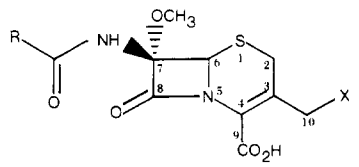
Sulfenyl Transfer Rearrangement of Sulfenimines (Thiooximes). A Novel Synthesis of 7α-Methoxycephalosporins and 6α-Methoxypenicillins[1a,2]

E. M. Gordon,*[1b] H. W. Chang,[1b] C. M. Cimarusti,[1b] B. Toeplitz,[1b] and J. Z. Gougoutas[1c]

Contribution from The Squibb Institute for Medical Research, P.O. Box 4000, Princeton, New Jersey 08540, and the Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received July 5, 1979*

Abstract: New methods were developed to transform cephalosporins into 7α-methoxycephalosporins. The critical step involves a previously unknown "sulfenyl transfer rearrangement" of 7-sulfeniminocephalosporin derivatives to 7α-aryl(alkyl)thio-7β-aminocephalosporins. Modification of this procedure affords 7α-methoxy-7β-aminocephalosporins directly. These rearrangements are performed under mild conditions and are both stereospecific and high yielding. The crystalline sulfenimine precursors are obtained from 7-aminocephalosporins by a novel sulfenyl chloride initiated oxidation. Free carboxylic acids of 7-sulfeniminocephalosporins are shown to be a new class of β-lactamase inhibitors. The structure of such sulfenimino-β-lactams has been elucidated by spectroscopic means and shown by an X-ray crystallographic analysis to have the sulfenimino sulfur atom anti to the α-azetidinone carbonyl group. The foregoing synthetic methodology has been generalized to include the penicillin nucleus. A high-yield synthesis of 6α-methoxypenicillin is reported. Further chemistry pertaining to oxidations of 6-sulfeniminopenicillins is also described. Speculations concerning the mechanisms of the above transformations are presented.

Discovery in the early 1970s of 7α-methoxycephalosporins (cephamycins) by workers at Eli Lilly[3] and Merck Sharp & Dohme[4] marked the beginning of another renaissance of research interest in the chemistry of β-lactam antibiotics. Since then, others have isolated similar naturally occurring cephalosporin nucleus confers β-lactamase resistance upon molecules which hitherto were substrates for such enzymes is of prime importance.[6d] This, combined with other factors, makes 7α-which hitherto were substrates for such enzymes is of prime importance.[6d] This, combined with other factors, makes 7α-methoxycephalosporins particularly effective antibiotics against gram-negative bacteria.[6] The past several years has seen an intensive worldwide effort expended to provide a viable synthetic route to this class of substances,[7] and also to search for antibiotics of this type with improved pharmacologic properties. A number of semisynthetic cephamycin derivatives have proved highly effective in this regard and one has recently been made available for clinical use.[8] At the time our study was initiated much of the published synthetic methodology bearing on introduction of a 7α-methoxy group into a preformed cephem nucleus required rigorously controlled experimental conditions (e.g., low temperature, strictly anhydrous solvents). The large-scale impracticality of such methods led us to undertake a program aimed at achieving a short, technically facile, stereospecific synthesis of 7α-methoxycephalosporin derivatives of general structure **1**.

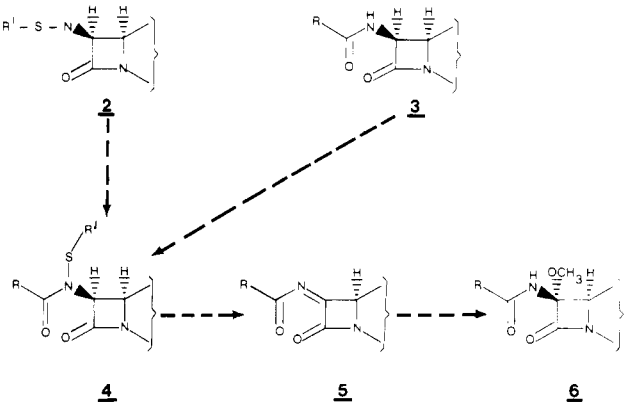


1

Synthesis of 7-Sulfeniminocephalosporins

Our original approach to this problem as outlined in Scheme I was predicated on the known base-catalyzed β-elimination of peptidic thiohydroxamic acid derivatives, which proceeds to yield acylimine intermediates.[9] Trapping of such reactive species with methanol in both the cephalosporin and penicillin series is precedented and proceeds with high stereochemical control.[7g,h] The requisite thiohydroxamate derivative **4** would

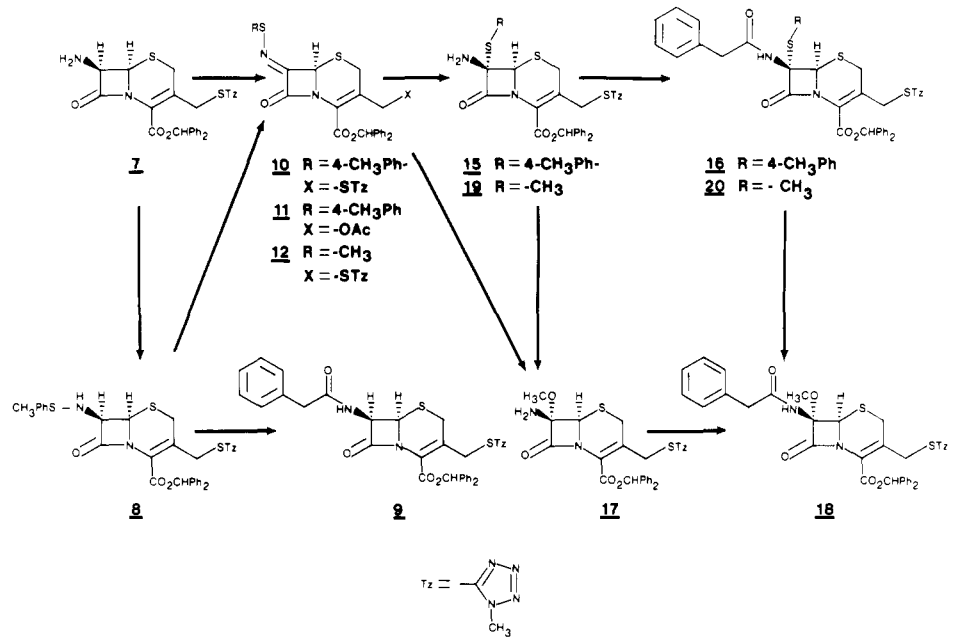
Scheme I



be formed by acylation of a sulfenamide (**2**) or N-sulfenylation of an appropriate amide (**3**). Hypothetical conversion of **4** to **6** bears strong analogy to earlier work by Baldwin[7g] and Koppel,[7h] who utilized *tert*-butyl hypochlorite to form *N*-chloroamides in situ. These intermediates were subsequently converted to type **6** β-lactams. The sensitivity of divalent sulfur to *tert*-butyl hypochlorite limits these methods, and requires either the generation of an amido anion (necessitating use of strong base and anhydrous solvents) or protection of sulfur as a sulfoxide or sulfone. We were attracted to Scheme I since it seemed to obviate these limitations. Cephalosporin amine ester **7**, when treated with a stoichiometric amount of *p*-toluenesulfenyl chloride (TSC) and an acid scavenger (such as potassium bicarbonate, or preferably propylene oxide and pulverized molecular sieves), afforded sulfenamide **8** in 92% yield (see Scheme II). Use of pyridine, triethylamine, or *N,N'*-diethylaniline as base was less satisfactory, initiating olefin isomerization and lower yields. Attempted synthesis of type **4** thiohydroxamates by acylation of sulfenamide **8** with phenylacetyl chloride and either potassium carbonate, triethylamine, *N,N*-diethylaniline, or propylene oxide all afforded amide **9**. Loss of tolylthio substituent in **9** is likely due to an extreme sensitivity of systems of type **4** to acids and nucleophiles. In the course of these experiments an observation was made which encouraged us to deviate from our original path. Treatment of amine ester **7** with more than a stoichiometric quantity of TSC gave rise to a new, bright yellow, higher R_f

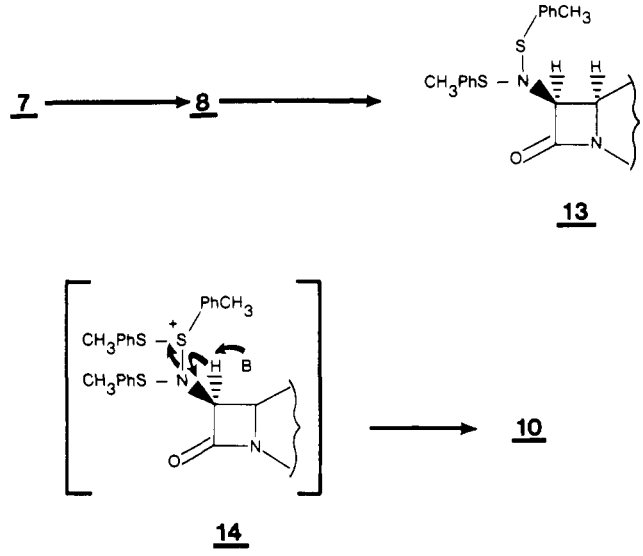
0002-7863/80/1502-1690$01.00/0 © 1980 American Chemical Society

Scheme II



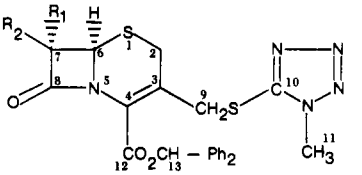
spot (TLC). Three equivalents of TSC (CH_2Cl_2, propylene oxide, pulverized molecular sieves,[11] 0 °C, 3 h) optimized production of this substance, which was crystallized (ethyl acetate/ether) directly from the reaction mixture in 80% yield (mp 154–155 °C). Infrared spectra of the new material indicated an intact β-lactam ring (1770, 1715 cm^{-1}), and ultraviolet spectra exhibited strong absorptions at 261 (ε 10 300) and 355 nm (ε 11 100) implicating introduction of a new chromophore. The presence of optical rotation ($[\alpha]^{25}_D$ −130.5°) demanded retention of at least one chiral center. ^1H and ^{13}C NMR spectra of the new substance were quite similar to those of sulfenamide **8**; however, a complex multiplet (^1H NMR) appearing at δ 4.87 (2 H) in **8** was absent and replaced by a sharp singlet at δ 5.25 (1 H). The C-7 carbon resonance (^{13}C NMR, see Table I) which appeared at δ 63.6 in amine **7**, and shifted to δ 73.2 in sulfenamide **8**, could not be observed, whereas the C-6 signal appearing at δ 58.8 in both **7** and **8** shifted slightly to δ 60.8. Combined with a combustion analysis of $C_{30}H_{26}N_6O_3S_3$, the above data led us to propose a 7-sulfenimino β-lactam structure (**10**) for this new substance. Verification of this assignment is borne out by additional chemical transformations described below, and by analogy to a similar penicillin derivative whose structure has been proven by X-ray crystallography (vide infra). Sulfenimine **10** is an unusual molecule which is apparently formed by further sulfenylation of sulfenamide **8**. Indeed treatment of **8** under similar reaction conditions (2 equiv of TSC) affords comparable yields of **10** and *p*-tolyl disulfide, a byproduct also formed in the synthesis of **10** from amine **7**. The mechanism we propose to rationalize these observations is illustrated in Scheme III. Further N-sulfenylation of **8** would lead to sulfenimide **13**, which could either directly eliminate to **10** or undergo S-sulfenylation (**14**) prior to forming **10**. Both pathways explain production of *p*-tolyl disulfide as a byproduct, the former route by trapping of eliminated *p*-tolylthiol with TSC and the latter by direct elimination of the disulfide. Welch[12] has prepared sulfenimides from 6-APA and arylsulfenyl chlorides by a modified Schotten–Baumann procedure. These sulfenimides were formed under aqueous conditions and both carboxylic acids and methyl ester derivatives of these materials appear to be stable compounds. The foregoing results encouraged us to attempt to isolate sulfenimide **13**. Treatment of amine **7** with 2 equiv of TSC under the above conditions gave, by silica gel chromatography, in order of increasing R_f, sulfenamide **8**

Scheme III. Mechanism of Sulfenimine Formation



(64.7%), sulfenimine **10** (5%), and sulfenimide **13** (16.4%). Compound **13** was isolated as an oil and displayed infrared absorptions at 1790 and 1730 cm^{-1}. Observation of a pair of one-proton doublets (J = 4 Hz) at δ 4.78 and 5.40 supports the proposed structure of this putative intermediate. Upon addition of TSC to a solution of **13** in deuteriochloroform, sulfenimine **10** was formed quickly and quantitatively (NMR, TLC). These observations substantiate the conclusion that a third equivalent of TSC activates sulfenimide **13** toward β-elimination through a type **14** intermediate. Such labilization of divalent sulfur toward elimination may be general. Conversions such as 2-methylthiocyclohexanone to 2-methylthiocyclohexenone with phenylsulfenyl chloride[13] and the similar transformation of cyclopentanone to 2-phenylthiocyclopentenone reported by Monteiro[14] may be carbon analogues of this process. The general nature of TSC-initiated oxidation of 7-amino-β-lactams is illustrated by conversion of 7-aminocephalosporanic acid benzhydryl ester to 7-sulfenimino-β-lactam **11**. This substance was isolated by chromatography as a yellow oil (77%). In a similar manner, treatment of **7** with freshly prepared methylsulfenyl chloride[15] led to methyl sulfenimine **12**. White, crystalline **12** could be isolated directly from the re-

Table I. ^{13}C NMR Assignments for β-Lactam Sulfenimines and Related Derivatives[a-c]



compd	2	11	9	6	7	13	10	12	8	others
7, R_1 = H; R_2 = NH_2	27.9	33.2	35.4	58.8	63.6	79.7	154.1	161.2	168.7	138.9, 139.1 -C=C-
8, R_1 = H; R_2 = $NHSC_6H_4CH_3$-*p*	28.4	33.2	35.2	58.8	73.2	79.8	n.o.	160.9	165.5	138.7 -C=C-; 138.9 -CS; 20.9 $-CH_3$
10, R_1, R_2 = $=NSC_6H_4CH_3$-*p*	29.3	33.3	35.2	60.8	n.o.	80.2	154.0	160.8	n.o.	138.8, 139.1 -C=C-; 138.4 =CS; 21.0 CH_3
12, R_1, R_2 = $=NSCH_3$	29.3	33.4	35.3	60.7	n.o.	80.1	154.2	160.9	n.o.	138.9, 139.3 -C=C-; 24.2 CH_3
15, R_1 = $-SC_6H_4CH_3$-*p*; R_2 = $-NH_2$	28.8	33.1	34.9	64.5	76.8	79.4	153.9	160.6	165.4	139.4, 139.1 -C=C-; 139.0 -CS; 21.0 CH_3
19, R_1 = SCH_3; R_2 = NH_2	28.8	33.0	34.6	65.2	76.9	79.6	153.8	160.6	165.7	138.9, 139.1 -C=C-; 12.1 CH_3

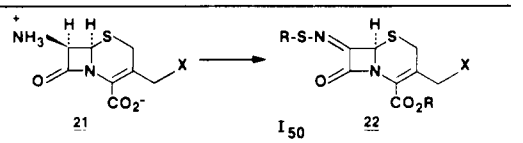
[a] Varian XL-100 NMR spectrometer, equipped with Nicolet Transform Technology Fourier Accessories, operating at a frequency of 25.2 MHz. The spectrometer was locked on ^2H frequency of the solvent. The chemical shifts were calculated from center frequency of $CDCl_3$ taken as 76.9 ppm. All spectra were taken in $CDCl_3$. [b] ^{13}C chemical shifts presented were obtained from proton noise decoupled spectra. The assignments of methyl, methylene, and methine carbons were made from nondecoupled or single frequency off-resonance spectra and chemical-shift assignments are consistent with literature data.[28] [c] The chemical shifts of the aromatic carbons are not listed. n.o. = not observed.

action mixture in 76% yield. Sulfenimines **10–12** have been stored for periods greater than 1 year at −5 °C without significant decomposition. These compounds are thus relatively stable materials and do not react at C-7 with nucleophiles such as thiols or alcohols under neutral conditions. The aforementioned sulfenimines all appear to form as single isomers as judged by ^1H NMR, ^{13}C NMR, TLC, and their sharp melting points. The aryl(alkyl)thio group is assigned as being anti to the azetidinone carbonyl, by analogy to a similar penicillin derivative (vide infra).

Use of the foregoing methodology has been extended to penicillins (vide infra) and to synthesis of α-amino acid derived sulfenimines.[17] In addition, we have sought free acids of 7-sulfenimino-β-lactams primarily for biological testing, but also for use as potential synthetic intermediates. Standard deprotection procedures (TFA/CH_2Cl_2/anisole, 0 °C) when applied to **10** caused extensive decomposition and consequently an alternative synthetic approach was required. Silylation (BSA/CH_2Cl_2/propylene oxide/pulverized molecular sieves, 26 °C) of 7-ACA (**21**, X = OAc) followed by treatment with TSC (0 °C, CH_2Cl_2) did provide the desired acid **23** (isolated as its sodium salt). In this way a series of free acids and sodium carboxylate salts were prepared by variation of sulfenyl chloride and β-lactam nucleus (see Table II). Sulfenimino-β-lactam acids and salts were obtained in 27–95% yields, although these yields were not maximized. Substances **23–33** displayed no significant antimicrobial activity; however, some of this group were shown to be β-lactamase inhibitors. The most potent inhibitor derived from this series is **29**, which possesses an I_{50} of 0.003 μg/mL vs. a cephalosporinase.[18] Other I_{50} values and yields are summarized in Table II.

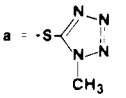
Although a number of reports dealing with sulfenimine chemistry have recently appeared, most notably by Davis and co-workers,[19] this area is still largely unexplored.[20] Rare use of the sulfenimine group in organic synthesis likely reflects a paucity of simple means of preparation,[21] rather than a lack

Table II. Synthesis of 7-Sulfenimino-β-lactam Acids and Salts



21 22

	R	X	R′	I_{50} (μg/ml)	Yield
23	4 CH_3Ph-	-OAc	Na	0.125	27%
24	4 CH_3Ph-	-H	Na	6.25	61%
25	4 CH_3Ph-	-STz[a]	-H	0.25	32%
26	CH_3-	-OAc	-H	10.0	76%
27	CH_3-	-H	-H	> 100	82%
28	CH_3-	-STz[a]	-H	12.5	95%[b]
29	Ph-	-OAc	Na	0.003	60% (28% Salt)
30	4 CH_3OPh-	-OAc	Na	0.44	83% (38% Salt)
31	4 ClPh-	-OAc	Na	0.38	73% (36% Salt)
32	$PhCH_2$-	-OAc	Na	0.88	62% (36% Salt)
33	CH_3Ph-[c]			0.5	60%[b]



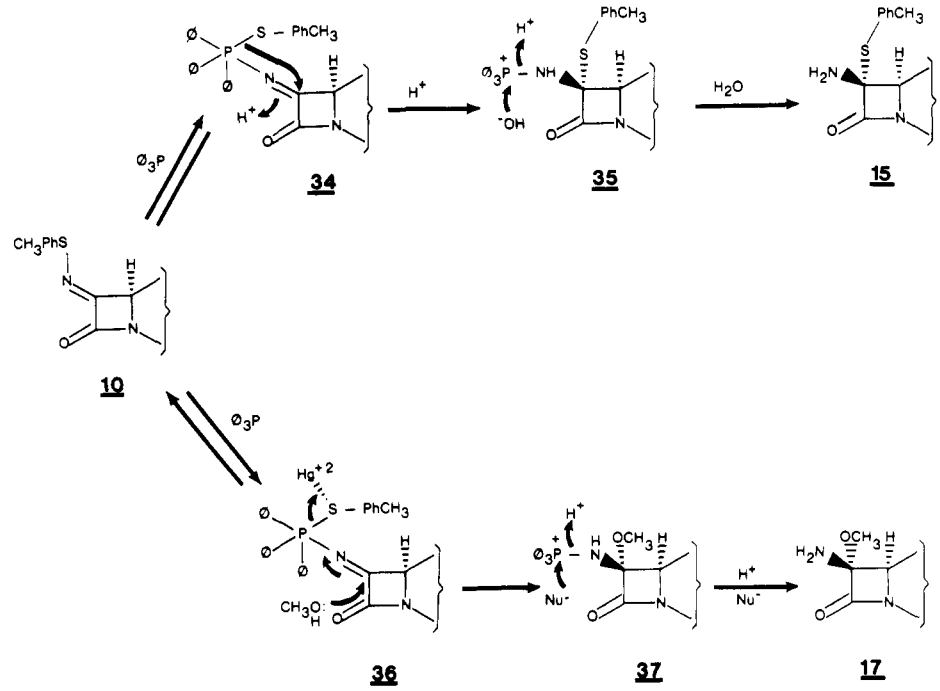
b = crude yield
c = 6-APA nucleus

of utility for this functionality. Interestingly, concurrent with our earlier report, workers at Sankyo Co. also reported a new synthesis of sulfenimino-β-lactams.[20] Their methodology involves manganese dioxide oxidation of type **8** sulfenamides, and provides an alternative approach to structures of type **10.** Utility of 7-sulfenimino-β-lactam acids (**22**) as synthetic intermediates has also been demonstrated by Slusarchyk and co-workers[23] in their synthesis of 7-oxocephalosporins.

Sulfenyl Transfer Rearrangement of 7-Sulfeniminocephalosporins

We were attracted to the idea of using 7-sulfenimino-cephems as synthetic precursors of 7α-methoxycephalosporins

Scheme IV. Mechanism of Sulfenyl Transfer Rearrangement



for several reasons: (1) although relatively little is known concerning the chemical behavior of sulfenimines, synthesis of **10** has already achieved the required oxidation at C-7, and eventual addition of a methoxyl group to this site seemed chemically reasonable; (2) the lone remaining chiral center in **10** enforces a molecular conformation which should be conducive to introduction of methoxyl in the desired stereochemical sense; (3) 7-sulfenimino-β-lactams are stable, crystalline materials which were easily prepared in good yield and consequently are desirable starting materials; (4) the concept of oxidation-reduction condensations, which has been elegantly researched and expounded on by Mukaiyama,[24] suggested to us thought-provoking analogies for the synthesis of 7α-methoxycephems.

When sulfenimine **10** was reacted with 3 molar equiv of triphenylphosphine in methylene chloride (26 °C), TLC indicated clean conversion of starting material (yellow) to a new (colorless), more polar compound. This substance was isolated by silica gel chromatography as a white foam (71%). Comparison of new material with **10** by ^{1}H NMR indicated an upfield shift of the singlet due to C-6 from δ 5.25 to 4.79. The only additional significant change was appearance of a broad two-proton singlet at δ 2.00 (exchanged with D_2O). ^{13}C NMR spectra also showed meaningful changes, the C-6 signal shifting from δ 60.8 to 64.5 consistent with the β effect of SR groups.[25] The resonance assigned to C-7, which could not be observed in spectra of starting **10**, appeared at δ 76.8 in the product. Assessment of the above and infrared spectral data (1775, 1715 cm^{-1}) suggest 7α-arylthioamine **15** to be the observed reaction product. Amine **15** appeared to be a single diastereomer (^{1}H NMR, ^{13}C NMR, TLC) and could be acylated (80%) to yield amide **16** (phenylacetyl chloride/diethylaniline/methylene chloride), also one diastereomer. Although this result was not entirely unexpected in view of Mukaiyama's earlier work, we decided to investigate this transformation more carefully. Rearrangement was again performed as described above (3 equiv of triphenylphosphine, CH_2Cl_2, 26 °C), and, after TLC indicated the absence of **10** and presence of **15**, the product was examined by ^{1}H and ^{13}C NMR *prior* to preparative chromatography. No 7α-arylthioamine was observed in either case. Instead the ^{1}H NMR (60 MHz, $CDCl_3$) spectrum appeared very similar to that of sulfenimine **10**; however, the arylmethyl group was present as a doublet. ^{13}C NMR spectra ($CDCl_3$) were also virtually identical with starting material except for the tolylmethyl carbon [δ 20.9 (new) and 21.1 (initial)]. Nevertheless, when this material was subsequently chromatographed, thioamine **15** was obtained in good yield. This observation implies formation of a complex between sulfenimine and triphenylphosphine, which, under chromatographic conditions (H^+, H_2O), is transformed to product **15**. It is noteworthy that silica gel buffered to pH 7 (Mallinckrodt SilicAR CC-7) was much less effective than acidic silica gel (pH 4, Mallinckrodt SilicAR CC-4) at achieving this conversion. Complex could be converted to arylthioamine by either plate or column chromatography; in fact, introduction of silica gel (Mallinckrodt SilicAR CC-4) directly into the reaction mixture did not interfere with complex formation, but was sufficient to convert **10** directly to **15**. Special silica gel is not a requirement since four randomly selected brands were equally effective.[26] The net effect of combining sulfenimine **10**, triphenylphosphine (3 equiv), and silica gel (CH_2Cl_2, 26 °C) is a rapid (<3 h) sulfenyl transfer from nitrogen to carbon resulting in stereospecific formation of 7α-arylthioamine **15** in excellent yield (>90% by ^{1}H NMR). We have termed reactions of this type "sulfenyl transfer rearrangements". In a similar manner, methylsulfenimine **9** undergoes sulfenyl transfer rearrangement to 7α-methylthioamine **19** (~90%, NMR). Substance **19** is identical with an authentic sample prepared via methylthiolation of the corresponding *p*-nitrobenzylidenaminocephalosporin.[27] Such procedures are known to afford 7α-sulfenylated products.[7d] Steric requirements and comparison (^{1}H NMR) of amines **15** and **19** strongly suggest that **15** is also α-substituted. Acylation (phenylacetyl chloride, propylene oxide, CH_2Cl_2, 0 °C) of **19** gave **20** (80%).

In order to explain the above observations we propose the mechanism outlined in Scheme IV (top). From ^{13}C data the extent of complex formation (**10** + 3 equiv of triphenylphosphine, $CDCl_3$, 26 °C, 3 h) was estimated at 40%. Thus, an equilibrium is established between sulfenimine **10** and triphenylphosphine leading to complex I, which may have a structure equivalent to **34**. A separate experiment showed that almost complete recovery of **10** can be made by crystallization of a mixture of **10** and triphenylphosphine (CH_2Cl_2), em-

phasizing the reversibility of complex formation. Similar insertions of trivalent phosphorus compounds within the S-N bond of sulfenamides are well documented[24] but the reversibility of such transformations is not well established. Attempted substitution of trimethyl phosphite, phosphorus trichloride, phosphorus tribromide, or hexamethylphosphorous triamide for triphenylphosphine was unsuccessful. Protonation of **34** initiates transfer of the sulfenyl moiety from phosphorus to carbon; subsequent hydrolysis of the resulting aminophosphorane (complex II) affords **15** and triphenylphosphine oxide, which is isolated as a byproduct in these reactions. The presence of silica gel satisfies a requirement of water in the final hydrolysis and provides a proton source for conversion of **34** to **35**. The intramolecularity of sulfenyl migration implied in this scheme is only tentative, and has not been proved by crossover experiments. Following this reaction by ^{31}P NMR may shed more light on the mechanism of thioamine formation.

Synthesis of 7α-Methoxycephalosporins

The synthetic methodology described above has been applied to preparation of 7α-methoxycephalosporins. Following the procedure of Slusarchyk and co-workers[7d] [$Hg(OAc)_2$/THF/MeOH], 7α-arylthioamide **16** was converted to a mixture of 7α-methoxycephalosporanic ester **18** and its 7β-methoxyl isomer in 73% yield (2/1, α/β). Amide **20** also underwent this transformation (67% yield); however, a more favorable ratio of α/β methoxy isomers was observed in this case (5/1, α/β). One simple explanation for this variance may be that the larger steric requirements of the tolylthio group (vs. $-SCH_3$) more severely shield the α face of C-7 from attack by methanol during solvolysis. Using a similar published procedure,[7e] 7α-methoxyamine **17** could be obtained by mercury(II)-catalyzed methanolysis of **15** or **19** (NMR, TLC). Acylation of this product (phenylacetyl chloride/propylene oxide/CH_2Cl_2) gave 7α-methoxy amide **18**.

Based on our aforementioned rationalization of the sulfenyl transfer rearrangement, it was recognized that judicious intervention in the reaction conditions might permit direct preparation of 7α-methoxy amine **17** from 7-sulfenimino-β-lactams. We have examined this possibility with positive results. Sulfenyl transfer rearrangement when performed on **10** in neat methanol yielded only a trace of 7α-methoxy amine **17**, as did addition of methanol to a preformed triphenylphosphine/sulfenimine mixture (CH_2Cl_2). Treatment, however, of this mixture with a methanolic solution of mercuric acetate (1 equiv) rapidly produced **17**. Further experimentation revealed that one need not wait for complex equilibrium to be established to effect this conversion. In practice, reaction of sulfenimine **10** with triphenylphosphine, mercuric acetate, methanol, and methylene chloride (26 °C, 3–5 h) affords **17** in high yield (NMR, TLC). Rather than attempting to separate the delicate methoxy amine from other reaction components, the crude reaction mixture was concentrated to remove methanol and immediately acylated (phenylacetyl chloride, propylene oxide, methylene chloride, −10 °C). After silica gel chromatography, 7α-methoxycephalosporin ester **18** was isolated as a white foam (80–90%). Similar results and yields were obtained with sulfenimine **12**. These reactions proceed with complete stereochemical control at C-7, since no products resulting from β-methoxylation are observed. The products (and those prepared by the method of Slusarchyk) were identical with an authentic sample synthesized according to Yanagisawa.[7k] In some cases small amounts of Δ^2 isomers were isolated after preparative chromatography; however, we feel that these are likely artifacts of isolation rather than true reaction products.[29] ^1H NMR spectra of the unchromatographed mixture did not show the presence of Δ^2 material. A number of potential mechanistic schemes may be envisioned for this rearrangement. One possible pathway, consistent with that proposed for sulfenyl transfer rearrangement, is illustrated in Scheme IV (bottom).

Trivalent phosphorus insertion within the S-N bond of **10** is followed by complexation of sulfur with mercury(II). It is noteworthy that sulfenimine **10** by itself does not react with mercuric acetate in methanol. Removal of the tolylthio group concomitant with methoxy introduction (**36**) affords aminophosphorane **37**, which is cleaved under the reaction conditions yielding 7α-methoxy amine **17**. This scheme, although it agrees fully with our knowledge of the sulfenyl transfer rearrangement, must be regarded as tentative. It is difficult to distinguish the proposed pathway from other possibilities such as mercuric acetate initiated methanolysis of complex **35** or amine **15**. In summary, coupling of 7-sulfenimino-β-lactam formation with the modified sulfenyl transfer rearrangement permits a stereospecific three-step conversion of amine ester **7** to 7α-methoxycephalosporin **18** in 64–72% overall isolated yield.

Synthesis of 6-Sulfeniminopenicillins

The synthetic methodology discussed above has been generalized to include the penam nucleus. Procedures for sulfenimine preparation from **38** are essentially the same as for cephalosporins; however, 1 equiv of tertiary amine is required in order to liberate the free base of 6-APA ester from its *p*-toluenesulfonate salt. Reaction of trichloroethyl 6-aminopenicillanate *p*-toluenesulfonate salt (**38**) with triethylamine (1 equiv) followed by TSC [3 equiv, propylene oxide, pulverized molecular sieves (4A), methylene chloride, 0 °C, 2.5 h] afforded after silica gel chromatography 6-sulfeniminopenam **40** as bright yellow crystals (80%). Likewise *p*-nitrobenzyl derivative **42** was obtained in 82% yield. Treatment of **38** with triethylamine and fresh methylsulfenyl chloride afforded methyl sulfenimine **41** as white crystals (42%). The yield of **41** is based on one run and is not maximized. 6-Sulfeniminopenams **40**, **41**, and **42** are formed as single geometric isomers (^{13}C NMR, ^1H NMR, TLC, melting point) and are stable entities at 26 °C. In order to elucidate the disposition of geometrical isomerization in this series (and by analogy the cephalosporin series), an X-ray crystallographic analysis of **42** was undertaken.

Molecular Structure of 42

As the sulfenimino moiety of **42** imposes the geometry of sp^2 carbon at C-6 of the β-lactam ring, its structure and the potential influence of its configuration on the conformation of the β-lactam and thiazolidine rings represent interesting points of departure from previous X-ray structural studies[30] of penicillin derivatives.

The sulfenimino sulfur atom S25 and the azetidinone carbonyl oxygen O8 are anti with respect to the C6–N24 imine bond, resulting in the S1–S25 intramolecular distance of 4.00 Å. Both N24 and S25 are displaced from the least-squares plane of the cyclic amide link (atoms N4, C7, O8, C6) in a sense opposite to that of C5, S1, and C3 (see Table IV, supplementary material), presumably in order to alleviate the repulsion of the adjacent C6 = N24 and C7 = O8 dipoles. The opposite displacements of N24–S25 and C5, as well as the essentially coplanar nature of atoms C5, C6, C7, and N24 (Table IV), suggest that the nonplanarity of the dipoles is attained primarily through a rotation about the C6–C7 bond (torsional angle O8–C7–C6–N24 = −7°).

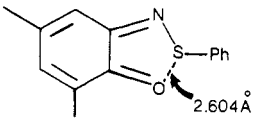
The distance of N4 from the plane of its substituents, C3, C5, C7, is 0.42 Å. In the thiazolidine ring, atoms C2, S1, C5, and N4 are nearly coplanar (torsional angle = −3°) with C3 displaced out of this plane and away from the β-lactam ring, resulting in axial carboxyl and β-CH_3(C10) substituents. The conformation of the bicyclic nucleus of **42**, therefore, is similar to that of pen-V.[31]

Table III. Intramolecular Distances and Bond Angles in **42**

Intramolecular Distances Less Than or Equal to 1.90 Å			
S(1)-C(2)	1.87(2)	C(16)-H(37)	1.11(2)
S(1)-C(5)	1.83(1)	C(17)-C(18)	1.38(3)
C(2)-C(3)	1.54(2)	C(17)-H(38)	1.11(2)
C(2)-C(9)	1.54(2)	C(18)-C(19)	1.35(2)
C(2)-C(10)	1.53(2)	C(18)-N(21)	1.53(2)
C(3)-N(4)	1.48(2)	C(19)-C(20)	1.35(2)
C(3)-C(11)	1.52(2)	C(19)-H(39)	1.09(2)
C(3)-H(33)	1.12(2)	C(20)-H(40)	1.09(2)
N(4)-C(5)	1.46(2)	N(21)-O(22)	1.18(3)
N(4)-C(7)	1.41 (2)	N(21)-O(23)	1.20(2)
C(5)-C(6)	1.51(2)	N(24)-S(25)	1.68(1)
C(5)-H(34)	1.12(2)	S(25)-C(26)	1.81(2)
C(6)-C(7)	1.52(2)	C(26)-C(27)	1.36(3)
C(6)-N(24)	25(2)	C(26)-C(31)	1.33(3)
C(7)-O(8)	1.19(2)	C(27)-C(28)	1.38(3)
C(11)-O(12)	1.21(2)	C(27)-H(41)	1.11(2)
C(11)-O(13)	1.32(2)	C(28)-C(29)	1.36(3)
O(13)-C(14)	1.44(2)	C(28)-H(42)	1.08(2)
C(14)-C(15)	1.51(2)	C(29)-C(30)	1.44(3)
C(14)-H(35)	1.13(2)	C(29)-C(32)	1.54(2)
C(14)-H(36)	1.10(2)	C(30)-C(31)	1.46(3)
C(15)-C(16)	1.43(2)	C(30)-H(43)	1.11(2)
C(15)-C(20)	1.40(2)	C(31)-H(44)	1.11(2)
C(16)-C(17)	1.38(2)		
Bond Angles (deg)			
C(2)-S(1)-C(5)	94.3(6)	C(15)-C(16)-C(17)	120.3(14)
S(1)-C(2)-C(3)	105.4(8)	C(15)-C(16)-H(37)	118.7(11)
S(1)-C(2)-C(9)	106.2(8)	C(17)-C(16)-H(37)	121.0(12)
S(1)-C(2)-C(10)	110.3(9)	C(16)-C(17)-C(18)	116.7(15)
C(3)-C(2)-C(9)	113.0(11)	C(16)-C(17)-H(38)	122.5(11)
C(3)-C(2)-C(10)	109.4(11)	C(18)-C(17)-H(38)	120.8(13)
C(9)-C(2)-C(10)	112.2(12)	C(17)-C(18)-C(19)	124.0(17)
C(2)-C(3)-N(4)	107.4(9)	C(17)-C(18)-N(21)	117.7(15)
C(2)-C(3)-C(11)	114.1(12)	C(19)-C(18)-N(21)	118.3(14)
C(2)-C(3)-H(33)	108.2(9)	C(18)-C(19)-C(20)	120.3(16)
N(4)-C(3)-C(11)	105.0(10)	C(18)-C(19)-H(39)	120.2(13)
N(4)-C(3)-H(33)	114.6(8)	C(20)-C(19)-H(39)	119.4(11)
C(11)-C(3)-H(33)	107.6(11)	C(15)-C(20)-C(19)	119.8(15)
C(3)-N(4)-C(5)	114.8(11)	C(15)-C(20)-H(40)	120.8(12)
C(3)-N(4)-C(7)	125.3(15)	C(19)-C(20)-H(40)	119.3(11)
C(5)-N(4)-C(7)	94.9(11)	C(18)-N(21)-O(22)	116.7(15)
S(1)-C(5)-N(4)	106.8(7)	C(18)-N(21)-O(23)	117.1(14)
S(1)-C(5)-C(6)	117.2(8)	O(22)-N(21)-O(23)	126.2(19)
S(1)-C(5)-H(34)	101.8(7)	C(6)-N(24)-S(25)	117.8(10)
N(4)-C(5)-C(6)	86.9(11)	N(24)-S(25)-C(26)	100.8(7)
N(4)-C(5)-H(34)	127.5(10)	S(25)-C(26)-C(27)	115.0(12)
C(6)-C(5)-H(34)	117.3(10)	S(25)-C(26)-C(31)	122.8(13)
C(5)-C(6)-C(7)	88.6(11)	C(27)-C(26)-C(31)	122.2(18)
C(5)-C(6)-N(24)	139.0(18)	C(26)-C(27)-C(28)	119.2(16)
C(7)-C(6)-N(24)	132.4(17)	C(26)-C(27)-H(41)	118.8(14)
N(4)-C(7)-C(6)	88.5(13)	C(28)-C(27)-H(41)	122.0(16)
N(4)-C(7)-O(8)	132.8(17)	C(27)-C(28)-C(29)	122.2(19)
C(6)-C(7)-O(8)	138.6(21)	C(27)-C(28)-H(42)	119.6(13)
C(3)-C(11)-O(12)	125.4(15)	C(29)-C(28)-H(42)	118.2(16)
C(3)-C(11)-O(13)	110.7(11)	C(28)-C(29)-C(30)	119.5(19)
O(12)-C(11)-O(13)	123.9(12)	C(28)-C(29)-C(32)	119.0(18)
C(11)-O(13)-C(14)	115.2(13)	C(30)-C(29)-C(32)	121.2(16)
O(13)-C(14)-C(15)	108.8(10)	C(29)-C(30)-C(31)	116.0(14)
O(13)-C(14)-H(35)	110.4(8)	C(29)-C(30)-H(43)	123.0(17)
O(13)-C(14)-H(36)	111.3(8)	C(31)-C(30)-H(43)	121.0(13)
C(15)-C(14)-H(35)	109.1(10)	C(26)-C(31)-C(30)	120.9(17)
C(15)-C(14)-H(36)	110.0(9)	C(26)-C(31)-H(44)	118.3(14)
H(35)-C(14)-H(36)	107.2(7)	C(30)-C(31)-H(44)	120.8(13)
C(14)-C(15)-C(16)	117.1(13)	C(14)-H(35)-H(36)	35.8(8)
C(14)-C(15)-C(20)	124.2(15)	C(14)-H(36)-H(35)	36.9(8)
C(16)-C(15)-C(20)	118.7(14)		

Except for the predictable shortening of the bonds to C6, the bond distances and angles of **42** (Table III) do not differ significantly from previously reported values.[30,32]

Exclusive formation of the anti stereoisomer of the sulfenimine, **42**, is in contradistinction to the result of Barton et al.[33] for the synthesis of several monosulfen- and selenoimines of aromatic *o*-quinones. One of these, **48** (and the selenoimine),



48

recently was shown by X-ray analysis[34] to have the syn configuration of sulfur and the α-keto group—a configuration which is stabilized relative to the (not formed) anti isomer, by virtue of the close (2.604 Å) through-space S···O interaction.

It is unlikely that similar stabilization is possible for the syn form of **42**, as the larger exocyclic N-C-C and C-C-O angles of four-membered rings results in an appreciable increase in the through-space S···O distance [assuming a planar arrangement of S25, N24, C6, C7, and O8, the observed N24-C6-C7, C6-C7-O8, and S25-N24-C6 angles of **42**, and the bond lengths S25-N24 = 1.641 and N24-C6 = 1.303 Å reported for **48**, we estimate that the syn form of **42** would have an S···O distance of 3.2 Å (sum of van der Waals radii = 3.25 Å)].

The extended conformation of **42** in the solid state is shown in Figure 1.

Sulfenyl Transfer Rearrangement of 6-Sulfeniminopenicillins

Penam sulfenimine **40**, in analogy with the cephalosporin case, underwent smooth sulfenyl transfer rearrangement (Ph_3P, silica gel, methylene chloride, 26 °C, 8 h) to 6α-arylthioamine **43**, which was isolated as a yellow foam (96%). In a similar manner sulfenimine **42** was converted to **44**. Amines **43** and **44** both formed stereospecifically (NMR, TLC, α configuration of -$SPhCH_3$ assumed) and, as further verification of the assigned structure, were converted ($HgCl_2$, dimethoxyethane, water) to 6-ketopenams **46** and **47** (99 and 95%, respectively). In addition, **43** could be acylated (phenylacetyl chloride, *N,N*-diethylaniline, methylene chloride, 0 °C) to yield amide **45**, also a single diastereomer (NMR, TLC).

Access to the relatively less explored 6α-methoxypenicillins was achieved via modified sulfenyl transfer rearrangement. Sulfenimine **40** was reacted with triphenylphosphine (3 equiv), mercuric acetate (1 equiv), methylene chloride, and methanol at 26 °C for 5 h. Removal of solvents under reduced pressure followed by acylation (phenylacetyl chloride, propylene oxide, methylene chloride, −15 °C) and subsequent silica gel chromatography yielded *N*-phenylacetyl-6α-methoxypenam **49** (92% from **40**) as a clear oil. The consistently high yields observed in this transformation recommend it as an excellent method for synthesis of 6α-methoxylated penicillins. Introduction of C-6 methoxyl occurs stereospecifically, the configuration of which is expected to be α on the basis of steric effects and analogy to the cephalosporin example. This was confirmed by conversion of **49** to a 7α-methoxycephem of known structure. Oxidation of **49** (*m*-chloroperbenzoic acid, methylene chloride, 10 °C) afforded isomerically pure β-sulfoxide **50** as white crystals (94%).[36] Classical Morin-type ring expansion[37] (2-picolinium dichloromethyl phosphonate,[38] toluene, reflux, 6 h) of **50** produced trichloroethyl-*N*-phenylacetyl-7α-methoxydeacetoxycephalosporanic acid (**51**,[7g] 24%, white crystals, mp 200–203 °C). Deesterification of **51** (Zn, THF, NH_4OAc, buffer, 25 min)[39] afforded the corresponding free acid **52**, which was identical by IR, NMR, and TLC with an authentic sample in which the configuration of

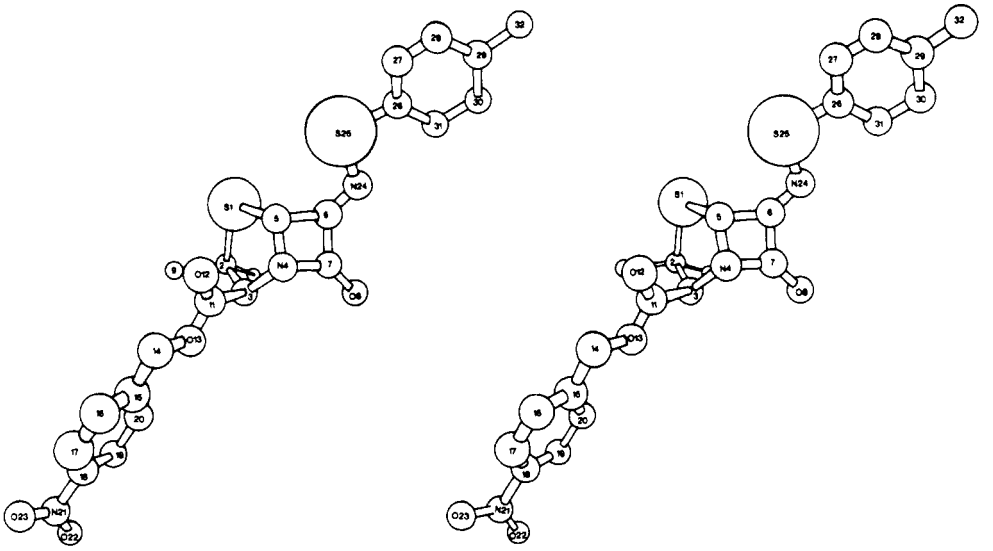
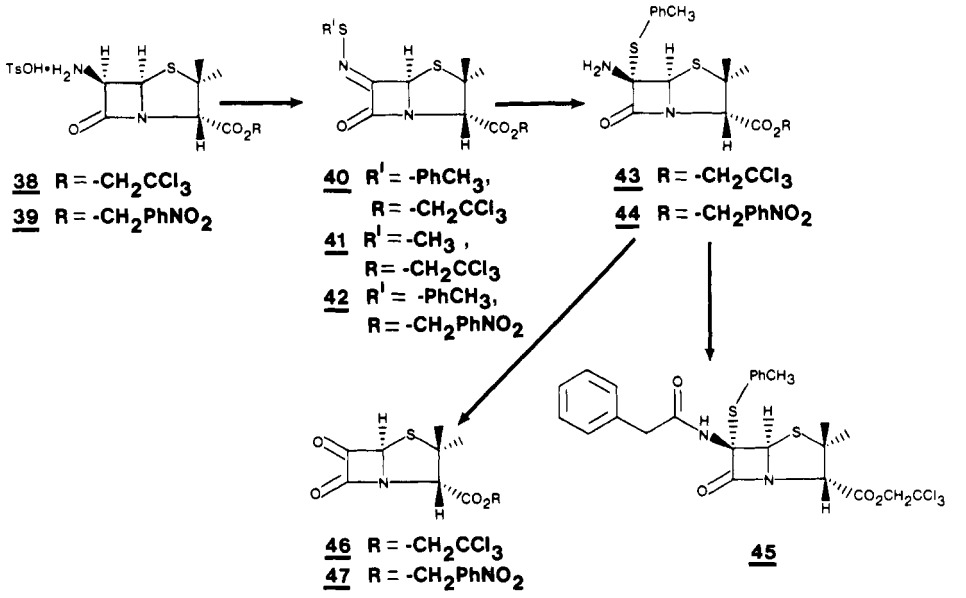
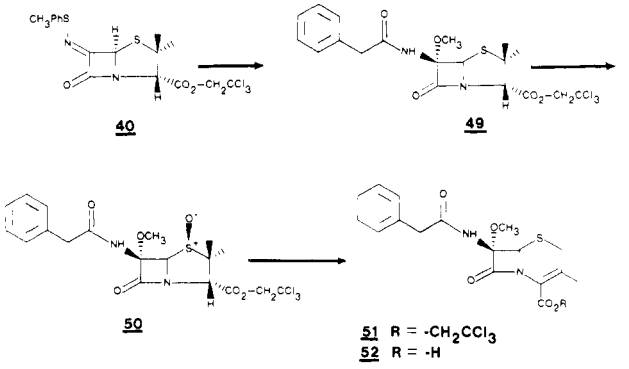


Figure 1.

Scheme V



Scheme VI



C-7 has been proven by X-ray crystallography.[40] The configuration of C-6 methoxyl introduction into penicillins by modified sulfenyl transfer rearrangement (**40** → **49**) is thus confirmed as α.

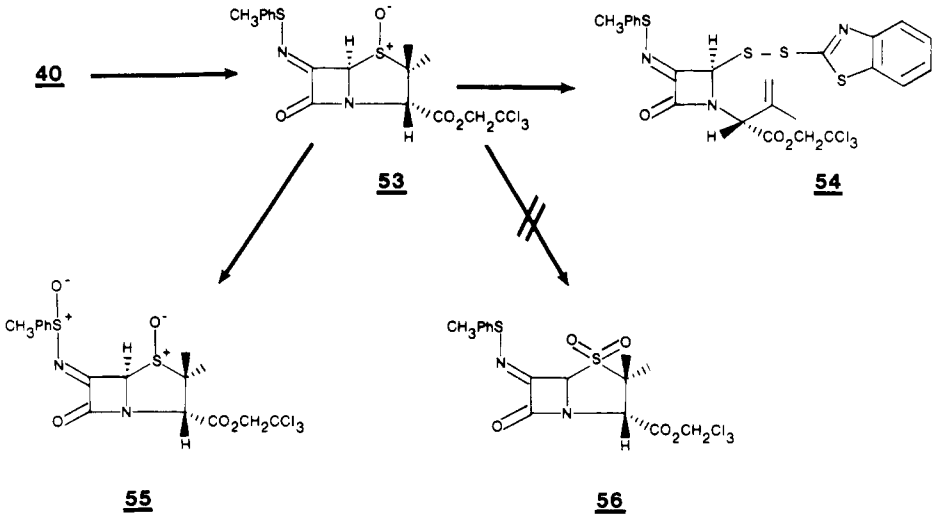
Additional Chemistry of 6-Sulfeniminopenams

The stability and structure of sulfenimino β-lactams suggest that these substances may prove to be valuable intermediates for further synthetic modification of penam and cephem nuclei. Workers at Sankyo Co. have demonstrated that such sulfenimines may be reconverted to the corresponding sulfenamides by stereoselective reduction.[20] This, coupled with the understanding that sulfenimino β-lactams are also actually latent 6- (7-) thio or alkoxy substituted amines, encouraged us to further investigate the chemistry of this functionality.

Oxidation (*m*-chloroperbenzoic acid, methylene chloride, 0 °C) of 6-sulfeniminopenam **40** was chemoselective for thiazolidine sulfur, affording sulfoxide **53** (58% isolated after chromatography) as a mixture of isomers (3:1 by ^{1}H NMR). Separation of these diastereoisomers by fractional crystallization yielded the major isomer in pure form (mp 141.5–142.5 °C). Support for the chemoselectivity of this process was found in mass spectral fragmentation patterns of **53**. Conclusive demonstration of the site of oxidation was obtained by reaction of **53** (mixture of *R,S* isomers) with 2-mercaptobenzothiazole in refluxing toluene (1.5 h). Disulfide **54** was isolated after silica gel chromatography as a clear, yellow oil. ^{1}H NMR of the crude reaction mixture clearly indicated a >90% yield of **54**; however, this material proved to be quite unstable during chromatography and isolated yields were low.

In contradistinction, further oxidation of sulfoxide **53**, was

Scheme VII



also chemoselective, but this time for sulfenimino sulfur. Thus reaction of *m*-chloroperbenzoic acid with **53** did not yield sulfone **56** but rather afforded a diastereoisomeric mixture of sulfinimine sulfoxides **55**. Mass spectra showed the addition of one oxygen atom to **53** (M^+ = 498). Fragments at *m/e* 450, 278, and 219 were diagnostic for structure **55**.[41] Further chemistry in this series is currently under investigation.

Conclusion

In summary we have found that 7-aminocephalosporins and 6-aminopenicillins undergo mild oxidation in the presence of sulfenyl chlorides. The resulting sulfenimino-β-lactams, which are obtained in good yields, are stable, versatile synthetic intermediates. Treatment of such substances with triphenylphosphine and silica gel in methylene chloride rapidly and stereospecifically affords high yields of 6α- (7α-) aryl(alkyl)-thioamine products by a process we have termed sulfenyl transfer rearrangement. A modified version of this reaction utilizing triphenylphosphine, mercuric acetate, methanol, and methylene chloride followed by acylation provides good yields of 7α-methoxycephalosporins and 6α-methoxypenicillins in a stereocontrolled fashion. Sulfenimino-β-lactam free acids have been shown to be a new class of β-lactamase inhibitors, and the overall structure and configuration of a 6-sulfeniminopenicillin have been established by X-ray crystallography. Additional chemistry of 6-sulfeniminopenicillins highlights the potential value of the sulfenimino group in further synthetic modifications of β-lactam antibiotics.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on Varian Associates Model T-60 and XL-100-15 spectrometers. Chemical shifts are reported as δ values (ppm) relative to tetramethylsilane as internal standard. Infrared spectra were determined on Perkin-Elmer Models 621 or 257 recording spectrophotometers. Analytical and preparative thin layer chromatography (PLC) was carried out using E. Merck F-254 silica gel plates. Column chromatography was performed with Mallinckrodt SilicAR CC-7 as adsorbant. Mass spectra were obtained from an AEI-MS 902 mass spectrometer.

Preparation of Sulfenamide 8. To a cold (0–5 °C) solution of amine ester **7** (2.46 g, 5 mmol) in dry methylene chloride (200 mL) and propylene oxide (20 mL), containing pulverized molecular sieves (4A, 25 g), was added, under nitrogen, dropwise with stirring, *p*-toluenesulfenyl chloride (0.795 g, 5 mmol). After 1 h, the reaction mixture was filtered, then washed with saturated sodium bicarbonate (2 × 100 mL) and brine (1 × 100 mL) and dried over sodium sulfate. The clear filtrate was concentrated under reduced pressure to an oil (2.81 g, 91.8%). PLC (methylene chloride) afforded a pure sample as a light yellow foam: NMR ($CDCl_3$) δ 2.34 (s, 3 H), 3.68 (m, 2 H), 3.80 (s, 3 H), 4.19, 4.40 (d of d, 2 H, *J* = 14 Hz), 4.87 (m, 2 H), 6.90 (s, 1 H), 7.30 (m, 14 H); IR ($CHCl_3$) 1795, 1730 cm^{-1}. Anal. ($C_{30}H_{28}N_6O_3S_3$) C, H, N, S.

Preparation of Sulfenimine 10 ((6*R*)-7-[[(4-Methylphenyl)thio]-imino]-3-[[(1-methyl-1*H*-tetrazol-5yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Diphenylmethyl Ester). To a cold (0–5 °C) solution of amine ester **7** (7.0 g, 14.15 mmol) in dry methylene chloride (600 mL) and propylene oxide (60 mL) containing pulverized molecular sieves (4A, 50 g) was added, under nitrogen, dropwise with stirring, *p*-toluenesulfenyl chloride (7.14 g, 45 mmol) in dry methylene chloride (50 mL). The reaction mixture was stirred as the temperature was allowed to rise to 25 °C over a 3-h period. The resulting mixture was filtered and the filtrate concentrated to an oil under reduced pressure. Crystallization (methylene chloride/ethyl ether, 0 °C) afforded fine, yellow needles (6.98 g, 80%): mp 154–155 °C; NMR ($CDCl_3$) 2.36 (s, 3 H), 3.68 (s, 2 H), 3.78 (s, 3 H), 4.13, 4.43 (d of d, 2 H, *J* = 13 Hz), 5.25 (s, 1 H), 6.90 (s, 1 H), 7.26 (m, 14 H); IR (KBr) 1770, 1715 cm^{-1}; UV (MeOH) 261 nm (ε 10 000), 355 (11 100);[42] $[\alpha]^{25}_D$ −130.5°. Anal. ($C_{30}H_{26}N_6O_3S_3$) C, H, N, S.

Conversion of Sulfenamide 8 to Sulfenimine 10. Sulfenamide **8** (0.204 g, 0.33 mmol) was treated under conditions described above for conversion of **7** to **10** except for the employment of only 2 molar equiv of *p*-toluenesulfenyl chloride (0.105 g, 0.66 mmol). Filtration followed by removal of solvents in vacuo afforded yellow, crystalline sulfenimine **10,** identical with the sample derived from **7**.

Preparation of Sulfenimine 12 ((6*R*)-3-[[(1-Methyl-1*H*-tetrazol-5-yl)thio]methyl]-7-(methylthioimino]-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic Acid Diphenylmethyl Ester). To a cold (0–5 °C) solution of amine ester **7** (4.94 g, 10 mmol) and propylene oxide (40 mL), containing pulverized molecular sieves (4A, 35 g), was added dropwise, under nitrogen, methylsulfenyl chloride[15] (2.49 g, 30 mmol) in dry methylene chloride (15 mL). The reaction mixture was stirred and allowed to rise to 26 °C over 8 h. The resulting mixture was filtered and the filtrate concentrated under reduced pressure to a semicrystalline solid. Crystallization (methylene chloride/ethyl ether, 0 °C) afforded white needles (4.08 g, 76.9%): mp 213–214 °C, NMR ($CDCl_3$) δ 2.88 (s, 3 H), 3.67 (s, 2 H), 3.81 (s, 3 H), 4.18, 4.46 (d of d, 2 H, *J* = 13 Hz), 5.23 (s, 1 H), 6.94 (s, 1 H), 7.36 (m, 10 H); IR ($CHCl_3$) 1770, 1710, 1350, 1050 cm^{-1}; UV ($CHCl_3$) 263 nm (ε 6900), 333 (12 000). Anal. ($C_{24}H_{22}N_6O_3S_3$) C, H, N, S.

Preparation of Sulfenimine 11. To a cold solution (0–5 °C) of 7-aminocephalosporanic acid diphenylmethyl ester (3.0 g, 6.84 mmol) in dry methylene chloride (100 mL) was added anhydrous potassium carbonate (3 g) followed by *p*-toluenesulfenyl chloride (3.24 g, 20.4 mmol). After 1 h the mixture was washed with water (2 × 100 mL) and brine (1 × 100 mL). The organic phase was dried over sodium sulfate and concentrated under reduced pressure to an oil. Column chromatography afforded **12** as a bright yellow oil (2.92 g, 77%): NMR ($CDCl_3$) δ 1.96 (s, 3 H), 2.33 (s, 3 H), 3.41 (m, 2 H), 4.70, 5.01 (d of d, 2 H, *J* = 13 Hz), 5.26 (s, 1 H), 6.91 (s, 1H), 7.28 (m, 14 H); IR ($CHCl_3$) 1775, 1730, 1720 (sh) cm^{-1}. Anal. ($C_{30}H_{26}N_2O_5S_2$) C, H, N, S.

Preparation of Amine 15 ((6*R*-*cis*)-7-Amino-7-[(4-methylphenyl)-thio]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Diphenylmethyl Ester). **Method A.** To a stirred solution of sulfenimine **10** (1.03 g, 1.66 mmol) in methylene chloride (100 mL) at 26 °C, under nitrogen, was added solid triphenylphosphine (1.30 g, 4.98 mmol). The solution was stirred for 5 h at 26 °C, at which time TLC indicated the absence of starting material. The reaction mixture was concentrated under reduced pressure and then applied directly onto a silica gel column (Baker silica gel). Elution with 5% ethyl acetate/methylene chloride afforded the product as a nearly colorless, clear oil (0.727 g, 70.7%): NMR ($CDCl_3$) δ 2.00 (br s, 2 H, exchange with D_2O), 2.30 (s, 3 H), 3.60 (s, 2 H), 3.76 (s, 3 H), 4.11, 4.38 (d of d, 2 H, J = 13 Hz), 4.73 (s, 1 H), 6.86 (s, 1 H), 7.30 (m, 14 H); IR ($CHCl_3$) 1775, 1715 cm^{-1}; UV (MeOH) 257 nm (ϵ 10:200), 288 (7800). Anal. ($C_{30}H_{28}N_6O_3S_3$) C, H, N, S.

Method B (Preferred). To a solution of sulfenimine **10** (2.90 g, 4.7 mmol) in methylene chloride (150 mL), at 26 °C, under nitrogen, was added Mallinckrodt SilicAR CC-4 (6 g) followed by triphenylphosphine (3.69 g, 14.10 mmol). The mixture was stirred at 26 °C for 3 h, at which point TLC (5% ethyl acetate/methylene chloride) indicated that starting material had been completely consumed. NMR spectra ($CDCl_3$) of a filtered aliquot indicated >90% conversion of **5**. The reaction mixture was concentrated under reduced pressure and applied directly onto a silica gel column (Mallinckrodt SilicAR CC-7). Elution with 5% ethyl acetate/methylene chloride afforded the product **15** (2.12 g, 73%), as described in method A.

Preparation of Amide 16. Method A (from 15). To a chilled solution (0 °C) of amine **15** (0.267 g, 0.432 mmol) in methylene chloride (20 mL) was added dimethylaniline (140 μL, 0.864 mmol) followed by phenylacetyl chloride (96 μL, 0.95 mmol). The mixture was stirred over 2.5 h, while the temperature was allowed to rise to 26 °C. The solvents were removed in vacuo and the resulting oil was chromatographed (TLC, 10% ethyl acetate/methylene chloride) to afford **16** as a foam (0.254 g, 80%): NMR ($CDCl_3$) δ 2.30 (s, 3 H), 3.50 (s, 2 H), 3.58 (s, 2 H), 3.76 (s, 3 H), 4.16, 4.45 (d of d, 2 H, J = 13 Hz), 4.91 (s, 1 H), 6.00 (s, 1 H, exchanges with D_2O), 6.85 (s, 1 H), 7.20 (m, 19 H); IR ($CHCl_3$) 1780, 1715, 1680 cm^{-1}. Anal. ($C_{37}H_{34}N_6O_4S_3$) C, H, N, S.

Method B (from 10). To a stirred solution of sulfenimine **10** (1.03 g, 1.66 mmol) in methylene chloride (50 mL), at 26 °C, under nitrogen, was added Mallinckrodt SilicAR CC-4 (4 g) followed by triphenylphosphine (1.30 g, 4.98 mmol). The mixture was well stirred for 3 h, then chilled to 0–5 °C, whereupon diethylaniline (532 μL, 3.32 mmol) and phenylacetyl chloride (334 μL, 3.32 mmol) were added. The temperature was allowed to rise to 26 °C over 18 h. After removal of solvents in vacuo, the resulting oil was chromatographed (SilicAR CC-7, 5% ethyl acetate/methylene chloride) to yield **16** as a foam (0.866 g, 71%).

Method C (from 10). To a chilled solution of sulfenimine **10** (1.23 g, 2 mmol) in methylene chloride (50 mL) at 26 °C, under nitrogen, was added Mallinckrodt SilicAR CC-4 (2.0 g), followed by triphenylphosphine (1.57 g, 3 mmol). The mixture was stirred for 4.5 h, whereupon propylene oxide was added (10 mL) and the mixture was cooled to −15 °C. Phenylacetyl chloride (3.1 mL, 20 mmol) was now added dropwise, and the reaction mixture was allowed to stir and come to 26 °C over 12 h. The solvents were removed in vacuo, and the residue was purified by column chromatography (ethyl acetate/methylene chloride) to afford product **16** as a light-colored foam (1.18 g, 80%).

Preparation of Sulfenimide 13. To a cold solution (0 °C) of amine ester **7** (0.494 g, 1 mmol) in dry methylene chloride (18 mL) and propylene oxide (2 mL), containing pulverized molecular sieves (4A, 2 g), was added *p*-toluenesulfenyl chloride (0.318 g, 2 mmol), dropwise under nitrogen. The mixture was stirred for 2 h and then concentrated under reduced pressure. Chromatography (PLC, methylene chloride) of the resulting oil afforded (in order of increasing R_f) sulfenamide **8** (0.398 g, 64.7%), sulfenimine **10** (0.031 g, 5.0%), and sulfenimide **13** (0.121 g, 16.4%) as an oil: NMR ($CDCl_3$) δ 2.33 (s, 6 H), 3.55 (s, 2 H), 3.76 (s, 2 H), 4.15, 4.51 (d of d, 2 H, J = 13 Hz), 4.78 (d, 1 H, J = 4 Hz), 5.40 (d, 1 H, J = 4 Hz), 6.92 (s, 1 H), 7.30 (m, 18 H); IR ($CHCl_3$) 1790, 1730 cm^{-1}.

Sulfenimide **13**, when treated in deuteriochloroform with a few drops of *p*-toluenesulfenyl chloride, was immediately converted to sulfenimine **10** (NMR, TLC).

Preparation of Amine 19 ((6*R*-*cis*)-7-Amino-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-7-(methylthio)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Diphenylmethyl Ester). To a cold solution (0–5 °C) of sulfenimine **12** (1.0 g, 1.85 mmol) in methylene chloride (50 mL) at 26 °C, under nitrogen, was added Mallinckrodt SilicAR CC-4 (2 g), followed by triphenylphosphine (1.46 g, 5.56 mmol). The mixture was stirred at 26 °C for 12 h, whereupon NMR indicated a >90% conversion to **19**. The reaction mixture was concentrated under reduced pressure to a reddish oil and chromatographed (SilicAR CC-7, 5% ethyl acetate/methylene chloride). Substance **19** was isolated as a clear oil (0.520 g, 52%) [NMR ($CDCl_3$) δ 1.96 (br s, 2 H), 2.31 (s, 3 H), 3.68 (s, 3 H), 3.80 (s, 3 H), 4.21, 4.51 (d of d, 2 H, J = 13 Hz), 4.76 (s, 1 H), 6.88 (s, 1 H), 7.33 (s, 12 H); IR ($CHCl_3$) 1775, 1715 cm^{-1}] identical with an authentic sample prepared by the method of Slusarchyk.[7d]

Methylthiolation of 7-(4-nitrobenzalimino)-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid diphenylmethyl ester (KO-*t*-Bu, $CH_3SSO_2CH_3$, dimethoxyethane, −60 °C, 30 min) gave amorphous 7α-methylthio-7-(4-nitrobenzalimino)-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl-Δ^3-cephem-2-carboxylic acid diphenylmethyl ester in 28% yield: NMR ($CDCl_3$) δ 2.27 (s, 3 H), 3.73 (s, 2 H), 3.82 (s, 3 H), 4.35 (d of d, 2 H, J = 13 Hz), 5.13 (s, 1 H), 6.95 (s, 1 H), 7.40 (m, 10 H), 8.00 (d, 2 H, J = 9 Hz), 8.33 (d, 2 H, J = 9 Hz), 10.17 (s, 1 H).

Treatment of the above with 1 equiv of *p*-toluenesulfonic acid (25 °C, 2 h, dimethoxyethane) afforded, after workup (methylene chloride/sodium bicarbonate), 7α-methylthioamine **19** in almost quantitative yield.

Preparation of Amide 20. To a cold solution (0 °C) of 7α-methylthioamine **19** (0.235 g, 0.434 mmol) in methylene chloride (30 mL) were added diethylaniline (0.130 g, 0.869 mmol) and phenylacetyl chloride (0.134 g, 0.869 mmol). After the mixture was stirred for 12 h at 26 °C, the solvents were removed in vacuo and the resulting oil was applied to PLC plates (10% ethyl acetate/methylene chloride). Amide **20** was isolated as a clear oil (0.229 g, 80%): NMR ($CDCl_3$) δ 2.20 (s, 3 H), 3.58 (m, 2 H), 3.75 (s, 3 H), 4.18, 4.48 (d of d, 2 H, J = 13 Hz), 4.90 (s, 1 H), 6.86 (s, 1 H), 7.40 (m, 15 H); IR ($CHCl_3$) 1770, 1705, 1670 cm^{-1}. Anal. ($C_{32}H_{30}N_6O_4S_3$) C, H, N, S.

Preparation of 18 ((6*R*-*cis*)-7-Methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo[(phenylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Diphenylmethyl Ester). 7α-Methoxy amide **18** was prepared from **2** by the method of Yanagisawa et al.,[7k] to give a white foam: NMR ($CDCl_3$) δ 3.48 (s, 3 H), 3.53 (s, 2 H), 3.66 (s, 2 H), 3.81 (s, 3 H), 4.16, 4.48 (d of d, 2 H, J = 13 Hz), 5.00 (s, 1 H), 6.33 (s, 1 H, exchanges with D_2O), 6.86 (s, 1 H), 7.3 (br s, 15 H); IR ($CHCl_3$) 1775, 1715, 1685 cm^{-1}.

Substance **18** could be isomerized to its Δ^2 analogue as follows. A solution of **18** (0.064 g, 0.1 mmol) in deuteriochloroform (0.4 mL) was treated with triethylamine (100 μL). NMR indicated complete conversion to Δ^2 after 5 min at 26 °C. The reaction mixture was evaporated in vacuo, and the base chased with toluene (2 × 10 mL), to afford the product as a clear oil (0.063 g, 99%): NMR ($CDCl_3$) δ 3.31 (s, 3 H), 3.58 (s, 2 H), 3.73 (s, 3 H), 4.01, 4.20 (d, of d, 2 H, J = 13 Hz), 5.26 (br s, 2 H), 6.40 (br s, 1 H), 6.71 (s, 1 H), 6.85 (s, 1 H), 7.3 (br s, 15 H); IR ($CHCl_3$) 1770, 1730, 1680 cm^{-1}.

Conversion of 16 to 18 and Its C-7 Epimer. To a stirred solution of amide **16** (0.100 g, 0.136 mmol) in dry methanol (5 mL) and tetrahydrofuran (1 mL), under argon, was added mercuric acetate (0.044 g, 0.136 mmol). The mixture was stirred at 26 °C for 3 h, then filtered and concentrated under reduced pressure. The resulting oil was chromatographed (PLC, 10% ethyl acetate/methylene chloride) to yield **18** and its C-7 epimer, 0.061 g [73%, α/β (2:1) by NMR analysis], accompanied by a small amount of Δ^2 isomers.

Conversion of 20 to 18 and Its C-7 Epimer. To a stirred solution of amide **20** (0.110 g, 0.166 mmol) in dry methanol (5 mL) and methylene chloride (1 mL) under nitrogen was added mercuric acetate (0.054 g, 0.166 mmol). The reaction mixture color darkened, and after 40 min TLC indicated the absence of starting material. The solvents were removed in vacuo, and the resulting foam was applied to PLC plates (10% ethyl acetate/methylene chloride) to afford the product as an oil [0.071 g, 67%, α/β (5:1) by NMR analysis], accompanied by some Δ^2 isomers as minor products.

Direct Conversion of Sulfenimine 10 to 7α-Methoxy Amide 18. To a chilled (0–5 °C) solution of sulfenimine **10** (0.617 g, 1 mmol) in dry methylene chloride (40 mL) under nitrogen was added solid triphenylphosphine (0.786 g, 3 mmol), followed by a solution of mercuric acetate (0.314 g, 1 mmol) in dry methanol (10 mL). After stirring for

3.5 h, the mixture was evaporated to dryness under reduced pressure and then redissolved in methylene chloride (40 mL) and propylene oxide (10 mL). The solution was cooled to −15 °C and phenylacetyl chloride (1.55 g, 10 mmol) was added dropwise. The temperature was allowed to rise to 26 °C over 3 h, and the mixture was concentrated in vacuo and applied to a chromatographic column (SilicAR CC-7, 5% ethyl acetate/methylene chloride). The product was isolated as a white foam (0.514 g, 80%). A small amount of Δ^2 was indicated by ^{1}H NMR.

Direct Conversion of Sulfenimine 12 to 7α-Methoxy Amide 18. To a chilled (0–5 °C) solution of sulfenimine **12** (1.086 g, 2 mmol) in dry methylene chloride (80 mL) under a nitrogen atmosphere was added triphenylphosphine (1.602 g, 6 mmol) followed by a solution of mercuric acetate (0.638 g, 2 mmol) in dry methanol (20 mL). The reaction mixture was allowed to stir for 3.5 h (0–26 °C) whereupon the solvents were removed in vacuo. The resulting residue was redissolved in methylene chloride (60 mL) and propylene oxide (15 mL), chilled to −15 °C, and treated dropwise with phenylacetyl chloride (2.55 g, 15 mmol). The mixture was stirred for 1 h at −15 °C, whereupon the cooling bath was removed and the temperature allowed to rise to 26 °C over 3 h. The reaction mixture was evaporated to an oil and chromatographed (SilicAR CC-7, 5% ethyl acetate/methylene chloride) to afford **18** as a white foam (1.04 g, 80.9%).

General Procedure for Preparation of 7-Sulfenimino-β-lactam Acids and Salts 23–33. A mixture of 7-ACA (20 mmol), dry methylene chloride (400 mL), molecular sieves (4A, 5 g), propylene oxide (20 mL), and bis(trimethylsilyl)acetamide (40 mmol) was stirred at 26 °C for 1.5 h under nitrogen, with exclusion of moisture. The reaction mixture was then cooled to 0 °C and sulfenyl chloride (80 mmol) in methylene chloride (30 mL) was added dropwise over 30 min. The reaction mixture was stirred over 3 h as the bath was allowed to warm to 26 °C. The resulting mixture was filtered and then extracted with saturated sodium bicarbonate solution (150 mL × 3). The combined extracts were acidified with 1 N HCl to pH 2.3 and extracted with ethyl acetate (250 mL × 3). The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure to afford the product.

For salt preparation, the acid was dissolved in acetone and treated with a solution containing 1 equiv of sodium 2-ethyl hexanoate in 1-butanol. The mixed solution was kept at 26 °C for 1 h, whereupon the salt was precipitated with ethyl ether. The slurry was cooled, filtered, and washed with ethyl ether to yield the sodium salt.

In the case of **24**, upon addition of the silyl ester to saturated sodium bicarbonate solution, yellow crystals form immediately. The crystals (which proved to be the sodium salt) were collected by filtration, and the filter cake was washed with brine and dried in vacuo.

Both acids and salts were crystallized from mixtures of methanol/acetone/ether/hexane.

Sulfenimine 23: pale yellow crystals (27%), mp 204–205.5 °C; NMR (Me_2SO-d_6) 2.04 (s, 3 H), 2.36 (s, 3 H), 3.30, 3.62 (d of d, 2 H, J = 18 Hz), 4.80, 5.03 (d of d, 2 H, J = 12 Hz), 5.67 (br s, 1 H), 7.30, 7.48 (d of d, 4 H, J = 8 Hz); IR (KBr) 1750 (br), 1730, 1615; TLC R_f 0.58 (Eastman cellulose 6064, *n*-butyl alcohol/ethanol/water, 4:1:5), R_f 0.63 (Merck silica gel 5765, *n*-butyl alcohol/acetic acid/water, 3:1:1). Anal. ($C_{17}H_{15}N_2O_5S_2Na$) C, H, N, S.

Sulfenimine 24: bright yellow crystals (61%); 186–187 °C; NMR (Me_2SO-d_6) δ 1.97 (s, 3 H), 2.33 (s, 3 H), 3.11, 3.35 (d of d, 2 H, J = 18 Hz), 5.52 (br s, 1 H), 7.25, 7.43 (d of d, 4 H, J = 8 Hz); IR (KBr) 1750 (br), 1600 (br) cm^{-1}; TLC R_f 0.60 (Eastman cellulose 6064 (*n*-butyl alcohol/ethanol/water, 4:1:5)), R_f 0.65 (Merck silica gel 5765 (*n*-butyl alcohol/acetic acid/water, 3:1:1)). Anal. $C_{15}H_{13}N_2O_3S_2Na$) C, H, N, S.

Sulfenimine 25: light tan solid (31.7%) material, judged by TLC and electrophoresis to be 85–90% pure; mp 74–76 °C; NMR ($CDCl_3$/CD_3OD) δ 2.33 (s, 3 H), 3.64, 3.85 (d of d, 2 H, J = 18 Hz), 3.93 (s, 3 H), 4.26, 4.42 (d of d, 2 H, J = 13 Hz), 5.70 (br s, 1 H), 7.26, 7.43 (d of d, 4 H, J = 8 Hz); IR (KBr) 1770, 1715 cm^{-1}; IR (KBr) 1770, 1715 cm^{-1}; TLC R_f 0.58 (Eastman cellulose 6064, *n*-butyl alcohol/ethanol/water, 4:1:5). R_f 0.58 (Merck silica gel 5765, *n*-butyl alcohol/acetic acid/water, 3:1:1).

Sulfenimine 26: yellow crystals (76%); mp 103–104 °C; NMR ($CDCl_3$) δ 2.10 (s, 3 H), 2.87 (s, 3 H), 3.34, 3.60 (d of d, 2 H, J = 18 Hz), 4.90, 5.12 (d of d, 2 H, J = 13 Hz), 5.28 (br s, 1 H); UV (MeOH) 243 nm (ε 4600), 324 (10 200); IR (KBr) 1765, 1730 cm^{-1}. Anal. ($C_{11}H_{12}N_2O_5S_2$) C, H, N, S.

Sulfenimine 27: yellow crystals (82%); mp 165–165.5 °C dec; NMR ($CDCl_3$/CD_3OD) δ 2.19 (s, 3 H), 2.87 (s, 3 H), 3.17, 3.48 (d of d, 2 H, J = 18 Hz), 5.25 (s, 1 H); UV (MeOH) 244 nm (ε 4900), 318 (9800); IR (KBr) 1760, 1700 cm^{-1}. Anal. ($C_9H_{10}N_2O_3S_2$) C, H, N, S.

Sulfenimine 28: off-white crystals (95%), greater than 90% pure by TLC and electrophoresis; mp 153–155 °C dec; NMR ($CDCl_3$/CD_3OD) δ 2.80 (s, 3 H), 3.50, 3.72 (d of d, 2 H, J = 18 Hz), 4.03 (s, 3 H), 4.35 (s, 2 H), 5.23 (s, 1 H): IR (KBr) 1765, 1720 cm^{-1}; UV (MeOH) 332 nm (ε 11 600).

Sulfenimine 29: greenish-yellow crystals (60% yield of acid, 27.5% yield of sodium salt); mp 146–148 °C; NMR (Me_2SO-d_6) δ 2.00 (s, 3 H), 3.30, 3.55 (d of d, 2 H, J = 18 Hz), 4.75, 5.00 (d of d, 2 H, J = 13 Hz), 5.65 (s, 1 H), 7.50 (br s, 5 H); IR (KBr) 1750 (br), 1605 (br) cm^{-1}; UV (MeOH) 326 nm (ε 10 800). Anal. ($C_{16}H_{13}N_2O_5S_2Na$) C, H, N, S, Na.

Sulfenimine 30: bright yellow crystals (82.7% of acid, 37.7% sodium salt); mp 144–146 °C, NMR (Me_2SO-d_6) δ 2.00 (s, 3 H), 3.26, 3.58 (d of d, 2 H, J = 18 Hz), 3.81 (s, 3 H), 4.78, 4.98 (d of d, 2 H, J = 12 Hz), 5.60 (br s, 1 H), 7.05, 7.54 (d of d, J = 10 Hz); IR (KBr) 1750 (br), 1605 (br) cm^{-1}. Anal. ($C_{17}H_{15}N_2O_6S_2Na$) C, H, N, S, Na.

Sulfenimine 31: yellow crystals (73.6% acid, 35.9% yield of sodium salt); mp 141–143 °C dec; NMR (Me_2SO-d_6) δ 2.00 (s, 3 H), 3.28, 3.60 (d of d, 2 H, J = 18 Hz), 4.80, 5.00 (d of d, 2 H, J = 13 Hz), 5.67 (br s, 1 H), 7.45 (m, 4 H); IR (KBr) 1750 (br), 1605 (br) cm^{-1}. Anal. ($C_{16}H_{12}N_2O_5S_2ClNa$) C, H, N, S, Cl, Na.

Sulfenimine 32: yellow solid (63.1% yield of acid, 27.2% yield of salt); mp 155–157 °C; NMR (Me_2SO-d_6) δ 2.00 (s, 3H), 3.25, 3.70 (d of d, 2 H, J = 18 Hz), 4.55 (s, 2 H), 4.80, 5.07 (d of d, 2 H, J = 13 Hz), 5.55 (s, 1 H), 7.40 (s, 5 H); IR (KBr) 1750 (br), 1610 (br) cm^{-1}. Anal. ($C_{17}H_{15}N_2O_5S_2Na$) C, H, N, S, Na.

Sulfenimine 33: orange-yellow solid (60%); 70% pure by TLC and electrophoresis; mp 142–147 °C; ^{1}H NMR ($CDCl_3$) δ 1.66 (s, 6 H), 2.41 (s, 3 H), 4.71 (s, 1 H), 5.83 (s, 1 H), 7.30, 7.54 (d of d, 4 H, J = 8 Hz); IR (KBr) 1770 (br), 1720 (br) cm^{-1}. Anal. ($C_{15}H_{16}N_2O_3S_2$) C, H, N.

Preparation of Sulfenimine 40 ([2*S*-(2α,5α)-3,3-Dimethyl-6-[[(4-methylphenyl)thio]imino]- 7 -oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid 2,2,2-Trichloroethyl Ester). To a cold (0 °C) mixture of 6-aminopenicillanic acid trichloroethyl ester *p*-toluenesulfonate salt (5.0 g, 9.40 mmol) in dry methylene chloride (400 mL) and propylene oxide (40 mL), containing crushed molecular sieves (4A, 50 g), was added dropwise, under nitrogen, triethylamine (1.31 mL, 9.40 mmol). After addition was complete, *p*-toluenesulfenyl chloride (4.46 g, 38.20 mmol) was added dropwise with stirring. The reaction mixture was stirred well at 0 °C for 2 h and then at 26 °C for 0.5 h. After filtration, the filtrate was concentrated under reduced pressure to an oil and chromatographed (SilicAR CC-7, hexane/methylene chloride, 1:2). Sulfenimine **40** was obtained as a clear, bright, yellow oil (3.53 g, 80.4%), which crystallized upon cooling: mp 77–79 °C; NMR ($CDCl_3$) δ 1.60 (br s, 6 H), 2.36 (s, 3 H), 4.73 (s, 1 H), 4.80 (s, 2 H), 5.73 (s, 1 H), 7.16, 7.46 (d of d, 4 H, J = 8 Hz); IR ($CHCl_3$) 1780, 1760 cm^{-1}; UV (MeOH) 226 nm (ε 15 400), 267 (8000), 338 (8800); mass spectrum *m/e* 466 (M^+); ^{13}C NMR ($CDCl_3$) δ 21.1, 25.5, 33.8, 63.6, 70.2, 71.4, 74.8, 93.9, 127.7, 129.8, 132.2, 138.5, 165.7. Anal. ($C_{17}H_{17}N_2O_3S_2Cl_3$) C, H, N, S, Cl.

Preparation of Sulfenimine 41 (2*S*-(2α,5α)-3,3-Dimethyl-6-[(methylthio)imino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid 2,2,2-Trichloroethyl Ester). To a cold (0 °C) mixture of 6-aminopenicillanic acid trichloroethyl ester *p*-toluenesulfonate salt (**38**, 3.00 g, 5.66 mmol), in dry methylene chloride (240 mL) and propylene oxide (24 mL), containing crushed molecular sieves (4A, 30 g) was added dropwise, under nitrogen, triethylamine (0.79 mL, 5.66 mmol). After addition was complete, freshly prepared methylsulfenyl chloride (1.40 g, 16.98 mmol) was added dropwise with stirring. The reaction mixture was stirred at 0 °C for 2 h and at 26 °C for 0.5 h. After filtration and water wash, the solvents were removed under reduced pressure and the residue crystallized from methylene chloride/ethyl ether (0.957 g, 42.9%). Recrystallization from the same solvents afforded an analytical sample as white crystals: mp 129–130 °C; NMR ($CDCl_3$) δ 1.60 (s, 6 H), 2.90 (s, 3 H), 4.76 (s, 1 H), 4.86 (s, 2 H), 5.81 (s, 1 H); IR (KBr) 1770, 1755 cm^{-1}; mass spectrum *m/e* 390 (M^+, weak); UV (MeOH) 247 nm (ε 1800), 313 (7700). Anal. ($C_{11}H_{13}N_2O_3S_2Cl_3$) C, H, N, S, Cl.

Preparation of Sulfenimine 42. By the above procedure 6-aminopenicillanic acid *p*-nitrobenzyl ester *p*-toluenesulfonate salt **39** (52.3 g, 0.1 M) was converted to sulfenimine **42** (38.7 g, 82%). Three

crystallizations from ether/hexane afforded an analytical sample as bright yellow needles: mp 84–86 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 3 H), 1.55 (s, 3 H), 1.55 (s, 3 H), 2.36 (s, 3 H), 4.66 (s, 1 H), 5.30 (s, 2 H), 5.70 (s, 1 H), 7.14 (m, 8 H); ^{13}C NMR (acetone-d_6) δ 20.9, 25.2, 33.8, 64.3, 66.2, 70.7, 71.8, 124.1, 127.7, 129.4, 130.5, 138.9, 143.3, 148.3, 167.4; IR (KBr) 1775, 1740 cm^{-1}; mass spectrum *m/e* 471 (M^+); UV($CHCl_3$) 267 nm (ε 17 000), 354 (8400). Anal. ($C_{22}H_{21}N_3O_2S_5$) C, H, N, S.

X-ray Analysis of 42. The unit cell parameters of the yellow crystals, a = 25.65 (1) Å, b = 6.016 (1) Å, c = 15.85 (3) Å, β = 97.08 (2)°, were determined diffractometrically from 15 well-centered reflections. The diffraction pattern and the measured crystal density, 1.26 g cm^{-3}, are consistent with four molecules of **42** ($C_{22}H_{21}N_3S_2O_5$) per unit cell of space group $C2$.

Of the 1686 intensities, measured diffractometrically out to $2\theta_{max}$ = 114.5° using the θ–2θ variable scan rate method with monochromatic Cu Kα radiation (λ = 1.542 Å), 1252 were "observed" with $I > 3\sigma$, where $\sigma = r(C + 4(B_1 + B_2))^{1/2}$. B_1 and B_2 are background counts measured for 1/4 of the scan time at each end of the scan range; C is the total reflection count and r is the scan rate.

The structure was solved by direct methods and refined through several cycles of least squares in which all atoms were treated anisotropically. Atomic scattering factors were taken from the International Tables for X-ray Crystallography.[35] The quantity minimized was $\Sigma w(|F_o|^2 - |F_c|^2)^2$ with $w = \sigma^{-2}$.

Some, but not all, of the hydrogens appeared as the only significant residuals in a difference map calculated after refinements of the other atoms had converged to $R = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.08$. All but the methyl hydrogens were then introduced at calculated positions though not refined in the subsequent, final cycles of refinements. The final R factor is 0.07.

The final atomic coordinates are given in Table V (see supplementary material).

Rearrangement of Sulfenimine 40 to Amine 43. [2*S*-(2α,5α,6α)]-6-Amino-3,3-dimethyl-6-[4-(methylphenyl)thio)-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid 2,2,2-Trichloroethyl Ester. To a stirred solution of sulfenimine **40** (4.66 g, 10 mmol) in methylene chloride (150 mL) at 26 °C, under nitrogen, were added triphenylphosphine (8.01 g, 30 mmol) and Mallinckrodt SilicAR CC-4 (5.0 g). The mixture was stirred for 18 h, then concentrated under reduced pressure and applied to a chromatographic column (SilicAR CC-7, methylene chloride). The product was obtained as a yellow foam (4.48 g, 96%): NMR ($CDCl_3$) δ 1.55 (s, 3 H), 1.63 (s, 3 H), 2.36 (s, 5 H, 3 H after D_2O exchange), 4.55 (s, 1 H), 4.76 (s, 2 H), 5.50 (s, 1 H), 7.30 (d of d, 4 H, J = 8 Hz); IR ($CHCl_3$) 1780, 1770 (sh) cm^{-1}; mass spectrum *m/e* 468 (M^+). The material appeared to be a pure diastereomer.

Conversion of Thioamine 43 to Amide 45. 6α-Arylthioamine **43** (86 mg, 0.184 mmol) was dissolved in methylene chloride (10 mL) and chilled at 0–5 °C under nitrogen. Phenylacetyl chloride (57 mg, 53 μL, 0.368 mmol) was added, followed by diethylaniline (55 mg, 66 μL, 0.368 mmol). After stirring for 2 h at 0 °C the reaction mixture was concentrated under reduced pressure to an oil. PLC (5% ethyl acetate/methylene chloride) afforded the product (**45**) as a clear oil (29.0 mg, 27%). The R_f of starting material and product were almost identical in this system: NMR ($CDCl_3$) δ 1.41 (s, 3 H), 1.45 (s, 3 H), 2.33 (s, 3 H), 3.51 (s, 2 H), 4.45 (s, 1 H), 4.73 (s, 2 H), 5.61 (s, 1 H), 7.2 (m, 10 H); IR ($CHCl_3$) 1790, 1770 (sh), 1690, 1500 cm^{-1}; mass spectrum *m/e* 586 (M^+). Anal. ($C_{25}H_{25}N_2O_4S_2Cl_3$) C, H, N, S, Cl.

Rearrangement of Sulfenimine 42 to Thioamine 44. Following the above procedure, sulfenimine **42** (7.0 g, 14.8 mmol) was converted to 6α-arylthioamine **44**. Purification by column chromatography (Baker silica gel, methylene chloride) afforded the product as a yellow foam (4.17 g, 59.5%): NMR ($CDCl_3$) δ 1.40 (s, 3 H), 1.60 (s, 3 H), 2.20 (br s, 2 H), 2.33 (s, 3 H), 4.46 (s, 1 H), 5.26 (s, 2 H), 5.45 (s, 1 H), 7.2–8.4 (m, 8 H); IR ($CHCl_3$) 1780, 1755 cm^{-1}.

Conversion of Thioamines 43 and 44 to 6-Ketopenams 46 and 47. A solution of 6α-arylthioamine **43** (0.468 g, 1 mmol) in 1,2-dimethoxyethane (25 mL) was treated with water (2.5 mL) and then solid mercuric chloride (0.271 g, 1 mmol) was added. After stirring at 26 °C for 30 min, the reaction mixture was diluted with ethyl acetate (120 mL), washed with water (5 × 20 mL) and brine (1 × 20 mL), and then dried over magnesium sulfate. After filtration, the filtrate was concentrated under reduced pressure to a semisolid. To this residue was added dry benzene (30 mL), and the solution filtered and evaporated. This process was repeated, to afford a semisolid yellow residue (0.335 g, 97%): NMR ($CDCl_3$) δ 1.65 (s, 6 H), 4.80 (s, 2 H), 4.91 (s, 1 H), 5.80 (s, 1 H); IR ($CHCl_3$) 1835, 1790, 1765 cm^{-1}; mass spectrum *m/e* 345 (M^+). This material could not be further purified by PLC.

In a similar manner, substance **44** (1.41 g, 3 mmol) afforded 6-ketopenam **47** as a yellow semisolid (1.00 g, 95%): NMR ($CDCl_3$) δ 1.51 (s, 3 H), 1.56 (s, 3 H), 4.93 (s, 1 H), 5.41 (s, 2 H), 5.86 (s, 1 H), 7.63 (d of d, 4 H, J = 10 Hz); IR ($CHCl_3$) 1835, 1785, 1750 cm^{-1}; mass spectrum *m/e* 350 (M^+).

Direct Conversion of Sulfenimine 40 to *N*-Phenylacetyl-6α-methoxypenam 49. Sulfenimine **40** (16.1 g, 34.5 mmol) and triphenylphosphine (27.6 g, 103.6 mmol) were dissolved in methylene chloride (600 mL) and stirred at 26 °C. A solution of mercuric acetate (11.0 g, 34.5 mmol) in dry methanol (150 mL) was immediately added and the reaction mixture stirred for 3.5 h. The mixture was evaporated to dryness under reduced pressure and then redissolved in methylene chloride (600 mL) and propylene oxide (150 mL). This solution was chilled to −10 °C and phenylacetyl chloride (26.7 g) in methylene chloride (80 mL) was added dropwise with stirring. After 3 h the residue was concentrated to an oil and chromatographed on silica gel (Mallinckrodt SilicAR CC-7) to yield **49** as a crystal clear, colorless oil (15.49 g, 91%): homogeneous by TLC; NMR ($CDCl_3$) 1.40 (s, 3 H), 1.46 (s, 3 H), 3.40 (s, 3 H), 3.63 (s, 2 H), 4.51 (s, 1 H), 4.81 (s, 2 H), 5.66 (s, 1 H), 7.30 (s, 5 H); IR ($CHCl_3$) 1765, 1760, 1675 cm^{-1}; mass spectrum *m/e* 494 (M^+).

Preparation of Sulfoxide 50. *N*-Phenylacetyl-6α-methoxypenam **49** (15.16 g, 30.68 mmol) was dissolved in methylene chloride (600 mL) and chilled to −10 °C. A solution of *m*-chloroperbenzoic acid (6.15 g) in methylene chloride (200 mL) was added dropwise over 20 min. After stirring further at −10 °C for 1 h, the mixture was warmed to 26 °C and stirred for 2 h. After removal of solvents under reduced pressure, the residue was redissolved in ethyl acetate (200 mL), washed with saturated sodium bicarbonate (2 × 100 mL) and brine (1 × 100 mL), and then dried over sodium sulfate. Evaporation under reduced pressure afforded the product as a white foam (14.70 g, 94%). A portion was crystallized from chloroform/ethyl ether/hexane (twice) to give an analytical sample: mp 140–141 °C; NMR ($CDCl_3$) δ 1.66 (s, 3 H), 1.76 (s, 3 H), 3.45 (s, 3 H), 3.73 (s, 2 H), 4.83 (s, 1 H), 4.70, 5.10 (d of d, 2 H, J = 12 Hz), 5.10 (s, 1 H), 7.10 (s, 1 H), 7.36 (s, 5 H); IR (KBr) 1785, 1770, 1680 cm^{-1}; mass spectrum *m/e* 510 (M^+). Anal. ($C_{19}H_{21}N_2O_6SCl_3$) C, H, N, S, Cl.

Conversion of Sulfoxide 50 to *N*-Phenylacetyl-7α-methoxycephem Trichloroethyl Ester (51). Sulfoxide **50** (0.988 g, 1.93 mmol) was dissolved in toluene (100 mL) and a catalytic amount of 2-picolinium dichloromethyl phosphonate (30 mg) was added. The mixture was heated under reflux in an argon atmosphere for 10 h. After cooling to 26 °C the reaction mixture was concentrated under reduced pressure to an oil which was chromatographed (PLC, 10% ethyl acetate/methylene chloride). The product was obtained as a tan solid (0.227 g, 24%) which crystallized from ethyl acetate/benzene: white crystals; mp 200–203 °C; NMR ($CDCl_3$) δ 2.23 (s, 3 H), 3.18 (s, 2 H), 3.45 (s, 3 H), 3.65 (s, 2 H), 7.30 (s, 5 H); IR ($CHCl_3$) 1770, 1730, 1680 cm^{-1}; mass spectrum *m/e* 492 (M^+). Anal. ($C_{19}H_{19}N_2O_5SCl_3$) C, H, N, S, Cl (block dried at 80 °C).

Preparation of Acid 52. Ester **51** (0.347 g, 0.70 mmol) was dissolved in dry THF (10 mL) and stirred well at 26 °C while powdered zinc (0.700 g) was added, followed by 1 M ammonium acetate buffer (2 mL, pH 7.6). After 25 min, the mixture was poured into pH 4 phosphate buffer which was extracted with ethyl acetate (2 × 50 mL). Drying over sodium sulfate, followed by removal of solvents in vacuo, gave material identified as starting ester **51** (52 mg). Acidification of the aqueous pH 4 buffer to pH 2 (HCl), followed by extraction with ethyl acetate (2 × 50 mL), gave acid **52** as a clear oil (60 mg, 16.5%): NMR ($CDCl_3$) δ 2.23 (s, 3 H), 3.16 (s, 3 H), 3.50 (s, 3 H), 3.73 (s, 2 H), 5.06 (s, 1 H), 7.33 (s, 5 H), 7.60 (br s, 2 H); IR ($CHCl_3$) 1780, 1710, 1695 cm^{-1}. This material was identical by NMR, IR, and TLC (1/1 acetone/chloroform with 1% acetic acid) with an authentic sample.[40]

Preparation of Sulfoxide 53. To a cold (0–5 °C) solution of sulfenimine **40** (27.93 g, 58.3 mmol) in dry methylene chloride (600 mL) was added dropwise with stirring a solution of 85% *m*-chloroperbenzoic acid (11.83 g, 58.3 mmol) in methylene chloride (300 mL). Upon completion of addition, the reaction mixture was stirred at 0–5 °C for 1 h and then at 26 °C for 12 h. The solvent was removed under reduced pressure at 26 °C and the resulting residue was chromatographed (SilicAR CC-7, methylene chloride) to afford **53** as a yellow foam

(16.86 g, 58.4%). This material was a mixture of diastereoisomers, 17:6 (NMR) epimeric at sulfinyl sulfur. PLC (1% ethyl acetate/methylene chloride) followed by fractional crystallization (CH_2Cl_2/hexane) yielded the major isomer in pure form: mp 141.5–142.5 °C; NMR ($CDCl_3$) δ 1.33 (s, 3 H), 1.80 (s, 3 H), 2.40 (s, 3 H), 4.71, 5.07 (d of d, 2 H, J = 11 Hz), 4.88 (s, 1 H), 5.41 (s, 1 H), 7.24, 7.52 (d of d, 4 H, J = 6 Hz); mass spectrum *m/e* 482; UV (MeOH) 257 nm (ϵ 5900), 348 (7900). Anal. ($C_{17}H_{17}N_2O_4S_2Cl_3$) C, H, N, S, Cl.

Preparation of Sulfenimine Disulfide 54. Sulfoxide **53** (0.254 g, 0.526 mmol, mixture of *R* and *S* isomers at sulfur) was mixed with 2-mercaptobenzothiazole (0.088 g, 0.526 mmol) in dry toluene (25 mL) under nitrogen and heated under reflux for 1.5 h. At this point TLC (2% ethyl acetate/methylene chloride) indicated the absence of starting sulfoxide and the appearance of a higher R_f yellow spot. The reaction mixture was cooled to 26 °C and concentrated under reduced pressure to a dark solid, which was >90% disulfide **54** by NMR. PLC (methylene chloride) afforded the product as a clear, rather unstable, yellow oil: NMR ($CDCl_3$) δ 2.00 (s, 3 H), 2.33 (s, 3 H), 4.61, 4.85 (d of d, 2 H, J = 12 Hz), 5.20 (m, 3 H), 5.76 (s, 1 H), 7.4 (m, 8 H), IR ($CHCl_3$) 1760, 1750 (sh) cm^{-1}. Material losses during isolation were high, presumably owing to instability of **54** on silica gel.

Preparation of Sulfoxide 55. Sulfoxide **53** (0.241 g, 0.50 mmol, mixture of *R* and *S* isomers at sulfur) was dissolved in methylene chloride (20 mL) and a solution of 85% *m*-chloroperbenzoic acid (0.099 g, 0.50 mmol) in the same solvent (5 mL) was added dropwise at 26 °C. The reaction mixture was stirred at 26 °C for 2 h, whereupon the original bright yellow color had faded to pale yellow. TLC indicated the conversion of starting material to a more polar substance. The mixture was partitioned between ethyl acetate and aqueous saturated sodium bicarbonate. The organic layer was washed with water, dried over sodium sulfate, and concentrated under reduced pressure to a clear oil. PLC (10% ethyl acetate/methylene chloride) afforded **55** (diastereoisomeric mixture) as a clear, pale yellow oil (0.129 g, 52%): NMR ($CDCl_3$) δ 1.36 (br s, 3 H), 1.75 (br s, 3 H), 2.40 (s, 3 H), 4.65, 5.01 (d of d, 2 H, J = 12 Hz), 4.93 (br s, 1 H), 6.13 (s, 0.5 H, C-5 one isomer), 6.21 (s, 0.5 H, C-5 one isomer), 7.43 (m, 4 H); IR ($CHCl_3$) 1800, 1765 cm^{-1}; mass spectrum *m/e* 498 (M^+), 450 (M − SO), 278, 219.[35] Anal. ($C_{17}H_{17}N_2O_5S_2Cl_3$) C, H, N, S, Cl.[35]

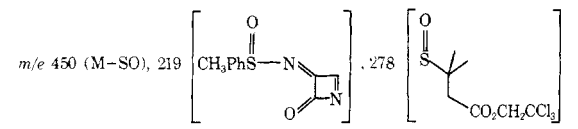
Acknowledgment. We wish to express our gratitude to The Squibb Institute Analytical Department for their assistance during the course of this research in obtaining spectral and microanalytical data. We also wish to acknowledge many helpful discussions with our colleagues Drs. William H. Koster and William A. Slusarchyk. We thank Dr. Jeff Schwartz for performing microbiological assays and Ms. Judith Melnik for help in preparation of the manuscript.

Supplementary Material Available: Tables of deviations from weighted least-squares planes of **42** (Table IV), fractional atomic coordinates and estimated errors (Table V), temperature factors (Table VI), and observed and calculated structure factors (Table VII) (11 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) A preliminary account of this study was presented at the 10th International Congress of Chemotherapy, Zurich, Switzerland, Sept 18–23, 1977. (b) The Squibb Institute for Medical Research. (c) University of Minnesota.
(2) E. M. Gordon, H. W. Chang, and C. M. Cimarusti, *J. Am. Chem. Soc.*, **99**, 5504 (1977).
(3) R. Nagarajan, L. D. Boeck, M. Gorman, R. L. Hamill, C. E. Higgens, M. M. Hoehn, W. M. Stark, and J. G. Whitney, *J. Am. Chem. Soc.*, **93**, 2308 (1971).
(4) E. O. Stapley, M. Jackson, S. Hernandez, S. B. Zimmerman, S. A. Currie, S. Mochales, J. M. Mata, H. B. Woodruff, and D. Hendlin, *Antimicrob. Agents Chemother.*, **2**, 122 (1972); T. W. Miller, R. T. Goegelman, R. G. Weston, I. Putter, and F. J. Wolf, *ibid.*, **2**, 132 (1972).
(5) (a) Japanese Patent 50-53597 (Takeda Chemical Ind.); (b) Japanese Patent 49-30593 (Fujisawa Co.); (c) Japanese Patent 49-42893 (Sankyo Co.); (d) Japanese Patent 50-69294 (Takeda Chemical Ind.); (e) Japanese Patent 51-110097 (Sanraku Ocean Co.); (f) H. Fukase, T. Hasegawa, K. Hatano, H. Iwasaki, and M. Yoneda, *J. Antibiot.*, **29**, 113 (1976); (g) H. Fukase and H. Iwasaki, *Bull. Chem. Soc. Jpn.*, **49**, 767 (1976); (h) Japanese Patent 49-26488 (Fujisawa Co.); (i) Japanese Patent 50-155696 (Meiji Seika Co.); (j) Japanese Patent 50-121488 (Meiji Seika Co.); (k) Japanese Patent 51-44694 (Yamanouchi Co.).
(6) (a) P. O. K. Ho, R. D. Towner, J. M. Idelicato, W. J. Wilham, W. A. Spitzer, and G. A. Koppel, *J. Antibiot.*, **26**, 213 (1973); (b) H. R. Onishi, D. R. Daust, S. B. Zimmerman, D. Handlin, and E. O. Stapley, *Antimicrob. Agents Chemother.*, **5**, 38 (1974), and references cited therein; (c) H. Nakao, H. Yanagisawa, B. Shimizu, M. Kaneko, M. Nagano, and S. Sugawara, *J. Antibiot.*, **29**, 554 (1976); (d) J. Birnbaum, E. O. Stapley, A. K. Miller, H. Wallick, D. Handlin, and H. B. Woodruff, *J. Antimicrob. Chemother., Suppl. B*, **4**, 15 (1978).
(7) (a) L. D. Cama, W. J. Leanza, T. R. Beatie, and B. G. Christensen, *J. Am. Chem. Soc.*, **94**, 1408 (1972); (b) W. A. Spitzer and T. Goodson, *Tetrahedron Lett.*, 273 (1973); (c) W. A. Slusarchyk, H. E. Applegate, P. Funke, W. Koster, M. S. Puar, M. Young, and J. E. Dolfini, *J. Org. Chem.*, **38**, 943 (1973); (d) H. E. Applegate, J. E. Dolfini, M. S. Puar, W. A. Slusarchyk, B. Toeplitz, and J. Z. Gougoutas, *ibid.*, **39**, 2794 (1974); (e) T. Jen, J. Frazee, and J. R. E. Hoover, *ibid.*, **38**, 2857 (1973); (f) L. D. Cama and B. G. Christensen, *Tetrahedron Lett.*, 2505 (1973); (g) J. E. Baldwin, F. J. Urban, R. D. G. Cooper, and F. L. Jose, *J. Am. Chem. Soc.*, **95**, 2401 (1973); (h) G. A. Koppel and R. E. Koehler, *ibid.*, **95**, 2403 (1973); (i) R. A. Firestone and B. G. Christensen, *J. Org. Chem.*, **38**, 1436 (1973); (j) P. H. Bentley and J. P. Clayton, *J. Chem. Soc., Chem. Commun.*, 278 (1974); (k) H. Yanagisawa, M. Fukushima, A. Ando, and H. Nakao, *Tetrahedron Lett.*, 2705 (1975); (l) Y. Sugimura, K. Iino, Y. Iwano, T. Saito, and T. Hiraoka, *ibid.*, 1307 (1976); (m) T. Saito, Y. Sugimura, Y. Iwano, K. Iwano, and T. Hiraoka, *J. Chem. Soc., Chem. Commun.*, 516 (1976); (n) H. Yanagisawa, M. Fukushima, A. Ando, and H. Nakao, *Tetrahedron Lett.*, 259 (1976) (o) H. Yanagisawa and H. Nakao, *ibid.*, 1811 (1976); (p) H. Yanagisawa and H. Nakao, *ibid.*, 1815 (1976); (q) Y. Sugimura, K. Iino, Y. Iwano, T. Saito, and T. Hiraoka, *Chem. Pharm. Bull.*, **25**, 369 (1977); (r) T. Saito and T. Hiraoka, *ibid.*, **25**, 784, 792 (1977); (s) T. Kobayashi, K. Iino, and T. Hiraoka, *J. Am. Chem. Soc.*, **99**, 5505 (1977); (t) T. Saito and T. Hiraoka, *Chem. Pharm. Bull.*, **25**, 1645 (1977); (u) Y. Sugimura, Y. Iwano, K. Iino, T. Saito, and T. Hiraoka, *Tetrahedron Lett.*, 2947 (1977).
(8) Merck Sharp and Dohme markets cefoxitin under the name Mefoxin. CS 1170 (Sankyo Co.) is presently undergoing clinical trials.
(9) (a) R. B. Morin, E. M. Gordon, T. McGrath, and R. Shuman, *Tetrahedron Lett.*, 2159 (1973); (b) E. M. Gordon, Ph.D. Dissertation, University of Wisconsin, Madison, 1973; (c) J. R. Lake, Ph.D. Dissertation, University of Wisconsin, Madison, 1974. (d) Hydroxamic acid derivatives are also known to undergo elimination yielding acylimine intermediates. See R. B. Morin, J. R. Lake, and E. M. Gordon, *Tetrahedron Lett.*, 2979 (1974).
(10) T. L. Cairns, *Org. Synth.*, **35**, 99 (1955).
(11) L. M. Weinstock, S. Karady, F. E. Roberts, A. M. Hoinowski, G. S. Brenner, T. B. K. Lee, W. C. Lumma, and M. Sletzinger, *Tetrahedron Lett.*, 3979 (1975).
(12) W. M. Welch, *J. Org. Chem.*, **41**, 2220 (1976).
(13) M. Oki and K. Kobayashi, *Bull. Chem. Soc. Jpn.*, **43**, 1223 (1970).
(14) H. J. Monteiro, *J. Org. Chem.*, **42**, 2324 (1977).
(15) I. B. Douglass, *J. Org. Chem.*, **24**, 2004 (1959).
(16) A. Atkinson, A. G. Brewster, S. V. Ley, R. S. Osborne, D. Rogers, D. J. Williams, and K. A. Woode, *J. Chem. Soc., Chem. Commun.*, 325 (1977).
(17) E. M. Gordon and Jelka Pluscec, *J. Org. Chem.*, **44**, 1218 (1979).
(18) The β-lactamase enzyme employed was a Richmond Type Ia cephalosporinase, available from Miles Laboratories. The test antibiotic was cephradine. Compounds were assayed by a disk agar diffusion plate technique, similar to one reported by H. Umezawa, S. Mitsuhashi, M. Hamada, I. Iyobe, S. Takahashi, R. Utahara, Y. Osato, S. Yamazaki, H. Ogawara, and K. Maeda, *J. Antibiot.* **26**, 51 (1973).
(19) (a) F. A. Davis and E. B. Skibo, *J. Org. Chem.*, **39**, 807 (1974); (b) F. A. Davis, A. J. Friedman, and E. W. Kluger, *J. Am. Chem. Soc.*, **96**, 5000 (1974); (c) F. A. Davis, J. M. Kaminski, E. W. Kluger, and H. S. Freilich, *ibid.*, **97**, 7085 (1975); (d) F. A. Davis and P. A. Mancinelli, *J. Org. Chem.*, **43**, 1797 (1978); (e) F. A. Davis, A. J. Friedman, and U. K. Nadir, *J. Am. Chem. Soc.*, **100**, 2844 (1978); (f) F. A. Davis and P. A. Mancinelli, *J. Org. Chem.*, **42**, 399 (1977).
(20) Sulfenimine literature prior to 1973 is summarized in a section of Davis's review on sulfenamides. F. A. Davis, *Int. J. Sulfur Chem.*, **8**, 71 (1973).
(21) In addition to those outlined in ref 18, sulfenimines may be prepared by methods described in (a) F. A. Davis, W. A. R. Slegeir, S. Evans, A. Schwartz, D. L. Goff, and R. Palmer, *J. Org. Chem.*, **38**, 2809 (1973); (b) F. A. Davis, A. J. Friedman, E. W. Kluger, E. B. Skibo, E. R. Fretz, A. P. Milicia, W. C. LeMasters, M. D. Bentley, J. A. Lacadie, and I. B. Douglass, *ibid.*, **42**, 967 (1977).
(22) T. Kobayashi, K. Iino, and T. Hiraoka, *J. Am. Chem. Soc.*, **99**, 5505 (1977). For related work see also ref 7r.
(23) H. E. Applegate, C. M. Cimarusti, and W. A. Slusarchyk, *Tetrahedron Lett.*, 1637 (1979).
(24) For an excellent review of this active area see T. Mukaiyama, *Angew. Chem., Int. Ed. Engl.*, **15**, 94 (1976).
(25) F. W. Wehrli and T. Wirthlin, "Interpretation of C-13 NMR Spectra", Heyden, London, 1976, p 36.
(26) Silica gel brands which were effective in this transformation include MCB grade 923, 100–200 mesh; ICN dry column; Baker 503405, 60–200 mesh; E. Merck silica gel 60, 9384, 230–400 mesh.
(27) We thank Dr. W. A. Slusarchyk of these laboratories for providing this sample.
(28) S. Kukolja, N. D. Jones, M. O. Chaney, T. K. Elzey, M. R. Gleissner, J. W. Pascha, and D. E. Dorman, *J. Org. Chem.*, **40**, 2388 (1975).
(29) Cephalosporin benzhydryl esters which contain large C-10 substituents (such as *N*-methyltetrazole) are notorious for facile olefin isomerization ($\Delta^3 \rightarrow \Delta^2$). Presumably the driving force for this shift is removal of ester and C-10 groups from coplanarity.
(30) R. M. Sweet in "Cephalosporins and Penicillins, Chemistry and Biology", E. H. Flynn, Ed., Academic Press, New York, 1972.
(31) S. Abrahamsson, D. C. Hodgkin, and E. N. Maslen, *Biochem. J.*, **86**, 514 (1963).

(32) E. H. W. Bohme, H. E. Applegate, B. Toeplitz, J. E. Dolfini, and J. Z. Gougoutas, *J. Am. Chem. Soc.*, **93**, 4324 (1971).
(33) D. H. R. Barton, I. A. Blair, P. D. Magnus, and R. K. Norris, *J. Chem. Soc., Perkin Trans. 1*, 1031 (1973).
(34) A. Atkinson, A. G. Brewster, S. V. Ley, R. S. Osborne, D. Rogers, D. J. Williams, and K. A. Woode, *J. Chem. Soc., Chem. Commun.*, 325 (1977).
(35) "International Tables for X-ray Crystallography", Vol. III, Kynoch Press, Birmingham, England, 1968.
(36) Based on the known tendency of 6β-amidopenams to undergo oxidation on sulfur syn to the neighboring amide group, the configuration of sulfinyl oxygen was expected to be *S* (β) [see R. D. G. Cooper, L. D. Hatfield, and D. O. Spry, *Acc. Chem. Res.*, **6**, 32 (1973), and references cited therein]. This was confirmed by examination of sulfoxide bond chemical shift perturbations and aromatic solvent induced shifts. In the *S* configuration (β) the sulfoxide bond of penicillin sulfoxide is known to exert deshielding effects on H-3 and 2β-CH_3–, and shielding effects on H-5 and 2α-CH_3, whereas in the *R* configuration (α) all protons are deshielded except H-3, which is shielded. The observed sulfoxide perturbation shifts (Δ_{SO}) for **50** where $\Delta_{SO} = \delta_{sulfide} - \delta_{sulfoxide}$ are H-3, −0.32; H-5, +0.56. This is in good agreement with Δ_{SO} for penicillin *S*-oxides in general, especially in comparison to the nonmethoxylated *S*-oxide derivative of **50**: Δ_{SO} H-3, −0.25; H-5, +0.56. See P. V. DeMarco and R. Nagarajan in ref 30, pp 349–353 and references cited therein.
(37) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Am. Chem. Soc.*, **91**, 1401 (1969).
(38) Belgium Patents 747 118, 747 119, and 747 120. We thank Dr. Roland Lau for providing this catalyst.
(39) G. Just and K. Grozinger, *Synthesis*, 457 (1976).
(40) See ref 7c,d. We thank Dr. W. A. Slusarchyk for providing this authentic sample.
(41) Mass spectral fragments of **55** are assigned as follows:



(42) The extinction coefficients reported for this compound in our preliminary communication were incorrect.

Streptonigrin Biosynthesis. 3. Determination of the Primary Precursors to the 4-Phenylpicolinic Acid Portion[1]

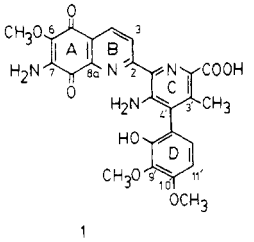
Steven J. Gould* and Chiu Chin Chang

Contribution from the School of Pharmacy, Section of Medicinal Chemistry and Pharmacognosy, University of Connecticut, Storrs, Connecticut 06268.
Received June 11, 1979

Abstract: The 4-phenylpicolinic acid portion of streptonigrin (**1**), a metabolite of *Streptomyces flocculus* with an unusual heterocyclic structure and striking anticancer activity, has been shown to be derived from tryptophan (**4**). All four methyls are derived from methionine, and C-3 of serine is likewise incorporated, although indirectly after apparently being degraded and entering the C-1 pool. From the serine findings and the results of specially timed feedings of [$^{13}CH_3$]-L-methionine, it was revealed that C-methylation occurs at a significantly earlier stage of the fermentation than does O-methylation; the dynamics of the pathway could be observed directly using ^{13}C NMR spectroscopy. The serine results also showed that no de novo biosynthesis of **4** was occurring, at the time of the incorporation experiments. A rational hypothesis for the formation of **1** is presented.

Introduction

Streptonigrin (**1**), a potent anticancer antibiotic, was first isolated from *Streptomyces flocculus* (ATCC 13852),[2] and subsequently from other actinomycetes, e.g., *S. albus* var. *bruneomycini*,[3] *S. echinatus*, and *S. rufochromogenes* 662.[4] The structure of **1** was originally deduced from chemical degradations,[5] and was confirmed by X-ray crystallography.[6] A partial assignment of the ^{13}C NMR spectrum has been reported.[7]



1

Streptonigrin is active against a variety of Gram-positive and Gram-negative bacteria, and has pronounced activity against numerous experimental tumors.[8,9] In the late 1960s testing progressed to a phase III clinical trial for the treatment of lymphoproliferative diseases; the antibiotic appeared to be as effective as chlorambucil but exhibited a somewhat greater degree of toxicity,[10] and interest in streptonigrin declined thereafter in the U.S. However, promising results from France[11] and the Soviet Union,[12] where streptonigrin has been used in combination therapies, have indicated a need to reassess the potential of this antibiotic.

The lethality of streptonigrin is apparently due to a redox process, involving the quinone portion of the molecule, that leads to uncoupling of oxidative phosphorylation and depletion of cellular ATP.[13,14] As a secondary effect, superoxide produced in the redox reaction reacts with its dismutation product, hydrogen peroxide, to yield hydroxyl radicals that cause single-strand breaks in DNA.[13]

A few simple derivatives of streptonigrin have been prepared by methylation of the carboxylic acid or modification of the quinone portion,[15,16] and a minimum structure required for activity has been suggested.[17] Although the synthesis of streptonigrin has not yet been reported, a number of analogues have been prepared.[18]

Our interest has focused on the biosynthesis of **1**.[1] The molecular structure, composed of a quinoline ring joined to a 4-phenylpyridine, is quite unlike that of any other known natural product.[2] While the quinoline quinone portion seemed reminiscent of the tryptophan-derived ommochromes (e.g., xanthomatin (**2**)),[19] pigments produced by various insects, the origin of the fully substituted pyridine ring seemed far less obvious. As a working hypothesis (shown in Figure 1) we considered that streptonigrin might be derived from the

0002-7863/80/1502-1702$01.00/0 © 1980 American Chemical Society