# Bicyclomycin: Synthetic, Mechanistic, and Biological Studies

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## Contents

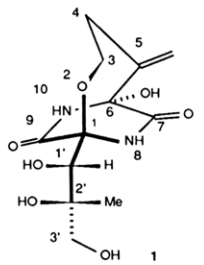


Robert M. Williams was born in New York in 1953. He obtained a B.A. degree from Syracuse University in 1975 and completed his doctoral study in 1979 at the Massachusetts Institute of Technology with Dr. W. H. Rastetter. After a 1-year postdoctoral fellowship at Harvard University in the laboratories of the late Professor R. B. Woodward, he joined the faculty at Colorado State University where he is currently Associate Professor of Chemistry. Professor Williams is the recipient of an NIH Research Career Development Award and an Eli Lilly Young Investigator Award and is a Fellow of the Alfred P. Sloan Foundation. His research interests are in the areas of bioorganic chemistry, natural products synthesis, asymmetric synthesis, and the design and synthesis of enzyme inhibitors.

Catherine Durham was born in Burbank, CA, in 1960. She received her B.S. in Chemistry from California Polytechnic State University, San Luis Obispo, in 1986, where she worked under the direction of Dr. John Marlier. During her undergraduate studies, she completed an internship in radiochemistry at Diablo Canyon Nuclear Power Plant in Avila Beach, CA. Catherine is presently studying the mechanism of action of bicyclomycin under the direction of Dr. Robert Williams at Colorado State University.

## I. Introduction

Bicyclomycin (1) is a commercially important antibiotic that was independently and simultaneous reported by two Japanese groups in 1972.[1,2] Bicyclomycin, obtained from cultures of *Streptomyces sapporonensis*,[1] is identical with aizumycin obtained from *Streptomyces aizunensis*.[2] This structurally unique antibiotic, now named bicozamycin,[3] is being produced on large scale from the fermentation harvest of an improved strain of *S. sapporonensis* at the Fujisawa Pharmaceutical Co.




*BICYCLOMYCIN*

The efficiency of the fermentation process and the low toxicity ($LD_{50}$ > 4 g/kg (mice)) of the antibiotic have made it possible to market bicyclomycin on a worldwide basis as an effective agent against nonspecific diarrhea in humans and bacterial diarrhea in calves and swine.[4]

Bicyclomycin is a weak antibiotic that has been shown to be effective against gram-negative bacteria such as *Escherichia coli*, *Klebsiella*, *Salmonella*, *Shigella*, *Citrobacter*, *Enterobacter cloacea*, and *Neisseria* but is inactive toward *Proteus*, *Pseudomonas aeruginosa*, and gram-positive bacteria. Bicyclomycin is a structurally unique antibiotic, bearing no structural resemblance to any of the other known classes of antibiotics. The mechanism of action of bicyclomycin is also thought to be distinct[5] from other known classes of antibiotics and is an area of intense interest. This review is intended to cover all of the published literature on bicyclomycin since 1972 and includes biosyn-

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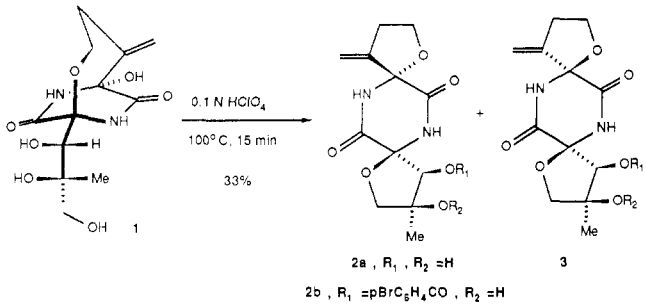
SCHEME 1




thesis, biological activity, mechanism of action, and synthetic work, which is emphasized.

## II. Isolation, Structural Elucidation, and Biosynthesis

Bicyclomycin is a crystalline, colorless, water-soluble, and weakly basic substance (mp 187–189 °C) with a molecular composition $C_{12}H_{18}N_2O_7$ (MW 302.28). The compound is soluble in water, in methanol, and sparingly in ethanol, is practically insoluble in most organic solvents, and is unstable in alkaline solution.

The structure and relative configuration of bicyclomycin was firmly established by Tokuma et al. by single-crystal X-ray analysis.[6] Later, Maag et al.[7] established the absolute configuration via synthesis and X-ray analysis (**2b**) of an acid-catalyzed dehydrative rearrangement product **2a**. The stereochemistry of bicyclomycin was thus determined to be 1*S*,6*R*,1′*S*,2′*S*. The synthesis of **2** will be described later (Scheme 1).

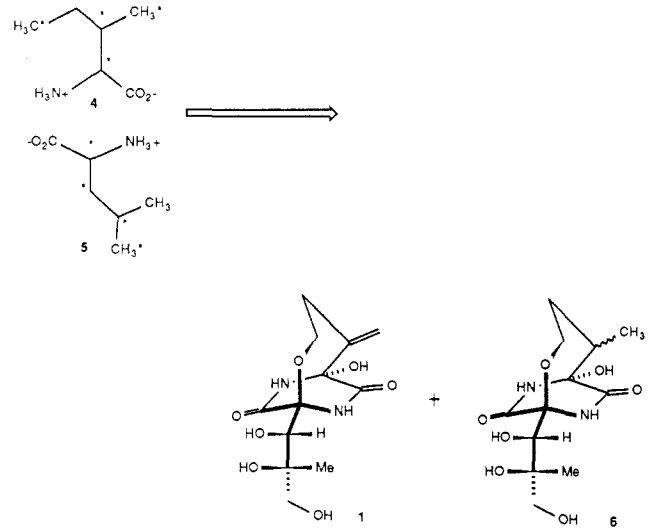
Bicyclomycin is biosynthetically derived[8] from the amino acids leucine and isoleucine. The results of $^{14}C$-labeled amino acid incorporation and nutrient constraints determined that *S. sapporonensis* synthesizes bicyclomycin from one molecule of leucine and one molecule of isoleucine and required $Fe^{2+}$ ion and nicotinamide as essential cofactors. Scheme 2 illustrates that all but two of the aliphatic carbons of these two amino acids must be oxidized (starred atoms) to produce bicyclomycin. The requirement of ferric ion and nicotinamide strongly suggest a cytochrome oxidase/nicotinamide cofactor system as obligate machinery for the biosynthesis. The involvement of the ribosomes in the biosynthesis has not been determined. Other than knowledge of the raw materials for the construction of antibiotic by the producing organism, virtually nothing is known with regard to the timing of events (hydroxylations, dioxopiperazine formation, bicyclo[4.2.2] ring closure, C-5 *exo*-methylene formation) that lead from Leu and Ile to **1**. The careful elucidation of this pathway is a fascinating and challenging problem. The only other available bit of information that may be related to this pathway is the observation[4] that dihydrobicyclomycin (**6**) is coproduced with **1** from the fermentation harvest. This suggests that the penultimate step is the oxidative elimination at C-5 of **6** to **1**; this speculation, however, has not been verified.

## III. Biological Activity

### A. General

Bicyclomycin is a relatively weak antibiotic that does not display in vitro or in vivo cross resistance with other

SCHEME 2



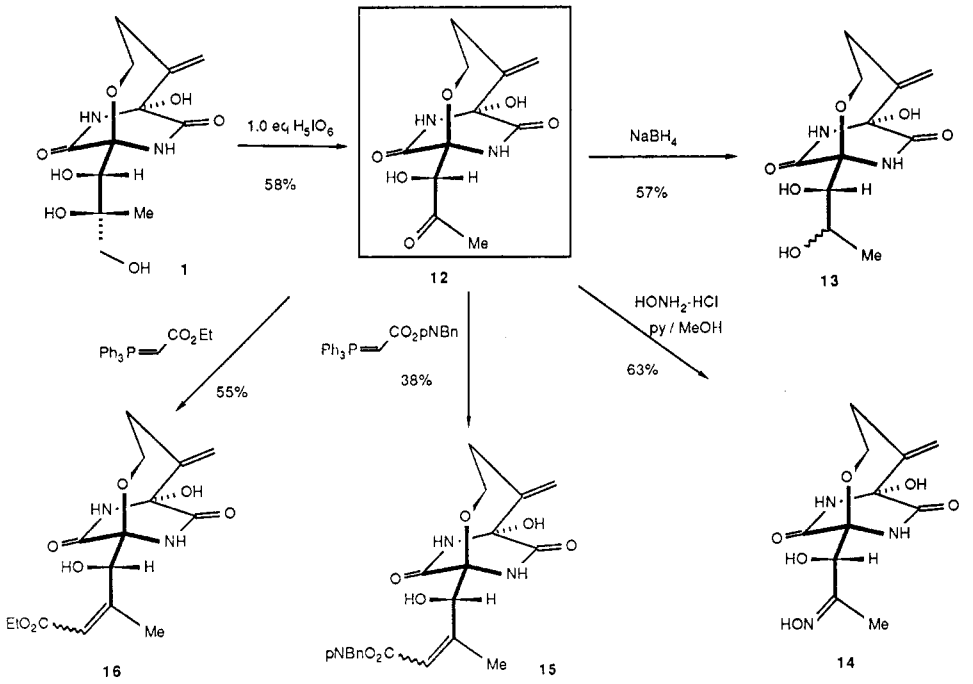
commercially available antibiotics. The MICs of bicyclomycin for various sensitive strains of *E. coli* are focused in a narrow range between 25 and 50 mcg/mL. Bicyclomycin is rapidly absorbed in test animals as well as man when given intramuscularly and is distributed in the body in high levels in various visceral organs as unchanged active antibiotic. This physiological stability seems to account for the significant high in vivo activity in spite of its relatively low in vitro antimicrobial spectrum. Bicyclomycin is apparently not metabolized in test animals or man since it is recovered unchanged in high yield from the urine. Bicyclomycin is poorly absorbed when administered orally and is therefore not effective against systemic infection when administered orally. The most practical application has therefore been to that bacterial infections of the gut (diarrhea) in both animals and man. In Japan, bicyclomycin is being developed as a feed additive for livestock.

### B. Semisynthetic Derivatives

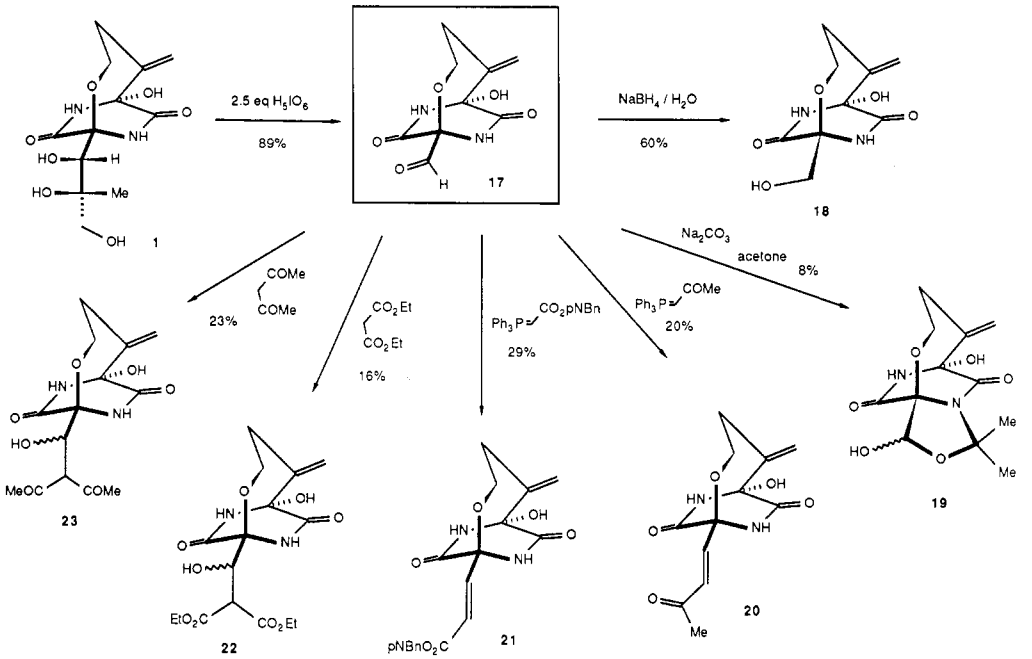
In general, alteration of the structure of bicyclomycin results in suppression or complete loss of biological activity. Müller and co-workers at Ciba-Geigy[9] prepared a number of semisynthetic derivatives of bicyclomycin and evaluated these for antibacterial activity. The synthesis of these derivatives is summarized herein since the methods utilized provide valuable insight as to chemical reactivity and stability of bicyclomycin.

Simple acylation of **1** with ethyl chloroformate in THF/pyridine at −10 °C furnished primarily the C-3′-carbonate (70%) and the C-1′/C-3′-dicarbonate (27%) (**7a,b**, Chart 1). Under the same conditions, trichloroethyl chloroformate furnished the C-2′/C-3′ cyclic carbonate (**7c**, 64%), which could be induced to rearrange to the thermodynamically more stable C-1′/C-2′ cyclic carbonate **7d** (MeOH, 3 days, 50%). Similarly, the C-3′-urethane **7e** is prepared by reaction with ethyl isocyanate in 60% yield. Reaction of **1** with DHP/TsOH in dioxane selectively provides the C-3′-OTHP derivative **7f** that suffers nonspecific benzoylation at C-1′ and C-6 to furnish a mixture of **7g** and **7h** in 17% and 23% yields, respectively. Acidic removal of the THP group from **7g** furnished the C-1′-benzoyl derivative **7i** (70%). The corresponding C-2′/C-3′-

SCHEME 3




SCHEME 4

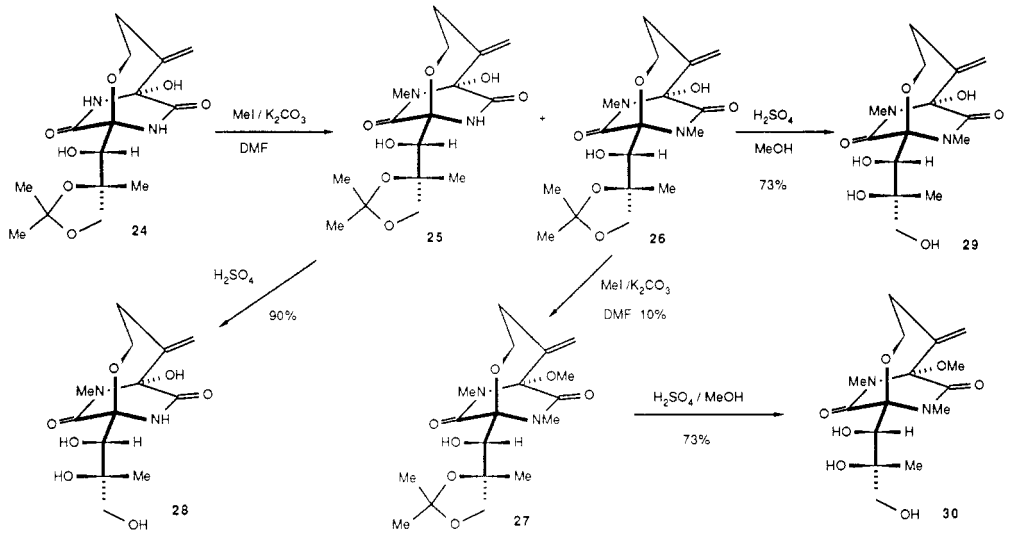



(OTHP)$_2$ derivative[10] **7j** could be selectively acylated (35%) at C-6 (**7k**) and deprotected (HOAc) to furnish the C-6 acetate **7l** (32%). Preparation of the 2′,3′-epoxide **8** was accomplished by selective mesylation of the C-3′-OH (**7m**, MsCl, py, −10 °C, 74%) and cyclization induced by $Et_3N$ (64%). Attempts at opening the epoxide with nitrogen nucleophiles and NaI instead resulted in the tricyclic material **9**. Ring opening of the epoxide did occur in the presence of $H_2S$ and mercaptoethanol to furnish the C-3′-mercapto analogues **10** (57%) and **11** (45%).

Further modification of the side chain was accomplished by partial oxidative cleavage with 1 equiv of periodic acid to furnish the methyl ketone **12** (58%). $NaBH_4$ reduction of **12** furnished the triols **13** as an epimeric mixture at C-2′. Other elaborations of **12** included formation of the oxime **14** (63%) with hydroxylamine hydrochloride and the two $\alpha,\beta$-unsaturated esters **15** (38%) and **16** (55%) via Wittig homologation (Scheme 3). Attempts to obtain the free acids from **15** or **16** were unsuccessful. Oxidation of bicyclomycin with 2.5 equiv of periodic acid cleanly furnished aldehyde **17** (89%).[10] Reduction of **17** with $NaBH_4$ provided the stable hydroxymethyl derivative **18** (60%). Aldehyde **17** could also be homologated in modest to poor yields to the condensation products **19**–**23** (Scheme 4).

A series of variously methylated derivatives were prepared from the 2′,3′-acetonide derivative **24** as shown in Scheme 5. The N-10-monomethyl derivative **28**, the N-8,N-10-dimethyl derivative **29**, and the trimethyl derivative **30** were all prepared by exhaustive methyl-

SCHEME 5



SCHEME 6

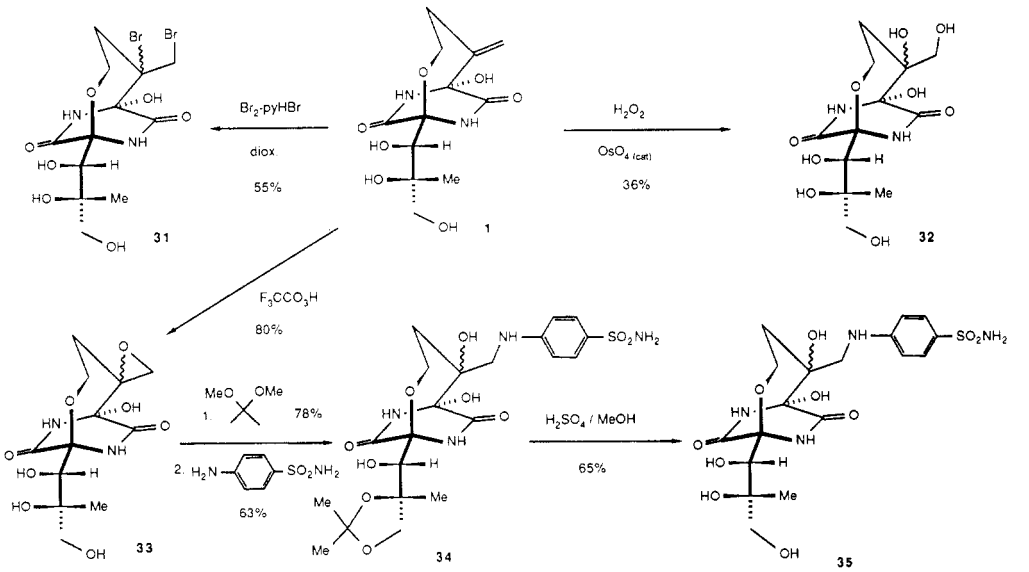
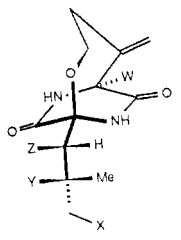
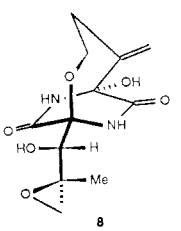


CHART I



| | X | Y | Z | W |
|---|---|---|---|---|
| 7 a | -OH | -OH | -OH | -OH |
| b | $-OCOOC_2H_5$ | -OH | -OH | -OH |
| c | $-OCOOC_2H_5$ | -OH | $-OCOOC_2H_5$ | -OH |
| d | -OCOO- | | -OH | -OH |
| e | -OH | -OCO-O- | | -OH |
| f | $-OCONHC_2H_5$ | -OH | -OH | -OH |
| g | -OTHP | -OH | -OH | -OH |
| h | -OTHP | -OH | $-OCOC_6H_5$ | -OH |
| i | -OTHP | -OH | $-OCOC_6H_5$ | $-OCOC_6H_5$ |
| j | -OH | -OH | $-OCOC_6H_5$ | -OH |
| k | -OTHP | -OH | -OTHP | -OH |
| l | -OTHP | -OH | -OTHP | $-OCOCH_3$ |
| m | -OH | -OH | -OH | $-OCOCH_3$ |
| | $-OSO_2CH_3$ | -OH | -OH | -OH |



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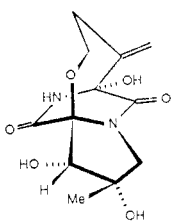

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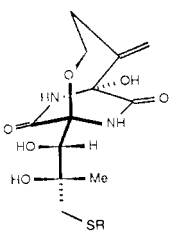


10, R = H
11, R = $CH_2CH_2OH$

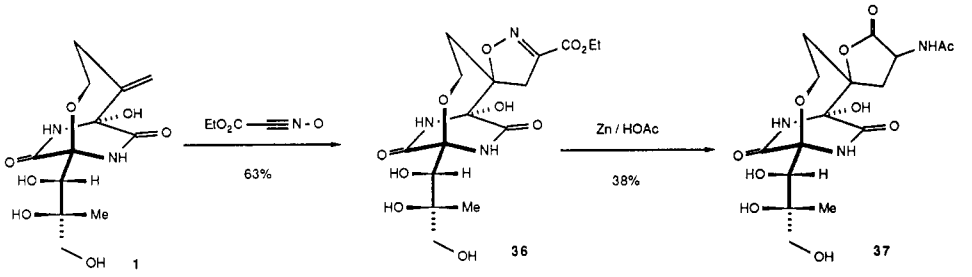
ation of **24**, separation, and acetonide hydrolysis in methanolic sulfuric acid in good yield.

Oxidation of the C-5-*exo*-methylene group furnished the dibromide **31**, the diol **32**, and the epoxide **33** (Scheme 6). Acetonide formation, oxirane opening (**34**), and deprotection furnished the sulfonamide **35**. Cycloaddition of carbethoxy nitrile oxide **36** followed by reductive cleavage/acylation furnished the spiro lactone **37** (Scheme 7).

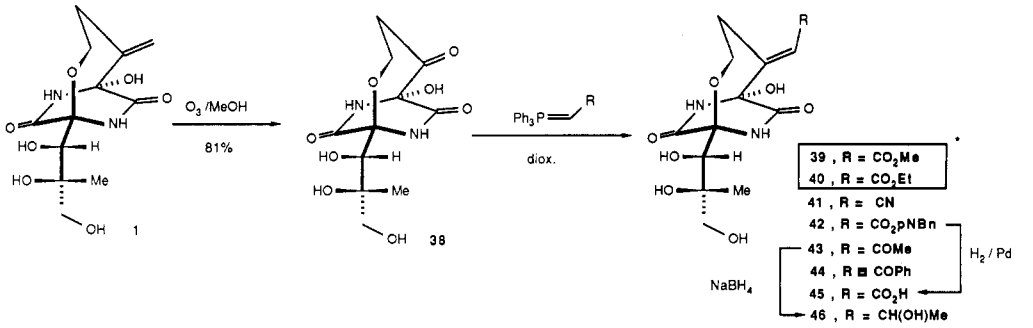
The most useful modification of the *exo*-methylene proved to be the Wittig homologation of ketone **38** obtained from **1** via ozonolysis. Both the carbomethoxy and carboethoxy derivatives **39** and **40** (Scheme 8) were found to be biologically active and displayed a broader spectrum of activity than bicyclomycin. Ketone **38** was also transformed into the oxime **47**, *O*-methyl oxime **48**, and phenylhydrazone **49**; of these, the *O*-methyl oxime **48** curiously displayed biological activity (Scheme 9).

Thus, of all the semisynthetic derivatives prepared in the Ciba-Geigy study, only **39**, **40**, and **48** displayed antimicrobial activity. The activities of these compounds and that of bicyclomycin are tabulated in Tables 1 and 2. Again, **1** and the active analogues are relatively weak antibiotics in vitro. Since bicyclomycin

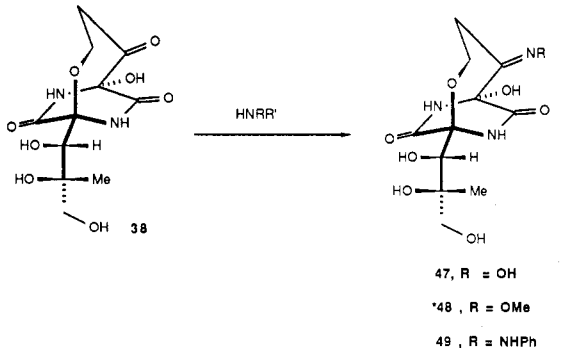
SCHEME 7




SCHEME 8




SCHEME 9




is poorly absorbed on oral administration, the Fujisawa group prepared a number of acyl derivatives[1b] in hopes of improving the lipophilicity and attendant penetration of the compound in the test organism. The C-3′-monoacyl derivatives all displayed slight activity or were inactive in vitro. When given orally to rats, bicyclomycin could be recovered in high yield from the urine, indicating that the acyl groups facilitated absorption and were readily hydrolyzed in the body.

The Ciba-Geigy work very clearly indicates that the C-1′,C-3′ side chain is essential for biological activity in vitro since even the slightest structural modification resulted in loss of activity. Alkylation of the amides and destruction of unsaturation at C-5 also resulted in loss of activity. The general lack of success in significantly improving the activity of bicyclomycin through semisynthesis seems to have led to the abandonment of additional chemical studies on this compound in the pharmaceutical industry. The only totally synthetic and biologically active compound based on the bicyclomycin structure was prepared in our laboratories and will be discussed in the following section.

## IV. Mechanism of Action

The morphological changes in *E. coli* induced by bicyclomycin include the formation of blebs on the cell surface, a highly undulated outer membrane, and the production of filamentous cells resulting in cell lysis.[11] These morphological changes are indicative of peptidoglycan disruption and resemble those changes induced by the β-lactam antibiotics. Several studies have appeared relating to this important and intriguing question. In the first, Tanka et al.[12] showed that bicyclomycin did not affect DNA synthesis or lipid synthesis in vivo. However, RNA synthesis and protein synthesis were inhibited by bicyclomycin in vivo but not in vitro. Bicyclomycin had no effect on cell-free protein synthesis, but in vivo envelope protein synthesis was significantly affected; there was not a significant inhibition of cytoplasmic proteins. These workers found that, of the envelope proteins examined, bicyclomycin inhibited the synthesis of the bound form of lipoprotein; a structure that is absent in gram-positive bacteria. However, an *E. coli* mutant JE5505(1po$^-$) lacking murein/lipoprotein has been isolated and grows well under a variety of conditions. This suggests that lack of murein/lipoprotein may not be fatal to *E. coli* and that the inhibition of the biosynthesis of this structure by bicyclomycin may not be the primary action, but rather a secondary action.

In a very important study, Iseki and collaborators[13] demonstrated that bicyclomycin binds seven inner-membrane (Sarkosyl-soluble) proteins (BBP's) of *E. coli* (ATCC 27166) that were shown to be distinct from the penicillin-binding proteins (PBP's 1–8). The BBP's ranged in molecular weight from 27 000 to 93 000, and each formed an irreversible, covalent, and stoichiometric complex with [$^{14}$C]bicyclomycin. The function of the BBP's and the nature of the BBP–bicyclomycin covalent complex remains unknown. These important results have led to the conclusion that the process of cell division is more complex than originally thought and that there exists inner membrane proteins (the BBP's) that are crucial for peptidoglycan assembly.

In a very recent study, Vazquez and collaborators[14] studied the structural modifications of *E. coli* (ATCC 27166) peptidoglycan induced by bicyclomycin. These

**TABLE I. Antibacterial Spectrum of Bicyclomycin**

| entry | test organism | min[a] inhib concn, mcg/mL |
|---|---|---|
| 1 | *Staphylococcus aureus* FDA 209P | 500 |
| 2 | *Sarcina lutea* PCI 1001 | 62.5 |
| 3 | *Streptococcus faecalis* 5 | >500 |
| 4 | *Bacillus antracis* 1 | >500 |
| 5 | *Bacillus subtilis* ATCC 6633 | >500 |
| 6 | *Pseudomonas aeruginosa* 35 | >500 |
| 7 | *Klebsiella pneumoniae* S | 15.6 |
| 8 | *Salmonella typhosa* 376 | 15.6 |
| 9 | *Salmonella derby* 3299 | 31.2 |
| 10 | *Salmonella enteritidis* NG 567 | 7.8 |
| 11 | *Escherichia coli* B | 31.2 |
| 12 | *E. coli* K-12 | 31.2 |
| 13 | *E. coli* H-3 | 31.2 |
| 14 | *Shigella flexneri* 3a 3196 | 15.6 |
| 15 | *Shigella flexneri* R-4 | 15.6 |
| 16 | *Shegella sonnei* R-1 | 15.6 |
| 17 | *Brucella melitensis* K-3 | 0.9 |
| 18 | *Vibrio comma* 384 | 3.9 |
| 19 | *Proteus vulgaris* X-19 | >500 |
| 20 | *Serratia marcescens* 2 | >500 |
| 21 | *Mycobacterium phlei* 607 | >500 |
| 22 | *Morganella* 3 | >500 |
| 23 | *Rettgerella* 15 | >500 |
| 24 | *Candida albicans* YU-1200 | >500 |
| 25 | *Aspergillus niger* N-1 | >500 |
| 26 | *E. coli* NIIIJ JC-2 | 25 |
| 27 | *Kl. pneumoniae* NCTC-418 | 100 |
| 28 | *Sh. flexneri* 1 a EW-8 | 25 |
| 29 | *Sh. flexneri* 1 b Showa 15 | 12.5 |
| 30 | *Sh. flexneri* 2 a EW-10 | 12.5 |
| 31 | *Sh. flexneri* 2 a Komagome BIII | 12.5 |
| 32 | *Sh. flexneri* 3a EW-14 | 12.5 |
| 33 | *Sh. flexneri* 4 a Saigon-Arai | 12.5 |
| 34 | *Sh. flexneri* 5 Komagome A | 25 |
| 35 | *Sh. sonnei* I EW-33 | 50 |
| 36 | *Sh. sonnei* Ohara | 12.5 |
| 37 | *Salm. typhosa* T-287 | 25 |
| 38 | *Salm. typhosa* O-901 | 25 |
| 39 | *Salm. paratyphi* A 1015 | 25 |
| 40 | *Salm. paratyphi* B 8006 | 25 |
| 41 | *Salm. typhimurium* 1406 | 25 |
| 42 | *Salm. enteritidis* 1891 | 12.5 |
| 43 | *Pr. vulgaris* IAM-1025 | >800 |
| 44 | *Ps. aeruginosa* IAM-1095 | >800 |
| 45 | *N. gonorrhoeae* Matuura | 25 |
| 46 | *N meningitidis* 68 | >800 |
| 47 | *Staph. aureus* 209-P JC-1 | >800 |
| 48 | *Staph. aureus* Newman | >800 |
| 49 | *Staph. aureus* Terashima | >800 |
| 50 | *Staph. aureus* Smith | >800 |
| 51 | *Strept. hemolyticus* S-23 | >800 |
| 52 | *Strept. faecalis* 6733 | >800 |
| 53 | *Dipl. pneumoniae* I | >800 |
| 54 | *Dipl. pneumoniae* II | >800 |
| 55 | *Dipl. pneumoniae* III | >800 |
| 56 | *B. subtilis* ATCC-6633 | >800 |
| 57 | *S. lutea* PCI-1001 | 250 |
| 58 | *Coryn. diphtheriae* PW8 | 800 |
| 59 | *Coryn. diphtheriae* A-7 | >800 |
| 60 | *Coryn. diphtheriae* AK O-222 | >800 |
| 61 | *Coryn. diphtheriae* M 406 MGL | >800 |
| 62 | *Coryn. diphtheriae* AK O-167 | G800 |
| 63 | *Mycob. tuberculosis* 607 | >800 |

[a] Entries 1–25 taken from ref 1; entries 26–63 taken from ref 2.

workers found that bicyclomycin-treated cells showed a significant *increase* in the diaminopimelic acid–diaminopimelic acid (DAP–DAP) linkage in peptidoglycan. The DAP–DAP bridge is a normal interpeptide cross-link that is rapidly produced by nascent peptidoglycan[15] and accounts for 12–17% of the total interpeptide cross-linking of peptidoglycan. The relative amount of DAP–DAP increased significantly in the

**TABLE II. Antibacterial Activity in Vitro of 5-Alkylene and 5-Imino Derivatives of Bicyclomycin**

| | MIC of compounds, mcg/mL | | | |
|---|---|---|---|---|
| organism | 1 (bicyclomycin) | 39 | 40 | 48 |
| *Haemophilis influenzae* NCTC 4560 | 3.1 | >100 | >100 | >100 |
| *Escherichia coli* 205 | 12.5 | 25 | 25 | 25 |
| *E. coli* 205 $R^{+}_{TEM}$ | 12.5 | 25 | 50 | 50 |
| *E. coli* 16 | 25 | 50 | 100 | 100 |
| *Salmonella typhimurium* 277 | 25 | 50 | 100 | 50 |
| *Enterobacter cloacae* P99 | 50 | >100 | >100 | >100 |
| *E. cloacae* 1404 | 50 | 100 | >100 | 100 |
| *Klebsiella pneumoniae* 327 | 25 | 100 | >100 | 100 |
| *Proteus mirabilis* 564 | >100 | 100 | >100 | 100 |
| *P. mirabilis* 1219 | >100 | 50 | 100 | 100 |
| *P. retigeri* 856 | >100 | 25 | 25 | >100 |
| *P. morganii* 2359 | >100 | 100 | >100 | >100 |
| *P. morganii* 1518 | >100 | 100 | >100 | >100 |
| *Pseudomonas aeruginosa* ATCC 12055 | >100 | >100 | >100 | >100 |
| *Serratia marcescens* 344 | >100 | 100 | >100 | >100 |

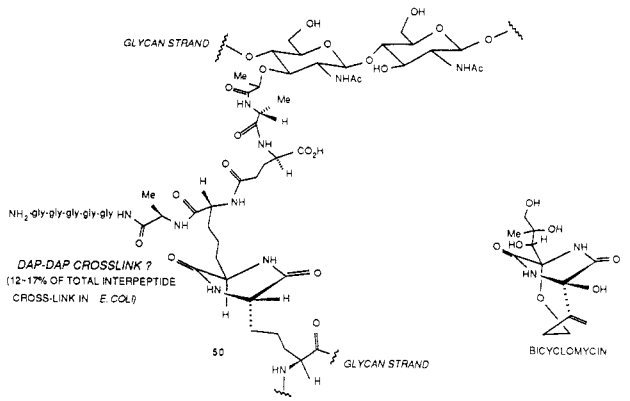
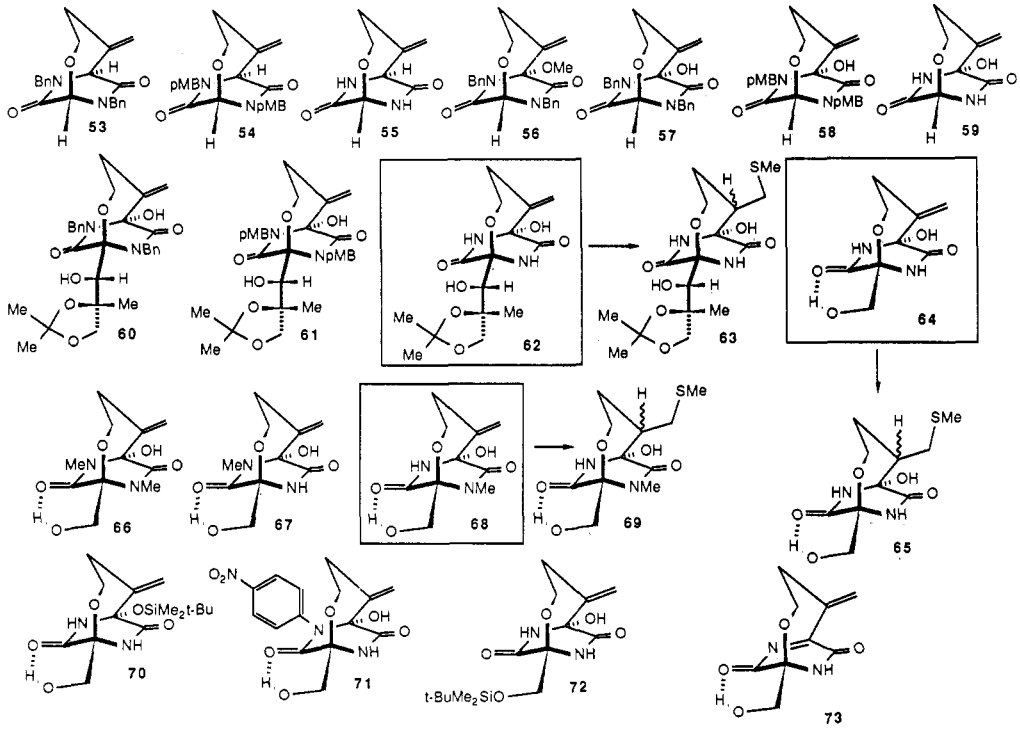



**Figure 1.** *E. coli* peptidoglycan.

presence of bicyclomycin, and a concomitant *decrease* in the amount of the more usual diaminopimelyl-D-alanine interpeptide linkages resulted. These results provide the provocative suggestion that bicyclomycin inhibits the amidase(s) that are normally responsible for cleaving the DAP–DAP bond in the final stages of peptidoglycan assembly that concomitantly results in an *increase* in the more abundant diaminopimelyl-D-alanine interpeptide bonds. The excess of the DAP–DAP linkage resulting from the hypothetical protease enzyme inhibition caused by bicyclomycin would, on the one hand, impede the normal remodeling of growing peptidoglycan whose structure would remain "tangled" by uncleavable DAP–DAP bonds and, on the other hand, impair the formation of the more usual diaminopimelyl-D-alanine interpeptide linkages. According to this hypothesis, the lack of sensitivity of gram-positive bacteria to bicyclomycin could be explained by the fact that most of these bacteria lack δ-*meso*-diaminopimelic acid! It is also significant to point out that the site of attachment of lipoprotein to the peptidoglycan, as noted above by Tanaka,[12] is impaired by bicyclomycin and occurs through a lysine residue of the lipoprotein and a diaminopimelic acid residue of the peptidoglycan. Vazquez has proposed a hypothetical DAP–DAP cell structure (diketopiperazine[16] (**50**); Figure 1) of which bicyclomycin may be a "substrate analogue". The crucial questions raised by these findings are the following: (1) Are the bi-

**CHART II. Bicyclomycin Analogues Examined for Reaction with NaSMe at pH 12.5**

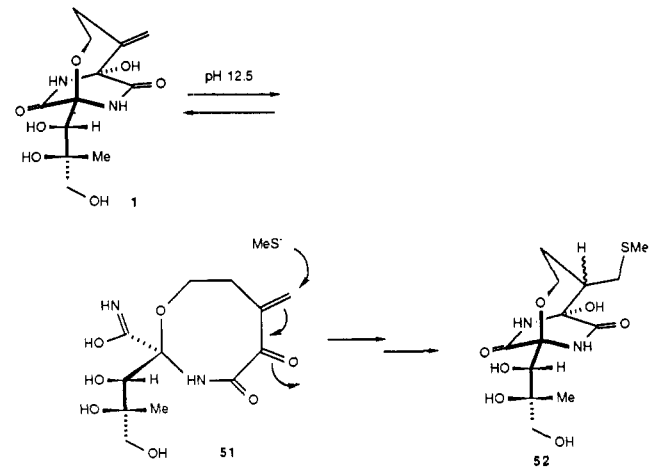


cyclomycin-binding proteins (BBP's) crucial *amidases* (i.e., proteases) that have a relationship to the formation/cleavage of DAP–DAP? (2) Do these proteins display *cis*-amidase (or diketopiperazinease) activity? (3) What is the normal cellular substrate for these enzymes? Is it **50** as suggested by Vazquez? (4) What is the chemical mechanism by which bicyclomycin becomes covalently and stoichiometrically attached to the BBP's?

Answers to the important former questions will have to await further investigation. Work has already been published relating to the latter on the chemical mechanism of action. The first paper dealing with this subject was a report in 1979 by Iseki and collaborators[17] who found that bicyclomycin undergoes a regiospecific addition of sodium methanethiolate at pH 12.5 to afford the sulfide **52** (Scheme 10). This reaction was proposed to be biologically significant since saturation of the C-5 olefinic residue (**6**, dihydrobicyclomycin) results in a biologically inactive species. In addition, the semisynthetic bicyclomycin derivatives prepared by Müller et al.[9] that retained biological activity also contained an unsaturated system at C-5. Accordingly, it has been suggested[17] that "...the terminal olefinic group reacts with the sulfhydryl groups of the inner-membrane proteins and covalent bonds are formed. Thus the olefinic double bond seems to be the reactive site or functional site of bicyclomycin... The thiol group or thiolate anion may attack the terminal olefinic group of bicyclomycin to form an enolate anion, which may then be protonated". Additionally, the binding of [$^{14}$C]bicyclomycin to whole cells of *E. coli* could be inhibited by the addition of thiols such as dithiothreitol and 2-mercaptoethanol.

The mechanism, structural requirements and biological relevance of this reaction has been carefully studied in our laboratories.[18,19] Careful inspection of the bicyclomycin structure and consideration of the regiochemistry of the mercaptan addition led to the

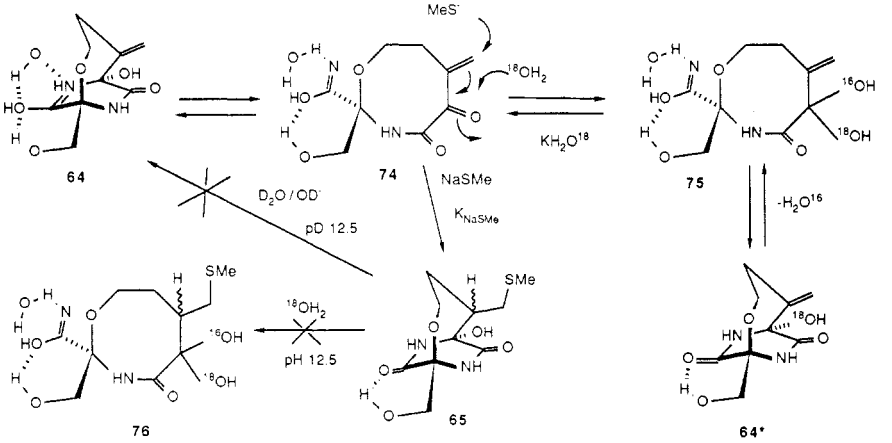
**SCHEME 10**



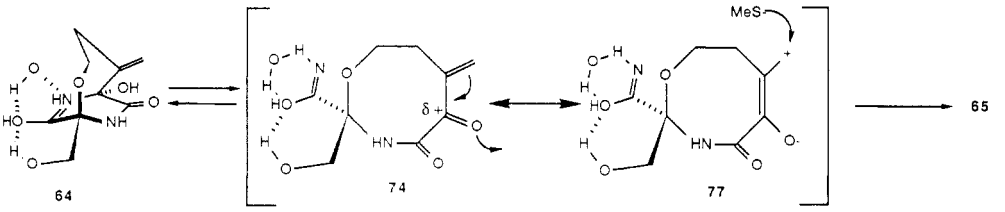
suggested[20] mechanistic pathway depicted in Scheme 10. Tautomeric ring opening of **1** was envisioned to produce the monocyclic eight-membered ring $\alpha,\beta$-unsaturated ketone **51**, which should function as a reactive Michael-type acceptor. Such a "latent Michael-acceptor" mechanism readily accounts for the regiochemistry of the adduct (**52**) formed. In order to understand the process represented in Scheme 10 and the relationship to the biomechanism, these workers set out (1) to establish the minimum structural requirements of the bicyclo[4.2.2] nucleus that allows for sulfide formation; (2) to demonstrate the intermediacy of the ring-opened, monocyclic eight-membered ring $\alpha,\beta$-unsaturated ketone **51**; and (3) to establish whether or not a correlation exists between the capacity for structures to undergo the addition of thiols at C-5 and the corresponding capacity of such reactive structures to display biological activity.

The totally synthetic and semisynthetic analogues shown in Chart 2 were subjected to reaction with NaSMe in THF/$H_2O$ at pH 12.5. It was found that

SCHEME 11



SCHEME 12



only **62**, **64**, and **68** reacted with NaSMe furnishing the corresponding sulfide adducts **63**, **65**, and **69**, respectively. These rather surprising observations defined the minimum structural requirements for thiolate addition: (1) a free (N–H) amide must be present at N-10 (compare **67** and **68**); (2) an *exo*-methylene moiety must exist at C-5; (3) a bridgehead hydroxyl must exist at C-6; and (4) a C-1′ hydroxyalkyl moiety must be present to allow for tautomeric ring opening and subsequent Michael addition.

For the thiolate-reactive substrates, the intermediacy of the ring-opened ketone could be demonstrated by incubating the substrate in (98% $^{18}O$) $^{18}OH_2$ at pH 12.5 and analysis of aliquots by mass spectroscopy. Structure **64** represents the minimum structural array for this reaction to proceed and was studied in the greatest detail. Thus, **64** incorporated 40–50% $^{18}O$ at C-6 after 30 min at pH 12.5. This experiment demonstrated that **64** undergoes ring opening to **74** (Scheme 11); hydration of the putative C-6 ketone (**75**) and loss of $^{16}OH_2$ by reversible mass action furnish the isotopically labeled **64**. The fact that derivatives **66**, **70**, and **72** do not incorporate $^{18}O$ under the same conditions clearly explains why these substrates do not react with NaSMe: they do not tautomerize to the ring-opened Michael acceptor. Kinetics for the reaction of **64** with NaSMe were measured over several half-lives ($t_{1/2} \approx 8$ min at 25 °C) at various temperatures (Table 3). The reaction displayed a significant temperature dependence from which the apparent Arrhenius activation parameters were calculated: $E_a = 18.1 \pm 0.6$ kcal/mol; $\Delta H^{\ddagger} = 17.5 \pm 0.6$ kcal/mol; ln $A = 28$; $\Delta S^{\ddagger} = -5$ eu ± 4 cal (mol·deg); $\Delta G^{\ddagger} = 19 \pm 1.0$ kcal/mol. A solvent deuterium isotope effect $K_{H_2O}/K_{D_2O} \approx 2.4$ indicates that a proton transfer from solvent occurs in the rate-limiting step. The rate at pH 7 (25 °C, $K = 3.2 \times 10^{-4}$ $M^{-1}$ $s^{-1}$) is ca. 600 times slower than that at pH 12.5; below pH 7, the reaction slows down considerably, and at pH 3.5 there is no observable formation of **65**.

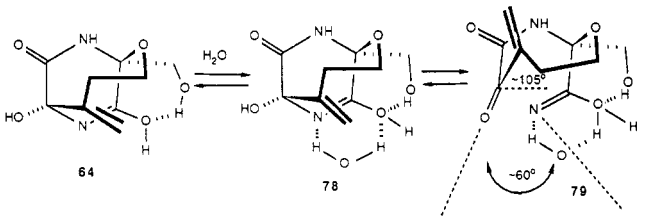
**TABLE III. Rate Constants for the Reaction of 64 with NaSMe in Aqueous THF (pH 12.5) at Various Temperatures**

| temp, °C | $T^{-1}$, $10^3$ | rate const ($k$), $M^{-1}$ $s^{-1}$ | ln $k$ |
|---|---|---|---|
| 0 | 3.663 | $1.29 \times 10^{-2}$ | −4.351 |
| 7 | 3.571 | $3.15 \times 10^{-2}$ | −3.458 |
| 15 | 3.472 | $8.09 \times 10^{-2}$ | −2.515 |
| 25 | 3.356 | $1.95 \times 10^{-1}$ | −1.635 |

Performing the reaction of **64** with NaSMe in (98% $^{18}O$) $^{18}OH_2$ at pH 12.5 resulted in the production of **65** with no $^{18}O$ incorporation. This result was very important for several reasons: (1) It is indicative that the rate of reaction of **64** with NaSMe is much faster than the rate of hydration and exchange of **74** via **75**. (2) The fact that the sulfide adduct (**65**) does *not* incorporate $^{18}O$ rigorously *excludes* the base-promoted expulsion of the C-6-OH forming a C-6/N-10-amidine (**73**) as a possible reactive intermediate since such an intermediate would *necessarily* incorporate a significant amount of $^{18}O$ from the solvent (98% $^{18}OH_2$) at C-6 in forming **65**. Exclusion of this pathway is important as it directly relates to one conceptual function of the obligate secondary (–NH–) amide at N-10 that can be rigorously excluded as a mechanistic alternative. (3) Once formed, the sulfide adduct (**65**) does *not* display reversible ring-opening behavior (as evidenced by lack of $^{18}O$ incorporation). This result can be interpreted by considering that the C-5 olefin of **64** can provide resonance stabilization to the highly electropositive C-6 ketone (canonical form **77**; Scheme 12) upon ring opening that is *not* enjoyed by the sulfide adduct **65**. Further evidence for the irreversibility of this reaction was obtained by incubating **65** in $D_2O$/OD at pD 12.5 showed no trace of retro-Michael reaction to **64**, nor was there any H/D exchange at C-5.

Last, it was demonstrated that a good H-bonding solvent is required for this reaction to proceed. Attempts at running the reaction in anhydrous THF containing 2.5 equiv of NaSMe (or a large molar excess)

SCHEME 13




resulted in no reaction. However, both anhydrous DMSO and formamide proved to be suitable solvents for the conversion of **64** → **65**. The fact that no reaction occurs in dry THF and the kinetics in $H_2O$ indicate a solvent deuterium isotope effect suggests that proton transfer *intermolecularly* is an obligate feature of this reaction. *Intramolecular* proton transfer (or H bonding) from the C-1′-hydroxyalkyl group is also a requirement since **72** is an unreactive substrate.

In order to explain the structural subtleties of the tautomeric ring opening and subsequent Michael addition, the authors proposed that the geometry for the ring-opening/ring-closing equilibria with respect to the C-6/N-10 $\sigma$ bond is confined to a ~60° vector cone (Scheme 13). The ideal Dunitz vector of ca 105° is precluded due to the rigidity of the amide bonds. Thus, to compensate for the strain energy required to cleave this bond in the transition state for ring opening, both *intramolecular* and *intermolecular* proton catalysis is required. Thus, only the secondary (–NH–) amides are capable of forming (through the imino alcohol tautomer shown in Scheme 13) the solvent H-bonded species **78**. The additional stabilization of the C-5 olefin for the forming carbonyl at C-6 (cf. **77**) is intimately associated with the requirement for proton catalysis.

The important question then was to evaluate the biological relevance of this reaction. Many of the compounds reported in this study[18-20] have been evaluated for antimicrobial activity. Only compounds **1** and **57** displayed activity; compounds **62** and **64** were reported as being biologically inactive, and compound **57** is unreactive toward NaDMe. The lack of correlation between simple thiolate susceptibility and antimicrobial activity indicates that this interesting and complex reaction *alone* cannot be used as the biomechanistic template. The fact that the synthetic compound **57** is active and the corresponding deoxy derivative **53** is not hints that **57** may represent the minimum *structural* array to obtain a biologically active compound of this general class. However, the spectrum of activity of **57** is distinct from that for bicyclomycin; these data are presented in Table 4. Compound **57** displays modest activity toward gram-positive organisms *Micrococcus luteus*, *Bacillus megaterium*, *Bacillus subtilus*, *Bacillus sp.* TA, and *Streptomyces cellulosae*, whereas bicyclomycin is only active toward gram-negative organisms. Since **57** was assayed as a racemate, the effective MIC's for the biologically active antipode (not established as of this writing) should be divided by 0.5. Clearly, **57** has a distinct cellular target from **1**, the mechanism of action of this compound being an equally unsolved and interesting problem.

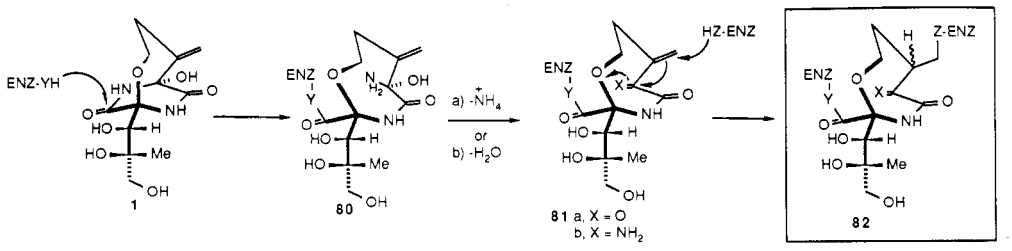
**TABLE IV. Minimal Inhibitory Concentration[a] (μg/mL)**

| | | **57** ($R^1$ = $CH_2Ph$, $R_2$ = OH, $R_3$ = H) | bicyclomycin Ro 21-7023 |
|---|---|---|---|
| G⁻ rods | *Pseudomonas aeruginosa* 56 | >1000 | >1000 |
| | *Proteus vulgaris* 101N | >1000 | >1000 |
| | *Escherichia coli* 94 | >1000 | 250 |
| | *Klebsiella pneumoniae* 369 | >1000 | 250 |
| | *Serratia marcescens* SM | >1000 | >1000 |
| | *Serratia sp.* 101 | >1000 | >1000 |
| | *Acinetobacter calcoaceticus* $PCI_3$ | >1000 | 1000 |
| G⁺ cocci | *Streptococcus faecium* ATCC 8043 | >1000 | >1000 |
| | *Staphylococcus aureus* 82 | >1000 | >1000 |
| | *Micrococcus luteus* PCI | 500 | >1000 |
| G⁺ rods | *Bacillus megaterium* 164 | 500 | >1000 |
| | *Bacillus sp.* E | >1000 | >1000 |
| | *Bacillus subtilis* | 250 | >1000 |
| | *Mycobacterium phlei* 78 | >1000 | 1000 |
| G⁺ filament molds | *Streptomyces cellulosae* 097 | 500 | 500 |
| | *Paecilomyces varioti* M16 | >1000 | >1000 |
| | *Penicillium digitatum* 0184 | >1000 | >1000 |
| yeasts | *Candida albicans* 155 | >1000 | >1000 |
| | *Saccharomyces cerevisiae* 90 | >1000 | >1000 |

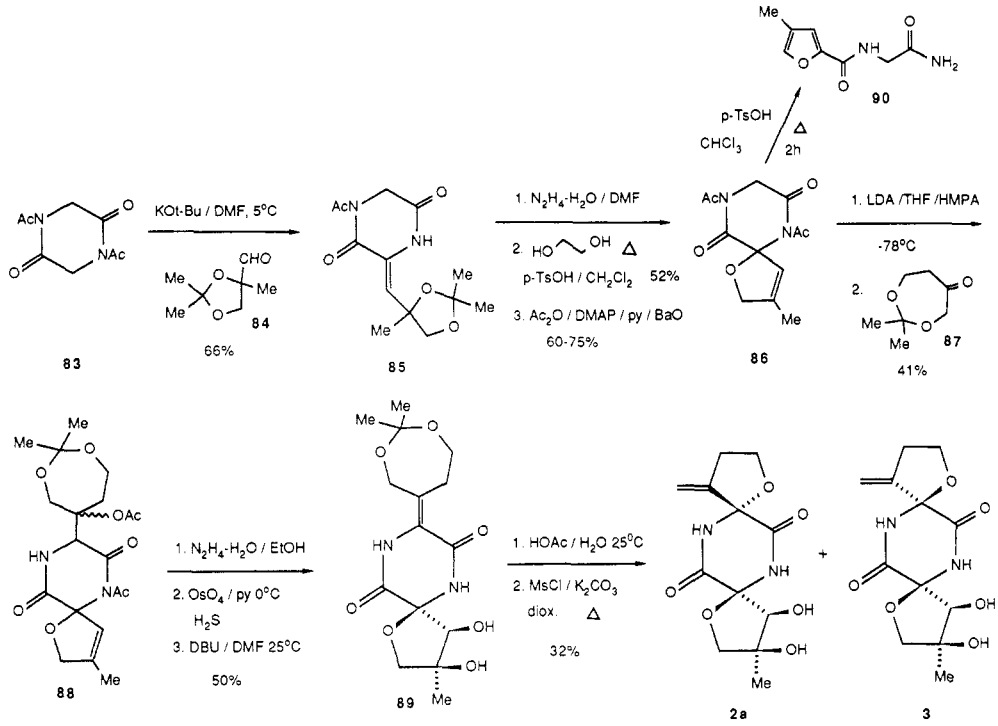
[a] Lowest concentration still showing zone of inhibition by the agar diffusion well method (serial dilutions up to 1000 μg/mL).

In 1985, these workers proposed[20] a similar yet distinct chemical mechanism of action for bicyclomycin that potentially embraces **1**, **57**, and the biochemical data of Vazquez appearing nearly 2 years later. As depicted in Scheme 14, the assumption was made that the bicyclomycin-binding proteins (BBP's) are crucial proteases that react at the 9,10-amide bond of **1** to initially produce the acyl-enzyme species **80**. This ring-opening process produces an unstable hemiamino hemiketal which should either lose $NH_4^+$ or $H_2O$ to furnish the electrophilic $\alpha,\beta$-unsaturated species **81a** or **81b**, which can similarly function as a Michael acceptor. The result of 1,4-addition to **81** is the alkylated enzyme **82** that should result in loss of catalytic activity. Thus, bicyclomycin would be viewed as a suicide ($K_{cat}$) inhibitor of a protease responsible for the critical chemical turnover of *amide bonds* during bacterial cell growth. The most recent findings of Vazquez and collaborators that implicate inhibition of a DAP–DAP protease render this mechanistic hypothesis all the more provocative and reasonable. The fact that compound **57** has a different spectrum of activity than bicyclomycin does not exclude the reasonable possibility that **57** may also

SCHEME 14



SCHEME 15



be interacting with a (distinct) protease(s) from 1 but may interact with this hypothetical protein via the same chemical mechanism depicted for 1 in Scheme 14.

The elucidation of the chemical mechanism of action of bicyclomycin and analogues remains an important and unsolved problem. The rigorous elucidation of the specific role of the BBP's, the cellular target(s) of **57** in gram-positive organisms and other semisynthetic species (i.e., **39**, **40**, and **48**), can be viewed as an unusual opportunity to further exploit the unique structural and mechanistic possibilities represented by **1**. It is expected that continued studies on these problems will open an exciting new chapter in bacterial cell wall chemistry and lead to the development of new chemotherapeutic materials. The low in vivo toxicity of bicyclomycin may be partially attributed to the general reluctance of this system to undergo reactions with biological nucleophiles at physiological pH as demonstrated above.[18,19] In a field where workers often struggle to "functionalize-out" the high cellular toxicity often accompanying biologically active substances, a property that often compromises or completely nullifies the eventual clinical utility of a given class, it seems unfortunate that a more intensive, focused, and rational approach to exploit the potential opportunities presented by bicyclomycin has not been evident.

## V. Synthetic Studies

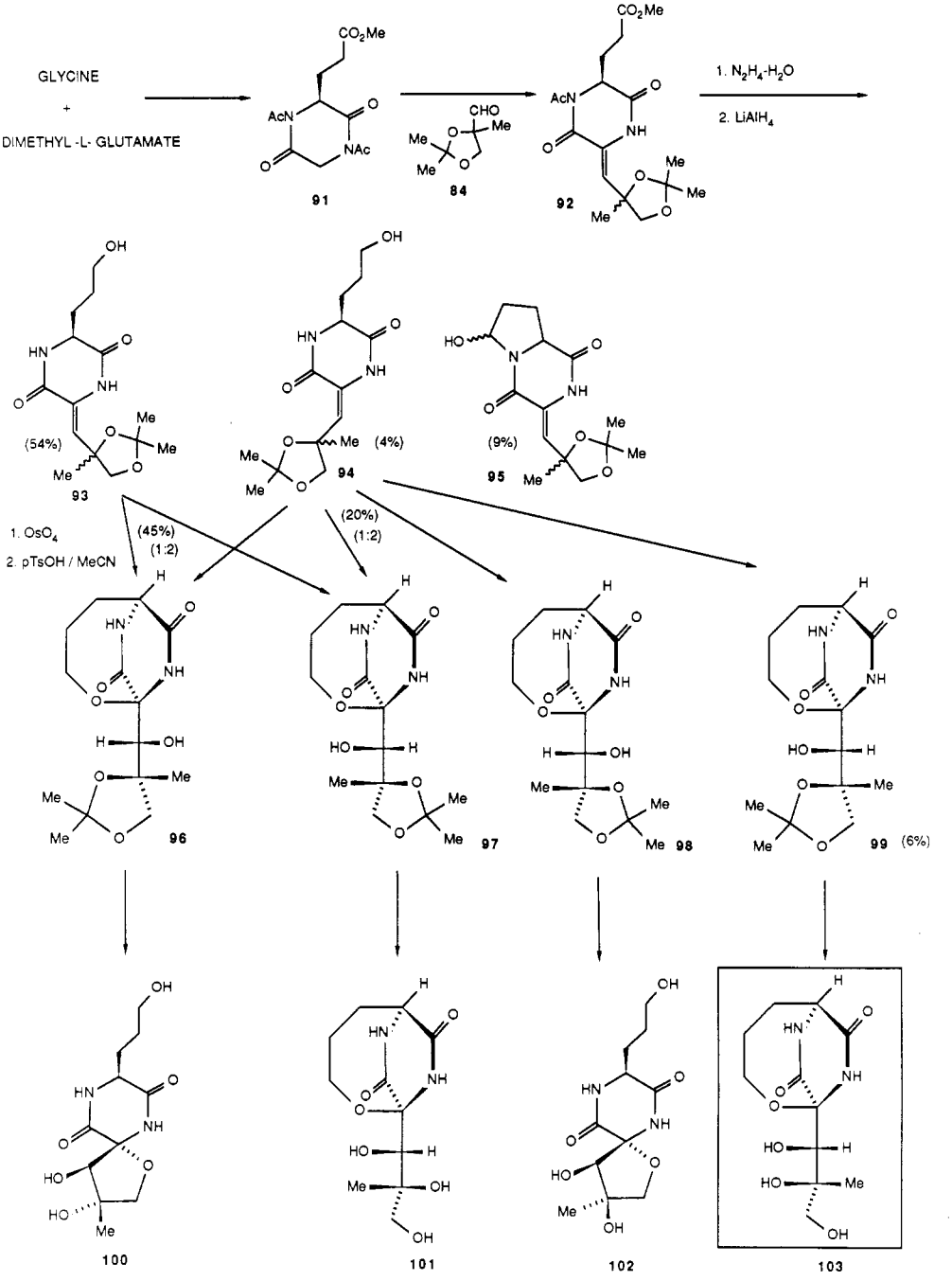
Not unlike the events following the disclosure of many a new antibiotic structure, the reports from Fujisawa and Niigata University in 1972 were soon followed by publications from the synthetic community to address the challenge posed by the unusual structure **1**. The history of synthetic approaches to bicyclomycin commenced with a landmark paper by Maag and associates at Hoffman-La Roche in 1978[7] wherein the absolute stereochemistry of **1** was established through an X-ray structure determination and total synthesis of the acid-catalyzed bis-spiro dehydration products **2** and **3**; the synthesis is detailed in Scheme 15. This account summarizes the synthetic papers as they appeared in chronological order to provide a sense of the excitement that ensued as the fundamental properties of this molecule emerged from the synthetic exercises.

*N*,*N*-Diacetylglycine anhydride (**83**) was condensed with the protected four-carbon aldehyde **84** in the presence of *t*-BuOK in DMF to furnish the unsaturated piperazinedione **85** (66%). Removal of the remaining acetyl group with hydrazine hydrate followed by trans ketalization and rearrangement (ethylene glycol, *p*-TSOH, $CH_2Cl_2$, reflux) furnished the spiro piperazinedione **86**. It is interesting to note that under slightly more vigorous conditions (*p*-TsOH, $CHCl_3$, reflux) **86** further rearranges to the furan **90**.

Reaction of the diacetyl derivative **86** with ketone **87** furnished the aldol product **88** (41%, mixture of isomers). Removal of the acetyl group, followed by osmylation and elimination, furnished **89** in 50% overall yield. The osmium tetroxide reaction proceeds stereospecifically from the face of the olefin proximal to the amide nitrogen. Removal of the ketal and mesylation of the allylic hydroxyl result in a concomitant intramolecular $S_N2'$ cyclization to give a mixture of the bis-spiro products **2a** and **3** in 32% yield. The absolute configuration of bicyclomycin was established as noted above, on the 2′(*R*)-bromobenzoate **2b**. The authors end this account by pointing out that "synthesis schemes for bicyclomycin should probably be contrived in a way that circumvents the energy minimum represented by **2** (and **3**)". This strategic warning is significant in that it will help define the general strategies that have evolved in tackling the bridged bicyclo[4.2.2] ring system.

Following this approach, a preliminary account that addressed preparation of bicyclo[4.2.2] ring system was presented[21,22] by Maag. As shown in Scheme 16, dimethyl-L-glutamate and glycine were condensed by

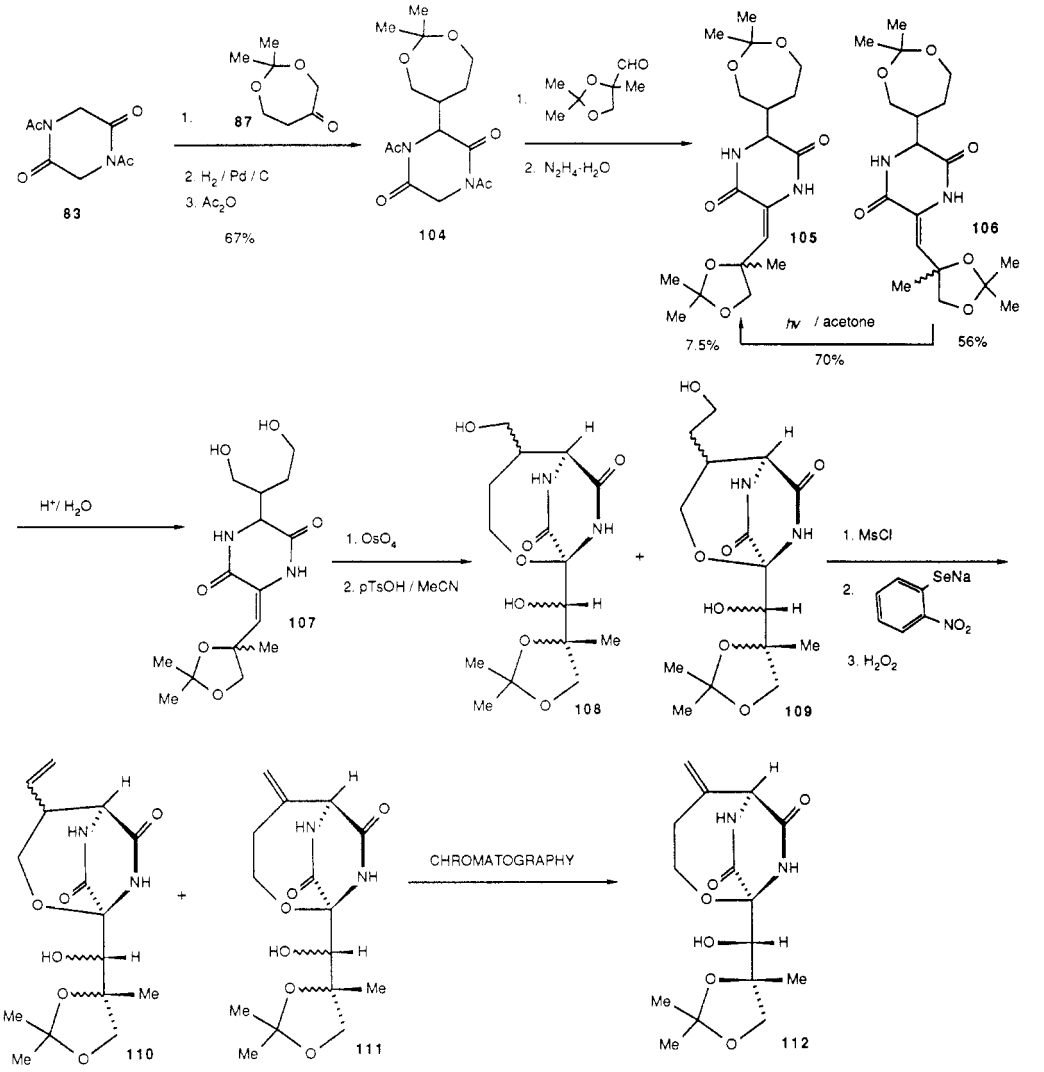
SCHEME 16



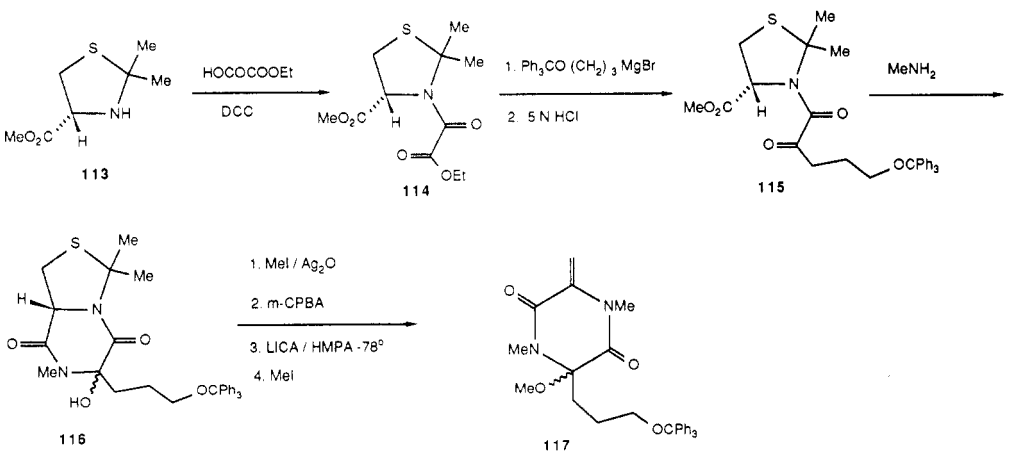

standard peptide coupling to furnish the piperazinedione **91**. Condensation with aldehyde **84** furnished a mixture of the unsaturated derivatives **92** (77%) with the *Z* isomer predominating. Removal of the acetyl group and LAH reduction furnished a mixture of **93–95**. Osmylation and acid-catalyzed cyclization of the incipient triols derived from **93** and **94** furnished the desired bicyclo[4.2.2] ring systems **96–99**. Unfortunately, the major isomer **93** gave rise to only **96** and **97** (1:2 ratio), bearing the incorrect relative stereochemistry. The minor isomer **94** gave rise to all four compounds (**96** + **97** to **98** + **99** 1:2 ratio in 20% combined yield). Only **99** bears the correct relative configuration and is obtained in only ca 6% yield from **94**. The structures of **97** and **98** were confirmed by X-ray analysis, and the $^1H$ NMR behavior of **99** when compared to **24** established the stereochemistry assigned for **99**. Interestingly, it was found that only the isomers bearing the 1′*S* configuration (**97** and **99**) could be hydrolyzed to the triols **101** and **103**, respectively. The 1′*R* isomers gave the corresponding spiro structures **100** and **102**.

This model study was extended to embrace the C-5-*exo*-methylene group as shown in Scheme 17. The requisite isoleucine moiety was introduced via condensation of **83** with ketone **87**. The intermediate dehydro derivative (cf. **89**, Scheme 15) was reduced, acetylated, and condensed with **84** to afford a mixture of (*Z*)-**106** and (*E*)-**105** isomers. On the basis of their experience with **93** and **94**, the minor isomer could be obtained in 70% yield by photoisomerization of the *Z* isomer in acetone. Selective removal of the seven-membered ring ketal, osmylation to the tetrol, and acid-catalyzed cyclization furnished a mixture of the bicyclo[4.2.2] system **108** and bicyclo[3.2.2] system **109**, with the undesired system **109** predominating. These were not separated at this stage but rather eliminated to **110** and

SCHEME 17




SCHEME 18

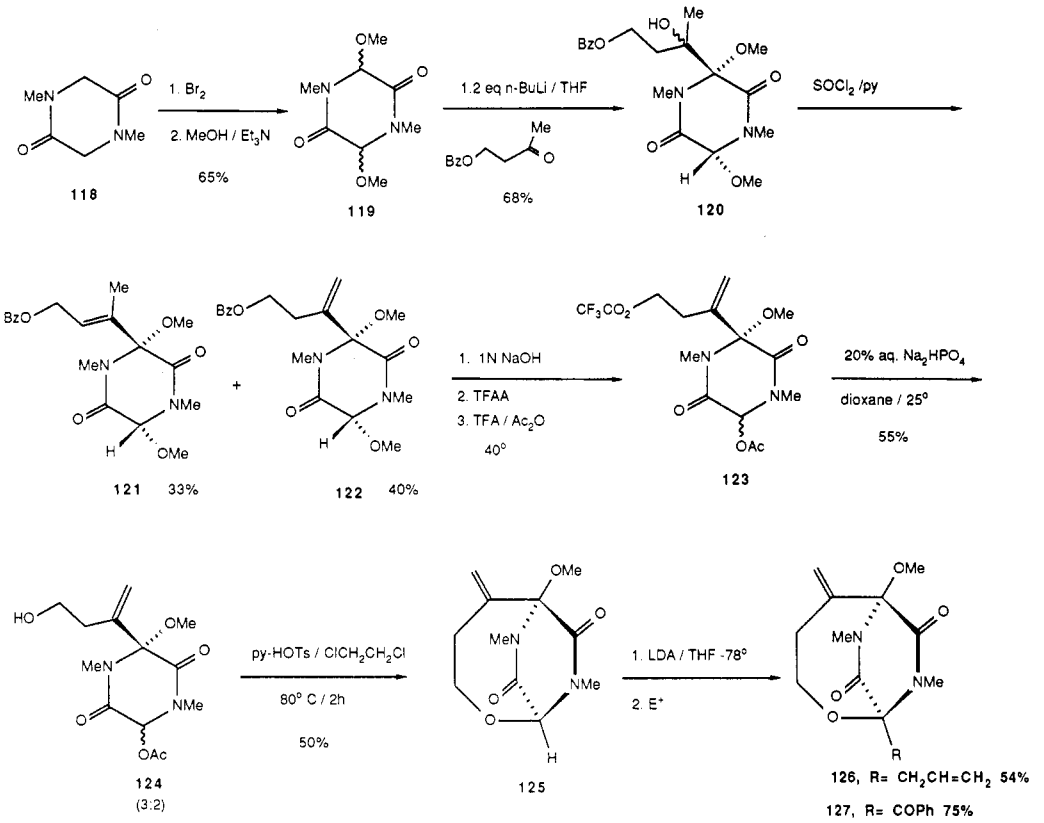



111 (isomer mixture), and compound **112** was separated by chromatography as one of four diastereomers of **111**. The final conversion of the C-6-desoxybicyclomycin derivative **112** to **1** would involve bridgehead hydroxylation but was not carried out successfully.

Dunkerton[23] reported the preparation of a potential precursor to a model bicyclo[4.2.2] system as shown in Scheme 18. Condensation of the cysteine derivative **113** with ethyl oxalate furnished **114** which was homologated to the α-keto amide **115** via a Grignard addition. Aminolysis with methylamine led to piperazinedione **116**. Methylation of the hydroxyl group, oxidation to the sulfoxide, and elimination promoted by lithium isopropylcyclohexylamide (LICA) and N-methylation provided **117**. Further conversion of **117** to bicyclic material has not been reported. However, as Maag had pointed out above, avoidance of spiro formation from **117** would have to be carefully avoided.

Shortly thereafter, two groups[24,25] independently and simultaneously discovered that a bicyclo[4.2.2]nucleus

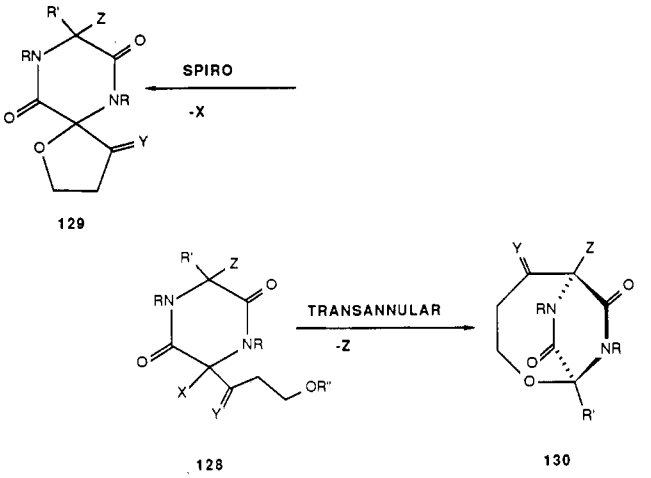
SCHEME 19




could be elaborated via bridgehead carbanion functionalization. Nakatsuka and co-workers[24] reported the model study illustrated in Scheme 19. Sarcosine anhydride is brominated and methanolized to furnish **119** as a syn–anti mixture. Enolate formation with *n*-BuLi and condensation with 4-*O*-benzoyl-2-ketobutane to afford exclusively the syn isomer as a 1:1 mixture of epimers at the tertiary hydroxyl carbon. Treatment of this material with thionyl chloride gave a mixture of *endo*-**121** and *exo*-**122** olefins in 33% and 40% yields, respectively. The desired isomer (**122**) was hydrolyzed and reprotected as the trifluoroacetate. In the key step, the secondary, methoxy group was selectively replaced with an acetate by treatment with acetic anhydride–trifluoroacetic acid to furnish **123**. The trifluoroacetate was removed and the alcohol (**124**, 3:2 stereoisomeric mixture) cyclized in the presence of pyridinium tosylate in dichloroethane at 80 °C for 2 h afforded the desired bicyclo[4.2.2] derivative **125** without formation of the isomeric spiro structure. Finally, these workers found that treatment of **125** with LDA in THF followed by quenching with allyl bromide or benzoyl chloride furnished the alkylated derivatives **126** and **127** in 54% and 75% yields, respectively. This successful model study formed the conceptual basis from which their total synthesis later emerged.

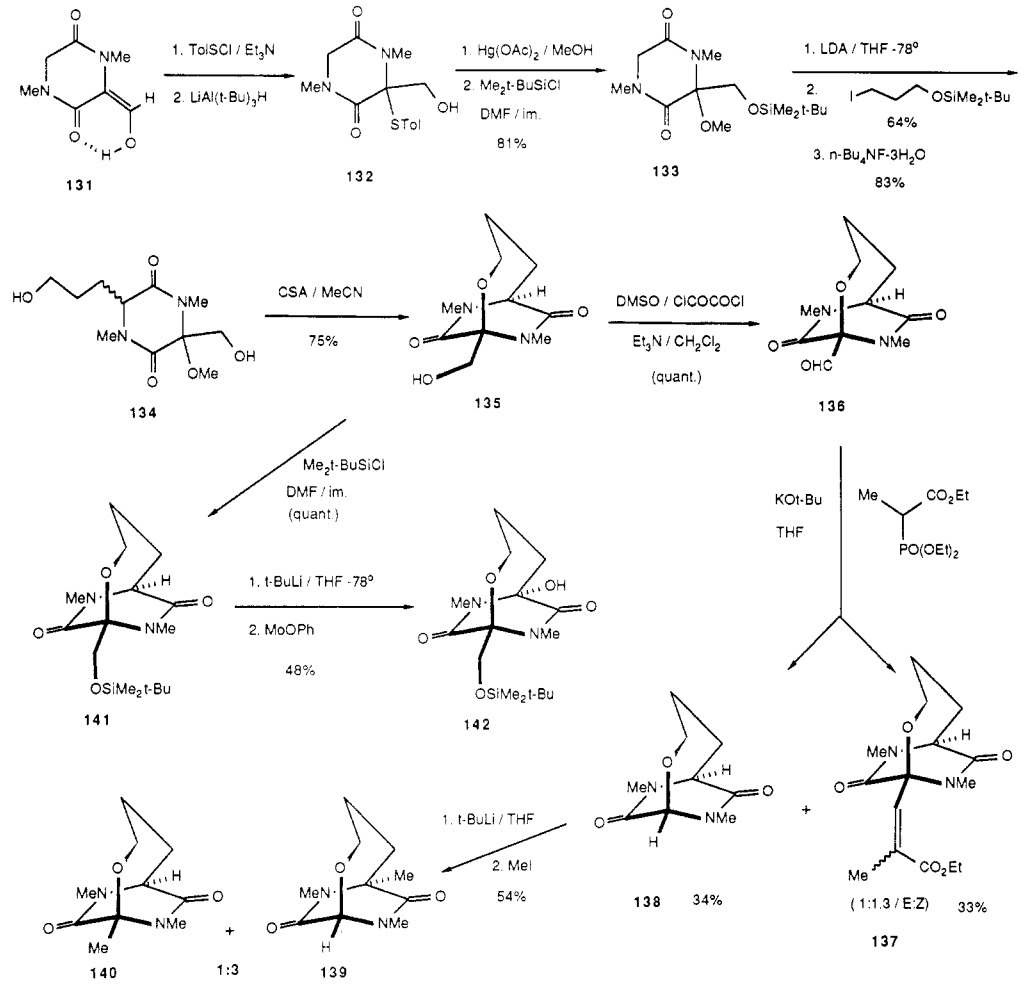
At this juncture, it is appropriate to point out that these workers were able to effectively circumvent the "spiro pitfall" alluded to by Maag.[7] By choosing a monocyclic precursor that has the same oxidation pattern as bicyclomycin such as **128** where X and Z are both heteroatoms (i.e., oxygen), spiro ring formation (**129**) would be expected to predominate where X and Z have the same leaving group ability or relative $pK_a$. The spiro mode of closure is both kinetically and thermodynamically favored over the desired transannular cyclization (**130**) (Scheme 20). However, as was shown by these workers, if Z is made into a much more powerful leaving group than X, the desired transannular cyclization can be carried out effectively. In Maag's second model study (Schemes 16 and 17), the spiro problem was completely avoided by choosing a substrate (**128**) where X is not a leaving group such as hydrogen. In this manner, only the transannular cyclization mode is possible as observed. However, this leaves in its wake the problem of introducing the bridgehead hydroxyl group. A solution to this dilemma originally posed by Maag[21,22] was accidentally discovered in the authors' laboratories.

SCHEME 20

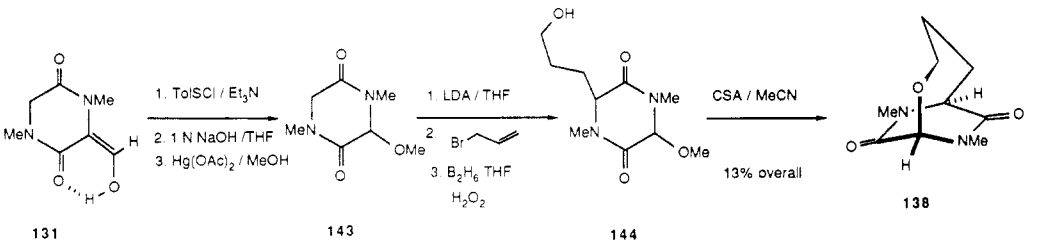



As shown in Scheme 21, formylpiperazinedione **131** is sulfenylated and reduced to **132**. Methanolysis in the presence of mercuric acetate and protection furnished **133**. Enolate alkylation and deprotection gave the polar diols **134** as a mixture of stereoisomers. These were

SCHEME 21



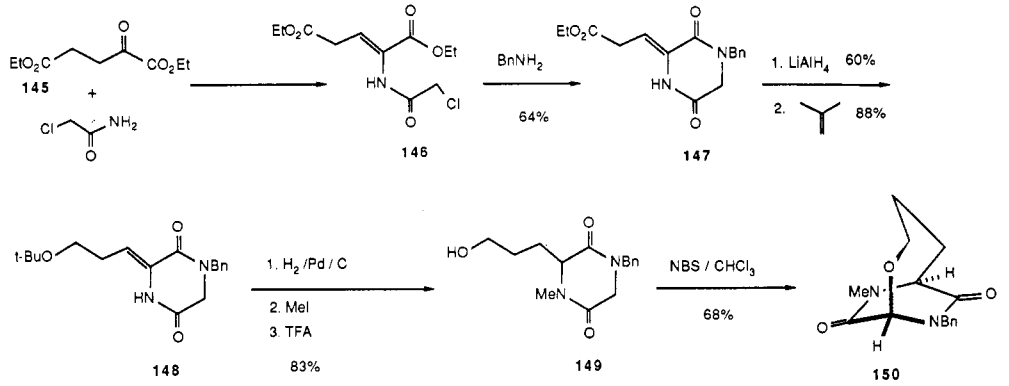
SCHEME 22



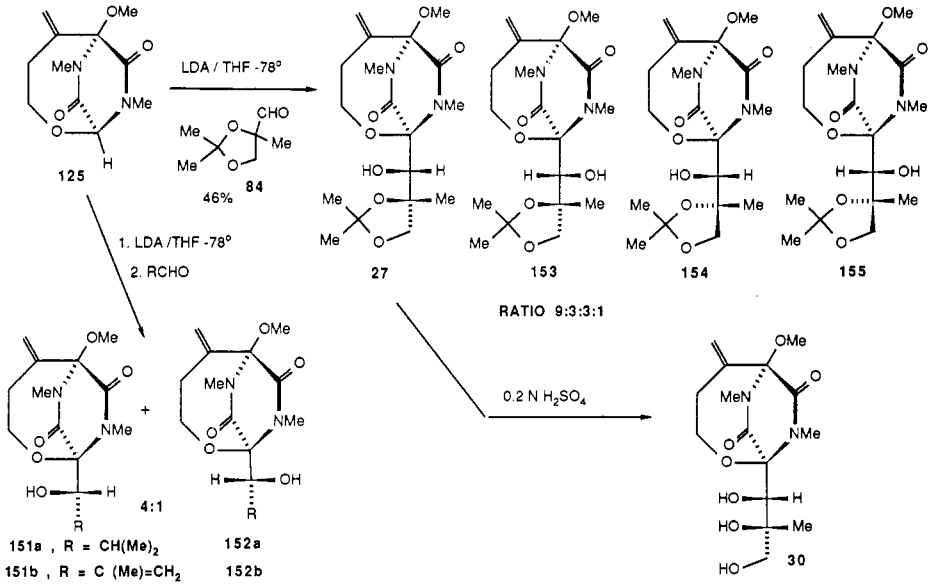
smoothly cyclized in the presence of camphorsulfonic acid (CSA) in warm acetonitrile to give a single bicyclic alcohol **135** in 75% yield. The original strategy was to elaborate the hydroxymethyl group of **135** (as a model) to the polyoxo side chain of **1**. Thus, oxidation to the aldehyde **136** using the Swern protocol and Wittig condensation furnished the desired olefins **137** as a 1:1.3 *E* to *Z* mixture in 33% yield. Surprisingly, the deformylated derivative **138** was also isolated from this reaction in 34% yield. The unexpected deformylation must have resulted from the retroaldol collapse of the intermediate oxyanion addition product of the aldehyde and the phosphorane expelling the corresponding bridgehead carbanion that is protonated upon workup to furnish **138**. The surprising ease of formation of the incipient bridgehead carbanion prompted an investigation of the bridgehead carbanion reactivity of **138**. Treatment of **138** with *t*-BuLi in THF at −78 °C and quenching with methyl iodide gave a mixture of **139** and **140** (54% combined) in a 3:1 ratio. This unexpected regioselectivity made obvious the possibility of introducing the required C-6 bridgehead hydroxyl group via bridgehead carbanion oxidation. Thus, silylation of **135** furnished **141**, which was treated with *t*-BuLi and quenched with oxodiperoxymolybdenum hexamethylphosphoric triamide pyridine complex (MoOPh) to furnish the bridgehead alcohol **142** in 48% yield. The structure of deformylation product **138** was unambiguously verified by the independent synthesis illustrated in Scheme 22. The fortuitous discovery of bridgehead carbanion reactivity, especially with regard to the regioselectivity observed in the alkylation of **138**, shaped the eventual strategy that led to the total synthesis of bicyclomycin from these laboratories.

Concomitant with these reports appeared an efficient one-step oxidative cyclization to construct the bicyclo[4.2.2] ring system by Shin, Sato, and Yoshimura.[26] As detailed in Scheme 23, chloroacetamide is condensed with ethyl 4-(ethoxycarbonyl)-2-oxobutanoate (**145**) to afford **146** followed by cyclization with benzylamine to

SCHEME 23




SCHEME 24

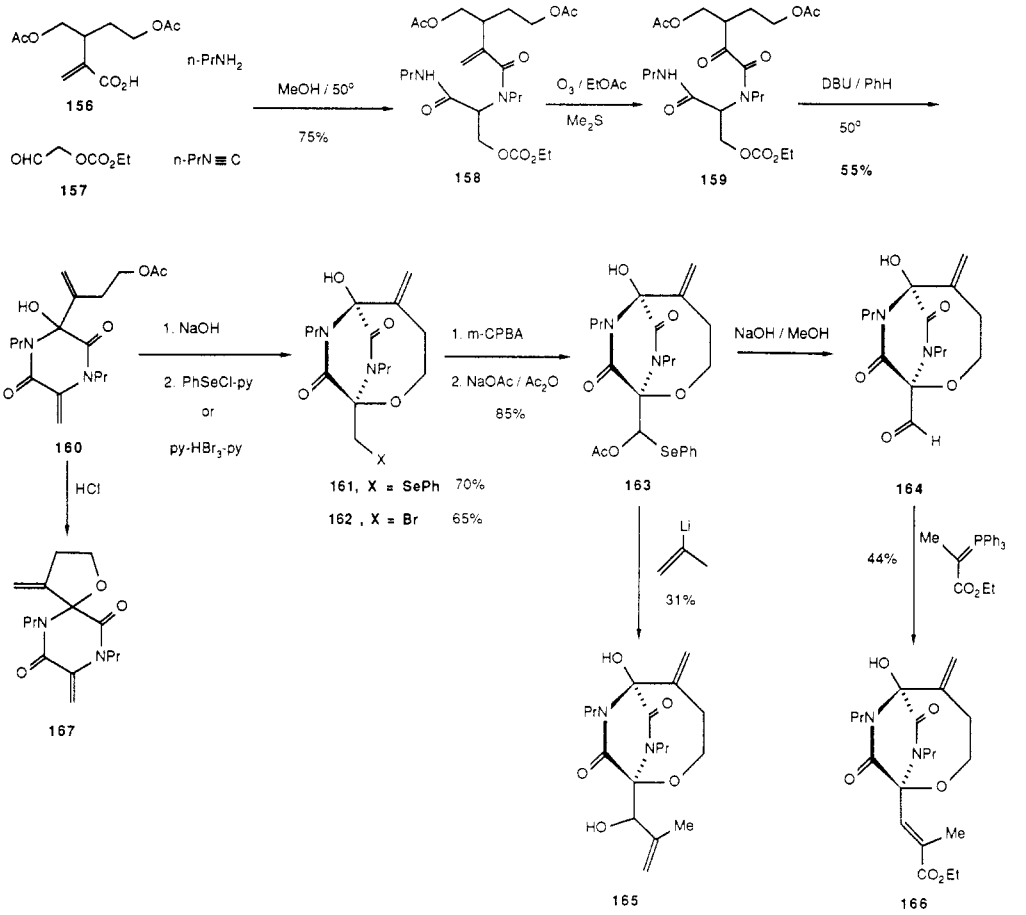



afford the unsaturated piperazinedione **147**. Reduction of the ester, protection of the resultant alcohol as the *tert*-butyl ether **148** followed by hydrogenation, N-methylation, and deprotection provided the key substrate **149**. It was found that **149** underwent selective monobromination with NBS in $CHCl_3$ at the unsubstituted position followed by concomitant ring closure to furnish the bicyclo[4.2.2] derivative **150** in 68% yield. No further elaboration of this ring system was reported, but the oxidative cyclization formed the basis for the subsequent total synthesis reported from this group.

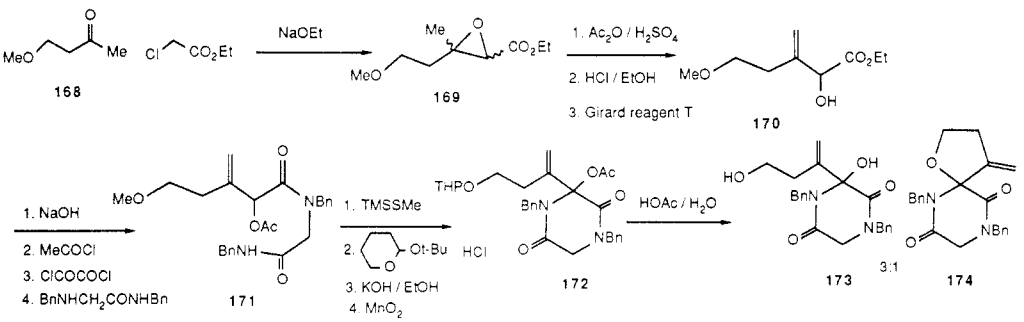
The Nagoya group quickly followed their initial report later the same year[27] with an examination of aldol condensations of **125** (Scheme 24). Thus, formation of the bridgehead carbanion of **125** with LDA in THF followed by quenching with isobutyraldehyde and methacrolein furnished the aldol products **151a,b** and **152a,b** in a 4:1 ratio favoring the 1′*S* relative configuration. By the same sequence, reaction with aldehyde **84** originally employed by Maag[7] produced the four aldol products **27**, **153**, **154**, and **155** in a 9:3:3:1 ratio in 46% combined yield. The major stereoisomer (**27**) was identical with the *N,N,O*-trimethyl-2′,3′-acetonide derivative reported above in the Ciba-Geigy study.[9] This double diastereodifferentiating aldol condensation favoring the natural relative configuration was exploited by all three groups that eventually completed the journey to **1**. Removal of the acetonide as was already known[9] for **27** furnished (±)-*N,N,O*-trimethylbicyclomycin.

Shortly prior to the above report, Fukuyama[28] and associates reported an approach to the bicyclomycin ring that shared some elements of all the above model studies (Scheme 25). By employing the Ugi four-component condensation, the unsaturated acid **156** provided the dipeptide **158** in 75% yield. Ozonolysis (**159**) followed by a double elimination/cyclization afforded the key precursor **160**. As in the case of the Nagoya[24] approach, proximal and distal α-positions of the piperazinedione had to be sufficiently differentiated to allow transannular closure (distal activation) to occur in the face of competing spiro cyclization. This was cleverly accomplished by either an intramolecular selenoetherification (**161**) or bromination (**162**) in good yield. Under these conditions, the spiro compound **167** was not formed. However, simply treating the diol derived from **160** with HCl in $CH_2Cl_2$ at 25 °C led to **167**. The selenide **161** was oxidized to the selenoxide and underwent an interesting Pummerer reaction to the aldehyde equivalent **163**. Hydrolysis furnished the labile aldehyde **164**, which underwent Wittig condensation in 44% to afford the *E* olefin **166**. Evidently, the plan for elaboration of the side chain paralleled that conceived in the authors' model study.[25] The hemiseleno acetal **163** reacted with isopropenyllithium to give a single stereoisomeric adduct (**165**) of unassigned relative

SCHEME 25



SCHEME 26



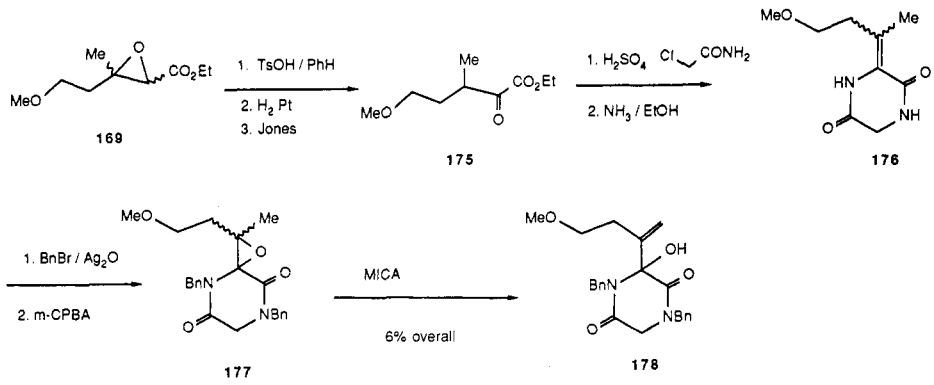
stereochemistry. It is interesting that it was noted that the aldehyde **164** did not successfully condense with alkyllithium or Grignard reagents; no mention of deformylation products, however, was made. Additional reports on the synthesis of bicyclomycin from this group have not appeared.

Yates and Hoare[29] prepared a potential precursor to bicyclomycin similar to the strategy developed by Dunkerton[23] and Fukuyama[28] involving piperazinedione formation via an $\alpha$-keto amide derivative (Scheme 26). Chloroethyl acetate was condensed with **168** to furnish the glycidate **169**, which was converted into olefin **170**. Hydrolysis, acetylation, and peptide coupling furnished **171**. Removal of the methyl ether was accomplished with (thiomethyl)trimethylsilane, followed by tetrahydropyranylation hydrolysis and $MnO_2$ oxidation to the $\alpha$-keto amide, which cyclized to **172**. Removal of the THP resulted in a mixture of **173** and the spiro compound **174** (3:1 ratio). A related approach to the same type of substrate (**178**) was achieved via the epoxypiperazinedione **177** as shown in Scheme 27. The only bicyclic materials reported by these workers are the spiro structures **174**.

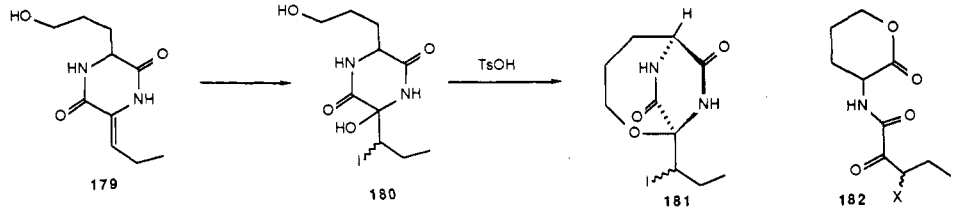
A preliminary report of a synthetic approach related to that by Fukuyama was presented by Dirlam and associates[30] of Pfizer, Inc. The unsaturated piperazinedione was converted into the iodohydrin (Scheme 28). Cyclization with *p*-TsOH afforded a small amount of **181**, the major type of byproducts being the lactone such as **182**. It is curious that other workers have not commented on the formation of similar degradation products, which seem to be reasonable structures to result from precursors in this oxidation state. No further reports from this group on their bicyclomycin approach have appeared.

Following the report[25] on the (accidental) discovery of the bridgehead carbanion chemistry of **138**, a very short and general synthesis of 3,6-unsubstituted bicyclo[4.2.2]piperazinediones was developed[31] as shown in Scheme 29. A series of N,N-dialkylated glycine an-

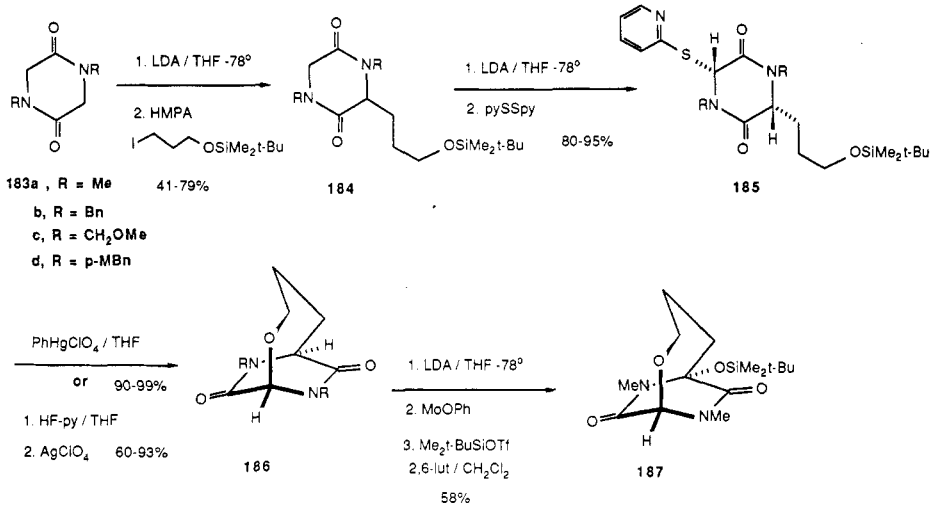
SCHEME 27




SCHEME 28




SCHEME 29




hydride derivatives **183** were lithiated and alkylated with 1-iodo-3-[(*tert*-butyldimethylsilyl)oxy]propane in moderate to good yields to furnish **184**. The major byproduct in these alkylations is the 3,6-dialkylated derivatives and is attributable to the relatively meager solubility of **183** compared to **184**. Subsequent enolate sulfenylation with dipyridyl disulfide proceeded in high yields to afford exclusively the syn isomers **185**. An X-ray crystal structure determination of **185a** was performed and also revealed that both substituents at the 3- and 6-positions adopt a pseudoaxial disposition and the piperazinedione adopts a boat conformation (Figure 2). Treatment of **185** with phenylmercuric perchlorate[32] in THF effected the concomitant silyl ether cleavage/cyclization in high yields to afford the bicyclo[4.2.2]piperazinediones **186**. The results from the initial model study[25] indicated that a regioselective bridgehead carbanion protocol was possible to elaborate **186** to incorporate the requisite functionality of **1**. Thus, generation of the bridgehead carbanion of **186a** and quench with the molybdenum peroxy reagent "MoOPh" followed by silylation afforded **187** in 58% yield. From this substrate, *N*,*N*′-dimethyl-4-desmethylenebicyclomycin (**191**) was prepared as illustrated in Scheme 30. Aldol condensation of aldehyde **84** with the carbanion generated from **187** afforded the three diastereomeric aldols **188**, **189**, and **190** in 9–3.7:1:1 ratio. The major aldol (**188**) obtained in 52% yield had the correct relative stereochemistry as was firmly established by single-crystal X-ray analysis.

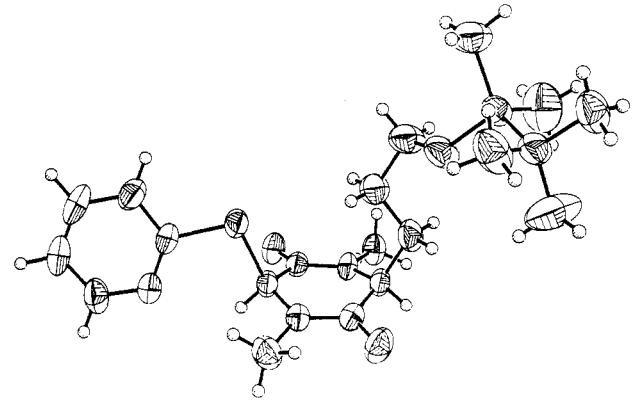
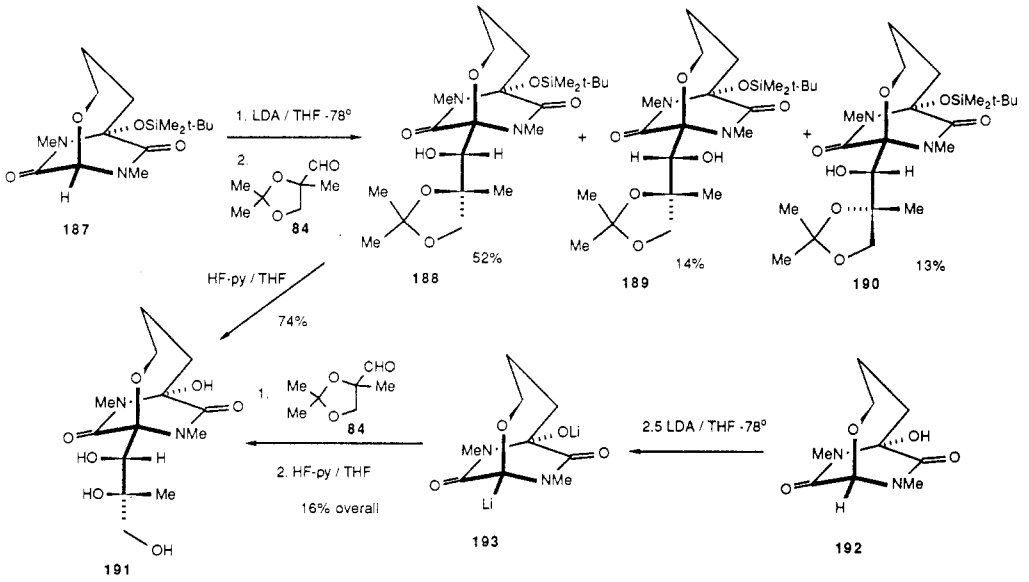
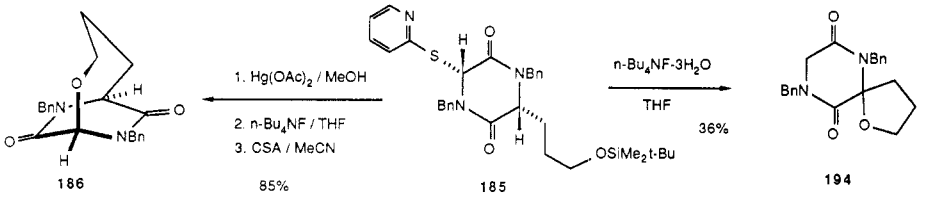


**Figure 2.** Structure of **185a**.

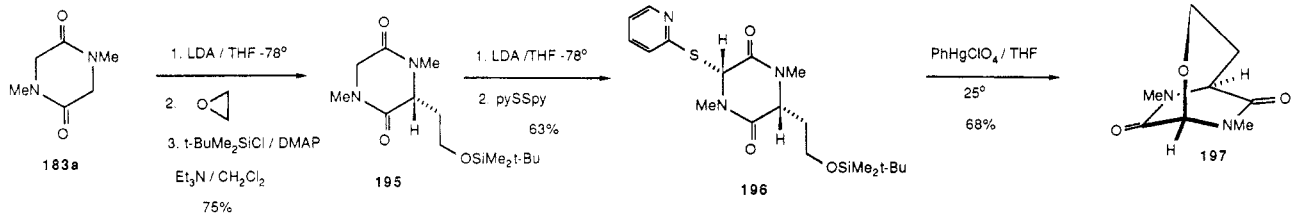
SCHEME 30



SCHEME 31



SCHEME 32



Treatment of **188** with HF–pyridine complex effected the removal of the silyl ether and the acetonide to furnish **191** in 74% yield. It was also found that the silyl ether was unnecessary since formation of the dianion **193** of **192** and aldol condensation with **84** followed by acetonide removal also furnished **191** (16% overall from **192**). However, the stereoselectivity in this case (4:3:3:2) was poorer than that for **187**. On the positive side, the total number of steps to synthesize **191** from commercially available sarcosine anhydride via the dianion aldol condensation was only six steps.

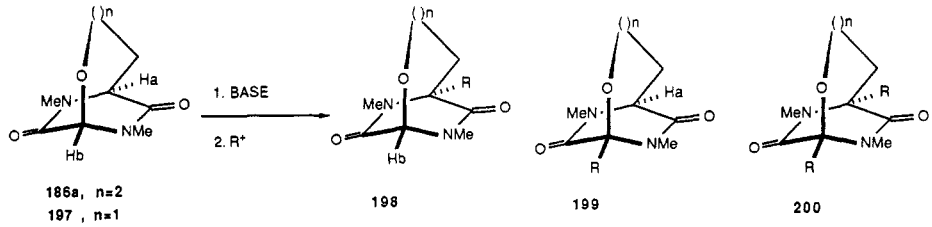
It was also found that **185** could be converted into the corresponding spiro derivative **194** upon treatment with tetra-*n*-butylammonium fluoride trihydrate in THF. Presumably the basic conditions of the medium generated a small equilibrium concentration of the enolate that resulted in intramolecular trans-sulfenylation and closure of the alkoxide to **194**. Although the yield was only 36%, this further demonstrates the thermodynamic energy well represented by the spiro compounds (Scheme 31).

Solvolysis of **185** in MeOH containing 1 equiv of mercuric acetate effected the clean displacement of the thiopyridyl residue by methanol without cleavage of the silyl ether. Subsequent fluoride removal of the silyl group and acid-catalyzed ring closure as above (for **144**) provide an alternate route to **186**.

The structure and reactivity of the bridgehead carbanions of the simple systems **186** were fundamentally interesting. For example, the bridgehead methine protons of **186** are each in very similar *steric* environments, the only difference being the presence of a bridging oxygen atom adjacent to C-1 and a bridging $CH_2$ adjacent to C-6. Was it a fundamental *electronic* effect on the stability/reactivity of the corresponding bridgehead carbanions that resulted in the *regioselectivity* observed? Are the bridgehead carbanions largely pyramidal or did they enjoy resonance stabilization from the adjacent amides (i.e. enolate character)? What are the relative kinetic and thermodynamic acidities of these methines?

In an effort to address these questions, Williams and co-workers[33] prepared the simple bicyclo[3.2.2]-piperazinedione **197** along the same lines used for **186**; the synthesis is illustrated in Scheme 32. Utilizing both ring systems, treatment with strong base followed by quenching with an electrophile resulted in a distribution of the two monoalkylated (**198**, **199**) and dialkylated products (**200**); in every case, **198** was the major, and sometimes exclusive, product (Scheme 33). The results are collected in Table 5. For both ring sizes, it was found the methine adjacent to bridging $CH_2$ ($H_a$) was thermodynamically more acidic than the methine adjacent to the bridging oxygen atom ($H_b$). This was

SCHEME 33




SCHEME 34

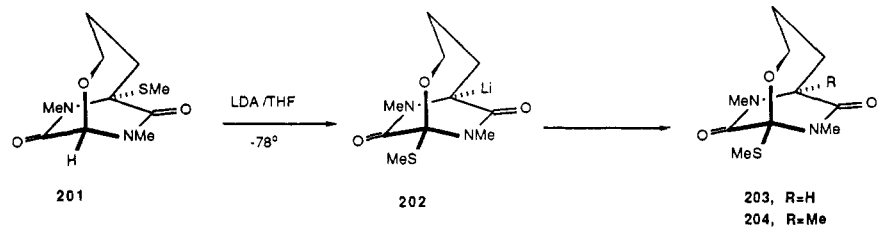



**TABLE V. Bridgehead Carbanion Functionalization of 186a and 197**

| entry | substrate | electrophile | reactn time[b] | 198, % | 199, % | 200, % |
|---|---|---|---|---|---|---|
| 1 | **186a** | $H_3COD$[a] | 1 min | 1 | 53 | 7 |
| 2 | **186a** | $H_3Cl$[a] | 1 min, −115 °C | 19 | 43 | |
| 3 | **197** | $H_3COD$[a] | 5 min | 28 | 11 | 11 |
| 4 | **186a** | PhCOCl[a] | 1 min | 50 | 41 | |
| 5 | **186a** | PhCOCl[a] | 1 h | 49 | 27 | |
| 6 | **197** | $H_3CI$[a] | 1 h | 60 | 25 | |
| 7 | **186a** | PhCHO[a] | 1 min | 45 | 47 | |
| 8 | | PhCHO[a] | 1 h | 40 | 26 | |
| 9 | | $H_3CI$ | 1 h | 74 | 3 | 5 |
| 10 | | PhCOCl | 1 h | 92 | 2.6 | |
| | | | | 66.5[c] | | |
| 11 | | $Me_3SiCl$ | 1 h | 53.3 | | |
| | | | | 80.5[c] | 6 | |
| 12 | | MeSSMe | 1 h | 72 | | |
| 13 | | MoOPh | 1 min | 65 | | |
| 14 | | PhCHO | 1 h | 82.1 | 10.5 | |
| 15 | | $BrCH_2CH{=}CH_2$ | 1 h | 67 | 1 | |
| 16 | **197** | $Me_3SiCl$ | 1 h | 65.6 | | 22 |
| 17 | | $H_3CI$ | 1 h | 75.1[c] | | 16[c] |
| | | | | 40.3 | | 8.6 |
| 18 | | $BrCH_2CH{=}CH_2$ | 1 h | 83 | | |
| 19 | | PhCOCl | 1 h | 82[c] | | |
| | | | | 44.3 | | |
| 20 | | MeSSMe | 1 h | 66[c] | | 33[c] |
| | | | | 33 | | 16 |

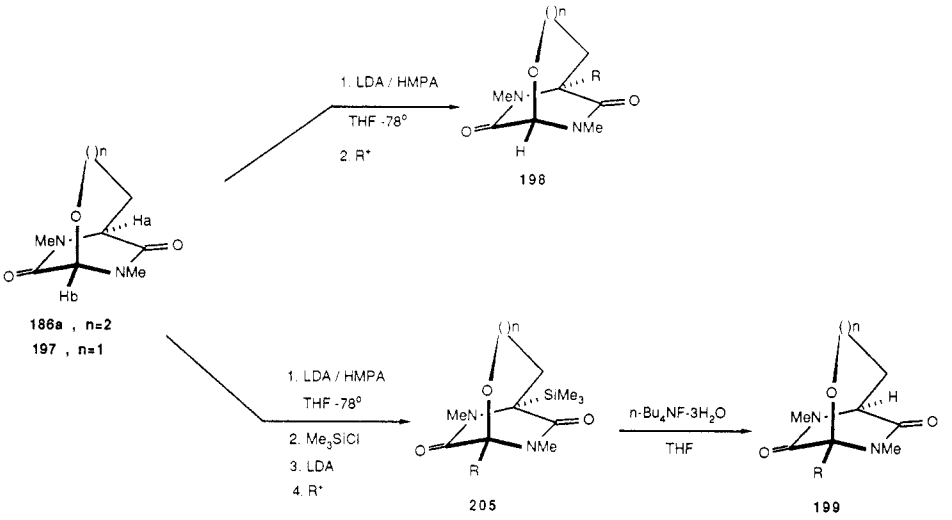
[a] These reactions were carried out with LDA in THF at −78 °C without HMPA. All other entries in the table were done with HMPA. [b] Reaction time refers to the time the anion was stirred at −78 °C before addition of the electrophile. [c] Yield is based on recovered starting material.

demonstrated by subjecting the anions to a rapid (kinetic) quench and then to longer reaction times in the presence of HMPA. By comparison of the product ratios as a function of time and concentration of HMPA, it was demonstrated that $H_b$ is deprotonated relatively faster than $H_a$ (kinetic conditions) but that the carbanions slowly equilibrate favoring the carbanion at $H_a$. Since each methine has identical environments with respect to the piperazinedione moiety, these results clearly demonstrate that the oxygen atom has a net *destabilizing* on the adjacent bridgehead anion, presumably through electrostatic repulsion. Although the structure(s) of the bridgehead carbanions themselves have not yet been rigorously elucidated, the close chemical reactivity behavior for both **186a** and **197** would argue in favor of predominantly pyramidal and not enolate structures for these species; this is particularly compelling for **197** whose enolate structure would be in violation of Bredts' rule. The question, however, is still open and important, particularly as the structure of the carbanions derived from the [4.2.2] systems relates to the aldol stereoselectivity.

Further evidence for the marked thermodynamic stability of the carbanion at the C-6 position relative to that at C-1 was obtained by the curious rearrangement of sulfide **201** to the carbanion **202** (Scheme 34); trapping of this species upon treatment of **201** with LDA afforded **203** and **204**.

The significant difference in the thermodynamic acidity of the bridgehead protons of these simple systems allowed for the development of the general regioselective protocol outlined in Scheme 35. As already noted, the regioisomers **198** can be directly accessed via bridgehead carbanion formation in the presence of HMPA and electrophilic quench (Table 5). The alternative regioisomers **199** can be accessed via a five-step one-pot protocol involving in situ generation of the trimethylsilyl species (at $H_a$) followed by carbanion

SCHEME 35




SCHEME 36

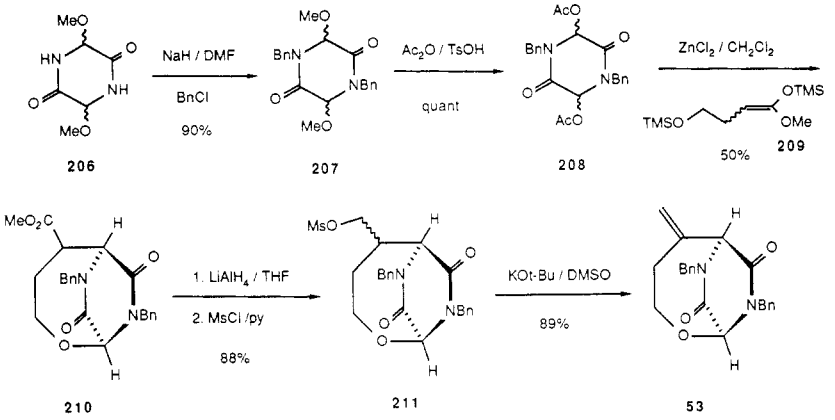



**TABLE VI. Regioselective Syntheses of 199**

| entry | substrate | electrophile | product | yield, % |
|---|---|---|---|---|
| 1 | **198**, $n$ = 1, R = $SiMe_3$ | $CH_3I$ | **199**, $n$ = 1, R = $CH_3$ | 59 (93a) |
| 2 | **186a** | $CH_3I$ | **199**, $n$ = 2, R = $CH_3$ | 56[b] |
| 3 | **186a** | CHO | **199**, $n$ = 2, R = CHOH | 80[b] |
| 4 | **198**, $n$ = 1, R = SiMe | $BrCH_2CH{=}CH_2$ | **199**, $n$ = 1, R = $CH_2CH{=}CH_2$ | 46 (52[a]) |

[a] Yield is based on recovered starting material. [b] The product **13** was directly obtained form **2** by a three-step, one-pot procedure.

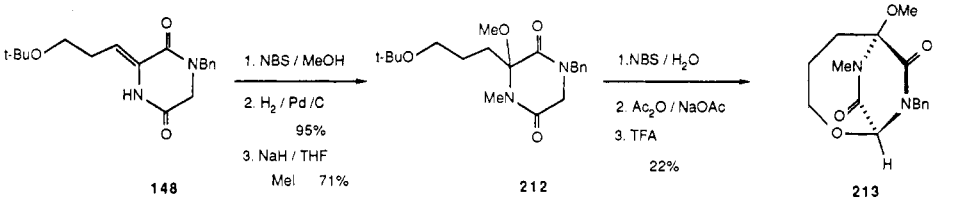
formation at $H_b$, electrophilic quench (to **205**), and fluoride removal of the *C*-trimethylsilyl protection. Table 6 lists some derivatives (**199**) prepared by this one-pot procedure.

Early in 1984, Sera and co-workers[34] reported an interesting approach to the bicyclomycin ring system involving a double electrophilic addition to a substrate similar to that used by the Nagoya group. 3,6-Dimethoxypiperazinedione (**206**)[35] was N-benzylated (**207**) and converted into the 3,6-diacetate (**208**) in excellent yield. Condensation of this material with the trimethylsilyl ether trimethylsilyl ketene acetal of 3-(hydroxymethyl)butyrate (**209**) in the presence of $ZnCl_2$ furnished the bicyclic system **210** in 50% yield (Scheme 36). Presumably this is formed as a mixture of stereoisomers at the carbomethoxy center, but this point is not mentioned.[34] Reduction to the ester, conversion to the mesylate **211**, and elimination provided the *N*,*N*′-dibenzyl derivative **53**. This same compound (**53**) had been reported in 1983[20,36] by a similar route to be discussed in the next section. No further transformations of **211** by this group[34] have been reported.

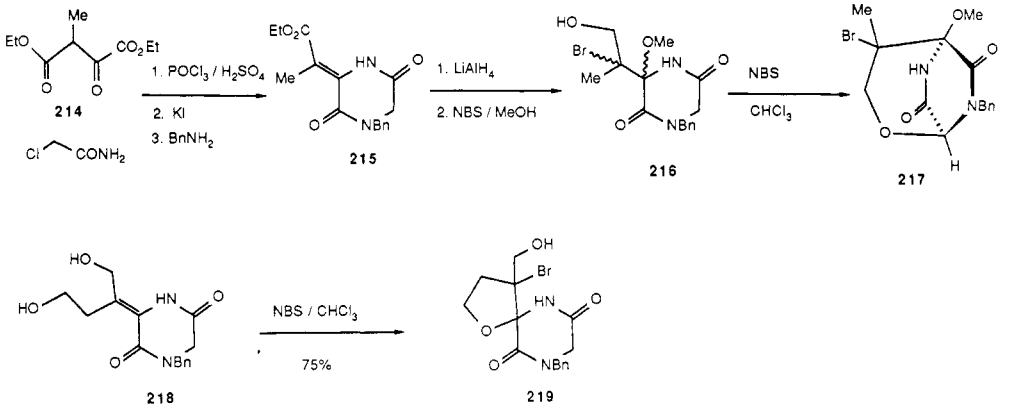
Following the report[26] detailed in Scheme 23, two papers appeared from the same group that addressed the incorporation of the bridgehead oxygen atom using the oxidative/cyclization methodology reported above. Thus, in Scheme 37, the dehydropiperazinedione **148** is oxidized with NBS in methanol followed by hydrogenation of the halogen and N-methylation (**212**). Oxidation of **212** furnished the corresponding hydroxylated material, which was acylated and treated with trifluoroacetic acid, effecting removal of the *tert*-butyl ether and cyclization to afford **213** in 22% overall yield from **212**. In a similar attempt, the dehydropiperazinedione **215** (Scheme 38) was converted into the diastereomeric mixture **216**. Oxidative/cyclization using NBS in $CHCl_3$ afforded the bicyclo[3.2.2] system **217** in high yield. Unfortunately, similar attempts to convert the bicyclo[4.2.2] precursor **218** into a homologue of **216** only resulted in the formation of spiro **219**.

Danishefsky and co-workers[37] have developed a very interesting (albeit incomplete) approach to the bicyclomycin system that is illustrated in Scheme 39. Condensation of methacrolein with (dimethoxymethyl)acetate afforded **220**. Bromolactonization with NBS in water afforded an 85:15 mixture of diastereomers (**221**). Subsequent conversion of **221** to the key amino lactone **222** proceded in good overall yield.

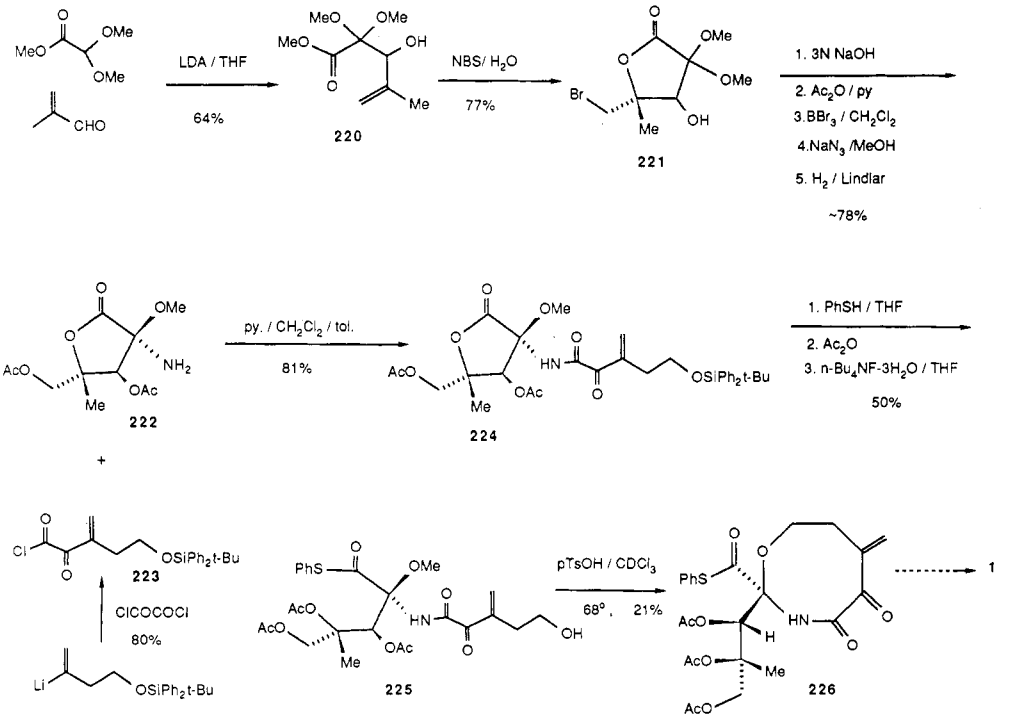
SCHEME 37




SCHEME 38



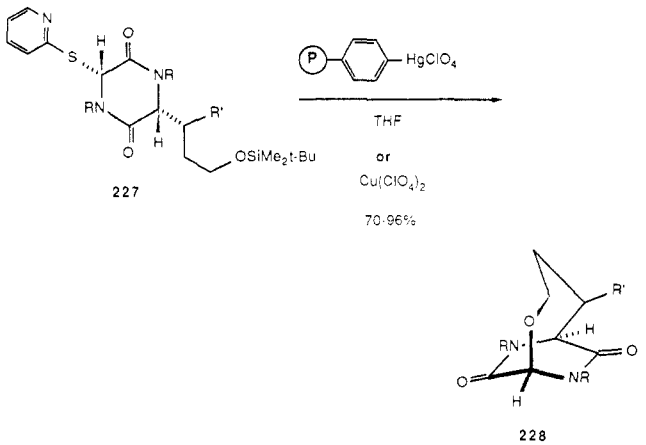

SCHEME 39




Acylation of **222** with the $\alpha$-keto acid chloride **223** afforded an 81% yield of **224**. Opening the lactone of **224** with thiophenol followed by acylation and fluoride removal of the silyl ether furnished compound **225**. Treatment of **225** with acid afforded a yellow substance in 21% yield tentatively identified as the eight-membered ring species **226**. The final conversion of **226** to **1** would involve aminolysis of the thio ester, which is expected to spontaneously cyclize to the piperazinedione and hydrolyze the acetates. It is worth mentioning that this rather daring approach to bicyclomycin is the only synthetic approach predicated on construction of the piperazinedione ring as the final synthetic transformation.

An improvement in the technology to construct the simple bicyclo[4.2.2] ring system via the metal perchlorate reaction outlined in Scheme 29 was developed as shown in Scheme 40. The authors[38] screened a

SCHEME 40

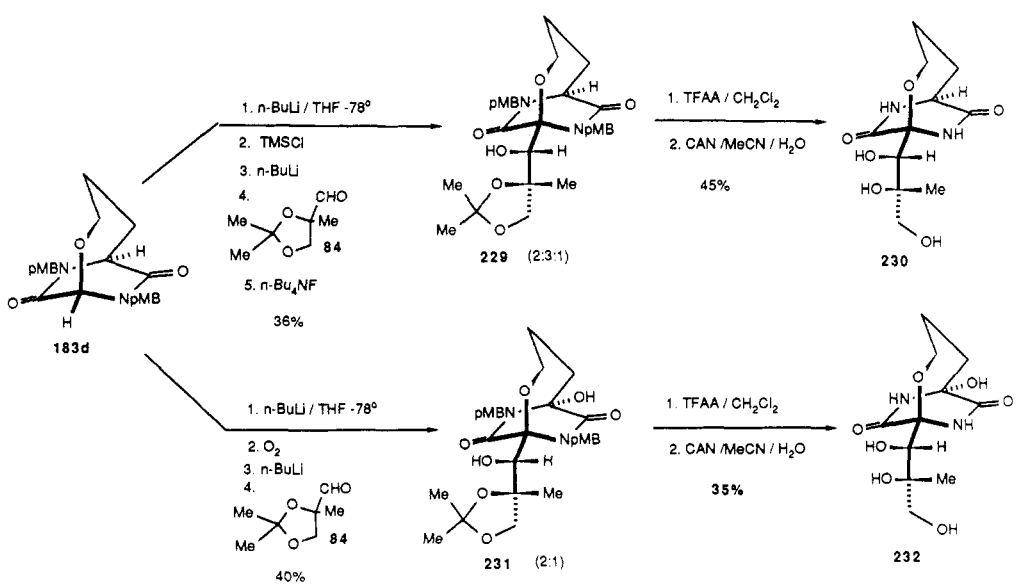



variety of metal salts to effect the one-step silyl ether cleavage/cyclization reaction. It was found that the perchlorate salts of Cu(II), Ag(I), Hg(II), Fe(III), Ni(II), Pb(II), and Tl(III) effect the conversion of **227** → **228** in good to excellent yields, the best reagent of this series being inexpensive $Cu(ClO_4)_2$. Other counterions were examined including triflate, hexafluoroantimonate, and tosylate (as their Ag(I) salts), which only worked with modest efficiency. Surprisingly, tetrafluoroborate and sulfate were completely ineffective. This paper also reported the synthesis and utility of a reusable polystyrene-bond mercury(II) perchlorate for this deprotection/cyclization, which precludes the necessity to chromatographically purify **228**.

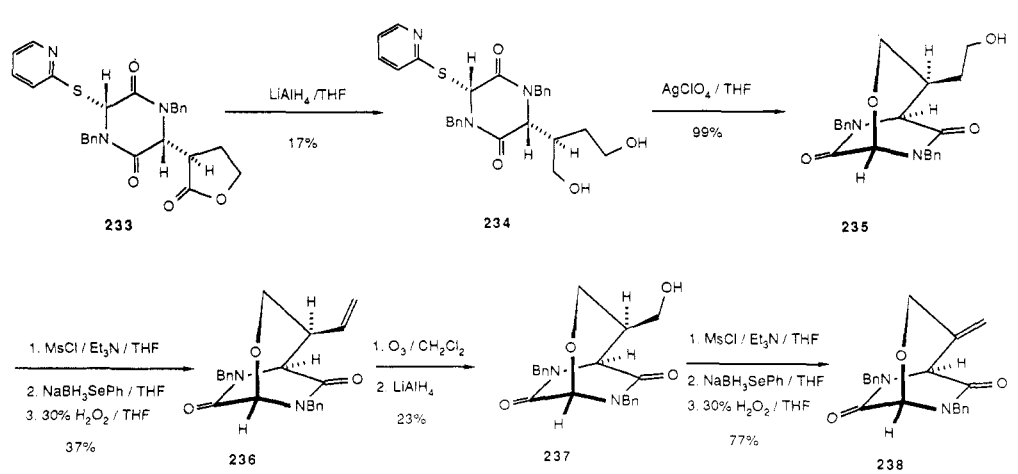
By exploiting the regioselective bridgehead carbanion protocol (Scheme 35), Williams and co-workers[20] synthesized 5-demethylene-6-deoxybicyclomycin (**230**) and 5-demethylenebicyclomycin (**232**) as shown in Scheme 41. The *N,N'*-(*p*-methoxybenzyl) substrate **183d** was chosen as a common precursor since it was found during these investigations that only the *p*-methoxybenzyl groups could be removed[39] under conditions mild enough that were compatible with the rest of the functionality. The aldol condensation for the deoxy series gave a 2:3:1 ratio, **229** having the natural relative configuration and **231** obtained along with another isomer in a 2:1 ratio. Trifluoroacetylation of the C-1'-hydroxyl and treatment with ceric ammonium nitrate furnished the water-soluble derivatives **230** and **232**. These two derivatives along with 22 additional bicyclomycin analogues were synthesized and submitted for antimicrobial assay. This point will be returned to at the end of the next section.

These workers also reported[20] the preparation of the bicyclo[3.2.2] homologue **238** as illustrated in Scheme

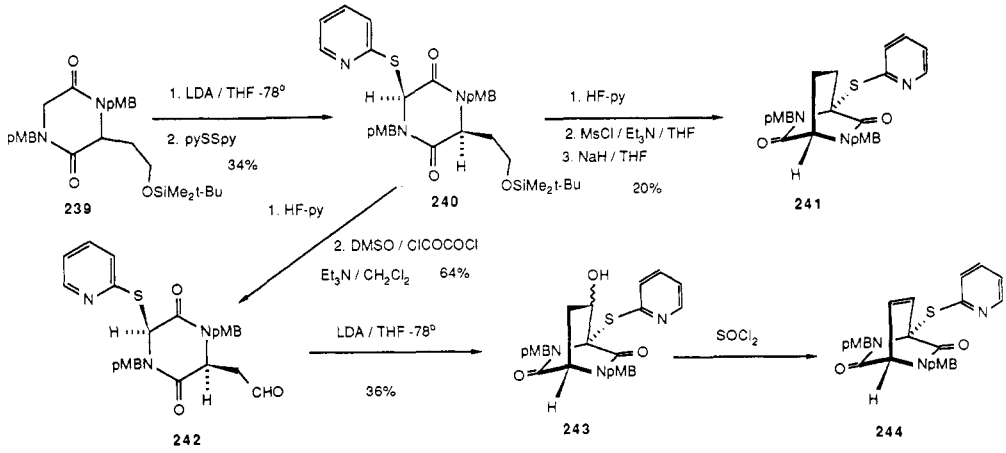
SCHEME 41



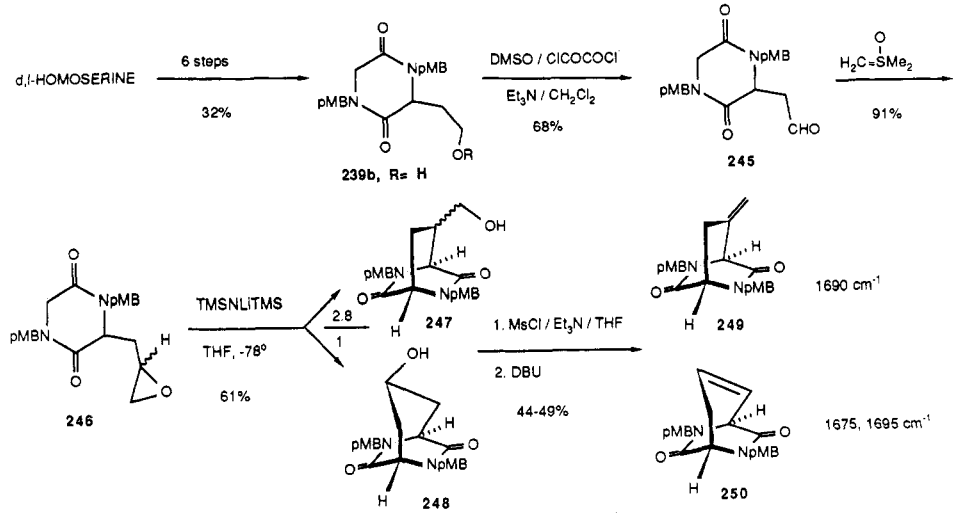

SCHEME 42



SCHEME 43




SCHEME 44




42. The lactone **233** (prepared and discussed in Scheme 46) was reduced in low yield to the diol **234**. Treatment of this material with silver(I) perchlorate afforded a nearly quantitative yield of the bicyclo[3.2.2] alcohol **235**. Elimination to **236** and ozonolysis removed the unwanted carbon atom (**237**). Dehydration of **237** furnished the bicyclo[3.2.2] system **238**.

In an effort to prepare additional, strained analogues of the bicyclomycin nucleus, Schemes 43 and 44 illustrate the preparation of several carbon-bridged systems. The piperazinedione **239a** derived from *d,l*-homoserine[40] was sulfenylated to afford **240**.[41] Removal of the silyl ether, mesylation, and cyclization[42] afforded the bicyclo[2.2.2] system[43] **247** in 20% overall yield from **240**. It might be noted in passing that all attempts at (vigorous) reductive desulfurization of **241** with Raney nickel to afford the parent system met with failure. Alternatively, desilylation and Swern oxidation afforded the aldehyde **242**, which suffered intramolecular aldol condensation to afford the alcohol **243**. Treatment of this material with thionyl chloride afforded the labile olefin **244** (IR, 1695 $cm^{-1}$) in low yield.

As shown in Scheme 44, piperazinedione **239b** was prepared from homoserine[40] and oxidized to the aldehyde **245**. Homologation with dimethylsulfoxonium methylide afforded a 1:1 mixture of diastereomeric epoxides **246** in high yield. Cyclization was effected by enolate generation, giving a 2.8:1 ratio of the bicyclo[2.2.2] isomers **247** to the bicyclo[3.2.2] alcohol **248**. Dehydration of each system afforded the olefins **249** and **250**, respectively.

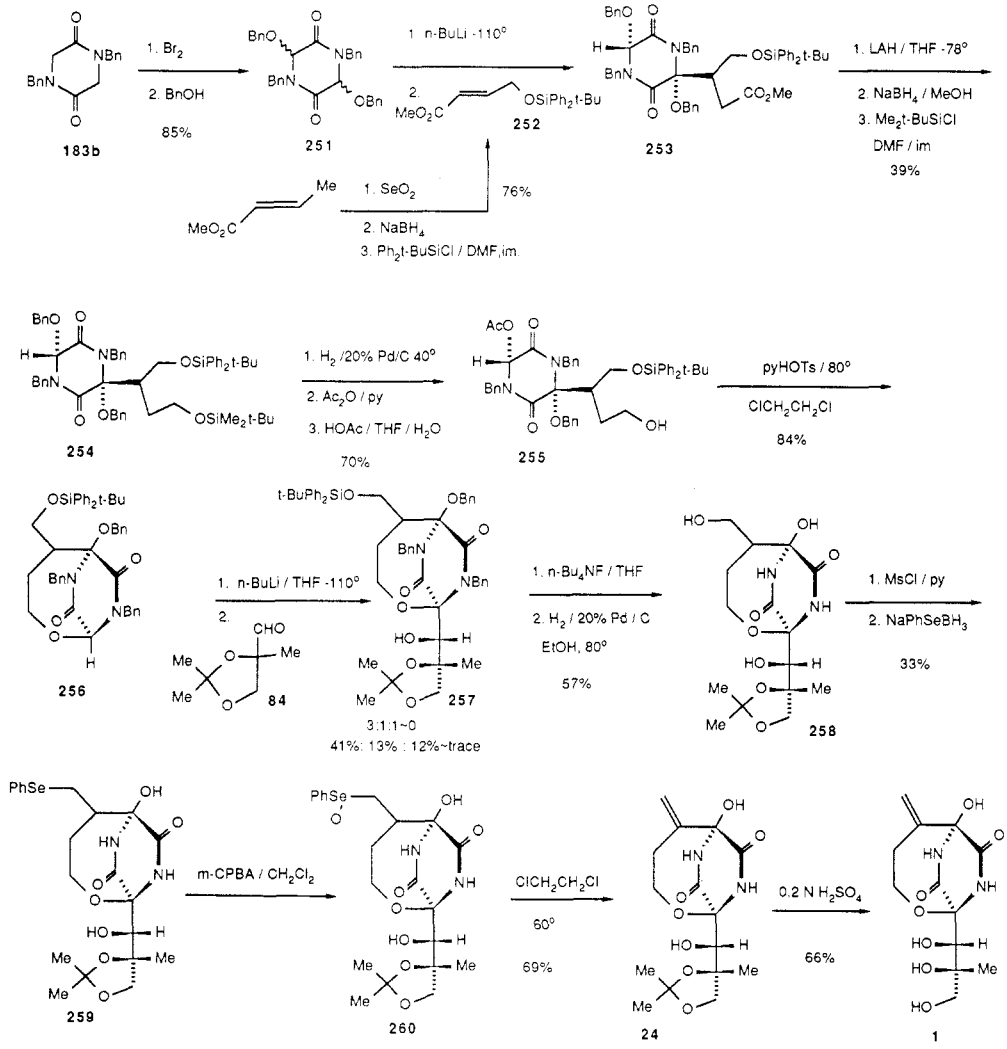
The functionalization and mechanistic and biological evaluation of these unusual bicyclo[3.2.2] and -[2.2.2] analogues based on **238**, **241**, **244**, **249**, and **250** are relatively recent areas of investigation that have the potential to provide rich and interesting chemistry of their own. It is also important to point out that a smaller (or larger) ring size homologue of bicyclomycin with all of the appropriate functionality has not, as of this writing, been prepared. It is expected that much of the fascinating synthetic, mechanistic, and biological insights that have resulted from the study of **1** should provide ample incentive to exploit and discover with bicyclomycin homologues.

## VI. Total Syntheses

The synthetic efforts expended by numerous groups on the model studies delineated above culminated in three independent total syntheses and one formal total synthesis of bicyclomycin.

The first success was achieved by Nakatsuka, Goto and collaborators in 1983; the results are summarized in Scheme 45. Following the protocol developed in the model study,[24,27] *N,N*-dibenzylglycine anhydride (**183b**) was brominated and solvolyzed with benzyl alcohol to afford the dibenzyl ether **251** as a 3:1, cis to trans mixture. Enolate generation and Michael addition to

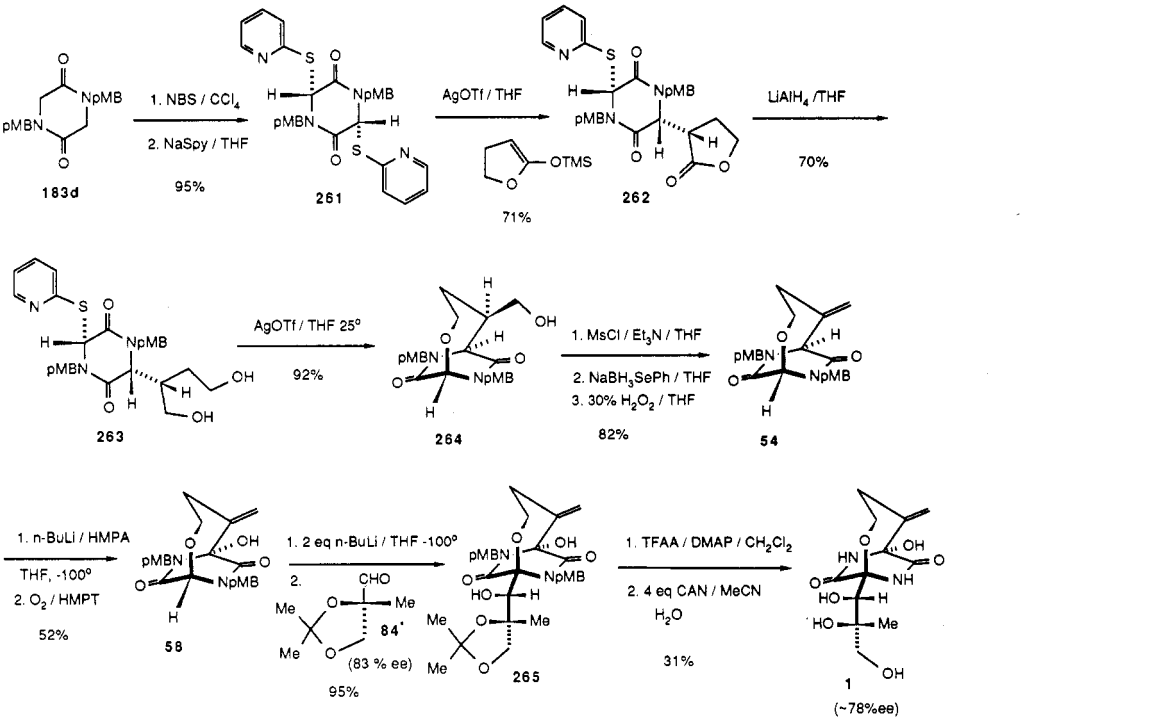
SCHEME 45




**252** afforded a syn-condensation product **253**. The stereochemistry $\beta$ to the carbomethoxy group was not assigned nor was the isomer distribution (if any) noted. Reduction of **253** and silylation furnished a 39% overall yield of **254**. In a key step, selective hydrogenation of the secondary benzyl ether followed by acetylation and removal of the *tert*-butyldimethylsilyl ether provided **255** in 70% yield. Cyclization with pyridinium tosylate in dichloroethane at 80 °C yielded the bicyclic compound **256** (84%). Aldol condensation of **84** with the bridgehead carbanion derived from **256** afforded the desired condensation product **257** in 41% yield along with three minor isomers. Chromatographic separation and desilylation was followed by a remarkable hydrogenation of all three benzylic groups to afford **258** in 57% overall yield from **257**. Mesylation of **258** followed by selenide displacement furnished **259**, which was converted into the stable selenoxide **260**. Chromatography and heating **260** afforded the racemic bicyclomycin 2,3′-acetonide derivative **24** which was hydrolyzed to give racemic **1**.

Scheme 46 details the synthesis of bicyclomycin reported by Williams and co-workers[45,48] in 1984. *N*,*N*′-(*p*-Methoxybenzyl)glycine anhydride (**183d**) was oxidized with NBS followed by reaction with sodio-2-thiopyridine to afford the crystalline *syn*-sulfide in 95% yield. Unlike the solvolyses of the 3,6-dibromide with alcohols (cf. **119** and **251**), which gave mixtures, the thiolate displacements resulted exclusively in the thermodynamically more stable syn isomers.[46] Precomplexation of **261** with AgOTf followed by condensation with the silyl ketene acetal of $\gamma$-butyrolactone afforded four stereoisomeric lactones **262**; both syn and anti isomers were obtained. The major isomers (syn major shown) were reduced with $LiAlH_4$ to afford the diols **263**. Cyclization in the presence of silver(I) triflate in THF at room temperature furnished the bicyclo[4.2.2] alcohol **264** in a 3:2 ratio with the corresponding bicyclo[3.2.2] system. The solution to this chemoselectivity problem is discussed separately below. Dehydration to the key olefin **54** proceeded in good overall yield. As in the simple bicyclic systems reported above in the model studies, the bridgehead positions of **54** could be regio- and stereoselectively functionalized, the methine proton adjacent to the bridging *exo*-methylene being more acidic than the methine adjacent to the bridging oxygen atom. Thus, bridgehead carbanion oxidation of **54** with molecular oxygen afforded a single hydroxylation product (**58**) in 52% yield. Formation of the dianion of **58** followed by aldol condensation with **84** at −100 °C furnished a single diastereomeric aldol **265** that possessed the desired relative configuration in high yield. It was noted that if the aldol condensation was carried out at higher temperature and/or quenched above −80 °C, a second diastereomeric aldol product appeared (presumed to be epimeric to **265** at C-1′). The

SCHEME 46

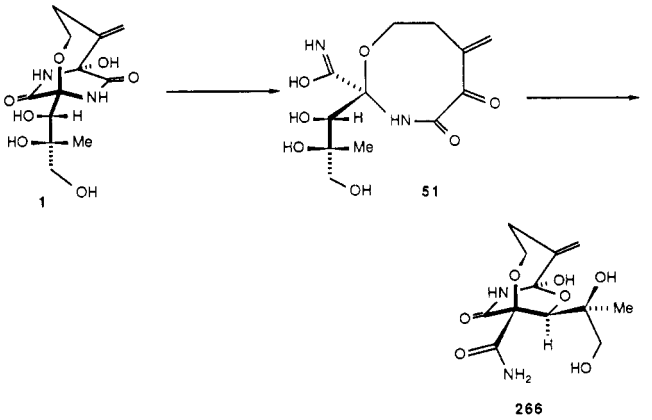



final transformation to **1** required prior protection of the C-1′-hydroxyl as the corresponding trifluoroacetate. Subsequent treatment with 4 equiv of ceric ammonium nitrate effected the removal of both *p*-methoxybenzyl groups and cleavage of the acetonide. Subjecting the reaction residue to methanolysis on silica gel then afforded racemic bicyclomycin in 31% overall yield from **265**. A comment regarding the obligate trifluoroacetylation is pertinent. It was known from a recent report from the Ciba-Geigy group[47] that bicyclomycin undergoes a rearrangement to the bicyclic hemiketal **266** in DMSO-$d_6$; this presumably occurs via the ring-opened tautomer **51** (Scheme 47). It was found that if **265** were directly treated with ceric ammonium nitrate, oxidation products with a structure based on **266** were isolated along with numerous other degradation products. Acylation of the C-1′-OH, which participates in this rearrangement precludes these undesired transformations and reliably permitted the deprotection to **1**.

The aldol condensation was also performed with optically active aldehyde **84** (83% ee) that was prepared via Sharpless technology.[50] Following the same protocol, optically active **1** was obtained in ca 78% ee. The optical purity of **1** obtainable via the double diastereodifferentiating aldol condensation is directly related to the optical purity of **84**. It was also noted in this account that the synthetic, racemic bicyclomycin obtained was evaluated for antimicrobial activity. The sample displayed the same spectrum of activity as natural **1**, but at half-potency. This result for the first time demonstrated that the enantiomer of **1** (in the racemate) was devoid of antimicrobial activity.

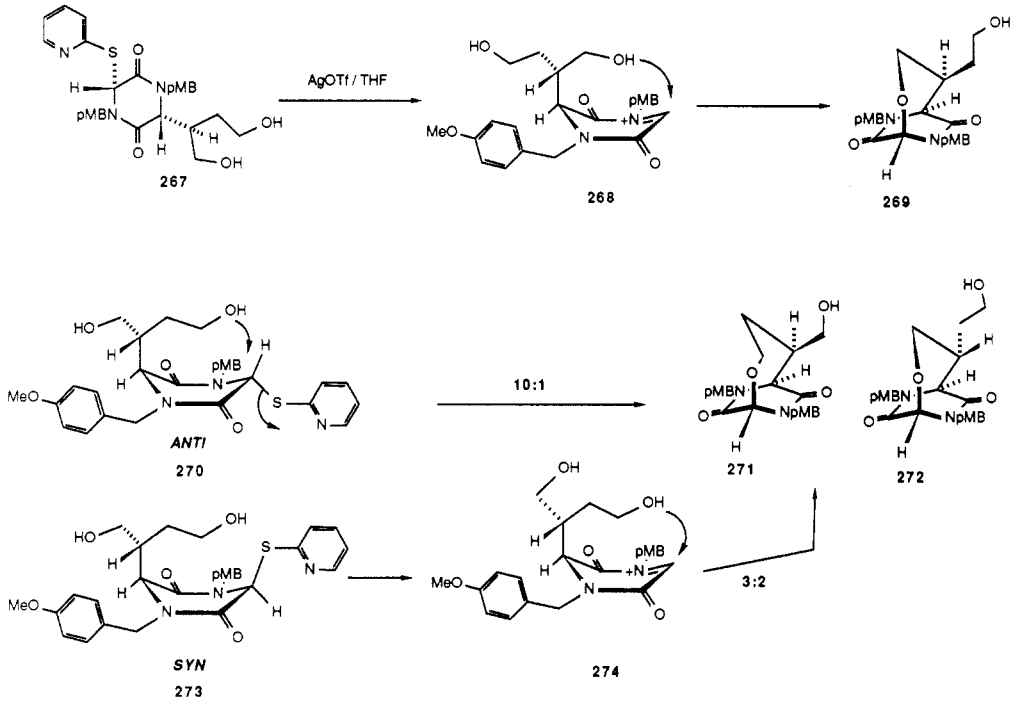
The primary problem encountered in this synthesis was the need to separate and manipulate the four diastereomeric lactones **262** produced in the crucial coupling reaction. It was found for example that diol **267** obtained as a minor isomer from the corresponding lactone furnished exclusively the undesired bicyclo-

SCHEME 47

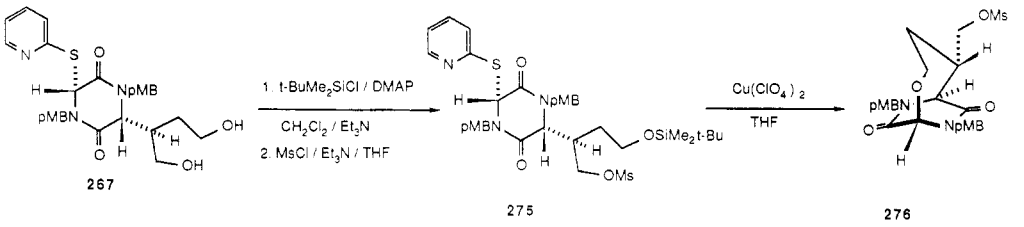


[3.2.2] system **269** (Scheme 48). This is reminiscent of Maag's related cyclization to **109** (Scheme 17). Apparently, the *p*-methoxybenzyl group exerts a steric influence favoring the conformation where the dihydroxybutyl residue is situated away from the proximal amide nitrogen (shown **268**). In this conformation, the hydroxymethyl residue should be in proximity to the reactive iminium center and would be predicted (as observed) to favor the [3.2.2] product. By the same rationale, inverting the stereochemistry of the dihydroxybutyl residue should favor the [4.2.2] ring system. This is indeed the case. The two major diol isomers (**270, 273**) upon cyclization give a mixture of the bicyclo[4.2.2] system **271** and bicyclo[3.2.2] system **272**, favoring the desired ring system **271**. Interestingly, the anti isomer **270** gave a 10:1 mixture of **271** and **272** and the syn isomer **273** gave a 3:2 mixture of **271** and **272**. The relatively poor selectivity displayed by **273** when compared to **270** was readily rationalized on the assumption that the syn isomer **273** must pass through the highly reactive (and less selective) iminium species **274**, whereas **270** has a lower energy transition state

SCHEME 48




SCHEME 49



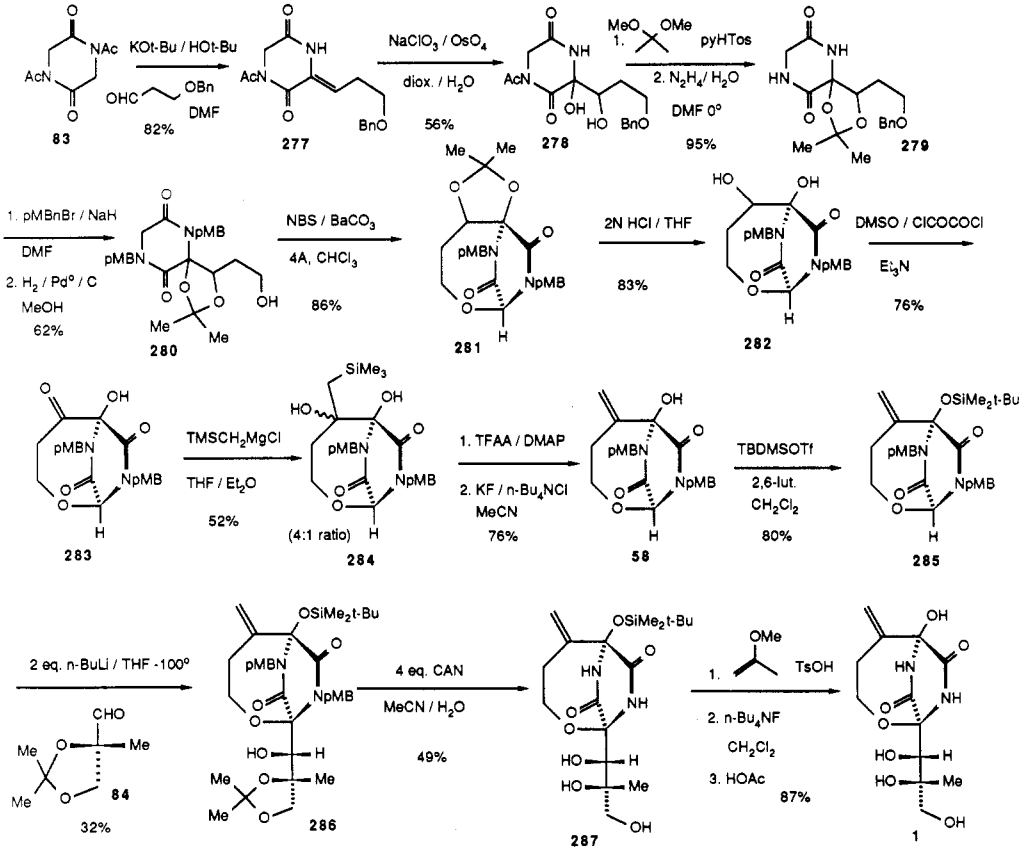

farther along the reaction coordinate ($S_N2$) and thus displays greater selectivity consistent with the conformational analysis.

A solution to this problem was engineered and is illustrated on the minor diol **267** in Scheme 49. Selective protection of **267** at the hydroxyethyl moiety followed by mesylation of the hydroxymethyl group furnished **275**. Cyclization of this material with copper(II) perchlorate in THF at room temperature afforded the desired bicyclo[4.2.2] mesylate **276** as the exclusive product in high yield. Compound **276** could then be subsequently converted into **54** by selenide displacement and oxidative/elimination. By applying the same protocol to any of the diol isomers such as **273**, the bicyclo[4.2.2] system **54** could be obtained exclusively without contamination of the bicyclo[3.2.2] ring system. This procedure only adds a single step to the overall synthesis of 1 (now 13 steps).

A point concerning amide protecting groups is pertinent. These workers[20,45] described a parallel series of compounds to that described in Scheme 46 that contained the *N*-benzyl protecting groups instead of the *N*-(*p*-methoxybenzyl) groups. It was found that catalytic hydrogenation did not effect the debenzylation on a range of substrates and conditions but rather resulted in the saturation of the aromatic rings to furnish *N*-methylcyclohexyl derivatives. Dissolving metal reductions in some cases did lead to debenzylation but were always accompanied by reductive opening of the bicyclic ring system. Only after extensive experimental failure to utilize the *N*-benzyl groups as amide protecting groups was the *N*-(*p*-methoxybenzyl) series investigated. The *N*-benzyl series culminated in the acetonide derivative identical with **265**. Thus, in spite of the Nagoya group's success in utilizing the *N*-benzyl group as an amide protecting group, the extensive and reliable success[20,45,49] of the *p*-methoxybenzyl group for amide protection in the bicyclomycin system would argue for use of the latter in future synthetic endeavors.[51]

Following the model studies outlined in Schemes 23, 37, and 38, Yoshimura and collaborators[49] completed a total synthesis of (+)-bicyclomycin as depicted in Scheme 50. The dehydropiperazinedione **277** was prepared from **83** and (benzyloxy)propanal. Conversion to the diol **278**, acetonide formation, deacylation, amide alkylation, and hydrogenation furnished the *N,N'*-bis-(*p*-methoxybenzyl) substrate **280**. Treatment of this alcohol with NBS in $CHCl_3$ furnished the desired bicyclo[4.2.2] system **281** in 86% yield. Acetonide cleavage and Swern oxidation gave ketone **283**, which was homologated by a Peterson olefination sequence to give a 4:1 mixture of diols **284**. Trifluoroacetylation and fluoride-induced elimination gave **58**, identical with that obtained by Williams.[45,52] The bridgehead hydroxyl was then silylated (**285**) and the corresponding bridgehead carbanion condensed with optically pure **84** (obtained from a sugar[53]). Three aldols were isolated (ratio unspecified), with the major product (32%) having the desired relative configuration. Removal of the *p*-methoxybenzyl groups and acetonide with ceric am-

SCHEME 50



monium nitrate afforded the triol **287** (49%). Apparently to avoid some rearrangement problems, **287** was reacetonized, the silyl group removed, and the resulting acetonide **24** hydrolyzed to optically active **1**. Since these workers passed through the same intermediate (**58**) utilized by Williams,[45] the final transformations to **1** could be formally reduced from six steps to three steps (see Scheme 46). This would formally shorten their overall synthesis from ~17 steps to ~14 steps.

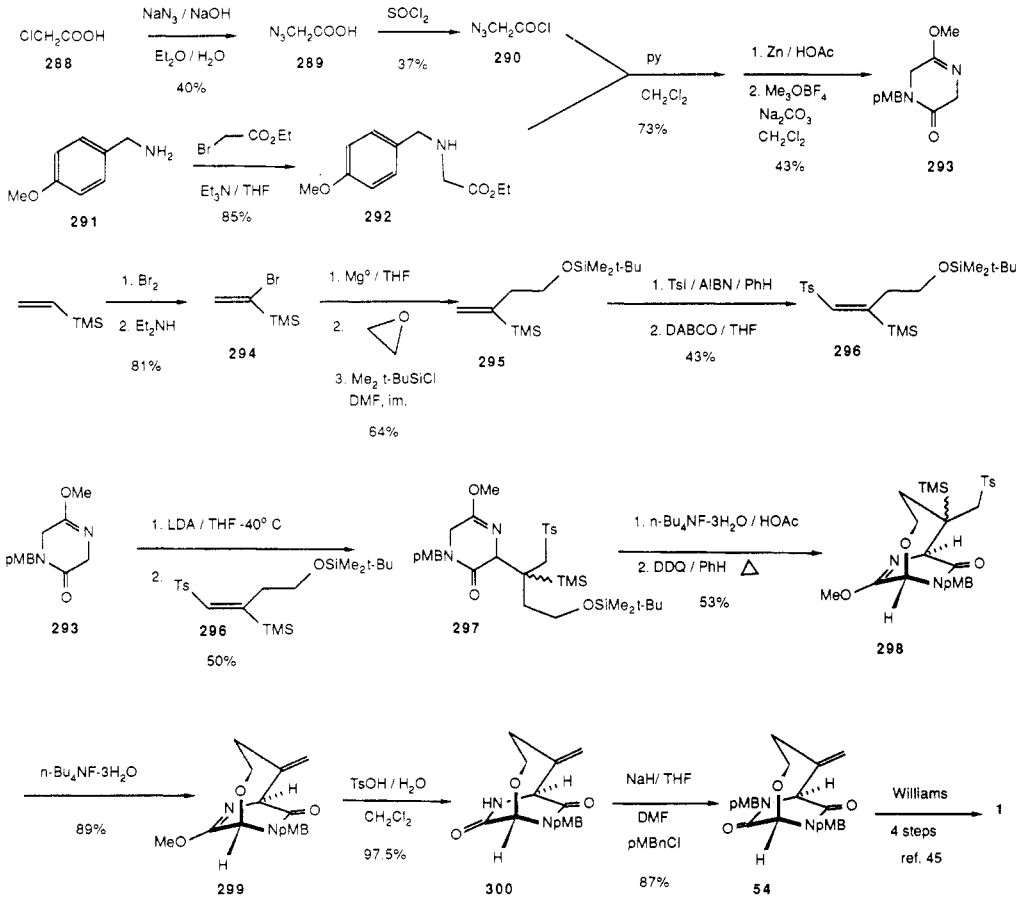
Very recently, a formal total synthesis of **1** was reported by Sammes and collaborators[54] culminating in the bicyclic olefin **54** that had previously been converted to **1**.[45] As shown in Scheme 51, lactim ether **293** is prepared from azidoacetic acid (**289**) and the glycine derivative **292**. Michael reaction of **293** with the unsaturated sulfone **296** furnished **297**. Silyl ether cleavage and oxidative/cyclization with DDQ in a manner similar to that developed by Yoshimura (Scheme 23) furnished the bicyclo[4.2.2] system **298** as a mixture of diastereomers (ratio unspecified). Elimination to **299** followed by lactim ether hydrolysis and amide alkylation with *p*-methoxybenzyl chloride furnished **54**, identical with that obtained previously.[45]

It is worth surveying all the closely related aldol condensations with the Maag aldehyde **84** and the bicyclic carbanions represented by **301** (Scheme 52). In almost every case, the naturally configured isomer **302** is obtained as the major product. The table collects all of the available data on this reaction. It can be seen that, functionally quite removed from the reacting carbanionic center at C-1, X/Y/Z have a profound effect on the stereoselectivity of these reactions. Most striking is the observation[45] that the dianion of **58** gives a single aldol but silylating this same substrate[49] (**285**) gives poorer selectivity. Additionally, as mentioned above, the temperature of the condensation is also important, better results being obtained at very low temperature. The degree of mutual kinetic resolution between **301** and **84** is, in most cases, remarkable. The condensation between **58** and **84** discussed above at −80 and +25 °C (quench temperature) indicates that above −80 °C the aldolate undergoes equilibrating retroaldol/aldol reaction resulting in the appearance of a second diastereomer (epimeric at C-1′). Thus, the *mutual* diastereoselectivity between **58** and **84** with respect to the existing stereogenic centers on each substrate therefore approaches 100%! It is reasonable then that the isomer distribution shown in the table is the result of partial retroaldol/aldol equilibration. The pertinent questions to ask in this regard concerns the structure of the bridgehead carbanion itself and the inherent stereofacial selectivity for nucleophilic addition[55] to **84**. Unfortunately, very little information is available on either point. For example, the "enolate" structure **306** would be expected to participate in a standard chelation-controlled kinetic aldolization. This interpretation has been favored by the Nagoya group.[27,44] The alternate pyramidal carbanion structure **307** (Scheme 53) can only undergo a direct (nonchelation-controlled) nucleophilic attack on **84**. Until additional experimental and perhaps theoretical work is performed to address these interesting questions concerning the structure of the bridgehead carbanions, only a hazy picture of this fascinating reaction will remain.

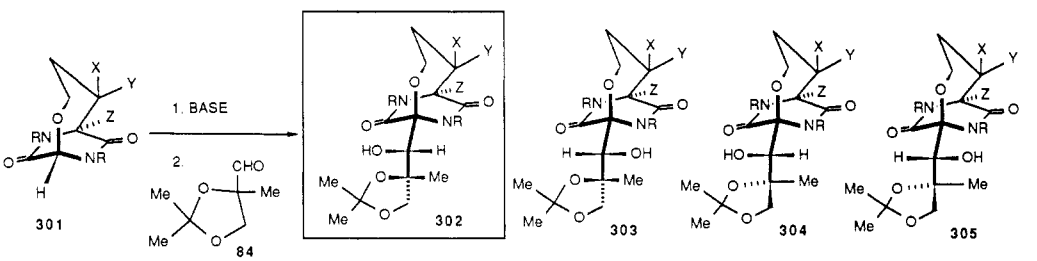
## VII. Concluding Remarks

Bicyclomycin has provided a unique opportunity to bring together biological, physical organic, and synthetic organic disciplines. The efficient production of this

**SCHEME 51**




**SCHEME 52**




| INVESTIGATOR (ref) | Z | X | Y | R | YIELD | TEMP °C | STEREOSELECTIVITY |
|---|---|---|---|---|---|---|---|
| WILLIAMS (31) | $OSiMe_2t$-Bu | H | H | Me | 79% | -78 | 4:1:1~0 |
| WILLIAMS (31) | OH | H | H | Me | 58% | -78 | 4:3:3:2 |
| WILLIAMS (20) | $SiMe_3$ | H | H | Bn | 36% | -78 | 2:3:1~0 |
| WILLIAMS (20) | OH | H | H | Bn | 64% | -78 | 2:1~0~0 |
| WILLIAMS (48) | H | H | $CH_2OSiMe_2t$-Bu | Bn | 80% | -100 | 1~0~0~0 |
| WILLIAMS (45) | OH | | $=CH_2$ | Bn | 73% | -100 | 1~0~0~0 |
| WILLIAMS (45) | OH | | $=CH_2$ | p-MBn | 95% | -100 | 1~0~0~0 |
| WILLIAMS (45) | OH | | $=CH_2$ | p-MBn | 70% | 25 | 1:1~0~0 |
| GOTO (27) | OMe | | $=CH_2$ | Me | 46% | -78 | 9:3:3:1 |
| GOTO (44) | OBn | H | $CH_2OSiPh_2t$-Bu | Bn | 66% | -78 | 3:1:1~0 |
| YOSHIMURA (49) | $OSiMe_2t$-Bu | | $=CH_2$ | p-MBn | 32% | -100 | 3 ISOMERS (UNSPECIFIED) |

**SCHEME 53**

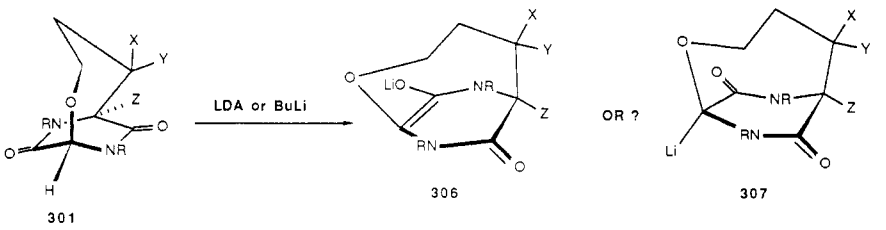
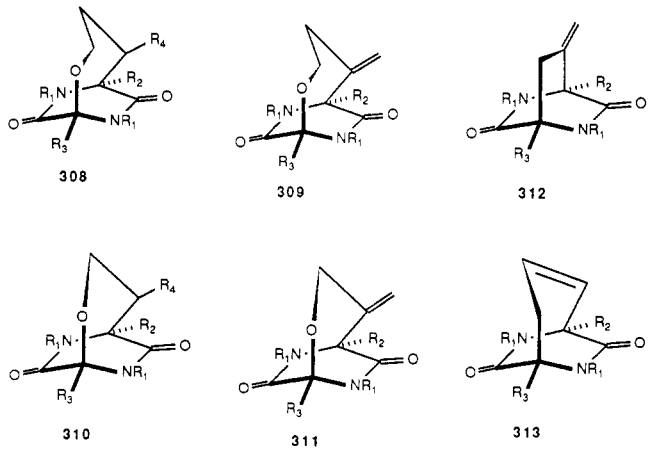


CHART III




natural product from fermentation harvests dwarfs, from a practical standpoint, the efforts of the synthetic workers to assemble this water-soluble antibiotic. Even the shortest linear route (12 steps, Williams[45]), which is completely regio- and stereocontrolled, only proceeds in ca. 4–5% overall yield. Obviously, even if this were improved substantially, the production of 1 or closely related analogues by total synthesis would be of dubious practical significance. The additional fact that bicyclomycin itself, and not even a trivially modified semisynthetic derivative, is sold commercially and has a relatively favorable pharmacological profile (particularly with respect to toxicity) has dampened the usual synthetic rationale to improve the antibiotic through total synthesis. This is indeed true of many conceivable structures based on the basic bicyclo[4.2.2] nucleus. However, the recent history of the β-lactam antibiotics, which has recently experienced a renaissance due to the discovery of numerous active nonclassical β-lactams, teaches us that the role of synthesis remains an indispensible vehicle for basic discovery. This potential era of discovery for bicyclomycin is only in its infancy. For example, Chart 3 lists the generic bicyclic nuclei that have been synthesized during the course of the bicyclomycin investigations. To date, extensive functionalization and biological testing have only been conducted on the bicyclomycin nucleus **309**. The demonstrated ability to functionalize the bridgehead positions of these bicyclic ring systems makes the overall approach quite appealing. This inherent versatility should render the construction of a relatively large set of designed structures from a small set of common bicyclic precursors a practical endeavor.

Of more pressing academic and ultimately utilitarian significance is the elucidation of the chemical mechanism of action of bicyclomycin and synthetic analogues that display activity (i.e., **57**). The exciting recent picture emerging vis-à-vis the DAP–DAP bond and the elucidation of specific functions of the bicyclomycin-binding proteins provides a compelling case that bicyclomycin can play a pivotal role in further defining the mechanisms and events in bacterial cell division and provide a vehicle for the discovery of new and interesting bioactive substances.

## VIII. Acknowledgment

Our investigations on the chemistry of bicyclomycin have been supported by NIH Grant AI 18957. It is a pleasure to acknowledge our collaborators whose names appear on the publications referenced from these laboratories; we also thank Dr. Hans Maag and Professor Samuel Danishefsky for providing us with material pertaining to their results. We are deeply indebted to Fujisawa Pharmaceutical Co. for providing generous samples of bicyclomycin and additional relevant information on this commercial product.

**Registry No.** 1, 38129-37-2.

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