Chemistry and Biology of the Tetrahydroisoquinoline Antitumor Antibiotics

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Contents

1. Introduction

The antitumor antibiotics belonging to the tetrahydroisoquinoline family have been studied thoroughly over the past 25 years starting with the isolation of naphthyridinomycin in 1974. The two core structures of this family are the quinone **1** and the aromatic core **2** (Figure 1). To date, 55 natural products in this family have been isolated. The tetrahydroisoquinolines include potent cytotoxic agents that display a range of antitumor activities, antimicrobial activity, and other biological properties to be discussed below depending on their structures.

These natural products are classified into the saframycin, naphthyridinomycin/ bioxalomycin, and quinocarcin/ tetrazomine families of natural products. Some of these natural products have been reviewed in the literature,[1] but this is intended to be the most comprehensive review to date. Pertinent structural, synthetic, semisynthetic, and biological studies reported in the open literature will be covered in this review.

2. Saframycin Family

2.1. Saframycins

2.1.1. Isolation and Structure Determination

Saframycins A, B, C, D, and E (**3**−**5**, **9**, **10**, respectively, Figure 2) were isolated from *Strepto-*

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Jack D. Scott was born in Grand Forks, ND, in 1972 and received his Bachelor of Science degree in Chemistry at the University of North Dakota in 1994. In 2001 he earned his Ph.D. degree in Synthetic Organic Chemistry under the supervision of Professor Robert M. Williams at Colorado State University. He currently holds the position of Senior Scientist at the Schering-Plough Research Institute in New Jersey.

Robert M. Williams was born in New York in 1953 and attended Syracuse University, where he received his B.A. degree in Chemistry in 1975. While at Syracuse, he did undergraduate research with Professor Ei-ichi Negishi in the area of hydroboration methodology. He obtained his Ph.D. degree in 1979 at MIT under the supervision of Professor William H. Rastetter. He joined the laboratories of the late Professor R. B. Woodward (subsequently managed by Professor Y. Kishi) in 1979 and joined the faculty at Colorado State University in 1980. He was promoted to Associate Professor with tenure in 1985 and Full Professor in 1988. Dr. Williams was the recipient of the NIH Research Career Development Award (1984–1989), The Eli Lilly Young Investigator Award (1986), Fellow of the Alfred P. Sloan Foundation (1986), the Merck, Academic Development Award (1991), The Japanese Society for the Promotion of Science Fellowship (1999), and The Arthur C. Cope Scholar Award (2002). He serves on the Editorial Board of the journal *Chemistry & Biology* and was an Editor for the journal *Amino Acids* from 1991 to 1998. He serves as a Series co-Editor for *The Organic Chemistry Series*, published by Pergamon Press/Elsevier. Dr. Williams was a member of the Scientific Advisory Board of Microcide Pharmaceutical Company from 1993 to 1998 located in Mountainview, CA, and is a founding scientist, Member of the Scientific Advisory Board, and Member of the Board of Directors of Xcyte Therapies, located in Seattle, WA. Dr. Williams' research results from the interplay of synthetic organic chemistry, microbiology, biochemistry, and molecular biology. He is the author of over 160 scientific publications. Dr. Williams' research interests have included the total synthesis of natural products, studies on drug–DNA interactions, design and synthesis of antibiotics and DNA-cleaving molecules, combinatorial phage libraries, and biosynthetic pathways. He has utilized natural products synthesis to probe and explore biomechanistic and biosynthetic problems with a particular emphasis on antitumor and antimicrobial antibiotics. He has developed technology for the asymmetric synthesis of α-amino acids and peptide isosteres, which have been commercialized by Aldrich Chemical Company, and he has written a monograph on this subject.

myces lavendulae in 1977 by Arai et al.[2] These were the first of many saframycins to be subsequently isolated in Nature. The structure of saframycin C was the first of this family to be determined. This was accomplished via X-ray crystallographic analysis.[3] From the comparison of the ^{13}C NMR data of saframycins B and C, the structure of saframycin B was determined. The structure of saframycin A, which contains a nitrile moiety at C-21, was determined through various spectroscopic techniques including high-field ^{1}H NMR analyses of saframycins A and C.[4] The structure of saframycin D was the next to be determined, once again by extensive NMR studies.[5] Saframycin E was found to be too unstable for spectroscopic studies, but it could be isolated and characterized as the corresponding triacetate.[2] The structure of saframycin E was determined by Kubo et al. via an intermediate in their synthetic studies of the saframycins.[6] This intermediate had identical spectroscopic properties to that of the triacetate derivative of saframycin E.

During studies of the optimization of saframycin A production, another saframycin was isolated, saframycin S.[7] Saframycin S was believed to be a biosynthetic precursor to saframycin A. It was found that treatment of saframycin S with sodium cyanide leads to the formation of saframycin A (Scheme 1). Treatment of saframycin A with aqueous acid lead to the formation of saframycin S and decyanosaframycin A.

Interestingly, the nitrile moiety of saframycin A was not observable by infrared spectroscopy. It was hypothesized that the extensive oxygenation in this substance quenches the nitrile absorption intensity. This characteristic was observed in all of the saframycins that contain a nitrile moiety.

Saframycin R was isolated in 1982 by Arai et al.[8] The structure was revised in 2000 by the use of HMQC and HMBC experiments on two acetate derivatives.[9] The main difference in structure between saframycin R and the previously isolated saframycins was that the E-ring was in the form of a hydroquinone rather than a quinone.[10] The isolation and structures of saframycins F, G, and H were determined in the study of the minor components of the saframycin mixture isolated from *Streptomyces lavendulae* No. 314.[11] The structures of saframycins F, G, and H were determined by comparison of spectroscopic data with that of saframycins C and D. In 1988, saframycins Mx1 (**13**) and Mx2 (**14**) were isolated.[12] Like saframycin R, one of the aromatic rings was in the hydroquinone form.

In the search for more biologically active saframycins, six new saframycins were produced by directed

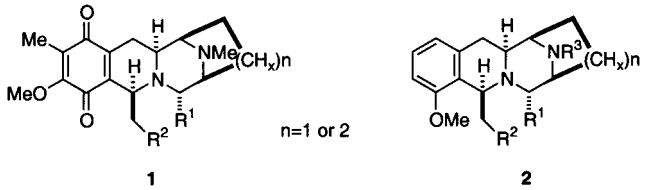

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Figure 1. General structures of the tetrahydroisoquinolines.

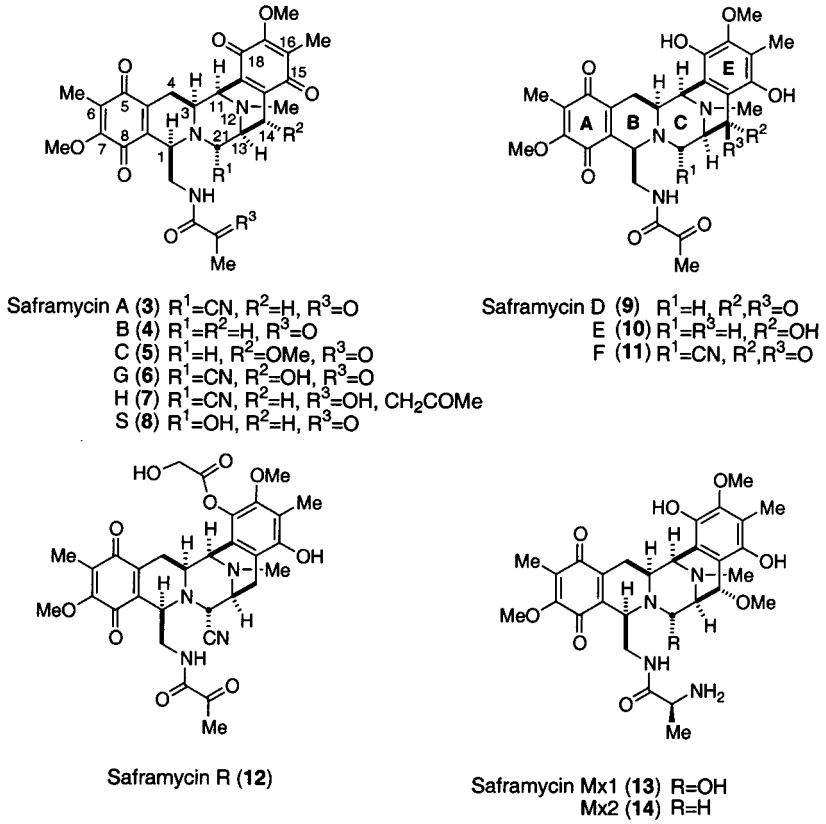
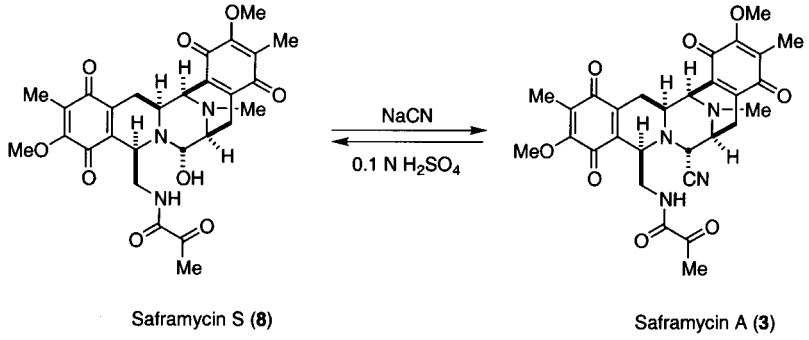


Figure 2. The saframycins.

Scheme 1. Interconversion of Saframycin S and Saframycin A



biosynthesis employing *Streptomyces lavendulae* No. 314 (Figure 3).[13] The supplementation of alanine and glycine or alanylglycine yielded saframycins Y3 (**15**) and the dimer Y2b (**19**). The addition of 2-amino-*n*-butyric acid and glycine or 2-amino-*n*-butyrylglycine produced saframycins Yd-1 (**16**), Ad-1 (**18**), and dimer Y2b−d (**20**). Saframycin Yd-2 (**17**) was produced by the supplementation of glycylglycine.

The separation of the saframycins by HPLC was reported by Fukushima et al.[14] Further studies on the quantitative and qualitative analysis of the saframycins by their polarographic and voltammetric behavior was reported by Bersier and Jenny.[15]

2.1.2. Biosynthesis

Mikami et al. showed that saframycin A was biosynthesized by the condensation of two ^{13}C-labeled tyrosine moieties (**21**)[16] (Figure 4), and glycine and alanine were also found to be incorporated into saframycin A.[17] To determine if the dipeptide was synthesized before or after coupling to the core, the dipeptide Ala-^{13}C-Gly (**23**) was synthesized and incorporated into the pyruvamide side chain. The five methyl groups of saframycin A were found to be derived from *S*-adenosylmethionine formed in vivo from the addition of labeled methionine (**22**).

Studies were also conducted on the biosynthesis of saframycin Mx1 by Pospiech et al. to determine what enzymes are involved in the biosynthesis.[18] These workers concluded that two multifunctional nonribosomal peptide synthetases and an *O*-methyltransferase are involved in the biosynthesis of this natural product.

2.1.3. Total Syntheses of the Saframycins

The total synthesis of (±)-saframycin B, which was reported by Fukuyama and Sachleben[19] in 1982, constitutes the first total synthesis of a member of the saframycin family (Scheme 2). Starting with aldehyde **24**, treatment with the lithium anion of

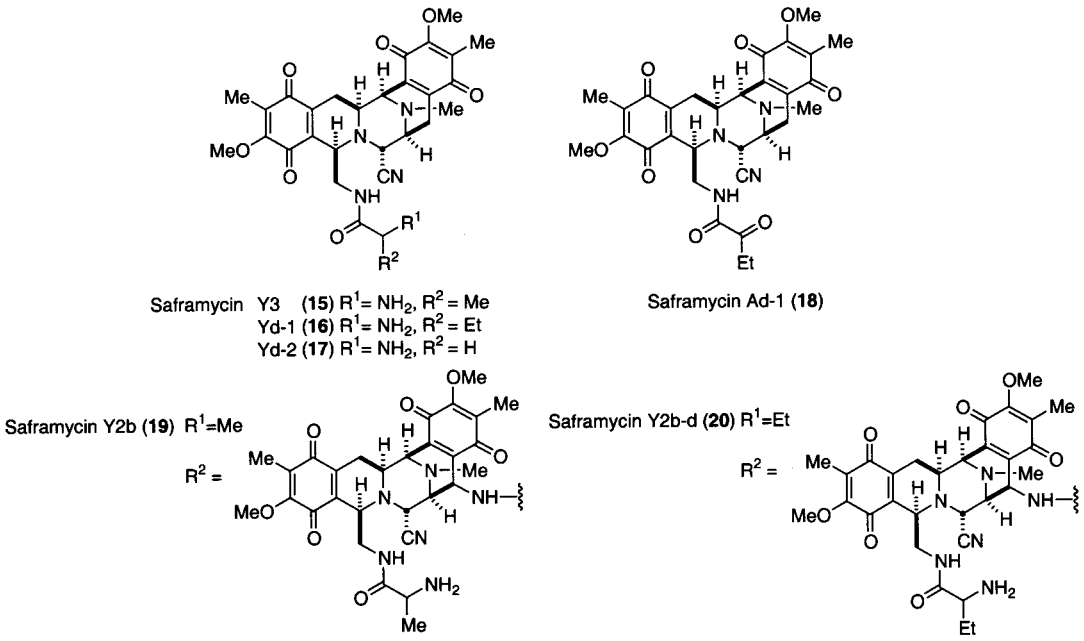


Figure 3. Saframycins obtained from directed biosynthesis.

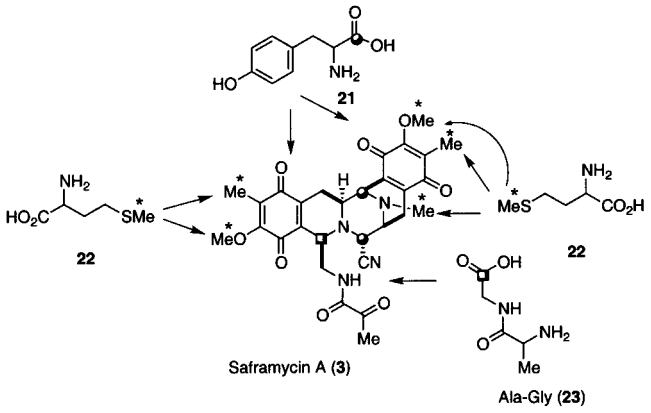


Figure 4. Primary biosynthetic precursors to saframycin A.

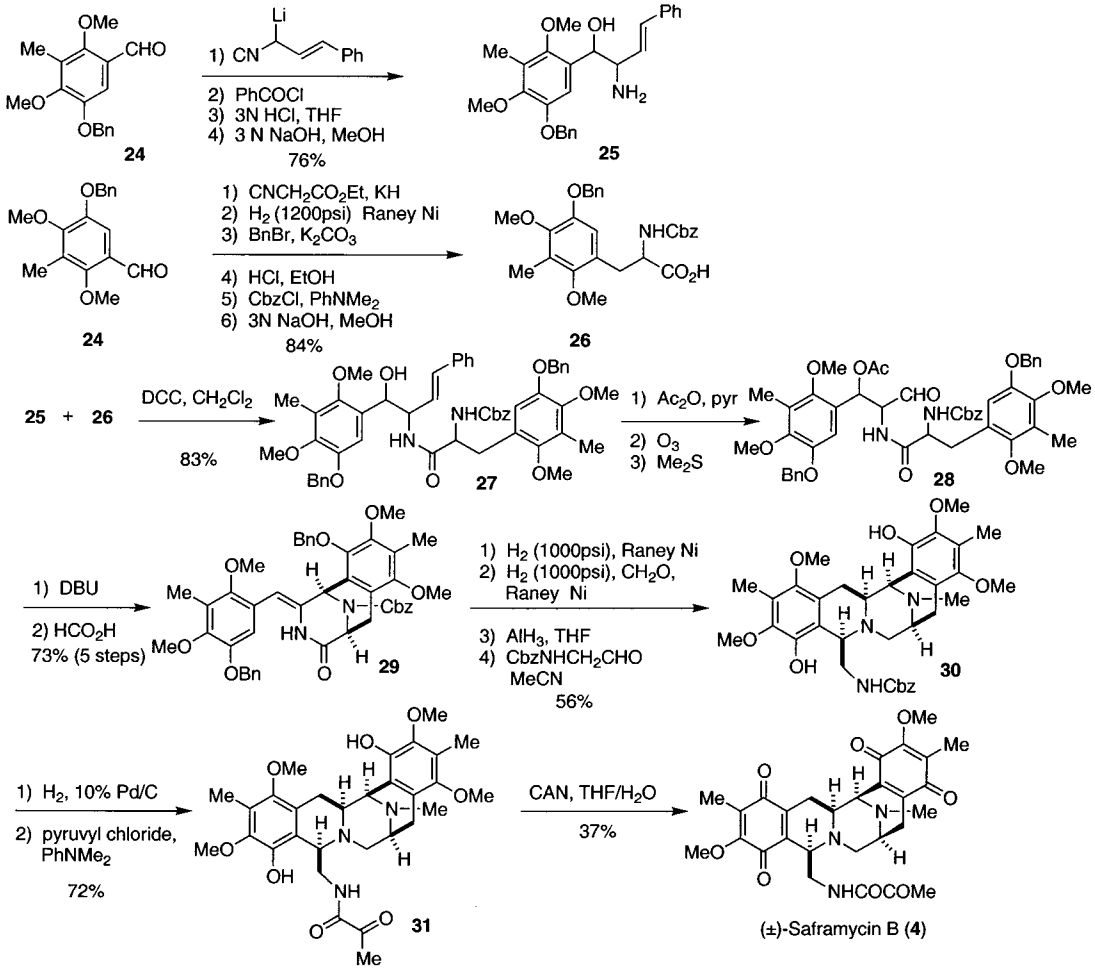
cinnamyl isocyanide afforded the benzylic alcohol that was esterified with benzoyl chloride. Hydration of the isocyanide followed by hydrolysis of the formamide afforded amino alcohol **25** in good yield. The A-ring of saframycin B was also synthesized from aldehyde **24**. Amino acid **26** was synthesized in six steps from aldehyde **24** in 84% overall yield through formation of the α,β-unsaturated isocyanide followed by subsequent reduction of the benzylic olefin. Coupling of amine **25** with the N-Cbz amino acid **26** yielded amide **27** in 83% yield. Acetylation of the secondary alcohol followed by careful ozonolysis and reductive workup yielded a diastereomeric mixture of unstable aldehydes **28**. Elimination of the acetate afforded a 1:1 diastereomeric mixture of olefins. Cyclization was accomplished using formic acid to form tetracycle **29** as a single diastereomer. The selectivity observed was rationalized on the fact that the two olefins were in equilibrium and only the *Z*-isomer could undergo cyclization.

A two-step sequence was used to reduce the benzylic olefin from the least hindered side, followed by removal of the Cbz protecting group and methylation of the amino group. Reduction of the lactam carbonyl using alane yielded the key Pictet−Spengler precursor. Upon treatment of the amine with N-Cbz-glycinal, the pentacycle **30** was formed in a 6:1 diastereomeric ratio at C-1 with the desired diastereomer as the major product. Removal of the Cbz group, followed by coupling with pyruvyl chloride, produced amide **31** in 72% yield. The final step was the oxidation of the two hydroquinones to quinones using ceric ammonium nitrate to afford saframycin B in 37% yield.

In 1990, Fukuyama et al. reported the first synthesis of (±)-saframycin A as shown in Scheme 3.[20] Aromatic aldehyde **24** was treated with the potassium enolate of the diketopiperazine **32** to form **33** in 86% yield. This aldol chemistry was first used by Kubo et al. in their saframycin B synthesis[21] (Scheme 4). Employment of these reaction conditions removed one acetate group, allowing for a selective protection of the amide as a Cbz carbamate to afford diketopiperazine **34**. Following a second aldol condensation with aldehyde **24**, the N-Cbz-protected amide was selectively reduced to the carbinolamine using sodium borohydride. This allowed for a cyclization via an iminium ion upon treatment with formic acid to afford tricycle **36.** High-pressure hydrogenation over Raney-Ni followed by amine methylation yielded **37** in 85% yield. The lactam was activated for ring opening via protection of the lactam nitrogen as the corresponding *tert*-butyl carbamate. The lactam carbonyl was then reduced under mild conditions to afford **38**. Removal of the *tert*-butyl carbamate was followed by a Pictet−Spengler reaction, affording the pentacyclic core.

Swern oxidation of the primary alcohol afforded the corresponding aldehyde, which condensed with the amine to form an intermediate carbinolamine that was trapped with sodium cyanide to form the stable aminonitrile **39**. The final steps of the synthesis involved cleavage of the *tert*-butyl carbamate, amide formation using pyruvyl chloride, and oxidation of the

Scheme 2. Fukuyama's Total Synthesis of D,L-Saframycin B



hydroquinones to quinones using DDQ, thus affording (±)-saframycin A.

In 1987, Kubo et al. reported their synthesis of (±)-saframycin B (Scheme 4).[21] Aromatic aldehyde **40** was condensed with diketopiperazine **32**, followed by hydrogenation of the benzylic olefin. A second aldol condensation provided **41** in 52% overall yield for the three steps. Activation of one of the lactam carbonyls was accomplished via the benzyl protection of the unprotected lactam followed by acetate removal and carbamate formation to afford **42**. Partial reduction of the activated lactam **42** was accomplished using lithium aluminum tri-*tert*-butoxyhydride. Cyclization of the carbinolamine was achieved using formic acid as in Fukuyama's syntheses.[19,20] Removal of the isopropyl carbamate followed by *N*-methylation yielded tricycle **43** in 50% yield.

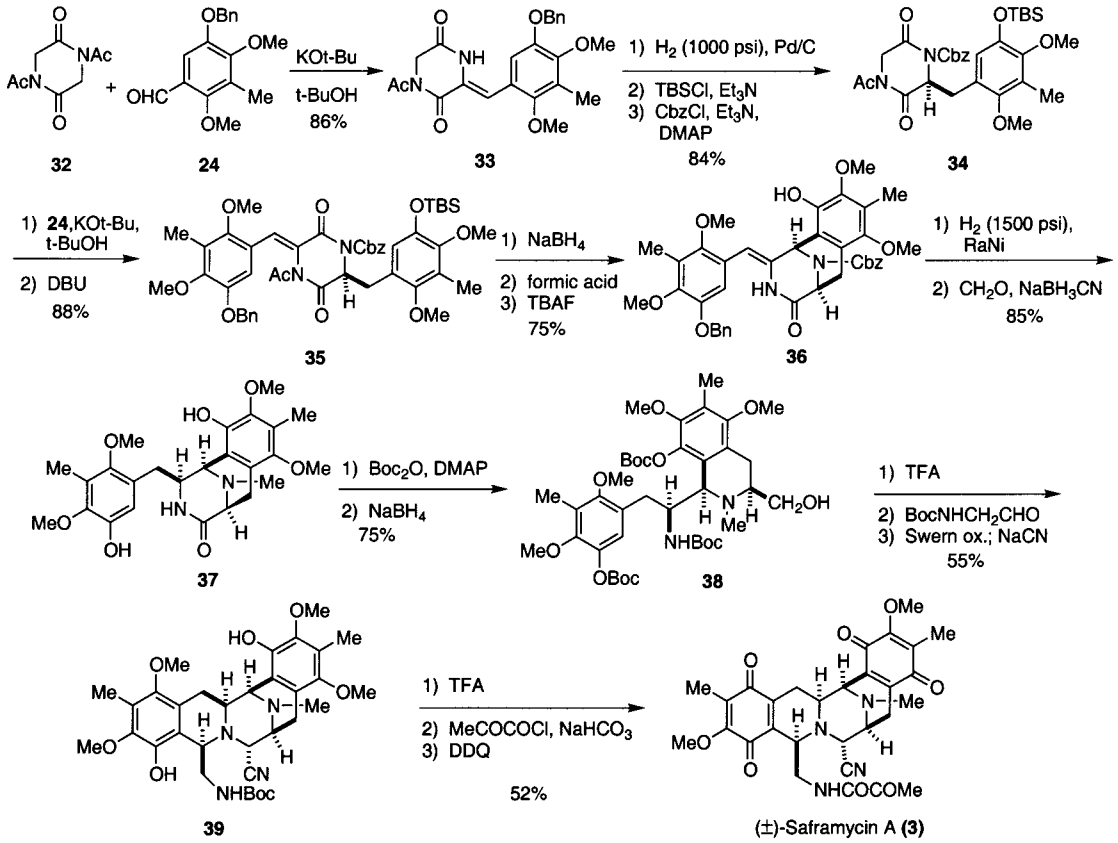
Tricycle **43** was converted to pentacycle **44** via reduction of the amide to the amine using alane followed by hydrogenolysis of the benzylic olefin and the benzylamine followed by a Pictet−Spengler cyclization. Unfortunately, the stereochemistry obtained at C-1 was undesired. Epimerization of this center was accomplished by oxidation of the amine to the imine using mercury(II) acetate followed by selective reduction of the imine from the least hindered face using $NaBH_4$. The butyl ester was reduced using LAH to afford **45** in 55% yield over the three steps. Amination of the alcohol was accomplished via a Mitsunobu reaction using phthalimide. The phthalimide protecting group was removed, and the amine was acylated with pyruvyl chloride to yield **46**. The final two steps were demethylation of the hydroquinones using boron tribromide followed by oxidation to the diquinone using 10 M HNO_3 to provide saframycin B in 41% yield for the last two steps.

Kubo et al. showed that (±)-saframycin B could be converted to saframycins C (**5**) and D (**9**) via a selective oxidation using SeO_2 (Scheme 5).[22] Using dioxane as the solvent, (±)-saframycin D was synthesized in 16% yield. The use of methanol as the solvent yielded (±)-saframycin C in 45% yield.

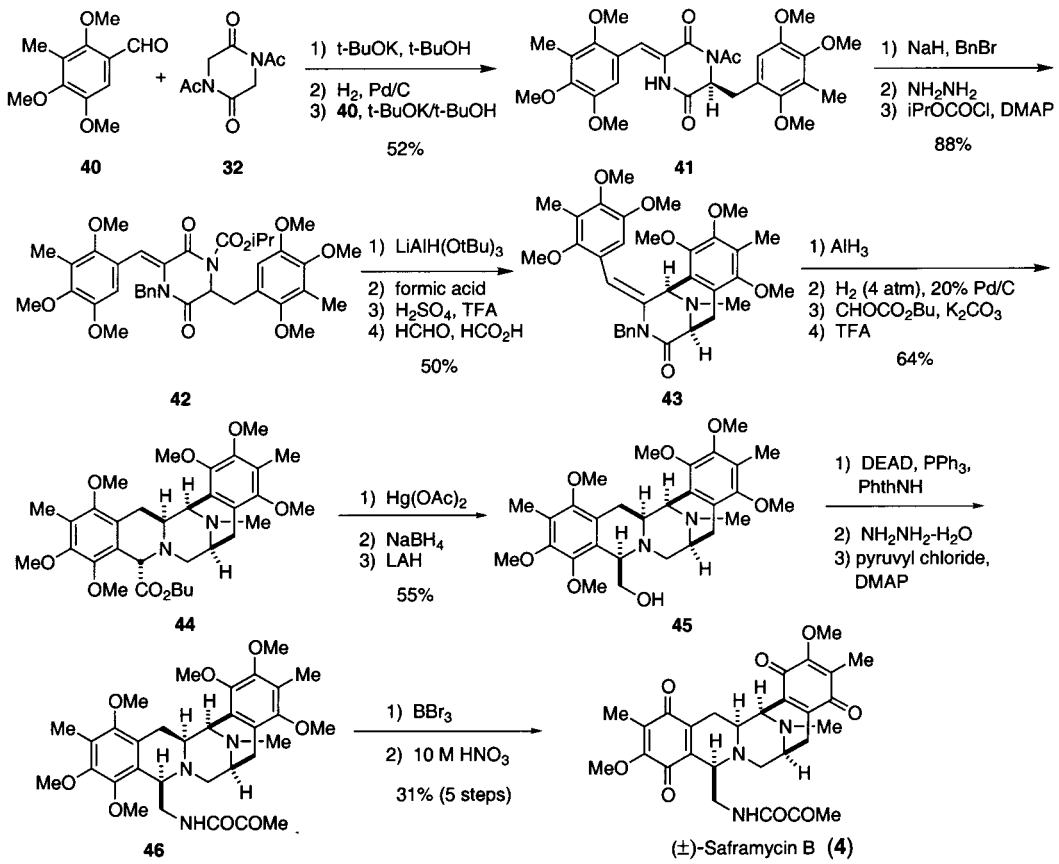
Kubo et al. also showed that (−)-saframycin A could be oxidized with SeO_2 to yield five saframycins (Scheme 6).[6,23] The highest yielding product was (−)-saframycin G in 30% yield. Saframycin G was then converted to the saframycin Mx series compound **49** by reduction of the two quinone rings to the hydroquinones under catalytic hydrogenation conditions. The A-ring was then regioselectively oxidized using silica gel in the presence of oxygen to provide **49** in 52% yield.

Using the same three-step sequence[6] as in Scheme 6, Kubo et al. transformed (±)-saframycin B into an unstable product that was acylated to form triacetate **50** (Scheme 7). The triacetate had identical spectroscopic data to that of the triacetate derivative of saframycin E (**10**).

Scheme 3. Fukuyama's Total Synthesis of D,L-Saframycin A

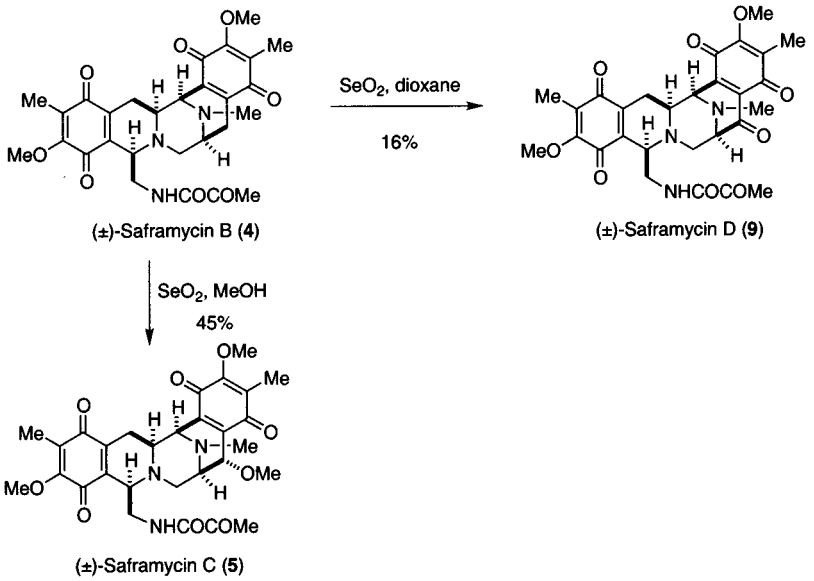


Scheme 4. Kubo's Total Synthesis of D,L-Saframycin B

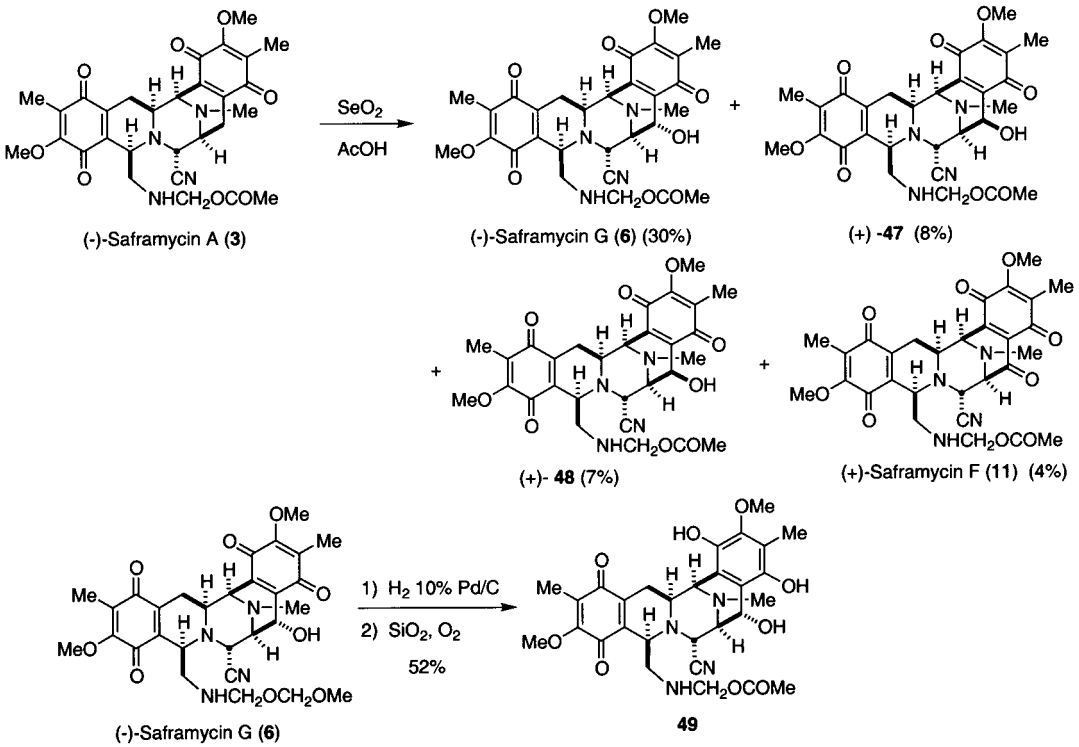


Racemic pentacycle **46**, an intermediate in the saframycin B synthesis, was used as a precursor for the synthesis of (−)-*N*-acetylsaframycin Mx2 (**55**) and *epi*-(+)-*N*-acetylsaframycin Mx2 (**56**) by Kubo et al.

Scheme 5. Conversion of Saframycin B to Saframycins C and D



Scheme 6. Selenium Dioxide Oxidation of Saframycin A

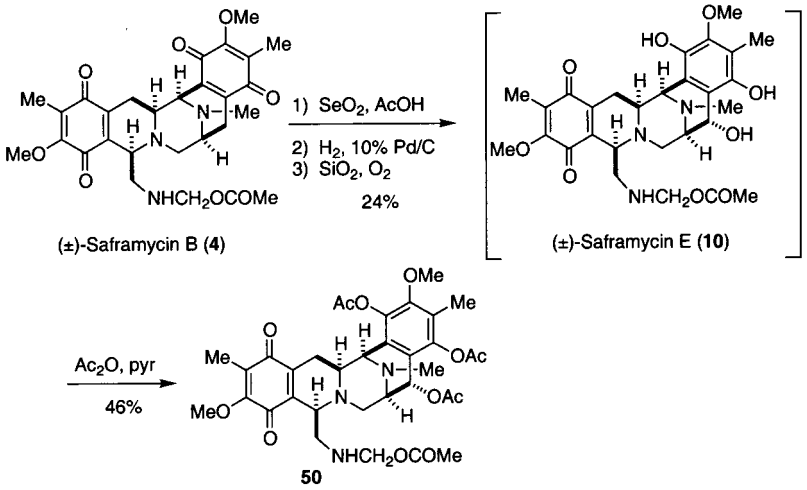


(Scheme 8).[24] The first step in the sequence involved the coupling of *N*-Cbz-L-alanine to the primary amine yielding the optically active amide **51** and its *epi*-enantiomer **52** in 42% and 37% isolated yields, respectively. Each diastereomer was subsequently carried on separately to the final Mx2-type compound.

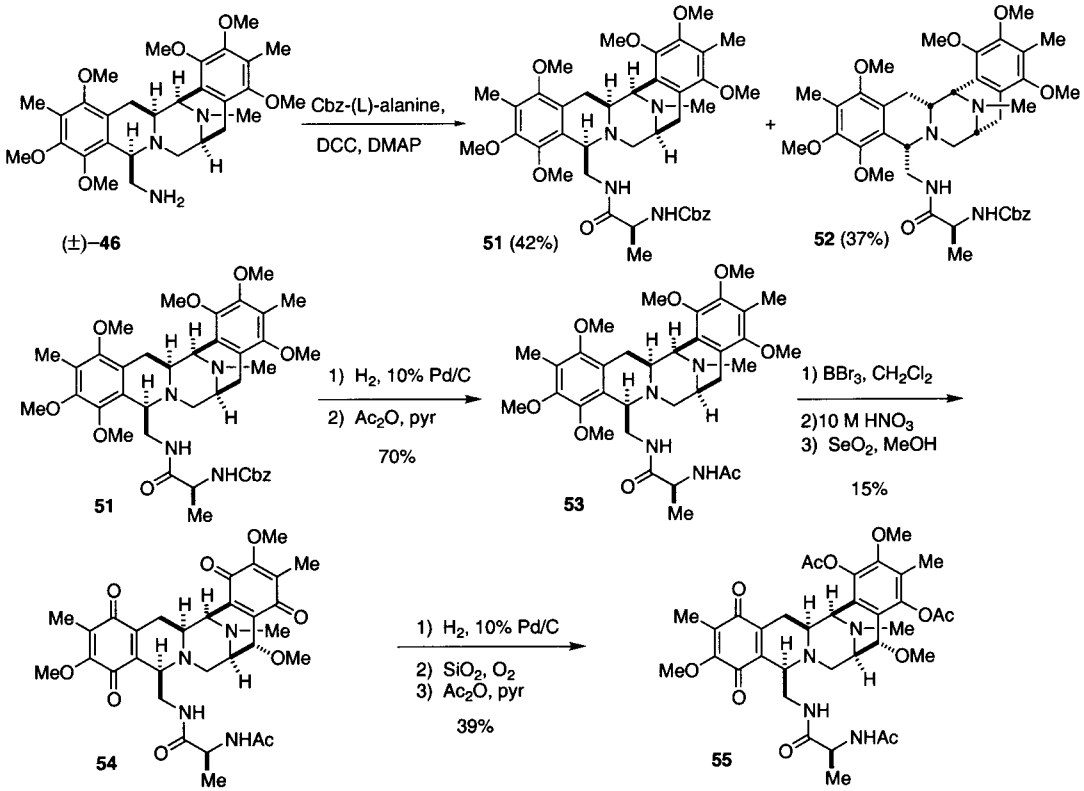
The *N*-Cbz group was removed from compound **51**, and the resultant amine was acylated to form **53** (Scheme 8). The hydroquinones were deprotected and oxidized to the corresponding quinones with SeO_2, which also effected selective oxidation of the D-ring, furnishing the desired methyl ether **54**. Reduction of the quinones followed by regioselective oxidation of the A-ring hydroquinone yielded the saframycin Mx2 derivative that proved to be both light and air sensitive. Acetylation of the hydroquinone portion yielded the stable triacetate **55**. Similarly, pentacycle **52** was transformed into **56** via the same sequence of steps in comparable yield (Scheme 9).

The first asymmetric synthesis of (−)-saframycin A was accomplished in 1999 by Myers and Kung.[25] This elegant and convergent synthesis focused on the hidden symmetry of saframycin A (Scheme 10). Alkylation of pseudoephedrine **57** with bromide **58** afforded the homobenzylic amine **59** in 80% yield.[25b] Cleavage of the auxiliary to form the amino alcohol was followed by amine protection and oxidation of the alcohol to the aldehyde **60**. This aldehyde was used to form both halves of saframycin A. Treatment

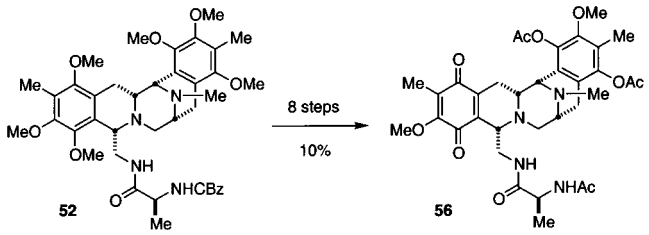
Scheme 7. Conversion of Saframycin B to Saframycin E



Scheme 8. Resolution and Transformations of Racemic Compound 46



Scheme 9. Synthesis of an *ent-epi*-Saframycin Derivative

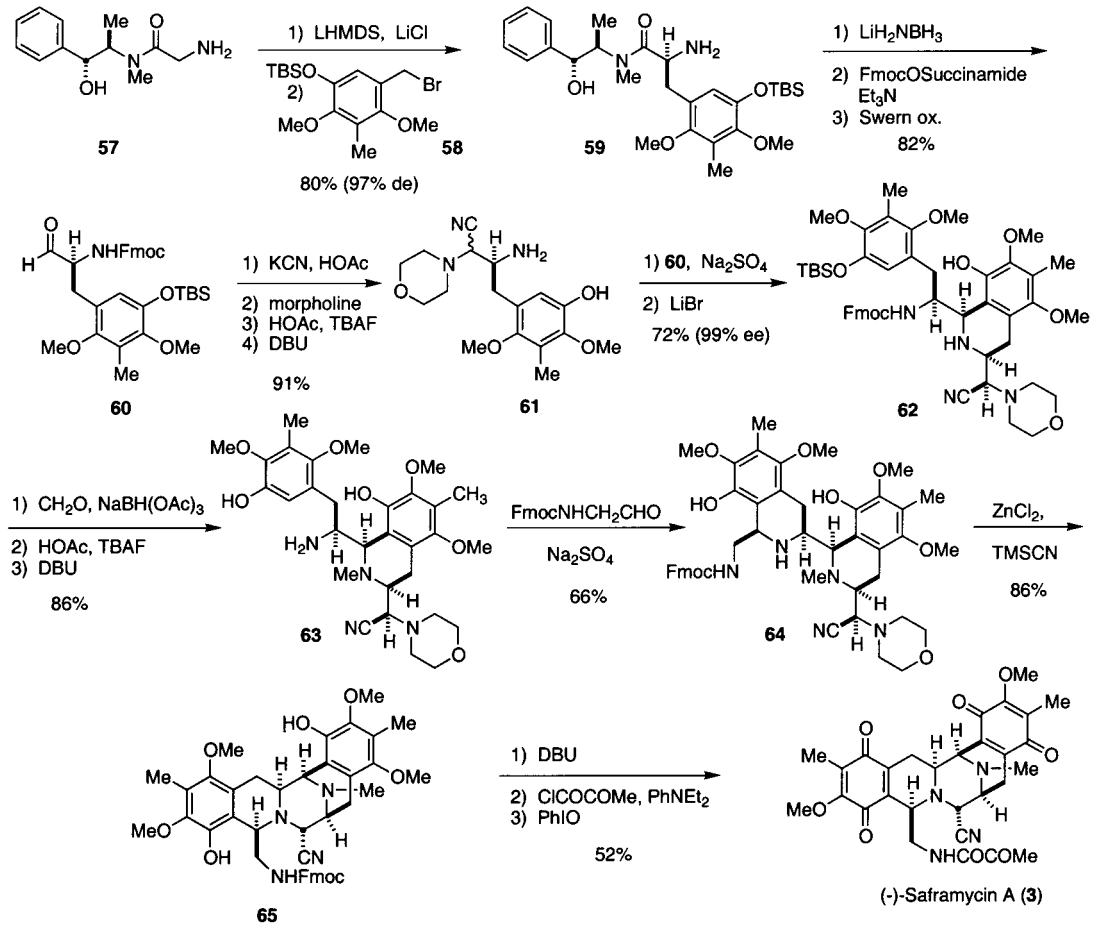


of the aldehyde with HCN formed the cyanohydrin, which was treated with morpholine to yield the corresponding amino nitrile,[25c] which served as a masked aldehyde. Removal of the TBS and Fmoc groups was accomplished in two steps in high yield to form amine **61**.

Pictet−Spengler cyclization of amine **61** with aldehyde **60** in the presence of Na_2SO_4 provided bicycle **62** in good yield and high enantiomeric excess. Reductive amination with formaldehyde followed by TBS and Fmoc deprotection afforded the *N*-methyl bicyclic substance **63**. A second Pictet−Spengler cyclization with *N*-Fmoc glycinal provided **64** in 66% yield. Treatment of **64** with anhydrous zinc chloride promoted iminium ion formation and cyclization, providing the pentacycle **65** in 86% yield. Removal of the Fmoc group was followed by acylation of the amine with pyruvoyl chloride. Finally, treatment with iodosobenzene provided (−)-saframycin A in 52% yield for the last three steps.

In 1999, Corey and Martinez published the second asymmetric synthesis of (−)-saframycin A as il-

Scheme 10. Myers' Total Synthesis of (−)-Saframycin A



lustrated in Schemes 11 and 12.[26] This synthesis started with hexacycle **77** (Scheme 12), an intermediate very similar to intermediate **76** originally published in their synthesis of ecteinascidin A[27] (the synthesis of **76** will be discussed here for clarity).

As shown in Scheme 11, arene **66** was methylated and formylated to form **67**. The methoxymethyl protecting group was swapped for the corresponding benzyl group yielding **68**. An aldol condensation between the mixed malonate **69** and aldehyde **68** yielded a mixture of *E*- and *Z*-olefin isomers **70**. This mixture was carried on, and the allyl ester was cleaved followed by a Curtius rearrangement in which the intermediate isocyanate was trapped with benzyl alcohol to form the carbamate **71** as a single stereoisomer.

The stereochemistry of the tetrahydroisoquinoline was set via a rhodium-catalyzed asymmetric hydrogenation of the benzylic olefin yielding the saturated compound in 96% ee. Deprotection of the aldehyde followed by an intramolecular Pictet−Spengler cyclization afforded tetracycle **72**. Amine **72** was then treated with aldehyde **73,** and the resultant carbinolamine was trapped with HCN to form the amino nitrile **74**. Reduction of the lactone yielded a lactol that was activated for iminium ion cyclization using methanesulfonic acid to afford hexacycle **75**. A six-step sequence featuring the selective activation of the least hindered phenol and methylation of the resultant triflate thus furnished compound **76**.

Allylation of phenol **77** (the only difference in structure between **76** and **77** is the silyl protecting group on the primary alcohol) followed by removal of the TBS groups provided the alcohol **78** in high yield (Scheme 12). The alcohol was converted into an amine, which was subsequently acylated with pyruvyl chloride. The phenol was then deprotected to afford **79**. An efficient one-step oxidation of the E-ring and MOM removal was accomplished using 1-fluoro-3,5-dichloropyridinium triflate. Methylation of the phenol followed by oxidation of the A-ring hydroquinone was accomplished using salcomine and oxygen to yield (−)-saframycin A.

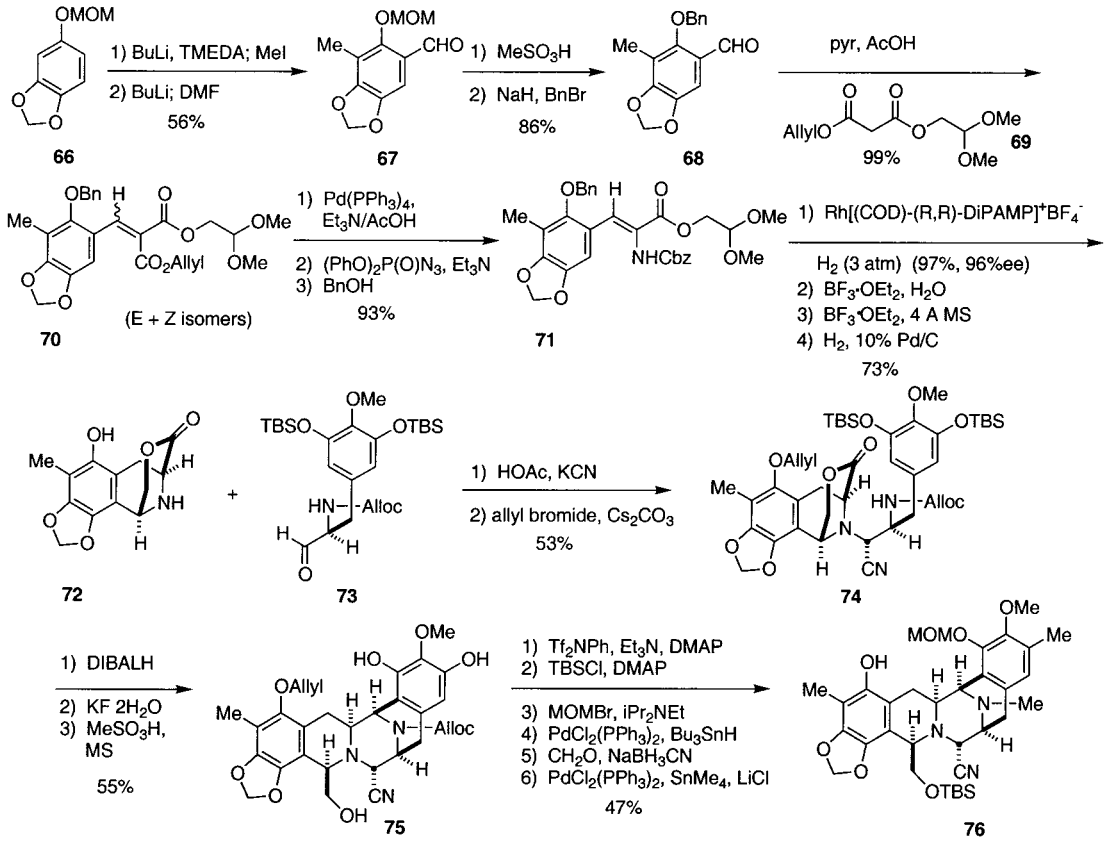
In 2000, Martinez and Corey reported an improved synthesis[28] of intermediate **75** that was utilized in their total syntheses of saframycin and ecteinascidin as shown in Scheme 13. This synthesis improved the yield of **75** from 11% in 13 steps to 57% in six steps.

The peptide coupling of **72** and **80** followed by phenol protection provided **81** in 81% yield. Reduction of the lactone to the aldehyde set up the intramolecular Pictet−Spengler cyclization, which afforded **82** in 85% yield. Finally, partial reduction of the amide to the carbinolamine was followed by treatment with HCN to form the aminonitrile **75** in very good yield.

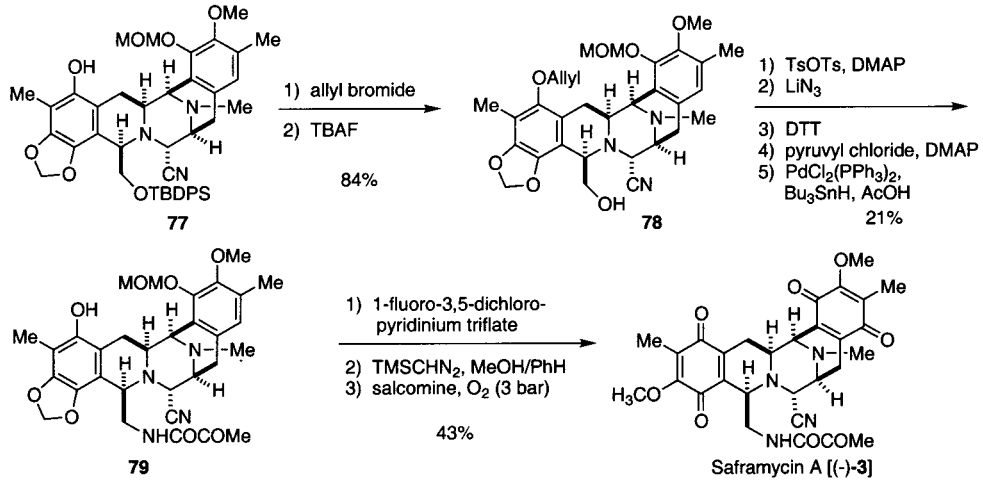
2.1.4. Synthetic Studies toward the Saframycins

In 1982, Kurihara et al. reported the first synthetic studies on the saframycins.[29] Starting with the ty-

Scheme 11. Corey's Synthesis of Saframycin A and Ecteinascidin Intermediate 76



Scheme 12. Corey's Saframycin Synthesis



rosine derivative **83**, the mixed anhydride was formed and condensed with aminoacetaldehyde dimethyl acetal to form **84** (Scheme 14). Heating **84** in trifluoroacetic acid afforded tricycle **85** via a double cyclization. After partial reduction of the amide using DIBALH, oxidation of the hydroquinone, followed by treatment with potassium cyanide, yielded a mixture of the desired tricycle **87** and **88** in 81% combined yield.

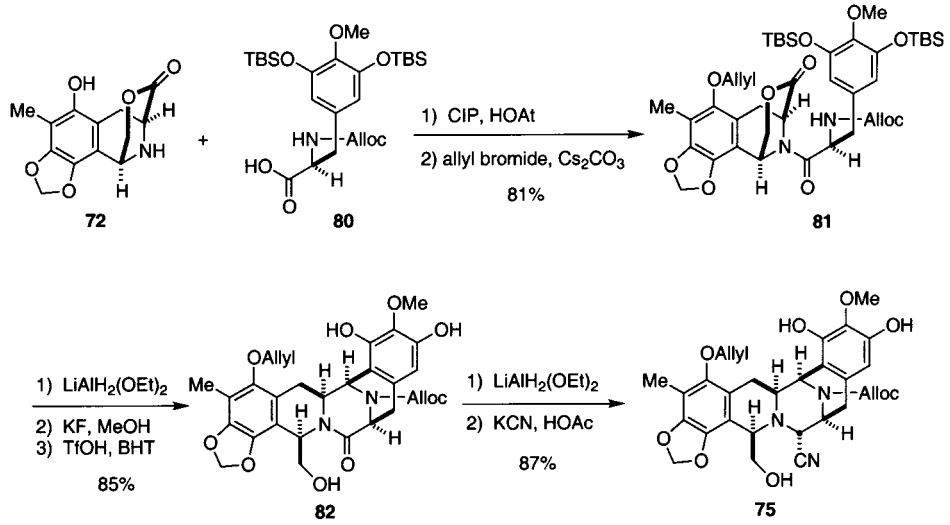
In 1989, Kubo et al. showed in synthetic studies toward saframycin A that tetracycle **91** could be formed with the amide carbonyl intact (Scheme 15).[30] This would allow for further functionalization to form the amino nitrile in saframycin A.

The first study on the asymmetric synthesis of the saframycins was published by Kubo et al. in 1997 (Scheme 16).[31] Aldol condensation between the optically active diketopiperazine (+)-**92** and aldehyde **40** yielded (−)-**93** after further elaboration. It was hoped that (−)-**93** could undergo a specific cyclization to form an optically active tricyclic compound. However, on a racemic model system, little diastereoselectivity was observed in the cyclization.

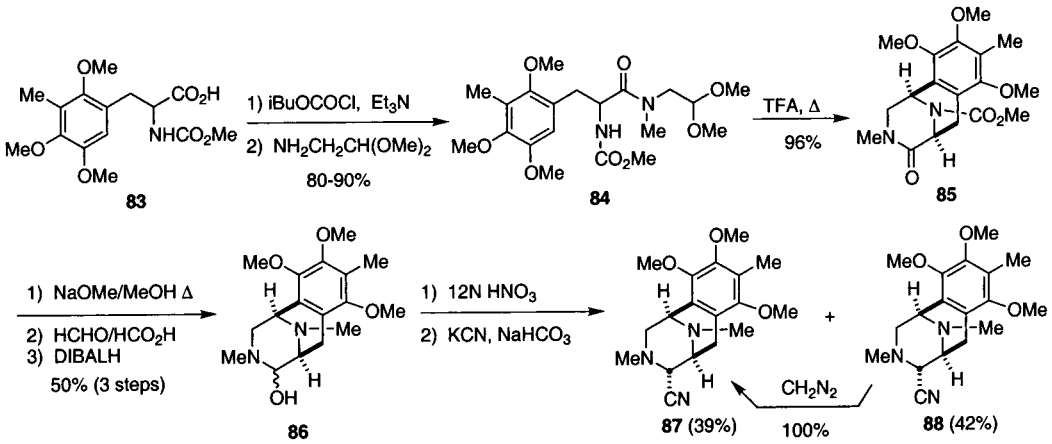
In 1990, Ong and Lee synthesized the tricycles **95** and **96** via a Pictet−Spengler cyclization using acetaldehyde on a diketopiperazine (Scheme 17).[32] The major drawback to this approach was that the stereogenic center constructed in the Pictet−Spengler reaction gave the unnatural relative stereochemistry.

In 1991, Liebeskind and Shawe took advantage of the hidden symmetry of saframycin B in their synthetic study illustrated in Scheme 18.[33] Condensation

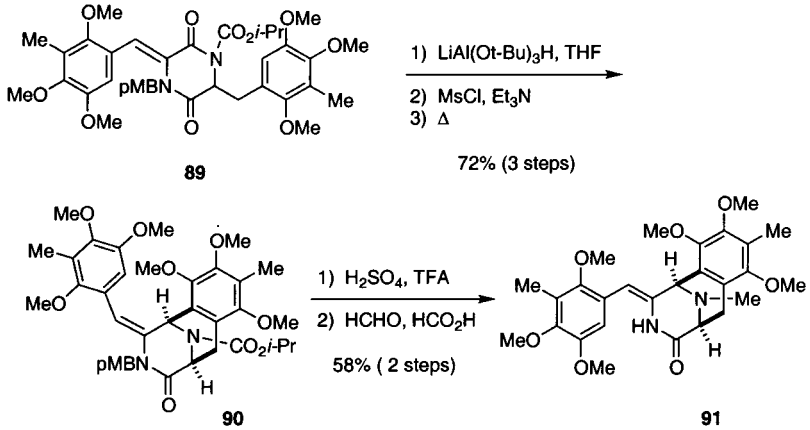
Scheme 13. Corey's Improved Synthesis of Intermediate 75



Scheme 14. Kurihara's Saframycin A Synthetic Studies



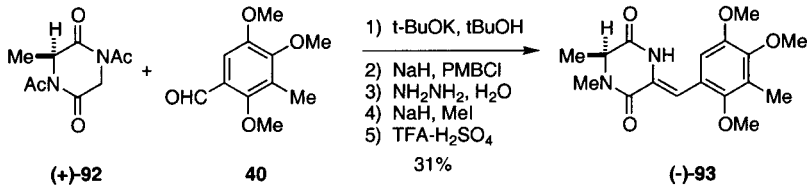
Scheme 15. Kubo's Synthetic Approach to Saframyicn A



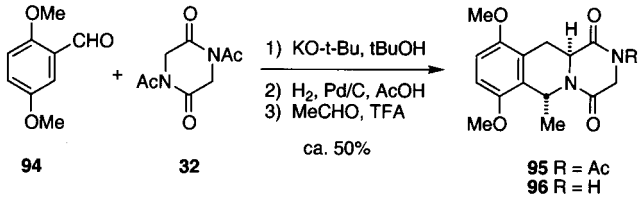
of 2 equiv of aldehyde **40** with diketopiperazine **32** yielded the symmetrical diketopiperazine **97**. After reduction of the olefins and protection of the amide nitrogens, a partial reduction of one of the amide carbonyls was accomplished using lithium diethoxyaluminum hydride. The carbinolamine was cyclized using TFA to form tricycle **99**. Unfortunately, the stereochemistry at C-3 was undesired and the authors note that attempts to invert the stereogenic center at C-3 of tricycle **100** were unsuccessful under various conditions.

In 2000, Danishefsky et al. published a route to the saframycins and ecteinascidins utilizing a convergent intramolecular Mannich cyclization strategy (Scheme 19).[34] The E-ring was constructed starting with aldehyde **101**. The phenolic hydroxyl group of **101** was alkylated, and the aldehyde residue was subjected to Bayer−Villiger oxidation to yield phenol **102**. Heating **102** in dimethylaniline at 210 °C effected Claisen rearrangement which, after protecting group manipulations, afforded **103**. Alkylation of the arene ring followed by protection of the hydroxyl

Scheme 16. Kubo's Asymmetric Studies toward the Saframycins



Scheme 17. Ong and Lee Synthetic Studies toward the Saframycins



groups and removal of the pivaloyl group provided **104** in high yield. Sharpless epoxidation followed by selective epoxide opening with azide and diol protection lead to dioxolane **105**. Azide reduction in the presence of di-*tert*-butyl dicarbonate afforded the corresponding carbamate. Methylation of the carbamate nitrogen was followed by cleavage of the silyl ether and *p*-methoxyl benzyl ether formation. Oxidative cleavage of the diol afforded the *N*-Boc amino acid **106** in 85% from **105**.

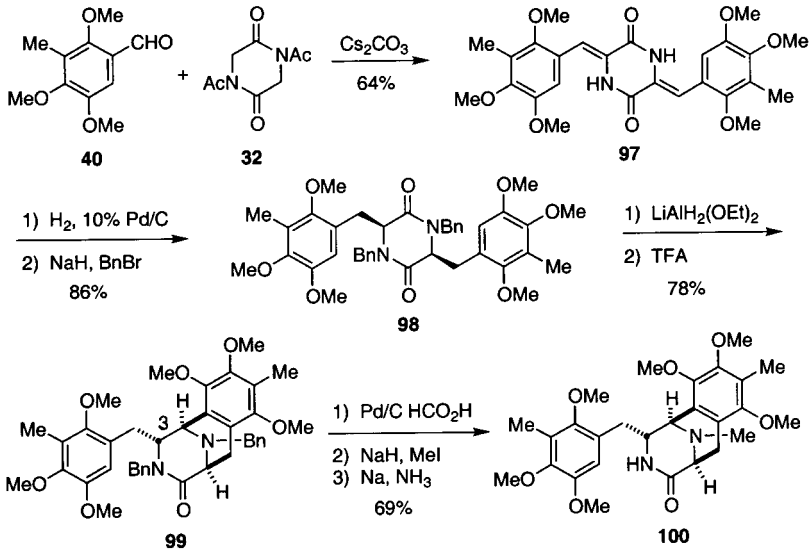
The A-ring was synthesized in high yield starting with the olefination of aldehyde **40** followed by Sharpless asymmetric dihydroxylation to furnish optically active material. The diol was converted to the optically pure epoxide **107** via tosylation and base-mediated ring closure. The epoxide was opened with sodium azide, and the azide was subsequently reduced and protected yielding **108**. Removal of the Boc group was followed by alkylation of the resultant amine with bromoacetaldehyde diethyl acetal. The acetal was then cyclized under acidic conditions to form the bicyclic substance **109**. The two fragments (**106** and **109**) were successfully coupled using BOPCl in 63% yield[34b] followed by a sequence of oxidations to furnish the precyclization substrate **110**. Treatment of **110** with formic acid effected removal of the *N*-Boc group and cyclization furnishing the desired pentacyclic substances **111** and **112** in 75% and 17% yields, respectively. Curiously, efforts to complete the total synthesis of a natural saframycin from **111** have not been reported.

Myers and Kung devised an extremely concise and elegant convergent approach to this family of alkaloids as illustrated in Scheme 20. The pentacyclic core of saframycin A (**65**) was constructed via a one-step cyclization from the amino aldehyde "trimer" **115** (Scheme 20).[35] The synthesis of **115** was accomplished utilizing the same components employed in their saframycin A synthesis. Thus, condensation of **60** with amine **113** in the presence of $H^{13}CN$ afforded **114** via a Strecker protocol. Removal of the TBS groups and Fmoc group followed by condensation with N-Fmoc-glycinal afforded **115** in 68% yield from **114**. The impressive formation of ^{13}CN-**65** was accomplished by the treatment of **115** with magnesium bromide etherate in refluxing THF for 5 h in 9% yield. Despite the low yield of this step, the formation of **65** constituted three consecutive cyclizations where three of the five stereogenic centers of **65** were formed in this single step. It was proposed that the aminal of **115** cleaved first followed by a Pictet−Spengler cyclization upon the resultant imine to form the B-ring. The D-ring was then formed by a second Pictet−Spengler cyclization followed by a Strecker reaction to form pentacycle **65**.

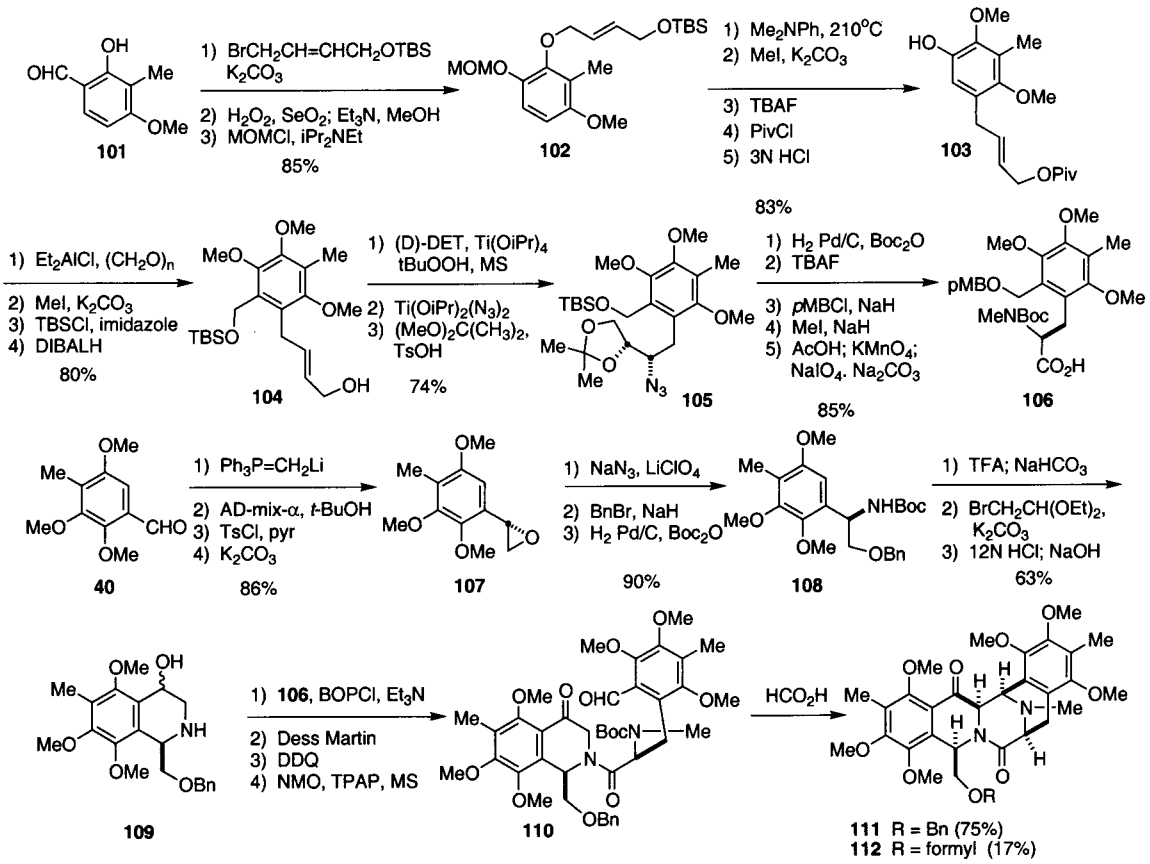
2.1.5. Analogue Syntheses

The first series of saframycin analogues was obtained by microbial bioconversions of natural (−)-

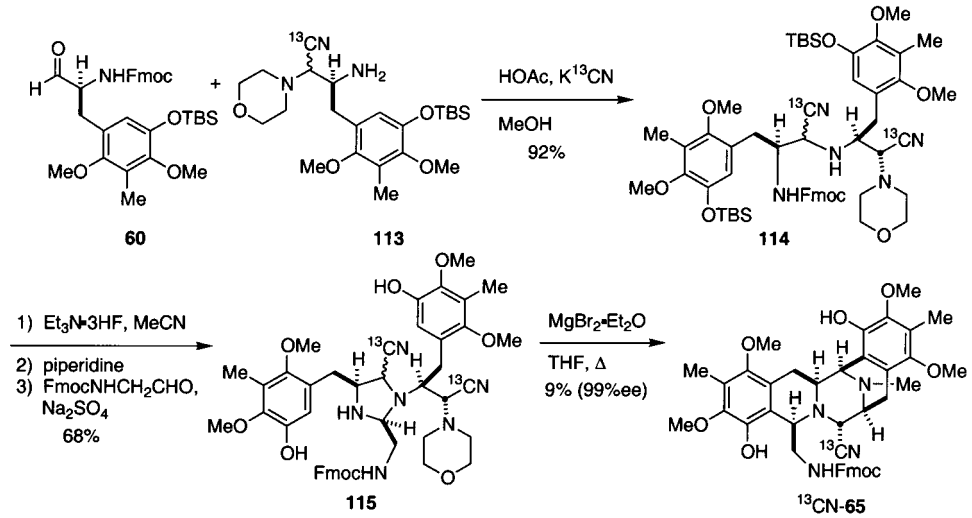
Scheme 18. Liebeskind's Synthetic Studies toward Saframycin A



Scheme 19. Danishefsky's Synthetic Studies toward the Saframycins



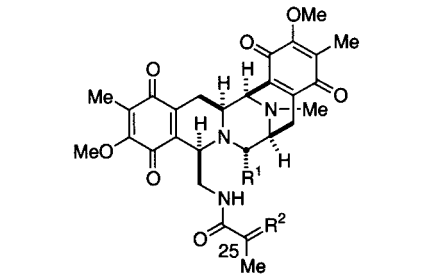
Scheme 20. Myers' Synthesis of Pentacycle 65



saframycin A (Figure 5).[36] Bioconversions using *Rhodococcus amidophilus* IFM 144 yielded three products, saframycins AR_1 (**116**), AR_2 (saframycin B), and AR_3 (**118**).[36a] This conversion was also seen with other species of actinomycetes.[36b] In this study saframycin A was also treated with sodium borohydride to reduce the carbonyl at C-25 to afford a mixture of diastereomeric alcohols AH_1 (**117**) and AH_2 (**116**) (same as AR_1). The reduced diastereomers **116** and **117** were then converted to their acetates forming AH_1Ac (**119**) and AH_2Ac (**120**).[36c]

Two simple amino nitrile analogues of saframycin A were synthesized by Kubo et al.[37] Scheme 21 illustrates the preparation of a diastereomeric pair of amino nitriles **124** and **125**. Condensation of aldehyde **40** with amine **121** yielded **122**. A four-step sequence featuring a Friedel−Crafts acylation afforded **123**. Deoxygenation was followed by reduction of the amide, in which the resultant carbinolamine was trapped with sodium cyanide. Finally, oxidation to the quinone afforded diastereomers **124** and **125**.

A second set of amino nitriles were also synthesized by Kubo et al. that contained a five-membered C-ring as shown in Figure 6.[38] The tricycles (**126**−**130**) were prepared utilizing the same chemistry as that above in Scheme 21.



Saframycin AR_1 = AH_2 (**116**) R^1 = CN, R^2 = H, OH
AH_1 (**117**)
AR_3 (**118**) R^1 = H, R^2 = H, OH
AH_1Ac (**119**)
AH_2Ac (**120**) R^1 = CN, R^2 = H, OAc

Figure 5. Saframycin analogues obtained via bioconversion and semisynthesis.

Table 1. Antimicrobial Activity of Saframycins A and S

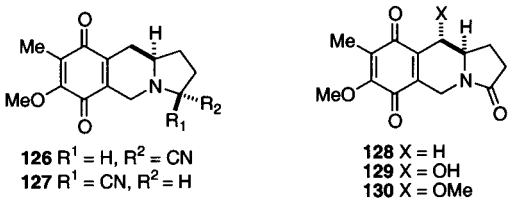
test organism	**3** MIC (μg/mL)	**8** MIC (μg/mL)
Staphylococcus aureus FDA 209P	0.1	0.025
Streptococcus faecalis	12.4	3.12
Bacillus subtilis PCI 219	0.1	0.025
Corynebacterium diphtheriae	0.003	0.004
Sarcina lutea	0.05	0.025

Myers and Plowright reported the synthesis of a series of saframycin A analogues that were synthesized from pentacycle **65** via the removal of the Fmoc group followed by coupling of several acids to the primary amine (Scheme 22).[39] For the structures of these analogues, see the Biological Activity section. These analogues were tested in the bishydroquinone oxidation state.

2.1.6. Biological Activity

All of the saframycins have been found to display antitumor and antimicrobial activity. Saframycin S displays the most potent antitumor activity,[40] while saframycins R^8 and A^7 exhibited similar but less potent antitumor and antimicrobial activities (Table 1). These three saframycins have either a nitrile or hydroxyl at C-21. Saframycins B and D, which lack a leaving group at C-21, as expected, displayed the lowest antitumor activity.[2]

The ID_{50} (50% inhibition dose) activities against L1210 leukemia of several saframycins are listed in Table 2.[36c] Saframycins A, S, AH_1, and AH_2 (**3**, **8**, **116**, and **117**, respectively) containing either a nitrile or hydroxyl group at C-21 possess the highest activities. Saframycins G, H, F, AH_1Ac, and AH_2Ac (**6**, **7**, **11**, **119**, and **120**, respectively) which contain a leaving group at C-21 also have sterically demanding side chains that apparently block the incipient iminium species from alkylating DNA. Saframycins B, C, D, and AR_3 (**4**, **5**, **9**, and **118**, respectively) which lack a leaving group at C-21 had much lower activities.



126 R^1 = H, R^2 = CN
127 R^1 = CN, R^2 = H

128 X = H
129 X = OH
130 X = OMe

Figure 6. Simple saframycin analogues.

Table 2. Antitumor Activity of Saframycins and Analogs versus L1210 Leukemia

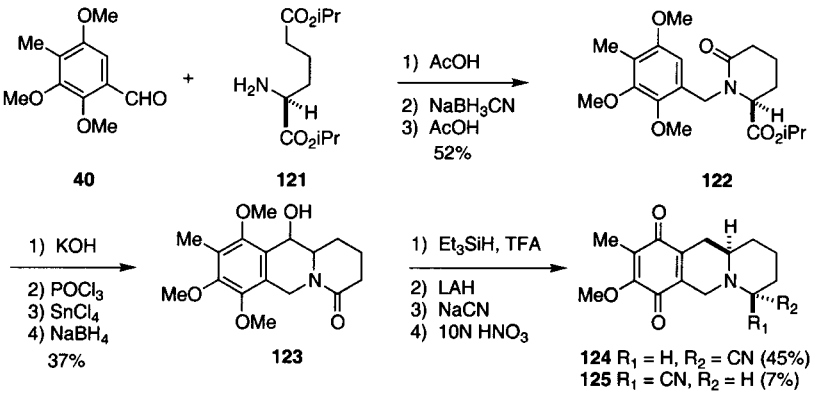
compound	ID_{50} (μM)	compound	ID_{50} (μM)
3	0.0056	119	0.025
8	0.0053	120	0.027
116	0.0061	4	0.80
117	0.0080	5	3.9
6	0.030	9	4.8
7	0.033	118	0.65
11	0.59		

Saframycin S had very potent in vivo activity against Ehrlich ascites tumors.[40] At the near optimum dose of 0.5 mg/kg/day the percentage of 40 day surviving mice was 80−90% versus all of the control mice that died within 18 days.

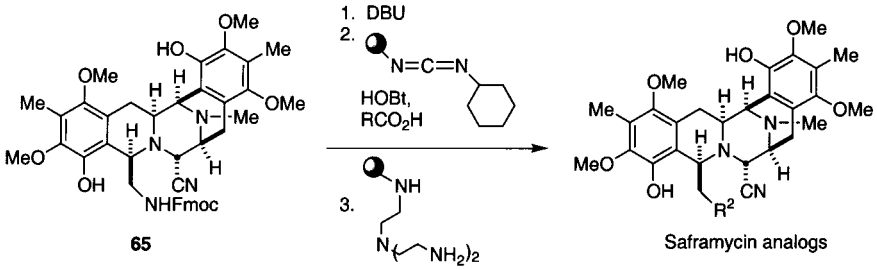
There was no difference in biological activity between saframycins Y3, Yd-1, Yd-2, and Ad-1 with respect to an amino group or a carbonyl at C-25.[41] Also, the dimers Y2b and Y2b−d had similar activities to the corresponding monomers. In a study to examine side chain effects on biological activity, Arai et al. synthesized 15 acyl, 9 alkyl, and 3 carbamoyl derivatives of the C-25 amino group of saframycin Y3.[42] It was found that the acyl derivatives had lower activity while the alkyl derivatives had similar activities to the natural product. Also, as the side chain became bulkier, the activity decreased.

Another study on the side chain involved the bioconverted saframycins AR_1 and AR_3 along with the semisynthetic saframycin AH_1.[34a] It was found that

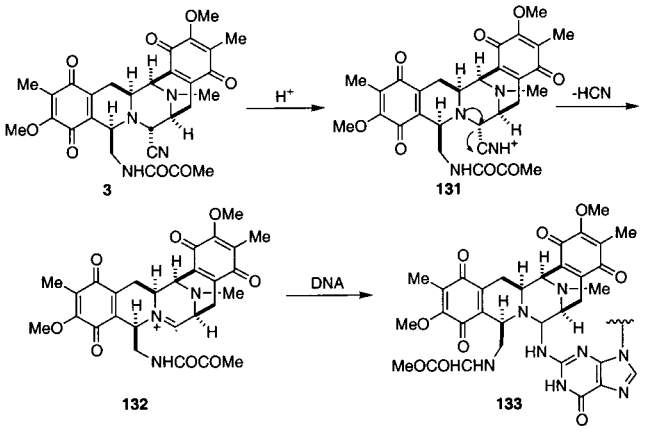
Scheme 21. Simple Saframycin A Analogs



Scheme 22. Myer's Synthesis of Saframycin A Analogs



Scheme 23. Proposed Mechanism of DNA Alkylation by Saframycin A



reduction of the ketone at C-25 had no impact on antitumor activity, but there was a marked loss of antimicrobial activity. The ED_{50} against L1210 leukemia was 0.003, 0.004, and 0.35 μg/mL for saframycins A, AR_1, and AR_3 respectively.

The simple saframycin A analogues **124**−**130** were also tested for biological activity;[37,38] however, none of these compounds displayed significant cytotoxicity exhibiting 2.0−4.0 μg/kg ED_{50} values against L1210 murine leukemia. However, the amino nitriles **126** and **127** possessed good bioactivity against fungi in which saframycin A had little activity.[38]

Saframycin A had been shown to inhibit RNA synthesis at 0.2 μg/mL, while DNA synthesis was inhibited at higher concentrations. Inhibition of nucleic acid biosynthesis was observed at lower concentrations when saframycin A was reduced to the corresponding hydroquinone prior to testing.[43] Saframycin S does not need to be reduced for antitumor activity, but the activity was enhanced when saframycin S was in the reduced form.[44] Reductants such as dithiothreitol (DTT) reduce the quinone moieties to the corresponding dihydroquinones that activate these substances for iminium ion formation and subsequent DNA alkylation. For example, saframycin A in the presence of DTT has been shown to release cyanide, indicating that the iminium species is readily formed from this oxidation state.

The presence of either a nitrile or hydroxyl group at C-21 allows for the formation of an electrophilic iminium species that alkylates DNA in the minor groove. The mechanism originally proposed by Lown et al. (Scheme 23) for alkylation invokes protonation of the nitrile (**131**) with expulsion of HCN to form the iminium ion species **132**.[45] The N-2 residue of guanine subsequently forms a covalent bond to the drug resulting in an adduct such as **133**.

Evidence to support the alkylation hypothesis was obtained by radiolabeling experiments in which ^{14}C-labeled tyrosine was biosynthetically converted to saframycin A.[44] Upon exposure of the ^{14}C-labeled saframycin to DNA in the presence of DTT, it was found that the DNA retained the ^{14}C label. When ^{14}CN was used to label the C-21 nitrile, under the same set of conditions, it was found that the ^{14}C label was not incorporated into DNA. Furthermore, footprinting studies on saframycin-treated DNA also provide direct experimental evidence for alkylation of DNA by the saframycins.

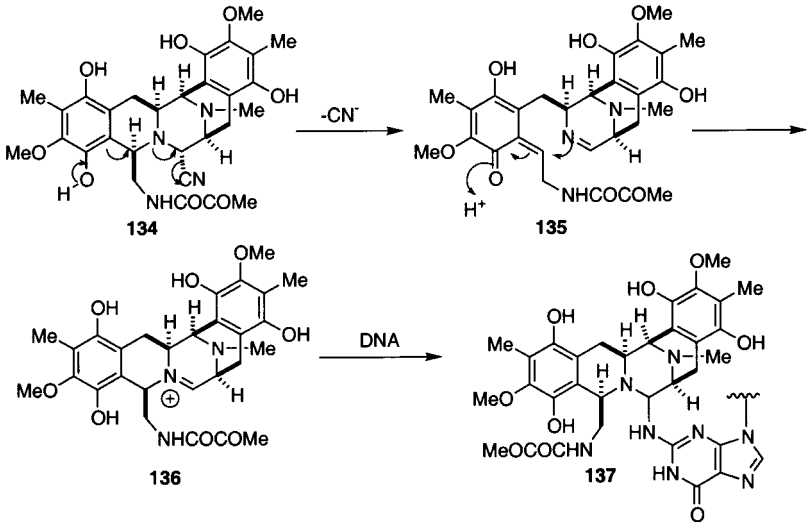
Another mechanism was proposed by Hill and Remers based on the fact that saframycin A does not alkylate DNA unless it was converted into the corresponding hydroquinone form (**134**) (Scheme 24).[46] These workers speculate that the phenol facilitates scission of the B-ring C−N bond, which in turn leads to the expulsion of cyanide. The resulting imine **135** subsequently re-attacks the *o*-quinone methide to form the iminium species **136**, which subsequently alkylates DNA to form the adduct **137**.

The characteristics of DNA binding by the saframycins thus appears to be a simple two-step process whereby (1) reversible noncovalent binding of the drug to the minor groove of DNA is immediately followed by (2) the formation of a covalent bond to DNA within the minor groove. Being a diamino aminal, this linkage is subject to thermal reversal. There is a second type of covalent binding that is promoted by a reducing agent and presumably proceeds through the more reactive dihydroquinones that more readily form the iminium ion species.

The bishydroquinone saframycin A analogues synthesized by Myers and Plowright were used to investigate if there would be increased activity in the reduced form of this natural product.[39] These analogues showed very potent activity against the A375 melanoma and A549 lung carcinoma tumor cell lines with some analogues having a 20-fold increase in activity over saframycin A (Table 3).

Saframycins A and S were found to be modestly sequence specific with respect to DNA alkylation, exhibiting a preference for 5′-GGG and 5′-GGC sequences by the use of MPE (methidium propyl EDTA) Fe(II) footprinting studies.[47a] Saframycin S also displayed a specificity for 5′-CGG, while saframycin A did not. Saframycins Mx1 and Mx3, which both contain the hydroxyl group at C-21, showed the same selectivity as saframycin S.[47b] It has been reasoned that the moderate sequence specificity

Scheme 24. Alternate Mechanism of DNA Alkylation by Saframycin A



observed is due to the molecular recognition of the saframycins for specific DNA sequences prior to iminium ion formation.

It has been argued that the cytotoxicity of the saframycins is not exclusively due to DNA alkylation, and it has also been demonstrated, for instance, that the saframycins cause DNA cleavage under aerobic conditions.[45] Mechanistic studies have provided evidence that superoxide and hydroxyl radical species are formed in the presence of saframycin A in the hydroquinone form while DNA cleavage was not observed in the presence of saframycin A in the quinone form. This is consistent with the well-known capacity of quinones to reduce molecular oxygen to superoxide. Saframycin R, which has an acyl group on the phenol, caused much less DNA cleavage than saframycin A, making it much less toxic without any loss in biological activity.[10]

2.2. Renieramycins

2.2.1. Isolation and Structure Determination

In 1982, Frincke and Faulkner isolated four new natural products from the sponge *Reniera* sp.[48] that possess structures similar to that of the saframycins. These compounds were named renieramycin A−D, **138**−**141,** respectively (Figure 7). The main difference between the saframycins and renieramycins is that the side chain is an angelate ester instead of a pyruvamide. Seven years later, He and Faulkner isolated renieramycins E and F, **142** and **143,** respectively;[49] both compounds proved to be unstable. Renieramycin G (**144**) was isolated in 1992 by Davidson from the Fijian sponge *Xestospongia caycedoi*,[50] and this renieramycin was also found to be unstable. Two different renieramycins were isolated in 1998 by Parameswaran et al. from the sponge *Haliclona cribricutis.*[51] The originally assigned structures for renieramycins H and I were **145** and **146,** respectively. Recently, the structure of renieramycin H has been revised to that of **147**,[52] which was also isolated from *Cribrochalina* sp. and given the name cribrostatin 4.[53] The structure of cribrostatin 4 (**147**) was determined by X-ray crystal analysis. The benzylic

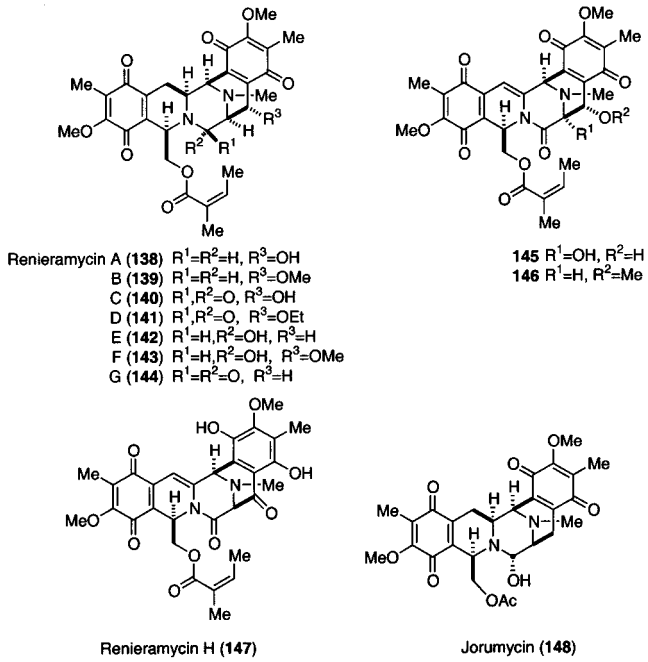


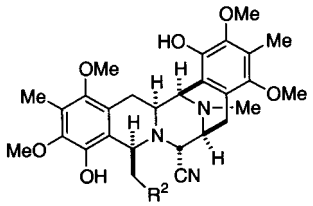
Figure 7.

olefin present is unique to renieramycin H. Due to this structural reassignment, the structure of renieramycin I is now in doubt. In 2000, Fontana et al. isolated jorumycin (**148**) from *Jorunna funebris*.[54] The structure of jorumycin is most similar to that of renieramycin F with exception of the acetate group on the alcohol versus the angelate ester on the renieramycins.

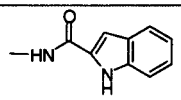
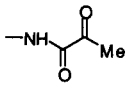
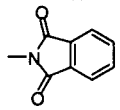
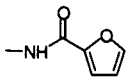
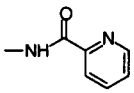
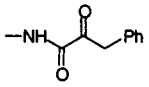
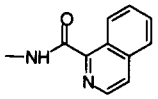
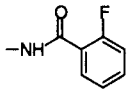
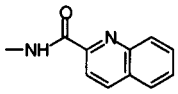
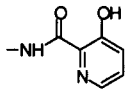
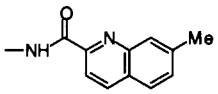
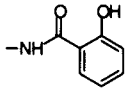
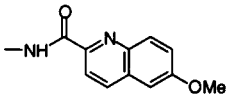
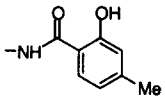
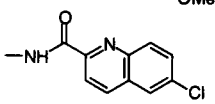
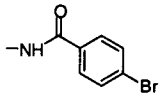
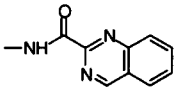
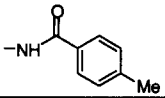
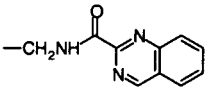
2.2.2. Total Synthesis of Renieramycin A

To date there has been only one total synthesis of a renieramycin. In 1990, Fukuyama et al. published the total synthesis of (±)-renieramycin A.[55] This synthesis used a similar strategy to that utilized in their saframycin A synthesis.[25] The main difference was that a different starting phenol was used in the E-ring to allow for the necessary benzylic oxidation at C-15. The phenol was protected as the corresponding 3-hydroxypropyl ether, which was further protected as the dimethylthexylsilyl (DMTS) ether.

Table 3. Antiproliferative Activities of Saframycin A Analogs



Saframycin analogs

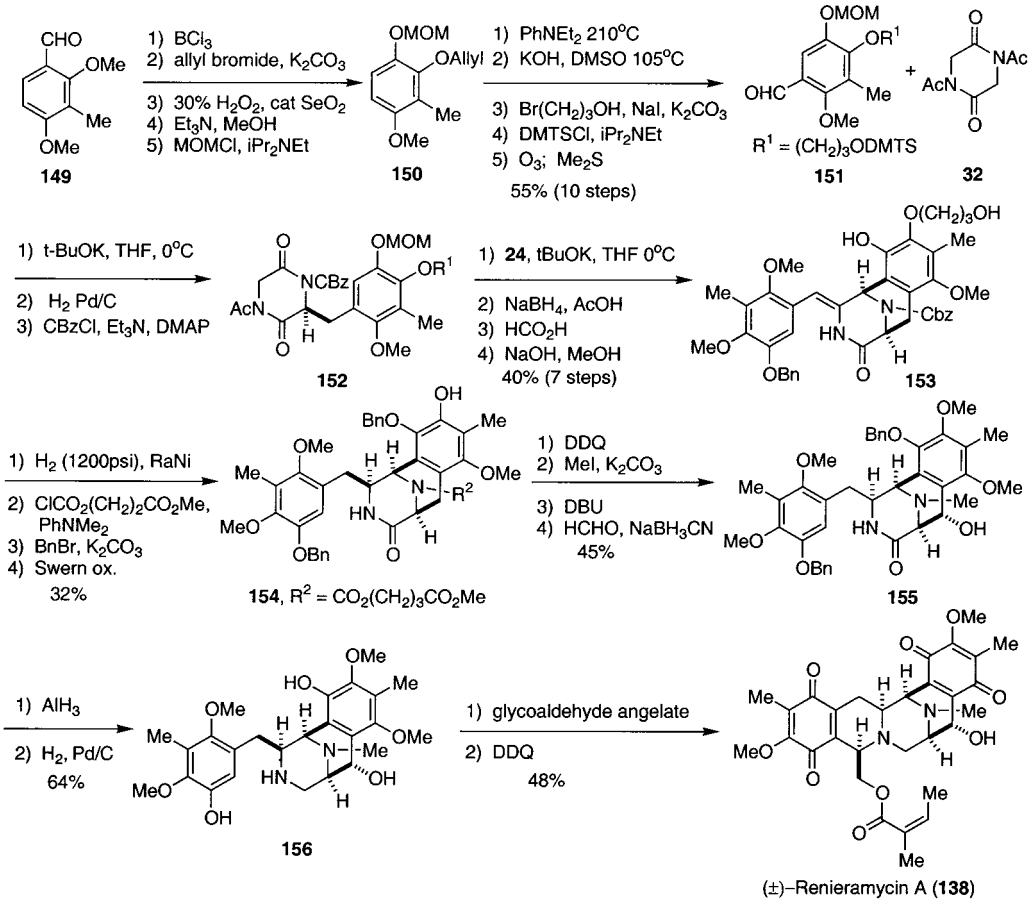
	IC_{50} (nM)			IC_{50} (nM)	
R^2 =	A375	A549	R^2 =	A375	A549
Saframycin A (**3**)	5.3	133		2.7	31
	4.5	160		1.7	9.2
	13	290		3.3	40
	2.4	39		2.5	32
	2.5	37		1.3	4.4
	1.4	14		1.4	4.6
	1.2	11		2.0	3.5
	1.2	6.5		1.5	4.1
	1.7	25		1.2	4.7
	1.9	37		3.6	78

The protected aldehyde **151** was synthesized in 10 steps from aldehyde **149**. Claisen condensation with diketopiperazine **32** followed by hydrogenation and carbamate formation yielded the diketopiperazine **152**. A second Claisen condensation followed by reduction of the amide with sodium borohydride yielded the carbinolamine, which when treated with formic acid cyclized upon the aromatic ring. Sodium hydroxide in methanol removed the DMTS group to provide **153**. High-pressure hydrogenation of the benzylic olefin along with removal of the Cbz and benzyl groups of **153** yielded a single tricyclic diastereomer. The bridgehead amine was then reprotected as a base-labile carbamate. Protection of the phenols followed by Swern oxidation to remove the hydroxy ether yielded **154**. Selective oxidation of the benzylic position with DDQ installed the necessary C-15 hydroxyl group. Following methylation of the phenol, the carbamate was removed using DBU and the methyl group was installed via a reductive amination to yield **155**. Alane reduction of the amide followed by benzyl group removal resulted in **156**. The final two steps to (±)-renieramycin A were a Pictet−Spengler cyclization using glycoaldehyde angelate and DDQ oxidation of the hydroquinones to quinones, which was accomplished in 48% yield.

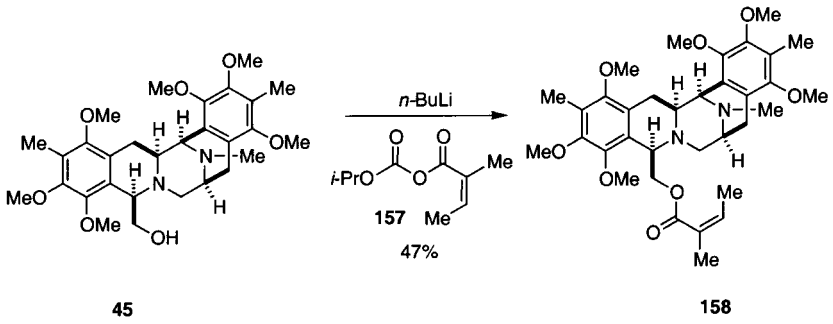
2.2.3. Synthetic Studies toward the Renieramycins

Kubo et al. synthesized some renieramycin congeners (Scheme 26).[56] Pentacycle **45**, culled from their saframycin B synthesis, was acylated with the mixed anhydride **157** to afford the angelate ester **158**. Unfortunately, these workers were unable to oxidatively demethylate the aromatic rings to form the corresponding quinones.

Scheme 25. Fukuyama's Synthesis of Renieramycin A



Scheme 26. Kubo's Synthesis of Renieramycin Congeners



2.2.4. Biological Activity

There has been scant data reported in the literature on the biological activity of the renieramycins. Reineramycins A−D,[48] H, and I[51] have moderate antimicrobial activities, while renieramycin G has shown moderate MIC activity against KB and LoVo cell lines with activities of 0.5 and 1.0 μg/mL, respectively.[50]

2.3. Safracins

2.3.1. Isolation and Structure Determination

Ikeda et al. isolated safracins A and B (**159** and **160**, respectively) from *Pseudomonas fluorescens* A2−2 in 1983 (Figure 8).[57] The safracins have structures very similar to that of the saframycins with the exception that the E-ring is a phenol instead of a quinone or hydroquinone as in the saframycins. The structures were determined by comparison to spectral data for saframycin B.[57b] Soon after that the absolute stereochemistry was determined by X-ray

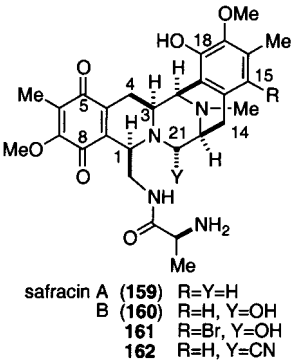
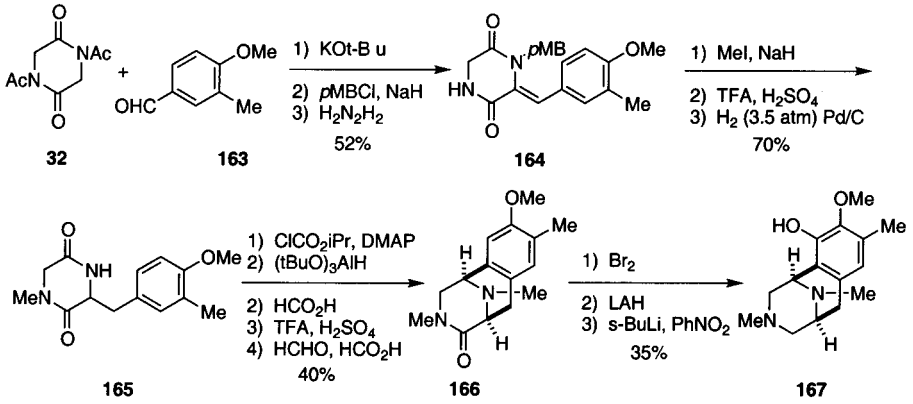
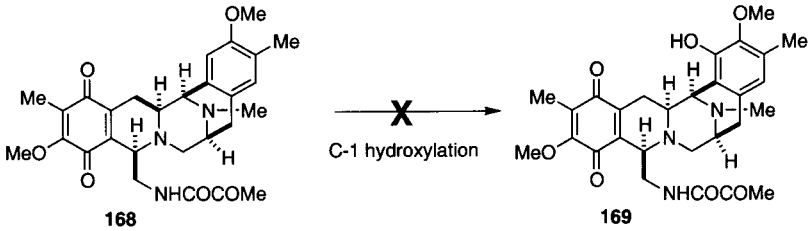


Figure 8. Safracins A and B.

Scheme 27. Kubo's Synthetic Studies on Safracin B



Scheme 28. Attempted Synthesis of Safracin A



crystallography using **161** (C-15 bromosafracin A).[58] Meyers et al. also isolated safracin B (they named it EM5519) from *Pseudomonas fluorescens* SC12695.[59]

In 1985, Ikeda found that addition of Fe(II) and Fe(III) to the fermentation broth increased the production of safracin B at a concentration of 0.01%.[60] Safracin A production was increased at higher iron concentration (0.1%). The cyano derivative of safracin B (**162**) was isolated on a multikilogram scale by Cuevas et al. for use in the semisynthetic synthesis of ecteinascidin 743 to be discussed below.[61]

2.3.2. Synthetic Studies toward the Safracins

Kubo et al. synthesized the ABC-ring of safracin B via the selective oxidation of the C-1 position (Scheme 27).[62] Using established chemistry, diketopiperazine **32** and aldehyde **163** were converted to **165** in six steps. Selective reduction of the activated carbonyl was followed by cyclization under acidic conditions to yield the tricyclic substance **166**. Bromination yielded the necessary functionality at C-1. The amide was then reduced to the amine using LAH. Treatment with *sec*-butyllithium in the presence of nitrobenzene installed the desired hydroxyl group in 53% for the final step to form **167**.

Kubo et al. then attempted the total synthesis of safracin A.[63] Unfortunately, hydroxylation was unsuccessful on the pentacycle **168** under several conditions including those used in previous model studies (Scheme 28).

2.3.3. Biological Activity

Safracin B was a more potent antibiotic than safracin A.[64] Interestingly, both safracins have antimicrobial activity against *Pseudomonas aeruginosa* and *Serratia marcencens* in which saframycin A was ineffective. Safracin B, possessing a C-21 carbinolamine, was much more active than safracin A against P388 and L1210 leukemia cell lines in vitro.

2.4. Ecteinascidins

2.4.1. Isolation and Structure Determination

The isolation of the ecteinascidins (Et's) was first reported by Reinhart et al. in 1990.[65] In this report, the isolation of six ecteinascidins including Et's 729, 743, 745, 759A, 759B, and 770 were reported (Figure 9). The structures for Et's 729 and 743 with the correct relative stereochemistry were reported by the Reinhart and Wright[66] groups simultaneously. The structures were determined by extensive NMR and mass spectral studies. In 1992, Reinhart et al. published the isolation of Et's 722, 736, and 734 N^{12}-oxide.[67a] Crystal structures for **175** and **176** (a synthetic derivative of **171**) were also obtained to confirm the structures of the ecteinascidins.[67] Four putative biosynthetic precursors (Et's 594, 597, 583, and 596) were isolated in 1996 by Reinhart et al.[68] In this report, the absolute stereochemistry of the ecteinascidins was determined via elucidation of the stereochemistry of the derivatized cysteine residue that was cleaved from **180**.

2.4.2. Biosynthesis

In 1995 Kerr and Miranda showed that ^{14}C-labeled tyrosine and ^{35}S-cysteine were incorporated into ecteinascidin 743 in a cell-free extract from *Ecteinascidia turbinata*.[69] These workers also found that labeled serine was not incorporated, however. Later, Kerr et al. synthesized three radiolabeled diketopiperazines (**183**−**185**) (Figure 10).[70] Using the same cell-free extract as above, it was found that the tyrosine-containing diketopiperazine **184** and the DOPA-containing diketopiperazine **185** were incorporated into Et 743. It was also found that **184** was

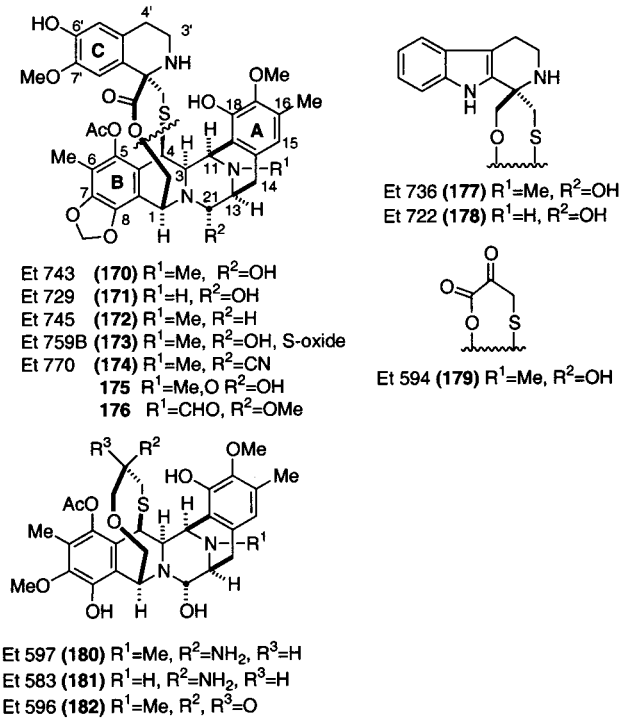


Figure 9. The ecteinascidins.

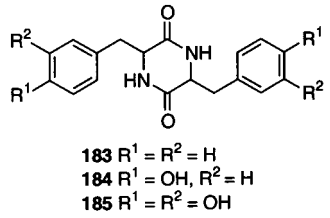


Figure 10. Biosynthetic precursors to ecteinascidin 743.

converted to **185** indicating that tyrosine first condenses to make **184** which then undergoes an oxidation to **185** in the biosynthetic route to Et 743. Being of marine origin, additional biosynthetic studies are likely to be very difficult and the elucidation of the more complex sequence of events is anticipated to be revealed slowly.

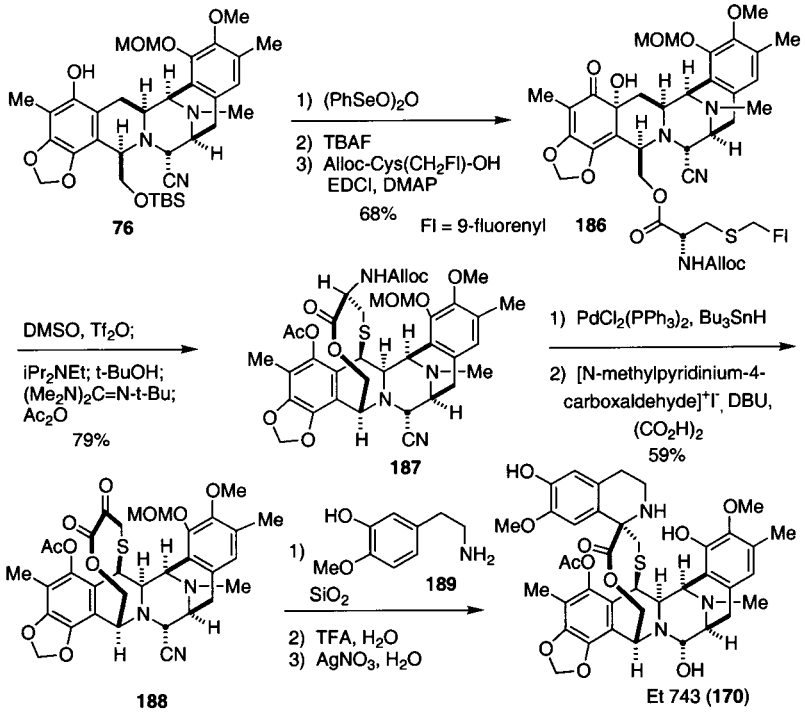
2.4.3. Total Syntheses of Ecteinsacidin 743

To date there have been two syntheses of ecteinascidin 743. Corey et al. published the first total synthesis of Et743 in 1996.[27] This was followed by a semisynthetic route involving the conversion of cyanosafracin B to Et743 by Cuevas et al. in 2000.[61]

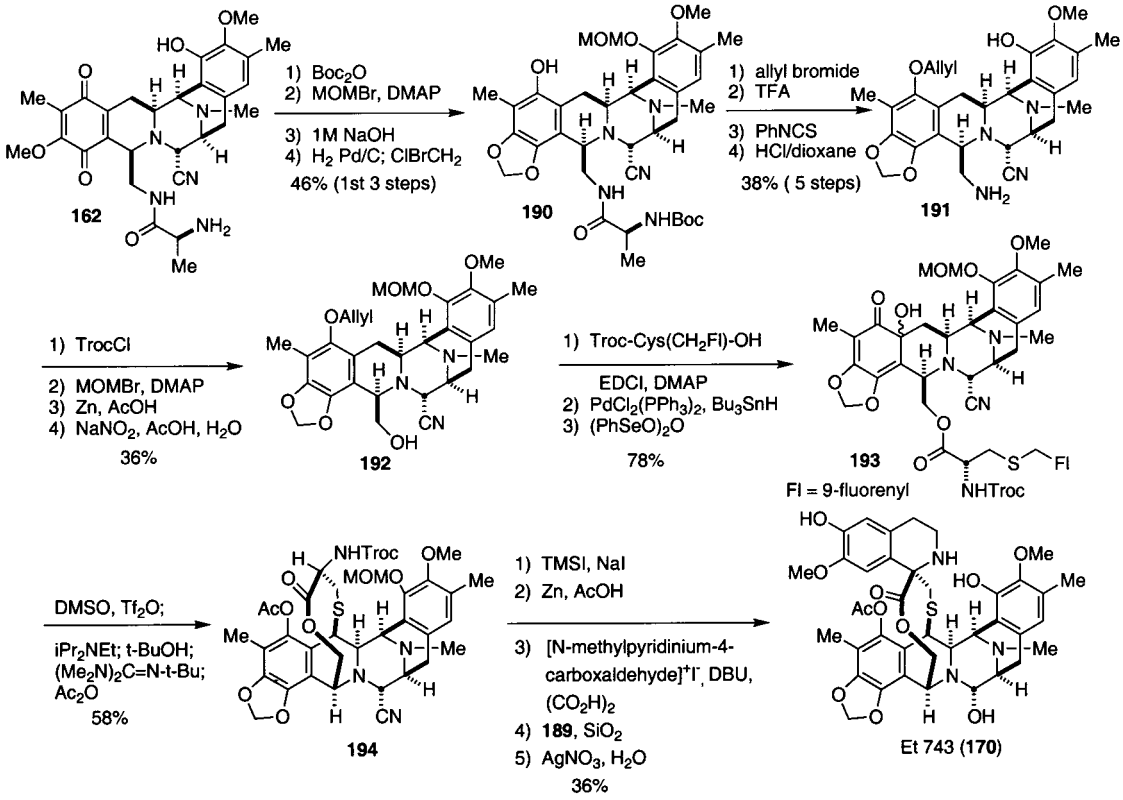
In 1996, Corey et al. synthesized Et 743 via a convergent synthesis employing the coupling of two optically active fragments as seen in their saframycin A synthesis[26] (Scheme 29). Starting with hexacycle **76**, a selective hydroxylation was accomplished using phenylselenic anhydride. Removal of the silyl ether followed by esterification with a diprotected cysteine derivative provided **186**. Elimination of the tertiary alcohol under Swern conditions allowed for cyclization of the thiol to form **187** in 79% yield. Removal of the Alloc carbamate followed by transamination afforded α-keto lactone **188** in 59% yield. The final three steps to Et 743 were the condensation of the homobenzylic amine **189** on the ketone followed by removal of the MOM group with TFA and finally conversion of the aminonitrile to the carbinolamine using silver(I) nitrate and water.

Starting with cyanosafracin B (**162**), which was available in kilogram quantities via fermentation, Cuevas et al. were able to synthesize Et 743 in a semisynthetic fashion (Scheme 30).[61] Cyanosafracin B was converted into **190** via a four-step sequence. Removal of the Boc group from **190** was followed by amide cleavage via an Edman degradation protocol

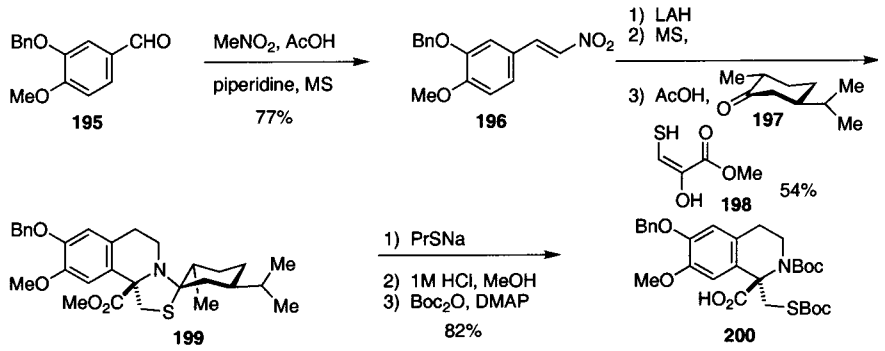
Scheme 29. Corey's Total Synthesis of Et 743



Scheme 30. Cuevas Semi-synthesis of Et 743



Scheme 31. Corey's Synthetic Studies toward Et 743



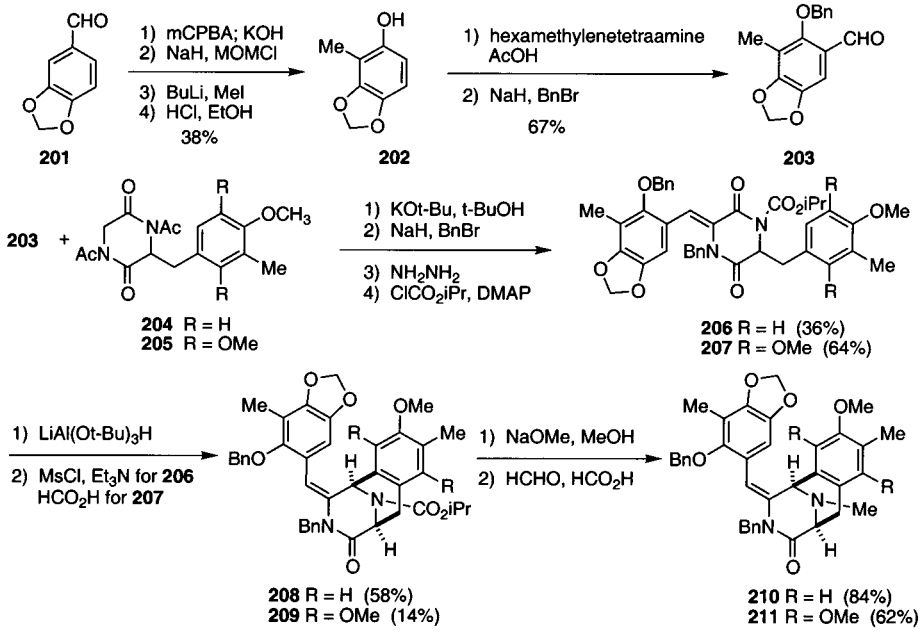
providing **191** in 68% yield. Protection of the phenol allowed for the diazotization of the primary amine for conversion to alcohol **192**. The synthesis of Et 743 was completed using the chemistry of Corey[27] on similar substrates. A three-step sequence was used to form **193**. Dehydration under Swern conditions allowed for the cyclization to afford **194**. Removal of the MOM and alloc protecting groups was followed by ketone formation. Finally, condensation with **189** and carbinolamine formation afforded Et 743.

2.4.4. Synthetic Studies toward the Ecteinascidins

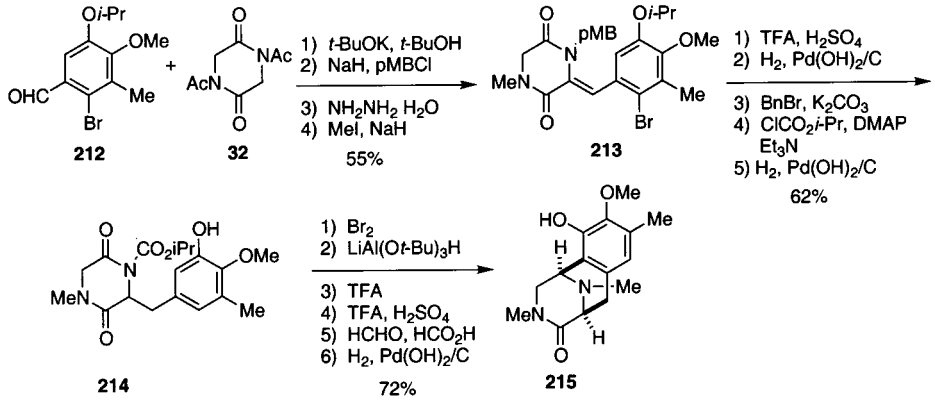
Corey and Gin reported an efficient synthesis of the tetrahydroisoquinoline C unit of Et 743 in 1996.[71] Aldehyde **195** was converted to the nitrostyrene **196** via a nitroaldol condensation as shown in Scheme 31. Reduction of the olefin and nitro group was followed by condensation of the resultant amine with (+)-tetrahydrocarvone (**197**). The resulting imine was treated with **198** to form **199** with a diastereoselectivity of 6.5:1. The inseparable mixture of diastereomers was treated with sodium propylmercaptide, which allowed for a selective hydrolysis of the methyl ester of the major diastereomer. Acidic cleavage of the auxiliary and protection of the amine and thiol afforded **200** in optically pure form.

In 1997, Kubo et al. published their synthetic studies toward the ecteinascidins that employed chemistry similar to that deployed in their saframycin syntheses.[72] Aldehyde **201**, was converted to phenol **202** in four steps featuring a Bayer−Villiger oxidation (Scheme 32). Formylation and phenol protection afforded **203** in 67% yield. An aldol condensation was performed on diketopiperazines **204** and **205** affording **206** and **207**, respectively. Each compound was carried through the synthesis. Partial reduction of the activated lactam followed by cyclization using two different conditions afforded the tricycles **208** and **209** in 58% and 14% yields, respectively, for the two steps. Carbamate cleavage was accomplished using sodium methoxide in methanol, and the secondary amine was methylated affording **210** and **211** in good

Scheme 32. Kubo's Synthetic Studies toward Et 743



Scheme 33. Kubo's Synthetic Studies toward Et 743

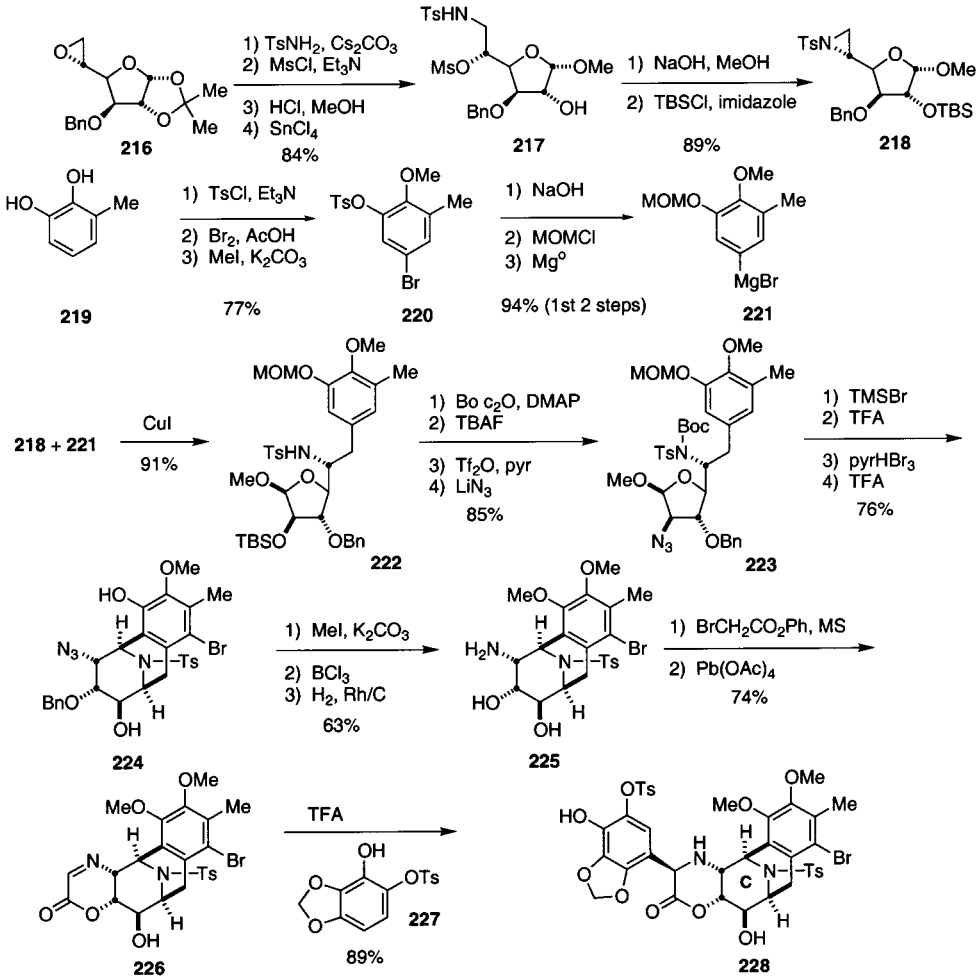


yields. These compounds were presented as possible precursors to the ecteinascidins.

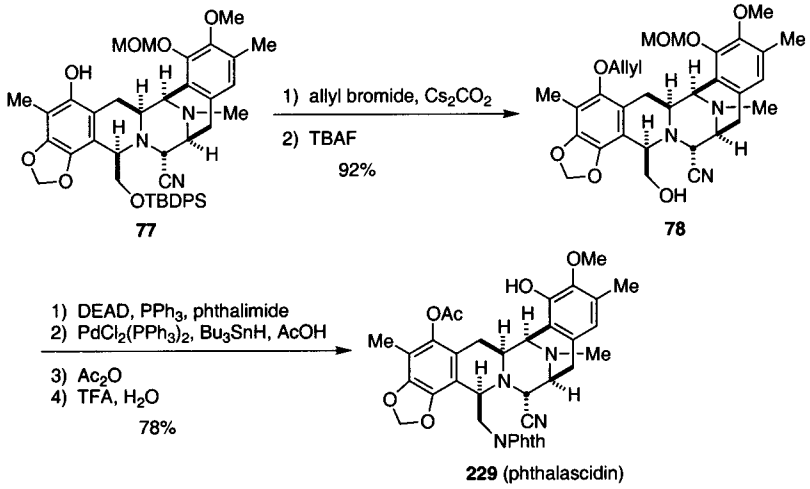
In 2000, Kubo et al. published a different route to the ABC-ring system of the ecteinascidins as shown in Scheme 33.[73] Aldol condensation of aldehyde **212** with diketopiperazine **32** afforded **213** after the protection of the lactams. Changing of the phenol protecting group and activation of the lactam for reduction afforded **214**. Bromination of the aromatic ring allowed for a regioselective cyclization under acidic conditions to form the tricycle. Cleavage of the carbamate was followed by amine methylation and removal of the bromine to afford the tricycle **215**.

In 1999, Fukuyama et al. published their synthetic studies toward Et 743 starting from D-glucose (Scheme 34).[74] Epoxide **216,** available in five steps from D-glucose, was subjected to selective epoxide ring opening followed by mesylation and acetonide deprotection to afford a 3:2 mixture of diastereomeric methyl glycosides. Treatment of this mixture with stannous chloride furnished **217** as a single diastereomer. Aziridine formation was accomplished using sodium hydroxide, followed by silyl ether formation, to afford **218** in high yield. The E-ring of Et 743 was introduced via a Grignard addition to the aziridine. Diphenol **219** was selectively protected with tosyl chloride and brominated para to the free phenol forming **220** after methylation of the phenol. After switching protecting groups, the Grignard **221** was formed allowing for addition to aziridine **218**. Subsequent copper-catalyzed aziridine ring opening by **221** afforded **222** in 91% yield. Protection of the sulfonamide followed by alcohol deprotection yielded the corresponding free alcohol. Activation and azide displacement of the triflate furnished compound **223**, which was subjected to deprotection of the MOM ether and Boc groups followed by bromination para to the phenol to block that position during the subsequent acidic cyclization. The cyclization reaction proceeded through an iminium ion intermediate affording the tricyclic compound **224** as a single stereoisomer. Protection of the phenol was followed by benzyl ether cleavage and azide reduction to afford amino diol **225**. Amino lactonization was followed by lead tetraacetate oxidation to form dehydrooxazinone **226** in 74% yield from **225**. Acidic alkylation of **226** with phenol **227** afforded **228** in 89% yield. The proposed completion of the synthesis from **228** involved the reduction of the lactone followed by oxidative cleavage of the resultant diol of the C-ring

Scheme 34. Fukuyama's Synthetic Approach to Et 743



Scheme 35. Corey's Synthesis of Phthalascidin-650 (229)



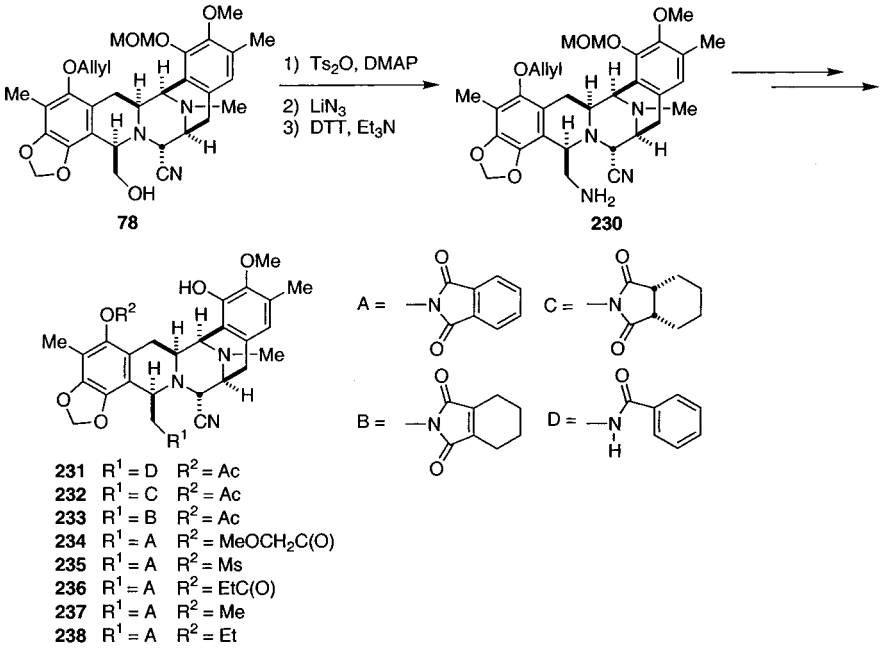
to the corresponding dialdehyde. Closure of the B- and C-rings would then afford the pentacyclic core of the ecteinascidins.

2.4.5. Analogue Syntheses

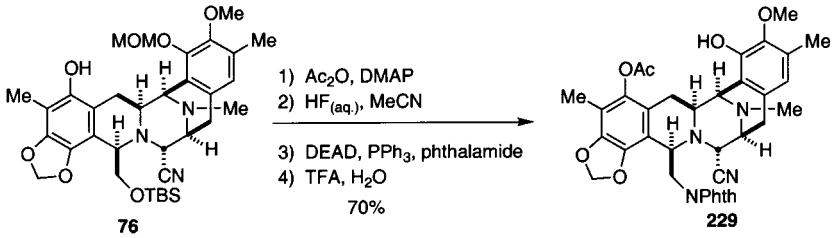
Corey et al. reported the synthesis and biological activity of potent analogues of Et 743 as shown in Scheme 35.[75] In this study, a compound named phthalascidin (**229** or Pt 650) was synthesized, which was surprisingly found to have comparable biological activity to that of Et 743. The synthesis of phthalascidin commenced with compound **77**, which was allylated followed by removal of the silyl protecting group to afford **78**. A Mitsunobu reaction using phthalimide followed by removal of the allyl group, acylation of the phenol, and removal of the MOM group provided **229** in six steps and 72% overall yield from **77**.

Several other ecteinascidin analogues were also prepared as described in Scheme 36. These analogues

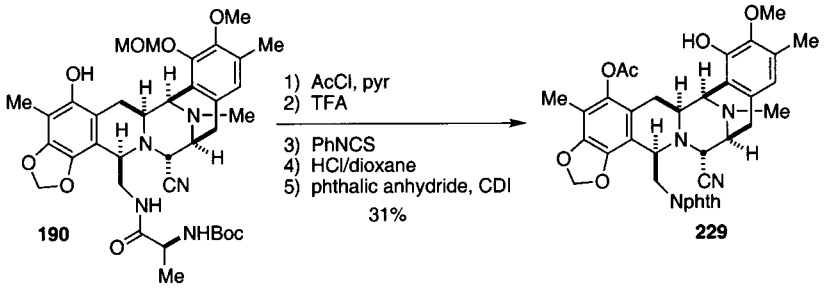
Scheme 36. Pthalascidin Analogs



Scheme 37. Corey's Improved Synthesis of Phthalascidin 650



Scheme 38. Cuevas' Synthesis of Phthalascidin (229)



were formed by the conversion of alcohol **78** to amine **230** followed by amide or succinimide formation affording **231**−**238**.

In 2000, Corey and Martinez published a shorter synthesis of phthalascidin (**229,** Scheme 37).[28] In this new route, a protection step and deprotection step were omitted, shortening the synthesis to four overall steps with a slightly lower yield of 70%.

In 2000, Cuevas et al. published a short synthesis of Pt-650 from an intermediate described in their Et 743 synthesis (Scheme 38).[61] Starting with **190**, Pt 650 was synthesized in five steps in 31% yield.

2.4.6. Biological Activity

The ecteinascidins have the most potent biological activities by a significant margin relative to that of any of the tetrahydroquinoline antitumor antibiotics. The activities of Et743 are orders of magnitude more potent than saframycin A against B16 melanoma.[1i] The exciting aspect of Et 743 is that it appears to have a unique mode of action, thus constituting a new subclass of antitumor agent that could be active against resistant cell lines. Et 743 is currently in phase II human clinical trials in the United States.[76] The in vitro activities of Et 743 against several common tumor cell lines were exceedingly high and are summarized in Table 4.[1i]

Et 729 exhibits higher in vivo activities against P 388 leukemia than Et 743 and Et 745 (Table 5).[77] The IC_{50}'s for Et 729 against L1210 cells in the absence and presence of 2.5% murine plasma were 37 and 72 pM, respectively.[78]

Et's 722 and 736 were found to also have high in vitro activities against L1210 with IC_{90}'s of 2.5 and 5.0 ng/mL, respectively.[67a] Et 722 was also highly

Table 4. Activity of Et 743 against Several Tumor Cell Lines

tumor type	IC_{50} (μM)
P388 leukemia	0.00034
L1210 leukemia	0.00066
A549 lung cancer	0.00026
HT29 colon cancer	0.00046
MEL-28 melanoma	0.00050

Table 5. Activities of Et's 729 (171), 743 (170), and 745 (172) against P388 Leukemia

compound	dose (μg/kg)	T/C[a]
171	3.8	214
170	15	167
172	250	111

[a] T/C = is the increased lifespan of mice treated with the drug versus the control group.

Table 6. Activity of Et 722 against Several Tumor Cell Lines

tumor type	dose (μg/kg)	T/C[a]
P388 leukemia	25	>265
B16 melanoma	50	200
Lewis lung carcinoma	50	0.27
LX-1 lung carcinoma	75	0.00

[a] T/C = is the increased lifespan of mice treated with the drug versus the control group.

active in vivo against a variety of cell lines (Table 6).

Valoti et al. treated several human ovarian carcinoma zenografts that were characterized by specific behavior and drug responsiveness versus *cis*-platinum (DDP) with Et-743.[79] Et 743 was found to be very active against the HO22−S cell line (sensitive toward DDP). Et-743 also induced long-lasting regressions against HOC18 (marginally sensitive to DDP). The HOC18 xenograft (nonresponsive to DPP) showed significant growth delay, but for MNB−PTX-1, a highly resistant tumor toward chemotherapy, Et-743 had no activity.

The mechanism of action of the ecteinascidins has been studied by several groups. It has been shown that Et 743 inhibits RNA, DNA, and protein synthesis with IC_{50} values of 8, 30, and 100 nM, respectively.[1i] Et 743 has a similar structure to that of saframycin S, indicating that DNA alkylation should indeed be possible. DNA alkylation has been studied by Pommier et al.[80] and Hurley et al.[81] The alkylation takes place in the minor groove, as does alkylation with the saframycins. The alkylated DNA substrate exhibits a bend or widening of the minor groove,[81e] presumably due to the C-subunit of the ecteinascidins. The C-subunit, which is perpendicular to the rest of the molecule, makes the ecteinascidins unique from the saframcyins, which are fairly flat. It has been postulated that this bend in DNA disrupts DNA−protein binding and may be, in part, the source of the enhanced biological activities of the ecteinascidins.

It has been demonstrated that the ecteinascidins alkylate DNA at the N-2 residue of guanine in GC-rich regions.[82] The alkylation has been shown to be reversible with DNA denaturization[80] and replacement of guanine with inosine abolishes DNA alkylation providing direct evidence for alkylation of the N-2 guanine residue in the minor groove. The unique sequence specificities of Et 743 have been shown to be 5′-GGG, 5′-GGC, and 5′AGC. Hurley et al. postulated that the sequence specificity arises from molecular recognition events dictated by the A and B subunits of Et 743.[81a] The rate of reversibility of Et 743 DNA covalent adducts was studied by Zewail-Foote and Hurley.[82] It was found that for the sequences 5′-AGT and 5′-AGC the rates of bond formation were similar; however, the rate of reversibility under nondenaturing conditions occurred faster for the 5′-AGT sequence. This reversibility was explained by the decreased stability of the Et 743 5′-AGT adduct compared to the Et 743 5′-AGC adduct. It was also shown that Et 743 would migrate from a 5′-AGT sequence to the 5′-AGC sequence.

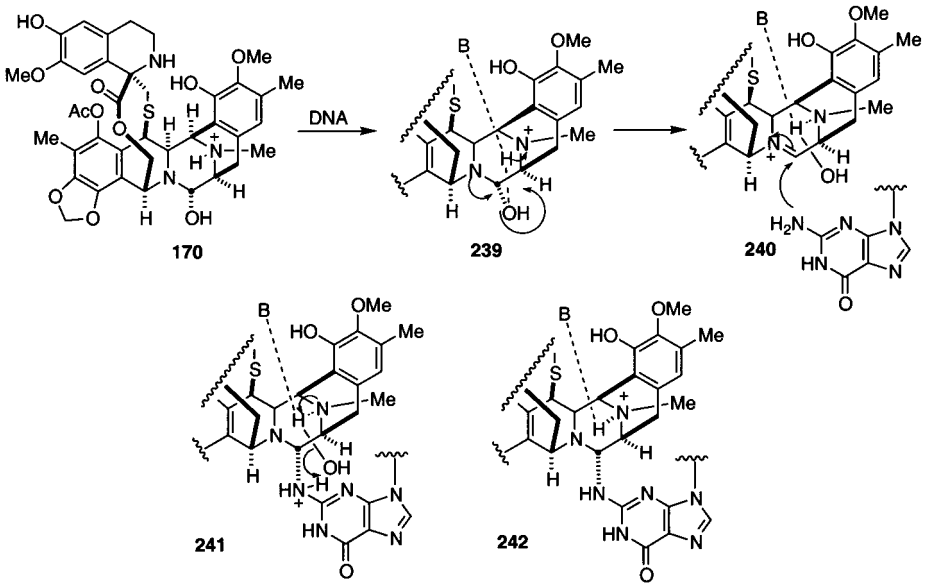
In 1998, Hurley et al. showed by NMR studies that the N-12 of Et 743 was protonated in the Et 743 DNA covalent adduct.[81b] From these data, a mechanism for DNA alkylation was suggested as illustrated in Scheme 39. The N-12 of Et 743 is protonated, which facilitates expulsion of the hydroxyl group in the form of water to form the iminium species **240**. The exocyclic nitrogen of guanine is then envisioned to attack this electrophilic species resulting in covalent adduct formation (**242**). NMR studies support the contention that the final DNA−Et743 adduct is protonated at N-12.

A molecular modeling study by Gago et al. of the DNA-Et 743 or Pt 650 adducts revealed widening of the minor groove and a positive roll in the DNA toward the major groove.[83] The widening of the minor groove was speculated to be due to specific hydrogen-bonding interactions that stabilized the binding of Et 743 to DNA. The AGC and CGG sequences were seen to have the best binding with CGA having poor binding to Et 743.

Recently two groups reported a mechanism of action that is unique to Et 743. It was reported by Pommier et al. that Et 743 halted the DNA excision repair (NER) system in cells.[84] It was shown that in two Et 743-resistant colon carcinoma cell lines, the repair mechanism had a defect with respect to the endonuclease *XPG* that is used for DNA repair. It was proposed that in Et 743 nonresistant cells, the NER would produce irreversible DNA strand breaks without effectively excising the Et 743 DNA covalent portion. This DNA cleavage would then lead to death of the cell. It was proposed that cisplatin-resitant ovarian carcinoma cells that have increased NER would be very suseptible to Et 743. It is also important to note that these observations were conducted at physiologically relevant concentrations of Et 743.

Hurley et al. also reported the effect of Et 743 upon the NER mechanism.[85] It was postulated that the Et 743 DNA covalent adduct trapped an intermediate in NER processing which would not allow the DNA to be fully repaired. Incubation of the UvrABC nuclease with DNA that was treated with Et 743 showed that the DNA-Et 743 site was recognized and incised. At high Et 743 concentrations, this incision was inhibited. It was noted that the incision fre-

Scheme 39. Hurley's Proposed Mechanism of DNA Alkylation by Et 743



quency was sequence related with the less stable adduct sequences of 5′-AGT and 5′-TGT which were incised with higher efficiency over the more stable adducts of 5′-AGC and 5′-TGC.

It has also been shown that Et 743 disrupts the microtubule network of tumor cells,[86] and this type of activity is apparently unique to the ecteinascidins within this family of alkaloids. Experiments have revealed that Et 743 does not react directly with tublin; however, a decrease in fibers was observed along with changes in microtuble distribution. Like taxol, the Et 743-treated microtubules were not anchored at the centrosome, but unlike taxol, Et 743 did not facilitate microtubule polymerization. Et's 735 and 736 were also shown to have the same effects at Et 743 but to a lesser extent.

In 1999, three groups showed that Et 743 formed a cross-link between DNA and topoisomerase I (Topo I).[75,81e,87] The cross-link was found to have a unique sequence specificity relative to that of other known Topo I cross-linking agents.[87] It was believed that the C subunit, which protrudes from the DNA, interacts with the protein.[81e] Significantly, the drug−protein cross-linking reaction occurs at much higher Et 743 concentrations than are necessary for the expression of its antitumor activity, indicating that the formation of a cross-link to topoisomerase I is not the primary mode of action. This was also observed in studies where camptothecin-resistant (a known Topo I cross-linking agent) mouse leukemia P388/CPT45 cells were susceptible to Et743.[88]

Another mode of action, which has been implicated at biological concentrations, was the interaction between the Et 743 DNA adduct and DNA transcription factors.[89] Three types of factors were studied: oncogene products, transcriptional factors regulated during the cell cycle, and general transcriptional factors. The NF−Y factor, a general transcription factor, was found to be inhibited most by Et 743. The other factors studied were either not inhibited or inhibited slightly. Due to the resemblance of NF−Y compared to histones H2A and H2B, nucleosome reconstitution was investigated in the presence of Et 743. It was found that Et 743 did affect the reconstitution at levels of 100 nM.

The binding of HSP70 promoter and NF−Y to DNA were also found to be inhibited at low concentrations of Et 743.[90] The NF−Y protein was found to still bind to the DNA in the presence of the drug, and it has been argued that the bound drug distorts the DNA−protein interactions. These interactions may not be disrupted directly but rather by the disruption of an unknown cofactor. This was demonstrated in the study of the binding of the MDR1 promoter with NF−Y.[91] These observations show that the mode of action of Et 743 was different than any known antitumor compound.

Pommier et al. showed that Et 743 caused protein-linked DNA single-strand breaks but at micromolar concentrations.[88] No sign of double-stranded breaks was observed. At 10 nM concentrations, Et 743 induced an accumulation of cells in the S and G_2-M cell cycle phases after 14 h. After 24 h there was an accumulation in the G_2-M phase. This profile was consistent with other DNA alkylating agents.

In 2000, Gago et al. reported a molecular modeling study in which it was found that the minor groove of a covalent DNA−Et 743 model was virtually superimposable with a model of the minor groove when DNA was bound to the zinc fingers of EGR-1, a transcriptional regulator. A model of the DNA bound to the zinc fingers showed that the N-2 of guanine was accessible to Et 743 without any further distortion of the DNA. This indicates that Et 743 may target specific sites on the chromosome where zinc fingers of a transcription factor such as Sp1 associate with DNA.[92]

In an attempt to make Et 743-resistant cancer cells, Erba et al. exposed Igrov-1 human ovarian cancer cells to Et 743 for differing amounts of time.[93] It was found that the most resistant cell line had IC_{50} values 50 times higher than the parent cell line. This resistance was found to be irreversible.

Table 7. Activities of Pthalascidin Analogs versus Various Tumor Cell Lines

compound	A-549 (nM)	A375 (nM)	PC-3 (nM)
229	0.95	0.17	0.55
231	3.2	0.35	0.64
232	1.5	0.27	1.1
233	1.2	0.35	0.75
234	1.6	0.31	0.90
235	1.7	0.29	0.86
236	2.1	0.51	2.9
237	3.1	0.55	3.1
238	3.0	0.97	2.4
170	1.0	0.15	0.70

The biological activity of several pthalascidin analogues were found to be similar to that of Et 743 (Table 7).[75] This was an important observation due to the fact that the phthalascidins are structurally less complex than the ecteinascidins and are also much easier to synthesize than the natural products.

The lethal biological target of Et 743 and the exact mechanism by which it kills cells at such extraordinarily low concentrations remains a partially unsolved and very fascinating problem despite the recent DNA repair inhibition mechanism. The intense interest in this clinical antitumor candidate is expected to continue to draw researchers to address the biological chemistry of Et 743.

3. Naphthyridinomycin Family

3.1 Naphthyridinomycin, Cyanocycline, and Bioxalomycins

3.1.1. Isolation and Structure Determination

The novel antitumor antibiotic naphthriydinomycin (**243**) was isolated in 1974 by Kluepfel et al. from *Streptomyces lusitanus* AYB-1026 as an unstable ruby red crystalline solid.[94] The structure was determined via single-crystal X-ray analysis.[95] In 1976, SF-1739 was isolated by Watanabe et al.[96] At the time the structure was not determined, but due to the analogue synthesized, SF-1739 HP (**244**), it has been assumed that SF-1739 was actually naphthyridinomycin.[97]

Treatment of the extraction broth of *Streptomyces lusitanus* with sodium cyanide afforded a more stable product cyanonaphthyridinomycin[98] (**245**) (cyanocycline A). Shortly thereafter, cyanocycline A was isolated from *Streptomyces flavogriseus*.[99] The structure was determined by single-crystal X-ray analysis along with the crystal structure of cyanocycline F (**246**).[100] The absolute stereochemistry of naphthyridinomycin was originally thought to be opposite that of **243**; however, synthetic and biosynthetic studies brought the assigned absolute stereochemistry into question. The asymmetric synthesis of (+)-cyanocyline A by Fukuyama confirmed that the originally assigned stereochemistry was indeed in error.[1f]

In 1993, Gould et al. isolated three minor antibiotics from the broth of *Streptomyces lusitanus*.[101] These minor unstable components were treated with sodium cyanide to afford stable cyanocyclines B (**247**) and C (**249**). It was assumed that the true natural products were actually compounds **248** and **250**, respectively. Cyanocycline D (**251**), an artifact of isolation, was also isolated.

In 1994, the bioxalomycins α_1, α_2, β_1, and β_2 (**252-255**) were isolated at Lederle laboratories from *Streptomyces viridostaticus* ssp *litoralis*,[102] bringing into question the true structure of the natural product originally believed to be that of naphthyridinomycin (**243**). The isolation procedures employed by the Lederle group were milder than that used for the original isolation of naphthyridinomycin. To address this issue, the Lederle group subjected the naphthyridinomycin-producing *Streptomyces lusitanus* (NRRL8034) to growth and isolation procedures employed to isolate the bioxalomycins, and under these conditions, bioxalomycin β_2 instead of naphthyridinomycin was obtained.[102b] Also, attempts to repeat the original naphthyridinomycin isolation procedure lead only to the procurement bioxalomycin β_2, indicating that naphthyridinomycin may in fact be an artifact of the original isolation procedures. Thus, the initial biosynthetic product, bioxalomycin β_2 suffered hydrolytic ring opening of the somewhat strained fused oxazolidine.

3.1.2. Biosynthesis

In 1982, Zmijewski et al. showed that ^{14}C-labeled L-tyrosine (**21**), L-methionine (**22**), glycine (**256**), and D,L-ornithine (**258**) were incorporated into cyanocycline A (Figure 12).[103] In 1985, Zmijewski et al. reported that glycine, after being converted into serine, labeled C-1 and C-2.[104] It had been shown that DOPA was not incorporated into cyanocycline A, but since tyrosine was incorporated, Gould and Palaniswamy showed that aromatic methylation takes place prior to hydroxylation to the catechol.[105] Labeled forms of *m*-methyl tyrosine and *m*-methyl-*m*-hydroxy tyrosine were synthesized (**259** and **260,** respectively) and both of these amino acids were shown to be biosynthetically incorporated into cyanocyline A. This indicated that tyrosine was methylated to form **259** followed by a hydroxylation to yield **260**, which undergoes further elaboration to form naphthyridinomycin.

3.1.3. Total Syntheses of Cyanocycline A

Two significant efforts toward the total synthesis of naphthyridinomycin, one by Evans[106] and the other by Fukuyama,[107] have been reported. However, naphthyridinomycin proved too unstable to succumb to total synthesis. In fact, there was some evidence suggesting that the final product in Fukuyama's total synthesis was actually bioxalomycin β_2.[1f]

Due to the difficulty encountered in synthesizing naphthyridinomycin, attention was turned to the synthesis of cyanocycline A by both of these groups with Evans publishing the first total synthesis of (±)-cyanocyline A in 1986.[108] Later, Fukuyama reported the asymmetric total synthesis of (+)-cyanocylcine A, thus elucidating the absolute stereochemistry of the natural product.[1f]

Evans' approach commenced with the synthesis of tricycle **269** as shown in Scheme 40.[106] Condensation of cyclopentadiene (**261**) with chlorosulfonyl isocyanate followed by reductive hydrolysis afforded β-lac-

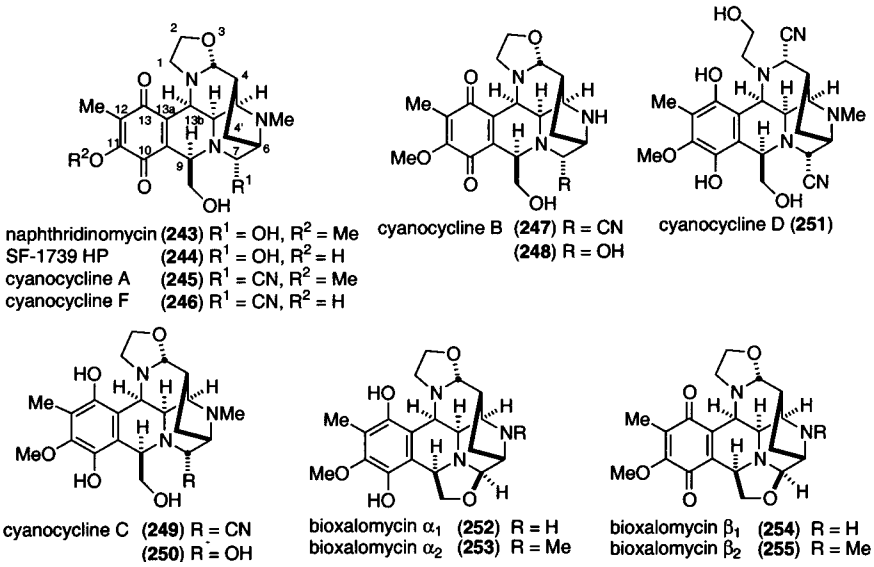


Figure 11. Structures of naphthyridinomycin, cyanocyclines, and bioxalomycins.

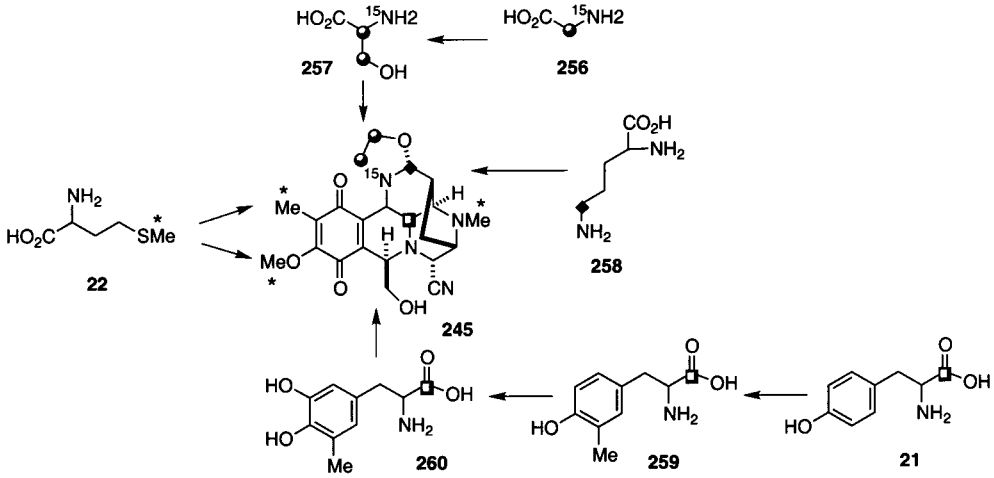
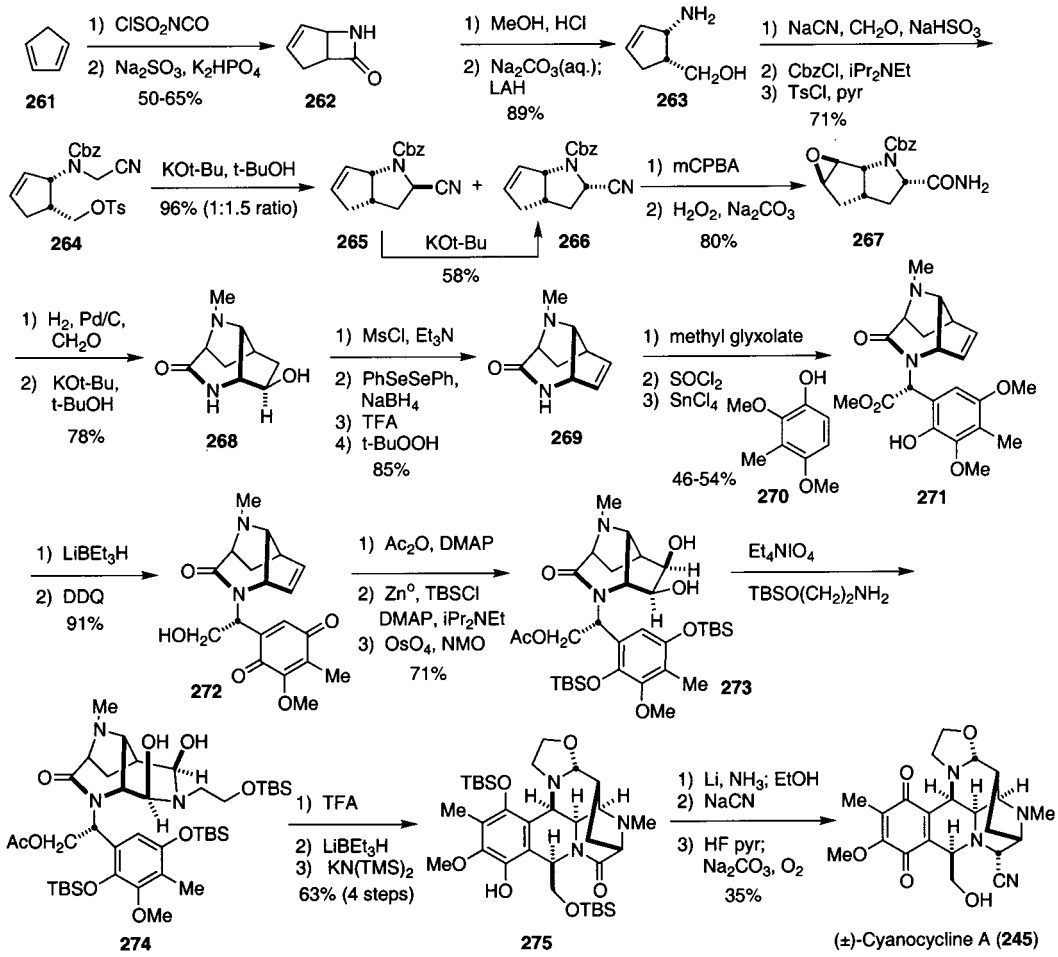


Figure 12. Biosynthetic precursors to cyanocycline.

tam **262**. Methanolysis of the lactam followed by ester reduction provided **263** in 89% yield. Treatment of the amine with formaldehyde and sodium cyanide followed by protection of the amine and alcohol afforded aminonitrile **264**. Cyclization was accomplished using potassium *tert*-butoxide to provide **265** and the desired bicyclic compound **266** in high yield but with poor diastereoselectivity. Fortunately **265** could be epimerized to **266** in 58% yield. Epoxidation was followed by amide formation to yield **267**. Carbamate cleavage and nitrogen methylation was followed by cyclization of the amide onto the epoxide to afford tricycle **268**. The final steps to intermediate **269** were the four manipulations required to install the olefin needed for later functionalization. Tricycle **269** was treated with methyl glyoxolate followed by thionyl chloride.[106b] Treatment of the resultant chloroamide with stannous chloride and phenol **270** afforded **271** in moderate yield. Subsequent reduction of the ester was followed by DDQ oxidation to give the quinone **272** in 91% yield. Following protection of the alcohol, the quinone was reduced and protected. Diol formation was accomplished using osmium tetroxide to yield **273**. Initial attempts to oxidatively cleave the diol were examined; however, the resulting dialdehyde could not be isolated due to facile hydration. This hydrated product was too stable for any further modification, so the oxidation was accomplished under anhydrous conditions using tetraethylammonium periodate in the presence of O-TBS protected ethanolamine to afford the bis-carbinolamine **274**. Treatment of **274** with trifluoroacetic acid afforded the hexacyclic core in high yield via two consecutive iminium ion cyclizations forming the B-, D-, and E-rings in one pot.[108] Cleavage of the acetate followed by silyl migration under basic conditions yielded **275**. Dissolving metal reduction converted the amide to the carbinolamine, which was trapped with sodium cyanide to afford the corresponding aminonitrile. The synthesis was completed by silyl deprotection, which was followed by hydroquinone oxidation to afford (±)-cyanocycline A in 35% yield from **275**.

Shortly after Evans' total synthesis of (±)-cyanocyline A was published, Fukuyama reported the second total synthesis of (±)-cyanocyline A as illustrated in Scheme 41.[109] The dihydropyrrole **277** was synthesized in three steps from the dehydroalanine **276.** The zinc enolate of **277** was formed and treated with aromatic aldehyde **24** to form the aldol

Scheme 40. Evan's Total Synthesis of D,L-Cyanocycline A

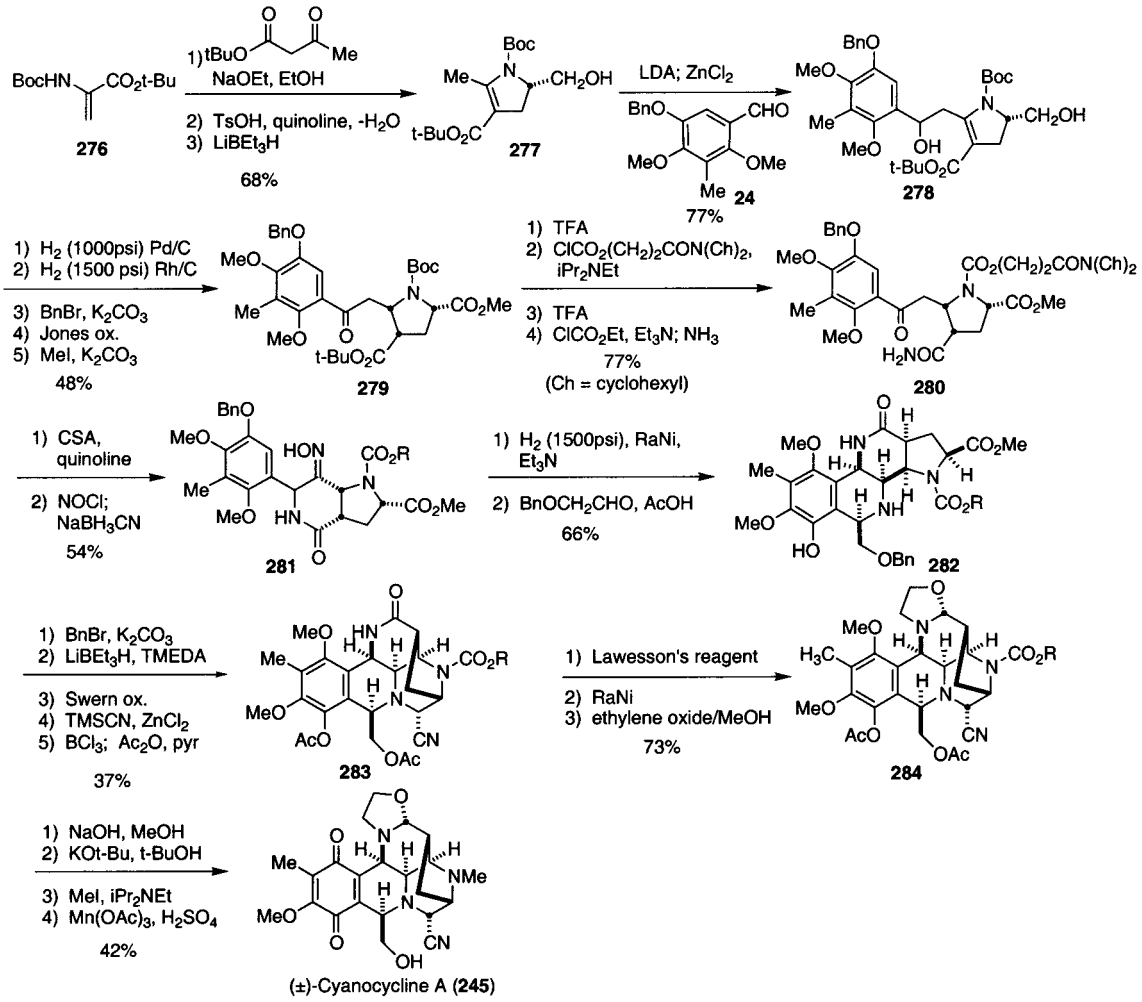


product **278**. Hydrogenation under two different conditions first cleaved the benzyl ether and subsequently saturated the olefin. Reprotection of the phenol was followed by oxidation of the primary alcohol to the corresponding acid, which was then converted to the methyl ester **279**. Selective Boc removal was accomplished using dilute TFA, which was followed by reprotection of the amine as a base-labile carbamate. This specific choice of protecting groups was based on their stability under a wide range of conditions. Finally, the *tert*-butyl ester was cleaved and transformed into the primary amide **280**, which was treated with camphor sulfonic acid to afford an enamine. Treatment of this substance with nitrosyl chloride followed by in situ reduction of the α-chloro oxime using sodium cyanoborohydride yielded the oxime **281**.

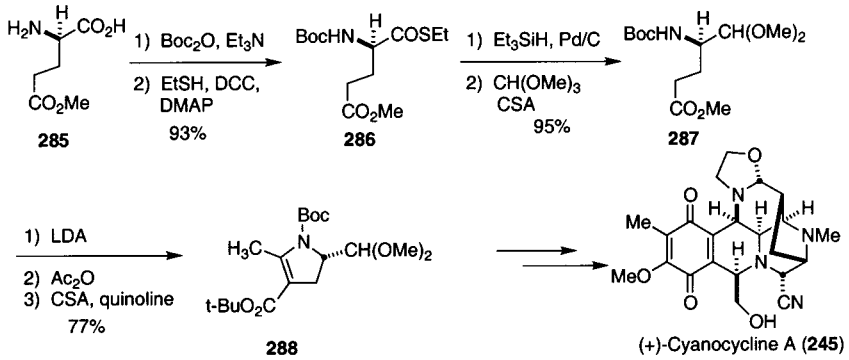
Selective reduction of the oxime followed by a Pictet−Spengler cyclization afforded tetracycle **282** in 66% yield. Reprotection of the phenol was followed by a two-step sequence to convert the methyl ester to an aldehyde which cyclized on the amine to form the corresponding carbinolamine. Conversion of the carbinolamine into the amino nitrile was accomplished using trimethylsilyl cyanide in the presence of zinc chloride. Treatment with boron trichloride cleaved the benzyl ether. The two hydroxyl groups were then reprotected as acetates to afford tetracycle **283**. Conversion of the amide to the oxazolidine A-ring was accomplished via a three-step sequence beginning with the formation of the thiolactam using Lawesson's reagent. Treatment with Raney-nickel lead to desulfurization of the thiolactam to afford an imine that was converted to oxazolidine **284** using ethylene oxide in methanol. The final steps in the total synthesis involved the cleavage of the acetates and carbamate followed by *N*-methylation. Finally, oxidation of the hydroquinone afforded (±)-cyanocyline A in 42% for the final four steps.

The Fukuyama laboratory accomplished the total synthesis of (+)-cyanocycline A via a similar route to that outlined in Scheme 41.[1f] Since the stereogenic center at C-6 was used to set all further stereocenters in the racemic total synthesis, the synthesis of an optically pure dihydropyrrole **277** or equivalent was necessary. Starting with commercially available L-glutamic acid methyl ester **285**, the amine was protected and the carboxylic acid converted to the thioester **286** (Scheme 42). The thioester was reduced under mild conditions to provide an aldehyde, which was protected as the dimethyl acetal **287**. Treatment of **287** with LDA followed by the addition of acetic anhydride effected Claisen condensation, which was immediately followed by dehydration in the presence of camphorsulfonic acid to afford the dihydropyrrole **288** in 77% yield. Dihydropyrrole **288** was converted to (+)-cyanocycline A utilizing the approach successfully employed in the racemic synthesis. This enantiospecific synthesis was used to confirm the absolute stereochemistry of the natural product.

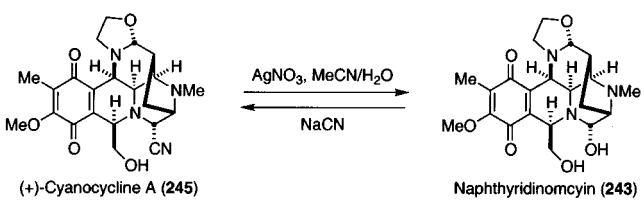
Scheme 41. Fukuyama's Total Synthesis of D,L-Cyanocycline A.



Scheme 42. Fukuyama's Total Synthesis of (+)-Cyanocycline A



Scheme 43. Interconversion of Naphthyridinomycin and Cyanocycline A

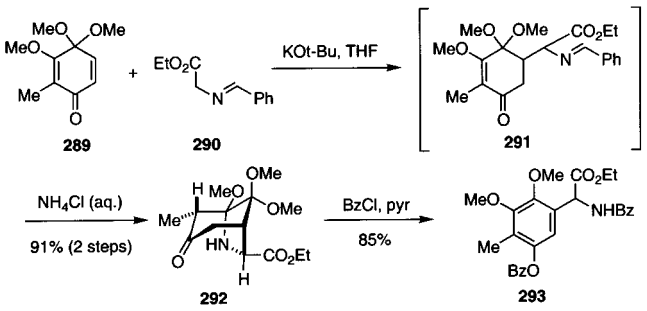


In addition, these workers accomplished the conversion of cyanocyline A to naphthyridinomycin using silver nitrate in water (Scheme 43).[1f] Under these conditions a new product was observed; however, this product was too unstable for purification or isolation. The crude ^{1}H NMR and mass spectral data were consistent with naphthyridinomycin. Treatment of the new product with sodium cyanide reformed cyanocycline A, indicating that indeed naphthyridinomycin had been formed.

3.1.4. Synthetic Studies toward the Naphthyridinomycins

The first published synthetic studies toward the synthesis of the A-ring of naphthyridinomycin were reported by Parker et al. in 1984 (Scheme 44).[110] Starting with quinone monoketal **289** a 1,4-addition was accomplished with the enolate of benzylidine glycine ethyl ester (**290**) to afford the unstable product **291**. Treatment of **291** with aqueous am-

Scheme 44. Parker's Synthetic Studies toward Naphthyridinomycin



monium chloride provided **292** in 91% yield overall from **289**. This bicyclic compound was then ring-opened using excess benzoyl chloride in pyridine to afford **293**. No further studies from this group have been reported.

In 1984, Danishefsky et al. reported their progress toward the total synthesis of naphthyridinomycin via a convergent strategy that consisted of coupling a bicyclic core (**296**) with an amino acid side chain (**302**) (Scheme 45).[111] The tetrahydroisoquinoline fragment **296** was synthesized in very high yield starting with phenol **294**. The phenol was allylated and subjected to a Claisen rearrangement followed by methylation of the phenolic group. Isomerization of the olefin into conjugation with the aromatic ring was accomplished using palladium dichloride bisacetonitrile. Ozonolysis followed by reductive workup yielded aldehyde **295** in 77% yield for the six steps. Imine formation followed by vinyl Grignard addition yielded an amino acetal which was cyclized under acidic conditions to afford the bicyclic substance **296**. Amino acid **302** was synthesized starting from *N*-Boc-L-serine (**297**) in eight steps in 40−45% overall yield. The carboxyl group of **297** was benzylated followed by hydroxyl activation and elimination to afford the dehydroalanine derivative **298**. Methylation of the carbamate nitrogen was followed by a Michael addition using the sodium salt of dimethyl malonate (**299**) to provide diester **300**. Anion formation followed by alkylation with chlorodithiane **301** afforded the cyclic dithioacetal, which was converted to the dimethyl acetal upon treatment with *N*-bromosuccinimide and silver nitrate in methanol. The final step to amino acid **302** was the cleavage of the benzyl ester under standard hydrogenolysis conditions. The overall yield of **302** was 40−45% for the eight steps from **297**.

Coupling of amine **296** and acid **302** was accomplished using BOPCl, and the authors noted that other coupling conditions were ineffective with this sterically hindered system.[111b] Oxidation of the benzylic alcohol using Collins' reagent afforded amide **303** (assumed to be a 1:1 mixture of diastereomers). Treatment with $BF_3 \cdot Et_2O$ afforded a mixture of two compounds, **304** and **305**. Only the *syn*-diastereomer of **303** underwent cyclization to form a tetracyclic compound, while the *anti*-diastereomer did not cyclize, and thus, bicyclic compound **305** was obtained as the resultant product.

In 1987, Joule et al. showed that the C−D-rings of naphthyridinomycin could be formed via a 1,3 dipolar cycloaddition.[112] A simple model study was examined using the piperazine *N*-oxide **306**. With the addition of a dipolarphile at either room temperature or in refluxing THF, a bicyclic product (**307**) was formed in moderate yields (Table 8). When methyl acrylate was used, the *exo*-product was formed in 51% yield. Interestingly, when acrylonitrile was used, virtually

Scheme 45. Danishefsky's Synthetic Approach to Naphthyridinomycin

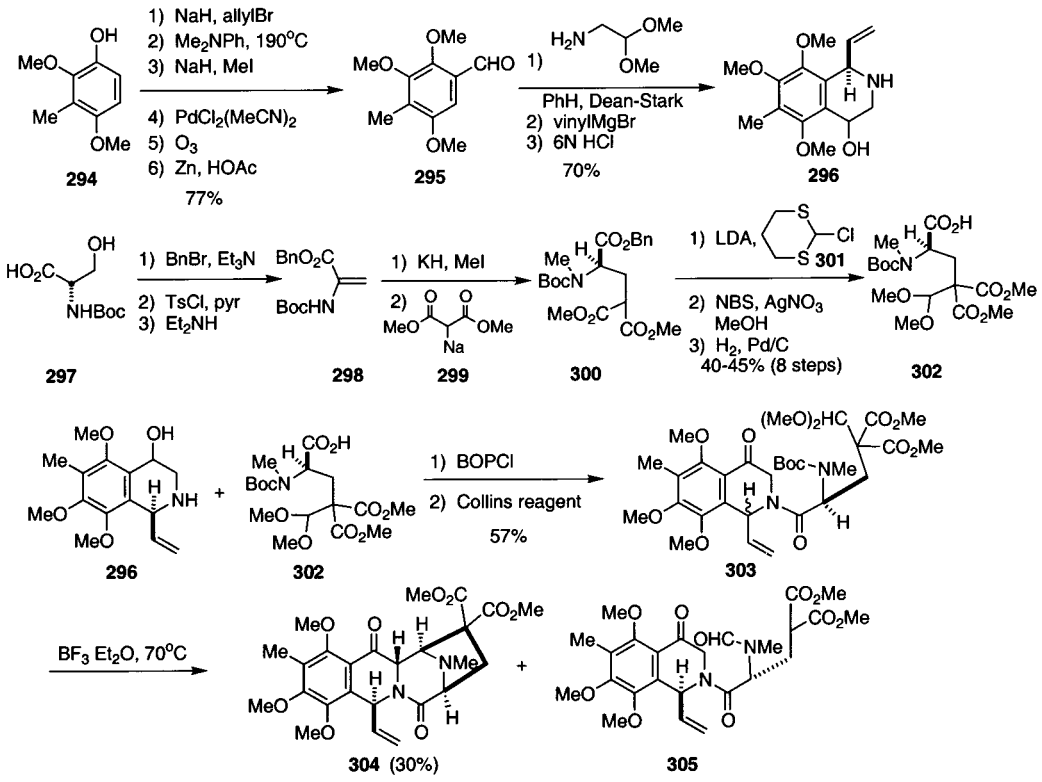
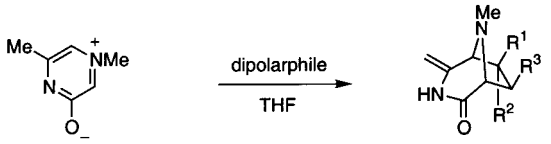


Table 8. Intermolecular 1,3-Dipolar Cycloadditions on 306 Using Various Dipolarphiles



306 307

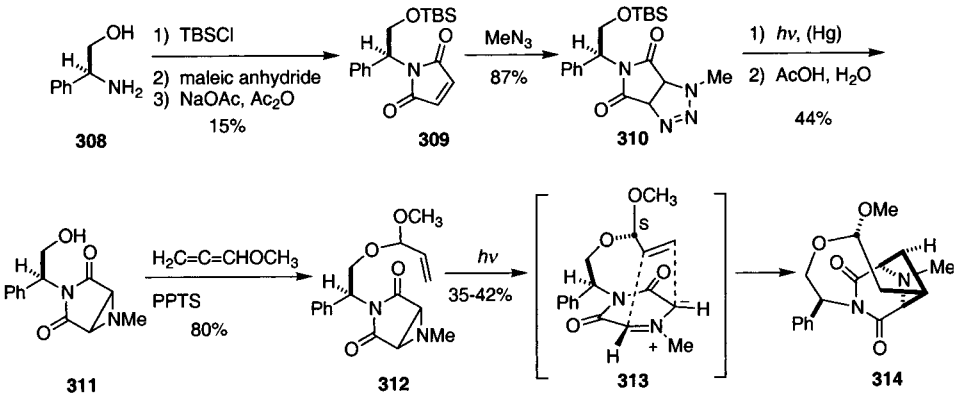
dipolarophile	temp/time	product (yield)
methyl acrylate	reflux/1 h	$R^1 = CO_2Me$, $R^2 = R^3 = H$ (51%)
acrylonitrile	20 °C/3 h	$R^1 = CN$, $R^2 = R^3 = H$ (25%)
diethyl maleate	reflux/2.5 h	$R^1 = R^3 = CO_2Me$, $R^2 = H$ (35%)

no *exo*/*endo* selectivity was observed. The use of the 1,3 dipolar cycloaddition was subsequently used by two other groups in the total synthesis of other members of the tetrahydroisoquinoline family of natural products.

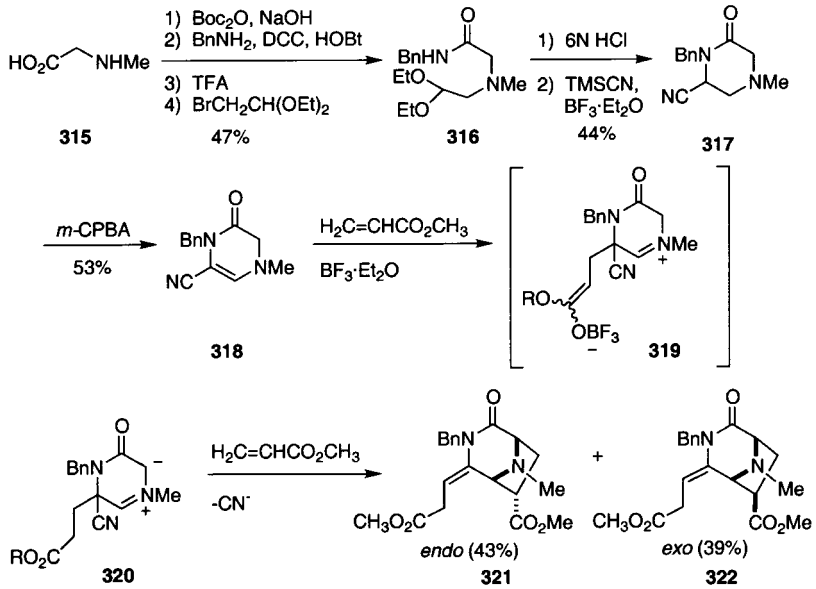
Garner et al. also published a strategy toward the synthesis of naphthyridinomycin via a 1,3-dipolar cycloaddition.[113] The dipolar species was generated by irradiation of aziridine **312** as outlined in Scheme 46, and good results were obtained when the cycloaddition was intramolecular. Intermolecular systems yielded good *endo*/*exo* selectivity; however, they yielded no diastereoselectivity with respect to *re*/*si* addition.[113b] Maleimide **309** was synthesized in three steps from alcohol **308**.[113a] Treatment with methyl azide yielded triazoline **310** in 87% yield. Irradiation with a Hg lamp followed by silyl ether cleavage yielded aziridine **311** followed by acetal formation affording **312**. Irradiation with a 2537 Å Rayonet source generated an azomethine ylide that cyclized with the olefin to yield tricyclic substance **314**. This compound possessed the desired stereochemistry (via *endo-re* attack); however, the yield was low due to cyclization of only one of the acetal diastereomers.

In studies on unsymmetrical azomethine ylides, Garner et al. showed that the bicyclic compounds could be formed by a tandem Michael addition/1,3-dipolar cycloaddition on tetrahydropyrazinone **318** (Scheme 47).[114] Treatment of **318** with methyl acrylate in the presence of $BF_3 \cdot Et_2O$ yielded the bicyclic compounds **321** and **322** in 43% and 39% yields, respectively. These substances were formed via a Michael addition followed by formation of the azomethine ylide **320** and cycloaddition. Slightly higher *endo*/*exo* selectivities were seen with other dipolarphiles, leading to the speculation that the addition could be directed by means of a BF_3 chelation.

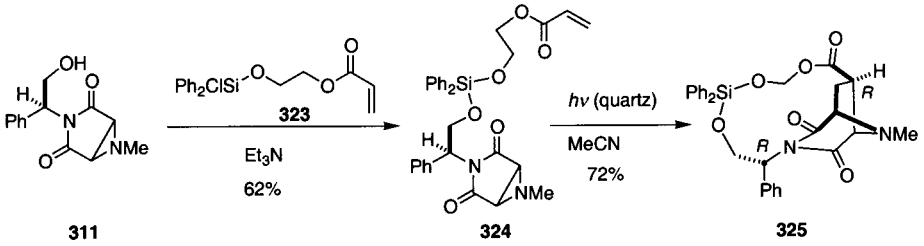
Scheme 46. Garner's Intramolecular 1,3-Dipolar Cycloadditions



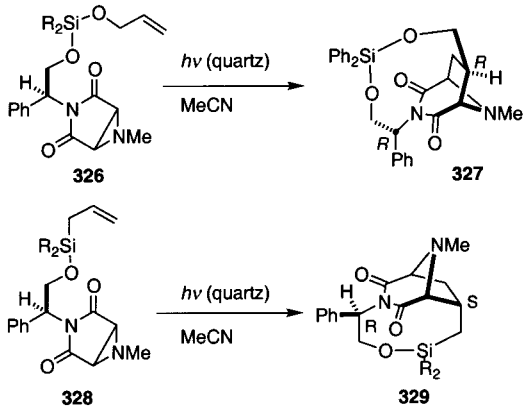
Scheme 47. Garner's Tandem Michael Additions/1,3-Dipolar Cycloadditions



Scheme 48. Effects of Tether Length on Intramolecular 1,3-Dipolar Cycloadditions



Scheme 49. Effects of Tether Length on Intramolecular 1,3-Dipolar Cycloadditions



In 1994, Garner et al. reported the effects of longer tethers with respect to the diastereoselectivity of the 1,3-dipolar cycloaddition (Scheme 48).[115] When alcohol **311** was silylated with chlorosilane **323**, compound **324** was formed. Irradiation afforded the tricycle **325** as the major diastereomer via an *endo-si* addition.

Shortening of the tether (**326**), thus creating a 10-membered transition state, also afforded an *endo-si* product **327** (Scheme 49).[115b] When the tether was shortened by one more atom, altering the cyclization to a nine-membered transition state, the desired *endo-re* addition product **329** was obtained.

In 2001, Williams et al. reported the synthesis of the tricyclic tetrahydroisoquinoline **336**, which is being utilized in an approach to the total synthesis of bioxalomycin α_2.[116] The approach involves a sequential Staudinger reaction followed by a Pictet−Spengler reaction. The efficient synthesis of this β-lactam started with the formation of an imine derived from aldehyde **330** and *O*-TBS-protected ethanolamine. The ketene of phthalimidoacetyl chloride was formed and treated with the imine to form the β-lactam in high yield. Cleavage of the phthalimide and benzyl ether afforded **331** in 64% overall yield. Pictet−Spengler cyclization with benzyloxyacetaldehyde afforded a single diastereomer; however, after amide coupling with Fmoc-sarcosine and cyclization, it was discovered that the tricyclic diketopiperazine **332** had the undesired *anti*-configuration at C-9.

The Pictet−Spengler cyclization was then performed using methyl glyoxylate to afford a single *anti*-diastereomer **333** that could undergo epimerization in the presence of DBU to afford the desired diastereomer **334**. Reduction of the methyl ester followed by protection of the resultant alcohol afforded **335**. Peptide coupling was followed by cleavage of the Fmoc carbamate; however, cyclization did not occur as in the anti diastereomer case.

3.1.5. Analogue Syntheses

SF-1739, which was initially believed to be naphthyridinomycin, was treated with concentrated HCl to afford a new product SF-1739 HP (**244**), which contained a phenol group at C-11 (Scheme 51).[97] The structural assignment of this material was secured by treatment of **244** with potassium cyanide to afford naphthocyanidine **337**.

3.1.6. Biological Activities

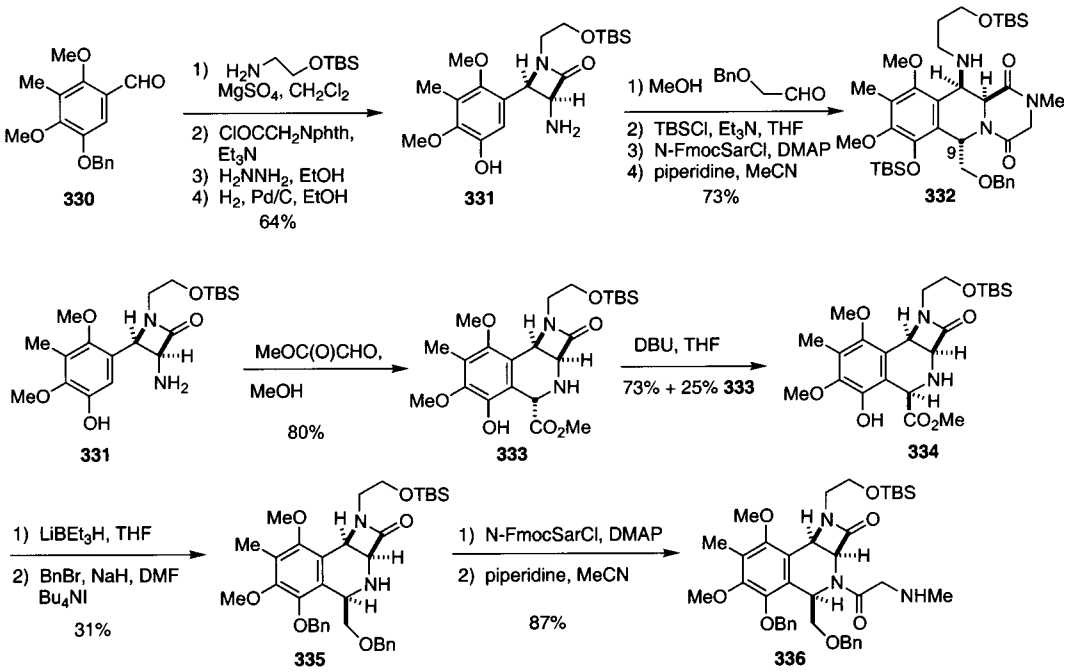
Naphthyridinomycin has potent antibiotic activity against both Gram-(−) and Gram-(+) bacteria.[94] Incorporation of ^{14}C-thymidine during DNA synthesis was inhibited by naphthyridinomycin in *E. coli* at low concentrations.[117] At higher concentrations, RNA and protein synthesis were also inhibited but to a lesser extent than DNA synthesis. The inhibition of DNA biosynthesis was found to be reversible at lower naphthyridinomycin concentrations, but at higher concentrations, inhibition was irreversible.

In studies by Zmijewski et al., ^{3}*H*-naphthridinomycin was found to bind covalently to DNA in small amounts.[118] Naphthyridinomycin that was reduced with DTT was found to covalently bind to DNA to a greater extent than that of natural product and was found to be irreversible under reductive activation conditions. Dithiothreitol has been shown to be the best reducing agent for naphthridinomycin.[118] There was a difference in the UV_{max} of the unreduced form (270 nm) versus the reduced form (287 nm). When glutathione was used as the reducing agent there was no change in the UV_{max} but binding of naphthyridinomycin to DNA was still enhanced, indicating a second possible mechanism exists for DNA binding. This behavior is similar to that described above for saframycin S, which also exhibits enhanced activity when reduced prior to DNA interaction.

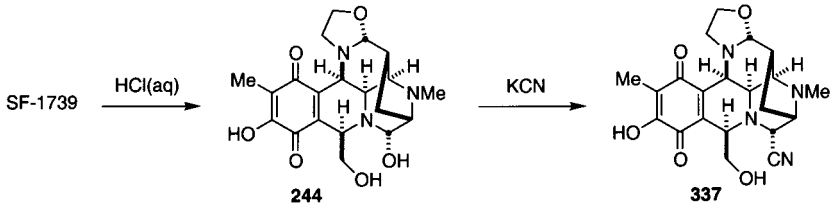
In experiments to determine the sequence specificity of DNA alkylation using poly(dG)-poly(dC) and poly(dA)-poly(dT) polydeoxyribonucleic acids, it was found that naphthyridinomycin binds preferentially to GC-rich regions. Substitution of inosine for guanine resulted in no detectable alkylation, suggesting that naphthyridinomycin covalently alkylates the exocyclic amine of guanine.

A study into the mechanism of binding to DNA by Zmijewski et al. showed that when treated with DTT naphthyridinomycin displays two distinct rates of DNA binding.[119] Initially when treated with DTT

Scheme 50. Williams' Synthetic Studies on Bioxalomycin α_2



Scheme 51. Analogues of SF-1739 (naphthyridinomycin)



there was a burst of fast binding to DNA, followed by a slower rate of binding that was similar in magnitude to that of the unreduced species. Reduction using DTT was shown to increase the rate of reaction 5−6-fold over that of naphthyridinomycin alone. The activated dihydroquinone form of naphthyridinomycin slowly reoxidized in the presence of oxygen to re-form the quinone moiety. A pH study revealed that the optimum pH range for the dihydroquinone form of naphthyridinomycin was 5−7.9, but the unreduced form exhibits maximal DNA binding at pH 5.

Two mechanisms were proffered for the DNA alkylation of naphthyridinomycin as shown in Scheme 52. The first (path A) was based on the previously described mechanism of DNA alkylation mediated by saframycin. Thus, reduction of the quinone moiety of naphthyridinomycin by thiols affords a structure corresponding to the dihydroquinone **338**. Formation of the dihydroquinone was invoked to assist loss of the hydroxyl group through scission of the benzylic C−N bond, thus forming imine **339**. The nonbonded electron pair of the imine then re-closes on the *o*-quinone methide, forming the iminium species **340**, which subsequently suffers alkylation with N-7 of guanine to form **341**. The second mechanism (path B) involves the protonation of the hydroxyl group to afford **342** followed by S_N2 displacement by DNA to form **341**.

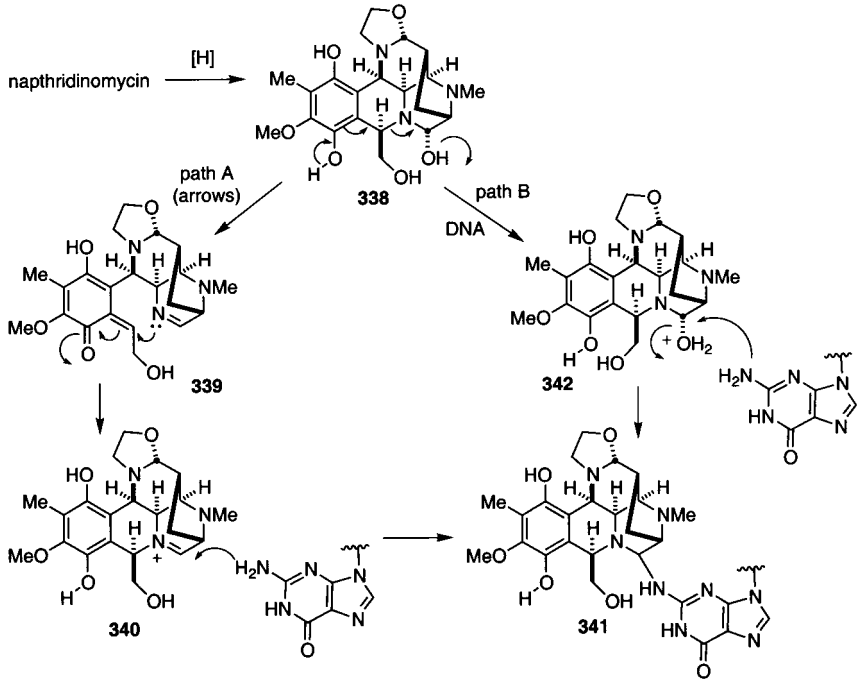
These two mechanisms were used to rationalize the two rates of alkylation. It was shown that the addition of SDS or Na^+ ions did not hinder the alkylation of DNA by naphthyridinomycin. These data were interpreted to infer that naphthyridinomycin does not chelate to DNA due to the presumption that naphthyridinomycin does not contain suitable intercalative functionality that has been emphasized for the saframycins and ecteinascidins. The reduction of naphthyridinomycin to the dihydroquinone was thus argued to provide a species (the dihydroquinone, **338**) that is capable of hydrogen bonding to DNA and consequently allowing for a higher rate of alkylation. This argument is specious however, and scant convincing evidence exists to support this mechanistic picture.

In the original paper that described the isolation of cyanocycline A, the biological activities of naphthyridinomycin and cyanocycline A were compared.[98] The MICs of naphthyridinomycin were better than or equal to that for cyanocyline A. However, an in vitro cytotoxicity study using HeLa cells revealed similar activity for the two compounds at the same concentrations.

In 1983, Hayashi et al.[120] reported that cyanocyline A reduced by DTT showed no enhancement in biological activity, indicating a different mechanism of action may be operative compared to naphthyridinomycin. In this report it was also mentioned that the aminonitrile moiety of cyanocyline A was more stable than that of saframycin A.

The semisynthetic derivatives SF-1739 HP and cyanocycline F exhibit reduced activity in most of the

Scheme 52. Proposed Mechanisms of DNA Alkylation by Naphthyridinomycin



antimicrobial screens compared to that of the natural substrates.[97] However, the chemical stability and toxicities were markedly increased over the parent SF-1739.

Cox et al. studied the X-ray structure and 2D NMR data along with molecular modeling of cyanocylcine A and molecular modeling of naphthyridinomycin to determine the best binding model to DNA.[121] Both partial intercalation and groove binding models were investigated. It was found that reduction of the quinone moiety to the hydroquinone was not necessary for DNA binding. The authors conclude that the major activation necessary for DNA binding was simply the formation of the iminium at C-7.

Remers et al. reported a molecular modeling study for the alkylation of naphthyridinomycin and cyanocycline A to DNA.[122] These studies suggested that another possible mode of DNA alkylation might involve opening of the oxazolidine ring and alkylation at C-3a; this third potential mechanism for DNA alkylation as suggested by Remers is shown in Scheme 53. After reduction to the hydroquinone **343**, ring opening of the oxazolidine would afford the *o*-quinone methide species **344**. Attack of the imine lone pair on the *o*-quinone methide would yield iminium **345**, which can undergo alkylation at C-3a by DNA to afford **346**. It was also suggested that DNA cross-linking of duplex DNA would not be possible via alkylation at the two oxazolidine moieties but that DNA−protein cross-linking might be possible.

The Lederle group reported that bioxalomycin α_2 displays excellent antimicrobial activity against Gram-(+) bacteria.[102a,123] The antimicrobial data for bioxalomycin α_2 is displayed in Table 9. These workers observed a slightly different profile of cellular macromolecule biosynthesis than that reported for naphthyridinomycin. Like naphthyridinomycin, bioxalomycin α_2 inhibited DNA synthesis drastically. However, both RNA synthesis and protein synthesis were inhibited to a more significant extent.

Scheme 53. Alternate Mechanism Proposed for DNA Alkylation by Naphthyridinomycin

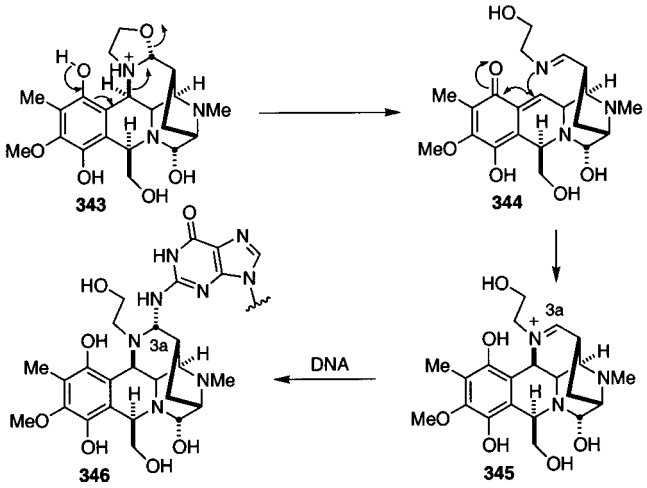


Table 9. Antimicrobial Activity of Bioxalomycin α_2 against Gram-(+) Isolates[119]

test organisms [no. of strains]	MIC (μg/mL)
MSSA[a] [4]	≤0.002−0.015
MRSA[b] [33]	0.004−0.015
SCN[c] [6]	≤0.002−0.004
Staphylococcus hemolyticus [1]	≤0.002
Streptococcus pyogenes [1]	≤0.002
Streptococcus agalactiae [1]	≤0.002
Streptococcus pneumoniae [1]	0.015
Enterococcus faecalis VS [4]	≤0.002−0.25
Enterococcus faecium VR [2]	0.03−0.06
Bacillus cereus [1]	0.12

[a] MSSA = methicillin-sensitive *Staphylococcus aureus.* [b] MRSA = methicillin-resistant *Staphylococcus aureus.* [c] SCN = coagulase-negative *Staphylococci.*

It was later shown by Williams and Herberich that bioxalomycin α_2 does indeed cross-link duplex DNA.[124]

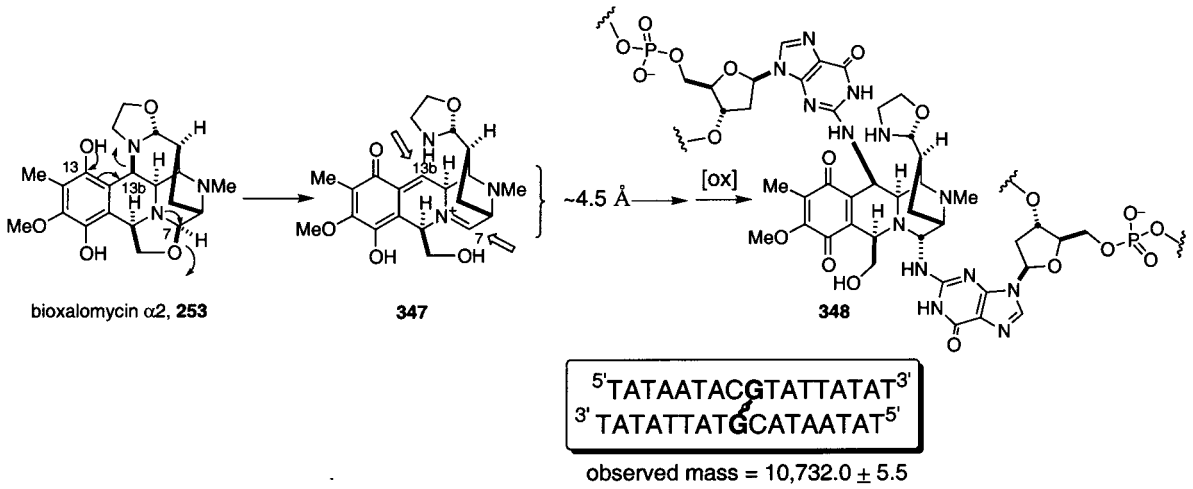


Figure 13. Interstrand cross-linking of DNA by bioxalomycin α_2 observed by Williams.

It was also noted that cyanocycline A formed DNA cross-links in low yield and only in the presence of DTT. Bioxalomycin α_2 DNA interstrand cross-linking showed $^{5'}$CpG$^{3'}$ selectivity as evidenced by footprinting studies. Substitution of guanine with inosine abolished the cross-linking, indicating that N-2 of guanine was alkylated. The calculated mass for the bioxalomycin cross-link = 10 766, and the authors note that the difference in the calculated and observed mass (10 732 ± 5.5) corresponds to a loss of the hydroxymethyl moiety at C-9. This facile fragmentation has been observed in related hydroxymethyl-substituted isoquinolines. The Colorado State researchers further note that the electrospray mass spectrum of cyanocycline observed under the same conditions gave the molecular ion peak (calculated mass = 426.2) minus the CH_2OH fragment (observed mass = 395.1) without detection of the parent ion peak to support the proposed structure (**348**). In addition, it was noted that the molecular mass of the drug−DNA cross-link on the DNA substrate shown in Figure 13 indicates that after alkylation the dihydroquinone suffers oxidation to the corresponding quinone as shown in the proposed structure **348**. Two possible sites of cross-linking on bioxalomycin were suggested (Figure 13), one at C-13b and C-9 (**347**) and the other at C-13b and C-7 (**348**). The authors note that their results point to the possible significance of benzylic (C-13b) oxidation in this family of antitumor antibiotics and that similar DNA interstrand and/or DNA−protein cross-linking behavior might be anticipated for the structurally related marine antitumor antibiotics, the ecteinascidins. This mode of action for the ecteinascidins has yet to be demonstrated, however.

Several bis-electrophilic species have been considered to arise from the bioxalomycin framework. Zmijewski proposed a mechanism (Scheme 52) that accounts for alkylation at C-3a or C-7 of bioxalomyicin α_2. Another mechanism for DNA cross-linking by naphthyridinomycin was postulated by Moore wherein it was proposed that a quinone methide, formed from the deprotonation of the dihydroquinone, might be a suitable alkylating agent and consequently places the alkylation sites at C-13b and C-9 of bioxalomyicin α_2. On the basis of the observed requirement for reductive activation for DNA interstrand cross-linking, Williams and Herberich contend that an *o*-quinone methide species which would result in alkylation at C-7 and C-13b via a partial intercalative presentation of the drug appears to be the most plausible. Previous modeling work in this area[4b,c,g] apparently only considered approach of the drug from the right-hand sector toward the minor groove in a "face on" approach and did not consider a partial intercalative approach. The authors note that positions C-9 and C-3a are also possible but seem unlikely in view of the well-established importance of the carbinolamine (C-7 for bioxalomycin) or functionally equivalent derivatives of the carbinolamine in DNA alkylation by these drugs. Identification of the exact molecular structure of the bioxalomycin α_2-mediated cross-link has not yet been established.

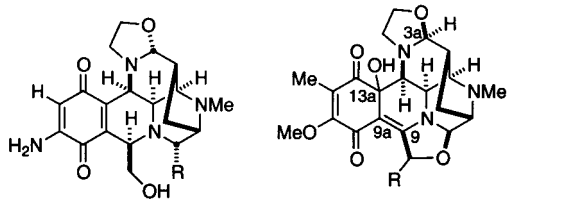


Figure 14. The Dnacins and aclindomycins.

3.2. Dnacins and Aclindomycins

3.2.1. Isolation and Structure Determination

In 1980, Tanida et al. published their report on the isolation of two new antitumor antibiotics from *Actinosynnema pretiosum* C-14482[125] but the structures were not determined until 1994 (Figure 14). The structure of dnacin B_1 was found to be very similar to that of naphthyridinomycin with the exception of the amino group at C-11 and the hydrogen at C-12. The structures of the dnacins were determined by NMR spectroscopy.

The most recent members to be added to the bioxalomycin family are the aclindomycins A and B (**350a**,**b**), which were isolated in 2001 from *Streptomyces halstedi* by Yoshimoto et al.[126] These structures contain the very unusual hydroxylated quinone at

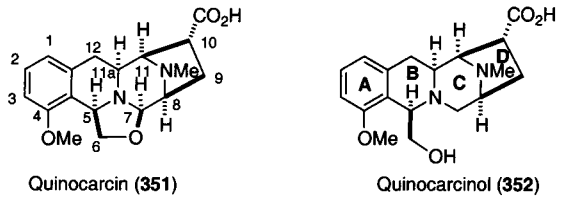


Figure 15. Quinocarcin and quinocarcinol.

C-13a and the unsaturation between C-9/C-9a and were reported to be epimeric at C-3a to all other known members of this family.

3.2.2. Biological Activity

Like naphthyridinomycin, dnacin B_1 was found to inhibit DNA synthesis.[127] Incorporation of ^{3}H-thymidine into DNA was inhibited, and incorporation of ^{14}C-uracil was somewhat inhibited, but protein synthesis was not affected. Along with DNA synthesis inhibition, dnacin B_1 (when first reduced) has been shown to cleave DNA via the formation of superoxide.

A more in-depth study of both dnacins A_1 and B_1 showed that the phosphatase activity of the cdc25B protein was inhibited.[128] This was noted to be important since the cdc25B gene was expressed at high levels in some human cell lines. Dnacin B_1 was approximately twice as effective as dnacin A_1 (IC_{50} values of 64 and 141 μM, respectively).

The aclindomycins were reported to display modest antimicrobial activity against the Gram-(+) organisms *Bacillus subtilis*, *Bacillus cereus*, and *Micrococcus luteus* but were inactive against Gram-(−) bacteria and fungi.

No synthetic work on these newest members of this family of natural products has been reported.

4. Quinocarcin Family

4.1 Quinocarcin and Quinocarcinol

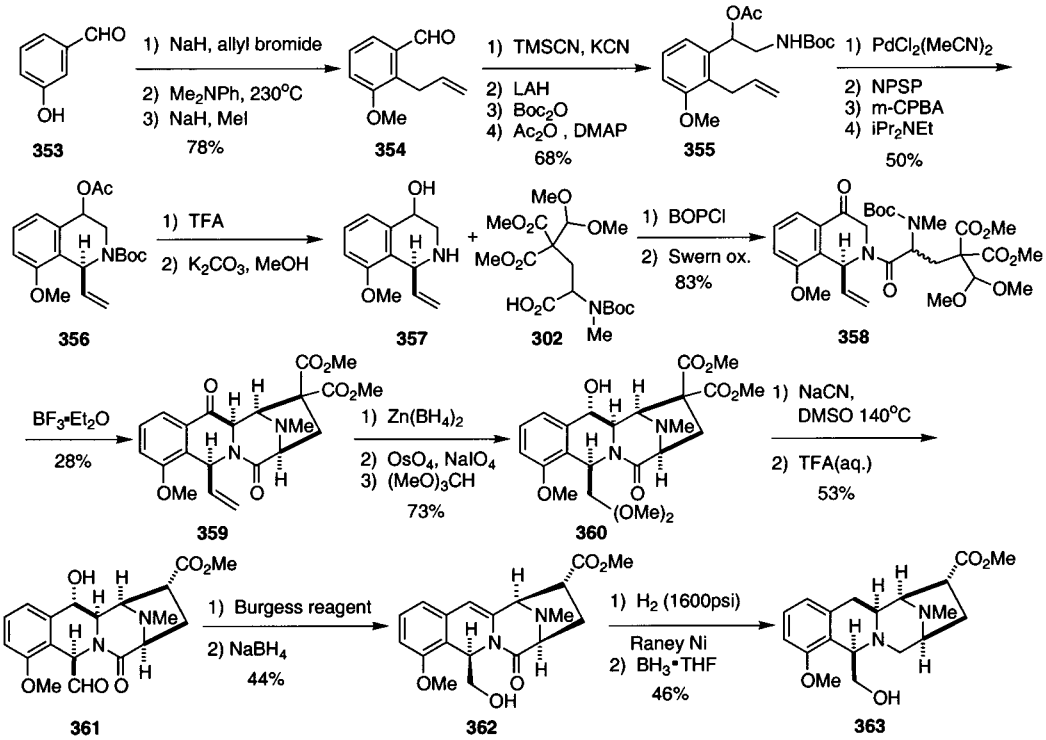
4.1.1. Isolation and Structure Determination

In 1983 Tomita et al. isolated the antitumor antibiotics quinocarcin (**351**) and quinocarcinol (**352**) from *Streptomyces melanovinaceus* nov. sp. (Figure 15).[129] The structure of quinocarcinol was determined by X-ray crystallography, but unfortunately, quinocarcin could not be crystallized for a similar analysis.[130] The structure of quinocarcin was determined by comparison of NMR spectra between the two natural products,[129b] and quinocarcin could be transformed into quinocarcinol via sodium borohydride reduction, thus confirming the assigned structure. The absolute stereochemistry was determined when the total synthesis of (−)-quinocarcin was reported by Garner et al. in 1992.[131]

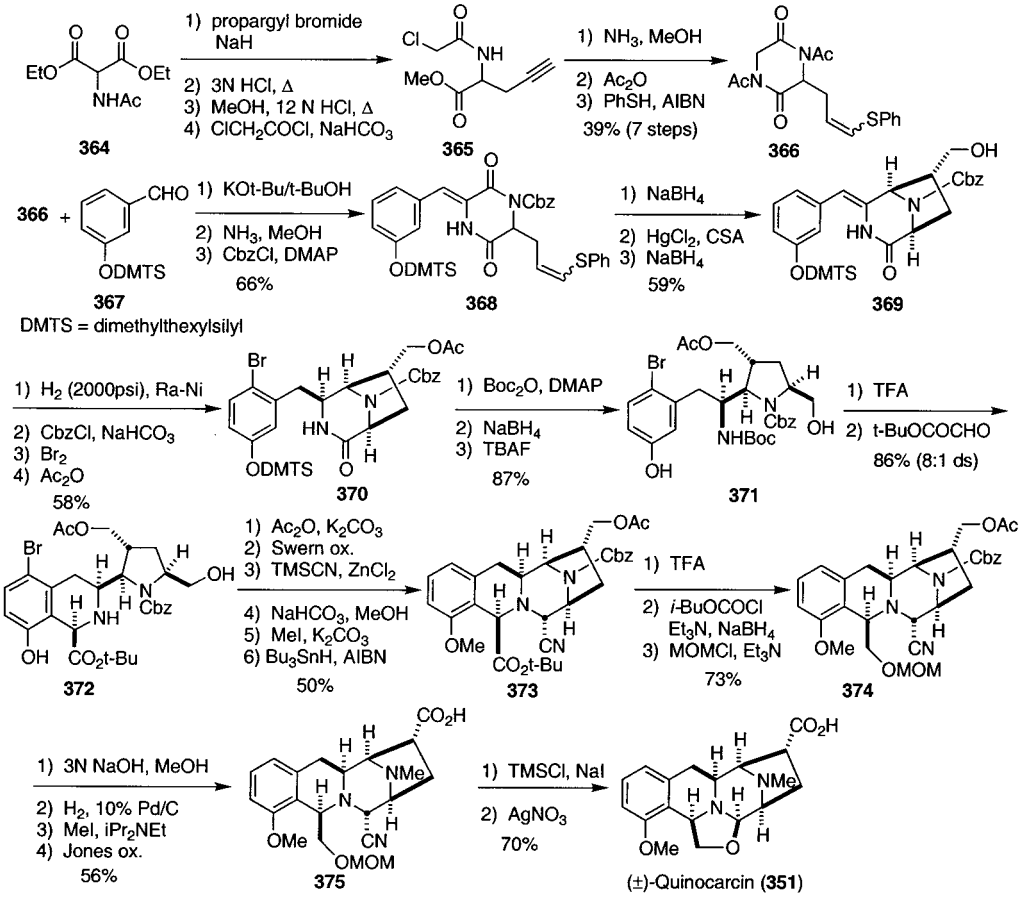
4.1.2. Total Syntheses of Quinocarcin, Quinocarcinol, and Quinocarcinamide

The first synthesis of quinocarcinol was accomplished by Danishefsky et al. in 1985.[132] Starting with aromatic aldehyde **353**, the phenol was allylated followed by a Claisen rearrangement and methylation of the phenol to afford **354** (Scheme 54). Conversion of the aldehyde to the cyanohydrin was followed by reduction of the nitrile using LAH. Protection of the amine and alcohol provided **355**, which was followed by treatment of the allyl group with $PdCl_2$-$(MeCN)_2$ complex to afford a 3.5:1 mixture of *E*:*Z* benzylic olefins. A three-step sequence was used to form the tetrahydroisoquinolone **356** using *N*-phenylselenophthalimide (NPSP) in the presence of camphorsulfonic acid followed by treatment with *m*-CP-

Scheme 54. Danishefsky's Synthesis of D,L-quinocarcinol Methyl Ester



Scheme 55. Fukuyama's Total Synthesis of D,L-quinocarcin

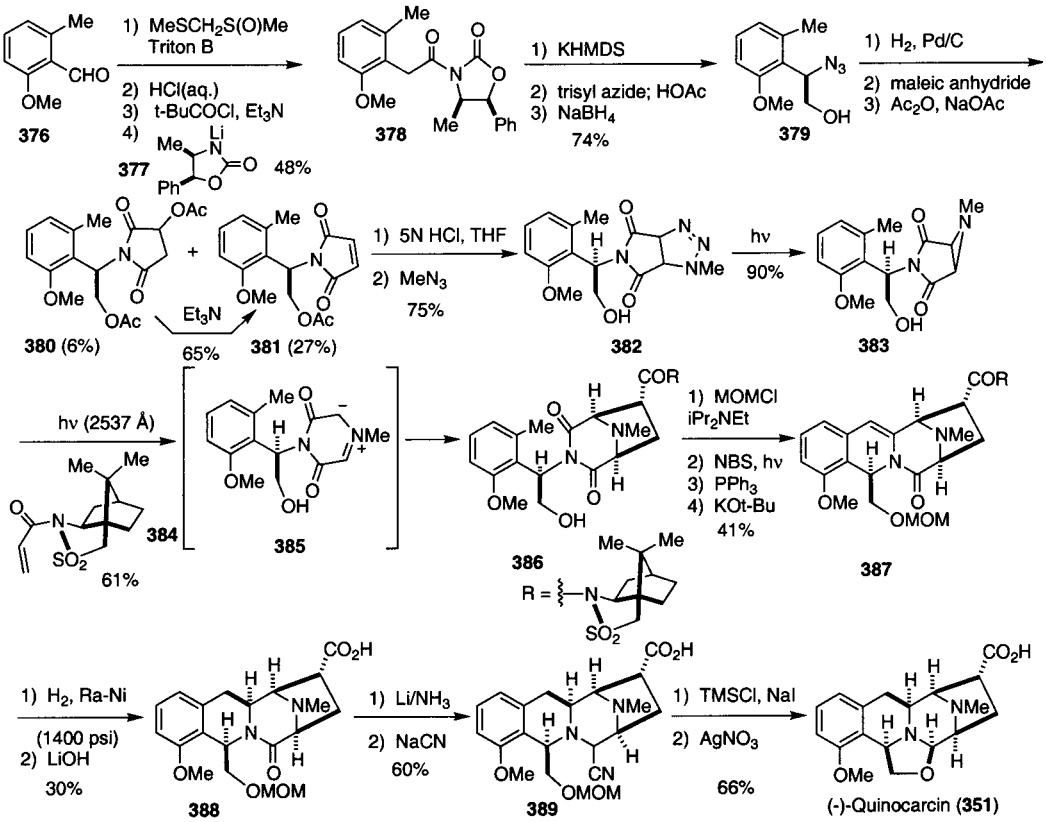


BA and Hunig's base. Removal of the Boc and acetate protecting groups afforded the secondary amine **357**, which was coupled to amino acid **302** using BOPCl. Swern oxidation provided ketone **358** as a 1:1 mixture of diastereomers. Treatment with $BF_3 \cdot Et_2O$ afforded tetracycle **359** in 28% yield as only one of the possible four diastereomers. Reduction of the benzylic ketone was followed by oxidative cleavage of the olefin to the corresponding aldehyde. Protection of the aldehyde as the dimethylacetal yielded **360**. Diastereoselective decarbomethoxylation was accomplished in 75% yield using sodium cyanide in DMSO at 140 °C. The acetal was then cleaved to afford aldehyde **361** in 73% yield from **360**. Elimination of the hydroxyl group was accomplished using the Burgess reagent followed by reduction of the aldehyde to afford **362**. The final steps to quinocarcinol methyl ester (**363**) were the reduction of the benzylic olefin using high-pressure hydrogenation over Raney-nickel followed by reduction of the amide to the amine using borane in THF. All attempts by these workers to synthesize quinocarcin from **362** by partial reduction of the amide were unsuccessful as was methylene oxidation of the amine **363**.

The first total synthesis of (±)-quinocarcin was accomplished by Fukuyama and Nunes in 1988 (Scheme 55).[133] Starting with the diethyl malonate **364**, diketopiperazine **366** was synthesized in seven steps in 39% overall yield. Aldol condensation with aromatic aldehyde **367**, followed by selective protection of one of the lactam nitrogen atoms, afforded diketopiperazine **368**. Reduction of the activated lactam carbonyl was followed by an acyliminium ion-mediated cyclization using $HgCl_2$ and camphorsulfonic acid. Reduction of the resultant aldehyde provided bicyclic compound **369** in 59% yield from **368**. Reduction of the benzylic olefin from the least hindered face was followed by reprotection of the secondary amine as a benzyl carbamate. Bromination para to the methoxy group prevented the formation of an undesired tetrahydroisoquinoline regioisomer later in the sequence. Subsequent acylation of the incipient alcohol afforded **370**. Ring opening was accomplished via activation of the lactam followed by treatment with sodium borohydride to afford the pyrrolidine **371** after silyl ether cleavage.

TFA cleavage of the Boc carbamate was followed by a Pictet−Spengler cyclization to afford tetrahydroisoquinoline **372** in 86% yield as a 8:1 mixture of diastereomers. Selective phenol acylation was followed by Swern oxidation of the primary alcohol. Treatment of the resultant amino aldehyde with TMS cyanide and zinc chloride afforded the tetracyclic core. Cleavage of the phenolic acetate was followed by phenol methylation and radical cleavage of the bromide to afford tetracycle **373** in 50% yield for the six steps. Cleavage of the *tert*-butyl ester and subsequent reduction of the carboxylic acid was followed by alcohol protection as the methoxymethyl ether (**374**). Removal of the acetate and Cbz groups was followed by *N*-methylation and oxidation of the alcohol to the carboxylic acid to afford the MOM-protected DX-52-1 derivative **375**. The final steps in the total synthesis were the removal of the MOM

Scheme 56. Garner's Total Synthesis of (−)-Quinocarcin



group using TMSI in situ and the closure of the oxazolidine ring using silver nitrate to afford (±)-quinocarcin in 70% yield for the final two steps.

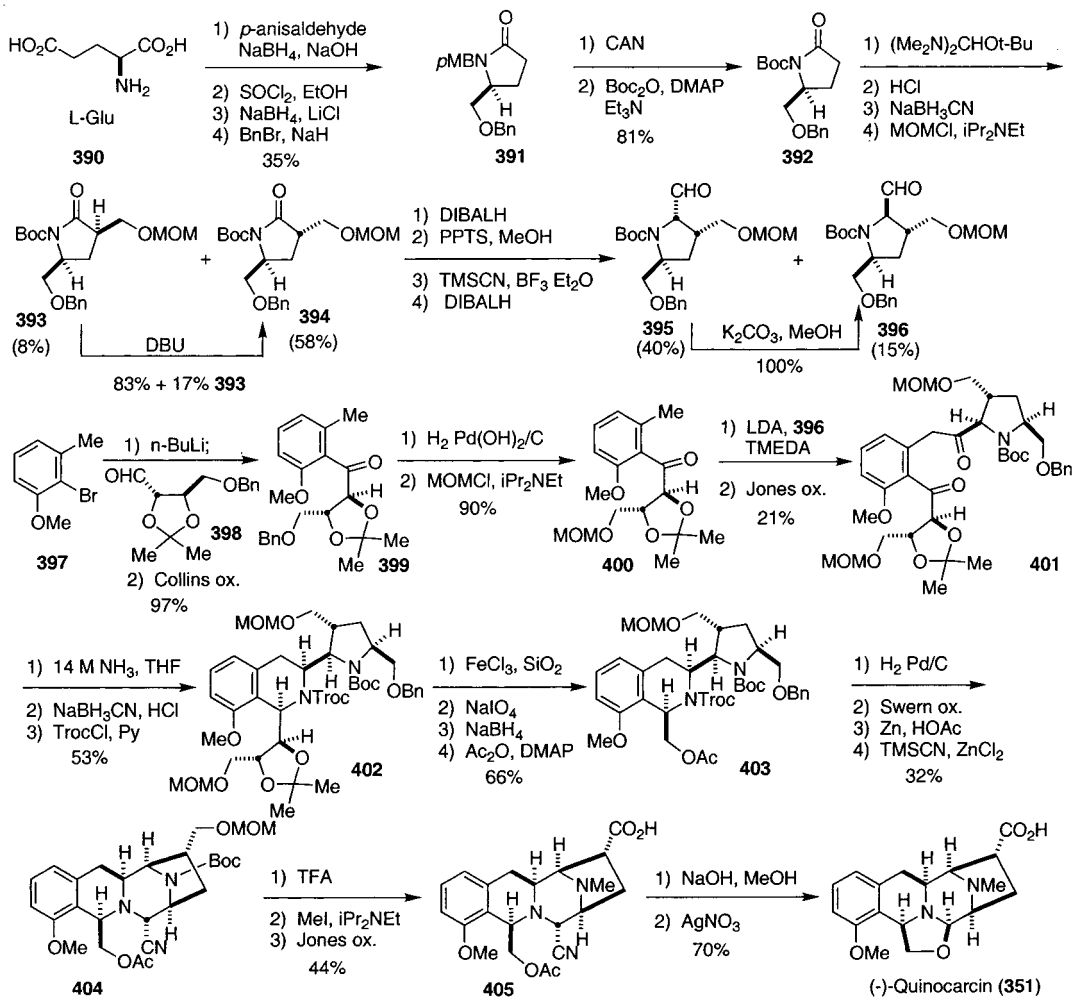
In 1992, Garner et al. published the first asymmetric synthesis of (−)-quinocarcin.[131] The key step involved an intermolecular 1,3-dipolar cycloaddition as shown in Scheme 56.[131,134] Starting with aldehyde **376**, treatment with methyl methylsulfinylmethyl sulfide in the presence of Triton B and acidic hydrolysis afforded a carboxylic acid. Formation of the mixed anhydride and treatment with chiral auxiliary afforded **378** in 48% overall yield. Deprotonation followed by treatment with trisyl azide provided the optically active azide.

Reductive cleavage of the chiral auxiliary afforded the azido alcohol **379** in 74% yield along with recovery of the chiral auxiliary **377** in 96% yield. Hydrogenolysis of the azide was followed by treatment with maleic anhydride. Cyclization to form the maleimide was accomplished using acetic anhydride to form **381** as the major product. Hydrolysis of the acetate of **381** was followed by treatment with methyl azide to afford the triazoline **382** in 75% yield. Irradiation using a high-pressure Hg lamp extruded nitrogen, affording aziridine **383** in high yield.

The azomethine ylide **385** was formed via irradiation of aziridine **383**, and the ylide was then trapped with Oppolzer's sultam **384** to afford the desired bicyclic cycloadduct **386** via an *exo-si* attack on the olefin. No other diastereomers were detected from this cycloaddition. Protection of the alcohol group of **386** was followed by benzylic bromination with NBS. Conversion to the phosphonium salt was followed by deprotonation to form the ylide, which suffered intramolecular Wittig cyclization to afford tetracycle **387** in 41% yield from **386**. High-pressure reduction of the benzylic olefin was followed by hydrolysis of the sultam to afford **388** in 30% yield. High-pressure hydrogenation provided a 1:1 mixture of the desired compound along with the product of reduction of the sultam imide to a primary alcohol. Partial reduction of the amide was accomplished using a dissolving metal reduction, followed by trapping of the carbinolamine with sodium cyanide to provide the stable amino nitrile **389** in 60% yield. The final two steps, as in Fukuyama's synthesis,[133] were the cleavage of the MOM group and closing of the oxazolidine ring to afford (−)-quinocarcin.

Terashima et al. reported, in a series of papers, quinocarcin and quinocarcin analogue syntheses that included the synthesis of both enantiomers of quinocarcin.[135] The total synthesis of (−)-quinocarcin started with the synthesis of the D-ring (Scheme 57).[135c] The conversion of (*S*)-glutamic acid (**390**) to the lactam **391** was accomplished via a four-step sequence in 35% overall yield. Replacement of the *p*-methoxybenzyl group with a Boc group provided **392** in 81% yield. Treatment of **392** with Brederick's reagent followed by acidic hydrolysis provided the *β*-amido aldehyde. Reduction of the aldehyde was followed by alcohol protection to afford two diastereomers **393** and **394**. Partial reduction of the lactam with DIBAl−H followed by treatment with acidic methanol resulted in the formation of the corresponding acetonide. Conversion to the diastereomeric amino aldehydes **395** and **396** was accomplished via treatment with TMSCN under Lewis acidic conditions followed by DIBAl-H reduction of the nitriles. Fortunately, the

Scheme 57. Terashima's Total Synthesis of (−)-Quinocarcin



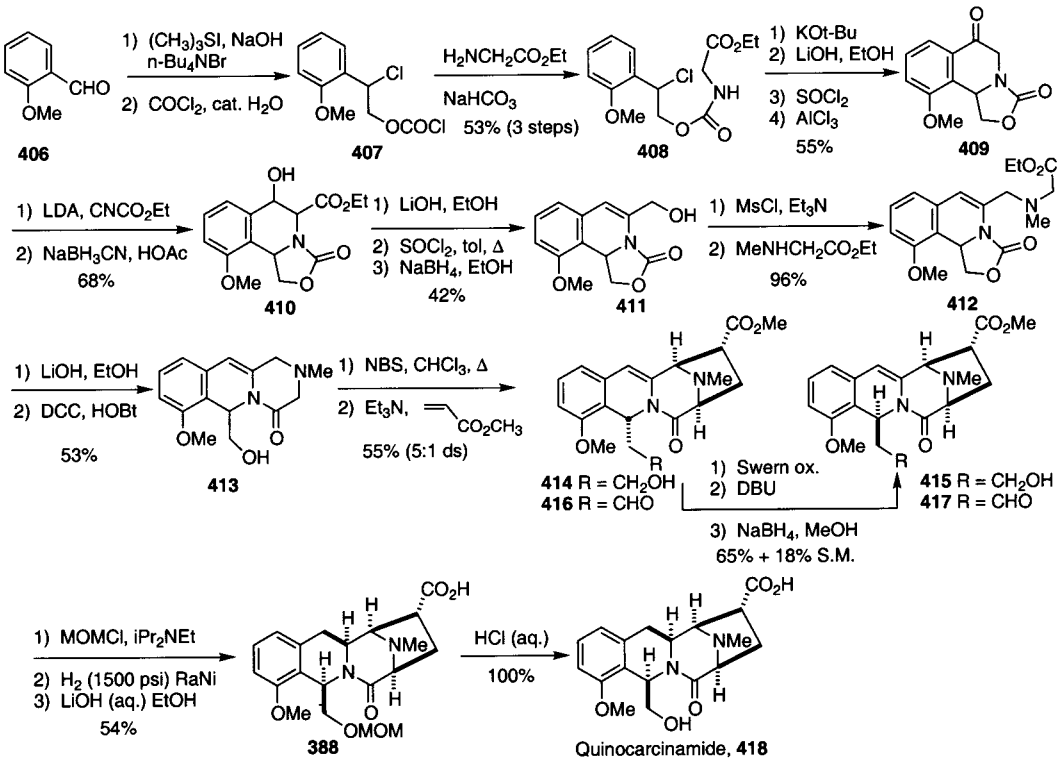
undesired diastereomer **395** could be epimerized in quantitative yield to **396**.

The A-ring was synthesized starting with lithiation of **397** followed by addition of protected threose **398** and Collins oxidation to afford **399** in high yield.[135d] Substitution of the MOM group for the benzyl ether yielded **400**. Lithiation of the benzylic position and condensation with **396** afforded **401** after oxidation. Treatment with ammonia promoted cyclization to the corresponding isoquinoline, and selective reduction to the tetrahydroisoquinoline was accomplished using $NaBH_3CN$ under acidic conditions. Protection of the resultant secondary amine as the Troc carbamate produced **402** in 53% yield from **401**. Deprotection of the 1,2-diol followed by oxidative cleavage afforded an aldehyde, which was subsequently reduced and protected to provide **403** in 66% yield from **402**. The cyclization strategy to afford the tetracycle was similar to that used by Fukuyama. Removal of the benzyl ether was followed by oxidation of the primary alcohol to an aldehyde. Removal of the Troc group allowed for cyclization and the resultant carbinolamine was converted to the amino nitrile **404**. Removal of the Boc and MOM groups was followed by *N*-methylation and oxidation of the primary alcohol to the acid **405**. The final two steps were the hydrolysis of the acetate and closing of the oxazolidine ring to afford (−)-quinocarcin in 70% yield. The asymmetric synthesis of (+)-quinocarcin was accomplished by the use of *ent*-**395** and *ent*-**397** in the same sequence of steps used in the (−)-quinocarcin synthesis.[135d]

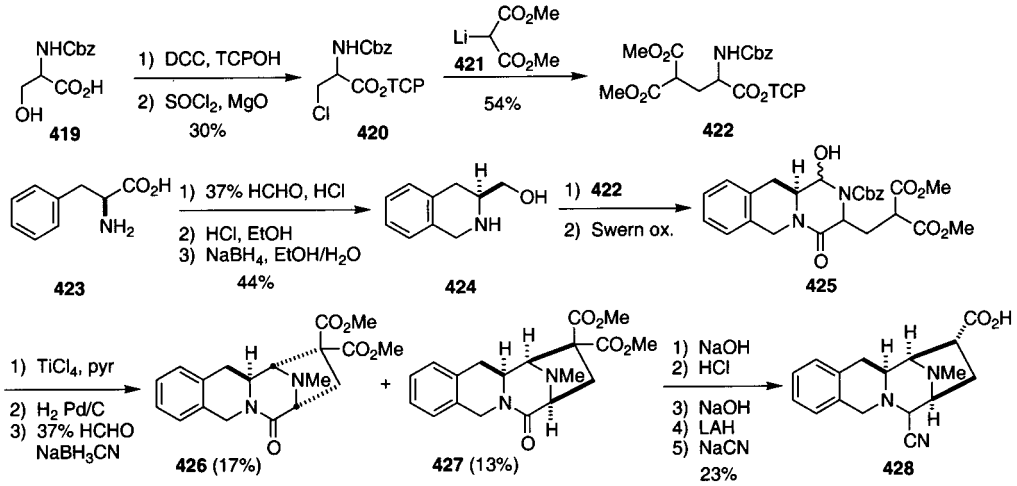
In 1995, Flanagan and Williams published the synthesis of (±)-quinocarcinamide (**418**).[136] A late stage intermediate **388** intersected Garner's total synthesis of quinocarcin, thus making this a formal total synthesis of D,L-quinocarcin. The key step in this synthesis was an intermolecular azomethine ylide 1,3-dipolar cycloaddition reaction where a new method for the formation of an azomethine ylide using NBS to oxidize an allylic amine was developed.

Thus, as shown in Scheme 58, treatment of *o*-anisaldehyde (**406**) with trimethylsulfonium iodide under phase-transfer conditions afforded the benzylic epoxide, which was opened with phosgene to form the chloroformate **407**. Conversion to the carbamate **408** was accomplished via treatment with glycine ethyl ester. Cyclization afforded the oxazolidinone, which upon saponification yielded the carboxylic acid, which was converted to the corresponding acid chloride. An intramolecular Friedel−Crafts acylation provided isoquinolone **409** in 55% overall yield.[137] Treatment with LDA and ethyl cyanoformate followed by reduction of the ketone afforded the β-hydroxy ester **410**. Conversion of **410** to the allylic alcohol **411** was accomplished by saponification of the ester followed

Scheme 58. Williams' Total Synthesis of D,L-Quinocarcinamide



Scheme 59. Saito's and Hirata's Synthetic Studies on Quinocarcin

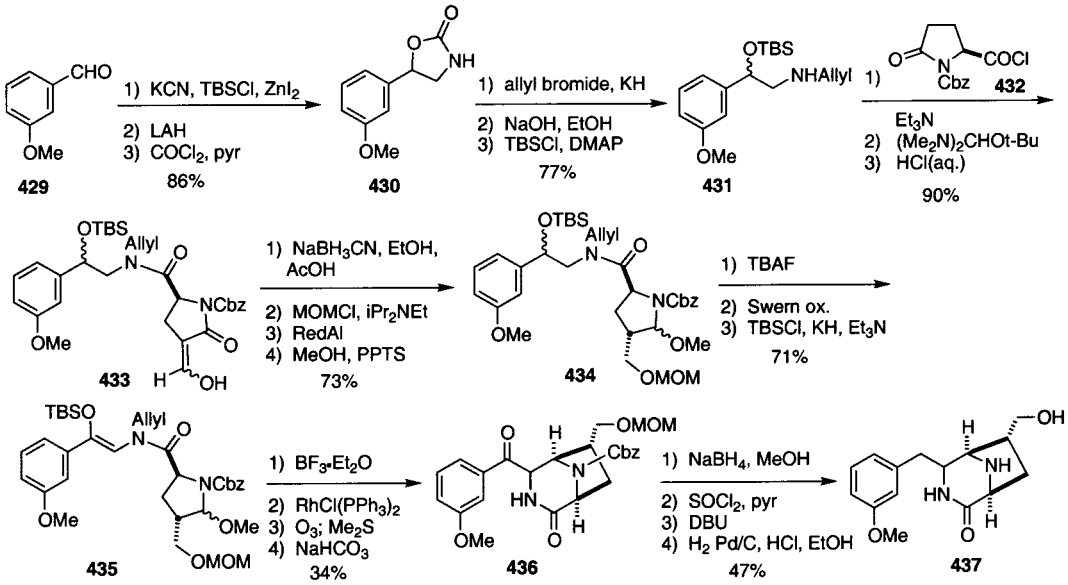


by conversion to the α,β-unsaturated acid chloride and finally reduction of the acid chloride. Formation of the allylic chloride was followed by treatment with sarcosine ethyl ester to afford the allylic amine **412**. Hydrolysis of the oxazolidinone was followed by coupling of the secondary amine upon the resultant acid to afford the tricyclic compound **413**. NBS oxidation of the allylic amine afforded a dark green solution of the incipient iminium salt, which upon deprotonation using triethylamine resulted in a dark blue solution of the azomethine ylide. Treatment of this substance with methyl acrylate afforded a 5:1 ratio of the cycloadducts **414** and **415**. Unfortunately, the desired diastereomer **415** was the minor product of the cycloaddition. The major product was efficiently epimerized to **415** via a three-step sequence of (1) oxidation to aldehyde **416** followed by (2) epimerization using DBU and finally (3) reduction of aldehyde **417** with sodium borohydride. Protection of the alcohol as the MOM ether was followed by high-pressure reduction of the benzylic olefin. Saponification of the ester afforded **388**, an intermediate in Garner's total synthesis. Removal of the MOM group afforded (±)-quinocarcinamide (**418**) in quantitative yield.

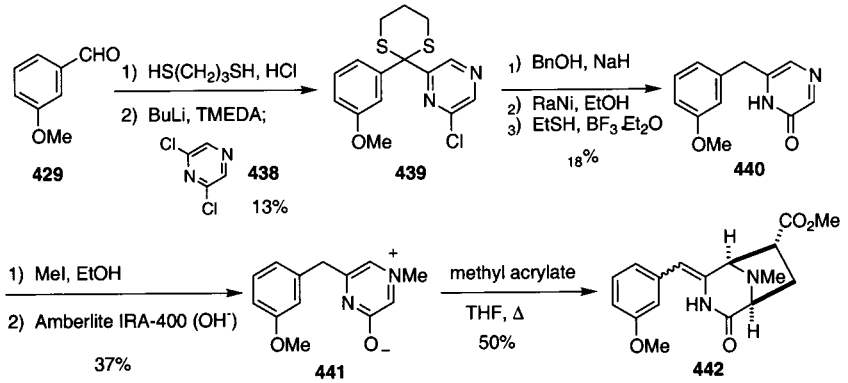
4.1.3. Synthetic Studies toward Quinocarcin

In 1987, Saito and Hirata published a synthetic approach to quinocarcin via the use of phenylalanine and a glutamic acid derivative as illustrated in Scheme 59.[138] The protected serine derivative (**419**) was converted to the glutamic acid derivative **422** using a three-step protocol in 16% overall yield. Phenylalanine (**423**) was treated with formalin to form the tetrahydroisoquinoline followed by conversion of the acid to the ethyl ester and subsequent

Scheme 60. Weinreb's Synthetic Studies on Quinocarcin



Scheme 61. Joule's Synthetic Approach to Quinocarcin

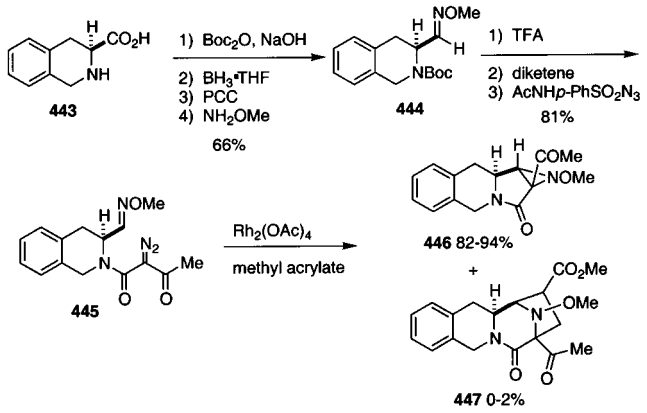


ester reduction to afford the amino alcohol **424**. Coupling with the active ester **422** followed by Swern oxidation yielded the carbinolamine **425**. Cyclization to yield the tetracyclic core was accomplished using titanium tetrachloride. Subsequent Cbz removal and *N*-methylation afforded the two diastereomers **426** and **427** in comparable yields. Saponification and decarboxylation of **427** afforded a single diastereomer. Partial reduction of the lactam was accomplished using LAH, and the resultant carbinolamine was converted to the stable aminonitrile **428** using sodium cyanide.

In 1990, Weinreb et al. reported a synthetic approach to quinocarcin using L-glutamic acid as a chiral, nonracemic starting material.[139] Starting with *m*-anisaldehyde aldehyde **429**, treatment of this substance with potassium cyanide followed by LAH afforded an amino alcohol, which was treated with phosgene to afford the oxazolidinone **430** in high yield (Scheme 60). Allylation of the carbamate nitrogen was followed by hydrolysis of the oxazolidinone and subsequent alcohol protection to afford **431**. Coupling to the acid chloride **432**, synthesized from L-glutamic acid, followed by treatment with Bredereck's reagent and hydrolysis of the enamine, yielded **433** in 90% yield. Reduction of the aldehyde tautomer afforded the *trans*-alcohol as the major product. This was followed by reduction of the activated lactam and conversion to the methoxy amine **434**. A three-step sequence was used to convert the TBS ether to the TBS enol ether **435**, which was treated with $BF_3 \cdot Et_2O$ to provide the desired bicyclic compound, along with some loss of the MOM group. Isomerization of the allylic olefin was followed by ozonolysis to afford the *N*-formyl group, which was hydrolyzed with $NaHCO_3$ to afford **436**. Reduction of the ketone was followed by elimination of the resultant alcohol, affording a benzylic olefin. Hydrogenation under acidic conditions afforded **437** as a single diastereomer. It is important to note that **437** is very similar to intermediate **370** in Fukuyama's total synthesis.[133] It is apparent that a direct Pictet−Spengler cyclization of **437** would give poor regioselectivity, and Fukuyama obviated this problem through the use of the *p*-bromo substituent on the aromatic ring.

Using the 1,3-dipolar cyclization methodology used in the naphthyridinomycin synthetic studies, Joule et al. synthesized a similar bicyclic compound to that described in Weinreb's study.[140] As detailed in Scheme 61, conversion of the aldehyde **429** to the dithiane was followed by alkylation with 2,6-dichloropyrazine **438** to afford **439**. Nucleophilic substitution with benzyl alcohol was followed by debenzylation and desulfurization to afford **440**. Quaternization of the

Scheme 62. McMills' Synthetic Studies on Quinocarcin



nitrogen followed by deprotonation yielded the dipolar species **441**. Cycloaddition using methyl acrylate afforded the bicyclic compound **442** in 50% yield.

In 1996, McMills et al. attempted to form an azomethine ylide similar to the Garner and Williams intermediates via a rhodium-catalyzed carbene cyclization (Scheme 62).[141] Conversion of commercially available **443** to the oxime **444** was accomplished via a four-step sequence in 66% overall yield. Removal of the Boc group from **444** was followed by α-diazoamide formation utilizing Davies protocol to afford **445**. Unfortunately, upon treatment of **445** with the rhodium catalyst, the desired tetracycle **447** could only be detected in very small amounts. Aziridine **446** was the major product in all attempts using both rhodium and copper catalysts.

4.1.4. Analogue Syntheses

There have been numerous quinocarcin analogues that have been synthesized over the years, and studies reported on this agent constitute the most in-depth structure−activity profile of this family of antitumor antibiotics. Kyowa Hakko Kogyo Company, Ltd., the discoverer of quinocarcin, has prepared a host of semisynthetic analogues of quinocarcin including quinone, hydroquinone, and other substituted quinocarcin derivatives.[142] Comparison of the biological activity of the ring-opened amino nitrile versus a parallel series of analogues with the fused oxazolidine ring intact was also performed.

It has been shown that treatment of quinocarcin with sodium cyanide affords DX52-1 (**448**), a stable and potent analogue (Scheme 63).[142a] Treatment of **448** with BBr_3 afforded demethyl quinocarcin **449**. Chlorination or iodination of **448** afforded the C-8-substituted analogues **450** or **451**, respectively. Treatment of the amino nitriles with concentrated hydrochloric acid effected closure of the oxazolidine ring of **450** to afford **452**. The oxazolidine ring of **451** was formed by treating this substance with silver nitrate, which afforded **453**.

Demethylation of **448** with BBr_3 followed by treatment with sodium cyanide afforded **454** (Scheme 64). Nitration yielded two regioisomers **455** and **456**. The nitro compounds **459** and **460** were then formed by methylation of the phenol with diazomethane and hydrolysis of the methyl ester to the corresponding carboxylic acids. Hydrogenation followed by protection of the resultant anilines provided **462** and **463**.

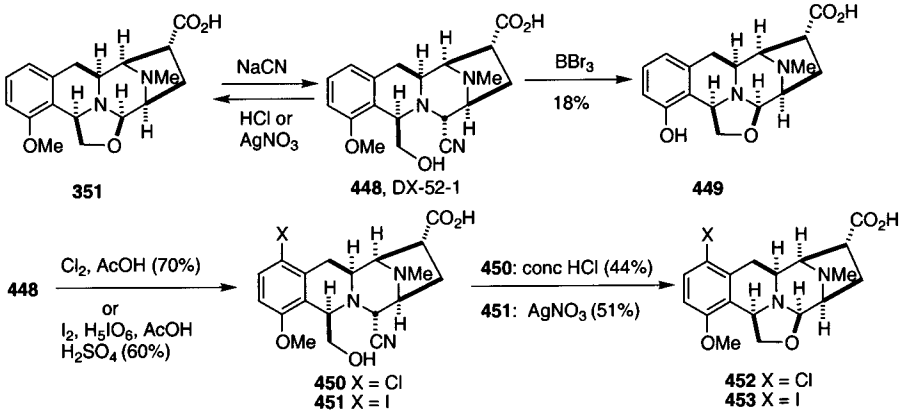
Several C-1 (quinocarcin numbering) analogues were prepared starting from DX52−1 (**448**) (Scheme 65). The formyl group was introduced using dichloromethyl methyl ether to afford **464** and **465**. Treatment with hydroxylamine hydrochloride provided oximes **466** and **467**. The C-1 cyano derivatives **468**−**470**, the phenol **471**, and the hydroxymethyl and aminomethyl (**472** and **473**, respectively) were prepared under standard conditions.

The oxidation of phenols **454**, **474**, and **475** with Fremy's salt provided quinones **476**−**478** in good yields (Scheme 66).[142b] Further A-ring functionalization afforded quinone analogues **482**−**486**.

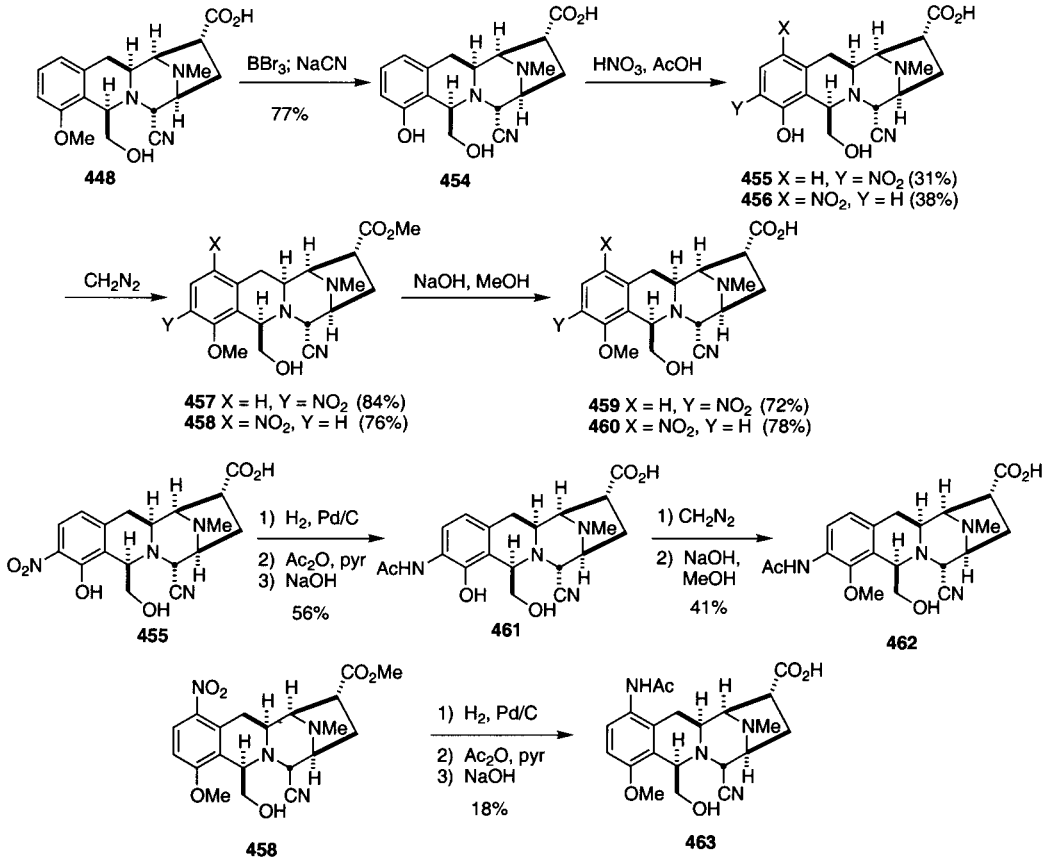
Several other quinone-containing analogues were synthesized via quinone substitution (Scheme 67). Dimethylaniline derivatives **487** and **488** were synthesized via copper-catalyzed addition of dimethylamine to **476**. Copper-catalyzed addition of methanol to **478** afforded **489** in 39% yield and subsequent treatment of **478** with other nucleophiles afforded **492** and **494**−**496**.

Several sulfide-substituted quinones were synthesized from quinone **476** via the addition of various mercaptans followed by reoxidation using Fremy's salt to afford the dithio quinones **497**−**502** (Scheme 68).[142c] Oxazolidine ring formation was accomplished by treatment of the amino nitriles with silver nitrate

Scheme 63. Quinocarcin C-1 Analogs



Scheme 64. C-1 and C-3 Analogs of Quinocarcin



to afford **503**−**506**. The methoxy sulfide-substituted quinones **507**−**512** were synthesized using similar chemistry starting from quinone **489**.

Some of the quinones prepared as described above were hydrogenated to afford the corresponding dihydroquinones **513**−**520** in high yields (Scheme 69).

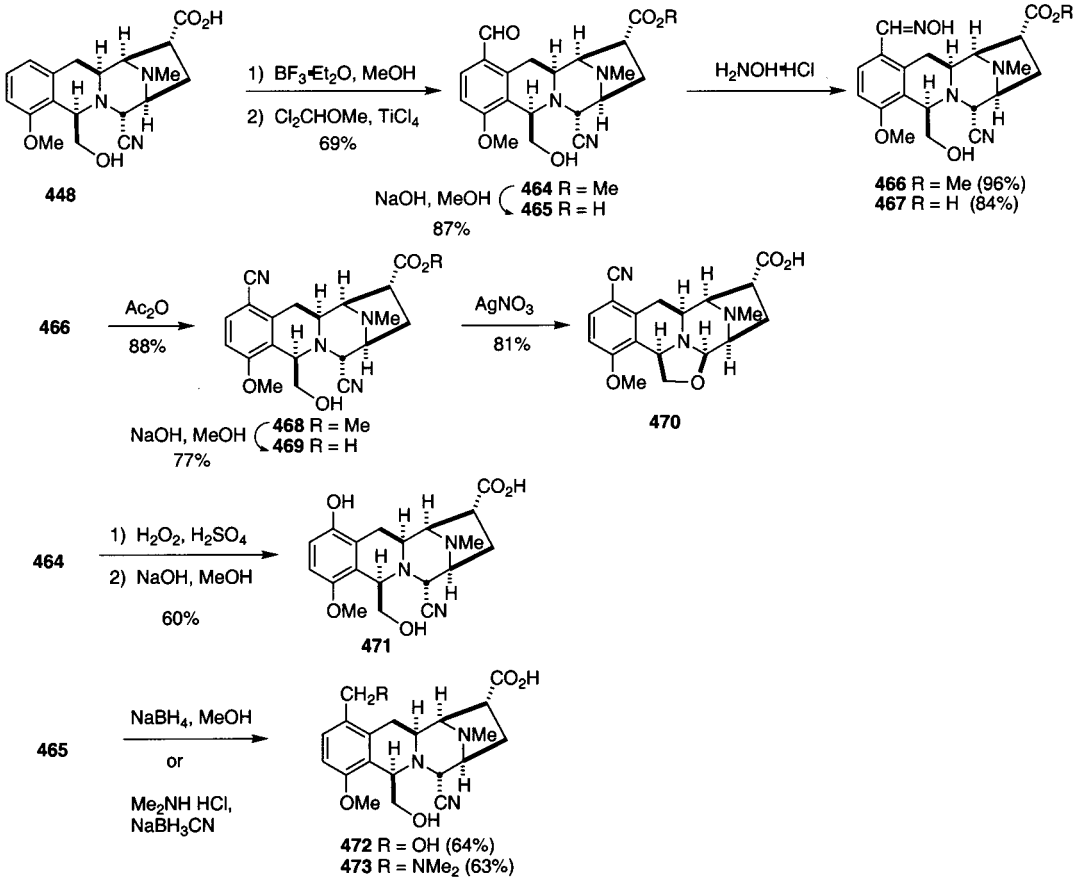
In an effort to probe the importance of the relative stereochemistry of the quinocarcin ring structure, two diastereomeric tetracyclic analogues were synthesized by Williams et al., as shown in Scheme 70.[143] Starting with tricyclic compound **410** (see Scheme 58), the ethyl ester was saponified followed by treatment with thionyl chloride in refluxing benzene to afford the corresponding α,*β*-unsaturated acid chloride. Treatment of this substance with the 2-methyl-2-*N*-methyl propanol afforded **521**. Hydrogenation of the benzylic olefin afforded a mixture of diastereomers **522** and **523** in a 2.4:1, *syn:anti* ratio, and each diastereomer was carried on separately. Reduction of the amide with diborane was followed by Swern oxidation of the primary alcohol to an aldehyde. Basic hydrolysis of the oxazolidinone effected ring closure of the incipient amino alcohol upon the aldehyde to afford the crystalline tetracycle analogues **524** and **525**. The relative stereochemistry of each compound was firmly established by X-ray crystallographic analysis.

Compounds **524** and **525** proved to be sparingly water-soluble, making an in-depth evaluation of their biochemical and biological activity relative to the freely water-soluble natural product problematic as will be discussed below. Thus, a more water-soluble tetracyclic analogue of quinocarcin was synthesized as detailed in Scheme 71.[144] Allylic alcohol **411** was converted to the allylic chloride and treated with 2-amino-2-methylpropanol to afford the allylic amine **526** in moderate yield. Hydrogenation of the benzylic olefin afforded a single *syn*-diastereomer. Oxidation of the alcohol was followed by treatment in refluxing lithium hydroxide to afford the tetracyclic secondary amine **527**. Alkylation of the secondary amine with ethyl bromoacetate and saponification of the ester afforded the water-soluble analogue **528**.

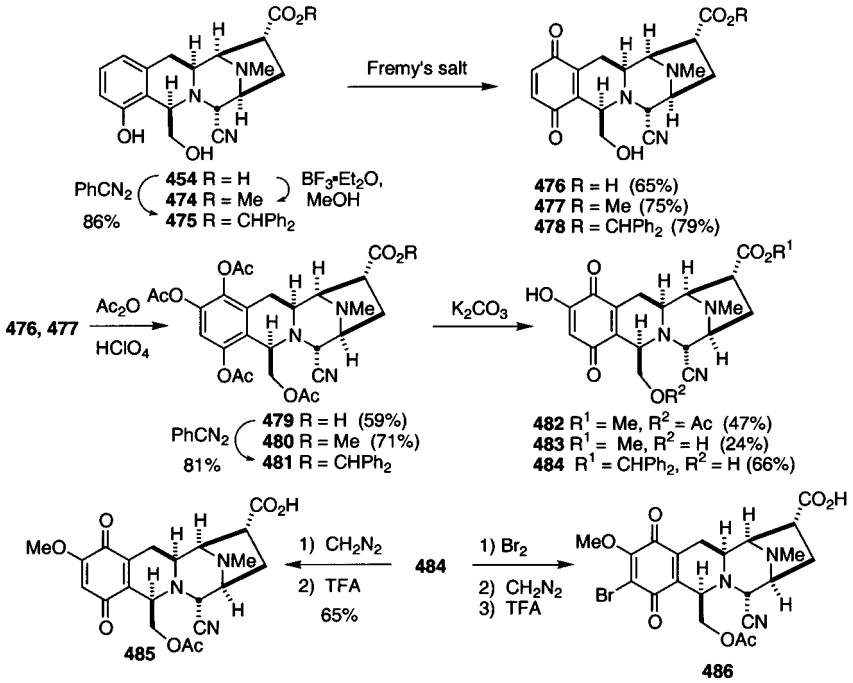
Tetracycle **528** was activated as the corresponding *p*-nitrophenyl ester (**529**) and coupled to the netropsin-like side chain (**530**), a well-known DNA-binding moiety, to afford the water-soluble netropsin conjugate **531** (Scheme 72). In addition, these workers prepared a symmetrical dimer by coupling spermine to 2 equiv of **528** to afford **532**.

The synthesis of the C-11a epimer of **528** was achieved in a diastereoselective manner as shown in Scheme 73.[145] Elimination of the hydroxyl group of **410** afforded α,*β*-unsaturated ester **533**. Hydrogenation yielded a mixture of diastereomers; however, saponification of the ester yielded only a single diastereomer **534**. Reduction of the acid was followed by activation of the resultant alcohol (**535**) for displacement with 2-amino-2-methyl propanol to provide amino alcohol **536**. Amine alkylation followed by Dess−Martin periodinane oxidation provided **537** in 77% yield from **536**. Finally, oxazolidinone ring opening and cyclization afforded the *anti*-quinocarcin analogue **539** in 67% yield.

Scheme 65. C-1 Analogs of Quinocarcin



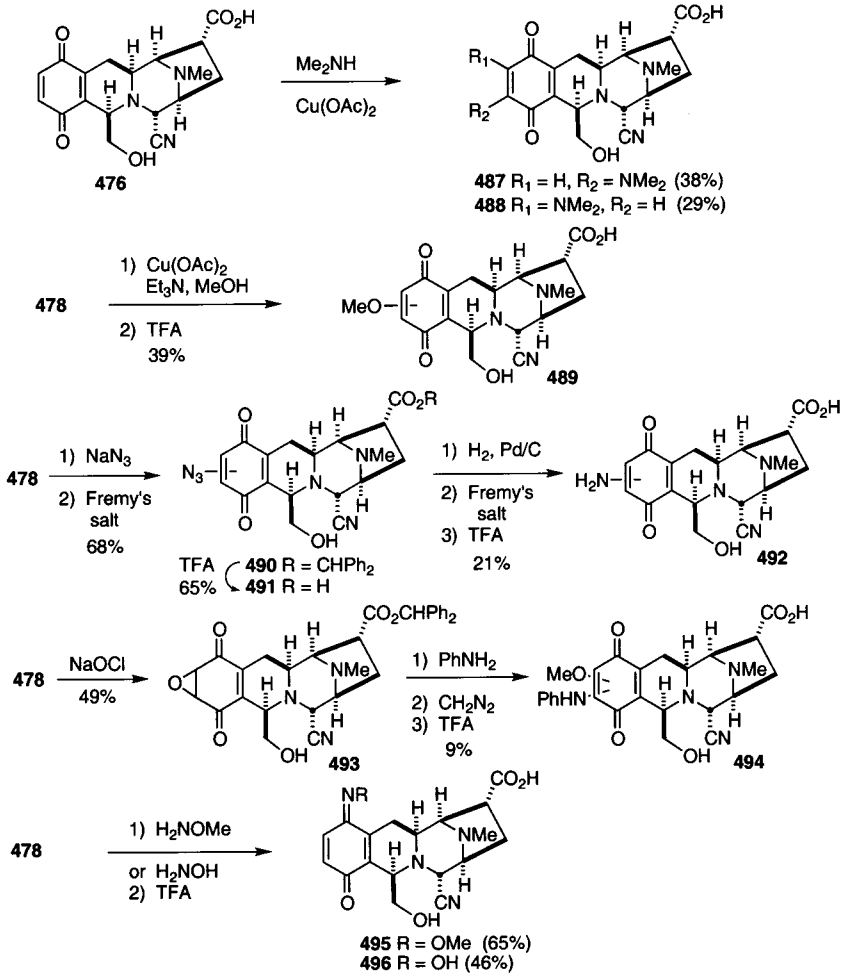
Scheme 66. Quinone Analogs of Quinocarcin



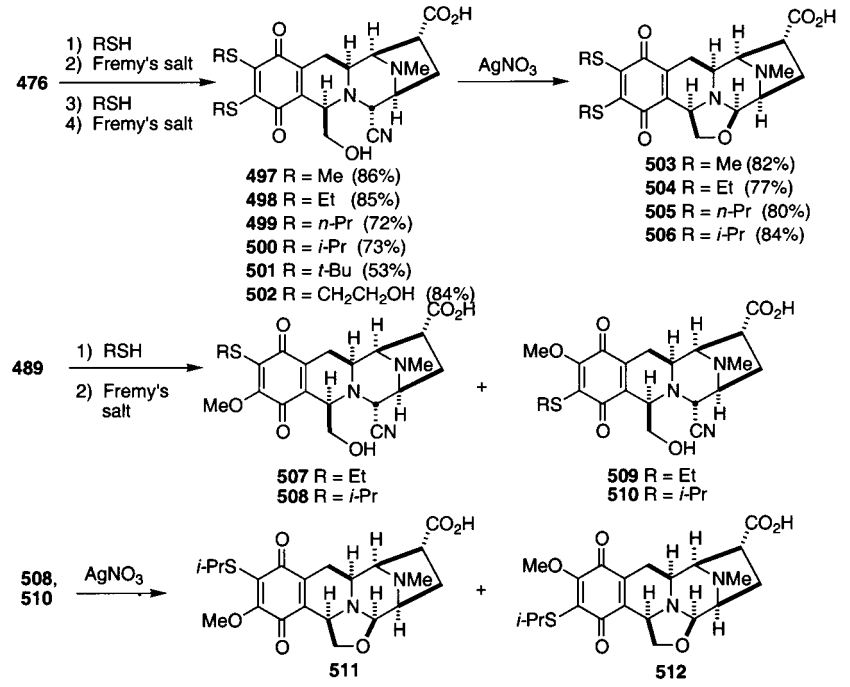
As in the case of the *syn*-analogue **528**, the *anti*-analogue **539** was coupled to netropsin to afford **541** as outlined in Scheme 74. Additionally, **539** was demethylated using BBr_3 to afford the phenol **542.**

Terashima et al. synthesized several analogues of quinocarcin including some simple ABE-ring analogues (Schemes 75 and 76).[135a] Arene **397** was functionalized at the benzylic position to yield **543** (Scheme 75) followed by alkylation to afford **544**. Removal of the trifluoroacetylamine protecting group effected cyclization on the benzylic ketone, and the resultant imine was reduced with sodium cyanoborohydride followed by reprotection of the amine to afford tetrahydroisoquinoline **545**. Oxidative cleavage

Scheme 67. Substituted Quinone Analogs of Quinocarcin



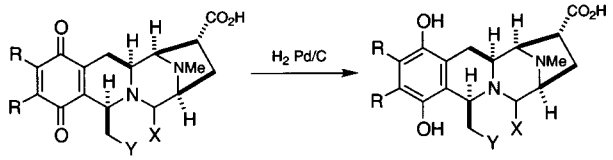
Scheme 68. Thiol-Substituted Quinone Analogs of Quinocarcin



of the diol afforded aldehyde **546** in 94% yield, which was reduced followed by oxazolidine formation to afford the tricyclic substance **547**.

Tricyclic compounds with six- and seven-membered E-rings (**549** and **551**, respectively) were also synthesized using similar chemistry (Scheme 76).

Scheme 69. Thiol-Substituted Hydroquinone Analogs of Quinocarcin



compound	R	X	Y	yield
513	H	OH	CN	29%
514	MeS	OH	CN	97%
515	EtS	OH	CN	79%
516	iPrS	OH	CN	100%
517	MeS	−O−		80%
518	EtO_2CCH_2S	OH	CN	100%
519	$HOCH_2CH_2S$	OH	CN	99%
520	EtS	OH	CN	100%

Several D-ring derivatives of quinocarcin were also synthesized by Terashima et al. as shown in Scheme 77.[135e] The carboxylic acid moieties of **405** and **552** were converted into the corresponding mixed anhydrides and reduced to afford the corresponding primary alcohols **553** and **554**, respectively. Compound **553** was deacylated and converted to the oxazolidine (**556**), which is the C-13 alcohol derivative of quinocarcin. Analogue **557** was prepared simply by acetylation of **553**, and the C-13-fluoro analogue **558** was prepared by treating **553** with DAST.

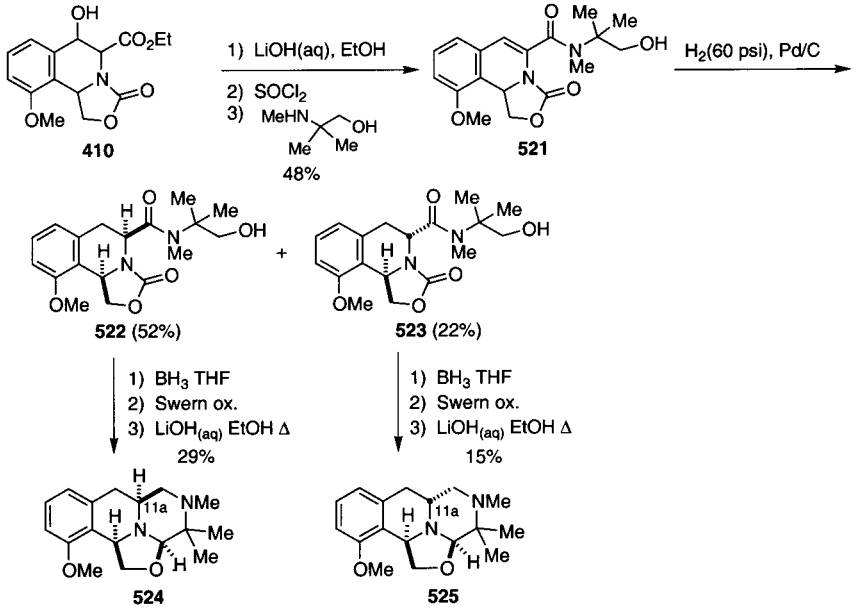
In the next section, the biochemical and biological activity of many of the quinocarcin analogues described above will be reviewed. An attempt will be made to provide mechanistic insight and speculation where appropriate.

4.1.5. Biological Activity

Quinocarcin has moderate activity against Gram-(+) bacteria such as *Staphylococcus aureus*, *Bacillus subtitlis*, and *Klebsiella pneumoniae* with MIC's of 12.5, 12.5, and 25 μg/mL, respectively.[129a] Quinocarcin has been shown to inhibit [^{3}H]-thymidine incorporation in *Bacillus subtilis*, and this was found to be due to inhibition of DNA polymerase and is also a manifestation of oxidative DNA cleavage.[146] No effect was seen on RNA or protein synthesis. Quinocarcinol had no activity against either Gram-(+) or Gram-(−) bacteria.

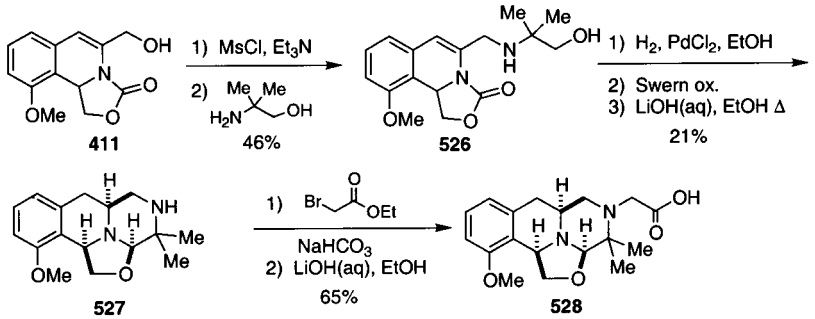
Quinocarcin as its citrate salt (named quinocarmycin citrate or KW2152), which was much more stable than free quinocarcin, has shown potent antitumor activity against several tumor cell lines including St-4 gastric carcinoma, Co-3 human colon carcinoma, MX-1 human mammary carcinoma, M5075 sarcoma, B16 melanoma, and P388 leukemia.[147] Quinocarcin citrate has also shown good activity against lung carcinoma cell lines that are resistant to either mitomycin C or cisplatin.[148] In P388 leuke-

Scheme 70. Williams' Synthesis of Tetracyclic syn- and *anti*-Analogues of Quinocarcin



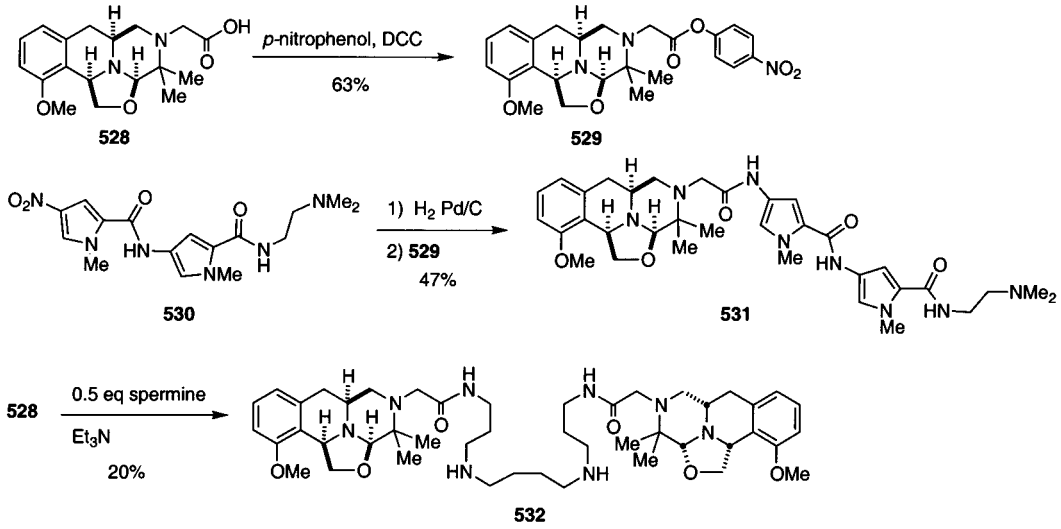
524 **525**

Scheme 71. Williams' Synthesis of a Water-Soluble Quinocarcin Analog

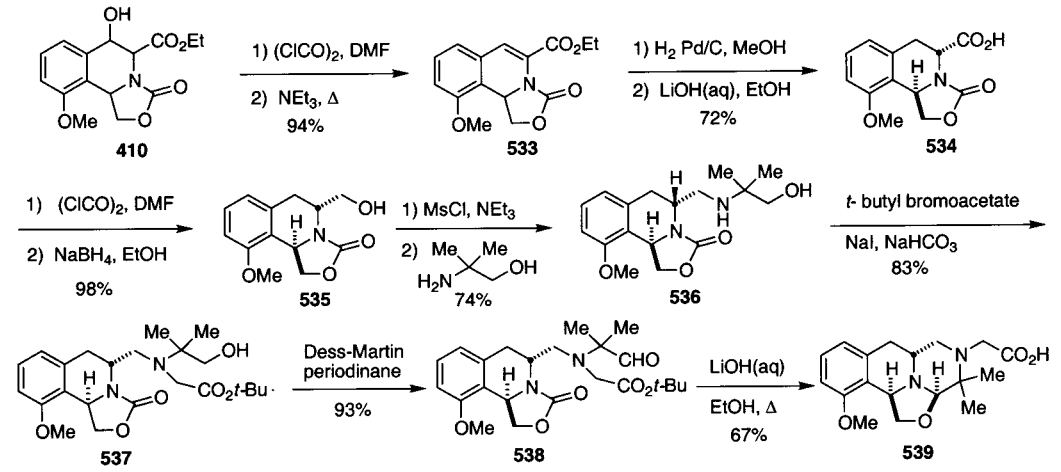


527 **528**

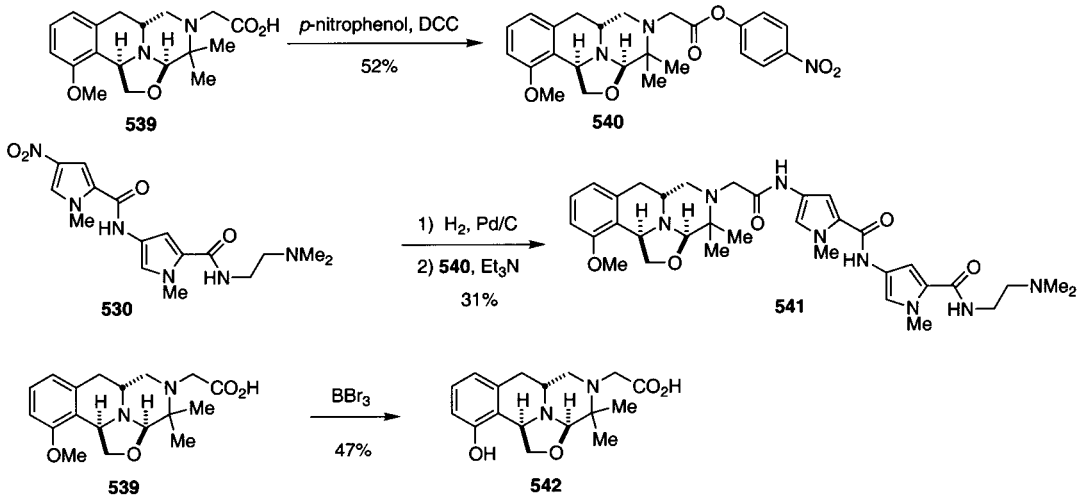
Scheme 72. Williams' Synthesis of Netropsin and Spermine Analogs 531 and 532



Scheme 73. Williams' Synthesis of *anti*-Quinocarcin Analog 539



Scheme 74. Williams' Synthesis of the *anti*-Netropsin and Phenolic Quinocarcin Analogs

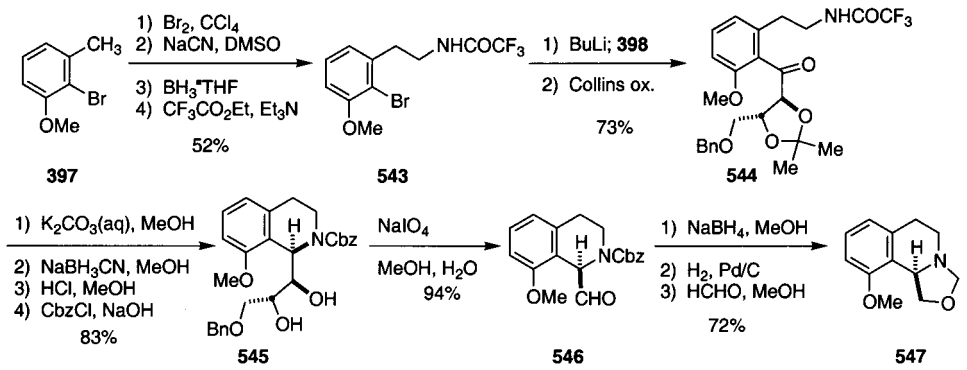


mia, quinocarcin was shown to inhibit RNA synthesis over DNA and protein synthesis.

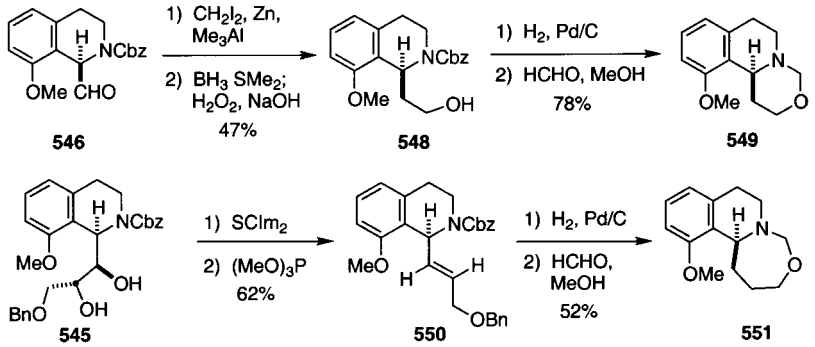
Quinocarcin citrate and DX-52−1 (**448**) were assayed by the National Cancer Institute in a screen of 60 tumor cell lines.[149] Both showed promising activity with DX-52-1 showing excellent activity against several melanoma cell lines. Quinocarcin citrate had been in clinical trials in Japan, but due to liver toxicity, the trials were discontinued. DX-52-1 does not display the toxicities associated with quinocarcin.

Quinocarcin has been reported to mediate oxidative cleavage of DNA and was found to be due to the formation of superoxide.[146,150] The addition of super-

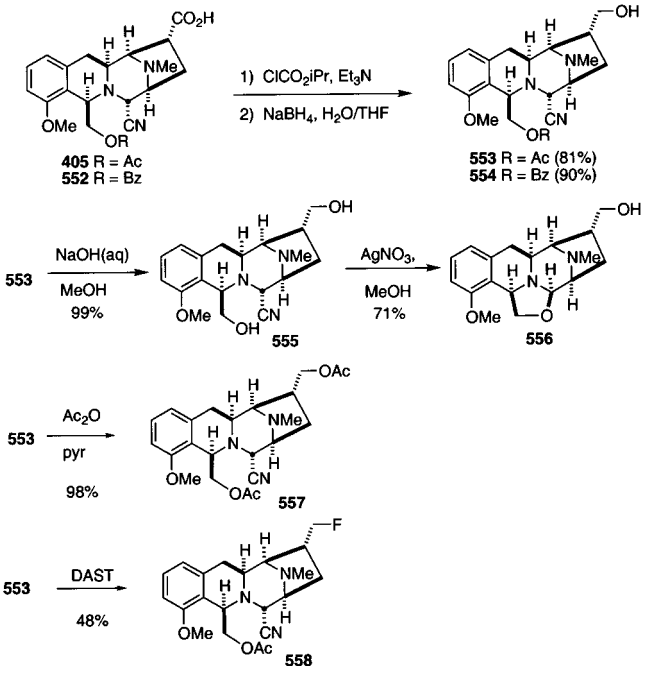
Scheme 75. Terashima's Synthesis of an ABE-Ring Analog of Quinocarcin



Scheme 76. Terashima's Synthesis of ABE-Ring Analogs of Quinocarcin



Scheme 77. Terashima's Semisynthesis of C-10 Analogs of Quinocarcin



oxide dismutase (SOD) inhibited DNA cleavage by quinocarcin, while the addition of DTT (dithiothreitol) enhanced DNA cleavage. Since the structure of quinocarcin is distinct from other known antitumor antibiotics that mediate the formation of superoxide, such as quinones, thiols, etc., the mechanism by which this drug mediates superoxide formation and DNA damage was not apparent and has been the subject of intense study.

In 1992, Williams et al. reported a study concerning the mechanism of superoxide formation by quinocarcin and quinocarcin analogues.[150] These workers note that since quinocarcin can exist in two distinct conformers (Figure 16), the two sets of tetracyclic quinocarcin analogues **524**/**528** and **525**/**539** were synthesized to study the stereoelectronic effect of the stereochemistry at the oxazolidine nitrogen atom. As illustrated above, these analogues were epimers at C-11a, mandating that the nonbonded pair of electrons on the oxazolidine nitrogen adopt an *anti*-configuration with respect to the methine hydrogen at C-7 for **524** and **528** and a *syn*-relationship in analogues **525** and **539**. The former arrangement mimics that for the natural product in the lowest energy conformation (shown, Figure 16), and the latter arrangement mimics the higher energy conformation calculated for quinocarcin (see Remers, below).

It was found that the *syn*-analogues **524** and **528** mediated superoxide production at rates comparable to that for quinocarcin, but the *anti*-analogues **525** and **539** were dormant in aerated water. This observation was explained by the fact that the *anti*-analogues assume a conformation in which the nonbonded electron pair at nitrogen is disposed *trans*-antiperiplanar to the methine hydrogen of the oxazolidine ring (Figure 16), allowing for the formation of a carbon-centered oxazolidinyl radical. These workers propose that this stereoelectronic arrangement is obligatory for the concomitant loss of the methine proton and a single nonbonded electron from nitrogen to form the oxazolidinyl radical. In contrast, the *syn*-analogues do not form a corresponding oxazolidinyl radical due to *syn*-clinal arrangement of

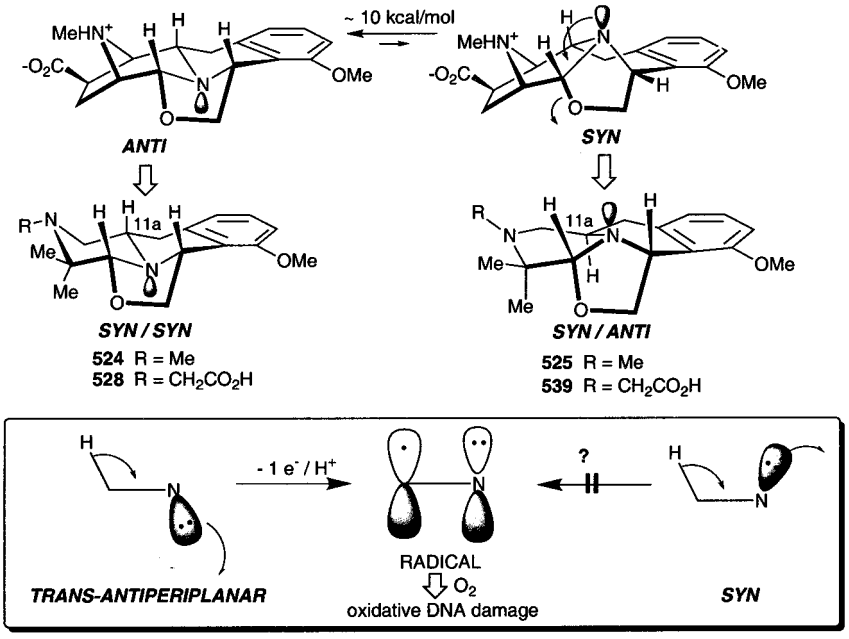


Figure 16. Importance of the stereochemistry at nitrogen of quinocarcin and analogues.

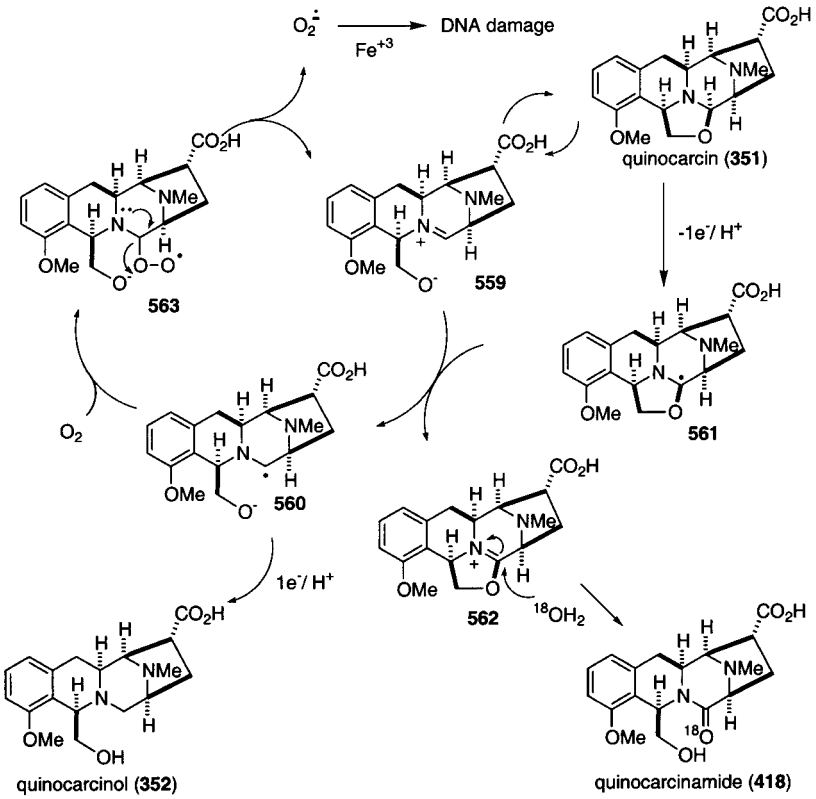
the nonbonded electron pair on nitrogen relative to the methine hydrogen. These workers performed extensive kinetic and mechanistic studies on this new reaction and found that the rate of superoxide formation for quinocarcin is well below (10^4-10^5 times slower) the rate-limiting step in the Fenton/Haber–Weiss cycle. This observation helps to explain the unusual observation that addition of either Fe(II) or Fe(III) does not enhance (or inhibit) DNA cleavage. Addition of the iron-chelator desferal had little effect on inhibiting oxidative DNA cleavage by quinocarcin at low concentrations but started to display inhibitory activity at very high concentrations (>10 mM). The authors postulated that due to the large chasm in the kinetics of superoxide production by quinocarcin versus the rate-limiting step in the Fenton/Haber–Weiss cycle (the Fenton reaction is the slow step with a rate = 76 M^{-1} s^{-1}), desferal only competes with DNA as a hydrocarbon substrate at high concentration for available oxidant and is not effectively sequestering trace metal from the sphere of the reaction. It was also observed that additives such as picolinic acid, a known hydroxyl radical scavenger, significantly inhibit DNA cleavage by quinocarcin. Finally, gel electrophoresis studies of drug-damaged DNA revealed a doublet at each nucleotide residue which is consistent with a diffusable (Fenton-derived) oxidant such as hydroxyl radical. A portion of their data is presented in Table 10.

Table 10. Effect of Additives on Cleavage of Supercoiled Plasmid DNA by Quinocarcin and Analogs

substrate	conditions	% inhibition	% enhancement
quinocarcin (1 mM)	10 μg/mL SOD	99	
quinocarcin (1 mM)	100 μg/mL catalase	83	
quinocarcin (1 mM)	0.1 mM H_2O_2		143
528 (1 mM)	10 μg/mL SOD	85	
528 (1 mM)	100 μg/mL catalase	65	
528 (1 mM)	0.1 mM H_2O_2		19
531 (0.2 mM)	10 μg/mL SOD	0	0
531 (0.2 mM)	100 μg/mL catalase	3	
531 (0.2 mM)	0.1 mM H_2O_2		95
532 (0.2 mM)	10 μg/mL SOD	83	
532 (0.2 mM)	100 μg/mL catalase	32	
532 (0.2 mM)	0.1 mM H_2O_2		289

Williams et al. proposed a unifying mechanism for superoxide formation that was primarily based on the redox self-disproportionation of quinocarcin that these workers discovered.[150] When quinocarcin was allowed to stand in deoxygenated pH = 7 water at 25 °C, two new products were obtained. One product was identified as quinocarcinol (**352**), a reduction product, and the other was identified and named quinocarcinamide (**418**), an oxidation product as illustrated in Scheme 78. Since the oxazolidine moiety of quinocarcin is a masked aldehyde, this reaction is similar to the well-known Cannizarro disproportionation reaction. However, unlike the Cannizarro reaction, which is believed to be a heterolytic, two-electron process, these workers invoked a single-electron-transfer process. Thus, transfer of a single, nonbonded electron from the oxazolidinyl nitrogen atom with concomitant proton loss to the ring-opened "no-bond tautomer" **559** would furnish radical anion **560** and oxazolidinyl radical **561**. Radical **561** would be capable of reducing another equivalent of **559** to afford the oxazolidinium species **562**, which would be captured by solvent water to afford quinocarcinamide (**418**). Radical anion **560** would undergo a second electron transfer with concomitant protonation to afford quinocarcinol (**352**). Under aerobic conditions, radical anion **560** and/or **561** would react with molecular oxygen to produce the peroxy radical **563** (from **560**), which would expel superoxide regenerating **559**. It has been demonstrated that superoxide alone does not cause strand scission of DNA and requires the presence of adven-

Scheme 78. Williams' Proposed Mechanism for Superoxide Formation via the Self-Redox Disproportionation Cycle for Quinocarcin



titious transition metals, such as iron or copper, to mediate oxidative strand scission of DNA. Thus, the superoxide produced goes through Fenton/Haber–Weiss cycling in the presence of adventitious Fe^{3+} leading to the formation of reactive oxygen radical species, such as hydroxyl radical, culminating in oxidative strand scission of DNA.

Surprisingly, netropsin analogue **531**, which did cause oxygen-dependent DNA cleavage, was not inhibited by SOD or catalase.[144] Also, the DNA cleavage patterns were not random, indicating a nondiffusable hydrogen atom abstractor. It was postulated that a carbon-centered oxazolidinyl radical derived from **531** or a peroxy radical derived from this species and molecular oxygen may directly abstract a hydrogen atom from drug-bound DNA causing the observed specific DNA cleavage pattern. Molecular modeling and DNA cleavage product analysis support this proposal. These workers employed a 516 base pair restriction fragment from pBR 322 to evaluate the unusual biochemical reactivity of **531**. To fully elucidate the pattern of DNA damage exhibited by compound **531**, the 516 base pair substrate was also labeled (5′-^{32}P) in an analogous manner to that used for the 3′-labeling process. Having the 516 base pair substrate labeled on either the 3′- and 5′-ends allowed for the determination of cleavage activity occurring on each individual strand of the double helix. Quinocarcin and spermine dimer **532** both gave rise to sequence-random cleavage at every nucleotide residue on this substrate, consistent with the production of a *non*-DNA-bound *diffusable* oxidant. The netropsin conjugate (**531**), however, exhibited a definite sequence-selective DNA cleavage pattern (Figure 17, histogram). The highest frequency of cleavage occurred around the $^{5'}$d(ATTT/TAAA)$^{3'}$. The actual sites of cleavage, however, were primarily two bases to the 3′-end of this four base recognition site. There was also evidence from the histograms (highlighted in boxes, Figure 17) that in some cases the drug may be able to bind in two orientations, which would also affect cleavage two bases to the 3′ end of the $^{5'}$d-(ATTT/TAAA)$^{3'}$ recognition sequence. Such an observation is not unexpected since it has been shown that many of the distamycin peptides actually bind in two orientations in the minor groove in a 1:1 or 2:1 drug to DNA ratio.[140] These workers proposed that it is therefore possible that proper orientation of **531** as dictated by the netropsin moiety might position a drug-centered radical in the proper geometry to abstract a hydrogen atom from the phosphoribose backbone that in the presence of oxygen would ultimately result in oxidative scission of the DNA. Experimental support for this hypothesis is presented Table 10, which shows that DNA damage caused by compound **531** was not readily inhibited by the addition of SOD and catalase; this situation is in marked contrast to that for quinocarcin and other members of this family of antitumor antibiotics.

In 1988, Remers et al. reported a molecular modeling study on the binding of quinocarcin to DNA.[151] By analogy to the mechanism of iminium formation described for the saframycins, the structurally related quinocarcin iminium species (**564**) was docked into the minor groove of DNA and several conformations were investigated (Scheme 79).

This study revealed that the original, arbitrarily assigned absolute stereochemistry of quinocarcin

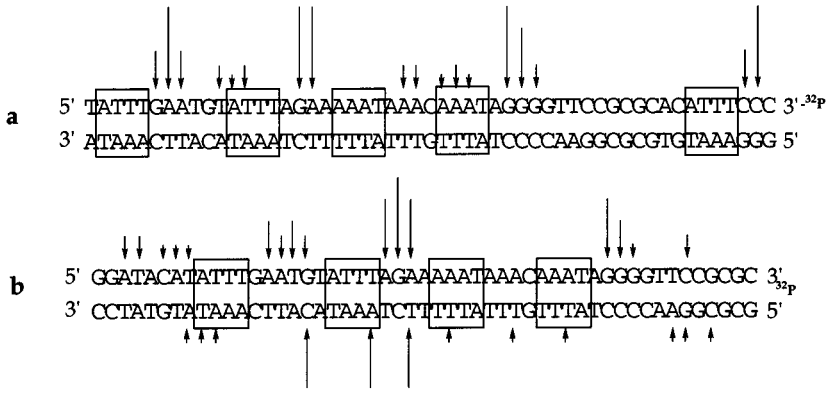


Figure 17. Histograms from gels in ref 140 depicting the selective DNA cleavage exhibited by **531**. (a) Histogram from Figure 6a, lane 5, ref 140. (b) Histogram from Figure 6b, lanes 5 and 6, ref 140. Histograms were prepared by measurements of the relative intensities of DNA bands from the autoradiograms. The length of the arrows approximate the relative intensities of the bands by scanning densitometry.

Scheme 79. Remers' Proposed DNA Alkylation by Quinocarcin

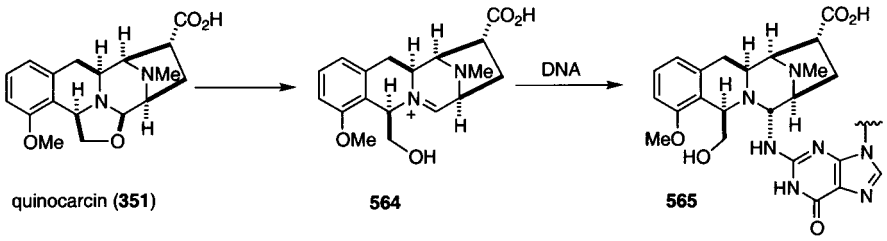
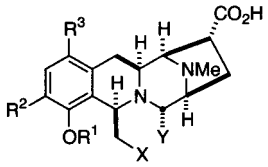


Table 11. In Vivo Studies for A-Ring Analogs of Quinocarcin[a]



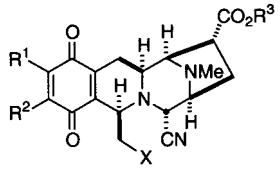
analog	R^1	R^2	R^3	X	Y	$HeLaS_3$ IC_{50}(μg/mL)	dose (mg/kg) x1 (P388)	ILS (%)	(R)
448	Me	H	H	OH	CN	0.05	20	26	0.59
449	H	H	H	−O−	−O−	3.03	6.25	14	0.35
454	H	H	H	OH	CN	5.32	3.13	18	0.43
450	Me	H	Cl	OH	CN	0.042	12.5	23	0.79
451	Me	H	Cl	−O−	−O−	0.04	12.5	40	0.93
452	Me	H	I	OH	CN	0.11	50	31	1.15
453	Me	H	I	−O−	−O−	0.04	25	24	0.56
455	H	NO_2	H	OH	CN	0.47	5	17	0.33
459	Me	NO_2	H	OH	CN	0.43	NT		
460	Me	H	NO_2	OH	CN	0.99	100	27	
461	H	NHAc	H	OH	CN	>10	NT		
462	Me	NHAc	H	OH	CN	2.76	NT		
463	Me	H	NHAc	OH	CN	>10	NT		
465	Me	H	CHO	OH	CN	0.56	200	38	0.95
467	Me	H	CH=NOH	OH	CN	1.1	200	38	0.68
469	Me	H	CN	OH	CN	0.3	25	40	0.74
470	Me	H	CN	−O−	−O−	0.51	20	22	0.67
351						0.05−0.11	10−20	27−56	1

[a] ILS = increase life span, (R) = ILS (analog)/ILS (quinocarcin), NT = not tested.

would be a poor DNA alkylating agent. The opposite absolute stereochemistry was suggested based on better DNA binding energies, and this was later confirmed by Garner through the asymmetric synthesis of (−)-quinocarcin as described above (Scheme 56).

Williams et al. originally suggested that the *syn*-quinocarcin analogue **539** would be expected to alkylate DNA in accordance with the modeling studies reported by Remers. However, DNA alkylation by compound **539** was not observed with this compound, the corresponding netropsin conjugate (**541**), nor the phenol analogue (**542**).[126] Two possible reasons were given for this observation. First, the *gem*-dimethyl group necessary for stability of the oxazolidine ring may be too sterically bulky to allow for the exocyclic amino group of a guanine residue of the DNA to achieve the transition state geometry for alkylation

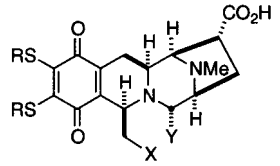
Table 12. In Vivo Studies for Quinone Analogs of Quinocarcin[a]



analog	R^1	R^2	R^3	X	HeLaS_3 IC_{50}(μg/mL)	dose (mg/kg) x1 (P388)	ILS(%)
476	H	H	H	OH	>10	NT	
471	H	H	Me	OH	0.12	20	18
482	OH	H	Me	OAc	>10	100	14
483	OH	H	Me	OH	>10	NT	
485	OMe	H	H	OAc	>10	25	22
486	OMe	Br	H	OAc	NT	9.38	20
487	H	NMe_2	H	OH	0.92	6.25	12
488	NMe_2	H	H	OH	0.79	3.13	15
491	N_3	H	H	OH	>10	3.13	2
492	NH_2	H	H	OH	>10	1.56	4
494	PhNH	OMe	H	OH	1.75	100	17
351					0.05−0.11	10−20	26
448					0.05	20	27−56

[a] ILS = increase life span, NT = not tested.

Table 13. In Vivo Studies of Dithiol-Substituted Quinone Analogs of Quinocarcin[a]



analog	R	X	Y	HeLaS_3 IC_{50}(μg/mL)	dose (mg/kg) x1 (P388	ILS (%)	(R)
497	Me	OH	CN	0.13	12.5	53	0.59
498	Et	OH	CN	0.11	12.5	50	0.42
499	n-Pr	OH	CN	0.05	25	56	0.48
500	i-Pr	OH	CN	0.012	25	65	1.35
501	t-Bu	OH	CN	0.004	25	48	0.50
502	$HOCH_2CH_2$	OH	CN	2.47	12.5	26	
503	Me	−O−	−O−	0.019	6.25	48	0.88
504	Et	−O−	−O−	0.08	6.25	64	0.93
505	n-Pr	−O−	−O−	0.03	12.5	58	0.98
506	i-Pr	−O−	−O−	0.0019	6.25	69	1.11
351				0.05−0.11	10−20	27−56	1

[a] ILS = increase life span, (R) = ILS (analog)/ILS (quinocarcin).

to proceed. A second more plausible possibility was that the alkylation may be reversible due to displacement of the DNA by the *trans*-antiperiplanar oxazolidinylamine lone pair.

The biological activities of the semisynthetic quinocarcin analogues prepared at the Kyowa Hakko Kogyo Company[142] are listed in Tables 11−15. All of these semisynthetic analogues were tested in vitro against the HeLa S_3 cell line along with in vivo studies against P388 leukemia.

The biological activities of the A-ring quinocarcin analogues are listed in Table 11.[142a] As can be seen from these data, analogues bearing the oxazolidine ring intact increase biological activity (cf., **451** versus **450** and **453** versus **452**), but the presence of the free phenolic group in place of the methoxy substituent lowers the activity.

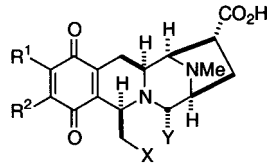
The quinone analogues, for the most part, showed reduced biological activities relative to that for quinocarcin (Table 12).[142b] The unsubstituted (**462**) and diamino (**473**) derivatives showed the best activities.

Surprisingly, the thioalkyl quinones showed some increased activity over that for quinocarcin (Table 13). Compounds **485** and **490** showed good activity in vitro, while **484** showed good activity in vivo.

Table 14 shows the activities for the mixed substituted quinone analogues.[142c] Once again, the oxazolidine-containing compounds display superior activity over that for the E-ring-opened congeners, and this phenomenon also held true with the corresponding hydroquinone derivatives as illustrated with the data presented in Table 15.

The analogues synthesized by Terashima et al. showed interesting biological activities, and some of these compounds displayed increased biological activity over that for quinocarcin against P388 murine leukemia (Table 16).[135e,f] Analogue **554** had the best activity by far, exhibiting approximately 2 orders of magnitude higher potency than quinocarcin (**351**).

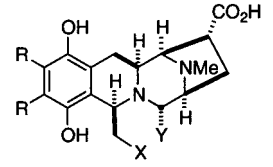
Table 14. In Vivo Studies of Thiol-Substituted Quinone Analogs of Quinocarcin[a]



analog	R^1	R^2	X	Y	HeLaS$_3$ IC_{50}(μg/mL)	dose (mg/kg) x1 (P388)	ILS (%)	(R)
507	EtS	MeO	OH	CN	2.42	6.25	29	0.67
509	MeO	EtS	OH	CN	2.88	25	31	0.72
508	i-PrS	MeO	OH	CN	1.12	12.5	21	0.58
510	MeO	i-PrS	OH	CN	0.56	12.5	17	
511	i-PrS	MeO	−O−	−O−	0.79	6.25	31	1.29
512	MeO	i-PrS	−O−	−O−	2.37	6.25	30	1.25
351					0.05−0.11	10−20	24−48	1

[a] ILS = increase life span, (R) = ILS (analog)/ILS (quinocarcin).

Table 15. In Vivo Studies of Thiol-Substituted Hydroquinone Analogs of Quinocarcin[a]



analog	R	X	Y	HeLaS$_3$ IC_{50}(μg/mL)	dose (mg/kg) x1 (P388)	ILS (%)	(R)
513	H	OH	CN	6.10	12.5	23	
514	MeS	OH	CN	0.09	6.25	47	1.09
515	EtS	OH	CN	<0.03	NT		
516	i-PrS	OH	CN	<0.03	12.5	51	1.00
517	MeS	−O−	−O−	0.13	12.5	65	1.51
518	EtO_2CCH_2S	OH	CN	>10	200	37	0.90
519	$HOCH_2CH_2S$	OH	CN	3.24	6.25	18	0.44
351				0.05−0.11	20	41−51	1

[a] ILS = increase life span, (R) = ILS (analog)/ILS (quinocarcin), NT = not tested.

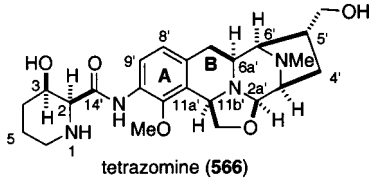
Table 16. In Vitro Toxicity of Quinocarcin Analogs against P388 Murine Leukemia

analog	IC_{50} (μg/Ml)	analog	IC_{50} (μg/Ml)
351	3.3×10^{-2}	**554**	1.0×10^{-5}
448	3.3×10^{-2}	**555**	3.2×10^{-3}
547	4.5	**556**	7.2×10^{-3}
549	0.66	**557**	1.4×10^{-3}
551	0.68	**558**	1.6×10^{-2}
553	3.4×10^{-3}		

4.2. Tetrazomine

4.2.1. Isolation and Structure Determination

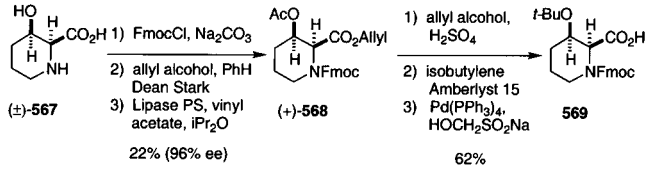
In 1991, Suzuki et al. at the Yamanouchi Pharmaceutical Company in Japan reported the isolation of tetrazomine (**566**) from *Saccharothrix mutabilis* subsp. *chichijimaensis*.[152] The structure was determined by NMR spectroscopy and relied heavily on 2D techniques.[153] The structure of tetrazomine is very similar to that of quinocarcin with respect to the pentacyclic core, the major difference being the presence of the amine at C-10′ bearing the unusual amino acid 3-hydroxy pipecolic acid, which is unique to tetrazomine. The relative and absolute stereochemistry of tetrazomine were not determined by the Yamanouchi group. The relative and absolute stereochemistry of the 3-hydroxy pipecolic acid moiety



tetrazomine (**566**)

Figure 18. Structure of tetrazomine.

Scheme 80. Williams' Synthesis of Protected *β*-Hydroxypipecolic Acid 569

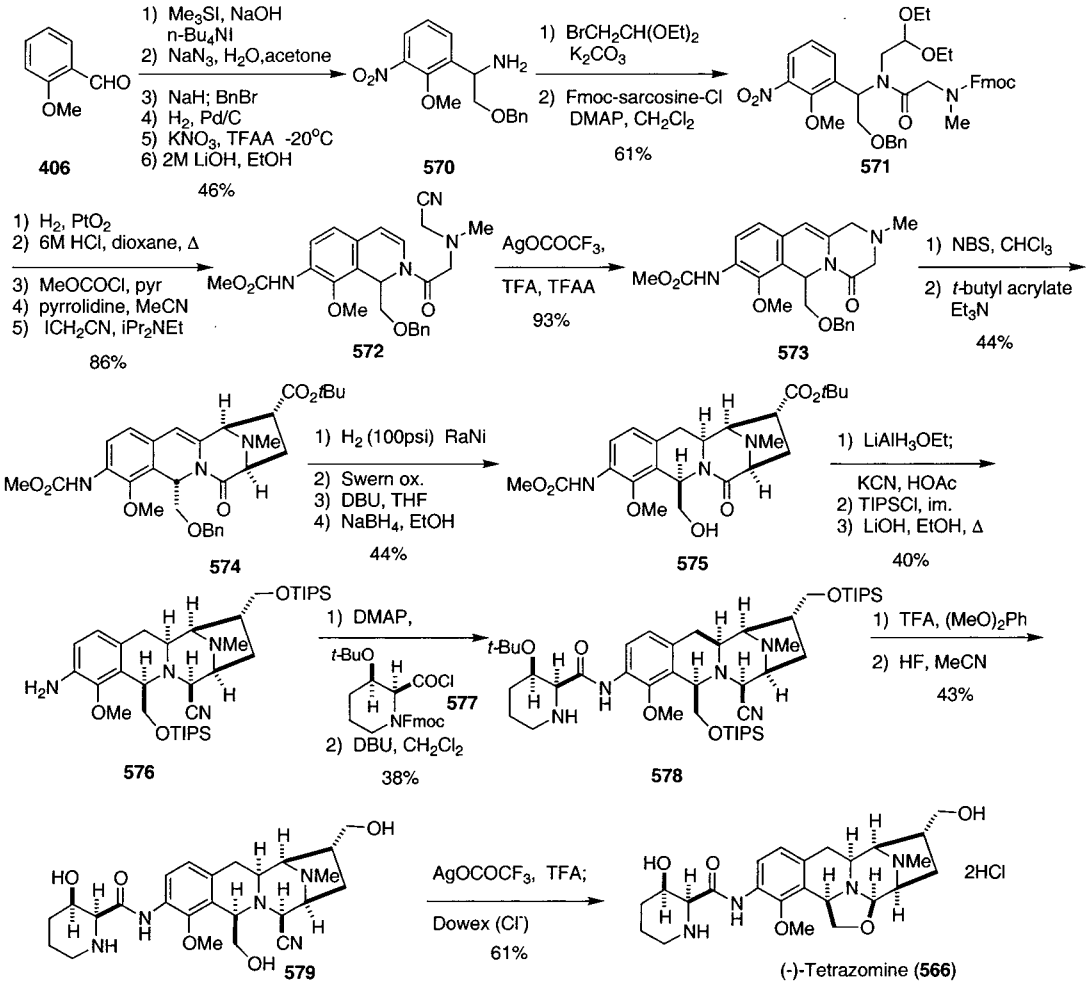


was determined by Williams et al. in 1998 as described below.[154] The total synthesis of (−)-tetrazomine by Scott and Williams in 2001 secured both the relative and absolute stereochemistry of the natural product and is that depicted in Figure 18.[155]

4.2.2. Total Synthesis of Tetrazomine

The only total synthesis of (−)-tetrazomine reported to date was that accomplished by Scott and Williams in 2001. Their synthesis featured an intermolecular 1,3-dipolar cycloadditon reaction that was

Scheme 81. Williams' Total Synthesis of (−)-Tetrazomine



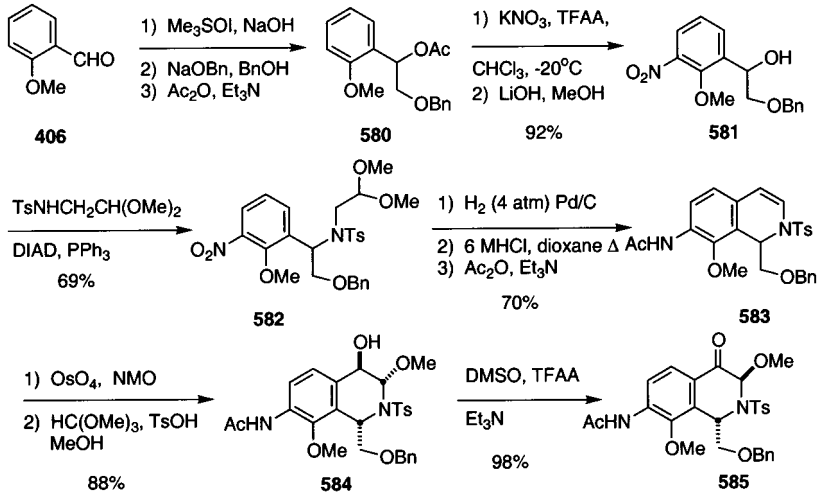
similar to that used in the quinocarcin synthesis reported by Williams and Flanagan.[136]

The synthesis of the protected optically active *cis*-*β*-hydroxy pipecolic acid **569** was accomplished via a Lipase PS-catalyzed resolution of the racemate **567** (Scheme 80).[156]

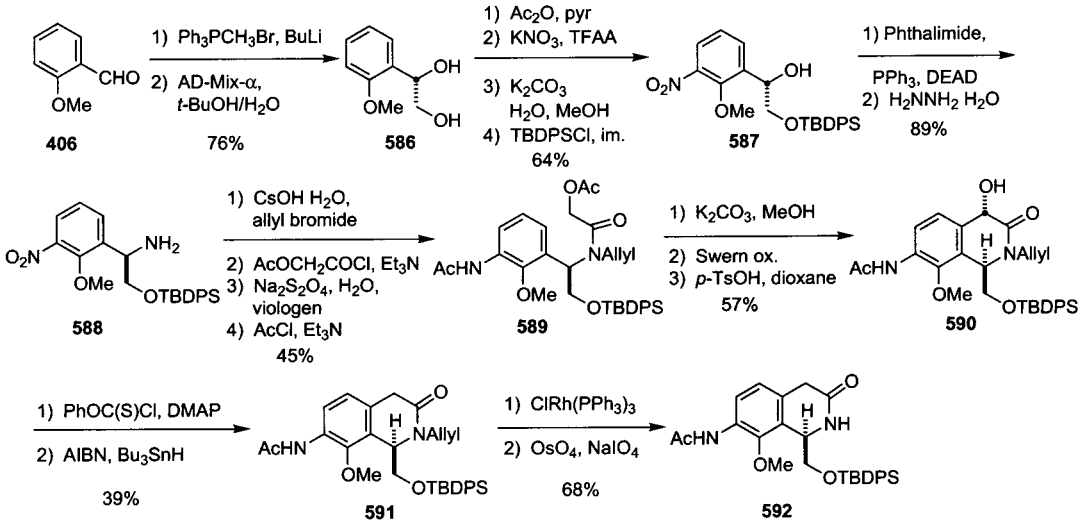
The synthesis of the tetrahydroisoquinoline core of tetrazomine started with *o*-anisaldehyde (**406**) as shown in Scheme 81. Treatment of *o*-anisaldehyde with trimethylsulfonium iodide under phase-transfer conditions provided the epoxide, which was subjected to regioselective ring opening with sodium azide. The resultant azido−alcohol was protected as the benzyl ether and the azide reduced to the primary amine. Aromatic nitration using the low-temperature conditions of Kaufman[157] afforded the desired *ortho*-nitration product with respect to the methoxy group. Hydrolysis of the resultant trifluoroacetamide yielded **570** in 49% overall yield. Alkylation of the amine with bromoacetaldehyde dimethyl acetal followed by coupling of *N*-Fmoc-sarcosine acid chloride to the secondary amine afforded **571**. Hydrogenation of the nitro group using platinum(IV) oxide was followed by acid-promoted cyclization and finally aniline protection to provide the bicyclic core **572** in 86% yield from **571**. Cleavage of the Fmoc group was followed by alkylation of the resulting amine, yielding the amino nitrile **572**. Cyclization using silver trifluoroacetate in the presence of TFAA and TFA afforded the tricycle **573** in high yield. Treatment of allylic amine **573** with NBS in refluxing chloroform yielded a dark green solution of the corresponding iminium ion species, which upon deprotonation with triethylamine afforded the dark blue azomethine ylide that was trapped by *tert*-butyl acrylate to afford a 3.9:1 mixture of separable cycloadducts **574** and a C-11b′ epimer, respectively. The major product from the cycloaddition (**574**) possessed the undesired configuration at C-11b′ as determined by ^{1}H NMR nOe analysis, and an epimerization at C-11b′ was thus executed.

Tetracycle **574** was hydrogenated in the presence of Raney-nickel at moderate pressure which effected removal of the benzyl group and concomitant reduction of the benzylic olefin from the least hindered face. The resultant alcohol was subjected to Swern oxidation conditions to afford the corresponding aldehyde, which was treated with DBU to afford a 1.4:1 mixture of epimers at C-11b with the desired isomer being predominant. These aldehydes were easily separated by column chromatography, allowing for recycling of the undesired epimer. Sodium borohydride reduction of the desired epimer afforded alcohol **575**. The simultaneous reduction of the *tert*-butyl ester and partial reduction of the amide were fortuitously accomplished in a single step using $LiAlH_3$-

Scheme 82. Kaufman's Synthesis of the AB-Ring System of Tetrazomine



Scheme 83. Wipf's Synthesis of an Optically Active AB-Ring System of Tetrazomine



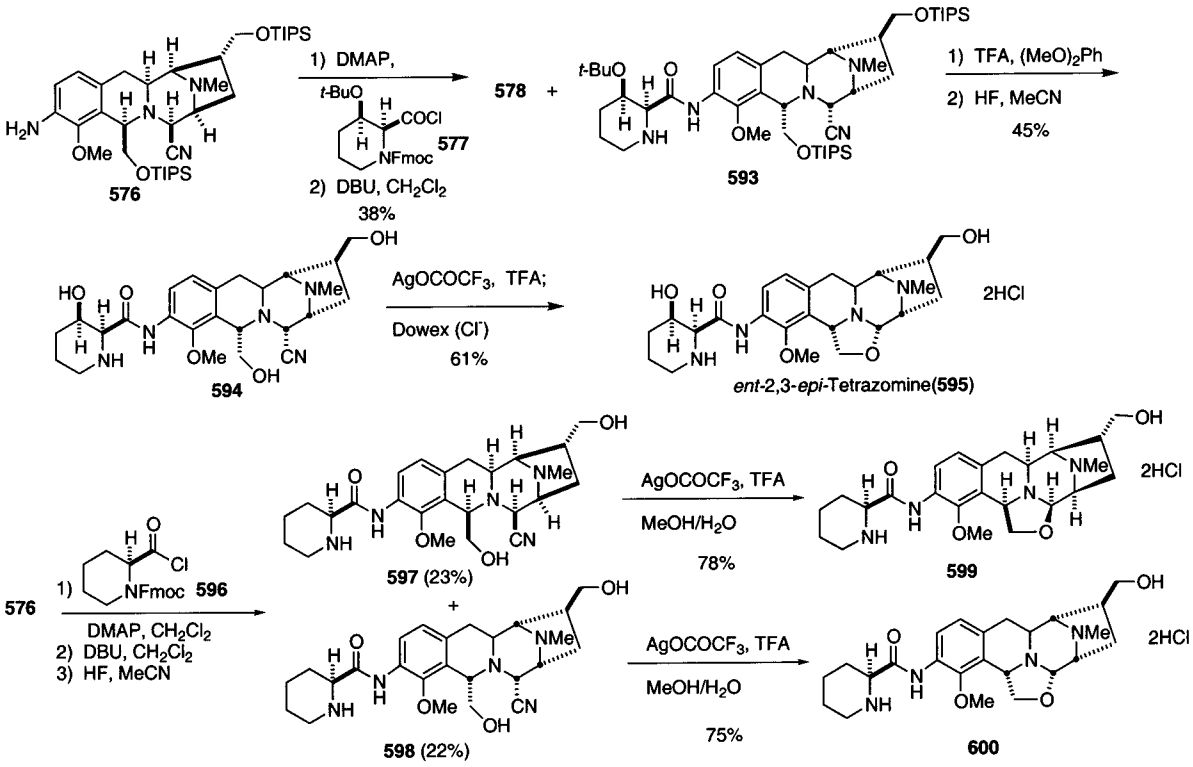
OEt in THF at 0 °C. The resultant carbinolamine was trapped with sodium cyanide under acidic conditions to afford the corresponding stable amino nitrile. The two primary alcohols were protected as their triisopropylsilyl ethers, and the methyl carbamate was hydrolyzed to afford aniline **576**. The optically active acid chloride **577** was prepared from **569** using oxalyl chloride and was coupled to **576** in the presence of DMAP to afford the corresponding pipecolamide (plus a separable diastereomer constituted with the *ent*-tetrahydroisoquinoline portion; obtained as a 1:1 mixture of optically active diastereomers), which was treated with DBU to cleave the Fmoc group furnishing **578**. Cleavage of the *tert*-butyl ether and the TIPS groups afforded 2a′-cyanotetrazomiol **579**. The final step to tetrazomine was the closure of the oxazolidine ring using silver trifluoroacetate in the presence of TFA to afford (−)-tetrazomine (**566**), thus confirming the relative and absolute stereochemistry.

4.2.3. Synthetic Studies toward Tetrazomine

Ponzo and Kaufman reported the synthesis of the AB-ring system of tetrazomine via an acid-catalyzed intramolecular Friedel−Crafts cyclization.[157] Starting with *o*-anisaldehyde (**406**), the epoxide was formed followed by selective opening with the sodium salt of benzyl alcohol and acetylation to afford **580** (Scheme 82). Selective nitration at low temperature afforded the desired regioisomer **581** selectively in high yield. A Mitsunobu reaction installed the desired benzylic amino functionality to afford **570** in 69% yield. Reduction of the nitro group was followed by intramolecular Friedel−Crafts cyclization under acidic conditions to afford the dihydroisoquinoline **583** following acylation of the aniline. Dihydroxylation of **583** was followed by methanolysis to afford **584**. The final step in this synthetic study involved Swern oxidation of the benzylic alcohol to afford the ketone **585**.

In 2001, Wipf and Hopkins reported the enantioselective synthesis of the AB-ring of tetrazomine.[158] This was accomplished via a Sharpless asymmetric dihydroxylation of 2-methoxy styrene to afford diol **586** (Scheme 83). Acylation of the diols followed by low-temperature nitration was followed by cleavage of the acetates and protection of the primary alcohol as the silyl ether **587**. A Mitsunobu inversion using phthalimide was followed by treatment with hydrazine to provide the amine **588**. Monoallylation of the amine was followed by acylation of the secondary

Scheme 84. Williams' Synthesis of Tetrazomine Analogs



amine to provide the amide. Reduction of the nitro group and acetate protection afforded **589** in 45% overall yield from **588**. Cleavage of the acetate followed by Swern oxidation afforded the aldehyde, which underwent a Friedel−Crafts hydroxyalkylation in the presence of *p*-toluenesulfonic acid to afford the bicycle **590**. Barton−McCombie deoxygenation provided the lactam **591** in which the allyl group was removed to afford **592**. Future plans called for the formation of the tetracyclic core via an intramolecular Heck cyclization.

4.2.4. Analogue Syntheses

Tetrazomine analogues were synthesized by Scott and Williams that had the enantiomer of the core tetrahydroisoquinoline nucleus of tetrazomine along with four 3-deoxy (pipecolic acid) analogues (Scheme 84).[155b] The coupling of the racemic aniline **576** with the optically active acid **577** (see Scheme 81) afforded **578** along with the diastereomer **593** which was carried on to *ent*-2,3-*epi*-tetrazomine **595**. The 3-deoxy tetrazomine analogues were synthesized in a similar fashion by coupling the protected L-pipecolic acid **596** followed by deprotection to afford the aminonitrile diastereomers **597** and **598**. Oxazolidine formation under the standard conditions afforded 3-deoxy tetrazomine **599** and *ent*-3-deoxy-2-*epi*-tetrazomine **600**.

4.2.5. Biological Activity

Tetrazomine has been shown to be active against both Gram-(+) and Gram-(−) bacteria as illustrated in Table 17.[133] The MIC's range from 0.78 to 25 μg/mL for Gram-(+) organisms and from 0.78 to 50 μg/mL for Gram-(−) organisms.

Tetrazomine has also been shown to be active against P388 leukemia and L1210 leukemia with IC_{50} values of 0.014 and 0.0427 μg/mL, respectively.[133] An in vivo study showed that tetrazomine has activity against P388 leukemia (Table 18). The optimal dose for tetrazomine was found to be 0.05 mg/kg, which yielded a T/C (treated vs control) of 173%.

Williams et al. showed that tetrazomine, like quinocarcin, undergoes a self-redox reaction to produce superoxide that can cleave DNA in a nonspecific manner.[159] The mechanism is essentially the same as that suggested for quinocarcin (Scheme 78) and

Table 17. Antimicrobial Activities of Tetrazomine

test organisms	MIC (μg/mL)
Bacillus subtilis ATCC 6633	6.25
Staphylococcus aureus FDA 209P JC-1	6.25
Staphylococcus epidermidis IID 866	25
Streptococcus pyogenes Cook	0.78
Enterococcus faecalis IID 682	6.25
Enterococcus faecium CAY 09−1	3.13
Mycobacterium smegmatis ATCC 607	12.5
E. coli NIHJ	1.56
Citrobacter freundii CAY 17−1	0.78
Klebsiella pneumoniae ATCC 10031	3.13
Proteus vulgaris OXK US	3.13
Pseudomonas aeruginosa NCTC 10490	6.25
Pseudomonas aerginosa ATCC 8689	50

Table 18. In Vivo Biological Activity of Tetrazomine against P388 Leukemia

antibiotic	dose (mg/kg/day)	MST (days)	T/C	survival (40 days)
Tetrazomine	0.0125 × 7 ip	11.0	100	0/8
	0.025	14.0	127	0/8
	0.05	19.0	173	0/8
	0.1	9.0	82	0/8
Mitomycin C	0.5 × 5 ip	27.0	245	2/8
	1.0	24.5	223	2/8
control		11.0	100	0/8

Scheme 85. Mechanism of Superoxide Formation Mediated by Tetrazomine

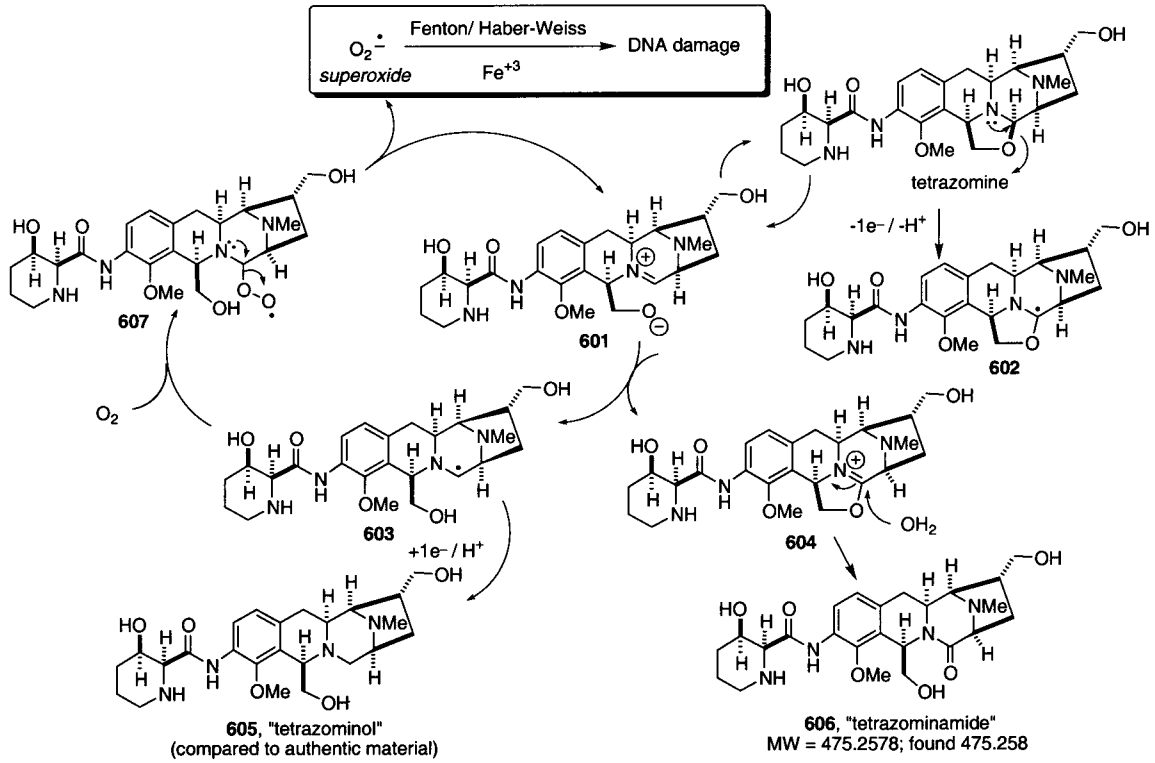


Table 19. Rates of Superoxide Formation for Bioxalomycin α_2, Tetrazomine, Quinocarcin, and Analogs

substratejl	concentration (mM)	pH	rate ($M\ s^{-1} \times 10^{-9}$)
bioxalomycin α_2	0.1	6.0	7.59
bioxalomycin α_2	0.1	7.0	38.8
bioxalomycin α_2	0.1	8.0	553
tetrazomine	1.0	6.0	2.46
tetrazomine	1.0	7.0	10.6
tetrazomine	1.0	8.0	17.5
tetrazomine + 10 mg/mL SOD	1.0	8.0	0
quinocarcin	1.0	8.0	1.1
524	1.0	8.0	0.41
525	1.0	8.0	0
DX-52−1 (**448**)	1.0	8.0	0

is shown in Scheme 85. These workers found that tetrazomine spontaneously disproportionated above neutral pH to give the Cannizarro-type products that were named tetrazominol (**605**) and tetrazominamide (**606**). Natural tetrazomine could be reduced to tetrazominol with $NaBH_4$ furnishing an authentic specimen of **605**. The structural assignment for **606** was based primarily on the exact mass spectrum for this material. Although this disproportionation reaction was not as clean as that observed for quinocarcin, in the presence of oxygen tetrazomine produced superoxide (as evidenced by the reduction of nitroblue tetrazolium) at a significant rate as illustrated in Table 19, where rates of superoxide formation are compared to that for quinocarcin, synthetic analogues **524**, **525**, DX-52-1 (**448**), and bioxalomycin α_2. The rate of superoxide formation was found to be pH-dependent, with the highest rate occurring above pH 8. The authors ascribed this to the requirement for the oxazolidine nitrogen atom to be in an unprotonated state as required by the mechanisms proffered in Schemes 78 and 85. A comparison of the pH profiles for DNA cleavage by quinocarcin and tetrazomine is illustrated in Figure 19.[141]

The rates for superoxide formation were measured spectrophotometrically by observing the reduction of nitroblue tetrazolium to formazan. The reduction of this dye in the presence of the various drugs was found to be completely inhibited by the addition of superoxide dismutase. Bioxalomycin was found to be several orders of magnitude more potent than any other members of this family with respect to the rate of superoxide formation. While the reasons for this have not yet been mechanistically ascertained, the presence of two fused oxazolidines and the dihydroquinone, which can participate in quinone/dihydroquinone redox cycling, in the bioxalomycin structure may all contribute to this molecule having several possible mechanistic manifolds for oxygen reduction. Unfortunately, at the time of this writing, a sufficient quantity of bioxalomycin α_2 could not be obtained to study the chemistry of the auto-redox chemistry of this natural product.

A variety of additives and conditions were examined in the presence of tetrazomine to determine the percent inhibition and enhancement of DNA cleavage as shown in Table 20.[154] The addition of the superoxide scavenger superoxide dismutase (SOD) and catalase inhibited DNA cleavage. As in the case of quinocarcin, addition of Fe^{2+} and Fe^{3+} had little effect. Desferal, an iron chelator, inhibited DNA cleavage but only at exceedingly high concentrations. All of these observations are similar to those seen with quinocarcin.

Williams and Scott reported the antimicrobial activity of a series of tetrazomine analogues that

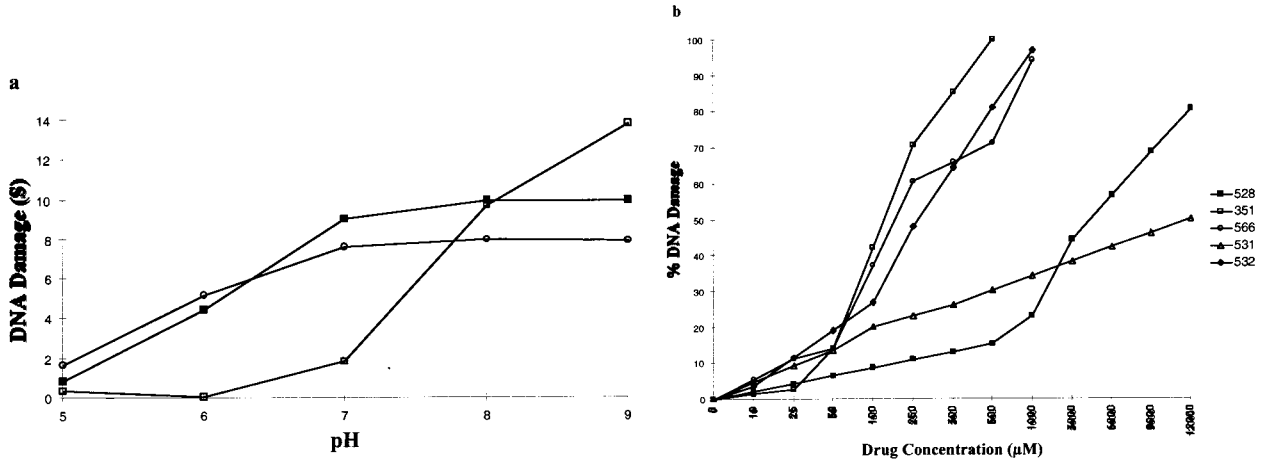


Figure 19. (a) Effects of pH on DNA cleavage (S) for **528** x 10 (○), quinocarcin (□), and tetrazomine (■). (b) Effects of concentration on DNA cleavage for **528**, quinocarcin **(351)**, tetrazomine, **531**, and **532**.[141]

Table 20. Effects of Additives on Plasmid DNA Cleavage by Tetrazomine

conditions	tetrazomine (mM)	% inhibition	% enhancement
0.1mM $Fe^{II}SO_4$	1.0	0	0
0.1mM $Fe^{III}NH_4SO_4$	1.0	5	0
0.1 mM desferal	1.0	0	0
1.0 mM desferal	1.0	37	
10 mM desferal	1.0	94	
deoxygenated	1.0	80	
0.1 mM H_2O_2	0.1		68
0.1 mM H_2O_2	1.0		29
1.0 mM picolinic acid	1.0	28	
10 mM picolinic acid	1.0	71	
10 ug/ mL catalase	1.0	55	
100 ug/mL catalase	1.0	54	
10 ug/mL SOD	1.0	94	

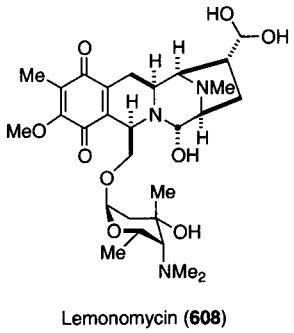
Table 21. Antimicrobial Activity of Tetrazomine Oxazolidine Analogs against *Klebsiella pneumoniae* and *Staphylococcus aureous*: R = Resistant

compound	amount (mg)	zone of inhibition Kleb (mm)	zone of inhibition Staph (mm)
566	0.2	28	12
	0.02	22	R
	0.002	10	R
595	0.12	15	R
	0.012	8	R
	0.0012	R	R
599	0.12	29	14
	0.012	21	9
	0.0012	19	R
600	0.12	24	7
	0.012	17	R
	0.0012	R	R
Penicillin G	10 units	NA	30
Streptomycin	0.01	14	NA

contained the oxazolidine ring intact (Table 21) along with the aminonitrile analogues (Table 22). Not surprisingly, the analogues that had the same absolute stereochemistry as the natural product were much more active than those analogues containing the *ent*-tetrahydroisoquinoline core. Also, the antimicrobial activities for the aminonitiriles were similar to that of the corresponding oxazolidine-containing analogues. The 3-deoxy analogues displayed slightly better antibiotic activity than those analogues that possessed the secondary alcohol at the 3-position.

Table 22. Antimicrobial Activity of Tetrazomine Aminonitrile Analogs against *Klebsiella pneumoniae* and *Staphylococcus aureous*: R = Resistant

compound	amount (mg)	zone of inhibition Kleb (mm)	zone of inhibition Staph (mm)
579	0.12	26	12
	0.012	20	R
	0.0012	16	R
594	0.12	18	R
	0.012	13	R
	0.0012	R	R
597	0.12	27	11
	0.012	23	R
	0.0012	13	R
598	0.12	16	R
	0.012	12	R
	0.0012	R	R
Penicillin G	10 units	NA	30
Streptomycin	0.01	14	NA



Lemonomycin (**608**)

Figure 20. Structure of lemonomycin.

4.3. Lemonomycin

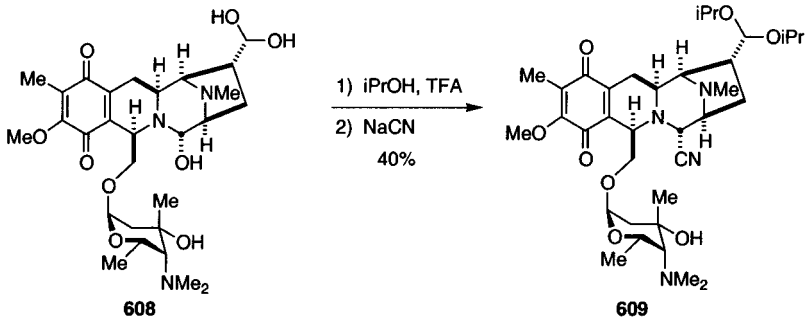
4.3.1. Isolation and Structure Determination

Lemonomycin (**608**) was isolated in 1964 from *Streptomyces candidus* (LL-AP191).[160] The structure was not determined until 2000, by He et al. via NMR spectroscopy (Figure 20).[161] Lemonomycin contains the unusual 2,6-dideoxo-4-amino sugar and is the only member in this family of tetrahydroisoquinoline antibiotics to bear a sugar residue.

4.3.2. Analogue Synthesis

Synthetic work on lemonomycin has yet to appear, but a single semisynthetic analogue has been pre-

Scheme 86. Semisynthesis of a Lemonomycin Analog



608 609

Table 23. Antimicrobial Activities of Lemonomycin

test organism	MIC (μg/mL)
Staphylococcus aureus	0.2
Bacillus subtilis	0.05
MR*Staphylococcus aureus*	0.4
Enterococcus faecium	0.2

pared as shown in Scheme 86. Treatment of lemonomycin with 2-propanol in TFA followed by sodium cyanide afforded **609** in 40% overall yield.[161]

4.3.3. Biological Activity

Lemonomycin has shown antimicrobial activity against several organisms (Table 23). Lemonomycin and the cyano analogue **609** also exhibit in vitro activity against the human colon cell line (HCT116) with IC_{50}'s of 0.36 and 0.26 μg/mL, respectively.[161]

5. Conclusion

The tetrahydroisoquinoline family of antitumor antibiotics constitutes a small yet growing and increasingly important family of chemotherapeutic agents. A diverse range of biochemical and biological activities are exhibited by this family of compounds, yet relatively little is known about the cellular biology and the interplay of cellular receptors with which these agents interact. In particular, the mode of cell death mediated by the extraordinarily potent ecteinascidins remains an unsolved and extremely important problem. A myriad of subtle structural and stereoelectronic issues have emerged as touched upon in this review, and these findings provide for an interesting mechanistic playing field for the future design and synthesis of potentially biologically significant agents. The known biochemical manifolds include (1) DNA alkylation, (2) DNA cross-linking, (3) oxidative nucleic acid damage, (4) topoisomerase inhibition, (5) superoxide formation, (6) inhibition of protein biosynthesis, and others. Nature has taken the relatively simple and innocuous tetrahydroisoquinoline ring system and endowed this simple heterocycle with a rich array of functionality and stereochemistry that has generated a bewildering manifold of biochemical and cellular reactivity.

The interesting structures manifest in this family have provided the synthetic chemist with a rich and challenging set of targets, and there is every expectation that synthetic work in this area will continue to provide the chemical community with a variety of new and interesting reactions as well as useful probes to penetrate the multiple modes of action that these agents display. It seems likely that additional members of this family of natural products will be discovered soon and will set the stage for new biochemical and biological investigations. Finally, knowledge concerning the biosynthesis of this family of compounds is not well advanced and constitutes yet another fascinating line of future investigation. The successful advancement of ecteinascidin and quinocarcin through advanced stages of human clinical trials should presage a bright future for this family of natural products, and it is hoped that this review has provided those skilled in the art with a useful guide.

6. Acknowledgment

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7. References

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