CHAPTER ONE

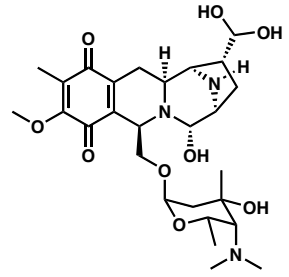
An Historical and Contextual Introduction to Lemonomycin and Cyanocycline A

1.1 The Tetrahydroisoquinoline Antitumor Antibiotics

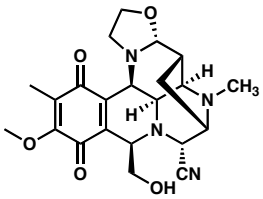
Representative Structures

Lemonomycin and cyanocycline A belong to a class of natural products known as the tetrahydroisoquinoline antitumor antibiotics.[1] These compounds are structurally characterized by a tetrahydroisoquinoline ring system fused to a diazabicyclic core with either a two- (**1**-**3**) or three-carbon (**4**-**6**) bridge (Figure 1.1). Variations of oxidation states, substitution patterns, and the presence of additional fragments distinguish several subfamilies of natural products. Of the two-carbon bridged subfamilies, lemonomycin (**1**) is distinguished by the presence of a unique glycosyl unit and an aldehyde hydrate. Cyanocycline A (**2**) and several closely related compounds exhibit an oxazolidine ring that connects the bridging carbons to the tetrahydroisoquinoline ring system. Quinocarcin (**3**) is characterized by an anisole ring in place of the typical quinone and a carboxylic acid attached to the two-carbon bridge. Among the subfamilies with a three-carbon bridge, the saframycins (e.g., saframycin A **4**) are bisquinone-containing compounds with a glyoxamide side chain. The renieramycins (e.g., renieramycin C **5**) manifest an angelate ester in place of the glyoxamide unit. The ecteinascidins (e.g., ecteinascidin 743 **6**) present a bridging thioether ring and an additional tetrahydroisoquinoline unit, as well as oxygenated aromatic rings in place of the typical quinones.

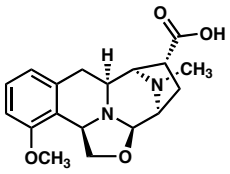
Figure 1.1 Representative Tetrahydroisoquinoline Antitumor Antibiotics



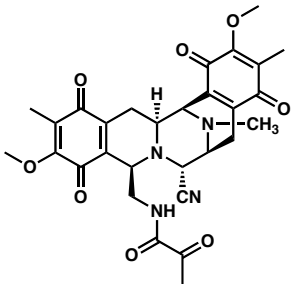
Lemonomycin (1)



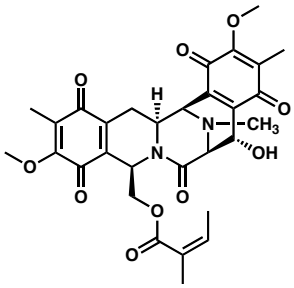
Cyanocycline A (2)



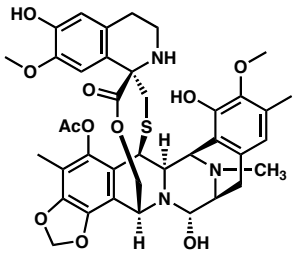
Quinocarcin (3)



Saframycin A (4)



Renieramycin C (5)

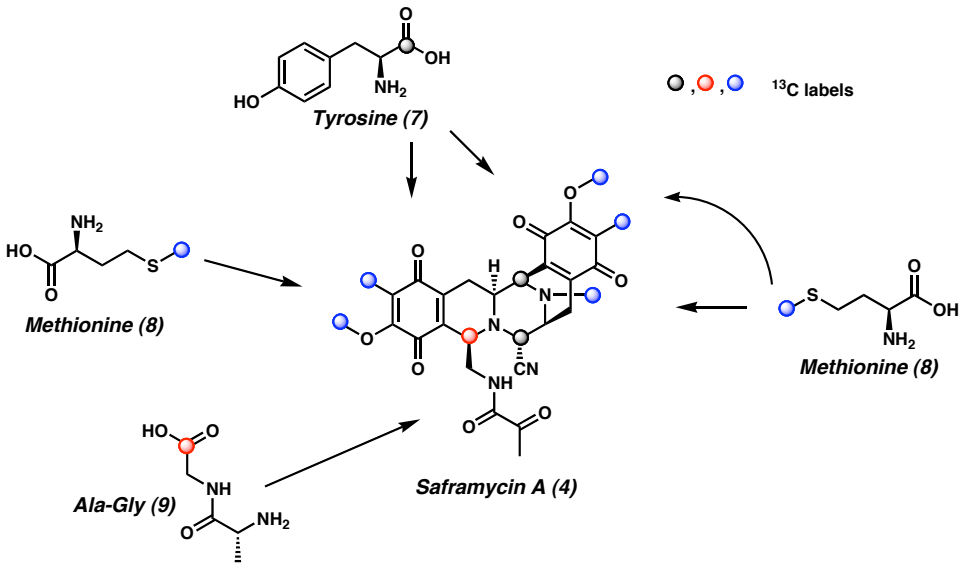


Ecteinascidin 743 (6)

Tetrahydroisoquinoline Biosynthesis

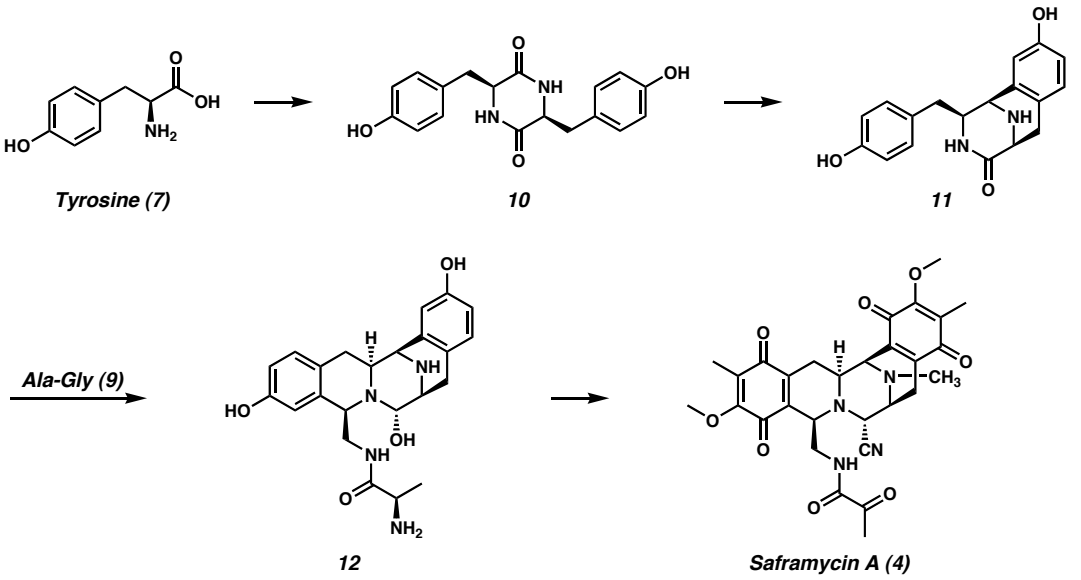
The tetrahydroisoquinoline antitumor antibiotics are biosynthetically derived from amino acids. The most intensely studied biosynthetic pathways are those leading to saframycin A and naphthyridinomycin (*vide infra*). The biosynthetic origins of saframycin A have been elucidated by the use of isotopically labeled substrates (Figure 1.2).[2] In this manner it has been determined that the central piperazine core and the two quinone rings are derived from two molecules of tyrosine (**7**), while the side chain and the remaining carbon of the tetrahydroisoquinoline system begin as the dipeptide Ala-Gly (**9**). The four methyl groups on the quinones and the *N*-methyl group arise from the *S*-methyl of methionine (**8**).

Figure 1.2 Labeling Studies on Saframycin A



The likely biosynthetic pathway begins with the dimerization of tyrosine to form diketopiperazine **10** (Scheme 1.1).[3] Amide reduction followed by iminium ion cyclization provides the diazabicyclic core structure (**11**). Reduction of the remaining amide and incorporation of Ala-Gly produces tetrahydroisoquinoline **12**. Subsequent arene oxidation and methylation yield saframycin A.[4] The biosyntheses of the renieramycins, safracins, and ecteinascidins are expected to proceed through similar pathways. Comparatively, the biosynthetic pathways leading to lemonomycin, quinocarcin, and the naphthyridinomycin (e.g., cyanocycline A) likely proceed through a similar diketopiperazine intermediate derived from tyrosine and either glutamic acid or an ornithine residue (*vide infra*).

Scheme 1.1 Biosynthetic Pathway of Saframycin A



Biological Activity of the Tetrahydroisoquinoline Antitumor Antibiotics

As befits the name, the tetrahydroisoquinoline antitumor antibiotics are generally potent, broad-spectrum cytotoxins effective against mammalian and bacterial cells. It is likely that these compounds are the end product of a cellular defense mechanism, and the wide variation of structures within the family may be indicative of a kind of microbial arms race. The various structures support several modes of action, of which three major categories have been proposed and studied.[1] The most common mode of action is DNA alkylation, which seems to be operative whenever carbinolamine or aminonitrile functionality is present. Oxidative degradation of DNA via the generation of superoxide from either semiquinone intermediates or α-amino radical intermediates is a second important pathway. Lastly, a unique mode of action involving interruption of the DNA excision repair system leading to double strand DNA cleavage has been observed for ecteinascidin 743.[5]

1.2 Lemonomycin

Isolation and Biological Activity

Lemonomycin was isolated in 1964 from a fermentation broth of the soil bacteria *Streptomyces candidus* as a mixture with two other minor-component antibiotic compounds.[6,7] In total, 4 g of lemonomycin hydrochloride were purified from 2,000 L of broth. The free base was prepared by treatment of the hydrochloride salt with aqueous sodium hydroxide and extraction into chloroform. This free base was precipitated from aqueous acetone "as lemon-yellow spheres," leading to the name of lemonomycin. The isolation chemists were not able to fully determine the structure of lemonomycin, but based on ^{1}H NMR and IR spectroscopy they were able to establish the presence of a quinone and of *N*-methyl and *O*-methyl functionality. Degradation experiments were found to liberate dimethyl amine. Potentiometric titration indicated that lemonomycin contained two basic sites.

The isolation chemists found lemonomycin to be a potent, broad-spectrum antibiotic with activity comparable or superior to penicillin G and erythromycin (Table ch1A). Both gram-positive and gram-negative bacteria were susceptible to the antibiotic. The authors note further that lemonomycin, administered either orally or subcutaneously, protects mice from staphylococcal and streptococcal infections. Unfortunately, the antibiotic was found to be lethal at levels only slightly higher than the therapeutic dose.

Table 1.1: Minimal Inhibitory Concentrations (μg/mL)

Organism	Lemonomycin	Tetracycline	Penicillin G	Erythromycin
Staphylococcus aureus (Lederle 4050B-122-7)	0.08	10	>100	1.5
S. aureus (Lederle 4050B-122-10)	0.15	>100	>100	3
S. aureus (Lederle 4050B-122-13)	0.15	2.5	0.08	3
S. aureus (Lederle 4050B-122-14)	0.15	>100	0.04	3

S. aureus Rose ATCC 14154	0.15	>100	>100	12.5
S. aureus Smith	0.04	2.5	0.8	1.5
Streptococcus faecalis ATCC 8043	0.4	2.5	2.5	0.4
S. pyogenes C203	<0.005	1.2	0.001	0.2
S. pyogenes (Lederle 8053B-40-2)	0.01	25	0.01	0.4
S. pyogenes (Lederle 8053B-40-3)	0.01	100	0.01	0.4
S. pyogenes NY5	<0.005	2.5	0.01	0.2
Streptococcus sp. λ-Strep. 11	5.0	>100	2.5	1.5
Streptococcus sp. β-Strep. 80	2.5	>100	2.5	1.5
Mycobacterium smegmatis ATCC 607	6.2			
Staphylococcus aureus ATCC 6548P	0.2			
Bacillus subtilis ATCC 6633	0.05			
Pseudomonas fluorescens ATCC 12633	1.6			
Proteus vulgaris ATCC 9484	0.4			
Escherichia coli ATCC9637	1.6			
Salmonella gallinarum (Lederle 604)	0.8			
Clostridium sporogenes ATCC7955	>100			

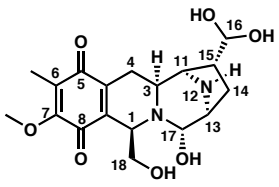
Structural Elucidation of Lemonomycin

The study of lemonomycin became dormant until 2000, when chemists at Wyeth-Ayerst Research reinvestigated the compound as part of a program aimed at testing older antibiotic compounds against newly-evolved, highly antibiotic-resistant bacterial strains.[8] These chemists found that lemonomycin is active against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* with minimal inhibitory concentrations of 0.4 and 0.2 μg/mL, respectively. The natural product also exhibited in vitro activity against a human colon tumor cell line (HCT116) with an IC_{50} of 0.36 μg/mL.

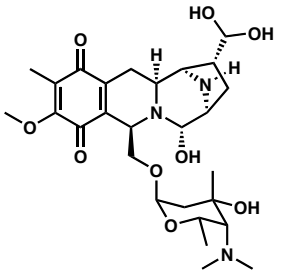
In addition to reinvestigating lemonomycin's biological activity, the Wyeth-Ayerst team determined the connectivity and relative stereochemistry of the natural product by NMR spectroscopic methods. The complex alkaloid exhibits a quinone portion reminiscent of the saframycin subfamily and a 3,8-diazabicyclo[3.2.1]octane core similar to quinocarcin (Figure 1.3). Contrastingly, lemonomycin is unique among the nearly 60 natural products and hundreds of synthetic analogues in this family in that it

bears glycosylation at C(18). This is particularly surprising in light of the extensive work toward derivatization of this position in the ecteinascidins,[9] saframycins, and quinocarcins. Furthermore, the 2,6-dideoxy-4-amino sugar (**14**) is rare in nature, having been isolated only as a substituent on glycothiohexide α,[10] nocathiacin I,[11] and MJ347-81F4 A,[12] and, in an oxidized form, as a substituent of the saccharocarcins.[13] All of these natural products are potent antibiotics, indicating that lemonose (**14**)[14] may be important to their biological activity.

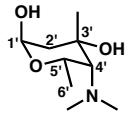
Figure 1.3 The Structure and Numbering of Lemonomycin



Lemonomycin Aglycon (13)



Lemonomycin (1)



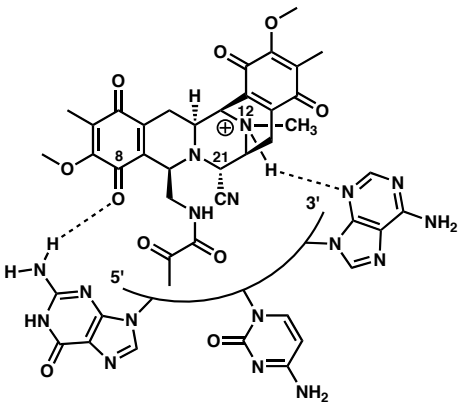
Lemonose (14)

In addition to the unique nature of the lemonose glycosylation, lemonomycin bears an aldehyde hydrate at C(16) that is unprecedented in the tetrahydroisoquinoline antitumor antibiotics. One OH group of the aldehyde hydrate likely forms an intramolecular hydrogen bond to N(12), in effect existing as a covalently bound water molecule. The incorporation of this hydrogen bond could affect the basicity of N(12), which might be important to lemonomycin's biological mode of action.

Proposed Mode of Action

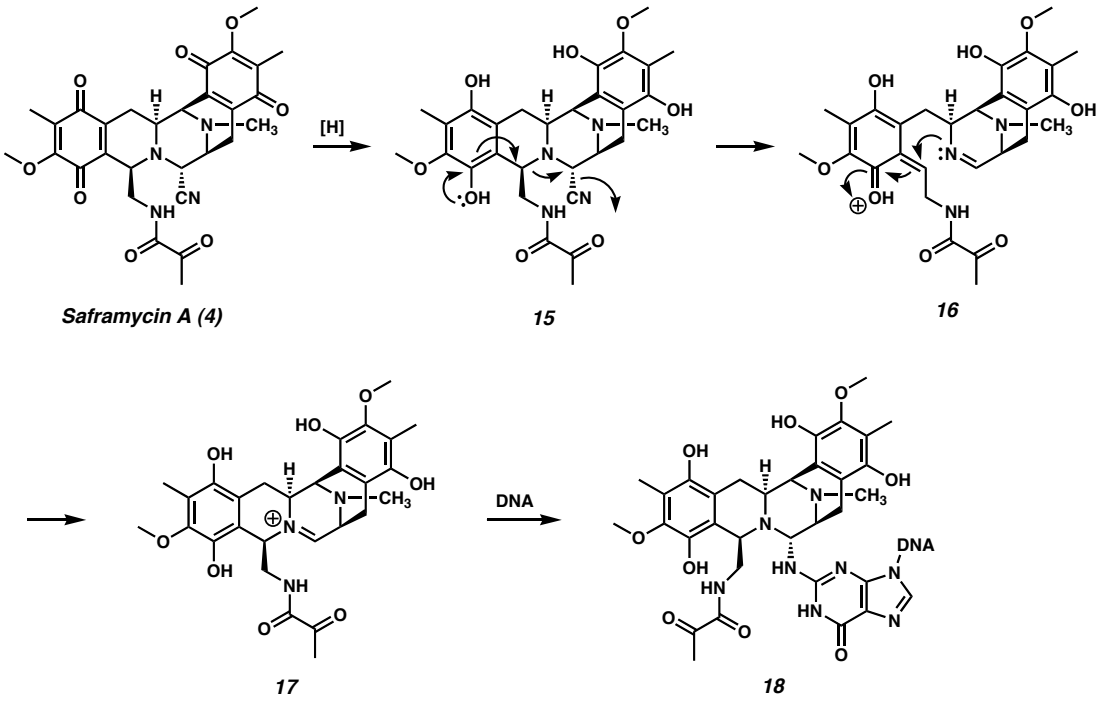
No studies have been reported concerning the mode of action responsible for the toxicity of lemonomycin. However, the structural similarities between lemonomycin and saframycin A, which has been extensively studied in this regard, indicate that the modes of action also may be similar. The toxicity of saframycin A occurs as a result of the natural product's interactions with DNA. Under mildly acidic conditions (pH 5-6), saframycin A exhibits non-covalent binding to the minor groove of DNA.[15] Calculations on the interaction of saframycin A with the DNA duplex oligomer d(GATGCATC)$_2$ have indicated that this binding is enforced by hydrogen bonding interactions of an N(12) ammonium proton with N(3) of adenine and of the C(8) oxygen with the NH_2 of an upstream guanine (Figure 1.4).[16] Additional hydrogen bonding interactions are available to the reduced (hydroquinone) form of saframycin A, which exhibits concomitantly stronger binding.

Figure 1.4 Hydrogen Bonding Interactions of Saframycin A with DNA



Saframycin A is also able to form covalent adducts with DNA. In vitro studies showed that reduction to the hydroquinone (**15**) was necessary to potentiate the alkylation activity of saframycin A (Scheme 1.2).[17] The fact that the saframycins are active in vivo without prior reduction indicates that saframycin A may undergo spontaneous conversion to hydroquinone **15** under the mild reducing conditions inside cells. Once formed, the hydroquinone readily loses HCN from C(21), generating iminium ion **17** via the intermediacy of quinone methide **16**. When bound to DNA, this iminium alkylates N(2) of guanine to form covalent complex **18**, thereby inhibiting RNA and DNA synthesis.[17]

Scheme 1.2 DNA Alkylation by Saframycin A



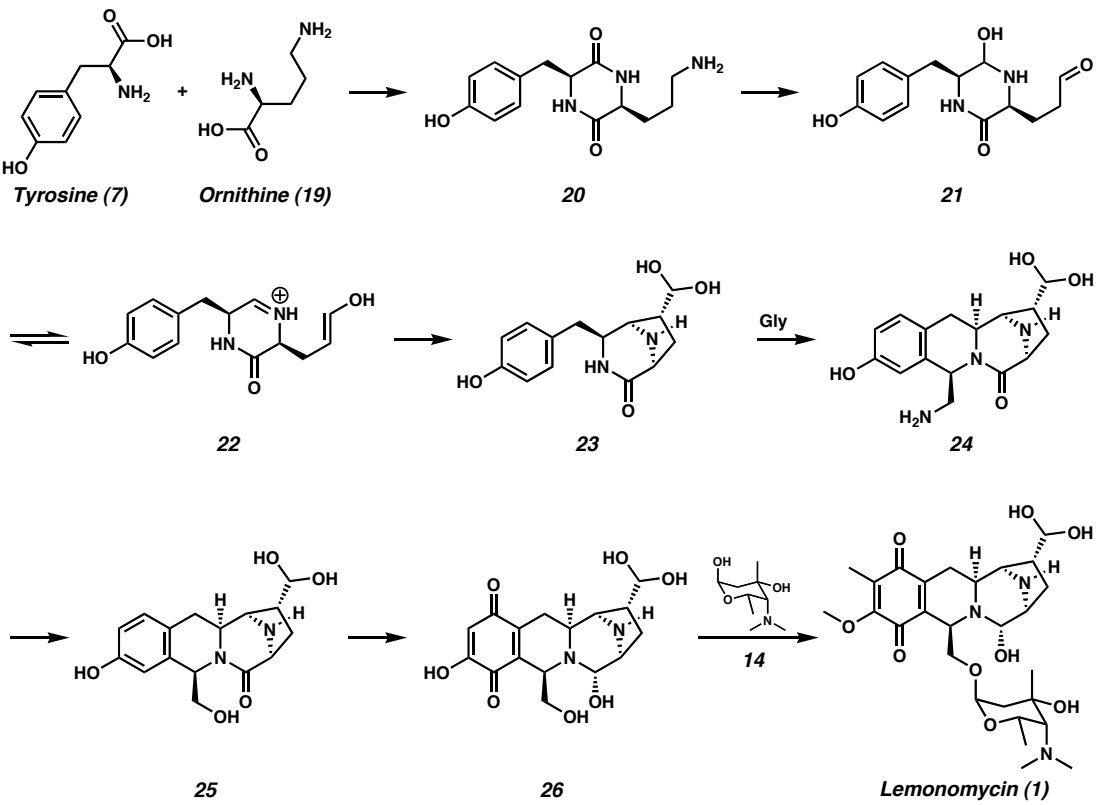
The saframycins also cause oxidative degradation of DNA via the production of oxygen radicals.[15,18] Hydroquinone **15** can undergo one electron oxidation to a

semiquinone, which reacts with dissolved oxygen to produce superoxide. Under protic conditions, superoxide rapidly disproportionates to form hydrogen peroxide and molecular oxygen.[19] Hydrogen peroxide then reacts with adventitious divalent iron to produce hydroxyl radical, which in turn causes oxidative single strand DNA cleavage.[15,20]

Biosynthetic Proposal for Lemonomycin

The biosynthesis of lemonomycin has not been studied. We expect, however, that it proceeds similarly to the biosynthesis of saframycin A (*vide supra*) and naphthyridinomycin (*vide infra*). Therefore, we propose that lemonomycin arises by the dimerization of tyrosine with ornithine to produce diketopiperazine **20** (Scheme 1.3).[21] Oxidation of the primary amine and reduction of one amide leads to carbinolamine **21**, which cyclizes to form diazabicyclooctane **23** through the intermediacy of iminium ion **22**. Incorporation of glycine generates tetrahydroisoquinoline **24**, the primary amine of which is converted to an alcohol by oxidation, hydrolysis, and reduction to produce **25**. Subsequent aromatic oxidation, methylation, and glycosylation with lemonose (**14**) yield lemonomycin. The biosynthesis of lemonose also has not been studied, but the sugar is likely derived from a common polyketide hexose by deoxygenation and amination.[22]

Scheme 1.3 Biosynthetic Proposal for Lemonomycin

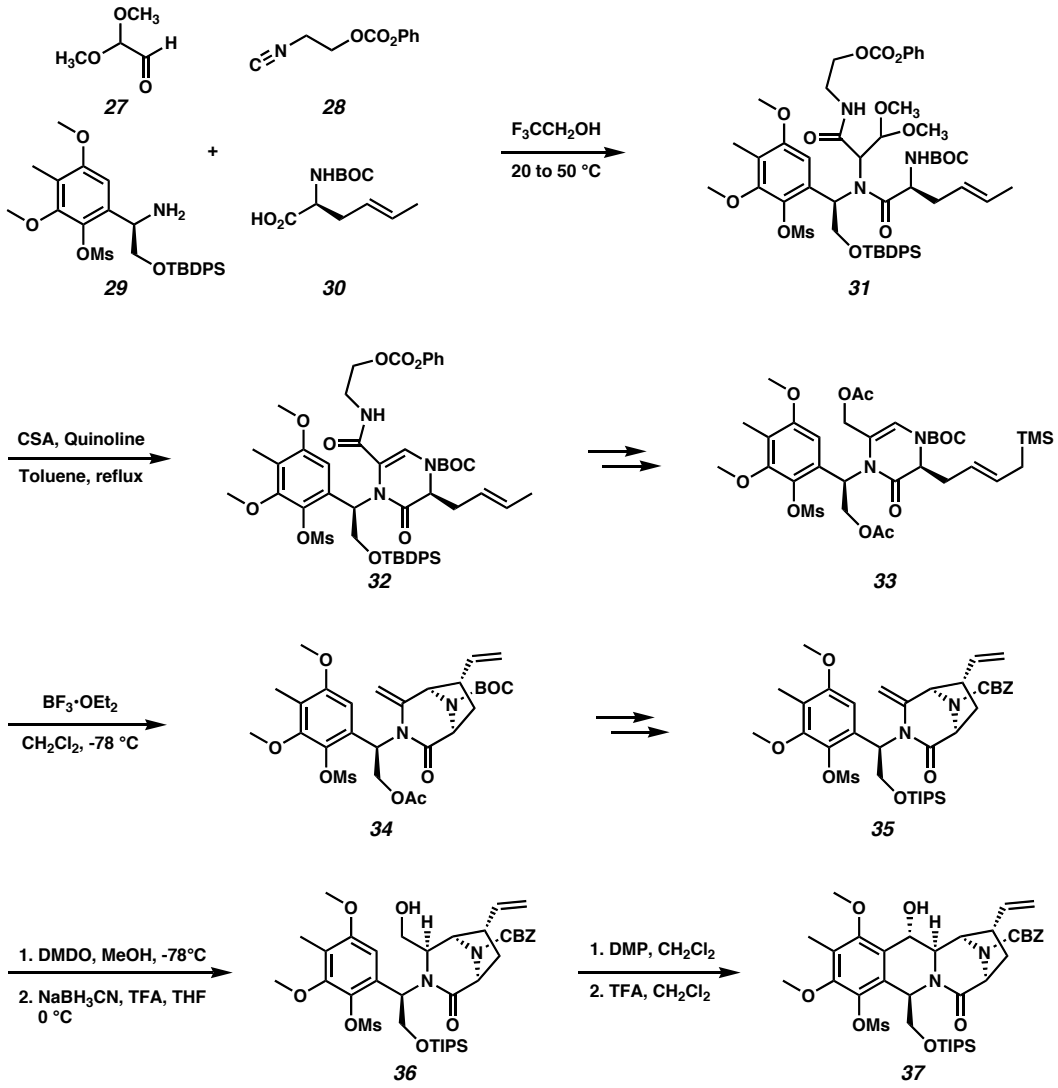


Synthetic Approaches to Lemonomycin

No synthetic work on lemonomycin was reported before our published total synthesis.[23] Subsequent to our work, however, two partial synthetic approaches were reported. The first, by the Fukuyama research group, features an Ugi four-component coupling reaction to form amide **31** (Scheme 1.4).[24] Acid catalyzed cyclization produces tetrahydroketopyrazine **32**, which is readily advanced to allylic acetate **33**. Under Lewis acidic conditions, acetate elimination followed by allyl silane cyclization provides enamide **34**. Protecting group manipulation followed by oxidation with dimethyldioxirane and reduction with sodium cyanoborohydride yields alcohol **36**, which

can be oxidized to the aldehyde and cyclized to tetrahydroisoquinoline **37** under acidic conditions. Further advancement of **37** has not been reported.

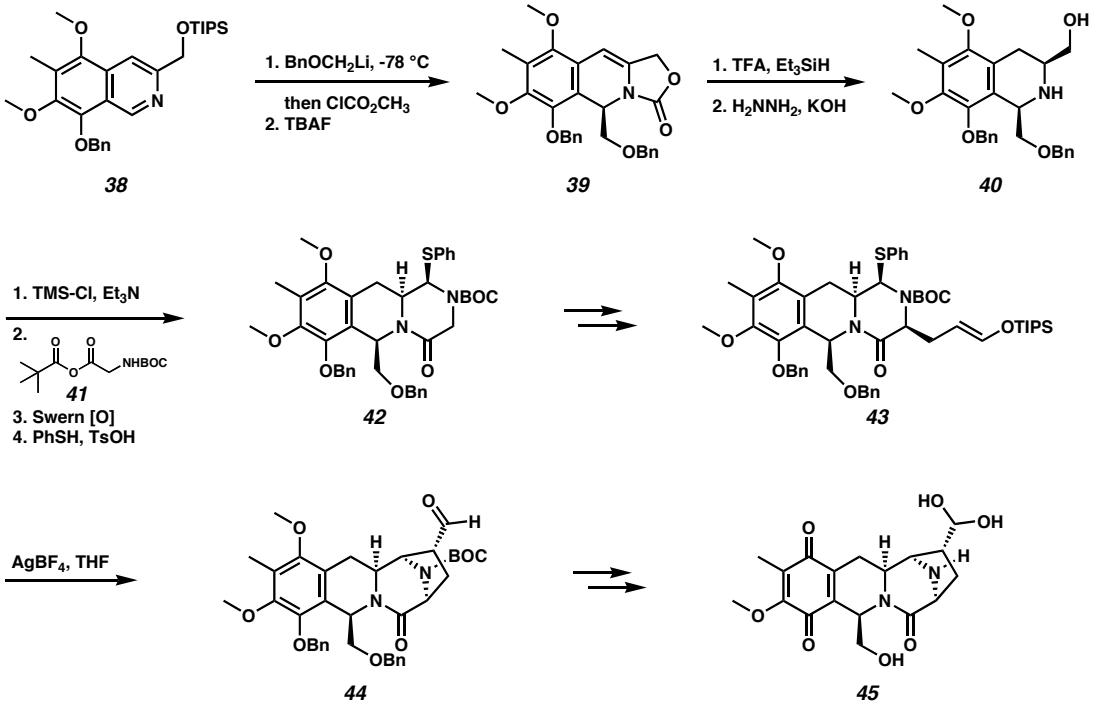
Scheme 1.4 Fukuyama's Approach Toward Lemonomycin



A similarly stepwise approach toward the bicyclic core of lemonomycin has been reported by Magnus et al. (Scheme 1.5).[25] Beginning from isoquinoline **38**,[26] alkyl lithium addition, acylation, and treatment with TBAF yielded racemic styrene **39**. Ionic

reduction and cleavage of the oxazolidinone provided cis-disubstituted tetrahydroisoquinoline **40**, which was advanced to α-thioamine **42** via acylation with *N*-BOC glycine equivalent **41** followed by oxidation and treatment with thiophenol. Lactam **42** was converted to silyl enol ether **43** by a five-step sequence involving a key alkylation and epimerization to the desired stereochemistry. Subsequent iminium ion cyclization mediated by silver tetrafluoroborate provided tetracycle **44**, which was uneventfully advanced to quinone **45**. Further work with **45** has not been reported.

Scheme 1.5 Magnus' Approach Toward Lemonomycin

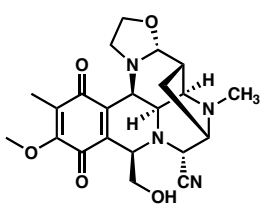


1.3 Cyanocycline A

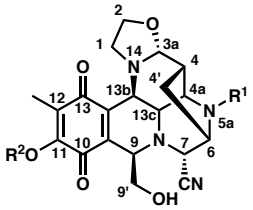
Isolation and Closely Related Compounds

Cyanocycline A (**2**, Figure 1.5) was isolated in 1982 from cultures of the soil bacterium *Streptomyces flavogriseus*.[27] The antibiotic was collected by Amberlite XAD-2 column chromatography of the culture filtrate followed by adjustment to pH 8.5 and extraction into ethyl acetate. The crude compound was purified by extensive chromatography followed by precipitation from chloroform with acetone to provide 65 mg of cyanocycline A from 150 L of culture broth. The structure was originally characterized spectroscopically by IR, UV, and NMR and was ultimately proven by X-ray analysis of single crystals obtained by recrystallization from acetone or ethanol.[28] Cyanocycline A exhibits a similar core structure to lemonomycin with a 3,8-diazabicyclo[3.2.1]octane moiety fused to a quinone-bearing tetrahydroisoquinoline ring system. However, the stereochemistry at C(4) is inverted, and the C(3a) aldehyde oxidation state is condensed into an oxazolidine ring. The oxazolidine nitrogen is bound to C(13b), forming a polycyclic, caged structure. Additionally, cyanocycline A bears its namesake cyano group as a C(7) aminonitrile, and the C(9') hydroxyl is unsubstituted.

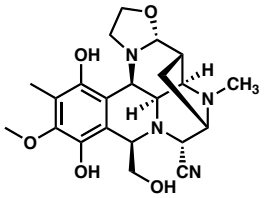
Figure 1.5 The Cyanocyclines, Bioxalomycins, and Naphthyridinomycin



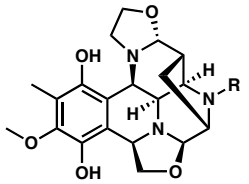
Cyanocycline A (2)



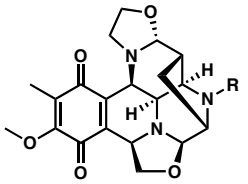
R^1=H, R^2=CH_3 Cyanocycline B (46)
R^1=CH_3, R^2=H Cyanocycline F (47)



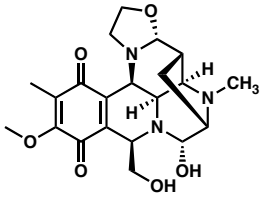
Cyanocycline C (48)



R=H Bioxalomycin α1 (49)
R=CH_3 Bioxalomycin α2 (50)



R=H Bioxalomycin β1 (51)
R=CH_3 Bioxalomycin β2 (52)



Naphthyridinomycin (53)

Prior to the discovery of cyanocycline A, a closely related structure was isolated from *Streptomyces lusitanus* AY B-1026, a species of soil bacteria collected on Easter Island.[29] This structure, named naphthyridinomycin, differs from cyanocycline A only in the presence of a carbinolamine in place of the C(7) aminonitrile. Almost simultaneously with the disclosure of the natural isolation of cyanocycline A, its structure was produced synthetically by treatment of naphthyridinomycin with sodium cyanide.[30] Later, in 1994, several closely related compounds were isolated from *Streptomyces viridostaticus* ssp. litoralis and named the bioxalomycins.[31,32] These compounds differ from the cyanocycline and naphthyridinomycin core structures only in that the C(9') hydroxyl is cyclized onto C(7) in the form of an oxazolidine ring. The isolation procedures utilized for the isolation of the bioxalomycins were milder than those utilized in the original isolation of naphthyridinomycin. When these milder procedures were applied to the

isolation of antibiotics from *Streptomyces lusitanus*, only bioxalomycin β2 was isolated, with no naphthyridinomycin being found. Synthetically, it was shown that bioxalomycin β2 could be converted to cyanocycline A upon treatment with potassium cyanide. The reverse reaction has also been accomplished by treating cyanocycline A with aqueous silver nitrate.[33] The interconversion of cyanocycline A and bioxalomycin β2, as well as the ready conversion of naphthyridinomycin to cyanocycline A, indicates that the naturally occurring form of all three natural products may in fact be the same.[34] The available evidence, however, does not allow a determination of which structure is the natural form.

Biological Activity of Cyanocycline A

Cyanocycline A exhibits potent, broad-spectrum antibiotic activity against both gram-positive and gram-negative bacteria (Table 1.2).[27] Cyanocycline A was found to have activity similar to naphthyridinomycin against several strains of bacteria and against Hela tumor cells, for which concentrations as low as 1 μg/mL were sufficient to prevent an increase in cell numbers for 48 hours.[30] Cyanocycline A is also active against Meth A cells in vitro and when grown as an ascites tumor.[35]

Table 1.2 Minimal Inhibitory Concentrations for Cyanocycline A

Test Organism	MIC (μg/mL)	Test Organism	MIC (μg/mL)
Bacillus subtilis PCI-219	0.005	*Proteus mirabilis* 1287	0.31
Bacillus cereus T-1	0.62	*Proteus mirabilis* 9'	0.31
Micrococcus luteus B	0.0025	*Serratia marcescens* TO-50	0.62
Staphylococcus epidermidis TO-3	0.005	*Serratia marcescens* FU-111	0.62
Staphylococcus aureus 209P	0.005	*Pseudomonas aeruginosa* J-272	0.62
Escherichia coli NIHJ	0.08	*Pseudomonas aeruginosa* NGB75	0.62
Escherichia coli 11	0.15	*Pseudomonas aeruginosa* M-57740	0.31

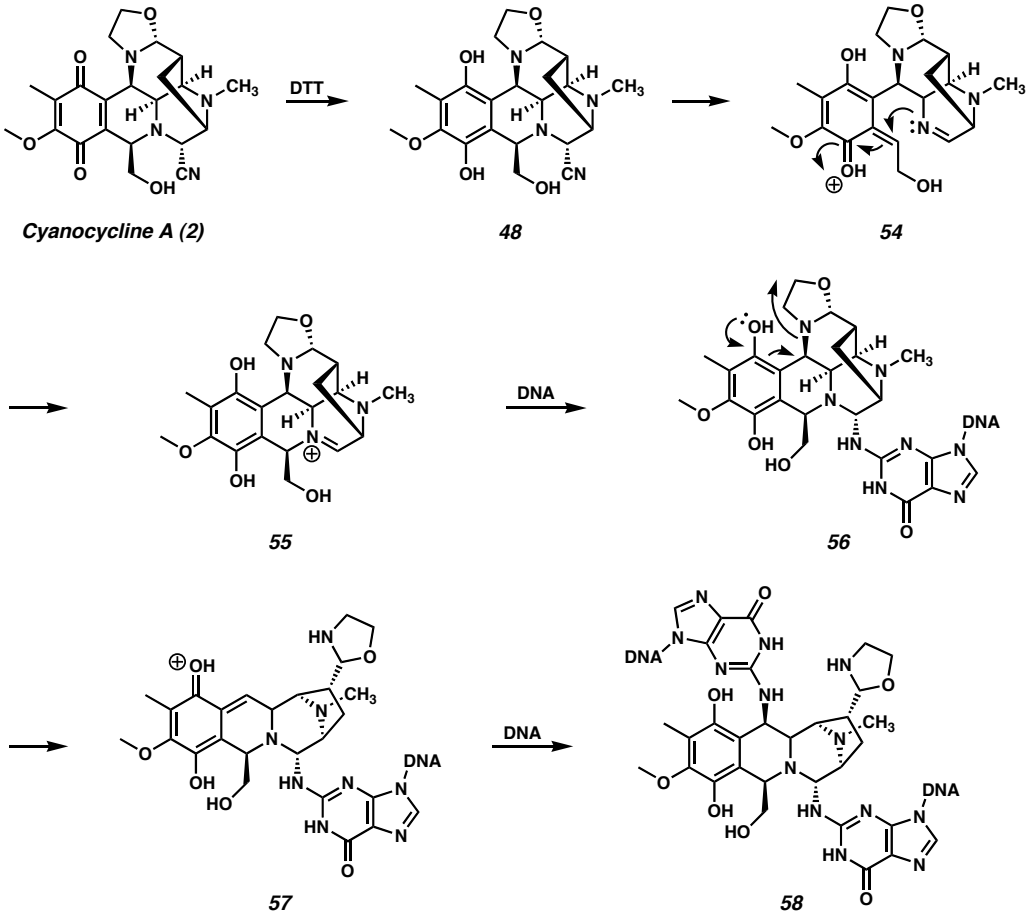
Salmonella enteritidis T-1	0.08	*Pseudomonas aeruginosa* J-162	0.31
Salmonella typhi Tanaka	0.04	*Pseudomonas aeruginosa* Ps-4	0.15
Klebsiella pneumoniae 3K25	0.31	*Candida albicans* IAM4888	50
Klebsiella pneumoniae 15C	0.31	*Saccharomyces cerevisiae* IAM4804	50
Shigella flexneri 2b TO-1	0.08	*Penicillium chrysogenum* IAM7305	50
Shigella sonnei TO-1	0.15	*Aspergillus niger* IAM2020	100

Cyanocycline A causes cell death by inhibiting the synthesis of DNA, RNA, and proteins.[35] This activity was found to be attenuated by the addition of exogenous DNA, indicating that DNA binding is the primary mode of action. In support of this indication, Zmijewski, et al. have shown that ^{3}H labeled naphthyridinomycin forms a covalent complex with DNA, and that this covalent complex is a poor substrate for DNA translation and transcription enzymes.[36]

Arora, et al. have used molecular modeling studies to analyze the binding mode of cyanocycline A and naphthyridinomycin with DNA.[37] These authors considered covalent binding of C(7) of the antibiotic to N(2) of guanine in either a groove-binding mode or a partially intercalated mode. The groove-binding conformation places the antibiotic in the minor groove of DNA with an edge-on approach, such that the carbonyl of C(10) and the C(9') hydroxyl form hydrogen bonds to DNA, while C(7) is covalently bound in the *R* configuration. This model was found to be substantially favored over the partially intercalated model, which places the quinone ring intercalated into DNA with C(7) again covalently bound in the *R* configuration. A similar study was carried out by Remers, et al., who considered DNA alkylation by both the C(7) aminonitrile and the C(3a) oxazolidine.[38] Both sites of alkylation were considered feasible, but the geometries of the bound complexes were not suitable for crosslinking duplex DNA. Instead, a crosslink between DNA and protein was proposed.

It was later determined, however, that both bioxalomycin α2 and cyanocycline A are capable of forming crosslinks with duplex DNA (cyanocycline A required prior reduction with dithiothreitol).[39] The crosslink was formed with $^{5'}CG^{3'}$ specificity, indicating alkylation of two guanine bases on N(2) in the minor groove. The authors report that preliminary molecular modeling studies support the covalent binding of C(7) and C(13b) to DNA with partial intercalation of the hydroquinone ring into the DNA helix. A mechanism for this crosslinking has been proposed (Scheme 1.6).

Scheme 1.6 DNA Crosslinking by Cyanocycline A

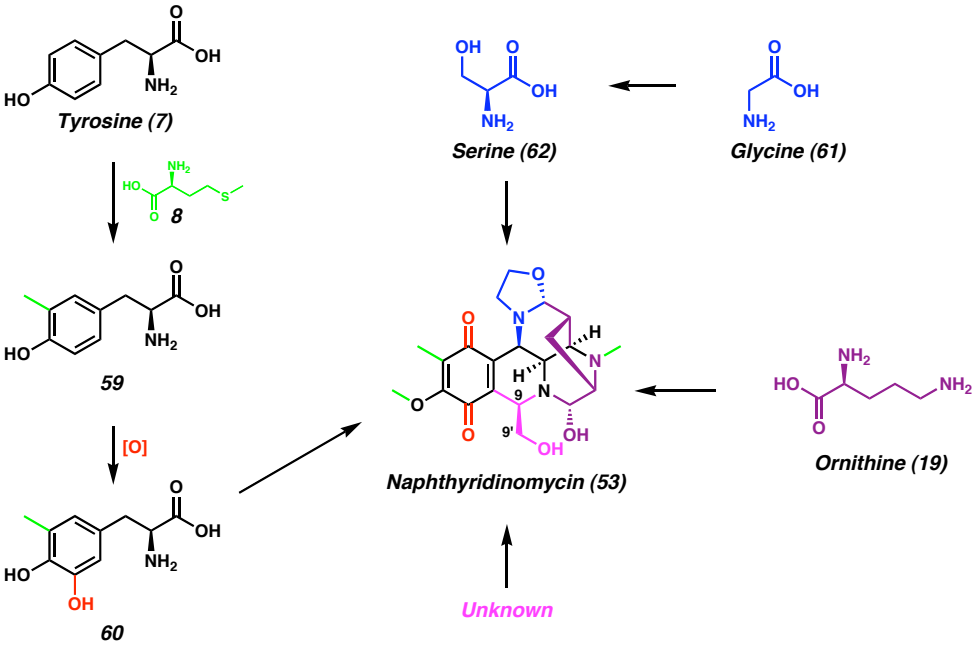


Biosynthesis of Cyanocycline A

While the biosynthesis of cyanocycline A has not been directly investigated, several studies have appeared regarding the biosynthesis of naphthyridinomycin. The close structural similarities of these two compounds indicate that the biosynthesis is likely the same for both antibiotics (*vide supra*). The biosynthesis of naphthyridinomycin by *Streptomyces lusitanus* NRRL 8034 was first investigated by feeding studies with labeled amino acids and other potential precursors.[40] This work showed that carbons C(13b), C(13c), and C(4a), as well as the quinone ring, were derived from tyrosine (Scheme 1.7). Labeled glycine was incorporated at C(1) and C(2), while the C(5'), C(11'), and C(12') methyl groups were labeled by *S*-$^{13}CH_3$ methionine. Ornithine was incorporated into the natural product, while glutamate, acetate, glucose, and dihydroxyphenylalanine were not incorporated.

It was later determined that glycine is converted to serine prior to incorporation into the natural product and that N(14) is derived from that serine.[41] The remaining carbons of the bicyclooctane moiety (3a, 4, 4', 6, and 7) are derived from ornithine. A final study determined that, while dihydroxyphenylalanine was not incorporated into naphthyridinomycin, 3'-methyltyrosine (**59**) and 5'-methyldihydroxyphenylalanine (**60**) were incorporated.[42] This information allowed the authors to propose that tyrosine is first methylated and then hydroxylated in the early stages of the biosynthetic pathway. The significant mystery that remains is the origin of C(9) and C(9').

Scheme 1.7 Biosynthesis of Naphthyridinomycin



Previous Syntheses of Cyanocycline A

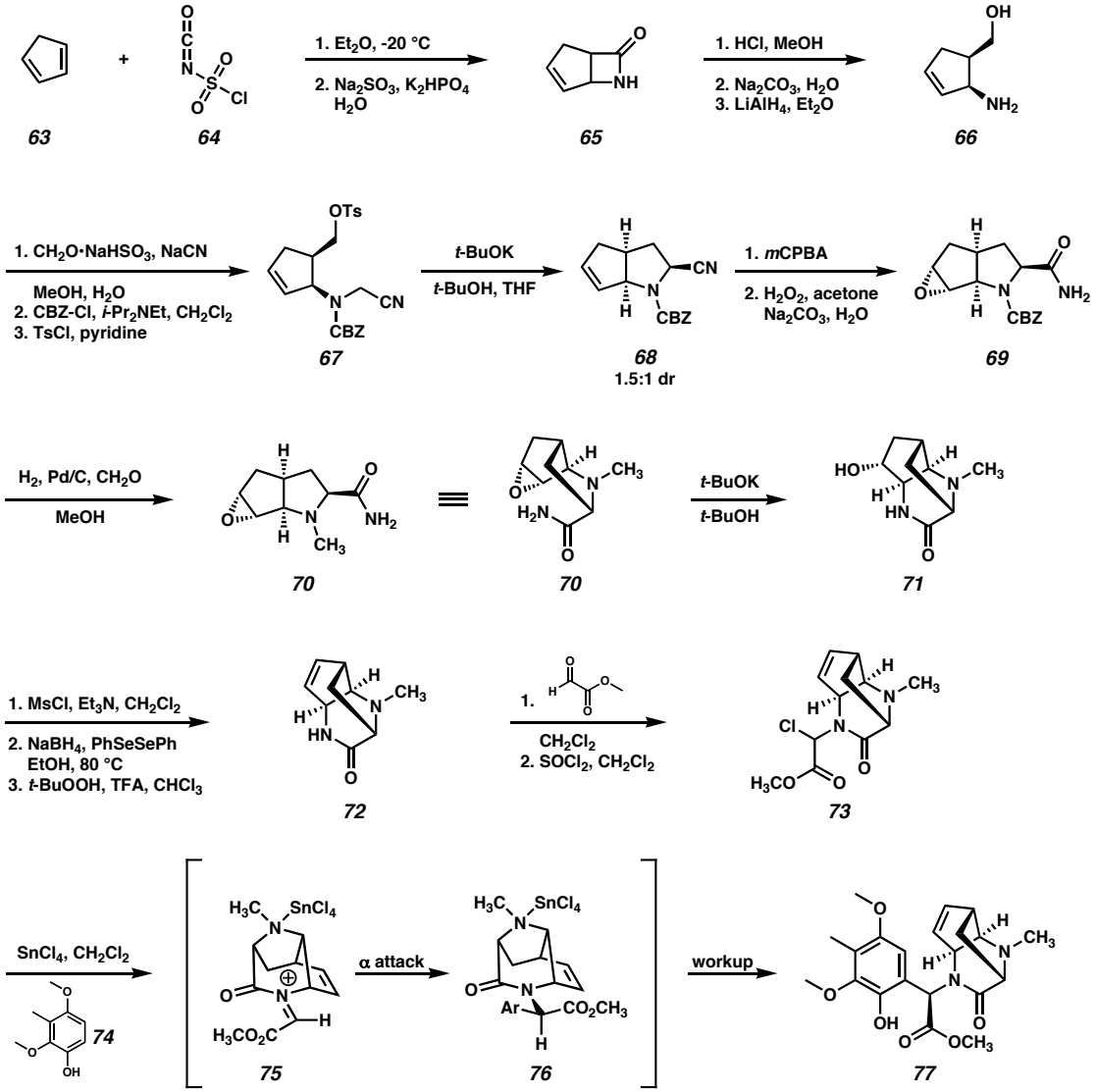
Several partial syntheses of the cyanocycline and naphthyridinomycin structures have been reported.[43] However, only two completed total syntheses have been reported, one by the Evans research group and one by the Fukuyama research group. Both of these synthetic efforts were originally targeted toward naphthyridinomycin, but were later focused on cyanocycline in light of the greater stability of the C(7) aminonitrile compared with the C(7) carbinolamine of naphthyridinomycin. Both syntheses were originally performed along racemic pathways, although each group was later able to develop an asymmetric route to cyanocycline A.

Evans' Total Synthesis of Cyanocycline A

The Evans synthesis of cyanocycline A began with the cycloaddition of cyclopentadiene and chlorosulfonyl isocyanate to yield β-lactam **65** (Scheme 1.8).[44] Methanolysis and reduction provided amino alcohol **66**, which was readily advanced to tosylate **67**. Treatment of the tosylate with potassium *tert*-butoxide allowed facile 5-exo cyclization to pyrrole **68**. The poor diastereocontrol in the formation of **68** could be partially addressed by separation of the diastereomers and epimerization of the undesired isomer under basic conditions, providing 58% yield of the pure desired isomer after one cycle. Epoxidation followed by hydrolysis of the nitrile produced amide **69**. The benzyl carbamate was replaced by a methyl group in a single step by hydrogenolysis in the presence of formaldehyde. The resulting epoxy amide (**70**) underwent facile *tert*-butoxide mediated 6-exo cyclization to generate ketopiperazine **71**,[45] which could be converted to alkene **72** by a three-step protocol.

To set up one of the key steps of the synthesis, amide **72** was condensed with methyl glyoxylate and then converted to α-chloroamine **73** with thionyl chloride. Under Lewis acidic conditions, the chloride was eliminated to form putative iminium ion **75**, which underwent Friedel-Crafts alkylation of arene **74** with high stereoselectivity and complete regiocontrol. The origin of the stereo- and regioselectivity is not completely clear, but Evans proposes that the *Z*-iminium ion is selectively formed, and the steric encumbrance of a Lewis acid coordinated to the amine of **75** prevents alkylation on the β face. The surprising regiocontrol may result from chelation of the ester carbonyl to an intermediate tin phenoxide.[46]

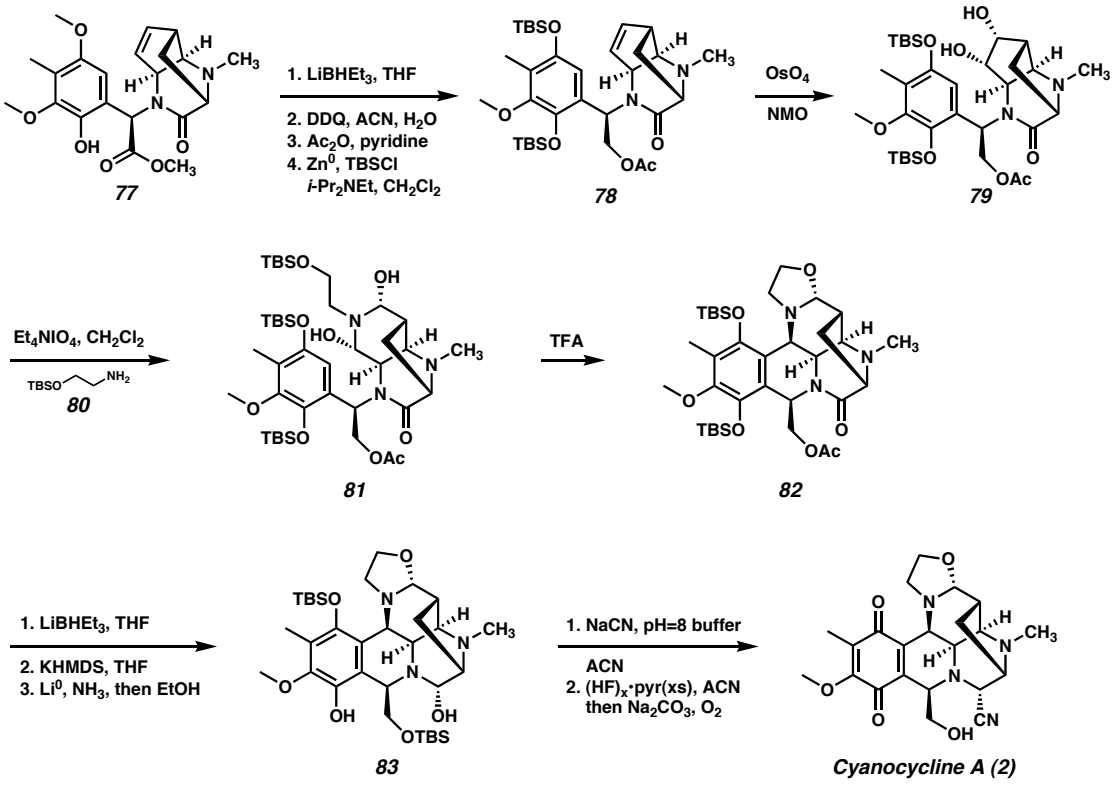
Scheme 1.8 Evans' Synthesis of Cyanocycline A



The key Friedel-Crafts alkylation product **77** was readily progressed to acetate **78**, which was converted to diol **79** under standard conditions (Scheme 1.9). The next challenge of the synthesis was the cleavage of this diol to an expected dialdehyde intermediate followed by incorporation of an ethanolamine unit. Unfortunately, this reaction was complicated by rapid hydration of the dialdehyde. The resulting dihydroxytetrahyropyran resisted all attempts to incorporate an ethanolamine unit. It was

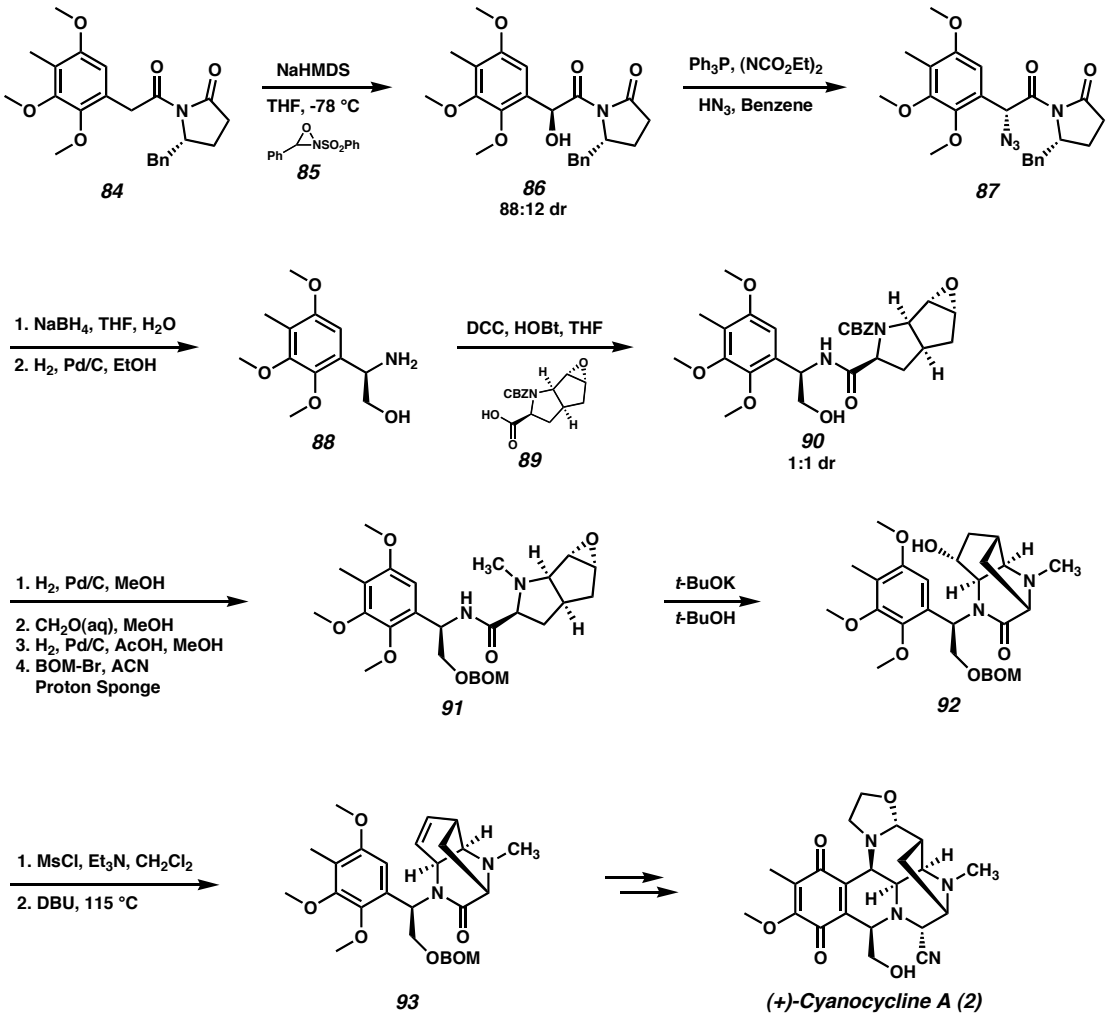
eventually discovered, however, that anhydrous cleavage of the diol with tetraethylammonium periodate in the presence of amine **80** yielded dihydroxypiperidine **81**. Treatment of this compound with neat trifluoroacetic acid potentiated an iminium ion cascade cyclization to hexacycle **82**. Amide reduction also proved challenging, and it was discovered that deacetylation of the C(9') hydroxyl and migration of a TBS group to this position was necessary before the amide could be reduced. Dissolving metal conditions were then employed for the generation of carbinolamine **83**. Incorporation of cyanide, desilylation, and oxidation provided cyanocycline A, which was identical to a natural sample in all respects other than optical rotation.

Scheme 1.9 Completion of the Evans Synthesis



The Evans group was later able to impart asymmetry to their synthesis through the use of a phenylalaninol-derived oxazolidinone chiral auxiliary (Scheme 1.10).[47] Oxidation of the sodium enolate of enantiopure **84** with Davis oxaziridine **85** delivered alcohol **86**, from which the minor diastereomer could be chromatographically removed. Mitsunobu inversion to azide **87** followed by two-step reduction yielded amino alcohol **88**. The amine of **88** could be coupled to racemic acid **89**[48] to provide a separable mixture of **90** and the diastereomer arising from the opposite enantiomer of **89**. Protecting group exchange revealed amide **91**, which was readily cyclized to tetracycle **92** under basic conditions. Elimination of the alcohol to alkene **93** was effected by mesylation and heating with neat DBU. This alkene was then advanced to (+)-cyanocycline A by a route thematically similar to that used in the racemic case, although the minor differences between alkene **93** and alkene **77** caused several technical challenges.

Scheme 1.10 Evans' Asymmetric Synthesis

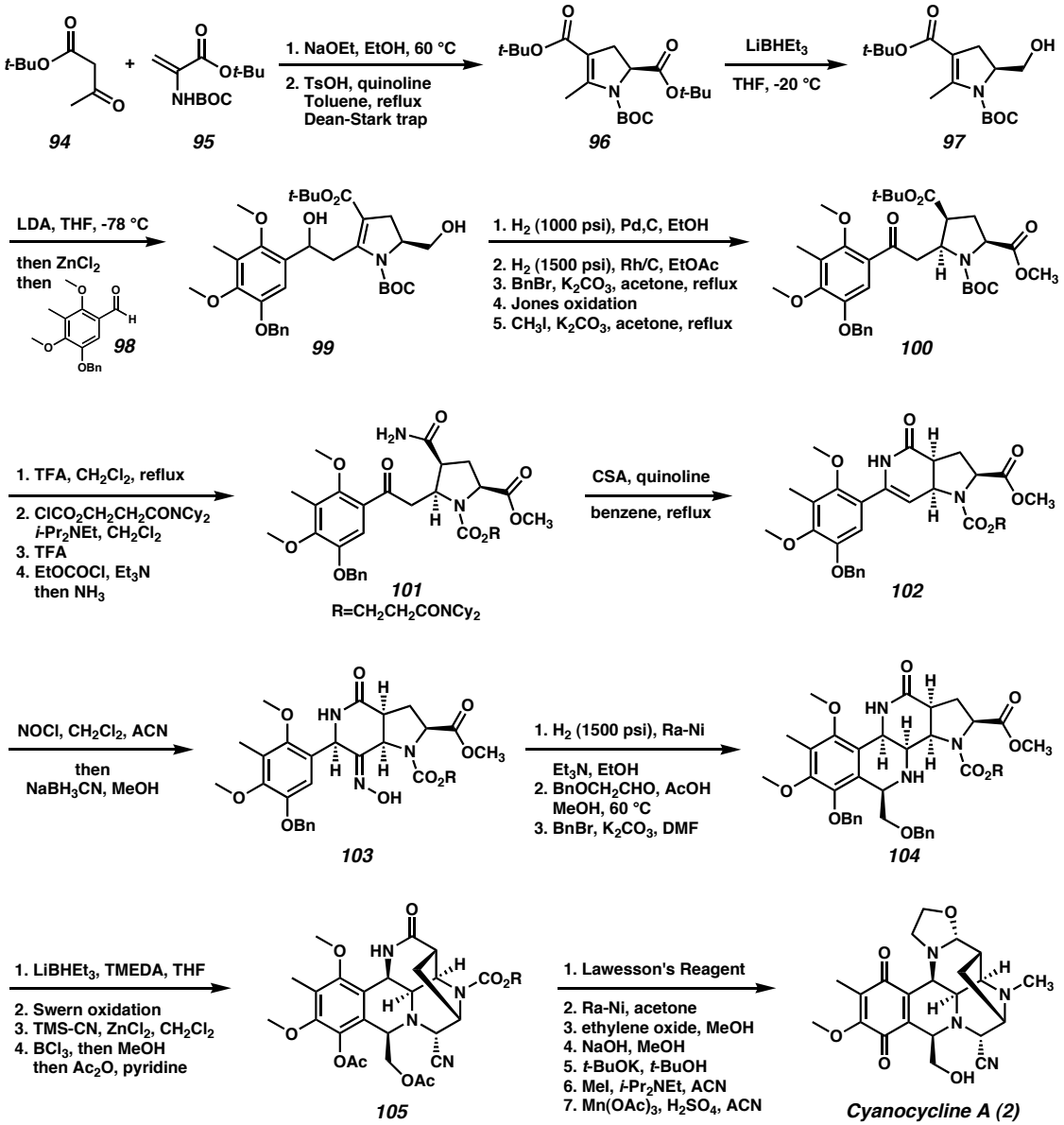


Fukuyama's Total Synthesis of Cyanocycline A

Fukuyama's synthesis of cyanocycline A began with the condensation of *tert*-butyl acetoacetate with dehydroalanine derivative **95** to provide pyrrolidine ring synthon **96** (Scheme 1.11).[49] Reduction followed by vinylogous aldol generated benzylic alcohol **99**, the enamide of which was diastereoselectively reduced to yield diester **100** after oxidation and methylation. This diester was selectively converted to monoamide **101**, which could then be condensed to enamide **102**. Nitrosylation and stereoselective

reduction supplied oxime **103**. Subsequent reduction of the oxime to an amine followed by Pictet-Spengler cyclization then generated key tetracycle **104**.

Scheme 1.11 Fukuyama's Total Synthesis of Cyanocycline A

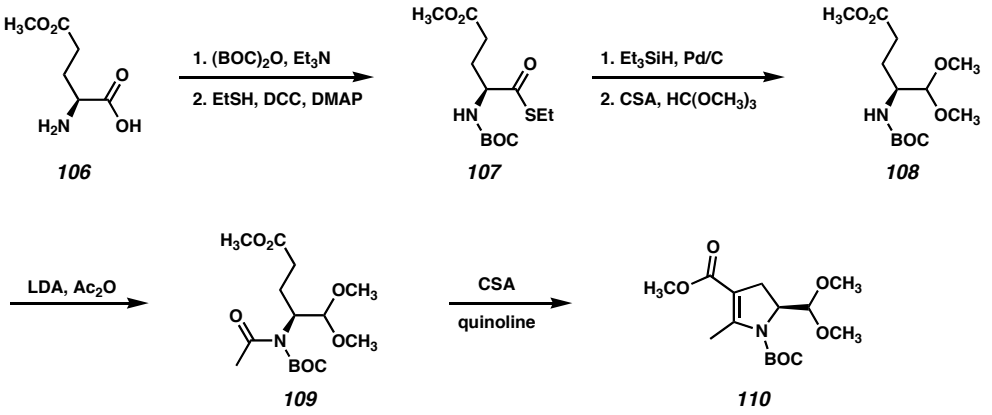


The ester of **104** was adjusted to the aldehyde oxidation state by superhydride reduction and Swern oxidation. The intermediate aldehyde underwent partial

condensation with the secondary amine and was trapped as the aminonitrile upon treatment with TMS-CN, producing pentacycle **105** after protecting group interchange. The remaining amide was converted to the oxazolidine by a three-step procedure consisting of conversion to the thioamide with Lawesson's reagent, Raney nickel reduction to the iminium ion, and reaction with ethylene oxide. Deprotection and oxidation then furnished racemic cyanocycline A.

The Fukuyama synthesis was rendered asymmetric by construction of dihydropyrrole **110** from L-glutamic acid methyl ester **106** (Scheme 1.12).[33] Reaction with di-*tert*-butyl-dicarbonate followed by coupling with ethanethiol provided thioester **107**, which could be reduced with palladium and triethylsilane to the aldehyde oxidation state and trapped as the dimethyl acetal (**108**). Acylation of the urethane nitrogen followed by condensative cyclization yielded dihydropyrrole **110**, which could be converted to (+)-cyanocycline A by the established route.

Scheme 1.12 Fukuyama's Asymmetric Route



1.4 Conclusion

The tetrahydroisoquinoline antitumor antibiotics are a structurally diverse, chemically interesting, and biologically active class of natural products isolated from bacterial and marine sources. The determined investigations of many chemists and biologists have elucidated the many structures, biosynthetic pathways, and modes of biological activity native to these compounds. Additionally, extensive synthetic work has led to the total syntheses of many of these complex natural products, particularly those characterized by their 3,9-diazabicyclo[3.3.1]nonane core. Cyanocycline A, as a representative of the subfamily characterized by a 3,8-diazabicyclo[3.2.1]octane core, has also garnered substantial synthetic attention, leading to two total syntheses. Lemonomycin, as the most recently characterized member of this family, had not been synthesized prior to our work.

1.5 Notes and Citations

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(14) Prior to this thesis, the glyscosyl unit common to lemonomycin and the glycothiohexides had not been individually named. Given that lemonomycin was the first of these compounds to be isolated and that we are the first chemists to synthesize the pyranose, we feel confident in bestowing the name lemonose upon this sugar. For the naming of the sugars vancosamine and callipeltose based on the names of their respective natural products, see: (a) Smith, R. M.; Johnson, A. W.; Guthrie, R. D. *J. Chem. Soc., Chem. Commun.* **1972**, 361-362. (b) Zampella, A.; D'Auria, M. V.; Minale, L.; Debitus, C.; Roussakis, C. *J. Am. Chem. Soc.* **1996**, *118*, 11085-11088.

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