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Orally Absorbable Cephalosporin Antibiotics. 2.[1] Structure-Activity Studies of Bicyclic Glycine Derivatives of 7-Aminodeacetoxycephalosporanic Acid

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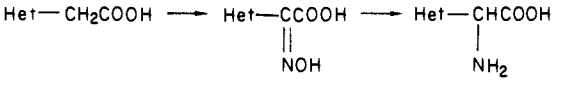
Three positional analogues (4-, 5-, and 7-) of benzothienylglycine and (*N*-acetylindolinyl)-5-glycine were prepared and coupled to 7-aminodeacetoxycephalosporanic acid (7-ADCA) to give the cephalosporins **17a–c**. In addition two isomeric (2,3-*b* and 3,2-*b*) thienothiopheneglycines were synthesized and coupled to 7-ADCA to yield cephalosporins **30d** and **30e**. In vitro testing of these new cephalosporins indicates good activity against Gram-positive bacteria. Against *Streptococcus pneumoniae* infections compound **25** displayed better mouse protection (both orally and subcutaneously) than cephalexin.

In the preceding paper we described some of our recent efforts in the field of orally absorbable cephalosporins.[1] The first part dealt mainly with functional derivatives of benzothienyl- and naphthylglycine side chains. Here we would like to report the synthesis and biological activities of the positional analogues (4-, 5-, and 7-) of benzothienylglycine and the (*N*-acetylindolinyl)-5-glycine derivative of 7-aminodeacetoxycephalosporanic acid (**17a–c** and **25**). In addition two thienothiophene-2-glycine cephalosporins **30d** and **30e** were prepared and tested.

Chemistry

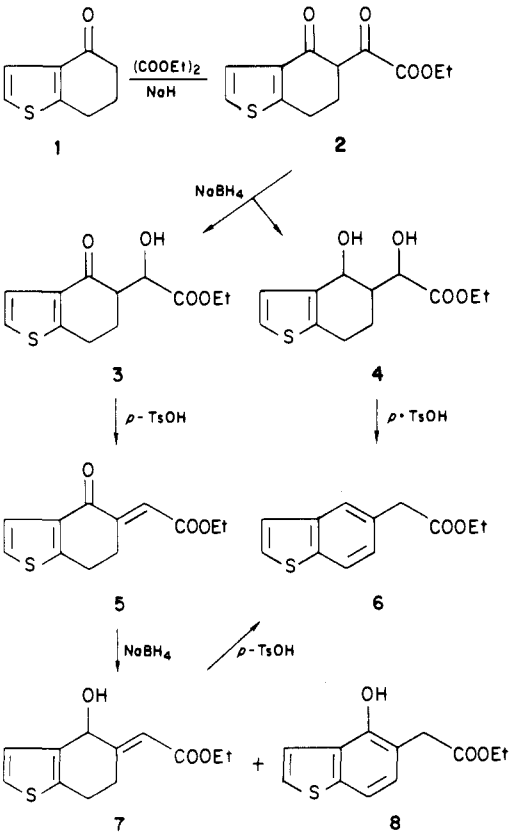
Among various possibilities for the synthesis of the described benzothienylglycines, we chose nitrosation of the pertinent heteroaromatic acetic acids to give the corresponding α-oximinoacetic acids and the subsequent reduction of the imino group to the desired amino acids, as illustrated in the following general scheme:



Het = heteroaromatic ring

The benzothienyl-4-acetate was commercially available, but the other two had to be synthesized. We began our synthesis of benzothienyl-5-acetate with 4-ketotetrahydrothianaphthene (**1**). By treatment of the ketone **1** with diethyl oxalate in the presence of sodium hydride the α,γ-diketo ester **2** was obtained in 88% yield. The reduction of **2** with 2.25 equiv of sodium borohydride gave a mixture of two products and the unreacted starting material. The mixture was separated by chromatography and three compounds were isolated: the starting diketone **2** (4%), a monoalcohol (31%), and a diol (27%). Physical chemical data indicate that in the case of the monoalcohol only the α-keto group was reduced to give **3** and further reduction of the 4-keto group gave the diol **4**. None of the 4-hydroxy α-keto ester was obtained. When the reduction of **2** was repeated with a larger excess of sodium borohydride (8 equiv), compound **4** was obtained in 87% yield without chromatography.

Scheme I



Our intended route from this point was a sequence starting with the monoalcohol **3** and leading to the benzothienyl-5-acetate **6** (Scheme I). Compound **3** was dehydrated by refluxing in toluene with *p*-toluenesulfonic acid and **5** was isolated in 88% yield. Treatment of **5** with sodium borohydride gave the olefinic alcohol **7**; interestingly, the phenolic benzothienyl-5-acetate **8** was also obtained. Reflux of **7** with *p*-toluenesulfonic acid afforded the benzothienyl-5-acetate **6**. However, similar reflux of the dihydroxy compound **4** gave 21% of **6** in one step.

(1) Kukolja, S.; Draheim, S. E.; Pfeil, J. L.; Cooper, R. D. G.; Graves, B. J.; Holmes, R. E.; Neel, D. A.; Huffman, G. W.; Webber, J. A.; Kinnick, M. D.; Vasileff, R. T.; Foster, B. J. *J. Med. Chem.*, preceding paper in this issue.

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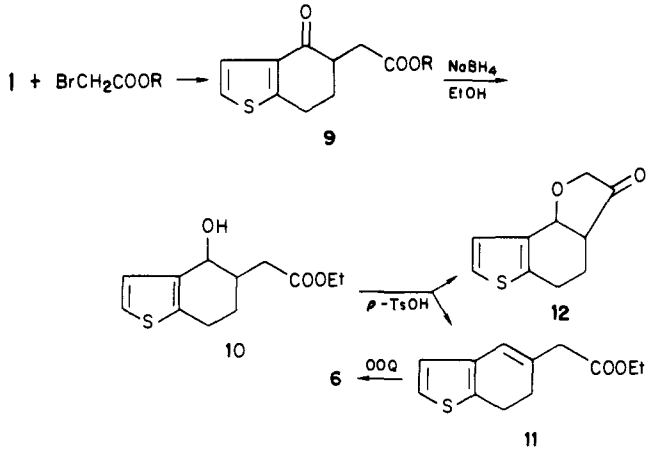
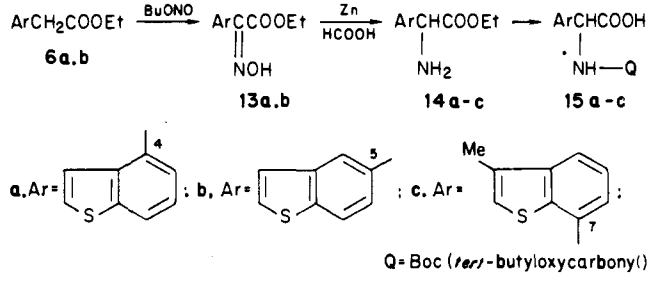
Scheme II



Scheme III



The aromatization of **4** and **7** most likely proceeds by the loss of 2 mol of water, followed by isomerization of allylic intermediates to the aromatic system.

Alternatively, the benzothienyl compound **6** can be prepared by the sequence of reactions shown in Scheme II. Alkylation of ketone **1** with methyl bromoacetate in the presence of lithium diisopropylamide gave the γ-keto ester **9**. Reduction of **9** with sodium borohydride in ethanol afforded the ethyl ester of alcohol **10**, which upon dehydration with *p*-toluenesulfonic acid yielded the β,γ-olefinic ester **11** in 29% yield, along with 23% of lactone **12**. Dehydrogenation of **11** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave the desired benzothienyl-5-acetate **6** in 44% yield.

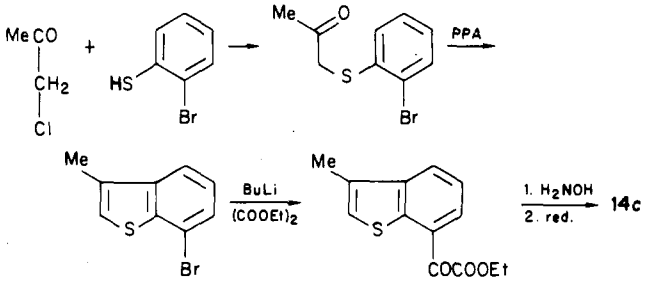
Treatment of the heteroaromatic acetates **6a,b** with butyl nitrite[2] in the presence of sodium ethoxide in ethanol gave the oximes **13a,b**, which on reduction with zinc and formic acid yielded the amino esters **14a,b**. These esters were hydrolyzed and the amino group was protected with di-*tert*-butyl dicarbonate in aqueous dioxane yielding the Boc-protected amino acids **15a–c** (Scheme III).

The 7-positional analogue **14c** was prepared with *o*-bromothiophenol as starting material (Scheme IV). The mercapto group was alkylated with chloroacetone and the alkylation product cyclized with polyphosphoric acid (PPA). The resulting 7-bromo-3-methylbenzothiophene was metalated with butyllithium and the lithio derivative treated with diethyl oxalate to give ethyl 3-methylbenzothiophene-7-glyoxalate. This derivative was later treated with hydroxylamine and the obtained oxime reduced to the amino ester **14c**. After hydrolysis and protection of the amino group, **15c** was used for coupling with 7-ADCA.

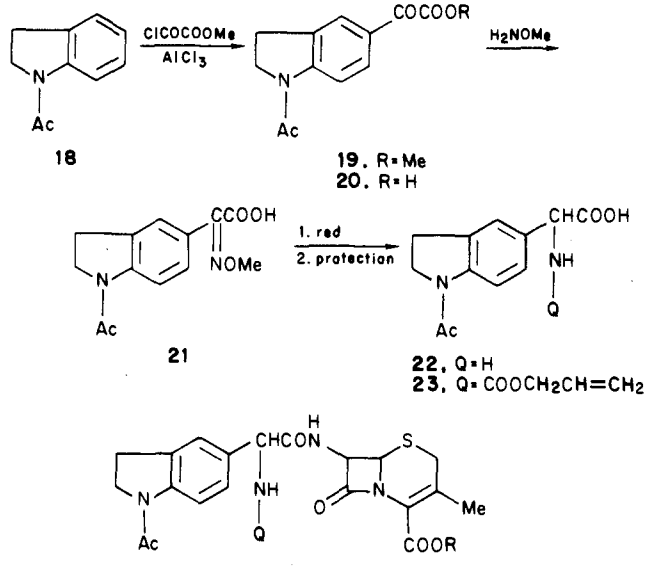
Acylation of the *p*-NB ester of 7-aminodeacetoxycephalosporanic acid (7-ADCA) with the Boc-protected heterocyclic glycines **15a–c** was achieved with *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) for activation of the carboxyl group, and the diprotected cephalosporins **16a–c** were isolated in good yields. The protecting groups in **16a** and **16b** were removed successively, the pNB ester group by catalytic hydrogenation, and the Boc group by hydrolysis with *p*-toluenesulfonic acid, yielding epimeric cephalosporins **17a,b**. The *R* and *S* epimers were separated by HPLC.

In the case of **16c** the diprotected *RS* mixture was chromatographed and the *R* and *S* epimers isolated. From the *R* epimer the Boc group was removed with *p*-toluenesulfonic acid and the free amine isolated in 73% yield. The protective pNB group was reduced in the presence of palladium on charcoal and the desired *R* epimer **17c** isolated in 65% yield.

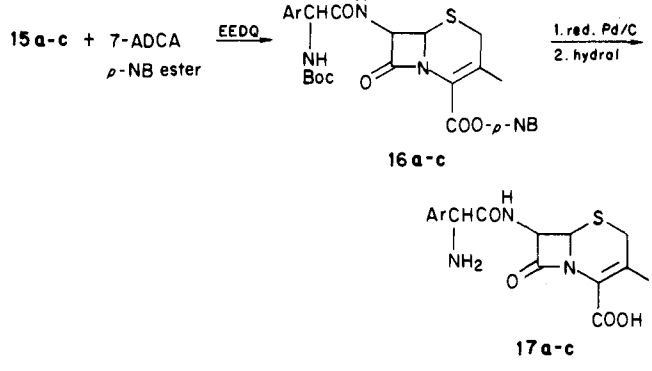
Scheme IV



Scheme V



24, Q = $COOCH_2CH{=}CH_2$; R = $CH_2CH{=}CH_2$
25, Q = and R = H



Synthesis of the (*N*-acetylindolinyl)-5-glycine **22** and subsequent coupling with the 7-ADCA nucleus is illustrated in Scheme V. The glyoxalate group in compound

(2) Levin, N.; Hartung, W. H. "Organic Syntheses"; Wiley: New York, 1955; Collect. Vol. III, p 191.

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

	Staphylococcus aureus				*Staphylococcus epidermidis*	*Streptococcus*			*Haemophilus influenzae*	
	penicillin G		methicillin							
no.	sens, X1.1	res, V41	S13E	X400	EPI 1	*pyogenes*, C203	*pneumoniae*, Park	group D, X66	sens, C.L.	res, 76
17a	2	16	64	64	16	0.25	1	64	4	2
17b	0.5	4	64	64	2	0.125	0.5	64	8	4
17c	2	16	64	64	8	1	2	64	8	1
25	2	64	64	64	8	1	1	64	16	8
30d	0.5	32	64	64+	16	0.25	1	64+	64	2
30e	0.5	32	16	64+	4	0.25	0.5	64+	8	2
31	2	16	128+	128+	32	2	2	128	8	8
cephalexin	4	128	128+	128	32	0.5	2	128	8	8

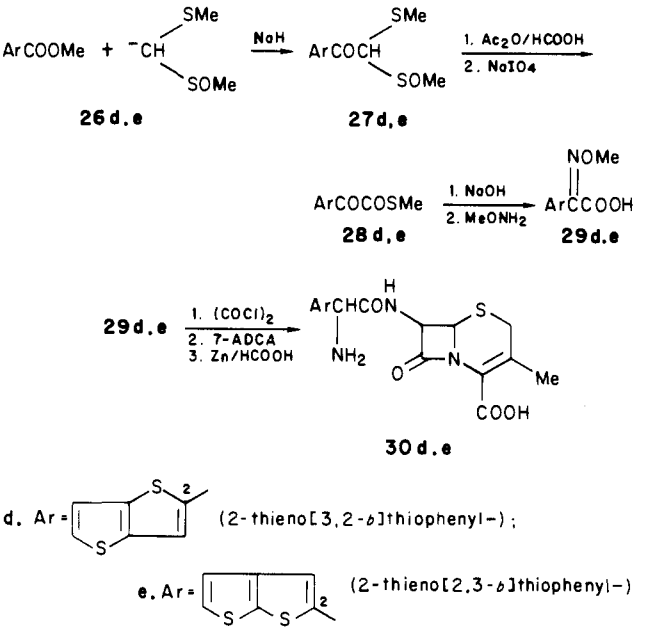
[a] Numerical values are MIC in μ/mL. Agar dilution method of Kirst et al.: Kirst, H. A.; Wild, G. M.; Baltz, R. H.; Hamill, R. L.; Ott, J. L.; Counter, F. T.; Ose, E. E. *Antibiot.* 1982, *35*, 1675.

19 was introduced in position 5 by treatment of the *N*-acetylindoline **18** with methoxalyl chloride. After hydrolysis, the α-keto acid **20** was isolated in 66% yield. Treatment of **20** with methoxylamine in an aqueous solution at pH 4–5 for 4 h afforded the corresponding oxime, which upon reduction with palladium on carbon gave the desired *RS*-amino acid **22**. The amino group in **22** was protected by treatment with allyl chloroformate to give compound **23** in 86% yield. Coupling of **23** with the allyl ester of 7-ADCA was achieved in 85% yield by stirring with EEDQ in THF and MeCN for 4.5 h. Removal of the allyl groups was effected according to the method of Jeffrey and McCombie[3] with tributyltin hydride, triphenylphosphine, and palladium acetate. The zwitterion **25** was isolated in 52% yield as an RS mixture. The mixture was separated by HPLC, and the *R* and *S* isomers were isolated as pure compounds and tested.

Since 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* isomer, the more potent one is designated as the *R* isomer.

Two isomers, the (2-thieno[3,2-*b*]thiophenylglycylamido)cephalosporin **30d** and the (2-thieno[2,3-*b*]thiophenylglycylamido)cephalosporin **30e**, were made as illustrated in Scheme VI.[4] The required methyl thienothiophene-2-carboxylates **26d–e** were prepared by oxidative cyclization of the appropriately substituted β-aryl-α-mercaptoacrylic acids, as reported by Chakrabarti et al.[5] and Gronowitz and Persson.[6] Homologation of the heterocyclic esters **26d–e** with the carbanion of methyl (methylthio)methyl sulfoxide was accomplished according to the procedure published by Ogura and co-workers[7] to afford the β-keto sulfoxides **27d,e**. These sulfoxides were heated with acetic anhydride and formic acid at 65 °C for 30 min and then with sodium periodate for 20 min to give the α-keto thio esters **28d,e**. The corresponding α-methoximes were made by treatment of the α-keto thio esters with methoxyamine followed by hydrolysis of esters to the α-methoximinoacetic acids **29d,e**. These acids were converted into acid chlorides, which were then used for acylation of 7-ADCA. Following the reduction of the methoximino group with zinc and formic acid, the new cephalosporins **30d,e** were isolated as mixture of epimers. The diastereomers were separated by preparative HPLC and

Scheme VI



the *R* epimers **30d** and **30e** were tested.

Biological Results and Discussion

The in vitro and in vivo testing was performed only with *R* enantiomers, since it is known that *S* epimers are less active.[8]

The antibiotic activities of the new cephalosporins were evaluated against several strains of *Staphylococcus aureus*, *Streptococcus* spp. and *Haemophilus influenzae* as shown in Table I. For comparison, the activities of cephalexin are included. The in vitro susceptibility of microorganisms in agar dilution tests indicates that compound **17b** is superior to cephalexin against staphylococcal and streptococcal strains, while compound **17a** is more potent against *H. influenzae*. It is known that bicyclic derivatives generally have somewhat better Gram-positive and less Gram-negative activity as compared with the simple monocyclic heteroaromatic derivatives.[9] A similar trend was also observed with the bicyclic thienothiophene-2-glycine derivatives **30d** and **30e** when they were compared with (2-thienylglycylamido)deacetoxycephalosporanic acid (**31**).

(3) Jeffrey, P. D.; McCombie, S. W. *J. Org. Chem.* 1982, *47*, 587.
(4) Litvinov, V. P.; Gol'gfarb, Ya. L. "Advances in Heterocyclic Chemistry"; Katritzky, A., Boulton, A. J., Eds.; Academic Press: New York, 1976; Vol. 19, p 123.
(5) Chakrabarti, P. M.; Chapman, N. B.; Clark, K. *Tetrahedron* 1969, *25*, 2781.
(6) Gronowitz, S.; Persson, B. *Acta Chem. Scand.* 1967, *21*, 812.
(7) Ogura, K.; Yamashita, M.; Tsuchihashi, G. *Tetrahedron Lett.* 1978, *1303*.
(8) Böhme, E. H. W.; Bambury, R. E.; Baumann, R. J.; Erickson, R. C.; Harrison, B. L.; Hoffman, P. F.; McCarty, F. J.; Schnettler, R. A.; Vaal, M. J.; Wenstrup, D. L. *J. Med. Chem.* 1980, *23*, 405 and ref 2 in the same paper.
(9) Gorman, M.; Ryan, C. W. "Cephalosporins and Penicillins: Chemistry and Biology"; Flynn, E. H., Ed.; Academic Press: New York, 1972; p 577.

Table II. Mouse Protection Tests: ED_{50} Values (mg/kg × 2)[a]

	S. aureus		S. pyogenes		S. pneumoniae	
compd	sc	po	sc	po	sc	po
25	2.11	0.85	0.79	2.6	10.6	9.3
cephalexin	1.66	0.9	2.9	1.77	30.5	37

[a] Compounds administered either subcutaneously (sc) or orally (po) to 20-g mice at 1- and 5-h intervals after intraperitoneal bacterial challenge.

Compound **25** was chosen for in vivo testing. A group of 10 mice were inoculated with *S. aureus*, *S. pyogenes*, and *S. pneumoniae* and then given oral and subcutaneous doses of cephalosporin **25** at 1- and 5-h intervals after inoculation. According to the ED_{50} values shown in Table II, compound **25** demonstrates both parenteral and oral activity in mice. In comparison with cephalexin against *S. pneumoniae* infections, compound **25** gives better mouse protection both orally and subcutaneously.

Experimental Section

Melting points are uncorrected. IR spectra were recorded on Beckman 1R-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 VA-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, 79% water, and 1% acetic acid as the mobile phase. Temperatures are reported in degrees centigrade. Solvent were dried over molecular sieves. Estimation of purity of analogues without elemental analyses was greater than 95% by analytical HPLC. In most cases the identity of the other components was determined to be unreacted side chain and/or cephem nucleus.

4,5,6,7-Tetrahydro-α,4-dioxobenzo[*b*]thiophene-5-acetic Acid Ethyl Ester (2). 4-Keto-4,5,6,7-tetrahydrothianaphthene (1; 10 g, 66 mmol) was dissolved in 13 mL of diethyl oxalate and added dropwise to a cooled solution of 3.3 g of NaH (50% dispersion in mineral oil), 130 mL of ether, and 0.35 mL of EtOH in an ice bath. The reaction was mechanically stirred, allowing the ice bath to warm gradually to room temperature. After 5 h, 1.3 mL of EtOH was added and the reaction mixture stirred an additional 15 min. The reaction mixture was diluted with water and extracted twice with ether. The aqueous portion was layered with fresh ether and acidified to pH 2.0. The ether layer was separated, dried, and evaporated, yielding an oil which crystallized upon stirring with cold EtOH. The crystals was filtered and dried to give 14.6 g (88%) of the desired compound: NMR ($CDCl_3$) δ 1.42 (t, 3 H, CH_3), 3.1 (m, 4 H, C_6 and C_7 H_2), 4.37 (q, 2 H, ethyl CH_2), 7.09 and 7.40 (2 d, 2 H, C_2 and C_3 H). Anal. ($C_{12}H_{12}O_4S$) C, H.

4,5,6,7-Tetrahydro-α-hydroxy-4-oxobenzo[*b*]thiophene-5-acetic Acid Ethyl Ester (3). 4,5,6,7-Tetrahydro-α,4-dioxobenzo[*b*]thiophene-5-acetic acid ethyl ester (2; (11.52 g, 46 mmol) was dissolved in 250 mL of EtOH and cooled to 0 °C. Sodium borohydride (1 g, 2.25 equiv) was added and the mixture was stirred 4 h at 0 °C. The EtOH was evaporated and the residue was dissolved in EtOAc, washed twice with 5% HCl, separated, and dried, yielding a mixture of **2**, **3**, and **4**. This material was chromatographed by applying to a column of silica gel and eluting with toluene, followed by 10% EtOAc in toluene: 417 mg (4%) of **2**, 3.16 g (27%) of 4, and 3.65 g (31%) of the desired product **3**. Compound **3**: NMR ($CDCl_3$) δ 1.28 (t, 3 H, CH_3), 2.38 (m, 2 H, α-H and C_5 H), 3.17 (m, 4 H, C_6 and C_7 H_2), 4. 29 (q, 2 H, ethyl CH_2), 7.03 and 7.33 (2 d, 2 H, C_2 and C_3 H). Anal. ($C_{12}H_{14}O_4S$) C, H.

4,5,6,7-Tetrahydro-α,4-dihydroxybenzo[*b*]thiophene-5-acetic Acid Ethyl Ester (4). 4,5,6,7-Tetrahydro-α,4-dioxobenzo[*b*]thiophene-5-acetic acid ethyl ester (2; 5.04 g, 20 mmol) was dissolved in 50 mL of EtOH and cooled to 0 °C in an ice bath. The ice bath was removed and 760 mg (4 equiv) of $NaBH_4$ was added, followed by two additional 380-mg portions (one after 2 h, the second after 4 h). The EtOH was then evaporated, the residue was dissolved in EtOAc/H_2O, and the pH was adjusted to 7.0 with 1 N HCl. The EtOAc layer contained 4.44 g (87%) of the desired compound: NMR ($CDCl_3$) δ 1.32 (t, 3 H, CH_3), 2.6 (m, 4 H, C_6 and C_7 H_2), 3.25 (d, 1 H, C_4 OH), 4.26 (m, 3 H, ethyl CH_2 and α-H), 4.92 (t, 1 H, C_4H), 7.03 (2 d, 2 H, C_2 and C_3 H). Anal. ($C_{12}H_{16}O_4S$) C, H.

(6,7-Dihydro-4-oxobenzo[*b*]thien-5(4*H*)-ylidene)acetic Acid Ethyl Ester (5). A mixture of 1.27 g (5 mmol) of **3**, 0.95 g of *p*-toluenesulfonic acid, and 50 mL of toluene was heated until the toluene began to reflux. The reaction was then diluted with EtOAc and washed with H_2O, 5% HCl, and 5% $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$, and evaporated, yielding 1.04 g (88%) of the desired product: NMR ($CDCl_3$) δ 1.35 (t, 3 H, CH_3), 3.16 and 3.55 (2 t, 4 H, C_6 and C_7 H_2), 4.26 (q, 2 H, ethyl CH_2), 6.84 (m, 1 H, α-H), 7.08 and 7.42 (2 d, 2 H, C_2 and C_3 H). Anal. ($C_{12}H_{12}O_3S$) C, H, O.

Benzothiophene-5-acetic Acid Ethyl Ester (6). A mixture of 1.92 g (7.2 mmol) of **4**, 0.15 g of *p*-toluenesulfonic acid, and 50 mL of toluene was refluxed for 2 h, then washed with water, and dried over $MgSO_4$ and the toluene evaporated. TLC (30% EtOAc/hexane) indicated several products. The mixture was chromatographed by applying to a silica gel column and eluting with a hexane to gradient. The compound that eluted first was the desired product **6**: 350 mg (21%); NMR ($CDCl_3$) δ 1.24 (t, 3 H, CH_3), 3.71 (s, 2 H, α-CH_2), 4.14 (q, 2 H, ethyl CH_2), 7.15–7.85 (m, 5 H, arom). Anal. ($C_{12}H_{12}O_2S$) C, H, O.

Alternative procedure: **7** (138 mg, 0.58 mmol) was dissolved in 5 mL of toluene. Then 11 mg of *p*-toluenesulfonic acid was added and the solution refluxed 2 h. TLC (30% EtOAc/hexane) of the product is identical with that of compound **6** above.

(6,7-Dihydro-4-hydroxybenzo[*b*]thien-5(4*H*)-ylidene)-acetic Acid Ethyl Ester (7, Scheme I) and 4-Hydroxybenzothiophene-5-acetic Acid Ethyl Ester (8). To a solution of **5** (500 mg, 2.1 mmol) in 5 mL of EtOH was added 45 mg (2 equiv) of $NaBH_4$ and the mixture was stirred 3 h at room temperature. The EtOH was evaporated, then the residue was dissolved in EtOAc/H_2O, and the mixture was neutralized to pH 7.0 with 1 N HCl. The EtOAc layer was separated, dried over $MgSO_4$, and evaporated. TLC (30% EtOAc/toluene) indicated two major products. The mixture was applied to a silica get column and eluted with toluene to give **7** and **8**. **7**: NMR ($CDCl_3$) δ 1.42 (t, 3 H, CH_3), 2.75 (d, 1 H, OH), 2.94–3.88 (m, 4 H, C_6 and C_7 H_2), 4.31 (q, 2 H, ethyl CH_2), 5.22 (d, 1 H, C_4 H), 6.22 (s, 1 H, α-H), 7.17 (2 d, 2 H, C_2 and C_3 H). Anal. ($C_{12}H_{14}O_3S$) C, H, O.

8: NMR ($CDCl_3$) δ 1.17 (t, 3 H, CH_3), 3.64 (s, 2 H, α-CH_2), 4.09 (q, 2 H, ethyl CH_2), 6.84–7.44 (m, 4 H, arom), 8.27 (s, 1 H, OH).

5-(Carbomethoxymethyl)-4-keto-4,5,6,7-tetrahydrothianaphthene (9). A solution of 2.8 mL (20 mmol) of diisopropylamine in 200 mL of THF was cooled to −20 °C under N_2 with stirring. A 12.5-mL sample of a 1.6 M solution of *n*-BuLi in hexanes (20 mmol) was added and stirring was continued for 0.5 h between −20 and −10 °C. The solution was cooled to −70 °C and stirred 1 h more. To this solution was added 3.04 g (20 mmol) of 4-keto-4,5,6,7-tetrahydrothianaphthene (1) in 50 mL of THF dropwise over 5–10 min. Stirring was continued for 0.5 h at −70 °C. A solution of 1.9 mL (20 mmol) of methyl bromoacetate in 20 mL of THF was then added dropwise over 5–10 min. After 15 min at −70 °C the cooling bath was removed and the reaction was allowed to warm to room temperature. Stirring was continued at room temperature under N_2 for 18 h. The THF was evaporated and the residue was dissolved in EtOAc/H_2O. The EtOAc was washed with 5% $NaHCO_3$ and 5% HCl, dried ($MgSO_4$), filtered, and evaporated to give 4.24 g of crude product. The crude product was purified by chromatography over silica gel,

eluting with EtOAc and toluene, yielding 2.2 g (49%) of the desired product (**9**): NMR ($CDCl_3$) δ 1.8–3.2 (m, 7 H, aliphatic), 3.72 (s, 3 H, OMe), 7.00 (d, J = 6 Hz, 1 H, aromatic), 7.30 (d, J = 6 Hz, 1 H, aromatic); MS, m/e 224 (M^+), 193, 192, 150, 124, 96; IR ($CHCl_3$) 1733 (CO_2Me), 1674 cm^{-1} (C=O).

5-(Carboethoxymethyl)-4-hydroxy-4,5,6,7-tetrahydrothianaphthene (10). The thianaphthene derivative **9** (1.34 g, 6.0 mmol) was dissolved in 30 mL of 2B EtOH at room temperature. $NaBH_4$ (120 mg, 3.2 mmol) was added and stirring at room temperature was continued overnight (~20 h). The EtOH was evaporated and the residue dissolved in EtOAc/5% HCl. The EtOAc layer was washed successively twice with 5% HCl, twice with 5% $NaHCO_3$, and once with saturated NaCl. The organic layer was dried ($MgSO_4$), filtered, and evaporated to give 1.31 g (91%) of the desired product **10**, as a colorless gum that crystallized on standing: NMR ($CDCl_3$) δ 1.25 (t, J = 7 Hz, 3 H), 1.5–3.0 (m, 7 H, aliphatic), 4.16 (q, J = 7 Hz, 2 H), 4.44 (br s, 1 H), 7.03 (m, 2 H, aromatic); MS, m/e 240 (M^+), 194, 152, 135, 126, 97; IR ($CHCl_3$) 1724 cm^{-1}; UV (EtOH) λ_{max} 232 nm (ε 7700).

5-(Carboethoxymethyl)-6,7-dihydrothianaphthene (11). The thianaphthene **10** (1.20 g, 5 mmol) was dissolved in 50 mL of toluene. p-TSOH (95 mg, 0.5 mmol) was added and the solution was heated briefly (1–2 min) to reflux. The toluene was evaporated and the residue was dissolved in EtOAc. The organic layer was washed twice with 5% HCl, twice with 5% $NaHCO_3$, and once with saturated NaCl, dried ($MgSO_4$), filtered, and evaporated to give 1.01 g of crude product. Chromatography over silica gel, eluting with toluene, gave 324 mg (29%) of the desired product **11** along with 223 mg (23%) of the lactone **12**. Spectral data for **11**: NMR ($CDCl_3$) δ 1.25 (t, J = 7 Hz, 3 H), 2.43 (m, 2 H), 2.90 (m, 2 H), 3.14 (br s, 2 H), 4.13 (q, J = 7 Hz, 2 H), 6.29 (br s, 1 H), 6.75 (d, J = 5 Hz, 1 H), 6.96 (d, J = 5 Hz, 1 H); MS, m/e 22 (M^+), 149, 147, 134; IR ($CHCl_3$) 1727 cm^{-1} (CO_2Et); UV EtOH) λ_{max} 225 nm (ε 17700). Spectral data for **12**: NMR ($CDCl_3$) δ 1.5–3.1 (m, 7 H), 5.33 (d, J = 5 Hz, 1 H), 7.00 (d, J = 5.3 Hz, 1 H), 7.11 (d, J = 5.3 Hz, 1 H); MS, m/e 194 (M^+, 150, 149, 135; IR ($CHCl_3$) 1769 cm^{-1} (C=O); UV (EtOH) λ_{max} 232 nm (ε 6600).

5-(Carboethoxymethyl)thianaphthene (6). The thianaphthene **11** (300 mg, 1.35 mmol) was dissolved in benzene. The solution was cooled to ~10 °C and DDQ (313 mg, 1.35 mmol) was added. The reaction immediately turned dark brown. After 30 min, the reaction was diluted with EtOAc, and the organic layer was washed twice with 5% HCl, three times with 5% $NaHCO_3$, and once with saturated HCl, dreid ($MgSO_4$), filtered, and evaporated to give 369 mg of crude product. Chromatography over silica gel, eluting with EtOAc/toluene, yielded 107 mg (36%) of the desired product: NMR ($CDCl_3$) δ 1.23 (t, J = 7 Hz, 3 H), 3.68 (s, 2 H), 4.11 (q, J = 7 Hz, 2 H), 7.1–7.9 (m, 5 H).

Oxime of Benzothiophene-5-acetic Acid Ethyl Ester (13b). A 0.43 M solution of NaOEt in EtOH was prepared and 21 mL was added to a solution of **6b** (725 mg, 3.3 mmol) in 6 mL of absolute EtOH. The reaction was cooled in a wet ice/acetone bath and 0.52 mL of butyl nitrite was added. After 10 min the cooling bath was removed and the reaction mixture stirred overnight. An additional 0.2 mL of butyl nitrite was then added and the reaction stirred another 4 h before addition of 0.7 mL of HOAc. The EtOH was evaporated and the product dissolved in EtOAc/H_2O. The EtOAc layer was separated and washed successively with 5% $NaHCO_3$, 5% HCl, and brine, then dried over $MgSO_4$, and evaporated. The syn and anti oximes formed were separated from unreacted **6b** by silica gel chromatography yielding 252 mg (31%) of the mixture of oximes: NMR ($CDCl_3$) δ 1.12 (t, 3 H, CH_3), 4.14 (q, 2 H, CH_2), 7.0–8.0 (m, 5 H, arom). Anal. ($C_{12}H_{12}NO_3S$) C, H, N.

Compound **13a** was obtained similarly from **6a**.

Benzothiophene-5-glycine Ethyl Ester (14b). The mixture of oximes **13b** (250 mg, 1 mmol) was dissolved in 5 mL of EtOH. Formic acid (5 mL) and water (3 mL) were added, and the solution was cooled to 0 °C. Powdered zinc (241 mg) was added and the reaction stirred at 0 °C for 3 h and then at room temperature overnight. The EtOH was evaporated and the product taken up in EtOAc/5% HCl. The aqueous portion was separated, layered with fresh EtOAc, and neutralized to pH 7.0 with 1 N NaOH. The EtOAc extract was dried over $MgSO_4$ and evaporated to give 129 mg (55%) of the desired compound **14b**: NMR ($CDCl_3$) δ 1.22 (t, 3 H, CH_3) 2.04 (br, 2 H, NH_2), 4.14 (m, 2 H, CH_2), 4.67 (s, 1 H, α-H), 7.12–7.92 (m, 5H, arom). Anal. ($C_{12}H_{13}NO_2S$) C, H. N.

Reduction of **13a** under similar conditions gave the amino ester, which was immediately converted to the Boc-protected **15a** as shown in the next experiment.

Boc-Protected Benzothiophene-5-glycine (15b). A suspension of 76 mg (0.32 mmol) of **14b** in 1 mL of EtOH and 0.3 mL of 2.22 N NaOH was stirred until TLC (4:1 EtOAc/HOAc) indicated complete hydrolysis of the ester. Water (1.6 mL) and dioxane (1.6 mL) were then added, and the pH was adjusted to 8.0 with 1 N HCl. Di-*tert*-butyl dicarbonate (0.08 mL) was added and the pH maintained at 8.0–8.5 with Et_3N. After the pH stabilized, the solution was neutralized, and the dioxane and EtOH were evaporated. The aqueous residue was layered with EtOAc and acidified to pH 2.5 with 1 N HCl. The EtOAc extract was separated, dried, and evaporated to give 85 mg (87%) of the Boc-protected compound **15b**: NMR (Me_2SO-d_6) δ 2.38 (s, 9 H, Boc CH_3), 5.19 (d, 1 H, α-H), 7.25–8.0 (m, 5 H, arom NH).

Coupling of Benzothiophene-5-glycine and 7-ADCA *p*-NB Ester. The Boc-protected amino acid (80.6 mg, 0.26 mmol) was dissolved in 3 mL of dry THF and 0.07 g of EEDQ was added. The solution was added in one portion to a solution of 0.1 g of 7-ADCA p-NB ester in 3 mL of dry CH_3CN and 3 mL of dry THF. The reaction was stirred 5.5 h at room temperature, and then the solvents were evaporated. The product was dissolved in EtOAc and washed successively with H_2O, 5% $NaHCO_3$ (2×) 5% HCl (2×), H_2O, and brine, then dried over $MgSO_4$, and evaporated to give 158 mg (95%) of an *RS* mixture of the desired cephalosporin **16b**. Another 150 mg of the *RS* mixture was obtained from a subsequent reaction, and the two were combined. The *RS*-mixture was chromatographed on a column of silica gel, eluted with 15% EtOAc/toluene to give 107 mg of *S* isomer and 100 mg of the desired *R* isomer (**16b**): NMR ($CDCl_3$) δ 1.38 (s, 9 H, Boc CH_3), 2.07 (s, 3 H, C_3CH_3), 3.20 (AB q, 2 H, C_2 H_2), 4.86 (d, 1 H, C_6 H), 5.23 (s, 2 H, p-NB CH_2), 5.34 (d, 1 H, α-H), 5.70 (m, 2 H, C_7 H, NH), 6.77 (d, 1 H, NH), 7.1–8.2 (m, 9 H, arom).

Coupling of **15a** was done under similar conditions to yield 87% of **16a**.

Removal of the Boc Group from 16b. The Boc-protected cephalosporin (R epimer, 100 mg, 0.16 mmol) was dissolved in 6 mL of dry CH_3CN, then 34 mg of p-toluenesulfonic acid was added, and the solution was stirred 30 min at room temperature. Upon standing at room temperature overnight, the tosylate salt crystallized out. The crystals were filtered and washed with CH_3CN to give 82.7 mg. The salt was taken up in EtOAc/5% $NaHCO_3$. The EtOAc layer was separated, dried over $MgSO_4$, and evaporated to give 61.3 mg (73%) of the free amine: NMR ($CDCl_3$) δ 1.78 (br, 2 H, NH_2), 2.16 (s, 3 H, C_3 CH_3), 3.38 (AB q, 2 H, C_2 H_2), 4.71 (s, 1 H, α-H), 4.98 (d, 1 H, C_6 H), 5.3 (s, 2 H, p-NB CH_2), 5.74 (dd, 1 H, C_7 H), 7.2–8.3 (m, 9 H, arom).

Removal of the *p*-NB Protective Group. Palladium on carbon (5%, 62 mg) was reduced in 10 mL of EtOH for 30 min at 60 psi H_2. The p-NB-protected cephalosporin (*R* epimer, 61.3 mg, 0.114 mmol) was dissolved in 40 mL of MeOH and 0.2 mL of 1 N HCl and added to the prereduced catalyst. The mixture was hydrogenated at 60 psi for 1.5 h. Before filtering the catalyst, another 0.2 mL of 1 N HCl was added and stirred 5 min. The filtered catalyst was washed with 4 mL of 1:1 MeOH/H_2O and then 20 mL of MeOH. The filtrates were combined, and the MeOH was evaporated. The aqueous residue was layered with EtOAc, an additional 20 mL of H_2O was added, and the pH was adjusted to 7.0 with 1 N NaOH. The EtOAc layer was separated and the aqueous extracted once more with EtOAc to remove the p-NB polymer. The aqueous portion was lyophilized after adjusting the pH to 4.25. The powder was suspended in 2 mL of H_2O, the pH adjusted to 4.25, and the mixture filtered to give 30 mg (65%) of the desired (*R*)-cephalosporin **17b**: NMR (D_2O/DCl) δ 1.96 (s, 3 H, 3-CH_3), 3.16 (AB q, 2 H, C_2 H_2), 5.02 (d, 1 H, C_6 H), 5.40 (s, 1 H, α-H), 5.58 (d, 1 H, C_7 H), 7.35–8.05 (m, 5 H, arom).

Removal of the Boc and p-NB groups from **16a** and **16c** was performed as described for **16b**. The racemic **17a** and **17b** were separated by preparative HPLC and the *R* epimers tested.

3-Methyl-7-(ethylglyoxyl)benzothiophene. Diethyl oxalate (13.5 mL, 0.1 mol) was dissolved in 500 mL of a 4:1:1 THF/pentane/ether solution and cooled to −120 °C in a pentane/N_2 bath. A second solution of 11.35 g (0.05 mol) of 7-bromo-3-

methylbenzothiophene and 7.6 mL of *N,N,N',N'*-tetramethylethylenediamine in another 500 mL of the 4:1:1 THF/pentane/ether solution was cooled to −120 °C and 31.3 mL of 1.6 M *n*-butyllithium was added. This second solution was transferred via canula into the first solution over a period of 40 min, and due to freezing, both flasks were allowed to warm to −105 to −110 °C. After the addition was complete, the reaction was quenched with 120 mL of saturated NH_4Cl, and the solvents were evaporated. The remaining aqueous solution was extracted twice with CH_2Cl_2, and the combined extracts were dried over $MgSO_4$ and evaporated to give 14 g of crude product, which was purified by HPLC to give 5.1 g (41%) of the desired product. Anal. ($C_{13}H_{12}O_3S$) C, H.

3-Methyl-7-benzothienylglycine Ethyl Ester (14c, Scheme IV). A solution of 5.1 g (0.021 mol) of 7-(ethylglyoxyl)-3-methylbenzothiophene, 1.57 g (1.1 equiv) of hydroxylamine hydrochloride, and 1.89 g of sodium acetate in 250 mL of ethanol was refluxed 2 h. A second 1.57-g portion of $H_2NOH{\cdot}HCl$ was then added and reflux continued for 2 h before addition of another 1.57-g portion and the reaction was stirred at room temperature overnight and then refluxed 90 min to complete the oximation. The crude reaction mixture was filtered, the filtrate was evaporated to an oil and the oil was redissolved in MeOH, and the solution was dried over $MgSO_4$ and evaporated. The crude oxime was dissolved in 100 mL of MeOH, and 100 mL of 50% formic acid was added and the solution was cooled to 0 °C. Zinc dust (1.34 g, 1 equiv) was added in portions and the reaction mixture stirred at 0 °C. After 90 min, 2 equiv more of Zn was added and the reaction mixture stirred 4 h, allowing the ice bath to warm to room temperature. Another 2 equiv of Zn was added, and after the mixture was stirred 4 more h all of the oxime had been reduced. The Zn salts were filtered off, and the MeOH was evaporated. The aqueous solution was adjusted to pH 8.5 and extracted with EtOAc. The EtOAc extracts were combined, dried, over $MgSO_4$, and evaporated to give 2.62 g (50%) of the amino ester.

***N*-Acetylindoline 5-Keto Ester 19 (Scheme V).** A solution of *N*-acetylindoline (18; 16.1 g, 0.1 mol) in 50 mL of carbon disulfide and 20 mL of methoxalyl chloride was stirred mechanically as 40 g of $AlCl_3$ was gradually added. The reaction was then heated on a water bath and stirred at 50 °C for 30 min at which time it solidified. The solid mass was broken up and poured over ice, yielding 15.2 g of yellow crystals which were by NMR approximately 50% *N*-acetylindoline (18). This material was triturated with EtOH, filtered, and dried to give 5.5 g of the methyl ester **19**, which was then recrystallized from EtOAc (5 mL/g): NMR (Me_2SO-d_6) δ 2.21 (s, 3 H, acetyl CH_3), 3.19 and 4.17 (2 t, 4 H, C_2 and C_3 H_2), 3.92 (s, 3 H, ester CH_3), 7.65–8.25 (m, 3 H, arom); mp 126–128 °C. Anal. ($C_{13}H_{13}NO_4$) C, H, N. O.

Hydrolysis of Ester 20 (Scheme IV). The methyl ester **19** (5.5 g, 22 mmol) was dissolved in 10 mL of hot EtOH and added to a solution of 1.43 g of 85% KOH in 10 mL of EtOH. The potassium salt immediately precipitated and was filtered, washed with EtOH, and dried. The salt was dissolved in a minimal amount of H_2O and layered with EtOAc. The pH was adjusted to 2.0 with HOAc, the brine was added, and the organic layer was separated, dried over $MgSO_4$, and evaporated to give 3.4 g (66%) of the desired acid **20**: NMR (Me_2SO-d_6) δ 2.20 (s, 3 H, CH_3), 3.19 and 4.16 (2 t, 4 H, C_2 and C_3 H_2), 7.65–8.25 (m, 3 H, arom); mp 216–217 °C. Anal. ($C_{12}H_{11}NO_4$) C, H, N, O.

Formation of Methoxime 21 (Scheme IV). Compound **20** (4.66 g, 20 mmol) was stirred with 2.5 g (30 mmol) of methoxylamine hydrochloride in 20 mL of H_2O and 20 mL of 1 N NaOH. Additional 1 N NaOH was added to bring the pH to 4.8 and then to maintain the pH at 4.0–5.0. After 4 h the pH was adjusted to 8.0 and the excess methoxylamine was extracted with 150 mL of ether. The aqueous portion was acidified with 1 N HCl to pH 2.0 and the resulting precipitate was filtered and dried to give 4.3 g (82%) of the desired methoxime **21**: NMR (Me_2SO-d_6) δ 2.16 (s, 3 H, acetyl CH_3), 3.15 and 4.11 (2 t, 4 H, C_2 and C_3 H_2), 3.90 (s, 3 H, methoxime CH_3), 7.2–8.2 (m, 3 H, arom); mp 183 °C. Anal. ($C_{13}H_{14}N_2O_4$) C, H, N, O.

***N*-Acetylindoline-5-glycine (22).** Palladium on carbon (5%, 4.3 g) was reduced in 40 mL of EtOH for 30 min at 60 psi H_2. The methoxime **21** (4.3 g, 16 mmol) was suspended in 200 mL of MeOH and added to the prereduced catalyst. The mixture was hydrogenated at 60 psi for 2.5 h. Then 20 mL of 1 N HCl was added and the reaction mixture stirred 5 min before the catalyst was filtered off. The filtrate was adjusted to pH 4.6 with the 2 N NaOH and refrigerated 1 h. The crystalline product was filtered and dried to give 3.53 g (92%) of the *RS*-amino acid **22**: NMR (D_2O/DCl) δ 2.2 (s, 3 H, acetyl CH_3), 3.15 and 4.08 (2 t, 4 H, C_2 and C_3 H_2), 5.2 (s, 1 H, α-H), 7.20–8.05 (m, 3 H, arom); mp 198–199 °C. Anal. ($C_{12}H_{14}N_2O_3$) C, H, N, O.

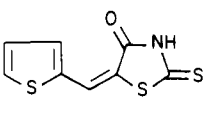
Protection of the Amino Group (23). The *RS*-amino acid **22** (1.17 g, 5 mmol) was suspended in 50 mL of H_2O and 50 mL of THF. Allyl chloroformate (0.65 mL, 6 mmol) was then added and the pH maintained at 8.0–8.5 by addition of 1 N NaOH until it stabilized. The solution was then neutralized to pH 7.0 with 1 N HCl and the THF evaporated. The aqueous solution was layered with EtOAc and the pH adjusted to 2.5 with 1 N HCl. The layers was separated, and the aqueous portion was extracted once more with EtOAc. The combined EtOAc extracts were dried over $MgSO_4$ and evaporated to give 1.37 g (86%) of the *RS*-protected amino acid **23**: NMR (Me_2SO-d_6) δ 2.14 (s, 3 H, acetyl CH_3), 3.11 and 4.06 (2 t, 4 H, C_2 and C_3 H_2), 4.47 (d, 2 H, allyl CH_2), 4.95–5.20 (m, 4 H, allyl and α-H), 7.0–8.2 (m, 4 H, arom, N H). Anal. ($C_{16}H_{18}N_2O_5$) C, H, N.

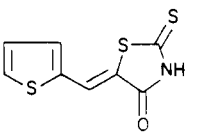
Coupling Reaction–Preparation of 24. Compound **23** (1.6 g, 5 mmol) was dissolved in 50 mL of dry THF. EEDQ (1.3 g, 5.2 mmol) was then added and the resulting solution added in one portion to a solution of 1.4 g (6 mmol) of 7-ADCA allyl ester in 50 mL of dry CH_3CN. The reaction was stirred 4.5 h at room temperature. The THF and CH_3CN were then evaporated, and the residue was taken up in EtOAc and washed successively with H_2O, 5% $NaHCO_3$ (2×), 5% HCl (2×), and H_2O, then dried over $MgSO_4$, and evaporated to give 2.35 g (85%) of the desired cephalosporin **24**. Anal. ($C_{27}H_{30}N_4O_7S$) C, H, N.

Deprotection Reaction. Palladium(II) acetate (11.6 mg) was dissolved in 7.5 mL of acetone, then triphenylphosphine (68 mg) was added, and the solution was stirred until $Pd(PPh_3)_4$ precipitated. The cephalosporin **24** (1.15 g, 2 mmol) was suspended in 50 mL of acetone and added to the $Pd(PPh_3)_4$ complex. The reaction was cooled in an ice bath and 1.2 mL of tributyltin hydride was added. After the mixture was stirred for 15 min, everything was in solution and the ice bath removed. One hour later the reaction was complete and 3.8 mL of 1 N HCl was added. Some of the desired product precipitated and was filtered off (333 mg). The filtrate was diluted with H_2O and excess tin extracted with hexane. The pH of the aqueous portion was adjusted to 4.3, the acetone evaporated, and the aqueous lyophilized to give 112 mg of the desired product **25**. The 445 mg (52%) obtained was a mixture of *R* and *S* isomers, which were separated by HPLC. *R* isomer: NMR (D_2O) δ 1.88 (s, 3 H, C_3 CH_3), 2.27 (s, 3 H, acetyl CH_3) 3.28 (AB q, 2 H, C_2 CH_2), 3.24 and 4.17 (2 d, 4 H, indoline C_2 and C_3 H_2), 5.05 (d, 1 H, C_6 H), 5.21 (s, 1 H, α-H), 5.66 (d, 1 H, C_7 H), 7.3–8.15 (m, 3 H, arom).

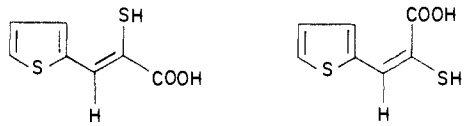
Methyl Thieno[3,2-*b*]thiophene-2-carboxylate (26d). The title compound was prepared in four steps as described below.

1. Condensation of Thiophene-2-carboxaldehyde with Rhodanine. Into a 3-L three-neck flask equipped with a mechanical stirrer, thermometer, reflux condenser, and heating mantle was charged 600 mL of glacial acetic acid, which was then heated to 80 °C. Thiophene-2-carboxaldehyde (100 g, 0.89 mol) and rhodanine (119 g, 0.89 mol) were added to the hot HOAc, and the mixture was stirred until a solution had formed. Anhydrous NaOAc (260 g, 3.17 mol) was added in one portion followed by heating the reaction to reflux for 30 min. The reaction mixture was cooled and poured into 3 L of H_2O upon which the product precipitated out as a bright yellow crystalline solid. This was collected on a suction filter and washed with H_2O, cold 1:1 H_2O/EtOH, and Et_2O. Vacuum drying at 40 °C gave 187 g (92.5%) of the desired product, mp 228 °C. The NMR shows several peaks in the aromatic region confirming the formation of both possible isomers. MS, *m/e* (227 (M^+). Anal. ($C_8H_5NOS_3$) C, H, N.





2. Hydrolysis of the Rhodanine Product. Into a 3-L three-neck flask equipped with mechanical stirrer, thermometer, N_2 inlet exhaust vent, and heating mantle was charged 950 mL of 8% NaOH solution under a slow steady stream of N_2. After heating of the base to 45 °C, the thiophenylrhodanine compound (113 g, 0.5 mol) was added in portions over 20 min under N_2. The reaction was then slowly heated over 30 min to 60 °C. TLC ($PhCH_3$/EtOAc, 1:1) indicated the reaction was complete. The reaction was cooled to 5 °C and the ice-cold solution of 3 N HCl (1 L) was added in a slow stream. The precipitated product was collected on a suction filter, washed several times with H_2O, and vacuum dried at 40 °C: yield 93 g; mp 151 °C; MS, *m/e* 186 (M^+). NMR ($CDCl_3$/Me_2SO-d_6) again shows a multitude of peaks in the aromatic reagion, indicating two isomers, although not in equal amounts. The ratio appears to be nearly 2:1, judging from the peak heights of the vinyl protons (8.0 and 8.2 ppm). Which peak belongs to which compound is not known at this time.



Desired Compound. 3. Cyclization of the α-Mercaptothiopheneacrylic Acid. Into a 22-L four-neck flask equipped with a mechanical stirrer, reflux condenser, 2-L addition funnel, and heating mantle were charged crude α-mercaptothiopheneacrylic acid (393 g, 2.1 mol) and 8 L of $CH_2ClCHCl_2$. A solution of Cl_2 (150 g, 2.1 mol) in 2400 mL of $CH_2ClCHCl_2$ was prepared and added at room temperature over 45 min to the slurry of the thiophene compound in Cl_2CHCH_2Cl. The formation of the sulfenyl chloride was immediate and was indicated by a sharp change in color to a deep orange. The reaction was stirred at room temperature for 1 h and then heated to reflux to effect the thermal cyclization which released large volumes of HCl gas. The refluxing was continued until no more HCl was evident (~1 h) and then the reaction mixture was cooled to room temperature. The solid was collected on a suction filter and washed with a small portion of Cl_2CHCH_2Cl to give 289 g of crude product. The filtrate was evaporated in vacuo to yield a second crop of 70 g. Purification by repeated recrystallizations from EtOH yielded the desired acid: mp 220 °C; NMR ($CDCl_3$/Me_2SO-d_6) δ 7.3 (AB q, 2 H, J = 5 Hz), 8.0 (s, 1 H), 9.5 (s, 1 H).

4. Esterification. Into a 22-L three-neck flask equipped with a mechanical stirrer, reflux condenser, and heating mantle were charged 667 g of the crude acids and 12 L of MeOH saturated with HCl (g). The mixture was stirred and heated to reflux and after 2 h, the color of the mixture was noticeably lighter. TLC (EtOAc/HCO_2H, 98:2) showed no acids remaining. The reaction was cooled and the precipitated solids were collected on a suction filter. The filtrate was evaporated to a solid weighing 400 g. This was chromatographed on silica gel to give 344 g of the desired ester **26d**. This was recrystallized from EtOAc to give a white crystalline solid: mp 98–99 °C; NMR ($CDCl_3$) δ 3.90 (s, 3 H), 7.3 and 7.6 (AB q, 2 H, J = 6 Hz), 8.0 (s, 1 H).

Homologation of 26d into the α-Keto Thio Ester 28d via the β-Keto Sulfoxide 27d. This homologation was done in two steps.

1. Addition of the Carbanion of Methyl (Methylthio)methyl Sulfoxide (MMTS) to Ester 26d. Into a 1-L four-neck round-bottom flask equipped with mechanical stirrer, N_2 inlet, $CaCl_2$ drying tube, addition funnel, and thermometer were charged 15 g (0.376 mol) of 60% NaH/mineral oil and 50 mL of dry hexane. This was stirred under N_2 for 2 min and allowed to settle for 2 min. The hexane/oil was removed by suction through a fitted filter stick. This procedure was repeated two times more and then the flask was evacuated to remove any residual hexane. To the dry NaH was added under N_2 100 mL of freshly distilled DMF. This mixture was stirred and chilled with an ice/water bath to 0–5 °C. Into the 125-mL addition funnel was mixed the thienothiophene methyl ester **26d** (19.8 g, 0.1 mol), 100 mL of DMF, and 14.5 mL (0.14 mol) of MMTS. This solution was added over 5 min to the cold NaH/DMF, and after the mixture was stirred at 0–5 °C for 30 min, the cold bath was removed and the reaction was stirred at room temperature for 3 h. TLC (EtOAc/HCO_2H, 98:2) indicated the reaction was complete. The reaction was cooled again and treated *cautiously* with MeOH to kill the excess NaH. After evaporating to a solid, the residue was treated with EtOAc and H_2O to form a two-phase solution. This was treated with concentrated HCl to pH 5.5, which caused some of the product to precipitate. After warming to dissolve the material, the layers were separated, and the aqueous was extracted four times with EtOAc. The combined EtOAc extracts were washed with H_2O, dried over $MgSO_4$, filtered, and evaporated to give the α-(methylthio)-β-keto sulfoxide **27d**: 18 g (62%); mp 144–145 °C; NMR (Me_2SO-d_6) δ 2.3 (2 s, 3 H), 2.8 (2 s, 3 H), 5.95 (2 s, 1 H) and 8.15 (AB q, 2 H, J = 5 Hz), 8.65 (2 s, 1 H).

2. Oxidation of the MMTS Product. Into a 1-L four-neck flask equipped with a mechanical stirrer, thermometer, heating mantle, and reflux condenser were charged 59.7 g (0.586 mol) of acetic anhydride and 330 g of 98% formic acid. The temperature rose from room temperature to 55 °C in 4–5 min. Heat was applied to raise the temperature to 70 °C, where it was held for no more than 15 min. The yellow sulfoxide **27d** (17 g, 0.0586 mol) was added in portions to the hot stirring mixed anhydride at 65 °C. After the mixture was stirred at 65 °C for 30 min, $NaIO_4$ (2.5 g, 0.0117 mol) was added in one portion and the reaction mixture was stirred for 20 min while the temperature was allowed to drop from 65 to 55 °C. The reaction mixture was then evaporated to a yellow slush and redissolved in EtOAc. This solution was cautiously washed with several portions of saturated $NaHCO_3$ solution followed by two treatments with 1 N $Na_2S_2O_3$ solution to remove excess I_2. After drying over $MgSO_4$, the solution was filtered and evaporated to yield a yellow solid, which was collected on a suction filter and washed with cold Et_2O; yield 10 g of the methylthio α-keto ester **28d**: mp 94–96 °C; NMR ($CDCl_3$) δ 2.4 (s, 3 H), 7.25–7.7 (AB q, 2 H, J = 6 Hz); 8.45 (s, 1 H).

α-Methoximinothieno[3,2-*b*]thiophene-2-acetic Acid (29d). Into a 1-L round-bottom flask were mixed the α-keto thio ester **28d** (7.2 g, 0.03 mol), 540 mL of MeOH, and 30 mL of 1 N NaOH and the mixture was allowed to stir at room temperature for 30 min. TLC (98:2 EtOAc/HCO_2H) indicated the hydrolysis to the acid was complete. Methoxyamine hydrochloride (2.5 g, 0.03 mol) was added in one portion and allowed to stir at room temperature overnight. (TLC indicated the reaction occurred rapidly and need not have gone overnight). The reaction mixture was evaporated to a gummy residue which was solubilized with H_2O and EtOAc. The aqueous solution was extracted three times with EtOAc and then acidified with 30 mL of N HCl to precipitate the free acid. EtOAc was used to extract the product, which was dried with $MgSO_4$, filtered, and evaporated to yield 1 g of the *E* and *Z* methoximino mixture: mp 116–117 °C; NMR ($CDCl_3$) δ 4.0 (s, 3 H), 7.4 (s, 1 H), 7.2–7.5 (AB q, 2 H, J = 6 Hz), 12.0 (s, 1 H).

The preparation of the isomeric thieno[2,3-*b*]thiophene derivatives followed the same general route but started with the thieno[2,3-*b*]thiophene-2-carbocyclic acid described by Gronowitz and Persson.[6]

Compound 26e: mp 95–97 °C; NMR ($CDCl_3$) δ 3.8 (s, 3 H), 7.2 and 7.35 (AB q, 2 H, J = 5 Hz), 7.85 (s, 1 H).

Compound 27e: NMR (Me_2SO-d_6) δ 2.3 (2 s, 3 H), 2.8 (2 s, 3 H), 6.05 (2 s, 1 H), 7.48 and 7.85 (2 AB q, 2 H), 8.5 (2 s, 1 H).

Compound 28e: NMR ($CDCl_3$) δ 2.42 (s, 3 H), 7.2–7.35 (AB q, 2 H), 8.35 (s, 1 H).

Compound 29e: NMR ($CDCl_3$) δ 4.01 (s, 3 H), 4.3 (s, 3 H), 7.0–7.4 (m, 3 H), 10.7 (s, 1 H).

Compound 30e: NMR (Me_2SO-d_6) δ 2.02 (s, 3 H), 3.2–3.7 (AB q, 2 H), 4.86 (br s, 1 H), 5.08 (d, 1 H), 5.68 (d, 1 H), 7.2–7.6 (m, 3 H); MS, *m/e* 409 (M^+), 365.

7-(2-Thieno[2,3-*b*]thiophenylglycylamido)deacetoxycephalosporanic Acid (30d). Oxalyl chloride (25 mL) was cooled to 0 °C and 723 mg of acid **29e** and 3 drops of DMF were added. After the mixture was stirred at 0 °C for 2 h, 50 mL of MeCN was added and the excess oxalyl chloride was evaporated under reduced pressure. This was repeated three more times with addition of MeCN. The acid chloride of **29e** was suspended in 15 mL of dry MeCN and the suspension was added to a cold (0 °C) solution of 1.28 g of 7-ADCA and 4.63 g of BSTFA in 100 mL of MeCN. Stirring at 0 °C was continued for 1 h and then the reaction mixture was left at room temperature overnight. The next day the solvent was removed under reduced pressure and the oily residue dissolved in water. The pH of the aqueous solution was adjusted to 2 and the desired compound extracted (3×) with

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).

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

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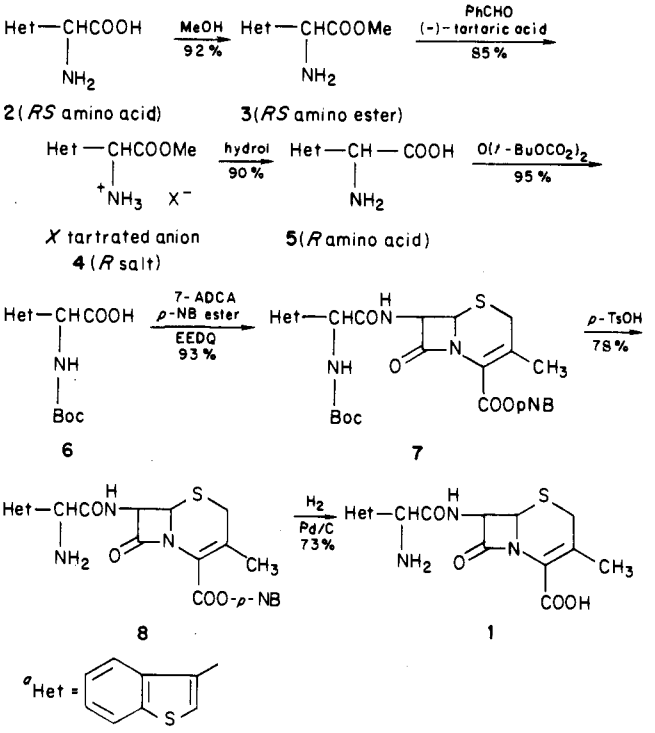
The methyl and isopropyl esters of (*RS*)-3-benzothienylglycine were resolved with (+)- and (−)-tartaric acid in acetonitrile to give the corresponding *R* and *S* salts. The *R*-salt 4 was hydrolyzed to (*R*)-3-benzothienylglycine (**5**). The amino group in **5** was protected with the Boc function 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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