DISSERTATION

SYNTHETIC AND PHARMACOPHORIC STUDIES OF QUINOCARCIN.

submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall, 1990

COLORADO STATE UNIVERSITY

September 17, 1990

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY **PAUL P. EHRLICH** ENTITLED *SYNTHETIC AND PHARMACOPHORIC STUDIES OF QUINOCARCIN* BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Adviser

Department Head

ABSTRACT

SYNTHETIC AND PHARMACOPHORIC STUDIES OF QUINOCARCIN.

A new synthetic approach to the stereoselective total synthesis of the structurally unique antitumor antibiotic quinocarcin (**1**) is described. The utilization of model studies in this approach has lead to novel methodologies concerning the construction of 1-(hydroxymethyl)-8-methoxy-1,2,3,4-tetrahydro-isoquinolin-4-one (**195**) and several variably substituted pyrrolidines (**180**, **181**, **182** and **183**). These methodologies are discussed in terms of their synthetic utility as well as their mechanistic aspects.

The synthetic approach to quinocarcin described herein allowed for the construction of several oxazolidine containing alkaloids which incorporate various aspects of the 8-11-iminoazepinotetrahydroiso-quinoline skeleton of quinocarcin. To this end the synthesis of a new tetracyclic oxazolidine moiety (**240**), which mimics quinocarcin's DNA nicking capabilities and represents the isolation of the pharmacophore of this novel antibiotic was achieved. The significance of the chemical stability and biological activity of **240** relative to quinocarcin is discussed.

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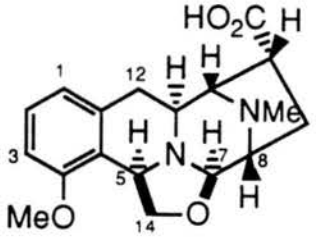
CHAPTER 1

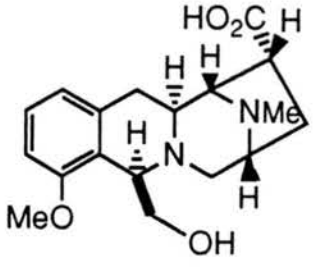
INTRODUCTION

Quinocarcin (**1**) is a novel antitumor antibiotic isolated by Tomita and coworkers from a new organism named *Streptomyces melanovinaceus*.[1] Quinocarcinol (**2**), was isolated from the same cell culture filtrates which, like quinocarcin, has the novel 8,11-iminoazepinotetrahydroisoquinoline skeleton, but lacks the oxygenation at C-7. However quinocarcinol lacks the antitumor activity that quinocarcin possess and is only a weak antibiotic (approximately 1000 times less active). With the above information in mind it seems obvious that the pharmacophore of quinocarcin is the C-5, C-7 fused oxazolidine moiety.[1,2] (Scheme 1)

Scheme 1



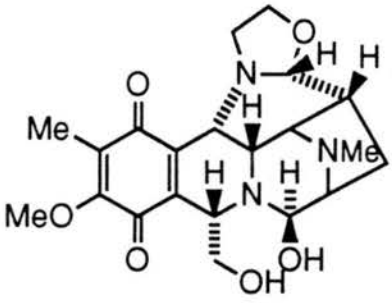
1 Quinocarcin (DC-52)



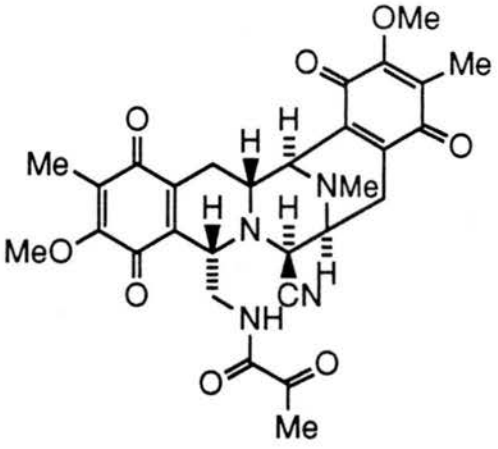
2 Quinocarcinol (DC-52d)

The structural relationship of quinocarcin to other antitumor antibiotics such as the naphthyridinomycin (**3**) and saframycins (**4**) is an interesting one. All three classes contain the oxazolidine (or its oxidative equivalent) moiety at C-5 and C-7 (quinocarcin numbering) (Scheme 2).

Scheme 2



3 Naphthyridinomycin



4 Saframycin A

Both naphthyridinomycin and saframycin A have been of considerable interest synthetically[3] and, in light of their potent antitumor activity, are also of biological interest.[4] These substances are members of the class of quinone-containing antitumor agents for which the quinone moiety has been implicated as an obligatory substructure for biological activity which participates in a $1e^-$ transfer mechanism (to be discussed in more detail in a later chapter) which, in turn reduces O_2 and generates superoxide. Superoxide has been implicated in the cleavage and/or nicking of nucleic acids. Although these antibiotics bear a common

quinone functionality, this does not necessarily confer a commonality of mechanism for DNA nicking and/or cleavage.[5]

Since quinocarcin does not contain this quinone moiety, its mode of action is, at best, speculative. The oxazolidine, as previously mentioned, is all important to its superoxide dependent cleavage of super coiled DNA, (CCC DNA). (There has been no study on the naphthyridomycins or saframycins with respect to their oxidation states at C-7 (quinocarcin numbering).)

Quinocarcin, being the simplest of the piperazine containing antitumor antibiotics, stands at the crossroads of several interesting theories regarding the mode of action of these antitumor agents.[5,6]

Biological Activities and Studies

Initial investigation by Tomita and coworkers[1] showed quinocarcin to have a broad spectrum of antibiotic and antibacterial activity. Quinocarcin has been shown to be moderately active against *Staphlococcus aureus, B. subtilis* and *Klebsiella pneumoniae*, while no activity was observed against Gram-negative bacteria tested. Quinocarcinol was almost devoid of antibiotic activity.

Quinocarcin inhibited the growth of *B. subtilis* at a concentration of 20 μg per ml, and inhibition increased with an increasing amount of the antibiotic. At a concentration of 50 μg/ml, lysis of cells was observed, indicating that quinocarcin acts as a bactericidal antibiotic.

Quinocarcin is effective against mouse lymphocylic leukemia P388 and, at a single injection of 12.5 mg/kg, it inhibited growth of

P388 with 47% ILS (increase of life span). The LD_{50} value of quinocarcin in mice was 27 mg/kg of body weight by intraperitoneal, (i.p.), injection.[1,7]

The effect of quinocarcin on the synthesis of macromolecules in *B. subtilis* was followed by Tomita and coworkers[7] by measuring the incorporation of labeled [methyl-^{3}H]thymidine, [2-^{14}C]uracil and [4,5-^{3}H]-L-leucine into acid soluble precipitates. Inhibition of DNA, RNA and protein synthesis was observed at 100 μg/ml concentration of quinocarcin. At a concentration of 25 μg/ml, inhibition of RNA and protein synthesis was slight and detected only after 20 minutes. However DNA synthesis was blocked completely after five minutes. These results indicate that quinocarcin primarily inhibits DNA synthesis and subsequently affects RNA and protein synthesis.[7]

Inhibition of DNA synthesis of *B. subtilis* was found to be due to both the inhibition of DNA polymerase and cleavage of double stranded DNA. Cleavage of DNA by quinocarcin was inhibited by the addition of radical scavangers superoxide dismutase, catalase, β-carotene and α-tocophenol. This suggests that DNA cleavage is caused by generation of oxygen and/or hydroxyl free radicals. Superoxide dependent cleavage is also supported by the fact that the DNA cleaving ability of quinocarcin is stimulated in the presence of a reducing agent, dithiothreitol, while no changes were observed in the presence of both ferrous ion and cuprous ion.[7]

Quinocarcin also inhibits the synthesis of DNA by DNA polymerase. Preincubation of the enzyme with quinocarcin for one hour at 37°C did not cause reduction of DNA synthesis compared with that of the control without quinocarcin, while the

preincubation of the template DNA with quinocarcin caused about a two-fold reduction of DNA synthesis compared with the control. These results suggest that quinocarcin inhibits DNA synthesis through the interaction with the template DNA. It has thus been suggested that quinocarcin binds to the minor groove of DNA, (presumably at a guanine residue) to produce its antitumor effect.[7,8] (The mode of action of quinocarcin will be postulated in greater detail in chapter 4.)

The aforementioned results concerning quinocarcin's ability to cleave CCC DNA promoted further evaluation by Morimoto and coworkers on the effect of quinocarcin on tumor growth.[9] Quinocarcin itself is not stable in aqueous solution; (a solution of quinocarcin in water at room temperature decomposes 60% after five days[2]), so as to evaluate the drug further, including clinical trials, a citrate salt of quinocarcin was utilized.[9] The citrate salt of quinocarcin: quinocarmycin citrate (KW2152), had much higher stability; more than 95% activity remained after 72 hours in phosphate buffer (pH 7.2) at 37 °C.

Quinocarmycin citrate showed marked activity against P388 leukemia but only marginal activity against L1210 leukemia, B16 melanoma, and M5076 sarcoma in the i.p.-i.p. system. These results suggests that the antitumor spectrum of KW2152 against murine tumor systems tested was not remarkable among established antitumor agents (i.e. mitomycin C and adriamycin).[9]

In spite of its narrow spectrum against murine tumor models, quinocarmycin citrate showed marked activity against human tumors transplanted into nude mice. At optimal doses, KW2152 suppressed

the growth of MX-1 human mammary carcinoma with all mice cured by daily administration for seven days. It was also active against Co-3 human colon carcinoma and St-4 gastric carcinoma, a strain insensitive to the established anticancer drugs: mitomycin C, adriamycin, *cis*-diaminodichloroplatinum, and bleomycin.[10,11] Quinocarcin citrate proved moderately effective against St-15 human stomach carcinoma.[9]

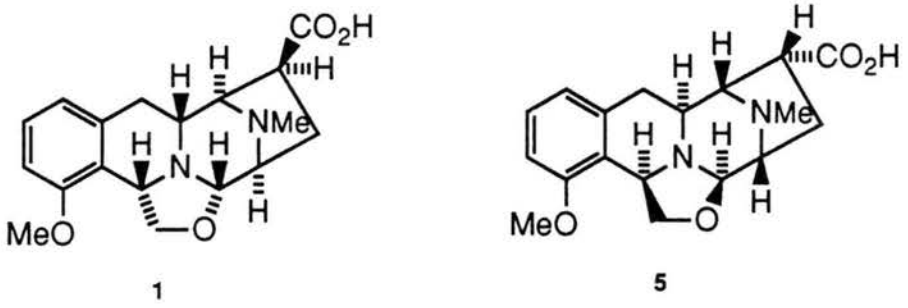
The most promising result obtained was the colony inhibition of KW2152 against human lung cancer cell lines.[12,13] Quinocarmycin citrate induced $\geq$70% colony inhibition of PC-7 (human adenocarcinoma), PC-10 (human squamous cell) and PC-13 (human large cell) at a drug concentration of 10 μg/ml. Significant activity (colony inhibition $\geq$70%) was not observed in PC-9 (human adenocarcinoma) and L929 (transformed mouse fibroblast). These results prompted the investigation of KW2152 as a new drug to be used against non-small cell lung cancer.[12,13] Currently KW2152 is in phase II clinical trials in Japan.[13]

Physical Characteristics

Quinocarcin is a colorless substance which becomes brown at 170 °C and does not show a clear melting point. It is freely soluble in water and methanol, easily soluble in ethanol and insoluble in chloroform, diethyl ether and n-hexane. Its specific rotation $[\alpha]_D^{22}$ is -32° (c 0.5, H_2O). The structure of quinocarcin could not be elucidated by ^1H NMR, ^{13}C NMR, UV, FD-MS and elemental analysis.[2] It was determined by direct correlation with quinocarcinol, (reduction of quinocarcin via $NaBH_4$ in ethanol produced

quinocarcinol),[2] and the single crystallographic x-ray determination of the structure quinocarcinol.[14] The proposed absolute configuration given by Hirayama has been the subject of some debate. Remers has argued that the absolute configuration is the opposite of that given by Hirayama.[8] He proposes the enantiomer **5** by observations with computer simulation of the binding of quinocarcin to a representative DNA fragment: $d(ATGCAT)_2$. (Scheme 3).[8]

Scheme 3



Stereoisomers proposed by Hirayama (**1**) and Remers (**5**).

This discrepancy has been recently resolved by Garner's asymmetric synthesis of quinocarcin.[24] He has determined that Remers' proposed stereoisomer, above, is the correct one.[15]

Synthetic Approaches to Quinocarcin and Quinocarcinol

The first approach to quinocarcin and the first successful synthesis of quinocarcinol was reported by Danishefsky and coworkers (Scheme 4).[16] Starting with *m*-hydroxy benzaldehyde **6** and subsequent O-allylation followed by Claisen rearrangement in N,N-dimethylaniline at 230° and O-methylation (MeI, K_2CO_3) produced **7**. Treatment of **7** with trimethylsilylcyanide followed by

$LiAlH_4$ reduction yielded the amino alcohol **8**. Protection of the amino functionality with $(tBuOCO)_2O$ followed by O-acetylation gave **9**. Conversion of the allyl group to a 3.5:1 mixture of E/Z isomers **10** was accomplished through the agency of $PdCl_2 \cdot (MeCN)_2$ in methanol.

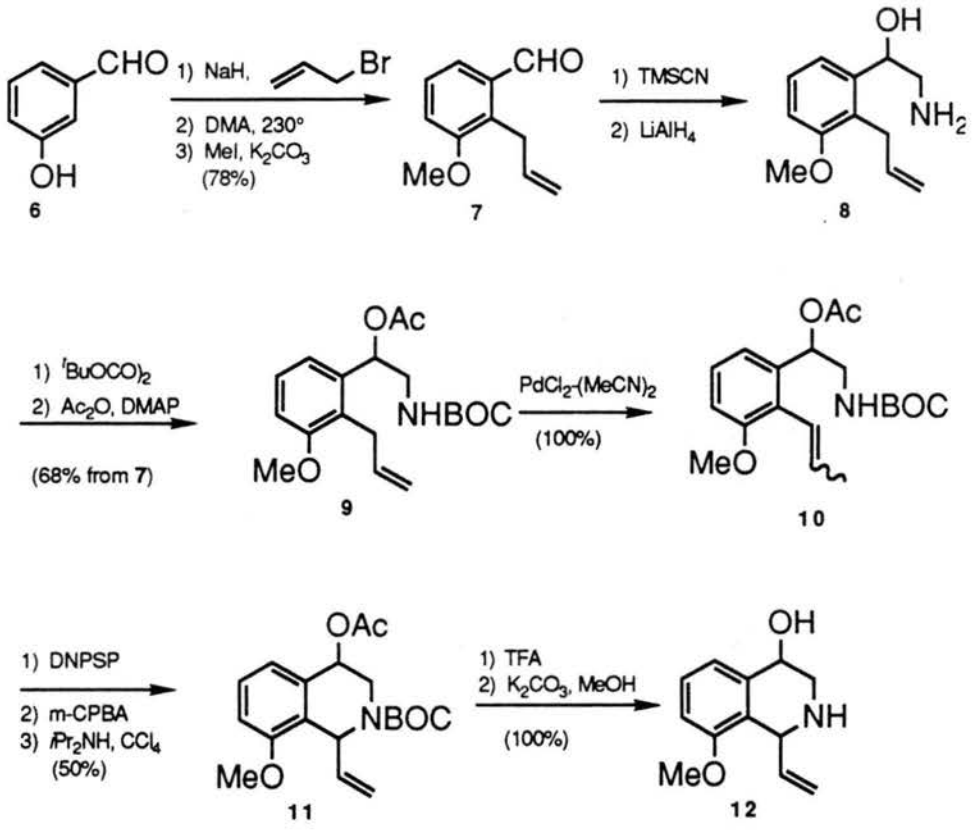
The tetrahydroisoquinoline ring was formed by the reaction of **10** with N-phenylselenophthalimide, in the presence of camphorsulfonic acid. Treatment of the resultant product with *m*-chloroperbenzoic acid followed by heating in the presence of diisopropylamine afforded **11**. Removal of the protecting groups by sequential treatment of **11** with trifluoroacetic acid followed by potassium carbonate furnished the amino alcohol **12**.

The coupling of **12** with the racemic differentiated γ-carboxyglutamate derivative[17] **13** was accomplished with BOP-Cl followed by Swern oxidation of the benzylic alcohol, producing ketone **14** as a 1:1 mixture of diastereomers. Cyclization of **14** mediated by $BF_3 \cdot Et_2O$ in refluxing chloroform gave the key tetracyclic intermediate **15**.

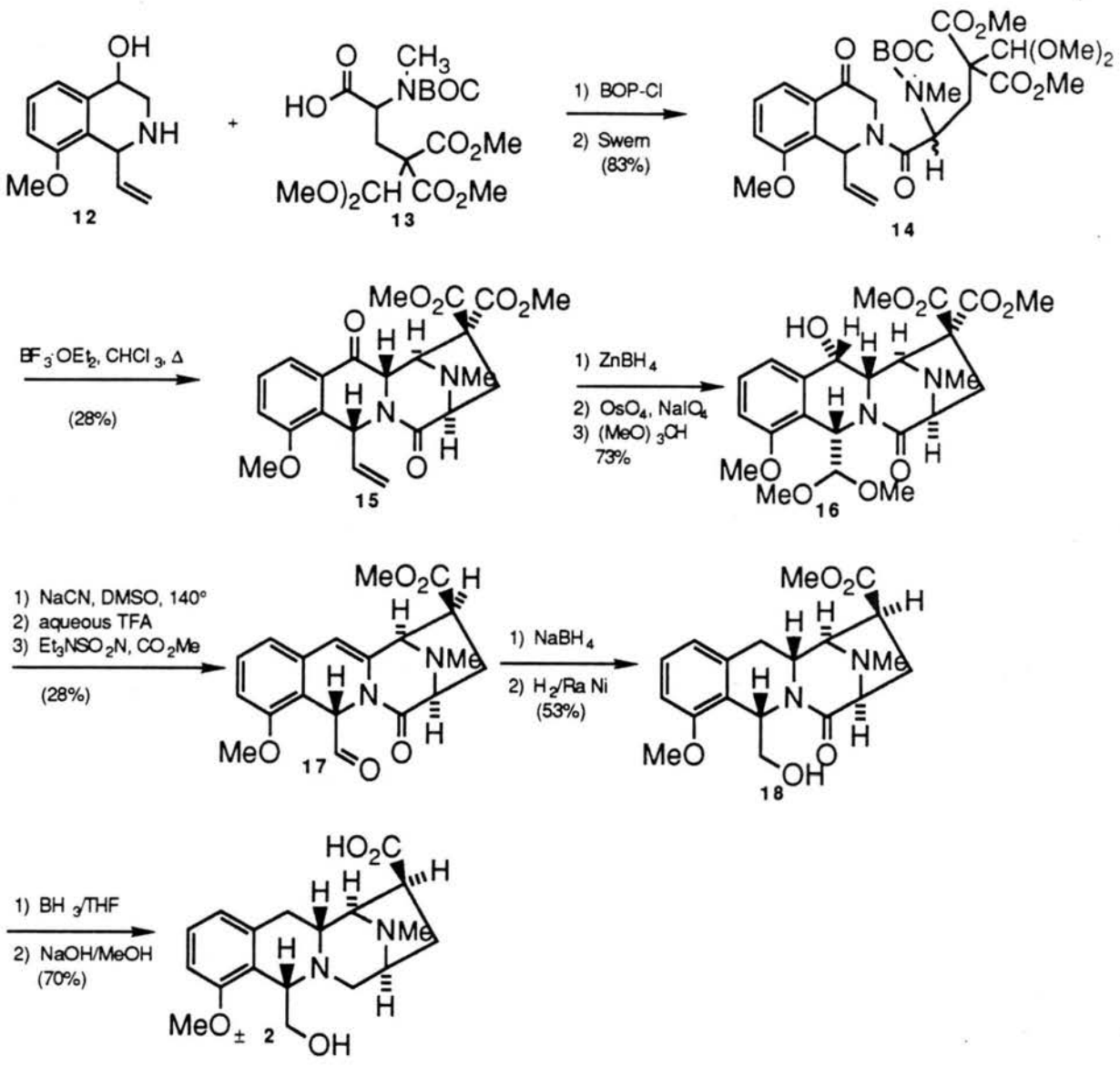
Stereoselective decarbomethoxylation of **16** with sodium cyanide in DMSO, acetal cleavage with aqueous trifluoroacetic acid followed by dehydration with the Burgess Reagent, $(Et_3N^+SO_2N^-CO_2Me)$ in benzene at reflux produced **17**. Reduction of **17** with sodium borohydride followed by hydrogenation with Raney nickel (1600 psi) at 60 °C produced lactam **18**. All attempts to reduce lactam **18** to the corresponding carbinolamine, which would presumably undergo conversion to quinocarcin, had been unsuccessful. However, reduction of lactam **18** with $BH_3 \cdot THF$

followed by hydrolysis of the methyl ester produced quinocarcinol **2**. Attempts by Danishefsky and coworkers to oxidize quinocarcinol to quinocarcin also proved fruitless. Thus, the first and only total synthesis of quinocarcinol was completed in 34 steps with an overall yield of 0.18% (Scheme 4).[16,17,18]

Scheme 4

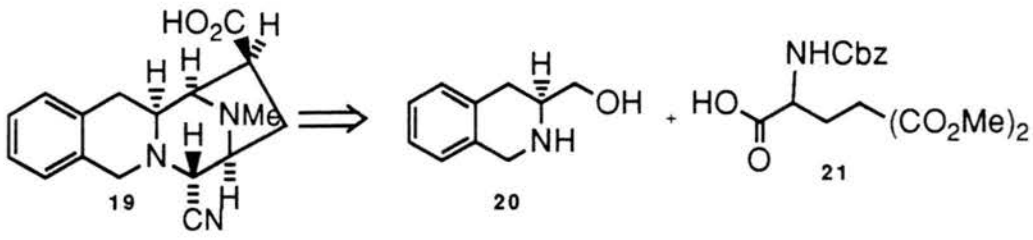


Scheme 4 (con't)



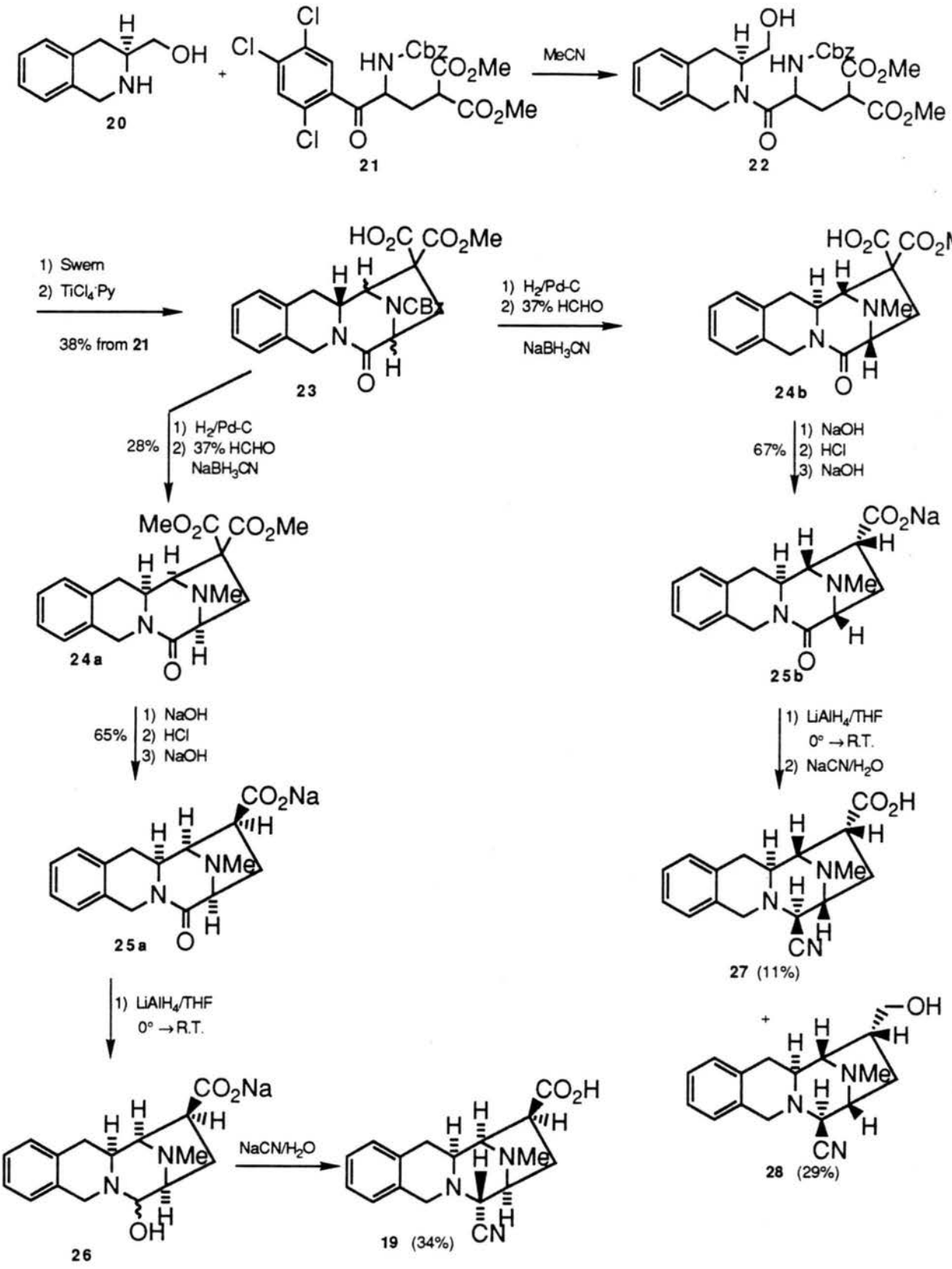
Hirata and Saito reported the synthesis of the optically active iminoazepinotetrahydroisoquinoline skeleton of quinocarcin, **19**.[19a,b] This was constructed from the tetrahydroisoquinoline[20] **20** and the glutamic acid derivative[21] **21** (Scheme 5).

Scheme 5



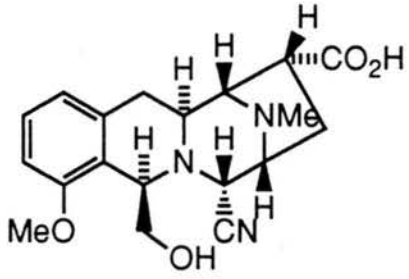
Coupling of the 3-hydroxymethyl-1,2,3,4-tetrahydroisoquinoline **20** with the trichlorophenyl activated glutamate **21** proceeds in acetonitrile to yield the amide **22**. Swern oxidation of **22** followed by titanium tetrachloride mediated ring closure furnishes the key tetracyclic intermediate **23**. It was interesting to note that without the additional carbomethoxy group activating the glutamate (i.e. starting with glutamic acid itself), the formation of the tetracycle was unsuccessful.[19b] The diastereomers of **23** were separated after hydrogenolysis and subsequent reductive alkylation gave rise to stereoisomers **24a** and **24b** in the ratio of 1:1.4. Ester hydrolysis followed by decarboxylation yielded the lactam acids **25a** and **25b**. Reduction of lactam **25a** to cyclic aminal **26** proceeded upon careful treatment with $LiAlH_4$ at 0°C to room temperature. Cyanation of unisolated aminal **26** afforded the desired product **19**. Similarly, stereoisomer **27** was obtained along with hydroxy methyl analog **28**. It is important to note that attempts to isolate aminal **26** failed, resulting in decomposition of the aminal[19a] (Scheme 6).

Scheme 6


20
21
22
MeCN
1) Swern
2) TiCl4·Py
38% from 21
23
1) H2/Pd-C
2) 37% HCHO
NaBH3CN
24b
28%
24a
67%
1) NaOH
2) HCl
3) NaOH
25b
65%
25a
1) LiAlH4/THF
0° → R.T.
2) NaCN/H2O
27 (11%)
26
NaCN/H2O
19 (34%)
28 (29%)

Stereochemical assignments of **27** and **19** were performed based on their relationship to the corresponding quinocarcin analog (DX-52-1, Scheme 7) and comparison of their analogous ^{13}C NMR chemical shifts.[19a,b] The ^{13}C NMR chemical shifts for diastereomer **27** most closely resembled those of DX-52-1, suggesting that the absolute configuration proposed by Remers[8] is the correct one. (Scheme 7)

Scheme 7



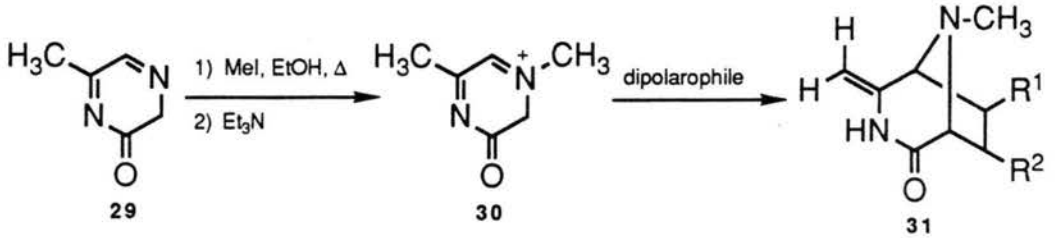
DX-52-1
Remers' Configuration

Cyano derivative DX-52-1 was first described by Hirata and coworkers before the discovery of quinocarmycin citrate.[22] DX-52-1 was synthesized in hopes of generating an analog of quinocarcin with increased stability and similar biological activity. Albeit more stable, DX-52-1 was made from quinocarcin in low yield (ca. 28%), and was found to be significantly less active than quinocarcin itself. Also, the LD_{50} of DX-52-1 versus quinocarcin by intraperitoneal administration in mice was found to be 24.5 mg/kg and 71.3 mg/kg respectively.[22]

Two recent reports concerning an interesting cycloaddition approach to the bicyclic skeleton of quinocarcin and quinocarcinol have appeared in the literature. The first, by Joule and coworkers demonstrated the cycloadditions that 1,5-dimethyl-3-

oxidopyrazinium, (**30**), underwent with methyl acrylate, acrylonitrile, diethyl maleate, maleimide methyl propiolate and diethyl acetylene dicarboxylate (Scheme 8).[23]

Scheme 8



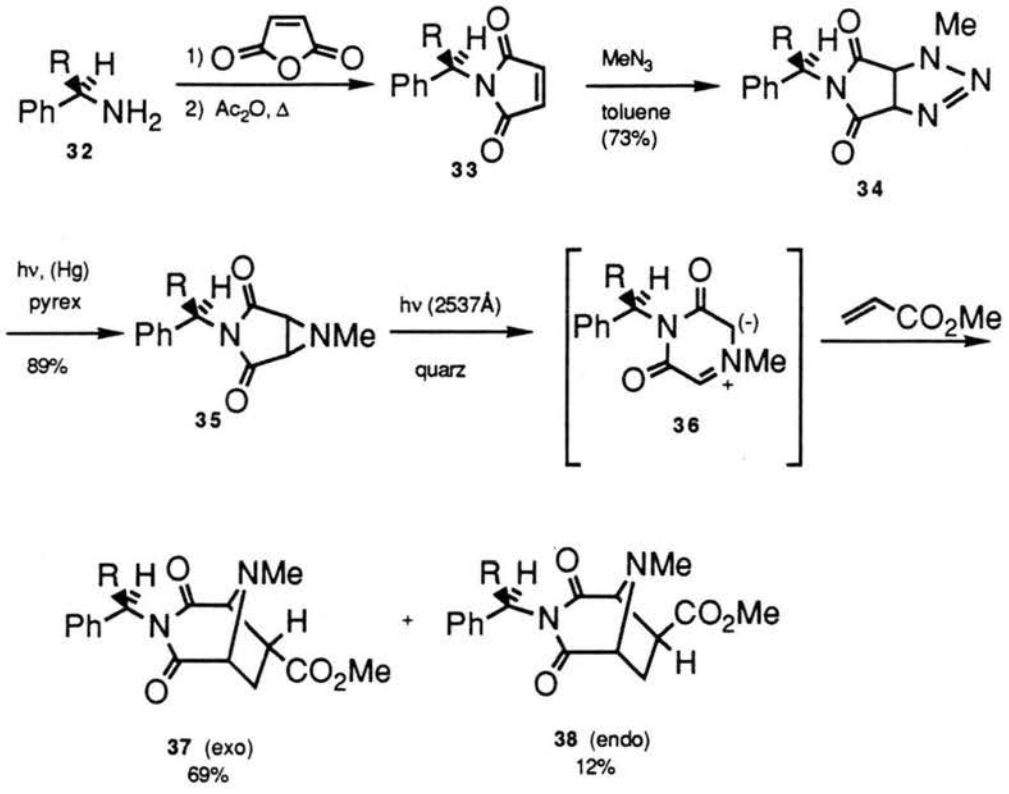
31	R^1	R^2
a)	exo-CO_2Me	H
b)	exo-CN	H
c)	endo-CN	H
d)	exo-CO_2Et	exo-CO_2Et
e)	CO_2Me (6,7 dihydro)	H
f)	CO_2Et (6,7 dihydro)	CO_2Et
g)	exo CH-CO-NH-CH exo	

6-Methylpyrazin-2-one was quaternized with methyliodide in refluxing ethanol, followed by zwitterion formation by treatment with triethylamine at room temperature. Dipolar cycloadditions of **30** with the aforementioned dipolarophiles were conducted in THF, at reflux which gave **31**, in unoptimized yields between 25 and 58%.[23]

In reaction with acrylonitrile, (the only anomalous cycloaddition), both exo-**31b** and endo nitrile **31c** were obtained in equal proportions from one reaction. (Temperature had no effect on the stereochemistry and the authors failed to mention any C-7 epimerization investigations.)

A similar approach to the diazabicyclo[3.2.1]octane moiety of quinocarcin via cycloaddition of photochemically generated azomethine ylides was reported by Garner and coworkers.[24] Their strategy is outlined below. (Scheme 9).

Scheme 9



a: R = H; b: R = CO_2Me; c: R = CH_2OH; d: R = CH_2OAc

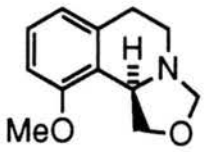
Preparation of **33** from benzylamine **32** via acetic anhydride mediated dehydration of the half acid and subsequent reaction with methyl azide in toluene resulted in the formation of triazoline **34**. Irradiation of **34** (0.04 M in dioxane) using a medium pressure Hanovia Hg lamp and a pyrex filter led to extrusion of nitrogen and subsequent formation of aziridine **35**. Photolysis of **35** (0.2 M dioxane solution) using a 2537 Å Rayonet source and a quartz vessel

resulted in the generation of azomethine ylide **36**, (via concerted disrotatory ring opening of **35**), which was "trapped" with methyl acrylate furnishing a mixture of exo (**37**) and endo (**38**) adducts in approximately a 5:1 ratio.[24]

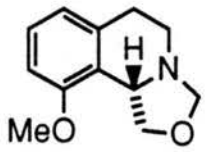
Examination of substrates that would lead to a chiral synthesis of **37** and **38** (i.e., phenyl glycine derivatives b, c, d) resulted in no diastereoselectivity in the cycloaddition of methyl azide (**34**), and the 1,3-dipolar cycloaddition of acrylate (**37** and **38**).[24]

The first enantioselective approach to quinocarcin was that by Terashima, which was reported within the scope of the enantioselective synthesis of the 1-hydroxymethyl-8-methoxytetrahydroisoquinoline portion of quinocarcin[25] (**39** and ent-**39**, Scheme 10).

Scheme 10



39



ent-**39**

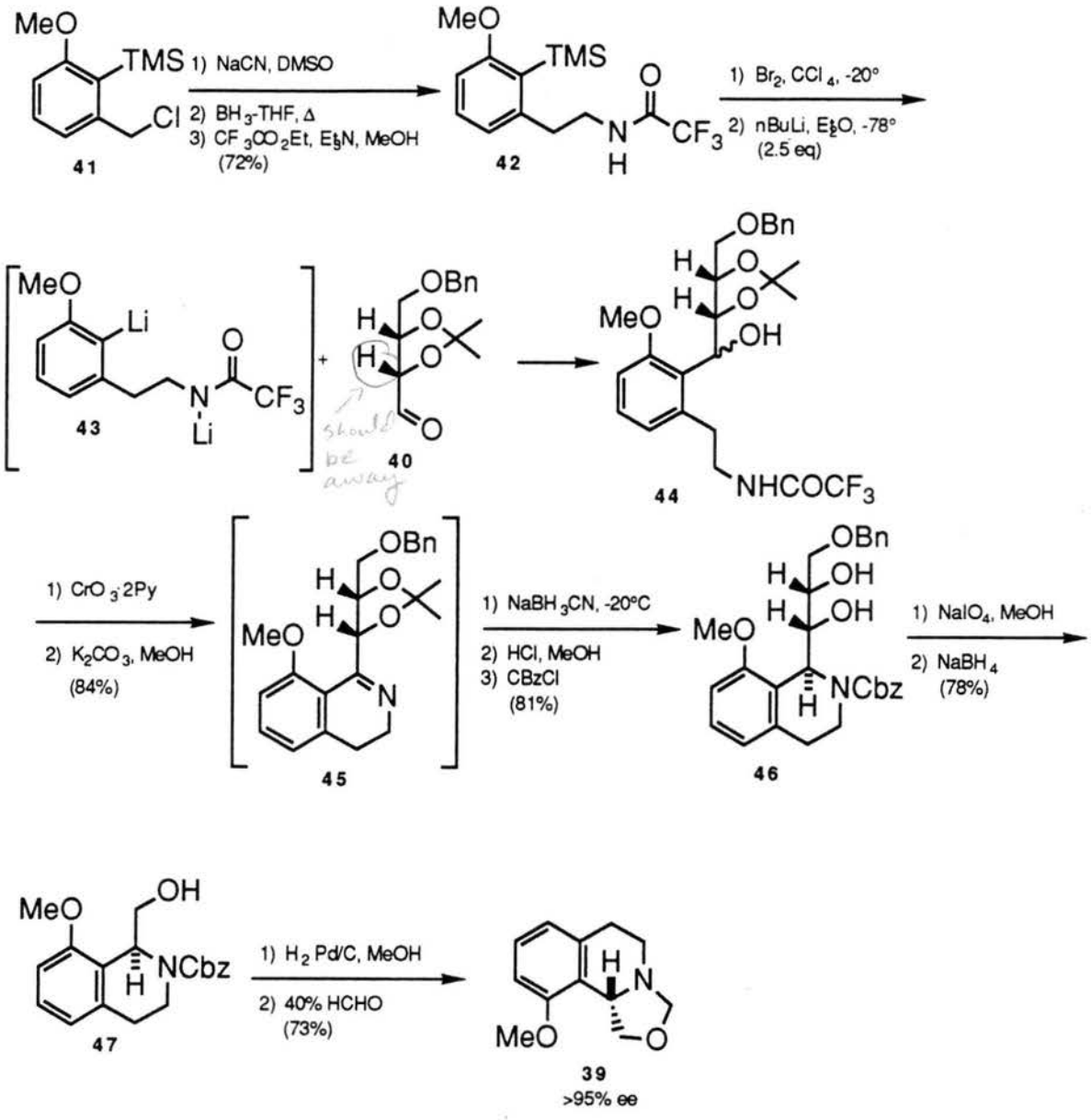
Terashima selected 4-O-Benzyl-2,3-O-isopropylidenethreose **40** as a chiral auxiliary since each enantiomer can be readily prepared from *l*- or *d*-tartaric acid.[25]

Starting with benzyl chloride **41** and subsequent reaction with NaCN in DMSO, which was then treated with BH_3·THF followed by protection of the resulting amine yielded **42**. The amide **42** was

then subjected to desilylation/brominating conditions. The resulting bromide was then lithiated and treated with threose **40** giving an epimeric mixture of benzylic alcohols (**44**). Oxidation of the mixture followed by deprotection yielded the dihydroisoquinoline **45** (not isolated) which was subjected to reduction with sodium cyanoborohydride. The resulting isoquinoline was isolated in the form of diol carbamate **46** as a single diastereomer, after sequential cleavage of the acetamide and protection of the amino group. The diastereoselectivity can be rationalized by chelation resulting from interaction with the alkoxy group adjacent to the C, N double bond.[26] Oxidative cleavage of the diol moiety of **46** followed by reduction of the resulting aldehyde produces protected amino alcohol **47**. Removal of the carbobenzyloxy group followed by acetal formation yields **39** in greater than 95% ee; determined via the Mosher ester of **47** (Scheme 11).[25] By employing 4-O-benzyl-2,3-O-isopropylidene-D-threose instead of the L isomer the enantiomeric amino acetal (ent-**39**) was prepared in the same manner as outlined above. The enantiomeric excess in ent-**39** was also >95%.[25,26]

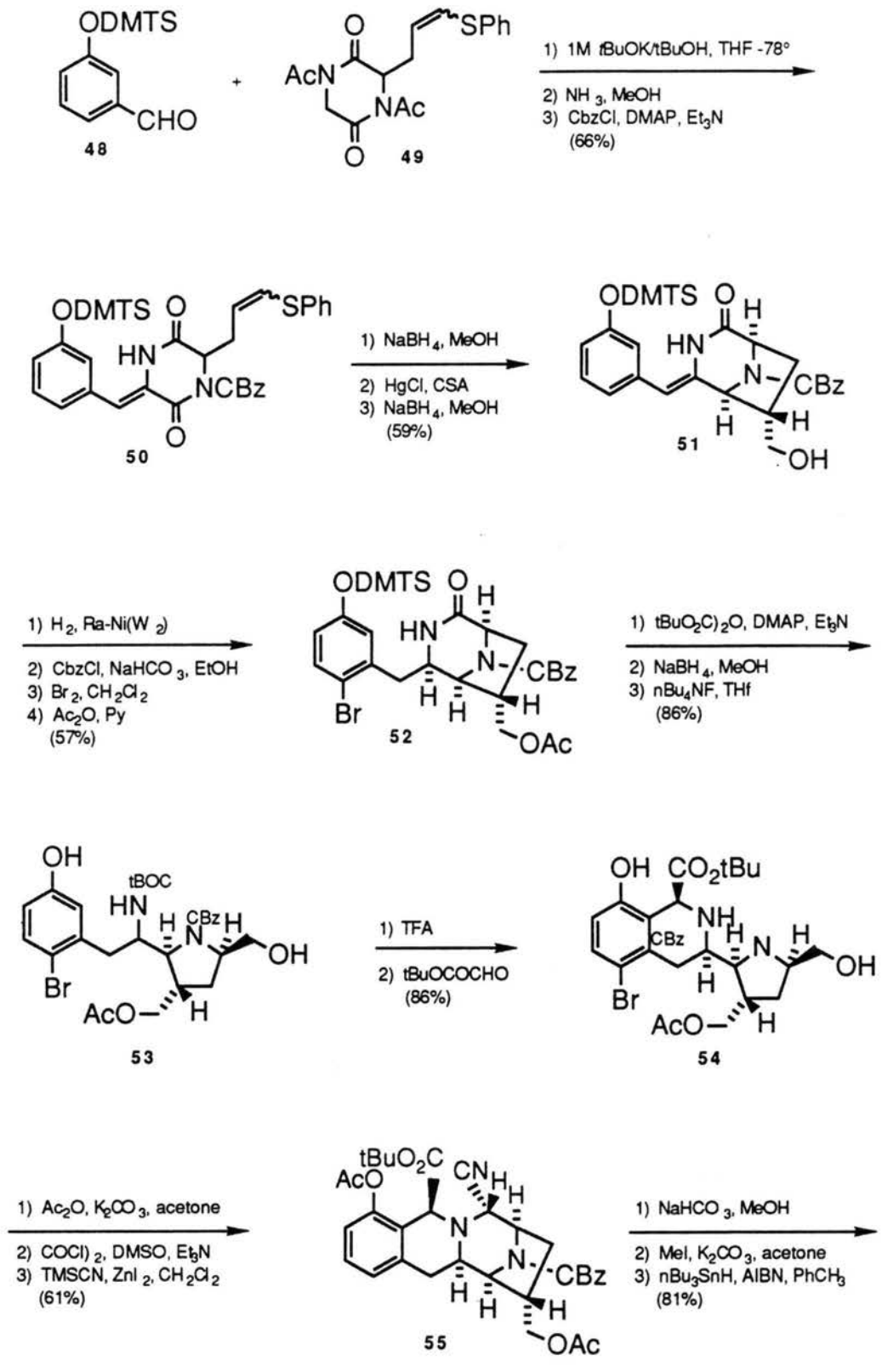
The cytotoxic activity of **39** and ent-**39** was studied against P388 murine leukemia (*in vitro*). Both enantiomers were found to have no significant cytotoxic activity; thus, Terashima suggested that this portion of quinocarcin has little to do with its antitumor activity.[25] (Scheme 11)

Scheme 11

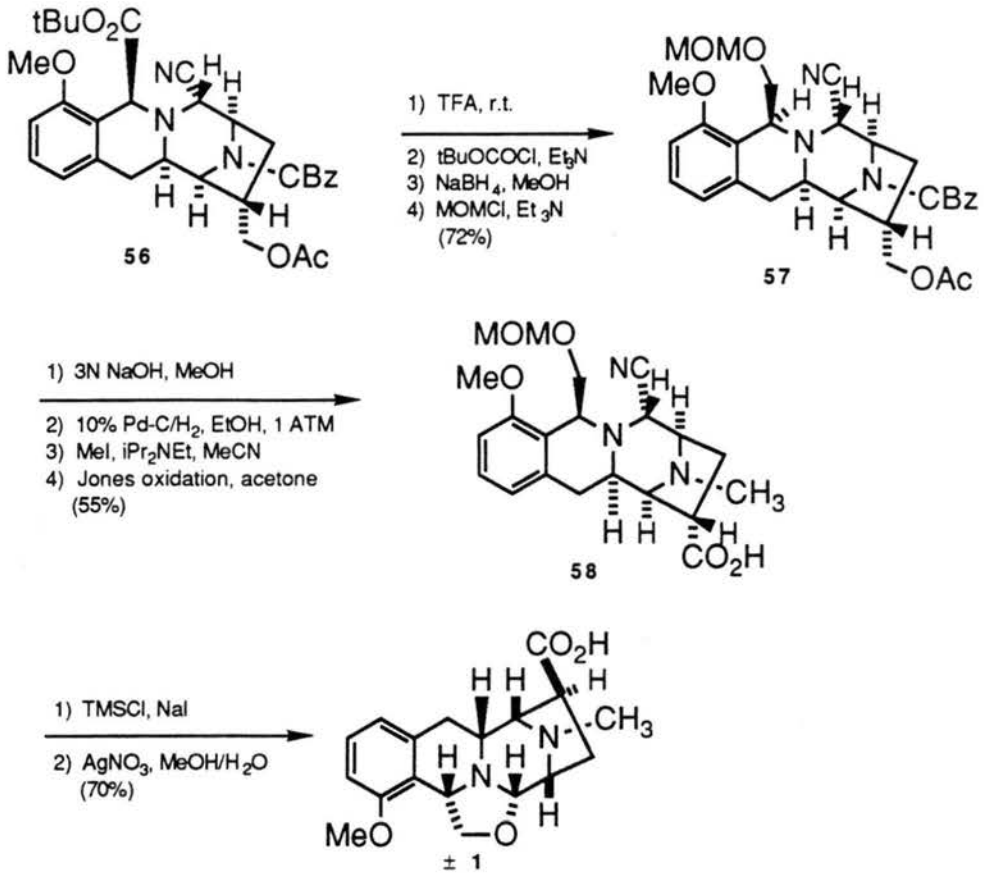


The first stereocontrolled total synthesis of (±) quinocarcin has recently been reported by Fukuyama and Nunes.[27] Their synthesis of quinocarcin utilized the stability of cyano derivative DX-52-1 for the crucial oxazolidine formation (Scheme 12).

Scheme 12


ODMTS
CHO
48
AcN
NAc
SPh
49
1) 1M tBuOK/tBuOH, THF -78°
2) NH 3, MeOH
3) CbzCl, DMAP, Et3N
(66%)
ODMTS
HN
NCBz
50
1) NaBH4, MeOH
2) HgCl, CSA
3) NaBH4, MeOH
(59%)
CBz
OH
51
1) H2, Ra-Ni(W 2)
2) CbzCl, NaHCO 3, EtOH
3) Br2, CH2Cl2
4) Ac2O, Py
(57%)
Br
OAc
52
1) tBuO2C)2O, DMAP, Et3N
2) NaBH4, MeOH
3) nBu4NF, THf
(86%)
tBOC
AcO
53
1) TFA
2) tBuOCOCHO
(86%)
CO2tBu
54
1) Ac2O, K2CO3, acetone
2) COCl) 2, DMSO, Et3N
3) TMSCN, ZnI 2, CH2Cl2
(61%)
tBuO2C
AcO
CN
55
1) NaHCO 3, MeOH
2) MeI, K2CO3, acetone
3) nBu3SnH, AIBN, PhCH3
(81%)

Scheme 12 (con't)



Condensation of aldehyde **48** with diketopiperazine **49** followed by ammonolysis and selective activation of the amide nitrogen furnished the unsymmetrically substituted piperazinedione **50**. Partial amide carbonyl reduction followed by acyliminium ion-mediated cyclization and subsequent reduction of the resultant aldehyde yielded the diazabicyclo[3.2.1] system (**51**). Reduction of the exocyclic double bond from the less hindered α-face followed by *in situ* reprotection of the amine with tandem bromination and O-acetylation furnished **52** as a single regioisomer. The bicyclic lactam was then converted to the pyrrolidine **53** by amide activation and reduction, then deprotection of the phenol. the isoquinoline

construction was then achieved via deprotection of the *t*-butylcarbamate followed by subjecting the amine salt to *t*-butyl glyoxylate. The Pictet-Spangler cyclization yielded an 8:1 ratio of desired isomer **54**. Selective protection of the phenol followed by Swern oxidation and subsequent reaction with trimethylsilylcyanide afforded the protected DX-52-1 skeleton **55**. Deprotection of the phenol, and subsequent methyl ether formation followed by radically induced aryl debromination yielded **56**. Next, a three step sequence accomplished the reduction of the *t*-butyl ester. First the *t*-butyl ester was deprotected, then reduced via the mixed anhydride. The resulting alcohol was then protected as the methoxymethyl ether to provide compound **57**. Acetate hydrolysis of **57** followed by hydrogenolysis of the carbobenzyloxy group with subsequent methylation and Jones oxidation of the alcohol gives rise to the MOM protected DX-52-1. Formation of quinocarcin is then achieved via tandem deprotection of the methoxymethyl ether followed by treatment with silver nitrate.[27] The synthesis required 31 steps with an overall yield of 2%.[27]

Our approach to the total synthesis of quinocarcin took into account three predominant features of this challenging target: 1) the lability of the oxazolidine ring, with respect to its formation as late as possible into the synthesis; 2) the formation of appropriately substituted pyrrolidines which could, in later steps be utilized to form the oxazolidine and diazabicyclo[3.2.1]ring system; and 3) the formation of the 8-substituted tetrahydroisoquinoline moiety.

The utilization of model studies for each of these fields of endeavor and subsequent combination of the methodologies involved

is the subject of this dissertation. It should be pointed out; however, that this approach has the distinct advantage of isolating the three main features of quinocarcin: 1) the oxazolidine moiety; 2) the pyrrolidine moiety; and 3) the tetrahydroisoquinoline moiety. This, in turn, allows one to establish and isolate the functions required in quinocarcin for its chemical stability and most importantly, its biological activity.

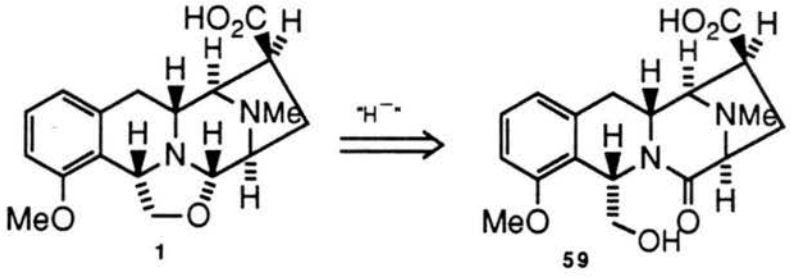
CHAPTER 2

CHAPTER 2.1

OXAZOLIDINE MODEL STUDIES

Initial investigations of oxazolidine construction stemmed from a preliminary proposal involving the total synthesis of quinocarcin,[28]. In this plan, a controlled reduction of the amide **59** utilizing intramolecular coordination of the reducing agent via the hydroxymethyl residue was deemed a crucial transformation to occur in the final stage of the total synthesis (Scheme 13).

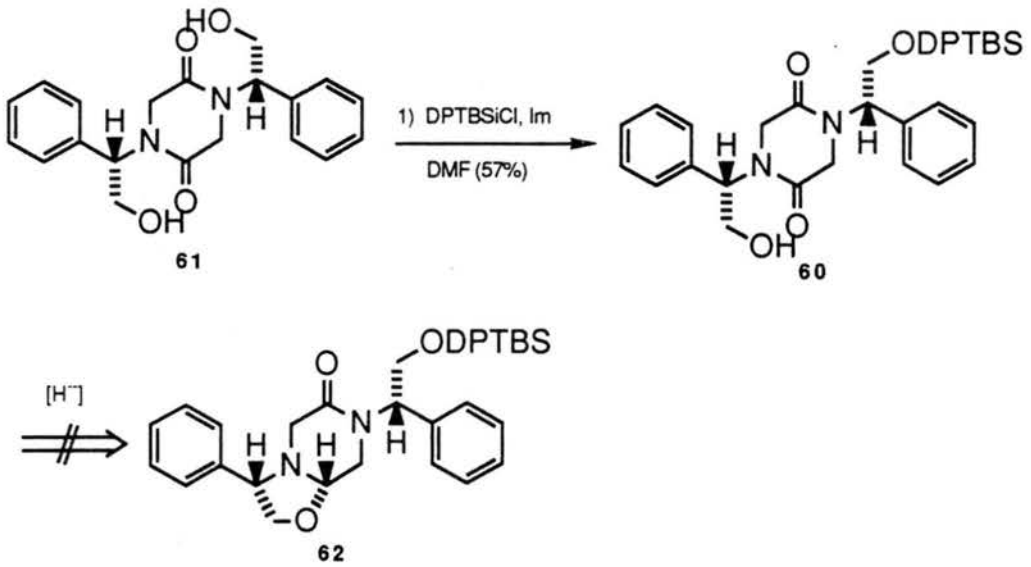
Scheme 13



To ascertain what conditions would be best suited for this transformation several model systems were investigated. The first system involved the monosilylated piperazinedione, **60**, derived from the dihydroxypiperazinedione, **61**, by treatment with chlorodiphenyl-t-butylsilane and imidazole in DMF at room temperature (57% yield). The reductive ring closure was attempted using Red-Al and $LiEt_3BH$, which yielded a variety of products. The

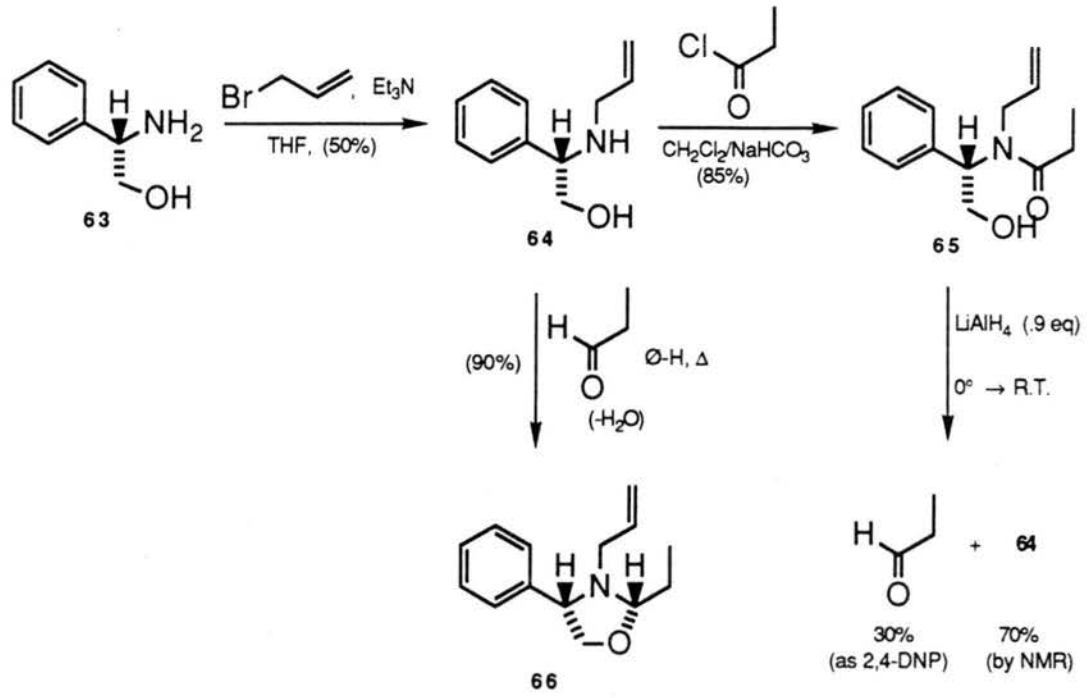
products obtained did not have the properties expected of **62** (by tlc and 270 MHz ^{1}H NMR of the crude reaction mixture). Since it was thought that approximately 6-8 products could be produced upon treatment of **60** with a reducing agent, (i.e., nonselective amide reduction and possible desilylation products), efforts for the attainment of **62** were abandoned. A new system was considered that was more characteristic of a quinocarcin "mimic" (Scheme 14).

Scheme 14



The phenyl glycinol model study was chosen for the purpose of having a system in which the desired product could be synthesized independently. This would simplify the isolation of the desired product from the amide reduction (Scheme 15).

Scheme 15

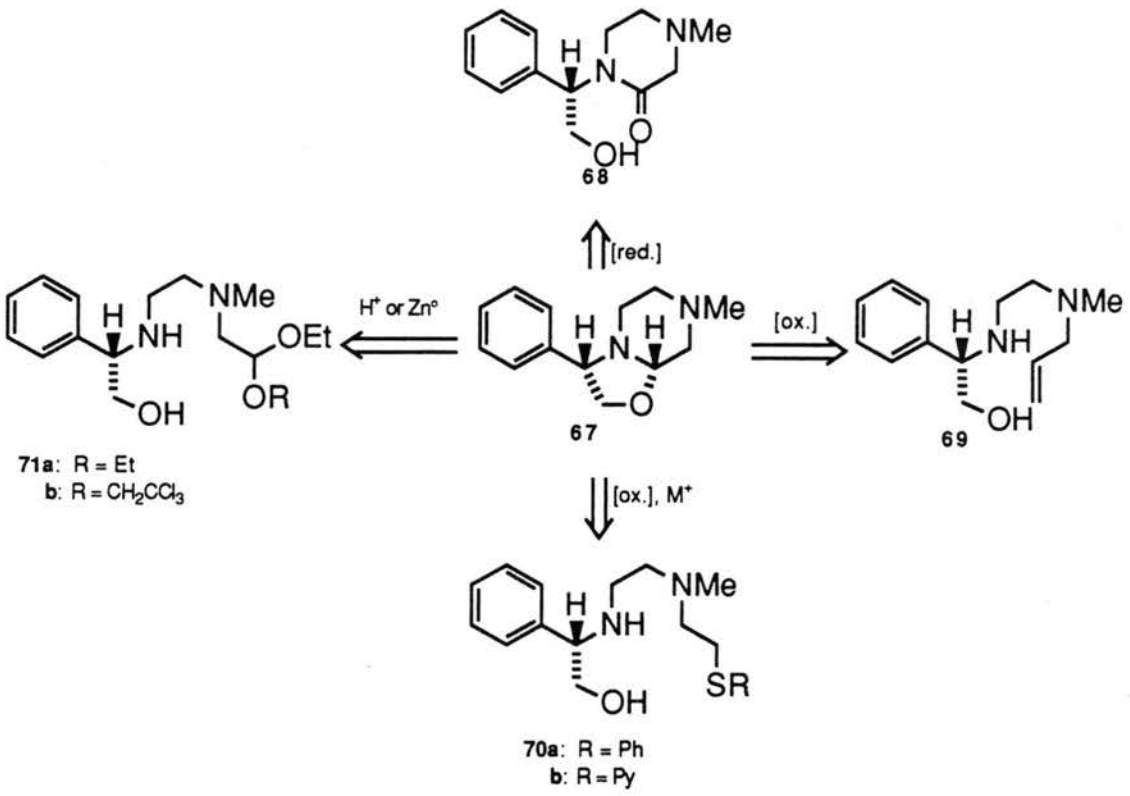


D-phenylglycinol **63** wa treated with allyl bromide to yield N-allylphenylglycinol **64**; subsequent amide formation with propionyl chloride gave **65** in 42% overall yield. Independent oxazolidine formation was achieved by condensation of **64** with propionaldehyde which furnished **66** as a colorless oil in 90% yield as a single diastereomer.[29,30] It was found that oxazolidine **66** was unstable to acid and exposure to silica gel resulted in hydrolysis of the oxazolidine to starting materials. Isolation of pure **66** was accomplished via microdistillation. Exposure of pure **66** to air also resulted in hydrolysis of the oxazolidine. These results were important in determining the best conditions to use in the isolation of **66** from the reaction of amide **65** with reducing agents. Treatment of **65** with Red-Al and $LiEt_3BH$ resulted in no reaction. Successful amide reduction occurred by using $LiAlH_4$

(less than one equivalent). Unfortunately very little of the oxazolidine was present by NMR, and the major product was the amino alcohol **64**. The propionaldehyde, being volatile, could be "trapped" as a 2,4-dinitrophenylhydrazine derivative to prove its existence from the amide reduction. The "trapped" propionaldehyde was compared with that made by standard methods.[31]

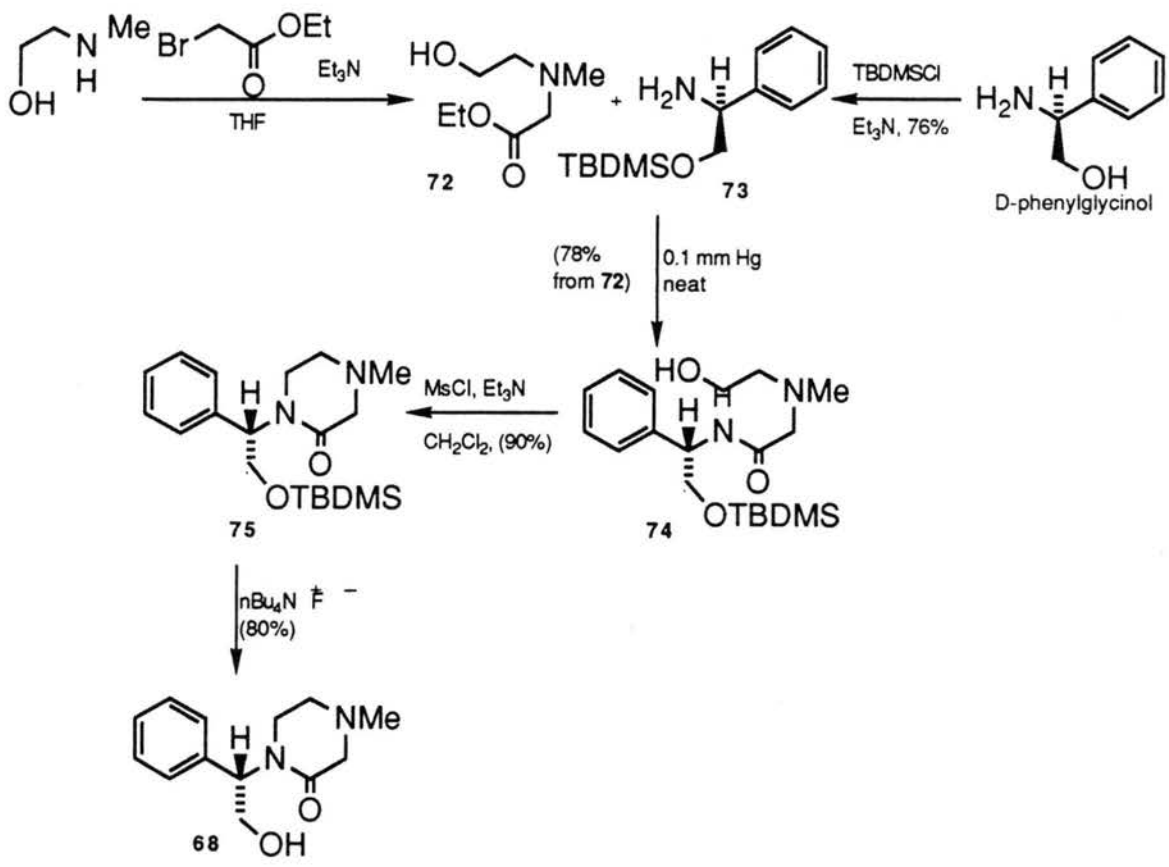
With these somewhat promising results it seemed necessary to test the reductive ring closure on a model system that would be less likely to undergo hydrolysis to starting materials. Again, maintaining similarity to quinocarcin itself the next model system that was investigated was the piperazine fused oxazolidine **67**. It was thought that this system would have the added advantage of intramolecularity; ie., that no disproportionation would be possible upon reduction of the monoketopiperazine **68**. Also investigated were three other aldehyde equivalents: 1) the allyl hydroxyl amine **69**, envisioned to produce **67** via oxidative dehomologation of the olefin; 2) The S-phenyl and thiopyridyl precursor **70a** and **70b**, which were envisioned to produce **67** upon treatment with N-chlorosuccinimide followed by metal induced oxazolidine formation; and 3) the acetal precursors **71a** and **71b**, expected to yield **67** via direct deprotection of the aldehyde with acid (**71a**), or utilizing metalic zinc under neutral conditions (**71b**), (Scheme 16).

Scheme 16



The first precursor to the piperazine fused oxazolidine which was investigated was the monoketo piperazine **68**. This substance was synthesized from N-methylethanolamine, **72**, by alkylation with ethyl bromoacetate followed by condensation with O-silylphenylglycinol **73**, which furnished the amide **74** in 60% overall yield. Intramolecular condensation of **74** via mesylate formation gave the O-silyl protected monoketopiperazine **75** in 90% yield. Desilylation with tetra-n-butylammonium fluoride then yielded the desired oxazolidine precursor **68** (Scheme 17).

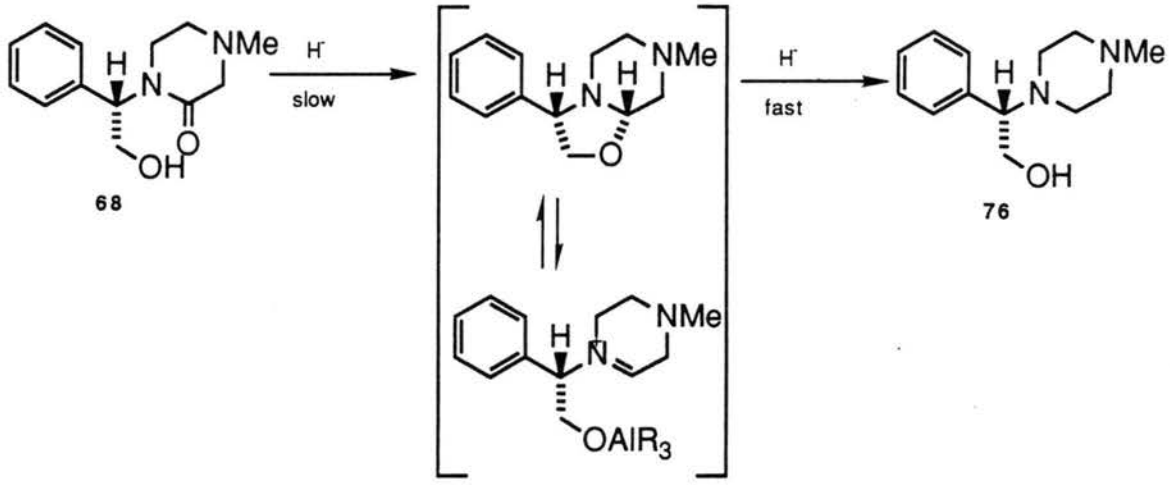
Scheme 17



Subjecting **68** to the same conditions previously employed for amide **66** did not have the desired results; the only product formed was over-reduced **68** (e.g., the quinocarcinol equivalent piperazine). The same result was realized when the reducing agents Red-Al or $LiEt_3BH$ were utilized. These results were rationalized by presuming that the desired oxazolidine would be the intermediate to the formation of the piperazine **76**. This conclusion was based on the mild reaction conditions used to achieve complete reduction of the amide **68**. The results of Danishefsky and coworkers in the aforementioned total synthesis of quinocarcinol[16] also suggested

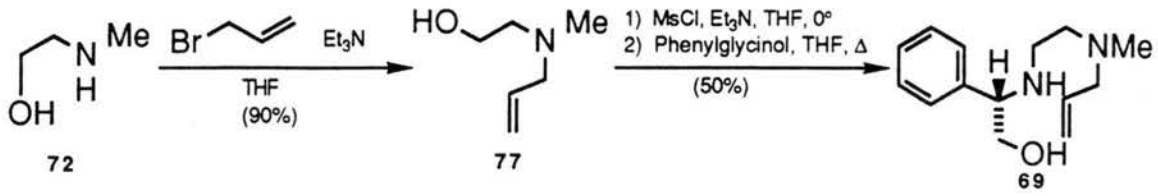
that over-reduction of such amides as **68** would be facilitated by participation of the hydroxymethyl group (Scheme 18).

Scheme 18



Attention was then turned to the N-allyl precursor **69** with the hope to avoid the use of hydride and to thereby have a precursor that would be hardy enough to withstand a multistep synthesis. The alkylation of N-methylethanolamine **72** with allyl bromide yielded the corresponding N-allyl-N-methylethanolamine **77** (isolated by distillation in 70% yield). Subjecting the amino alcohol **77** to mesylating conditions followed by the addition of *d*-phenyglycinol yielded **69** (Scheme 19).

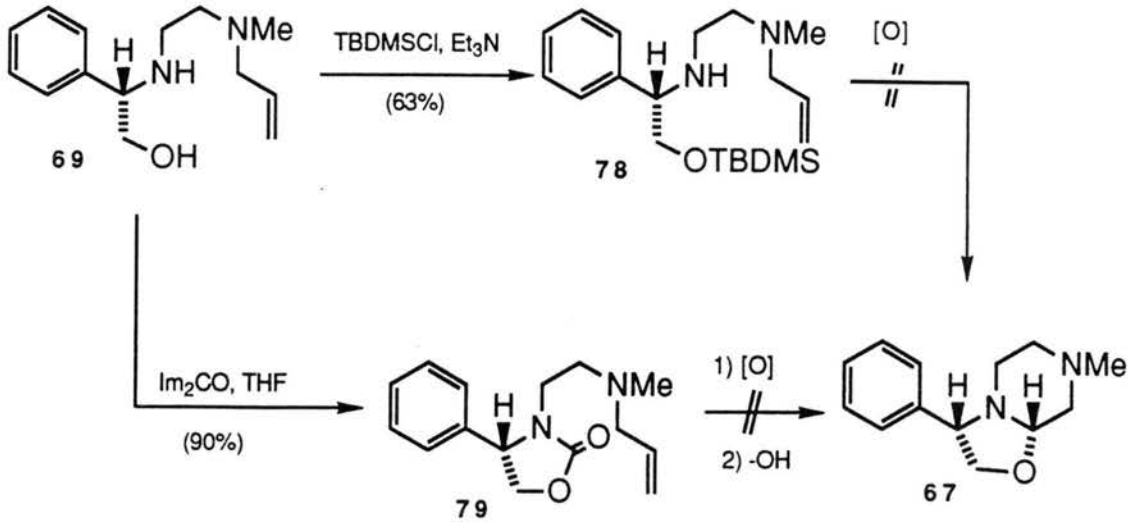
Scheme 19



Attempts to oxidize **69** to produce **67** proved futile. Ozonolysis resulted in a variety of reaction products none of which could be identified. Ozonolysis was performed under acidic conditions and protic neutral conditions in attempts to stabilize the formation of **67**, along with aprotic neutral conditions (i.e., 1M HCl MeOH, MeOH, and CH_2Cl_2 reaction solutions).

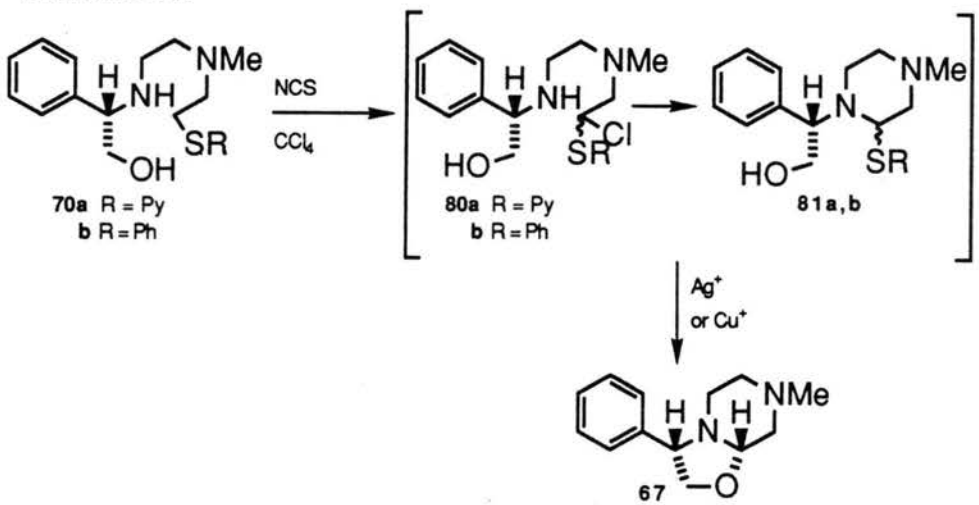
Periodate-osmium tetraoxide cleavage in 80% acetic acid also gave complex reaction products. (This procedure was adapted from that of Dvornik who used these conditions to cleave a terminal olefin in the presence of an imine.[32]) Protection of **69** by O-silylation (**78**), or urethane formation (**79**), followed by treatment with OsO_4, with the hope of isolating the diol, also was unsuccessful. The failure of the above described reaction was quite possibly due to the β-heteroatoms which have been implicated to chelate to periodate and may subsequently allow for oxidative cleavage between any and/or all β-heteroatoms, not just the diol presumably formed from OsO_4[32] (Scheme 20).

Scheme 20



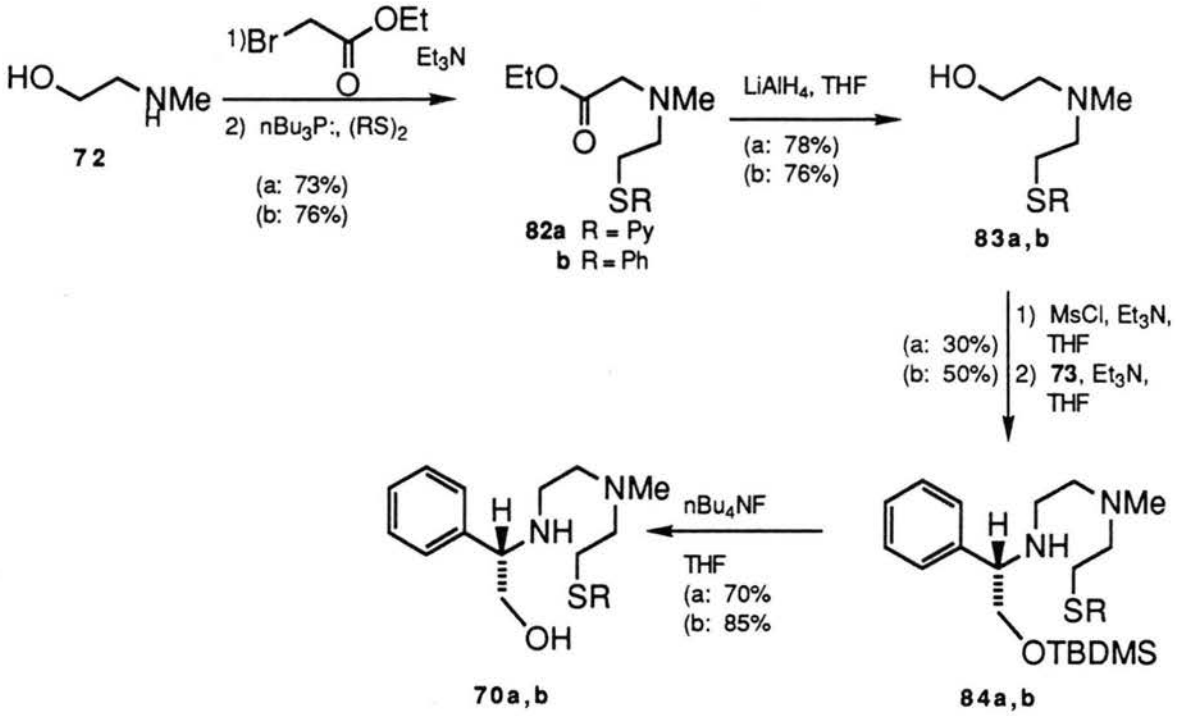
The next approach to **67** was via sulfides **70a** and **70b**. It was rationalized that upon treatment with NCS the α-chlorosulfides **80a** and **80b** could spontaneously ring close to **67** via **81**. If **81** were the major product then treatment of such a species with silver or copper salts would be expected to result in the formation of **67** (Scheme 21).

Scheme 21



The first in this series to be investigated was **70a**, the 2-thiopyridyl derivative. Sequential treatment of N-methylethanolamine **72** with ethylbromoacetate followed immediately with tri-n-butylphosphine and 2,2'-dipyridyldisulfide furnished the thiopyridyl glycinate **82a**. Reduction to the amino alcohol **83a** by $LiAlH_4$ followed by subsequent mesylation and treatment with O-silyl-*D*-phenylglycinol **73** gave the O-silyl protected precursor **84a**. Deprotection of **84** with nBu_4NF resulted in the formation of **70a**. The synthesis of **70b** followed precisely the same pathway (Scheme 22).

Scheme 22

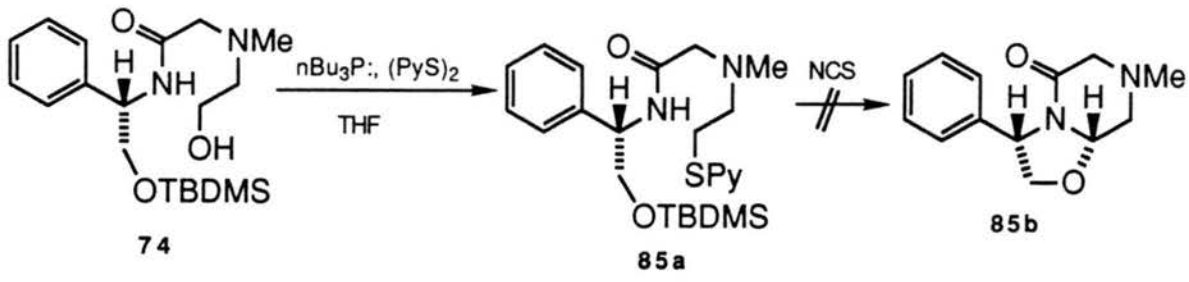


To see whether **81a** would be a stable intermediate, **84a** was subjected to treatment with NCS in CCl_4, resulting in immediate

decomposition of **84a**. It was thought that the thiopyridyl moiety might have been the reason for this surprising result. Unfortunately, the same spontaneous decomposition of **84b** resulted upon treatment with NCS. Deprotection of the O-silyl group was effected to see whether **67** spontaneously forms from **84a,b**. Treatment of this compound with NCS resulted in the same disappointing result: immediate decomposition.

To try and understand the shortcomings of this approach, synthesis of **85** was undertaken by treatment of **74** with tri-n-butylphosphine and 2,2'-dipyridyldisulfide. It is reasonable to speculate that N-chlorination of the amine in **84a,b** could have occurred.[33] This would result in imine formation and subsequent decomposition. However, treatment of amide **85a** with NCS/CCl_4 did not produce the desired oxazolidine **85b** demonstrating that there was an inherent problem with this approach that was not amenable to the total synthesis of quinocarcin (Scheme 23).

Scheme 23



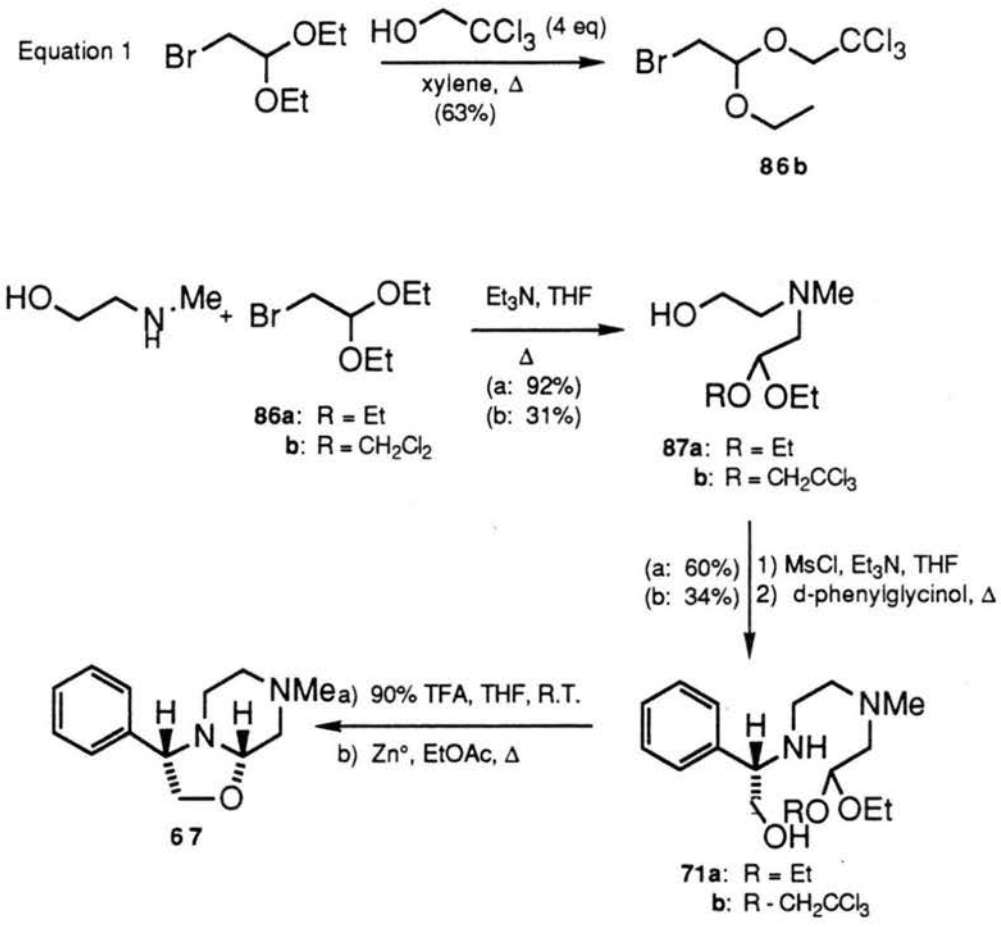
The next approach to **67** was the obvious: utilizing an aldehyde protected as its acetal. Synthesis of **71a** and **71b** was then undertaken. Synthesis of **71a** was achieved via alkylation of

N-methylethanolamine, **72**, with bromoacetaldehyde diethylacetal, **86a**, to produce **87a** (Scheme 24). Alkylation of *D*-phenylglycinol via the mesylate of **87a** furnished the desired precursor **71a**.

Hydrolysis of diethylacetal **71a** proved problematic.[34] Due to the unusual hardiness of the acetal and the instability of the product itself, it was found that the reaction mixtures could not be heated. Thus, the reaction conditions which were found to work in cleaving the acetal and yielding the desired product were strongly acidic and performed at room temperature (90% aq. TFA, THF) under relatively high dilution (.01M). The product itself (and oxazolidines in general) was also unstable to the Bronstead acid required to cleave the acetal. The desired **67** was only found to be present in trace amounts by NMR. Attempted isolation resulted in complete decomposition. The only fortunate aspect of the reaction is that **67** was the only product of hydrolysis that didn't reside at the origin of a TLC plate, therefore it could be easily ascertained that it was indeed present.

The low yield of **67** using the diethyl acetal seemed to be predominantly due to the strongly acidic conditions required to hydrolyze it. For this reason the mixed acetal **71b** was synthesized, in hopes that neutral aprotic conditions would lead to an isolable product (Scheme 24).

Scheme 24

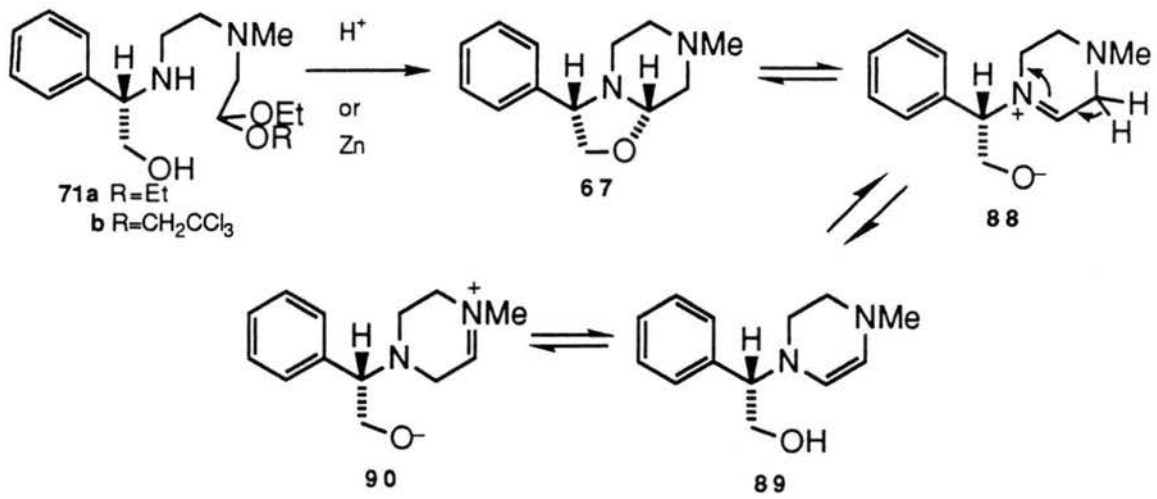


Synthesis of **71b** followed the same protocol employed in the synthesis of **71a**, with the exception of the required synthesis of **81b**, (Scheme 12, equation 1), by transacetylation of bromoacetaldehyde diethylacetal with 2,2,2-trichloroethanol in xylenes at reflux.[35] Only the mixed acetal **81b** could be obtained, even with extended reaction times and more equivalents of 2,2,2-trichloroethanol (up to 10 equivalents were used in attempts to generate the di-2,2,2-trichloroethyl acetal.) This was presumably due to the "deactivation" of the incipient oxonium ion by the 2,2,2-

trichloroethyl moiety. This "deactivation" was also apparent in the synthesis of **71b** with the yields of the respective reactions being substantially less than those utilizing bromoacetaldehyde diethylacetal. Treatment of **71b** with Zn° in ethylacetate at reflux for 30 minutes generated a sole product identical to that previously generated using the diethyl acetal **71a**. Unfortunately, upon attempted isolation of pure **67** severe decomposition resulted. The NMR was much like that previously observed upon treatment of **71a** under acidic protic conditions. This result suggested that decomposition of **71a** was primarily due to the inherent instability of **67** and not on the conditions used to create **67**.

A rationalization for the instability of **67** is presented in Figure 7. Upon formation of **67**, equilibrium between **67** and its iminium tautomer **88** exists.[19] Tautomerization to **89** yields the tetrahydropyrazine. This very unstable species[36] could subsequently revert to **88** or convert to iminium **90**. It is reasonable that the above equilibrations form very reactive intermediates by which **67** could decompose (Scheme 25).

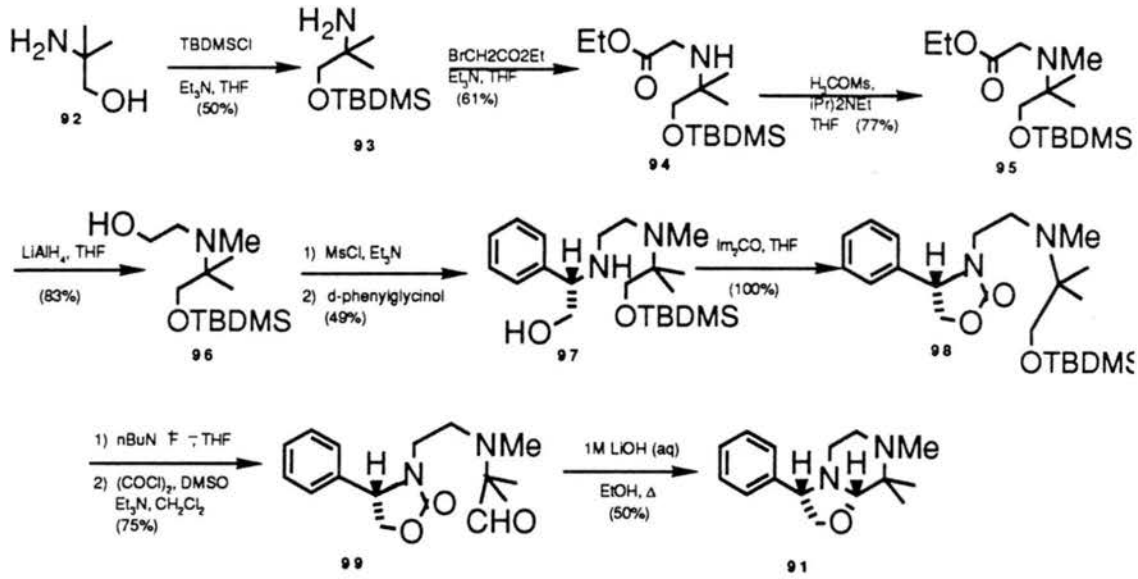
Scheme 25



It was rationalized that if the tautomerization of **88** to **89** could be prevented, stabilization of **67**, or its equivalent, could be achieved. To support this theory, the synthesis of the gem-dimethyl derivative **91** was undertaken which is incapable of tautomeric decomposition as proposed for **67** (Scheme 26).

Beginning with amino alcohol **92** and protecting the alcohol with *t*-butyldimethylchlorosilane resulted in the formation of **93** in 50% yield. The moderate yield is due to the volatility of the silane (bp: 63°C/18 mmHg). Alkylation of **93** with ethylbromoacetate furnishes **94**. N-methylation of the severely hindered amine with methylmesylate furnished **95**. Lithium aluminum hydride reduction of glycinate **95** then gave **96**.

Scheme 26

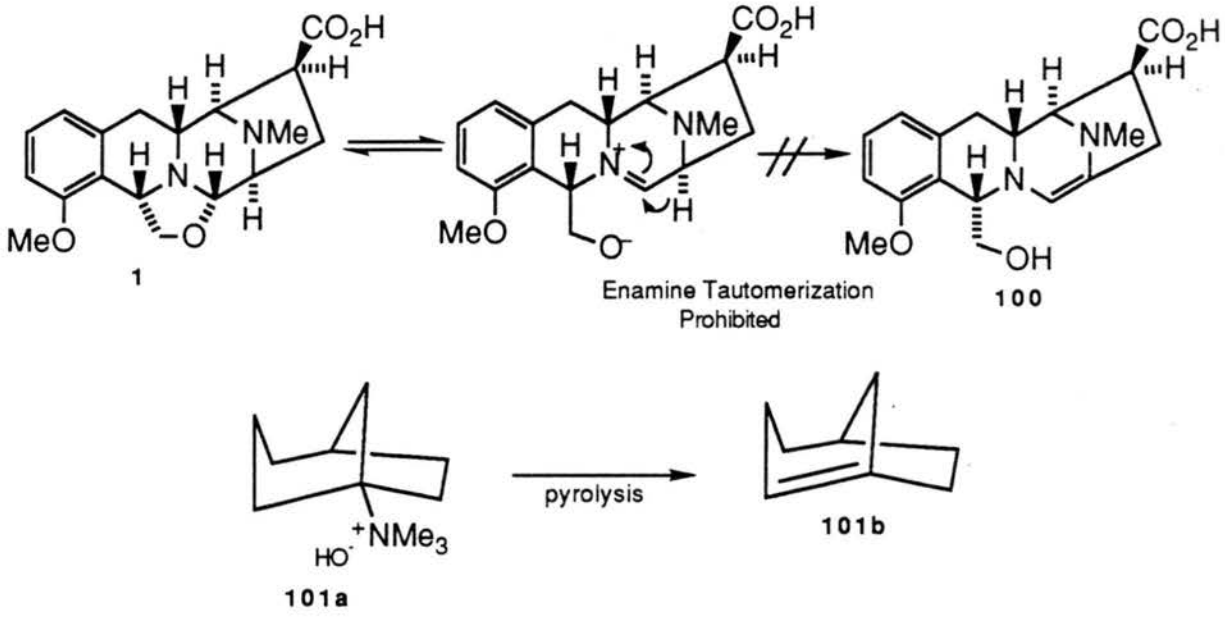


Using previously developed methodology **96** was mesylated and subsequently treated with *d*-phenylglycinol to give the amino alcohol **97**. Urethane formation mediated by 1,1'-carbonyldiimidazole then yielded **98**. Deprotection of **98** with tetra-n-butylammonium fluoride followed by Swern oxidation[37] furnished the stable amino aldehyde **99**. Oxazolidine formation of **91** was then accomplished via treatment of **99** with 4 equivalents of aqueous 1M LiOH in ethanol. The resulting oxazolidine **91** was a very stable entity supporting the aforementioned mode of decomposition of **67**. More importantly, however, is what this result implies for the reactivity of quinocarcin itself.

Since quinocarcin has the bicyclic iminoazepino[3.2.1] skeleton it seems unlikely that tautomerization to the "anti-Bredt" bridgehead dienamine **100**, (corresponding to **89**), is possible. This

is especially true if a qualitative comparison to the corresponding "anti-Bredt" bicyclo[3.2.1]oct-1-ene (**101**) is made (Scheme 27).

Scheme 27



Maier and Schleyer have postulated the following empirical rules relating the calculated olefinic strain (O.S.) energy and predicted experimental observability: a) isolable bridgehead olefins OS$\leq$17 kcal/mole; b) observable bridgehead olefins 17 kcal/mol $\leq$ OS $\leq$ 21 kcal/mol; and, c) unstable bridgehead olefins OS $\geq$ 21 kcal/mol.[38]

The bicyclo[3.2.1]oct-1-ene **101** has been implicated to have been formed in small amounts from pyrolysis of the trimethylammonium hydroxide salt, **101a**, by Chong and Wiseman;[39] the existence of **101b** was indirectly confirmed via a Diels-Alder trapping adduct. The olefin strain energy for **101b** has

been determined to be 28.6 kcal/mol[39] which places this ring system in the most strained catogory.

With the above information in mind, it seems unlikely that formation of **100** is a mode by which decomposition of quinocarcin can occur. This raises the question of whether the incapacity of enamine tautomerization of the bicyclic oxazolidine provides chemical stability to quinocarcin as well as being obligatory for biological activity.

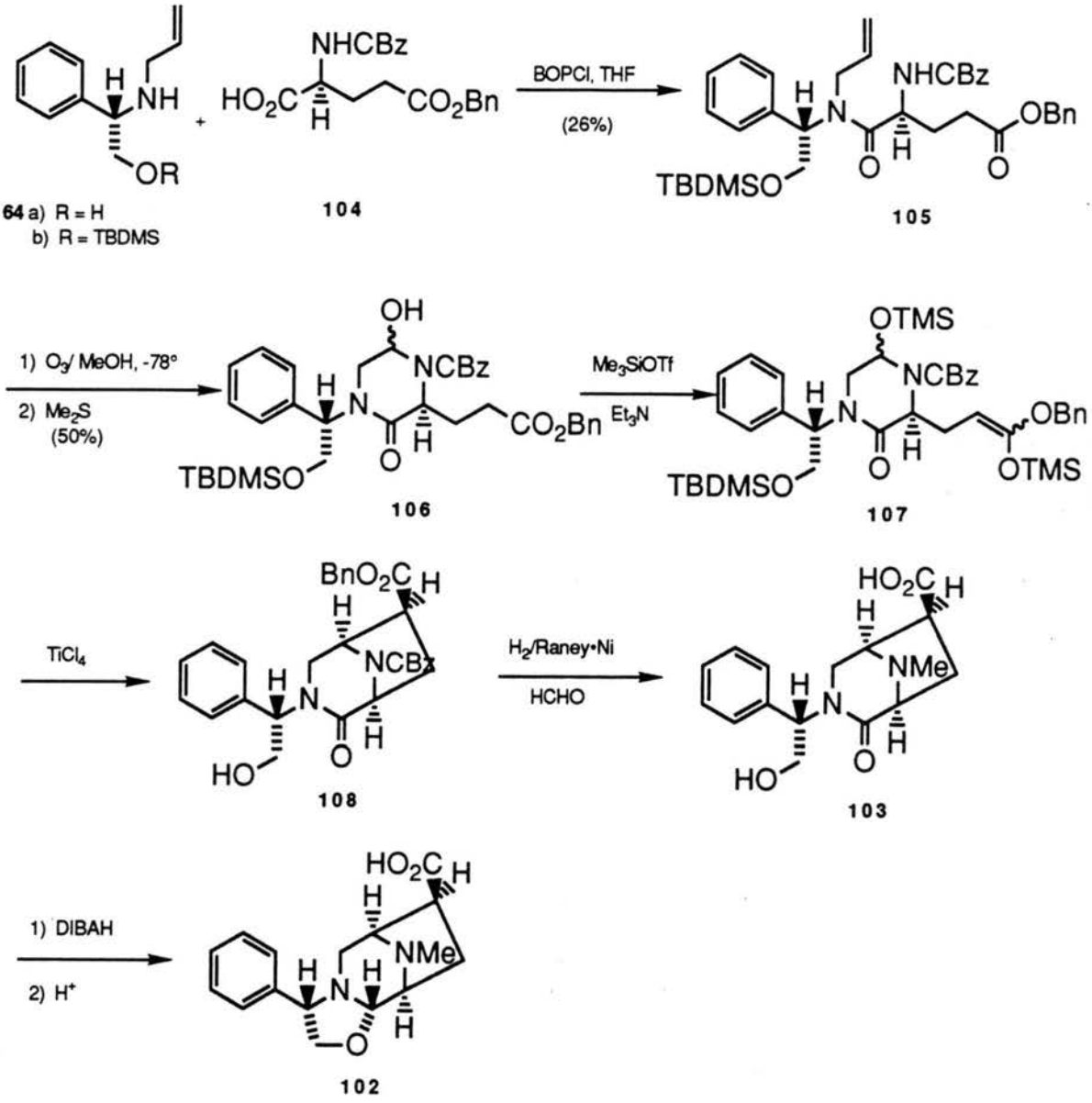
CHAPTER 2.2

STUDIES TOWARD TRICYCLIC BICYCLO[3.2.1]IMINOAZEPINO FUSED OXAZOLIDINES

From the aforementioned studies on the construction of the oxazolidine portion of quinocarcin, it seemed that this moiety could be constructed by the utilization of three separate reaction conditions: 1) from the diethylacetal, (i.e., **71a** → **67**), using protic acidic conditions; 2) from the 2,2,2-trichloroethylacetal, (i.e., **71b** → **67**), using non-polar aprotic conditions; or 3) from the urethane aldehyde (i.e., **99** → **91**), utilizing basic conditions. Thus, it seemed that all the possibilities for this conversion were considered.

The next study that was undertaken was the synthesis of tricyclic oxazolidine **102**. The proposed synthesis of **102** was designed in such a manner as to mimic the prospective final stages of the synthesis of quinocarcin. The initial approach was the construction of the bicyclo[3.2.1]iminoazepino lactam **103**, and then oxazolidine formation via selective hydride delivery to the lactam[28] (Scheme 28).

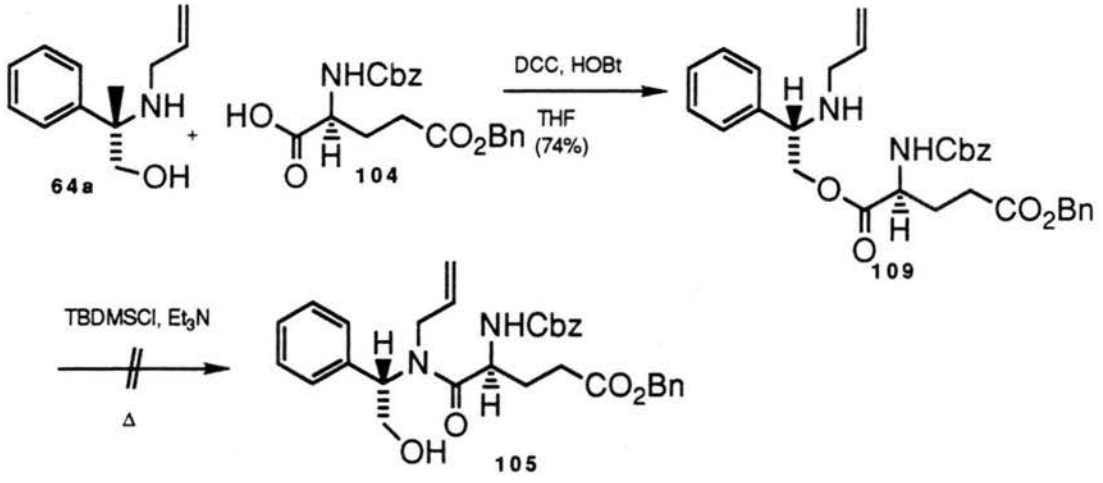
Scheme 28



Initial investigations on the coupling of N-allyl-phenylglycinol **64a** to the known glutamate[40] **104** proved problematic. Utilizing a variety of peptide forming conditions,[41] formation of the ester **109** was the only product observed.

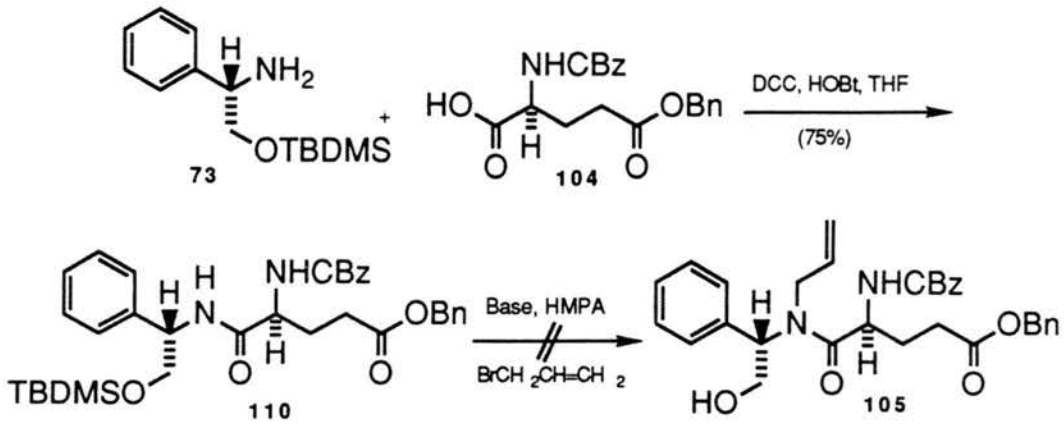
Attempted conversion of the ester **109** to the corresponding amide **105** was also unsuccessful (Scheme 29).

Scheme 29



Protection of **64a** was then required (TBDMSCl, Im, DMF, 63%), to form the O-silyl protected **64b**. Coupling of **64b** also proved problematic since intermolecular peptide bond formation was thwarted by intramolecular side reactions of the glutamate **104** via the activated carbonyl carbon.[42] The single reagent that was found to mediate coupling between **64b** and **104** was N,N-bis[2-oxo-3-oxazolidinyl]phosphordiamide chloride, (BOP-Cl),[43] which afforded **105** in a 26% yield (Scheme 28). With the realization that a low yield such as this would be detrimental to the total synthesis of quinocarcin a slightly different approach to **106** was attempted via the coupling of O-*t*-butyldimethylsilylphenyl glycinol, **73**, to glutamate **104** followed by N-allylation of the resulting amide, **110**, to produce the desired **105** (Scheme 30).

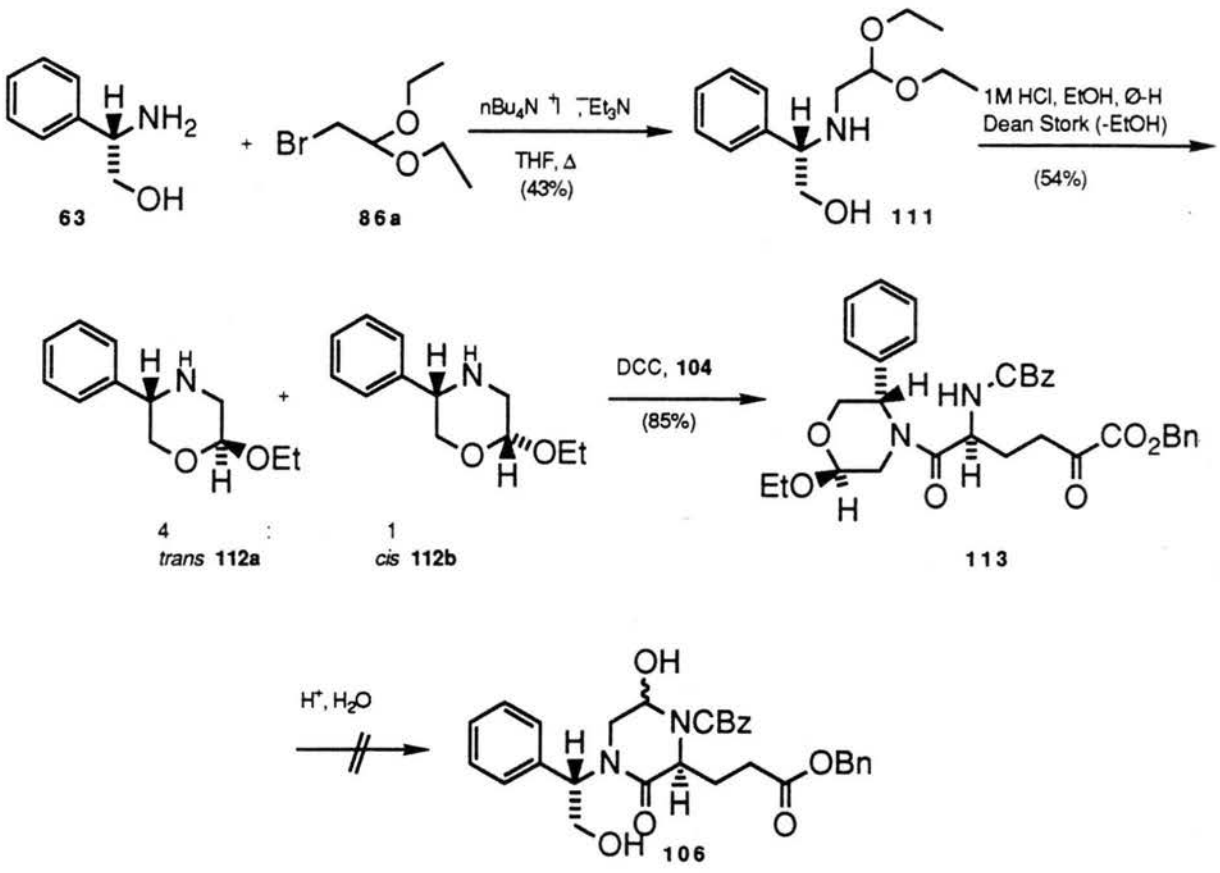
Scheme 30



Coupling of **64b** with **104** occurred smoothly yielding **110**. Unfortunately N-allylation of the amide to produce **105** was hindered by intramolecular cyclizations of the carbamate, (furnishing a urea), and the benzyl ester, (furnishing an imide), in approximately equal proportions. Ozonolysis of **105** (obtained in low yield, Scheme 28), in methanol resulted in conversion to the cyclic hemiaminal **106** in 50% yield.

Since the ozonolysis occurred in modest yield, and since the previous step also occurred in low yield, the formation of **106** via an alternate route was explored (Scheme 31).

Scheme 31



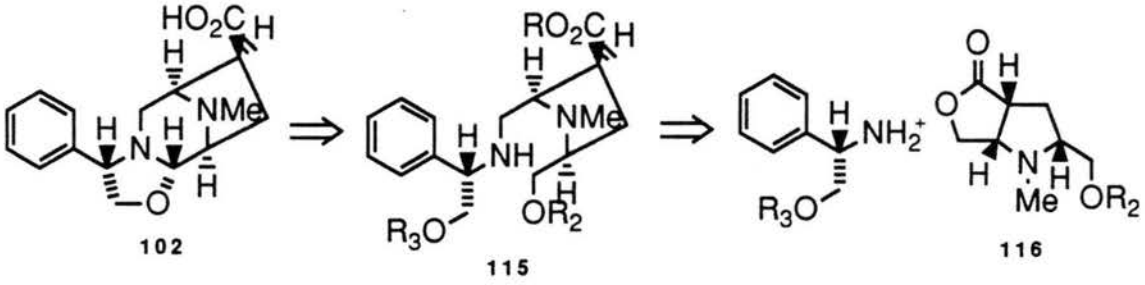
Alkylation of *d*-phenylglycinol, **63**, with **86a** furnished **111**. Morpholine formation resulted in a 4:1 ratio of **112a** and **112b**. Coupling of **112a** with **104** proceeded smoothly utilizing DCC to yield **113** in 85% yield. Attempted rearrangement to **114** was unsuccessful. The major product from treatment of **113** with various acids was hydrolysis of the ethoxy hemiacetal to the corresponding hydroxy acetal. Under more forcing conditions decomposition of **113** resulted.

At this point the results from the aforementioned piperazine fused oxazolidine model studies were complete (c.f. Chapter 2,

section 1). A conclusion of the foregoing studies was that the amide route involving a reductive ring closure to form the oxazolidine moiety would not be a viable approach. This information along with that of Danishefsky's[16] unsuccessful attempts at a reductive ring closure and the less than promising synthetic approach to the amide precursor **103** strongly suggested that the design of an alternate synthetic pathway was appropriate.

The approach that was subsequently designed took into account what had been learned from the studies directed towards oxazolidine formation and separated pyrrolidine formation from bicycloc[3.2.1]iminoazepino construction (Scheme 32).

Scheme 32



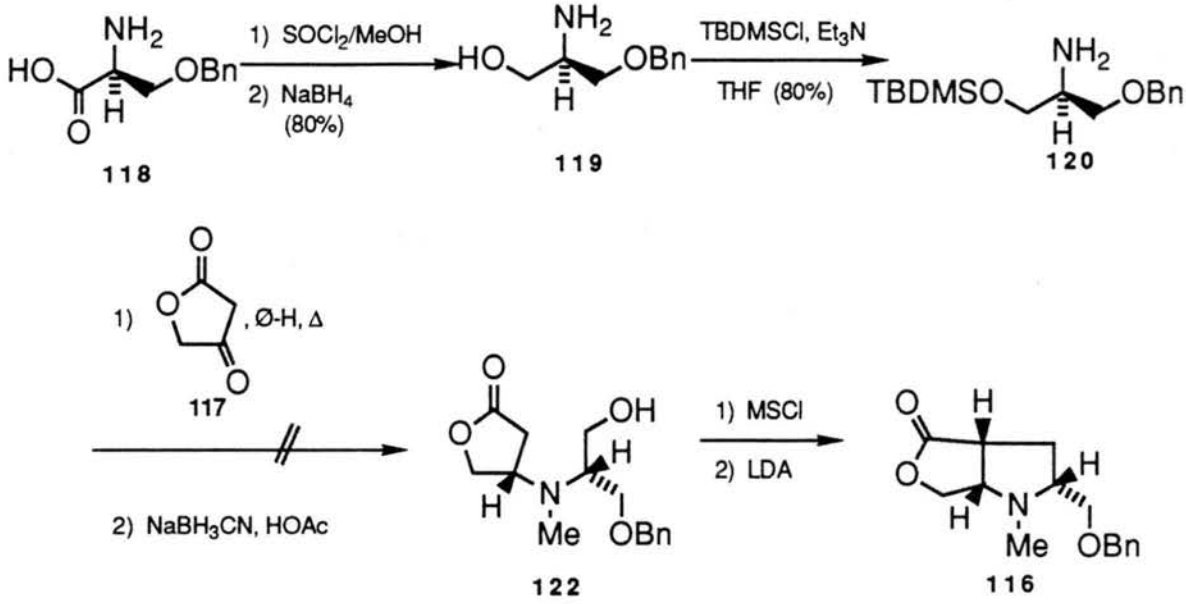
Retrosynthesis for **102**; second generation.

It was envisioned that the 2-hydroxymethyl, (pyrrolidine numbering) group of **115** would serve as a desirable precursor to **102**, in that its oxidative manipulation would be highly suited for oxazolidine formation. Furthermore, direct ring closure would furnish the corresponding quinocarcinol derivative. Construction of **115** was thought to occur via the appropriately protected

phenylglycinol and the properly substituted ring fused pyrrolidine lactone **116**.

The synthetic route to pyrrolidine **115** involved reductive amination of **120** to tetronic acid[44] **117** as the key step (Scheme 33).

Scheme 33

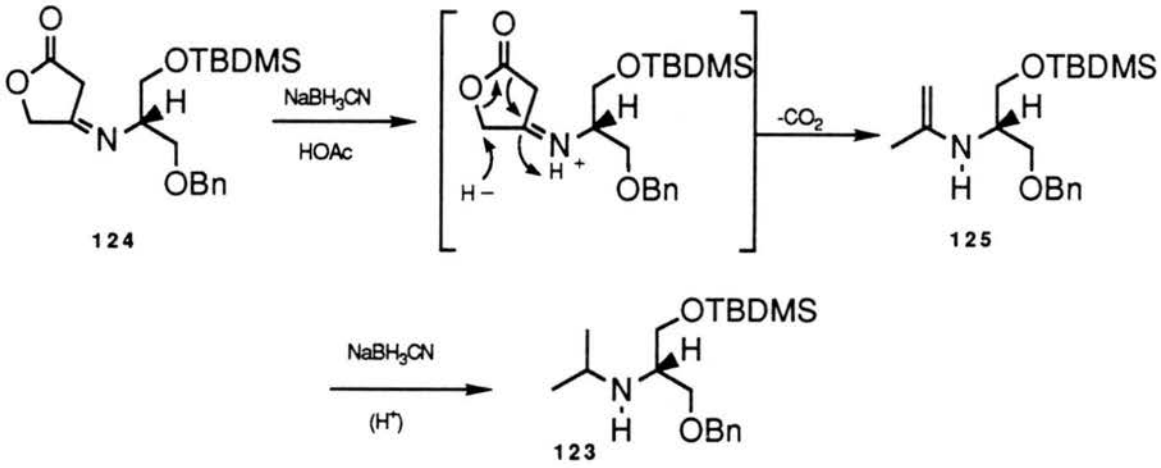


O-Benzyl-L-serine, **118**, was reduced to the corresponding amino alcohol **119** with $NaBH_4$ via its methyl ester hydrochloride. The alcohol **119** was then protected with t-butyldimethylchlorosilane to afford the differentially protected amine **120**. The amine **120** was chosen as an intermediate because one could generate either antipode depending on deprotection. Also, one could generate an "unnatural" D center from an L amino acid.[45] Unfortunately reductive amination with tetronic acid mediated by

sodium cyanoborohydride in acetic acid (or HCl/methanol)[46] did not furnish the desired amino lactone **121** but, instead, furnished the corresponding isopropyl alkylated amine **123**.

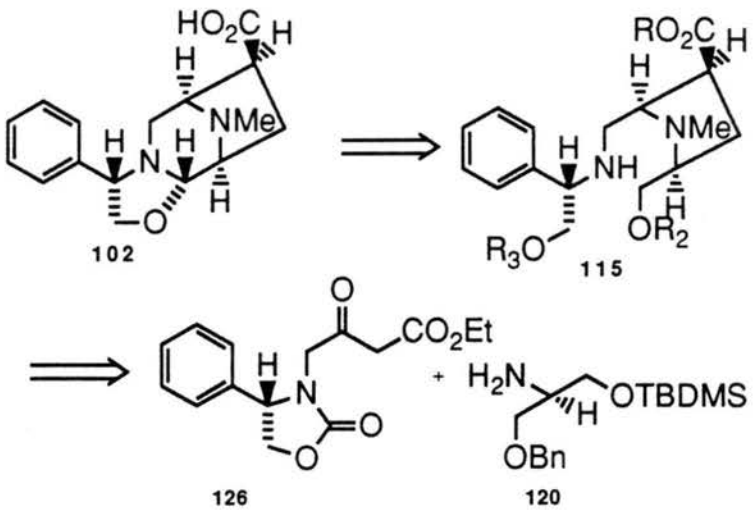
The reductive amination of tetronic acid with amine **120** was abandoned; however, the concept was not. It was thought that the mechanism for the formation of **123** from the imine **124** was a hydride mediated decarboxylation followed by enamine (**125**) reduction (Scheme 34).

Scheme 34



It was reasoned that if the lactone, or more precisely the tetronic acid portion of **124** were opened and an ethyl or t-butyl ester installed no decarboxylation would take place. Furthermore, the b-keto ester could be directly attached to the phenyl glycinol portion of **102** (Scheme 35).

Scheme 35



Retrosynthesis for **102**; third generation.

Synthesis of b-keto ester **126** was then undertaken starting with the known ethyl(phenylglycinol)-N-acetate **127**.[47] Urethane formation with 1,1'-carbonyldiimidazole provided **128**, from **127**. Hydrolysis of the ethyl ester then yielded the very crystalline acid **129**, in 75% overall yield from phenyl glycinol with no chromatography required. b-Keto ester formation was attempted utilizing Meldrum's acid,[48] with poor results. Better results were obtained by using the method of Brooks, Masumune and Lu, in which the magnesium salt of the malonate mono-ester acted as the ethyl acetate anion equivalent.[49] With **126** in hand, reductive amination was performed with **120** which furnished the coupled product **130** as a 1:1 mixture of inseparable diastereomers.

The lack of diastereoselectivity in the formation of **130** was not a major concern. It was thought that in the quinocarcin total synthesis the tetrahydroisoquinoline would provide the added

rigidity required for an increase in diastereoselectivity. In addition, the adjacent stereocenter would increase the possibility for the desired diastereomer to be formed.

Nevertheless, one attempt at a diastereoselective coupling was performed utilizing the amino alcohol, **119**. It was reasoned that the free alcohol could induce diastereoselectivity through the intermediacy of oxazolidine formation with the b-keto ester **126**.[50] Added diastereoselectivity was modest, however, yielding the coupled product **131** as an inseparable (3:2) mixture of diastereomers. The aforementioned result was encouraging since a more rigid system, such as the actual isoquinoline, should impart greater selectivity in this respect.

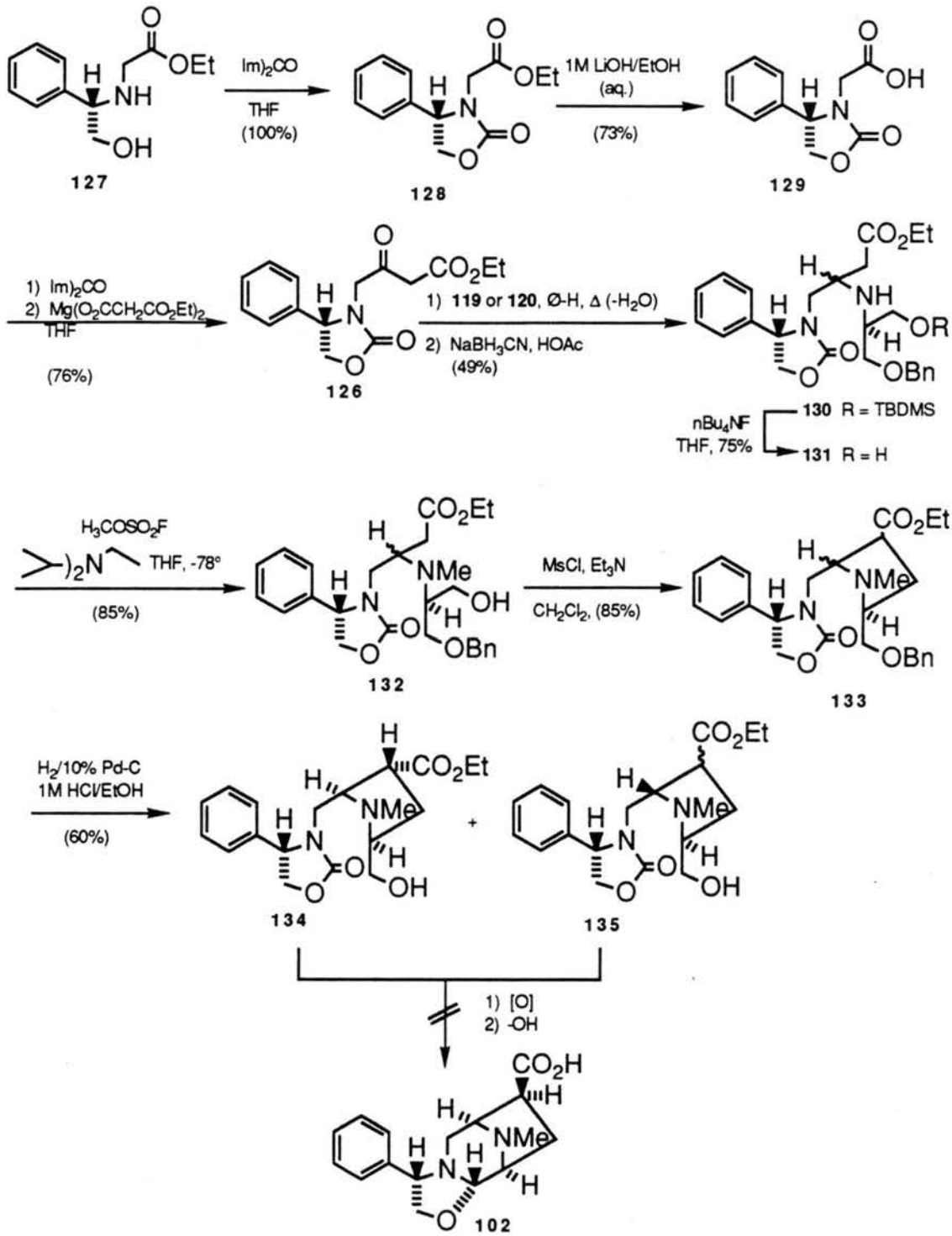
N-Methylation of **131** proved to be problematic and required forcing conditions (fluoromethanesulfonate at -78°) to furnish the desired pyrrolidine precursor **132**. Initial experimentation in the ring closure reaction showed that the desired transformation could be achieved via treatment of **132** with mesyl chloride followed by pyrrolidine formation with LDA at -78°C.[51] It was later discovered that the desired transformation would also occur using excess triethylamine (2.5 equivalents) and performing the reaction in anhydrous methylene chloride at 0°C. This novel mode of pyrrolidine formation under such mild conditions was investigated more thoroughly and was found to be a general method with synthetic utility and will be the sole subject of the next section of this chapter.

Pyrrolidine **133**, as an inseparable mixture of three diastereomers, was then subjected to hydrogenolysis conditions to

afford a 1:1 mixture of alcohols **134** and **135**. Alcohol **134** was a single diastereomer which was assigned the cis stereochemistry at the C-2 and C-5 positions, (pyrrolidine numbering). Alcohol **135** was a mixture of two diastereomers, presumably epimers at the carboethoxy center at the 4-position (pyrrolidine numbering). These are reasonable assignments based on the fact that in **134** the thermodynamic stereoisomer at position 4 should be anti to the cis C-2, C-5 centers to minimize any steric constraints. In alcohol **135** the trans C-2, C-5 centers do not allow any relief from the C-1, C-2 or C-1, C-3 interactions on the pyrrolidine ring; thus, the carboethoxy center is epimeric.

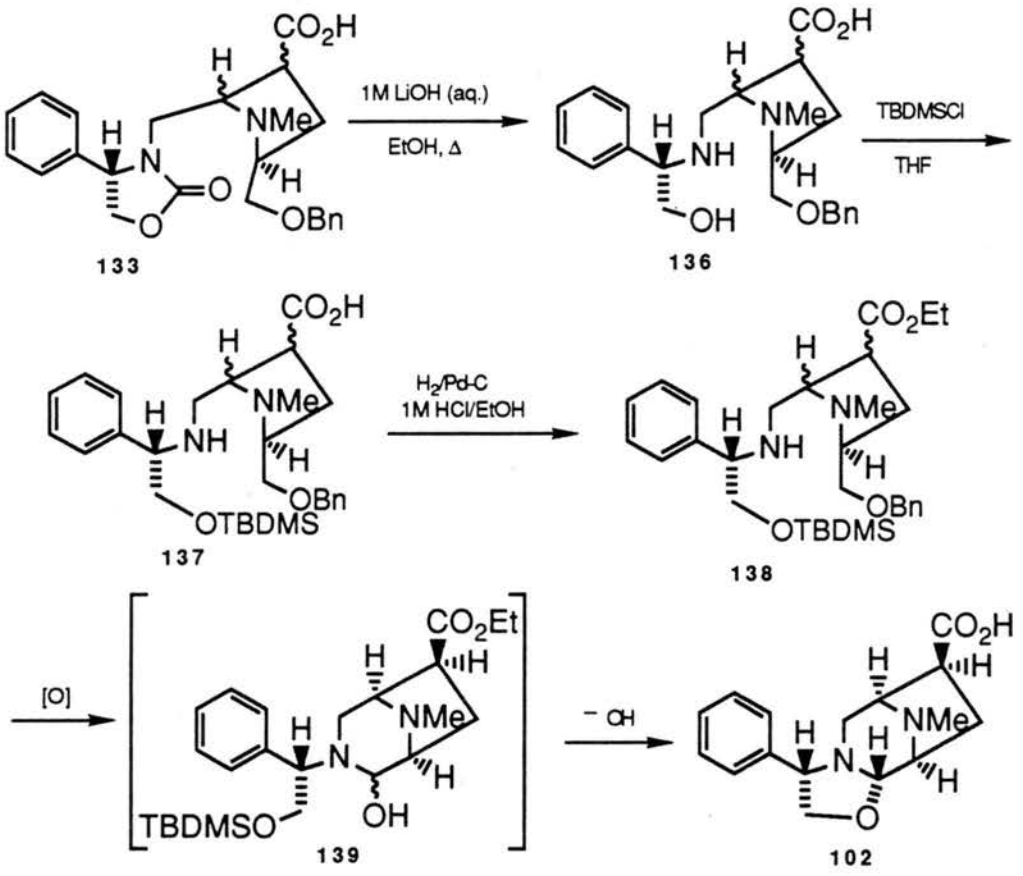
Oxazolidine formation to produce **102** proved to be the pitfall of this approach. Under a variety of oxidizing conditions a stable aldehyde of **134** or **135** could not be formed. It was thought that this was due to the inherent instability of a-amino aldehydes which are enolizable.[52] (When not protected at nitrogen with bulky groups to hinder the approach of base, a-amino aldehydes readily racemize and decompose.[53]) Tandem oxidation followed by treatment with base (LiOH/EtOH) with the objective of trapping the aldehyde as the oxazolidine (as per the formation of **91**) also resulted in the decomposition of the alcohols **134** and **135** (Scheme 36).

Scheme 36


Im)2CO
THF
(100%)
1M LiOH/EtOH
(aq.)
(73%)
1) Im)2CO
2) Mg(O2CCH2CO2Et)2
THF
(76%)
1) 119 or 120, Ø-H, Δ (-H2O)
2) NaBH3CN, HOAc
(49%)
nBu4NF
THF, 75%
130 R = TBDMS
131 R = H
H3COSO2F
THF, -78°
(85%)
MsCl, Et3N
CH2Cl2, (85%)
H2/10% Pd-C
1M HCl/EtOH
(60%)
1) [O]
2) -OH
127
128
129
126
132
133
134
135
102

With the aforementioned results in mind it was rationalized that the urethane protecting group should be removed. This would provide a better chance of trapping the aldehyde as the hemiaminal, followed by deprotection of the hydroxymethyl group to form the bicyclic oxazolidine **102** (Scheme 37).

Scheme 37

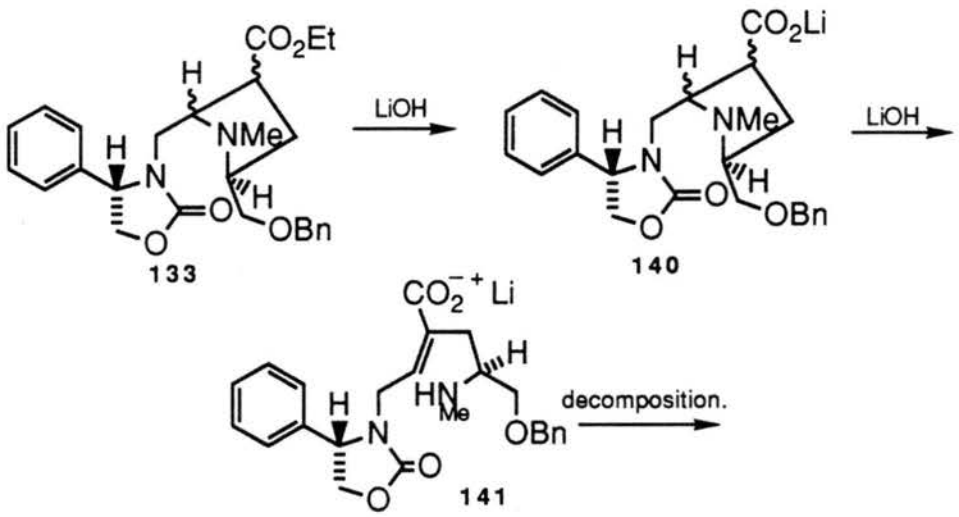


The above scheme represents the envisioned transformations that would be required to obtain the desired hemiaminal, **139**, en route to the formation of **102**. Unfortunately, treatment of **133** under the strongly basic conditions required for urethane deprotection, resulted in decomposition of the starting material.

Hydrazine was also utilized in this attempted deprotection, however, only amide formation resulted, and no urethane deprotection was evident.

Treatment of **133** with hydroxide at room temperature resulted in immediate saponification of the ethyl ester. Prolonged reaction times, however, produced a variety of products. The mode by which **133** decomposed was thought to have proceeded through the amino acid salt **140** (Scheme 38).

Scheme 38

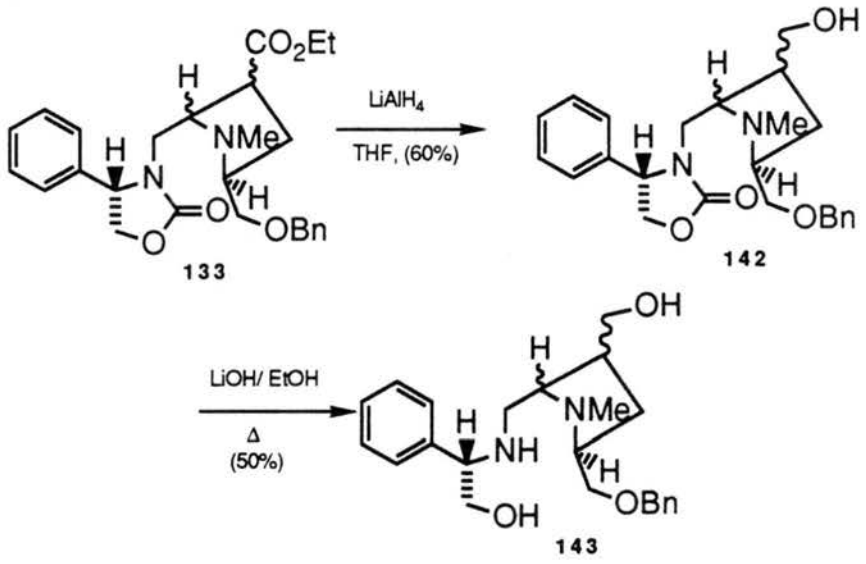


Upon hydrolysis of the ethyl ester of **133** to form the amino acid salt **140** the proton adjacent to the carboxylate increases in pKa to 35 (or greater). It then becomes thermodynamically favorable to eliminate the amino function of the pyrrolidine (pKa = approximately 30) which subsequently could protonate from the solvent, pKa = 16 (ethanol). (The pKa cascade would be the driving force for this event: 35 Æ 30 Æ 16). The ring opened pyrrolidine is

then a sufficiently nucleophilic entity to undergo ring closure to the carbonyl or further base initiated degradation of **141**.

Evidence for this type of base promoted decomposition of **133** was supported by the reduction of the ethyl ester to the corresponding hydroxy methyl compound **142**. Deprotection of the urethane then proceeded smoothly to yield **143** (Scheme 39).

Scheme 39

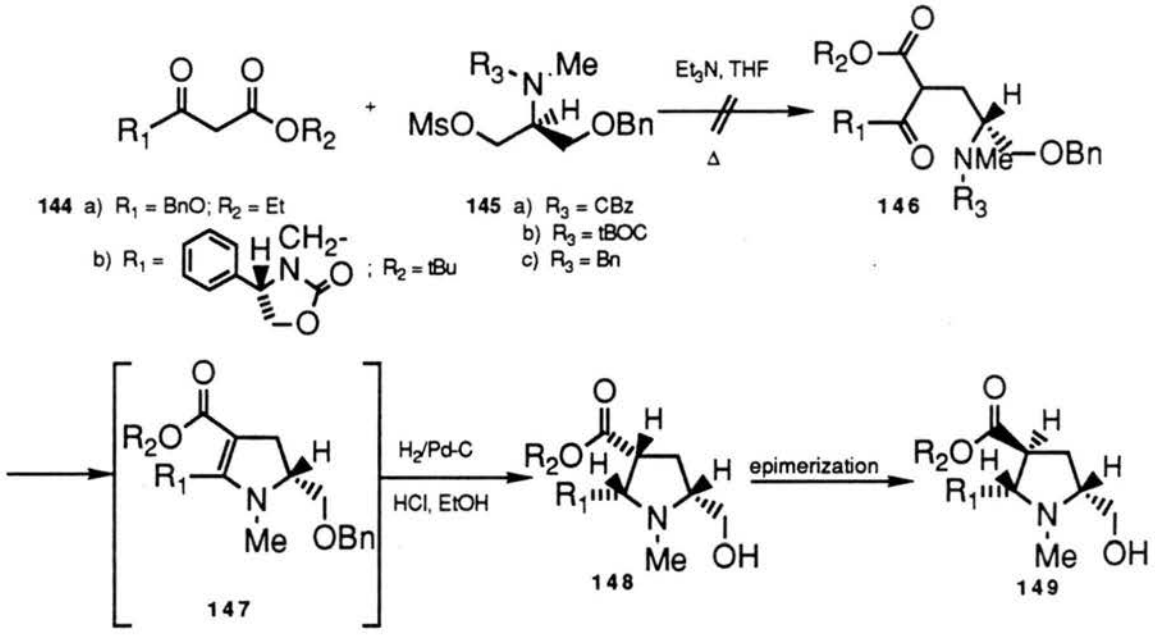


The above results led to the conclusion that it was necessary to replace the urethane as a protecting group for the amino alcohol function. Unfortunately, the urethane moiety was introduced into the synthesis in the second step of the twelve step sequence to **102** (c.f. Scheme 19). Thus, it was very important to consider the selection of new protecting groups that would be able to survive the anticipated synthetic transformations.

Another concern in the synthesis of **102** that would directly bear on the synthesis of quinocarcin was the poor diastereomeric selectivity encountered in the reductive amination step (c.f.

Scheme 19, conversion of **126** Æ **131**). It was resoned that the reductive amination would proceed with better diastereoselectivity if there was a more rigid transition state and catalytic reduction was utilized.[54,55](Scheme 40)

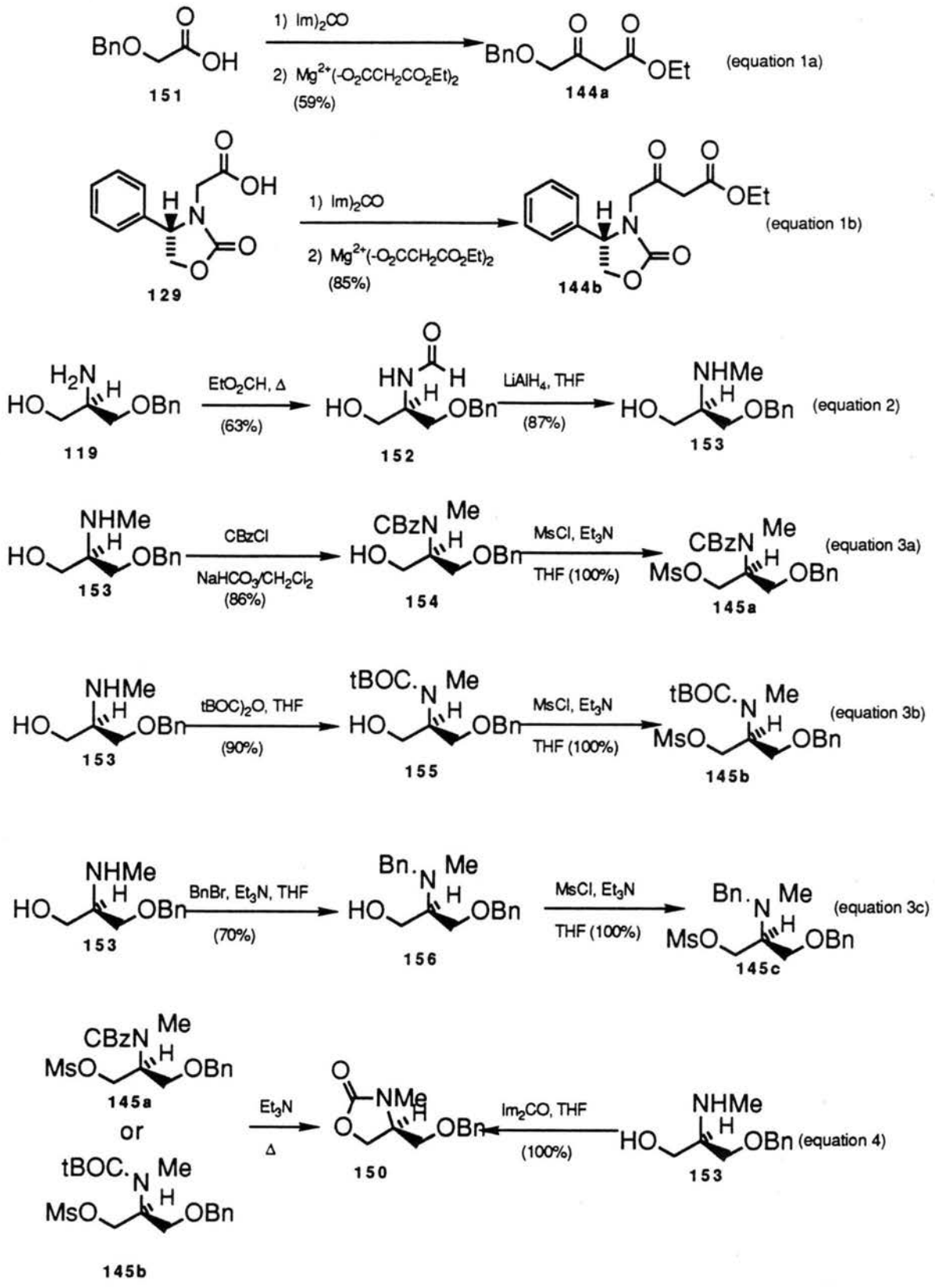
Scheme 40



Unfortunately, upon reaction of the b-keto esters **144a** or **144b** with the protected amino mesylates, **145a-c**, in the presence of base, no desired coupling was observed. With **145a** and **145b**, cyclic urethane **150** formed exclusively. When the N-benzyl amine **145c** was utilized, decomposition of the starting material resulted, presumably through the formation of alkyl aziridinium intermediates.[56]

Scheme 41 shows the syntheses of the aforementioned starting materials utilized in the attempted asymmetric pyrrolidine synthesis outlined in Scheme 40.

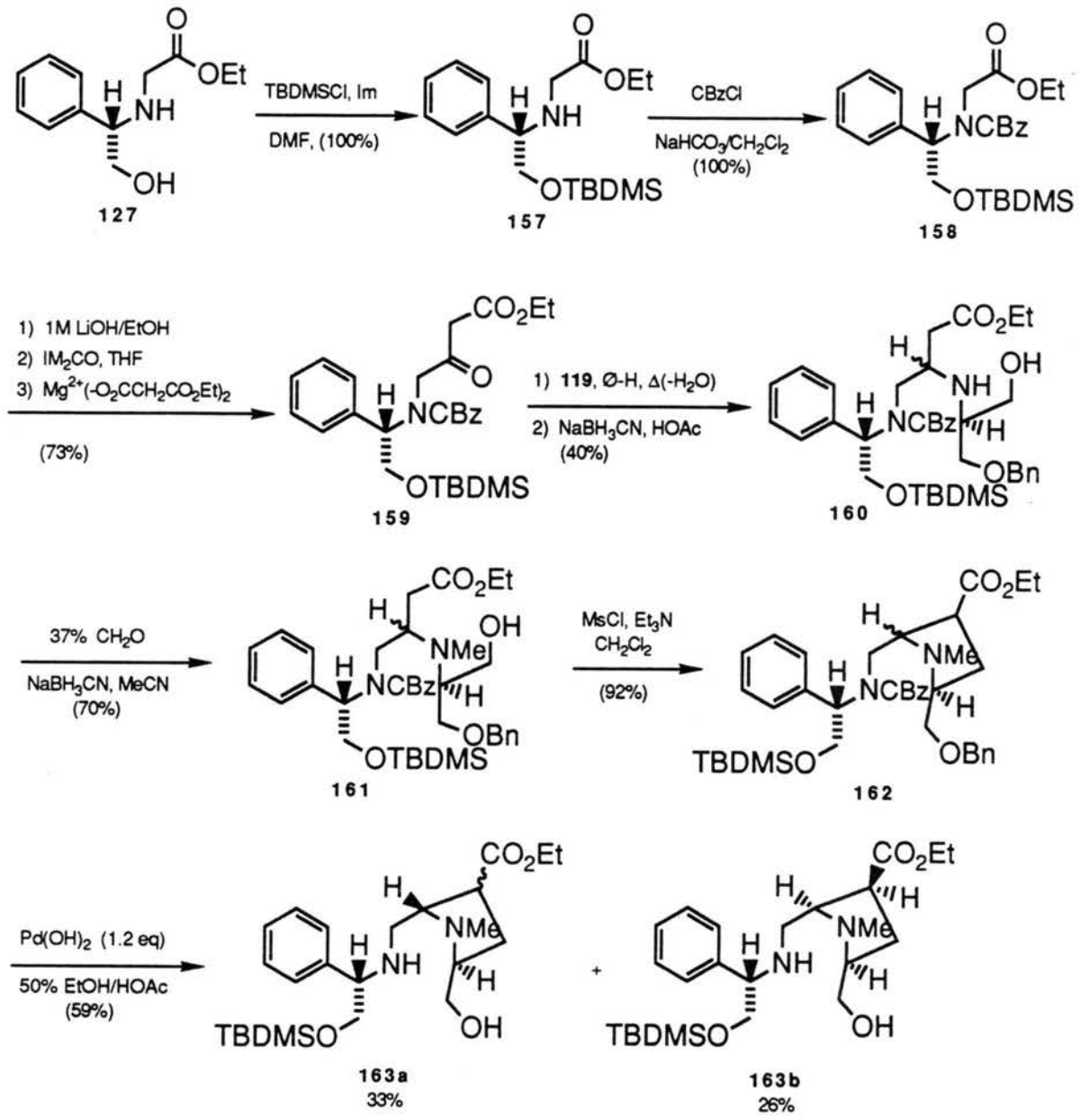
Scheme 41


1) Im)2CO
2) Mg2+(-O2CCH2CO2Et)2
(59%)
151
144a
(equation 1a)
129
1) Im)2CO
2) Mg2+(-O2CCH2CO2Et)2
(85%)
144b
(equation 1b)
119
EtO2CH, Δ
(63%)
152
LiAlH4, THF
(87%)
153
(equation 2)
CBzCl
NaHCO3/CH2Cl2
(86%)
154
MsCl, Et3N
THF (100%)
145a
(equation 3a)
tBOC)2O, THF
(90%)
155
MsCl, Et3N
THF (100%)
145b
(equation 3b)
BnBr, Et3N, THF
(70%)
156
MsCl, Et3N
THF (100%)
145c
(equation 3c)
or
Et3N
Δ
Im2CO, THF
(100%)
150
(equation 4)

b-Keto esters **144a** and **144b** were constructed via the method of Brooks, Masumune and Lu from the benzyloxyacetic acid **151**[57] and the previously described phenylglycinol based urethane **129** (Scheme 24 equations 1a and 1b). N-Methylation of serinol **119** was achieved via the formate **152** by $LiAlH_4$ mediated reduction which furnished exclusively monomethylated serinol **153**, (Scheme 24, equation 2). Upon utilization of **153** as a common starting material for equations 3a-c (Scheme 24), **145a-c** were synthesized in excellent overall yields, using the appropriate electrophile under standard conditions. Equation 4 depicts the formation of cyclic urethane from the mesylates **145a** or **145b**. The structure of **150** was proven by its facile synthesis from **153** utilizing 1,1'-carbonyldiimidazole. Formation of **150** from **145a** or **145b** presumably proceeded through the nucleophilic attack of the carbonyl of the carbamates.[58]

With the failure of an alternate pyrrolidine synthesis it was decided that the low diastereoselectivity previously observed would be tolerated. The synthesis was still envisioned to go through the amino alcohol **163b** en route to the oxazolidine **102** (Scheme 42).

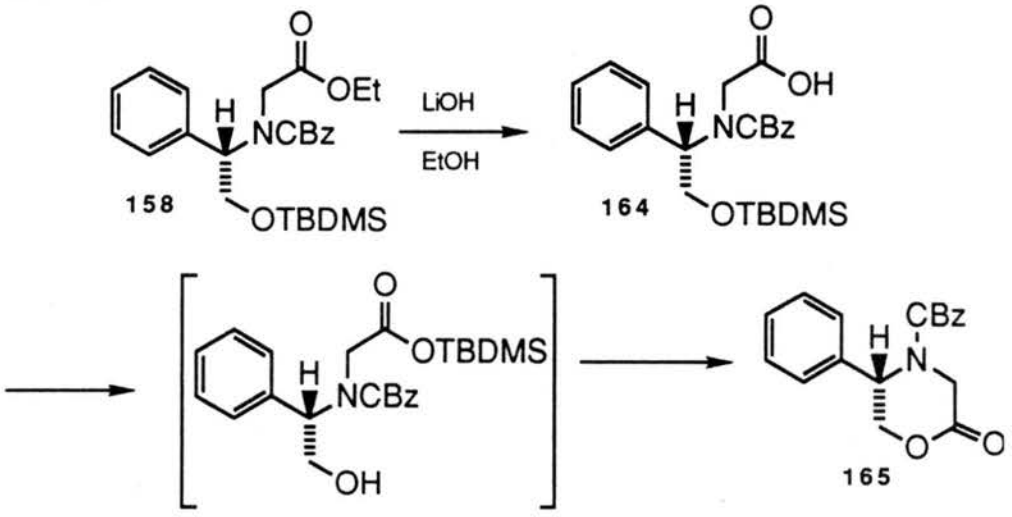
Scheme 42



Synthesis of the amino alcohol **163b** followed the previously devised methodology (c.f. Scheme 19), with only moderate

difficulties encountered. Beginning with the N-(carboxyethyl)-methylphenyl glycinol **127** and subsequently protecting the alcohol with t-butylchlorodimethylsilane produced **157**; nitrogen protection with benzylchloroformate furnished **158**. Tandem ethyl ester hydrolysis followed by b-keto ester formation was necessary due to competing lactone formation. When isolated and allowed to stand at room temperature, the acid **164** underwent a silyl migration-mediated rearrangement to the lactone **165**[59] (Scheme 43).

Scheme 43



In order to minimize this event, the acid **164** was treated with 1,1'-carbonyldiimidazole immediately upon isolation. With b-keto ester **159** in hand, reductive amination with **119** was then performed under standard conditions which produced **160** as a mixture of diastereomers in approximately a 3:2 ratio. Unfortunately these diastereomers were inseparable by HPLC, and were therefore carried on as a mixture. N-Methylation was

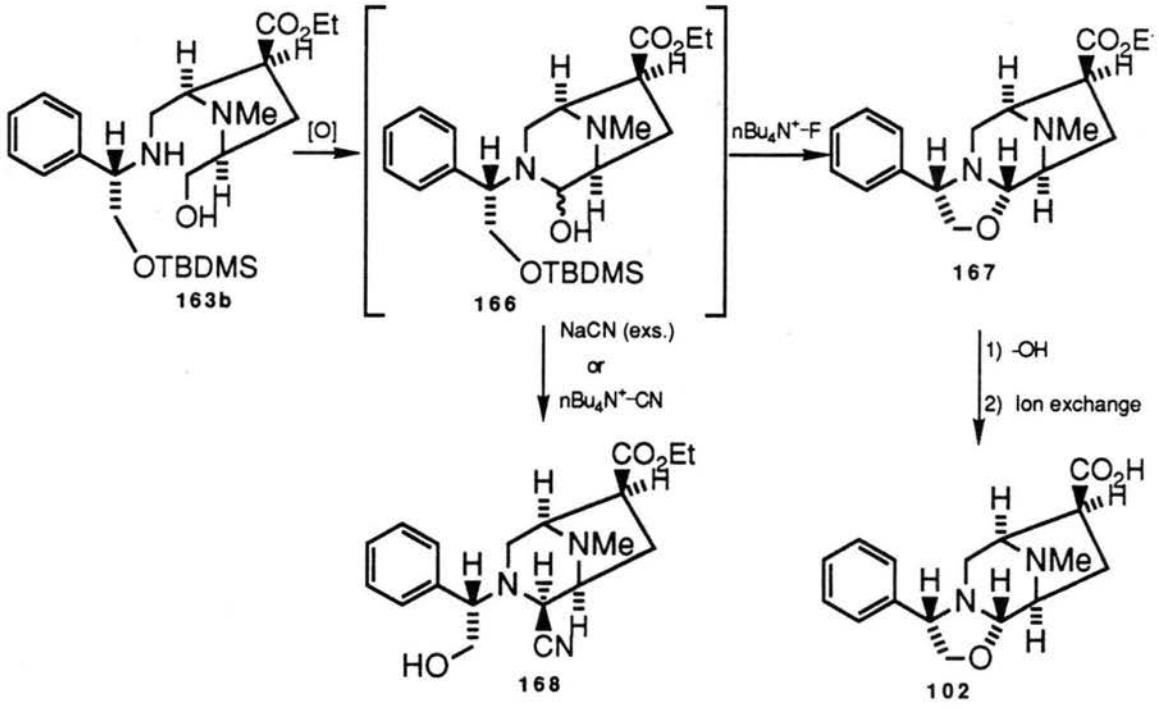
performed with formaldehyde and sodium cyanoborohydride rather than fluoromethanesulfonate because of the anticipated lability of an intermediate oxazolidine to reduction. This was indeed the case as the hindered oxazolidine can, in fact, be isolated. With **161** in hand, pyrrolidine formation proceeded under standard conditions in high (92%) yield to furnish **162** as a mixture of 3 diastereomers in a 1.5:1.5:2 ratio. At this point, it was realized how valuable this novel ring closure was (predominantly due to the extensive functionality present in **161**); therefore, several pyrrolidines were synthesized exploiting this novel methodology. (Which will be presented in the following section.)

After extensive study it was found that it was necessary to use greater than one equivalent of $Pd(OH)_2$ to afford the desired deprotection of **162**. With less than one equivalent of $Pd(OH)_2$, the hydrogenolysis stopped after Cbz deprotection. This was presumably due to the ethylenediamine functionality in **162** which was viewed as an excellent bidentate chelater, which in turn poisoned the catalytic activity of the palladium.[60] Upon hydrogenolysis of both N and O protecting groups, the separation of the products **163a** and **163b** was realized. Amino alcohol **163a** was an inseparable mixture of two diastereomers whereas **163b** was a single diastereomer. It was presumed that the steric interaction (C-2, C-4 and C-2, C-5) of **163b** was minimized with the carboethoxy group trans to the two and five positions (pyrrolidine numbering). If these two positions were cis then **163b** would have been a single diastereomer. Conversely, the trans C-2 - C-5 orientation of **163a** would not allow for this type of bias when

the C-3 - C-4 bond was constructed in the conversion of **161** Æ **162**, and; therefore, it seems reasonable to assign the stereochemistry illustrated.

With **163b** in hand the aforementioned conversion to **102** via the hemiaminal **166** was attempted (Scheme 44).

Scheme 44



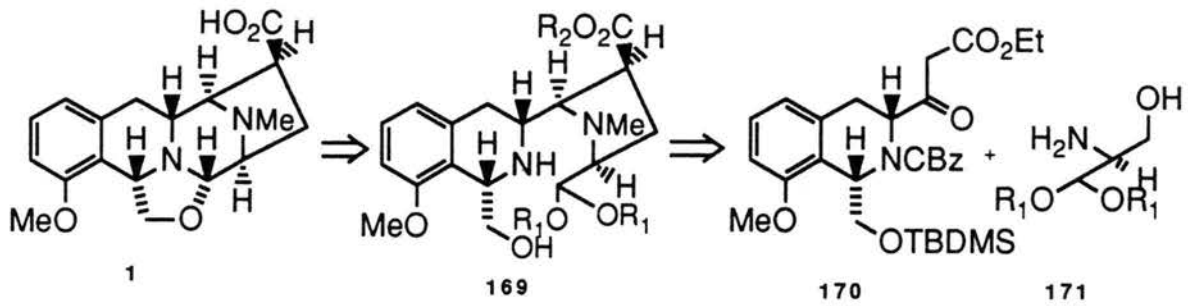
Under a variety of oxidative conditions (acidic, basic, and radical),[61] hemiaminal **166** could not be isolated. Sequential deprotection of the crude oxidation reaction products, with tetrabutylammonium fluoride, in an effort to "trap" the product also failed to produce **167**.

When hindsight was used to rationalize the failure to isolate **167**, one needed only to consider the attempts outlined in Chapter

1 in the syntheses of quinocarcin analogs containing the bicycloimino-azepino[3.2.1]ring system. In all cases cited, the hemiaminal was trapped *in situ* with cyanide and not isolated. Furthermore the hemiaminal in these syntheses was deemed too unstable to isolate.[62]

Attempted trapping with cyanide ion in the case of **166** did not yield the desired **168**. Whether this failure was due to the *in situ* oxidative conditions or not remains moot as the literature procedure was under reducing conditions.[63] In any event, the formation of **102** from **163b** could not be realized, which, in and of itself, was not necessarily bad news. What the aforementioned study did imply, however, was that the acetal retrosynthetic approach to quinocarcin was the most likely to produce the desired results (Scheme 45).

Scheme 45



It was envisioned that amino acetal **169** would be derived from the tetrahydroisoquinoline b-keto ester **170** and the appropriately protected serinal moiety **171**.

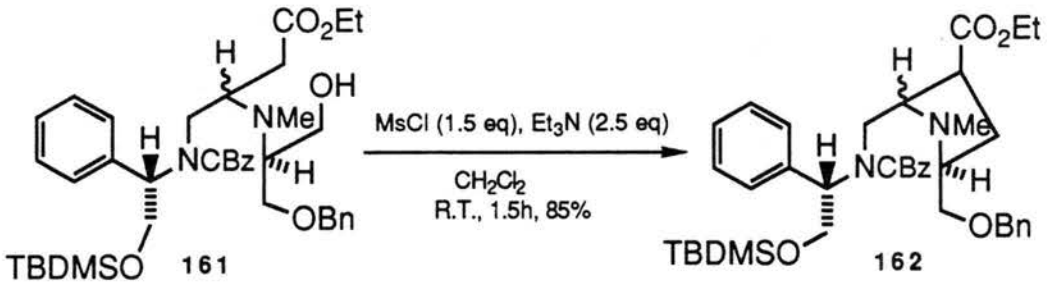
Further studies with the synthesis of **102** were abandoned, predominantly due to the piperazine fused oxazolidine investigations previously reviewed (c.f. Chapter 2, Section 1). These studies implied that the acetal function, when appropriately protected, serves as a viable functionality for oxazolidine synthesis. This information, coupled with the methodology developed for pyrrolidine synthesis, led to the retrosynthetic approach outlined above, and was thought to be one that had a high probability for success.

CHAPTER 2.3

PYRROLIDINE SYNTHESES - AN INTERLUDE

As stated in the preceeding section, the unusually facile ring closure forming the pyrrolidine **162** from the amino alcohol **161** in the presence of diverse functionality, prompted us to explore this novel methodology (Scheme 46).

Scheme 46

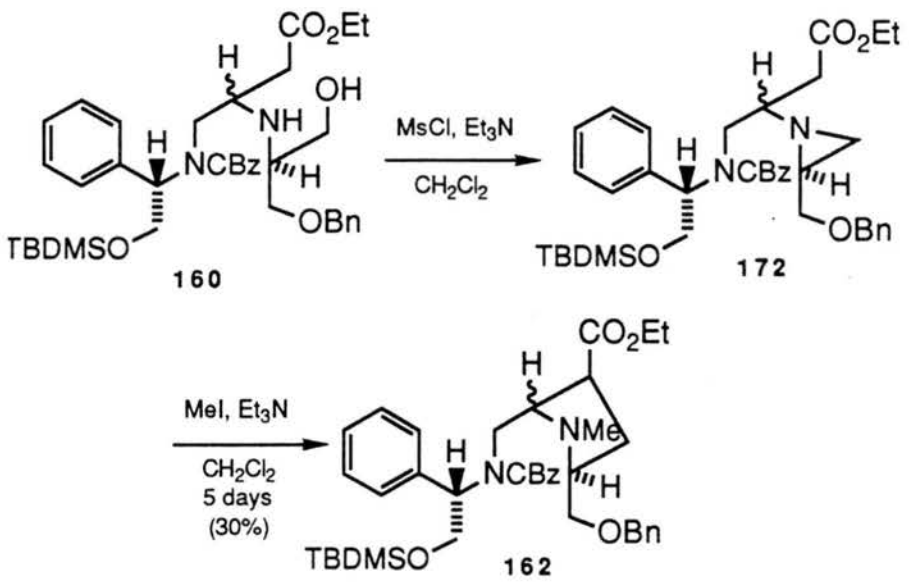


Under the conditions used for the formation of the pyrrolidine ring system, it was assumed that a very reactive (electrophilic) intermediate was responsible for the facility of this carbon-carbon bond forming reaction.

A plausible mechanism that accounts for the facility of this reaction invokes an alkyl aziridinium salt (or its electrophilic equivalent) as the reactive intermediate. There are several pieces of evidence in support of such a mechanism. Preliminary investigations utilizing **160** and subjecting it to the same reaction

conditions cited above (Scheme 47) lead to the formation of the aziridine **172**.[64] Upon treatment of **172** with methyl iodide at room temperature over a period of several days, the product pyrrolidine **162** was isolated in modest yield (Scheme 47).

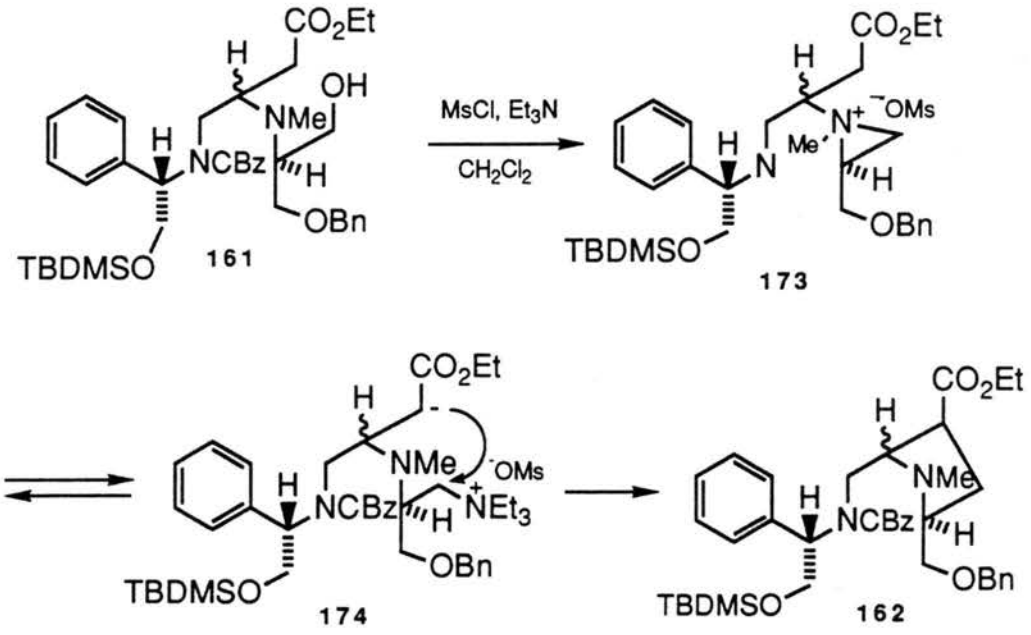
Scheme 47



The intermediacy of an alkyl aziridinium salt was also supported by the use of a different solvent in which salt solvation was suppressed (i.e., THF). When the reaction was performed in THF the product, **162**, was not formed from **161**. This piece of data suggested that the putative alkyl aziridinium was <u>not</u> the species which undergoes ring closure. This conjecture was reasonable based on the required geometric orientation that the enolate would have to assume to achieve an S_N2 like transition state with an aziridinium species.[65] However, if a dynamic equilibrium between the opened and closed aziridine (mediated by triethylamine) existed, a very

reactive primary alkyl ammonium ion intermediate would be present that would be capable of adopting the requisite transition state geometry (Scheme 48).

Scheme 48

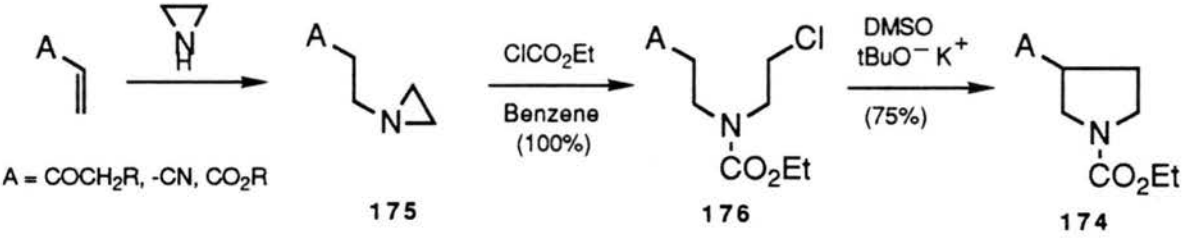


A species such as **174** as the reactive intermediate was also supported by the aforementioned study with **172** and methyl iodide (Scheme 47). It seemed that the quaternization of **172** with methyl iodide would produce the intermediate alkyl aziridinium species (equivalent to **173**), which would also be prone to elimination as well as ring opening to **174**; this would explain the modest yield of **162** from **172**. Also, with the utilization of methyl iodide, the iodine itself presented a problem in that it promoted the formation of the primary iodide rather than the triethylammonium species **174**.[65] Thus, with the agency of methyl mesylate instead of methyl

iodide in the conversion of **172** to **162,** one would expect to more closely mimic the reaction conditions that were present in the conversion of **161** to **162**. Indeed, when **172** was treated with methyl mesylate the yield of **162** doubled to 62%, thus supporting the existence of a dynamic equilibrium between salts such as **173** and **174**. Of these two species, **174** was most likely the carbon-carbon bond forming precursor.

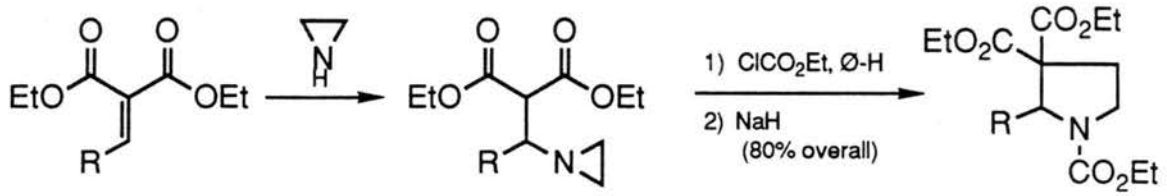
There exists other reports of pyrrolidine syntheses utilizing aziridines as intermediates in a similar manner to that described above (i.e., not as 1,3-dipolar substrates, which is the classical use of aziridines in the synthesis of pyrrolidines[65]). One such report by Dolfini[51] utilized ethyleneamine additions to a variety of electrophilic olefins which formed compounds such as **175**. Upon treatment of **175** with a chloroformate, quarternization took place followed by ring cleavage providing **176**. Exposure of **176** to potassium tert-butoxide in DMSO furnished the pyrrolidine **177**, which completed the heteroannulation sequence (Scheme 49).

Scheme 49



Koning, in a very similar approach, used the Michael addition of ethyleneamine to α,β-unsaturated malonates to generate the chloroethylamine carbamate precursor **178**[66] (Scheme 50).

Scheme 50



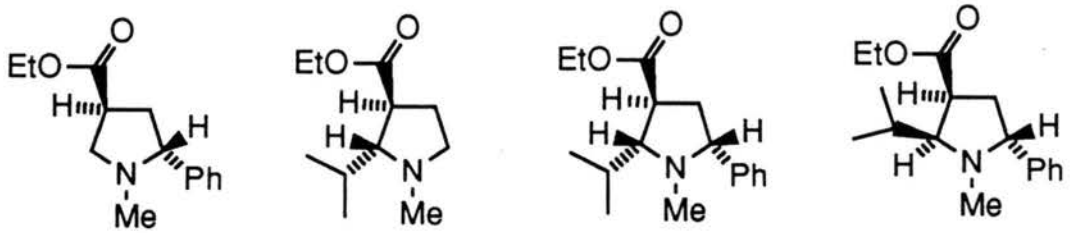
178 **179**

R = $(CH_2)_6CN$, $(CH_2)_6CO_2Me$, $(CH_2)_6CO_2Et$

Tandem exposure of **178** to ethylchloroformate followed by sodium hydride furnished **179** in 80% overall yield from the α,β-unsaturated malonate.

Utilizing the ring closure methodology that we had developed, pyrrolidines **180**, **181**, **182**, and **183** were synthesized (Scheme 51).

Scheme 51

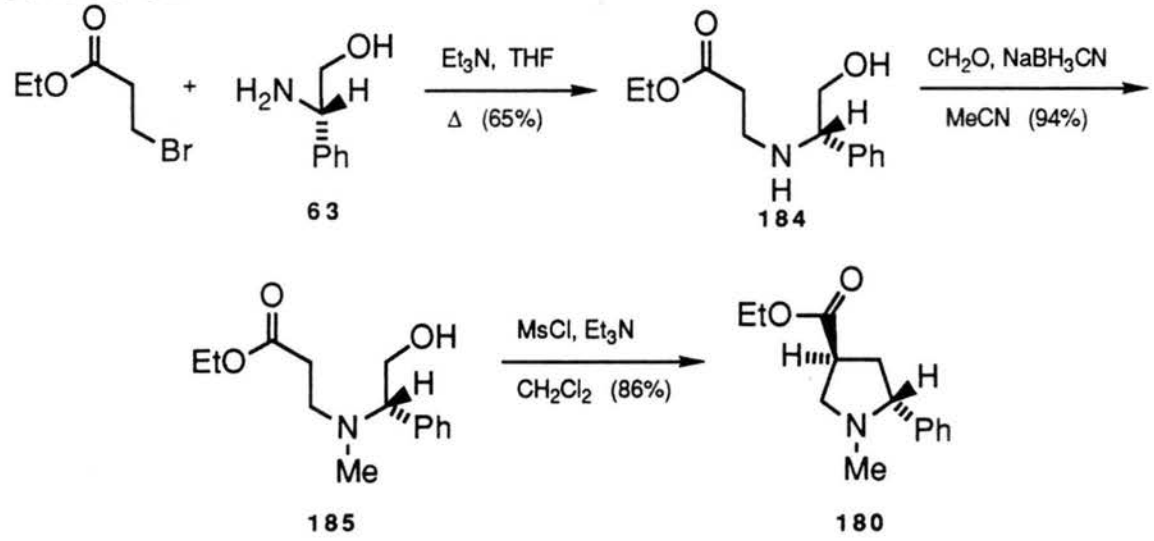


180 **181** **182** **183**

The same ring closure methodology was used to form the three different substitution patterns of pyrrolidines in Scheme 51. The precursors utilized were all intentionally different in order to explore the scope and limitations of this mode of pyrrolidine synthesis.

The synthesis of the 1,2,4-substituted pyrrolidine **180** was accomplished via the alkylation of *d*-phenylglycinol, **63**, with ethyl-3-bromopropionate which furnished **184**. N-Methylation then produced the desired pyrrolidine precursor **185**. Subjecting **185** to the standard ring closure conditions yielded **180** as a single diastereomer (Scheme 52).

Scheme 52

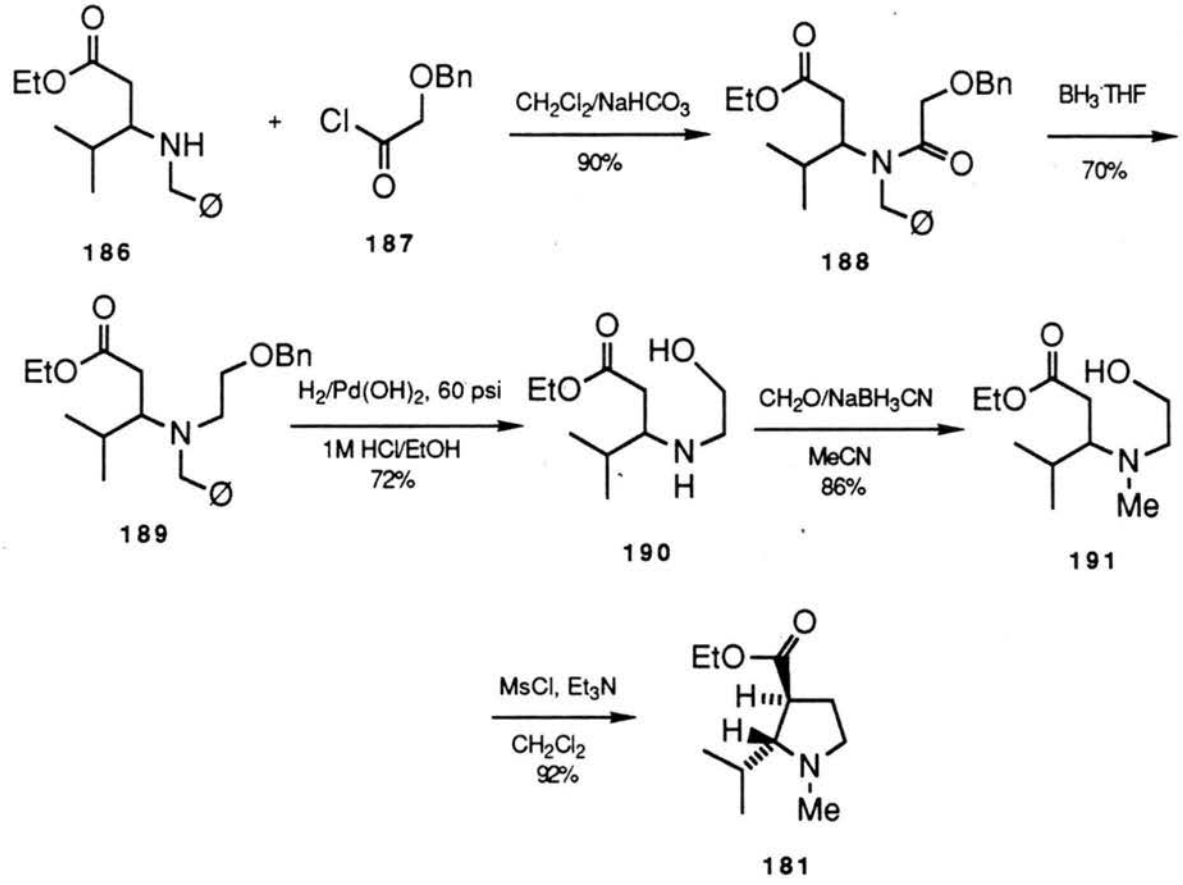


Treatment of **180** with a catalytic amount of ethoxide ion in ethanol failed to yield a different compound and resulted in a 90% recovery of parent **180**. Therefore, this substance was assigned the trans orientation since this should be the thermodynamic product. The synthesis of **180** complemented that of Dolfini's work on

Michael additions of ethylene amine to acrylates as previously described.

The synthesis of **181** was from the known β-amino ester **186**.[67] Coupling of **186** to benzyloxyacetyl chloride[57] **187** furnished the amide **188**. Reduction to the tertiary amine yielded **189** which was deprotected using palladium hydroxide in 1M HCl/EtOH which furnished the amino alcohol **190**. N-Methylation under standard conditions then gave **191** which was subjected to standard ring closure conditions furnishing **181** (Scheme 53).

Scheme 53

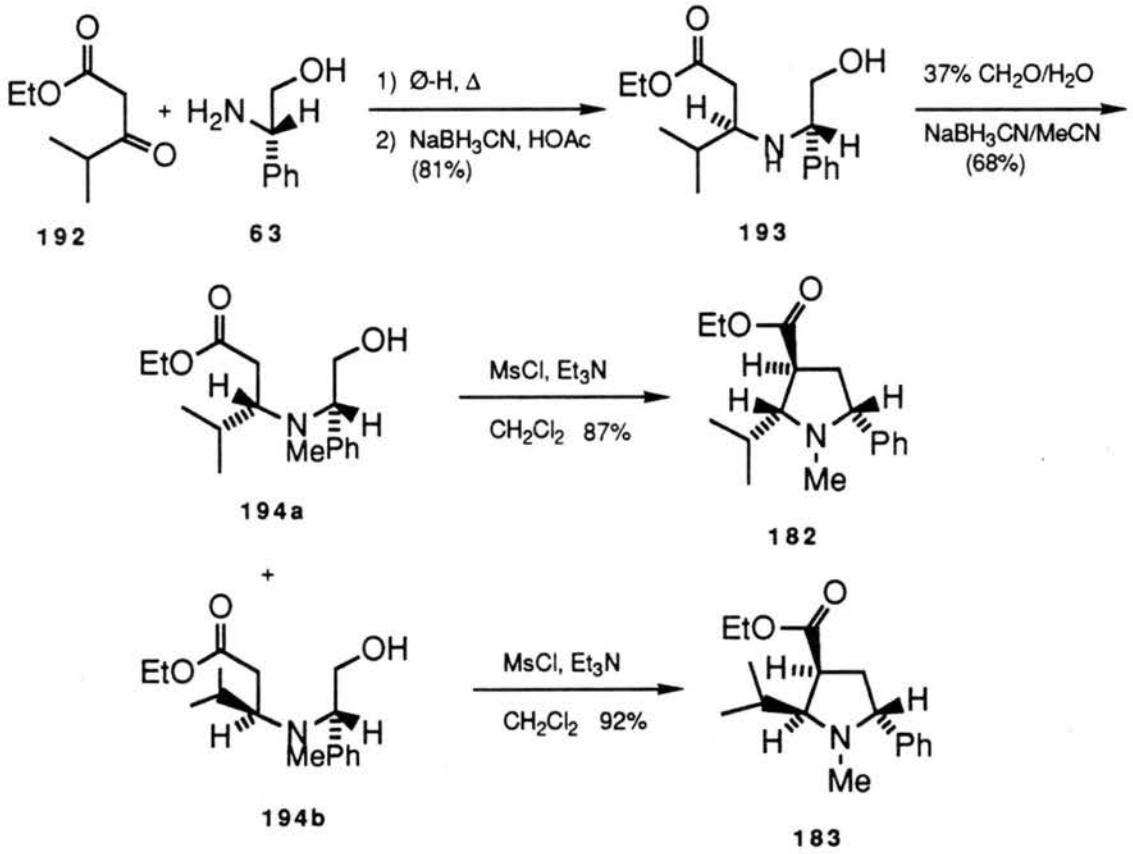


The stereochemical assignment of **181** was based on an analogy to **180**. Exposure of **181** to a catalytic amount of ethoxide

resulted in no change and, therefore, **181** was assigned the *trans* C-2, C-3 stereochemistry.

Commercially available β-keto ester **192**, and *d*-phenyl glycinol, **63**, were subjected to the reductive amination conditions previously employed; **193** was created as a 1:1 mixture of inseparable diastereomers. Fortunately, upon N-methylation **194a** and **194b** were furnished and readily separated. Using standard ring closure methodology, **194a** and **194b** yielded **182** and **183**, respectively (Scheme 54).

Scheme 54



192 **63** **193**

194a **182**

194b **183**

Stereochemical assignments of **182** and **183** were based on three factors: 1) NOE experiments showed the proper enhancement[68] (Appendix I); 2) upon treatment of **183** with a catalytic amount of ethoxide no change in R_f of the pyrrolidine was evident; however, NMR showed the presence of the C-4 epimer as a 1:1 mixture; 3) upon treatment of **182** with a catalytic amount of ethoxide, no change was evident by NMR. With the aforementioned data, the stereochemical assignments of **182** and **183** seemed reasonable.

With the synthesis of **180**, **181**, **182**, and **183**, the utility of this novel pyrrolidine synthesis has been demonstrated. It has been shown that, by utilizing this methodology, a variety of precursors can be used, including (but not limited to): β-hydroxy amines, β-amino acids, α-hydroxy acids and β-keto esters, to introduce functionality on the pyrrolidine ring.

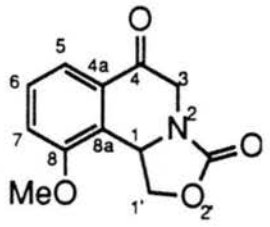
CHAPTER 3

CHAPTER 3.1

1-HYDROXYMETHYL-8-METHOXY-1,2,3,4-TETRAHYDROISOQUINOLINE-4-ONE SYNTHESIS

Initial investigations into the synthesis of quinocarcin required the construction of the "left hand side" of the molecule; namely, the synthesis of the urethane protected 1-hydroxymethyl-8-methoxy-1,2,3,4-tetrahydroisoquinoline-4-one, **195** (Scheme 55).

Scheme 55



195

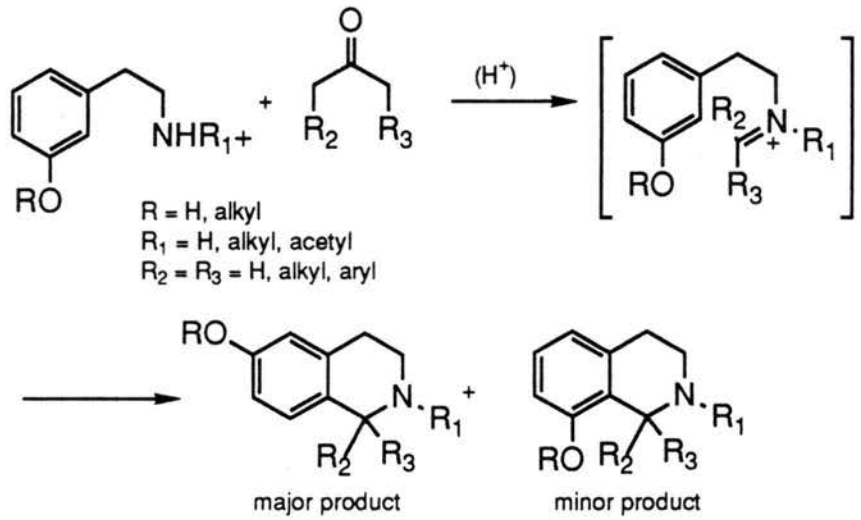
Selection of this molecule as a target upon which to base the total synthesis of quinocarcin required careful consideration. The presence of the keto functionality in the 4-position of **195** allows for the necessary elaboration at the 3-position. The 1-hydroxymethyl moiety is an obvious requirement for later oxazolidine formation, and the 8-methoxy substituent is a functional requirement that is present in quinocarcin itself.

Upon surveying the literature for methods to construct the appropriately substituted 8-methoxy isoquinoline it was found that classical approaches could not be utilized. This was predominantly due to the regiochemical requirement posed by the 8-methoxy substituent in **195**.

Perhaps the most widely used synthetic construction of the isoquinoline skeleton is the Picket-Spengler approach. This method utilizes β-arylethylamines and, upon condensation with carbonyl compounds, the tetrahydroisoquinoline is formed through the intermediacy of the putative Schiff base. The rate accelerating effect of an electron donating group generally induces cyclization (ortho, para) to occur at the less hindered para position (Scheme 56).

Scheme 56

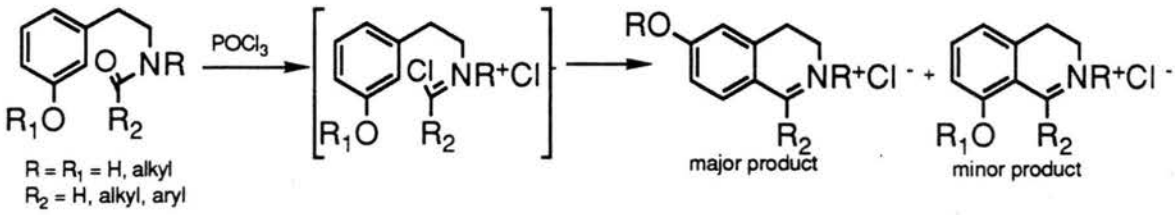
The Picket-Spengler Cyclization



The related Bischler-Napieralski reaction furnishes the 3,4-dihydroisoquinolines through an electronically similar electrophilic aromatic substitution, which also results in the formation of the 6-oxygenated regioisomer as the major product (Scheme 57).

Scheme 57

The Bischler-Napieralski Cyclization

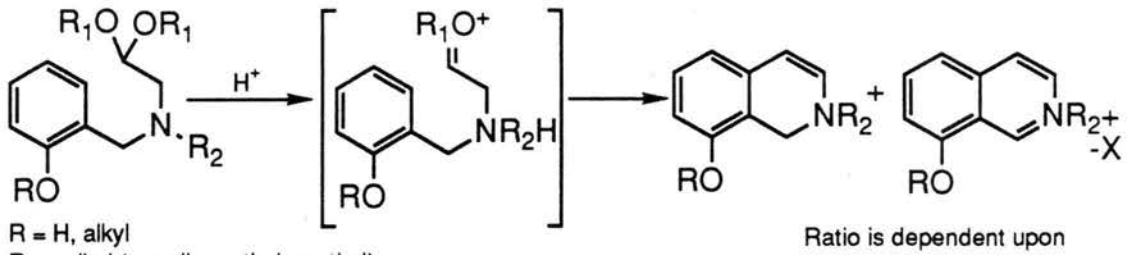


R = R_1 = H, alkyl
R_2 = H, alkyl, aryl

The Pomeranz-Fritsch approach constructs the 1,2-dihydroisoquinoline by utilizing α-amino acetal precursors. Regiochemistry is not a problem in this case. However, oxygenation in the meta position results in lowered yields, presumably due to the forcing conditions required to affect ring closure on systems which have minimum activation. Products, in general, tend to aromatize to the isoquinoline or to their corresponding isoquinolinium salts (Scheme 58).

Scheme 58

The Pomeranz-Fritsch Cyclization



R = H, alkyl
R_1 = alkyl (usually methyl or ethyl)
R_2 = alkyl, aryl

Ratio is dependent upon "H^+" and nature of R_2.

Among the aforementioned classical isoquinoline syntheses there exists an additional problem associated with using these methodologies for the synthesis of **195**, namely, the oxygenation in the C-4 position. The Picket-Spengler and Bischler-Napieralski cyclizations will not tolerate the deactivating keto group at C-4. The 1,2-dihydroisoquinoline formation by the Pomeranz-Fritsch approach does give rise to functionality which can be viewed as a masked keto group, but is rather sensitive to substituents at the C-1 and C-3 position. In this case, the forcing conditions required to affect C-4/C-4a bond construction would be incompatible with the functionality that we required within the tetrahydroisoquinoline skeleton.

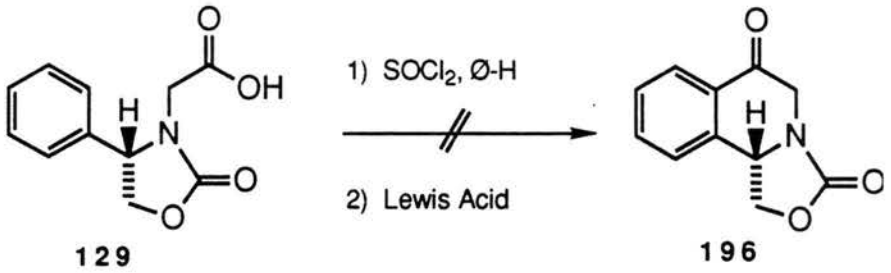
Thus, two choices remained in the construction of **195**: 1) to develop novel methodology that would be a reliable and unambiguous synthetic protocol that would embrace the 8-oxygenated 1,2,3,4-tetrahydroisoquinoline nucleus; or 2) block undesired regioisomer formation via halogenation para- to the

methoxy group in the Picket-Spengler and/or Bischler-Napieralski approaches.

Of these choices, the second was not considered; thus, a new synthetic approach to **195** was devised.[69]

Initial investigations began with **129** using an approach that was related to the Pomeranz-Fritsch cyclization (Scheme 59).

Scheme 59



129 **196**

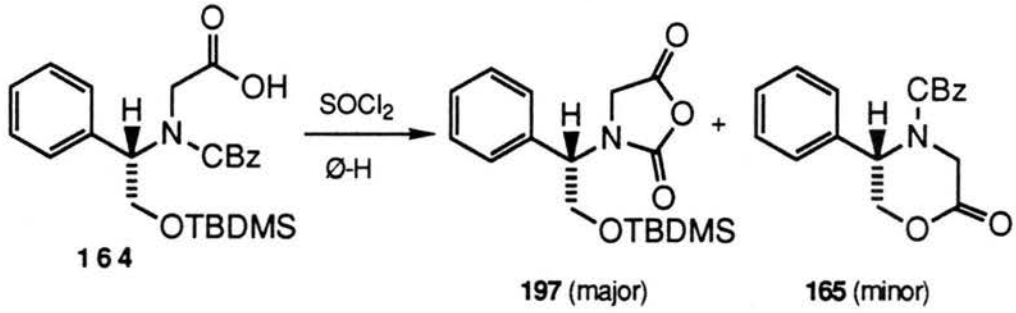
Lewis Acid	Solvent (R.T.)	Product	Yield (%)
1) $AlCl_3$ (cat.)	benzene	no reaction	- -
2) $AlCl_3$ (1 eq.)	benzene	phenyl ketone	10-13
3) SbF_5 (1 eq.)	benzene	phenyl ketone	6
4) $FeCl_3$ (1 eq.)	benzene	phenyl ketone	10
5) $AlCl_3$ (1 eq.)	nitrobenzene	no reaction	--
6) $AlCl_3$ (1 eq.)	nitromethane	no reaction	--
7) AgOTf (1 eq.)	benzene	phenyl ketone	45
8) AgOTf (1 eq.)	acetonitrile	dimer of **129**	15
9) PPA	PPA/100°C	decomposition	--

As indicated in Scheme 59, the desired cyclization failed. It seems that the reason for this failure was due to the lack of nucleophilicity in the aryl group of **129**. As can be seen in entries 2, 3, 4, 7, when the solvent was benzene the intermolecular

Friedel-Crafts reaction predominated. This was an indication that the presumed oxonium intermediate was forming but not affording the desired intramolecular reaction. The dimerization of **129** (entry 8, Scheme 59) was a puzzling result and seemed to be an indication that ring strain played a role in the reluctance of **129** to undergo the desired conversion.

The cyclization of **164** was then attempted to probe if ring strain associated with the cyclic urethane was the reason for the unsuccessful cyclization of **129** (Scheme 60).

Scheme 60

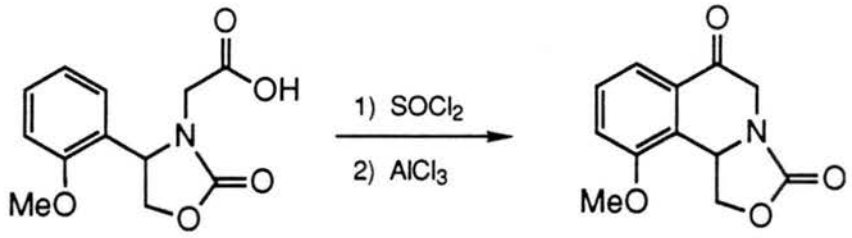


164 **197** (major) **165** (minor)

Upon treatment of **164** with $SOCl_2$ in benzene at room temperature the formation of the anhydride, **197**, was the major product along with **165**. Not surprisingly, upon exposure of **197** to a Lewis acid, decomposition of the starting material resulted, with no evidence of isoquinoline formation.

Even with these discouraging results, the synthesis of **195** was nonetheless undertaken, in anticipation that the added electron density provided by the meta-methoxy group in **198** would be enough to afford the cyclized product **195** (Scheme 61).

Scheme 61



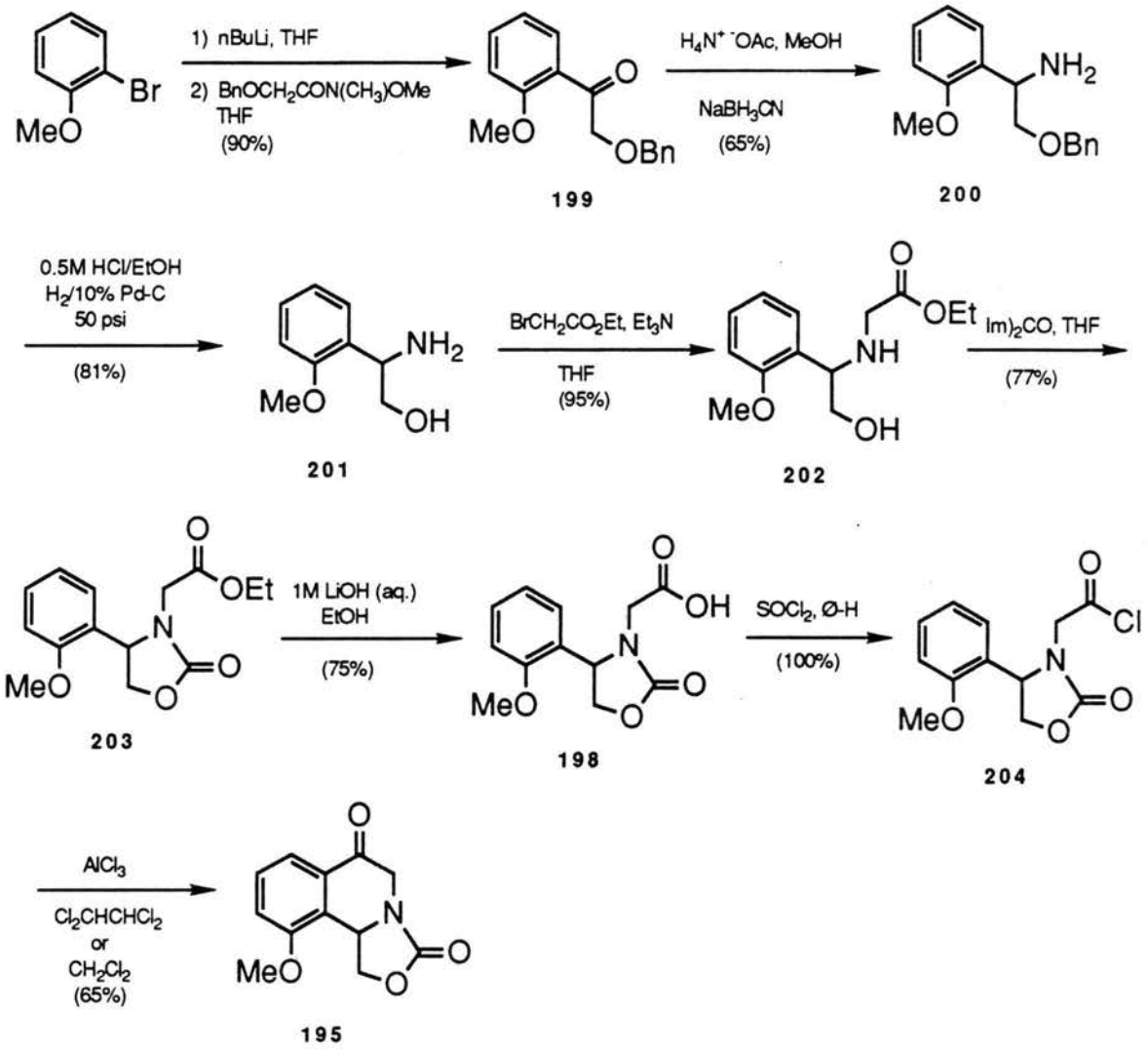
198 **195**

The construction of **198** was envisioned to follow the methodology developed in the synthesis of **129** (c.f. Chapter 2, Section 2, Scheme 36). Thus, the synthesis of (2'-methoxy)phenylglycinol, **201**, was undertaken (Scheme 62).

Lithiation of o-bromoanisole followed by addition to a solution of (N-methoxy-N-methyl)benzyloxy acetamide furnished the ketone, **199**. This coupling proved significantly superior to condensations of o-lithioanisole with benzyloxyacetyl chloride or benzyloxyacetic acid. In addition, attempted coupling of the corresponding Grignard of o-bromoanisole or the organocadmium reagent generated from o-lithioanisole[70] with benzyloxyacetyl chloride or benzyloxyacetic acid also proved inferior. The major products in these condensations was the tertiary alcohol resulting from further reaction of **199** with the corresponding organometallic reagent.

Reductive amination of the ketone, **199**, using the Borch procedure[71] furnished the o-benzyl protected (2'-methoxy)phenyl glycinol **200**. Hydrogenolysis of **200** then furnished amino alcohol **201**. Utilizing previously established protocol, amino alcohol **201**

Scheme 62



was alkylated with ethylbromoacetate yielding **202**, followed by urethane formation with 1,1'-carbonyldiimidazole to give **203**. Selective basic hydrolysis of the ethyl ester provided the crystalline acid **198**. Conversion of **198** to the acid chloride **204** was accomplished utilizing thionyl chloride. The crucial intramolecular Friedel-Crafts acylation proved to be extremely difficult and required extensive experimentation. Low yields (<20%)

were obtained utilizing $AlCl_3$ with a variety of solvents, but eventually the conditions reported by Uggeri,[72] using 1,1,2,2-tetrachloroethane as solvent at room temperature, provided **195** in 65% yield. Further investigation showed that methylene chloride could also be used as the solvent, which yielded **195** in the same yield.

Performing the aforementioned series of reactions the "left hand side" of quinocarcin was synthesized in 10 steps in 17% overall yield furnishing **195** as a valuable precursor to allow for further functionalization at the C-3 position via alkylation of the ketone moiety.

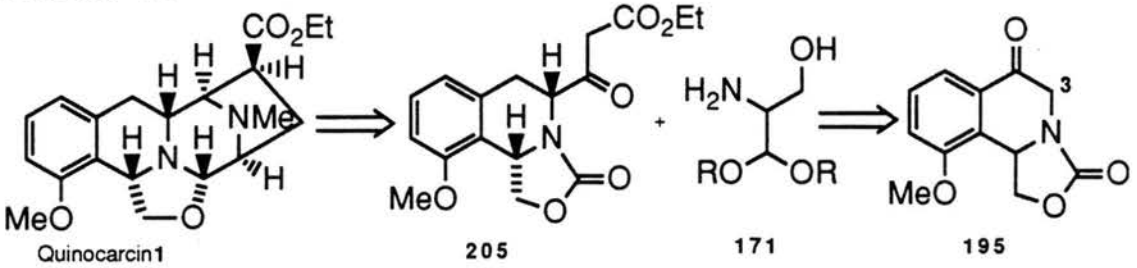
It is of interest to note that the acid chloride (prepared from **129**, c.f. Scheme 35), **204** prepared from phenyl glycinol, did not furnish the homologous tetrahydroisoquinoline. Thus, it seems that some electronic activation of the aromatic ring is required to effect closure in the modified Pomeranz-Fritsch approach.

CHAPTER 3.2

SYNTHETIC STUDIES DIRECTED TOWARDS THE TOTAL SYNTHESIS OF QUINOCARCIN

With isoquinolone **195** in hand, the stage was set to functionalize at C-3 (isoquinoline numbering) to generate the β-keto ester **205** which would serve as the pyrrolidine precursor (Scheme 63).

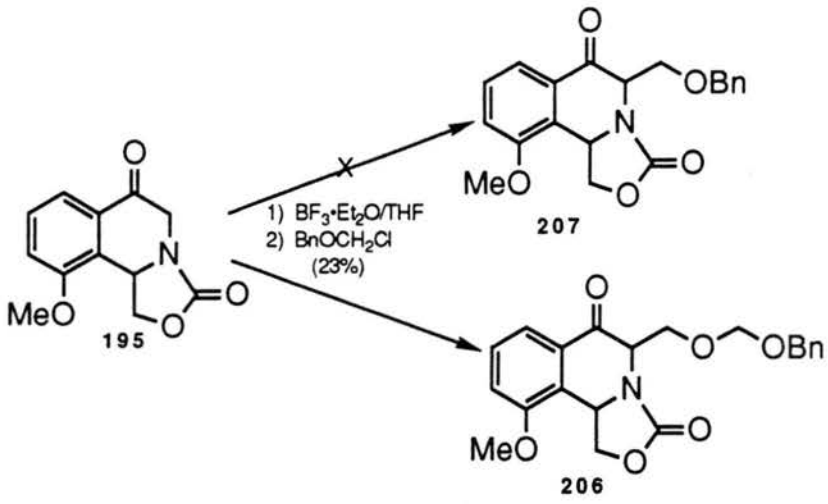
Scheme 63



Quinocarcin1 205 171 195

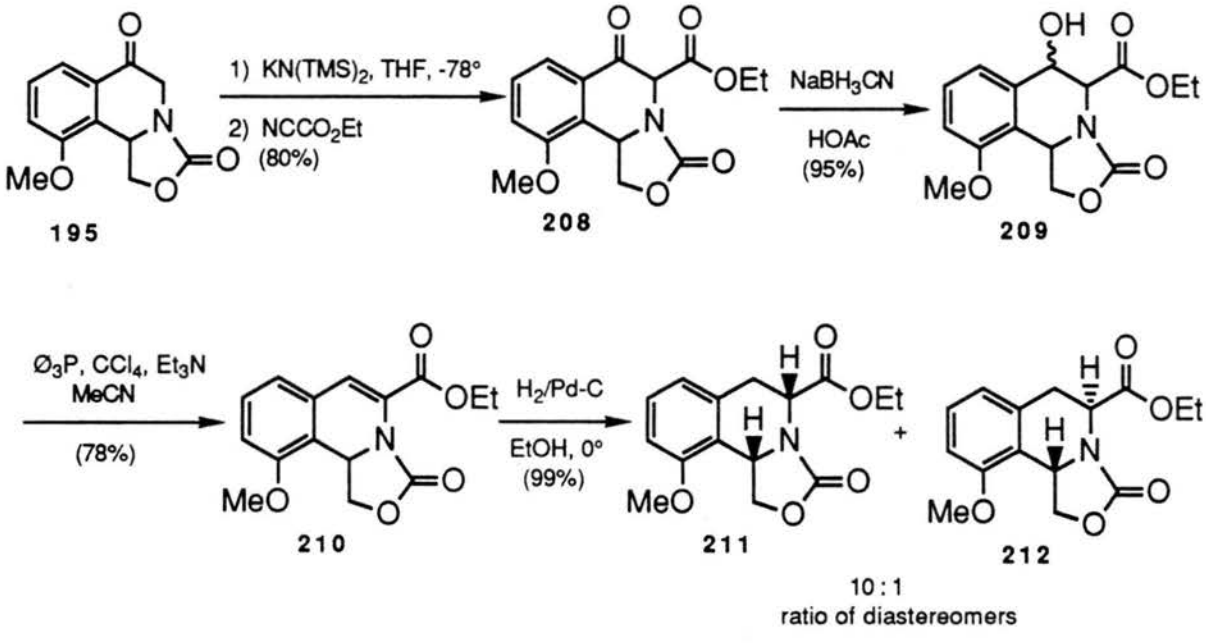
Initial attempts at C-3 functionalization of **195** proved problematic. Enolate chemistry with a variety of electrophiles (benzylchloromethyl ether, benzylchloroformate, ethylchloroformate, and methylcarbonate) and a variety of counterions (sodium, lithium, and potassium) resulted in O-alkylated products. Conditions which utilized $BF_3 \cdot OEt_2$ and benzylchloromethyl ether in an acid catalyzed alkylation resulted in the formation of **206** and not the expected **207** (Scheme 64).

Scheme 64



It was thought that the chloride counterion in the aforementioned reaction(s) was the reason for the difficulties encountered. Returning to enolate chemistry, utilizing ethylcyanoformate (Mander's reagent),[73] the desired conversion to **208** was achieved (Scheme 65).

Scheme 65

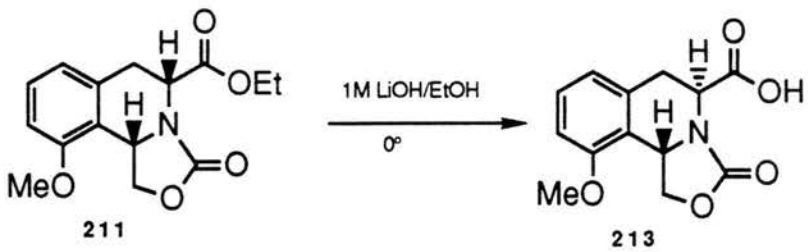


Compound **208** was subjected to reduction with $NaCNBH_3$ which provided the β-hydroxy ester **209** as a mixture of diastereoisomers (ca. 1:1). Initial experimentation with methylcyanoformate resulted in decarboxylation at this step, yielding only the corresponding 4-hydroxy-1-hydroxymethyl-8-methoxy urethane protected tetrahydroisoquinoline. Dehydration of **209** to afford **210** proceeded with the agency of triphenylphosphine with carbon tetrachloride and triethylamine. These conditions were arrived at after the initial observation that tosic acid in benzene at reflux provided only 50% conversion to **210**. This difficulty was attributed to the problems associated with syn elimination from the trans benzylic alcohol. The triphenylphosphine mode of elimination circumvents this problem by means of inversion of the anti-alcohol to the syn chloride.

With the α,β-unsaturated ester **210** in hand, hydrogenation with palladium was found to be ideal in providing the desired diastereomer **211** (major to minor ratio of 10:1). This reaction was found to be temperature dependent with respect to diastereomeric excess; optimal conditions were at 0-10°C which provided a 10:1 ratio.

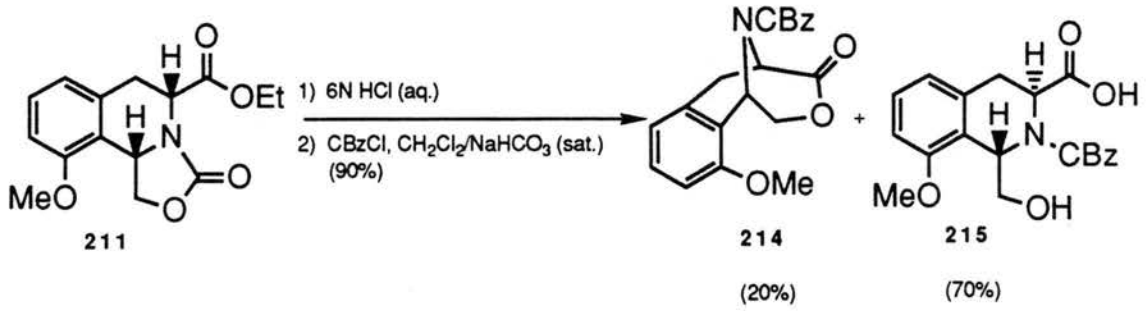
It was at this time that the ongoing model studies mentioned above indicated that the urethane protecting group would most likely be incompatible with the total synthesis of quinocarcin. Initial investigations utilizing base in a controlled hydrolysis of **211** to the corresponding acid **213** with lithium hydroxide in cold EtOH resulted in epimerization at C-3 (Scheme 66).

Scheme 66



Attention was then turned to acid mediated hydrolysis of **211**. Stringent conditions were required for the hydrolysis of the urethane. Thus, treatment of **211** in 6N HCl at reflux for 18 hr followed by exposure to benzylchloroformate furnished two products: bicyclic lactone **214** and acid alcohol **215** (Scheme 67).

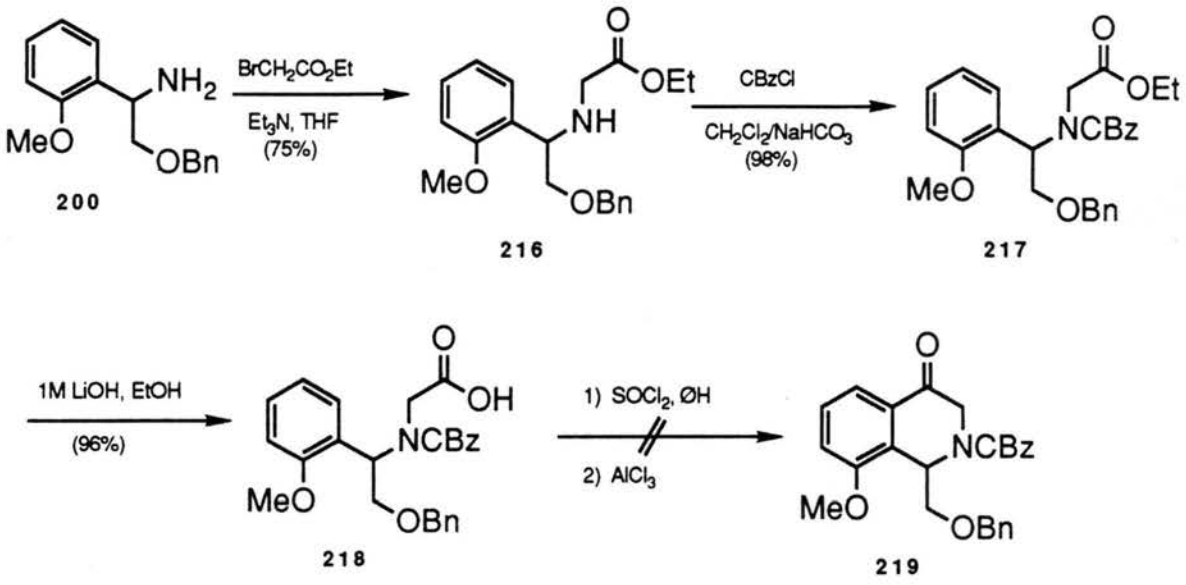
Scheme 67



Unfortunately, by starting with the desired diastereomer one generates a mixture of desired lactone, **214**, and the undesired acyclic diastereomer **215**. Recycling of **215** did not, however, regenerate **214** in the same ratio; only a 5% yield of **214** was realized. Because of the low yield in generating **214** in this manner other alternatives were explored.

The first alternative began with O-benzyl-(2'-methoxy)phenyl glycinol, **200**. Exposure of **200** to ethylbromoacetate furnished **216**. Protection of the amine gave **217**, which was hydrolysed to afford the acid **218** (Scheme 68).

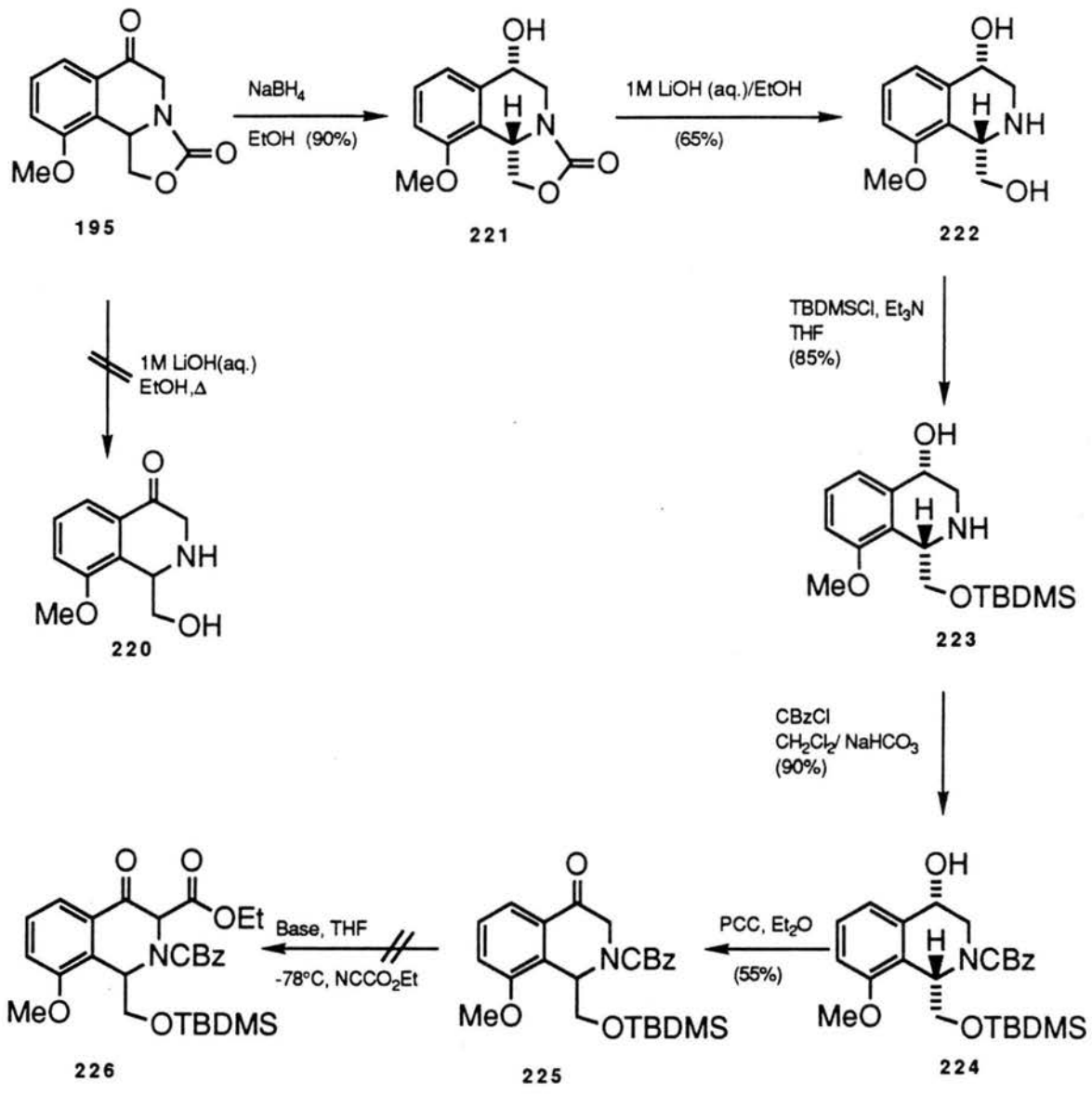
Scheme 68



Attempts at ring closure of **218** using the conditions employed above for **204** unfortunately failed and resulted in complex reaction mixtures.

The next alternative that was undertaken to circumvent problems related to the urethane protecting group was to start with the isoquinolin-4-one **195** itself (Scheme 69).

Scheme 69



Initial attempts to directly deprotect **195** failed, resulting in decomposition. It was thought that this was due to the inherent instability of α-amino ketones[74] under basic conditions. Thus, reduction of **195** to the benzylic alcohol **221** proceeded smoothly to yield a single diastereomer (presumably the <u>syn</u> isomer). Upon treatment of **221** with 1M LiOH (aq.) in absolute ethanol at reflux,

complete deprotection occurred giving the amine diol **222**. Subsequent selective protection of the primary alcohol with t-butylchlorodimethyl silane furnished the silyl ether **223**. Carbamate formation under standard conditions provided the alcohol **224**. Attempted oxidation utilizing the conditions of Swern[37] resulted in a very poor conversion to the ketone **225**. Pyridinium chlorochromate as oxidation agent, however, proceeded in moderate yield to furnish **225**.

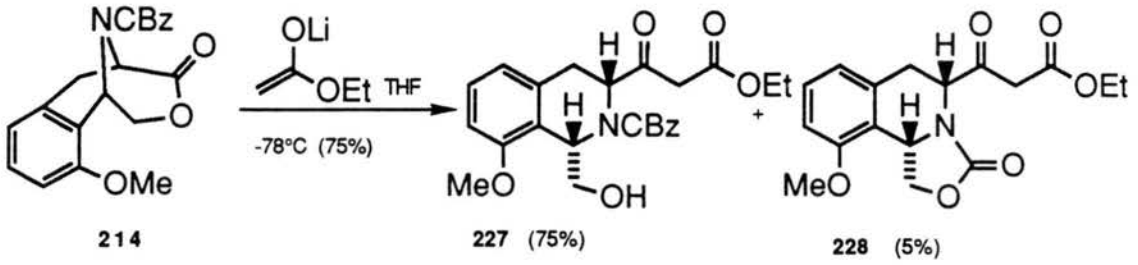
With **225** in hand, the primary objective of switching the urethane protecting group for ones more suitable for the total synthesis was achieved. Unfortunately, attempted alkylation of **225** under standard conditions ($K^{+-}N(TMS)_2$, $NCCO_2Et$, THF, -78 °C) failed, as did a variety of other reactions with alternate bases (Na^+ and Li^+ counterions). Upon examination of the Drieding model of **225** it was apparent that the problem encountered was steric in nature. It seemed that the t-butyldimethylsilyl ether prevented the approach of base from one face of the molecule and the carbobenzyloxy group the other.

The difficulties associated with the synthesis of **226** from **225**, encouraged re-examination of the synthesis of the bicyclic lactone **214**.

With the lactone **214** in hand, a two carbon homologation was required to convert it to the β-keto ester **227**. This was accomplished by the addition of the lithium enolate of ethyl acetate (1 equivalent) to a cold solution of **214**. Concern about racemization at the C-3 center (isoquinoline numbering) was determined to be unwarranted. When the β-keto ester formed, the

pKa of the resulting methylene of the acetoacetate derivative was approximately 10 and the alcohol that was liberated was approximately 16; any racemization at C-3 (with a pKa of about 26) was therefore deemed to be rather unlikely[75] (Scheme 70).

Scheme 70



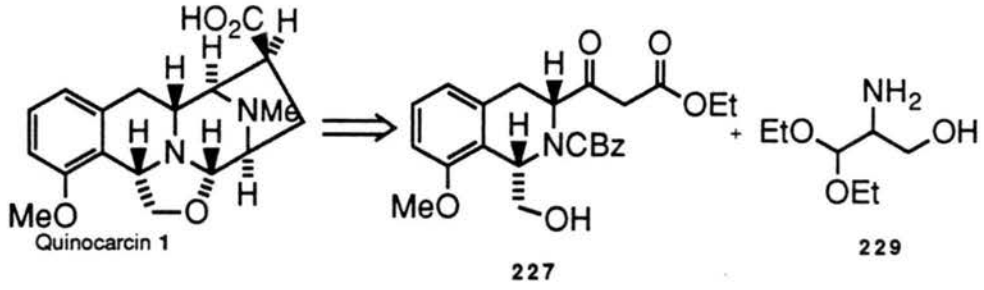
214 **227** (75%) **228** (5%)

The argument for retention of stereochemistry about C-1 and C-3 was further supported by the formation of **228** as a by-product in the reaction. This was primarily due to past experience with the N-CBz protected amino alcohols, (c.f. Chapter 2, Section 2), and their facile conversion to the cyclic urethane under basic conditions.

With **227** in hand, the next step was to protect the primary alcohol as a t-butyldimethylsilyl ether. This could not be achieved under a variety of conditions, presumably due to the β-keto ester moiety. Most conditions attempted gave no reaction and when more forcing conditions were utilized cyclization to **228** occurred.

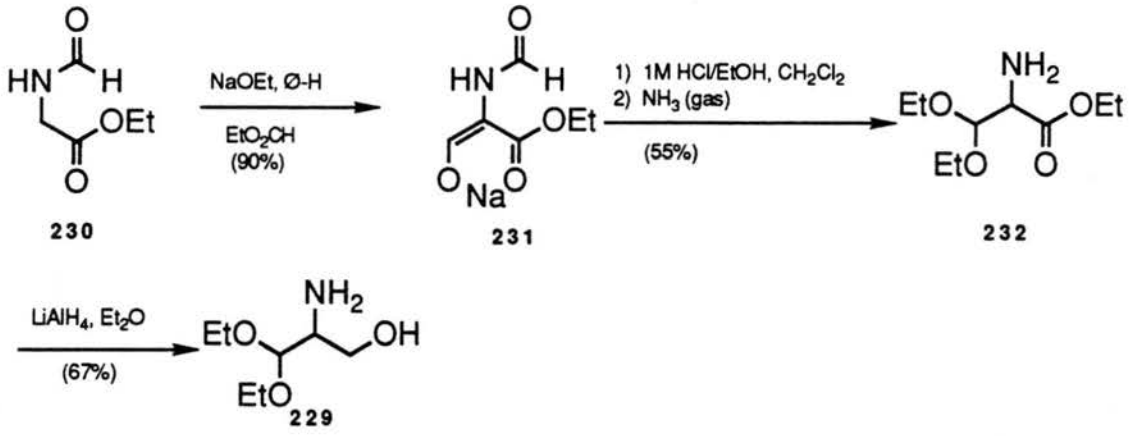
The inability to protect **227** was deemed not to be a serious setback as the next step in the synthesis was coupling of **227** with **229** (Scheme 71).

Scheme 71



The synthesis of **229** followed previously established protocol[76] (Scheme 72).

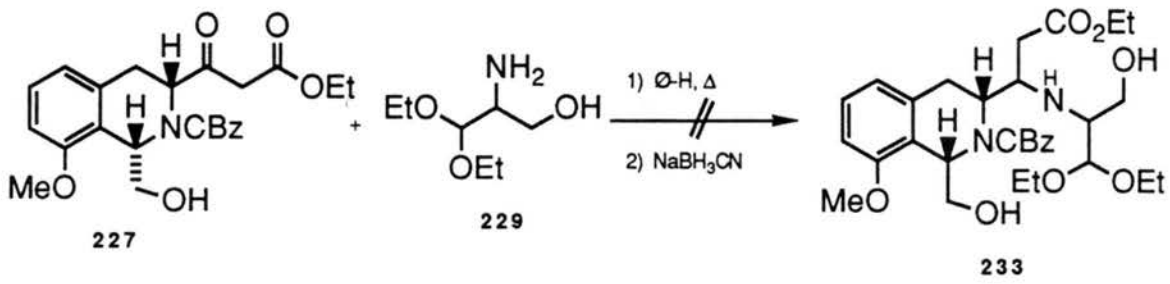
Scheme 72



Treatment of ethyl-N-formyl glycinate **230** with sodium ethoxide in the presence of ethylformate resulted in the formation of the sodium salt **231** in excellent yield. Acetal formation with 1M HCl/EtOH in methylene chloride followed by deformylation with ammonia yielded the diethoxy alanine derivative **232**. Reduction of **232** with hydride then furnished the desired serinal diethyl acetal **229**.[76]

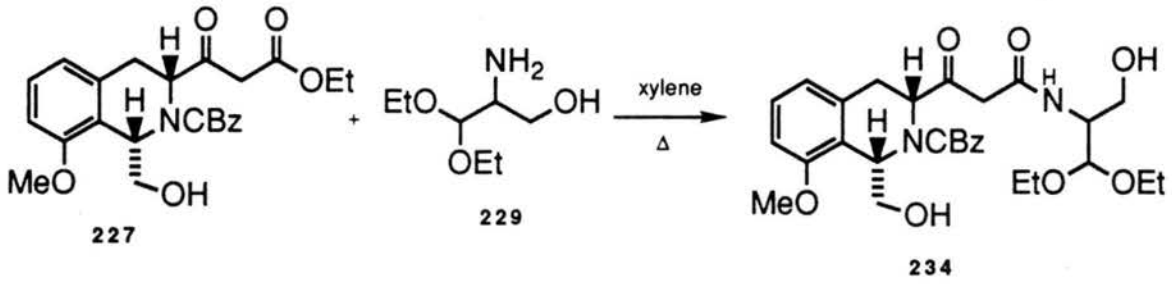
With both **227** and **229** in hand the stage was set for the crucial coupling of the left hand side of quinocarcin with the right to afford the multifunctional **233** (Scheme 73).

Scheme 73



Unfortunately, under standard[69] or more forcing conditions the desired transformation could not be achieved. The problem was clearly with imine formation between **227** and **229**. Thus, the solvent was changed from benzene to xylenes. This, however, resulted in the formation of amide **234** (Scheme 74).

Scheme 74

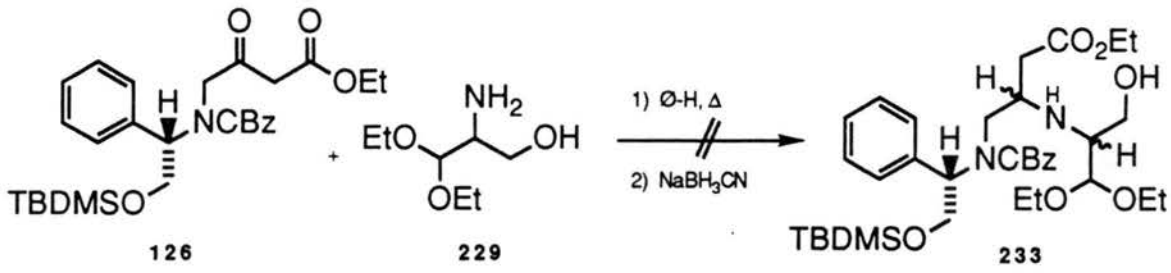


The failure of **227** and **229** to couple properly was a puzzling result. An attempt was made to acertain if the problem was due to

the β-keto ester's electrophilicity or the serinal diethyl acetal's nucloephilicity.

Thus, coupling of a previously useful β-keto ester, **126** with **229** under standard conditions was attempted (Scheme 75).

Scheme 75

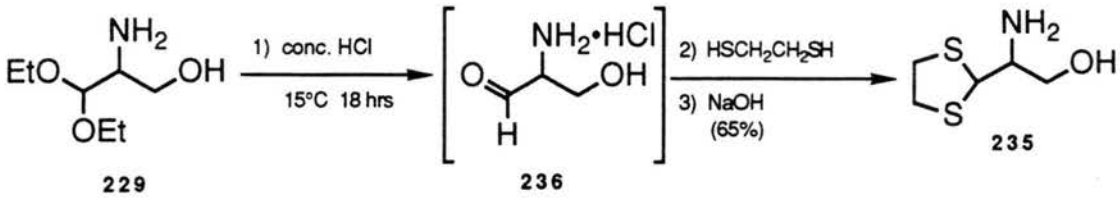


126 229 233

The failure of the above coupling suggested that the problem in the coupling of **227** with **229** was inherent to **229**. The reasons for this conclusion were as follows: 1) the previous success of **126** to couple with a variety of amino alcohols (i.e. **119** and **120** which were both sterically encumbered and variably protected); and, 2) the presence of the silane in **126** was thought to aid **229** in the coupling (primarily because of the reduced chances of hydroxyl mediated interferences).

From the information cited above, it was deemed appropriate to alter **229** to make it sterically less encumbered; thus the synthesis of **235** was undertaken[76] (Scheme 76).

Scheme 76

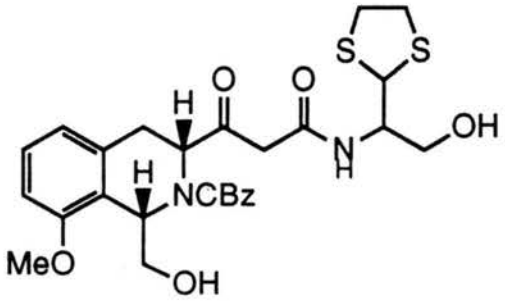


229 236 235

Diethylacetal **229** was exposed to concentrated hydrochloric acid at 15 °C followed by rapid high vacuum removal of solvent. The amorphous, extremely hygroscopic amino aldehyde salt **236** was formed and could be isolated. Alternatively, addition of 1,2-ethanedithiol to the acid solution and continued stirring formed the thioacetal hydrochloride which was basified and distilled which provided pure **235**.

With **235** in hand, coupling with **227** was again attempted. The results for these couplings were much the same as those employing **229**, that is, the formation of the intermediate imine was not evident and the formation of amide **237** was observed (Scheme 77).

Scheme 77

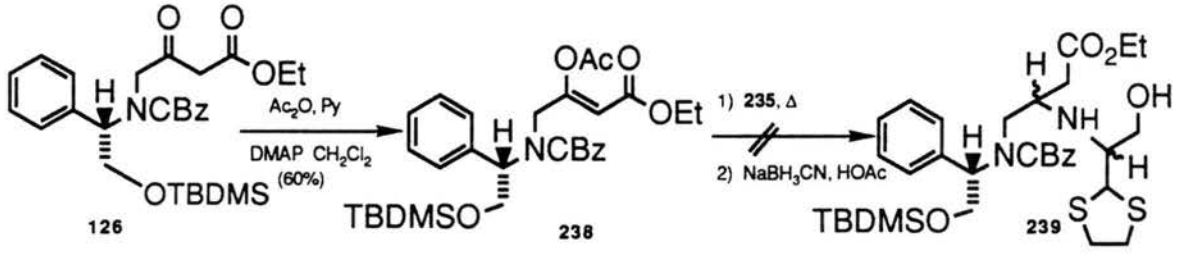


237

The failure of **235** to undergo the desired coupling with **227** was reevaluated and thought not to be due to steric constraints (bearing in mind that **119** and **120** did couple with **126** without difficulty), but, perhaps due to the basicity of the amino functionality in **229** and **235**. This would help to explain why amide formation precluded imine formation in the aforementioned coupling experiments.

Presumably one way to avoid this problem would be to activate the β-keto ester as a Michael-retro-Michael acceptor. Experimentation along these lines was performed with the analogous **126** and not the precious **235** (Scheme 78).

Scheme 78



Treatment of **126** with acetic anhydride under basic conditions afforded **238**. Attempts were made to couple **238** to **235** to no avail. The reaction products were predominantly N-acetyl **235** and **126**.

The above results concerning the attempts at coupling **229** or **235** with either **126** or **227** indicated that to overcome the problems encountered, an extensive study would be required, and perhaps a re-evaluation of the synthetic approach to quinocarcin

would be appropriate. Accordingly, at this point, studies directed towards the total synthesis of quinocarcin were terminated and our attention was turned to pharmacophoric studies in relation to quinocarcin's antitumor antibiotic activity.

CHAPTER 4

CHAPTER 4.1

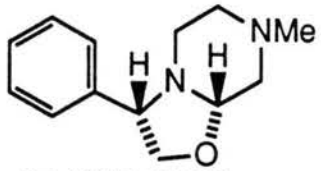
PHARMACOPHORIC STUDIES OF QUINOCARCIN

Studies concomitant with the synthetic approach to quinocarcin previously outlined were inquiries into the structural requirements necessary for the biological activity of this unique antitumor antibiotic.

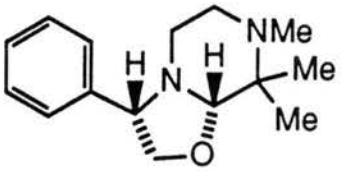
By comparison of the biological activities of quinocarcin (**1**) and quinocarcinol (**2**), (c. f. Chapter 1), one can safely assume that the existence of the oxazolidine portion, (or its hydrated equivalent), is imperative to impart any, (if not all), antitumor activity. Thus, the C-5, C-7 fused oxazolidine must be the predominant pharmacophore responsible for the aforementioned biological activity.

Observations made thus far concerning chemical stability can be summized by the comparison of the relative stabilities of the previously described model compounds **67** and **91**, (c.f. Chapter 2, section 1). These comparisons raised questions concerning the bicyclic nature of quinocarcin: Does the bicyclic oxazolidine with a bridgehead methine provide chemical stability? Is this bicyclic moiety necessary for biological activity? (Scheme 79).

Scheme 79



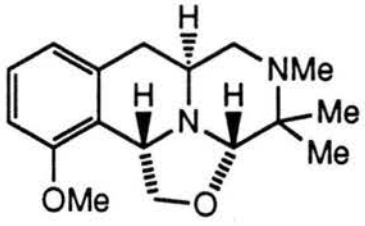
67 (UNSTABLE)



91 (STABLE)

In order to answer questions regarding the bicyclo[3.2.1]piperazine ring system of quinocarcin and its function concerning chemical stability and biological activity, another model compound was proposed and synthesized: **240**. This compound took into account the stability of **91** over **67** and more closely resembled the ridged nature of quinocarcin itself by incorporating the isoquinoline nucleus. (Scheme 80).

Scheme 80

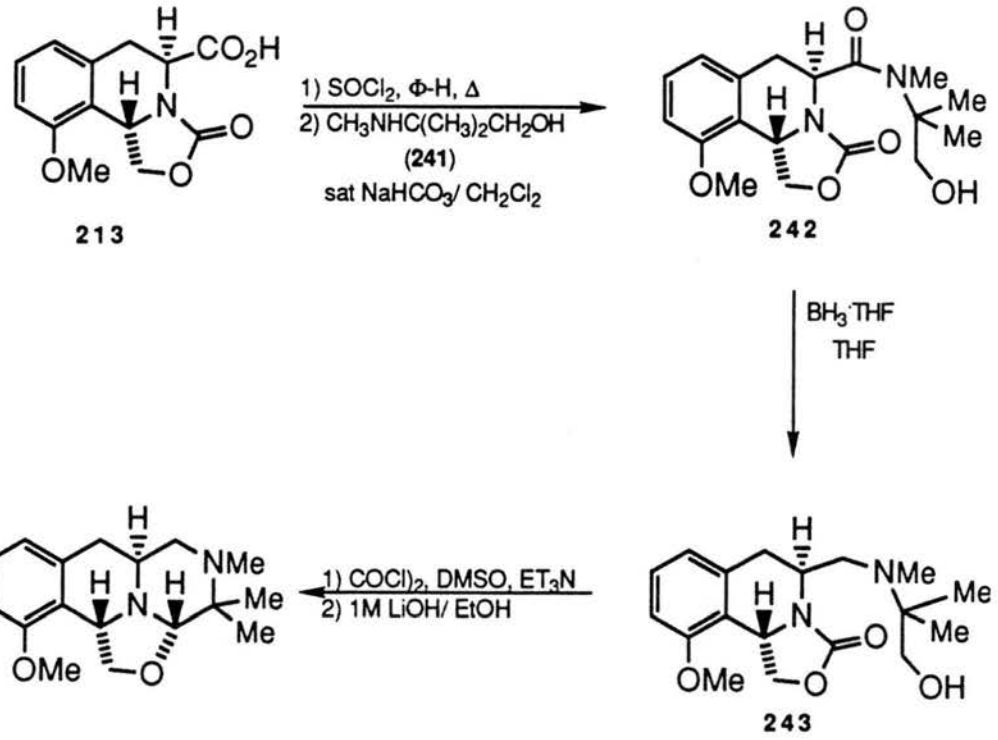


240

Tetracycle **240** was chosen as a target because of the aforementioned reasons and because it also served as a link between **91** and quinocarcin. Both compounds, **91** and **240**, were envisioned to have possible antitumor/ antibiotic activity. We also planned to ascertain the ability of these compounds to nick and /or cleave DNA.

The synthesis of **240** started from the previously described isoquinoline **213**. (Scheme 81)

Scheme 81



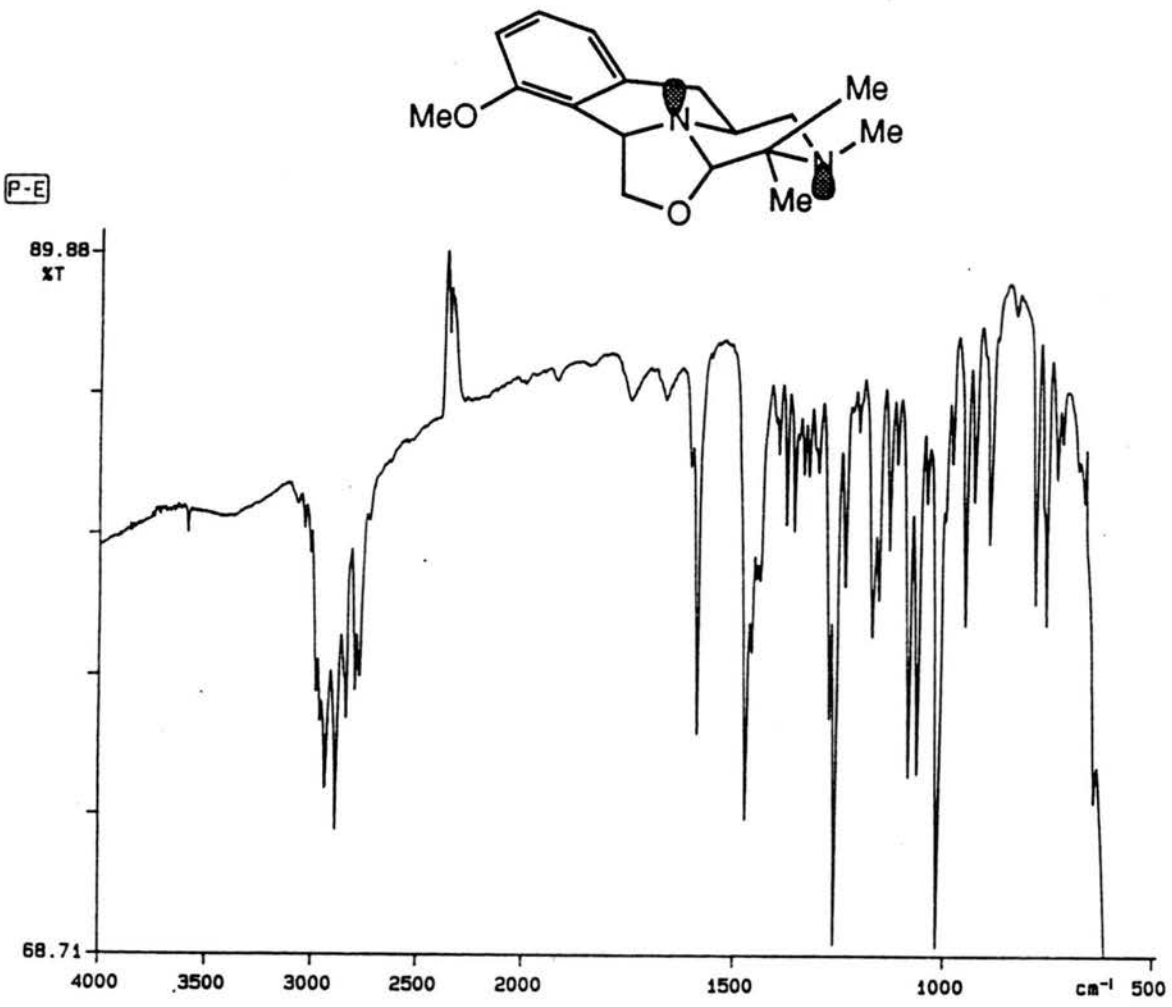
213 242 243

Treatment of **213** with thionyl chloride followed by reaction with 2-(N-methyl)amino-2-methyl-1-propanol, (**241**), gave the amide **242**. Reduction of amide **242** to the corresponding amine was readily accomplished with BH_3·THF. With amine **243** in hand, the stage was set to use the previously developed method of oxazolidine formation under basic conditions. Thus, oxidation of amino alcohol **243**, using the method of Swern followed by exposure to 1M LiOH in absolute ethanol yielded the desired tetracyclic oxazolidine **240**.

Characterization of **240** was initially performed with the utilization of NMR and IR techniques. NOE data and heteronuclear

decoupling experiments were utilized to confirm the stereostructure assigned, (Appendix 1). This lead to the following assignment of conformation which was supported by IR Bohlmann[77] absorptions. (Figure 1)

Figure 1



Infrared spectrum of **240** (NaCl, neat), showing the Bohlmann absorptions at 2774, 2796 and 2838cm^{-1}.

This assignment of conformation of **240** was ultimately supported by a single X-Ray crystallographic experiment, (Appendix 2).

With **240** in hand, the assessment of biological activity relative to quinocarcin remained to be ascertained.

CHAPTER 4.2

BIOLOGICAL ACTIVITY OF **240**-MODE OF ACTION OF QUINOCARCIN

With **240** in hand, Investigations were immediately directed towards assessing its ability to nick CCC DNA compared with that of quinocarcin (**1**). Once this was established more refined experimentation could be designed to further define the mode of action of this unique antitumor antibiotic. A speculative comparison of the origin of biological activity of quinocarcin to the presumed modes of action of saframycin and napthyridinomycin will then conclude this dissertation.

Initial investigations were conducted with quinocarcin itself to confirm the observations of Tomita, et.al.[1,2] Natural quinocarmycin, obtained from Kyowa Hakko Co., Japan, was separated from citric acid by ion exchange chromatography (HP20) and purified to homogeneity by reverse phase HPLC. Pure quinocarcin was allowed to react with phage PM2 CCC superhelical DNA between pH 6.5 and 9.5 at 37°C for 1h in the presence of air at various concentrations (0.01 mM-50mM). Nicking of the DNA was visualized by 0.8% agarose gel electrophoresis; ethidium bromide solution (0.5 μg/mL) was added to the gel *after* the gel was run in the dark. (This protocol was followed to minimize photo-induced nicking of the DNA by the potent intercalating agent ethidium.) Quinocarcin showed significant nicking of the DNA at 0.1 mM concentration (ca. 50% conversion of F-I to F-II (open circular forms) and complete nicking at 1.0mM (at pH 8.5). At lower pH values, (pH 6.5, 7.0, 7.5 and 8.0) nicking was

observed, but was significantly less than that between pH 8.0 and 9.5. Exclusion of oxygen significantly inhibited this reaction as previously recorded;[2] DTT enhanced the reaction at 0.1 mM and SOD and catalase inhibited the reaction. Quinocarmycin displayed a markedly inferior relative ability to nick the DNA at the same concentrations as quinocarcin. It had previously been established that the addition of Fe^{+3}, Fe^{+2}, and Cu^{+2} has no stimulatory effect on the ability of quinocarcin to nick DNA.[78] Also, the addition of the potent Fe^{+3} sequestering reagent desferal initially seemed to display no inhibitory effect on the ability of quinocarcin to nick DNA, thus, metal-mediated Fenton chemistry was excluded.[78] (Figure 2, lanes 1-12 and 16-25).

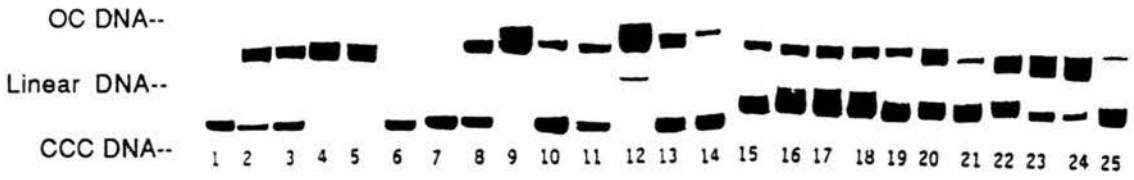


FIGURE 2: **Lane 1**: Drug free control; **Lane 2**:1.0 mM pure **1**; **Lane 3**: 1.0 mM **1** + 0.01 mM desferal; **Lane 4**: 3.0 mM **1**; **Lane 5**: 3.0 mM 1 + 0.1 mM desferal; **Lane 6**: 0.1 mM desferal; **Lane 7**: drug free control; **Lane 8**: 0.1 mM **1**; **Lane 9**: 1.0 mM 1; **Lane 10**: 1.0 mM 1 + SOD (10 μg/mL); **Lane 11**: 1.0 mM **1** + 5.0 mM citric acid; **Lane 12**: 1.0 mM **1** + 0.1 mM DTT; **Lane 13**: 5.0 mM **240**-2/3 citrate + 0.1 mM DTT; **Lane 14**: 5.0 mM **240**-2/3 citrate; **Lane 15**: drug free control at pH 6.5; **Lane 16**: 1.0 mM **1** at pH 6.5; **Lane 17**: 1.0 mM **1** at pH 7.0; **Lane 18**: 1.0 mM **1** at pH 7.5; **Lane 19**: drug free control at pH 8.0; **Lane 20**: 1.0 mM **1** at pH 8.0; **Lane 21**: drug free control at pH 8.5; **Lane 22**: 1.0 mM **1** at pH 8.5; **Lane 23**: 1.0 mM **1** at pH 9.0; **Lane 24**: 1.0 mM **1** at pH 9.5; **Lane 25**: drug free control at pH 9.5.

However, more careful examination of metal dependent cleavage revealed that desferal did cause partial inhibition of the cleavage event.

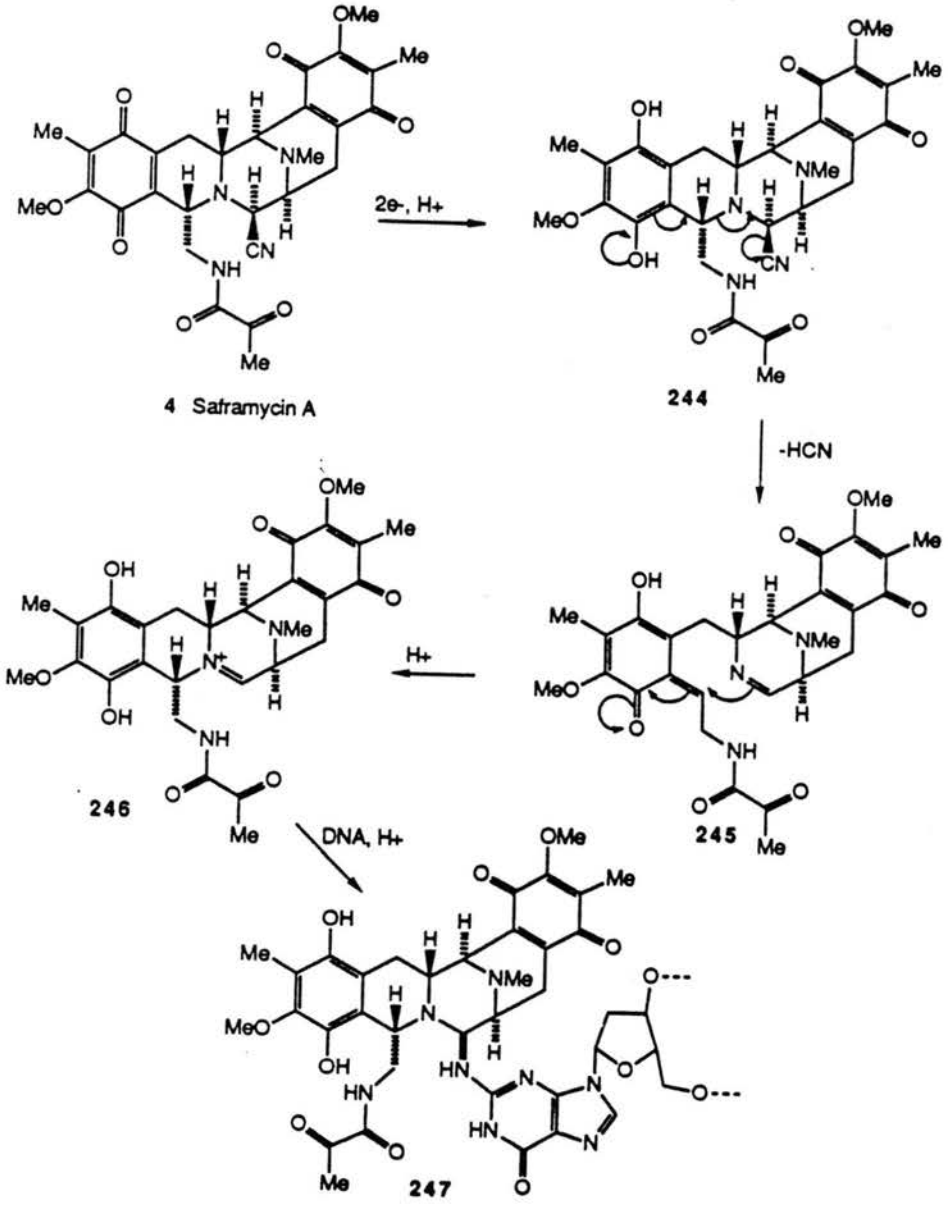
Treatment of the plasmid DNA with the tetracyclic analog **240** as the 2/3 citrate salt[79] demonstrated nicking of the DNA at 5.0 mM concentration. This effect was enhanced by the addition of DTT. (Figure 2, lanes 13, 14 and 15)

Several rationalizations can be made with respect to the nicking ability of **240** being inferior to that of quinocarcin. Before any stipulations can be made concerning quinocarcin one must consider at least two factors: 1) the accepted modes of action of the related, albeit more complex, families of antitumor antibiotics; the saframycins and napthyridinomycins; and 2) conformational and structural distinctions between quinocarcin and **240**. Comparative SAR data for compound **91** in this study proved problematic due to the inherent insolubility of **91** as its free base and as a variety of salt complexes.

The mechanism of binding and DNA scission by saframycin A (**4**) was investigated by Lown et al.[5] They found that saframycin A was protonated on N(12) at low pH, but N(2) was not protonated. Elevations in the transition melt temperatures (T_m) of calf thymus and T4 DNAs showed that the protonated species bound weakly and reversibly. The fluorescence of ethidium bound to these DNAs was quenched immediately, showing that the ethidium was extruded from its intercalation sites. The slow reversible binding of saframycin A toward heat and lower pH is characteristic of an aminal link, (i.e. **246** to **247**), presumably at the minor grove of the DNA to the 2-

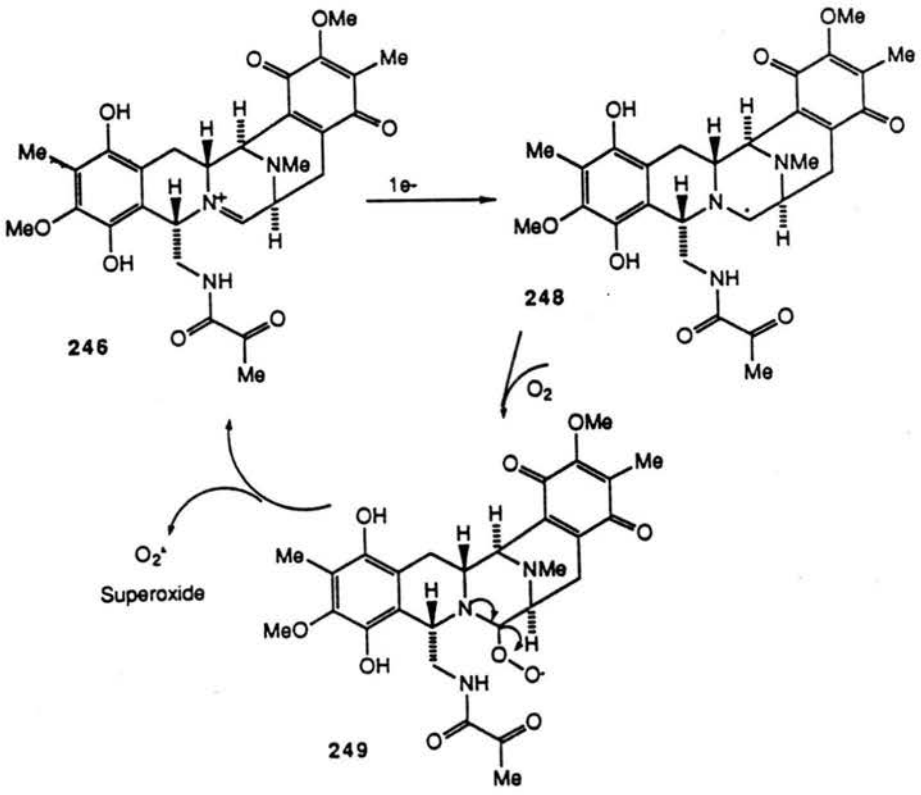
amino group of guanine (**247**). These results support, but do not confirm, the mode of action outlined in Scheme 82.[5]

Scheme 82



The above mode of action seems at first cumbersome with the formation of the iminium species **246** from the hydroquinone **244**, rather than directly from the parent (i.e. **4** to **246**). (However, it is possible that the phenol residue of **244** can contribute to the formation of **246**.) This sequence of events is substantiated by the fact that the binding of saframycin to DNA increased substantially after reduction of the quinone ring. Electrochemical evidence including E^1_0 and values for the quinone rings, pyruvamide side chains, glutathione, and NAD^+/NADH, in addition to an EPR spectrum for the quinhydrone radical, was proposed to support this hypothesis. Reduction of saframycin A in the presence of oxygen results in single-strand cleavage. The pathway by which this event is thought to occur is via the imminium species **246**. A one electron reduction of **246** may produce the radical **248**. In the presence of oxygen, the peroxide **249** can form via an electron transfer mecanism. The species **249** then may undergo elimination to produce superoxide and subsequently regenerate **246**. Additionally, it is thought that traces of adventitious Fe^{+3} in the aqueous system may catalyze the formation of hydroxyl radical via the Haber-Weiss redox cycling event involving the Fenton chemistry previously discussed (Scheme 83).

Scheme 83



Haber-Weiss redox cycling:

$$Fe^{+3} + O_2^{\bullet} \longrightarrow Fe^{+2} + O_2$$

$$2O_2^{\bullet} \xrightarrow{2H^+} H_2O_2 + O_2$$

(Fenton): $Fe^{+2} + H_2O_2 \longrightarrow HO^- + HO^{\bullet} + Fe^{+3}$

Lower pH promotes the noncovalent and covalent binding of saframycin A, but decreases strand cleavage. Lown has suggested that increased groove binding makes the saframycin less accessible to reducing agents.[5] He did not ascertain, however, whether or not the lower pH resulted in protonation of N(2) and thus rendering the saframycin substantially less active by inhibiting the formation of **246**.

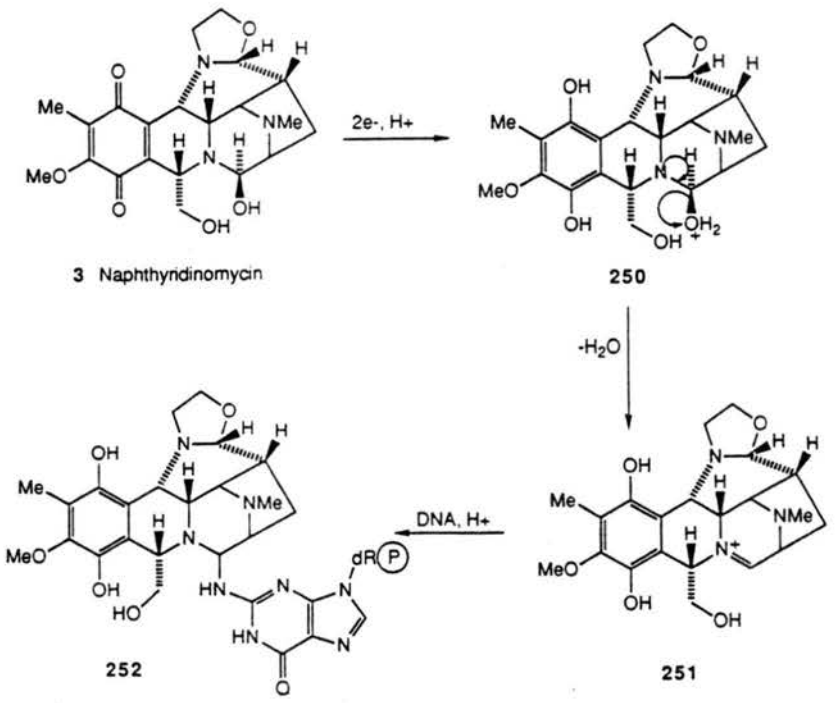
Zmijewski and coworkers studied the binding of napthyridinomycin to calf thymus DNA and T4 DNAs under a variety of conditions.[80,81] They found that napthyridinomycin covalently binds to the minor groove of DNA via a guanine 2-amino group, (as Lown presumed with saframycin A[5]). They also found that certain reducing agents converted **3** into the corresponding hydroquinone and markedly stimulated binding to DNA. The most effective reducing agents were DTT and penicillamine, cystine was effective at higher concentrations, and glutathione, sodium dithionite and sodium borohydride were ineffective.[81] Dialysis experiments showed that only a small amount of napthryidinomycin bound irreversibly in the absence of reducing agent, but substantial binding occurred when DTT or penicillamine were present at 1.0mM. The napthyridinomycin-DNA complex formed in the presence or absence of DTT was isolated by gel chromatography on sephedex G-25. No difference was found in the nature of the complexes formed under reducing and non-reducing conditions, but five to six-fold more complex was produced when DTT was present.

The time course, pH dependency, and reversibility of napthyridinomycin-DNA complex formation was studied.[81] At pH 7.9 the presence of 1.0mM DTT caused a rapid burst of binding followed by a slower phase. Binding also occurred in the absence of DTT, but at a slower rate that resembled the slower phase of the DTT activated drug. Below pH 5.0 and above pH 7.9 very little binding occurred in the presence of DTT. In the absence of DTT, the reaction displayed maximum binding kinetics at pH 5.0 which decreased as the pH increased. The UV absorption of the drug in the presence of DNA and DTT was monitored. At pH 7.9 the hydroquinone chromophore at 287nm formed rapidly, but after 4 to 6 h the quinone chromophore at 270nm reappeared. The addition of fresh DTT restored the hydroquinone form. When this experiment was repeated at pH 5.0, the hydroquinone form remained throughout. Bound drug was released slowly and constantly from the DNA complex at pH 7.9 with or without DTT, but 58% of it remained on day twelve. At pH 5.0 with DTT all of the drug that could be released went in the first six days. This release was more constant in the absence of DTT, but faster at pH 5.0 than at pH 7.9. The melt transition temperature (T_m) of calf thymus DNA increased in proportion to the amount of bound drug, but the melt profiles were not reversible, indicating that there was no thermostable crosslinking.[81]

The foregoing evidence allowed Zmijewski to propose two mechanisms for the interactions of napthyridinomycin with DNA. One was based on the mechanism advanced for saframycin A (Scheme 83). Reduction of napthyridinomycin (**3**) to the hydroquinone, which facilitates the formation of an iminium ion (**251**) by the loss of

water from C(7). (Scheme 84). This ion alkylates the 2-amino group of guanine in the minor groove to produce **252**. The enhanced reactivity of napthyridinomycin at pH 5.0 is explained by protonation of the carbinolamine to facilitate formation of the imminium ion. In the second proposed mechanism it is assumed that formation of the hydroquinone does not activate the carbinolamine, but provides initial hydrogen-bonding near the reactive site on DNA. Additional noncovalant interactions with DNA would be provided by protonation of the amines at pH 5.0. The carbinolamine functionality at C(7) is considered to be more reactive than the oxazolidine functionality, based on the facile conversion of the carbinolamine to cyanoamine (cyanocycline), by cyanide ion. The two reactive functionalities in the structure of this antibiotic suggest the capability of bisalkylation, but the presence of interstrand crosslinks was ruled out by the irreversibility of the T_m curves of DNA-bound napthyridinomycin. (Scheme 84)

Scheme 84



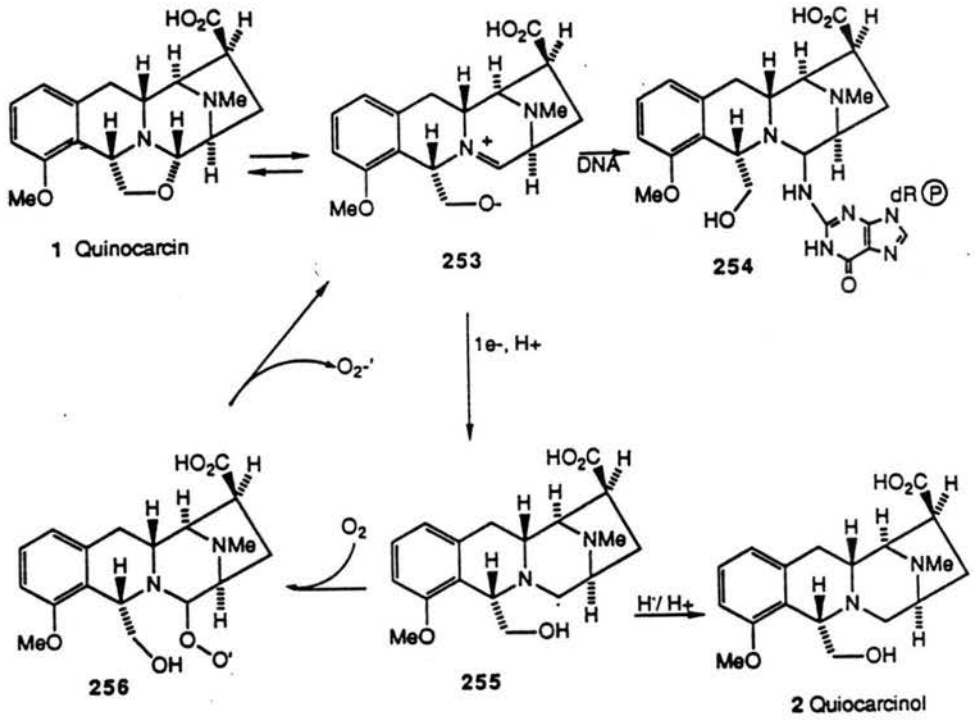
The most obvious difference between quinocarcin and the saframycins and napthridinomycins is the absence of the quinone moiety in quinocarcin. Thus, the modes of action that involve this portion of the drugs' interaction with DNA and its subsequent participation must be ruled out. However, iminium ion formation and minor grove alkylation by **3**and **4** would indicate that a similar mode of DNA binding by quinocarcin is very reasonable. However, the absence of the quinone moiety in compound **240** and quinocarcin indicates that a distinct mechanism of DNA nicking is operative.

There has been some preliminary work published on the mode of action of quinocarcin, (c.f. Chapter 1). Most studies with this drug have been *in vivo* and not *in vitro*. With compound **240** as a quinocarcin analog one can draw certain conclusions based on their differences in reactivity. The observation that **240** is inferior to quinocarcin in cleavage of DNA can be rationalized by several factors: 1) The solubility differences between the two compounds; quinocarcin is soluble in water and **240** is not. 2) The decrease in DNA-cleavage activity of quinocarmycin vs that of quinocarcin may be directly related to the relative decrease in reactivity between **240** and quinocarcin. 3) The absence of the pyrrolidine and carboxylic acid functions of quinocarcin in **240** may influence the noncovalant interactions with DNA that have been described for the saframycins and the napthridinomycins. These interactions have been shown to play an integral part in the DNA nicking ability of this class of antitumor antibiotics.[5]

Irrespective of the subtle differences between **240** and quinocarcin, the modes of action that have been proposed from the

studies concerning the saframycins and napthyridinomycins[5] may still apply to quinocarcin. The possibility of the formation of an iminium ion, such as **253**, which may be capable of binding to the 2-amino group of guanine in the minor groove of DNA. This would produce a species such as **254** in an analogous mode as that proposed for the saframycins and napthrydinomycins.[7,8] (Scheme 85).

Scheme 85

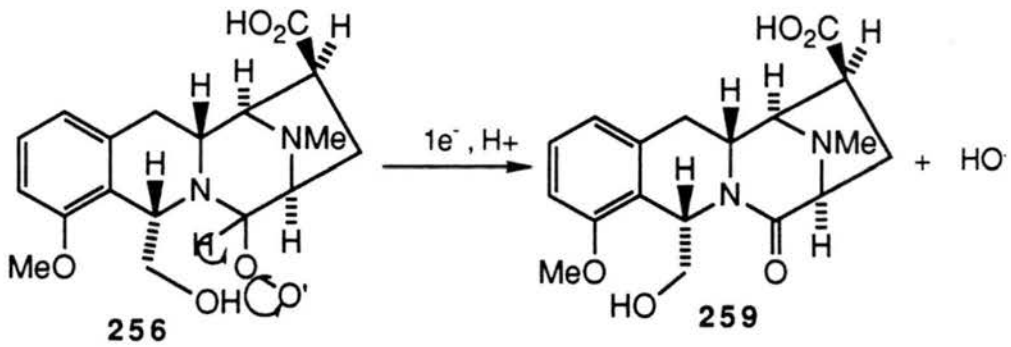


The generation of hydroxyl or peroxy radicals that have been implicated in the oxygen dependent DNA cleavage by quinocarcin[7] may be generated during the reversibile binding of quinocarcin to DNA. One electron reduction of the iminium ion **253** produces **255**. Superoxide production can be accomplished under aerobic conditions via an intermediate such as peroxy radical **256**. Upon elimination of superoxide, species **253** is regenerated and the cycle can continue

provided a one electron reductant is present. The possibility exists that radical **255** could be quenched by abstraction of a hydrogen from DNA thus producing the cycle-terminating species quinocarcinol **2**. (Scheme 85).

When purified quinocarcin was allowed to stand in water at room temperature, two new products were produced. Upon isolation and characterization one was found to be quinocarcinol and the other was the amide **259**. The sequence of events outlined in scheme 85 can also account for the generation of amide **259**. (Scheme 86)

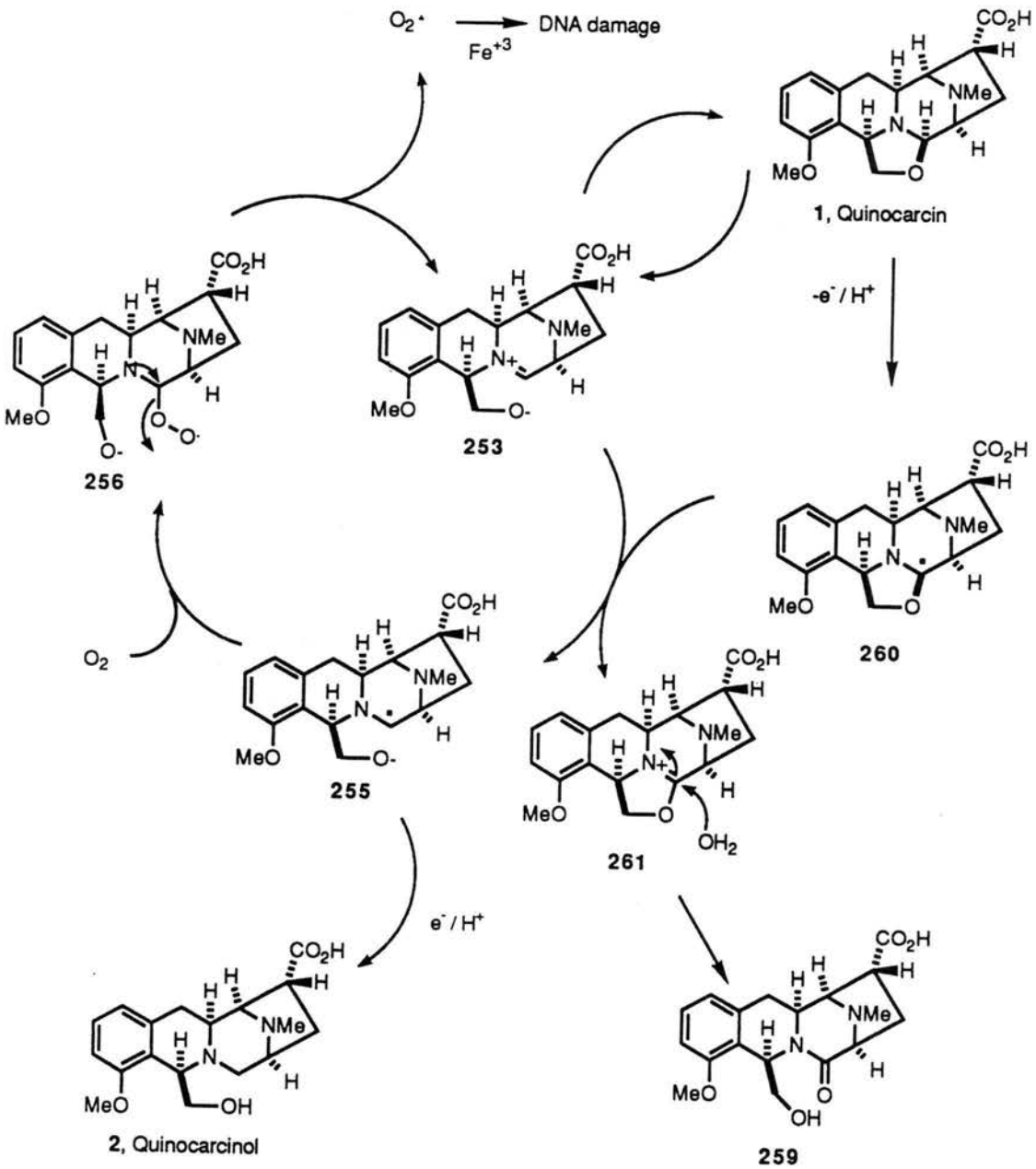
Scheme 86



The formation of peroxy radical **256** is common to both mechanisms, but not the production of amide **259**. The possibility of differentiating between the two mechanisms resides in the capacity to observe **259**. In water saturated with $^{18}O_2$, one would expect that the ^{18}O could be incorporated into the amide carbonyl. When this experiment was performed, the amide **259** was found not to incorporate ^{18}O. Further experimentation was performed utilizing 98%$^{18}OH_2$ and exposing quinocarcin under anaerobic conditions. This resulted in the incorporation of greater than 40% ^{18}O in the amide

carbonyl.[82] With the aforementioned results, the mechanism of scheme 86 seems unlikely. However, a mechanism that does account for the self-redox disproportionation of quinocarcin to the amide **259** and quinocarcinol is the Cannizzaro-driven reduction of molecular oxygen outlined below. (Scheme 87)

Scheme 87.



As was outlined in scheme 85, quinocarcin may, by one electron reduction of the iminium **253**, form **255**. The species **255** may then be quenched to produce quinocarcinol or interact with oxygen to generate **256**. Upon elimination of superoxide, **256** can regenerate the iminium species **253**. An alternative pathway to the superoxide generating precursor, **255** is through the oxazolidnyl radical **260**. Radical **260** should be capable of reducing a second equivalent of **253**, ultamately becoming oxazolidium ion **261** which should hydrolize to the amide **259**.

It is clear from the foregoing discussion that the mode of action of quinocarcin is unique but should receive further study to be fully elucidated. Research directed towards the synthesis of quinocarcin analogs; DNA binding studies; (i.e. alkylation vs oxidative cleavage of DNA), and ascertaining which of the two modes of action proposed is important for anti-tumor activity are worthy of study. Research in this area is in its infancy, and there are more questions than answers. Hopefully, this dissertation has laid the foundation for future work to to continue.

CHAPTER 5

EXPERIMENTAL SECTION

A. General Information

Melting points were determined in open-ended capillary tubes on a "Mel-Temp" apparatus and are uncorrected. Infrared spectra were recorded on a Beckman Model 4240 spectrophotometer and were obtained on NaCl pellets. Absorption are reported in cm^{-1}. ^1H NMR spectra were recorded on the following instruments. Varian T-60 spectrometer without lock, Brucker WP-200SY 200 MHz spectrometer with lock, or Brucker WP-270 MHz spectrometer with lock. The field strength (MHz) is indicated for each spectrum in the experimental section. Chemical shifts are reported in parts per million downfield from the internal standard, which is specifically indicated for each compound in the experimental section as δ-standard. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = doublet of doublets.

Low resolution mass spectra were obtained on a V. G. Micromass Ltd., Model 16F spectrometer. Elemental analyses were performed by M-H-W Laboratories, Phoenix, Arizona and by Spang Microanalytical Laboratories, Eagle Harbor, Michigan.

Optical rotations were obtained on a Perkin-Elmer 24 polarimeter at wavelength 589 nm (sodium D line) using a 1.0

decimeter cell with a total volumne of one mL. Specific rotations, $[\alpha]_D$, were reported in degrees per decimeter at the specified temperature and the concentration (c) given in grams per 100 mL in the specified solvent.

The single crystal x-ray analysis was obtained on a Nicolet R3m/E diffractometer.

B. CHROMATOGRAPHY

Analytical thin layer chromatography was performed on E. Merck 0.25 mm or 0.50 mm silica gel 60 F-254 layers backed by glass. Visualization on TLC was achieved with ultraviolet light, I_2 developing chamber, Dragondorf reagent,[77] and/or heating the TLC plates submerged in a 5% (by weight) solution of phosphomoloybdic acid in 95% ethanol. Preparative chromatography was performed by the following methods. Column and flash chromatography were performed using Woelm (32-62 μm) silica gel, in which the mixtures were pre-absorbed on silica gel.

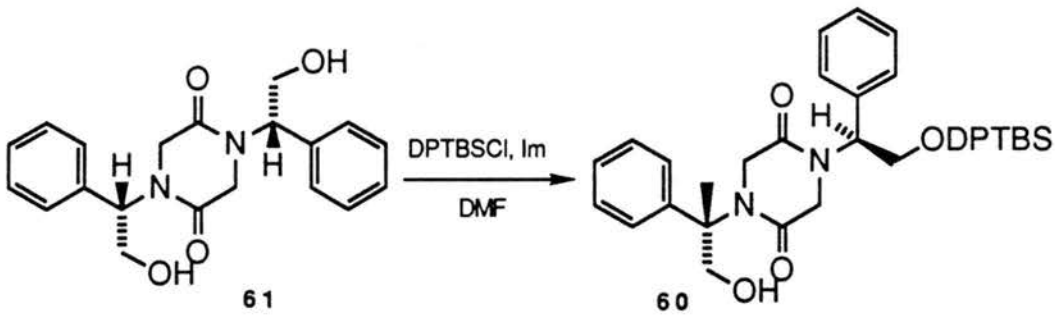
C. REAGENTS AND SOLVENTS

Reagents and solvents were commercial grades and were used as supplied with the following exceptions. Tetrahydrofuran was freshly distilled from sodium benzophenone ketyl. Diisopropylamine was distilled from CaH_2 and kept under N_2 over activated 4Å molecular sieves. *n*-Butyllithium was obtained from Ventron and was titrated (diphenylacetic acid, -78 °C, THF) prior to use. Lithium diisopropyl amide (LDA) was freshly prepared by dropwise addition of *n*-butyllithium in hexane to a stirred solution of diisopropylamine

in THF at 0 °C and was used after stirring 10 min. LDA solutiosn were transferred via cannula to the reaction vessel using N_2 pressure. Diethyl ether was freshly distilled from sodium benzophenone butyl under N_2 atmosphere. Dry methylene chloride, chloroform, and carbon tetrachloride were obtained by distillation over P_2O_5. When required, dry DMF, DMSO, pyridine, HMPA, oxalyl chloride, acetonitrile, trifluoroacetic anhydride were taken via dry syringe from storage over activated 3Å and 4Å sieves after distillation from an appropriate reagent. All organic materials and intermediates were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin.

D. General Experimental Considerations

All moisture or oxygen sensitive reactions were conducted in glassware that was flame dried under high vacuum (0.05-1.0 mmHg) and then purged with N_2. All reactions were stirred with Teflon coated stir bars. The following low temperature baths were used: 0 °C (ice water), -10 °C (ice methanol), -78 °C (acetone, dry ice), -105 to -100 °C (4% water in methanol, liquid nitrogen). When reaction temperatures are given it refers to the temperature maximum indicated by a thermometer inside the reaction vessel. The term concentrated refers to solvent removed under the vacuum achieved by a water aspirator attached to a Buchi rotary-evaporator, (ca. 26 mmHg). Residual solvent was removed at reduced pressure (0.05-0.50 mmHg) using a vacuum pump.

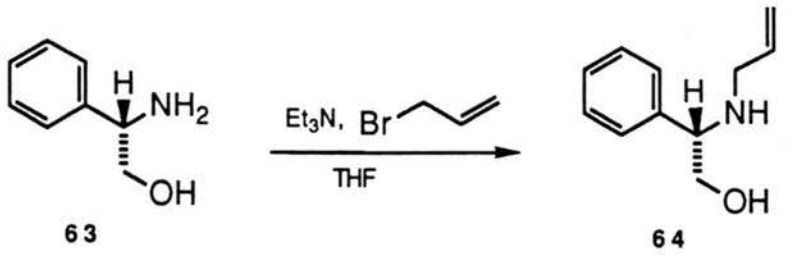


O-Diphenyl(t-butylsilyl-1,4-(2-phenylethanol)-2,5-piperazinedione. (**60**). To a stirred solution of **61**, (100 mg, 0.28 mmol, 1.0 equiv) in dry DMF, (6 mL) was added imidazole, (19 mg, 0.28 mmol, 1.0 equiv) in one portion. To this clear solution was added diphenyl-t-butylchlorosilane (77 mg, 0.28 mmol, 1.0 equiv) via a syringe. The clear solution was allowed to stir at room temperature for 18 h, diluted with 30 mL CH_2Cl_2 and washed with H_2O (3 x 10 mL). The water layers were back extracted with CH_2Cl_2 (1 x 10 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, evaporated, and separated (silica gel column eluted with 1:1 ethylacetate/hexanes) yielding **60**, (62 mg, 57%) as an oil.

$[\alpha]_D^{20}$ = -90.7 (c = 10 mg/mL/CH_2Cl_2).

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.03 (9H, s), 3.73 (2H, d, 1/2AB J=19.3Hz, 4.08 (6H, m), 5.66 (1H, t, J=7.7Hz), 5.83 (1H, t, J=7.8Hz), 7.30 (2H, m).

IR(NaCl, CH_2Cl_2): 3400, 1650, 1450, 1100 cm^{-1}.

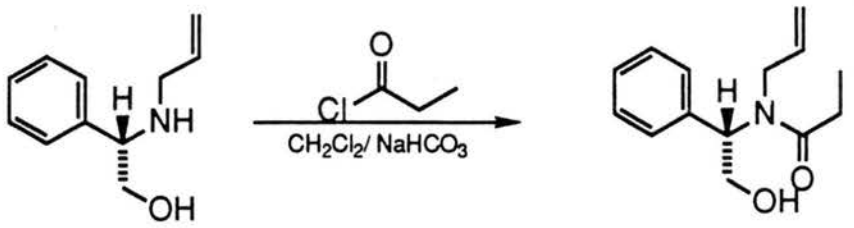


63 64

N-Allylphenylglycinol. (**64**). To a stirred solution of **63** (1.5 g, 10.93 mmol, 1.0 equiv) and triethylamine (1.5 mL, 10.93 mmol, 1.0 equiv) in dry THF (45 mL) was added allyl bromide (1.3 mL, 10.93 mmol, 1.0 equiv) in a steady stream via syringe. The resulting solution was allowed to stir at room temperature for 36 h (or until diallylated compound becomes detectable by TLC), diluted with 100 mL CH_2Cl_2, washed with H_2O (2 x 25 mL), 1M NaOH (2 x 25 mL) and again with H_2O (1 x 25 mL), dried over Na_2SO_4, filtered, evaporated, and purified by chromatatron (4 mm silica gel plate, eluted with 2% MeOH/CH_2Cl_2) yielding **64** (721 mg, 50%) and diallylated product (75 mg) as oils.

[α] of **64**: -77.8 (10 mg/mL; CH_2Cl_2).

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 3.20 (2H, M), 3.70 (3H, m), 5.10 (1H, d, J=11.5Hz), 5.15 (1H, d, J=21.2Hz), 5.80 (1H, m), 7.30 (5H, m). IR(NaCl, neat): 3300, 1645, 1050 cm^{-1}.



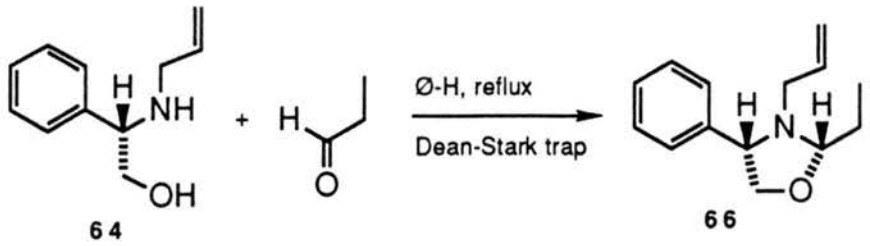
64 65

(N-Allylphenylglycinol)propionamide, (**65**). To a stirred solution of **64** (50 mg, 0.28 mmol, 1.0 equiv) in CH_2Cl_2 was added saturated $NaHCO_3$. To this vigorously stirred two phase mixture was added propionyl chloride (33 mg, 0.35 mmol, 1.25 equiv) in a steady stream via syringe. The interfacial reaction was stirred at room temperature for 2h when the CH_2Cl_2 layer was separated and diluted with 10 mL CH_2Cl_2, washed with H_2O (1 x 5 mL) dried over $MgSO_4$, filtered, and separated by PTLC (silica gel eluted with 5% MeOH/CH_2Cl_2) yielding **65** (56 mg, 85%) as a clear oil.

$[\alpha]_D$ = -69.25 (c = 12.75 mg/mL/CH_2Cl_2).

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.15 (3H, t, J=7.7Hz), 2.40 (2H, m), 3.75 (1H, m), 4.10 (2H, m), 5.15 (2H, m), 5.85 (1H, m), 7.28 (5H, m).

IR(NaCl, neat): 3400, 1640, 1075 cm^{-1}

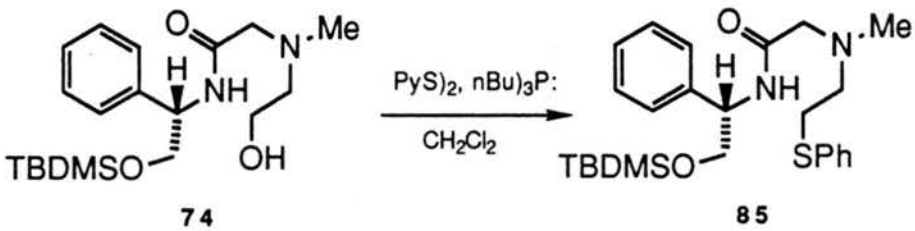


64 66

((N-allyl)cis-2-propyl-5-phenyl)oxazolidine, (**66**). To a stirred solution of **64** (125 mg, 0.71 mmol, 1.0 equiv), in dry benzene (9 mL), was added propionaldehyde (82 mg, 1.4 mmol, 2.0 equiv), via syringe. The cloudy solution was heated to relfux under a nitrogen atmosphere for 2h, evaporated to an oil, then vacuum distilled to yield **66** (141 mg, 90%) as a colorless oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.00 (3H, t, J=7.6Hz), 1.67 (2H, m), 3.1 (1H, 1/2AB, J=7.4Hz), 3.25 (1H, 1/2AB, J=7.4Hz), 3.65 (1H, t, J=7.9Hz), 3.89 (1H, t, J=7.9Hz), 4.14(1H, t, J=7.9Hz), 4.28 (1H, m), 5.02 (2H, m), 5.80 (1H, m), 7.35 (5H, m).

IR(NaCl, neat): 1645, 1600, 1170, 1025, 920, 750, 700 cm^{-1}.



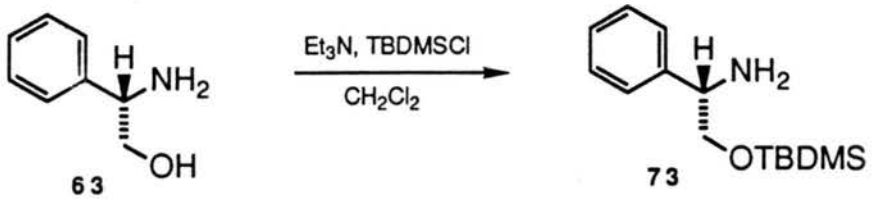
74 85

O-dimethyl-^{t}butylsilylphenylglycinol-2-(N-methyl-2-thiophenyl ethane)-acetamide, (**85**). To a stirred solution of **74** (50 mg, 0.14 mmol, 1.0 equiv) and 2,2'-dipyridyldisulfide (45 mg, 0.2 mmol, 1.5 equiv) in dry CH_2Cl_2 (1.5 mL) was added tri-n-butylphosphine (41 mg, 0.2 mmol, 1.5 equiv) at room temperature.

The resulting yellow solution was evaporated to an oil and separated (PTLC, silica gel, 1:1 EtOAc/hexanes) yielding **85** as a light yellow oil (53 mg, 86%).

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): -0.06 (3H, s), 0.11 (3H, s), 0.85 (9H, s), 2.65 (3H, s), 2.81 (2H, t, J=5.4Hz), 3.12 (2H, s), 3.30 (2H, m), 3.80 (2H, m), 5.03 (1H, m), 6.93 (1H, m), 7.23 (7H, m), 8.07 (1H, d, J=7.1Hz), 8.39 (1H, m).

IR(NaCl, neat): 2900, 1750, 1690, 1590, 1125, 850 cm^{-1}.

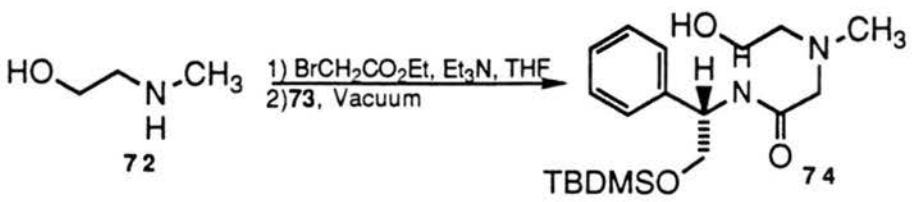


O-Dimethyl-^{t}butylsilylphenyl glycinol, (**73**). To a stirred solution of **63** (100 mg, 0.73 mmol, 1.0 equiv) and Et_3N (89 mg, 09 mmol, 1.2 equiv) in dry CH_2Cl_2 (4 mL) was added ^{t}butyldimethylchlorosilane in one portion. The reaction solution was allowed to stir at room temperature for 5h, diluted with 30 mL CH_2Cl_2, washed with H_2O (2 x 5 mL), 1.0 M NaOH (1 x 5 mL), and again with H_2O (1 x 5 mL), dried over $MgSO_4$, filtered, evaporated, and separated via chromatatron (3 mm plate silica gel eluted with 1:1 ethyl acetate/hexanes) affording **73** (140 mg, 76%) as a clear oil.

$[\alpha]_D^{20}$ = -29.38 (c, 7.25 mg/mL, CH_2Cl_2).

^{1}H NMR (270 MHz, $CDCl_3$) δ($CHCl_3$): 0.00 (6H, s), 0.85 (9H, s), 3.50 (1H, t, J=11.5Hz), 3.71 (2H, m), 7.30 (5H, m).

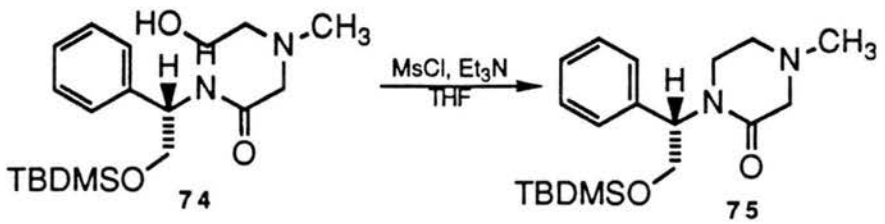
IR(NaCl, neat): 3800, 1100 cm^{-1}.



O-dimethyl-^{t}butylsilylphenylglycinol-2-(N-methyl-2-thiophenyl ethanol)-acetamide, (**74**). To a stirred solution of **72** (500 mg, 6.66 mmol, 1 equiv) and Et_3N (1.35 g, 13.31 mmol, 2 equiv) in dry THF was added ethylbromoacetate (1.33 g, 7.99 mmol, 1.2 equiv) at 0°C. This solution was left to stir at room temperature for 2 h, when it was cooled to 0°C and then filtered. The filtrate was then evaporated to a residue, which was dissolved in CH_2Cl_2 (75 mL), washed with H_2O (2 x 20 mL), 1M NaOH (1 x 20 mL) and brine, dried over $MgSO_4$ and filtered. To the colorless filtrate was added **73** (1.67 g, 6.66 mmol, 1 equiv). This solution was then evaporated to an oil which was put on a vacuum for 48h, the resulting solid was recrystallized from EtOAc/hexanes yielding **74** as colorless rectangular plates (1.9 g, 78% from **72**).Analysis calculated for $C_{19}H_{34}O_3N_2Si$: C, 62.25; H, 9.35; N, 7.64. Found: C, 62.29; H,9.32; N,7.73.

^{1}H NMR (270 MHz, $CDCl_3$) δ($CHCl_3$): 0.06(s, 6H), 0.85(s, 9H), 2.38(s, 3H), 2.60(t, 2H, J=4.2Hz), 3.07(m, 2H), 3.65(t, 2H, J=5.7Hz), 3.82(m, 2H), 5.05(m, 1H), 7.28(m, 4H), 8.05(d, 1H, J=7.1Hz).

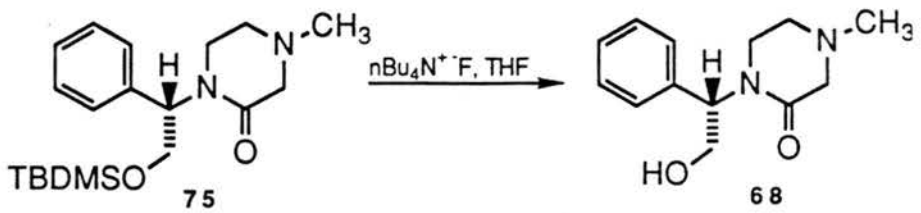
IR(NaCl, neat): 3360, 1650, 1540, 1255, 1150, 1075 cm^{-1}.



O-Diphenyl(t-butylsilyl-1-N-(d-2-phenylethanol)-2-ketopiperazine, (**75**). To a stirred solution of **74** (50 mg, 0.14 mmol, 1 equiv) and Et_3N (27.5 mg, 0.27 mmol, 2 equiv) in dry THF (5 mL) was added mesyl chloride (15 mg, 0.14 mmol, 1 equiv) at 0°C. The reaction mixture was allowed to stir for 18h when another equivalent of MsCl was added along with two equivalents Et_3N. The reaction mixture was then allowed to stir for 48h, then evaporated, dissolved in 40 mL CH_2Cl_2, washed with H_2O (2 x 10 mL) and 1M NaOH (2 x 10 mL) dried over $MgSO_4$, filtered, evaporated to an oil, then separated (PTLC, silica gel, 2:1 EtOAc/hex) yielding **75** as a viscous oil (36 mg, 70%).

^1H NMR (270 MHz, $CDCl_3$) $\delta(CHCl_3)$: 0.11(s, 3H), 0.06(s, 3H), 0.85(s, 9H), 2.31(s, 3H), 2.77(t, 2H, J=5.31Hz), 3.12(s, 2H), 3.56(t, 2H, J=5.62Hz), 3.80(m, 2H, 5.05(m, 1H), 7.28(m, 5H).

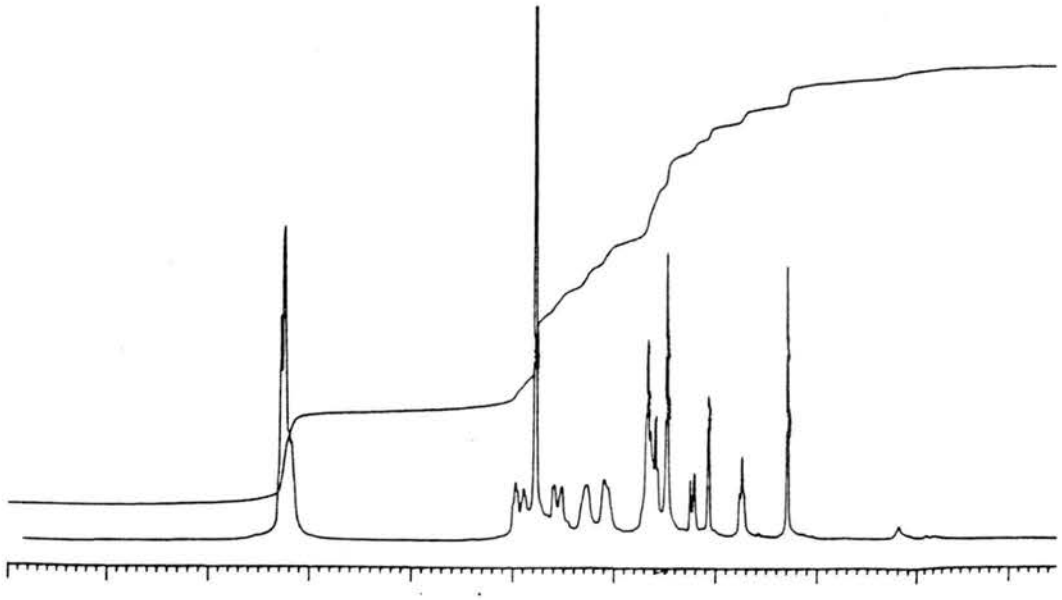
IR(NaCl, neat): 1680, 1505, 1250, 1100 cm^{-1}.



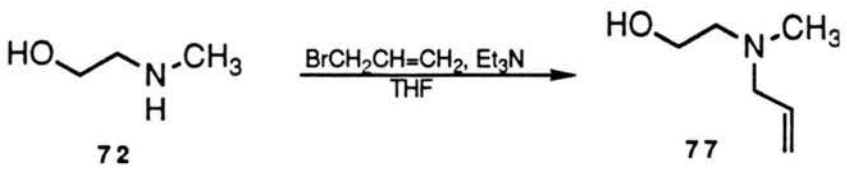
N-(d-2-phenylethanol)-4-N-methyl-2-ketopiperazine, (**68**). To a stirred solution of **75** (280 mg, 0.80 mmol, 1 equiv) in dry THF (10 mL) was added tetrabutylammonium fluoride monohydrate (325 mg, 1.21 mmol, 1.5 equiv) at room temperature. The colorless solution was then allowed to stir at room temperature for 2h. The mixture was diluted with CH_2Cl_2 40 mL, washed with H_2O (2 x 10 mL) and NaOH (1 x 10 mL), dried over $MgSO_4$, and evaporated to an oil which was separated on PTLC (silica gel, 2:1 EtOAc/hexanes) yielding **68** (150 mg, 80%) as a colorless oil.

^1H NMR (270 MHz, CD3OD) δ TMS: unassignable.

IR(NaCl, neat): 3300, 1700, 1050 cm^{-1}.



^1H NMR (270 MHz) of **68** in CD_3OD at 295°K

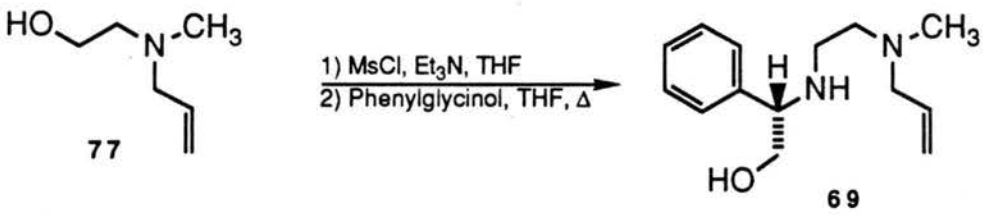


72 77

(N-methyl-N-allyl)ethanolamine, (**77**). To a stirred solution of **72** (1.0 g, 13.31 mmol, 1 equiv) and triethylamine (2.7 g, 26.62 mmol, 2 equiv) in dry THF (45 mL) was added allyl bromide (1.6 g, 13.31 mmol, 1 equiv) at 0°C. After 2h the precipitated $Et_3N \cdot HBr$ was filtered off and the solvent distilled. The resulting oil was distilled under reduced pressure (12 mmHg) yielding 1.4 g **77** as a colorless oil (bp 80-82°C/12 mmHg) (90%).

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 2.20(s, 3H), 2.47(t, 2H, J=7.6Hz), 3.0(d, 2H, J=7.7Hz), 3.25(s, 1H), 3.55(t, 2H, J=7.5Hz), 5.12(m, 2H), 5.77(m, 1H).

IR(NaCl, neat): 3400, 1645, 1030, 910 cm^{-1}.



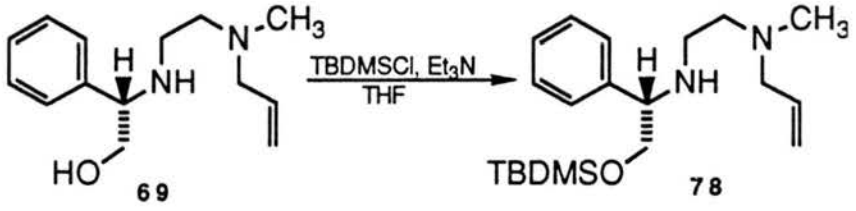
77 69

N-(N-methyl-N-allyl-2-ethyl)phenylglycinol, (**69**). To a stirred solution of **77** (1.0 g, 8.7 mmol, 1 equiv) and Et_3N (1.76 g, 17.4 mmol, 2 equiv) in dry THF (45 mL) was added mesyl chloride (1.0 g, 8.7 mmol, 1 equiv) at 0°C. The reaction was stirred at 0°C for 1h, then allowed to warm to room temperature for an additional hour.

The $Et_3N{\cdot}HCl$ was then filtered off. Phenylglycinol was then added to the filtrate (1.3 g, 9.6 mmol, 1.1 equiv) along with Et_3N (0.88 g, 8.7 mmol, 1 equiv). The resultant solution was refluxed for 20h; the solvent evaporated and the resultant oil washed with ether (75 mL). The ether was then washed with water (1 x 25 mL); dried over Na_2SO_4, evaporated to a white crystalline solid, (**69**), 1.10 g (50% from **77**), recrystallization from hexane/pet. ether yielded colorless needles, mp:92-94°C

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 2.12(s, 3H), 2.38(m, 1H), 2.56(m, 3H), 2.90(t, 2H, J=7.5Hz), 3.65(m, 3H), 5.12(m, 2H), 5.80(m, 1H), 7.27(m, 5H).

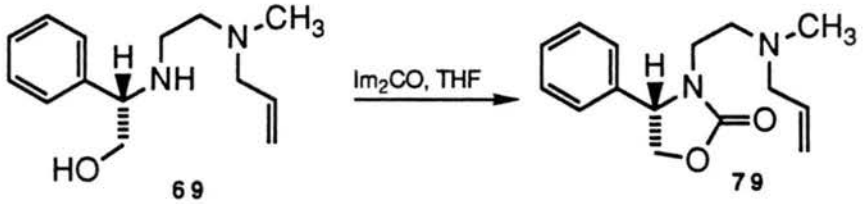
IR(NaCl, neat): 3270, 3100, 1645, 1600, 910 cm^{-1}.



O-tButyldimethylsilyl-N-(N-methyl-N-allyl-2-ethyl)phenylglycinol, (**78**). To a stirred solution of **69** (50 mg, 0.20 mmol, 1 equiv) and Et_3N (30 mg, 3.0 mmol, 1.5 equiv) in 1.5 mL dry THF was added the ^{t}butyldimethylchlorosilane, (57 mg, 0.22 mmol, 1.1 equiv) at room temperature. After stirring for 1h at room temperature, the reaction was diluted with 40 mL CH_2Cl_2 and washed with H_2O (2 x 10 mL) and 1M NaOH (1 x 10 mL); dried over $MgSO_4$, filtered, evaporated, and separated (PTLC, silica gel 5% MeOH, CH_2Cl_2) yielding **78** as a colorless oil (45 mg, 63%).

^{1}H NMR (270 MHz, $CDCl_3$) $\delta(CHCl_3)$: 0.00(s, 6H), 0.85(s, 9H), 2.13(s, 3H), 2.47(m, 4H), 2.95(d, 2H, J=7.15Hz), 2.63(m, 3H), 5.12(m, 2H), 5.82(m, 1H), 7.30(m, 5H).

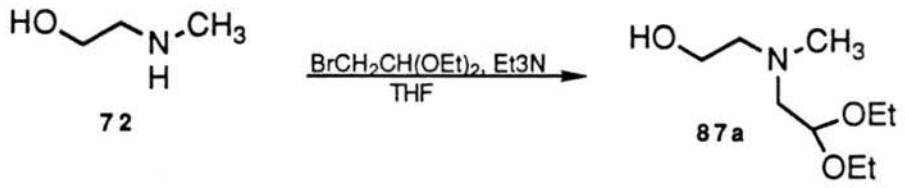
IR(NaCl, neat): 3100, 1645, 1600, 910 cm^{-1}.



N-(2-N-methyl-N-allyl)ethyl-5-phenyl-2-oxazolidinone, (**79**). To a stirred solution of **69** (50 mg, 0.20 mmol, 1 equiv) in THF (5 mL) was added 1,1'-carbonyldiimidazole (65 mg, 0.40 mmol, 2.0 equiv) at room temperature. The reaction solution was allowed to stir for 2h when the solvent was removed *in vacuo*. The resulting slurry was washed with CH_2Cl_2 (40 mL), the organic layer was then washed with H_2O (2 x 5 mL), $NaHCO_3$(2 x 5 mL) and brine, dried over $MgSO_4$, filtered, evaporated, and separated yielding **79** as a colorless oil (5 0 mg, 91%).

^{1}H NMR (270 MHz, $CDCl_3$) $\delta(CHCl_3)$: 2.15(s, 3H), 2.42(m, 2H), 2.87(m, 3H), 3.60(m, 1H), 4.07(t, 1H, J=7.5Hz), 4.61(t, 1H, J=7.3Hz), 4.93(t, 1H, J=7.4Hz), 5.12(m, 2H), 5.78(m, 1H), 7.32(m, 5H).

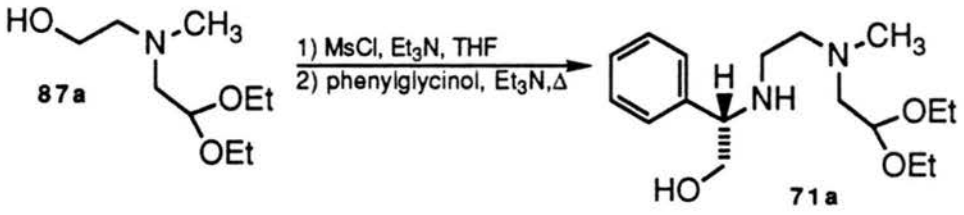
IR(NaCl, neat): 1750, 1645, 1415, 1240, 1060, 760, 700 cm^{-1}.



N-(2,2-diethoxyethyl)-N-methylethanolamine, (**87a**). To a stirred solution of **72** (1.0 g, 13.31 mmol, 1 equiv) and triethylamine (2.7 g, 26.62 mmol, 2 equiv) in dry THF (45 mL) was added bromoacetaldehyde diethyl acetal (2.6 g, 13.31 mmol, 1 equiv) at room temperature. The resulting solution was refluxed for 36 hr. The $Et_3N \cdot HBr$ was then filtered off and the solvent removed by distillation. The resulting red oil was distilled under reduced pressure (12 mmHg) yielding 2.3 g (92%, bp 110-112°C/12 mmHg) of **87a** as a colorless oil.

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.07(t, 6H, J=7.1Hz), 2.22(s, 3H), 2.47(t, 2H, J=5.0Hz), 3.50(m, 8H), 4.46(t, 1H, J=5.1Hz).

IR(NaCl, neat): 3460, 1460, 1375, 1130, 1050 cm^{-1}.

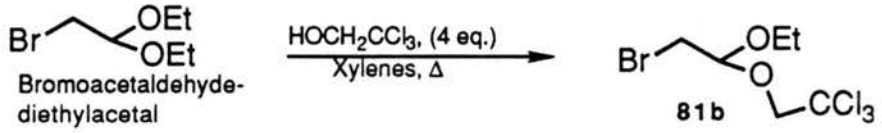


d-N-2-(N-methyl-2-N-(diethoxyethyl)-ethyl)phenylglycinol, (**71a**). To a stirred solution of **87a** (1.0 g, 5.2 mmol, 1 equiv) and Et_3N (1.05 g, 10.47 mmol, 2 equiv) in 45 mL dry THF was added mesyl chloride (0.59 g, 5.2 mmol, 1.0 equiv) at 0°C. The reaction was left

to stir at 0°C for 1h, then at room temperature for an additional hour. The $Et_3N{\cdot}HCl$ was filtered off. *d*-Phenylglycinol was added to the filtrate (0.71 g, 5.2 mmol, 1.0 equiv) along with Et_3N (0.53 g, 5.2 mmol, 1 equiv). The resulting solution was refluxed for 12h. The solvent was removed *in vacuo* and the resulting oil was triturated with ether (75 mL). The ether was then washed with H_2O (1 x 25 mL), $NaHCO_3$ (1 x 25 mL), and brine (1 x 25 mL); dried over Na_2SO_4 evaporated to an oil and separated by MPLC (silica gel eluted with 10% $MeOH/CH_2Cl_2$) yielding 0.95 g of **71a** as an oil (60% from **87a**).

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.15(t, 6H, J=7.6Hz), 2.15(s, 3H), 2.45(m, 6H), 3.55(m, 7H), 4.5(t, 1H, J=5.6Hz), 7.27(m, 5H).

IR(NaCl, neat): 3400, 1600, 1450, 1375, 1125, 1055, 750cm^{-1}.

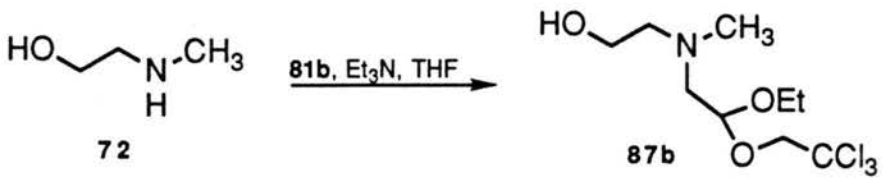


Bromoacetaldehyde-2,2,2-trichloroethyl-ethylacetal, (**81b**). To a solution of 2,2,2-trichloroethanol (29.8 g, 200 mmol, 4 equiv) in 75 mL xylene heated to reflux was added a catalytic amount of p-TsOH, followed by the addition of a solution of bromoacetaldehyde diethylacetal (9.8 g, 50 mmol, 1 equiv) in 15mL xylene. The acetal was added at a rate equal to the distillation of xylene from the reaction mixture. After the addition was complete, and reflux temperature was equal to the boiling point of xylene, the reaction was distilled for an additional hour (additional xylene was added). The xylene was then removed *in vacuo* yielding a dark reddish

solution from which the product was distilled under 15 mmHg, yielding pure **86b** (9.52 g, 63%); bp: 125-127°C/15 mmHg.

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.27(t, 3H, J=7.3Hz), 3.46(d, 2H, J=5.9Hz), 3.78(m, 2H), 4.19(s, 2H), 4.99(t, 1H, J=5.5Hz).

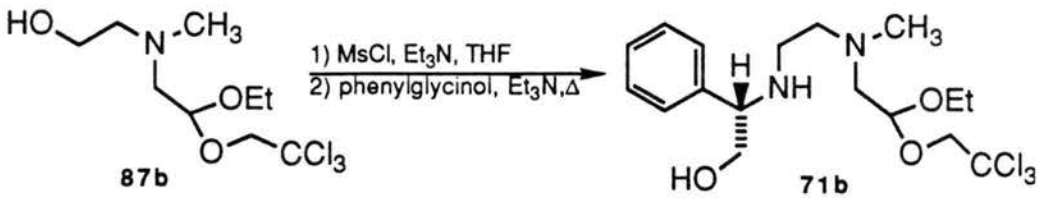
IR(NaCl, neat): 2950, 1645, 1075, 800, 720 cm^{-1}.



N-methyl-2-N-(ethoxy-2',2',2'-trichloroethoxy-ethyl)-ethanolamine, (**87b**), To a stirred solution of **72** (0.5 g, 6.66 mmol, 1 equiv) and Et_3N (1.35 g, 13.31 mmol, 2 equiv) in 50 mL THF was added the bromoacetaldehyde ethyl-2,2,2-trichloroethyl acetal (2.0 g, 6.657 mmol, 1 equiv). The resulting colorless reaction solution was refluxed for 48h, cooled and the solvent removed *in vacuo*. The resulting oil was dissolved in 50 mL CH_2Cl_2, washed with H_2O (2 x 10 mL), 1 M NaOH (2 x 10 mL) and brine, dried over $MgSO_4$, filtered, evaporated, and separated (MPLC, silica gel, 5% MeOH/CH_2Cl_2) affording **87b** (0.6 g, 31%) as a light yellow oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.25(t, 3H, J=7.1Hz, 2.39(s, 3H), 2.64(t, 2H, J=5.2), 2.71(dd, 2H, J=5.3Hz), 3.62(m, 3H), 3.85(m, 1H), 4.17(ABq, 2H, J=14.7Hz), 4.90(t, 1H, J=5.4Hz).

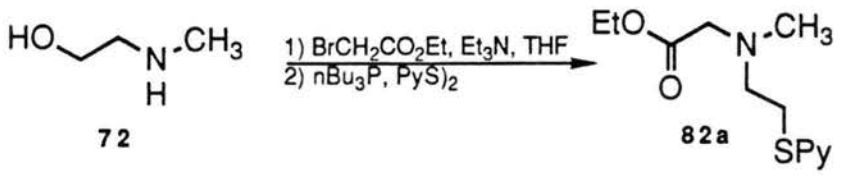
IR(NaCl, neat): 3440, 1450, 1130, 1050, 785, 715 cm^{-1}.



d-N-2-(N-methyl-2-N-(ethoxy-2',2',2'-trichloroethoxyethyl)-ethyl phenylglycinol, (**71b**). To a stirred solution of **87b** (300 mg, 1.02 mmol, 1 equiv) and Et_3N (0.3 mL, 2.0 mmol, 2 equiv) in 25 mL dry THF was added mesyl chloride (174 mg, 1.53 mmol, 1.5 equiv) at 0°C. The reaction was left stirring for 2 hr at 0°C, then filtered. To the colorless filtrate was added D-phenylglycinol (140 mg, 1.02 mmol, 1 equiv) and Et_3N (0.15 mL, 1.02 mmol, 1 equiv). The resulting clear solution was refluxed for 4h when the solvent was removed *in vacuo*. The resulting oil was triturated with Et_2O (2 x 30 mL). The Et_2O was washed with H_2O (2 x 5 mL), 10% $NaHCO_3$ (2 x 5 mL), H_2O (1 x 5 mL) brine, and dried over $MgSO_4$. Filtration and evaporation yielded an oil which was separated (PTLC, silica gel, 3 mm), 89:9:1 CH_2Cl_2:MeOH:NH_4OH) yielding **71b** as a light yellow oil (150 mg, 34%).

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.23(3H, m), 2.25(s, 3H), 2.60(m, 6H), 2.85(bs, 2H), 3.72(m, 5H), 4.19(s, 2H), 4.88(m, 1H), 7.30(m, 5H).

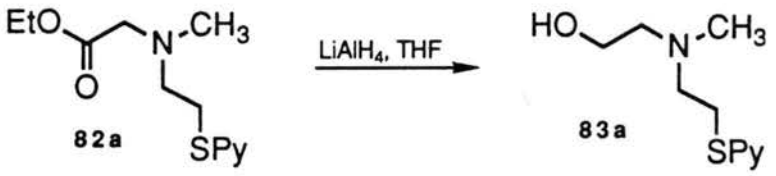
IR(NaCl, neat): 3300, 2900, 1450, 1140, 1050, 800, 720cm^{-1}.



Ethyl(N-methyl-2-N-thiopyridylethyl)glycinate, (**82a**). To a stirred solution of **72** (1.0 g, 13.31 mmol, 1 equiv) and Et_3N (2.7 g, 26.62 mmol. 2 equiv) in 50 mL dry THF was added ethylbromoacetate (3.33 g, 19.97 mmol, 1.5 equiv) at 0°C. The reaction was allowed to stir for 2h when it was filtered and the $Et_3N \cdot HBr$ salt washed with 10 mL cold THF. To this clear filtrate was added 2,2'-dipyridyldisulfide (4.4 g, 19.97 mmol, 1.5 equiv) and $nBu)_3P$ (4.05 g, 19.97 mmol, 1.5 equiv). The resulting yellow solution was evaporated then separated (MPLC, silica gel, 2:1 hex/EtOAc) affording **82a** as a pale yellow oil (2.48 g, 73%).

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.23(t, 3H, J=7.2Hz), 2.43(s, 3H), 2.83(t, 2H, J=6.6Hz), 3.27(t, 2H, J=6.9Hz), 3.33(s, 2H), 4.15(q, 2H, J=7.3Hz).

IR(NaCl, neat): 1740, 1575, 1175, 1050, 750, 710 cm^{-1}.

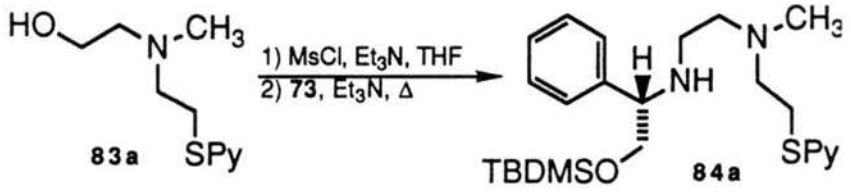


(N-2-thiopyridylethyl)ehtanolamine, (**83a**). To a stirred solution of **82a** (100 mg, 0.39 mmol, 1 equiv) in 2 mL dry THF was added $LiAlH_4$ (45 mg, 1.18 mmol, 3 equiv) at 0°C. The reaction

suspension was stirred for 2h and quenched with 250 μL H_2O. The reaction was diluted with CH_2Cl_2 (40 mL) and washed with H_2O (1 x 10 mL), 1M NaOH (1 x 10 mL) and brine, dried over $MgSO_4$, filtered, evaporated, and separated (PTLC, silica gel, 10% MeOH/CH_2Cl_2) yielding **83a** as a pale yellow oil (60 mg, 78%).

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 2.31(s, 3H), 2.57(t, 2H, J=5.1Hz), 2.73(t, 2H, J=6.8Hz), 3.28(t, 2H, J=7.1Hz), 3.54(t, 2H, J=5.4Hz), 6.93(m, 1H), 7.15(d, 1H, J=7.7Hz), 7.42(t, 1H, J=6.8Hz), 8.47(d, 1H, J=5.1Hz).

IR(NaCl, neat): 3400, 2950, 1585, 1560, 1050, 755 cm^{-1}.

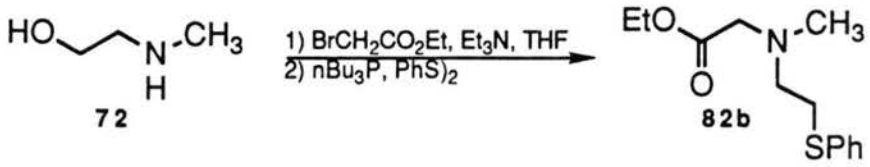


d-N-2-(N-methyl-2-N-(thiopyridylethyl)ethyl phenylglycinol, (**84a**). To a stirred solution of **83a** (300 mg, 1.53 mmol, 1 equiv) and Et_3N (309 mg, 3.06 mmol, 1 equiv) in 25 mL dry THF was added mesyl chloride (263 mg, 2.30 mmol, 1.5 equiv) at 0°C. The reaction was allowed to stir for 2h, and filtered. To the colorless filtrate was added the phenyl glycinol derivative, **73**, (380 mg, 1.5 mmol, 1 equiv) and Et_3N (154 mg, 1.5 mmol, 1 equiv). The clear solution was refluxed for 5h and the solvent was removed *in vacuo*. The resulting oil was triturated with Et_2O (2 x 50 mL). The Et_2O was then washed with H_2O (1 x 20mL), 1M NaOH (1 x 20 mL), and brine; dried over

Na_2SO_4, filtered, evaporated, and separated (PTLC, silica gel, 89:9:1 CH_2Cl_2:MeOH:NH_4OH) yielding **84a** as a yellow oil (194 mg, 30%).

^1H NMR (270 MHz, $CDCl_3$) δ: 0.00(s, 6H), 0.85(s, 9H), 2.23(s, 3H), 2.60(m, 5H), 3.23(t, 2H, J=5.78Hz), 3.65(m, 3H), 6.90(m, 1H), 7.31(m, 7H), 8.33(m, 1H).

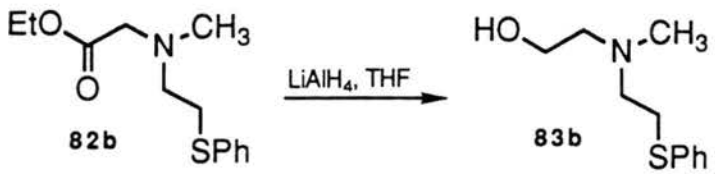
IR(NaCl, neat): 3330, 1580, 1260, 1100, 840 cm^{-1}.



Ethyl(N-methyl-2-N-thiophenylethyl)glycinate, (**82b**). To a stirred solution of **72** (1.0 g, 13.31 mmol, 1.0 equiv) and Et_3N (2.7 g, 26.62 mmol, 2.0 equiv) in 50 mL dry THF was added ethylbromoacetate (3.33 g, 19.97 mmol, 1.5 equiv) at 0°C. The reaction was allowed to stirring at 0°C for 2h when it was filtered and the Et_3N·HBr salt washed with 10 mL cold THF. To this clear filtrate was added phenyl disulfide (4.35 g, 19.97 mmol, 1.5 equiv) and tri-n-butyl phosphine (4.05 g, 19.97 mmol, 1.5 equiv). The resulting yellow solution was evaporated then separated (MPLC, silica gel, 3:1 hex/EtOAc) yielding **82b** as a pale yellow oil (2.58 g 76%).

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.25(t, 3H, J=7.3Hz), 2.43(s, 3H), 2.80(t, 2H, J=6.7Hz), 3.05(t, 2H, J=6.6Hz), 3.30(s, 2H), 4.15(q, 2H, J=7.2Hz).

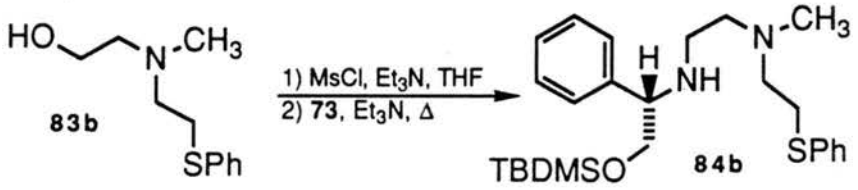
IR(NaCl, neat): 1740, 1580, 1175, 1050, 730, 680 cm^{-1}.



(N-2-thiophenylethyl)ehtanolamine, (**83b**). To a stirred solution of **82b** (1.0 g, 3.95 mmol, 1 equiv) in 25 mL dry THF was added $LiAlH_4$ (0.45 g, 11.84 mmol, 3 equiv) at 0°C. The reaction mixture was stirred for 2h at 0°C when it was quenched with H_2O (3 mL). The reaction was then diluted with 100 mL CH_2Cl_2 and washed with H_2O (2 x 25 mL), 1M NaOH (2 x 25 mL) and brine, dried over $MgSO_4$, filtered, evaporated, and separated (MPLC, silica gel, 5% $MeOH/CH_2Cl_2$) yielding **83b** as a pale yellow oil (635 mg, 76%).

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 2.31(s, 3H), 2.56(t, 2H, J=5.4Hz), 2.70(t, 2H, J=6.6Hz), 3.05(t, 2H, J=6,0Hz), 3.56(t, 2H, J=5.3Hz), 7.33(m, 5H).

IR(NaCl, neat): 3400, 2960, 1580, 1050 cm^{-1}.

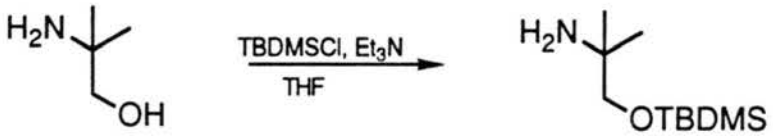


d-N-2-(N-methyl-2-N-(thiophenylethyl)ethyl phenylglycinol, (**84a**). To a stirred solution of **83b** (300 mg, 1.42 mmol, 1 equiv) and Et_3N (0.40 mL, 2.84 mmol, 2 equiv) in 25 mL dry THF was added mesyl chloride (263 mg, 2.3 mmol, 1.5 equiv) at 0°C. The reaction was stirred for 1h, and filtered. To the colorless filtrate solution

was added the phenyl glycinol derivative, **73** (352 mg, 1.42 mmol, 1 equiv) and Et_3N (0.20 mL, 1.42 mmol, 1 equiv). The reaction was then stirred at room temperature for 4h and the solvent was removed *in vacuo*. The resulting oil was triturated with ether (2 x 50 mL). The ether was then washed with H_2O (1 x 10 mL), 5% NH_4CO_3((1 x 10 mL) and dried over $MgSO_4$, filtered, evaporated, and separated by MPLC (silica gel, 89:9:1, CH_2Cl_2:MeOH:NH_4OH) yielding **84b** as a clear oil (310 mg, 50%).

^1H NMR (270 MHz, $CDCl_3$) $\delta(CHCl_3)$: 0.00(d, 6H, J=0.5Hz), 0.874(s, 9H), 2.207(s, 3H), 2.466(m, 5H), 2.61(t, 2H, J=5.3Hz), 3.0(t, 2H, J=5.2Hz), 3.60(m, 3H), 7.35(m, 10H).

IR(NaCl, neat): 3330, 1580, 1260, 1100, 840 cm^{-1}.

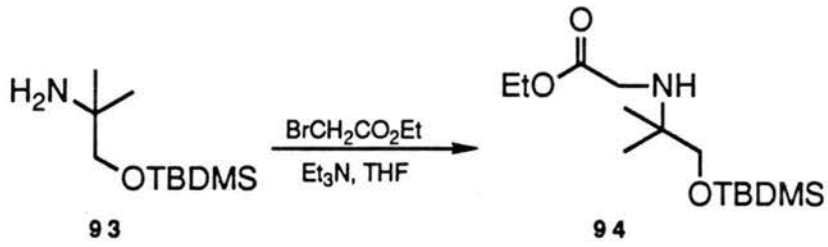


92 **93**

O-^{t}Butyldimethylsilyl-2-amino-2-methylpropanol, (**93**). To a stirred solution of **92** (2 g, 22.4 mmol, 1 equiv) and Et_3N (2.72 g, 26.9 mmol, 1.2 equiv) in 45 mL dry THF was added ^{t}butyldimethylchlorosilane (3.40 g, 22.4 mmol, 1 equiv) in one portion at room temperature. The reaction was allowed to stir overnight. The resulting suspension was filtered and the THF removed *in vacuo*. The resulting oil was distilled yielding 2.3 g (50%) of **93** bp = 63° / 18 mmHg.

^{1}H NMR (270 MHz, $CDCl_3$) δ TMS: 0.10(s, 6H), 0.82(s, 9H), 0.97(s, 6H), 2.15(bs, 2H), 3.22(s, 2H);

IR(NaCl, neat): 3300, 2920, 1590, 1250, 1100, 850 cm^{-1}.

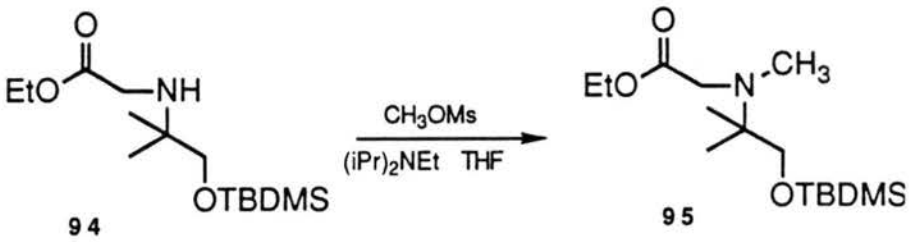


93 94

O-^{t}Butyldimethylsilyl-2-(N-carboethoxymethyl)amino-2-methylpropanol, (**94**). To a stirred solution of **93** (1.0 g, 4.9 mmol, 1.0 equiv) and Et_3N (1.40 mL, 9.8 mmol, 2.0 equiv) in 10 mL dry THF was added ethylbromoacetate (0.93 mL, 8.4 mmol, 1.7 equiv) in one portion at room temperature. The reaction was allowed to stir at room temperature for 36h, diluted with 70 mL CH_2Cl_2 washed with water (3 x 20 mL), sat. $NaHCO_3$ (1 x 20 mL), dried over $MgSO_4$, filtered and evaporated to a pale yellow oil. The oil was purified by chromatography (silica gel, 3:1 hexanes:EtOAc) yielding 0.860 g of **94** as a colorless oil (61%).Analysis calculated for $C_{14}H_{31}O_3NSi$: C, 58.09; H, 10.79; N, 4.84. Found: C, 58.31; H,10.50; N,4.78.

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 0.02(s, 6H), 0.88(s, 9H), 1.01(s, 6H), 1.25(t, 3H, J=7.2Hz), 3.35(s, 4H), 4.16(q, 2H, J=7.2Hz);

IR(NaCl, neat): 2950, 1750, 1250, 1100 cm^{-1}.

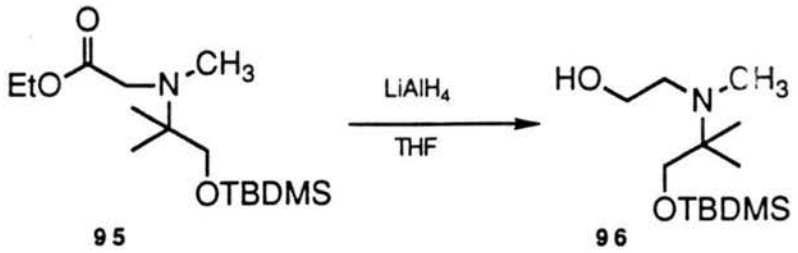


94 95

O-^{t}Butyldimethylsilyl-2-(N-carboethoxymethyl-N-methyl)amino-2-methylpropanol, (**95**). To a stirred solution of **94** (0.86 g, 2.97 mmol, 1.0 equiv) and diisopropylethylamine (3.11 mL, 17.83 mmol, 6 equiv) in 12 mL dry THF was added methanemethylsulfonate (1.26 mL, 14.86 mmol, 5 equiv) at room temperature. The reaction was allowed to stir at room temperature for 36h, diluted with 85 mL CH_2Cl_2, washed with water (5 x 20 mL) and sat. $NaHCO_3$, dried over $MgSO_4$, filtered and evaporated to an oil. The oil was purified by column chromatography (silica gel, 4:1 hexane:EtOAc) yielding 700mg of **95** as a colorless oil (77%).Analysis calculated for $C_{15}H_{33}O_3NSi$: C, 59.36; H, 10.96; N, 4.62. Found: C, 59.16; H,10.68; N,4.49.

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 0.03(s, 6H), 0.86(s, 9H), 1.02(s, 6H), 1.23(t, 3H, J=7.2Hz), 2.33(s, 3H), 3.37(s, 2H), 3.45(s, 2H, 4.13(q, 2H, J=7.1Hz);

IR(NaCl, neat): 2920, 1750, 1250, 1180, 1100, 850 cm^{-1}.

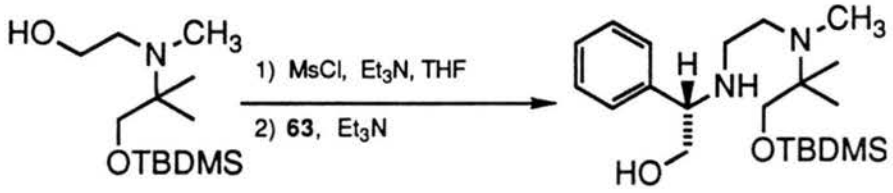


95 96

O-^{t}Butyldimethylsilyl-2-(N-(2-hydroxy)ethyl-N-methyl)amino-2-methylpropanol, (**96**). To a stirred solution of **95** (700mg, 2.3 mmol, 1.0 equiv) in 50 mL dry THF was added $LiAlH_4$ (87 mg, 2.3 mmol, 1.0 equiv) at 0°C. The reaction was stirred at 0°C for 2h, quenched with $Na_2SO_4 \cdot 10\ H_2O$, filtered and evaporated yielding pure **96** as a colorless oil (0.498 g, 83%).

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 0.01(s, 6H), 0.85(s, 9H), 0.97(s, 6H), 2.20(s, 3H), 2.58(t, 2H, J=5.7Hz), 3.30(bs, 1H), 3.40(s, 2H), 3.46(t, 2H, J=5.1Hz);

IR(NaCl, neat): 3420, 2940, 1250, 1100, 850 cm^{-1}.



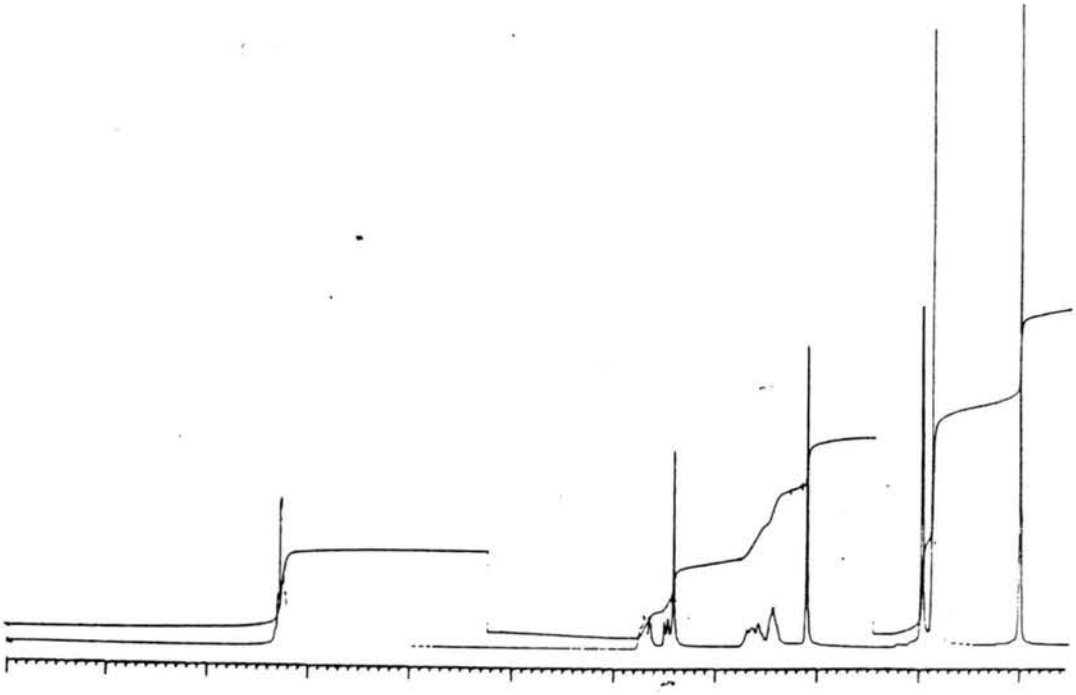
96 97

D-N-2-(N-methyl-N-(2-(O-^{t}butyldimethylsilyl)hydroxy-1,1-dimethylethyl))ethyl phenylglycinol, (**97**). To a stirred solution of **96** (50 mg, 0.19 mmol, 1.0 equiv) in 2 mL dry THF cooled to 0°C was added the Et_3N (40 μL, 0.23 mmol, 1.2 equiv). The reaction was allowed to stir at 0°C for 1h, filtered to remove the $Et_3N \cdot HCl$ salt. To

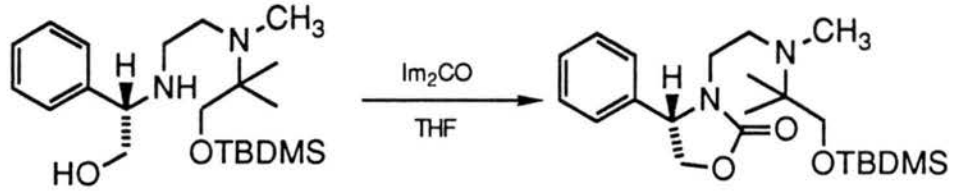
the colorless filtrate was added phenyl glycinol (26 mg, 0.19 mmol, 1.0 equiv) and Et_3N (26.6 μL, 0.19 mmol, 1.0 equiv). The reaction solution was refluxed for 3h, cooled to room temperature, diluted with 25 mL CH_2Cl_2, washed with H_2O (2 x 5 mL), 1M NaOH (1 x 5 mL) and sat. NaCl (1 x 5 mL) dried over $MgSO_4$, filtered, evaporated and separated by PTLC (silica gel, 89:9:1, CH_2Cl_2:MeOH:NH_4OH) yielding **97** as a colorless oil (35 mg, 49%).

^1H NMR (270 MHz, $CDCl_3$) δ TMS: 0.00(s, 6H), 0.86(s, 9H), 0.97(s, 3H), 0.98(s, 3H), 2.10(s, 3H), 2.57(m, 4H), 3.42(s, 2H), 3.45(m, 1H), 3.72(m, 2H), 7.27(m, 5H);

IR(NaCl, neat): 3300, 2900, 1600, 1250, 1085, 845, 765cm^{-1}.



^1H NMR (270 MHz) of **97** in $CDCl_3$ at 295°K

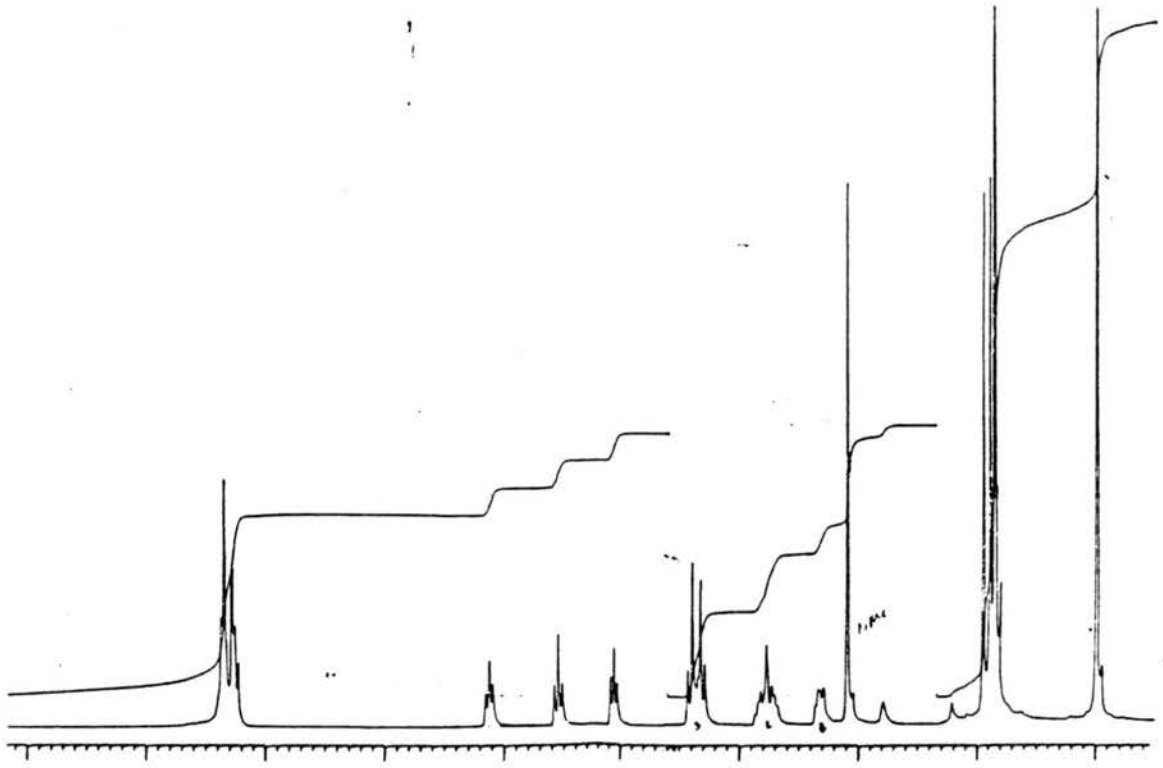


97 98

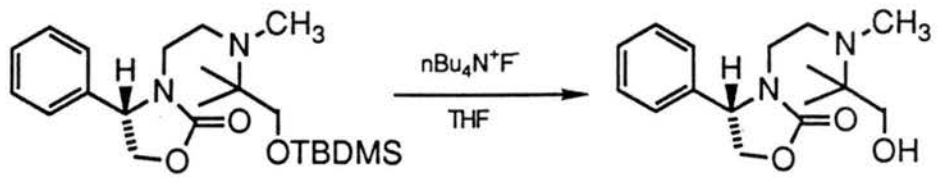
D-N-(2-N-methyl-N-(2-(O-^{t}butyldimethylsilyl)hydroxy-1,1-dimethylethyl)ethyl-5-phenyl-2-oxazolidinone, (**98**). To a stirred solution of **97** (26 mg, 0.07 mmol, 1.0 equiv) in 1.0 mL dry THF was added 1,1'-carbonyldiimidazole (22 mg, 0.14 mmol, 2.0 equiv) at room temperature. The reaction was allowed to stir at room temperature overnight, then diluted with 15 mL CH_2Cl_2, washed with 15% NaOH (2 x 5 mL), H_2O (2 x 5 mL) and sat. NaCl (1 x 5 mL). The organic phase was dried over $MgSO_4$, filtered, evaporated and separated by PTLC (silica gel, 3:2 hexane:EtOAc) yielidng **98** as a colorless oil (28 mg, 100%).Analysis calculated for $C_{22}H_{38}O_3N_2Si$: C, 64.98; H, 9.42; N, 6.89. Found: C, 64.38; H,8.78; N,7.84.

^{1}H NMR (270 MHz, $CDCl_3$) δ ($CHCl_3$): 0.00(s, 6H), 0.85(s, 9H), 0.89(s, 3H), 0.96(s, 3H), 2.10(s, 3H), 2.30(m, 1H), 2.75(m, 2H), 3.37(m, 3H), 4.06(m, 1H), 4.55(m, 1H), 5.121(t, 1H, J=8.1Hz);

IR(NaCl, neat): 2900, 1750, 1250, 1090, 840, 765, 690 cm^{-1}.



^{1}H NMR (270 MHz) of **98** in $CDCl_3$ at 295^{o}K



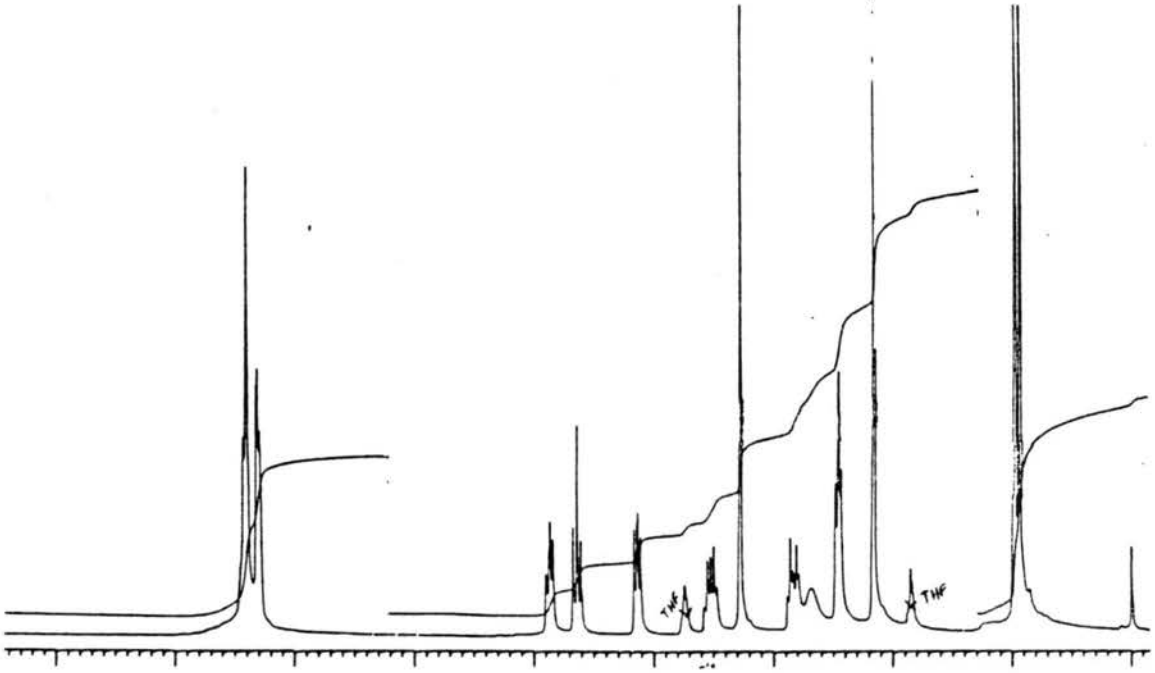
98

D-N-(2-N-methyl-N-(2-hydroxy-1,1-dimethylethyl)ethyl-5-phenyl-2-oxazolidinone. To a stirred solution of **98** (92 mg, 0.23 mmol, 1.0 equiv) in 2.5 mL dry THF was added tetrabutylammonium fluoride trihydrate (107 mg, 0.34 mmol, 1.5 equiv) at room temperature. The reaction was allowed to stir for 1h, evaporated and separated by PTLC (silica gel, 92:7:1, CH_2Cl_2:MeOH:NH_4OH) yielding 60 mg of the intermediate alcohol (91%) as a colorless oil.

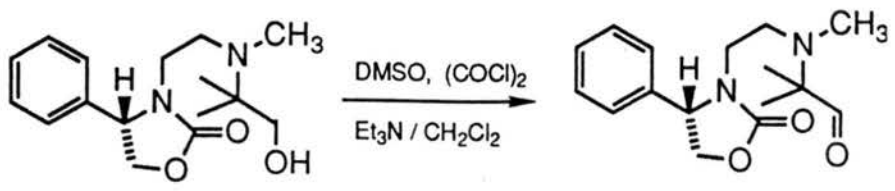
Analysis calculated for $C_{16}H_{24}O_3N_2$: C, 65.73; H, 8.27; N, 9.58. Found: C, 63.93; H,8.17; N,9.15.

^1H NMR (270 MHz, $CDCl_3$) δ TMS: 0.95(s, 3H), 0.99(s, 3H), 2.17(s, 3H), 2.47(t, 2H, J=6.3Hz), 2.67(bs, 1H), 2.84(m, 1H), 3.28(s, 2H), 3.53(m, 1H), 4.14(dd, 1H, J=8.7Hz), 4.65(dd, 1H, J=8.9Hz), 4.87(dd, 1H, J=8.8Hz), 7.29(m, 2H), 7.44(m, 3H);

IR(NaCl, neat): 3460, 2940, 1750, 1410, 1050, 760, 695 cm^{-1}.



^1H NMR (270 MHz) of **98** in $CDCl_3$ at 295°K



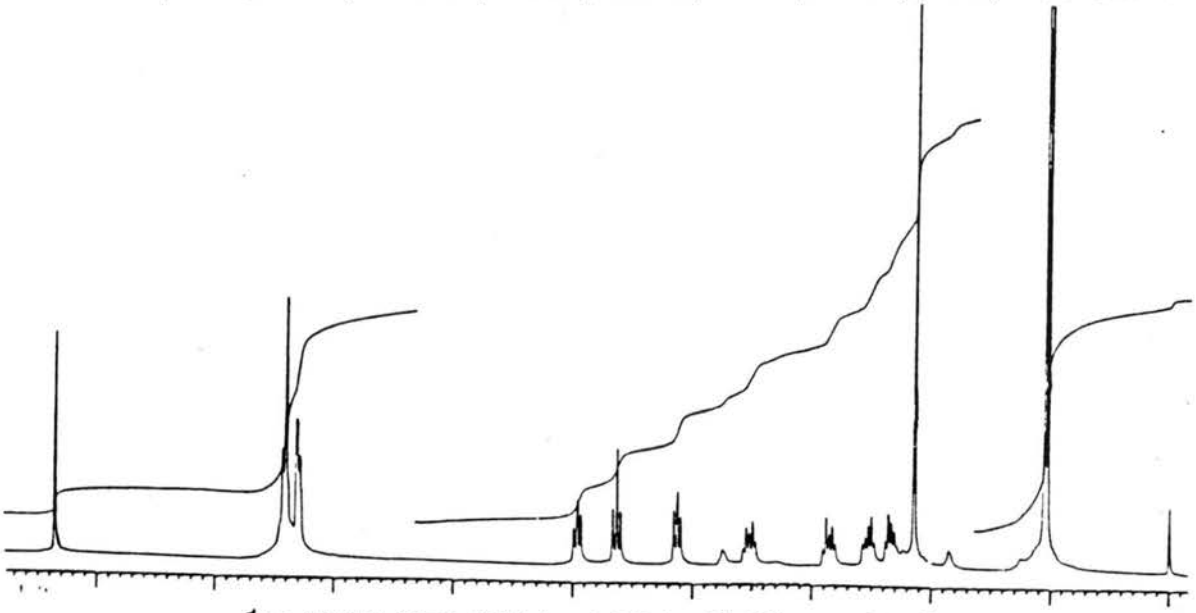
99

D-N-(2-N-methyl-2-N-(dimethylacetaldehyde)ethyl-5-phenyl-2-oxazolidinone, (**99**). To a solution of dry DMSO (28 μL, 0.40 mmol, 3.0 equiv) in 1.5 mL dry CH_2Cl_2 cooled to -78°C was added oxalyl

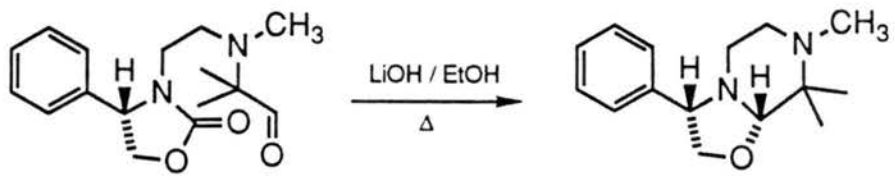
chloride (17 μL, 0.20 mmol, 1.5 equiv). The resulting solution left stirring at -78°C for 1h. To this solution was added the intermediate alcohol (obtained above; 39 mg, 0.13 mmol, 1.0 equiv) as a solution in 1.5 mL dry CH_2Cl_2. The reaction was stirred at -78°C for 1.5h and Et_3N (93 μL, 0.67 mmol, 5.0 equiv) was added. The resulting suspension was allowed to warm to room temperature, diluted with 20 mL CH_2Cl_2, washed with H_2O (1 x 5 mL), 15% NaOH (1 x 5 mL) and sat. NaCl (1 x 5 mL). The organic layer was dried over $MgSO_4$, filtered, evaporated, and separated (silica gel, 3% $MeOH/CH_2Cl_2$) yielding **99** (32 mg, 82%) as a colorless oil.Analysis calculated for $C_{16}H_{22}O_3N_2$: C, 66.18 H, 7.64; N, 9.65. Found: C, 65.91; H,7.67; N,9.52.

^1H NMR (270 MHz, $CDCl_3$) δ TMS: 1.04(s, 3H), 1.06(s, 3H), 2.15(s, 3H), 2.31(m, 1H), 2.50(m, 1H), 2.85(m, 1H), 3.62(m, 1H), 4.13(dd, 1H, J=8.1Hz), 4.63(t, 1H, J=8.8Hz), 4.96(t, 1H, J=8.6Hz), 7.31(m, 2H), 7.45(m, 3H), 9.36(s, 1H);

IR(NaCl, neat): 2980, 1800, 2695, 1750, 1175, 760, 700 cm^{-1}.



^1H NMR (270 MHz) of **99** in $CDCl_3$ at 295°K

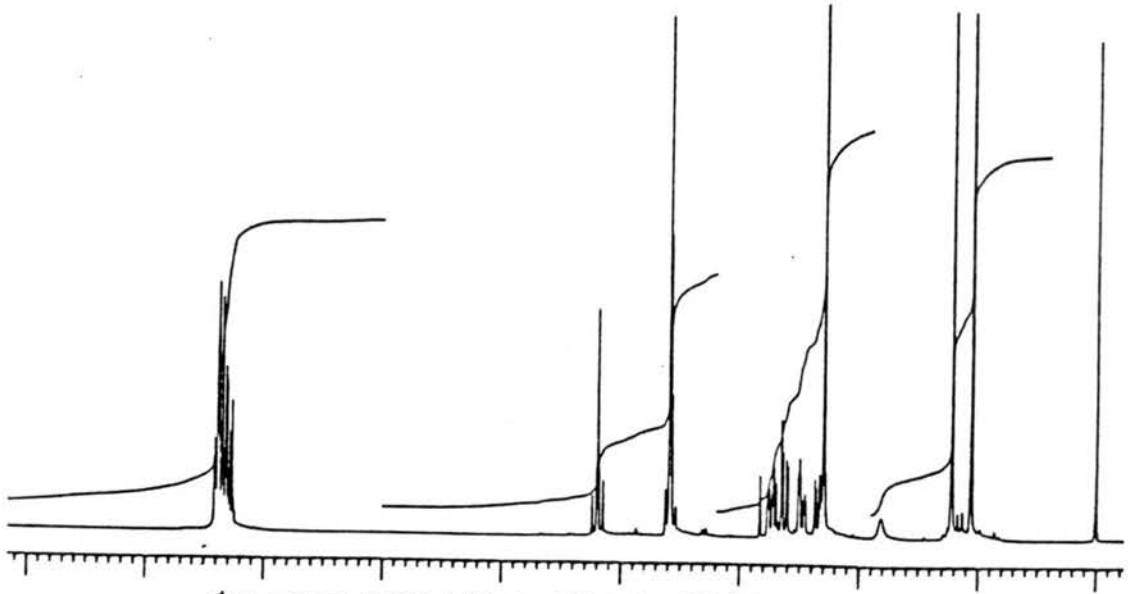


99 **91**

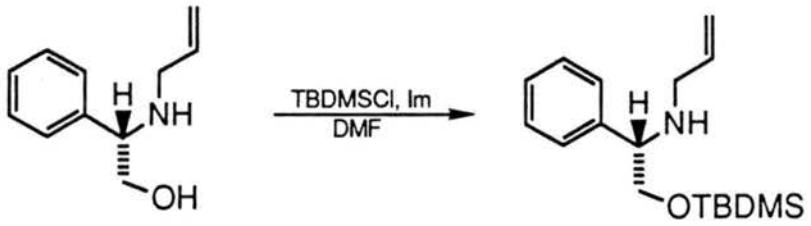
Cis-1,2-(3,3,4-trimethyl)piperazine-fused-(5-phenyl) oxazolidine, (**91**). To a stirred solution of **99** (32 mg, 0.11 mmol, 1.0 equiv) in 1 mL absolute ethanol was added a 1M solution of LiOH (0.44 mL, 0.44 mmol, 4.0 equiv). The resulting solution was refluxed for 12h then diluted with CH_2Cl_2 (5 mL). The organic phase was separated and dried over $MgSO_4$, filtered, evaporated and separated by PTLC (silica gel, 89:9:1, CH_2Cl_2:MeOH:NH_4OH) yielding 7 mg (23%) of **91** as a colorless oil.Analysis calculated for $C_{15}H_{22}ON_2$: C, 73.13; H, 9.00; N, 11.37. Found: C, 72.89; H,9.19; N,11.39.

^1H NMR (100 MHz, $CDCl_3$) δTMS: 1.06(s, 3H), 1.20(s, 3H), 2.30(s, 3H), 2.60(m, 4H), 3.57(m, 3H), 4.20(t, 1H, J=1.1Hz), 7.35(m, 5H).

IR(NaCl, neat): 2940, 2800, 1760, 1455, 1140, 1065, 820cm^{-1}.



^1H NMR (270 MHz) of **91** in $CDCl_3$ at 295°K

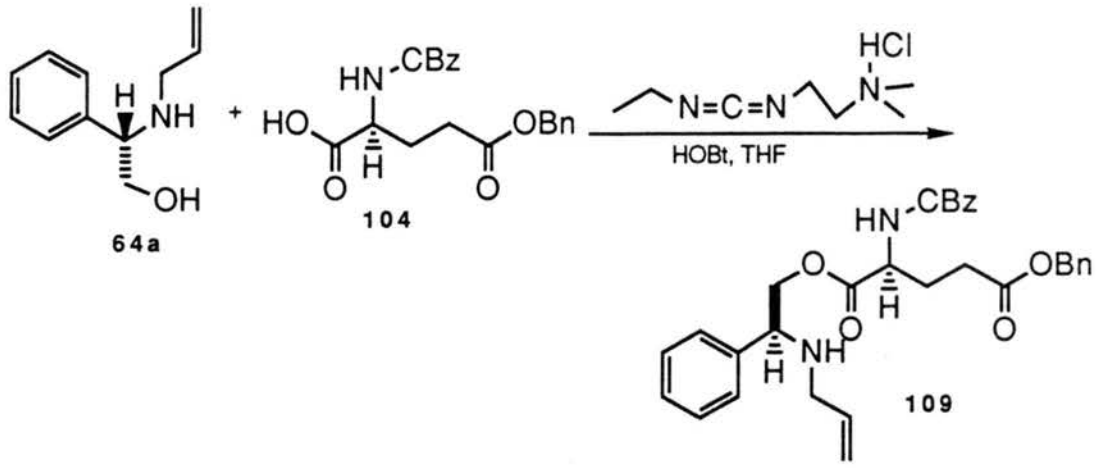


64a **64b**

N-allyl-O-^{t}butyldimethylsilyl phenylglycinol, (**64b**). To a stirred solution of **64a** (50 mg, 0.28 mmol, 1.0 equiv), and imidazole (23 mg, 0.34 mmol, 1.2 equiv) in DMF (2 mL) was added ^{t}butyldimethylchlorosilane (51 mg, 0.34 mmol, 1.2 equiv) in one portion at room temperture. The resulting solution was stirred at room temperature for 1h, diluted with 40 mL CH_2Cl_2 and washed with H_2O (1 x 10 mL) 1.0 M NaOH (1 x 10 mL) and again with H_2O (1 x 10 mL), dried over $MgSO_4$, filtered, evaporated and separated on PTLC silica gel (eluted with 20% ethylacetate/hexanes) to afford **64b** (52 mg, 63%) as a clear oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 0.00 (6H, s); 0.85 (9H, s); 3.05 (2H, m); 3.65 (3H, m); 5.10 (2H, m); 5.85 (1H, m); 7.30 (5H, m).

IR(NaCl, neat): 3340, 1645, 110 cm^{-1}.

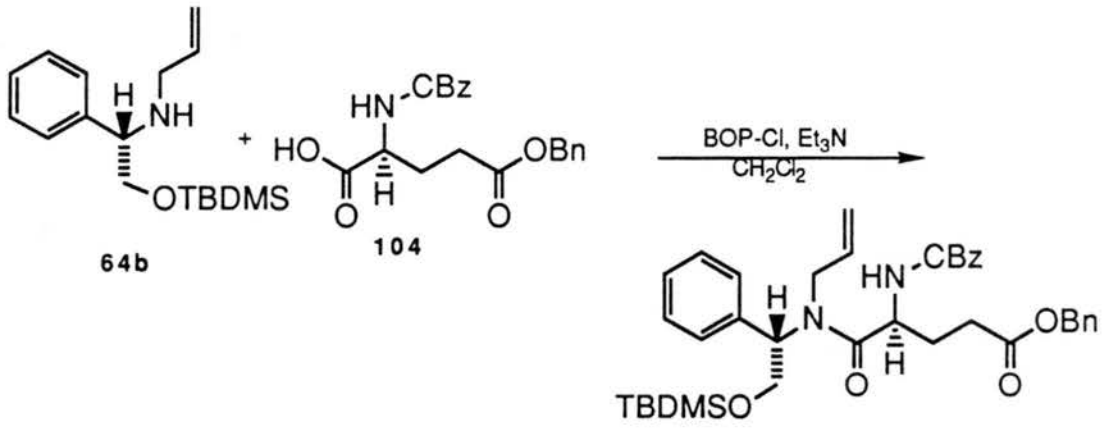


α-O-(N-allyl)phenylglycinyl-δ-benzyl-N-carbobenzyloxy glutamate, (**109**). To a stirred solution of **64a** (50mg, 0.28 mmol, 1.0 equiv) **104** (105mg, 0.28, 1.0 equiv) and HOBt (76mg, 0.56 mmol, 2.0 equiv) in dry THF (2 mL) was added the N-ethyl-2(N-N-dimethyl)ethylcarbdiimide hydrochloride (50mg, 0.28 mmol, 1.0equiv) at 0 °C. The resulting solution was stirred at 0 °C for 1h, diluted with ethylacetate (40 mL), washed with H_2O (1 x 10 mL) 10% HCl (1 x 10 mL) dried over Na_2SO_4, filtered, evaporated and separated on PTLC (silica gel, eluted with 5% MeOH/CH_2Cl_2) to yield **109** (36 mg, 24%) as a clear oil.

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.90 (1H, m); 2.15 (1H, m); 2.40 (2H, m); 3.10 (2H, m); 3.95 (1H, t, J=6.6Hz); 4.15 (1H, m); 4.28 (1H, m); 4.37 (1H, m); 5.05 (6H, m); 5.40 (1H, d, J=8.7Hz); 5.80 (1H, m); 7.28 (15H, m).

IR(NaCl, neat): 3400, 1740, 1640, 110 cm^{-1}.

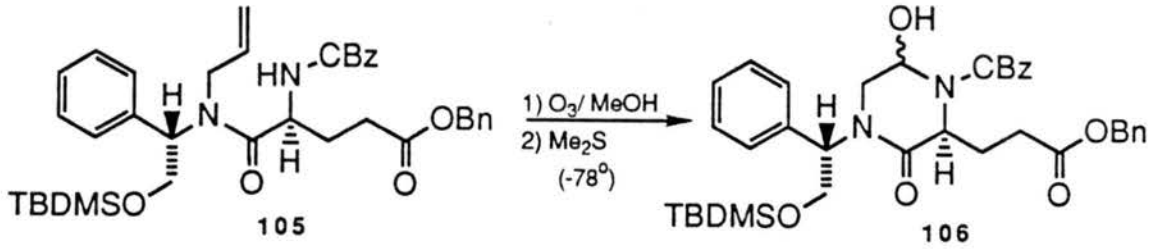
Mass spectrum, m/e = 218 (M^+, 0.1%), 107 (5.8), 91 (34), 77 (34.8), 41 (26).



α-(N-allyl-O-tbutyldimethylsilyl)phenylglycinol-δ-benzyl-N-carbobenzyloxy glutamide, (**105**). To a stirred solution of **64b** (20 mg, 0.07 mmol, 1.0 equiv) **104** (25 mg, 0.07 mmol, 1.0 equiv) and triethylamine (14 mg, 0.14 mmol, 2.0 equiv) in dry CH_2Cl_2 (0.5 mL) was added N,N-bis[2-oxo-3-oxazolidinyl)phosphordiamidic chloride (17.5 mg, 0.07 mmol, 1.0 equiv) at 0 °C. The resulting solution was allowed to stir at room temperature for 3 days, diluted with CH_2CL_2 (20 mL) washed with H_2O (2 x 5 mL) $NaHCO_3$ (2 x 5 mL), and 1M HCl (2 x 5 mL), dried over $MgSO_4$ filtered, evaporated and separated on PTLC (silica gel, eluted with 5% MeOH/CH_2Cl_2) to yield **105** (13 mg, 26%) as a colorless oil.

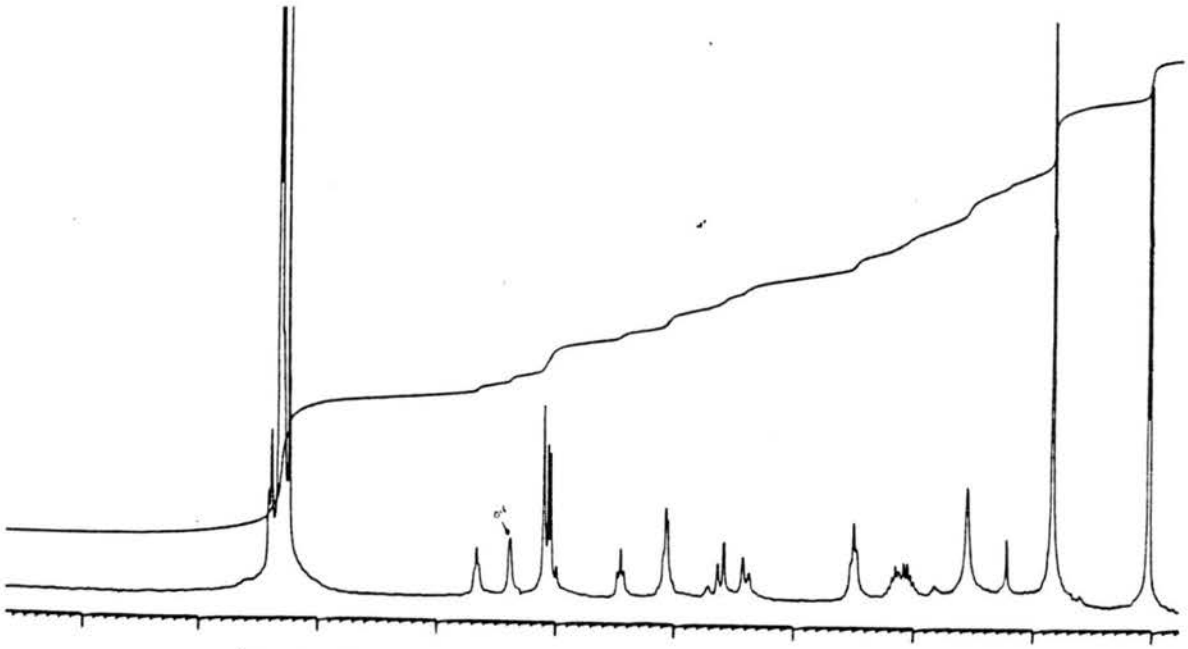
^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 0.05 (6H, s); 0.8 (9H, s); 1.8 (1H, m); 2.05 (1H, m); 2.40 (2H, m); 4.07 (2H, m); 4.72 (1H, m); 5.05 (6H, m); 5.34 (1H, m); 5.65 (2H, m); 7.35 (15H, m).

IR(NaCl, Neat): 1730, 1640 cm^{-1}.

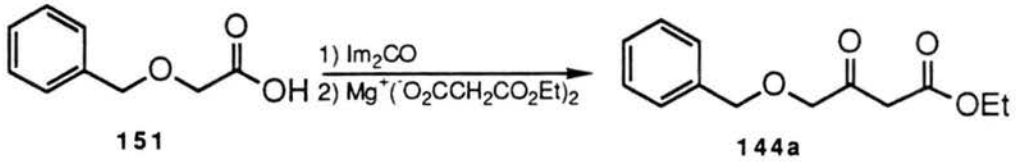


1-(1'-phenyl(2'-O-^{t}butyldimethylsilyl)hydroxy)-2-keto-3-(2"-carbobenzoxy)ethyl-4-carbobenzyloxy-5-hydroxy piperazine, (**106**). A stirred solution of **105** (20 mg, 0.03 mmol, 1 equiv) in dry methanol (5 mL) was subject to ozonolysis for 45 min at -78 °C. The clear solution of ozonide was then reduced with Me_2S (20 mg, 0.31 mmol, 10 equiv). The clear solution was stirred at -78 °C for an additional hour. The solvent was then evaporated and the clear oil was separated on PTLC (silica gel eluted with 2:1 EtOAc/hex) yielding **106** (9 mg, 50%) as a colorless oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: unassignable.



^{1}H NMR (270 MHz) of **105** in $CDCl_3$ at 295^{o}K



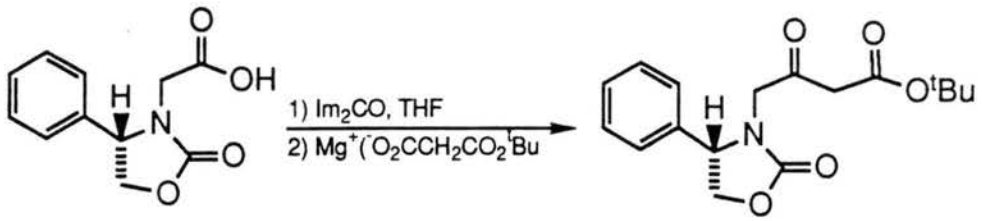
151 **144a**

1-benzyloxy-ethyl acetoacetate, (**144a**). To a stirred solution of **151** (3 g, 18.12 mmol, 1.0 equiv) in 180 mL THF was added 1,1'-diimidazole carbonyl (3.52 g, 21.74 mmol, 1.2 equiv) at room temperature. The reaction was allowed to stir at room temperature for 6h. Ethylmagnesium malonate (3.11 g, 10.87 mmol, 0.6 equiv) was added, the suspension was allowed to stir at room temperture for 16h when the reaction solution was evaporated to an oil, then triturated with EtOAc, filtered, and the filtrate evaporated to an oil which was separated (silica gel, 2:1 hexane/EtOAc) yielding 2.5 g of **144a** (59%) as a free flowing volatile oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ TMS: 1.24 (3H, t, J=7.0Hz); 3.53 (2H, s); 4.15 (4H, m); 4.57 (2H, s); 7.33 (5H, s).

IR(NaCl, neat): 1735, 1650, 1240, 1100, 680 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 236 (M^+, 7.7%), 207 (11), 190 (65), 91 (100).

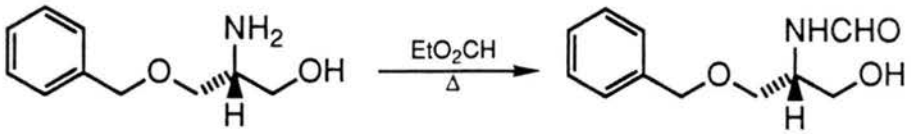


129 130

1-(1'-^{t}butylacetylacetate)-5-phenyl-2-oxazolidinone, (**130**). To a stirred solution of **129** (200 mg, 0.91 mmol, 1.0 equiv) in 9 mL dry THF is added 1,1'-carbonyldiimidazole (147 mg, 0.91 mmol, 1.0 equiv) at room temperature. The reaction was allowed to stir at room temperature for 3h and ^{t}butylmagnesium malonate (310 mg, 0.9049 mmol, 1.0 equiv) was added. The resulting suspension was allowed to stir at room temperature overnight. The reaction solution was evaporated to an oil and then triturated with ethyl acetate, filtered, evaporated and separated (silica gel 2:1 hexane:EtOAc) yielding the β-ketoester as a viscous colorless oil (245 mg, 85%).

^{1}H NMR (270 MHz, $CDCl_3$)δ $CHCl_3$: 1.38 (9H, s); 3.31 (2H, s); 3.62 (1H, d, J=18.7Hz); 4.14 (1H, t, J=8.6Hz); 4.44 (1H, t, J=18.7Hz); 4.72 (1H, t, J=8.3Hz); 5.02 (1H, t, J=8.4Hz); 7.28 (2H, m); 7.40 (3H, m).

IR(NaCl, neat): 2980, 2910, 1750, 1415, 1250, 1150, 1080, 840, 760, 700 cm^{-1}.



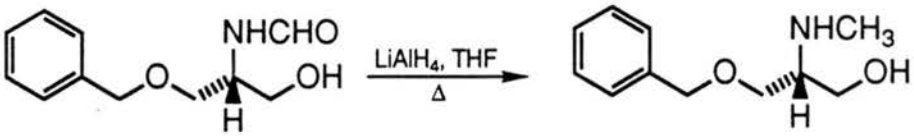
119 152

D-1-benzyl-2-(N-formyl)amino-3-hydroxy propane, (**152**). A solution of **119** (2 g, 11.04 mmol, 1.0 equiv) in 50 mL ethylformate was refluxed for 18h; the ethylformate was subsequently removed in vacuo. The resulting residue was chromatographed (silica gel, 5% $MeOH/CH_2Cl_2$) yielding **152** (1.455 g, 63%) as a colorless oil. $[\alpha]^{25}_D$ = -17.08 ° (c = 1.58 in CH_2Cl_2).

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 3.655 (5H, m); 4.17 (1H, m); 4.51 (2H, s); 6.54 (1H, bs); 7.36 (5H, m); 8.13 (1H, s).

IR(NaCl, neat): 3320, 1675, 1050 cm^{-1}.

Mass spectrum CI(NH_3), m/e = 209 (M^+, 15.7%), 191 (100), 106 (77).



152 153

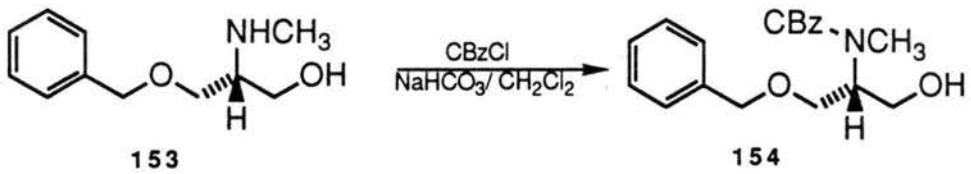
D-1-benzyl-2-(N-methyl)amino-3-hydroxy propane, (**153**). To a stirred solution of **152** (1.455 g, 6.96 mmol, 1.0 equiv) in 30 mL dry THF cooled to -10 °C was added $LiAlH_4$ (264 mg, 6.96 mmol, 1.0 equiv) the reaction was allowed to warm to room temperature and allowed to stir for an additional 14h. The mixture was quenched with 10 mL 1M HCl, basified to pH >10 with 1M NaOH and extracted

with CH_2Cl_2 (3 x 30 mL). The organic phases were combined and dried over $MgSO_4$, filtered, evaporated and separated (silica gel, 89:9.1, CH_2Cl_2:MeOH:NH_4OH) yielding 1.2 g **153** (87%) as a colorless oil; $[\alpha]^{25}_D$ = -21.54 °, c = (2.3/CH_2Cl_2.)

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 2.43 (3H, s); 2.81 (2H, s); 3.53 (3H, m); 3.70 (1H, 1/2AB, J=11.2Hz); 4.51 (2H, s); .733 (5H, m).

IR(NaCl, neat): 3300, 1020, 680 cm^{-1}.

Mass spectrum CI(NH_3), m/e = 211 (M^+, 0.3%), 195 (100), 86 (45).



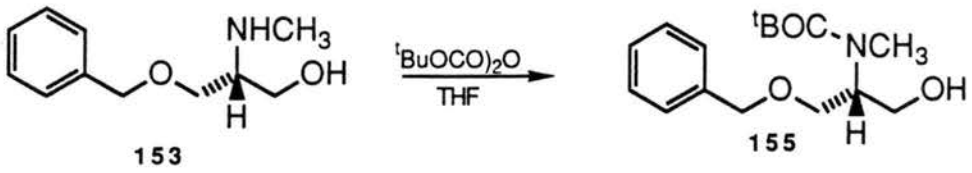
153 154

D-1-benzyl-2-(N-methyl-N-carbobenzyloxy)amino-3-hydroxy propane, (**154**). To a vigorously stirred bilayer of sat. $NaHCO_3$ (2 mL) and CH_2Cl_2 (2 mL) containing **153** (60 mg, 0.31 mmol, 1.0 equiv) was added benzylchloroformate (43 μL, 0.31 mmol, 1.0 equiv). The reaction was stirred at room temperature for 1h and diluted with 15 mL CH_2Cl_2. The organic layer was separated and washed with 1M NH_4HCO_3 (1 x 5 mL) 1M HCl (1 x 5 mL), H_2O (1 x 5 mL) and brine. The organic layer was then dried over $MgSO_4$, filtered, evaporated and separated (PTLC, silica gel, 2.5% MeOH/CH_2Cl_2) yielding **154** (85 mg, 86%) as a viscous colorless oil, $[\alpha]^{25}_D$ = -2.52° (c= 1.30, CH_2Cl_2).

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 2.80 (1H, bs); 2.91 (3H, s); 3.65 (4H, m); 4.27 (1H, m); 4.51 (2H, s); 5.12 (2H, s); 7.35 (10H, m).

IR(NaCl, neat): 3440, 1680, 1040, 680 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 329 (M^+, 0.7), 238 (100), 221 (77).

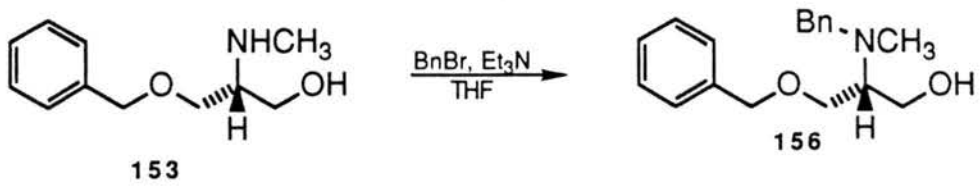


D-1-benzyl-2-(N-methyl-N-carbob^{t}butyloxy)amino-3-hydroxy propane, (**155**). To a stirred solution of **153** (66 mg, 0.34 mmol, 1.0 equiv) in 4 mL dry THF was added di-t-butyl dicarbonate (117 μL, 0.51 mmol, 1.5 equiv) at room temperature. The reaction was allowed to stir at room temperature for 3h. The reaction was then diluted with CH_2Cl_2 (30 mL), washed with H_2O (2 x 5 mL), brine (1 x 5 mL), dried over $MgSO_4$, filtered, evaporated and separated (PTLC, silica gel, 2% MeOH/CH_2Cl_2) yielding 90 mg **155** (90%) as a colorless oil, $[\alpha]^{25}_D$ = -1.88° (c, 2.73, CH_2Cl).

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.45 (9H, s); 2.60 (1H, bs); 2.85 (3H, s); 3.62 (2H, m); 3.74 (2H, m); 4.16 (1H, t, J=6.1Hz); 4.47 (2H, m); 7.30 (5H, m).

IR(NaCl, neat): 3440, 1680, 1150, 1040, 680 cm^{-1}.

Mass spectrum CI(NH_3) m/e = (M^+, 100), 174 (21.5), 149 (18), 74 (21.5).



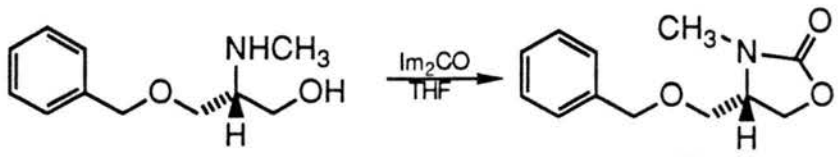
153 156

D-1-benzyl-2-(N-methyl-N-benzyl)amino-3-hydroxy propane, (**156**). To a stirred solution of **153** (20 mg, 0.10 mmol, 1.0 equiv) in 2 mL dry THF was added 1,1'-carbonyldiimidazole (25 mg, 0.15 mmol, 1.5 equiv). The reaction solution was stirred at room temperature for 1h. The mixture was diluted with 15 mL CH_2Cl_2, washed with 1M HCl (1 x 5 mL), 1M NH_4HCO_3 (1 x 5 mL), H_2O (1 x 5 mL), brine (1 x 5 mL), dried over $MgSO_4$, filtered, evaporated, and separated (PTLC silica gel 1:1 EtOAc/hexane) yielding 23 mg **156** (100%); $[\alpha]^{25}_D$ = -18.06° (c, 0.7/CH_2Cl_2).

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 2.87 (3H, s); 3.55 (2H, d, J=4.6Hz); 3.85 (1H, m); 4.08 (1H, t, J=7.9Hz); 4.35 (1H, t, J=8.1Hz); 4.55 (2H, s); 7.33 (5H, m).

IR(NaCl, neat): 1750, 1100, 1030, 680 cm^{-1}.

Mass spectrum, CI(NH_3), m/e = 222 (M^+, 100%), 106 (27); 91 (6).



153 150

1-methyl-5-(O-benzyl)hydroxymethyloxazolidin-2-one, (**150**). To a solution of **153** (88 mg, 0.45 mmol, 1.0 equiv) and Et_3N (125 μL, 0.90 mmol, 2.0 equiv) in 5 mL dry THF was added benzylbromide (91 μL, 0.77 mmol, 1.7 equiv). The reaction solution was allowed to stir at room temperature for a period of 18h. The mixture was diluted with CH_2Cl_2 (40 mL), washed with H_2O (2 x 10 mL) and brine (1 x 10 mL), dried over $MgSO_4$, filtered, evaporated and separated (silica gel, 2% MeOH/CH_2Cl_2) yielding 89 mg **150** (70%) as a colorless oil, $[\alpha]^{25}_D$ = -31.41° (c = 2.92, CH_2Cl_2).

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 2.24 (3H, s); 3.14 (2H, m); 3.68 (7H, m); 4.51 (2H, s); 7.33 (10M, m).

IR(NaCl, neat): 3440, 1030, 680 cm^{-1}.

Mass spectrum CI(NH_3), m/e = 285 (M^+, 42), 175 (26), 122 (82), 105 (100).

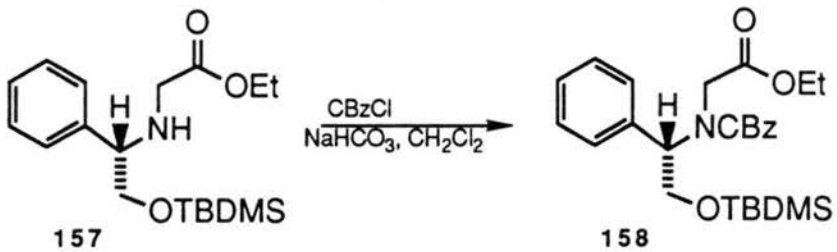
127 157

N-carboethoxymethyl-O-^{t}butyldimethylsilyl phenylglycinol, (**157**). To a stirred solution of **127** (1.5 g, 6.73 mmol, 1.0 equiv) and

imidazole (0.915 g, 13.45 mmol, 2.0 equiv) in 20 mL dry DMF was added dimethyl^{t}butylchlorosilane (1.20 g, 7.96 mmol, 1.2 equiv). The resulting reaction solution was allowed to stir at room temperature for 3h. The reaction was then diluted with 100 mL CH_2Cl_2. The organic phase was washed with H_2O (2 x 25 mL), $NaHCO_3$ (2 x 25 mL), and brine, dried over $MgSO_4$, filtered, and evaporated yielding **157** as a colorless oil (2.3 g, 100%).

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 0.003 (3H, s); 0.005 (3H, s); 0.86 (9H, s); 1.20 (3H, t, J=7.3Hz); 3.14 (1H, d, J=17.2Hz); 3.31 (1H, d, J=17.2Hz); 3.57 (2H, m); 3.75 (1H, m); 4.11 (2H, q, J=7.2Hz); 7.28 (5H, m).

IR(NaCl, neat): 3340, 1645, 110 cm^{-1}.



157 158

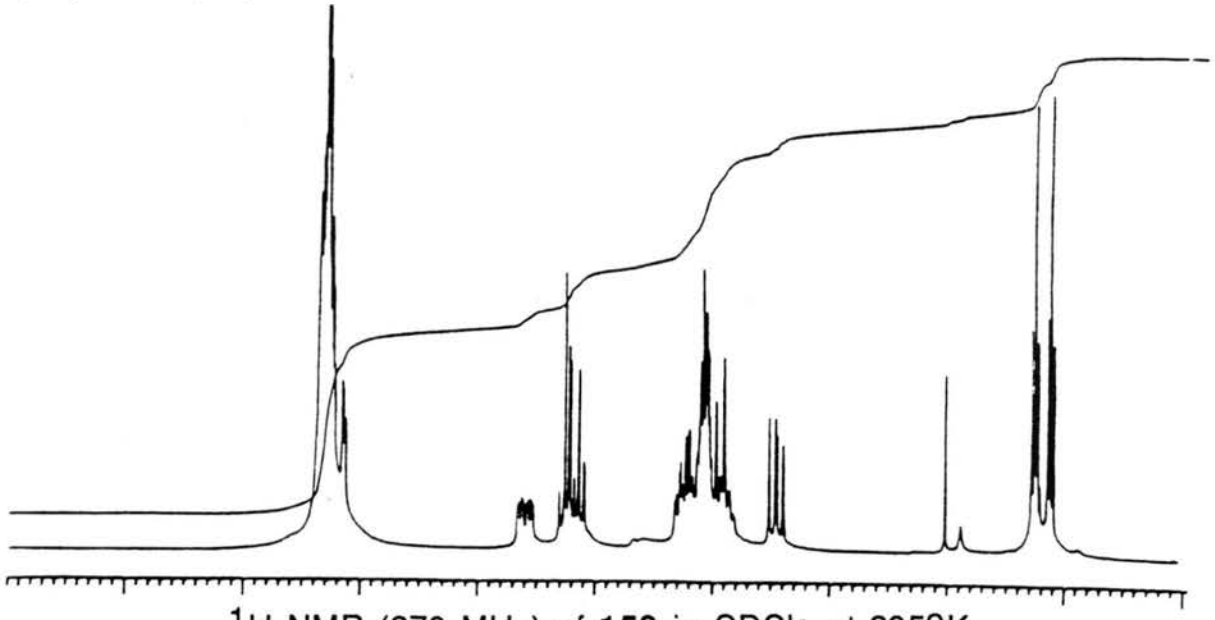
N-carboethoxymethyl-N-carbobenzyloxy-O-^{t}butyldimethyl-silyl phenylglycinol, (**158**). To a stirred solution of **157** (3.18 g, 9.45 mmol, 1.0 equiv) in 20 mL CH_2Cl_2 was added 20 mL sat. $NaHCO_3$ followed by benzylchloroformate (1.35 mL, 9.45 mmol, 1.0 equiv). The reaction was stirred vigorously for 1h, diluted with 100 mL CH_2Cl_2 and the organic layer separated and washed with 1M NH_4HCO_3 (2 x 20 mL), 1M HCl (2 x 20 mL), H_2O (1 x 20 mL) and brine (1 x 20 mL). The organic extracts were then dried over $MgSO_4$, filtered and

evaporated yielding pure **158** (4.43 g, 99%) as a colorless oil, $[\alpha]^{25}_D$ = -18.94° (c, 1.05, CH_2Cl_2).

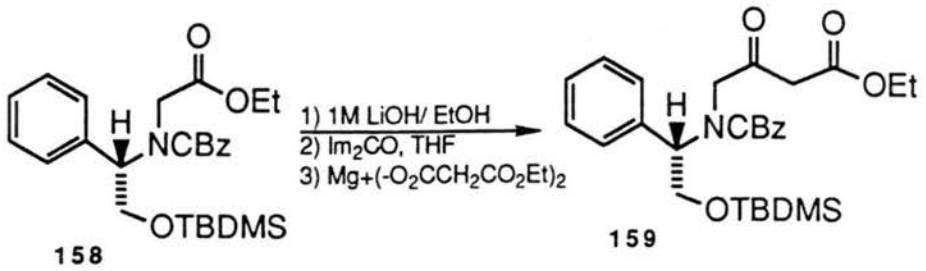
^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: unassignable, but presence of silane and CBz and ethyl ester obvious.

IR(NaCl, neat): 1750, 1705, 1190, 1100, 680 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 471 (M^+, 71%), 235 (74), 132 (29), 108 (41).



^1H NMR (270 MHz) of **158** in $CDCl_3$ at 295°K



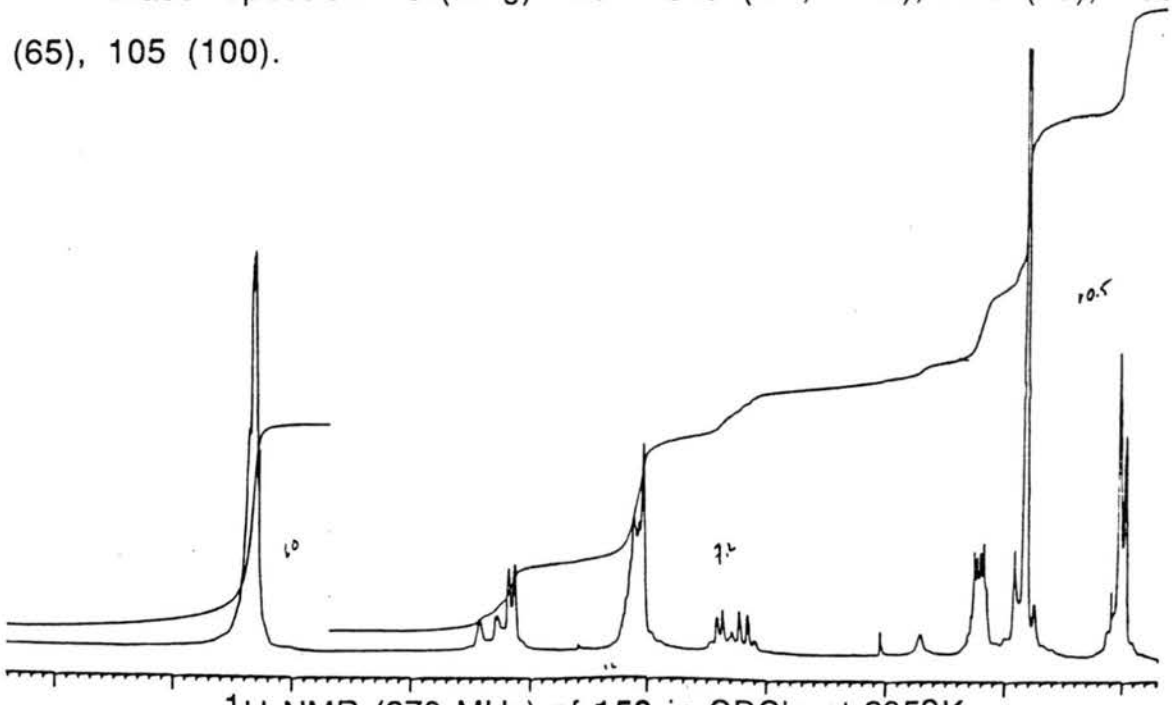
N-1-ethylacetylacetate-N-carbobenzyloxy-O-^{t}butyldimethyl-silyl phenylglycinol, (**158**). To a stirred solution of **158** (4.32 g, 9.19 mmol, 1.0 equiv) in 30 mL absolute EtOH was added 9.2 mL of 1M LiOH (9.20 mmol, 1.0 equiv) at 0°. The reaction was allowed to

stir for 7h, neutralized with 9.2 mL 1M HCl and the solvent evaporated. To the clear residue was added 10 mL 1M HCl and 100 mL CH_2Cl_2; the organic layer was separated and washed with H_2O (1 x 10 mL), brine (1 x 10 mL), dried over $MgSO_4$, filtered and evaporated to a clear oil which was dissolved in 30 mL THF. To this clear solution was added 1,1'-carbonyldiimidazole (1.79 g, 11.02 mmol, 1.2 equiv) and allowed to stir for 8h at room temperature. Ethylmagnesium malonate was added (2.10 g, 7.35 mmol, 0.8 equiv) in one portion and the reaction solution was left stirring for 18 hr. The solvent was then evaporated and the residue triturated in EtOAc, filtered, evaporated and separated (silica gel, 4:1 hexane/EtOAc) yielding **159** 3.45 g (73%), $[\alpha]^{25}_D$ = -21.675 (c, 1.02, CH_2Cl_2).

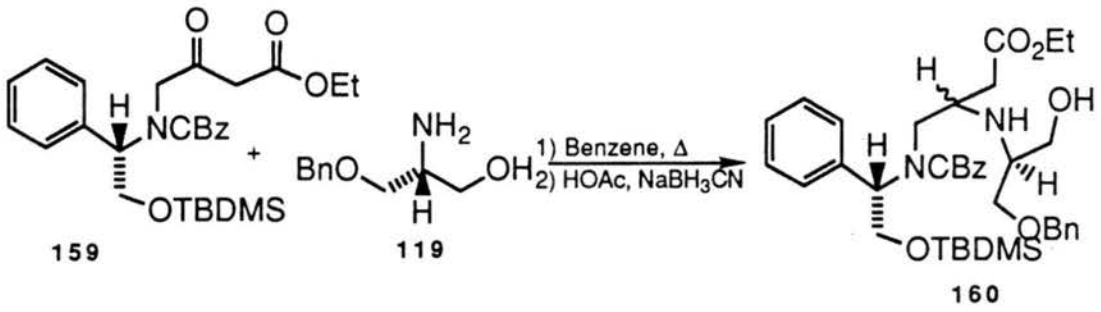
^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: unassignable.

IR(NaCl, neat): 1750, 1730, 1705, 1250, 1120, 830, 680 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 513 (M^+, 17%), 278 (45), 235 (65), 105 (100).



^{1}H NMR (270 MHz) of **159** in $CDCl_3$ at 295°K

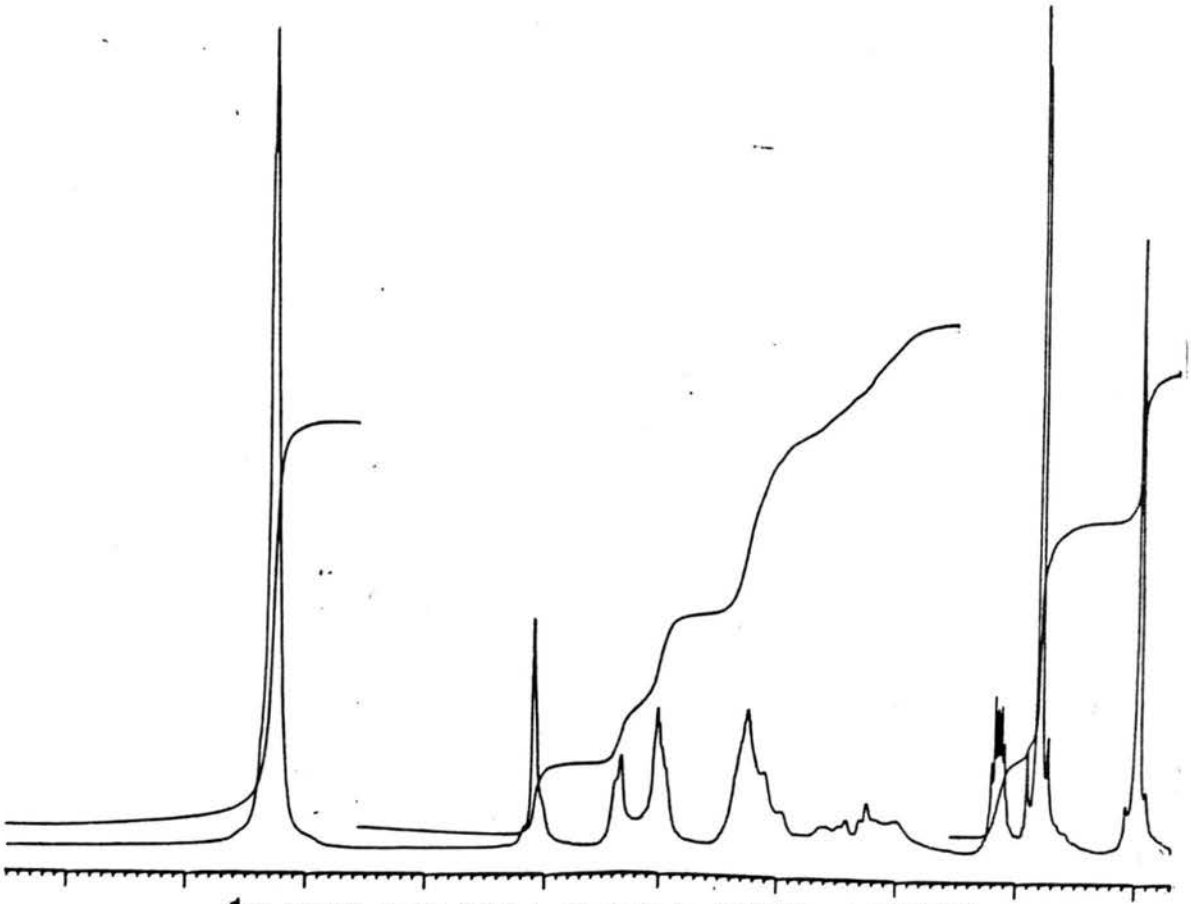


4-N-ethyl(3-amino(N'-(1-hydroxy-3-benzyloxy)isopropyl)) butanoate-N-carbobenzyloxy-O-^{t}butyldimethyl-silyl phenylglycinol, (**160**). A solution of **159** (1.50 g, 2.92 mmol, 1.0 equiv) and **119** (0.530 g, 2.92 mmol, 1.0 equiv) in dry benzene was refluxed with a Dean-Stark trap to remove water for 4.5h. The solvent was removed in vacuo yielding a viscous light amber oil. This oil was dissolved in HOAc (25 mL) and $NaBH_3CN$ (184 mg, 2.92 mmol, 1.0 equiv) was added in one portion. The resulting reaction solution was allowed to stir at room temperature overnight. The reaction was then diluted with H_2O and the product extracted with CH_2Cl_2 (5 x 30 mL), the combined organics were washed with $NaHCO_3$ (2 x 20 mL), H_2O (1 x 20 mL), brine (1 x 15 mL), dried over $MgSO_4$, filtered, evaporated, and separated (silica gel, 3:2 hexane/EtOAc) yielding 800 mg of **160** as a colorless oil which was a 1:1 mxiture of diastereomers.

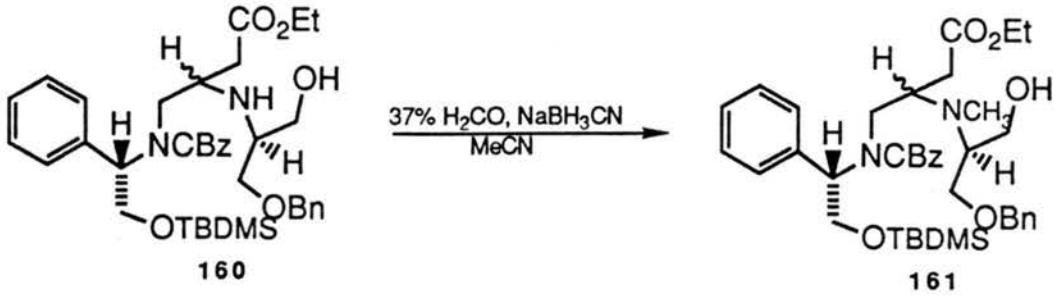
^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: unassignable.

IR(NaCl, neat(: 3485, 3330, 1730, 1695, 1100, 825, 680 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 678 (11.5), 631 (100), 523 (35), 496 (42), 385 (29).



^{1}H NMR (270 MHz) of **160** in $CDCl_3$ at 295^{o}K



160 **161**

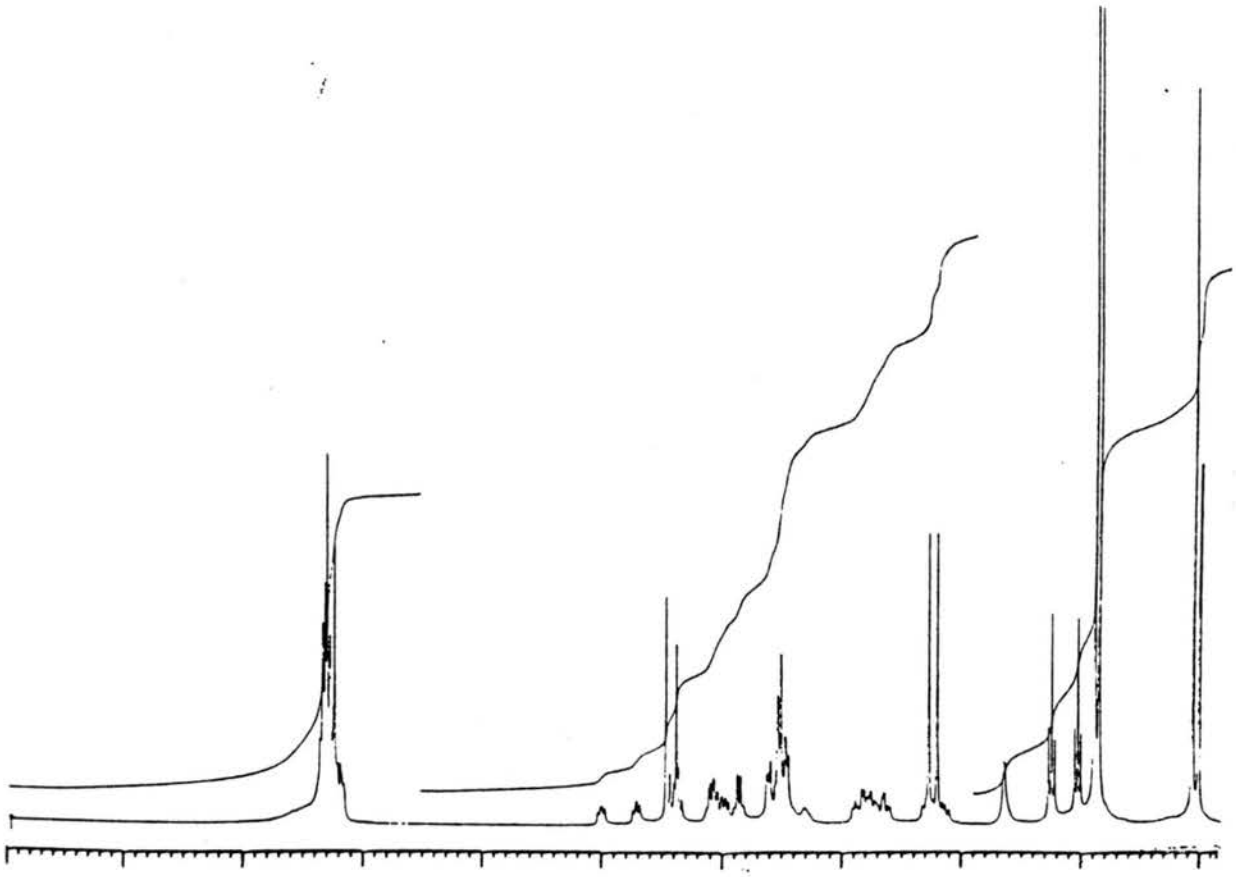
4-N-ethyl(3-amino(N'-methyl-N'-(1-hydroxy-3-benzyloxy) isopropyl))butanoate-N-carbobenzyloxy-O-^{t}butyldimethyl-silyl phenylglycinol, (**161**). To a stirred solution of **160** (0.745 g, 1.1

mmol, 1.0 equiv) in acetonitrile (3 mL) was aded 37% CH_2O/H_2O (0.445 mL, 5.5 mmol, 5.0 equiv) followed by $NaBH_3CN$ (138 mg, 2.2 mmol, 2.0 equiv). The resulting turbid solution was allowed to stir at room temperature for 18h, and diluted with CH_2Cl_2 (50 mL). The mixture was then washed with H_2O (2 x 10 mL), 1M NH_4HCO_3 (1 x 25 mL), sat. $NaHCO_3$, H_2O (1 x 10 mL), brine (1 x 10 mL), dried over $MgSO_4$, filtered, evaporated and purified (PTLC silica gel, 4:1 hexanes:EtOAc) yielding 526 mg (70%) of **161** as a colorless oil.

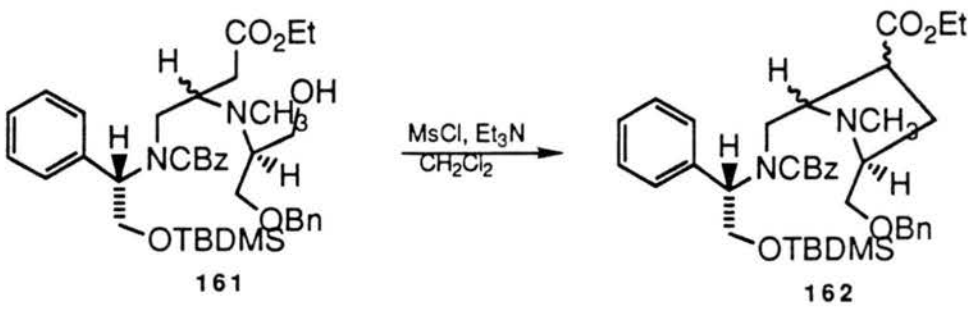
^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: unassignable.

IR(NaCl, neat): 3460, 1720, 1690, 1250, 1090, 820, 680 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 692 (M^+, 1.7%), 644 (76), 496 (32), 385 (28), 247 (98).



^1H NMR (270 MHz) of **161** in $CDCl_3$ at 295°K



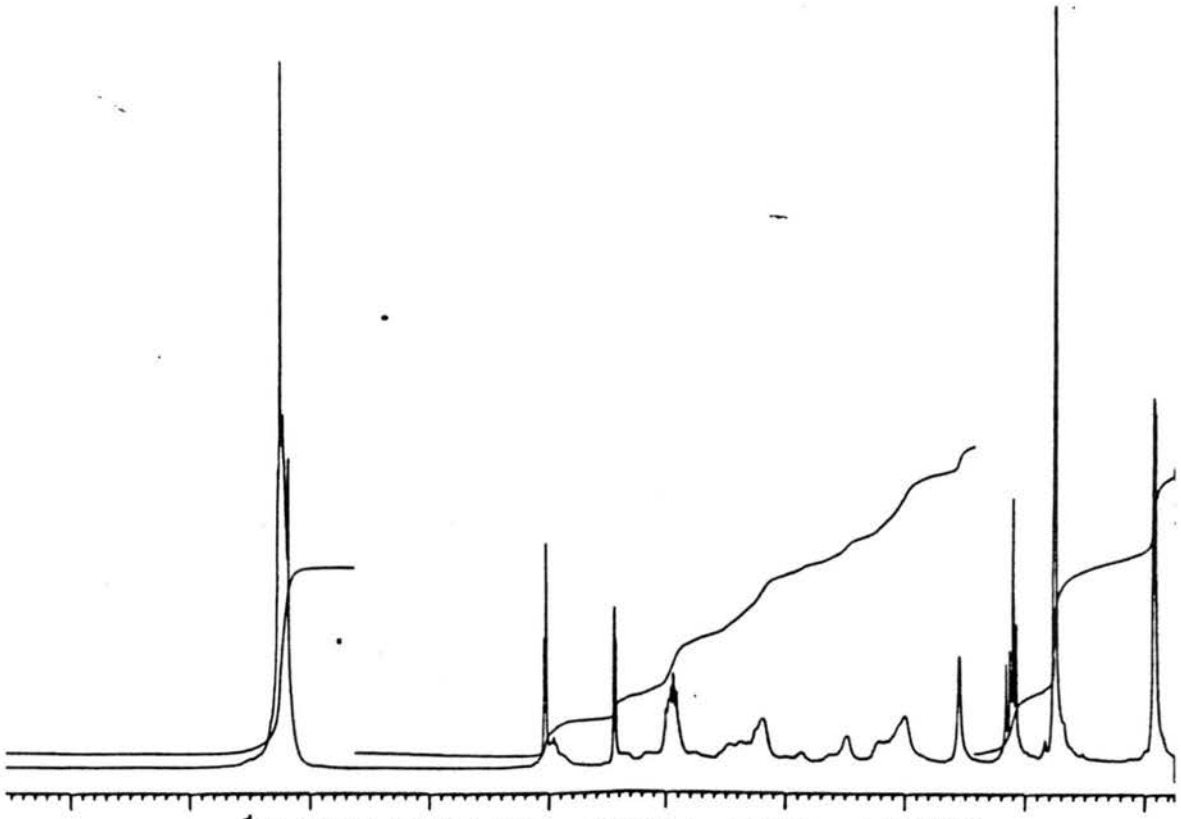
161 **162**

1-methyl-2-(N-carbobenzyloxy-N(2-O-^{t}butyldimethylsilyl hydroxy-1'-phenyl)ethyl)methylamino-3-carboethoxy-5-(O_benzyl) hydroxymethyl pyrrolidine, (**162**). To a stirred solution of **161** (392 mg, 0.57 mmol, 1.0 equiv) and Et_3N (197 μL, 1.42 mmol, 2.5 equiv) in 5.5 mL dry CH_2Cl_2 was added MsCl (66 μL, 0.8499 mmol, 1.5 equiv). The resulting solution was allowed to stir at room temperature for 1h, diluted with 50 mL CH_2Cl_2, washed with 1M NH_4HCO_3 (1 x 10 mL), H_2O (1 x 10 mL), brine (1 x 10 mL), dried over $MgSO_4$, filtered, evaporated and purified (silica gel, 4:1 hexane:EtOAc) yielding 350 mg of **162** (92%) as a colorless oil.

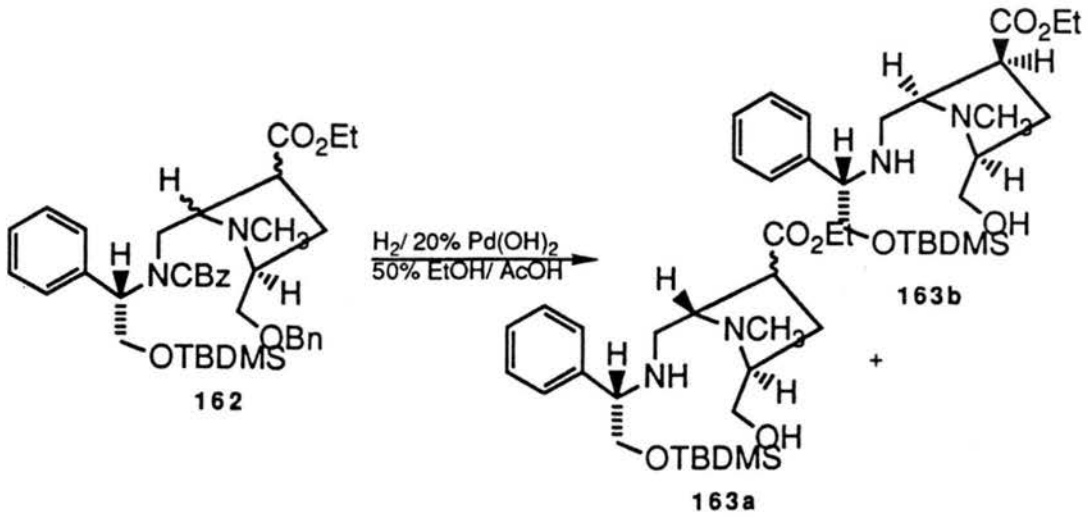
^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: unassignable but obviously containing silane, CBz, OBn, OCH_2CH_3 and NMe.

IR(NaCl, neat): 1725, 1695, 1250, 1100, 820, 680 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 674 (M^+, 4%), 527 (37), 311 (94), 234 (80).



^{1}H NMR (270 MHz) of **162** in $CDCl_3$ at 295^{o}K



1-methyl-2-(N(2-O-^{t}butyldimethylsilyl)hydroxy-1'-phenyl)ethyl)methylamino-3-carboethoxy-5-hydroxymethyl pyrrolidine, (**163a**) and *1-methyl-2-(N(2-O-^{t}butyldimethylsilyl) hydroxy-1'-phenyl)ethyl)methylamino-3-epi-carboethoxy-5-hydroxymethyl pyrrolidine,* (**163b**). To a stirred solution of **162**

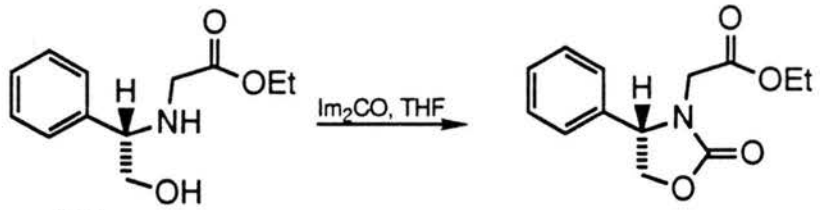
(200 mg, 0.29 mmol, 1.0 equiv) in 5 mL 50% EtOH/AcOH was added 20% $Pd(OH)_2$ on carbon (250 mg, 0.35 mmol, 1.2 equiv). This suspension was purged with hydrogen and hydrogenolysis was continued for 6h after which the palladium was filtered off and the filtrate evaporated and purified (PTLC silica gel, 3:2 hexane/EtOAc) yielding **163b** (45 mg) and **163a** (35 mg) as colorless oils.

163a (lower mixture of diastereomers by TLC Rf=0.45): ^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$): 0.00 (3H, s); 0.05 (3H, s); 0.86 (9H, s); 1.20 (3H, m); 2.05 (1H, m); 2.22 (3H, s); 2.40 (3H, m); 2.67 (1H, m); 2.97 (1H, m); 3.18 (1H, m); 3.57 (2H, m); .3.77 (3H, m); 4.25 (3H, m); 7.29 (5H, m).

IR(NaCl, neat): 3300, 1735, 1080 cm^{-1}.

163b (upper diastereomer by TLC Rf=0.6): ^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 0.00 (6H, s); 0.87 (7H, s); 1.15 (3H, t, J=7.1Hz); 2.07 (1H, dd, J=7.5Hz); 2.23 (3H, s); 2.43 (1H, dd, J=6.0Hz); 2.65 (2H, m); 2.87 (1H, dd, J=9.7Hz); 3.18 (1H, m); 3.70 (6H, m); 2.78 (1H, dd, J=6.9Hz); 3.93 (1H, dd, J=4.3Hz); 4.05 (2H, q, J=7.4Hz); 7.28 (5H, m).

IR(Nacl, neat): 3340, 1740, 1090 cm^{-1}.



127 128

1-carboethoxymethyl-5-phenyl-2-oxazolidinone, (**128**). To a stirred solution of **127** (1.6 g, 7.18 mmol, 1.0 equiv) in dry THF (60 mL) was added 1,1'-carbonyldiimidazole (2.30 g, 14.35 mmol, 2.0 equiv) at room temperature. The reaction solution was allowed to stir for 2h, and concentrated under reduced pressure to an oil. The oil was dissolved in CH_2Cl_2 (150 mL), washed with H_2O (2 x 25 mL), 1M HCl (2 x 25 mL), and 1 x 25 mL 1M NH_4CO_3, dried over $MgSO_4$, filtered and evaporated to give pure **128** (1.75 g, 100%) as a colorless oil.

^1H NMR (270 MHz, $CDCl_3$) δ ($CHCl_3$): 1.23 (3H, t, J=7.5Hz), 3.31 (1H, d, J=14.3Hz); 4.12 (4H, m); 4.69 (1H, t, J=7.6Hz); 5.03 (1H, t, J=7.3Hz); 7.28 (5H, m).

IR(NaCl, neat): 2980, 1760, 1200, 1050, 755, 695 cm^{-1}.

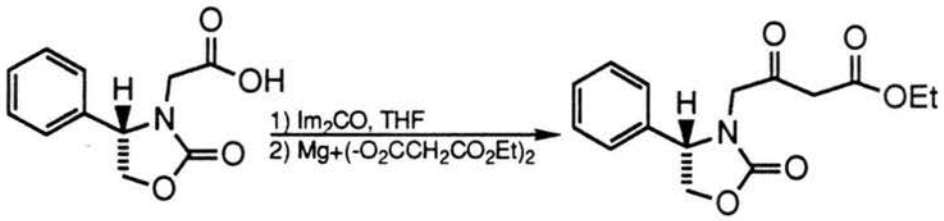
128 129

1-carboxymethyl-5-phenyl-2-oxazolidinone, (**129**). To a stirred solution of **128** (1.0 g, 4.11 mmol, 1.0 equiv) in 18 mL

absolute EtOH, cooled to 0 °C, was added 7.5 mL 1.0M LiOH. The reaction was allowed to stir for 2h at 0°C. The reaction was neutralized with 2 mL 4M HCl and the bulk of the EtOH removed *in vacuo*. The mixture was diluted with EtOAc (100 mL), 3 mL 1M HCl, the organic phase was washed with 2 x 10 mL water and brine, dried, and concentrated to afford crude **129** as an oil. Recrystallization (EtOAc/hexane) afforded pure **129** as white crystals mp: 176-178°C (670 mg, 75%). Analysis calculated for $C_{11}H_{11}NO_4$: C, 59.72; H, 5.01; N, 6.33. Found: C, 59.92; H, 5.02; N, 6,44.

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 3.37 (1H, d, J=18.3Hz); 4.13 (1H, t, J=8.5Hz); 4.27 (1H, d, J=18.3Hz); 4.69 (1H, t, J=8.8Hz); 5.03 (1H, t, J=8.5Hz); 7.30 (5H, m); 9.29 (1H, bs).

IR(NaCl, CH_2Cl_2): 3400, 3000, 1740, 1430, 1200, 725 cm^{-1}



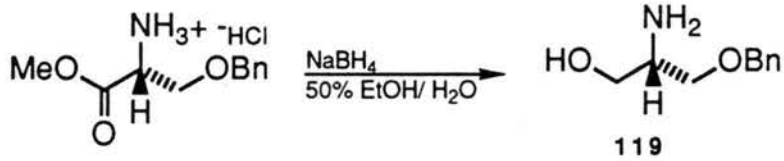
129 **126**

1-ethylacetylacetate-5-phenyl-2-oxazolidinone, (**126**). To a stirred solution of **129** (250 mg, 1.13 mmol, 1.0 equiv) in 12 mL dry THF was added 1,1'-diimidazole carbonyl (201 mg, 1.24 mmol, 1.1 equiv) at room temperature. The reaction was allowed to stir at room temperature for 3h. Ethylmagnesium malonate (324 mg, 1.13 mmol, 1.0 equiv) was added, and the suspension was allowed to stir overnight at room temperature. The reaction solution was evaporated to an oil and then triturated with dry EtOAc. The

supernatent was filtered, evaporated to an oil and purified (silica gel, 3:2 hexane:EtOAc) yielding **126** 925 mg, 76%) as a viscous colorless oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.20 (3H, t, J=7.2Hz); 3.40 (2H, s); 3.30 (1H, d, J=18.7Hz); 4.12 (3H, m); 4.45 (1H, d, J=18.7Hz); 4.73 (1H, t, J=8.8Hz); 5.01 (1H, t, J=7.9Hz); 7.285 (2H, m); 7.40 (3H, m).

IR(NaCl, neat): 2980, 2920, 1750, 1410, 1250, 1170, 1080, 1020, 750, 695 cm^{-1}.

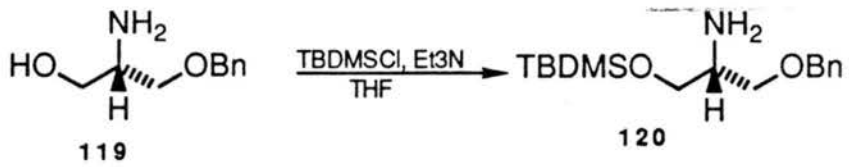


119

D-2-amino-1-benzyloxy-3-hydroxy propane, (**119**). To a stirred solution of $NaBH_4$ (1.457 g, 38.673 mmol, 5.0 equiv) in 26 mL 50% $EtOH/H_2O$ was added a solution of D-O-benzylserine methyl ester hydrochloride,(Aldrich Chemical Co.) (1.90 g, 7.74 mmol, 1.0 equiv) in 15 mL of 50% $EtOH/H_2O$ at 0°C. After the addition was complete the reaction was stirred 3.5 hr at room temperature, then refluxed for 6 hr. After cooling, the EtOH was decanted and the remaining white emulsion was washed with absolute ethanol (3 x 20 mL). The combined EtOH washings were then concentrated *in vacuo.* The resulting oil was dissolved in CH_2Cl_2 and washed with 1M NaOH (1 x 10 mL), sat. NaCl (1 x 10 mL), dried over $MgSO_4$, filtered, and evaporated yielding **119** (1.12 g, 80%) as a clear oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 2.20 (3H, bs); 3.075 (1H, m); 3.50 (4H, m); 4.487 (2H, s); 7.28 (5H, m).

IR(NaCl, neat): 3360, 3200, 2900, 1590, 1100, 1050, 735cm^{-1}.

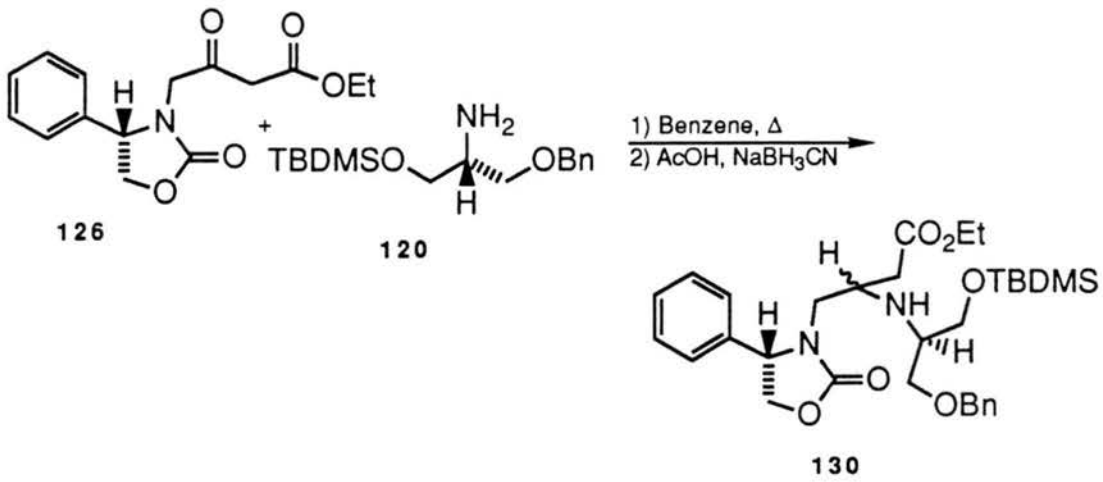


119 120

D-2-amino-1-benzyloxy-3-(O-tbutyldimethylsilyl)hydroxy propane, (**120**). To a stirred solution of **119** (0.826 g, 4.6 mmol, 1.0 equiv) and Et_3N (0.95 mL, 6.84 mmol, 1.5 equiv) in 45 mL dry THF was added t-butyldimethylchlorosilane (0.687 g, 0.45 mmol, 1.0 equiv) at room temperature. The reaction was allowed to stir at room temperature overnight. The reaction was filtered to remove Et_3N•HCl, and evaporated, diluted with CH_2Cl_2 (75 mL) and washed with H_2O (2 x 10 mL) 15% NaOH (1 x 10 mL), and sat. NaCl (1 x 10 mL). The CH_2Cl_2 extract was dried over $MgSO_4$, filtered, evaporated and purified by column chromatography (silica gel, 5% MeOH/CH_2Cl_2) yielding 1.08 g of **120** (80%) as a colorless oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 0.038 (6H, s); 0.8748 (9H, s); 1.413 (2H, s); 2.975 (1H, m); 3.310 (1H, m); 3.50 (3H, m); 4.435 (2H, s); 7.27 (5H, m).

IR(NaCl, neat): 3400, 3040, 2940, 1600, 1475, 1260, 1100, 835, 780, 730, 700, 665cm^{-1}.



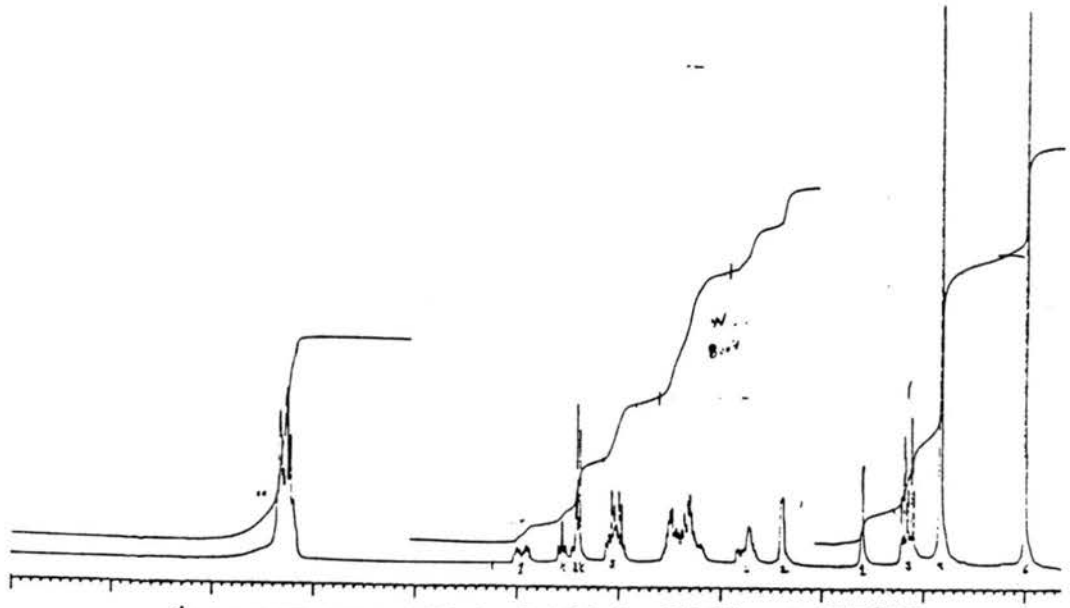
126 120 130

1-(4-N-ethyl(3-amino(1-O-tbutyldimethylsilyl)hydroxy-3-benzyloxy) isopropyl))butanoate-N-5-phenyl-2-oxazolinone, (**130**). A solution of **126** (156 mg, 0.54 mmol, 1.0 equiv) and **120** (158.4 mg, 0.54 mmol, 1.0 equiv) in 4 mL dry benzene was refluxed with a Dean Stark trap to remove water for 2h. The benzene was then removed in vacuo yielding 251 mg (82%) of the imine as a viscous oil. The imine was dissolved in 3.7 mL glacial acetic acid; to this solution was added $NaCNBH_3$ (27.7 mg, 0.44 mmol, 1.0 equiv relative to imine) at room temperature. The reaction was allowed to stir at room temperature for 2h, and quenched by pouring into 5 mL 0.1M NaOH. Saturation with NaCl followed by extraction with CH_2Cl_2 (4 x 15 mL), washing the CH_2Cl_2 layer with sat. $NaHCO_3$ (2 x 10 mL), drying over $MgSO_4$, filtered, evaporated and purified by column chromatography (silica gel 3:2 hexane:EtOAc) yielded 150 mg (49% from **126**) of **130** as a viscous colorless oil, which was a 1:1 mixture of diastereoisomers.

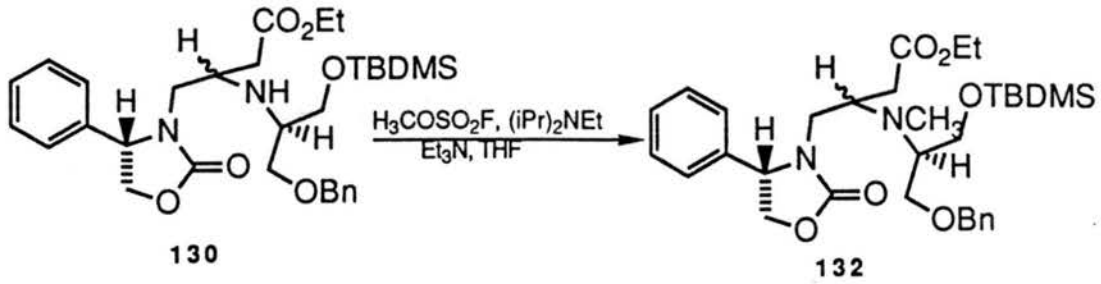
^1H NMR (270 MHz, $CDCl_3$) δ TMS: unassignable.

IR(NaCl, neat): 3320, 3040, 2910, 1760, 1740, 1410, 1250, 1090, 1030, 830, 770, 690 cm^{-1}.

Mass spectrum m/e = 570 (M^+, 4.0); 276 (5.5); 181 (5.9); 106 (100); 91 (34.8).



^1H NMR (270 MHz) of **130** in $CDCl_3$ at 295°K



130 **132**

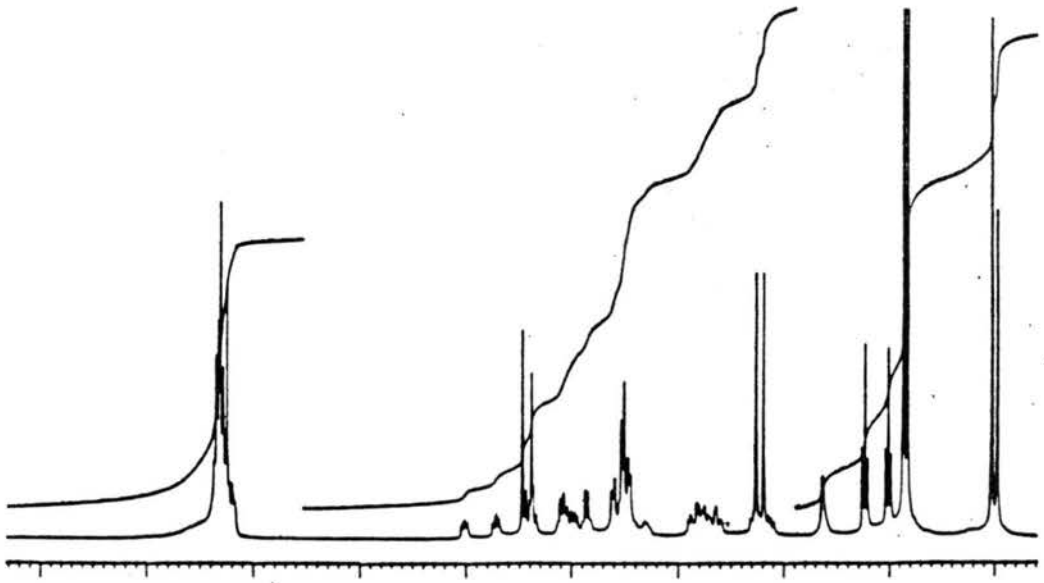
1-(4-N-ethyl(3-N-methylamino(1-O-^{t}butyldimethylsilyl) hydroxy-3-benzyloxy)isopropyl))butanoate-N-5-phenyl-2-oxazolinone, (**132**). To a stirred solution of **130** (150 mg, 0.26 mmol, 1.0 equiv) and diisopropylethylamine (183 μL, 1.05 mmol, 4.0 equiv) in 1.3 mL dry THF was added methane fluorosulfonate (47 μL, 0.58 mmol, 2.2 equiv) at 0°C in one portion. The reaction was allowed to stir at 0°C for 1h, warmed to room temperature and

diluted with CH_2Cl_2 (40 mL). The mixture was washed with H_2O (4 x 5 mL), and sat. NaCl (1 x 10 mL). The CH_2Cl_2 was dried over $MgSO_4$, filtered, evaporated, and purified by column chromatography (3:1 hexane:EtOAc; silica gel) yielding 131 mg **132** (85%).

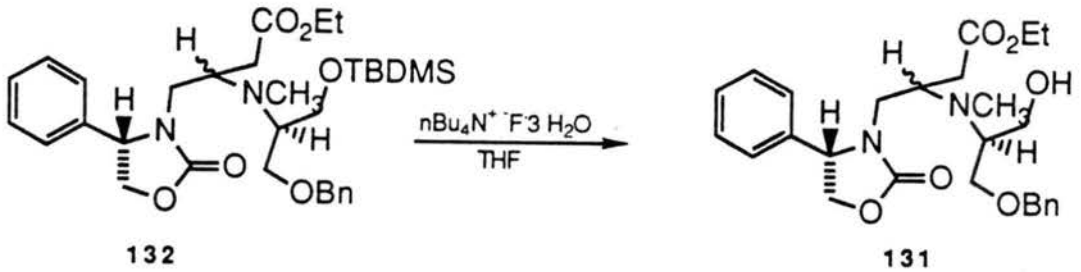
^1H NMR (270 MHz, $CDCl_3$) δ TMS: unassignable.

IR(NaCl, neat): 3020, 2920, 1750, 1730, 1400, 1250, 1150, 1080, 825, 765, 690 cm^{-1}.

Mass spectrum m/e = 584 (M^+, 2.0); 407 (0.5); 310 (4.5); 276 (2.7); 293 (2.9); 181 (3.5); 164 (2.0); 132 (2.0); 106 (100); 91 (2.7).



^1H NMR (270 MHz) of **132** in $CDCl_3$ at 295°K



132 **131**

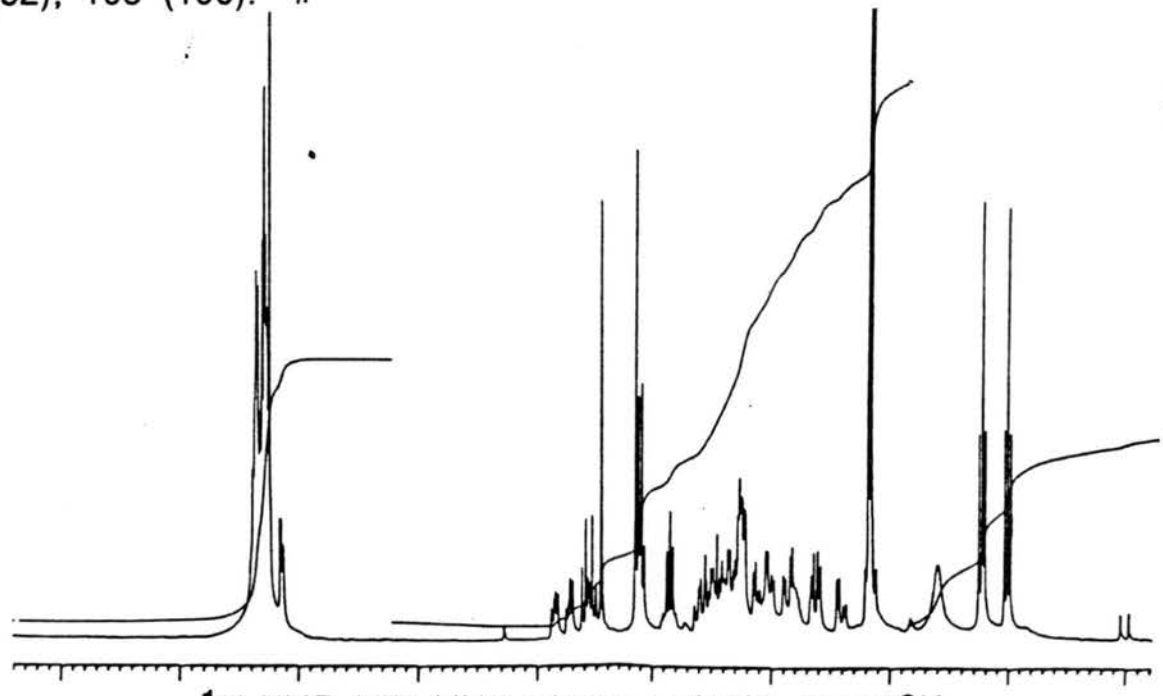
1-(4-N-ethyl(3-N-methylamino(1-hydroxy-3-benzyloxy) isopropyl))butanoate-N-5-phenyl-2-oxazolinone, (**131**). To a stirred

solution of **132** (118 mg, 0.20 mmol, 1.0 equiv) in 3 mL dry THF was added tetrabutylammonium fluoride trihydrate (96 mg, 0.30 mmol, 1.5 equiv) at room temperature. The reaction solution was allowed to stir at room temperture for 1h, diluted with CH_2Cl_2 (25 mL), washed with H_2O (1 x 5 mL) sat. $NaHCO_3$ (1 x 5 mL); dried over $MgSO_4$, filtered, evaporated and purified (silica gel, 1:1 EtOAc/hexane) yielding **131** (72 mg, 75%) as a colorless oil.

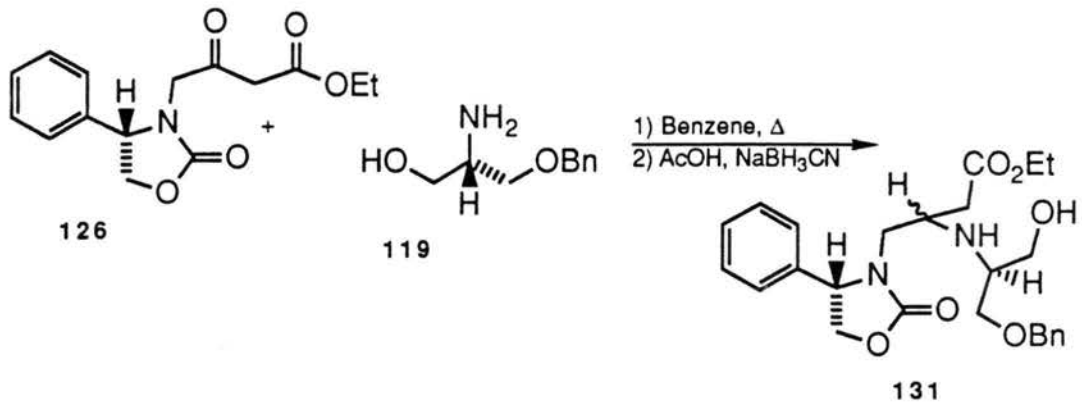
^1H NMR (270 MHz, $CDCl_3$) δ ($CHCl_3$): unassignable.

IR(NaCl, neat): 3450, 1750, 1730, 1030, 680 cm^{-1}.

Mass spectrum, CI(NH_3), m/e = 470 (M^+, 2.4); 424 (29); 181 (52); 106 (100).



^1H NMR (270 MHz) of **131** in $CDCl_3$ at 295°K

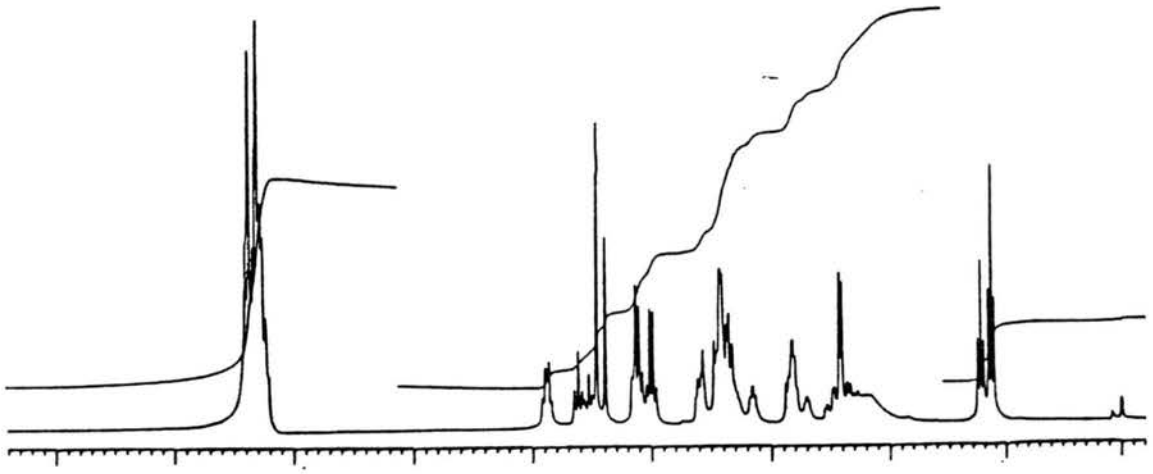


1-(4-N-ethyl(3-amino(1-hydroxy-3-benzyloxy)isopropyl)) butanoate-N-5-phenyl-2-oxazolinone, (**131**). A solution of **126** (64 mg, 0.22 mmol, 1.0 equiv) and **119** (40 mg, 0.22 mmol, 1.0 equiv) in benzene was heated to reflux with a Dean-Stark trap to remove water for 2h. The benzene was evaporated yielding the imine as a viscous oil (100 mg, 100%). The imine was then dissolved in 2 mL of acetic acid. To this solution was added $NaBH_3CN$ (13 mg, 0.22 mM, 1.0 equiv) in one portion at room temperature (gas evolution). After 20 min the reaction was diluted with CH_2Cl_2 (50 mL) and carefully neutralized with 1M NH_4HCO_3. The organic layer was separated and washed with 1M NH_4CO_3 (1 x 10 mL), H_2O (1 x 10 mL), brine (1 x 10 mL), dried over $MgSO_4$, filtered, evaporated and purified (silica gel 1:1 EtOAc/hexane) yielding 57 mg of **131** (60%) as a colorless oil and a 1.5:1 mixture of diastereoisomers.

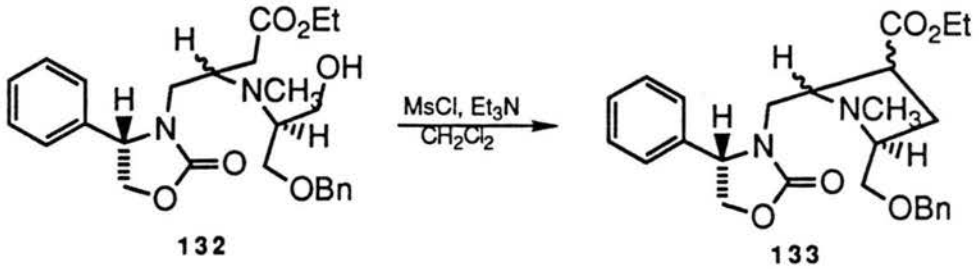
^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: unassignable.

IR(Nacl, neat): 3440, 3320, 1740, 1025, 680 cm^{-1}.

Mass spectrum, CI(NH_3), m/e = 456 (M^+, 11); 410 (13); 292 (27); 163 (29); 91 (15).



^{1}H NMR (270 MHz) of **131** in $CDCl_3$ at 295^{o}K



132 **133**

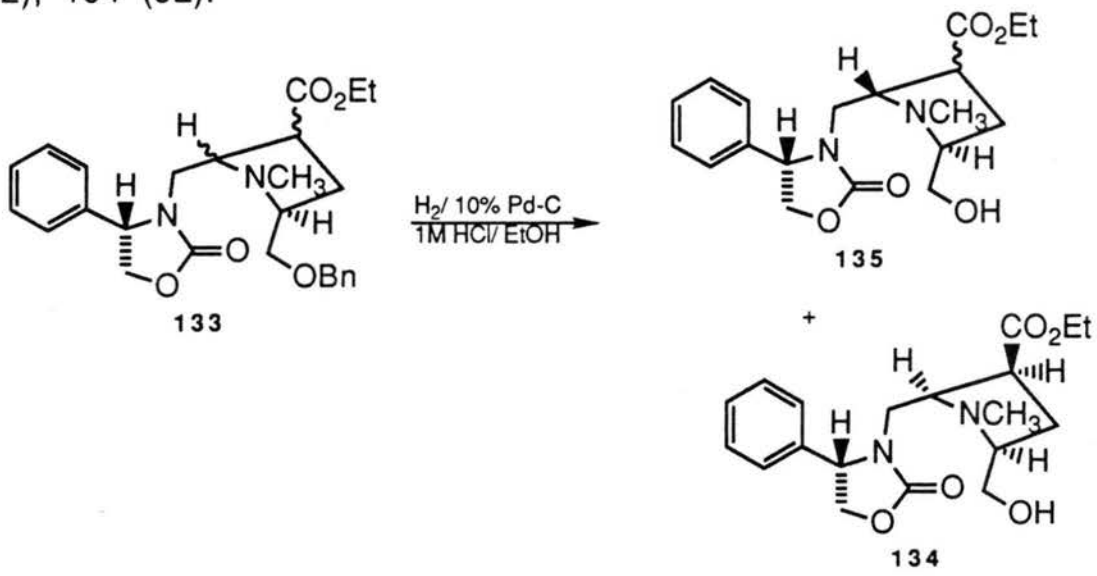
1-methyl-2-methylamino(5-phenyl-2-oxazolinone)-3-carboethoxy-5-(O-benzyl)hydroxymethyl pyrrolidine, (**133**). To a stirred solution of **132** (185 mg, 0.39 mmol, 1.0 equiv) in 3 mL of dry CH_2Cl_2 was added Et_3N (137 µL, 0.98 mmol, 2.5 equiv) followed by mesyl chloride (34 µL, 0.43 mmol, 1.1 equiv). The resulting reaction solution was allowed to stir at room temperature for 30 min, diluted with 15 mL CH_2Cl_2, washed with 1M NH_4HCO_3 (1 x 5 mL), H_2O (1 x 5 mL) and brine. The colorless solution was then dried over $MgSO_4$, filtered, evaporated and purified (silica gel, 2:1

hexane/EtOAc) yielding **133** as a mixture of three stereoisomers, (150 mg, 85%) as a colorless oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: unassignable.

IR(NaCl, neat): 1755, 1730, 1600, 1030, 680 cm^{-1}.

Mass spectrum, CI(NH_3), m/e = 452 (M^+, 0.2); 312 (19); 130 (32); 104 (92).



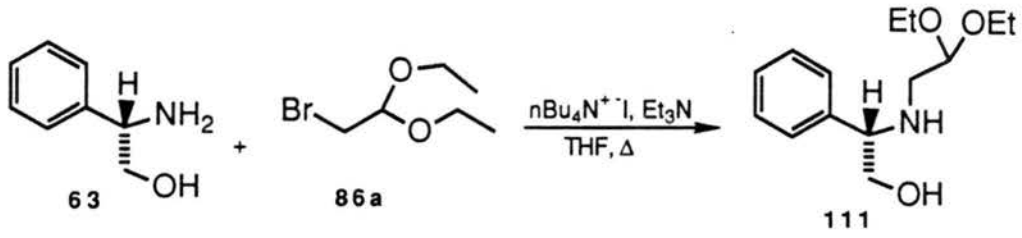
1-methyl-2-methylamino(5-phenyl-2-oxazolinone)-3-carboethoxy-5-hydroxymethyl pyrrolidine, (**133**). To a solution of **133** (15 mg, 0.03 mmol, 1.0 equiv) in 0.5 mL 1M AcOH/EtOH was added 10 mg of 10% Pd-C (0.01 mmol, 0.3 equiv) in a pressure vessel. The vessel was evacuated and flushed several times with H_2 then charged to 50 psi and hydrogenated for 2 days. The Pd-C was then filtered over Celite. Evaporation of the filtrate followed by separation of the diastereoisomers (PTLC silica gel, 3:2 hexane/EtOAc) furnished 3 mg of **135** as a mixture of diastereomers and 3 mg of the desired diastereomer **134** as colorless oils.

NMR of desired diastereomer **134:**

^{1}H NMR (270 MHz, $CDCl_3$) δ ($CHCl_3$): 1.25 (3H, t, J=7.1Hz); 2.13 (3H, s); 2.25 (1H, 1/2AB, J=15.6Hz); 2.44 (1H, 1/2AB, J=15.6Hz); 2.68 (2H, d, J=7.1Hz); 2.80 (1H, m); 3.28 (1H, m); 3.40 (1H, dd, J=8.2Hz); 3.75 (2H, d, J=4.9Hz); 3.95 (1H, m); 4.12 (3H, m); 4.59(1H, t, J=8.9Hz); 4.93 (1H, dd, J=8.9Hz); 7.37 (5H, m).

IR(NaCl, neat): 3420, 1750, 1730, 680 cm^{-1}.

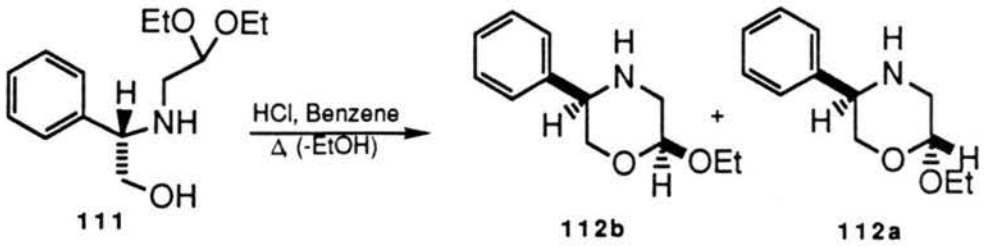
Mass spectrum, CI(NH_3), m/e = 363 (M+1, 22.9); 307 (100); 276 (16.8).



N-2,2-diethoxyethyl-D-phenylglycinol, (**111**). To a stirred solution of phenylglycinol, **63** (100 mg, 0.73 mmol, 1.0 equiv), tetrabutylammonium iodide (537 mg, 1.46 mmol, 2.0 equiv), triethylamine (147 mg, 1.46 mmol, 2.0 equiv) in dry THF (10 mL) was added **86a** (287 mg, 1.46 mmol, 2.0 equiv) at room temperature. The resulting solution was refluxed for 3 days, allowed to cool to room temperature, diluted with dry Et_2O (20 mL), filtered, washed with water (2 x 5 mL), 10% $NaHCO_3$ (2 x 5 mL) and brine (1 x 5 mL), dried over $MgSO_4$, filtered, evaporated and purified on PTLC (silica gel eluted with 2:1 ethylacetate/hexanes) yielding **111** (80 mg, 43%) as a colorless oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ ($CHCl_3$): 1.17 (6H, t, J=6.8Hz); 2.63 (2H, m); 3.57 (7H, m); 4.52 (1H, t, J=5.9Hz); 7.26 (5H, m).

IR(NaCl, neat): 3300, 1600, 1130, 1060, 750, 700 cm^{-1}.



111 112b 112a

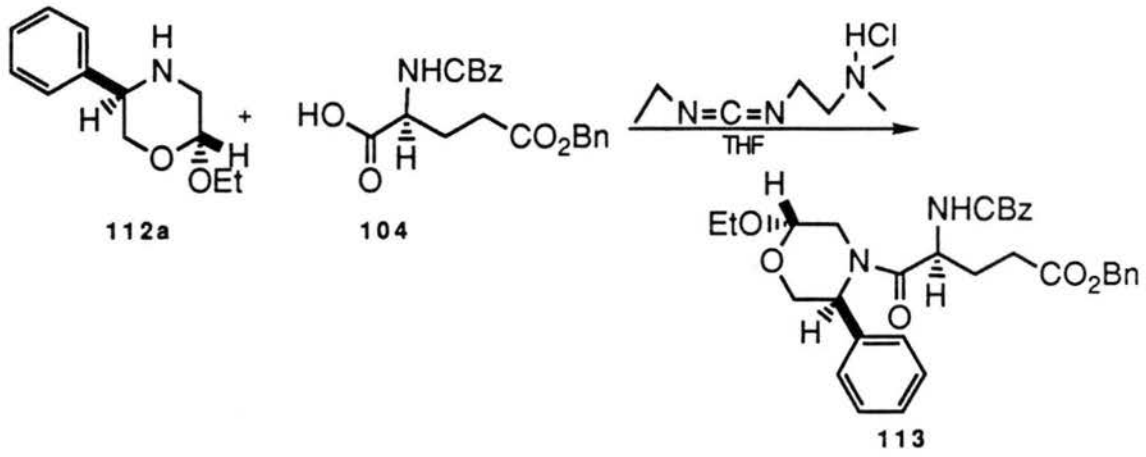
Trans-2-phenyl-5-ethoxy morpholine, (**112a**) and *cis-2-phenyl-5-ethoxy morpholine,* (**112b**). To a stirred solution of **111** (40 mg, 0.16 mmol, 1.0 equiv) in 2 mL of 1.0M HCl/absolute ethanol was added 20 mL of dry benzene. The reaction was heated to reflux and 20 mL of benzene/ethanol azeotrope was removed. Heating was ceased and the reaction cooled to room temperature. The mixture was diluted with 20 mL Et_2O and washed with H_2O (1 x 5 mL), saturated $NaHCO_3$ (1 x 5 mL) and brine (1 x 5 mL), dried over $MgSO_4$, filtered, evaporated, and separated on PTLC (silica gel eluted twice with 50% Et_2O/CCl_4) to afford **112b** and **112a** in a 1:4 ratio (3 mg:14.7 mg, 54%) as oils.

112b: ^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.23 (3H, t, J=6.9Hz); 3.13 (2H, m); 3.56 (2H, m); 3.77 (1H m); 3.95 (1H, m); 4.66 (1H, s); 7.34 (5H, m).

IR(NaCl, CH_2Cl_2): 3300, 1600, 1440, 1120, 1040, 975cm^{-1}.

112a: ^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.24 (3H, t, J=7.1Hz); 2.80 (1H, 1/2AB, J=8.3Hz); 3.15 (1H, 1/2AB, J=2.5Hz); 3.55 (2H, m); 3.90 (2H, m); 4.6 (1H, dd, J=2.5Hz).

IR(NaCl, CH_2Cl_2): 3310, 1600, 1445, 1150, 1060, 915, 820, 745, 690 cm^{-1}.

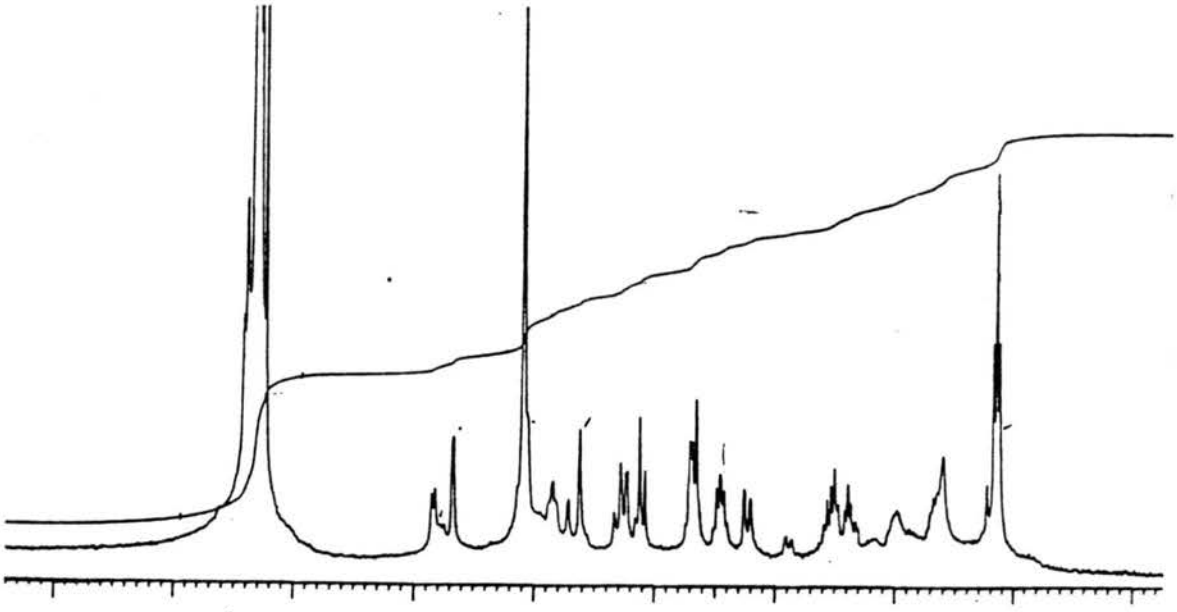


112a 104 113

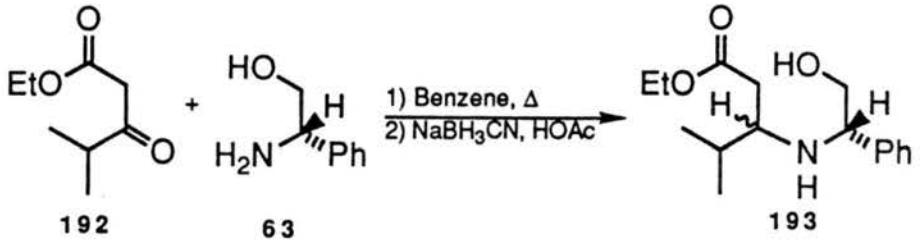
α-(trans-2-phenyl-5-ethoxy morpholine)-N-carbobenzyloxy-δ-benzyl glutamide, (**113**). To a stirred solution of **112a** (20 mg, 0.1 mmol, 1.0 equiv) and **104** (36 mg, 0.1 mmol, 1.0 equiv) in dry THF (1 mL) was added the N,N-dimethylethyl-N'-ethyl carbodiimide hydrochloride salt (37 mg, .2 mmol, 2.0 equiv) at room temperature. The resulting solution was allowed to stir for 4h, diluted with CH_2Cl_2 (25 mL), washed with H_2O (2 x 5 mL), 1M HCl (1 x 5 mL), dried over $MgSO_4$, evaporated and purified (PTLC silica gel, 2:1 ethylacetate/hexanes) yielding **113** (45.5 mg, 85%) as a clear viscous oil.

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: unassignable.

IR(NaCl, CH_2Cl_2): 1745, 1650.



^{1}H NMR (270 MHz) of **113** in $CDCl_3$ at 295^{o}K



192 63 193

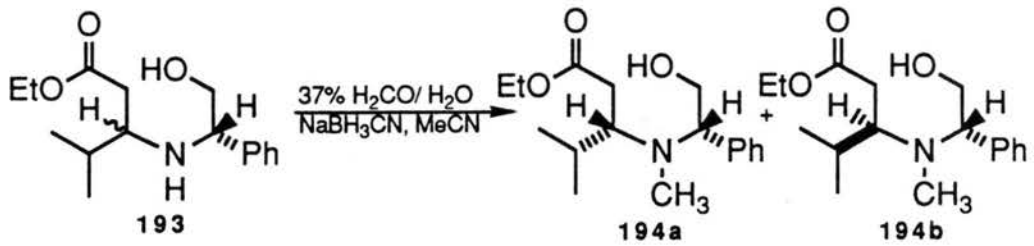
Ethyl(2-R,S-amino(N-(R-1-phenyl-2-hydroxy)ethyl)-4-methyl pentanoate. (**193**). To a stirred solution of phenylglycinol, **63** (30 mg, 0.66 mmol, 1.0 eqiv) in 6 mL benzene was added the β-keto ester, **192** (105 μL, 0.66 mmol, 1.0 equiv) and the resulting solution refluxed with a Dean Stark trap to remove water for 4h. The benzene was then evaporated and the resulting residue (190 mg) was dissolved in HOAc (6 mL) to which was added $NaBH_3CN$ (41 mg, 0.66 mmol, 1.0 equiv). The resulting solution was allowed to stir for 3h, diluted with CH_2Cl_2 (60 mL) and washed with 15% NaOH (2 x 10 mL), H_2O (1 x 10 mL), sat. $NaHCO_3$ (1 x 10 mL), brine (1 x 10 mL), dried

over $MgSO_4$, filtered, evaporated and purified (PTLC silica gel, 1:1 hex./EtOAc) yielding (50 mg **193** (81%) as a colorless oil. (1:1 mixture of diastereomers.)

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.19 (3H, m), 2.15 (3H, s), 2.40 (4H, m), 2.77 (2H, m), 3.55 (1H, m), 3.71 (1H, m), 3.92 (1H, m), 4.10 (2H, m), 7.10 (2H, m), 7.25 (3H, m).

IR(NaCl, net): 3440, 3340, 1730, 1030 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 279 (19.4), 233 (100), 160 (29), 106 (42).



Ethyl(S-2-amino(N-methyl-N-(R-1-phenyl-2-hydroxy)ethyl)-4-methyl pentanoate. (**194a**), and *Ethyl(R-2-amino(N-methyl-N-(R-1-phenyl-2-hydroxy)ethyl)-4-methyl pentanoate.* (**194b**). To a stirred solution of **193** (70 mg, 0.25 mmol, 1.0 equiv) in 1.5 mL acetonitrile was added 37% CH_2O/H_2O (60 μL, 0.75 mmol, 3.0 equiv) immediately followed by $NaBH_3CN$ (31 mg, 0.50 mmol, 2.0 equiv). The resulting reaction mixture was allowed to stir for 1h, diluted with 25 mL CH_2Cl_2 and washed with H_2O (2 x 5 mL), brine (1 x 5 mL), dried over $MgSO_4$, filtered, evaporated and separated (PTLC, silica gel, 2:1 hexane/EtOAc) yielding the diastereomer **194a** (25 mg) and **194b** (25 mg, 68% combined yield).

194a (Lower Diastereomer by TLC Rf=.35): Analysis calculated for $C_{17}H_{27}NO_3$: C, 69.59; H, 9.28; N, 4.77. Found: C, 69.79; H, 9.10; N, 4.82.

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 0.77 (3H, d, J=6.7Hz), 0.90 (3H, d, J=6.8Hz), 1.20 (3H, t, J=5.7Hz), 1.67 (1H, m), 2.15 (1H, m), 2.22 (3H, s), 3.03 (1H, m), 3.75 (3H, m), 4.10 (2H, q, J=6.7Hz), 7.31 (5H, m).

IR(NaCl, neat): 3450, 1725, 1015 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 293 (2.8), 242 (100), 120 (24).

194b (Upper Diastereomer by TLC Rf=.5): ^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 0.83 (3H, d, J=6.6Hz), 0.97 (3H, d, J=6.6 Hz), 1.21 (3H, t, J=5.5Hz), 1.70 (1H, m), 2.08 (3H, s), 2.13 (2H, m), 3.05 (1H, m), 3.58 (1H, m), 3.85 (3H, m), 4.08 (2H, q, J=6.6Hz), 7.25 (5H, m).

IR(NaCl, neat): 3430, 1725, 1020 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 292(2.7), 248 (100), 204(12).

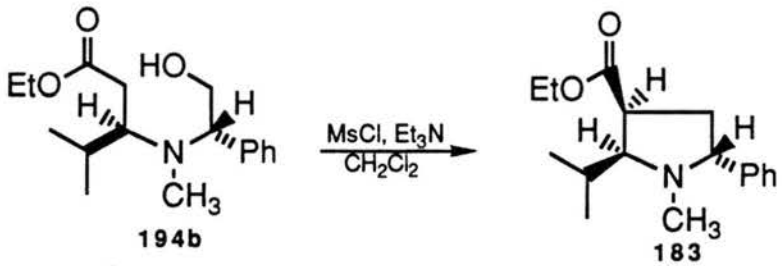
N-methyl-R-2-phenyl-R-4-carboethoxy-S-5-isopropyl pyrrolidine, (**182**). To a stirred solution of **194a** (27 mg, 0.09 mmol, 1.0 equiv) and Et_3N (32 μL, 0.23 mmol, 2.5 equiv) in 1 mL dry CH_2Cl_2

was added mesyl chloride (9 μL, 0.15 mmol, 1.5 equiv) at room temperature. The reaction was diluted with 25 mL CH_2Cl_2, washed with 1H NH_4HCO_3 (2 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL), dried over $MgSO_4$, filtered, and evaporated yielding **182** as a colorless oil (20 mg, 87%).Analysis calculated for $C_{17}H_{26}ClNO_3$: C, 74.18; H, 9.08; N, 5.09. Found: C, 71.53; H, 9.06; N, 5.85.

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 0.88 (3H, d, J=6.6Hz), 1.02 (3H, d J=6.6Hz), 1.20 (3H, t, J=7.2Hz), 1.78 (1H, m), 2.31 (3H, s), 2.40 (1H, dd, J=7.2Hz), 2.75 (1H, dd, J=6.8Hz), 2.87 (1H,m), 4.17 (2H, q , J=7.2Hz) 4.67 (1H,m) 7.35 (5H, m).

IR(NaCl, neat): 1720, 1245 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 275 (34), 230 (100), 201 (45).

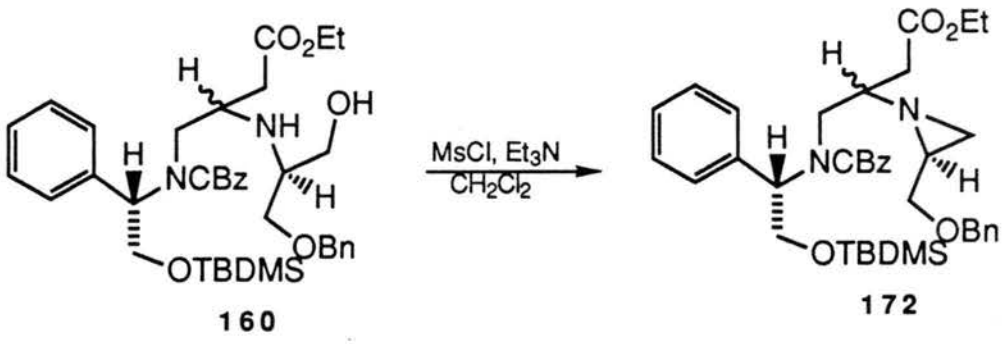


N-methyl-R-2-phenyl-R-4-carboethoxy-R-5-isopropyl pyrrolidine, (**183**). To a stirred solution of **194b** (36 mg, 0.12 mmol, 1.0 equiv) and Et_3N (43 μL, 0.31 mmol, 2.5 equiv) in 1 mL dry CH_2Cl_2 was added mesyl chloride (12 μL, 0.15 mmol, 1.5 equiv) at room temperature. The reaction was diluted with 25 mL CH_2Cl_2, washed with 1H NH_4HCO_3 (2 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL), dried over $MgSO_4$, filtered, and evaporated yielding **183** as a colorless oil (26mg, 78%).

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 0.85 (2H, d, J=4.3Hz), 0.96 (2H, d, J=3.9Hz), 1.26 (3H, t, J=7.2Hz), 1.80 (1H, m), 2.36 (3H, s), 2.43 (1H, dd, J=6.1Hz), 2.68 (1H, dd, J=7.1Hz), 2.80 (1H,m), 4.14 (2H, q , J=7.1Hz) 4.60 (1H,m) 7.35 (5H, m).

IR(NaCl, neat): 1730, 1245 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 275 (48), 230 (100), 201 (38).



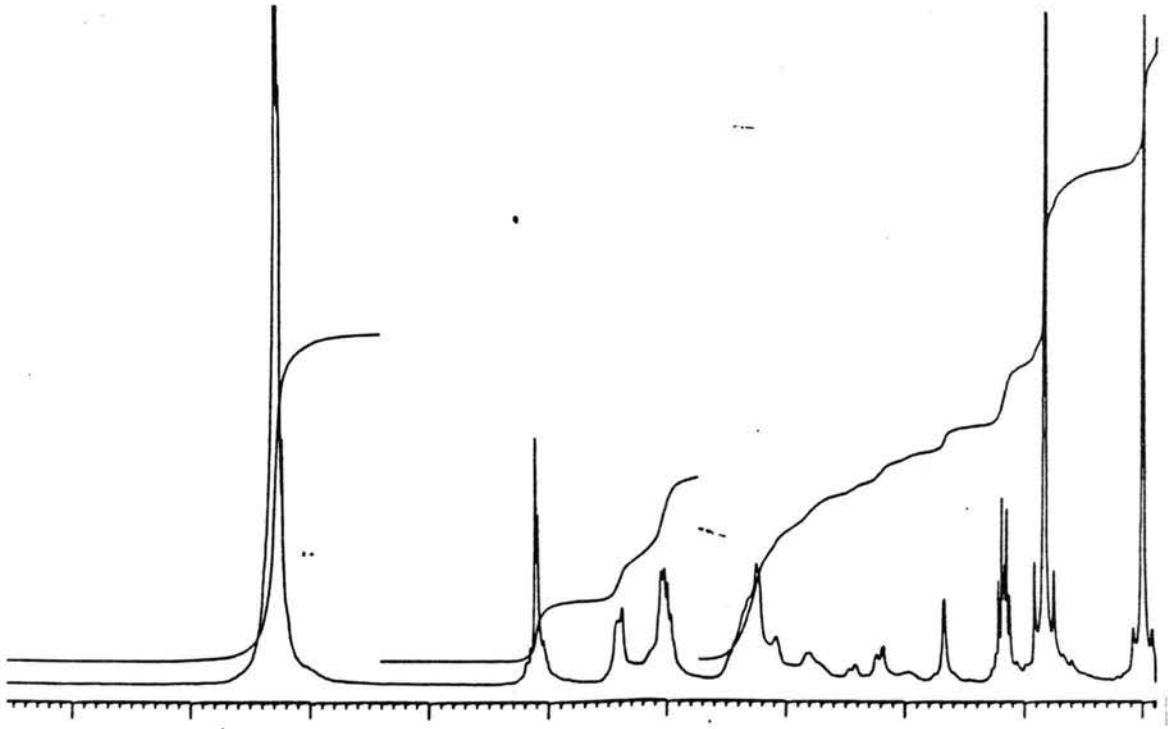
160 **172**

Ethyl-2-(2'-benzyloxymethyl)aziridine-4-amino(N-carbo-benzyloxy-N-(1-phenyl-2-(O-^{t}butyldimethylsilyl)hydroxy)ethyl butanoate, (**172**). To a stirred solution of **160** (113 mg, 0.17 mmol, 1.0 equiv) and Et_3N (58 μL, 0.42 mmol, 2.5 equiv) in 1.5 mL dry CH_2Cl_2 was added mesyl chloride (15.4 μL, 0.2 mmol, 1.2 equiv). The resulting solution was allowed to stir at room temperature for 1h, diluted with 20 mL CH_2Cl_2, washed with H_2O (1 x 5 mL), 1M NH_4HCO_3 (1 x 5 mL), brine, dried over $MgSO_4$, filtered, evaporated and purified (PTLC, silica gel, 4:1 hexane/EtOAc) yielding 85 mg **172** (77%) as a colorless oil.

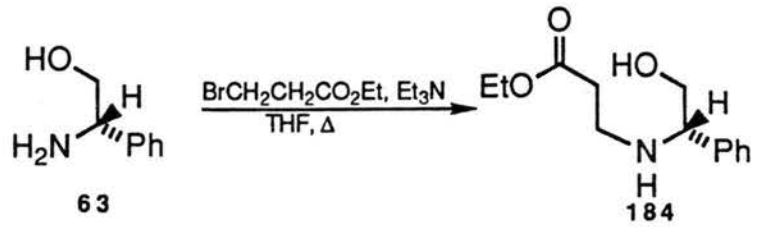
^{1}H NMR (270 MHz, $CDCl_3$) δ($CHCl_3$): unassignable. INEPT 2 ^{13}C NMR (200 MHz, $CDCl_3$) δ(TMS): 26.5, 52.0, 56.8, 127.7, 128.0, 128.5, 129.2, 130.1, 130.9.

IR(NaCl, neat): 1730, 1700, 680 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 660 (M^+, 3), 498 (3.2), 364 (9.6), 235 (27), 164 (53), 106 (100).



^{1}H NMR (270 MHz) of **172** in $CDCl_3$ at 295°K



63 184

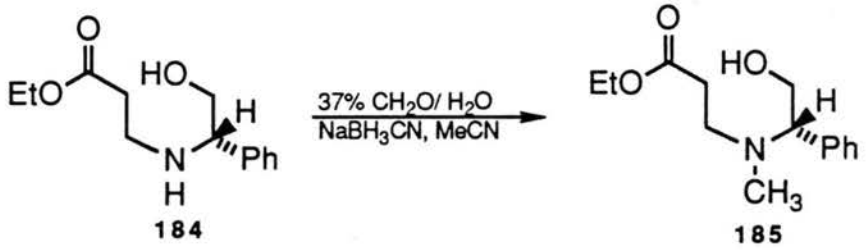
Ethyl (2-amino(N-(R-1-phenyl-2-hydroxy)ethyl) propionoate. (**184**). To a stirred solution of ethyl-3-bromopropionate (0.66 mL, 5.2 mmol, 1.2 equiv), Et_3N (0.9 mL, 6.5 mmol, 1.5 equiv) in 25 mL dry THF, was added L-phenylglycinol (0.59 g, 4.31 mmol, 1.0 equiv). The resulting solution was refluxed for 18h, cooled to room temperature, filtered, evaporated, and purified (silica gel, 89:9:1, CH_2Cl_2:MeOH:NH_4OH) yielding **184** as a colorless oil (0.862 g, 65%),

$[\alpha]_D$ = -50.81 (c = 2.13, CH_2Cl_2). Analysis calculated for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.67; H, 7.95; N, 5.72.

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.18 (3H, t, J=7.3Hz), 2.41 (2H, m), 2.65 (3H, m), 2.77 (1H, m), 3.43 (1H, t, J=8.8Hz), 3.62 (2H, m), 4.05 (2H, q, J=7.2Hz), 7.25 (5H, m).

IR(NaCl, neat): 3400, 3300, 1725, 1170, 1035 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 238 (32), 206 (100), 118 (28), 106 (49).



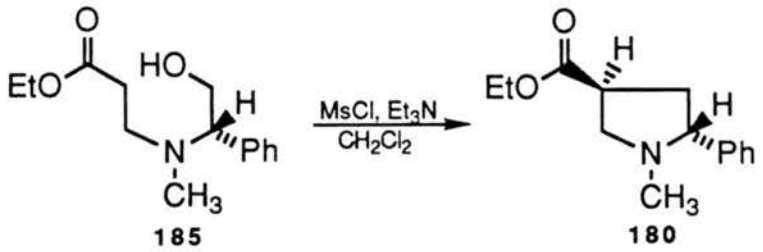
184 185

Ethyl(2-N-methylamino(N-(R-1-phenyl-2-hydroxy)ethyl) propionoate. (**185**). To a stirred solution of **184** (120 mg, 0.51 mmol, 1.0 equiv) in acetonitrile (1.5 mL) was added 37% CH_2O/H_2O (205 μL, 2.52 mmol, 5.0 equiv) immediately followed by $NaBH_3CN$ (63 mg, 1.0 mmol, 2.0 equiv). The resulting turbid solution is let stir at room temperature for 1h, diluted with CH_2Cl_2 (25 mL), washed with H_2O (2 x 5 mL) and brine (1 x 5 mL) dried over $MgSO_4$, filtered, and evaporated yielding pure **185** as a colorless oil (120 mg, 94%), $[\alpha]_D$ = -27.51 (c = 2.23, CH_2Cl_2). Analysis calculated for $C_{14}H_{21}NO_3$: C, 66.90; H, 8.42; N, 5.57. Found: C, 66.77; H, 8.45; N, 5.39.

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.25 (3H, t, J=7.2Hz), 2.17 (3H, s), 2.45 (3H, m), 2.83 (1H, m), 3.61 (1H, dd, J=10.8, 4.7Hz), 3.75 (1H, dd, J=10.7, 4.70Hz), 3.99 (1H, t, J=10.3Hz), 4.12 (2H, q, J=7.2Hz).

IR(NaCl, neat): 3420, 1730, 1250, 1025 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 252 (34), 206 (100), 152 (7.5), 106 (42).



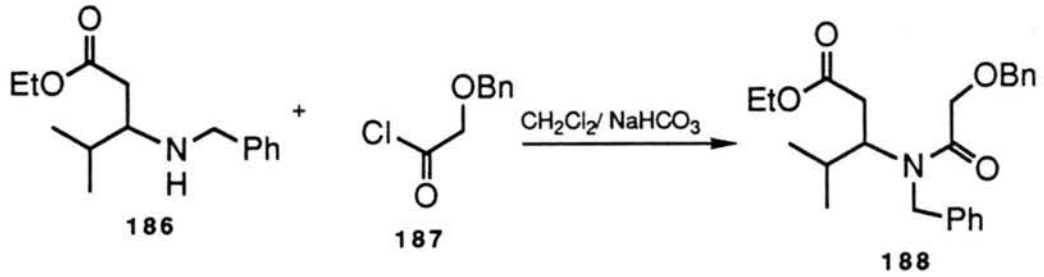
185 **180**

N-methyl-2-R-phenyl-3-S-carboethoxypyrrolidine, (**180**). To a stirred solution of **185** (25 mg, 0.1 mmol, 1.0 equiv) and Et_3N (35 μL, 0.25 mmol, 2.5 equiv) in 1 mL dry CH_2Cl_2 was added mesyl chloride (12 μL, 0.15 mmol, 1.5 equiv) at room temperature. The reaction was diluted with 25 mL CH_2Cl_2, washed with 1H NH_4HCO_3 (2 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL), dried over $MgSO_4$, filtered, and evaporated yielding **180** as a colorless oil (20 mg, 86%), $[\alpha]_D$ = -10.35 (c = 1.20, CH_2Cl_2). Analysis calculated for $C_{14}H_{20}ClNO_3$: C, 62.33; H, 7.47; N, 5.19. Found: C, 62.37; H, 7.49; N, 5.87.

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.22 (3H, t, J=7.0Hz), 2.28 (3H, s), 2.43 (2H, t, J=7.5Hz), 2.75 (2H, m), 3.01 (1H, dd, J=7.6, 13.6Hz), 4.52 (2H, q, J=7.0Hz), 4.87 (1H, t, J=6.6Hz), 7.30 (5H, m).

IR(NaCl, neat): 1735, 1250 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 233 (60.8), 132 (100), 106 (35).



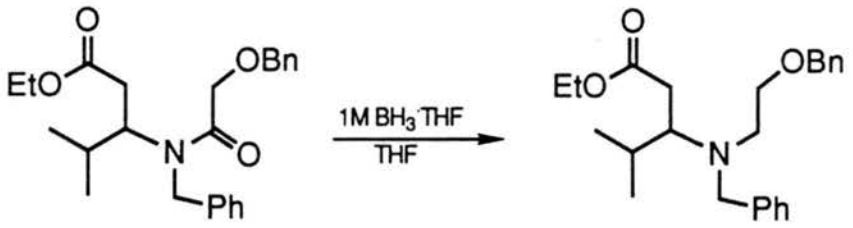
186 **187** **188**

Ethyl-3-amino(N-benzyl-N-benzyloxyacetamide)4-methyl pentanoate, (**188**). To a stirred solution of CH_2Cl_2 (25mL) was added

186 (1.0g, 4.02mmol, 1.0 equiv) followed by the addition of 25mL sat. $NaHCO_3$. To this vigorously stirred bilayer was added **187** (0.738g, 4.02mmol, !.0 equiv) at room temperture. The reaction was allowed to stir for 1h. The organic layer was separated and washed with 1M HCl (1 x 10mL), 1M NaOH (1 x 10mL) and water (1 x 10mL), dried (MgSO4), filtered and evaporated to yield pure**188** (1.43g, 90%). Analysis calculated for $C_{24}H_{31}NO_4$: C, 72.51; H, 7.86; N, 3.52. Found: C, 72.39; H, 7.88; N, 3.44.

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 0.85 (3H, d, J=7.2Hz), 0.95, (3H, d, J=7.3Hz), 1.10 (3H, t, J=7.3Hz),1.93, (1H, m), 2.43 (2H, m), 3.95 (5H, m), 4.65 (4H, m) 7.35 (5H, m).

IR(NaCl, neat): 1725, 1675, 1250, 1080 cm^{-1}.



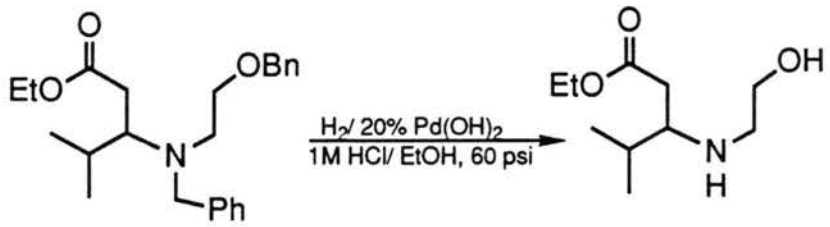
188 189

Ethyl-3-amino(N-benzyl-N-2-benzyloxyethyl)4-methyl pentanoate, (**189**). To a stirred solution of **188** (1.40g, 3.50 mmol, 1.0 equiv) in 20 mL anhydrous THF was added 7mL of BH_3 THF complex (1M soln. in THF, 7.00 mmol, 2.0 equiv) at 0°. The resulting reaction was allowed to warm to room temperature, quenched with 10 mL 1M HCl then diluted with 75 mL CH_2Cl_2 and 20mL 1M NaOH. The organic layer was separated and washed with 1M NaOH (1 x 15mL), H_2O (1 x 15mL) and brine (1 x 20mL). The resulting clear solution was dried (MgSO4) and filtered to furnish **189** (0.95g, 70%), as a colorles oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 0.85 (3H, d, J=6.5Hz), 0.98 (3H, d J=6.6Hz), 1.24 (3H, t, J=6.9Hz), 1.78 (1H, m), 2.30 (1H, m), 2.75 (4H, m), 3.47 (2H,m), 3.60 (2H, d, J=13.1Hz) 3.75 (2H, d, J=12.6Hz), 4.10, (2H, q, J=7.1Hz), 4.41 (2H,s) 7.35 (5H, m).

IR(NaCl, neat): 1740, 1085 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 384 (14), 106 (100).



189 190

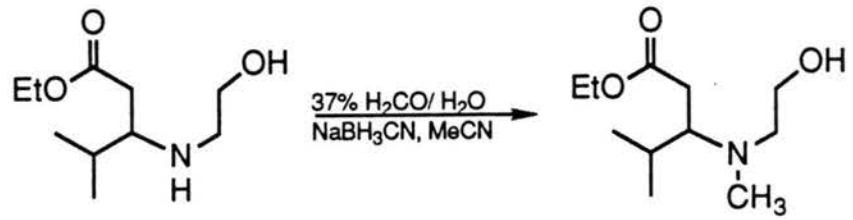
Ethyl-3-amino(N-2-hydroxyethyl)4-methyl pentanoate, (**190**). A solution of **189** (900mg, 2.35 mmol, 1.0 equiv) in 20mL 1M HCl/EtOH was placed in a Parr pressure vessel and purged with N_2. To this solution was added 20% $Pd(OH)_2$ on carbon (900mg, 0.47 mmol, 0.2 equiv). The system was sealed and purged with hydrogen then charged to 60 psi. (H_2)and stirred at room tempurature for 18h. The pressure was released, the vessel purged with N_2, and the reaction filtered over celite. The mixture was evaporated to an oil which was dissolved in 75mL CH_2Cl_2 followed by washing with 2M NH_4OH, (3 x 20mL), H_2O, (1 x 15mL), and brine (1 x 15ml), dried, ($MgSO_4$), filtered and evaporated to yield **190**, (342mg, 72%) as a colorless oil. Analysis calculated for $C_{10}H_{21}NO_3$: C, 59.11; H, 10.34; N, 6.80. Found: C, 61.07; H, 8.04; N, 7.51.

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 0.88 (3H, d, J=6.8Hz), 0.93 (3H, d, J=6.9Hz), 1.27 (3H, t, J=7.3Hz), 1.84 (1H, m), 2.15 (2H, bs),

2.26 (1H, dd, J=8.8), 2.45 (1H, dd, J=5.8Hz), 2.77 (2H, t, J=5.2Hz), 2.84 (1H, m) 3.57 (2H, m,), 4.14, (2H, q, J=7.2Hz).

IR(NaCl, neat): 3380, 1730, 1160, 1035 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 203 (100), 158 (54).



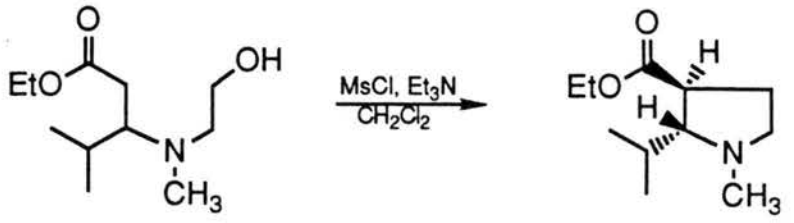
190 **191**

Ethyl-3-amino(N-methyl-N-2-hydroxyethyl)4-methyl pentanoate, (**191**). To a stirred solution of **190** (300 mg, 1.47 mmol, 1.0 equiv) in acetonitrile (10.0 mL) was added 37% CH_2O/H_2O (600 µL, 7.39 mmol, 5.0 equiv) immediately followed by $NaBH_3CN$ (185 mg, 2.96 mmol, 2.0 equiv). The resulting turbid solution was allowed to stir at room temperature for 1h diluted with CH_2Cl_2 (25 mL), washed with H_2O (2 x 5 mL) and brine (1 x 5 mL) dried over $MgSO_4$, filtered, and evaporated yielding pure **191** as a colorless oil (275 mg, 86%).

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 0.80 (3H, d, J=6.8Hz), 0.89 (3H, d, J=6.7Hz), 1.17 (3H, t, J=7.1Hz), 1.70 (1H, m), 2.16 (3H, s), 2.30 (2H, m), 2.63 (2H, m), 2.87 (1H, bs), 3.44 (2H, t, J=5.1Hz) , 4.03, (2H, q, J=7.1Hz).

IR(NaCl, neat): 3460, 1730, 1025 cm^{-1}.

Mass spectrum CI(NH_3) m/e = 217 (10.4), 172 (100), 160 (21.6).



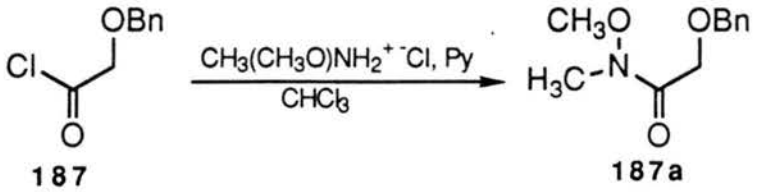
191 **181**

N-methyl-2-isopropyl-3-carboethoxy pyrrolidine, (**181**). To a stirred solution of **191** (230 mg, 1.06 mmol, 1.0 equiv) and Et_3N (150 μL, 2.65 mmol, 2.5 equiv) in 10 mL dry CH_2Cl_2 was added mesyl chloride (52 μL, 1.58 mmol, 1.5 equiv) at room temperature. The reaction was stirred for 2h, diluted with 25 mL CH_2Cl_2, washed with 1M NH_4HCO_3 (2 x 5 mL), H_2O (1 x 5 mL) and brine (1 x 5 mL), dried over $MgSO_4$, filtered, and evaporated yielding **181** as a colorless oil (194 mg, 92%). Analysis calculated for $C_{11}H_{22}ClNO_3$: C, 56.03; H, 9.41; N, 5.94. Found: C, 56.17; H, 9.41; N, 5.82.

^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 0.83 (3H, d, J=7.1Hz), 0.93 (3H, d, J=7.2Hz), 1.22 (3H, t, J=7.3Hz), 1.65 (1H, m), 2.26 (3H, s), 2.30 (1H, m), 2.72 (3H, m), 3.42 (2H, t, J=7.5Hz), 4.11, (2H, q, J=7.3Hz).

IR(NaCl, neat): 1730, 1225 cm^{-1}.

Mass spectrum CI(NH_3) m/e =$(M + NH_4)^+$ 217 (100), 143 (19).



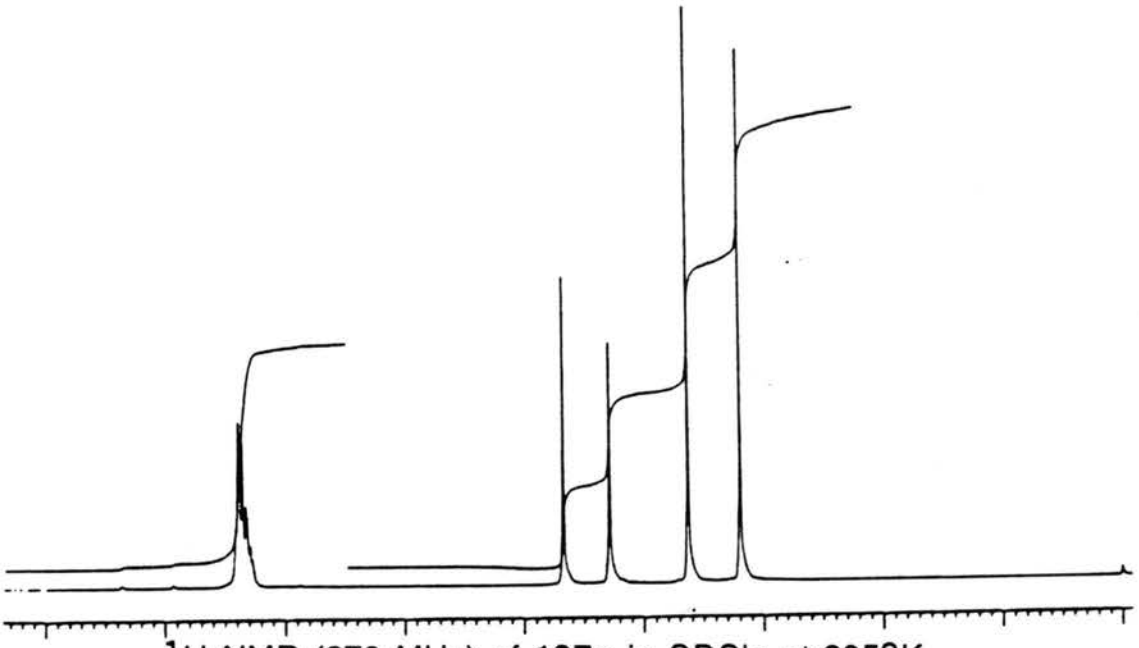
187 187a

N-Methoxy-N-methyl-(2-benzyloxy)acetamide, (**187a**). To a stirred solution of benzyloxyacetyl chloride, **187**, (3.227g, 17.54 mmol, 1.0 equiv) and methoxymethylamine hydrochloride (1.93 g, 19.29 mmol, 1.1 equiv) in dry $CHCl_3$ (175 mL) cooled to 0°C was added pyridine (3.12 mL, 3.858 mmol, 2.2 equiv). The resulting solution was stirred at room temperature for 12h when the $CHCl_3$ was subsequently evaporated yielding a white residue. The residue was partitioned between brine and a 1:1 mixture of CH_2Cl_2/Et_2O. The organic layer was separated and dried over Na_2SO_4, filtered and evaporated yielding the title compound (3.64 g, 99.5%) as a colorless oil, bp = 132°C/.2 mmHg.

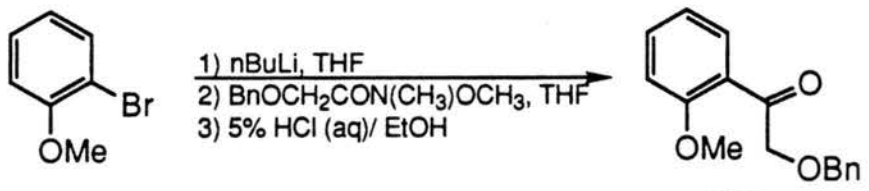
^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 3.20 (3H, s), 3.63 (3H, s), 4.29 (2H, s), 4.67 (2H, s), 7.36 (5H, m).

IR(NaCl, neat): 3020, 3060, 2940, 1675, 1450, 1325, 1130, 1080, 980, 730, 690 cm^{-1}.

Mass spectrum, $CI(NH_3)$, m/z = 209.8 (M+, 0.7%), 197(3.1), 180(9.0), 108(5.8), 106(10.4), 91(2.4), 74(5.9), 44(4.5), 35(100).



^{1}H NMR (270 MHz) of **187a** in $CDCl_3$ at 295°K.



199

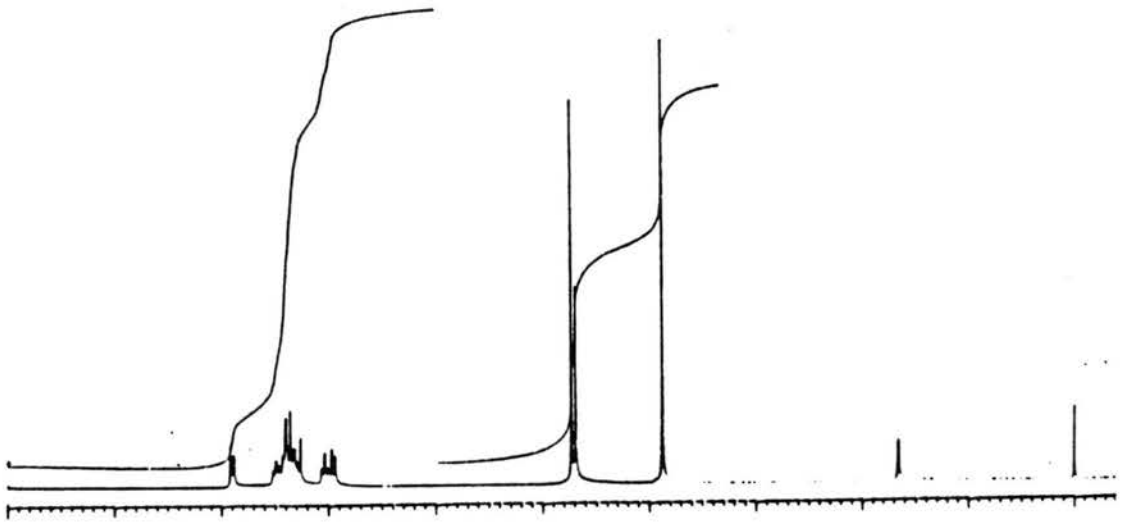
Benzyloxymethyl(2'-methoxyphenyl)ketone, (**199**). To a stirred solution of o-bromoanisole (4.56 mL, 36.68 mmol, 3.0 equiv) in dry THF (12.5 mL) cooled to -15°C was added n-BuLi (23.7 mL of a 1.54M solution in hexanes, 3.0 equiv). The resulting solution was allowed to stir for 1h at -15°C and added to a solution of (N-methoxy-N-methyl)benzyloxy acetamide (2.55 g, 12.23 mmol, 1.0 equiv) in dry THF (125 mL), cooled to -15°C, via cannula. The resulting solution was stirred for 30 min and poured into 50 mL of 5% HCl/EtOH at 0°C. This solution was then partitioned between brine and a 1:1 mixture of CH_2Cl_2/Et_2O. The organic layer was separated and dried over

Na_2SO_4, filtered and evaporated yielding **199** as a colorless oil (2.82 g, 90%).

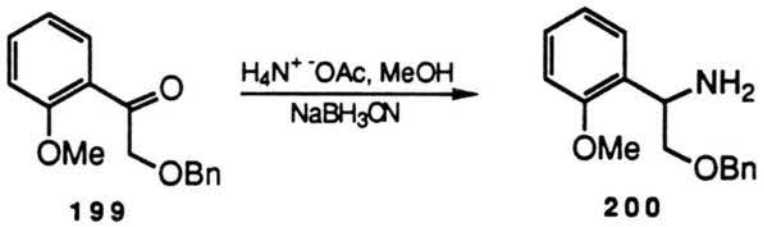
^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 3.87 (3H, s), 4.68 (2H, s), 4.72 (2H, s), 6.93 (2H, m), 7.39 (6H, m), 7.89 (1H, dd, J=7.73Hz);

IR(NaCl, neat): 3020, 3060, 2930, 1680, 1595, 1480, 1280, 1235, 1100, 1010, 940, 740, 685 cm-1.

Mass spectrum, CI(NH_3), m/z = 257(M+, 14.5%), 151(100), 135(6.6), 106(6.0), 91(2.4), 35(100).



^1H NMR (270 MHz) of **199** in $CDCl_3$ at 295°K



199 **200**

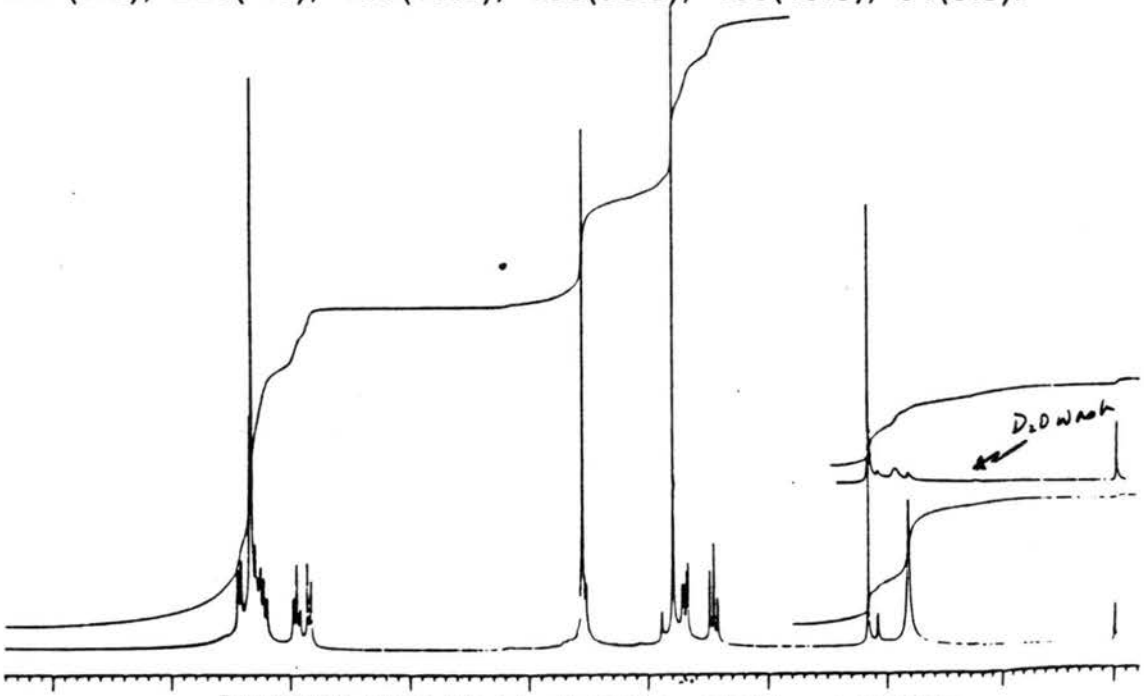
O-Benzyl(2'-methoxy)phenylglycinol, (**200**). To a stirred solution of **199** (2.82 g, 11.03 mmol, 1.0 equiv) and ammonium acetate (8.50 g, 110.3 mmol, 10 equiv) in absolute methanol (35 mL)

was added sodium cyanoborohydride (0.485 g, 7.72 mmol, 0.7 equiv) in one portion. The resulting solution was stirred at room temperature for 36h. Conc. HCl was added until pH <2. The MeOH was then evaporated and the resulting white residue was dissolved in H_2O (10 mL) and washed with Et_2O (2 x 10 mL). The aqueous phase was then basified with powdered KOH to pH >10, saturated with NaCl and extracted with CH_2Cl_2(4 x 10 mL). The combined CH_2Cl_2 extracts were dried over $MgSO_4$, filtered and evaporated to a colorless oil (1.83 g, 65%).

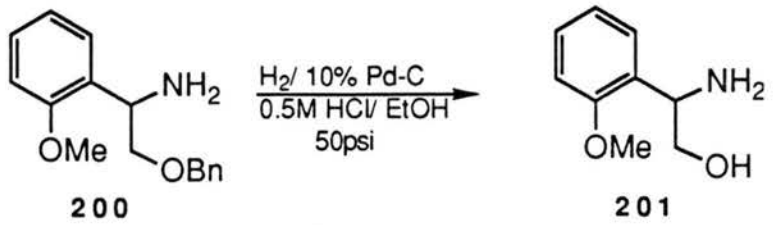
^1H NMR (270 MHz, $CDCl_3$), δ(TMS): 1.82 (2H, bs), 3.46 (1H, t, J=8.5Hz), 3.69 (1H, dd, J=9.2Hz), 3.79 (3H, s), 4.56 (3H, m), 6.92 (2H, m), 7.32 (7H, m);

IR(NaCl, neat): 3380, 3300, 3020, 3060, 2900, 2840, 1580, 1485, 1450, 1230, 1080, 1115, 850, 735, 680 cm-1.

Mass spectrum, CI(NH_3), m/z = 258(M+,100), 256(210), 241(2.5), 228(1.8), 150(19.2), 136(38.5), 106(19.5), 91(6.8).



^1H NMR (270 MHz) of **200** in $CDCl_3$ at 295°K



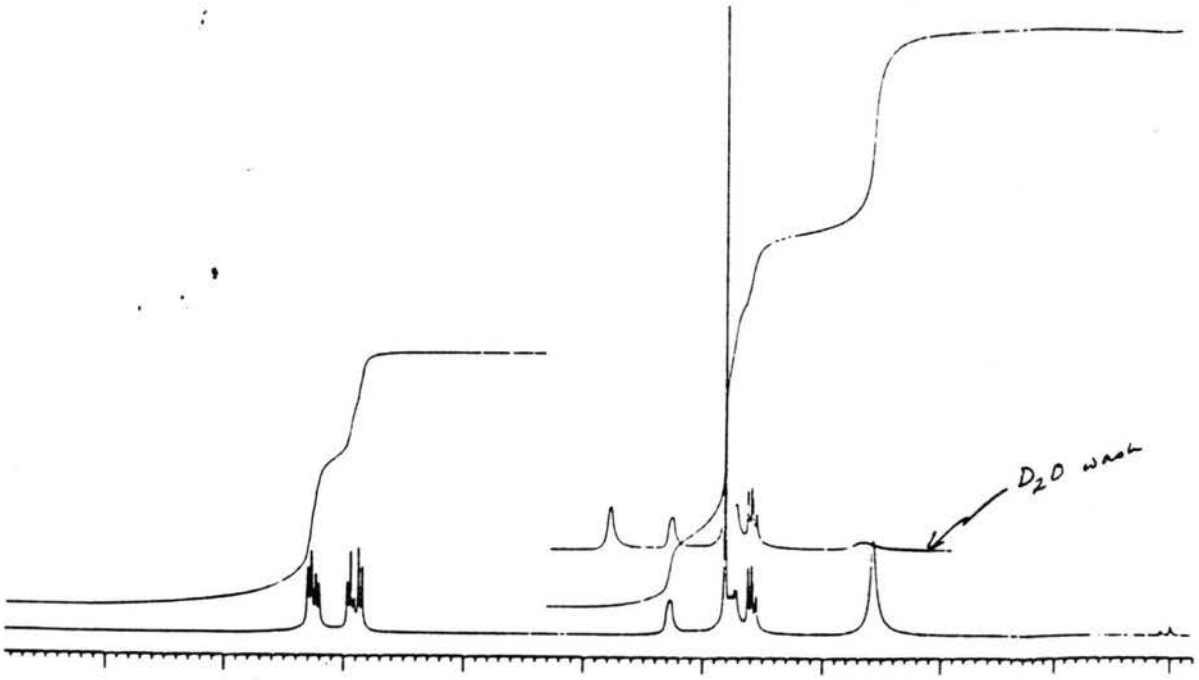
200 **201**

2'-Methoxyphenylglycinol, (**201**). To a solution of **200** (2.88 g, 11.22 mmol, 1.0 equiv) in 60 mL 0.5M HCl/EtOH contained in a Parr pressure vessel was added 10% Pd/C (2.98 g, 2.8037 mmol, 0.25 equiv). The vessel was purged with hydrogen several times then charged to 50 psi (H_2) and hydrogenated for 20h. The Pd/C was filtered off over celite and the filtrate evaporated to a white solid. The solid was dissolved in water and washed once with Et_2O then basified to pH >10 with solid KOH, saturated with NaCl and extracted with CH_2Cl_2 (4 x 20 mL). The organic phase was then dried over $MgSO_4$, filtered and evaporated yielding **201** (1.52 g, 81%) as a colorless oil.

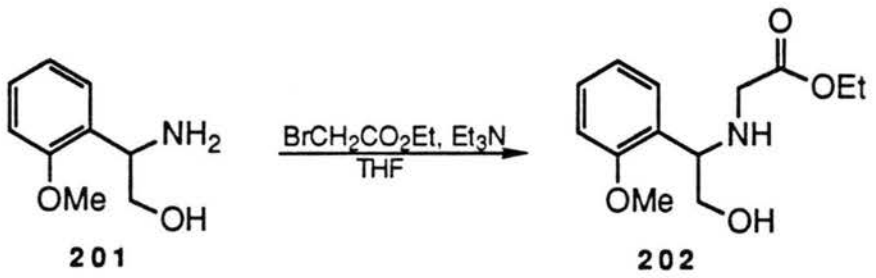
^1H NMR (270 MHz, $CDCl_3$), δ(TMS): 2.57 (3H, bs), 3.59 (1H, m), 3.73 (1H, m), 3.81 (3H, s), 4.27 (1H, m), 6.69 (1H, d, J=8.2Hz), 6.91 (1H, m), 7.23 (2H, m).

IR(NaCl, neat): 3360, 3280, 2920, 2830, 1590, 1490, 1235, 1140, 1120, 740 cm^{-1}.

Mass spectrum, CI(NH_3), m/z = 168(M+, 5.8%), 151(10.9), 136(23.6), 44(6.0), 35(100).



^{1}H NMR (270 MHz) of **201** in $CDCl_3$ at 295°K



201 **202**

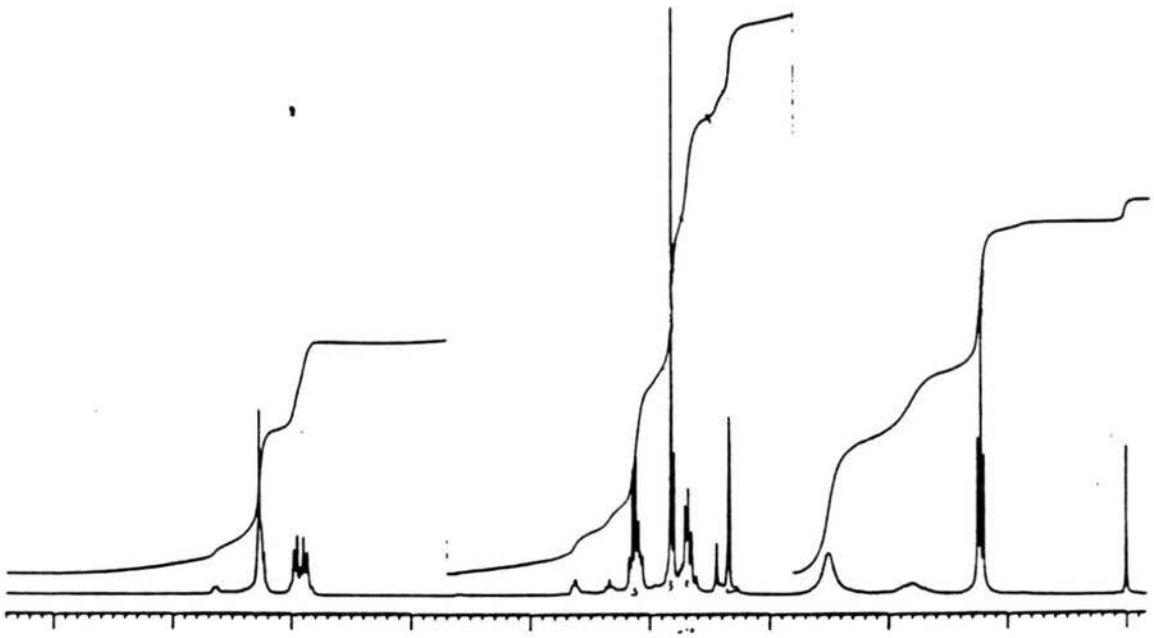
N-(Ethoxyacetyl)(2'-methoxy)phenylglycinol, (**202**). To a stirred solution of **201** (1.16 g, 6.95 mmol, 1.0 equiv) and triethylamine (1.45 mL, 10.44 mmol, 1.5 equiv) in dry THF (60 mL) was added ethylbromoacetate (1.00 mL, 9.05 mmol, 1.3 equiv). The reaction solution was stirred at room temperature for 20 h. The $Et_3N.HBr$ was filtered off and washed with THF. The filtrate was evaporated to a clear residue which was taken up in 70 mL CH_2Cl_2, washed with H_2O (3 x 20 mL) and brine (1 x 20 mL), dried over

$MgSO_4$, filtered and evaporated yield **202** (1.665 g, 95%) as a colorless oil.

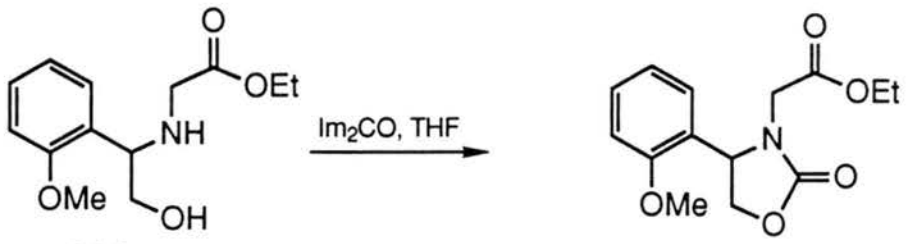
^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.23 (3H, t, J=7.45Hz), 2.50 (2H, bs), 3.35 (2H, d, J=5.4Hz), 3.70 (2H, m), 3.82 (3H, s), 4.13 (3H, m), 6.92 (2H, m), 7.28 (2H, m).

IR(NaCl, neat): 3310, 2910, 1735, 1595, 1485, 1455, 1230, 1180, 1020, 740 cm-1.

Mass spectrum, CI(NH_3), m/z = 254(M+, 1.9), 236(1.8), 208(18.9), 168(2.5), 150(6.7), 130(61.1), 104(11.3), 72(7.2), 55(100).



^1H NMR (270 MHz) of **202** in $CDCl_3$ at 295°K



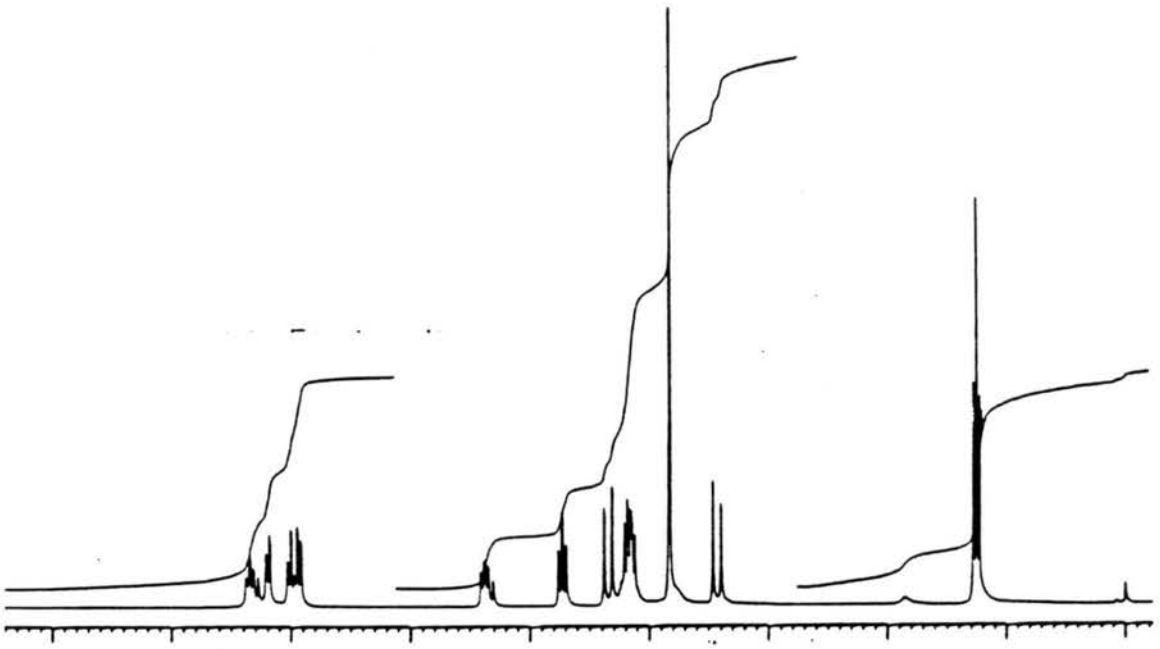
202 **203**

1-(carboethoxy)methyl-5-(2'-methoxy)phenyl oxazolidin-2-one, (**203**). To a stirred solution of **202** (1.665 g, 6.59 mmol, 1.0 equiv) in dry THF (60 mL) was added 1,1'-carbonyldiimidazole (1.60 g, 9.87 mmol, 1.5 equiv). The resulting solution was stirred at room temperature for 2 h and evaporated to a white residue. The residue was taken up in CH_2Cl_2 (100 mL) and washed with 1M HCl (3 x 25 mL), H_2O (2 x 25 mL) and brine (1 x 25 mL), dried over $MgSO_4$, filtered and evaporated yielding **203** as a colorless oil (1.41 g, 77%).

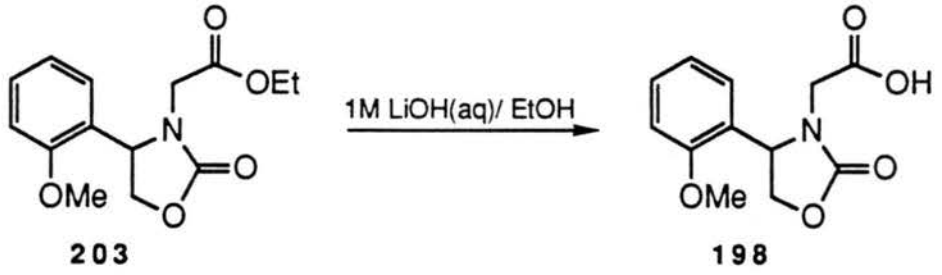
^1H NMR (270 MHz, $CDCl_3$) δ(TMS): 1.26 (3H, t, J=7.0Hz), 3.43 (1H, d, J=18.0Hz), 3.83 (3H, s), 4.16 (3H, m), 4.35 (1H, d, J=18.0Hz), 4.72 (1H, t, J=8.7Hz), 5.35 (1H, m), 6.97 (2H,m), 7.28 (2H, m).

IR(NaCl, neat): 2960, 2920, 2820, 1750, 1600, 1580, 1485, 1460, 1415, 1240, 1195, 1080, 1115, 745 cm-1.

Mass spectrum, CI(NH_3), m/z = 280(M+, 54.9%), 250(3.1), 235(1.2), 220(1.4), 162(1.8), 148(1.7), 133(2.0), 104(1.7), 35(100).



^{1}H NMR (270 MHz) of **203** in $CDCl_3$ at 295^{o}K



203 **198**

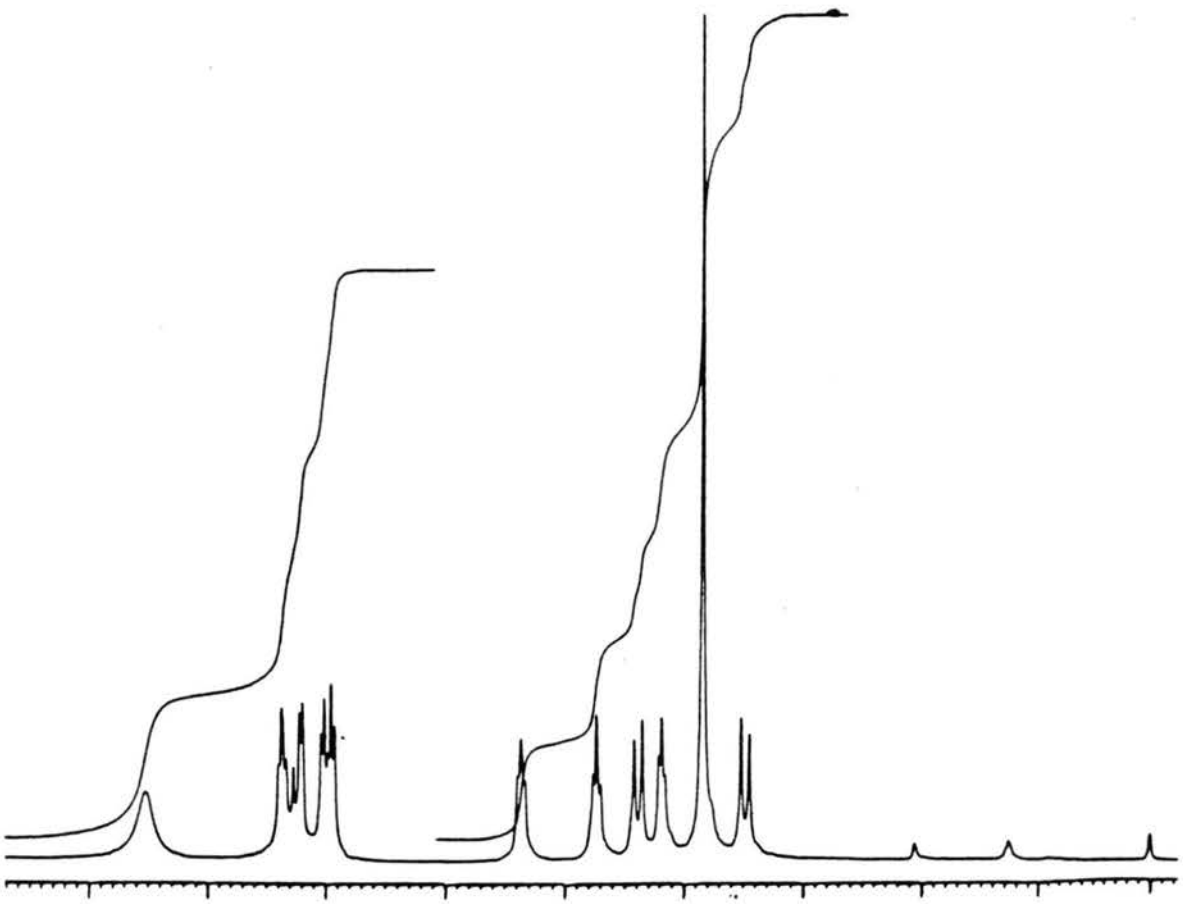
1-(carboxy)methyl-5-(2'-methoxy)phenyloxazolidin-2-one, (**198**). To a stirred solution of **203** (1.41 g, 5.06 mmol, 1.0 equiv) in 16 mL absolute ethanol at -10^{o}C was added 6.7 mL of 1M LiOH (6.7 mmol, 1.32 equiv). The reaction was allowed to stir for 1.5h at -10^{o}C, neutralized with 6M HCl (1.11 mL, 6.7 mmol, 1.32 equiv). The ethanol was evaporated and the resulting residue was partitioned between 1M HCl and CH_2Cl_2. The organic layer was separated and washed with H_2O (1 x 10 mL), and brine (1 x 10 mL), dried over $MgSO_4$, filtered and evaporated to a white solid. Recrystallization

from EtOHAc/hexanes afforded 957 mg of pure **198** (75%), mp 165-166°C.

^{1}H NMR (270 MHz, $CDCl_3$) δ(TMS): 3.48 (1H, d, J=18.3Hz), 3.83 (3H, s), 4.19 (1H, t, J=8.0Hz), 4.39 (1H, d, J=18.4Hz), 4.73 (1H, t, J=9.2Hz), 5.36 (1H, m), 6.95 (2H, m), 7.36 (2H, m), 8.52 (1H, bs).

IR(NaCl, neat): 2900, 2810, 2700, 2585, 2500, 1750, 1675, 1595, 1580, 1450, 1240, 1200, 1190, 1110, 940, 850, 750, 735, 700, 630 cm-1.

Mass spectrum, CI(NH_3), m/z = 251(M+, 13.8%), 236(3.5), 208(7.9), 194(6.6), 164(2.5), 150(5.5), 135(4.1), 102(3.7), 76(3.2), 44(8.1), 35(100).



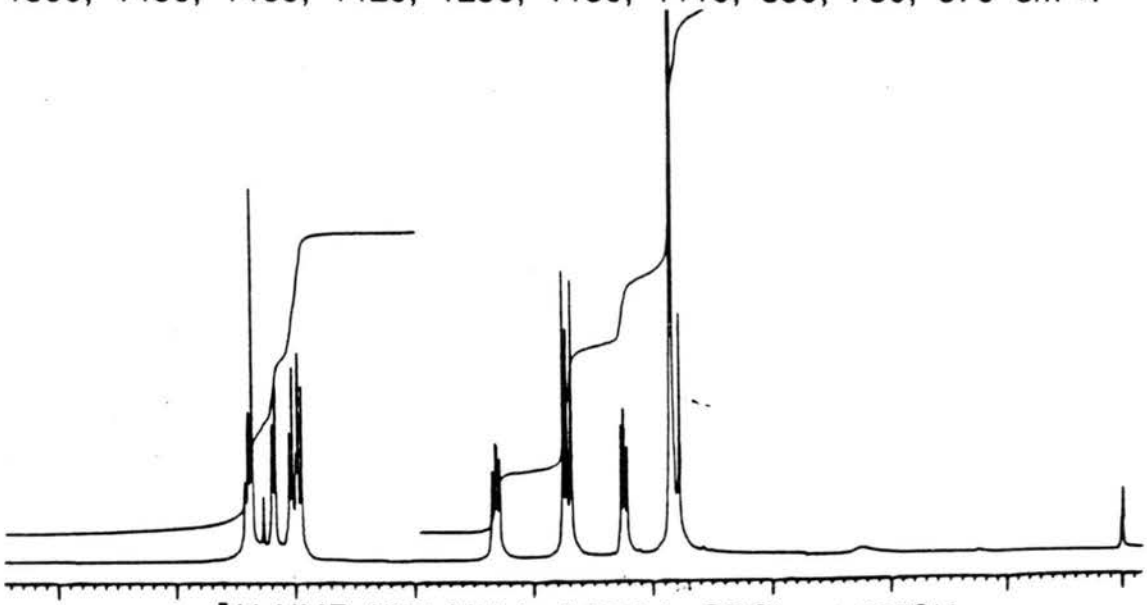
^{1}H NMR (270 MHz) of **198** in $CDCl_3$ at 295°K

198 **204**

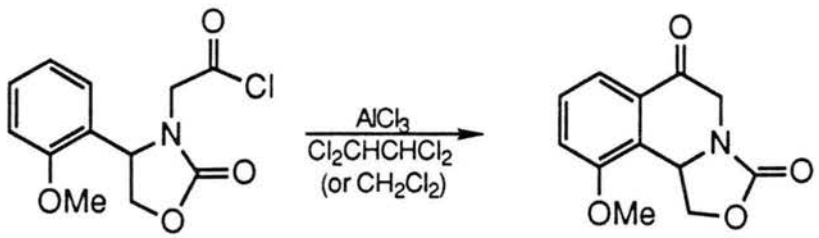
1-(carbochloro)methyl-5-(2'-methoxy)phenyloxazolidin-2-one, (**203**). To a suspension of **198** (408 mg, 1.63 mmol, 1.0 equiv) in dry benzene (8 mL) was added $SOCl_2$ (0.358 mL, 4.91 mmol, 3.02 equiv). The suspension was then heated to mild reflux for 3h and the benzene and $SOCl_2$ were evaporated under reduced pressure. The resulting light amber residue (438 mg, 100%) was used directly for the next step without purification.

^1H NMR (270 MHz, $CDCl_3$), δ(TMS): 3.78 (1/2H, s), 3.84 (3.5H, s), 4.25 (1H, dd, J=8.6Hz), 4.73 (2H, m), 5.32 (1H, dd, J=9.0Hz), 6.97 (2H, m), 7.27 (2H, m).

IR(NaCl, neat): 3060, 3020, 2940, 2830, 1800, 1760, 1600, 1590, 1490, 1460, 1420, 1250, 1180, 1110, 850, 750, 670 cm^{-1}.



^1H NMR (270 MHz) of **204** in $CDCl_3$ at 295°K



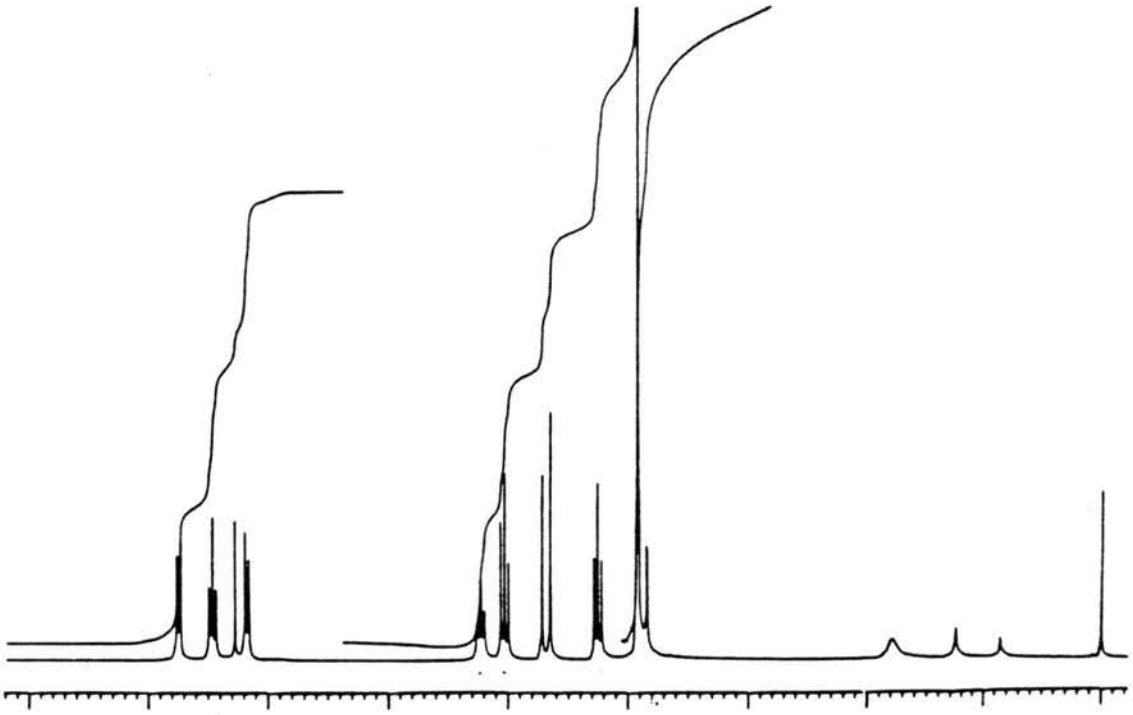
204 **195**

1-hydroxymethyl(2,2'-carbonyl)-4-keto-8-methoxytetrahydro-isoquinoline, (**195**). To a stirred solution of **204** (438 mg, 1.63 mmol, 1.0 equiv) in 16 mL dry 1,1,2,2-tetrachloroethane was added $AlCl_3$ (867 mg, 6.5 mmol, 4.0 equiv). The reaction was stirred at room temperature for 24h, poured into 40 mL ice water and acidified to pH <2 with conc. HCl. The resulting slurry was extracted with CH_2Cl_2 (4 x 20 mL) and the combined organic extracts were washed with 1M NaOH (1 x 10 mL), and brine (1 x 10 mL), dried over $MgSO_4$, filtered and evaporated to an oil which was purified by column chromatography (silica gel, 3:2 hexanes/EtOAc) yielding **195** (246 mg, 65%), mp 157-159°C (dec). (Recrystalized from EtOAc/Hexanes.)

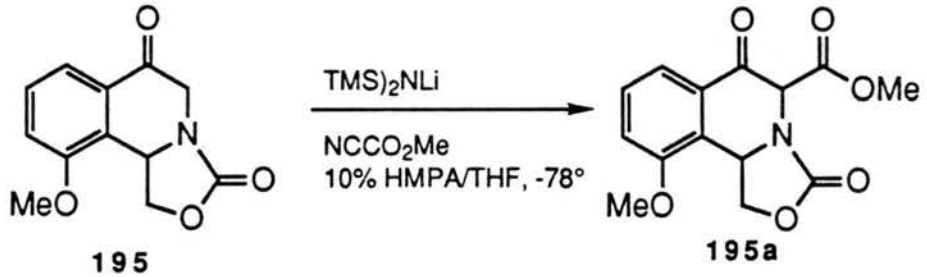
1H NMR (270 MHz, $CDCl_3$), δ(TMS): 3.83 (1/2H, s), 3.91 (3.5H, s), 4.25 (1H, t, J=8.5Hz), 4.68 (1H, d, J=18.2Hz), 5.03 (1H, t, J=8.9Hz), 5.23 (1H, t, J=8.6Hz), 7.16 (1H, dd, J=8.3Hz), 7.46 (1H, t, J=8.4Hz), 7.73 (1H, dd, J=8.1Hz).

IR(NaCl, neat): 3080, 3020, 2940, 2870, 1765, 1695, 1595, 1580, 1430, 1280, 1250, 1120, 1030, 785, 740, 670 cm-1.

Mass spectrum, CI(NH_3), m/z = 233(M+, 16.9%), 219(7.9), 189(2.1), 174(7.4), 159(2.8), 132(1.3), 35(100).



^{1}H NMR (270 MHz) of **195** in $CDCl_3$ at 295°K



195 **195a**

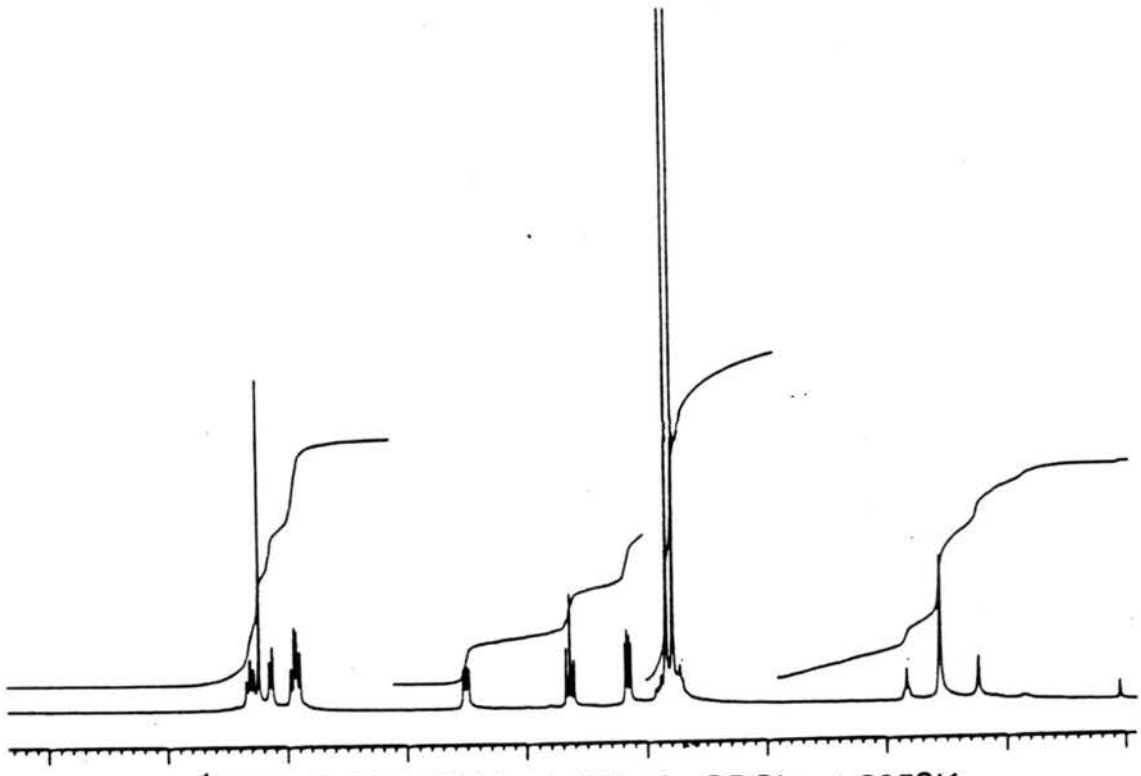
1-hydroxymethyl(2,2'-carbonyl)-3-carbomethoxy-4-keto-8-methoxytetrahydroisoquinoline, (**195a**).To a stirred solution of **195** (25 mg, 0.11 mmol, 1.0 equiv) in 1.1 mL 10% HMPA/THF cooled to -78 °C was added 120 µL lithium bis(trimethylsilyl)amide (1M solution in THF; 0.12 mmol, 1.15 equiv). This solution was allowed to stir at -78 °C for 45 min when methyl cyanoformate was added in the portion (9 mg, 0.11 mmol, 1.0 equiv). The resulting solution was allowed to stir at -78 °C for 2h, the reaction was quenched with sat. aq. NH_4Cl (25 mL), diluted with 25 mL CH_2Cl_2, and washed with H_2O

(2 x 5 mL) and brine (1 x 5 mL). The organics were then dried over $MgSO_4$, filtered, evaporated and purified (PTLC silica gel 1:1 hex:EtOAc) yielding 25 mg of the methyl β-keto ester as a colorless viscous oil (78%).

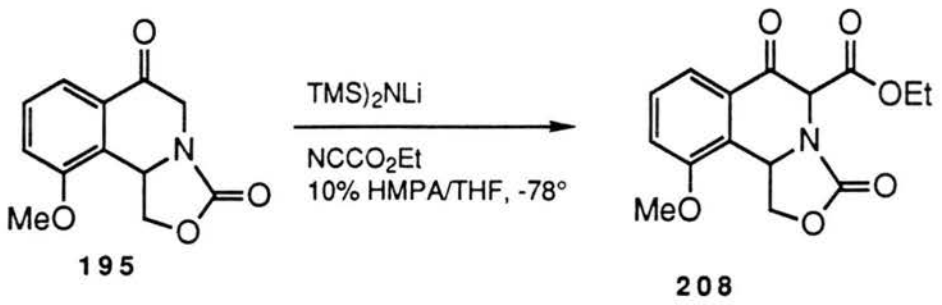
^1H NMR (270 MHz, $CDCl_3$) δ($CHCl_3$): 3.73 (3H, s), 3.86 (3H, s), 4.17 (1H, t, J = 7.1Hz), 5.07 (1H, t, J = 7.2Hz), 5.32 (1H, s), 5.54 (1H, t, J = 7.1Hz), 7.13 (1H, d, J = 5.2Hz), 7.41 (1H, t, J = 5.3Hz), 7.76 (1H, d, J = 5.2Hz).

IR(NaCl, neat): 1760, 1745, 1700, 1250 cm^{-1}.

Mass spectrum, CI(NH_3), m/e = 291 (M^+, 100%), 250 (71), 233 (48).



^1H NMR (270 MHz) of **195a** in $CDCl_3$ at 295°K



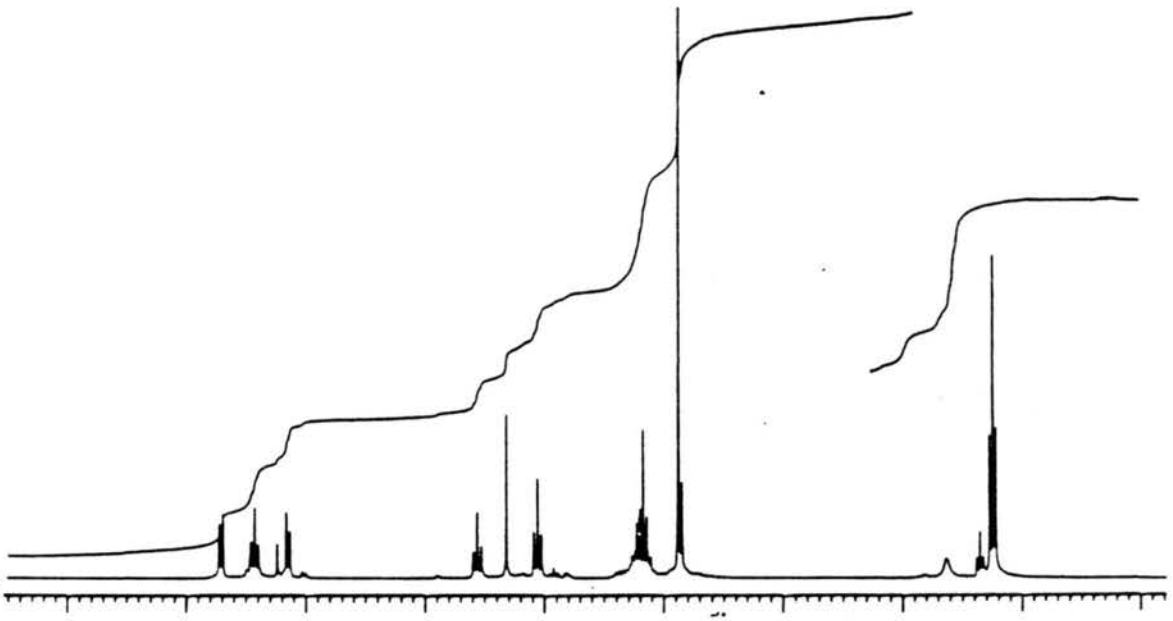
195 **208**

1-hydroxymethyl(2,2'-carbonyl)-3-carboethoxy-4-keto-8-methoxytetrahydroisoquinoline, (**208**). To a stirred solution of **195** (120 mg, 0.52 mmol, 1.0 equiv) in 8.8 mL 10% HMPA/THF cooled to -78 °C was added 0.60 mL of 1.0M lithium bis(trimethylsilyl)amide in THF (0.6 mmol, 1.15 equiv). The resulting solution was alowed to stir at -78 °C for 45 min and ethylcyanoformate (51 mg, 0.52 mmol, 1.0 equiv) was added in one portion. The reaction was allowed to stir at -78 °C for 2h, quenched with sat. aq. NH_4Cl (1.5 mL), diluted with CH_2Cl_2 (50 mL), washed with H_2O (2 x 10 mL) and brine (1 x 10 mL). The organics were then dried, ($MgSO_4$), filtered, evaporated and chromatographed (silica gel, 3:2 hex:EtOAc) yielding **208** (126 mg, 80%) as an amorphous solid. mp: 91-92°C (Recrystalized from EtOAc/hexanes). Analysis calculated for $C_{15}H_{15}NO_6$: C, 59.01; H, 4.95; N, 4.59. Found: C, 59.04; H, 5.08; N, 4.61.

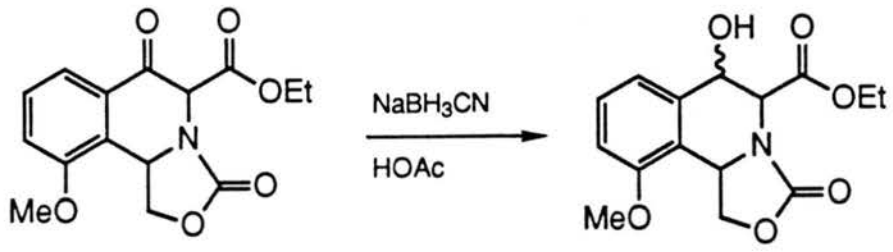
^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.24 (3H, t, *J* = 8.23 Hz), 3.87 (3H, s), 4.18 (3H, m), 5.05 (1H, t, *J* = 8.50Hz), 5.32 (1H, s), 5.57 (1H, t, *J* = 8.55Hz), 7.16 (1H, d, *J* = 7.72 Hz), 7.43 (1H, t, *J* = 8.0Hz), 7.71 (1H, d, *J* = 7.85Hz).

IR(NaCl, CH_2Cl_2): 1765, 1750, 1700, 1250 cm^{-1}.

Mass spectrum, CI(NH_3), m/e = 305 (100), 233 (12).



^{1}H NMR (270 MHz) of **208** in $CDCl_3$ at 295°K



208 **209**

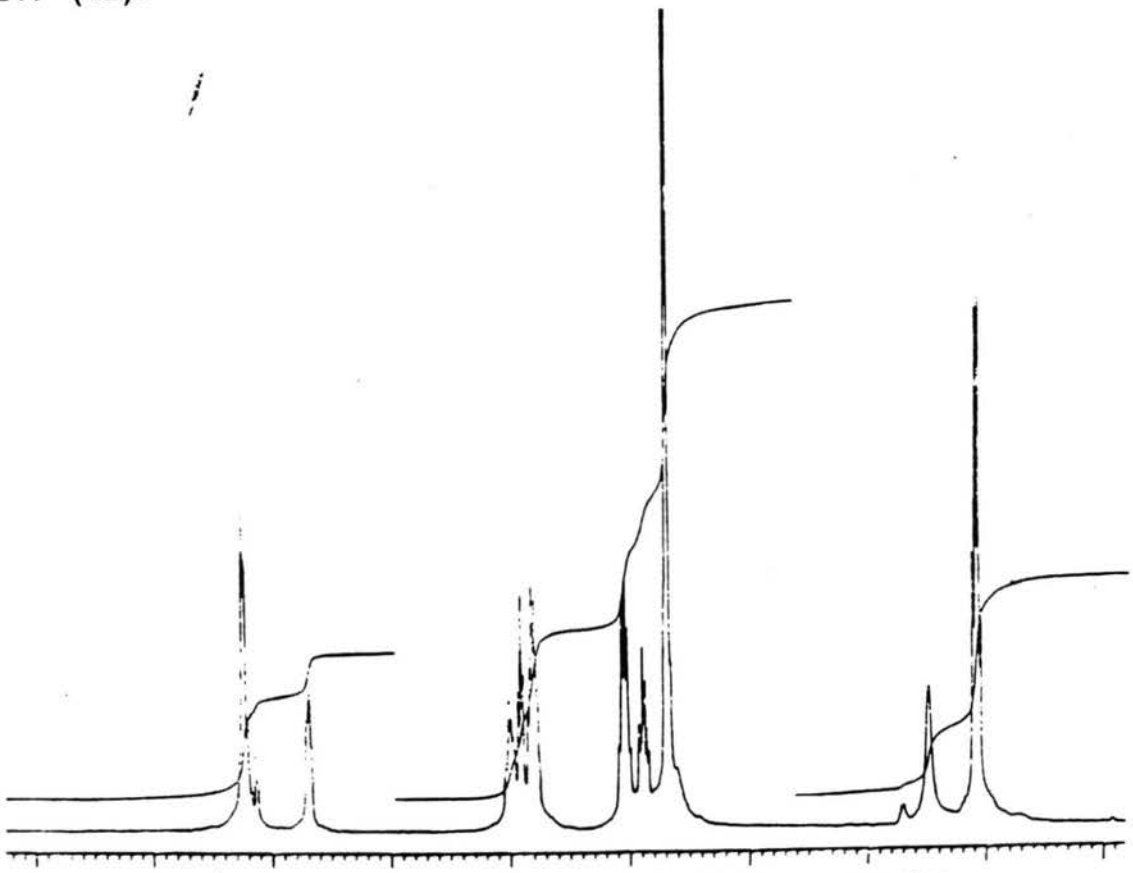
1-hydroxymethyl(2,2'-carbonyl)-3-carboethoxy-4-hydroxy-8-methoxytetrahydroisoquinoline, (**209**). To a stirred solution of **208** (125 mg, 0.41 mmol, 1.0 equiv) in 4 mL acetic acid (glacial) was added $NaBH_3CN$ (27 mg, 0.43 mM, 1.05 equiv). The reaction was allowed to stir for 3h, diluted with CH_2Cl_2 (60 mL) washed with H_2O (2 x 15 mL), brine (1 x 15 mL), dried over $MgSO_4$, filtered and evaporated yielding **209** (120 mg, 95%) as a white foam which was crystallized from EtOAc/hexanes. mp: 127-128°C. Analysis

calculated for $C_{15}H_{17}NO_6$: C, 58.62; H, 5.58; N, 4.56. Found: C, 58.80; H, 5.71; N, 4.60.

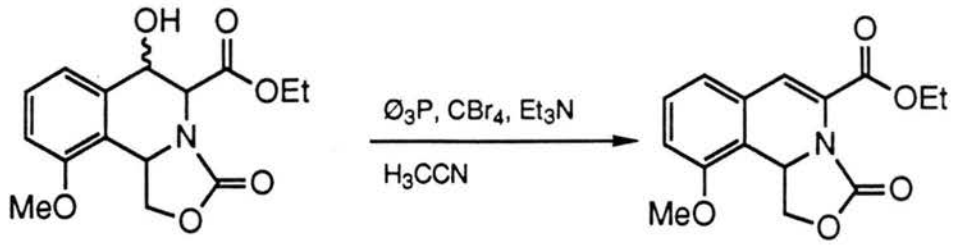
^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.08 (3H, m), 1.49 (1H, bs), 3.68 (1.5H, s), 3.70 (1.5H, s), 3.85 (1H, m), 4.05(2H, m), 4.78 (2H,), 4.87 (1H, m), 4.97 (1H, m), 6.67 (1H, m), 7.20 (2H, m).

IR(NaCl, neat): 3450, 1760, 1735, 1250 cm^{-1}.

Mass spectrum, CI(NH_3), m/e = 307 (100), 289 (71), 234 (21), 217 (42).



^1H NMR (270 MHz) of **209** in $CDCl_3$ at 295°K

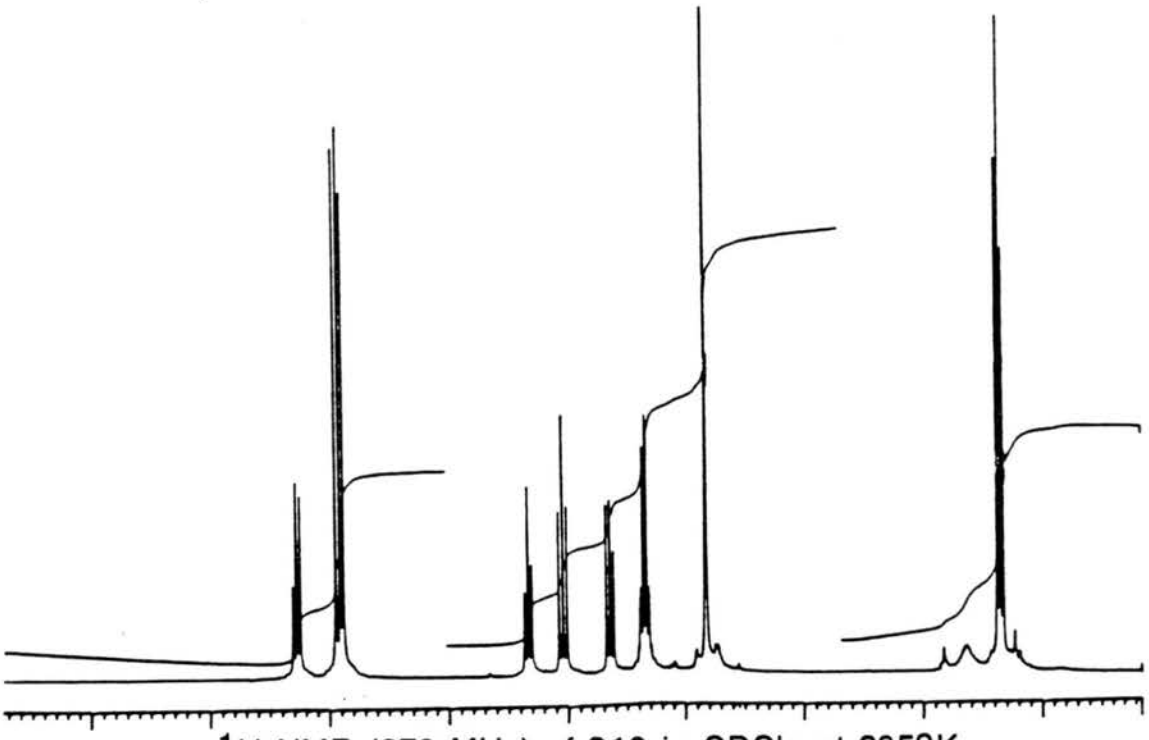


209 **210**

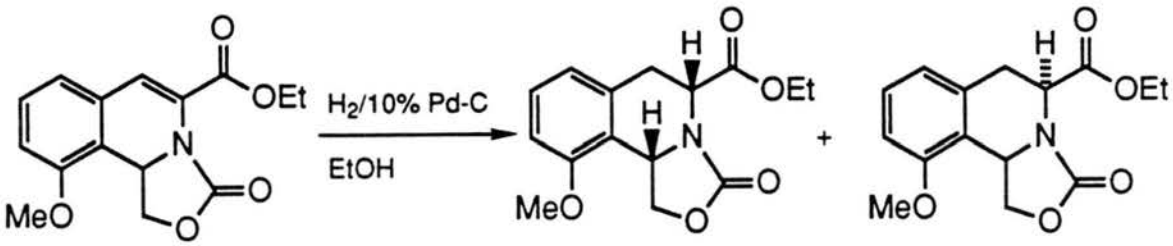
1-hydroxymethyl(2,2'-carbonyl)-3-carboethoxy-8-methoxy-1,2-dihydroisoquinoline, (**210**). To a stirred solution of **209** (120 mg, 0.39 mmol, 1.0 equiv) in 4 mL acetonitrile was added Et_3N (163 μL, 1.1726 mM, 3.0 equiv), CBr_4 (390 mg, 1.17 mmol, 3.0 equiv) and triphenylphosphine (310 mg, 1.17 mmol, 3.0 equiv). The resulting orange reaction mixture was allowed to stir at room temperature for 18h, diluted with CH_2Cl_2 (80 mL), washed with H_2O (3 x 15 mL), 1M HCl (1 x 15 mL), brine (1 x 15 mL), dried over $MgSO_4$, filtered, evaporated, and chromatographed (silica gel 10% EtOH/benzene) yielding **210** (90 mg, 78%) as a crystalline solid mp: 110-112°C (recrystallized from EtOAc/hexanes). Analysis calculated for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.16; H, 5.21; N, 4.80.

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.34 (3H, t, J = 7.1Hz), 3.82 (3H, s), 4.34 (2H, qq, J = 1.9Hz), 4.64 (1H, dd, J = 8.3Hz), 5.04 (1H, t, J = 9.0Hz), 5.32 (1H, t, J = 8.4Hz), 6.89 (2H, d, J = 8.0Hz), 6.94 (1H, s), 4.24 (1H, dd, J = 8.0Hz).

IR(NaCl, CH_2Cl_2): 1765, 1725, 1635 cm^{-1}.



^{1}H NMR (270 MHz) of **210** in $CDCl_3$ at 295^{o}K



210 **211** **212**

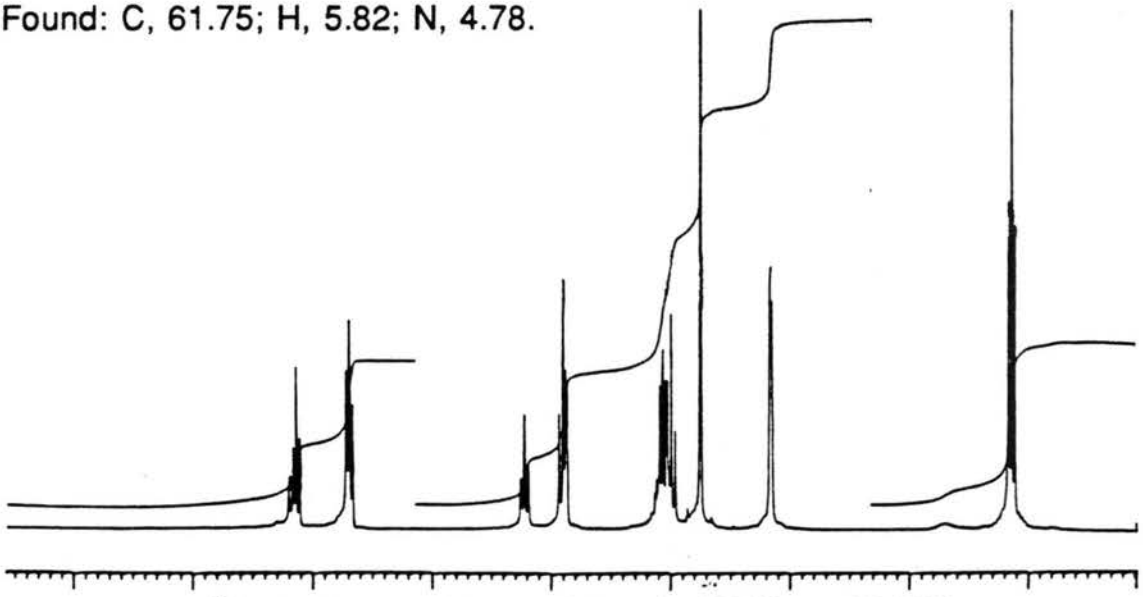
Cis-1-hydroxymethyl(2,2'-carbonyl)-3-carboethoxy-8-methoxy tetrahydroisoquinoline, (**211**) and *trans-1-hydroxymethyl (2,2'-carbonyl)-3-carboethoxy-8-methoxy tetrahydroisoquinoline*, (**212**). To a stirred solution of **210** (60 mg, 0.17 mmol, 1.0 equiv) in 3 mL absolute EtOH was added 10% Pd-C (46 mg, 0.04 mmol, 0.25 equiv). The resulting suspension was purged with hydrogen then hydrogenation was continued for 4h at one atmosphere H_2 pressure (balloon). The suspension was then filtered over celite, evaporated

and separated (PTLC, silica gel, 10% EtOHc/benzene) yielding two diasteroemers in a 10:1 ratio , 50 mg **211** and 5 mg **212** (99% combined yield).

Major diastereomer (*cis*) mp: 95-96°C (recrystallized from EtOAc/hexanes): ^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.24 (3H, tt, *J* = 7.0Hz, 1.2Hz), 3.0 (1H, dd, *J* = 16.3Hz, 4.8Hz), 3.30 (1H, dd, *J* = 16.3Hz, 8.3Hz), 3.80 (3H, s), 4.20 (3H, m), 4.38 (1H, t, *J* = 6.5Hz), 4.83 (1H, t, *J* = 7.0Hz), 5.05 (1H, t, *J* = 7.1Hz), 6.77 (2H, m), 7.231 (1H, m).

IR (NaCl, neat): 1760, 1740 cm^{-1}.

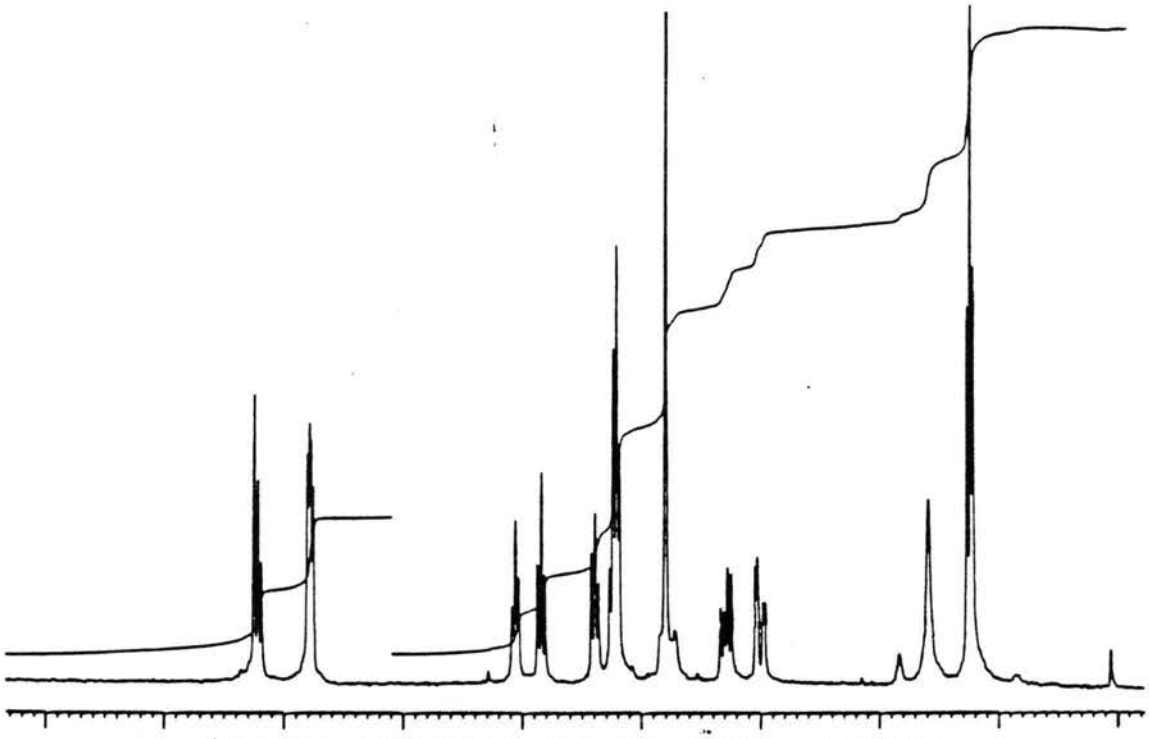
Analysis calculated for $C_{15}H_{17}NO_5$: C, 61.84; H, 5.88; N, 4.81. Found: C, 61.75; H, 5.82; N, 4.78.



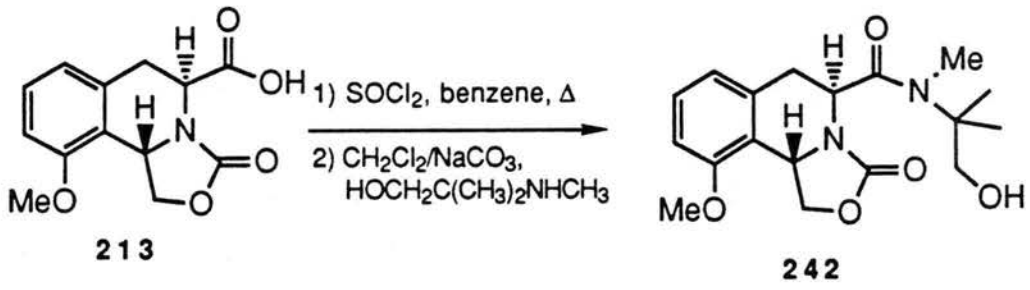
^{1}H NMR (270 MHz) of **211** in $CDCl_3$ at 295°K

Minor diastereomer (*trans*): ^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.14 (3H, t, *J* = 7.2Hz), 3.15 (2H, d, *J* = 4.1Hz), 3.74 (3H, s), 4.05 (3H, m), 4.87 (2H, m), 5.21 (1H, t, *J* = 8.9Hz), 6.67 (2H, m), 7.12 (1H, m).

IR(NaCl, neat): 1760, 1740 cm^{-1}.



^{1}H NMR (270 MHz) of **212** in $CDCl_3$ at 295^{o}K



213 **242**

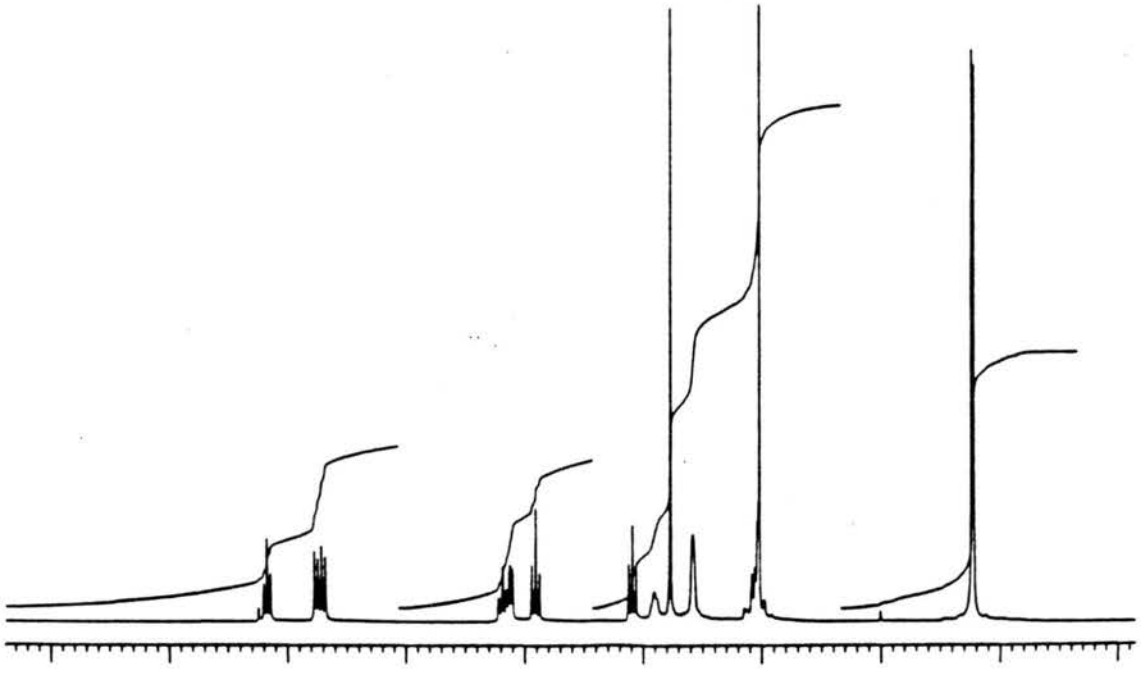
trans-1-hydroxymethyl(2,2'-carbonyl)-3-(N-methyl-N-2,2-dimethylethoxy)carboxamide-8-methoxytetrahydroisoquinoline, (**242**). To a stirred suspension of acid (**213**), (500 mg, 1.90 mmol, 1.0 eq.) in 20 mL dry benzene was added thionyl chloride (680 mg, 5.70 mmol, 3.0 eq) in one portion. The resulting suspension was refluxed for 3h. The resulting solution was cooled to room temperature and evaporated to a solid. The solid (crude) acid chloride was taken up in 40mL methylene chloride to which was

added 30 mL of sat. $NaHCO_3$ followed by amine (200 mg, 1.90 mmol, 1.0 eq.). The reaction was stirred vigorously for 2 h, diluted with 50 mL methylene chloride, separated, dried over $MgSO_4$ and evaporated to an off white solid Recrystalization from ethylacetate/hexanes yielded 520 mg (78%) of the amide (**242**). Mp:138-140°C.

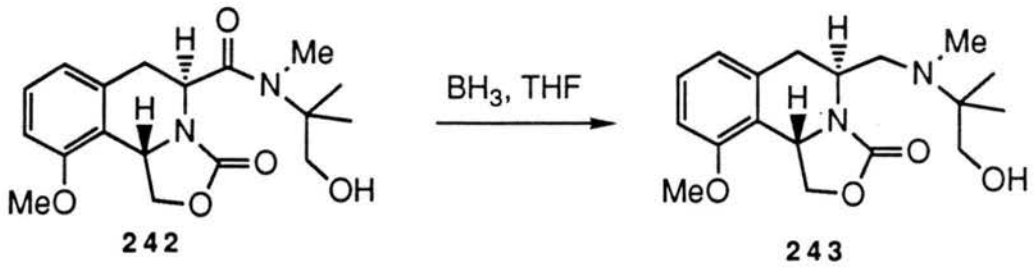
^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.22 (s, 3H), 1.23 (s, 3H), 3.02 (s, 3H), 3.06 (m, 1H), 3.59 (bs, 2H), 3.78 (s, 3H), 3.91 (m, 1H), 4.09 (t, 1H, *J*=8.7Hz), 4.90 (t, 1H, *J*=8.8Hz), 5.11 (dd, 1H, *J*=4.6, 7.5Hz), 5.18 (t, 1H, *J*=8.6Hz), 6.68 (d, 1H, *J*=8.2), 6.75 (d, 1H, *J*=8.2Hz), 7.16 (t, 1H, *J*=7.9Hz).

IR (NaCl, neat): 3459, 1745, 1648, 1591, 1084, 731 cm^{-1}.

Mass spectrum (CI, NH_3): m/e 349(M+1), 331, 218.



^1H NMR (270 MHz) of **242** in $CDCl_3$ at 295°K



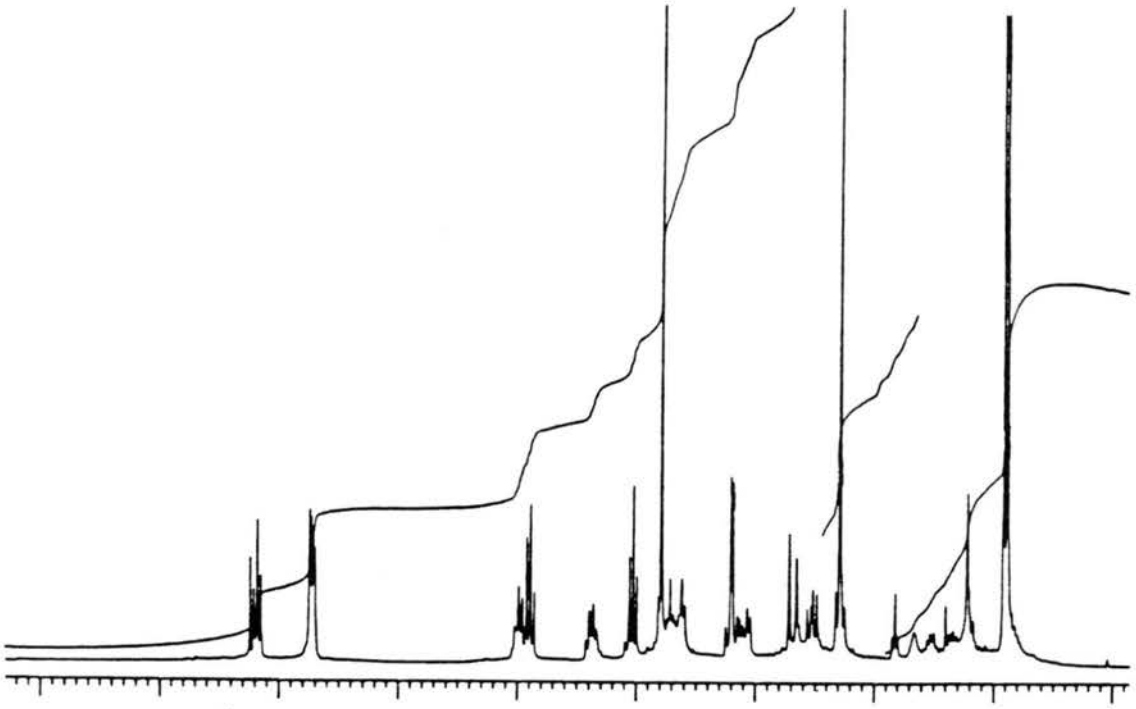
242 **243**

trans-1-hydroxymethyl(2,2'-carbonyl)-3-(N-methyl-N-2,2-dimethylethoxy)aminomethyl-8-methoxytetrahydroisoquinoline, (**243**).To a stirred solution of amide (**242**), (600 mg, 1.72 mmol, 1.0 eq) in 15mL of dry THF, under a nitrogen atmosphere, was added a 1M solution of borane in THF (3.4 mL, 3.44 mmol, 2.0 eq). The resulting colorless solution was allowed to stir at room temperature for 4h, quenched with 1M NH_4CO_3, and diluted with 50 mL methylene chloride. The organic layer was separated and washed with water (10 mL) and brine. The organic extracts were then dried over $MgSO_4$, filtered, and evaporated to a colorless oil. 420 mg (73%).

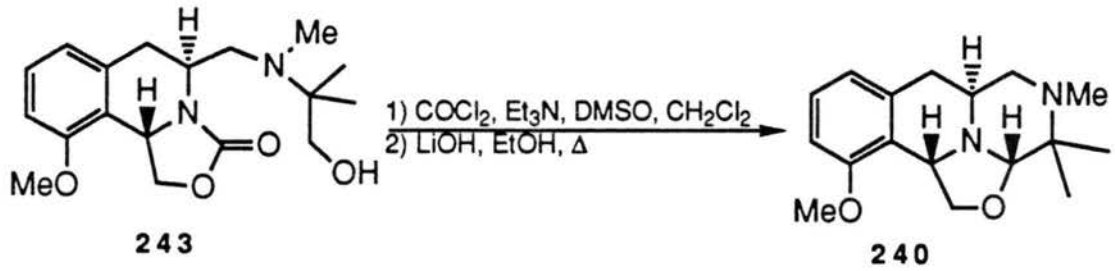
^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 0.89 (s, 3H), 0.92 (s, 3H), 2.29 (s, 3H), 2.31 (m, 1H), 2.53 (dd, 1H, *J*=8.8, 13.1Hz), 2.71 (d, 1H, *J*=12.9Hz), 3.18 (m, 3H), 4.10 (t, 1H, *J*=7.9Hz), 4.39 (m, 1H), 4.90 (t, 1H, *J*=8.1Hz), 4.06 (t, 1H, *J*=8.1Hz), 6.71 (d, 1H, *J*=4.1Hz), 6.74 (d, 1H, *J*=3.6Hz), 7.18 (t, 1H, *J*=8.0Hz).

IR (NaCl, neat): 3455, 1751, 1587, 1077, 778, 744 cm^{-1}.

Mass spectrum (CI, NH_3): m/e 335 (M+1), 333 (M-1), 303, 263.



^{1}H NMR (270 MHz) of **243** in $CDCl_3$ at 295^{o}K



243 **240**

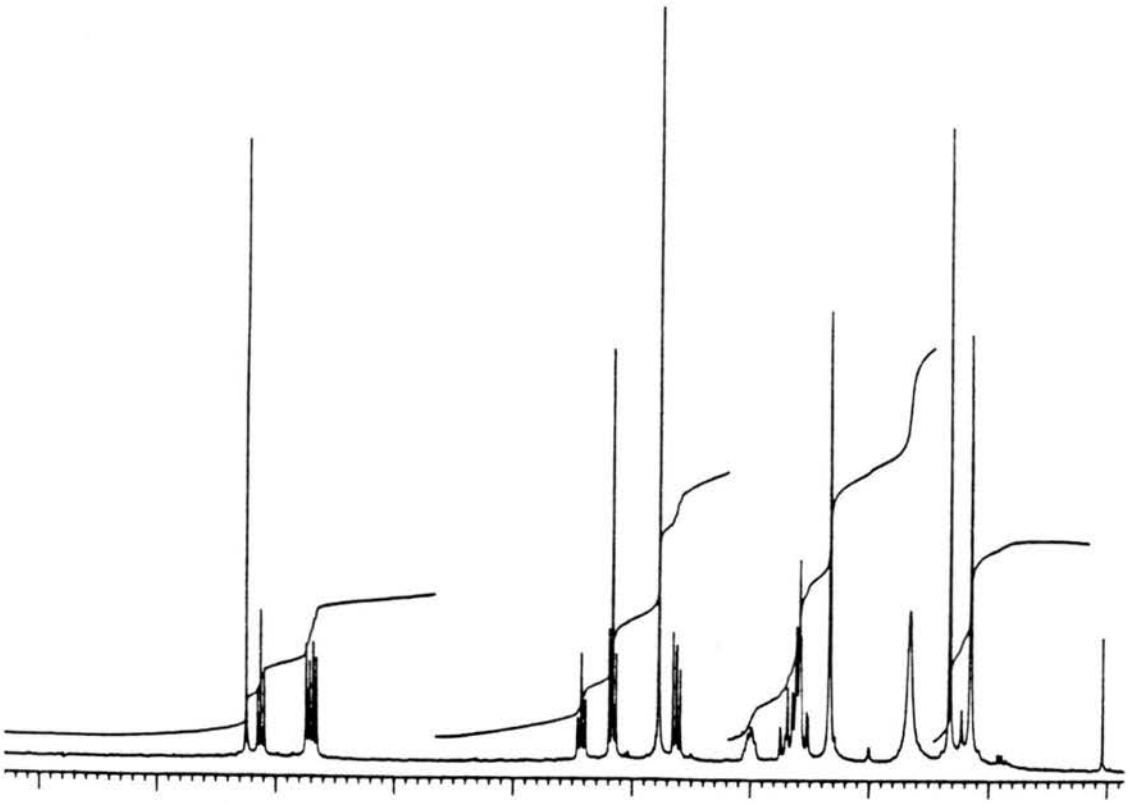
Tetracyclic oxazolidine, (**240**).To a stirred solution of DMSO, (9 μL, 0.13 mmol, 3.0 eq.) in 0.3 mL dry methylene chloride, cooled to -78 °C, was added oxalyl chloride, (6 μL, 0.06 mmol, 1.5 eq.) in one portion. The resulting colorless solution was allowed to stir at -78 °C for 15 min. A solution of alcohol (**243**), (14 mg, 0.04 mmol, 1.0 eq) in 0.2 mL dry methylene chloride was added in one portion. The flask containing the alcohol **243** was rinsed with another 0.1 mL dry methylene chloride and added to the reaction solution. The reaction was stirring at -78°C for 45 min., and quenched with triethylamine

(29 μL, 0.210 mmol, 5.0 eq.). After stirring at -78°C for 30 min. the reaction suspension was filtered thru a plug of silica gel (prewetted with methylene chloride), and rinsed with 2.0 mL dry methylene chloride. The resulting colorless filtrate was diluted with 2.0 mL dry ethanol and the methylene chloride was carefully evaporated, not letting the temperature exceed room temperature, while adding more ethanol making sure not to let the solvent evaporate completely. When all the methylene chloride was exchanged for ethanol a total of 5.0 mL of ethanol was present. To this colorless solution was added 1M LiOH (0.420 mL, 0.420 mmol, 10 eq.) and the resulting light yellow solution was refluxed for 6h and diluted with methylene chloride (25mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered, evaporated and purified (PTLC silica gel 5% methanol/ethylacetate, then HPLC 5% methanol ethyl acetate) yielding 3 mg (25%) of oxazolidine **240**.

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.16 (s, 3H), 2.65 (m, 4H), 3.00 (m, 1H), 3.62 (dd, 1H, *J*=7.3, 6.3Hz), 3.77 (s, 1H), 4.16 (t, 2H, *J*=7.4Hz), 4.42 (t, 1H, *J*=7.7Hz), 6.71 (d, 1H, *J*=7.5Hz), 6.74 (d, 1H, *J*=7.6Hz), 7.18 (t, 1H, *J*=7.9Hz).

IR (NaCl, neat): 2886, 2838, 2796, 2774, 1588, 1473, 1260, 1018, 786, 744 cm^{-1}.

Mass spectrum (CI, NH_3): m/e 327 (M+1), 363, 160.



^{1}H NMR (270 MHz) of **240** in $CDCl_3$ at 295^{o}K

CHAPTER 6

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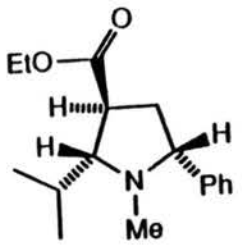
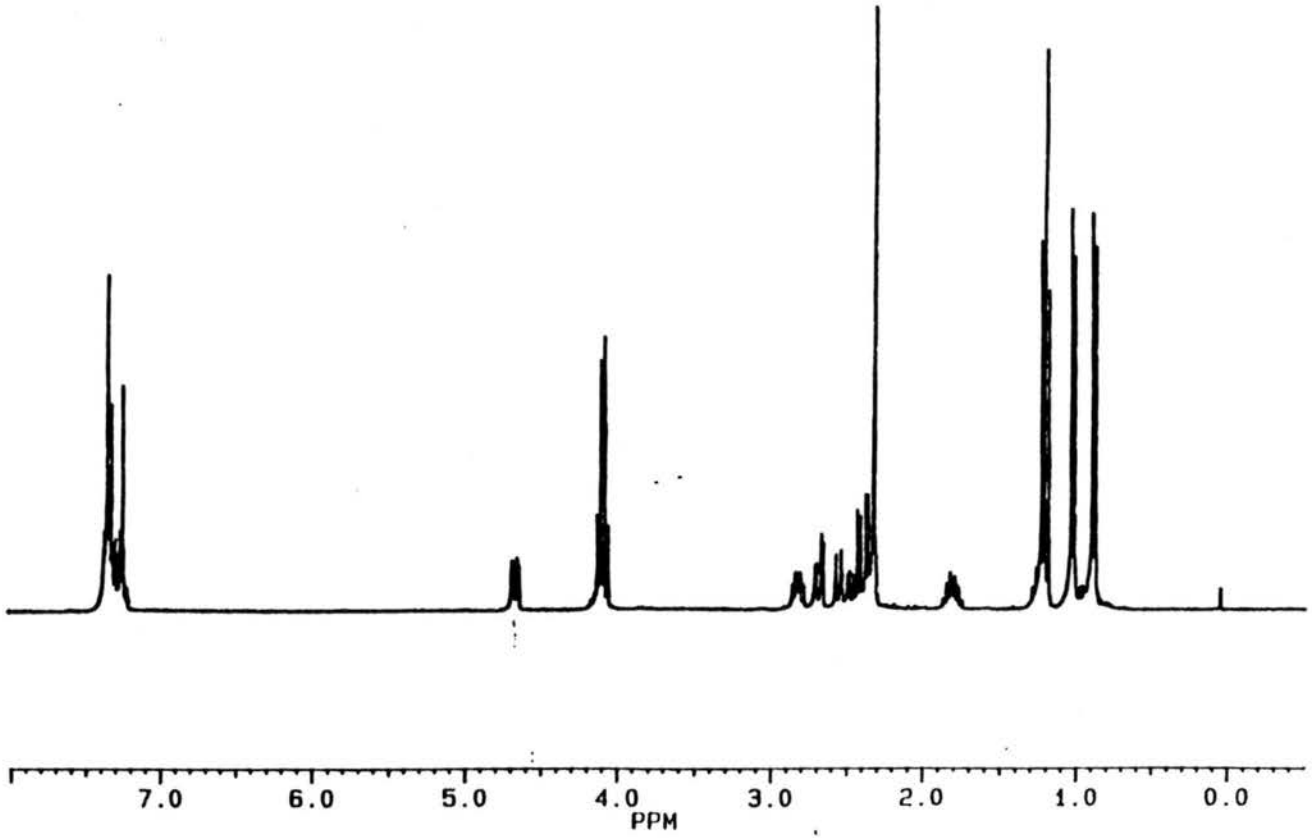
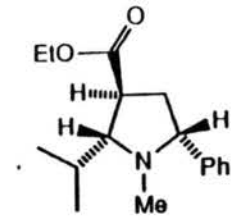
APPENDIX I



182

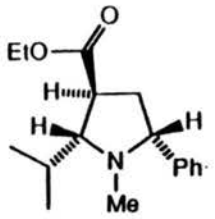
'H NMR spectra of **182** (360 MHz) obtained in $CDCl_3$ at 25°C





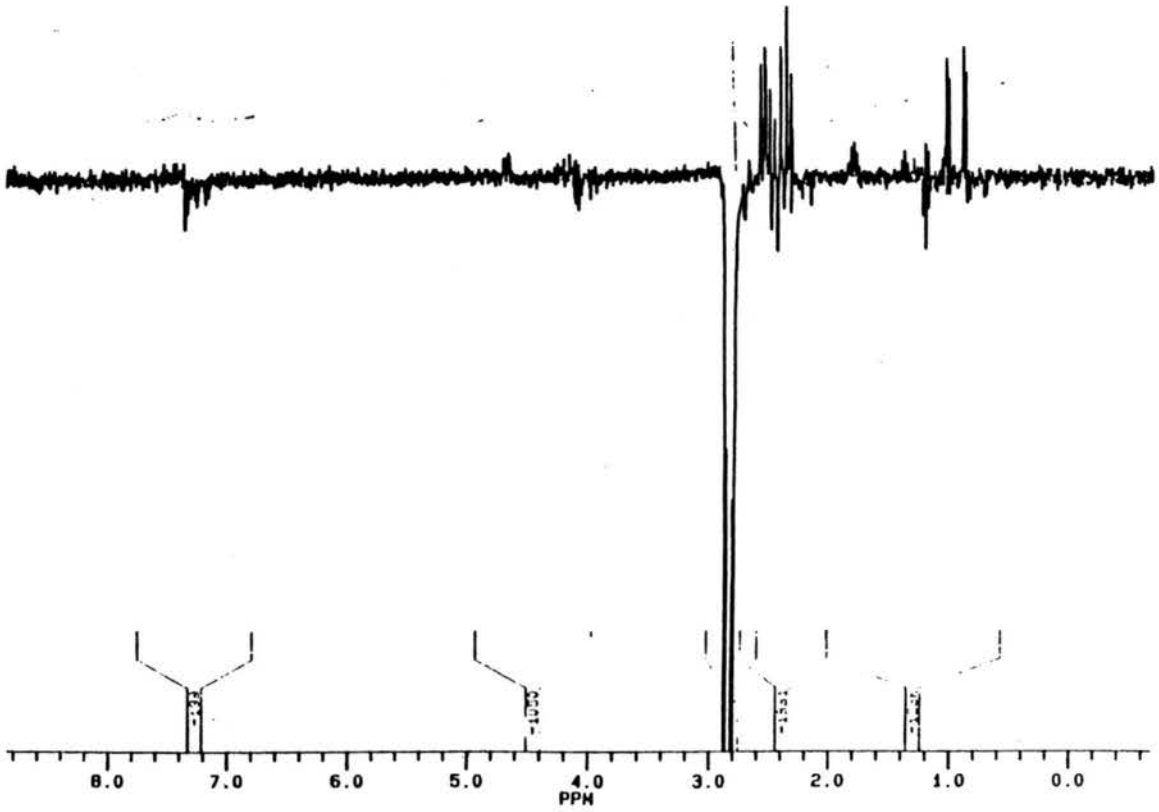
182

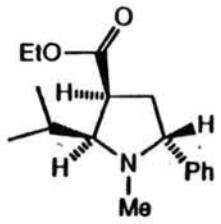
'H NMR NOE difference spectrum obtained upon irradiation of **182** at the δ 4.70 resonance



182

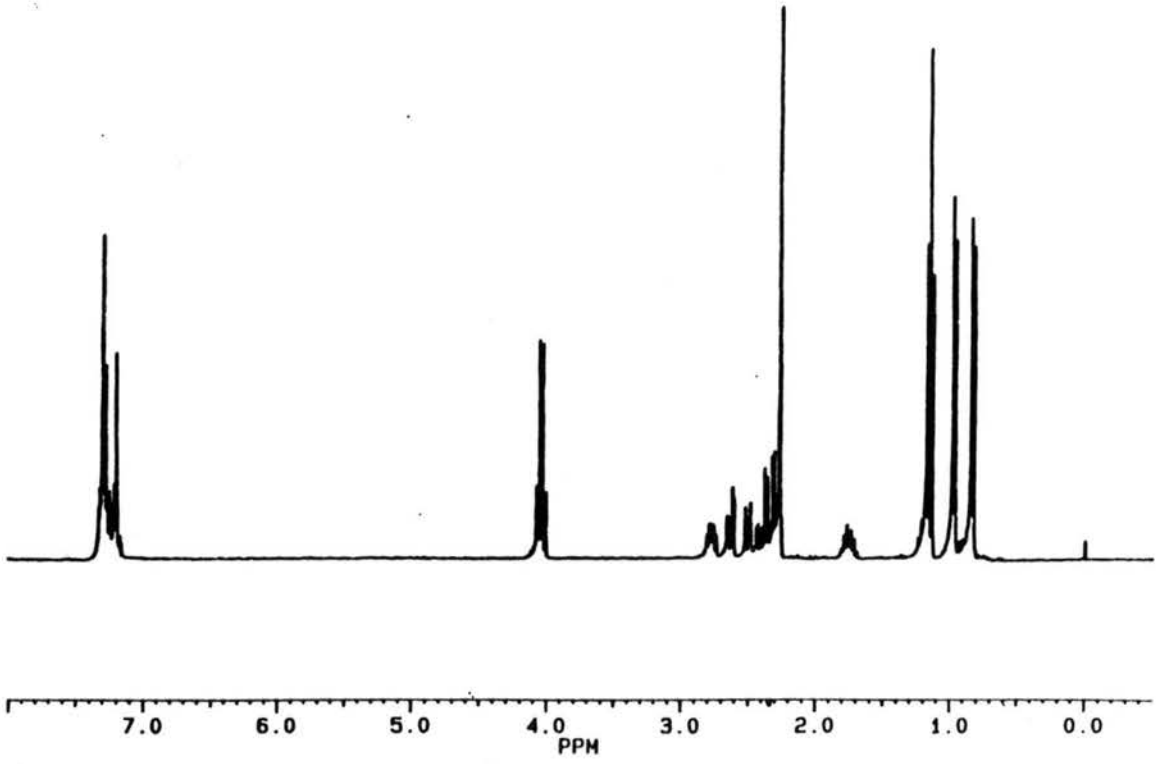
'H NMR NOE difference spectrum obtained upon irradiation of **182** at the δ 2.87 resonance

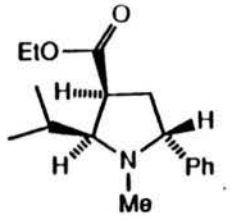




183

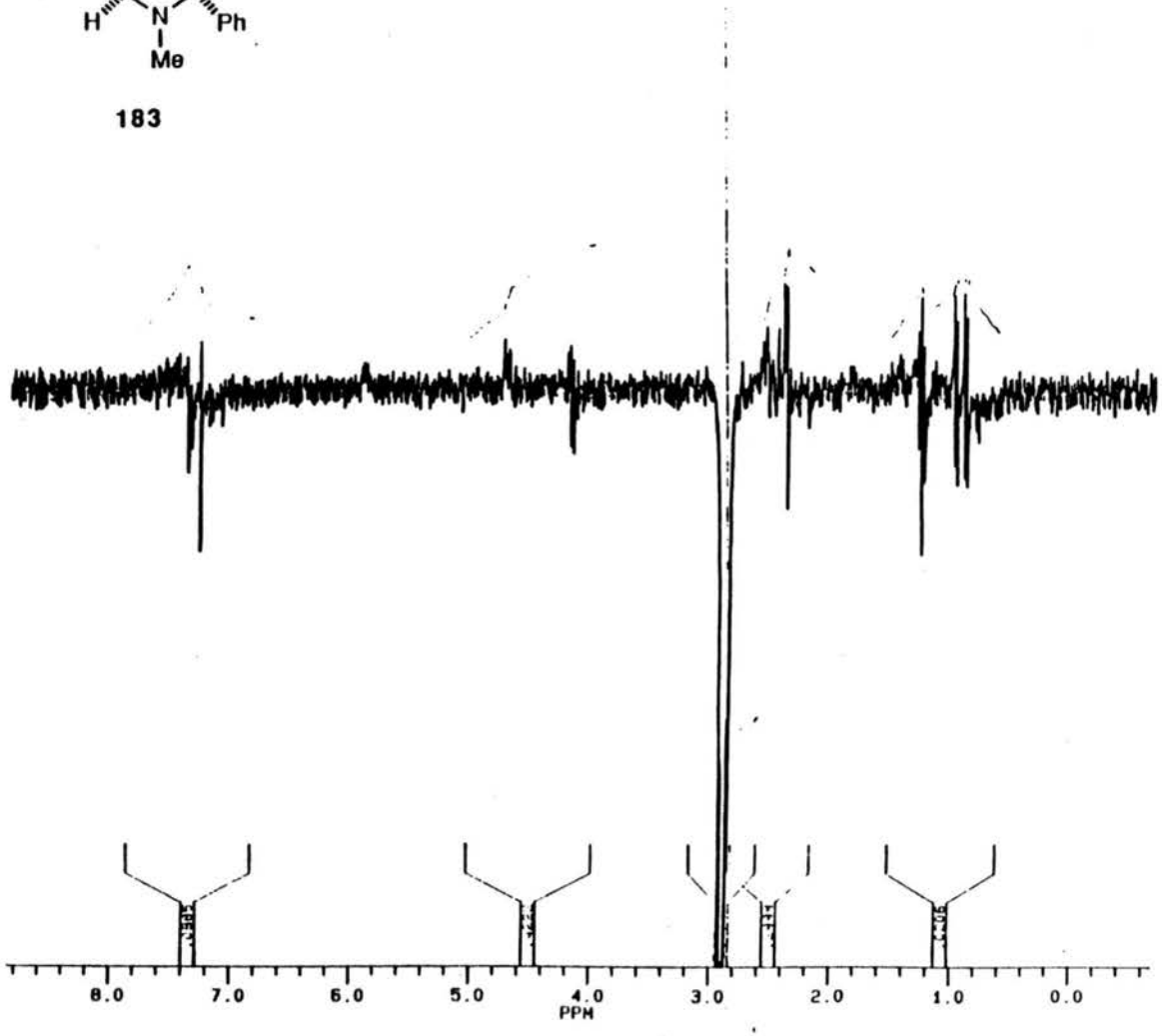
'H NMR spectra of **183** (360 MHz) obtained in $CDCl_3$ at 25°C

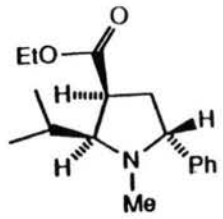




183

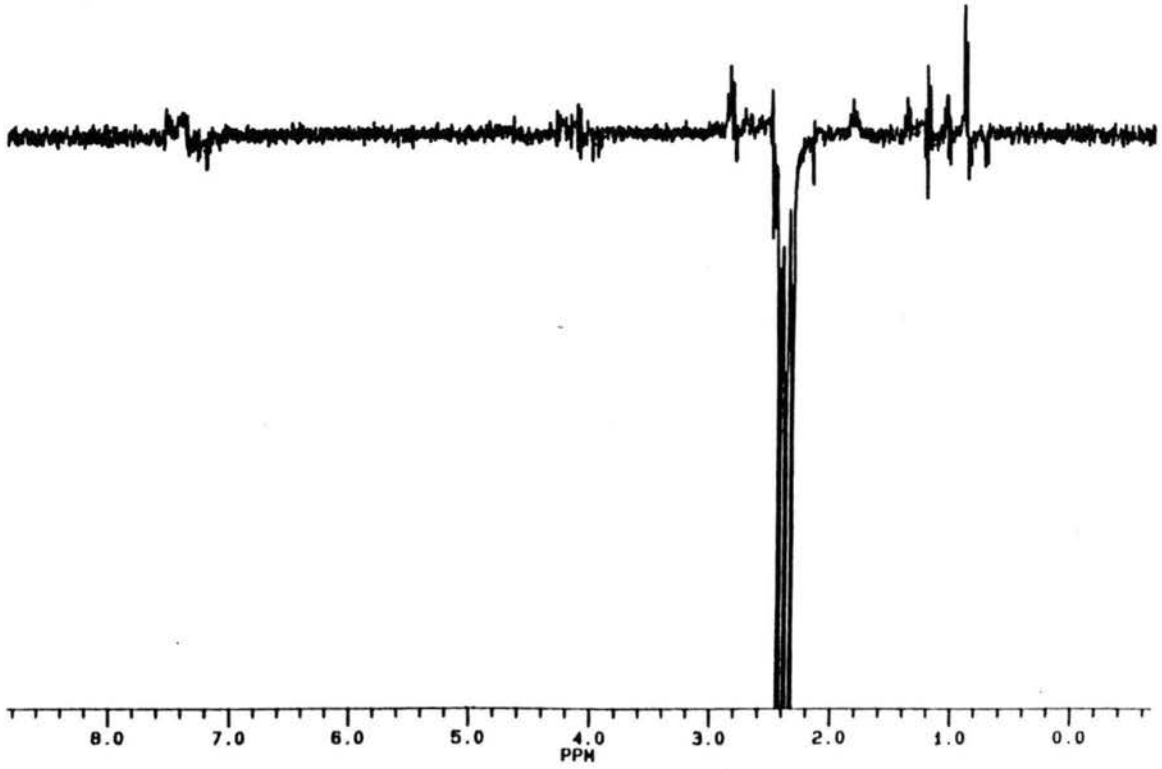
'H NMR NOE difference spectrum obtained upon irradiation of **183** at the δ 2.92 resonance

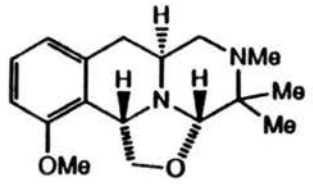




183

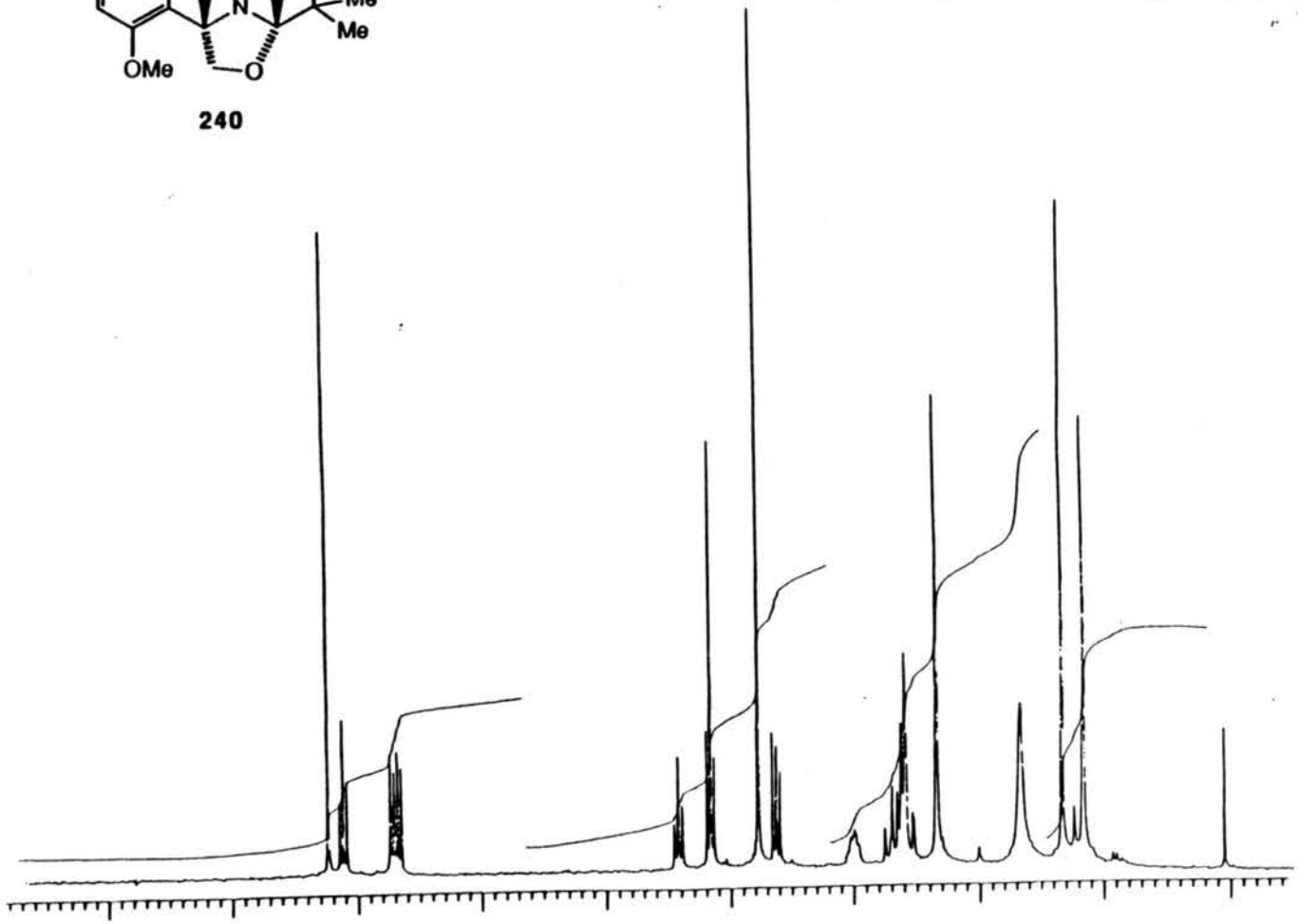
'H NMR NOE difference spectrum obtained upon irradiation of **183** at the δ 2.20 resonance

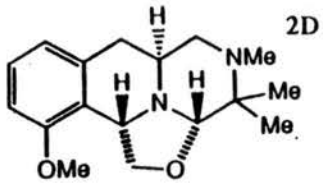




240

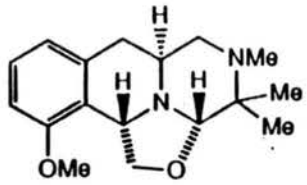
'H NMR spectra of **240** (360 MHz) obtained in $CDCl_3$ at 25°C





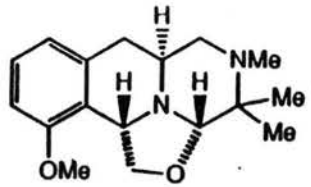
240

2D Heteronuclear Corralation spectra (360 MHz) of **240** at 25°C



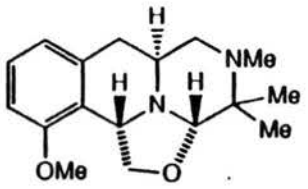
240

^{1}H NMR NOE difference spectrum obtained upon irradiation of 240 at the δ 4.42 resonance



240

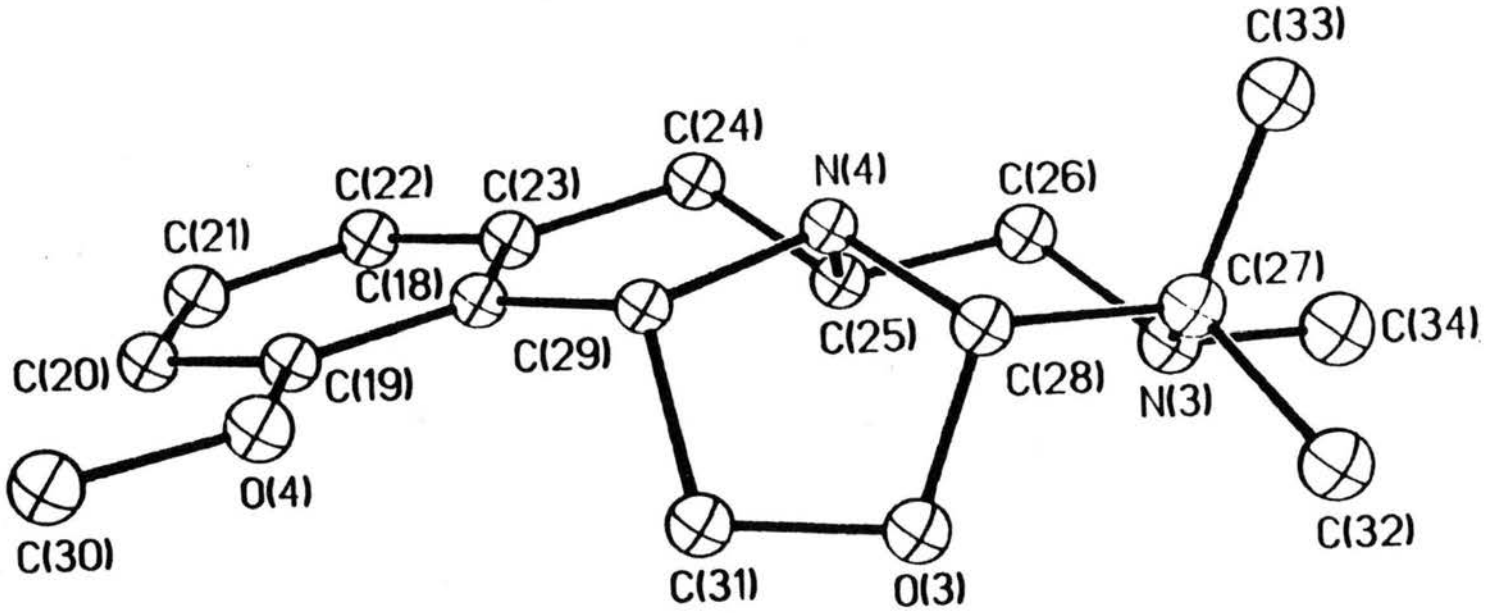
'H NMR NOE difference spectrum obtained upon irradiation of 240 at the δ 3.77 resonance



240

^{1}H NMR NOE difference spectrum obtained upon irradiation of 240 at the δ 4.16 resonance

APPENDIX II



X-ray stereostructure of **240**. Spheres are of fixed, arbitrary radii. Hydrogen atoms have been ommitted for clarity.

TABLE 1 Atomic coordinates ($\times 10^4$) and isotropic thermal parameters ($Å^2 \times 10^3$)[a] for $C_{17}H_{24}N_2O_2$

atom	x	y	z	U_{iso}[b]
C1	10641(2)	8316(2)	2293(2)	21(1)*
C2	9948(2)	8834(2)	2656(2)	23(1)*
C3	9373(2)	9360(2)	2054(2)	28(1)*
C4	9511(3)	9371(2)	1054(2)	32(1)*
C5	10194(2)	8877(2)	676(2)	29(1)*
C6	10760(2)	8338(2)	1285(2)	23(1)*
C7	11451(2)	7747(2)	881(2)	26(1)*
C8	11509(2)	7027(2)	1515(2)	21(1)*
C9	12150(3)	6382(2)	1141(2)	29(1)*
C10	12603(2)	5885(2)	2819(2)	24(1)*
C11	12046(2)	6590(2)	3184(2)	21(1)*
C12	11215(2)	7743(2)	2985(2)	20(1)*
C13	9195(3)	9268(2)	4090(3)	41(1)*
C14	10500(2)	7153(2)	3441(2)	24(1)*
C15	12410(3)	5202(2)	3487(2)	32(1)*
C16	13784(2)	6061(2)	2910(2)	32(1)*
C17	12600(3)	5037(2)	1356(3)	40(1)*
N1	12157(2)	5714(1)	1793(2)	26(1)*
N2	11966(2)	7256(1)	2520(2)	19(1)*
O1	11006(2)	6419(1)	3385(1)	24(1)*
O2	9891(2)	8764(1)	3661(1)	29(1)*
C18	5655(2)	9340(2)	2412(2)	20(1)*
C19	4952(2)	8800(2)	2713(2)	20(1)*
C20	4385(2)	8319(2)	2041(2)	23(1)*
C21	4551(2)	8360(2)	1050(2)	27(1)*
C22	5253(2)	8883(2)	740(2)	24(1)*
C23	5809(2)	9379(2)	1416(2)	21(1)*
C24	6541(2)	9972(2)	1086(2)	23(1)*
C25	6614(2)	10655(2)	1789(2)	21(1)*
C26	7317(2)	11297(2)	1511(2)	26(1)*
C27	7660(2)	11726(2)	3228(2)	23(1)*
C28	7075(2)	11002(2)	3499(2)	21(1)*
C29	6232(2)	9865(2)	3175(2)	18(1)*
C30	4223(3)	8221(2)	4069(2)	30(1)*
C31	5511(2)	10435(2)	3666(2)	24(1)*
C32	7423(3)	12380(2)	3922(2)	31(1)*

TABLE 1 (continued)

C33	8841(2)	11544(2)	3393(2)	31(1)*
C34	7782(3)	12625(2)	1832(3)	39(1)*
N3	7284(2)	11942(1)	2197(2)	25(1)*
N4	7013(2)	10370(1)	2788(2)	19(1)*
O3	6021(2)	11171(1)	3660(1)	24(1)*
O4	4874(2)	8794(1)	3712(1)	25(1)*

(a) Estimated standard deviations in the least significant digits are given in parentheses.

(b) For values with asterisks, the equivalent isotropic U is defined as 1/3 of the trace of the U_{ij} tensor.

TABLE 2 Bond lengths (Å)[a] for $C_{17}H_{24}N_2O_2$

C1-C2	1.394(4)	C1-C6	1.399(4)
C1-C12	1.503(4)	C2-C3	1.381(4)
C2-O2	1.384(4)	C3-C4	1.393(5)
C4-C5	1.369(4)	C5-C6	1.395(4)
C6-C7	1.500(4)	C7-C8	1.512(4)
C8-C9	1.510(4)	C8-N2	1.480(3)
C9-N1	1.455(4)	C10-C11	1.525(4)
C10-C15	1.529(4)	C10-C16	1.541(4)
C10-N1	1.479(4)	C11-N2	1.461(3)
C11-O1	1.427(4)	C12-C14	1.549(4)
C12-N2	1.477(4)	C13-O2	1.422(4)
C14-O1	1.432(3)	C17-N1	1.458(4)
C18-C19	1.394(4)	C18-C23	1.395(4)
C18-C29	1.509(4)	C19-C20	1.381(4)
C19-O4	1.376(3)	C20-C21	1.392(4)
C21-C22	1.378(4)	C22-C23	1.395(4)
C23-C24	1.496(4)	C24-C25	1.515(4)
C25-C26	1.507(4)	C25-N4	1.483(3)
C26-N3	1.458(4)	C27-C28	1.526(4)
C27-C32	1.526(4)	C27-C33	1.544(4)
C27-N3	1.481(4)	C28-N4	1.456(4)
C28-O3	1.429(3)	C29-C31	1.555(4)
C29-N4	1.473(4)	C30-O4	1.419(4)
C31-O3	1.433(3)	C34-N3	1.456(4)

(a) Estimated standard deviations in the least significant digits are given in parentheses.

TABLE 3 Bond angles (deg)[a] for $C_{17}H_{24}N_2O_2$

C2-C1-C6	118.6(3)	C2-C1-C12	119.3(3)
C6-C1-C12	122.1(3)	C1-C2-C3	122.2(3)
C1-C2-O2	113.8(2)	C3-C2-O2	124.0(3)
C2-C3-C4	118.0(3)	C3-C4-C5	121.3(3)
C4-C5-C6	120.4(3)	C1-C6-C5	119.5(3)
C1-C6-C7	118.6(2)	C5-C6-C7	121.8(3)
C6-C7-C8	110.0(2)	C7-C8-C9	114.1(2)
C7-C8-N2	107.2(2)	C9-C8-N2	109.6(2)
C8-C9-N1	110.2(2)	C11-C10-C15	107.9(2)
C11-C10-C16	108.2(2)	C15-C10-C16	109.3(2)
C11-C10-N1	108.8(2)	C15-C10-N1	109.6(2)
C16-C10-N1	112.9(2)	C10-C11-N2	115.4(2)
C10-C11-O1	112.6(2)	N2-C11-O1	106.3(2)
C1-C12-C14	114.4(2)	C1-C12-N2	113.9(2)
C14-C12-N2	104.2(2)	C12-C14-O1	105.4(2)
C9-N1-C10	113.0(2)	C9-N1-C17	111.3(2)
C10-N1-C17	114.8(2)	C8-N2-C11	110.8(2)
C8-N2-C12	109.8(2)	C11-N2-C12	100.7(2)
C11-O1-C14	105.6(2)	C2-O2-C13	117.4(2)
C19-C18-C23	119.1(2)	C19-C18-C29	119.0(2)
C23-C18-C29	121.8(3)	C18-C19-C20	121.4(3)
C18-C19-O4	114.8(2)	C20-C19-O4	123.9(3)
C19-C20-C21	118.9(3)	C20-C21-C22	120.6(3)
C21-C22-C23	120.3(3)	C18-C23-C22	119.6(3)
C18-C23-C24	119.3(2)	C22-C23-C24	121.1(3)
C23-C24-C25	109.9(2)	C24-C25-C26	114.2(2)
C24-C25-N4	108.2(2)	C26-C25-N4	108.7(2)
C25-C26-N3	109.9(2)	C28-C27-C32	108.5(2)
C28-C27-C33	107.6(2)	C32-C27-C33	109.0(2)
C28-C27-N3	108.6(2)	C32-C27-N3	109.5(2)
C33-C27-N3	113.4(2)	C27-C28-N4	116.4(2)
C27-C28-O3	111.9(2)	N4-C28-O3	105.9(2)
C18-C29-C31	113.9(2)	C18-C29-N4	114.3(2)
C31-C29-N4	104.2(2)	C29-C31-O3	105.2(2)
C26-N3-C27	112.6(2)	C26-N3-C34	110.8(2)
C27-N3-C34	114.8(2)	C25-N4-C28	110.4(2)
C25-N4-C29	110.2(2)	C28-N4-C29	101.2(2)
C28-O3-C31	105.3(2)	C19-O4-C30	117.2(2)

(a) Estimated standard deviations in the least significant digits are given in parentheses.

TABLE 4 Anisotropic thermal parameters ($Å^2 \times 10^3$)[a,b] for $C_{17}H_{24}N_2O_2$

atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C1	16(2)	20(2)	25(2)	4(1)	-0(1)	-6(1)
C2	18(2)	22(2)	29(2)	2(1)	0(1)	-1(1)
C3	23(2)	21(2)	38(2)	0(1)	-5(1)	1(1)
C4	27(2)	28(2)	38(2)	9(2)	-10(2)	1(1)
C5	28(2)	31(2)	25(2)	10(1)	-4(1)	-4(2)
C6	16(2)	24(2)	28(2)	4(1)	-1(1)	-4(1)
C7	26(2)	33(2)	19(2)	6(1)	-0(1)	-2(1)
C8	16(2)	29(2)	19(2)	1(1)	2(1)	-0(1)
C9	28(2)	36(2)	21(2)	-3(1)	2(1)	0(2)
C10	24(2)	26(2)	20(2)	-1(1)	-1(1)	1(1)
C11	17(2)	25(2)	19(2)	1(1)	-2(1)	-1(1)
C12	20(2)	20(2)	19(1)	-2(1)	-1(1)	-4(1)
C13	39(2)	43(2)	41(2)	-5(2)	7(2)	13(2)
C14	25(2)	21(2)	26(2)	4(1)	6(1)	4(1)
C15	34(2)	28(2)	33(2)	6(1)	0(2)	5(2)
C16	25(2)	35(2)	34(2)	-1(2)	-2(1)	6(2)
C17	50(2)	34(2)	36(2)	-6(2)	-1(2)	10(2)
N1	30(2)	25(1)	22(1)	-4(1)	-0(1)	3(1)
N2	18(1)	20(1)	17(1)	1(1)	-2(1)	1(1)
O1	22(1)	22(1)	28(1)	4(1)	6(1)	2(1)
O2	31(1)	29(1)	26(1)	-1(1)	1(1)	9(1)
C18	19(2)	18(2)	22(2)	1(1)	-1(1)	1(1)
C19	18(2)	19(2)	21(2)	-3(1)	-0(1)	7(1)
C20	20(2)	19(2)	29(2)	-0(1)	-2(1)	-2(1)
C21	29(2)	23(2)	27(2)	-8(1)	-6(1)	1(1)
C22	26(2)	24(2)	21(2)	-3(1)	1(1)	5(1)
C23	20(2)	19(2)	22(2)	-0(1)	1(1)	5(1)
C24	22(2)	27(2)	20(2)	1(1)	3(1)	5(1)
C25	17(2)	25(2)	20(2)	0(1)	1(1)	2(1)
C26	27(2)	30(2)	21(2)	1(1)	4(1)	-2(1)
C27	18(2)	31(2)	21(2)	1(1)	1(1)	-2(1)
C28	17(2)	26(2)	19(1)	-2(1)	-2(1)	-0(1)
C29	18(2)	18(1)	19(1)	1(1)	3(1)	0(1)
C30	29(2)	33(2)	28(2)	-2(1)	6(1)	-8(2)
C31	24(2)	26(2)	24(2)	-6(1)	3(1)	-7(1)
C32	34(2)	28(2)	31(2)	-2(1)	2(1)	-10(2)
C33	22(2)	30(2)	41(2)	1(2)	-2(2)	-6(2)
C34	51(3)	31(2)	36(2)	5(2)	4(2)	-14(2)
N3	32(2)	22(1)	21(1)	1(1)	4(1)	-5(1)
N4	18(1)	21(1)	16(1)	-0(1)	-1(1)	-2(1)
O3	22(1)	23(1)	27(1)	-7(1)	7(1)	-5(1)
O4	27(1)	27(1)	23(1)	-3(1)	5(1)	-9(1)

(a) Estimated standard deviations in the least significant digits are given in parentheses.

(b) The anisotropic thermal parameter exponent takes the form:

$$-2\pi^2(h^2a^{*2}U_{11}+k^2b^{*2}U_{22}+ \ldots +2hka^*b^*U_{12})$$

TABLE 5 Hydrogen coordinates ($x10^4$) and thermal parameters ($Å^2 x10^3$) for $C_{17}H_{24}N_2O_2$

atom	x	y	z	U_{iso}
H3	8892	9708	2316	35
H4	9118	9732	623	39
H5	10285	8901	-14	35
H7A	11170	7616	218	32
H7B	12141	7959	877	32
H8	10814	6823	1503	27
H9A	12854	6558	1120	35
H9B	11853	6236	488	35
H11	12491	6723	3774	21
H12	11559	8080	3480	25
H13A	9232	9128	4775	53
H13B	8491	9207	3783	53
H13C	9406	9798	4036	53
H14A	9819	7143	3071	26
H14B	10436	7284	4117	26
H15A	12568	5376	4157	36
H15B	12870	4785	3364	36
H15C	11698	5026	3384	36
H16A	14172	5637	2681	35
H16B	14021	6161	3593	35
H16C	13888	6514	2525	35
H17A	12549	4599	1781	49
H17B	13316	5109	1242	49
H17C	12180	4948	737	49
H20	3884	7963	2254	28
H21	4172	8021	579	35
H22	5360	8905	54	31
H24A	7221	9747	1078	29
H24B	6284	10146	434	29
H25	5925	10872	1764	27
H26A	7083	11469	852	30
H26B	8021	11108	1537	30
H28	7497	10834	4088	26
H29	6556	9496	3640	22
H30A	4244	8279	4772	36
H30B	4477	7716	3921	36
H30C	3515	8278	3767	36
H31A	5440	10277	4331	29
H31B	4832	10458	3292	29
H32A	7515	12178	4583	37
H32B	6715	12552	3765	37
H32C	7891	12807	3873	37
H33A	9226	11983	3200	38
H33B	9002	11101	3012	38
H33C	9032	11440	4082	38
H34A	7701	13046	2276	47
H34B	7414	12739	1195	47
H34C	8511	12552	1769	47

Appendix III

The following abbreviations are used throughout this dissertation:

AcOH	acetic acid
Ac_2O	acetic anhydride
BOC or Boc	*tert-* Butoxycarbonyl
Bn	benzyl
CBZ or Cbz	benzyloxycarbonyl
CSA	camphor sulfonic acid
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
Dibal	diisobutylaluminum hydride
DMAP	4-(dimethylamino)pyridine
ED_{50}	effective dose in 50% of test subjects
ee	enantiomeric excess
EtOH	ethanol
Et_3N	triethylamine
HPLC	high performance liquid chromatography
Hz	hertz
IR	infrared
LAH	lithium aluminum hydride
LD_{50}	lethal dose for 50% of test subjects
MeOH	methanol

MHz	megahertz
MOM	methoxymethyl
MsCl	methansulfonyl (mesyl) chloride
NBS	N-bromosuccinamide
NCS	N-chlorosuccinamide
NOE	nuclear Overhauser effect
nBuLi	n-butyl lithium
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldipenylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC or tlc	thin layer chromatography
TMS	trimethylsilyl, tetramethylsilane
TsOH	tosyl acid
$[\alpha]$	specific rotation
Φ	phenyl
Φ–H	benzene

Appendix IV

Reprinted from The Journal of Organic Chemistry, Vol. 52, page 2615, June 12, 1987

A New Synthetic Approach to 1-(Hydroxymethyl)-8-methoxy-1,2,3,4-tetrahydroisoquinolin-4-one

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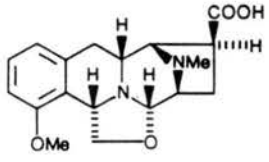
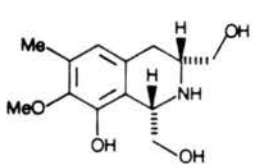
Received October 21, 1986

The tetrahydroisoquinoline moiety occurs as the structural nucleus of a wide variety of naturally occurring alkaloids.[1] As a result, numerous methods[2] have been developed and employed in the construction of natural alkaloids constituted of this ring system. Perhaps the most widely used synthetic construction is the classic Pictet–Spengler isoquinoline synthesis,[1] which involves the condensation of β-arylethylamines and carbonyl compounds. Cyclization occurs via the intermediacy of the putative Schiff base, furnishing the tetrahydroisoquinoline. The related Bischler–Napieralski reaction furnishes the corresponding 3,4-dihydroisoquinolines through an electronically similar electrophilic aromatic substitution. In both instances, rate-accelerating electron-releasing substituents generally induce cyclization to occur (ortho/para) at the less hindered (para) position to a significant extent. In the case of a *m*-methoxy-substituted β-arylamine, cyclization occurs to give the 6-methoxy regioisomer as the major and, often times, exclusive product.[1]

As part of a program to construct and study the rare tetrahydroisoquinoline antitumor alkaloid quinocarcin (DC-52, **1**)[3] and the β-adrenergic receptor antagonist MY



1, quinocarcin



2, MY 336-a

336-a,[4] we needed a reliable and unambiguous synthetic protocol that would embrace the 8-oxygenated 1,2,3,4-tetrahydroisoquinoline nucleus.[5] Our approach is related to the classic Pomeranz–Fritsch reactions, wherein an appropriately substituted benzylic amine serves as the template for the penultimate C-4a/C-4 bond construction.[6]

2-Bromoanisole is lithiated (*n*-BuLi, THF) and condensed with the *N*-methoxy-*N*-methylamide[7] of (benzyloxy)acetic acid[8] (**4**) to furnish the ketone **5** in 90% yield (Scheme I). This coupling proved to be significantly superior to condensations of **3** with (benzyloxy)acetyl chloride,[8] the corresponding tertiary alcohol resulting from further reaction of **5** and **3** being the predominant product. However, preparatively useful quantities of **5** could also be obtained by coupling (benzyloxy)acetyl chloride and **3** in the presence of $CdCl_2$.[9]

Reductive amination of the ketone using the Borch[10] procedure (65%) followed by hydrogenolytic removal of the benzyl ether furnished the amino alcohol **7** (81%). Alkylation of the amine with ethyl bromoacetate (**8**; 95%) and formation of the cyclic urethane furnished the ethyl ester **9** (77%). Selective basic hydrolysis of the ethyl ester furnished the crystalline acid (75%; mp 165–166 °C), which was converted to the acid chloride with thionyl chloride. The crucial intramolecular Friedel–Crafts acylation proved to be extremely difficult and required extensive experimentation. Low yields (<10%) were obtained under classical conditions (hot CS_2, $AlCl_3$), but eventually the conditions reported by Uggeri[11] ($AlCl_3$, $Cl_2CH_2CH_2Cl_2$, 25

†Fellow of the Alfred P. Sloan Foundation 1986–1988. NIH Research Career Development Awardee 1984–1989. Eli Lilly Grantee 1986–1988.

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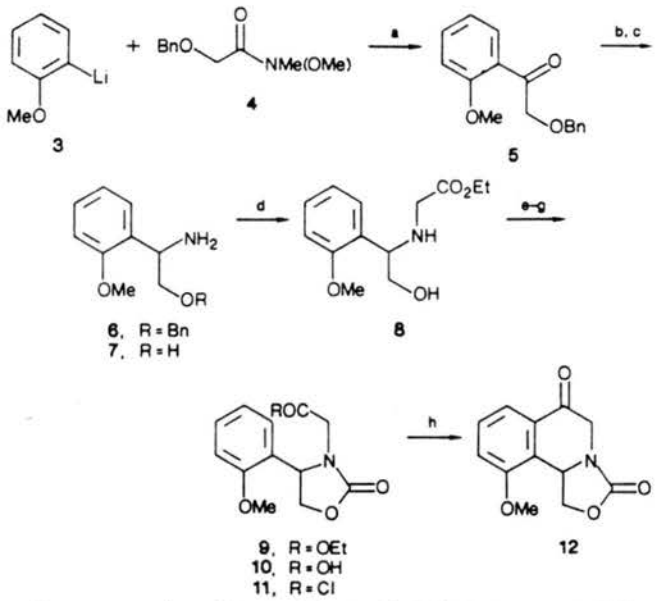
Scheme I[a]



[a] Reagents and conditions: (a) −15 °C, THF, 30 min, 5% HCl/EtOH, 90%; (b) $AcO^-N^+H_4$, $NaBH_3CN$, MeOH, 36 h, 65%; (c) 10% Pd/C, 0.5 M HCl/EtOH, 50 psi, 20 h, 81% (d) $BrCH_2CO_2Et$, Et_3N, THF, 20 h, 95%; (e) Im_2CO, THF, 2 h, 77%; (f) 1.0 M LiOH, EtOH, 1.5 h, 75%; (g) $SOCl_2$, C_6H_6, 80 °C, 3 h, 100%; (h) $AlCl_3$, $Cl_2HCCHCl_2$, 24 h, 65%.

°C; 65% yield) proved satisfactory to furnish the crystalline 1,2,3,4-tetrahydroisoquinoline **12**.

In a parallel series of experiments, the acid chloride corresponding to **11** prepared from phenylglycinol did *not* react intramolecularly to furnish the homologous tetrahydroisoquinoline. Instead, only *intermolecular* acylation products resulting from solvent incorporation or dimerization were obtained. Indeed, it seems that some electronic activation of the aromatic ring is required to effect closure in the modified Pomeranz–Fritsch approach.[12]

Experimental Section

(Benzyloxy)methyl 2-Methoxyphenyl Ketone (5). To a stirred solution of *o*-bromoanisole (1.28 mL, 10.0 mmol, 1.0 equiv) in dry pentane (15 mL) was added a 1.60 M solution of *n*-butyllithium in hexanes (6.25 mL, 10.0 mmol, 1.0 equiv) at room temperature in a nitrogen atmosphere. After 30 min, the solvent was removed in vacuo, and freshly distilled benzene (15 mL) was added immediately, followed by the addition of cadmium chloride (0.916 g, 5.0 mmol, 1.0 equiv) at room temperature. The resulting vigorously stirred suspension was heated to reflux in a nitrogen atmosphere for 6.5 h, at which time the mixture gave a negative Gilman's test. The mixture was allowed to cool to room temperature, (benzyloxy)acetyl chloride (1.845 g, 10.0 mmol, 1.0 equiv) was added, and the mixture was heated to reflux in a nitrogen atmosphere. After 2 h, the vigorously stirred mixture was cooled to room temperature, added to an equal volume of 10% HCl solution, and stirred for at least 30 min. The mixture was then separated, and the aqueous layer was washed with ether. The combined organic layers were then washed with 5% $NaHCO_3$ followed by saturated NaCl, dried over $MgSO_4$, concentrated, and separated by silica gel (eluted with 2.5% EtOAc/benzene) to afford 0.994 g (39%) of **5** as a yellow oil: ^1H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 3.87 (3 H, s), 4.68 (2 H, s), 4.72 (2 H, s), 6.93 (2 H, m), 7.39 (6 H, m), 7.89 (1 H, dd, J = 7.73 Hz); IR (NaCl, neat) 3024, 2938, 1685, 1240, 1104 cm^{-1}.

(Note: The same procedure carried out with CdI_2 gave a 25% yield, and the same procedure carried out with the aryl Grignard reagent with $CdCl_2$ gave a 20% yield.)

***N*-Methoxy-*N*-methyl-2-(benzyloxy)acetamide (4).** To a stirred solution of (benzyloxy)acetyl chloride (3.226 g, 17.54 mmol, 1.0 equiv) and methoxymethylamine hydrochloride (1.93 g, 19.29 mmol, 1.1 equiv) in dry $CHCl_3$ (175 mL) cooled to 0 °C was added pyridine (3.12 mL, 38.58 mmol, 2.2 equiv). The resulting solution was stirred at room temperature for 12 h, when the $CHCl_3$ was evaporated, yielding a white residue. The residue was partitioned between brine and a 1:1 mixture of CH_2Cl_2/Et_2O. The organic layer was separated, dried over Na_2SO_4, filtered, and evaporated, yielding **4** (3.64 g, 99.5%) as a colorless oil: bp 132 °C (0.2 mmHg); ^1H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 3.19 (3 H, s), 3.63 (3 H, s), 4.29 (2 H, s), 4.67 (2 H, s), 7.36 (5 H, m); IR (NaCl, neat) 3020, 3060, 2940, 1675, 1450, 1325, 1130, 1080, 980, 730, 690 cm^{-1}; mass spectrum, CI (NH_3) m/z 209.8 (M^+, 0.7%), 197 (3.1), 180 (9.0), 108 (5.8), 106 (10.4), 91 (2.4), 74 (5.9), 44 (4.5), 35 (100).

(Benzyloxy)methyl 2-Methoxyphenyl Ketone (5). To a stirred solution of *o*-bromoanisole (4.56 mL, 36.68 mmol, 3.0 equiv) in dry THF (12.5 mL) cooled to −15 °C was added *n*-BuLi (23.7 mL of a 1.54 M solution in hexanes, 3.0 equiv). The resulting solution was allowed to stir for 1 h at −15 °C, when it was added to a solution of **4** (2.55 g, 12.23 mmol, 1.0 equiv) in dry THF (125 mL), cooled to −15 °C, via cannula. The resulting solution was stirred for 30 min and poured into 50 mL of 5% HCl/EtOH at 0 °C. This solution was then partitioned between brine and a 1:1 mixture of CH_2Cl_2/Et_2O. The organic layer was separated, dried over Na_2SO_4, filtered, and evaporated, yielding **5** as a colorless oil (2.82 g, 90%): ^1H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 3.87 (3 H, s), 4.68 (2 H, s), 4.72 (2 H, s), 6.93 (2 H, m), 7.39 (6 H, m), 7.89 (1 H, dd, J = 7.73 Hz); IR (NaCl, neat) 3020, 3060, 2930, 1680, 1595, 1480, 1280, 1235, 1100, 1010, 940, 740, 685 cm^{-1}; mass spectrum, CI (NH_3) m/z 257 (M^+, 14.5%), 151 (100), 135 (6.6), 106 (6.0), 91 (2.4), 35 (100).

***O*-Benzyl(2-methoxyphenyl)glycinol (6).** To a stirred solution of **5** (2.82 g, 11.029 mmol, 1.0 equiv) and ammonium acetate (8.50 g, 110.3 mmol, 10 equiv) in absolute methanol (35 mL) was added sodium cyanoborohydride (0.485 g, 7.72 mmol, 0.70 equiv) in one portion. The resulting solution was stirred at room temperature for 36 h. Concentrated HCl was added until pH <2. The MeOH was then evaporated, and the resulting white residue was dissolved in H_2O (10 mL) and washed with Et_2O (2 × 10 mL). The aqueous phase was then basified with powdered KOH to pH >10, saturated with NaCl, and extracted with CH_2Cl_2 (4 × 10 mL). The combined CH_2Cl_2 extracts were dried over $MgSO_4$, filtered, and evaporated to a colorless oil (1.832 g, 65%): ^1H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 1.82 (2 H, br s), 3.45 (1 H, t, J = 8.52 Hz), 3.69 (1 H, dd, J = 9.24 Hz), 3.79 (3 H, s), 4.56 (3 H, m), 6.92 (2 H, m), 7.32 (7 H, m); IR (NaCl, neat) 3380, 3300, 3020, 3060, 2900, 2840, 1580, 1485, 1450, 1230, 1080, 1115, 850, 735, 680 cm^{-1}; mass spectrum, CI (NH_3) m/z 258 (M^+, 100), 256 (210), 241 (2.5), 228 (1.8), 150 (19.2), 136 (38.5), 106 (19.5), 91 (6.8), 35 (100).

(2-Methoxyphenyl)glycinol (7). To a solution of **6** (2.885 g, 11.21 mmol, 1.0 equiv) in 60 mL of 0.5 M HCl/EtOH contained in a Parr pressure vessel was added 10% Pd/C (2.98 g, 2.8 mmol, 0.25 equiv). The vessel was purged with hydrogen several times, charged to 50 psi, and hydrogenated for 20 h. The Pd/C was filtered off over Celite and the filtrate evaporated to a white solid. The solid was dissolved in water and washed once with Et_2O, basified to pH >10 with solid KOH, saturated with NaCl, and extracted with CH_2Cl_2 (4 × 20 mL). The organic phase was then dried over $MgSO_4$, filtered, and evaporated, yielding **7** (1.52 g, 81%) as a colorless oil: ^1H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 2.57 (3 H, br s), 3.59 (1 H, m), 3.73 (1 H, m), 3.81 (3 H, s), 4.27 (1 H, m), 6.68 (1 H, d, J = 8.24 Hz), 6.91 (1 H, m), 7.23 (2 H, m); IR (NaCl, neat) 3360, 3280, 2920, 2830, 1590, 1490, 1235, 1140, 1120, 740 cm^{-1}; mass spectrum, CI (NH_3) m/z 168 (M^+, 5.8%), 151 (10.9), 136 (23.6), 44 (6.0), 35 (100).

***N*-(Carboxymethyl)(2-methoxyphenyl)glycinol (8).** To a stirred solution of **7** (1.16 g, 6.935 mmol, 1.0 equiv) and triethylamine (1.45 mL, 10.437 mmol, 1.5 equiv) in dry THF (60 mL) was added ethyl bromoacetate (1.00 mL, 9.04 mmol, 1.3 equiv). The reaction solution was stirred at room temperature for 20 h. The Et_3N·HBr was filtered off and washed with THF. The filtrate was evaporated to a clear residue, which was taken up in 70 mL of CH_2Cl_2, washed with H_2O (3 × 20 mL) and brine (1 × 20 mL), dried over $MgSO_4$, filtered, and evaporated to yield

(11) Uggeri, F.; Giordano, C.; Brambilla, A. *J. Org. Chem.* **1986**, *51*, 97.
(12) Some notable exceptions are included in ref 2f–h; see also ref 6.

8 (1.665 g, 95%) as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 1.23 (3 H, t, J = 7.45 Hz), 2.50 (2 H, br s), 3.35 (2 H, d, J = 5.43 Hz), 3.70 (2 H, m), 3.82 (3 H, s), 4.13 (3 H, m), 6.92 (2 H, m), 7.28 (2 H, m); IR (NaCl, neat) 3310, 2910, 1735, 1595, 1485, 1455, 1230, 1180, 1020, 740 cm^{-1}; mass spectrum, CI (NH_3) m/z 254 (M^+, 1.9), 236 (1.8), 208 (18.9), 168 (2.5), 150 (6.7), 130 (61.1), 104 (11.3), 72 (7.2), 55 (100).

Cyclic Urethane 9. To a stirred solution of **8** (1.665 g, 6.59 mmol, 1.0 equiv) in dry THF (60 mL) was added *N,N'*-carbonyldiimidazole (1.60 g, 9.87 mmol, 1.5 equiv). The resulting solution was stirred at room temperature for 2 h and evaporated to a white residue. The residue was taken up in CH_2Cl_2 (100 mL), washed with 1 M HCl (3 × 25 mL), H_2O (2 × 25 mL), and brine (1 × 25 mL), dried over $MgSO_4$, filtered, and evaporated, yielding **9** as a colorless oil (1.41 g, 77%): ^{1}H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 1.25 (3 H, t, J = 7.03 Hz), 3.43 (1 H, d, J = 17.96 Hz), 3.82 (3 H, s), 4.16 (3 H, m), 4.34 (1 H, d, J = 17.98 Hz), 4.72 (1 H, t, J = 8.67 Hz), 5.35 (1 H, m), 6.97 (2 H, m), 7.27 (2 H, m); IR (NaCl, neat) 2960, 2920, 2820, 1750, 1600, 1580, 1485, 1460, 1415, 1240, 1195, 1080, 1115, 745 cm^{-1}. Mass spectrum, CI (NH_3) m/z 280 (M^+, 54.9%), 250 (3.1), 235 (1.2), 220 (1.4), 162 (1.8), 148 (1.7), 133 (2.0), 104 (1.7), 35 (100).

Carboxylic Acid 10. To a stirred solution of **9** (1.41 g, 5.059 mmol, 1.0 equiv) in 16 mL of absolute ethanol at −10 °C was added 6.7 mL of 1 M LiOH (6.7 mmol, 1.32 equiv). The reaction was allowed to stir for 1.5 h at −10 °C and was then neutralized with 6 M HCl (1.11 mL, 6.7 mmol, 1.32 equiv). The ethanol was evaporated, and the resulting residue was partitioned between 1 M HCl and CH_2Cl_2. The organic layer was separated, washed with H_2O (1 × 10 mL) and brine (1 × 10 mL), dried over $MgSO_4$, filtered, and evaporated to a white solid. Recrystallization from EtOAc/hexanes afforded 957 mg of pure **10** (75%): mp 165–166 °C; ^{1}H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 3.48 (1 H, d, J = 18.25 Hz), 3.83 (3 H, s), 4.19 (1 H, t, J = 8.02 Hz), 4.39 (1 H, d, J = 18.438 Hz), 4.73 (1 H, t, J = 9.174 Hz), 5.36 (1 H, m), 6.95 (2 H, m), 7.36 (2 H, m), 8.52 (1 H, br s); IR (NaCl, neat) 2900, 2810, 2700, 2585, 2500, 1750, 1675, 1595, 1580, 1450, 1240, 1200, 1190, 1110, 940, 850, 750, 735, 700, 630 cm^{-1}; mass spectrum, CI (NH_3) m/z 251 (M^+, 13.8%), 236 (3.5), 208 (7.9), 194 (6.6), 164 (2.5), 150 (5.5), 135 (4.1), 102 (3.7), 76 (3.2), 44 (8.1), 35 (100). Anal. ($C_{12}H_{13}NO_5$) C, H, N.

Acid Chloride 11. To a suspension of **10** (408 mg, 1.626 mmol, 1.0 equiv) in dry benzene (8 mL) was added $SOCl_2$ (0.36 mL, 4.91 mmol, 3.02 equiv). The suspension was then heated to mild reflux for 3 h, and the benzene and $SOCl_2$ were evaporated under reduced pressure. The resulting light amber residue (438 mg, 100%) was used directly for the next step without purification: ^{1}H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 3.78 ($^1/_2$ H, s), 3.84 (3.5 H, s), 4.25 (1 H, dd, J = 8.63 Hz), 4.73 (2 H, m), 5.32 (1 H, dd, J = 9.02 Hz), 6.97 (2 H, m), 7.27 (2 H, m); IR (NaCl, neat) 3060, 3020, 2940, 2830, 1800, 1760, 1600, 1590, 1490, 1460, 1420, 1250, 1180, 1110, 1090, 1020, 950, 920, 850, 750, 670 cm^{-1}.

Isoquinolone 12. To a stirred solution of **11** (438 mg, 1.626 mmol, 1.0 equiv) in 16 mL of dry 1,1,2,2-tetrachloroethane was added $AlCl_3$ (867 mg, 6.5 mmol, 4.0 equiv). The reaction was stirred at room temperature for 24 h, when it was poured into 40 mL of ice water and acidified to pH <2 with concentrated HCl. The resulting slurry was extracted with CH_2Cl_2 (4 × 20 mL), and the combined organic extracts were washed with 1 M NaOH (1 × 10 mL) and brine (1 × 10 mL), dried over $MgSO_4$, filtered, and evaporated to an oil, which was separated by column chromatography (silica gel, 3:2 hexanes/EtOAc), yielding **12**: 246 mg, 65%; mp 157–159 °C dec (recrystallized from EtOAc); ^{1}H NMR (270 MHz, $CDCl_3$, Me_4Si) δ 3.83 ($^1/_2$ H, s), 3.91 (3.5 H, s), 4.25 (1 H, t, J = 8.54 Hz), 4.67 (1 H, d, J = 18.15 Hz), 5.03 (1 H, t, J = 8.94 Hz), 5.23 (1 H, t, J = 8.61 Hz), 7.16 (1 H, dd, J = 8.28 Hz), 7.46 (1 H, t, J = 8.41 Hz), 7.73 (1 H, dd, J = 8.14 Hz); IR (NaCl, neat) 3080, 3020, 2940, 2870, 1765, 1695, 1595, 1580, 1430, 1280, 1250, 1120, 1030, 785, 740, 670 cm^{-1}; mass spectrum, CI (NH_3) m/z 233 (M^+, 16.9%), 219 (7.9), 189 (2.1), 174 (7.4), 159 (2.8), 132 (1.3), 35 (100). Anal. ($C_{12}H_{11}NO_4$) C, H, N.

Acknowledgment. We acknowledge The National Institutes of Health, The National Science Foundation, The Alfred P. Sloan Foundation, and Eli Lilly Corp. for their generous support of our programs.

Tetrahedron Vol. 47, No. 14/15, pp. 2629-2642, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

SYNTHESIS, CONFORMATION, CRYSTAL STRUCTURES AND DNA CLEAVAGE ABILITIES OF TETRACYCLIC ANALOGS OF QUINOCARCIN

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(*Received in USA* 20 *September* 1990)

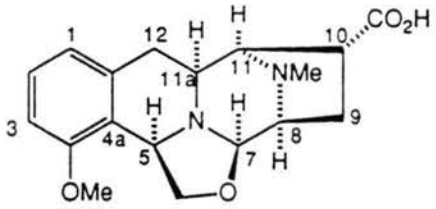
Abstract. Two totally synthetic, racemic analogs of quinocarcin have been designed and their crystal structures determined. Both substances effect the modest cleavage of plasmid DNA. Alteration of the conformation of the reactive oxazolidine fused to the piperazine ring by selecting the stereochemistry at C-11a through synthesis drastically attenuates the relative ability of these substances to cleave DNA.

Introduction

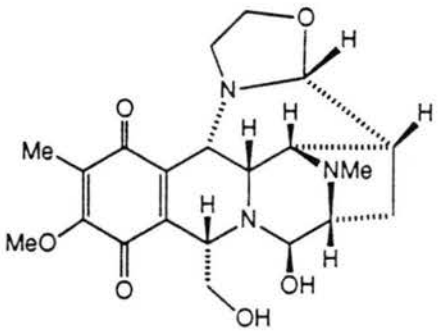
Quinocarcin (**1**) is a natural secondary metabolite produced by *Streptomyces melanovinaceus* and is the simplest member of the naphthyridinomycin (**3**)/saframycin (**4**) class of anti-tumor agents.[1,2] Quinocarcin has been shown[1a,3] to display weak antimicrobial activity against several Gram-positive microbes but is inactive toward Gram-negative bacteria. As its citrate salt, quinocarcin (named quinocarmycin citrate or KW2152) displays promising anti-tumor activity[3] against several lines of solid mammalian carcinomas including St-4 gastric carcinoma; Co-3 human colon carcinoma; MX-1 human mammary carcinoma; M5076 sarcoma; B16 melanoma and P388 leukemia. This substance is currently under evaluation in human clinical trials by the Kyowa Hakko Kogyo Co., Japan.

Our interest in this substance stems from a report by Tomita, et. al.[4] that recorded the remarkable observation that **1** cleaves plasmid DNA in an O_2-dependent fashion that was reported: 1) to not require metal ions (Fe^{2+} or Cu^{2+}); 2) to be stimulated by dithiothreitol; 3) to be inhibited by oxygen free radical scavengers such as methanol, *tert*-butanol, α-tocopherol and; 4) to be inhibited by superoxide dismutase (SOD) and catalase. Quinocarcin blocks RNA synthesis in preference to DNA and protein synthesis in P388 leukemia cells.[3d] On the other hand, in *Bacillus subtilis*, quinocarcin inhibited [^{3}H] thymidine incorporation suggesting inhibition of DNA polymerase; therefore, DNA synthesis is thought[4] to be preferentially inhibited in *Bacillus subtilis*. It has also been reasonably proposed[3d,4,5] that quinocarcin alkylates DNA in the minor groove[5] through the ring-opened form of the oxazolidine (imminium **5**); similar DNA alkylation has been invoked for **3** and **4**. Indirect support[3d,4] for the involvement of the oxazolidine ring in the above context comes from the lack of biological activity displayed by quinocarcinol (**2**) which is coproduced with **1** by *Streptomyces melanovinaceus*. Quinocarcinol also does not cleave plasmid DNA[4] which forces the conclusion that the oxazolidine moiety is also responsible for the oxidative degradation of DNA by a unique mechanism.

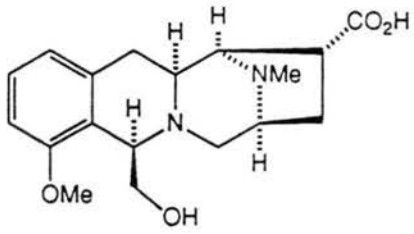
While it is not yet clear whether the anti-tumor properties of quinocarmycin citrate are a manifestation of only one mode of action (i.e., DNA alkylation) or both (DNA alkylation and oxidative DNA cleavage), we were intrigued by the oxidative cleavage observations of the Kyowa-Hakko group[4] since **1** does not contain any readily recognizable functionality that would be associated with the capacity for oxidative DNA cleavage,[6] such as metal



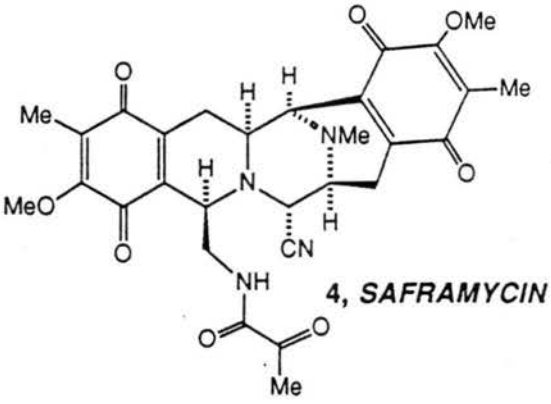
1, *QUINOCARCIN, DC-52*



3, *NAPHTHYRIDINOMYCIN*



2, *QUINOCARCINOL, DC-52d*

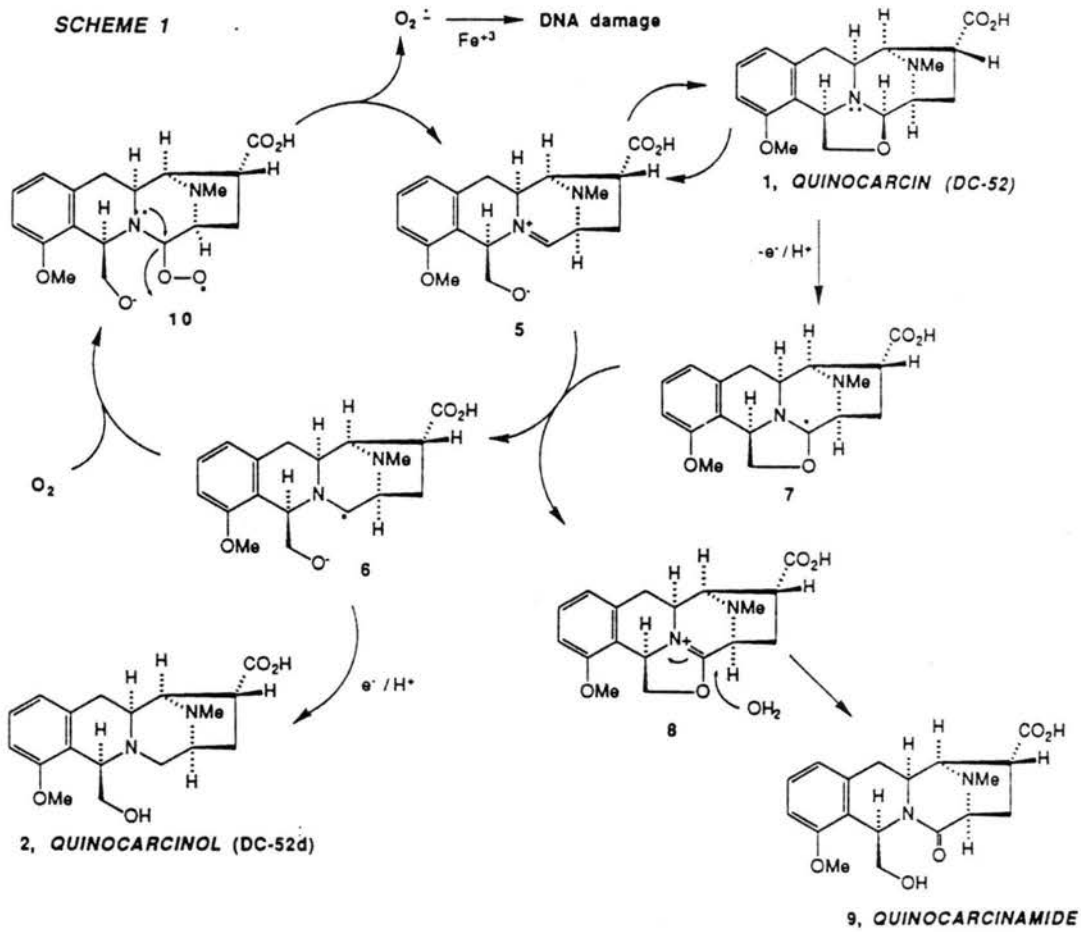


4, *SAFRAMYCIN A*

chelation sites, quinones, and ene-diynes amongst others. Most likely, the efficacy of this drug is a delicate and intimate combination of multiple effects that are brought to bear on its macromolecular targets. We have recently found[7] that quinocarcin undergoes a redox self-disproportionation reaction that we have invoked is coupled to the capacity of this substance to effect the production of superoxide in the presence of molecular oxygen and results, at least in part, to Fenton-mediated lesions in DNA; a mechanism for this process is reviewed[7] in Scheme 1. At the heart of this process, the oxazolidine ring is functioning as its own reductant which ultimately results in the reduction of oxygen and the cleavage of DNA. Such a process would also presumably be relevant to possible oxidative damage to RNA *in vitro* and *in vivo*. In the present study, we wished to examine the intrinsic capacity of simpler oxazolidine-containing analogs to effect the DNA cleavage reaction. Most significantly, we wished to experimentally determine whether pre-designed and synthetically[8] incorporated stereoelectronic control elements into simpler analogs could attenuate the capacity of this ring system to oxidatively damage DNA relative to **1**.

Design Criteria

Remers[5] has conducted molecular mechanics calculations on quinocarcin by docking the drug in the minor groove. From this study, it was concluded that the absolute configuration of quinocarcin is most likely that depicted in Scheme 1. The calculations suggested that the lowest energy conformer of **1** orients the piperazine ring in a chair-like conformation which therefore places the oxazolidine nitrogen lone pair in an antiperiplanar



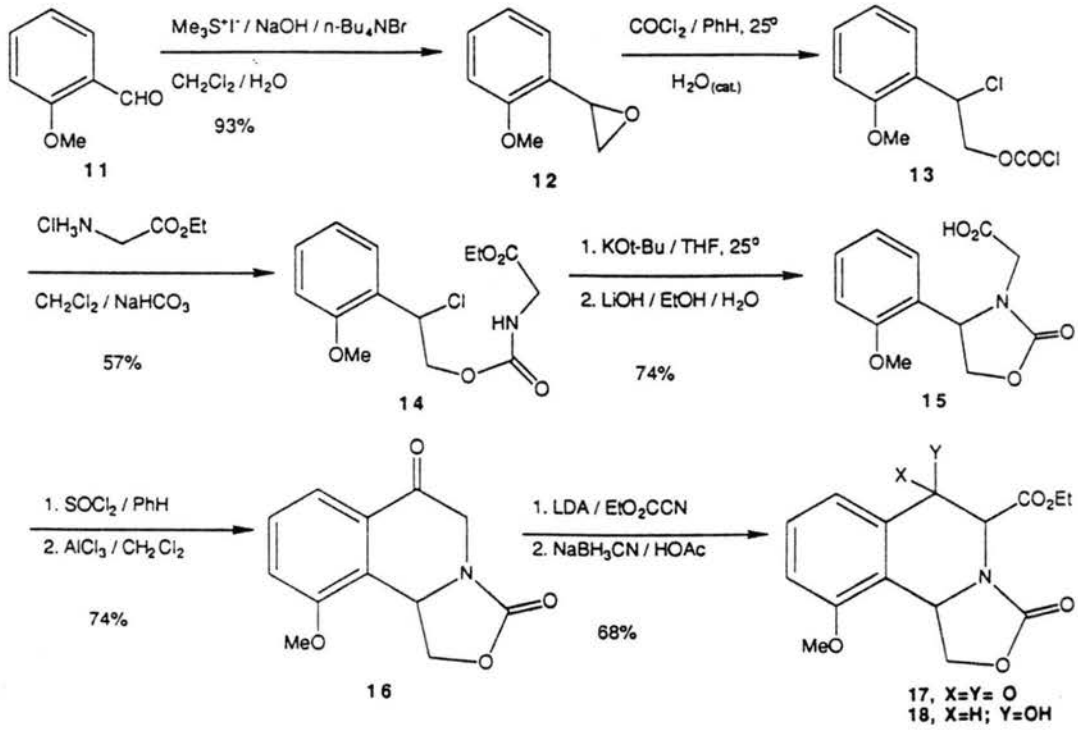
orientation to the oxazolidine methine (Figure 1, *anti*-**1**). Ring opening of the oxazolidine to the imminium species (see **5**, Scheme 1) requires nitrogen pyrimidal inversion to a higher energy twist boat conformer (Figure 1, *syn*-**1**) that was calculated to lie ~10 kcal mol^{-1} above the other conformer. In this situation, the oxazolidine nitrogen lone pair is *syn*- to the methine and antiperiplanar to the C–O bond. It was postulated[5] that the imminium species should be a good alkylator for N-2 of guanine in the minor groove of the sequence $d(ATGCAT)_2$. Based on the similarity to **3** and **4**,[2k] this is a reasonable expectation. In the present study, we wished to ask a different question regarding the conformational significance of the oxazolidine moiety. As shown in Scheme 1, the initial step in the electron-transfer between the oxazolidine and the imminium species involves 1-electron loss from the oxazolidine nitrogen with loss of the oxazolidine methine as a proton producing the reduction and oxidation radicals **6** and **7**, respectively. It is reasonable to expect that the *trans*, antiperiplanar arrangement of the oxazolidine methine and nitrogen lone pair in the lower energy conformer predicted by calculation, should also be the most favorable geometry for concomitant electron and proton loss in the redox self-disproportionation. We hoped to test this idea by synthesizing two simple analogs of quinocarcin that would each mirror one of the two conformational states of the natural product depicted in Figure 1. Thus, analog **23a** which has all three methines

oriented *syn* (same as **1**) would be expected to have the same relative conformation as *anti*-**1** with respect to the oxazolidine and piperazine rings. Analog **23b**, on the other hand, which has inverted stereochemistry at C-11a (quinocarcin numbering) is predicted to exist in a conformation that mirrors *syn*-**1**. Both of these predictions were based on examination of Dreiding stereomodels and molecular mechanics calculations.[9] From this stereoelectronic analysis, **23a** should be much faster at effecting oxidative DNA cleavage relative to **23b**. The synthesis, structures and relative DNA cleavage ability of these materials is presented below.

Results

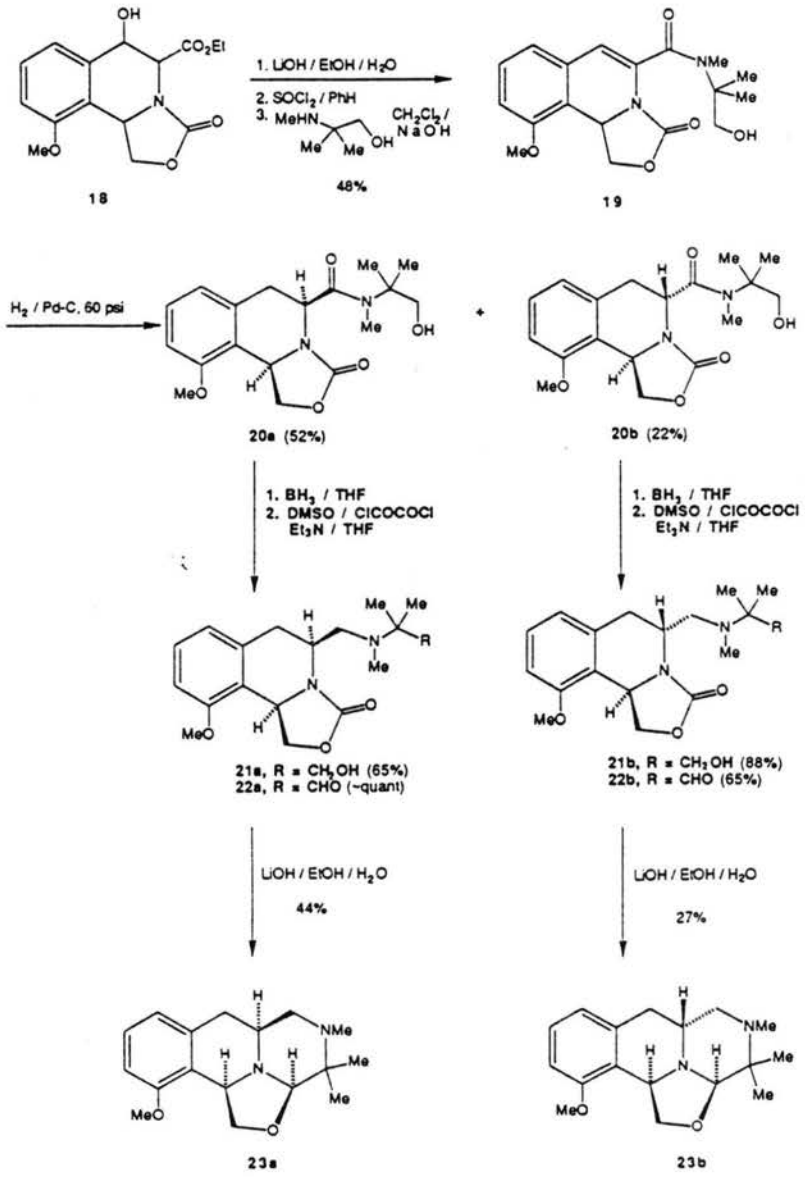
The preparation of the key isoquinoline **18** is detailed in Scheme 2 and was made by a modification of a known procedure.[10] *Ortho*-anisaldehyde (**11**) was treated with trimethylsulfonium iodide under phase-transfer conditions to afford the epoxide **12** in high yield. The epoxide was regioselectively opened with phosgene as a solution in benzene containing a catalytic amount of water to afford the chloroformate **13**. Without purification, **13** was subjected to acylation under Schotten-Baumann conditions to provide the urethane **14** in 57% overall yield from **12**. Treatment of **14** with potassium t-butoxide in THF at room temperature effected cyclization to the corresponding oxazolidinone which was saponified to the acid[10] **15** (74%, two steps). Acid chloride formation and intramolecular Friedel-Crafts acylation provided the isoquinolone **16** in 74% yield from **15**. The procedure described herein is an optimized preparation (from **15**) based on our previously reported[10] synthesis of **16**. The overall sequence from **11** is considerably more efficient and is amenable to multi-gram scale (10 gm scale is described in the experimental section).

SCHEME 2



C-Homologation of **16** proved to be troublesome and required extensive examination of various electrophilic species and reaction conditions due to the propensity of the ketone enolate to undergo O-acylation. Eventually, it was found that the lithium enolate condensed smoothly with ethyl cyanoformate to provide the β-ketoester **17**. Reduction of the ketone with sodium cyanoborohydride gave a single diastereomer (**18**) of unknown relative stereochemistry. This substance served as the key substrate from which various stereoselective and non-stereoselective routes to the target oxazolidine analogs (**23**) were examined. We found two parallel,

SCHEME 3



stereoselective routes[11] to **23a** and **23b** from **18**, wherein the relative stereochemistry at C-11a and the oxazolidine methine could be controlled. More recently, we have found it to be more efficient in terms of man-hours, to employ a common, non-stereoselective route that furnishes both stereochemical series and requiring only a simple chromatographic separation as described in Scheme 3.

Saponification of **18** furnished a crude carboxylic acid which was directly treated with thionyl chloride in benzene. The crude product was directly subjected to Schotten-Baumann acylation with 2,2,3-trimethyl ethanolamine to give the unsaturated amide **19** in 48% overall yield from **18**. This substance was then hydrogenated to give 52% of the *syn*-isomer **20a** and 22% of the *anti*-isomer **20b**. These materials were separated by silica gel chromatography and carried on separately to the final oxazolidines.

For **20a**, borane reduction furnished the tertiary amine **21a** in 65% yield without loss of stereochemical integrity. Swern oxidation to the aldehyde **22a** proceeded in essentially quantitative yield. The crucial oxazolidine-forming step proved somewhat capricious, but could be achieved in 44% yield by refluxing **22a** in basic ethanol for two days. Silica gel purification and crystallization furnished a single oxazolidine diastereomer for which a single crystal x-ray analysis[12] has been secured (Figure 2).

Similarly, **20b** was converted into **23b** in modest overall yield. The nicely crystalline **23b** also proved amenable to x-ray crystallographic analysis[12] as shown in Figure 2.

As is evident from the crystal structures, **23a** and **23b** differ with respect to the orientation of the oxazolidine nitrogen lone pair relative to the oxazolidine methine in the crystal. Substance **23a** positions the nitrogen lone pair *trans*-antiperiplanar to the oxazolidine methine; whereas **23b** positions the nitrogen lone pair *trans*-antiperiplanar to the oxazolidine C–O bond. These conformations are exactly those predicted from examination of Dreiding molecular models and molecular mechanics calculations.[9] The inversion of stereochemistry at C-11a (quinocarcin numbering) in **23b** from the all *syn*-situation in **23a** induces sufficient ring strain to favor nitrogen pyramidal inversion and results in a geometry similar to that evident in the crystal structure. These results confirm our predictions alluded to above that **23a** should mirror the ground state conformation (*anti*-**1**, Figure 1) of the oxazolidine for quinocarcin that was postulated in Remers computational study.[5] Similarly, **23b** can be thought of as mirroring the higher energy conformer of quinocarcin (*syn*-**1**, Figure 1) that Remers postulated as being the reactive conformation that precedes oxazolidine ring-opening to the imminium species (see **5**, Scheme 1) which should (quite reasonably) alkylate DNA.

Reactions with DNA

Compounds **23a** and **23b** as the free bases were virtually insoluble in aqueous buffers and were used as either water-soluble citrate or hydrochloride salts. Reaction of these materials with supercoiled plasmid DNA (pBR 322) were examined at various concentrations and conditions; the results are collected in Table 1 and Figure 3. Reactions were conducted in pH 8, 20 mmol phosphate buffer at 37°C for 2 hours and were analyzed by 0.8% agarose gel electrophoresis. The DNA bands were visualized by staining with ethidium bromide after running the gel and were quantitated by scanning densitometry. The supercoiled plasmid (form I, ccc DNA, fastest band) when nicked is first converted to open circular plasmid (form II, cc DNA, slowest band) and after extensive scission, linear DNA (form III, intermediate band) was observed. Both analogs required fairly high concentrations relative to **1** to produce observable damage to the DNA. As predicted, compound **23b** is significantly inferior to **23a** in effecting DNA cleavage and required dithiothreitol (DTT) even at concentrations as high as 5 mmol (entries 2 and 3, Table 1, lanes 2 and 3, Figure 3; compare entry 22). Since **23a** displayed

Figure 1

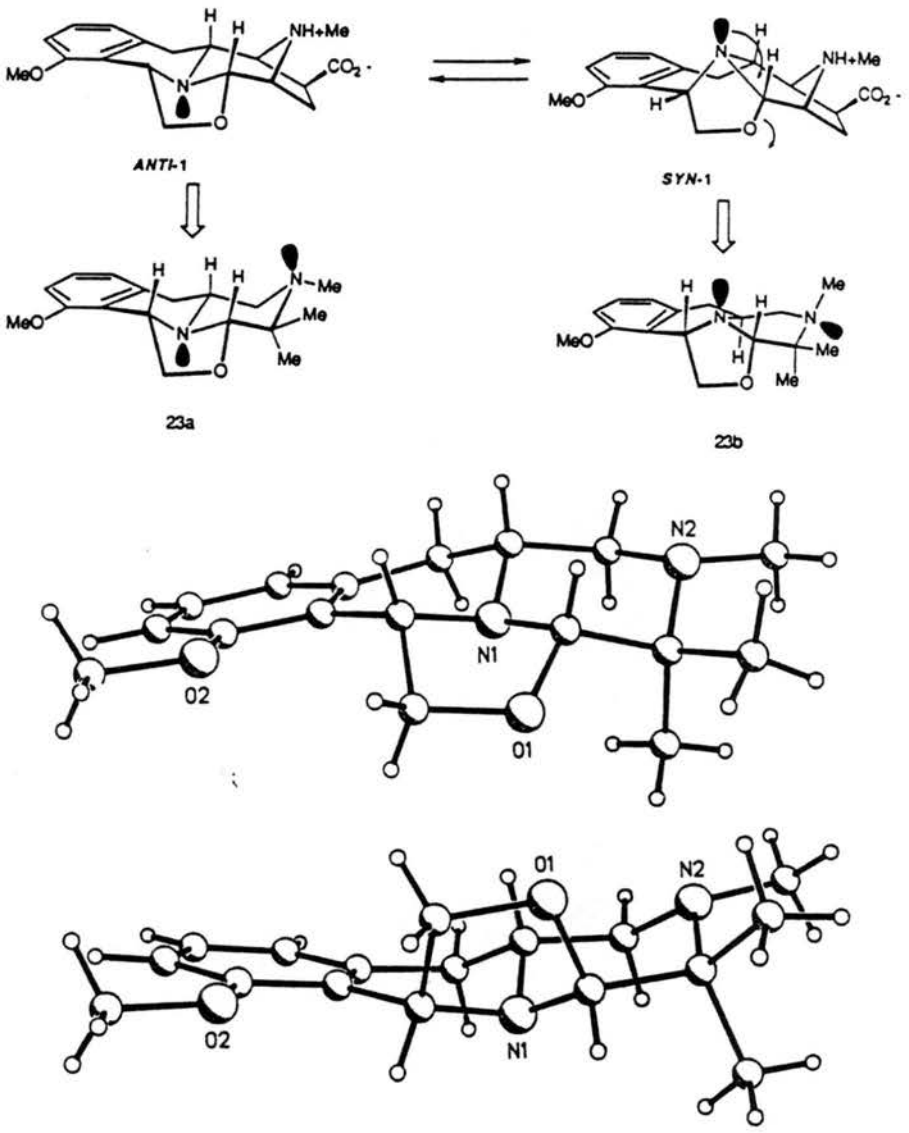


Figure 2. X-Ray Molecular Structures of 23a and 23b. Spheres are of fixed arbitrary radius.

superior DNA cleavage relative to **23b**, the reactivity of this material was examined in more detail and compared to that of **1**. As with quinocarcin, the DNA cleavage by **23a** is enhanced by the addition of DTT (compare entries 7, 16 and 17, Table 1; lanes 7, 16, and 17, Figure 3). It is significant to note that DTT by itself is capable of modest DNA cleavage (entry 17, Table 1, lane 17, Figure 3) *via* Fenton-mediated production of hydroxyl radical; this reaction is a manifestation of superoxide production during thiol autoxidation.[13] The DNA cleavage observed by **23a** in the presence of DTT is at least an order of magnitude greater than DTT alone; the DNA cleavage is thus clearly not due to DTT alone (compare lanes 16 and 17, Figure 3). Superoxide dismutase and catalyse both inhibit

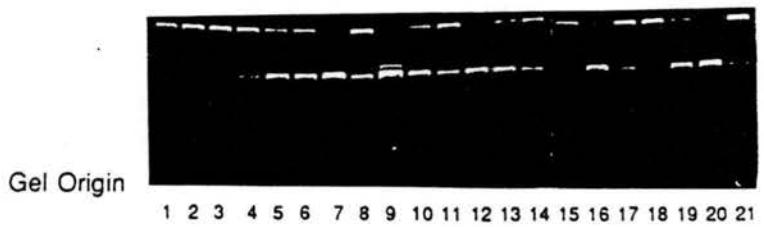


Figure 3. 0.8% Agarose gel electrophoresis of plasmid pBR322 DNA cleavage experiments. Lanes 1-21 correspond to entries 1-21 from Table I. See Table I for visualization details.

the cleavage; SOD being the more potent inhibitor (entries 10 and 11, Table 1; lanes 10 and 11, Figure 3). This is consistent with superoxide production and subsequent Fenton-mediated DNA cleavage.[14] Hydrogen peroxide strongly stimulates the DNA cleavage by **23a** (compare entries 7 and 20, Table 1; lanes 7 and 20, Figure 3). Quinocarcin is significantly better than either **23a** or **23b** at effecting DNA cleavage and gives comparable DNA scission at 1 mmol without external reductants that **23a** gives at 5 mmol with hydrogen peroxide (compare entries 9 and 20, Table 1; lanes 9 and 20, Figure 3).

Quinocarcin has been shown[4,7] to produce superoxide (see Scheme 1) and therefore, Fenton-mediated production of hydroxyl radical with adventitious iron must be invoked for the DNA cleavage event by these molecules. However, hydrogen peroxide alone at 0.1 mmol causes virtually no significant DNA damage (entry 21, Table 1). Since hydrogen peroxide is reduced by Fe(II) in the Fenton reaction, producing hydroxyl radical, the oxazolidine can be functioning indirectly in cycling adventitious Fe(III) to Fe(II) *via* superoxide production or may directly effect the redox cycling of the metal. This point has not yet been addressed. Attempts to sequester adventitious iron and uncouple[15] the presumed Haber-Weiss/Fenton reaction was performed by the addition of the potent Fe(III) chelator desferal (log k_f = 30.7). Addition of desferal to the reaction of **23a** with DNA showed very little inhibition at 0.1 mmol (entry 12, Table 1; lane 12, Figure 3) and partial protection at 10 mmol (entry 14, Table 1; lane 14, Figure 3). However, since the citrate salt of these materials proved to be less effective than the hydrochloride salts in effecting DNA cleavage, we suspect that both citrate and desferal are functioning as competitive CH substrates for the reactive oxidant with DNA (present in very low concentration relative to citrate or desferal) rather than as efficient metal sequestering agents. Additional experimental evidence for direct metal mediation in the DNA cleavage event is not yet available. Ascorbate, a powerful oxygen reductant, is very effective at mediating DNA cleavage which is completely inhibited by catalase (entries 23 and 24, Table 1). The incomplete inhibition of DNA cleavage by **23a** with either catalase of SOD (entries 10 and 11, Table 1) and the incomplete protection afforded by desferal suggests that the mechanism of DNA cleavage by this heterocycle may involve other pathways that are distinct from most recognized DNA oxidants. These possibilities are currently being pursued.

However, indirect experimental evidence points to a significant difference in the capacity of **23a** and **23b** to produce superoxide, paralleling their DNA cleavage abilities. Reduction of nitroblue tetrazolium (NBT)[18] by the HCl salts of **23a** and **23b** were determined at pH 8.0 (20 mM phosphate buffer) containing 1% Triton X-100 at 25°C. For **23a** (1.0 mM) ΔOD_{500nm}/minute = 0.0003 and for **23b** (1.0 mM) ΔOD_{500nm}/minute = 0.0000. For reference[4] 1.0 mM quinocarcin has a ΔOD_{500nm}/minute = 0.002 in the absence of any external reductant

Table I

Entry	Substrate[a]	Concentration [mmol]	% DNA Form I	II	III	S	DNA Cleavage Yield[b] ppm
1	DNA Control		75	25	---	0.29[c]	---
2	**23b** citrate	1.0	77	23	---	-0.03	0.0
3	**23b** citrate	5.0	69	31	---	0.08	0.08
4	**23a** citrate	1.0	63	37	---	0.17	0.85
5	**23a** citrate	5.0	46	54	---	0.49	0.51
6	**23a** hydrochloride	1.0	51	49	---	0.38	2.0
7	**23a** hydrochloride	5.0	19	81	---	1.37	1.4
8	quinocarcin (**1**)	0.1	62	38	---	0.19	9.9
9	quinocarcin (**1**)	1.0	---	84	16	9.5	49
10	**23a** hydrochloride + catalase (10 μg/mL)	5.0	39	61	---	0.65	0.68
11	**23a** hydrochloride + SOD (10 μg/mL)	5.0	60	40	---	0.22	0.23
12	**23a** hydrochloride + desferal	5.0 0.1	27	73	---	1 0	1.0
13	**23a** hydrochloride + desferal	5.0 1.0	37	63	---	0.70	0.73
14	**23a** hydrochloride + desferal	5.0 10	59	41	---	0.24	0.25
15	DNA Control		81	19	---	0.21[c]	---
16	**23a** hydrochloride + DTT	5.0 0.1	10	87	3	3.8	3.9
17	DTT	0.1	61	39	---	0.28	---
18	DTT + catalase (10 μg/mL)	0.1	78	22	---	0.04	---
19	**23a** hydrochloride + DTT + catalase (10 μg/mL)	5.0 0.1	32	68	---	0.93	0.97
20	**23a** hydrochloride + H_2O	5.0 0.1	3.5	89	7.5	6.0	6.2
21	H_2O_2	0.1	74	26	---	0.08	---
22	**23b** citrate + DTT	5.0 0.1	50	50	---	0.45	0.47
23	ascorbic acid	0.1	15	79	6	5.4	280
24	ascorbic acid + catalase (10 μg/mL)	0.1	80	20	---	0.02	1.0

a. Reaction mixtures were 20 mmol in pH 8 phosphate buffer and contained 0.15 μg of pBR 322 plasmid DNA. b. The cleavage yield is expressed by the term S[ccc DNA]/[substrate] and describes the number of single hits per cleavage substrate molecule and allows for a comparison of the relative efficiency of DNA cleavage. c. The S value for the DNA control represents the amount of oc (form II) DNA present in the starting plasmid DNA solution and was subtracted from the S values calculated for the individual cleavage reactions. Measurements of the relative intensity of DNA bands were obtained by scanning densitometry of black and white (Polaroid instant) photographs of the gels (0.8% agarose) visualized by ethidium bromide staining and UV illumination. The mean number of single strang scissions (S)[16] per supercoiled DNA substrate was calculated using the Poisson distribution. When only forms I (ccc or covalently closed circular supercoiled) and forms II (oc or open circular) are present, the equation simplifies to $S = -\ln f_I$, where f_I is the fraction of form I molecules. In those cases where form III (linear) DNA was present, S was calculated from $f_I + f_{II} = [1 - S(2h + 1)/(2L)]^{S/2}$ where h is the distance between hits on opposite strands to produce a linear molecule (16 base pairs)[17] and L is the total number of base pairs in pBR 322 (4362 base pairs). The film used to photograph the gels is assumed to have a linear response to the range of DNA quantities used.[16] Supercoiled DNA is restricted with respect to its ability to bind ethidium bromide and the densitometry values obtained for form I were multiplied by 1.22 as described by Dervan.[16]

(such as DTT). Finally, these substances cleave both double-stranded and single-stranded DNA in a non-sequence specific manner with essentially equal efficacy[7] which argues against the significance of any relative difference in the capacity of these compounds to dock to DNA as a mechanistic determinant relevant to DNA cleavage.

This work demonstrates that simple oxazolidine-containing isoquinolines based on the quinocarcin structure are intrinsically capable of cleaving plasmid DNA and that stereoelectronic elements can markedly attenuate the capacity of these systems to damage nucleic acids. Efforts to attach DNA-binding domains to the synthetic analog nucleus and evaluation of the relative ability of these materials to alkylate DNA as well as their biological activities is under study.

Acknowledgement. This work was supported by the National Institutes of Health (CA43969). We are grateful to Kyowa Hakko Kogyo Co., Japan, for a generous gift of quinocarmycin citrate. We acknowledge Ciba Geigy Corp. and Dr. Peter G. Wuts of the Upjohn Co. for gifts of desferal. We are especially indebted to Professor Tony K. Rappé (CSU) for performing molecular mechanics calculations on **23**. Mrs. Maria Ludwikow and Mr. Robert Hyta are acknowledged for providing technical assistance. Dr. Jed Fisher of the Upjohn Company is thanked for helpful discussions.

EXPERIMENTAL

DNA Nicking Experimentals

DNA nicking reaction mixtures were made up by addition at 0° of appropriate amounts of reagent stock solutions to a stock solution of pBR 322 DNA plasmid (Boehringer-Mannheim Biochemical Co.) containing 0.15 μg DNA per reaction (20 μM base pair concentration). The total volumes of the reaction mixtures were brought up to 10 μl with distilled and deionized water when necessary and the reaction mixtures were incubated at 37° for 2 hours in tightly capped plastic tubes. Stock solutions for DNA including experiments were prepared using distilled, deionized water and commercially available reagents: DTT - Sigma; sodium phosphate monobasic - EM Science; sodium phosphate dibasic, 30% hydrogen peroxide - Malinckrodt; superoxide dismutase, beef liver catalase (suspension in water) - Boehringer Mannheim Biochemical. Desferal was the generous gift from Ciba-Geigy Co. From quinocarcin citrate which was a generous gift from Kyowa Hakko Kogyo Co., Japan, free quinocarcin was obtained by passing it through HP-20 ion exchange resin (Mitsubishi Corp.) at 4°. Citric acid was eluted with water and subsequently free quinocarcin was eluted in methanol/water - 3/1 fraction. Free quinocarcin was further purified by HPLC on C-18 Resolve Column (Waters) using 5% methanol/5% acetonitrile in 6.0mM, pH 6.8 potassium phosphate buffer. To remove the phosphate buffer from lyophilized quinocarcin fraction it was passed through HP-20 column in the same manner as described above.

The degree of DNA nicking was monitored by horizontal gel electrophoresis on 0.8% agarose gel onto which the whole volumes of the reaction mixtures were loaded after prior addition of 3 μl of loading buffer (0.25% bromophenol blue, 40% sucrose). The electrophoreses were run for 2 h at 55V and the gels were submerged for 15 min in ethidium bromide solution. The electrophoresis gels were immediately visualized on a UV transilluminator and photographed using black and white instant films (Polaroid T667). The measurements of the relative intensities of DNA bands were performed on the photographs using the Dell System 325 computer and Technology Resources Inc. image processing software. The average number of nicks per DNA molecule S was calculated according to the method described by Dervan.[16]

Synthesis of Quinocarcin Analogs

(2-Methoxyphenyl)oxirane **12**. A nonhomogenous mixture of o-anisaldehyde (20.0 g, 0.147 mol, 1.0 eq.), trimethylsulfonium iodide (37.0 g, 0.177 mol, 1.2 eq.), tetra n-butylammonium iodide (0.52 g, 0.0014 mol, 0.01 eq.), CH_2Cl_2 (500 mL) and aqueous NaOH (50%, 330 mL) was vigorously stirred at room temperature for 5 days. After dilution with water the organic layer was separated, washed with water, dried over Na_2SO_4, concentrated in vacuum and the residue was Kugelrohr distilled to yield the pure product in form of colorless liquid (20.4 g, 92.5%).

12. ^1H NMR (270 MHz) ($CDCl_3$) TMS: 2.69 (1 H, q, J = 2.4 Hz), 3.12 (1 H, q, J = 4.6 Hz), 3.85 (3 H, s), 4.20 (1 H, t, J = 2.7 Hz), 6.91 (2 H, m), 7.15 (1 H, q, J = 1.6 Hz), 7.25 (1 H, m). IR (NaCl, neat):

3051, 3002, 2941, 2838, 1689, 1602, 1496, 1466, 1439, 1391, 1287, 1256, 1103, 1048, 1027, 989, 880, 755 cm^{-1}.

Glycine ethyl ester N-carbamate **14**. A solution of epoxide **12** (0.69 g, 4.60 mmol, 1.0 eq.) and phosgene (0.85 g, 8.58 mmol, 1.86 eq.) in benzene (10 mL) was kept in sealed flask for 48 h. The solvent was removed under reduced pressure (prior to this with larger scale runs the reaction mixture has to be purged with the flux of dry nitrogen and the excessive phosgene should be deactivated by passing through aqueous solution of alkali). The oily residue of crude chloroformate **13** was redissolved in CH_2Cl_2 (15 mL), and a solution of saturated $NaHCO_3$ was added (15 mL), followed by a solution of glycine ethyl ester hydrochloride (0.64 g, 4.6 mmol, 1.0 eq.) in small volume of water. After 10 min of vigorous stirring at room temperature the organic layer was separated, washed with water, dried over $MgSO_4$ and concentrated to yield crude product as yellow oil from which pure **14** was isolated by radial chromatography (CH_2Cl_2/CH_3OH - 10/1) 0.83 g (57%). Analytical sample was obtained by recrystallization from isopropyl alcohol, mp = 61-63°.

13. ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 3.85 (3 H, s); 4.55 (1 H, dd, J = 11.3 Hz, J = 5.0 Hz); 4.67 (1 H, dd, J = 11.3 Hz, J = 7.9 Hz); 5.62 (1 H, dd, J = 7.9 Hz, J = 5.0 Hz); 6.90 (1 H, d, J = 8.3 Hz); 6.98-7.03 (1 H, m); 7.30-7.36 (1 H, m); 7.48 (1 H, dd, J = 7.6 Hz, J = 1.6 Hz); IR (NaCl, neat): 1779, 1492, 1252, 1145, 755 cm^{-1}.

14. ^1H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.28 (1 H, t, J = 7.2 Hz); 3.85 (3 H, s); 3.95 (2 H, d, J = 5.5 Hz); 4.21 (2 H, q, J = 7.2 Hz); 4.44-4.48 (2 H, m); 5.32 (1 H, br s); 5.58 (1 H, t, J = 6.3 Hz); 6.88 (1 H, d, J = 8.4 Hz); 6.98 (1 H, t, J = 7.2 Hz); 7.30 (1 H, m); 7.48 (1 H, dd, J = 7.7 Hz, J = 1.6 Hz). IR (NaCl, neat): 3357, 1729, 1533, 1495, 1252, 1201, 1052, 1026, 757 cm^{-1}. Anal. Calcd for $C_{14}H_{18}ClNO_5$: C, 53.25; H, 5.74; N, 4.43. Found: C, 53.40; H, 5.81; N, 4.39.

Cyclic urethane **15**. To a solution of **14** (15.7 g, 50.0 mmol, 1.0 eq.) in THF (150 mL) cooled to 0°C a solution of potassium t-butoxide (6.13 g, 55.0 mmol, 1.1 eq.) in THF (75 mL) was added slowly with stirring. After 0.5 h the reaction mixture was diluted with water, slightly acidified with dilute HCl and extracted with CH_2Cl_2. The organic extract was dried over Na_2SO_4, filtered and concentrated in vacuum to yield crude **11**[5] as brownish oil (14.1 g, quant.). Ethanol (150 mL) was added followed by LiOH monohydrate (2.8 g, 66.7 mmol, 1.3 eq.) in water (60 mL) at 0°C. After 0.5 h the reaction mixture was concentrated under reduced pressure to a half volume at room temperature, diluted with water, acidified and extracted with ethyl acetate. The extract was dried over Na_2SO_4, filtered and concentrated. From the oily residue pure **12**[5] was obtained by crystallization from ethyl acetate (9.3 g, 74%).

Isoquinolone **16**. **14** was obtained from acid **12** as previously described.[5] Substituting CH_2Cl_2 for tetrachloroethane as a solvent for Friedel-Crafts cyclization improved the yield to 74% (on 40.0 mmol scale).

β-Ketoester **17**. To a stirred solution of **16** (120 mg, 0.515 mmol, 1.0 eq.) in 10% HMPA/THF (8.8 mL) cooled to -78°C was added $(TMS)_2NLi$ in THF (0.60 mL, 1.0 M, 0.60 mmol, 1.15 eq.). The resulting solution was stirred at -78°C for 45 min when cyanoethylformate (51 mg, 0.515 mmol, 1.0 eq.) was added in one portion. The reaction was stirred at -78°C for 2 h and quenched with saturated aqueous NH_4Cl (0.60 mL, 1.0 M, 1.5 mL), diluted with CH_2Cl_2 (50 mL) and washed with water and brine. The organic layer was dried over $MgSO_4$, filtered, evaporated and the residue was chromatographed on silica gel (hexane/ethyl acetate - 3/2) yielding **17** as an amorphous solid (126 mg, 80%).

17. ^1H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$: 1.24 (3 H, t, J = 8.2 Hz), 3.87 (3 H, s); 4.11-4.25 (3 H, m); 5.05 (1 H, t, J = 8.5 Hz); 5.32 (1 H, s); 5.57 (1 H, t, J = 8.5 Hz), 7.16 (1 H, d, J = 7.7 Hz), 7.43 (1 H, t, J = 8.0 Hz); 7.71 (1 H, d, J = 7.8 Hz); IR (NaCl, CH_2Cl_2): 1765, 1750, 1700, 1250 cm^{-1}; mass spectrum, $CI(NH_3)$, m/e = 305 (100), 233 (12).

β-Hydroxyester **18**. To a solution of ketoester **17** (267 mg, 0.87 mmol, 1.0 eq.) $NaBH_3CN$ (89 mg, 1.50 mmol, 1.72 eq.) was added at room temperature and the reaction mixture was stirred for 4 h. After diluting with CH_2Cl_2 the organic layer was washed with water, Na_2CO_3 solution, water and dried over Na_2SO_4. Evaporation of the solvent yielded yellowish oily product which crystallized on standing (226 mg, 84%). Analytical sample was obtained by recrystallization from ethyl acetate/hexane mp = 119-121°.

18. ^1H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.23 (3 H, t, J = 7.0); 3.83 (3 H, s); 4.03 (1 H, t, J = 8.8 Hz); 4.92-5.18 (4 H, m); 6.81-6.84 (1 H, m); 7.26-7.37 (2 H, m). IR (KBr pellet): 3520, 1753, 1717, 1413, 1221. Anal. Calcd for $C_{15}H_{17}NO_6$: C, 58.62; H, 5.57; N, 4.56. Found: C, 58.72; H, 5.60; N, 4.44.

Hydroxyamide **19**. To a solution of crude β-hydroxyester **18** (148 mg, 0.48 mmol, 1.0 eq.) in ethanol (5.0 mL) aqueous LiOH (2.0 M, 0.36 mL, 0.72 mmol, 1.5 eq.) was added at room temperature. After 0.5 h at room temperature the reaction mixture was diluted with water and washed with CH_2Cl_2. The aqueous layer was acidified with HCl (2.0 M, 0.4 mL) and extracted with CH_2Cl_2. Drying of the organic extract over Na_2SO_4 and

evaporation of the solvent yielded slightly yellowish solid (105 mg) which was refluxed in benzene (2.0 mL) with thionyl chloride (134 mg, 1.13 mmol, 3.0 eq.) for 1.5 h. The reaction mixture was concentrated in vacuum, the oily residue was redissolved in dry CH_2Cl_2 and excess of 2,2,3-trimethylethanolamine (118 mg, 1.13 mmol, 3.0 eq., obtained from 2,2-dimethylethanoloamine by treatment with methylchloroformate and subsequent reduction of the methylurethane with excess of lithium aluminum hydride) in CH_2Cl_2 (2.0 mL) was added with ice-water cooling. After 20 min at room temperature the reaction mixture was washed with water and the hydroxyamide **19** (63 mg, 48%) was isolated by radial chromatography on silica gel (ethyl acetate/hexane - 2/1) as crystalline colorless solid. Analytical sample was obtained by recrystallization from methanol mp = 188-190°.

19. ^{1}H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.43 (3 H, s); 1.46 (3 H, s); 3.06 (3 H, s); 3.83 (3 H, s); 3.80-3.92 (2 H, m); 4.56 (1 H, dd, J = 10.8, J = 9.3 Hz); 5.09 (1 H, dd, J = 8.8 Hz, J = 8.1 Hz); 5.39 (1 H, dd, J = 10.9 Hz, J = 8.2 Hz); 6.03 (1 H, br s); 6.77-6.85 (2 H, m); 7.23-7.30 (1 H, m). IR (NaCl, neat): 3500, 1755, 1633, 1575 cm^{-1}. Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.41; H, 6.40; N, 8.09. Found: C, 62.21; H, 6.44; N, 7.89.

Hydroxyamides **20A,B**. A solution of unsaturated amide **19** (252 mg, 0.728 mmol) in ethanol (100 mL) was hydrogenated under 60 psi H_2 at room temperature with 5% palladium on charcoal catalyst (240 mg) for 12 h. The mixture of diastereoisomeric products was separated by PTLC chromatography on silica gel (CH_2Cl_2/THF - 20/1) to yield amides **20A** (131 mg, 52%) and **20B** (55 mg, 22%) as colorless oils. Analytical samples were obtained by recrystallization. **20A** (ethyl acetate) mp= 197.5-198.5°; **20B** (ethyl acetate/hexane) mp = 131-132°.

20A. ^{1}H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.39 (3 H, s); 1.42 (3 H, s); 2.88 (1 H, dd, J = 16.9 Hz, J = 3.5 Hz); 3.06 (3 H, s); 3.20 (1 H, br s); 3.41 (1 H, dd, J = 16.3 Hz, J = 11.6 Hz); 3.83 (3 H, s); 4.12 (1 H, dd, J = 11.3 Hz, J = 3.6 Hz); 4.23 (1 H, dd, J = 9.0 Hz, J = 6.3 Hz); 4.63 (1 H, dd, J = 11.4 Hz, J = 1.2 Hz); 4.85 (1 H, t, J = 8.9 Hz); 5.14-5.20 (1 H, m); 6.75-6.82 (2 H, m); 7.20-7.27 (1 H, m). IR (NaCl, neat): 3474, 1731, 1645, 1583 cm^{-1}. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 61.92; H, 6.75; N, 7.73.

20B. ^{1}H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$: 1.22 (3 H, s); 1.23 (1 H, s); 3.02 (3 H, s); 2.98-3.12 (2 H, m); 3.59 (2 H, br s); 3.78 (3 H, s); 3.87-3.94 (1 H, m); 4.09 (1 H, t, J = 8.7 Hz); 4.90 (1 H, t, J = 8.8 Hz); 5.11 (1 H, dd, J = 7.5 Hz, J = 4.6 Hz); 5.18 (1 H, t, J = 8.6 Hz); 6.68 (1 H, d, J = 8.2 Hz); 6.75 (1 H, d, J = 8.2 Hz); 7.16 (1 H, t, J = 7.9 Hz). IR (NaCl, neat): 3459, 1745, 1648, 1591, 1084, 731 cm^{-1}. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 61.99; H, 6.86; N, 7.82.

Hydroxyamines **21A,B**. To a suspension of **20A** (129 mg, 0.37 mmol, 1.0 eq.) in dry THF (10 mL) solution of borane in THF (1.0 M, 1.85 mL, 5.0 eq.) was added at room temperature under N_2. After 5 h at room temperature 1.0 M aqueous $(NH_4)_2CO_3$ was added and stirring was continued for another 5 h. The reaction mixture was concentrated and partitioned between water and methylene chloride. Separation on silica gel by radial chromatography (CH_2Cl_2/MeOH - 10/1) yielded starting amide (13 mg, 10%) and amine **21A** (80 mg, 65%) as colorless oil.

21A. ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.03 (3 H, s); 1.07 (3 H, s); 2.31 (3 H, s); 2.70 (1 H, br s); 2.76-2.85 (2 H, m); 3.06 (1 H, dd, J = 15.8 Hz, J = 8.8 Hz); 3.16 (1 H, dd, J = 13.3 Hz, J = 5.3 Hz); 3.30 (1 H, 1/2 ABq, J = 10.8 Hz); 3.45 (1 H, 1/2 ABq, J = 10.8 Hz); 3.58-3.67 (1 H, m); 3.82 (3 H, s); 4.31 (1 H, dd, J = 9.0 Hz, J = 6.6 Hz); 4.76 (1 H, t, J = 8.7 Hz); 5.00 (1 H, t, J = 7.2 Hz); 6.78 (1 H, d, J = 8.1 Hz); 6.82 (1 H, d, J = 7.6 Hz); 7.23 (1 H, t, J = 8.1 Hz). IR (NaCl, neat): 3457, 1747, 1586, 1070 cm^{-1}.

By analogous procedure **21B** was obtained from **20B** in 88% yield as colorless oil.

21B. ^{1}H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$: 0.89 (3 H, s); 0.92 (3 H, s); 2.24-2,34 (1 H, m); 2.29 (3 H, s); 2.53 (1 H, dd, J = 13.1 Hz, J = 8.8 Hz); 2.71 (1 H, d, J = 12.9 Hz); 3.05-3.25 (3 H, m); 3.80 (3 H, s); 4.10 (1 H, t, J = 7.9 Hz); 4.35-4.43 (1 H, m); 4.90 (1 H, t, J = 8.1 Hz); 4.96-5.02 (1 H, m); 6.71-6.75 (2 H, m); 7.18 (1 H, t, J = 8.0 Hz). IR (NaCl, neat): 3455, 1751, 1587, 1077 cm^{-1}; mass spectrum m/e = 335 (M^+ +1), 333, 303, 263.

Aldehydes **22A,B**. To a solution of DMSO (7 mg, 0.09 mmol, 3.0 eq.) in dry methylene chloride (0.2 mL) at -78° oxalyl chloride (5.7 mg, 0.045 mmol, 1.5 eq.) was added and after 15 min at -78° addition of hydroxyamine **21A** (11 mg, 0.03 mmol, 1.0 eq.) in methylene chloride (0.2 mL) followed. After 1.5 h at -78°C triethylamine (30 mg, 0.30 mmol, 1.0 eq.) was added and stirring was continued for 30 min. The reaction mixture was concentrated under reduced pressure, diluted with methylene chloride and washed with water. The organic layer was dried over Na_2SO_4 and concentrated to furnish pure aldehyde **22A** (11 mg, quant.) as colorless oil.

22A. ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.09 (3 H, s); 1.12 (3 H, s); 2.30 (3 H, s); 2.70 (1 H, dd, J = 13.3 Hz, J = 9.2 Hz); 2.88 (1 H, dd, J = 15.7 Hz, J = 4.1 Hz); 3.02 (1 H, dd, J = 13.2 Hz, J = 4.5 Hz);

3.09 (1 H, dd, J = 15.8 Hz, J = 8.1 Hz); 3.62-3.68 (1 H, m); 3.82 (3 H, s); 4.30 (1 H, dd, J = 8.9 Hz, J = 7.2 Hz); 4.77 (1 H, dd, J = 9.0 Hz, J = 8.1 Hz); 4.98 (1 H, t, J = 7.4 Hz); 6.78 (1 H, d, J = 8.3 Hz); 6.82 (1 H, d, J = 7.7 Hz); 7.24 (1 H, t, J = 7.7 Hz); 9.47 (1 H, s). IR (NaCl, neat): 1730, 1586, 1470, 1070 cm^{-1}

By analogous procedure aldehyde **22B** was obtained from **21B** in 65% yield as colorless oil.

22B. ^1H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$: 0.93 (3 H, s); 0.99 (3 H, s); 2.23-2.42 (5 H, m); 2.88 (1 H, d, J = 16.3 Hz); 3.06 (1 H, dd, J = 16.2 Hz, J = 5.8 Hz); 3.80 (3 H, s); 3.98-4.07 (1 H, m); 4.37-4.45 (1 H, m); 4.86-4.94 (1 H, m); 6.71-6.75 (2 H, m); 7.20 (1 H, t, J = 7.9 Hz); 9.25 (1 H, s). IR (NaCl, neat): 1756, 1587, 1472, 1258, 1078 cm^{-1}.

Quinocarcin analogs **23A,B**. To a solution of crude **22A** (11.0 mg, 0.03 mmol) in ethanol (2 mL) aqueous LiOH solution (2.0 M, 0.2 mL) was added and the mixture was refluxed under N_2 for 48 h. The reaction mixture was diluted with methylene chloride and washed with brine. The organic layer was dried over Na_2SO_4, concentrated and the oily residue was separated by silica gel PTLC (CH_2Cl_2/MeOH - 10/1) to yield starting aldehyde **22A** (2.2 mg, 20%), oxazolidine **23A** (4.2 mg, 44%) and alcohol **21A** (1.0 mg, 10%) as colorless oils. Recrystallization from pentane produced crystalline **23A** mp = 111-113° which was used for X-ray structure determination.

23A. ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 0.95 (3 H, s); 1.23 (3 H, s); 2.32 (3 H, s); 2.39 (1 H, dd, J = 11.5 Hz, J = 9.5 Hz); 2.73-2.98 (4 H, m); 3.52-3.67 (3 H, m); 3.77 (3 H, s); 4.55 (1 H, t, J = 6.2 Hz); 6.67 (1 H, d, J = 8.2 Hz); 6.77 (1 H, d, J = 7.6 Hz); 7.14 (1 H, t, J = 8.1 Hz). IR (NaCl, neat): 1581, 1470, 1260, 1087, 1018, 779 cm^{-1}.

By analogous procedure **23B** was obtained from **22B** as colorless oil with 27% yield. Recrystallization from ethyl acetate/hexane produced crystalline product mp = 159-160° which was used for X-ray structure determination.

23B. ^1H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.16 (3 H, s); 1.33 (3 H, s); 2.34 (3 H, s); 2.52-2.75 (4 H, m); 2.95-3.05 (1 H, m); 3.62 (1 H, d, J = 7.3 Hz); 3.77 (3 H, s); 4.16 (2 H, t, J = 7.4 Hz); 4.42 (1 H, t, J = 7.7 Hz); 6.71 (1 H, d, J = 7.5 Hz); 6.74 (1 H, d, J = 7.6 Hz); 7.18 (1 H, t, J = 7.9 Hz). IR (NaCl, neat): 1588, 1473, 1260, 1018, 786, 744 cm^{-1}.

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