was allowed to warm to 0 °C and was concentrated in vacuo. The residual oil was dissolved in $CHCl_3$ and washed twice with saturated aqueous $NaHCO_3$. The $CHCl_3$ was dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed over dry-column silica gel (Waters Associates). The product was eluted with 1:1 EtOAc–cyclohexane. Cuts of 50 mL were taken. Fractions 8–26 contained the product; these were combined and concentrated. The residue was crystallized from Et_2O to yield 1.22 g (36%) of **6a**: mp 108–110.5 °C dec; UV max (EtOH) 270 nm (ϵ_M 13 775); NMR ($CDCl_3$) δ 1.4 (s, 9 H), 2.13 (s, 3 H), 2.71 (s, 3 H), 3.60 (br s, 2 H), 4.1 (d, 1 H, J = 13.5 Hz), 4.68 (d, 1 H, J = 13.5 Hz), 4.87 (d, 1 H, J = 5 Hz), 5.25 (d, 1 H, J = 6 Hz), 5.65–6.0 (m, 4 H), 6.75 (d, 1 H, J = 9 Hz), 7.35 (s, 5 H). Anal. ($C_{27}H_{31}N_5O_8S_3$) C, H, N.

Pivaloyloxymethyl 7-[D(−)-2-*tert*-Butyloxycarbonylamino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylate (6b). Phosphorus trichloride (0.274 g, 2.0 mmol) was added to a methylene chloride solution (100 mL) of **5b** (0.353 g, 0.5 mmol) and the mixture was stirred at reflux for 16 h. The mixture was then allowed to cool to room temperature and poured into saturated aqueous $NaHCO_3$. The layers were separated and the methylene chloride layer was washed twice with saturated aqueous sodium chloride solution. The methylene chloride layer was dried (anhydrous $MgSO_4$) and concentrated in vacuo. The residue was chromatographed over dry-column silica gel. The desired product was eluted with 1:1 EtOAc–cyclohexane. The product was crystallized from Et_2O (0.103 g, 30%): mp 163–165 °C dec; UV max (EtOH) 272 nm (ϵ_M 13 791); NMR ($CDCl_3$) δ 1.23 (s, 9 H), 1.40 (s, 9 H), 2.46 (s, 3 H),

3.64 (s, 2 H), 4.15 (d, 1 H, J = 14 Hz), 4.64 (d, 1 H, J = 14 Hz), 4.87 (d, 1 H, J = 5 Hz), 5.23 (d, 1 H, J = 6.5 Hz), 5.70 (dd, 1 H, J = 5, 9 Hz), 5.85 (d, 1 H, J = 5.5 Hz), 5.95 (d, 1 H, J = 5.5 Hz), 6.67 (d, 1 H, J = 9 Hz), and 7.35 (s, 6 H). Anal. ($C_{30}H_{37}N_5O_8S_3$) C, H, N.

Phthalidyl 7-[D(-)-2-*tert*-Butyloxycarbonylamino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylate (6c). The dicyclohexylamine (DCHA) salt of **3** (7.0 g, 9.24 mmol) (prepared by reaction of equimolar amounts of **3** and dicyclohexylamine in EtOAc) was suspended in DMF (150 mL) and α-bromophthalide (2.16 g, 10.16 mmol) was added. The mixture was stirred for 16 h at room temperature and then filtered to remove 1.35 g of DCHA·HBr (56%). The filtrate was poured into 750 mL of 2:1 hexane–Et_2O. The solvent was decanted and the residual oil was triturated with an additional 750 mL of 2:1 hexane–Et_2O. The solvent was decanted and the remaining oil was triturated with EtOAc and filtered. The filtrate was concentrated in vacuo and the residue was crystallized twice from MeCN to yield 0.385 g (5.2%) of **6c** as a white crystalline solid: mp 192–195 °C dec; UV max (EtOH) 228 and 273 nm (ϵ_M 18924 and 13834, respectively); NMR ($CDCl_3$) δ 1.39 (s, 9 H), 2.72 (s, 3 H), 3.68 (br s, 2 H), 4.19 (d, 1 H, J = 13.5 Hz), 4.63 (d, 1 H, J = 13.5 Hz), 4.85 (d, 1 H, J = 5 Hz), 5.26 (d, 1 H, J = 7 Hz), 5.6–5.9 (m, 2 H), 6.97 (d, 1 H, J = 10 Hz), 7.34 (s, 5 H), 7.42 (s, 1 H), and 7.55–7.97 (m, 4 H). Anal. ($C_{32}H_{31}N_5O_8S_3$) C, H, N.

Acetoxymethyl 7-[D(-)-2-Amino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylate Hydrochloride Salt (1b). A trifluoroacetic acid (50 mL) solution of **6a** (1.17 g, 1.8 mmol) was allowed to stand at 0 °C for 10 min. The TFA was removed in vacuo and the residual oil was triturated with Et_2O. The Et_2O was decanted and the white residue was dissolved in EtOAc and washed twice with 5% aqueous sodium bicarbonate solution. The EtOAc was dried (anhydrous $MgSO_4$) and concentrated in vacuo. The residual oil was dissolved in THF and treated with HCl–Et_2O and again concentrated to yield **1b** as a white amorphous powder which was crystallized from MeOH–Et_2O (0.35 g, 33%): mp 122.5–125 °C dec; UV max (EtOH) 270 nm (ϵ_M 12810); NMR (Me_2SO-d_6) δ 2.07 (s, 3 H), 2.67 (s, 3 H), 3.49 (d, 1 H, J = 18.5 Hz), 3.74 (d, 1 H, J = 18.5 Hz), 4.13 (d, 1 H, J = 13.5 Hz), 4.55 (d, 1 H, J = 13.5 Hz), 5.06 (d, 2 H, J = 6 Hz), 5.82 (m, 3 H), 7.5 (s, 5 H), 9.57 (d, 1 H, J = 8.5 Hz), and 9.0 (s, 3 H). Anal. ($C_{22}H_{24}ClN_5O_6S_3$) C, H, N.

Pivaloyloxymethyl 7-[D(-)-2-Amino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylate Hydrochloride Salt (1c). A trifluoroacetic acid (50 mL) solution of **6b** (1.03 g, 1.49 mmol) was cooled to 0 °C for 10 min and worked up in the same manner described above for **1b**. Evaporation of the THF–Et_2O solution yielded **1c** as a white amorphous solid (0.45 g, 48%): UV max 270 nm (ϵ_M 13200); NMR (Me_2SO-d_6) δ 1.15 (s, 9 H), 2.67 (s, 3 H), 3.49 (d, 1 H, J = 18.5 Hz), 3.74 (d, 1 H, J = 18.5 Hz), 4.13 (d, 1 H, J = 13.5 Hz), 4.55 (d, 1 H, J = 13.5 Hz), 5.06 (d, 2 H, J = 6 Hz), 5.82 (m, 3 H), 7.5 (s, 5 H), 9.57 (d, 1 H, J = 8.5 Hz), and 9.0 (s, 3 H). Anal. ($C_{25}H_{30}ClN_5O_6S_3$) C, H, N.

Phthalidyl 7-[D(-)-2-Amino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylate Tosylate Salt (1e). A mixture of **6c** (0.325 g, 0.458 mmol) and *p*-toluenesulfonic acid monohydrate (0.087 g, 0.458 mmol) in MeCN (75 mL) was allowed to stand at room temperature for 48 h during which time white needles deposited. These needles were collected by filtration to yield 0.195 g (55%) of **1e**: mp 170–175 °C dec; NMR (Me_2SO-d_6) δ 2.58 (s, 3 H), 2.66 (s, 3 H), 3.50 (d, 1 H, J = 18 Hz), 3.80 (d, 1 H, J = 18 Hz), 4.27 (d, 1 H, J = 14 Hz), 4.54 (d, 1 H, J = 14 Hz), 5.07 (m, 2 H), 5.85 (m, 2 H), 7.1 (d, 2 H, J = 8 Hz), 7.3–8.05 (m, 11 H), 8.7 (br s, 3 H), and 9.58 (d, 1 H, J = 9 Hz). Anal. ($C_{34}H_{31}N_5O_9S_4$) C, H, N.

Phthalidyl 7-[D(-)-2-Amino-2-phenylacetamido]-3-[5-methyl-(1,3,4-thiadiazol-2-yl)thiomethyl]-3-cephem-4-carboxylate Hydrochloride Salt (1d). A suspension of **1e** (0.321 g, 0.41 mmol) in water (50 mL) was layered with EtOAc (50 mL) and chilled to 0 °C with an ice bath. The pH of the aqueous phase was adjusted to 6.8 by the dropwise addition of 10% aqueous $NaHCO_3$. The EtOAc layer was separated, dried (Na_2SO_4), and concentrated in vacuo (temperature <25 °C). The amino ester was dissolved in EtOAc and Et_2O–HCl was added. Amorphous **1d** (0.146 g, 55%) was collected by filtration: UV max (EtOH) 228 and 270 nm (ϵ_M 17624 and 12588, respectively); NMR (Me_2SO-d_6) δ 2.67 (s, 3 H), 3.49 (d, 1 H, J = 18.5 Hz), 3.74 (d, 1 H, J = 18.5 Hz), 4.13 (d, 1 H, J = 13.5 Hz), 4.55 (d, 1 H, J = 13.5 Hz), 5.06 (d, 2 H, J = 6 Hz), 5.82 (m, 3 H), 7.3–8.0 (m, 10 H), 9.57 (d, 1 H, J = 8.5 Hz), and 9.0 ppm (s, 3 H).

Absorption Studies in Mice. Cox Standard male mice [Lai:COX (Standard) BR] were allowed water ad libitum and a liquid diet for 18 h before use. The purpose of the liquid diet was to provide nutritional intake and to prevent coprophagy. The result was a GI tract free of solids, yet without the nutritional shock that accompanies overnight starvation. The diet consisted of 40 g of glucose, 5 g of casein hydrolysate, 2 drops of Vi-Mix, Lilly (a pediatric vitamin preparation), and 100 mL of water. Groups of mice prepared in this way were dosed with 2.0 mg/mL solutions of the parent compound or esters at 17 mg/kg either orally or subcutaneously. After dosing, the mice were housed in individual ventilated wide mouth glass jars (1-lb ointment jars) fitted with a wire screen floor elevated 0.5 in. above the bottom of the jar. Beneath the screen was a layer of 0.1 M, pH 6.0, sodium phosphate buffer into which urine could drop. At intervals after dosing, mice were removed from the jars, anesthetized with ether, and the abdomen and chest cavity opened. Whole blood was aspirated from the heart in heparinized syringes and frozen for later dilution and assay. The urinary bladder was removed and its contents were added to the urine which had been voided into the buffer in the bottom of the housing jar. The stomach and small intestine were removed separately, cut open to empty the contents, and each organ and its contents placed in measured amounts of saline for later assay. In this way, the fraction of the dose of drug present in the stomach, small intestine, and urine at various time intervals after dosage could be determined as well as the concurrent blood level.

Bioassay. Bioassays were by the conventional disk plate technique using *Micrococcus luteus* ATCC 9341 as the test organism. Standard curves of the parent compound were prepared in 0.9% NaCl directly from dilutions of an aliquot of the dose solution itself. The esters required hydrolysis prior to bioassay since the intact ester is inactive. Standard curve dilutions of the ester dose were treated for 15 min at room temperature with 1 mg/mL of a preparation of freeze-dried intestinal villi.[23] These conditions were found to result in complete hydrolysis of these esters to the bioactive parent compound. GI tract and urine samples from the animals were treated with villi prior to assay in the same way as standard curve samples. It was later found that the treatment of urine samples was unnecessary since no intact ester survived the passage through the animal. Blood samples were not treated with villi. The treatment of whole blood samples by freezing, thawing, and dilution with water resulted in lysis of the red cells.

Decomposition Studies. The nonenzymatic decomposition studies on esters and parent compound were carried out at 50 μg/mL in 0.1 M sodium phosphate buffer at pH 4.5, 6.5, and 7.5 at 37 °C. Periodic samples were removed from incubations, cooled to ice bath temperature, and adjusted with phosphoric acid to pH 6.0, conditions at which the compounds were known to be stable for several hours. Prior to assay, these samples were treated with the freeze-dried intestinal preparation[23] as described in the absorption studies section to release the surviving bioactive parent compound. They were assayed as described in the assay section.

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Synthesis and Biological Activity of Some Broad-Spectrum *N*-Acylphenylglycine Cephalosporins[1]

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The synthesis and the in vitro and in vivo antibacterial activities of a series of N-acylated phenylglycine cephalosporins are described. These compounds exhibit activity against a broad spectrum of gram-positive and gram-negative bacteria including some strains of *Pseudomonas aeruginosa*, a bacterial species normally insensitive to the cephalosporin antibiotics. The cephalosporins were prepared by acylation of cephaloglycin or its 3-tetrazolylthiomethyl analogue. In several cases, the acylations produced mixtures of diastereomeric cephalosporins, the components of which, when separated, showed different levels of antibiotic activity. Optimum activity was obtained when the acyl moiety on the phenylglycine nitrogen contained an oxygen atom centrally located between the amide carbonyl and a carboxyl substituent, preferably in a three- or five-membered ring. Replacement of acetoxymethyl by (1-methyl-1*H*-tetrazol-5-yl)thiomethyl at the 3 position resulted in overall improvement in activity both in vitro and in vivo. Against a group of *P. aeruginosa* strains, the best compounds of this series showed activity on the order of carbenicillin.

Chemical modifications of the cephalosporin structure have produced numerous derivatives which are active against a broad spectrum of gram-positive and gram-negative bacteria. In spite of intensive synthetic efforts, the overwhelming majority of these derivatives show no significant antibacterial activity against *Pseudomonas aeruginosa*. Recently several laboratories have described three types of semisynthetic cephalosporins of diverse structure which are active against this species: 3-(substituted)vinyl cephalosporins;[2] 7-(α-sulfocephalosporins);[3] and 7-(α-ureidophenylacetyl) cephalosporins.[4] This report describes the synthesis and the in vitro and in vivo activities of a series of N-acylated phenylglycine cephalosporins which are active against a broad spectrum of gram-positive and gram-negative bacteria including some strains of *P. aeruginosa* which are normally insensitive to most cephalosporin antibiotics.

Chemistry. The cephalosporins listed in Table I were prepared by acylation of cephaloglycin **1**[5] or its 3-tetrazolylthiomethyl analogue **2**[6] (Scheme I). These zwitterionic cephalosporins (**1** and **2**) were relatively insoluble in most organic solvents but they could be acylated under very mild conditions by stirring a suspension of them in an inert solvent such as acetone with a suitably activated carboxylic acid. Cephalosporins **12–14** and **20–22** were obtained using commercially available anhydrides **3–5**. Cephalosporins **15–18** and **23–26** were prepared using cyclic anhydrides **6–8** of the corresponding dicarboxylic acids which were obtained by known procedures.[7-10]

Biology. In this series, substitution of 3-tetrazolylthiomethyl for acetoxymethyl exerts no significant influence on the level of gram-positive activity. The MIC's against a penicillin-G resistant strain of *Staphylococcus aureus* [*S.a.*(R)] for the tetrazole-containing analogues (Table III) are all within one twofold dilution of those obtained for the corresponding acetoxy analogues (Table II). On the other hand, this change results in an overall improvement of the MIC's against the gram-negative bacteria for the majority of compounds tested. These results are consistent with other structure–activity relationship studies which have shown the same general trends reported here.[12]

Significant changes in MIC's are observed with variation of the *N*-acyl group attached to the phenylglycine portion of the molecule within each series. Compounds **12–14** (Table II) and **20–22** (Table III) show the effects of altering