Recent Chemistry of the β-Lactam Antibiotics

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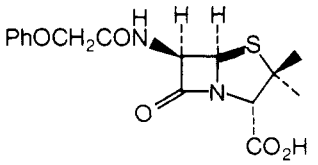
I. Introduction

Even over 30 years after their introduction, the β-lactam drugs are still the most widely prescribed antibiotics used in medicine. Initial chemical studies centered on degradative and structural investigations.[1] Apart from the classical work of Sheehan on the total synthesis of penicillin V (**1**)[2] few attempts to modify these compounds were made until the isolation of 6-aminopenicillanic acid in 1959[3] and the discovery and structural elucidation of the cephalosporins, a related group of antibiotics, around 1960.[4]



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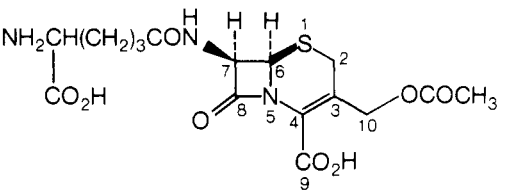
In the last decade there has been a spate of publications concerning these compounds and there are several reasons for this current impetus. First and foremost is the constant need for new antibiotics with either different and/or broader antibacterial activities. A second spur is the search for β-lactam antibiotics to combat bacteria which have built up a resistance against the more traditional penicillins. To be superior, any new antibiotic must also possess low toxicity and good absorption, distribution, and metabolic characteristics—a combination of properties often difficult to achieve.

Much of the current highlighting of the β-lactam drugs came with the revelation that modified cephalosporin antibiotics have a much wider spectrum of activity than the penicillins. Since cephalosporin C (**2**), the parent metabolite, is costly to extract from culture media, considerable effort has been expended in attempting production of the cephem-type antibiotics from the more readily available penicillins by semisynthetic processes.



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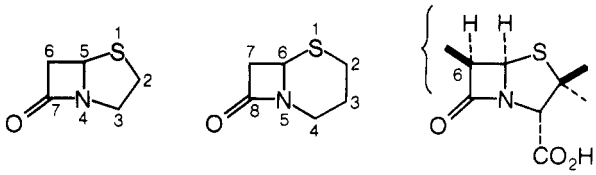
Resistance to the penicillins and cephalosporins is mainly caused by formation of enzymes capable of opening the β-lactam ring common to these antibiotics, viz. the β-lactamases. The recent discovery that various species of *Streptomyces* produce cephalosporin derivatives bearing a 7α-methoxy substituent[5] which show enhanced stability to the β-lactamases has further stimulated chemical studies.

Chemical advances in manipulating these sensitive materials have depended heavily on the use of modern analytical and purification techniques and on the development of more selective reagents and protecting groups. For example, a considerable boost to chemical morale was given by Woodward's approach to the total synthesis of cephalosporin C.[6]

This review is restricted to two main aspects of β-lactam chemistry: those describing attempts to modify and vary naturally available antibiotics, while retaining the β-lactam ring (partial synthesis), and recent trends in totally synthetic approaches. Recent biosynthetic studies have included some model reactions, and the chemistry of some of these results is also described. The literature is selected from that published up to the beginning of 1974, and this review is intended to complement several recent books[7-9] and reviews.[10-16]

The nomenclature generally employed in this area is as follows. The penam (**3**) and cepham (**4**) skeletons are the basic structures commonly encountered among the β-lactam antibiotics, the latter one often bearing a double bond at position 3 as the ceph-3-em system. The penicillanic acid structure (**5**)



3 4 5

generally bears an acylamido group at position 6 and has the natural configuration 3*S*, 5*R*, 6*R*. The chirality about the β-lactam (azetidinone) ring in the cephalosporins is the same as for the penicillins. Reference is often made to stereochemical centers in terms of the trivial α,β convention, the α-face being the less hindered side of the folded, bicyclic penam of cepham skeleton. The substituted methyl group in cephalosporins will be referred to as position 10.[17]

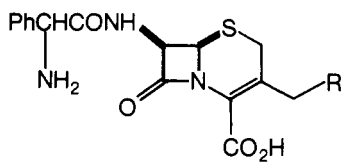
II. Chemical Modifications of Penicillins and Cephalosporins

A. Sulfoxide Reactions

1. Penam–Cephem Conversion

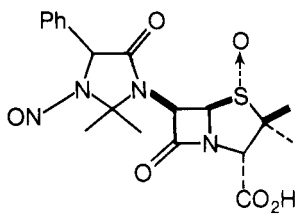
Modern large-scale fermentation methods have enabled penicillin production to reach a level of several thousand tons annually. As a consequence, penicillins can be regarded as a commercially available raw material for transformations into other, more useful compounds.

Since the cephalosporin group of antibiotics are costlier to produce than the penicillins and yet are often clinically more useful, one prime objective of recent chemical studies has been the conversion of the penam nucleus into the cephem system. Such a transformation requires an oxidation of the methyl groups of penicillin and a ring expansion of the thiazolidine function. These stringent requirements were fulfilled as a result of a novel process originally described by Morin and coworkers.[18] Oxidation of the penicillin ester **6** with sodium metaperiodate in aqueous dioxane,[19] or other oxidants,[20-22] formed the sulfoxide **7.** Treatment with acetic anhydride, under normal Pummerer conditions (a reaction used to convert sulfoxides bearing α-hydrogen atoms to α-acetoxy sulfides[23]) left the sulfoxide **7** unaffected. In refluxing acetic anhydride, however, two major products (ratio 2:1) formed in 60% yield. These were the 2β-acetoxymethylpenam (**8**) and the 3β-acetoxycepham (**9**). Treatment of the latter acetate with triethylamine gave the ceph-3-em **10** (Scheme I). The latter compound had previously been obtained by palladium-catalyzed reduction of the cephalosporin **11**,[24] and this chemical correlation was the first between the penicillin and cephalosporin antibiotics. The ceph-3-em **10** could also be obtained directly (10–15%) from the sulfoxide by heating it in xylene containing a trace of *p*-toluenesulfonic acid. Other catalysts are also effective,[25] and the use a pyridine phosphate buffer in dioxane has enabled conversions in the order of 90% to be achieved routinely.[26] This ring expansion reaction is now the basis of the preferred route to the commercial derivative cephalexin (**12**).[27]



12, R = H
14, R = OAc

The Morin reaction is extremely general for penicillin sulfoxides; even hetacillin, as its nitrososulfoxide **13,** reacts, the product being used for the preparation of cephaloglycine (**14**).[28] During the ring-expansion step, the 3-carboxyl group is



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generally protected, the free acid undergoing decarboxylation. An exception is claimed for the derivative **13,** which can apparently be rearranged without recourse to protection of the carboxyl group.

The serendipitous nature of the sulfoxide–acetic anhydride rearrangement reaction demanded a novel mechanistic interpretation, and this was also provided by Morin, who postulated initial formation of a sulfenic anhydride (**15**) followed by formation of the sulfonium ion **16.** Collapse of the sulfonium ion (Scheme II) leads to the observed products.[18]

Many aspects of the reaction sequence have been examined in more detail. Of initial interest was the stereoselective oxidation of the penicillin derivatives into only one of the two possible sulfoxides by a variety of reagents, including sodium

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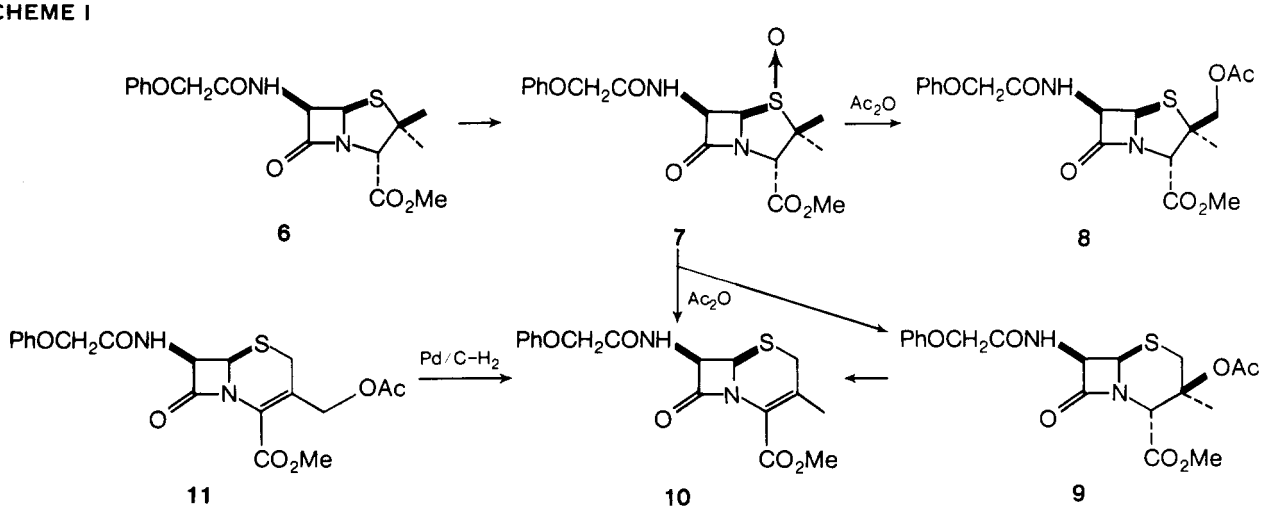
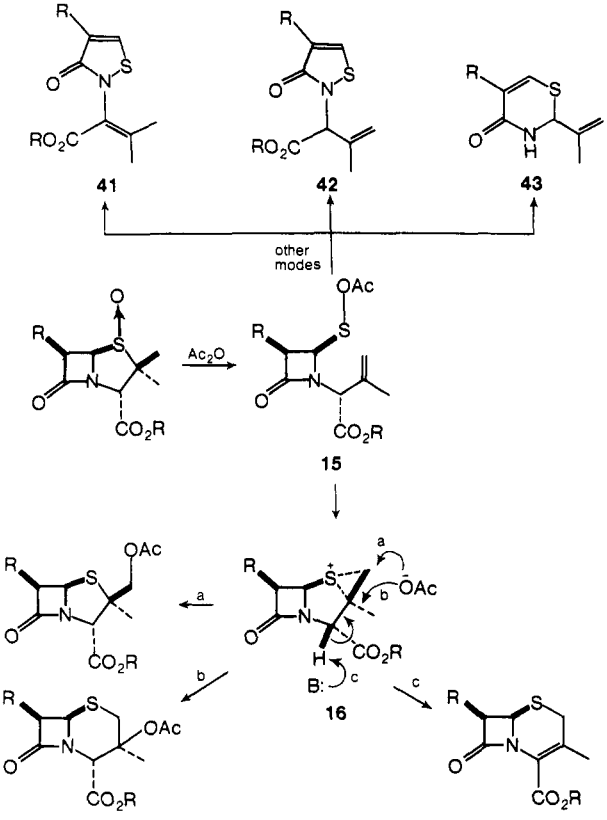
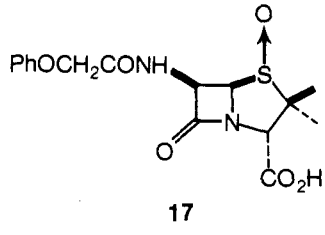


SCHEME II

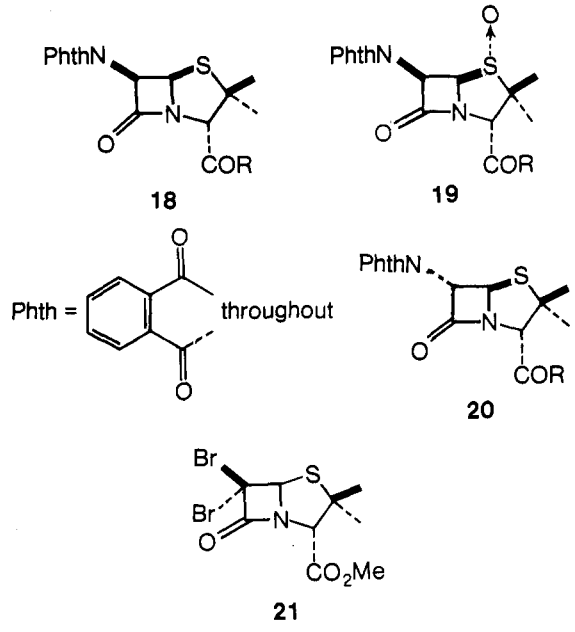


metaperiodate, hydrogen peroxide,[22] and *m*-chloroperbenzoic acid.[29] The configuration of the sulfoxide bond was shown to be (*S*) by extensive NMR studies[29-31] and by a subsequent x-ray crystallographic analysis on penicillin V sulfoxide (**17**).[30] Among the NMR techniques employed were aro-



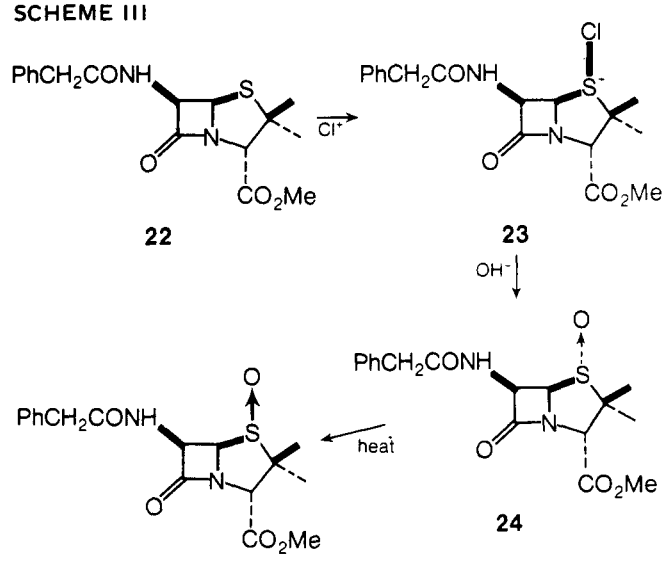
matic solvent induced shifts[29,31] and nuclear Overhauser enhancements.[30] The preferential formation of the (*S*)-sulfoxide isomer is attributed to the directing influence of the amido side chain, which is considered to form a hydrogen bond with the oxidant. This allows "reagent approach control", despite the fact that the oxygen enters the more hindered (β face) side of the molecule. Moreover, the product, (*S*)-sulfoxide, is stabilized by formation of a strong intramolecular hydrogen bond with the N–H group of the 6β-acylamido group, which also makes the product the thermodynamically most stable isomer. A different situation exists in the absence of the secondary amide group. Thus the phthalimido derivatives (**18**)[29,31] do *not* yield (*S*)-sulfoxides but rather the *R* isomers **19.** In these cases no hydrogen bonding is available to direct the oxidant, allowing steric effects to predominate. That steric shielding effects are important was established by oxidizing the epimeric 6α-phthalimide derivative **20,** which afforded both of the two possible sulfoxides.[29] As expected from these arguments, the 6,6-dibromopenam **21** gave a mixture of two sulfoxides.[32]

Methods for overcoming the directing influence of the secondary amide group in the 6β position have been developed.



Since substitution at tervalent sulfur proceeds with inversion of configuration,[33] it was argued that chlorination of the sulfur atom in the ester **22** (Scheme III) would give the chlorosulfonium ion **23** and that subsequent hydrolysis would produce the hitherto unknown (*R*)-sulfoxide **24.** This scheme was realized

SCHEME III

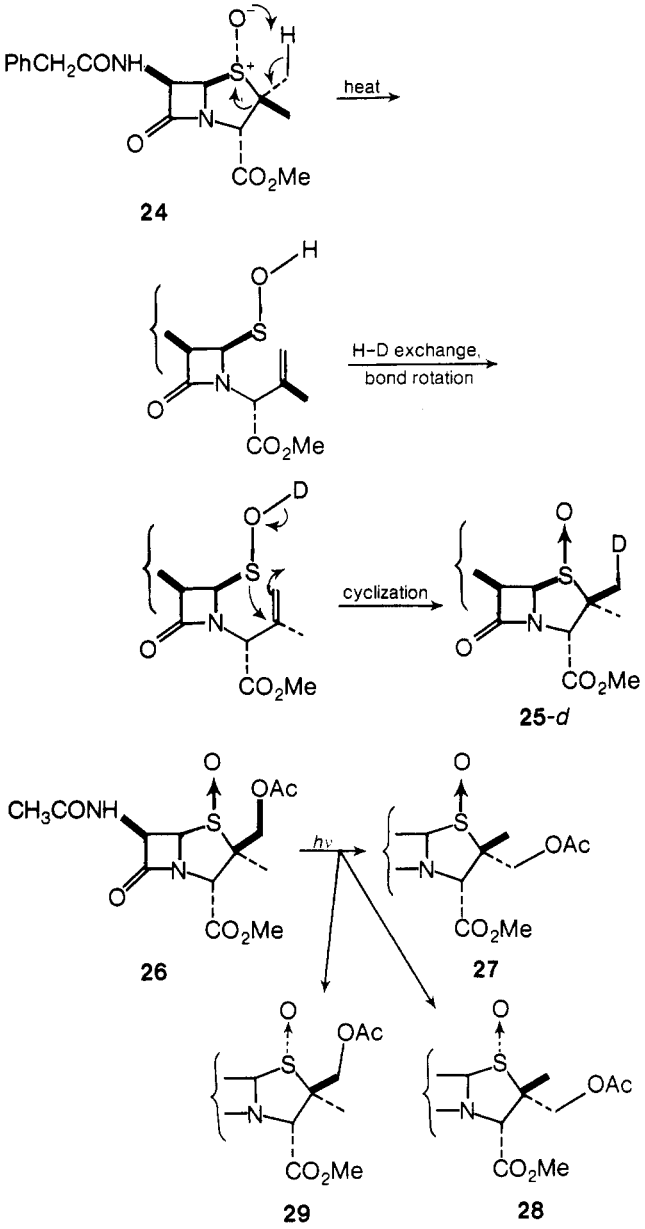


by use of iodobenzene dichloride in aqueous pyridine,[31,34] a reagent known to react by such a two-step mechanism.[35] Two alternative methods have also been invented. Use of the sterically sensitive oxidant ozone in protic solvents, such as aqueous acetone, in order to offset the hydrogen bonding tendency of the side-chain amide group, afforded a 1:1 mixture of the two isomeric sulfoxides from the acid **1.**[36] Furthermore, (*S*)-sulfoxides have been photoequilibrated using acetone-sensitized irradiation.[37] By the latter method the substituted sulfoxide **26** was photolyzed to give a mixture of all four possible acetoxy sulfoxides **26–29,** isomeric about positions 1 and 2,[36] thus establishing that the photochemical reaction proceeds via homolytic cleavage of the S(1)–C(2) bond, in accordance with literature precedent.[38]

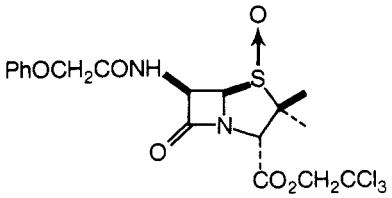
2. Sulfoxide–Sulfenic Acid Equilibrium

It was soon found that the (*R*)-sulfoxides (e.g., **24**) were very unstable to heat, rapidly reverting to the thermodynami-

SCHEME IV



cally more stable *S* isomers.[31,37] Evidence for the formation of a sulfenic acid in this reaction was first obtained by heating the (*R*)-sulfoxide **24** in the presence of deuterated *tert*-butyl alcohol.[39] The product was the corresponding (*S*)-sulfoxide in which only one atom was incorporated (Scheme IV) into the 2β-methyl group, i.e., cis to the resultant sulfoxide bond. Under identical conditions, no incorporation was observed for the (*S*)-sulfoxide. Use of prolonged periods, however, showed that the (*S*)-sulfoxide **30** did undergo slow incorporation of



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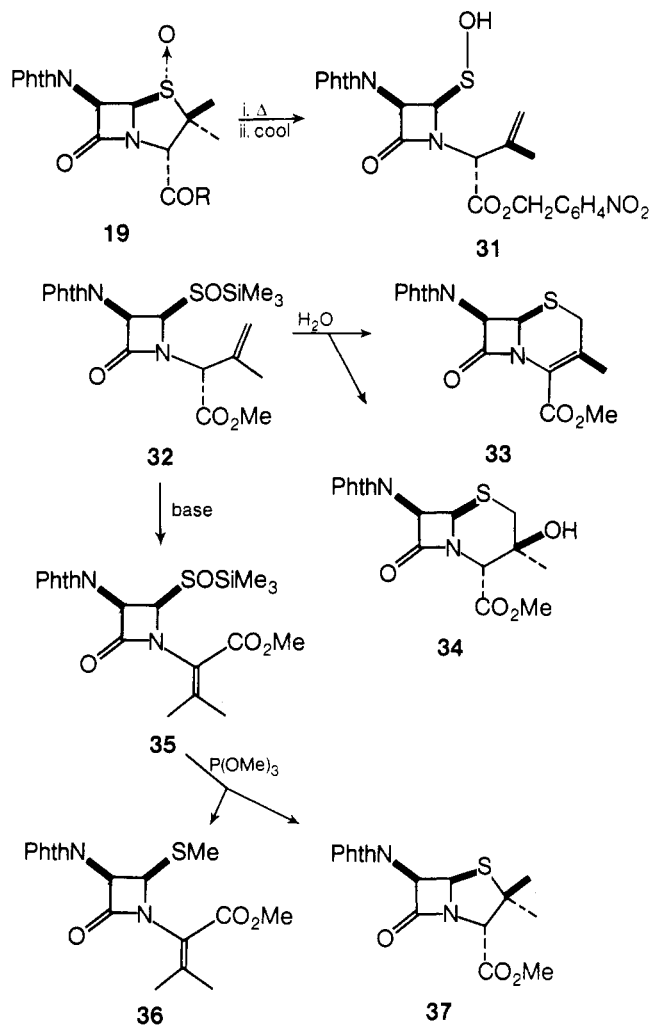
deuterium specifically into the cis-disposed methyl group when heated in solution in the presence of deuterium oxide,[40] thus demonstrating the reversibility of the sulfoxide–sulfenic acid equilibrium.

Such six-electron sigmatropic processes have precedent in that di-*tert*-butyl sulfoxide readily dissociates at 80° into isobutylene and *tert*-butylsulfenic acid.[41] The beauty of the penicillin sulfoxide–sulfenic acid lies in its intramolecular nature. This demands that the olefin and sulfenic acid components are held close together so that, even at 80°, the absolute concentration of the unstable sulfenic acid intermediates is generally low and, consequently, in the absence of catalysts or external reagents, they do not readily disproportionate. The isolation of one sulfenic acid has been reported. Rapid cooling of a hot solution of the phthalimido derivative (**19**, R = $OCH_2C_6H_4$-*p*-NO_2) gave some of the corresponding sulfenic acid **31**, the equilibration being frozen.[42] Alternatively, when **19** (R = MeO) was heated in benzene in the pres-

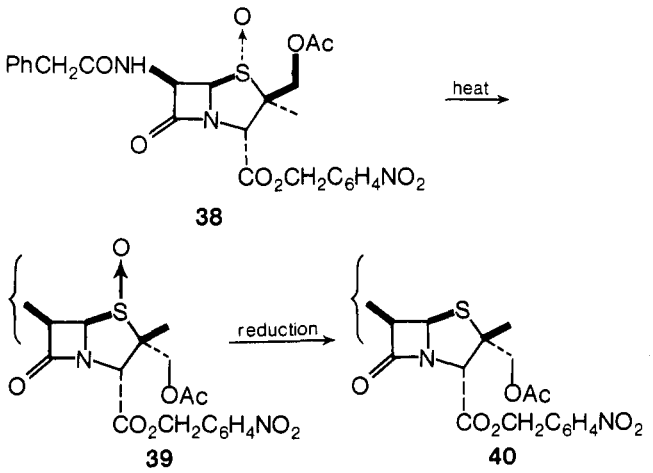


ence of a 2:1 ratio of trimethylsilyl chloride and hexamethyldisilazane, the trimethylsilyl ester **32** formed.[43] Brief treatment of this derivative with water gave the sulfenic acid **19a**,[42] the starting penam sulfoxide, as well as some of the cephem **33** and the hydroxycepham **34**.[43] Base catalyzed the conjugation of the double bond to give the ester **35**, which gave the methyl sulfide **36** and some of the penam **37** by reduction with trimethyl phosphite. The latter was assumed to arise by a Michael-type recyclization of the intermediate thiol (but see also section IV).

These electrocyclic reactions occur in a stereospecific manner, the hydrogen atom always being abstracted from the adjacent *cis*-methyl group. An examination of Dreiding models shows that, for the (*S*)-sulfoxides, the conformations of which have been established by both NMR and x-ray analysis,[29-31] the *cis*-methyl hydrogens are in the order of 2 Å

away from the sulfoxide oxygen, while the *trans*-methyl hydrogens and the proton at position 3 are at least 4 Å distant.

A pertinent example of the stereospecific nature of the electrocyclic process was observed for the 2β-acetoxypenicillanic ester sulfoxide (**38**).[34] Heating this in toluene converted it into the isomeric (*S*)-sulfoxide **39**. Because during this isomerization, rotation must occur about the C(2)–C(3) bond (cf. Scheme IV), the acetoxy group ended in the 2α position. Reduction of the sulfoxide product **39**, using phosphorus tribromide in dimethylformamide,[34,44] gave the new sulfide **40**.



The rate at which the sulfenic acid–sulfoxide equilibrium is attained depends on steric as well as electronic factors. Buttressing of the sulfoxide group against the adjacent *gem*-dimethyl group can be achieved either by the use of bulky side chains at the 6 position, e.g., the phthalimido residue,[45] or by the use of bulky carboxylic acid derivatives.[46,47]

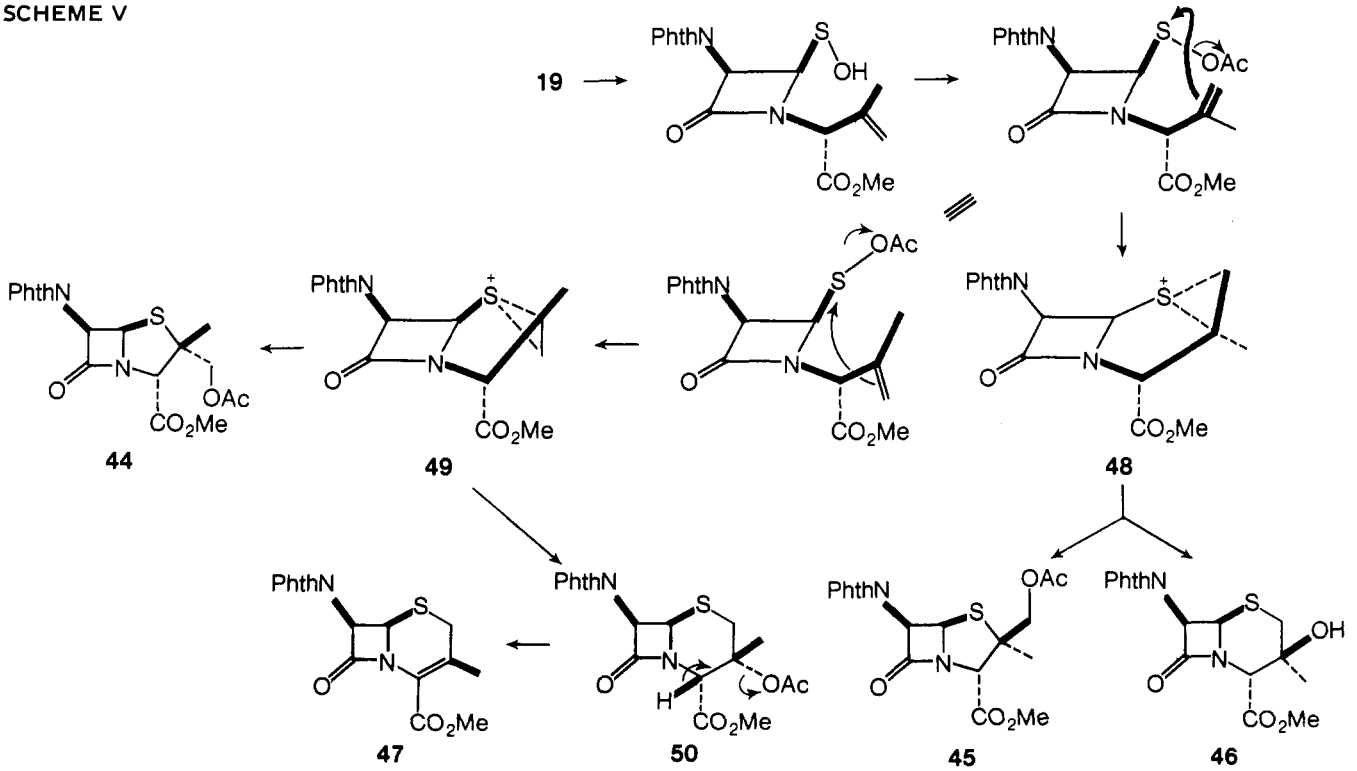
3. Mechanism of the Ring-Expansion Reaction

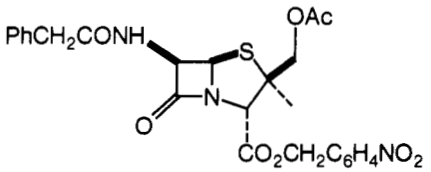
Morin and his coworkers envisaged that the penam to cephem reaction proceeded by intermediate formation of the sulfenic acid derivatives (e.g., **15**) followed, mainly, by formation of an episulfonium ion of the type **16** (Scheme II). The small amounts of products **41, 42,** and **43** were explained by alternative fates of the mixed sulfenic–acetic anhydride. For most penicillin substrates only one of the two possible sulfonium ions appears to form, since subsequent addition of acetate ion only produces the β-substituted acetoxy derivatives (paths a and b, Scheme II). Cooper et al.[13] have discussed this mechanism at length and conclude that the direction described by the leaving group (acetate in **15**) must be sterically controlled, leaving from the less hindered α face, the remaining sulfur species encountering attack from the opposite (β) side by the olefinic bond. They found that for the phthalimido derivative **19** (R = MeO), rearrangement with acetic anhydride produced both isomeric acetoxy penams **44** and **45,** as well as the acetoxycepham **46** and the cephem **47,** as expected if both the sulfonium ions **48** and **49** had formed (Scheme V). In this case, it was argued, steric hindrance by the bulkier phthalimido group restricts approach of the double bond from the β face, allowing some reaction to proceed by the less favorable β departure of the leaving group, probably in a pseudo-equatorial direction. No isomeric acetoxycepham **50** was detected since it can undergo trans elimination to the ceph-3-em **47.**

The point of opening of the sulfonium ion is dictated by the nucleophilicity of the counterion, the kinetic process leading to the penam system and thermodynamic control yielding the cepham derivatives. For example, although the acetoxypenam **51** is the major product from reaction of the corresponding sulfoxide in acetic anhydride, use of chloroacetic anhydride gave only the cepham **52,** acetate ion being more nucleophilic than chloroacetate ion.[34]

Use of strong acids in the ring expansion reaction tends to yield ceph-3-em derivatives as the major products, but the type of acid used is important. Whereas methanesulfonic acid and *p*-toluenesulfonic acid gave the desired cephem products, use of sulfuric acid on the ester **30** afforded the 3β-hydroxycepham **53** in good yield.[48,49] When these reactions were applied to the free acid, rather than the trichloroethyl ester **30,** the sulfonic acids caused decarboxylation to give the cephem **55,** presumably via the indicated fragmentation of the intermediate **54.** In contrast, sulfuric acid gave the hy-

SCHEME V





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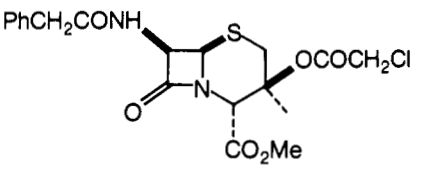

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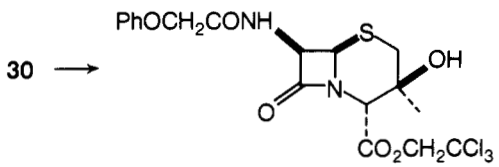

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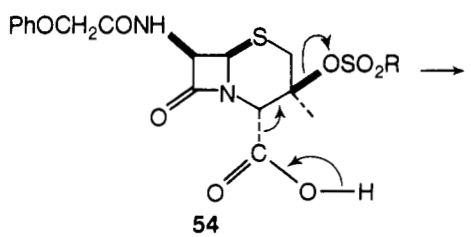
droxy acid **57** since the alternative fragmentation pathway, as in **56**, predominates.



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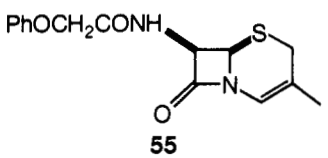

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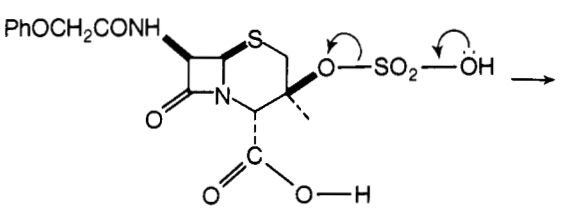

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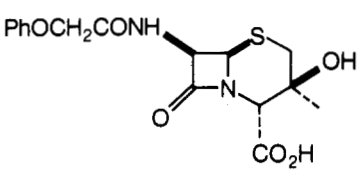

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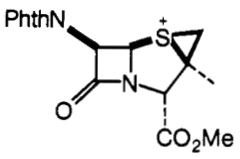
In principle, formation of the thiiranium intermediate (e.g., **58**) should be possible from both the appropriate penam and cepham systems. Provided such a species can collapse by attack at either carbon atom of the sulfonium ion, this provides a method for the ring *contraction* of the cepham to the penam system. This route has been traversed by treating the hydroxycepham **59** with thionyl chloride and triethylamine in carbon tetrachloride.[50] A mixture of the chlorides **60** and **61**, as well as the cephem **47**, was obtained. Furthermore, heating the penam chloride **61** with silver acetate in acetic acid for 5 min gave the acetates **62** and **63** as well as more of the cephem **47**.



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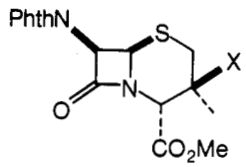
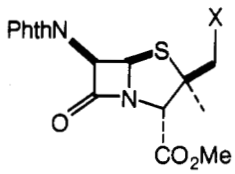
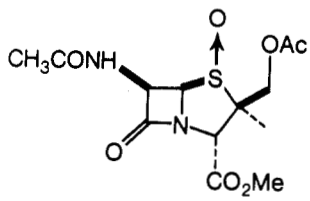


59, X = OH
60, X = Cl
62, X = OAc



61, X = Cl
63, X = OAc

One of the original aims of Morin was the conversion of penicillin into useful cephalosporin-type antibiotics.[18] As mentioned above, cephalexin (**12**) can be prepared in an economically viable manner using the sulfoxide rearrangement.[27]



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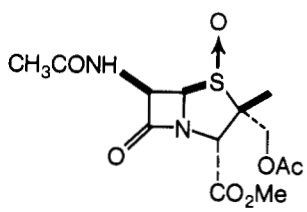

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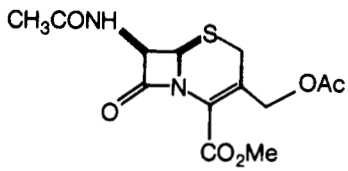

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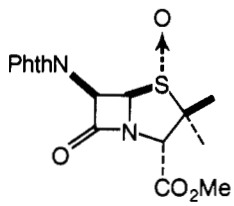

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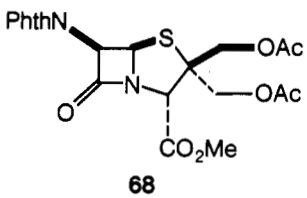

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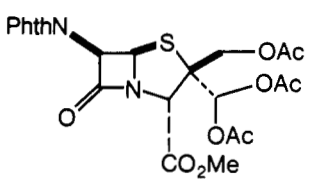

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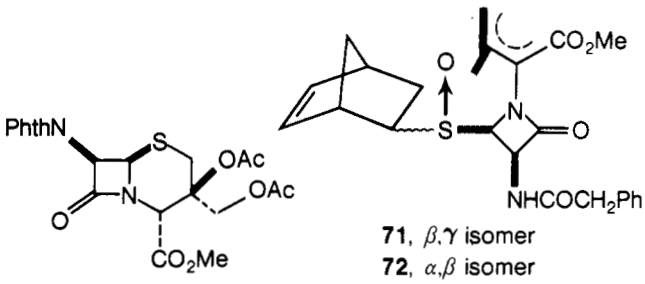
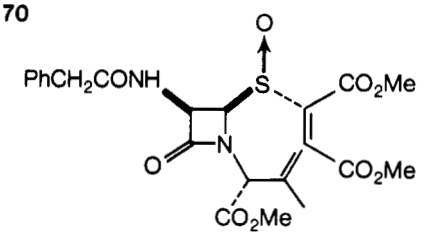


70

71, β,γ isomer
72, α,β isomer



73

More recently this rearrangement has also been used as an entry into the 10-substituted cephem systems.[36] While acid-catalyzed rearrangement of the acetoxypenam sulfoxide **64** was abortive, presumably because of unfavorable conformational interactions between the sulfoxide and acetate functions, ring expansion was observed with the isomeric sulfoxide **65** to give the cephalosporin derivative **66.**

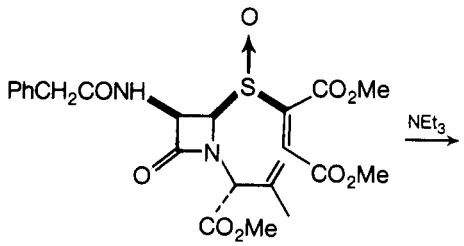
A detailed examination of multiple rearrangements, using the 6β-phthalimido derivative **67** as starting material, has yielded the diacetoxy- (**68**) and triacetoxypenam (**69**),[51] as well as some of the disubstituted cepham **70.**

4. *Trapping of the Sulfenic Acid Intermediates*

The facile thermal equilibration of the penicillin sulfoxides with their complementary sulfenic acid isomers permits a study of the chemistry of the latter, rather labile species. Interest has largely centered on the formation of new carbon–sulfur bonds such that new structures can be built up between the sulfur atom and the nitrogen of the β-lactam ring. These studies have also shown that the sulfenic acid group is a very versatile species, and the types of reactions involved may be classified according to the following types.

a. Additions to Acetylenes and Olefins

Competition for the intramolecular recyclization of the sulfenic acid with an external olefin has been achieved between the sulfoxide **25** and norbornadiene.[52,53] A stereoisomeric mixture of the adducts **71** was obtained; the β,γ isomers initially obtained are readily isomerized into the corresponding α,β-unsaturated system **72** by brief treatment with triethylamine. Use of dimethyl butynedioate gave the corresponding vinyl sulfoxides **73** and **74.**[54] Both of these adducts were also isomerized by base, the former to the conjugated derivative and the latter to the cyclic compound **75.** Ethyl propiolate afforded the related adducts **76.** The trans orientation of the protons across the olefinic bond of these adducts reflects the cis mode of addition of the sulfenic acid to the triple bond.



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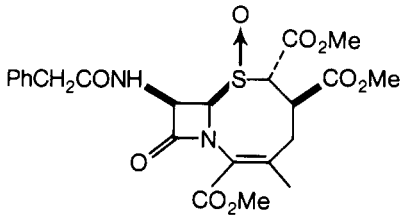

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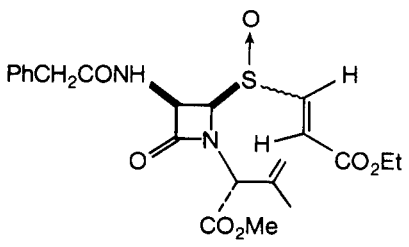

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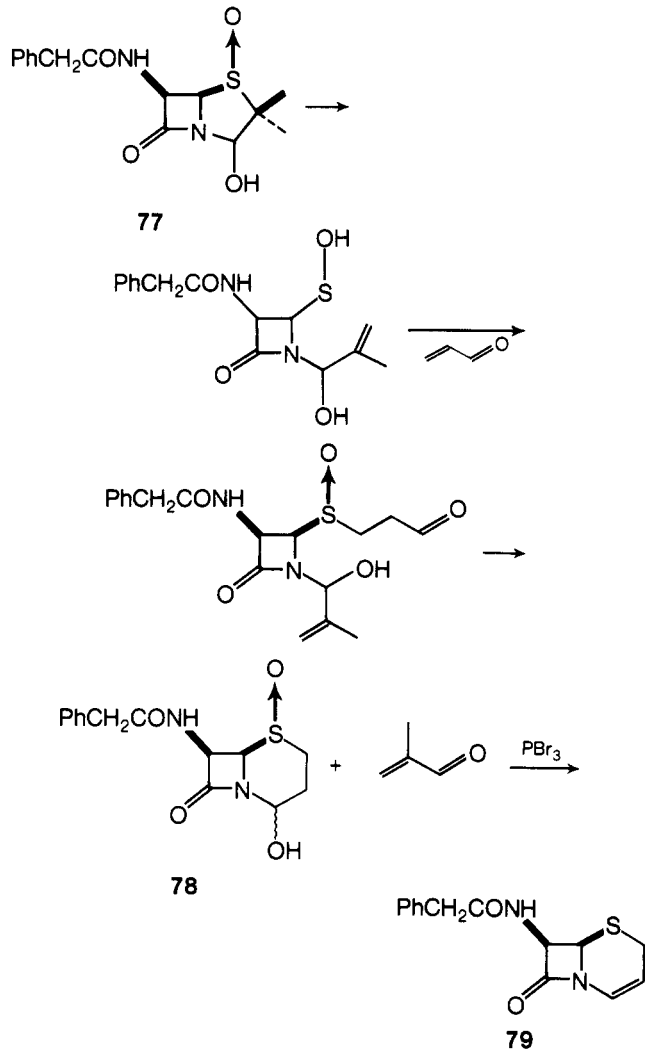
SCHEME VI



Further transformations of these adducts have been described, the conjugated sulfoxide system undergoing Michael-type addition reactions.[55]

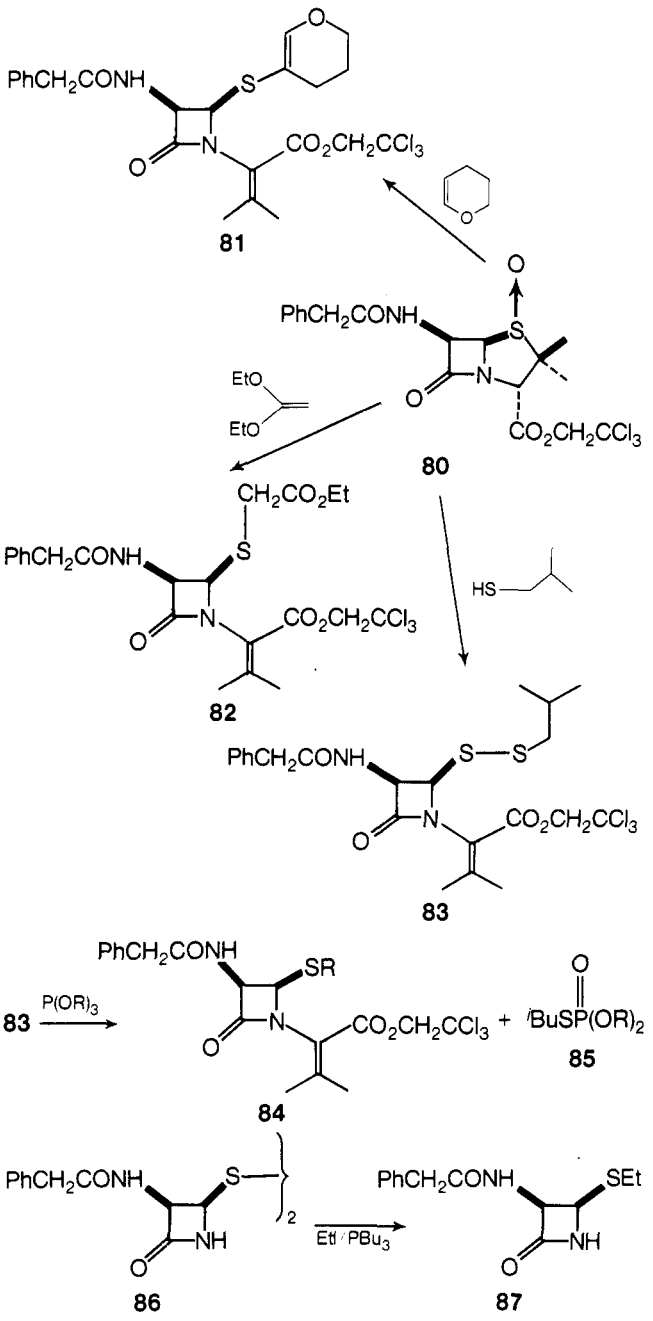
Reaction of the hydroxypenam **77** with acrolein effects a net exchange process, the hydroxycepham **78** resulting and which can be easily dehydrated to give the cephem **79.** A possible reaction scheme is depicted (Scheme VI).[53]

b. Electrophilic Reactions

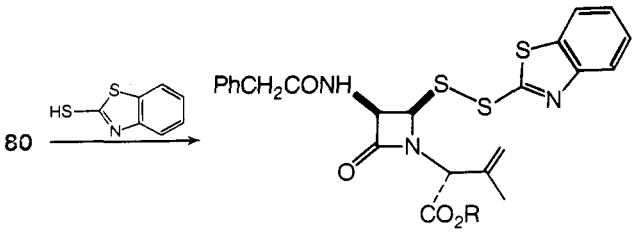
Olefins bearing oxygen substituents react in a different manner with sulfenic acids, a displacement of the hydroxyl group occurring with formation of a sulfide derivative. Dihydropyran[56] and vinyl ethers[52] were found to react in this manner. Heating the sulfoxide **80** with dihydropyran, for example, followed by treatment with triethylamine gave the conjugated sulfide **81.** With 1,1-diethoxyethene the product was not the expected vinyl sulfide but, instead, the ester **82,** produced by in situ hydrolysis with the extruded water.

Other nucleophiles can also be used to cleave the sulfur–oxygen bond including thiols.[57] 2-Methylpropane-1-thiol reacted with the sulfoxide **80** to give the crystalline disulfide **83.** These disulfides lend themselves to further manipulation, allowing the attachment of other functional groups onto the sulfur. An example is reaction of the disulfide **83** with trialkyl phosphites, which affords the alkylated sulfide **84** and the thiolphosphate ester **85.**[58] Use of trialkyl- or triarylphosphines in the presence of an alkylating agent gives alkyl sulfide (e.g., **86** to **87**).[57]

Related trapping reactions have been observed with het-

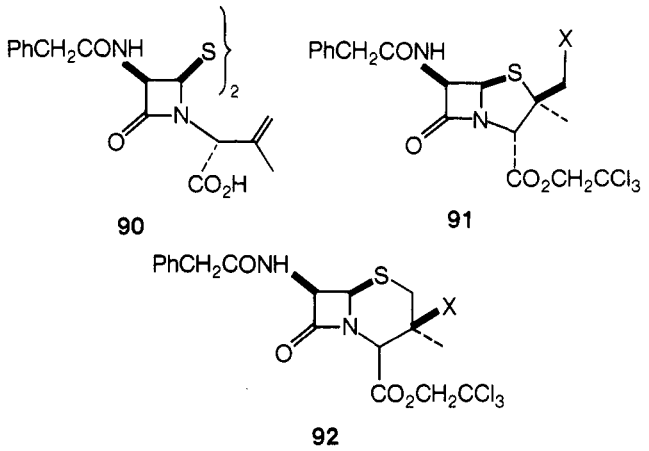


eroaromatic thiols, 2-mercaptobenzothiazole, for example, reacting with the sulfoxide **80** to give the disulfide **88**.[59] Even

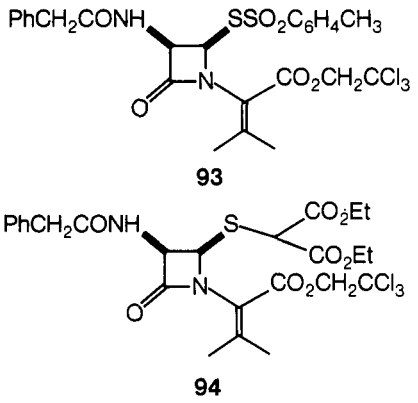


88, R = CH_2CCl_3
89, R = H

the free acid could be trapped to give the disulfide **89** without decarboxylation. Treatment of the former disulfide with base merely caused conjugation of the double bond, but in the latter case a disproportionation to the symmetrical disulfide **90** was observed. When the disulfide **88** was treated with either bromine or chlorine, the corresponding 2β-halomethylpenam system **91** formed. In dimethylformamide these gave the thermodynamically more stable 3β-halocepham **92**, which, on treatment with base, gave the ceph-3-em. These processes can be rationalized in terms of a common sulfonium ion, as discussed above. It was also found that direct treatment of the sulfoxide **80** with pyridine hydrochloride in tetrachloroethane afforded some of the chlorinated penicillin **91** (X = Cl), together with some of the expected cephem.



More recently the sulfenic acids have also been trapped with sulfinate ions to produce the thiosulfonate. These intermediates (e.g., **93**) are also of potential synthetic use since they allow the introduction of a variety of substituents onto the sulfur atom. A typical reaction is the preparation of the substituted malonate **94**.[60]

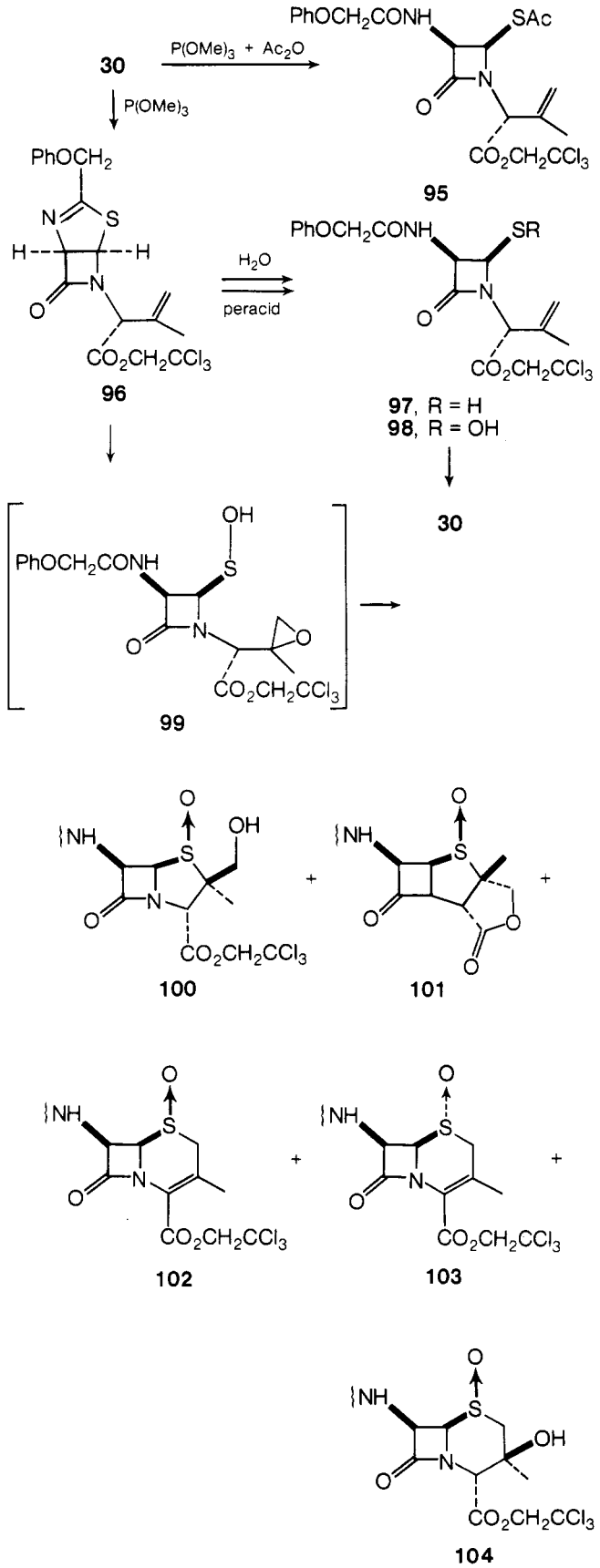


c. Reduction and Oxidation Reactions

Reduction of sulfenic acids should produce the corresponding thiols. This has been achieved in an elegant manner using trivalent phosphorus.[61] Heating the sulfoxide **30** in acetic anhydride in the presence of trimethyl phosphite gave the corresponding thioacetate **95** by acylation of the intermediate thiol.[62] In the absence of the acylating agent the thiol group tends to interact with the side chain 6β-amide group. Subsequent dehydration accounts for the thiazoline product **96**. The thiazoline is ostensibly a protected (dehydrated) form of the intermediate thiol **97**. It is a valuable derivative since the thiazoline ring can be cleaved under very mild conditions. For instance, treatment with peracid can regenerate the sulfenic acid species **98** (Scheme VII),[63] which can recyclize to the penam sulfoxide **30** in nonpolar solvents or, under more polar conditions, react to give the products **100** to **104**. Selective oxidation of the double bond was not observed in these experiments, which required acid catalysis, the products probably arising from the epoxide–sulfenic acid (**99**) as indicated.

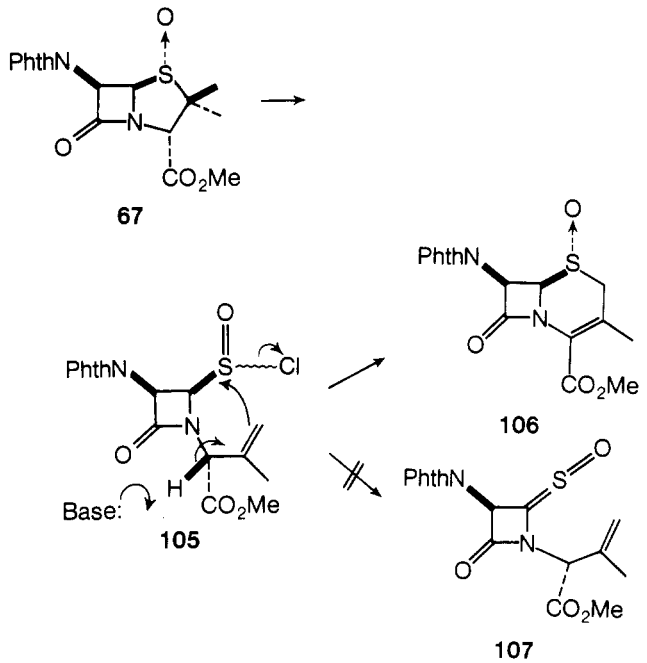
Oxidation of the sulfenic acid itself has also been recorded.[64] Heating the sulfoxide **67** in carbon tetrachloride in the presence of sulfuryl chloride gave the diastereoisomeric mixture of sulfinyl chlorides **105**, epimeric about the sulfur atom.

SCHEME VII

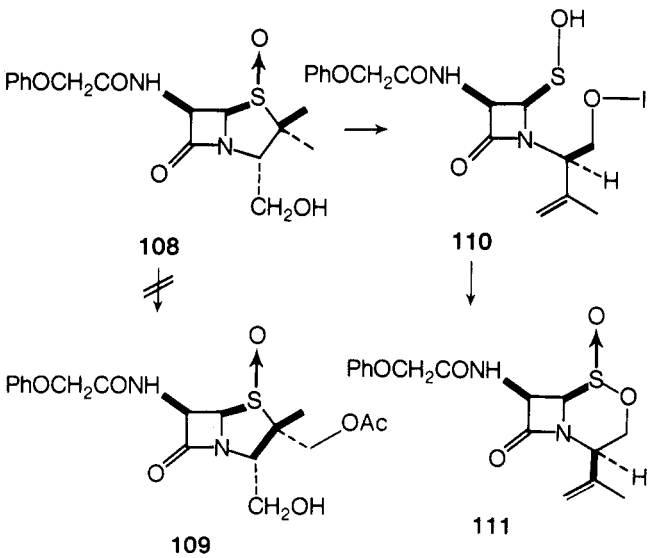


Consequent treatment with triethylamine converted these isomers into the deacetoxycephalosporin sulfoxide (**106**), presumably through the mechanism shown since there was no evidence for formation of the sulfene **107.**

Another oxidation reaction involved an attempt to activate



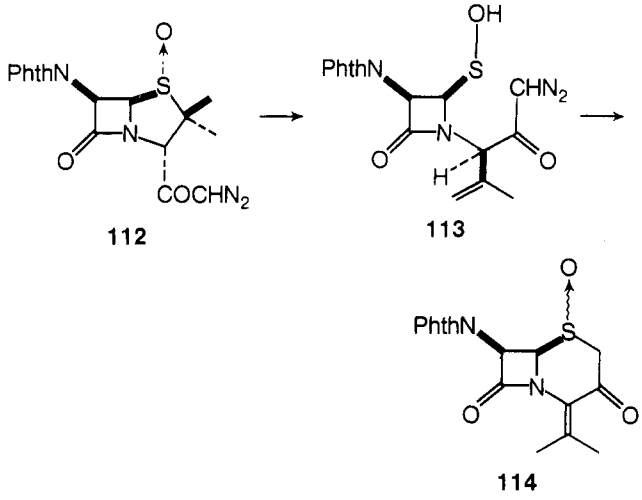
the methyl groups of the penicillanyl alcohol **108** by use of the hypoiodite reaction, viz., formation of the alkoxy radical and abstraction of a hydrogen atom from a proximate methyl group.[65] Reaction of the sulfoxide with lead tetraacetate and iodine afforded not the expected acetoxymethyl derivative **109** but, instead, the sulfinate ester **111.** Intramolecular nucleophilic attack of the intermediate sulfenic acid onto the hypoiodite **110** accounts for the product.[66]



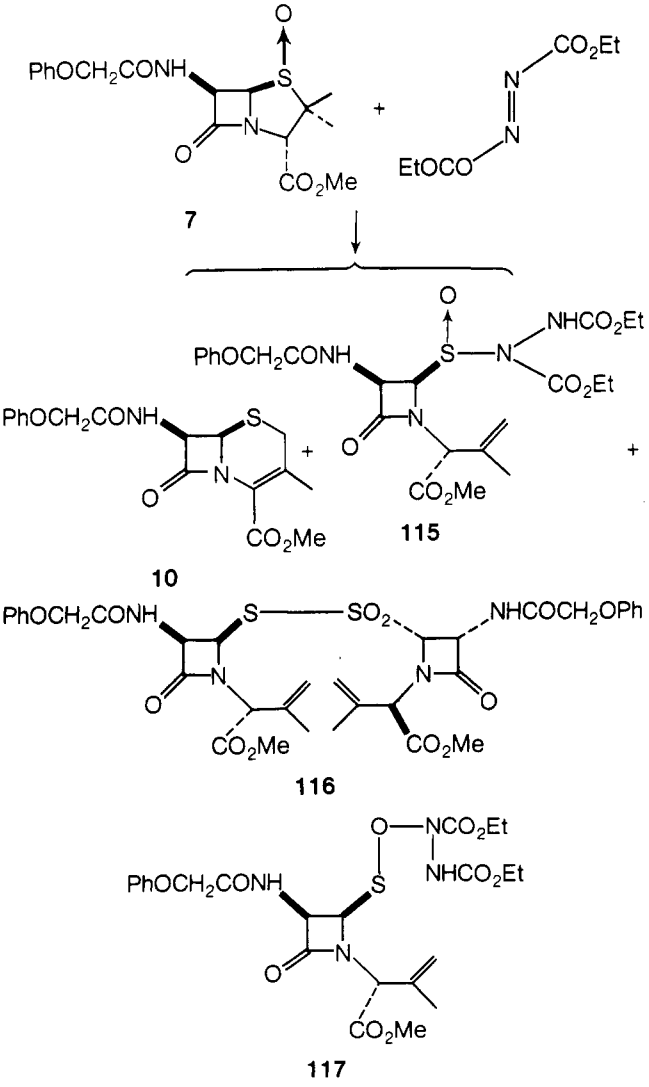
d. Nucleophilic Reactions

The oxidations referred to in the preceding section can be considered as nucleophilic reactions of the sulfenic acid. In order to extend this class of reaction, Cooper and his colleagues reacted the diazo ketone **112** in the presence of copper sulfate as a catalyst.[67] The sulfenic acid **113,** produced on warming, reacted in the anticipated manner to give the epimeric keto sulfoxides **114.**

An unusual array of products was produced by treating the sulfoxide **7** with diethyl azodicarboxylate in refluxing toluene.[68] These included the deacetoxycephalosporin **10,** the adduct **115,** and the oxidized dimer **116.** The cepham **10** is probably formed by the normal type of ring expansion reaction, the reagent possibly, but not necessarily, activating the

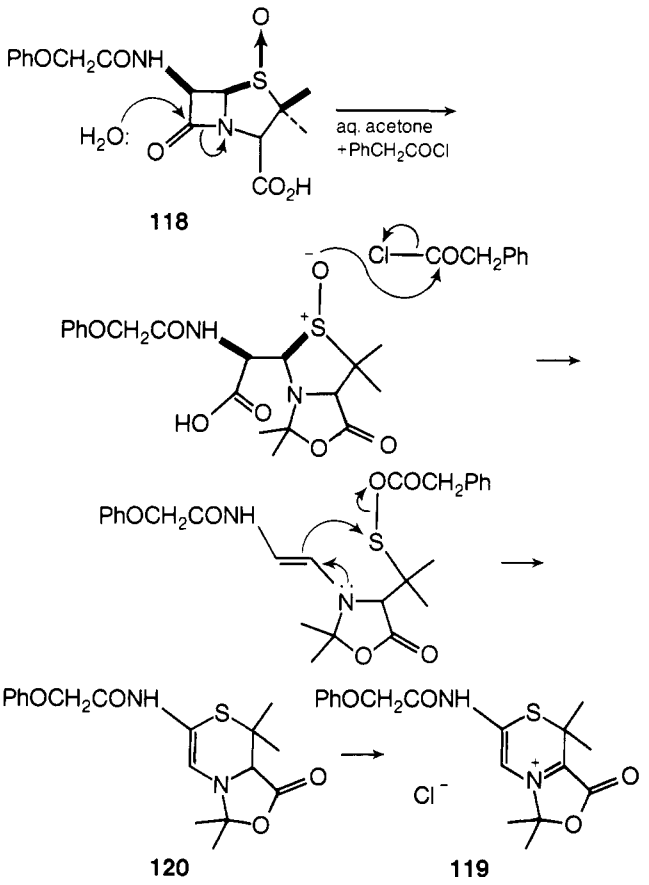


process by formation of intermediates such as **117.** The adduct **115** arises either by nucleophilic addition to the azo linkage or by an electrocyclic process (as in the case of addition to an olefin), while the thiosulfonate must be produced by further oxidation either prior to or after the expected dimerization of the sulfenic acid intermediate.



A fascinating reaction occurs on treating the sulfoxide acid **118** with phenylacetyl chloride in acetone. Structural elucidation of the product (**119**) by x-ray crystallography showed an oxidation had occurred.[69] A proposed route, which involves the intermediate formation of a dihydrothiazine[70] (**120**) is outlined in Scheme VIII.

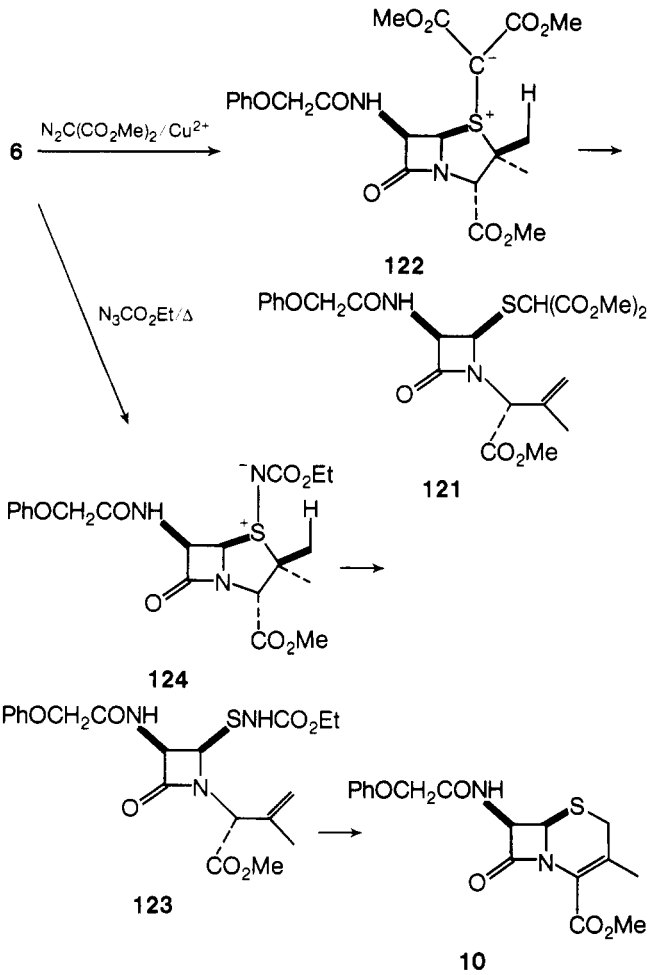
SCHEME VIII



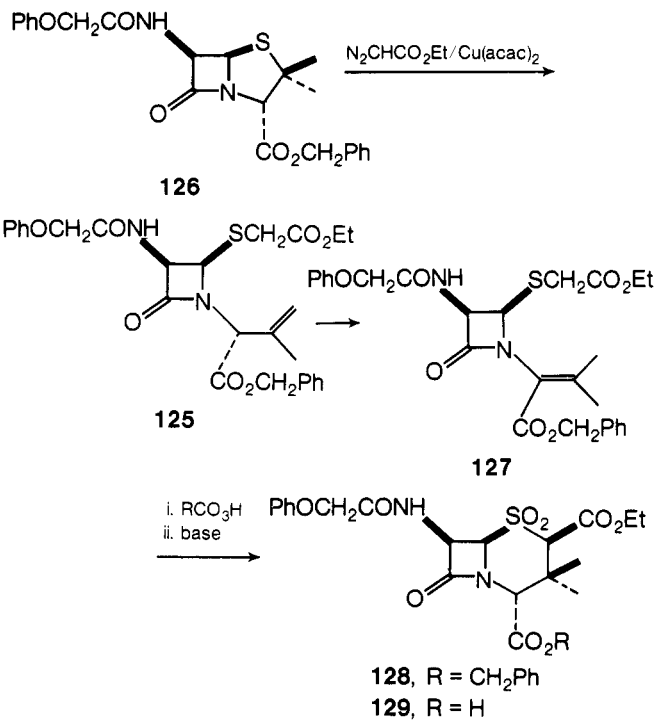
B. Reactions Related to Sulfoxide Transformations

The electrocyclic nature of the process sulfoxide–sulfenic acid has provided a variety of new reactions for opening the thiazolidine ring of the penicillins. Related electrocyclic processes involving sulfonium ylides[70] and sulfilimines[71] bearing β-hydrogen atoms are also well established, and it was soon revealed that, despite the steric encumbrance around the sulfur atom, penicillins can also be used as substrates.[72] Treating the sulfide **6** with an excess of dimethyl diazomalonate in diethyl carbonate at 110° in the presence of copper sulfate afforded the azetidinone **121** in 46% yield. Presumably the copper complexed carbene, initially formed from the diazo compound, attacks the sulfur atom in an electrophilic manner to generate the ylide **122,** which, at the temperature used, immediately produces the β-lactam product **121.** Other diazo compounds can also be used. In an analogous manner, the ester **6** was also heated in diethyl carbonate with an excess of ethyl azidoformate. A low yield of the azetidinone **123** formed via intermediacy of the sulfilimine **124.** Treating the sulfenamide product **123** with a weak acid (diethylamine hydrochloride) in dimethylacetamide afforded some of the ceph-3-em derivative **10.**

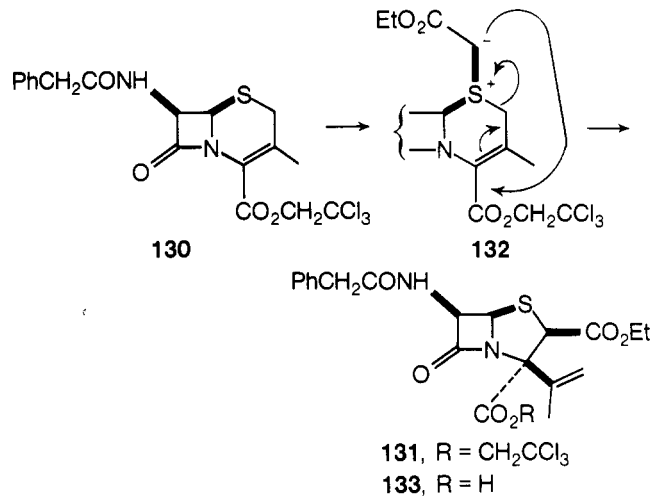
In a related study, formation of the azetidinone **125** was achieved by reaction of ethyl diazoacetate on the penicillin **126** using $Cu^{II}(acac)_2$ as the catalyst.[73] Diazoacetaldehyde, diazoacetonitrile, diazoacetophenone, and methyl diazopyruvate have also been employed to give the corresponding 1,2-secopenicillins. Treatment of the derivative **125** with triethylamine simply caused conjugation of the double bond to give **127** which could then be oxidized to the sulfone and cy-



clized to the cepham compound **128** with 1,4-diazabicyclooctane. Liberation of the free acid **129** by hydrogenolysis showed that this was an inactive bacteriacide.



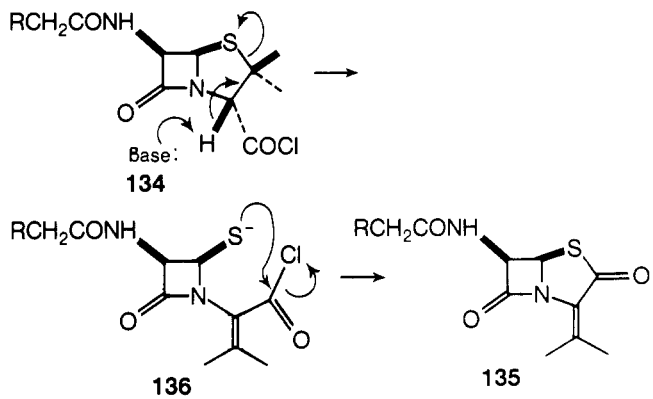
The reaction of a carbene with the deacetoxycephalosporin **130** has resulted in a novel ring contraction method for producing the penam skeleton.[74] It is known that allylic sulfonium ylides can undergo two types of reaction,[75,76] either a Stevens rearrangement or a [2,3] sigmatropic migration. Copper-catalyzed reaction of ethyl diazoacetate with the ester **130** gave the penam **131,** which is a result of the sigmatropic process on the intermediate ylide **132.** Zinc–acetic acid reduction of the trichloroethyl ester[77] afforded the free acid **133,** but this showed reduced antibacterial properties compared to the normally substituted penicillins.



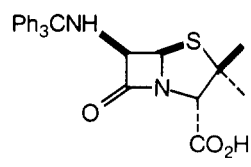
C. Anhydropenicillin Rearrangement and Its Ramifications

In 1963 Wolfe and his colleagues found that treatment of an activated ester or the acid chloride of penicillins with triethylamine catalyzed a rearrangement into a thiol ester, the reaction occurring with the formal loss of 1 mol of water from the starting penicillanic acid.[78,79] Thus the penicillin **134** afforded the anhydro derivative **135.** The rearrangement was explained in terms of the thiolate intermediate **136** (Scheme IX) which can then react intramolecularly with the acyl chloride function. Although the reaction is fairly general, yields are

SCHEME IX

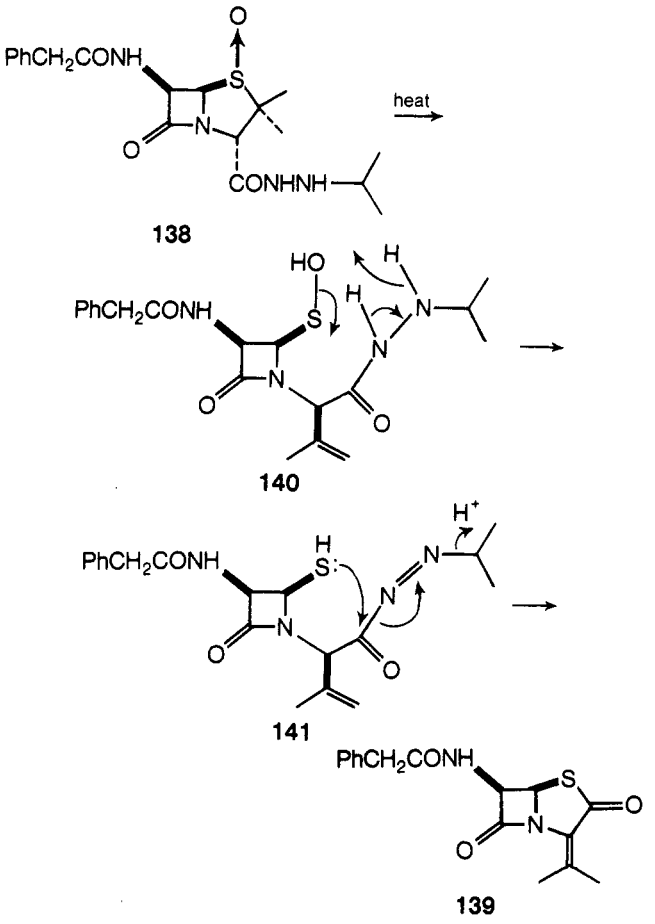


often poor (<20%), probably because of interfering reactions involving the side-chain acylamido group. Better yields are attained using 6β-amino derivatives such as the trityl compound **137.**



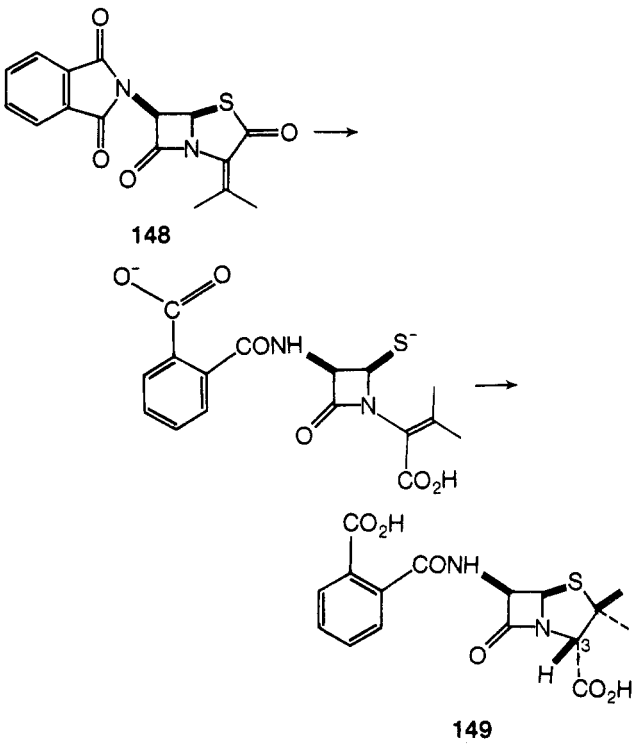
A different method for making anhydropenicillin derivatives has also been described.[80] Heating the monoisopropylhydrazide of the sulfoxide **138** gave the anhydropenicillin **139.** An intramolecular redox reaction must be involved through the intermediates **140** and **141** (Scheme X).

SCHEME X



Although anhydropenicillins are potentially promising intermediates for the preparation of novel β-lactam derivatives, little direct success have been achieved with them. Attempts to activate the vinylic methyl groups with reagents such as lead tetraacetate and selenium dioxide have failed, and other oxidants lead to reaction at the sulfur atom. Treatment of the compound **142** with mercury(II) acetate in refluxing benzene was fruitful, initially providing the mercury derivative **143.**[81] Under these reaction conditions, this species reacts further (Scheme XI) to eventually give the tautomeric mixture of

SCHEME XII



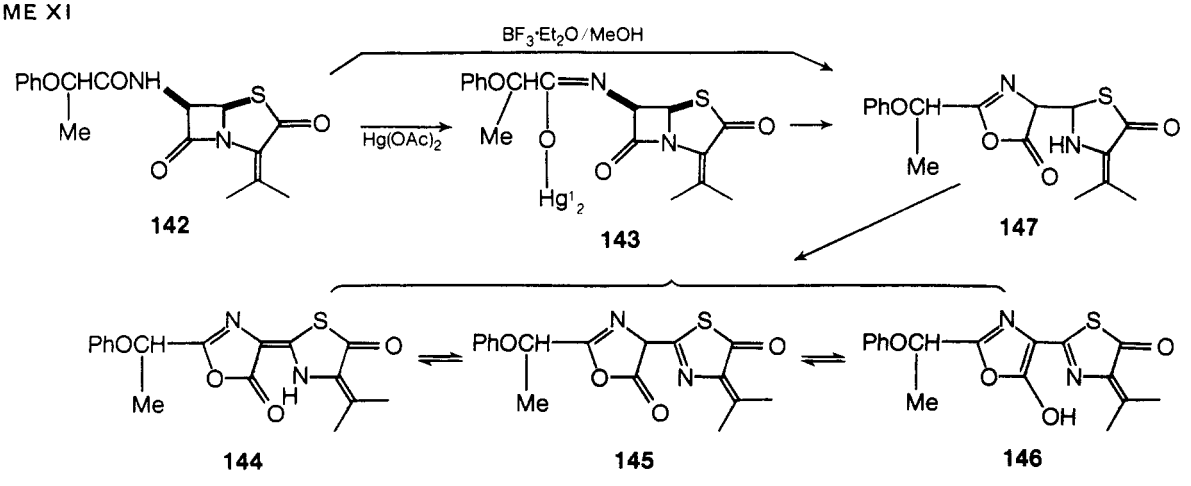
anhydropenicillenes **144** to **146,** probably via the second intermediate, the oxazolone **147.**

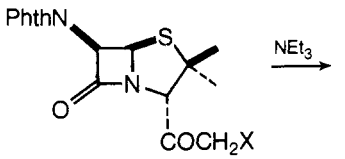
A reversal of the anhydropenicillin rearrangement has been claimed.[82] Treating compound **148** with aqueous dimethyl sulfoxide at pH 7.4 gave the acid **149** (Scheme XII). Strikingly, the Michael-type addition step results in formation of only the natural epimer about position 3. Steric factors probably control the direction of reprotonation at this point.

The above reactions proceed through the formation of free thiolate anions, and these intermediates are potentially useful in developing new reactions of the penicillins. The general value of these species was first recognized by Ramsay and Stoodley,[83] who treated the chloro ketone **150** with strong base. Intramolecular alkylation of the chloro ketone moiety afforded the new ketones **151** and **152,** the latter arising from concomitant epimerization about position 6 (see section II.F.2). Use of a strong base, such as 1,5-diazabicyclononene, on the corresponding iodo ketone minimized the epimerization.

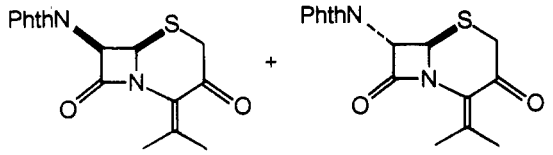
A further important result was also obtained by Clayton,[84] who observed that, on treating the 6,6-dibromopenicillanate (**153**) with ethyl chloroformate and base, two products

SCHEME XI



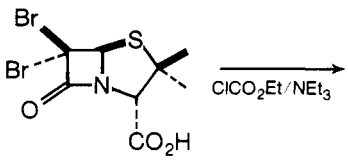


150, X = Cl



151 (ratio 2:3) 152

formed, the expected anhydropenicillin **154** together with a small amount of the thiol carbonate **155,** arising from *intermolecular* transacylation. This result suggested that *intermolecular* reactions of the thiolate might be general. Subsequent experiments have elegantly supported this prediction.[84] Thus, treatment of trityl derivatives such as **156** with methyl iodide and a strong base (sodium hydride or potassium *tert*-butoxide) gave the sulfide **157.** Removal of the protecting trityl and anisyl groups and acylation of the 6-amino function with phenoxyacetyl chloride gave the penicillin analogue **158,** although this had no appreciable antibiotic properties.



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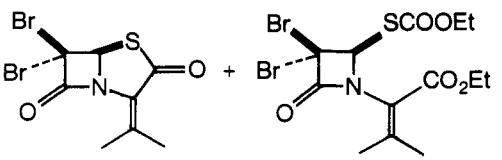
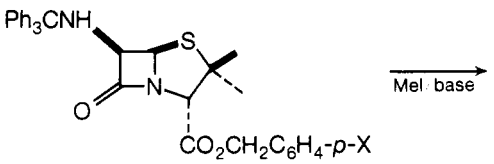
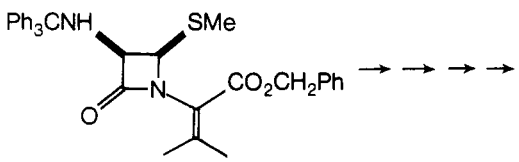


154 155

Comparable alkylations with α-chloro ketones failed, but allylic halides could be employed.[85] In one case reaction of the thiolate anion from **159** with 3-phenylprop-2-ynyl bromide gave the compound **160.** Subsequent transformation of this product, using the Woodward route (see section III.B) and hydration of the triple bond gave a ketone which was converted into the ceph-3-em **161.**[86] Treatment of the corresponding propynyl derivative **162** with piperidine, either with or without mercuric salt catalysis, gave the corresponding ketone **163.** This probably forms by prior formation of the allene **164** followed by addition of the amine at the β position to the sulfur substituent and hydrolysis of the transient enamine to the carbonyl structure during the work-up procedure. The corresponding sulfoxide of **162** reacted similarly. The conjugate addition of nucleophiles to allenic sulfides and sulfoxides is well established, as is the tautomerism of acetylene–allene systems.[87,88]



156, X = OMe
159, X = H



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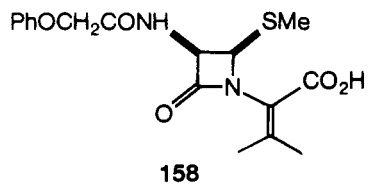

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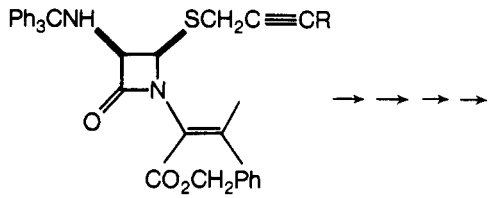
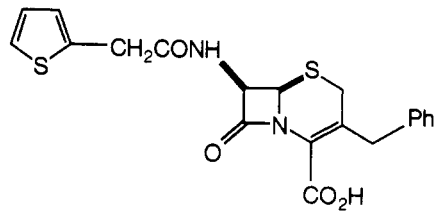


160, R = Ph
162, R = H



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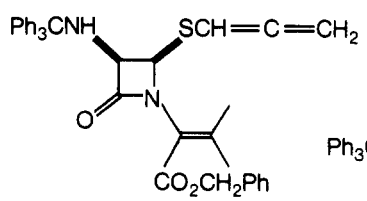
162 →



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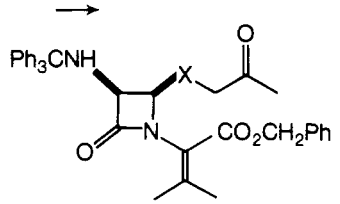


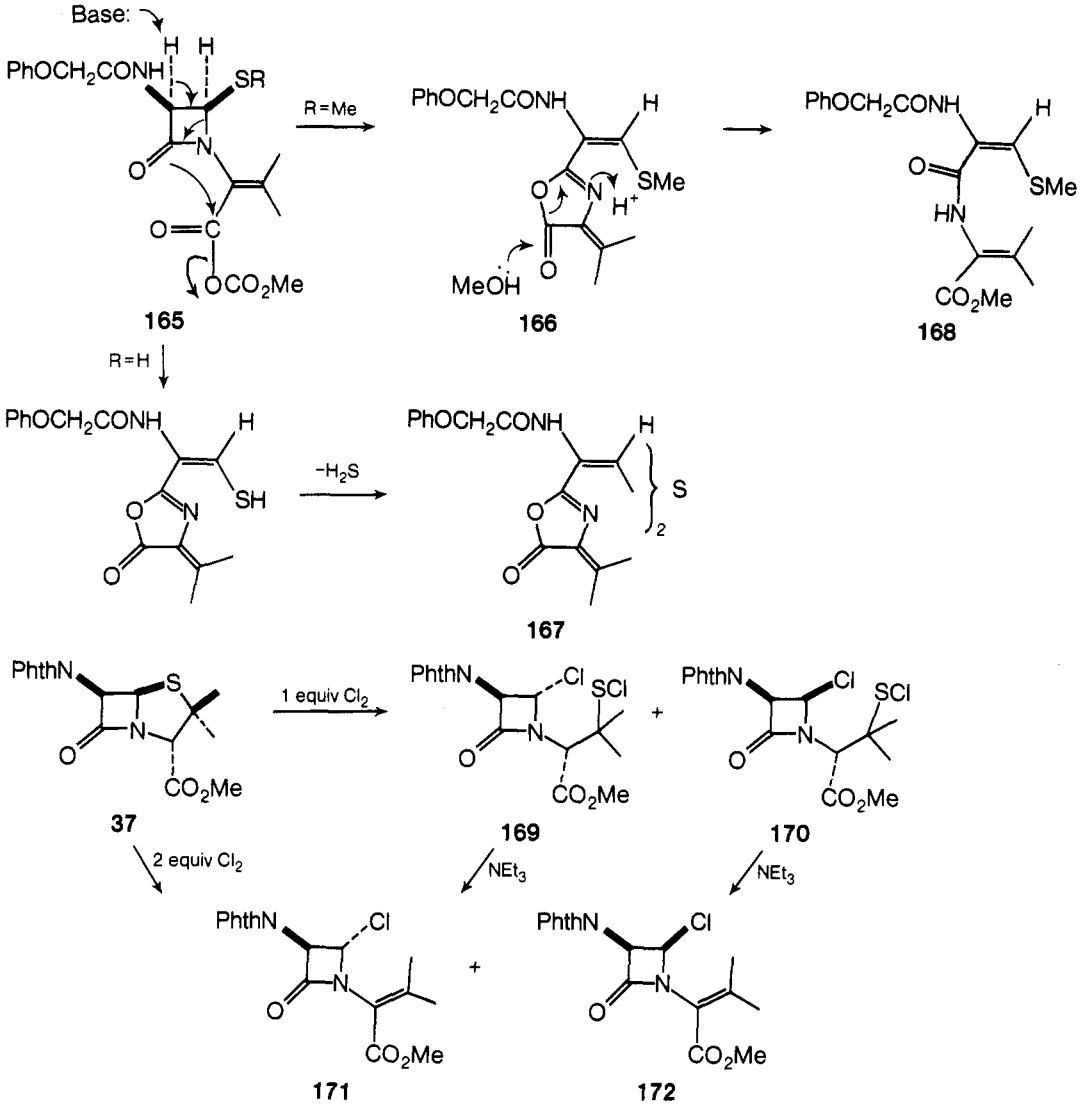
163

Failure of the 6β-acylamino derivatives of penicillins to undergo efficient anhydropenicillin rearrangements was referred to above. One example, where the nature of the side reaction was determined, involves the reaction of 6β-phenoxyacetamidopenicillanic acid (penicillin V) with methyl chloroformate, and then methyl iodide and triethylamine gave, as the major product, the sulfides **166** and **167,** presumably by collapse of the intermediate **165** as indicated (Scheme XIII). Further treatment of the product **166** with methoxide ion gave the ester **168.**[89]

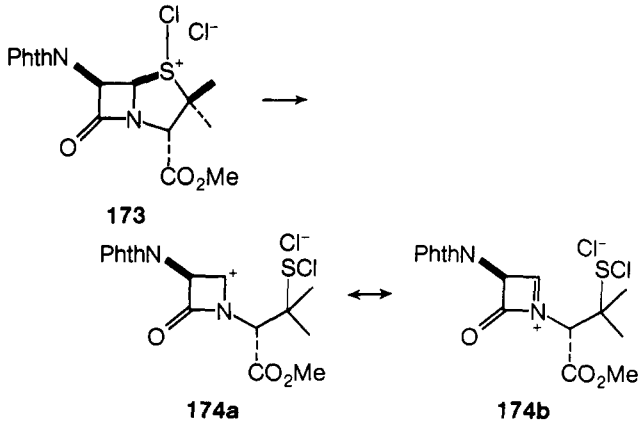
D. Other Thiazolidine Ring-Opening Reactions

In the last section most reactions involved cleavage of the 1,2 carbon to sulfur bond of the penam skeleton. Recently alternative means for opening the thiazolidine ring have been invented which involve prior 1,5-bond cleavage. Kukolja found that electrophilic attack onto the thiazolidine ring of **37** with 1 equiv of chlorine gave an almost quantitative yield of the sulfenyl derivatives **169** and **170.**[90] If 2 equiv of chlorine was employed, the penam system was converted into the olefinic azetidinones **171** and **172,** which could also be obtained by further treatment of the initial sulfenyl chlorides with either more chlorine or exposure to triethylamine. The major isomer was the trans-substituted β-lactam. A possible reaction course proceeds by initial formation of the sulfonium ion **173**

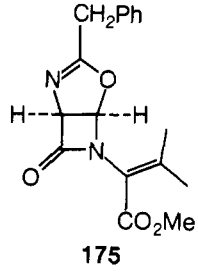
SCHEME XIII



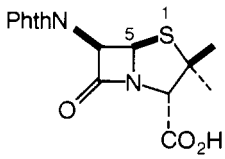
which can then cleave to generate the resonance-stabilized carbonium ion **174.** Quenching of this ion with the chloride anion can probably take place from either face of the β-lactam ring, steric shielding by the phthalimido ring favoring the trans-oriented product.



The carbonium ion (e.g., **174**) appears to be an important species. It is probably also involved in formation of the oxazoline **175** during oxidation of the appropriate penicillin with either *tert*-butyl hypochlorite[91] or *iodobenzene dichloride*.[31] Anhydropenicillins undergo a similar cleavage reaction with chlorine (vide infra).



Opening of the thiazolidine ring can also be achieved by oxidation of substrates with mercury(II) acetate.[92] In acetic acid at 85° 6β-phthalimidopenicillanic acid (**176**) is oxidized with both extrusion of sulfur and decarboxylation to produce the acetate **177.** Ester derivatives, which cannot decarboxylate, also react, the methyl series giving the acetate **178,** although the corresponding sulfoxides are not affected. Either the 1,2 bond can initially cleave to give the derivative **179,** which then undergoes elimination–addition, via the species **180,** or 1,5 bond breaking precedes the decarboxylation step. A closer examination of this reaction showed that the potassium salt of 6β-phenylacetamidopenicillanic acid initially produces the salt **181,** i.e., initial 1,5 cleavage. On leaving, this compound changes into the oxazoline (**182**), while on heating to 85° it eventually forms the acetate **183** (Scheme XIV).[93] The reactive oxazoline can also be opened by reagents such as alcohols.[94]



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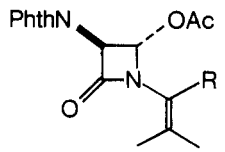
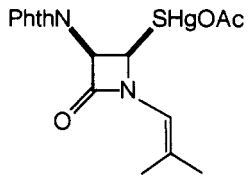


177, R = H
178, R = CO_2Me



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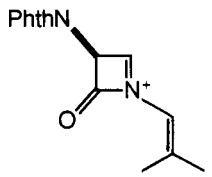
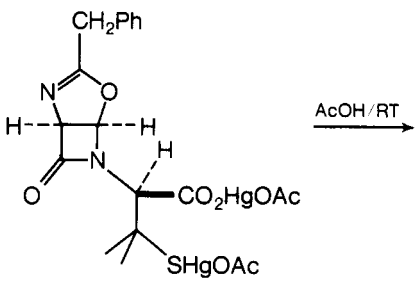


180

SCHEME XIV



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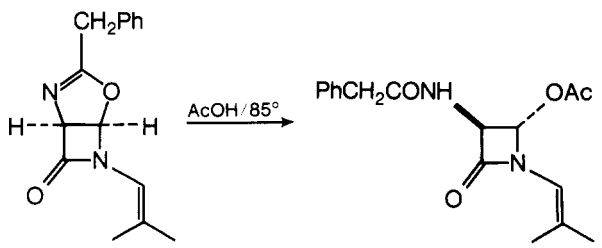
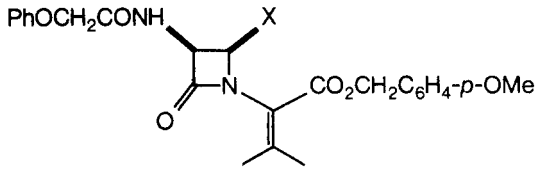


182 183

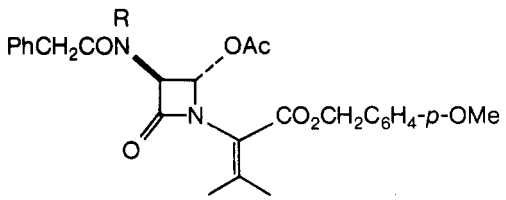
Compounds of the type **178** may also be prepared by oxidation of the 1,2-secopenicillin derivatives of the type **184** with lead tetraacetate.[95] A range of products form, including the sulfoxide **185**, the acetoxymethyl sulfide **186,** and the acetate **187.** An unusual product (**188**), which is also observed, is claimed to arise by an unprecedented migration of the thiomethyl group.

Some elegant synthetic work has been carried out with the chloro derivatives **169** and **170.**[96] Reduction of the sulfenyl chloride function with stannous chloride produced its precursor **37,** together with some of the 5-epi isomer **189** (ratio 1:4 respectively from compound **169**), which is the first example of a naturally derived penicillin epimeric about this center. The free acid of this system was, as expected, devoid of antibacterial properties.

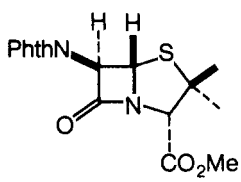
Chlorination of the trichloroethyl ester of 6β-phenylacetamidopenicillanic acid mainly afforded the 2(*S*)-chloroazetidinone **190.** Reaction of this compound with phosphorus pentachloride, known to convert the side chain into the corresponding imino chloride, followed by reaction with hydrogen sulfide and triethylamine, gives the thiazoline **191,** identical in chirality with the material obtained by treatment of the sulfoxide **80** with trimethyl phosphite in benzene.[97] Direct treatment of the chlorosulfenyl chloride **169** with thiols, e.g., 2-methylpropane-2-thiol, gave the corresponding disulfide **192,** which, under the influence of refluxing trifluoroacetic acid, gave four new compounds, the β-lactam derivatives **193** and **194** and an epimeric mixture of the degradation products **195.**[98] Again initial formation of a carbonium ion of the type **180** is intimated. The



184, X = SMe
185, X = S(O)Me
186, X = SCH_2OAc



187, R = H
188, R = SMe



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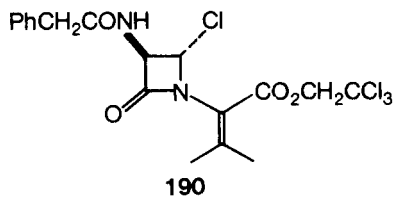

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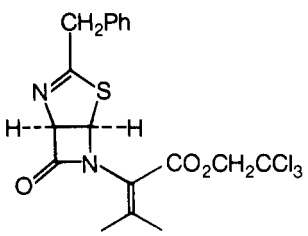

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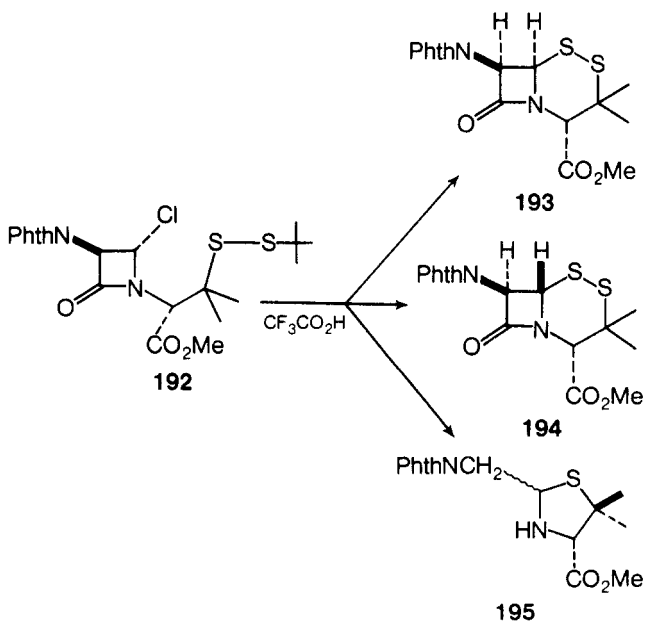
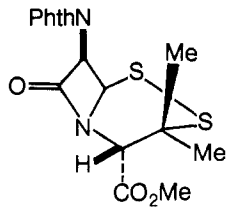


192 193 194 195

degradation products **195** appear to arise from the corresponding penam systems, which are known to collapse by further treatment with trifluoroacetic acid.[99] The disulfide **193** has been shown to possess the conformation indicated **196.**[100] The free acid is less active as an antibiotic compared to the corresponding penam system.

Chlorination of anhydropenicillins **197** in halogenated solvents has also been investigated, the products being the acid chlorides **198** and **199.**[101] The ratio of the epimers varied



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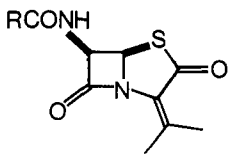

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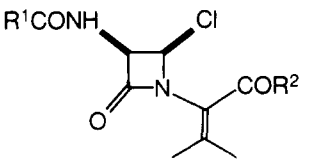
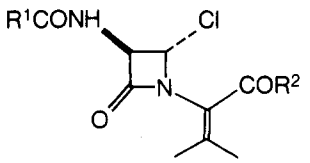
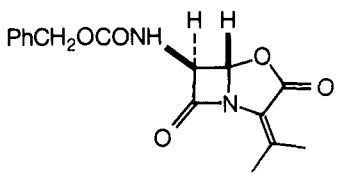


198, R^2 = Cl
201, R^1 = $PhCH_2$; R^2 = MeO
202, R^1 = $PhCH_2O$; R^2 = OH



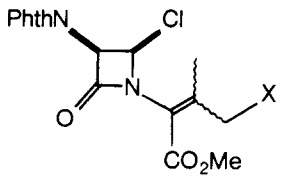
199, R^2 = Cl
200, R^1 = $PhCH_2$; R^2 = MeO

with the conditions used but generally favored the *R* isomer **198.** Careful hydrolysis or alcoholysis gave the corresponding acid or ester. Treatment of the chloride **200** with bicarbonate gave the oxazoline **175,** explained by anti displacement of the chlorine by the side-chain amide group. The other epimer (**201**) was stable to reaction with bicarbonate under similar conditions, but both isomers reacted on silica gel or alumina to give the oxazoline **175,** the reaction proceeding under these conditions via intermediate formation of the carbonium ion.[102] The free acid **202** afforded the epi-oxa analogue of anhydropenicillin **203** by treatment with sodium hydrogen carbonate in aqueous acetone.

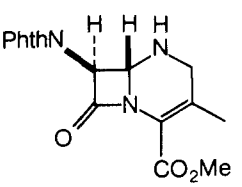


203

The methyl groups of the ester **204** undergo allylic bromination with *N*-bromosuccinimide, mono- and dibromination occurring.[103] Treatment of the monobromide **205** with azide ion, followed by reduction and treatment with base, gave entry into the hetero analogue of cephalosporin, the aza-cephem **206,** and this method can probably be extended by incorporation of other heteroatoms as well.

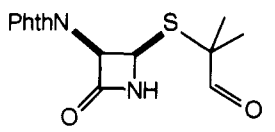


204, X = H
205, X = Br



206

Some important chlorination reactions have been reported with the nor-penam system. For example, treatment of the aldehyde **207** with an excess of chlorine gave the *trans*-chloro derivative **208** in high yield. The chlorine atom undergoes a variety of displacement reactions, methanol, for example, giving the methoxy derivative **209.** In contrast to the behavior of compound **207** and to the chlorination reactions described above, phthalimido derivative **210** reacted with 2 equiv of chlorine by 1,2-bond cleavage to give the sulfenyl halide **211.** Presumably, in this instance, the presence of the *N*-trifluoroacetyl group inhibits participation of the nitrogen lone pair electrons in the cleavage of the azetidinone–sulfur bond, hence favoring the observed, alternative cleavage step.[104] Both compounds **208** and **211** are potentially useful compounds for the reconstruction of modified rings fused to the β-lactam group. The intermediate **211,** for example, reacted with ethyl vinyl ether to give the aldehyde **212,** which, with acetic anhydride in pyridine, afforded the closed acetate **213.**[105]



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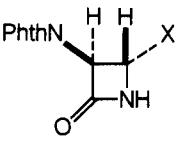
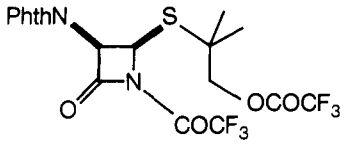


208, X = Cl
209, X = OMe



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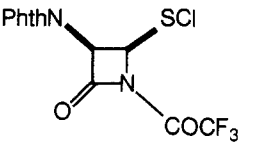

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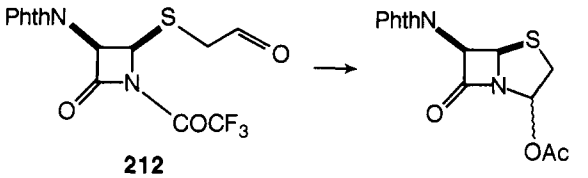


212 **213**

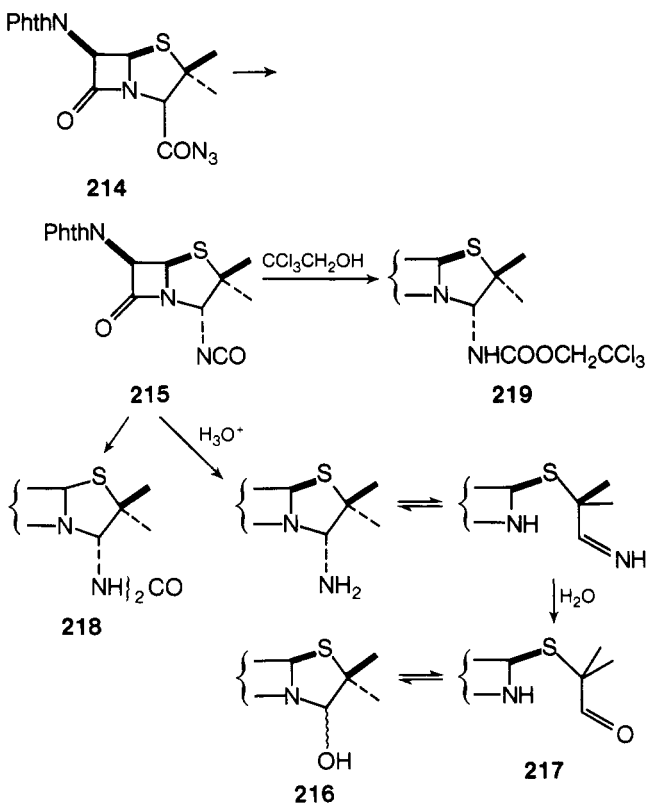
E. Liberation of the β-Lactam Nitrogen

1. Decarboxylation

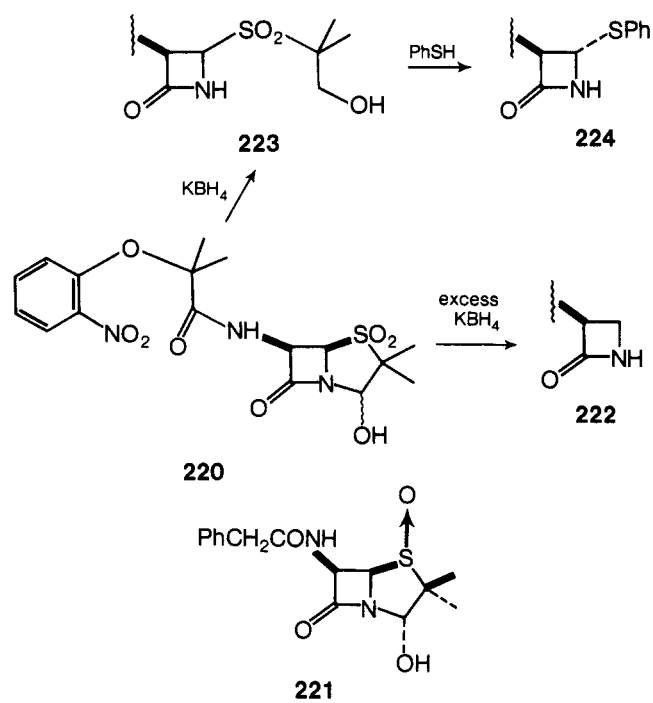
Sheehan and Brandt[106] found that performing a Curtius degradation on the acid azide **214** followed by acid hydrolysis of the resulting isocyanate **215** afforded not the amine but the corresponding alcohol **216.** In the phthalimido series this was shown to be in equilibrium with some of the corresponding aldehyde **217.** Hydrolysis also afforded considerable quantities of the urea **218.** Although high dilution helped to increase the yield of the alcohol, Woodward and Heusler[107,108] found it expedient to first add trichloroethanol to give the carbamate **219,** followed by reduction with zinc in acetic acid to form the corresponding aldehyde. The equilibrium between the open and closed form depends on the bulk of the 6β-amino substituent as well as electronic effects, the proportion of aldehyde increasing in the series $PhCH_2CONH$, $PhOCH_2CONH$, CCl_3CH_2OCONH < $(CH_3)_3COCONH$ < phthalimido.[109]

The oxidation level of the sulfur atom is also important since, for the sulfone **220,** none of the aldehyde form was present.[110] Again, only alcohol was observed for the sulfoxide **221.**[111] In these last two cases the side chain amide group is known to hydrogen-bond to the sulfur–oxygen group. This interaction would restrict the freedom of bond rotation in the open, aldehyde form, thus holding the aldehyde group nearer to the β-lactam nitrogen and hence favoring the closed, carbinolamine structure.

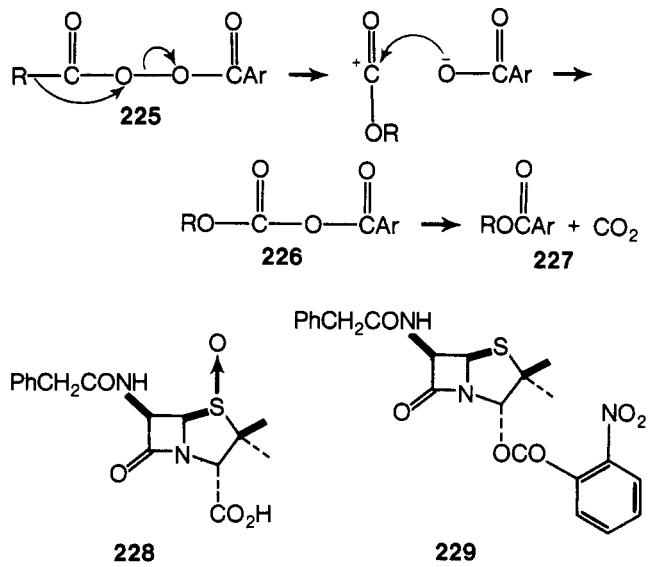
Reduction of the hydroxy sulfone **220** with an excess of potassium borohydride gave the intact, but reduced, β-lactam derivative **222,** while use of a limited amount of the reagent



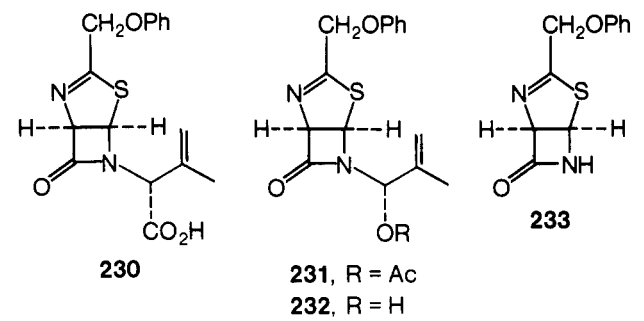
gave the sulfone **223.** Substitution of the sulfone was accomplished by reaction with thiols, thiophenol, for example, giving the sulfide **224** by elimination of the sulfinic acid.[110,112]



As an alternative to the Curtius reaction the penicillanic acids can be decarboxylated by rearrangement of their mixed anhydrides with aroyl peroxides.[111] These mixed peroxides **225** initially rearrange into the isomeric inversion product **226,** with net retention of configuration at the alkyl (penam) center.[113] On warming, the inversion products decarboxylate to produce the corresponding esters **227.**[114] Thus *o*-nitroperbenzoic acid and the acid **228** gave the *o*-nitrobenzoate **229.** Removal of the aroyl group can be effected, in this case by mild reduction using zinc dust and ammonium chloride, to give the alcohol **221.**

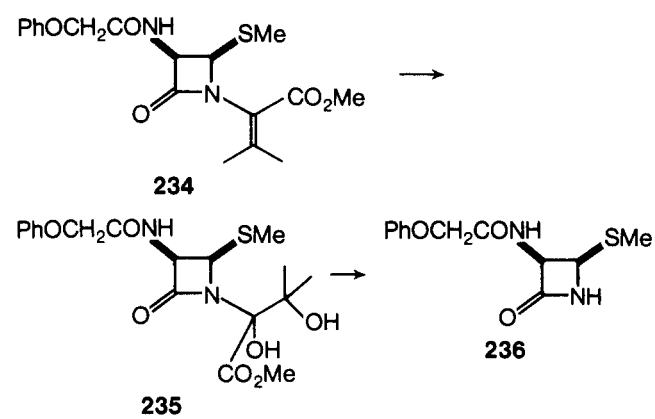


Decarboxylation of certain derivatives can also be achieved with lead tetraacetate.[115] For example, the acid **230** was converted with this reagent into the acetate **231.** Selective hydrolysis of the acetate group is followed by spontaneous liberation of the β-lactam derivative **233** via the unstable carbinol **232.**

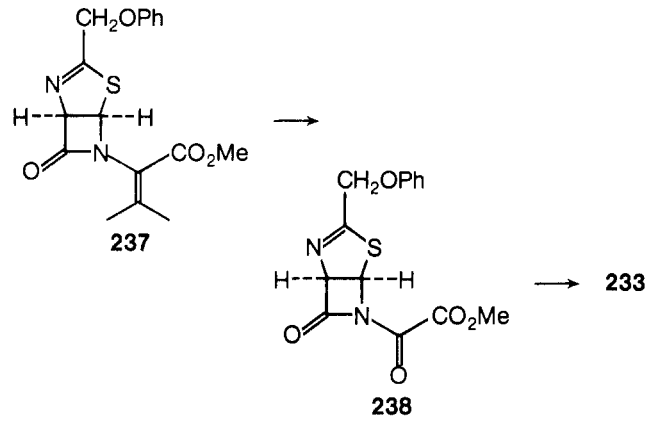


2. *Oxidation of the Nitrogen Substituent*

Cleavage of the thiazolidine ring of the penam system about bonds 1,2 (or 1,5) leaves a C_5 appendage attached to the β-lactam nitrogen atom. Removal of this substituent has been achieved by oxidation in several ways.[95] One method employs cis-hydroxylating agents; the sulfide **234,** for example, is expected to lead to the diol intermediate **235,** but this carbinolamine collapses directly into the β-lactam component **236.** Either osmium tetroxide or potassium permanganate

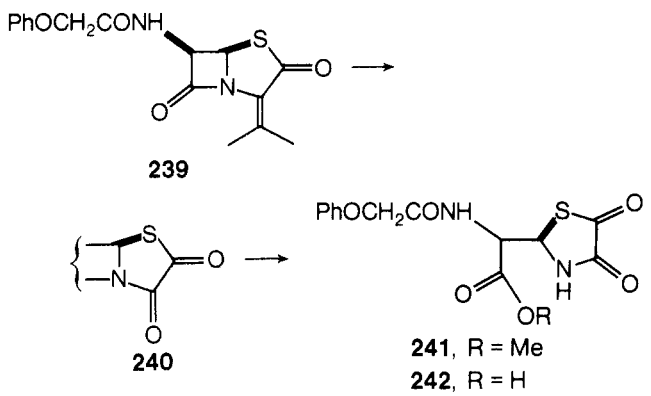


buffered at pH 7 can be employed. An alternative oxidant is ozone.[116] In this case the initial product is the oxamide, e.g., **237** to **238.** Dilute base or acid hydrolyzes this at the oxamide–β-lactam junction to liberate the intact β-lactam ring.



237 238 233

Similar treatment of the anhydropenicillin **239** with ozone in methanol gave, instead of the expected azetidinone, the methyl ester **241** by hydrolysis of the intermediate **240.**[117] Simple *N*-acyl derivatives of β-lactams are also cleaved by opening of the β-lactam ring. Presumably, in the bicyclic intermediate **240,** the extra ring strain is reflected mainly in the β-lactam ring which is now more susceptible to solvolysis than the oxamide carbonyl group, in contrast to the situation with the acyclic oxamides.

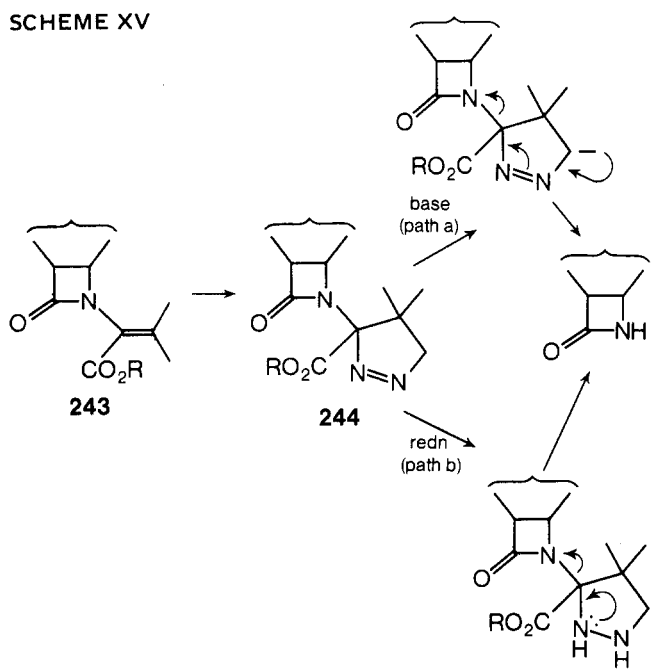


239 240 241, R = Me 242, R = H

3. Pyrazoline Formation

In the presence of sensitive groups, such as disulfides, oxidation cannot be tolerated as a method for the removal of the nitrogen substituent. For these cases an alternative procedure has been developed which depends on the introduction of an internal nucleophilic center capable of displacing the β-lactam nitrogen[118] Addition of diazomethane across the conjugated bond of derivatives such as **243** produces a mixture of the epimeric pyrazolines **244.** Either brief treatment of this mixture with potassium *tert*-butoxide (Scheme XV, path a) or reduction, either with zinc dust in acetic acid or with chromous acetate, (path b), liberates the β-lactam ring, generally in high yields.

SCHEME XV



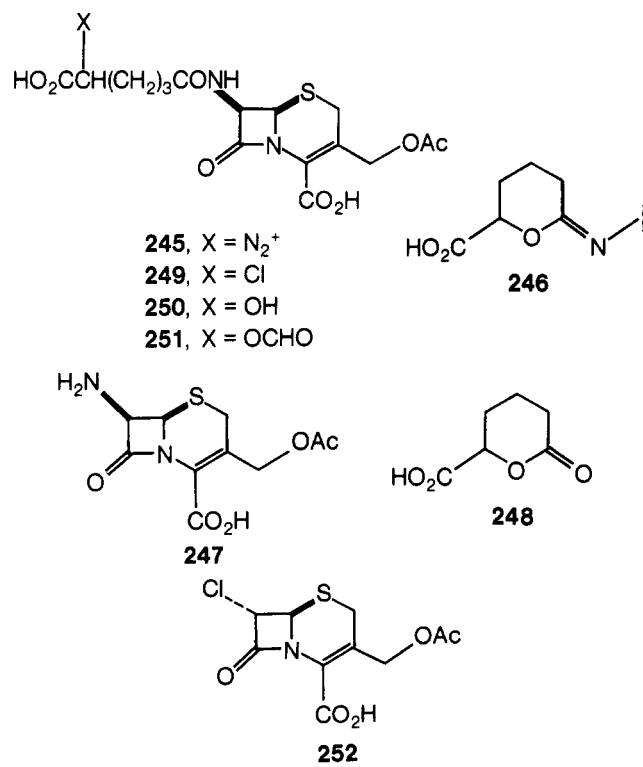
243 244

F. Transformations about the 6(7)-Penam (Cephem) Position

1. Acylation and Deacylation of the Amino Group

That modification of the side-chain acyl group has a profound effect on the potency of both the penicillin and cephalosporin antibiotics is well established.[119,120] Since reviews on methods used to deacylate and reacylate penicillins and cephalosporins are abundant,[14,15,121] only principal methods will be considered in this section. It should be remembered that, whereas the removal of the acyl function (position 6) is not a prerequisite for penicillins, since 6-aminopenicillanic acid is available from fermentations,[3] only cephalosporin C is produced in culture broths, and removal of the D-α-aminoadipoyl group is necessary to obtain the deacylated material.

Cleavage of the aminoadipoyl group has been managed by several methods, including direct hydrolysis.[122] An interesting scheme involves an intramolecular process with nitrosyl chloride in formic acid.[123] Diazotization of cephalosporin C occurs, and the diazonium salt **245** collapses to form the iminolactone **246,** which is rapidly hydrolyzed into the amine **247** and the lactone **248.** The side products **249** to **251** form by competing reactions of the diazonium species. The chloride

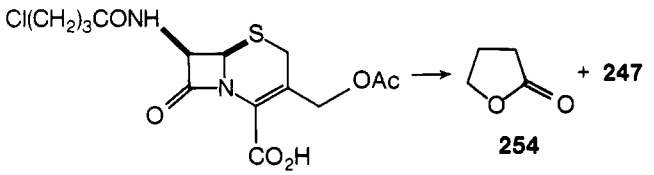


245, X = N_2^+
249, X = Cl
250, X = OH
251, X = OCHO

246 247 248 252

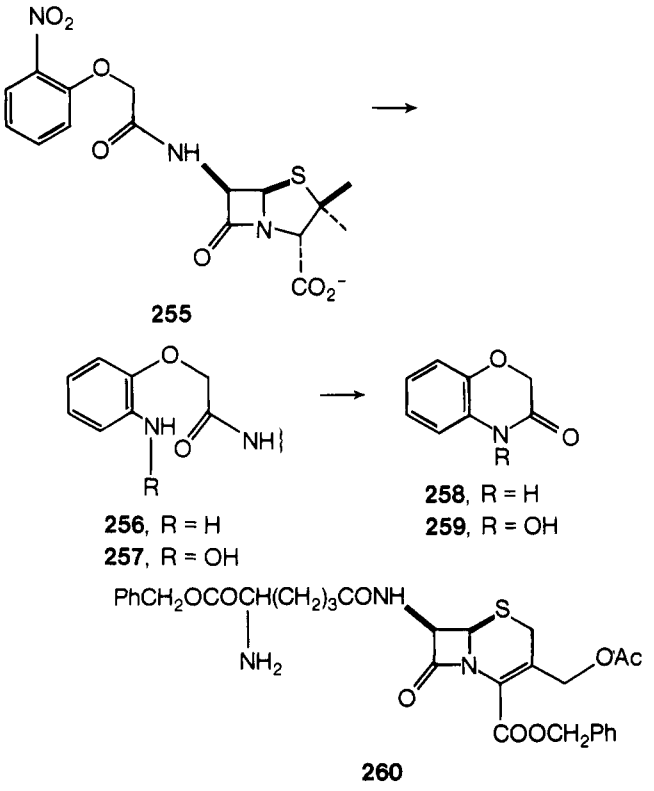
252 is also formed in small amounts by further reaction of the amine **247** with the slight excess of nitrosyl chloride present.[124] Intramolecular assistance also occurs with certain ω-haloacylamides. For example, the 4-chlorobutanamide de-

rivative **253** reacted spontaneously to liberate the amine **247** and the lactone **254.**[125]



253 **254** + **247**

A slightly different form of intramolecular assistance pertains with *o*-nitrophenoxyacetamides (e.g., **255**) after reduction.[126] The resulting aniline **256** or hydroxylamine **257** interacts with the adjacent amide bond at room temperature in aqueous solution to release the amine and form the benzoxazine (**258** or **259**). A similar process occurs with the dibenzyl ester of cephalosporin C (**260**), the reaction taking place in pyridine–acetic acid to liberate the free amine.[127]



255

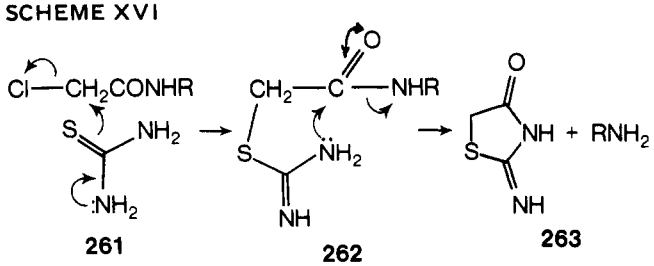
256, R = H
257, R = OH

258, R = H
259, R = OH

260

An interesting reaction was noted between thiourea and the chloroacetyl derivative of 7-aminocephalosporanic acid.[125] Initial attack occurred with displacement of the chlorine (e.g., **261** to **262**). Subsequent interaction produced the amine and the thiazolidone **263** (Scheme XVI).

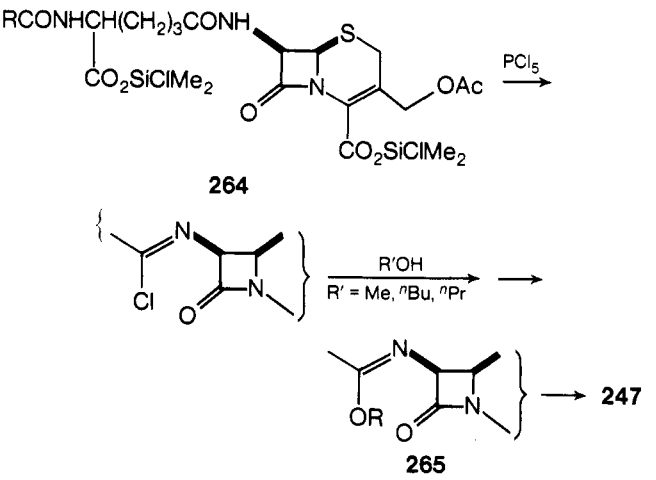
SCHEME XVI



261 **262** **263**

Such intramolecular reactions can be enhanced by first preparing the imino ether derivatives of the amide group.[121] One of the more general methods of amide cleavage, however, involves the use of phosphorus pentachloride on a protected system, such as the silylated cephalosporin C ester

SCHEME XVII



264

265

(**264**) (Scheme XVII); penicillins also react in this manner.[121] The reaction is presumed to follow initial formation of the imino chloride. Generally a weak tertiary base is used, such as *N,N*-dimethylaniline, since stronger bases such as triethylamine can cause epimerization about the β-lactam junction, or can give rise to ketenimines.[128] Reaction with an alcohol then gives the corresponding imino ether **265.** NMR studies have shown that subsequent hydrolysis of the imino ether is not essential since collapse of the (protonated) imino ether can occur smoothly by *O*-alkyl cleavage in the absence of water.[129] Protonated (quaternized) imino ethers are known to be powerful alkylating agents.[130]

Several methods are available for exchange of acyl groups on the side chain without prior liberation of the amine. A specific example is due to Sheehan (Scheme XVIII) in which a net exchange of the phenylacetyl group of compound **266** to give the oxaloyl derivative **267** is achieved.[131] A more general method involves modification of the amide group by either formation of an imino ether,[132,133] or by silylation,[134] followed by reaction with an acyl halide. The exchanged amide **269** is formed from the starting imino ether **268,** probably via an intermediate of the type **270.**

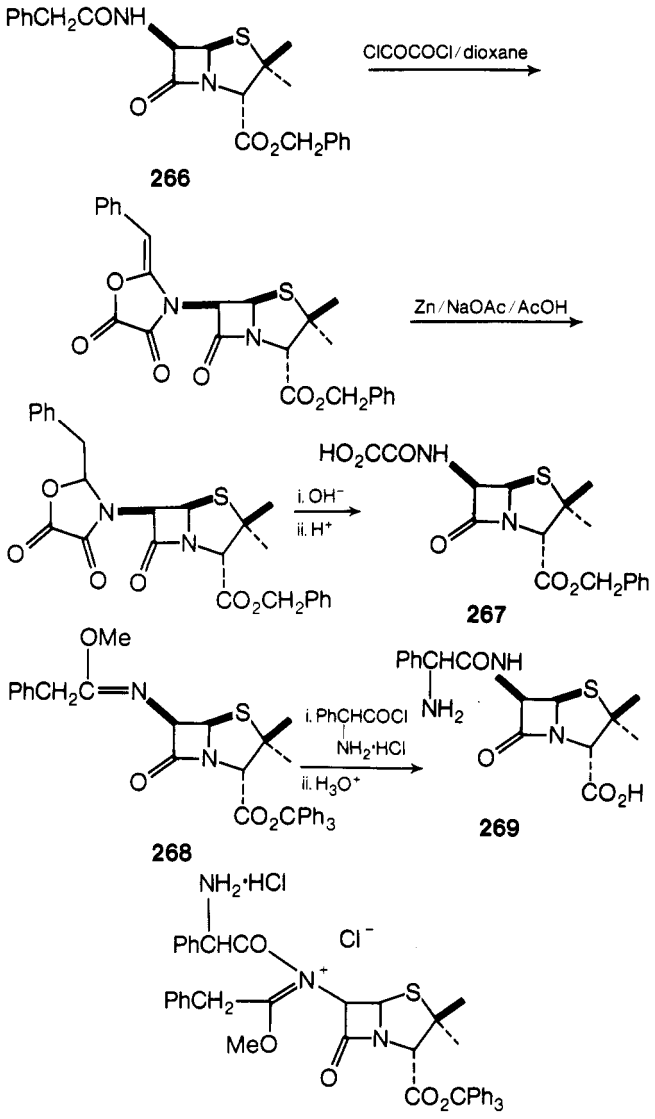
2. Epimerization

Penicillins and cephalosporins act upon microorganisms by interfering with production of their protective cell wall.[135-137] The synthesis of the microbial coating is a multistage process involving formation of a three-dimensional polymer consisting of polysaccharide strands cross-linked with peptides.[138] There is a considerable body of evidence which suggests that the β-lactam antibiotics inhibit the cross-linking process by blocking one (or more) of the transpeptidase enzymes.[139-141]

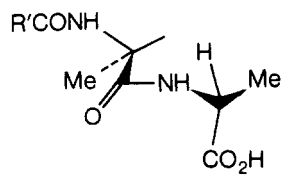
The natural substrate involved in the cross-linking reactions is an *N*-acyl-R-alanyl-R-alanine and, in 1965, Tipper and Strominger[139] suggested that the lactam antibiotics mimic the natural substrate in complexing with the transpeptidase enzyme, which is then inhibited by acylation with the β-lactam function, i.e., the antibiotics acting as enzyme inhibitors. A comparison of *N*-acyl-R-alanyl-R-alanine (**271**) with penicillin (**272**), allowing the former to adopt the most comparable conformation, indicates a close similarity. It was suggested by Tipper and Strominger that the presence of a 6α-methyl group in the penam nucleus (e.g., **273**) would provide an even closer similarity and, as a consequence, that such derivatives might prove to be even more effective antibiotics.[142]

The above hypothesis provided a strong incentive for the examination of the reactivity about position 6 in the penam system, as well as at position 7 in the ceph-3-em structure. A further boost to these studies was provided by the revelation

SCHEME XVIII



that the 7α-methoxycephalosporins, e.g., **274,** are very effective antibiotics.[5,143] Finally, a problem encountered in many approaches to the total synthesis of these antibiotics is the need to convert the more readily available trans-substituted β-lactam system into the corresponding cis-oriented isomers.[144]



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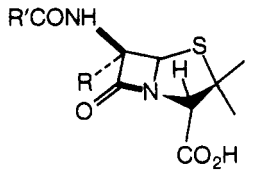
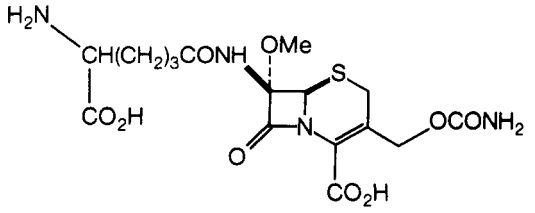


272, R = H
273, R = Me



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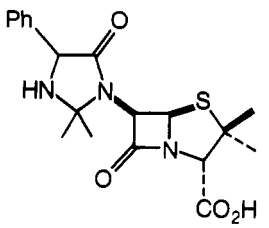
The first observation of epimerization about position 6 of the penam skeleton was noticed almost simultaneously by Johnson and coworkers[145] and by Wolfe and Lee.[146,147] The former observed that hetacillin (**275**) gave the isomer **276** by treatment with base, and the latter observed the same type of behavior with the phthalimido derivative **37.** Subsequently many examples have been recorded.



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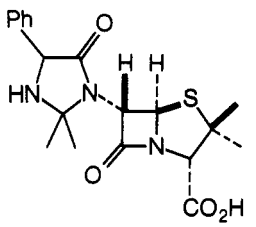

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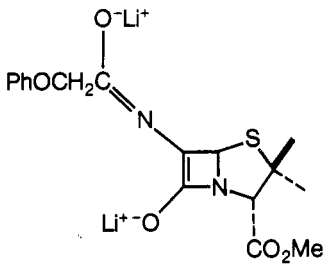
Direct epimerization of penicillins bearing a free NH bond on the acylamido substituent is difficult to effect, probably because this proton is more acidic than the 6α-hydrogen.[148] In protic solvents formation of the amide anion can also lead to intramolecular attack onto the β-lactam ring, thus destroying it. Removal of the amide proton is thus an advantage in epimerization studies, and this can be achieved by silylation. As a result, trimethylsilyl derivatives readily undergo epimerizations.[149-152]

Very recently a direct method for the formation of epimers has been recorded which involves treatment of a penicillin ester (**6**) with lithium diisopropylamide at −80° in tetrahydrofuran, followed by the addition of some methanol; in the absence of the protic solvent, epimerization is not observed. Transient formation of the vicinal dianion **277** was invoked to explain this result.[153]



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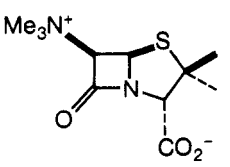
The electronegativity of the 6-substituent also appears to be important. Thus 6β-amino-, 6β-dimethylamino-, and 6β-triphenylmethylaminopenicillins fail to epimerize at pH 11, whereas the betaine **278** does. The 6α-bromo derivative (**279**) undergoes deuterium exchange for the 6β-hydrogen at a similar pH.[148]



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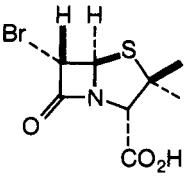

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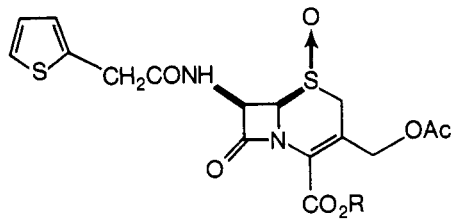
An important method for effecting epimerization is to convert the free 6β-amine into a Schiff's base with an aromatic aldehyde. These derivatives then undergo very ready epimerization even with tertiary amine bases.[154,155]

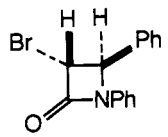
The presence of a sulfoxide function facilitates the epimerization step.[156] The cephalosporin sulfoxide **280,** for example, equilibrates even with triethylamine in dimethyl sulfoxide. Even more remarkable is the epimerization of the penicillin sulfoxide **25** catalyzed by diethylamine.[110] It is assumed that



280, R = fluorenyl

the reagent is too bulky to attack the β-lactam ring at a rate comparable with the equilibration.

A preponderance of the 6α-(trans)-substituted penicillins (>90%) is generally observed at equilibrium. The relative instability of the 6β isomers can be attributed to the steric interaction with the *syn*-2β-methyl group. In the absence of such interactions, as in the compound **281,** the cis isomer becomes more favored (30% at equilibrium).[157] Similarly, a net reduction in the steric bulk of the 6β-substituent also increases the proportion of the cis isomer at equilibrium, as for the Schiff's bases (15–30%).



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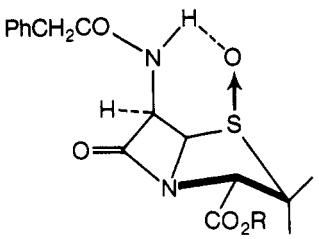
The presence of a (*S*)-sulfoxide function in the thiazolidine ring introduces two further parameters of importance in determining the equilibrium values. Hydrogen bonding between the sulfoxide bond and the side chain amide group favors the 6β orientation. Furthermore, the presence of the sulfoxide group induces a conformational change in the thiazolidine ring, the envelope configuration of the thiazolidine ring having position 2 bent away from the β-lactam ring, hence relieving steric interactions between the geminal methyl groups and the side chain (see **282**).



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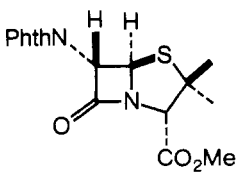
Mechanistic details of the epimerization reaction have been thoroughly investigated.[11] Sjöberg and his group found that treatment of the methyl ester of 6β-phthalimidopenicillanate (**37**) with triethylamine in dichloromethane gave both the α epimer **283** and the 1,4-thiazine **284.**[158] The latter



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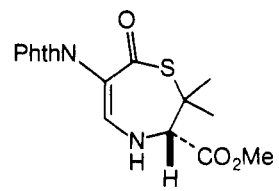
arises by cleavage of the 1,5-bond of the penam nucleus to give an enethiolate species (**285**) followed by intramolecular attack on the β-lactam ring to break the 4,7-bond. The question then arises as to whether or not the enethiolate species is either a common or necessary intermediate for formation of both the thiazepine and the 6α isomer, a proposal originally formulated by Wolfe et al.[147] In making a careful examination of the process, Stoodley subsequently found that, for the homopenicillanate (**286**) strong bases gave mainly the epimer while weak bases produced more of the thiazepine.[155] Furthermore, with strong bases, exchange of hydrogen at this position was possible. Stoodley argued that the ground state free energy of the 6α epimer must be lower than that of the 6β isomer because of steric compression of the latter. With strong bases the transition states leading to the corresponding enolate anion must have considerably more sp^3 character about position 6 and hence bear closer resemblance to the starting compounds (cf. the ion pairs **287** and **288**), also reflecting the ground state differences. With a weak base other factors are introduced such as formation of the thiazepine. With these, the 6β to 6α isomerization is essentially irreversible since the 6α isomer is converted into the thiazepine more *slowly* by a factor of ca. 300 than the rate at which the 6β epimer is changed into the thiazepine and the 6α compound. With the weak bases the transition states would be with the proton almost completely removed from the substrates and would hence be similar, having increased sp^2 character. In order to complete epimerization the anion intermediates have to be reprotonated. The free energy of activation for reprotonation would be expected to be *lower* with a stronger conjugate acid (weaker base) but, since thiazepine formation increases with weaker bases, the free energy of formation of the thiazepine must decrease to a greater degree. Thiazepine formation is, therefore, a good example of an E1cB process.[159]



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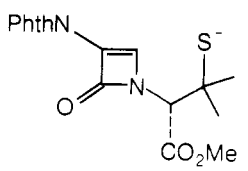

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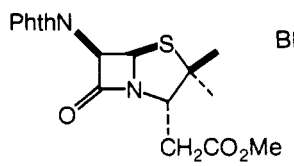

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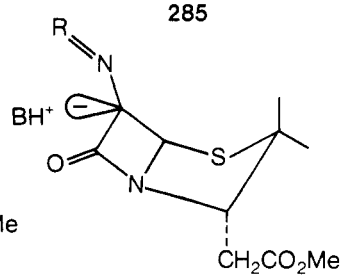

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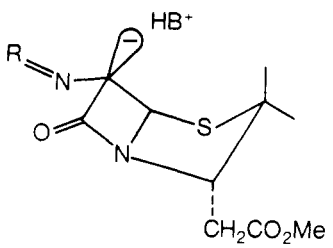

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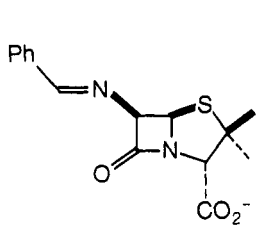
3. Substitutions

Initial approaches to the 6-substituted penams failed. Methyl 6β-phthalimidopenicillanate, for example, could not be alkylated with a combination of sodium hydride and various alkylating agents.[146]

The first successful reaction involving an electrophile other than deuterium ions was achieved by Reiner and Zeller,[160] who, in 1968, reported the reaction of 6-aminopenicillanic acid with benzaldehyde at pH 7.5. The benzylidenimine **289** was initially produced, and this subsequently condensed with more benzaldehyde to give the adduct **290.** Subsequent hydrolysis of the imine, with aqueous acid, and acylation with

phenylacetyl chloride gave one acid specifically, of undefined stereochemistry (**291**), but this showed no activity in subsequent bioassays.



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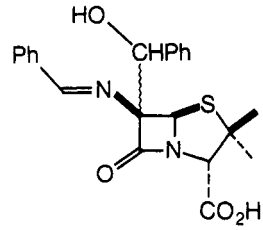

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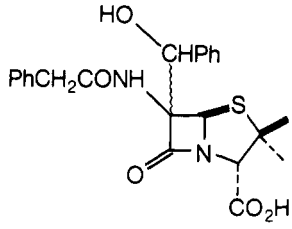
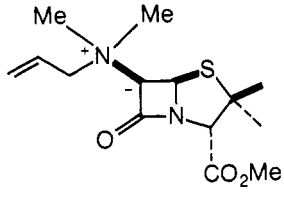


291

Soon afterwards a stereospecific method for alkylation at position 6 was developed which involved the nitrogen ylide **292.** Heating this induced a [2,3] sigmatropic rearrangement of the allyl substituent to form the isomer **293.** Although the reaction was reasonably efficient, it is of limited applicability. The stereochemistry of the rearrangement product was again confirmed by nuclear Overhauser effects on the derived quaternary salt **294.**[161]



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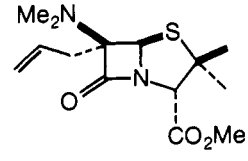

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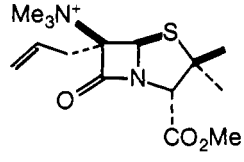

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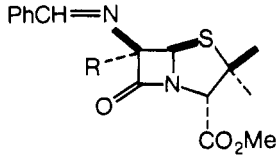
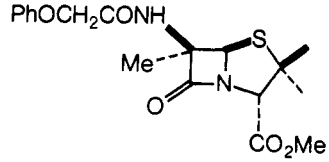
The above exploratory work was soon followed by a flood of communications in this area. In 1971 workers from the Squibb Institute described a method for the discrete alkylation of methyl *N*-benzylidene-6-aminopenicillanate (**295**) by initial treatment with sodium hydride followed by addition of an excess of methyl iodide.[162] Both epimers of the 6-methyl derivative were obtained (ratio 6α:6β 18:1). The major isomer was hydrolyzed and phenoxyacetylated to give the amide **296,** the structure of which was confirmed by an x-ray crystallographic analysis. In a similar manner the cephalosporins **297** and **298** were prepared (ratio 85:15). Against the prediction made earlier by Strominger, both the penam ester **296** and the cephem acid **299** showed reduced antibiotic activities compared to the unsubstituted systems.



295, R = H or Me



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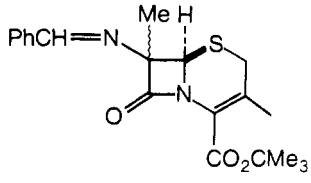
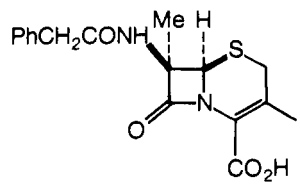
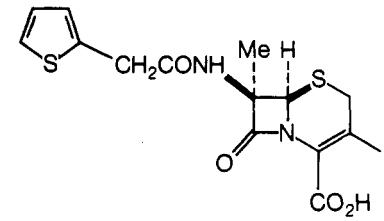


297, α-imine
298, β-imine



299

In order to increase the acidity of the 6α proton of the penam nucleus, Firestone and his colleagues employed the *p*-nitrobenzylidene derivative **300.**[163] Phenyllithium gave the enolate which could also be alkylated. In this way the free penam acid **273** was made. Removal of the nitrobenzylidene group was best effected by an exchange process involving either aniline hydrochloride or 2,4-dinitrophenylhydrazine as its *p*-toluenesulfonic acid salt. Further work also gave the 6α-ethyl analogue and the cephem acid **301;** all of these showed reduced biological activities compared to the unsubstituted system.



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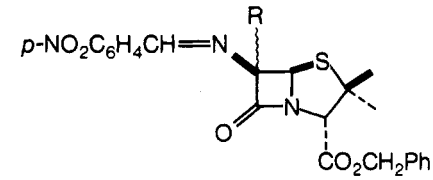
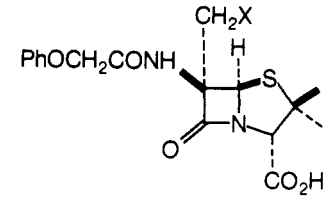


300, R = H
302, R = CH_2OH
303, R = $CH_2OSO_2C_6H_4Me$
309, R = $CH_2OSO_2CF_3$

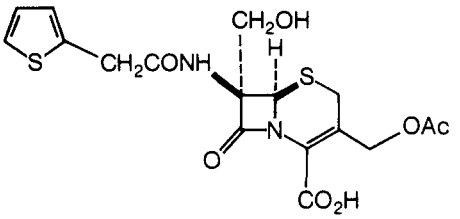


304, X = OH
305, X = NH_2
306, X = H
307, X = Cl
308, X = F

Condensation of the anion from the nitrobenzylidenimine with formaldehyde afforded the 6α-hydroxymethylene adduct **302.** By making the derived *p*-toluenesulfonate (**303**), a variety of displacement reactions were effected, leading to the derivatives **304** to **307.** The fluoromethyl compound **308** was also prepared, but from the triflate derivative **309.**[164] As expected, a similar reaction sequence gave the cephem acid **310.**

Oxidation of the lithium enolate (cf. **300,** R = Li) with air, in the hope of preparing the 6α alcohol, gave instead two different products, the dimer **311** and the nitrone **312.**[165] A variety of alternative oxidants, such as alkyl hydroperoxides, also gave the dimer. A one-electron oxidation of the anion was postulated to explain dimer formation. The nitrone is stabilized

by resonance of the oxygen charge over the β-lactam carbonyl, an effect reflected in its infrared spectrum, the carbonyl absorption decreasing to 1760 cm^{-1}. Similar shifts are noticed for the hydrazone **313**[166] and the diazo ketone **314.**[167]



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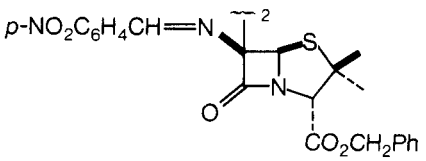

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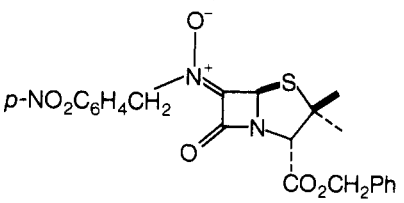

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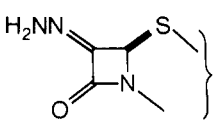

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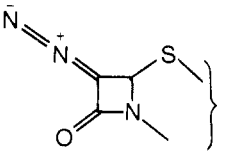

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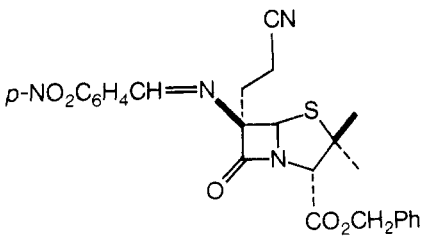
More recent ramifications of the substitution reactions involving the *p*-nitrobenzylidene derivatives include Michael additions, for example, to acrylonitrile, which gives the expected adduct **315** and the spiro product **316.**[168] Reaction with methyl chloroformate gave the carbomethoxy derivative, released as the acid **317,** while benzyl chloroformate gave the salt **318.** Oxidation of the hydroxymethyl derivatives (e.g., **304**) has produced the aldehyde **319,** and the ketone **320** was similarly prepared. Tests for antibacterial activity against *B. subtilis* showed the sequence 6α-CHO ≈ $COCH_3$ > CH_2CH_2CN ≈ CH_2OH > CO_2Me ≈ $CO_2^-Na^+$ > $CHOHCH_3$, although all were less active than the unsubstituted system.

7α-Carboxycephems have also been described.[169] An x-ray crystallographic analysis of the simple methyl derivative **321** has confirmed the earlier structural assignment.[170] It may be concluded from these findings, therefore, that a general entry into the appropriately substituted 6α-penam and 7α-cephem systems has been attained. None of the derivatives described above, however, had the desirable β-lactamase resistance and antibacterial spectrum observed for the 7α-methoxy-substituted cephem system (e.g., **274**). Although introduction of the methoxy function was eventually achieved using a benzylidene derivative to protect the side-chain amino group,[171a] an alternative scheme was also devised (Scheme XX).[171b] The method involves the 6(7)-diazopenams (cephems). Such species were recognized since 1962 when Cignarella and his colleagues described the deamination of 6-aminopenicillanic acid in the presence of hydrogen chloride or bromide to form the corresponding 6α-halopenicillanic acids.[172] Later the 6α-acetoxy and 6α-hydroxy compounds were also made and, in the methyl ester series,[167,173] a reduction gave the unsubstituted lactam **322.**[174] Intermediate formation of the diazo ketone (e.g., **323**) was established



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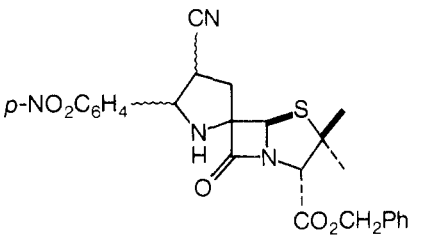

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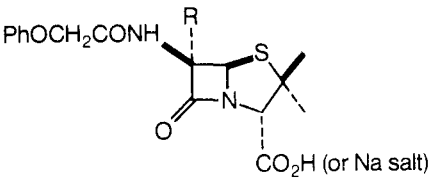
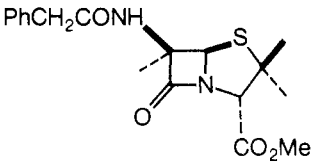


317, R = CO_2Me
318, R = CO_2Na
319, R = CHO
320, R = $COCH_3$



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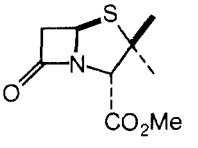


322

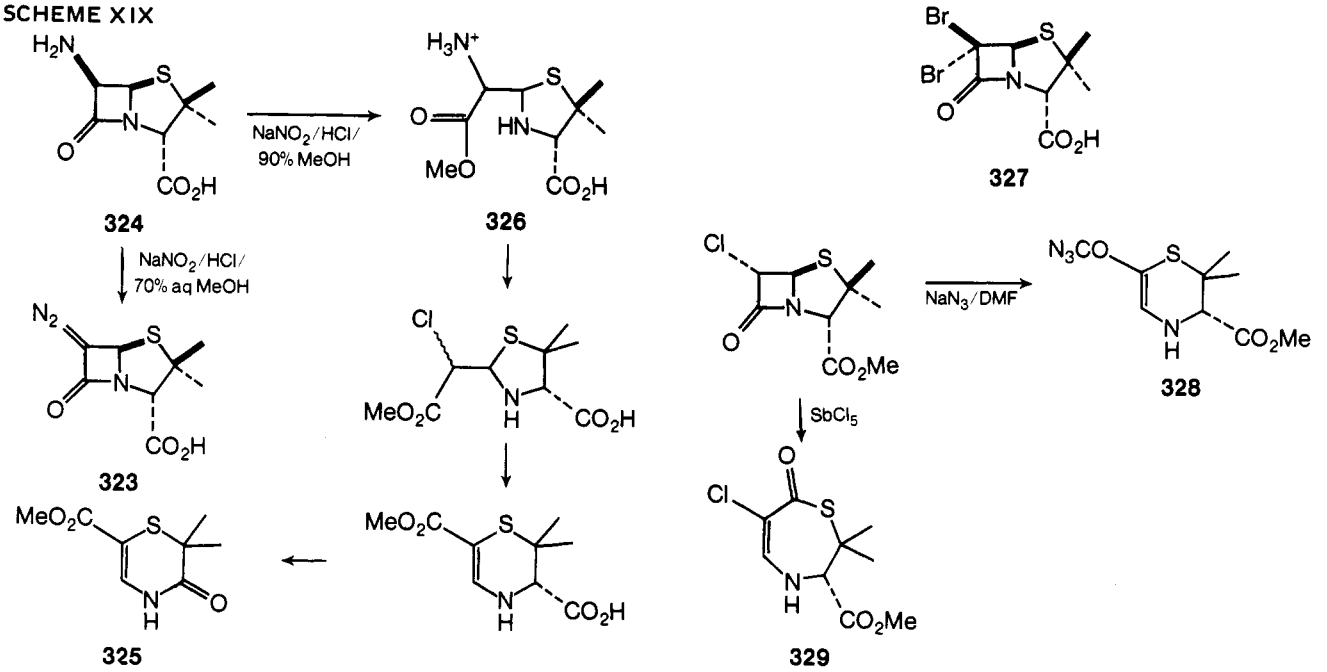
since with deuterium chloride both a deuterium atom and chlorine were introduced.[175] The free diazo compounds can be isolated in both the penam and cephem series using carefully controlled conditions, such as nitrosation of an amide group with dinitrogen tetroxide (cf. ref 173), to form the nitrosoamide, followed by reaction with pyridine.[176]

With methanol as solvent the amine **324** also gave the thiazine **325.** McMillan and Stoodley concluded that methanolysis of the β-lactam ring preceded the deamination since the same thiazine was produced by treatment of the ester **326;** a possible reaction path is given in Scheme XIX.[175] Treatment of the amino acid **324** with sodium nitrite in the presence of bromine gives the dibromide **327** and the corresponding diiodide has also been made.[32]

Initial attempts to displace the 6-halopenicillins with nucleophiles were unsuccessful.[173] With sodium azide in dimethylformamide, methyl 6α-chloropenicillanate only gave the dihydrothiazine **328** (cf. Scheme XIX).[175] Attempts to boost the reactivity of the halo group by using Lewis acids also failed; for example, antimony pentachloride caused 1,5 bond cleavage and formation of the thiazepine **329.**[177]

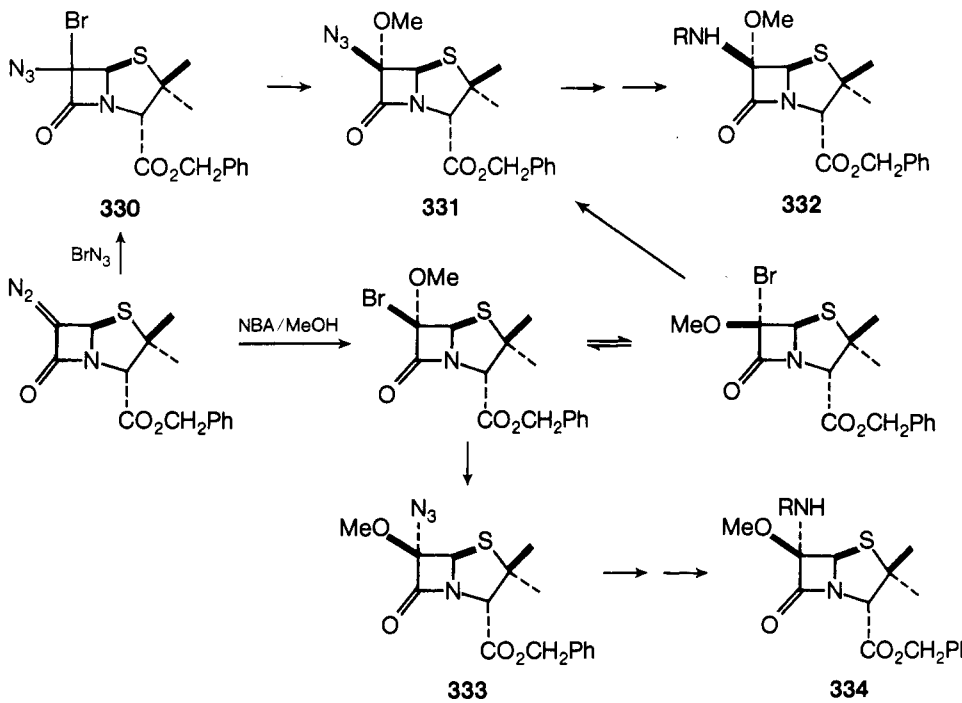
On considering these results Christensen and coworkers[171] realized that bromo azide would react with the diazo derivatives[173] in a similar manner to bromine and hence prepared the bromo azides **330** (Scheme XX). These reacted with silver fluoroborate in methanol to form the corresponding methoxy azides **331,** further chemical manipulation leading to the desired methoxy-substituted penams **332.** An alternative route involved initial reaction with *N*-bromoacetamide in

SCHEME XIX

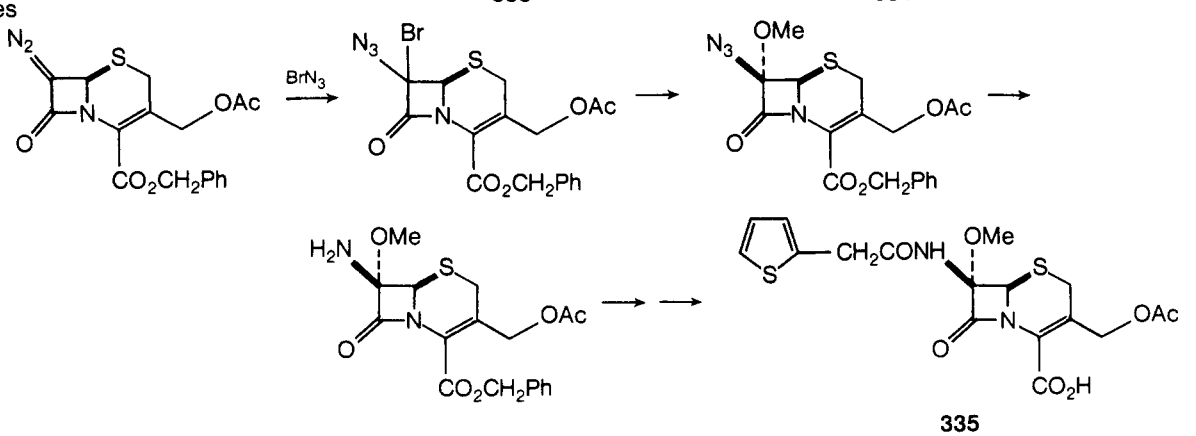


SCHEME XX

Penam series

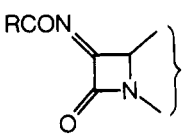


Cephem series



methanol followed by treatment with azide ion to give the isomers **331** and **333,** the latter eventually yielding the isomer **334.** Similar conversions were effected in the cephem series. The acid **335** showed enhanced antimicrobial properties.

This chemical entry into the methoxy-substituted β-lactam systems was immediately followed by alternative, more direct routes involving oxidation of the substituent acylamido group into the corresponding acylimine (e.g., **336**), known to undergo rapid nucleophilic addition reactions. For example, oxidation of the anhydropenicillin **337** with *tert*-butyl hypochlorite in methanol led, via the acylimine, to the methoxylated derivative **338,** the structure of which was confirmed by an x-ray crystallographic analysis. In the penam system the use of these conditions led to concurrent oxidation of the sulfide function so that this group had to be protected as the corresponding sulfoxide.[178]



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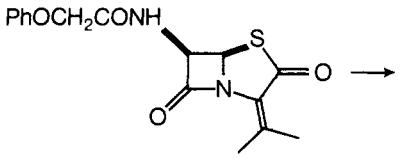

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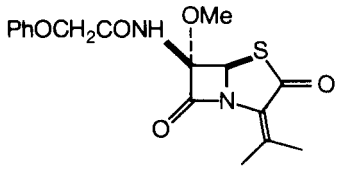

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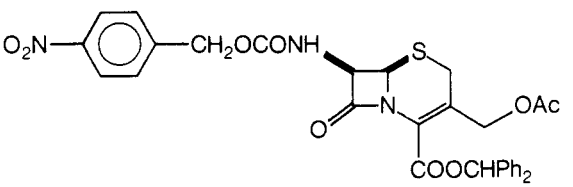
A method for overcoming the need to protect the sulfide function was independently published at the same time. Oxidation of the amide function to the acylimine intermediate is speeded up by initial formation of the amide anion, for example, use of lithium methoxide at low temperature (−60 to −80°) followed by addition of the *tert*-butyl hypochlorite oxidant. The acylimine so formed is immediately captured by the methanol and liberated from the base, to give the desired methoxylated intermediates.[179]

With the use of lithium *tert*-butoxide or phenyllithium as alternative bases, alternative nucleophiles can be added to the acylimine intermediates. In this way 6α-hydroxy-, benzyloxy-, and formyloxypenicillins could be made.[180]

Methoxylation has also been achieved in an indirect manner using arylidene derivatives of aminoazetidinones. Reaction of the Schiff's base with sodium hydride followed by reaction with methyl methylthiosulfonate forms the corresponding 6- (or 7-) methylthio derivatives. Hydrolysis of the imine produces the amino derivative (e.g., **339** to **341**), and treatment of this with mercuric chloride in methanol gave the amine **342,**[181] species that can also be obtained from the cephamycins,[129] while acylation gave the required product **343** (Scheme XXI). Acylation can also precede the exchange process, but in the penam derivatives **344** exchange is more difficult and needs to be activated by initial chlorination of the new sulfide group, to give the chlorosulfonium ion **345** which immediately collapses to the acylimine and, hence, in methanol, the adduct **343.**[182] In the cephem series, however, such vigorous activation was not necessary, and exchange could be effected by the action of mercuric salts in methanol on the intermediates of the type **344.**[183]

An alternative path to the important aminomethoxy derivatives has also been obtained from a protected 7-aminocephalosporin. Thus the *p*-nitrobenzyl carbamate **346** could be methoxylated by the lithium methoxide/*tert*-butyl hypochlorite process and then hydrogenolyzed to give the amine **347.**[184]



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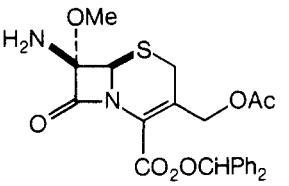

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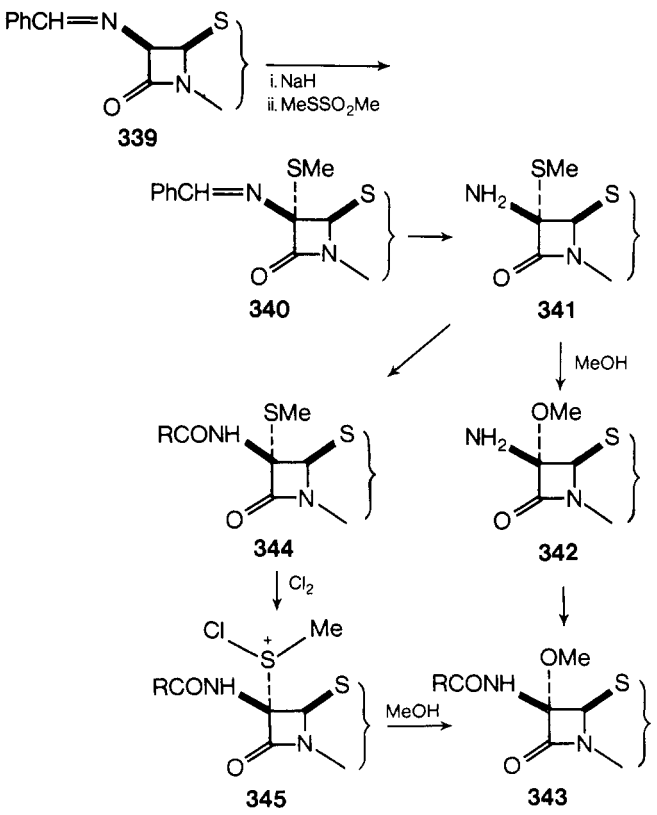
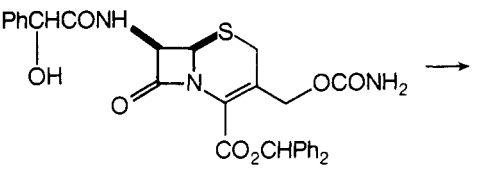
SCHEME XXI



An interesting reaction involving oxidation of the D-mandeloamidocephem (**348**) with *tert*-butyl hypochlorite in methanol afforded, not the methoxy derivative but, instead, the intramolecularly trapped spiro derivative **349,** the free acid of which showed considerable biological activity.[185]



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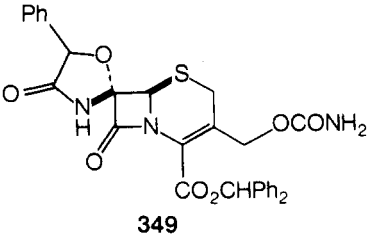

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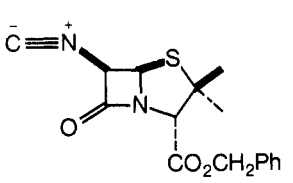
As an alternative to either the diazo or arylideneamino functions for the activation of the 6 position in penicillins the isonitrile group may be used. This is easily prepared by dehydration of the formyl derivative using phosgene and a tertiary base. Dehydration gives an epimeric mixture of the isonitriles (e.g., **350**), both of which can either be alkylated or thiomethylated under mild conditions. The major product from both epimers is the 6β-amine. The 6α-methylthio derivative **351** can be converted into the amine by treatment with *p*-toluenesulfonic acid and the thiomethyl group exchanged with methoxyl groups as described above (Scheme XXI).[186]



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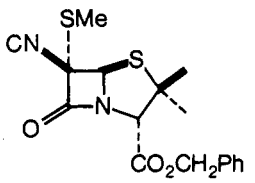

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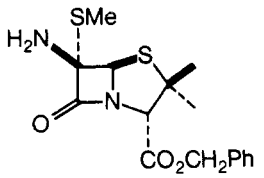
During reactions aimed at the exchange of the 6α-methylthio group of derivatives of the type **352** under the influence of mercuric chloride, it was found that in aqueous dimethylformamide the 6-oxopenam **353** formed. This compound had also been prepared by Sheehan and Lo by oxidation of the known[173] 6α alcohol with carbodiimides and dimethyl sulfoxide.[187]



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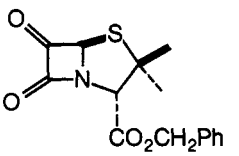

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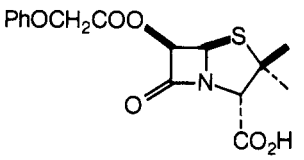

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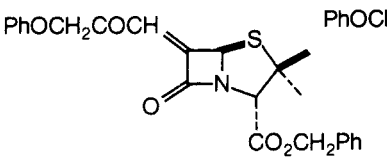
The ketone **353** has proven to be quite versatile. Reduction, followed by acylation, gave, after cleavage of the acid protecting group, 6β-phenoxyacetoxypenicillanic acid (**354**). The 6α analogue has also been prepared from the previously known 6α-acetoxy derivative, using an enzymic hydrolysis to liberate the 6α alcohol prior to reacylation.[188]

The ketone **353** can also be made to react with Wittig reagents, such as phenoxyacetylmethylenetriphenylphosphorane.[189] Reduction of the resulting olefin **355** gave a mixture of the cis (**356**) and trans reduction products, the former predominating. The cis product was converted into the corresponding acid **357**, which, rather surprisingly, showed considerable antimicrobial activity and which was also resistant to the β-lactamase from *B. cereus*.



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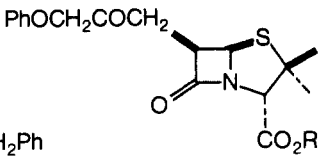


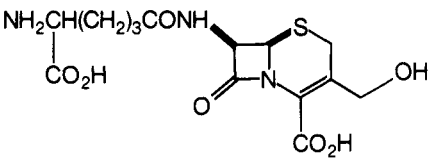
356, R = CH_2Ph
357, R = H

G. Reactions of the Dihydrothiazine Ring

1. *Modifications of the 10-Substituent*

a. Hydrolysis

Early work on isolating cephalosporin C from culture filtrates showed that it was often accompanied by considerable amounts of a more polar, but less effective, antibiotic.[190] Subsequent work showed that this was the deacetyl derivative **358**. Although this was not readily isolated from the cul-



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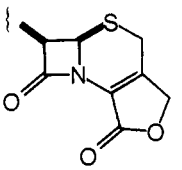
ture filtrates, it could be formed quite easily by enzymic hydrolysis of cephalosporin C itself, for example, with citrus acetylesterase.[191] A similar deacetylation accounts for much of the 10-acetoxycephems in animals. Large amounts of the alcohol have been obtained in this manner, and it has been converted into a wide range of carboxylic and related acid esters. Mild alkaline hydrolysis of the system is not a viable process since undesirable hydrolytic reactions predominate.[191] With acid, hydrolysis is effected but, instead of the hydroxy acid, ceph-3-ems afford large quantities of the corresponding lactone **359**.[192-194] Compared to the open forms, the lactones are often fairly insoluble in aqueous and organic solvents. They generally show considerably diminished antibiotic



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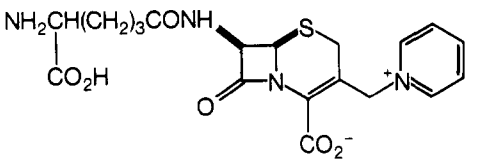
activity. Nevertheless, because of their stability, relative to the open forms, they have attracted attention in synthetic schemes.[195] Initial attempts to reopen the lactone ring met with considerable difficulties, and it is only recently, using carefully controlled conditions, that this has been achieved. The lactone is often an undesirable product from acylation, for example, in attempts to functionalize the 7-amino group.

A method for overcoming the lactonization is to use, instead, the ceph-2-em isomers, which do not readily lactonize.[196] Subsequent isomerization of the double bond back into the 3 position can be effected by mild oxidation to the corresponding sulfoxide, which occurs with concomitant migration of the double bond, and then reduction to the sulfide. It has also been found that 3-hydroxymethylceph-3-em 1-oxides show a decreased tendency to lactonize, compared to the corresponding sulfides.

b. Displacement of the 10-Substituent

During the course of cephalosporin C isolation, pyridine–acetic acid buffers were initially used. It was soon found that the buffer also reacted with the antibiotic to form a new derivative which was a more effective substance.[197] This betaine (**360**) was produced by displacement of the acetoxy group by pyridine. This reaction is general, and a wide range of derivatives have been produced with tertiary amines. Other nucleophiles also displace the acetoxy group, including the thiosulfate ion, which produces the Bunte salt **361**.[198] In general,



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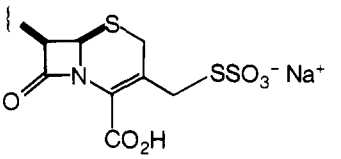

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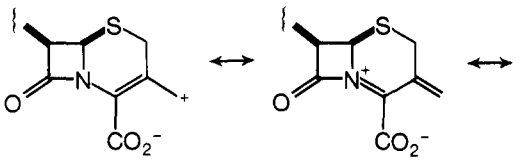
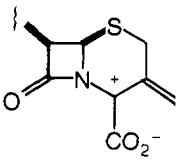


362 363



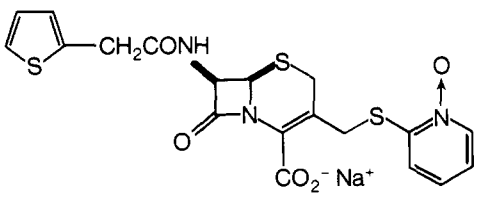
364

any soft nucleophile reacts,[199] including nitrogen heterocycles, such as pyridines,[197,200] thiols,[201] xanthates,[202] and anilines.[203] The reaction only occurs with the free 4-carboxylate salts and not with esters of this function.[204] Sulfoxide acids are not used in the displacement process since, in these cases, decarboxylation competes with the substitution. Resonance forms of the types **362** to **364** are indicated, the reaction proceeding according to first-order kinetics.

Azide ion can also be used as a nucleophile, the resulting dipolar species being then employed in either cycloaddition reactions or, after reduction, production of the 10-amino group, which can then be acylated.[201]

Some carbon nucleophiles have also been employed, including enamines of the indole and pyrrole type, and enols such as resorcinol.[201]

By prior conversion of the 10-hydroxy function into the corresponding chloro, bromo, or iodo compound, an even wider range of derivatives has been made, and thus direct substitution at this position raises no insuperable difficulties.[199] The introduction of the harder oxygen nucleophile, however, can lead to side reactions, but a novel, indirect process for oxygen substitution has been developed.[205] Substitution of the 10-acetoxy group with 2-mercaptopyridine *N*-oxide gives the sulfide (e.g., **365**). In the presence of copper(II) salts facile



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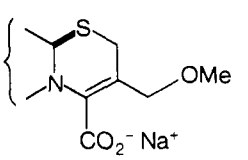

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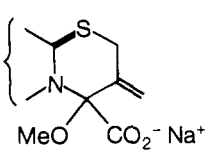
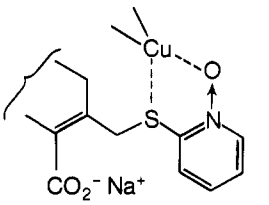


367

displacement of this function occurs, methanol, for example, producing the 10-methoxy derivative **366** together with some of the isomer **367**. The latter could be rearranged into the more stable isomer **366** with methanol and acid. The copper is presumed to activate the pyridine derivative by complex formation of the type **368.** Other alcohols, phenols, and anilines have also been introduced in this manner.

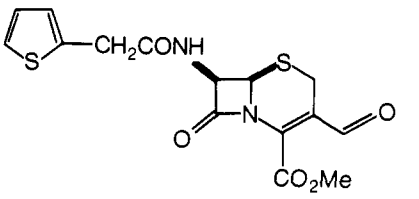


368

Ceph-2-em isomers also undergo nucleophilic displacement of 10-acetoxy groups, but less readily than for the ceph-3-em systems.[206]

c. Oxidation and Reduction

Although oxidation of the 10-hydroxycephems into the corresponding aldehyde (e.g., **369**) has been reported,[207] other work on this aspect of cephem chemistry has not been fully described. The ester **370** has been prepared during a total synthesis of cephalosporins.[6] The bromination of the ceph-2-em ester **371** with *N*-bromosuccinimide, under free radical conditions, gave the unstable 10-bromide **372** which rapidly afforded the aldehyde **373.**[208] In the ceph-3-em series, bromination occurs mainly at position 2.



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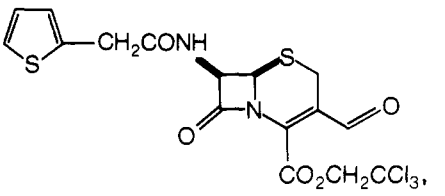

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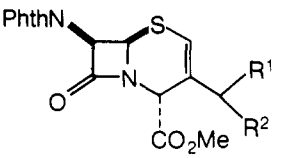
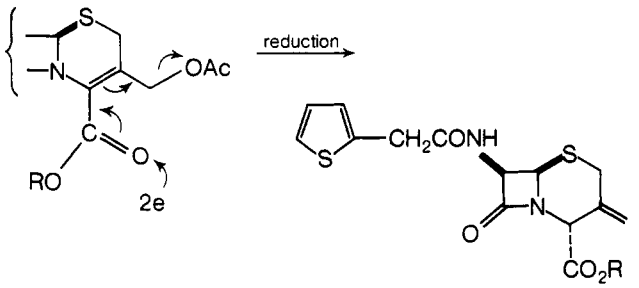


371, R^1 = H; R^2 = OAc
372, R^1 = Br; R^2 = OAc
373, R^1, R^2 = O

In contrast to oxidation, several studies on the reduction of the 10-substituted systems into the deacetoxy series have been made. Formerly hydrogenolysis was the preferred method,[24,209] but recently electrochemical reduction, using a mercury cathode at pH 6.9, has succeeded, producing the 3-*exo*-methylene system **374.**[210,211] In this way both cephalosporin C and 7-aminocephalosporanic acid have been reduced. Isomerization to the more familiar ceph-3-ems is catalyzed by pyridine and trimethylsilyl chloride.

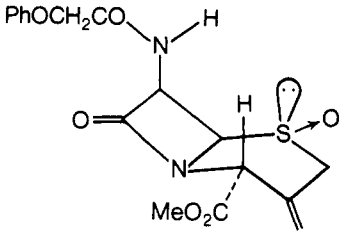
Metal reductants, such as chromium(II) salts, are also effective.[212] Yet another route to the exocyclic olefin involves



374

initial transformation of the 10-acetoxycephems with sulfur nucleophiles. Selective desulfurization of these derivatives with Raney nickel then affords the exocyclic olefin as major product; a combined zinc dust–formic acid–dimethylformamide brew also effects the latter transformation. Similar reductions on the acetoxy derivatives only give small quantities of the exomethylene isomer.[213]

Determination of the stereochemistry about position 4 of these 3(10)-olefins showed that the carboxylate group had the α configuration, as for the ceph-2-ems.[214-216] Furthermore, by preparing the two sulfoxides and using the known anisotropic effects of the sulfoxide group in NMR experiments, the preferred conformation **375** was deduced.[215] The α-sulfoxide **376** was prepared by use of *N,N*-dichlorourethane in aqueous tetrahydrofuran as oxidant. Whereas the α-sulfoxide showed a large shift of the amide proton in going from nonpolar to polar solvents, the corresponding proton of the β-sulfoxide **377** remained almost unaffected, an observation consistent with previous assignments of configuration among the cephalosporin sulfoxides.[217] Use of lanthanide shift reagents allowed the deduction of a similar conformation for the sulfide.[216]



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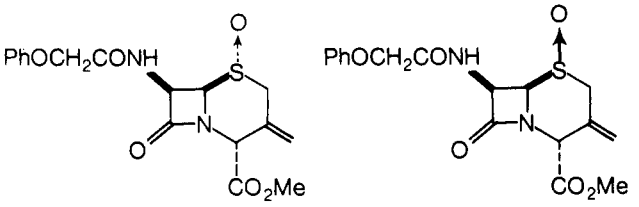
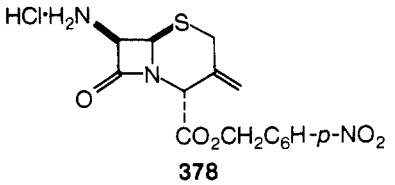


376 377

The 3-methylenecephems available by these methods have their own particular chemistry. They can be ozonized at low temperature to produce the corresponding ketone, which exists mainly as the corresponding enol (e.g., **378** to **379**). For the amine **379**, selective acylation of the side chain could be effected. The enol function can be alkylated, e.g., with diazomethane to give the methyl ether **380** or converted into



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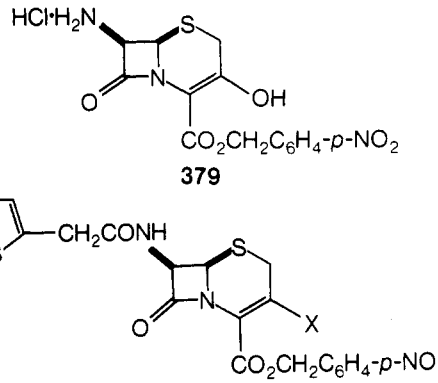


379

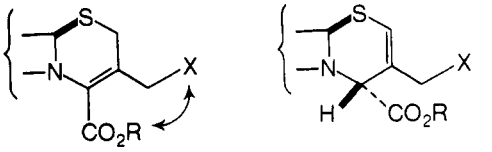
380, X = OMe
381, X = Cl

halo derivatives, such as the chloride **381**, both derived acids having considerable biological activity.[218]

2. *Reactions of the Double Bond*

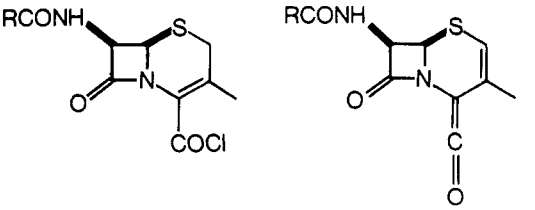
a. Isomerization

The ceph-3-ems can readily equilibrate under basic conditions with the ceph-2-em isomers.[206,219,220] The double bond migration is facilitated by electronegative substituents at position 4, such as the esters and in mixed anhydrides.[220] The free acids can also equilibrate but do so at a much slower rate. The 2-em isomer is preferred over the more normal system when the 10 position bears bulky substituents; in the unsubstituted series, for example, the 3-ene is preferred, whereas for the 10-acetoxy compound the 2-ene isomer predominates at equilibrium. Release of steric interference between the cis substituents of the ceph-3-em system can be invoked to explain these results (cf. **382** and **383**), a 4α-configuration forming.

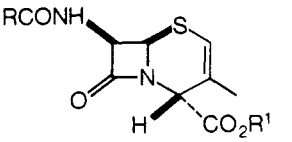


382 383

A method for the preparation of pure ceph-2-em compounds has been invented. Treatment of the acid chloride **384** with a strong tertiary amine base gives the ketene **385**, which is quenched by alcohols to form the pure ceph-2-em esters **386**.[221]



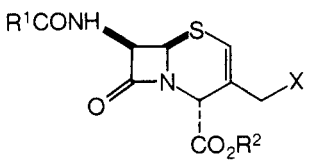
384 385



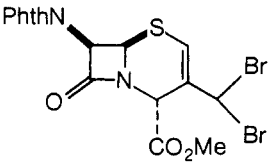
386

Preparation of the (*S*)-sulfoxide again introduces a new stereoelectronic constraint,[340] and the system overwhelmingly prefers the 3-ene structure.[222] This effect has been used to

advantage in several synthetic reactions using the ceph-2-em systems as intermediates. Thus, whereas allylic bromination of the deacetoxyceph-3-em derivatives proceeds only with great difficulty, high yields of the 3-bromomethyl compounds **387** are obtained from esters of the 2-ene isomers with *N*-bromosuccinimide,[223] a method superior to the alternative route involving photoinduced bromination of the ceph-3-em isomers.[199] After displacement of the bromine by other groups, including sulfur and nitrogen,[224] oxygen, and even carbon (cyanide ion) nucleophiles,[225] a simple oxidation–reduction sequence regenerates the biologically active ceph-3-em series.[224] Use of an excess of *N*-bromosuccinimide can lead to the dibrominated derivative as for the compound **388.** Subsequent hydrolysis forms the aldehyde.[209]



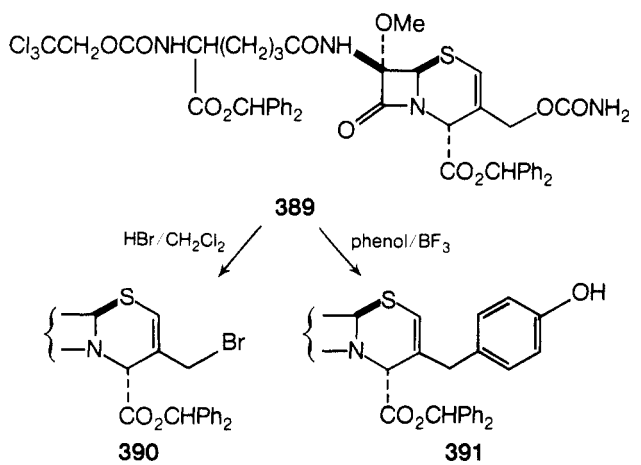
387, X = Br



388

As referred to above, whereas 10-hydroxycephalosporins undergo lactone formation with ease, the corresponding alcohols of the 2-ene series do not.[196] This allows ready acylation of the alcohol group, which can also be introduced by hydrolysis of the corresponding brominated material. Nucleophilic displacement of the acetoxy group in the ceph-2-ems has also been reported,[206] but these are less efficient than for the 3-ene-4-carboxylic acids.

Of interest is the observation that 10-halo-ceph-2-ems (e.g., **390**) can be obtained by treating the cephamycin isomers (e.g., **389**) with hydrogen halides in nonpolar aprotic solvents.[226] Similar displacements can also be catalyzed by boron trifluoride. In the presence of enols or phenols substitution occurs (e.g., **389** to **391**).[227]



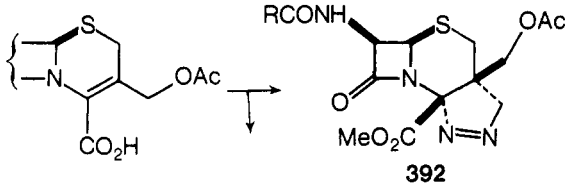
389

390 **391**

b. Additions

The 1,3-dipolar addition of diazomethane to ceph-3-ems has been reported,[228,229] but reaction is slow. The 1-pyrazoline isomers (**392**) form in dichloromethane, and these can be isomerized into the 2-pyrazoline adducts **393** by subsequent chromatography; reaction in dimethylformamide produces the 2-pyrazolines directly. So far no other examples of dipolar addition across this olefinic bond, which is highly substituted, have been observed.

A novel type of addition reaction was encountered during displacement reactions of the acetate group of cephalosporanic acids with ambident nucleophiles.[230] Reaction of the salt **394** with *N,N*-diethylthiourea gave the product **395,** in which the characteristic ultraviolet absorption associated with



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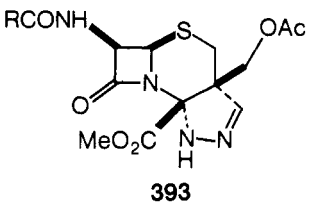

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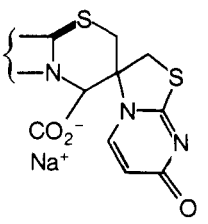
the ceph-3-em chromophore (λ_{max} 270 nm) had disappeared. Other nucleophiles, such as pyridinethione and 2-thiouracil gave the corresponding adducts **396** and **397.** The former de-



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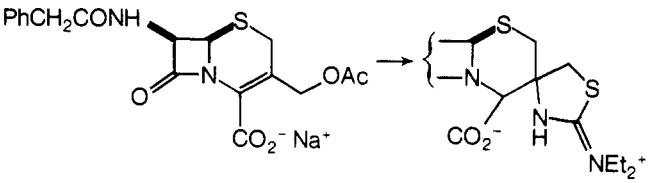
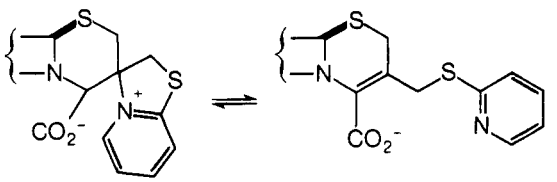
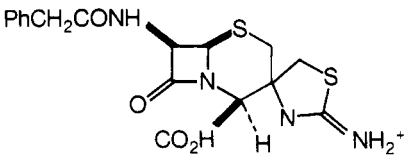


394 **395**



396 **398**

rivative (**396**) was in equilibrium with the open form (**398**), base favoring the latter isomer and acid the closed form. Confirmation of the structural assignment, for the *N*-ethylthiourea adduct (**399**) has been obtained by an x-ray crystallographic analysis.[231]



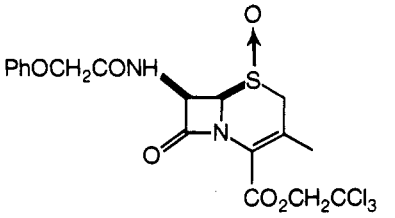
399

Catalytic reduction of the double bond has also been attempted, and claimed, but proceeds with difficulty and competing desulfurization.[199] Reduction of the ceph-2-em chromophore can be achieved, but it gives a mixture of reduced products.[232]

3. Reactions at Position 2

The presence of the adjacent sulfur and double bonds about position 2 tends to make the methylene group acidic,

enabling it to accommodate negative charges. This tendency is enhanced in the corresponding sulfoxides. Reactions involving activation of carbon atoms adjacent to sulfide functions are manifold and many have been applied to the cephalosporins. A classical case is the Pummerer rearrangement on the sulfoxide **400** with acetic anhydride to give the 2-acetoxy derivative **401.**[16] This reaction can also be achieved directly by treating the corresponding sulfide with lead tetraacetate.[217]



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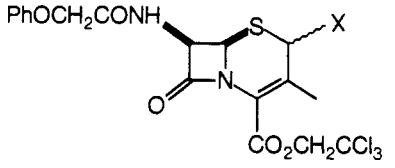
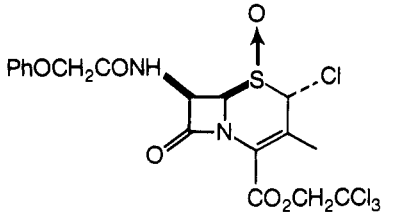
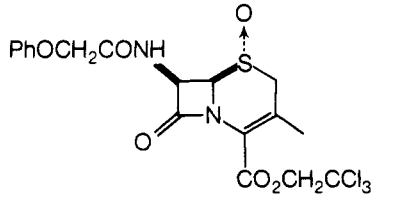


401, X = OAc
403, X = Cl
404, X = OEt



402



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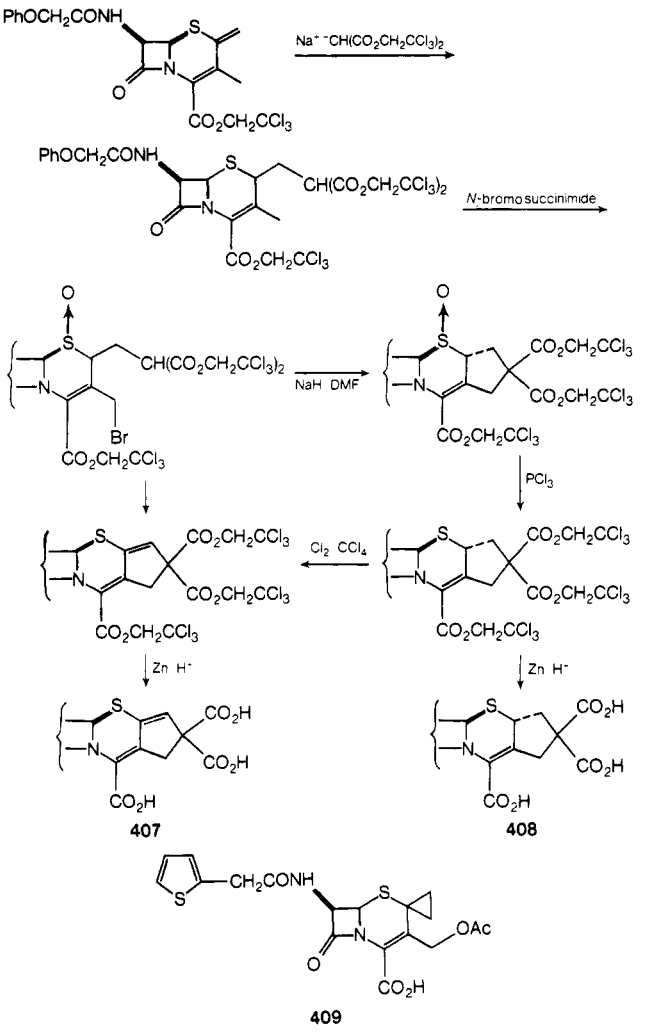
Use of α-chlorinating agents, such as sulfuryl chloride or chlorine in pyridine, on the sulfoxide **400** gives the chlorinated product **402,** easily reduced to the corresponding sulfide.

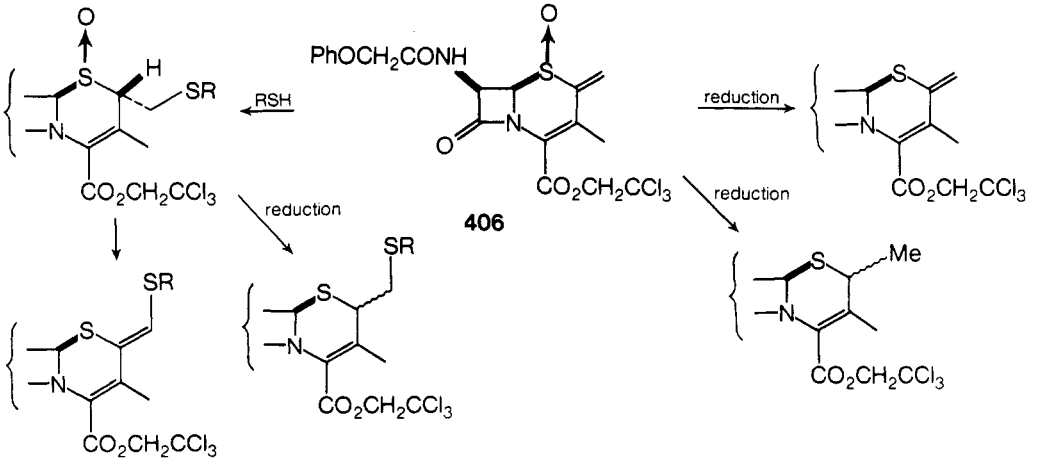
SCHEME XXIII



Chlorination of the sulfide, however, formed the highly reactive α-chloro sulfide **403,** which was immediately converted in situ into a series of 2-ethoxy-substituted compounds **404,** assigned the α configuration by NMR studies.[233] When a hydrogen carbonate buffer was used in the reaction, the product of chlorination was mainly the α-sulfoxide **405.**[233]

Mannich reactions on the sulfoxides, using formaldehyde, generates the 2-methylene derivatives (e.g., **406**).[234] These can either be reduced, to the corresponding isomeric methylated products, or be utilized in subsequent Michael addition reactions, e.g., with thiols (Scheme XXII). Although such de-

SCHEME XXII



rivatives do possess antibacterial properties, they do not appear to be superior to the unsubstituted systems.[235]

Spry has used the 2-substituted cephems to generate the novel tricyclic cephalosporins **407** and **408**,[236] as well as the spiro derivatives **409** (Scheme XXIII).[237]

III. Total Syntheses

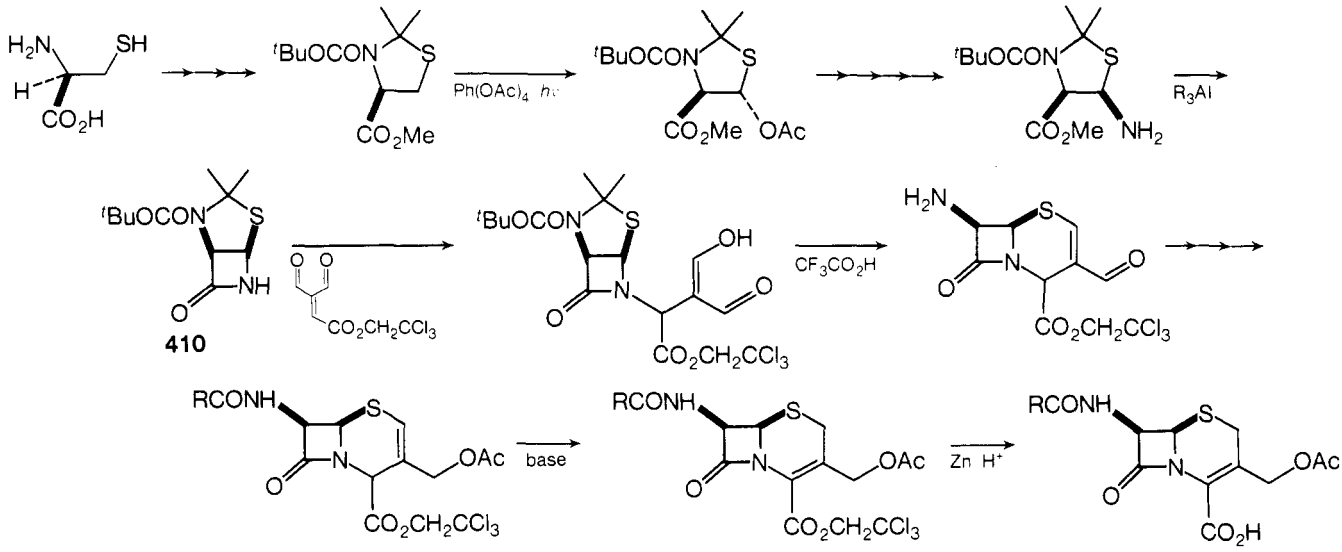
A. Introduction

Current objectives in designing total synthetic routes to the β-lactam antibiotics do not include competition with the relatively efficient microbiological methods but rather the attainment of profound skeletal variations in order to recognize the influence of structure on activity relationships. At the time of writing, a host of synthetic schemes are under scrutiny, and any sweeping deductions on the latter point have, as yet, only a hazy form. This section will therefore only emphasize recent developments in total syntheses. The classical work of Sheehan and his collaborators will not be discussed. Furthermore, the synthesis of azetidinones has been reviewed elsewhere, and for this reason will not be included here.[238] The logistics behind the preparation of β-lactam systems have been discussed by Heusler.[239]

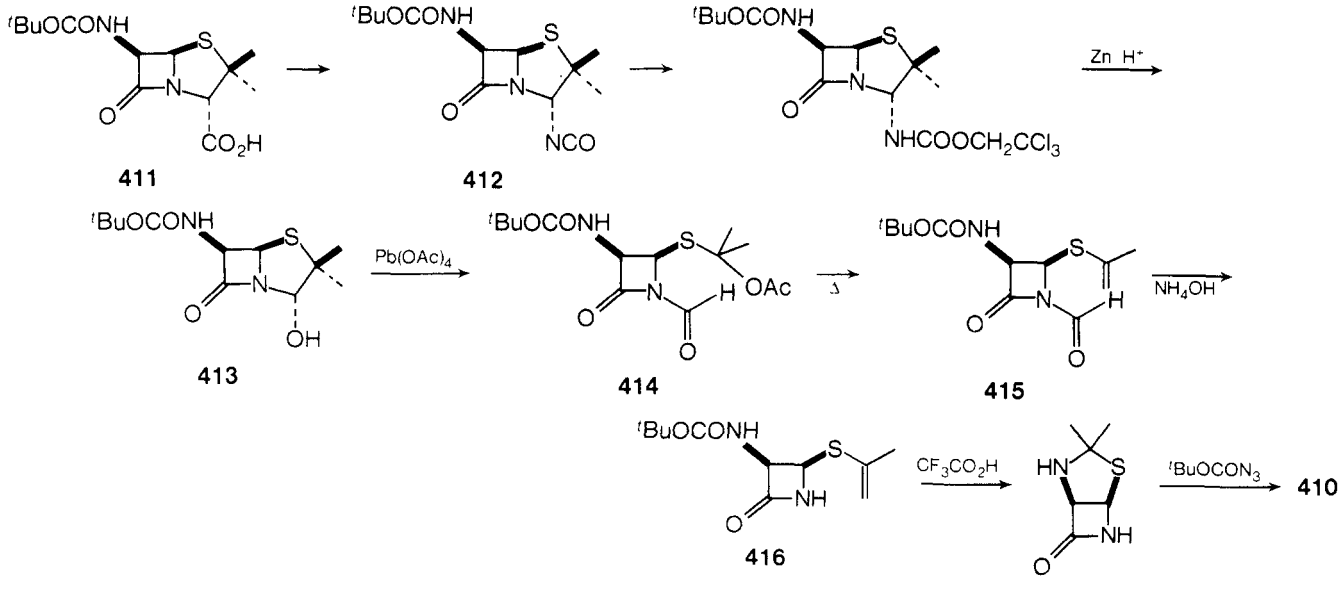
B. Woodward Approach

Woodward's general method is outlined in Scheme XXIV. Originally the method started with L-cysteine, and the route incorporated many original features.[6] One of the points that became evident as the work unfolded was the preparation of several compounds which can be described as key intermediates. One of the most important of these was the fused thiazolidine **410**, since it was subsequently prepared more directly from the naturally formed 6-aminopenicillanic acid.[239] Curtius degradation of the acid **411** gave the isocyanate **412** (Scheme XXV) which was smoothly converted into the alcohol **413**. A two-step procedure (as illustrated) was found to be more efficient than direct hydrolysis. Oxidation of the carbinolamine **413** with lead tetraacetate in benzene, upon photoly-

SCHEME XXIV

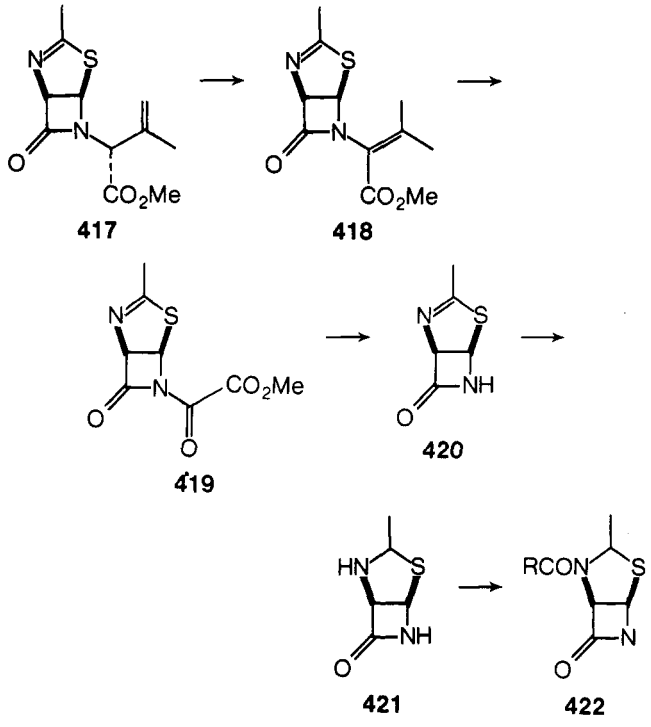


SCHEME XXV



sis, gave the acetate **414,** which smoothly eliminated acetic acid at 50–80° to give the thiovinyl ether **415.** Selective hydrolysis of the formyl derivative with dilute aqueous ammonia afforded the free β-lactam compound **416.** Treatment of **416** with trifluoroacetic acid followed by reacylation gave the thiazolidine **410.**

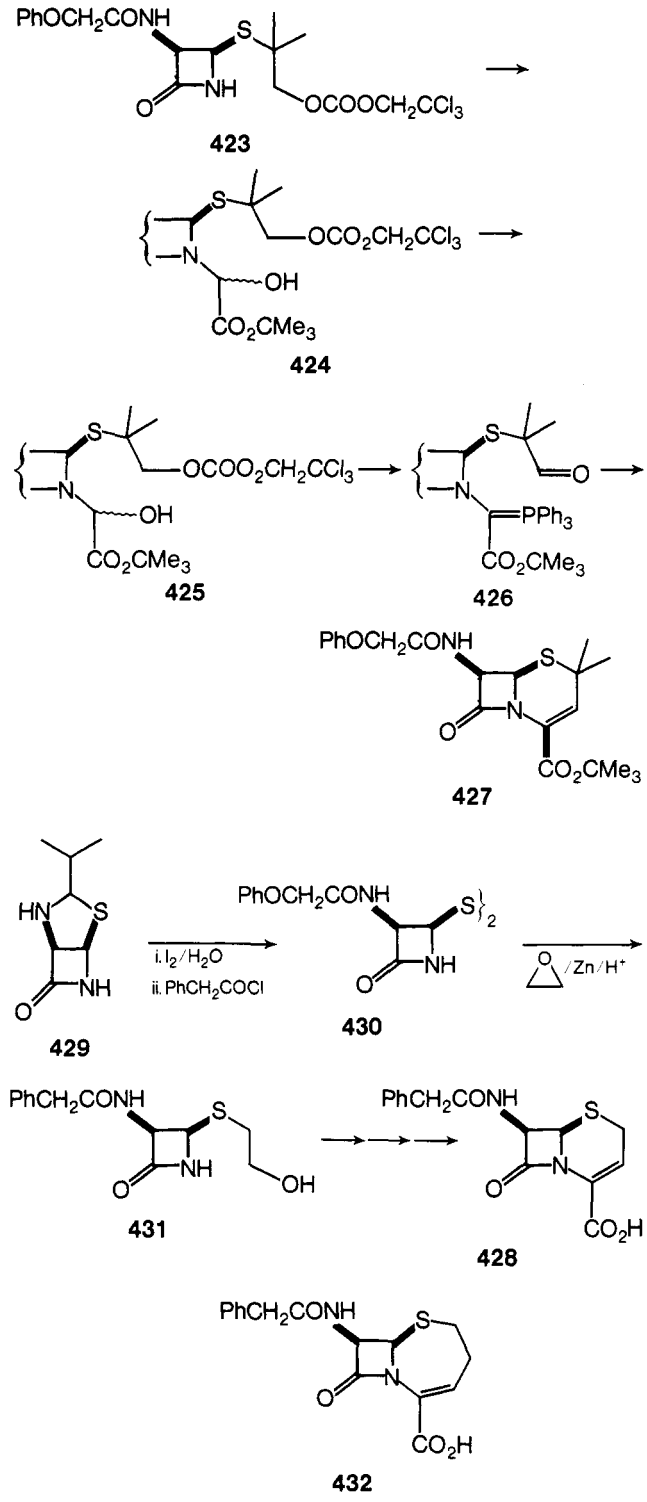
An even more simple route to the thiazolidines (e.g., **410**) is available from the thiazolines (e.g., **417**) obtained from the trapping of penicillin sulfoxides with trimethyl phosphite. The derivative **418,** obtained by conjugation of the double bond with base, can be ozonized to give the oxamide **419,** which can then be hydrolyzed to give the thiazoline **420.**[116] Reduction with aluminum amalgam produces the thiazolidine **421.** Reduction and acylation prior to ozonolysis affords the amide derivative **422.**



A method for annelating new rings to the β-lactam group which is more general than that outlined in Scheme XXIV has also been invented.[240] This capitalizes on the observation that glyoxylic esters add to the β-lactam nitrogen to give an epimeric mixture of the hydroxyamides (e.g., **423** to **424**). Subsequent reaction with thionyl chloride produces the chlorides **425** which can both be converted into the corresponding phosphorane **426.** Intramolecular cyclization, for example, with the aldehyde **426,** leads to the 2,2-dimethylcephem **427.** The generality of this scheme is further illustrated by the synthesis of the related system **428.** Treatment of the disulfide **430,** itself obtained by oxidation of the thiazolidine **429** with iodine followed by acylation, with ethylene oxide and zinc dust in dilute acetic acid gave the alcohol **431,** which was subjected to the latter scheme to produce the cephem **428.**[241] The homocephem **432** was made in a similar manner but was reported to be inactive.[242]

Heteroatom analogues of the ceph-3-em system have also been made, in particular the disulfides **433** and **434.** The key step in the synthesis of the latter compound (Scheme XXVI) was the photochemical Norrish type II cleavage of the phenacyl residue to give the thione **435.** The intermediate **436** was again made by the phosphorane route.

A further variant utilizing the phosphorane route produced compounds of the type **437,** where R represents a variety of functional groups, including substituted aromatic derivatives.

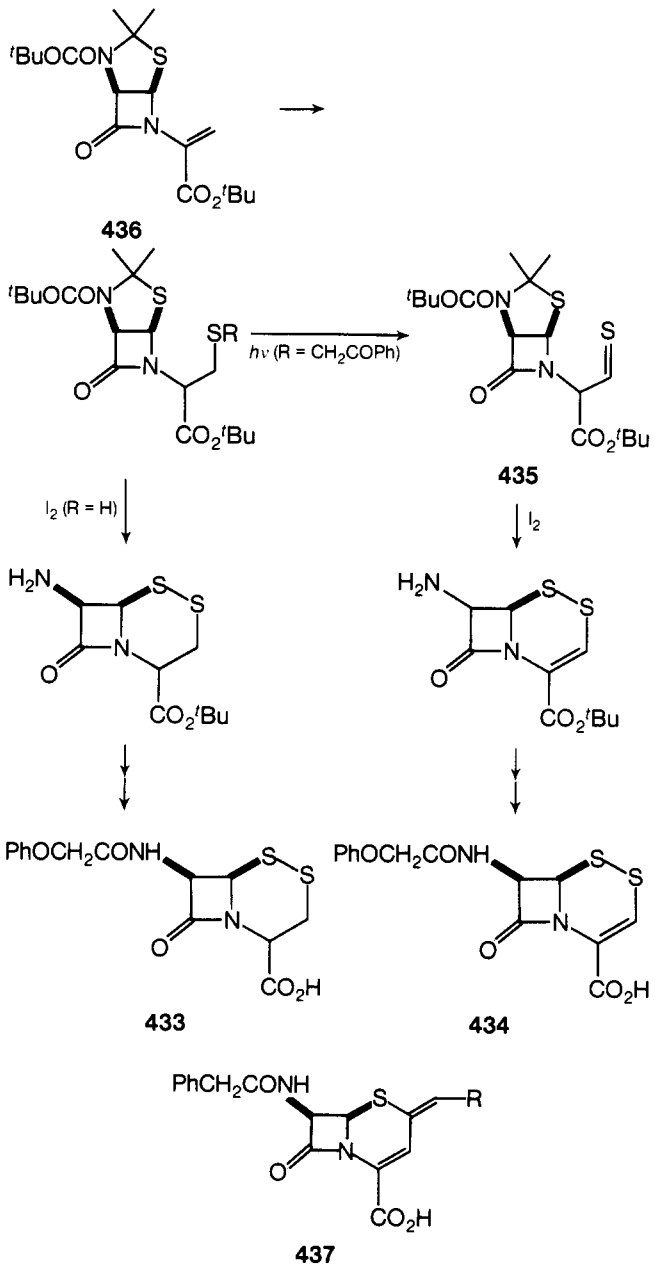


No clear-cut structure–activity pattern was observed although such compounds did exhibit considerable microbiological activity.[243]

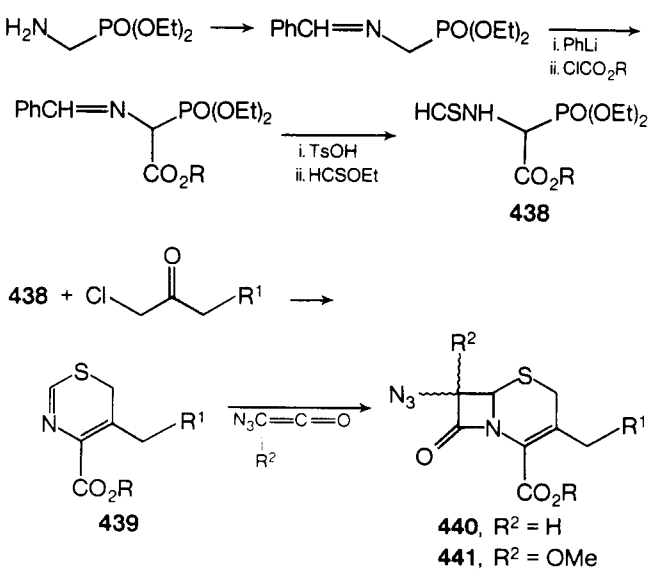
C. Merck and Syntex Approach

These methods are based on the well-known cycloaddition reaction of ketenimine precursors with imines,[244] a procedure originally adapted to the preparation of penicillin analogues by Bose and coworkers.[245] A relatively straightforward entry into the cephem and 7α-methoxycephem structures has been achieved by the Merck group. The critical starting material in their work was the diethyl α-thioformamidophosphonoacetate (**438**) (Scheme XXVII).[246] Condensation with

SCHEME XXVI

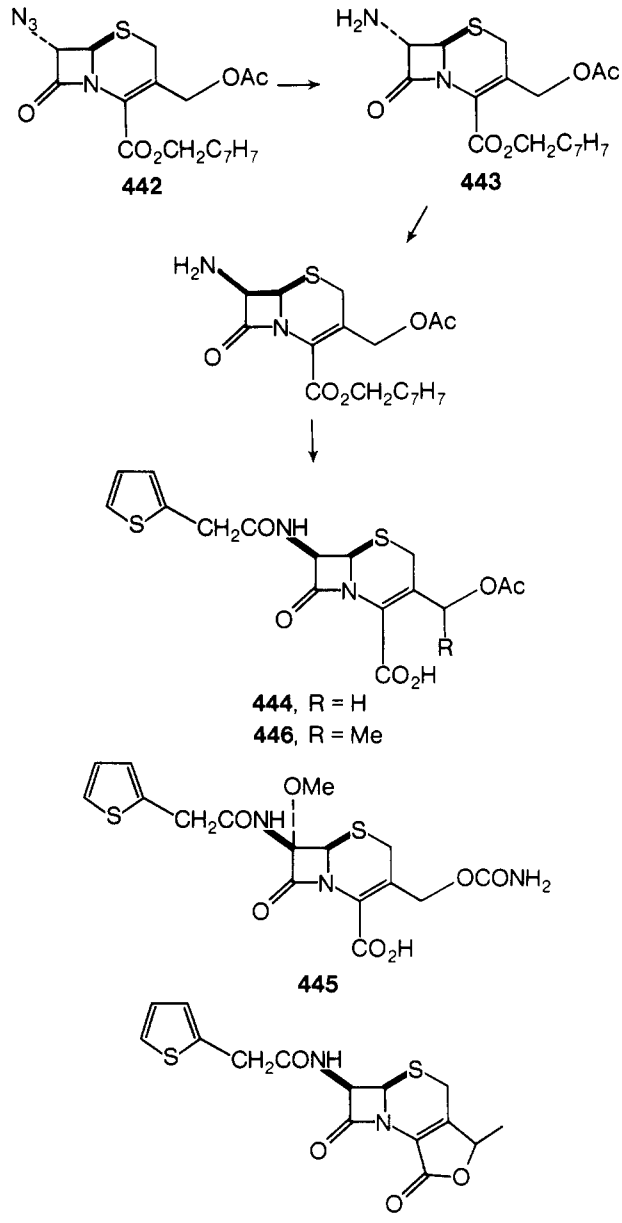


SCHEME XXVII



1-chloro-2-propanones produces the 6*H*-1,3-thiazine-4-carboxylates (**439**).[247] These react smoothly with azidoacetyl chloride and 1 equiv of triethylamine to give a stereoisomeric mixture of the 7-azidocephems, of general structure **440.** Use of methoxyazidoacetyl chloride gave the analogous methoxy-substituted system **441.**

The cycloaddition reaction proceeds through ketene intermediates. In the particular case of the azido adduct **442** hydrogenation gave the racemic 7α-amino compound **443** which was epimerized by formation of the Schiff's base with *p*-nitrobenzaldehyde and treatment with phenyllithium in tetrahydrofuran at −78°.[248] Kinetically controlled quenching of the

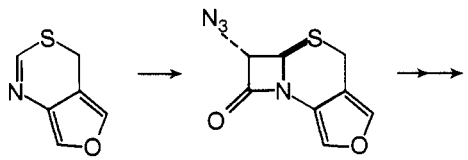


free anion, ensured by prior addition of dimethylformamide, with aqueous acetic acid gave a 55:45 ratio of the 7β:7α epimers.[248] Regeneration of the amine, by exchange of the imine with 2,4-dinitrophenylhydrazine and *p*-toluenesulfonic acid in ethanol, followed by isolation of the cis-substituted isomer and acylation afforded authentic, but racemic, cephalothin (**444**) of exactly half the antimicrobial activity of the naturally derived material. Cephoxitin (**445**) has been similarly prepared.[249]

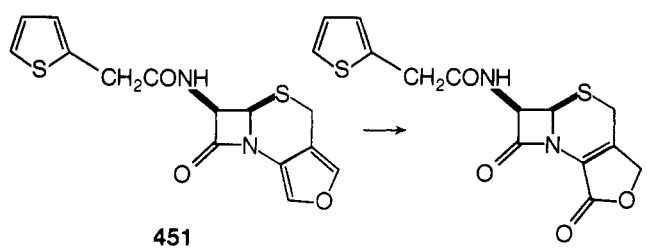
The generality of this route is exemplified by the preparation of the 10-methylcephalothin (**446**). It was expected that this would be more stable than the normal antibiotic. It was

found, however, that this material rapidly cyclized into the corresponding lactone **447.**[250]

Workers at the Syntex laboratories have independently produced a similar route to the lactone **448.**[251] A straightforward synthesis of the furanothiazine (**449**) was followed by addition of the ketene from azidoacetyl chloride to give the racemic adduct **450.** By using a similar procedure to that described above, the cephem **451** was made. Bromination in acetic acid and subsequent treatment with acid gave the lactone **448.**



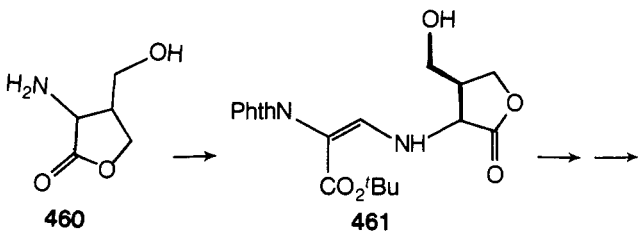
449 **450**



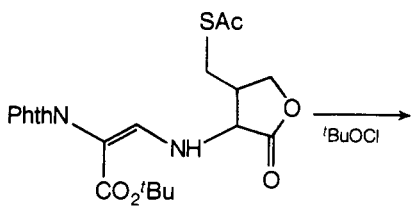
451 **448**

D. Roussel–Squibb Route

Both of these preparative routes were mainly studied in the 1960's and show similarities. The starting material was the condensation product of pyruvic acid, formaldehyde, and dimethylamine, viz. **452,** first described by Mannich in 1924.[252] Displacement with either thioacetic acid (Roussel)[253] or triphenylmethylmercaptide (Squibb)[254] introduced the sulfur atom to give **453** or **454,** respectively. Acidic hydrolysis of the acetate group and condensation with the phthalimido-protected malonyl derivative **455** gave the condensate **456** as a mixture of epimers (Scheme XXVIII). Fortunately, treatment of the mixture with acid equilibrated the mixture to give, mainly,



460 **461**



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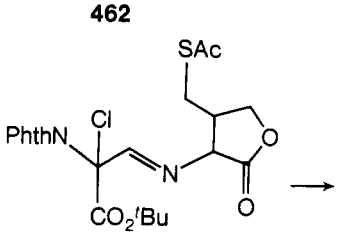

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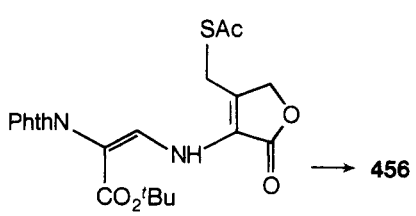
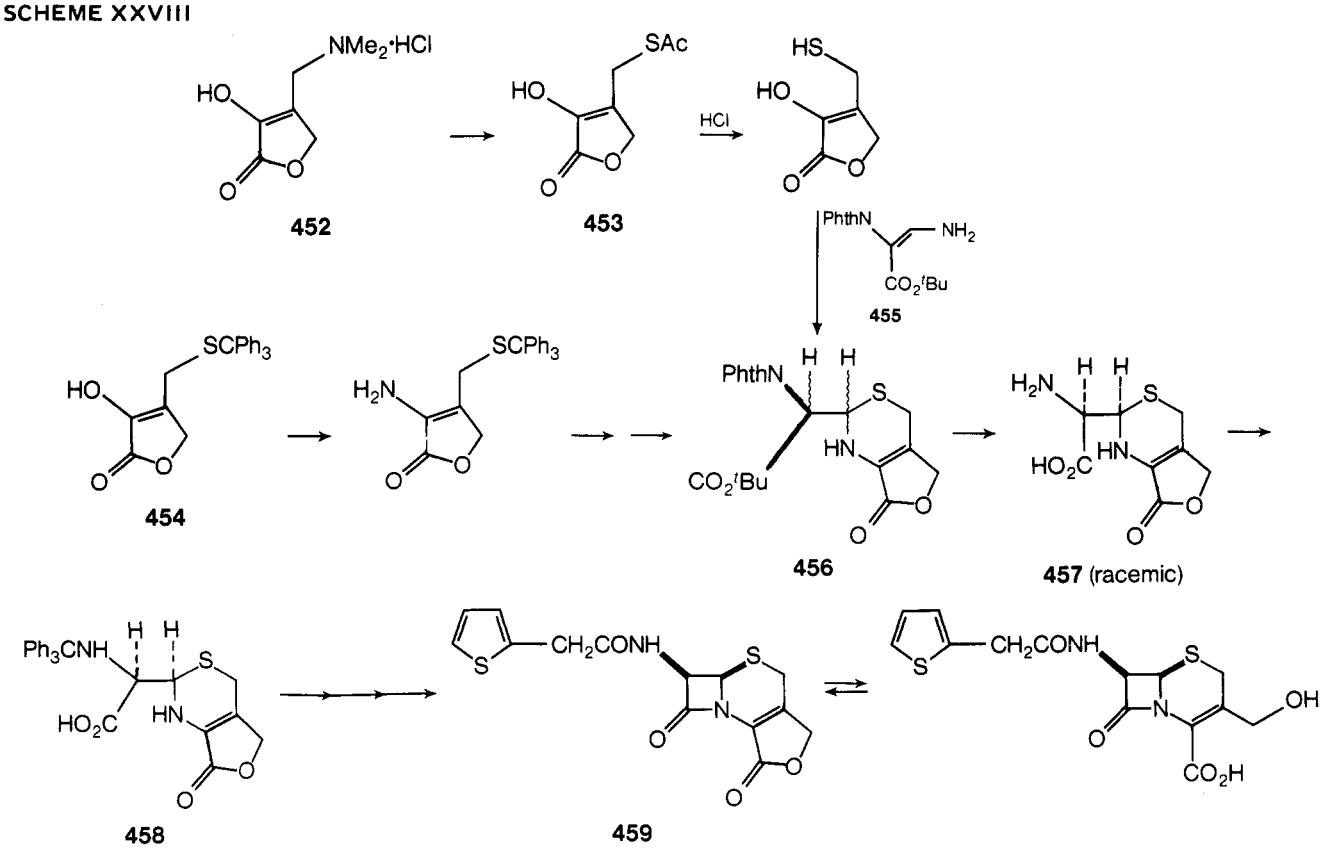


464 **456**

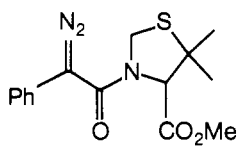
SCHEME XXVIII



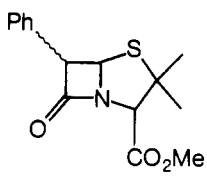
the required threo epimer **457.** Removal of the protecting groups gave the amino acid **458.** Reprotection of the amine as its trityl derivative was followed by coupling to form the β-lactam ring, using reagents such as dicyclohexylcarbodiimide. Although the lactone **459** can be obtained in high overall yield, this route suffers from the weakness that subsequent opening of the lactone ring is difficult to achieve in an efficient manner; maximum yields of 20% have been reported by the Squibb group.[195] Despite this disadvantage, this route has recently been given a boost by the introduction of a new method for making the key lactone **456** in overall 50% yield.[255] This method uses the γ,γ′-dihydroxyvaline lactone (**460**) which was condensed with α-phthalimidomalonaldehydic acid, as its *tert*-butyl ester, to form the enamine **461.** Oxidation of the lactone **462** with *tert*-butyl hypochlorite gave the chloride **463** which eliminated hydrogen chloride with triethylamine to give the ester **464,** and which was converted into the required lactone **456** by aqueous potassium carbonate in methanol.

E. Lowe Syntheses

One of the most original approaches to annelated β-lactams has been developed by Lowe and his collaborators and allows entry into a variety of modified cephem structures as well as the more difficult penam analogues. The work is based on an earlier method in which α-diazoamides, on photolysis, generate a carbene species which can abstract hydrogen from the carbon adjacent to the amide nitrogen with subsequent coupling to form a β-lactam ring (e.g., **465** to **466**).[256,257] In order to introduce the appropriate functional groups Lowe used α-diazomalonyl derivatives.

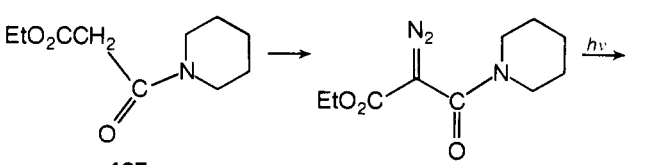


465

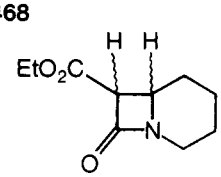


466

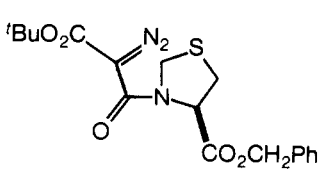
In plotting this route, model precursors of the type **467** were treated with the diazo transfer reagent *p*-toluenesulfonyl azide to give the diazo ester **468.** [258] Photolysis gave a 1:2 ratio of the ring-fused products **469.** The thiazoline **470** also reacted to give the products **471** and **472.** The pipecolic acid derivative **473** was similarly prepared.[259] Selective removal



467 **468**



469



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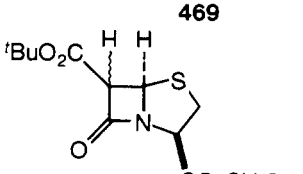
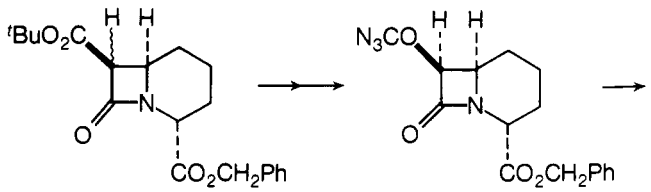
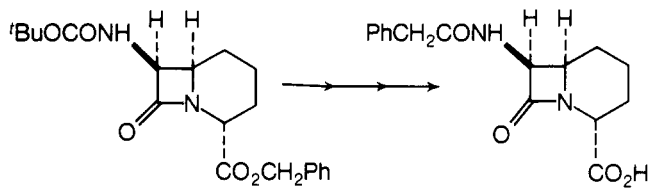


471, cis
472, trans

SCHEME XXIX

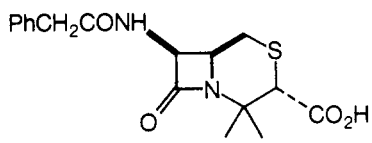


473 **474**



475 **476**

of the *tert*-butyl group afforded the acid which could be converted into the acid azide **474** (Scheme XXIX). Curtius rearrangement of this material gave the appropriately substituted amine **475** and, eventually, the nuclear analogue **476.**[260] Considerable variation in this route has been achieved, and the racemic cephalosporin analogue **477** has been prepared;[261] the relative stereochemistry of the latter compound has been confirmed by an x-ray crystallographic analysis on the intermediate **478.**[262]



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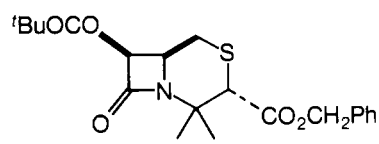
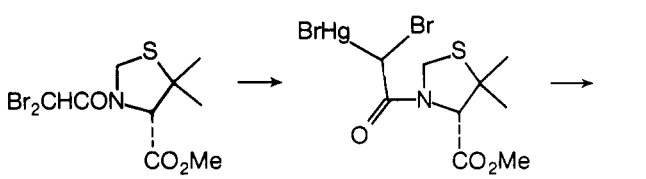
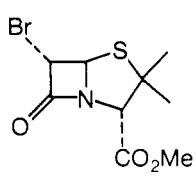


478

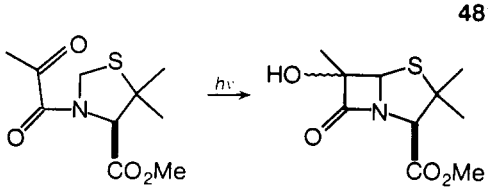
Several related methods for generating the four-membered ring, by an intramolecular cyclization process, have been reported. Using the dibromide **479** to make the masked car-



479 **480**



481



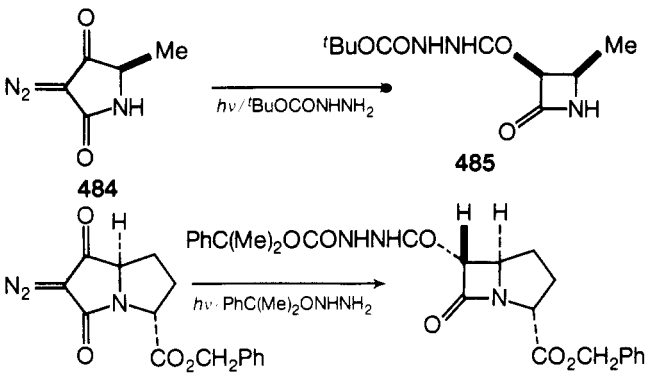
482 **483**

bene **480,** followed by thermolysis, yielded the 6α-bromopenicillanate **481** in low yield.[263,264] Alternatively, photolysis of α-ketoamides can result in formation of the β-lactam ring. In this manner the thiazolidine derivative **482** was converted into the β-lactam **483,** isolated as an epimeric mixture. The corresponding sulfoxide and sulfone both react more efficiently.[265,266]

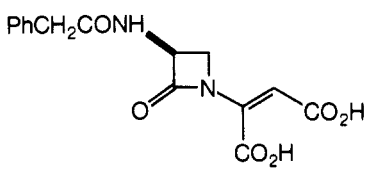
A more recent innovation, introduced by the Oxford group, is to make use of a Wolff rearrangement in order to effect a ring contraction and hence to generate β-lactam rings from γ-lactam precursors.[267] This method has the advantage over the earlier method in that ring contraction proceeds stereoselectively with kinetic control and proceeds in higher overall yields.

When applied to the pyrrolidine derivative **484** (Scheme XXX) in the presence of *tert*-butyl carbazate, the β-lactam **485** formed.[267] Similarly, the fused system **486** gave the β-lactam **487.**[268] This modified route has also produced the

SCHEME XXX

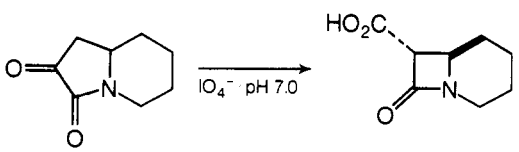


novel system **488,** but this compound exhibited no antimicrobial properties.[269]



488

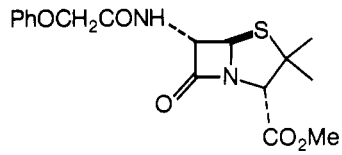
Some other means for preparing β-lactam rings by ring-contraction methods are currently under investigation.[270-272] The most promising of these involves the alkaline oxidation of cyclic α-ketoamides.[272,273] The compound **489** gives the product **490** upon oxidation with periodate.



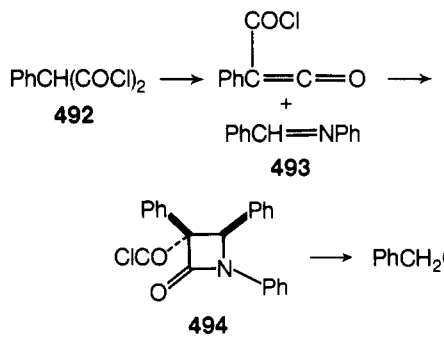
489 490

F. Other Methods

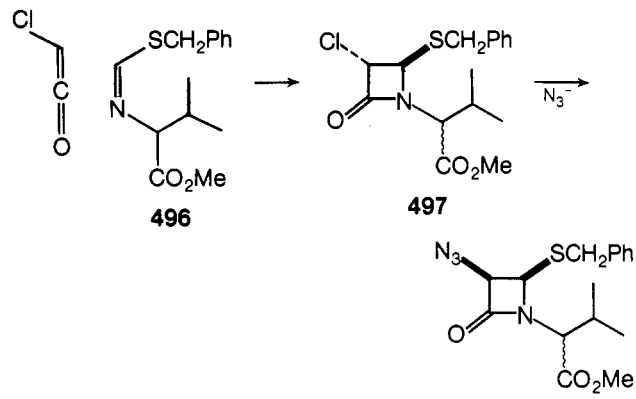
The successful Merck synthesis of the cephalosporins described above relied on the net (2 + 2) cycloaddition of a ketene (either free or potential) with an imine. Earlier work on the cycloaddition reaction involved use of a ketene precursor containing a protected nitrogen group, such as α-azidoacetyl chloride. In this way 6-epipenicillin derivatives of the type **491** were obtained, but the yields of the cycloaddition product were generally low, partly because of the formation of stereoisomeric mixtures.[268] By using the innovation introduced by Lowe, viz. the use of substituted malonyl derivatives, the scope of this method has increased considerably.[274] Substituted malonyl chlorides react with imines via ketenimine intermediates to give, principally, one cycloadduct. Thus the substituted malonyl chloride **492** reacted with the anil **493** to give the amide **494,** and a following Curtius rearrangement introduced the required side chain in the product **495.**



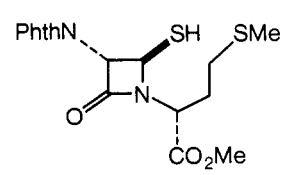
491



Use of the cycloaddition method for the preparation of β-lactam derivatives more closely related to penicillins have also been reported.[275,276] Reaction of chloroacetyl chloride and triethylamine with the thioimidate **496** gave the trans-substituted chloroazetidinone **497** in 45% yield. Unlike bicyclo

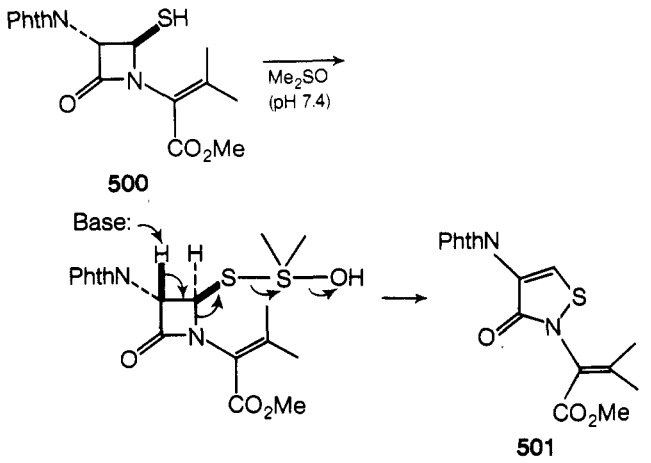


systems of the penam type, the chlorine could be displaced by azide ion to give the diastereoisomeric mixture of cis-substituted β-lactams **498.** In extensions of this work β-lactam systems bearing a free mercapto substituent were isolated. This was achieved by initial protection of the mercaptan as either its *p*-nitrobenzyl or trityl derivatives. In this manner both



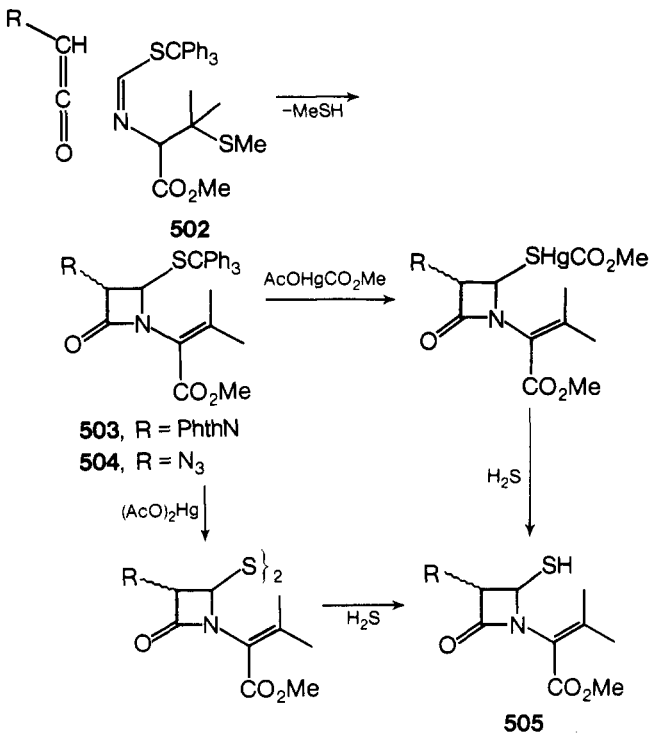
499

the phthalimido derivatives **499**[277] and **500**[278] were isolated. The latter did not cyclize when subjected to the conditions reported by Wolff for the reversal of the anhydropenicillin rearrangement, viz. use of a borax buffer at pH 7.4 in aqueous dimethyl sulfoxide.[82] Instead, the isothiazoline **501** was formed,



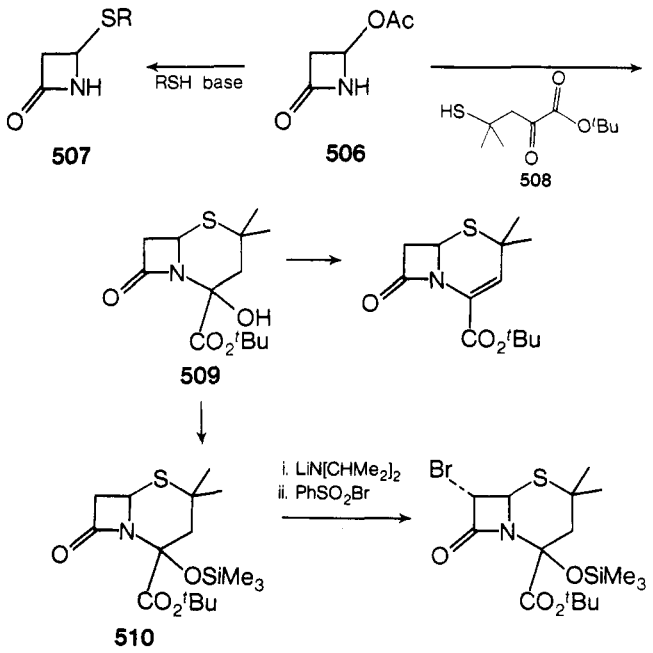
probably via an oxidative reaction involving the dimethyl sulfoxide as indicated. A similar approach has also been recorded by an independent group.[279,280] In this latter work the thioimidate **502** was reacted with either phthalimido- or azidoacetyl chloride in the presence of triethylamine. The products **503** and **504**, respectively, were isolated. Mixtures of cis and trans adducts were formed in these cases but, when phenylacetamidoacetyl chloride was used, the product was the cis-substituted lactam. Removal of the trityl group was accomplished with mercury(II) salts, regeneration of the mercaptan **505** being effected with hydrogen sulfide (Scheme XXXI).

SCHEME XXXI



The Hoechst group have also developed a route to novel cepham systems which commences with simple azetidinones followed by formation of the remaining heterocyclic ring.[281] This work has capitalized on the observation that 4-acetoxyazetidin-2-ones react with thiols under mild base catalysis to form the thioethers (e.g., **506** to **507**). Using more complex mercaptans, such as **508**, the azetidinone **506** gives the cepham system **509** directly. Dehydration (trace of iodine in hot xylene) produces the cephem, unsubstituted in position 7. Methods for introducing the 7-acylamino group have been worked out for the (protected) hydroxy intermediates (e.g., **510**) (Scheme XXXII).[282]

SCHEME XXXII



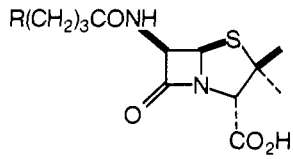
IV. Recent Biosynthetic Studies

Much work has been aimed at unravelling the intricacies of the biosynthetic pathway to the β-lactam antibiotics.[283] Although studies on the mode of antibiotic action have been carried out to a sophisticated level,[135-142] biosynthetic experiments are hampered by the fact that conventional feeding methods cannot be used, biosynthesis occurring inside the cell into which the largest unit entering seems to be monopeptides.[284] Although attempts to produce active cell-free systems have so far failed, such experiments may soon be possible. It has recently been shown, for example, that protoplast systems from both *P. chrysogenum* and *C. acremonium* strains can be obtained by an ultrasonic method and that these protoplasts are still capable of metabolizing β-lactam antibiotics.[285]

Only a few microorganisms produce β-lactam antibiotics and the role of these compounds is unknown.[283] Although various theories to explain the presence of these metabolites have been suggested, their principal function is probably as internal enzyme inhibitors.[286]

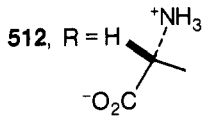
The biosynthesis of these compounds has been examined both by looking for likely β-lactam precursors among the cell constituents and by deductions from incorporation experiments of simple labeled precursors. Besides these studies several biomimetic experiments have been designed for in vitro studies. Earlier work, up to 1971,[283] has been reviewed and is only considered in brief.

β-Lactam antibiotics are secondary metabolites. Various studies have shown their formation to be dependent on the presence of three amino acids, L-α-aminoadipic acid, L-cysteine, and L-valine. The evidence that the α-aminoadipic acid is an obligatory intermediate for the synthesis of *all* penicillins (and cephalosporins) is at first sight somewhat surprising when considering the wide range of acylated penicillins that can be produced. It is thought that the penicillin initially produced is isopenicillin N (**511**) and that this can either be enzymically transacylated or isomerized, to penicillin N (**512**) before being deacylated to give the ubiquitous 6β-aminopenicillanic acid before reacylation.[283] L-α-Aminoadipic acid is incorporated into isopenicillin N, which has the L-α-adipoyl side chain, and this is the precursor of the more familiar D-α-ami-



511, R = H_3N^+ H

^-O_2C



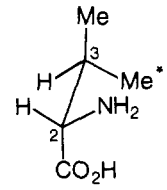
512, R = H $^+NH_3$

^-O_2C

noadipic acid group.[283,287,288] A similar state of affairs exists in the cephalosporin series for which a more efficient incorporation of the L-amino acid than for the D isomer has been demonstrated.[289] To date only the α-aminoadipoyl side chain has been found in β-lactam antibiotics isolated from *Cephalosporium* species.[283]

In 1959, Arnstein et al. isolated the tripeptide δ-(L-α-aminoadipoyl)-L-cysteinyl-L-valine from *P. chrysogenum*, and this was recognized as a possible precursor of penicillin N,[290] which had been isolated earlier. Isolation of isopenicillin N boosted this suggestion.[291] Although there is much circumstantial evidence for the tripeptide theory,[290,293] which is consistent with all the available biochemical data, experimental evidence for this theory, e.g., via direct incorporation experiments, is still lacking. In order to be converted into the penam nucleus the tripeptide must undergo an inversion of the valine configuration since, in the penicillin antibiotics, this has the D configuration. Although other tripeptides have not been reported from *Penicillium* species, several, including δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine, have been found in *Cephalosporium* strains.[294] The presence of the D-valine unit in this tripeptide is of considerable interest since it suggests an early inversion at the valine center in the biosynthesis of the cephalosporins. It is possible, however, that these peptides are merely by-products unrelated to the main antibiotic-producing route and that the D-valine unit is formed by a subsequent reduction of, for example, an α,β-dehydrovaline intermediate.

Recently two groups have carried out complementary studies on the incorporation of ^{13}C-labeled valine, using *Cephalosporium acremonium*. Use of the labeled L isomer, (2*S*,3*S*)-[4-^{13}C]valine (**513**) afforded the specifically labeled penicillin N (**514**),[295] as well as the cephalosporin C (**515**), with the label in the *exo*-methylene group (position 10). In the other study (2*S*,3*R*)-[4-^{13}C]valine[296] gave the isomeric derivatives, the cephalosporin C containing the label in the ring carbon (position 2).[297] These results indicate that incorporation of the L-valine proceeds with net inversion about position 2 and net retention at position 3.



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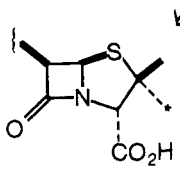

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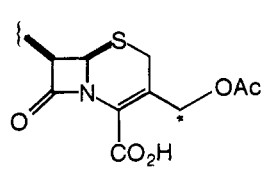

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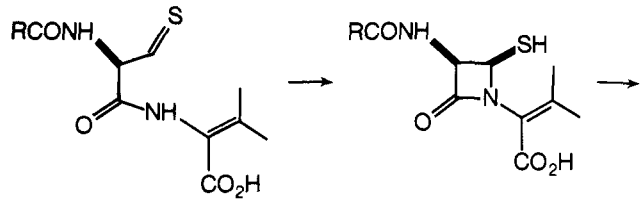
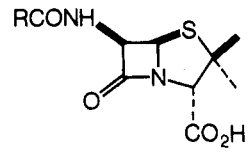
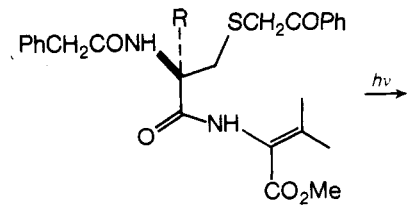
In order for the tripeptide to be converted into the penicillins and cephalosporins, a variety of oxidative transformations have to be envisaged, in particular of the cysteinyl and valinyl residues. A variety of speculative chemical schemes have so far been proposed and tested in in vitro experiments. In older theories the participation of a thioaldehyde intermediate, of type **516**, was postulated, which could interact with a dehydrovalinyl unit.[298] This was expected to undergo rapid formation of the β-lactam ring to produce the thiol **517**, followed by an enzyme mediated addition to the dehydrovalinyl unit to form the required penam nucleus. In support of this scheme it could be claimed that the thioaldehydes are very reactive species, known to undergo addition reactions with ease, and that anhydropenicillins can be reconverted into penams under carefully controlled conditions, the latter reaction exemplifying the β addition of the thiol unit to the dehydrovaline group.[82] Although this biosynthetic scheme has been criticized,[299] the chemistry of such a route is attractive. Largely because of the difficulty in preparing thioaldehydes in vitro, however, an experimental test of this scheme has only recently been reported.



516 **517**

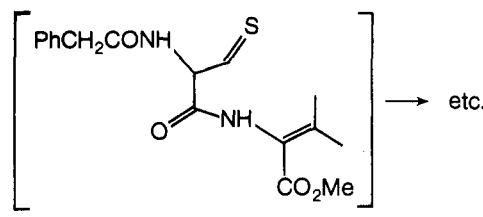


The thioaldehyde **518** was prepared by several methods, including use of a photochemical cleavage of the precursor **519**.[300] In the event, no cyclization of the type expected was observed, even though intermediates with the structure **517** have been shown to be isolable.[279,280] Instead of the desired β-lactam formation the thioaldehyde group undergoes preferential thioenolization, followed by other reactions. In an attempt to inhibit this side reaction, the corresponding methyl derivative **520** was also prepared, but on photolysis no thioaldehydes but only polymers were produced. Treating these polymers in a variety of ways did not produce any β-lactam-containing compounds. Although these results do not disprove the occurrence of some similar enzyme-mediated pro-



519, R = H

520, R = Me



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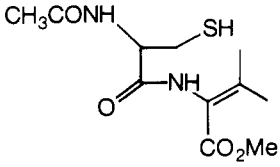
cess in vivo, the complete absence of any β-lactam compounds in these studies and the known lack of cyclization with species such as **512** cast a shadow on the specific involvement of this intermediate. More work is needed to delineate the exact conformational characteristics and conditions of this reaction.

What seemed an attractive alternative to the above route has also been ruled out on the results of labeling experiments. This involved the β,γ-unsaturated valinyl peptide (**96**) (Scheme VII). As a possible model the intermediate **96** has been shown to undergo oxidation to both the penam and cephem structures and hence that the intermediate **96** may be the point along the biosynthetic pathway at which the paths to the two distinct groups of antibiotics diverge.[63] Although this hypothesis cannot be completely ignored, it has recently been shown that [Me_2-$^2H_6$]-D,L-valine is incorporated into penicillin V, in a *P. chrysogenum* strain, with retention of all the deuterium atoms, which suggests that, for the penicillins at least, biosynthesis does not involve chemical modification of the methyl groups.[301]

Another, earlier, theory for the production of the penam skeleton suggested that the cysteinyl group added to a dehydrovaline residue before formation of the β-lactam ring. Some model reactions along these lines have been successful, the thiol **521** giving the cyclic peptide **522.**[302] The intermediate thiazepinone (e.g., **522**) was then assumed to undergo an oxidation of the sulfur group, followed by formation of the β-lactam ring in a transannular manner. Again all efforts to reduce this scheme to reality have failed.[303] In a more recent attempt the *N*-chloro derivative **523** was prepared and treated with silver ion, but no lactam formation was observed.[304] Photochemical methods of cyclizing the thiazepine type derivatives have also been unsuccessful.[305]



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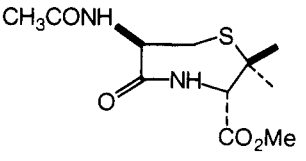

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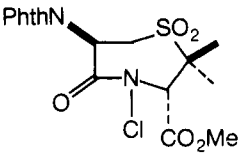

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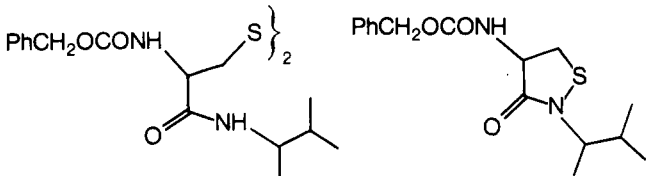
Undaunted by this catalogue of failures, two groups have independently suggested an alternative means for the oxidation of the tripeptide precursor.[306,307] This involves the possible intermediacy of isothiazolidinones in the oxidative sequence. The systems could be made in vitro either by treatment of the disulfide precursor **524** with bromine in pyridine, to give the compound **525**,[306] or by a more esoteric route using the thioselenide **526** before oxidation with *m*-chloroperbenzoic acid followed by treatment with ammonia at −60° to produce the dehydro derivative **527.**[307] The structures **525** and **527** are formally intramolecularly protected forms of the thioaldehyde but, so far, they have not been converted into this species. Oxidation of the intermediate **525** with peracid yielded a mixture of the isomeric oxides **528** which react with thiols to give thiolsulfinates.[306] Thermolysis of the sulfoxides



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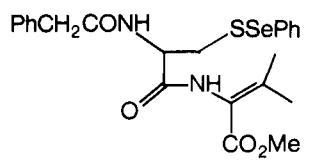

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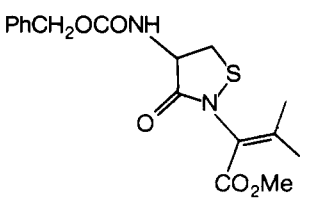

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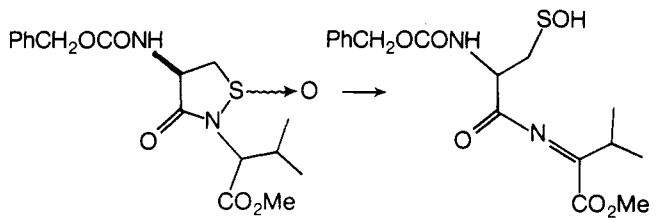
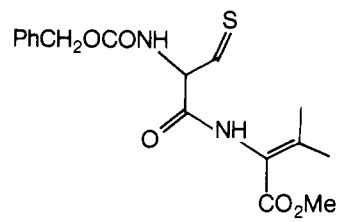
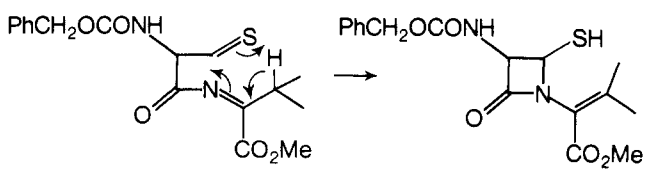


528 **529**

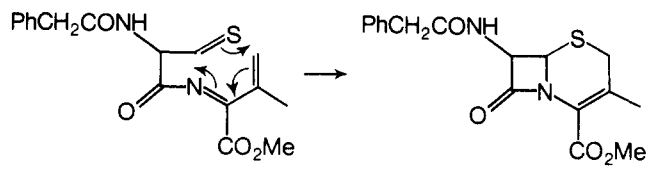


530

may cause rearrangement into the intermediates **529** and the thioaldehyde **530,** but again, a method which ensures reliable conversion of the latter into the lactam, and hence the penicillin, is required. An "ene" type mechanism (**531** to **532**) might also achieve the same process, while the dehydro intermediate **533** might be able to undergo an electrocyclic addition to give the cephem **534.**[307] These schemes are, as yet, speculative and definite results are still awaited.



531 **532**



533 **534**

The ceph-3-em nucleus is at a higher oxidation level than that of the penicillins. There is no evidence, however, that the penicillin structure can act as an in vivo precursor for the formation of cephalosporins, and it is generally considered that the point of divergence for the biosynthetic pathways lies before the cyclization step. There is evidence that the deacetoxycephems are the primary product of the cyclization step and that hydroxylation to the deacetyl derivative is finally followed by acetylation.[308] Deacetoxycephalosporins have been detected in some *Streptomyces* species and in a variety of fungi.[309] Work with mutants of *C. acremonium* strongly indicates that acetylation by an acetyl transferase follows the hy-

droxylation.[310] There is also considerable biosynthetic evidence that methoxylation of the cephem nucleus at position 7 also occurs at a late stage in the biosynthesis. It will be interesting to see if samples of 7-methoxy-10-deacetoxycephalosporins are eventually detected in the relevant species of *Streptomyces.*

V. Addendum

A general method for the direct conversion of penicillin sulfoxide acids into ceph-3-ems has been reported.[311] Whereas attempted sulfoxide rearrangements with the free acids normally lead to either decarboxylation[18] or 3-hydroxy-3-methylcephams,[49] temporary protection as trimethylsilyl esters is possible. Use of *N,O*-bis(trimethylsilyl)acetamide as both dehydrating agent and protecting group is preferred, and the cephem acid can be isolated directly from the reaction in yields greater than 80%.

More reactions of the sulfur atom have been recorded. Methylation of the ceph-3-em system with methyl fluorosulfonate gives a methylsulfonium salt but with the inverted configuration at position 6 (i.e., 6*S*) owing to a rapid equilibration which probably occurs by intermediate fission and reclosure, in the unnatural sense, of the 1,6 bond.[312] A similar ring cleavage–closure sequence is probably involved in the reaction of penicillins with chloramine T. A variety of products form including a ring-expansion product involving the insertion of nitrogen between positions 1 and 5 to give a fused thiadiazine.[313-315] The stereochemistry of the chlorination products of penicillins and the penam–cepham interconversions have been published;[316] ^{13}C NMR spectroscopy has been used in assigning structures to these products.[317]

Recent variations accomplished at position 10 of the cephalosporin nucleus include the direct displacement of the 10-acetoxy substituent by halogens, using boron halides[318] and the introduction of aromatic groups, such as 4′-methoxyphenyl, by using trifluoroacetic acid as catalyst.[319]

3-Formylceph-3-ems are highly active antibiotics but of limited use because of their lability.[320] More stable derivatives, such as oximes, can be made[321] and the formyl group can be decarbonylated by use of tris(triphenylphosphine)rhodium chloride, to give the 10-nor compounds.[320] Removal of C-10 can also be accomplished by oxidative cleavage of 3-*exo*-methylenecephams.[218] Further interest in these important derivatives (e.g., **380**) has been shown[322] and the 3-fluorocephem system has also been described.[323]

Chemical manipulations about positions 6 (7) of the penam (cephem) nucleus continue. The imino chloride derivatives of side-chain amides are known to form ketenimines with base,[128] but side reactions can also occur.[324] 7-Isocyanatocephalosporins have been described and used for the direct insertion of new side chains such as the α-carboxyphenylacetamido group.[325] Introduction of the 6α(7α)-methoxy group can be efficiently accomplished by initial preparation of the 4-hydroxy-3,5-di-*tert*-butylbenzylidene derivative followed by oxidation with lead dioxide and treatment with methanol.[326] Attempts to make 6α-fluoropenicillin derivatives are frustrated by their enhanced reactivity.[327]

Considerable progress with total syntheses of cephalosporin-type antibiotics and their analogues has been accomplished.[328-332] The flexibility of the Merck route has been demonstrated by the successful syntheses of 3-arylcephalosporins,[333] 10-methylcephalothin,[334] 1-oxacephalothin,[335] and 1-carbacephalothin.[336] The relatively high antibiotic activity of the latter two compounds is encouraging since they are the first which demonstrate that sulfur is not an essential ingredient for active analogues.

Among a growing number of metabolites isolated from relevant culture broths has been a 3-methylthiomethylcephem, which was isolated from a mutant *Cephalosporium* species.[337]

Labeling experiments have shown that the inversion of L-valine during its incorporation into the tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV) probably does not involve the α,β-dehydro amino acid, since only the α proton is lost.[338] Furthermore, it has also been shown that the incorporation of ACV into penicillin N, which is carried out by protoplast lysates of *C. acremonium* occurs *without* loss of the α-valinyl proton since material tritiated in this position is retained without loss of the label.[339] This implies that the formation of the carbon–sulfur bond also does not involve an α,β-dehydrovalinyl intermediate. This leaves formation of a β-radical or carbenium ion (e.g., β-hydroxylation) as possible means for activating this position.

VI. References and Notes

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