concentrations.) The dose–response curves obtained were subjected to probit analysis, using the Fortran program ISOBOL, to compare parallelism of slopes; the slopes of the common regression line of the response metameter on the logarithm of the dose and the 5-HT concentrations required for half-maximal contraction were calculated. The apparent affinities were calculated as $pA_2$ values by the method of Arunlakshana and Schild.[21]

**Hydrolysis Experiments.** In order to determine the extent of ester hydrolysis, solutions of the esters in Tyrode's buffer were incubated at 37 °C for 10–15 min, both with and without a freshly cut portion of fundus strip (ca. 1 g wet weight). At the end of the incubation time, the fundus strip was immediately removed. The amount of bufotenine present was determined by a modification of the thin-layer chromatographic procedure of Narasimhachari and Plaut.[18] The procedure involved spotting 1–5 μL of the ester solutions on silica gel G TLC plates along with standard quantities of bufotenine. After development with $CHCl_3$–MeOH–$NH_4OH$ (12:7:1) and spraying with *o*-phthalaldehyde reagent, the size and fluorescence intensity of the bufotenine spots from the ester solutions were compared with the standard spots in order to obtain quantitative estimates of the amount of hydrolysis.

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# Cephalosporin Derivatives with 2- and 4-Pyridone Groups at Carbon-3

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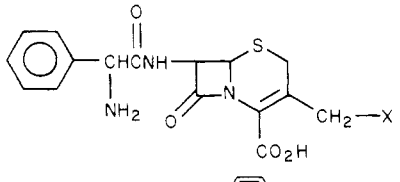
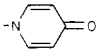
Two compounds, analogues of cephalexin with 2- and 4-pyridone groups at C-3, were prepared. Biological evaluation found the compounds to exhibit activity against Gram-positive and Gram-negative organisms in vitro and in vivo. The compounds were only active in vivo on subcutaneous administration.

One approach to the design of orally effective cephalosporins has been the modification of C-3 methylene of cephalexin (**1c**). The phenylglycine group at C-7 seems



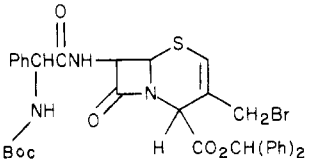
**1a**, X = 

**b**, X = 

**c**, X = H

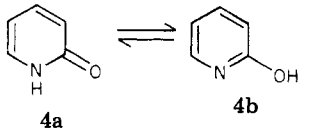
a requisite for oral activity in cephalosporin antibiotics. This paper reports the synthesis and activity of compounds incorporating the 2- and 4-pyridone moiety at C-3 of cephalexin (**1a,b**).

As a general route to these compounds we decided on the reaction of the allylic bromide **2** with the trimethylsilyl



**2**

ether of a hydroxy-substituted pyridine.[1a,b] The nitrogen atom of 2-hydroxypyridine is amide-like due to the predominance of the keto form **4a** in the equilibrium **4a–4b**.[2]



**4a** **4b**

The nonbasic character of 2-hydroxypyridine accounts for

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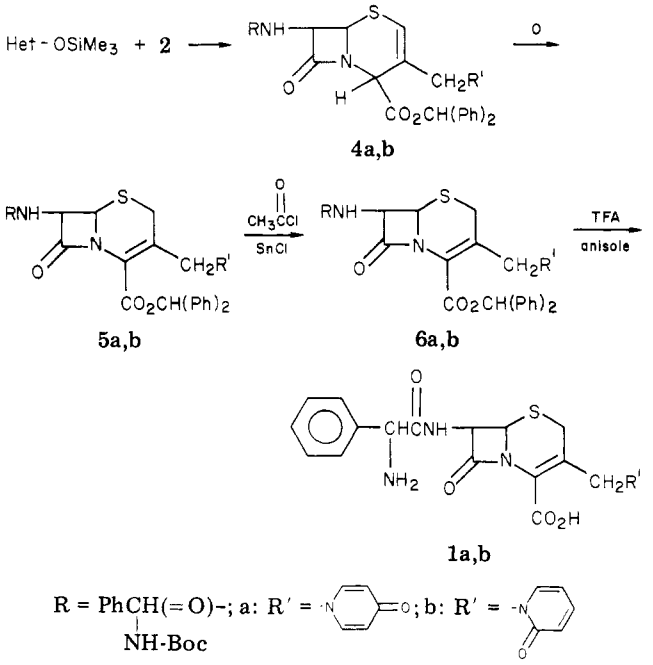
Table I. Activity in Vitro of Compounds 1a, 1b, and Cephalexin (1c)

| | minimal inhibitory concn, μg/mL,[a] of microorganism[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| no. | *S.a.* | *S.ar.* | *S.e.* | *S.f.* | *S.p.* | *St.p.* | *E.c.* | *K.p.* | *P.m.* | *S.s.* |
| 1a | 3.1 | 50 | 25 | 100 | 0.4 | 1.6 | 12.5 | 12.5 | 50 | 12.5 |
| 1b | 1.6 | 6.2 | 6.2 | 50 | 0.2 | 0.8 | 25 | 6.2 | >100 | 12.5 |
| 1c | 1.6 | 6.2 | 12.5 | >100 | 1.6 | 0.8 | 6.2 | 3.1 | 25 | 6.2 |

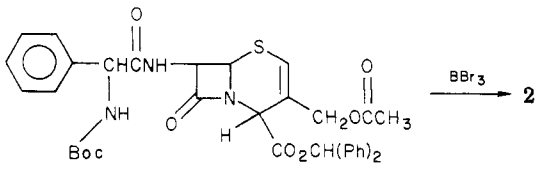
[a] Determined by serial twofold dilution of compound in Mueller-Hinton agar or trypticase soy broth (for *Streptococcus pyogenes* only) and inoculation of the agar surface or broth with an appropriately diluted 18-24-h broth culture. Agar plates and tubes of broth were incubated at 37 °C for 17 h and the lowest concentration causing inhibition of visible growth was considered to be the minimal inhibitory concentration. [b] Abbreviations used: *S.a., Staphylococcus aureus; S.ar., Staphylococcus aureus* (benzylpenicillin resistant); *S.e., Staphylococcus epidermidis* (benzylpenicillin resistant); *S.f., Streptococcus pneumoniae; St.p., Streptococcus pyogenes; P.m., Proteus mirabilis; S.s., Salmonella schottmuelleri.*

Scheme I




our synthesis of **1b** via the bromide **2** rather than the solvolysis reaction used in the preparation of cephaloridine.[3] Attempts to utilize 4-hydroxypyridine in a solvolysis reaction were unsuccessful and **1a** was also prepared via **2**.

The allylic bromide **2** was prepared from **3** by a published procedure.[4] Attempts to utilize the $\Delta^3$ isomer of **3** resulted in cleavage of the diphenylmethyl ester (as discussed in ref 4) by $BBr_3$. The bromomethyl compound **2** from this reaction was utilized without further purification in the reaction sequence outlined in Scheme I. We started with **3** derived from D-phenylglycine, so **1a** and **1b** are D isomers. The TFA salts of the products were converted to the zwitterions for biological evaluation.

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## Biological Results and Discussion

Compounds **1a** and **1b** were compared with cephalexin in vitro against a variety of Gram-positive and Gram-negative bacteria. As shown in Table I, both compounds displayed a broad spectrum of activity, with cephalexin being generally more active against the Gram-negative bacteria. Against Gram-positive bacteria, compound **1b** was more active than **1a** and as active or more active than cephalexin for this group of organisms. It also is of interest to note that compound **1b** is significantly more potent than **1a** against the two strains of penicillin G resistant staphylococci tested. Neither **1a** nor **1b** was active against *Enterobacter aerogenes, Enterobacter cloacae, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa* or *Serratia marcescens*, organisms that also are resistant to clinically established cephalosporins.

Table II. Activity in Vivo of Compounds 1a, 1b, and Cephalexin (1c)

| | $ED_{50}$, (mg/kg)/dose,[a] of microorganism[b] | | | | |
|---|---|---|---|---|---|
| | *St.p.* | | *S.p.*, | *S.s.*, | *E.c.*, |
| no. | po | sc | sc | sc | sc |
| 1a | >8 | >0.5 | nt | 5 | 4.6 |
| 1b | >8 | >0.5 | 14 | 10 | 8.4 |
| 1c | 1.2 | 3.4 | 38.7 | 8.2 | 5.6 |

[a] Male albino CD-1 mice weighing 20 (±1) g were infected by intraperitoneal injection of a bacterial suspension to produce uniformly lethal infections. Groups of ten mice each were treated orally (po) or subcutaneously (sc) with appropriate concentrations of antibiotic at 1 and 4 h after infection. The number of mice in each group surviving the challenge of 4 days was recorded, and the $ED_{50}$ (the dose in mg/kg required to protect 50% of the infected mice) was determined by the method of Reed and Muench [*Am. J. Hyg.*, 27, 493 (1938)]. [b] See footnote *b*, Table I, for microorganism names.

With regard to activity in vivo, oral efficacy was not observed, as evidenced by the results obtained against *Streptococcus pyogenes* infections in mice when the compounds were administered both orally and subcutaneously (Table II). When administered subcutaneously, good protection was obtained against both Gram-positive and Gram-negative infections.

In summary, these compounds, while exhibiting limited broad-spectrum antibacterial activity, lack activity on oral administration.

## Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. IR spectra were determined in pressed KBr disks. All compounds gave NMR spectra in agreement with the proposed structure. $Me_4Si$ was the internal standard for NMR spectra determined in $CDCl_3$ and $Me_2SO$-$d_6$. Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. All temperatures are in degree Celsius.

**Diphenylmethyl 3-(Acetoxymethyl)-7-[(*tert*-butoxycarbonylphenylglycyl)amido]-2-cephem-4-carboxylate (3).** 7-[(*tert*-Butoxycarbonylphenylglycyl)amido]cephalosporanic acid (20 g, 0.04 mol) was dissolved in THF (600 mL), and a solution of diphenyldiazomethane (8 g, 0.04 mol) in THF (75 mL) was added. The mixture was stirred for 5 h, $NEt_3$ (1.5 mL, 0.01 mol) was added, and the mixture was stirred overnight to effect the isomerization of the cephalosporin ($\Delta^3$ to $\Delta^2$). The reaction mixture was evaporated and the residue was chromatographed

on a silica gel column (Ph/EtOAc, 9:1) to give 15 g of a mixture ($\Delta^2/\Delta^3$ isomers). This mixture was dissolved in anhydrous ether and the $\Delta^2$ isomer crystallized to give 8.4 g of white solid: mp 151–152 °C; IR (KBr) 1780, 1740, 1700, 1500, 1380, 1240, 1170, 770, 710 $cm^{-1}$; NMR ($CDCl_3$–$Me_4Si$) 1.35 (s, 9 H), 1.85 (s, 3 H), 4.5 (s, 2 H), 4.8–5.0 (m, 3 H), (br s, 1 H), 6.93 (s, 1 H), 7.28 (s, 16 H). Anal. ($C_{36}H_{37}N_3O_8S$) C, H, N.

**Diphenylmethyl 3-(Bromomethyl)-7-[(*tert*-butoxycarbonylphenylglycyl)amido]-2-cephem-4-carboxylate (2).** Compound **3** (2 g, 3 mmol) was dissolved in $CH_2Cl_2$ (70 mL) and the solution was chilled to −25 °C. With the mixture under a $N_2$ atmosphere, a solution of $BBr_3$ (750 mg, 3 mmol) in $CH_2Cl_2$ (10 mL) was added at a rate which kept the temperature at −25 to −30 °C. The mixture was stirred for 30 min after completion of the $BBr_3$ addition and then poured into aqueous $NaHCO_3$. The layers were separated and the aqueous layer was extracted with a second portion of $CH_2Cl_2$. The combined organic extracts were dried ($Na_2SO_4$) and evaporated to give 1.8 g of **5** as a pale tan foam. An NMR spectrum of the foam ($CDCl_3$) showed disappearance of the methyl group of the C-3 acetate and a quartet at $\delta$ 4.28 ($J$ = 3 Hz) for the $-CH_2Br$ group. The material was used without further purification.

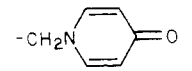
**Diphenylmethyl 7-[(*tert*-Butoxycarbonylphenylglycyl)amido]-3-[(1,4-dihydro-4-oxopyridin-1-yl)methyl]-2-cephem 4-carboxylate (4a).** Compound **2** (0.9 g, 1.3 mmol) was dissolved in acetonitrile (70 mL), and 0.9 mL (5 mmol) of the trimethylsilyl ether of 4-hydroxypyridine was added. The mixture was stirred at room temperature under a $N_2$ atmosphere for 18 h. The mixture was evaporated and redissolved in $CHCl_3$, and the solution was washed with aqueous $NaHCO_3$ and water. The organic layer was dried ($Na_2SO_4$) and evaporated. The residue was purified by preparative TLC ($CH_3CN$/EtOH, 4:1) to give 189 mg of product: mp 129–131 °C; IR (KBr) 1790, 1720, 1680, 1650, 1570, 1500, 1175, 850, 750, 700 $cm^{-1}$; NMR ($CDCl_3$–$Me_4Si$) $\delta$ 1.32 (s, 9 H), 4.2–5.6 (m, 5 H), 5.9–6.4 (m, 5 H), 6.7–7.9 (m) and 7.35 (s) together = 19 H. Anal. ($C_{39}H_{38}N_4O_7S$) C, H, S; N: calcd. 7.93; found, 7.15.

In an analogous manner, compound **4b** was prepared and purified by column chromatography [silica gel, benzene/ethyl acetate (1:1)] and isolated as a foam: IR (KBr) 1775, 1720, 1650, 1490, 1165, 760, 705 $cm^{-1}$; NMR ($CDCl_3$–$Me_4Si$) $\delta$ 1.4 (s, 9 H), 4.48 (s, 2 H), 4.9–5.4 (m, 3 H), 5.5 and 5.68 (d, $J$ = 1.5 Hz, 1 H), 5.8–6.15 (m, 3 H), 6.48 (d, $J$ = 4 Hz, 1 H), 6.9 (s, 1 H), 7.3 (s, 18 H), 7.55–8.0 (m, 1 H). Anal. ($C_{39}H_{38}N_4O_7S$) H, N, S; C: calcd, 66.27; found, 64.60.

**Diphenylmethyl 7-[(*tert*-Butoxycarbonylphenylglycyl)amido]-3-[(1,4-dihydro-4-oxopyridin-1-yl)methyl]-3-cephem-4-carboxylate Sulfoxide (5a).** Compound **4a** (707 mg, 1 mmol) was dissolved in $CHCl_3$ (30 mL) and the solution was chilled in an ice bath. A solution of *m*-chloroperbenzoic acid (203 mg of 85% peracid, 1 mmol) in $CHCl_3$ (10 mL) was added dropwise. The ice bath was removed and the mixture was stirred for 2 h. The mixture was extracted with aqueous $NaHCO_3$, dried, and evaporated. Recrystallization from ethyl acetate gave **5a** (276 mg): mp 178 °C dec; IR (KBr) 1790, 1720, 1680, 1640, 1560, 1490, 1370, 1245, 1168, 855, 750, 705 $cm^{-1}$. The compound was insoluble in $Me_2SO$-$d_6$ and $CD_3OD$; no NMR was obtained. Anal. ($C_{39}H_{38}N_4O_8S$) C, H, N, S.

In a similar manner, **5b** was prepared: mp 194–195 °C dec; IR (KBr) 1800, 1730, 1700, 1670, 1590, 1540, 1500, 1370, 1260, 1235, 1170, 1030, 760, 700 $cm^{-1}$; NMR ($CDCl_3$–$Me_4Si$) $\delta$ 1.4 (s, 9 H), 3.4 (d, $J$ = 4 Hz, 2 H), 4.35 (d, $J$ = 3 Hz, 2 H), 5.1–5.45 (m, 2 H), 5.85–6.15 (m, 2 H), 6.51 (d, $J$ = 5 Hz, 1 H), 6.93 (s, 1 H), 7.33 (s, 20 H), 7.7–8.0 (m, 1 H). Anal. ($C_{39}H_{38}N_4O_8S$) C, H, N, S.

**Diphenylmethyl 7-[(*tert*-Butoxycarbonylphenylglycyl)amido]-3-[(1,4-dihydro-4-oxopyridin-1-yl)methyl]-3-cephem-4-carboxylate (6a).** Compound **5a** (500 mg, 0.7 mmol) was dissolved in a mixture of acetonitrile (14 mL) and dimethylformamide (3.5 mL), and the solution was chilled in an ice bath. Acetyl chloride (3.5 mL, 4.9 mmol) and $SnCl_2$ (500 mg, 2.2 mmol) were added, and the mixture was stirred at ice bath temperature for 2.5 h. The mixture was poured into excess aqueous $NaHCO_3$, and the aqueous mixture was extracted with ethyl acetate. The organic extracts were dried and evaporated. Recrystallization of the residue from ethyl acetate gave **6a** (300 mg): mp 156–157° C; IR (KBr) 1790, 1720, 1680, 1640, 1560, 1490, 1370, 1245, 1178, 855, 755, 705 $cm^{-1}$; NMR ($CD_3OD$–$Me_4Si$) $\delta$ 1.5 (s, 9 H), 5.01 (d, $J$ = 3 Hz, 1 H), 5.35 (s, 1 H), 5.87 (d, $J$ = 3 Hz, 1 H), 6.4 (d, $J$ = 4 Hz, 2 H), 7.08 (s, 1 H), 7.4 (s) and 7.65 (d, $J$ = 2 Hz) together = 18 H. The $-CH_2-$ at $C_2$ ($\delta$ 3.4) and i ($\delta$ 4.7)



i

are found with $CD_3OD$ peaks. Anal. ($C_{39}H_{38}N_4O_7S$) H, N, S; C: calcd, 66.27; found, 65.59.

In a similar manner, compound **5b** (669 mg) was converted to **6b** (387 mg), isolated as a foam: IR (KBr) 1803, 1720, 1700, 1670, 1590, 1540, 1500, 1375, 1260, 1235, 1170, 1030, 760, 700 $cm^{-1}$; NMR ($CDCl_3$–$Me_4Si$) $\delta$ 1.4 (s, 9 H), 3.29 (s, 2 H) (m, 2 H), 5.37 (d, $J$ = 4 Hz, 1 H), 5.7–6.17 (m, 3 H), 6.58 (d, $J$ = 4 Hz, 1 H), 6.98 (s, 1 H), 7.42 (s, 17 H), 7.8 (d, $J$ = 4 Hz, 1 H). Anal. ($C_{39}H_{38}N_4O_7S$) C, H, N.

**3-[(1,4-Dihydro-5-oxopyridin-1-yl)methyl]-7-[(phenylglycyl)amido]-3-cephem-4-carboxylate (1a).** Compound **6a** (433 mg, 0.61 mmol) was dissolved in anisole (5 mL) and the solution was chilled in an ice bath. Trifluoroacetic acid (25 mL), which had been previously chilled in an ice bath, was added and the reaction mixture was stirred at ice bath temperature ($N_2$ atmosphere) for 30 min. The mixture was poured into ether (700 mL), and the precipitate was filtered and vacuum dried to give 400 mg of the TFA salt of the product. This material was dissolved in water (15 mL) and the solution was filtered to remove a small amount of insoluble material. The filtrate was treated with amberlite IR-45 resin to pH 4.5. The mixture was filtered and the filtrate was concentrated to 5 mL, at which point crystallization of product began. Ethanol (15 mL) was added and the mixture was filtered to give 187 mg of **1a**: mp 172–176 °C; IR (KBr) 1770, 1670, 1640, 1540, 1480, 1470, 1430, 1175, 860, 705 $cm^{-1}$. Anal. ($C_{21}H_{20}N_4O_5S$) H, N, S; C: calcd, 57.26; found, 55.55.

Using the same procedure, **6b** was converted to **1b**: mp 167–172 °C; IR (KBr) 1770, 1690, 1650, 1570, 1390, 1350, 1245, 1145, 770, 700 $cm^{-1}$. Anal. ($C_{21}H_{20}N_4O_5S$) H, N; C: calcd, 5.76; found, 55.24.

A high-pressure LC assay of **4a** and **4b** indicated purities of 82.6 and 82.3%, respectively.[5]

## References and Notes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(5) This assay, developed by Dr. W. Fike of our Analytical Chemistry Department, compares the UV absorbance of the major peak of the sample with that of the unchromatographed sample.