EtOAc. After drying ($MgSO_4$) and evaporation of the solvent, 1.5 g of the product obtained. This material was dissolved in 25 mL of MeOH and 25 mL HCOOH (50%) and the solution was cooled to 0 °C. The zinc dust (690 mg) was added in portions over 30 min and the reaction mixture stirred for 2 h. The zinc salts were filtered off, and the solvent was removed in vacuo. The residue was suspended in 150 mL of water and 20 mL of MeOH the pH adjusted to 7.0, and the insoluble dark material filtered off. The product from the filtrate was purified by HPLC. The isomers were separated by using 8-L gradient 0–10% MeCN, 1% AcOH/H_2O, and 2 L of 20% MeCN/1% AcOH/H_2O. NMR (Me_2SO-d_6) of the *R* isomer **30e**: δ 2.0 (s, 3 H), 3.25 and 3.55 (AB q, 2 H, J = 18 Hz), 4.8 (s, 1 H), 5.08 (d, 1 H, J = 5 Hz), 5.58 (dd, 1 H, J = 5 Hz), 7.3–7.8 (m, 3 H).

Acknowledgment. We express our appreciation to Dr. Lowell D. Hatfield for valuable discussions and guidance, to Donnis M. Berry and Lyell Huckstep for analytical and preparative HPLC, and to the analytical and physical chemistry sections for elemental analyses, UV, IR, and NMR spectroscopy. One of us (S.K.) is grateful to Robert R. Chauvette for his counsel and critical reading of this manuscript.

Orally Absorbable Cephalosporin Antibiotics. 3.[1] Preparation of Biologically Active *R* Isomer of 7-(3-Benzothienylglycylamido)deacetoxycephalosporanic Acid

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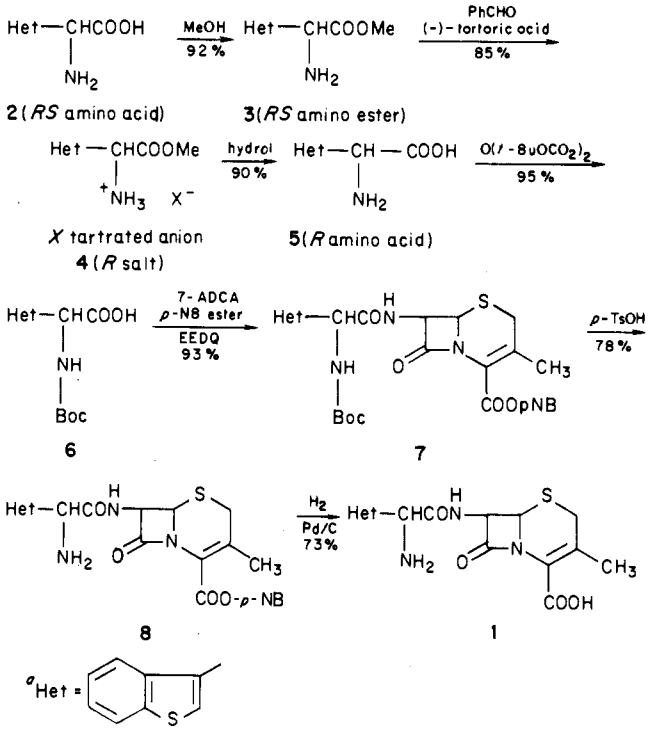
The methyl and isopropyl esters of (*RS*)-3-benzothienylglycine were resolved with (+)- and (−)-tartaric acid in acetonitrile to give the corresponding *R* and *S* salts. The *R*-salt **4** was hydrolyzed to (*R*)-3-benzothienylglycine (**5**). The amino group in **5** was protected with the Boc function and the protected *R* amino acid **6** coupled with the *p*-NB ester of 7-ADCA to give the diprotected cephalosporin **7**. After removal of the Boc and *p*-NB groups, the *R* isomer of 7-(3-benzothienylglycylamido)deacetoxycephalosporanic acid (**1**) was obtained. The *p*-NB ester of epimeric cephalosporin **7** was separated by preparative chromatography into *R* and *S* isomers. After removal of the protective groups, the *S* epimer was isolated. The comparison of antibacterial activity of the *R* and *S* epimers and the *RS* mixture of cephalosporin **1** is reported.

Preliminary bioligical evaluations of the *R* isomer of the 3-benzothienylglycyl derivative of 7-aminodeacetoxycephalosporanic acid (**1**) have been very encouraging. This new cephalosporin has both oral and parenteral activity in mice against commonly encountered Gram-positive bacteria.[2] Initially, this compound was prepared as an epimeric mixture by coupling of Boc-3-benzothienylglycine with the *p*-NB ester of 7-aminodeacetoxycephalosporanic acid (7-ADCA). After removal of protective groups the diastereomeric mixture was separated into *R* and *S* isomers by HPLC. This procedure provided a small amount of material for early testing. In order to proceed with complete biological and clinical evaluations of **1**, larger quantities of the *R* epimer were required. Therefore, we decided to pursue the chemical resolution of the 3-benzothienylglycine side chain before it was acylated on the cephalosporin nucleus.

Scheme I[a]



It is known that (*R*)-phenylglycine, which is incorporated in the antibiotics ampicillin, cephalexin, and cefaclor, can be resolved efficiently by chemical methods. Clark et al.[3] reported the resolution of *RS* esters of phenylglycine with tartaric acid. Their method involves conversion of a ra-

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0022-2623/85/1828-1903$01.50/0

Table I. Antibacterial Activity of Cephalosporins Having Benzothienylglycyl Side Chains[a]

	Staphylococcus aureus				*Staphylococcus epidermidis*, EPI 1	*Streptococcus*			*Haemophilus influenzae*	
	penicillin G		methicillin resistant			*pyogenus*, C203	*pneumoniae*, Park	group D, X66		
compd	sens, X1.1	res, V41	S13E	X400					sens, C.L.	res, 76
1R	1	4	4	32	4	0.5	0.5	128	2	1
1S	32	64	128	>128	64	16	16	>128	64	16
1RS	2	8	16	64	4	2	4	>128	4	2
cephalexin	4	128	>128	128	32	0.5	2	128	8	8

[a] Numerical values are MIC's in μ/mL; agar dilution method of Kirst et al.: Kirst, H. A.; Wild, G. M.; Baltz, R. H.; Hamill, R. L.; Ott, J. L.; Counter, F. T.; Ose, E. E. *J. Antibiot.* **1982**, *35*, 1675.

cemic aryl amino ester into one isomer through continuous racemization of its Schiff base and crystallization of a diastereomeric salt with an optically active acid. Formation of the Schiff base intermediate is important because it is more easily racemized than the amino ester. Which isomer is isolated depends on several factors, but the main factor is the solubility (or crystallinity) of the particular diastereomeric salt in a particular solvent.

This was nicely illustrated when a published procedure[3] for (*RS*)-phenylglycine was applied to the methyl ester of (*RS*)-3-benzothienylglycine. By employing (+)-tartaric acid and benzaldehyde, the *S* enantiomer of 3-benzothienylglycine was isolated, not the *R* enantiomer as expected by analogy with phenylglycine. However, when in a similar experiment (−)-tartaric acid was used [instead of (+)-tartaric acid], using CH_3CN as the solvent, the *R* salt crystallized out. After hydrolysis and neutralization, the *R* amino acid **5** was isolated in good yield.

An impressive example of how the ester funtionality influences the solubility of a diastereomeric salt was observed upon changing the ester moiety from methyl to isopropyl. Thus, when the methyl (*RS*)-3-benzothienylglycinate (**3**) was treated with benzaldehyde and (−)-tartaric acid in acetonitrile, the *R* salt precipitated. After neutralization the *R* isomer of methyl 3-benzothienylglycinate was isolated. However, when the same experiment was repeated with the corresponding isopropyl ester, the enantiomeric *S* isopropyl 3-benzothienylglycinate was obtained.

From the yields quoted in Scheme I, it is obvious that an effective and economical preparation of side chain **5** was achieved. With the availability of the optically pure *R* isomer **5**, we proceeded with the synthesis of compound **1**. The amino group in **5** was protected by treatment with di-*tert*-butyl dicarbonate, affording the Boc-protected amino acid **6**. Coupling of **6** with the *p*-NB ester of 7-aminodeacetoxycephalosporanic acid (7-ADCA) by means of EEDQ yielded the diprotected cephalosporin **7**. From **7** the Boc group was removed by hydrolysis with *p*-toluenesulfonic acid and the tosylate salt crystallized out. Since the isolation of **1** after hydrogenation proved to be much easier if the *p*-toluenesulfonic acid was removed prior to hydrogenation, the tosylate salt was treated with a solution of sodium bicarbonate and the free amine **8** isolated. Removal of the ester protective group in **8** was accomplished by hydrogenation in the presence of palladium on carbon and the optically pure *R* isomer **1** was isolated as a colorless zwitterion.

Early in the course of our synthetic work, we noted the separation of the *RS* diprotected (Boc and *p*-NB) derivative of **1** by thin-layer chromatography. This observation encouraged us to explore the separation of the epimers by preparative chromatography. After initial experimentation we found that indeed *R* and *S* epimers were separable on a silica gel column. For example, chromatography of 4 g of the *RS* mixture on 300 g of silica gel using a toluene/ethyl acetate gradient afforded 1.44 g of the *R* epimer, 1.23 g of the *S* epimer, and 1.13 g of a *RS* mixture. The total recovery of *R*, *S*, and *RS* fractions was usually more than 90%.

After removal of the protecting groups by procedures described earlier, both epimers were available in gram quantities. In order to compare directly the antibacterial activity of the two epimers, we submitted both **1R** and **1S** for testing. In addition the *RS* mixture of compound **1** was also tested; the results are shown in Table I. It is a well-established fact that cephalosporins with the *R* configuration of the amino acid side chain are biologically more active than the corresponding *S* epimer.[4] From the MIC values of **1R** and **1S**, it is obvious that the *R* epimer is 16–32 times more potent as an antibiotic than the *S* epimer against *Staphylococcus* spp., *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Klebsiella*.

Experimental Section

Melting points are uncorrected. IR spectra were recorded on Beckman IR-7 or Perkin-Elmer Model 21 or Infracord instruments. NMR spectra were determined on Varian HA-60 and T-60, JEOL FX-90Q, and Brucher WM270 instruments with Me_4Si as the internal reference. TLC was done with Merck silica gel plates. Preparative HPLC was carried out on a Waters Associates Prep LC System 500A fitted with an ISCO Model UA-5 280-nm UV detector. Elution was performed at flow rate 200 mL/min with 8-L gradient starting with H_2O/MeCN/AcOH (95:5:2) with an increase to the ratio 80:20:2. The progress of separation (*R* and *S* epimers) and the check of purity was monitored on an analytical Waters μ-Bondapak C_{18} column with 20% MeCN, 70% water, and 1% acetic acid as the mobile phase. Solvents were dried over molecular sieves; temperatures are reported in degrees centigrade.

(*RS*)-3-Benzothienylglycine (2). To a stirred suspension of 4.0 g of 5% palladium on carbon in 200 mL of MeOH was added in one portion 3.93 g of [2-(hydroxyimino)-3-benzothienyl]acetic acid.[5] The reaction mixture was stirred at 24 °C for 3 h under H_2 at 60 psi. The reaction mixture was then diluted by addition of 21 mL of 1 N HCl and stirring was continued for 5 min. After filtration the pH of the reaction mixture was adjusted to 4.3 with concentrated NaOH and the precipitate that formed was collected and air-dried to give 1.35 g of 3-benzothienylglycine: mp 195–198 °C; IR (KBr) 1643 cm^{-1}; UV λ_{max} (MeOH) 258 nm (ϵ 5300); MS, *m/e* 207 (M^+); titration (66% DMF/H_2O) 3.8, 9.4; NMR (Me_2SO-d_6/DCl) δ 5.54 (s, 1 H), 7.3–8.1 (m, 5 H). Anal. ($C_{10}H_9NO_2S$) C, H, N.

(*RS*)-*N*-(*tert*-Butoxycarbonyl)-3-benzothienylglycine (6). (*RS*)-3-Benzothienylglycine (20.7 g, 0.1 mmol) was suspended in 1 L of 1:1 H_2O/THF. The pH was adjusted to 8.0 with Et_3N, then di-*tert*-butyl dicarbonate (24 mL) was added, and the pH was maintained at 8.0–8.5 with Et_3N. After it stabilized, the pH was adjusted to 7.0 with 1 N HCl and the THF was evaporated. The remaining aqueous solution was layered with EtOAc and acidified to pH 2.5 with 1 N HCl. The EtOAc was separated and

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(5) Cook. M. C.; Gregory, G. I.; Bradshaw, J. British Patent 1 399 089, 1975; *Chem. Abstr.* **1975**, 83, 147306.

the aqueous extracted once more with EtOAc. The combined EtOAc extracts were dried over $MgSO_4$ and evaporated to give 28.2 g (92%) of the Boc-protected amino acid as a white foam: NMR (M_2SO-d_6) δ 1.39 (s, 9 H, Boc CH_3), 5.50 (d, 1 H, α-H), 7.2–8.0 (m, 6 H, arom, NH); IR ($CHCl_3$) 1723 cm^{-1}; UV λ_{max} (EtOH) 228 nm (ϵ 24 000); MS, m/e 307 (M^+). Anal. ($C_{15}H_{17}NO_4S$) C, H, N, O, S.

Methyl (*RS*)-3-Benzothienylglycinate (3). (*RS*)-3-Benzothienylgycine (**2**; 5.0 g, 24.2 mM) was suspended in 50 mL of MeOH and cooled in an ice bath. Thionyl chloride (2.72 mL, 36.2 mM) was added dropwise over 10 min. The ice bath was removed and the solution refluxed for 2 h. The MeOH was evaporated and the residue was stirred with 100 mL of Et_2O. The colorless hydrochloride salt of ester **3** precipitated and was filtered and dried; mp 180–182 °C. Anal. ($C_{11}H_{12}NO_2SCl$) C, H, N, Cl. The salt was dissolved in H_2O, the pH was adjusted to 9.0 with 5 N NaOH, and the amino ester was extracted with EtOAc. The H_2O layer was washed with additional EtOAc, and the combined EtOAc layers were dried over Na_2SO_4, filtered, and evaporated to give 4.94 g (92%) of **3** as a yellow oil: NMR ($CDCl_3$) δ 7.2–8.0 (m, 5 H), 5.02 (s, 1 H), 3.71 (s, 3 H), 2.03 (br s, 2 H). Anal. ($C_{11}H_{11}NO_2S$) C, H, N.

Methyl (*R*)-3-Benzothienylglycinate (the Free Amine of 4). A suspension of methyl (*RS*)-3-benzothienylglycinate (**3**); 4.30 g, 19.4 mM), (−)-tartaric acid (2.88 g, 19.4 mM), and benzaldehyde (2.6 mL, 25.7 mM) in CH_3CN (75 mL) was heated in a boiling water bath for 20 min. The suspension was stirred at room temperature for 26 h, then filtered, and dried in vacuo 18 h at 40 °C. Yield of the tartrate salt **4**, 6.10 g (85%), mp 174–176 °C. Anal. ($C_{15}H_{17}NO_8S$) C, H, N. This solid was stirred in 200 mL of CH_2Cl_2 + 250 mL 5% $NaHCO_3$ in H_2O until it dissolved, in about 10 min. The layers were separated, and the H_2O layer was extracted with 50 mL of CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over $MgSO_4$, filtered, and evaporated to give 3.42 g of the title compound (80% from **3**) as a colorless oil: NMR ($CDCl_3$) δ 2.10 (br s, 2 H), 3.68 (s, 3 H), 5.00 (s, 1 H), 7.2–8.0 (m, 5 H); $[\alpha]_D$ − 173.8° (*c* 1, EtOH).

(*R*)-3-Benzothienylglycine (5). To methyl (*R*)-3-benzothienylglycinate (3.30 g, 14.9 mM) was added 13.7 mL of 2.22 N NaOH. This reaction was stirred for 15 min, while the temperature was maintained at about 25 °C by periodic immersion in cold water. The reaction was acidified to pH 4.6 by the addition of 2 N HCl, along with addition of about 100 mL of H_2O to maintain stirring. The aqueous suspension was cooled to about 5 °C for 1.5 h, then filtered, and dried in vacuo at 60 °C for 16 h to give 2.97 g of colorless crystals. A second crop (0.124 g) was obtained upon reducing the volume of the filtrate to ~25 mL: total yield 3.09 g of **5** that is 96% pure by elemental analysis; $[\alpha]_D$ −182.6° (*c* 1, 5% 1 N HCl in MeOH); mp 203–207 °C.

(*R*)-*N*-(*tert*-Butoxycarbonyl)-3-benzothienylglycine (6). (*R*)-3-Benzothienylglycine (**5**; 1.035 g, 5.0 mM) was suspended in THF and H_2O (25 mL each) and the pH adjusted to 8.0 with Et_3N. Di-*tert*-butyl dicarbonate (1.2 mL, 5.2 mM) was added and the pH maintained between 8 and 8.5 with Et_3N. After 30 min, the pH was adjusted to 7.0 with 1 N HCl, and the THF was removed by rotary evaporation. EtOAc was added to the H_2O layer and the pH adjusted to 2.0 with 1 N HCl. The layers were separated, and the H_2O layer was extracted with fresh EtOAc. The combined EtOAc layers were washed with saturated NaCl, dried over $MgSO_4$, filtered, and evaporated to give 1.51 g (98%) of the desired product **6** as a white foam: NMR (Me_2SO-d_6) δ 1.40 (s, 9 H), 5.52 (d, J = 9 Hz), 7.2–8.1 (m, 6 H).

***p*-Nitrobenzyl (*R*)-7-[*N*-(*tert*-Butoxycarbonyl)-3-benzothienylglycylamido]-3-methyl-3-cephem-4-carboxylate (7).** The protected glycine **6** (1.38 g, 4.5 mM) was used to acylate the *p*-NB ester of 7-ADCA according to the procedure described by Kukolja et al.[1] to give 2.67 (93%) of **7** as a colorless solid.

The Boc group was removed from **7** (2.55 g, 4.0 mM) to give 2.26 g (78%) of *p*-nitrobenzyl (*R*)-7-(3-benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylate *p*-toluenesulfonate monohydrate as a colorless solid following the procedure described by Kukolja et al.[1]

(*R*)-7-(3-Benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylic Acid (1). Methyl (*RS*)-3-benzothienylglycinate (**3**; 500 mg, 2.26 mM), (+)-tartaric acid (339 mg, 2.26 mM), and benzaldehyde (0.30 mL, 2.94 mM) were suspended in CH_3CN (6.7 mL) and refluxed for 10 min. The *p*-nitrobenzyl group was removed from **8** (2.20 g, 3.03 mM) according to the procedure described by Kukolja et al.[1] to give 875 mg (73%) of **1** as a white amorphous powder. The product was 100% *R* isomer by quantitative analytical HPLC (UV detection); no *S* isomer was detected. Compound **1**: $[\alpha]_D$ +100.8°; UV λ_{max} (MeOH); 260 nm (ϵ 11 500); NMR (D_2O/DCl) δ 2.03 (s, 3 H), 3.08, 3.35 (ABq, 2 H, J = 19 Hz), 5.03 (d, 1 H, J = 5 Hz), 5.69 (d, 1 H, J = 5 Hz), 5.76 (s, 1 H), 7.45–8.10 (m, 5 H). Anal. ($C_{18}H_{17}N_3O_4S_2$) C, H, N.

Methyl (*S*)-3-Benzothienylglycinate. Methyl (*RS*)-3-benzothienylglycinate (**3**; 500 mg, 2.26 mM), (+)-tartaric acid (339 mg, 2.26 mM), and benzaldehyde (0.30 mL, 2.94 mM) were suspended in CH_3CN (6.7 mL) and refluxed for 10 min. The suspension was cooled to room temperature and stirred for 72 h. The suspension was filtered and dried to give 657 mg of the tartrate salt as a white solid. This material was stirred in CH_2Cl_2 and 5% $NaHCO_3$ in H_2O (50 mL each) until it dissolved. The layers were separated, the H_2O layer was extracted with 25 mL of CH_2Cl_2, and the combined CH_2Cl_2 layers were dried over $MgSO_4$. After filtration and evaporation, 337 mg (67%) of the title compound was obtained as a colorless gum; $[\alpha]_D$ +165.4° (*c* 1, EtOH).

Isopropyl (*RS*)-3-Benzothienylglycinate. To *i*-PrOH (35 mL) were carefully added concentrated H_2SO_4 (6.6 mL, 0.126 M) and then (*RS*)-3-benzothienylglycine (10.35 g, 0.05 M). The solution was refluxed for 2 h and then cooled to room temperature. The pH was adjusted to 7.0 with concentrated NH_4OH and the MeOH was evaporated. The residue was taken up in CH_2Cl_2 and H_2O, the layers were separated, and the CH_2Cl_2 layer was washed with H_2O. The combined CH_2Cl_2 layers were dried over $MgSO_4$, filtered, and evaporated to give 8.73 g (70%) of the desired ester as a gold oil: NMR ($CDCl_3$) δ 1.09, 1.14 (dd, J = 6 Hz, 6 H), 2.06 (br s, 2 H), 4.92 (s, 1 H), 5.06 (m, J = 6 Hz, 1 H), 7.1–8.0 (m, 5H).

Isopropyl (*S*)-3-Benzothienylglycinate. A suspension of isopropyl (*RS*)-3-benzothienylglycinate (500 mg, 2.0 mM), (+)-tartaric acid (300 mg, 2.0 mM), and benzaldehyde (0.30 mL, 2.94 mM) was suspended in CH_3CN (4.7 mL) and refluxed for 2 min. The suspension was cooled to room temperature and stirred for 24 h. The suspension was filtered and dried to give 723 mg (90%) of the tartrate salt as a white solid. One hundred milligrams of this material was stirred in CH_2Cl_2 (40 mL) and 5% $NaHCO_3$ (50 mL) until it dissolved. The layers were separated, the H_2O layer was washed with 20 mL CH_2Cl_2, and the combined CH_2Cl_2 layers were dried over $MgSO_4$. After filtration and evaporation, 53 mg (85%) of the title compound were obtained as a colorless gum; $[\alpha]_D$ +48.6° (*c* 1, EtOH).

Isopropyl (*R*)-3-Benzothienylglycinate. A suspension of isopropyl (*RS*)-3-benzothienylglycinate (500 mg, 2.0 mM), (+)-tartaric acid (300 mg, 2.0 mM), and benzaldehyde (0.60 mL, 5.9 mL) in 13.4 mL of CH_3CN was heated to reflux and then stirred at room temperature for 24 h. The suspension was filtered and dried to give 649 mg (81%) of the tartrate salt as a yellow solid. A 160-mg sample of this material was stirred in CH_2Cl_2 and 5% $NaHCO_3$ and the CH_2Cl_2 layer was dried over $MgSO_4$. After filtration and evaporation 59 mg (62%) of the title compound was obtained as a colorless gum; $[\alpha]_D$ −35.6° (*c* 1, EtOH).

***p*-Nitrobenzyl (*RS*)-7-[*N*-(*tert*-Butoxycarbonyl)-3-benzothienylglycylamido]-3-methyl-3-cephem-4-carboxylate (7).** To a stirred solution of 19.25 g (55 mmol) of *p*-nitrobenzyl 7-amino-3-methyl-3-cephem-4-carboxylate in 350 mL of THF and 350 mL of CH_3CN was added in one portion a solution of 15.35 g (50 mmol) of (*RS*)-*N*-(*tert*-butoxycarbonyl)-3-benzothienylglycine in 350 mL of THF containing 13 g of EEDQ. The reaction mixture was stirred at 25 °C for 5.5 h and then the solvent was removed by evaporation under reduced pressure to give an oil. The oil was dissolved in 500 mL of EtOAc and washed with 150 mL of H_2O, twice with 150-mL portions of 5% $NaHCO_3$, twice with 150-mL portions of 5% HCl, again with 150 mL of H_2O, and finally with 150 mL of brine. The solution was dried and the solvent was removed by evaporation to afford 30.45 g (95% yield) of *p*-nitrobenzyl (*RS*)-7-[*N*-*tert*-(butoxycarbonyl)-3-benzothienylglycylamido]-3-methyl-3-cephem-4-carboxylate as a colorless solid: IR (KBr) 1774, 1522, 1348 cm^{-1}; UV (CH_3OH) λ 225

nm (ε 32 000), 265 (ε 21 000); NMR (Me_2SO-d_6) δ 1.4 (s, 9 H), 2.01 and 2.05 (2 s, 3 H), 3.48 (m, 2 H), 5.10 (2 d, 1 H), 5.38 (s, 2 H), 5.70 (m, 2 H), 7.3–8.3 (m, 9 H), 9.18 (m, 2 H). Anal. ($C_{30}H_{30}N_4O_8S_2$) C, H, N.

Separation of *R* and *S* Isomers of 7 by Chromatography. Four grams of *p*-nitrobenzyl (*RS*)-7-[*N*-(*tert*-3-butoxycarbonyl)-3-benzothienylglycylamido]-3-methyl-3-cephem-4-carboxylate (7) was dissolved in 500 mL of dichloromethane and slurried with 20 g of silica gel 60. The solvent was removed and the mixture was added to an 8 cm × 15 cm column packed with 400 g of silica gel 60 in toluene. The column was eluted with a gradient of 2 L of 5% ethyl acetate in toluene (v/v) to 2 L of 15% ethyl acetate in toluene and finally with 3 L of 15% ethyl acetate in toluene. Twenty-five-milliliter fractions were collected every 2 min. NMR and thin-layer chromatographic analysis showed partial separation of the *R* and *S* isomers, resulting in 1.44 g of *p*-nitrobenzyl (*R*)-7-[*N*-(*tert*-butoxycarbonyl)-3-benzothienylglycylamido]-3-methyl-3-cephem-4-carboxylate (72% yield), 1.135 g of the *RS* mixture, and 1.23 g of the *S* isomer, all as colorless solids. *R* isomer: IR ($CHCl_3$) 1785 cm^{-1} (β-lactam carbonyl); UV λ_{max} (MeOH) 264 nm (ε 21 000); MS, *m/e* 538, 539 (M + 1, + 2); NMR (Me_2SO-d_6) δ 1.39 (s, 9 H), 2.01 (s, 3 H), 3.2–3.7 (AB q, *J* = 18.4 Hz, 2 H), 5.07 (d, *J* = 4.8, Hz 1 H), 5.38 (s, 2 H), 5.65–5.85 (m, 2 H), 7.2–8.3 (m, 10 H), 9.21 (d, *J* = 7.5 Hz, 1 H). Anal. ($C_{30}H_{30}N_4O_8S_2$) C, H, N. *S* isomer: IR ($CHCl_3$) 1783 cm^{-1} (β-lactam carbonyl); UV λ_{max} (MeOH) 262 nm (ε 20 100); MS, *m/e* 539 (M + 1); NMR (Me_2SO-d_6) δ 1.39 (s, 9 H), 2.05 (s, 3 H), 3.3–3.8 (AB q, 2 H), 5.13 (d, *J* = 4.8, Hz, 1 H), 5.36 (s, 2 H), 5.6–5.8 (m, 2 H), 7.15–8.3 (m, 10 H), 9.12 (d, *J* = 7.9 Hz, 1 H). Anal. ($C_{30}H_{30}N_4O_8S_2$) C, H, N.

(*S*)-7-(3-Benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylic Acid (1S). Following the procedure described for the *R* epimer,[1] the Boc and *p*-NB groups were removed from the diprotected *S* epimer of 7, yielding a colorless solid: NMR (Me_2SO-d_6) δ 2.0 (s, 3 H, 3-CH_3), 3.45 (AB q, 2 H, C_2 H_2), 4.95 (s, 1 H, α-H), 5.04 (d, 1 H, C_6 H), 5.57 (d, 1 H, C_7 H), 7.25–8.06 (m, 5 H, arom). Anal. ($C_{18}H_{17}N_3O_4S_2$) C, H, N.

***p*-Nitrobenzyl (*RS*)-7-(3-Benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylate (8).** A solution of 9.6 g (15 mmol) of *p*-nitrobenzyl 7-[*N*-(*tert*-butoxycarbonyl)-3-benzothienylglycylamido]-3-methyl-3-cephem-4-carboxylate in 210 mL of acetonitrile containing 3.42 g (18 mmol) of *p*-toluenesulfonic acid monohydrate was stored at 25 °C for 3 days. The precipitate that formed was collected by filtration and identified as *p*-nitrobenzyl (*RS*)-7-(3-benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylate *p*-toluenesulfonic acid salt monohydrate: IR (KBr) 1777cm^{-1}; UV λ_{max} (EtOH) 263 nm (ε 18 500); MS, *m/e* 539; NMR (Me_2SO-d_6) 2.0 (s, 3 H), 2.3 (s, 3 H), 3.15–3.65 (AB q, *J* = 18, Hz, 2 H), 3.32 (s, 2 H), 5.0 d, 1 H), 5.35 (s, 2 H), 5.5 (s, 1 H), 5.8 (dd, 1 H), 7.0–8.3 (m, 13 H), 9.55 (d, 1 H). Anal. ($C_{32}H_{30}N_4O_9S_3 \cdot H_2O$) C, H, N, O, S.

The salt was dissolved in 60 mL of 10% aqueous sodium bicarbonate and the solution was extracted several times with ethyl acetate. The extracts were combined, washed with water, dried, and concentrated to dryness by evaporation under reduced pressure to give 7.9 g (76% yield) of *p*-nitrobenzyl (*RS*)-7-(3-benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylate as a colorless gum: NMR (Me_2SO-d_6) β 1.99 and 2.04 (2 s, 3 H, *R* and *S* isomers), 3.26–3.6 (m, 2 H), 4.9 (s, 1 H), 5.1 (d, 1 H), 5.36 (s, 2 H), 5.7 (m, 1 H), 7.3–8.25 (m, 10 H).

(*RS*)-7-(3-Benzothienylglycylamido)-3-methyl-3-cephem-4-carboxylic Acid (1). A solution of 5.2 g of *p*-nitrobenzyl 7-(3-benzothienylglycylamido)-3-cephem-4-carboxylate in 150 mL of CH_3OH containing 10 mL of 1 N HCl and 5.2 g of 5% palladium on carbon was stirred at 25 °C for 90 min under 60 psi H_2. The reaction mixture was filtered and the filtrate was concentrated to give a gum. The gum was dissolved in 40 mL of water and 40 mL of EtOAc. The mixture was neutralized to pH 7.0 by addition of 1 N NaOH, and the organic layer was removed and discarded. The aqueous layer was acidified to pH 4.25 by addition of 1 N HCl. The aqueous acid solution was lyophilized to afford 1.62 g of (*RS*)-7-(3-benzothienylglycylamido-3-methyl-3-cephem-4-carboxylic acid. Separation of the isomers was effected by high-performance liquid chromatography to give 299.2 mg of *R*, 131.7 mg of *S*, and 106.6 mg of *RS* isomers as colorless amorphous solids. NMR (Me_2SO-d_6) of *R* isomer: δ 1.94 (s, 3 H), 3.20 and 3.43 (AB q, *J* = 19.5 Hz, 2 H), 4.96 (d, *J* = 4.84 Hz, 1 H), 5.07 (s, 1 H), 5.62 (dd, *J* = 4.4 Hz, 1 H), 7.2–8.1 (m, 6 H). Anal. ($C_{18}H_{17}N_3O_4S_2$) C, H, N.

Acknowledgment. We express our appreciation to Dr. Lowell D. Hatfield for valuable discussions and guidance, to Donnis M. Berry and Lyell Huckstep for analytical and preparative HPLC, and to the analytical and chemistry sections for elemental analyses, UV, IR, and NMR spectroscopy.

[[(4,5-Dihydro-2-oxazolyl)phenoxy]alkyl]isoxazoles. Inhibitors of Picornavirus Uncoating†

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A series of [[(4,5-dihydro-2-oxazolyl)phenoxy]alkyl]isoxazoles has been synthesized and evaluated as antipicornavirus agents. The effect of alkyl groups in the 4- and 5-position of the oxazoline ring, as well as the alkyl chain length, on antiviral activity was examined. Compound 14 was evaluated in vivo and was found to significantly reduce mortality at an oral dose of 4 mg/kg in mice infected intracerebrally with poliovirus-2. Compound 14 was also effective in preventing paralysis when administered intraperitoneally to mice infected subcutaneously with a lethal dose of ECHO-9 virus. On the basis of the results of these studies, compound 14 is a strong candidate for clinical evaluation as a systemic agent for the treatment of picornavirus infections.

We recently reported the synthesis and antipicornavirus activity of a new class of compounds whose mode of action was determined to involve the prevention of viral uncoating.[1] In continuation of our pursuit of compounds with this mode of action, we have prepared several modifications of our original series with increased potency both in vitro and in vivo.

As a result of our previous work, the isoxazole 1 was shown to have broad spectrum activity against several serotypes of rhino and enteroviruses with MICs as low as

† Presented in part at the VIIIth International Symposium on Medicinal Chemistry, Upsalla, Sweden, Aug 1984.

(1) Diana, G. D.; McKinlay, M. A.; Brisson, C. J.; Zalay, E. S.; Miralles, J. V.; Salvador, U. J. *J. Med.* 1985, *28*, 748.

0022-2623/85/1828-1906$01.50/0