DISSERTATION

TOTAL SYNTHESIS OF (-)-TETRAZOMINE AND BIOCHEMICAL STUDIES

Submitted by

Jack David Scott

In partial fulfillment of the requirments

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JACK DAVID SCOTT ENTITLED TOTAL SYNTHESIS OF (-)-TETRAZOMINE AND BIOCHEMICAL STUDIES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Advisor

Department Head

ABSTRACT OF DISSERTATION

TOTAL SYNTHESIS OF (-)-TETRAZOMINE AND BIOCHEMICAL STUDIES

The total synthesis of (-)-tetrazomine is presented in which the relative and absolute stereochemistry of the natural product has been determined. The synthesis features a unique iminium cyclization followed by a 1,3-dipolar cylcoaddition. The stereochemistry of advance intermediates were determined by extensive 2D NMR techniques.

The asymmetric synthesis of all four stereoisomers of β-hydroxy pipecolic acid has also been described. These stereoisomers were compared to the amino acid isolated from the hydrolysis of tetrazomine to determine the absolute stereochemistry of the amino acid moiety on the natural product.

The route used to complete the total synthesis allowed for pipecolic acid analogs to be synthesized in order to study the effect of structure on the biochemical characteristics of these compounds. Along with oxazolidine ring containing analogs, ring opened amino nitrile analogs were also studied.

Jack D. Scott

Department of Chemistry

Colorado State University

Fort Collins, Colorado 80523

Summer 2001

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List of Abbreviations

Ac	acetyl
AIBN	2,2'-azobisisobutyronitrile
Alloc	allyloxycarbonyl
Bn	benzyl
Boc	*tert*-butoxycarbonyl
BOPCl	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
Bz	benzoyl
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
CDI	carbonyldiimidazole
CIP	2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate
CSA	camphorsulfonic acid
DAST	(diethylamino)sulfur trifluoide
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DIBALH	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Fmoc	9-fluorenylmethoxycarbonyl
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
Im	imidazol-1-yl
KHMDS	potassium bis(trimethylsilyl)amide
LAH	lithium aluminum hydride
LDA	lithium diisoproplyamide
LHMDS	lithium bis(trimethylsilyl)amide
mCPBA	*m*-chloroperoxybenzoic acid
MOM	methoxymethyl
Ms	methanesulfonyl
NBS	*N*-bromosuccinimide
NBT	nitro blue tetrazolium chlroride
NMM	4-methylmorpholine
NMO	4-methylmorpholine *N*-oxide
NPSP	*N*-phenylselenophthalimide
PCC	pyridinium chlorochromate
phth	phthalimidyl
piv	pivoyl
pMB	*p*-methoxybenzyl

PPTS	pyridinium *p*-toluenesulfonate
pyr	pyridine
Red Al	sodium bis(2-methoxyethoxy)aluminum hydride
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TCP	2,4,5-trichlorophenyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TMAD	1,1'-azobis(*N,N*-dimethylformamide)
TMEDA	*N,N,N',N'*-tetramethylethylenediamine
TMS	trimethylsilyl
TPAP	tetra-*n*-propylammonium perruthenate
Troc	2,2,2,-trichloroethoxycarbonyl
Ts	*p*-toluenesulfonyl

Acknowledgements

I would like to thank my advisor, Professor Robert (Bob) Williams for the guidance and financial support throughout my graduate career at Colorado State University. Bob gave me the flexibility to take my project wherever I chose making me an independent worker which is very important in the world of science.

My parents, Dr. Thomas and Mary Scott, were also very important in my college career, giving me the support throughout the 10 plus years through the good times and a few not so good ones. From the time that I was told that fraternities were a really bad choice to the time when I treated my first year of grad school as if I were attempting to cram four years of frat life into one year, they always were behind me no questions asked.

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Last and definitely not least, I would like to thank my immediate family. To my wife, Lisa I would like thank for all of the love and support that was given to me throughout the final five years of graduate school. I became a much better person and chemist since Lisa entered my life during "the summer of Love". Lisa literally put her life and career on hold to raise our two children during my final years at CSU and for that and all that she put up with being married to a grad student, I say thank you. To my daughter Taylor I would like to thank her for her ability to cheer me up when it was necessary, for her ability to show me that learning is fun, and that there is much more to life than any given chemical reaction. To my son Isaac I would like to thank for his ability to show me that there are many worse things that can go wrong besides a poor yielding reaction. And for both kids I say thanks for the nightly WWF wrestling that took the edge off from a day in the lab.

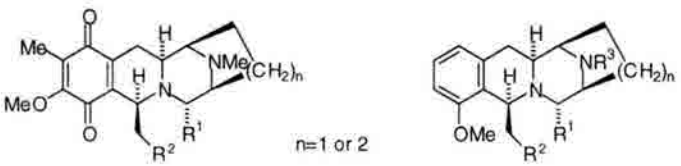
I am sure that there are others that I have slighted by not mentioning them in these pages, so I will give a broad thanks to all and to all a good night.

Chapter 1

A Review of the Chemistry and Biology of the Tetrahydroisoquinoline Antitumor Antibiotics

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** (Fig 1). To date, 55 natural products in this family have been isolated. Their biological activities range from potent antitumor compounds to benign depending on their structures.



1 2

Figure 1 General Structures of the tetrahydroisoquinolines

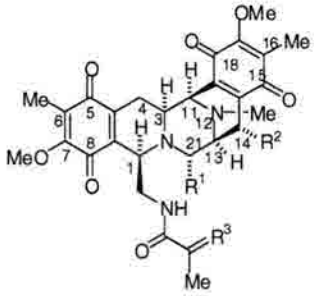
These natural products are classified into the saframycin, naphthyridinomycin/ bioxalomycin, and quinocarcin/ tetrazomine families of natural products. Some of these natural products have been reviewed in the past[1], but this is intended to be the most comprehensive review to date.

2. *Saframycin Family*

2.1. Saframycins

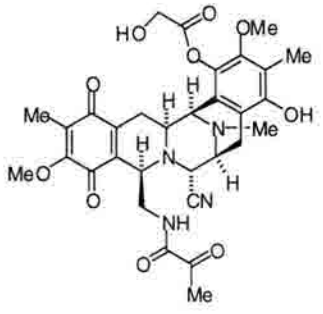
2.1.1. Isolation and structure determination

Saframycins A, B, C, D, and E (**3-5**, **9**, **10** respectively) were isolated from *Streptomyces lavendulae* in 1977 by Arai *et al.*[2] These were the first of many saframycins to be isolated. The structure of saframycin C was the first 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 as the 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 data as the triacetate derivative of saframycin E.

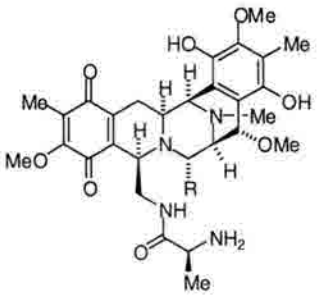


Saframycin A (**3**) R^1=CN, R^2=H, R^3=O
B (**4**) R^1=R^2=H, R^3=O
C (**5**) R^1=H, R^2=OMe, R^3=O
G (**6**) R^1=CN, R^2=OH, R^3=O
H (**7**) R^1=CN, R^2=H, R^3=OH, CH_2COMe
S (**8**) R^1=OH, R^2=H, R^3=O

Saframycin D (**9**) R^1=H, R^2,R^3=O
E (**10**) R^1=R^3=H, R^2=OH
F (**11**) R^1=CN, R^2,R^3=O



Saframycin R (**12**)



Saframycin Mx1 (**13**) R=OH
Mx2 (**14**) R=H

Figure 2 The saframycins

In some studies of the optimization of saframycin A production, another saframycin was isolated, saframycin S.[7] Saframycin S was believed to be the precursor to saframycin A. It was found that treatment of saframycin S with sodium cyanide lead 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 observed in the IR. It was hypothesized that the oxygenated functionality quenches the nitrile absorption intensity. This characteristic was seen in all of the saframycins that contain a nitrile moiety.

Saframycin S (**8**) Saframycin A (**3**)

Scheme 1 Interconversion of saframycin S and saframycin A.

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 the 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, however it is the E ring that was in the hydroquinone oxidation state.

In the search for more biologically active saframycins, six new saframycins were produced by directed biosynthesis employing *Streptomyces lavendulae* No. 314[13] (Figure 3). 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 in the quantitative and qualitative analysis of the saframycins by their polarographic and voltammetric behavior was reported by Bersier and Jenny.[15]

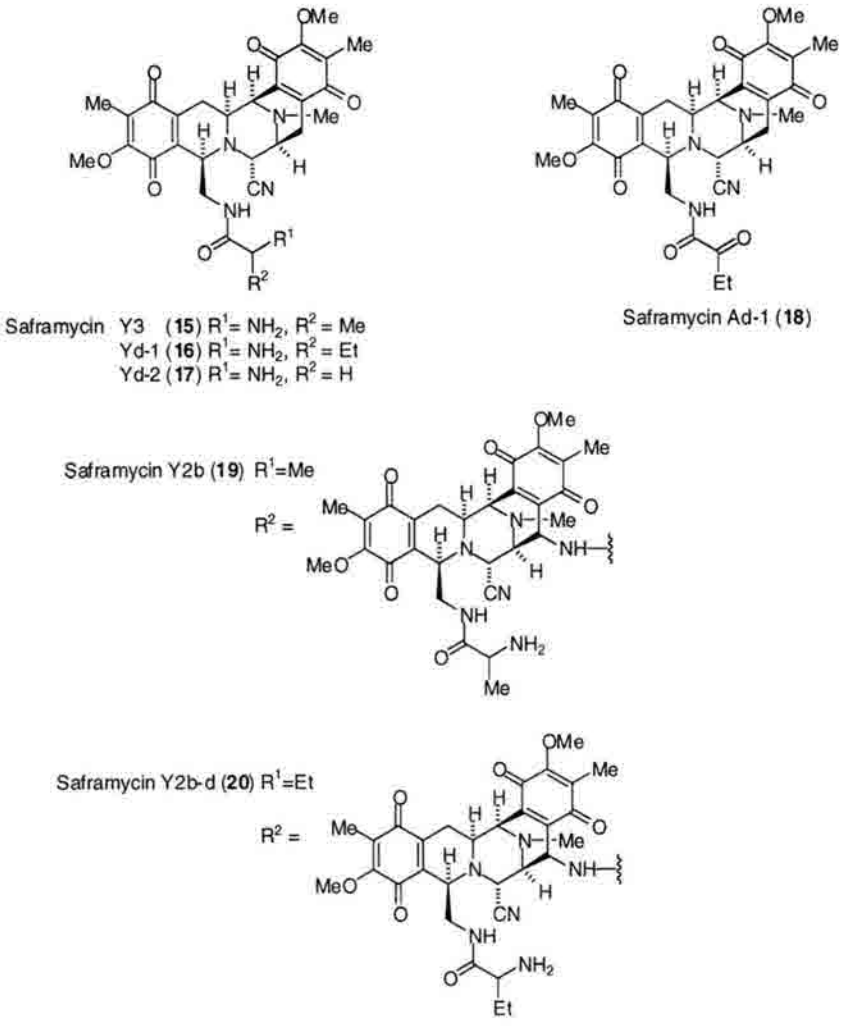


Figure 3 Saframycins from directed biosynthesis

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). Glycine and alanine were also 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 was incorporated into the pyruamide side chain. The five methyl groups of saframycin A were found to be derived from methionine (**22**).

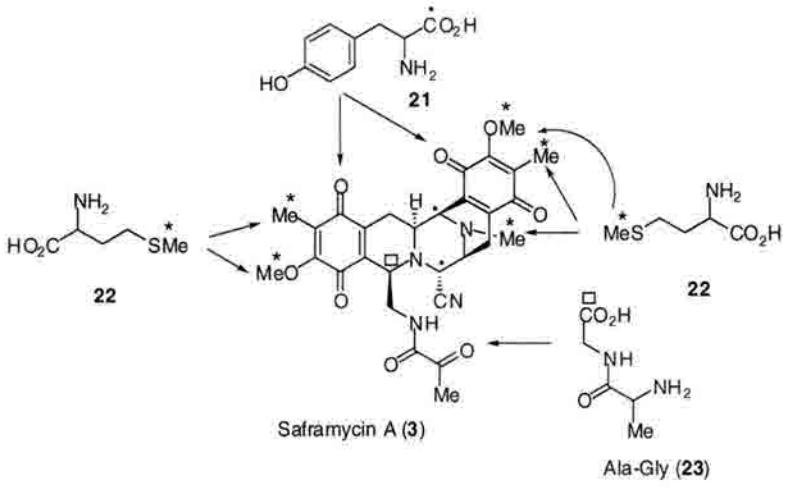


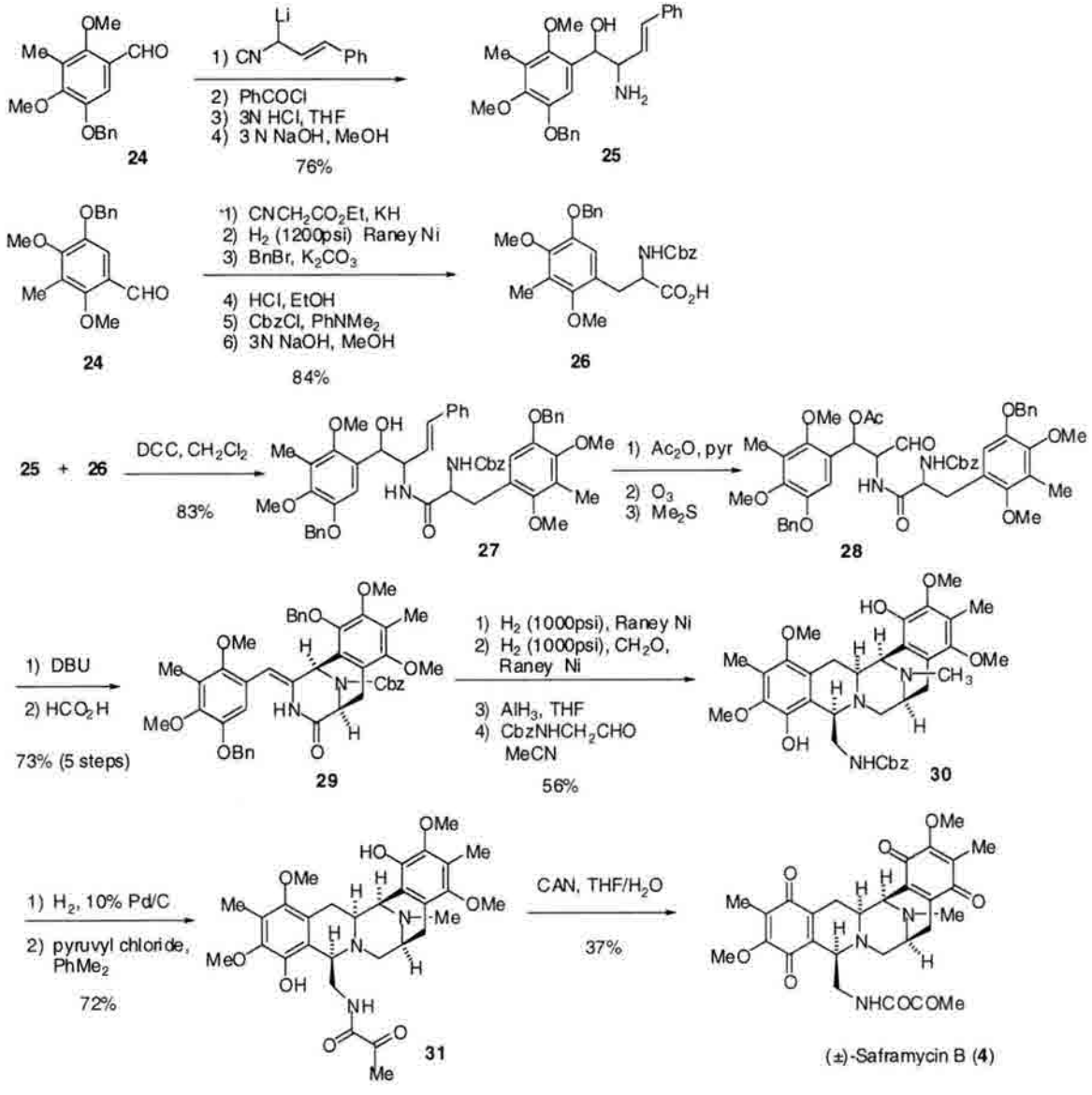
Figure 4 Biosynthetic precursors to Saframycin A

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

2.1.3. Total Synthesis

The total synthesis of (±)-saframycin B by Fukuyama and Sachleben[19] in 1982 was the first total synthesis of a member of the saframycin family. Starting with

aldehyde **24**, treatment with the lithium anion of cinnamyl isocyanide afforded the benzylic alcohol that was esterified with benzoyl chloride (Scheme 2).



Scheme 2 Fukuyama's total synthesis of (±)-saframycin B

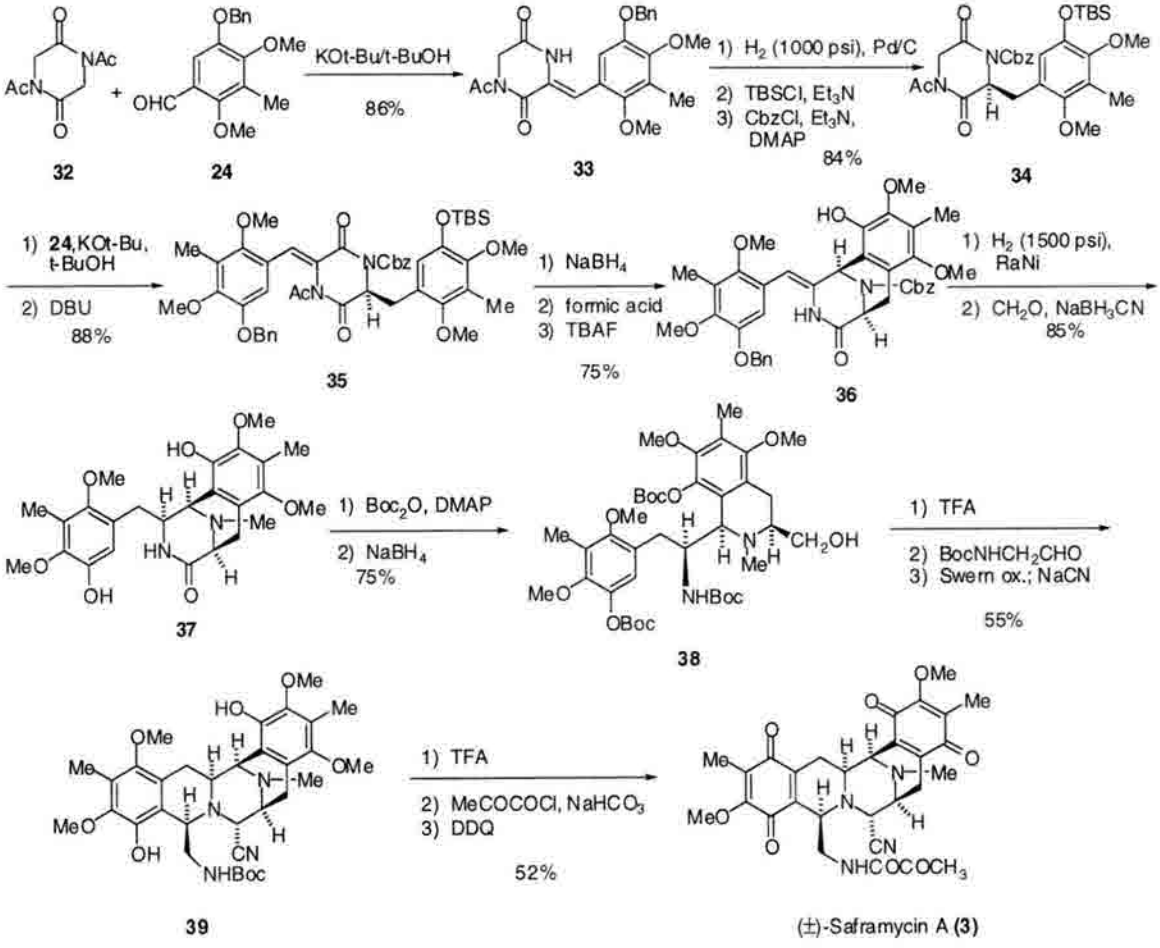
Hydration of the isocyanide followed by hydrolysis of the formamide afforded amino alcohol **25** in good yield. The A ring of saframycin B was also synthesized from aldehyde **24**. Amino acid **26** was synthesized in six steps from aldehyde **24** in 84%

overall yield through formation of the α,ß 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 tricycle **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, removal of the Cbz protecting group, and methylation of the amino group. Reduction of the lactam carbonyl using alane yielded the 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, Fukyama *et al.* were also the first to synthesize (±)-saframycin A.[20] Aromatic aldehyde **24** was treated with the potassium enolate of the diketopiperazine **32** to form **33** in 86% yield (Scheme 3). This aldol chemistry was first used by Kubo *et al.* in their saframycin B synthesis[21] (Scheme 4). These 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 a t-butyl carbamate. The lactam carbonyl was then reduced under mild conditions to afford **38**. Removal of the *t*-butyl carbamate was followed by a Pictet-Spengler reaction affording the pentacyclic core. Swern oxidation of the primary alcohol afforded the 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 total synthesis include cleavage of the *t*-butyl carbamate, amide formation using pyruvyl chloride, and oxidation of the hydroquinones to quinones using DDQ to afford (±)-saframycin A.

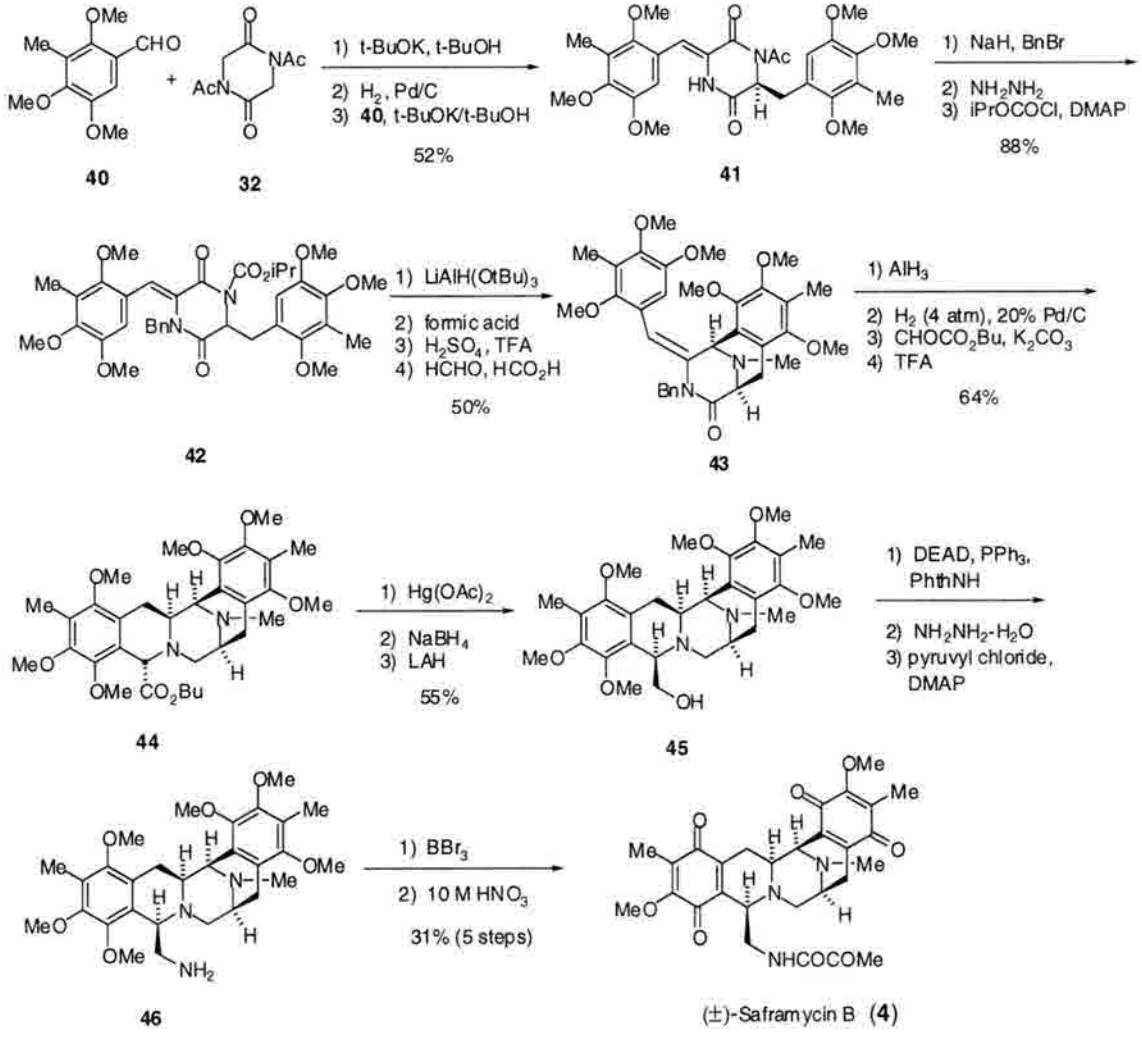
In 1987, Kubo *et al.* reported their synthesis of (±)-saframycin B (Scheme 4)[21]. Aromatic aldehyde **40** was condensed with diketopiperazine **32**, followed by hydrogenation of the benzylic olefin. A second aldol condensation provided **41** in 52% overall yield for the three steps. Activation of 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-*t*-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.



Scheme 3 Fukuyama's total synthesis of saframycin A

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 benzyl amine 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

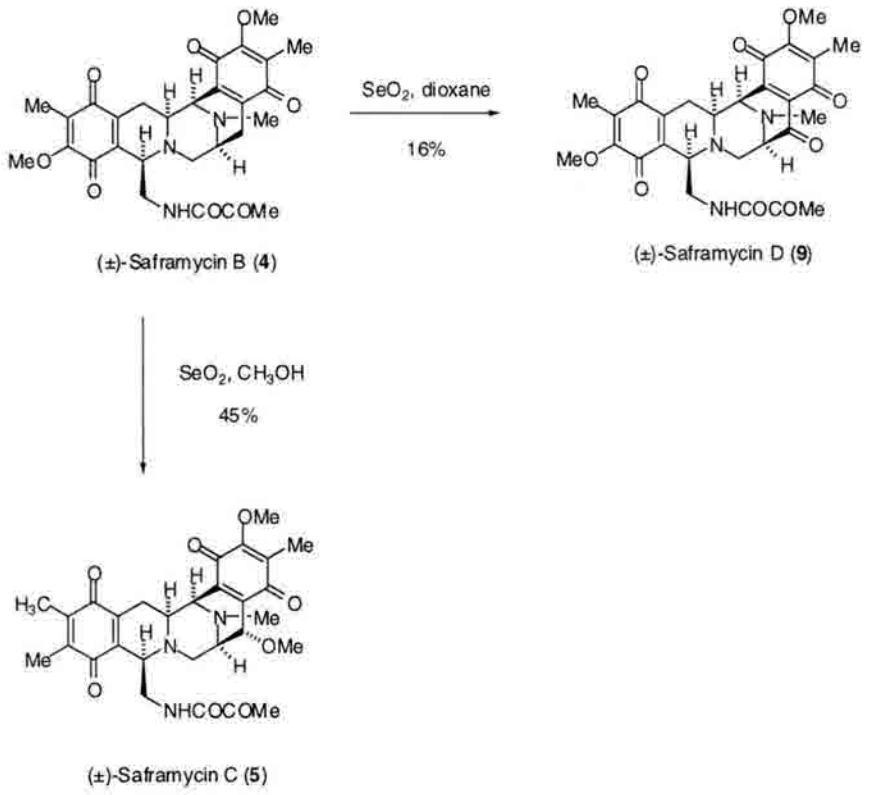
and the amine was acylated with pyruvyl chloride to yield **46**. The final two steps were demethylation of the hydroquinones using boron tribromide followed by oxidation to the diquinone using 10 M HNO_3 to provide saframycin B in 41% yield for the last two steps.



Scheme 4 Kubo's total synthesis of Saframycin B

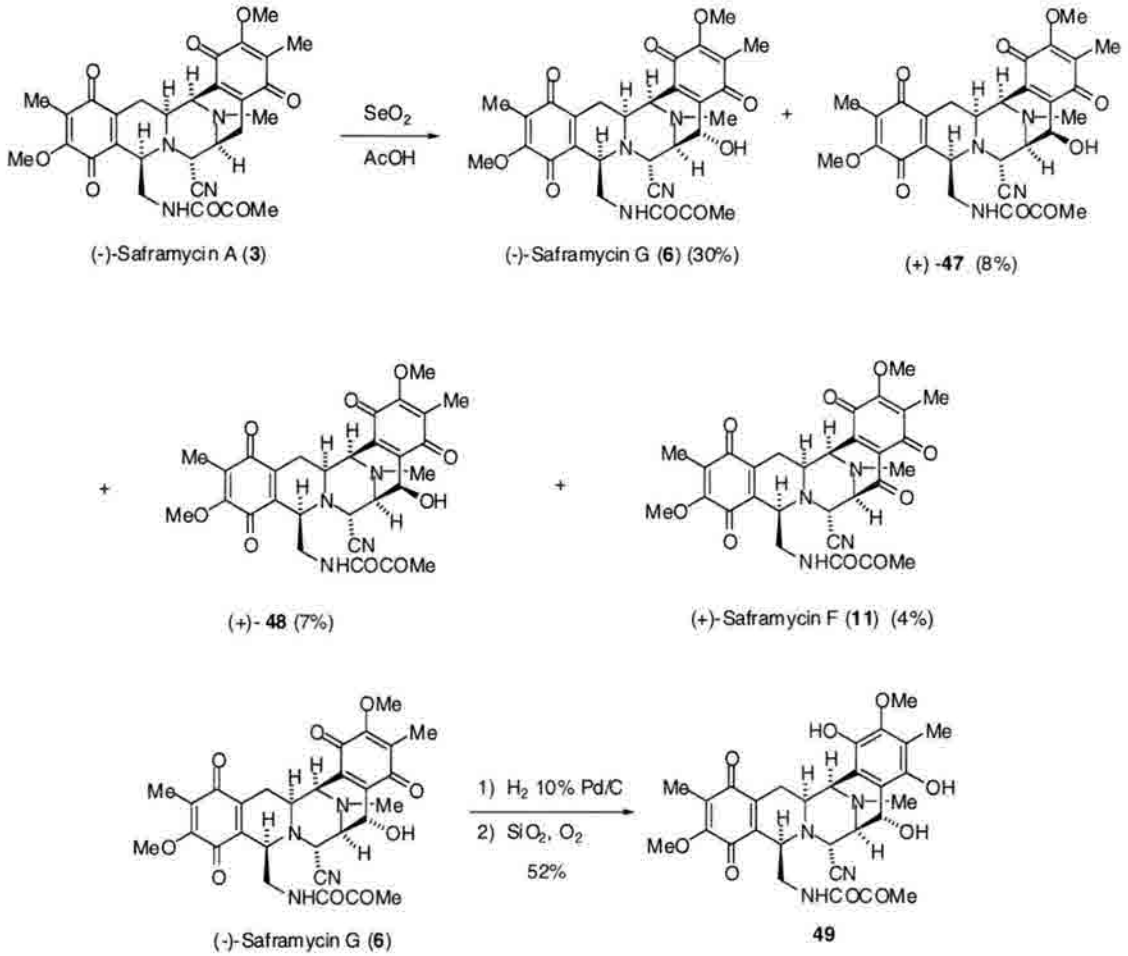
Kubo *et al.* showed that (±)-saframycin B could be converted to saframycins C (**5**) and D (**9**) via a selective oxidation using SeO_2 (Scheme 5)[22]. Using dioxane as the

solvent (±)-saframycin D was synthesized in 16% yield. The use of methanol as the solvent yielded (±)-saframycin C in 45% yield.



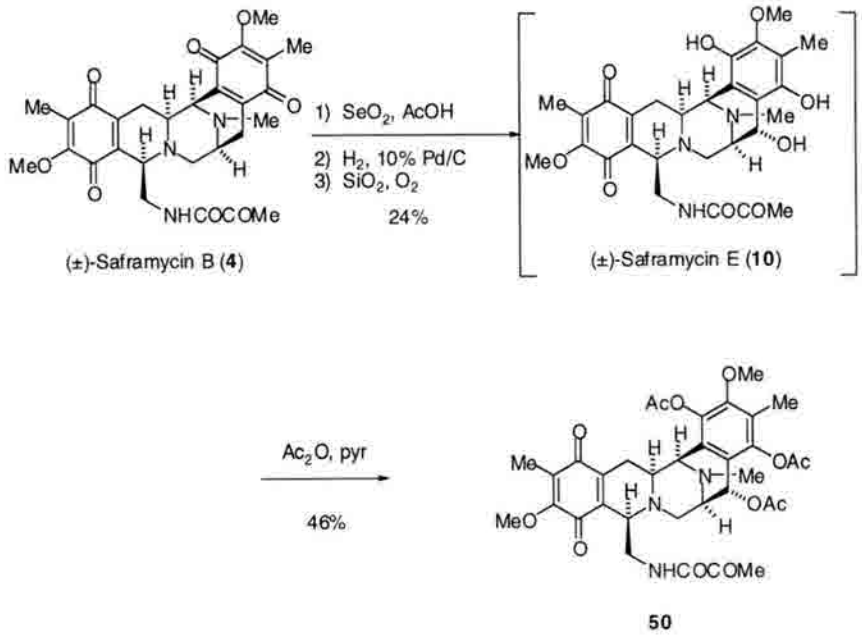
Scheme 5 Conversion of Saframycin B to Saframycins C and D

Kubo *et al.* also showed that (-)-saframycin A could be oxidized with SeO_2 to yield five saframycins (Scheme 6).[7,23] The highest yielding product was (-)-saframycin G in 30% yield. Saframycin G was then converted to the saframycin Mx type 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.



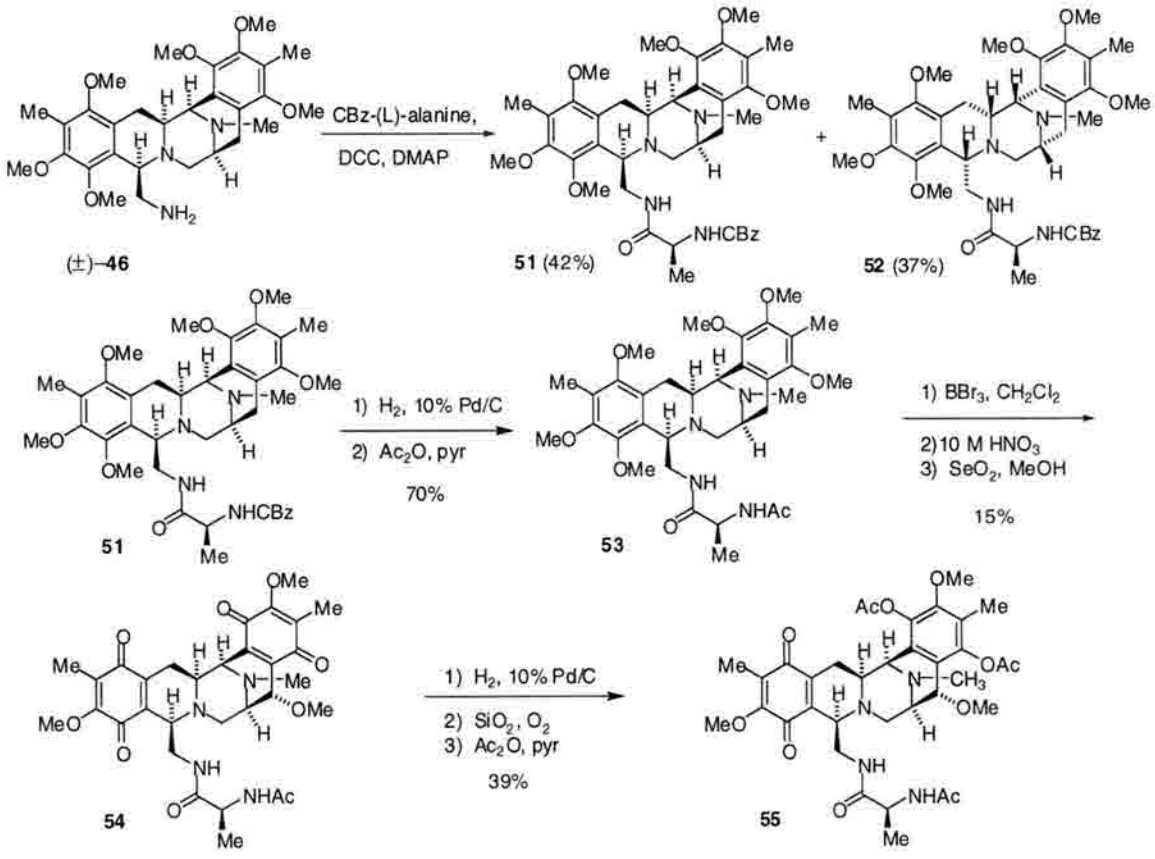
Scheme 6 Selenium dioxide oxidaiton of Saframycin A

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 7 Conversion of Saframycin B to Saframycin E

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 8).[24] The first step in the sequence was the coupling of 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 carried on separately to the final Mx2 type compound.



Scheme 8 Resolution of racemic compound **46**

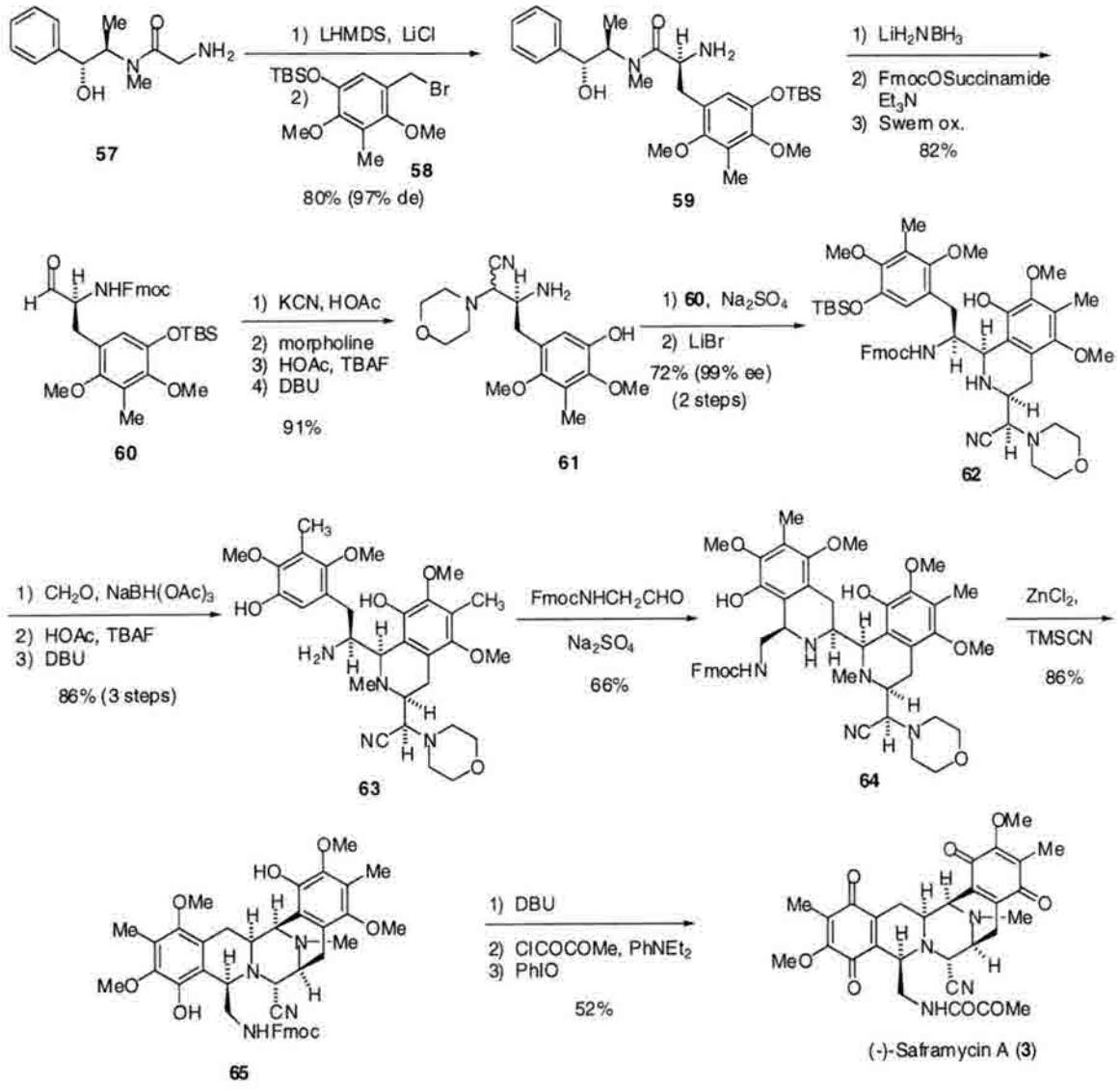
The CBz group was removed from compound **51** and the resultant amine was acylated to form **53** (Scheme 8). The hydroquinones were deprotected and oxidized to the quinones. Selective oxidation of the D ring was accomplished using SeO_2 in methanol to form the desired methyl ether **54**. Reduction of the quinones followed by regioselective oxidation of the A ring hydroquinone yielded the saframycin Mx2 type compound which was light and air sensitive. Acetylation of the hydroquinone portion yielded the stable triacetate **55**.

Pentacycle **52** was transformed into **56** via the same sequence of steps in similar yield (Scheme 9).

Scheme 9 Synthesis of ent-*epi*-Saframycin type compound

The first asymmetric synthesis of (-)-saframycin A was accomplished in 1999 by Myers and Kung.[25] This convergent synthesis focused on the 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 of the aldehyde with HCN formed the cyanohydrin which was treated with morpholine to yield the amino nitrile.[25c] This amino nitrile was used as an aldehyde protecting group. Removal of the TBS and Fmoc groups was accomplished in two steps in high yield to form amine **61**.

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



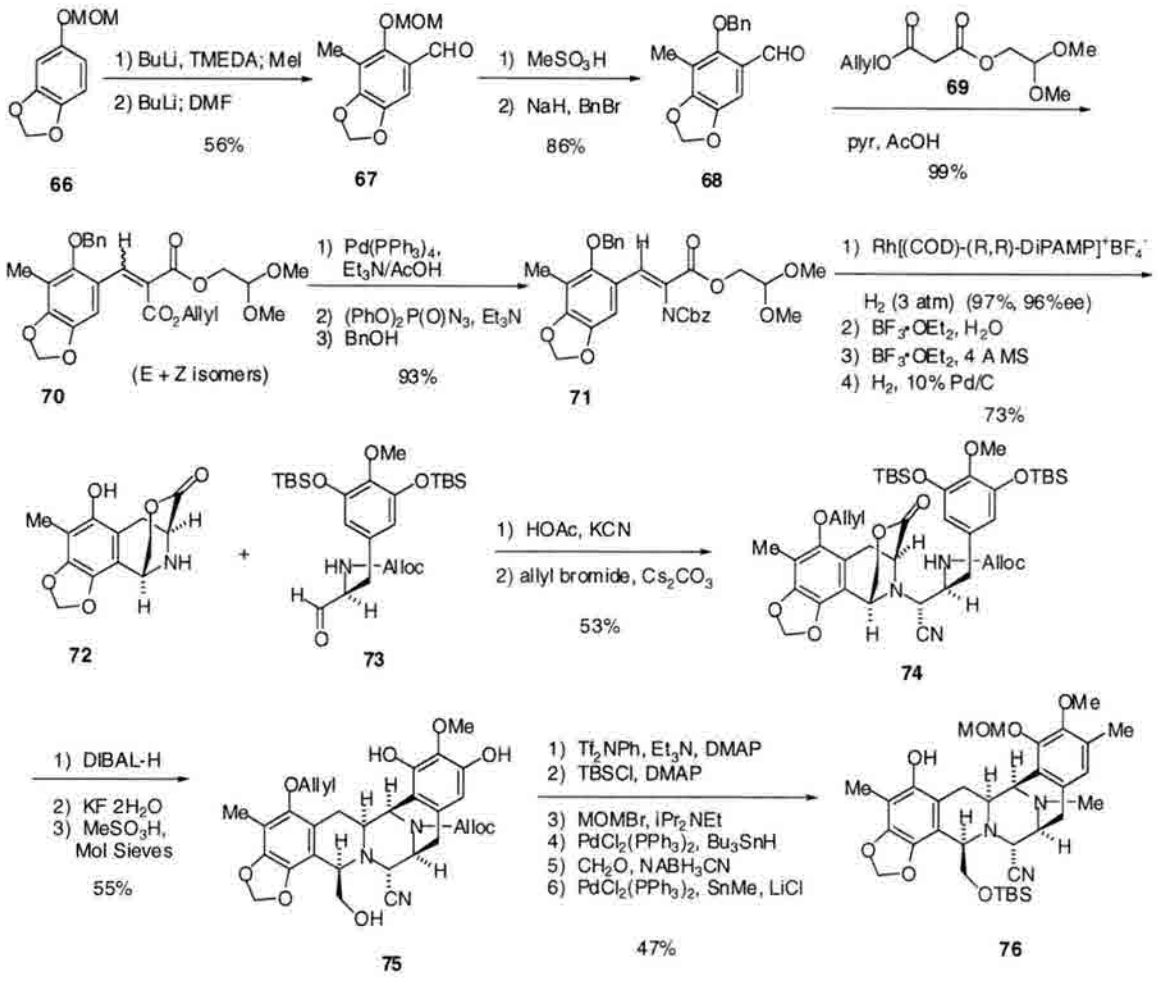
Scheme 10 Myers' total synthesis of (-)-Saframycin A

In 1999, Corey and Martinez published the second asymmetric synthesis of (-)-saframycin A.[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).

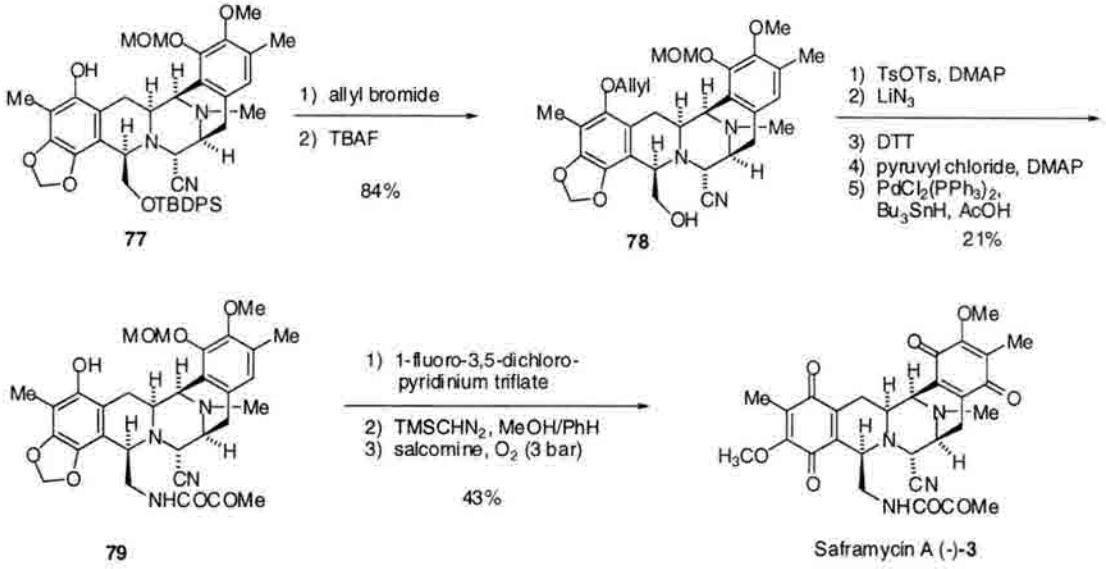
Arene **66** was methylated and formylated to form **67** (Scheme 11). Changing of the methoxymethyl protecting group to the benzyl group yielded **68**. An aldol condensation between the mixed malonate **69** and aldehyde **68** yielded a mixture of E and

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 an asymmetric hydrogenation of the benzylic olefin. This reduction yielded 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 afforded **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 that was 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.

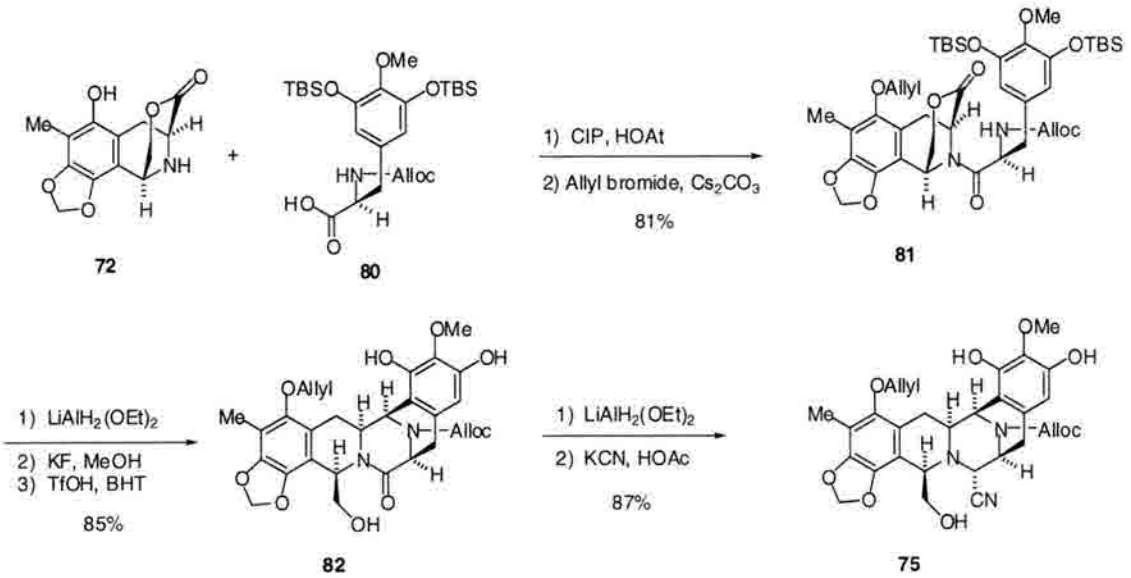


Scheme 11 Corey's synthesis of Saframycin A and Ecteinascidin intermediate **76**.



Scheme 12 Corey's Saframycin synthesis.

In 2000, Martinez and Corey reported an improved synthesis[28] of intermediate **75** used in the saframycin and ecteinascidin syntheses (Scheme 13). This synthesis improved the yield of **75** from 11% in 13 steps to 57% in 6 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.

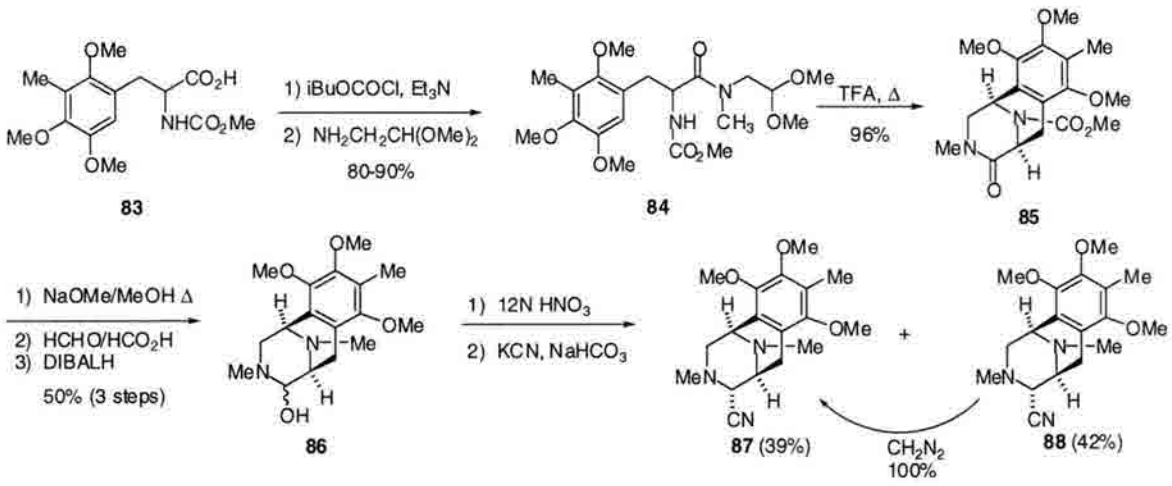


Scheme 13 Corey's improved synthesis of intermediate **75**.

2.1.4. Synthetic studies toward the saframycins

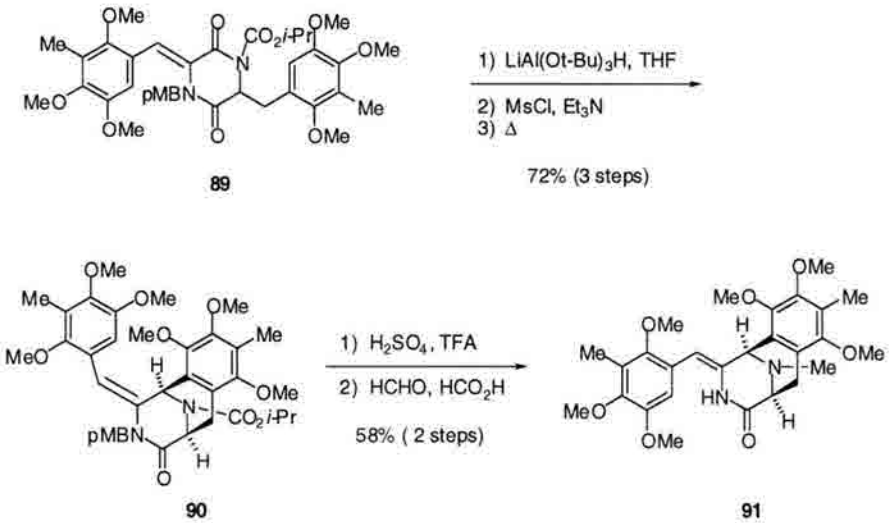
In 1982, Kurihara *et al.* reported the first synthetic studies on the saframycins.[29] Starting with the tyrosine derivative **83** the mixed anhydride was formed and condensed with aminoacetaldehyde dimethyl acetal to form **84** (Scheme 14). Heating **84** in trifluoroacetic acid afforded tricycle **85** via a double cyclization. After partial reduction of the amide using DIBALH, oxidation of the hydroqinone, followed by treatment with

potassium cyanide yielded a mixture of the desired tricycle **87** and phenol **88** in 81% combined yield.



Scheme 14 Kurihara's Saframycin A synthetic Studies

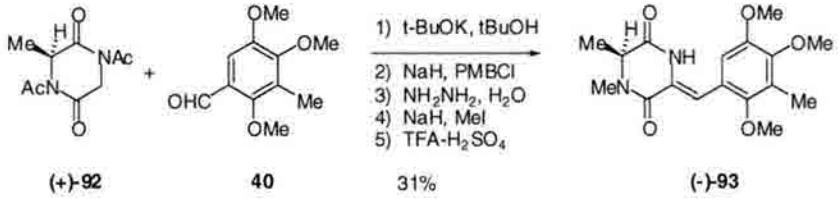
In 1989, Kubo *et al.* showed in synthetic studies towards saframycin A that tricycle **91** could be formed with the amide carbonyl intact (Scheme 15).[30] This would allow for further functionalization to form the amino nitrile in saframycin A.



Scheme 15 Kubo's synthetic studies towards Saframyicn 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

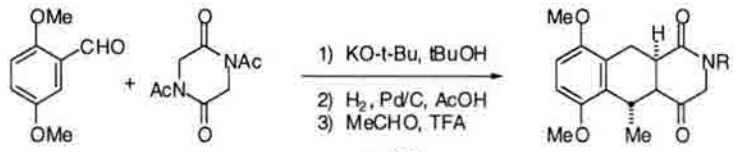
diketopiperazine (+)-**92** and aldehyde **40** yielded (-)-**93** after further elaboration. It was hoped that (-)-**93** could undergo a specific cyclization to form an optically active tricyclic compound. However, on a racemic model system, little diastereoselectivity was observed in the cyclization.



(+)-92 40 31% (-)-93

Scheme 16 Kubo's asymmetric studies towards the saframycins.

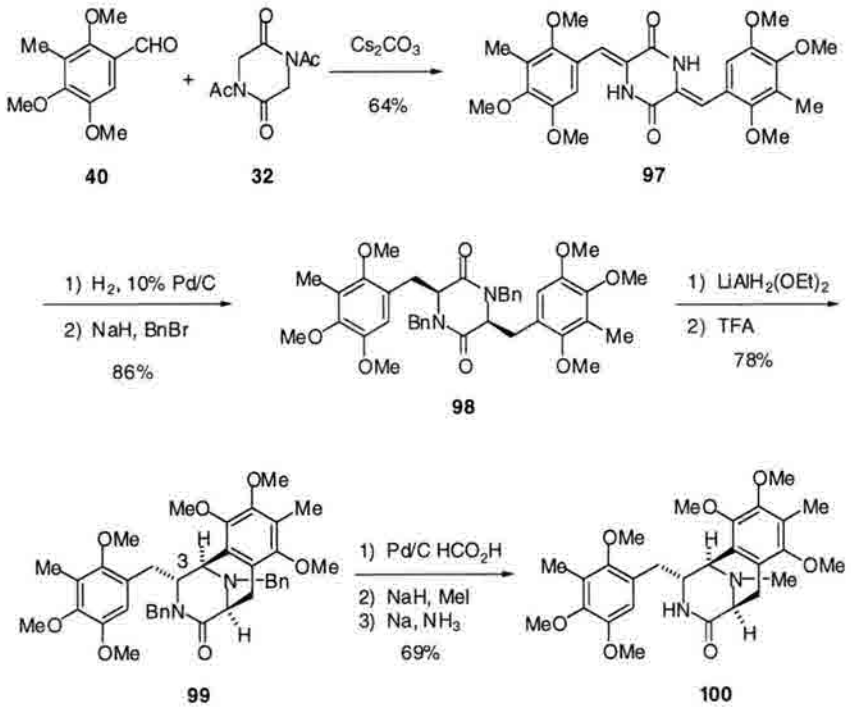
In 1990, Ong and Lee synthesized the tricycles **95** and **96** via a Pictet-Spengler cyclization on an amide (Scheme 17).[32] The major drawback to this approach was that the stereochemistry at the benzylic methine was opposite that of the saframycins.



94 32

Scheme 17 Ong and Lee synthetic studies towards the saframycins.

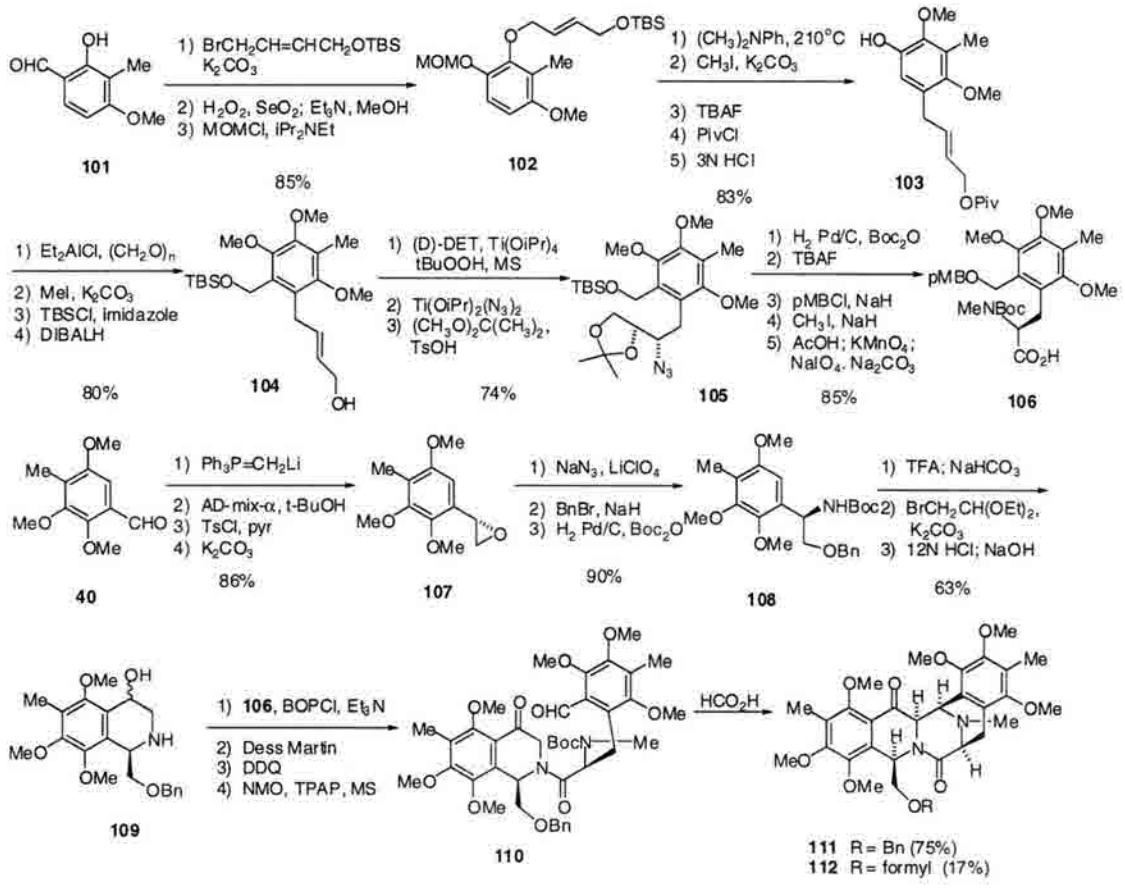
In 1991, Liebeskind and Shawe took advantage of the symmetry of saframycin B in their synthetic study (Scheme 18).[33] Condensation of two equivalents of aldehyde **40** with diketopiperazine **32** yielded the symmetrical diketopiperazine **97**. After reduction of the olefins and protection of the 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 incorrect. Attempts to invert the stereogenic center at C-3 of tricycle **100** were unsuccessful under various conditions.



Scheme 18 Liebeskind's synthetic studies towards Saframycin A.

In 2000, Danishefsky *et al.* published a route to the saframycins and ecteinascidins via a convergent intramolecular Mannich approach (Scheme 19).[34] The E ring portion was formed starting with aldehyde **101**. The phenol was alkylated and the aldehyde converted to a phenol via a Bayer-Villiger oxidation to form **102**. A Claisen rearrangement was the key step in forming **103**. Alkylation of the arene ring followed by protection of the alcohols and removal of the pivoloyl 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 carbamate. Methylation of the carbamate nitrogen was followed by the cleavage of the silyl ether and *para*-methoxyl benzyl ether formation. Oxidative cleavage of the diol afforded the N-Boc amino acid **106** in 85% from **105**.

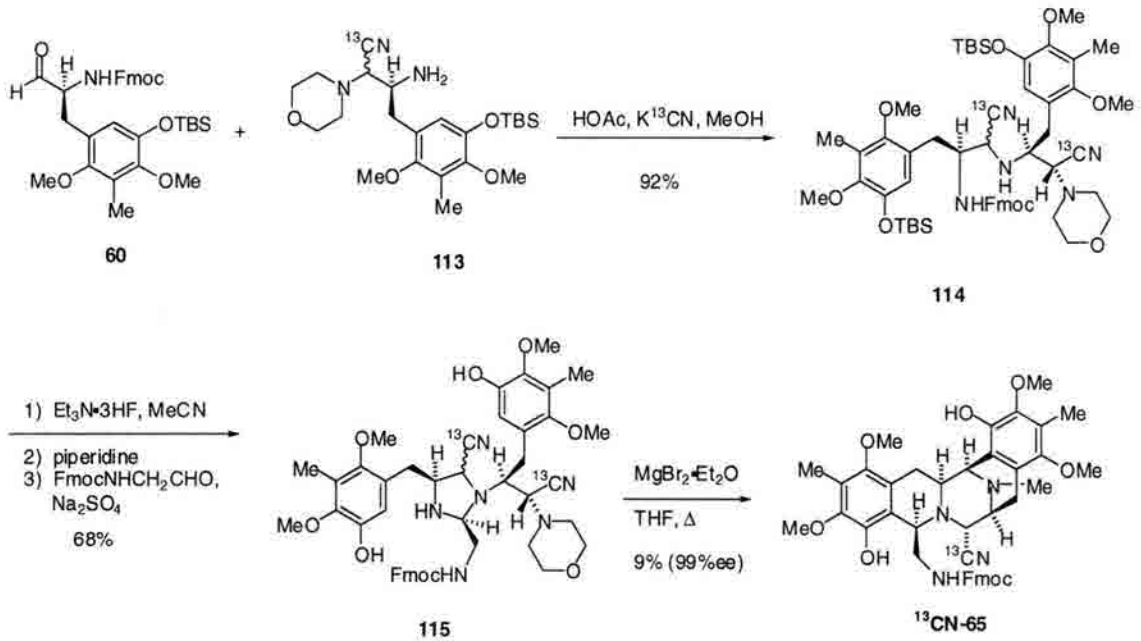
The A ring portion was synthesized in high yield starting with the olefination of aldehyde **40**. A Sharpless asymmetric dihydroxylation was used to install the desired functionality and stereochemistry. The diol was converted to the optically pure epoxide **107** via tosylation and basic ring closure. The epoxide was opened with sodium azide and the azide was reduced and protected to form the protected amino alcohol **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 bicycle **109**. The two fragments (**106** and **109**) were coupled using BOPCl in 63% yield.[34b] A sequence of oxidations lead to the formation of **110**. The final step in this synthetic study was the acidic Boc deprotection and cyclization of the resultant



Scheme 19 Danishefsky's synthetic studies towards the saframycins.

amine upon the benzylic aldehyde to form the pentacycles **111** and **112** in 75% and 17% yields respectively.

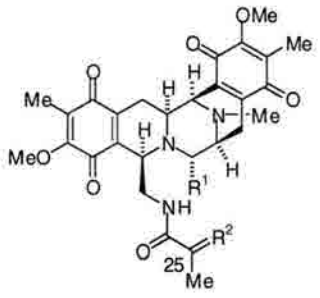
Myers and Kung showed that the pentacyclic core of saframycin A (**65**) could be constructed via a one-step cyclization from an amino aldehyde "trimer" **115** (Scheme 20).[35] The synthesis of **115** was accomplished using the same components used in their saframycin A synthesis. The condensation of **60** with amine **113** in the presence of $H^{13}CN$ afforded **114** via a Strecker reaction. 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 hours, albeit at a yield of 9%. The formation of **65** occurs via three cyclizations. Three of the five stereocenters of **65** were formed in this single step.



Scheme 20 Myers' one step synthesis of pentacycle **65**.

2.1.5. Saframycin Analogs

The first saframycin analogs were obtained by microbial bioconversions of natural (-)-saframycin A (Fig 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]

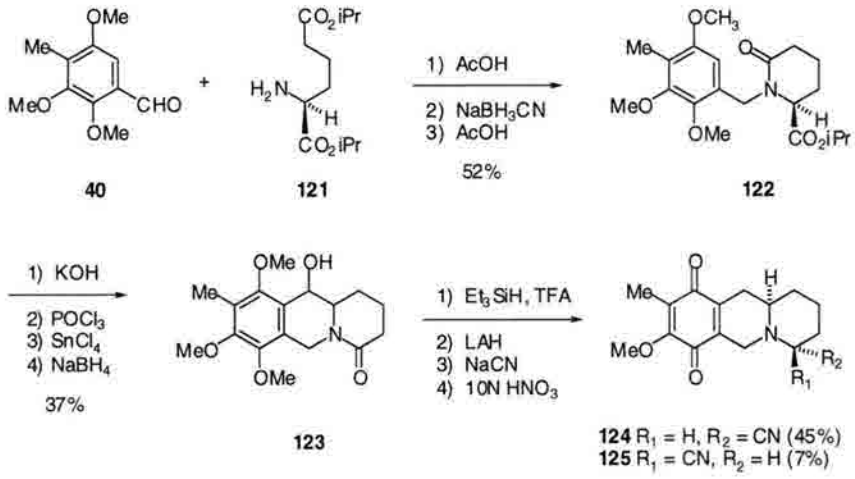


Saframycin $AR_1 = AH_2$ (**116**) R^1 = CN, R^2 = H, OH
AH_1 (**117**)
AR_3 (**118**) R^1 = H, R^2 = H, OH
AH_1Ac (**119**)
AH_2Ac (**120**) R^1 = CN, R^2 = H, OAc

Figure 5 Saframycin analogs via bioconversion and semi-synthesis.

Two simple amino nitrile analogs of saframycin A were synthesized by Kubo *et al.*[37] The first analogs synthesized were a diastereomeric pair of amino nitriles **124** and **125** (Scheme 21). 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**.



Scheme 21 Simple Saframyicin A analogs.

A second set of amino nitriles were also synthesized that contained a five membered C ring as shown in Figure 6.[38] The tricycles (**126-130**) were synthesized using the same chemistry as above.

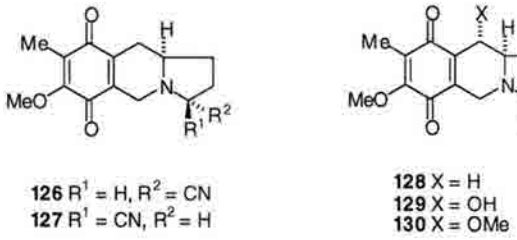


Figure 6 Simple Saframycin analogs.

2.1.6. Biology

All of the saframycins have been found to display some antitumor and antimicrobrial activities. Saframycin S had the most potent antitumor acitvity,[39] while saframycins R[8] and A[7] exhibited similar but less potent antitumor and antimicrobrial 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, displayed the lowest antitumor activity.[2]

Test Organism	3 MIC (μg/mL)	8 MIC (μg/mL)
Staph. 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

Table 1 Antimicrobrial activity of Saframycins A and S.

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

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		

Table 2 Antitumor Activity of Saframycins and analogs vs. L1210 leukemia.

Saframycin S had very potent *in vivo* activity against Ehrlich ascites tumors. 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.[40] Also, the dimers Y2b and Y2b-d had similar activities to the monomers. In a study on side chain affects on biological activity Arai *et al.* synthesized 15 acyl, 9 alkyl, and 3 carbamoyl derivatives of the C-25 amino group of saframycin Y3.[41] 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.

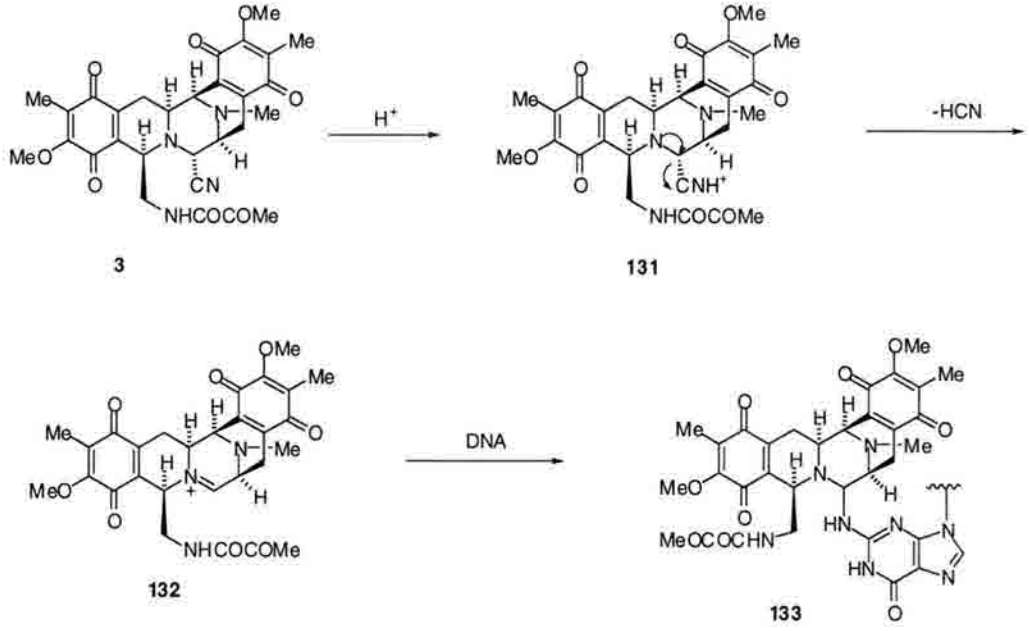
Another study on the side chain involved the bioconverted saframycins AR_1 and AR_3 along with the semisynthetic saframycin AH_1.[34a] It was found that reduction of the ketone at C-25 had no impact on antitumor activity, but there was a marked loss of antimicrobrial activity. The ED_{50} against L1210 leukemia was 0.003, 0.004, and 0.35 μg/mL for saframycins A, AR_1, and AR_3 respectively.

The simple saframycin A analogs **124-130** were also tested for their biological activity.[37,38] None of these compounds had any significant cytotoxicity with the activities of 2.0-4.0 μg/kg (ED_{50}) for L1210 murine leukemia. However, the amino nitriles **126** and **127** had 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. These inhibitions were seen at lower concentrations when saframycin A was reduced to the corresponding hydroquinone prior to testing.[42] Saframycin S does not need to be reduced for antitumor activity, but

the activity was enhanced when saframycin S was in the reduced form.[43] Reductants such as dithiothreitol (DTT) were used to activate the saframycins in DNA studies. Saframycin A in the presence of DTT would release cyanide indicating that the iminium species may have formed.

Having either a nitrile or hydroxyl group at C-21 allowed for the formation of an electrophillic iminium species that could alkylate DNA. The originally proposed mechanism (Scheme 22) for alkylation invoked the nitrile being protonated (**131**) and leaving as HCN to form the iminium ion species **132**.[44] The N-2 of guanine was then allowed to form a covalent bond resulting in a covalent adduct such as **133**.

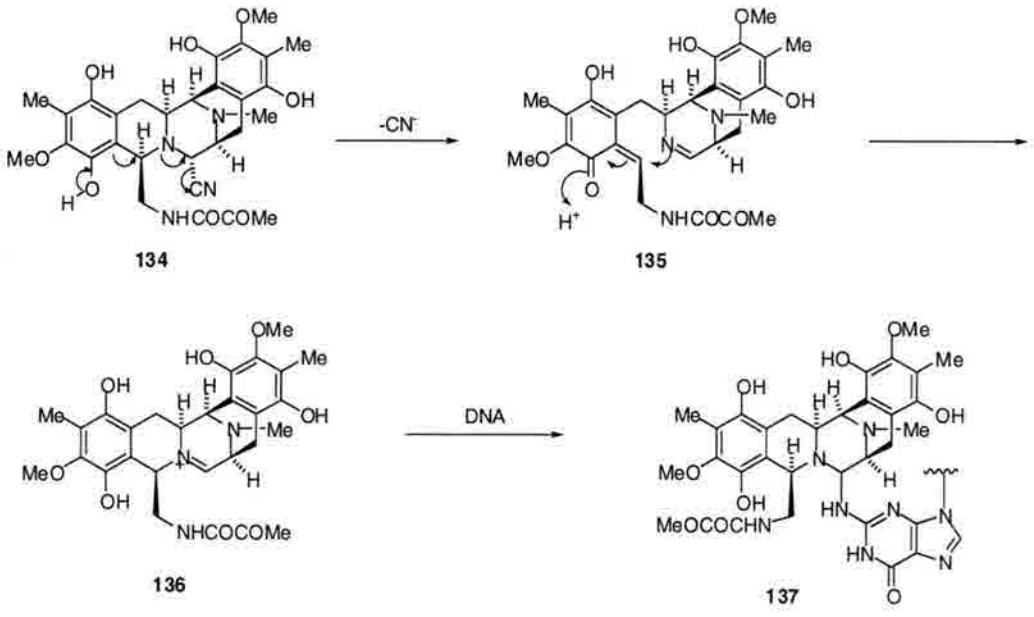


Scheme 22 Proposed mechanism of action of Saframycin A by DNA.

Evidence to support the alkylation hypothesis was obtained by radiolabling experiments in which ^{14}C-labeled tyrosine was converted to saframycin A.[43] When treated with DTT and DNA the ^{14}C label was incorporated into the DNA. When ^{14}CN was used to label the C-21 nitrile, the ^{14}C label was not incorporated into DNA. Footprinting studies have also indicated alkylation of DNA by the saframycins.

Another mechanism was proposed by Hill and Remers based on the fact that saframycin A would not alkylate DNA unless it was converted into the hydroquinone form (**134**) (Scheme 23).[45] The phenol could lose a proton causing the B ring to open which in turn eliminated cyanide. The imine **135** could attack the *ortho*-quinone methide iminium species to form iminium species **136** which could alkylate DNA to form **137**.

The characteristics of DNA binding by the saframycins can be summarized as follows: first there is reversible non-covalent binding. Secondly, there is reversible covalent binding within the minor groove of DNA. There is a second type of covalent binding that is promoted by a reducing agent.



Scheme 23 Alternate mechanism of DNA alkylation by Saframycin A.

Saframycins A and S were found to be somewhat sequence specific with preference for 5'-GGG and 5'-GGC sequences by the use of MPE (methidium propyl EDTA) Fe(II) footprinting studies.[46a] Saframycin S also had a specificity for 5'-CGG while saframycin A did not. Saframycins Mx1 and Mx3, which both contain the hydroxyl at C-21, showed the same selecitivities as saframcyin S.[46b] It was believed that the

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

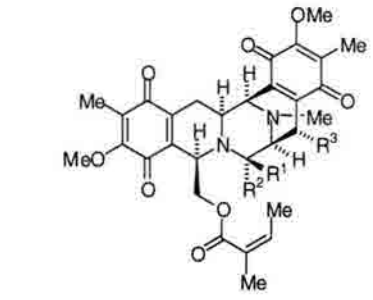
It had been argued that the cytotoxicity of the saframycins was not exclusively due to DNA alkylation. It had also been shown that the saframycins caused DNA cleavage under aerobic conditions.[44] Studies had shown that superoxide and hydroxyl radicals were formed in the presence of saframycin A in the hydroquinone form while DNA cleavage was not observed in the presence of saframycin A in the quinone form. 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 activity.[10]

2.2. Renieramycins

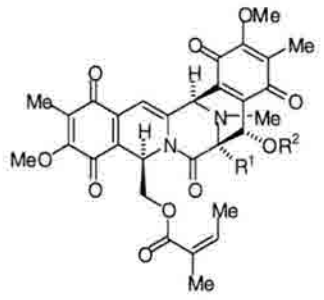
2.2.1. Isolation and structure determination

In 1982, Frincke and Faulkner isolated four new natural products from the sponge *Reniera* sp.[47] that had structures similar to the saframycins. These compounds were named renieramycin A-D **138-141** respectively (Fig. 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.[48] Renieramycins E and F were both unstable compounds. Renieramycin G (**144**) was isolated in 1992 by Davidson from the Fijian sponge *Xestospongia caycedoi.*[49] This renieramycin was found to be unstable. Two different renieramycins were isolated in 1998 by Parameswaran *et al.* from the sponge *Haliclona cribricutis.*[50] The originally assigned structures for renieramycins H and I were **145** and

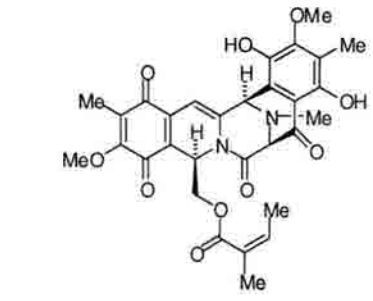
146 respectively. Recently, the structure of renieramycin H has been revised to that of **147**[51], which was also isolated from *Cribrochalina* sp. and given the name cribrostatin 4[52]. The structure of cribrostatin 4 (**147**) was determined by X-Ray crystal analysis. The benzylic olefin present is unique to renieramycin H. Due to this change in structure, the structure of renieramycin I comes in doubt. In 2000, Fontana *et al.* isolated jorumycin (**148**) from *Jorunna funebris.*[53] 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.



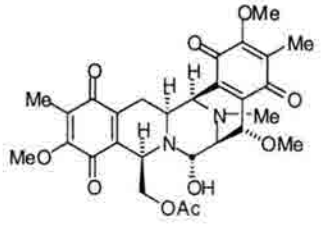
renieramycin A (**138**) $R^1=R^2=H$, $R^3=OH$
B (**139**) $R^1=R^2=H$, $R^3=OMe$
C (**140**) $R^1,R^2=O$, $R^3=OH$
D (**141**) $R^1,R^2=O$, $R^3=OEt$
E (**142**) $R^1=H,R^2=OH$, $R^3=H$
F (**143**) $R^1=H,R^2=OH$, $R^3=OMe$
G (**144**) $R^1=R^2=O$, $R^3=H$



145 $R^1=OH$, $R^2=H$
146 $R^1=H$, $R^2=Me$



renieramycin H (**147**)

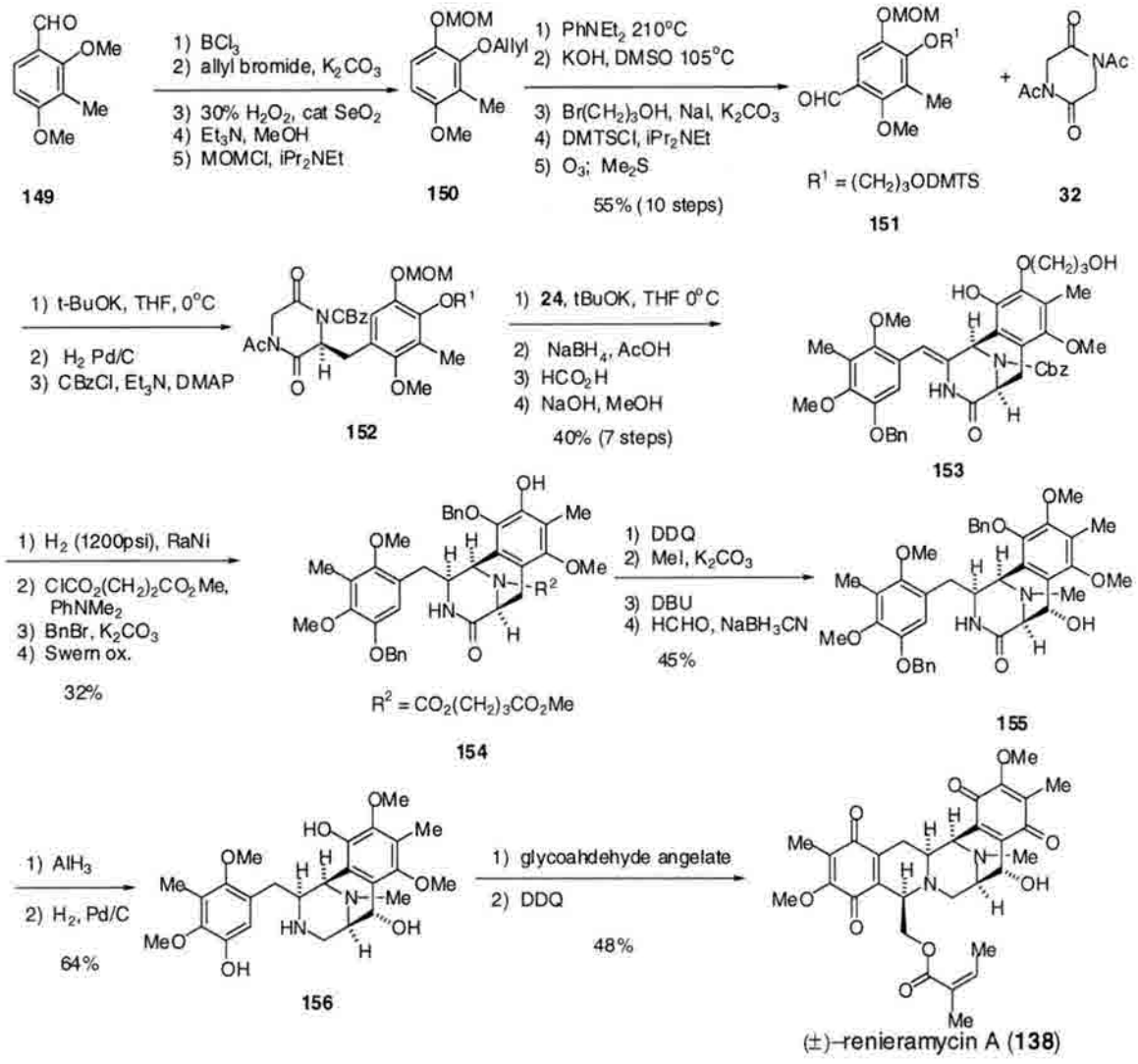


jorumycin (**148**)

Figure 7

2.2.2. Synthesis of renieramycins

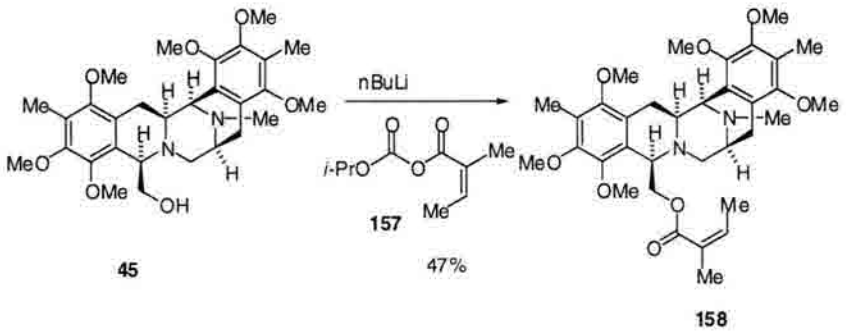
To date there has been only one total synthesis of a renieramycin. In 1990, Fukuyama *et al.* published the total synthesis of (±)-renieramycin A.[54] This synthesis used a similar strategy to that utilized in their saframycin A synthesis (Scheme 24). The main difference was that a different starting phenol was used in the E ring to allow for the necessary benzylic oxidation at C-15. The phenol was protected as a 3-hydroxypropyl ether that was further protected as the dimethylthexylsilyl (DMTS) ether. The protected aldehyde **151** was synthesized in ten 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, that when treated with formic acid cyclized with 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.



Scheme 24 Fukuyama's synthesis of Renieramycin A.

2.2.3. Synthetic Studies of the Reineramycins

Kubo *et al.* synthesized some reineramycin congeners (Scheme 25).[55] Pentacycle **45**, from their saframycin B synthesis, could be acylated with the mixed anhydride **157** to afford the angelate ester **158**. Unfortunately, they were unable to oxidatively demethylate the aromatic rings to form the quinones.



Scheme 25 Kubo's synthesis of Renieramycin Congeners.

2.2.4. Biological activity

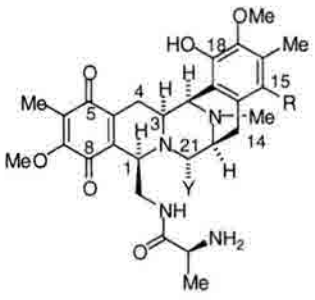
There has been little biological data reported in the literature on the biological acitivties of the renieramycins. Reineramycins A-D[47], H, and I[50] have moderate antimicrobial activities. Reineramycin G has shown moderate MIC activity against KB and LoVo cell lines with activities of 0.5 and 1.0 μg/mL respectively.[49]

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 (Fig. 8).[56] The safracins have structures very similar to the saframycins with the exception of the E ring being a phenol instead of a quinone or hydroquinone as in the saframycins. The structures were determined by comparison to spectral data for saframycin B.[56b] Soon after that, the absolute stereochemistry was determined by X-ray crystallography using **161** (C-15 bromosafracin A).[57] Meyers *et al.* also isolated safracin B (they named it EM5519) from *Pseudomonas fluorescens* SC12695.[58]

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%.[59] 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.[60]

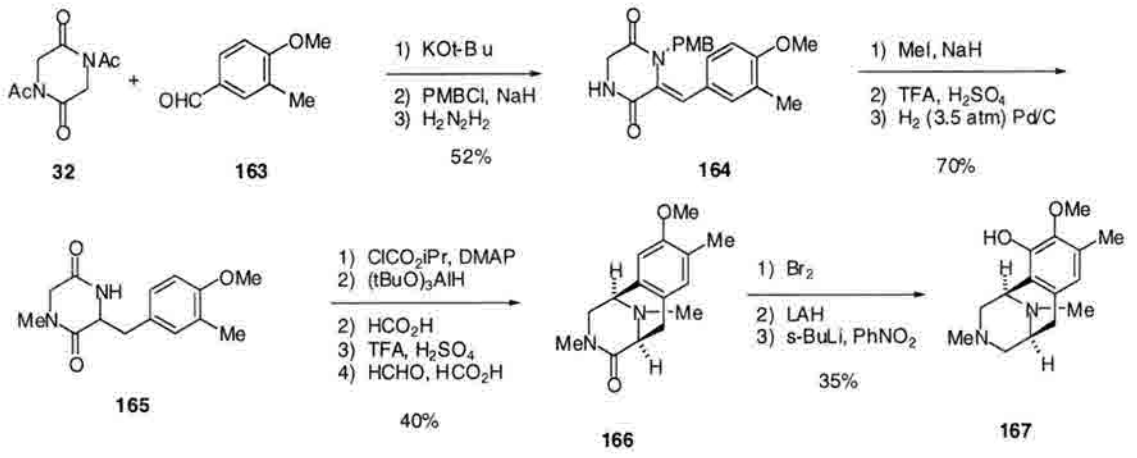


safracin A (**159**) R=Y=H
B (**160**) R=H, Y=OH
161 R=Br, Y=OH
162 R=H, Y=CN

Figure 8 Safracins A and B

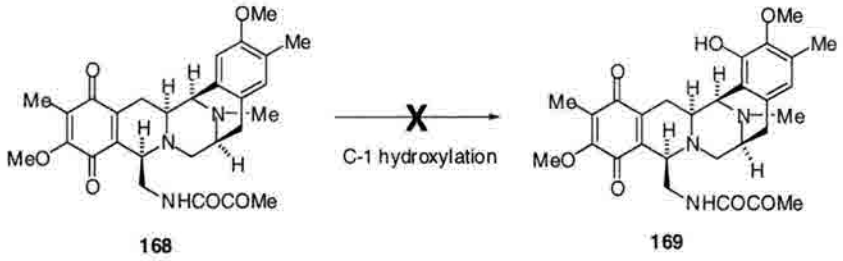
2.3.2. Synthetic studies towards the safracins

Kubo *et al.* synthesized the ABC ring of safracin B via the selective oxidation of the C-1 position (Scheme 26).[61] 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 tricycle **166**. Bromination yielded the necessary functionality at C-1. The amide was then reduced to the amine using LAH. Treatment with *sec*-butyl lithium in the presence of nitrobenzene installed the desired hydroxyl group in 53% for the final step to form **167**.



Scheme 26 Kubo's synthetic studies of Safracin B.

Kubo *et al.* then attempted the total synthesis of safracin A.[62] Unfortunately, hydroxylation was unsuccessful on the pentacycle **168** under several conditions including those used in previous model studies (Scheme 27).



Scheme 27 Attempted synthesis of Safracin A.

2.3.3. Biological activity

Safracin B was a more potent antibiotic than safracin A.[63] Interestingly, both safracins have 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*.

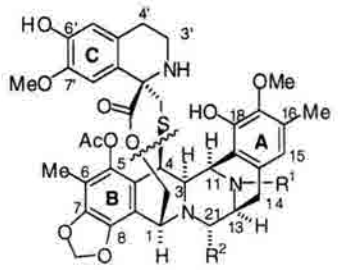
2.4. Ecteinascidins

2.4.1. Isolation and structure determination

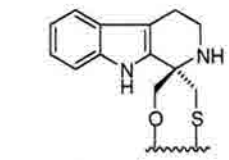
The isolation of the ecteinascidins were first reported by Reinhart *et al.* in 1990.[64] In this report, the isolation of six ecteinascidins including Et's 729, 743, 745, 759B, and 770 were reported (Figure 9). The structures for Et's 729 and 743 with the correct relative stereochemistry were reported by the Reinhart and Wright[65] 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.[66a] Crystal structures for **175** and **176** (a synthetic derivative of **171**) were also obtained to confirm the structures of the ecteinascidins.[66] Four putative biosynthetic precursors (Et's 594, 597, 583, and 596) were isolated in 1996 by Reinhart *et al.*[67] In this report, the absolute stereochemistry of the ecteinascidns was determined via determination of the stereochemistry of the derivatized cysteine residue that was cleaved from **180**.

2.4.2. Biosynthesis

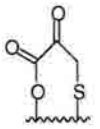
In 1995 Kerr and Miranda showed that ^{14}C labeled tyrosine and ^{35}S-cysteine were incorporated into ecteinascidin 743 via a cell free extract from *Ecteinascidia turbinata.*[68] Labeled serine was not incorporated, however.



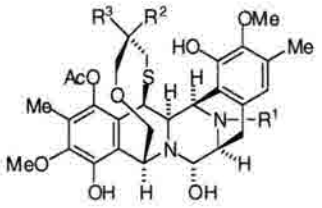
Et 743 **(170)** R^1=CH_3, R^2=OH
Et 729 **(171)** R^1=H, R^2=OH
Et 745 **(172)** R^1=Me, R^2=H
Et 759B **(173)** R^1=Me, R^2=OH, S-oxide
Et 770 **(174)** R^1=Me, R^2=CN
175 R^1=Me,O R^2=OH
176 R^1=CHO, R^2=OMe



Et 736 **(177)** R^1=Me, R^2=OH
Et 722 **(178)** R^1=H, R^2=OH



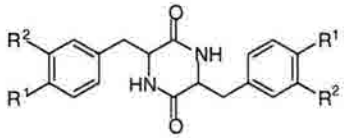
Et 594 **(179)** R^1=Me, R^2=OH



Et 597 **(180)** R^1=Me, R^2=NH_2, R^3=H
Et 583 **(181)** R^1=H, R^2=NH_2, R^3=H
Et 596 **(182)** R^1=CH_3, R^2, R^3=O

Figure 9 The Ecteinascidins

Later, Kerr *et al.* synthesized three radiolabeled diketopiperazines (**183-185**) (Figure 10).[69] 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 converted to **185** indicating that tyrosine first condenses to make **184** that then undergoes an oxidation to **185** in the biosynthetic route to Et 743.



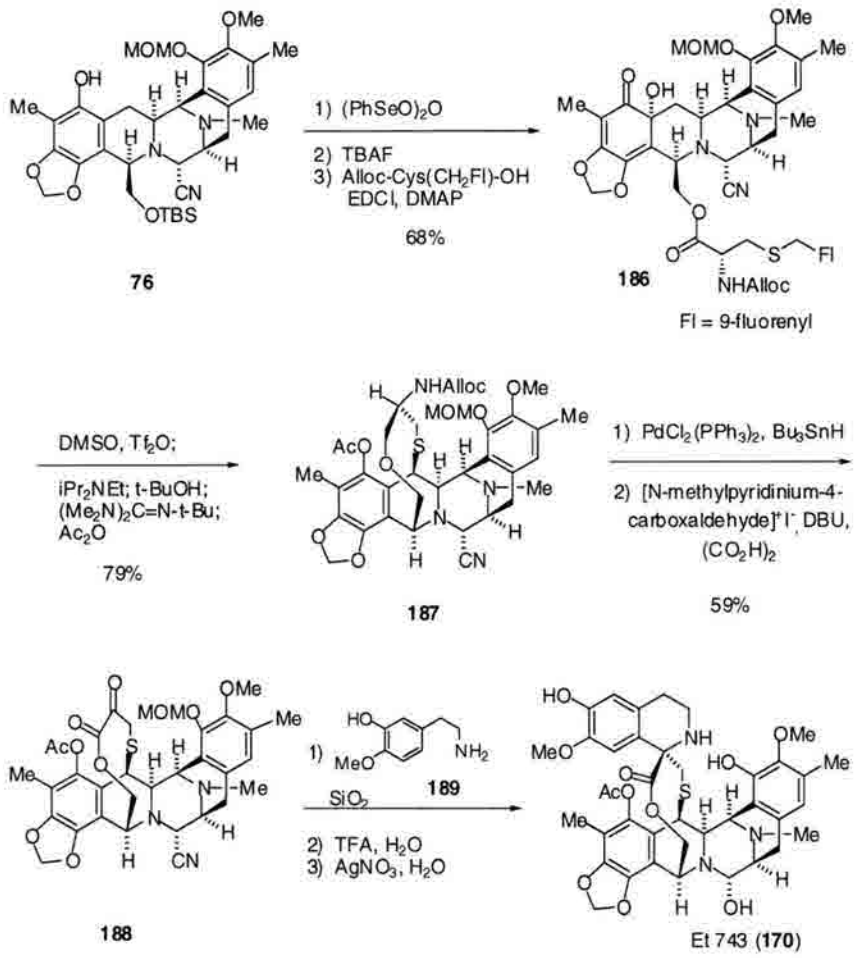
183 R^1 = R^2 = H
184 R^1 = OH, R^2 = H
185 R^1 = R^2 = OH

Figure 10 Biosynthetic Precursors to Ecteinascidin 743.

2.4.3. Synthesis 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 semi-synthetic route involving the conversion of cyanosafracin B to Et743 by Cuevas *et al* in 2000.[60]

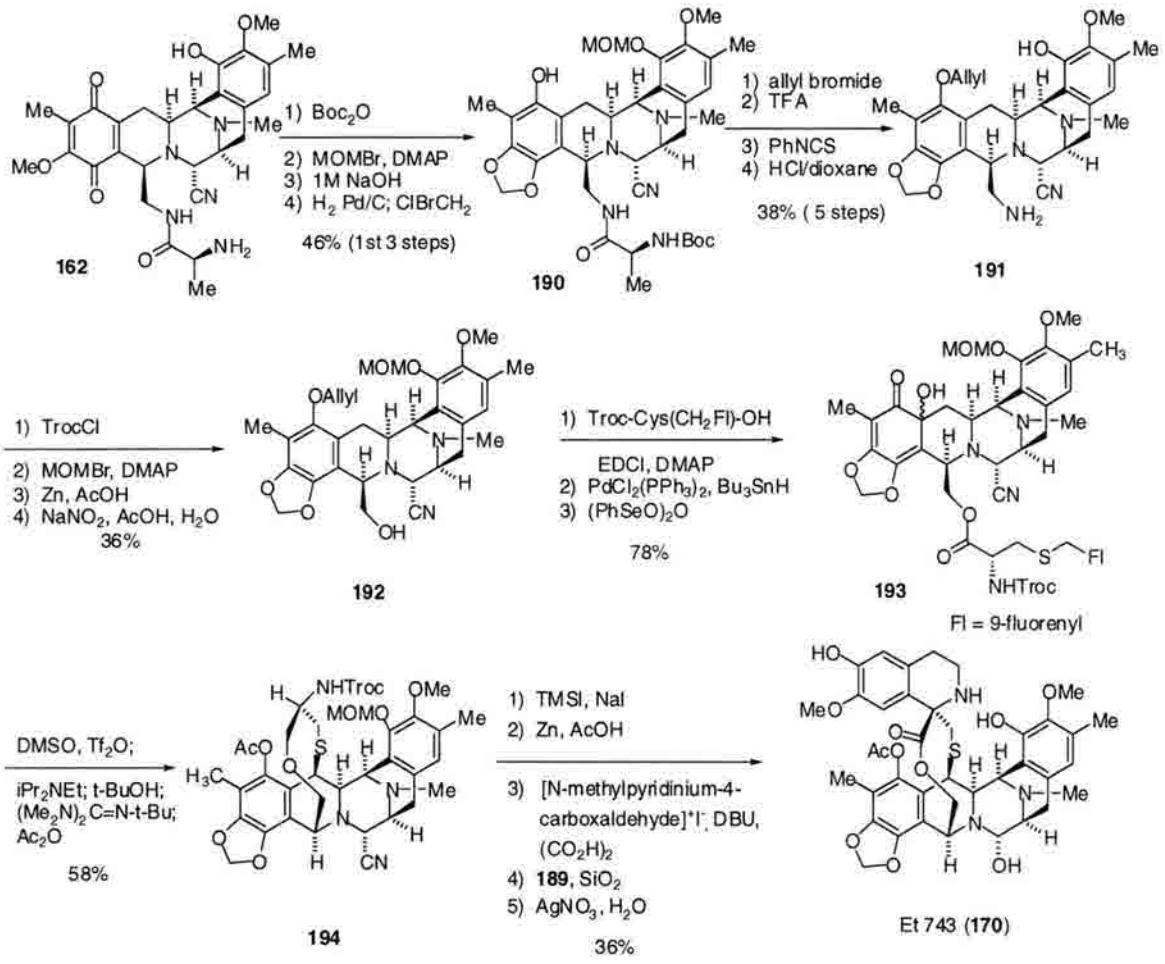
In 1996, Corey *et al.* synthesized Et 743 via a convergent synthesis employing the coupling of two optically active fragments as seen in their saframycin A synthesis[26] (Scheme 28). Starting with hexacycle **76**, a selective hydroxylation was accomplished using phenylselenic anhydride. Removal of the silyl ether followed by esterification with the diprotected cysteine 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.



Scheme 28 Corey's total Synthesis of Et 743.

Starting with cyanosafracin B (**162**), which was available in kilogram quantities via fermentation, Cuevas *et al.* were able to synthesize Et 743 (Scheme 29).[60] Cyanosaframcin B was converted to **190** via a four step sequence. Removal of the Boc group from **190** was followed by amide cleavage via an Edman degradation 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 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.

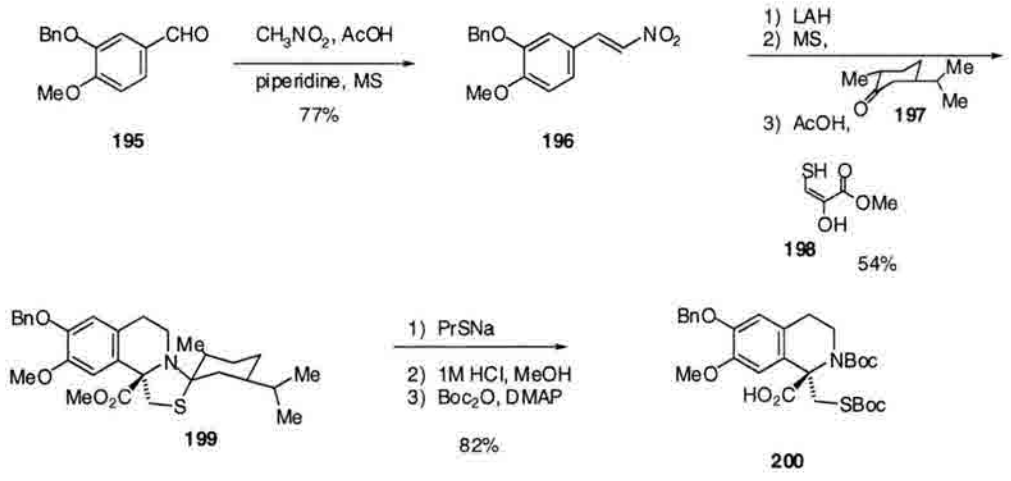


Scheme 29 Cuevas Semisyntheis of Et 743.

2.4.4. Synthetic studies

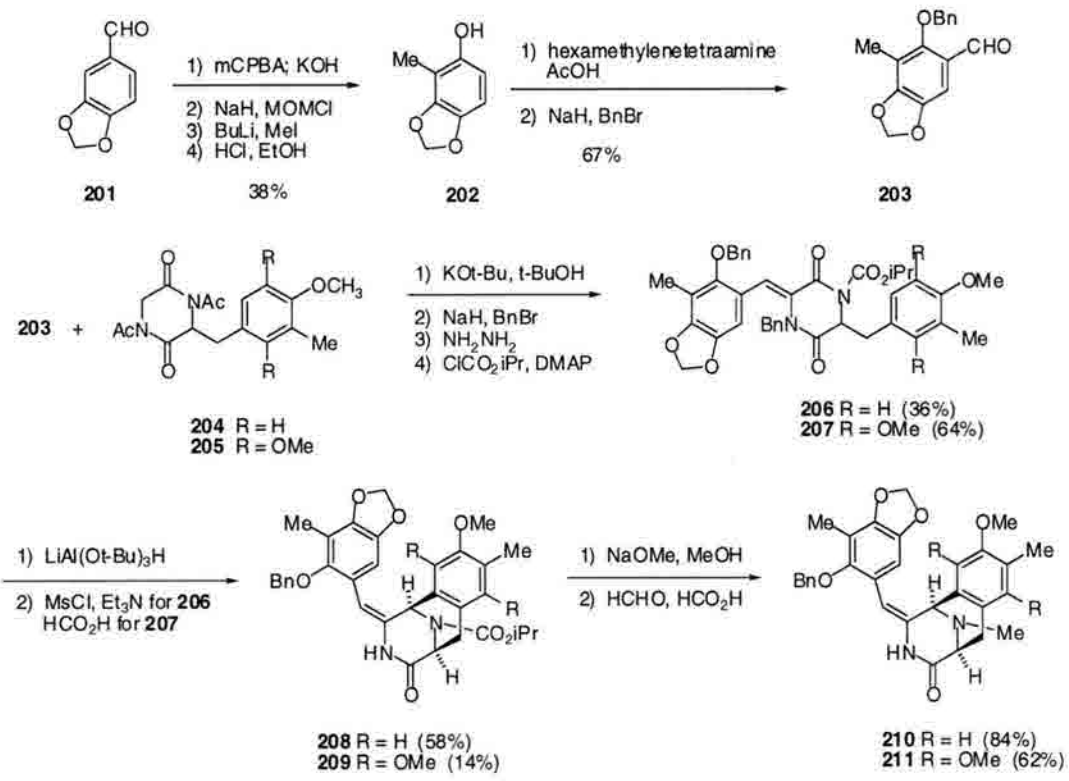
Corey and Gin reported an efficient synthesis of the tetrahydroisoquinoline C unit of Et 743 in 1996.[70] Aldehyde **195** was converted to the nitrostyrene **196** via a nitroaldol condensation (Scheme 30). Reduction of the olefin and nitro group was followed by condensation of the resultant amine with (+)-tetrahydrocarvone (**197**). This 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 auxilary and protection of the amine and thiol afforded **200** in optically pure form.



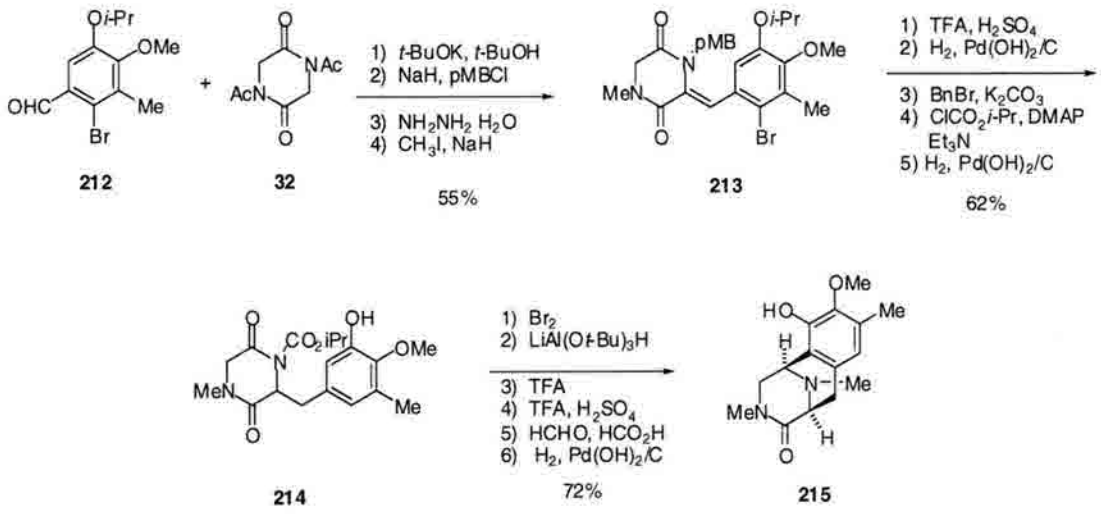
Scheme 30 Corey's synthetic studies towards Et 743.

In 1997, Kubo *et al.* published their synthetic studies towards the ecteinascidins that used chemistry similar to that deployed in their saframycin syntheses.[71] Aldehyde **201**, was converted to phenol **202** in four steps featuring a Bayer-Villiger oxidation (Scheme 31). 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 yields. These compounds were presented as possible precursors to the ecteinascidins.



Scheme 31 Kubo's synthetic studies towards Et 743.

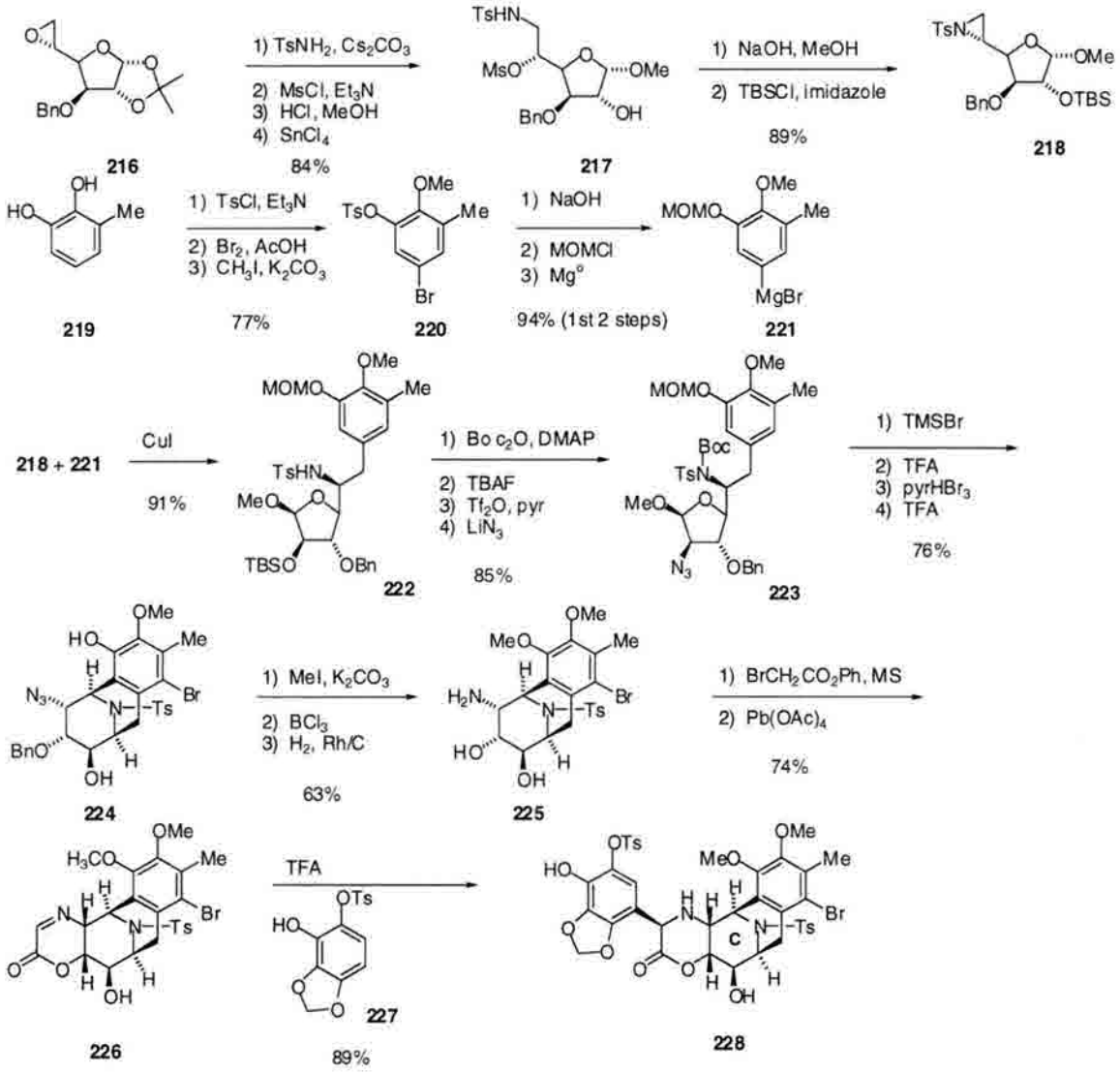
In 2000, Kubo *et al.* published a different route to the ABC ring system of the ecteinascidins.[72] Aldol condensation of aldehyde **212** with diketopiperazine **32** afforded **213** after the protection of the lactams (Scheme 32). Changing of the phenol protecting group and activation of the lactam for reduction afforded **214**. Bromination of the aromatic ring allowed for a regioselective cyclzation 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**.



Scheme 32 Kubo's synthetic studies towards Et743.

In 1999, Fukuyama *et al.* published their synthetic studies towards Et 743 starting from D-glucose (Scheme 33).[73] Epoxide **216** was available in five steps from D-glucose. Selective epoxide opening followed by mesylation and acetonide deprotection afforded a 3:2 mixture of diastereomers. Treatment with stannous chloride furnished **217** as a single diastereomer. Aziridine formation was accomplished using sodium hydroxide, and 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**. Copper catalyzed azridine ring-opening by **221** afforded **222** in 91% yield. Protection of the sulfonamide followed by alcohol deprotection yielded the free alcohol. Activation and azide displacement of the triflate resulted in **223**. Deprotection of the MOM ether and Boc groups was followed by bromination *para* to the phenol to block that position during the subsequent acidic cyclization. This cyclization proceeded through an iminium intermediate affording tricycle **224** as a single

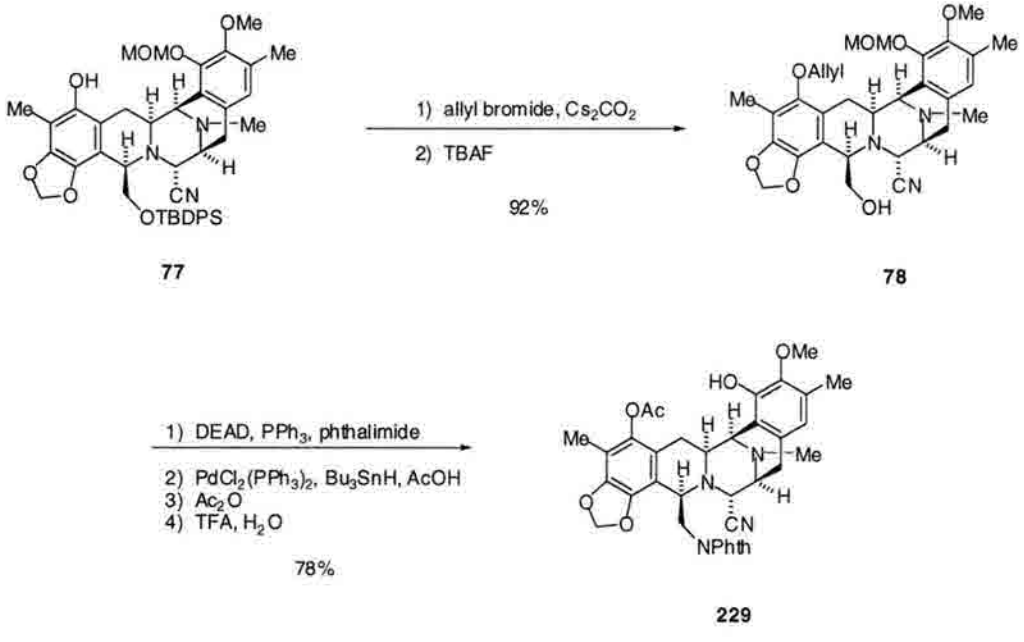
stereoisomer. Protection of the phenol was followed by benzyl ether cleavage and azide reduction to afford amino diol **225**. Amino lactonization was followed by lead tetracetate oxidation to form dehydrooxazinone **226** in 74% from **225**. Acidic alkylation of **226** with phenol **227** afforded **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 to the dialdehyde. Closure of the B and C rings would then afford the pentacyclic core of the ecteinascidins.



Scheme 33 Fukuyama's synthetic studies towards Et 743.

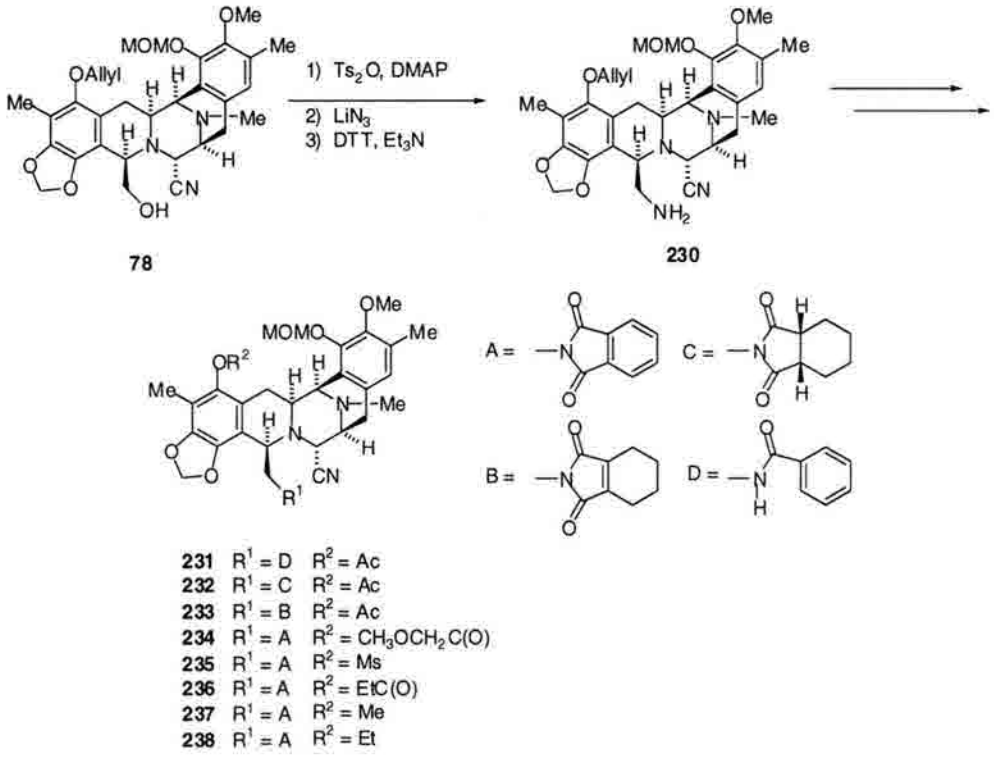
2.4.5. Ecteinascidin Analogs

Corey *et al.* reported the synthesis and biological activity of analogs of Et 743 (Scheme 34).[74] In this study phthalascidin **229** (Pt 650) was synthesized, which was surprisingly found to have comparable biological activity to Et 743. The synthesis of phthalascidin started from **77**. Allylation and removal of the silyl protecting group afforded **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**.



Scheme 34 Corey's synthesis of phthalascidin 650.

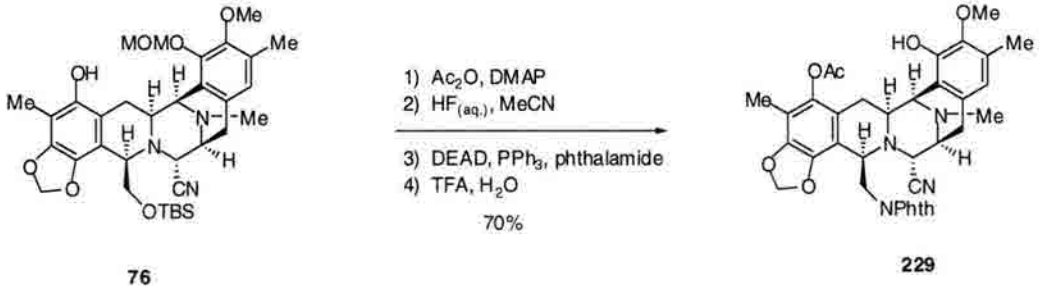
Several other ecteinascidin analogs were also synthesized (Scheme 35). These analogs were formed by the conversion of alcohol **78** to amine **230** followed by amide or succinimide formation affording **231-238**.



231 R^1 = D R^2 = Ac
232 R^1 = C R^2 = Ac
233 R^1 = B R^2 = Ac
234 R^1 = A R^2 = $CH_3OCH_2C(O)$
235 R^1 = A R^2 = Ms
236 R^1 = A R^2 = EtC(O)
237 R^1 = A R^2 = Me
238 R^1 = A R^2 = Et

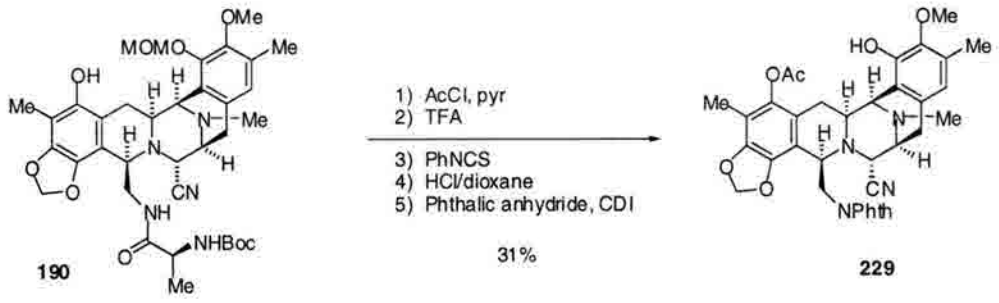
Scheme 35 Pthalascidin Analogs

In 2000, Corey and Martinez published a shorter synthesis of **229** (Scheme 36).[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%.



Scheme 36 Corey's improved synthesis of phthalascidin 650

In 2000, Cuevas *et al.* along with their Et 743 synthesis published a short synthesis of Pt-650 from an intermediate in the Et 743 synthesis (Scheme 37).[60] Starting with **190**, Pt 650 was synthesized in five steps in 31% yield.



Scheme 37 Cuevas synthesis of phthalascidin 650

2.4.6. Biology

The ecteinascidins have the most potent biological activities by far 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 making for a new class of antitumor agent that could be active against resistant cell lines. Et 743 is currently in phase II clinical trials.[75]

The *in vitro* activities of Et 743 against several common tumor cell lines were very high (Table 3).[1i]

Tumor type	**IC50 (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 3 Activity of Et 743 against several tumor cell lines

Et 729 has shown higher *in vivo* activities against P 388 leukemia than Et 743 and Et 745 (Table 4).[76] 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.[77]

Compound	Dose (μg/kg)	T/C
171	3.8	214
170	15	167
172	250	111

Table 4 Activities of Et's 729 (**171**), 743 (**170**), and 745 (**172**) against P388 leukemia

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.[66a] Et 722 was also highly active *in vivo* against a variety of cell lines (Table 5).

Tumor type	Dose (μg/kg)	T/C
P388 leukemia	25	>265
B16 melanoma	50	200
Lewis lung carcinoma	50	0.27
LX-1 lung carcinoma	75	0.00

Table 5 Activity of Et 722 against several tumor cell lines.

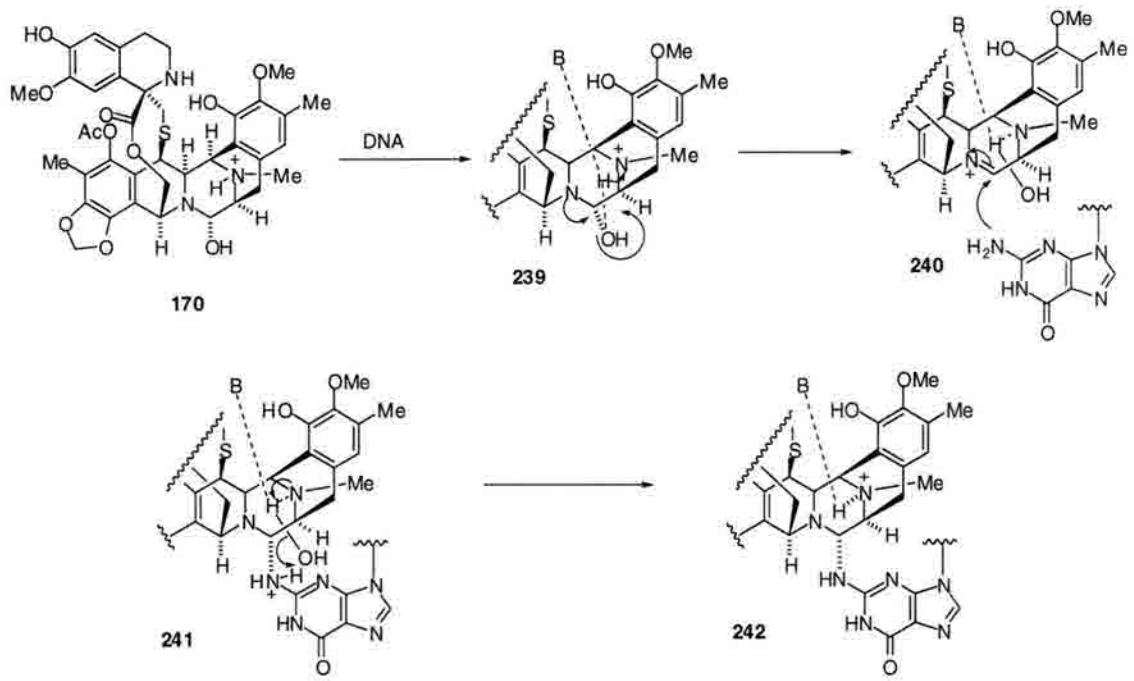
Valoti *et al.* treated several human ovarian carcinoma zenografts that were chacterized by behaviors and drug responsiveness vs. cisplatinum (DDP) with Et-743.[78] Et 743 was found to be very active against the HO22-S cell line (sensitive towards DDP). Et-743 also induced long-lasting regressions against HOC18 (marginally sensitive to DDP). The HOC18 xenograft (nonresponsive to DPP) showed significant growth delay, but for MNB-PTX-1, a highly resistant tumor towards 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 nM, 30 nM, and 100 nM respectively.[li] Et 743 has a similar structure to that of

saframycin S indicating that DNA alkylation would indeed be possible. DNA alkylation has been studied by Pommier *et al.*[79] and Hurley *et al.*[80] The alkylation takes place in the minor groove, as does alkylation with the saframycins. This alkylation causes a bend or widening of the minor groove[80e] in the DNA presumably due to the C-subunit of the ecteinascidins. This 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 acitivties of the ecteinascidins.

DNA alkylates the ecteinascidins via the N-2 of guanine in GC rich regions.[72] The alkylation has been shown to be reversible with DNA denaturization.[79] Replacement of guanine with inosine eliminates DNA alkyation. The unique sequence specificities of Et 743 has been shown to be 5'-GGG, 5'-GGC and 5'AGC. Hurley *et al.* have postulated that the sequence specificity arises from the A and B subunits of Et 743.[80a]

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.[80b] From this a mechanism for DNA alkylation was suggested (Scheme 38). The N-12 of Et 743 was protonated which allowed for explusion of the hydroxyl group in the form of water to form the iminium **240**. The guanine could then attack the iminium intermediate. Following proton loss of the guanine the covalent adduct **242** was formed. NMR studies agree with this final DNA-Et743 adduct being protonated at N-12.



Scheme 38 Proposed alkylation of Et 743 by DNA

A molecular modeling study by Gago *et al.* of the DNA Et 743 or Pt 650 adduct revealed widening of the minor groove and a positive roll in the DNA towards the major goove.[81] The widening of the minor groove was speculated to be due to specific hydrogen bonding 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.

It has also been shown that Et 743 disorganizes the microtubule network of tumor cells.[82] This type of microtubule disorganization was apparently unique to the ecteinascidins. Et 743 did not react directly with tublin; however, a decrease in fibres 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).[74,80e,83] The cross link was found to have a different sequence specificity than that of other known Topo I cross linking agents.[83] It was believed that the C subunit, which protrudes from the DNA, interacts with the Topo I.[80e] The crosslinking was seen at much higher Et 743 concentrations than are necessary for its antitumor activity indicating that the crosslink to Topo I was not the primary mode of action. This was also observed in studies where camptothecin-resistant (a known Topo I crosslinking compound) mouse leukemia P388/CPT45 cells were suceptible to Et743.[84]

Another mode of action, which has been implicated at biological concentrations, was the interaction between the Et 743 DNA adduct and DNA transcription factors.[85] 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 resemblence of NF-Y compared to histones H2A and H2B, nuclesome 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 were also found to be inhibited at low concentrations of Et 743.[86] The NF-Y protein still bound to the DNA, but it was believed that the Et 743 distorts the DNA protein interactions. These interactions may not be disrupted directly but rather by the distruption of an unknown cofactor. This was demonstrated in the study of the binding of the MDR1 promoter with NF-Y.[87] These observations show that the mode of action of Et 743 was different than any known antitumor compound.

Another study by Pommier *et al.* showed at micromolar concentrations Et 743 caused protein-linked DNA single strand breaks.[84] No sign of double stranded breaks was observed. At 10nM concetrations, Et 743 induced an accumulation of cells in the S and G_2-M cell cycle phases after 14 hours. After 24 hours there was an accumulation in the G_2-M phase. This profile was consistent with other DNA alkyating 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 Et743 without any further distortion of the DNA. This indicates that Et743 may target the sites where DNA was bound to zinc fingers of a transcription factor such as Sp1.[88]

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.[89] 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.

The biological activity of the pthalascidin analogs were similar to that of Et 743 (Table 6).[74] This was an important observation due to the fact that **229** was much easier to synthesize than Et 743.

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

Table 6 Activities of pthalascidin analogs vs various tumor cell lines.

3. Naphthyridinomycin Family

3.1 Naphthyridinomycin, Cyanocycline, and Bioxalomycin

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.[90] The structure was determined via single crystal X-ray analysis.[91] In 1976, SF-1739 was isolated by Watanabe *et al.*[92] At the time the structure was not determined, but due to the analog synthesized, SF-1739 HP (**244**), it has been assumed that SF-1739 was actually naphthyridinomycin.[93]

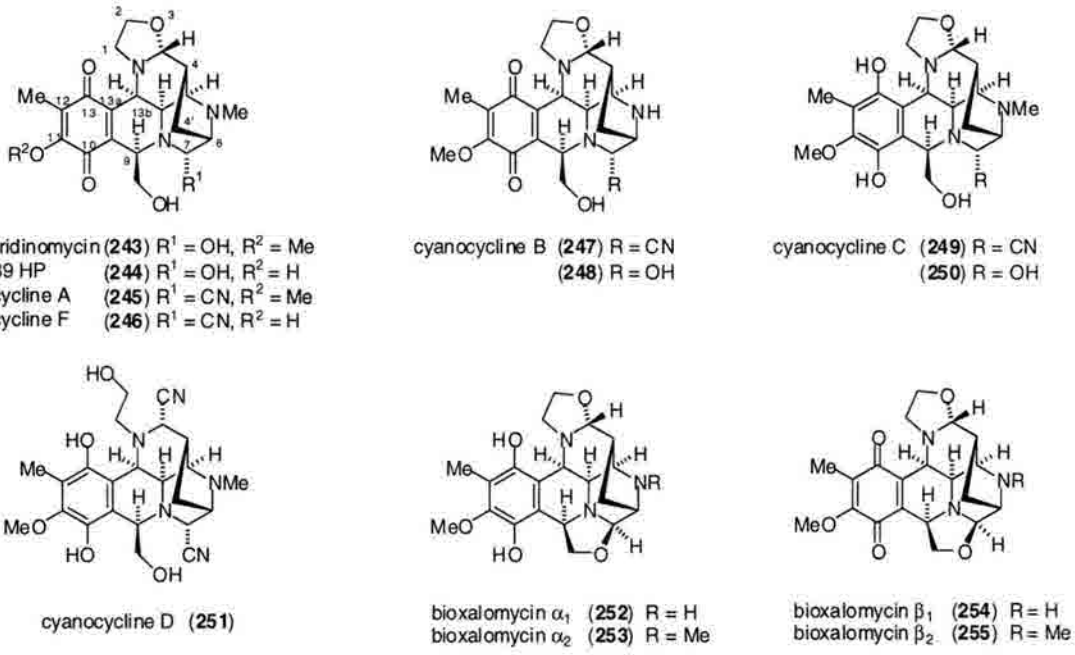


Figure 11 Naphthyridinomycin, Cyanocyclines, and Bioxalomycins.

Treatment of the extraction broth of *Streptomyces lusitanus* with sodium cyanide afforded a more stable product cyanonapthyridinomycin[94] (**245**) (cyanocycline A). Shortly thereafter, cyanocycline A was isolated from *Streptomyces flavogriseus.*[95] The structure was determined by single crystal X-ray analysis along with the crystal structure of cyanocycline F (**246**).[96] 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.*[97] 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 from *Streptomyces viridostaticus* ssp *litoralis*[98] bringing into question the true structure of the natural product originally believed to be that of **243**. The isolation procedure was milder than that used for the original isolation of napthyridinomycin. Isolation of natural products from *Streptomyces lusitanus* (NRRL8034) under these conditions did not result in the isolation of naphthridinomycin.[98b] Also, attempts to repeat the original naphthyridinomycin isolation procedure lead only to the procurement bioxalomycin β_2 indicating that the true natural product may be in fact bioxalomycin β_2 instead of naphthyridinomycin. It was thus postulated that naphthyridinomycin may in fact be an artifact of isolation.

3.1.2. Biosynthesis

In 1982, Zmijewski *et al.* showed that ^{14}C labeled L-tyrosine (**21**), L-methionine (**22**), glycine (**256**), and dl-ornithine (**258**) were incorporated into cyanocycline A (Figure 11).[99] In 1985, Zmijewski *et al.* reported that glycine was being converted to form serine that ended up at C1 and C2.[100] 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.[101] Labeled forms of *m*-methyl tyrosine and *m*-methyl-*m*-hydroxy tyrosine were synthesized (**259** and **260** respectively). Both of these amino acids were shown to be incorporated into cyanocyline A. This

indicated that tyrosine was methylated to form **259** followed by a hydroxylation to yield **260** which undergoes further manipulation to form napthyridinomycin.

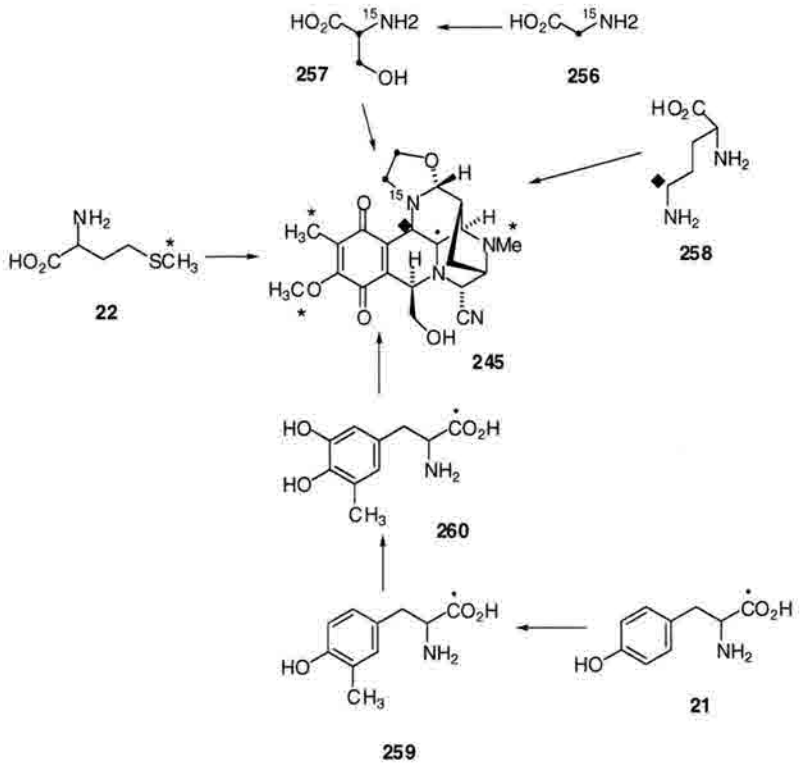


Figure 12 Biosynthetic precursors to Cyanocycline.

3.1.3. Total Syntheses of Cyanocycline A

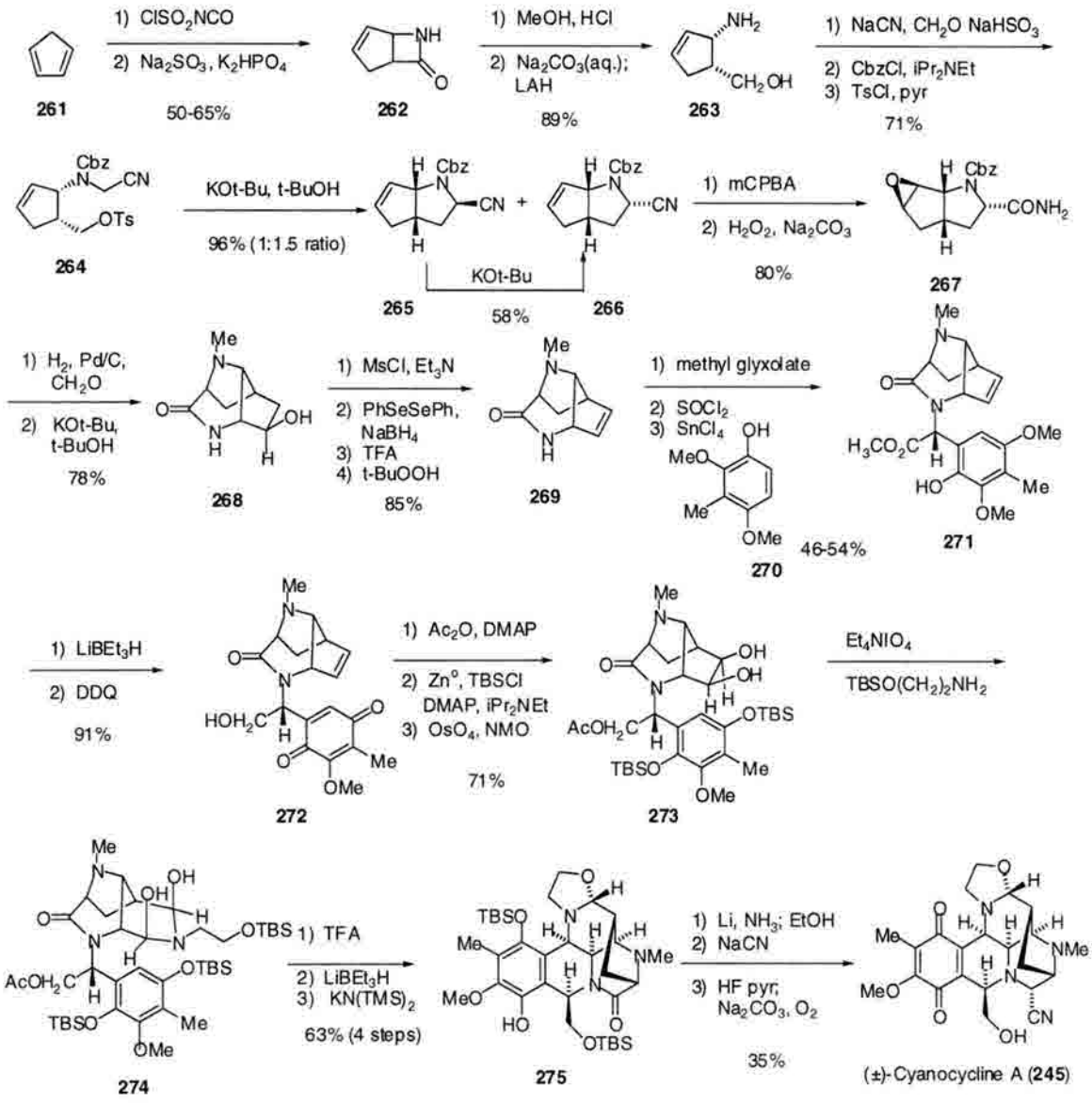
Two significant efforts towards the total synthesis of napthyridinomycin, one by Evans[102] and the other by Fukuyama[103] have been reported. However, napthyridinomycin proved too unstable to succumb to total synthesis. In fact, there was some evidence suggesting that the final product in Fukuyama's total synthesis was actually bioxalomycin β_2.

Due to the difficulty 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.[104] Later, Fukuyama reported the asymmetric total synthesis of (+)-cyanocylcine A thus determining the absolute stereochemistry of the natural product.[1f]

The Evans' approach began with the synthesis of tricycle **269**. (Scheme 39).[102] Condensation of cyclopentadiene (**261**) with chlorosulfonyl isocyanate followed by reductive hydrolysis afforded β-lactam **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 *t*-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.[102b] 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**. Attempts were originally made to oxidatively cleave the diol. 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 the TBS protected ethanolamine to afford the amino diol **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.[104] 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. Silyl deprotection was followed by hydroquinone oxidation afforded (±)-cyanocycline A in 35% yield from **275**.

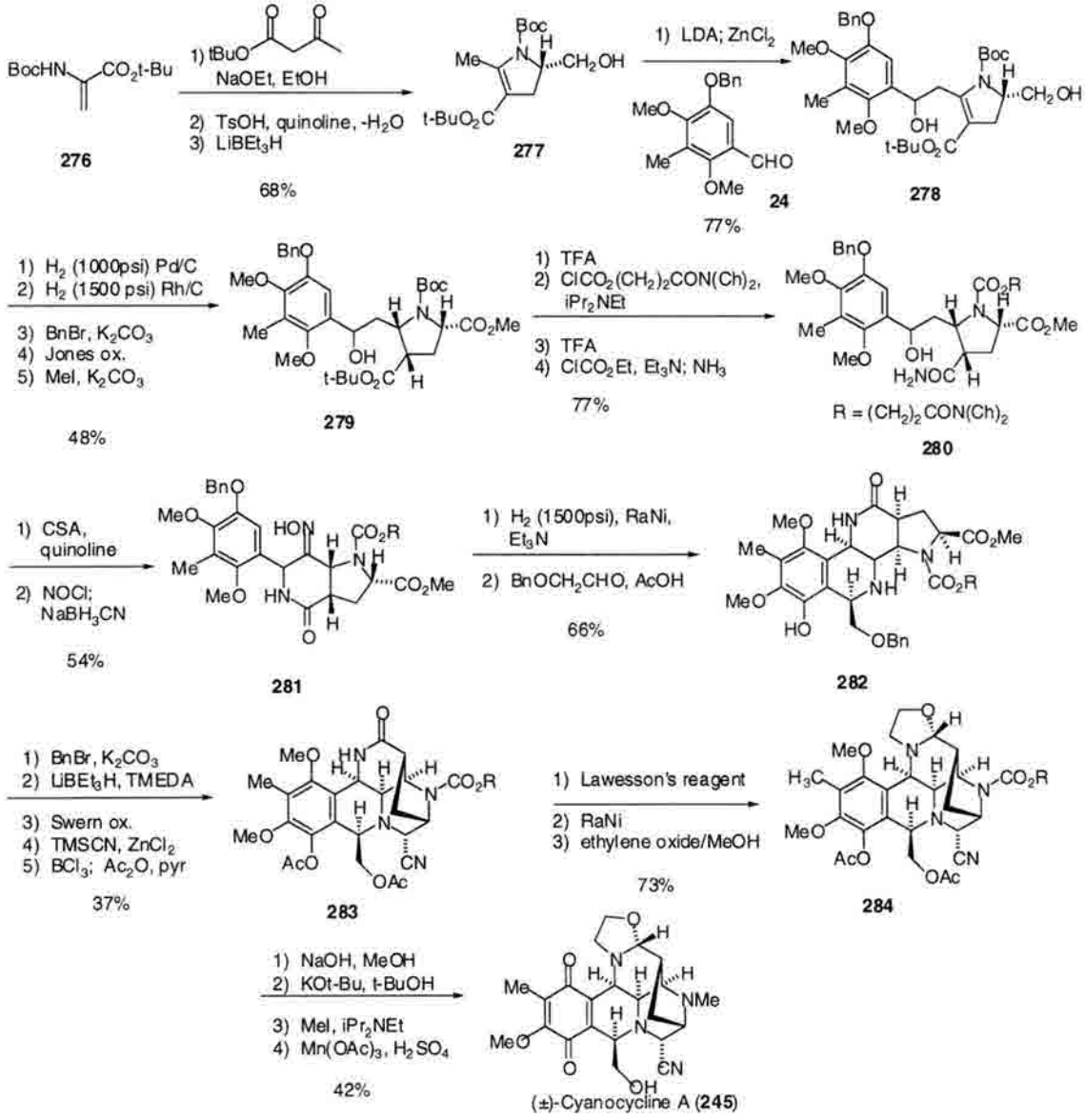
Shortly after Evans' total synthesis of (±)–cyanocyline A was published, Fukuyama reported the second total synthesis of (±)–cyanocyline A.[105] The dihydropyrrole **277** was synthesized in three steps from the dehydroalanine **276** (Scheme 40). The zinc enolate of **277** was formed and treated with aromatic aldehyde **24** to form the aldol product **278**. Hydrogenation under two different conditions first cleaved the benzyl ether then reduced the olefin. Reprotection of the phenol was followed by oxidation of the primary alcohol. The resultant acid was then converted to the methyl ester **279**. Selective Boc removal was accomplished using dilute TFA. This 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 t-butyl ester was cleaved and transformed into the primary amide **280**. Amide **280** was treated with camphor sulphonic acid to afford an enamine.



Scheme 39 Evan's total synthesis of Cyanocycline A.

Treatment 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. Reproteciton 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 a carbinolamine. Conversion of

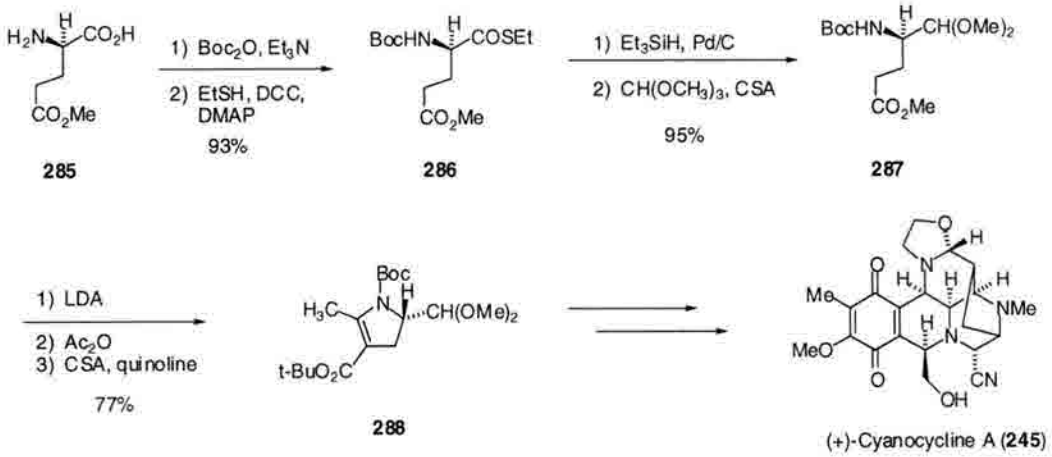
the carbinolamine to the amino nitrile was accomplished using trimethylsilyl cyanide in the presence of zinc chloride. Treatment with boron trichloride cleaved the benzyl ether. The two hydroxyl groups were then reprotected as acetates to afford tetracycle **283**. Conversion of the amide to the oxazolidine 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



Scheme 40 Fukuyama's total synthesis of Cyanocycline A.

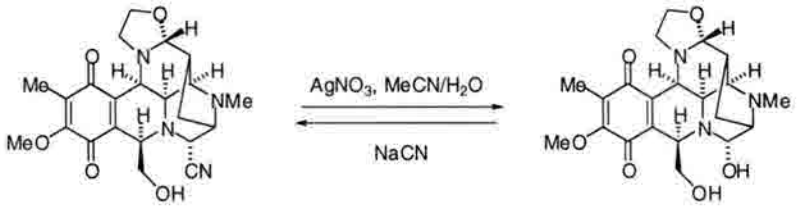
that was converted to oxazolidine **284** using ethylene oxide in methanol. The final steps in the total synthesis involved the cleavage of the acetates and carbamate followed by N-methylation. Oxidation of the hydroquinone afforded (±)-cyanocyline A in 42% for the final four steps.

The total synthesis of (+)-cyanocycline A was accomplished via a similar route.[1f] Since the stereogenic center at C-6 was used to set all further stereocenters during 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 41). The thioester was reduced under mild conditions to provide an aldehyde that was protected as the dimethyl acetal **287**. A three step sequence was undertaken to afford the dihydropyrrole **288** in 77% yield. Dihydropyrrole **288** was converted to (+)-cyanocycline A using similar chemistry to that utilized in the racemic synthesis. This enantioselective synthesis was used to confirm the absolute stereochemistry of the natural product.



Scheme 41 Fukuyama's total synthesis of (+)-Cyanocycline A.

Also, in these studies, the conversion of cyanocyline A to napthyridinomycin was attempted using silver nitrate in water (Scheme 42).[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 napthyridinomycin. Treatment of the new product with sodium cyanide reformed cyanocycline A indicating that indeed napthyridinomycin had indeed been formed.

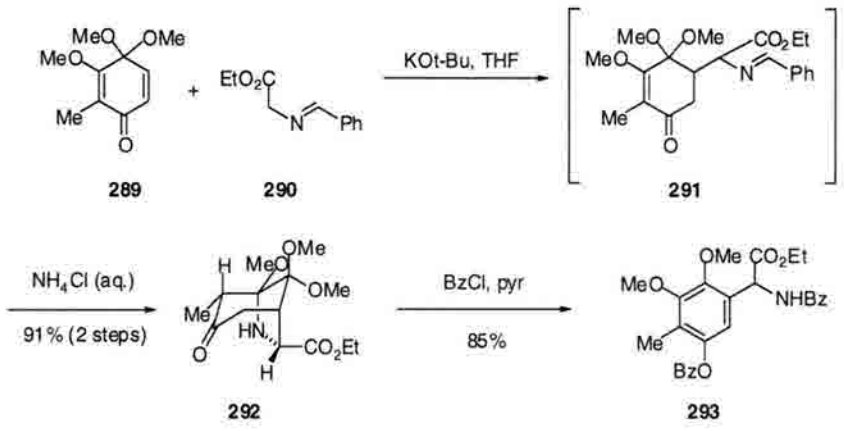


(+)-Cyanocycline A (**245**) Naphthyridinomcyin (**243**)

Scheme 42 Interconversion of Naphthyridinomycin and Cyanocycline A.

3.1.4. Synthetic studies

The first published synthetic studies towards the synthesis of the A ring of napthyridinomycin were reported by Parker *et al.* in 1984 (Scheme 43).[106] 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 ammonium 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.

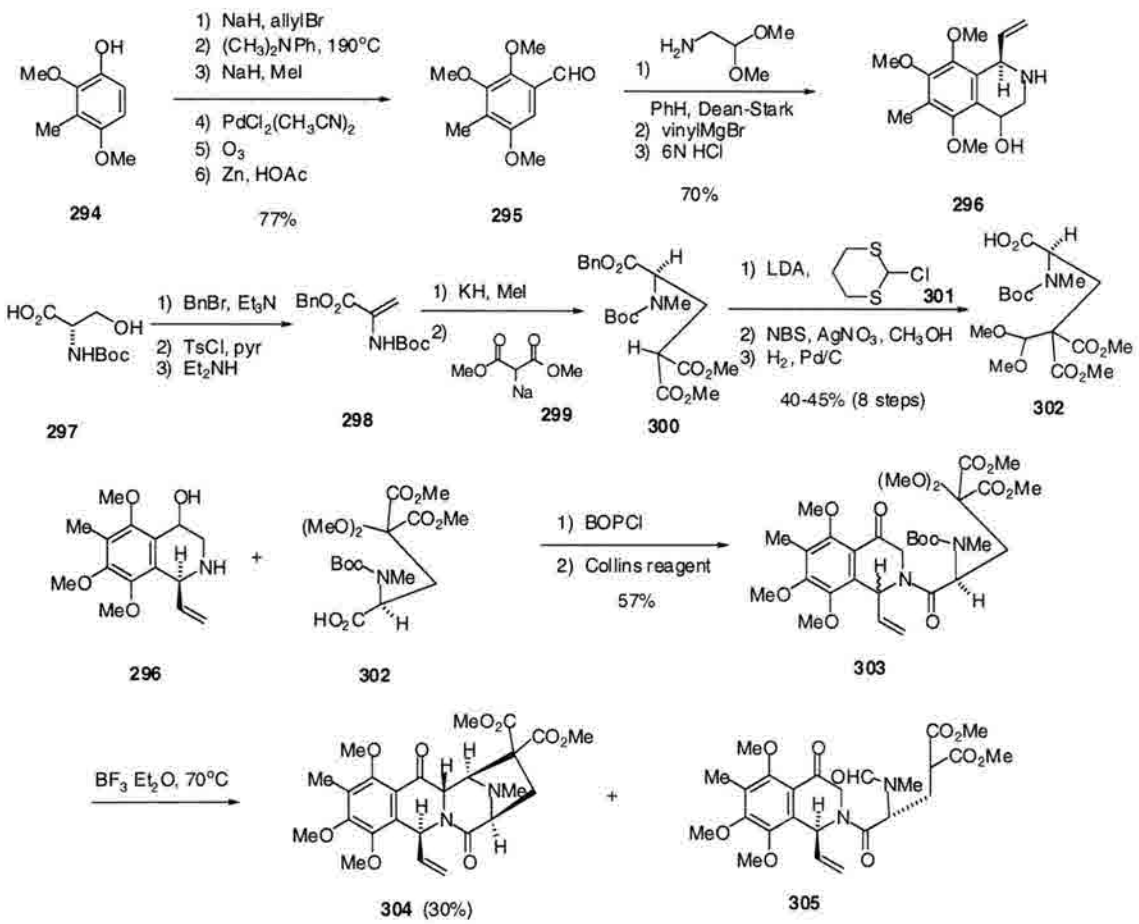


Scheme 43 Parker's synthetic studies toward naphthyridinomycin.

In 1984, Danishefsky *et al.* reported their progress towards the total synthesis of napthyridinomycin via a convergent strategy that consisted of coupling a bicyclic core (**296**) with an amino acid side chain (**302**) (Scheme 44).[107] The tetrahydroisoquinoline fragment **296** was synthesized in very high yield starting with phenol **294**. The phenol was allylated followed by a Claisen rearrangement. The phenol was then methylated and the olefin was migrated into conjugation with the aromatic ring using palladium dichloride bisacetonitrile. Ozonolysis followed by reductive workup yielded aldehyde **295** in 77% yield for the five steps. Imine formation followed by vinyl grignard addition yielded an amino acetal which was cyclized under acidic conditions to afford the bicycle **296**. Amino acid **302** was synthesized starting from N-Boc-L-serine (**297**). The acid 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 chloro-dithiane **301** afforded the cyclic dithioacetal which was converted to the dimethyl acetal upon treatment with N-

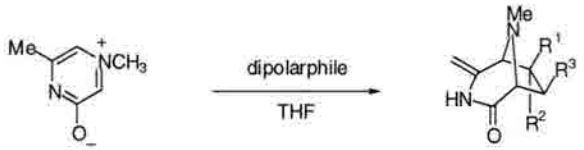
bromosuccinimide and silver nitrate in methanol. The final step to amino acid **302** was the cleavage of the benzyl ester under standard hydrogenolysis conditions. The overall yield of **302** was 40-45% for the eight steps from **297**.

Coupling of amine **296** and acid **302** was accomplished using BOPCl. No other coupling conditions were effective with this system.[107b] 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 was unable to cyclize and bicycle **305** was the resultant product.



Scheme 44 Danishefsky's synthetic study towards Naphthyridinomycin.

In 1987, Joule *et al.* showed that the C-D rings of naphthyridinomycin could be formed via a 1,3 dipolar cycloaddition.[108] A simple model study was studied using the zwitterion **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 7). When methyl acrylate was used, the exo product was formed in 51% yield. Interestingly, when acrylonitrile was used there was almost no *exo/endo* selectivity was seen. The use of the 1,3 dipolar cycloaddition was then used by two other groups in the total synthesis of other members of the tetrahydroisoquinoline natural products.



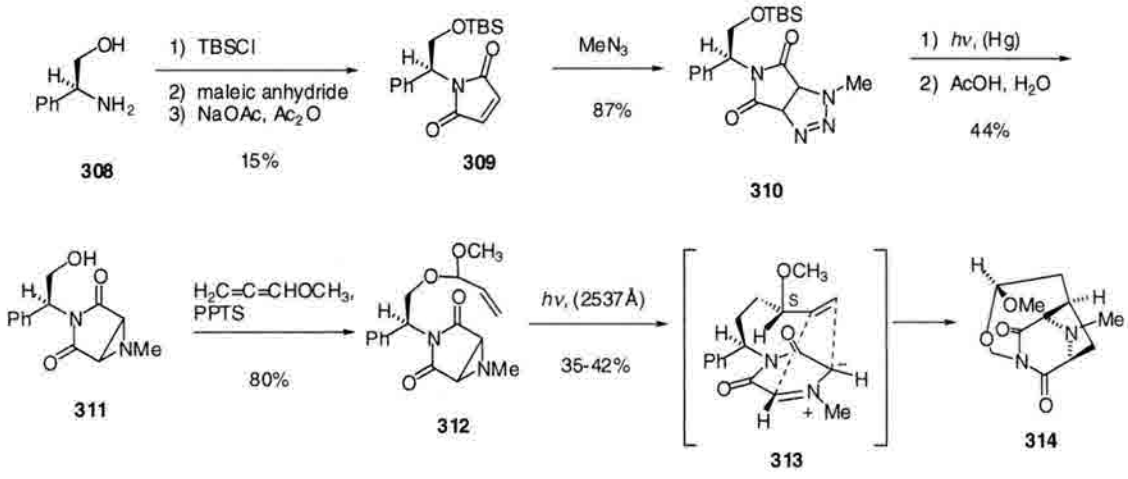
306 307

Dipolarphile	Temp/time	Product (yield)
methyl acrylate	reflux/ 1 hr	$R^1 = CO_2CH_3$, $R^2 = R^3 = H$ (51%)
acrylonitrile	20°C/ 3 hr	$R^1 = CN$, $R^2 = R^3 = H$ (25%)
diethyl maleate	reflux/ 2.5 hr	$R^1 = R^3 = CO_2CH_3$, $R^2 = H$ (35%)

Table 7 Intermolecular 1,3-dipolar cycloaddition using various dipolarphiles

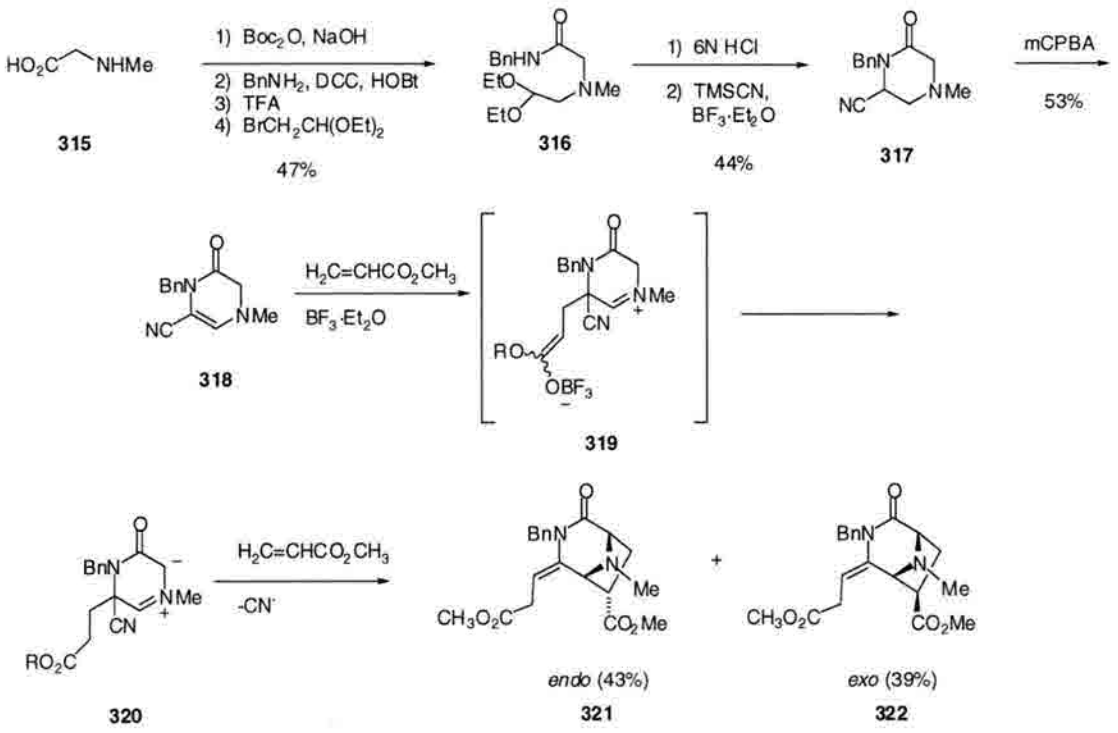
Garner *et al.* also published a strategy towards the synthesis of naphthyridinomycin via a 1,3 dipolar cycloaddition.[109] The dipolar species was generated by irridiation of an aziridine **312** (Scheme 45). Good results were obtained when the cycloaddition was intramolecular. Intermolecular systems yielded good *endo/exo* selectivity, however no diastereoselectivity with respect to *re/si* addition.[109b] Maleimide **309** was synthesized in three steps from alcohol **308**.[109a] 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 2537Å Rayonet source generated an azomethine ylide that cyclized with the olefin to yield tricycle **314**. This tricycle posessed the desired stereochemistry (via endo-*re* attack), however the yield was low due to cyclization of only one of the acetal diastereomers.



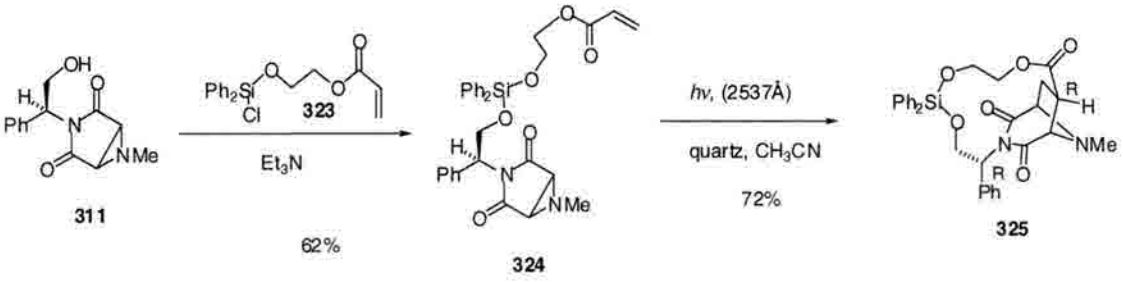
Scheme 45 Intramolecular 1,3-dipolar cycloadditions.

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 46).[110] Treatment of **318** with methyl acrylate in the presence of $BF_3 \cdot Et_2O$ yielded bicycles **321** and **322** in 43% and 39% yields respectively. These bicycles were formed via a Michael addition followed by formation of the ylide **320** and cycloaddition. Slightly higher *endo/exo* selecitivities were seen with other dipolarphiles leading to the speculation that the addition could be directed by means of a BF_3 chelation.



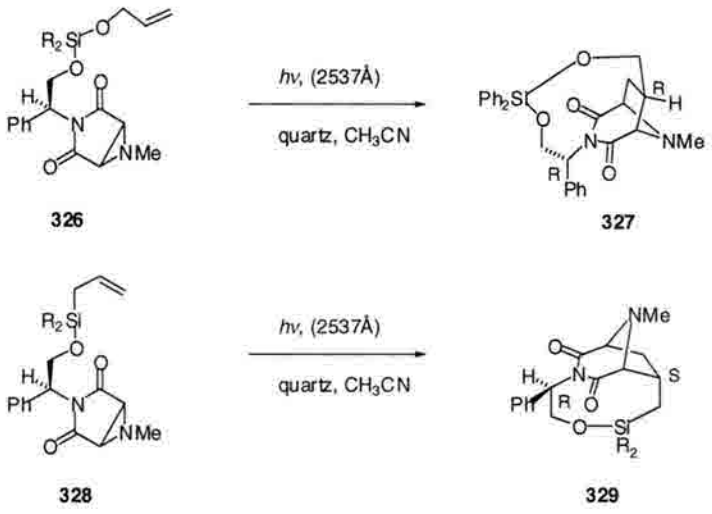
Scheme 46 Tandem Michael additions/1,3 dipolar cycloadditions.

In 1994, Garner *et al.* reported the effects of longer tethers with respect to the diastereoselectivity of the 1,3-dipolar cycloaddition (Scheme 47).[111] 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.



Scheme 47 Effects of tether length on intramolecular 1,3-dipolar cycloadditions.

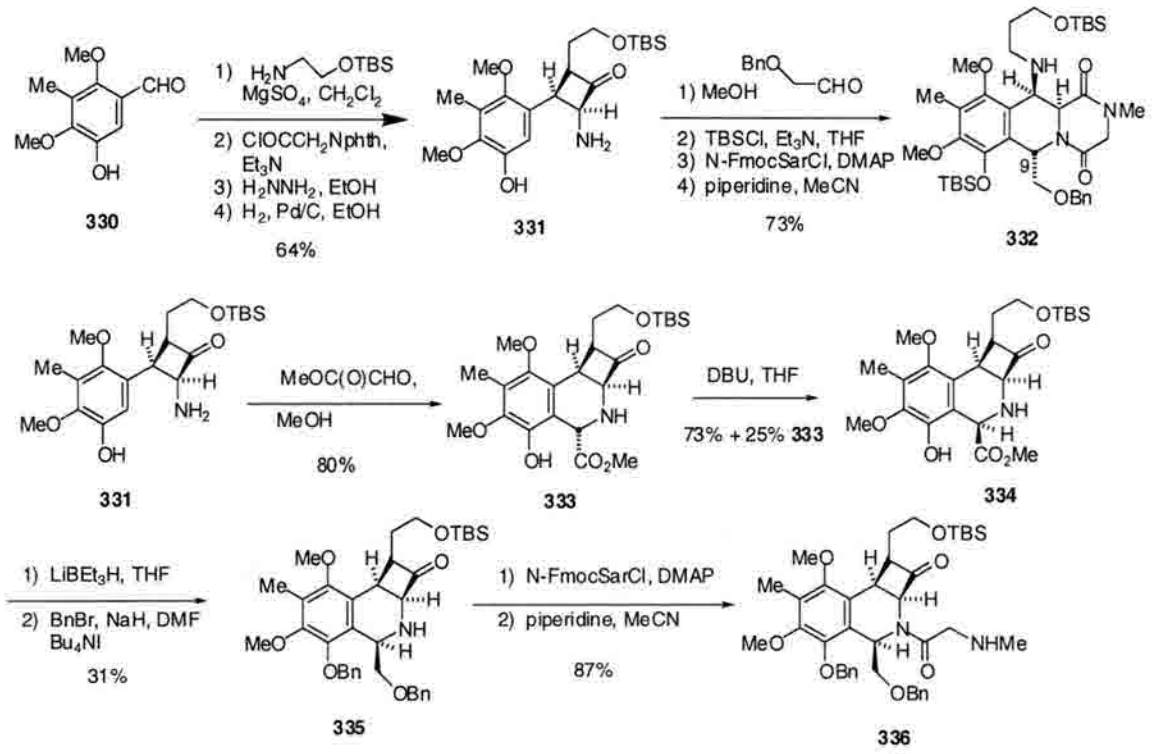
Shortening of the tether (**326**) thus creating a 10 membered transition state also afforded a *endo-si* product **327** (Scheme 48).[111b] Changing to a 9-membered transisition state, the desired *endo-re* addition product **329** was obtained.



326 327 328 329

Scheme 48 Effects of tether length on intramolecular 1,3-dipolar cycloadditions.

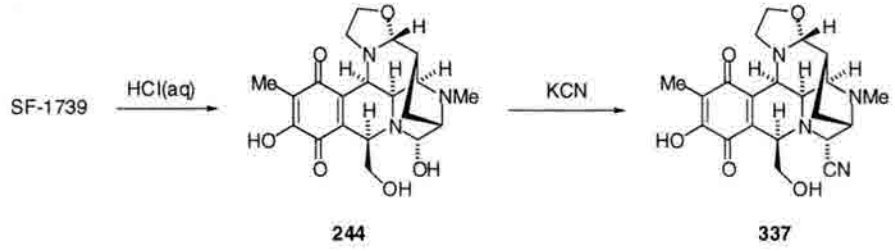
In 2001, Williams *et al.* reported the synthesis of tricycle **336** that could be used in the total synthesis of bioxalomycin α_2.[112] The efficient synthesis of this beta lactam started with the imine formation of aldehyde **330** with *O*-TBS-protected ethanolamine. The ketene of phthalimidoacetyl 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. A Pictet-Spengler cyclization with benzyloxyacetaldehyde afforded a single diatereomer, however after amide coupling with Fmoc-sarcosine and cyclization it was discovered that the tricycle **332** had the undesired *anti*-configuration at C-9. The Pictet-Spengler cyclization was then preformed using methyl glyoxylate to afford a single 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.



Scheme 49 Williams' synthetic studies towards Bioxalomycin α_2.

3.1.5. Analogs of Naphthridinomcycin

SF-1739, which was 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 50).[93] Treatment of **244** with potassium cyanide afforded naphthocyanidine **337**.



Scheme 50 Analogs of SF-1739 (naphthyridinomycin).

3.1.6. Biological Activities

Naphthyridinomycin has potent antibiotic activity against both Gram-(-) and Gram-(+) bacteria.[90] Incorporation of ^{14}C-thymidine in DNA synthesis was inhibited in by naphthyridinomycin *E. coli* at low concentrations.[113] At higher concentrations, RNA and protein synthesis were also inhibited, but to a lesser extent than DNA synthesis. The DNA synthesis inhibition was reversible at lower naphthyridinomycin concentrations, but at higher concentrations the inhibition was irreversible.

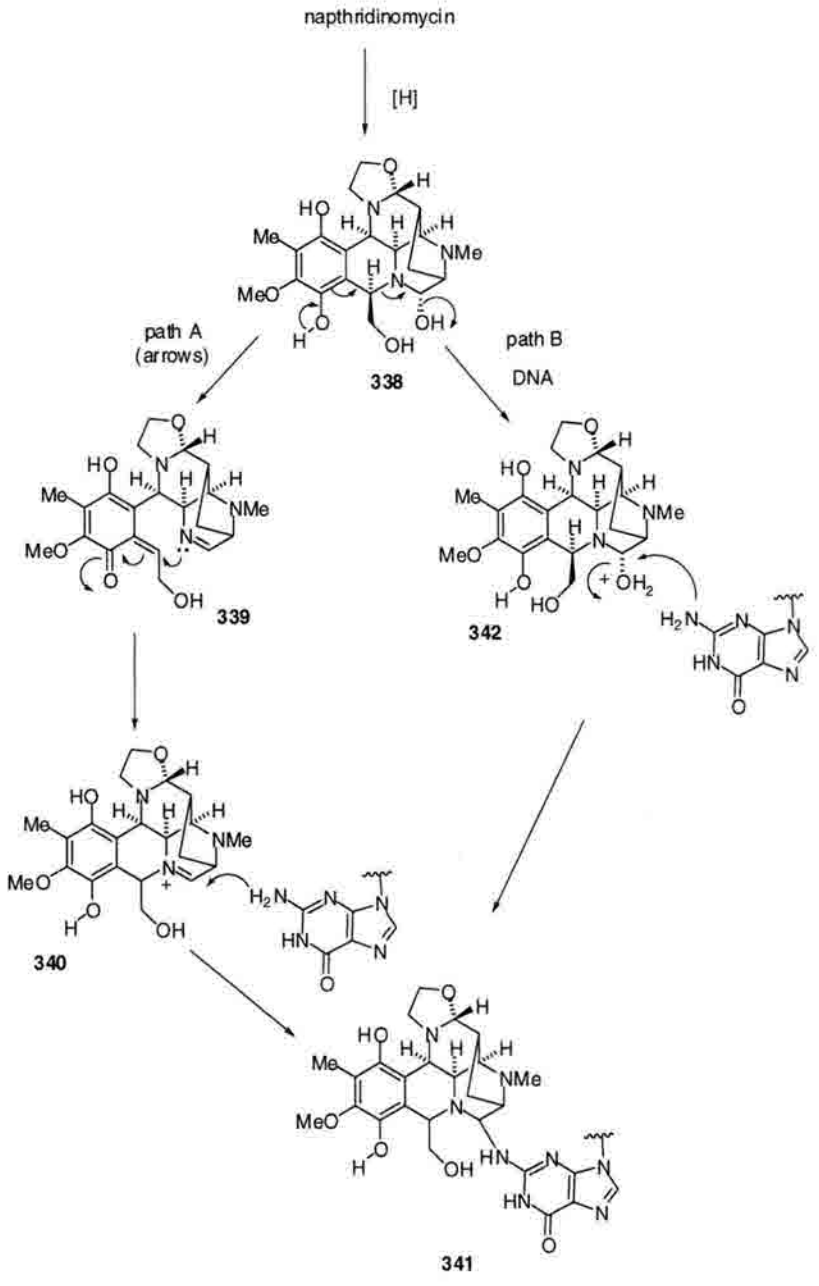
In studies by Zmijewski *et al.*, ^{3}H-naphthridinomycin was found to bind covalently to DNA in small amounts.[114] Naphthyridinomycin that was reduced with DTT was found to covalently bind to DNA to a greater extent than the unreduced naphthyridinomycin. This binding was found to be irreversible.

In experiments to determine sequence specificity 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 alkylation by naphthyridinomycin, suggesting that naphthyridinomycin was covalently bound to the exocyclic amine of guanine.

Being similar to saframycin S, naphthyridinomycin also has an enhanced activity when reduced prior to DNA interaction. Dithiothreitol has been shown to be the best reducing agent for napthridinomycin.[114] There was a difference in the UV_{max} of the unreduced form (270nm) vs. the reduced form (287nm). 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.

A study into the mechanism of binding to DNA by Zmijewski *et al.* showed that when treated with DTT naphthyridinomycin has two rates of DNA binding.[115] Initially when treated with DTT there was a burst of fast binding to DNA, followed by a slower rate of binding that was similar in magnitude to unreduced naphthyridinomycin. Reduction using DTT was shown to increase the reactivity 5-6 fold over naphthyridinomycin alone. The activated form of naphthyridinomycin will reoxidize with time to reform naphthyridinomycin. A pH study showed that the pH range for active reduced naphthyridinomycin was 5-7.9, but the unreduced form showed highest DNA binding at pH 5. Two mechanisms were given for the DNA alkylation of naphthyridinomycin (Scheme 51). The first (path A) was based on the saframycin binding mechanism. Naphthyridinomycin was reduced to the dihydroquinone **338**. Formation of the semiquinone allows for loss of the hydroxyl group, thus forming imine **339**. The imine non-bonded electron pair then closes on the olefin forming the iminium **340** that can be alkylated 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 explain 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. This indicated that naphthyridinomycin does not chelate to DNA due to the fact that naphthyridinomycin did not contain the DNA intercalative functionality that was present on the saframycins and ecteinascidins. The reduction of naphthyridinomycin may give a species that will hydrogen bond with DNA allowing for a higher rate of alkylation.



Scheme 51 Proposed mechanisms of DNA alkylation by naphthyridinomycin.

In the isolation paper of cyanocycline A, the biological activities of naphthyridinomycin and cyanocycline A were compared.[94] The MICs of naphthyridinomycin were better than or equal to the cyanocyline A values. An *in vitro*

study using HeLa cells showed similar activities for the two compounds at the same concentrations.

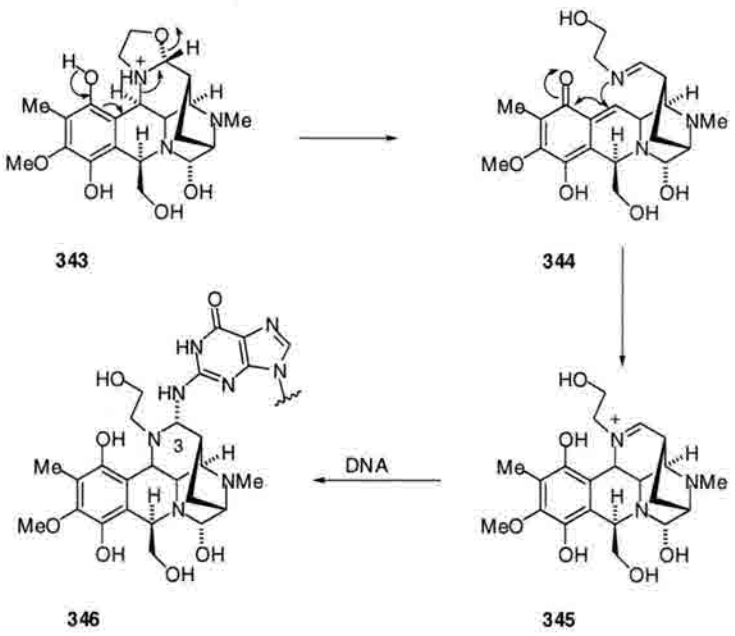
In 1983, Hayashi *et al.*[116] reported that cyanocyline A reduced by DTT showed no enhancement in biological activity indicating a different mechanism of action compared to naphthyridinomycin. In this report it was also mentioned that the aminonitrile moiety of cyanocyline A was more stable than that of saframycin A.

The semi synthetic derivatives SF-1739 HP and cyanocycline F had demonstated reduced activity in most of the antimicrobial screens compared to the natural substrates.[93] 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.[117] 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 major activation necessary was 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.[118] These studies suggested that one other possible mode of alkylation could be opening of the oxazolidine ring and alkylation at C3a, this third potential mechanism for DNA alkylation was suggested as shown in Scheme 52. After reduction to the hydroquinone **343**, tautomerization would afford the ring-opened oxazolidine **344**. Attack of the imine lone pair on the cyclic olefin would yield iminium **345** that can undergo alkylation by DNA to afford **346**. It was also

suggested that DNA crosslinking of duplex DNA would not be possible via alkylation at the two oxazolidine moieties, but that DNA- protein crosslinking might be possible.

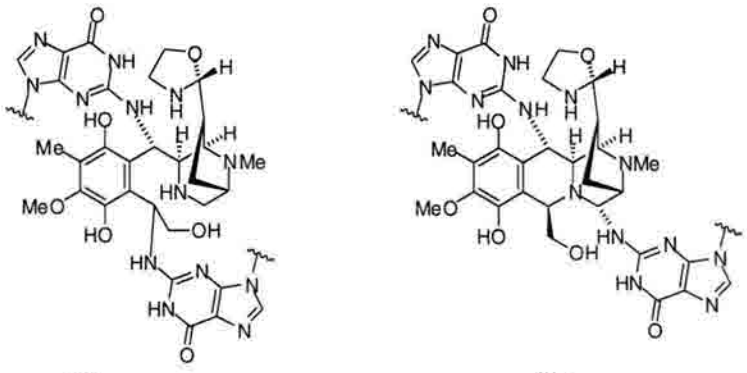


Scheme 52 Alternate proposed mechanism of DNA alkylation by naphthyridinomycin.

Bioxalomycin α_2 was shown to have excellent antimicrobial activity against Gram-(+) bacteria.[119,98a] Also, there was a slight difference in the mechanism of action when compared to naphthyridinomycin. Like naphthyridinomycin, bioxalomycin $\alpha 2$ inhibited DNA synthesis drastically. However, both RNA synthesis and protein synthesis were also inhibited significantly.

It was later shown by Williams and Herberich that bioxalomycin α_2 does indeed crosslink duplex DNA.[120] It was also noted that cyanocycline A formed DNA crosslinks in low yield only in the presence of DTT. Bioxalomycin α_2 crosslinking showed $^{5'}GC^{3'}$ selectivity as evidenced by foot-printing studies. Substitution of guanine with inosine eliminated the crosslinking, indicating that N-2 of guanine was alkylated. Two possible

sites of crosslinking on bioxalomycin were suggested (Figure 13), one at C-13b and C-9 (**347**) and the other at C-13b and C-7 (**348**).



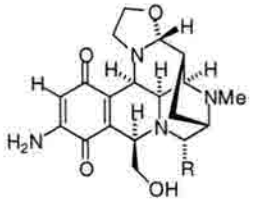
347 **348**

Figure 13 Proposed crosslinking sites on Bioxalomycin α_2.

3.2. Dnacins

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,[121] but the structures were not determined until 1994 (Figure 14). The structure of dnacin B_2 was very similar to that of naphthyridinomycin with the exception of the amino group at C-11 and the hydrogen at C-12.[122] The structures of the dnacins were determined by NMR spectroscopy.



Dnacin A_1 (**349**) R = CN
B_1 (**350**) R = OH

Figure 14 The Dnacins.

3.2.2. Biological Activity

Like naphthyridinomycin, dnacin B_1 was found to inhibit DNA synthesis.[123] 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.[124] 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 μM and 141 μM respectively).

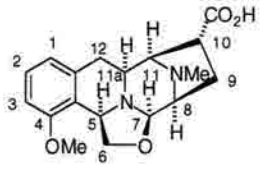
4. Quinocarcin Family

4.1 Quinocarcin and Quinocarcinol

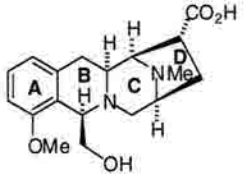
4.1.1. Isolation and structure determination

In 1983 Tomita *et al.* isolated the antitumor antibiotics quinocarcin (**351**) and quinocarcinol (**352**) from *Streptomyces melanovinaceus* nov. sp. (Figure 15).[125] The structure of quinocarcinol was determined by X-ray crystallography.[126] The structure of quinocarcin was determined by comparison of NMR spectra between the two natural products.[125b] Also, 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.[127]



Quinocarcin (**351**)



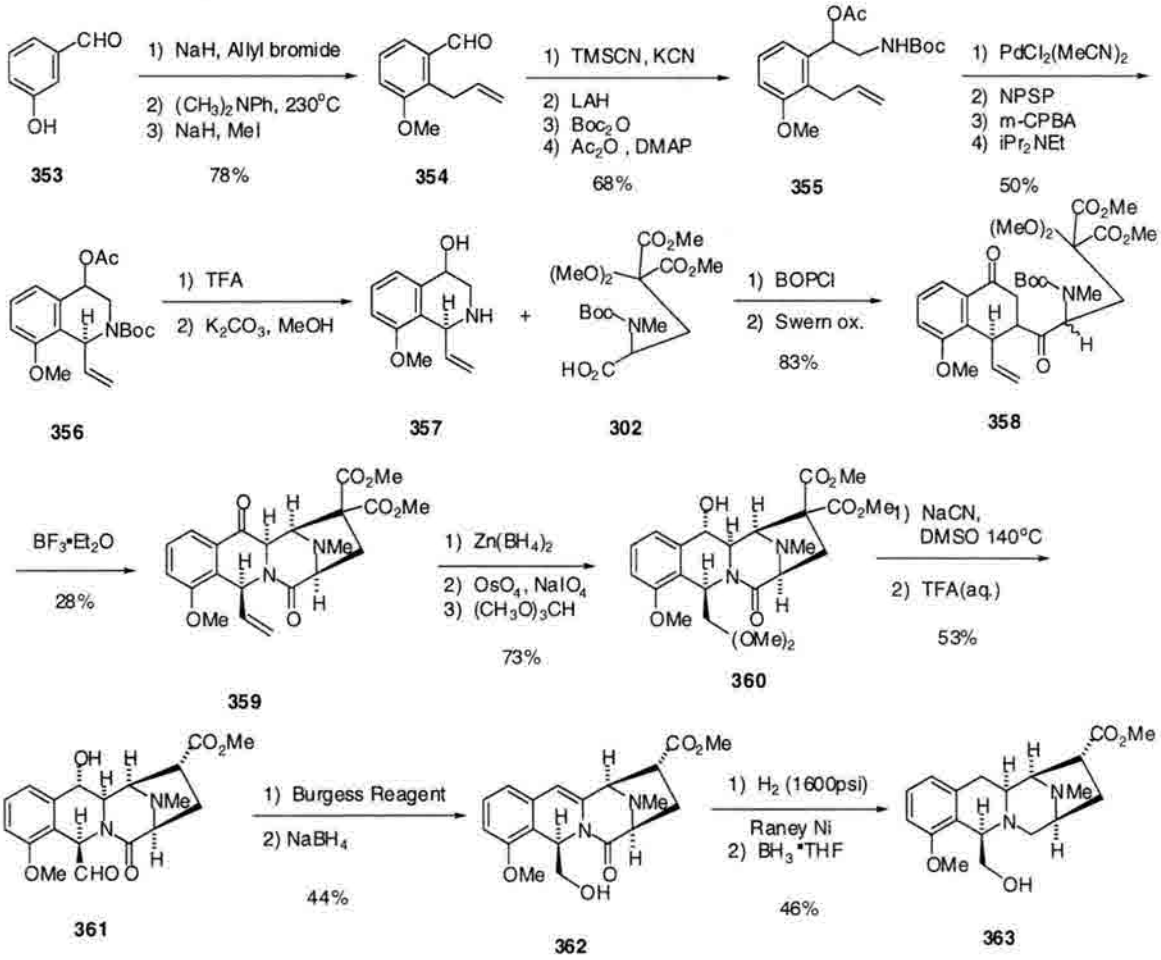
Quinocarcinol (**352**)

Figure 15 Quinocarcin and quinocarcinol.

4.1.2. Total Syntheses of Quinocarcin, Quinocarcinol, and Quinocarcinamide

The first synthesis of quinocarcinol was accomplished by Danishefsky *et al.* in 1985.[128] Starting with aromatic aldehyde **353**, the phenol was allylated followed by a Claisen rearrangement and methylation of the phenol to afford **354** (Scheme 53). Conversion of the aldehyde to the cyanohydrin was followed by reduction of the nitrile using LAH. Protection of the amine and alcohol provided **355**. Treatment of the allyl group with $PdCl_2(MeCN)_2$ afforded 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-CPBA and Hunig's base. Removal of the Boc and acetate protecting groups afforded the secondary amine **357** that 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

8 diastereomers. Reduction of the benzylic ketone was followed by oxidative cleavage of the olefin to the 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

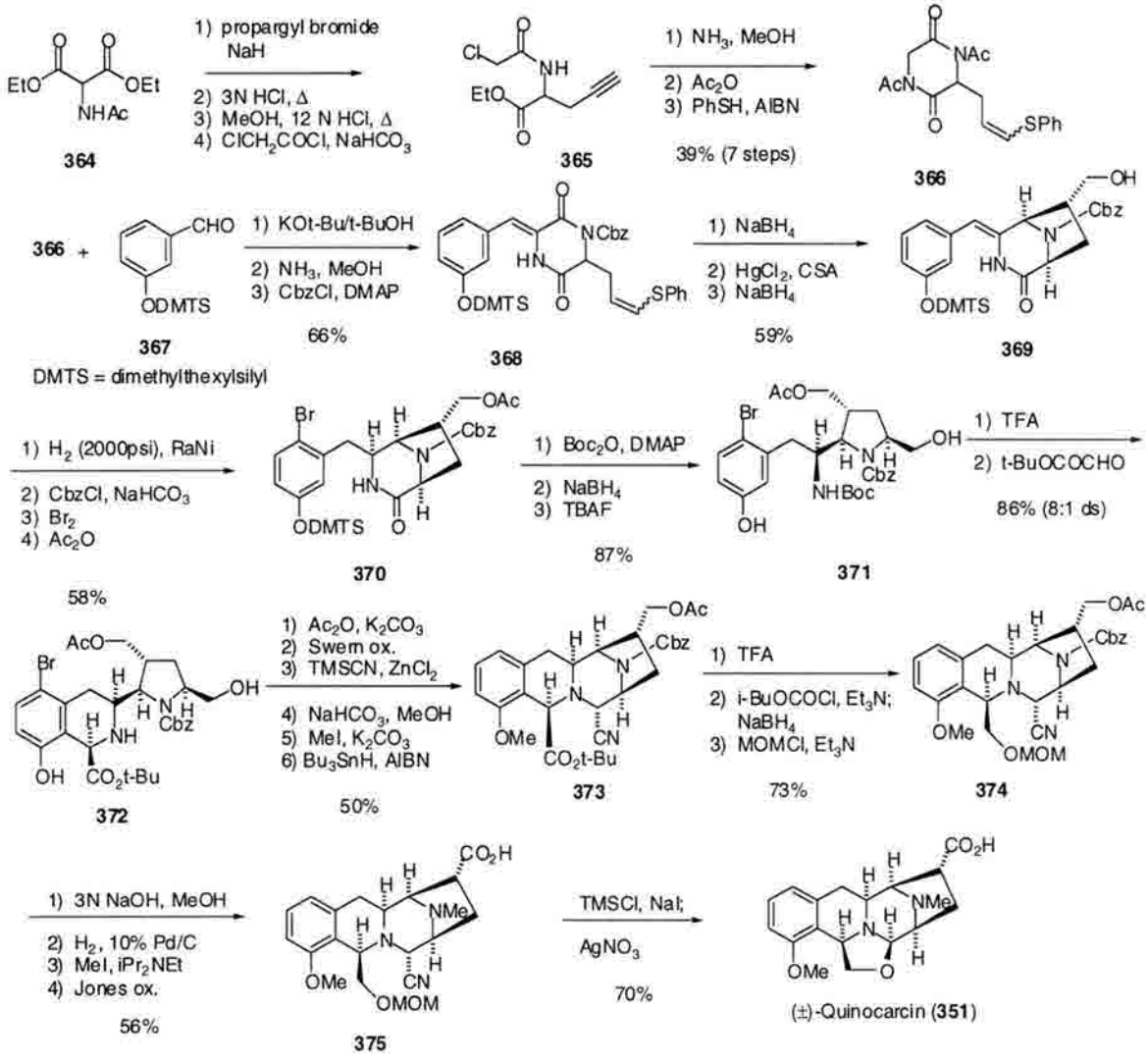


Scheme 53 Danishefsky's total synthesis of quinocarcinol methyl ester.

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 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 54).[129] 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 nitrogens 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 bicycle **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 isomer 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

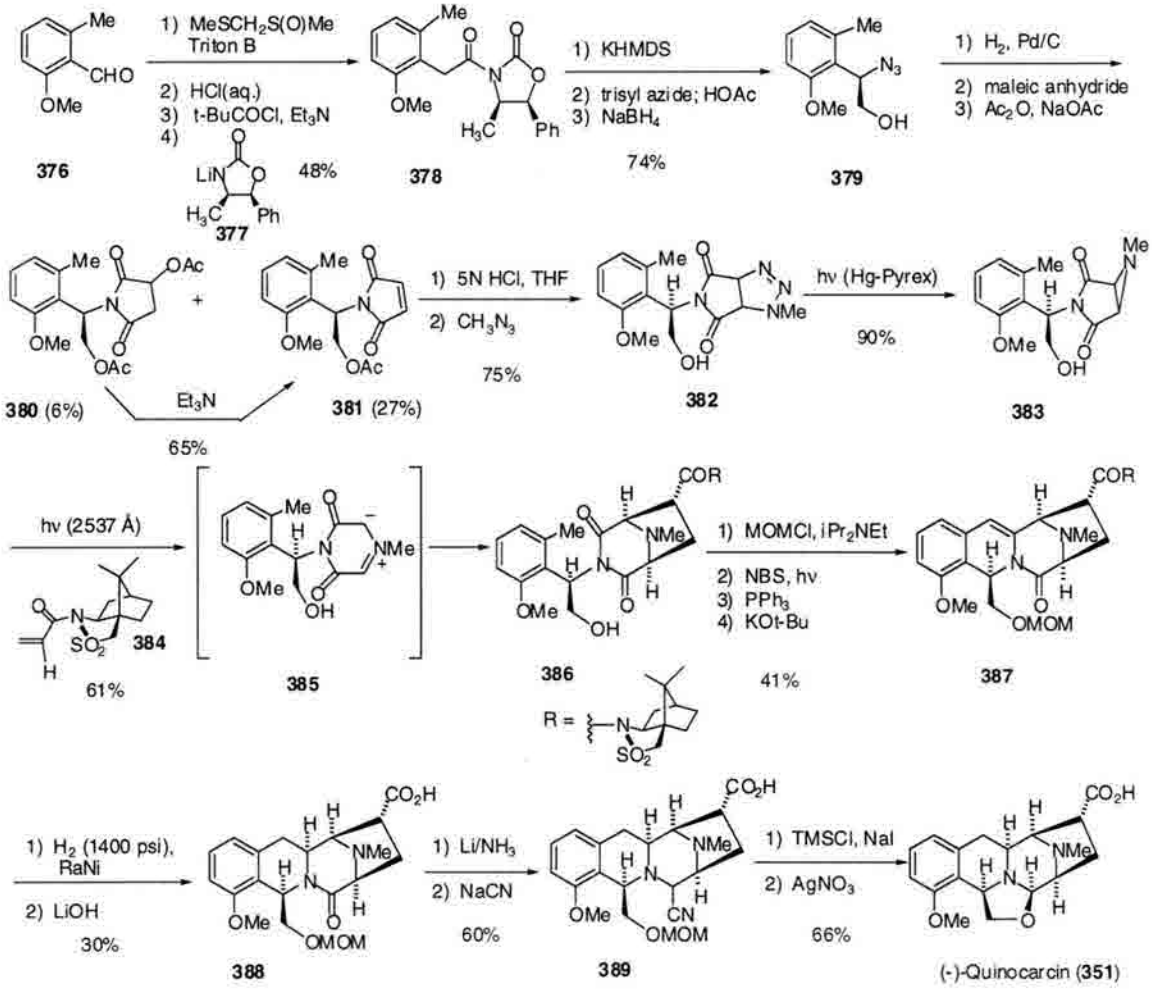
of the *tert*-butyl ester and subsequent reduction of the carboxylic acid was followed by alcohol protection as the methoxymethyl ether **374**. Removal of the acetate and Cbz groups was followed by N-methylation and oxidation of the alcohol to the carboxylic acid to afford the MOM protected DX-52-1 derivative **375**. The final steps in the total synthesis were the removal of the MOM 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.



Scheme 54 Fukuyama's total synthesis of quinocarcin.

In 1992, Garner *et al.* published the first asymmetric synthesis of (-)-quinocarcin.[127] The key step involved an intermolecular 1,3-dipolar cycloaddition (Scheme 55).[127,130] Starting with aldehyde **376**, treatment with methyl methylsulfinylmethylsulfide in the presence of Triton B, and acidic hydrolysis afforded a carboxylic acid. Formation of the mixed anhydride and treatment with chiral auxilary **377** afforded **378** in 48% overall yield. Deprotonation followed by treatment with trisyl azide provided the optically active azide. Reductive cleavage of the chiral auxilary afforded the azido alcohol **379** in 74% yield along with recovery of the chiral auxilary **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**. The ylide was then trapped with Oppolzer's sultam **384** to afford the desired bicyclic cycloadduct **386** via the *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. An intramolecular Wittig cyclization afforded 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[129], were the cleavage of the MOM group and closing of the oxazolidine ring to afford (-)-quinocarcin.



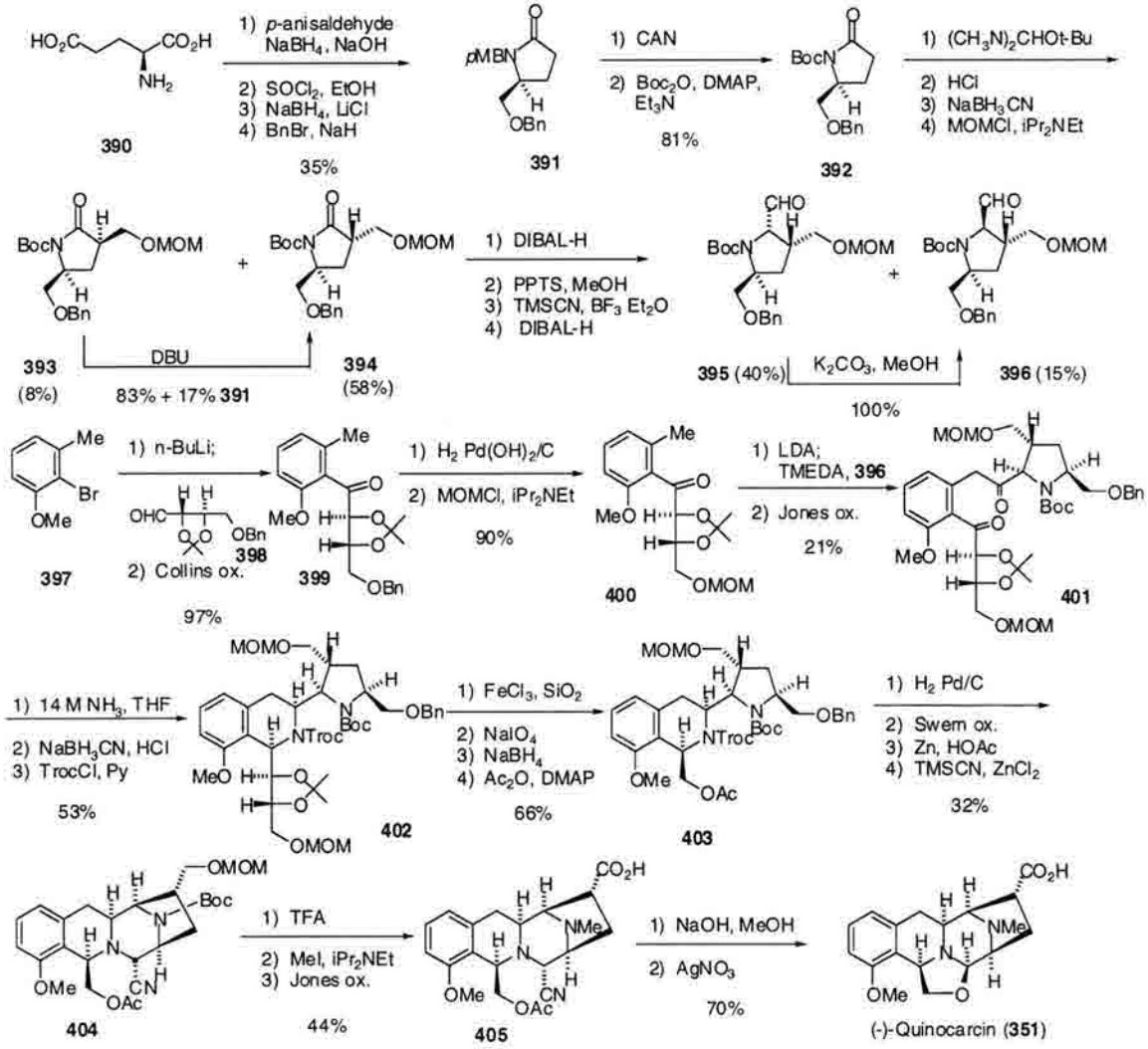
Scheme 55 Garner's total synthesis of (-)-quinocarcin.

Terashima *et al.* reported, in a series of papers, quinocarcin and quinocarcin analog syntheses that included the synthesis of both enantiomers of quinocarcin.[131] The total synthesis of (-)-quinocarcin started with the synthesis of the D ring (Scheme 56).[131c]

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

The A ring was synthesized starting with lithiation of **397** followed by addition of protected threose **398** and Collins oxidation afforded **399** in high yield.[131d] Substitution of the MOM group for the benzyl ether yielded **400**. Lithiation of the benzylic position and condensation with **396** afforded **401** following oxidation. Treatment with ammonia promoted cyclization to the isoquinoline. 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 that was subsequently reduced and protected to provide **403** in 66% yield from **402**. The cyclization to afford the tetracycle was similar to that used by Fukuyama. Removal of the benyzl 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.



Scheme 56 Terashima's total synthesis of (-)-quinocarcin.

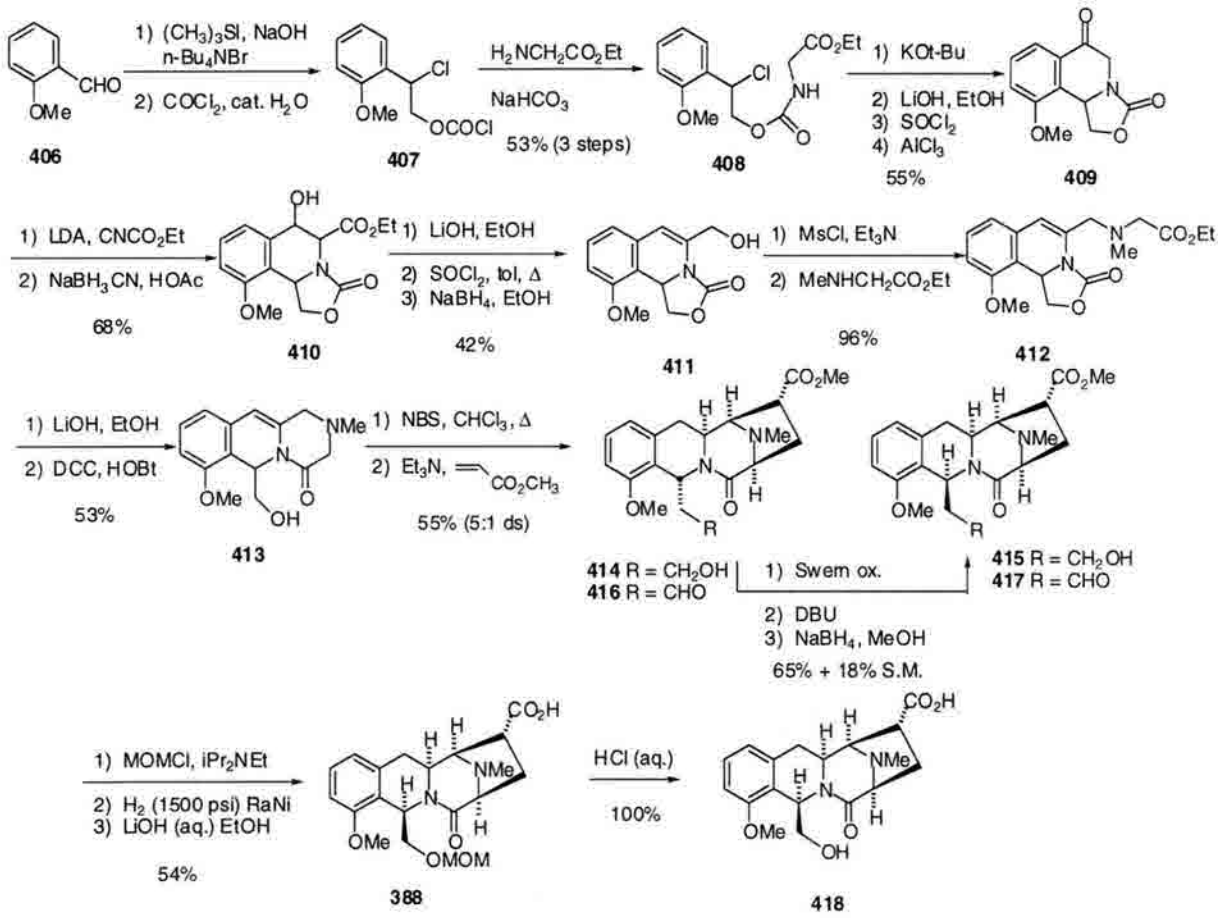
The asymmetric synthesis of (+)-quinocarcin was accomplished by the use of *ent*-**395** and *ent*-**397** in the same sequence of steps used in the (-)-quinocarcin synthesis.[131d]

In 1995, Flanagan and Williams published the synthesis of (±)-quinocarcinamide (**418**).[132] A late stage intermediate **388** intersected Garner's total synthesis of quinocarcin

thus making this a formal total synthesis of quinocarcin. The key step in this synthesis was a 1,3 dipolar cycloaddition via the formation of an azomethine ylide using NBS to oxidize an allylic amine.

Treatment of *o*-anisaldehyde (**406**) with trimethylsulphonium iodide under phase transfer-conditions afforded the benzylic epoxide that was opened with phosgene to form the chloroformate **407** (Scheme 57). Conversion to the carbamate **408** was accomplished via treatment with glycine ethyl ester. Cyclization afforded the oxazolidinone which uppon saponification yielded the carboxylic acid that was converted to the acid chloride An intramolecular Friedel Crafts acylation provided isoquinolone **409** in 55% overall yield.[133] 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 saponfication of the ester followed 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 tricycle **413**. NBS oxidation of the allylic amine afforded a dark green solution of the iminium salt. Deprotonation using triethylamine resulted in a dark blue solution of the azomethine ylide, which in the presence of 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 quantative yield.

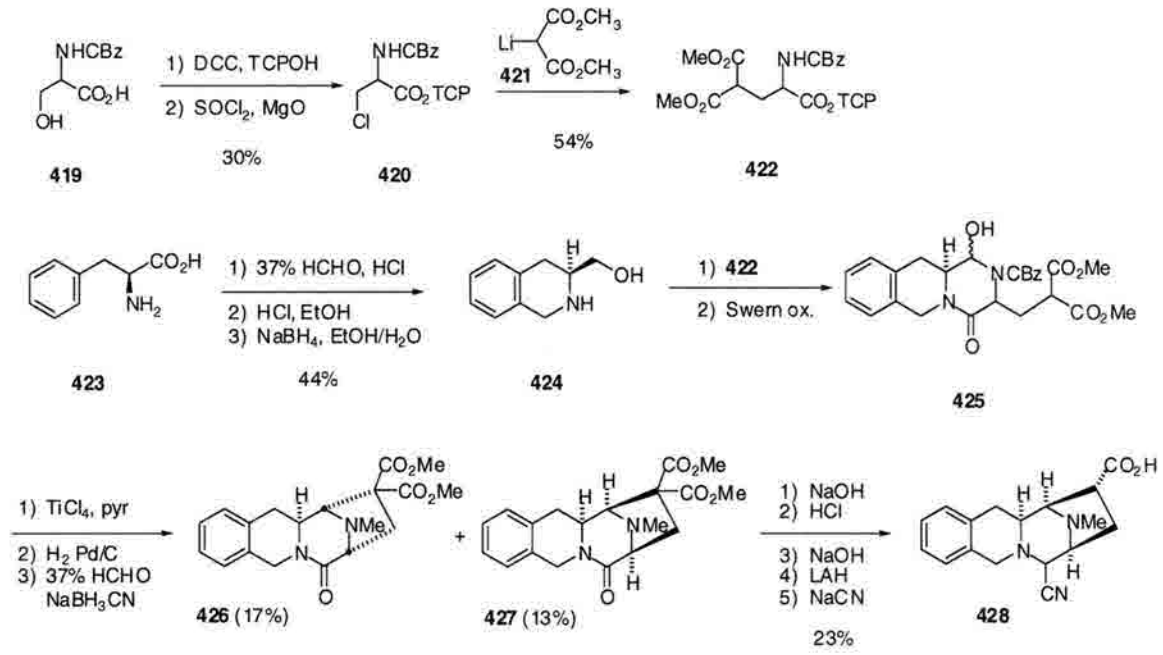


Scheme 57 Williams' total synthesis of quinocarcinamide.

4.1.3. Synthetic studies

In 1987, Saito and Hirata published a synthetic approach to quinocarcin via the use of phenylalanine and a glutamic acid derivative (Scheme 58).[134] The protected serine **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

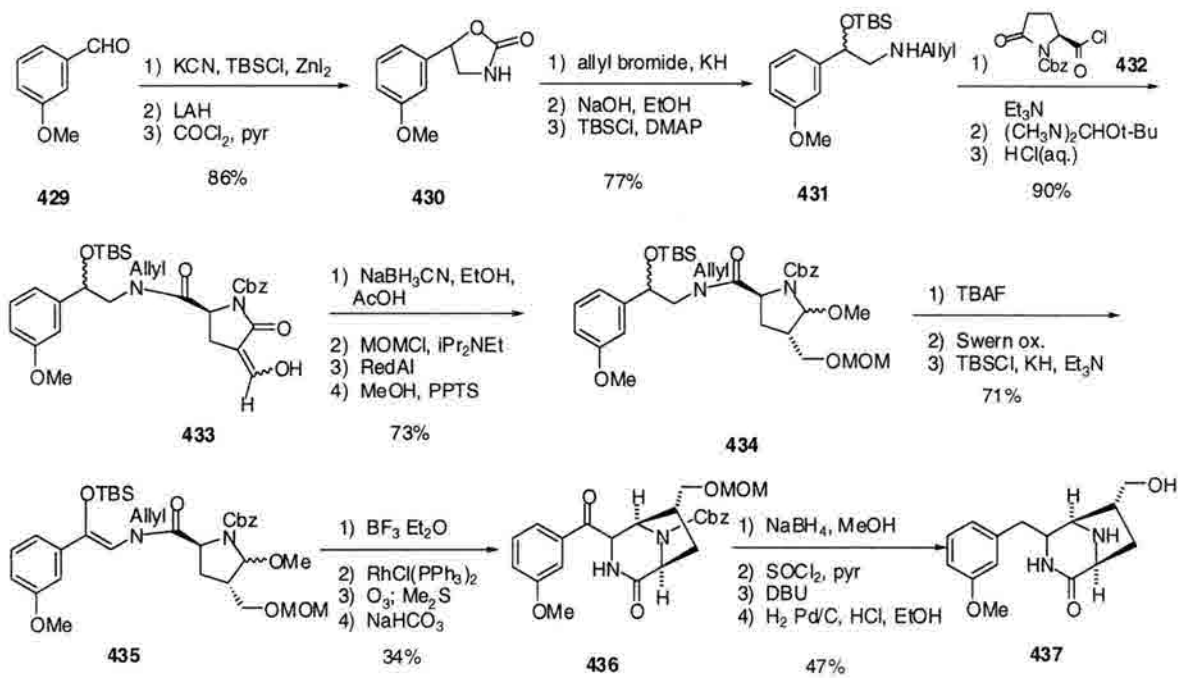
tetrahydroisoquinoline followed by conversion of the acid to the ethyl ester and subsequent ester reduction to afford the amino alcohol **424**. Coupling with the active ester **422** followed by Swern oxidation yielded the carbinolamine **425**. Cyclization to yield the tetracyclic core was accomplished 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.



Scheme 58 Saito's and Hirata's synthetic studies on quinocarcin.

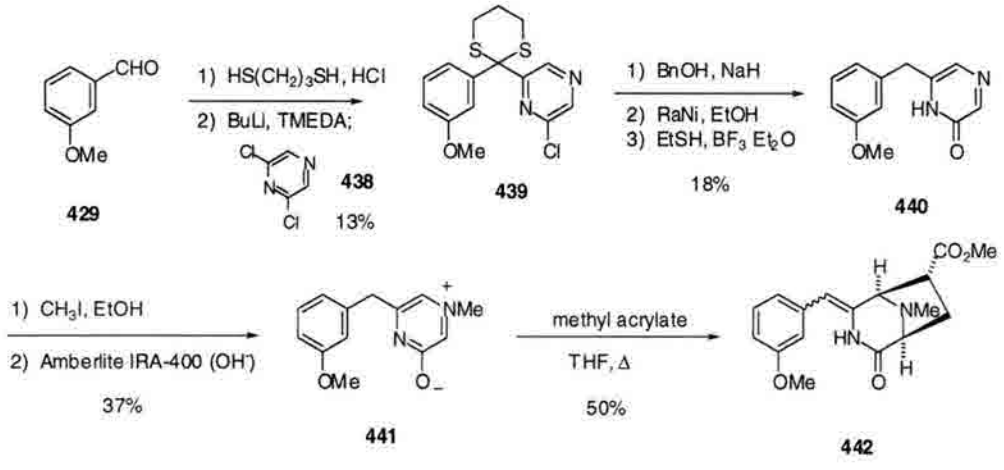
In 1990, Weinreb *et al.* reported a synthetic study towards quinocarcin using L-glutamic acid as a chiral, non-racemic starting material.[135] Starting with the aromatic aldehyde **429**, treatment with potassium cyanide followed by LAH afforded an amino alcohol that was treated with phosgene to afford the oxazolidinone **430** in high yield

(Scheme 59). 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**. Treatment with $BF_3 \bullet Et_2O$ provided 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 that 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 **364** in Fukuyama's total synthesis.



Scheme 59 Weinreb's synthetic studies on quinocarcin.

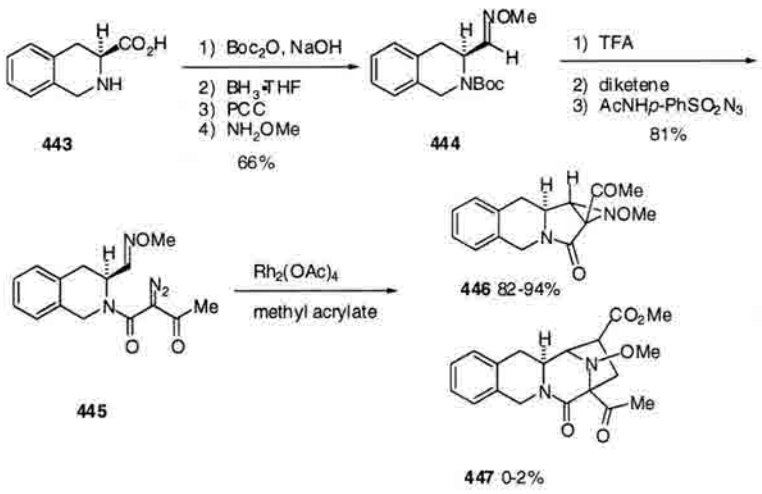
Using the 1,3 dipolar cyclization methodology used in the naphthyridinomycin synthetic studies, Joule *et al.* synthesized a similar bicyclic compound as in Weinreb's study.[136] Conversion of the aldehyde **429** to the dithiane was followed by alkylation with 2,6-dichloropyrazine **438** to afford **439** (Scheme 60). Nucleophilic substitution with benzyl alcohol was followed by debenzylation and desulfurization to afford **440**. Quaternization of the nitrogen followed by deprotonation yielded the dipolar species **441**. Cycloaddition using methyl acrylate afforded the bicyclic compound **442** in 50% yield.



Scheme 60 Joule's synthetic studies on quinocarcin.

In 1996, McMills *et al.* attempted to form an azomethine ylide similar to the Garner and Williams intermediates via a rhodium carbene cyclization (Scheme 61).[137] 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 was followed by α-diazoamide formation via Davies protocol to afford **445**. Unfortunately, upon treatment of **445** with the rhodium catalyst, the desired tetracycle **447** could only be detectected in

very small amounts. Aziridine **446** was the major product in all attempts using both rhodium and copper catalysts.

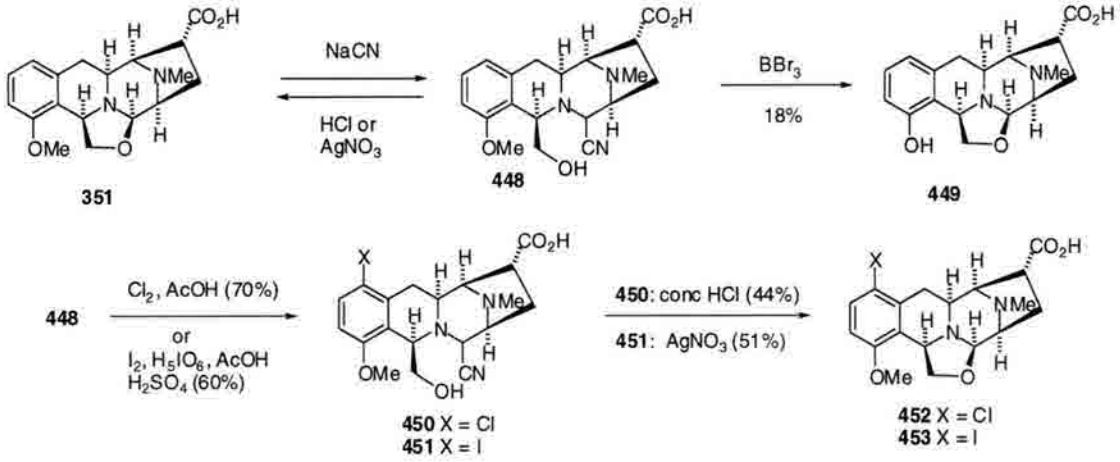


Scheme 61 McMills' synthetic studies on quinocarcin.

4.1.4. Quinocarcin Analogs

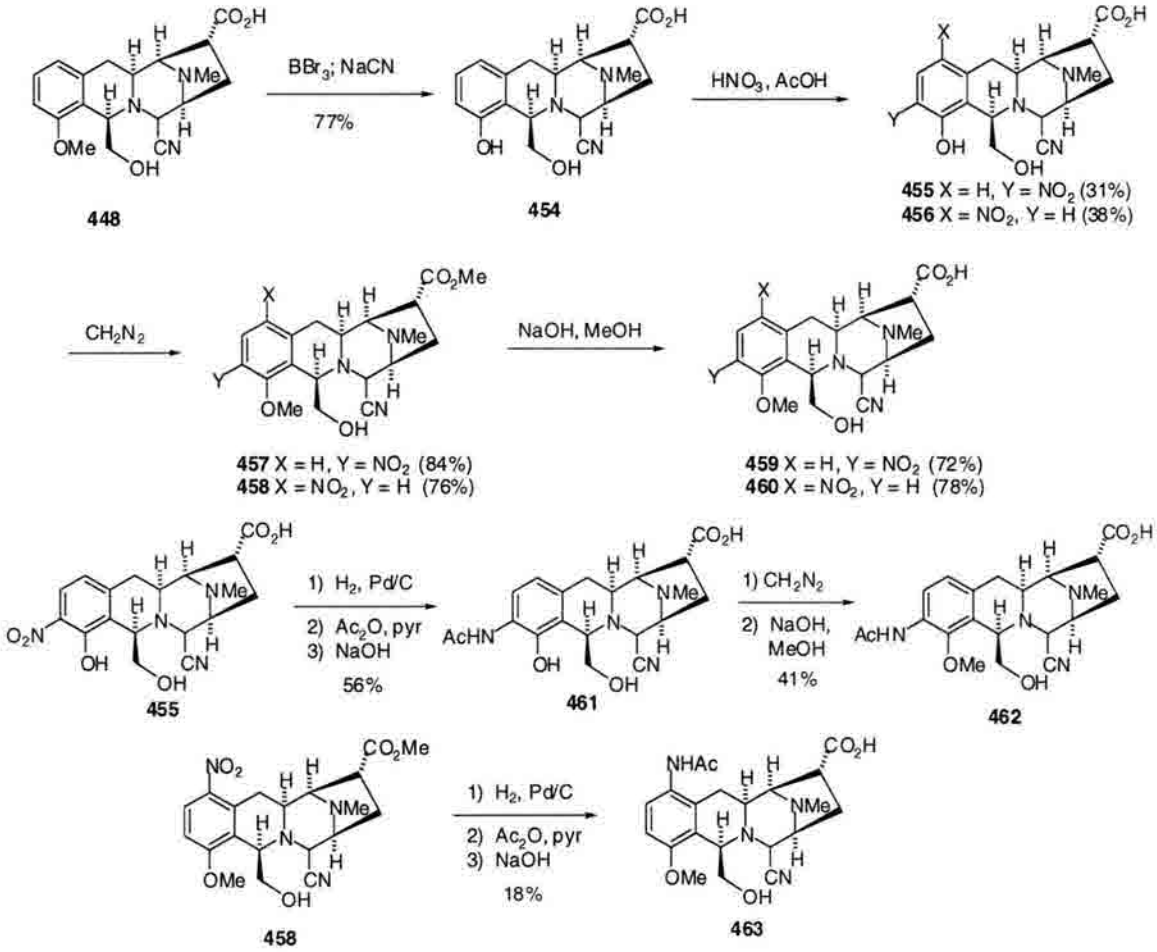
There have been numerous quinocarcin analogs that have been synthesized over the years. These analogs have been used for SAR studies and for studying the biological mechanism of action of this family of cytotoxic agents.

Kyowa Hakko Kogyo Company, Ltd., the discoverer of quinocarcin, has prepared a host of semi-synthetic analogs of quinocarcin including quinone, hydroquinone, and other substituted quinocarcin derivatives.[138] Comparison of the ring opened amino nitrile *versus* the closed oxazolidine ring was also preformed.



Scheme 62 Quinocarcin C-1 analogs.

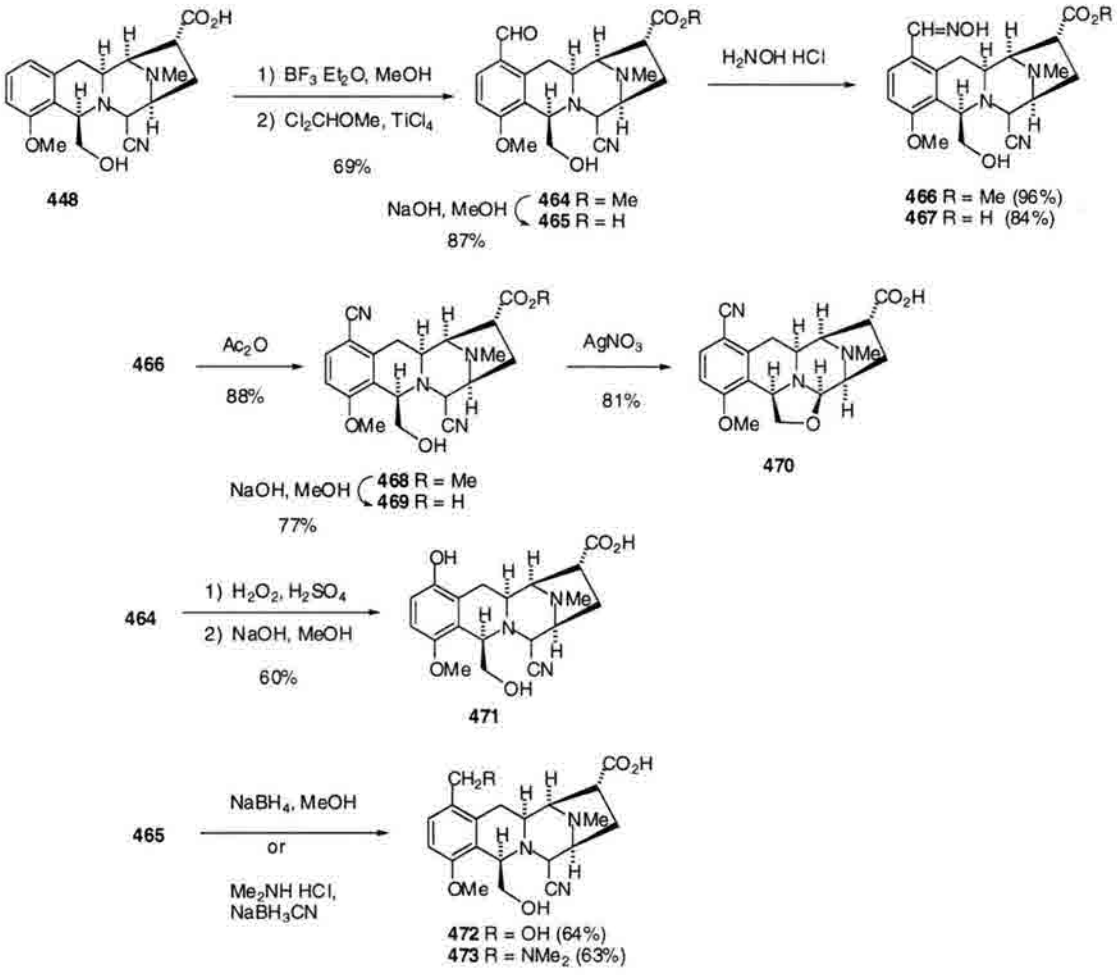
Demethylation of **448** followed by treatment with sodium cyanide afforded **454**



Scheme 63 C-1 and C-3 analogs of quinocarcin.

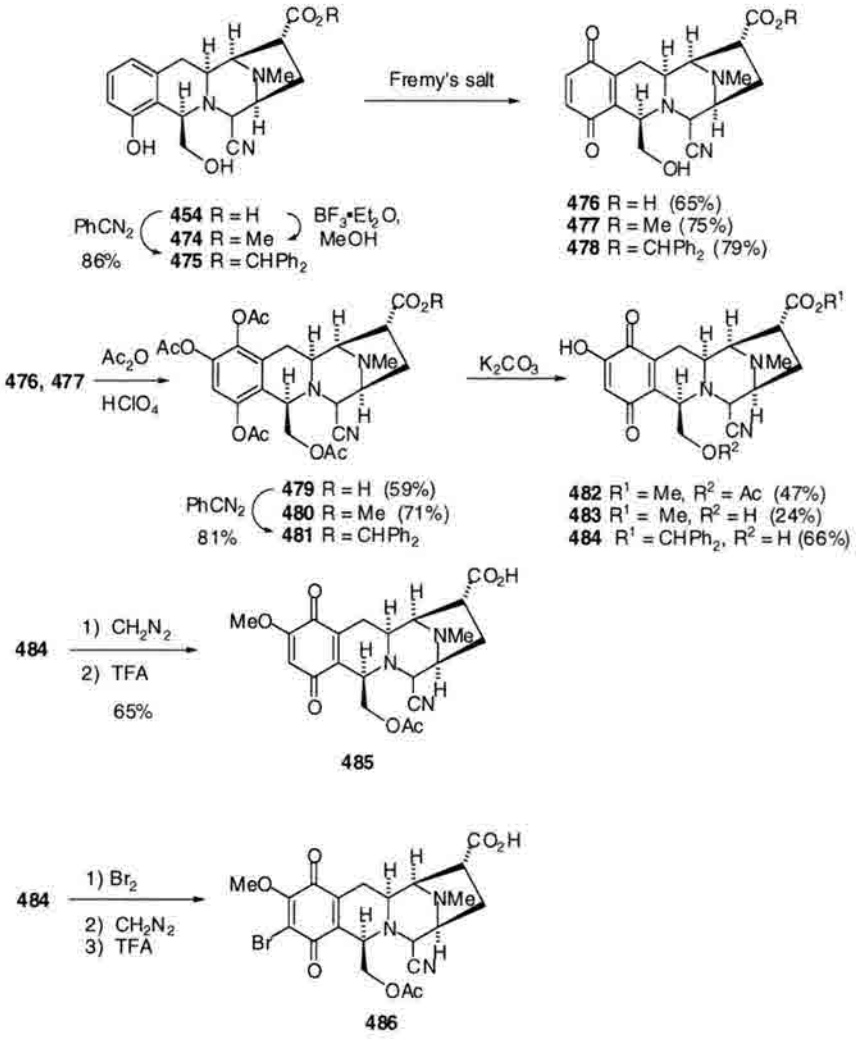
(Scheme 63). Nitration yielded two regioisomers **455** and **456**. The nitro compounds **459** and **460** were then formed by methylation of the phenol. Hydrogenation followed by protection of the resultant anilines provided **462** and **463**.

Several C-1 (quinocarcin numbering) analogs were prepared starting from DX52-1 (**448**) (Scheme 64). 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.



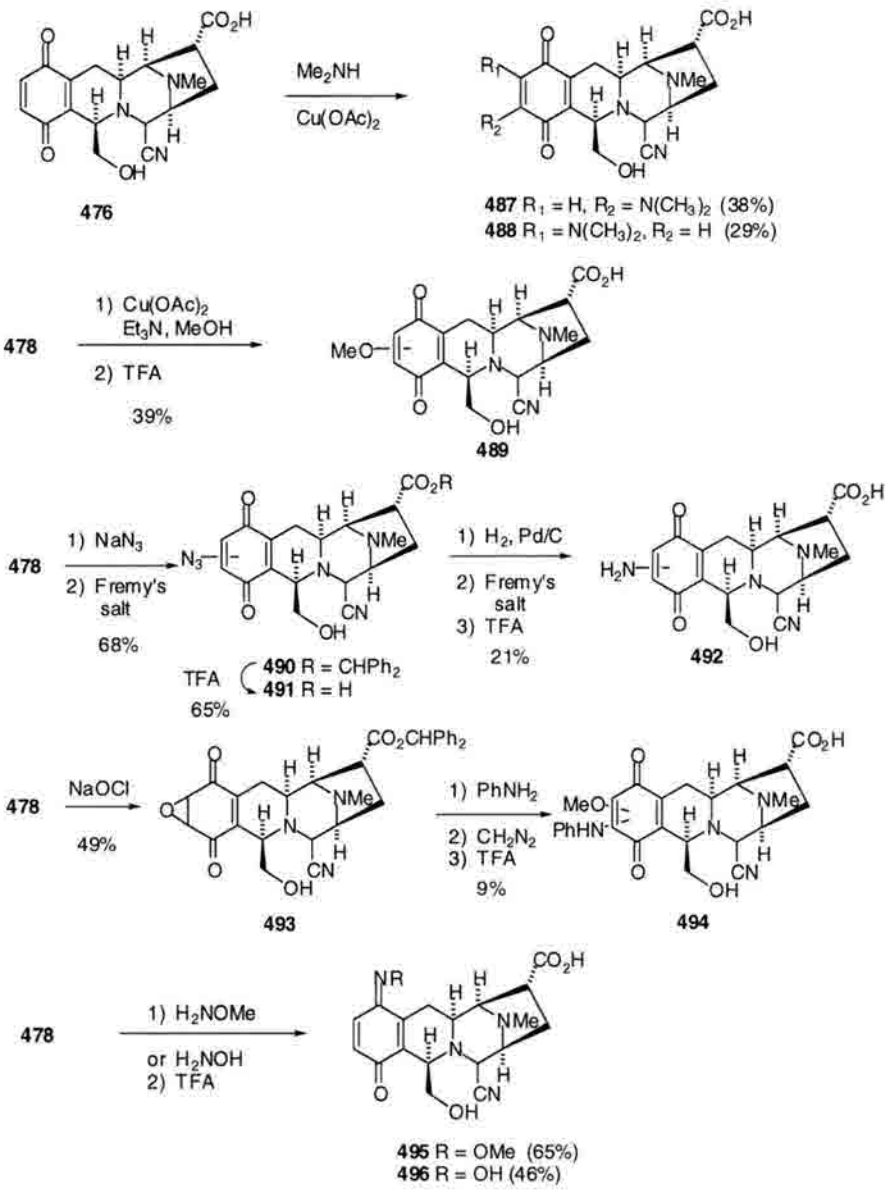
Scheme 64 C-1 analogs of quinocarcin.

The oxidation of phenols **454**, **474**, and **475** with Fremy's salt provided quinones **476-478** in good yields (Scheme 65).[138b] Further A ring functionalization afforded quinone analogs **482-486**.



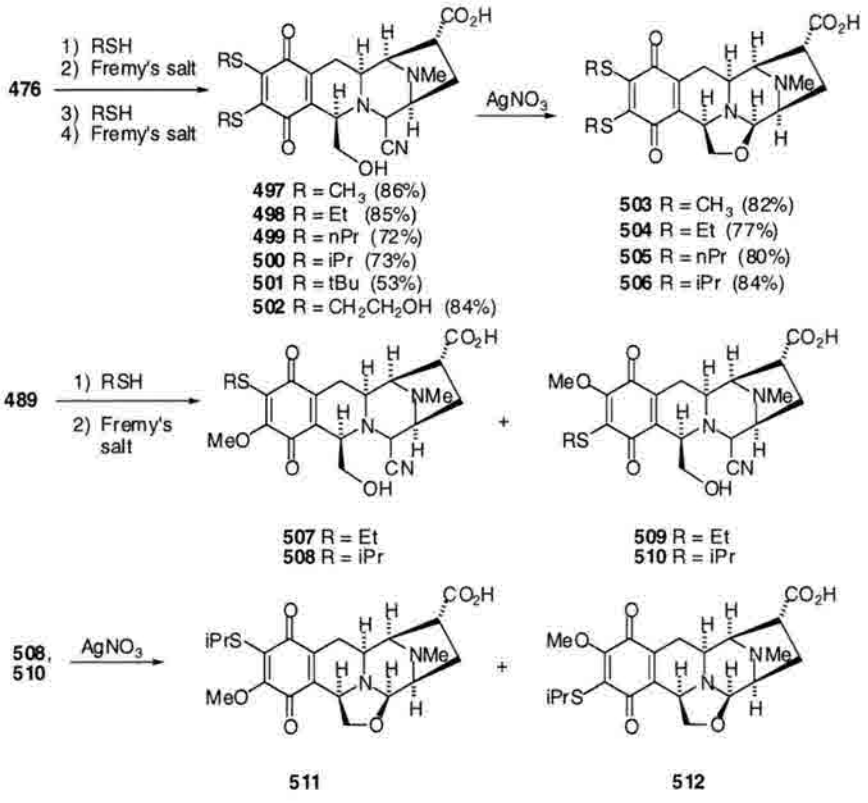
Scheme 65 Quinone analogs of quinocarcin.

Several other quinones were synthesized via quinone substitution (Scheme 66). Dimethylaniline derivatives **487** and **488** were synthesized via copper catalyzed addition of dimethyl amine to **476**. Copper catalyzed addition of methanol to **478** afforded **489** in 39% yield. Treatment of **478** with other nucleophiles afforded **492**, **494-496**.



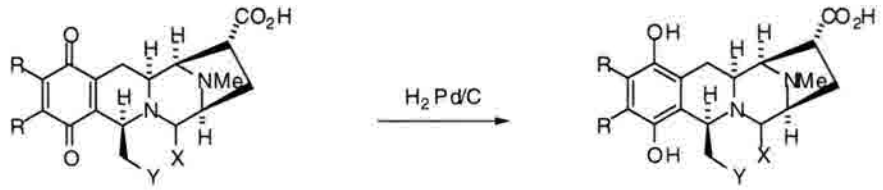
Scheme 66 Substituted quinone analogs of quinocarcin.

Several thiol quinones were synthesized from quinone **476** via the addition of thiols followed by reoxidation using Fremy's salt to afford the dithio quinones **497-502** (Scheme 67).[138c] Oxazolidine ring formation was accomplished using silver nitrate affording **503-509**. The methoxy thiol quinones **507-512** were synthesized using similar chemistry starting from quinone **489**.



Scheme 67 Thiol substituted quinone analogs of quinocarcin.

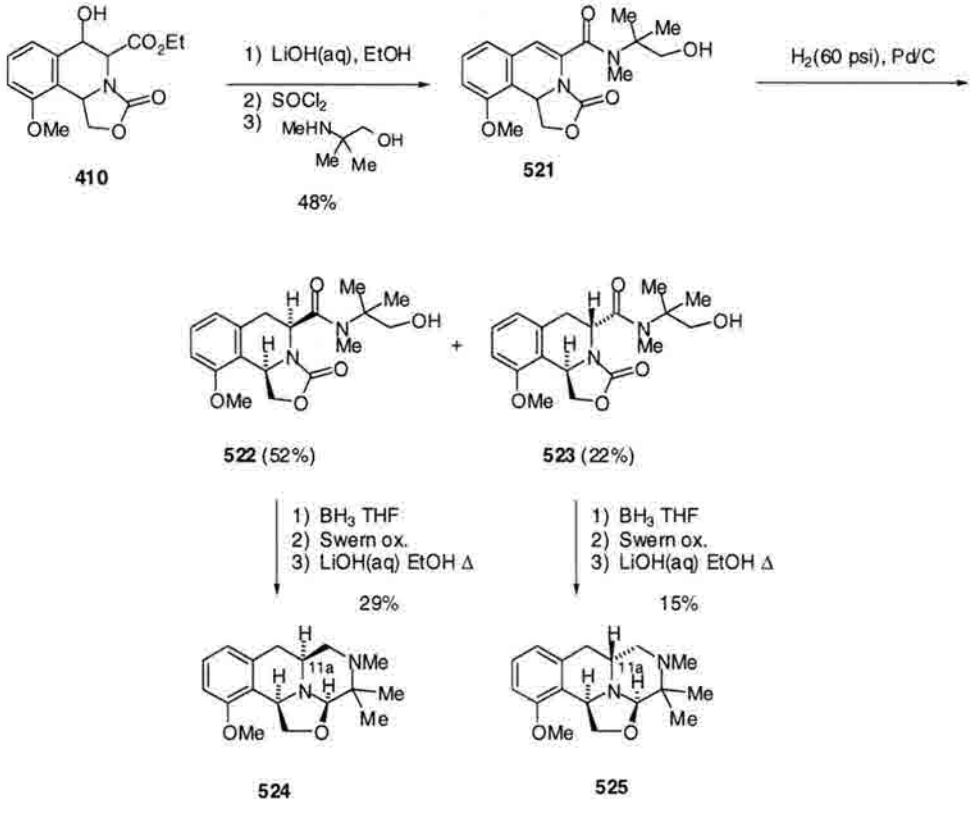
Some of the quinones were hydrogenated to afford the hydroquinones **513**-**520** in high yields (Scheme 68).



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%

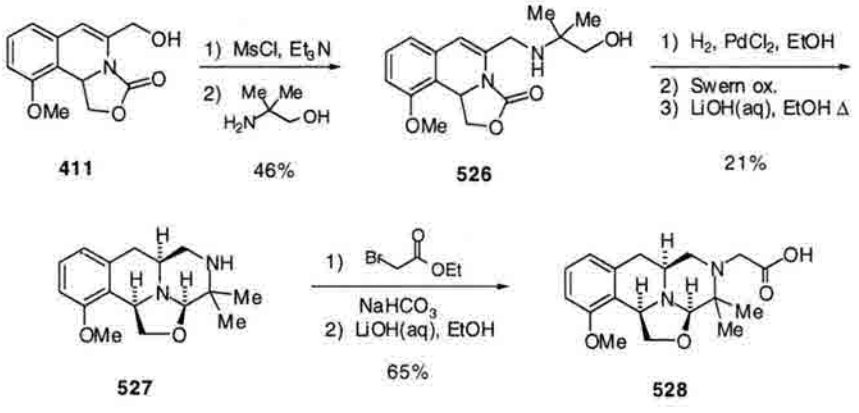
Scheme 68 Thiol substituted hydroquinone analogs of quinocarcin.

In order to study the importance of the stereochemistry of quinocarcin, two tetracyclic diastereomers were synthesized by Williams *et al.*[139] These diastereomers were epimers at C-11a (Scheme 69). Starting with tricycle **410**, the ester was hydrolyzed followed by treatment with thionyl chloride in refluxing benzene to afford the α,β-unsubstituted acid chloride. Treatment with the 2-methyl-2N-methyl propanol afforded **521**. Hydrogenation of the benzylic olefin afforded a mixture of diastereomers **522** and **523**. Each diastereomer was carried on separately. Reduction of the amide was followed by oxidation of the primary alcohol to an aldehyde. Hydrolysis of the oxazolidinone allowed for cyclization upon the aldehyde to afford the tetracycle analogs **524** and **525**.



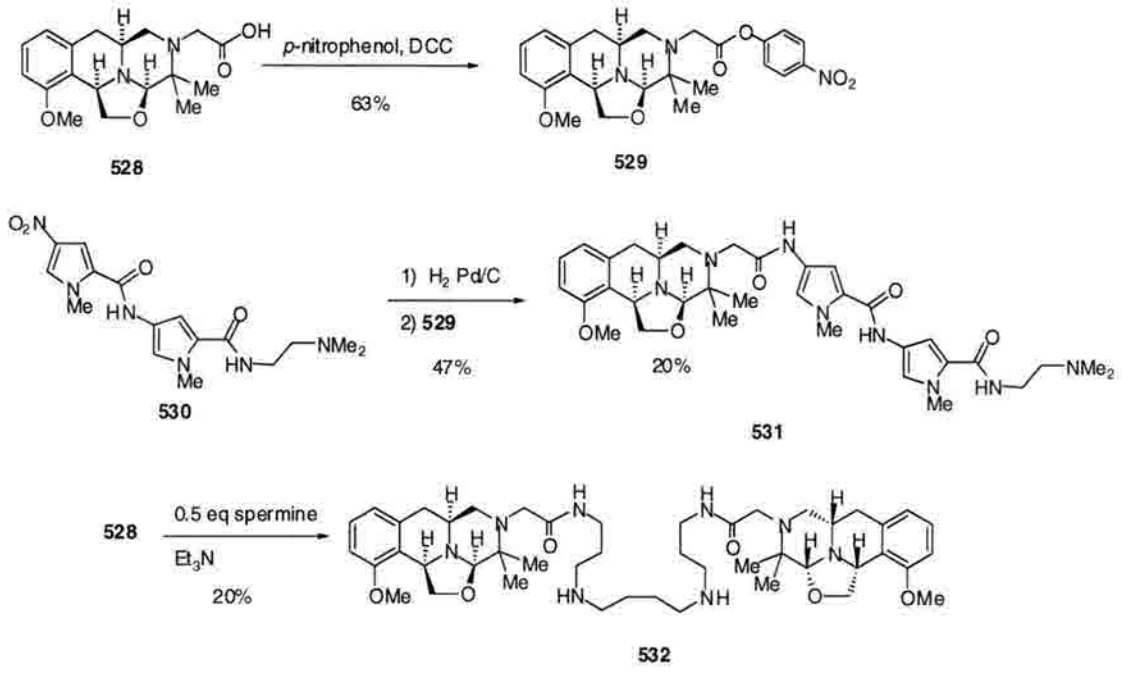
Scheme 69 Tetracyclic *syn* and *anti* analogs of quinocarcin.

These analogs were only sparingly water soluble, so a more soluble tetracyclic quinocarcin analog was synthesized (Scheme 70).[140] Allylic alcohol **411** was converted to the allylic chloride and treated with 2-amino-2-methylpropanol to afford the allylic amine **526**. Hydrogenation of the benzylic oleflin afforded a single diastereomer. Oxidation of the alcohol was followed by treatment in refluxing lithium hydroxide to afforded tetracycle **527**. Alkylation of the secondary amine with ethyl bromoacetate and saponification of the ester afforded the water soluble analog **528**.



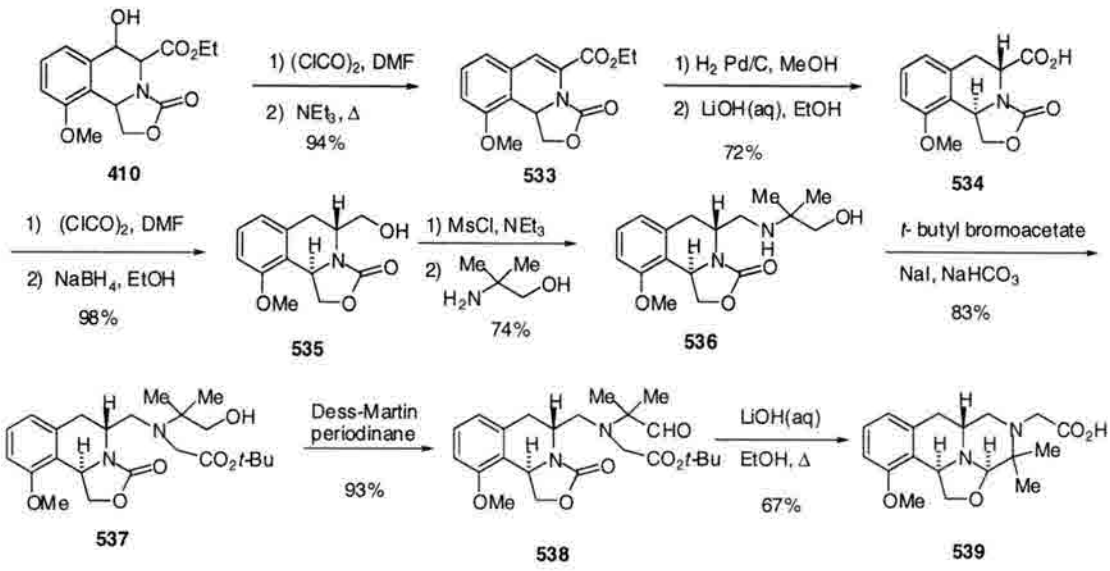
Scheme 70 Synthesis of water soluble quinocarcin analog

Tetracycle **528** was coupled to neutropsin (**530**), a DNA selective moiety, to afford **531** (Scheme 71). Also a quinocarcin analog dimer was prepared via the coupling of spermine to two equivalents of **528** to afford **532**.



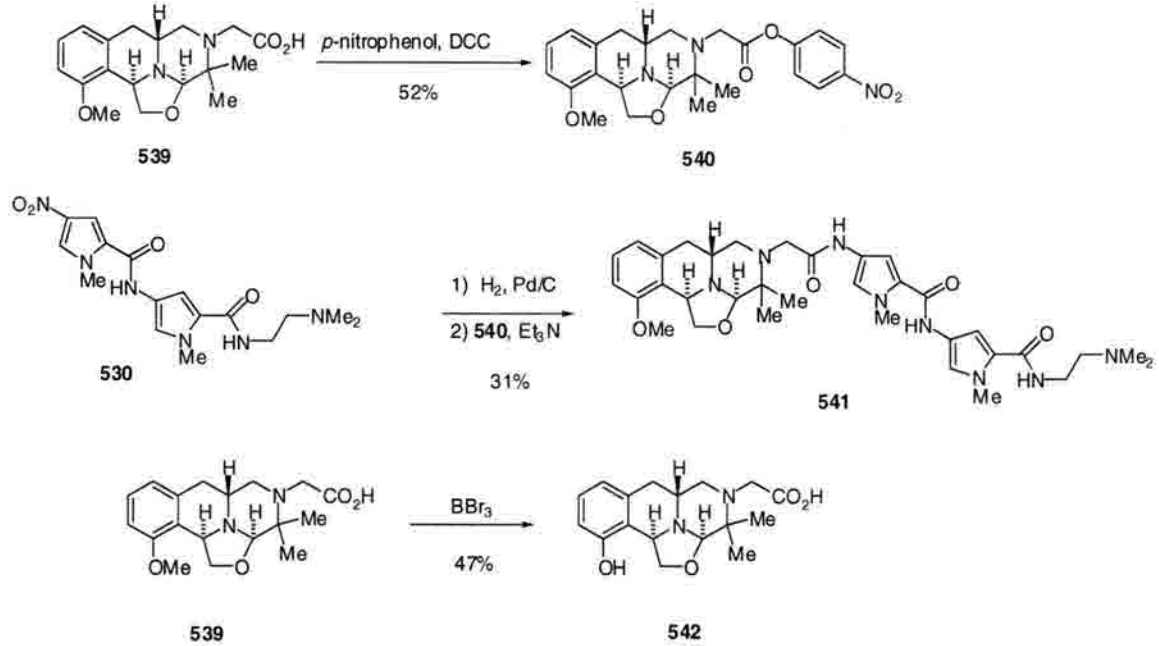
Scheme 71 Synthesis of neutropsin and spermine analogs of **529**.

The synthesis of the C-11a diastereomer of **528** was also diastereoslective (Scheme 72).[141] 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 diasteomer **534**. Reduction of the acid was followed by activation of the resultant alcohol (**535**) for displacement 2-amino-2-methyl propanol to provide amino alcohol **536**. Amine alkylation followed by Dess-Martin oxidation provided **537** in 77% yield from **536**. Finally, oxazolidinone opening and cyclization afforded the *anti* quinocarcin analog **539** in 67% yield.



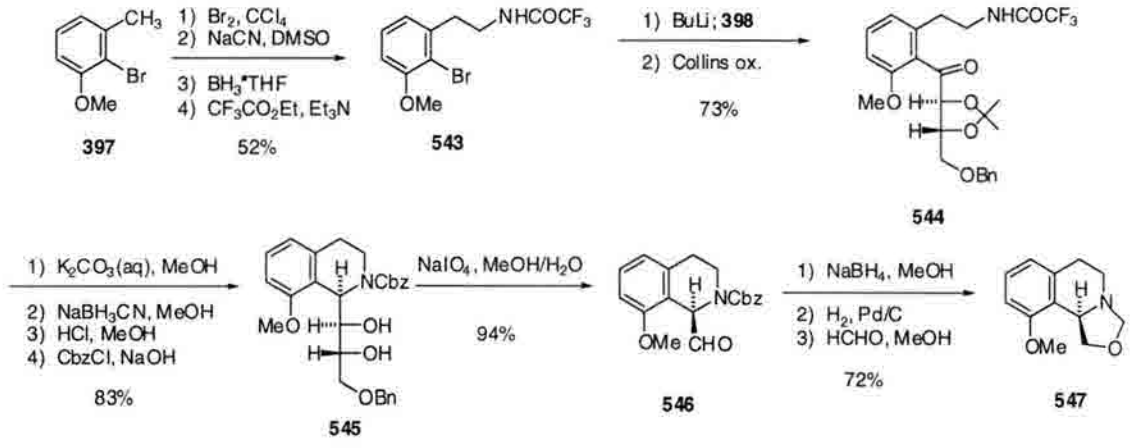
Scheme 72 Synthesis of *anti* quinocarcin analog **539**.

As in the case of the syn analog **528**, the anti analog **539** was coupled to neutropsin to afford **541** (Scheme 73). Also, **539** was demethylated using BBr_3 to afford the phenol **542.**



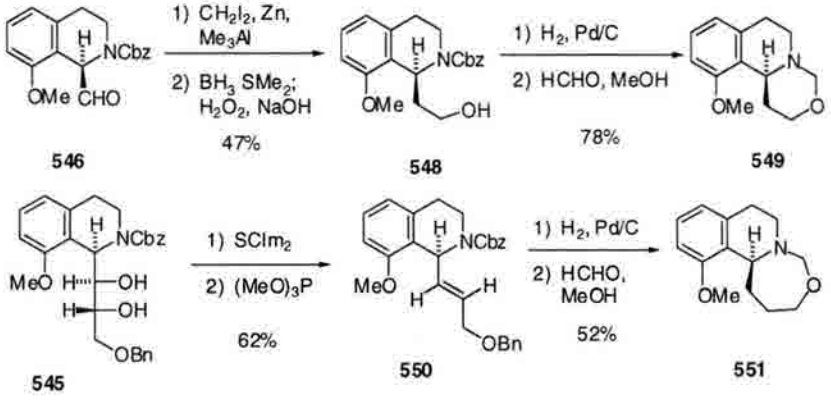
Scheme 73 Synthesis of neutropsin *anti* and phenol quinocarcin analogs.

Terashima *et al.* synthesized many analogs of quinocarcin including some simple ABE ring analogs (Schemes 74-75).[131a] Arene **397** was functionalized at the benzylic position to yield **543** (Scheme 74). Alkylation was accomplished to afford **544**. The amine was deprotected and it closed on the benzylic ketone. The resultant imine was reduced with sodium borohydride and the amine protected to afford bicylce **545**. Diol cleavage afforded the aldehyde **546** in 94% yield. Reduction of the aldehyde followed by reductive amination yielded tricycle **547**.



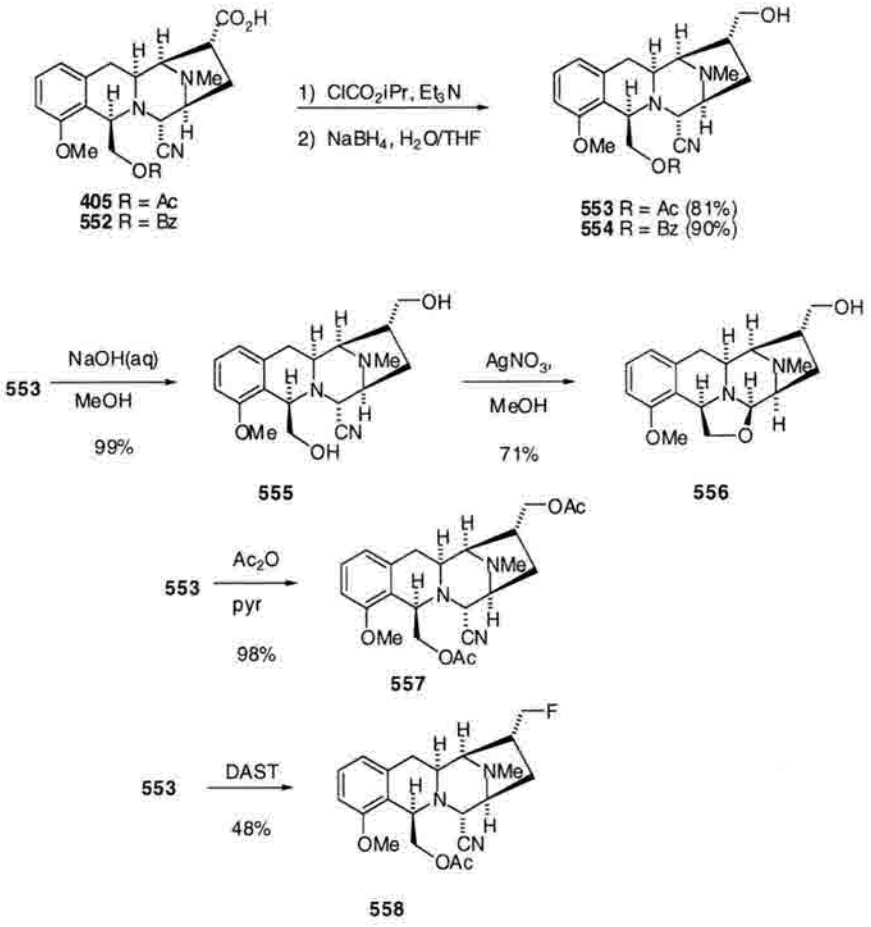
Scheme 74 Synthesis of ABE ring analog of quinocarcin.

Tricycles with 6 and 7-membered E rings (**549** and **551** respectively) were also synthesized using similar chemistry (Scheme 77).



Scheme 75 Synthesis of ABE ring analogs of quinocarcin.

D ring derivatives of quinocarcin were also synthesized by Terashima *et al.* (Scheme 76).[131e] The acid moiety was reduced to afford tetracycles **553** and **554**. Tetracycle **553** was then converted to the C-13 alcohol of quinocarcin (**556**). Analogs **557** and **558** were synthesized by treating **553** with acetic anhydride and DAST respectively.



Scheme 76 C-10 analogs of quinocarcin.

4.1.5. Biological Activity of quinocarcin and quinocarcin analogs

Quinocarcin has moderate activity against Gram-(+) bacteria such as *Staphylococcus aureus, B. subtitlis,* and *AAKlebsiells pneumoniae* with MIC's of 12.5,

12.5, and 25 μg/mL respectively.[125a] Quinocarcin has been shown to inhibit [^{3}H]-thymidine incorporation in *B. subtilis* this was found to be due to inhibition of DNA polymerase and DNA cleavage.[142] 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.[143] Quinocarcin citrate has also shown good activity against lung carcinoma cell lines that are resistant to either mitomycin C or cisplatin. [144] In P388 leukemia quinocarcin was shown to inhibit RNA synthesis over DNA and protein synthesis.

Quinocarcin citrate and DX-52-1 (**448**) were assayed in the National Cancer Institute screen of 60 tumor cell lines.[145] 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 have the toxicities associated with quinocarcin.

The DNA cleavage from quinocarcin was found to be due to the formation of superoxide.[142,146] The addition of superoxide dismutase (SOD) inhibited DNA cleavage by quinocarcin, while addition of DTT increased DNA cleavage. With the structure of quinocarcin being different than other known antitumor antibiotics that form superoxide, *i.e.* quinones, the mode of action was unknown.

In 1992, Williams *et al.* reported a study of superoxide formation by quinocarcin and quinocarcin analogs.[146] Since quinocarcin can exist in two distinct confomers (Figure

15) the four tetracyclic quinocarcin analogs **524**, **525**, **528**, and **539** were synthesized to study the effect of the stereochemistry of the nitrogen lone pair. These analogs were epimers at C-11a causing the nitrogen lone pair to adopt an *anti* configuration with the methine hydrogen at C-7 for **524** and **528** and a *syn* relationship in analogs **525** and **539**. It was found that the *syn* analogs **524** and **528** could produce superoxide, but the *anti* analogs **525** and **539** could not produce superoxide. This observation can be explained by the fact that the *anti* analogs can form a carbon centered in which the nitrogen lone pair is antiperiplanar to the methine hydrogen (Figure 15). While the *syn* analogs cannot form the radical because the nitrogen lone pair is periplanar to the methine hydrogen. The previous observations of superoxide formation were confirmed along with the fact that picolinic acid, a known hydroxyl radical scavenger, inhibited DNA cleavage. In addition, gel electrophoresis studies of the cleaved DNA showed a doublet for each base cleaved, indicating a diffusible DNA cleaving agent such as hydroxyl radical.

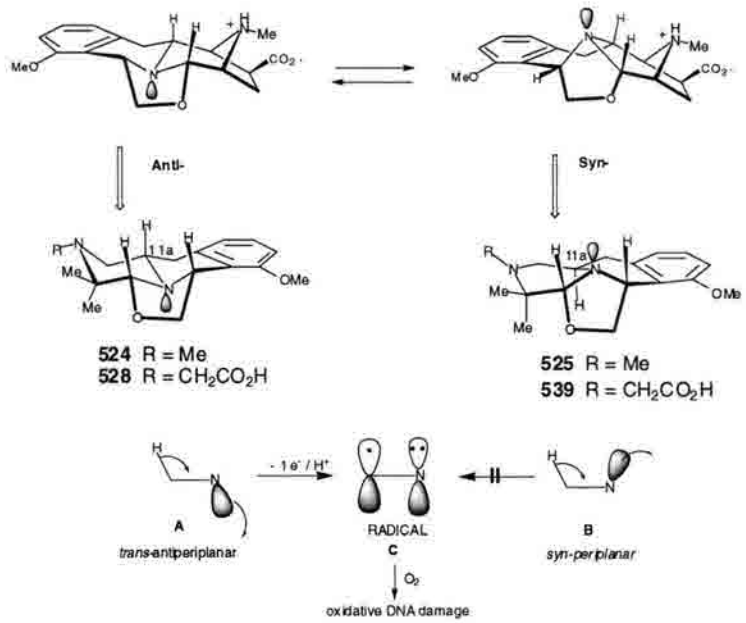
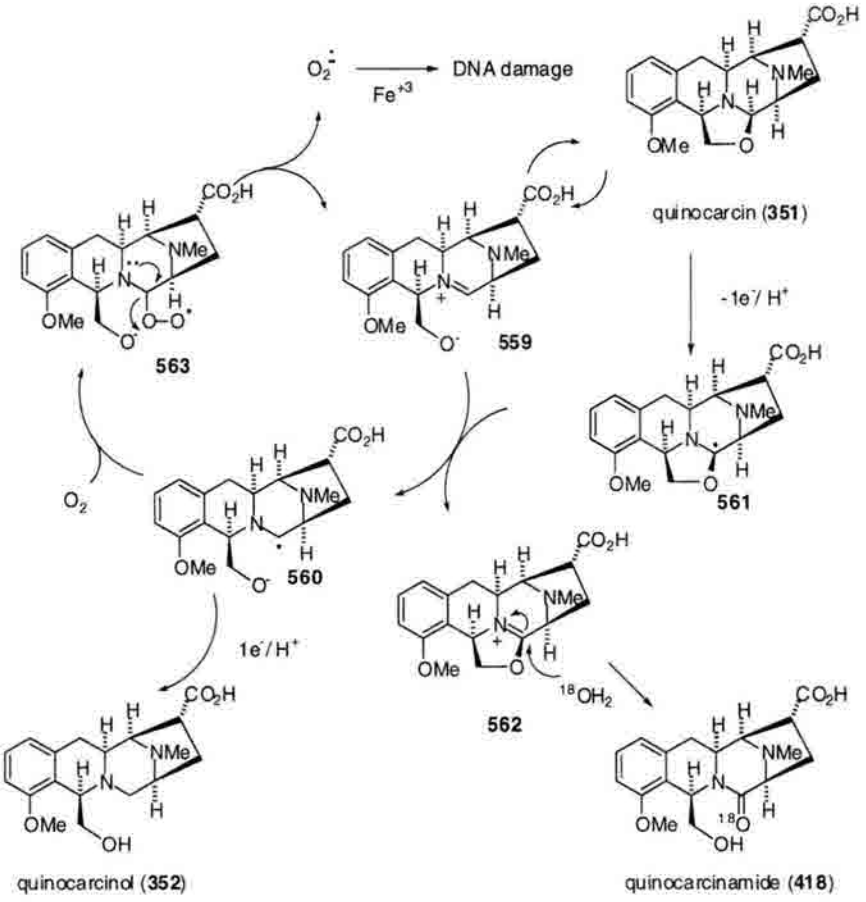


Figure 15 Importance of the stereochemistry of the nitrogen of quinocarcin and analogs.

A mechanism based on the redox self-disproportionation of quinocarcin was proposed based on the following experimental results.[146] When quinocarcin was allowed to stand in deoxygenated water at 25°C two new products were obtained. One product was quinocarcinol (**352**) the other was quinocarcinamide (**418**) (Scheme 77). A single electron transfer from quinocarcin with concomitant proton loss from the oxazolidine nitrogen to the ring-opened tautomer (**559**) would furnish radical anion **560** and oxazolidinyl radical **561**. Radical **561** would be capable of reducing another equivalent of **559** to afford oxazolidinium **562** which would be hydrolyzed by water to afford quinocarcinamide (**418**). Radical anion **560** would undergo a second electron transfer

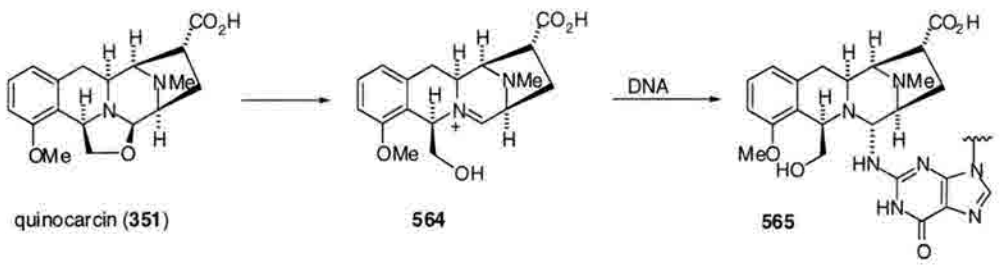


Scheme 77 Self redox cycle for quinocarcin.

with concomitant protonation to afford quinocarcinol (**352**). Under aerobic conditions, radical anion **560** would react with molecular oxygen to produce the peroxy radical **563** which would expel superoxide regenerating **559**. The superoxide would then go through Fenton cycling in the presence of Fe^{+3} to cause DNA damage via hydroxyl radicals.

Surprisingly, neutropsin analog **531**, which did cause DNA cleavage, was not inhibited by SOD or catalase.[140] Also, the DNA cleavage patterns were not random indicating a nondiffusable hydrogen atom abstractor. It was postulated that a carbon centered radical on **531** may directly abstract a hydrogen atom from DNA causing the observed specific DNA cleavage pattern.

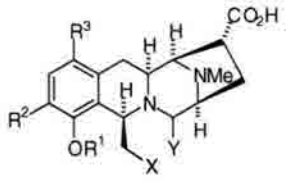
In 1988, Remers *et al.* reported a molecular modeling study on the binding of quinocarcin to DNA.[147] Using the mechanism of iminium formation from the saframycins, the quinocarcin iminium (**564**) was docked into the minor groove of DNA and several conformations were investigated (Scheme 78). This study indicated that that the original arbitrarily assigned absolute stereochemistry of quinocarcin would be a poor DNA alkylating agent. The opposite absolute stereochemistry was suggested based on better DNA binding energies and this was proven to be correct with the asymmetric synthesis of (-)-quinocarcin.



quinocarcin (**351**) **564** **565**

Scheme 78 Proposed DNA alkylation of quinocarcin.

It was originally believed by Williams *et al.* that the *syn*-quinocarcin analog **539** would alkylate DNA. However, no DNA alkylation was observed with this compound , the neutropsin analog (**541**), nor the phenol analog (**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 bulky to allow for DNA to approach and to be alkylated. A second possiblity was that the alkyation maybe reversible due to displacement of the DNA by the amine lone pair.



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-		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-		0.04	12.5	40	0.93
452	Me	H	I	OH	CN	0.11	50	31	1.15
453	Me	H	I	-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-		0.51	20	22	0.67
351						0.05-0.11	10-20	27-56	1

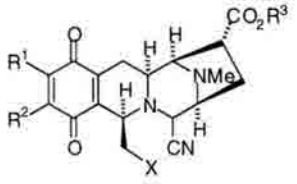
ILS = increase life span, (R) = ILS (analog)/ILS (quinocarcin), NT = not tested

Table 8 *In vivo* studies for C-1 analogs of quinocarcin.

The biological activities of the Kyowa Hakko Kogyo compounds[138] are listed in tables 8-12. All 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 analogs are listed in table 8.[138a] As can be seen having the oxazolidine intact increases the activity (**451** vs. **450** and **453** vs. **452**) Also the presence of the free phenol lowers the activity.

The quinone analogs, for the most part, showed much lower activities than quinocarcin (Table 9).[138b] The unsubstituted (**462**) and diamino (**473**) derivatives showed the best activities.

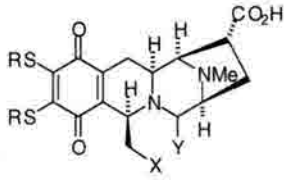


Analog	R^1	R^2	R^3	X	$HeLaS_3$ IC_{50}(μg/mL)	Dose (mg/kg) x1 (P388)	ILS(%)
476	H	H	H	OH	>10	NT	
471	H	H	Me	OH	0.12	20	18
482	OH	H	Me	OAc	>10	100	14
483	OH	H	Me	OH	>10	NT	
485	OMe	H	H	OAc	>10	25	22
486	OMe	Br	H	OAc	NT	9.38	20
487	H	NMe_2	H	OH	0.92	6.25	12
488	NMe_2	H	H	OH	0.79	3.13	15
491	N_3	H	H	OH	>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

ILS = increase life span, NT = not tested

Table 9 *In vivo* studies for quinone analogs of quinocarcin.

Surprisingly the thioalkyl quinones showed some increased activity over quinocarcin (Table 10). Compounds **485** and **490** showed good acitivity *in vitro* while **484** showed good activity *in vivo*.

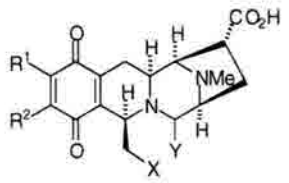


Analog	R	X	Y	$HeLaS_3$ $IC_{50}(\mu g/mL)$	Dose (mg/kg) x1 (P388)	ILS(%)	(R)
497	Me	OH	CN	0.13	12.5	53	0.59
498	Et	OH	CN	0.11	12.5	50	0.42
499	n-Pr	OH	CN	0.05	25	56	0.48
500	i-Pr	OH	CN	0.012	25	65	1.35
501	t-Bu	OH	CN	0.004	25	48	0.50
502	$HOCH_2CH_2$	OH	CN	2.47	12.5	26	
503	Me	-O-		0.019	6.25	48	0.88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

ILS = increase life span, (R) = ILS (analog)/ILS (quinocarcin)

Table 10 *In vivo* studies of dithiol substituted quinone analogs of quinocarcin.

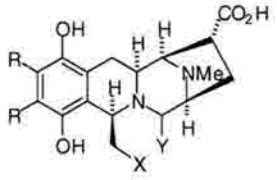
Table 11 shows the activities for the mixed substituted quinone analogs.[138c] Once again, the oxazolidine compounds have better activity than the E ring opened forms. This is also true with the hydroquinone derivatives (Table 12).



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-		0.79	6.25	31	1.29
512	MeO	i-PrS	-O-		2.37	6.25	30	1.25
351					0.05-0.11	10-20	24-48	1

ILS = increase life span, (R) = ILS (analog)/ILS (quinocarcin)

Table 11 *In vivo* studies of thiol substituted quinone analogs of quinocarcin.



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-		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

ILS = increase life span, (R) = ILS (analog)/ILS (quinocarcin), NT = not tested

Table 12 *In vivo* studies of thiol substituted hydroquinone analogs of quinocarcin.

The analogs synthesized by Terashima *et al.* showed some increased biological activity, over quinocarcin, against P388 murine leukemia (Table 13).[131e,f] Analog **554** had the best activity by far with two orders of magnitude more potent than quinocarcin (**351**).

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}		

Table 13 *In vitro* toxicity of quinocarcin analogs against P388 Murine Leukemia.

4.2. Tetrazomine

4.2.1. Isolation and structure determination

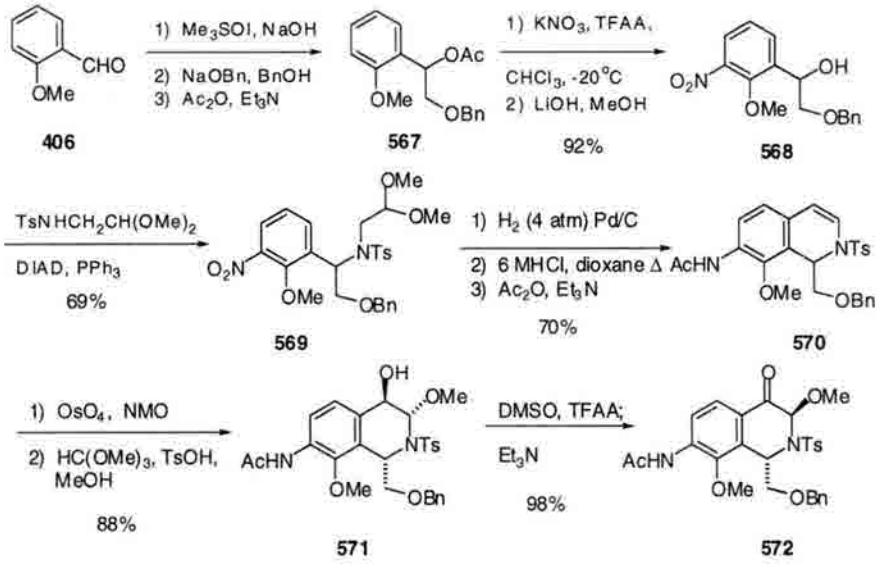
In 1991, Suzuki *et al.* isolated tetrazomine (**566**) from *Saccharothrix mutabilis* subsp. *chichijimaensis*.[148] The structure was determined by NMR spectroscopy relying heavily on 2-D techniques.[149] The structure is very similar to that of quinocarcin with respect to the pentacyclic core. The major difference being the amine at C-10' with the unusual amino acid 3-hydroxy pipecolic acid, which is unique to tetrazomine.

Figure 16 Structure of tetrazomine.

4.2.2. Synthetic Studies

To date there have been no total synthesis of tetrazomine. In a synthetic study towards tetrazomine, Ponzo and Kaufman reported the synthesis of the AB ring system of tetrazomine via acidic cyclization.[150] Starting with *o*-anisaldehyde (**406**) the epoxide was

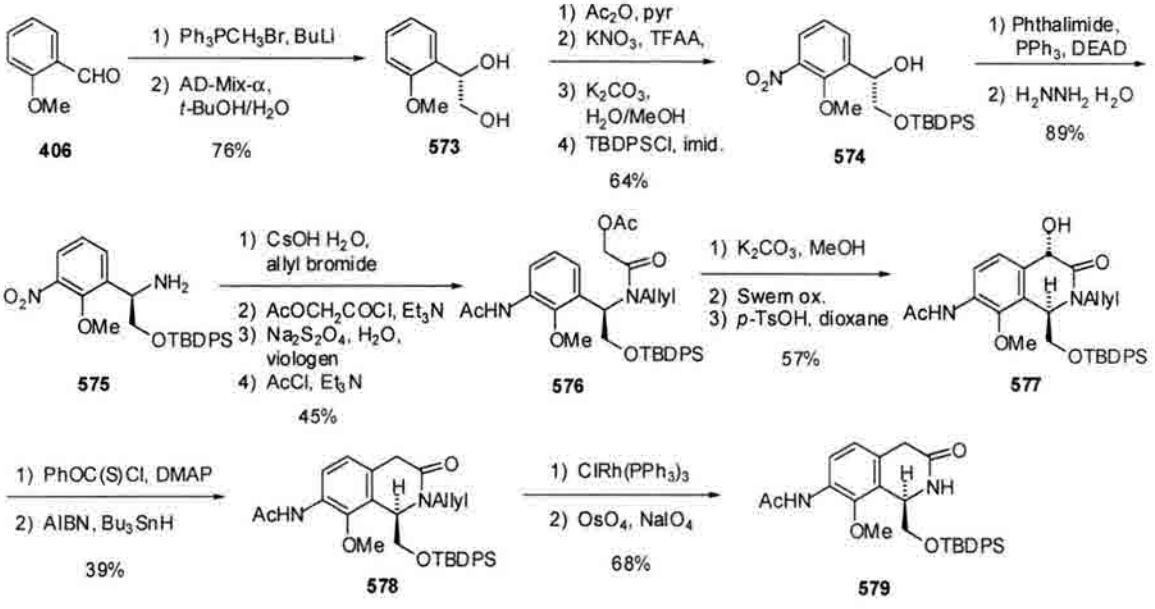
formed followed by selective opening with the sodium salt of benzyl alcohol to afford **567** (Scheme 79). Selective nitration at low temperature afforded the desired regioisomer **568** selectively in high yield. A Mitsunobu reaction installed the desired benzylic amino functionality to afford **569** in 69% yield. Reduction of the nitro group was followed by cyclization under acidic conditions to afford the dihydroquinone **570** following acylation of the aniline. Dihydroxylation was followed by methanolysis to afford **571**. The final step in the synthetic study was the oxidation of the benzylic alcohol to the ketone to afford **572**.



Scheme 79 Kaufman synthesis of AB ring of tetrazomine.

In 2001, Wipf and Hopkins reported the enantioselective synthesis of the AB ring of tetrazomine.[151] This was accomplished via a Sharpless asymmetric dihydroxylation of 2-methoxy styrene to afford diol **573** (Scheme 80). 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 **574**. A Mitsunobu inversion using phthalimide was followed by treatment with hydrazine to provide the amine **575**. Mono allylation of the

amine was followed by acylation of the secondary amine to provide the amide. Reduction of the nitro group and acetate protection afforded **576** in 45% overall yield from **575**. Cleavage of the acetate followed by Swern oxidation afforded the aldehyde that underwent a Friedel Crafts hydroxyalkylation in the presence of *para*-toluene sulfonic acid to afford the bicycle **577**. Barton-McCombie deoxygenation provided the lactam **578** in which the allyl group was removed to afford **579**. Future plans called for the formation of the tetracyclic core via an intramolecular Heck cyclization.



Scheme 80 Wipf's syntheis of optically active AB ring of tetrazomine.

4.2.3 Biological Activity of Tetrazomine

Tetrazomine has been shown to be active against both Gram-(+) and Gram-(-) bacteria (Table 14).[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.

Test organisms	MIC (μg/mL)
Bacillus subtilis ATCC 6633	6.25
Staphylococcus aureus FDA 209P JC-1	6.25
S. epidermidis IID 866	25
Streptococcus pyogenes Cook	0.78
Enterococcus faecalis IID 682	6.25
E. 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
P . aerginosa ATCC 8689	50

Table 14 Antimicrobrial activities of tetrazomine.

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 15). The optimal dose for tetrazomine was found to be 0.05 mg/kg that yielded a T/C (Treated vs. Control) of 173%.

Antibiotic	Dose (mg/kg/day)	MST (days)	T/C	Survival (40 days)
Tetrazomine	0.0125 x 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 x 5 ip	27.0	245	2/8
	1.0	24.5	223	2/8
Control		11.0	100	0/8

Table 15 *In vivo* biological activity of tetrazomine against P388 leukemia.

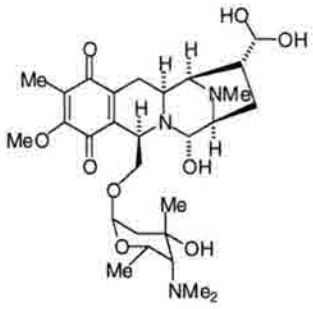
Williams *et al.* has shown that tetrazomine, like quinocarcin, undergoes a self redox reaction to produce superoxide that can cleave DNA in a non-specific manner.[152] The mechanism is the same of that of quinocarcin (Scheme 77). The rate of superoxide formation was pH dependent, with the highest rate at pH 8. A variety of conditions were

used in the presence of tetrazomine to determine the percent inhibition and enhancement. The addition of superoxide inhibitors SOD and catalase inhibited DNA cleavage. Addition of Fe^{2+} and Fe^{3+} had little effect. Desferal, an iron chelator, inhibited the cleavage at higher concentrations. All of these observations are similar to those seen with quinocarcin.

4.3. Lemonomycin

4.3.1. Isolation and structure determination

Lemonomycin (**580**) was isolated in 1964 from *Streptomyces candidus* (LL-AP191).[153] The structure was not determined until 2000, by He *et al.* via NMR spectroscopy (Figure 17).[154] Lemonomycin contains the unusual 2,6-dideoxo-4-amino sugar.

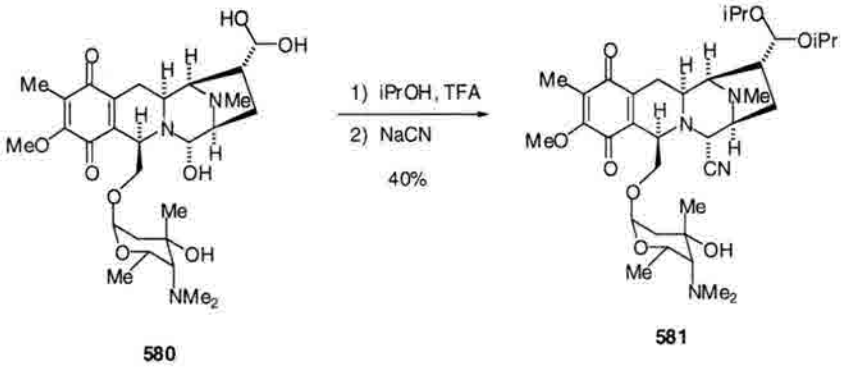


Lemonomycin (**580**)

Figure 17 Structure of lemonomycin.

4.3.2. Analog of Lemonomycin

The treatment of lemonomycin with isopropanol in TFA followed by sodium cyanide afforded **581** in 40% overall yield (Scheme 81).[154]



580 581

Scheme 81 Synthesis of lemonomycin analog.

4.3.3. Biological Activity

Lemonomycin has shown antimicrobrial activity against several organisms (Table 16). Lemonomycin and the cyano analog **581** also have shown *in vitro* activity against the human colon cell line (HCT116) with IC_{50}'s of 0.36 and 0.26 μg/mL respectively.[154]

Test organism	MIC (μg/mL)
Staphylococcus aureus	0.2
Bacillus subtilis	0.05
MRStaphylococcus aureus	0.4
Enterococcus faecium	0.2

Table 16 Antimicrobrial activites of lemonomycin.

Conclusion

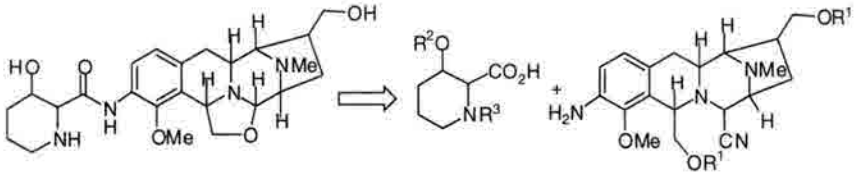
The tetrahydroisoquinoline antitumor antibiotics have been studied throughly over the years. A diverse range of biological activity and structure fuels this ongoing research. Recently, with the evidence for a new class of antitumor compounds being reported, the ecteinascidins are generating interest of both chemists and biologists. The synthetic routes to these compounds have provided the chemical community with a variety of new and interesting reactions. It is certain that the future of these natural products will continue to provide much more useful information for both the chemist and biologist.

Chapter 2

Synthesis of the β-Hydroxypipecolic Acids

2.1. Introduction

The total synthesis of (-)-tetrazomine (**566**) required the synthesis and determination of the correct stereoisomer of the β-hydroxypipecolic acid moiety. The total synthesis was envisioned to be completed by coupling of a protected β-hydroxypipecolic acid (**582**) to aniline **583** (Scheme 82). This coupling would occur late in the synthesis. After a series of deprotections, the closure of the oxazolidine ring would be the final step in the total synthesis.

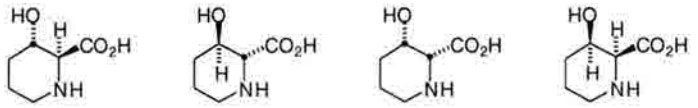


566 582 583

Scheme 82 Original late stage retrosynthetic analysis of tetrazomine.

Since neither the absolute nor relative stereochemistry of the β-hydroxypipecolic acid moiety of tetrazomine was known, the synthesis of all four stereoisomers was undertaken (Figure 18). The synthetic amino acids would then be compared to the amino acid that can be hydrolyzed from the natural product (our laboratory had 80 mg of natural

tetrazomine from Yamanouchi Pharmaceutical Co.) to determine the absolute stereochemistry of the β-hydroxypipecolic acid on tetrazomine.

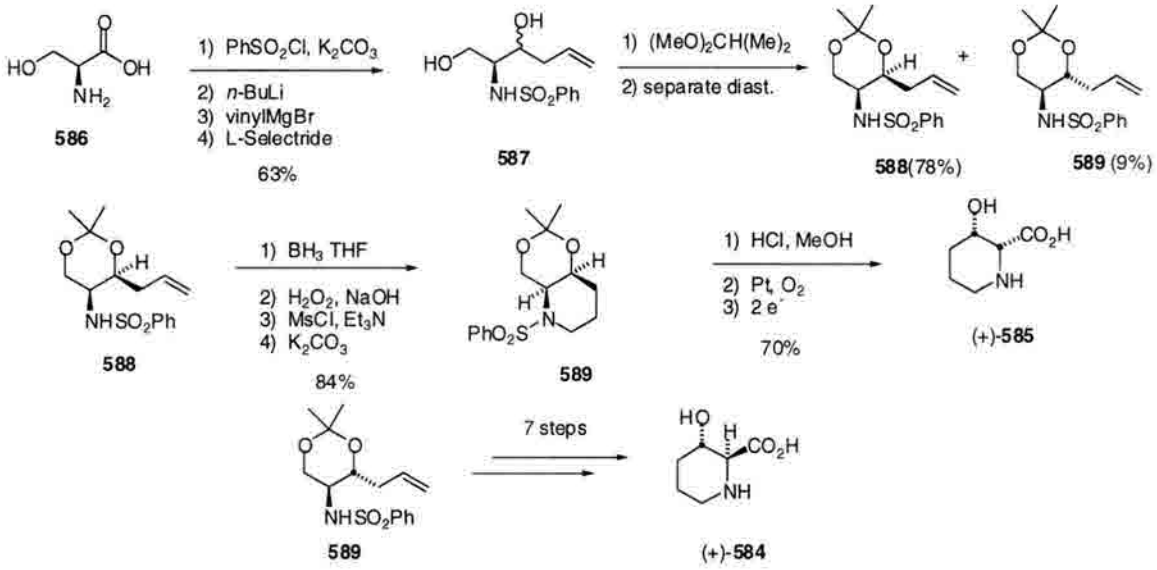


(+)-**584** (2S, 3S) (-)-**584** (2R, 3R) (+)-**585** (2R, 3S) (-)-**585** (2S, 3R)

Figure 18 Structures of β-hydroxypipecolic acid stereoisomers.

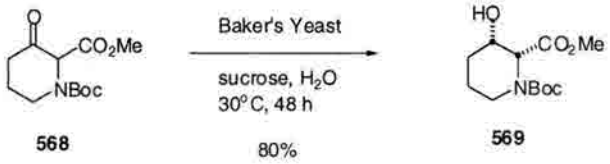
2.2. Previous Syntheses of β-hydroxypipecolic acid

Prior to our work on the syntheses of the β-hydroxypipecolic acids there was only one asymmetric synthesis of each of the diastereomers of β-hydroxypipecolic acid. Roemmele and Rapoport reported the synthesis of both *cis*– and *trans*-β-hydroxypipecolic acids starting from serine.[155] This synthesis, however, was 10 steps in length for the *cis*- diastereomer including a difficult separation of diastereomers requiring MPLC (Medium Pressure Liquid Chromatography) (Scheme 83). Protection of the serine amine as a sulfonamide was followed by conversion of the acid to a vinyl ketone and ketone reduction to afford a 9:1 mixture of diastereomers **587**. Protection of the 1,3-diol as an isopropylidene ketal was followed by separation of the diastereomers by MPLC to afford **588** and **589**. Each diastereomer was carried on separately to the respective β-hydroxypipecolic acids. In the case of the synthesis of the *cis* amino acid, hydroboration of the alkene of **588** was followed by formation of the mesylate and cyclization under basic conditions to afford the bicyclic substance **590**. Cleavage of the isopropylidene ketal was followed by oxidation of the primary alcohol to the acid using oxygen in the presence of platinum. The cleavage of the sulfonamide was accomplished under electrochemical conditions to afford (+)-**585**. The same chemistry was used to synthesize (+)-**584** from **589**.



Scheme 83 Rapoport's syntheses of both *cis* and *trans* β-hydroxypipecolic acids.

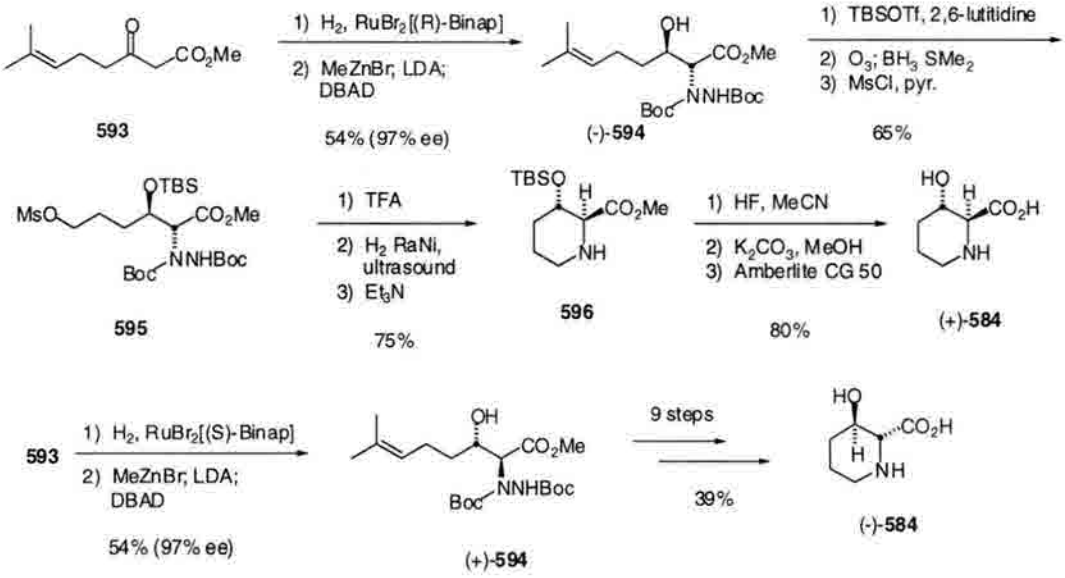
Knight *et al.* reported the Baker's yeast reduction of racemic β-keto ester **591** to afford the optically active β-hydroxy ester **592** (Scheme 84).[156] This reaction is an example of a dynamic kinetic resolution. Deprotection of the Boc group and hydrolysis of the ester to afford (+)-**585** were not reported by these workers.



Scheme 84 Knight's Baker's yeast reduction.

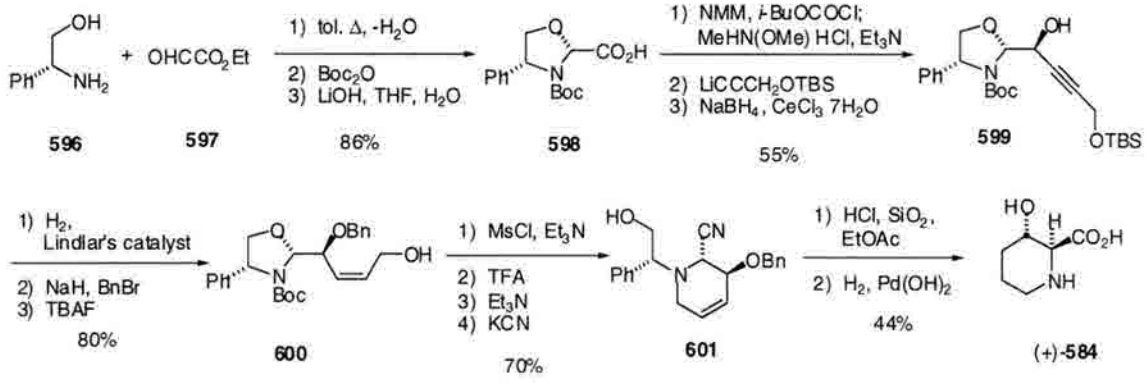
During the work on the syntheses of the four stereoisomers of β-hydroxypipecolic in our laboratories, several other syntheses of the two diastereomers were reported. In 1996, Ferreira *et al.* reported the synthesis of both enantiomers of *trans*- **584**.[157] Starting from the β-ketoester **593**, asymmetric reduction of the ketone was followed by diastereoselective amination using di-*t*-butylazodicarboxylate (DBAD) as the amine source to afford **594** (Scheme 85). Protection of the alcohol was followed by ozonolysis of the olefin and activation of the resultant alcohol as a mesylate to provide

595. Deprotection of the hydrazine was accomplished using TFA. The hydrazine was then hydrogenated to afford the amine which cyclized upon the mesylate under basic conditions to afford **596** in 75% yield from **595**. Deprotection of the secondary alcohol and hydrolysis of the ester afforded the free amino acid (+)-**584**. The use of the (*S*)-Binap catalyst afforded (+)-**594** which was carried onto (-)-**584**.



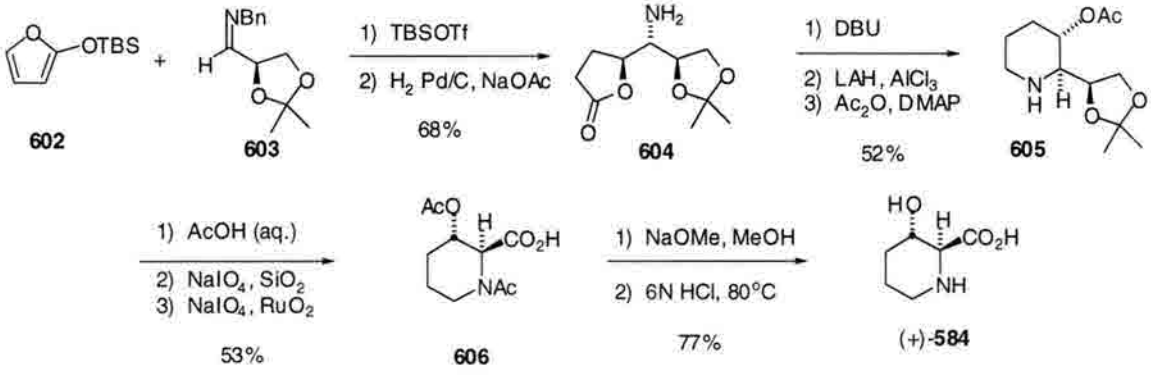
Scheme 85 Ferreira's syntheses of both enantiomers of *trans* β-hydroxypipecolic acid.

Agami *et al.* reported the synthesis of (+)-**584** starting from (R)-phenylglycinol (**596**) and ethyl glyoxylate (**597**) (Scheme 86).[158] Oxazolidine formation was followed by amine protection and ester hydrolysis to afford **598**. The Weinreb amide of **598** was formed and then treated with the lithium salt of TBS-protected propargyl alcohol. The resultant ketone was then reduced to provide **599**. Partial reduction of the alkyne using Lindlar's catalyst was followed by protection of the secondary alcohol and deprotection of the primary alcohol to yield **600**. The cyclic amino nitrile **601** was formed via a four step sequence. Hydrolysis of the nitrile and hydrogenation afforded (+)-**584**.



Scheme 86 Agami's synthesis of (2*S*,3*S*) β-hydroxypipecolic acid.

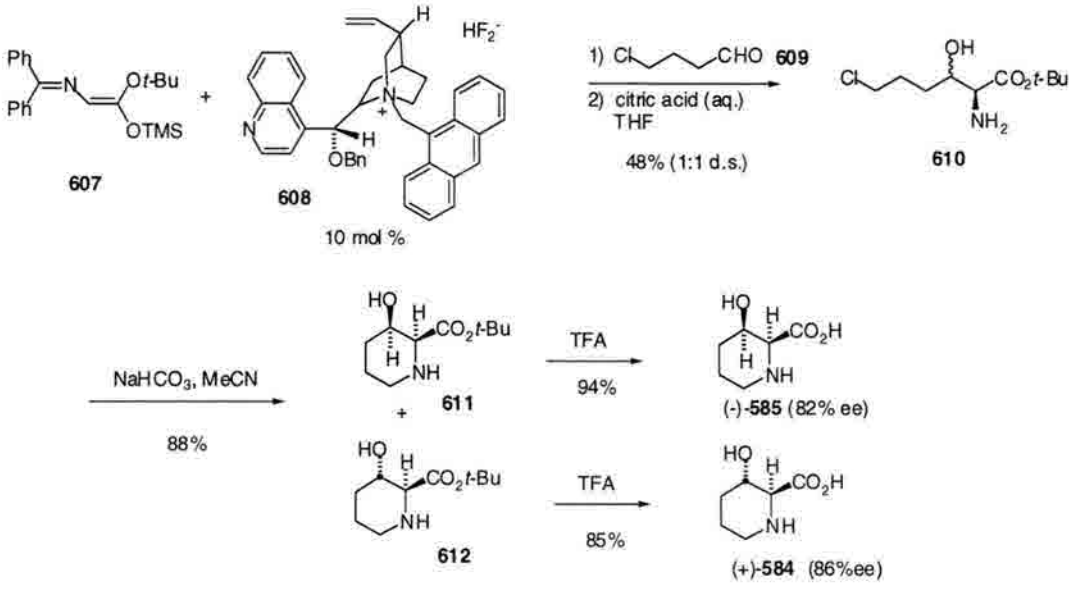
In 1997, Battistini *et al.* reported the synthesis of both enantiomers of **584** via the addition of furan **602** with imine **603** to **604** after hydrogenation (Scheme 87).[159] Lactam formation was followed by LAH reduction of the lactam to the amine and alcohol protection to afford **605**. Hydrolysis of the acetonide followed by diol cleavage and oxidation of the resultant aldehyde to the acid **606** was accomplished in 53% overall yield. Cleavage of the acetate groups afforded (+)-**584** in 14% overall yield for the synthesis. Starting from *ent*-**603**, (-)-**584** was synthesized in 10 steps in 15% overall yield.



Scheme 87 Battistini's syntheses of both enantiomers of *trans* β-hydroxypipecolic acid.

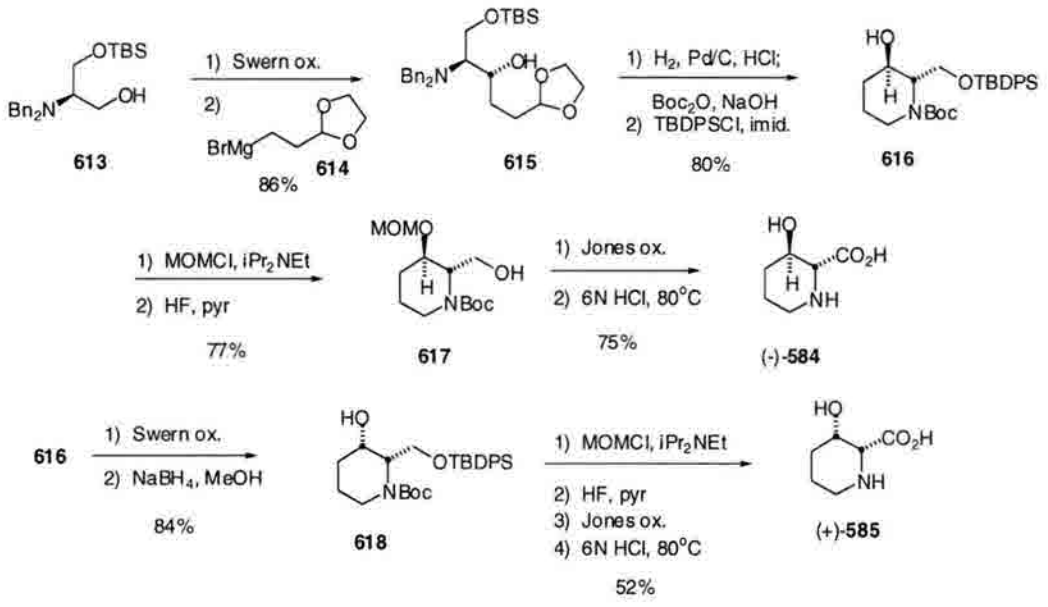
Corey *et al.* reported the synthesis of (+)-**584** and (-)-**585** via the addition of TMS enol ether **607** to aldehyde **609** in the presence of ammonium salt catalyst **608** (Scheme

88)[160] to afford **610** as a 1:1 mixture of diastereomers. Amine displacement of the chloride followed by diastereomer separation afforded **611** and **612**. Each diastereomer was deprotected to yield the free amino acids in moderate enantiomeric excess.



Scheme 88 Corey's syntheses of (2*S*,3*S*) and (2*S*,3*R*) β-hydroxypipecolic acids.

In 2000, Jourdant and Zhu reported the synthesis of (-)-**584** and (+)-**585** via the addition of Grignard **614** to the protected glycinol **613** to provide **615** (Scheme 89).[161] Hydrogenation followed by selective protection of the primary alcohol afforded the piperidine diol **616**. Protection of the secondary alcohol as a methoxymethyl ether was followed by cleavage of the silyl ether to yield **617**. Jones' oxidation of the alcohol was followed by deprotection of the amine and alcohol to afford (-)-**584**. Oxidation of the secondary alcohol of **616** followed by diastereoselective reduction of the resultant ketone afforded **618** that was carried on to (+)-**585**.

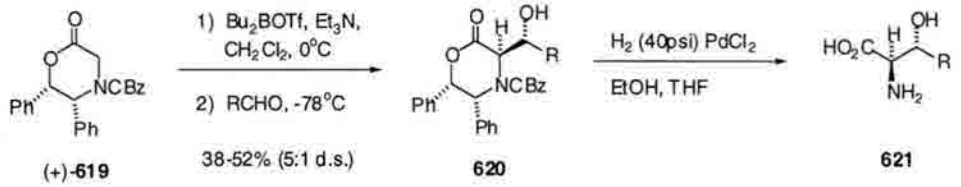


Scheme 89 Zhu's syntheses of (2*R*,3*R*) and (2*R*,3*S*) β-hydroxypipecolic acids

With the existing chemistry not being efficient to synthesize these four amino acids (the later chemistry was developed as our efforts were underway), it was desired to develop an efficient route to all four stereoisomers of β-hydroxypipecolic acid.

2.3. Aldol Condensations using Williams' Glycine Template:

Miller *et al.* reported that the boron enolate of the Cbz-protected Williams' glycine template (+)-**619** would add to an aldehyde to form the *anti*-β-hydroxy lactone **620** in moderate yields and good diastereoselectivity (Scheme 90).[162] These products could then be hydrogenated to afford the β-hydroxy amino acids **621**.



Scheme 90 Miller's synthesis of β-hydroxy amino acids.

The diasetereoselectivity arises from the aldol addition proceeding through a six membered Zimmerman-Traxler chair transition state (Figure 19).[162]

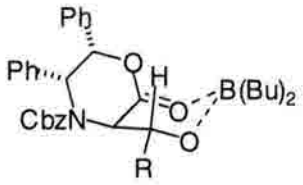
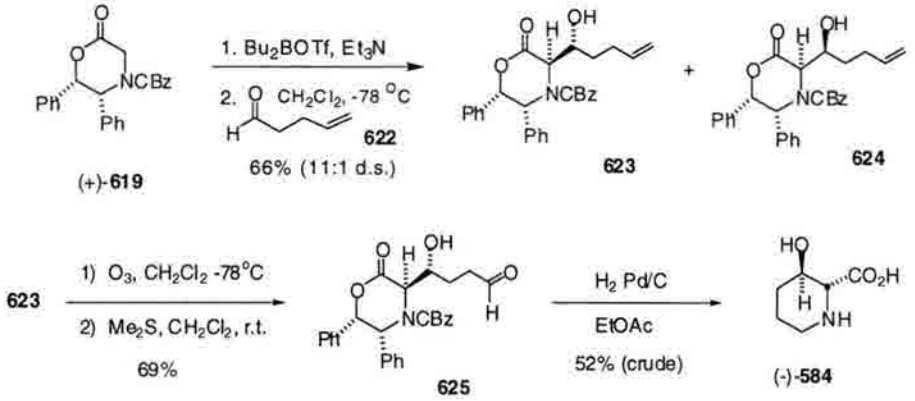


Figure 18

2.4. Tippie's Synthesis of *trans*-β-Hydroxypipecolic Acid:

In our laboratories, Tracy Tippie reported that the boron enolate of (+)-**619** would add to 4-pentenal to afford an 11:1 mixture of diastereomers **623** and **624** (Scheme 91).[163] The major diastereomer **623** could be separated from **624**, then treated with ozone with a reductive workup to afford aldehyde **625**. A one pot Cbz removal, reductive amination, and bibenzyl cleavage afforded (-)-**584** in 52% crude yield. However, the amino acid was contaminated with an undetermined side product.



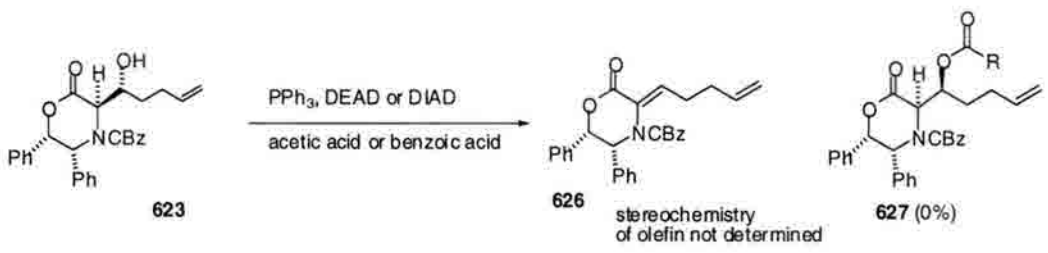
Scheme 91 Tippie's synthesis of (2*R*,3*R*) β-hydroxypipecolic acid.

2.5. Synthesis of all Four stereoisomers of β-hydroxy pipecolic acid

Using the existing aldol chemistry, there were two goals to be achieved in order to synthesize all four stereoisomers of β-hydroxypipecolic acid. First, the route needed to be improved to eliminate the need for HPLC purification of the final compound to make

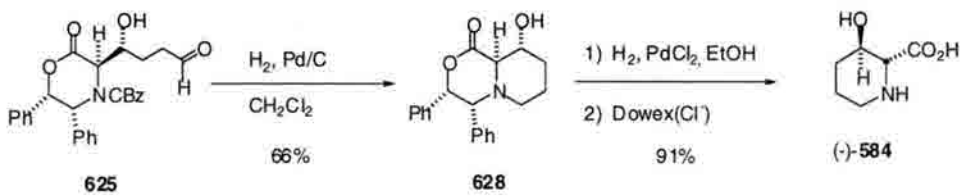
the route more efficient. It was also necessary to invert the secondary alcohol on compound **623** to access the two *cis* enantiomers of β-hydroxypipecolic acid.

Attempts were made at inverting the secondary alcohol of **623** under standard Mitsunobu conditions[164] (Scheme 92). However, only elimination product **626** and starting materials were recovered. The desired product **627** was not observed.



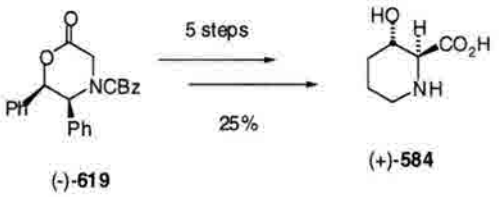
Scheme 92 Attempted Mitsunobu inversion of secondary alcohol.

Since the activated alcohol was capable of undergoing rotation allowing for elimination, it was deemed desirable to have a rigid system to attempt the Mitsunobu inversion. Hydrogenation of aldehyde **625** in dichloromethane afforded the bicycle **628** in moderate yield (Scheme 93).[165] This allowed for the removal of any side products from the Cbz cleavage and reductive amination. Cleavage of the bibenzyl group followed by ion exchange chromatography afforded pure (-)-**584** in 91% yield.



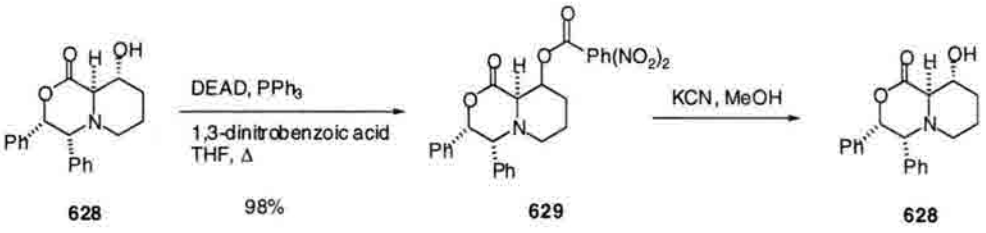
Scheme 93 Improved synthesis of (2*R*,3*R*) β-hydroxypipecolic acid.

With the other enantiomer of the glycine template also available, (+)-**584** could be synthesized in 5 steps in comparable yield (Scheme 94).[165]



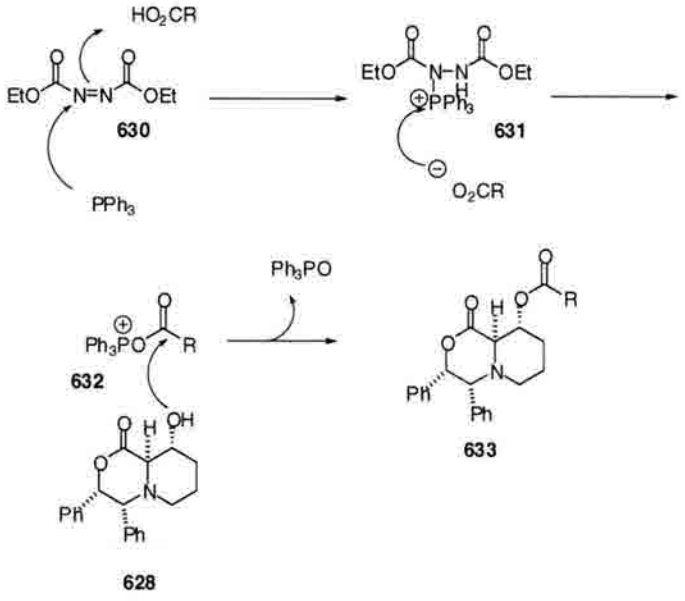
Scheme 94 Synthesis of (2*S*,3*S*) β-hydroxypipecolic acid.

With **628** in hand, a Mitsunobu inversion was again attempted (Scheme 95). The benzoylated product **629** was isolated in 98% yield. However, when the benzoate was cleaved, the major product obtained was the starting material **628**, indicating inversion did not take place at all.



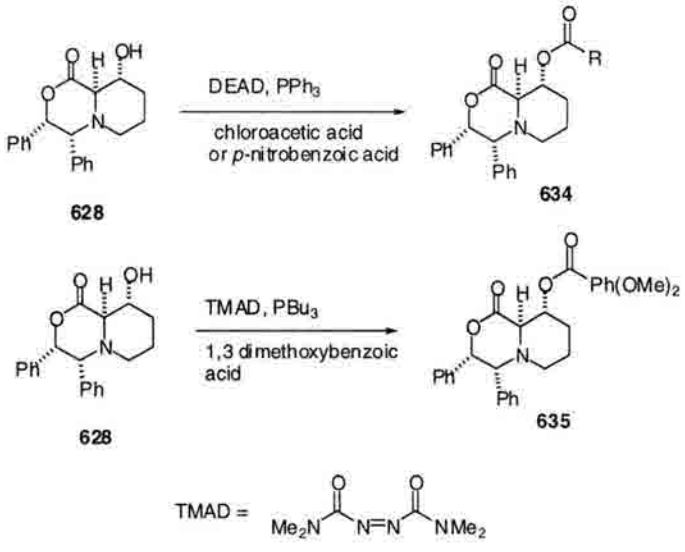
Scheme 95 Acylation with retention of configuration.

The literature revealed that the typical cause of acylation with retention of configuration was due to steric hindrance of the alcohol.[166] This would cause the activated triphenylphosphine **631** to be attacked by the carboxylate affording a very active acylating agent **632** that subsequently acylates the alcohol **628** resulting in the formation of **633** (Scheme 96).



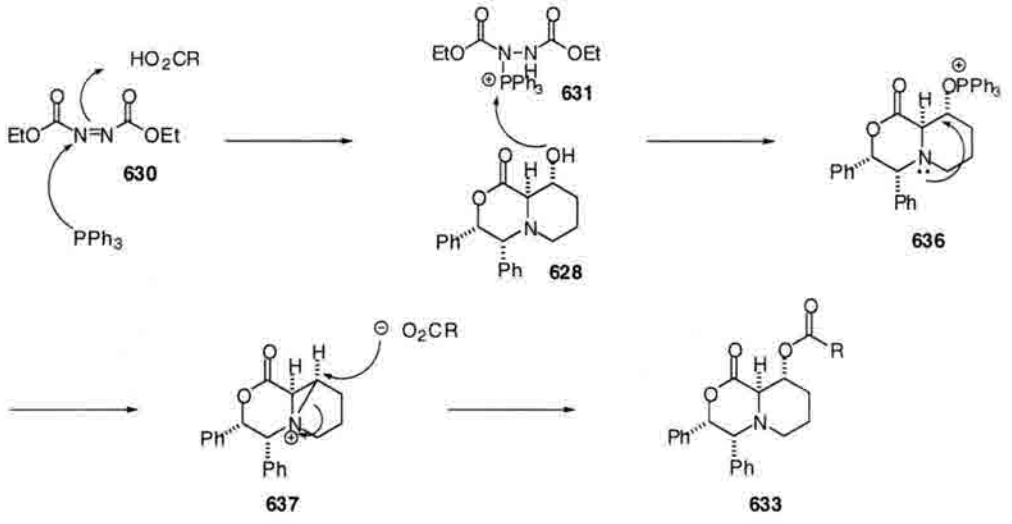
Scheme 96 Originally proposed mechanism of acylation with retention of configuration.

Use of chloroacetic acid[167] or *para*-nitrobenzoic acid[168], which were more acidic (i.e. the free carboxylate was more stable and less prone to attacking **631**), were also unsuccessful (Scheme 97). Attempts at an inversion using TMAD[169], a reagent that was reported to be superior at inverting sterically hindered alcohols, also led to acylation with retention of configuration.



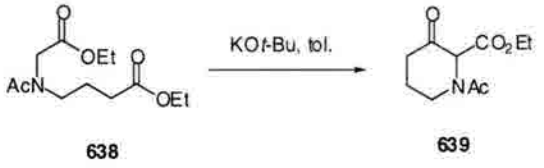
Scheme 97 Further failed attempts at inversion of secondary alcohol.

With all attempts at inverting the secondary alcohol leading to acylation with retention of configuration, a new mechanism was postulated (Scheme 98). If the alcohol **628** was activated as in a standard Mitsunobu to form **636** the lone pair of the tertiary amine could displace the activated alcohol inverting that stereocenter forming aziridinium ion **637**. This aziridinium species could then be attacked by the carboxylate, inverting that stereocenter a second time, yielding ester **633** with net retention of configuration.



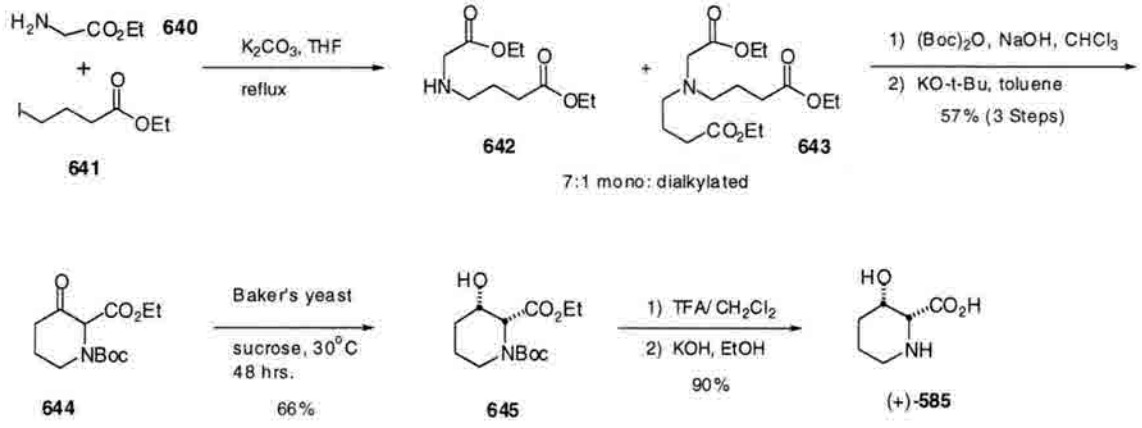
Scheme 98 Revised proposed mechanism for acylation with retention of configuration.

Due to the unsuccessful syntheses of both enantiomers of *cis*-β-hydroxypipecolic acid, for comparison purposes, it was desired to synthesize at least one of the enantiomers of **585**. The shortest method in the literature entailed the Baker's yeast reduction[156] of the racemic β-ketoester **638**. No experimental details were given for the synthesis of **639**, but a reference to the Dieckmann cyclization below was given (Scheme 99).[170]



Scheme 99 β-ketoester via Dieckmann cyclization.

An efficient route was then devised for the synthesis of the β-ketoester **644**. Alkylation of glycine ethyl ester (**640**) with the iodobutyrate **641** afforded a mixture of monoalkylated (**642**) and dialkylated (**643**) products (Scheme 100). This mixture was treated with di-*tert*-butyldicarbonate and the dialkylated side-product was removed by chromatography. Dieckmann cyclization yielded a single product **644** in 57% yield from **640**. Treatment of **644** with Baker's Yeast for 48 hours[156] afforded the optically active β-hydroxy ester **645** in 66% yield. Removal of the Boc group and hydrolysis of the ester afforded the free amino acid (+)-**585**.

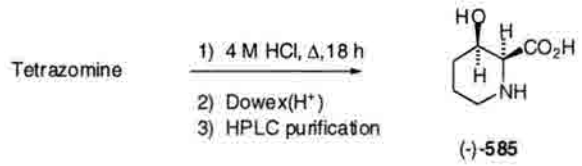


Scheme 100 Synthesis of (2*R*, 3*S*) β-hydroxypipecolic acid.

2.5.1. Hydrolysis of Natural Tetrazomine

With three of the four stereoisomers of β-hydroxy pipecolic acid in hand, it was possible to determine the absolute stereochemistry of the β-hydroxy pipecolic acid moiety of tetrazomine. Unfortunately, under basic hydrolysis conditions a mixture of *cis* and *trans* amino acids were observed in the crude hydrosylate of tetrazomine.[165] Under acidic hydrolysis conditions, only a *cis* amino acid was observed. This amino acid was purified by HPLC and was found to have identical spectral data to that of the synthetic *cis*

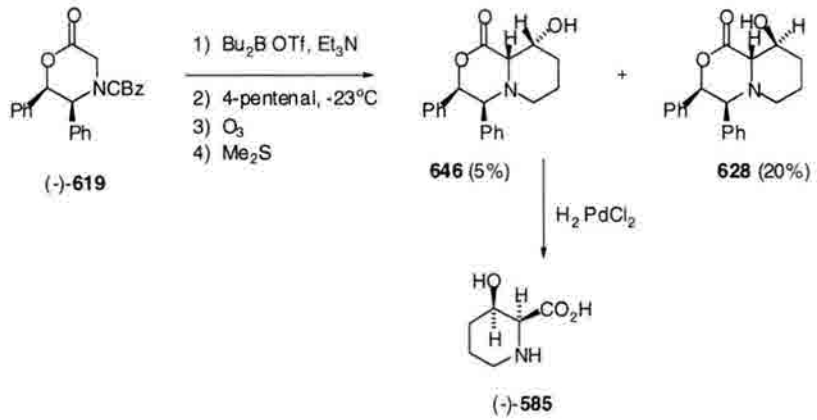
amino acid (+)-**585** with the exception of an opposite sign in the optical rotation indicating that (-)-**585** was the amino acid isolated from tetrazomine.



Scheme 101 Hydrolysis of natural tetrazomine.

2.5.2. High Temperature Aldol Condensation

Due to the fact that (-)-**585** was the only stereoisomer that was not readily available, it was desired to find a route for the synthesis of (-)-**585**. The aldol condensation with the Williams glycine template afforded a 11:1 diastereomeric ratio at –78°C. Running the condensation at higher temperature (–23°C) afforded a 4:1 diastereoselectivity (Scheme 102). Unfortunately, the minor diastereomer could not be purified, so the mixture of diastereomers was carried on to the bicyclic stage at which point the diastereomers could be separated. Hydrogenation of the minor bicycle **646** afforded (-)-**585** in 82% yield.

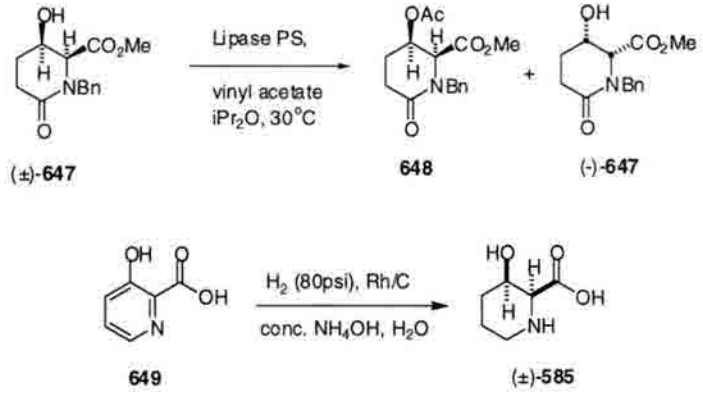


Scheme 102 High temperature aldol condensation on Williams' lactone.

Even though (-)-**585** was now available the synthesis was very inefficient. Also, more material would be necessary to complete the total synthesis of tetrazomine and thus a new route to (-)-**585** was investigated.

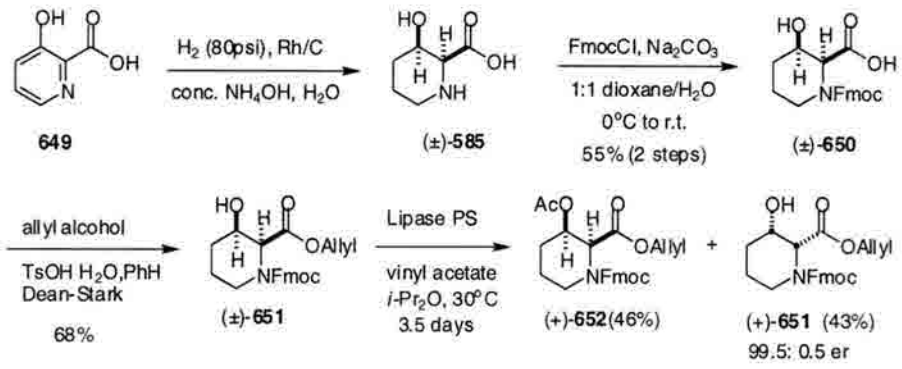
2.5.3. Lipase PS Resolution of Racemic cis-β-Hydroxypipecolic Acid

Toyooka *et al.* reported the Lipase PS resolution of (±)-**647** afforded the optically active acetate **648** and alcohol (-)-**647** in good yields and high ee's (Scheme 103).[171] Due to the fact that racemic **585** was available in one step from 3-picolinic acid[172] **649**, the Lipase PS resolution was attempted.



Scheme 103 Toyooka's Lipase PS resolution of β-hydroxy ester **647**.

As shown in Scheme 104, the amine of (±)-**585** was protected as the Fmoc carbamate to afford (±)-**650**. The carboxyl group was converted to the allyl ester (±)-**651** that was subjected to the Lipase PS conditions of Toyooka.[171] When the reaction reached 50% conversion by HPLC analysis, the products were isolated affording (+)-**652** in 46% yield and 98:2 enantiomeric ratio (er) and (+)-**651** in 43% yield and 99.5:0.5 er.[173]

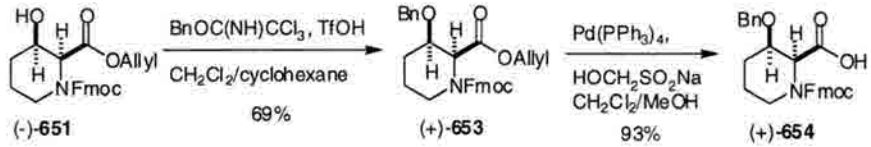


Scheme 104 Lipase PS resolution of racemic *cis*-β-hydroxypipecolic acid.

Scheme 105 Completion of synthesis of (2*S*,3*R*) β-hydroxypipecolic acid.

The absolute stereochemistry of the two protected β-hydroxypipecolic acids were determined by cleavage of the acetate of (+)-**652** to afford (-)-**651** which was treated with lithium hydroxide to afford (-)-**585** (Scheme 105).[173]

It was also necessary to protect the secondary alcohol prior to coupling to the aniline **583**. A benzyl group was initially used for this purpose (Scheme 106). Treatment with benzyl trichloroacetamide in the presence of triflic acid[174] afforded (+)-**653**. Cleavage of the allyl ester under the conditions of Honda[175] provided the free acid (+)-**654** in high yield.



Scheme 106 Completion of synthesis of protected (2*S*,3*R*) β-hydroxypipecolic acid.

2.6. Conclusion

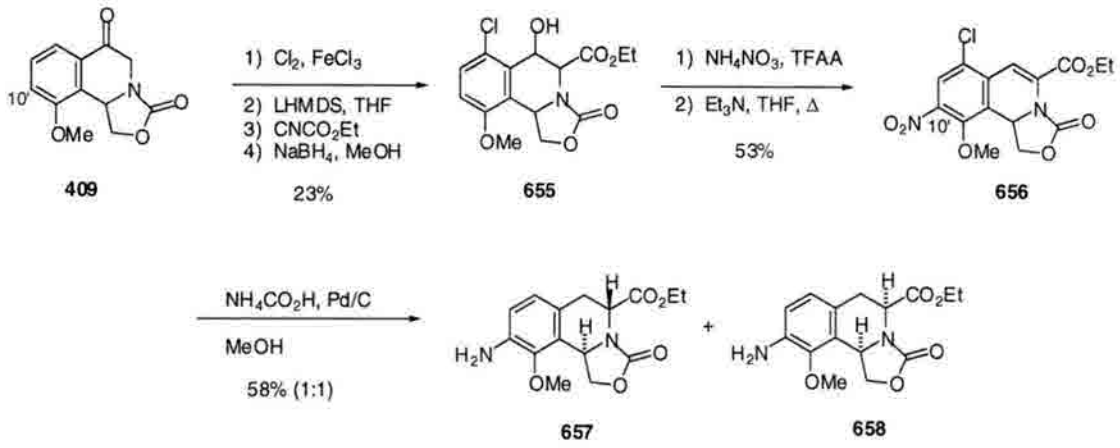
The asymmetric synthesis of the two *trans*-β-hydroxypipecolic acids has been presented along with the Lipase PS resolution of the two *cis* enantiomers allowing for preparative access to all four stereoisomers. These amino acids were then used to determine the absolute stereochemistry of the β-hydroxypipecolic moiety of tetrazomine, which was necessary for completion of the total synthesis.

Chapter 3

Total Synthesis of (-)-Tetrazomine and Analogs

3.1 Introduction

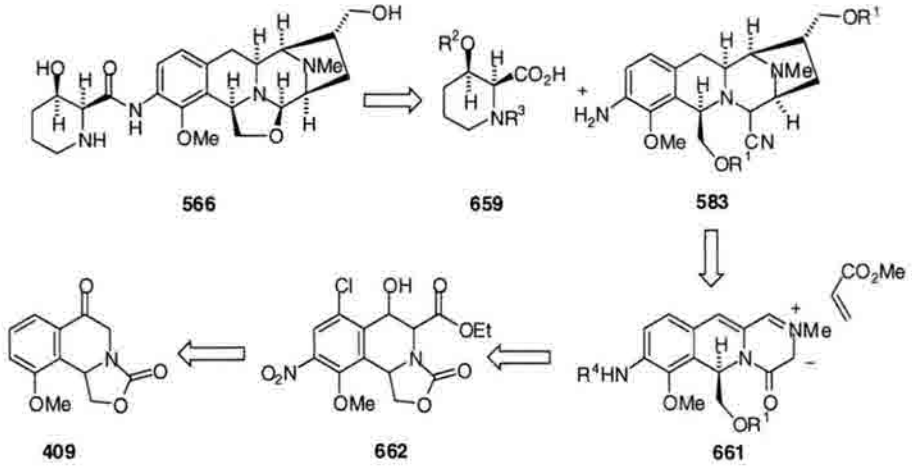
The originally proposed synthetic route towards tetrazomine paralleled that of Mark Flanagan's quinocarcinamide synthesis.[132,176] Tracy Tippie laid the ground work on this route,[163] with the introduction of the amino functionality at C-10' (Scheme 107). Isoquinoline **409** was chlorinated *para* to the methoxy group in order to block that position from the ensuing aromatic nitration. Acylation of the ketone was followed by ketone reduction using sodium cyanoborohydride to yield β-hydroxyester **655**. Aromatic nitration under mild conditions was followed by elimination of the resultant trifluoroacetate group to afford **656** in 53% yield over two steps. Reduction of the nitro group was accompanied by cleavage of the chloro group and reduction of the benzylic olefin to provide diastereomers **657** and **658** in 58% combined yield. Reduction of the olefin was an unwanted consequence under these hydrogenation conditions. In this approach, the olefin was found to be necessary for the oxidation of the amine for the 1,3-dipolar cycloaddition. Therefore, the route needed to be adjusted such that the olefin remains intact.



Scheme 107 Tippie's installation of the aniline portion of tetrazomine.

3.2 Original Synthetic Route to Tetrazomine

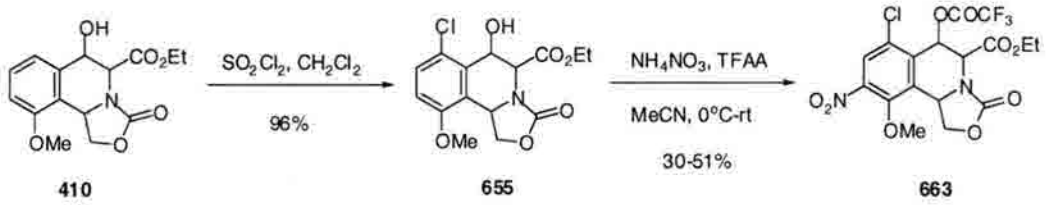
The original retrosynthetic analysis for the tetrazomine synthesis is shown in Scheme 108. Tetracycle **583** could come from a 1,3-dipolar cycloadditon of methyl acrylate upon the dipolar species **661**. Tricycle **661** could be arrived at from **662** with careful planning to keep the benzylic olefin intact. Finally, Tippie had shown that **662** could be synthesized from isoquinoline **409**.[163]



Scheme 108 Original retrosynthetic analysis towards tetrazomine.

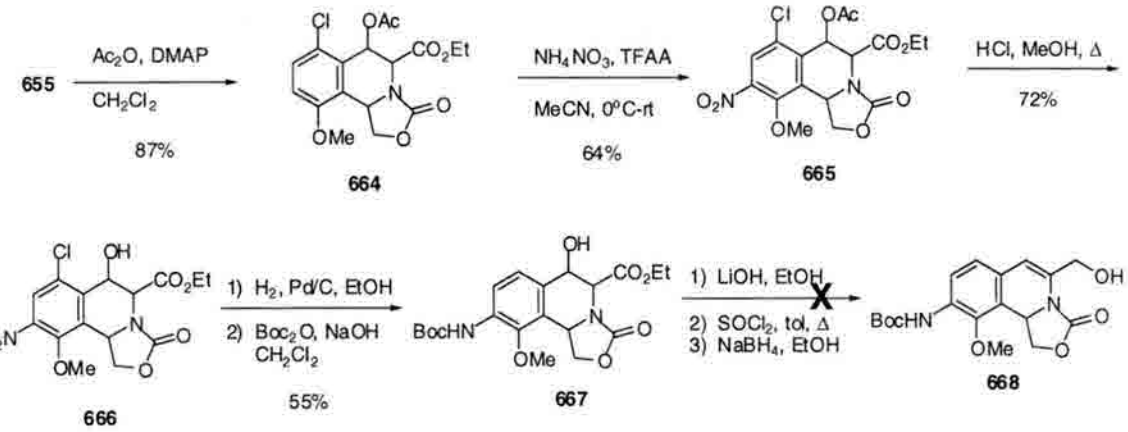
Due to the low yields encountered in the previous synthesis of **662**, a more efficient route was desired. First, the use of chlorine gas to introduce the aromatic chloro

group was less than desirable due to the low yields and difficulty in measuring out exact amounts of chlorine. Secondly, the aromatic nitration was low yielding. Starting from β-hydroxy ester **410,** chlorination was accomplished in high yield using sulfuryl chloride[177] to afford **655** (Scheme 109). Nitration under mild conditions[178] lead to the desired nitro compound **663** in moderate yields.



Scheme 109 Nitration of aromatic ring.

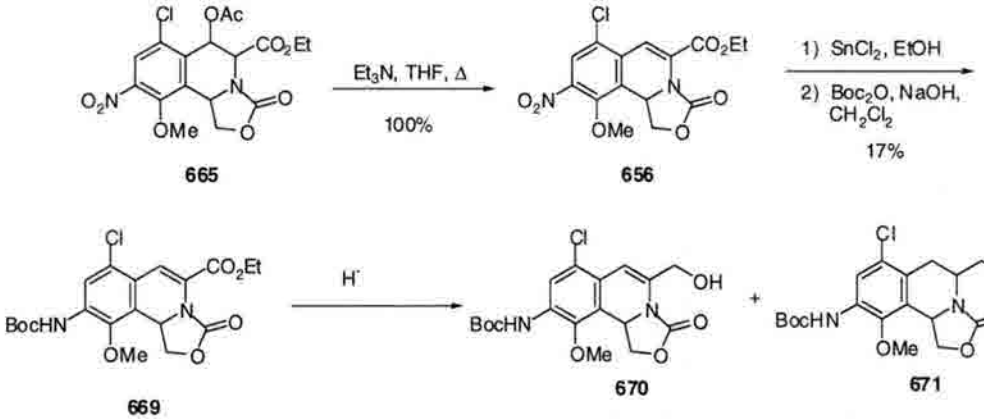
It was found that if the secondary alcohol was protected with an acetate prior to nitration, the yield of **665** was improved (Scheme 110). Acidic methanolysis of the acetate group afforded the β-hydroxyester **666**. Reduction of the nitro group and cleavage of the chloro group afforded aniline **667.** Unfortunately, under the conditions to form the allylic alcohol **668** no desired product was obtained.



Scheme 131 Failed attempt to get allylic alcohol **668**.

A milder method for elimination of the benzylic alcohol followed by reduction of the ester was then examined (Scheme 111). Elimination of the benzylic acetate

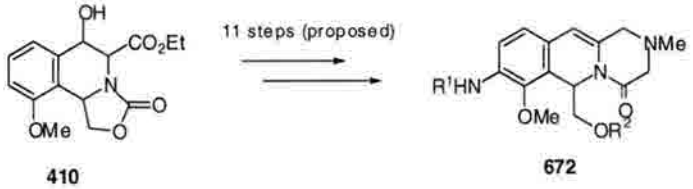
proceeded in quantative yield to afford **656**. Reduction of the nitro group and protection of the aniline was accomplished in low yield to afford **669**. Several attempts[179] to selectively reduce the ester moiety of the α,β-unsaturated ester provided only the saturated alcohol **671** or decomposition. The presence of the electron-donating amino group at C-10' was apparently responsible for the observed 1,4-reduction in all attempts.



Reagent	Solvent	Products
$LiBH_4$	MeOH	**671** (20%) + decomp
$LiBH_4$	THF, MeOH	**671** (25%)+ decomp
$NaBH_4$, $CeCl_3$	EtOH	**671** (< 10%)+ decomp
Dibal, n-BuLi	THF	**671** (33%)+ decomp

Scheme 111 Attempt to obtain allylic alcohol via 1,2 reduction of α,β-unsaturated ester.

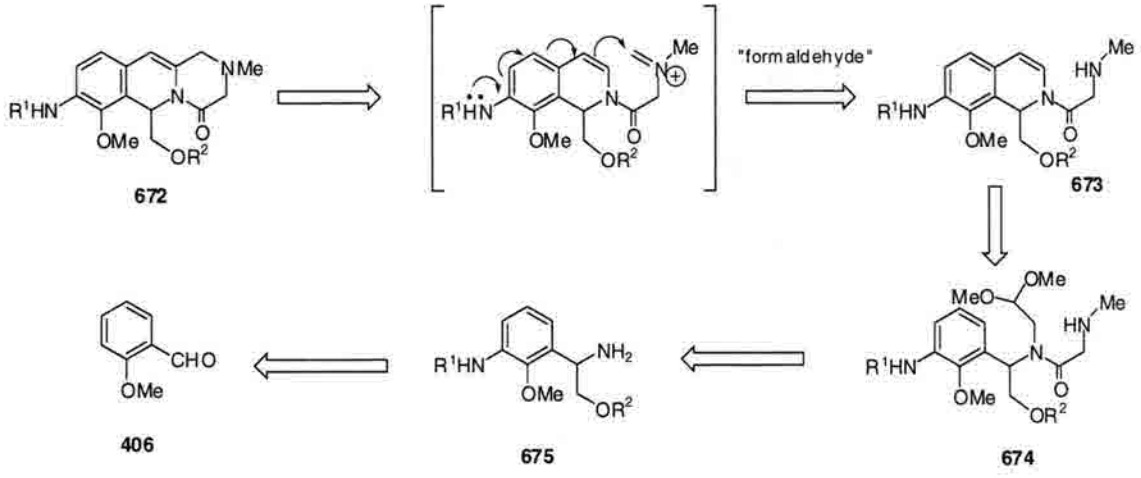
The original retrosynthetic analysis towards tetrazomine would require 11 steps to synthesize the key intermediate **672** from the β-hydroxyester **410** (Scheme 112). With the problems seen above and the uncertain chemistry to come, including the low yield expected in the cleavage of the oxazolidinone, a new route was investigated for the synthesis of **672**.



Scheme 112 Amount of steps originally proposed to form tricycle **672** from **410**.

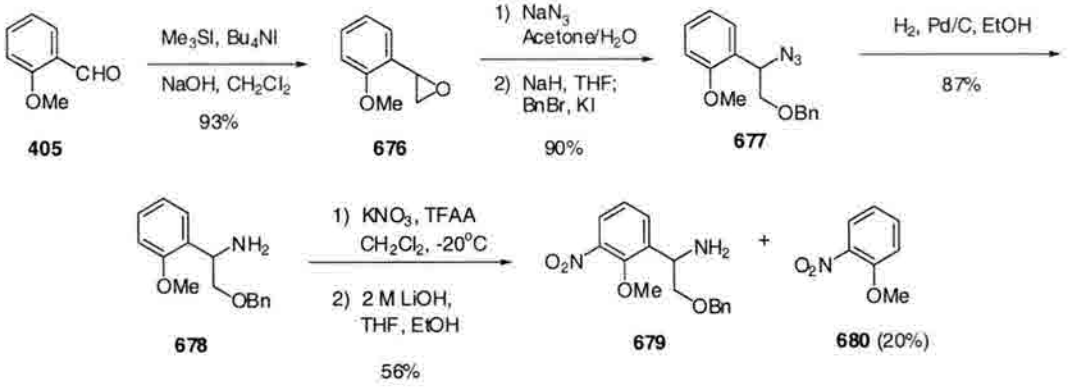
3.3 Revised Synthetic Route to Allylic Amine

The new route to tricycle **672** was based on the cyclization of a benzylic olefin onto an iminium formed by the treatment of a formaldehyde equivalent on secondary amine **673** (Scheme 113). Bicycle **673** could be formed by the acid-catalyzed cyclization of the aromatic ring on acetal **674** as shown by Danishefsky[128] and Kaufman[150]. Acetal **674** could be derived from the protected amino alcohol **675** that could be obtained from *o*-anisaldehyde.



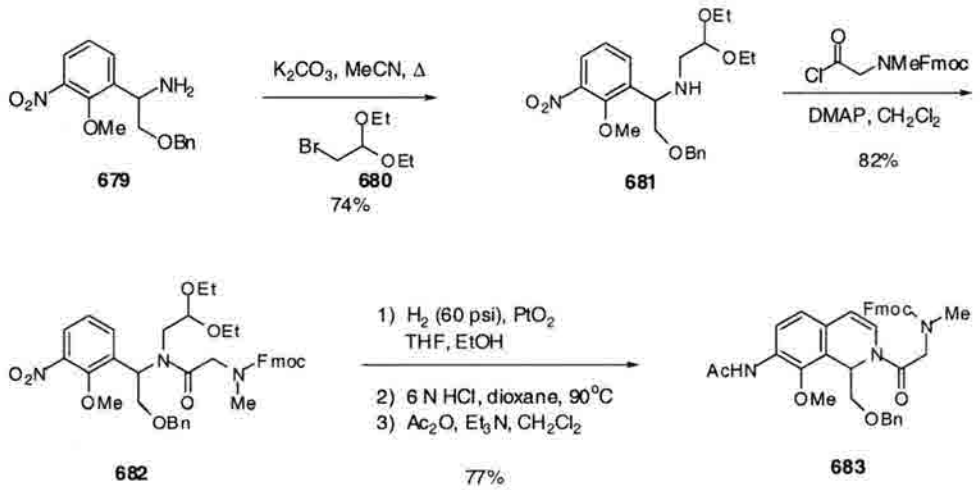
Scheme 113 Revised retrosynthetic analysis towards tricycle **672**.

The synthesis of the bicyclic Mannich precursor proceeded smoothly and in good yields (Scheme 114). Regioselective opening of epoxide **676** with sodium azide afforded the azido alcohol that was immediately protected as the benzyl ether to afford **677** in 90% yield. Hydrogenation of the azide yielded amine **678**. Nitration under the low temperature conditions of Kaufman.[150] followed by hydrolysis of the resultant trifluoroacetamide afforded **679** in 55% overall yield. A major side product was *o*-nitro anisole (**680**) which that was obtained via an unknown mechanism. Several attempts were made to reduce the amount of **680** formed, such as changing the solvent and using different amine protecting groups; however, the yields did not improve.



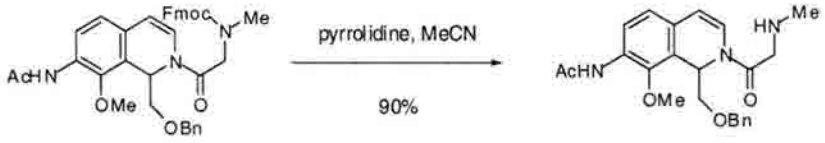
Scheme 114 Low temperature regioselective nitration of aromatic ring.

Alkylation of amine **679** with bromoacetaldehyde diethyl acetal (**680**) in refluxing acetonitrile for five days[34a] afforded the secondary amine **681** (Scheme 115). Coupling of the secondary amine with N-Fmoc-sarcosine acid chloride in the presence of DMAP afforded the amide **682** in 82% yield. Hydrogenation of the nitro group was accomplished using platinum oxide at moderate hydrogen pressure. The use of platinum prevented the Fmoc group from being cleaved. Acidic cyclization afforded the desired, but unstable aniline bicycle. Protection of the aniline as the acetamide afforded the stable bicycle **683** in 77% yield from **682**.



Scheme 115 Acid mediated formation of AB ring of tetrazomine.

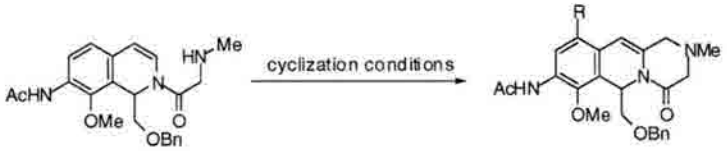
With **683** in hand, the Fmoc group was cleaved in good yield to provide the secondary amine necessary for the iminium cyclization (Scheme 116).



683 **684**

Scheme 116 Synthesis of Mannich precursor

Several attempts to form tricycle **685** were unsuccessful. The use of both formalin and paraformaldehyde afforded very little desired product (Table 17). Starting material was often recovered. With the use of excess paraformaldehyde, a dimer was formed along with **686** in which the aromatic ring added a hydroxymethyl group. With the benzylic olefin having electron donating groups from each direction, it was postulated that the acetate was a poor activating group (Figure 20).

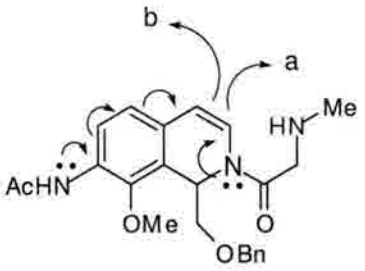


684

685 R = H
686 R = CH_2OH

Reagents	Solvent	Temp/Time	Products
10 eq. formalin	AcOH	100°C, 16 h	S.M.
10 eq. formalin, 10 eq. AcOH	MeOH	reflux, 16 h	S.M.
10 eq. formalin, 1M HCl	MeOH	reflux, 40 h	S.M.
3.5 eq. $(CH_2O)_n$, Na_2SO_4	MeCN	reflux, 16 h	dimer, **686** (major), **685** (trace)
1.1 eq. $(CH_2O)_n$, Na_2SO_4	MeCN (high dilution)	reflux, 24 h	S.M., **686**, **685** (5%)
1.1 eq. $(CH_2O)_n$, Na_2SO_4	MeCN (high dilution)	reflux, 40 h	S.M., **686**, **685** (10%)

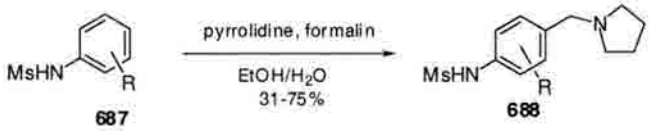
Table 16 Attempted Mannich cyclization.



661

Figure 20 Two directions of resonance of benzylic olefin.

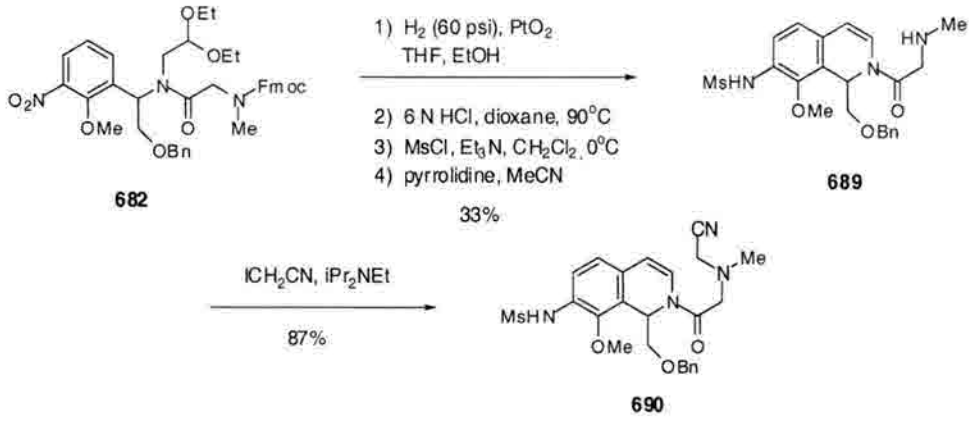
The Mannich addition of iminium species to mesylate-protected anilines (**687**) in the presence of excess base was reported by Lis and Marisca[180] (Scheme 117). An electron-withdrawing group is contrary to activation of the aromatic ring towards nucleophilic attack, but the excess base could deprotonate the sulfonamide (pKa < 10) allowing for addition to the iminium.



687 **688**

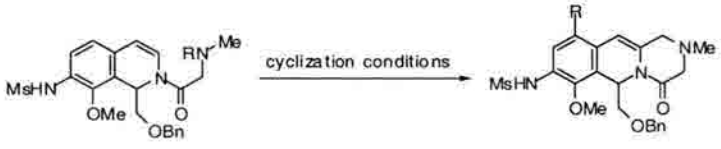
Scheme 117

A mesylate-protected bicyclic compound **689** was synthesized in a manner similar to the acetamide (Scheme 118). Also, a new formaldehyde equivalent was used. The amino nitrile[181] **690** was formed by treatment of the secondary amine **689** with iodoacetonitrile in 87% yield. The amino nitrile could be treated with a silver salt to form the requisite iminium ion species thus obviating the direct use of formaldehyde.



Scheme 118 Synthesis of mesyl protected Mannich precursors.

As was the case for the acetate-protected bicycle **684**, addition of paraformaldehyde afforded both the desired tricycle and a product resulting from formaldehyde addition to the aromatic ring, **691** and **692** respectively, in low yields (Table 18). Also, treatment of the aminonitrile **667** with silver nitrate[181] or silver tetrafluoroborate[182] yielded the desired tricycle **691** in low yields. The major product isolated from the silver-promoted reactions was the secondary amine **689**, indicating that the iminium ion species did indeed form, but was hydrolyzed during workup. If the silver-promoted cyclization reaction was allowed to proceed for significantly longer periods of time, only decomposition was observed.



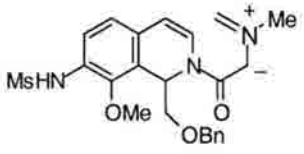
689 R = H
690 R = CH_2CN

691 R = H
692 R = CH_2OH

Substrate	Reagents	Products
687	1.1 eq. $(CH_2O)_n$,	S.M., **691** (trace),**692**
687	1.1 eq. $(CH_2O)_n$, iPr_2NEt	S.M., **691**
687	1.1 eq. $(CH_2O)_n$, K_2CO_3	S.M., **691 (<10%), 692**
688	$AgNO_3$	**691 (7%)**
688	$AgNO_3$, iPr_2NEt	S.M., **691 (<10%)**
688	$AgBF_4$, Et_3N	S.M., **691 (<10%)**

Table 18 Attempted Mannich cyclization of mesyl protected bicycle.

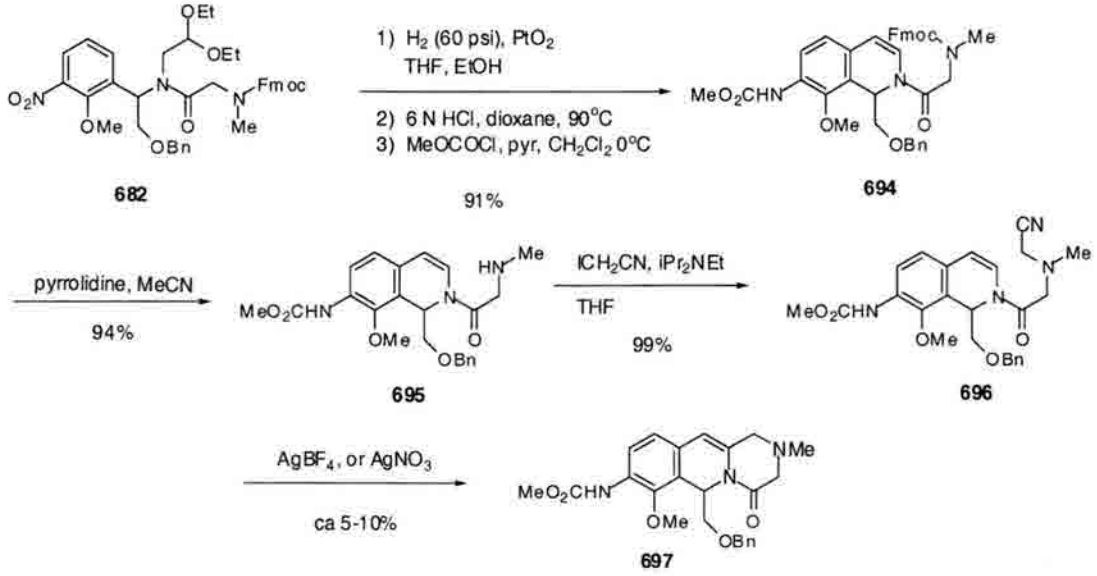
A possible problem with the use of excess base on the sulfonamide system derives from the fact that formation of the iminium ion species caused the protons alpha to the amide to become very acidic. In the total synthesis of quinocarcinamide by Williams and Flanagan,[132] triethylamine was used to deprotonate the alpha-position of the iminium, thus forming the dipolar species. If this dipolar species were to form (Figure 21) from the bicyclic compounds **689** or **690**, the iminium ion species would not be as electrophillic as a free iminium ion, thus lowering the reactivity significantly.



670

Figure 21 Proposed unreactive azomethine ylide intermediate.

Switching to an electron-donating protecting group was investigated since this would provide a better nucleophile (Scheme 119). The synthesis of a methyl carbamate-protected aniline was accomplished in high yield to form **694**.

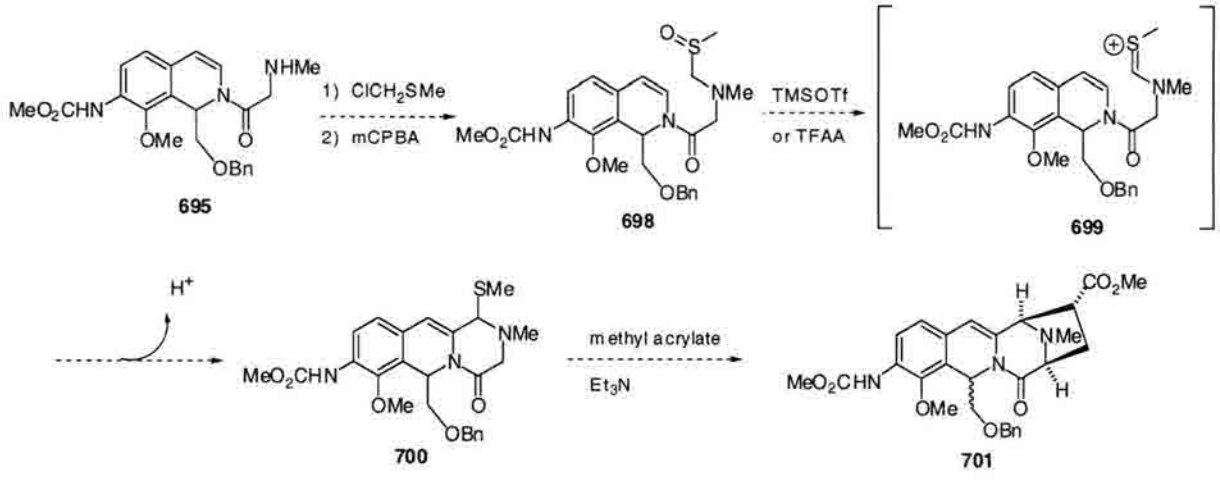


682 **694** **695** **696** **697**

Scheme 119 Methyl carbamate protected Mannich attempted cyclization.

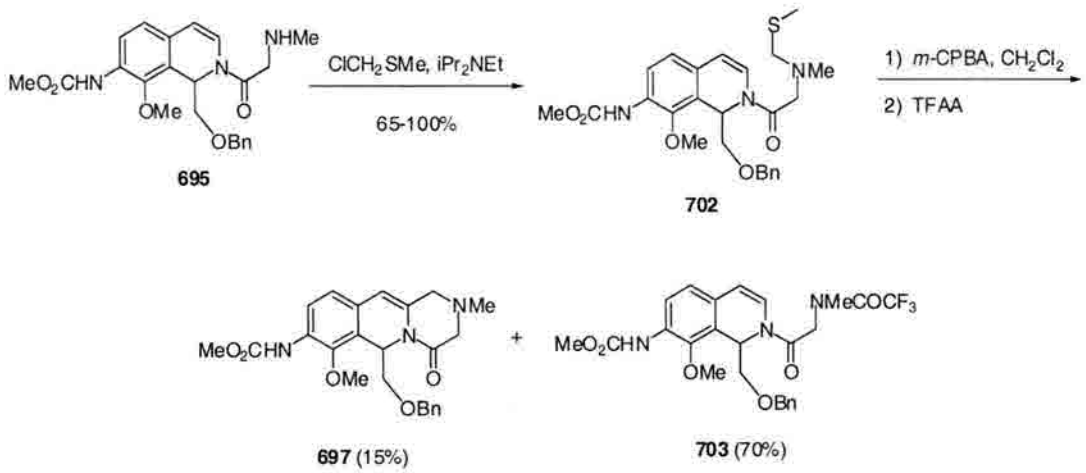
Removal of the Fmoc group afforded secondary amine **695** that was converted to the amino nitrile **696** in 99% yield. Unfortunately, under silver-promoted cyclization conditions,[181,182] the desired tricycle **697** was isolated in low yields.

Due to problems with the iminium ion cyclization, a new method was investigated. The Pummerer reaction[183] involves the treatment of a sulfoxide with an acid anhydride to form a sulfonium species that can be attacked by a nucleophile to yield a sulfide (Scheme 120). In the context of this synthesis, the amine could be alkylated with chloromethylmethyl sulfide followed by oxidation of the sulfide to the sulfoxide **698**. Treatment with TMSOTf or TFAA would afford the sulfonium ion **699** that could cyclize, forming the unstable allylic amino sulfide **700**. Treatment with base in the presence of methyl acrylate should then result in tetracycle **701**.



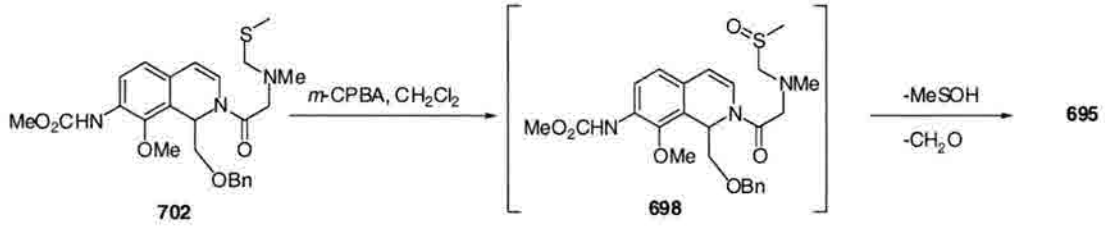
Scheme 120 Proposed formation of tetracycle **701** via a Pummerer cyclization.

Treatment of **695** with chloromethyl methyl sulfide afforded the labile aminosulfide **702** (Scheme 121). Oxidation of the sulfide followed by treatment with TFAA afforded two products. The minor product was tricycle **697** and the major product was the trifluoroacetamide **703**.



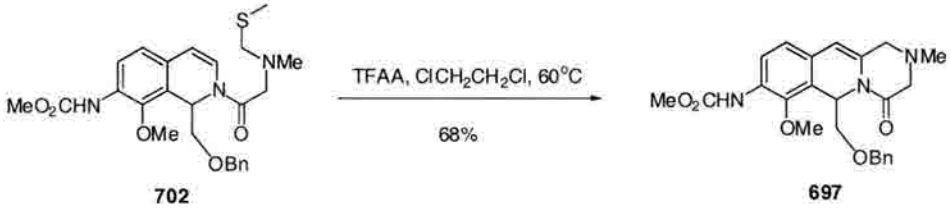
Scheme 121 Attempted Pummerer sequence.

This result suggested that the sulfoxide probably formed but was unstable. Elimination of sulfinic acid followed by hydrolysis of the resultant iminium ion species would form the bicycle **695** which, in the presence of TFAA, would form trifluoroacetamide **703** (Scheme 122). It was hypothesized that tricycle **697** resulted from the cyclization of unoxidized sulfide **702**.



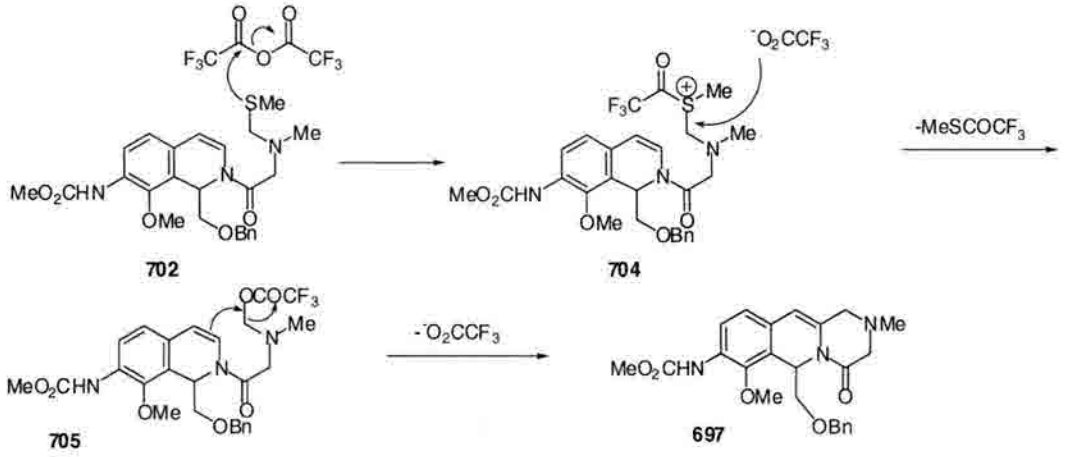
Scheme 122 Proposed decomposition of sulfoxide **698**.

To test this hypothesis, aminosulfide **702** was treated with TFAA to afford tricycle **697** in 68% yield (Scheme 123).



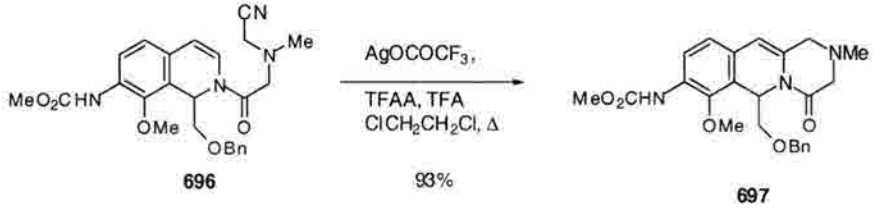
Scheme 123 Cyclization of amino sulfide using TFAA.

The proposed mechanism for this cyclization is shown below. Acylation of the sulfide would form the very unstable sulfonium species **704** that would be displaced by trifluoroacetate anion to afford **705** (Scheme 124). This trifluoroacetyl hemi-aminoacetal then cyclized to provide tricycle **697**.



Scheme 124 Proposed cyclization of amino sulfide using trifluoroacetic anhydride.

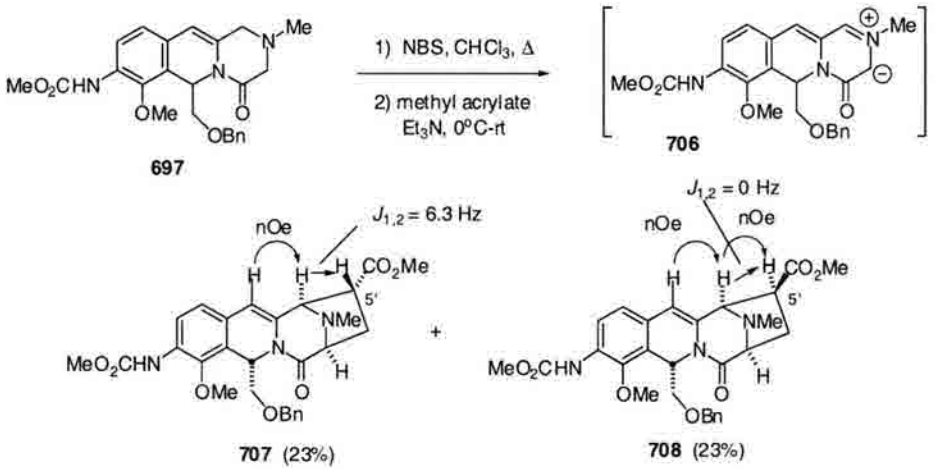
If this were the correct mechanism for this cyclization, then the treatment of aminonitrile **696** with silver trifluoroacetate should form tricycle **697** (Scheme 125). Indeed this was found to be true. Treatment of **696** with silver acetate in the presence of TFA and TFAA afforded the tricycle **697** in 93% yield. The major advantage of this route was the fact that the aminonitrile **696** was much more stable than the aminosulfide **702** allowing for much higher yields during the cyclization. The fact that the trifluoroacetate anion is necessary indicates that the cyclization proceeds through a 6-*exo*-tet mechanism and not a 6-*endo*-trig mechanism.[184]



Scheme 125 Cyclization of amino nitrile using trifluoroacetic anhydride.

3.4. 1,3-Dipolarcycloadditions

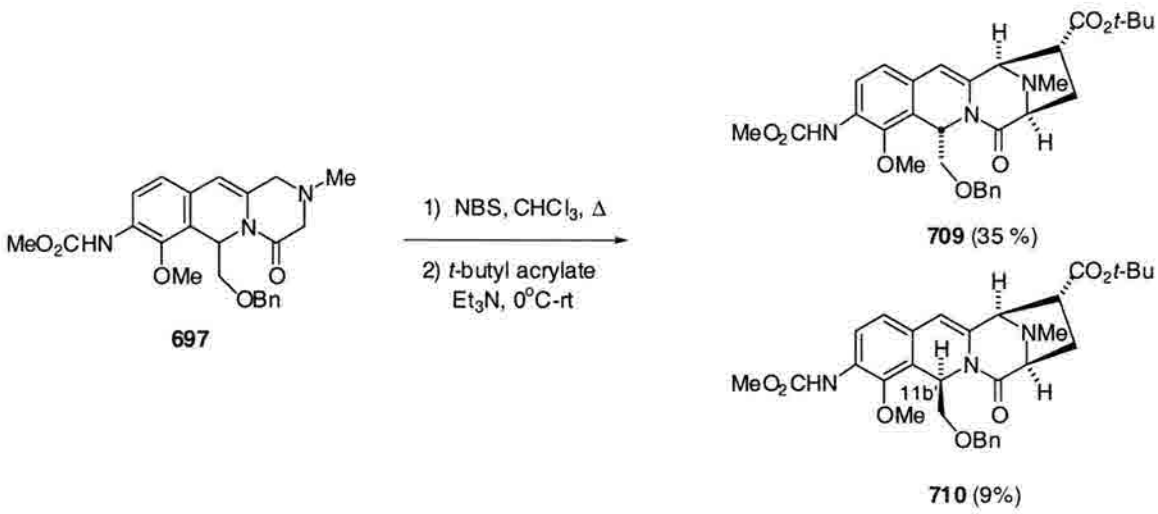
With tricycle **697** in hand the 1,3-dipolar cycloaddition was attempted using the conditions of Flanagan.[132] Heating **697** to reflux in chloroform in the presence of one equivalent of NBS afforded a dark green solution of the allylic iminium ion species (Scheme 126). Addition of methylacrylate and triethylamine afforded a dark blue solution of the azomethine ylide **706**. Two products were isolated in a 1:1 ratio. ^{1}H-NMR analysis of the products revealed that they were diastereomers at C-5'. The signal in the ^{1}H-NMR for the proton at C-5' was a doublet with a coupling constant of 6.3 Hz which indicated that the proton was *trans* to the proton at C-6'. The *cis* diastereomer **708** had a singlet for the C-5' proton indicating that the protons were *cis*. These values were consistent with those calculated for both diastereomers by MacroModel (6.6 Hz for **707** and 0.6 Hz for **708**). Similar observations of coupling constants were noted by Garner in his quinocarcin synthesis.[127] This lack of diastereoselectivity at C-5' was unexpected due to the fact that Flanagan obtained only one diastereomer at C-5' in the quinocarcinamide 1,3-dipolarcycloaddition.



Scheme 126 1,3-dipolar cycloaddition via NBS oxidation of allylic amine.

The stereochemistry of natural tetrazomine at C-5' was unknown so a 1:1 mixture would have been advantageous in determining the relative stereochemistry of the natural product. However, a 1:1 mixture would require two times the amount of material to finish the total synthesis, so new conditions were attempted to obtain solely the *R* configuration at C-5' (same configuration as quinocarcin).

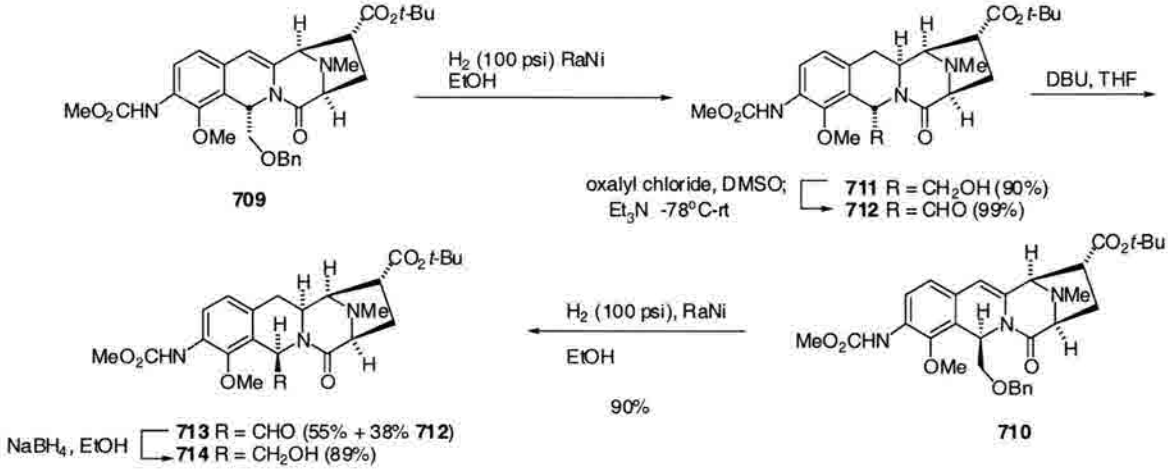
The *endo/exo* addition of the dipolarophile to the azomethine ylide determined the stereoselectivity with the *exo* addition yielding **707** and *endo* addition yielding **708**. Hence, a larger dipolarophile would have a higher selectivity for *exo* addition (R configuration). *Tert*-butylacrylate was the dipolarophile chosen. This yielded a single diastereomer at C-5' along with a 4:1 mixture of diasteromers at C-11b' (**709** and **710** respectively) both products arose from *exo* addition of the acrylate (Scheme 127). As in the quinocarcinamide synthesis, the major product from the cycloaddition (**709**) possessed the undesired configuration at C-11b' and an epimerization at C-11b' was thus executed.



Scheme 127 1,3-dipolar cycloaddition using *tert*-butyl acrylate as dipolarphile.

3.5. Completion of Total Synthesis of (-)-Tetrazomine

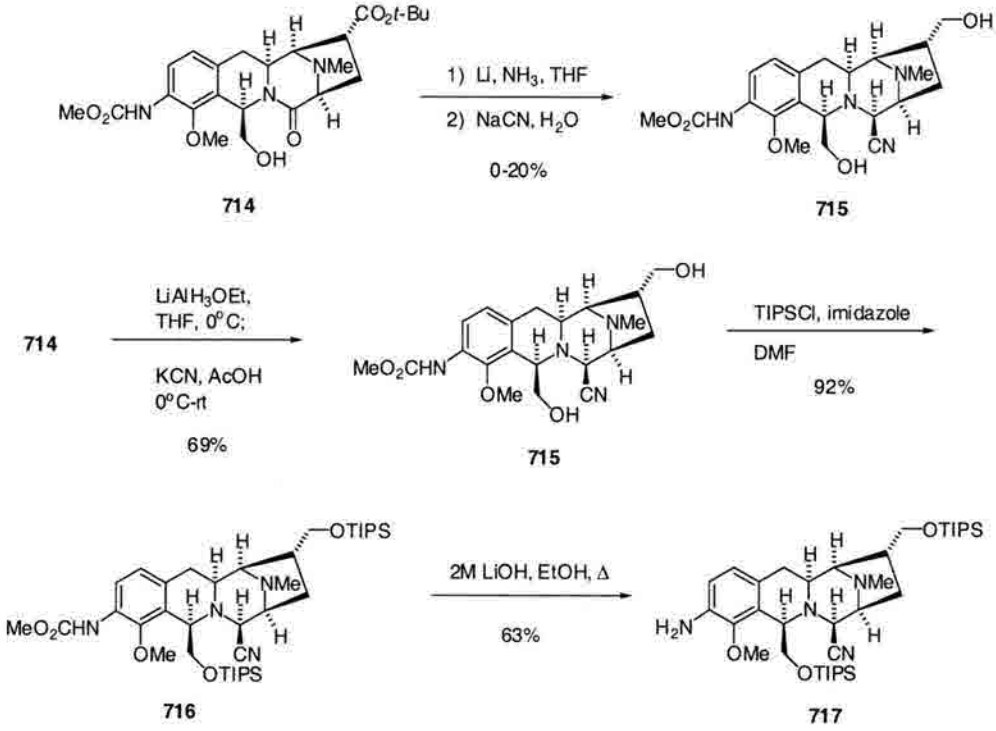
The epimerization was accomplished first by hydrogenation of the benzyl ether in the presence of Raney Nickel at moderate pressure (100 psi). Under these conditions the benzylic olefin was also reduced to afford **711** (Scheme 128). It is important to note that the reduction of the benyzlic olefin was unexpected since in several similar systems high pressure and elevated temperatures[127-129,132] (1600-2000 psi, 60°C) were necessary for reduction of the olefin. Alcohol **711** was oxidized to aldehyde **712** via a Swern oxidation. Epimerization was accomplished with the use of DBU to afford a 1.4:1 mixture of epimers at C-11b' with the desired isomer (**713**) being predominant. These aldehydes were easily separated by column chromatography, allowing for recycling of the undesired epimer. Sodium borohyride reduction of the desired epimer afforded alcohol **714**. Hydrogenation of the minor cycloadduct **710** afforded **714** in one step in 90% yield.



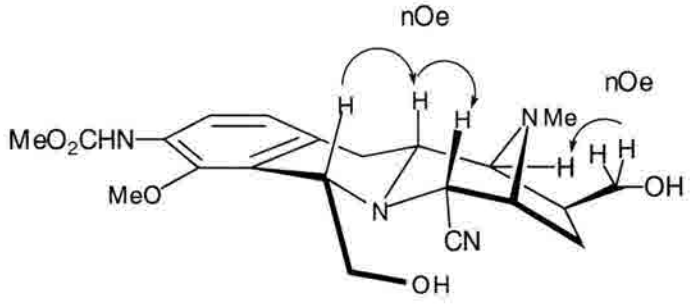
Scheme 128 Epimerization of C-11b'.

Attempted simultaneous reduction of the *tert*-butyl ester along with partial reduction of the amide to afford **715** was accomplished using a dissolving metal

reduction[104,127] followed by treatment with sodium cyanide in very low yields (Scheme 129). Corey had shown on a similar system[28] that lithium aluminum diethoxy hydride provided the carbanolamine in high yields (Scheme 13). Reduction of the *tert*-butyl ester required the use of lithium aluminum ethoxy hydride that provided **715** in 69% yield. The relative stereochemistry of **715** was determined via 2D NMR techniques (TOXCY, ROSEY, and dQ-COSY see Figure 22 and Appendix 2). The two primary alcohols were then protected as the triisopropylsilyl ethers to afford **716**. The choice of the triisoproplysilyl protecting group was made due to the fact that they are more stable to the basic hydrolysis conditions[185] necessary for the cleavage of the methyl carbamate. Treatment of **716** with lithium hydroxide in refluxing ethanol provided the free aniline **717** in 63% yield allowing for coupling of the activated amino acid.



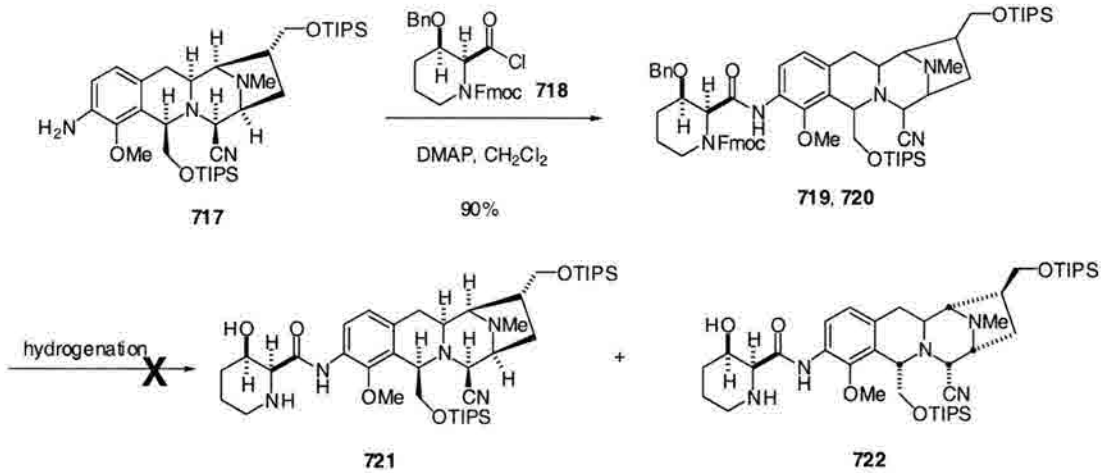
Scheme 129 Synthesis of aniline **717**.



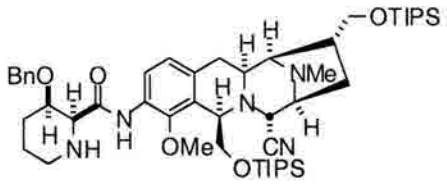
692

Figure 22 2D NMR determination of relative stereochemistry of tetracycle **715.**

Coupling of racemic aniline **717** to the optically active acid chloride **718** provided a 1:1 mixture of diastereomers **719** and **720** (Scheme 133). Due to the likely cleavage of the Fmoc group in the presence of the tertiary amine on the tetrahydroisoquinoline portion, the cleavage of the Fmoc and benzyl ether groups were attempted without separation of the diastereomers. Unfortunately, under several hydrogenation conditions only the Fmoc group could be cleaved effectively yielding compound **721** (Table 19). No benzyl ether cleavage was observed prior to decomposition.



Scheme 130 Coupling of β-hydroxypipecolic acid derivative to aniline core.

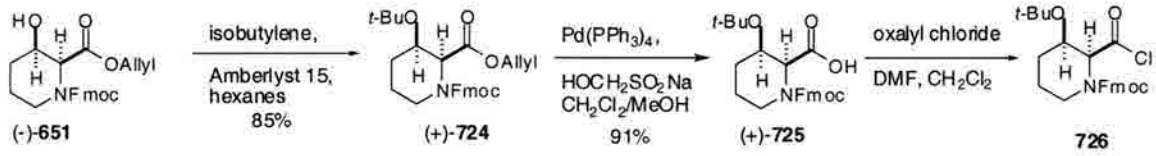


723

Hydrogenation Conditions	Products
H_2 (1 atm), 10% Pd/C	**723**
H_2 (60 psi), 10% Pd/C	**723**, decomp.
H_2 (60 psi), 10% Pd/C, AcOH	**723**, decomp
H_2 (60 psi), 20% $Pd(OH)_2$/C	**723**, decomp
H_2 (40 psi), $PdCl_2$	decomp
H_2 (40 psi), RaNi	decomp

Table 19 Attempted deprotection of benzyl and Fmoc groups.

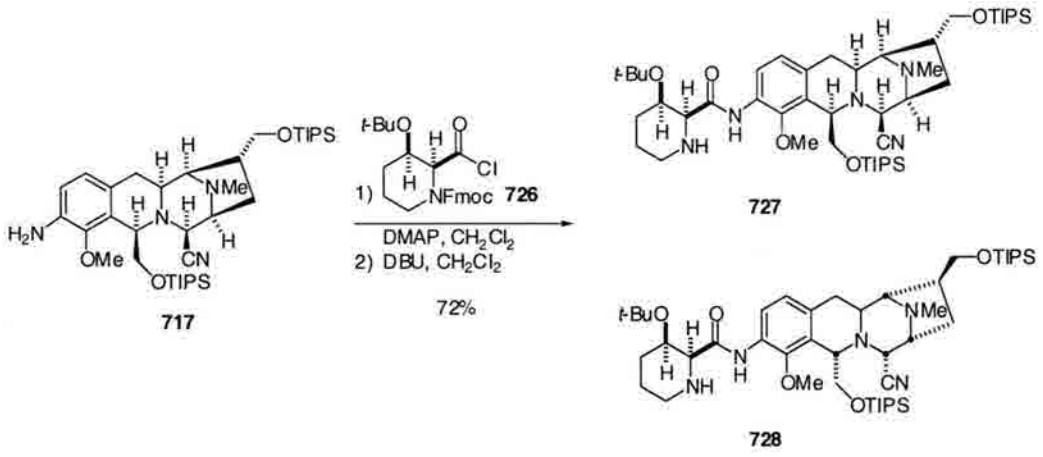
The inability to cleave the benzyl ether made it necessary to use a different protecting group on the hydroxyl group of the amino acid. A *tert*-butyl ether was chosen due to the fact that the amino nitrile was shown to be stable towards treatment with TFA.[138] Treatment of (-)-**651** with isobutene in the presence of Amberlyst 15 ion exchange resin[179] afforded the *tert*-butyl ether (+)-**724** in 85% yield (Scheme 131). Cleavage of the allyl ester was accomplished under the previous conditions to afford **725**.



Scheme 131 Protection of secondary alcohol as a *tert*-butyl ester.

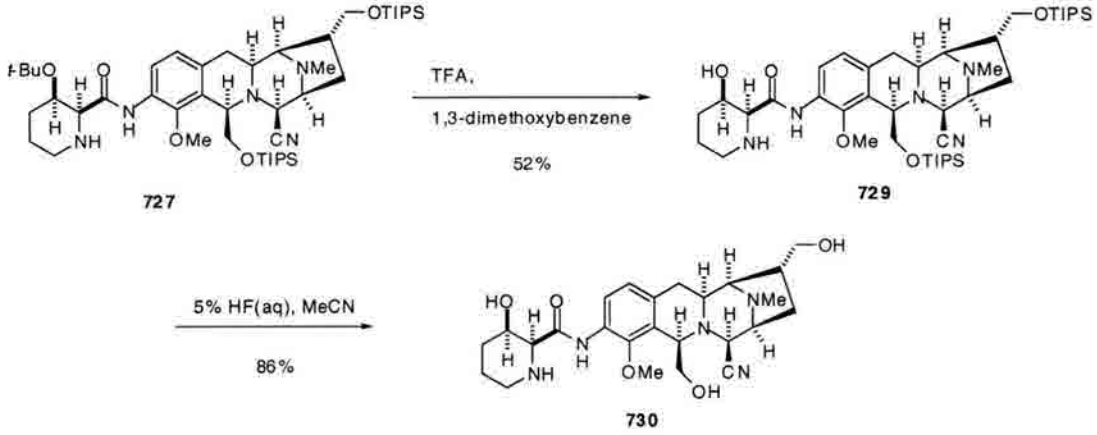
Acid chloride **726** was coupled to aniline **717** in the presence of DMAP to afford the corresponding diastereomeric amides (Scheme 132). This mixture was treated with DBU to cleave the Fmoc groups to provide **727** and **728** which were separable by flash

chromatography. Since the relative stereochemistry of the diastereomers was unknown, each compound was carried on through the rest of the synthesis to tetrazomine.

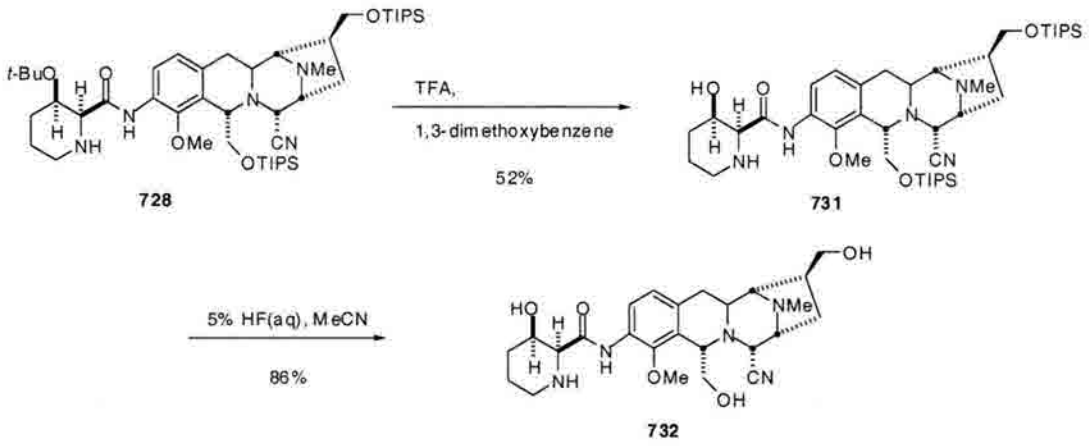


Scheme 132 Coupling of *tert*-butyl ether β-hydroxypipecolic acid derivative to aniline.

Cleavage of the *tert*-butyl ether was accomplished using TFA in the presence of the cation scavenger 1,3-dimethoxybenzene in 52% yield for each diastereomer. The TIPS groups were cleaved using aqueous HF in MeCN to afford the diastereomers **730** and **732** (Schemes 133 and 134).

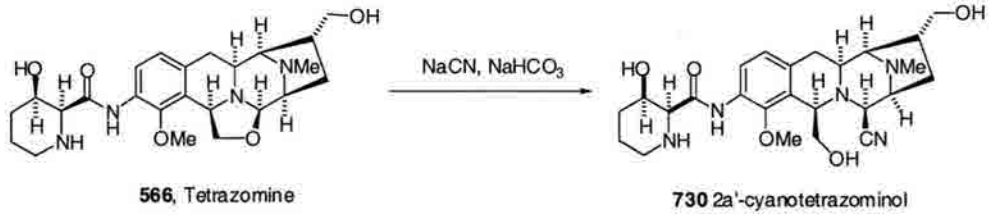


Scheme 133 Deprotection of *tert*-butyl and triisopropylsilyl ethers.



Scheme 134 Deprotection of *tert*-butyl and triisopropylsilyl ethers.

To determine which diastereomer would lead to tetrazomine, tetrazomine was treated with sodium cyanide to form 2a'-cyanotetrazominol. The spectral data of this derivative of tetrazomine was identical to tetracycle **730** (Scheme 135). With this comparison, the relative stereochemistry of the pentacyclic core of tetrazomine was thus established. The absolute configuration of the tetrahydroisoquinoline core was assumed to be that depicted in structure **566** based on biosynthetic considerations since quinocarcin, bioxalomycin, the saframycins and ecteinascidins all possess the same absolute configuration of the tetrahydroisoquinoline moiety.



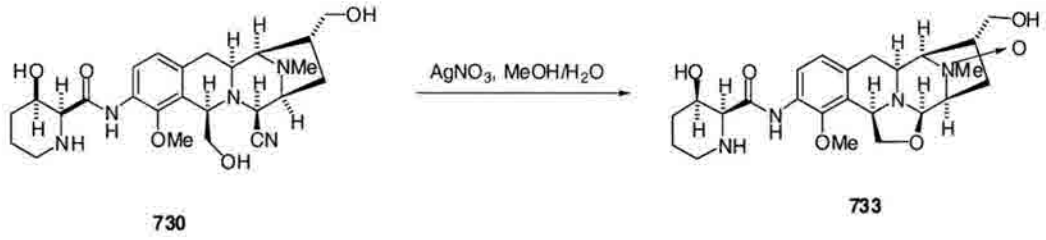
566, Tetrazomine

730 2a'-cyanotetrazominol

Scheme 135 Opening of tetrazomine oxazolidine with cyanide.

Treatment of **730** with silver nitrate under the conditions reported by Fukuyama provided a single compound by ^{1}H-NMR; however, it was not tetrazomine (Scheme 136). The mass spectrum indicated a new product with a mass of 16 higher than that of

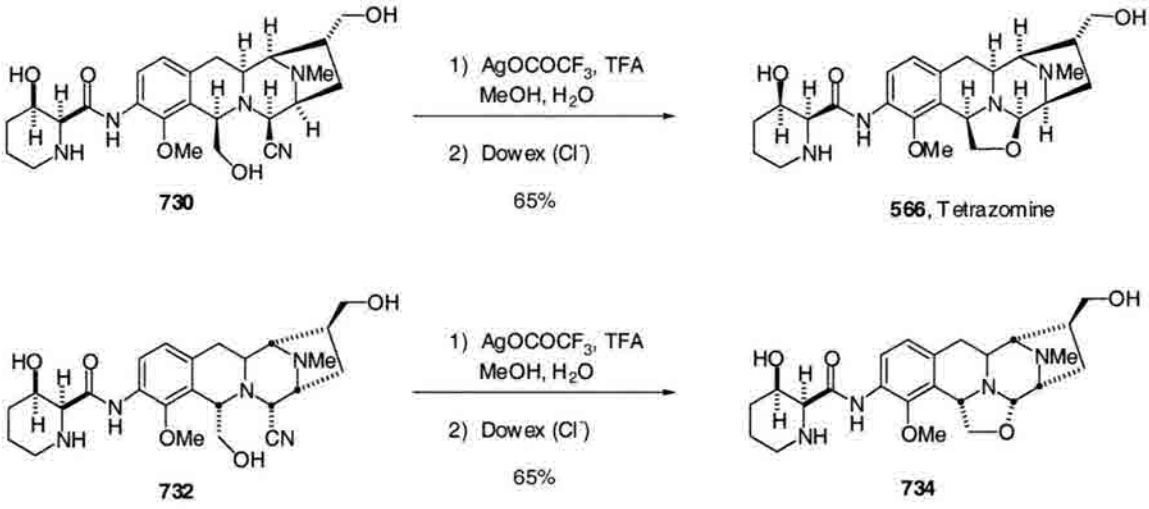
tetrazomine indicating that an extra oxygen was present. The structure of this compound was not determined, but the product was probably **733**, the N-oxide of tetrazomine.



730 733

Scheme 136 Attempted oxazolidine ring formation using silver(I) nitrate.

The completion of the total synthesis of (-)-tetrazomine was accomplished by the treatment of **730** with silver(I)trifluoroacetate in the presence of TFA. The presence of acid prevented the oxidation of the amine. The cyclization was followed by treatment with basic Dowex ion exchange resin and filtration to afford (-)-tetrazomine•2HCl which had identical spectral data (Figure 23) to the natural product (Scheme 137). Aminonitrile **732** was treated under the same conditions to afford *ent*-2,3-*epi*-tetrazomine **734**.

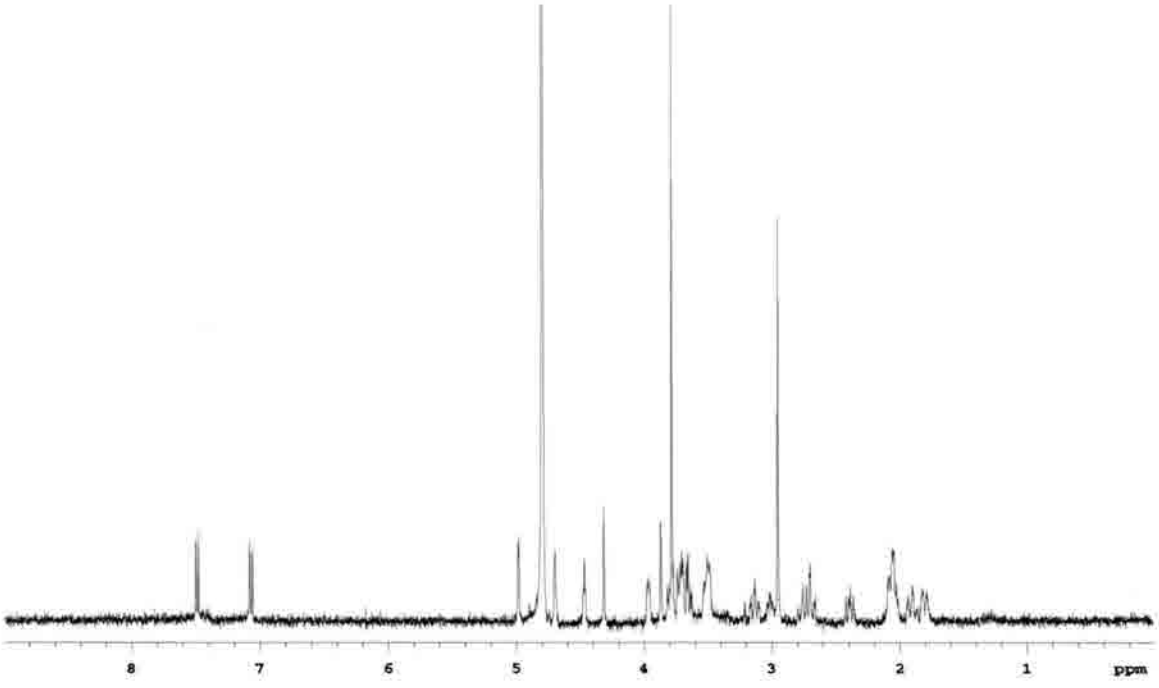


730 566, Tetrazomine

732 734

Scheme 137 Synthesis of (-)-tetrazomine and *ent-epi*-tetrazomine.

Synthetic Tetrazomine (400 MHz, D_2O)



Natural Tetrazomine (400 MHz, D_2O)

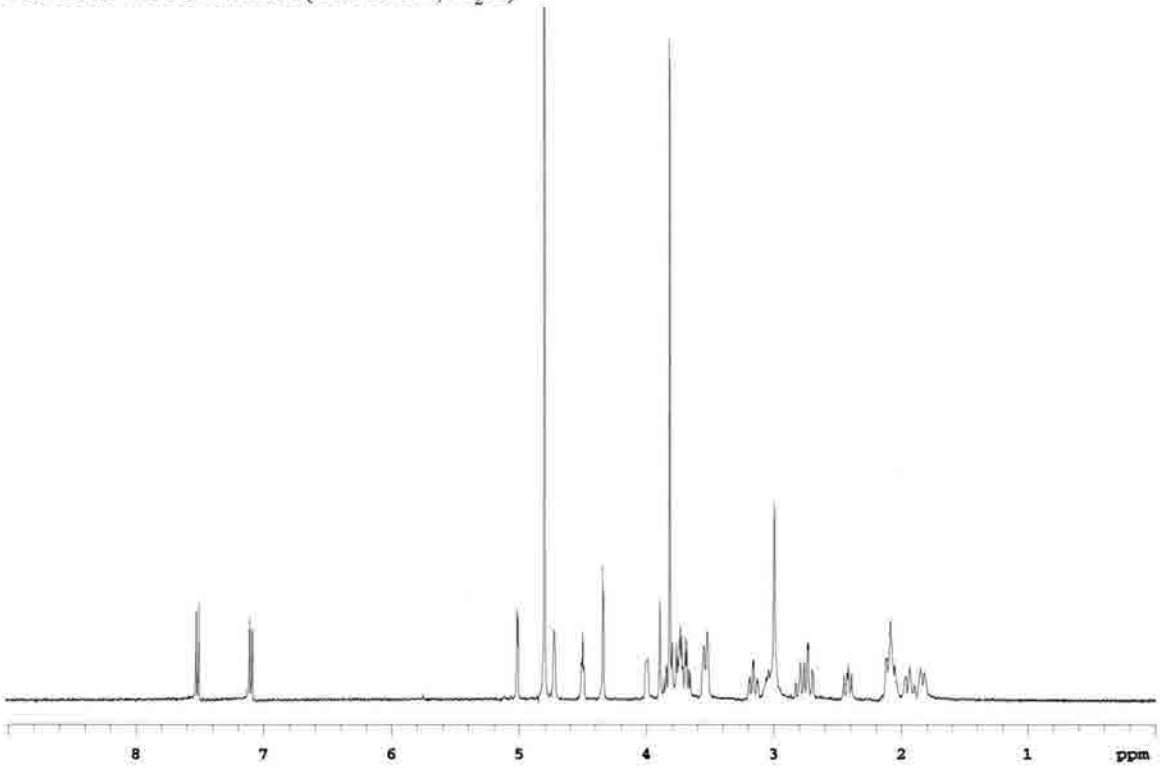
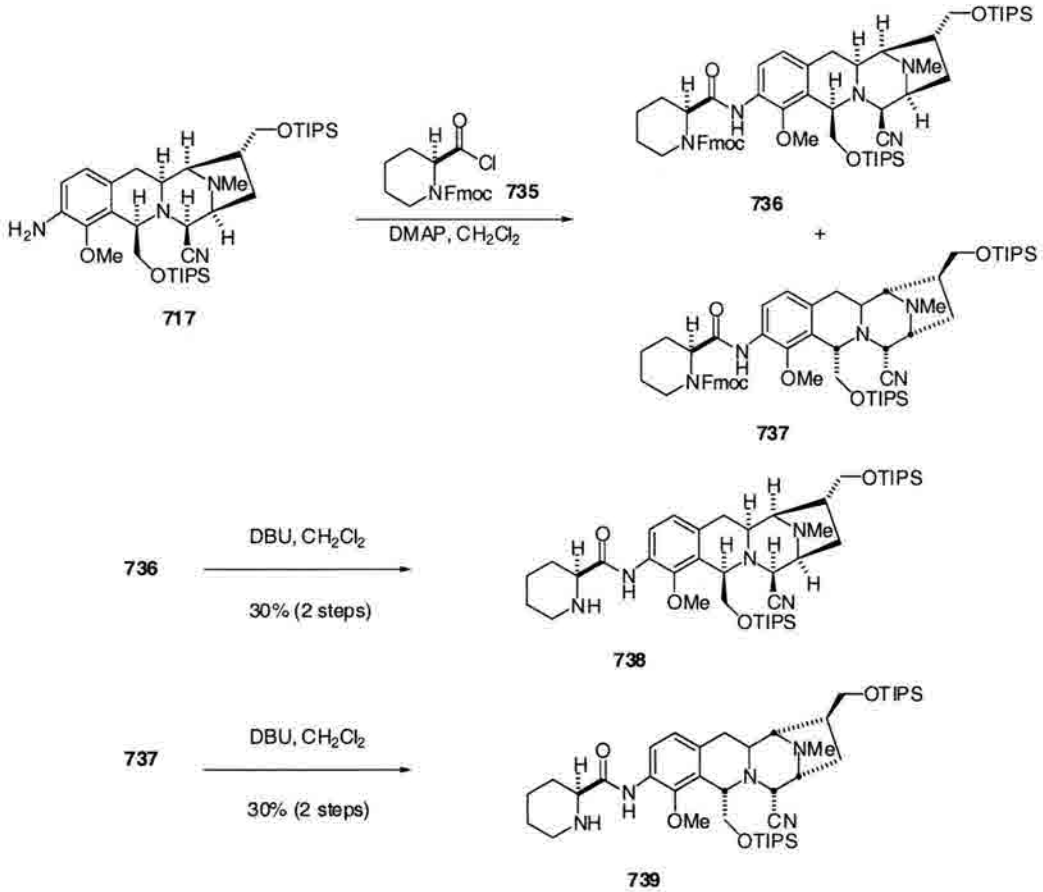


Figure 21 Comparison of ^{1}H-NMR's of synthetic and natural tetrazomine.

3.6. Synthesis of Tetrazomine Analogs

To help determine the role of the β-hydroxy pipecolic acid moiety of tetrazomine in DNA recognition and superoxide formation, two pipecolic acid analogs were synthesized. This was easily accomplished using acid chloride **735** that was readily available from commercially available L-pipecolic acid (Scheme 138). The coupling yielded a 1:1 mixture of diastereomers were separated by preparative TLC. Removal of the Fmoc groups afforded **738** and **739** in 30% yield overall for each diastereomer.



Scheme 138 Coupling of L-pipecolic acid derivative to aniline core.

The relative stereochemistry of each diastereomer was unknown at this time so each was treated with HF in acetonitrile to afford the diols **740** and **741** (Scheme 139).

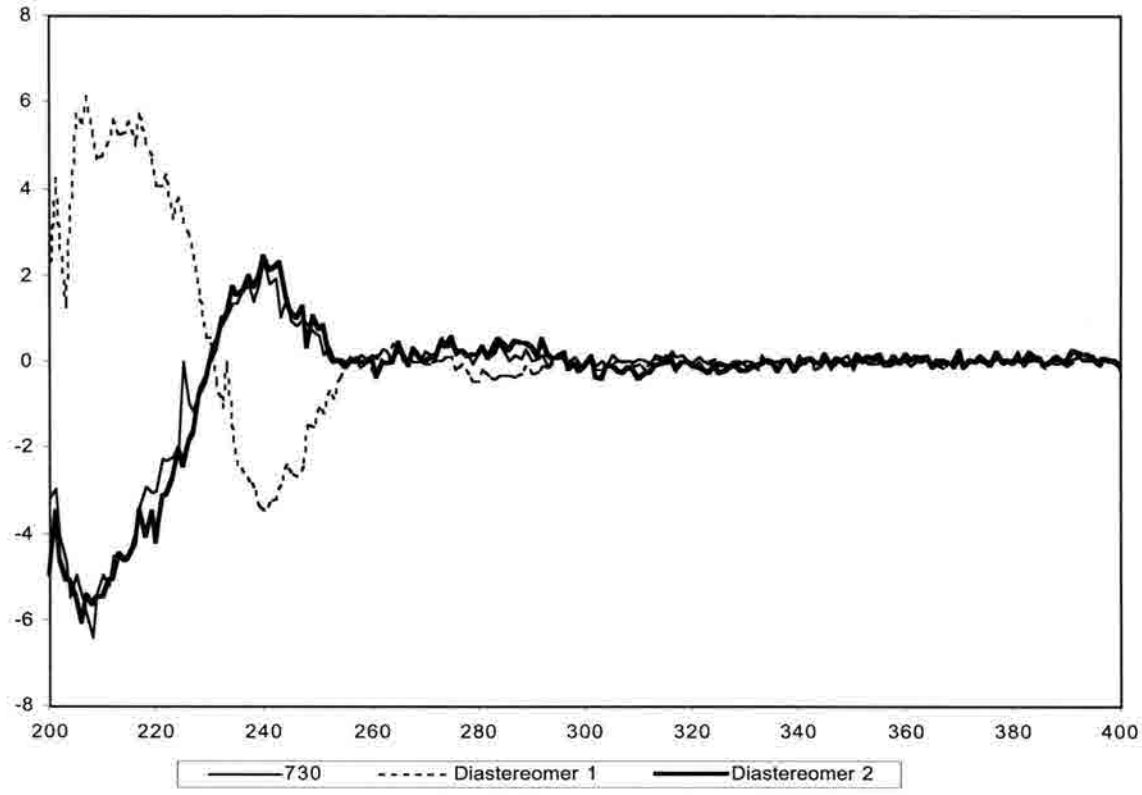
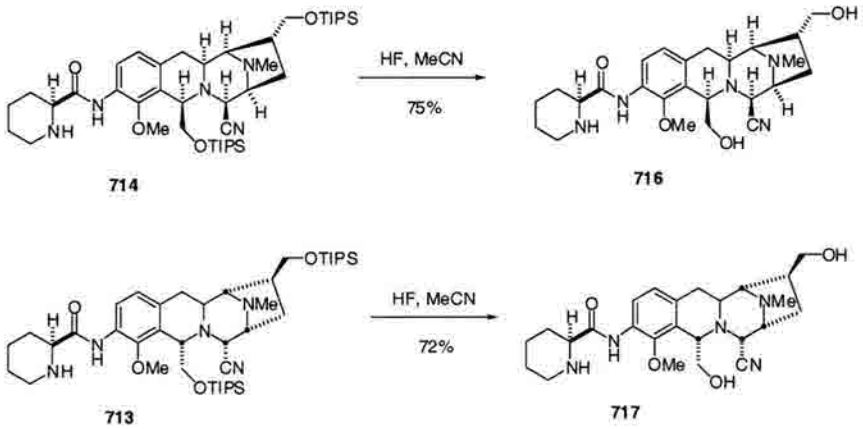


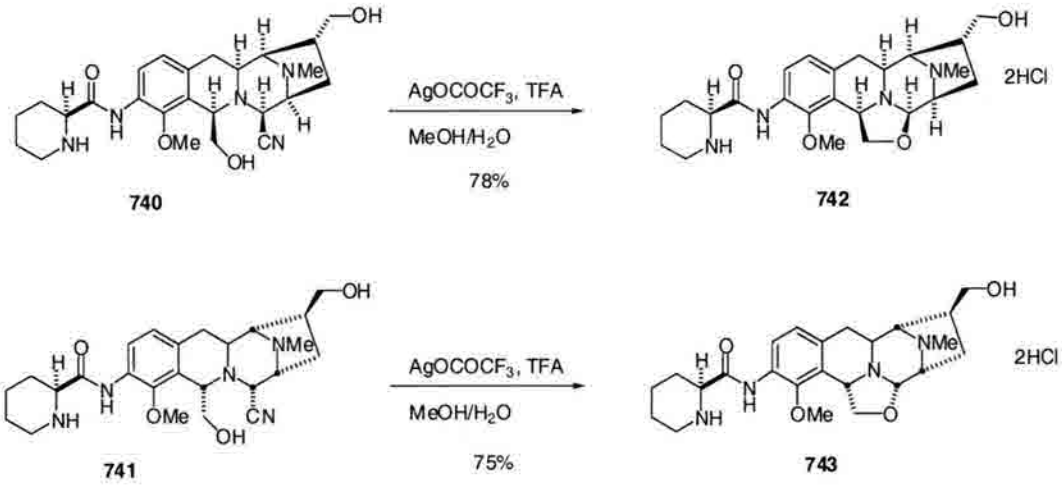
Figure 24 CD spectra of pipecolic acid analogs vs. **730**.

The CD spectra of each of these diastereomers was obtained and compared to the CD spectra of **730** (Figure 24). It was determined that the higher R_f (Diastereomer 1) following the coupling was amide **739** and the lower R_f (Diastereomer 2) was amide **738**.



Scheme 139 Deprotection of triisopropyl ethers of pipecolic acid analogs.

The completion of the synthesis of analogs **742** and **743** was accomplished using the same conditions used for the oxazolidine ring formation of tetrazomine (Scheme 140).



Scheme 140 Completion of synthesis of pipecolic acid analogs of tetrazomine.

3.7. Conclusion

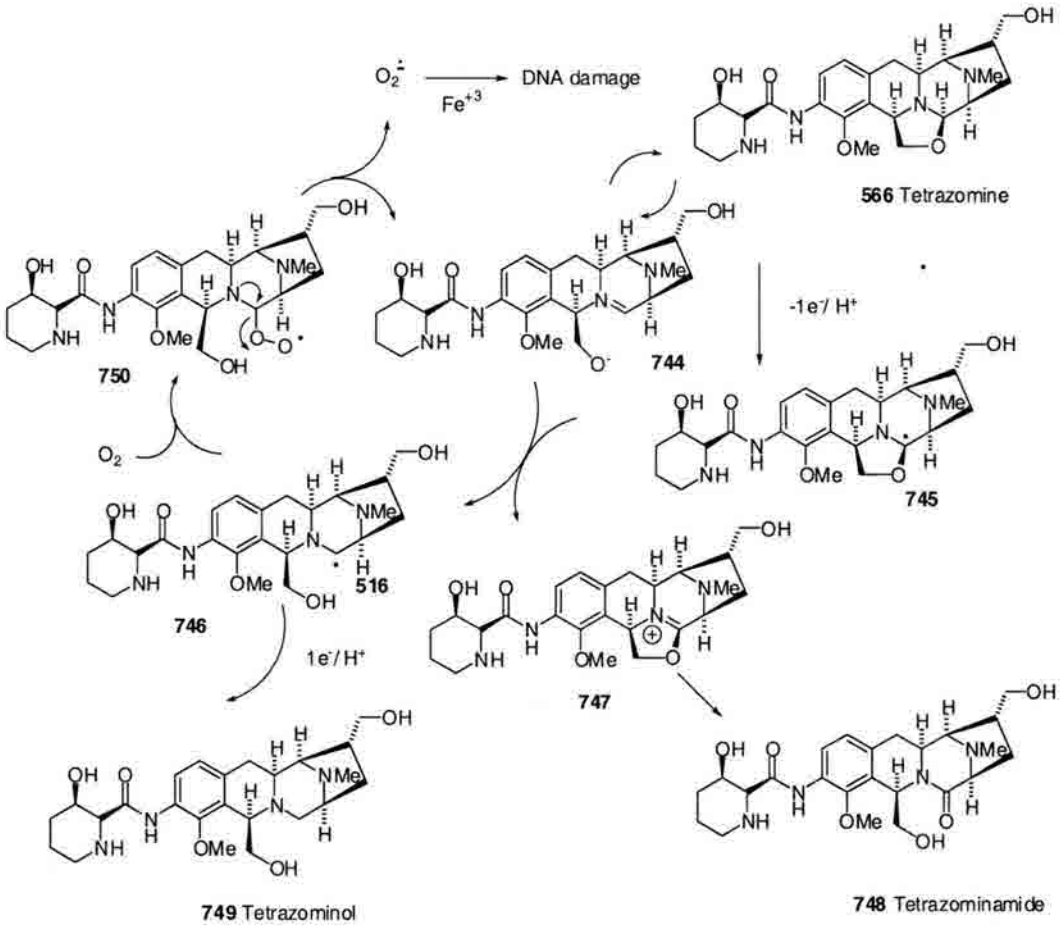
In conclusion, the total synthesis of tetrazomine has been presented using a new and efficient route to synthesize the core tetrahydroisoquinoline. The route used to couple the optically active amino acid also allowed for the synthesis of other amino acid containing analogs with the pipecolic acid analogs presented. These analogs can be used to study the DNA selectivity and reactivity of this type of antitumor antibiotics.

Chapter 4

Biochemical Studies of Tetrazomine and Analogs

4.1. Introduction

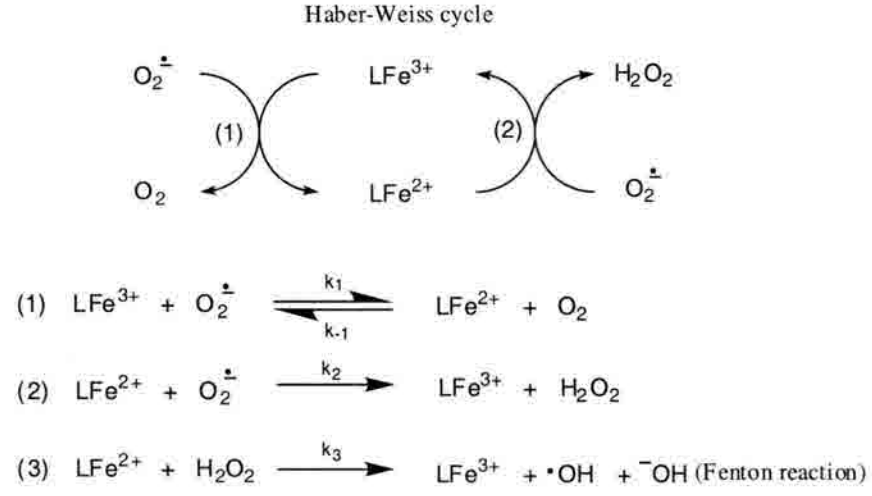
As stated in Chapter 1, Williams *et al.* had shown that tetrazomine produces superoxide via a Cannizaro-type self redox reaction. [152] The products that are formed in this process are tetrazominol (**749**), tetrazominamide (**748**), and superoxide. It was also noted that there were several minor products in the disproportionation of tetrazomine.



Scheme 141 Canizzaro self redox reaction of tetrazomine.

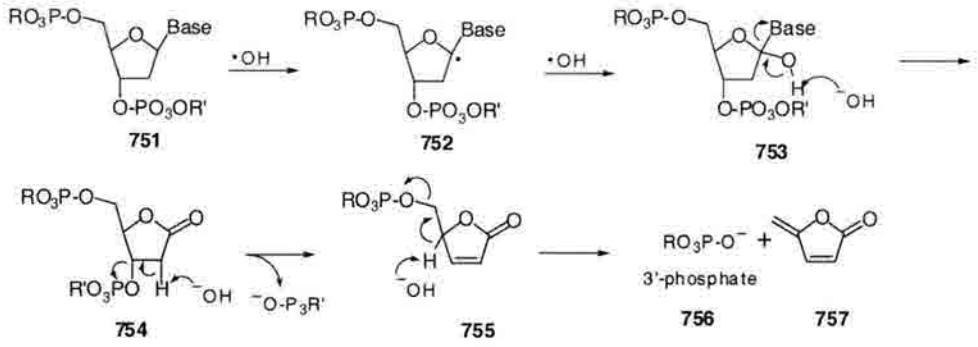
These products were not able to be identified on the scale that this disproportionation was conducted. The anaerobic self redox reaction of quinocarcin however was a much cleaner reaction producing only quinocarcinol and quinocarcinamide.

Superoxide has been shown to cleave DNA via the formation of hydrogen peroxide via the Haber-Weiss cycle followed by Fenton-mediated generation of hydroxyl radical (Reaction 3, Scheme 143).[187] Hydroxyl radicals abstract hydrogen atoms from the sugar backbone of DNA causing the cleavage in a sequence non-specific manner.



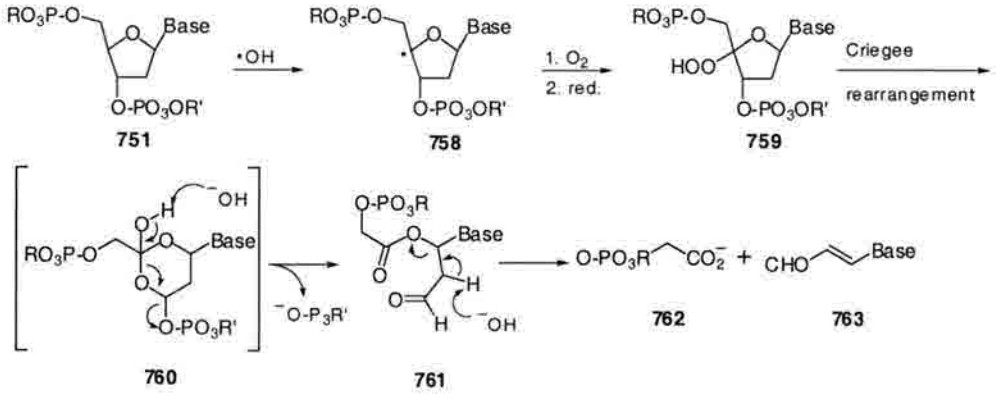
Scheme 142 Haber Weiss cycle and Fenton formation of hydroxyl radical.

There are two major products observed from the abstraction of a hydrogen atom from DNA.[188] Abstraction of the C-1' hydrogen atom results in the radical **752**. Capture of **752** by another hydroxyl radical forms the hemi-ketal **753** which forms lactone **754** after loss of the base. Alpha deprotonation causes strand scission of the 3'-phosphate. Deprotonation at the 4' position affords the unstable unsaturated lactone **757** with the 3'-phosphate product **756** (Scheme 143).



Scheme 143 Hydroxyl radical cleavage of DNA at C-1'.

Abstraction of the C-4' hydrogen atom is the other main site for the hydroxyl radical (Scheme 144).[188] Abstraction of the C-4' hydrogen atom forms the radical **758**. Addition of oxygen followed by reduction yields the peroxide **734**. Criegee rearrangement followed by deprotonation and strand scission forms the ring opened aldehyde **761**. Subsequent deprotonation affords the 3'-phosphoglycolate **762** and the base propenal **763**.



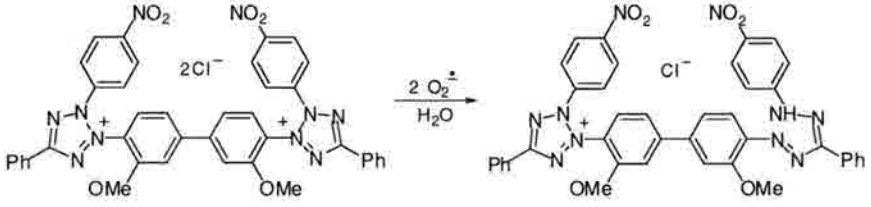
Scheme 144 Hydroxyl radical cleavage of DNA at C-4'.

In DNA/tetrazomine reactions, non-sequence specific cleavage was observed. Every cleavage band appeared as a doublet due to the two products that were formed from DNA cleavage (Schemes 143 and 144). The observed ratio of 3'-phosphate to phosphoglycolate for tetrazomine was 8:2 while the ratios for quinocarcin and Fe/EDTA

were 6:4 and 4:6 respectively. It was speculated that tetrazomine, and to a lesser extent quinocarcin, protect the 4'-hydrogen atom from being abstracted. DNA that had been incubated with tetrazomine showed virtually no 3'-phosphoglycolate formation upon treatment with Fe/EDTA providing further evidence for some tetrazomine DNA binding.

4.2. Superoxide Formation by Tetrazomine and Analogs

The rate of superoxide formation for tetrazomine has been determined by measuring the UV/VIS absorbance of the reaction between nitroblue tetrazolium (NBT) **764** and the superoxide formed by the drug over a 30 minute period. The two-electron reduction of **764** yields the monoformazan compound **765** that can be detected spectrophotometrically in the visible range (500nm).



764 765

Scheme 145 Reduction of NBT by superoxide.

Tetrazomine has been shown to have superoxide formation rates of 10.6 x 10^{-9} M s^{-1} and 17.5 x 10^{-9} M s^{-1} (1.0 mM tetrazomine) at pH 7 and 8 respectively. As was expected, the oxazolidine ring-opened 2a'-cyanotetrazominol (**730**) produced no superoxide under these conditions.

4.3. DNA Cleavage Studies of Tetrazomine and Analogs

Since tetrazomine cleaves DNA in a non-sequence specific manner, but in a specific product manner (8:2 3'-phosphate to phosphoglycolate ratio), it was desired to investigate the cleavage pattern of the tetrazomine oxazolidine analogs **734**, **739**, and **741**. The cleavage studies were preformed on a synthetic 45-mer duplex DNA (Figure 23). A publication quality gel was obtained for the *ent-epi*-tetrazomine **734**, but not for the deoxycompounds **739** and **741**. The cleavage pattern observed for **734** was 4.5:3.5 for the 3´-phosphate to phosphoglycolate ratio was 4.1: 1.6 for tetrazomine as measured by the use of a phosphoimager. The Fe EDTA standard yielded a 3.8:4.1 ratio. These

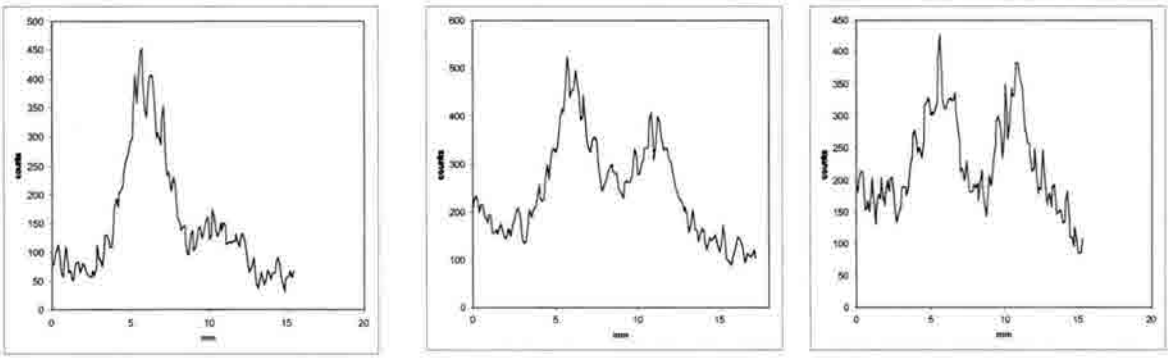


Figure 25 graphed ratios for cleavage pattern of **566** (left), **734** (center), and Fe EDTA (right).

ratios were consistent with those measured in our research group by the use of densitometry.[152]

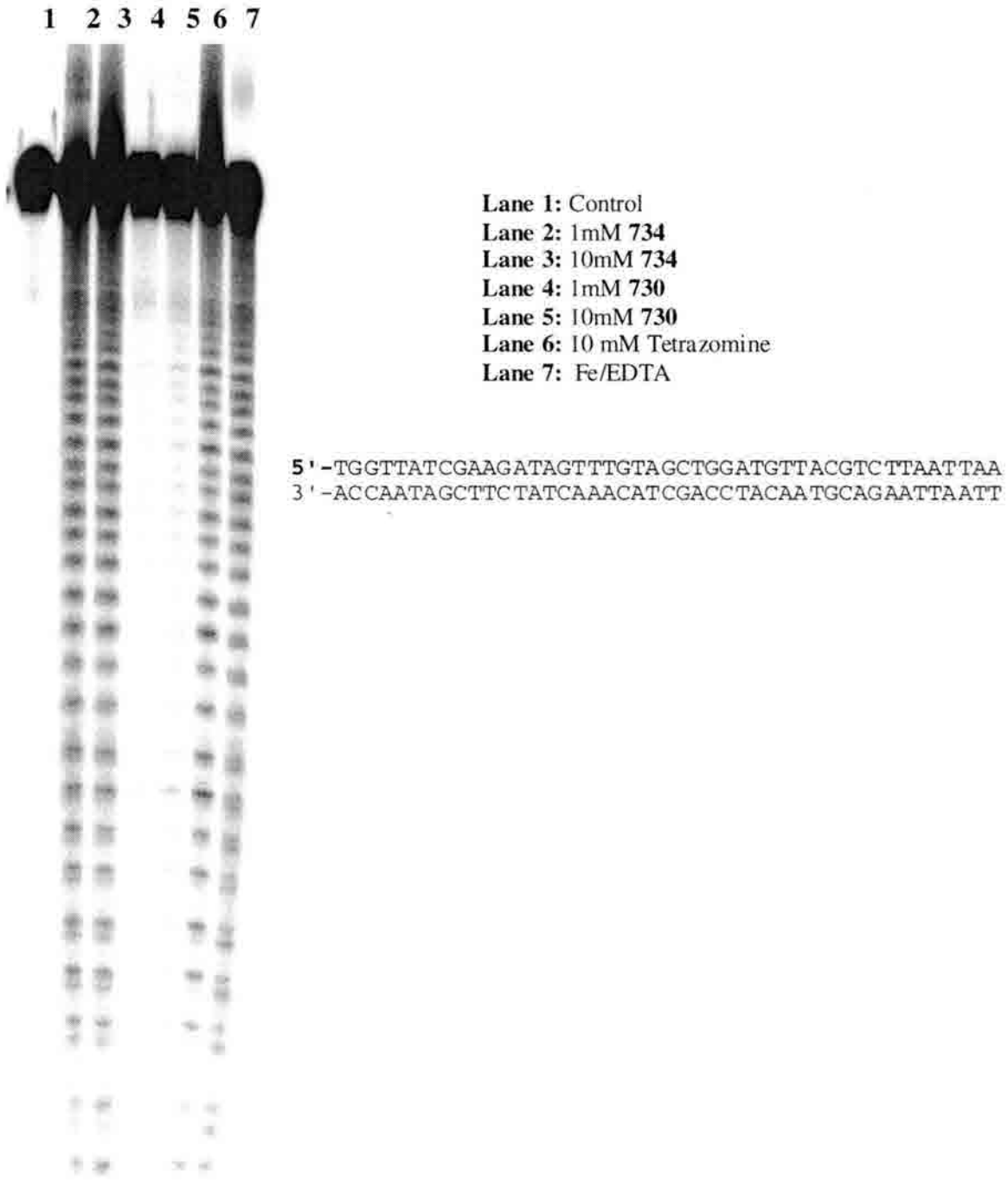


Figure 26 Polyacrylamide Gel of tetrazomine and *ent-epi*-tetrazomine.

It had also been postulated that tetrazomine and quinocarcin could alkylate DNA. It could also be assumed that cyanotetrazominol (**730**) and its analogs could also alkylate DNA. To date there have been no reports of DNA alkylation in the literature by any of these compounds. As stated in Chapter 1, several other tetrahydroisoquinoline antitumor

antibiotics have been shown to mono alkylate or crosslink DNA. It was difficult to observe DNA alkylation on the synthetic 45-mer, but there were two bands and a streak observed in the *ent-epi*-tetrazomineand tetrazomine lanes (Lanes 2,3, and 6 respectively). This could be a result of DNA alkyation, but since there were many possible sites of alkyation (i.e. many guanine residues) many products could have formed. Interestingly no retardation of the DNA was observed with the aminonitrile **730**. The use of a DNA sequence that contains only one GC base pair would be ideal for the future study of such an alkylation.

4.4 Antimicrobrial Studies of Tetrazomine and Analogs

In order to investigate the structure activity relationship of the tetrazomine analogs, all four oxazolidine and all four amino nitrile analogs were studied for their antimicrobrial activity against one Gram-(+) bacteria (*Staphylococcus aureus*) and a Gram-(-) bacteria (*Klebsiella pneumoniae*). These studies were run via the disc diffusion method. It was found that the deoxy compounds **739** and **741** were slightly more active than the C-3 alcohol containing tetrazomine (**566**) and **734**. Also the oxazolidine compounds had similar activities to the ring opened amino nitriles.

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
734	0.12	15	R
	0.012	8	R
	0.0012	R	R
739	0.12	29	14
	0.012	21	9
	0.0012	19	R
741	0.12	24	7
	0.012	17	R
	0.0012	R	R
Penicillin G	10 units	NA	30
Streptomycin	0.01	14	NA

Table 20 Antimicrobrial activities of oxazolidine analogs (R = Resistant)

Compound	Amount (mg)	Zone of inhibition Kleb (mm)	Zone of inhibition Staph (mm)
730	0.12	26	12
	0.012	20	R
	0.0012	16	R
732	0.12	18	R
	0.012	13	R
	0.0012	R	R
738	0.12	27	11
	0.012	23	R
	0.0012	13	R
740	0.12	16	R
	0.012	12	R
	0.0012	R	R
Penicillin G	10 units	NA	30
Streptomycin	0.01	14	NA

Table 21 Antimicrobrial activities of amino nitrile analogs (R = Resistant)

4.5 Conclusion

It had been previously seen that tetrazomine cleaved DNA in a non specific manner. *Ent-epi*-tetrazomine has now been shown to cleave DNA also, along with some possible evidence for DNA alkylation by tetrazomine and the analog. This evidence

warrants further investigation. Surprisingly, cyanotetrazominol (**730**) did not show any evidence of DNA alkylation. From the antimicrobrial standpoint, the tetrazomine analogs with the same absolute stereochemistry of natural tetrazomine had significantly higher activities compared to the *ent* analogs. Also a slight improvement of biological activity was seen in the 3-deoxy pipecolic acid analogs when compared to the natural product.

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Experimental Section

5.1 General Procedures

Unless otherwise noted, materials were obtained from commercially available sources and used without futher purification. Diethyl ether and THF were distilled from sodium benzophenone ketyl under an Ar atmosphere. Methylene chloride and triethylamine were distilled from calcium hydride under an Ar atmosphere. Dimethyl formamide was dried over activated 4Å molecular sieves.

All reactions involving hygroscopic substances were conducted in flame dried glassware under and Ar atmosphere.

Infrared spectra were obtained on a Perkin-Elmer 1600 series FTIR as thin films from CH_2Cl_2.

Nuclear magnetic resonance (NMR) spectra were obtained using a Varian Mercury or Inova spectrometer. NMR chemical shifts are given in ppm relative to internal $CHCl_3$, TMS, DMSO, or methanol. Proton (^{1}H)NMR are tabulated in the following order: number of protons, multiplicity (s = singlet; d = doublet; t = triplet; q = quartet; and m = multiplet), and coupling constant(s) in hertz. When appropriate, the multiplicity of a signal is denoted as "br" in indicate that the signal was broad.

Desalting of amino acids were performed using Dowex (50WX2-100) strongly acidic ion exchange resin. The resin was prewashed with ddH_2O followed by 2M NaOH, ddH_2O, 4M HCl and ddH_2O. The amino acid (pH 7) was loaded in ddH_2O and the salt

was washed by eluting with ddH_2O (3 volumes). The amino acid was eluted using 2% NH_4OH and lyophillized to afford the free amino acid.

Desalting of water soluble organic compounds was accomplished using HP20 absorption resin. The resin was equilibrated at in 1:1 MeOH/ddH_2O at 4°C for 16h. The column was rinsed with ddH_2O (10 volumes) at 4°C. The compound was loaded in ddH_2O and the column was eluted with ddH_2O (5 volumes) to remove the salt. The organic compound was eluted with 90:10 MeOH:H_2O. The solvent was removed by rotary evaporation followed by lyophilization to afford the desalted compound.

5.2 Labeling (5'-^{32}P) of synthetic oligonucleotides for high resolution polyacrylamide gel electrophoresis

To a solution of 1.22 $X10^{-4}$ M deoxyoligonucleotide (36 μL) was added ddH_2O (43.9 μL), PNK Buffer (10 μL), γ^{32}P ATP (5 μL, 50 μCi, Amersham), and T4 kinase (New England Biolabs). The reaction was incubated at 37°C for 1.5 h. The DNA was precipitated (3x) using ethanol/ 3M NaOAc (pH 5.2). Annealing was performed by mixing equimolar amounts of this 5'-^{32}P end labeled strand and its complimentary strand in ddH_2O to total volume of 100μL. The mixture was heated to 80°C for 15 min then slowly cooled to rt over 2h.

Reactions with 5'-^{32}P-labeled double stranded DNA.

To an eppendorf containing dsDNA (2μL, 42mM) was added the drug (1μL of either 10mM or 100mM). The final volume was brought to 10μL using ddH_2O (5.4 μL) and 100mM phosphate buffer (1.6 μL). The reactions were incubated at 37°C for 16 h. The reactions were ethanol precipitated and the solvent was removed. Each reaction was diluted with ddH_2O and an appropriate amount (to get a count of 25,000 CPMA) was

removed and brought to a volume of 5μL with loading dye. The sample was loaded to the polyacrylamide gel and heated with a heat gun for 5 min and the denaturing gel was run at 1600 volts for 4 h. The bands were visualized using a phosphoimager.

Preparation of Compounds

(+)-**619** (+)-**623**

(3S,5S,6R)-4-Morpholinecarboxylic acid, 3-[(1S)-1-hydroxy-4-pentenyl]-2-oxo-5,6-diphenyl-phenylmethyl ester [(+)-623].

To a solution of (+)-**619** (387 mg, 1.0 mmol) in CH_2Cl_2 (18 mL) at 0°C was added dibutylboron triflate (1M soln. in CH_2Cl_2, 2.9 mL, 2.9 mmol, 2.9 eq.). This solution was stirred for 5 min at 0°C. Triethylamine (418 μL, 3.0 mmol, 3.0 eq.) was added and the solution was stirred for 15 min at 0°C. The solution was then cooled to –78°C and 4-pentenal (158 μL, 1.5 mmol, 1.5 eq.) (Lancaster) in CH_2Cl_2 (5 mL) was added slowly and allowed to stir for 1 h at –78°C. Excess phosphate buffer (0.025 M, pH 7) was added and the mixture was allowed to warm to rt. The aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic fractions were dried over $MgSO_4$ and concentrated. The crude product was purified via flash chromatography (gradient 1-2% EtOAc/CH_2Cl_2) to afford 340 mg (+)-**623** (72%) as a white foam. (+)-**623:** TLC (40% EtOAc/hex) R_f = 0.43 (UV and PMA). ^{1}H-NMR (300 MHz) (d_6-DMSO vs TMS 120°C) 1.69-1.85 (2H, m), 2.14-2.28 (2H, m), 4.17 (1H, m), 4.84 (1H, d, J = 2.25 Hz), 4.95-5.18 (4H, m), 5.25 (1H, d, J = 3.18Hz), 5.47 (1H, d, J = 5.34 Hz), 5.82-5.88 (1H, m), 6.53 (1H, d, J = 3.16 Hz), 6.60 (2H, d, J = 7.24 Hz), 6.99-7.39 (12H, m). IR (NaCl, neat) 3526, 3064, 3031, 2947, 1744, 1704, 1082, 1057 cm^{-1}. Anal calcd. for $C_{29}H_{28}NO_5$: C, 73.87; N 2.97; H 6.23 found: C, 74.04; N,2.85; H, 6.14. $[\alpha]_D^{20}$ = +13.7 (c=1.3, $CHCl_3$).

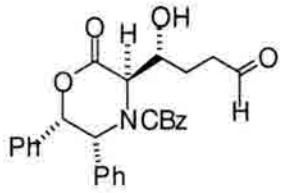
(-)-**623**: $[\alpha]_D^{20}$ = -12.4 (c=1.1, $CHCl_3$).

(+)-**623** (+)-**625**

(3S,5S,6R)-4-Morpholinecarboxylic acid, 3-[(1S)-1-hydroxy-4-oxobutyl]-2-oxo-5,6-diphenyl-phenylmethyl ester [(+)-625].

To a solution of (+)-**623** (78 mg, 0.17 mmol) in CH_2Cl_2 (10 mL) at –78°C ozone was bubbled through until the solution turned blue. The solution was degassed with Ar and excess dimethyl sulfide was added. The solution was allowed to warm to rt and was stirred for 16 h. The solvent was removed *in vacuo* and the crude material was purified via flash chromatograhy (25% EtOAc/hex) to afford 61 mg (+)-**625** (77%) as a clear oil. TLC (40% EtOAc/hex) R_f = 0.39 (UV and PMA).^{1}H-NMR (300MHz) (d6-DMSO vs TMS, 120°C) δ 2.04 (2H, m), 2.29 (2H, m), 4.53 (1H, m), 4.99 (1H, d, J = 2.52 Hz), 5.03 (4H, m), 5.24 (2H, m), 6.71 (1H, d, J = 3.24 Hz), 7.02 (14H, m) 9.70 (1H, s). IR(NaCl, neat) 3467, 3063, 3033, 2953, 1751, 1670, 1455, 1404, 1269, 1062 cm^{-1}. HRMS (FAB) calcd. for $C_{28}H_{28}NO_6$ (MH^+): 474.1917. found: 474.1914. $[\alpha]_D^{20}$ = + 3.1 (c = 1.25, $CHCl_3$).

(-)-**625**: $[\alpha]_D^{20}$ = - 3.7 (c = 1.25, $CHCl_3$).



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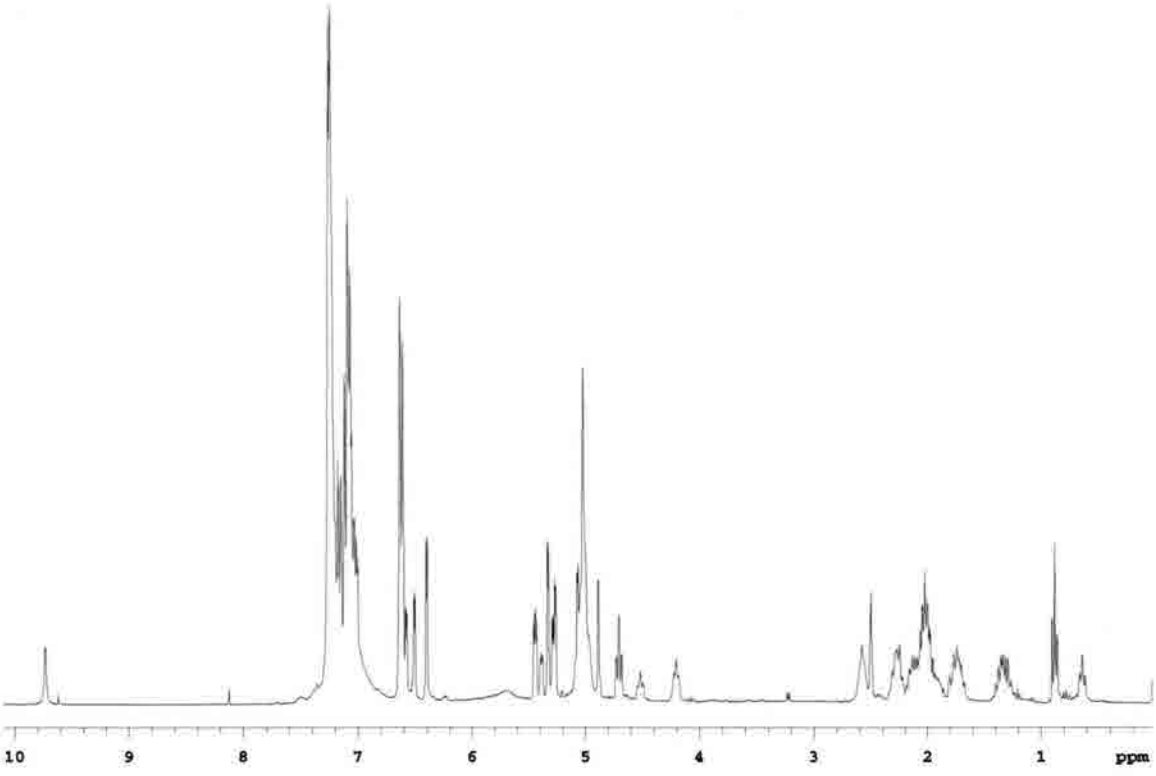


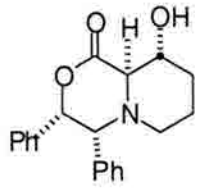
Compound (+)-**625**: ^{1}H-NMR (300 MHz) in d_6-DMSO at 120°C

(+)-**625** (+)-**628**

(3R,4S,9S,9aS)- hexahydro-9-hydroxy-3,4-diphenyl-pyrido[2,1-c][1,4]oxazin-1(6H)-one [(+)-628].

To a solution of (+)-**625** (130 mg, 0.27 mmol) in CH_2Cl_2 (8 mL) was added 5% Pd/C (117 mg, 0.055 mmol, 0.2 eq). To this solution H_2 was bubbled through for 15 min. A H_2 balloon was then attached. After the starting material was consumed by TLC (2.25 h) the solution was purged with Ar. The mixture was filtered through Celite and the solvent was removed. The crude product was purified via flash chromatography (20% EtOAc/hex) to afford 58 mg (+)-**628** (66%) as a white foam. TLC (30% EtOAc/hex) R_f = 0.30 (UV and PMA). ^{1}H-NMR (300MHz, $CDCl_3$ vs TMS) δ 1.20 (1H, m), 1.70 (2H, m), 2.06 (2H, m), 2.86 (1H, d, *J* = 11.4Hz), 3.01 (1H, d, *J* = 8.7Hz), 3.96 (1H, ddd, *J* = 2.1, 3.9, 4.8Hz), 4.24 (1H, d, *J* = 3.9Hz), 4.9 (1H, s, D_2O exchangeable), 6.17 (1H, d, *J* = 3.9Hz), 7.02-7.30 (10H, m). ^{13}C-NMR (75 MHz) δ 22.89, 32.42, 52.04, 62.95, 62.95, 69.11, 83.29, 125.3, 125.4, 127.6, 128.0, 128.2, 129.9, 130.7, 135.6, 172.1. IR (NaCl, neat) 3500, 2943, 1714, 1453, 1040 cm^{-1}. HRMS (FAB) calcd. for $C_{20}H_{22}NO_3$ (MH^+): 324.1600. found: 324.1586. $[\alpha]_D^{20}$ = +21.4 (c = 1.9 $CHCl_3$).

(-)-**628** $[\alpha]_D^{20}$ = -20.9 (c = 1.6 $CHCl_3$).



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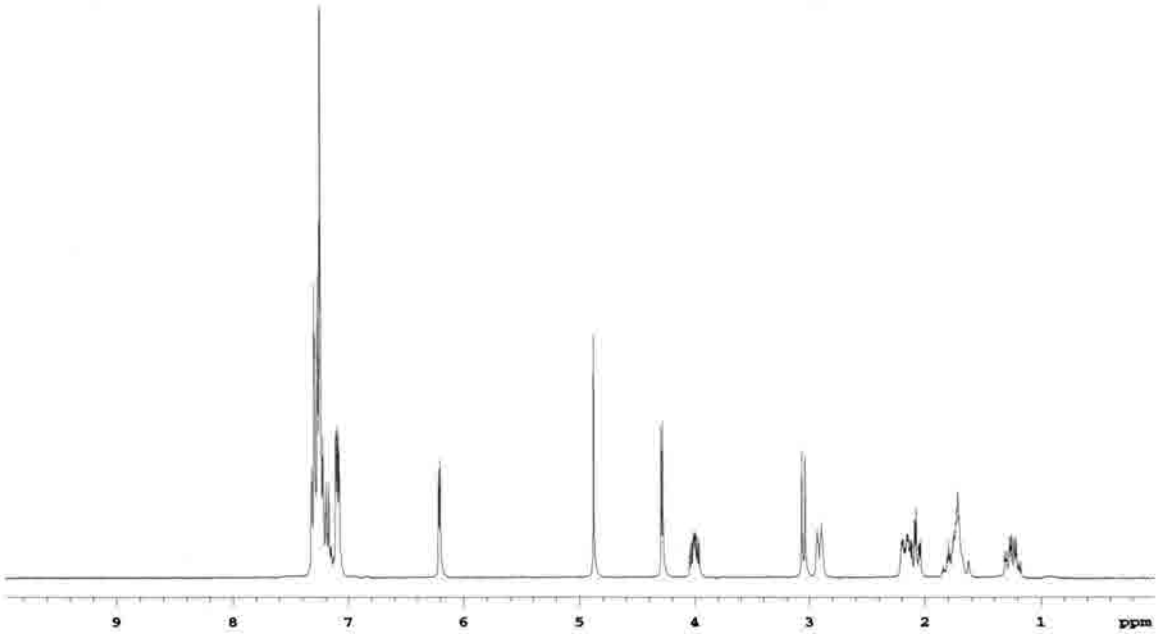
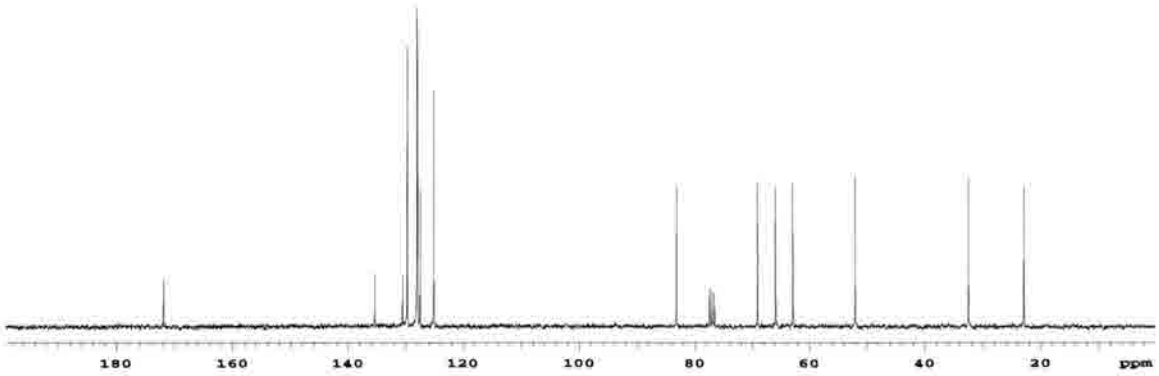
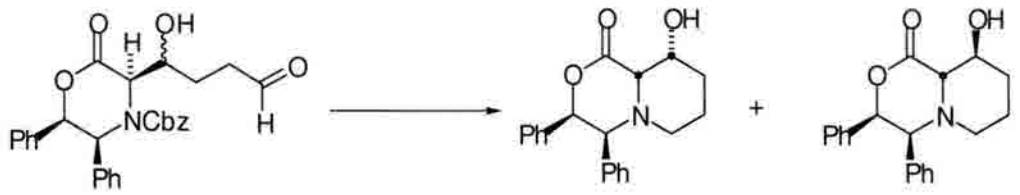




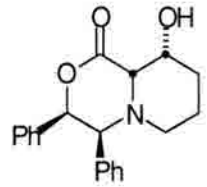
Compound **628**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$



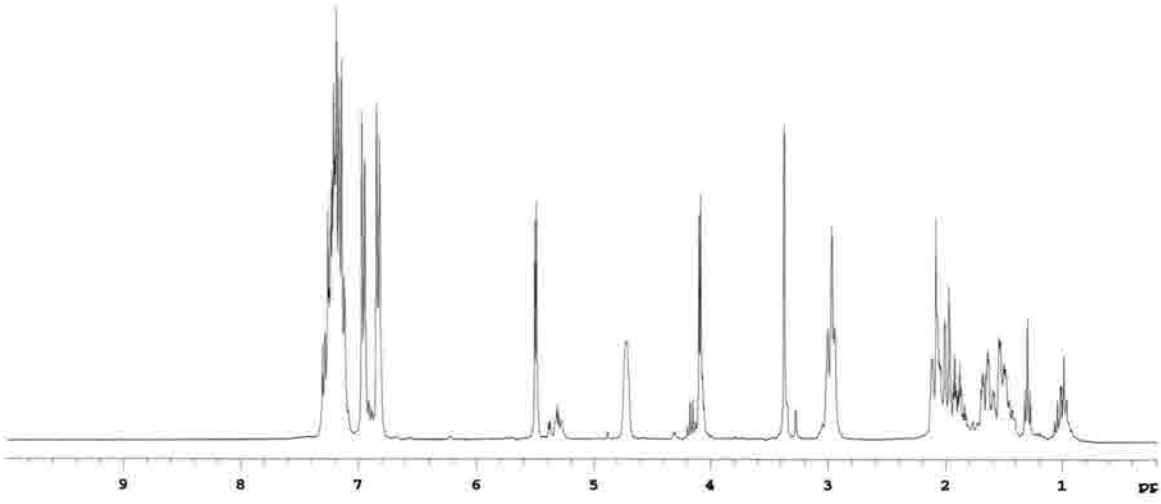
625, *epi*-625 (+)-**646** (-)-**628**

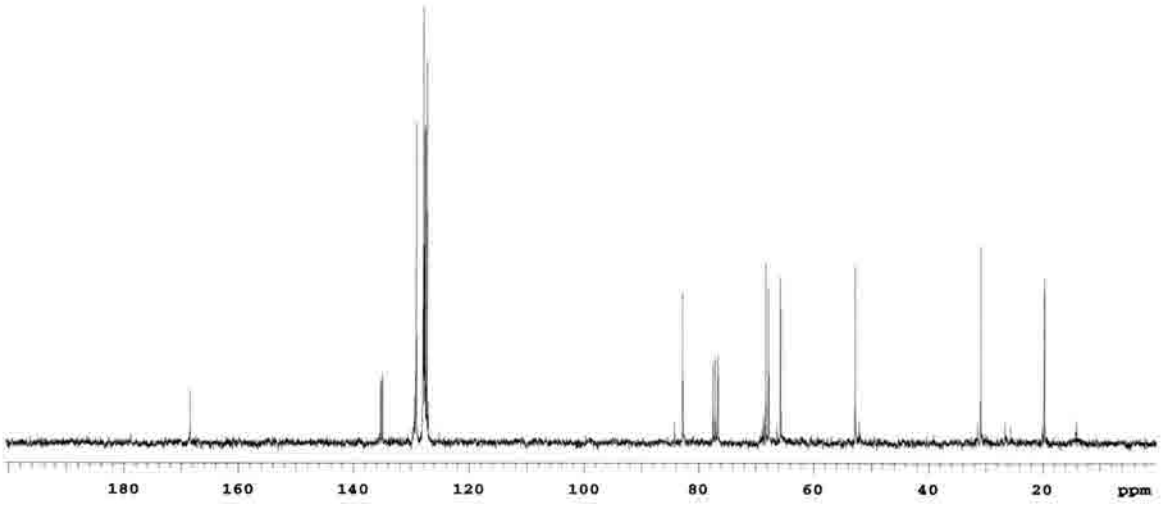
(3R,4S,9R,9aS)- hexahydro-9-hydroxy-3,4-diphenyl-pyrido[2,1-c][1,4]oxazin-1(6H)-one [(+)-646].

To an argon degassed solution of a mixture of **625** and *epi*-**625** (1.06g, 2.2 mmol) in CH_2Cl_2 (65 mL) was added 5% Pd/C (938 mg, 0.44 mmol, 0.2 eq). Hydrogen was bubbled through this mixture for 20 min. A hydrogen balloon was attached and the mixture was stirred for 5 h. The mixture was purged with Ar and filtered through Celite. The solvent was removed and the crude product was purified via flash chromatography (20% EtOAc/hex) to afford 123 mg **646** (17%) as a white solid and 403 mg (-)-**628** (60%) as a white foam. **646:** TLC (30% EtOAc/hex) R_f = 0.20 (UV and PMA). ^{1}H-NMR (300MHz, $CDCl_3$ vs TMS) δ 1.60 (2H, m); 1.97 (3H, m); 2.97 (2H, m); 3.37 (1H, s); 4.09 (1H, d, *J* = 4.2 Hz); 4.72 (1H, s, broad); 5.50 (1H, d, *J* = 4.5 Hz); 6.83 (2H, d, *J* = 6.9 Hz); 6.96 (2H, d, *J* = 6.9 Hz); 7.18 (6H, m). ^{13}C-NMR (75 MHz) δ 19.68, 30.87, 52.78, 65.67, 67.72, 68.22, 82.77, 127.31, 127.52, 127.63, 127.83, 127.96, 129.17, 134.99, 135.33, 168.49. IR (NaCl, neat) 3466, 2933, 1723, 1456, 1349, 1047 cm^{-1}. HRMS (FAB) calcd. for $C_{20}H_{22}NO_3$ (MH^+): 324.1600. found: 324.1583. $[\alpha]_D^{20}$ = +136 (c = 1.0 $CHCl_3$).



(+)-**646**



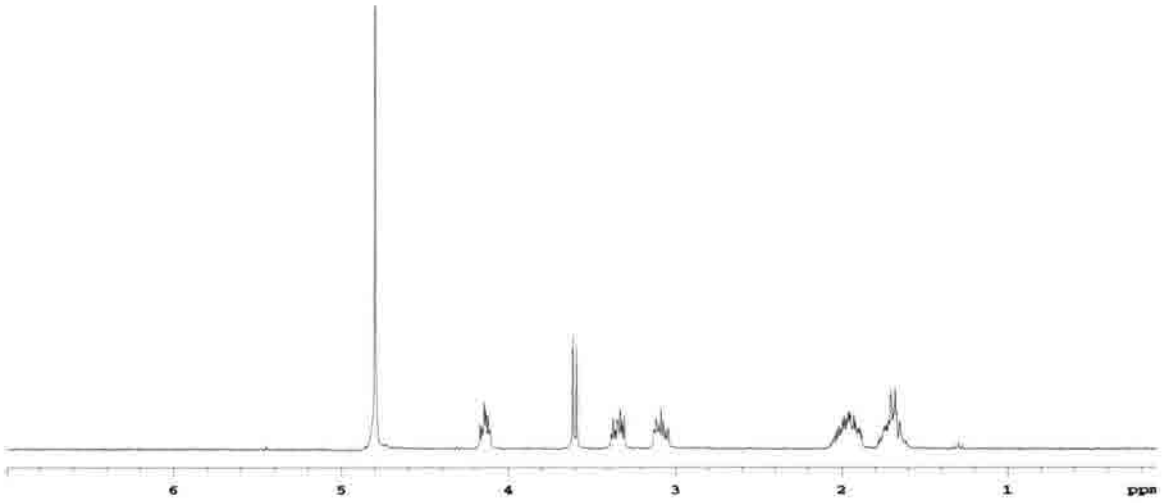


Compound **646**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$

628 (-)-584

(2R,3R)-β-Hydroxypipecolic acid [(-)-584]

To an Ar degassed solution of (+)-**628** (41mg, 0.13 mmol) in 1:1 EtOH/THF (2 mL) in a pressure tube was added $PdCl_2$ (4.4 mg, 0.025 mmol, 0.2 eq). This mixture was pressurized to 60 psi with H_2 and stirred for 16 h. The pressure was released and the mixture was degassed with Ar. The mixture was filtered through Celite and the solvent was removed. The crude product was triturated with Et_2O and the remaining product was dissolved in ddH_2O and run through a Dowex (50WX2-100) column (washed with ddH_2O then eluted with 2% NH_4OH). The NH_4OH fraction was lyophilized to afford 17 mg (-)-**584** (91%) as a white powder. ^{1}H-NMR (300 MHz, D_2O) δ 1.55 (2H, m), 1.81 (2H, m), 2.91 (1H, m), 3.18 (1H, m), 3.44 (1H, d, J = 6.96 Hz), 3.97 (1H, m). HRMS (FAB) calcd. for $C_6H_{12}NO_3$ (MH^+): 146.0817, found 146.0822. The optical purity of the amino acids (+)-**584** and (-)-**584** were determined to be > 99.5 : 0.5 er by chiral HPLC analysis (Daicel Chiral Pak WH, column temperature 40 °C, 0.25 mM $CuSO_4$, Waters 600 HPLC, UV detector 210 nm).



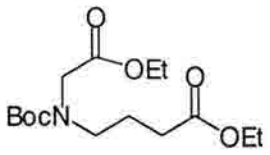
Compound **584**: ^{1}H-NMR (300 MHz) in D_2O.

642

4-(*tert*-Butoxycarbonyl-ethoxycarbonylmethyl-amino)-butyric acid ethyl ester (642).

Glycine ethyl ester hydrochloride (8.34 g 60 mmol) was dissolved in a 10% Na_2CO_3 solution (64 mL, 60 mmol). This solution was extracted with CH_2Cl_2 (3x). The combined organic layers were dried over $MgSO_4$ and the solvent was removed. A solution of ethyl bromobutyrate (4.29 mL, 30 mmol) in acetone (50 mL) was treated with NaI (13.5 g, 60mmol), this solution was allowed to stir for 1.5 h at room temperature. The acetone was removed and the solid taken up in water. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$ and the solvent was removed. The glycine ethyl ester was dissolved in THF (150 mL) and K_2CO_3 (8.28 g, 60 mmol) was added followed by the iodobutyrate. This solution was then heated to reflux and stirred for 24 h. Water was added and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$ and the solvent was removed. The crude product was dissolved in $CHCl_3$ (120 mL). Di-*tert*-butyl dicarbonate (6.54 g, 30 mmol) was added followed by 5% NaOH (60 ml). This mixture was stirred at room temperature for 3 h. The mixture was extracted with CH_2Cl_2 (3x) and the combined organic layers were washed with dilute HCl and water, dried over $MgSO_4$, and concentrated. The crude product was purified via flash chromatography (gradient 1-3% MeOH/CH_2Cl_2) to afford 5.72 g **642** (60%) as a light yellow oil. TLC (5% MeOH/CH_2Cl_2) R_f = 0.65 (UV and PMA). ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ

1.26 (3H, t, J = 6.9 Hz); 1.28 (3H, t, J = 6.9 Hz); 1.54 (9H, s); 1.84 (2H, d, J = 7.5 Hz); 2.35 (2H, t, J = 6.9 Hz); 3.32 (2H, t, J = 6.9 Hz); 3.94 (2H, s); 4.13 (2H, q, J = 7.5 Hz); 4.19 (2H, q, J = 7.2 Hz). ^{13}C-NMR (d_6-DMSO, 120°C) δ 13.19, 22.78, 27.31, 27,51, 30.45, 46.82, 48.60, 58.87, 59.52, 78.61, 154.19, 168.94, 171.64. IR (NaCl, neat) 2979, 1733, 1696, 1367, 1050 cm^{-1}. HRMS (FAB) calc. for $C_{15}H_{28}NO_6$ (MH^+) 318.1916; found 318.1922.



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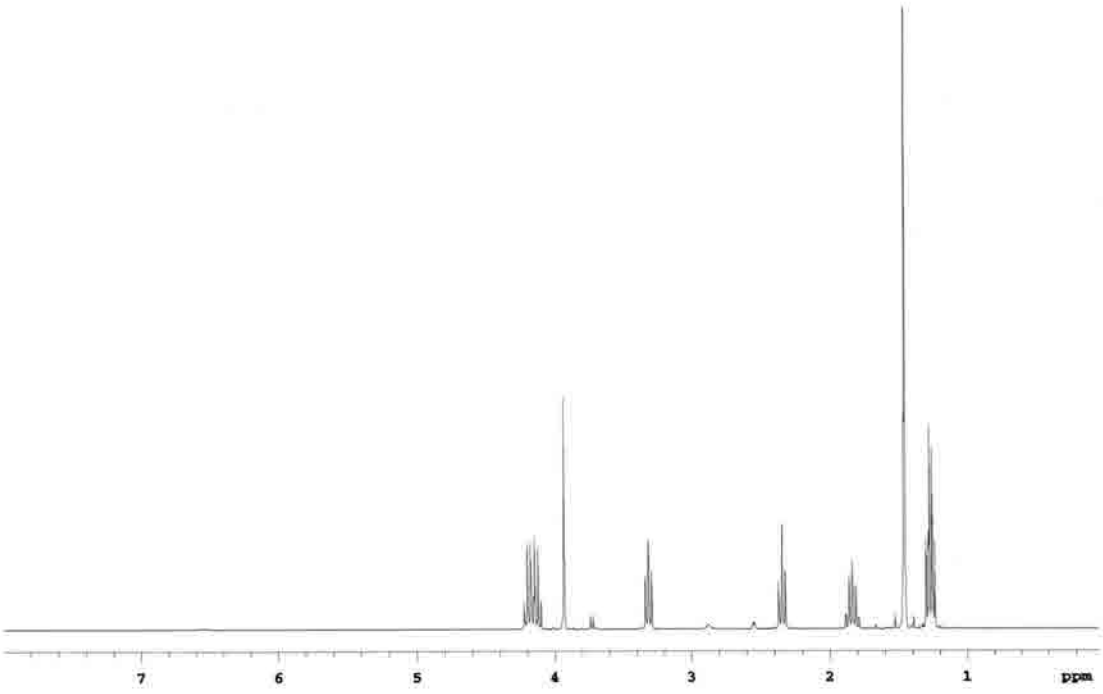
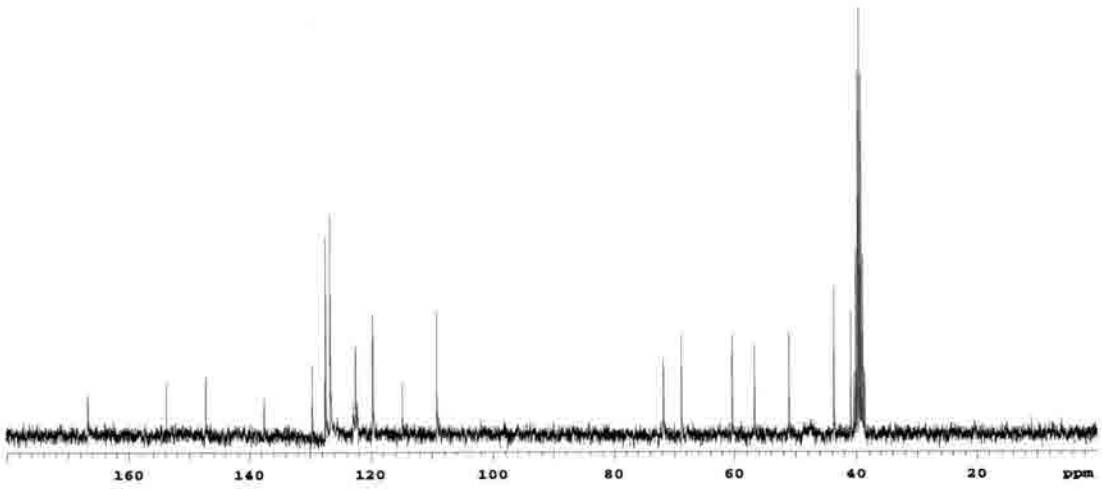


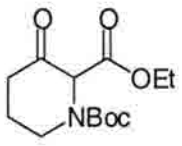


Compound **642**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

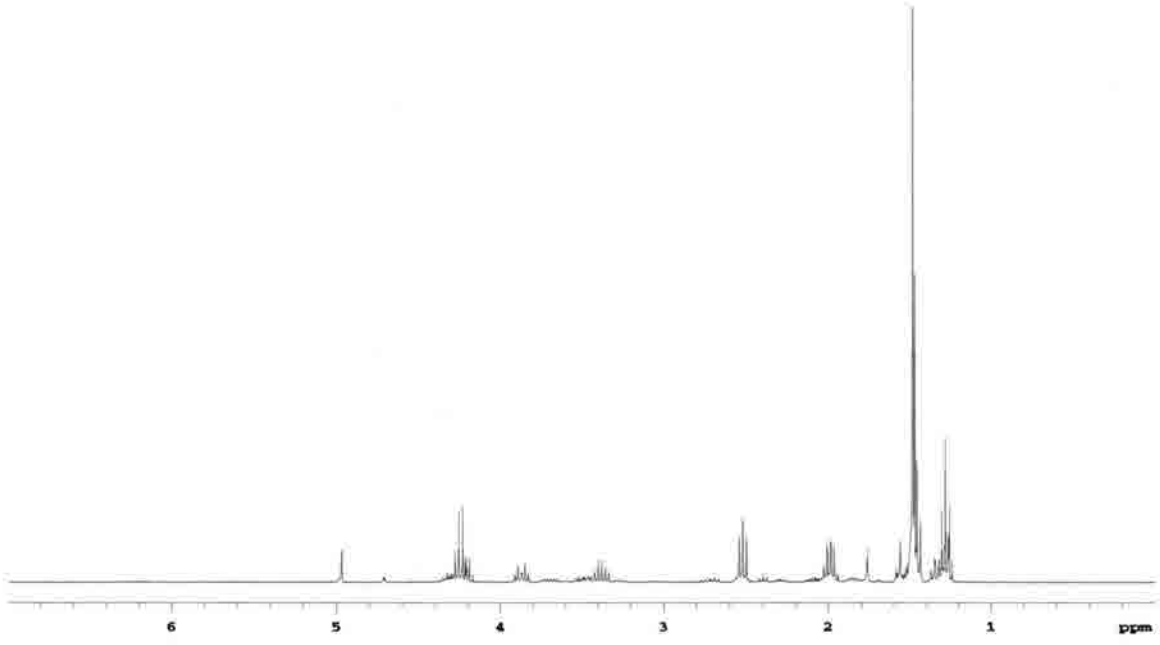
642 644

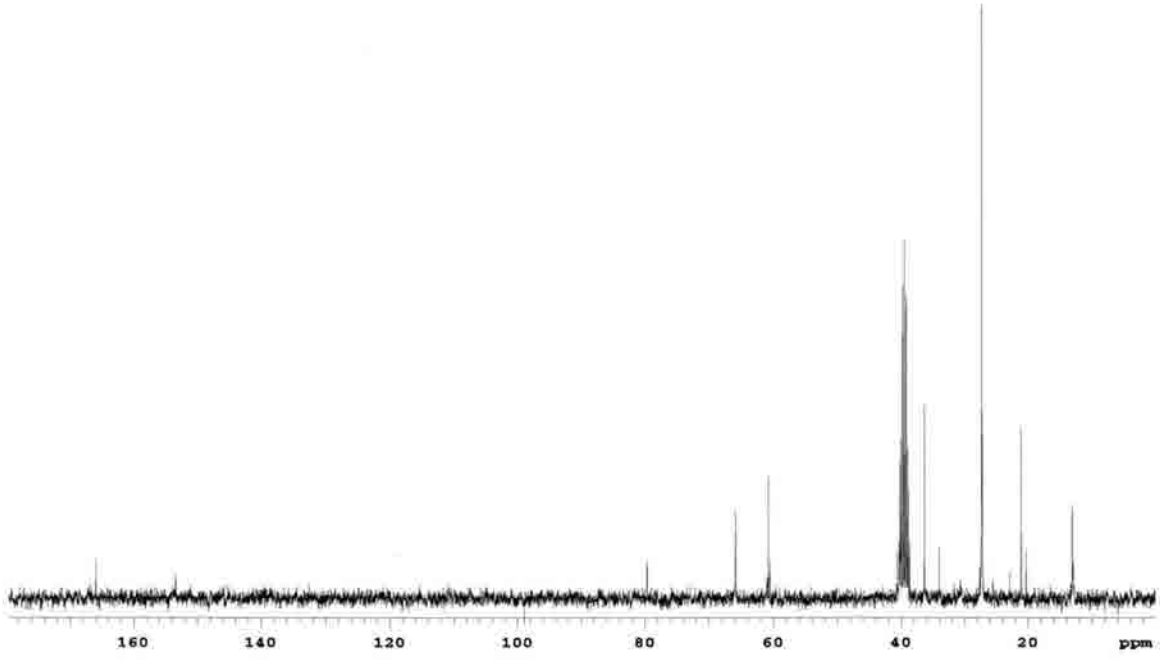
3-Oxo-piperidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-ethyl ester (644).

To a solution of **642** (5.7 g, 18 mmol) in toluene (100 mL) at 0°C was added a slurry of potassium t-butoxide (3.43 g, 31 mmol, 1.7 eq) in toluene (200 mL). This solution was allowed to stir in the ice bath 18 h (the reaction slowly came to room temperature). Water was added and the reaction extracted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$ and the solvent removed to yield 4.62 g **644** (95%). This material was pure by ^{1}H-NMR and GC analysis. TLC (40% EtOAc/hex) R_f = 0.40 (UV and PMA). ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.29 (3H, t, J = 6.9 Hz); 1.52 (9H, s); 2.01 (2H, t, J = 6.6 Hz); 2.52 (2H, t, J = 6.0 Hz); 3.40 (1H, m); 3.89 (1H, m); 4.23 (2H, q, J = 6.9 Hz); 4.97 (1H, s). ^{13}C-NMR (d_6-DMSO, 120°C) δ 13.06, 20.26, 21.05, 27.62, 36.34, 60.52, 60.80, 65.91, 79.64, 153.43, 165.96. IR (NaCl, neat) 3383, 2979, 1732, 1713, 1454, 1252, 1163 cm^{-1}. HRMS (FAB) calc. for $C_{13}H_{22}NO_5$ (MH^+) 270.1341; found 270.1342.



644



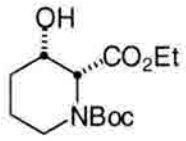


Compound **644**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

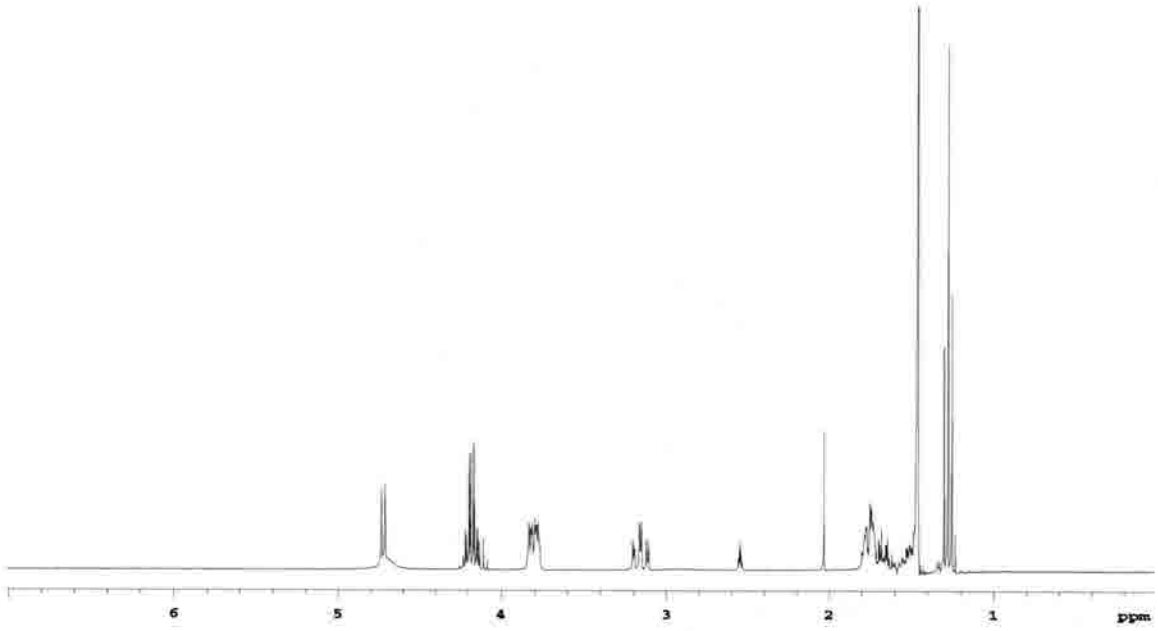
644 (+)-645

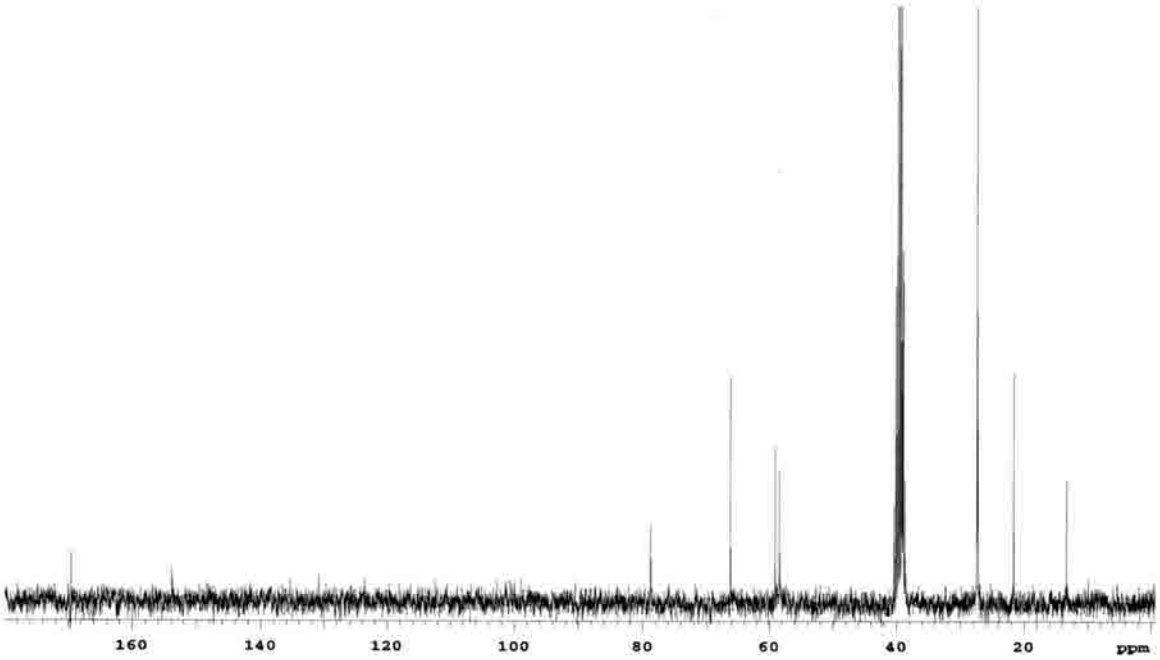
(2R,3S)—N(*tert*-butyloxycarbonyl)-2-carboethoxy-3-hydroxypiperidine [(+)-645].

To a suspension of **644** (1.00 g, 3.7 mmol) in tap water (85 mL) at 30°C was added sucrose (15.3 g) and Baker's yeast (10.3 g). This mixture was allowed to stir at 30°C for 48 h. The mixture was filtered through Celite and the filtrate was washed with water. The water was saturated with NaCl and extracted with EtOAc (5x). The combined organic layers were dried over $MgSO_4$ and the solvent removed. The crude product was purified via flash chromatography (1% MeOH/CH_2Cl_2) to yield 740 mg **645** (73%). TLC (50% EtOAc/hex) R_f = 0.42 (UV and PMA). ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.25 (3H, t, J = 6.3 Hz); 1.73 (3H, m); 2.00 (9H, s); 3.16 (2H, ddd, J = 6.9, 3.3, 3.3 Hz); 3.75 (2H, m); 4.17 (2H, m); 4.72 (2H, d, J = 6.3 Hz). ^{13}C-NMR (d_6-DMSO, 120°C) δ 13.32, 21.64, 27.26, 27.39, 30.09, 58.40, 59.10, 66.19, 78.74, 153.88, 169.54. IR (NaCl, neat) 3452, 2949, 1703, 1682, 1450, 1427, 1257, 1074, 1042 cm^{-1}. HRMS (FAB) calc. for $C_{13}H_{24}NO_5$ (MH^+) 274.1654; found 274.1665. $[\alpha]_D^{20}$ = +33.5 (c = 1.07, $CHCl_3$).



645



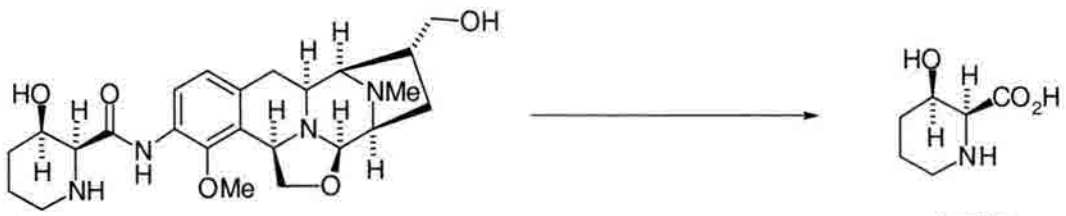


Compound **645**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

645 (+)-**585**

(2S,3R)-3-hydroxypipecolic acid [(+)-585].

To a soluton of **645** (39 mg, 0.14 mmol) in CH_2Cl_2 (1 mL) was added TFA (0.25 mL). This solution was stirred at room temperature for 3 h. The solvent was removed *in vacuo* and the crude product was dissolved in 5:1 EtOH/H_2O (0.6 mL). Postassium hydroxide (24 mg, 0.43 mmol, 3 eq.) was added and this solution was stirred at room temperature for 10 h. The solution was adjusted to pH 7 and the crude product was purified via ion exchange chromatography (Dowex 50WX2-100, washed with ddH_2O then eluted with 2% NH_4OH). The NH_4OH fraction was lyophilized to afford 17 mg (+)-**585** (81%) as a white powder. ^{1}H-NMR (300 MHz, D_2O) δ 1.85 (2H, m), 2.12 (2H, m), 3.07 (1H, ddd, J= 12.9, 3.6, 2.7 Hz), 3.47(1H, ddd, J= 12.6, 2.1, 1.8 Hz), 3.75 (1H, d, J = 1.5 Hz), 4.57 (1H, s, broad). ^{13}C-NMR (75.47 MHz vs. d_4 MeOD) δ 16.89, 29.72, 44.56, 63.20, 65.04, 173.06. HRMS (FAB) calcd. for $C_6H_{12}NO_3$ (MH^+) 146.0817 found: 146.0821. $[\alpha]_D^{20} = +82.1^O$ (c=0.12, 1M HCl).



566, Tetrazomine

(-)-**585**

(2R,3S)-3-hydroxypipecolic acid [(-)-585].

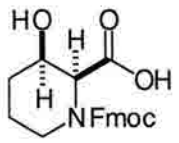
Tetrazomine(2HCl) (7.3 mg, 0.015 mmol) was dissolved in 4 M HCl (750 μL). This solution was heated to 80°C for 20 h. The solvent was removed and the crude product was redissolved in ddH_2O and desalted by ion exchange chromatography (Dowex 50WX2-100, washed with ddH_2O then eluted with 2% NH_4OH). The semi crude product was purified via HPLC (Waters Resolve C_{18}, 5% MeOH/H_2O, isocratic, UV 210 nm) to afford 1.2 mg (-)-**585** as a white powder. All spectroscopic data matched (+)-**585.** (-)-**562** $[\alpha]_D^{20}$ = -72.0 (c=0.10, 1M HCl).

649 (±)-650

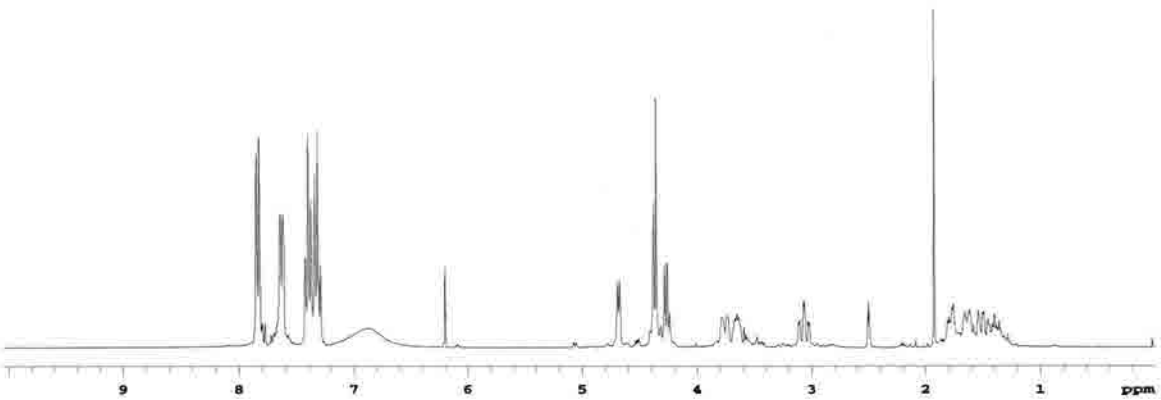
***cis*-3-Hydroxy-1-(9H-fluoren-9-ylmethyl)ester-2-piperidinecarboxylic acid (650).**

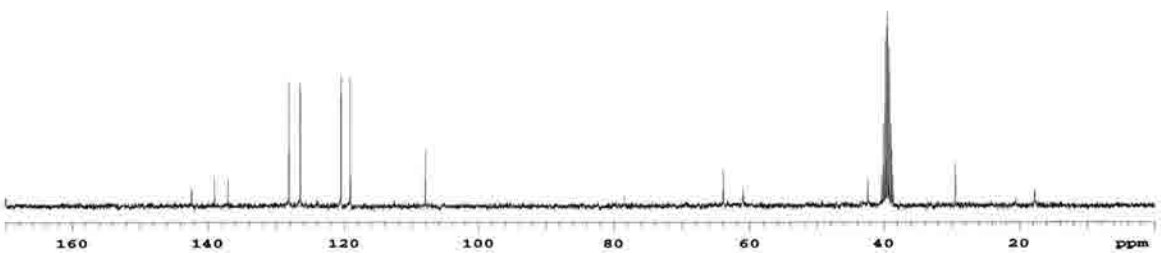
A solution of picolinic acid (**649**) (1.00 g, 7.2 mmol) in 15 mL ddH_2O in a pressure vessel was degassed with Ar for 10 min. Ammonium hydroxide (2 mL) was added along with 5% Rh/C (400 mg). The vessel was sealed and purged with H_2 (3x). The vessel was then pressurized to 80 psi with H_2. This mixture was stirred for 72 h. The pressure was released and the mixture was degassed with Ar. The catalyst was filtered through Celite and the solvent was removed *in vacuo*. The crude product was dissolved in 1:1 dioxane/H_2O (30 mL). The solution was then cooled to 0°C. Sodium carbonate(1.91 g, 18.0 mmol, 2.5 eq.) was added followed by FmocCl (1.86g, 7.2 mmol). This solution was stirred at 0°C for 3 h then warmed to room temp. and stirred for 16 hours. The solution was adjusted to pH 1 with 6N HCl and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, and the solvent was removed. The crude material was purified via flash chromatography (gradient 2.5/97.5/0 to 10/88/2 MeOH/CH_2Cl_2/AcOH) to afford 1.45 g of **650** (55%) as a clear oil. ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.35-1.80 (4H, m); 1.93 (1H, s); 3.07 (1H, ddd, J = 12.9, 12.9, 3.3 Hz); 3.66 (1H, m); 3.76 (1H, m); 4.26 (1H, d, J = 6.3 Hz); 4.37 (2H, d, J = 5.7 Hz); 4.68 (1H, d, J = 5.7 Hz); 7.32 (2H, t, J = 7.5 Hz); 7.40 (2H, t, J = 7.5 Hz); 7.63 (2H, t, J = 7.5 Hz); 7.84 (2H, d, J = 7.5 Hz). ^{13}C-NMR (d_6-DMSO, 120°C) δ 17.75, 29.43, 42.44, 60.96, 63.90, 78.43, 107.94, 119.11, 120.48, 126.48, 128.15, 137.06, 139.03, 142.36, 169.88. IR (NaCl, neat) 3452, 3064, 1703, 1681,

1450, 1427, 1257, 1074, 1041 cm^{-1}. HRMS (FAB) calc. for $C_{21}H_{22}NO_5$ (MH^+) 368.1498; found 368.1502.



(±)-**650**



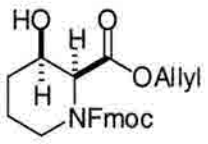


Compound **650**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

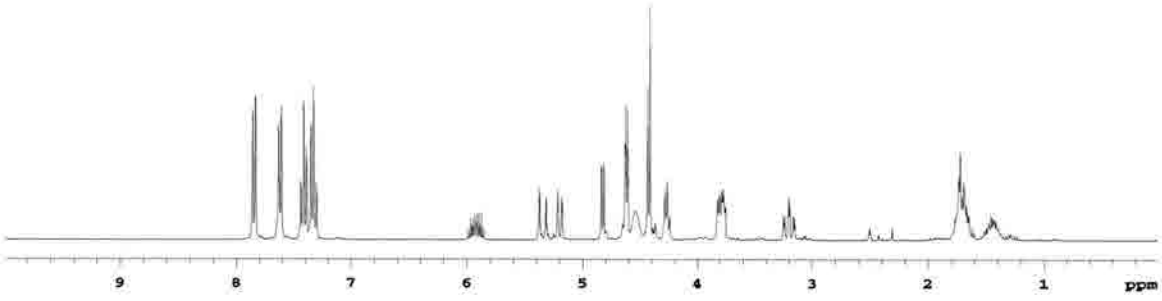
(±)-**650** (±)-**651**

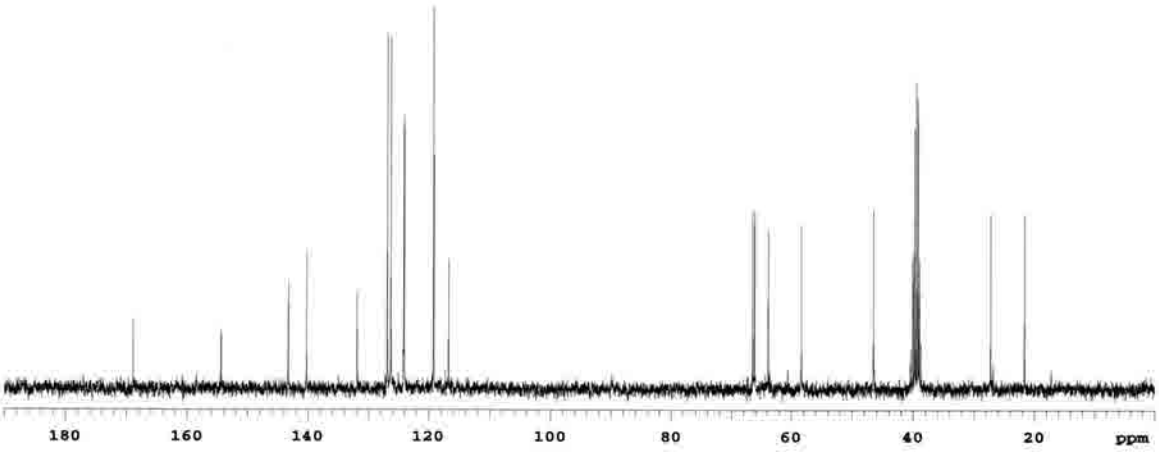
***cis*-3-Hydroxy-1-(9H-fluoren-9-ylmethyl)ester-2-piperidinecarboxylic acid allyl ester (651).**

To a solution of **650** (1.10g, 3.0 mmol) in benzene (30 mL) in a round bottomed flask fitted with a Dean Stark trap was added $TsOH{\cdot}H_2O$(571 mg, 3.0 mmol, 1.0 eq.) and allyl alcohol (2.1 mL, 30.0 mmol, 10 eq.). This solution was heated to reflux and stirred for 16 h. The solution was cooled to room temp and the solvent was removed *in vacuo*. The crude product was partitioned in EtOAc/$NaHCO_3$. The aqueous layer was then extracted with EtOAc (3x). The combined organic layers were washed with brine and dried over Na_2SO_4. The mixture was filtered and the solution concentrated. The crude product was purified via flash chromatography (gradient 30-50% EtOAc/hex) to afford 830 mg **651** (68%) as a clear oil. ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.44 (1H, m); 1.71 (3H, m); 3.20 (1H, ddd, J = 12.3, 12.3, 3.3 Hz); 3.78 (2H, m); 4.27 (1H, dd, J = 6.0 Hz); 4.42 (2H, d, J = 6.6 Hz); 4.54 (1H, s, broad); 4.62 (2H, m); 4.82 (1H, d, J = 7.2 Hz); 5.21 (1H, dd, J = 10.2, 1.2 Hz); 5.35 (1H, dd, J = 17.1, 1.8 Hz); 5.91 (1H, m); 7.33 (2H, t, J = 7.5 Hz); 7.41 (2H, t, J = 7.5 Hz); 7.62 (2H, d, J = 7.5 Hz); 7.84 (2H, d, J = 7.5 Hz). ^{13}C-NMR (d_6-DMSO, 120°C) δ 21.60, 27.21, 46.47, 58.33, 63.83, 66.10, 66.44, 116.75, 119.23, 124.12, 126.29, 126.87, 131.85, 140.26, 143.26, 154.41, 168.89 Note: One carbon signal is not observed. IR (NaCl, neat) . HRMS (FAB) calc. for $C_{24}H_{26}NO_5$ (MH^+) 408.1811; found 408.1820.

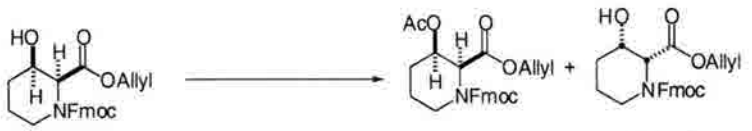


651





Compound **651**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

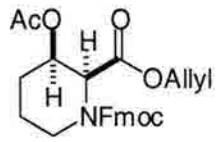


(±)-**651** (+)-**652** (+)-**651**

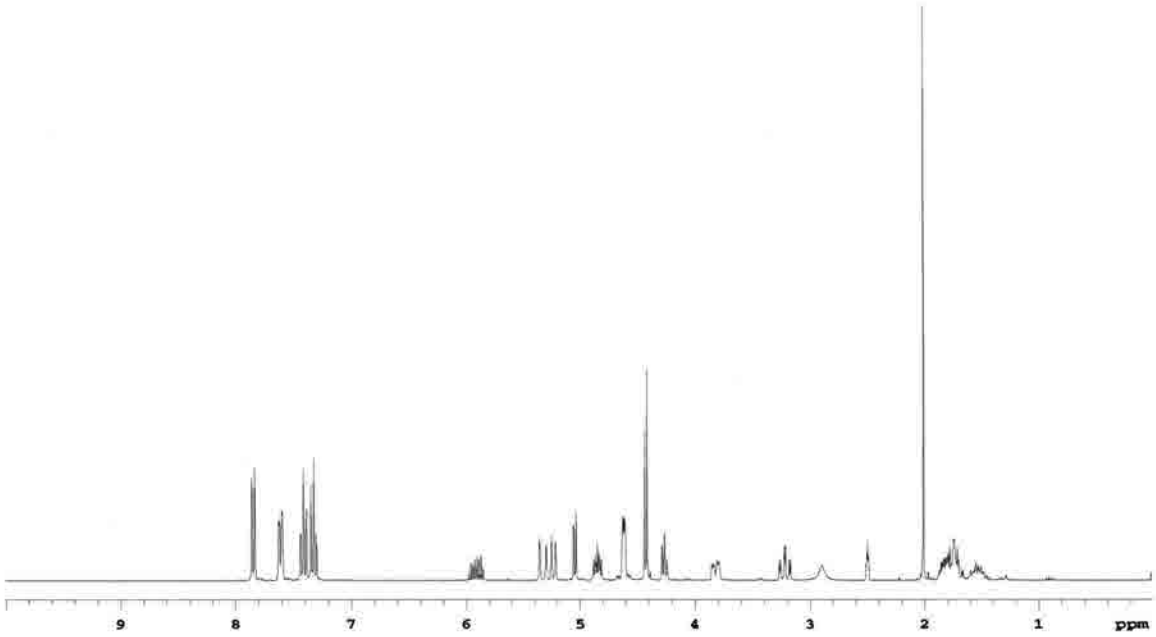
(2R,3S)-3-acetoxy-1-(9H-fluoren-9-ylmethyl)ester-2-piperidinecarboxylic acid allyl ester [(+)-652].

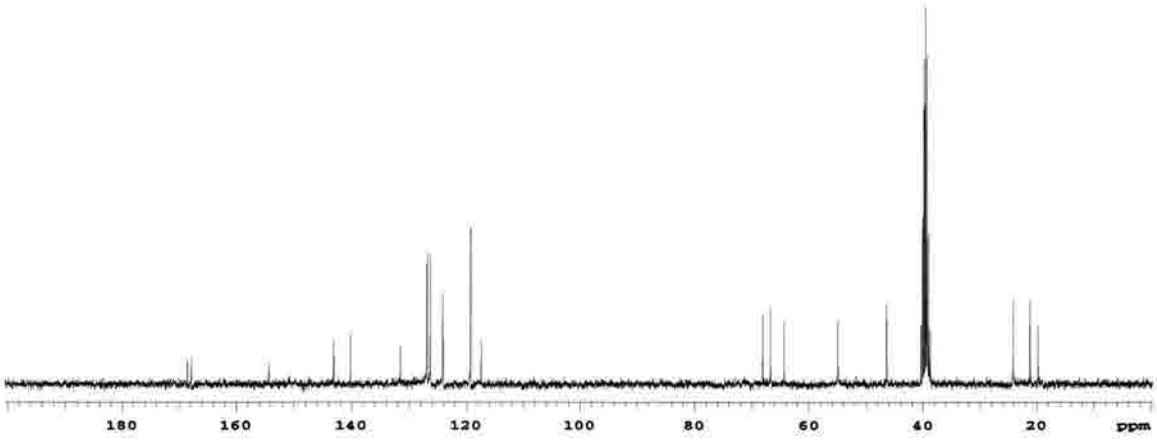
(2S,3R)- 3-hydroxy-1-(9H-fluoren-9-ylmethyl)ester-2-piperidinecarboxylic acid allyl ester [(+)-651].

To a solution of (±)-**651** (380 mg, 0.93 mmol) in iPr_2O (20 mL) was added lipase PS (Amano) (1.52g). This mixture was stirred at 30°C until HPLC (2 mL/min, gradient 90/10 3 min initial to 70/30 hex/EtOAc over 5 min. NovaPak HP silica column, UV 254 nm) showed 50% conversion to the acetate (ca 3.5 days). The mixture was filtered through celite and the filtrate was washed with iPr_2O. The solvent was removed *in vacuo* and the crude material was purified via flash chromatography (gradient 25-50% EtOAc/hex) to afford 191 mg (+)-**652** (46%) as a white foam followed by 165 mg (+)-**651** (43%) as a white solid. (+)-**652**: ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.53 (1H, m); 1.78 (3H, m); 2.10 (3H, s); 3.22 (1H, ddd, J = 12.9, 12.9, 3.6 Hz); 3.82 (1H, dd, J = 12.9, 5.1 Hz); 4.27 (1H, t, J = 6.3 Hz); 4.42 (2H, dd, J = 17.3, 1.5 Hz); 4.63 (2H, m); 5.04 (1H, d, J = 6.3 Hz); 5.24 (1H, dd, J = 11.7, 1.5 Hz); 5.33 (1H, dd, J = 17.3, 1.5 Hz); 5.91 (1H, m); 7.32 (2H, t, J = 7.5 Hz); 7.41 (2H, t, J = 7.5 Hz); 7.61 (2H, d, J = 7.5 Hz); 7.84 (2H, d, J = 7.5 Hz). ^{13}C-NMR (d_6-DMSO, 120°C) δ 19.74, 21,15, 24.13, 46.36, 54.91, 64.34, 66.66, 68.05, 117.36, 119.23, 124.08, 124.14, 126.30, 126.87, 131.49, 140.22, 143.15, 154.31, 167.82, 168.54. IR (NaCl, neat) 2950, 1741, 1705, 1450, 1421, 1234, 1045 cm^{-1}. HRMS (FAB) calc. for $C_{26}H_{28}NO_6$ (MH^+) 450.1917; found 450.1916.



(+)-**652**





Compound **652**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

(+)-**652** (-)-**651**

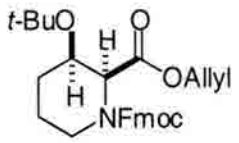
(2R,3S)- 3-hydroxy-1-(9H-fluoren-9-ylmethyl)ester-2-piperidinecarboxylic acid allyl ester [(-)-651].

To a solution of (+)-**652** (265 mg, 0.59 mmol) in allyl alcohol (10 mL) was added H_2SO_4 (5 drops). This solution was heated to 80°C for 15 hours. The solvent was removed *in vacuo* and the crude material was partitioned in EtOAc/$NaHCO_3$(aq.). The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. The solution was concentrated and the crude product was purified via flash chromatography (30% EtOAc/hex) to afford 219 mg (-)-**651** (91%) as a white solid. For spectral data see (+)-**651**. (-)-**651**: $[\alpha]_D^{20} = -33.6$ (c=1.5 CH_2Cl_2).

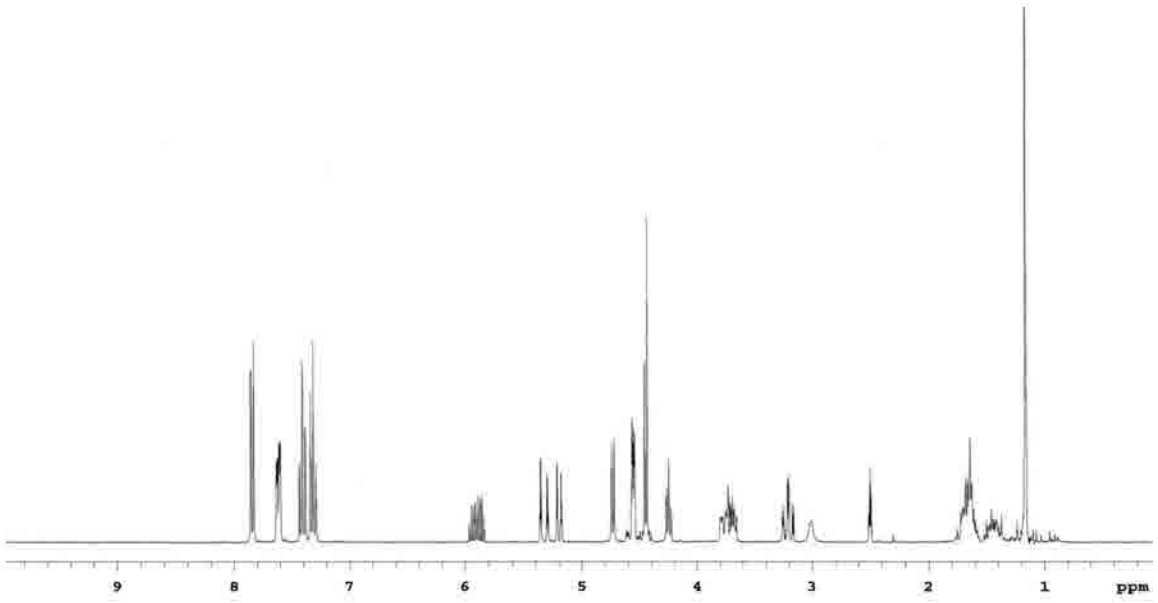
(-)-**651** (+)-**724**

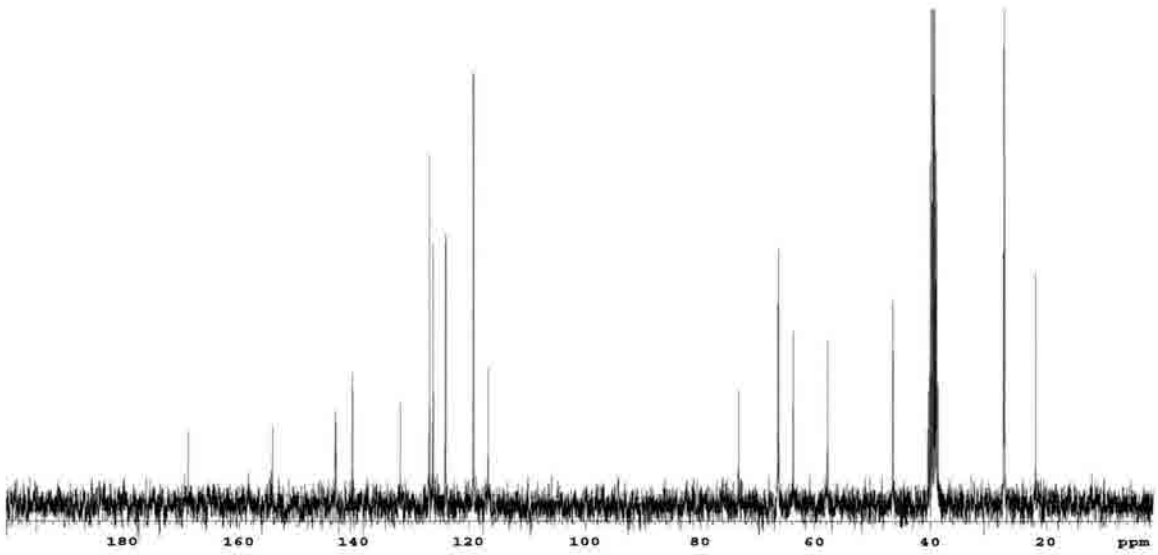
(2S,3R)- 3-*tert*-butoxy-1-(9H-fluoren-9-ylmethyl)ester-2-piperidinecarboxylic acid allyl ester [(+)-724].

To a slurry of (-)-**651** (145 mg, 0.36 mmol) and Amberlyst 15 (120 mg) in hexanes (8 mL) in an open tube was bubbled isobutene for 10 min. The tube was sealed and the mixture stirred for 48 h. The mixture was filtered through Celite and the filtrate was washed with hexanes. The solvent was removed *in vacuo* and the crude oil was purified via flash chromatography (10% EtOAc/hex) to afford 142 mg (+)-**724**(86%) as a clear oil. ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.17 (9H, s); 1.45 (1H, m); 1.67 (3H, m); 3.01 (1H, s, broad); 3.21 (1H, ddd, *J* = 12.6, 3.3, 3.3 Hz); 3.71 (2H, m); 4.25 (1H, t, *J* = 6.3 Hz); 4.44 (2H, d, *J* = 6.0 Hz); 4.55 (2H, m); 4.73 (1H, d, *J* = 6.6 Hz); 5.19 (1H, m); 5.90 (1H, m); 7.32 (2H, t, *J* = 7.5 Hz); 7.41 (2H, t, *J* = 7.5 Hz); 7.62 (2H, dd, *J* = 7.5, 3.3 Hz); 7.84 (2H, d, *J* = 7.5 Hz). ^{13}C-NMR (d_6-DMSO, 120°C) δ 21.80, 27.17, 27.31, 46.47, 57.72, 63.69, 66.24, 66.37, 73.26, 116.71, 119.22, 124.02, 126.21, 126.24, 126.82, 131.85, 140.27, 143.24, 154.23, 168.75. IR(NaCl, neat) 2972, 1734, 1701, 1265, 1210, 1049 cm^{-1}. HRMS (FAB) calc. for $C_{28}H_{34}NO_5$ (MH^+) 464.2437; found 464.2437. $[\alpha]_D^{20}$ = +5.5 (c=0.55 $CHCl_3$).



724



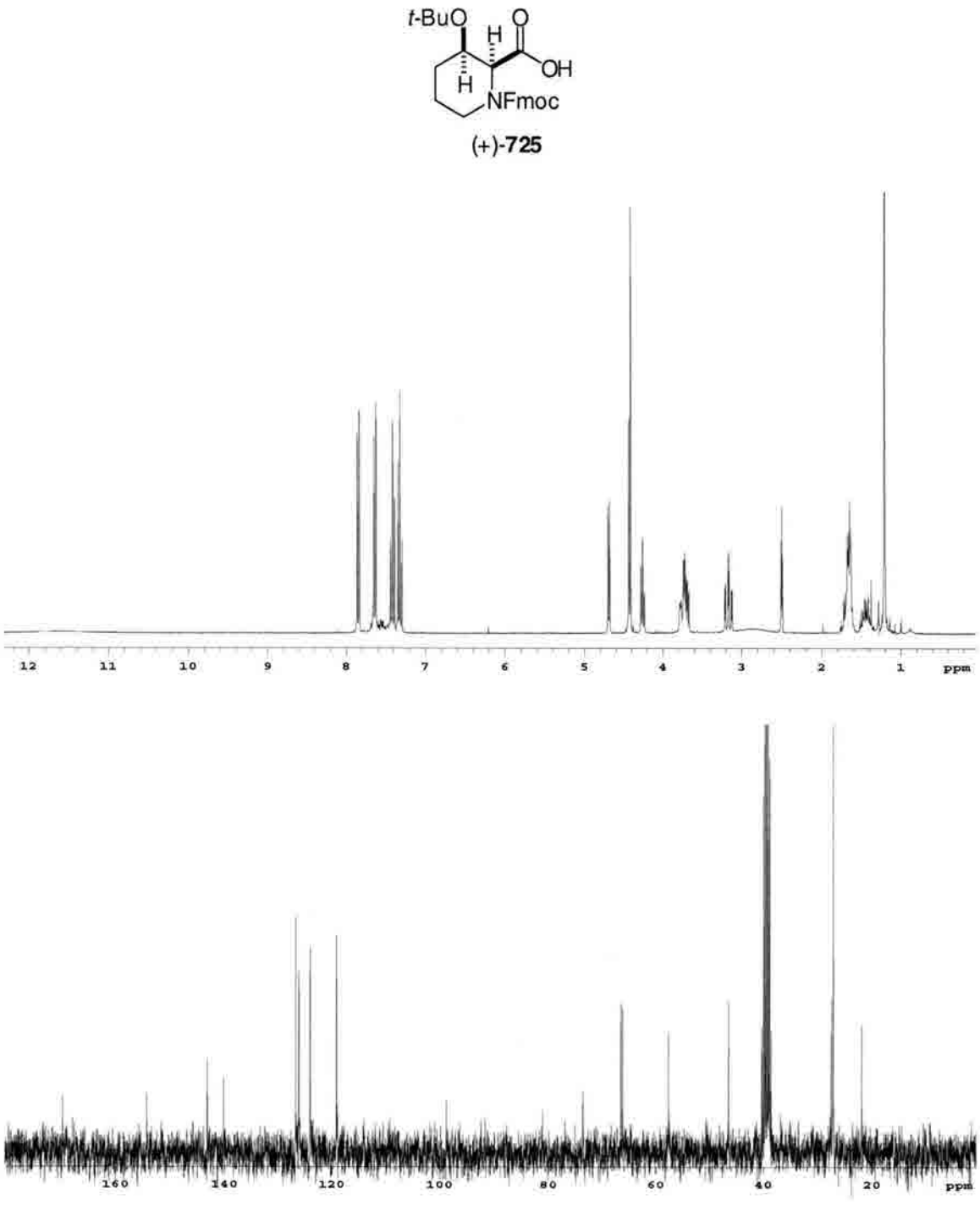


Compound **724**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

724 (+)-**725**

(2S,3R)- 3-*tert*-butoxy-1-(9H-fluoren-9-ylmethyl)ester-2-piperidinecarboxylic acid [(+)-725].

To a solution of (+)-**725** (142 mg, 0.32 mmol) and palladium tetrakis triphenyl phosphine (29 mg, 0.025 mmol, 0.08 eq.) in CH_2Cl_2 (8 mL) was added hydroxymethanesulfinic acid sodium salt (53 mg, 0.35 mmol, 1.1 eq.) in MeOH (4 mL) and the solution was stirred for 3 h. Hydrochloric acid (1 M) was added to adjust to pH 2 and the mixture was extracted with EtOAc (3x). The combined organic layers were washed with brine and dried over $MgSO_4$ and concentrated. The crude product was purified via flash chromatography (gradient 1-2.5% MeOH/CH_2Cl_2) to afford 123 mg (+)-**725** (95%) as a white foam. ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.21 (9H, s); 1.44 (1H, m); 1.66 (3H, m); 3.17 (1H, ddd, J = 12.3, 3.3, 3.3 Hz); 3.72 (2H, m); 4.26 (1H, t, J = 6.3 Hz); 4.42 (2H, d, J = 5.7 Hz); 4.68 (1H, d, J = 6.3 Hz); 7.32 (2H, t, J = 7.5 Hz); 7.41 (2H, t, J = 7.5 Hz); 7.63 (2H, d, J = 7.5 Hz); 7.84 (2H, t, J = 7.5 Hz); 11.6 (1H, s, broad). ^{13}C-NMR (d_6-DMSO, 120°C) δ 21.98, 27.22, 27.57, 46.48, 57.7, 66.29, 66.61, 73.69, 98.84, 119.22, 124.15, 126.26, 126.85, 140.25, 143.27, 154.35, 169.82. IR (NaCl, neat) 3066, 2971, 1768, 1701, 1420, 1150, 1059 cm^{-1}. HRMS (FAB) calc. for $C_{25}H_{30}NO_5$ (MH^+) 424.2124; found 424.2121. $[\alpha]_D^{20}$ = +19.1 (c=0.23 $CHCl_3$).

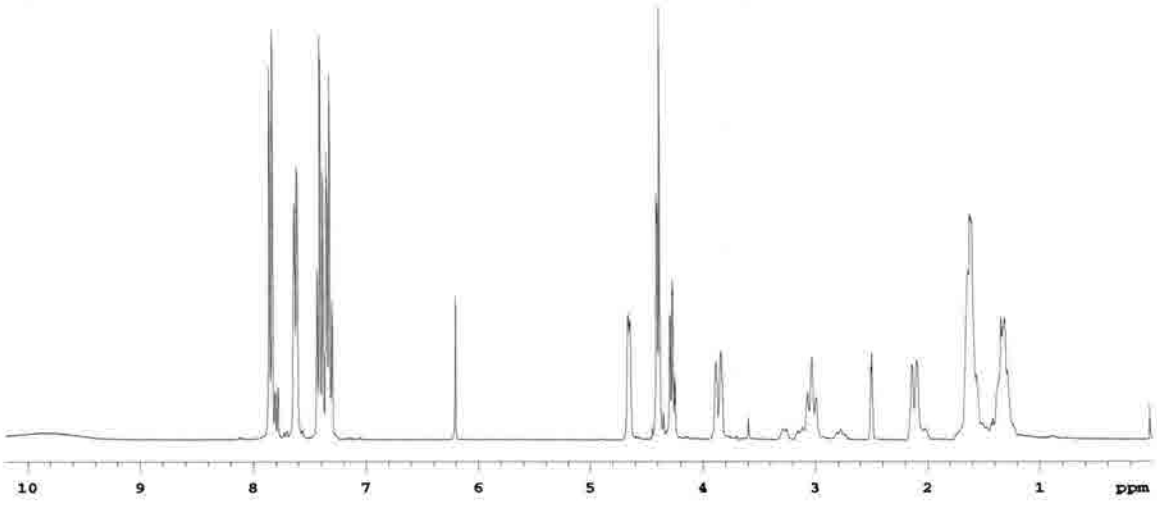


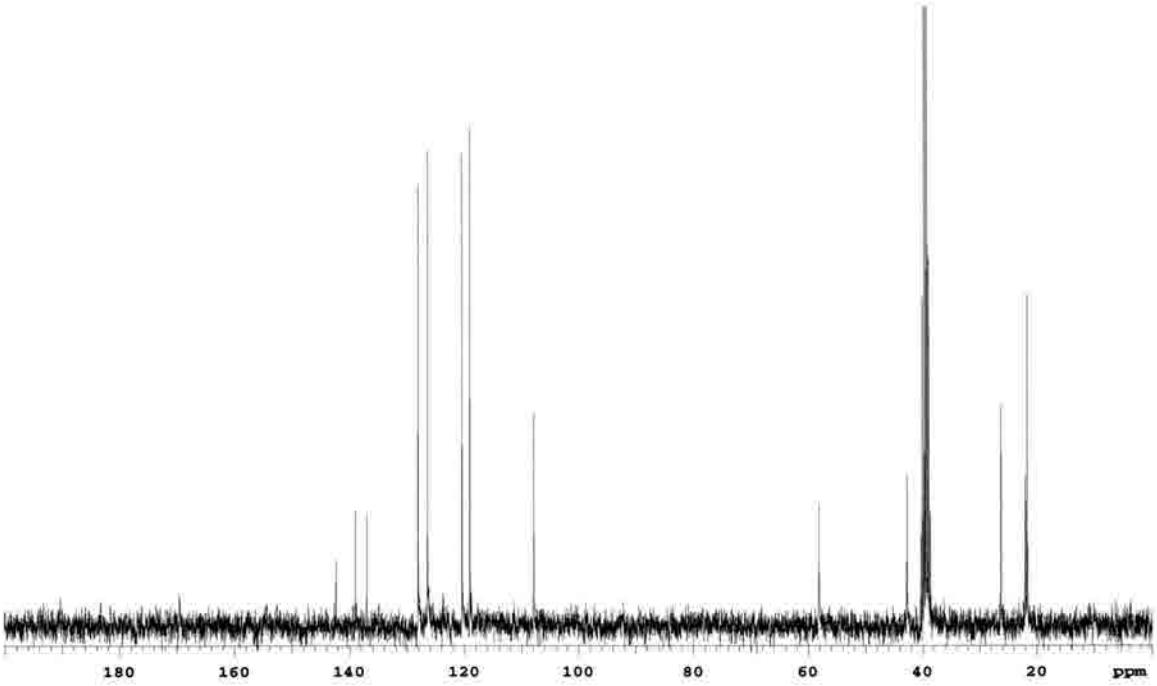
(+)-**725**

Compound **725**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

(2S)-N-Fmoc-pipecolic acid:

To a solution of L-pipecolic acid (Aldrich) (100 mg, 0.77 mmol, 1 eq.) in 1:1 H_2O:dioxane (10 mL) at 0°C was added Na_2CO_3 (122 mg, 1.16 mmol, 1.5 eq.) and fluorenylmethyl chloroformate (200 mg, 0.77 mmol, 1.0 eq). This mixture was allowed to slowly warm to rt over 16 h. The mixture was acidified to pH 2 using 1M HCl and the mixture was extracted with EtOAc (3x). The combined organic layers were washed with brine and dried over $MgSO_4$ and concentrated. The crude product was purified via flash chromatography (1-2.5-5% MeOH/CH_2Cl_2) to afford 192 mg N-Fmoc-pipecolic acid (71%) as a white foam. ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.34 (2H, m); 1.62 (3H, m); 2.12 (1H, d, *J* = 6.0 Hz); 3.03 (1H, t, *J* = 12.0 Hz); 3.87 (1H, d, *J* = 13.2 Hz); 4.27 (1H, m); 4.39 (2H, m); 4.65 (1H, d, *J* = 3.9 Hz); 7.32 (2H, t, *J* = 7.8 Hz); 7.41 (2H, t, *J* = 7.8 Hz); 7.62 (2H, d, *J* = 7.8 Hz); 7.84 (2H, d, *J* = 7.8 Hz); 9.85 (1H, s, broad). ^{13}C-NMR (d_6-DMSO, 120°C) δ 21.66, 21.80, 26.26, 42.79, 58.13, 107.90, 119.08, 120.45, 123.81, 126.45, 128.11, 137.04, 139.00, 142.35, 169.61. HRMS (FAB) calc. for $C_{21}H_{22}NO_4$ (MH^+) 352.1549, found: 352.1560. $[\alpha]_D^{25}$ –29 (c = 1.0, $CHCl_3$).





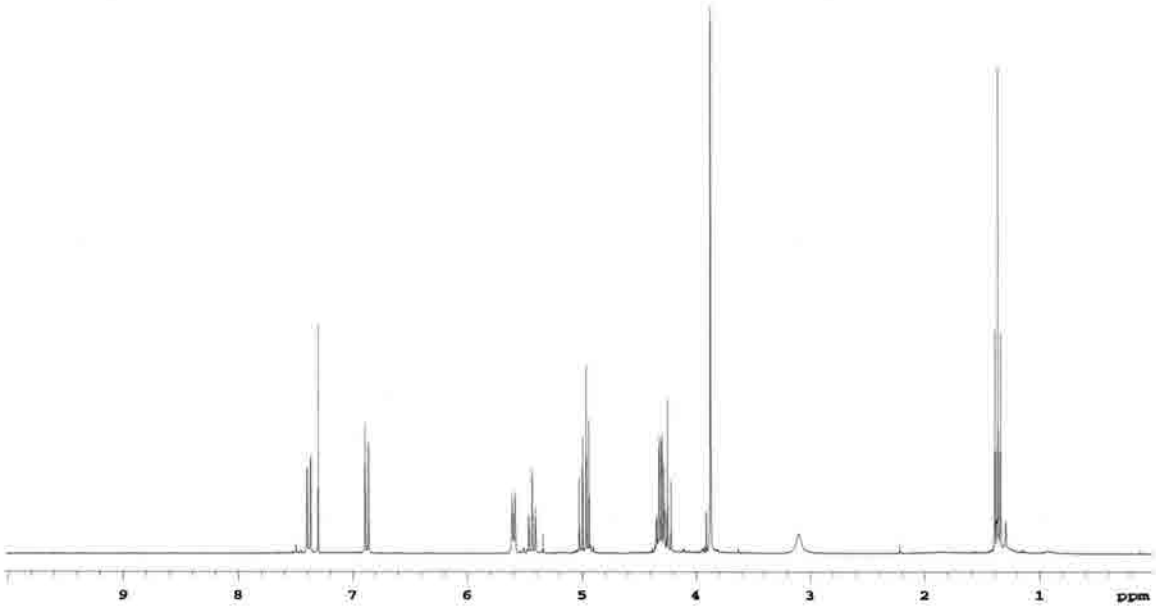
^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

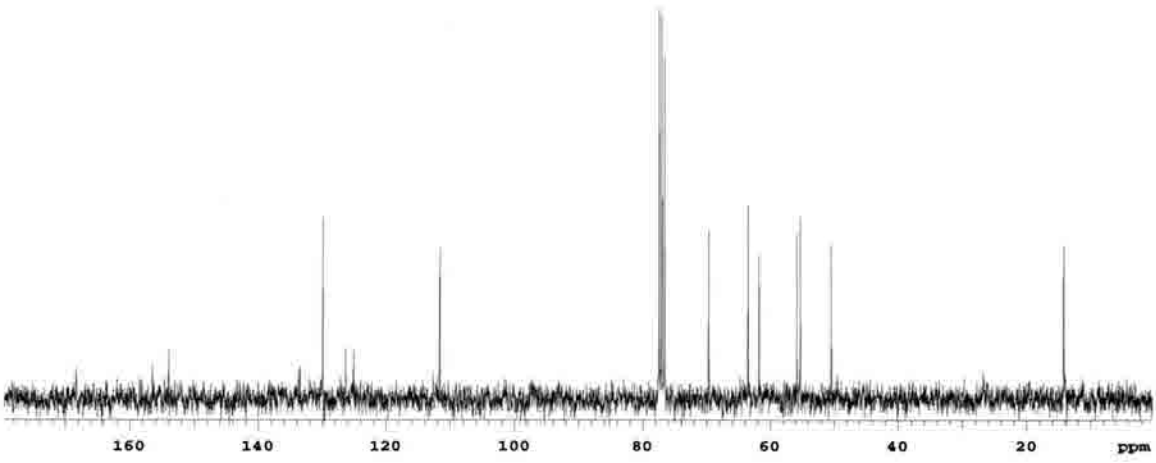
410 **655**

1,5,6,10b-tetrahydro-7-chloro-6-hydroxy-10-methoxy-3-oxo-3H-Oxazolo[4,3a]isoquinoline-5-carboxylic acid ethyl ester (655).

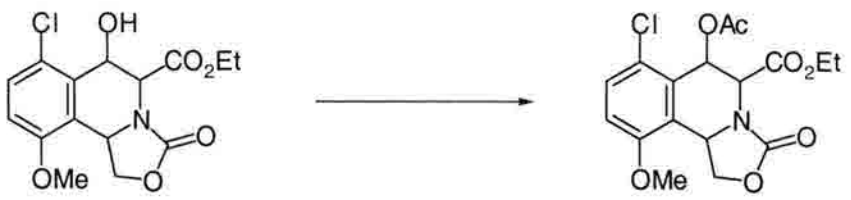
To a solution of **410** (920 mg, 3.0 mmol) in CH_2Cl_2 (15 mL) was added sulfuryl chloride (265 μL, 3.3 mmol, 1.1 eq.). This solution was allowed to stir at room temperature for 2 hours. The solution was poured into water and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated to afford 985 mg **655** (96%) as a white foam. The product was sutibly pure for continued use. TLC (50% EtOAc/hex) R_f = 0.33 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.27 (3H, t, *J* = 6.9 Hz); 3.05 (1H, d, *J* = 4.5 Hz, D_2O exch.); 3.81 (3H, s); 4.15-4.29 (3H, m); 4.98 (2H, m); 5.34 (1H, t, *J* = 8.7 Hz); 5.52 (1H, q, *J* = 4.5 Hz); 6.79 (1H, d, *J* = 8.9 Hz); 7.30 (1H, d, *J* = 8.9 Hz). ^{13}C-NMR (100 MHz) ($CDCl_3$) δ 14.12, 50.40, 55.17, 55.83, 61.74, 63.36, 69.66, 111.66, 125.25, 126.47, 130.07, 133.58, 154.14, 156.55, 168.46. IR (Neat, NaCl) 3398, 2937, 2843, 1738, 1584, 1060, 1027 cm^{-1}. HRMS (FAB) calcd. for $C_{15}H_{17}NO_6Cl$ 342.0744; found 342.0753.

655





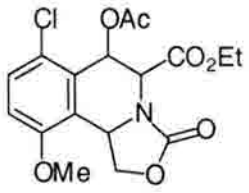
Compound **655**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$



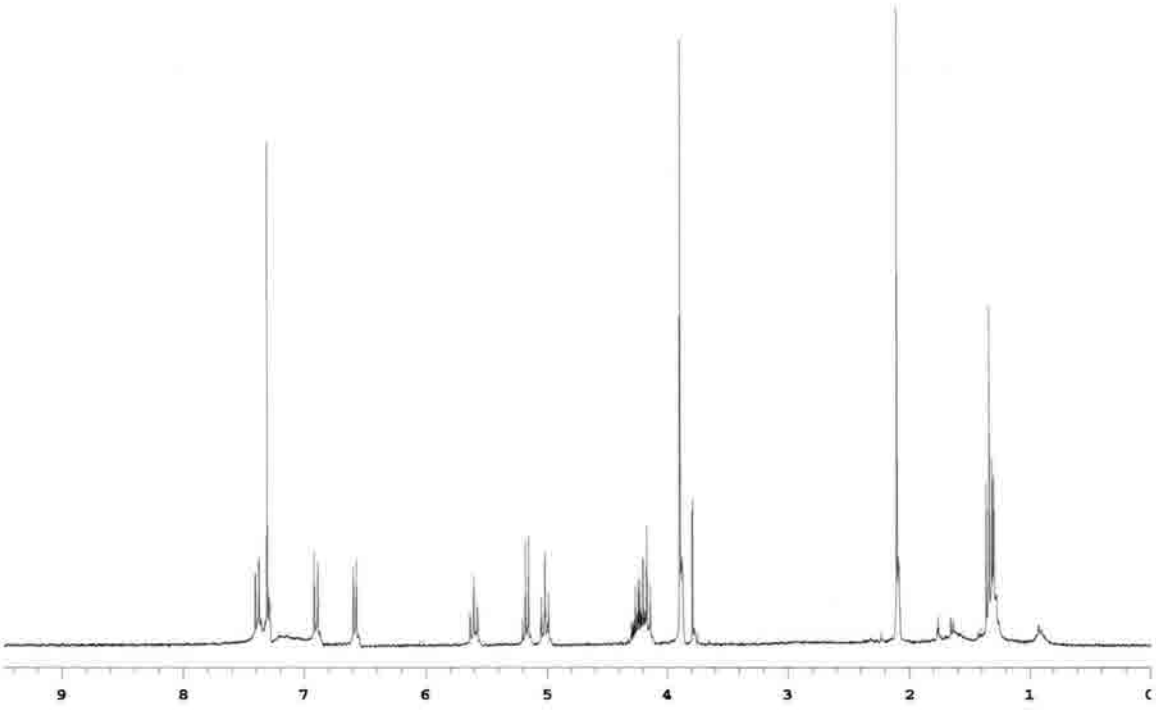
655 **664**

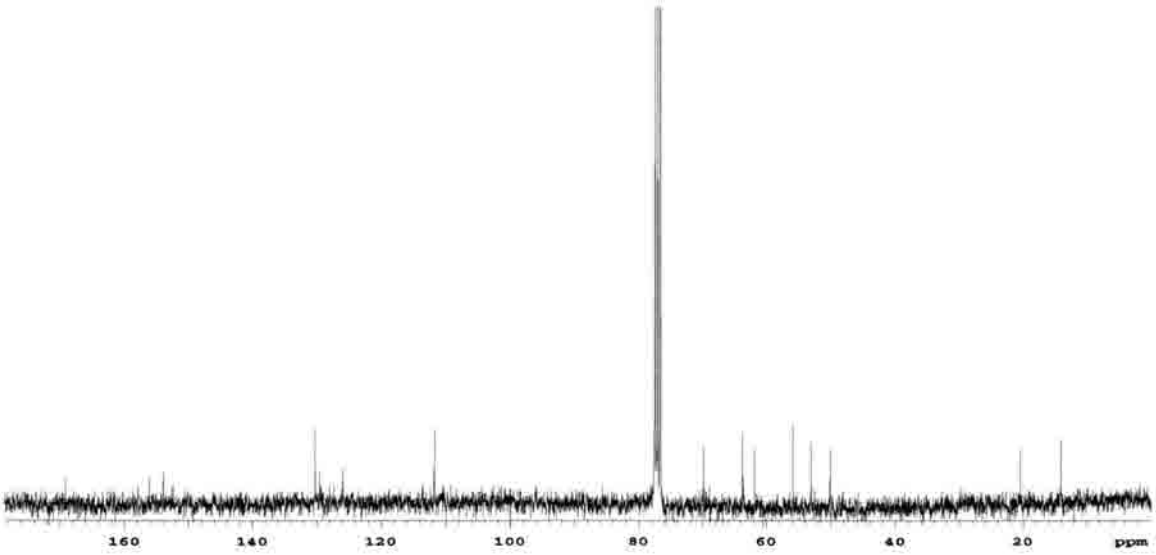
1,5,6,10b-tetrahydro-7-chloro-6-acetoxy-10-methoxy-3-oxo-3H-Oxazolo[4,3a]isoquinoline-5-carboxylic acid ethyl ester (664).

To a solution of **655** (993 mg, 2.9 mmol) in CH_2Cl_2 (50 mL) acetic anhydride (547 μL, 5.8 mmol, 2 eq.) was added followed by Et_3N (808 μL, 5.8 mmol, 2 eq.) and DMAP (35 mg, 0.29 mmol, 0.1 eq.). This solution was allowed to stir at rt for 18 h. The solution was poured into 10% NH_4Cl(aq.) and extracted with CH_2Cl_2 (3x). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified via flash chromatography (50% EtOAc/hex) to afford 870 mg **664** (78%) as a white solid. TLC (50% EtOAc/hex) R_f = 0.38 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.43 (3H, t, *J* = 7.5 Hz); 2.08 (3H, s); 3.87 (3H, s); 4.20 (3H, m); 4.99 (1H, t, *J* = 8.7 Hz); 5.12 (1H, d, *J* = 8.1 Hz); 5.57 (1H, t, *J* = 8.7 Hz); 6.54 (1H, d, *J* = 8.1 Hz); 6.88 (1H, d, *J* = 8.7 Hz), 7.36 (1H, d, *J* = 8.7 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 14.06, 20.41, 49.47, 50.10, 53.04, 55.84, 61.79, 63.65, 69.75, 100.00, 111.75, 125.92, 129.51, 130.24, 153.92, 156.11, 169.14. IR (Neat, NaCl) 2943, 1760, 1472, 1525, 1025 cm^{-1}. HRMS (FAB) calcd. for $C_{17}H_{19}NO_7Cl$ 384.0850; found 384.0837.

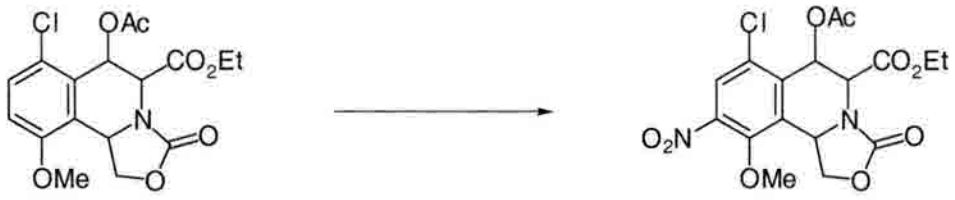


664





Compound **664**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$

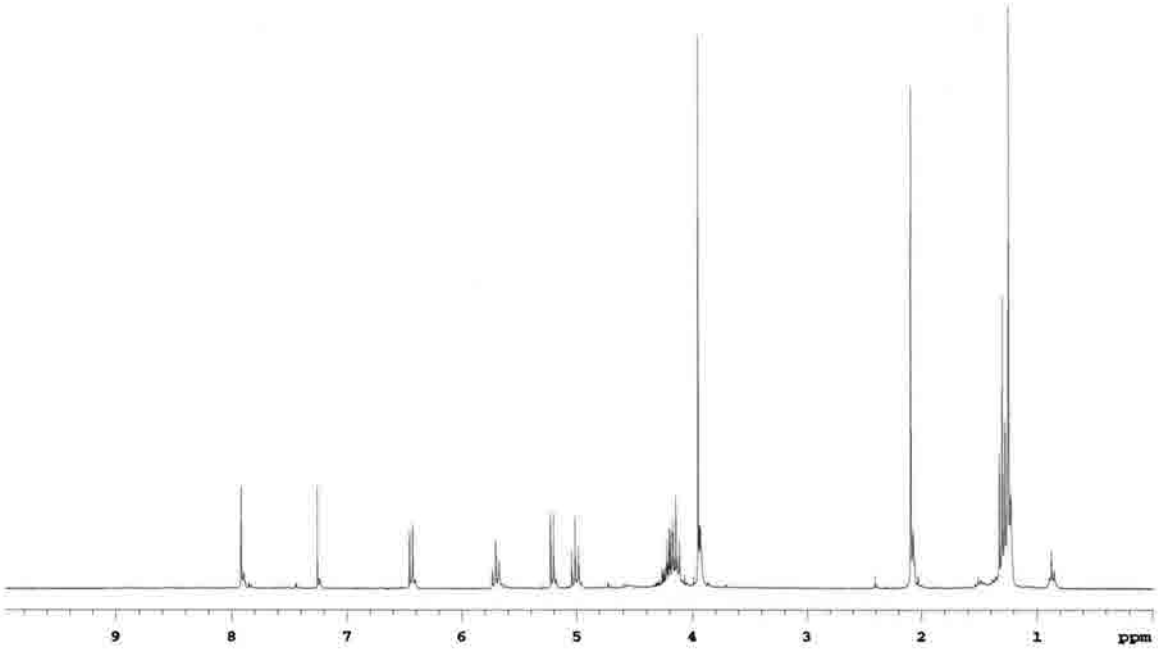


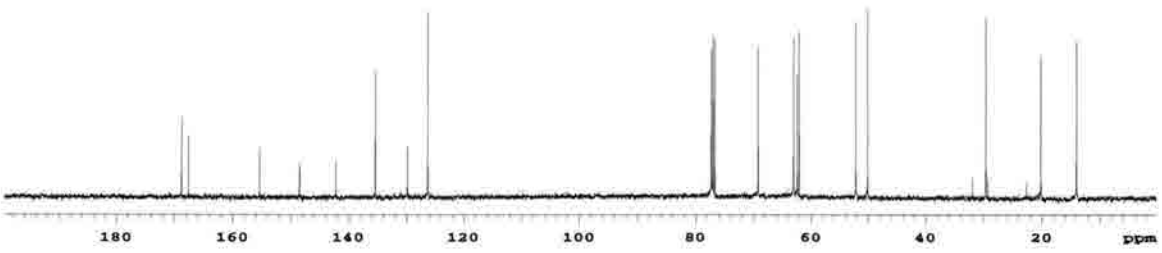
664 665

1,5,6,10b-tetrahydro-7-chloro-6-acetoxy-10-methoxy-9-nitro-3-oxo-3H-Oxazolo[4,3a]isoquinoline-5-carboxylic acid ethyl ester (665).

To a solution of **664** (680 mg, 1.77 mmol) in CH_2Cl_2 (5 mL) at 0°C was added ammonium nitrate (480 mg, 6.0 mmol, 3 eq.) and trifluroacetic anhydride (2.8 mL, 20 mmol, 10 eq.). This mixture was stirred at 0°C for 30 min and warmed to rt and allowed to stir for 2 h. The mixture was poured into sat $NaHCO_3$ and extracted with CH_2Cl_2 (3x). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified via flash chromatography (gradient 30-50 % EtOAc/hex) to afford 480 mg (64%) **665** as a white foam. TLC (50% EtOAc/hex) R_f = 0.29 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.35 (3H, t, *J* = 7.2 Hz); 2.14 (3H, s); 4.00 (3H, s); 4.26 (3H, m); 5.06 (1H, t, *J* = 9.3 Hz); 5.26 (1H, d, *J* = 8.4 Hz); 5.70 (1H, t, *J* = 8.4 Hz); 6.49 (1H, d, *J* = 8.4 Hz); 7.31 (1H, s). ^{13}C-NMR (100 MHz) ($CDCl_3$) δ 13.99, 20.15, 29.61, 50.11, 52.17, 62.06, 62.39, 63.03, 69.19, 126.28, 129.83, 135.41, 142.23, 148.45, 155.36, 167.59, 168.76. IR (NaCl, neat) 2985, 1767, 1750, 1533, 1229, 1023 cm^{-1}. HRMS (FAB) calcd. for $C_{17}H_{18}N_2O_9Cl$ 429.0700 found 429.0693.

665





Compound **665**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$

665 656

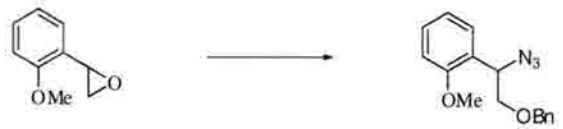
1,10b-dihydro-7-chloro-10-methoxy-9-nitro-3-oxo-3H-Oxazolo[4,3a]isoquinoline-5-carboxylic acid ethyl ester (656).

To a solution of **665** (266 mg, 0.62 mmol) in THF (5 mL) was added Et_3N (862 μL, 6.2 mmol, 10 eq.) and heated to reflux for 24h. The solvent was removed *in vacuo* and the crude product was purified via flash chromatography (40% EtOAc/hex) to afford 232 mg **656** (100%) as a yellow foam. TLC (50% EtOAc/hex) R_f = 0.38 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.35 (3H, t, *J* = 7.2 Hz); 3.85 (3H, s); 4.34 (2H, q, *J* = 7.2 Hz); 4.67 (1H, t, *J* = 9.5 Hz); 5.12 (1H, t, *J* = 9.5 Hz); 5.45 (1H, t, *J* = 8.7 Hz); 7.12 (1H, s); 7.91 (1H, s). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 8.56, 14.15, 45.81, 53.52, 59.65, 62.09, 62.59, 70.24, 115.52, 126.65, 127.47, 130.82, 131.62, 142.10, 158.57. IR (NaCl, neat) 2933, 1772, 1734, 1546, 1260, 1022 cm^{-1}. HRMS (FAB) calcd. for $C_{15}H_{14}N_2O_7Cl$ 369.0490 found 369.0478.

656 669

1,10b-dihydro-9-amino-(carbamic acid *tert*-butyl ester)-7-chloro-10-methoxy-3-oxo-3H-Oxazolo[4,3a]isoquinoline-5-carboxylic acid ethyl ester (669).

To a solution of **656** (40 mg, 0.11 mmol) in EtOH (1 mL) was added stannous chloride (139 mg, 0.54 mmol, 5 eq.). This mixture was heated to 70°C for 30 min. Sodium hydroxide was added to adjust to pH 8. The mixture was extracted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$ and concentrated. This crude product was partitioned in 2:1 CH_2Cl_2/5% $NaOH_{(aq.)}$ (1.5 mL) and Boc_2O (24 mg, 0.11 mmol, 1 eq) was added. This mixture was stirred at room temp. for 3 hours. The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated to afford 8 mg **669** (17%) as a yellow oil. TLC (60% EtOAc/hex) R_f = 0.27 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.34 (3H, t, *J* = 7.2 Hz); 1.50 (9H, s); 3.65 (3H, s); 4.34 (2H, q, *J* = 7.2 Hz); 4.74 (1H, t, *J* = 7.8 Hz); 5.03 (1H, d, *J* = 7.8 Hz); 5.34 (1H, t, *J* = 7.5 Hz); 6.72 (1H, s); 7.33 (1H, s). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 14.20, 53.69, 59.24, 61.62, 69.89, 77.43, 115.34, 115.54, 117.76, 121.11, 124.15, 125.14, 125.68, 130.06, 130.24, 142.80, 155.13, 162.41. IR (NaCl, neat) 3360, 2928, 1762, 1717, 1403, 1230, 1040 cm^{-1}. HRMS (EI^+) calc for $C_{20}H_{23}N_2O_7Cl$ (MH^+) 438.1194, found 438.1187.

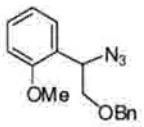


676 677

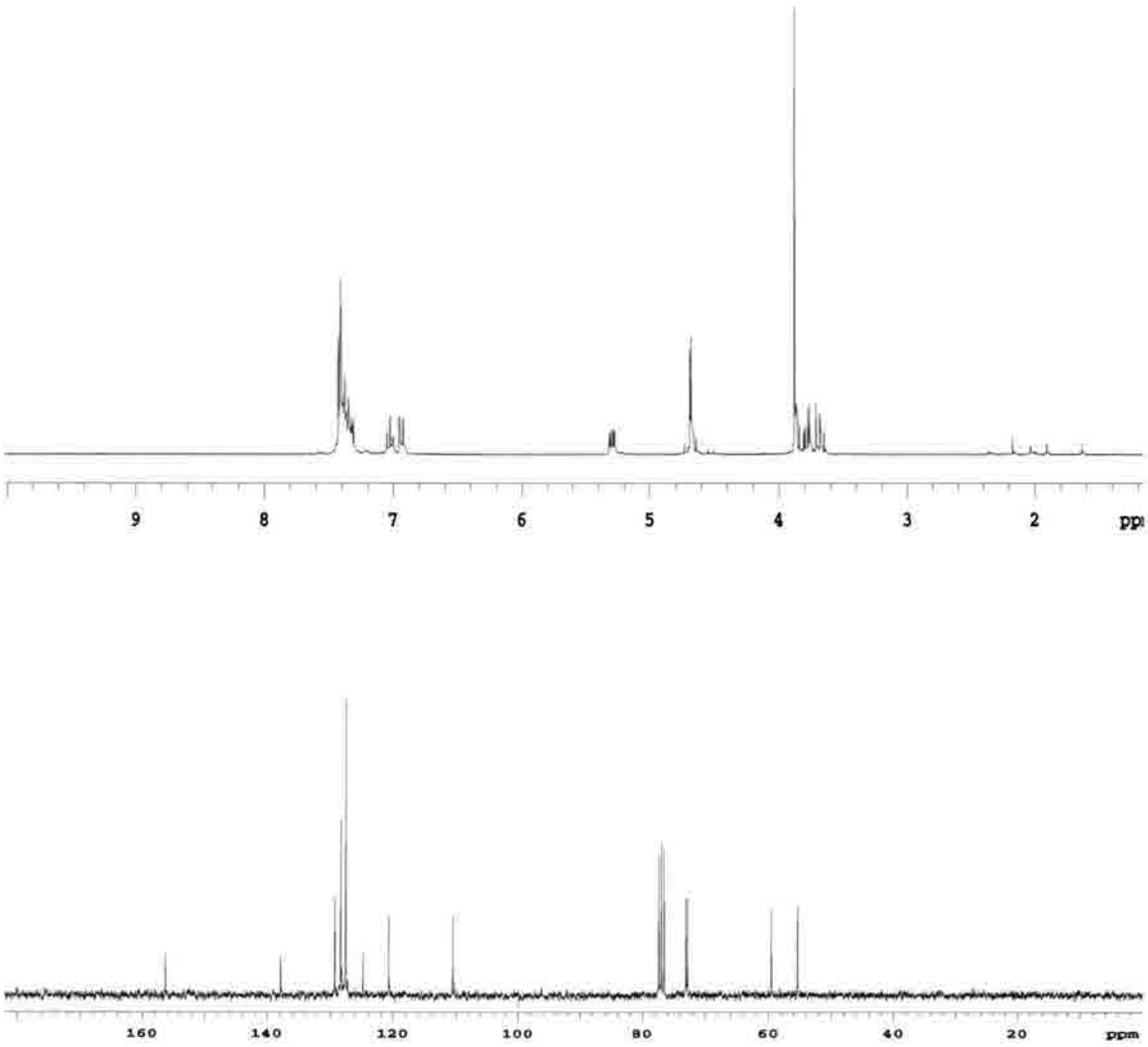
2-Azido-O-benzyl-1-(2-methoxy-phenyl)-ethanol (677).

To a solution of **676** (22.0 g, 147 mmol) in 1:1 acetone/H_2O (400 mL) was added sodium azide (14.3 g, 221 mmol, 1.5 eq.) and this solution was heated to reflux for 3 h. The acetone was removed via rotary evaporation the aqueous layer was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and concentrated to afford a clear oil. This oil was taken up in THF (100 mL) and added via cannula to a solution of NaH (6.68 g, 153 mmol, 1.05 eq, 55% dispersion in oil) in THF (100 mL) and this solution was allowed to stir at rt for 15 min. To this solution, benzyl bromide (22.6 mL, 190 mmol, 1.3 eq.) was added dropwise and postassium iodide (100mg) was added in one portion and stirred for 2 h. The solution was poured onto ice and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The crude oil was purified by flash chromatography (10% EtOAc/hex) to afford 13.18 g of **677** (94%) as a light yellow oil. TLC (10% EtOAc/hex) R_f = 0.35 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 3.68 (1H, dd, *J* = 9.9, 8.4 Hz); 3.78 (1H, dd, *J* = 9.9, 3.6 Hz); 3.89 (3H, s); 4.66 (1H, 1/2 ABq, *J* = 12.0 Hz); 4.70 (1H, 1/2 ABq, *J* = 12.0 Hz); 5.29 (1H, dd, *J* = 8.7. 3.6 Hz); 6.94 (1H, d, *J* = 7.8 Hz); 7.03 (1H, t, *J* = 6.9 Hz); 7.31-7.42 (7H, m). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 55.35, 59.51, 72.84, 73.12, 110.43, 120.43, 124.75, 127.17, 127.53, 128.12, 128.30, 129.24, 137.83, 156.26. IR (NaCl, neat) 2937,

2860, 2359, 2097, 1602, 1028 cm^{-1}. HRMS (FAB) calc. for $C_{16}H_{18}N_3O_2$ (MH^+) 284.1399; found 284.1398.



677

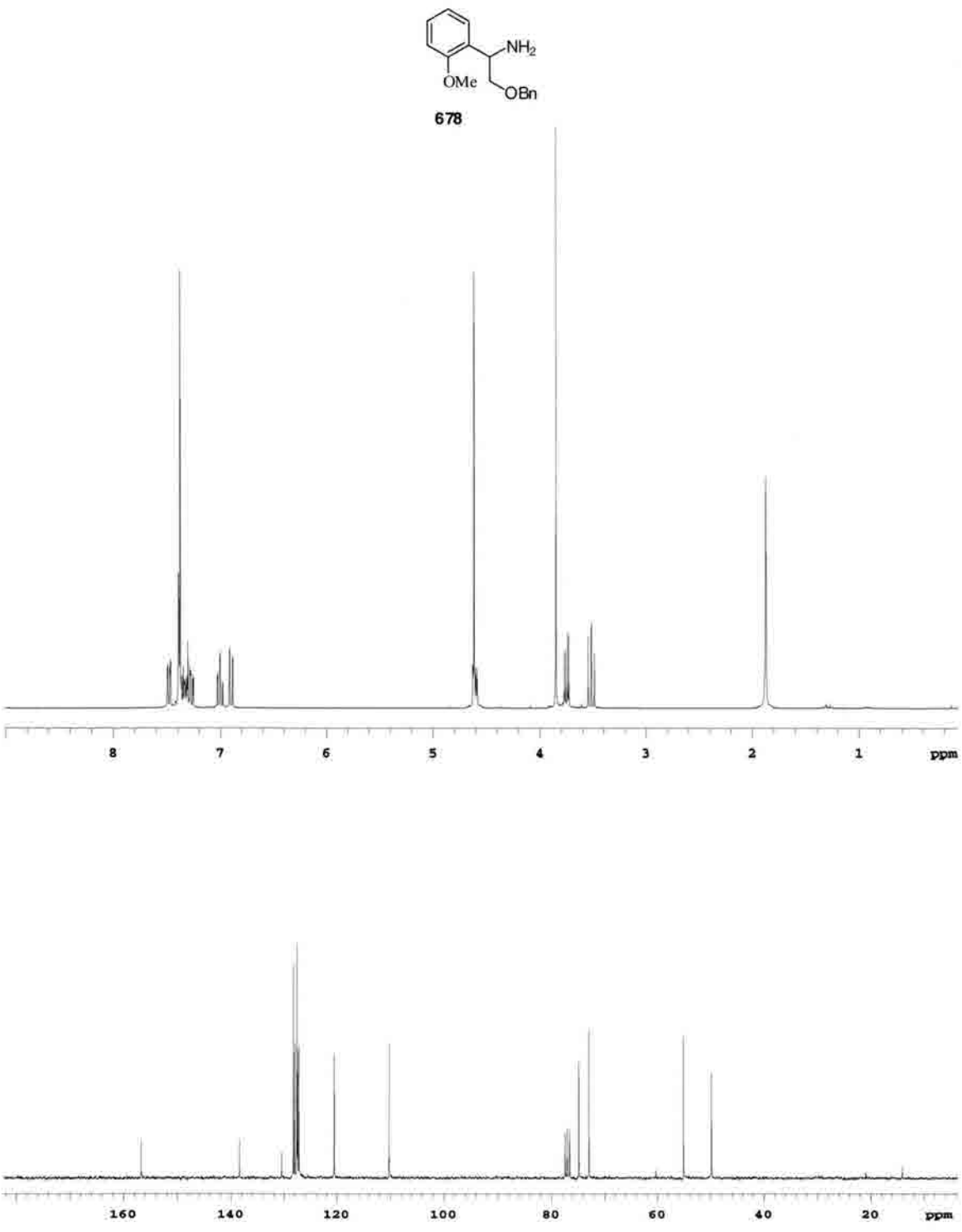


Compound **677**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$

677 678

2-Benzyloxy-1-(2-methoxy-phenyl)-ethylamine (678):

To an argon degassed solution of **677** (11.78 g, 41.6 mmol) in EtOH (140 mL) was added 5% Pd on carbon (4.42 g, 2.08 mmol, 0.05 eq.). Hydrogen was bubbled through the mixture for 10 min and a hydrogen balloon was attached. The solution was stirred for 3 h at rt. The mixture was purged with argon and the solution was filtered through Celite. The crude oil was purified via flash chromagraphy (gradient 4 – 6% $MeOH/CH_2Cl_2$) to afford 9.30 g of **678** (87%) as a clear oil. TLC (5% $MeOH/CH_2Cl_2$) R_f = 0.25 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.87 (2H, s, broad, D_2O exch.); 3.53 (1H, t, J = 9.3 Hz); 3.76 (1H, dd, J = 9.3, 3.9 Hz); 3.86 (3H, s); 4.63 (3H, m); 6.91 (1H, d, J = 8.1Hz); 7.02 (1H, t, J = 7.4Hz); 7.26-7.40 (6H, m); 7.49 (1H, dd, J = 7.4, 1.5 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 49.81, 55.10, 72.92, 74.85, 110.20, 120.54, 127.20, 127.43, 127.58, 127.98, 128.24, 103.45, 138.38, 156.74. IR (NaCl, neat) 3378, 2857, 2360, 1600, 1049, 1028 cm^{-1}. HRMS (FAB) calc. for $C_{16}H_{20}NO_2$ (MH^+) 258.1494; found 258.1496.



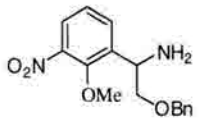
Compound **678**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$

678 679

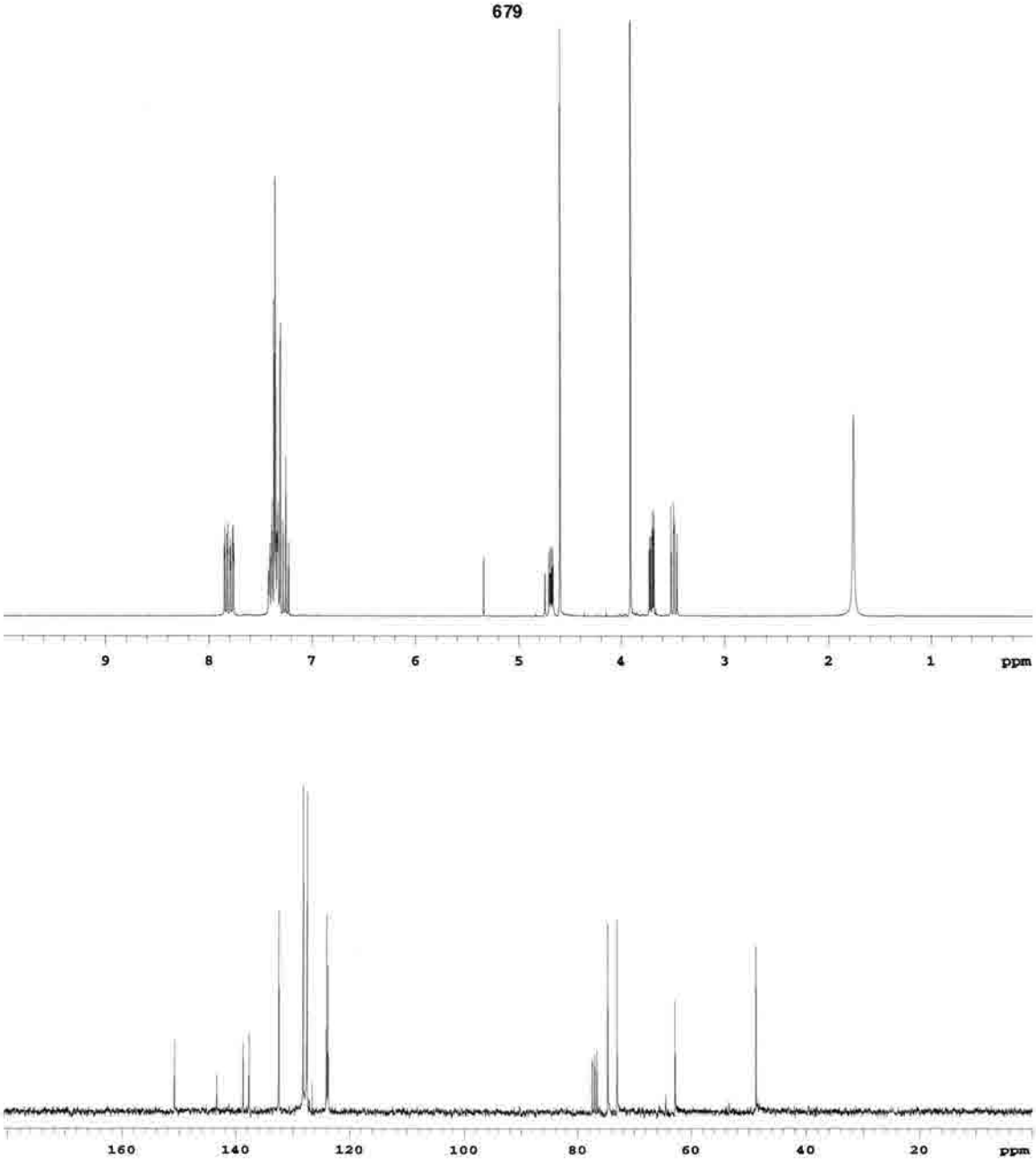
2-Benzyloxy-1-(2-methoxy-3-nitro-phenyl)-ethylamine (679).

A solution of **678** (16.02 g, 62.4 mmol) in CH_2Cl_2 (250 mL) was cooled to –20°C. Potassium nitrate (6.62 g, 65.5 mmol, 1.05 eq.) was added followed by the slow addition of TFAA (44 mL, 312 mmol, 5 eq.). This solution was stirred at –20°C for 48 h. Saturated $NaHCO_3$ was added slowly to adjust to pH 7. The aqeous layer was extracted with CH_2Cl_2 (3x) and the combined organic layers were dried over $MgSO_4$, and concetrated. The crude oil was purified via a short column (25% EtOAc/hex). The semi crude product was dissolved in 1:1 THF/EtOH (200 mL) and 2 M LiOH (100 mL) was added. This solution was allowed to stir for 14 h. Acetic acid was slowly added until the pH was 7-8. The solvent was reduced to approx 1/2 original volume via rotary evaporation and the product was partitioned in EtOAc/H_2O. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. Purification via flash chromatography (2.5% MeOH/CH_2Cl_2) afforded 10.5 g of **679** (56%) as a yellow oil. TLC (5% MeOH/CH_2Cl_2) R_f = 0.27 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.78 (2H, s, broad); 3.49 (1H, dd, *J* = 8.7, 7.8 Hz); 3.70 (1H, dd, *J* = 8.7, 3.9 Hz); 3.91 (3H, s); 4.60 (2H, s); 4.68 (1H, dd, *J* = 7.9, 3.9 Hz); 7.25 (1H, t, *J* = 8.1 Hz); 7.33-7.42 (5H, m); 7.77 (1H, dd, *J* = 8.1, 1.5 Hz); 7.83 (1H, dd, *J* = 8.1, 1.5 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 48.75, 62.82, 73.03, 74.67, 123.74, 124.04, 127.44, 127.53, 128.19, 132.42. 137.69, 138.76,

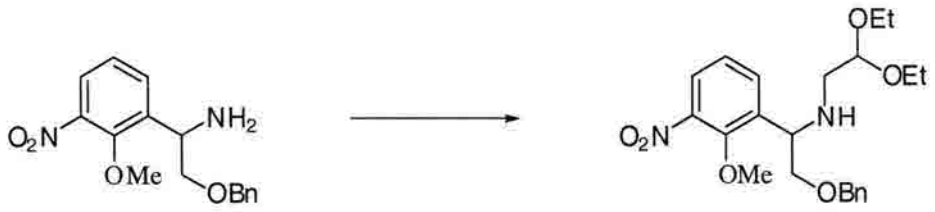
143.38, 150.84. IR (NaCl, neat) 3378, 3312, 2916, 1528, 1355, 1089, 1027 cm^{-1}. HRMS (FAB) calc. for $C_{16}H_{19}N_2O_4$ (MH^+) 303.1345; found 303.1349.



679



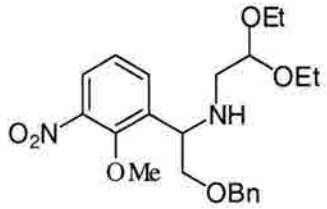
Compound **679**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$



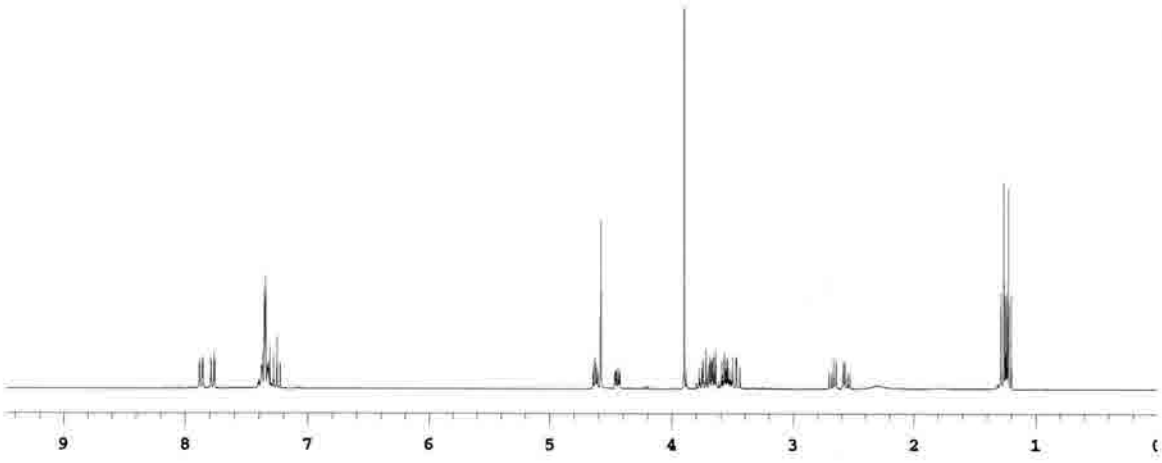
679 **681**

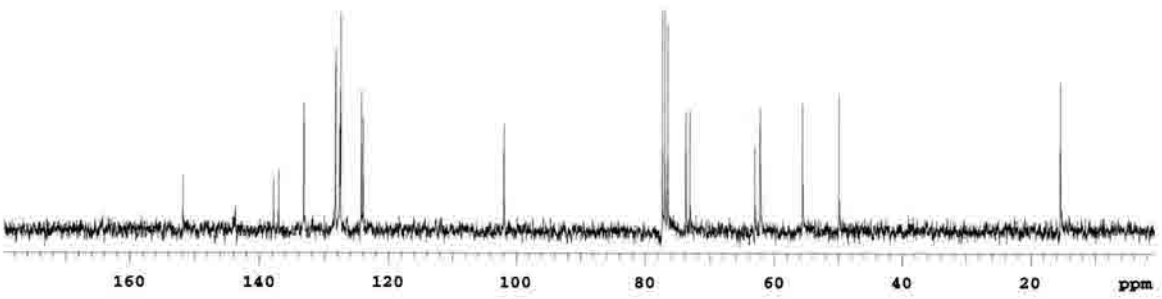
[2-Benzyloxy-1-(2-methoxy-3-nitro-phenyl)-ethyl]-(2,2-diethoxy-ethyl)-amine (681).

To a solution of **679** (6.00 g, 19.8 mmol) in acetonitrile (50 mL) was added bromoacetaldehyde diethyl acetal (15.0 mL, 99.3 mmol, 5 eq.) and potassium carbonate (10.9g, 79.2 mmol, 4 eq.). The solution was heated to reflux for 5 days. The solvent was removed *in vacuo* and the crude mixture was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified via flash chromatography (25% EtOAc/hex) to afford 6.11 g **681** (74%) as a yellow oil. TLC (5% MeOH/CH_2Cl_2) R_f = 0.70 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.22 (3H, t, *J* = 6.9 Hz); 1.26 (3H, t, *J* = 6.9 Hz); 2.27 (1H, s, broad, D_2O exch.); 2.55 (1H, dd, *J* = 11.7, 4.8 Hz); 2.67 (1H, dd, *J* = 11.7, 6.0 Hz); 3.47 (1H, dd, *J* = 9.6, 8.7 Hz); 3.56 (2H, m); 3.64-3.77 (3H, m); 3.90 (3H, s); 4.44 (1H, dd, *J* = 8.4, 4.2 Hz); 4.58 (2H, s); 4.62 (1H, dd, *J* = 6.3, 4.8 Hz); 7.25 (1H, t, *J* = 7.8 Hz); 7.35 (5H, m); 7.77 (1H, dd, *J* = 7.8, 1.2 Hz); 7.87 (1H, dd, *J* = 7.8, 1.2 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 15.39, 49.89, 55.60, 62.18, 62.23, 63.07, 73.12, 73.76, 101.91, 124.02, 124.32, 127.54, 127.65, 128.31, 133.20, 137.06, 137.83, 143.72, 151.79. IR (NaCl, neat) 3340, 2975, 2688, 1602, 1530, 1356, 1064 cm^{-1}. HRMS (FAB) calc. for $C_{22}H_{31}N_2O_6$ (MH^+) 419.2182; found 419.2184.



681

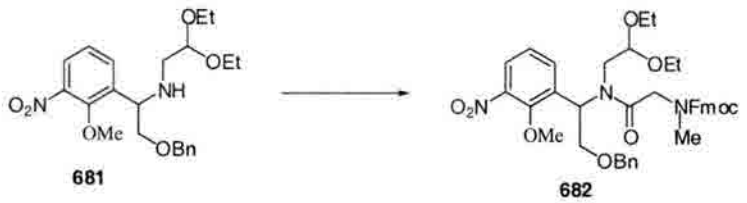




Compound **681**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$

N-Fmoc-Sarcosine

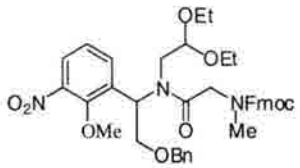
To a solution of sarcosine (1.00g, 11.2 mmol) in 9% Na_2CO_3 (25 mL) cooled to 0°C was added a slurry of fluorenylmethyl hydroxy succinimide (3.78 g, 11.2 mmol, 1 eq) in dioxane (25 mL). This solution was warmed to rt and stirred for 3 h. The mixture was extracted with EtOAc and Et_2O. Hydrochloric acid (6N) was added to the aqueous layer until pH = 1. The solution was extracted with EtOAc (3x). The combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was removed and the crude product was recrystallized from EtOAc/hex to afford 3.40 g of N-Fmoc sarcosine (97%) as a white solid. ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 2.97 (1.5 H, s); 3.01 (1.5 H, s); 3.97 (1H, s); 4.17 (1H, s); 4.28 (1H, m); 4.49 (2H, m); 7.32-7.45 (4H, m); 7.56 (1H, d, *J* = 7.5 Hz); 7.65 (1H, d, *J* = 7.5 Hz); 7.78 (1H, d, *J* = 7.5 Hz); 7.81 (1H, d, *J* = 7.5 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 35.41, 35.99, 47.11, 50.67, 50.52, 67.77, 68.02, 119.84, 124.64, 124.88, 126.93, 127.52, 127.58, 141.12, 143.61, 155.94, 156.74, 174.51. (Note: Due to rotomers there are more carbon signals all are listed as seen in the room temp spectrum.) IR (NaCl, neat) 3300, 3065, 1701, 1684, 1230, 1155 cm^{-1}. HRMS (FAB) calc. for $C_{18}H_{17}NO_64(MH^+)$ 311.1158; found 311.1158.



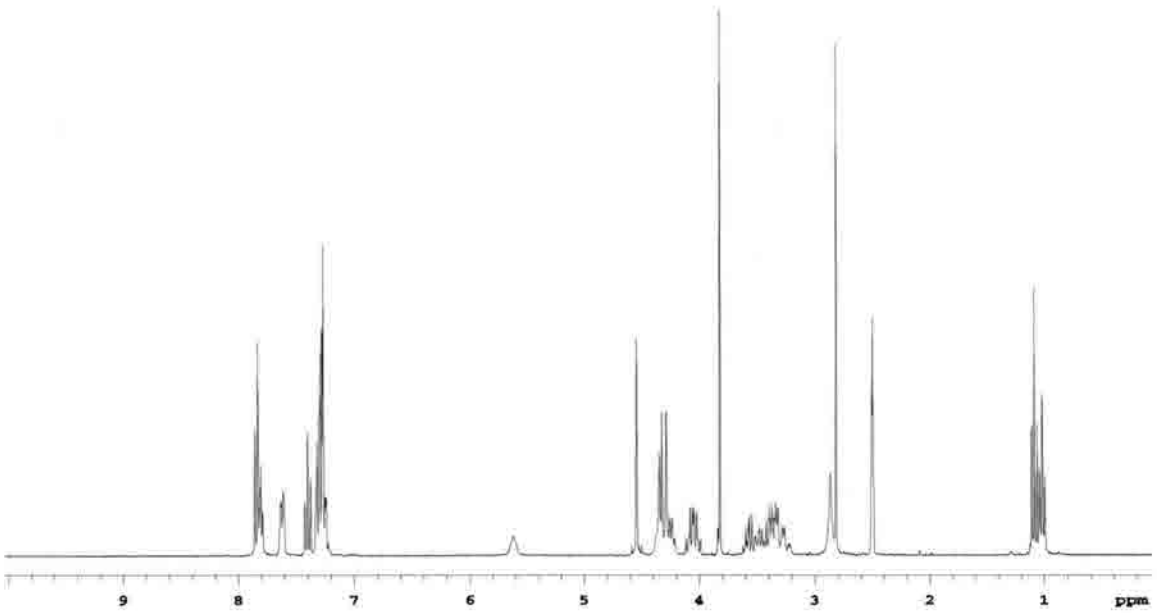
{[[2-Benzyloxy-1-(2-methoxy-3-nitro-phenyl)-ethyl]-(2,2-diethoxy-ethyl)-carbamoyl]-methyl}-methyl-carbamic acid 9*H*-fluoren-9-ylmethyl ester (682).

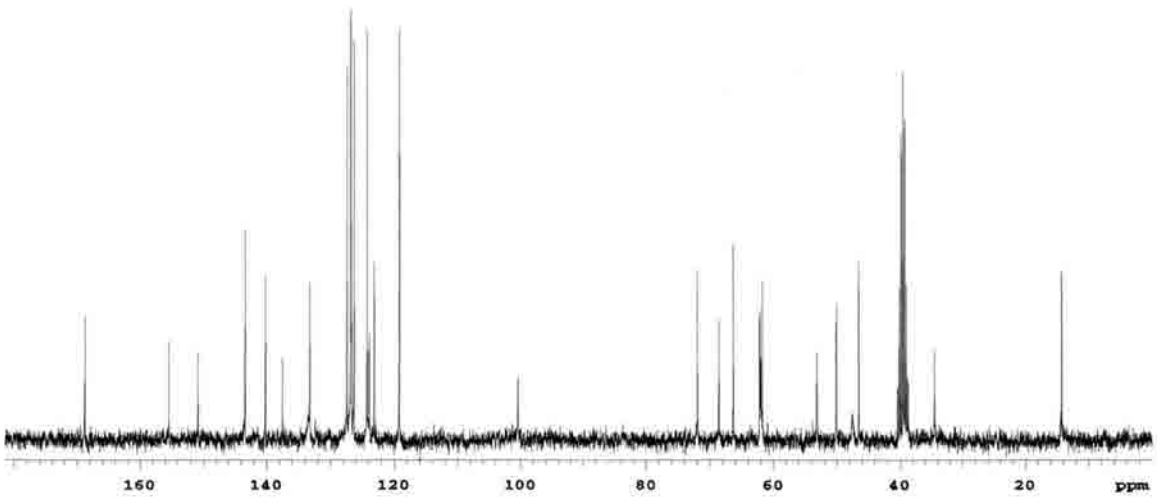
To a solution of N-Fmoc-sarcosine (6.70g, 21.4 mmol, 1.25 eq.) in CH_2Cl_2 (100 mL) was added oxalyl chloride (2.0 mL, 23.0 mmol, 1.35 eq.) and DMF (159 μL, 2.1 mmol, 0.12 eq.) and was stirred at rt for 1 h. Hexanes (100 mL) were added and the solution was filtered through a cotton plug and concentrated. A solution of amine **681** (7.15 g, 17.1 mmol), pyridine (4.15 mL, 51.3 mmol, 3.0 eq.) and DMAP (209 mg, 1.71 mmol, 0.10 eq.) in CH_2Cl_2 (100 mL) at 0°C was added to the acid chloride and the solution was allowed to stir at 0°C for 45 min. Dilute HCl(aq.) was added and the aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were washed with sat $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$ and concetrated. The crude product was purified via flash chromatrography (30-40% EtOAc/hex) to afford 10.0 g **682** (82%) as a light yellow foam. TLC (50% EtOAc/hex) R_f = 0.60 (UV and PMA). ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.02 (3H, t, *J* = 7.2 Hz); 1.09 (3H, t, *J* = 6.9 Hz); 2.82 (3H, s); 2.87 (1H, s); 3.37-3.60 (6H, m); 3.82 (3H, s); 4.05 (2H, ddd, *J* = 10.2, 6.9, 6.9 Hz); 4.21-4.34 (5H, m); 4.54 (2H, s); 5.62 (1H, m); 7.24-7.32 (8H, m); 7.40 (2H, t, *J* = 7.5 Hz); 7.63 (2H, dd, *J* = 7.5, 2.7 Hz); 7.84 (4H, m). ^{13}C-NMR (75 MHz) (d_6-DMSO, 120°C) δ 14.23, 14.28, 34.45, 46.51, 47.57, 50.08, 53.14, 61.71, 61.91, 62.11, 66.32, 68.56, 71.94, 100.42, 119.17, 123.12, 123.90, 124.25, 126.28,

126.70, 126.77, 126.81, 127.42, 133.24, 137.51, 140.21, 143.39, 150.82, 155.45, 168.81. IR (NaCl, neat) 2959, 1735, 1716, 1697, 1153, 1079 cm^{-1}. HRMS (FAB) calc. for $C_{40}H_{46}N_3O_9$ (MH^+) 712.3234; found 712.3233.



682



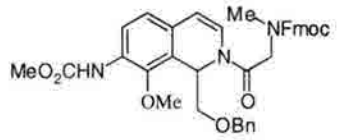


Compound **682**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

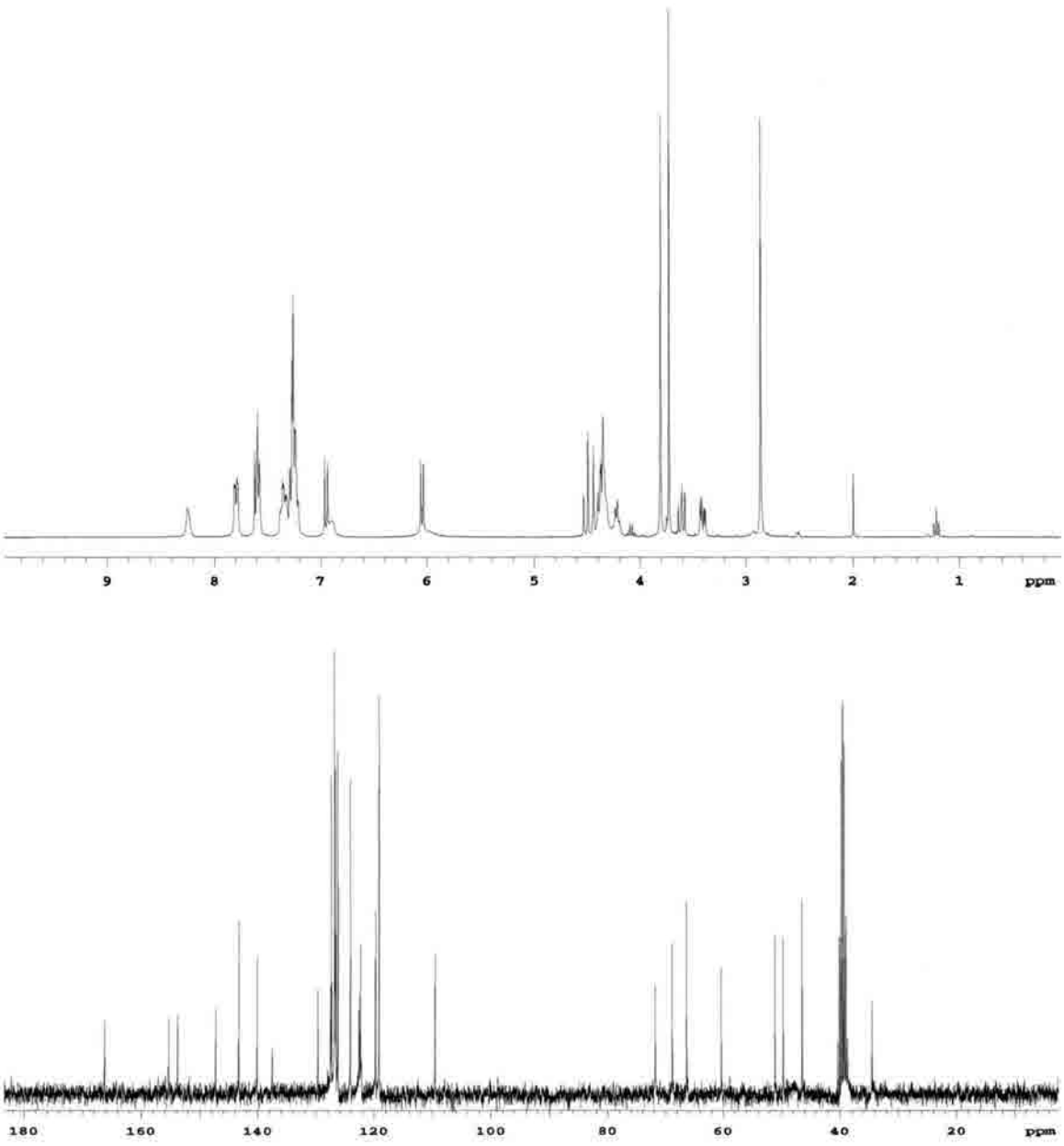
(1-Benzyloxymethyl-2-{[(9*H*-fluoren-9-ylmethoxycarbonyl)-methyl-amino]-acetyl}-8-methoxy-1,2-dihydro-isoquinolin-7-yl)-carbamic acid methyl ester (694).

To an argon degassed solution of **682** (6.45 g, 9.07 mmol) in 1:1 THF/EtOH (140 mL) in a pressure vessel was added PtO_2 (102 mg, 0.45 mmol, 0.05 eq.) and the vessel was sealed and pressurized with 80 psi H_2. The solution was stirred at rt for 16 h. The vessel was depressurized and the solution was purged with Ar. The catalyst was removed by filtering through celite and the solution was concentrated. The crude product was dissolved in dioxane (75 mL) to this solution was added 6M HCl (4.48 mL, 26.9 mmol, 3 eq.) and the solution was stirred in a oil bath at 90°C for 15 min. The solution was allowed to cool to room temp. Excess sat. $NaHCO_3$ was added and the mixture was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The crude product was redissolved in CH_2Cl_2 (50 mL) and cooled to 4°C. Methyl cholorfomate (2.07 mL, 26.9 mmol, 3 eq.) was added followed by pyridine (724 μL, 9.0 mmol, 1 eq.) and this solution was stirred at 4°C for 18 hr. Saturated $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2 (3x). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified via flash chromatography (45% EtOAc/hex) to afford 5.22 g **694** (89%) as a light yellow foam. TLC (50% EtOAc/hex) R_f = 0.29 (UV and PMA). ^{1}H-NMR (300

MHz) (d_6-DMSO, 120°C) δ 2.83 (3H, s); 3.38 (1H, dd, *J* = 10.2, 3.4 Hz); 3.58 (1H, dd, *J* = 10.5, 8.7 Hz); 3.71 (3H, s); 3.78 (3H, s); 4.29 (5H, m); 4.37 (1H, 1/2 ABq *J* = 12.3 Hz); 4.48 (1H, 1/2 ABq, 12.3 Hz); 6.03 (1H, d, *J* = 7.5 Hz); 6.87 (1H, s, broad); 6.94 (1H, d, *J* = 7.5 Hz); 7.20-7.38 (10 H, m); 7.58 (3H, m); 7.80 (2H, dd, *J* = 7.7, 2.9 Hz); 8.23 (1H, s, broad). ^{13}C-NMR (d_6-DMSO, 120°C) δ 34.50, 46.42, 49.69, 51.07, 60.32, 66.32, 68.84, 71.79, 109.54, 119.15, 119.76, 122.20, 122.33, 122.58, 124.10, 126.24, 126.58, 126.70, 126.80, 127.35, 127.51, 129.68, 137.55, 140.17, 143.31, 147.26, 153.80, 155.31, 166.25 Note: one carbon resonance not observed. HRMS (FAB) calcd for $C_{38}H_{38}N_3O_7$ (MH^+) 648.2710; found 648.2698.



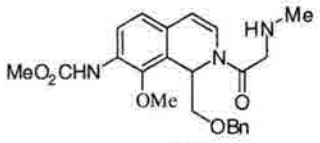
694



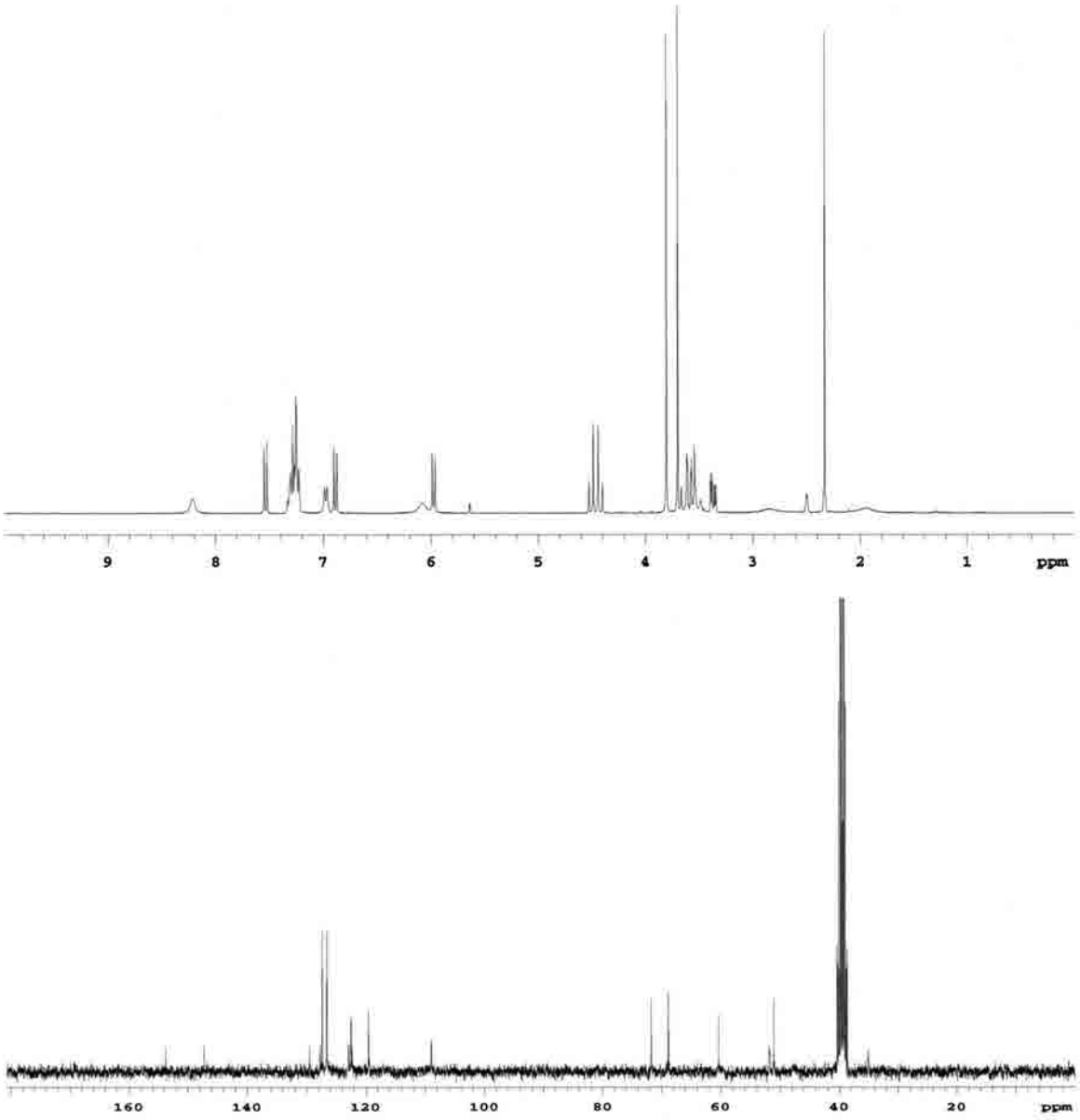
Compound **694**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

(1-Benzyloxymethyl-8-methoxy-2-methylaminoacetyl-1,2-dihydro-isoquinolin-7-yl)-carbamic acid methyl ester (695).

To a solution of **694** in acetonitrile (50 mL) was added pyrrolidine (5 mL) and this was allowed to stir at rt for 1 h. The solution was concentrated and the crude product was purified via flash chromatography (3-7.5 % MeOH/CH_2Cl_2) to afford 3.12 g **695** (94%) as a white foam. TLC (7.5% MeOH/CH_2Cl_2) R_f = 0.18 (UV and PMA). ^{1}H-NMR (300 MHz) (d_6-DMSO, 120°C) δ 2.37 (3H, s); 3.00 (1H, s, broad, D_2O exchangeable); 3.39 (1H, dd, *J* = 10.5, 4.5 Hz); 3.61 (3H, m); 3.68 (3H, s); 3.71 (3H, s); 4.44 (1H, 1/2 ABq, *J* = 12.0 Hz); 4.51 (1H, ABq, *J* = 12.0 Hz); 5.99 (1H, d, *J* = 7.8 Hz); 6.05 (1H, s, broad); 6.89 (1H, d, *J* = 8.1 Hz); 6.98 (1H, d, *J* = 7.2 Hz); 7.24-7.35 (5H, m); 7.54 (1H, d, *J* = 8.1 Hz); 8.22 (1H, s). ^{13}C-NMR (75 MHz) (d_6-DMSO, 120°C) δ 35.11, 51.04, 51.82, 60.33, 68.85, 71.74, 108.96, 119.44, 119.58, 122.23, 122.50, 122.94, 126.09, 126.56, 126.62, 127.36, 127.70, 129.49, 147.30, 153.79 (Note: one carbon resonance not observed) IR (NaCl, neat) 3421, 3328, 2945, 2860, 1729, 1671, 1628, 1526, 1229, 1088 cm^{-1}. HRMS (FAB) calc. for $C_{23}H_{28}N_3O_5$ (MH^+) 426.2029; found 426.2025.



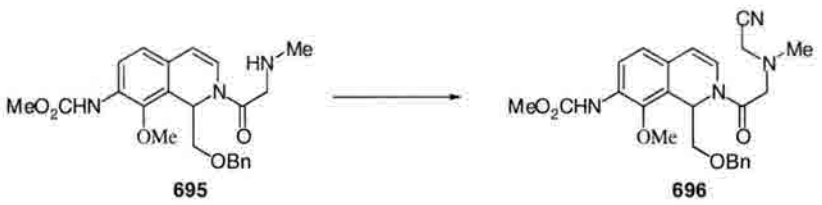
695



Compound **695**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

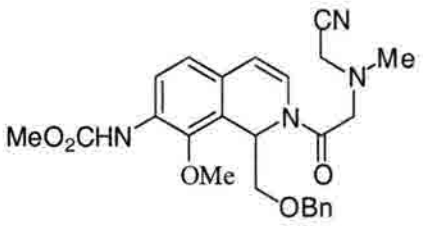
{1-Benzyloxymethyl-8-methoxy-2-[(methyl-methylsulfanylmethyl-amino)-acetyl]-1,2-dihydro-isoquinolin-7-yl}-carbamic acid methyl ester (702).

To a solution of **695** (246mg, 0.579 mmol) in THF (10 mL) was added chloromethyl methyl sulfide (63μL, 0.752 mmol, 1.3 eq.), diisopropylethyl amine (131 μL, 0.75 mmol, 1.3eq.) and potassium iodide (5 mg, 0.029 mmol 0.05eq). The solution was heated to 45°C for 24 h. Aqueous $NaHCO_3$ was added and the mixture was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The crude oil was run through short column (96:3:1 CH_2Cl_2:MeOH:Et_3N) to afford 284mg (100%) **702** as a yellow foam. ^{1}H-NMR (d_6-DMSO, 120°C) δ 2.11 (3H, s); 2.38 (3H, s); 3.39 (1H, dd *J* =10.8, 4.5 Hz); 3.53 (1H, d *J* =16.2 Hz), 3.59(1H, dd, *J* =10.8, 9.0 Hz); 3.71 (3H, s); 3.81(3H, s), 3.95(1H, 1/2 ABq, *J* = 12.9 Hz); 3.99 (1H, 1/2 ABq, *J* = 12.9 Hz); 4.44 (1H, 1/2 ABq, *J* = 12.0 Hz); 4.51 (1H, 1/2 ABq, *J* = 12.0 Hz); 5.98 (1H, d, *J* = 7.8 Hz); 6.90 (1H, d, *J* = 8.4 Hz); 7.06 (1H, d, *J* = 7.5 Hz); 7.24-7.34 (5H, m); 7.55 (1H, d, *J* = 8.4 Hz); 8.21(1H, s, broad). IR (NaCl, neat) 3423, 3318, 2944, 2859, 1733, 1667, 1626, 1524, 1415, 1091, 1053 cm^{-1}. HRMS (FAB) calc. for $C_{25}H_{30}N_3O_5S$ (MH^+) 484.1906. found: 484.1903.

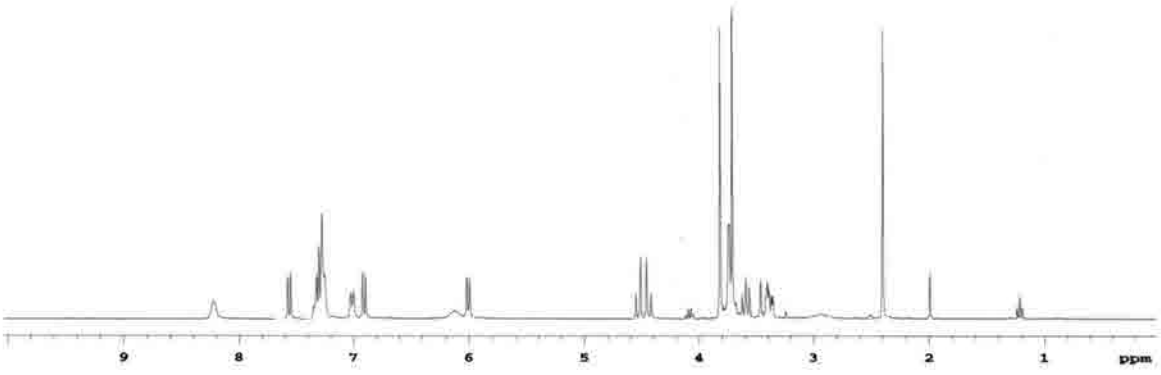


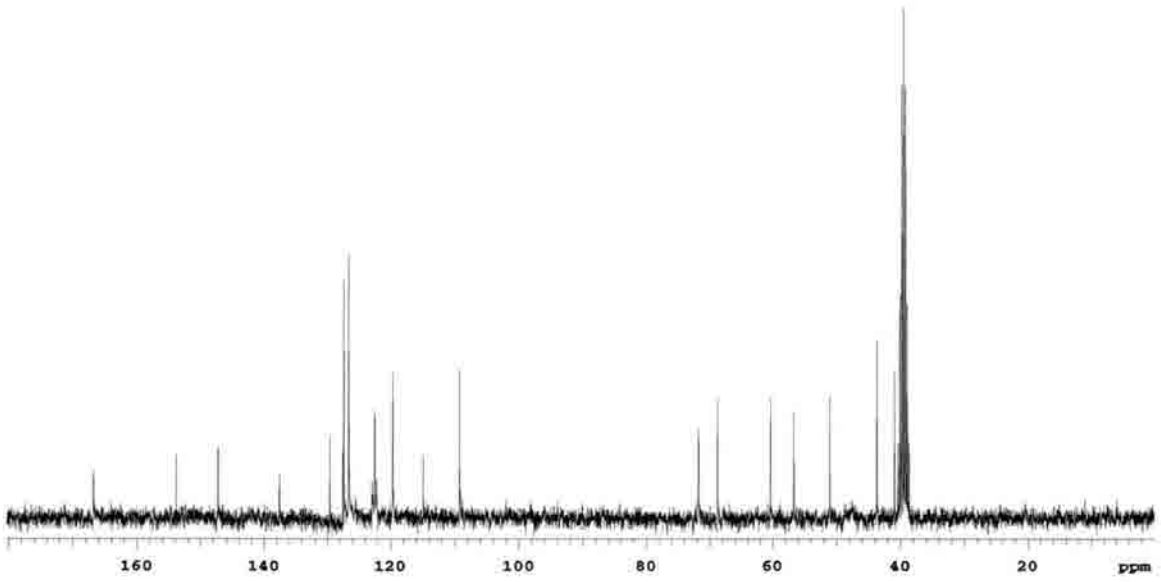
{1-Benzyloxymethyl-2-[(cyanomethyl-methyl-amino)-acetyl]-8-methoxy-1,2-dihydro-isoquinolin-7-yl}-carbamic acid methyl ester (696).

To a solution of **695** (1.50 g, 3.53 mmol) in THF (40 mL) was added iodoacetonitrile (281 μL, 3.88 mmol, 1.1 eq.) and diisopropylethyl amine (675 μL, 3.88 mmol, 1.1 eq.). This solution was stirred at rt for 18 h. The solution was diluted with EtOAc and washed with sat. $NaHCO_3$ and brine then dried over $MgSO_4$ and concentrated. The crude product was purified via flash chromatography (50-60 % EtOAc/hex) to afford 1.64 g **696** (99%) as a white foam. TLC (50% EtOAc/hex) R_f = 0.25 (UV and PMA). ^{1}H-NMR (300MHz) (d_6DMSO, 120°C) δ 2.40 (3H, s); 3.40 (2H, m); 3.59 (1H, t, *J* = 10.2 Hz); 3.71 (3H, s) 3.74 (3H, s); 3.82 (3H, s); 4.45 (1H, 1/2 ABq, *J* = 12.0 Hz); 4.52 (1H, 1/2 ABq, *J* = 12.0 Hz); 6.00 (1H, d, *J* = 7.5 Hz); 6.12 (1H, s, broad); 6.91 (1H, d, *J* = 8.4 Hz); 7.01 (1H, d, *J* = 7.5 Hz); 7.25-7.25 (5H, m); 7.56 (1H, d, *J* = 8.4 Hz); 8.22 (1H, s). ^{13}C-NMR (75 MHz) (d_6-DMSO, 120°C) δ 40.92, 43.68, 51.06, 56.71, 60.35, 68.77, 71.77, 109.24, 114.86, 119.69, 122.22, 122.50, 122.91, 126.63, 126.68, 127.40, 127.55, 129.60, 129.94, 137.57, 147.25, 153.79, 166.76. IR (NaCl, neat) 2925, 1733, 1669, 1524, 1093, 1047 cm^{-1}. HRMS (FAB) calc. for $C_{25}H_{29}N_4O_5$ (MH^+) 465.2138; found 265.2127.

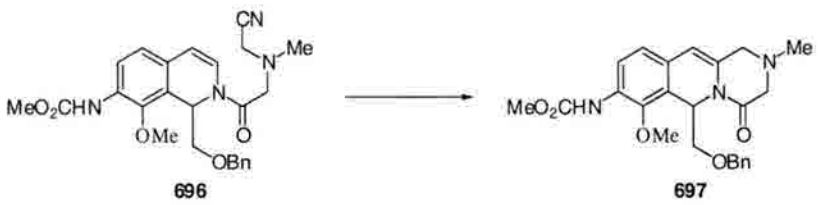


696



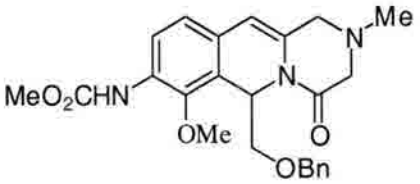


Compound **696**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in d_6-DMSO at 120°C

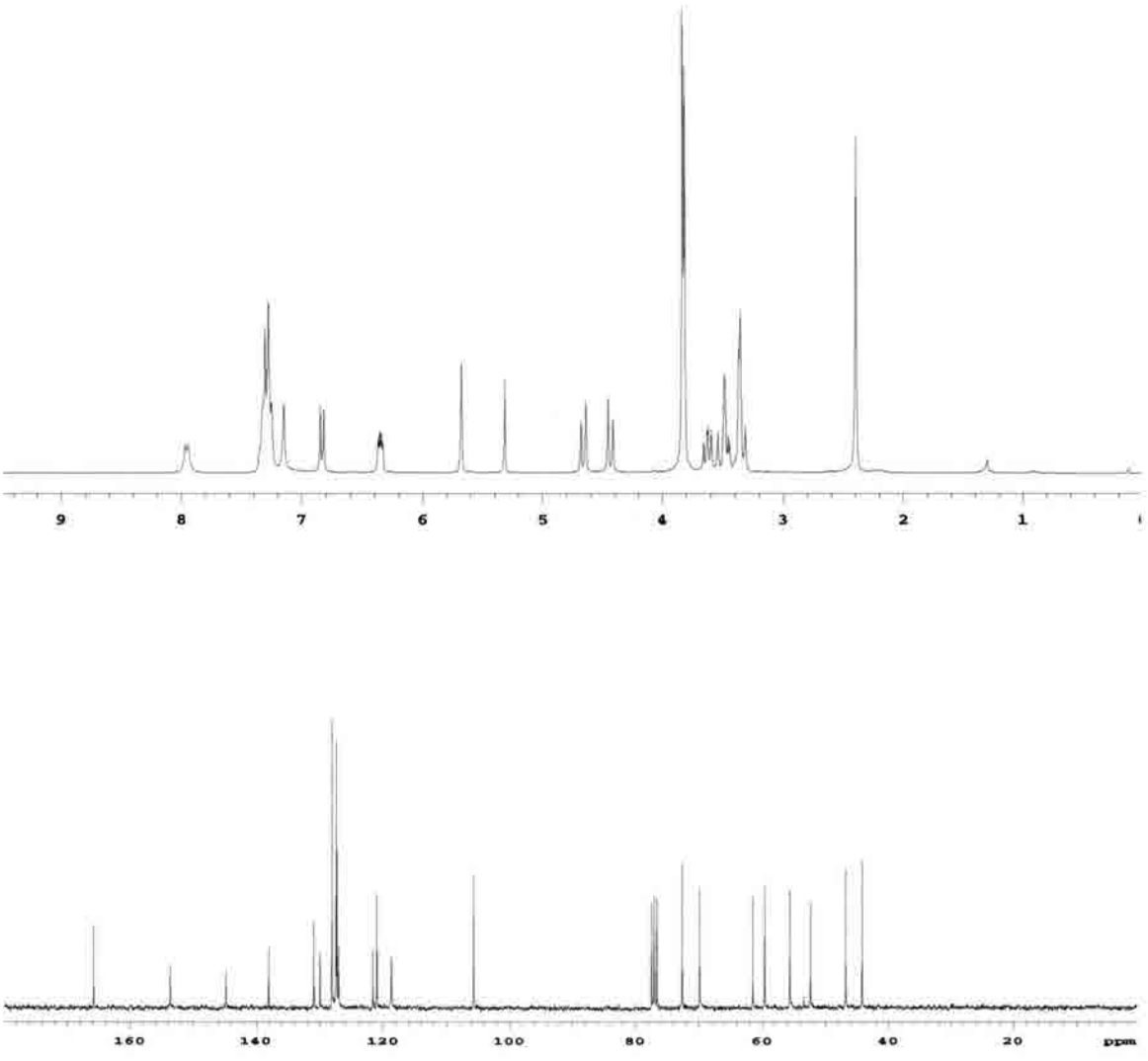


(6-Benzyloxymethyl-7-methoxy-2-methyl-4-oxo-1,3,4,6-tetrahydro-2*H*-pyrazino[1,2-*b*]isoquinolin-8-yl)-carbamic acid methyl ester (697).

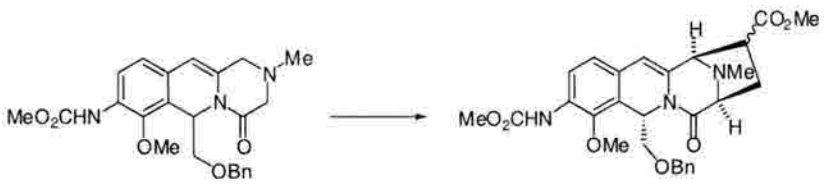
To a solution of **696** (2.65 g, 5.71 mmol) in dichloroethane (220 mL) was added trifluoroacetic anhydride (806 μL, 5.71 mmol, 1.0 eq.), trifluoroacetic acid (660 μL, 8.57 mmol, 1.5 eq.), and Silver(I)trifluoroactate (1.32 g, 5.71 mmol, 1.05 eq.) The mixture was heated to reflux for 45min. The mixture was cooled to rt. Excess sat. $NaHCO_3$ was added and the mixture was extracted with CH_2Cl_2 (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was purified via flash chromatography (3% MeOH/CH_2Cl_2) to afford 2.32 g **697** (93%) as a yellow foam. TLC (5% MeOH/CH_2Cl_2) R_f = 0.36 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 2.42 (3H, s); 3.38 (2H, m); 3.50 (2H, m); 3.63 (2H, dd, *J* = 10.5, 8.4 Hz); 3.83 (3H, s); 3.85 (3H, s); 4.45, (1H, 1/2 ABq, *J* = 12.3Hz), 4.67 (1H, 1/2 ABq, *J* = 12.3Hz); 5.70 (1H, s); 6.35 (1H, dd, *J* = 8.1, 3.4 Hz); 6.86 (1H, d, *J* = 8.4 Hz); 7.07(1H, s); 7.30(5H, m), 7.98 (1H, d, *J* = 8.1Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 44.16, 46.77, 52.34, 55.60, 59.58, 61.45, 69.83, 72.59, 105.68, 118.67, 120.98, 121.62, 127.01, 127.30, 127.44, 128.10, 129.96, 130.91, 138.02, 144.77, 153.01, 165.81. IR(NaCl, neat) 3420, 3318, 2926, 2854, 1731, 1682, 1645, 1526, 1234, 1204, 1096 cm^{-1}. HRMS (FAB) calcd for $C_{24}H_{28}N_3O_5$ 438.2029; found 438.2024.



697



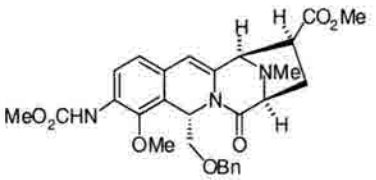
Compound **697**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$



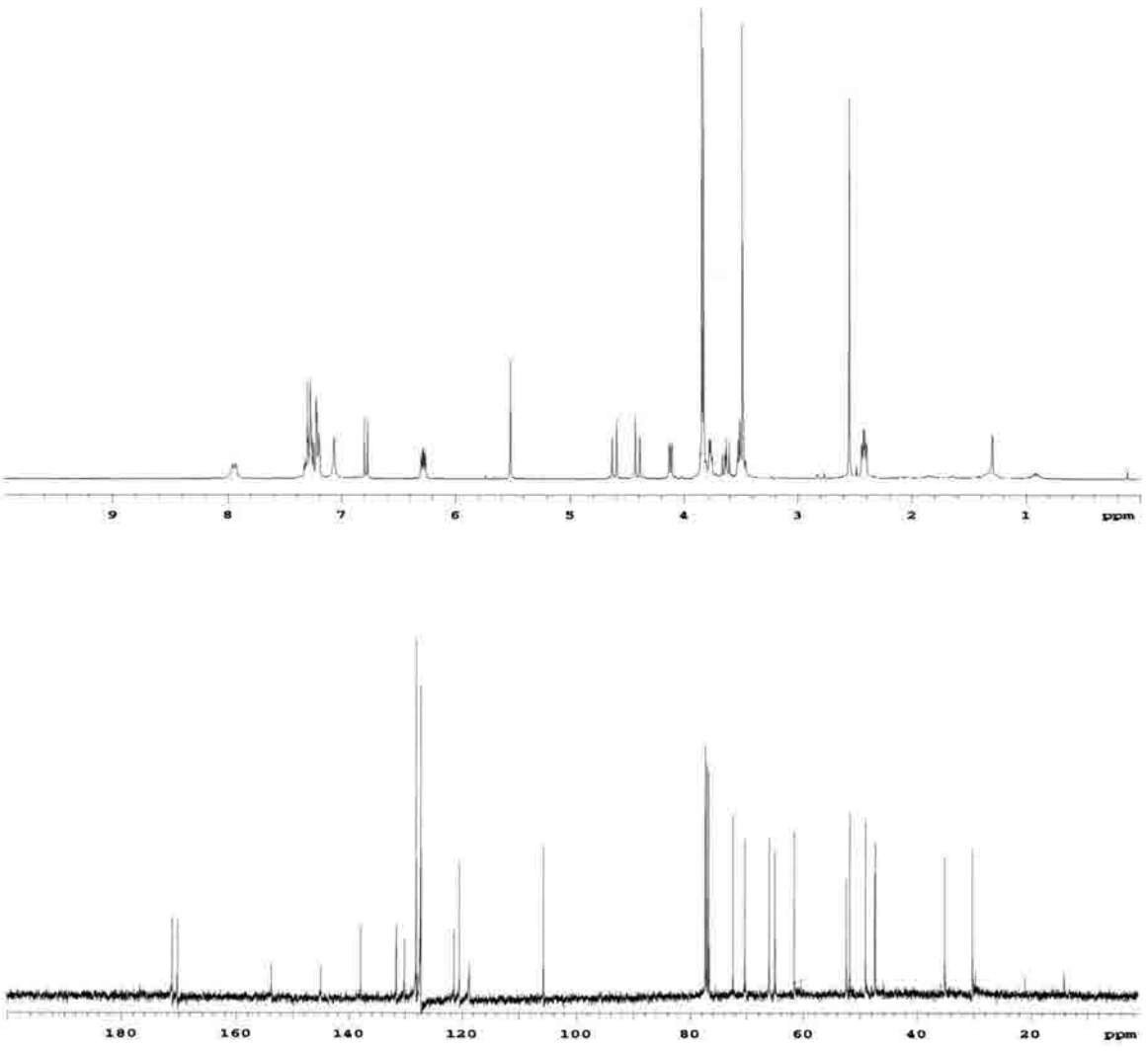
697 **707, 708**

To a solution of **697** (222 mg, 0.50 mmol) in $CHCl_3$ (15 mL) was added NBS (90 mg, 0.50 mmol, 1.0 eq.) and the solution was heated to reflux for 45 min (a dark green color formed). The solution was cooled to 0°C and methyl acrylate (2.25 mL, 25 mmol, 50 eq.) was added followed by the dropwise addition (over 10 min.) of a solution of Et_3N (696 μL, 5.0 mmol, 10eq.) in CH_2Cl_2 (10 mL) (the solution turned dark blue). This solution was stirred at rt for 1.5h. The solvent was removed under reduced pressure. The crude material was dissolved in CH_2Cl_2 and washed with sat. $NaHCO_3$. The organic layer was dried over $MgSO_4$ and the solvent was removed. The crude product was purified via flash chromatography (gradient 50-70% EtOAc/hex) to afford 58mg **707** (23%) that was slightly contaminated with a third unknown diastereomer. Tetracycle **708** had to be repurified via flash chromatrography (4% MeOH/CH_2Cl_2) to afford 59 mg **708** (23%). TLC (**707**) (80% EtOAc/hex) R_f = 0.47 (UV and PMA). TLC (**708**) (50% EtOAc/hex) R_f = 0.27 (UV and PMA). ^{1}H-NMR (300MHz) ($CDCl_3$) δ 2.43 (2H, dd *J* = 11.4, 7.8 Hz); 2.56 (3H, s); 3.49 (3H,s); 3.53 (1H, m); 3.61 (1H, d, *J* = 7.8 Hz); 3.64 (1H, d, *J* = 7.8 Hz); 3.78 (1H, m); 3.84 (3H, s); 3.85(3H, s); 4.12 (1H, d, *J* = 6.3 Hz); 4.42 (1H, 1/2 ABq, *J* = 12.3Hz); 4.61 (1H, 1/2 ABq *J* = 12.3Hz); 5.53 (1H, s); 6.29 (1H, dd, *J* = 7.2, 3.6 Hz); 6.79 (1H, d J= 8.4Hz); 7.07 (1H, s); 7.23-7.34 (5H, m), 7.95 (1H, d *J* = 8.1Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 30.27, 35.11, 47.39, 49.04, 51.80, 52.41, 61.59, 65.05, 66.10, 70.36, 72.45, 105.77, 118.83, 120.48, 121.44, 127.30, 127.38, 127.47, 128.13, 130.20, 131.68,

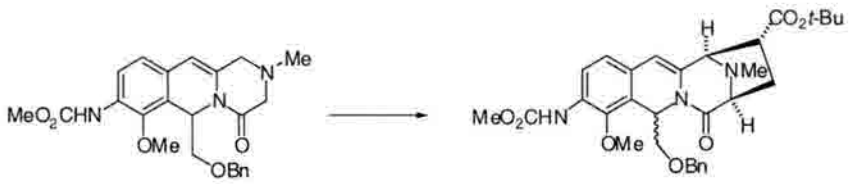
137.99, 145.07, 153.78, 170.29, 171.18. IR (NaCl, neat) 3502, 2950, 2840, 1729, 1473, 1262, 1041 cm^{-1}. $C_{28}H_{32}N_3O_7$ (MH^+): 522.2240 found: 522.2240.



708



Compound **707**: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$



697 709, 710

(5α,8α,10α, 11α)-5,7,8,9,10,11--hexahydro-5-(benzyloxymethyl)-3-amino(carbamic acid methyl ester)-4-methoxy-13-methyl-7-oxo, 8,11-Iminoazepino[1,2-b]isoquinoline-10-carboxylic acid, *tert*-butyl ester (709).

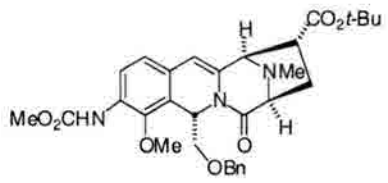
(5β,8α,10α, 11α) 5,7,8,9,10,11--hexahydro-5-(benzyloxymethyl)-3-amino(carbamic acid methyl ester)-4-methoxy-13-methyl-7-oxo, 8,11-Iminoazepino[1,2-b]isoquinoline-10-carboxylic acid, *tert*-butyl ester (710).

To a solution of **697** (2.31 g, 5.3 mmol) in $CHCl_3$ (75 mL) was added NBS (943 mg, 5.3 mmol, 1.0 eq.) and the solution was heated to reflux for 45 min (a dark green color formed). The solution was cooled to 0°C and t-butyl acrylate (15.5 mL, 106 mmol, 20 eq.) was added followed by the dropwise addition (over 10 min.) of a solution of Et_3N (5.9 mL, 42 mmol, 8 eq.) in CH_2Cl_2 (20 mL) (The solution turned dark blue). This solution was stirred at rt for 3h. The solvent was then removed under reduced pressure. The crude material was taken up in CH_2Cl_2 and washed with sat. $NaHCO_3$. The organic layer was dried over $MgSO_4$ and the solvent was removed. The crude product was purified via flash chromatography (50-60% EtOAc/hex) to afford 1.04 g **709** (35%) as a white foam and recovered 211 mg **697** (9%). Diastereomer **710** had to be repurified via flash chromatography (gradient 1-1.5% MeOH/CH_2Cl_2) to afford 200 mg **710** (7%).

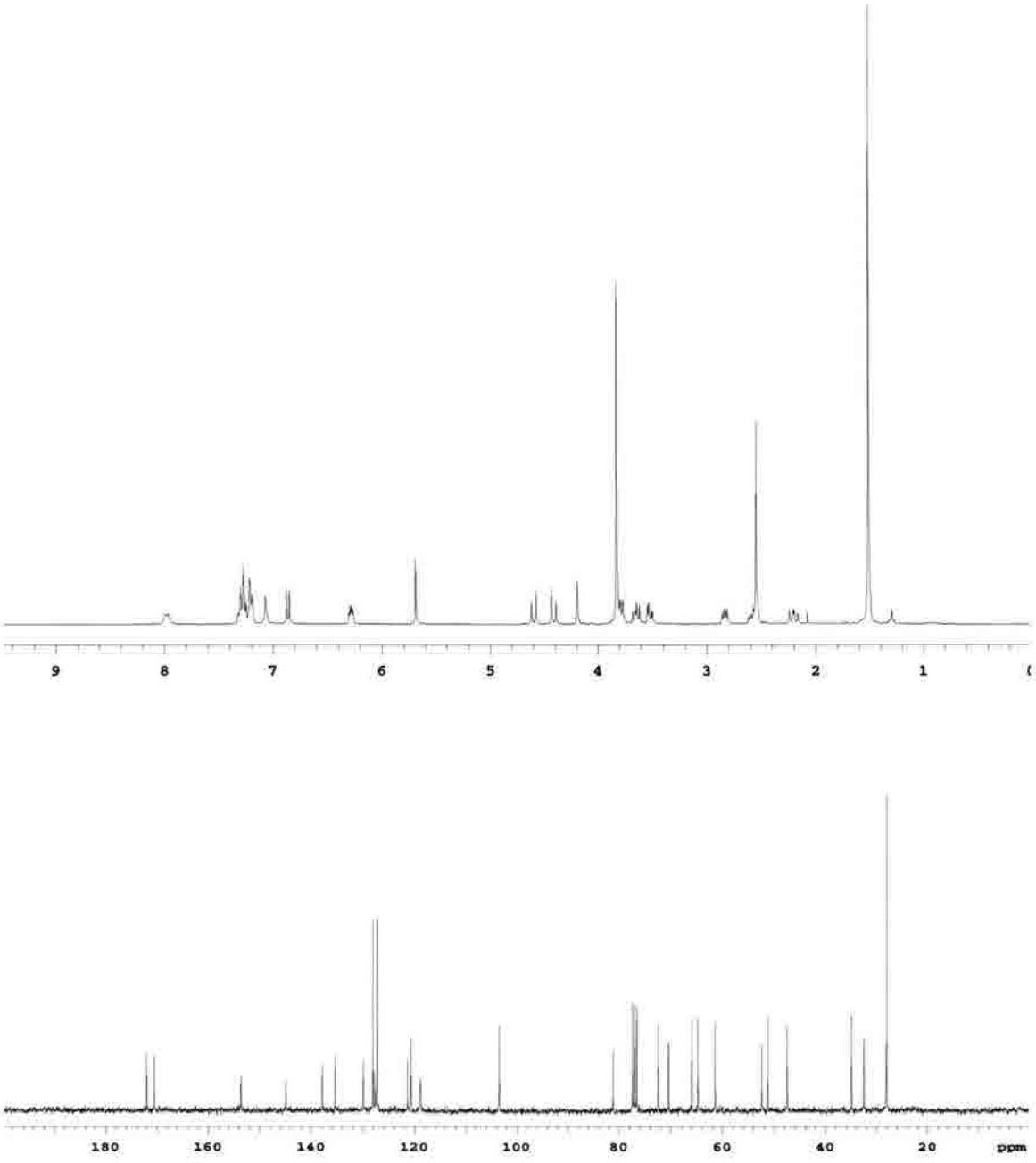
709: TLC (80% EtOAc/hex) R_f = 0.65 (UV and PMA).^{1}H-NMR (300 MHz) ($CDCl_3$ vs. TMS) δ 1.51 (9H, s); 2.21 (1H, dd, *J* = 13.2, 9.9 Hz); 2.55 (3H, s); 2.60 (1H, m); 2.83 (1H, dd, *J* = 9.9, 5.1 Hz); 3.52 (1H, dd, *J* = 10.8, 4.2 Hz); 3.65 (1H, dd, *J* = 10.8, 7.5 Hz);

3.78 (1H, m); 3.80 (6H, s); 4.19 (1H, s); 4.42 (1H, 1/2 ABq, J = 12.0 Hz); 4.58 (1H, 1/2 ABq, J = 12.0 Hz); 5.69 (1H, s); 6.28 (1H, dd, J = 7.2, 4.2 Hz); 6.86 (1H, d, J = 8.4 Hz); 7.07 (1H, s); 7.21-7.33 (5H, m); 7.98 (1H, d, J = 8.4 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 28.05, 32.48, 34.92, 47.48, 51.26, 52.39, 61.41, 64.76, 65.92, 70.40, 72.39, 81.16, 103.48, 118.83, 120.64, 121.34, 127.21, 127.25, 127.67, 128.05, 129.80, 135.34, 137.83, 144.93, 153.63, 170.50, 171.98. HRMS (FAB) calcd. for $C_{31}H_{38}N_3O_7$ (M+H) 564.2710, found 564.2693.

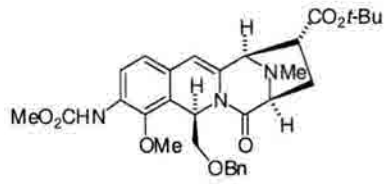
710: TLC (80% EtOAc/hex) R_f = 0.35 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs. TMS) δ 1.52 (9H, s); 2.33 (1H, dd, J = 12.9, 9.9 Hz); 2.48 (3H, s); 2.54 (1H, m); 3.09 (1H, dd, J = 9.9, 6.3 Hz); 3.44 (1H, dd, J = 10.5, 3.9 Hz); 3.62 (1H, dd, J = 10.5, 8.4 Hz); 3.68 (1H, d, J = 6.6 Hz); 3.84 (3H, s); 3.87 (3H, s); 3.98 (1H, s); 4.42 (1H, 1/2 ABq, J = 11.7 Hz); 4.65 (1H, 1/2 ABq, J = 11.7 Hz); 5.72 (1H, s); 6.26 (1H, dd, J = 8.4, 3.6 Hz); 6.88 (1H, d, J = 8.4 Hz); 7.06 (1H, s); 7.31 (5H, m); 7.98 (1H, d, J = 8.4 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 28.07, 34.55, 35.91, 45.86, 48.21, 52.46, 61.58, 65.31, 67.13, 70.31, 73.00, 81.20, 105.24, 118.91, 121.53, 121.97, 126.57, 127.67, 127.96, 128.31, 130.20, 133.75, 137.77, 144.78, 153.75, 169.10, 172.42. IR (NaCl, neat) 2949, 1724, 1687, 1525, 1230, 1205, 1096 cm^{-1}. HRMS (FAB) calcd. for $C_{31}H_{38}N_3O_7$ (M+H) 564.2710, found 564.2699.



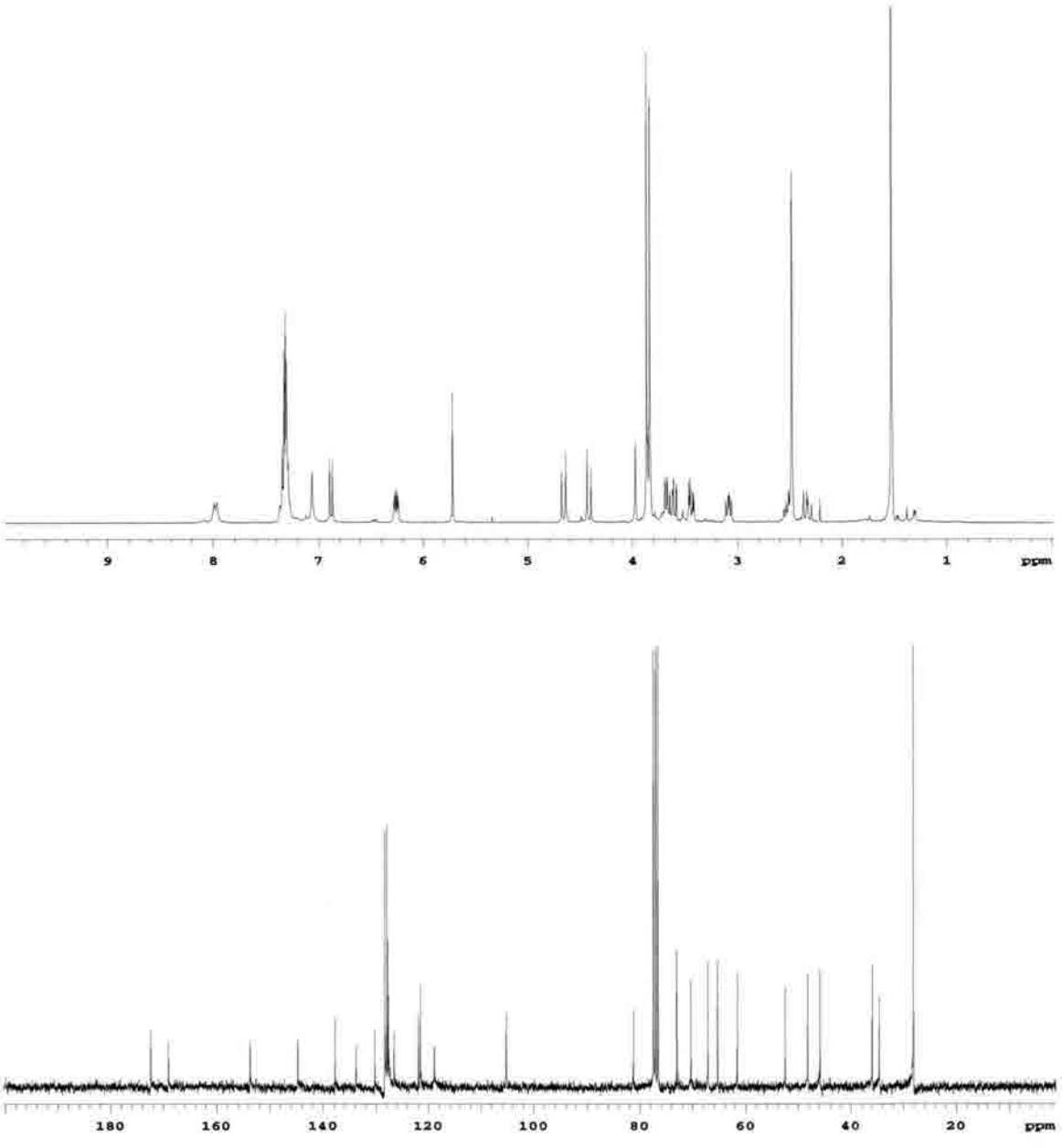
709



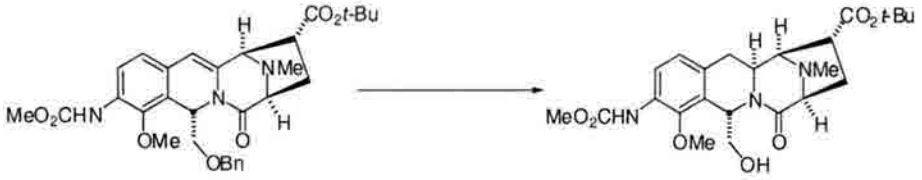
Compound **709**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$



710

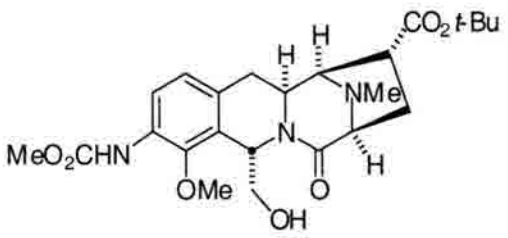


Compound **710**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$

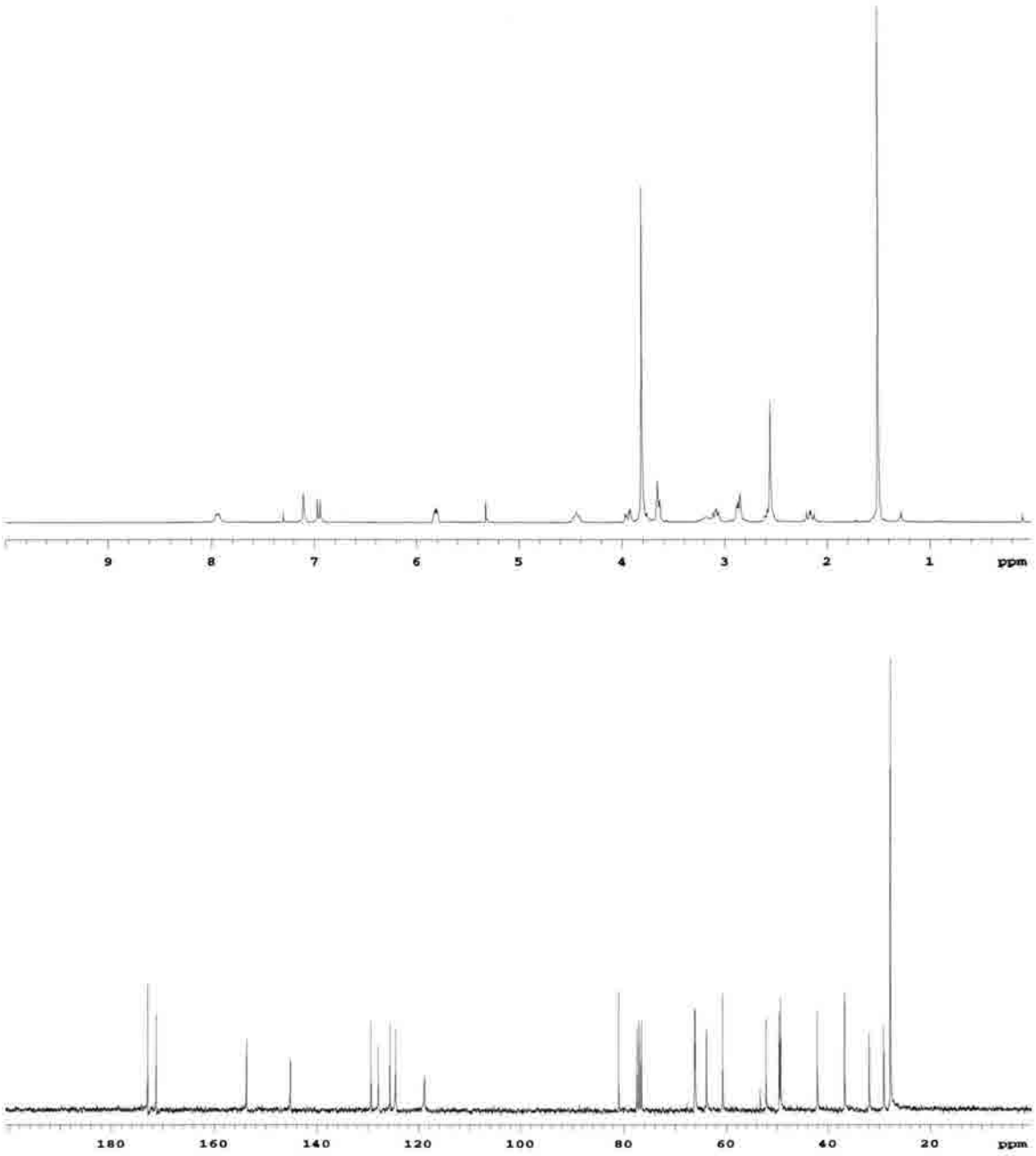


(5α,8α,10α,11α,11aα)-5,7,8,9,10,11,11a,12-octahydro-5-(hydroxymethyl)-3-amino(carbamic acid methyl ester)-4-methoxy-13-methyl-7-oxo 8,11-Iminoazepino[1,2-b]isoqunoline-10-carboxylic acid, *tert*-butyl ester (711).

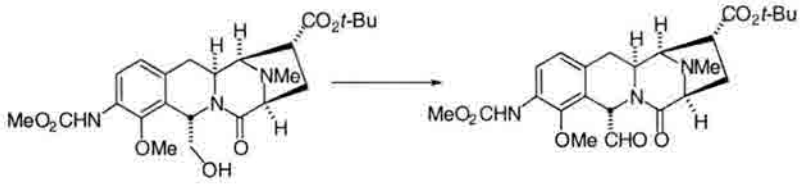
To an argon degassed solution of **709** (930 mg, 1.65 mmol) in absolute EtOH (40 mL) in a pressure vessel, Raney Nickel (W-2, Aldrich) (4 mL) was added and the vessel was pressurized to 100 psi with H_2. This mixture was stirred for 24 h. The pressure was released and the mixture was degassed with Ar. The catalyst was removed by filtering through celite. The solvent was removed *in vacuo* and the crude product was purified via flash chromatography (5% MeOH/CH_2Cl_2) to afford 710 mg **711** (90%) as a white foam. TLC (7.5% MeOH/CH_2Cl_2) R_f = 0.22 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.46 (9H, s); 2.12 (1H, dd, *J* = 13.2, 9.6 Hz); 2.51 (3H, s); 2.53 (1H, m); 2.83 (2H, m); 3.04 (1H, dd, *J* = 9.0, 6.6 Hz); 3.15 (1H, s, broad, D_2O exchangeable); 3.60 (2H, m); 3.72 (1H, m); 3.77 (6H, s); 3.90 (1H, dd, *J* = 7.8, 3.7 Hz); 4.40 (1H, m); 5.77 (1H, dd, *J* = 6.9, 3.3 Hz); 6.91 (1H, d, *J* = 8.4 Hz), 7.07 (1H, s); 7.90 (1H, d, *J* = 8.4 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 27.79, 29.03, 31.90, 36.72, 42.07, 49.28, 49.55, 52.16, 60.67, 63.83, 66.06, 66.17, 80.91, 118.85, 124.52, 125.63, 127.90, 129.36, 145.18, 153.70, 171.21, 172.86. IR (NaCl, neat) 3431, 2948, 1727, 1648, 1066 cm^{-1}. HRMS (FAB) calcd. for $C_{24}H_{34}N_3O_7$ (MH^+) 476.2397; found 476.2388.



711



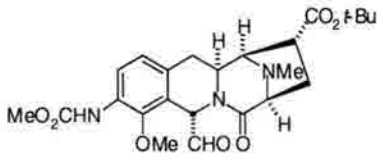
Compound **711**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$



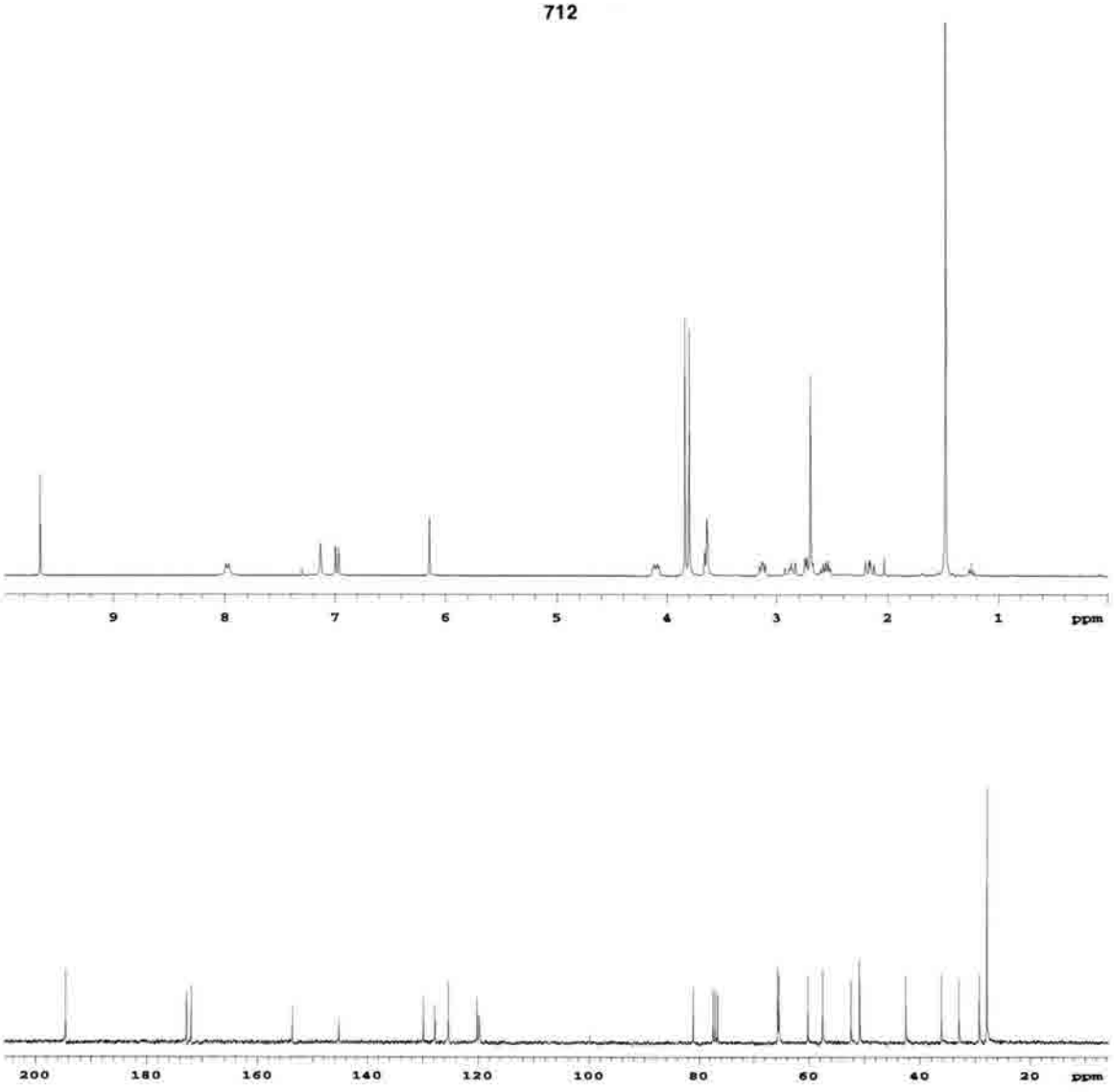
(5α,8α,10α,11α,11aα)-5,7,8,9,10,11,11a,12-octahydro-5-formyl-3-amino(carbamic acid methyl ester)-4-methoxy-13-methyl-7-oxo, 8,11-Iminoazepino[1,2-b]isoqunoline-10-carboxylic acid *tert*-butyl ester (712).

To a solution of DMSO (424 μL, 5.98 mmol, 4 eq.) in CH_2Cl_2 (35 mL) at –78°C was added oxalyl chloride (260 μL, 2.98 mmol, 2 eq.). The solution was stirred at –78°C for 10 min. To this solution **711** (710 mg, 1.49 mmol, 1 eq.) in 20 mL CH_2Cl_2 cooled to –78°C was added via a cannula. The resulting solution was stirred at –78°C for 1 h. Triethylamine (2.07 mL, 14.9 mmol, 10 eq.) was added slowly and the solution was allowed to warm to rt. The solvent was removed *in vacuo* and the crude material was partitioned between $NaHCO_3$ (aq) and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography (70% EtOAc/hexanes) to afford 694 mg **712** (98%) as a white foam. TLC (80% EtOAc/hex) R_f = 0.20 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.48 (9H, s); 2.18 (1H, dd, *J* = 13.2, 9.9 Hz); 2.57 (1H, m); 2.71 (3H, s); 2.74 (1H, m); 2.87 (1H, m); 3.13 (1H, dd, *J* = 9.0, 6.3 Hz); 3.65 (2H, m); 3.81 (3H, s); 3.85 (3H, s); 4.12 (1H, m); 6.15 (1H, s); 6.98 (1H, d, *J* = 8.4 Hz); 7.10 (1H, s); 8.00 (1H, d, *J* = 8.4 Hz); 9.67 (1H, s). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 27.93, 29.28, 32.93, 36.10, 42.52, 50.85, 52.39, 57.48, 60.17, 65.35, 65.58, 81.04, 119.77, 120.20, 125.32, 127.73, 129.84, 145.20, 153.58, 172.01, 172.85, 194.57. IR (NaCl, neat)

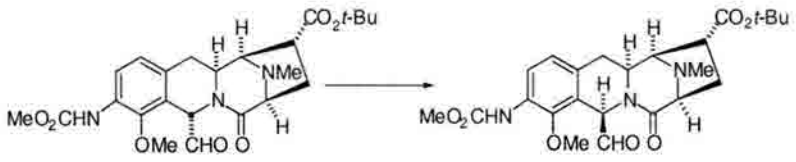
2932, 1737, 1703, 1472, 1031 cm^{-1}. HRMS (FAB) calcd. for $C_{24}H_{32}N_3O_7$ (MH^+) 474.2240; found 474.2241.



712

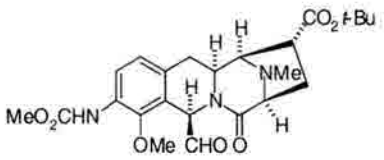


Compound **712**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$

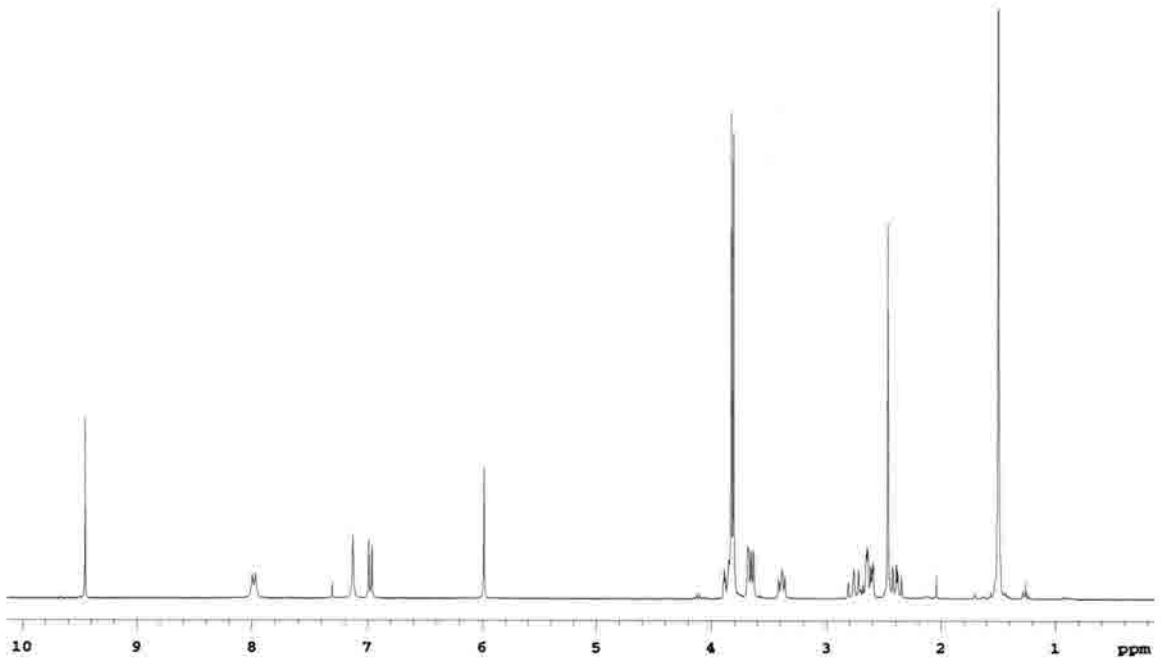


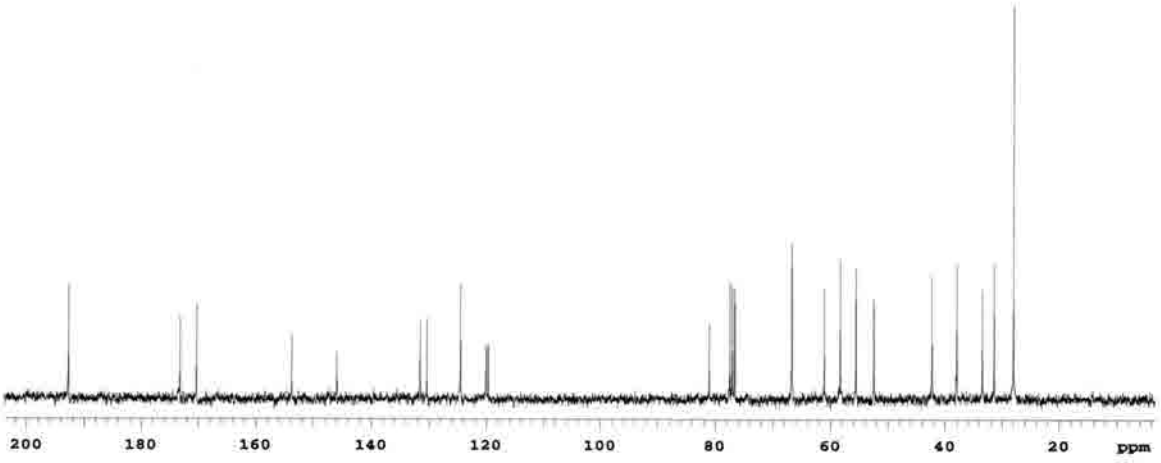
(5α,8β,10β,11β,11aβ)-5,7,8,9,10,11,11a,12-octahydro-5-formyl-3-amino(carbamic acid methyl ester)-4-methoxy-13-methyl-7-oxo, 8,11-Iminoazepino[1,2-b]isoqunoline-10-carboxylic acid *tert*-butyl ester (713).

To a solution of **712** (290 mg, 0.613 mmol) in THF (20 mL) was added DBU (84 μL, 0.613 mmol, 1 eq.) and the solution was allowed to stir at room temperature for 24 h. Sat. $NaHCO_3$ was added and the aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified via flash chromatography (gradient 80/20/0 - 90/10/0 - 94/0/4 EtOAc/hexanes/MeOH) to afford 160 mg **713** (55%) as a white foam along with starting aldehyde 109 mg **712** (38%). TLC (80% EtOAc/hex) R_f = 0.08 (UV and PMA). ^{1}H-NMR (300 MHz) (CDCl3 vs TMS) δ 1.49 (9H, s); 2.40 (1H, dd, *J* = 12.9, 9.9 Hz); 2.47 (3H, s); 2.64 (2H, m); 2.74 (1H, m); 3.39 (1H, t, *J* =8.1 Hz); 3.66 (3H, m); 3.81 (3H, s); 3.83 (3H, s); 5.99 (1H, s); 6.98 (1H, d, *J* = 8.4 Hz); 7.09 (1H, s); 7.99 (1H, d, *J* = 8.4 Hz); 9.46 (1H, s). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 27.97, 31.35, 33.43, 37.93, 42.25, 52.47, 55.60, 58.30, 61.06, 66.66, 66.72, 81.00, 119.65, 120.06, 124.49, 130.36, 131.53, 145,96, 153.72, 170.30, 173.22, 192.70. IR (NaCl, neat) 2977, 2948, 1729, 1659, 1525, 1078 cm^{-1}. HRMS (FAB) calcd. for $C_{24}H_{32}N_3O_7$ (MH^+) 474.2240; found 474.2237.

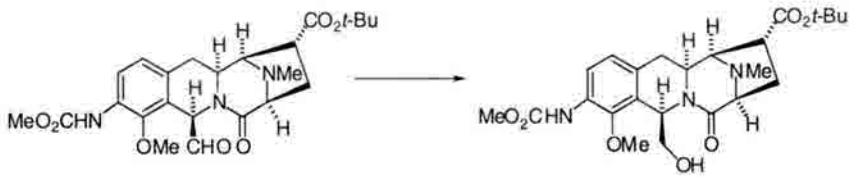


713





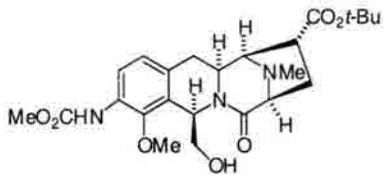
Compound **713**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$



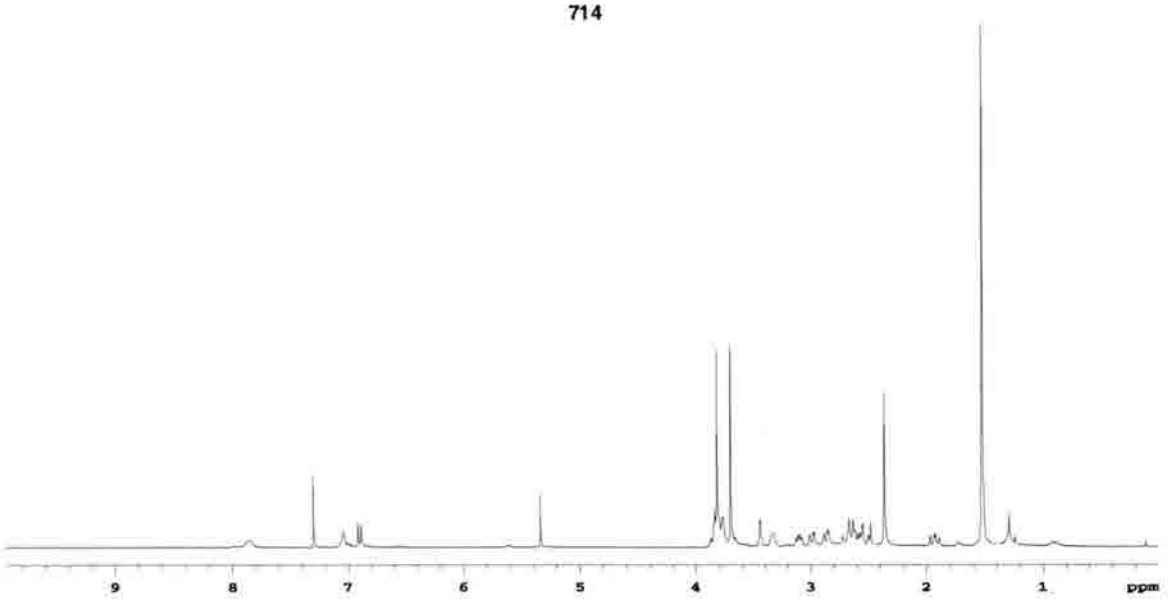
713 714

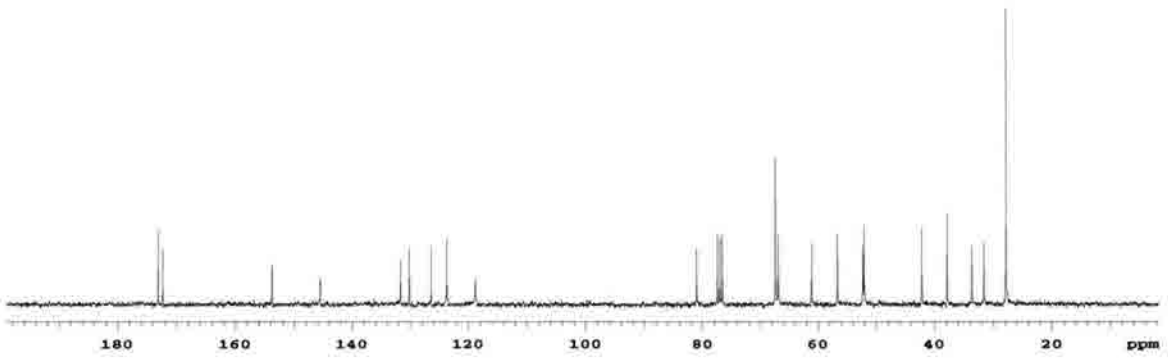
(5α,8β,10β,11β,11aβ)-5,7,8,9,10,11,11a,12-octahydro-5-(hydroxymethyl)-3-amino(carbamic acid methyl ester)-4-methoxy-13-methyl-7-oxo, 8,11-Iminoazepino[1,2-b]isoqunoline-10-carboxylic acid, *tert*-butyl ester (714).

To a solution of **713** (174 mg, 0.368 mmol) in absolute EtOH (15 mL) at 0°C was added sodium borohydride (56mg, 1.47mmol, 4 eq.). This mixture was stirred at 0°C for 1 h. Aqueous 1 M HCl was added slowly until there was no more H_2 evolution. Excess sat. $NaHCO_3$ was added and the ethanol was removed by rotary evaporation. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified via flash chromatography (5% MeOH/CH_2Cl_2) to afford 156 mg **714** (89%) as a white solid. TLC (7.5% MeOH/CH_2Cl_2) R_f = 0.29 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$ vs TMS) δ 1.44 (9H, s); 2.25 (1H, dd, *J* = 12.9, 9.3 Hz); 2.37 (3H, s); 2.55 (2H, m); 2.87 (1H, t, *J* = 12.9 Hz); 3.12 (1H, dd, *J* = 9.0, 6.9 Hz); 3.20 (1H, t, *J* = 5.7 Hz); 3.50 (3H, m, 1 H is D_2O exchangeable); 3.70 (3H, s); 3.73 (3H, s); 3.79 (2H, m); 5.45 (1H, dd, *J* = 5.1, 3.6 Hz); 6.88 (1H, d, *J* = 7.8 Hz); 7.15 (1H, s); 7.84 (1H, d, *J* = 7.8 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 27.92, 31.63, 33.64, 37.93, 42.20, 52.08, 52.31, 56.68, 61.10, 66.90, 67.39, 67.45, 81.00, 118.77, 123.68, 126.39, 130.14, 131.65, 145.57, 153.79, 172.27, 173.05. IR (NaCl, neat) 3388, 2977, 2948, 1725, 1638, 1527, 1064 cm^{-1}. HRMS (FAB) calcd. for $C_{24}H_{34}N_3O_7$ (MH^+) 476.2397; found 476.2400.

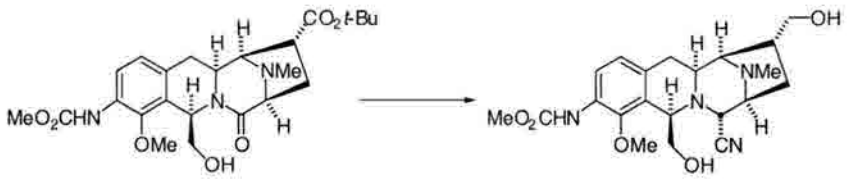


714





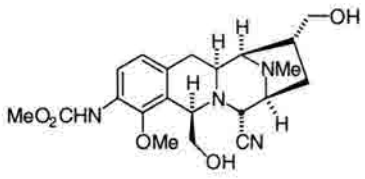
Compound **714**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in CDCl



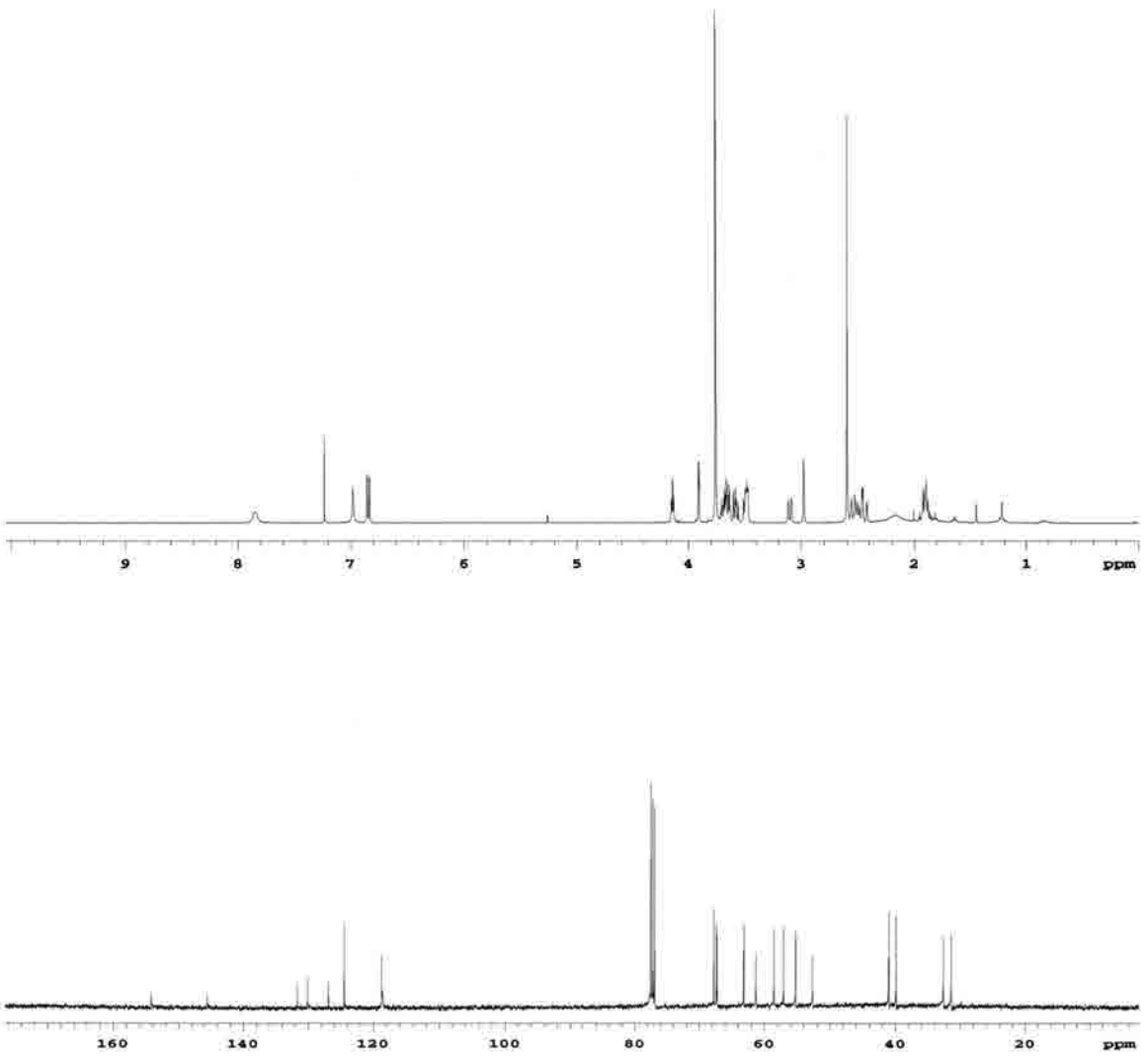
(5α,8β,10β,11β,11aβ)-5,7,8,9,10,11,11a,12-octahydro-5-(hydroxymethyl)-3-amino(carbamic acid methyl ester)-7-cyano--10-(hydroxy methyl)-4-methoxy-13-methyl-8,11-Iminoazepino[1,2-b]isoqunoline (715).

To a solution of lithium aluminum hydride (1M soln. in hexanes) (160 μL, 0.16mmol, 8 eq.) in THF (750 μL) at 0°C, ethyl acetate (7.8 μL, 0.08 mmol, 4 eq.) was added. This solution was allowed to stir at 0°C for 2 hours. To this solution, a solution of **714** (9.5 mg, 0.020 mmol) in THF (1 mL) was added dropwise. This solution was allowed to stir at 0°C for 45 min. Acetic acid (34 μL, 0.60 mmol, 30 eq.) was added slowly followed by an aqueous solution of KCN (4.5 M, 27 μL, 6 eq.). The resulting solution was stirred at room temperature for 16 hours. Excess sat. $NaHCO_3$ was added and the solution was extracted with 1:1 EtOAc/THF (3 x 10 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude material was purified by flash chromatography (2.5-5% MeOH/CH_2Cl_2) to yield 5.7mg **715** (69%) as an oil. TLC (7.5% MeOH/CH_2Cl_2) R_f = 0.15 (UV and PMA). ^{1}H-NMR (400 MHz) ($CDCl_3$) δ 1.93 (3H, m); 2.19 (1H, s, broad); 2.46 (1H, dd, *J* = 11.1, 1.8 Hz); 2.51-2.56 (2H, m); 2.63 (3H, s); 3.01 (1H, s); 3.13 (1H, d, *J* = 8.7 Hz); 3.50-3.54 (2H, m); 3.60 (1H, dd, *J* = 7.5, 5.4 Hz); 3.66-3.73 (2H, m); 3.78 (3H, s); 3.79 (3H, s); 3.93 (1H, d, *J* = 1.8 Hz); 4.17 (1H, dd, *J* = 3.3, 3.3 Hz); 6.87 (1H, d, *J* = 6.3 Hz); 7.01 (1H, s); 7.87 (1H, s, broad). ^{13}C-NMR (100 MHz) ($CDCl_3$) δ 31.40, 32.63, 39.91, 41.00, 52.63, 55.23, 57.02, 58.11, 61.24, 63.09, 61.20, 67.33, 67.70, 118.63,

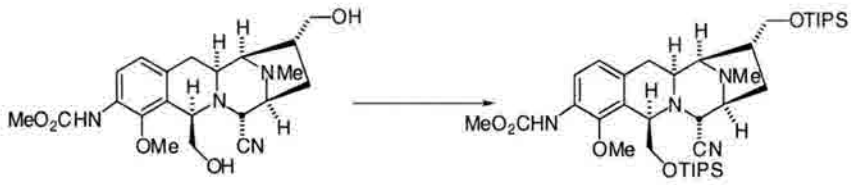
118.75, 124.46, 126.91, 130.08, 131.68, 145.57, 154.18. IR (NaCl, neat) 3418, 2979, 2248, 1724, 1048. HRMS (FAB) calcd. for $C_{21}H_{29}N_4O_5$ (MH^+) 417.2138; found 417.2135.



715



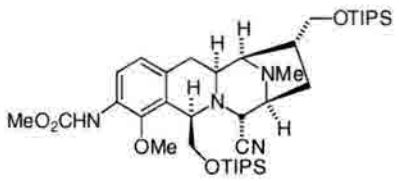
Compound **715**: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$



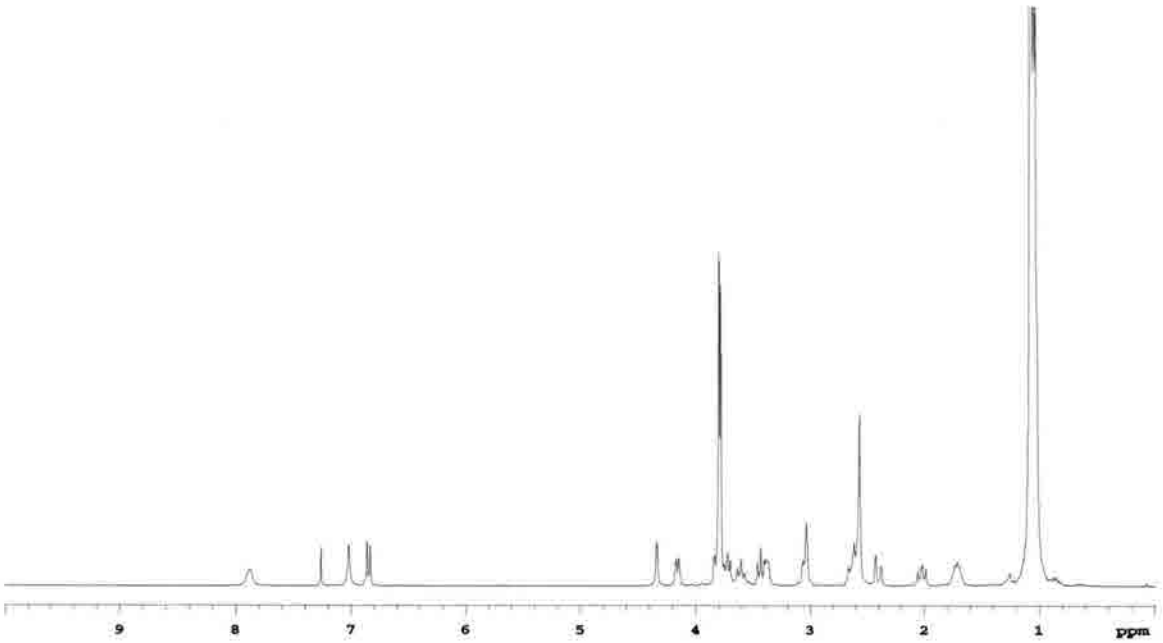
715 716

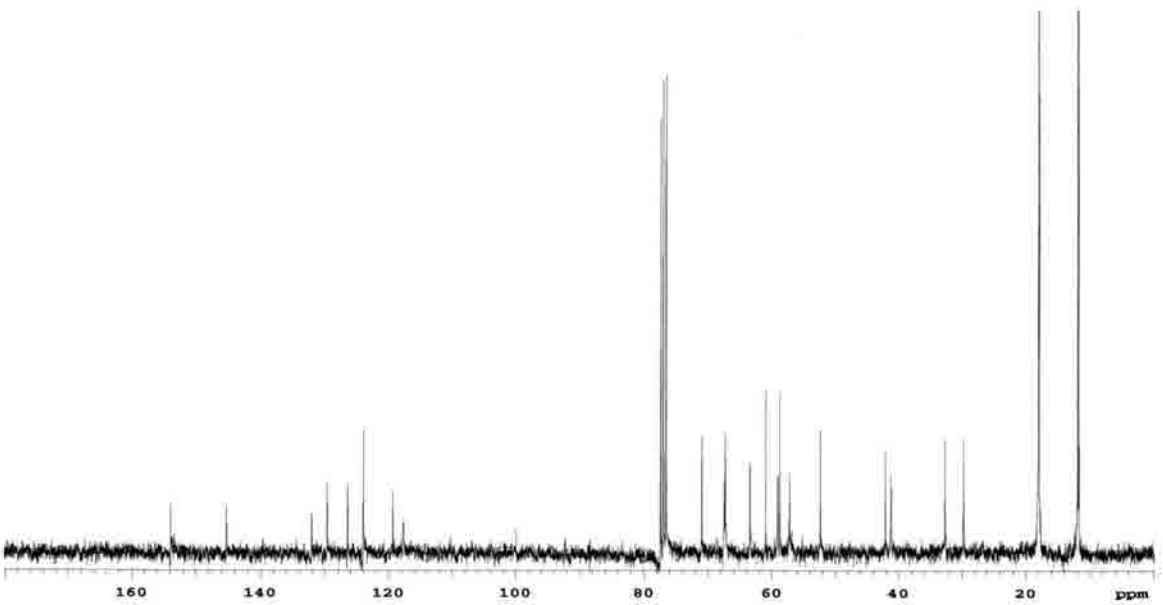
(5α,8β,10β,11β,11aβ)-10-(triisopropylsilyloxymethyl) 5,7,8,9,10,11, 11a,12-octahydro-5-(triisopropylsilyloxymethyl)-3-amino(carbamic acid methyl ester)-7-cyano-4-methoxy-13-methyl-8,11-Iminoazepino[1,2-b]isoqunoline (716).

Aminonitrile **715** (28 mg, 0.67 mmol), triisopropylsilyl choride (58 μL, 0.269 mmol, 4 eq.), imidazole (37 mg, 0.536mmol, 8 eq.), and Et_3N (93 μL, 0.67 mmol, 1.0 eq.) were dissolved in a minimum amount of DMF (ca. 500 μL). This solution was allowed to stir for 18 hours. The solution was partitioned in water and Et_2O. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromagraphy (2.5 % MeOH/CH_2Cl_2) to yield 45 mg **716** (92%) as a clear oil. TLC (5% MeOH/CH_2Cl_2) R_f = 0.44 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$) δ 0.95-1.20 (42H, m); 1.71 (1H, ddd, *J* = 12, 6, 6 Hz); 2.03 (1H, m); 2.40 (1H, m); 2.57 (3H, s); 2.61 (2H, m); 2.95 (3H, m); 3.36 (1H, m); 3.43 (1H, dd, *J* = 9.0, 9.0 Hz); 3.60 (1H, dd, *J* = 9.6, 9.6 Hz); 3.72 (1H, m); 3.78 (3H, s); 3.80 (3H, s); 4.15 (1H, dd, *J* = 8.4, 2.1 Hz); 4.33 (1H, d, *J* = 2.1 Hz); 6.85 (1H, d, *J* = 8.0 Hz); 7.02 (1H, s); 7.88 (1H, dd, *J* = 8.0 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 11.79, 11.94, 17.94, 18.04, 29.77, 32.64, 41.19, 42.07, 52.32, 57.22, 58.76, 59.09, 60.95, 63.42, 67.29, 67.49, 70.94, 117.76, 119.40, 124.03, 126.44, 129.68, 132.06, 145.28, 153.92. . IR (NaCl ,neat) 2939, 2865, 1727, 1498, 1461, 1095, 1064 cm^{-1}. HRMS (FAB) calcd. For $C_{39}H_{69}N_4O_5Si_2$ (MH^+) 729.4807; found 729.4805.

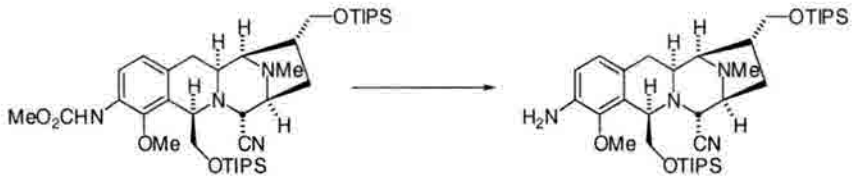


716





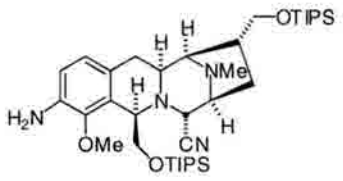
Compound **716**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) in $CDCl_3$



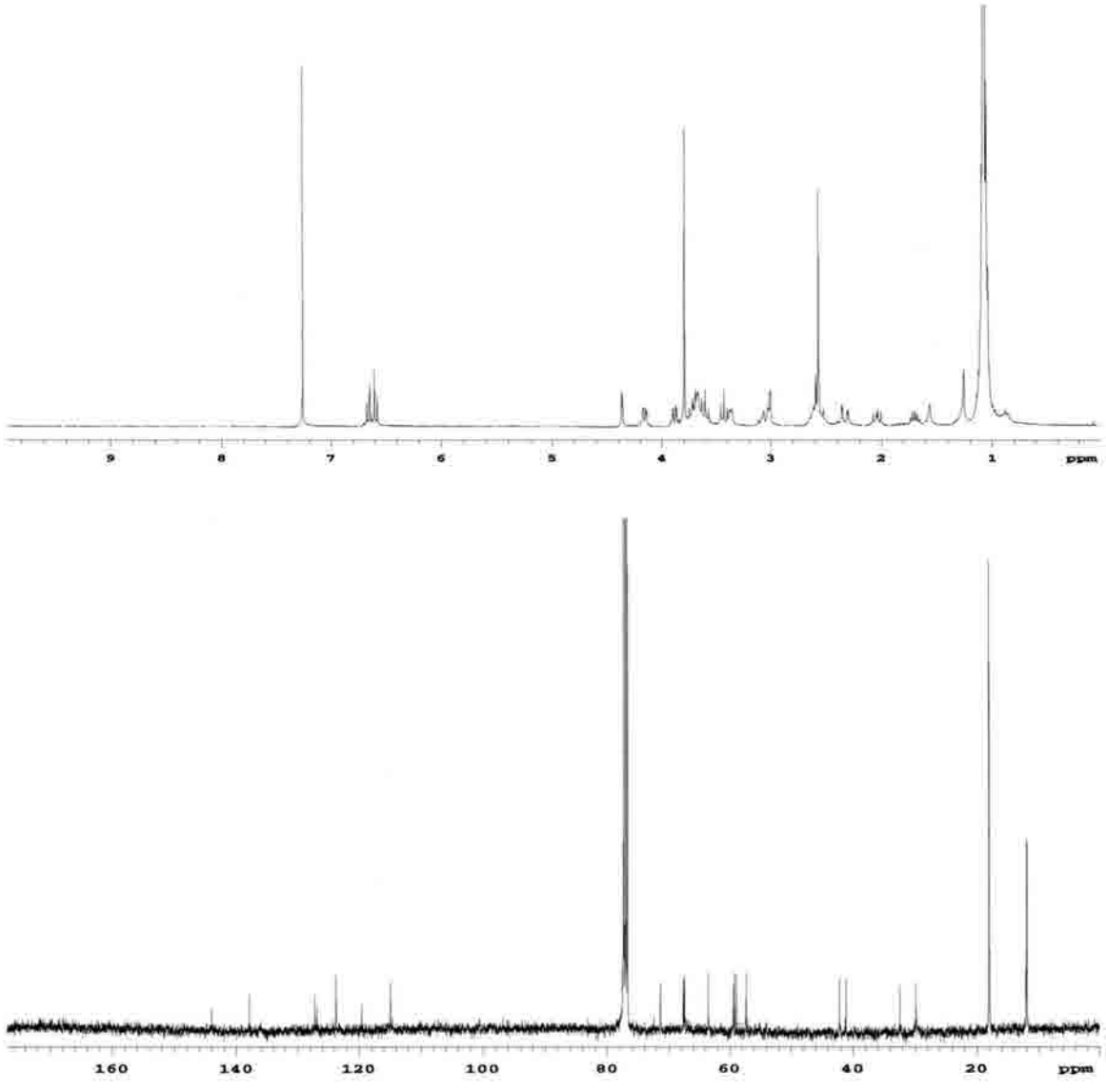
716 717

(5α,8β,10β,11β,11aβ)-10-(triisopropylsilyloxymethyl) 5,7,8,9,10,11, 11a,12-octahydro-5-(triisopropylsilyloxymethyl)-3-amino-7-cyano-4-methoxy-13-methyl-8,11-Iminoazepino[1,2-b]isoqunoline (717).

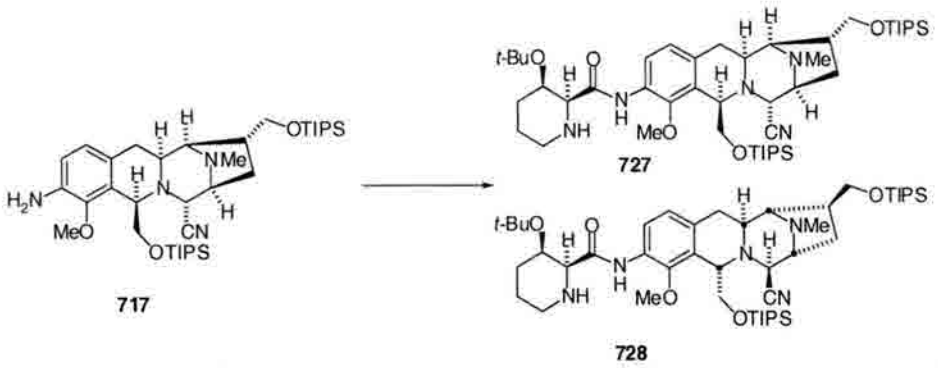
To a solution of **716** (12 mg, 0.17 mmol) in EtOH (2 mL) was added 2M LiOH (200 μL) and the solution was heated to reflux for 5.5 h. The solvent was removed *in vacuo* and the crude product was partitioned between CH_2Cl_2 and water. The pH was adjusted to 7 with dilute HCl and the aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were dried over Na_2SO_4 and the solvent was removed. The crude product was purified via flash chromatograhy (2.5% MeOH/CH_2Cl_2) to afford 7 mg **717** (63%) as a clear oil. TLC (5% MeOH/CH_2Cl_2) R_f = 0.31 (UV and PMA). ^{1}H-NMR (300 MHz) ($CDCl_3$) δ 1.11 (42H, m); 1.61 (1H, s); 1.76 (1H, dd, *J* = 12.0, 5.7 Hz); 2.08 (1H, dd, *J* = 12.3, 9.0 Hz); 2.37 (1H, dd, *J* = 14.4, 2.4 Hz); 2.61 (3H, s); 2.64 (1H, m); 3.07 (2H, m); 3.40 (1H, d, *J* = 6.3 Hz); 3.47 (1H, t, *J* = 9.3 Hz); 3.71 (4H, m); 3.84 (3H, s); 3.92 (1H, dd, *J* = 9.3, 2.4 Hz); 4.20 (1H, dd, *J* = 9.0, 2.4 Hz); 4.41 (1H, d, *J* = 2.4 Hz); 6.63 (1H, d, *J* = 8.1 Hz); 6.71 (1H, d, *J* = 8.1 Hz). ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 12.04, 12.17, 18.20, 18.26, 30.03, 32.64, 41.33, 42.33, 57.49, 59.00, 59.34, 59.56, 63.69, 67.59, 67.79, 71.52, 115.06, 119.79, 123.96, 127.06, 127.41, 138.14, 144.20. IR (NaCl ,neat) 3437, 3368, 2942, 2865, 1734, 1498, 1461, 1097, 1064 cm^{-1}. HRMS (FAB) calcd. For $C_{37}H_{67}N_4O_3Si_2$ (MH^+) 671.4752; found 671.4769.



717



Compound **717**: ^{1}H-NMR (300 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$



(5S,7R,8S,10R,11R,11aS)-5,7,8,9,10,11,11a,12-octahydro-5-(triisopropylsilyloxymethyl)-3-amino [(2'S,3'R)-piperidine-2'-carboxy-3'- *tert*-butyloxy]-7-cyano-4-methoxy--10-(triisopropylsilyloxymethyl)-13-methyl-8,11-Iminoazepino[1,2-b]isoqunoline [(+)-727].

(5R,7S,8R,10S,11S,11aR)-5,7,8,9,10,11,11a,12-octahydro-5-(triisopropylsilyloxymethyl)-3-amino [(2'S,3'R)-piperidine-2'-carboxy-3'-*tert*-butyloxy]-7-cyano--10-(triisopropylsilyloxymethyl)-4-methoxy-13-methyl-8,11-Iminoazepino[1,2-b]isoqunoline [(-)-728].

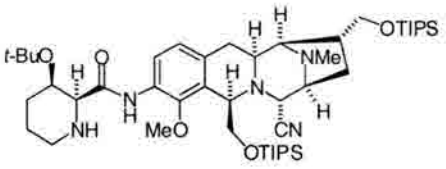
To a solution of (+)-**725** (44 mg, 1.04 mmol, 1.8 eq) in CH_2Cl_2 (4 mL) was added oxalyl chloride (10 μL, 0.116 mmol, 2.0 eq) and DMF (0.8 μL, 0.01 mmol, 0.18 eq) and the resultant solution was stirred at rt for 1 h. Hexanes (4 mL) were added and the solution was filtered through a cotton plug and the solvent removed *in vacuo*. A solution of aniline **717** (39 mg, 0.058 mmol, 1 eq) and DMAP (7.1 mg, 0.058 mmol, 1 eq) in CH_2Cl_2 (1 mL) was added to the acid chloride and the solution was stirred at rt for 24 h. The solution was diluted with CH_2Cl_2 and washed with $NaHCO_3$(aq). The organic layer was dried over Na_2SO_4 and the solvent was removed. The crude product was redissolved in CH_2Cl_2 (4 mL) and DBU (10 μL, 0.075 mmol, 1.3 eq) was added and the solution was stirred at rt for 15 h. The solution was washed with $NaHCO_3$ and dried over Na_2SO_4.

The solvent was removed and the crude product was purified via flash chromatography (gradient 2.5-4.0% MeOH/CH_2Cl_2) to afford 17 mg (+)-**727** (35%) and 17 mg (-)-**728** (35%).

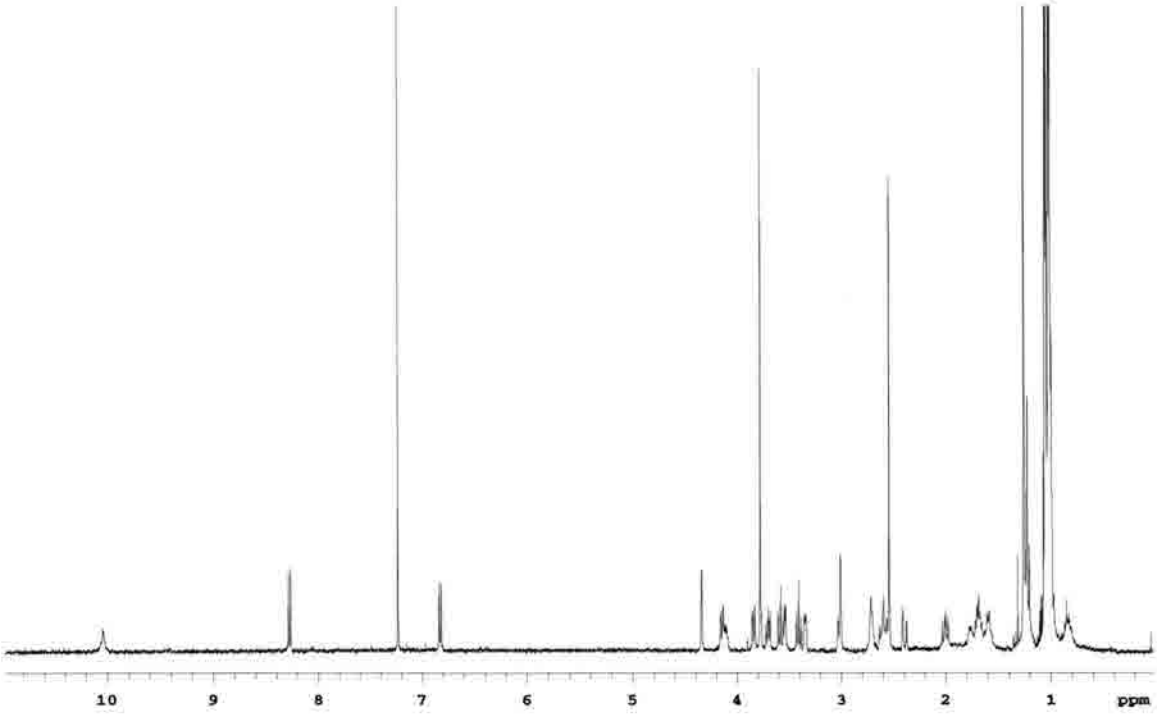
(+)-**727**: ^{1}H-NMR (400 MHz) ($CDCl_3$) δ 1.07 (42H, m); 1.25 (3H, s); 1.28 (6H, s); 1.63 (2H, m); 1.71 (3H, m); 1.80 (1H, m); 2.01 (1H, d, *J* = 8.0 Hz); 2.04 (1H, d, *J* = 9.2 Hz); 2.42 (1H, d, *J* = 12.8 Hz); 2.57 (3H, s); 2.62 (1H, m); 2.74 (2H, m); 3.04 (2H, m); 3.37 (1H, d, broad, *J* = 6.4 Hz); 3.43 (1H, t, *J* = 8.8 Hz); 3.57 (1H, d, *J* = 4.0 Hz); 3.61 (1H, t, *J* = 9.6 Hz); 3.73 (1H, dd, *J* = 9.6, 5.8 Hz); 3.80 (3H, s); 3.86 (1H, dd, *J* = 9.2, 1.5 Hz); 4.16 (2H, m); 4.37 (1H, d, *J* = 2.8 Hz); 6.85 (1H, d, *J* = 8.0 Hz); 8.30 (1H, d, *J* = 8.0 Hz); 10.06 (1H, s, broad). ^{13}C-NMR (100 MHz) ($CDCl_3$) δ 11.78, 11.95, 17.98, 18.05, 23.96, 28.17, 29.75, 31.24, 32.68, 41.25, 42.12, 42.69, 57.28, 58.81, 59.20, 61.10, 62.24, 63.49, 67.35, 67.54, 68.64, 71.15, 75.67, 119.13, 119.39, 123.81, 126.41, 130.36, 132.42, 146.08, 170.24. IR (NaCl, neat) 2928, 2864, 1733, 1652, 1559, 1049 cm^{-1}. . HRMS (FAB) calcd. For $C_{47}H_{84}N_5O_5Si_2$ (MH^+) 854.6011; found 854.6006. $[\alpha]_D^{25}$ = +26.7 (c = 0.08 $CHCl_3$).

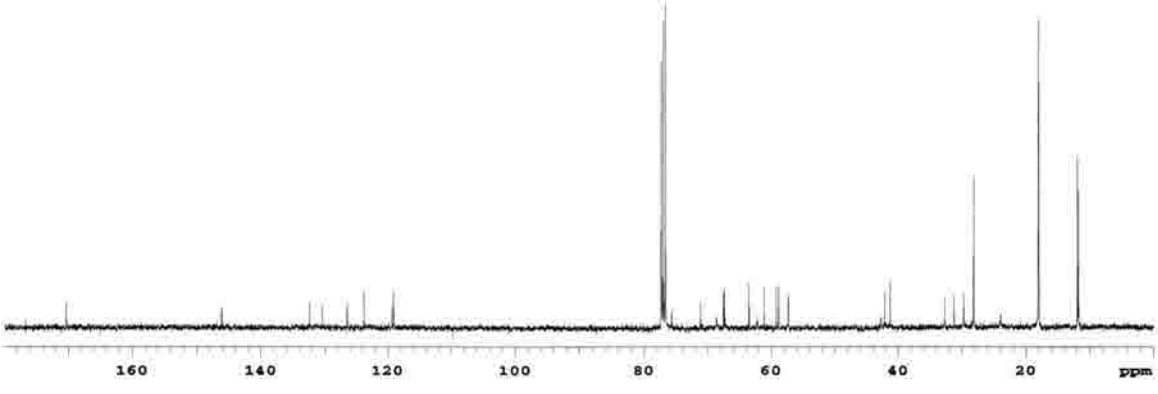
(-)-**728**: ^{1}H-NMR (400 MHz) ($CDCl_3$) δ 1.02-1.13 (42H, m); 1.26 (9H, s); 1.58-1.80 (7H, m); 2.03 (1H, dd, *J* = 10.0, 9.2 Hz); 2.42 (1H, dd, *J* = 15.2, 2.0 Hz); 2.57 (3H, s); 2.62 (2H, m); 2.84 (1H, m); 3.03 (2H, m); 3.36 (1H, d, broad, *J* = 7.6 Hz); 3.44 (1H, t, *J* = 8.8 Hz); 3.61 (2H, t, *J* = 9.6 Hz); 3.72 (1H, dd, *J* = 9.2, 6.9 Hz); 3.80 (3H, s); 3.85 (1H, dd, *J* = 9.2, 2.4 Hz); 4.17 (1H, dd, *J* = 6.4, 2.0 Hz); 4.28 (1H, m); 4.35 (1H, d, *J* = 2.8 Hz); 6.85 (1H, d, *J* = 8.8 Hz); 8.06 (1H, d, *J* = 8.8 Hz); 9.51 (1H, s, broad). ^{13}C-NMR (100 MHz) ($CDCl_3$) δ 11.80, 11.95, 17.98, 18.05, 22.92, 28.52, 29.68, 29.82, 31.33, 32.75, 41.13, 42.09, 57.10, 58.73, 59.10, 60.73, 63.43, 67.26, 67.31, 67.52, 71.08, 74.69, 74.88, 119.45,

120.29, 123.78, 126.45, 129.57, 132.94, 146.69, 169.97. IR (NaCl, neat) 2928, 2865, 1733, 1653, 1457, 1047 cm^{-1}. HRMS (FAB) calcd. For $C_{47}H_{84}N_5O_5Si_2$ (MH^+) 854.6011; found 854.6003. $[\alpha]_D^{25} = -8.2$ (c = 0.11 $CHCl_3$).

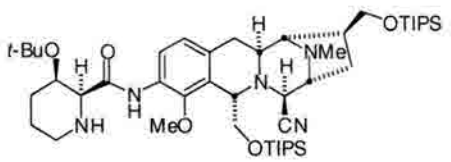


(+)-**727**

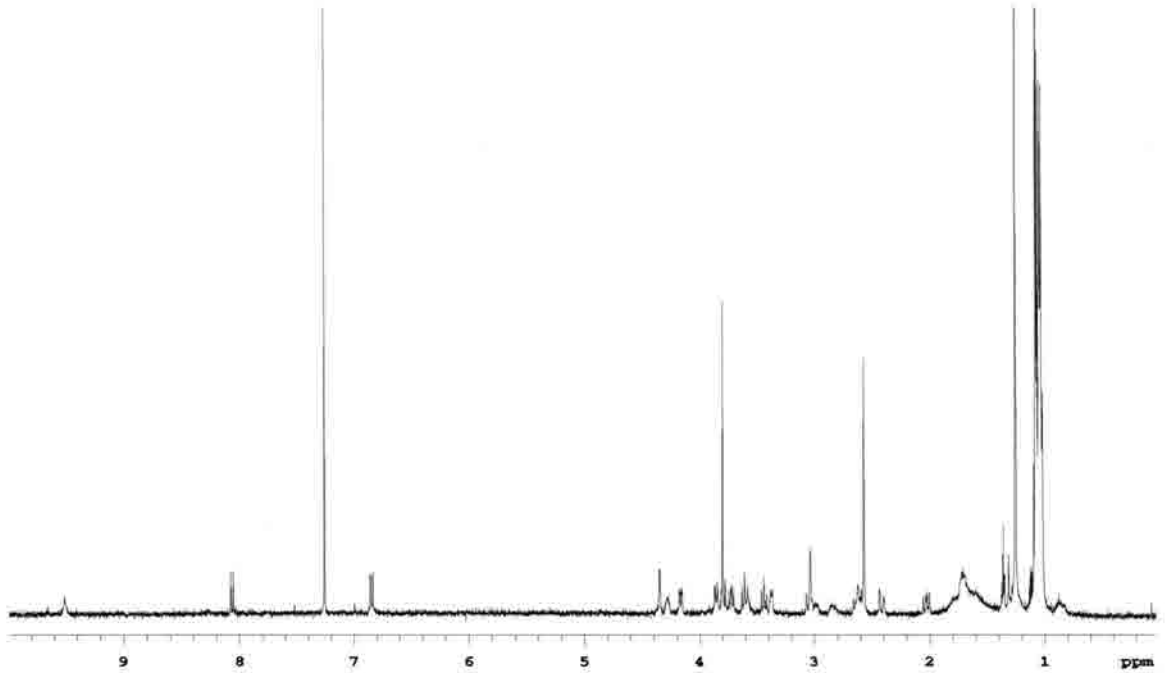


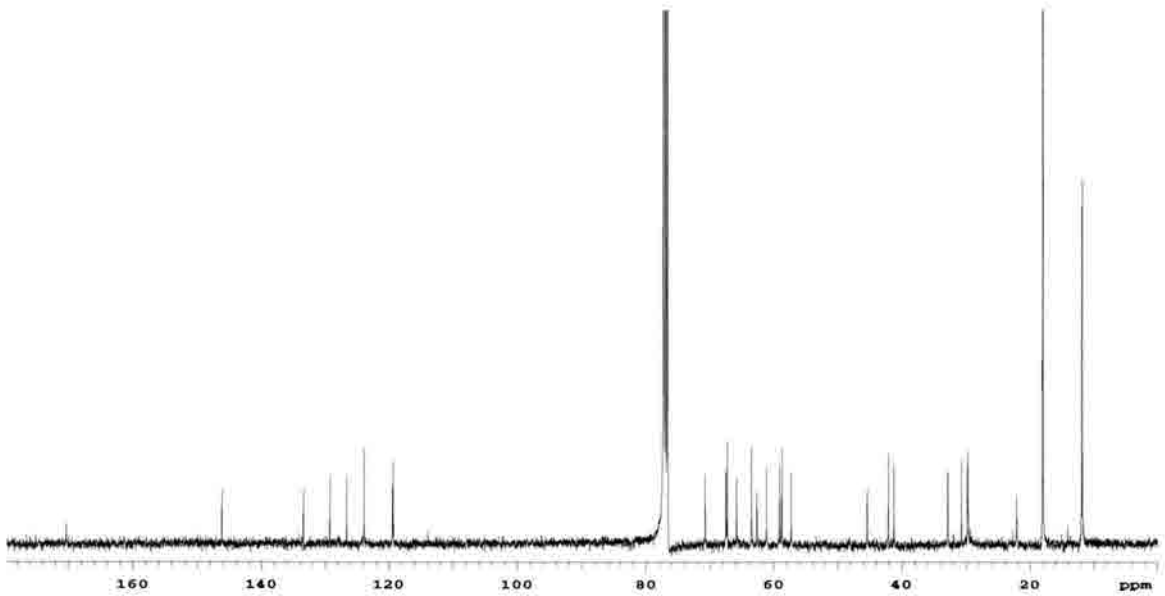


Compound **727**: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$

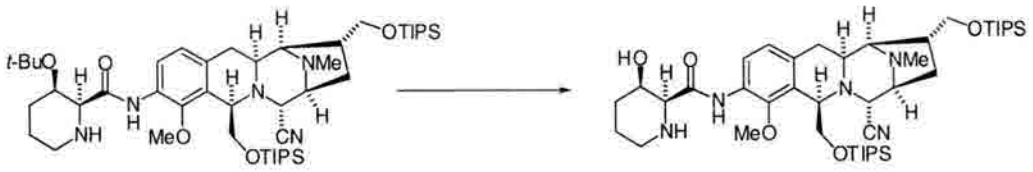


(-)-**728**





Compound **728**: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$

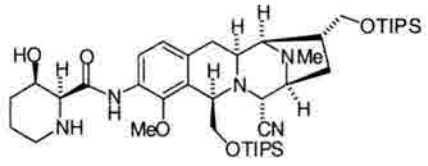


(+)-**727** (+)-**729**

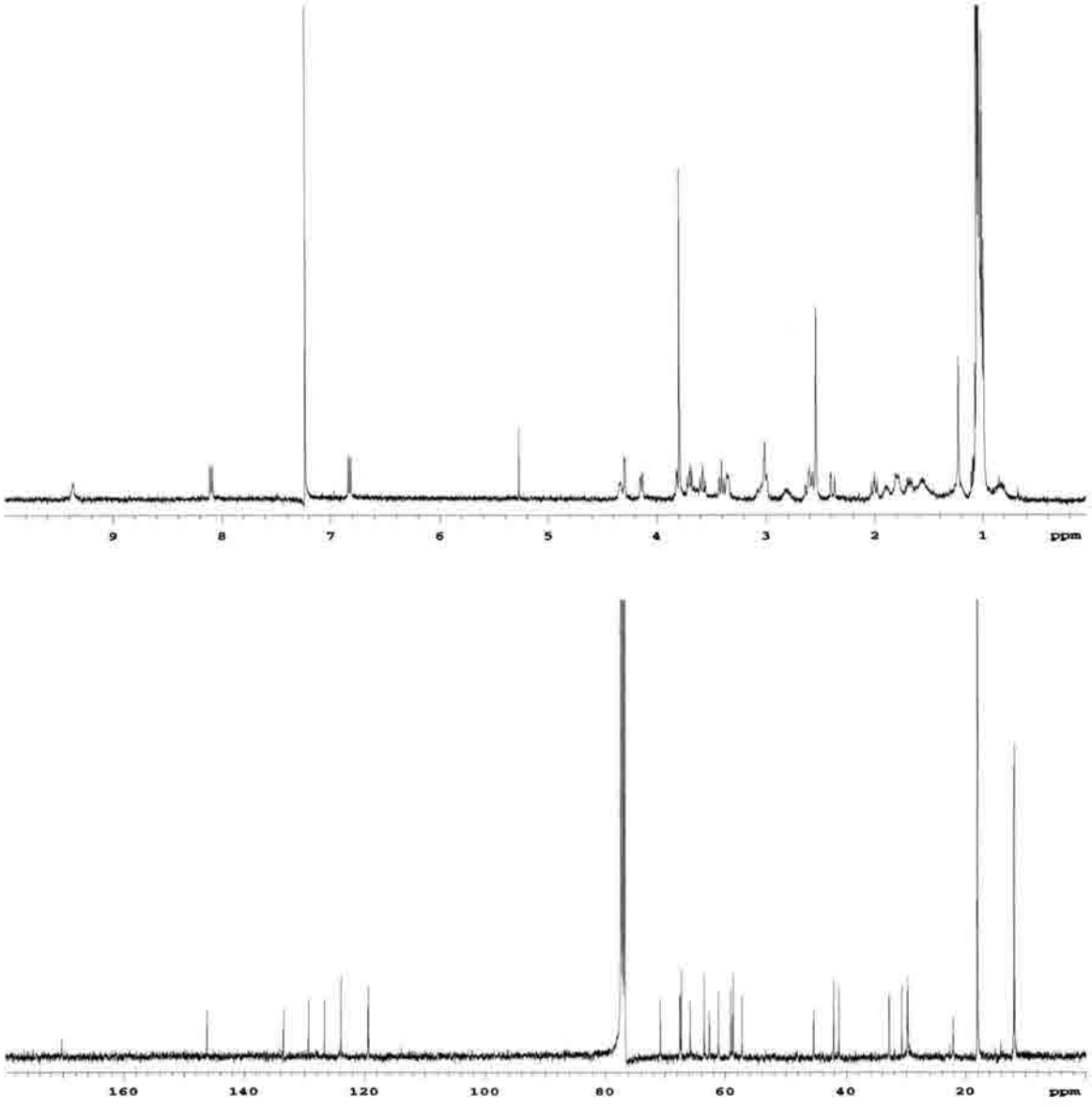
(5S,7R,8S,10R,11R,11aS)-5,7,8,9,10,11,11a,12-octahydro-5-(triisopropylsilyloxymethyl)-3-amino [(2'S,3'R)-piperidine-2'-carboxy-3'-hydroxy]-7-cyano--10-(triisopropylsilyloxymethyl)-4-methoxy-13-methyl-8,11-Iminoazepino[1,2-b]isoqunoline [(+)-729).

To a mixture of (+)-**727** (9 mg, 0.011 mmol) and 1,3-dimethoxybenzene (50μL) at 0°C was added TFA (1 mL) and this solution was stirred at 4°C for 26h. The solvent was removed *in vacuo* and the crude product was partitioned in CH_2Cl_2 and $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were dried over Na_2SO_4 and the solvent was removed. The crude product was purified via flash chromatography (gradient 1-2.5-5.0 % MeOH/CH_2Cl_2) to afford 4.5 mg (+)-**729** (53%) as a clear oil. ^{1}H-NMR (400 MHz) ($CDCl_3$) δ 1.02-1.10 (42H, m); 1.58 (2H, m); 1.71 (2H, m); 1.83 (2H, m); 1.91 (1H, m); 2.03 (1H, dd, *J* = 13.2, 8.8 Hz); 2.41 (1H, d, *J* = 12.4 Hz); 2.57 (3H, s); 2.63 (2H, m); 2.83 (1H, m); 3.04 (2H, m); 3.36 (1H, m); 3.43 (1H, t, *J* = 8.8 Hz); 3.60 (2H, t, *J* = 10.0 Hz); 3.71 (1H, d, *J* = 7.2 Hz); 3.73 (1H, d, *J* = 8.8 Hz); 3.82 (3H, s); 3.83 (1H, d, *J* = 4.4 Hz); 4.16 (1H, d, *J* = 6.0 Hz); 4.32 (1H, d, *J* = 2.0 Hz); 4.36 (1H, m); 6.85 (1H, d, *J* = 8.0 Hz); 8.12 (1H, d, *J* = 8.0 Hz); 9.39 (1H, s, broad). ^{13}C-NMR (100 MHz) ($CDCl_3$) δ 11.83, 11.96, 17.99, 18.07, 22.13, 29.79, 30.72, 32.84, 41.21, 42.09, 45.38, 57.25, 58.73, 59.10, 61.14, 62.64, 63.46, 65.84, 67.31, 67.52, 70.74, 119.34, 119.45, 123.92, 126.64, 129.29, 133.44, 146.17, 170.30. IR (NaCl, neat) 3220,

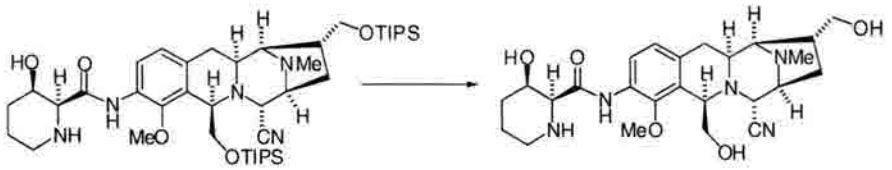
3066, 2944, 1716, 1528, 1167, 1044 cm^{-1}. HRMS (FAB) calcd. For $C_{43}H_{76}N_5O_5Si_2$ (MH^+) 798.5385; found 798.5373. $[\alpha]_D^{25} = +31.0$ (c = 0.33 $CHCl_3$).



(+)-**729**



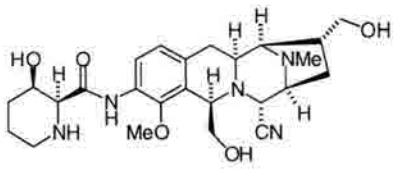
Compound **729**: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$



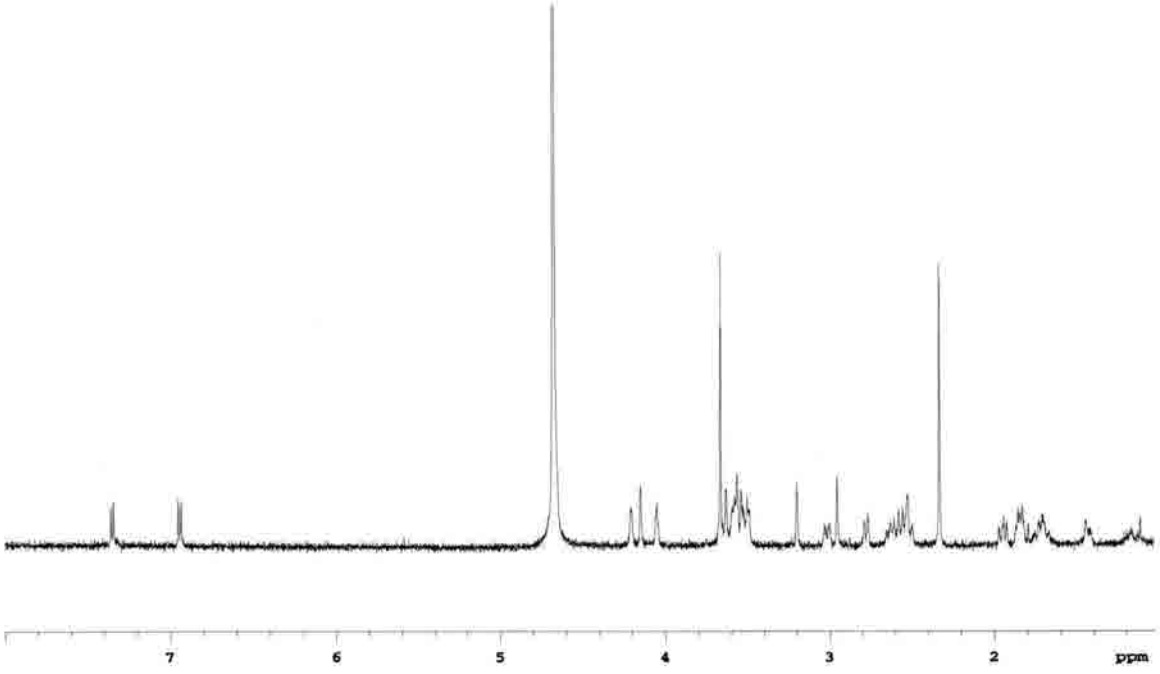
729 730

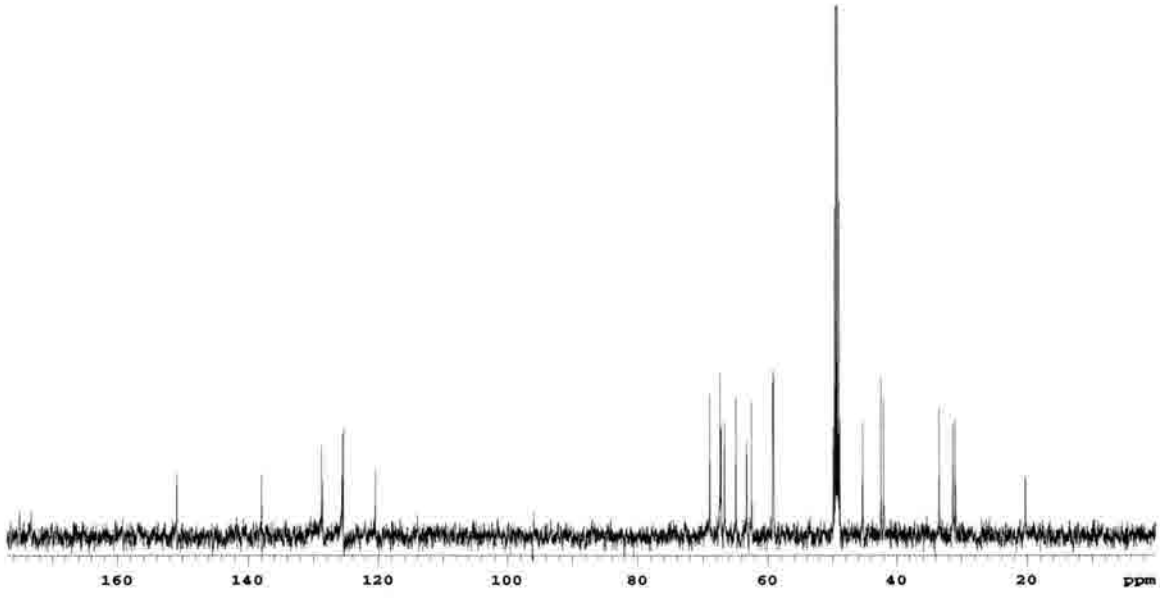
2a'-cyanotetrazominol (730):

To a solution of **727** (6.2 mg, 0.0078 mmol) in MeCN (1 mL) was added 5% HF(aq.)/MeCN (150 μL) and the solution was stirred for 4 h. Excess sat. $NaHCO_3$ was added and the mixture was lyophillized. The crude product was taken up in dd H_2O and filtered through a Nalgene syringe filter (0.2 μm, nylon). The solution was then purified using a HP-20 column (100:0-10:90 H_2O:MeOH). The MeOH was removed from the organic fractions by rotary evaporation and the remaining water was removed by lyophillization to afford 3 mg **729** (79%) as a white solid. TLC (9:90:1 MeOH/CH_2Cl_2/conc. NH_4OH) R_f = 0.31 (UV and PMA). ^{1}H-NMR (400 MHz) (D_2O) δ 1.52 (1H, m); 1.81 (2H, m); 1.96 (2H, m); 2.05 (1H, dd, *J* = 14.0, 8.4 Hz); 2.44 (3H, s); 2.60-2.75 (4H, m); 2.88 (1H, d, *J* = 10.4 Hz); 3.07 (1H, s); 3.11 (1H, d, *J* = 14.0 Hz); 3.59-3.70 (6H, m); 3.78 (3H, s); 4.17 (1H, t, *J* = 1.2 Hz); 4.26 (1H, d, *J* = 1.2 Hz); 4.33 (1H, s, broad); 7.05 (1H, d, *J* = 8.4 Hz); 7.50 (1H, d, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz) (D_2O vs. d_4-MeOH) δ 20.15, 30.82, 31.19, 33.25, 42.28, 45.12, 48.63, 58.93, 58.99, 59.04, 62.30, 63.07, 64.74, 66.49, 67.05, 67.20, 68.75, 120.29, 125.24, 125.41, 128.35, 128.59, 137.75, 150 70, 172.49. HRMS (FAB) calcd. for $C_{25}H_{36}N_5O_5$ (MH^+) 486.2716; found 486.2722. IR (KBr) 3430, 2933, 1738, 1731, 1574, 1384, 1136 cm^{-1}.



730





Compound **730**: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) in D_2O (vs. d_4-MeOH)

730 **566**, Tetrazomine

Tetrazomine (566).

To a solution of **730** (1.5 mg, 0.003 mmol) in 4:1 MeOH/ddH_2O (500 μL) was added TFA (1.2 μL, 0.015 mmol, 5 eq.) followed by silver trifluoroacetate (2.1 mg, 0.009 mmol, 3 eq). This solution was allowed to stir at rt for 4 h. Excess Dowex (Cl^-) ion exchange resin was added and the mixture was stirred for 15 min. The mixture was filtered though a cotton plug and the resin was washed with ddH_2O. The filtrate was then lyophilized to afford crude tetrazomine that was purified via HPLC (Waters Resolve C_{18}, isocratic 90/10/0.1 H_2O/MeOH/TFA) to afford 1.0 mg tetrazomine•2HCl (61%). ^{1}H-NMR (400 MHz) (D_2O) δ 1.84 (1H, d, *J* = 12.4 Hz); 1.93 (1H, t, *J* = 9.6 Hz); 2.09 (3H, m); 2.42 (1H, dd, *J* = 14.8, 10.4 Hz); 2.75 (2H, m); 2.99 (3H, s); 3.04 (1H, m); 3.15 (1H, ddd, *J* = 12.4, 12.4, 2.8 Hz); 3.52 (1H, s, broad); 3.55 (1H, s, broad); 3.69 (1H, m); 3.75 (2H, m); 3.82 (3H, s); 3.83 (1H, m); 3.90 (1H, s); 3.99 (1H, m); 4.34 (1H, d, *J* = 1.6 Hz); 4.50 (1H, t, *J* = 4 Hz); 4.73 (1H, s, broad); 5.01 (1H, d, *J* = 2.8 Hz); 7.10 (1H, d, *J* = 8.8 Hz); 7.51 (1H, d, *J* = 8.8 Hz). HRMS (FAB) calcd. For $C_{24}H_{35}N_4O_5$ (MH^+) 459.2607; found 459.2612. $[\alpha]_D^{25}$ = -57 (c = 0.04 MeOH); $[\alpha]_D^{25}$(natural tetrazomine) = -59 (c = 0.1 MeOH) Lit. $[\alpha]_D^{25}$= –62 (c = 1.0, MeOH).

566, Tetrazomine

730

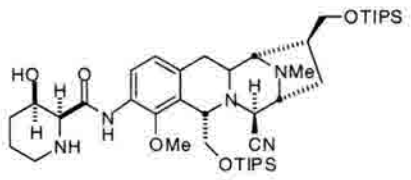
Cyanotetrazominol (730).

To a solution of natural tetrazomine•2HCl (4.3 mg, 0.0081 mmol) in ddH_2O (300 μL) was added sat. $NaHCO_3$ (20 μL) followed by a 0.5 M NaCN (32 μL, 2 eq) and the solution was stirred at rt for 3 h. The solution desalted via a HP20 column (1:0 to 10:90 ddH_2O: MeOH). The MeOH fractions were combined and most of the solvent was removed by rotary evaporation. The remaining solvent was lyophillized to afford 3.5 mg **730** (89%) as a white foam. The spectroscopic data matched that of synthetic **730**.

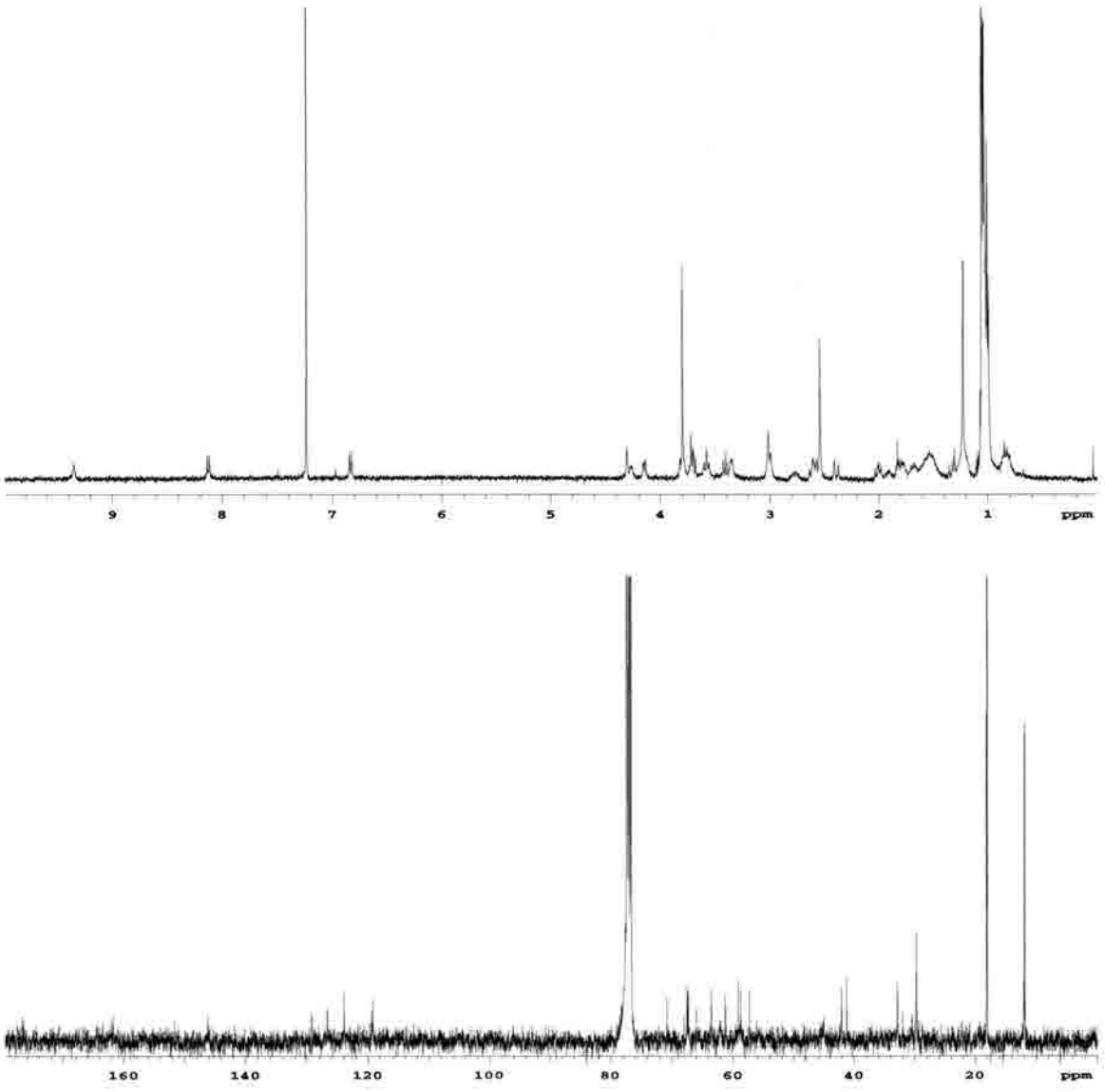
(5S,7R,8S,10R,11R,11aS)-8,11-5,7,8,9,10,11,11a,12-octahydro-5-(triisopropylsilyloxymethyl)-3-amino [(2'S,3'R)-piperidine-2'-carboxy-3'-hydroxy]-7-cyano-4-methoxy-13-methyl--10-(triisopropylsilyloxymethyl)Iminoazepino[1,2-b]isoqunoline [(+)-731].

To a mixture of **728** (8 mg, 0.0094 mmol) and 1,3-dimethoxybenzene (50μL) at 0°C was added TFA (1 mL) and this solution was stirred at 4°C for 26 h. The solvent was removed *in vacuo* and the crude product was partitioned in CH_2Cl_2 and $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were dried over Na_2SO_4 and the solvent was removed. The crude product was purified via flash chromatography (gradient 1-2.5-5.0 % MeOH/CH_2Cl_2) to afford 4 mg **731** (54%) as a clear oil. ^{1}H-NMR (400 MHz) ($CDCl_3$) δ 0.85 (3H, m); 1.07 (35H, m); 1.25 (4H, m); 1.56 (4H, m); 1.70 (2H, m); 1.85 (2H, m); 2.03 (1H, dd, *J* = 14.0, 8.4 Hz); 2.42 (1H, d, *J* = 14.0 Hz); 2.57 (3H, s); 2.63 (2H, m); 2.79 (1H, m); 3.02 (2H, m); 3.38 (1H, m); 3.43 (1H, t, *J* = 8.4 Hz); 3.60 (2H, t, *J* = 8.8 Hz); 3.73 (2H, m); 3.82 (3H, s); 4.17 (1H, dd, *J* = 7.6, 0.8 Hz); 4.29 (1H, m); 4.33 (1H, d, *J* = 1.2 Hz); 6.86 (1H, d, *J* = 8.0 Hz); 8.14 (1H, d, *J* = 8.0 Hz); 9.38 (1H, s, broad). ^{13}C-NMR (100 MHz) ($CDCl_3$) δ 11.81, 11.95, 17.98, 18.05, 29.68, 29.72, 30.45, 32.83, 41.24, 42.09, 45.01, 57.28, 58.72, 59.11, 61.22, 63.48, 65.95, 67.32, 67.51, 68.00, 70.76, 119.20, 119.54, 123.93, 126.67, 129.22, 146.13, 151.61, 161.80. IR (NaCl, neat) 3220, 3066, 2944, 1716, 1528, 1167, 1044 cm^{-1}. HRMS (FAB)

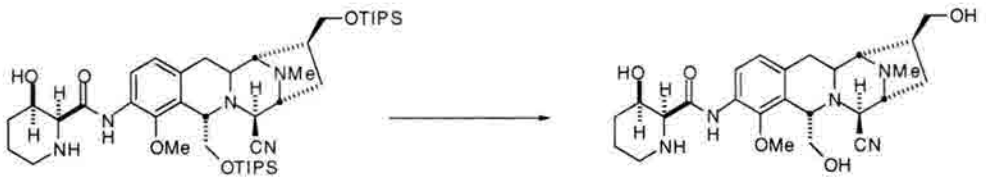
calcd. for $C_{43}H_{76}N_5O_5Si_2$ (MH^+) 798.5385; found 798.5375. $[\alpha]_D^{25}$ = -21.9 (c = 0.29 $CHCl_3$).



731

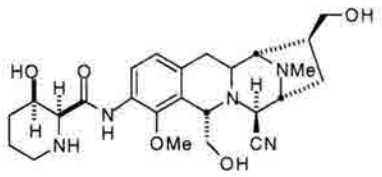


Compound **731**: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$

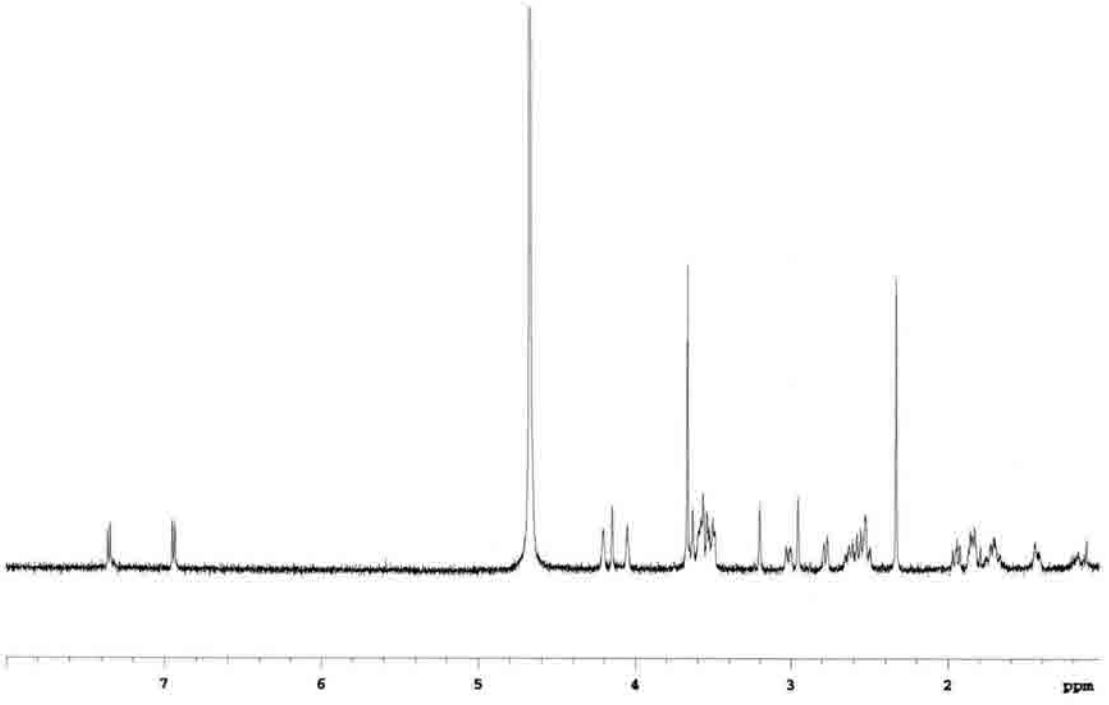


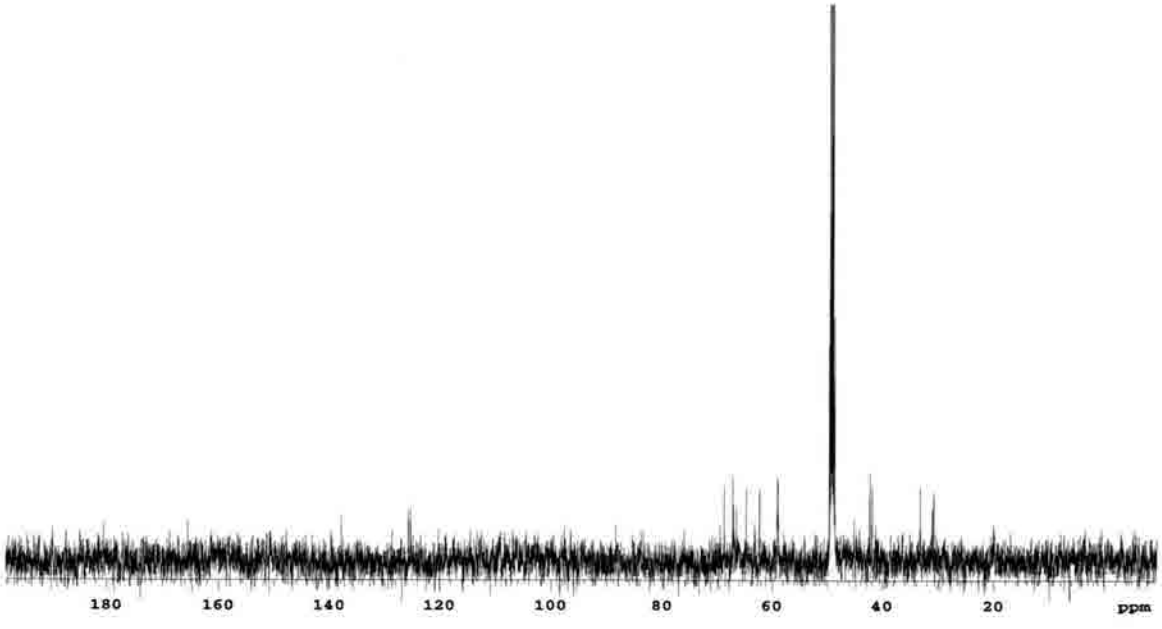
2',3'-*epi*-ent cyanotetrazominol (732):

To a solution of **731** (3.5 mg, 0.0044 mmol) in MeCN (500 μL) was added 5% HF(aq.)/MeCN (100 μL) and the solution was stirred for 3 h. Excess sat. $NaHCO_3$ was added and the mixture was lyophillized. The crude product was taken up in dd H_2O and filtered through a Nalgene syringe filter (0.2 μm, nylon). The solution was then purified using a HP-20 column (100:0-10:90 H_2O:MeOH). The MeOH was removed from the organic fractions by rotary evaporation and the remaining water was removed by lyophillization to afford 1.5 mg **732** (70%) as a white solid. TLC (9:90:1 MeOH/CH_2Cl_2/conc. NH_4OH) R_f = 0.25 (UV and PMA). ^{1}H-NMR (300 MHz) (D_2O) δ 1.49 (2H, m); 1.78-2.07 (5H, m); 2.42 (3H, s); 2.57-2.75 (4H, m); 2.86 (1H, d, *J* = 11.1 Hz); 3.04 (1H, s); 3.09 (1H, d, *J* = 14.1 Hz); 3.59–3.71 (4H, m); 3.75 (3H, s); 4.14 (1H, t, *J* = 3.0 Hz); 4.23 (1H, d, *J* = 1.2 Hz); 4.29 (1H, m); 7.03 (1H, d, *J* = 8.1 Hz); 7.42 (1H, d, *J* = 8.1 Hz). HRMS (FAB) calcd. for $C_{25}H_{36}N_5O_5$ (MH^+) 486.2716; found 486.2739. $[\alpha]_D^{25}$ = -22.0 (c = 0.09 MeOH). IR (KBr) 3428, 2934, 1667, 1537, 1455, 1134 cm^{-1}.

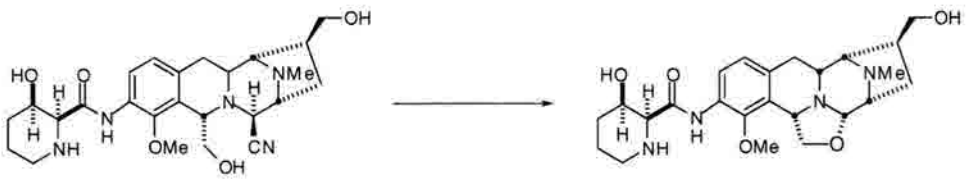


732



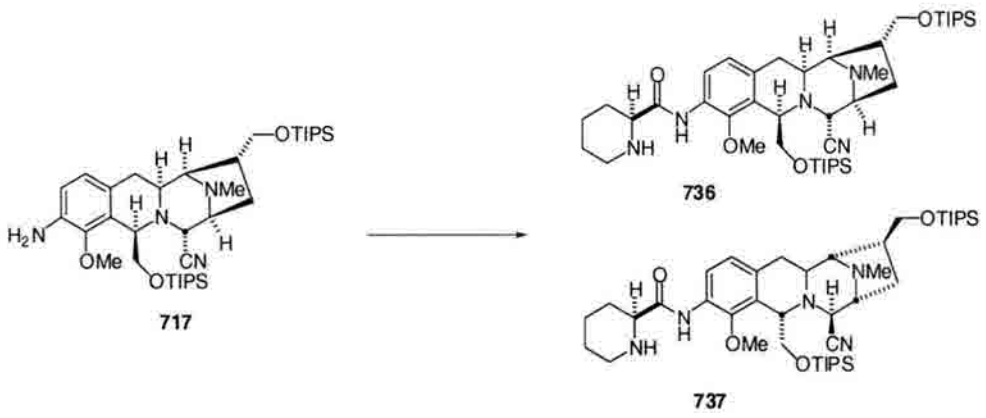


Compound **732**: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) in D_2O (vs. d_4-MeOH)



***ent*-2',3'-Tetrazomine (734):**

To a solution of **732** (1mg, 0.0021 mmol) in 4:1 MeOH/H_2O (250 μL) was added TFA (1μL, 0.011 mmol, 5 eq) followed by silver trifluoroacetate (1.4 mg, 0.0062 mmol, 3 eq.). The mixture was stirred at room temperature for 4 h. Excess Dowex (Cl^-) was added and the mixture was stirred at rt for 15 min. The mixture was filtered through a cotton plug followed by filtration through a nylon (0.2 μM) syringe filter. The product was then lyophilized to afford 1 mg (90%) **734** as a white foam.



(5S,7R,8S,10R,11R,11aS)-5,7,8,9,10,11,11a,12-octahydro-5-(triisopropylsilyloxymethyl)-3-amino (2'S-piperidine-2'-carboxy-)-7-cyano--10-(triisopropylsilyloxymethyl) 8,11-Iminoazepino[1,2-b]isoqunoline-4-methoxy-13-methyl- (736).

(5R,7S,8R,10S,11S,11aR)-5,7,8,9,10,11,11a,12-octahydro-5-(triisopropylsilyloxymethyl)-3-amino-(2'S-piperidine-2'-carboxy-)7-cyano--10-(triisopropylsilyloxymethyl)-4-methoxy-13-methyl-8,11-Iminoazepino[1,2-b]isoqunoline (737).

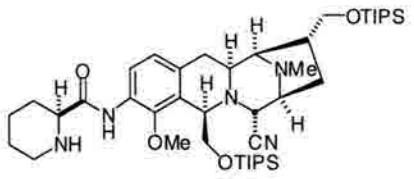
To a solution of Fmoc-pipecolic acid (10 mg, 0.029 mmol, 1.5 eq.) in CH_2Cl_2 (1 mL) was added oxalyl chloride (3 μL, 0.033 mmol, 1.7 eq.) followed by DMF (0.25 μL, 0.0029 mmol, 0.15 eq). This solution was stirred at rt for 1 h. Hexanes (1 mL) was added and the solution was filtered through a cotton plug and the solvent was removed. Aniline **717** (13 mg, 0.0194 mmol, 1 eq) was dissolved in a minimum amount of CH_2Cl_2 and added to the acid chloride with DMAP (2.4 mg, 0.0194 mmol, 1 eq). This solution was stirred at rt for 18 h. The solution was diluted with CH_2Cl_2 and washed with $NaHCO_3$, dried over Na_2SO_4, and the solvent was removed. The crude product was purfied by preparative TLC (20% EtOAc/hex) to afford 6 mg of each diasteromer. Each

diastereomer was seperately dissolved in CH_2Cl_2 (1 mL) and DBU (0.7 μL, 0.0050 mmol, 1 eq) was added and the solution was stirred at rt for 1 h. The solvent was removed and the crude product was purified via column chromatography (1-5% MeOH/CH_2Cl_2; extracted with THF) to afford 3.8 mg **736** (30%) as a clear oil and 3.5 mg **737** (28%) as a clear oil.

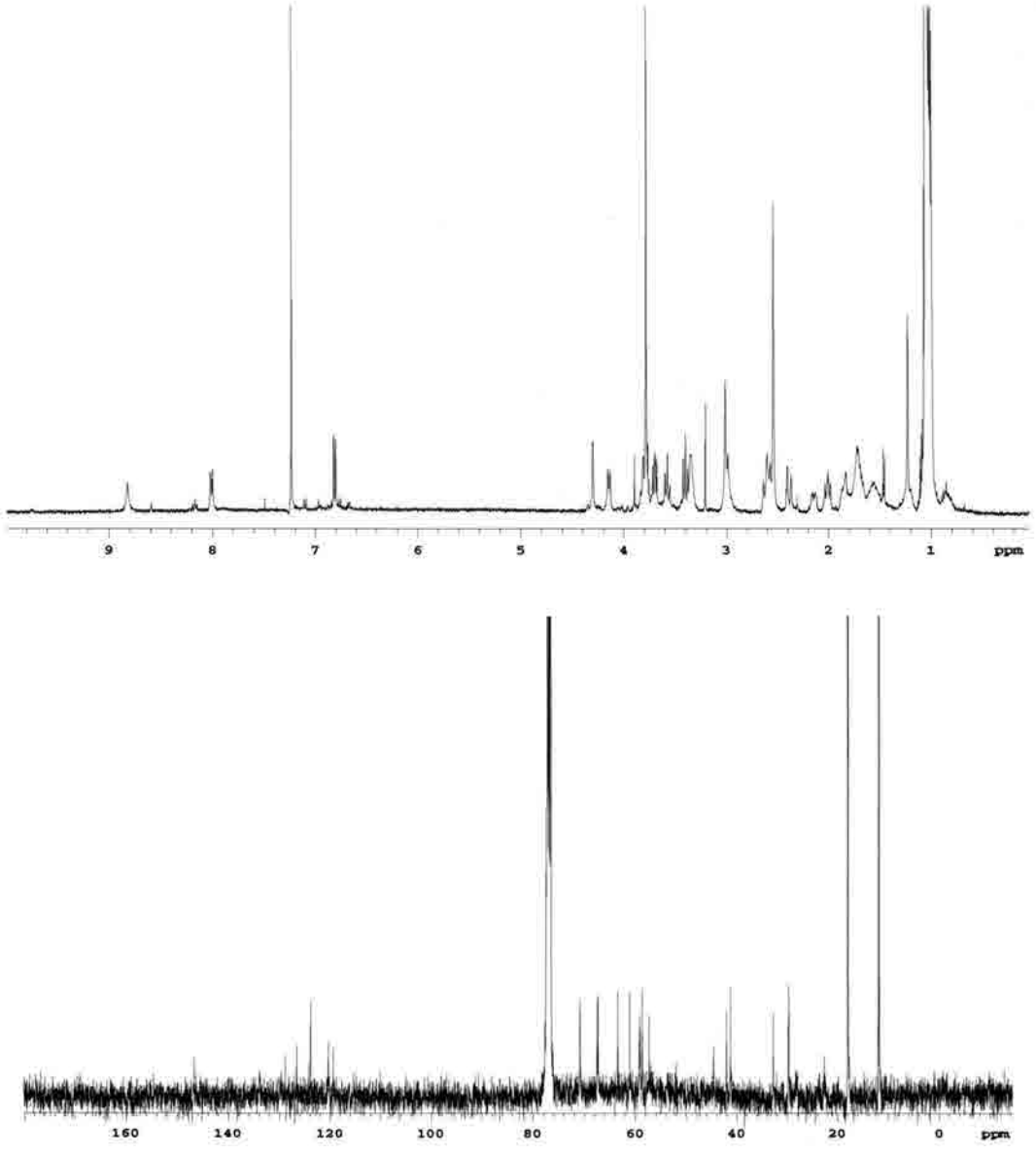
736: ^{1}H-NMR (400 MHz) ($CDCl_3$) δ 1.04 (42H, m); 1.48-1.71 (5H, m); 1.80 (1H, m); 2.03 (2H, m); 2.38 (1H, d, *J* = 14.4 Hz); 2.54 (3H, s); 2.59 (2H, m); 2.87 (1H, t, *J* = 6.4 Hz); 3.01 (2H, m); 3.24 (1H, d, *J* = 8.0 Hz); 3.38 (1H, d, *J* = 4.0 Hz); 3.43 (1H, d, *J* = 12.0 Hz); 3.58 (1H, t, *J* = 9.6 Hz); 3.67 (1H, m); 3.73 (1H, d, *J* = 10.0 Hz); 3.79 (1H, s, broad); 3.82 (3H, s); 4.14 (1H, d, *J* = 8.8 Hz); 4.30 (1H, s); 6.81 (1H, d, *J* = 8.8 Hz); 8.06 (1H, d, *J* = 8.8 Hz); 8.99 (1H, s, broad). ^{13}C-NMR (100 MHz) ($CDCl_3$) δ 11.83, 11.96, 17.99, 18.07, 23.09, 28.89, 29.70, 29.78, 32.84, 41.24, 42.11, 45.01, 57.27, 58.72, 59.11, 59.81, 61.33, 63.49, 67.32, 67.52, 70.82, 119.42, 119.74, 123.90, 126.59, 129.34, 133.43, 146.39, 159.36. HRMS (FAB) calcd. for $C_{43}H_{76}N_5O_4Si_2$ (MH^+) 782.5436; found 782.5399. $[\alpha]_D^{25}$ = -31.9 (c = 0.32, $CHCl_3$).

737: ^{1}H-NMR (400 MHz) ($CDCl_3$) δ 1.06 (42H, m); 1.25 (2H, s); 1.58-1.85 (7H, m); 2.03 (1H, dd, *J* = 12.4, 9.2 Hz); 2.36 (1H, d, *J* = 9.2 Hz); 2.41 (1H, d, *J* = 15.2 Hz); 2.57 (3H, s); 2.63 (2H, m); 3.02 (3H, m); 3.38 (1H, s, broad); 3.43 (1H, t, *J* = 8.8 Hz); 3.60 (1H, t, *J* = 9.2 Hz); 3.72 (1H, dd, *J* = 9.2, 6.8 Hz); 3.81 (3H, s); 3.84 (1H, m); 4.17 (1H, dd, *J* = 6.4, 1.2 Hz); 4.33 (1H, d, *J* = 1.2 Hz); 6.83 (1H, d, *J* = 8.4 Hz); 8.03 (1H, d, *J* = 8.4 Hz); 8.85 (1H, s, broad). ^{13}C-NMR (100 MHz) ($CDCl_3$) δ 11.81, 11.95, 17.99, 18.05, 22.65, 28.10, 29.60, 29.76, 32.84, 41.25, 42.09, 44.64, 57.30, 58.68, 59.14, 59.22, 61.16, 63.48, 67.31, 67.51, 70.85, 119.43, 120.38, 123.87, 124.29, 126.64, 133.92, 146.72, 159.16.

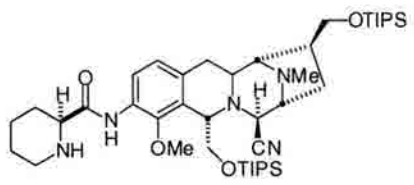
HRMS (FAB) calcd. for $C_{43}H_{76}N_5O_4Si_2$ (MH^+) 782.5436; found 782.5443. $[\alpha]_D^{25}$ = +14.4 (c = 0.29 $CHCl_3$).



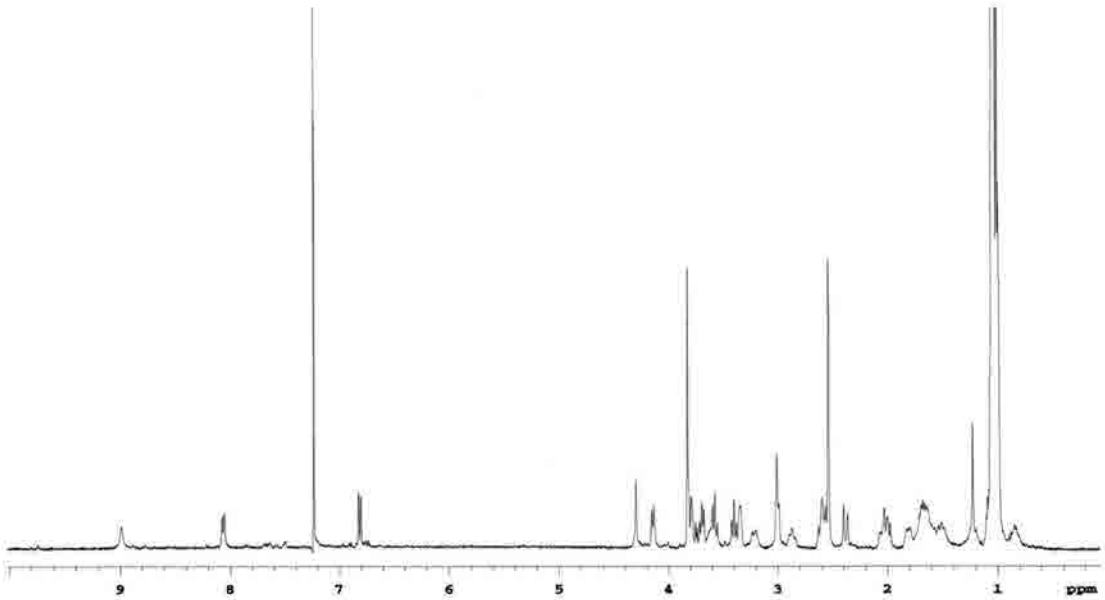
736

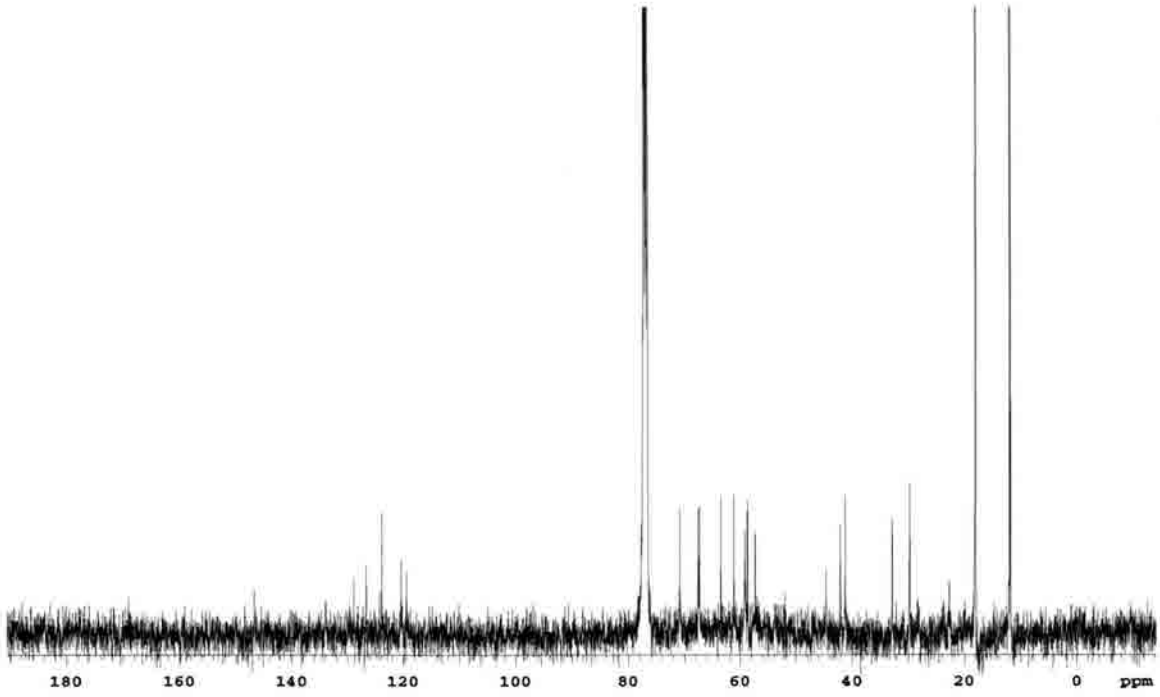


Compound **736**: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$



737

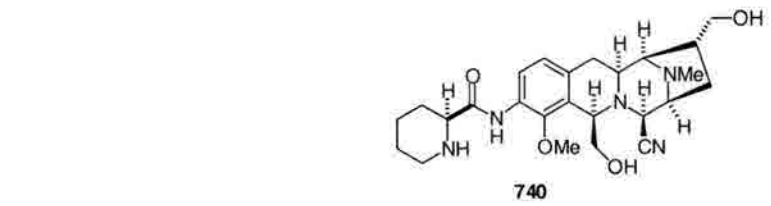




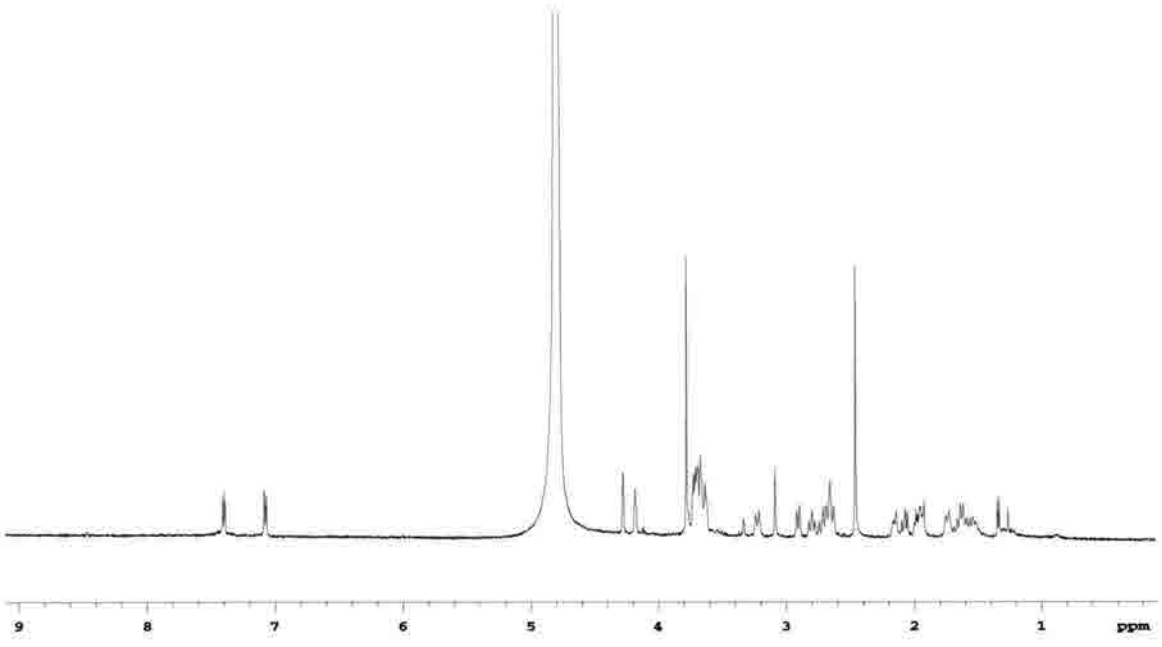
Compound **737**: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$

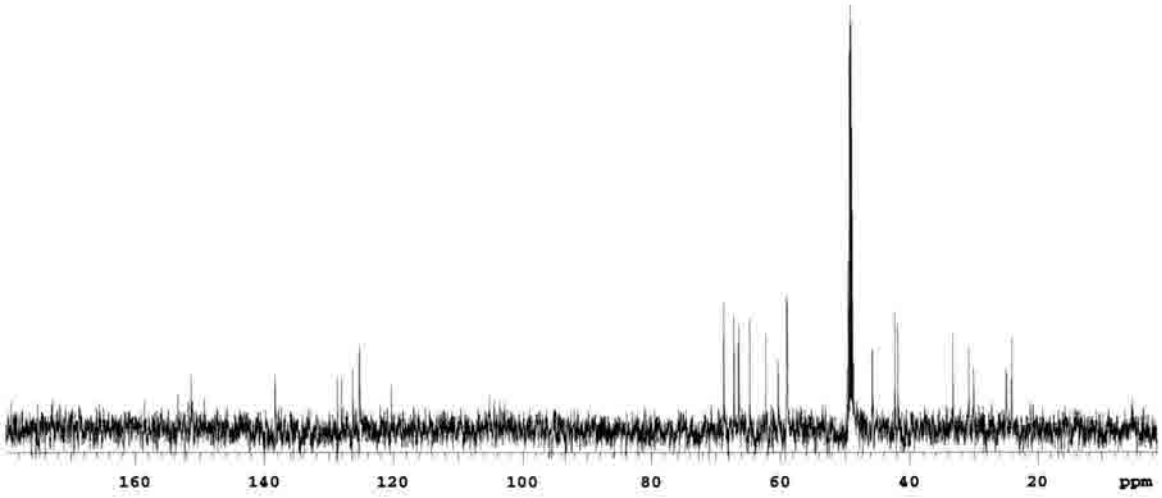
3-deoxy-2'cyanotetrazominol (740):

To a solution of **738** (4 mg, 0.0051 mmol) in MeCN (500 μL) was added 5% HF/MeCN (100 μL). This solution was stirred at rt for 4 h. Excess sat $NaHCO_3$ was added and the solution was lyophillized. The crude product was taken up in ddH_2O and filtered through a cotton plug followed by a syringe filter (nylon 2μM). The product was desalted using a HP20 column (1:0 to 10:90 H_2O:MeOH) and the solvent was removed by rotary evaporation follwed by lyohpillization to afford 1.8 mg (75%) **740** as a white foam. ^{1}H-NMR (300 MHz) (D_2O) δ 1.49 (2H, m); 1.78-2.07 (5H, m); 2.42 (3H, s); 2.57-2.75 (4H, m); 2.86 (1H, d, *J* = 11.1 Hz); 3.04 (1H, s); 3.09 (1H, d, *J* = 14.1 Hz); 3.59-3.71 (4H, m); 3.75 (3H, s); 4.14 (1H, t, *J* = 3.0 Hz); 4.23 (1H, d, *J* = 1.2 Hz); 4.29 (1H, m); 7.03 (1H, d, *J* = 8.1 Hz); 7.42 (1H, d, *J* = 8.1 Hz). ^{13}C-NMR (125 MHz) (D_2O vs. d_4-MeOH) δ 24.08, 24.95, 30.07, 30.80, 33.28, 41.84, 42.28, 45.75, 58.91, 58.98, 59.26, 60.32, 62.23, 64.72, 66.43, 67.18, 68.73, 120.27, 125.26, 128.05, 128.69, 135.50, 138.31, 151.38, 153.38. HRMS (FAB) calcd. for $C_{25}H_{36}N_5O_4$ (MH^+) 470.2756; found 470.2767. $[\alpha]_D^{25}$=-17.4 (c = 0.17 MeOH). IR (KBr) 3550, 2942, 1698, 1537, 1454, 1043 cm^{-1}.

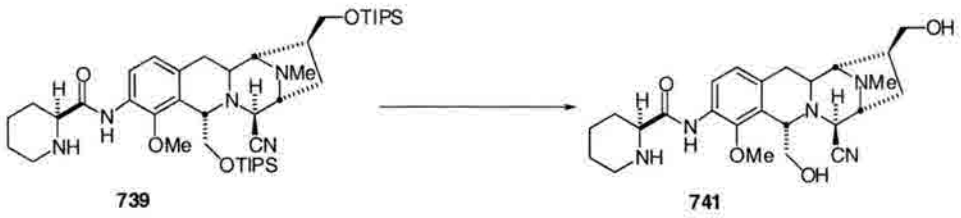


740





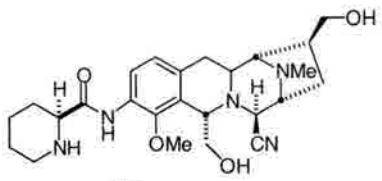
Compound **740**: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) in D_2O (vs. d_4MeOH)



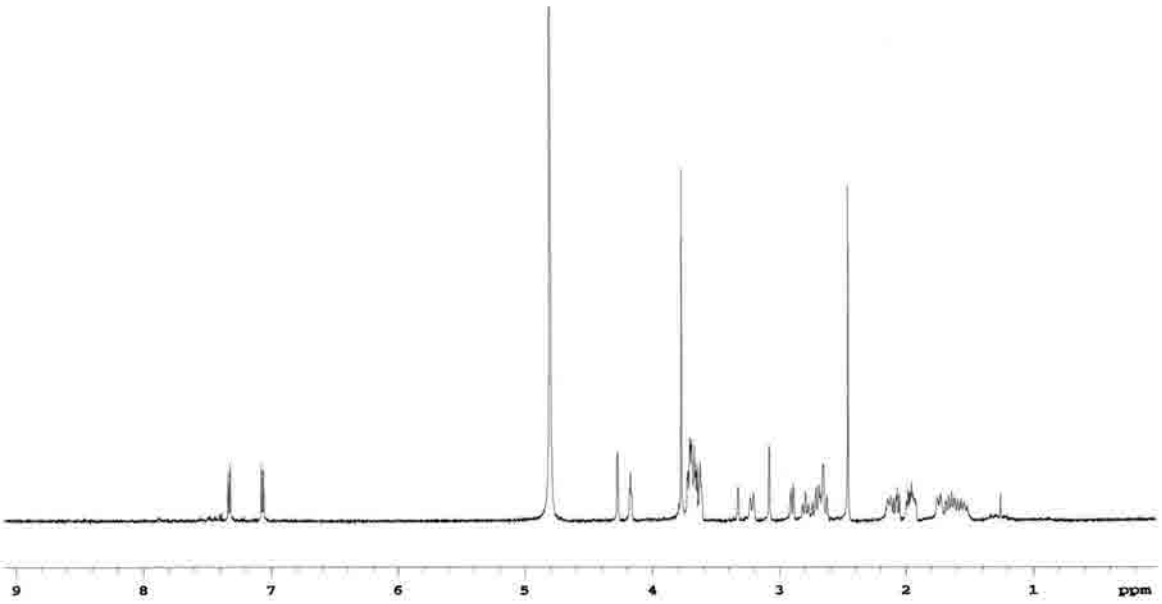
739 741

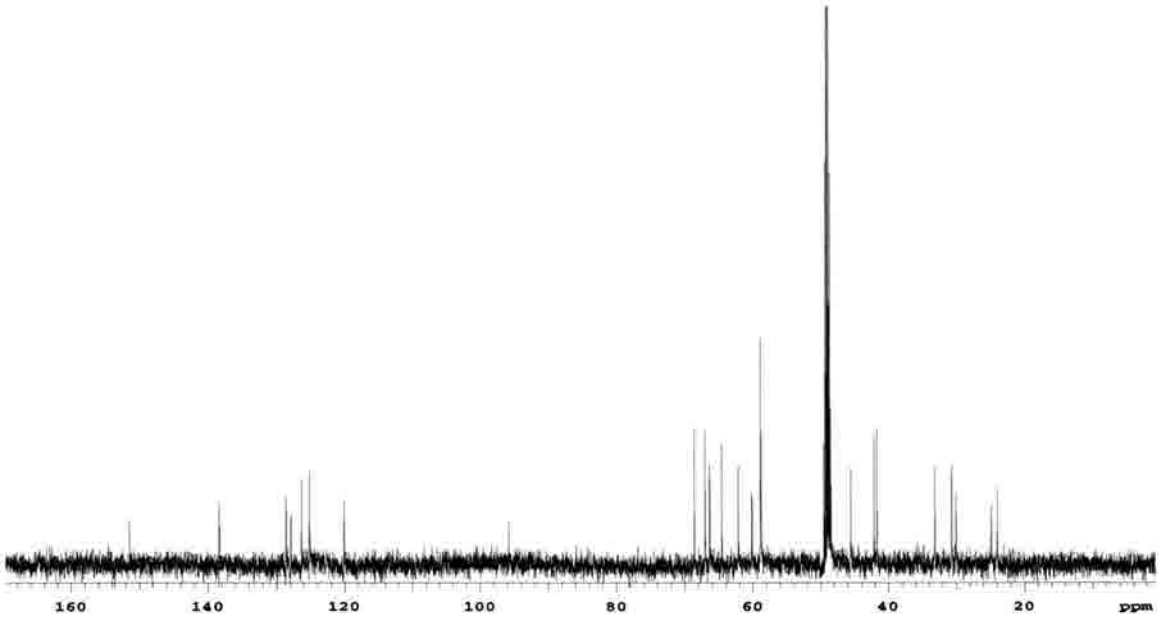
3-deoxy-2-*epi*-*ent*-2'-cyanotetrazominol (741):

To a solution of **739** (4 mg, 0.0051 mmol) in MeCN (500 μL) was added 5% HF/MeCN (100 μL). This solution was stirred at rt for 4 h. Excess sat $NaHCO_3$ was added and the solution was lyophillized. The crude product was taken up in ddH_2O and filtered through a cotton plug followed by a syringe filter (nylon 2μM). The product was desalted using a HP20 column (1:0 to 10:90 H_2O:MeOH) and the solvent was removed by rotary evaporation follwed by lyohpillization to afford 1.8 mg (75%) **741** as a white foam. ^{1}H-NMR (300 MHz) (D_2O) δ 1.67 (2H, m); 1.88-2.04 (4H, m); 2.36 (1H, m); 2.42 (3H, s); 2.63 (3H, m); 2.87 (1H, d, *J* = 10.2 Hz); 3.05 (2H, s, broad); 3.49 (1H, d, *J* = 10.5 Hz); 3.55 (4H, m); 3.73 (3H, s); 4.06-4.16 (2H, m); 4.24 (1H, d, *J* = 1.2 Hz); 7.04 (1H, d, *J* = 7.8 Hz); 7.38 (1H, d, *J* = 7.8 Hz). ^{13}C-NMR (125 MHz) (D_2O vs. d_4-MeOH) δ 24.13, 25.04, 30.20, 30.81, 33.30, 41.86, 42.29, 45.72, 58.95, 59.04, 60.29, 60.39, 62.32, 64.74, 66.49, 67.19, 68.75, 120.26, 125.36, 126.54, 128.06, 128.76, 138.53, 151.67, 154.00. HRMS (FAB) calcd. for $C_{25}H_{36}N_5O_4$ (MH^+) 470.2767; found 470.2765. IR (KBr) 3407, 2939, 1687, 1538, 1460, 1030 cm^{-1}. $[\alpha]_D^{25}$ = +17.0 (c = 0.1 MeOH).

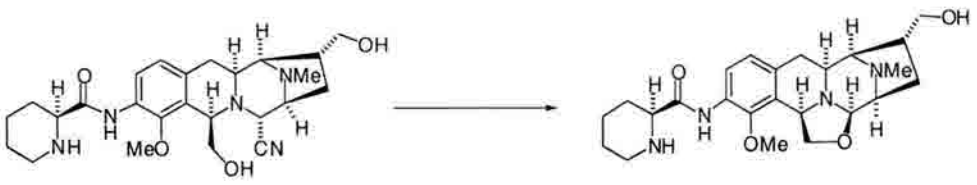


741





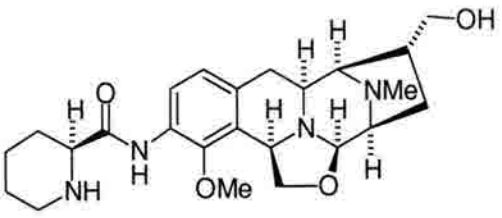
Compound **741**: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) in D_2O (vs. d_4MeOH)



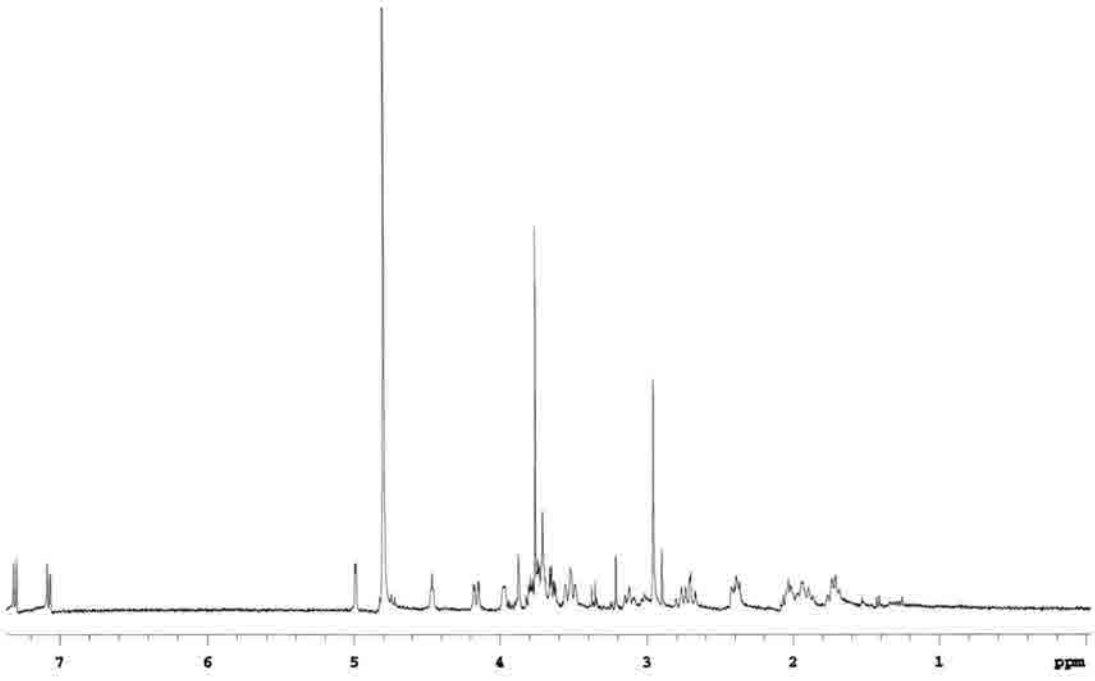
740 742

3-Deoxy-tetrazomine (742)

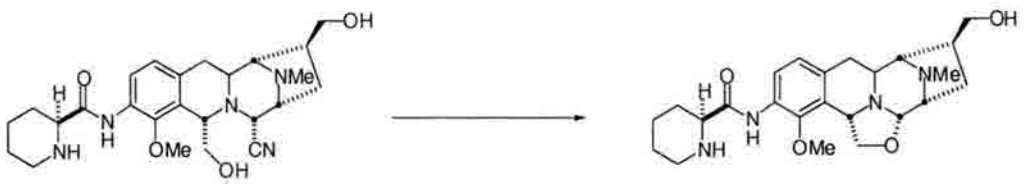
To a solution of **740** (0.7 mg, 0.0015 mmol) in 4:1 MeOH/H_2O (250 μL) was added TFA (0.6 μL, 0.0075 mmol, 5 eq) followed by $AgOCOCF_3$ (1mg, 0.0045 mmol, 3 eq) and the solution was allowed to stir at rt for 4 h. Excess Dowex (Cl^-) in ddH_2O (1 mL) was added and the slurry was stirred for 15 min. The reaction mixture was filtered through a cotton plug followed by filtration through a syringe filter (Gelman GHP 0.45 μM). The solvent was removed via lyophilization to afford pure 0.6 mg **742**•2HCl (78%) as a white foam. ^{1}H-NMR (400 MHz) (D_2O) 1.72 (2H, m); 1.85-2.09 (3H, m); 2.41 (2H, m); 2.68-2.80 (2H, m); 2.96 (3H, s); 3.02 (1H, m); 3.13 (1H, m); 3.52 (2H, m); 3.67 (2H, m); 3.77 (3H, s); 3.97 (1H, d, J = 3.2 Hz, broad); 4.16 (1H, dd, J = 12.0, 3.2 Hz); 4.47 (1H, t, J = 3.2 Hz); 4.99 (1H, d, J = 3.2 Hz); 7.08 (1H, d, J = 8.0 Hz); 7.39 (1H, d, J = 8.0Hz). HRMS (FAB) calcd. for $C_{24}H_{35}N_4O_4$ (MH^+) 443.2658; found 443.2667. $[\alpha]_D^{25}$ = +36.0 (c = 0.033 MeOH).



742

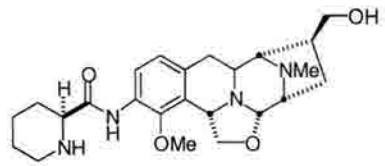


Compound **742**: ^{1}H-NMR (400 MHz)) in D_2O

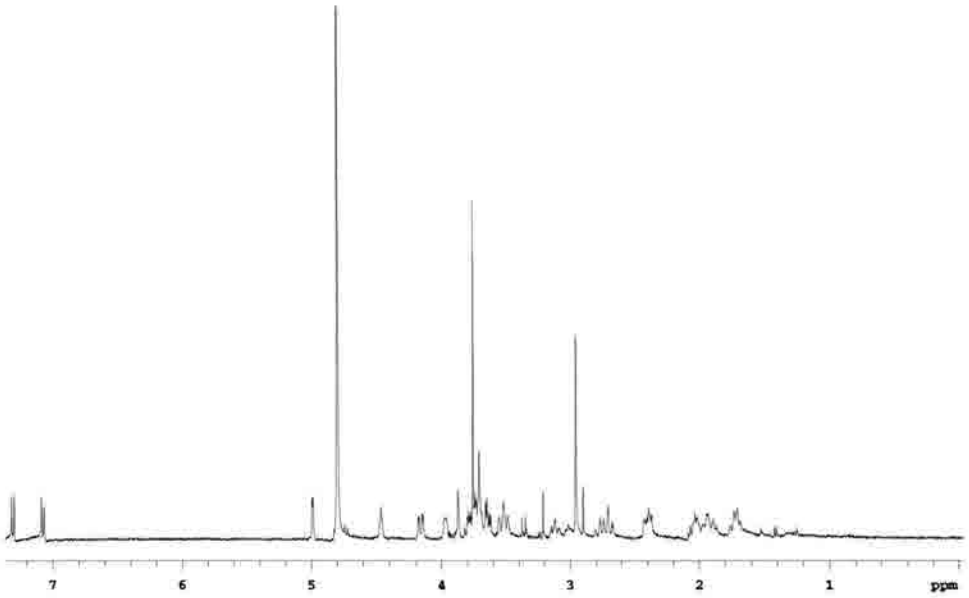


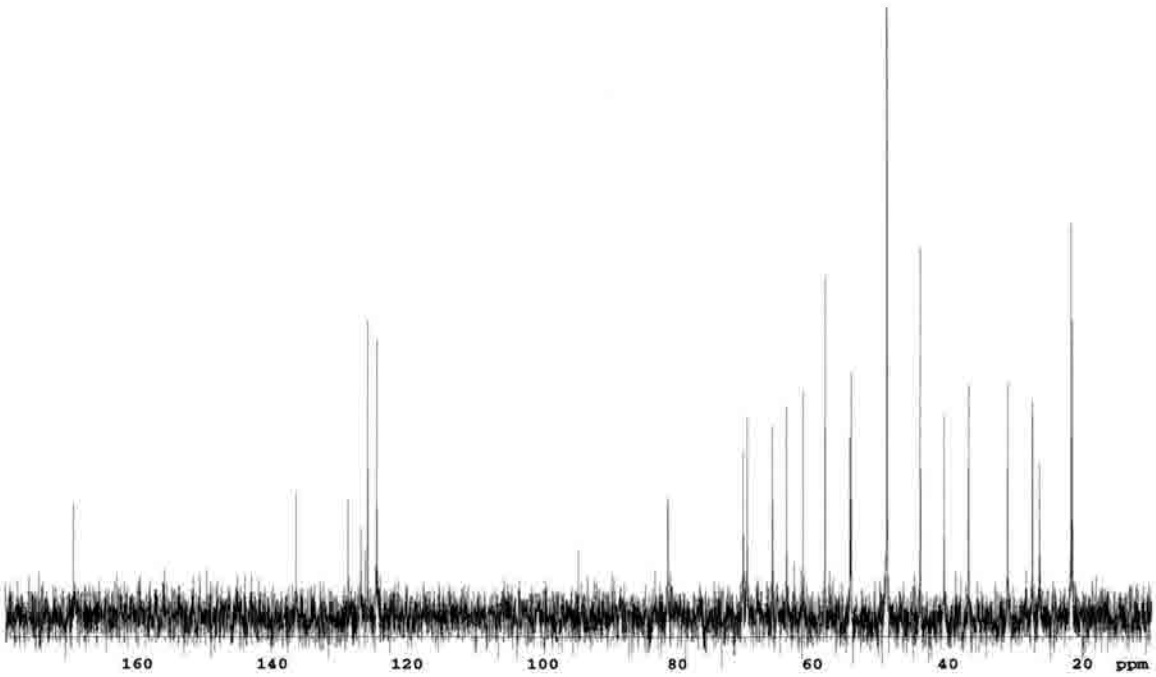
***ent*,-2-*epi*-3-Deoxy-tetrazomine (743)**

To a solution of **741** (0.7 mg, 0.0015 mmol) in 4:1 MeOH/H_2O (250 μL) was added TFA (0.6 μL, 0.0075 mmol, 5 eq) followed by $AgOCOCF_3$ (1mg, 0.0045 mmol, 3 eq) and the solution was allowed to stir at rt for 4 h. Excess Dowex (Cl^-) in ddH_2O (1 mL) was added and the slurry was stirred for 15 min. The reaction mixture was filtered through a cotton plug followed by filtration through a syringe filter (Gelman GHP 0.45 μM). The solvent was removed via lyophilization to afford pure 0.6 mg **743**•2HCl (75%) as a white foam. ^{1}H-NMR (400 MHz) (D_2O) 1.71 (2H, m); 1.85-2.10 (3H, m); 2.39 (2H, m); 2.74 (2H, m); 2.96 (3H, s); 3.02 (1H, m); 3.12 (1H, m); 3.52 (2H, t, J = 13.6 Hz); 3.66 (2H, m); 3.76 (3H, s); 3.78 (1H, m); 3.87 (1H, s); 4.16 (1H, dd, J = 12.0, 3.6 Hz); 4.64 (1H, t, J = 3.6 Hz); 4.99 (1H, d, J = 3.2 Hz); 7.07 (1H, d, J = 8.0 Hz); 7.30 (1H, d, J = 8.0Hz). ^{13}C-NMR (100 MHz) (D_2O vs. d_4MeOH) δ 21.6, 21.8, 26.5, 27.6, 31.2, 37.1, 40.7, 44.2, 54.1, 54.6, 58.2, 61.6, 63.9, 66.0, 69.7, 70.3, 73.4, 124.6, 125.9, 126.2, 126.9, 128.8, 136.5, 169.5. HRMS (FAB) calcd. for $C_{24}H_{35}N_4O_4$ (MH^+) 443.2658; found 443.2667. $[\alpha]_D^{25}$ = -27.0 (c = 0.033 MeOH).



743

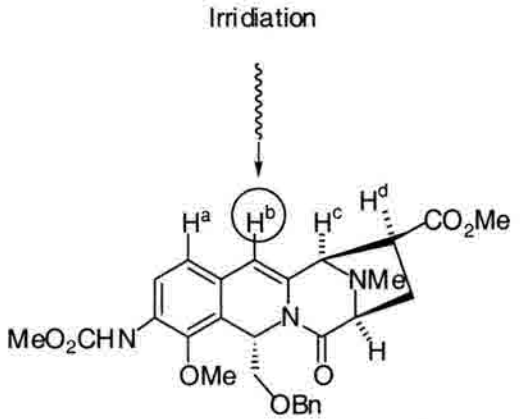




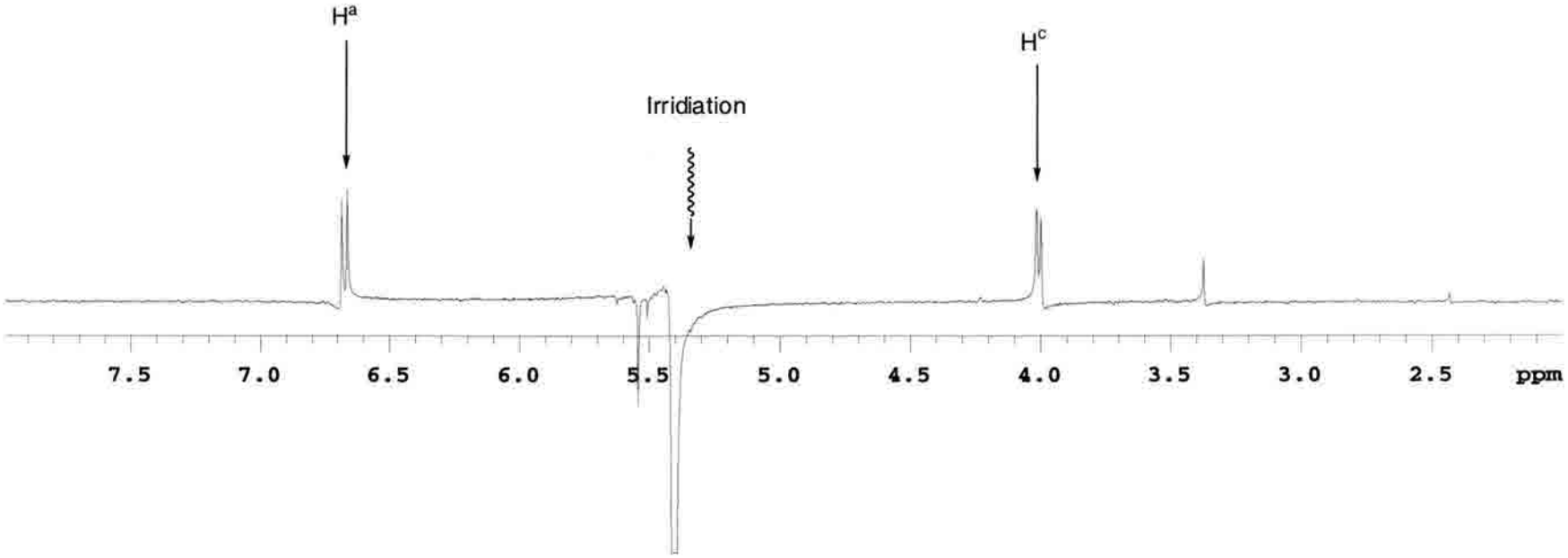
Compound **743**: ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in D_2O (vs. d_4MeOH)

Appendix 1

nOe data on compound **708**


Irridiation
Ha
Hb
Hc
Hd
CO2Me
NMe
N
MeO2CHN
OMe
O
H
OBn

708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Irridiation
Hc
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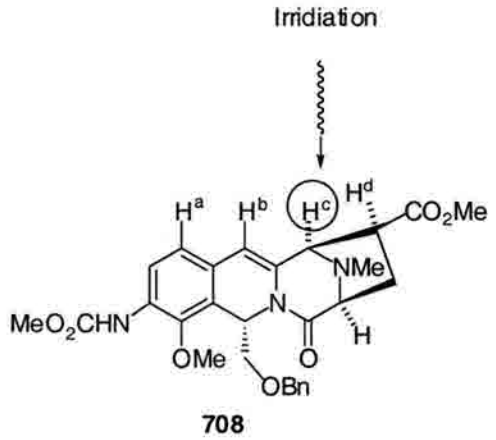
2.5
ppm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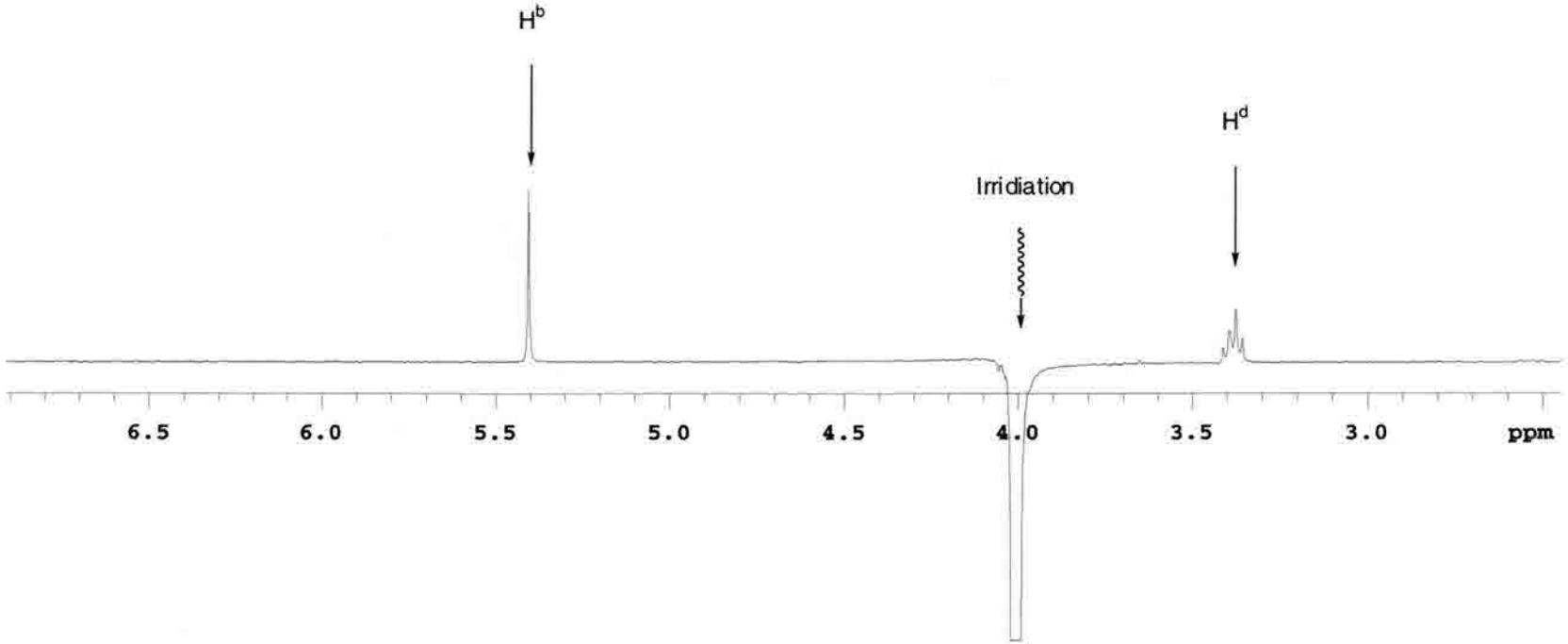
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H^d
Irridiation
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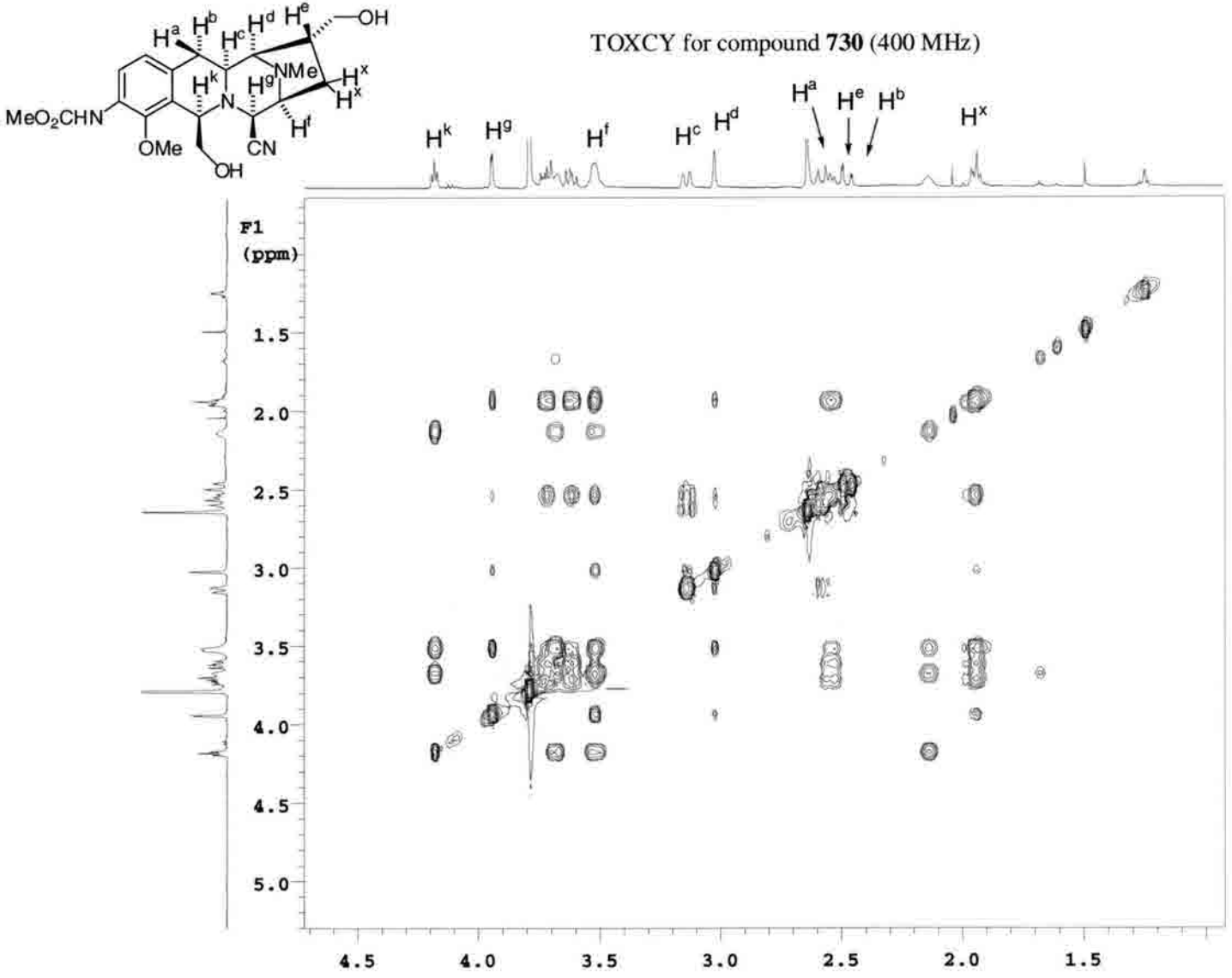
5.0
4.5
4.0
3.5
3.0
ppm

Appendix 2

TOXCY and ROSEY for compound **730**


TOXCY for compound 730 (400 MHz)
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OMe
CN
OH
NMe
F1
(ppm)
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2.0
2.5
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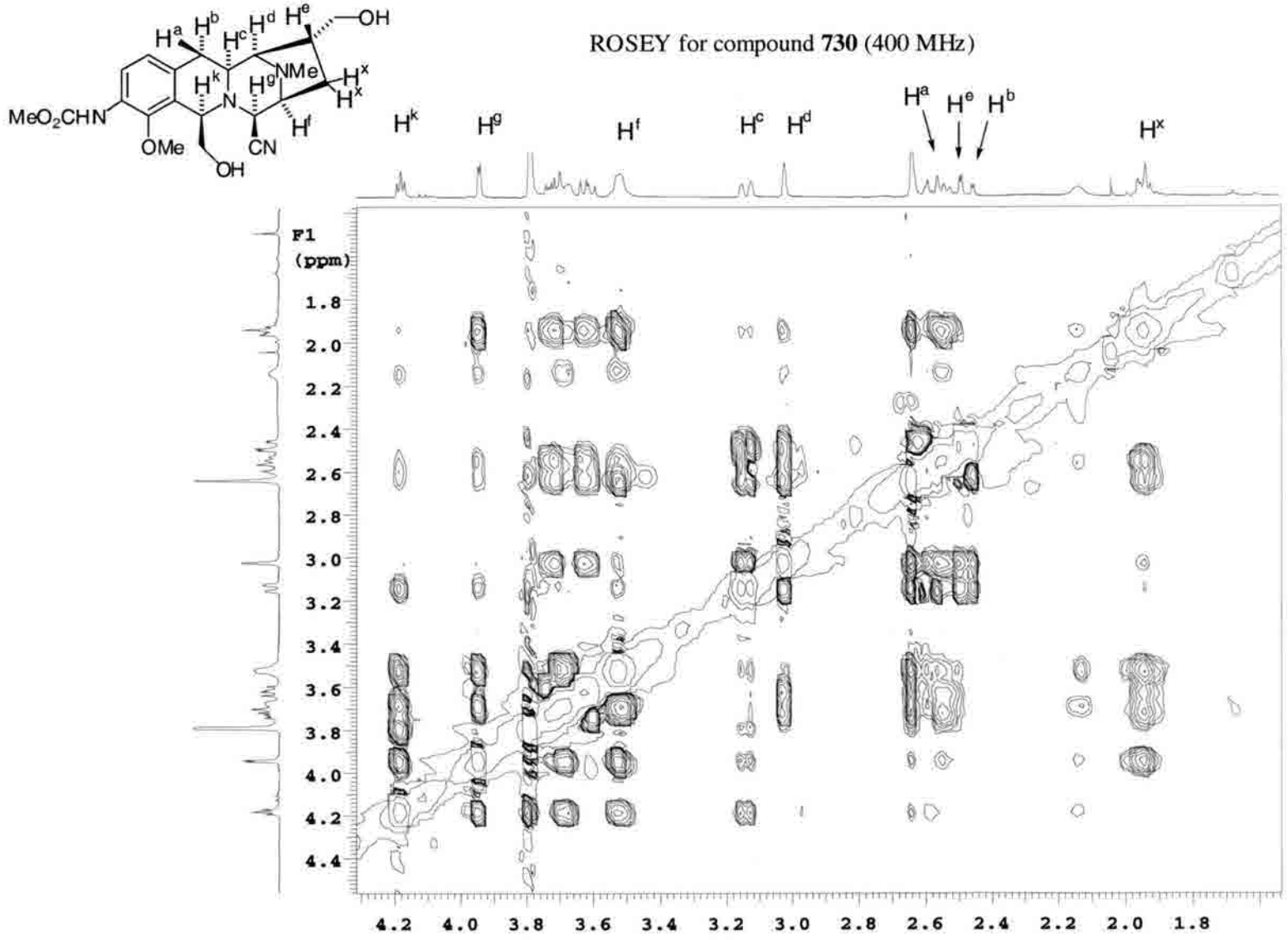
ROSEY for compound 730 (400 MHz)
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OMe
OH
CN
NMe
N
OH
F1
(ppm)
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2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8

Appendix 3

Publications

1. *Synthetic Studies on Tetrazomine: Assignment of Stereochemistry of the β-Hydroxypipecolic Acid.* Scott, J.D.; Tippie, T.N.; Williams, R.M., *Tetrahedron Lett.* **1998**, *39*, 3659~3662.
2. *Synthesis of a Netropsin Conjugate of a Water-Soluble Epi-quinocarcin Analog: The Importance of Stereochemistry at Nitrogen.* Herberich, B.; Scott, J.D.; Williams, R.M., *Bioorg. Med. Chem.*, **2000**, *8*, 523~532.
3. *Synthetic Studies on Tetrazomine: Lipase PS Resolution of Racemic cis-β-Hydroxypipecolic Acid.* Scott, J.D.; Williams, R.M., *Tetrahedron Lett.* **2000**, *41*, 8413~8416.
4. *Total Synthesis of (-)-Tetrazomine and Determination of Its Stereochemistry.* Scott, J.D.; Williams, R.M. *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 1463~1465.
5. *Total Synthesis of (-)-Tetrazomine. Determination of the Stereochemistry of Tetrazomine and the Synthesis and Biological Activity of Tetrazomine Analogs.* Williams, R.M.; Scott, J.D., *J. Am. Chem. Soc.* **2002**, *124*, 2951~2956.
6. *Chemistry and Biology of the Tetrahydroisoquinoline Antitumor Antibiotics.* Scott, J.D.; Williams, R.M., *Chem. Rev.* **2002**, *102*, 1669~1730.

Pergamon

Tetrahedron Letters 39 (1998) 3659–3662

TETRAHEDRON LETTERS

Synthetic Studies on Tetrazomine: Stereochemical Assignment of the β-Hydroxypipecolic Acid

Jack D. Scott, Tracy N. Tippie and Robert M. Williams*

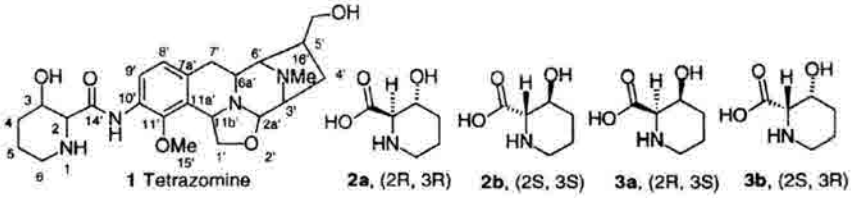
Department of Chemistry, Colorado State University

Fort Collins, Colorado 80523

Received 10 February 1998; accepted 16 March 1998

Abstract. The asymmetric syntheses of 2(R), 3(R)- and 2(S), 3(S)-β-hydroxypipecolic acids are described, including the determination of the absolute stereochemistry of the β-hydroxypipecolic acid moiety of tetrazomine.

Tetrazomine (1), is an antitumor antibiotic that was isolated from *Saccharothrix mutabilis*[1] by the Yamanouchi Pharmaceutical Co. in Japan. Preliminary antitumor/antimicrobial assays of this substance indicate that tetrazomine displays potent antitumor activity against P388 leukemia *in vivo* and displays good antimicrobial activity against both Gram-negative and Gram-positive organisms. Tetrazomine is structurally similar to the quinocarcin and bioxalomycin class of antitumor antibiotics and the mechanism of oxidative DNA cleavage mediated by tetrazomine has been reported from these laboratories.[2] Tetrazomine contains the unusual amino acid β-hydroxypipecolic acid. The relative and absolute stereochemistry of this amino acid and that of the polycyclic framework of tetrazomine have not yet been determined. Here we report the determination of the absolute stereochemistry of the β-hydroxypipecolic acid unit of tetrazomine.



1 Tetrazomine **2a**, (2R, 3R) **2b**, (2S, 3S) **3a**, (2R, 3S) **3b**, (2S, 3R)

In connection with studies on the structure elucidation, total synthesis[3] and mechanism of action of tetrazomine, we required stereochemically unambiguous syntheses of 2(R),3(R)- and 2(S),3(S)-β-hydroxypipecolic acids **2a** and **2b** (Figure 2). The synthesis of the 2(R),3(S)-stereoisomer **3a** has been completed by two groups[4,5] while the 2(S),3(R)-β-hydroxypipecolic acid stereoisomer **3b** has not been previously reported. Several groups have recently reported syntheses of **2a** and **2b**[6] and in this account, we describe an efficient asymmetric synthesis of the two *anti*-isomers of β-hydroxypipecolic acid.

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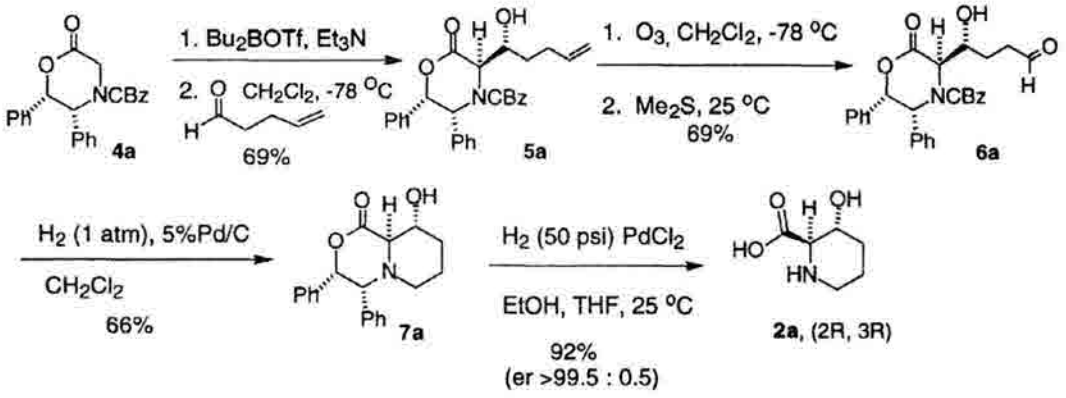
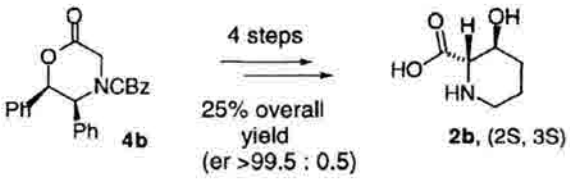
PII: S0040-4039(98)00642-X

As shown in Scheme 1, the commercially available lactone **4a** (Aldrich) was converted into the corresponding boron enolate with di-*n*-butyl boron triflate.[7] Diastereoselective aldol condensation with 4-pentenal (Lancaster) provided the desired *anti*-β-hydroxy aldol product **5a**[8] in 69% yield. Ozonolysis of the olefin furnished the aldehyde **6a**.[9] Mild catalytic hydrogenation of **6a** afforded bicyclic **7a**[10] via sequential N-CBz deprotection and reductive amination. Finally, the amino acid 2(R),3(R) β-hydroxy pipecolic acid **2a**[11] was produced through catalytic hydrogenation over palladium-black.



Scheme 1

The corresponding 2(S),3(S) β-hydroxy pipecolic acid **2b** was synthesized in the same manner using the commercially available lactone **4b** in the aldol condensation (Scheme 2). Both enantiomers of β-hydroxy pipecolic acid were produced with enantiomeric ratios (er) of >99.5 : 0.5. The synthesis reported here is five steps from commercially available **4** with an overall yield of 25-28% and compares very favorably with existing syntheses reported in the literature which range from 9-15 steps in overall yields less than 25%.[5,6]

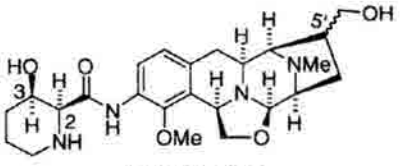


Scheme 2

To determine the relative and absolute stereochemistry of the β-hydroxy pipecolic acid of tetrazomine the amide of natural tetrazomine (Yamanouchi Pharmaceutical Co.) was subjected to hydrolysis. The hydrolysis product was compared to synthetic amino acids **2a**, **2b**, and **3a**.[12] Unfortunately, under basic hydrolysis conditions (2M LiOH, reflux 6 h) both *syn*- and *anti*-β-hydroxy pipecolic acids were detected in the crude hydrolysate by HPLC analysis indicating epimerization had occurred. Under acidic hydrolysis conditions, (4M HCl, 80 °C, 20 h) the only β-hydroxypipecolic acid detected by HPLC possessed the *syn*-relative stereochemistry. This amino acid was isolated from the hydrolysate by reverse-phase HPLC (20% MeOH/H_2O, Waters Resolve column, Waters 600 HPLC, uv 210nm) and was found to have physical data

(^{1}H-NMR, FAB HRMS) identical to that of the *syn*-β-hydroxy pipecolic acid **3a** which was prepared according to the procedure of Knight, et al., via Baker's yeast reduction of N-t-BOC 3-keto methyl pipecolate.[12] However, the β-hydroxy pipecolic acid obtained from the tetrazomine hydrolysis exhibited an optical rotation with the opposite sign to that of the authentic, synthetic sample of **3a**.[13] Thus, the β-hydroxy pipecolic acid moiety of tetrazomine must correspond to **3b** with the (2S, 3R)-configuration.

Based on the structural similarity of tetrazomine to quinocarcin and the bioxalomycins, biogenetic and stereoelectronic[2] considerations lead us to propose an almost complete stereostructure for tetrazomine as depicted below. The only stereogenic center that is still reasonably suspect, is C5′ which differs in relative configuration between quinocarcin and the bioxalomycins.



1 Tetrazomine

Efforts to elucidate the complete stereostructure of tetrazomine as well as defining the role that the β-hydroxy pipecolic acid unit plays in DNA recognition, transport and biological activity are under study in this laboratory.

Acknowledgment. We are grateful to Yamanouchi Pharmaceutical Co. for providing the generous gift of natural tetrazomine. This work was supported by the National Institutes of Health (CA 43969) and the National Science Foundation (CHE-9320010).

References and Footnotes

1. (a) Sato, T.; Hirayama, F.; Saito, T., *J. Antibiotics* **1991**, *44*, 1367~1370; (b) Suzuki, K., *et al.*, *J. Antibiot.* **1991**, *44*, 479~485.

2. (a) Williams, R.M.; Flanagan, M.E.; Tippie, T., *Biochemistry* **1994**, 33, 4086~4092; (b) Flanagan, M.E.; Rollins, S.B.; Williams, R.M., *Chemistry and Biology* **1995**, 2, 147~156.

3. Flanagan, M.E., Williams, R.M., *J.Org.Chem.* **1995**, *60*, 6791~6797.

4. Knight, D.W.; Lewis, N.; Share, A.C.; Haigh, D., *Tetrahedron Asymmetry* **1993**, **4**, 625~628. This paper does not describe the synthesis of the free amino acid but the protected version: N-t-BOC (2R, 3S)-3-hydroxy-methyl pipecolate which has been converted to **3a** (this work, ref. 11).

5. (a) Roemmele, R.C.; Rapoport, H., *J.Org.Chem.* **1989**, *54*, 1866~1875; (b) for a synthesis of the racemic *syn*-isomer, see: Makara, G.; Marshall, G.R., *Tetrahedron Lett.* **1997**, *38*, 5069~5072.

6. For other syntheses of the *anti*-isomers (**2a** and **2b**), see: (a) Greck, C.; Ferreira, F.; Genet, J.P., *Tetrahedron Lett.* **1996**, *37*, 2031~2034; (b) Ferreira, F.; Greck, C.; Genet, J.P., *Bull.Soc.Chim.Fr.* **1997**, *134*, 615~621; (c)

Battistini, L.; Zanardi, F.; Rassu, G.; Spanu, P.; Pelosi, G.; Fava, G.G.; Ferrari, M.B.; Casiraghi, G., *Tetrahedron Asymmetry* **1997**, *8*, 2975~2987; (d) Agami, C.; Couty, F.; Mathieu, H., *Tetrahedron Lett.* **1996**, *37*, 4001~4002.

7. For general experimental procedures for carrying out the boron enolate aldolization, see: Williams, R.M.; Yuan, C., *J.Org.Chem.* **1992**, *57*, 6519~6527.

8. **5a**: ^{1}H-NMR (300 MHz, d_6-DMSO vs TMS 393K) 1.69-1.85 (2H, m), 2.14-2.28 (2H, m), 4.17 (1H, m), 4.84 (1H, d, J=2.25 Hz), 4.95-5.18 (4H, m), 5.25 (1H, d, J = 3.18Hz), 5.47 (1H, d, J= 5.34 Hz), 5.82-5.88 (1H, m), 6.53 (1H, d, J = 3.16 Hz), 6.60 (2H, d, J=7.24 Hz), 6.99-7.39 (13 H, m). Anal calcd. for $C_{29}H_{28}NO_5$: C, 73.87; N 2.97; H 6.23 found: C, 74.04; N,2.85; H, 6.14. $[\alpha]_D^{20}$ = +13.7 (c=1.3, $CHCl_3$). **5b**: $[\alpha]_D^{20}$ = -12.4 (c=1.1, $CHCl_3$).

9. **6a**: ^{1}H-NMR (300MHz, d6-DMSO vs TMS, 393 K) δ 2.04 (2H, m), 2.29 (2H, m), 4.53 (1H, m), 4.99 (1H, d, J = 2.52 Hz), 5.03 (4H, m), 5.24 (2H, m), 6.71 (1H, d, J = 3.24 Hz), 7.02 (15H, m) 9.70 (1H, s). HRMS (FAB) calcd. for $C_{28}H_{28}NO_6$ (MH^+): 474.1917. found: 474.1914. $[\alpha]_D^{20}$ = + 3.1 (c = 1.25, $CHCl_3$). **6b**: $[\alpha]_D^{20}$ = - 3.7 (c = 1.25, $CHCl_3$).

10. **7a**: ^{1}H-NMR (300MHz, $CDCl_3$ vs TMS) δ 1.20 (1H, m), 1.70 (2H, m), 2.06 (2H, m), 2.86 (1H, d, J=11.4Hz), 3.01 (1H, d, J=8.7Hz), 3.96 (1H, ddd, J=2.1, 3.9, 4.8Hz), 4.24 (1H, d, J=3.9Hz), 4.9 (1H, s, D_2O exchangeable), 6.17 (1H, d, J=3.9Hz), 7.02-7.30 (10H, m). ^{13}C-NMR (75.47 MHz) δ 22.89, 32.42, 52.04, 62.95, 66.02, 62.95, 66.02, 69.11, 83.29, 125.3, 125.4, 127.6, 128.0, 128.2, 129.9, 130.7, 135.6, 172.1. HRMS (FAB) calcd. for $C_{29}H_{22}NO_3$ (MH^+): 324.1600. found: 324.1586. $[\alpha]_D^{20}$ = +21.4 (c = 1.9 $CHCl_3$) **7b** $[\alpha]_D^{20}$ = -20.9 (c = 1.6 $CHCl_3$).

11. **(2R,3R)-β-Hydroxypipecolic acid (2a)**. ^{1}H-NMR (300 MHz, D_2O) δ 1.55 (2H, m), 1.81 (2H, m), 2.91 (1H, m), 3.18 (1H, m), 3.44 (1H, d, J = 6.96 Hz), 3.97 (1H, m). HRMS (FAB) calcd. for $C_6H_{12}NO_3$ (MH^+): 146.0817, found 146.0822. The optical purity of the final amino acids **2a** and **2b** were determined to be > 99.5 : 0.5 er by chiral HPLC analysis (Daicel Chiral Pak WH, column temperature 40 °C using 0.25 mM $CuSO_4$ on a Waters 600 HPLC with a UV detector at 210 nm).

12. **(2R,3S)-β-Hydroxypipecolic acid (3a)**. This amino acid was synthesized using the Baker's yeast method of Knight, et al., (see ref. 4.) ^{1}H-NMR (300 MHz, D_2O) δ 1.85 (2H, m), 2.12 (2H, m), 3.07 (1H, ddd, J= 12.9, 3.6, 2.7 Hz), 3.47(1H, ddd, J= 12.6, 2.1, 1.8 Hz), 3.75 (1H, d, J = 1.5 Hz), 4.57 (1H, s, broad). ^{13}C-NMR (75.47 MHz vs. d_4 MeOD) δ 16.89, 29.72, 44.56, 63.20, 65.04, 173.06. HRMS (FAB) calcd. for $C_6H_{12}NO_3$ (MH^+) 146.0817 found: 146.0821. The final product reported by Knight, et al., (ref. 4) N-t-BOC (2R, 3S)-3-hydroxy-methyl pipecolate, was converted to the free amino acid by sequential treatment with TFA in CH_2Cl_2 followed by removal of the solvent and treatment of the amino methyl ester with KOH in water at room temperature. Acidic Dowex-50WX2-100 ion exchange resin treatment (eluted within 2% ammonium hydroxide) afforded the free amino acid **3a** (81% for two steps). To our knowledge, physical data for the free amino acid (**3a**) have not been previously reported .

13. The isolated amino acid (**3b**) exhibited an $[\alpha]_D^{20}$ = -72.3° (c=0.10, 1M HCl) whereas, **3a** (see ref. 12) exhibited an $[\alpha]_D^{20}$ = +82.1° (c=0.12, 1M HCl).

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Bioorganic & Medicinal Chemistry 8 (2000) 523–532

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of a Netropsin Conjugate of a Water-Soluble *epi*-Quinocarcin Analogue: the Importance of Stereochemistry at Nitrogen

Brad Herberich, Jack D. Scott and Robert M. Williams*

Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA

Received 9 August 1999; accepted 15 October 1999

Abstract—The efficient synthesis of a water-soluble C11a-*epi*-analogue (**6b**) of quinocarcin is described. This substance, and a netropsin amide conjugate (**8**) lack the capacity to inflict oxidative damage on DNA due to the stereoelectronic geometry of their oxazolidine nitrogen atoms. The capacity of these substances to alkylate DNA through the generation of an iminium species has been examined. Both compounds were found to be unreactive as DNA alkylating agents. The results of this study are discussed in the context of previous proposals on the mode of action of this family of antitumor alkaloids. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Quinocarcin,[1–3] tetrazomine[4,5] and bioxalomycin[6–8] are cytotoxic oxazolidine-containing natural products obtained from various *Actinomycetes* sp. These substances have been shown to display potent antitumor activity in vitro and in vivo.[9–15] We have previously shown that the fused oxazolidine ring of these naturally occurring substances undergoes a spontaneous redox disproportionation reaction that, in the presence of molecular oxygen, results in the net reduction of dioxygen to superoxide radical anion culminating in strand scission of DNA.[16–19] During our studies on the mechanism of this novel auto redox disproportionation reaction, we postulated an unusual stereoelectronic requirement of the non-bonded electron pair on the oxazolidine nitrogen atom to adopt a *trans*- antiperiplanar geometry with respect to the adjacent oxazolidine methine hydrogen atom (**A**, Fig. 1) as an obligate structural parameter for inflicting oxidative damage on DNA and other cellular macromolecules (Fig. 1).[20,21] Experimental support for the stereoelectronic control of this reaction has been obtained and reported in the literature from this laboratory[17–21] through the synthesis of the C11a-*epi*-analogue **6a** which lacked the capacity to produce superoxide or cleave DNA. In contrast, the naturally configured analogues **5a**, **5b** and **7** retained the capacity to reduce molecular oxygen to superoxide and mediated oxidative cleavage of DNA. An X-ray structural determination on compound **5a** confirmed the geometry of the oxazolidine ring as that depicted in Figure 1.[20,21]

In 1988, Remers and co-workers reported the results of computer simulations on the covalent and non-covalent binding of quinocarcin to DNA and postulated that the nitrogen atom of the oxazolidine ring would have to undergo pyramidal inversion via a boat/twist conformational change to adopt a *trans*-antiperiplanar relationship to the oxazolidine C–O bond for ring-opening to the putative iminium species **9**, which was invoked as the electrophilic, DNA-alkylating reactive intermediate leading to the proposed adduct **10** (Fig. 2).[22] This conformational change (**1a**→**1b**) was calculated to require ~10 kcal/mol and suggested that iminium ion formation should occur readily via conformer **1b**. To further probe the stereoelectronic requirements for ring-opening of a fused oxazolidine to a putative DNA-alkylating iminium species, we have designed the C11a-*epi*-series **6** (quinocarcin numbering, Fig. 1) which disposes the non-bonded electron pair of the oxazolidine nitrogen atom *trans*-antiperiplanar to the C7–O bond.

The stereochemistry of the C11a-*epi*-system was previously secured through an X-ray structural determination

*Corresponding author. Tel.: +1-970-491-5610; fax: +1-970-491-5610; e-mail: rmw@chem.colostate.edu

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PII: S0968-0896(99)00314-4

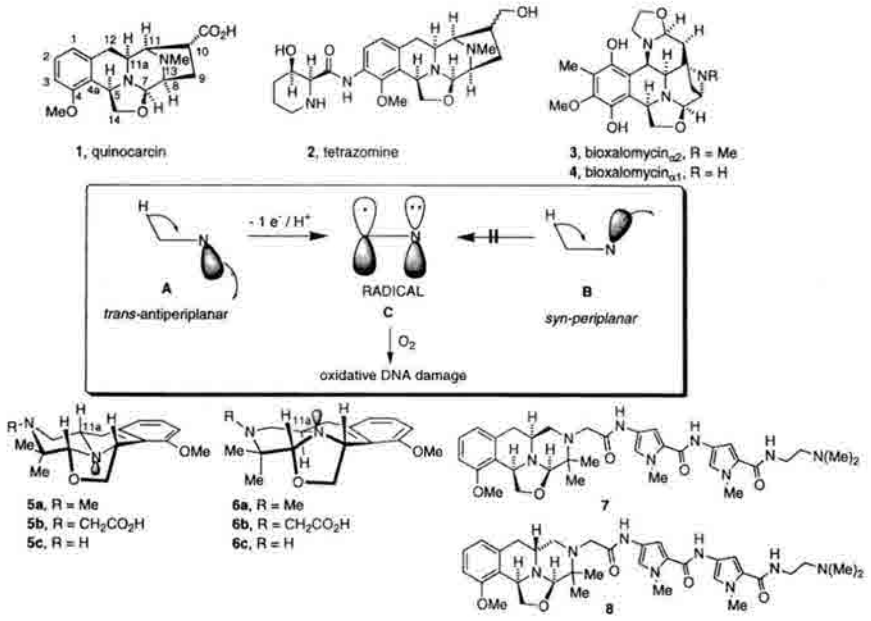


Figure 1.

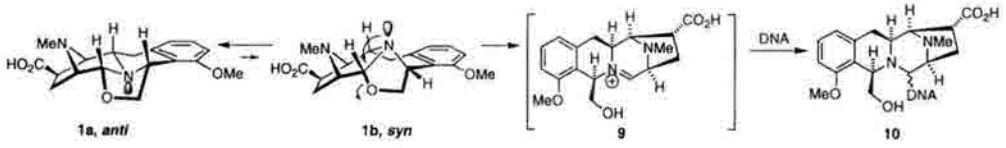


Figure 2.

on compound **6a**.[21] As discussed above, this stereoelectronic arrangement in the oxazolidine ring also renders such compounds very stable to the auto-redox disproportionation manifested in the natural series. Thus, the expectation was that the C11a-*epi*-series (**6**), should readily form an iminium ion and exhibit DNA alkylation reactivity. As discussed previously for compounds **5a** and **6a**, limited water solubility of these substances precluded an in-depth study of their interactions with DNA. The synthesis of the water-soluble analogues in the naturally configured series **5b** and **7** have been previously reported and their biochemical reactivity discussed.[19] The purpose of the present study, was to examine the stereochemically complementary analogues **6b** and **8**; the synthesis and reactivity of these substances are reported herein.

Results and Discussion

The retrosynthetic plan for the synthesis of **6b** was initially envisioned to follow a similar route to that employed in the construction of the related quinocarcin analogues **5b** and **7** which proceeded through alkylation of the secondary amine of tetracycle **5c**.[19] However, in the event, we were unable to effect the cyclization of the amino alcohol-aldehyde precursor to **6c** in synthetically useful yields and had to deploy a somewhat different strategy. Previous experience with this type of ring-closure dictated that *N*-alkylation (at N13, quinocarcin numbering) to a tertiary amine gave improved yields in the key oxazolidine-forming cyclization reaction. The *anti*-saturated acid **13** was envisioned to be readily available from the previously reported β-hydroxy ester **11**[20] via elimination to **12**, saturation of the olefin and epimerization to the kinetically favored *anti*-stereochemistry. Reduction of the acid of compound **13** to the corresponding alcohol **14**, secondary amine formation **16** and alkylation with *t*-butylbromoacetate would furnish the tertiary amino alcohol **17**. Oxidation of the alcohol to the corresponding aldehyde **18** followed by the crucial concomitant oxazolidinone hydrolysis and oxazolidine ring closure was anticipated to provide the key tetracyclic acid **6b**. Homologation of the acid of **6b** to the corresponding netropsin amide **8** using our previously established protocol[19] involving the in situ reductive acylation with **19** would complete the synthesis.

As shown in Scheme 1, conversion of **11** to the unsaturated ester **12** proceeded in excellent yield through treatment of **11** with oxalyl chloride. The incipient labile β-chloro ester was immediately treated with triethylamine at reflux temperature to provide **12**. Catalytic hydrogenation of **12** provides the corresponding saturated ethylester as a ~1:1 mixture of *syn*- : *anti*-diastereomers. Without separation this mixture was subjected to saponification with lithium hydroxide in ethanol, epimerization of the *syn*-ester to the *anti*-ester and concomitant hydrolysis of the ester to the acid ensues to yield *anti*-**13** in 72% overall yield from **12**. Next, treatment of acid **13** with oxalyl chloride provides the acid chloride which was immediately reduced with sodium borohydride to give the desired *anti*-alcohol **14** in excellent yield. Mesylation of **14** and alkylation with 2-amino-2-methyl-1-propanol (**15**) provided the desired secondary amine **16** in 74% yield for the two steps.[23]

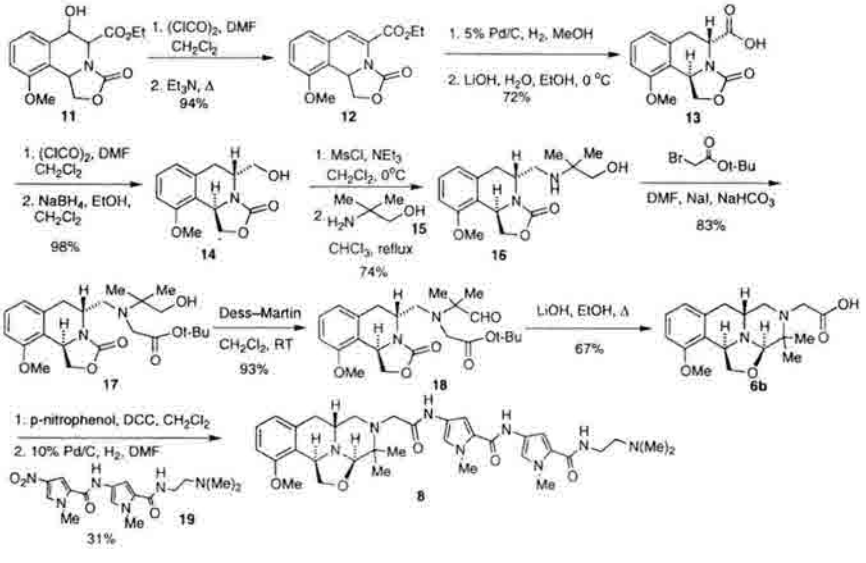
As mentioned above, we examined the conversion of **16** to **6c** by Dess–Martin oxidation to the aldehyde (57%) followed by attempted ring closure under basic conditions as previously described for **5c**.[19] However, in this case, only decomposition attended many attempts to effect the closure to **6c**. Thus, alkylation of **16** with *t*-butylbromoacetate provided the tertiary amine **17** in 83% yield without any δ-lactone formation which, we observed to occur with less hindered bromoacetate derivatives. Oxidation of the primary alcohol of **17** with Dess–Martin periodinane gave the key aldehyde **18** in 93% yield. We were very pleased to see that treatment of **18** with lithium hydroxide in hot ethanol effected the cleavage of the oxazolidinone, closure of the putative amino alcohol to the desired oxazolidine and hydrolysis of the *t*-butyl ester to the acid **6b** in 67% overall yield. It is worth mentioning that this transformation represents one of the highest yielding ring closure reactions to a fused oxazolidine ring system thus far reported in this family of alkaloids.

The final conversion of **6b** to **8** proceeded smoothly and was accomplished by transforming the acid of **6b** to the corresponding active *p*-nitrophenyl ester. Reduction of the known nitro compound **19**[24] to the corresponding primary amine and direct acylation with the *p*-nitrophenyl ester provided **8** in 31% overall yield from **6b**.

Evaluation of 6b and 8 for superoxide production

The capacity of **6b** to produce superoxide was measured by following the reduction of nitroblue tetrazolium (NBT). In the reduction process NBT is reduced to monoformazan which can be detected spectrophotometrically in the visible range. We have previously shown that this reduction is completely inhibited by the addition of superoxide dismutase (SOD).[17–19]

The assay was carried out as described previously in measuring the superoxide production of quinocarcin and analogues **5a**, **5b** and **7**.[19] Compound **6b** was added to an aerated solution of NBT (0.12 mM) in a 20 mM phosphate buffer (pH 7) such that the final concentration of **6b** was 2.0 mM. The optical absorbance was recorded over 15 min and the change in optical density was the average slope for the linear change in optical density over the reaction time. The rates of superoxide production were calculated from the molar extinction coefficient of formazan at 500 nm (12,200) and by assuming the reaction is pseudo first order in O_2. As expected based on the stereoelectronic parameters previously established via **6a**, nitroblue tetrazolium in the presence of **6b** was not reduced, demonstrating the inability of **6b** to produce superoxide. By comparison,



Scheme 1.

under the same conditions the all *syn*–analogue **5b** had a rate of superoxide production of 0.82×10^{-9} M s^{-1},[19] while a 1.0 mM solution of quinocarcin exhibits a rate of 4.2×10^{-9} M s^{-1} and a 0.1 mM solution of bioxalomycin α_2 demonstrates a rate of 3.88×10^{-8} M s^{-1}.

Evaluation of DNA cleavage and alkylation reactions

To assay the DNA alkylating abilities of **6b** and **8**, band shift assays were conducted using ^{32}P-5′-end-labeled deoxyoligonucleotides. In these experiments, solutions of the analogues and 5′-^{32}P-labeled DNA were incubated at 37 °C for 12 h. The DNA was then precipitated with ethanol and dried. The samples were suspended in water and loading dye, and loaded onto a denaturing or non-denaturing electrophoresis gel. After the gels were run, they were stored with photographic film at −80 °C for 12 h. If alkylation of the DNA occurred, the mobility of the alkylated DNA should be retarded due to the increased mass.

In the event, when **6b** was incubated under a wide range of concentrations (5 μM~5 mM) with a number of DNA templates (templates A–C, Fig. 3) at different concentrations (0.1–10 mM), no evidence for DNA alkylation was observed. As expected from the lack of capacity of either **6b** or **8** to produce superoxide, neither substance effected the oxidative cleavage of DNA.

The netropsin conjugate **8** was incubated (at high concentrations (2.0–4.0 mM)) with DNA templates D, E and F (0.85–2.45 mM), which contained netropsin binding sequences (Fig. 4).[19] Despite many alterations of the reaction parameters, we did not detect any evidence for DNA alkylation with this substance.

While we expected compounds **6b** and **8** to be incapable of causing oxidative damage to DNA based on the stereoelectronic grounds detailed above, we were somewhat surprised at the lack of reactivity of these substances toward DNA alkylation. The stereoelectronic configuration of the non-bonded electron pair on the oxazolidine nitrogen atom in **6b** and **8** is disposed *trans*-antiperiplanar to the C–O bond of the oxazolidine ring and, in accordance with the modeling predictions of Remers,[22] should facilitate ring-opening to an iminium species **20** which in principle, should be capable of forming the DNA alkylation adduct **22** (Scheme 2).

We examined an alternative, indirect probe for the capacity of this system to undergo ring-opening. Quinocarcin, when treated with NaCN under basic conditions, gives the corresponding α–aminonitrile derivative DX–52–1, which displays antitumor activity.[25–29] However,

Template A- 5′-TTTATTAACGTAATGCTTAATCGCAATGGGATT-3′
3′-AAATAATTGCATTACGAATTAGCGTTACCCTAA-5′

Template B- 5′-CCCACATCACTATACACGCGCGAAATTTCTGCGTAT-3′
3′-GGGTGTAGTGATATGAGCGCGCTTTAAAGACGCATA-5′

Template C- 5′-ATACCCACATCACGCGCGTATACCAAATTTCTCATTACT-3′
3′-TATGGGTGTAGTGCGCGCATATGGTTTAAAGAGTAATGA-5′

Figure 3. DNA templates incubated with **6b**.

Template D- 5′-ACAGCATGTCCTACG**AATT**CGTAGGACATGCTGT-3′
3′-TGTCGTACAGGATGC**TTAA**CGATCCTGTACGACA-5′

TemplateE- 5′-GGAATTGGGC**AATT**GCCCAATTCC-3′
3′-CCTTAACCCG**TTAA**CGGGTTAAGG-5′

Template F- 5′-GGGCCC**AAATTT**GGGCCC-3′
3′-CCCGGG**TTTAAA**CCCGGG-5′

Figure 4. DNA templates incubated with **8**. The netropsin binding regions are in bold face.

stirring **6b** with NaCN under basic conditions[30] gave mostly recovered starting material with no detectable production of the corresponding α-aminonitrile **21**. Conversely, the exact same reaction conditions when applied to **5b** cleanly afforded the α-aminonitrile **23** (Scheme 3).[31]

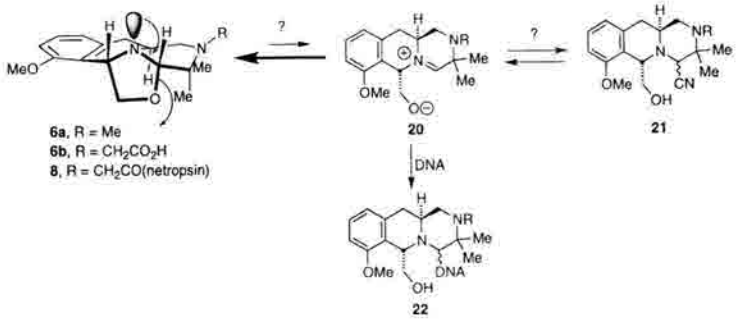
The difference in reactivity between **5b** and **6b** towards the ring-opening/cyanation reaction raises interesting questions concerning the intrinsic, relative stability and facility of ring-opening of the oxazolidine rings of these two substances. Based on stereoelectronic considerations, it is possible that the oxazolidine ring of **6b** and **8** might be destabilized by an $n\rightarrow\sigma^*$ (anomeric effect) interaction and rendered more reactive to ring-opening to the corresponding iminium species. However, comparison of the C–O bond lengths for compounds **5a** and **6a** as revealed from their respective X-ray structures, shows that both compounds have C–O bond lengths of 1.424 and 1.427Å, respectively.[20,21] An anomeric effect would typically lengthen the C–O bond which is not observed in the solid state for **6a** relative to **5a** to any significant extent.[32]

Due to the lack of reactivity of **6b** and **8**, one final derivative of **6b** was synthesized to probe a previously proposed mechanism of DNA alkylation. The reductive activation of naphthyridinomycin was proposed by Zmijewski et al., to involve formation of an incipient *ortho*-quinone methide species with participation of the phenolic residue to yield an iminium ion upon re-closure.[33,34] A similar mechanism depicted for analogues **6b** or **8** is shown in Scheme 4. In this mechanism the phenol of **24** would be deprotonated, resulting in opening of the oxazolidine ring and formation of imine **25**. The non-bonded pair of electrons on the nitrogen of the imine could then add to the electrophilic quinone methide to restore aromaticity and form iminium ion **26**, which is the proposed DNA alkylating agent.

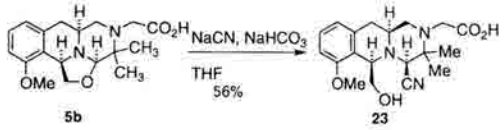
The demethylation of **6b** to produce phenol **24** was readily achieved using BBr_3 in 47% yield. To assay for DNA alkylation, phenol **24** and DNA template **A** were incubated at 37 °C in a phosphate buffer (pH 8) for 12 h and the samples were evaluated as described earlier using gel electrophoresis. Again, substance **24** demonstrated no evidence for DNA alkylation by the gel shift assay.

Conclusions

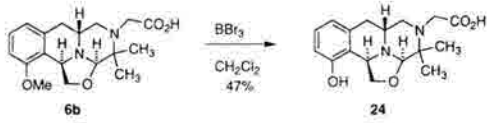
The results of this study underscore the subtle yet significant changes in chemical and biochemical reactivity



Scheme 2.



Scheme 3.



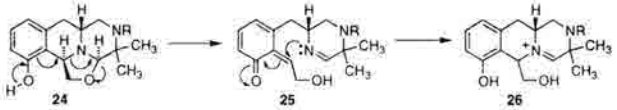
Scheme 5.

rendered by the stereoelectronic geometry of the nitrogen atom in the simple fused oxazolidine ring of this family of alkaloids. The lack of DNA alkylation products with analogues that possess the *epi*-quinocarcin stereochemistry (**6b**, **8** and **24**) may be due to steric congestion around C-7 caused by the *gem*-dimethyl groups or may be a manifestation of the intrinsic instability of the alkylated product. The proximity of the reaction center (C-7) to a *gem*-dimethyl group may make the alkylation difficult due to steric interactions. On the other hand, the bioxalomycins, which are sterically congested around C-7, both alkylate and covalently cross-link DNA.[35]

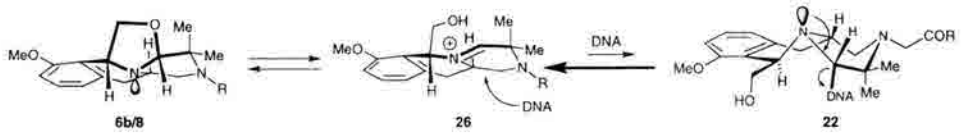
Another explanation for the absence of DNA alkylation and cyanation by the *epi*-quinocarcin analogues is illustrated in Scheme 6. If **6b**/**8** is alkylated via the ring-opened iminium species **26** from the more accessible α-face by DNA, the developing non-bonded electron pair on the nitrogen atom in the resulting product (**22**) would be expected to initially adopt an *anti*-periplanar geometry with respect to the DNA base residue due to repulsion in the transition state. Although this configuration at nitrogen (*cis*-decalin-type) is expected to be slightly less stable than the alternative configuration at nitrogen (*trans*-decalin-type),[36] if this is the geometry initially formed at nitrogen, the DNA residue can be easily expelled to give back iminium **26** and then **6b**/**8**. The relative instability of such adducts would also explain the failure to prepare α-aminonitrile product **21** from **6b**.

A vector analysis on the X-ray crystal structure[21] of compound **6a** was carried out and this analysis revealed that the dihedral angle between the C–O bond of the oxazolidine and the non-bonded electron pair on the adjacent nitrogen atom in the oxazolidine ring is approximately 162° (or ~18° off normal; Fig. 5). While this angle is not an optimal 180° for a perfectly aligned *trans*-antiperiplanar disposition of the non-bonded electron pair on the nitrogen atom and the scissile C–O bond, this relatively small deviation from normal would not, a priori, be expected to preclude ring-opening of the oxazolidine. It can be assumed that the geometries of compounds **6b** and **6c** closely mimic that for **6a** which have the same stereochemistry surrounding the oxazolidine ring. Thus, although this analysis supports the notion that the oxazolidine ring of **6a**~**c** should readily suffer ring-opening to an iminium species such as **26**, the initially formed intermolecular adducts such as **21** and **22** may rapidly revert back to **6**.

As discussed above, trapping of **5b** with sodium cyanide gave the α-aminonitrile adduct **23** and the relative configuration of this substance has been assigned by ^{1}H NMR/NOE studies. Although the pyramidal configuration at nitrogen in this substance has not been assigned, it appears very reasonable that the nitrile group in this substance is in an equatorial orientation as shown in Scheme 7 and that the bridgehead nitrogen adopts a *trans*-decalin-type of geometry. If the incipient iminium species (**28**) is attacked from the β-face, *anti*- to the hydroxymethyl group, the initially formed



Scheme 4.



Scheme 6.

α-aminonitrile **29** would place the nitrile group in an axial orientation and *trans*-antiperiplanar to the bridgehead nitrogen atom and would be expected to be readily expelled regenerating **28**. The thermodynamic product **23**, would therefore result from cyanide attack *syn*- to the hydroxymethyl group from the α-face, (which is *anti*- to the methine hydrogen at C-11a) but produces the stable adduct **23**. Applying this analysis to iminium species **26**, the alternative β-face attack of cyanide is more hindered than the complementary α-face attack on **28** (in other words, *syn*- to the hydroxymethyl group) since, both the hydroxymethyl group and the C-11a methine in **26** are situated in an axial orientation rendering this a more sterically demanding approach. In addition, the β-face adduct, once readjusted to the more stable *trans*-decalin-type of conformation would situate the nitrile group axial and *trans*-antiperiplanar to the non-bonded electron pair on the bridgehead nitrogen atom and thus labile to expulsion.

The stereoelectronic geometry of the oxazolidine ring in the *natural* series may therefore be obligate for *both* the redox disproportionation manifold culminating in oxidative DNA strand scission *and* for DNA alkylation reactions. Efforts to further clarify and define these parameters are under study in these laboratories.[37]

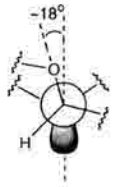
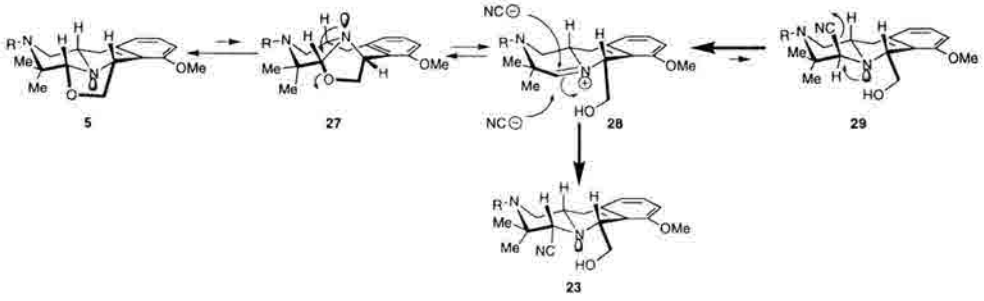


Figure 5. Dihedral angle between the non-bonded electron pair on the oxazolidine nitrogen atom and the adjacent scissile C–O bond of compound **6a** as revealed from a vector analysis of the X-ray structure.[21]

Experimental

General experimental methods

All drug concentrations were made up to 54 mM in water immediately prior to use. Deoxyoligonucleotides ('oligos') were synthesized on the Applied Biosystems 380B DNA synthesizer using standard phosphoramidite chemistry (reagents and phosphoramidites were purchased from GLEN Research). Deoxyoligonucleotides were deprotected by heating 15 h at 55 °C in NH_4OH, followed by filtering of the CPG resin and concentration of supernatant in vacuo. All oligos were purified by 20% denaturing polyacrylamide gel electrophoresis (DPAGE). Oligos of interest were 5′-end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs). Labeled oligos were then hybridized to their corresponding blunt-ended complements in 20 mM phosphate buffer (pH 7 and 8) by heating the equimolar mixture of oligos to 80 °C for 15 min, and cooling to room temperature over 2 h. Gel-loading buffer contained 0.03% bromophenol blue and 0.03% xylene cyanole in formamide. Centrex MF 0.45 μM cellulose acetate spin filters were obtained from Schleicher & Schuell. Samples were counted on a Packard 1500 Tri–Carb liquid scintillation analyzer. Unless otherwise noted, materials were obtained from commercially available sources and used without further purification. Diethyl ether and THF were distilled from sodium benzophenone ketyl under a nitrogen atmosphere. Methylene chloride and triethylamine were distilled under a nitrogen atmosphere from calcium hydride. Dimethyl formamide was dried over 4 Å molecular sieves. The molecular sieves were activated by heating at 150 °C at 1 mm Hg for 3 h in a vacuum oven. Chromatographic separations were performed with EM Science TLC plates (silica gel 60, F254, 20×20 cm×250 μm) or with EM Science 230–400 mesh silica gel under positive air pressure. Reactions and chromatographic fractions were



Scheme 7.

monitored and analyzed with EM Science TLC plates. Visualization on TLC were achieved with ultraviolet light and heating of TLC plates submerged in a 5% solution of phosphomolybdic acid in 95% ethanol (PMA) or 2,4-dinitrophenylhydrazine in 2 M HCl (DNP) or *p*-anisaldehyde in 95% ethanol or Dragendorff solution. Melting points were determined in open capillary tubes with a Mel-Temp apparatus and are uncorrected. Infared spectra were recorded on a Perkin–Elmer 1600 series FTIR as thin films from methylene chloride and are reported as λ_{max} in wavenumbers (cm^{-1}). Elemental analyses are accurate to within 0.4% of the calculated values. Mass spectra were obtained on a 1992 Fisions VG Autospec at the Chemistry Department at Colorado State University. Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker AC–300, Varian 300 or 400 spectrometer. NMR chemical shifts are given in parts per million (ppm) relative to internal $CHCl_3$, DMSO, or methanol. Proton (^1H) NMR are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant in hertz, and number of protons. When appropriate, the multiplicity of a signal is denoted as 'br' to indicate that the signal was broad. All compounds without elemental analysis data were determined to have purity of at least 95% by ^1H and ^{13}C NMR.

Reductions of nitroblue tetrazolium (NBT)

Each reaction was performed in triplicate by adding an appropriate amount of drug stock solution to an aerated solution of nitroblue tetrazolium (0.12 mM) in 20 mM phosphate buffer (at pH 7) containing 1% Triton X100 detergent and the final volumes brought to 750 μL with deionized water. The optical absorbance was measured at 25 °C over a 15 min period at 500 nm (Varian DMS 80 UV–vis spectrophotometer) and the ΔOD was the average slope for the linear OD change over the reaction time. The rates for superoxide production were calculated by assuming that $[O_2]$ does not appreciably change over this time period and is in excess (zero order in oxygen). The rates were calculated from the ΔOD measurements and based on a molar extinction coefficient (ϵ_o) of 12,200 for the monoformazan product of NBT at 500 nm.

1,10b-Dihydro-10-methoxy-3*H*-oxazolo[4,3-α]isoquinolin-3-one-5-carboxylic acid ethyl ester (12). DMF (1.06 mL, 13.6 mmol) followed by oxalyl chloride (1.33 mL, 20.5 mmol) was added to a solution of **11** (4.19 g, 13.6 mmol) in 50 mL of CH_2Cl_2 at room temperature. After 30 min the solvent was stripped off and the residue dissolved in NEt_3 (30 mL) and heated at reflux for 2 h. After cooling to room temperature the reaction was diluted with Et_2O, washed with water, and dried over $MgSO_4$ to give 3.70 g (94%) of **12**. Mp 117–119 °C (recryst. Et_2O). TLC (1:1 EtOAc:hexane) R_f 0.26 (UV and PMA). ^1H NMR (300 MHz) ($CDCl_3$) δ 1.32 (t, J=7.2 Hz, 3H), 3.80 (s, 3H), 4.32 (dq, J=2.5, 7.2 Hz, 2H), 4.59 (dd, J=8.4, 9.3 Hz, 1H), 5.01 (dd, J=8.7, 9.3 Hz, 1H), 5.29 (dd, J=8.4, 8.7 Hz, 1H), 6.86 (dd, J=7.8, 8.7 Hz, 2H), 6.90 (s, 1H), 7.25 (dd, J=7.4, 8.7 Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 14.2, 53.5, 55.7, 61.8, 70.7, 112.6, 120.6, 122.1, 123.0, 127.5, 129.7, 130.3, 155.3, 155.6, 162.6. IR (NaCl, neat) 2981, 2360, 2343, 1767, 1724, 1635, 1575, 1476, 1405, 1306, 1263, 1213, 1092 cm^{-1}. Anal. calcd for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.18; H, 5.40; N, 4.89.

***trans*-1,5,6,10b-Tetrahydro-10-methoxy-3*H*-oxazolo[4,3-α]isoquinolin-3-one-5-carboxylic acid (13).** Ester **12** (5.65 g, 19.5 mmol) was dissolved in 325 mL of EtOH and 5% Pd/C (10.4 g, 4.9 mmol) was added. Hydrogen was bubbled through the solution for 5 min and the reaction was allowed to stir under an atmosphere of H_2 overnight. Argon gas was bubbled through the reaction and the mixture was filtered through a pad of Celite to give the saturated ethyl ester as a mixture of diastereomers (4.46 g, 78%). To a stirred solution of the ethyl ester (2.25 g, 8.12 mmol) in 100 mL of EtOH and 40 mL of H_2O at 0 °C was added $LiOH–H_2O$ (511 mg, 12.2 mmol). After 3 h at 0 °C the volume of the reaction was reduced by half and acidified with 1 M HCl. The reaction was extracted with ethyl acetate, dried over $NaSO_4$ and concentrated to afford acid **13** (92% yield). The acid was purified for analytical sample by recrystallization. Mp 230–232 °C (recryst. EtOAc–EtOH). ^1H NMR (300 MHz) (DMSO-d_6) δ 3.14 (d, J=4.1 Hz, 2H), 3.78 (s, 3H), 3.97 (t, J=8.7 Hz, 1H), 4.76 (dd, J=4.1, 4.6 Hz, 1H), 4.93 (t, J=8.6 Hz, 1H), 5.10 (t, J=8.8 Hz, 1H), 6.83 (d, J=7.7 Hz, 1H), 6.99 (d, J=8.2 Hz, 1H), 7.23 (dd, J=8.1, 7.9 Hz, 1H), 13.20 (bs, 1H); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 29.6, 49.5, 50.1, 55.6, 69.5, 108.9, 121.3, 121.5, 128.4, 132.4, 155.7, 156.3, 171.3. Anal. calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.81; H, 5.12; N, 5.31.

***trans*-1,5,6,10b-Tetrahydro-5-hydroxymethyl-10-methoxy-3*H*-oxazolo[4,3-α]isoquinolin-3-one (14).** To a solution of acid **13** (3.31 g, 12.57 mmol) in 200 mL of CH_2Cl_2 was added oxalyl chloride (1.40 mL, 22.00 mmol) and a drop of DMF. After 2 h the solvent was stripped off. The acid chloride was dissolved in 150 mL of CH_2Cl_2 and cooled to −78 °C. A slurry of $NaBH_4$ (2.38 g, 62.85 mmol) in 75 mL of EtOH was made, cooled to 0 °C, and added to the reaction. The cooling bath was removed and the reaction was allowed to warm up to room temperature. After 2 h the reaction was quenched by careful addition of 1 M HCl at 0 °C. The reaction was washed with CH_2Cl_2 and the organic phase dried over $MgSO_4$ and concentrated. The crude was purified with column chromatography (SiO_2, 3:1 EtOAc:hexane) giving 3.10 g (98%) of **14** as yellow plates. Mp 108–110 °C (recryst. EtOAc). TLC (3:1 EtOAc:hexane) R_f 0.18 (UV). ^1H NMR (300 MHz) ($CDCl_3$) δ 2.24 (t, J=6.1 Hz, 1H, D_2O exch.), 2.69 (d, J=16.9 Hz, 1H), 3.13 (dd, J=7.0 Hz, 16.8 Hz, 1H), 3.59–3.64 (m, 2H), 3.78 (s, 3H), 4.08 (t, J=8.3 Hz, 1H), 4.36 (dd, J=7.0, 14.1 Hz, 1H), 4.92 (dd, J=8.5, 8.7 Hz, 1H), 5.02 (dt, J=8.3, 8.5 Hz, 1H), 6.73 (dd, J=6.0, 8.0 Hz, 2H), 7.20 (t, J=8.0 Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 28.8, 49.5, 50.0, 55.5, 61.7, 70.2, 108.3, 122.2, 128.7, 133.4, 156.0, 158.4, 169.6. IR (NaCl, neat) 3421, 2942, 1737, 1586, 1473, 1257, 1094 cm^{-1}. Anal. calcd for $C_{13}H_{17}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.49; H, 5.94; N, 5.66.

***trans*-1,5,6,10b-Tetrahydro-5-[(2-hydroxy-1,1-dimethylethyl)amino]methyl-10-methoxy-3*H*-oxazolo[4,3-α]-isoquinolin-3-one (16).** Methanesulfonyl chloride (116 μL, 1.5 mmol) and NEt_3 (348 μL, 2.5 mmol) were added to a solution of alcohol **14** (250 mg, 1 mmol) in 2 mL of CH_2Cl_2 at 0 °C. After 2 h the reaction was run through a short plug of silica with 10:1 CH_2Cl_2:MeOH as the eluent. The mesylate was redissolved in 3 mL of $CHCl_3$ and 2-amino-2-methyl-1-propanol (**15**) (0.95 mL, 10 mmol) was added. The reaction was allowed to reflux for 2 days. It was diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd), dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (SiO_2, 10:1 CH_2Cl_2:MeOH) affording **11** (236 mg, 70%) as slightly-yellow plates. TLC (10:1 CH_2Cl_2:MeOH) R_f 0.28 (UV and Dragendorff). Mp 111–115 °C (recryst. MeOH). ^1H NMR (300 MHz) ($CDCl_3$) δ 0.94 (s, 3H), 0.97 (s, 3H), 2.50 (br s, 1H), 2.51 (dd, J=5.3, 11.7 Hz, 1H), 2.56–2.67 (m, 2H), 3.11 (dd, J=6.8, 16.7 Hz, 1H), 3.24 (dd, J=10.8, 17.9 Hz, 2H), 3.39 (s, 1H, D_2O exch.), 3.76 (s, 3H), 4.02 (t, J=5.7 Hz, 1H), 4.22–4.29 (m, 1H), 4.87–4.97 (m, 2H), 6.69 (d, J=8.0 Hz, 2H), 7.15 (t, J=8.0 Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 23.6, 23.7, 30.8, 42.3, 49.0, 49.0, 53.8, 55.4, 69.1, 70.0, 108.1, 122.0, 122.1, 128.6, 133.4, 155.8, 158.3. IR (NaCl, neat) 3441, 2964, 1748, 1586, 1472, 1258, 1094 cm^{-1}. Anal. calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.74. Found: C, 63.53; H, 7.46; N, 8.66.

***trans*-1,5,6,10b-Tetrahydro-5-[(2-hydroxy-1,1-dimethylethyl)aceticacid]amino]methyl-10-methoxyl-3*H*-oxazolo-[4,3-α]isoquinolin-3-one-*tert* butyl ester (17).** To a solution of **16** (183 mg, 0.54 mmol) in 4 mL of DMF was added $NaHCO_3$ (454 mg, 5.4 mmol), NaI (809 mg, 5.4 mmol) and *t*-butyl bromoacetate (0.88 mL, 5.4 mmol) at room temperature. After 12 h the reaction was diluted with CH_2Cl_2 and washed with water. The layers were separated and the organic layer dried over $MgSO_4$. Column chromatography (3:1 EtOAc:hexane) gave **17** (195 mg, 83%) as a yellow solid. TLC (3:1 EtOAc:hexane) R_f 0.50 (UV and Dragendorff). Mp 152–154 °C (recryst. EtOAc). ^1H NMR (300 MHz) ($CDCl_3$) δ 0.91 (s, 3H), 0.92 (s, 3H), 1.46 (s, 9H), 2.61 (dd, J=6.9, 13.0 Hz, 1H), 2.76 (dd, J=8.5, 13.0 Hz, 1H), 2.99 (d, J=3.5 Hz, 2H), 3.05 (1/2 ABq, J=11.6 Hz, 1H), 3.20 (1/2 ABq, J=11.6 Hz, 1H), 3.27 (1/2 ABq, J=18.1 Hz, 1H), 3.45 (1/2 ABq, J=18.1 Hz, 1H), 3.73 (br s, 1H, D_2O exch.), 3.80 (s, 3H), 4.04 (dd, J=8.0, 9.0 Hz, 1H), 4.22 (m, 1H), 4.88 (dd, J=8.0, 9.0 Hz, 1H), 4.98 (dd, J=8.0, 9.0 Hz, 1H), 6.72 (d, J=8.0 Hz, 1H), 6.78 (d, J=8.0 Hz, 1H), 7.19 (t, J=8.0 Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 21.1, 23.1, 28.2, 29.4, 47.5, 49.5, 49.6, 51.7, 55.5, 59.0, 69.1, 70.1, 82.0, 108.2, 122.0, 122.7, 128.8, 133.6, 155.9, 157.9, 173.7. IR (NaCl, neat) 3466, 2976, 1755, 1587, 1473, 1258, 1152 cm^{-1}. Anal. calcd for $C_{23}H_{34}N_2O_6$: C, 63.58; H, 7.93; N, 6.45. Found: C, 63.71; H, 7.93; N, 6.22.

***trans*-1,5,6,10b-Tetrahydro-5-[(2-formyl-1,1-dimethylethyl)aceticacid]amino]methyl-10-methoxy-3*H*-oxazolo-[4,3-α]isoquinolin-3-one *tert*-butyl ester (18).** Dess–Martin periodinane (48 mg, 0.11 mmol) was added to **17** (32 mg, 0.076 mmol) in 1 mL of CH_2Cl_2. The reaction stirred for 30 min at room temperature after which a solution of 1 mL of $NaHCO_3$ (1 M) with sodium thiosulfate (273 mg) dissolved in it was added. The quenched reaction was allowed to stir for 30 min and was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and concentrated to yield **18** (30 mg, 90%) as a yellow oil. TLC (3:1 EtOAc:hexane) R_f 0.65 (UV and Dragendorff). ^1H NMR (300 MHz) ($CDCl_3$) δ 1.03 (s, 3H), 1.04 (s, 3H), 1.45 (s, 9H), 2.61 (d, J=7.5 Hz, 2H), 3.05 (m, 2H), 3.37 (1/2 ABq, J=17.7 Hz, 1H), 3.44 (1/2 ABq, J=18.0 Hz, 1H), 3.78 (s, 3H), 4.01 (t, J=12.0 Hz, 1H), 4.33 (dd, J=7.0, 16.0 Hz, 1H), 4.87 (dd, J=9.0, 12.0 Hz, 2H), 6.71 (d, J=7.0 Hz, 1H), 6.75 (d, J=8.0 Hz, 1H), 7.18 (t, J=8.0 Hz, 1H), 9.26 (s, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 19.0, 20.3, 28.2, 29.3, 46.9, 49.5, 50.9, 51.1, 55.5, 66.4, 70.2, 81.5, 108.4, 121.8, 122.4, 129.0, 132.9, 156.0, 158.0, 171.9, 203.7. IR (NaCl, neat) 2977, 1755, 1587, 1473, 1393, 1368, 1258, 1155, 1096 cm^{-1}. HRMS (FAB) calcd. for $C_{23}H_{32}N_2O_6$ (M+H) 433.2339; found 433.2345.

4α,6α,11aβ-2-Aza-2-carboxyacetyl-1,3,4,6,11,11a-hexahydro-7-methoxy-5,4-oxazolo-3,3-dimethyl-2*H*-benzo[*b*]-quinolizine (6b). To a solution of **18** (120 mg, 0.27 mmol) in 17 mL of EtOH was added 1.66 mL of 2 M LiOH. This solution was degassed by bubbling through a stream of $N_{2(g)}$ and heated at reflux for 24 h. After cooling to room temperature, the reaction was concentrated and chromatographed (10:1 CH_2Cl_2:MeOH) to give 60 mg (67%) of **6b** as a yellow foam. TLC (10:1 CH_2Cl_2:MeOH) R_f 0.20 (UV and Dragendorff). ^1H NMR (300 MHz) (MeOH-d_4) δ 1.55 (s, 3H), 1.59 (s, 3H), 2.73 (m, 2H), 3.16 (m, 1H), 3.46 (m, 2H), 3.50 (1/2 ABq, J=16.0 Hz, 1H), 3.62 (dd, J=8.0, 9.0 Hz, 1H), 3.82 (s, 3H), 3.91 (1/2 ABq, J=16.0 Hz, 1H), 4.26 (dd, J=8.0, 9.0 Hz, 1H), 4.47 (s, 1H), 4.54 (t, J=9.0 Hz, 1H), 6.78 (d, J=8.0 Hz, 1H), 6.83 (d, J=8.0 Hz, 1H), 7.19 (t, J=8.0 Hz, 1H); ^{13}C NMR (75 MHz) (MeOH-d_4) δ 170.3, 158.2, 135.5, 129.2, 123.9, 121.9, 109.3, 96.5, 69.6, 62.5, 60.7, 56.1, 54.3, 53.8, 32.9, 22.5, 20.2. IR (NaCl, neat) 3381, 2953, 1651, 1472, 1383, 1340, 1261 cm^{-1}. HRMS (FAB) calcd for $C_{18}H_{25}N_2O_4$ (M+H) 333.1814; found 333.1816.

Netropsin conjugate (8). Preparation of 4α,6α,11aβ-2-aza-3,3-dimethyl-1,3,4,6,11,11a-hexahydro-2-(4-nitrophenoxyacetyl)-7methoxy-5,4-oxazolo-2*H*-enzo[*b*]quinolizine. To a stirred mixture of **6b** (12.0 mg, 0.036 mmol) and *p*-nitrophenol (5.5 mg, 0.040 mmol) in 2 mL of CH_2Cl_2 at 0 °C was added 1,3-dicyclohexylcarbodiimide (8.2 mg, 0.040 mmol). The reaction stirred at 0 °C for 1 h, and was then warmed to room temperature and stirred for an additional 15 h. The reaction mixture was then concentrated under reduced pressure and purified by PTLC (10:1 CH_2Cl_2:MeOH) to afford 8.5 mg (52%) of the corresponding *p*-nitrophenylester derivative of **6b** as a yellow oil. TLC (10:1 CH_2Cl_2:MeOH) R_f 0.76 (UV and Dragendorff). ^1H NMR (300 MHz) ($CDCl_3$) δ 1.28 (s, 3H), 1.40 (s, 3H), 2.57 (dd, J=2.7, 15.6 Hz, 1H), 2.71 (dd, J=11.1, 15.6 Hz, 1H), 2.89–2.92 (m, 2H), 3.01–3.08 (m, 1H), 3.51 (1/2 ABq, J=17.1 Hz, 1H), 3.60 (dd, J=7.8, 8.7 Hz, 1H), 3.78 (s, 3H), 3.84 (1/2 ABq, J=17.1 Hz, 1H), 4.15–4.20 (m, 2H), 4.44 (t, J=8.7 Hz, 1H), 6.67 (d, J=8.1 Hz, 1H), 6.72 (d, J=7.5 Hz, 1H), 7.12

(dd, J=7.5, 8.1 Hz, 1H), 7.28 (d, J=9.0 Hz, 2H), 8.25 (d, J=9.0 Hz, 2H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 169.5, 156.7, 155.2, 145.3, 135.4, 127.3, 125.2, 123.5, 122.3, 120.7, 107.4, 97.6, 67.7, 59.4, 55.3, 55.2, 51.9, 51.7, 48.5, 32.8, 25.1, 19.3. IR (NaCl, neat) 2936, 1781, 1590, 1523, 1472, 1346, 1261, 1207, 1105, 1012, 914, 864 cm^{-1}. To a stirred solution of **19** (10.2 mg, 0.028 mmol) in 0.5 mL of DMF and degassed with argon was added 5% Pd/C (6 mg, 0.0028 mmol) and the resulting mixture saturated with hydrogen. The mixture was then stirred at room temperature under 1 atm of hydrogen for 24 h. The reaction mixture was then filtered through Celite into a solution of the *p*-nitrophenyl ester obtained above (8.5 mg, 0.029 mmol) dissolved in 0.5 mL of DMF. To the reaction was added NEt_3 (12 μL, 0.087 mmol) and the resulting solution stirred at room temperature for 7 h. The reaction mixture was then concentrated and the crude product purified by PTLC (3% concd NH_4OH in methanol) to give 5.6 mg (31%) of netropsin conjugate **8** as a yellow oil. TLC (3% NH_4OH in MeOH) R_f 0.59 (UV and Dragendorff). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.25 (s, 3H), 1.30 (s, 3H), 2.23 (s, 6H), 2.44 (t, J=5.7 Hz, 2H), 2.52–2.61 (m, 2H), 2.67–2.72 (m, 1H), 2.81 (t, J=11.3 Hz, 1H), 2.89 (d, J=17.4 Hz, 1H), 2.98–3.05 (m, 1H), 3.40 (q, J=5.7 Hz, 2H), 3.52 (d, J=17.4 Hz, 1H), 3.66 (dd, J=7.8, 8.4 Hz, 1H), 3.80 (s, 3H), 3.88 (s, 3H), 3.90 (s, 3H), 4.20 (s, 1H), 4.24 (dd, J=7.8, 8.4 Hz, 1H), 4.48 (t, J=8.7 Hz, 1H), 6.43 (m, 1H), 6.51 (d, J=1.8 Hz, 1H), 6.59 (d, J=1.8 Hz, 1H), 6.71 (dd, J=7.8, 8.4 Hz, 2H), 7.09 (d, J=1.8 Hz, 1H), 7.10 (d, J=1.8 Hz, 1H), 7.14 (dd, J=7.8, 8.4 Hz, 1H), 7.38 (br s, 1H), 9.34 (br s, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 169.1, 161.7, 158.9, 156.7, 135.3, 127.4, 123.6, 123.1, 121.2, 121.1, 120.7, 118.9, 118.6, 107.6, 103.3, 103.2, 97.4, 77.2, 68.0, 59.4, 57.9, 55.4, 55.0, 53.5, 52.7, 49.8, 45.1, 36.6, 36.5, 32.8, 24.8, 18.8. IR (NaCl, neat) 3293, 2935, 2845, 2775, 1654, 2586, 1541, 1467, 1437, 1402, 1261, 1203, 1163, 1063 cm^{-1}. HRMS (FAB) calcd for $C_{34}H_{47}N_8O_5$ (M+H) 647.3669; found 647.3677.

6α,11aβ-2-Aza-2-carboxyacetyl-1,3,4,6,11,11a-hexahydro-7-methoxy-4-cyano-3,3-dimethyl-5-hyroxyl-2*H*-benzo[*b*]quinolizine (23). A solution of **5b** (6 mg, 0.018 mmol) in 0.45 mL of 1:2 THF:$NaHCO_3$ (satd) was adjusted to pH 7 using 1 M HCl. The solution was treated with 0.5 M NaCN (0.036 mmol) and stirred for 18 h. The pH of the solution was adjusted to 6–7 using 1 M HCl and the solution was extracted with 1:1 EtOAc:THF. The organic phase was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, 100:1 EtOAc:AcOH) affording **23** (3.7mg, 56%) as a slightly-yellow foam. TLC (9:1 CH_2Cl_2:MeOH) R_f 0.36 (UV and Dragendorff). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.36 (s, 3H), 1.38 (s, 3H), 2.64 (m, 3H, 1H is D_2O exchangeable), 2.74 (m, 2H), 2.87 (1/2 ABq, J=18.0 Hz, 1H), 3.10 (m, 1H), 3.52 (1/2 ABq, J=18.0 Hz, 1H), 3.63 (dd, J=5.1, 11.1 Hz, 1H), 3.79 (m, 1H), 3.84 (s, 3H), 3.95 (s, 1H), 4.24 (m, 1H), 6.72 (d, J=8.1Hz, 1H), 6.77 (d, J=8.1Hz, 1H), 7.18 (t, J=8.1Hz, 1H); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 170.60, 155.83, 135.56, 128.07, 121.88, 120.19, 116.31, 108.79, 99.92, 66.15, 65.27, 58.95, 56.52, 54.74, 52.69, 52.15, 33.52, 26.08, 16.89. IR (NaCl, neat) 3422, 2926, 2253, 1774, 1382, 1060 cm^{-1}. HRMS (FAB) calcd for $C_{19}H_{26}N_3O_4$ (M+H) 360.1929; found 360.1923.

4α,6α,11aβ-2-Aza-2-carboxyacetyl-1,3,4,6,11a-hexahydro-7-hydroxy-5,4-oxazolo-3,3-dimethyl-2*H*-benzo[*b*]quinolizine (24). To a solution of **6b** (10 mg, 0.030 mmol) in 0.5 mL of CH_2Cl_2 in an oven-dried three-neck flask fitted with a septum, argon line, and drying tube containing $CaCl_2$ was added BBr_3 (1.0 M soln, 180 μL, 0.18 mmol) at −78 °C. The reaction was then allowed to warm up to room temperature and stirred overnight. The reaction was quenched with water and extracted with CH_2Cl_2. After concentration the residue was chromatographed (PTLC, 10:1 CH_2Cl_2:MeOH) to give 3.3 mg of **6b** (33%) and 4.5 mg of phenol analogue **24** (47%) as a clear oil. TLC (10:1 CH_2Cl_2:MeOH) R_f 0.07 (UV and PMA). ^{1}H NMR (300 MHz) (MeOH-d_4) δ 1.55 (s, 3H), 1.59 (s, 3H), 2.69–2.73 (m, 2H), 3.14–3.24 (m, 1H), 3.35 (s, 1H), 3.40–3.52 (m, 2H), 3.64–3.73 (m, 1H), 3.85–3.94 (m, 1H), 4.32 (t, J=8.4 Hz, 1H), 4.49 (s, 1H), 4.56 (t, J=8.4 Hz, 1H), 6.65 (dd, J=2.7, 7.8 Hz, 2H), 7.03 (t, J=7.8 Hz, 1H); ^{13}C NMR (100 MHz) (MeOH-d_4) δ 174.0, 156.1, 135.5, 128.9, 122.5, 120.5, 113.5, 96.6, 69.8, 62.4, 60.9, 54.3, 53.9, 32.9, 22.6, 20.1. IR (NaCl, neat) 3409, 2926, 1640, 1466, 1380, 1277, 1160, 1123 cm^{-1}. HRMS (FAB) calcd for $C_{17}H_{23}N_2O_4$ (M+H) 319.1658; found 319.1655.

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TETRAHEDRON LETTERS

Synthetic studies on tetrazomine: lipase PS resolution of racemic *cis*-β-hydroxypipecolic acid

Jack D. Scott and Robert M. Williams*

Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA

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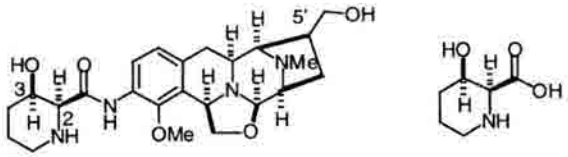
Abstract

An efficient enzymatic resolution of racemic *cis*-β-hydroxypipecolic acid is described affording both the (2*S*,3*R*) and (2*R*,3*S*) protected amino acids in good yield and high enantiomeric ratios.

Tetrazomine (**1**, Fig. 1) is an antitumor antibiotic that was isolated from *Saccharothrix mutabilis* and reported by the Yamanouchi Pharmaceutical Co. in Japan.[1] Preliminary antitumor/antimicrobial assays of this substance indicate that tetrazomine displays potent antitumor activity against P388 leukemia in vivo and displays good antimicrobial activity against both Gram-negative and Gram-positive organisms. Tetrazomine is structurally related to the quinocarcin and bioxalomycin classes of antitumor antibiotics, but is distinct from these agents by virtue of containing the unusual amino acid β-hydroxypipecolic acid. The absolute stereochemistry of the β-hydroxypipecolic acid stereoisomer present in tetrazomine has been determined in these laboratories to be (2S,3*R*), (–)-**2**.[2]



1 Tetrazomine (-)-**2**, (*2S, 3R*)

Figure 1.

As part of a program directed at tackling the total synthesis of tetrazomine, we required a practical synthesis of (–)-**2**. The previous synthesis[3] of (–)-**2** was accomplished recently by Corey

* Corresponding author.

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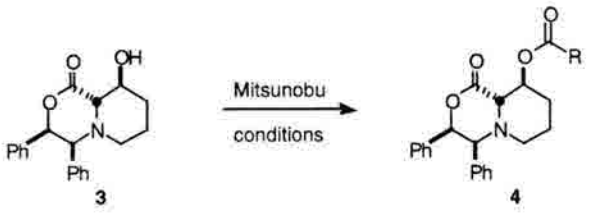
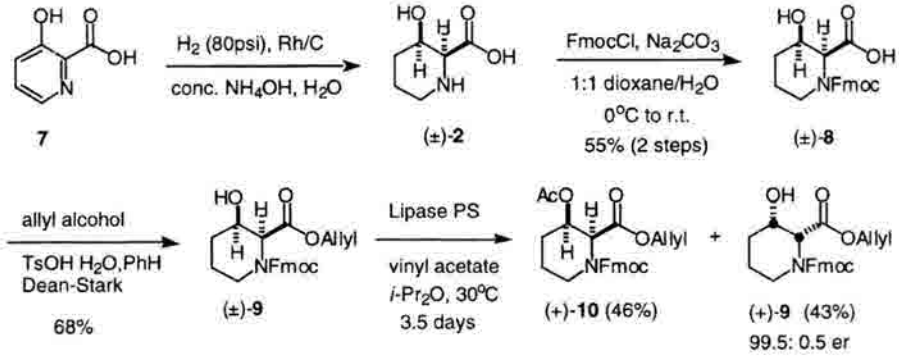
et al. This synthesis entailed an aldol condensation using an ammonium salt catalyst, yielding a 1:1 mixture of *syn*/*anti* aldol products. Initially, we attempted a Mitsunobu[4] inversion of the secondary alcohol of previously reported **3**[2] (Scheme 1). Unfortunately, under various conditions the only product that was obtained was acylation with retention of configuration (**4**). The mechanism of this acylation was not investigated and an alternative route was explored.



Scheme 1.

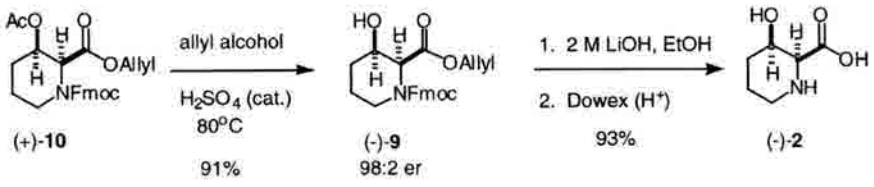
The literature revealed that the racemic *cis*-β-hydroxypipecolic acid [(±)-**2**] was available in one-step from picolinic acid (**7**) via reduction using Rh/C.[5] Since (±)-**2** is thus readily available, we endeavored to resolve the enantiomeric pair of amino acids. The use of Lipase PS has been reported by Toyooka et al.[6] for the resolution of a structurally related pipecolic acid derivative and this method was examined in the present context.

As shown in Scheme 2, picolinic acid was reduced as previously described[5] and the amine protected as the corresponding Fmoc derivative (±)-**8**[7] in 55% yield for the two steps. Esterification of the acid to the allyl ester afforded (±)-**9**[8] in 68% yield. Enzymatic resolution of this protected amino acid racemate[9] using Lipase PS (Amano) proceeded smoothly forming the acetate of the (2*S*,3*R*) isomer, providing (+)-**10**[10] in 46% yield. The (2*R*,3*S*) amino acid (+)-**9** was recovered in 43% yield and an enantiomeric ratio (er) of 99.5:0.5 was determined by chiral HPLC analysis.[11]



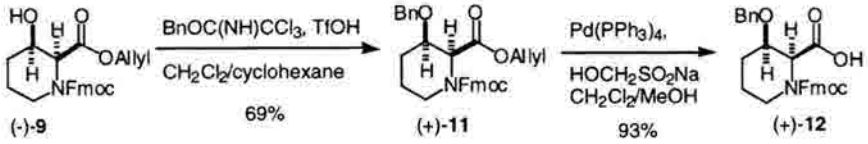
Scheme 2.

The acetate (+)-**10** was easily cleaved to form (−)-**9** in 91% yield (Scheme 3). The er of (−)-**9** was found to be 98:2 by chiral HPLC analysis.[11] To confirm the absolute stereochemistry, (−)-**9** was converted to the free amino acid (−)-**2** via treatment with lithium hydroxide. The optical rotation matched that of the literature value.[2] With these amino acids in hand, we next investigated the conversion of (−)-**9** to a suitably protected form that would be compatible with peptide coupling strategies.



Scheme 3.

The hydroxyl group on the amino acid (−)-**9** was protected as the benzyl ether using benzyl trichloroacetimidate and triflic acid[12] to afford (+)-**11**[13] in 69% yield (Scheme 4). Finally, the allyl ester was cleaved in 93% yield using palladium tetrakistriphenylphosphine and the sodium salt of hydroxymethanesulfinic acid[14] to afford the amino acid (+)-**12**.[15] This substance should be a useful protected form of (−)-**2** suitable for incorporation into peptides as well as synthetic strategies directed toward tetrazomine. This route to (−)-**2** compares favorably to the previous synthesis due to higher er (98:2 versus 91:9).



Scheme 4.

Acknowledgements

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7. (±)-**8**: ^{1}H NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.35–1.80 (4H, m); 1.93 (1H, s); 3.07 (1H, ddd, J=12.9, 12.9, 3.3 Hz); 3.66 (1H, m); 3.76 (1H, m); 4.26 (1H, d, J=6.3 Hz); 4.37 (2H, d, J=5.7 Hz); 4.68 (1H, d, J=5.7 Hz); 7.32 (2H, t, J=7.5 Hz); 7.40 (2H, t, J=7.5 Hz); 7.63 (2H, t, J=7.5 Hz); 7.84 (2H, d, J=7.5 Hz). HRMS (FAB) calcd for $C_{21}H_{22}NO_5$ (MH^+) 368.1498; found 368.1502.
8. (±)-**9**. ^{1}H NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.44 (1H, m); 1.71 (3H, m); 3.20 (1H, ddd, J=12.3, 12.3, 3.3 Hz); 3.78 (2H, m); 4.27 (1H, dd, J=6.0 Hz); 4.42 (2H, d, J=6.6 Hz); 4.54 (1H, s, broad); 4.62 (2H, m); 4.82 (1H, d, J=7.2 Hz); 5.21 (1H, dd, J=10.2, 1.2 Hz); 5.35 (1H, dd, J=17.1, 1.8 Hz); 5.91 (1H, m); 7.33 (2H, t, J=7.5 Hz); 7.41 (2H, t, J=7.5 Hz); 7.62 (2H, d, J=7.5 Hz); 7.84 (2H, d, J=7.5 Hz). HRMS (FAB) calcd for

$C_{24}H_{26}NO_5$ (MH^+) 408.1811; found 408.1820. (−)-**9**. $[\alpha]_D^{20} = -33.6$ ($c = 1.5$ CH_2Cl_2). (+)-**9**. $[\alpha]_D^{20} = +36.8$ ($c = 1.2$ CH_2Cl_2).

9. Procedure for lipase PS resolution: to a solution of (±)-**9** (380 mg, 0.93 mmol) in 20 mL iPr_2O at 30°C was added 1.52 g lipase PS. The reaction progress was monitored by HPLC until 50% completion (Waters novapak HR silica, isocratic 70/30 hexanes/EtOAc, 2 mL/min, UV 254 nm). When complete, the mixture was filtered through Celite and the solvent was removed in vacuo. The crude product was purified via flash chromatography (gradient 25–50% EtOAc/hex) to afford 191 mg (+)-**10** (43%) and 165 mg (+)-**9** (46%).
10. (+)-**10**: ^1H NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.53 (1H, m); 1.78 (3H, m); 2.10 (3H, s); 3.22 (1H, ddd, $J = 12.9, 12.9, 3.6$ Hz); 3.82 (1H, dd, $J = 12.9, 5.1$ Hz); 4.27 (1H, t, $J = 6.3$ Hz); 4.42 (2H, d, $J = 6.6$ Hz); 4.63 (2H, m); 4.84 (1H, m); 5.04 (1H, d, $J = 6.3$ Hz); 5.24 (1H, dd, $J = 11.7, 1.5$ Hz); 5.33 (1H, dd, $J = 17.3, 1.5$ Hz); 5.91 (1H, m); 7.32 (2H, t, $J = 7.5$ Hz); 7.41 (2H, t, $J = 7.5$ Hz); 7.61 (2H, d, $J = 7.5$ Hz); 7.84 (2H, d, $J = 7.5$ Hz). HRMS (FAB) calcd for $C_{26}H_{28}NO_6$ (MH^+) 450.1917; found 450.1916. $[\alpha]_D^{20} = +12.8$ ($c = 1.1$ CH_2Cl_2).
11. Chiral HPLC conditions: Pirkle Whelk-O 2 (*R,R*), isocratic 85/15 hex/*i*PrOH, 1 mL/min, UV 254 nm.
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15. (+)-**12**: ^1H NMR (300 MHz) (d_6-DMSO, 120°C) δ 1.41 (1H, m); 1.72 (3H, m); 3.26 (1H, ddd, $J = 12.6, 12.6, 3.9$ Hz); 3.65 (1H, m); 3.76 (1H, m); 4.05 (1H, s); 4.28 (1H, t, $J = 6.3$ Hz); 4.42 (1H, m); 4.55 (1H, 1/2ABq, $J = 11.7$ Hz); 4.67 (1H, 1/2ABq, $J = 11.7$ Hz); 4.87 (1H, d, $J = 6.3$ Hz); 7.18–7.43 (8H, m); 7.50–7.86 (5H, m). HRMS (FAB) calcd for $C_{28}H_{28}NO_5$ (MH^+) 458.1967; found 458.1959. $[\alpha]_D^{20} = +12.2$ ($c = 0.9$ CH_2Cl_2).

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[12] For **4b**: A red plate of **4b** ($0.25 \times 0.2 \times 0.08$ mm) was grown from dichloromethane. Data were obtained on a Bruker SMART APEX CCD diffractometer at 211 K. The structure was solved and refined by the programs SAINT+ and SHELXTX by using heavy atom (Patterson) methods. Hydrogen atoms were localized and refined in the riding mode. The crystal was fixed in a capillary. $CoC_{65}H_{53} \cdot CH_2Cl_2$ ($M_r = 977.93$); $Mo_{K\alpha}$ radiation $\lambda = 0.71073$ Å, graphite monochromator; $2\Theta_{max} = 22.50°$, tetragonal, space group $I4_1$; $a = 19.156(5)$, $b = 19.156(5)$, $c = 15.842(7)$ Å, $V = 5814(3)$ Å^3, $Z = 48$, $\rho_{calcd} = 1.117$ g cm^{-3}, $\mu = 0.424$ mm^{-1}, 12129 reflections were measured and 4807 reflections with $I > 2\sigma(I)$ observed, $R = 0.0491$, $R_w = 0.1031$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-154093. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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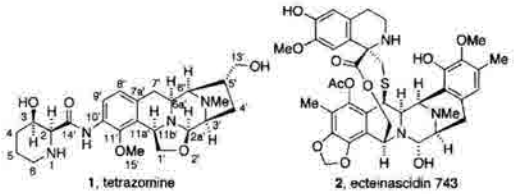
Total Synthesis of (−)-Tetrazomine and Determination of Its Stereochemistry**

Jack D. Scott and Robert M. Williams*

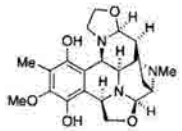
Dedicated to Professor K. Barry Sharpless on the occasion of his 60th birthday

The antitumor antibiotic tetrazomine **1** was isolated from *Saccharothrix mutabilis* at the Yamonouchi Pharmaceutical company by Suzuki et al.[1] Tetrazomine is a member of the tetrahydroisoquinoline family of antitumor antibiotics that

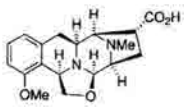


1, tetrazomine

2, ecteinascidin 743



3, bioxalomycin α2



4, quinocarcin

includes ecteinascidin 743 (**2**),[2] bioxalomycin α2 (**3**),[3] and quinocarcin (**4**).[4] Tetrazomine most closely resembles quinocarcin, except for the amino functionality at C10′, the unusual β-hydroxy pipecolic acid moiety, and the oxidation state of C13′. Neither the relative nor the absolute stereochemistry of tetrazomine were determined when the structure was initially reported.[1b] We have since determined that the absolute stereochemistry of the pipecolic acid moiety is 2*S*,3*R*.[5]

Preliminary antitumor/antimicrobial assays of tetrazomine revealed that this substance possesses activity against P388 leukemia in vivo and good antimicrobial activity against both Gram-negative and Gram-positive bacteria.[1a] Tetrazomine exerts its cytotoxic activity through oxidative damage to DNA by the superoxides formed in the auto-redox disproportionation of the fused oxazolidine, and possibly through DNA alkylation.[6]

The total synthesis of tetrazomine has not been reported in the literature,[7] although the synthesis of the AB-ring system of tetrazomine has been discussed by Ponzo and Kaufman.[8] Herein, we describe the first total synthesis of (−)-tetrazo-

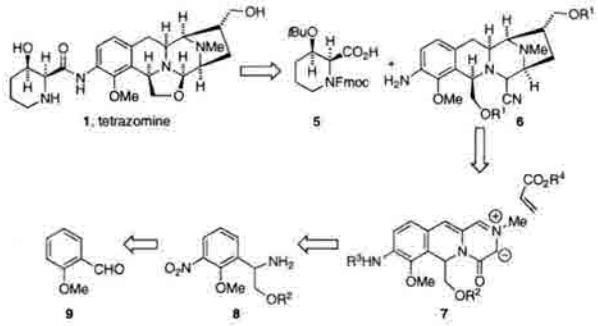
[*] Prof. R. M. Williams, J. D. Scott
Department of Chemistry
Colorado State University
Fort Collins, CO 80523 (USA)
Fax: (+1) 970-491-3944
E-mail: rmw@chem.colostate.edu

[**] This work was supported by the National Institutes of Health (Grant CA85419). We are grateful to Yamanouchi Pharmaceutical Co. for providing a generous gift of natural tetrazomine.

Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.

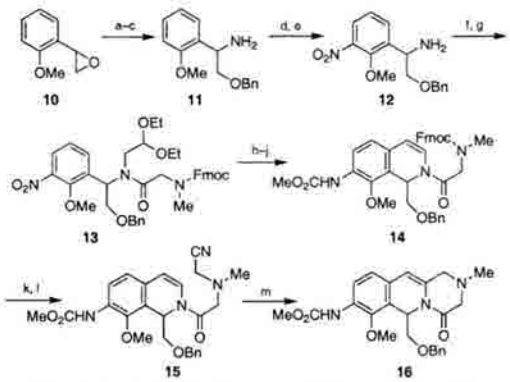
mine and the determination of its relative and absolute stereochemistry.

A retrosynthetic disconnection of tetrazomine included the late-stage coupling of the protected β-hydroxypipecolic acid **5**[9] to the aniline **6** (Scheme 1). The aniline was to be derived from a 1,3-dipolar cycloaddition to the azomethine ylide of tricycle **7** using a methodology developed in our laboratories.[7e] Substance **7** could in turn be obtained from the trisubstituted nitroaromatic **8**, which would ultimately be derived from *ortho*-anisaldehyde (**9**).



Scheme 1. Retrosynthesis of tetrazomine (**1**). Fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl.

The synthesis began with the regioselective opening of the previously described[10] epoxide **10** with sodium azide (Scheme 2). The resulting primary alcohol was protected as the benzyl ether, and the azide was hydrogenated to afford amine **11**. Selective nitration *ortho* to the methoxy group was accomplished at low temperature by using potassium nitrate and trifluoroacetic anhydride (TFAA).[8] Hydrolysis of the trifluoroacetamide afforded amine **12**. Alkylation of the primary amine with bromoacetaldehyde diethylacetal was followed by coupling with *N*-Fmoc-sarcosine acid chloride to yield amide **13** in 82% yield. The nitro group was hydrogenated by using platinum oxide to afford the aniline derivative necessary for acid-promoted cyclization onto the acetal.[7a, 8] The aniline was then protected as the methyl carbamate to afford **14** in 92% yield from **13**. Removal of the Fmoc group was followed by amine alkylation with iodoacetonitrile to yield aminonitrile **15** in 93% yield over the two steps. Attempted iminium cyclization to form tricyclic **16** by using $AgNO_3$[11] or $AgBF_4$[12] afforded no desired product. After extensive experimentation, it was found that treatment of **15** with silver(I) trifluoroacetate in the presence of trifluoroacetic acid (TFA) and trifluoroacetic anhydride afforded the tricycle **16** in 93% yield.

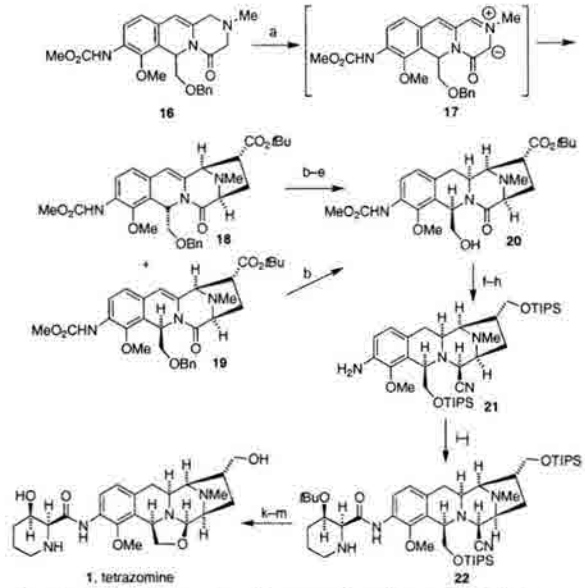


Scheme 2. Synthesis of tricyclic precursor **16**. Reagents and conditions: a) NaN_3, acetone/H_2O, Δ; b) NaH, THF; BnBr, KI (94%, two steps); c) H_2, Pd/C, EtOH (87%); d) KNO_3, TFAA, CH_2Cl_2, $-20\,^{\circ}C$; e) LiOH (2M), EtOH, (56%, two steps); f) bromoacetaldehyde diethylacetal, K_2CO_3, MeCN, Δ (74%); g) *N*-Fmoc-sarcosine-Cl, DMAP, pyridine, CH_2Cl_2 (82%); h) H_2 (60 psi (414 kPa)), PtO_2, EtOH/THF; i) HCl (6M), dioxane, $90\,^{\circ}C$; j) MeOCOCl, pyridine, CH_2Cl_2, $4\,^{\circ}C$ (92%, three steps); k) pyrrolidine, MeCN (94%); l) ICH_2CN, iPr_2NEt (99%); m) AgO-$COCF_3$, TFA, TFAA, $ClCH_2CH_2Cl$, Δ (93%).

Tetracycle **19** was hydrogenated in the presence of Raney-nickel at a moderate pressure, which resulted in the removal of the benzyl group and reduction of the benzylic olefin from the least hindered face to afford **20**. The major product from the cycloaddition, **18**, possessed the undesired configuration at C11b', thus an epimerization at C11b' was executed. After hydrogenation, the resulting alcohol was oxidized to the aldehyde. Treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded a 1.4:1 mixture of epimers at C-11b' with the desired isomer as the predominant product. These aldehydes were easily separated by column chromatography, thus allowing the undesired epimer to be recycled. Sodium borohydride reduction of the desired epimer afforded alcohol **20**.

The simultaneous reduction of the *tert*-butyl ester group and partial reduction of the lactam were fortuitously accomplished in a single step by using $LiAlH_3OEt$ in THF at $0\,^{\circ}C$. The resulting methanolamine was trapped with potassium cyanide under acidic conditions[13] to afford the corresponding stable aminonitrile. The relative stereochemistry of this product was determined by extensive 2D NMR studies. The two primary alcohols were protected as their triisopropylsilyl (TIPS) ethers, and the methyl carbamate was hydrolyzed to afford aniline **21**. The optically active acid chloride of **5**[9] was prepared by using oxalyl chloride, and was coupled to **21** in the presence of 4-dimethylaminopyridine to afford the corresponding pipecolamide (plus a separable diastereomer, which was identified as the *ent*-tetrahydroisoquinoline portion; obtained as a 1:1 mixture of optically active diaster-

  1433-7851/01/4008-1464 $ 17.50+.50/0 *Angew. Chem. Int. Ed.* **2001**, *40*, No. 8



Scheme 3. Synthesis of tetrazomine (**1**). Reagents and conditions: a) NBS, $CHCl_3$, Δ; *tert*-butyl acrylate, Et_3N, 0 °C to room temperature, **18** (35 %), **19** (9 %); b) H_2 (100 psi (689 kPa)), Raney-Ni, EtOH (90 %); c) Swern oxidation (98 %); d) DBU, THF, (55 % + 38 % starting material); e) $NaBH_4$, EtOH (89 %); f) $LiAlH_3OEt$, THF, 0 °C; KCN, AcOH, 0 °C to room temperature (69 %); g) TIPSCl, imidazole, DMF (92 %); h) 2 M LiOH, EtOH, Δ, (63 %); i) **5**, oxalyl chloride, DMF, CH_2Cl_2; **21**, DMAP, CH_2Cl_2; j) DBU, CH_2Cl_2 (72 %, two steps); k) TFA, 4 °C, (53 %); l) HF, MeCN (82 %); m) $AgOCOCF_3$, TFA, MeOH/H_2O; Dowex (Cl^-) (61 %).

eomers). The intermediate pipecolamide was treated with DBU to cleave the Fmoc group, thus furnishing **22**.

To determine which diastereomer would lead to tetrazomine, natural tetrazomine was treated with sodium cyanide to afford 2a′-α-cyanotetrazominol, which was compared to the synthetic diastereomeric products of the cleavage of the *tert*-butyl ether and silyl ethers of **22** and its diastereomer. As expected, one diastereomer afforded a product that had identical spectral data to the naturally derived substance. With this comparison, the relative stereochemistry of the pentacyclic core of tetrazomine was established. Based on biosynthetic considerations, the absolute configuration of the tetrahydroisoquinoline core is assumed to be that depicted in structure **1**, since quinocarcin, bioxalomycin, and ecteinascidin possess the same absolute configuration of the tetrahydroisoquinoline moiety.

After removal of the protecting groups, precursor **22** was treated with silver(I) trifluoroacetate in the presence of TFA for four hours to furnish the intact oxazolidine ring. The addition of Dowex (Cl^-), followed by filtration and lyophilization afforded (−)-tetrazomine·2HCl, which after purification by HPLC exhibited identical spectral characteristics to those of the natural product. The chemistry described herein is currently being extended to the preparation of several mechanistically inspired analogues for biochemical and biological evaluation, and will be reported in due course.

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Total Synthesis of (−)-Tetrazomine. Determination of the Stereochemistry of Tetrazomine and the Synthesis and Biological Activity of Tetrazomine Analogues

Jack D. Scott and Robert M. Williams*

Contribution from the Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

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Abstract: The first total synthesis of the potent antitumor antibiotic (−)-tetrazomine has been accomplished. A new method for the formation of the allylic amine precursor to an azomethine ylide has been developed and exploited in an efficient [1,3]-dipolar cycloaddition to afford the key tetracyclic intermediate used in the synthesis of (−)-tetrazomine. Several analogues of tetrazomine have been synthesized and tested for antimicrobial and biochemical activity.

Introduction

The antitumor antibiotic tetrazomine **1** is a natural secondary metabolite isolated from *Saccharothrix mutabilis* subsp. *chichijimaensis* subsp. nov. by Suzuki et al.[1] Tetrazomine is a member of the tetrahydroisoquinoline family of antitumor antibiotics including ecteinascidin 743 (Et 743)[2] (**2**), bioxalomycin[3] (**3**), and quinocarcin[4] (**4**). Tetrazomine most closely resembles quinocarcin with the exception of the amino functionality at C-10′, the unusual β-hydroxy pipecolic acid moiety, and the oxidation state of C-5′. Neither the relative nor the absolute stereochemistry of tetrazomine was determined when the structure was initially reported.[1] The absolute stereochemistry of the pipecolic acid moiety has since been determined in these laboratories to be 2(*S*),3(*R*) as depicted in Figure 1.[5] The relative stereochemistry at C-5′ then remained as the only stereogenic center to be in question and was solved through a total synthesis that we recently communicated.[6]

Preliminary antitumor and antimicrobial assays of tetrazomine revealed that this substance possesses potent cytotoxicity with activity against P388 leukemia in vivo and good antimicrobial activity against both Gram-negative and Gram-positive bacteria.[1a] Tetrazomine and quinocarcin exert their cytotoxic activity through the expression of multiple mechanisms that include the mediation of oxidative damage to DNA via the reduction of molecular oxygen to superoxide by the auto-redox disproportionation of the fused oxazolidine and, possibly, DNA alkylation.[7] DNA alkylation has not been observed for tetrazomine or quinocarcin; however, alkylation has been observed with bioxalomycin α_2[8] and Et 743.[9]

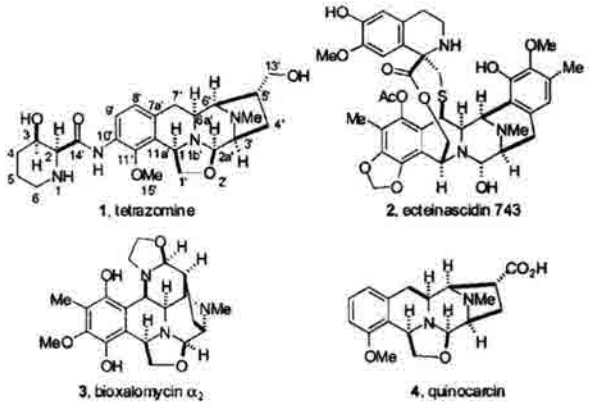

Figure 1. Structures of tetrazomine, ecteinascidin 743, bioxalomycin a2, and quinocarcin.

Prior to our communication,[6] the total synthesis of tetrazomine had not been reported in the literature[10] although two syntheses of the AB ring system of tetrazomine have been reported.[11] Ponzo and Kaufman[11a] reported a racemic synthesis followed

* To whom correspondence should be addressed. E-mail: rmw@chem.colostate.edu. FAX: (970)-491-3944.

(1) (a) Suzuki, K.; Sato, T.; Morioka, M.; Nagai, K.; Abe, K.; Yamaguchi, H.; Saito, T. *J. Antibiot.* **1991**, *44*, 479−485. (b) Sato, T.; Hirayama, F.; Saito, T. *J. Antibiot.* **1991**, *44*, 1367−1370.
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(3) (a) Zaccardi, J.; Alluri, M.; Ashcroft, J.; Bernan, V.; Korshalla, J. D.; Morton, G. O.; Siegel, M.; Tsao, R.; Williams, D. R.; Maiese, W.; Ellestad, G. A. *J. Org. Chem.* **1994**, *59*, 4045−4047. (b) Bernan, V. S.; Montenegro, D. A.; Korshalla, J. D.; Maiese, W. M.; Steinberg, D. A.; Greenstein, M. *J. Antibiot.* **1994**, *47*, 1417−1424.
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(5) Scott, J. D.; Tippie, T. N.; Williams, R. M. *Tetrahedron Lett.* **1998**, *39*, 3659−3662.
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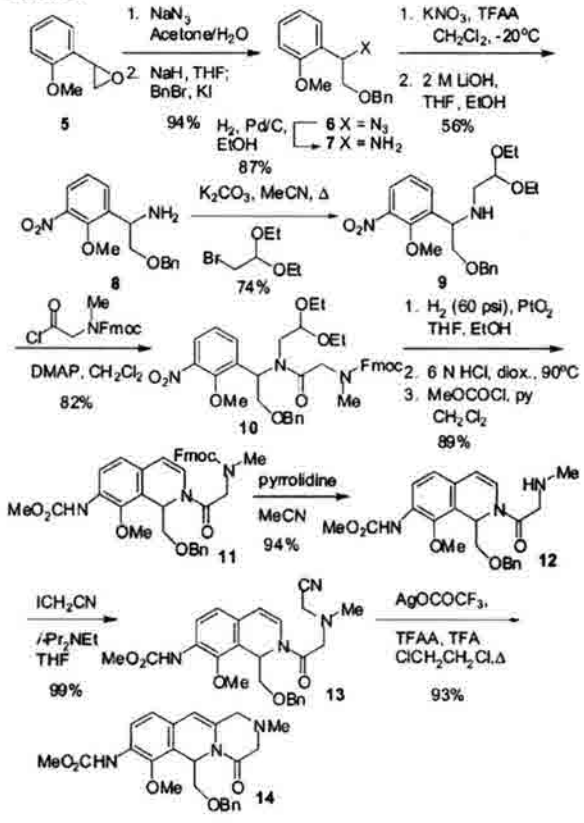
10.1021/ja0174027 CCC: $22.00

Scheme 1



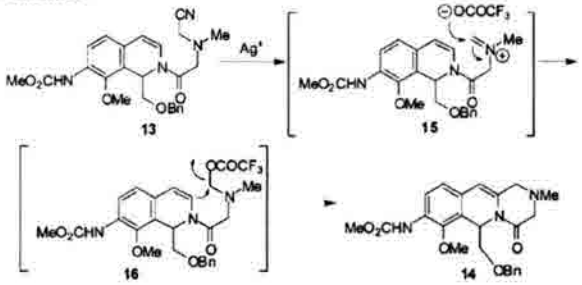
by an enantioselective synthesis by Wipf and Hopkins.[11b] Herein, the first total synthesis of (−)-tetrazomine is described along with the determination of its relative and absolute stereochemistry.[6] In addition, the chemistry developed for the total synthesis of tetrazomine was conscripted for the synthesis of several analogues of tetrazomine and their biochemical and biological activity have been examined.

Results and Discussion

The synthesis began with the regioselective opening of the previously described[12] epoxide **5** with sodium azide (Scheme 1). The resultant primary alcohol was protected as the corresponding benzyl ether and the azide was hydrogenated to afford amine **7**. Selective nitration ortho to the methoxy group was accomplished at low temperature by using potassium nitrate and trifluoroacetic anhydride.[11a] Hydrolysis of the trifluoroacetamide afforded amine **8**.

Alkylation of the primary amine with bromoacetaldehyde diethylacetal[13] provided the secondary amine **9**, which was coupled with N-Fmoc-sarcosine acid chloride to provide amide **10** in 82% yield.

Scheme 2



Hydrogenation of the nitro group was accomplished by using platinum oxide to afford the aniline derivative necessary for acid-promoted cyclization upon the acetal.[10a,11a] Cleavage of the Fmoc group was not observed during the hydrogenation with the use of platinum oxide. The aniline was then protected as the methyl carbamate affording **11** in 89% overall yield for the three steps from **10**. Cleavage of the Fmoc group with pyrrolidine in acetonitrile afforded the secondary amine **12** in high yield. Attempts to convert the secondary amine **12** to the tricyclic substance **14** under standard Mannich conditions with formalin or paraformaldehyde afforded little or none of the desired tricyclic product and a two-step sequence was chosen. First, the amine was converted to the aminonitrile **13** by alkylation with iodoacetonitrile.

Attempted iminium ion cyclization to form the tricyclic substance **14** with $AgNO_3$ or $AgBF_4$[14] afforded none of the desired product. After extensive experimentation, it was found that treatment of **13** with silver(I)trifluoracetate in the presence of TFA and TFAA afforded the desired tricycle **14** in 93% yield. Due to the fact that this cyclization did not yield tricycle **14** when other silver salts were used, we speculate that this cyclization does not proceed through a *6-endo-trig* ring closure[15] on the "free" iminium ion **15** (Scheme 2). Alternatively, the trifluoroacetate anion appears obligatory to trap the incipient iminium ion species to form **16** that allows for the cyclization to occur via a *6-exo-tet* process.[15]

One of the key steps of our synthesis involves an azomthine ylide dipolar cycloaddition[10c,e] to form the piperizine ring. Treatment of allylic amine **14** with NBS in refluxing chloroform yielded a dark green solution of the corresponding iminium ion species that upon deprotonation with triethylamine afforded the dark blue azomethine ylide that was trapped by *tert*-butyl acrylate to afford a 5:1 mixture of separable cycloadducts **17** and **18**, respectively (Scheme 3). The diastereoselectivity observed for the dipolar cycloaddition results from approach of the acrylate from the least hindered face of the azomethine ylide. A similar bias was also observed in our quinocarcinamide synthesis.[10e]

Surprisingly, the use of methyl acrylate as the dipolarphile afforded a 1:1 mixture at C-5′ that is a consequence of poor *endolexo* selectivity during the addition. By contrast, a similar dipolar cycloaddition utilized in our quinocarcin synthesis[10e] provided product only from *exo*-addition with the use of methyl acrylate.

(10) For total syntheses of quinocarcin see: (a) Danishefsky, S. J.; Harrison, P. J.; Webb, R. R.; O'Neill, B. T. *J. Am. Chem. Soc.* **1985**, *107*, 1421−1423. (b) Fukuyama, T.; Nunes, J. J. *J. Am. Chem. Soc.* **1988**, *110*, 5196−5198. (c) Garner, P.; Ho, W. B.; Shin, H. *J. Am. Chem. Soc.* **1993**, *115*, 10742−10753. (d) Katoh, T.; Kirihara, M.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron* **1994**, *50*, 6239−6258. (e) Flanagan, M. E.; Williams, R. M. *J. Org. Chem.* **1995**, *60*, 6791−6797.

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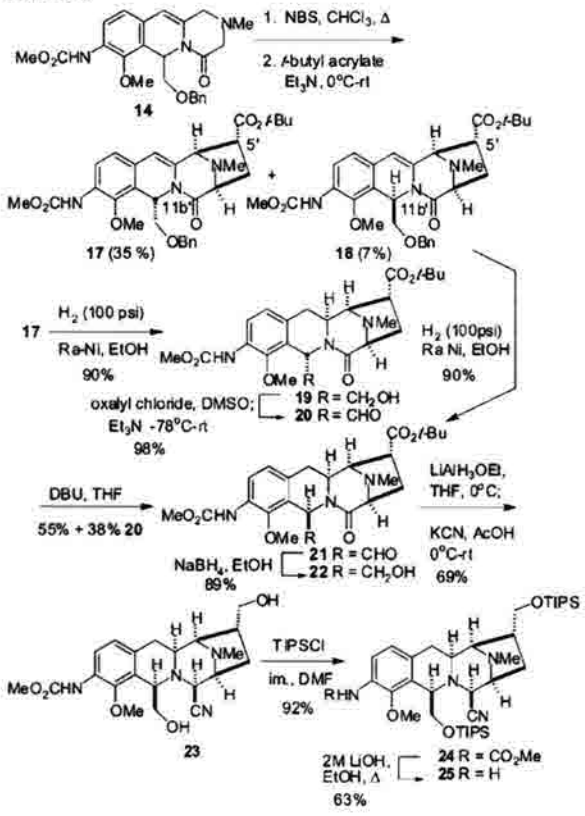
(15) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734−736.

Scheme 3



Tetracycle **18** was hydrogenated in the presence of Raney Nickel at moderate pressure to remove the benzyl group and reduce the benzylic olefin from the least-hindered face to afford **22**. The ease of reduction of the olefin was unexpected in light of literature precedent, where catalytic hydrogenation of similar benzylic olefins required much higher pressure (1000−2000 psi) and elevated temperatures.[10b,c] The major product from the cycloaddition (**17**) possessed the undesired configuration at C-11b′ as determined by ^{1}H NMR nOe analysis and an epimerization at C-11b′ was thus executed.

The benzyl ether and the olefin of **17** were reduced as above for **18** with Raney Nickel. The resultant alcohol was subjected to Swern oxidation conditions[16] to afford the corresponding aldehyde **20** that was treated with DBU to afford a 1.4:1 mixture of epimers at C-11b with the desired isomer **21** 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 **22**.

The simultaneous reduction of the *tert*-butyl ester and partial reduction of the amide were fortuitously accomplished in a single step with $LiAlH_3OEt$ in THF at 0 °C. The resultant carbinolamine was trapped with sodium cyanide under acidic conditions[17] to afford the corresponding stable aminonitrile **23**. The stereochemistry of the aminonitrile was assigned via 2D ^{1}H NMR analysis (ROSEY, TOCSY, and gDQCOSY). The relative stereochemistry of the nitrile was found to be opposite

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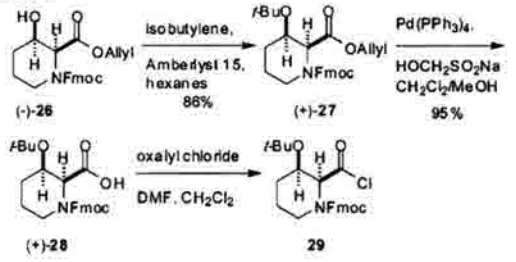
Scheme 4



to that expected based on the known stereochemistry of cyanocycline and the carbinolamine of Et 743. This raises an important question concerning the possible influence of this stereogenic center on the biological reactivity of the aminonitrile versus what has been observed with cyanocycline and Et 743 with respect to DNA alkylation. Further studies to penetrate this issue, however, are warranted. The two primary alcohols were protected as their triisopropylsilyl ethers and the methyl carbamate was hydrolyzed to afford aniline **25** that was ready to couple to a protected β-hydroxy pipecolic acid derivative.

A fully protected β-hydroxy pipecolic acid was prepared in order to couple to the free aniline as illustrated in Scheme 4. Since the amino nitrile moiety has been shown to be stable toward TFA, the secondary alcohol of the previously described amino acid **26**[18] was protected as the corresponding *tert*-butyl ether. Treatment of (−)-**26** with isobutylene in the presence of Amberlyst 15 ion-exchange resin[19] afforded the *tert*-butyl ether (+)-**27** in 85% yield. Cleavage of the allyl ester[20] was accomplished in high yield with $Pd(PPh_3)_4$ to afford **28**. Attempts at coupling the free carboxylic acid (**28**) to aniline **25** with reagents such as EDCI, BOP reagent, or BOPCl were all uniformly unsuccessful. It was thus found that acid chloride **29**, prepared from **28** with oxalyl chloride, resulted in a successful condensation with aniline **25** without epimerization.

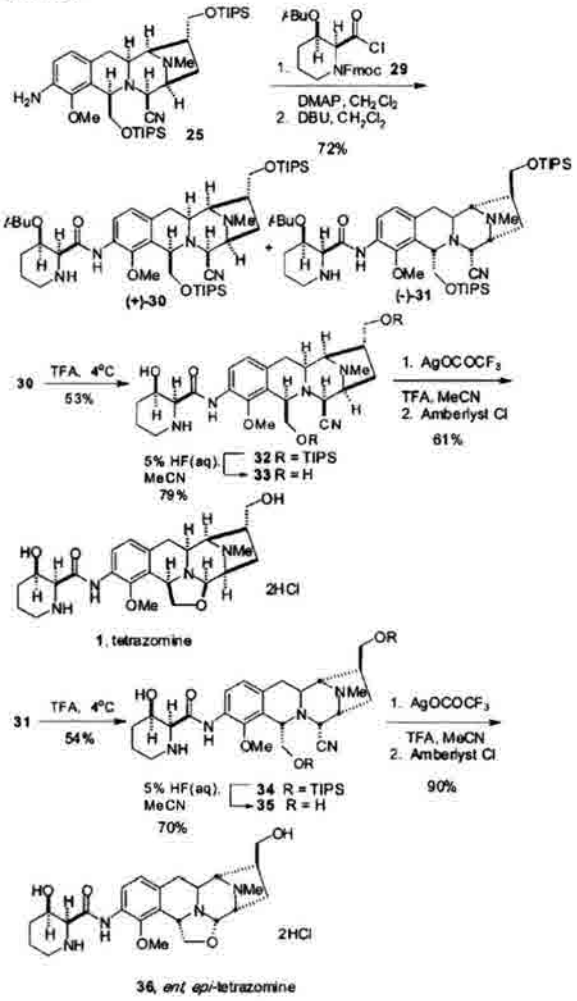
Coupling of acid chloride **29** to aniline **25** in the presence of DMAP followed by cleavage of the Fmoc group with DBU afforded the corresponding pipecolamide **30** (plus a separable diastereomer **31** constituted as the *ent*-tetrahydroisoquinoline portion; obtained as a 1:1 mixture of optically active diastereomers) (Scheme 5).

Each diastereomer was carried on separately, through the end of the synthesis. The *tert*-butyl ether was cleaved with TFA at 4 °C followed by cleavage of the TIPS groups with HF in acetonitrile to afford diastereomers **33** and **35**. To finish the synthesis of tetrazomine, the oxazolidine ring was closed upon treatment of the amino nitrile with silver trifluoroacetate in the presence of excess TFA. Addition of Amberlyst ion-exchange resin (Cl− form) followed by filtration and lyophillization afforded tetrazomine and *ent,epi*-tetrazomine (**36**) as their di-HCl salts. After purification by reversed phase HPLC, the synthetic tetrazomine exhibited identical spectroscopic properties to that of natural tetrazomine.[21]

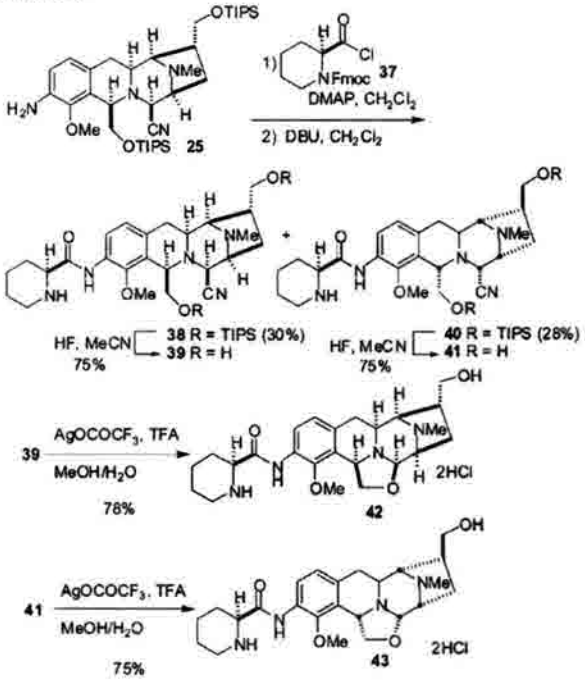
This series of manipulations served to confirm the relative stereochemistry of the pentacyclic tetrahydroisoquinoline core of the natural product. The absolute configuration of the

(18) Scott, J. D.; Williams, R. M. *Tetrahedron Lett.* **2000**, *41*, 8413−8416.
(19) Alexakis, A.; Gardette, M.; Colin, S. *Tetrahedron Lett.* **1988**, *29*, 2951−2954.
(20) Honda, M.; Morita, H.; Nagakura, I. *J. Org. Chem.* **1997**, *62*, 8932−8936.
(21) Provided by Yamanouchi Pharmaceutical Company.

Scheme 5



Scheme 6



tetrahydroisoquinoline core is therefore assumed to be that depicted in structure **1** based on biosynthetic considerations since quinocarcin, bioxalomycin, and ecteinascidin all possess the same absolute configuration of the tetrahydroisoquinoline core.

In an effort to probe the biochemical and biological activity of tetrazomine, several analogues of tetrazomine were prepared as shown in Scheme 6. Aniline **25** was coupled to N-Fmoc-L-pipecolic acid chloride (**37**) followed by cleavage of the Fmoc and TIPS groups to afford the two aminonitrile diastereomers **38** and **40**. The absolute stereochemistry of the tetracyclic core of these compounds was assigned by comparison of the CD spectra of **38** and **40** to that of compound **32**. Removal of the TIPS groups with HF in acetonitrile from **38** and **40** afforded **39** and **41**, respectively. Finally, the oxazolidine rings were installed as described above for tetrazomine to afford the two deoxytetrazomine analogues **42** and **43**.

Biochemical and Biological Activity

We have previously demonstrated that tetrazomine cleaves DNA in an O_2-dependent manner[7] and that the DNA cleavage reaction is inhibited by the addition of free radical scavengers such as picolinic acid and is also inhibited by superoxide dismutase (SOD). The mechanism of DNA cleavage arises from the self-disproportionation of tetrazomine to form a carbon-centered radical that can react with molecular oxygen eventually to be expelled as superoxide radical anion. Superoxide released from the drug subsequently undergoes Fenton/Haber-Weiss redox cycling leading to the formation of various oxygen-based radicals, including hydroxyl radical, that can damage DNA.

It was further demonstrated that tetrazomine cleaves DNA in a non-sequence-specific manner with cleavage observed at every nucleotide residue, which is consistent with a freely diffusable oxidant.[7] In addition, each nucleotide residue of the tetrazomine-damaged DNA was observed as a doublet on denaturing polyacrylamide gel electrophoresis indicative of the production of both the 3′-phosphate and 3′-phosphoglycolate products. The observed product ratios of 3′-phosphate:3′-phosphoglycolate were 8:2 for tetrazomine, 6:4 for quinocarcin, and 4:6 for Fe/EDTA. It was hypothesized that the difference in ratios of the 3′-phosphate to 3′-phosphoglycolate products formed from tetrazomine or quinocarcin and Fe/EDTA might be attributed to the capacity of the drugs to noncovalently bind, thus affecting the presentation of the oxidant for hydrogen atom abstraction on the ribose−phosphate backbone. Further evidence supporting this notion was observed when DNA that was preincubated with tetrazomine and then treated with Fe/EDTA showed virtually no 3′-phosphoglycolate product formation.

It has also been postulated that quinocarcin can noncovalently bind to DNA and alkylate via an iminium ion species generated from ring-opening of the oxazolidine.[22] However, despite compelling experimental precedent for this mode of reactivity on ecteinascidin 743 and saframycin, there is no published experimental evidence for DNA alkylation by either quinocarcin or tetrazomine.

To examine the interaction of tetrazomine and analogues **33** and **36** with DNA, these compounds were incubated with a

(22) Hill, G. C.; Wunz, T. P.; Remers, W. A. *J. Comput.-Aided Mol. Des.* **1988**, *2*, 91−106.

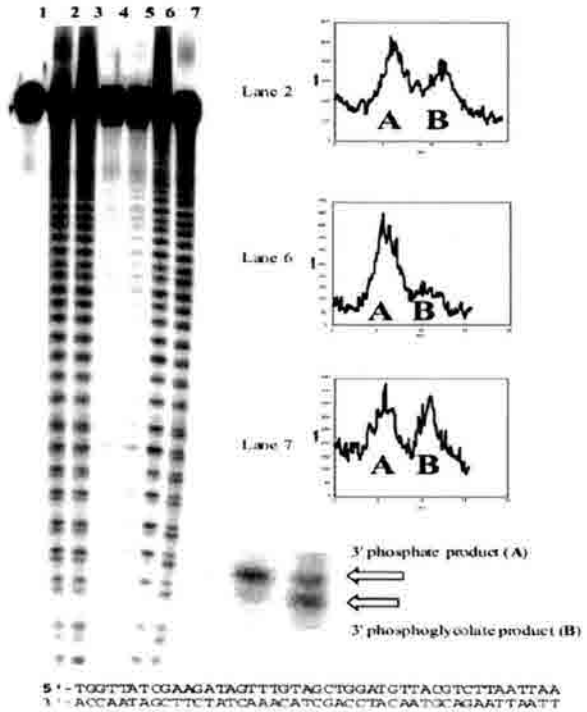


Figure 2. Lane 1: DNA (control). Lane 2: DNA + 1 mM **36**. Lane 3: DNA + 10 mM **36**. Lane 4: DNA + 1 mM **33**. Lane 5: DNA + 10 mM **33**. Lane 6: DNA + 10 mM tetrazomine. Lane 7: DNA + Fe(II)/EDTA.

synthetic ^{32}P-5′-end-labeled 45 bp duplex at pH 7 in 20 mM phosphate buffer (Figure 2). As expected, cyanotetrazominol (**33**) did not exhibit any capacity to inflict oxidative DNA damage. On the other hand, *ent,epi*-tetrazomine did mediate DNA cleavage in a non-sequence-specific manner, with a 3′-phosphate:3′-phosphoglycolate ratio of 4.5:3.5 as measured by the use of a phosphoimager.

In an effort to investigate a preliminary structure−activity relationship with respect to the antimicrobial activities of the tetrazomine analogues, all four oxazolidine and all four aminonitrile analogues were assayed against a Gram-(+) bacteria (*Staphylococcus aureus*) and a Gram-(−) bacteria (*Klebsiella pneumoniae*) via the disk diffusion method. It was found that the deoxy compounds **42** and **43** possessed slightly better activity than either tetrazomine or compound **36** (Table 1).

The analogues that lacked an oxazolidine ring but contained the aminonitrile moiety displayed comparable antimicrobial activities to that for the oxazolidine-containing compounds (Table 2).

Conclusion

In summary, we have developed the first total synthesis of the natural antitumor antibiotic (−)-tetrazomine and have established the stereochemistry of the natural product. In particular, the relative stereochemistry at the C-5′ position, which was the only stereogenic center remaining in question following our elucidation of the relative and absolute stereochemistry of the β-hydroxypipecolic acid moiety, has now been firmly secured. The absolute stereochemistry of the tetrahydroisoquinoline core has been assigned based on biogenetic considerations relative to all other known members of this family of natural products,

Table 1. Antimicrobrial Activities of Oxazolidine-Containing Analogues[a]

compd	amount (mg)	zone of inhibition *Kleb* (mm)	zone of inhibition *Staph* (mm)
1	0.2	28	12
	0.02	22	R
	0.002	10	R
36	0.12	15	R
	0.012	8	R
	0.0012	R	R
42	0.12	29	14
	0.012	21	9
	0.0012	19	R
43	0.12	24	7
	0.012	17	R
	0.0012	R	R
Penicillin G	10 units	NT	30
Streptomycin	0.01	14	NT

[a] R = resistant, NT = not tested.

Table 2. Antimicrobial Activities of Aminonitrile-Containing Analogues[a]

compd	amount (mg)	zone of inhibition *Kleb* (mm)	zone of inhibition *Staph* (mm)
33	0.12	26	12
	0.012	20	R
	0.0012	16	R
35	0.12	18	R
	0.012	13	R
	0.0012	R	R
39	0.12	27	11
	0.012	23	R
	0.0012	13	R
41	0.12	16	R
	0.012	12	R
	0.0012	R	R
Penicillin G	10 units	NT	30
Streptomycin	0.01	14	NT

[a] R = resistant, NT = not tested.

all of which possess the same absolute stereochemistry (Figure 1). The synthesis features a novel method to construct the monoketopiperazine ring fused to the dihydroisoquinoline nucleus (**13** → **14**, Schemes 1 and 2). The coupling of the optically pure β-hydroxypipecolic acid moiety (**29**) to the racemic tetrahydroisoquinoline nucleus (**25**) has permitted the simultaneous synthesis of the natural product and the *ent,epi*-tetrazomine structure (**36**). Unexpectedly, *ent,epi*-tetrazomine retained some antimicrobial activity against *Klebsiella pneumoniae*, a Gram-(−) organism, but was inactive against *Staphylococcus aureus*, a Gram-(+) organism. More surprising was the observation that *both* pipecolic acid analogues **42**, which possesses the natural stereochemistry, and **43**, which possesses the entantiomorphic tetrahydroisoquinoline nucleus, displayed antimicrobial activity against *Klebsiella pneumoniae Staphylococcus aureus*. The preparation of key aniline derivative **25** provides an intermediate that can be exploited for the attachment of other amino acid or peptide residues to this structure that may prove to be useful for enhancing the sequence-specificity of binding to and alkylating DNA. In a preliminary study exhibiting the utility of compound **25**, we have prepared pipecolic acid analogues of tetrazomine (**42** and **43**) that, somewhat surprisingly, proved to be slightly more potent antimicrobial agents than the natural product. Further studies to prepare derivatives of tetrazomine to probe the biochemistry and biology of this

important family of tetrahydroisoquinoline antitumor antibiotics are in progress in these laboratories.

Acknowledgment. This work was supported by the National Institutes of Health (Grant CA85419). We thank Yamanouchi Pharmaceutical Co. for providing the generous gift of natural tetrazomine.

Supporting Information Available: Complete experimental procedures and spectroscopic and analytical data including copies of NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Chemistry and Biology of the Tetrahydroisoquinoline Antitumor Antibiotics

Jack D. Scott and Robert M. Williams*

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523

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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-*

* To whom correspondence should be addressed. E-mail: rmw@chem.colostate.edu.

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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

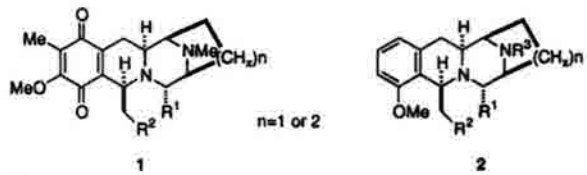


Figure 1. General structures of the tetrahydroisoquinolines.

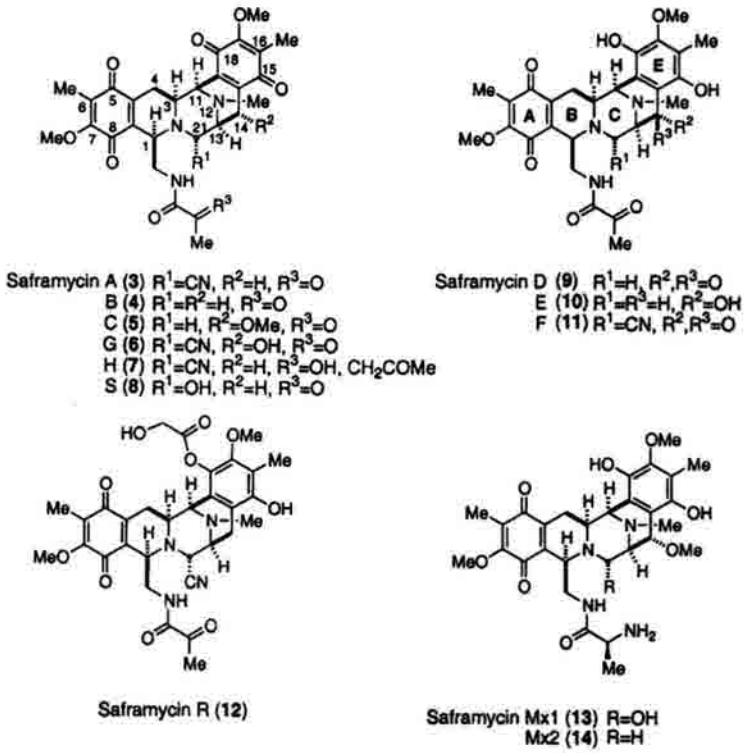


Figure 2. The saframycins.

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

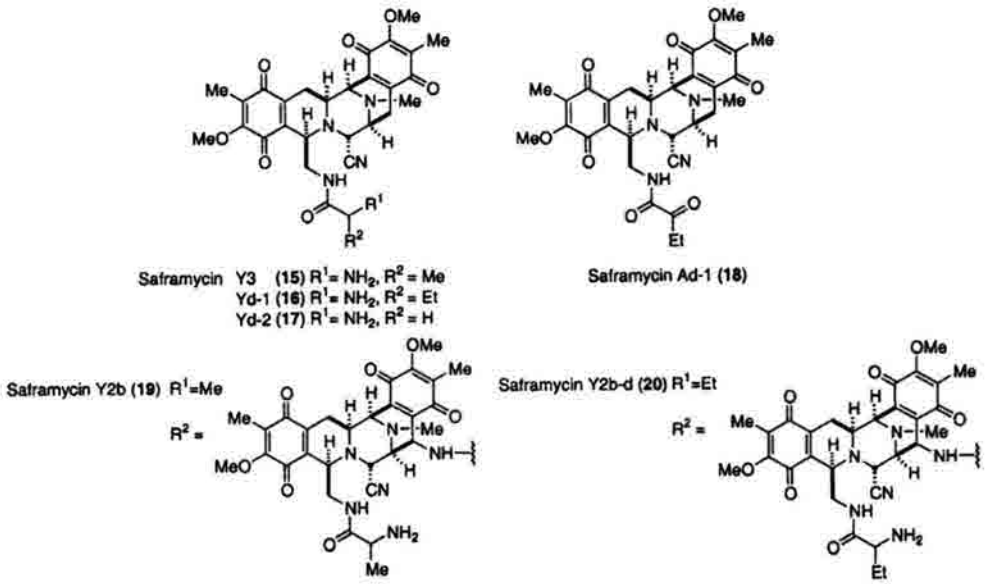


Figure 3. Saframycins obtained from directed biosynthesis.

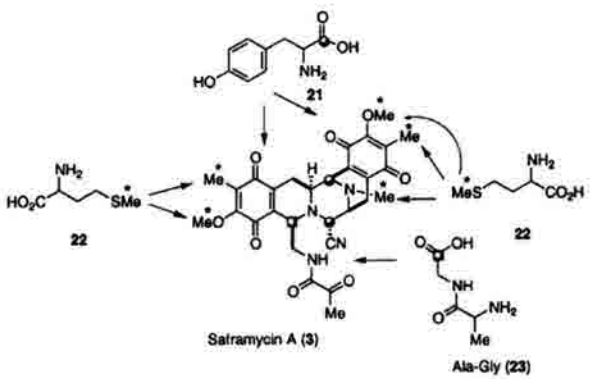


Figure 4. Primary biosynthetic precursors to saframycin A.

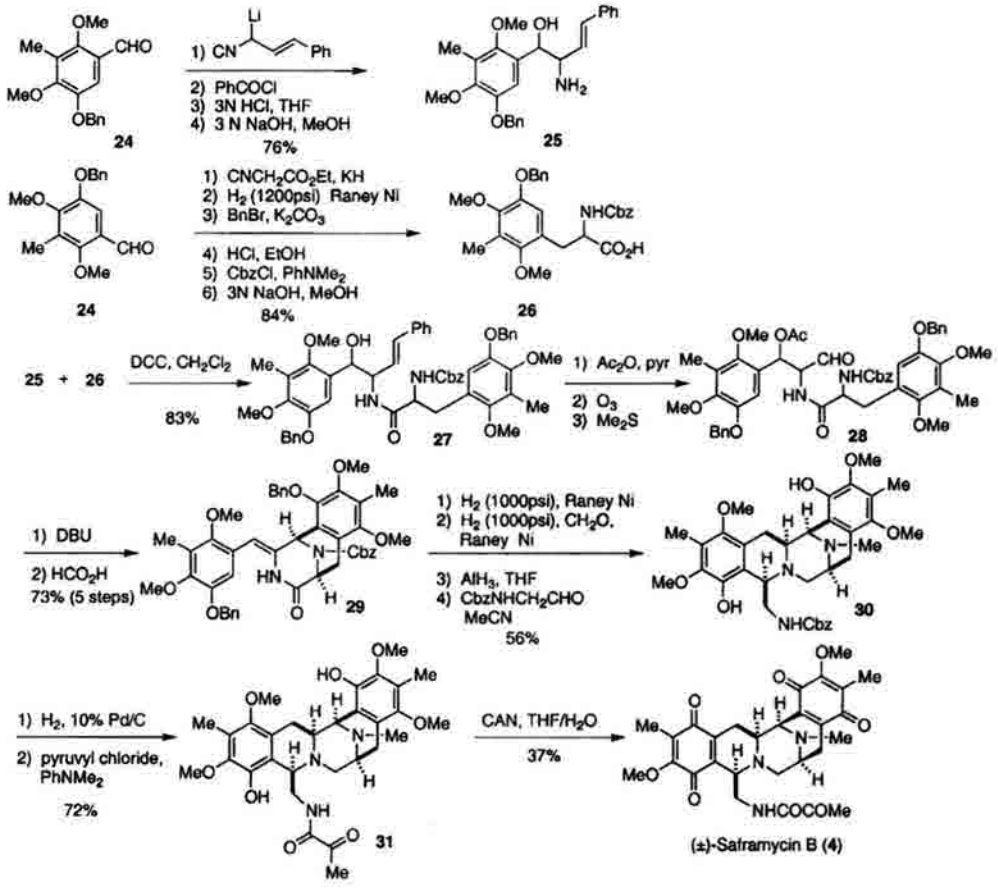
cinnamyl isocyanide afforded the benzylic alcohol that was esterified with benzoyl chloride. Hydration of the isocyanide followed by hydrolysis of the formamide afforded amino alcohol **25** in good yield. The A-ring of saframycin B was also synthesized from aldehyde **24**. Amino acid **26** was synthesized 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.

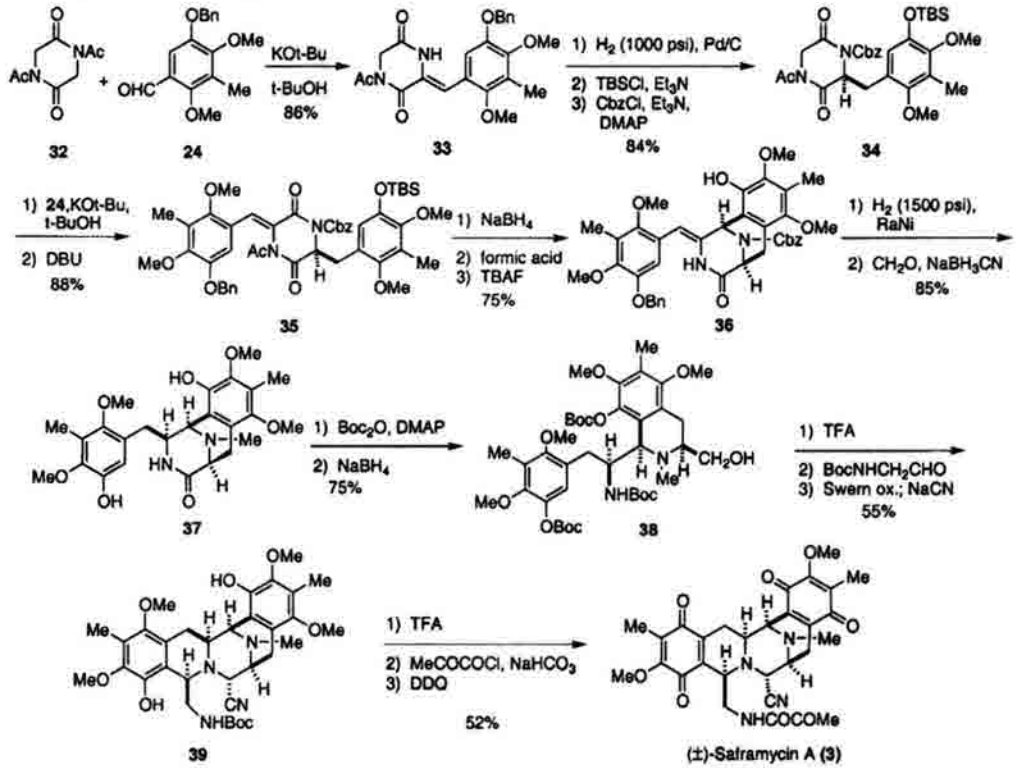
Tricycle **43** was converted to pentacycle **44** via reduction of the amide to the amine using alane followed by hydrogenolysis of the benzylic olefin and the benzylamine followed by a Pictet–Spengler cyclization. Unfortunately, the stereochemistry obtained at C-1 was undesired. Epimerization of this 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.

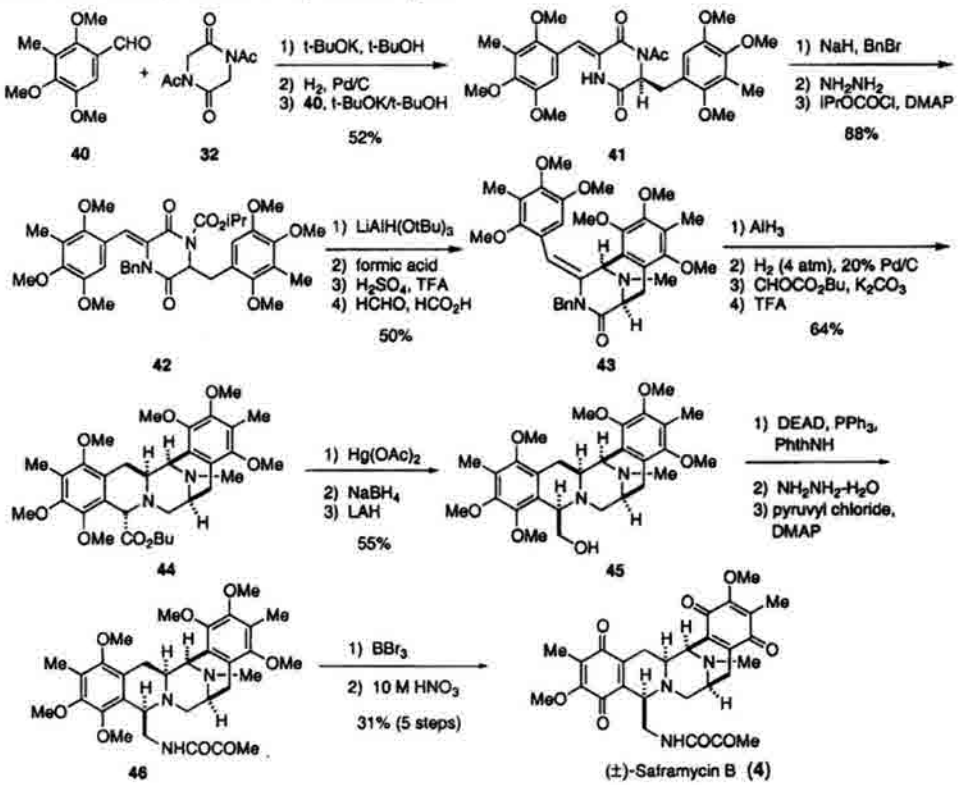
Kubo et al. also showed that (−)-saframycin A could be oxidized with SeO_2 to yield five saframycins (Scheme 6).[6,23] The highest yielding product was (−)-saframycin G in 30% yield. Saframycin G was then converted to the saframycin Mx series compound **49** by reduction of the two quinone rings to the 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

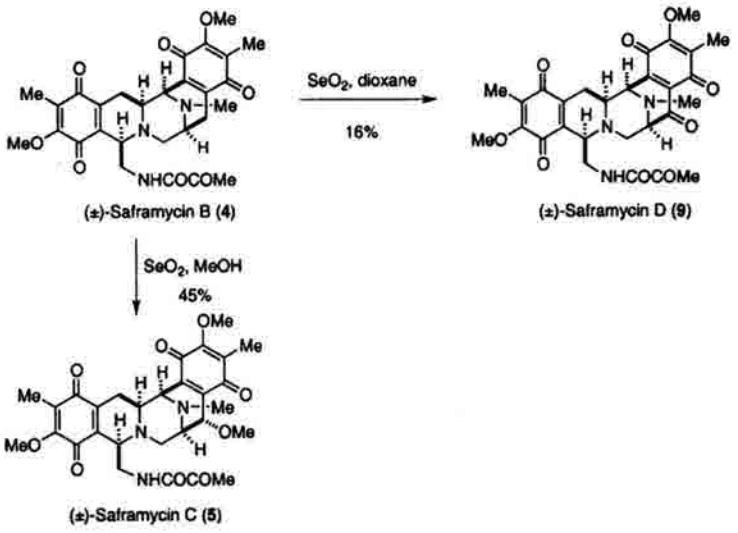


Scheme 4. Kubo's Total Synthesis of D,L-Saframycin B

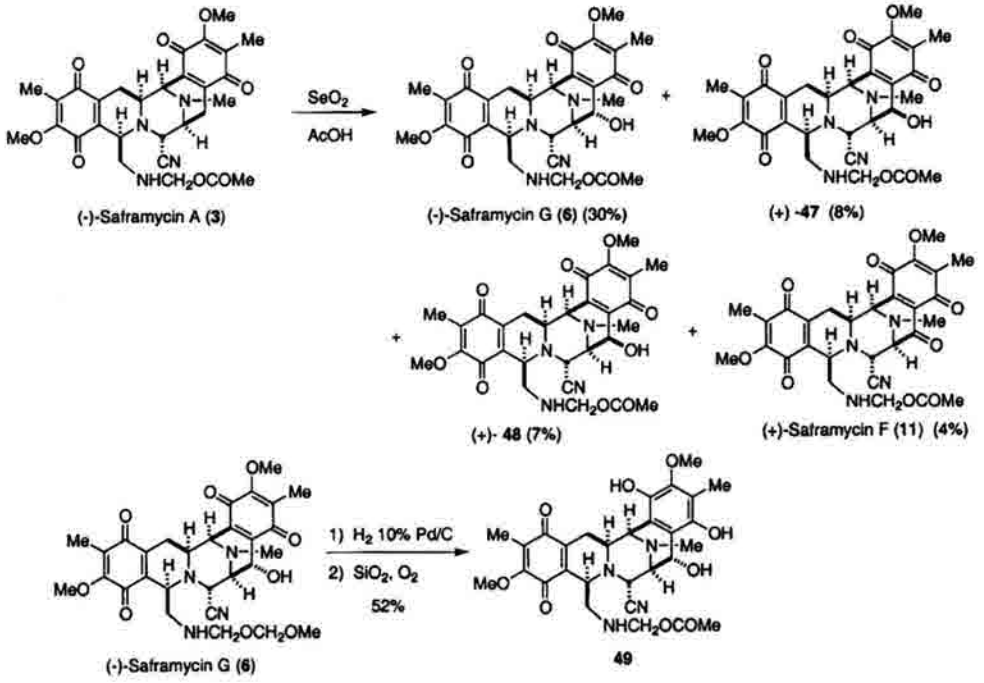


Racemic pentacycle **46**, an intermediate in the saframycin B synthesis, was used as a precursor for the synthesis of (−)-*N*-acetylsaframycin Mx2 (**55**) and *epi*-(+)-*N*-acetylsaframycin Mx2 (**56**) by Kubo et al.

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



Scheme 6. Selenium Dioxide Oxidation of Saframycin A

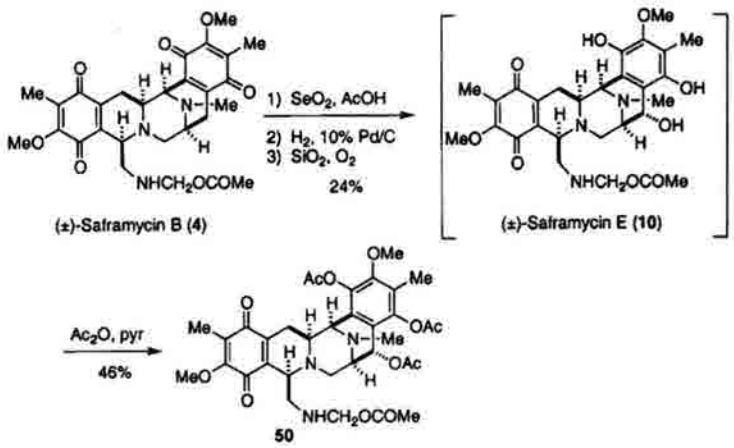


(Scheme 8).[24] The first step in the sequence involved the coupling of *N*-Cbz-L-alanine to the primary amine yielding the optically active amide **51** and its *epi*-enantiomer **52** in 42% and 37% isolated yields, respectively. Each diastereomer was subsequently carried on separately to the final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

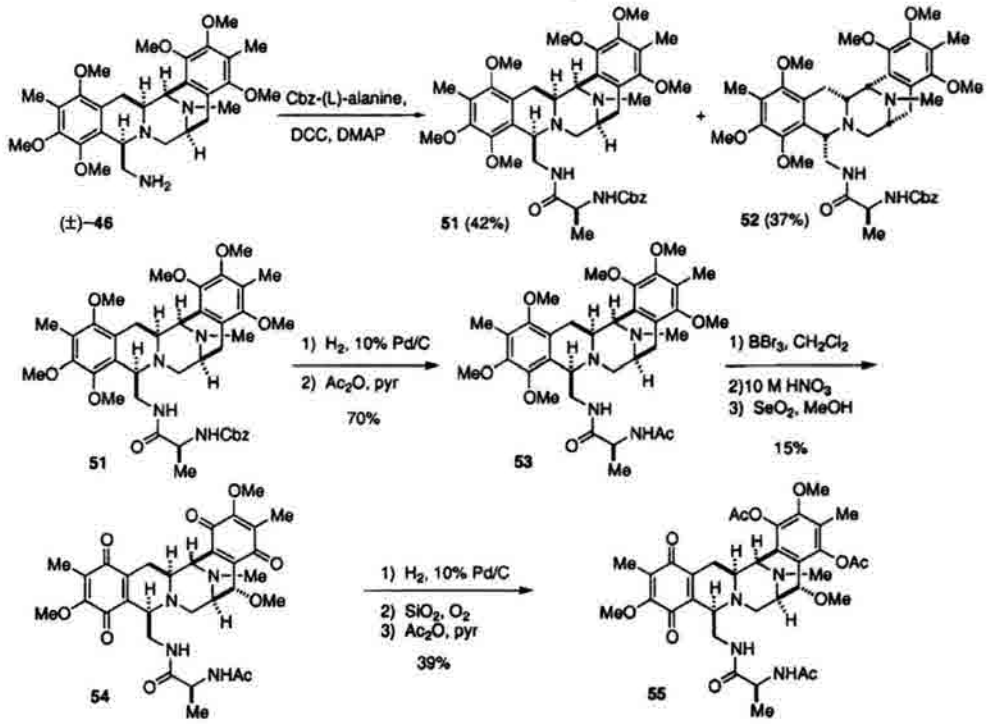
The *N*-Cbz group was removed from compound **51**, and the resultant amine was acylated to form **53** (Scheme 8). The hydroquinones were deprotected and oxidized to the corresponding quinones with SeO_2, which also effected selective oxidation of the D-ring, furnishing the desired methyl ether **54**. 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

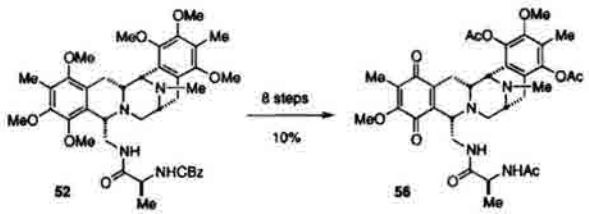
Scheme 7. Conversion of Saframycin B to Saframycin E



Scheme 8. Resolution and Transformations of Racemic Compound 46



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

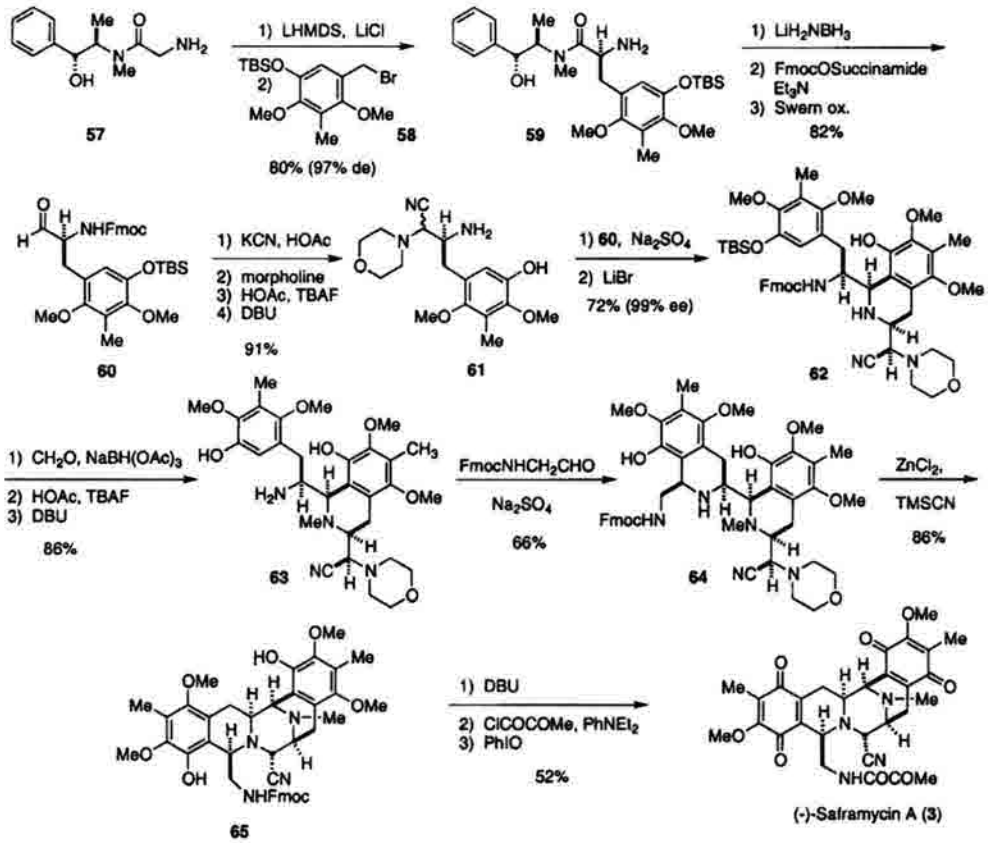


of the aldehyde with HCN formed the cyanohydrin, which was treated with morpholine to yield the corresponding amino nitrile,[25c] which served as a masked aldehyde. Removal of the TBS and Fmoc groups was accomplished in two steps in high yield to form amine **61**.

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.

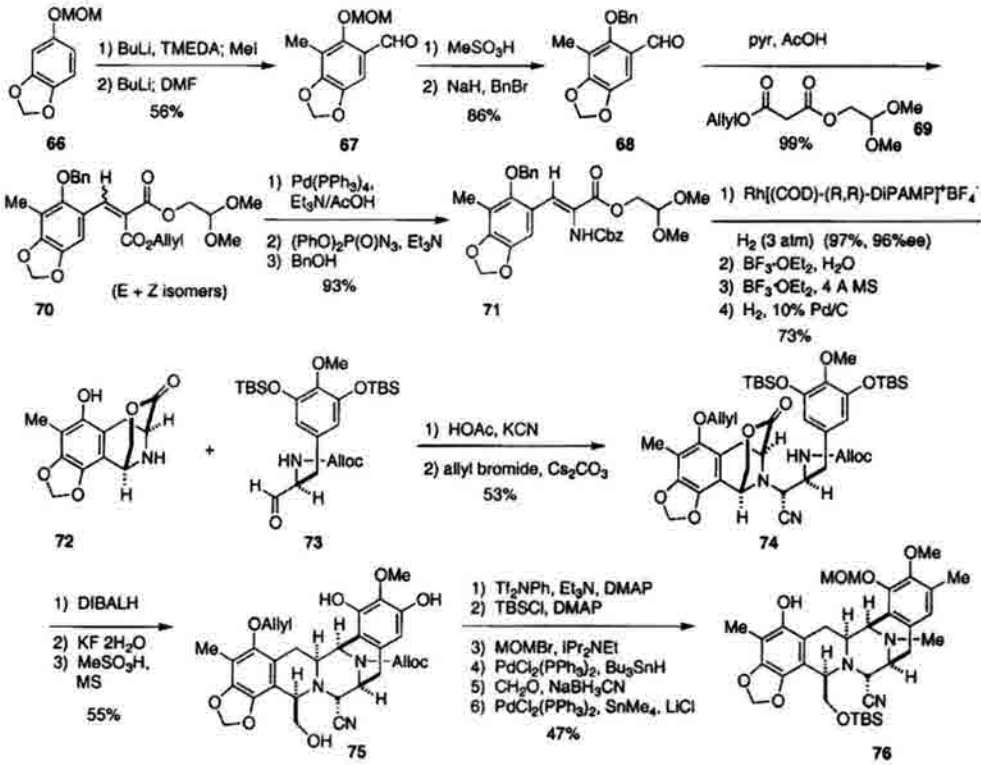
In 2000, Martinez and Corey reported an improved synthesis[28] of intermediate **75** that was utilized in their total syntheses of saframycin and ecteinascidin as shown in Scheme 13. This synthesis improved the yield of **75** from 11% in 13 steps to 57% in six steps.

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.

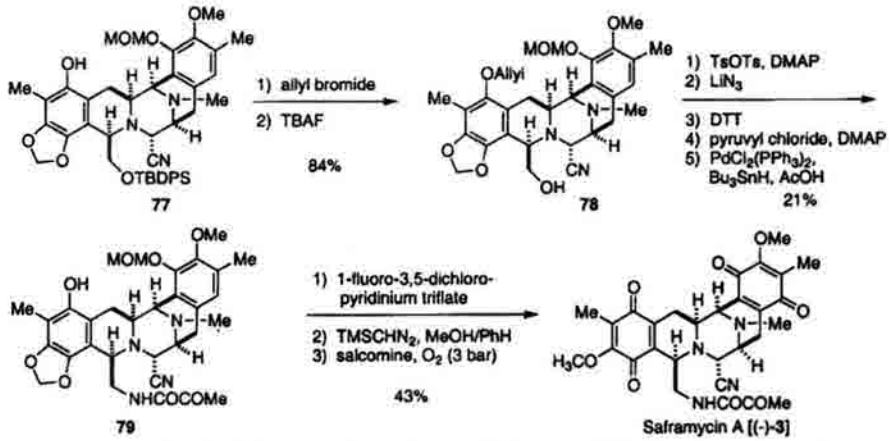
2.1.4. Synthetic Studies toward the Saframycins

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.

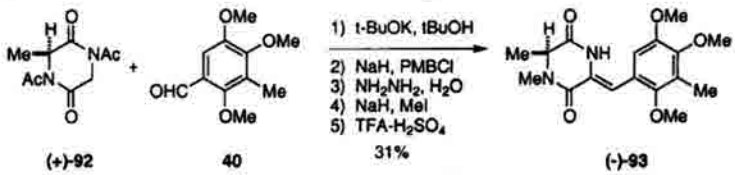
In 1989, Kubo et al. showed in synthetic studies toward saframycin A that tetracycle **91** could be formed with the amide carbonyl intact (Scheme 15).[30] This would allow for further functionalization to form the amino nitrile in saframycin A.

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.

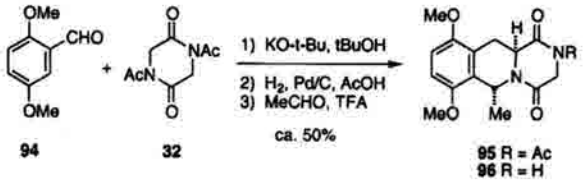
In 1990, Ong and Lee synthesized the tricycles **95** and **96** via a Pictet−Spengler cyclization using acetaldehyde on a diketopiperazine (Scheme 17).[32] The major drawback to this approach was that the stereogenic center constructed in the Pictet−Spengler reaction gave the unnatural relative stereochemistry.

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 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**.

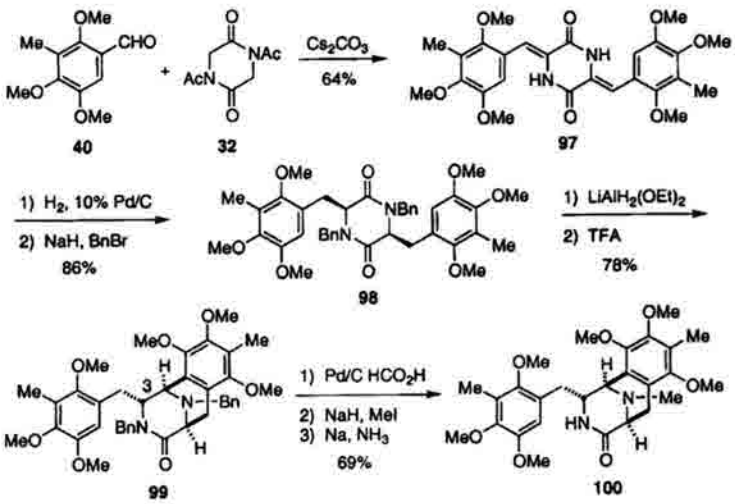
The A-ring was synthesized in high yield starting with the olefination of aldehyde **40** followed by Sharpless asymmetric dihydroxylation to furnish optically active material. The diol was converted to the optically pure epoxide **107** via tosylation and base-mediated ring closure. The epoxide was 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**.

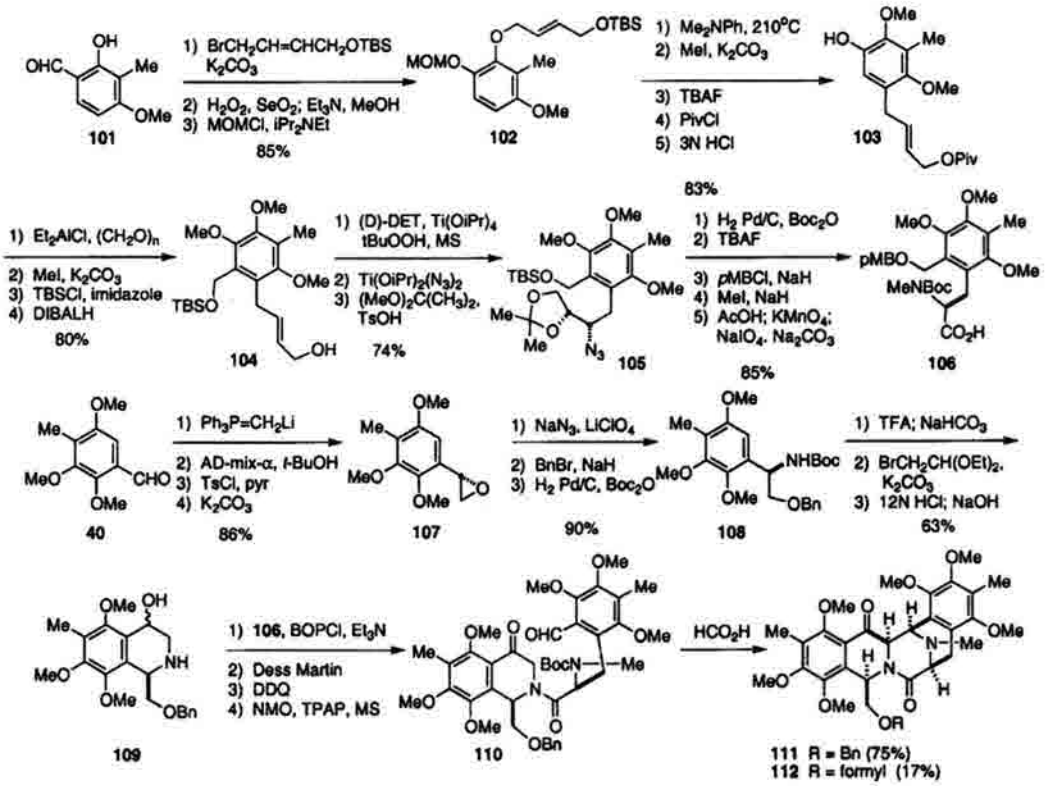
2.1.5. Analogue Syntheses

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

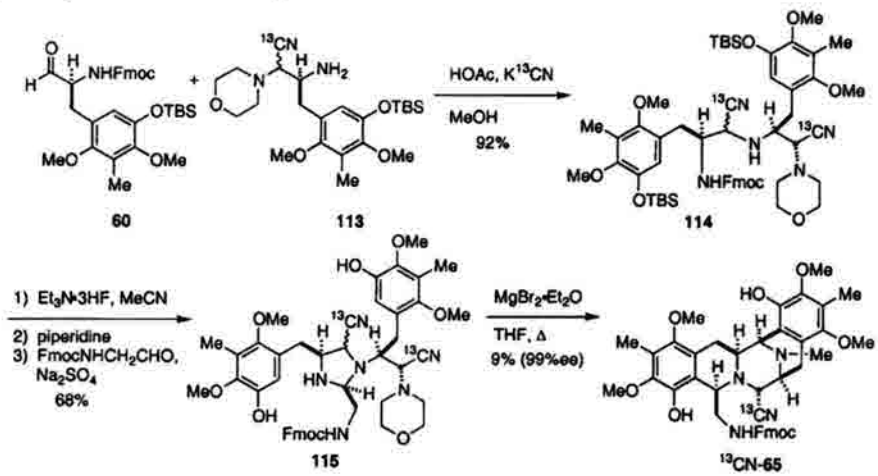
Scheme 18. Liebeskind's Synthetic Studies toward Saframycin A



Scheme 19. Danishefsky's Synthetic Studies toward the Saframycins



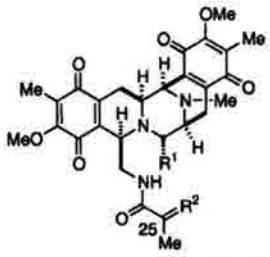
Scheme 20. Myers' Synthesis of Pentacycle 65



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

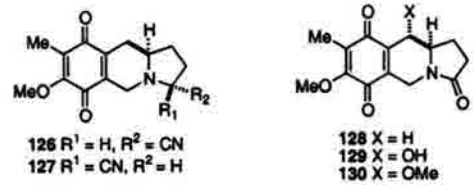
test organism	3 MIC (μg/mL)	8 MIC (μg/mL)
Staphylococcus aureus FDA 209P	0.1	0.025
Streptococcus faecalis	12.4	3.12
Bacillus subtilis PCI 219	0.1	0.025
Corynebacterium diphtheriae	0.003	0.004
Sarcina lutea	0.05	0.025

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**, **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

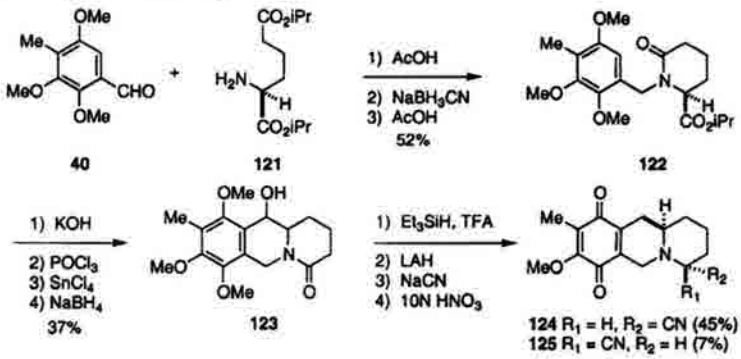
compound	ID_{50} (μM)	compound	ID_{50} (μM)
3	0.0056	119	0.025
8	0.0053	120	0.027
116	0.0061	4	0.80
117	0.0080	5	3.9
6	0.030	9	4.8
7	0.033	118	0.65
11	0.59		

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.

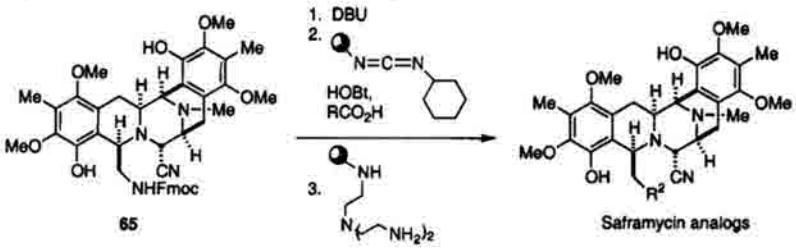
There was no difference in biological activity between saframycins Y3, Yd-1, Yd-2, and Ad-1 with respect to an amino group or a carbonyl at C-25.[41] Also, the dimers Y2b and Y2b- d had similar activities to the corresponding monomers. In a study to examine side chain effects on biological activity, 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

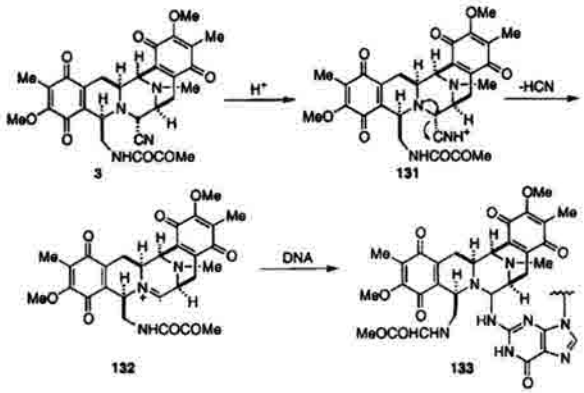
Scheme 21. Simple Saframycin A Analogs



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.

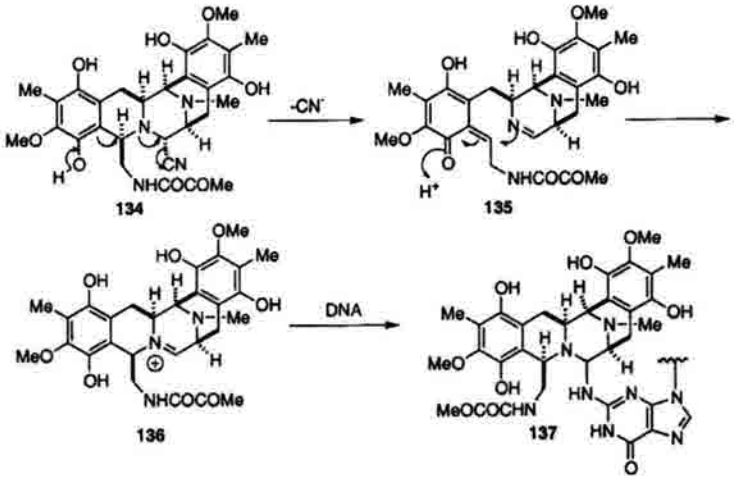
Another mechanism was proposed by Hill and Remers based on the fact that saframycin A does not alkylate DNA unless it was converted into the corresponding hydroquinone form (**134**) (Scheme 24).[46] These workers speculate that the phenol facilitates scission of the B-ring C- N bond, which in turn leads 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

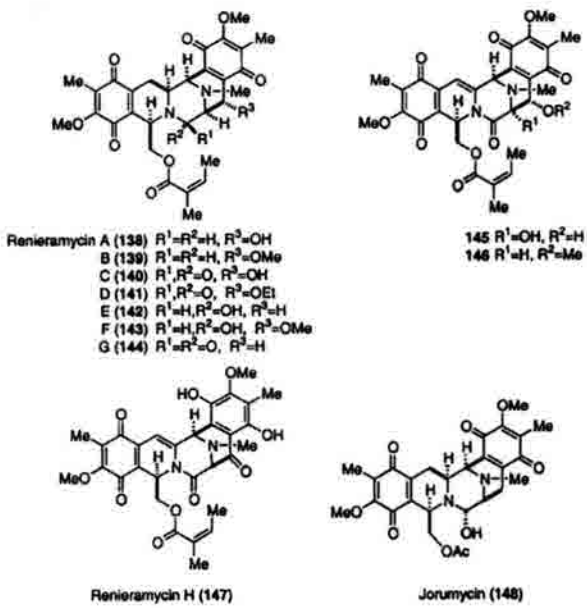


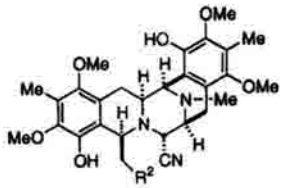
Figure 7.

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.

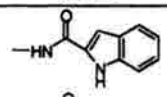
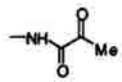
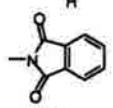
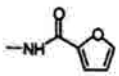
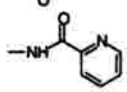
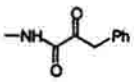
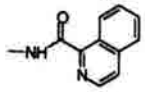
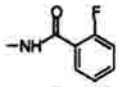
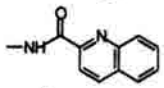
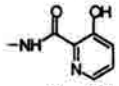
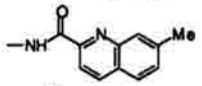
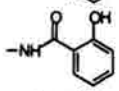
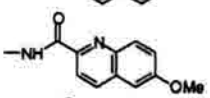
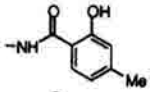
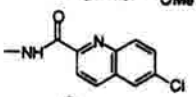
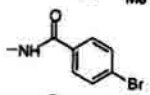
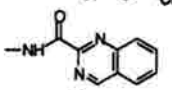
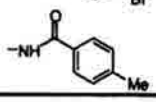
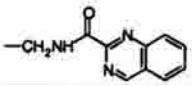
2.2.2. Total Synthesis of Renieramycin A

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

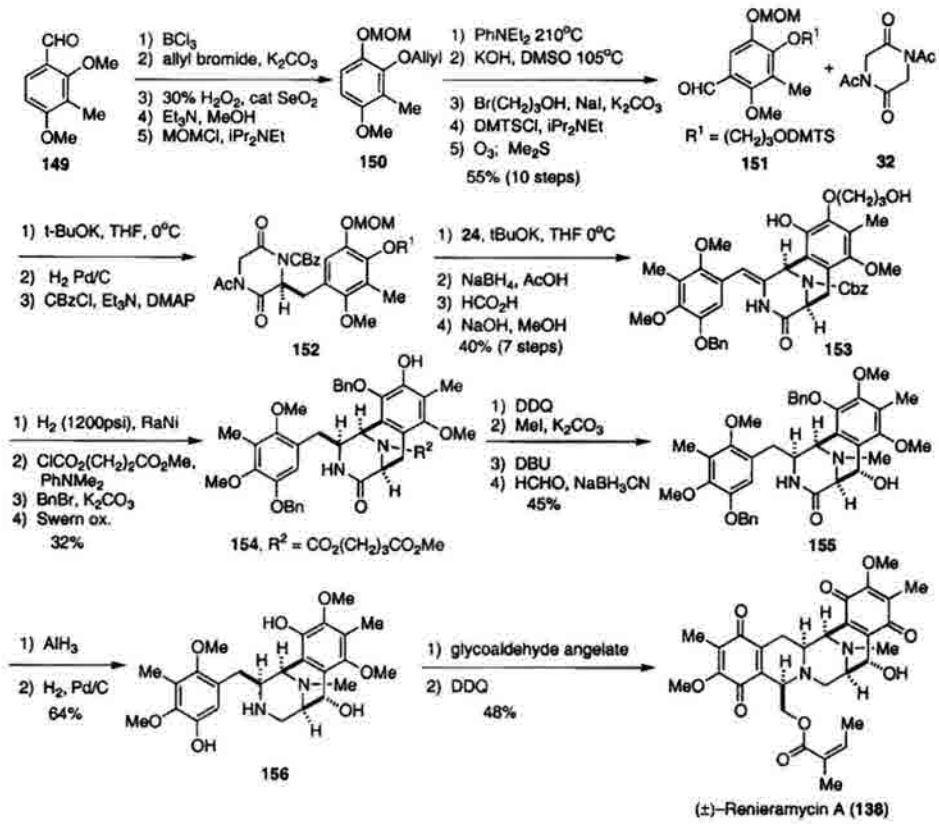
R^2 =	IC_{50} (nM) A375	A549	R^2 =	IC_{50} (nM) A375	A549
Saframycin A (3)	5.3	133		2.7	31
	4.5	160		1.7	9.2
	13	290		3.3	40
	2.4	39		2.5	32
	2.5	37		1.3	4.4
	1.4	14		1.4	4.6
	1.2	11		2.0	3.5
	1.2	6.5		1.5	4.1
	1.7	25		1.2	4.7
	1.9	37		3.6	78

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.

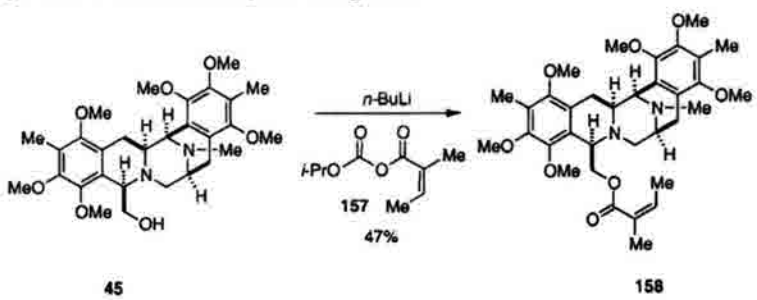
2.2.3. Synthetic Studies toward the Renieramycins

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

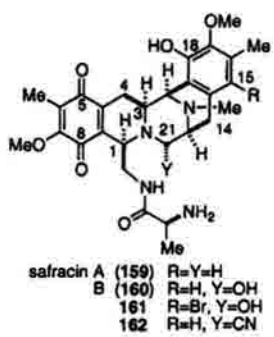
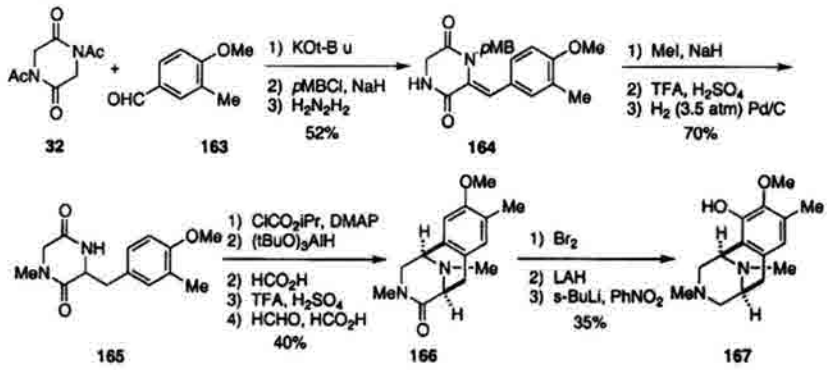
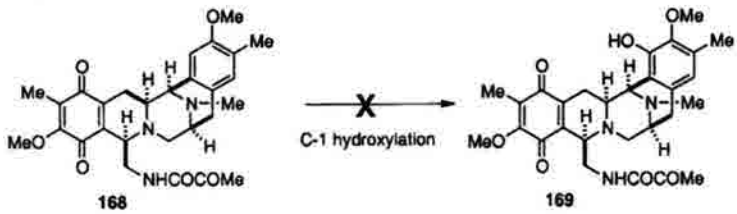


Figure 8. Safracins A and B.

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

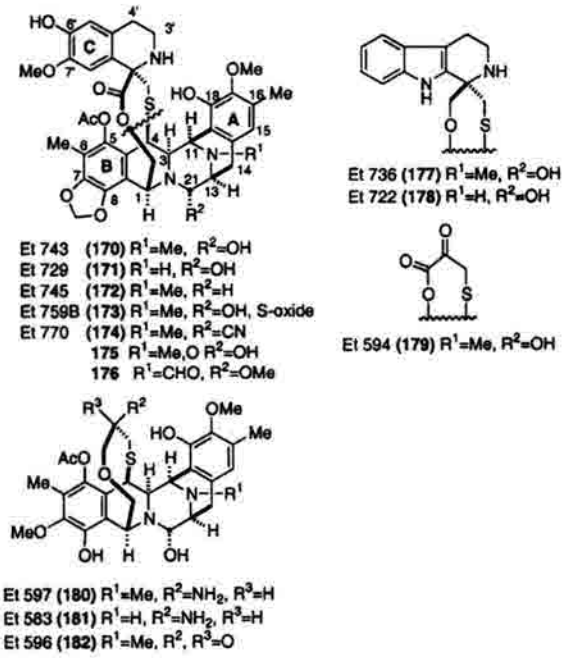


Figure 9. The ecteinascidins.

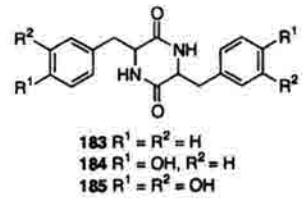


Figure 10. Biosynthetic precursors to ecteinascidin 743.

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.

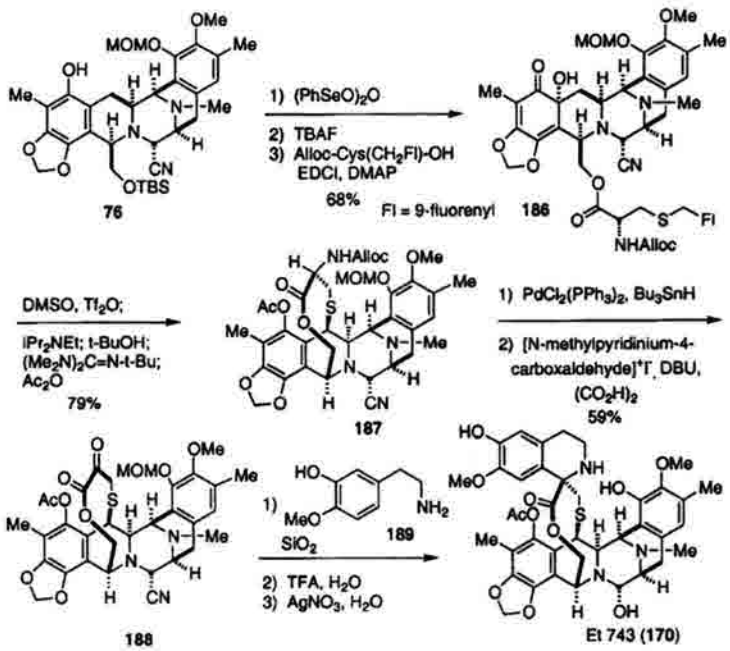
2.4.3. Total Syntheses of Ecteinsacidin 743

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]

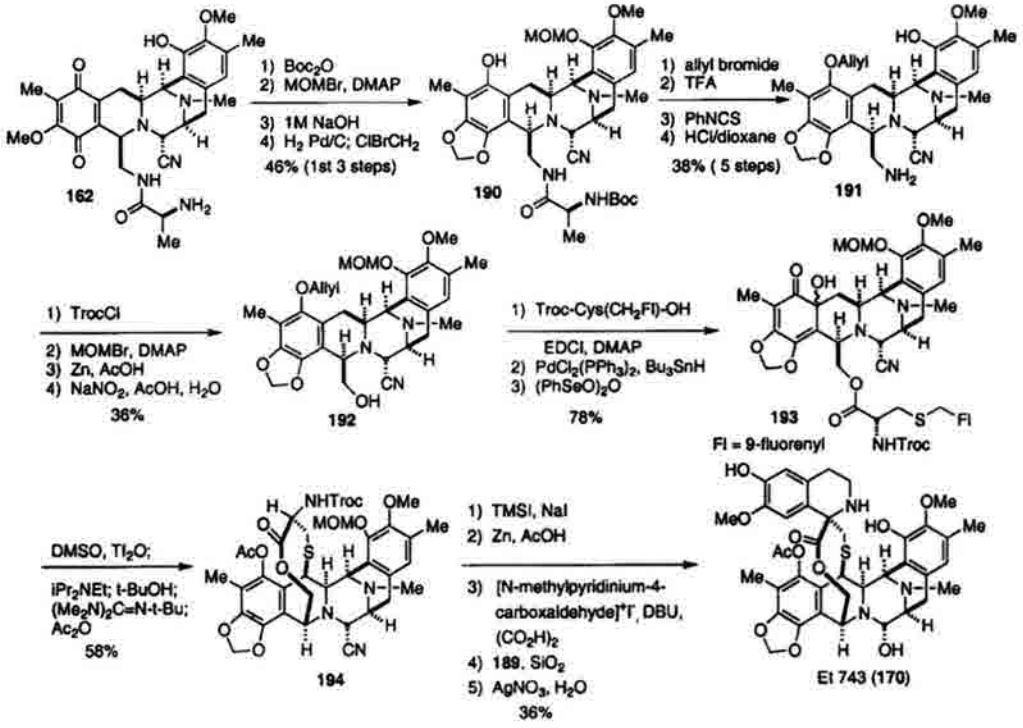
In 1996, Corey et al. synthesized Et 743 via a convergent synthesis employing the coupling of two optically active fragments as seen in their saframycin A synthesis[26] (Scheme 29). Starting with hexacycle **76**, a selective hydroxylation was accomplished using phenylselenic anhydride. Removal of the 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 R-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

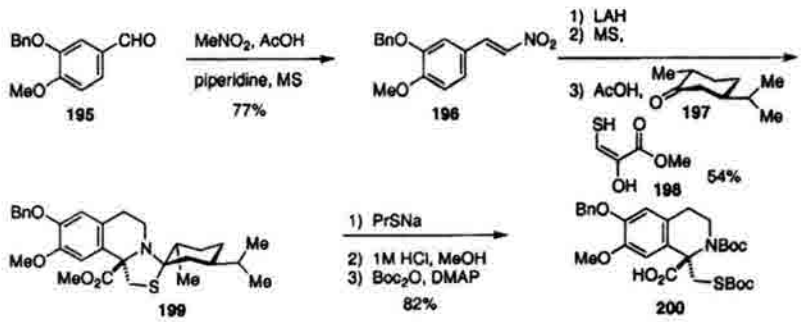
Scheme 29. Corey's Total Synthesis of Et 743



Scheme 30. Cuevas Semi-synthesis of Et 743



Scheme 31. Corey's Synthetic Studies toward Et 743



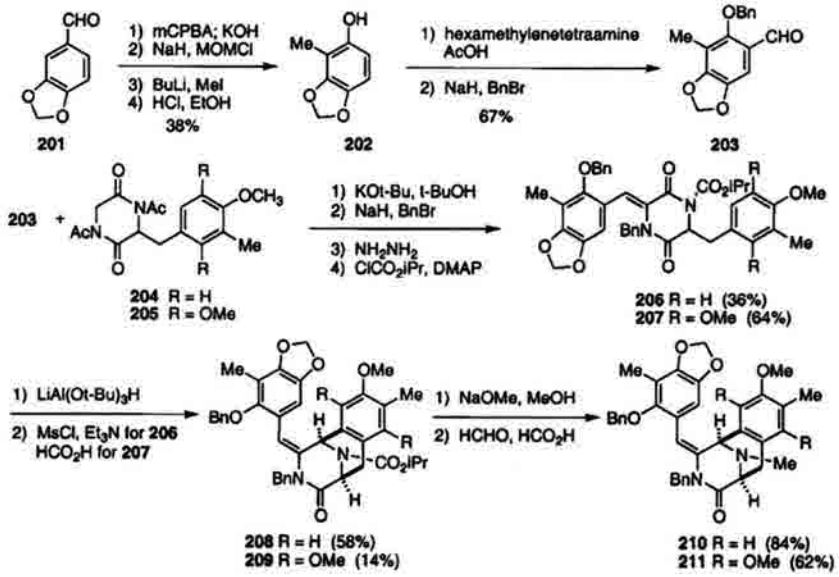
providing **191** in 68% yield. Protection of the phenol allowed for the diazotization of the primary amine for conversion to alcohol **192**. The synthesis of Et 743 was completed using the chemistry of Corey[27] on similar substrates. A three-step sequence was used to form **193**. Dehydration under 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

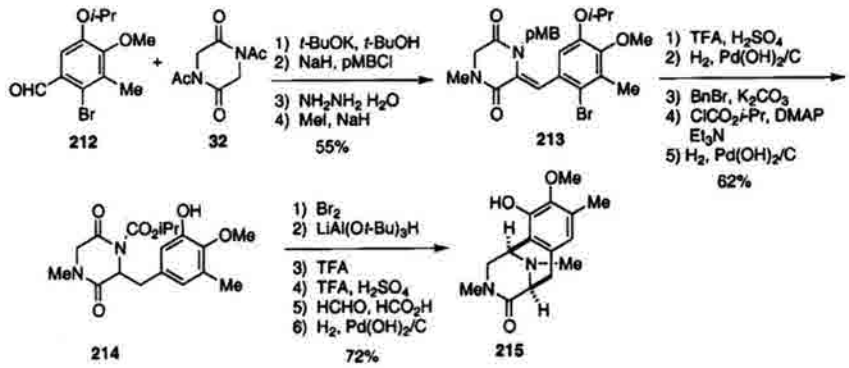
Corey and Gin reported an efficient synthesis of the tetrahydroisoquinoline C unit of Et 743 in 1996.[71] Aldehyde **195** was converted to the nitrostyrene **196** via a nitroaldol condensation as shown in Scheme 31. Reduction of the olefin and nitro group was followed by condensation of the resultant 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

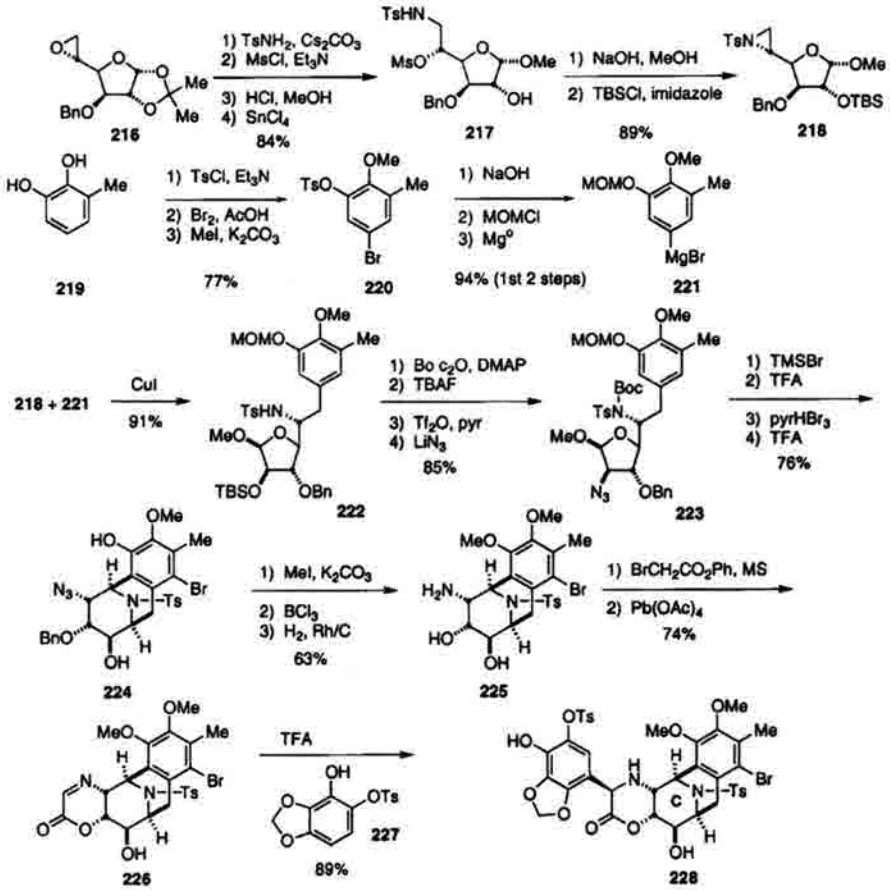


yields. These compounds were presented as possible precursors to the ecteinascidins.

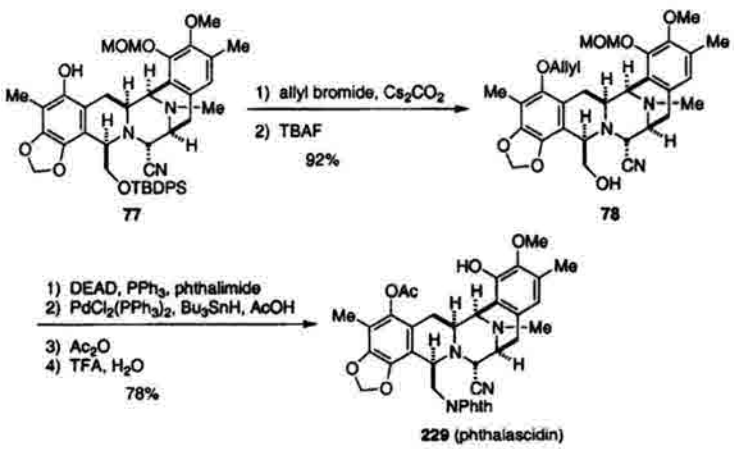
In 2000, Kubo et al. published a different route to the ABC-ring system of the ecteinascidins as shown in Scheme 33.[73] Aldol condensation of aldehyde **212** with diketopiperazine **32** afforded **213** after the protection of the lactams. Changing of the phenol protecting group and activation of 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)



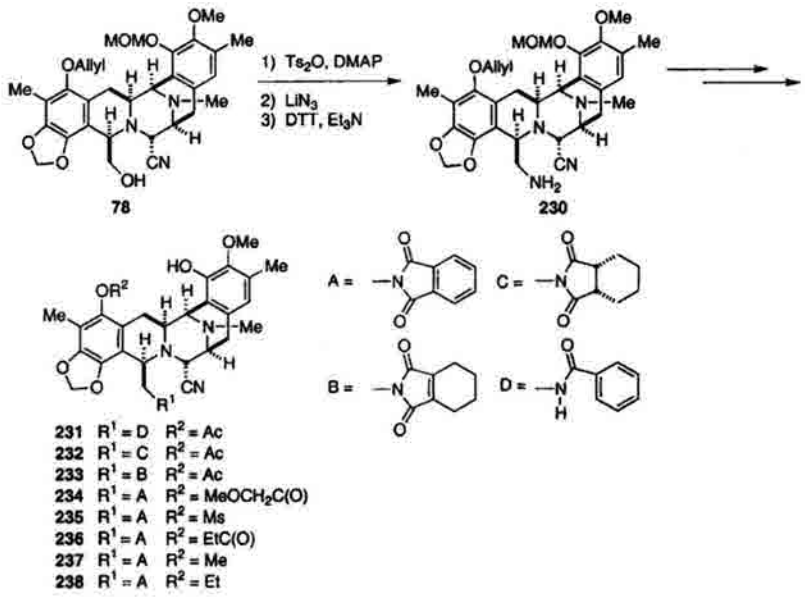
to the corresponding dialdehyde. Closure of the B- and C-rings would then afford the pentacyclic core of the ecteinascidins.

2.4.5. Analogue Syntheses

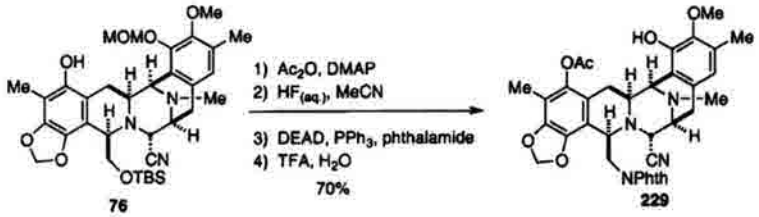
Corey et al. reported the synthesis and biological activity of potent analogues of Et 743 as shown in Scheme 35.[75] In this study, a compound named phthalascidin (**229** or Pt 650) was synthesized, which was surprisingly found to have comparable biological activity to that of Et 743. The synthesis 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

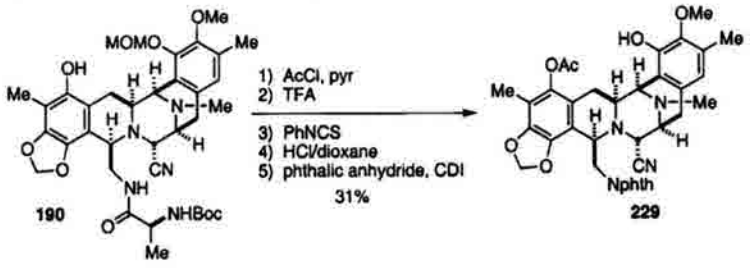
Scheme 36. Pthalascidin Analogs



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.

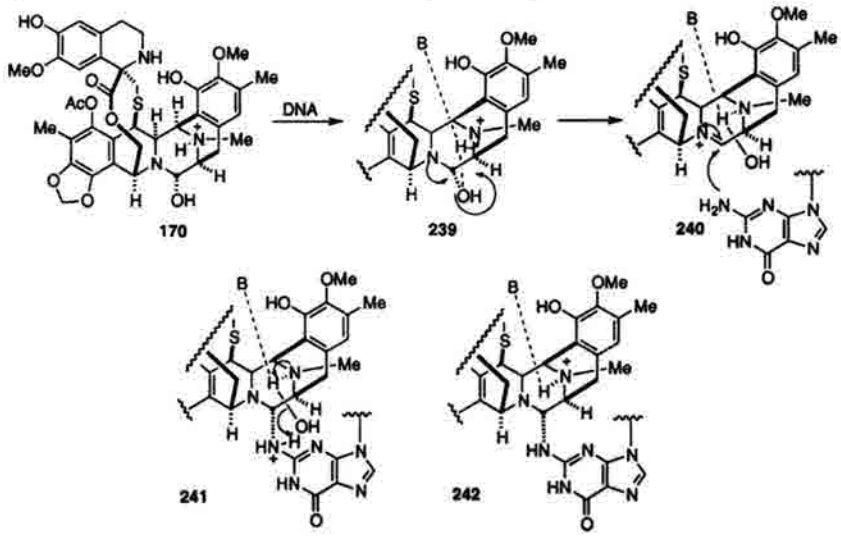
In 1998, Hurley et al. showed by NMR studies that the N-12 of Et 743 was protonated in the Et 743 DNA covalent adduct.[81b] From these data, a mechanism for DNA alkylation was suggested as illustrated in Scheme 39. The N-12 of Et 743 is protonated, which facilitates expulsion of the hydroxyl group in 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 R_1, R_2, $_1$, and $_2$ (**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 $_2$ instead of naphthyridinomycin was obtained.[102b] Also, attempts to repeat the original naphthyridinomycin isolation procedure lead only to the procurement bioxalomycin $_2$, indicating that naphthyridinomycin may in fact be an artifact of the original isolation procedures. Thus, the initial biosynthetic product, bioxalomycin $_2$ 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 $_2$.[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 -lac-

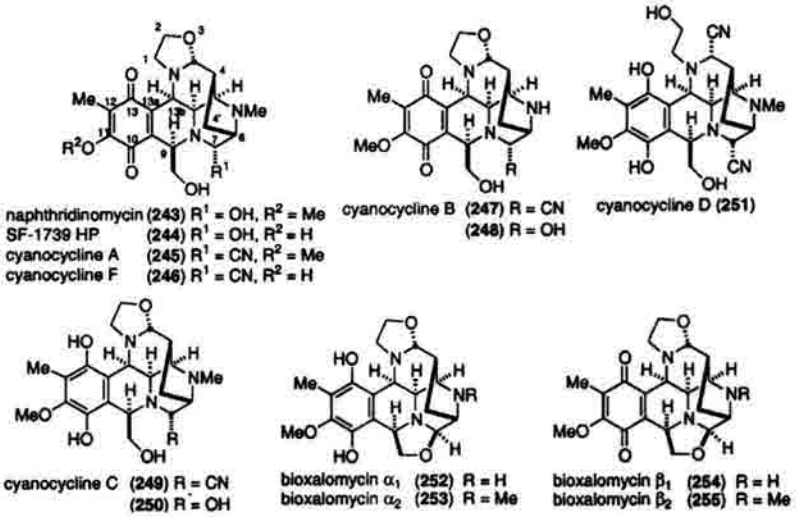


Figure 11. Structures of naphthyridinomycin, cyanocyclines, and bioxalomycins.

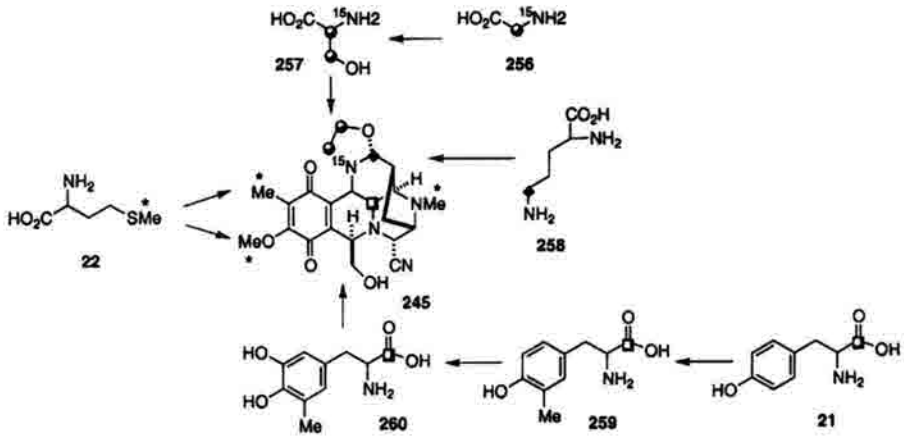
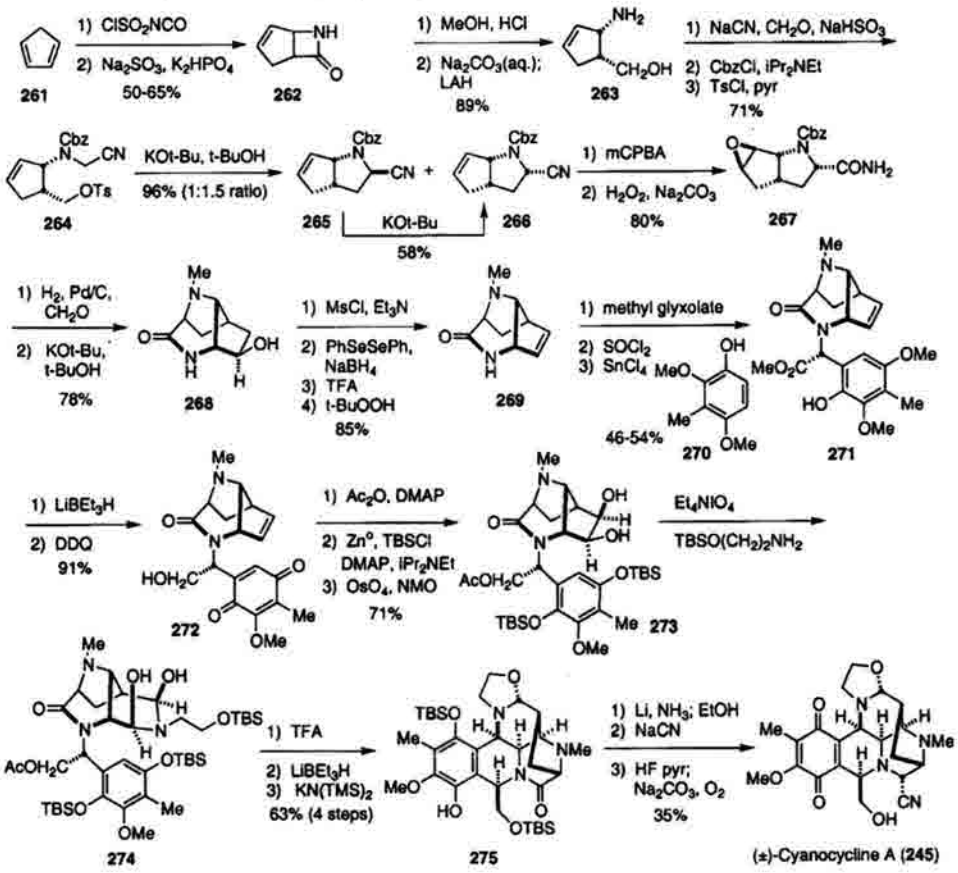


Figure 12. Biosynthetic precursors to cyanocycline.

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

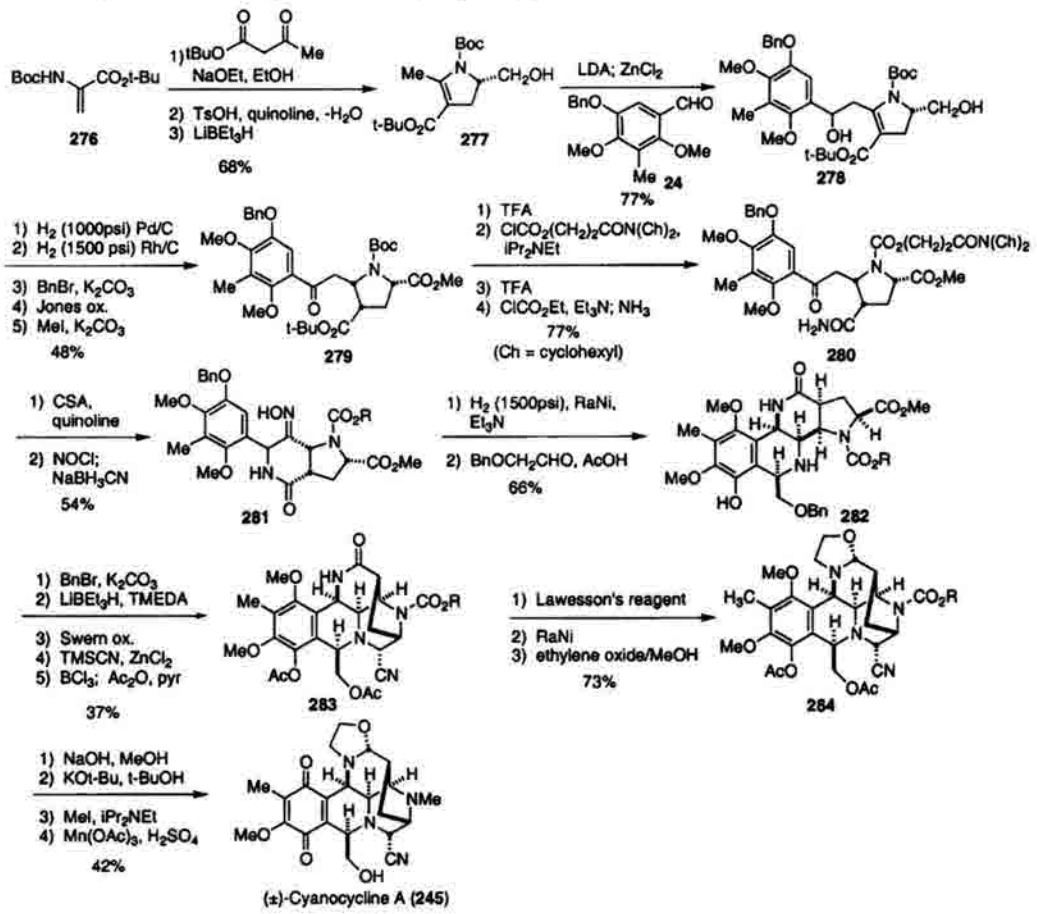


product **278**. Hydrogenation under two different conditions first cleaved the benzyl ether and subsequently saturated the olefin. Reprotection of the phenol was followed by oxidation of the primary alcohol to the corresponding acid, which was then converted to the methyl ester **279**. Selective Boc 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 R-chloro oxime using sodium cyanoborohydride yielded the oxime **281**.

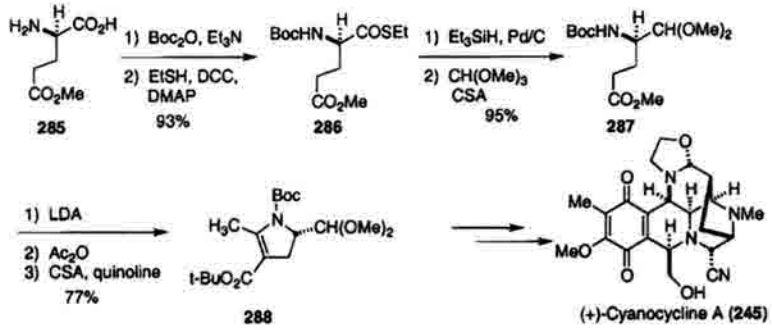
Selective reduction of the oxime followed by a Pictet-Spengler cyclization afforded tetracycle **282** in 66% yield. Reprotection of the phenol was followed by a two-step sequence to convert the methyl ester to an aldehyde which cyclized on the amine to form the corresponding carbinolamine. Conversion 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



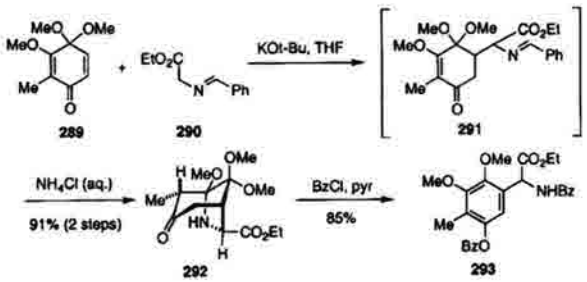
Scheme 43. Interconversion of Naphthyridinomycin and Cyanocycline A

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,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

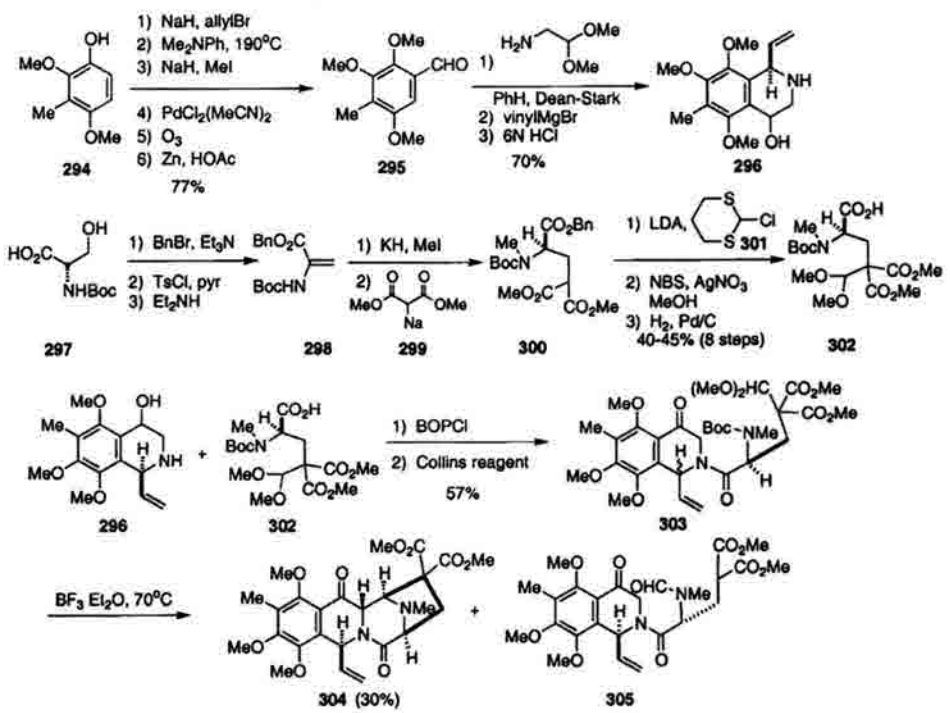
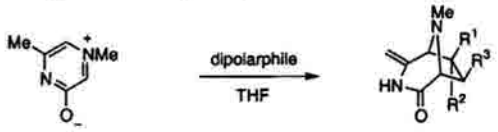


Table 8. Intermolecular 1,3-Dipolar Cycloadditions on 306 Using Various Dipolarphiles



306 307

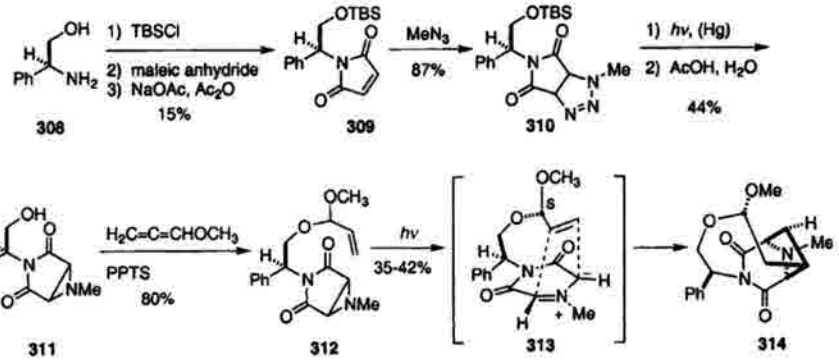
dipolarophile	temp/time	product (yield)
methyl acrylate	reflux/1 h	R¹) CO_2Me, R²) R³) H (51%)
acrylonitrile	20 °C/3 h	R¹) CN, R²) R³) H (25%)
diethyl maleate	reflux/2.5 h	R¹) R³) CO_2Me, R²) H (35%)

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.

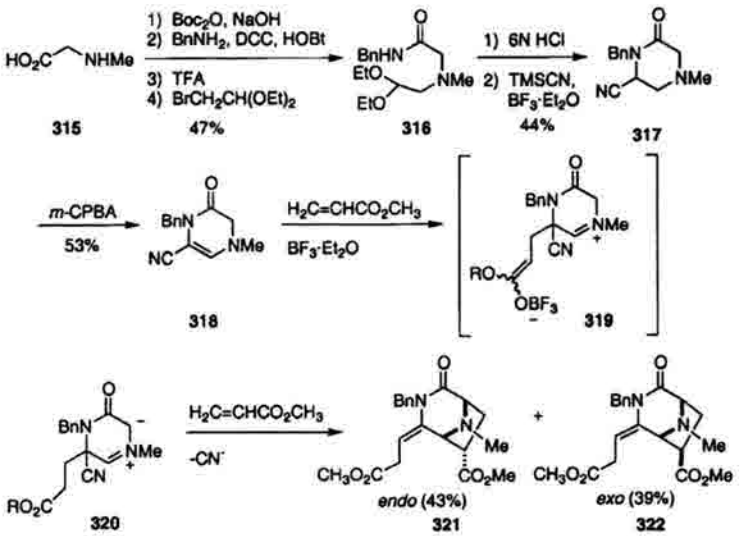
Garner et al. also published a strategy toward the synthesis of naphthyridinomycin via a 1,3-dipolar cycloaddition.[113] The dipolar species was generated by irradiation of aziridine **312** as outlined in Scheme 46, and good results were obtained when the cycloaddition was intramolecular. Intermolecular 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,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.

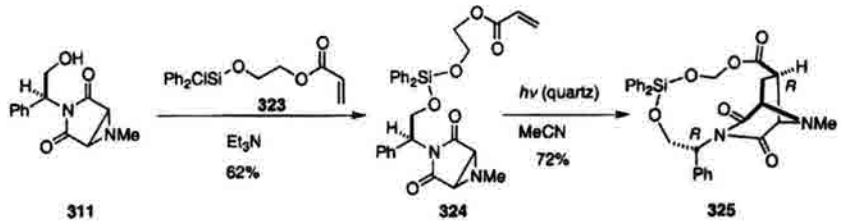
Scheme 46. Garner's Intramolecular 1,3-Dipolar Cycloadditions



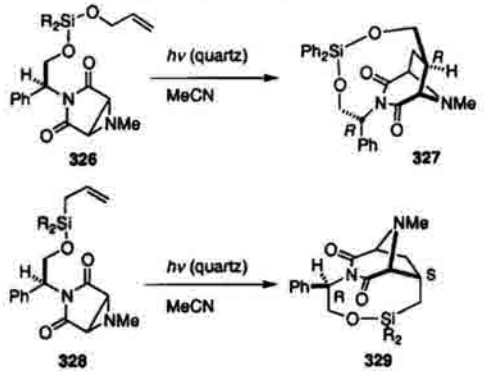
Scheme 47. Garner's Tandem Michael Additions/1,3-Dipolar Cycloadditions



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 R_2.[116] The approach involves a sequential Staudinger reaction followed by a Pictet-Spengler reaction. The efficient synthesis of this -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 -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

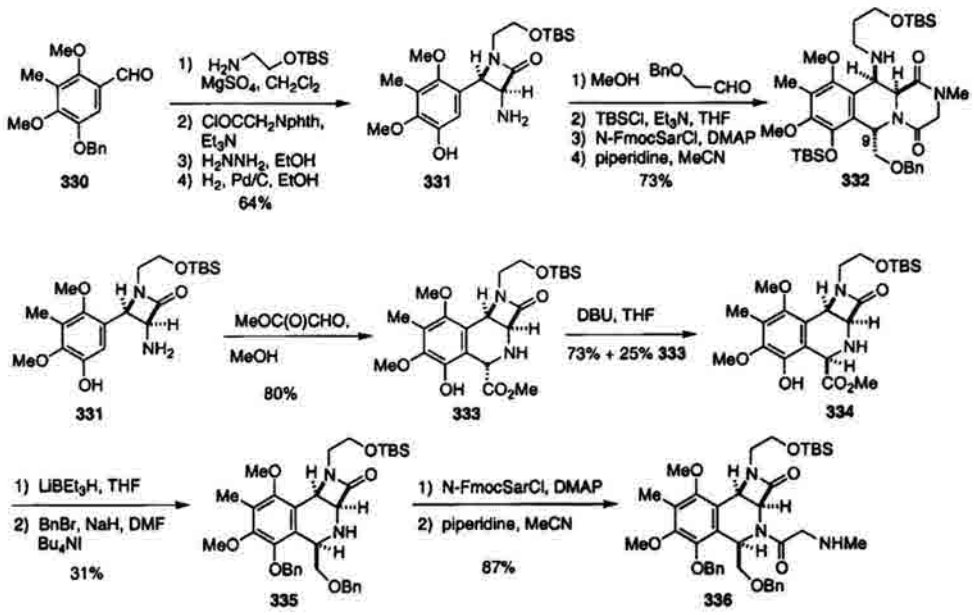
Naphthyridinomycin has potent antibiotic activity against both Gram-(-) and Gram-(+) bacteria.[94] Incorporation of ^{14}C-thymidine during DNA synthesis was inhibited by naphthyridinomycin in *E. coli* at low concentrations.[117] At higher concentrations, RNA and protein synthesis were also inhibited 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.

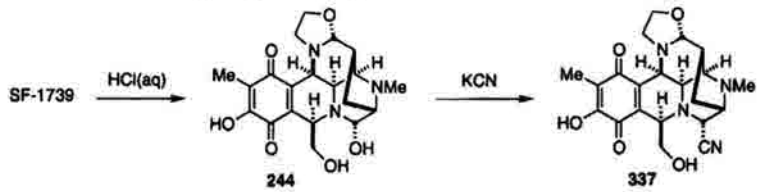
In experiments to determine the sequence specificity of DNA alkylation using poly(dG)-poly(dC) and poly(dA)-poly(dT) polydeoxyribonucleic acids, it was found that naphthyridinomycin binds preferentially to GC-rich regions. Substitution of inosine for guanine resulted in no detectable alkylation, suggesting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

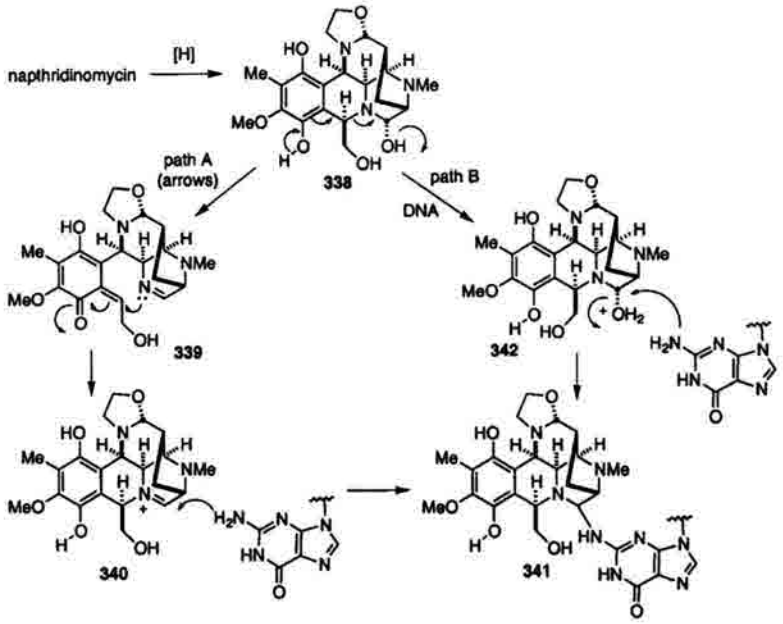
These two mechanisms were used to rationalize the two rates of alkylation. It was shown that the addition of SDS or Na^+ ions did not hinder the alkylation of DNA by naphthyridinomycin. These data were interpreted to infer that naphthyridinomycin does not chelate to DNA due to the presumption that naphthyridinomycin 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 R_2 displays excellent antimicrobial activity against Gram-(+) bacteria.[102a,123] The antimicrobial data for bioxalomycin R_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 R_2 inhibited DNA synthesis drastically. However, both RNA synthesis and protein synthesis were inhibited to a more significant extent.

It was later shown by Williams and Herberich that bioxalomycin R_2 does indeed cross-link duplex DNA.[124]

Scheme 53. Alternate Mechanism Proposed for DNA Alkylation by Naphthyridinomycin

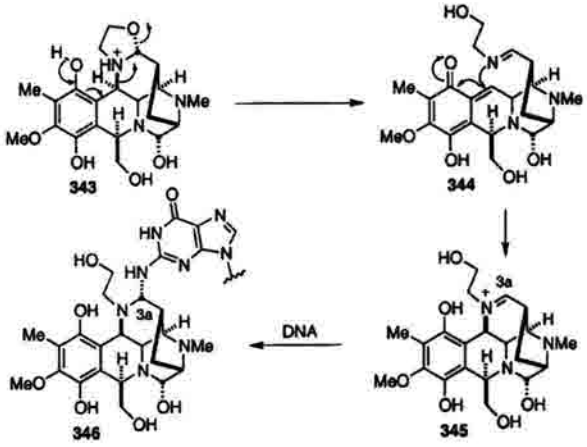


Table 9. Antimicrobial Activity of Bioxalomycin R_2 against Gram-(+) Isolates[119]

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*.

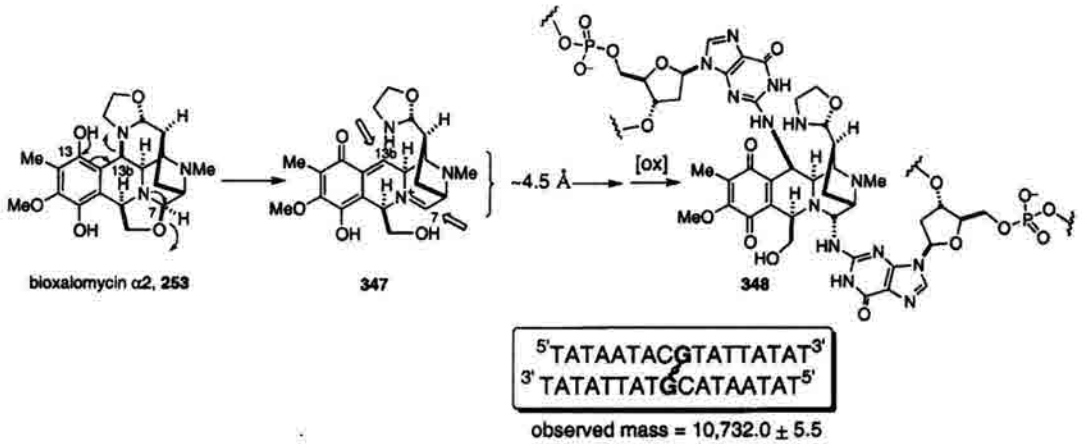


Figure 13. Interstrand cross-linking of DNA by bioxalomycin R_2 observed by Williams.

It was also noted that cyanocycline A formed DNA cross-links in low yield and only in the presence of DTT. Bioxalomycin R_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 R_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 R_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 R_2-mediated cross-link has not yet been established.

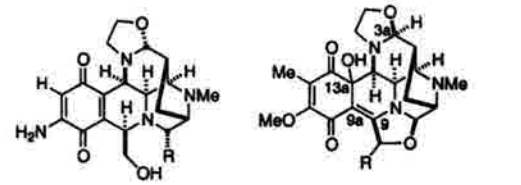


Figure 14. The Dnacins and aclindomycins.

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

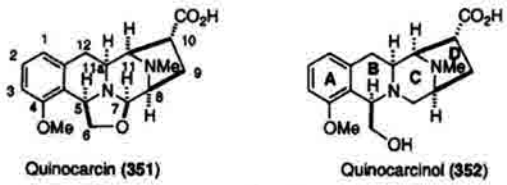


Figure 15. Quinocarcin and quinocarcinol.

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

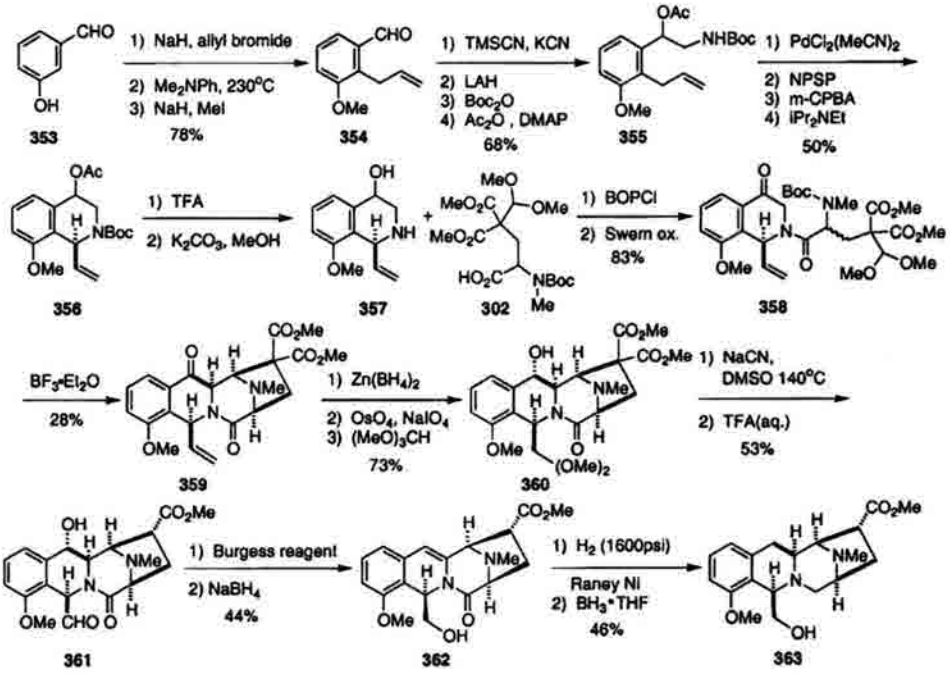
4.1.1. Isolation and Structure Determination

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]

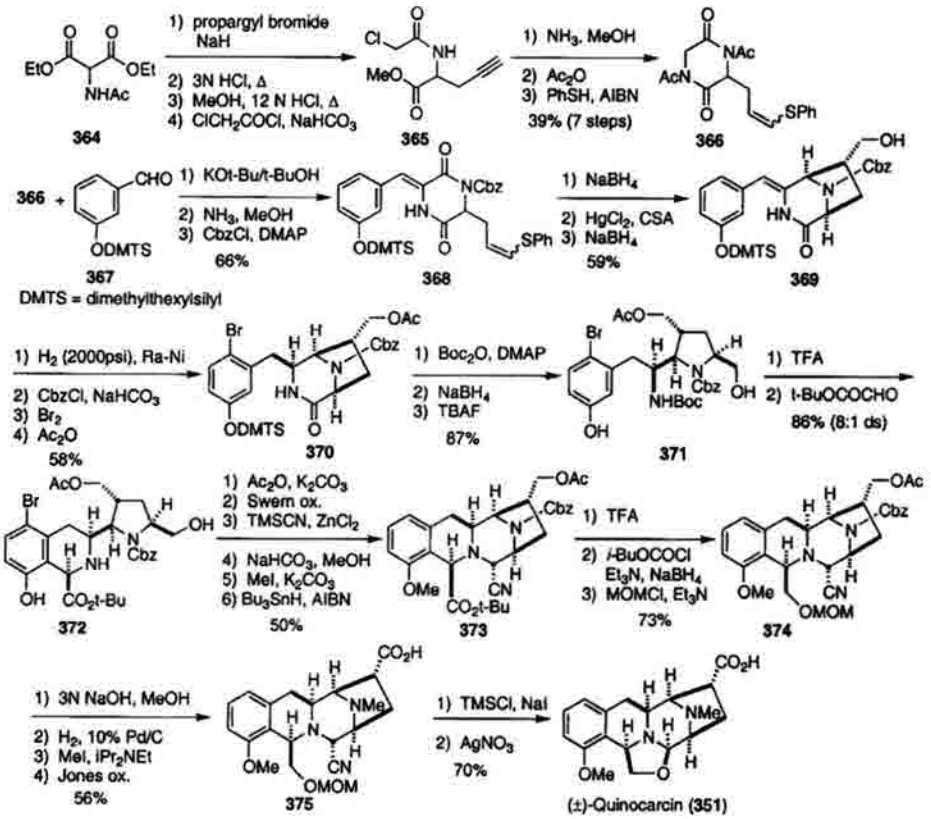
4.1.2. Total Syntheses of Quinocarcin, Quinocarcinol, and Quinocarcinamide

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

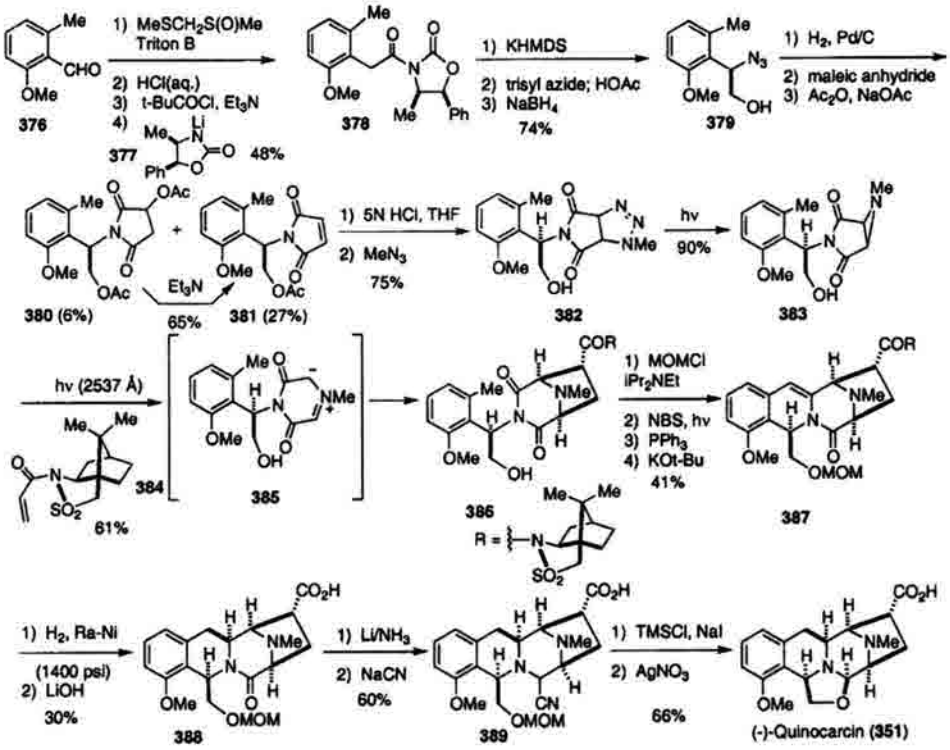


BA and Hunig's base. Removal of the Boc and acetate protecting groups afforded the secondary amine **357**, which was coupled to amino acid **302** using BOPCl. Swern oxidation provided ketone **358** as a 1:1 mixture of diastereomers. Treatment with BF_3,Et_2O afforded tetracycle **359** in 28% yield 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.

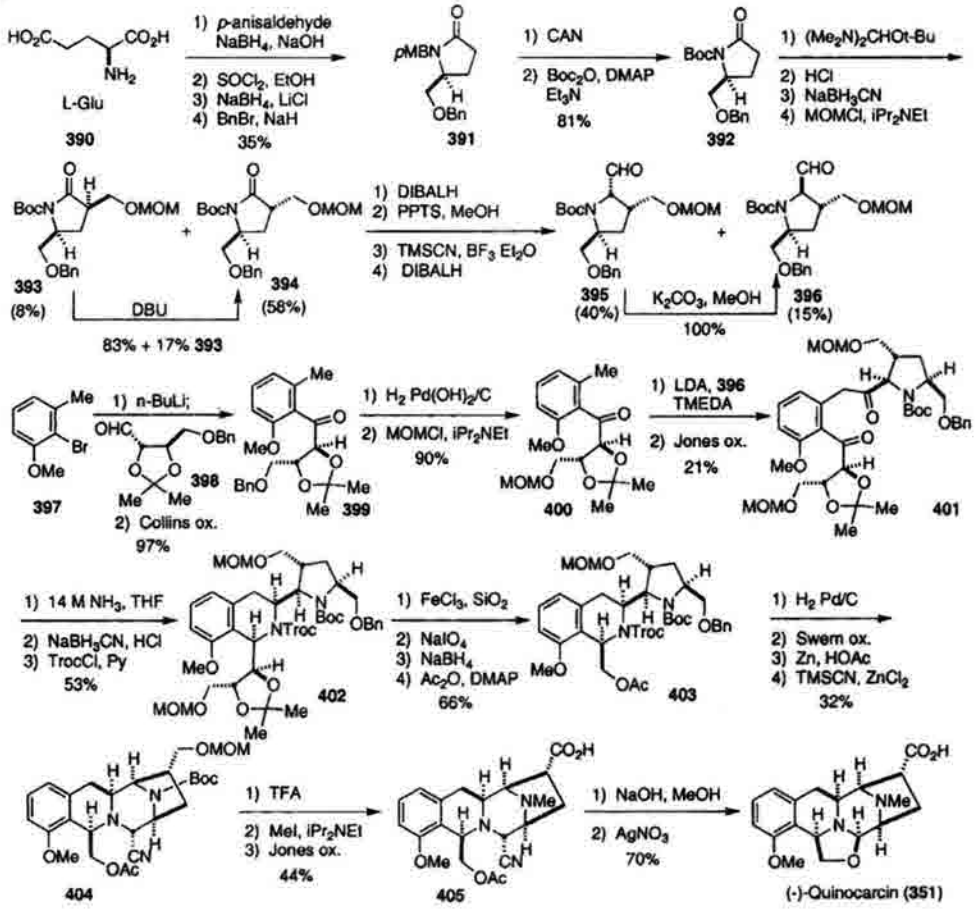
In 1992, Garner et al. published the first asymmetric synthesis of (-)-quinocarcin.[131] The key step involved an intermolecular 1,3-dipolar cycloaddition as shown in Scheme 56.[131,134] Starting with aldehyde **376**, treatment with methyl methylsulfinylmethyl sulfide in the presence of Triton B and 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 -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



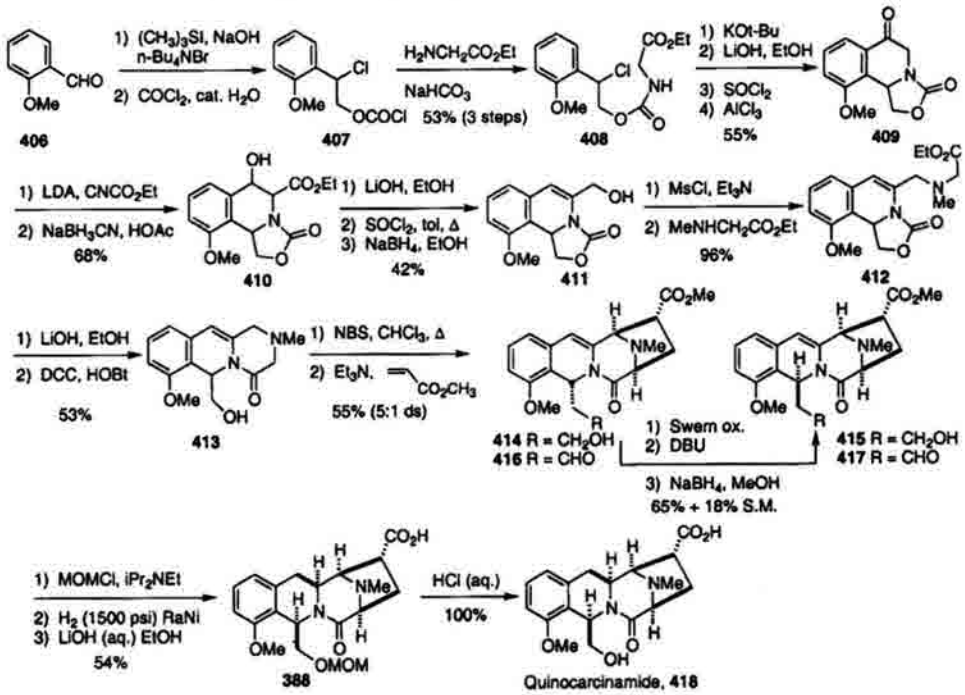
undesired diastereomer **395** could be epimerized in quantitative yield to **396**.

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]

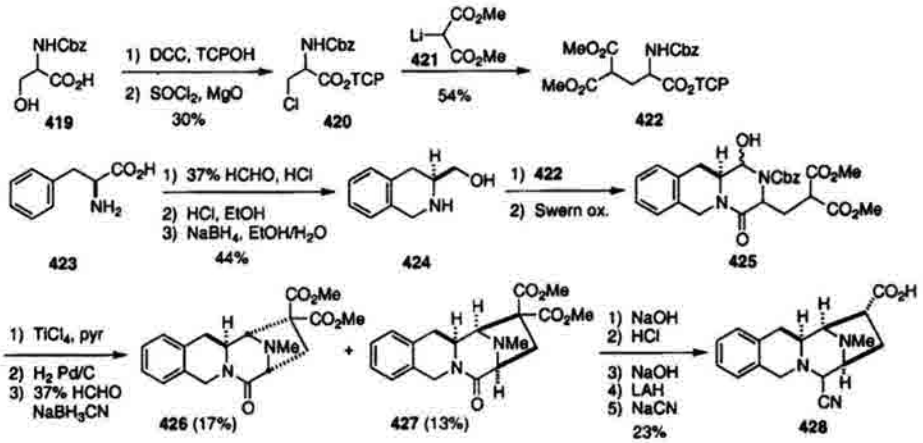
In 1995, Flanagan and Williams published the synthesis of (±)-quinocarcinamide (**418**).[136] A late stage intermediate **388** intersected Garner's total synthesis of quinocarcin, thus making this a formal total synthesis of D,L-quinocarcin. The key step in this synthesis was an intermolecular azomethine 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 -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

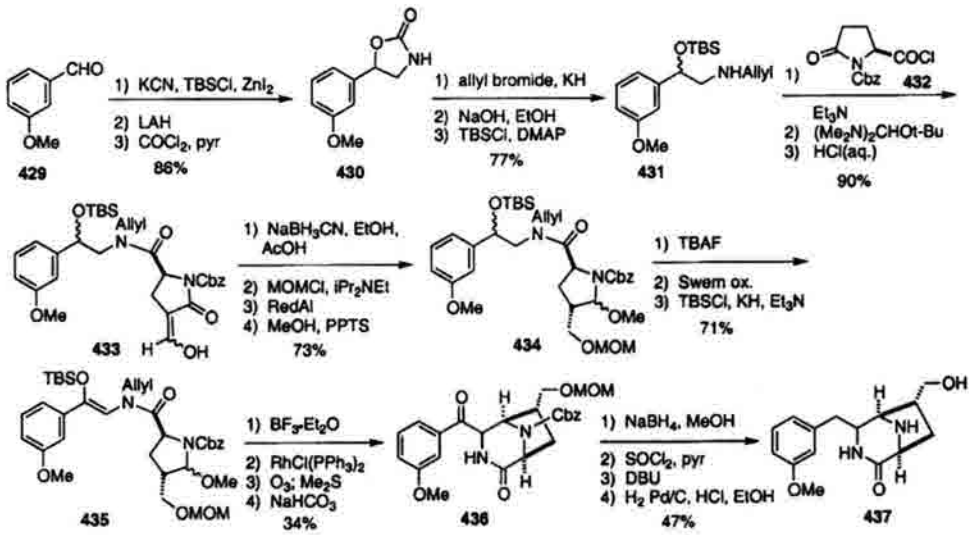


by conversion to the R, -unsaturated acid chloride and finally reduction of the acid chloride. Formation of the allylic chloride was followed by treatment with sarcosine ethyl ester to afford the allylic amine **412**. Hydrolysis of the oxazolidinone was followed by coupling of the secondary amine upon 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.

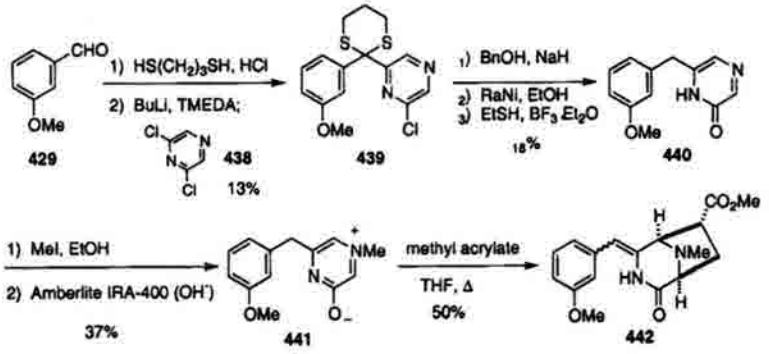
4.1.3. Synthetic Studies toward Quinocarcin

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

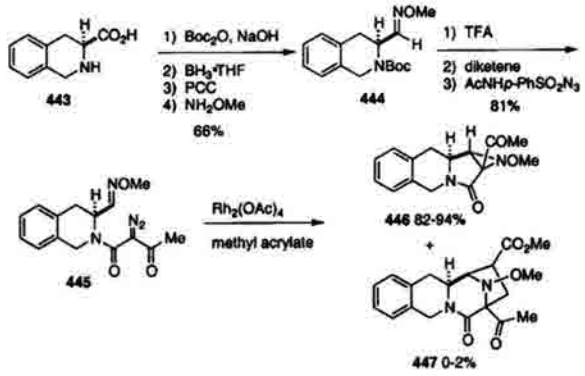


ester reduction to afford the amino alcohol **424**. Coupling with the active ester **422** followed by Swern oxidation yielded the carbinolamine **425**. Cyclization to yield the tetracyclic core was accomplished using titanium tetrachloride. Subsequent Cbz removal and *N*-methylation afforded the two 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, 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 R-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**.

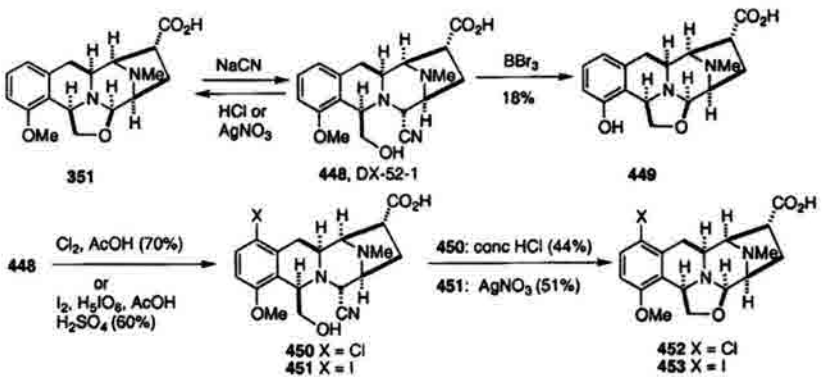
Several C-1 (quinocarcin numbering) analogues were prepared starting from DX52-1 (**448**) (Scheme 65). The formyl group was introduced using dichloromethyl methyl ether to afford **464** and **465**. Treatment with hydroxylamine hydrochloride provided oximes **466** and **467**. The C-1 cyano derivatives **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**.

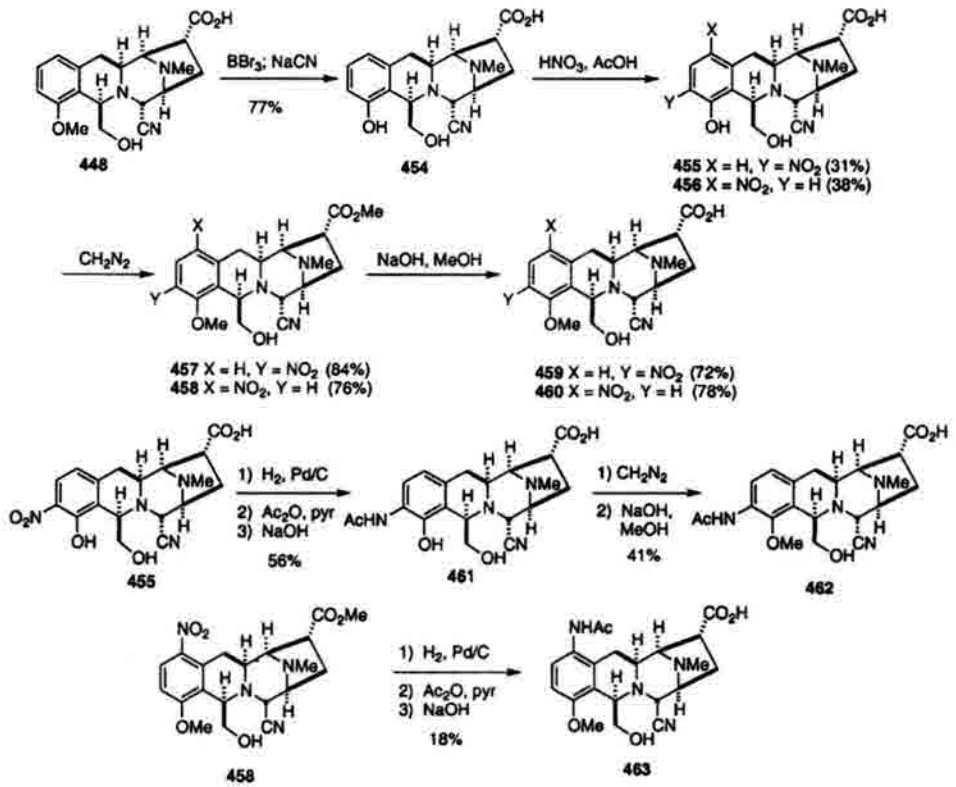
Several other quinone-containing analogues were synthesized via quinone substitution (Scheme 67). Dimethylaniline derivatives **487** and **488** were synthesized via copper-catalyzed addition of dimethylamine to **476**. Copper-catalyzed addition of methanol to **478** afforded **489** in 39% yield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

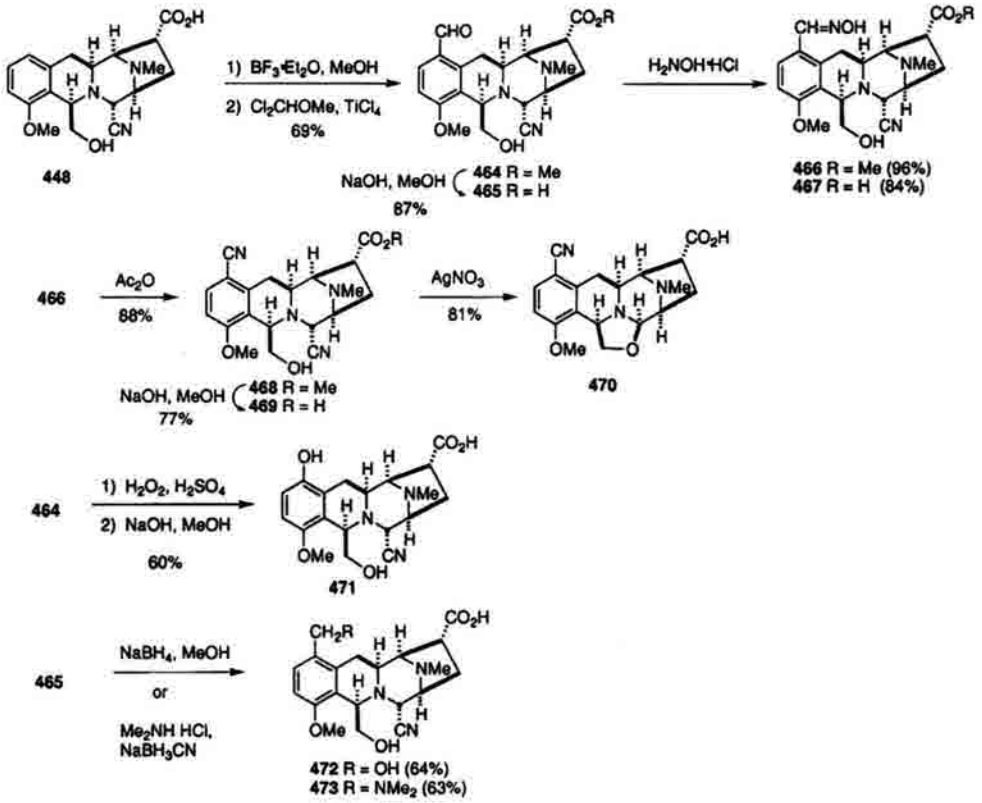
In an effort to probe the importance of the relative stereochemistry of the quinocarcin ring structure, two diastereomeric tetracyclic analogues were synthesized by Williams et al., as shown in Scheme 70.[143] Starting with tricyclic compound **410** (see Scheme 58), the ethyl ester was saponified followed by treatment with thionyl chloride in refluxing benzene to afford the corresponding R, -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**.

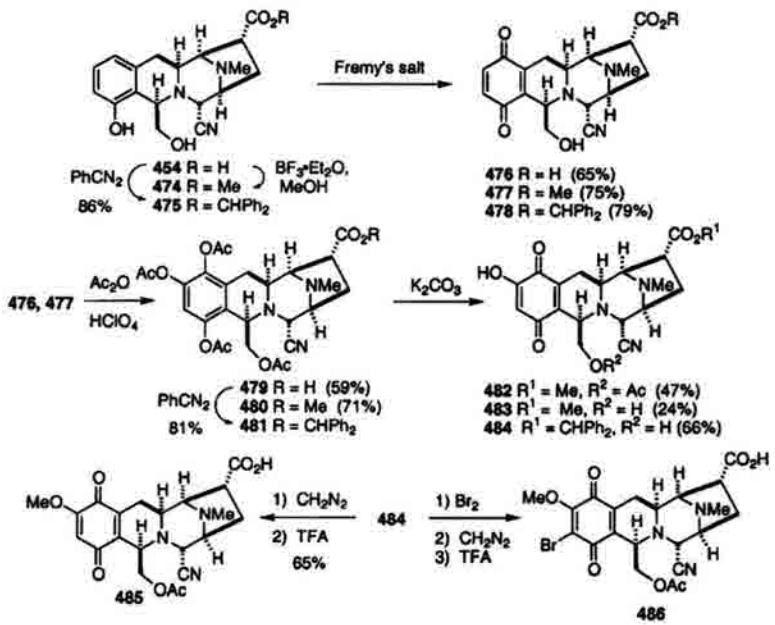
Tetracycle **528** was activated as the corresponding *p*-nitrophenyl ester (**529**) and coupled to the netropsin-like side chain (**530**), a well-known DNA-binding moiety, to afford the water-soluble netropsin conjugate **531** (Scheme 72). In addition, these workers prepared a symmetrical dimer by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



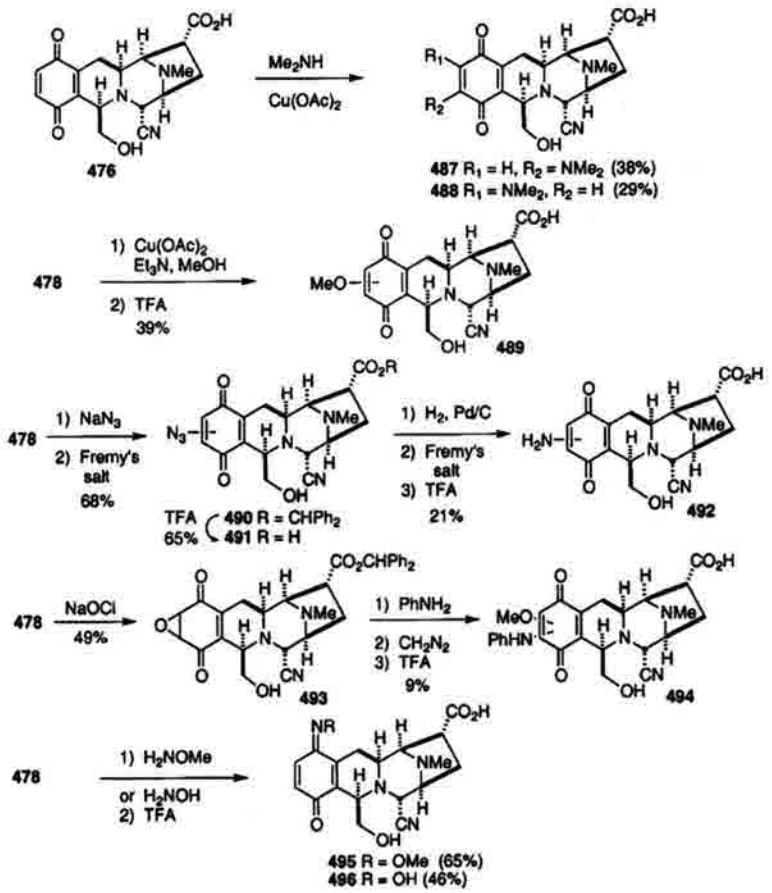
Scheme 66. Quinone Analogs of Quinocarcin



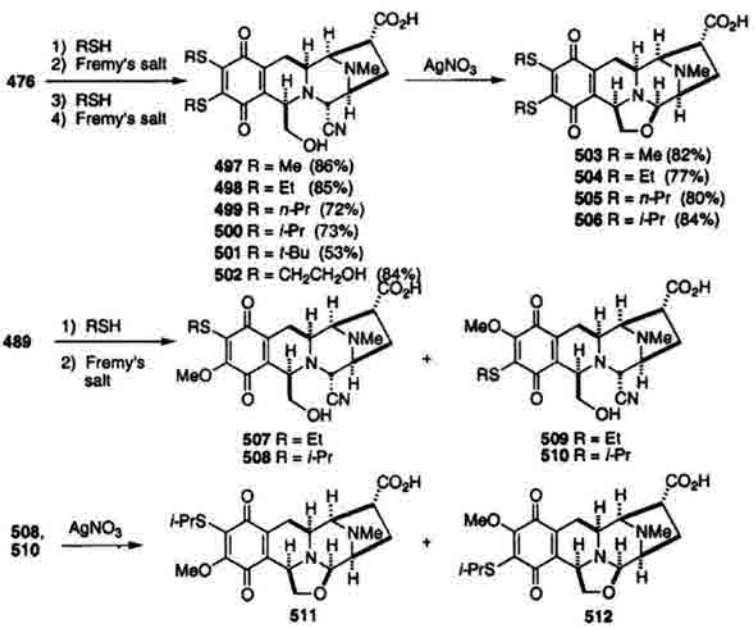
As in the case of the *syn*-analogue **528**, the *anti*-analogue **539** was coupled to netropsin to afford **541** as outlined in Scheme 74. Additionally, **539** was demethylated using BBr_3 to afford the phenol **542.**

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



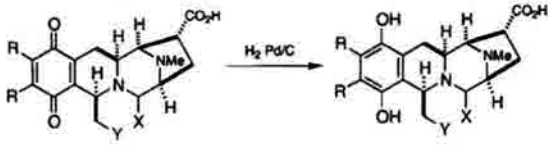
Scheme 68. Thiol-Substituted Quinone Analogs of Quinocarcin



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.

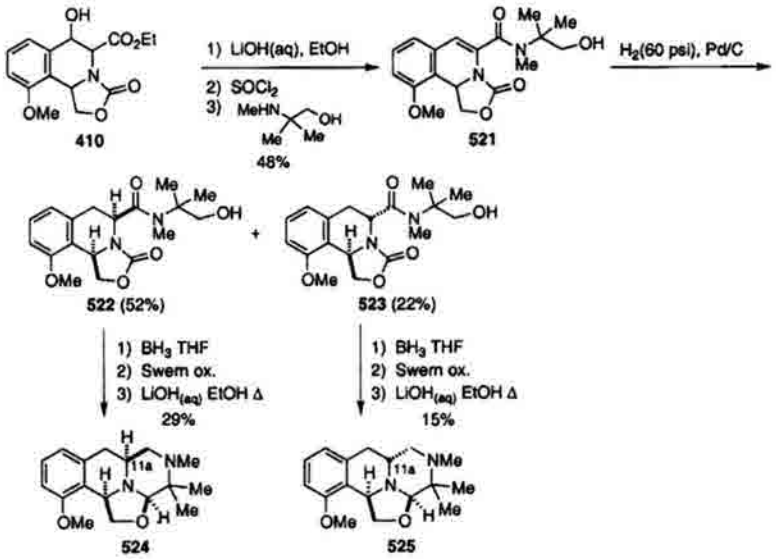
In the next section, the biochemical and biological activity of many of the quinocarcin analogues described above will be reviewed. An attempt will be made to provide mechanistic insight and speculation where appropriate.

4.1.5. Biological Activity

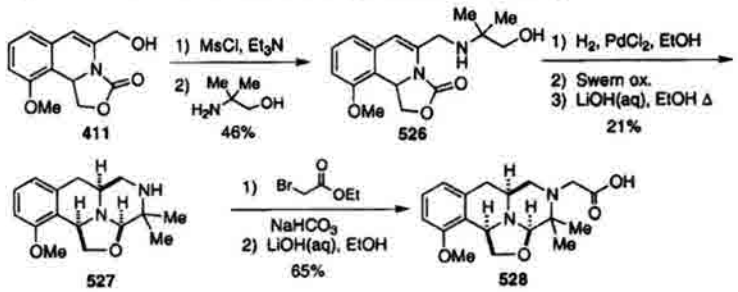
Quinocarcin has moderate activity against Gram-(+) bacteria such as *Staphylococcus aureus, Bacillus subtitlis,* and *Klebsiella pneumoniae* with MIC's of 12.5, 12.5, and 25 μg/mL, respectively.[129a] Quinocarcin has been shown to inhibit [^{3}H]-thymidine incorporation in *Bacillus subtilis,* and this 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-

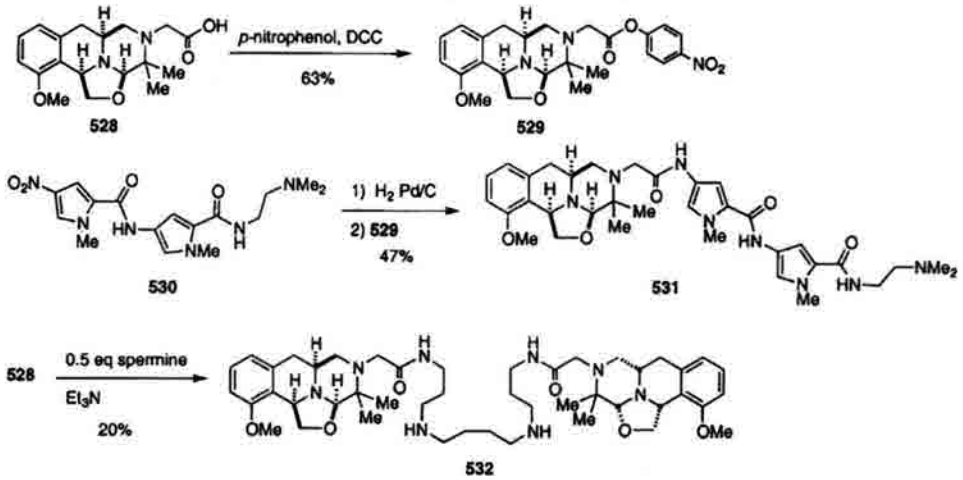
Scheme 70. Williams' Synthesis of Tetracyclic syn- and *anti*-Analogues of Quinocarcin



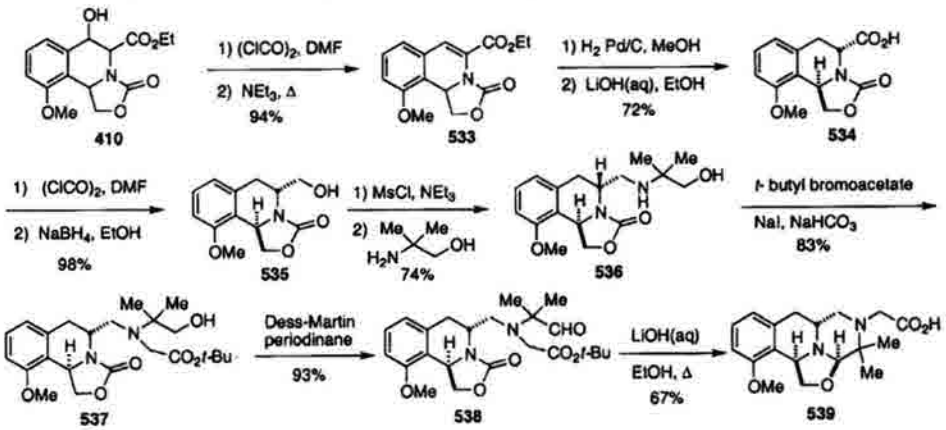
Scheme 71. Williams' Synthesis of a Water-Soluble Quinocarcin Analog



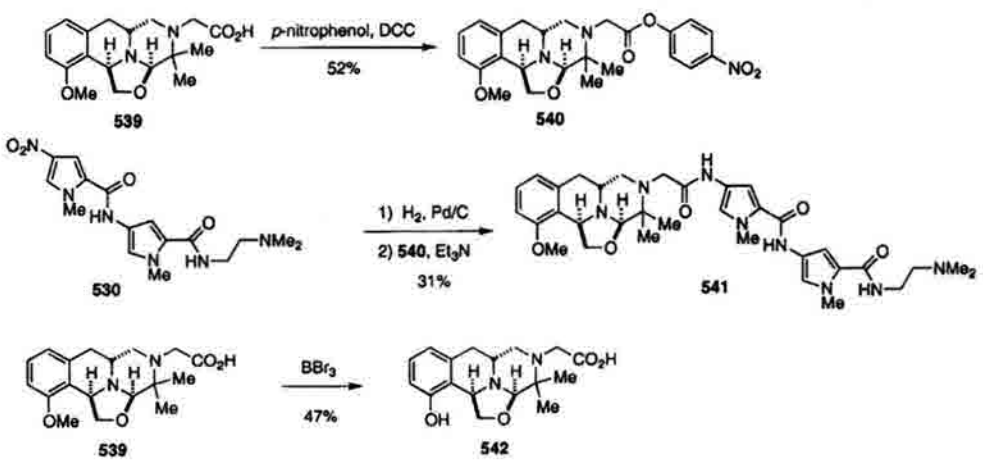
Scheme 72. Williams' Synthesis of Netropsin and Spermine Analogs 531 and 532



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



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

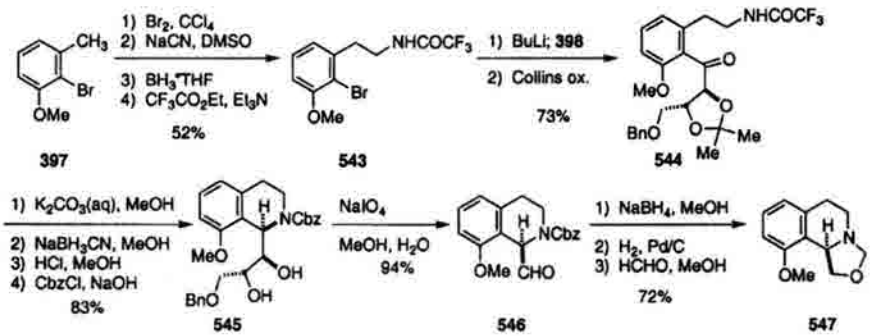


mia, quinocarcin was shown to inhibit RNA synthesis over DNA and protein synthesis.

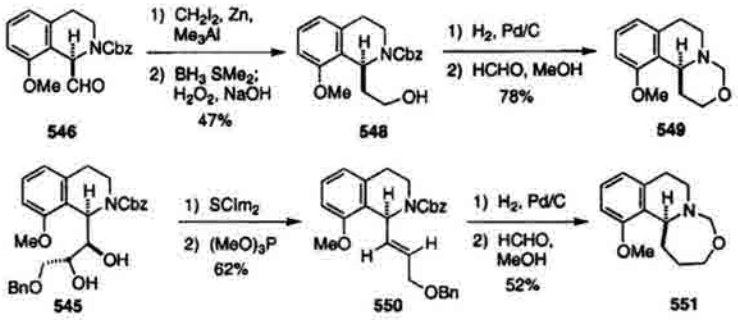
Quinocarcin citrate and DX-52-1 (**448**) were assayed by the National Cancer Institute in a screen of 60 tumor cell lines.[149] Both showed promising activity with DX-52-1 showing excellent activity against several melanoma cell lines. Quinocarcin citrate had been in clinical trials in Japan, but due 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-

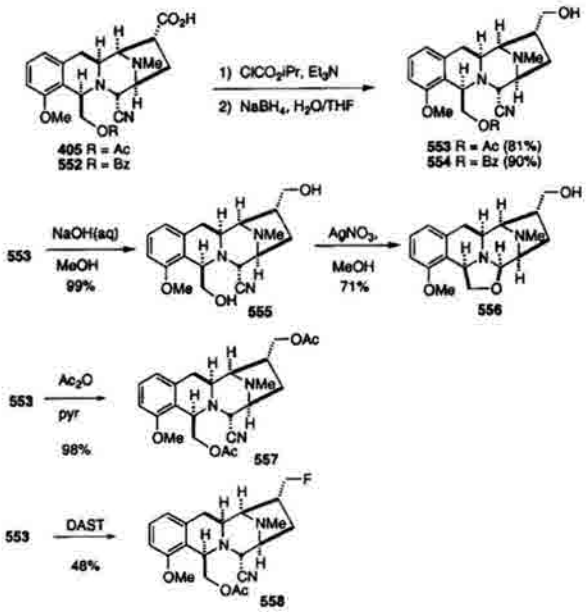
Scheme 75. Terashima's Synthesis of an ABE-Ring Analog of Quinocarcin



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

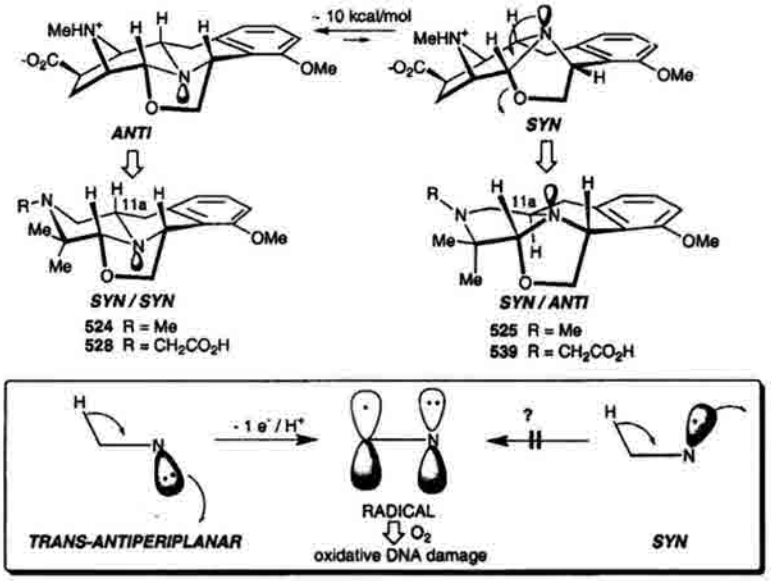


Figure 16. Importance of the stereochemistry at nitrogen of quinocarcin and analogues.

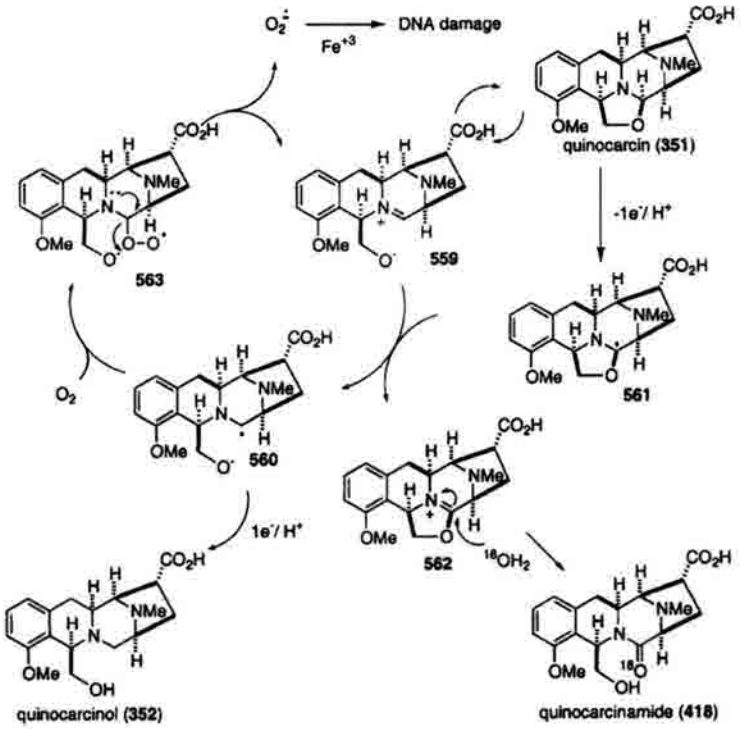
the nonbonded electron pair on nitrogen relative to the methine hydrogen. These workers performed extensive kinetic and mechanistic studies on this new reaction and found that the rate of superoxide formation for quinocarcin is well below (10^4- 10^5 times slower) the rate-limiting step in the Fenton/Haber-Weiss 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

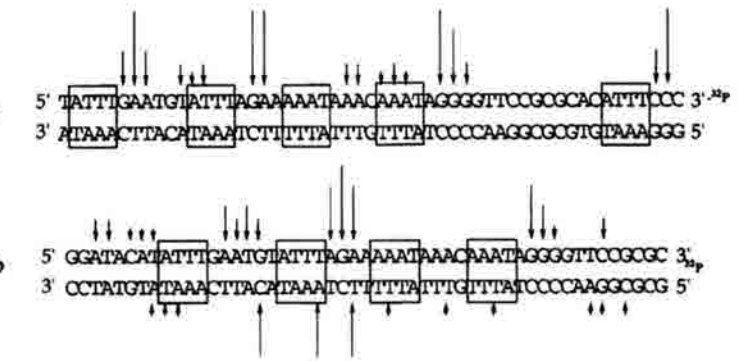


Figure 17. Histograms from gels in ref 140 depicting the selective DNA cleavage exhibited by **531**. (a) Histogram from Figure 6a, lane 5, ref 140. (b) Histogram from Figure 6b, lanes 5 and 6, ref 140. Histograms were prepared by measurements of the relative intensities of DNA bands from the autoradiograms.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

Scheme 79. Remers' Proposed DNA Alkylation by Quinocarcin

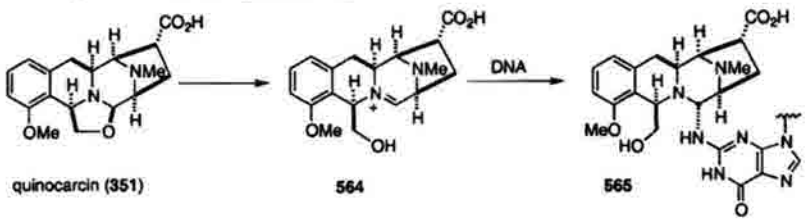
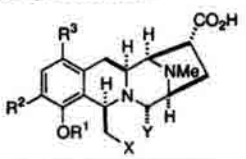


Table 11. In Vivo Studies for A-Ring Analogs of Quinocarcin[a]



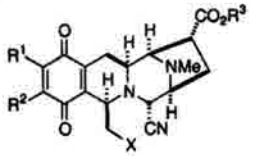
analog	R^1	R^2	R^3	X	Y	HeLaS$_3$ IC_{50}(μg/mL)	dose (mg/kg) x1 (P388)	ILS (%)	(R)
448	Me	H	H	OH	CN	0.05	20	26	0.59
449	H	H	H	-O-	-O-	3.03	6.25	14	0.35
454	H	H	H	OH	CN	5.32	3.13	18	0.43
450	Me	H	Cl	OH	CN	0.042	12.5	23	0.79
451	Me	H	Cl	-O-	-O-	0.04	12.5	40	0.93
452	Me	H	I	OH	CN	0.11	50	31	1.15
453	Me	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

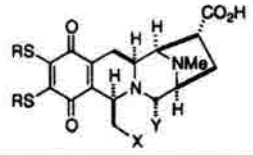
Table 12. In Vivo Studies for Quinone Analogs of Quinocarcin[a]



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.

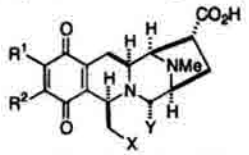
The quinone analogues, for the most part, showed reduced biological activities relative to that for quinocarcin (Table 12).[142b] The unsubstituted (**462**) and diamino (**473**) derivatives showed the best activities.

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**).

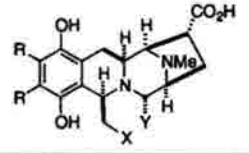
Table 14. In Vivo Studies of Thiol-Substituted Quinone Analogs of Quinocarcin[a]



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}		

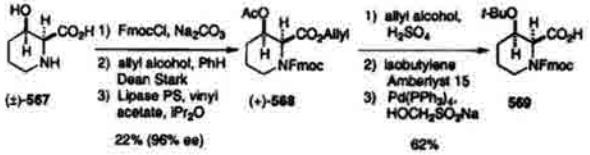
4.2. Tetrazomine

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

Figure 18. Structure of tetrazomine.

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

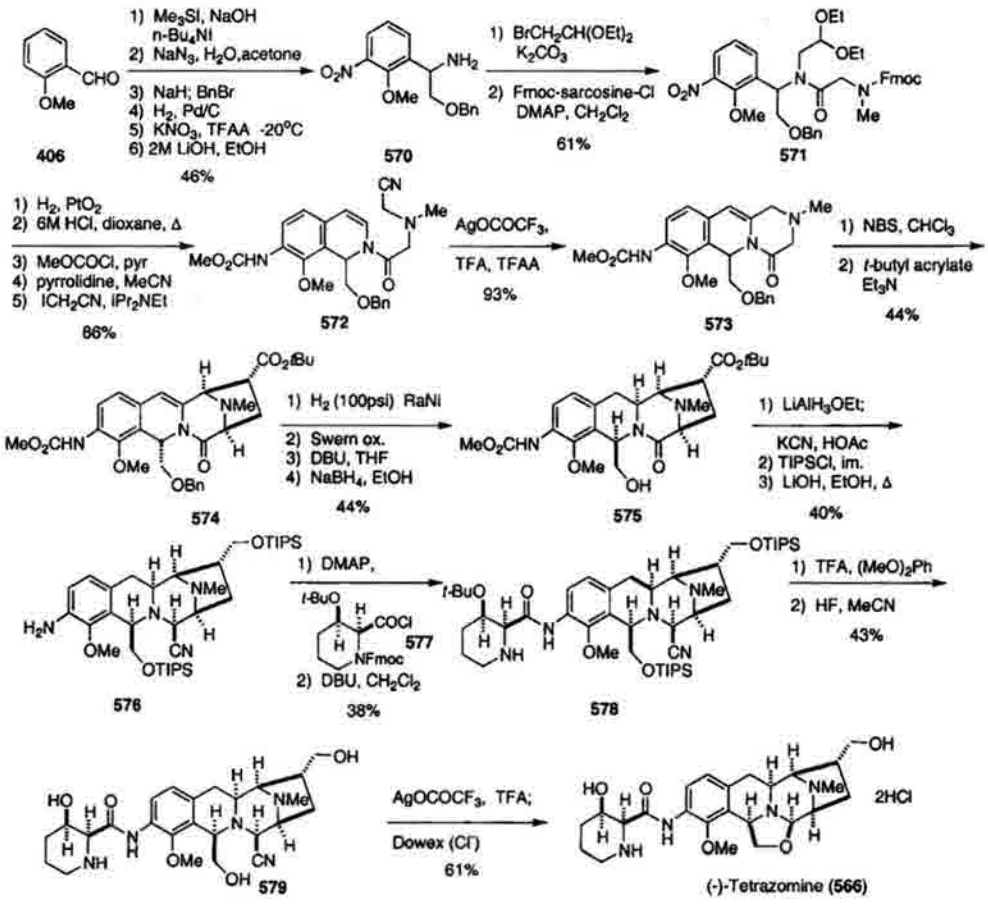


was determined by Williams et al. in 1998 as described below.[154] The total synthesis of (-)-tetrazomine by Scott and Williams in 2001 secured both the relative and absolute stereochemistry of the natural product and is that depicted in Figure 18.[155]

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



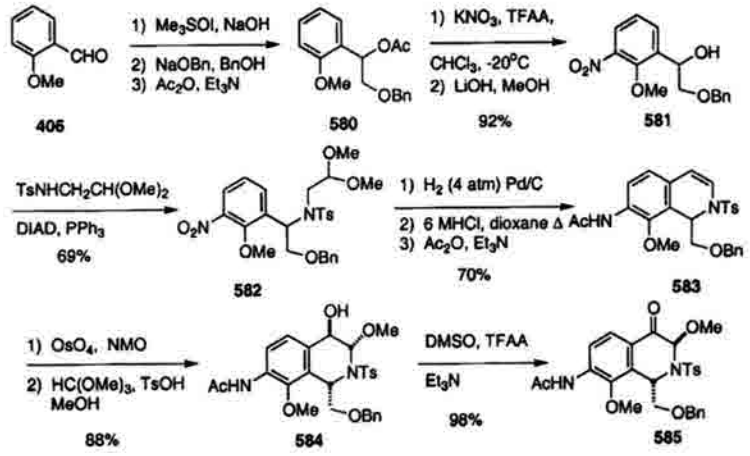
similar to that used in the quinocarcin synthesis reported by Williams and Flanagan.[136]

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

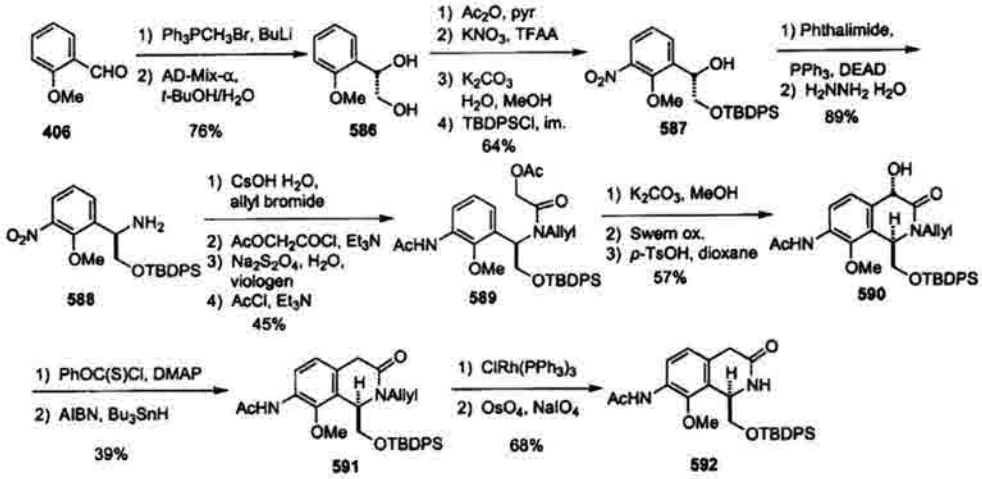
The synthesis of the tetrahydroisoquinoline core of tetrazomine started with *o*-anisaldehyde (**406**) as shown in Scheme 81. Treatment of *o*-anisaldehyde with trimethylsulfonium iodide under phase-transfer conditions provided the epoxide, which was subjected to regioselective ring opening with sodium 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



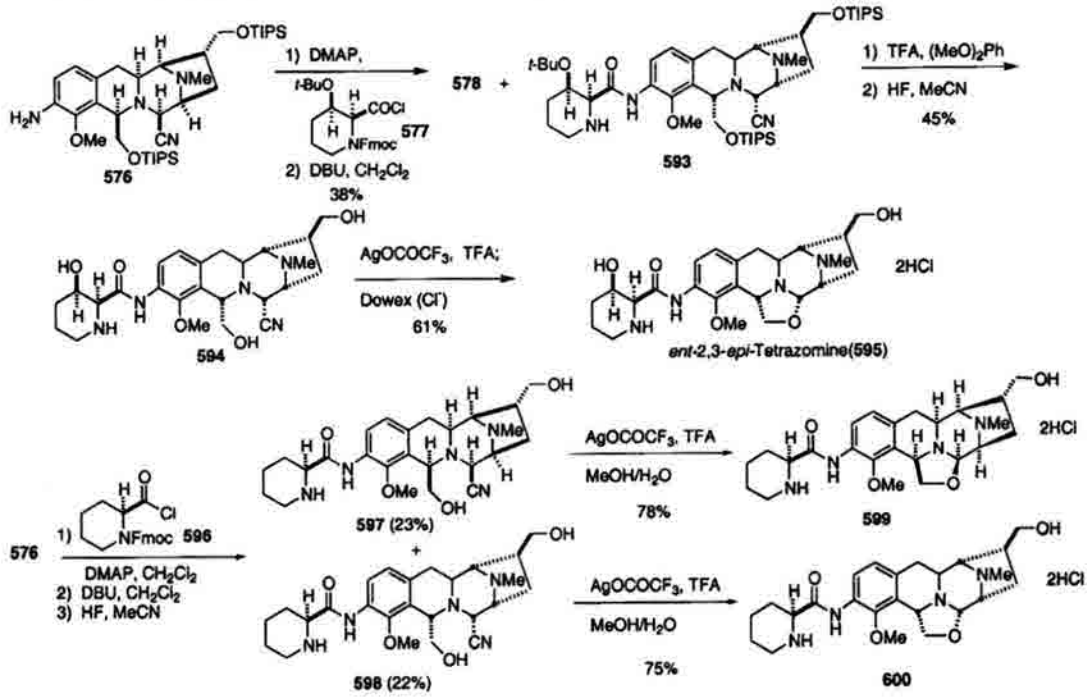
OEt in THF at 0 °C. The resultant carbinolamine was trapped with sodium cyanide under acidic conditions to afford the corresponding stable amino nitrile. The two primary alcohols were protected as their triisopropylsilyl ethers, and the methyl carbamate was hydrolyzed to afford aniline **576**. The optically 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

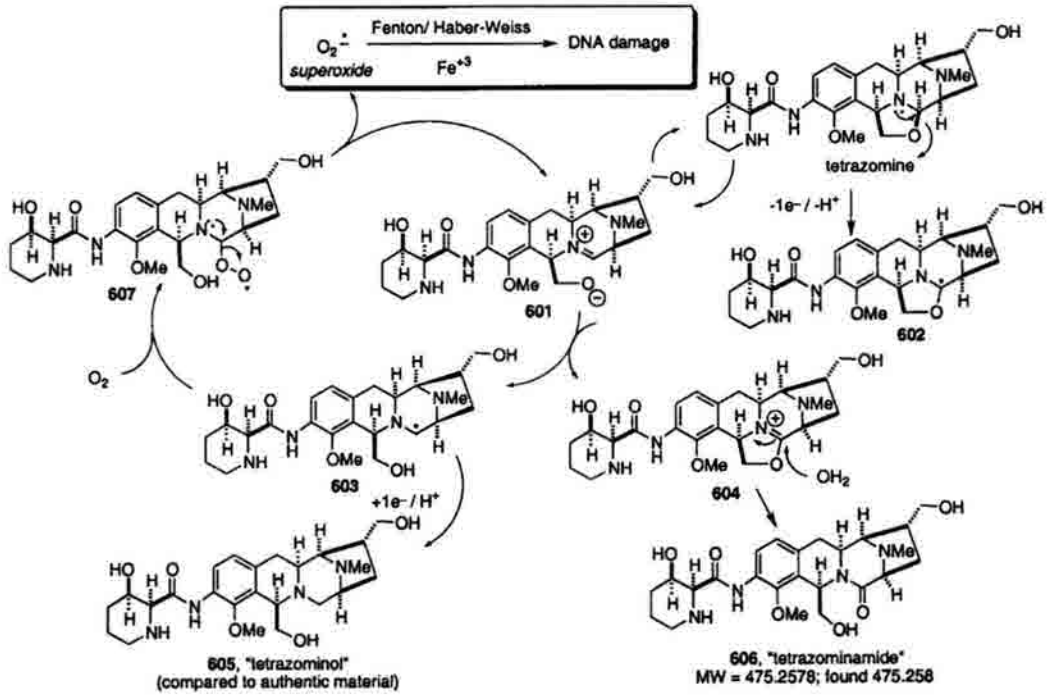


Table 19. Rates of Superoxide Formation for Bioxalomycin r_2, Tetrazomine, Quinocarcin, and Analogs

substratejl	concentration (mM)	pH	rate ($M\ s^{-1} \times 10^{-9}$)
bioxalomycin R_2	0.1	6.0	7.59
bioxalomycin R_2	0.1	7.0	38.8
bioxalomycin R_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 R_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 R_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

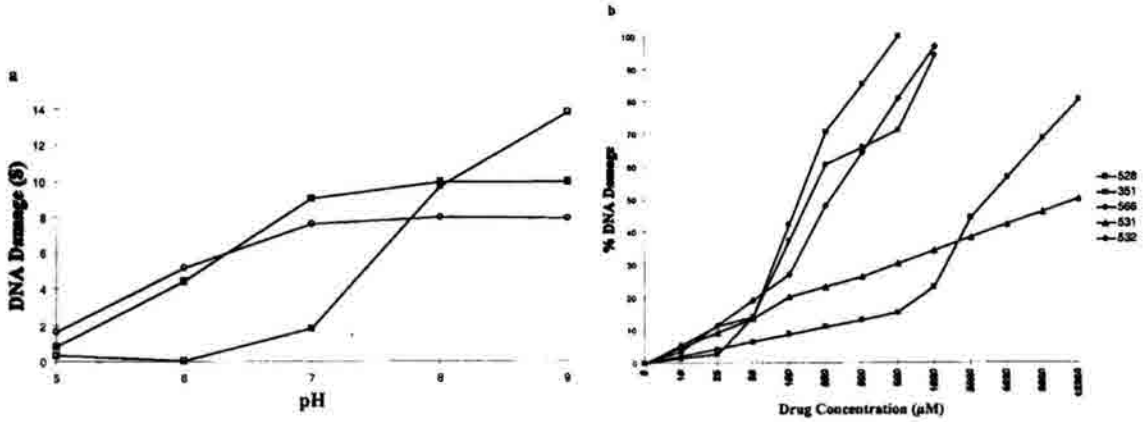


Figure 19. (a) Effects of pH on DNA cleavage (S) for **528** x 10 (○), quinocarcin (□), and tetrazomine (■). (b) Effects of concentration on DNA cleavage for **528**, quinocarcin (**351**), tetrazomine, **531**, and **532**.[141]

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	

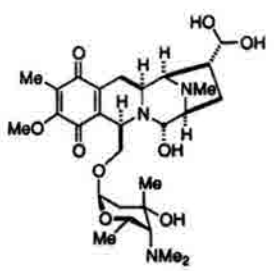
Table 21. Antimicrobial Activity of Tetrazomine Oxazolidine Analogs against *Klebsiella pneumoniae* and *Staphylococcus aureous*: R) Resistant

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

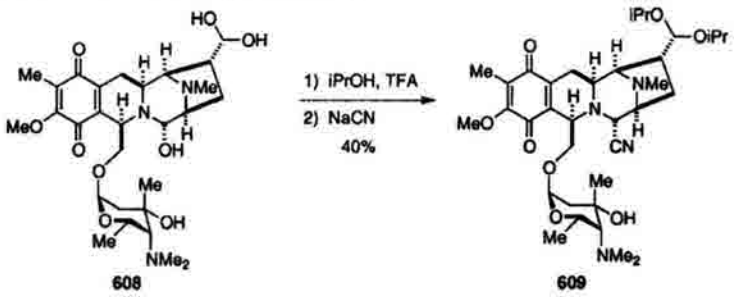


Table 23. Antimicrobial Activities of Lemonomycin

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.

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