DISSERTATION

PART I. ASYMMETRIC SYNTHESIS OF 2,6-DIAMINOPIMELIC ACIDS (DAP) AND γ-D(L)-GLUTAMYL-L-*meso*-DIAMINOPIMELIC ACID DIPEPTIDE

PART II. TOTAL SYNTHESIS OF TAN-1057 AND ANALOGUES

Submitted by

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Department of Chemistry

In partial fulfillment of the requirements

for the degree of Doctor of Philosophy

Colorado State University

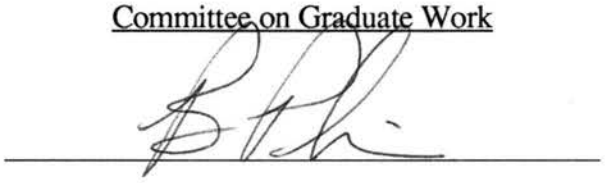
Spring 1997

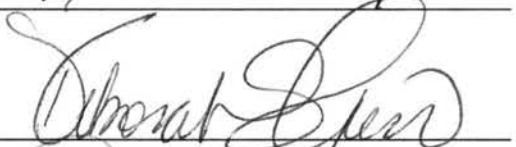
COLORADO STATE UNIVERSITY

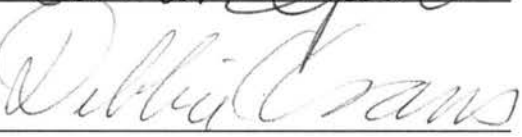
January 28, 1997

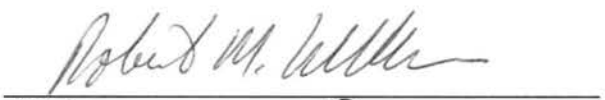
WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY CHENGUANG YUAN ENTITLED "PART I. ASYMMETRIC SYNTHESIS OF 2,6-DIAMINOPIMELIC ACIDS (DAP) AND γ-D(L)-GLUTAMYL-L-*meso*-DIAMINOPIMELIC ACID DIPEPTIDE; PART II. TOTAL SYNTHESIS OF TAN-1057 AND ANALOGUES" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work









Adviser

Department Head

ABSTRACT OF DISSERTATION

PART I. ASYMMETRIC SYNTHESIS OF 2,6-DIAMINOPIMELIC ACIDS (DAP) AND γ-D(L)-GLUTAMYL-L-*meso*-DIAMINOPIMELIC ACID DIPEPTIDE

An asymmetric and stereochemically unambiguous construction of diaminopimelic acid and related system using the chiral, non-racemic diphenyl oxazinones glycinate templates has been developed. The preparations of (R,R)-DAP, (S,S)-DAP, (S,S)-2,7-diaminosuberic acid, and mono-N-protected (S,R)-DAP are described. The synthesis of γ-D(L)-glutamyl-L-*meso*-diaminopimelic acid dipeptide, a subunit of both FK-156 and FK-565, is also described. The availability of both optical antipodes of the glycinate templates renders this chemistry adaptable to prepare all possible diastereoisomers of substances based on the DAP skeleton in optically pure form.

PART II. TOTAL SYNTHESIS OF TAN-1057 AND ANALOGUES

The first total synthesis of anti-MRSA dipeptides TAN-1057 A~D has been achieved. A new efficient method for preparation of amidinoureas has been developed and successfully applied to the total synthesis of TAN-1057. More importantly, this concise total synthesis paves the way for access to analogues that are not available from natural sources. Eight new analogues of TAN-1057 were designed, synthesized and assayed against MRSA. A new synthetic analogue **102** (**CY-1800**) showed very similar activity to that of TAN-1057. Other analogues prepared showed weak or no activity against *Staphylococcus aureus* up to 1 mg/mL. The success on discovery of a new potent anti-MRSA analogue proved the usefulness of this highly flexible strategy for the development of better analogues than the natural ones for the potential use as antibiotics.

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I would like to recognize and thank the following persons for their contribution to the success of this project: Dr. Chris for assistance in performing NMR analysis, Don Dick for conducting most the mass spectral analyses and Renè Gallegos for tutoring the author the technique of agar diffusion assay. Takeda Chemical Industries, LTD. is acknowledged for providing authentic TAN-1057 A and B. Microcide Pharmaceuticals Inc. was kind enough to perform the bioassay. The author is indebted to professor Marc Greenberg for writing letters of recommendation.

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Finally, I would like to thank Dr. Williams for providing laboratory space, chemical, equipment and financial support during my stay at CSU. More importantly, Dr. Williams provided me freedom to pursue my own intellectual interests. I have learned an incredible amount of science from him and thank him for the opportunity of working in his laboratories.

DEDICATION

I wish to dedicate this thesis to my parents and my wife Min Liu. Without their love and constant support none of this work would have been possible.

Table of Contents

Abbreviations

aq.	aqueous
AZIDAP	2-(4-amino-4-carboxybutyl)-2-aziridinecarboxylic acid
t-BOC	*tert*-butoxycarbonyl
$(BOC)_2O$	di-*tert*-butyl dicarbonate
BOC-ON	[2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitile]
BOP-Cl	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
CBz	carbobenzyloxy
CBz-Cl	benzyl chloroformate
CD	circular dichroism
(S,S)-CHIRAPHOS	(bicyclo[2.2.1]hepta-2,5-diene)[(2S,3S)-bis(diphenylphosphino)butane]rhodium perchlorate
DAP	2,6-diaminopimelic acid
DCC	1,3-dicyclohexylcarbodiimide
de	diastereomeric excess
DIEA	diisopropylethylamine
DMAP	4-dimethylamino pyridine
DMF	N,N-dimethylformamide
ED	effective dose
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ee	enantiomeric excess
eq	eqivalent
h	hour
HMPA	hexamethylphosphoramide
HOAt	1-hydroxy-7-azabenzotriazole

Abbreviations

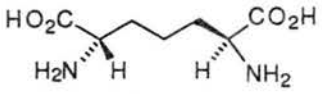
HOBt	1-hydroxybenzotriazole
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectrum
LD_{50}	Median lethal dose
MIC	minimal inhibitory concentration
min	minute
mmol	mini mole
MNNG	1-methyl-3-nitro-1-nitrosoguanidine
mol	mole
MRSA	methicillin-resistant *Staphylococcus aureus*
MTPA	α-methoxy-α-(trifluoromethyl)phenyl acetic acid (Mosher's acid)
NBS	N-bromosuccinimide
NCS	N-chlorosuccinimide
NMM	4-methylmorpholine
pht	phthaloyl
TBTU	*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMP	2,4,6-trimethylpyridine
TMS	tetramethylsilane
TsOH	*para*-toluenesulfonic acid

PART 1 Asymmetric Synthesis of 2,6-Diaminopimelic Acids (DAP) and γ-D(L)-Glutamyl-L-*meso*-Diaminopimelic Acid Dipeptide

Chapter 1. Introduction

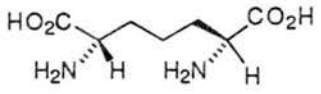
1.1 The Biology of DAP and DAP Analogues

2,6-Diaminopimelic acid (DAP), an important, naturally occurring amino acid was first isolated from the hydrolysates of *Corynebacterium diphtherine* by Work in the 1950's[1], and later found to exist in a wide range of higher plant and related organisms. Interestingly, DAP, as a mixture of all three possible stereoisomers, was first synthesized by Sorensen and Andersen in 1908, much earlier than when it was discovered in nature. [1]



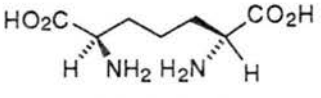
L,L-DAP, 1a

$[\alpha]^{24}_D$ = + 8.14(c 5, H_2O)
$[\alpha]^{24}_D$ = + 45.1(c 2.6, 5N HCl)



***meso*-DAP, 1b**

$[\alpha]^{24}_D$ = 0(c 5, H_2O)



D,D-DAP, 1c

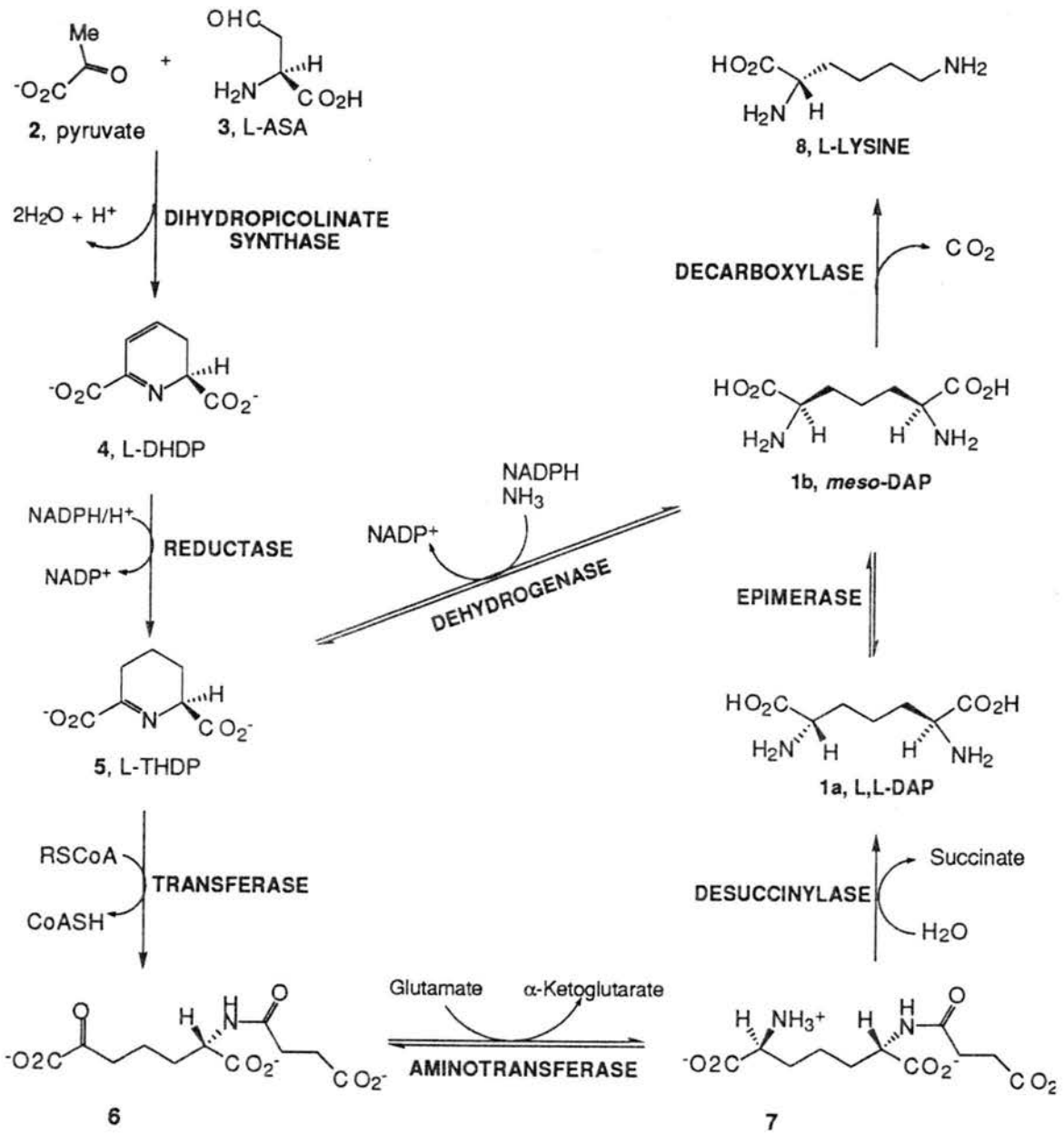
$[\alpha]^{24}_D$ = - 8.45(c 5, H_2O)
$[\alpha]^{24}_D$ = - 44.6(c 2.6, 5N HCl)

FIGURE 1.

After successful separation of the three stereoisomers from a synthetic DAP mixture, the naturally occurring stereoisomers were identified as L,L-DAP and *meso*-L,D-DAP, as predicted D,D-DAP was not found in nature. [1,2]

In plant and bacterial metabolism, L,L-DAP and *meso*-DAP are pivotal amino acids serving as the conduit through which L-lysine, an essential amino acid for protein synthesis, is biosynthesized (Scheme 1). L-Lysine is formed in bacteria via two main avenues from L-tetrahydrodipicolinic acid (**5**, L-THDPA). Most organisms synthesize L,L-DAP and employe an epimerase to convert it into *meso*-L,D-DAP. However, some

organisms, such as *Bacillus sphacericus*, have evolved a more direct route that bypasses L,L-DAP employing a dehydrogenase-mediated reductive amination of L-THDPA directly to *meso*-L,D-DAP. [3]



SCHEME 1

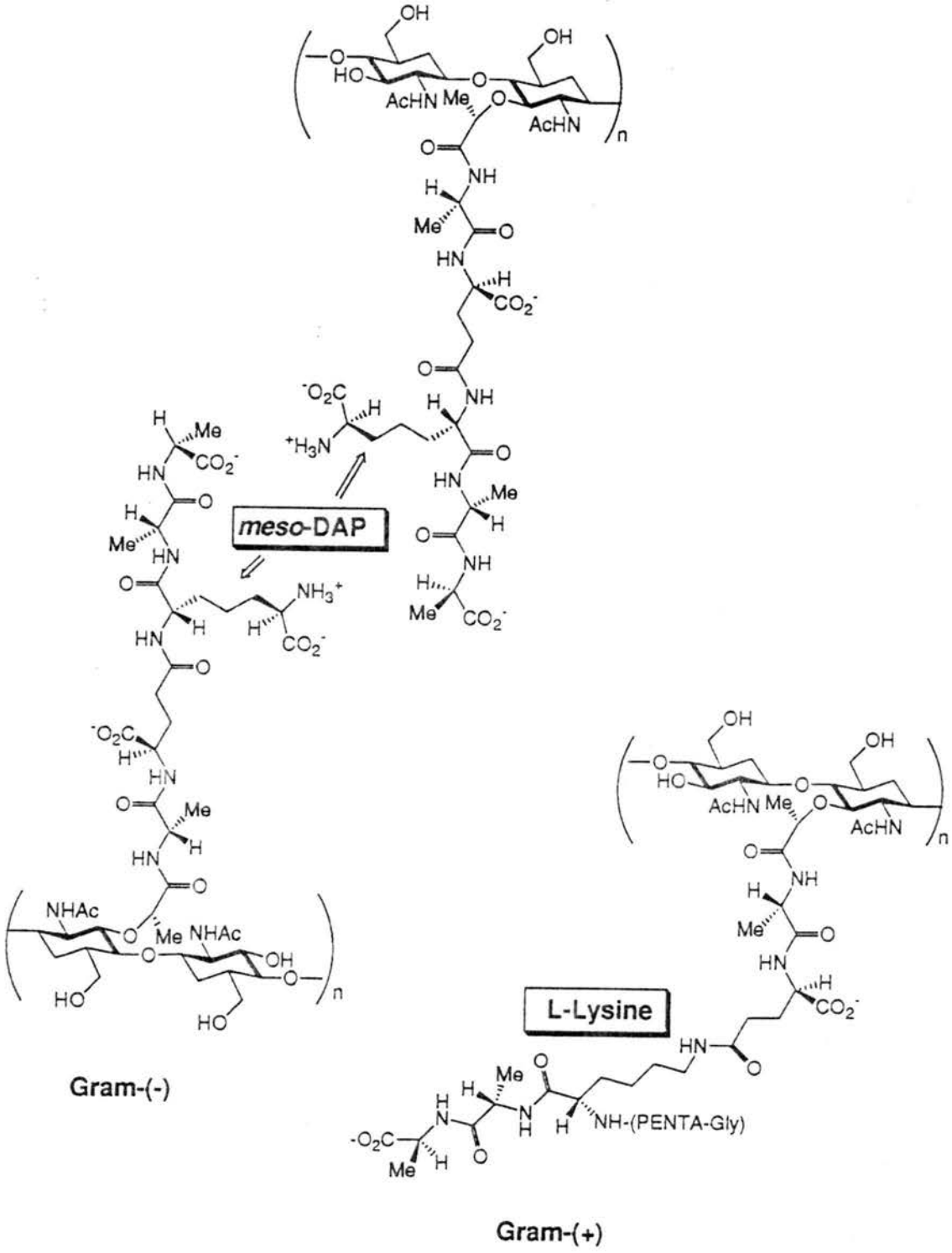


FIGURE 2

TABLE 1 Enzymological and Biological Properties of DAP Analogues

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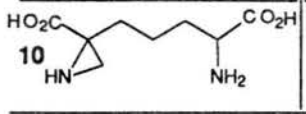
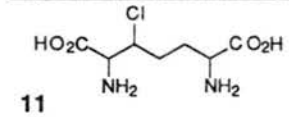
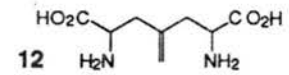
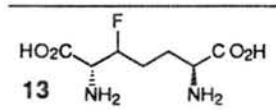
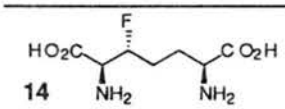
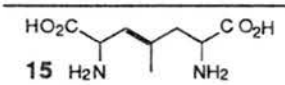
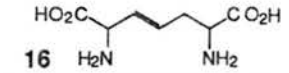
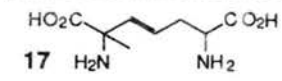
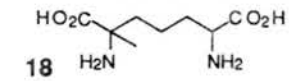
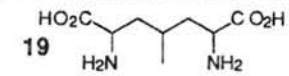
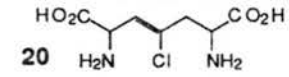
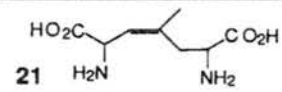
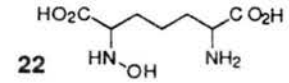
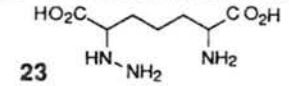
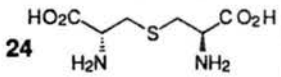
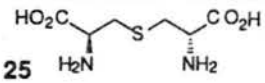
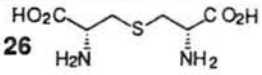
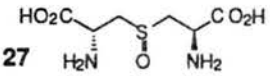
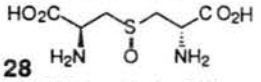
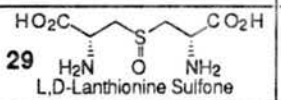
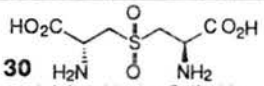
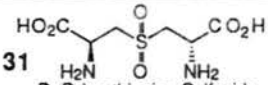
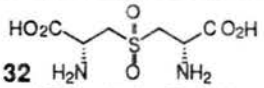
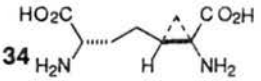
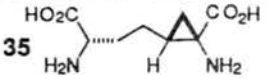
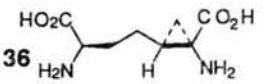
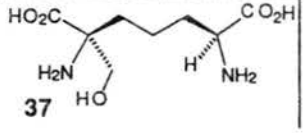
 **10**	++	nd	nd	++	5
 **11**	++	nd	nd	++	9
 **12**	++	-	+	++	8
 **13**	++	nd	nd	-	7
 **14**	++	nd	nd	+	7
 **15**	nd	+	nd	+	6
 **16**	nd	++	nd	-	6
 **17**	nd	-	nd	+	6
 **18**	nd	+	nd	+	6
 **19**	nd	+	nd	-	6
 **20**	nd	+	nd	+	6
 **21**	nd	+	nd	+	6
 **22**	++	-	++	++	8
 **23**	+	–	+	++	8

TABLE 1 (con't.)

DAP ANALOGUE	Epimerase Inhibition	Decarboxylase Inhibition	Dehydrogenase Substrate	Antimicrobial Activity	Ref.
24 	+	-	-	-	8
25  D, D-Lanthionine	+	-	-	-	8
26  L,D-Lanthionine	-	-	-	-	8
27  L,L-Lanthionine Sulfone	-	+	-	-	8
28  D, D-Lanthionine Sulfone	-	-	-	-	8
29  L,D-Lanthionine Sulfone	-	-	-	-	8
30  L,L-Lanthionine Sulfoxide	-	-	-	-	8
31  D, D-Lanthionine Sulfoxide	-	-	-	-	8
32  L,D-Lanthionine Sulfoxide	-	-	-	-	8
34  2S,3S,6S-cyclopropylDAP	nd	nd	nd	-	10
35  2R,3R,6S-cyclopropylDAP	nd	nd	nd	-	10
36  2S,3S,6R-cyclopropylDAP	nd	nd	nd	-	10
37 	nd	nd	nd	-	11

LEGEND: ++ = significant antibiotic activity
+ = weak antibiotic activity
- = no antibiotic activity
nd = not determined (unknown)
* For the cases of the dehydrogenase, the above legends refer to capacity to act as a substrate.

Significantly, *meso*-DAP or its biosynthetic product, L-lysine, is a ubiquitous cross-linking constituent of the peptidoglycan layer of virtually all Gram-negative and some Gram-positive bacterial cell walls and serves to anchor various membrane-associated macromolecules, such as lipoprotein to the cell wall. The peptidoglycan, a polymeric compound, consists of chains of alternating N-acetylglucosamine and N-acetylmuramic acid, cross linked by short peptides. The cross links give the cell wall the strength to resist lysis caused by high intracellular osmotic pressures.

Inhibitors of L-lysine or DAP biosynthesis could therefore be very effective antibiotics, like other successful drugs targeted towards cell wall biosynthesis, such as β-lactams and glycopeptides.

Mammalian organisms on the other hand, lack the lysine/DAP biosynthetic pathway and require ingestion of L-lysine as an essential dietary constituent. Furthermore, when administered to mammals, DAP or small peptides containing DAP are excreted. This offers the potential for the development of novel antibiotics with low mammalian toxicity.

Recognition of the pivotal roles that DAP plays in microbial metabolism [2] and cell wall structure has resulted in an increased level of interest in possible means to disrupt the DAP biosynthetic pathway.

A number of DAP-analogues had been synthesized and assayed against the epimerase, decarboxylase, and dehydrogenase enzymes involved in the DAP/lysine biosynthesis (Table 1). [4]

Of the compounds examined, the AZIDAP **10**, 4-methylene DAP derivative **12**, 3-chloro-DAP **11**, and the interesting substances **22** and **23** displayed significant antimicrobial activity. Weak antimicrobial activity was displayed by **14, 15, 17, 18, 20**

and **21**. Of these compounds, several showed inhibition of one or more enzymes involved in DAP biosynthesis.

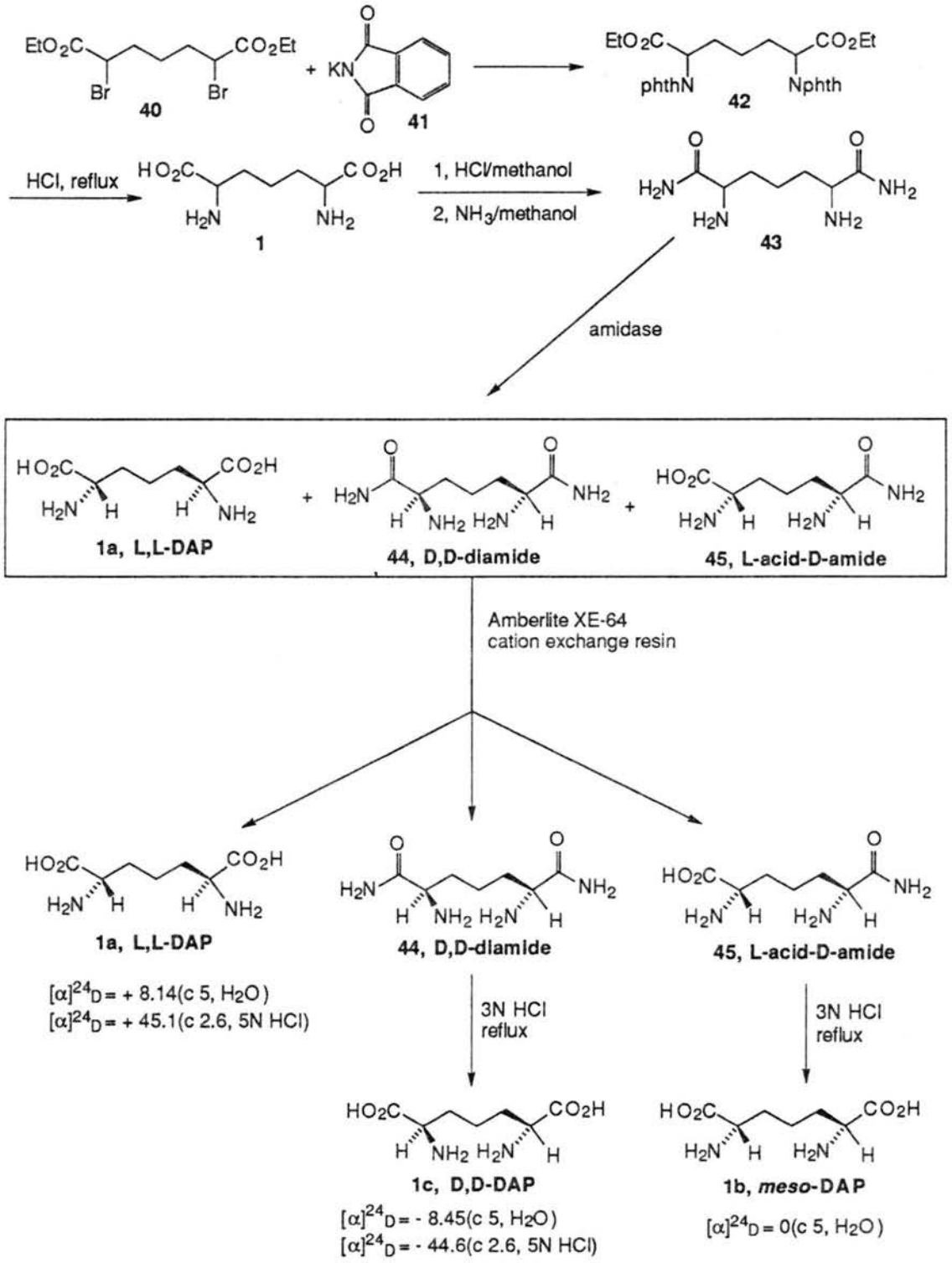
So far, none of the synthesized DAP-analogues presently have commercial value. This is primarily due to the limited number of compounds that have been synthesized, the incomplete knowledge of the mechanism of action of active derivatives and the most promising target enzymes whose inhibition would be manifested as antibiotic activity.

Until recently attention has been focused mainly on those enzymes of the pathway where the substrates were available. Development of specific inhibitors for lysine biosynthetic enzymes rely heavily on novel syntheses. The lack of readily available substrates has stimulated efforts towards the synthesis of the DAP-analogues.

1.2 The Syntheses of DAP and DAP-analogues

Soon after the discovery of DAP, the desire to identify the stereochemistry of naturally occurring DAP stimulated efforts to improve the original synthesis and the separation of the stereoisomers of DAP. As shown in Scheme 2, Work and associates [12] improved the synthesis of DAP by mixing of 1,3-dibromopimelate **40** with potassium phthalimide **41** in DMF, followed by acidic hydrolysis to give a mixture of stereoisomers of DAP. They developed a resolution process for DAP by converting DAP into the corresponding diamide followed by treatment with a hog kidney amidase-Mn^{++}. The action of this exclusively L-directed enzyme led to a mixture of the free L,L-DAP, the D,D-diamide **44**, and the L-diaminopimelic acid D-monoamide **45**. Paper chromatography of the reaction mixture revealed the three components as distinct ninhydrin-reactive spots and was subsequently employed to follow their separation on an Amberlite XE-64 cation exchange resin. The separated amides were hydrolyzed to the respective free DAP isomers. However, during this separation process, some overlap of the mono- and di-amide

fractions occurred which resulted in reduced yields of the corresponding DAP isomers subsequently obtained therefrom.

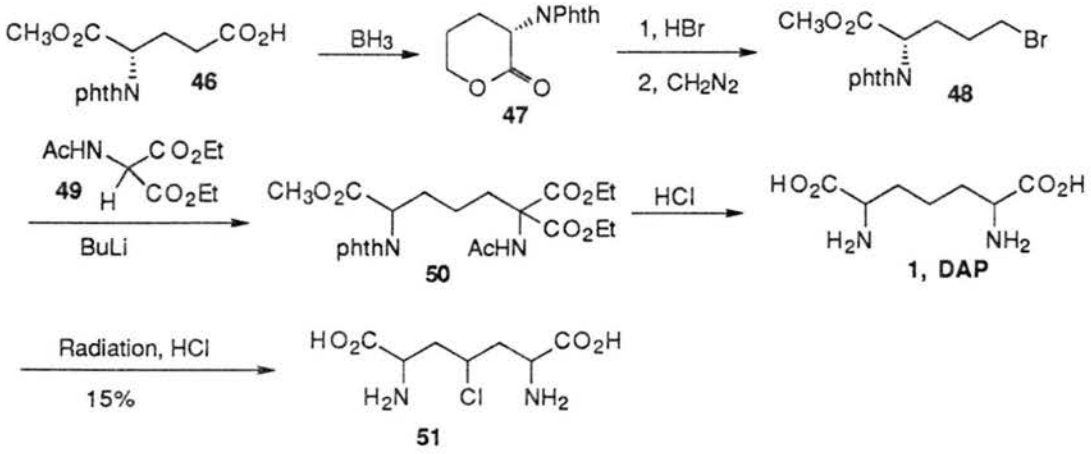


SCHEME 2

Wade and associates [13] improved this resolution process by treating the DAP mixture with CBz-Cl to convert them into corresponding CBz-derivatives. The *meso*-di-CBz-DAP can be separated from the mixture by recrystallization twice from ethyl acetate. The enzymatic resolution between LL-DAP and DD-DAP was much easier.

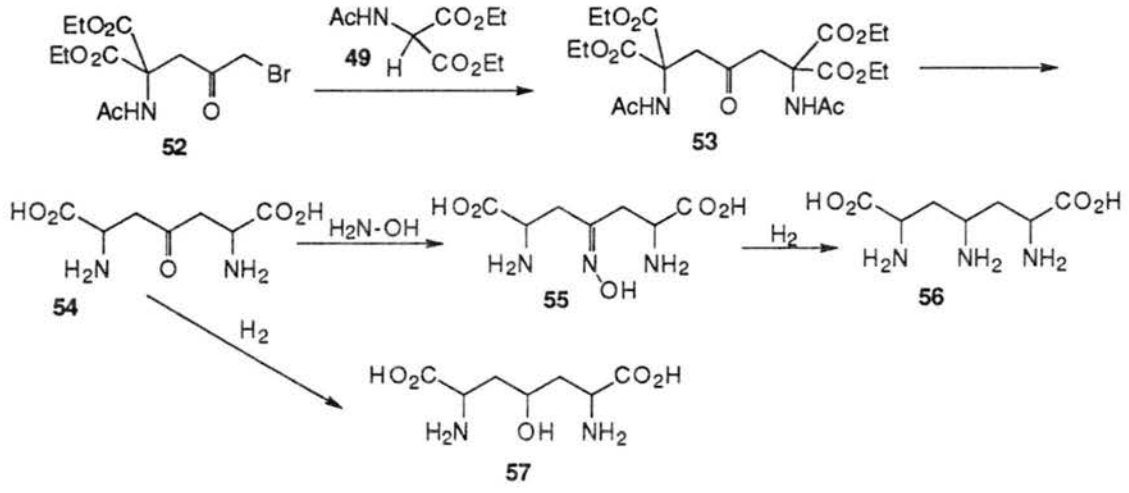
As prior mention, the naturally occurring stereoisomers of DAP were identified as L,L-DAP and *meso*-L,D-DAP respectively by comparison with isolated isomers by paper chromatography.

Hanus and associates, [14] in the 1970's, attempted to synthesize partially stereo-defined DAP (on one α-carbon) by using protected L-glutamic acid as a starting material with no success (Scheme 3). When the 1-methyl ester of N-phthaloyl-L-glutamic acid **46** was treated with BH_3, 3-phthalimido-L-valerolactone **47** was obtained. This lactone was converted into 5-bromo-N-phthaloyl-L-norvaline methyl ester **48** by treatment with HBr followed by diazomethane. The alkylation between 5-bromo-N-phthaloyl-L-norvaline methyl ester **48** and diethyl acetamidomalonate **49** required a strong base, such as butyl lithium, causing almost complete racemization. The resulting triester **50** was hydrolyzed and afforded all three possible stereoisomers of DAP. By using the radical chlorination of the free amino acid, 4-chloro-DAP **51** was obtained in low yield.



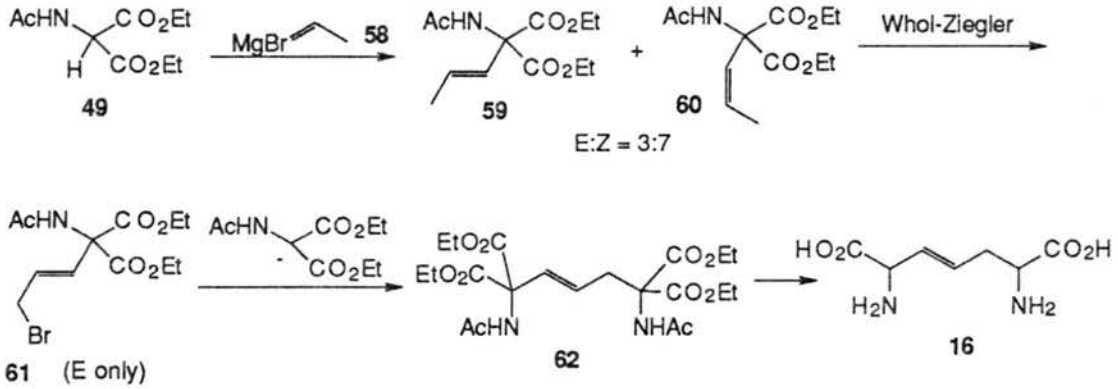
SCHEME 3

Hanus' group further developed a common approach for the preparation of 4-substituted-DAP analogues (Scheme 4). The alkylation of diethyl acetanidomalonate **49** with ethyl 2-acetamido-5-bromo-2-ethoxycarbonyllevulinate **52** gave the desired product **53** in 65% yield. The acidic hydrolysis of **53** gave 2,6-diamino-4-oxopimelic acid **54**. Upon reaction with hydroxylamine and hydrogenation, 2,6-diamino-4-oxopimelic acid **54** was successfully converted into 2,4,6-triaminopimelic acid **56**. Also, 4-hydroxy-DAP **57** was obtained upon hydrogenation of 2,6-diamino-4-oxopimelic acid **54**.

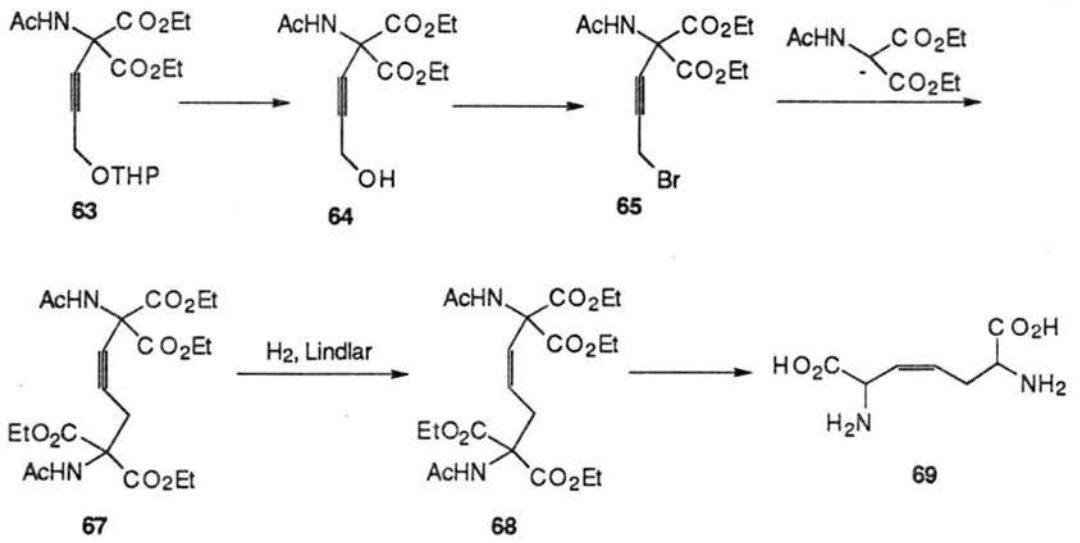


SCHEME 4

The same research group, in 1990, contributed more to DAP-chemistry by publishing the synthesis of (Z)-3,4-didehydro-DAP **69** and (E)-3,4-didehydro-DAP **16** (Scheme 5 and 6). [14b]

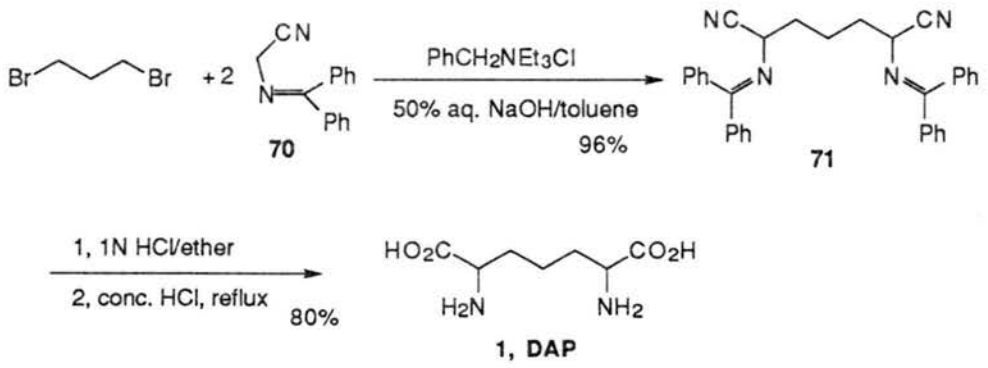


SCHEME 5



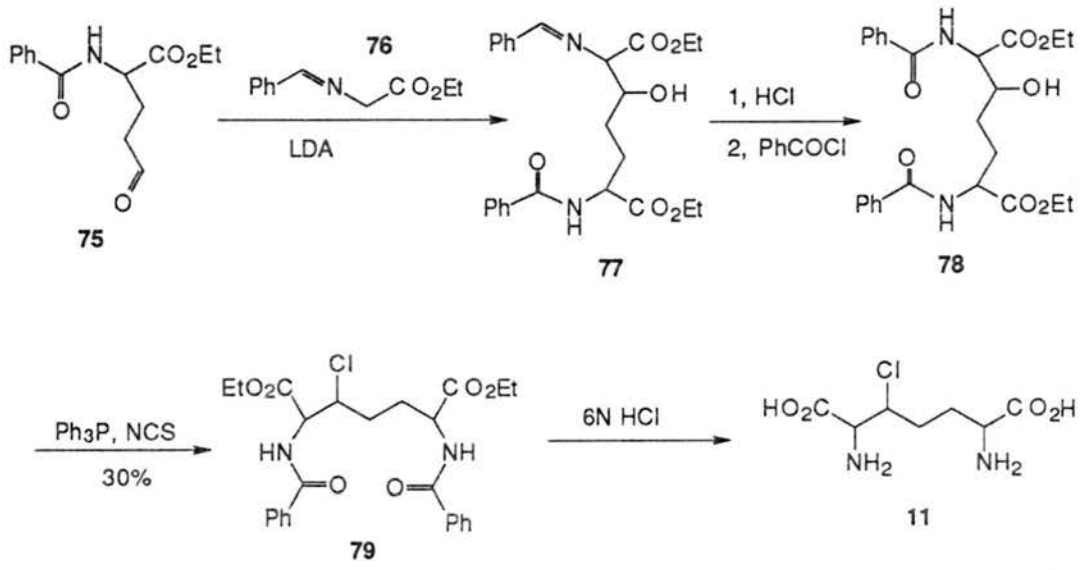
SCHEME 6

O'Donnell and associates [15] employed a phase-transfer alkylation procedure in the preparation of DAP (Scheme 7). The glycine synthon **70** is readily alkylated with 1,3-dibromopropane (0.5 eqalent) in a single step using a catalytic phase-transfer procedure with benzyltriethylammonoium chloride, 50% aqueous sodium hydroxide, and toluene to afford the corresponding protected dicarboxylic acid derivative **71**. Hydrolysis produced 2,6-diaminopimellic acid (a mixture of all three stereoisomers) in 77% overall yield from 1,3-dibromopropane.



SCHEME 7

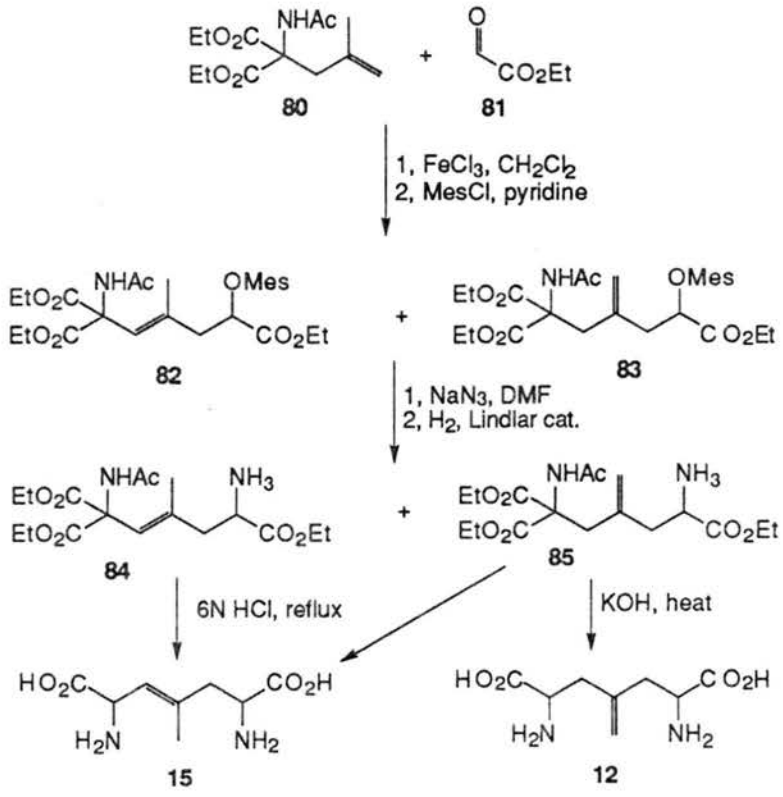
As shown in Scheme 8, 3-chloro-DAP **11**, a potent inhibitor of DAP-epimerase, was synthesized by condensation of the aldehyde **75** with the benzylidene Schiff base **76** of ethylglycinate with lithium diisopropylamide as a base in THF at -78 °C gave alcohol **77**. The alcohol **78** was chlorinated with N-chlorosuccinimide and triphenylphosphine in THF. Finally, 3-chloro-DAP **11**, as a mixture of all possible stereoisomers, was obtained by refluxing the tetra-protected precursor **79** in 6 N HCl overnight. [9]



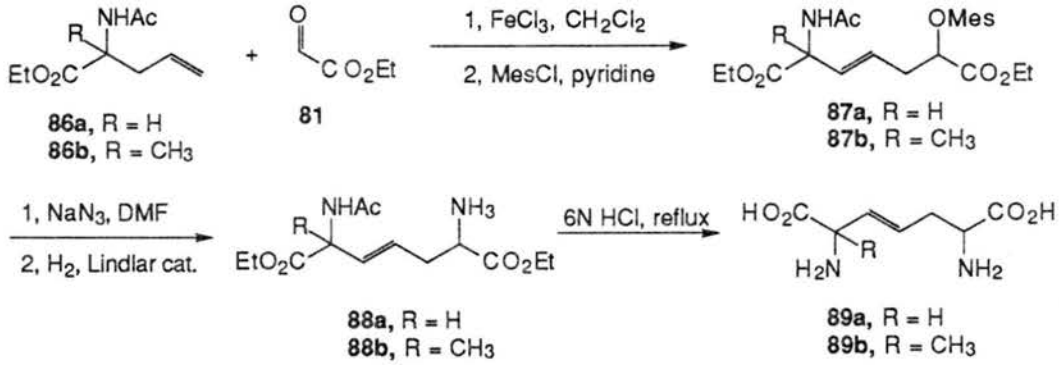
SCHEME 8

Girodeau and associates [6] designed and prepared a number of unsaturated analogues of DAP with the intention of possibly inhibiting L,L-DAP empimerase and *meso*-DAP decarboxylase. These DAP-analogues were assayed against *meso*-DAP decarboxylase. *E*-3,4-didehydrodiaminopimelic acid **16**, as a mixture of four stereoisomers, was found to be the most potent inhibitor (Ki = 180 uM), but this potent inhibitor showed no antibacterial activity. On the other hand, a moderate inhibitor of L,L-DAP epimerase (Ki = 0.95 mM), 4-methelene-DAP **12**, shows strong antibacterial activity.

The syntheses of these DAP-analogues are depicted in Schemes 9 and 10. The DAP skeleton was formed in one step concomitant with the desired unsaturation by an



SCHEME 9



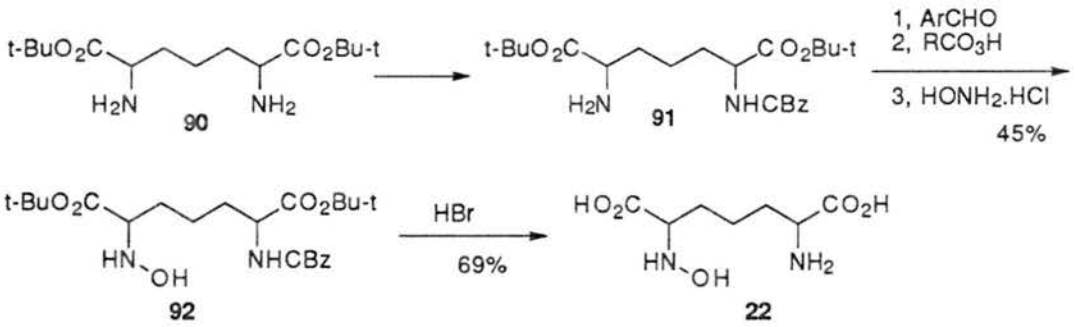
SCHEME 10

intermolecular ene reaction between an allylic amino acid and ethyl glyoxylate **81**. The allylic alcohols thus obtained were mesylated and the mesylates (**82** and **83**) were treated with sodium azide in DMF to give the azido derivatives, which were isolated and purified. Chemospecific reduction of the azides with hydrogen in the presence of Lindlar catalyst at

atmospheric pressure gave the amines (**84** and **85**). Treatment of the amines (**84** and **85**) in refluxing 6 N HCl gave the DAP analogues (**12** and **15**).

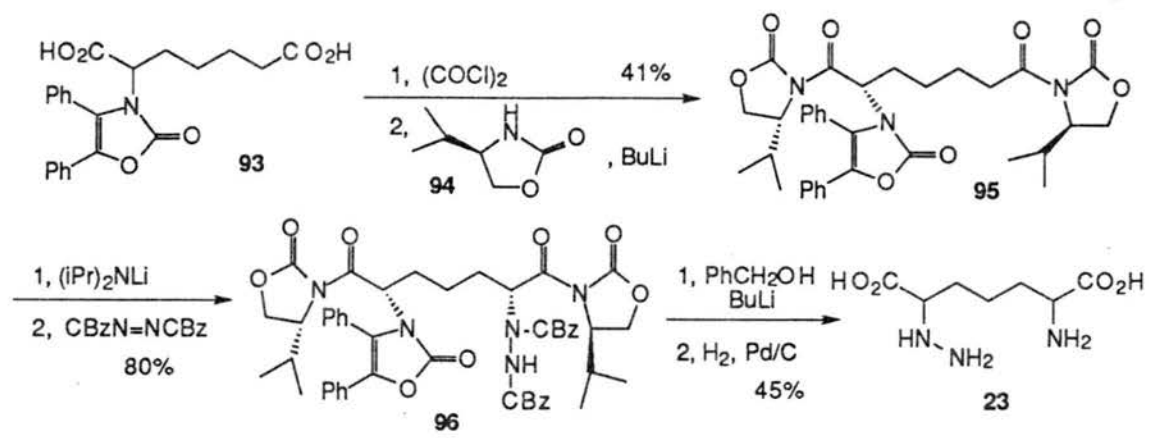
Two N-modified DAP-analogues inhibitors, N-hydroxy-DAP **22** and N-amino-DAP **23**, targeting *meso*-DAP decarboxylase were synthesized and assayed by Vederas and associates.[8] Both compounds were found to be effective competitive inhibitors of the enzyme from *B. sphaericus* (K_i = 84 and 100 uM respectively) and wheat germ (K_i = 910 and 710 uM respectively).

Synthesis of N-hydroxy-DAP **22** was accomplished as shown in Scheme 11. The mono-N-CBz-derivative of di-tert-butyl diaminopimelate **91** was oxidized to give N-hydroxy compound **92**. Deprotection of **92** with HBr in the acetic acid produced the desired N-hydroxy-DAP **22** as a mixture of all possible stereoisomers.



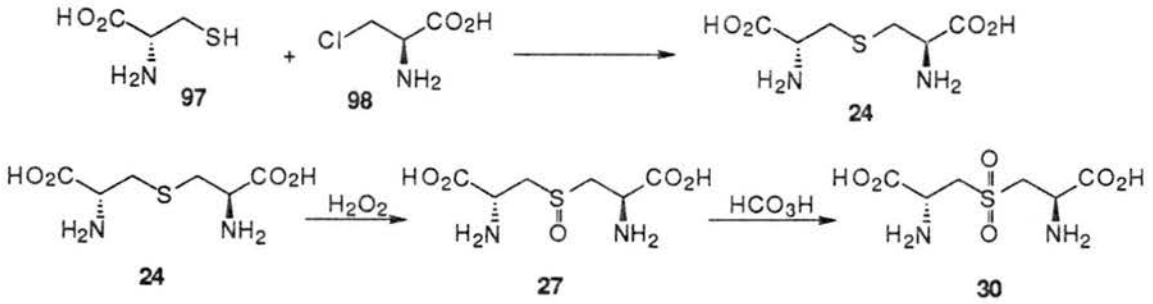
SCHEME 11

The N-amino-DAP **23** was prepared as illustrated in Scheme 12. Racemic α-aminopimelic acid was N-protected, converted into diacid chloride and treated with the lithium derivative of the R-oxazolidinone **94** developed by Evans *et al.* A pair of diastereomers of **95** was easily separated and the reaction with dibenzyl azodicarboxylate afforded **96** with good stereoselectivity, but epimerization occurred during basic cleavage of the chiral oxazolidinone auxiliary. Hydrogenolysis of the protecting groups produced the N-amino-DAP **23** as an optically inactive mixture of stereoisomers.



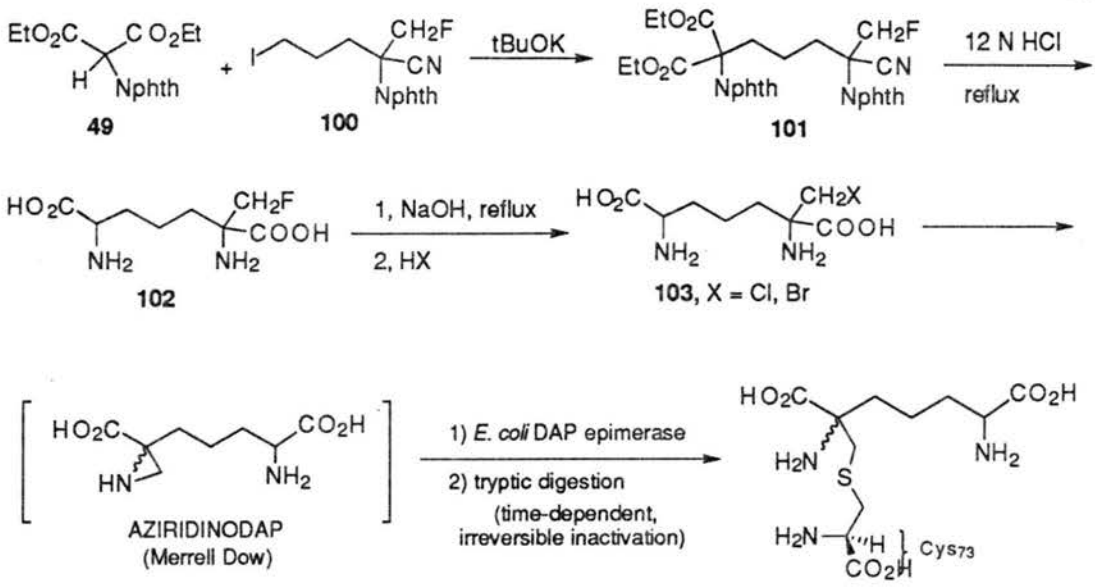
SCHEME 12

As shown in Scheme 13, the pure *meso*-, LL-, and DD-isomers of lanthionine (**24-26**, Table 1) [8] were prepared by condensation of D or L-cysteine with the appropriate D- or L-isomer of β-chloroalanine. Each lanthionine isomer was then individually oxidized with hydrogen peroxide to the corresponding sulfoxides (**27-29**). Since no epimerization occurred at C-2 or C-6, the sulfoxides were stereochemically pure. The isomerically pure *meso*-, LL-, and DD-isomers of lanthionine sulfones (**30-32**) were obtained by performic acid oxidation of the corresponding lanthionine sulfoxides.



SCHEME 13

One of the most potent DAP-epimerase inhibitor, AZIDAP **10**, was reported by the Marrion-Merrell Dow research group. [5] However, this compound was obtained as a mixture of all stereoisomers from a stereo-random synthesis (Scheme 14).

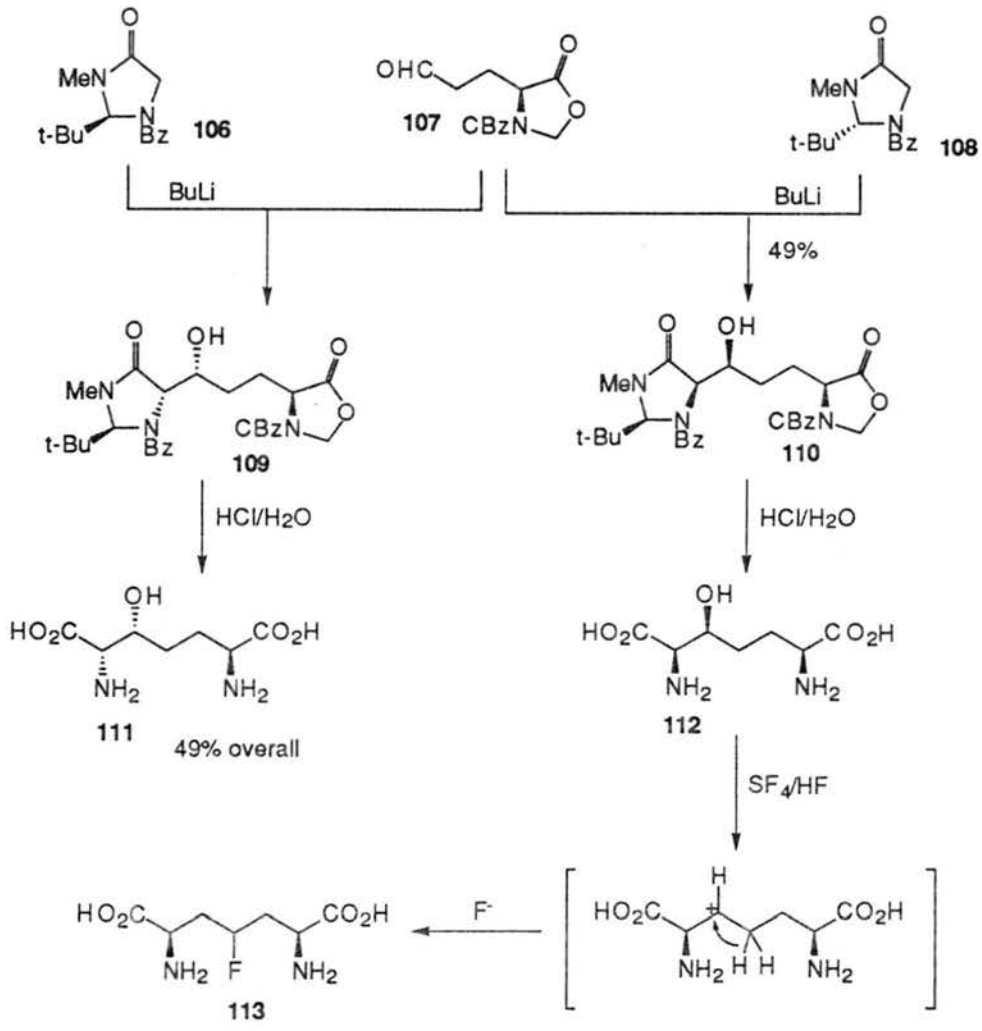


SCHEME 14

Since 1990, the lack of stereochemically unambiguous syntheses of DAP and DAP-analogues has prompted a number of research groups including our group to develop an asymmetric synthesis of DAP and DAP-analogues.

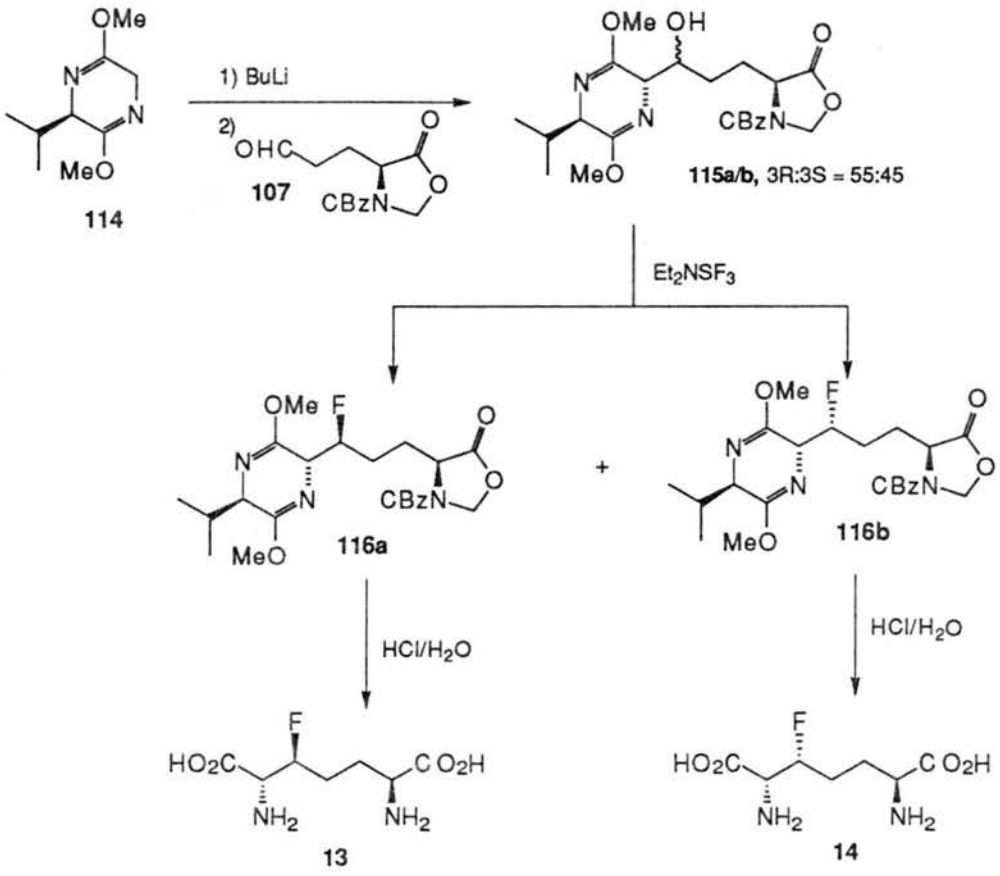
Realizing the 3-substituted DAP analogues have potential to become potent inhibitors of *meso*-DAP epimerase, Gelb and associates [7] developed the stereospecific approaches for synthesizing this class of analogues (Scheme 15).

As shown in Scheme 15, 2S,3R,6S-3-hydroxy-DAP **111** was obtained in 49% overall yield by condensation of the Seebach's chiral imidazolidinone **106** with the aldehyde **109** derived from L-glutamic acid and subsequent hydrolysis. The ^{1}HNMR and ^{13}CNMR spectra indicate that the condensation is highly stereospecific (>95% de). Unfortunately, treatment of (2R,3S,6S)-3-hydroxy-2,6-diaminopimelic acid **112** with sulfur tetrafluoride in hydrogen fluoride did not produce any of the desired 3-fluoro-DAP but generated instead the symmetrical 4-fluoro-DAP **113** in 50% yield, presumably via a 1,2 hydride shift of a carbocationic intermediate.



SCHEME 15

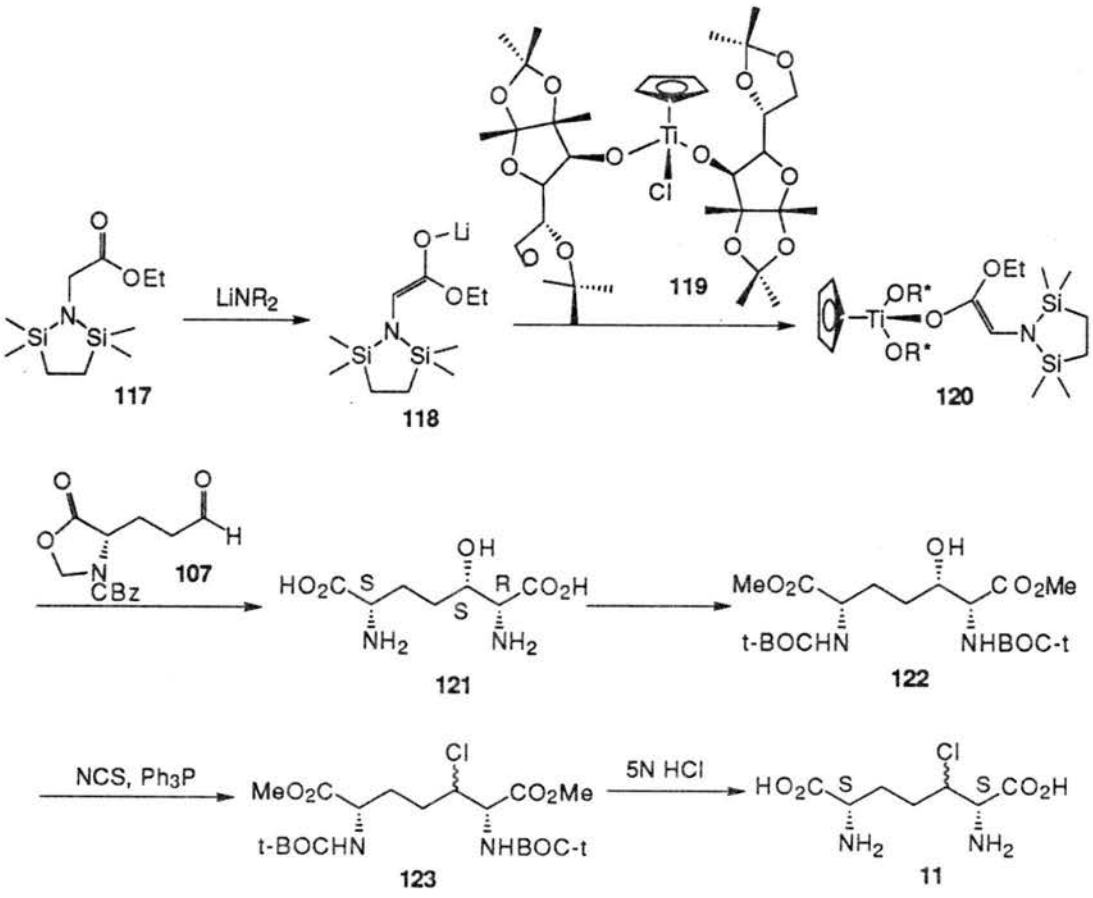
An alternative route employing condensation of the Schöllkopf chiral bis(lactim ethers) **114** with aldehyde **107** proved to be successful (Scheme 16). However, the aldol reaction was much less stereoselective and yielded a 55:45 ratio for **115 a/b** (3R/3S). Although attempts at the separation of the mixture of **115a/b** were unsuccessful, treatment with DAST gave the expected fluoro derivatives **116a/b**, respectively, in low yield (8-15%). Separation of the isomeric mixture **116a/b** affords pure **116a** (major component) and impure **116b**, which upon hydrolysis yield (2R,3S,6S)-3-fluoro-2,6-diaminopimelic acid **13** and the (2R,3R,6S)-3-fluoro-2,6-diaminopimelic acid **14**, respectively.



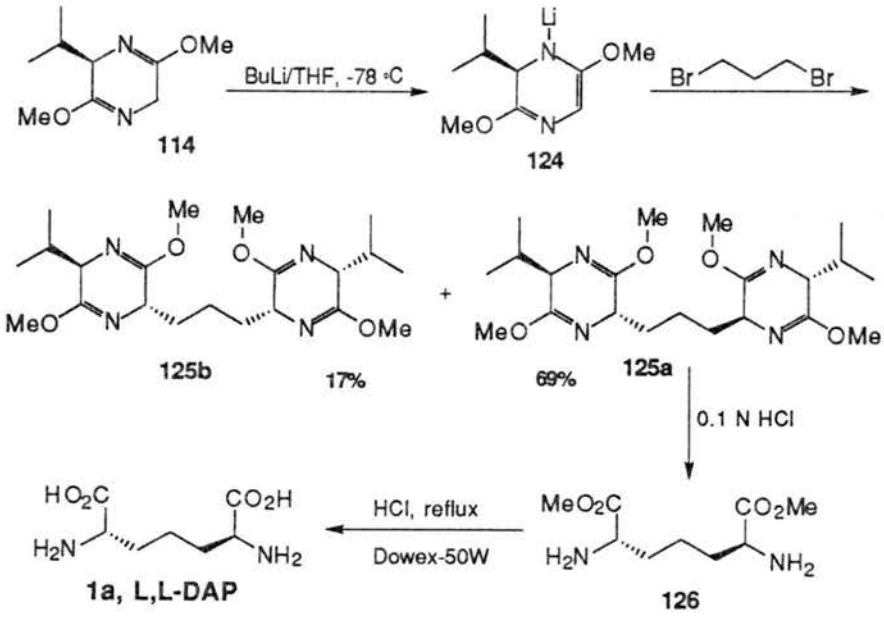
SCHEME 16

Bold and associates [16,17] attempted to prepare 3-chloro-DAP **11** enantioselectively without success (Scheme 17), even though (2R,3S,6S)-3-hydroxy-DAP **121** was obtained through the condensation of enolate of the titanium-carbohydrate complex **120** with aldehyde **107**. Unfortunately, the chlorination process of the protected 3-hydroxy-DAP **122** epimerized the C-3 chiral center and a pair of diastereomers of (2R,3S,6S)-3-chloro-DAP and (2R,3R,6S)-3-chloro-DAP was obtained as a mixture.

Using Schöllkopf's chiral auxiliary, Bold and associate reported the enantioselective synthesis of L,L-DAP as shown in Scheme 18.[17] When the Li-enolates of the chiral bislactim ether was treated with 1,3-dibromopropane, the alkylated products were obtained in 69% yield for the *anti*-stereoselective isomer and 17% yield for the minor isomer. The acidic hydrolysis of the major isomer afforded pure L,L-DAP.

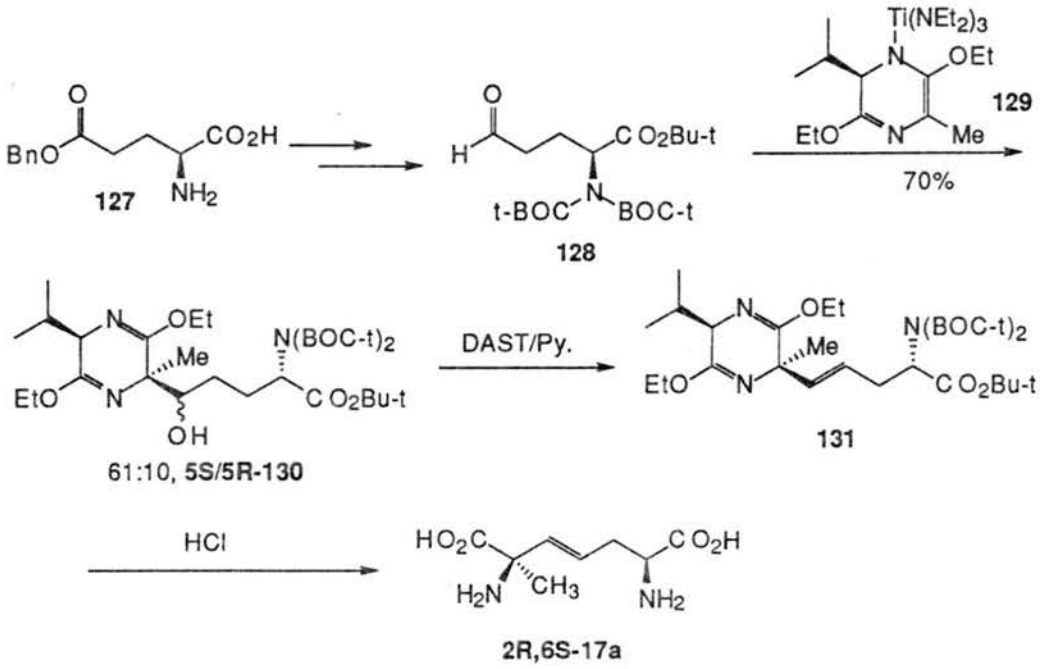


SCHEME 17



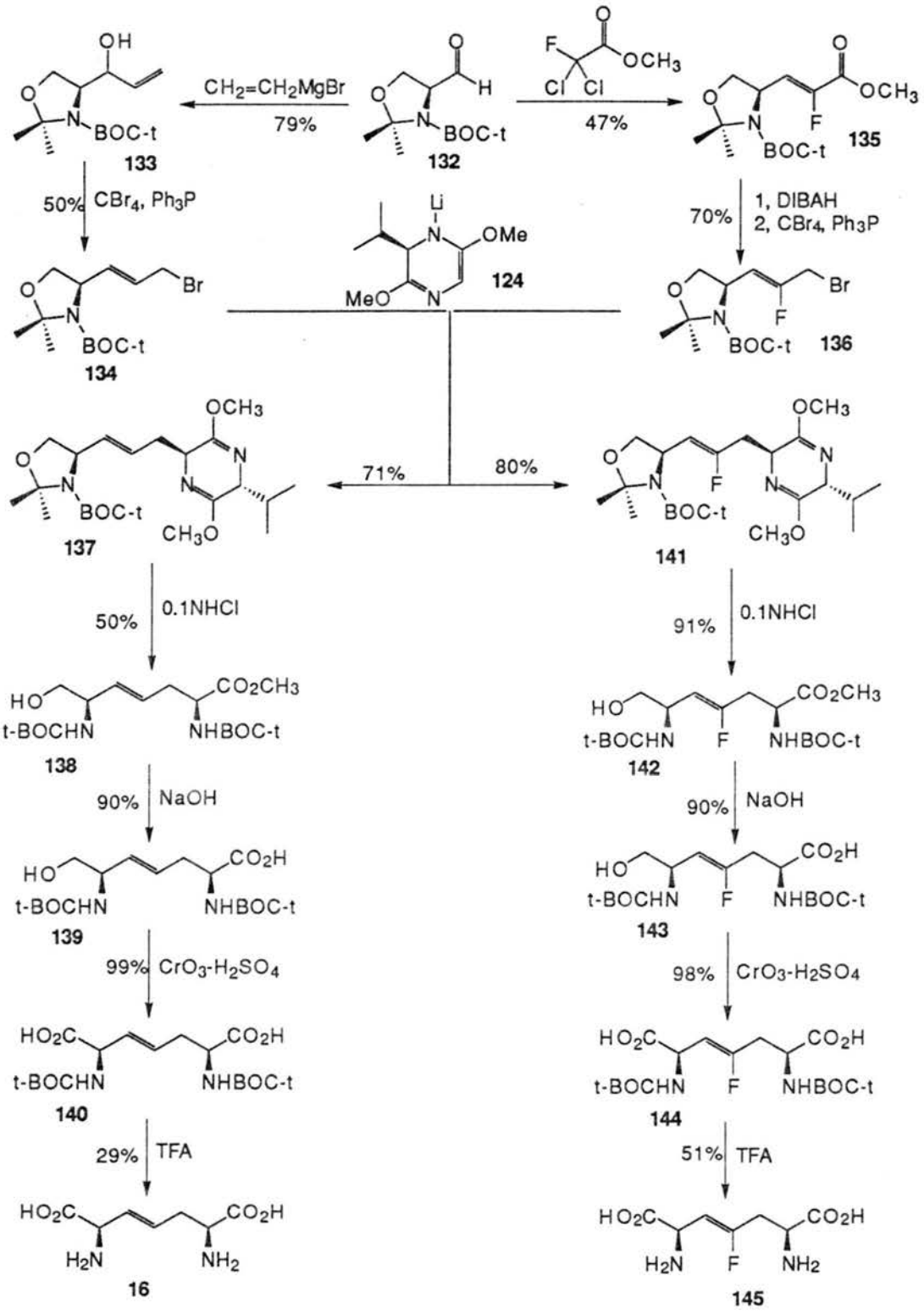
SCHEME 18

The aldol condensation between the enolate of bislatim ethers **129** and aldehyde **128** gave 61:10 of 5S/5R-hydroxy product **130**. After dehydration and acidic deprotection, the (2R,6S)-2,6-diamino-2-methylhept-3-enedionic acid **17a** was prepared in good yield (Scheme 19).



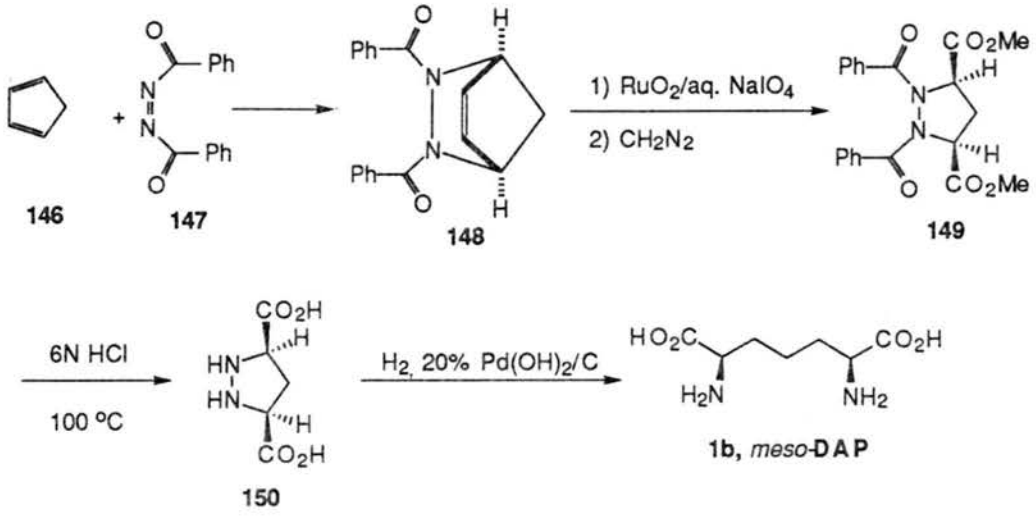
SCHEME 19

With the moderate success realized in controlling the stereochemistry of the C-2 and C-3 stereogenic centers of the DAP skeleton by Schöllkopf's chiral auxiliary, they further explored the alkylation reaction with chiral, non-racemic halides derived from Garner aldehyde **132**, as shown in Scheme 20. These alkylations proceeded with good yield and high stereoselectivity. After manipulation of protecting groups, the primary alcohols were oxidized to carboxylic acids to afford the corresponding DAP-analogues, (2R,6S)-2,6-diaminohept-3-enedionic acid **16** and (2R,6S)-2,6-diamino-4-fluorohept-3-enedionic acid **145**, respectively.


CH2=CH2MgBr
79%
47%
50% CBr4, Ph3P
70% 1, DIBAH 2, CBr4, Ph3P
71%
80%
50% 0.1NHCl
91% 0.1NHCl
90% NaOH
90% NaOH
99% CrO3-H2SO4
98% CrO3-H2SO4
29% TFA
51% TFA
BOC-t
t-BOCHN
NHBOC-t
132
133
134
135
136
137
138
139
140
141
142
143
144
145
16
124

SCHEME 20

Recently, Arakawa and coworkers [18] converted stereospecific hetero Diels-Alder adduct **148** to *meso*-DAP **1b** (Scheme 21). The hetro Diels-Alder adducts **148** was oxidized by ruthenium tetroxide in EtOAc at 0 °C to afford *cis*-dicarboxylic acid **150**. After hydrolysis and hydrogenation, the *meso*-DAP **1b** was obtained.

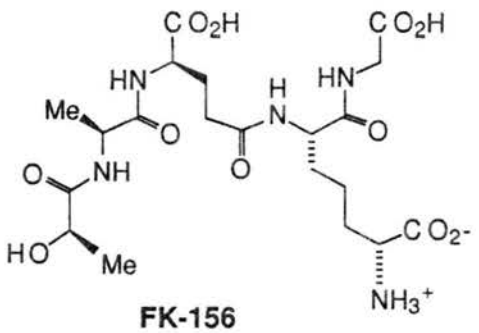


146 **147** **148** **149** **150** **1b**, *meso*-**DAP**

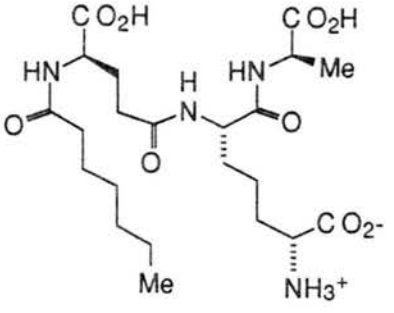
SCHEME 21

1.3 FK-156 and FK-565

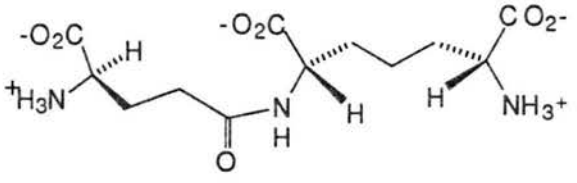
In recent years, considerable attention has been focused on the peptidoglycan fragments of bacterial cell walls because of their unique immunostimulating activity [19]. Several recently isolated metabolites of peptidoglycan, FK-156 [20,21] and its synthetic analogue FK-565 [22], both containing the γ-D-glutamyl-*meso*-L-diaminopimelic acid **162** substructure, revealed that these substances enhanced host defense against microbial infections, exhibited strong antiviral activity [23] and remarkable antitumor potency. [24,25,26] It was found that FK-565 alone and in combination with zidovudine (AZT) inhibited retroviral infections by Friend leukemia virus in mice.[27] It is expected that FK-565 could be used not only in conjunction with cancer surgery and radiation therapy but, it may also have potential for use in the treatment of human Acquired Immunodefiency Syndrome (AIDS).



FK-156



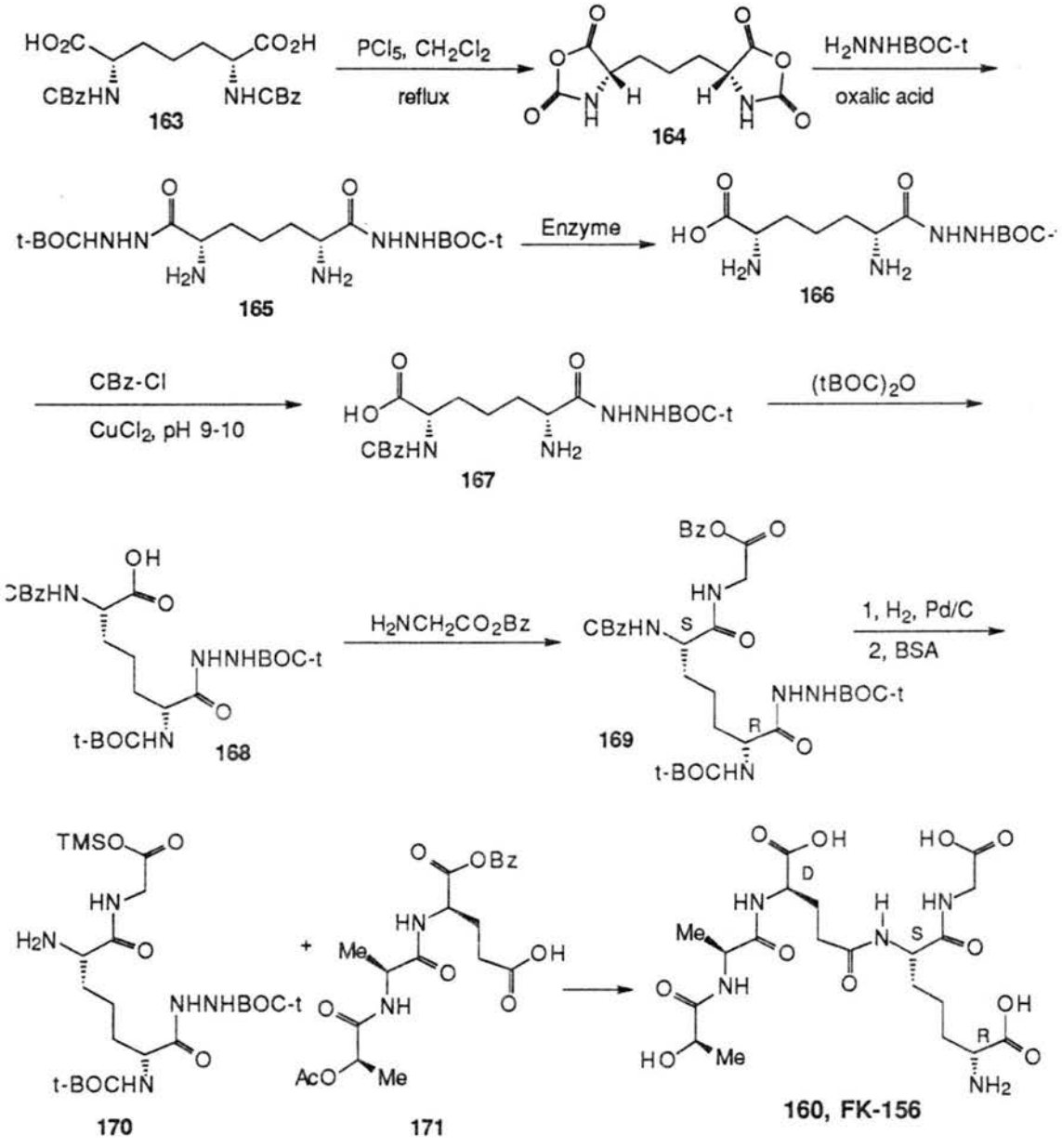
FK-565



162, γ-D-glutamyl-*meso*-L-diaminopimelic acid

Figure 3.

The unique biological activity of these compounds renders the synthesis of this class of peptides an attractive and worthy synthetic problem.[19]

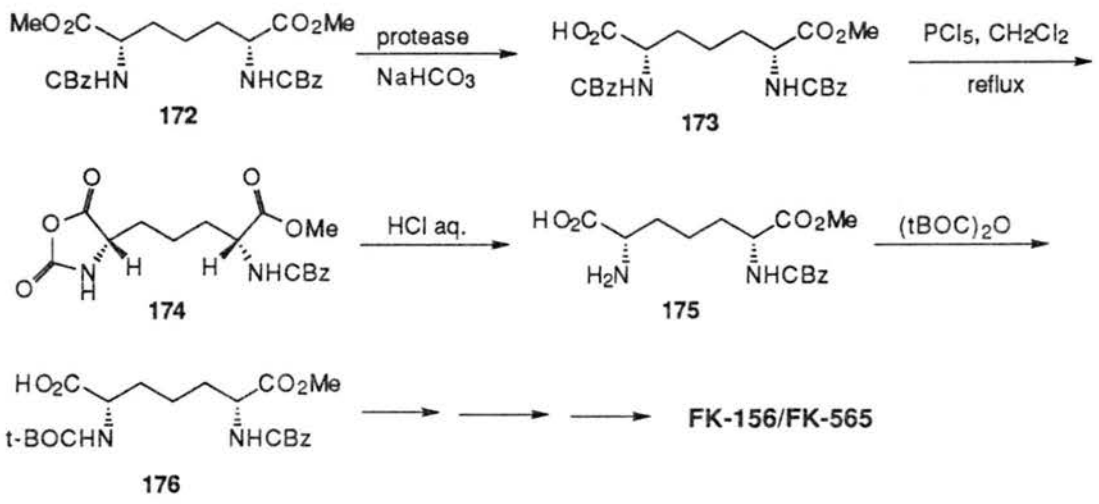


SCHEME 22

Soon after the isolation of FK-156 from *Streptomyces Olivaceogriseus* sp. nov., the research group at Fujisawa Pharmaceutical Company developed the first synthesis of FK-156 (Scheme 22). [28] The key step of the synthesis is to prepare the differentially

protected *meso*-DAP **166** through an asymmetric hydrolysis of the ester of *meso*-DAP **165** using hog kidney leucine aminopeptidase.

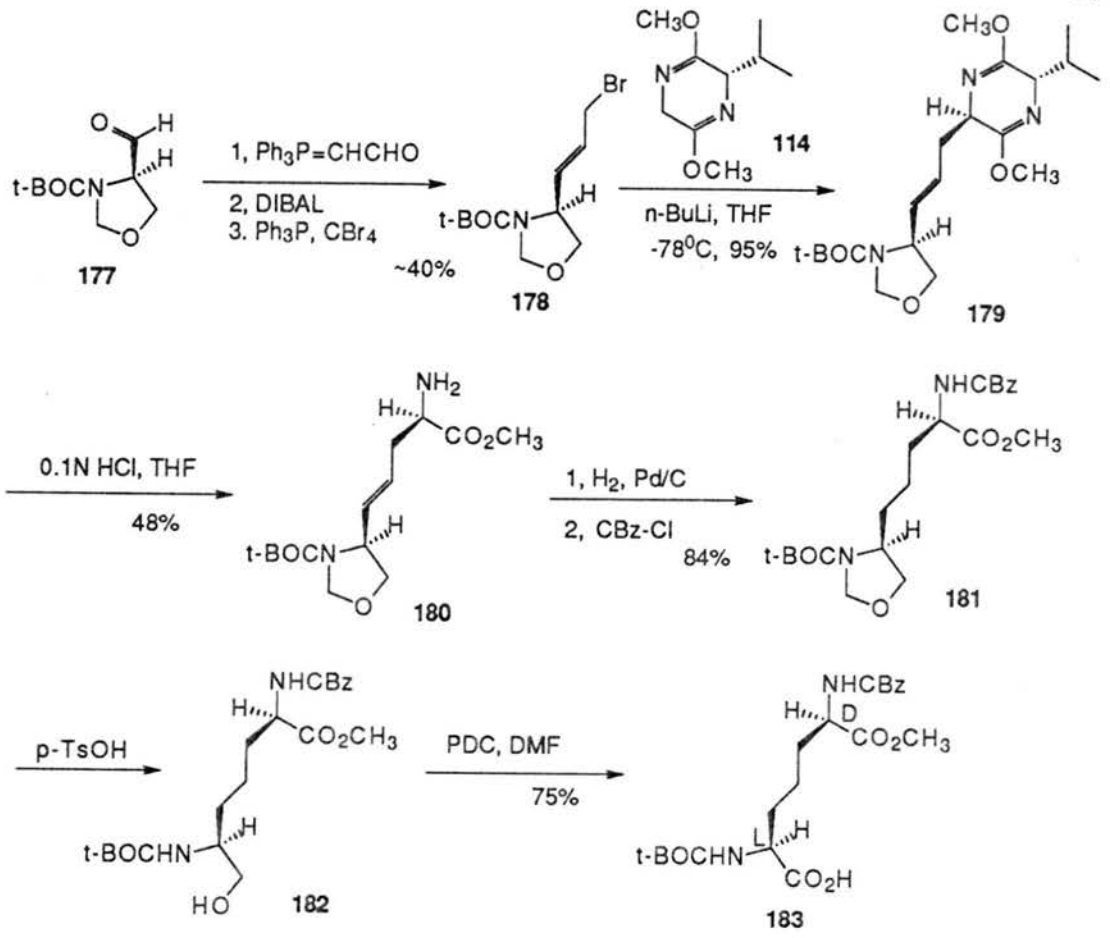
Kolodziejczyk and associates reported an improved synthesis of FK-156 and FK-565 by using selective enzymatic hydrolysis of the methyl ester group of the L-centre of Z_2-*meso*-A2pm(OMe) **172** as the key step (Scheme 23).[29]



SCHEME 23

However, both approaches shown above are limited in accessing only *meso*-dimainopimelic acid (DAP) containing peptides.

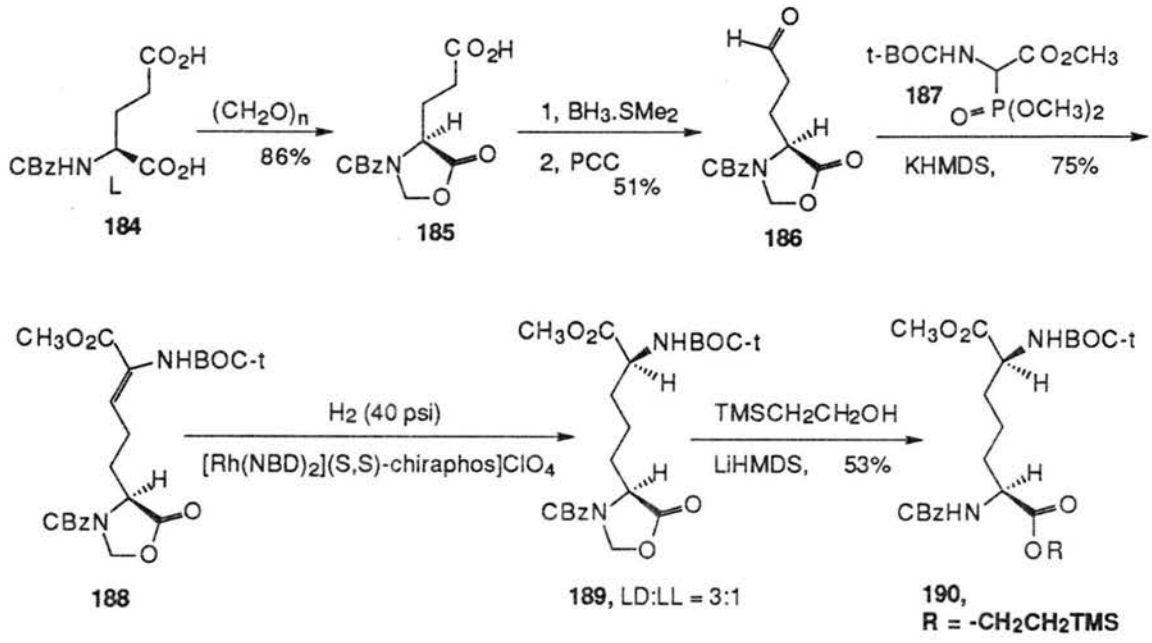
Very recently, more attention has been focused on the asymmetric synthesis of differentially protected *meso*-2,6-diaminopimelic acid. As shown in Scheme 24, [30] the versatile, D-serine derived, Garner oxazoline **177** was converted to the bromide **178** in three steps in about 40% overall yield. The bromide **178** underwent a facial alkylation with Schollkopf's chiral auxiliary **114** to provide **179** in good yield and high stereoselectivity. After selective deprotection, hydrogenation and oxidation, differentially protected *meso*-2,6-diaminopimelic acid **183** was obtained.



SCHEME 24

Differentially protected *meso*-2,6-diaminopimelic acid was also prepared by Holcomb and coworkers [31] using a different approach (Scheme 25), which is very similar to our initial approach for the asymmetric synthesis of DAP (see Chapter 2). As shown in Scheme 25, the aldehyde **186**, derived from L-glutamic acid, and the amino phosphinate **187** underwent an Hornor-Emmons-Wadsworth reaction to provide the acrylate **188** as a mixture of geo-isomers (6.4:1, Z:E), which was separated by flash chromatography. The Z-isomer was subjected to a catalytic asymmetric hydrogenation using the chiral rhodium catalyst (S,S)-chiraphos $Rh(NBD)_2ClO_4$ to give an inseparable mixture of diastereomers at

a roughly 3:1 ratio (LD/LL-**189**). After protecting group manipulation, a differentially protected *meso*-2,6-diaminopimelic acid **190** was prepared in about 50% de.



SCHEME 25

Chapter 2, Results and Discussion

2.1 Background: The Williams Synthesis of Optically Active Amino Acids

Williams' research group has previously reported [32] on the utility of the diphenyl oxazinones (**191a**, **191b**, **191c** and **191d**), now commercially available, as versatile chiral templates from which both electrophilic [33] and nucleophilic [34] C-C bond-forming strategies can be employed to access a variety of non-proteinogenic α-amino acids in optically active form.

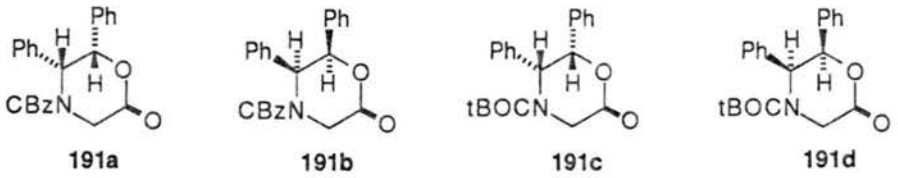
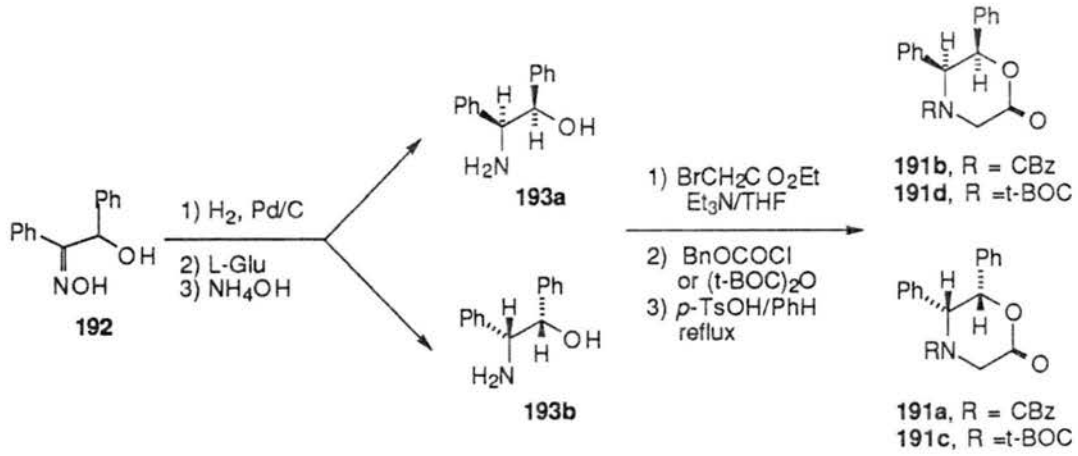


Figure 4

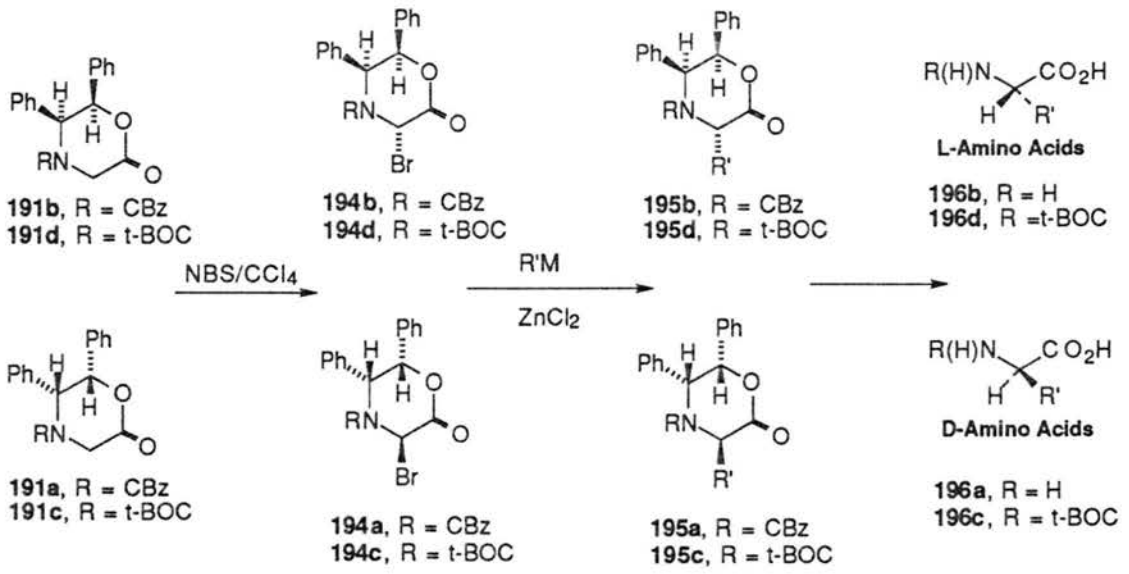


SCHEME 26.

These optically pure glycinate auxiliaries were prepared via a six-step procedure shown in Scheme 26. [32a] Inexpensive benzoin is converted into the oxime **192** and stereospecifically hydrogenated to the racemic *erythro*-amino alcohols **193**; they are subsequently resolved through the derived L-glutamate salts on large scale, providing each

optical isomer **193a** and **193b** of > 98% ee. Each isomer is then separately alkylated with ethyl bromoacetate, acylated with either benzyl chloroformate or di-*t*-butyl dicarbonate, and, finally, lactonized with catalytic *p*-TSOH in hot benzene or toluene to afford the crystalline lactones **191** in ~65% overall yield from the amino alcohols.

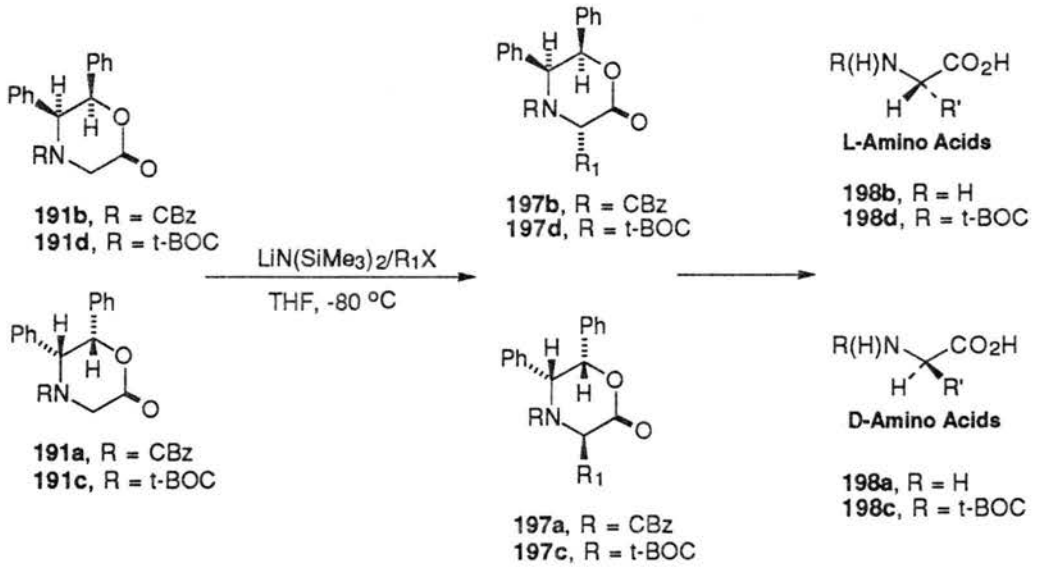
As shown in Scheme 27, these chiral oxazinones are used as electrophilic glycinates. [33] Bromination of these oxazinones with NBS in reflux carbon tetrachloride proceeds in essentially quantitative yield. The relative stereochemistry of the bromide **194** is *anti* to the two phenyl groups and only a single diastereomer is observed. The bromides are unstable to silica gel purification and are used directly for the subsequent coupling reactions. Reaction of the bromides **194** with various organometallic reagents in the presence of zinc chloride results in displacement of the halogen providing the homologated oxazinones **195**. In most cases, the relative stereochemstry of the coupling reaction proceeds with net retention providing *anti*-**195**.



SCHEME 27

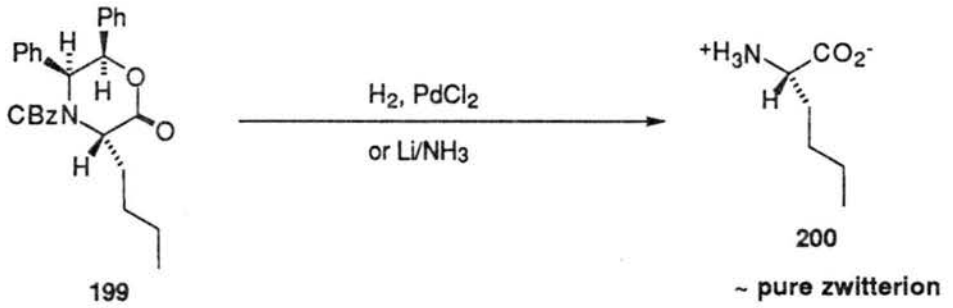
These chiral oxazinones can also be used as nucleophilic glycinates, as shown in Scheme 28. [34] Generation of the enolates of the chiral oxazinones with lithium or sodium hexamethyldisilylamide for 30-40 min in THF at low temperature, followed by addition of an alkyl halide, results in the formation of highly diastereoselective (typically 99% de) *trans*-alkylation products **197**.

Four different deprotection protocols were devised to convert the oxazinones into amino acids, depending on which protecting group (CBz or t-BOC) was employed in the oxazinone and type of alkyl group (R' and R1). In the case of CBz as protecting group and a saturated R, the Kagan-type reductive cleavage is typically chosen (Scheme 29).

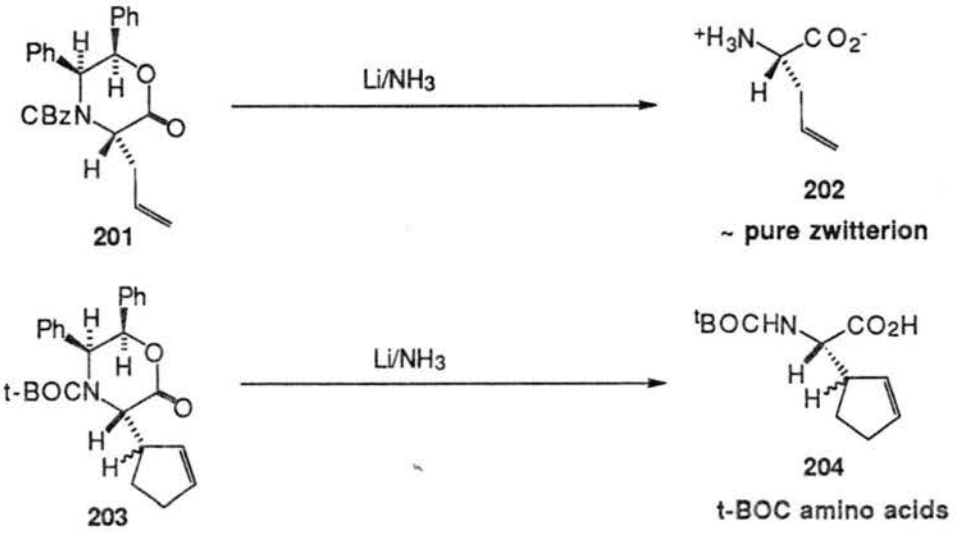


SCHEME 28

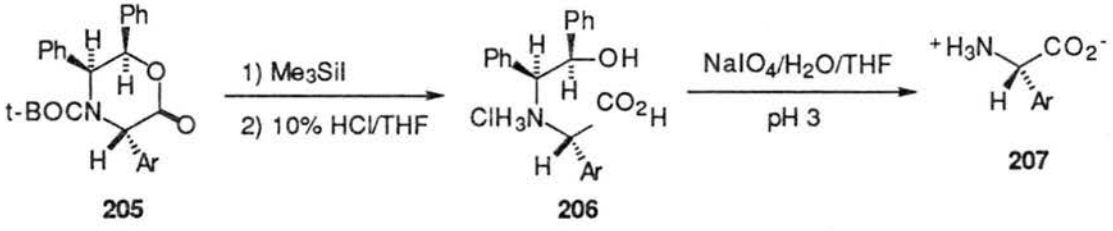
In the case of the unsaturated or hydrogenolizable "R", the dissolving metal reduction protocol is employed (Scheme 30). This protocol is also the choice for t-BOC substrate deprotection, and the corresponding t-BOC amino acids were obtained directly . As shown in Scheme 31, the third method for the removal of the chiral auxiliary is used in the special cases where based-induced empimerization at α-center is problematic. Finally, a new method was developed during this study to selectively deprotect t-BOC-oxazinone over CBz-oxazinone, and it will be discussed in detail later (see Scheme 44).



SCHEME 29



SCHEME 30



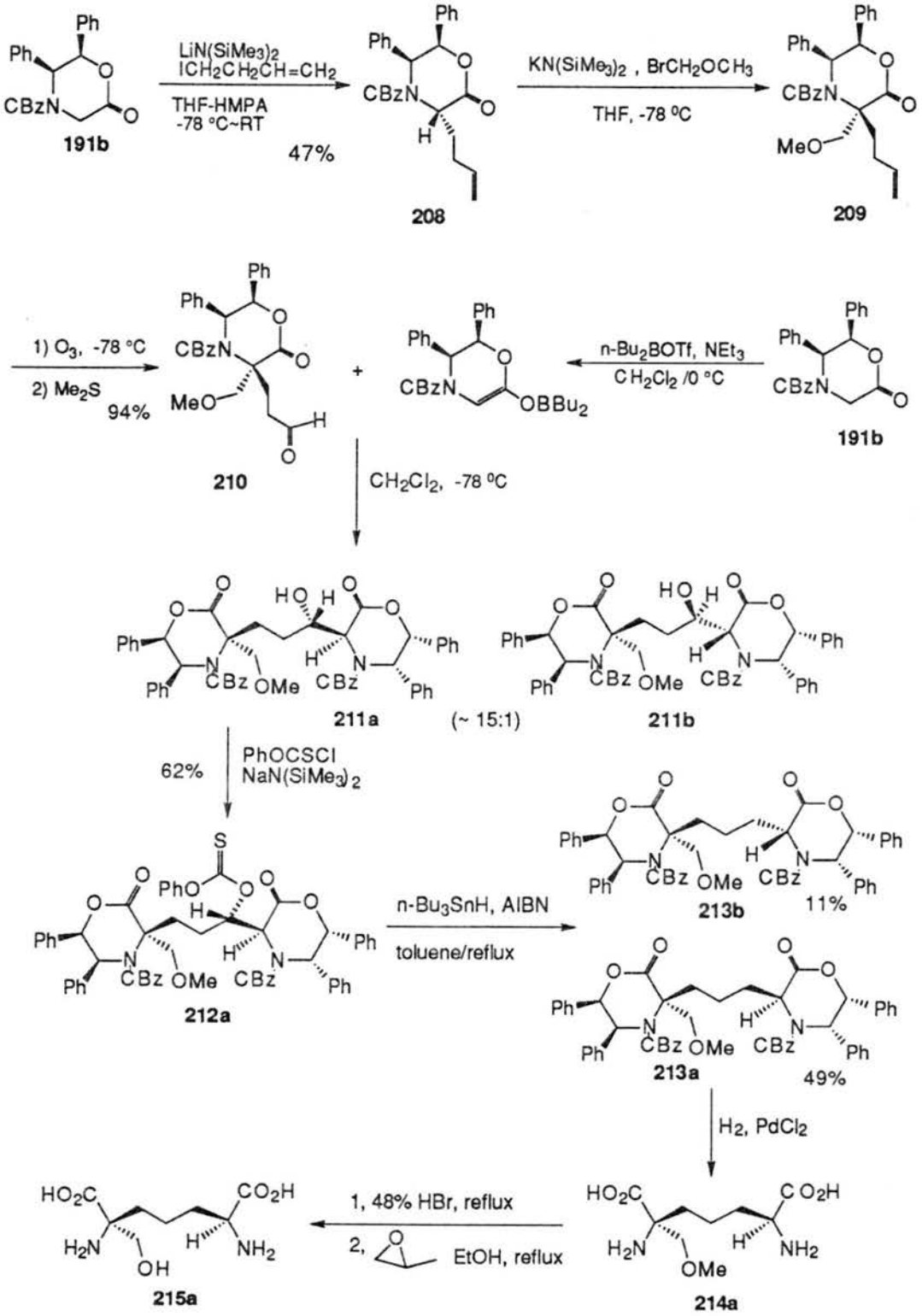
SCHEME 31

During the last decade, Williams' research group has exploited the rich chemistry of the glycine framework to carry out a variety of useful homologation reaction at the α-position of the simplest amino acid building block. This has resulted in the practical

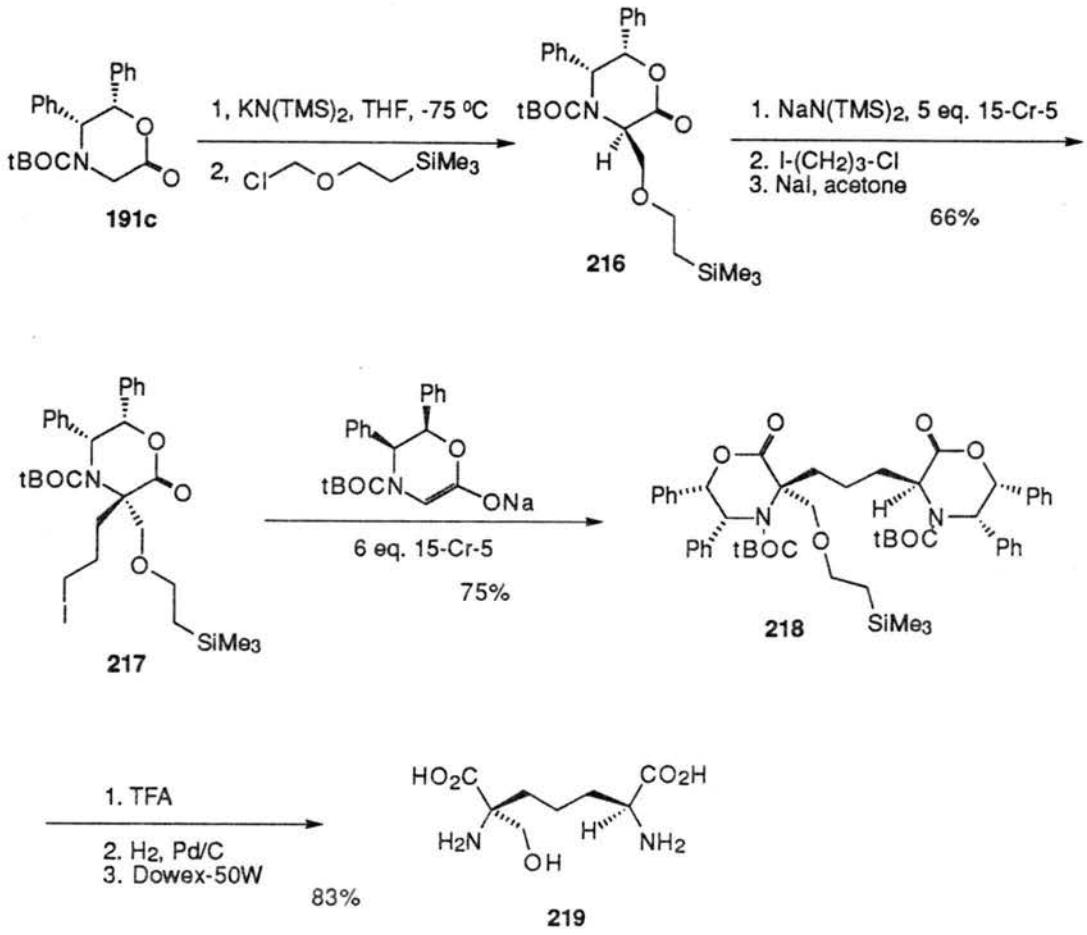
synthesis of a large structural array of amino acids in optically active form. The rigid geometry of the six-membered ring glycinate templates allows for both diastereocontrolled C-C bond-forming processes and convenient spectroscopic determinations of relative and absolute configurations and diastereochemical ratios of the homologated products. These properties render these glycinates predictable and powerful tools for accessing a rich functional array of amino acids in either the D- or L-configurations.

As part of the ongoing project on developing an asymmetric synthesis of diaminopimelic acids, Williams and Im reported the first asymmetric synthesis of (2*S*,6*S*)-2,6-diamino-6-(hydroxymethyl)pimelic acid **215a** (Scheme 32). [11] The key coupling of aldehyde **210a** and boran enolate generated from chiral oxazinone **191b** gave the *anti*-β-hydroxy aldol **211a** as the major product along with a minor diastereomer **211b** (25:1 ratio). The Barton deoxygenation reaction [40] proved to be quite troublesome due to competing elimination reactions of the activated thionocarbonate **212a**. The modest yield (38%) of the esterification process on this substrate reflects the difficulty associated with activating this hindered alcohol in the presence of base that mediates the subsequent competing elimination. After tin hydride reduction, the major bis-lactone **213a** was cleanly hydrogenated in nearly quantitative yield to **214a**. Cleavage of the methyl ether in concentrated HBr gave the (2*S*,6*S*)-2,6-diamino-6-(hydroxymethyl)pimelic acid **215a**.

Recently, Baldwin and associates [44] have described an improved procedure for asymmetric synthesis of (2*S*,6*S*)-2,6-diamino-6-(hydroxymethyl)pimelic acid **215a** using the enolate alkylation of glycinates **191**. As shown in scheme 33, the direct enolate coupling between 3'-halopropyl derivatized glycinate **217** and a second glycinate enolate **218** can be realized in the presence of 6 eqivlent of 15-crown-5 to give **219**. Deprotection with trifluoroacetic acid followed by hydrogenation and ion exchange chromatography gave (2*S*,6*S*)-2,6-diamino-6-(hydroxymethyl)pimelic acid **215a**.


191b
LiN(SiMe3)2
ICH2CH2CH=CH2
THF-HMPA
-78 °C~RT
47%
208
KN(SiMe3)2 , BrCH2OCH3
THF, -78 °C
209
1) O3, -78 °C
2) Me2S
94%
210
n-Bu2BOTf, NEt3
CH2Cl2 /0 °C
CH2Cl2, -78 °C
211a
(~ 15:1)
211b
62%
PhOCSCl
NaN(SiMe3)2
212a
n-Bu3SnH, AIBN
toluene/reflux
213b
11%
213a
49%
H2, PdCl2
214a
1, 48% HBr, reflux
2, EtOH, reflux
215a

SCHEME 32

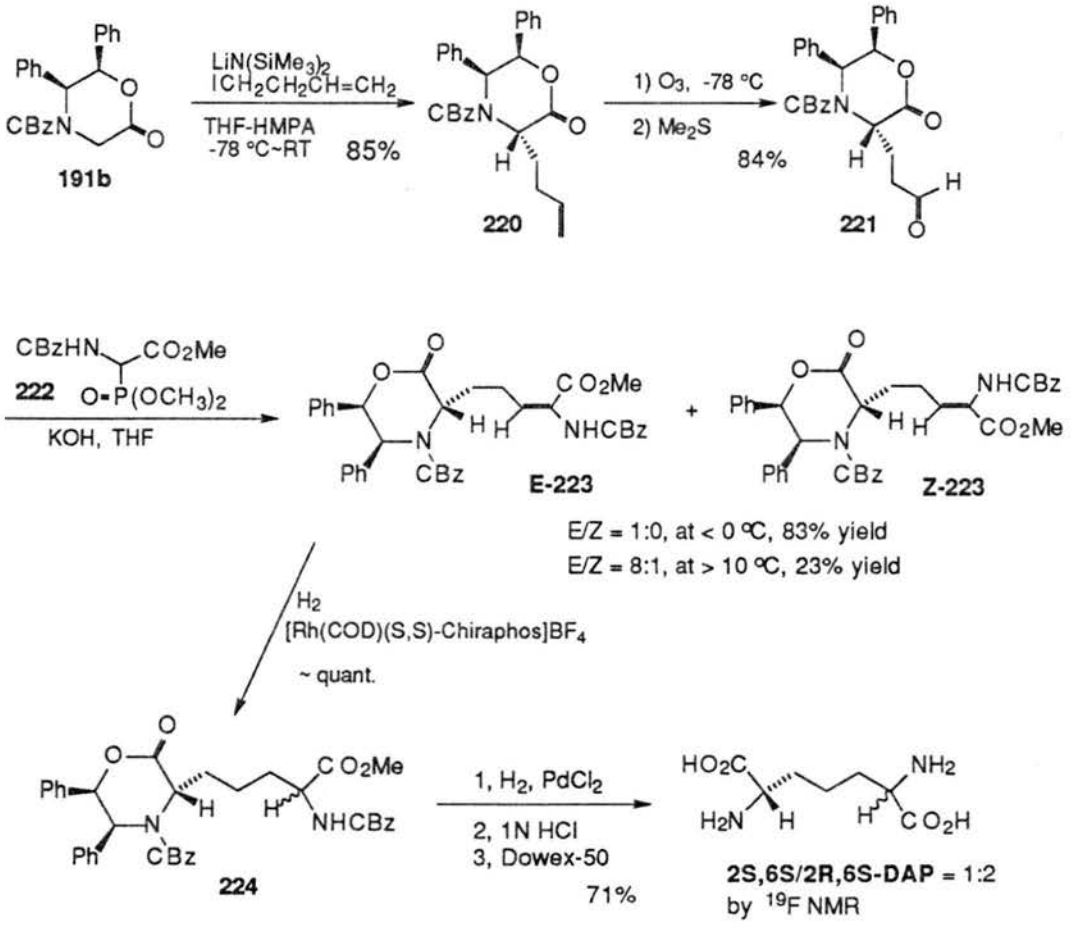

Ph
Ph
tBOCN
O
O
191c
1, KN(TMS)2, THF, -75 °C
2, Cl O SiMe3
tBOCN
H
216
SiMe3
1. NaN(TMS)2, 5 eq. 15-Cr-5
2. I-(CH2)3-Cl
3. NaI, acetone
66%
217
SiMe3
tBOCN
ONa
6 eq. 15-Cr-5
75%
218
SiMe3
tBOC
1. TFA
2. H2, Pd/C
3. Dowex-50W
83%
HO2C
CO2H
H2N
NH2
OH
219

SCHEME 33

2.2 Asymmetric Synthesis of 2,6-Diaminopimelic Acid (DAP)

In the attempts to accomplish the asymmetric synthesis of DAP by using the Williams' lactones **191**, two approaches were explored.

As shown in Scheme 37, the key reaction of the first approach was the catalytic asymmetric hydrogenation of the 2,3-dehydroaminoacids. [32b,35,36] Thus, the lactone **191b** was treated with homoallyl iodide in the presence of lithium bis(trimethylsilyl)amide to give the homoallyl oxazinone **220** in 85% yield. This substance was ozonized and then quenched with dimethyl sulfide to afford the aldehyde **221** in 79-84% yield.



SCHEME 37

Hornor-Emmons-Wadsworth condensation of aldehyde **221** and amino phosphinate **222** [37] using KOH in MeOH/THF as base at -78 °C to 0 °C, provided the *E*-alkene **223** as one exclusive diastereomer in high yield (83%). When the reaction temperature was allowed to raise above 10 °C, the reaction produced a mixture of two geo-isomers (8:1) in low combined yield (about 20%). The two isomers were separated by flash chromatography. After comparison of the ^{1}HNMR of the two isomers, [36,38] the major isomer was assigned as the undesired (E)-alkene (δ_E = 6.50 ppm and δ_Z = 6.10 ppm for the vinyl proton). In the attempts to increase the production of the (Z)-isomer, various reaction conditions were explored with no success. In most cases, when other bases were employed, such as lithium bis(trimethylsilyl)amide, LDA, NaH and t-BuOK, no condensation products were obtained. This might be due to the lability of aldehyde **221** under the basic conditions.

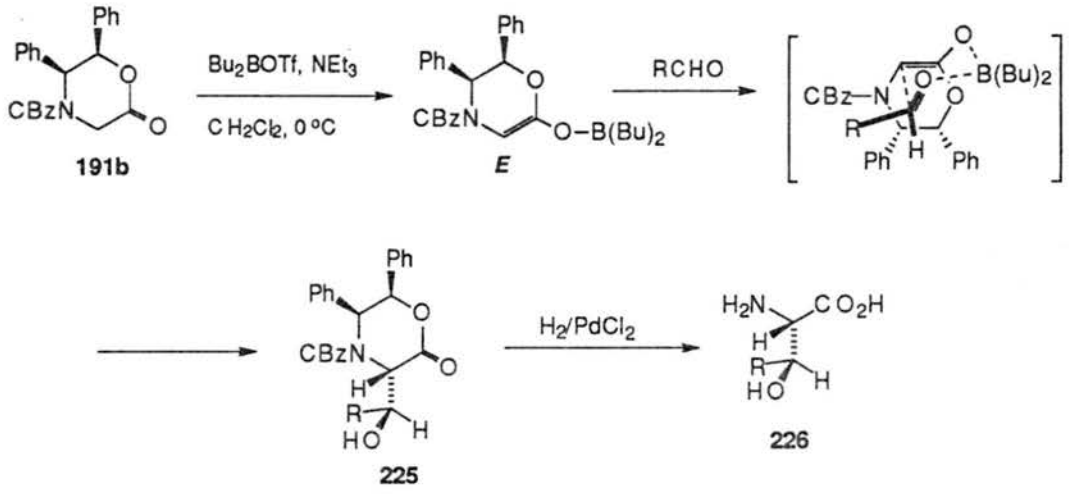
The catalytic asymmetric hydrogenation of (E)-alkene **E-223** with [Rh(COD)(s,s)-chirophos]BF_4 as the asymmetric catalyst produced an inseparable mixture of diastereomers in a roughly 2:1 ratio based on the stereochemical outcome of the final product (DAP).

The low enantioselectivity of the catalytic asymmetric hydrogenation might be due to two reasons: (1) the catalytic asymmetric hydrogenation of the (E)-alkene usually gave lower product enantiomeric ratios; and (2) the chiral auxiliary on the other side of molecule might interfere with the asymmetric induction from the chiral catalyst. Interestingly, a similar result was observed by Helcomb and coworkers (Scheme 25) later. [31]

After simple deprotection and hydrolysis of the methyl ester, DAP, as a mixture of two diastereomers, was obtained. The optical purity of the diaminopimelic acid product was determined by Mosher-amide methodology, which will be discussed in detail later.

In selecting a strategy to accomplish the key coupling of two optically pure glycinates to a three-carbon tether, we found that employment of the enol borane aldol couplings (scheme 38) reported by Miller [39] and subsequently by Williams and Im [11] on

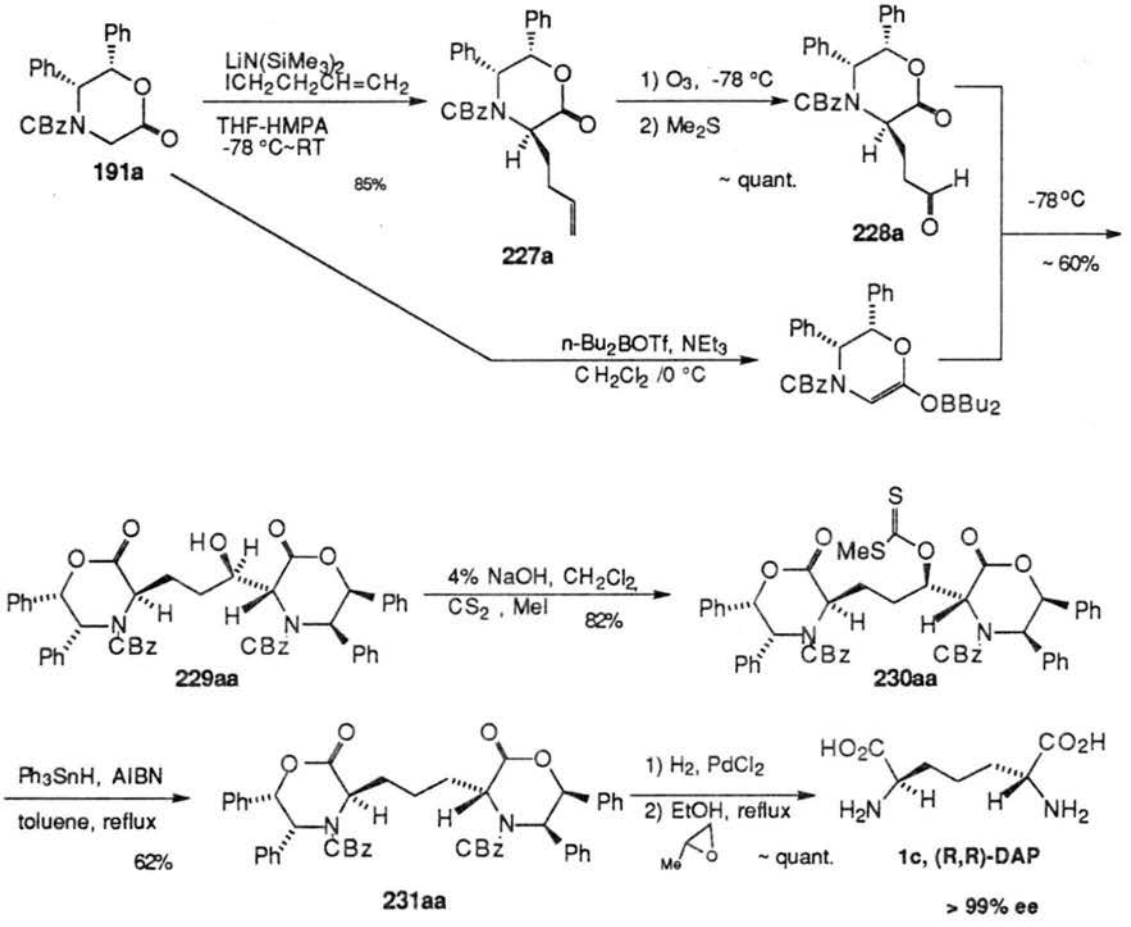
these oxazinone systems proved to be attractive. While this approach mandates the deoxygenation of a β-hydroxy construct to obtain the parent DAP systems, we desired an approach that would also install functionality in the connecting propyl chain for the ultimate purpose of providing starting materials for further elaboration into potential k_{cat} inhibitors of DAP biosynthesis.



SCHEME 38

As shown in Scheme 39, preparation of the boron enolate of **191a** according to Miller [39] followed by aldol condensation with the aldehyde **228a** in methylene chloride at low temperature gave the β-hydroxy dilactone **229a** (55-62%). Although ultimately unimportant for the synthesis of DAP, the diastereoselectivity of the aldol condensation was excellent. Out of a total of four possible diastereoisomers, only two were observable in the crude reaction mixture. The small vicinal coupling constants (~1.9 Hz) for the C-2/C-3 (DAP numbering) methines for **229aa** is in accord with the *anti*-selectivity observed by Miller and subsequently by us. Both sets of aldolizations support a Zimmerman-Traxler chair-type transition state predominantly from the face of the oxazinone *anti*-to the two phenyl rings with the aldehyde methine oriented toward the inside of the oxazinone ring.

The stereoselective preparation of such β-hydroxy DAP derivatives should provide useful substrates from which additional DAP analogues might be prepared.

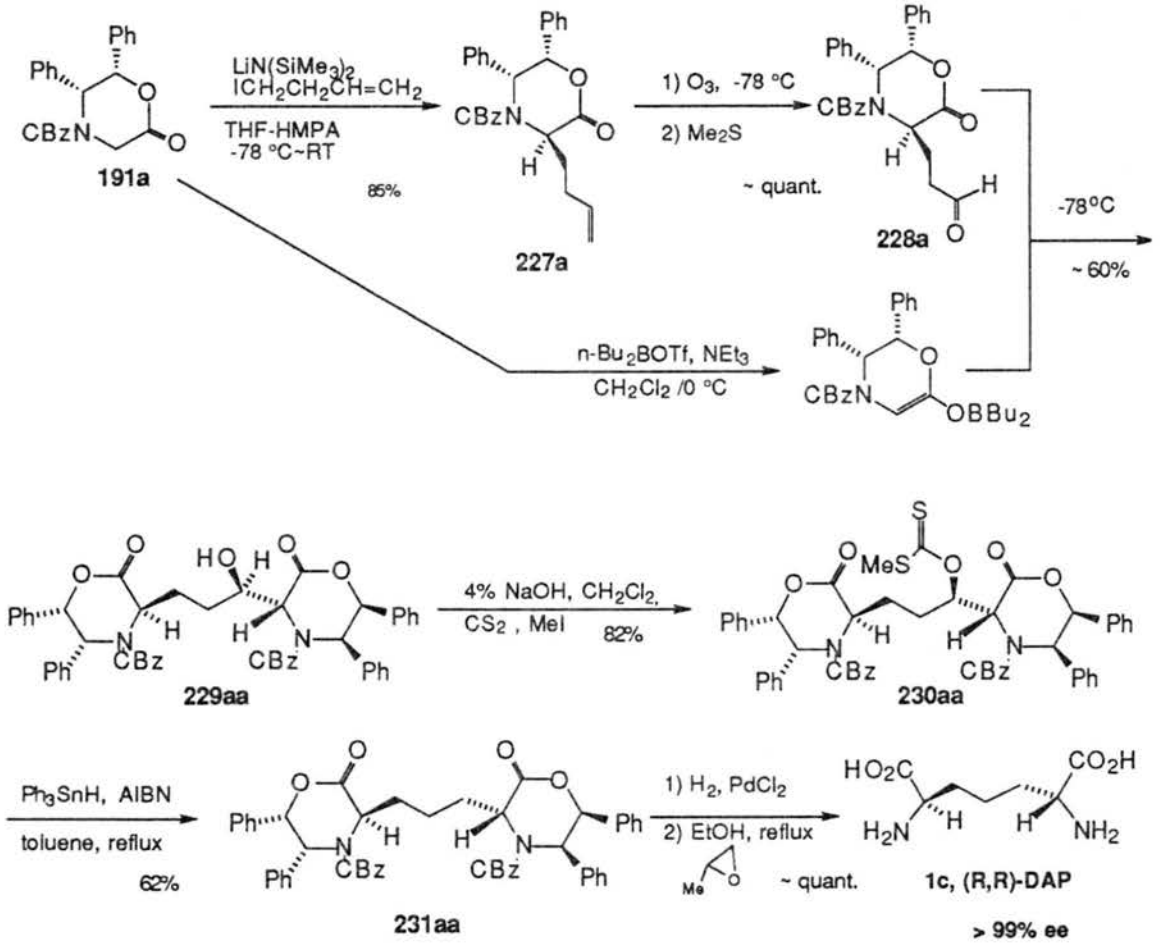


SCHEME 39

Next, the reductive functional group transformation of the β-hydroxy group was examined to obtain the requisite deoxygenation products. As in the synthesis of **37**, this proved to be very difficult since this alcohol moiety was very hindered and was prone to β-elimination. Many attempts at activating the hydroxyl for hydride displacement resulted in either no reaction or α,β-dehydrogenation.[11] After extensive experimentation, an improved procedure was found which, simply involved stirring **229aa** in 4% NaOH / methylene chloride in the presence of carbon disulfide and methyl iodide; the xanthate ester **230aa** was obtained in good yield with little or no detectable elimination product. Radical

reduction [40] of **230aa** with triphenyltin hydride in hot toluene afforded the reduction product **231aa** in 62-83% yield with no detectable loss of stereochemical integrity at the adjacent methine position. Finally, catalytic hydrogenolysis of **231aa** proceeded in essentially quantitative yield to afford R,R-diaminopimelic acid (**1c**).

Following the same protocol, S,S-diaminopimelic acid **1a** was synthesized by employing two equivalents of the antipodal lactone **191b**, as shown in Scheme 40.

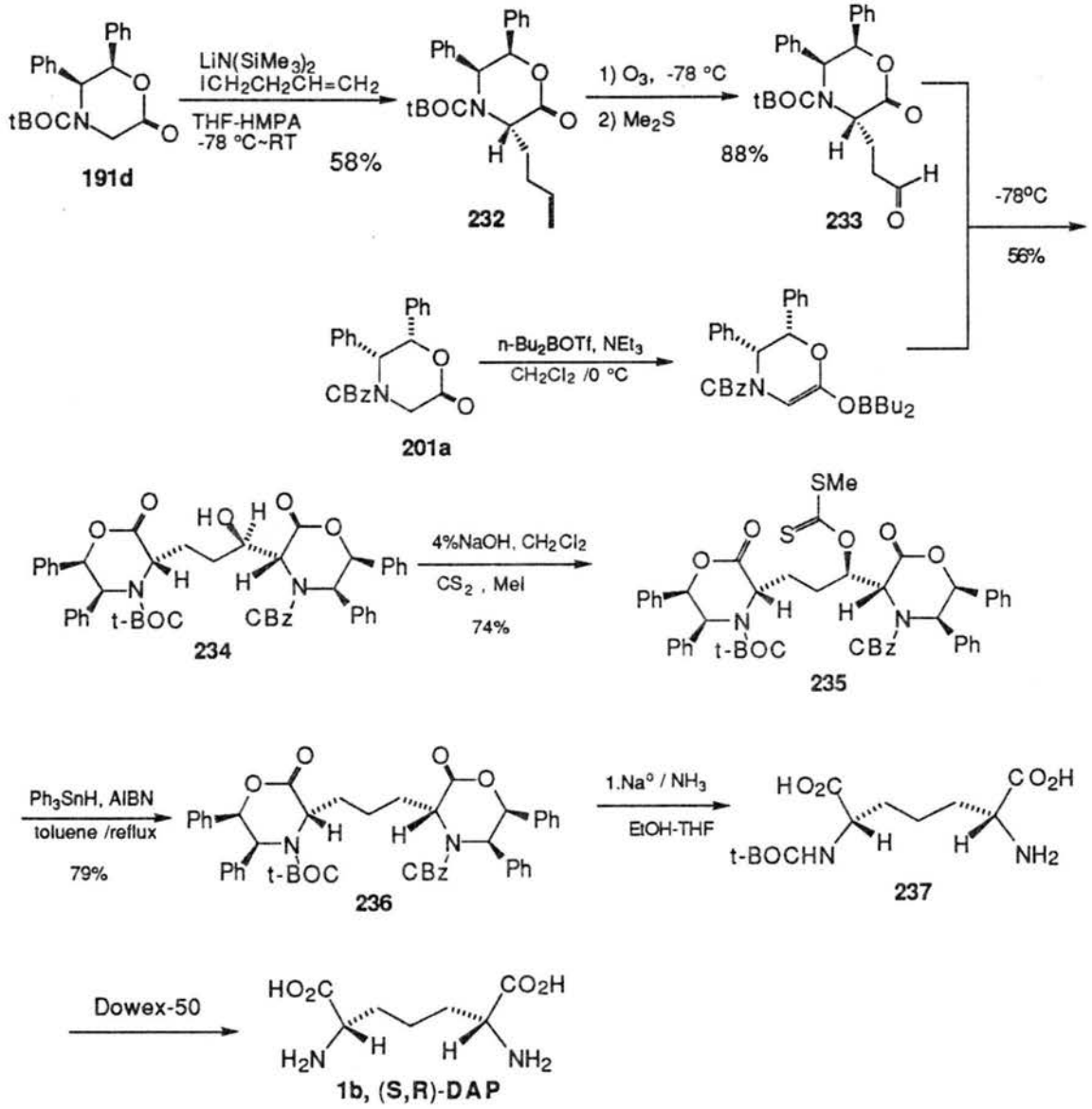


SCHEME 40

Syntheses of potent immunostimulants [41] derived from substructures of bacterial cell walls, such as FK-156 and FK-565, and small peptides containing DAP or analogues of DAP require the selective manipulation of the terminal amino and carboxyl residues.

This is a particularly difficult regiochemical problem for the synthesis of peptides derived from *meso*-DAP. The synthesis of *meso*-diaminopimelic acid can be realized by

SCHEME 2

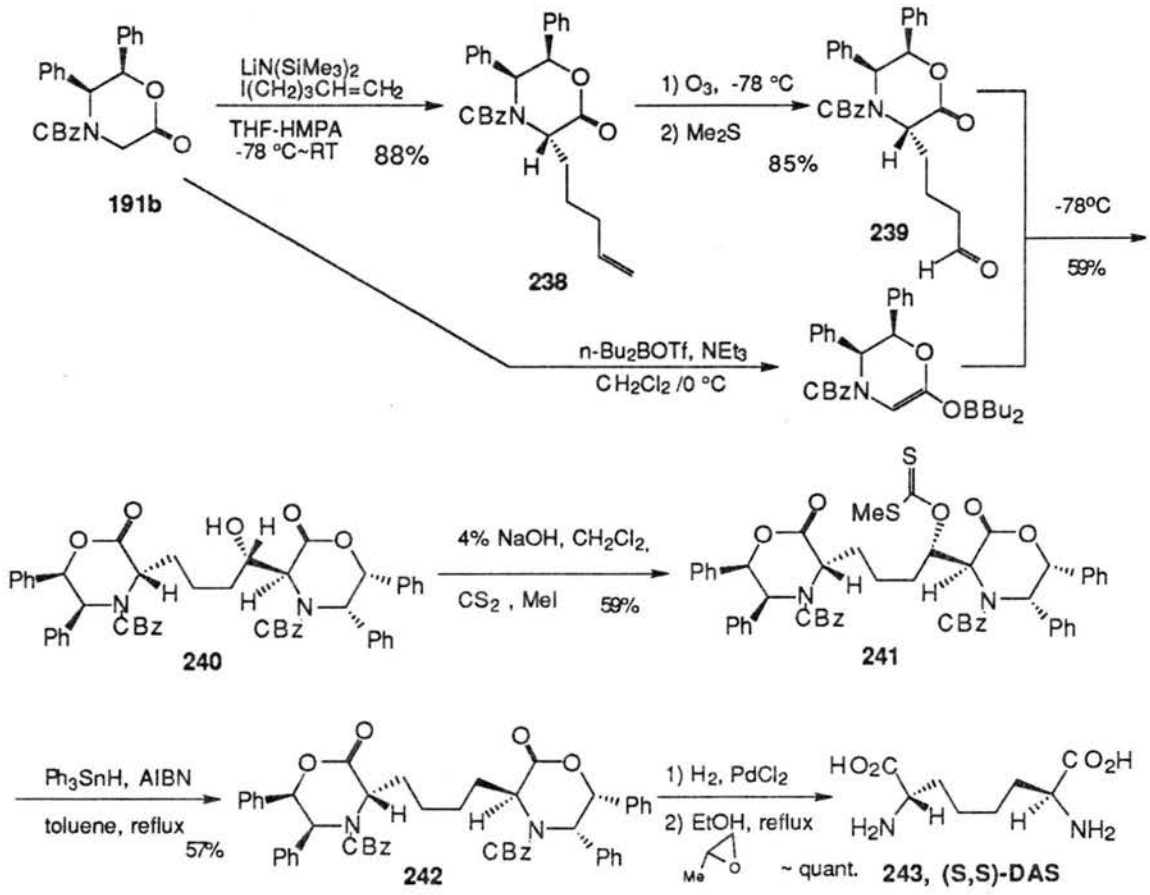


SCHEME 41

essentially the same protocol used above, except that both **191a** and **191b** being employed. As an approach to solving the general problem of selectively manipulating the *meso*-DAP system, we have synthesized a differentially protected N-t-BOC-R,S-DAP isomer by employing the N-t-BOC glycinate **191d**. By selecting the appropriate absolute stereochemistry and N-protection from the glycinates **191a-d**, in principle, any

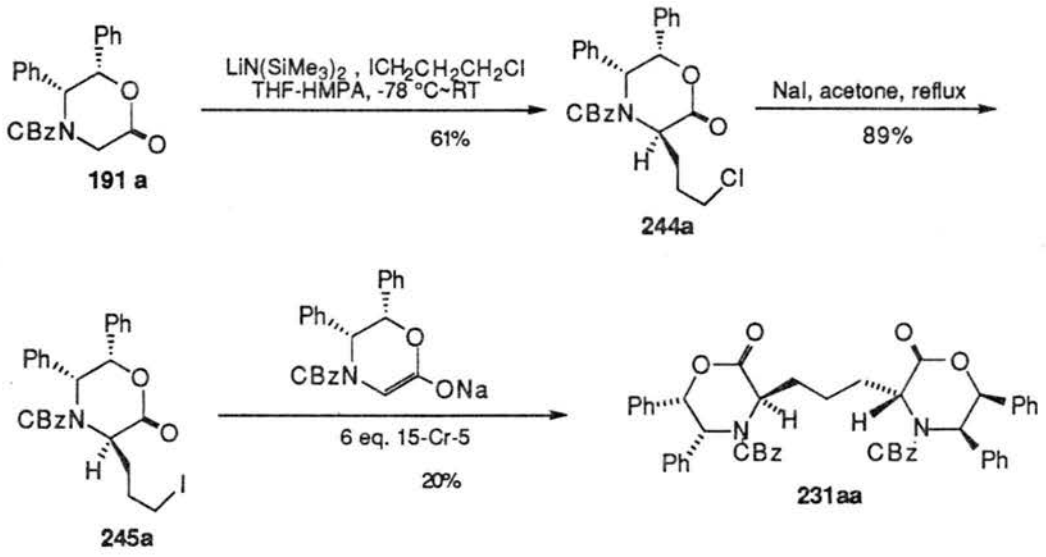
differentially protected DAP stereoisomer can be synthesized by following the general protocol described. As shown in Scheme 41, the corresponding mono-N-t-BOC DAP isomer S,R-**237** has been prepared by following essentially the same experimental protocol as that outlined above. The only difference between these routes (Schemes 39 and 41), is the final dissolving metal reduction of bis-lactone **236** to the corresponding mono-N-t-BOC product **237**. The t-BOC group can be removed to afford *meso*-DAP.

Application of the same basic protocol to higher homologs of DAP can be readily envisioned. For example, α,α'-diaminosuberic acid [1a] (**243**, Scheme 42) has been used as a nonreducible analogue of dicystine.[42] Previous stereodefined syntheses of this amino acid have relied on Kolbe electrolytic coupling of glutamic acid derivatives. As shown in Scheme 42, enolate alkylation of **191b** with 1-iodo-4-pentene furnished **238** in high yield (88%). Ozonolysis and aldol coupling of the resulting aldehyde **239** with enolborane provided **240** in good yield. Radical deoxygenation and reductive cleavage furnished S,S-2,7-diaminosuberic acid **243** in high optical purity.



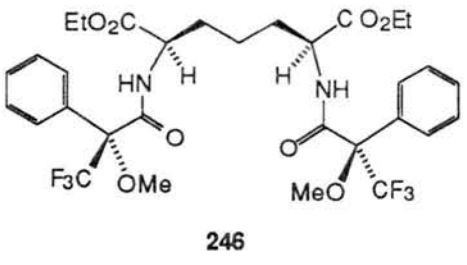
SCHEME 42

Recently, Baldwin and associates,[44] have described an improved procedure for the enolate alkylation of glycinates **191**. In applying this method to the DAP syntheses, the direct enolate coupling between a 3'-halopropyl derivatized glycinate and a second glycinate enolate can be realized, albeit in moderate yields. An example is illustrated in Scheme 43. Enolate alkylation of **191** with 3-chloro-1-iodopropane followed by Finkelstein replacement provides the iodides **245** in moderate yield as single (*anti*-) diastereomers. Addition of the iodide to a preformed solution of enolate derived from **191** in the presence of 15-crown-5 ether proceeds with excellent diastereofacial selectivity, but in modest yield to afford the bis-lactones **231** or **236**. These substances were found to be identical to the products obtained from the radical deoxygenation protocol described in Schemes 39, 40 and 41. As such, these substances can be processed to the corresponding DAP isomers as described above.

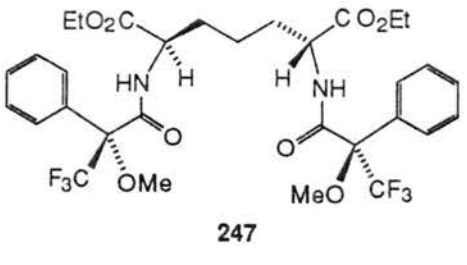


SCHEME 43

The optical purity of each diaminopimelic acid product was ascertained by comparing the ^{19}F nmr spectrum of the bis-N-acylated (+)-α-methoxy-α-trifluoromethyl phenylacetamide (**246, 247, 248**) with that of an authentic mixture of (+)-α-methoxy-α-trifluoromethyl phenylacetamides obtained from a commercially available mixture consisting of S,S- ; R,R-; and S,R-diaminopimelic acids (Figure 6). The optical purity of each isomer was >98% ee.



246



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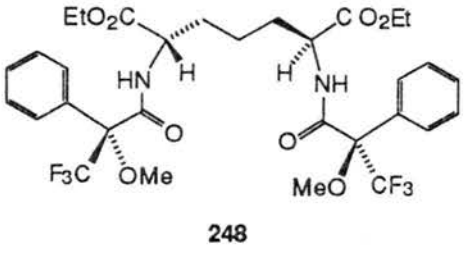

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FIGURE 5

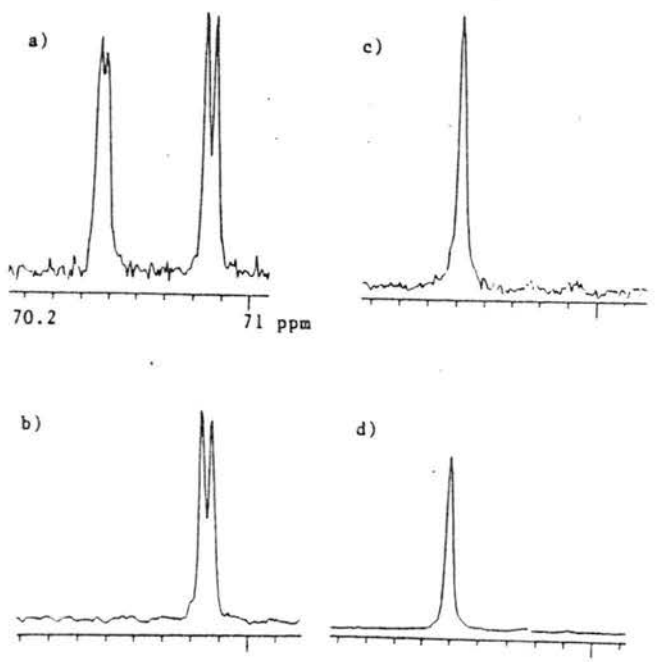


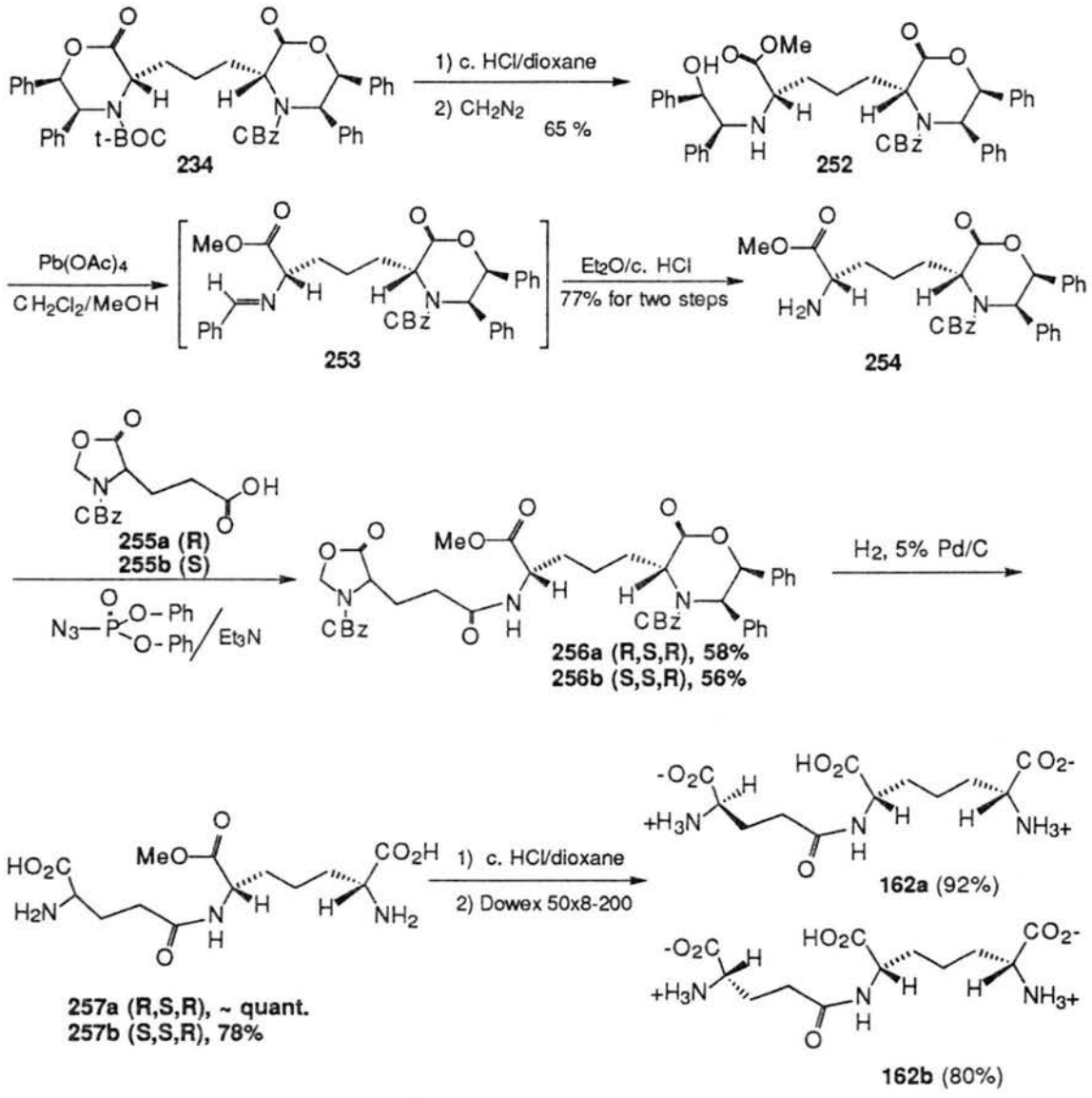
FIGURE 6. Comparision of ^{19}F NMR of (+)-MTPA amides of DAP

(a) stereorandom mixture of DAP isomers (b) synthetic *meso*-R,S-DAP

(c) synthetic S,S-DAP (d) synthetic R,R-DAP

2.3 Asymmetric Synthesis of γ-D(L)-Glutamyl-L-*meso*-Diaminopimelic Acid Dipeptide

The unique biological activity of these metabolites of peptidoglycans, FK-156 (**1**)[2,3] and its synthetic analogue FK-565 (**2**), [4] renders the synthesis of this class of peptides an attractive and worthy synthetic problem.[10] The key problem faced in preparing peptides of *meso*-DAP, is the unambiguous, selective protection of the respective amino and carboxy termini suitable for standard peptide coupling chemistry.



SCHEME 44

As shown in scheme 44, we have developed a new asymmetric and efficient synthesis of γ-D-glutamyl-L-*meso*-diaminopimelic acid peptide, a subunit of both FK-156 and FK-565. The approach employed should allow the preparation of peptides containing all of the possible DAP stereoisomers in an unambiguous fashion.

As previously discussed, the differentially N-protected DAP precursor **234** was prepared by employing selected diphenyloxazinones carrying the requisite N-t-BOC and N-CBz protecting groups. The N-t-BOC group of **234** was removed by treatment with concentrated hydrochloric acid in dioxane to give the regioselectively ring-opened product which was esterified with diazomethane furnishing **252** in 65% yield. There was no detectable racemization of this product as evidenced by inspection of the proton NMR. Amino alcohol **252** was treated with lead tetraacetate forming Shiff base **253**. The Shiff base was converted to the free amine (**254**, 77% from **252**) by treatment with concentrated hydrochloric acid in ether at ice bath temperature. Peptide coupling between the free amine of **254** and the protected D-glutamic acid derivative **255a** or **255b** [44] was accomplished using diphenylphosphoryl azide in DMF affording the desire peptide **256** in 61% yield. The fully protected peptide was unmasked by simple catalytic hydrogenation to give the methyl ester **257a** in 91% yield. After the hydrolysis of the methyl ester and the purification on Dowex-50, the free dipeptides **162a** and **162b** were obtained seperately.

Since all stereochemical variations of differentially N-protected DAP precursors corresponding to **234** are readily accessible, strategies to access many different N-terminal and C-terminal DAP-containing peptides can be easily envisioned.

In summary, an asymmetric and stereochemically unambiguous construction of diaminopimelic acid and related system has been developed. The availability of both optical antipodes of the glycinate templates renders this chemistry adaptable to prepare all possible diastereoisomers of substances based on the DAP skeleton in optically pure form.

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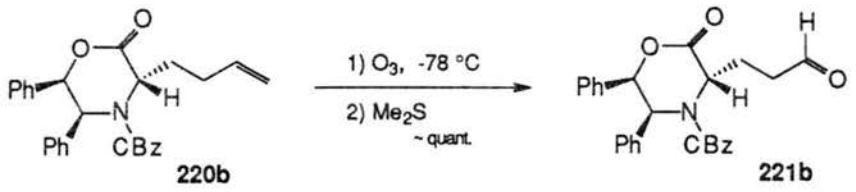
Experimental Section

General Information

^1H NMR spectra were obtained on a Bruker AC 300 MHz spectrometer or on an IBM WP200 or WP270 MHz FT NMR and chemical shifts are reported in parts per million downfield from TMS. NMR data collected in methanol-d_4 or D_2O are reported relative to the methanol peak at 3.30 ppm or HOD peak at 4.63 ppm respectively. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR either as KBr pellets or as thin films (NaCl plates) from dichloromethane and are reported as λmax in cm^{-1}. Melting points were determined in open-ended capillary tubes on a Mel-Temp apparatus and are uncorrected. Optical rotations were obtained on a Rudolph research Autopol III automatic polarimeter at a wavelength of 589 nm (sodium "D" line) with a 1.0-dm cell with a volume of 1 mL. Specific rotations, $[\alpha]_D$, are reported in degrees per decimeter at the specified temperature and the concentration (c) given in grams per 100 mL in the specified solvent. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are accurate to within $\pm$ 0.4% of the calculated values. Mass spectra were obtained on a 1992 Fisons VG AutoSpec at the Chemistry Department (CSU) or on a VG-7070 at UC Riverside. Column chromatography was performed using Merck silica gel grade 60, 230-400 mesh, 60Å. Both analytical and preparative thin-layer chromatography was performed using Merck Kieselgel 60 F_{254} plates. Visualization on TLC was achieved with ultraviolet light, or heating of TLC plates submerged in a 5% solution of phosphomolybdic acid in 95% ethanol. Reagents and solvents were commercial grades and were used as supplied with the following exceptions. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl. Dry methylene chloride was obtained by distillation over CaH_2. DMF and HMPA were dried over activated 4Å molecular sieves.

Determination of Optical Purity, General Procedure

The amino acids (5-10 mg) were converted into the corresponding ester hydrochloride salts as follows: The diaminopimelic acids were refluxed for 2 h in EtOH containing 5 eq. oxalyl chloride. All the resulting reaction mixtures were cooled, concentrated, and dried *in vacuo*. The amino ester hydrochloride salts were treated with (±) or (+)- -α-methoxy-α-trifluoromethyl phenylacetyl chloride (1.2 eq) in THF in the presence of excess propylene oxide at 50°C. After 1 h, the solvent was evaporated and the residue was dried *in vacuo*. The crude Mosher amides were analyzed by ^{1}H and ^{19}F NMR spectroscopy and compared to spectra of an authentic diastereomeric mixture of Mosher amides prepared by the same protocol from the corresponding commercial, stereoisomeric mixture of DAP isomers (Sigma Chemical Co.)



(3S,5S,6R)-4-(Benzyloxycarbonyl)-5,6-diphenyl-3-(2'-carbonylethyl)-2,3,5,6-tetrahydro-4H-1,4-oxain-2-one 221b.

Ozone was bubbled through a solution of **220b** (740 mg, 1.68 mmol, 1.0 eq.) in MeOH-CH_2Cl_2 (200 mL, 1:1) until the solution turned blue (ca. 15 min) at -78 °C. Nitrogen gas was then passed through the reaction mixture to remove excess O_3 until the solution became colorless, and then the solution was allowed to warm up to room temperature. The resulting solution was quenched with excess dimethylsulfide. After 12 h of stirring, the reaction mixture was concentrated. Purification via column chromatography (silica gel, hexane: CH_2Cl_2: EtOAc; 5:4:1) provided 622 mg (84%) of **221b** as a white solid.

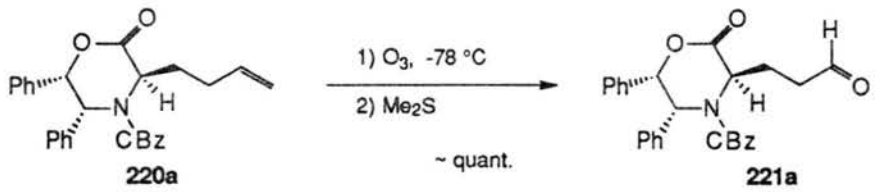
^{1}H NMR (200 MHz, DMSO-d_6, 393K vs TMS): δ 2.42 (2H, q, J = 7.7 Hz), 2.71 (2H, t, J = 6.9 Hz), 4.86 (1H, t, J = 7.3 Hz), 4.98 (2H, s), 5.27 (1H, d, J = 2.9 Hz), 6.24 (1H, d, J = 2.9 Hz), 6.54 (1H, s), 6.58 (1H, s), 6.95-7.31 (13H, m), 9.71 (1H, s)ppm;

IR (NaCl, film): 2732, 1752, 1700cm^{-1};

mp: 144-5 °C (recryst. hexane/ CH_2Cl_2/ EtOAc);

$[\alpha]_D^{25}$ = - 37.3 (c 0.67, CH_2Cl_2);

Anal. Calcd. for $C_{27}H_{25}NO_5$: C, 73.12; H, 5.68; N, 3.16. Found: C, 72,87; H, 5.87; N, 3.18.



(3R,5R,6S)-4-(Benzyloxycarbonyl)-5,6-diphenyl-3-(2'-carbonylethyl)-2,3,5,6-tetrahydro-4H-1,4-oxain-2-one 221a.

Ozone was bubbled through a solution of **220a** (2.40 g, 0.187 mol, 1.0 eq) in MeOH-CH_2Cl_2 (200 mL, 1:1) until the solution turned blue (ca. 15 min) at -78 °C. Nitrogen gas was then passed through the reaction mixture to remove excess O_3 until the solution become colorless, and then the solution was allowed to warm to room temperature. The resulting solution was quenched with excess dimethylsulfide. After 12 h of stirring, the reaction mixture was concentrated. Purification via column chromatography (silica gel, hexane: CH_2Cl_2: EtOAc; 5:4:1) provided 1.90g (79%) of **221a** as a white solid.

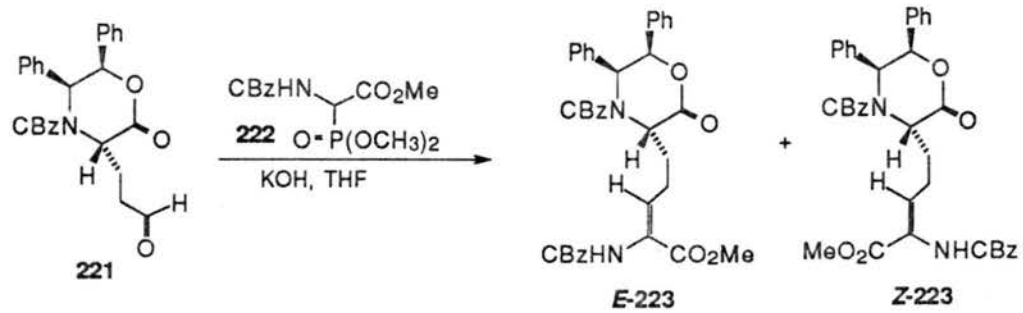
^1H NMR (200 MHz, 393K, DMSO-d_6 vs TMS) δ 2.42 (2H, q, J =7.0 Hz), 2.68 (2H, t, J = 6.7 Hz), 4.84 (H, t, J = 7.1 Hz), 4.97 (2H, s), 5.26 (1H, d, J = 2.9 Hz), 6.25 (1H, d, J = 2.9 Hz), 6.53 (1H, s), 6.57 (1H, s), 7.02-7.33 (13H, m), 9.70 (1H, s)ppm;

IR (NaCl, film): 2725, 1745, 1702 cm^{-1};

mp: 148-149 °C (recryst. hexane:CH_2Cl_2: EtOAc; 5:4:1);

$[\alpha]_D^{25}$ = + 42.5° (c = 0.50, CH_2Cl_2).

Combustion analytical data obtained in the antipodal series **221b**.

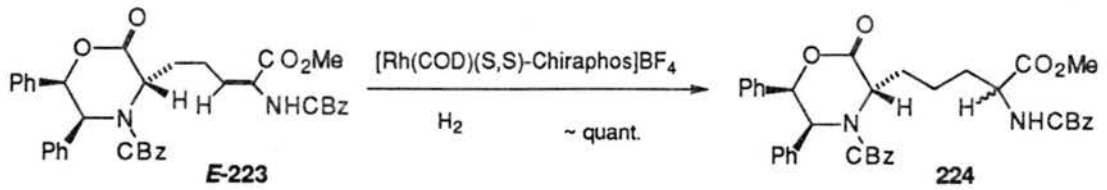


Alkene 223.

To a stirring solution of KOH(124 mg, 2.22 mmol, 3.0 eq) in MeOH(0.5 mL)/THF(4 mL) was added SM-1 (328 mg, 0.75 mmol, 1.0 eq) and SM-2(294 mg, 0.82 mmol, 1.1 eq)/THF (20 mL) at -78 °C. The cold bath was removed and allowed the temperature to warm up slowly. The reaction mixture was poured into EtOAc/H_2O the temperature of the reaction mixture reach 0 °C. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated and separated by column chromatography on silica gel (eluted with CH_2Cl_2: EtOAc; 10:1) to afford 400 mg (83%) of **E-223** as a white solid.

E-223: ^1H NMR (300 MHz, 393K, DMSO-d_6 vs TMS) δ 2.27(2H, m); 2.36(2H, m); 3.69(3H, s); 4.87(1H, t, J = 7.5 Hz); 5.01(2H, d, J = 3.0 Hz); 5.08(2H, s); 5.29(1H, d, J = 3.0 Hz); 6.21(1H, d, J = 2.8 Hz); 6.52(1H, t, J = 7.2 Hz); 6.59(2H, d, J = 7.5 Hz); 7.00-7.40(18H, m); 8.21(1H, br, D_2O exchanged)ppm; IR (NaCl, film): 3416, 3339, 2975, 1755, 1695, 1511, 1180cm-^{1}; mp: 115-8 °C (recryst. hexane:CH_2Cl_2: EtOAc; 5:4:1); $[\alpha]_D^{25}$ = - 46.6 (c = 0.50, CH_2Cl_2). Anal. Calcd. for $C_{38}H_{36}N_2O_8$: C, 70.35; H, 5.59; N, 4.32. Found: C, 70.48; H, 5.66; N, 4.11.

Z-223: ^1H NMR (300 MHz, 393K, DMSO-d_6 vs TMS) δ 2.24(2H, q, J = 7.5 Hz); 2.59(2H, q, J = 7.8 Hz); 3.71(3H, s); 4.87(1H, t, J = 7.5 Hz); 5.01(2H, d, J = 3.0 Hz); 5.10(2H, s); 5.29(1H, d, J = 3.0 Hz); 6.10(1H, t, J = 7.5 Hz); 6.22(1H, d, J = 2.7 Hz); 6.60(2H, d, J = 7.5 Hz); 7.00-7.40(18H, m); 8.42(1H, br, D_2O exchanged)ppm; IR (NaCl, film): 3418, 3341, 2978, 1756, 1698, 1514, 1367, 1243, 1163cm-^{1};



(3R,5S,6R)-α-[(phenylmethoxy)carbonylamino]-2-oxo-5,6-diphenyl-4-[(phenylmethoxy)carbonyl]-3-morpholinepentanoic acid, methyl ester, 224.

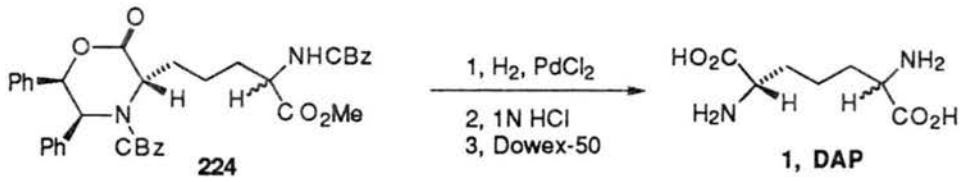
To a solution of ***E*-223** (388 mg, 0.60 mmol) in CH_2Cl_2-CH_3OH (8 mL, 1:1) was added [Rh(COD)(s,s)-chirophos]BF_4 (80 mg). The reaction vessel was charged with H_2 gas and the mixture was hydrogenated at 50 psi for 12h. The mixture was then purged with nitrogen, concentrated and filtered through a short silica column to give 386 mg (99%) of product **224** as a white solid.

^{1}H NMR (200 MHz, 393K, DMSO-d_6 vs TMS) δ 1.67(2H, m); 1.80(2H, m); 2.13(2H, q, J = 7.6 Hz); 3.63(3H, s); 4.12(1H, m); 4.80(1H, t, J = 7.3 Hz); 4.96/4.98(2H, s); 5.05(2H, s); 5.25(1H, d, J = 3Hz); 6.20(1H, d, J = 3Hz); 6.56(2H, d, J = 7.0Hz); 6.95-7.40(18H, m)ppm;

mp: 118-122 °C;

$[\alpha]_D^{25}$= -15.3° (c = 0.85, CH_2Cl_2).

Anal. Calcd. for $C_{38}H_{38}N_2O_8$: C, 70.14; H, 4.31; N, 5.89. Found: C, 70.24; H, 4.16; N, 5.95.

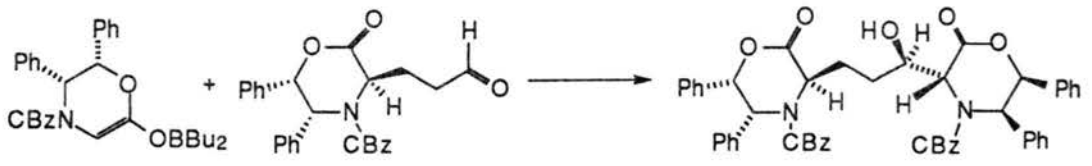


(2S/2R,6S)-2,6-Diaminopimelic acid.

To a solution of **224** (264 mg, 0.42 mmol, 1.0 eq) in THF-EtOH (4 mL, 3:1) was added $PdCl_2$ (60 mg, 0.028 mmol, 3.0 eq). The reaction vessel was degassed by N_2 for 10 min., then was charged with hydrogen and the mixture was hydrogenated at 60 psi for 48h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and trituraed with Et_2O and CH_2Cl_2. The crude product was dissolved in 1 N HCl aqeous solution and heated to reflux for 2 hours. The resulting mixture was concentrated to dryness and purified on Dowex 50x8-200 to give 57 mg (71.4%) product as a white solid.

(2R,6S)-DAP: (2S,6S)-DAP = 2:1

^1H NMR (300 MHz, D_2O): δ 1.40 (2H, m), 1.79 (4H, m), 3.75 (2H, m)ppm.



221a **229aa**

(3R,3'R,5R,5'R,6S,6'S)-3,3'-(1-hydroxy-1,3-propanediyl)-bis[2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 229aa.

To a stirred solution of **191a** (388 mg, 1.00 mmol, 1.0 eq) in CH_2Cl_2 (10 mL) was added di-n-butylborontriflate (2.0 mL, 2.00 mmol, 2.0 eq of a 1M solution in CH_2Cl_2) followed by the addition of Et_3N (421 uL, 2.0 mmol, 3.0 eq) at -5 °C. After 15 min the reaction mixture was cooled to -78 °C and a CH_2Cl_2 (5 mL) solution of aldehyde **220a** (664 mg, 1.5 mmol, 1.5 eq) was added. After 1 h the reaction mixture was quenched with phosphate buffer solution (0.025 M, pH = 7) and poured into water. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic solution was dried over anhydrous Na_2SO_4, filtered, concentrated and separated by column chromatography (silica gel, hexane: CH_2Cl_2: EtOAc, 5:4:1) to afford 460 mg (55%) of **229aa** as a white solid.

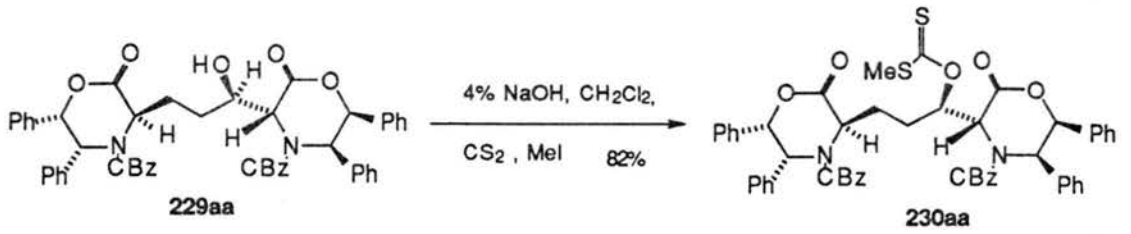
^1H NMR (200 MHz, DMSO-d6, 393K vs TMS): δ 1.91-2.02 (2H, m), 2.32-2.38 (2H, m), 4.27 (1H, m), 4.91(2H, s), 4.99 (2H, s), 4.85-5.06 (2H, m), 5.25 (1H, d, J = 2.9 Hz), 5.30 (1H, d, J = 2.9 Hz), 5.67 (1H, br, D2O exchanged), 6.23 (1H, d, J = 1.9 Hz), 6.51 (1H, d, J = 3.1 Hz), 6.58 (2H, s), 6.61 (2H, s), 7.00-7.30 (26H, m)ppm;

IR (NaCl, film): 3545, 1747, 1738, 1700cm^{-1};

mp: 231-4 °C (recryst. hexane: CH_2Cl_2: EtOAc; 5:4:1);

$[\alpha]_D^{25}$ = + 19.1 (c 0.48, CH_2Cl_2);

Anal. Calcd. for $C_{51}H_{46}N_2O_9$: C, 73.72; H, 5.58; N, 3.37. Found: C, 73.61; H, 5.59; N, 3.21.



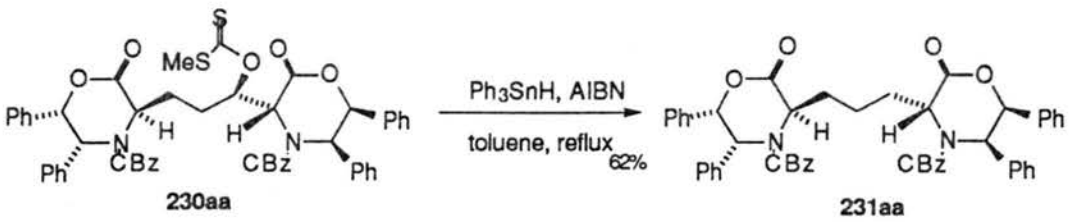
(3R,3'R,5R,5'R,6S,6'S)-3,3'-[1-(methylthio)thioxomethoxy]propyl-bis[2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 230aa.

To a stirred solution of **229aa** (420 mg, 0.505 mmol, 1.0 eq) in CH_2Cl_2 (200 mL) containing CS_2 (30 mL) and MeI (2 mL) was added 4% NaOH aq. solution (50 mL) and TBAHS (5 mg). The reaction mixture was cooled to 0 °C and excess MeI was added. The reaction mixture was stirred for 5 h at 0-15° C. The organic layer was separated and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic extracts were washed with water and sat. NaCl aq., and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography (silica gel, hexane: CH_2Cl_2: EtOAc, 5:4:1) to yield 340 mg (73%) of product **230aa** as a greenish oil and 30 mg of unreacted **229aa** as a white solid.

^{1}H NMR (200 MHz, DMSO-d_6, 393k vs TMS) δ 2.11(4H, m), 2.63 (3H, s), 4.88 (H, t, J = 7.2 Hz), 4.94 (3H, s), 5.02 (2H, s), 5.03 (2H, s), 5.28 (1H, d, J = 3.0 Hz), 5.33 (1H, d, J = 3.0 Hz), 5.33 (1H, d, J = 2.0 Hz), 6.25 (1H, d, J = 2.6 Hz), 6.54 (1H, d, J = 3.1 Hz), 6.61 (2H, s), 6.64 (2H, s), 6.95-7.25 (26H, m)ppm;

IR (NaCl, film): 3033, 1757, 1706, 1229cm^{-1};

$[\alpha]_D^{25}$ = -16.0 (c 0.5, CH_2Cl_2).



(3R,3'R,5R,5'R,6S,6'S)-3,3'-(1,3-propanediyl)bis[2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 231aa.

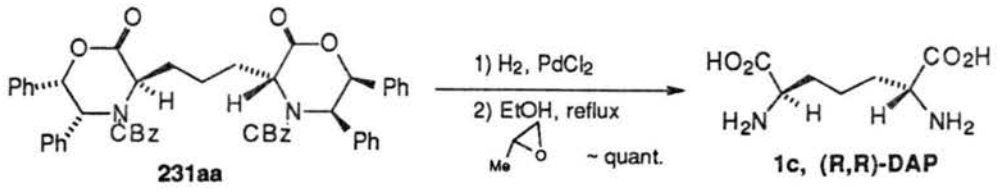
To a solution of **230aa** (340 mg, 0.38 mmol, 1.0 eq) in toluene (15 mL) was added AIBN (6 mg, 0.038 mmol, 0.1 eq) follow by the addition of triphenyltin hydride (296 mg. 0.76 mmol, 2.0 eq). The resulting solution was brought to reflux temperature. After 1h the toluene was evaporated and the residue was separated by column chromatography on silica gel (eluted with hexane: CH_2Cl_2: EtOAc; 5:4:1) to afford 226 mg (73.0%) of **231aa** as a white solid. Combustion analytical data obtained in the antipodal series **231bb**.

^{1}H NMR (200 MHz, DMSO-d_6, 393K vs TMS) δ 1.73-1.83 (2H, m), 2.19-2.29 (4H, m), 4.91 (2H, t, J = 6.7 Hz), 4.95 (2H, s), 4.98 (2H, s), 5.29 (2H, d, J = 2.9 Hz), 6.21 (2H, d, J = 2.9 Hz), 6.57 (2H, s), 6.61 (12H, s), 6.99-7.25 (26H, m)ppm.

IR (NaCl, film): 1749, 1706 cm-1;

mp: 284-8 °C (recryst. EtOAc);

[a]D25 = + 36.7 (c 0.54, CH_2Cl_2).

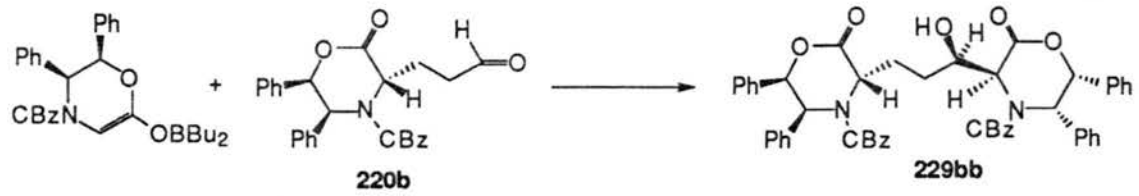


(2R, 6R)-2,6-Diaminopimelic acid (R,R-1c).

Method A: To a solution of **231aa** (76 mg, 0.093 mmol, 1.0 eq) in CH_2Cl_2-EtOH (8 mL, 5:2) was added palladium chloride (50 mg, 0.028 mmol, 2.0 eq). The reaction vessel was charged with H_2 gas and the mixture was hydrogenated at 60 psi for 48 h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. The crude product was dissolved in dry EtOH(2 mL) and heated to reflux. To this refluxing solution was added excess propylene oxide, and stirring was continued for 30 min. at reflux. The white precipitate was filtered to give 18 mg (100%) of **R,R-DAP 1c** as a white solid.

Method B: To a solution of **231aa** (80 mg, 0.098 mmol, 1.0 eq) in CH_2Cl_2 (4 mL)-MeOH (2 mL)-H_2O (0.1 mL) was added 20% $Pd(OH)_2$ on carbon (80 mg). After degassed with N_2, the reaction vessel was charged with H_2 gas and the mixture was hydrogenated at 60 psi for 16 h. After the pressure was released, the reaction mixture was transferred onto a celite column. This celite column was first eluted with MeOH to wash off the byproduct, then eluted with water to collect product. The aqueous eluate was concentrated on lyophilizer to afford 18 mg (96%) of **R,R-DAP 1c** as a white solid.

^1H NMR (270 MHZ, D_2O): δ 1.40 (2H, m), 1.85 (4H, m), 3.72 (2H, m)ppm.
$[\alpha]_D^{25}$ = - 20.0 (c 0.50, H_2O); > 99% ee.



(3S,3'S,5S,5'S,6R,6'R)-3,3'-(1-hydroxy-1,3-propanediyl)bis[2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 229bb.

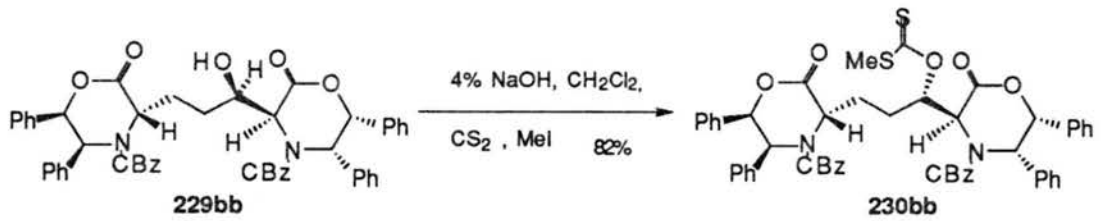
To a stirred solution of **191b** (170 mg, 0.43 mmol, 1.0 eq) in CH_2Cl_2 (5 mL) was added di-n-butylborontriflate (860 uL, 0.86 mmol, 2.90 eq, 1M solution in CH_2Cl_2) followed by the addition of Et_3N (181 uL, 1.29 mmol, 3.0 eq.) at -5 °C. After 15 min the reaction mixture was cooled to -78 °C and a 5 mL CH_2Cl_2 solution of aldehyde **220b** (290 mg, 0.65 mmol, 1.5 eq.) was added. After 1 h the reaction mixture was quenched with phosphate buffer solution (0.025 M, pH = 7) and poured into water. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, concentrated and separated by column chromatography on silica gel (eluted with hexane: CH2Cl2: EtOAc; 5:4:1) to afford 220 mg of **229bb** (62%) as a white solid.

^{1}H NMR (200 MHz, DMSO-d_6, 393k vs TMS): δ 1.87-2.00 (2H, m), 2.26-2.37 (2H, m), 4.26 (H, m), 4.92 (2H, s), 4.97 (2H, s), 4.89-5.07 (2H, m), 5.26 (1H, d, J = 2.8 Hz), 5.31 (1H, d, J = 2.8 Hz), 5.66 (1H, br D_2O exchanged), 6.24 (1H, d, J = 1.8 Hz), 6.51 (1H, d, J = 2.9 Hz), 6.59 (2H, s), 6.62 (2H, s), 7.02-7.31 (26H, m)ppm;

IR (NaCl, film): 3528, 1734, 1702 cm^{-1};

mp: 244-7 °C (recryst.hexane/ CH_2Cl_2/ EtOAc);

$[\alpha]_D^{25}$ = - 20.3 (c 0.5, $CHCl_3$).



(3S,3'S,5S,5'S,6R,6'R)-3,3'-[1-(methylthio)thioxomethoxy-1,4-propanediyl-bis[2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 230bb.

To a stirred solution of **229bb** (200 mg, 0.24 mmol, 1.0 eq.) in CH_2Cl_2 (100 mL) containing CS_2 (15 mL) was added 4% NaOH aq. solution (15 mL) and TBAHS (5 mg). After stirring for 5 h at room temperature, the reaction mixture was cooled down to 0 °C and excess MeI was added. The reaction mixture was then stirred for 1 h at 0° C. The organic layer was separated and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic extracts were washed with water, saturated NaCl solution, and dried over anhydrous Na_2SO_4. The residue was purified by column chromatography on silica gel (eluted with hexane: CH_2Cl_2: EtOAc; 5:4:1) to yield 177 mg of product **230bb** (82%) as a white solid.

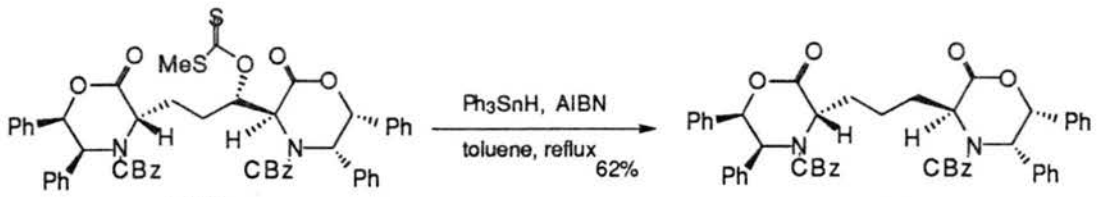
^{1}H NMR (200 MHz, DMSO-d_6, 393k vs TMS): δ 2.22-2.41 (4H, m), 2.64 (3H, s), 4.88 (3H, m), 4.99 (4H, s), 5.22 (1H, d, J = 3.2 Hz), 5.30 (1H, d, J = 2.8 Hz), 5.38 (1H, d, J = 2.8 Hz), 6.01 (1H, d, J = 2.8 Hz), 6.59 (2H, s), 6.62 (2H, s), 6.95-7.25 (26H, m)ppm;

IR (NaCl, film): 3033, 1759, 1703, 1229cm^{-1};

mp: 95-105 °C (recryst.hexane/ CH_2Cl_2/ EtOAc);

$[\alpha]_D^{25}$ = + 16.0 (c 0.62, $CHCl_3$).

Anal. Calcd. for $C_{53}H_{48}N_2O_9S_2$: C, 69.11; H, 5.25; N, 3.04; S, 6.96. Found: C, 68.89; H, 5.47; N, 3.13; S, 6.76.



230bb 231bb

(3S,3'S,5S,5'S,6R,6'R)-3,3'-(1,3-propanediyl)-bis[2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 231bb.

To a solution of **230bb** (500 mg, 0.55 mmol, 1.0 eq) in toluene (20 mL) was added AIBN (6 mg, 0.038 mmol, 0.1 eq) followed by addition of triphenyltin hydride (436 mg, 1.11 mmol, 2.0 eq). The resulting solution was brought to reflux temperature. After 1 h the toluene was evaporated off and the residue was separated by column chromatography on silica gel (eluted with hexane: CH2Cl2: EtOAc; 5:4:1) to afford 280 mg (62%) of **231bb** as a white solid.

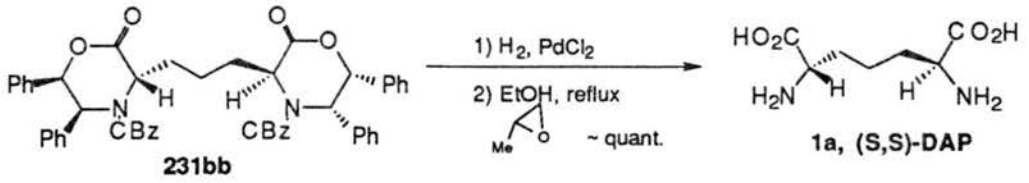
^{1}HNMR(200 MHz, 393k, DMSO-d_6 vs TMS): δ 1.71-1.79 (2H, m), 2.23-2.30 (4H, m), 4.89 (2H, m), 4.92 (2H, s), 4.98 (2H, s), 5.29 (2H, d, J = 2.5 Hz), 6.24 (2H, d, J = 2.5 Hz), 6.57 (2H, s), 6.60 (2H, s), 7.00-7.25 (26 H, m)ppm.

IR (NaCl, film): 1748, 1701cm^{-1};

mp: 270 °C (recryst. EtOAc);

$[a]_D^{25}$ = - 36.8 (c 0.5, CH_2Cl_2).

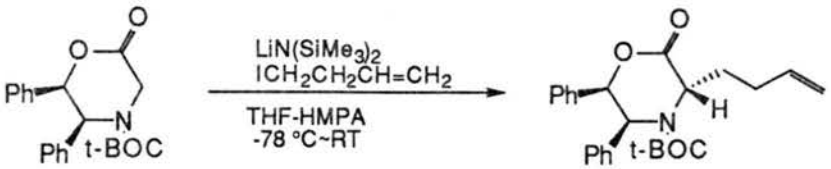
Anal. Calcd. for $C_{51}H_{46}N_2O_8$: C, 75.16; H, 5.69; N, 3.44. Found: C, 75.31; H, 5.88; N, 3.39.



(2S,6S)-2,6-Diaminopimelic acid (1a).

To a solution of **231bb** (100 mg, 0.12 mmol, 1.0 eq) in CH_2Cl_2-EtOH (8 mL, 5:2) was added $PdCl_2$ (60 mg, 0.028 mmol, 3.0 eq). The reaction vessel was charged with hydrogen and the mixture was hydrogenated at 60 psi for 48h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. The crude product was dissolved in dried EtOH (2 mL) and heated to reflux. To this refluxing solution was added excess propylene oxide, and stirring was continued for 30 min at reflux. The white precipitate was filtered to give 19 mg (100%) of product S,S-**DAP 1a** as a white solid.

^{1}HNMR (270 MHz, D_2O): δ 1.40(2H, m), 1.85(4H, m), 3.72(2H, m)ppm.
$[\alpha]_D^{25}$ = + 20.0 (c 0.506, H_2O); $[\alpha]_D^{25}$ = + 44.5 (c 0.95, 1N HCl); lit.$[\alpha]_D^{25}$ = + 45 (c 1, 1N HCl). > 99% ee.



201d **220d**

(3S,5S,6R)-4-(tert-butyloxycarbonyl)-5-6-diphenyl-3-(3'-butenyl)-3-(2,3,5,6-tetrahydro)-4H-1,4-oxazin-2-one 220d.

To a stirred solution of **191d** (7.06g, 20 mmol, 1.0eq) and 4-iodobutene (18.2 g, 100 mmol, 5 eq) in THF (150 mL) and HMPA (15 mL) was added lithium bis(trimethylsilyl)amide (30 mL, 30 mmol, 1.5eq, 1.0M in methylene chloride) dropwise via syringe at -78 °C. After 10 min the dry ice bath was removed. After an additional 1 h, the reaction mixture was poured into ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (eluted with hexane/ethyl acetate; 3:2) to afford 4.75 g (58.4%) of **220d** as a white solid. The antipode was similarly obtained from **5c** in 56% yield.

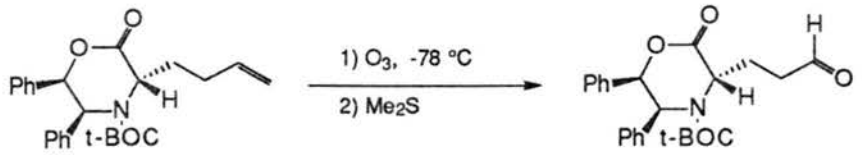
^{1}HNMR (200 MHz, 393k, DMSO-d_6 vs TMS): δ 1.17 (9H, s), 2.11-2.39 (4H, m), 4.80 (1H, t, J = 6.8 Hz), 5.01-5.24 (2H, m), 5.14 (1H, d, J = 2.9 Hz), 5.81-6.00 (1H, m), 6.18 (1H, d, J = 3.2 Hz), 6.54 (1H, d, J = 1.9Hz), 6.58 (1H, d, J = 1.4Hz), 7.00-7.38 (8H, m)ppm;

IR (NaCl, film): 2978, 1747, 1703 cm^{-1};

mp: 158-160 °C (recryst. hexane/ CH_2Cl_2/ EtOAc);

$[\alpha]_D^{25}$ = - 56.7 (c 0.54, CH_2Cl_2);

Anal. Calcd. for $C_{25}H_{29}NO_4$: C, 76.46, H, 6.42, N, 3.09. Found: C, 76.49; H, 6.59; N, 3.09.



220d **221d**

(3S,5S,6R)-4-(tert-butyloxycarbonyl)-5-6-diphenyl-3-(2'-carbonylethyl)-3-(2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one (221d).

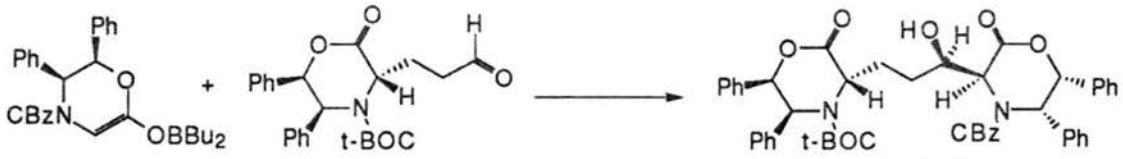
Ozone was bubbled through a solution of **220d** (1.52g, 3.68 mmol, 1.0eq) and $NaHCO_3$ (100mg) in MeOH-CH_2Cl_2 (100mL, 1:1) until the solution turned blue (ca. 15 min) at -78° C. Nitrogen gas was then passed through the reaction mixture to remove excess O_3 until the solution become colorless, and then the solution was allowed to warm up to room temperature. The resulting solution was quenched with excess dimethylsulfide. After 12 h the reaction mixture was concentrated and separated by column chromatography on silica gel (eluted with hexane: CH2Cl2: EtOAc; 5:4:1) to afford 1.32g (88%) of **221d** as a white solid. The anitpode was similarly obtained in 90% yield.

^1H NMR (200 MHz, 393k, DMSO-d_6 vs TMS) δ 1.17 (9H, s), 2.39 (2H, m), 2.69 (2H, m), 4.83 (1H, t, J = 6.5 Hz), 5.14 (1H, d, J = 2.5), 6.20 (1H, d, J = 2.6 Hz), 6.55 (1H, s), 6.59 (1H, s), 7.04-7.24 (8H, m), 9.75 (1H, s)ppm;

IR (NaCl, film): 2720, 1757, 1699 cm^{-1}; mp: 170-4 °C;

$[\alpha]_D^{25}$ = - 45.7 (c 0.83, CH_2Cl_2);

Anal. Calcd. for $C_{25}H_{27}NO_5$: C, 70.39; H, 6.65; N, 3.42. Found: C, 70.38; H, 6.71; N, 3.43.



(3S,3'S,5S,5'S,6R,6'R)-3-[3-[4-[(1,1-dimethylethoxy)carbonyl]-2-oxo-5,6-diphenyl-3-morpholinyl]-1-(hydroxy)propyl]-2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 234db.

To a stirred solution of **191b** (630mg, 1.60 mmol, 1.0 eq) in CH_2Cl_2 (10 mL) was added di-n-butylborontriflate (2.4 mL, 2.40 mmol, 2.0 eq, 1M solution in CH_2Cl_2) followed by the addition of Et_3N (670 uL, 4.8 mmol, 3.0 eq) at -5 °C. After 15 min the reaction mixture was cooled to -78° C and a CH_2Cl_2 (5 mL) solution of aldehyde **220d** (1000 mg, 2.4 mmol, 1.5 eq) was added. After 1h the reaction mixture was quenched with phosphate buffer solution (0.025 M, pH = 7) and poured into water. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, concentrated and separated by column chromatography on silica gel (eluted with hexane: CH_2Cl_2: EtOAc, 5:4:1) to afford 728 mg (61%) of **234db** as a white solid.

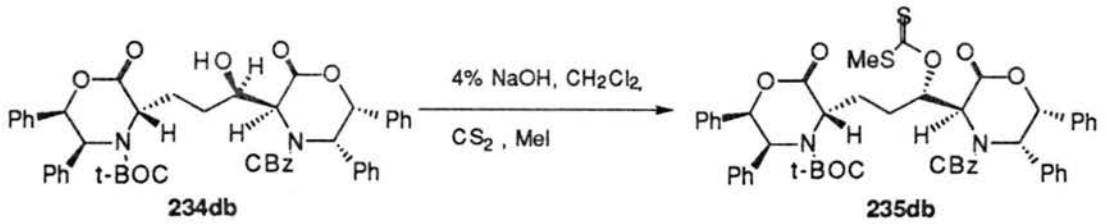
^1H NMR (200 MHz, 393k, DMSO-d_6 vs TMS): δ 1.18 (9H, s), 1.86-1.97 (2H, m), 2.17-1.33 (2H, m), 4.21-4.32 (1H, m), 4.81-5.08 (4H, m), 5.18 (1H, d, J = 2.8 Hz), 5.25 (1H, d, J = 2.9 Hz), 5.75 (1H, br, D_2O exchanged), 6.19 (1H, d, J = 1.3 Hz), 6.52 (1H, d, J = 3.1 Hz), 6.57 (2H, s), 6.61 (2H, s), 6.97-7.34 (21H, m)ppm;

IR (NaCl, film): 3523, 1757, 1701cm^{-1};

mp: 223-5 °C (recryst.hexane/ CH_2Cl_2/ EtOAc);

$[\alpha]_D^{25}$ = - 22.5 (c 0.32, CH_2Cl_2);

Anal. Calcd. for $C_{48}H_{48}N_2O_9$: C, 72.41; H, 6.06; N, 3.51. Found: C, 72.19; H, 6.04; N, 3.47.



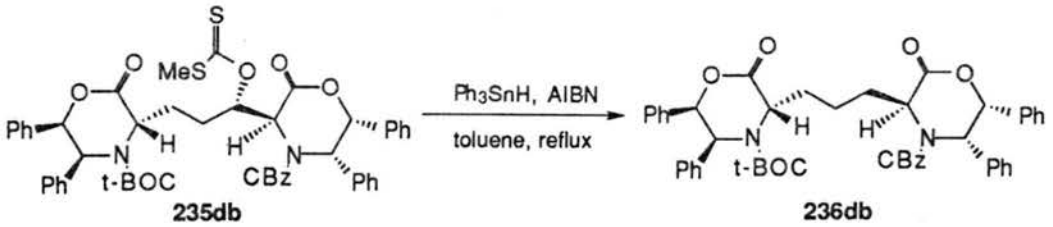
(3S,3'S,5S,5'S,6R,6'R)-3-[3-[4-[(1,1-dimethylethoxy)carbonyl]-2-oxo-5,6-diphenyl-3-morpholinyl]-1-[(methylthio)thioxomethoxy]propyl]-2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 234db.

To a stirred solution of **234db** (728 mg, 0.91 mmol, 1.0 eq) and methyliodide (2mL) in CH_2Cl_2 (100 mL) and CS_2 (15 mL) was added 4% NaOH aq. solution (15 mL) and TBAHS (5 mg). The reaction mixture was stirred for 5 h at 0-15 °C. The organic layer was separated and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic extracts were washed with water; sat. NaCl aq., and then dried over anhydrous Na_2SO_4. The residue was purified by column chromatography on silica gel, eluted with hexane: CH_2Cl_2: EtOAc (5:4:1) to yield 606 mg (74%) of product **235db** as a white solid.

^1H NMR (300 MHz, 393k, DMSO-d_6 vs TMS): δ 1.21 (9H, s), 2.27 (4H, m), 2.65 (3H, s), 4.83-5.02 (5H, m), 5.19 (1H, d, J = 2.8 Hz), 5.22 (1H, d, J = 3.1 Hz), 5.38 (1H, d, J = 2.4 Hz), 6.01 (1H, d, J = 3.1 Hz), 6.61 (2H, s), 6.64 (2H, s), 6.97-7.26 (21H, m)ppm; IR (NaCl, film): 3019, 2976, 1759, 1704, 1229 cm^{-1};

mp: 191-7 °C (recryst.hexane/ CH_2Cl_2/ EtOAc);

$[\alpha]_D^{25}$ =+31.0 (c 0.42, CH_2Cl_2).



(3S,3'S,5S,5'S,6R,6'R)-3-[3-[4-[(1,1-dimethylethoxy)carbonyl]-2-oxo-5,6-diphenyl-3-morpholinyl]-propyl]-2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 236db.

To a solution of **235db** (570 mg, 0.64 mmol, 1.0 eq) in toluene (20 mL) was added AIBN (6 mg, 0.9038 mmol, 0.1 eq) followed by the addition of triphenyltin hydride (740 mg, 1.89 mmol, 2.0 eq). The resulting solution was brought to reflux. After 1h the toluene was evaporated off and the residue was separated by column chromatography on silica gel (eluted with hexane: CH_2Cl_2 : EtOAc, 5:4:1) to afford 410 mg (79%) **236db** as a white solid.

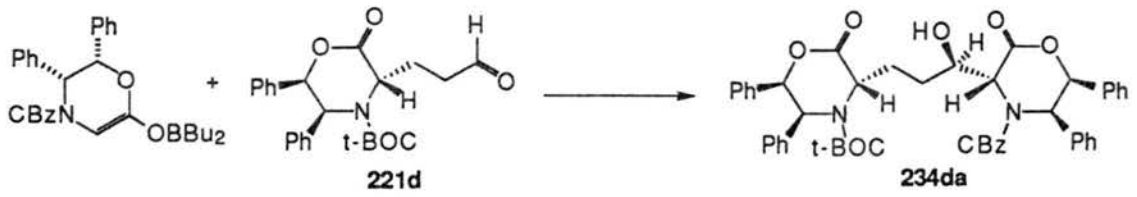
^{1}HNMR (200 MHz, 393k, DMSO-d_6 vs TMS) 1.18 (9H, s), 1.72-1.85 (2H, m), 2.18-2.29 (4H, m), 4.86-5.02 (4H, m), 5.16 (1H, d, J = 2.9 Hz), 5.30 (1H,d J = 2.9 Hz), 6.19 (1H, d, J = 2.7 Hz), 6.26 (1H, d, J = 3.00 Hz), 6.56 (2H, s), 6.60 (2H, s), 7.04-7.25 (21H, m)ppm;

IR (NaCl, film): 1749, 1703cm^{-1};

mp: 214-6 °C (recryst. EtOAc);

$[\alpha]_D^{25}$ = - 43.4 (c 0.5, CH_2Cl_2);

Anal. Calcd. for $C_{48}H_{48}N_2O_8$; C, 73.82; H, 6.20; N, 3.59. Found: C, 73.90; H, 6.14; N, 3.58.



(3S,3'R,5S,5'R,6R,6'S)-3-[3-[4-[(1,1-dimethylethoxy)carbonyl]-2-oxo-5,6-diphenyl-3-morpholinyl]-1-(hydroxy)propyl]-2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 234da.

To a stirred solution of **191a** (949 mg, 2.44 mmol, 1.0 eq) in CH_2Cl_2 (20 mL) was added di-n-butylborontriflate (3.66 mL, 3.66 mmol, 1.5 eq of a 1M solution in CH_2Cl_2) followed by the addition of Et_3N (1026 uL, 7.32 mmol, 3.0 eq) at -5 °C. After 15 min the reaction mixture was cooled to -78° C and a CH_2Cl_2 (10 mL) solution of aldehyde **221d** (1.5 g, 3.67 mmol, 1.5 eq) was added. After 1h the reaction mixture was quenched with phosphate buffer solution (0.025 M, pH = 7) and poured into water. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, concentrated and separated by column chromatography on silica gel (eluted with hexane: CH_2Cl_2: EtOAc; 5:4:1) to afford 1.63g (56%) **234da** as a white solid.

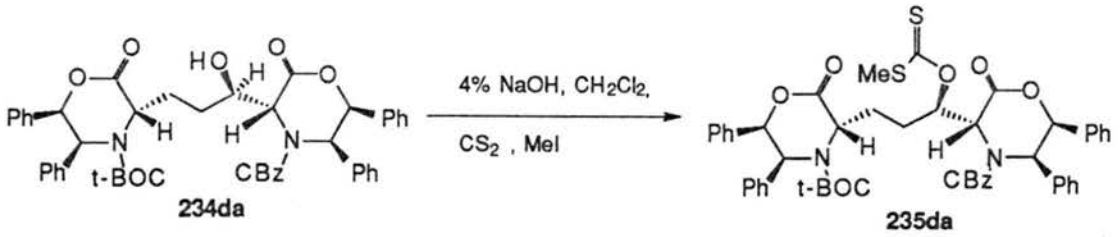
^1H NMR (200 MHz, 393k, DMSO-d_6 vs TMS) δ 1.21 (9H, s), 1.76-1.85 (2H, m), 2.20-2.32 (2H, m), 4.24-4.26 (1H, m), 4.84-5.04 (2H, m), 4.92 (2H, s), 5.19 (1H, d, J = 2.5 Hz), 5.25 (1H, d, J = 2.7 Hz), 5.65 (1H, br, D_2O exchanged), 6.19 (1H, d, J = 2.5 Hz), 6.51 (1H, d, J = 2.9 Hz), 6.58 (2H, s), 6.62(2H, s), 6.97-7.34 (21H, m)ppm;

IR (NaCl, film): 3421, 1752, 1701, 1663 cm^{-1};

mp: 204-211 °C (recryst.hexane / CH_2Cl_2 / EtOAc);

$[\alpha]_D^{25}$ = + 15.2 (c 0.67, CH_2Cl_2);

Anal. Calcd. for $C_{48}H_{48}N_2O_9$: C, 72.41; H, 6.06; N, 3.51. Found: C, 72.21; H, 6.19; N, 3.26.



(3S,3'R,5S,5'R,6R,6'S)-3-[3-[4-[(1,1-dimethylethoxy)carbonyl]-2-oxo-5,6-diphenyl-3-morpholinyl]-1-[(methylthio)thioxomethoxy]propyl]-2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 235da.

To a stirred solution of **234da** (1.50 g, 1.88 mmol, 1.0 eq) in CH_2Cl_2 (100 mL) containing CS_2 (30 mL) was added 4% NaOH aq. solution (30 mL) and TBAHS (20 mg). After stirring for 5h at room temperature, the reaction mixture was cooled to 0° C and excess MeI was added. The reaction mixture was then stirred for 1h at 0° C. The organic layer was separated and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic extracts were washed with water ; sat. NaCl aq., and was dried over anhydrous Na_2SO_4. The residue was purified on column chromatography on silica gel, (eluted with hexane: CH_2Cl_2: EtOAc; 5:4:1) to yield 1.178g (71%) of product **235da** as a white solid.

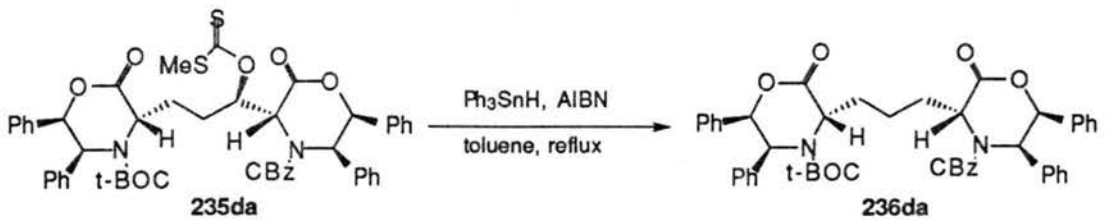
^1H NMR (200 MHz, 393k, DMSO-d_6 vs TMS) δ 1.20 (9H, s), 2.19-2.39 (4H, m), 2.62 (3H, s), 4.85-5.04 (4H, m), 5.17 (1H, t, J = 3.4 Hz), 5.25 (1H, d, J = 2.4 Hz),5.42 (1H, d, J = 4.1 Hz), 6.02 (1H, d, J = 3.1 Hz), 6.17 (1H, d, J = 2.7 Hz), 6.51-6.64 (4H, m), 6.97-7.26 (21H, m)ppm;

IR (NaCl, film): 1748, 1700 cm^{-1};

mp: 219-220 °C (recryst. hexane / CH_2Cl_2 / EtOAc);

$[\alpha]_D^{25}$ = + 32.6 (c 0.50, CH_2Cl_2);

Anal. Calcd. for $C_{50}H_{50}N_2O_9S_2$: C, 67.70; H, 5.63; N, 3.16; S,7.23; Found: C, 67.81; 5.63; 3.15; S, 7.42.



235da 236da

(3S,3'R,5S,5'R,6R,6'S)-3-[3-[4-[(1,1-dimethylethoxy)carbonyl]-2-oxo-5,6-diphenyl-3-morpholinyl]-propyl]-2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 236db.

To a solution of **235da** (1131 mg, 1.29 mmol, 1.0 eq) in toluene (50 mL) was added AIBN (20 mg) followed by the addition of triphenyltin hydride (1000 mg, 2.58 mmol, 2.0 eq). The resulting solution was brought to reflux temperature. After 1h the toluene was evaporated off and the residue was separated by column chromatography on silica gel (eluted with hexane: CH_2Cl_2: EtOAc, 5:4:1) to afford 841mg (83%) **236da** as a white solid.

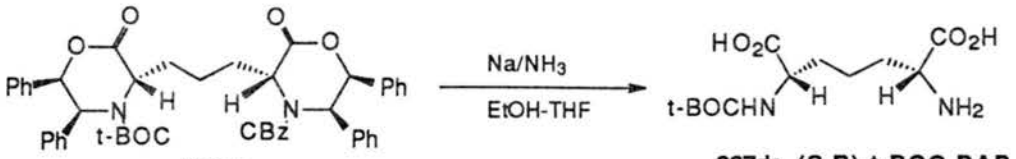
^{1}H NMR (200 MHz, 393k, DMSO-d_6 vs TMS) δ 1.15 (9H, s), 1.75 (2H, m), 2.25 (4H, m), 4.20-4.92 (2H, m), 4.97 (2H, s), 5.16 (1H, d, J =2.5 Hz), 5.29 (1H,d J =2.7 Hz), 6.18 (1H, d, J =2.7 Hz), 6.23 (1H, d, J =2.7 Hz), 6.56 (2H, s), 6. 59 (2H, s), 6.96-7.23 (21H, m)ppm;

IR (NaCl, film): 1750, 1699 cm^{-1};

mp: 225-7 °C (recryst.hexane / CH_2Cl_2 / EtOAc);

$[\alpha]_D^{25}$ = + 1.7(c 0.6, CH_2Cl_2);

Anal. Calcd. for $C_{48}H_{48}N_2O_8$; C, 73.82; H, 6.20; N, 3.59. Found: C, 74.00; H, 6.21; N, 3.42.



236da **237da, (S,R)-t-BOC-DAP**

Mono-N-Boc-DAP 237da.

To a mixture of **236da** (81mg, 0.1mmol, 1.0 eq), abs. EtOH(300uL), and THF (4 mL) in liquid ammonia (30 mL, distilled from Li at -33 °C) was added Li (28 mg, 4 mmol, 40 eq). The resulting blue solution was stirred for 30min. at -33 °C, then was quenched with NH_4Cl. The mixture was allowed to warm. After the NH_3 was evaporated, the residue was diluted with water (2 mL) and was carefully acidified with 1N HCl to pH of 5. The aqueous solution was extracted with EtOAc, and was then concentrated to dryness. The resulting white precipitates was triturated in abs. EtOH (2 mL), filtered out the the precipitates, and the filtrate was concentrated, and recrystallized in THF to yield 20 mg (72%) product **237da** as a white solid.

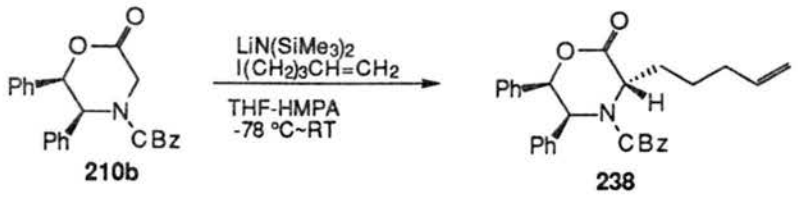
^1H NMR (270MHz, D_2O) δ 1.30 (9H, s), 1.50-1.58 (2H, m), 1.61-1.85 (4H, m), 3.64 (1H, t, J = 6.2 Hz), 3.76 (1H, m)ppm;

IR (NaCl, film): 3422, 1638, 1404 cm^{-1};

mp: 264 °C decomp. (recryst. THF);

$[\alpha]_D^{25}$ = + 2.2 (c 0.45, H_2O);

MS (FAB): Cacld for ($C_{12}H_{22}N_2O_6$ + Na) = 313, Found (M + Na) = 313.



(3S,5S,6R)-4-(Benzyloxycarbonyl)-5,6-diphenyl-3-(4'-pentenyl)-2,3,5,6-tetrahydro-4H-1,4-oxain-2-one 238.

To a stirred solution of **191b** (1.94 g, 5 mmol, 1.0 eq) and 5-iodopentene (4.90 g, 25 mmol, 5.0 eq) in warm THF and HMPA was added lithium bis(trimethylsilyl)amide (5.5 mL, 5.5 eq, 1.0 M in CH_2Cl_2) dropwise via syringe at -78 °C. After 10 min the dry ice bath was removed. After an additional 1 h, the reaction mixture was poured into ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (eluted with hexane: ethyl acetate; 3:2) to afford 2.00g (87.7%) of **238** as a white solid.

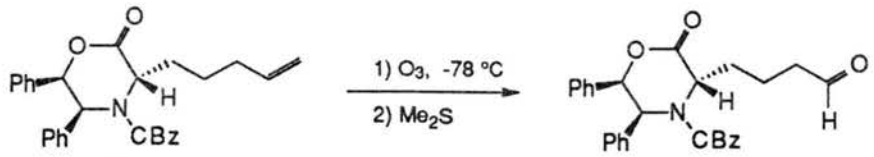
^1H NMR (200 MHz, 393k, DMSO-d_6 vs TMS) δ 1.55-1.63 (2H, m), 2.07-2.19 (4H, m), 4.81 (1H, t, J = 7.2 Hz), 4.97 (2H, s), 4.94-5.06 (2H, m), 5.27 (1H, d, J =2.9 Hz), 5.72-5.93 (1H, m), 6.19 (1H, d, J = 3.0 Hz), 6.55 (1H, s), 6.58 (1H, s), 7.03-7.32 (13H, m)ppm;

IR (NaCl, film): 1750, 1705, 1454, 1401cm^{-1};

mp: 153-4 °C (recryst.hexane / CH_2Cl_2 / EtOAc);

$[\alpha]_D^{25}$ = - 20.0 (c 0.5, CH_2Cl_2);

Anal. Calcd. for $C_{29}H_{29}NO_4$: C, 73.67; H, 7.33; N, 3.44. Found C, 73.81; H, 7.33; N, 3.44.



(3S,5S,6R)-4-(Benzyloxycarbonyl)-5,6-diphenyl-3-(4'-carbonylbutyl)-2,3,5,6-tetrahydro-4H-1,4-oxain-2-one 239.

Ozone was bubbled through a solution of **238** (2.00 g, 4.49 mmol, 1.0 eq) and $NaHCO_3$ (100mg) in MeOH-CH_2Cl_2 (100 mL, 1:1) until the solution turned blue (ca. 15 min) at -78 °C. Nitrogen gas was then passed through the reaction mixture to remove excess O_3 until the solution become colorless, and then the solution was allowed to warm up to room temperature. The resulting solution was quenched with excess dimethylsulfide. After 12 h the reaction mixture was concentrated and separated by column chromatography on silica gel (eluted with hexane: CH_2Cl_2: EtOAc; 5:4:1) to afford 1.71g (85%) of **239** as a white solid.

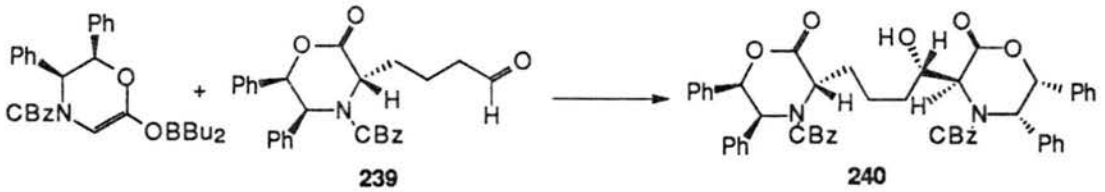
^1H NMR (200 MHZ, 393k, DMSO-d_6 vs TMS) δ 1.72-1.821(2H, m), 2.09-2.21 (2H, m), 2.51-2.62 (2H, m), 4.81 (1H, t, J = 7.3 Hz), 4.98 (2H, s), 5.26 (1H, d, J =3.0 Hz), 6.19 (1H, d, J =3.1 Hz), 6.55 (1H, s), 6.58 (1H, s), 7.02-7.25 (13H, m), 9.68 (1H, s)ppm;

IR (NaCl, film): 2722, 1755, 1703 cm^{-1};

mp: 162-5 °C (recryst.hexane / CH_2Cl_2 / EtOAc);

[a]D25 = - 26.8 (c 0.5, CH_2Cl_2);

Anal. Calcd. for $C_{29}H_{27}NO_5$: C, 73.50; H, 5.95; N, 3.06. Found C, 73.36; H, 6.14; N, 3.06.

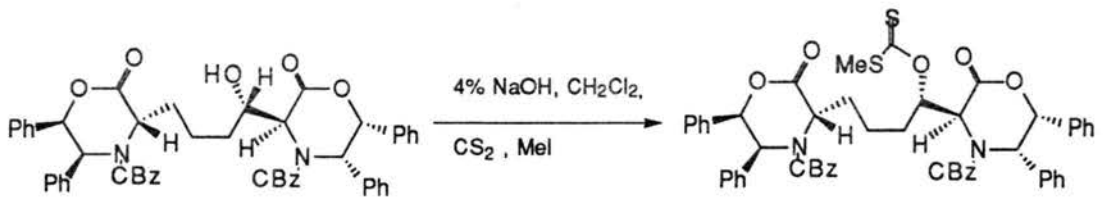


239 240

(3S,3'S,5S,5'S,6R,6'R)-3,3'-(1-hydroxy-1,4-butanediyl)bis[2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 240.

To a stirred solution of **191b** (155 mg, 0.40 mmol, 1.0 eq) in CH_2Cl_2 (4 mL) was added dibutylborontriflate (800 uL, 0.80 mmol, 2.0 eq of a 1M solution in CH_2Cl_2) followed by the addition of Et_3N (168 uL, 1.2 mmol, 3.0 eq.) at -5 °C. After 15 min the reaction mixture was cooled to -78° C and a CH_2Cl_2 (5 mL) solution of aldehyde **239** (260 mg, 0.57 mmol, 1.5 eq) was added. After 1h the reaction mixture was quenched with phosphate buffer solution (0.025 M, pH = 7) and poured into water. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (eluted with hexane : CH_2Cl_2 : EtOAc; 5:4:1) to afford 200 mg (59%) of **240** as a white solid.

^1H NMR (200 MHz, 393k, DMSO-d_6 vs TMS) δ 1.62-1.89 (4H, m), 2.13-2.27 (2H, m), 4.16-4.21(1H, m), 4.88(H, t, J = 8.8 Hz), 4.95-5.10 (3H, m), 5.02 (2H, s), 5.25(H, d, J = 3.0 Hz), 5.28 (H, d, J = 2.9 Hz), 5.57 (H, br, D_2O exchanged), 6.25(1H, d, J = 2.4 Hz), 6.52(1H, d, J = 2.8 Hz), 6.55 (2H, s), 6.58 (2H, s), 6.95-7.26 (26H, m)ppm;
IR (NaCl, film): 3526, 1752, 1734, 1699 cm^{-1};
mp: 186-9 °C (recryst.hexane / CH_2Cl_2 / EtOAc);
$[\alpha]_D^{25}$ = - 20.8 (c 0.26, CH_2Cl_2);
Anal. Calcd. for $C_{52}H_{48}N_2O_9$: C, 73.91; H, 5.73; N, 3.32. Found C, 73.78; H, 5.92; N, 3.26.



(3S,3'S,5S,5'S,6R,6'R)-3,3'-[1-(methylthio)thioxomethoxy-1,4-butanediyl]-bis[2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 241.

To a stirred solution of **240** (98 mg. 0.12 mmol, 1.0 eq) in CH_2Cl_2 (10 mL) and CS_2 (5 mL) was added 4% NaOH aq. solution (2 mL) and TBAHS (1 mg). After stirring for 5h at room temperature, the reaction mixture was cooled to 0 °C and excess MeI was added. The reaction mixture was then stirred for 1h at 0 °C. The organic layer was separated and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic extracts were washed with water; sat. NaCl aq., and dried over Na_2SO_4. The residue was purified by column chromatography on silica gel, (eluted with hexane: CH_2Cl_2: EtOAc; 5:4:1) to yield 62 mg (58.6%) product (**241**) as a white solid.

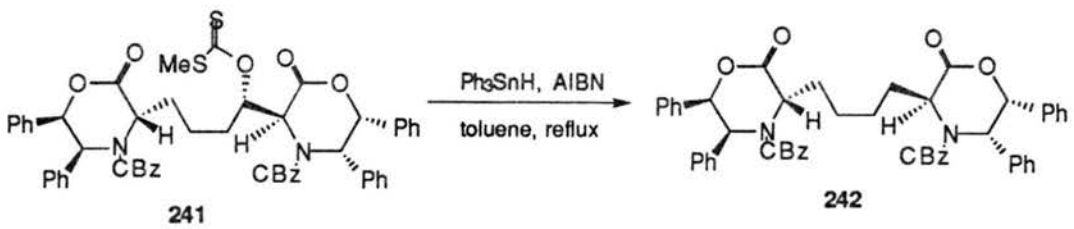
^{1}H NMR (200 MHz, 393K, DMSO-d_6 vs TMS): δ 1.63-1.82 (2H, m), 2.05-2.29 (4H, m), 2.61 (3H, s), 4.84-5.04 (4H, m), 4.88 (H, t), 5.00 (2H, s), 5.21(1H, d, J = 2.8 Hz), 5.29 (1H, d, J = 2.5 Hz), 5.96 (1H, d, J = 3.5 Hz), 6.20 (1H, d, J = 3.0 Hz), 6.56-6.64 (4H, m), 6.94-7.35 (26H, m)ppm;

IR (NaCl, film): 1744, 1703, 1281, 1229, 1195cm^{-1};

mp: 234-6 °C (recryst. hexane:CH_2Cl_2:EtOAc);

$[\alpha]_D^{25}$ = - 5.6 (c 0.43, CH_2Cl_2);

Anal. Calcd. for $C_{54}H_{50}N_2O_9S_2$: C, 69.36; H, 5.39; N, 3.00; S, 6.86. Found C, 69.04; H, 5.44; N, 2.86; S, 6.67.



(3S,3'S,5S,5'S,6R,6'R)-3,3'-(1,4-butanediyl)-bis[2-oxo-5,6-diphenyl-4-morpholinecarboxylic acid], bis(phenylmethyl) ester 242.

To a solution of **241** (96 mg, 0.105 mmol, 1.0 eq) in toluene (20 mL) was added AIBN (2 mg, 0.0 mmol, 0.1 eq) followed by the addition of triphenyltin hydride (412 mg, 1.05 mmol, 10 eq). The resulting solution was brought to reflux temperature. After 1h the toluene was evaporated and the residue was separated by column chromatography on silica gel (eluted with hexane: CH2Cl2: EtOAc; 5:4:1) to afford 50 mg (57%) of **242** as a white solid.

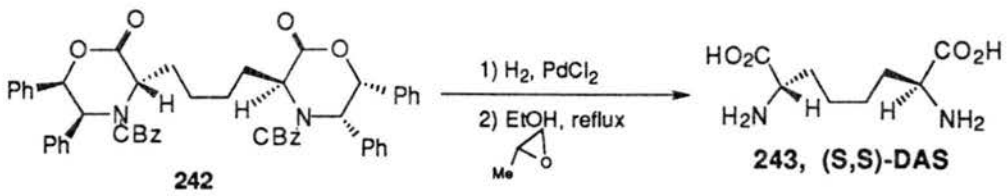
^{1}H NMR (200 MHZ, 393k, DMSO-d_6 vs TMS): δ 1.609 (4H, m), 2.14 (4H, m) 4.80 (2H, t, J = 7.3 Hz), 4.97 (2H, s), 4.99 (2H, s), 5.27 (2H,d, J = 6.3 Hz), 6.22 (2H,d,J = 2.8 Hz), 6.55 (2H, s), 6.59 (2H, s), 6.95-7.78 (26H, m)ppm.

IR (NaCl, film): 1749, 1700 cm^{-1};

mp:. 291-9 °C (recryst. EtOAc);

$[\alpha]_D^{25}$ = -31.9 (c 0.26, $CHCl_3$).

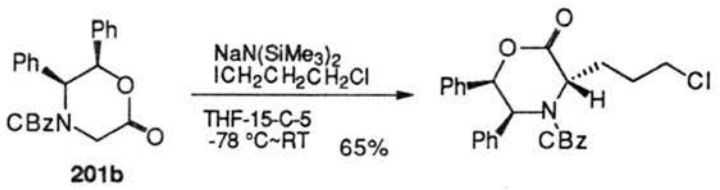
Anal. Calcd. for $C_{52}H_{48}N_2O_8$: C, 75.34; H, 5.84; N, 3.38; Found: C, 75.12; H, 6.00; N, 3.17.



S,S-2,7-Diaminosuberic acid (S,S-243).

To a solution of **242** (23 mg, 0.028 mmol, 1.0 eq) in CH_2Cl_2-95% EtOH (8 mL, 5:2) was added $PdCl_2$ (10 mg, 0.056 mmol, 2.0 eq). The reaction vessel was charged with H_2 and the mixture was hydrogenated at 60 psi for 48h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. The crude product was dissolved in dry EtOH (2 mL) and heated to reflux. To this refluxing solution was added excess propylene oxide, and the mixture was stirred for 30 min at reflux. The white precipitate was filtered to give 6 mg (100%) of product (**243**) as a white solid.

^{1}HNMR (270 MHz, D_2O): δ 1.41 (4H, m), 1.90 (4H, m), 3.95 (2H, m)ppm. $[\alpha]_D^{25}$ = + 24.8 (c 0.25, H_2O).

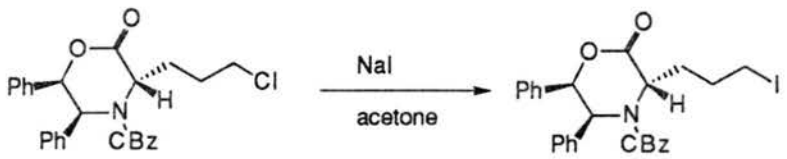


201b

(3S,5S,6R)-4-(Benzyloxycarbonyl)-5,6-diphenyl-3-(3'-chloropropyl)-2,3,4,5-tetrahydro-4H-1,4-oxazin-2-one 244. (Representative Procedure).

To a stirred solution of **191b** (3.88 g, 10 mmol, 1.0 eq) THF (80 mL) and HMPA (40 mL) was added sodium bis(trimethylsilyl)amide (15 mL, 1.5 eq, 1.0M in methylene chloride) dropwise via syringe at -78 °C. After 10 min., 3-chloro-1-iodopropane (6.12 g, 50 mmol, 5.0 eq) was added. After an additional 2 h, the reaction mixture was poured into ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (eluted with hexane:CH_2Cl_2 : EtOAc; 5:4:1) to afford 2.86 g (61%) of the 3'-chloride as a white solid. This material was used directly for the Finkelstein reaction without further purification.

^{1}H NMR (270 MHZ, $CDCl_3$ vs TMS): δ 2.00-2.40 (4H, m), 3.60 (2H, t, J = 6.8 Hz), 4.85-5.30 (3H, m), 5.98 (1H, d, J = 2.8 Hz), 6.55 (1H, d, J = 3.1 Hz), 6.95 (2H, s), 7.00-7.40 (13H, m)ppm.



244b

(3S,5S,6R)-4-(Benzyloxycarbonyl)-5,6-diphenyl-3-(3'-iodopropyl)-2,3,4,5-tetrahydro-4H-1,4-oxazin-2-one 244b.

The 3'-chloride (330 mg, 0.71 mmol, 1.0 eq) and NaI (1.06 g, 7.1 mmol, 10 eq) in acetone (15 mL) were stirred overnight at reflux temperature. After the solvent was evaporated, the resulting residue was dissolved in ethyl acetate (50 mL), washed with brine, and dried over Na_2SO_4. The residue was purified by column chromatography on silica gel, (eluted with hexane: CH_2Cl_2: EtOAc; 5:4:1) to yield 350 mg (89%) of **244b** as a white solid.

^{1}H NMR (200 MHZ, DMSO-d_6 vs TMS): δ 1.90 (2H, m), 2.23 (2H, m), 3.30(2H, t, J = 7.3 Hz), 4.85-5.35 (3H, m), 6.27 (1H, d, J = 3.0 Hz), 6.52 (2H, s), 6.70 (1H, d, J = 3.3 Hz), 7.00-7.45(13H, m) ;

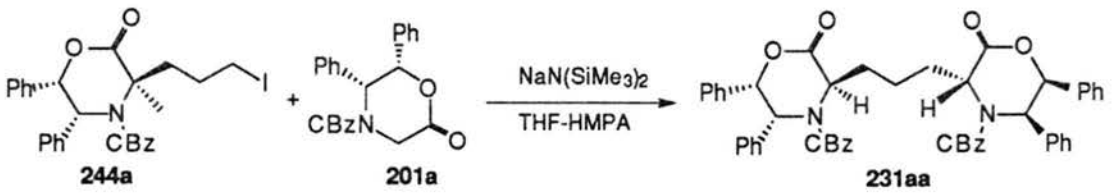
IR (NaCl, film): 1754, 1704, 1245, 1207, 699 cm^{-1};

mp: 167-169 °C (recryst. Hexane/EtOAc, 5:1);

$[\alpha]_D^{25}$ = -26.4 (c 0.5, CH_2Cl_2).

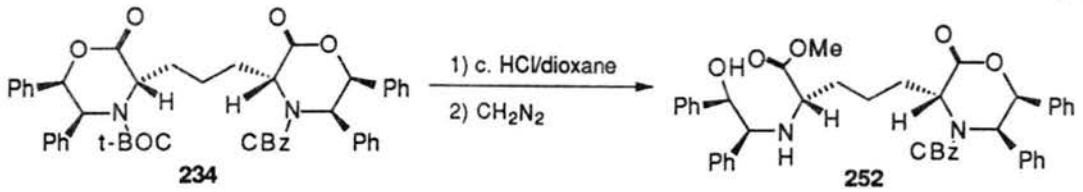
The antipode (**244a**) was similarly obtained from **191a** in 45% yield (two steps).

mp: 168-71 °C (recryst. Hexane/EtOAc, 5:1); $[\alpha]_D^{25}$ = + 25.6 (c 0.5, CH_2Cl_2).



Representative Enolate Alkylation of 191 to Produce 231.

To a stirred solution of **244a** (146 mg, 0.38 mmol, 1.5 eq) in HMPA/THF (7 mL, 1:6) was added sodium bis(trimethylsilyl)amide (375 uL, 1.0 eq, 1.0 M in CH_2Cl_2) dropwise via syringe at -78 °C. After 10 min a solution of **191a** (140 mg, 0.25 mmol, 1.0 eq) was added. After an additional 2 h, the reaction mixture was allowed to warm to room temperature, and was quenched by phosphate buffer solution (pH = 7), extracted with methylene chloride, washed with water and brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and recrystallized in ethyl acetate to afford 62 mg (20%) of **231aa** as a white solid. This material was identical to that obtained above from the reduction of **230aa**.



234 252

[3R,αS(1'S,2'R),5S,6R]-α-[(2'-hydroxy-1',2'-diphenylethyl)amino]-2-oxo-5,6-diphenyl-4-[(phenylmethoxy)carbonyl]-3-Morpholinepentanoic acid, methyl ester, 252.

To a solution of the **234** (1.558 g, 2.0 mmol, 1.0 eq) in dioxane (20 mL) was added c. HCl at 0oC. The resulted mixture was stirred for 3 days at room temperature, and then was concentrated and dried on vacuum overnight to yield white solid residue. The residue was treated with ethyl acetate (200 mL) and phosphor buffer (PH = 7). The organic phase was separated and washed with sat. NH_4Cl aq. solution, dried over anhydrous $MgSO_4$. Then, after filtration, the mixture was concentrated to afford white solid residue. The residue was treated with dry THF (50 mL) and CH_2Cl_2 (20 mL). To this solution was added diazomathane/ether (generated from MNNG), and the solution was stirred overnight at room temperature. The resulting mixture was concentrated to dryness and separated by column chromatography (silica gel, dicholomethane: hexane:ethyl acetate, 5:4:1) to give 926 mg (65%) product **252** as a white solid.

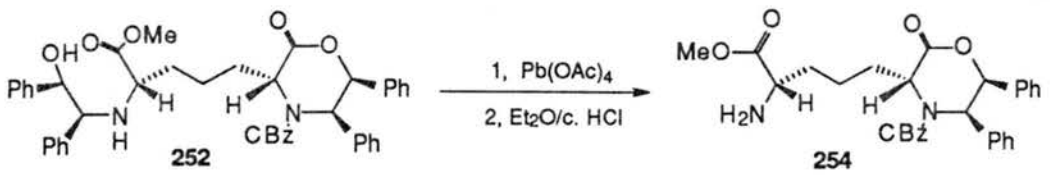
^1H NMR (300 MHz, 393K, DMSO-d_6 vs TMS): 1.43-1.61 (4H, m); 2.05 (2H, m); 3.12 (1H, m); 3.45 (3H, s); 3.81 (1H, d, J = 5.53 Hz); 4.77 (1H, t, J = 7.44 Hz); 4.80 (1H, d, J = 5.52 Hz); 5.00 (2H, s); 5.27 (1H, d, J = 3.16 Hz); 6.15(1H, d, J = 3.06 Hz); 6.59 (2H, d, J = 7.35 Hz); 7.03-7.26 (23H, m)ppm;

IR (NaCl, film): 3498, 3031, 2949, 1755, 1703, 1496, 1453, 1268, 1112, 1060cm-^{1};

$[\alpha]_D^{25}$ = + 8.1 (c = 1.0, CH_2Cl_2).

mp = 188-9 ^oC;

Anal. Calcd for $C_{44}H_{44}N_2O_7$: C, 74.13; H, 6.22; N, 3.93. Found: C, 74.08, H, 6.38; N, 3.83.

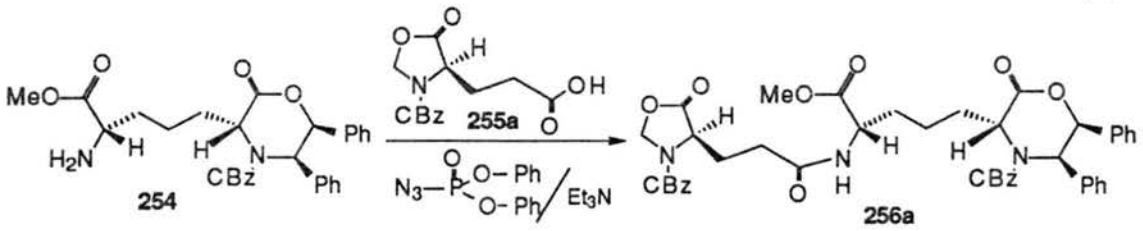


(3R,αS,5S,6R)-α-amino-2-oxo-5,6-diphenyl-4-[(phenylmethoxy)carbonyl]-3-morpholinepentanoic acid, methyl ester, 252.

To a solution of **252** (895 mg, 1.25 mmol, 1.0 eq) in CH_2Cl_2/MeOH was added $Pb(OAc)_4$ (583 mg, 1.32 mmole, 1.05 eq) at 0 °C. The mixture was stirred for 5 min. at 0 °C and was quenched by sodium bicarbonate aq. solution, the yellow precipitate was filtered off, and the residue partitioned between CH_2Cl_2 (3x30 mL), and sat. NaCl solution. The combined organic extracts were dried over anhydrous MgSO4, filtered, concentrated, and dried on vacuum line to give oil residue of Schiff base **253** that was used directly for the next step without further purification.

The crude product obtained from above was treated with conc. HCl/ether (20:30 mL) at 0 °C, and was stirred at room temperature for 1 h. The organic phase was separated, and the aqueous phase was concentrated to dryness at room temperature to give a white solid residue that was treated with phosphate buffer (pH = 7) and extracted with CH_2Cl_2 (2x100 mL). The combined organic layer was washed with aqueous NaCl and dried over anhydrous MgSO4, filtered, concentrated, and separated by column chromatography (silica gel, dicholomethane:hexane:ethyl acetate, 5:4:1) to give 500 mg (77.4%) of **254** as a colorless oil.

^1H NMR (200 MHz, 393K, DMSO-d_6 vs TMS): 1.55 (2H, m); 1.85 (2H, m); 2.15 (2H, m); 3.78 (3H, s); 3.87 (1H, t, J = 6.49 Hz); 4.83 (1H, t, J = 7.16 Hz); 4.99 (2H, d, J = 6.49 Hz); 5.28 (1H, d, J = 2.91 Hz); 6.21 (1H, d, J = 3.99 Hz); 6.58 (2H, d, J = 7.49 Hz); 7.02-7.25 (13H, m)ppm; IR (NaCl, film): 3382, 3063, 2950, 1754, 1704, 1641, 1603, 1498, 1402, 1297, 1269, 1113, 1081, 975, 699 cm^{-1}; $[\alpha]_D^{25}$ = + 4.2 (c = 1.0, CH_2Cl_2); HRMS (FAB): Calcd for ($C_{30}H_{32}N_2O_6$ + H) = 517.2339, Found (M + H) = 517.2364.



[3R,αS(4'S),5S,6R]-2-oxo-α-[[1-oxo-3-[5-oxo-3-[(phenylmethoxy) carbonyl]-4-oxazolidinyl]propyl]amino]-5,6-diphenyl-4-[(phenylmethoxy) carbonyl]-3-Morpholinepentanoic acid, methyl ester 256a,

To a mixture of **254** (190 mg, 0.37 mmol., 1.0 eq) and **255a** (216 mg, 0.74 mmol., 2.0 eq) in DMF (5 mL) was added diphenylphosphoryl azide (203 mg, 0.74 mmol., 2.0 eq) at 0oC. Et_3N (156 uL, 1.11 mmol., 3 eq) was then added to the reaction mixture. The mixture was stirred for 6 h at 0 °C. The resulting mixture was poured into water and extracted with CH_2Cl_2 (3x30 mL). The combined organic layer was washed with sat. NaCl solution, dried over anhydrous MgSO4 , filtered, concentrated, and separated by column chromatography (silica gel, dicholomethane:ethyl acetate:MeOH, 5:1:0.2) to give 170 mg (58%) of **256a** as a colorless oil.

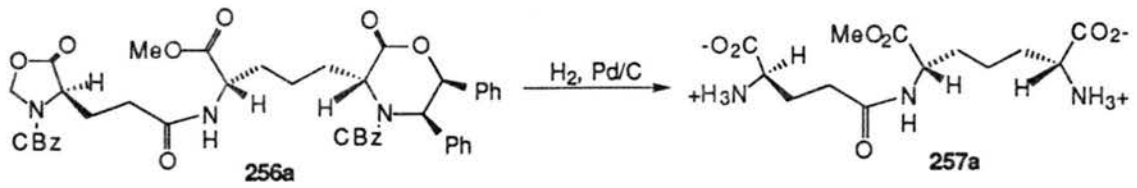
^1H NMR (300 MHz, 393K, DMSO-d_6 vs TMS): 1.57(2H, m); 1.80(2H, m); 2.19(6H, m); 3.66 (3H, s); 4.37(2H, t, J = 6.11 Hz); 4.83(1H, t, J = 7.26 Hz); 5.01(2H, d, J = 4.29 Hz); 5.18(2H, s); 5.27(2H, dd, J = 4.29, 3.12 Hz); 5.47(1H, d, J = 4.17 Hz); 6.19(1H, d, J = 3.07 Hz); 6.60(2H, d, J = 7.23 Hz); 7.05-7.73(18H, m); 7.73(1H, d, J = 7.00 Hz)ppm;

IR (NaCl, film): 3354, 3063, 2952, 1799, 1749, 1705cm-^{1};

$[\alpha]_D^{25}$ = + 70.0° (c = 0.55, CH_2Cl_2);

HRMS (FAB): Calcd for ($C_{44}H_{45}N_3O_{11}$ + H) = 792.3132. Found: (M + H) = 792.3148.

Anal. Calcd for $C_{44}H_{45}N_3O_{11}$: C, 66.74; H, 5.73; N, 5.31. Found: C, 66.60; H, 6.00; N, 5.14.



γ-(D)-Glutamyl-L-*meso*-diaminopimelic acid dipeptide methyl ester 257a:

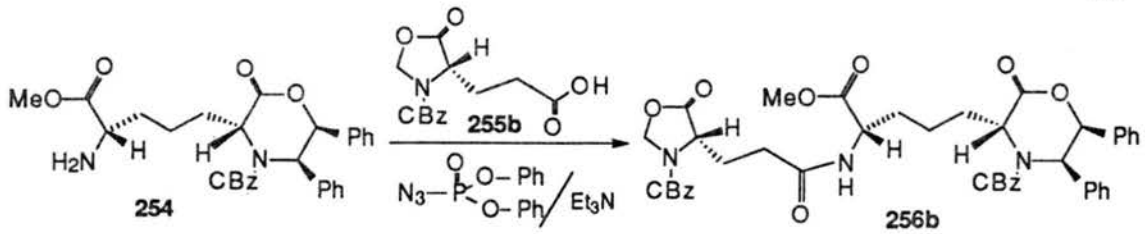
A mixture of substrate **256a** (83 mg, 0.105 mmol) and 86 mg of 5% Pd/C in CH_2Cl_2/MeOH (1:1, 6 mL) was hydrogenated under 60 psi H_2 for 24 h at room temperature. After filtering off the catalyst, the clear solution was concentrated to afford a crude residue. The residue was triturated with hexane to remove the bibenzyl yielding 35 mg (100%) of **257a** as a white amorphous solid.

^{1}H NMR (300 MHz, D_2O vs DOH) : 1.30 (2H, m); 1.71 (4H, m); 1.97 (2H, m); 2.32 (2H, m); 2.40(1H, m); 3.58(1H, m); 3.62 (3H, s); 4.27 (1H, m)ppm; IR (KBr, pellet): 3380, 3246, 2954, 1730, 1638, 1438, 1226 cm-^{1}; $[\alpha]_D^{25}$ = - 13.9 (c = 1.6, MeOH); HRMS (FAB): Calcd for ($C_{13}H_{23}N_3O_7$ + H) = 334.1614. Found (M + H) = 334.1614.

γ-(D)-Glutamyl-L-*meso*-diaminopimelic acid dipeptide 162a.

To the suspension of **257a** (17 mg, 0,051 mmol) in dioxane (3 mL) was added conc. HCl at room temperature. The mixture was stirred for 2 h at 80 °C. The resulting mixture was concentrated in *vecuo* to give the oily residue. The residue was purified on Dowex 50x8-200 (eluted with 1.5% aqueous NH_4OH solution) to yield 15mg (92 %) of **162a** as a semi-solid.

^{1}H NMR (300 MHz, D_2O): δ 1.23(2H, m); 1.49-2.08(6H, m); 2.27(2H, m); 3.37(1H, m); 3.59(1H, m); 3.96(1H, m)ppm; IR (KBr, pellet): 3342 (br), 2941, 1638, 1586,1448, 1408, 1317, 1086, 825 cm-^{1}; $[\alpha]_D^{25}$ = + 8.4 (c = 0.5, MeOH).



[3R,αS(4'R),5S,6R]-2-oxo-α-[[1-oxo-3-[5-oxo-3-[(phenylmethoxy) carbonyl]-4-oxazolidinyl]propyl]amino]-5,6-diphenyl-4-[(phenylmethoxy) carbonyl]-3-Morpholinepentanoic acid, methyl ester 256b.

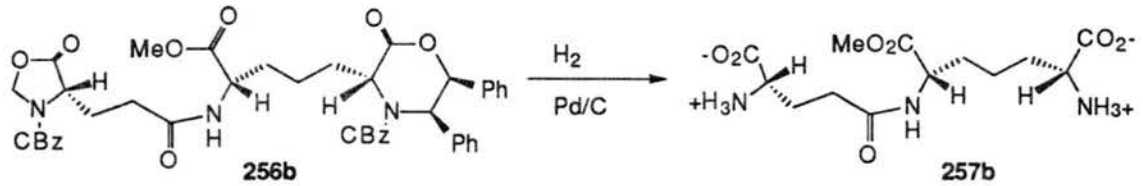
To a mixture of **254** (300 mg, 0.58 mmol., 1.0 eq) and **255b** (341 mg, 1.16 mmol, 2.0 eq) in DMF (5 mL) was added diphenylphosphoryl azide (319 mg, 1.46 mmol, 2.0 eq) at 0oC. Et_3N (220 uL, 1.74 mmol, 3 eq) was then added to the reaction mixture. The mixture was stirred for 6 h at 0 °C. The resulting mixture was poured into water and extracted with CH_2Cl_2 (3x30 mL), washed with sat. NaCl solution, dried over anhydrous MgSO4 , filtered, concentrated, and separated by column chromatography (silica gel, dicholomethane:ethyl acetate:MeOH, 5:1:0.2) to give 256 mg (56%) of **256b** as colorless oil.

1H NMR (300 MHz, 393K, DMSO-d_6 vs TMS): δ 1.57(2H, m); 1.79(2H, m); 2.08-2.47(6H, m); 3.65 (3H, s); 4.37(2H, t, J = 5.92 Hz); 4.83(1H, t, J = 7.21 Hz); 5.00(2H, d, J = 4.27 Hz); 5.26(1H, d, J = 4.18 Hz); 5.28(1H, d, J = 3.07 Hz); 5.46 (1H, d, J = 7.10 Hz); 6.19(1H, d, J = 3.08 Hz); 6.59(2H, d, J = 7.25 Hz); 7.04-7.40(18H, m); 7.80(1H, J = 3.08 Hz)ppm;

IR (NaCl, film): 3355, 3064, 2952, 1799, 1749, 1706, 1674, 1586, 1531, 1296, 1245, 1121, 1058, 1002, 699 cm^{-1};

$[a]_D^{25}$ = + 58.5 (c = 1.6, CH_2Cl_2);

HRMS (FAB): Calcd for ($C_{44}H_{45}N_3O_{11}$ + H) = 792.3132. Found (M + H) = 792.3139.



γ-(L)-Glutamyl-L-*meso*-diaminopimelic acid dipeptide methyl ester 257a.

A mixture of substrate **256b** (73 mg, 0.092 mmol) and 70 mg of 5% Pd/C in CH_2Cl_2/MeOH (1:1, 6 mL) was hydrogenated under 60 psi H_2 for 24 h at room temperature. After filtering off the catalyst, the clear solution was concentrated to afford a crude residue. The residue was triturated with hexane to remove the bibenzyl yielding 24 mg (78%) of **257b** as a white, amorphous solid.

^1H NMR (300 MHz, D_2O): 1.18 (2H, m); 1.38-1.71 (4H, m); 1.90 (2H, m); 3.41 (3H, s); 3.54(1H, m); 3.77(1H, m); 4.07 (1H, m)ppm; IR (KBr, pellet): 3575, 3324, 2918, 1740, 1724, 1703, 1625 cm^{-1}; $[\alpha]_D^{25}$ = - 11.7 (c = 1.2, MeOH);

γ-(L)-Glutamyl-L-*meso*-diaminopimelic acid dipeptide 162b.

To the suspension of **257b** (16 mg, 0,048 mmol.) in dioxane (3 mL) was added conc. HCl at room temperature. The mixture was stirred for 2 h at 80 °C . The resulting mixture was concentrated in *vecuo* to give oily residue. The residue was purified on Dowex 50x8-200 (eluted with 1.5% aqueous NH_4OH solution) to yield 12 mg(80 %) of **162b** as a semi-solid.

^1H NMR (300 MHz, D_2O vs HOD): δ 1.19(2H, m); 1.40-1.72(4H, m); 1.91(2H, m); 2.23(2H, q, J = 7.76 Hz); 3.64(1H, m); 3.73(1H, m); 4.02(1H, m)ppm;

IR (KBr, pellet): 3412, 3252, 3045, 2945, 1723, 1633 cm^{-1};

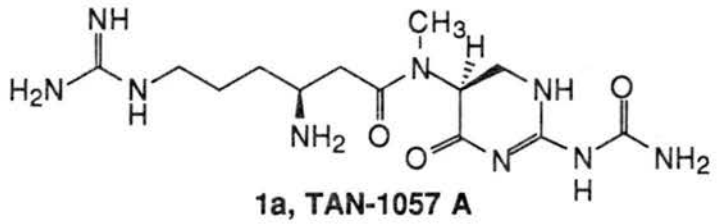
$[\alpha]_D^{25}$ = - 6.4 (c = 0.5, H_2O).

PART II. Total Synthesis of TAN-1057 and Analogues

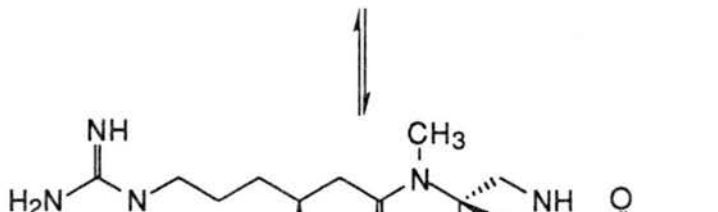
Chapter 1. Introduction

Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have become a very serious clinical problem.[1] MRSA has developed resistance to most β-lactam antibiotics as well as numerous other antibiotics due to the presence of the *mec A* gene.[1] MRSA produces an altered penicillin-binding protein, PBP2a for which most clinically significant ß-lactam antibiotics have low affinity. A desperate search has been initiated very recently to find so-called fourth generation cephalosporins (such as FK-037) that possess affinity for PBP2a.

Recently, four new *anti*-MRSA dipeptides, TAN-1057 A, B, C & D (Figure 1, 2), were isolated from the culture broth of the microorganism identified as *Flexibacter* sp. PK-74 and PK-176 by Takeda Chemical, Japan.[2]

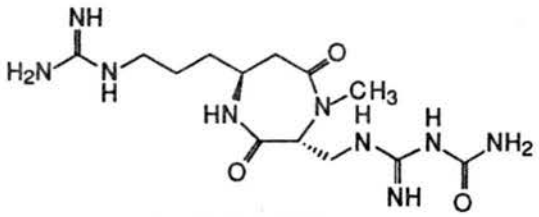


1a, TAN-1057 A

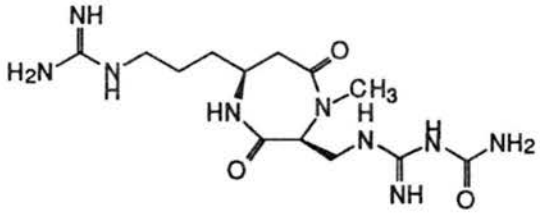


1b, TAN-1057 B

Figure 1 Structures of TAN-1057 A and B



2a, TAN-1057C (5R)



2b, TAN-1057D (5S)

Figure 2. Structure of TAN-1057 C and D

TABLE 1 Antibacterial Activity of TAN-1057A, B, C and D [2]

MIC (ug/mL)

Organism	A	B	C	D
Staphylococcus aureus FDA 209P	6.25	25	50	6.25
S. epidermidis IFO 3762	0.78	3.13	6.25	0.78
Micrococcus luteus IFO 12708	3.13	12.5	12.5	3.13
Bacillus subtilis NIHJ PCI 219	12.5	50	>100	12.5
B. cereus FDA 5	25	100	>100	25
B. megaterium IFO 12108	12.5	50	>100	12.5
Streptococcus faecalis IFO 3989	12.5	50	50	12.5
S. faecium IFO 3181	25	100	>100	25
Escherichia coli NIHJ JC-2	>100	>100	>100	>100
E. coli LD-2	12.5	25	50	12.5
Serratia marcescens IFO 12648	>100	>100	>100	>100
Proteus vulgaris IFO 3988	>100	>100	>100	>100
Pseudomonas aeruginosa IFO 3080	>100	>100	>100	>100
Alcaligenes faecalis IFO 13111	25	100	>100	25
Acinetobacter calcoacetius IFO 13006	50	100	>100	25

The assay medium was DYAB medium (pH 9).

TAN-1057 A, B, C and D were found to be dipeptide antibiotics with potent activity against MRSA. TAN-1057A~D displayed better activity against Gram-positive bacteria than against Gram-negative bacteria (Table 1). TAN-1057 A and D, which have the S-configuration in the heterocyclic portion of the molecule, were more active than TAN-1057 B and C which possess the R-configuration.

There was no cross-resistance between TAN-1057 and methicillin, or erythromycin or gentamycin (Table 2). It is significant to note that TAN-1057A displays potent activity against all the MRSA strains evaluated and is very comparable to vancomycin. The Takeda group concluded that the therapeutic effects of TAN-1057 A, as determined in mice, were superior to vancomycin and imipenem, especially against MRSA (Table 3).

TABLE 2 Susceptibility of *S. aureus* to TAN-1057A [2]

MIC (ug/mL)

Strain	TAN-1057A	Methicillin	Gentamicin	Erythromycin	Vancomycin
FDA 209P	0.39	1.56	<0.20	0.39	1.56
N8	0.78	1.56	<0.20	>100	1.56
N235	1.56	1.56	>100	>100	1.56
C3	0.78	50	100	>100	0.78
N262	0.78	100	50	>100	0.78
N267	<0.20	100	100	>100	1.56
N129	0.39	400	50	0.39	0.78
C10	0.78	800	>100	>100	1.56
N28	0.78	1,600	0.39	>100	1.56
N326	0.78	>1,600	>100	>100	1.56

The assay medium was Mueller-Hinton medium (pH 9).

The antibacterial activity of TAN-1057 is affected by the assay medium. The antibacterial activity of TAN-1057 proved to be about ten times stronger at pH 9 than at pH 7; for TAN-1057 A the antibacterial activity against *S. aureus* in synthetic media was more than ten times stronger than it was in the standard assay media; and the antibacterial activity of TAN-1057 A measured in complete defined synthetic medium (CDSM) was decreased with the addition of peptone, yeast extract or casamino acids.

The preliminary acute toxicity (LD_{50}) data obtained for TAN-1057A was ca. 100 mg/kg upon intraperitoneal injection and 50 mg/kg upon intravenous injection in mice.

TABLE 3 Therapeutic Effect of TAN-1057A in Mice [2]

Organism	Agent	Route	ED_{50} (mg/kg)	MIC(ug/mL)
S. aureus 308A-1	TAN-1057A	sc	**0.027**	12.5
		po	1.2	
	IMP/CS[b]	sc	0.1	0.025
	Vancomycin	sc	**2.2**	0.78
S. aureus N133A[a]	TAN-1057A	sc	**0.026**	6.25
		po	0.56	
	IMP/CS[b]	sc	4.20	>25
	Vancomycin	sc	**2.3**	1.56
S. aureus N295A[a]	TAN-1057A	sc	**0.064**	12.5
		po	1.56	
	IMP/CS[b]	sc	15.1	25
	Vancomycin	sc	**3.36**	0.78
S.pneumoniae type 1	TAN-1057A	sc	>25	6.25
Escherichia coli O-111	TAN-1057A	sc	>12.5	100

a) MRSA.
b) Imipenem/cilastatin.
c) MIC values were determined by a broth dilution method using Mueller-Hinton broth.

So far, the mechanism of action of these substances is not yet clear. The Takeda group found that TAN-1057A did not inhibit the incorporation of tritiated thymidine and uridine into *S. aureus* FDA 209P or *E. coli* LD-2. However, TAN-1057A inhibited the incorporation of leucine into macromolecules in these organisms at concentrations below the MIC. In addition, poly-A and poly-U-directed protein synthesis was inhibited in an *E. coli* cell-free system at 40 mg/mL and 10 mg/mL, respectively. TAN-1057A did not inhibit aminoacyl-tRNA synthetase; thus this drug appears to interfere with protein biosynthesis after the formation of aminoacyl-tRNA. The Takeda group did not mention any data concerning the morphological characteristics of susceptible strains treated with TAN-1057; thus, it is not presently known if TAN-1057 inhibits bacterial cell wall protein biosynthesis or disrupts the assembly of the cell wall.

TAN-1057 A and B are basic, water soluble substances existing in an equilibrium with each other. Analyses of their physicochemical properties and spectral data showed that the molecular formulas of TAN-1057 A and B are $C_{13}H_{25}N_9O_3$. They are dipeptides consisting of L-β-homo-arginine and a unique heterocyclic amidinourea derivative of 2,3-diaminopropionic acid.

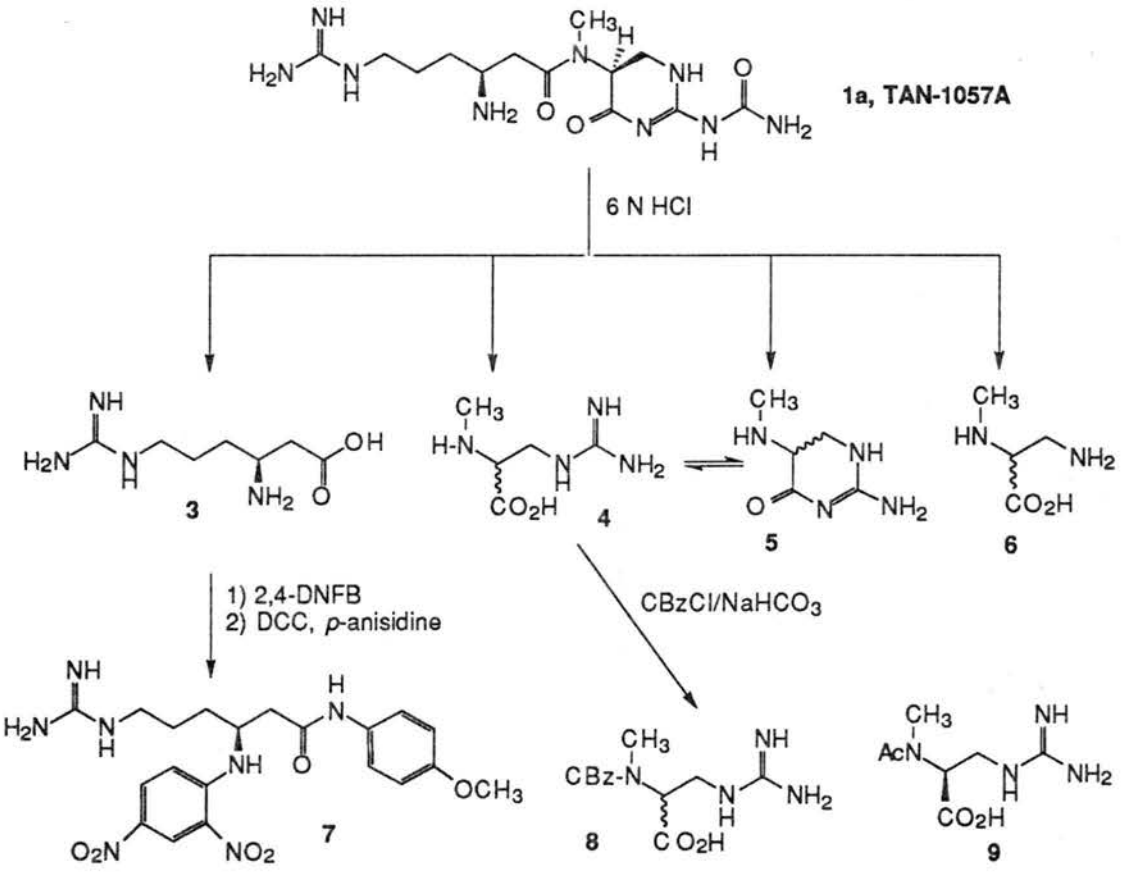
TAN-1057 A and B, as a mixture, was isolated from the broth filtrates through chromatography. The Takeda group has achieved a kinetic separation of TAN-1057 A via preparative HPLC with low recovery.

However, detailed HPLC studies revealed that TAN-1057A and B converted to each other very quickly in an aqueous solution. Especially, when TAN-1057 A or B was treated in either acidic or basic conditions, epimerization was observed and usually a 1:1 mixture of 5S and 5R products was obtained.

Treatment of TAN-1057 A with NaOMe in MeOH gave a 1:1 mixture of TAN-1057 A and B.

After an acidic degradation of TAN-1057 A, as shown in Scheme 1, all three products, **4**, **5** and **6**, containing C-5 chiral center were obtained as racemic mixtures.

TAN-1057 A and B gradually lost their antibacterial activities in basic aqueous solutions due to the hydrolytic opening of the six-membered ring system (Scheme 2). Hydrolysis of TAN-1057 A occurs in both acidic and basic media to afford the acyclic form **10** with attendant racemization of the α-amino acid stereogenic center. However, the acidic hydrolysis product seemed to retain most of the chirality at the C-5 position while the basic hydrolysis caused complete racemization. The Takeda group also reported that the acyclic form of the molecule can be converted back into the cyclic form via the methyl ester intermediate resulting in a diastereomeric mixture (1:1) of TAN-1057 A and B.

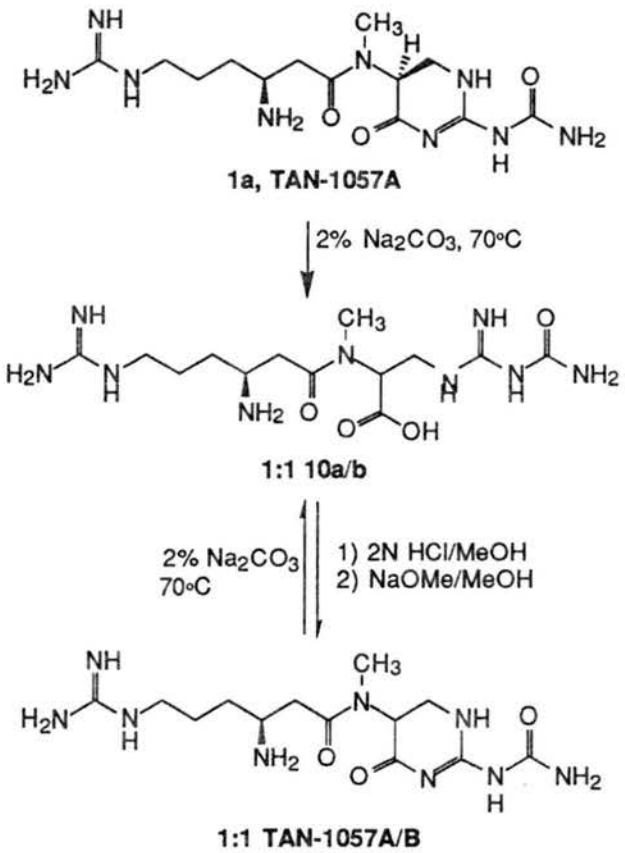


SCHEME 1, Acidic Hydrolysis of TAN-1057 A

The stereochemistry of the 3'-position of the β-homo-arginine moiety was determined by the CD method. Amino acid **3** obtained from the acidic degradation of TAN-1057 A was converted to N-dinitrophenyl-β-homoarginine *p*-methoxyanilide **7**, which

showed a positive Cotton effect at 402 nm. This indicated the absolute configuration of the 3'-position of **3** to be "S".

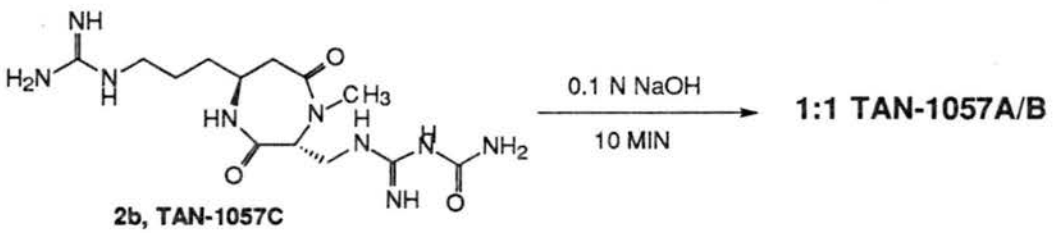
Epimerization during the acidic degradation made it impossible to determine the absolute configuration of the C-5 position from the degradation products. The C-5 chiral center was assigned to be "S" only because of the similarity of the CD spectrum of synthetic S-**9** and one isomer of the hydrolysis product **10a**.



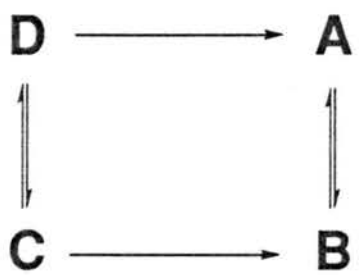
SCHEME 2, Hydrolysis and Cyclization of TAN-1057 A and B

TAN-1057 C and D, as minor components, were found and isolated only from the culture broth of *Flexibacter* sp. PK-176. The isolation and separation procedures were similar to those used in the isolation and separation of TAN-1057 A and B, except that TAN-1057 C and D were not as stable as TAN-1057 A and B in aqueous solution. TAN-1057 C and D have the same molecular formulas and C-C bond sequences as A and B, but,

TAN-1057 C and D have a 7-membered amide ring as shown in Figure 1, instead of the 6-membered ring in A and B. However, the amide carbonyl and the endo-guanidino =NH groups are located at the 1, 6-position, making it easy for annulation to a 6-membered ring in basic solution. Indeed, TAN-1057 C converted into TAN-1057 A and B in basic solution, as shown in Scheme 3, and B was detected as the major product in the early stage of the reaction. Thus, the stereochemistry at the C-5 position of TAN-1057 C was concluded to be "R", and TAN-1057 D has "S" configuration at C-5. They are therefore epimers of each other.



SCHEME 3, The Conversion of TAN-1057 C to B and A



SCHEME 4, The interconversion of TAN-1057

The relationship among TAN-1057 A, B, C and D in aqueous solution is summarized in Scheme 4. A and B exist in an equilibrium with each other and they do not convert to C and D. On the other hand, C and D can convert to a mixture of A and B very rapidly, even through C and D exist in an equilibrium with each other as well.

Chapter 2. Results and Discussion

TAN-1057 has several features that make synthesis of this class of molecules challenging. Foremost among the synthetic challenges is to develop a general approach for synthesis of this new class of peptide antibiotics containing the unique heterocyclic amidinourea derivative of 2,3-diaminopropionic acid which has not been reported before this study.

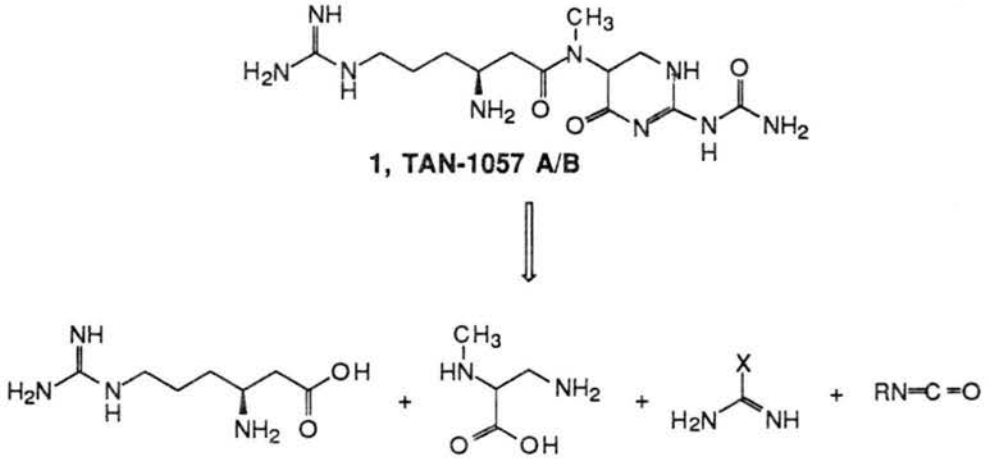
The lability toward racemization of the C-5 chiral center in TAN-1057 made it difficult to obtain these compounds in optically pure form. In spite of this, the Takeda research group claimed that a kinetic separation of A and B from the equilibrium mixture was achieved. However, our attempts to separate A and B through HPLC (conditions similar to Takeda's) did not produce satisfactory results. In this study, it appeared that the corresponding epimer appeared as soon as the "pure" fraction was collected from the HPLC column. Compounds TAN-1057 C and D are highly labile, not only toward racemization, but also toward chemical rearrangement into a mixture of TAN-1057 A and B. Therefore, our efforts in this study were focused on developing a general approach for the synthesis of TAN-1057.

The first part of this chapter describes the preparation of two basic subunits, β-*homo*-Arginine and 2,3-Diaminopropionic Acid. The second part of this chapter is focused on the development of a new and efficient method for the preparation of the amidinoureas. The third part is devoted to the first total synthesis of TAN-1057 and the discovery of new synthetic anti-MRSA analogues of TAN-1057.

2.1. Retro-synthetic Analysis

As shown in Scheme 5, a very simple and straightforward retrosynthetic analysis consisting of dissecting the molecule into (1): the β-homoarginine subunit; (2): the 2,3-diaminopropionic acid portion and (3): the amidinourea homologation and six-membered ring formation was considered.

Thus, peptide coupling, followed by guanidino, ureido homologation and heterocycle formation was expected to give the desired product. Several variants of this approach have been extensively investigated.



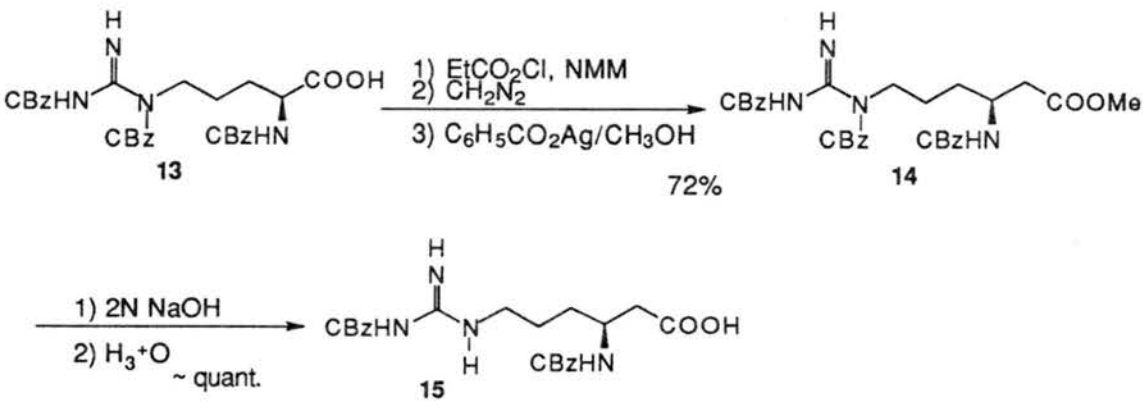
Scheme 5. Retro-synthetic analysis

In planning synthetic routes, the choice of the protecting groups was viewed as being critical to an ultimately successful approach. In addition, the unusual and labile cyclic amidinourea was targeted to be constructed in the late stages of the synthesis. It was deemed crucial to orchestrate the selective assembly of blocking groups so that (1) the guanidino group of the homo-Arginine moiety would be kept inert, (2) the amino group at C-5 could be methylated selectively, (3) the C-1 and C-5 amino groups could be differentially unblocked.

2,2 Synthesis of the β-*homo*-Arginine Subunit:

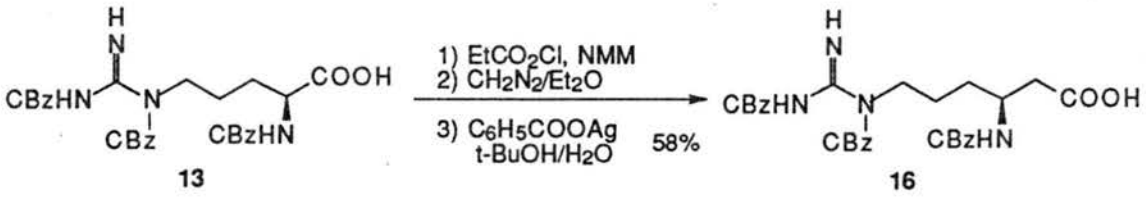
The guanidino group usually needs to be protected to reduce its basicity (pKa = 13.6) and increase solubility in organic solvents. [3] Commercially available L-N^{α},N^{δ},N^{ω}-tri-CBz-arginine **13** was chosen as starting material since it was expected that catalytic hydrogenation would be compatible with the final unmasking of the fully derivatized, protected structure.

Protected L-β-homoarginine subunits were prepared from the corresponding L-arginines through the Arndt-Eister reaction. [4] As shown in Scheme 6, L-N^{α},N^{δ},N^{ω}-tri-CBz-arginine **13** was first converted to the corresponding ethoxycarbonic anhydride in the presence of N-methylmorpholine and ethylchloroformate. The mixed anhydride was immediately allowed to react with diazomethane generated from MNNG to furnish the corresponding diazoketone. Wolff rearrangement in methanol gave N^{α}, N^{δ}, N^{ω}-tri-CBz-β-homoarginine methyl ester **14** as a white solid, which was saponified in 2N LiOH or NaOH to give the carboxylic acid derivative **15**. However, the base labile N^{δ}- CBz group [5] on the guanidine was lost during saponification, and N^{α},N^{ω}-di-CBz-β-homoarginine **15** was thus obtained.



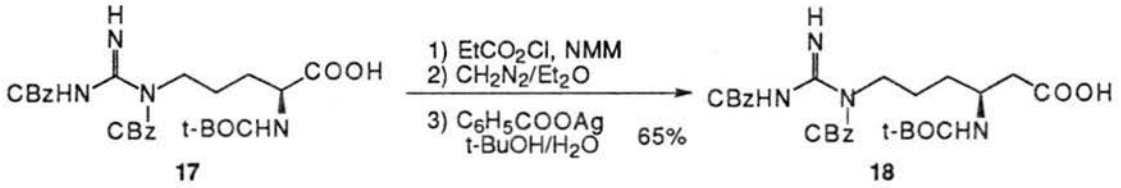
SCHEME 6, Syhthesis of N^{α},N^{ω}-di-CBz-β-homoarginine

The N^{α},N^{ω}-di-Cbz-β-homoargine was used as a subunit in the synthesis of TAN-1057 with some success. However, it was realized later that more sufficient protection was needed to reduce the basicity of the guanidino group further.



SCHEME 7, Synthesis of L-N^{α},N^{δ},N^{ω}-tri-CBz-β-homoarginine

As shown in Scheme 7, a modified Arndt-Eistert synthesis of β-amino acids was developed. When a 1:1 mixture of t-butyl alcohol and water instead of methanol was used as solvent in the Wolff rearrangement step, the free carboxylic acid, *L*-N^{α},N^{δ},N^{ω}-tri-CBz-β-homoarginine **16**, was prepared directly without a saponification step, obviating loss of the N^{δ}-CBz group.

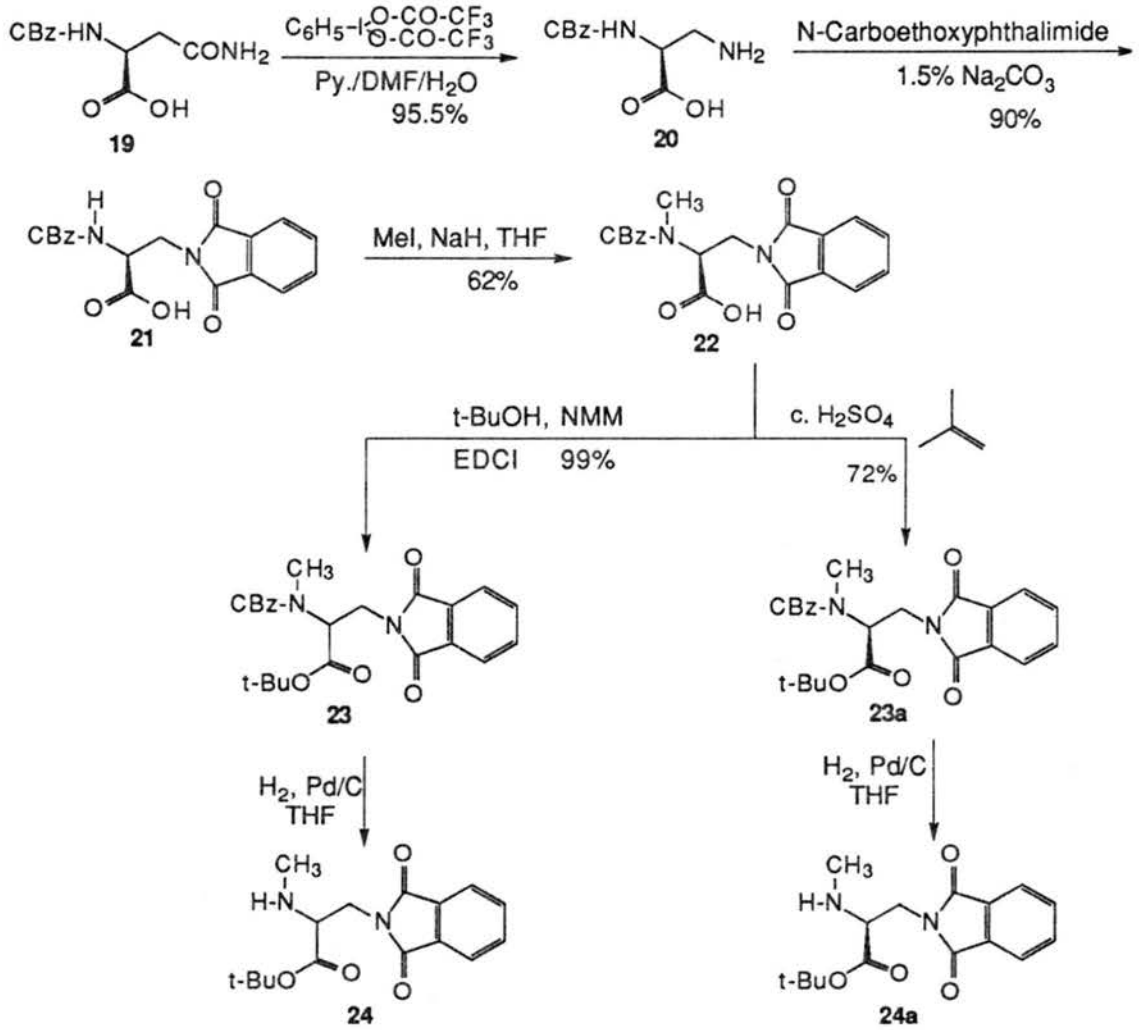


SCHEME 8, Synthesis of *L*-N^{α}-t-BOC- N^{δ}, N^{ω}-di-CBz-β-homoarginine

Similarly, as shown in Scheme 8, L-N^{α}-t-BOC- N^{δ}, N^{ω}-di-CBz-β-homoarginine **18** was prepared from N^{α}-t-BOC- N^{δ}, N^{ω}-di-CBz-arginine **17** in 65% yield. The use of L-N^{α}-t-BOC- N^{δ}, N^{ω}-di-CBz-β-homoarginine **18** in the synthesis of TAN-1057 C/D will be discussed later.

2.3 Synthesis of the 2,3-Diaminopropionic Acid Subunit:

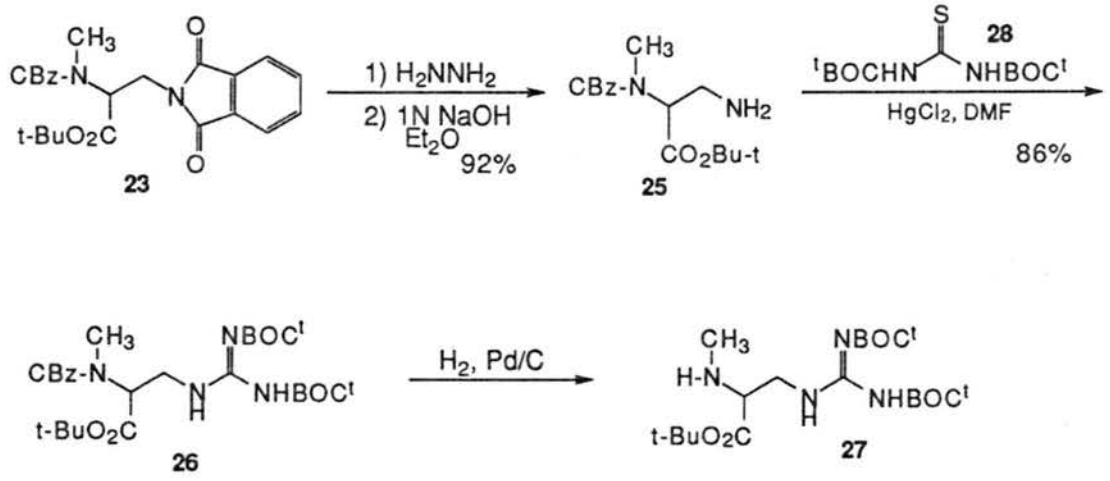
(2S)-N^2-CBz-N^2-methyl-N^3-Pht-2,3-diaminopropionic acid **22** was prepared according the literature procedure [2b] using L-N-CBz-asparagine as starting material (Scheme 9). L-N-CBz-asparagine was converted into L-N^2-CBz-2,3-diaminopropanoic acid through Hofmann rearrangement in high yield. [6] After the 3-amino group in **20** was protected with the phthaloyl group, the 2-amino group of L-N^2-CBz-N^3-pht-2,3-diaminopropanoic acid **21** was methylated with iodomethane and NaH in THF to afford **22** in good yield.



SCHEME 9, Synthesis of the 2,3-Diaminopropionic Acid Subunit

The lability of the α-chiral center of 2,3-diaminopropionic acid derivative was soon encountered. When the acid **22** was treated with EDCI / t-BuOH, [7] the corresponding t-butyl ester **23** was obtained in excellent chemical yield with complete racemization of the α-chiral center. However, optically pure t-butyl (2S)-N^2-CBz-N^2-methyl-N^3-Pht-2,3-diaminopropionate **23a** was provided by using an alternative method employing conc. H_2SO_4 and isobutene [8]. Reductive removal of the N-CBz groups of **23** and **23a** provided the amine components **24** and **24a** separately. Unfortunately, the attempts to purify optically pure amine **24a** via silica gel column chromatography caused racemization. Thus, purification of crude **24a** through chromatography should be avoided.

The optical purity of **24** and **24a** was determined by the Mosher amide method. Thus, the crude samples of **24** and **24a** obtained from hydrogenation were treated with (+)-MTPA-Cl separately to provide corresponding Mosher amides. Comparison of the ^{1}HNMR spectrum of the Mosher amides indicated that **24** was a 1:1 mixture of two enantiomers, and the crude amine **24a** was one pure isomer.



SCHEME 10

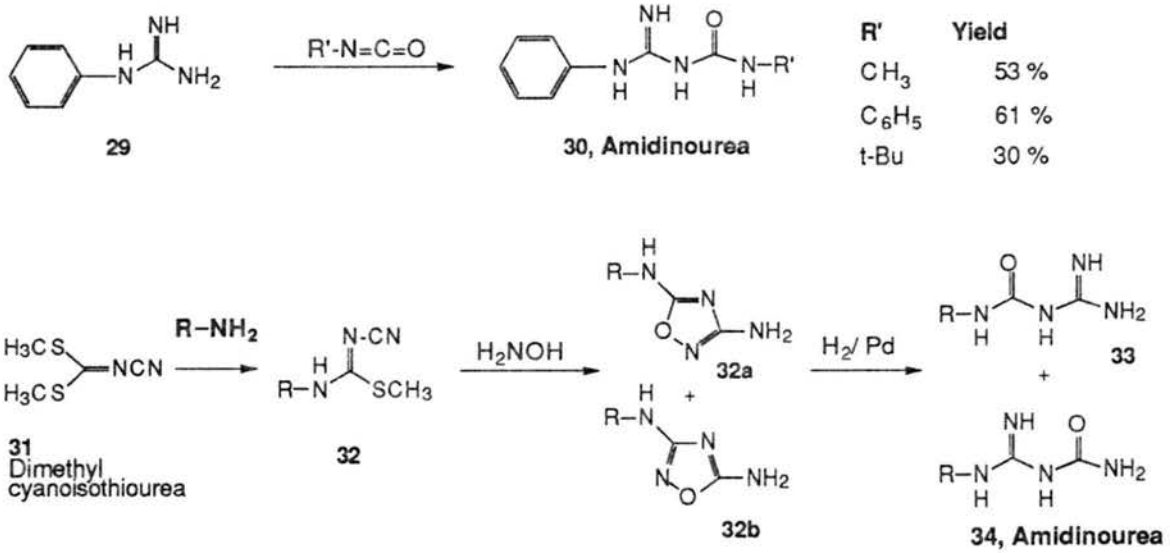
The conversion of the phthaloyl amino group into the bis-t-BOC guanidino group was then accomplished. As shown in Scheme 10, the phthaloyl group was cleaved by hydrazine accompanied by a basic workup to afford free amine **25**. The 3-amino group

was guanidinylated by N,N'-di(t-BOC)thiourea [9] to give **26** in excellent yield. Reductive removal of the N-CBz group provided the amine components **27**.

2.4 An Efficient Method for the Preparation of Amidinoureas

The success of developing a total synthesis of TAN-1057 relies heavily on a mild and efficient preparation of the amidinourea moiety.

Amidinoureas have been known for their pharmaceutical uses. [10] However, they are rarely found in natural products, especially in peptides. Most amidinoureas prepared before this study had relatively simple structures, such as aryl amidinoureas **30** and alkyl amidinoureas. Their preparation methods included the reaction of guanidines with isocyanates, [11] hydrogenation of 5-amino-3-amino-1,2,4-oxadiazoles (Scheme 11) [12] and the hydrolysis of cyanoguanidines under strongly acidic conditions. [13]

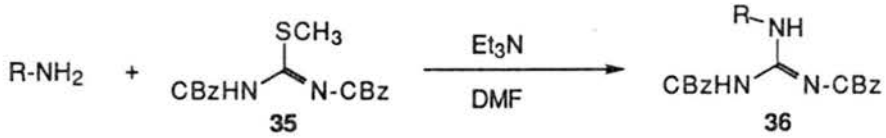


SCHEME 11, The Preparation of Amidinoureas

After extensive exploration of existing methods in the synthesis of the recently discovered anti-MRSA peptide antibiotics TAN-1057, it was determined that these methods were inefficient to incorporate the amidinourea group into the molecule (see section **2.5**).

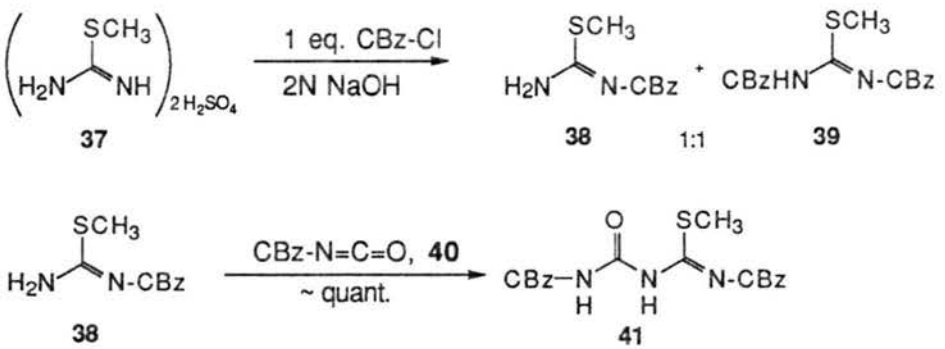
The efficient synthesis of N, N'-bis(t-BOC or CBz)- protected guanidines **36** via reaction of diacyl-S-methylisothioureas **35** [14] with amines in weakly basic media (Scheme 12) prompted us to explore the preparation of aminidoureas from acylureido-S-

methylisothioureas. It was reasoned that the selection of a suitable acyl protecting group, such as CBz or t-BOC, would allow the direct preparation of amidinoureas from amines that would be compatible with peptide synthesis strategies.



SCHEME 12.

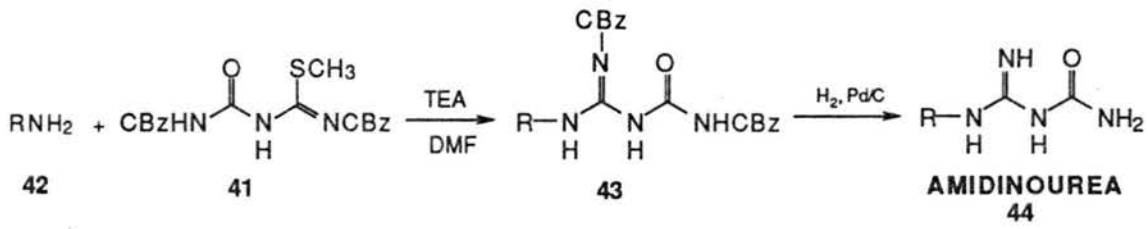
As shown in Scheme 13, N-(benzyloxycarbonyl)ureido-N'-benzyloxycarbonyl-S-methylisothiourea **41** was prepared from mono-benzyloxycarbonyl-S-methylisothiourea **38** and benzyloxycarbonylisocyanate **40** [15] (THF, 100%). Mono-benzyloxycarbonyl-S-methylisothiourea **38** was readily obtained by the slow addition of one equivalent of a solution of benzylchloroformate in CH_2Cl_2 to a cold mixture of S-methylisothiourea semisulfate in CH_2Cl_2/ 2N NaOH [14b]. The bis-acylated product, N,N'-bis(benzyloxycarbonyl)-S-methylisothiourea **39** was also produced, but can be easily separated from **38** by silica gel column chromatography.



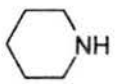
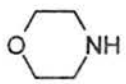
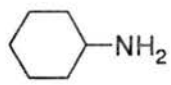
SCHEME 13

When N-(benzyloxycarbonyl)ureido-N'-benzyloxycarbonyl-S-methylisothiourea **41** was condensed with amines **42a-d** (Scheme 14) in the presence of triethylamine in DMF at room temperature, the N^G, N^U-bis(benzyloxycarbonyl)-amidinoureas **43a-d** were produced in good yield. Removal of the N-CBz groups were achieved by

hydrogenolysis (H_2 / 20% $Pd(OH)_2$ on carbon) to provide the amidinoureas **44a-d** in excellent yields.[16]

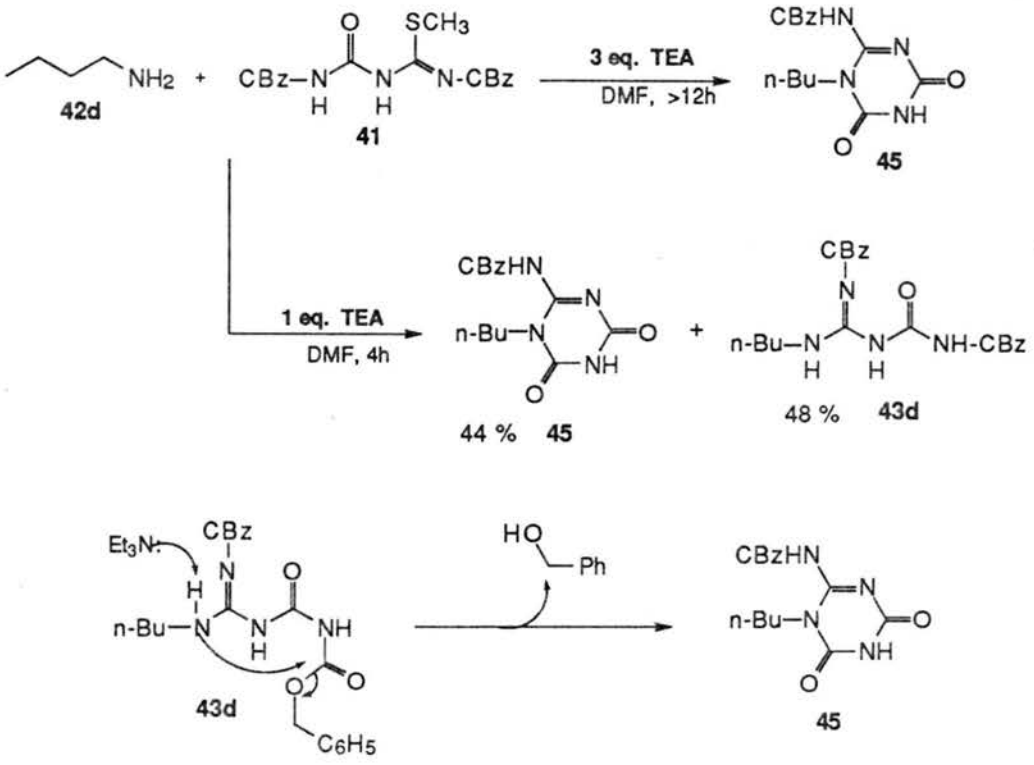


42 41 43 AMIDINOUREA 44

Entry	Amines, 42	Yield of 43	Yield of AMIDINOUREA, 44
a		81 %	96 %
b		75 %	97 %
c		100 %	93 %
d	n-BuNH$_2$	48%	99%

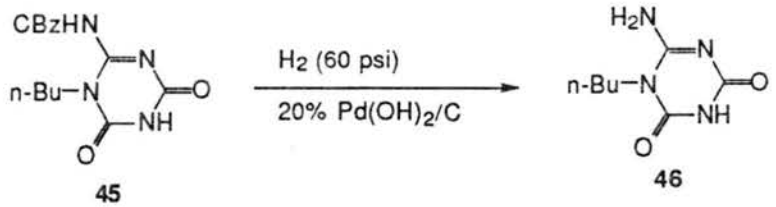
SCHEME 14

An interesting observation was made in the case of n-butylamine **42d**. Under the same reaction conditions as those employed for the other substrates, this substrate produced about a 1:1 ratio of the expected product **43d** and a cyclic by-product, triazine **45** (Scheme 15). If the reaction was allowed to proceed longer (12 hours instead of 4 hours), trazine **45** was the only product isolated. It is believed that the initially formed **43d** cyclizes to triazine in the presence of excess triethylamine to form the more stable 1,3,5-triazine-2,4-dione. The formation of the triazine by-product is, of course, precluded from the secondary amines (entry **a** and **b**), and the more sterically hindered cyclohexylamine (entry **c**) does not readily cyclize to this system.



SCHEME 15

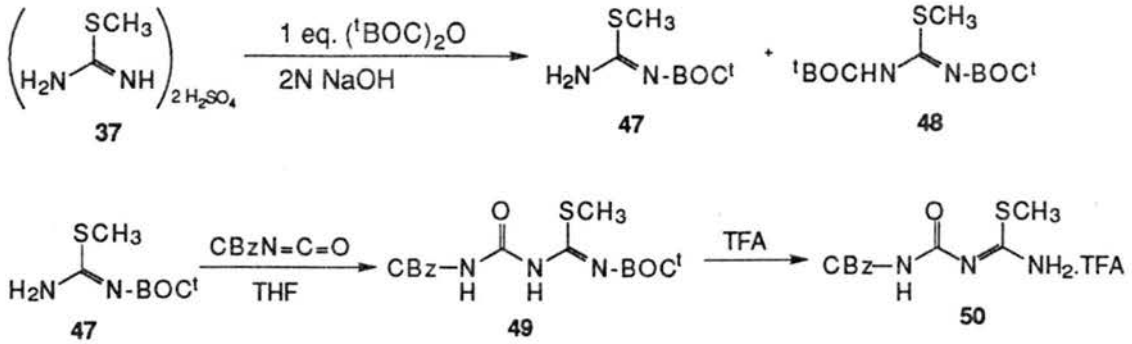
The N-CBz group of the protected triazine **45** can be simply removed by catalytic hydrogenation to give 2-amino-1-n-butyl-4,6-dioxo-tetrahydro-*s*-triazine **46** (Scheme 16).



SCHEME 16

As an alternative of **41**, N-(benzyloxycarbonyl)ureido-N'-butyloxycarbonyl-S-methylisothiourea **49** was prepared as shown in Scheme 17.

Mono-butyloxycarbonyl-S-methylisothiourea **47** was also readily obtained by the slow addition of one equivalent of a solution of $(t\text{-BOC})_2O$ in CH_2Cl_2 to a cold mixture of S-methylisothiourea semisulfate **37** in CH_2Cl_2/ 2N NaOH.[14b] The bis-acylated product, N,N'-bis(t-BOC)-S-methylisothiourea **48** was also produced, but can be easily separated from **47** by silica gel column chromatography.

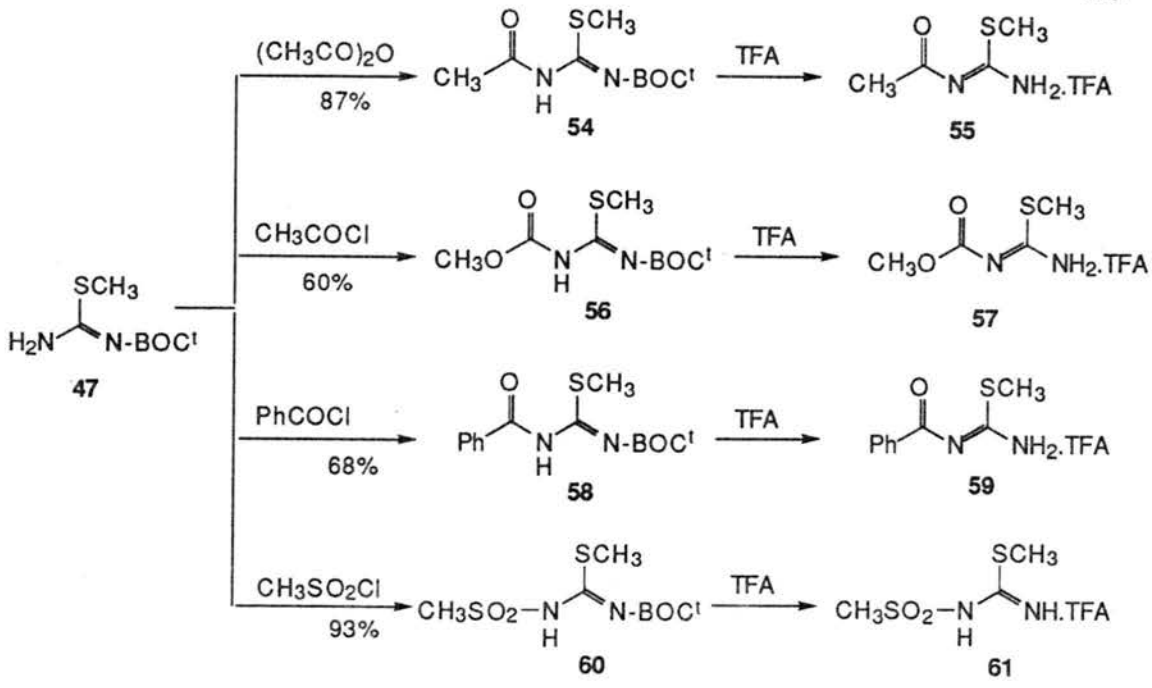


SCHEME 17

Treatment of mono-butoxycarbonyl-S-methylisothiourea **47** with benzyloxy -carbonylisocyanate **40** provided N-(benzyloxycarbonyl)ureido-N'-butyloxycarbonyl-S-methyl -isothiourea **49** in excellent yield. Using this reagent for preparation of amidinoureas will be discussed in the section on the total synthesis of TAN-1057 C and D.

When N-(benzyloxycarbonyl)ureido-N'-butyloxycarbonyl-S-methyl -isothiourea **49** was treated with TFA, the t-BOC group was selectively removed to afford N-(benzyloxycarbonyl)ureido-S-methylisothiourea **50**. The application of this compound in the synthesis of TAN-1057 will be discussed later.

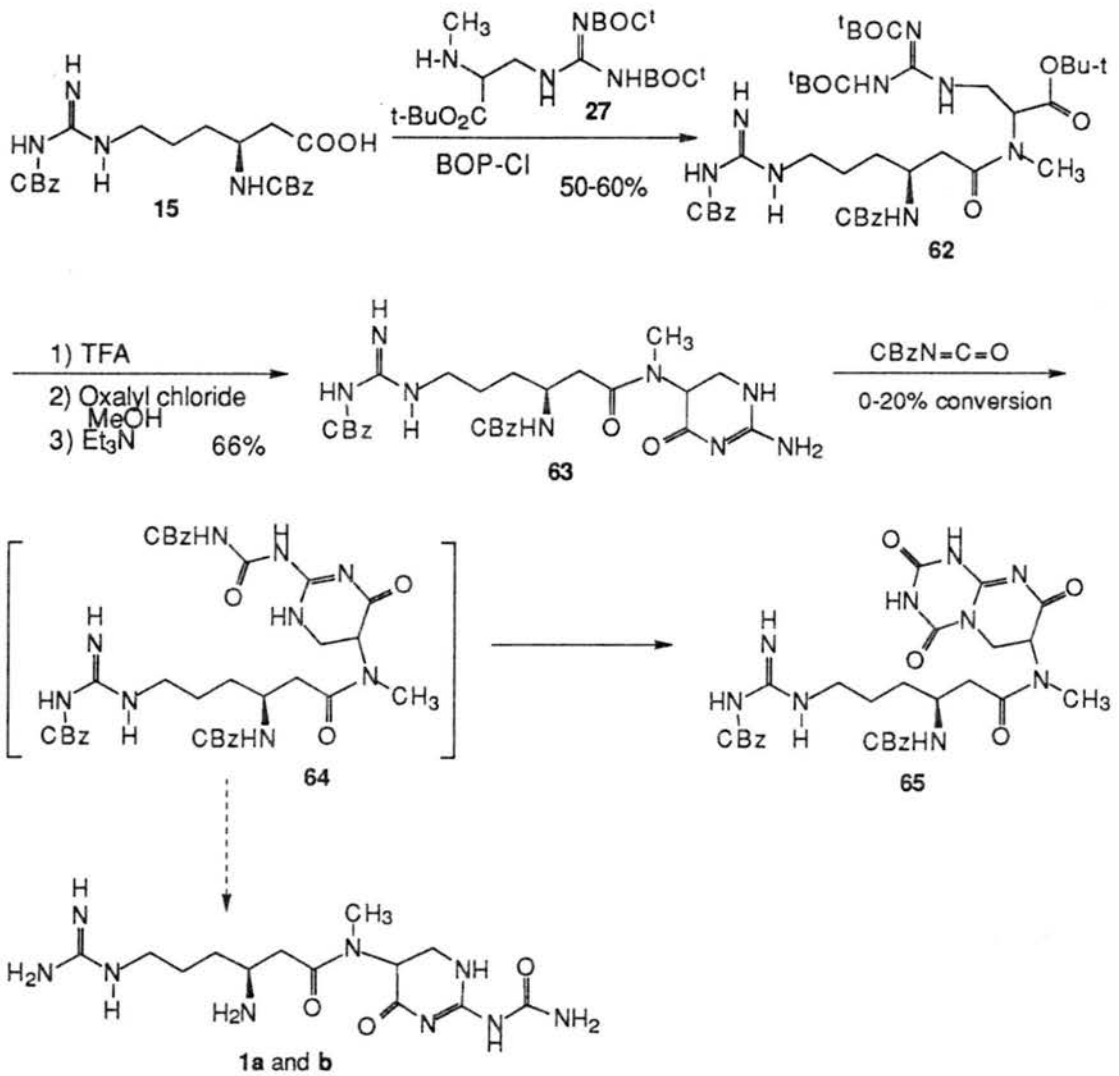
Using a similar approach, a few analogues of **50** were prepared as shown in Scheme 18. These analogues were expected to provide us with access to the preparation of the analogues of TAN-1057.


SCH3
H2N
N-BOCt
47
(CH3CO)2O
87%
CH3
O
SCH3
N
H
N-BOCt
54
TFA
CH3
NH2.TFA
55
CH3COCl
60%
CH3O
56
57
PhCOCl
68%
Ph
58
59
CH3SO2Cl
93%
CH3SO2–N
60
NH.TFA
61

SCHEME 18

2.5 The Initial Approaches For Total Synthesis of TAN-1057 A/B

Our first approach for the total synthesis of TAN-1057A/B is shown in Scheme 19. In this approach, the preparation of amidinourea moiety of TAN-1057 relied on the reaction of guanidine with isocyanates. [11]



SCHEME 19

The peptide coupling between L-N$^{\alpha}$,N$^{\omega}$-di-CBz-β-homoarginine **15** and N-methyl amine **27** was achieved in moderate yield with BOP-Cl as coupling reagent. [18] Other coupling reagents, such as DCC, EDCI and TBTU, proved inefficient. The two t-BOC

groups and the t-butyl ester of the peptide **61** were cleaved efficiently by treatement with TFA at room temperature. After conversion of the free carboxy to methyl ester, the six-membered ring was formed when treated with excess triethylamine. However, the purification of cyclized product **62** was problematic. This is primarily due to the low solubility of **62** in organic solvents. A partially purified sample was obtained after being triturated in THF and CH_2Cl_2 respectively.

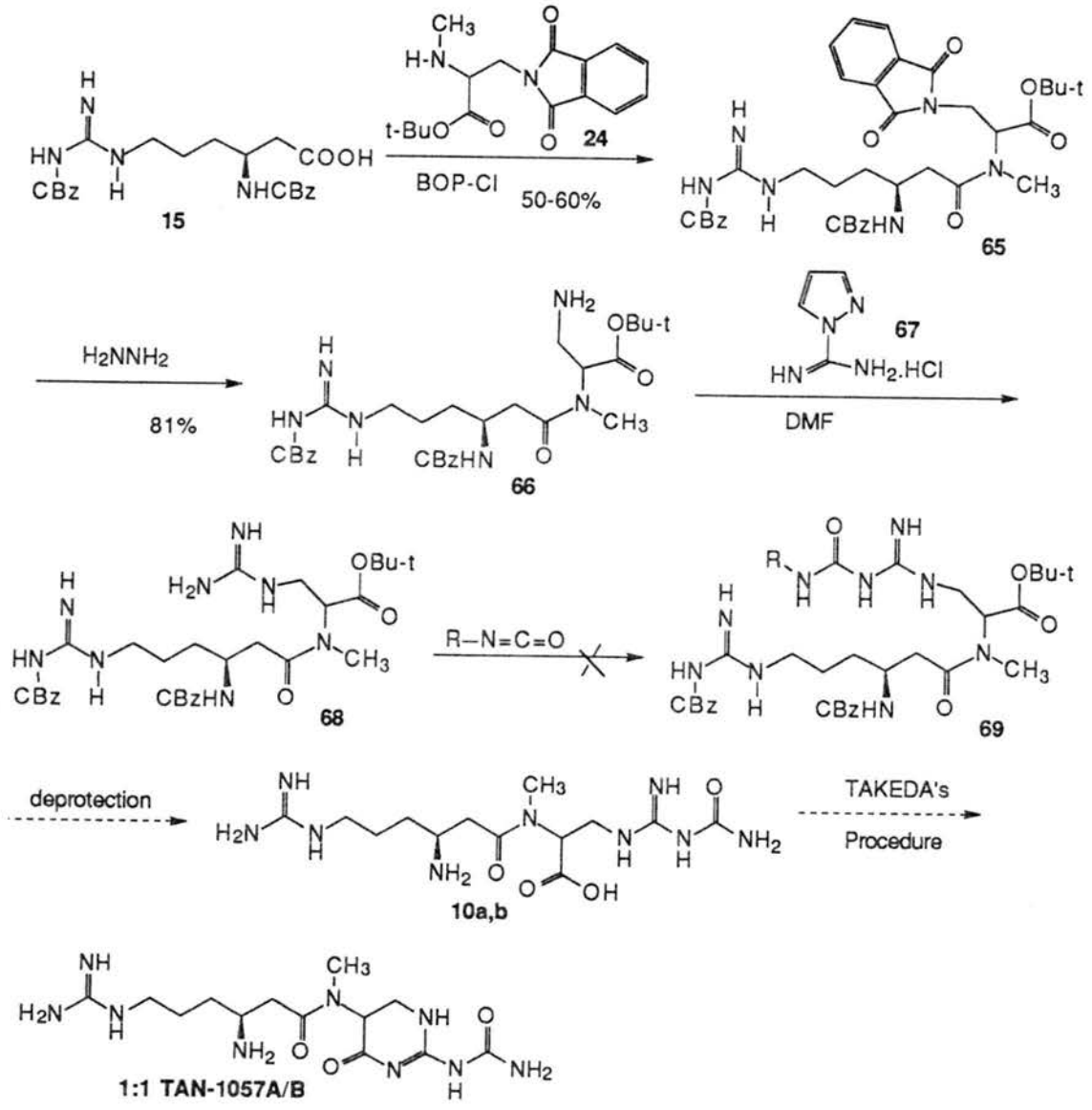
Treatment of **62** with benzyloxycarbonylisocyanate **40** did not provide desired results. This reaction proceeded with low conversion in both THF and DMF, and a complex mixture was produced. All attempts to isolate the desired product **63** from this crude mixture were unsuccessful, although the mass spectrum indicated its existence. After careful examination, it was clear that the desired product **63** was unstable with a tendency to form a bicyclic byproduct **64**. Treatment of **62** with chlorosulfonylisocyanate did not give expected results either.

It was believed that the low reactivity of **62** toward isocyanates is primarily due to the weak nucleophilicity of the acetoguanidine in compound **62**. The ureado homologation of simple guanidines was believed to be possible. Therefore, a second approach for the synthesis of TAN-1057, as shown in Scheme 20, was explored.

The peptide coupling between L-N^{α},N^{ω}-di-CBz-β-homoarginine **15** and t-butyl N^2-methyl-N^3-Pht-2,3-diaminopropionate **24** was achieved in moderate yield with BOP-Cl as coupling reagent. The phthaloyl group was cleaved by treating the peptide **65** with excess hydrazine accompanied by a basic workup to afford free amine **66** in good yield. However, amine **66** decomposed into multiple spots (detected by TLC) upon standing at room temperature for a few hours. Therefore, it should be used for the next step of the reaction as soon as it is purified from a column chromatography. Treatment of amine **66** with 1H-pyrazole-1-carboxaminedine hydrochloride **67** [17] in DMF provided guanidine **68** in good yield.

Unfortunately, treatment of guanidine **68** with isocyanates, such as benzyloxycarbonyl isocyanate and chlorosulfonylisocyanate, did not provide expected amidinourea products **69**.

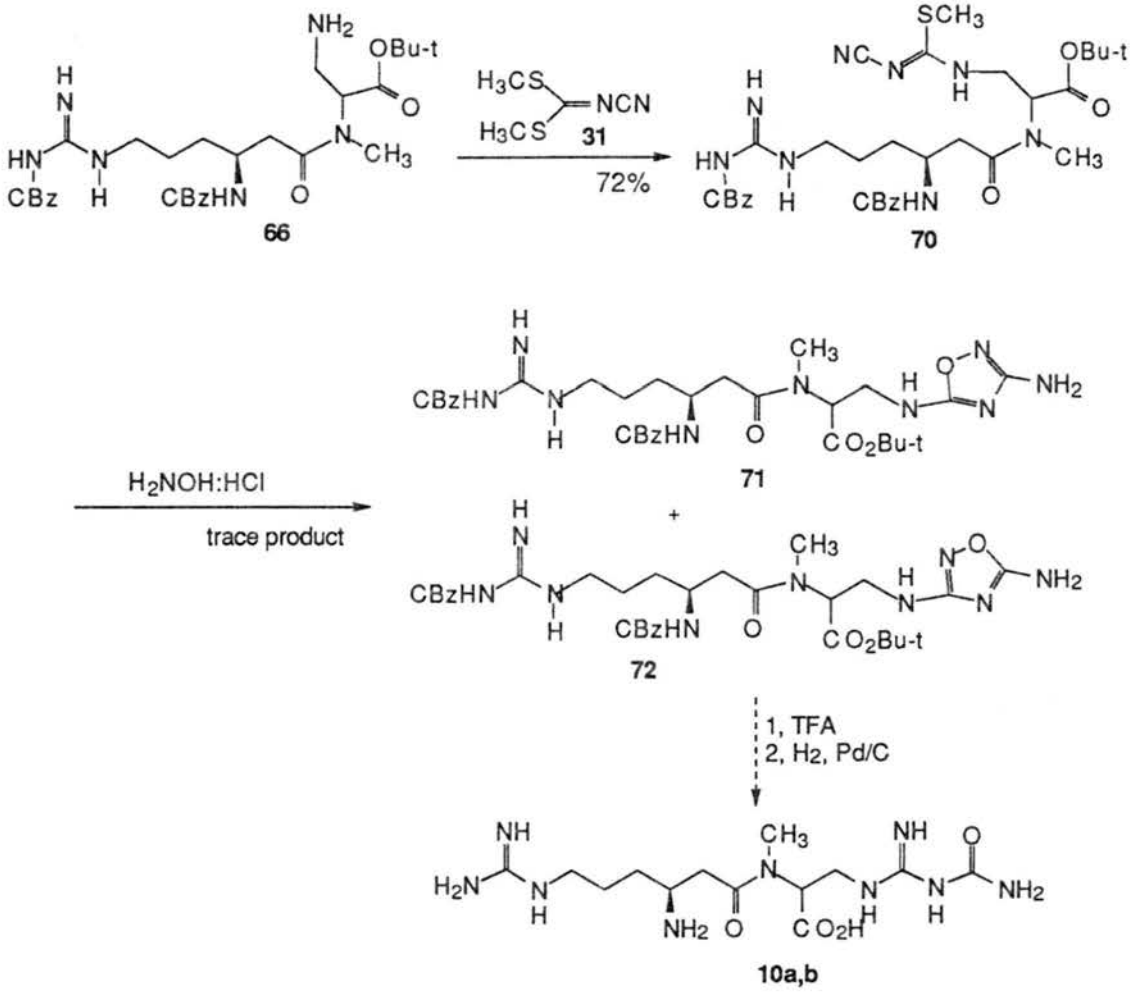
In conclusion, from these two approaches, it is believed that the guanidino groups in this type of peptide system have low reactivity toward isocyanates, even the highly reactive chlorosulfonylisocyanate.



SCHEME 20

The third approach for total synthesis of TAN-1057 A/B was explored as shown in Scheme 21. Treatment of amine **66** with dimethyl N-cyanodithioimino carbonate **31** in ethanol provided cyanoisothiourea **70** in good yield. [12] The conversion of **70** to the corresponding 5-amino-3-amino-1,2,4-oxadiazole derivatives **72** did not proceed as expected. Only a trace amount of the product was detected in the reaction mixture.

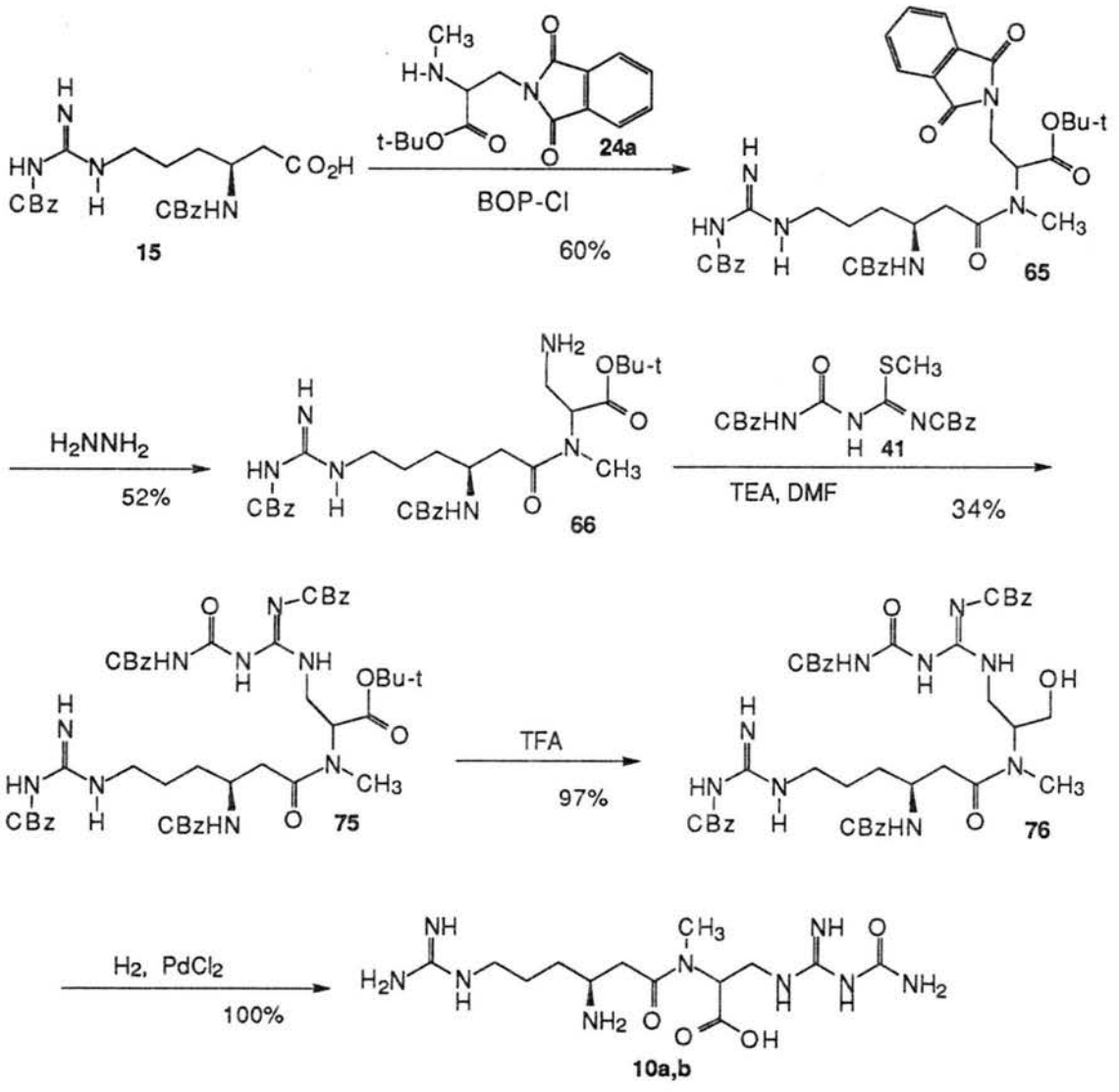
From all these failures, it became clear that a mild and efficient method for the preparation of amidinoureas from peptide amine residues must be developed to accommodate the attempt for the total synthesis of TAN-1057.



SCHEME 21

2.6 The Formal Total Synthesis of TAN-1057 A and B

Having firmLy established an efficient methodology for the preparation of amidinoureas (see Section 2.4), we further employed this methodology in the total synthesis of TAN-1057.



SCHEME 22

As shown in Scheme 22, this methodology was first applied to the construction of the fully functionalized acyclic hydrolysis product **10** of TAN-1057A/B. Treatment of amine **66** with N-(benzyloxycarbonyl)ureido-N'-benzyloxycarbonyl-S-methylisothiourea **41** gave the desired product **75** in 34% yield. Compound **75** has a tendency to form a triazinedione byproduct, so it was converted into the more stable acid **76** by being treated with TFA immediately after purification. Upon hydrogenation, the acyclic product **10**:2HCl was obtained in good yield. This approach has provided a formal total synthesis of TAN-1057 A and B, base on the Takeda group's procedure of transformation of **10** to a mixture of TAN-1057 A and B. [2b]

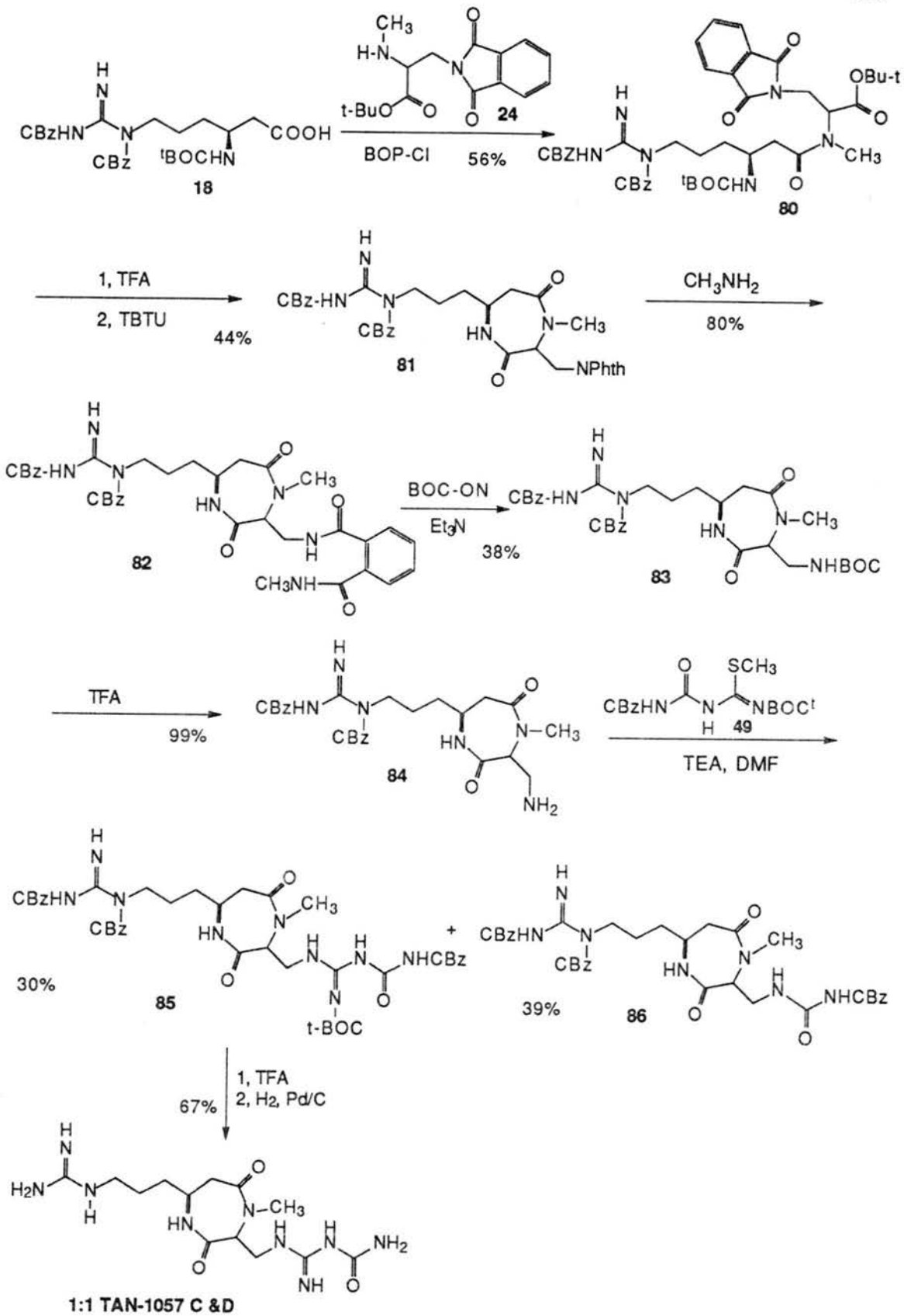
2.7 Total Synthesis of TAN-1057 C and D

After successfully applying N-(benzyloxycarbonyl)ureido-N'-benzyloxycarbonyl-S-methylisothiourea **41** in the first formal total synthesis of TAN-1057 A/B, the focus of thsi study shifed to developing a total synthesis of TAN-1057 C/D.

As mentioned in Chapter 1, TAN-1057 C and D have similar anti-MRSA activity to A and B respectively, but are rarely found in nature. TAN-1057 C and D are highly unstable in aqueous solution with a tendency to convert into a mixture of TAN-1057 A and B. [2b]

The total synthesis of TAN-1057 C and D was achieved as shown in Scheme 23. The fully protected peptide **80** was obtained through a peptide coupling reaction between L-N^{α}-t-BOC- N^{δ}, N^{ω}-di-CBz-β-homoarginine **18** and t-butyl N^{2}-methyl-N^{3}-Pht-2,3-diaminopropionate **24** in moderate yield with BOP-Cl as a coupling reagent. When treated with TFA, both t-BOC-amine and t-butyl ester in peptide **80** were cleaved, and the intramolecular coupling was accomplished by using TBTU as a coupling reagent. The desired cyclic-peptide **81** was obtained in about 40% yield. The dimer was also found at about 1:1 ratio to the desired product. Dilution of the reaction concentration did not improve the ratio of the cyclization products. The separation between the desired product **81** and the dimer was difficult through silica gel column chromatography. However, they were separated through preparative TLC.

The deprotection of the phthaloyl group with hydrazine was not suitable here because it caused a N^{δ}-CBz group cleavage. An alternative procedure using methylamine, followed by BOC-ON was developed (see next section) and used to convert the phthaloyl amine to t-BOC-amine. After the cleavage of the t-BOC group, amine **82** was condensed with N-(benzyloxycarbonyl)ureido-N'-butyloxycarbonyl-S-methylisothiourea **49** to provide product **83**, which is a fully protected precursor of TAN-1057 C and D. The idea of choosing **49** over **41** in this reaction was to avoid the formation of the triazindione

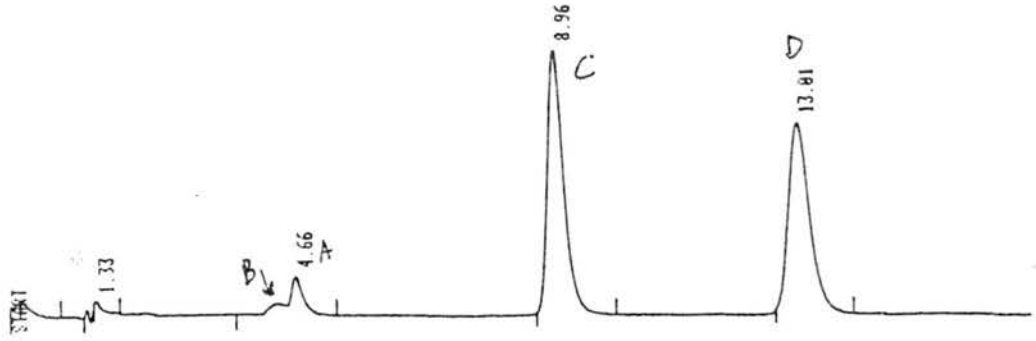

18
24
BOP-Cl
56%
80
1, TFA
2, TBTU
44%
81
CH3NH2
80%
82
BOC-ON
Et3N
38%
83
TFA
99%
84
49
TEA, DMF
30%
85
39%
86
1, TFA
2, H2, Pd/C
67%
1:1 TAN-1057 C &D

SCHEME 23

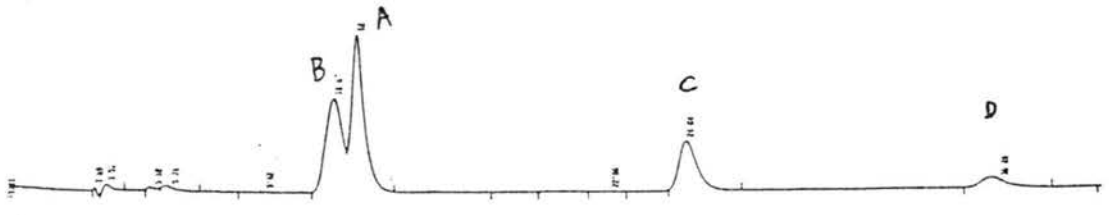
byproduct. However, an unexpected byproduct **84** (39% yield) was isolated from the reaction mixture. Hydrogenation of (1 atm. H_2, $PdCl_2$, 10 min) the byproduct **84** produced an unidentified product.

After treatment with TFA and hydrogenation (1 atm. H_2, $PdCl_2$, 10 min), peptide **83** was converted into a 1:1 mixture of TAN-1057 C and D (as 2HCl salt). The synthetic product was identified by ^{1}HNMR, MS and HPLC mobility.

A detailed HPLC analysis revealed, however, that TAN-1057 C/D was highly unstable in aqueous solution (Figure 4), not only were TAN-1057 C and D interconverting to each other, they were also converting even more quickly into a mixture of A and B in both water and 0.1 N phosphate buffer (pH 5) (>70% conversion within 30 min). Based on this result, it is very unlikely that C and D can be separated and purified under this condition.



(1) A fresh sample of synthetic TAN-1057 C/D [flow rate, 3 mL/min]



(2) Immidiate reinjection of the "C" fraction collected from (1) [flow rate, 1 mL/min]

Column: ODS, YMC Pack A-312. Mobil phase: 0.1 M phosphate buffer (pH 5.0). UV detector wave length = 215 nm.

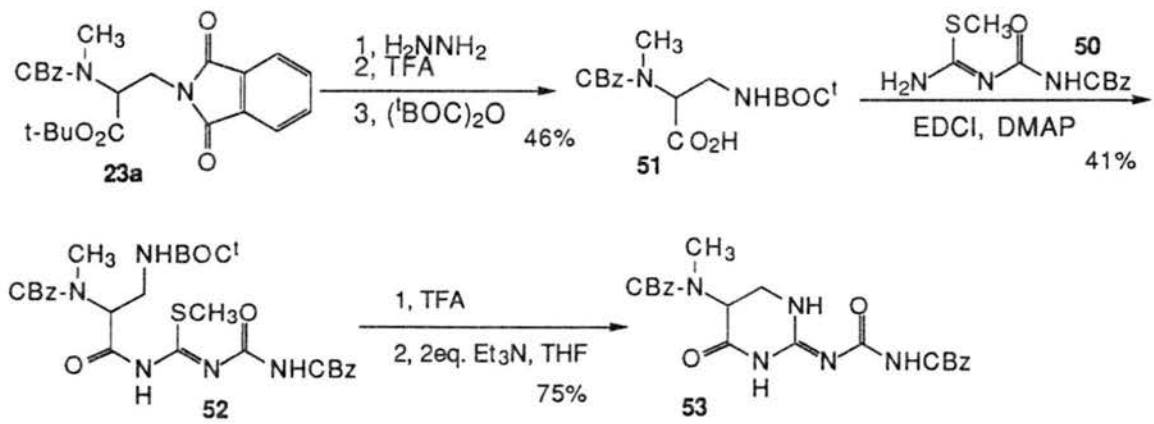
Figure 4. HPLC Analysis of synthetic TAN-1057 C/D

2.8 Total Synthesis of TAN-1057 A and B

Having successfully achieved the formal total synthesis of TAN-1057 A/B and the total synthesis of TAN-1057 C/D, we set our focus on developing a new general approach for the total synthesis of TAN-1057 A/B and analogues.

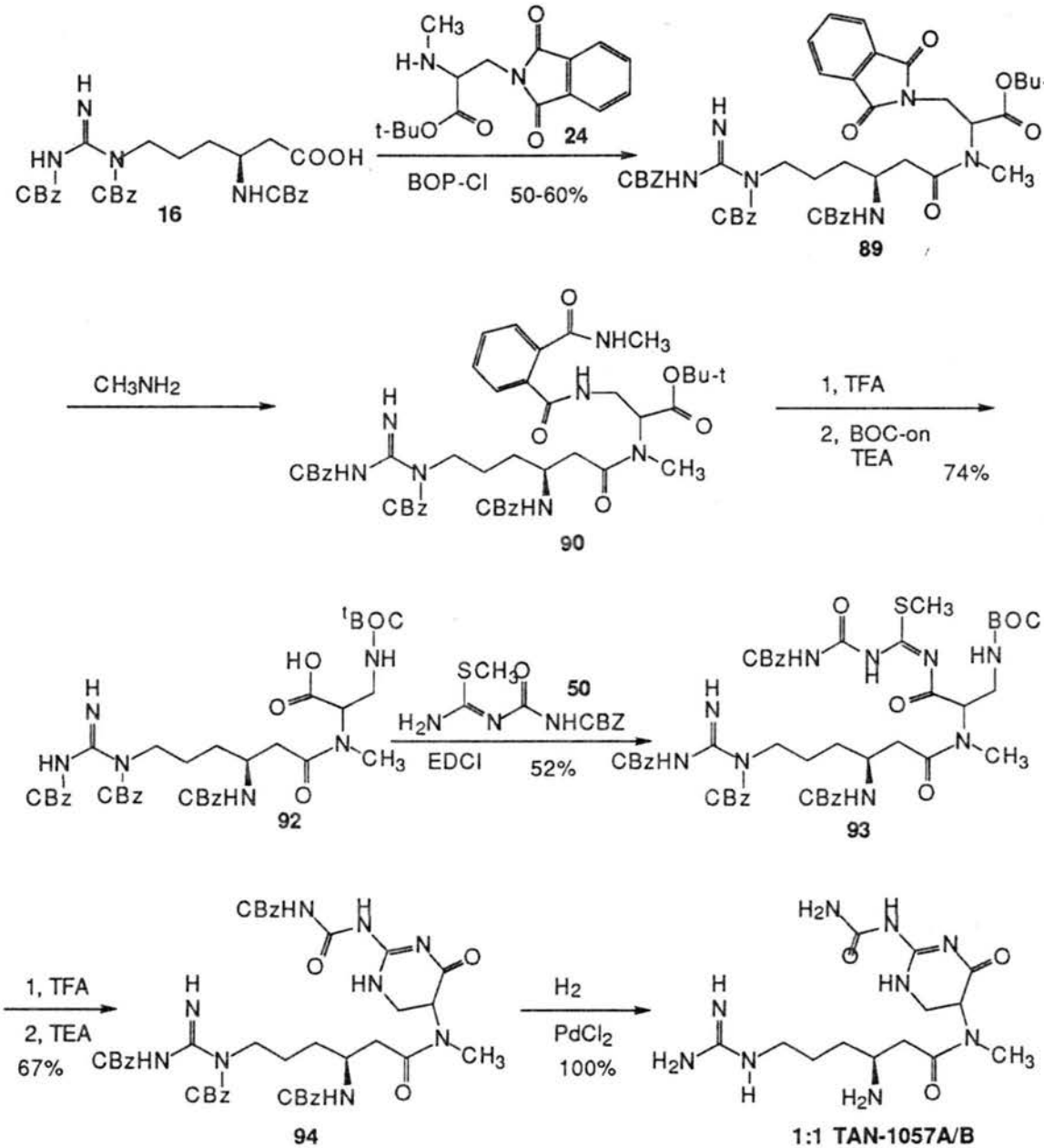
The Takeda group's procedure of converting acyclic **10** into TAN-1057A/B required using strong acidic and basic conditions and difficult chromatography, which may not be tolerated in the synthesis of some modified analogues containing sensitive functional groups.

The new idea for constructing the unique heterocyclic amidinourea substructure of TAN-1057 A/B was first demonstrated in the model system as shown in Scheme 24. After protection group transformation, compound **23** was converted to acid **51**. Coupling of acid **51** with N-(benzyloxycarbonyl)ureido-S-methylisothiourea **50** produced the desired compound **52** in 41% yield with EDCI as coupling reagent. After selective removal of the t-BOC group, the intramolecular guanilation was triggered with the addition of triethylamine. The six-membered ring was constructed efficiently to provide compound **53**, which possesses the unique structural feature of the natural product TAN-1057 A and B.



SCHEME 24

Having demonstrated the feasibility of the new strategy for constructing the six-membered ring of TAN-1057 A/B in the model study, we undertook the task of applying this approach toward the first total synthesis of TAN-1057 A/B.

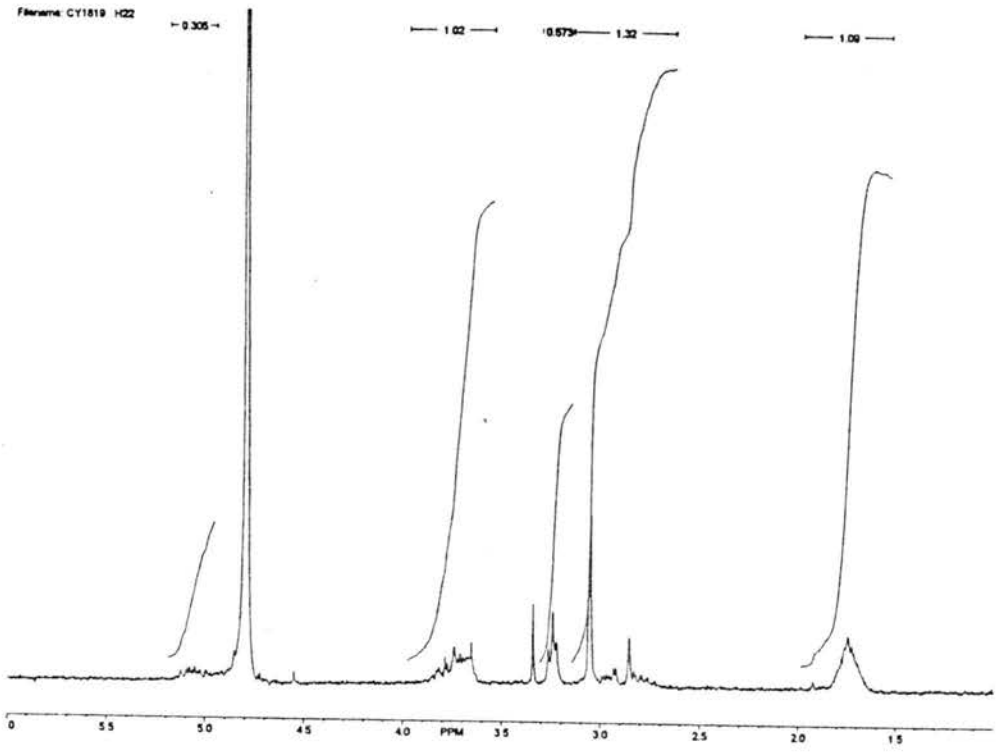


SCHEME 25

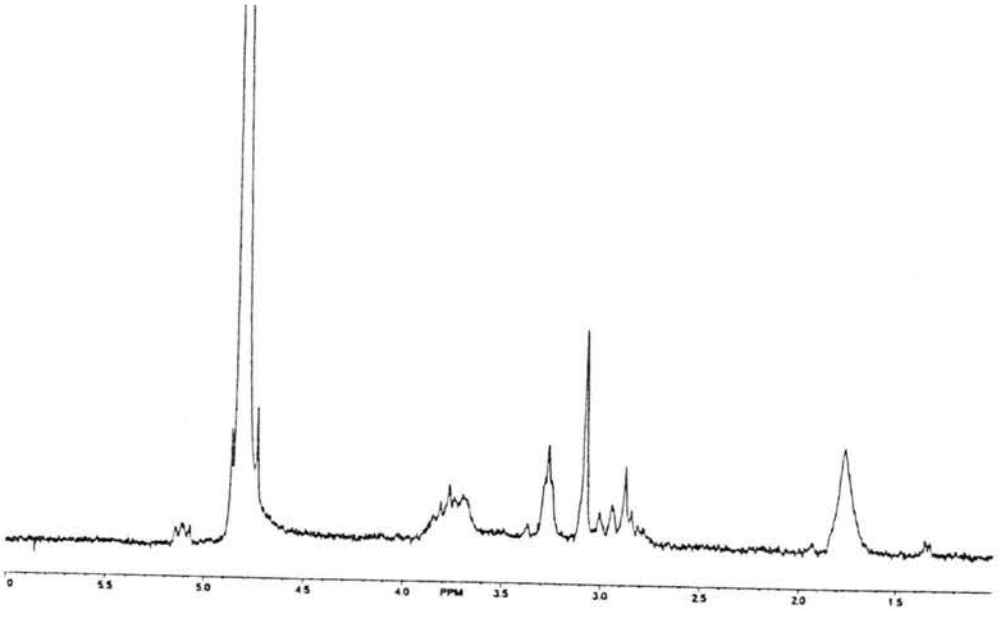
Drawing on the experiences from the model study, the approach shown in Scheme 25 was devised. Coupling of tri-N-CBz-homoarginine **16** and **24** with BOP-Cl gave the

desired peptide **89** in good yield. Attempts to deprotect the phthalimido group with hydrazine in methanol resulted in the loss of one of the N-CBz groups from the guanidine. An alternative procedure was developed in order to overcome this problem. Through tthe reatment of peptide **89** with methylamine in ethanol, a partially deprotected compound **90** was obtained. When peptide **89** was treated with 30% methylamine in EtOH for more than five minutes, the N^{δ}-CBz group from the guanidino group was removed. However, if the reaction was stopped in 5 min, the partially deprotected **90** was obtained without concomitant loss of the N^{δ}-CBz group. Compound **90** was then treated with TFA to provide the mono-acylated amino acid **91**. After treatment of the mono-acylated amino acid **91** with excess triethylamine and BOC-ON in dioxane/water, the corresponding t-BOC-protected amine **92** was obtained in good yield.

The coupling reaction between acid **92** and N-(benzyloxycarbonyl)ureido-S-methylisothiourea **50** gave the desired product **93** in moderate yield. Deprotection of the N-t-BOC group with TFA produced the corresponding amine-TFA salt. When this substance was treated with triethylamine (2.0 eq.), cyclization ensued to furnish the desired, fully protected TAN-1057A/B derivative **94**. This substance proved to be labile undergoing a second cyclization to a bicyclic byproduct. Fortunately, this problem can be circumvented by converting **94** into TAN-1057A/B immediately after PTLC purification. The deprotection was very efficient with hydrogen (1 atm.) and $PdCl_2$ in methanol for 30 minutes. A mixture of 1:1 TAN-1057 A and B was obtained after a simple workup. The synthetic material proved to be identical to the natural sample in ^1H nmr (Figure 5), mass spec fragmentation (ES^+), IR, mobility by HPLC (Figure 6), and bioassay.

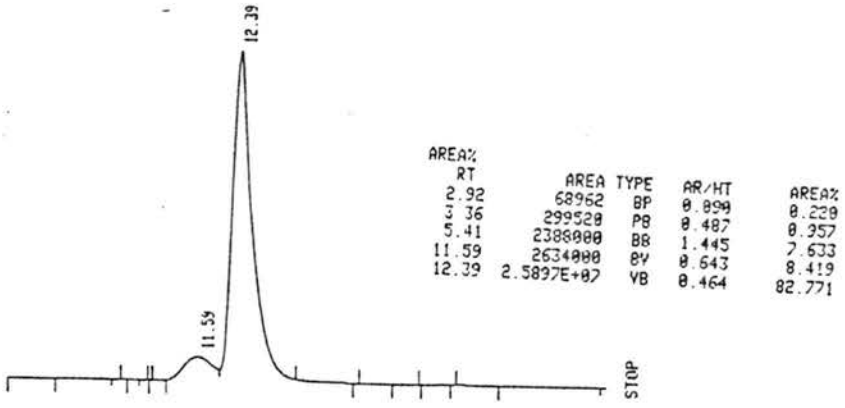


(a) Synthetic TAN-1057 A/B



(b) Authentic TAN-1057 A

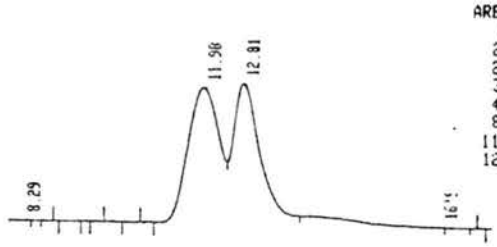
Figure 5. Comparison of ^{1}H NMR of (a) Synthetic and (b) natural TAN-1057 A



AREA%

RT	AREA	TYPE	AR/HT	AREA%
2.92	68962	BP	0.899	0.229
3.36	299528	PB	0.487	0.357
5.41	2388000	BB	1.445	7.633
11.59	2634000	BV	0.643	8.419
12.39	2.5897E+07	VB	0.464	82.771

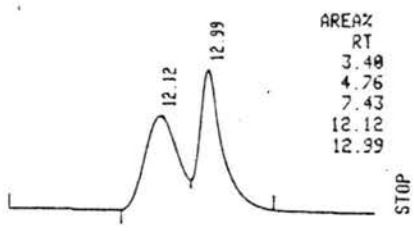
(1) TAN-1057 A (Takeda)



AREA%

RT	AREA	TYPE	AR/HT	AREA%
2.57	786240	BV	0.600	7.111
2.93	206050	VV	0.136	1.864
3.29	443670	VB	0.374	4.013
4.88	619530	PB	0.466	5.603
8.29	657920	BV	1.209	5.951
11.98	4346100	PV	0.743	39.309
12.81	3996700	VV	0.664	36.149

(2) TAN-1057 A (Takeda, after epimerization)



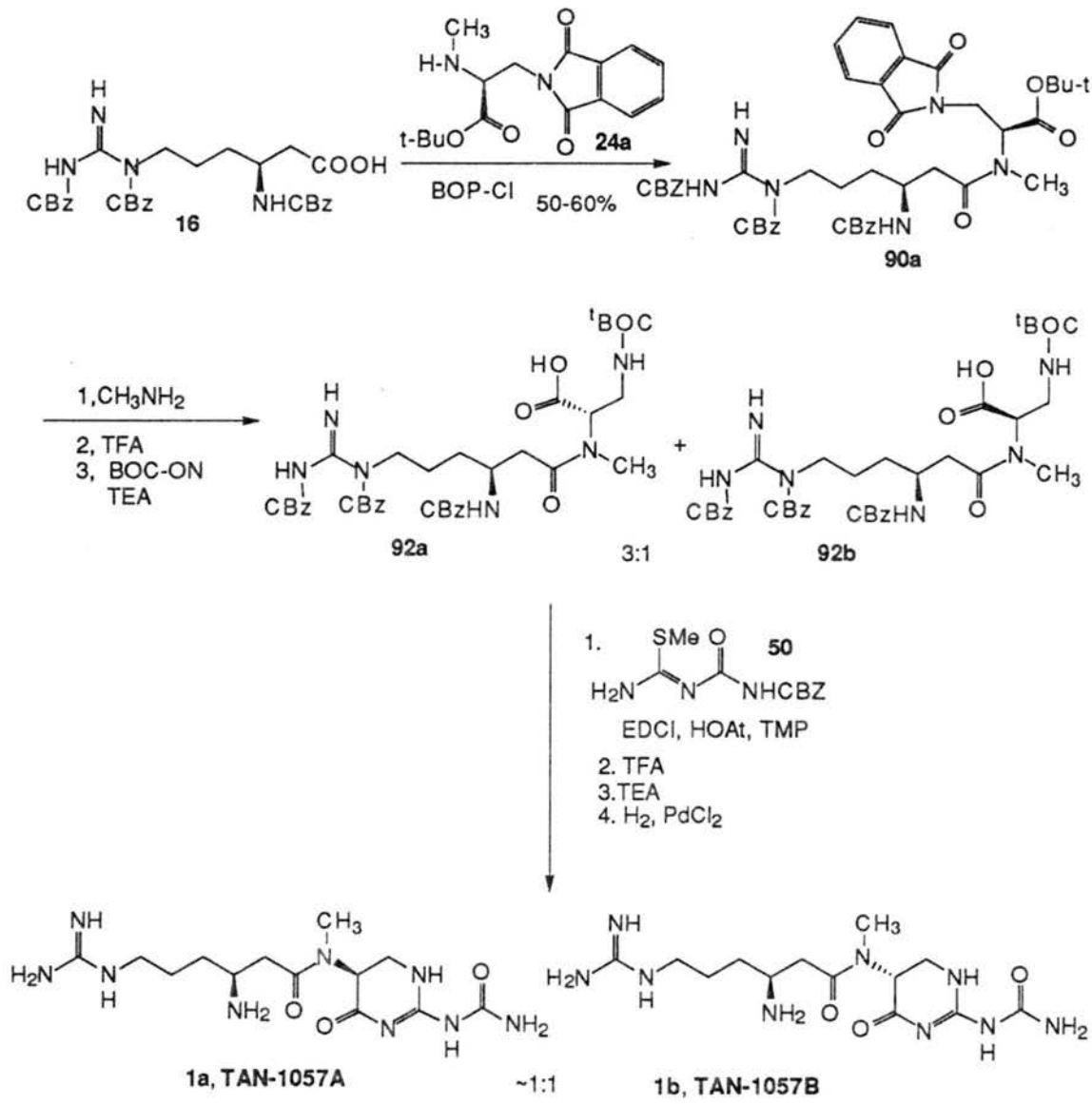
AREA%

RT	AREA	TYPE	AR/HT	AREA%
3.40	732750	PB	0.325	4.650
4.76	4000100	PB	0.296	25.384
7.43	274670	PB	0.554	1.743
12.12	5289500	PV	0.695	33.566
12.99	5461600	VB	0.483	34.658

(3) TAN-1057 A/B (Synthetic)

Column: ODS, YMC Pack A-312. Mobil phase: 0.1 M phosphate buffer (pH 5.0).
Flow rate: 1 mL/min. UV detector wave length = 215 nm.

Figure 6. HPLC Analysis of synthetic and natural TAN-1057 A/B



SCHEME 26

In an attempt to obtain optically pure TAN-1057 A through this approach, optically pure amine **24a** was used as starting material. The peptide coupling with BOP-Cl afforded the desired peptide **90a** with no detectable racemization as indicated by ^{1}H nmr. However, during the protection group adjustment, empimerization was observed, and the N^3-t-BOC acid **92** was obtained as a mixture of two diastereomers at approximately 3:1 ratio through nmr study. The attempts to separate the diastereomers through chromatography were unsuccessful. The coupling of the acid **92** (3:1 mixture) with N-(benzyloxycarbonyl) ureido-S-methylisothiourea **50** proceeded with a combination of EDCI and HOAt as

coupling reagent to avoid racemization.[19] The optical rotation and ^{1}HNMR spectrum of the resulting product seem to suggest that the chirality of the C-5 chiral center was retained during this step. Again, attempts to separate the diastereomers through chromatography were unsuccessful. After converting this intermediate to the final product, a mixture of approximately 1:1 ratio of TAN-1057 A and B was obtained as before. It is believed that the complete empimerization occurred during the last two steps (cyclization and deprotection). This is primarily due to the lability of C-5 chiral centers in both **93** and **94** toward racemization.

2.9 The Syntheses of TAN-1057 Analogues

Having firmly established the first total synthesis of TAN-1057, we designed and synthesized a few analogues of TAN-1057 that are not available from fermentation. Our interest in preparation of TAN-1057 analogues is two folds. First, we would like to explored the scope and limitation of our approach for the total synthesis of TAN-1057. Second, we are also interested in discovery new potent anti-MRSA compounds.

TAN-1057, with its potent anti-MRSA activity and the unique structure feature, is well qualified as a lead compound for the discovery of new antibiotics. There are several approaches (Figure 7), including (1) identification of the active part of the molecule (the pharmacophore); (2) modification of functional groups; (3) homologation of side chain, are used in pharmaceutical industry to modify a lead compound into drugs. [20]

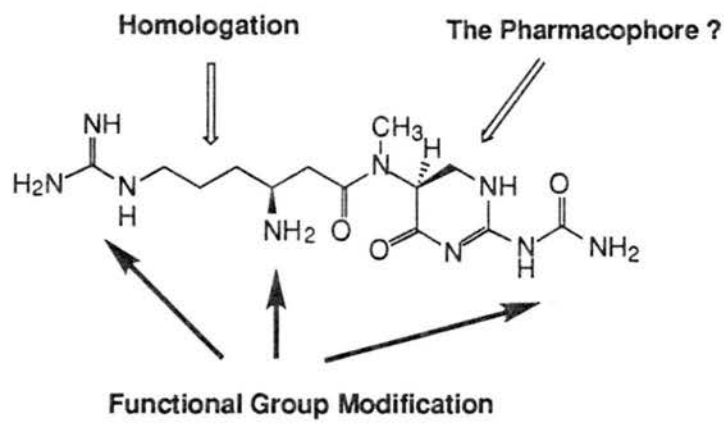


Figure 7

In the attempt to elucidate the pharmacophore of TAN-1057, four new compounds were designed (Figure 8). Removal of the ureado group from TAN-1057 A/B gives analogue **105**. Trimming off the 3'-amino group leads to analogue **121**. Cutting away the side chain of the β-homoarginine moiety affords compound **128** and **130**.

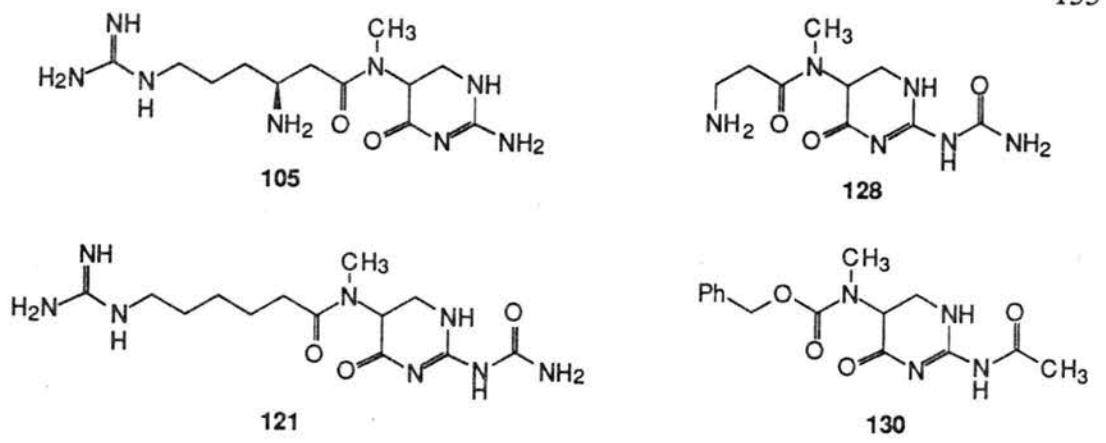


Figure 8

The ureado group is our first target for functional group modification approach. There are many nonclassical bioisosteres of ureado group. [20a] Of them, acetyl, methyloxycarbonyl, bezoyl and methansulphonyl groups were chosen, and four new TAN-1057 analogues (Figure 9, **102**, **108**, **111** and **114**) were created.

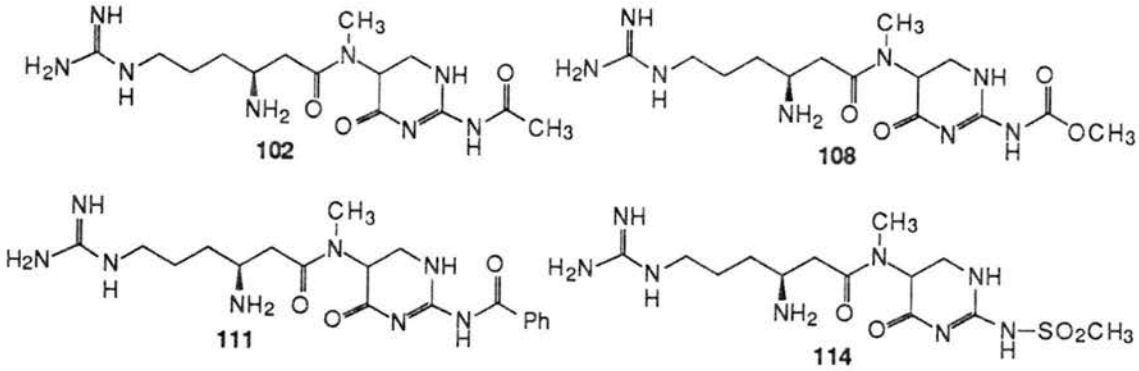
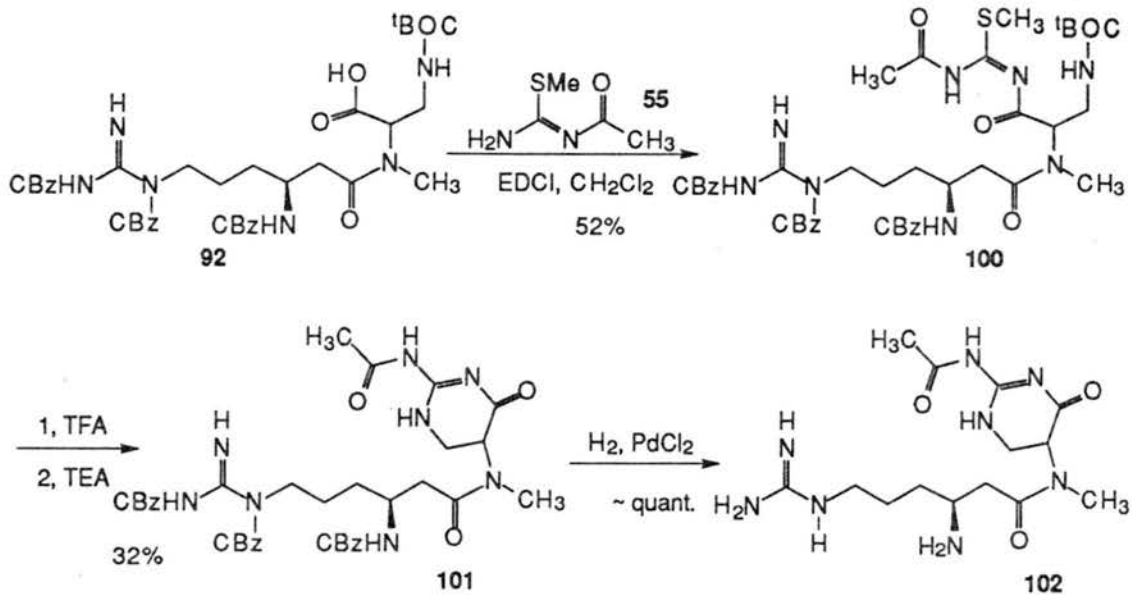


Figure 9

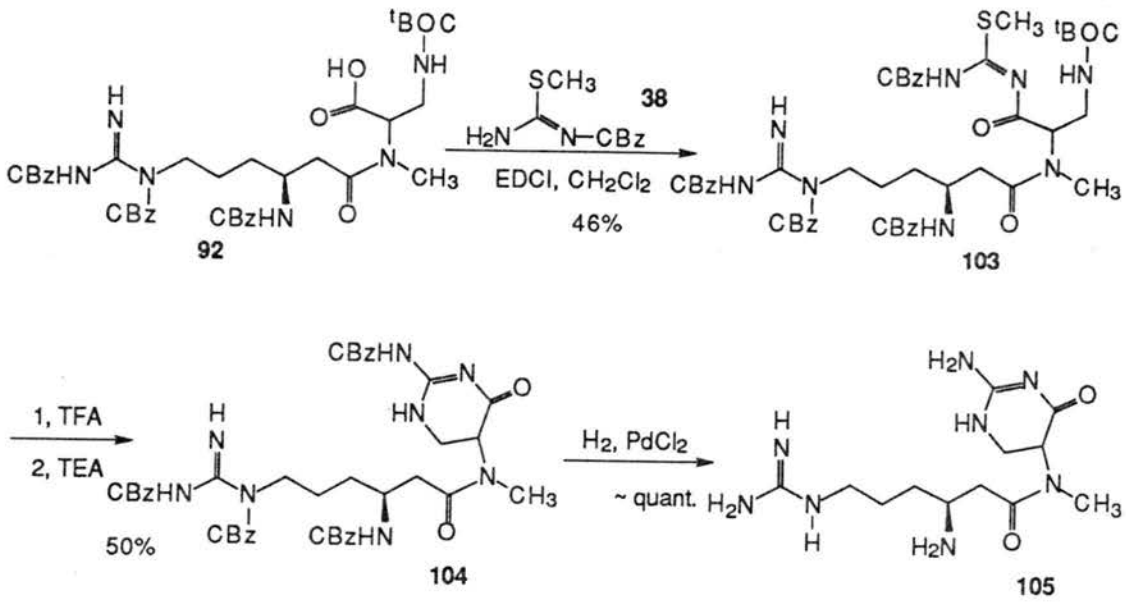
Using a similar approach, these analogues of TAN-1057 were synthesized and tested against MRSA. All the analogues were prepared and tested as mixtures of two possible diastereomers or enantiomers.



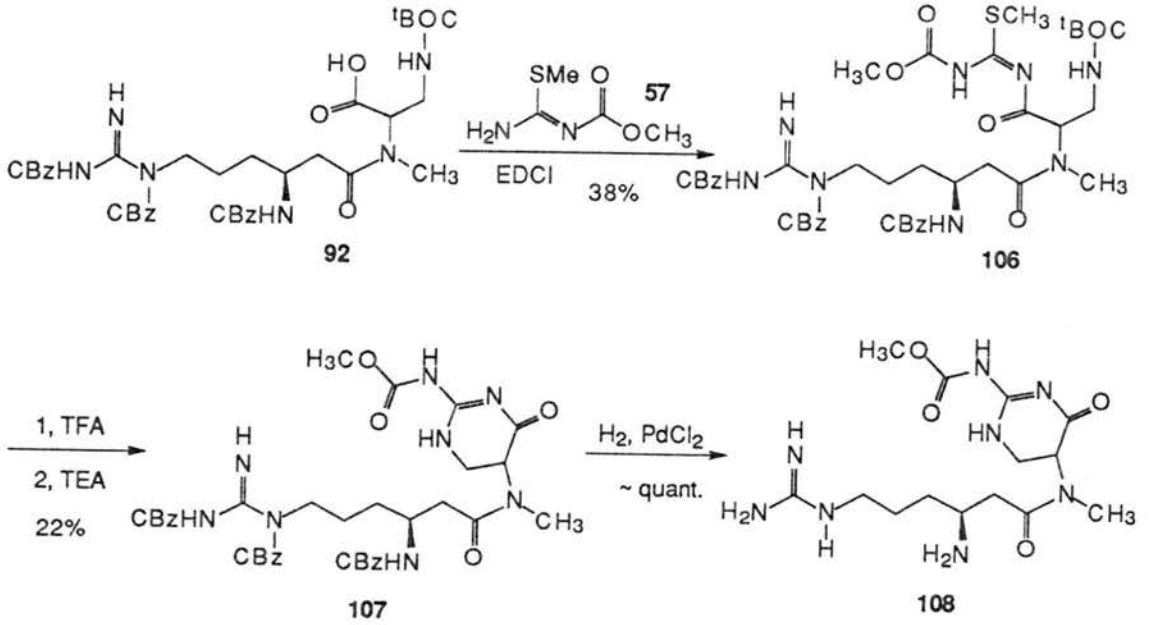
SCHEME 27

Thus, the acetyl analogue **102** was prepared as described in Scheme 27. The acetyl-S-methylisothiourea **55** was prepared from acidic deprotection following acetylation of N-t-BOC-S-methylisothiourea (Scheme 15). The coupling of acid **92** with N-acetyl-S-methylisothiourea **55** provided the desired product **100** in moderate yield. Cleavage of the t-BOC group with TFA and cyclization of the six-membered ring produced the protected precursor of the acetyl-analogue **101** in 32% yield. The deprotection was very efficient with hydrogen (1 atm.) and $PdCl_2$ in methanol for 10 minutes to provide analogue **102**. Longer reaction time resulted in the loss of the acetyl group.

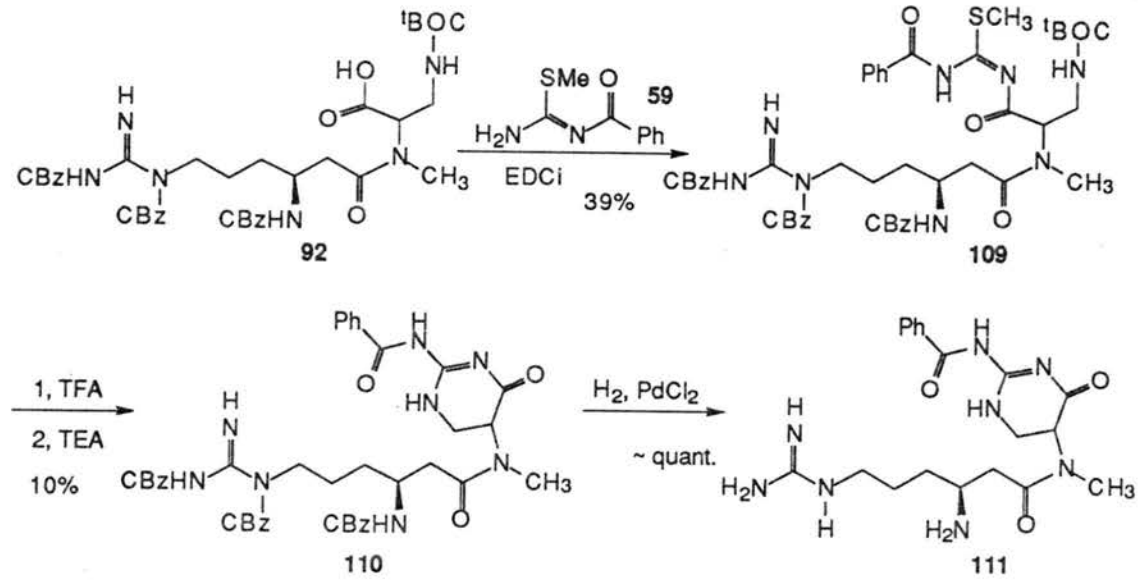
Similarly, analogues **105** (Scheme 28), **108** (Scheme 29), **111** (Scheme 30) and **114** (Scheme 31) were synthesized.



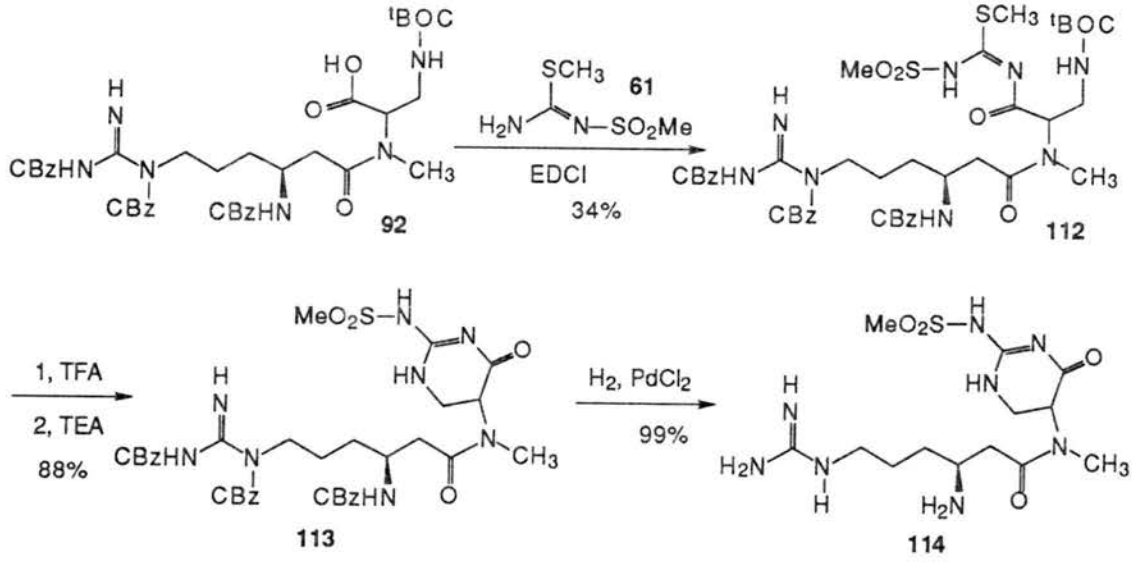
SCHEME 28



SCHEME 29



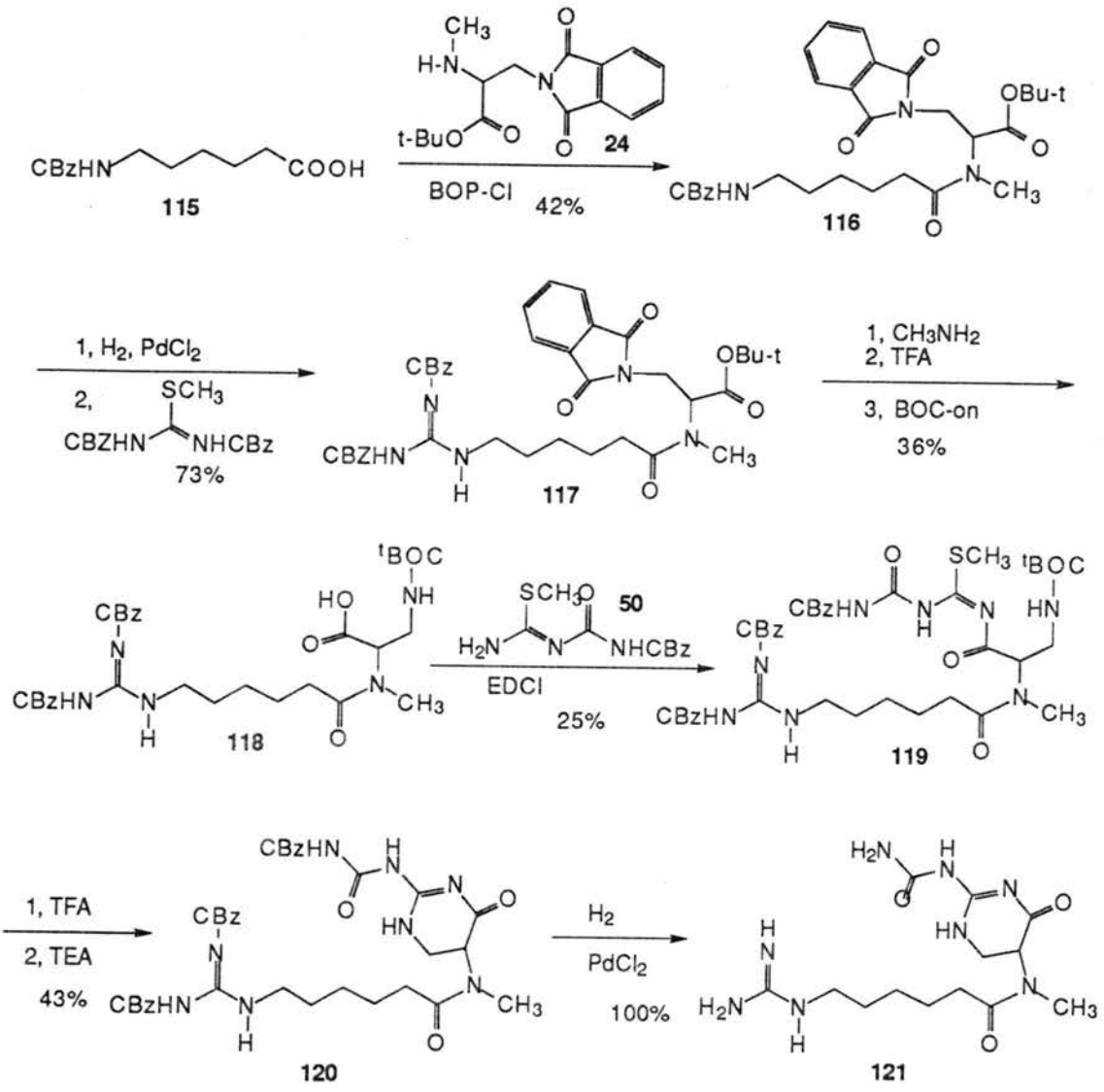
SCHEME 30



SCHEME 31

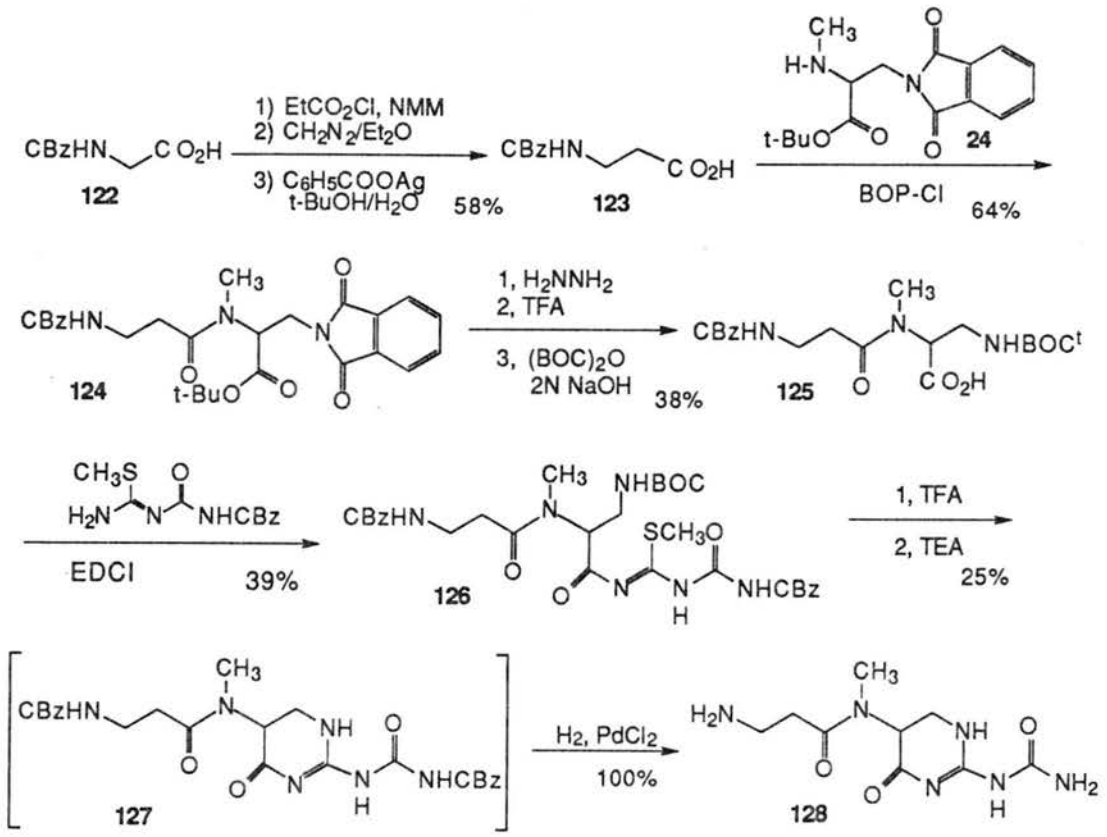
The side chain modified analogues **121**was prepared as shown in Scheme 32. Commercially available N-CBz-7-aminoheptonic acid was chosen as starting material. After peptide coupling and the removal of the CBz group, the 7-amino group was converted into

N,N-di-CBz-guanidino group in high yield. Following similar procedures to those used in the total synthesis of TAN-1057A/B, analogue **121** was synthesized.

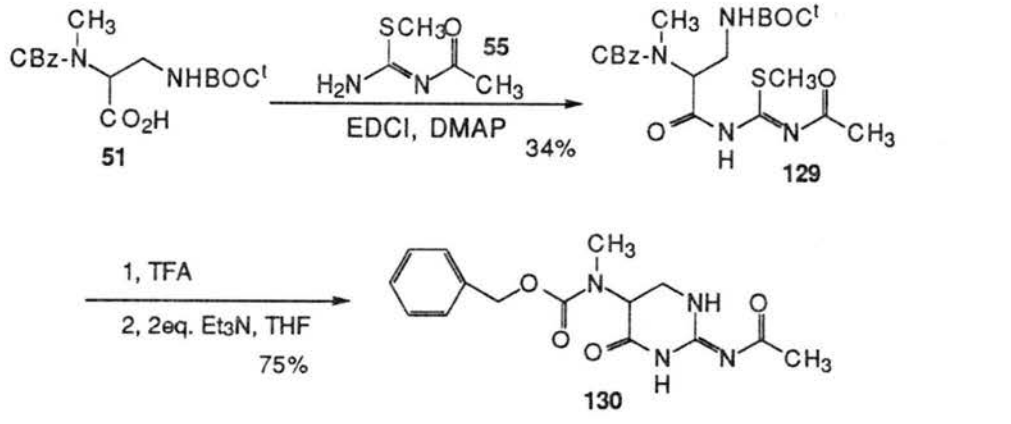


SCHEME 32

As shown in Scheme 33, analogue **128** was synthesized through a similar procedure. A similar analogue, **130**, was also synthesized (Scheme 34).



SCHEME 33



SCHEME 34

2.10 The Anti-MRSA Assay of TAN-1057 and Analogues

Ten compounds (Figure 10) were synthesized during this study, including the natural products TAN-1057 A/B, C/D and eight structural analogues. All of them were assayed as 1:1 mixture of two possible diastereomers or enantiomers related to C-5 chiral center.

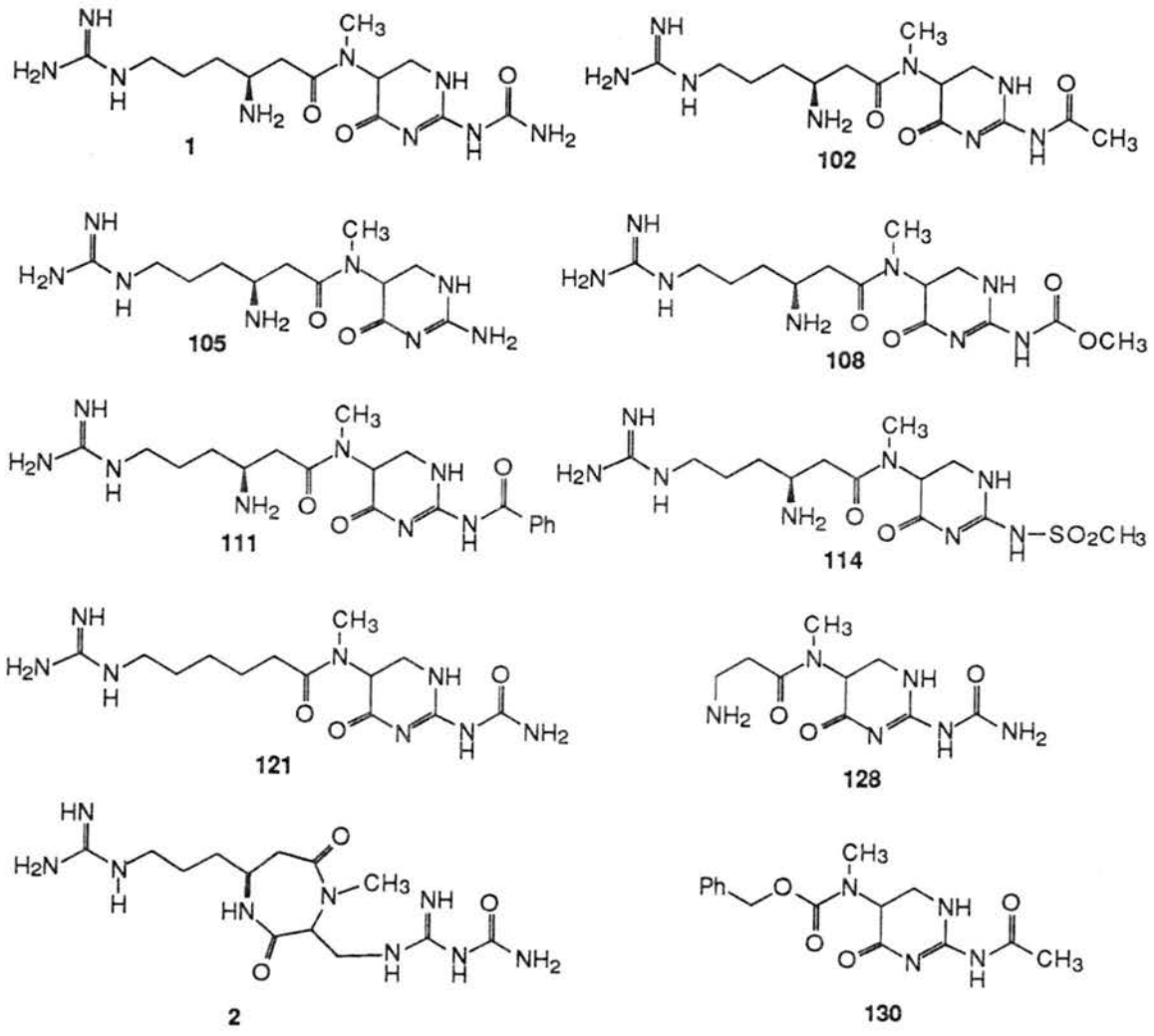


Figure 10

All the compounds synthesized were first screened by the 10-fold agar diffusion method on BHI plates (pH 7 and 9) against *Staphylococcus aureus* ACTT 25923) (Table 4). Synthetic TAN-1057 showed same activity against MRSA as reported. Two synthetic analogues, compound **102** and **108**, showed potent activity against MRSA. Further testing on synthetic TAN-1057A/B and analogue **102** (Table 5) revealed that they had very similar activities against microorganisms tested including MRSA strains. Analogue **108** showed weaker activity. Other analogues showed no activity against *Staphylococcus aureus* up to 1 mg/mL.

Table 4. Anti-MRSA Activity of Synthetic Compounds

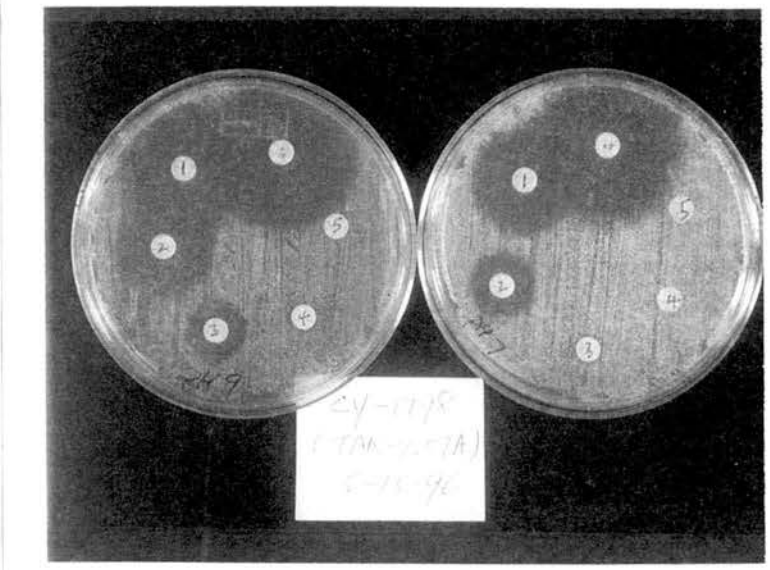
(Against *Staphylococcus aureus* ACTT 25923)

	MIC (ug/mL)	
Compounds	**BHI plate** (pH = 7)	**BHI plate** (pH = 9)
1a (Takeda)	100	nd
1b(Takeda)	100	nd
1	100	10
2	100	10
102	100	10
105	1000	100
108	1000	100
111	R	1000
114	R	R
121	R	R
127	R	R
130	R	R

BHI = Brain Heart Infusion Media
R = resistant

Table 5. Antibacterial Activity of Synthetic Compounds

	MIC (ug/mL)				
ORGANISM	**1**	**102**	**108**	Imipenem	Vancomycin
MSSA 29213	16	16	64	<0.25	0.25
MSSA COL8A	16	16	64	<0.25	<0.25
MSSA PC1	8	8	32	<0.25	<0.25
MSSA mouse 027	16	16	32	<0.25	<0.25
MRSA COL	16	16	64	16	0.5
MRSA 76	16	16	64	16	<0.25
MRSA ATCC 33593	16	8	64	16	0.5
MRSA Spain #356	16	16	64	16	<0.25
MRS. *haemolyt.* 05	32	128	256	64	1
Efs ATCC 29212	32	32	64	<0.25	1
Efm ATCC 35667	32	64	128	2	<0.25
EfmVan A	32	128	64	4	>128
Efm A491	128	256	>256	128	<0.25
***E. coli* ATCC 25992**	256	128	>256	<0.25	>128
***p. aer.* ATCC 27853**	256	256	>256	1	>128
solvent (1:1)	H_2O DMSO	H_2O DMSO	MeOH	H_2O	H_2O



(a) BHI, pH = 9. (b) BHI, pH = 7

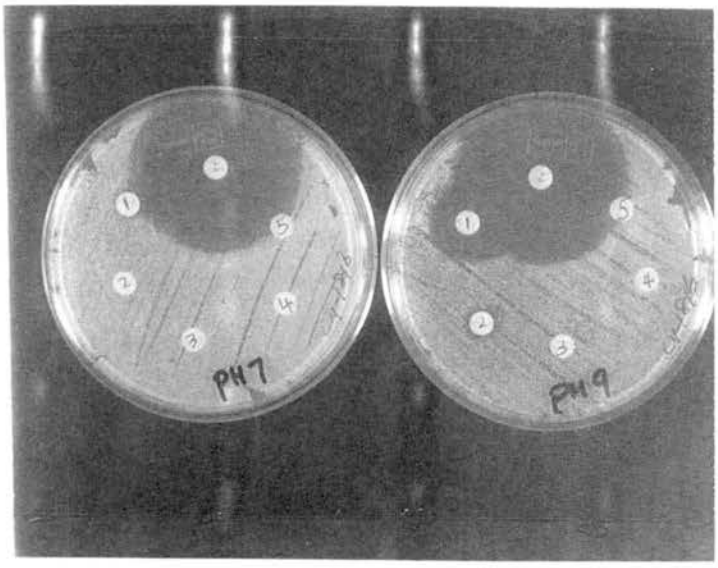
Figure 11. The Agar Diffusion Test of Synthetic TAN-1057 A/B

(a) BHI, pH = 9. (b) BHI, pH = 7

Figure 12. The Agar Diffusion Test of Synthetic TAN-1057 C/D

(a) BHI, pH = 9. (b) BHI, pH = 7

Figure 13. The Agar Diffusion Test of Synthetic Analogue **102**



(a) BHI, pH = 7. (b) BHI, pH = 9

Figure 14. The Agar Diffusion Test of Synthetic Analogue **105**

2.11 Conclusion

The first total synthesis of TAN-1057 has been achieved in 13 steps from N-CBz-asparagine. A new efficient method for preparation of amidinoureas was developed and successfully applied to the total synthesis of TAN-1057. More importantly, this concise total synthesis paves the way for access to analogues not available from natural sources. Eight analogues of TAN-1057 were designed, synthesized and tested. The agar diffusion assay of synthetic TAN-1057 and analogues revealed that the synthetic TAN-1057 had same activity as reported and the synthetic analogue **102** had very similar activity to TAN-1057. Other analogues showed no activity against *Staphylococcus aureus* up to 1 mg/mL.

The success in the discovery of a new anti-MRSA analogue proved the usefulness of this highly flexible strategy for the synthesis of TAN-1057 and its analogues. It shows the potential to create molecular diversity--in other words, to make a library of a large number of TAN-1057 analogues for biological screening to find better analogues than the natural ones for potential use as drugs.

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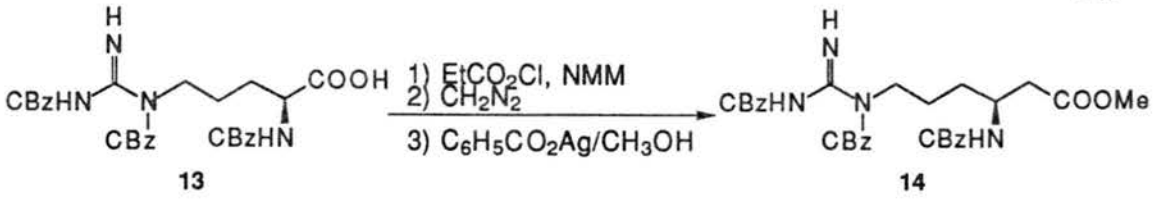
Chapter 3. Experimental Section

General Information

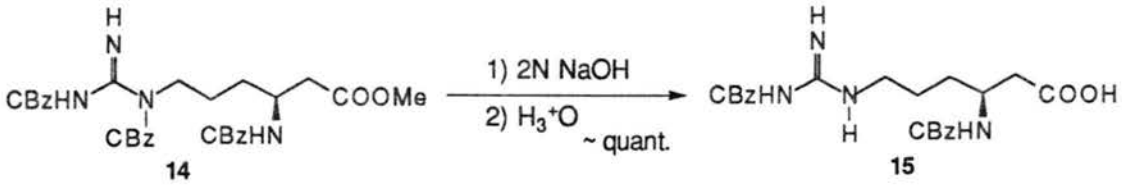
^1H NMR spectra were obtained on a Bruker AC 300 MHz spectrometer or on an IBM WP270 MHz FT NMR and chemical shifts are reported in parts per million downfield from TMS. NMR collected in methanol-d_4 or D_2O are reported relative to the methanol peak at 3.30 ppm or HOD peak at 4.63 ppm respectively. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR either as KBr pellets or as thin films from dichloromethane and are reported as λmax in cm^{-1}. Melting points were determined in open-ended capillary tubes on a Mel-Temp apparatus and are uncorrected. Optical rotations were obtained on a Rudolph research Autopol III automatic polarimeter at a wavelength of 589 nm (sodium "D" line) with a 1.0-dm cell with a volume of 1 mL. Specific rotations, $[\alpha]_D$, are reported in degrees per decimeter at the specified temperature and the concentration (c) given in grams per 100 mL in the specified solvent. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are accurate to within $\pm$ 0.4% of the calculated values. Mass spectra were obtained on a 1992 Fisons VG AutoSpec at Chemistry department (CSU) or on a VG-7070 at UCR. Visualization on TLC was achieved with ultraviolet light, or heating of TLC plates submerged in a 5% solution of phosphomolybdic acid in 95% ethanol. Column chromatography was performed using Merck silica gel grade 60, 230-400 mesh, 60Å. Both analytical and preparative thin-layer chromatography was performed using Merck Kieselgel 60 F_{254} plates. HPLC analysis of TAN-1057 was carried out using Water 6000 pump equipped with a UV detector, utilizing an ODS, YMC Pack A-312 column, using 0.1 M phosphate buffer (pH 5.0) mobil phase. Reagents and solvents were commercial grades and were used as supplied with the following exceptions. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl. Dry methylene chloride was obtained by distillation over CaH_2. DMF and HMPA were dried over activated 4Å molecular sieves.



***L*-N$^{\alpha}$,N$^{\delta}$,N$^{\omega}$-tri-CBz-β-homoarginine methyl ester 14.**

To a solution of tri-CBz-L-arginine **13** (1.15 g, 2.0 mmol, 1.0 eq) in EtOAc (30 mL) was added NMM (250 uL) and ethyl chloroformate (200 uL) at 0 °C. The resulting mixture was stirred for 3 h at 0 °C. Then the precipitated amine hydrochloride was rapidly filtered off in the cold. To this clear solution was added CH_2N_2/ether solution (generated from MNNG).The solution was stirred overnight at room temperature and concentrated to give oily diazoketone. The oily diazoketone was dissolved in MeOH (30 mL), and to this solution was added silver benzoate (250 mg, 1 mmol, 0.5 eq) and triethyl amine (1 mL). The resulting mixture was stirred overnight in the dark at room temperature and then concentrated *in vacuo*. The residue was dissolved in ethyl acetate and insoluble material was filtered off. The filtrate was washed with saturated sodium bicarbonate solution, saturated sodium chloride solution, 1M hydrochloric acid, and then finally saturated sodium chloride solution to neutral. Organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. Purification via column chromatography (silica gel, 5:4:0.2 methylene chloride:EtOAc: MeOH) yield 873 mg (74%) **14** as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$ vs TMS): 1.49(2H, m); 1.53(2H, m), 2.48(2H, d, J = 5.40 Hz), 3.58(3H, s), 3.93(1H, m), 5.04(2H, s); 5.10(2H, s); 5.19(2H, s); 5.43(1H, d, J = 7.0 Hz, D_2O exchanged); 7.31(15H, m); 9.30(1H, br, D_2O exchanged); 9.46(1H, br, D_2O exchanged)ppm. IR (NaCl, film): 3342, 2936, 1697, 1525, 1455, 1248, 1056, 1024, 667cm-^{1}; mp: 139.5-140.5 °C; $[\alpha]_D^{25}$ = - 1.43 (c 0.7, CH_2Cl_2); Anal. Calcd. for $C_{32}H_{36}N_4O_8$: C, 63.57; H, 6.00; N, 9.27. Found: C, 63.71; H, 5.81; N, 9.20.



14 15

***L*-N$^{\alpha}$,N$^{\omega}$-di-CBz-β-homoarginine 15.**

To a solution of **14** (873 mg, 1.44 mmol) in dioxane (16 mL) was added 2 M NaOH (16 mL). The resulting mixture was stirred for 1h at room temperature, diluted with water and extracted with ethyl acetate (50 mL). The aqueous layer was adjusted to PH=5 by 1 M HCl solution and extracted with dichloromethane (2x100 mL). The combined dichloromethane solution was washed with saturated ammonium chloride, dried over $MgSO_4$, filtered and concentrated to give a colorless oil. After triturated in anhydrous ethyl ether, 656 mg (100%) of **15** was obtained as a white solid.

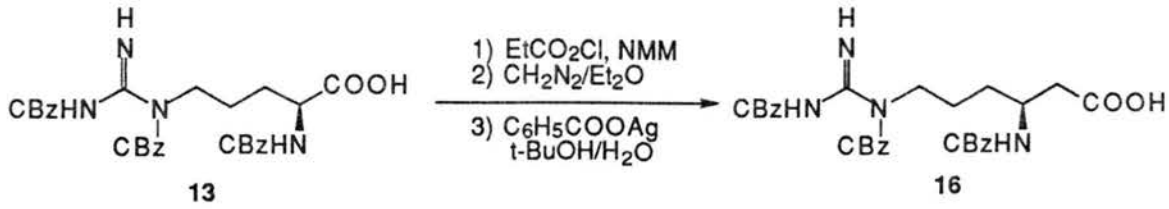
^{1}H NMR (300 MHz, CD_3OD, vs TMS) δ 1.60 (4H, m), 2.37 (2H, m), 3.20 (2H, m), 3.94 (1H, m), 5.07 (2H, s), 5.16 (2H, s), 7.32 (10H, m)ppm;

IR (NaCl, film): 3314, 2947, 1693, 1613, 1503, 1402, 1259, 1065cm^{-1};

mp: 95-98 °C;

$[\alpha]_D^{25}$ = -14.0(c 1.0, CH_3OH);

Anal. Calcd. for $C_{23}H_{28}N_4O_6$: C, 60.52; H, 6.18; N, 12.27. Found: C, 60.80; H, 5.80; N, 12.06.



L-N$^{\alpha}$,N$^{\delta}$,N$^{\omega}$-tri-CBz-β-homoarginine 16.

To a solution of tri-CBz-L-arginine **13** (1.15 g, 2.0 mmol, 1.0 eq) in THF (30 mL) was added NMM (250 uL, 2.2 mmol, 1.1 eq) and ethyl chloroformate (200 uL, 2.2 mmol, 1.1 eq) at 0 °C. The resulting mixture was stirred for 1 h at 0 °C. Then the precipitated amine hydrochloride was rapidly filtered off in the cold. To this clear solution was added CH_2N_2/ether solution (generated from MNNG) at 0 °C. The solution was stirred overnight at room temperature and concentrated to give oily diazoketone.

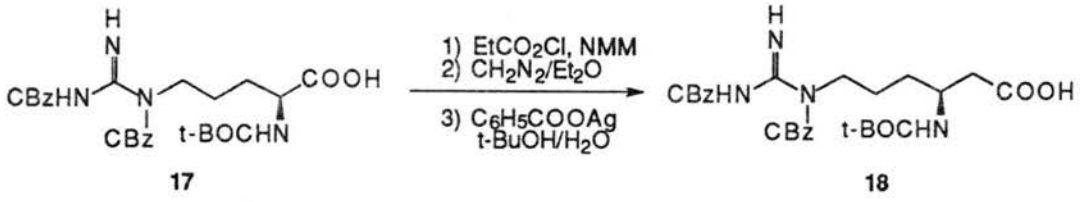
The oily diazoketone was dissolved in t-BuOH/H_2O (20 mL, 1:1), and to this solution was added silver benzoate (250 mg, 1 mmol, 0.5 eq) and triethyl amine (1 mL). The resulting mixture was stirred overnight in the dark at room temperature and then concentrated *in vacuo*. The residue was treated with CH_2Cl_2/sat. NaH_2PO_4. The organic layer was separated, dried over anhydrous sodium sulfate and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:4:0.5) provided 717 mg (58%) of **16** as a semi-solid.

^{1}H NMR (300 MHz, CD_3OD): 1.47 (2H, m); 1.62 (2H, m); 2.40 (2H, d, J = 3.84 Hz); 3.91 (3H, m); 5.01 (2H, s); 5.13 (2H, s); 5.23 (2H, s); 7.32 (15H, m) ppm.

IR (NaCl, film): 3380, 2954, 1676, 1544, 1451, 1204, 1144, 848, 798 cm-^{1};

$[\alpha]_D^{25}$ = - 2.5 (c 1.1, CH_2Cl_2/CH_3OH);

Anal. Calcd. for $C_{31}H_{34}N_4O_8 \cdot H_2O$: C, 61.18; H, 5.96; N, 9.21. Found: C, 61.34; H, 5.83; N, 9.16.



17 18

L-N$^{\alpha}$-t-BOC- N$^{\delta}$, N$^{\omega}$-di-CBz-β-homoarginine 18.

To a solution of N$^{\alpha}$-t-BOC-di-CBz-L-arginine **17** (2.72 g, 5.0 mmol, 1.0 eq) in THF (50 mL) was added NMM (604 uL, 5.5 mmol, 1.1 eq) and ethyl chloroformate (524 uL, 5.5 mmol, 1.1 eq) at 0 °C. The resulting mixture was stirred for 3h at 0 °C. Then the precipitated amine hydrochloride was rapidly filtered off in the cold. To this clear solution was added CH_2N_2/ether solution (generated from MNNG). The solution was stirred overnight at room temperature, concentrated to give oily diazoketone.

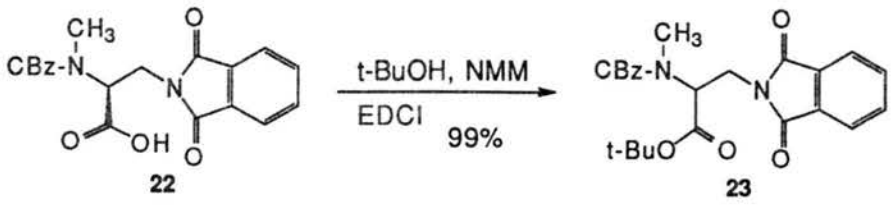
The oily diazoketone was dissolved in t-BuOH/H_2O (60 mL, 1:1), and to this solution was added silver benzoate (1.0 g) and triethyl amine (5 mL). The resulting mixture was stirred overnight in the dark at room temperature and then concentrated *in vacuo*. The residue was treated with ethyl acetate/sat. NaH_2PO_4 aqueous solution. The organic layer was separated, dried over anhydrous sodium sulfate and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:4:0.5) provided 1.80 g (65%) of **18** as a semi-solid.

^{1}H NMR (300 MHz, CD_3OD): δ 1.39(9H, s); 1.42(2H, m); 1.63(2H, m); 2.36(2H, m); 3.83(1H, m); 3.93(2H, t, J = 7.2 Hz); 5.12(2H, s); 5.27(2H, s); 7.36(10H, m)ppm.

IR (NaCl, film): 3386, 3286, 2975, 1718, 1610, 1508, 1456, 1380, 1253, 1174, 1098, 1006, 738, 698cm-^{1};

$[\alpha]_D^{25}$ = - 1.8 (c 2.0, CH_2Cl_2);

Anal. Calcd. for $C_{28}H_{36}N_4O_8$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.30; H, 6.62; N, 10.06.



t-Butyl N^2-CBz-N^2-methyl-N^3-Pht-2,3-diaminopropionate 23.

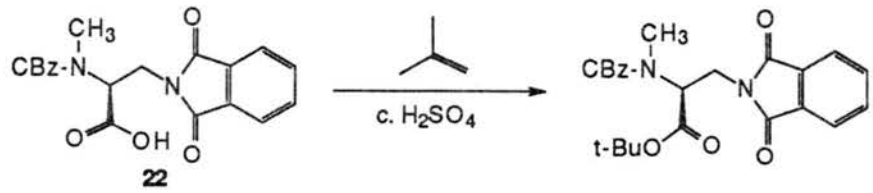
To a solution of **22** (1.23 g, 3.12 mmol, 1.0 eq), DMAP (190 mg, 1.56 mmol, 0.5 eq) and t-BuOH (277 mg, 3.74 mmol, 1.2 eq) in methylene chloride was added EDCI.HCl (717 mg, 3.74 mmol, 1.2 eq) at 0 °C. The mixture was stirred for 2 hr at 0 to 10 °C, and then overnight at room temperature. Poured the mixture into EtOAc (300 mL) and washed with brine and water, dried over anhydrous $MgSO_4$, filtered and concentrated. Purification via column chromatography (silica gel, 5:1:0.2 methylene chloride:EtOAc: MeOH) provided 1.44 g (99%) of **23** as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.41/1.47 (9H, s), 2.91 (3H, s), 4.13 (2H, m), 4.97 (2H, m), 5.11 (1H, dd, J = 5.46 Hz, J = 5.43 Hz), 7.26 (5H, m), 7.71 (2H, m), 7.78 (2H, m)ppm;

IR (NaCl, film): 2978, 1774, 1718, 1394, 1306, 1149, 1000, 732cm^{-1};

$[\alpha]_D^{25}$ = - 6.7 (c 1.2, CH_2Cl_2);

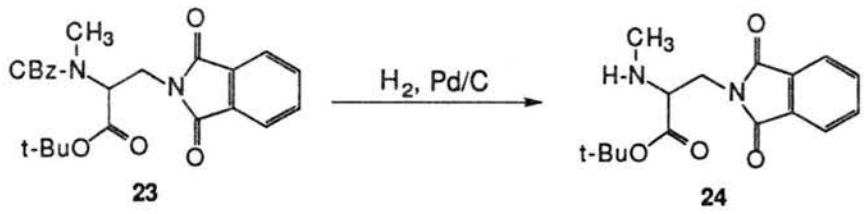
Anal. Calcd. for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.52; H, 5.72; N, 6.35.



t-Butyl S-N^2-CBz-N^2-methyl-N^3-Pht-2,3-diaminopropionate 23a.

To a solution of **22** (3.3 g, 8.64 mmol, 1.0 eq) in CH_2Cl_2 (200 mL) in a pressure vessel was added H_2SO_4 (0.4 mL) at - 5 °C. Isobutene (10 g) was passed into the solution and the vessel was sealed. The resulting mixture was stirred for 60 h at room temperature. After releasing the pressure, the organic solution was washed with water, 5% $NaHCO_3$ and water. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc: MeOH, 5:1:0.2) provided 2.84 g (73%) of product **23a** as a colorless oil.

$[\alpha]_D^{25}$ = - 77.7 (c 1.0, CH_2Cl_2)



23 24

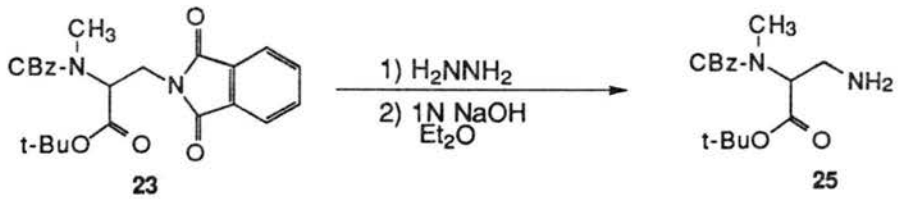
t-Butyl N^2-methyl-N^3-Pht-2,3-diaminopropionate 24.

The mixture of SM (1.30 g, 2.97 mmol) and 10% Pd on carbon (430 mg) in THF (90 mL) was degassed for 10 min by N_2. The reaction vessel was then charged with H_2 and the mixture was hydrogenated at 18 psi for 48 h. The mixture was purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo* to give 780 mg (87%) of **24** as a semi-solid. The crude product can be carried on without further purification. The analytically pure sample was obtained from column chromatography [silica gel, methylene chloride:EtOAc: MeOH, 5:1:0.1]. However, the chromatography on silica gel caused racemization.

24: ^{1}H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.39(9H, s), 1.63(1H, br, D_2O exchanged) 2.37(3H, s), 3.52(1H, t, J = 7.59 Hz), 3.86(2H, d, J = 7.47 Hz), 7.28(2H, m), 7.85(2H, m) ppm. IR (NaCl, film): 3388, 2929, 1778, 1750, 1718, 1631, 1503, 1376, 1062cm^{-1}; mp 132 °C(decomp.); $[\alpha]_D^{25} = 0$ (c 1.0, CH_2Cl_2);

Anal. Calcd. for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.20. Found: C, 63.30; H, 6.49; N, 9.2.

24a: obtained from hydrogenation of optically pure **23a**. $[\alpha]_D^{25} = -18.3$ (c 0.76, CH_2Cl_2, with crude product)



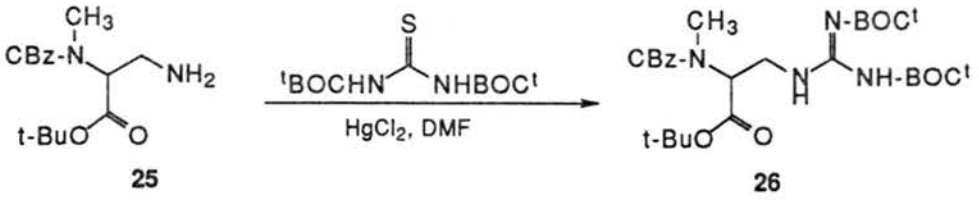
t-Butyl N^2-CBz-N^2-methyl-2,3-diaminopropionate 25.

To a solution of **23** (4.17 g, 9.27 mmol, 1.0 eq) in MeOH (80 mL) was added hydrazine (1.45 mL, 46.33 mmol, 5.0 eq) at 0 °C. The mixture was stirred overnight at room temperature, concentrated and treated the residue with ether (300 mL) and 0.5 N NaOH (50 mL). The organic phase was washes with brine and dried over anhydrous $MgSO_4$, filtered, concentrated and separated on column chromatography (silica gel, eluted with methylene chloride:EtOAc:MeOH, 5:1:0.2) to give 2.66 g (92%) of **25** as an oil.

^1H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.36/1.39(9H, s); 2.87(3H,s); 3.15(1H, m); 4.39(1H, t, J = 5.23 Hz); 4.55(1H, t, J = 5.76 Hz); 5.12(2H, m); 7.30(5H, m); 8.52(1H, br) ppm.

^{13}C NMR (75 MHz, $CDCl_3$ vs TMS): 27.40, 31.00,62.20, 66.73, 81.12, 127.18, 127.40, 136.10, 156.30, 168.81ppm.

IR (NaCl, CH_2Cl_2): 3374, 3307, 2977, 1730, 1704, 1455, 1318, 1154 cm^{-1}.



t-Butyl N^2-CBz-N^2-methyl-N^3-bis(t-BOC-amino)methyl-2,3-diaminopropionate 26.

To a solution of **25** (710 mg, 2.30 mmol, 1.0 eq), triethylamine (968 uL, 6.90 mmol, 3.0 eq) and bis(t-BOC)thiourea (637 mg, 2.30 mmol, 1.0 eq) in DMF (3.0 mL) was added $HgCl_2$ (686 mg, 2.53 mmol, 1.1 eq) at 0 °C. The mixture was stirred for 30 min at 0 °C. Poured it into EtOAc (200 mL) and passed through a short packed celite column, washed with brine and dried over over anhydrous $MgSO_4$, filtered, concentrated and separated on column chromatography (silica gel, hexane:methylene chloride:EtOAc, 5:4:1) to give 1.02 g (81%) of **26** as an oil.

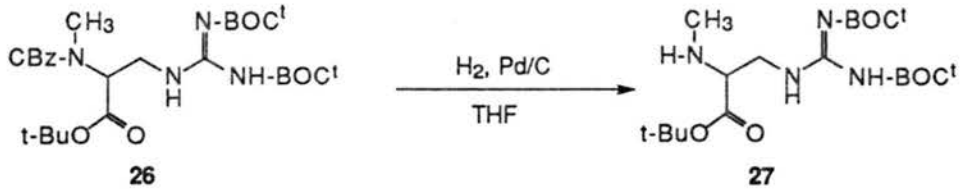
^1H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.45(27H, m); 2.94(3H,s); 3.81(2H, m); 4.57(1H, m); 5.12(2H, m); 7.32(5H, m); 8.52(1H, br, D_2O exchanged); 11.41(1H, br, D_2O exchanged)ppm.

^{13}C NMR (75 MHz, $CDCl_3$ vs TMS): δ 27.68, 27.75, 27.81, 32.66, 39.47, 59.55, 67.30, 79.06, 82.25, 83.03, 127.45, 127.71, 128.27, 156.05, 163.21, 168.37 ppm.

IR (NaCl, film): 3332, 2978, 1795, 1640, 1617, 1363, 1140, 1103, 770 cm^{-1};

$[\alpha]_D^{25}$ = -2.3 (c 1.3, CH_2Cl_2);

Anal. Calcd. for $C_{27}H_{42}N_4O_8$: C, 58.89; H, 7.69; N, 10.17. Found: C, 58.63; H, 7.30; N, 10.08.



26 27

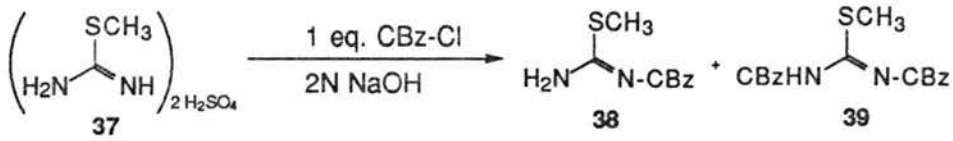
t-Butyl N^2-methyl-N^3-[bis(t-BOC-amino)]methyl-2,3-diaminopropionate

27. The mixture of **26** (605 mg, 1.1 mmol) and 20% $Pd(OH)_2/C$ (300 mg) in THF (10 mL) was stirred under H_2 (1 atm) for 20 h. The catalyst was filtered off by passing though a short pack celite column. The filtrate was concentrated to give the crude **27** as a white solid. After recrystallization in hexane /CH_2Cl_2 /EtOAc (4:4:1), pure **27** (366 mg, 80%) was obtained as white crystals.

^1H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.48(27H, m); 2.83(3H, s); 3.88(1H, m); 4.28(2H, m). 8.69(1H, br); 10.98(2H, br)ppm;

mp 137-9 °C (recryst. hexane /CH_2Cl_2 /EtOAc);

Anal. Calcd. for $C_{19}H_{36}N_4O_6$: C, 54.79; H, 8.71; N, 13.45. Found: C, 55.00; H, 8.47; N, 13.66.



N-CBz-S-methylisothiourea 38.

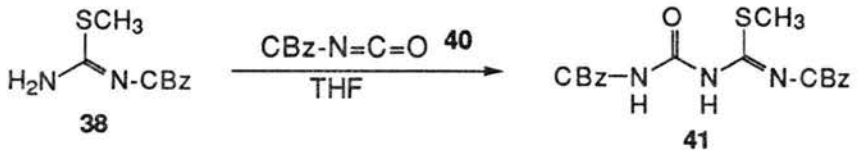
To a stirring mixture of S-methylisothiourea semisulfate **37** (5.57 g, 40 mmole, 1.0 eq) in CH_2Cl_2 (60 mL)/2N NaOH (20 mL) was added a solution of benzyloxychloroformate (5.71 mL, 40 mmol, 1.0 eq) in CH_2Cl_2 (10 mL) dropwise at 0 °C over 1 h period. The mixture was stirred overnight at room temperature. The organic layer was separated and washed with brine twice, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (CH_2Cl_2 : ethyl acetate, 80:20) to afford 3.3 g of **38** and 3.0 g **39** (67 %, conbined) as white solids.

38: ^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 2.47 (3H, s), 5.16 (2H, s), 7.35 (5H, m); ^{13}CNMR (75 MHZ, $CDCl_3$) d 13.58, 67.37, 127.03, 127.59, 128.10, 128.35, 128.53, 136.58, 161.77, 174.13 ppm;

IR (NaCl, film): 3423, 3284, 3032, 2944, 1657, 1587, 1496, 1378, 1256, 1109, 1059, 1003 cm^{-1};

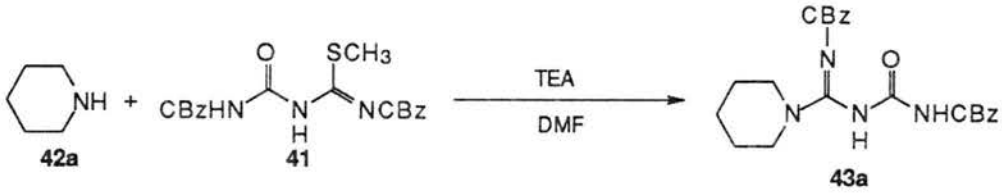
mp: 70-75 °C (recryst. CH_2Cl_2 / EtOAc);

Anal. Calcd. for $C_{10}H_{12}N_2O_2S$: C, 53.55, H, 5.39, N, 12.49. Found: C, 53.69; H,5.48; N, 12.57.



N-(benzyloxycarbonyl)ureido-N'-benzyloxycarbonyl-S-methylisothiourea 41. To a solution of **38** (1.12 g, 5.0 mmole, 1.0 eq) in THF (20 mL) was added benzyloxycarbonylisocyanate **40** (1.06 mL, 6.0 mmol, 1.2 eq) at room temperature. After 20 min, the solvent was evaporated and the residue was triturated in anhydrous ethyl ether twice to afford 2.0 g (100%) of **41** as a white solid.

^{1}HNMR (300 MHz, DMSO-d_6 vs TMS): δ 2.29 (3H, s), 5.15 (2H, s), 5.22 (2H, s), 7.40 (10H, m) ppm; IR (NaCl, film): 3226, 3159, 2925, 1749, 1715, 1558, 1469, 1261, 1205 cm^{-1}; mp: 165-6 °C (recryst. CH_2Cl_2 / EtOAc); Anal. Calcd. for $C_{10}H_{12}N_2O_2S$: C, 56.85, H, 4.77, N, 10.47, S 7.99. Found: C, 57.00; H,4.97; N, 10.36; S, 7.76.

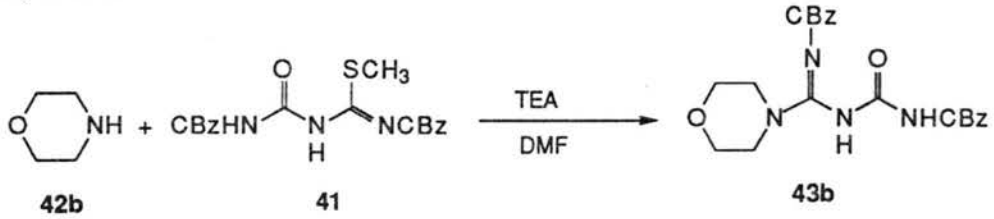


5-(Piperidinyl)-3,7-dioxo-1-phenyl-2-oxa-4,6,8-triazanon-4-en-9-oic acid, phenylmethyl ester 43a.

To a mixture of **41** (401 mg, 1.0 mmol, 1.0 eq) in DMF (9 mL) was added piperidine **42a** (148 uL, 1.5 mmol, 1.5 eq) and triethylamine (421 uL, 3.0 mmol, 3.0 eq). The resulting mixture was stirred overnight at room temperature. The reaction mixture was poured into CH_2Cl_2. The organic layer was washed with 1N HCl, sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (eluted with CH_2Cl_2:ethyl acetate: MeOH, 75:20:5) to afford 358 mg (81.6 % yield) of **43a** as an colorless oil.

^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 1.62 (6H, m); 3.53 (4H, m), 5.14 (2H, s), 5.19 (2H, s), 7.33 (10H, m), 10.85 (1H, br, D2O exchanged), 11.33 (1H, br, D2O exchanged)

ppm; IR (NaCl, film): 3257, 2918, 2845, 1730, 1604, 1455, 1270, 1218, 1154, 1108cm^{-1}; Anal. Calcd. for $C_{23}H_{26}N_4O_5$: C, 6300, H, 5.98, N, 12.78. Found: C, 63.10; H, 6.13; N, 12.56.



42b 41 43b

5-(4-Morpholinyl)-3,7-dioxo-1-phenyl-2-oxa-4,6,8-triazanon-4-en-9-oic acid, phenylmethyl ester 43b.

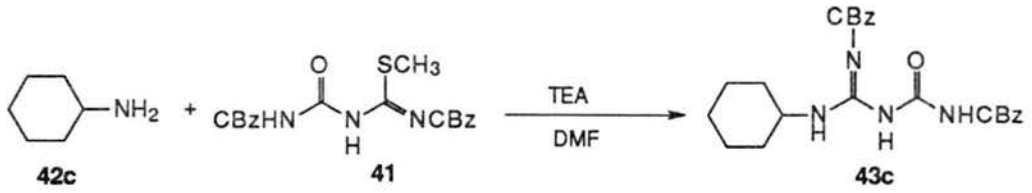
To a mixture of **41** (401 mg, 1.0 mmol, 1.0 eq) in DMF (9 mL) was added morpholine (131 mg, 1.5 mmole, 1.5 eq.) and triethylamine (303 mg, 3.0 mmol, 3.0 eq). The resulting mixture was stirred overnight at room temperature. The reaction mixture was poured into CH_2Cl_2. The organic layer was washed with 1N HCl, sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (eluted with CH_2Cl_2:ethyl acetate: MeOH, 75:20:5) to afford 332 mg (75 % yield) of **43b** as a white solid.

^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 3.59 (4H, m), 3.71 (4H, m), 5.14 (2H, s), 5.20 (2H, s), 7.36 (10H, m), 7.75 (1H, br, D_2O exch.), 11.43 (1H, br, D2O exch.).

IR (NaCl, film): 3244, 2963, 1731, 1652 1605, 1472, 1361, 1288, 1252, 1217, 1186, 1111, 1026 cm^{-1};

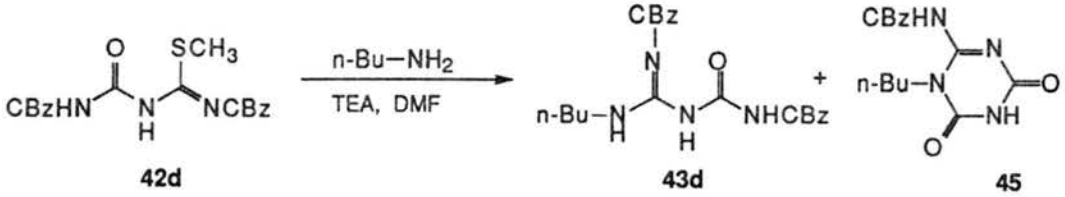
mp 121-2 °C (recryst. MeOH / CH_2Cl_2 / EtOAc);

Anal. Calcd. for $C_{22}H_{24}N_4O_6$: C, 59.99, H, 5.49, N, 12.72. Found: C, 60.02; H,5.49; N, 12.70.



5-Cyclohexylamino-3,7-dioxo-1-phenyl-2-oxa-4,6,8-triazanon-4-en-9-oic acid, phenylmethyl ester 43c.

To a mixture of **41** (200 mg, 0.5 mmol, 1.0 eq) in DMF (4.0 mL) was added cyclohexylamine (86 uL, 0.75 mmol, 1.5 eq) and triethylamine (69 uL, 0.5 mmol, 1.0 eq). The resulting mixture was stirred for 4 hours at room temperature. The reaction mixture was poured into CH_2Cl_2. The organic layer was washed with 1N HCl, sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (eluted with CH_2Cl_2:ethyl acetate: MeOH, 75:20:5) to afford 226 mg (100 % yield) of **43c** as a colorless oil. ^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 1.25 (5H, m), 1.60 (1H, m), 1.68 (2H, m), 1.89 (2H, m), 3.80 (1H, m), 5.16 (2H, s), 5.17 (2H, s), 7.33 (10H, m), 8.24 (1H, br, D2O exchanged), 8.85 (1H, br, D2O exchanged), 12.04 (1H, br, D2O exchanged) ppm; IR (NaCl, film): 3295, 2933, 2855, 1753, 1705, 1650, 1614, 1568, 1453, 1378, 1304, 1219, 1171, 1126, 1094, 1014 cm^{-1}; Anal. Calcd. for $C_{24}H_{28}N_4O_5$: C, 63.70, H, 5.6.24, N, 12.38. Found: C, 63.56, H, 6.22, N, 12.38.



42d 43d 45

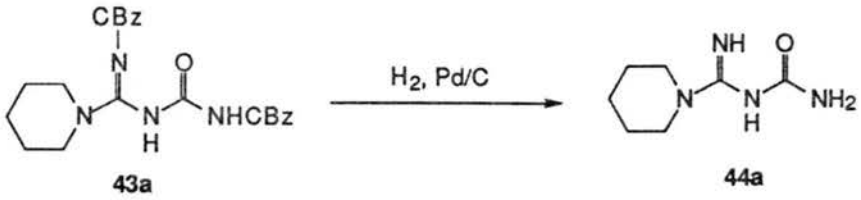
5-Butylamino-3,7-dioxo-1-phenyl-2-oxa-4,6,8-triazanon-4-en-9-oic acid, phenylmethyl ester 43d.

To a mixture of **41** (200 mg, 0.50 mmole, 1.0 eq.) in DMF (2.0 mL) was added n-butylamine (50 uL, 0.50 mmol, 1.0 eq) and triethylamine (36 uL, 0.5 mmol, 1.0 eq). The resulting mixture was stirred for 4 h at room temperature. The reaction mixture was poured into CH_2Cl_2. The organic layer was washed with 1N HCl, sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (eluted with Hexane:CH_2Cl_2:ethyl acetate, 40:40:20) to afford 100 mg (47 % yield) of **43d** as a colorless oil and 44% of **45** as a white solid.

43d: ^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 0.92 (3H, m), 1.35 (2H, m), 1.52 (2H, m), 3.31 (2H, m), 5.16 (2H, S), 5.17 (2H, S), 7.34 (10H, m), 8.30 (1H, br, D2O exchanged), 8.86 (1H, br, D2O exchanged), 12.02 (1H, br, D2O exchanged) ppm; IR (NaCl, film): 3302, 2957, 1750, 1706, 1618, 1570, 1453, 1379, 1319, 1218, 1172, 1172, 1128, 1067cm^{-1}; an colorless oil; Anal. Calcd. for $C_{22}H_{26}N_4O_5$: C, 61.96, H, 6.15, N, 13.14. Found: C, 62.28; H,5.80; N, 13.15.

45: ^{1}HNMR (300 MHz, CD_3Cl vs TMS): δ 0.93 (3H, t, J = 7.34 Hz), 1.38 (2H, p, J = 7.2 Hz), 1.63(2H, p, J = 7.2 Hz), 4.00 (2H, t, J = 7.8 Hz), 5.21 (2H, s), 7.38 (5H, m), 9.18 (1H, br, D2O exchanged), 11.86 (1H, br, D2O exchanged) ppm; ^{13}CNMR (75 MHZ, CD_3OD): δ 13.83, 19.96, 29.54, 42.93, 68.42, 128.53, 128.59 (2C) 128.69 (2C), 135.74, 146.26, 148.96, 152.91, 163.18 ppm; IR (NaCl, film): 3216, 3106, 2960, 1730, 1654, 1608, 1464, 1379, 1318, 1242, 1169, 1083, 754 cm^{-1};

HRMS: Calcd. for ($C_{15}H_{18}N_4O_4$ + H) = 319.1406. Found (M + H) = 319.1408.



43a 44a

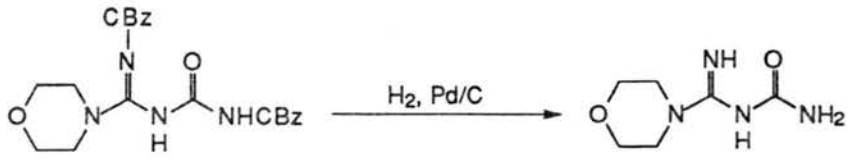
(Piperidino)iminomethylurea 44a.

To a solution of **43a** (279 mg, 0.64 mmol, 1.0 eq) in MeOH (3 mL) was added 20% $Pd(OH)_2/C$ (100 mg). The reaction vessel was charged with H_2 and the mixture was hydrogenated at 60 psi for 24h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give 105 mg (96% yield) of product **44a** as a semi-solid.

^{1}HNMR (300 MHz, CD_3OD): δ 1.70 (6H, m), 3.53 (4H, m) ppm; ^{13}CNMR (75 MHz, CD_3OD): δ 24.9, 26.5, 47.8, 156.4, 160.1 ppm;

IR (NaCl, film): 3296, 3161, 2941, 2858, 1730, 1654, 1608, 1541, 1457, 1342, 1298, 1077 cm^{-1};

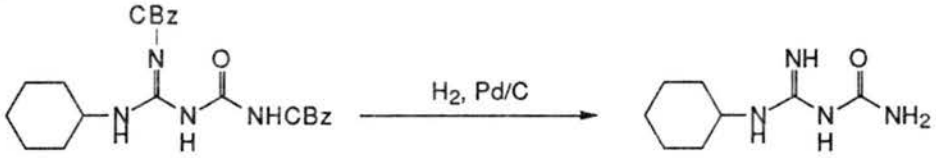
mp 128-131 °C (recryst. CH_3OH); Lit.[11] mp = 131-132 °C



43b 44b

(Morpholino)iminomethylurea 44b.

To a solution of **43b** (277 mg, 0.63 mmol, 1.0 eq.) in MeOH (3 mL)/ THF (6 mL) was added 20% $Pd(OH)_2/C$ (30 mg). The reaction vessel was charged with H_2 and the mixture was hydrogenated at 60 psi for 24h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give 105 mg (97% yield) of product **44b** as a semi-solid.
^{1}HNMR (300 MHz, CD_3OD): δ 3.51 (4H, t., J = 5.0 Hz), 3.68 (4H, t., J = 5.1 Hz) ppm; ^{13}CNMR (75 MHz, CD_3OD) d 46.56, 67.58, 161.5, 168.7 ppm. IR (NaCl, film): 3459, 3359, 2966, 2865, 1644, 1600, 1566, 1513, 1397, 1369, 1273, 1117, 1002 cm^{-1}. Anal. Calcd. for $C_6H_{12}N_4O_2$: C, 41.85, H, 7.02, N, 32.54. Found: C, 41.75; H, 6.85; N, 32.23.



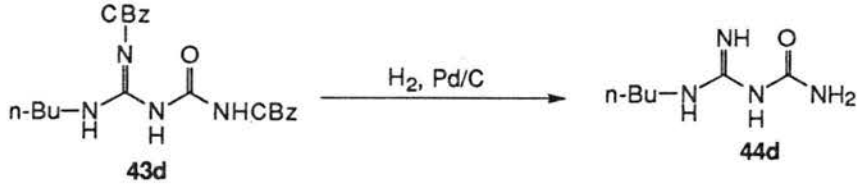
43c 44c

(Cyclohexylamino)iminomethylurea 44c.

To a solution of **43c** (180 mg, 0.40 mmol, 1.0 eq) in MeOH (2 mL) was added 20% $Pd(OH)_2/C$ (120 mg). The reaction vessel was charged with H_2 and the mixture was hydrogenated at 60 psi for 24h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give 68 mg (93% yield) of product **44c** as a semi-solid. [11]
^{1}HNMR (300 MHz, CD_3OD): δ 1.38 (5H, m), 1.62 (1H, m), 1.75 (2H, m), 1.93 (2H, m), 4.53 (1H, m) ppm. ^{13}CNMR (75 MHz, CD_3OD): δ 25.51, 26.24, 33.31, 51.22,

156.8, 171.2 ppm. IR (NaCl, film): 3326, 2934, 2858,1717, 1682, 1614, 1453, 1416, 1345, 1152, 1102, 1046 cm^{-1}.

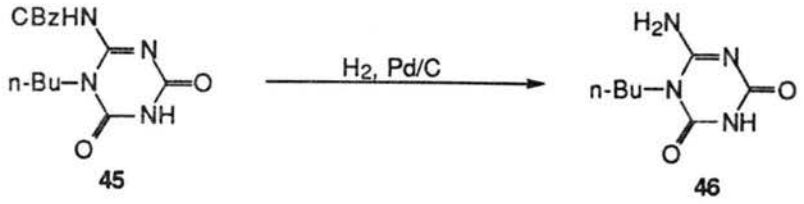
HRMS: calcd. for ($C_8H_{16}N_4O$ + H) = 185.1402. Found: (M + H) = 185.1408.



43d 44d

(Butylamino)iminomethylurea 44d.

To a solution of **43d** (71mg, 0.17 mmol, 1.0 eq) in MeOH (1.5 mL) was added 20% $Pd(OH)_2/C$ (50 mg). The reaction vessel was charged with H_2 and the mixture was hydrogenated at 60 psi for 24h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give 35 mg (99% yield) of product **44d** as a semi-solid. ^{1}HNMR (300 MHz, CD_3OD): δ 0.99 (3H, t, J = 7.35Hz), 1.44 (2H, m), 1.64 (2H, m), 3.30 (2H, t, J = 6.9 Hz) ppm; ^{13}CNMR (75 MHz, CD_3OD): δ 14.31, 21.23, 32.62, 41.78, 161.9, 170.8 ppm; IR (NaCl, film): 3339, 2960, 2862, 1610, 1558, 1394, 1140 cm^{-1}.



Triazinedione 46:

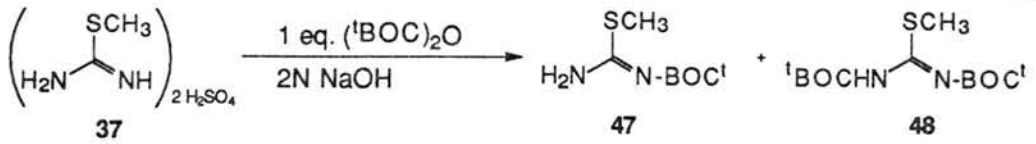
To a solution of **45** (100 mg, 0.31 mmol, 1.0 eq) in MeOH (2 mL)/THF (2 mL) was added 20% $Pd(OH)_2/C$ (50 mg). The reaction vessel was charged with H_2 and the mixture was hydrogenated at 60 psi for 24h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give 56 mg (98% yield) of product **46** as a white solid.

^{1}HNMR (300 MHz, CD_3OD vs TMS): δ 0.99 (3H, t, J = 7.50 Hz), 1.41 (2H, m), 1.64(2H, m), 3.87 (2H, m) ppm;

^{13}CNMR (75 MHz, CD_3OD): δ 16.93, 19.13, 29.16, 41.25, 150.20, 153.00, 156.40 ppm;

IR (NaCl, film): 3353, 3186, 3089, 2957, 1744, 1644, 1548, 1514, 1416, 1023 cm^{-1};

HRMS: calcd. for ($C_7H_{12}N_4O_2$ + H) = 185.1039. Found (M+ H) = 185.1041.

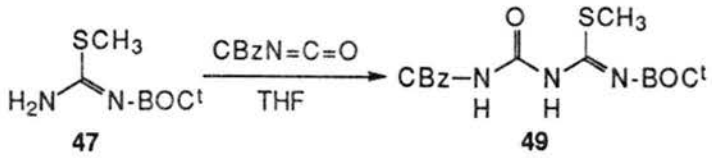


37 47 48

mono-t-BOC-S-methylisothiourea 47. [14a]

To a stirring mixture of S-methylisothiourea semisulfate **37** (5.57 g, 40 mmole, 1.0 eq) in CH_2Cl_2 (60 mL) / 2N NaOH (20 mL) was added a solution of Di-*tert*-butyl dicarbonate (8.73 g, 40 mmol, 1.0 eq) in CH_2Cl_2 (10 mL) dropwise at 0 °C over 1 h period. The mixture was stirred overnight at room temperature. The organic layer was separated and washed with brine twice, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by column chromatography on silica gel (CH_2Cl_2:ethyl acetate 80:20) to afford 5.48 g (72 %) of **47** as a white solid.

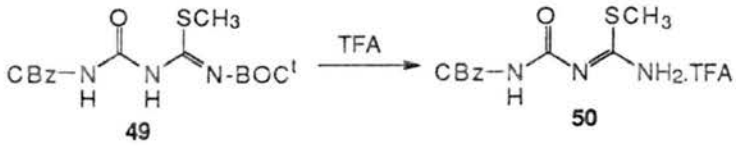
^{1}NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.50(9H, s); 2.46(3H, s)ppm.



5-Methylthio-3,7-dioxo-1-phenyl-2-oxa-4,6,8-triazanon-4-en-9-oic acid, t-butyl ester 49. To a solution of **37** (1.69 g, 8.91 mmol, 1.0 eq) in THF (40 mL) was added CBZ-N=C=O (1.90g, 10.73 mmol, 1.2 eq). The resulting mixture was stirred for 10 min at room temperature and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:4:0.5) provided 3.25 g (100%) of **49** as a white solid.

^1H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.48 (9H, s); 2.32(3H, s); 5.22(2H, s); 7.37(5H, m); 7.50(1H, br, D_2O exchanged); 11.96(1H, br, D_2O exchanged)ppm.

IR (NaCl, film): 3270, 2979, 1747, 1664, 1570, 1476, 1370, 1275, 1207, 1138, 1072, 1056cm^{-1}. mp: 120-3 °C; Anal. Calcd. for $C_{16}H_{21}N_3O_5S$: C, 52.30 H, 5.76 N, 11.44. Found: C, 52.15; H, 5.73 N, 11.38.

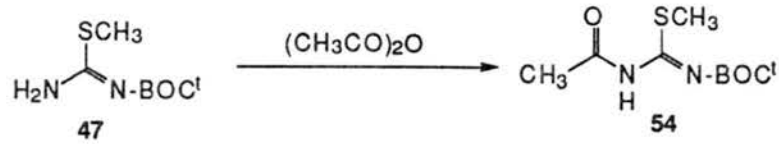


N'-CBzureido-S-methylisothiourea 50.

The **49** (401 mg, 1.0 mmol) was treated with TFA (1.0 mL). The resulting mixture was stirred for 30 min at room temperature, evaporated to dryness and put on vacume line for 2 h, triturated with anhydrous Et_2O to give 400 mg of **50** as a solid. This crude product was carried on without further purification.

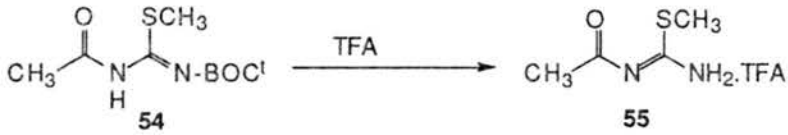
^1H NMR (300 MHz, CD_3OD): δ 2.64(3H, m); 5.21(2H, m); 7.31(5H, m)ppm.

IR (NaCl, CH_2Cl_2): 3252, 2959, 1790, 1682, 1203, 1137, 1025, 778, 722cm^{-1}



N-Acetyl-N'-butyloxycarbonyl-S-methylisothiourea 54.

To a solution of **47** (350 mg, 1.84 mmol, 1.0 eq) in CH_2Cl_2 (5.0 mL) was added acetic anhydride (190 ul/207 mg, 1.1 mmol, 1.1 eq) and TEA (388 uL, 2.76 mmol, 1.5 eq). The mixture was stirred for 16 h at room temperature. The resulting mixture was diluted with CH_2Cl_2 (50 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 9:1) provided 372 mg (87%) of **54** as a semi-solid. ^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 1.53(9H, s); 2.21(3H, s); 2.39(3H, s); 12.45(1H, br, D_2O exchanged)ppm. IR (NaCl, film): 3090, 2986, 2926, 1726, 1650, 1584, 1406, 1291, 1234, 1151cm^{-1}. Anal. Calcd. for $C_9H_{16}N_2O_3S$: C, 45.56; H, 6.94; N, 12.06. Found: C, 46.41; H, 6.99; N, 12.23.

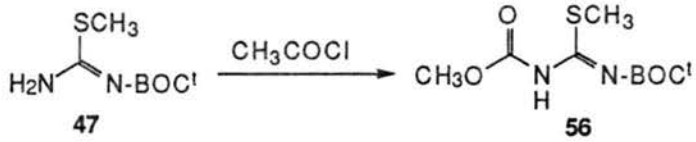


N-Acetyl-S-methylisothiourea 55.

The **54** (133 mg, 0.57 mmol) was treated with TFA (1.0 mL). The resulting mixture was stirred for 30 min at room temperature, evaporated to dryness and put on vacume line for 2 h, triturated with anhydrous Et_2O to give 130 mg of **55** as a semi-solid. This crude product was carried on without further purification.

^{1}HNMR (300 MHz, CD_3OD): δ 2.24(3H, s); 2.71(3H, s)ppm.

IR (NaCl, film): 3265, 2885, 1740, 1650, 1435, 1242, 1188cm^{-1}.



47 56

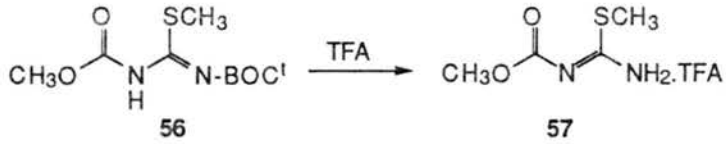
N-Methylcarbonyl-N'-butyloxycarbonyl-S-methylisothiourea 56.

To a solution of **47** (190 mg, 1.0 mmol, 1.0 eq) in CH_2Cl_2 (5.0 mL) was added methyl chloroformate (170 uL/208 mg, 2.2 mmol, 2.2 eq) and TEA (842 uL, 6.0 mmol, 6.0 eq). The mixture was stirred for 16 hours at room temperature. The resulting mixture was diluted with CH_2Cl_2 (50 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 4:1) provided 150 mg (60%) of **56** as an oil.

^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 1.50(9H, s); 2.41(3H, s); 3.79(3H, s); 11.59(1H, br, D_2O exchanged)ppm.

IR (NaCl, film): 3466, 3187, 1981, 1748, 1659, 1651, 1574, 1416, 1254, 1145cm^{-1}.

HRMS: Anal. Calcd. for ($C_9H_{16}N_2O_4S$ + H) = 249.0926. Found: (M + 1) = 249.0916.



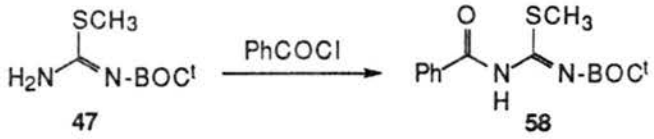
56 57

N-Methylcarbonyl-S-methylisothiourea 57.

To a mixture of **56** (140 mg, 0.55 mmol.) and anisole (0.1 mL) was added TFA (1.0 mL). The resulting mixture was stirred for 30 min at room temperature, evaporated to dryness and put on vacume line for 2 hours, triturated with anhydrous Et_2O to give 140 mg of product as a semi-solid. This crude **57** was carried on without further purification.

^{1}HNMR (300 MHz, CD_3OD): δ 2.70(3H, s); 3.88(3H, s)ppm.

IR (NaCl, film): 3387, 3283, 3012, 2930, 1672, 1589, 1505, 1433, 1257, 1112cm^{-1}.



47 58

N-Benzoyl-N'-butyloxycarbonyl-S-methylisothiourea 58.

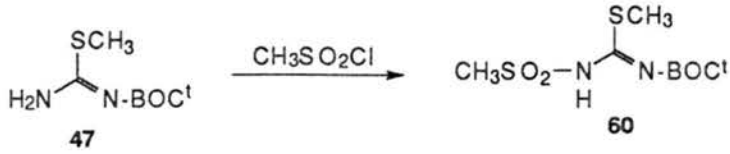
To a solution of **47** (190 mg, 1.0 mmol, 1.0 eq) in CH_2Cl_2 (5.0 mL) was added PhCOCl (128 uL/154 mg, 2.0 mmol, 2.0 eq) and TEA (308 uL, 2.2 mmol, 2.2 eq). The mixture was stirred for 16 h at room temperature. The resulting mixture was diluted with CH_2Cl_2 (50 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 4:1) provided 200 mg (68%) of **58** as a white solid. ^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 1.45(9H, s); 2.50(3H, s); 7.39(3H, m); 8.09(2H, m); 12.49(1H, br, D_2O exchanged)ppm. IR (NaCl, film): 3067, 2980, 2929, 1746, 1612, 1538, 1392, 1312, 1280, 1141cm^{-1}. mp: 99-101°C. HRMS: Anal. Calcd. for ($C_{14}H_{18}N_2O_3S$ + H) = 295.1133. Found: (M + 1) = 295.1110.

58 59

N-Benzoyl-S-methylisothiourea 59.

To a mixture of **58** (160 mg, 0.54 mmol.) and anisole (0.1 mL) was added TFA (1.0 mL). The resulting mixture was stirred for 30 min at room temperature, evaporated to dryness and put on vacume line for 2 hours, triturated with anhydrous Et_2O to give 15 mg of **59** as a semi-solid. This crude product was used directly in next step without further purification.

^{1}HNMR (300 MHz, CD_3OD): δ 2.74(3H, m); 7.55(2H, t, J = 7.8 Hz); 7.69(1H, t, J = 7.5 Hz); 8.02(2H, d, J = 7.5 Hz)ppm. IR (NaCl, film): 3226, 2936, 1695, 1680, 1540, 1188cm^{-1}.

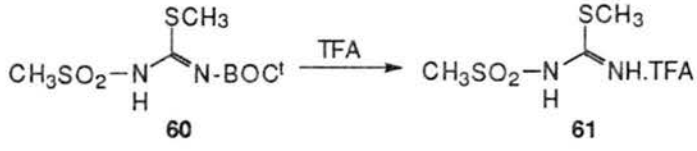


N-Methylsulfonyl-N'-butyloxycarbonyl-S-methylisothiourea 60.

To a solution of **47** (380 mg, 2.0 mmol, 1.0 eq) in CH_2Cl_2 (5.0 mL) was added CH_3SO_2Cl (310 ul/458 mg, 2.0 mmol, 2.0 eq) and TEA (842 uL, 6.0 mmol, 3.0 eq). The mixture was stirred for 2 hours at room temperature. The resulting mixture was diluted with CH_2Cl_2 (50 mL), washed with brine, dried (Na_2SO_4), filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 4:1) provided 500 mg (93%) of **60** as a yellow oil.

^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 1.50(9H, s); 2.34(3H, s); 3.09(3H, s); 10.05(1H, br, D_2O exchanged)ppm. IR (NaCl, film): 3242, 2981, 2934, 1752, 1572, 1455, 1371, 1298, 1234, 1154, 1115, 1066, 966, 850, 768cm^{-1}.

HRMS: Anal. Calcd. for ($C_8H_{16}N_2O_4S_2$ + H) = 269.0663. Found: (M + 1) = 269.0623

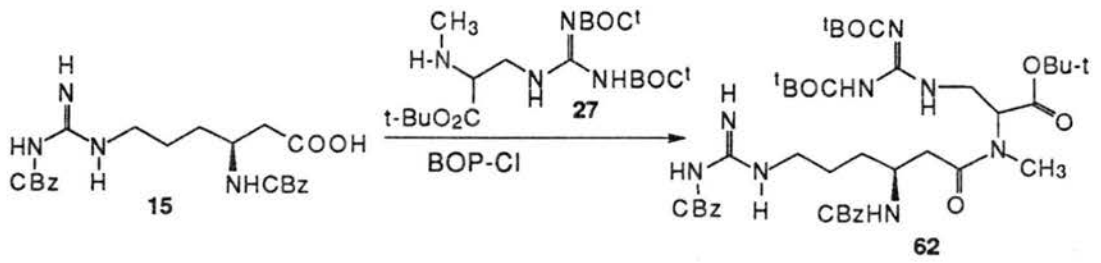


N-Methylsulfonyl-S-methylisothiourea 61.

To a mixture of **60** (300 mg, 1.12 mmol) and anisole (0.1 mL) was added TFA (1.0 mL). The resulting mixture was stirred for 30 min at room temperature, evaporated to dryness and put on vacuum line for 2 hours, triturated with anhydrous Et_2O to give 300 mg of **61** as a semi-solid. This crude product was carried on without further purification.

^{1}HNMR (300 MHz, CD_3OD): δ 2.42(3H, s); 3.05(3H, s)ppm.

IR (NaCl, film): 3405, 3306, 3018, 2925, 1622, 1540, 1340, 1266, 1119cm^{-1}.



Peptide 62.

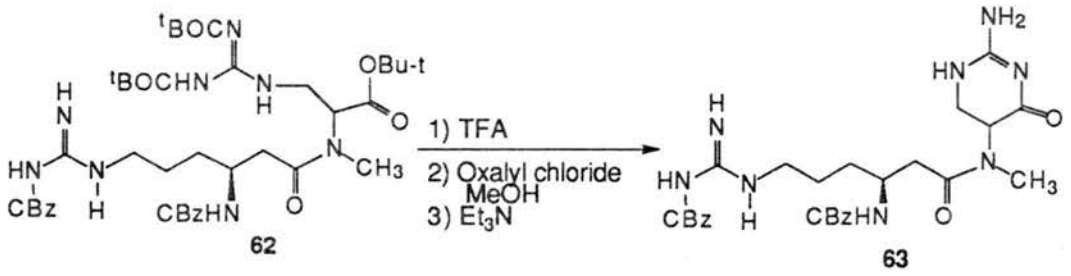
To a mixture of the acid **15** (400 mg, 0.88 mmol, 1.0 eq) and NMM (106 ul, 0.97 mmol, 1.1 eq) in CH_2Cl_2 (2 mL.) was added BOP-Cl (268 mg, 1.05 mmol, 1.2 eq) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C. Then, to the resulting mixture was added amine (450 mg, 0.88 mmol, 1.0 eq) in CH_2Cl_2 (2 mL). The mixture was stirred overnight at room temperature, diluted with CH_2Cl_2 (200 mL.), washed with brine, dried over anhydrous Na_2SO_4 and concentrated. Purification via column chromatography (silica gel, methylene chloride:EtOAc: MeOH, 5:4:0.2) provided 302 mg (40%) of **62** as an oil.

^1H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.45(27H, m); 1.55(2H, m); 1.67(2H, m); 2.41(2H, m); 3.00/3.01(3H, s); 3.30(1H, m); 3.66(1H, m); 3.81(1H, m); 3.91(1H, m); 4.05(1H, m); 4.70(1H, m); 5.06(2H, s); 5.11(2H, s); 6.00(1H, br, D_2O exchanged); 6.09(1H, br, D_2O exchanged); 6.65(1H, br, D_2O exchanged); 7.32(10H, m); 7.95(1H, br, D_2O exchanged); 8.52(1H, br, D_2O exchanged); 11.39(1H, br, D_2O exchanged)ppm.

IR (NaCl, film): 3327, 2978, 2935, 1724, 1641, 1619, 1413, 1368, 1289, 1140, 1057 cm^{-1};

$[\alpha]_D^{25}$ = -16.4 (c 1.0, CH_2Cl_2);

HRMS(FAB): Anal. Calcd. for ($C_{42}H_{62}N_8O_{11}$ + H) = 855.4616. Found: (M + H) = 855.4547.



Cyclization product 63.

To a solution of **62** (156 mg, 0.18 mmol, 1.0 eq) in methylene chloride (2.0 mL) was added TFA (1.5 mL) at 0 °C. The mixture was stirred overnight at room temperature, concentrated and triturated in dry ether to give 118 mg (92%) of crude product as an oil.

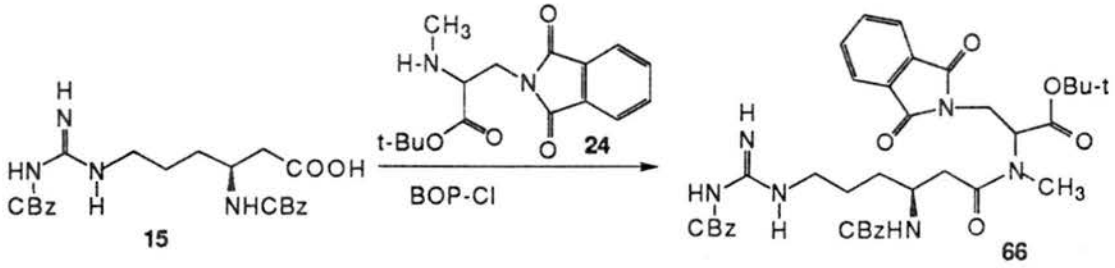
To a solution of crude product obtained above (83 mg, 0.38mmole) in MeOH (2 mL) was added oxalyl chloride (400 ul) at room temperature. The mixture was stirred overnight at room temperature. It was then concentrated and dried on vacuo to give white solid. This crude product was dissolved in MeOH(1.0 mL) and to this solution was added Et_3N (0.2 mL). The resulting mixture was stirred overnight at room temperature, concentrated to give solid residue, triturated in anhydrous THF and methylene chloride to give 53 mg (66%) of **63** as a solid.

^1H NMR (300 MHz, CD_3OD) 1.61(4H, m); 2.50(1H, m); 2.62 (1H, m); 2.92/2.96 (3H, s); 3.19 (2H, m); 3.32 (1H, m); 3.60 (2H, m); 3.99 (1H, m); 5.05 (4H, s); 7.32 (10H, m).

IR (NaCl, film): 3336, 3184, 2947, 1697, 1645, 1621, 1531, 1517, 1455, 1403, 1285,1118cm^{-1}.

$[\alpha]_D^{25}$ = - 3.4 (c 1.3, CH_3OH);

HRMS(FAB): Calcd. for ($C_{28}H_{37}N_8O_6$ + H) = 581.2836. Found: (M + H) = 581.2833.



Peptide 66.

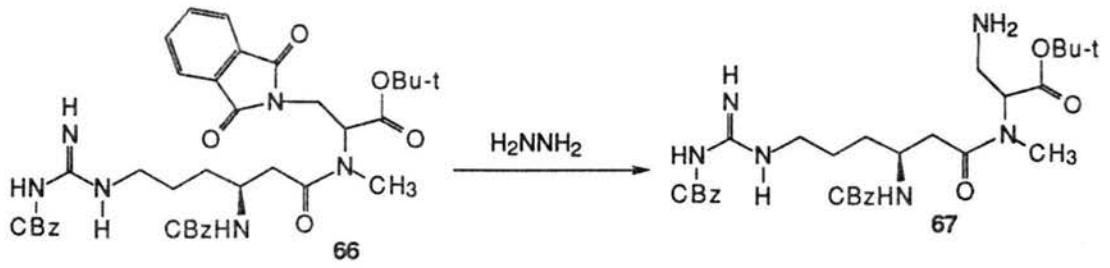
To a mixture of the acid **315** (540 mg, 1.19 mmol, 1.0 eq) and NMM (200 uL, 1.43 mmol, 1.2 eq) in CH_2Cl_2 (2 mL) was added BOP-Cl (456 mg, 1.82 mmol, 1.2 eq) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C. Then, to the resulting mixture was added amine **24** (360 mg, 1.19 mmol, 1.0 eq) in CH_2Cl_2 (2 mL). The mixture was stirred overnight at room temperature, diluted with CH_2Cl_2 (200 mL.), washed with brine, dried over anhydrous Na_2SO_4 and concentrated. Purification via column chromatography (silica gel, methylene chloride:EtOAc: MeOH, 5:4:0.2) provided 650 mg (74%) of **66** as an oil.

^{1}H NMR (300 MHz, DMSO-d6, 393K vs TMS): δ 1.43 (13H, m); 2.38 (2H, m); 2.93 (3H, s); 3.05 (1H, m); 3.15 (1H, m), 3.77 (1H, m); 4.04 (2H, m); 5.00 (2H, s); 5.03 (2H, s); 5.15 (1H, m); 7.32 (10H, m); 7.82 (4H, m)ppm.

IR (NaCl, film): 3396, 2939, 1774, 1716, 1636, 1395, 1287, 1155, 1065 cm^{-1};

$[\alpha]_D^{25}$ = - 17.5 (c 1.65, CH_2Cl_2);

HRMS(FAB): Calcd. for ($C_{39}H_{46}N_6O_9$ + H) = 743.3405. Found: (M + H) = 743.3439.



Amine 67.

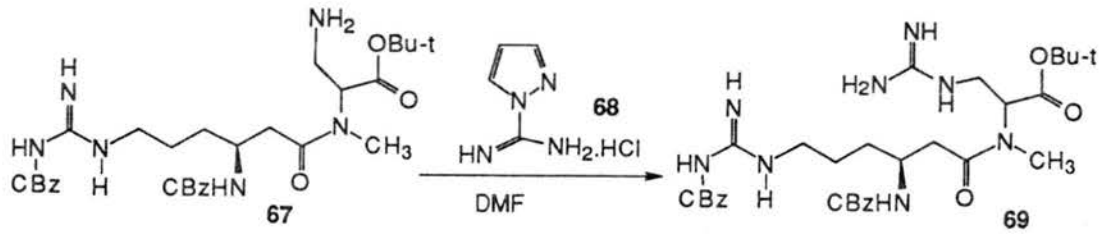
To a solution of **66** (700 mg, 0.94 mmol, 1.0 eq) in MeOH (20 mL) was added hydrazine (0.4 mL, 12.5 mmol, 13 eq) at 0 °C. The mixture was stirred overnight at room temperature, concentrated and treated with ethyl acetate (200mL) and sat. $NaHCO_3$ (50 mL). The organic phase was separated, washes with brine, dried over anhydrous $MgSO_4$, filtered and concentrated. Purification via column chromatography (silica gel, methylene chloride:MeOH, 10:1-10:4) provided 300 mg (52%) of **67** as an oil.

^1H NMR (300 MHz, $CDCl_3/D_2O$ vs TMS): 1.43 (9H, s); 1.51 (4H, m); 2.56 (2H, m); 2.84 (1H, m); 2.92 (3H, s); 3.06 (1H, m); 3.18 (2H, m); 3.67 (1H, m); 4.85 (1H, m); 5.06 (4H, s); 7.32 (10H, m)ppm.

IR (NaCl, film): 3374, 3310, 2933, 1718, 1636, 1595, 1285, 1149, 1061cm^{-1};

$[\alpha]_D^{25}$ = - 19.0 (c 0.7, CH_2Cl_2);

HRMS(FAB): Calcd. for ($C_{31}H_{44}N_6O_7$ + H) = 613.3350. Found: (M + 1) = 613.3366



Guanidine 69.

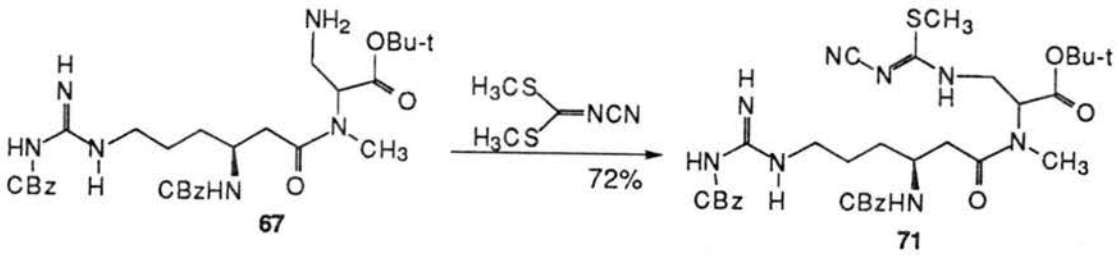
To a solution of **67** (260mg, 0.42 mmol, 1.0 eq) and DIEA (81 uL, 0.46 mmol, 1.0 eq) in DMF (1.5 mL) was added 1H-pyrazole-1-carboxamidine hydrochloride **68** (81mg, 0.55 mmol, 1.1 eq). The mixture was stirred for 20 h at room temperature. The solvent was evaporated in vacuo to give an oil, triturated in anhydrous ethyl ether to afford product as white solid, separated on PTLC (silica plate, eluted with CH_3CN:water:ethyl acetate, 3:1:1) to give 126 mg (46%) of **69** as a semi-solid.

^1H NMR (300 MHz, CD_3OD): δ 1.45(9H, s); 1.65 (4H, m); 2.62 (2H, m); 3.04/3.06(3H, s); 3.23 (2H, m); 3.47 (1H, m); 3.68 (1H, m); 3.99 (1H, m); 4.53 (1/2H, t, J = 6.15 Hz); 4.77(1/2H, t, J = 6.15 Hz); 5.07 (2H, s); 5.11(2H, s); 7.33 (10H, m)ppm.

IR (NaCl, film): 3156, 2936, 1734, 1651, 1539, 1281, 1155cm^{-1};

$[\alpha]_D^{25}$ = - 10.0 (c 1.0, CH_2Cl_2);

HRMS(FAB): Calcd. for ($C_{32}H_{46}N_8O_7$ + H) = 655.3568. Found: (M + 1) = 655.3568.



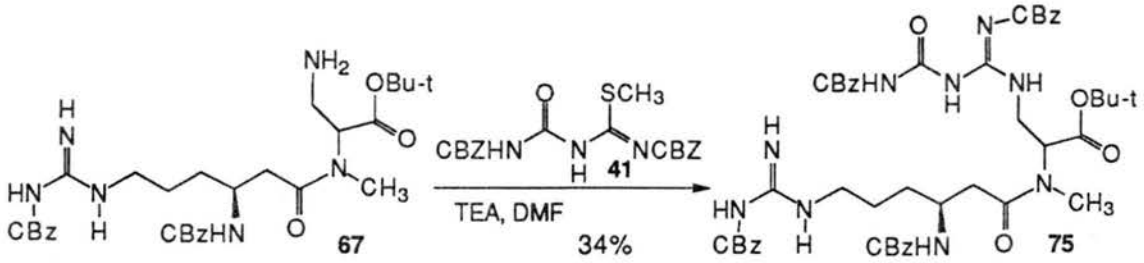
N-cyano-S-methylisothiourea 71.

The mixture of **67** (53 mg, 0.086 mmol, 1.0 eq) and dimethyl N-cyanodithioiminocarbonate (14 mg, 0.086 mmol, 1.0 eq) in EtOH (0.5 mL) was stirred for 24 h at room temperature. The resulting mixture was concentrated and separated on column chromatography (silica gel, methylene chloride: MeOH, 9:1) to give 45 mg (72%) of product **71** as an oil.

^{1}H NMR (300 MHz, $CDCl_3/D_2O$ vs TMS): δ 1.43 (9H, s); 1.59 (4H, m); 2.42/2.44 (3H, s); 2.52 (2H, m); 2.98 (3H, s); 3.23 (2H, m); 3.75 (2H, m); 3.97 (1H, m); 4.43 (1H, m); 5.05 (2H, s); 5.07 (2H, s); 7.32 (10H, m)ppm.

IR (NaCl, film): 3313, 2934, 2180, 1723, 1702, 1634, 1557, 1435, 1286, 1155, 1027, 737 cm^{-1}.$[\alpha]_D^{25}$ = + 4.6 (c 0.65, CH_2Cl_2);

HRMS(FAB): Calcd. for ($C_{34}H_{46}N_8O_7S$ + H) = 711.3288. Found: (M + 1) = 711.3283.



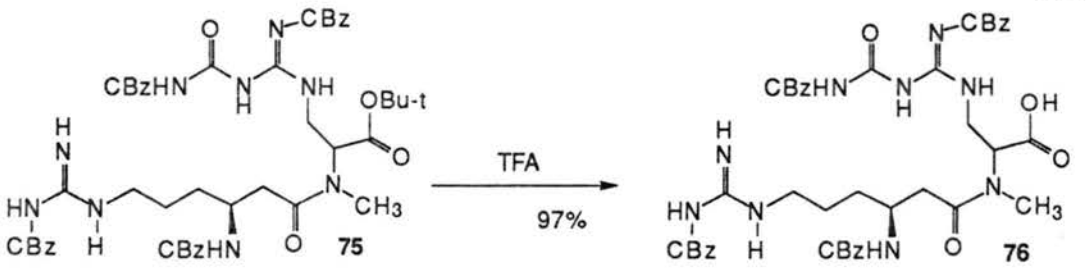
Condensation Product 75.

To a mixture of **41** (315 mg, 0.79 mmol, 1.5 eq) in DMF (5 mL) was added **67** (320 mg, 0.52 mmol, 1.0 eq) and TEA (73 uL, 0.52 mmol, 1.0 eq). The resulting mixture was stirred for 4 h at room temperature. The reaction mixture was concentrated, and dried on vacuo to give a white solid. The crude product was triturated in EtOAc and the solid was filtered off. The filtrate was concentrated and separated via column chromatography (silica gel, CH_2Cl_2:ethyl acetate: MeOH, 75:20:5) to afford 167 mg (33.8 %) of **75** as a semi-solid.

^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 1.44 (9H, m); 1.56 (4H, m), 2.56 (1H, m); 2.75 (1H, m); 2.91/2.94 (3H, s), 3.12 (2H, m); 4.11(3H, m); 5.11 (9H, m); 7.31 (20H, m) ppm;

IR (NaCl, film): 3307, 2946, 1715, 1629, 1449, 1373, 1293, 1218, 1152, 1097, 731, 695cm^{-1}; $[\alpha]_D^{25}$ = - 20.9 (c 0.65, CH_2Cl_2);

HRMS: Calcd. for ($C_{49}H_{59}N_9O_{12}$ + H) = 966.4361. Found (M + H) = 966.4361.



Acid 76.

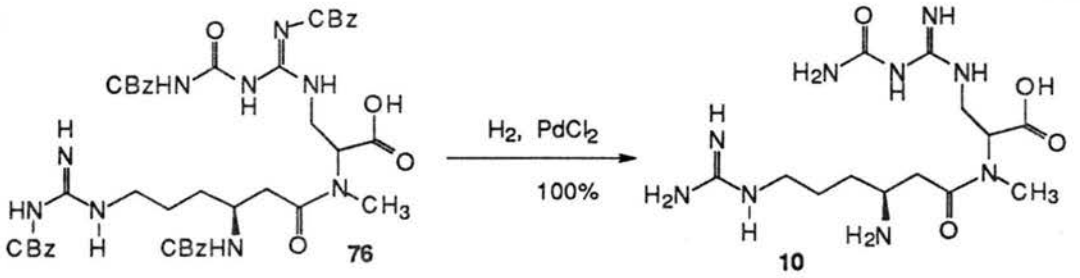
To a mixture of **75** (167 mg, 0.18 mmol, 1.0 eq) and anisole (0.1 mL) was added TFA (3.0 mL) at 0 °C. The mixture was stirred for 4 h at room temperature, concentrated and dried on vacuo. Trituration in dry ethyl ether provided 153 mg (97%) of **76** as an amorphours powder.

^{1}H NMR (300 MHz, CD_3COOD vs TMS): δ 1.64 (4H, m); 2.70 (2H, m); 3.15 (3H, m); 3.30 (2H, m); 3.96 (3H, m); 5.13 (9H, m); 7.32 (20H, m)ppm.

IR (NaCl, film): 3310, 2934, 1734, 1700, 1636, 1494, 1454, 1383, 1260, 1187, 1097, 695 cm^{-1};

$[\alpha]_D^{25}$ = - 10.22 (c 1.8, CH_2Cl_2);

Anal. Calcd. for $C_{45}H_{51}N_9O_{12}H_2O$: C, 58.25; H, 5.76; N, 13.58.Found: C, 58.25; H, 5.76; N, 13.71.



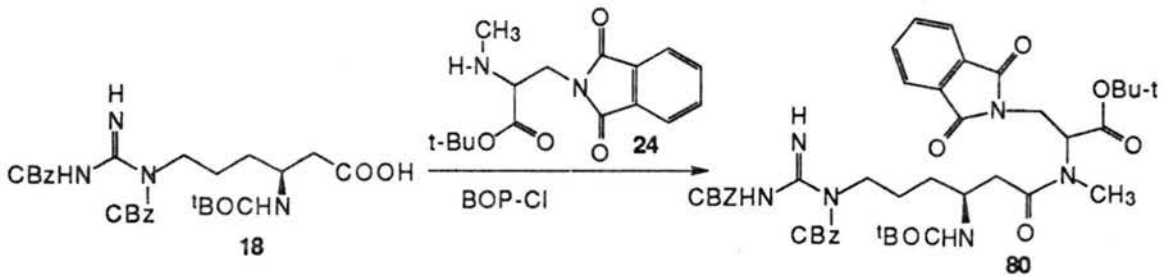
To a solution of **76** (110 mg, 0.123 mmol, 1.0 eq) in MeOH (2 mL)/ THF (2 mL) was added 20% $Pd(OH)_2/C$ (100 mg). The reaction vessel was charged with H_2 and the mixture was hydrogenated at 60 psi for 24 h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **10** (46 mg, 100% yield) as an amorphous solid.

^{1}HNMR (300 MHz, D_2O): δ 1.57(4H, m); 2.66(1H, m); 2.79(1H, m); 2.88/2.89(3H, s), 3.05(2H, t, J = 5.7 Hz); 3.53(2H, m); 3.73(1H, m); 4.79(1H, m)ppm;

IR (KBr, pellet): 3350, 3179, 2955, 1696, 1622, 1395, 1205, 1135cm^{-1};

$[\alpha]_D^{25}$ = + 0.26 (c 0.35, H_2O);

MS (ES^+): Cacld. ($C_{13}H_{28}N_9O_4$ + H) = 374.2, Found (M + H) = 374.2; (M- $CONH_2$) = 331.2.



Peptide 80.

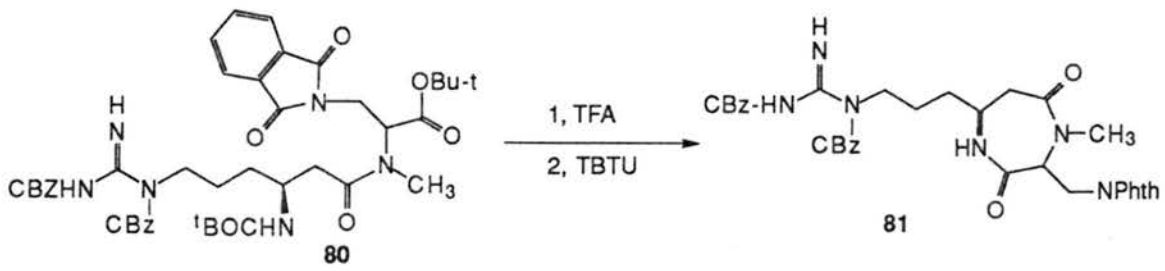
To a mixture of the acid **18** (1.0 g, 1.79 mmol, 1.0 eq) and NMM (255 uL, 2.33 mmol, 1.3 eq) in CH_2Cl_2 (2 mL) was added BOP-Cl (593 mg, 2.33 mmol, 1.3 eq) at 0 °C, and amine **24** (708 mg, 2.33 mmol, 1.3 eq) in CH_2Cl_2 (3 mL) at 10 min later. The resulting mixture was stirred overnight, diluted with CH_2Cl_2 (200 mL), washed with brine, dried over anhydrous Na_2SO_4, and concentrated. Purification via column chromatography (silica gel, methylene chloride:EtOAc, 8:2) provided 388 mg (56%) of **80** as an oil.

^{1}H NMR (300 MHz, CD_3OD): δ 1.16(2H, m); 1.35(9H, s); 1.43(2H, m); 1.45(9H, s); 2.10-2.55(2H, m); 2.87/2.91(3H, s); 3.72(3H, m); 4.08(1H, m); 5.10(2H, s); 5.17(2H, m); 5.24(2H, m); 7.38(10H, m); 7.67(2H, m); 7.74(2H, m)ppm.

IR (NaCl, film): 3390, 2974, 1716, 1647, 1609, 1507, 1368, 1252, 1169, 1099, 1006, 719, 696cm-^{1};

$[\alpha]_D^{25}$ = - 6.2 (c 1.2, CH_2Cl_2);

Anal. Calcd. for $C_{44}H_{54}N_6O_{11}$: C, 62.70; H, 6.46; N, 9.97. Found: C, 62.60; H, 6.31; N, 9.79.



Cyclic Peptide 81.

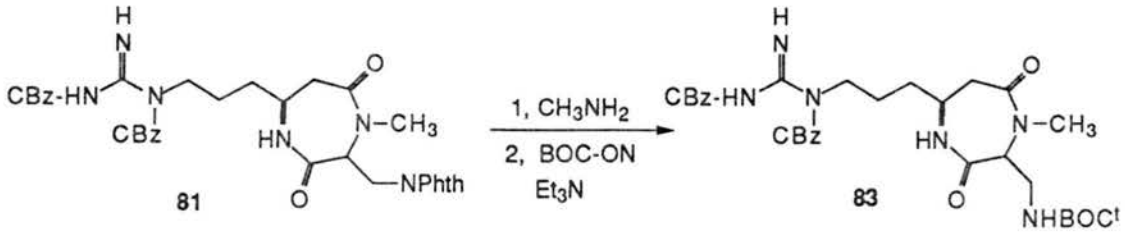
The **80** (179 mg, 0.212 mmol, 1.0 eq) was treated with anisole (0.1 mL) and TFA (2.0 mL) at 0 °C. The mixture was stirred for 2 hours at room temperature, concentrated and triturated in dry ether to give 140 mg of product as a white solid. This solid was taken into CH_2Cl_2(350 mL). To this solution was added TBTU (84 mg, 0.26 mmol, 1.5 eq) and DIEA (92 uL, 0.51 mmol, 3.0 eq). The resulting mixture was stirred 48 h, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Separation on PTLC (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.1) provided 50 mg (43%) of **81** as a semi-solid.

^{1}H NMR (300 MHz, CD_3OD): δ 1.40(1H, m); 1.55(1H, m); 1.68(2H, m); 2.64(1H, m); 2.81/2.86(3H, s); 3.13(1H, m); 3.59(1H, m); 3.93(2H, m); 4.18(2H, m); 4.45(1H, m); 5.09/5.11(2H, s); 5.26/5.30(2H, s); 7.34(10H, m); 7.78(4H, m)ppm.

IR (NaCl, film): 3389, 3280, 2948, 1773, 1716, 1659, 1608, 1513, 1394, 1253, 1097, 1006, 910, 724cm-^{1};

$[\alpha]_D^{25}$ = - 18.4 (c 1.5, CH_2Cl_2);

HRMS(FAB): Calcd. for ($C_{35}H_{36}N_6O_8$ + H) = 669.2672. Found: (M + H) = 669.2690.



t-BOC Amine 83.

To a solution of **81** (100 mg, 0.15 mmol, 1.0 eq) in CH_2Cl_2 (2.0 mL) was added 2 N methylamine/MeOH (2.0 mL) at 0 °C. The mixture was stirred for 10 min at 0 °C, concentrated and separated on column chromatography (silica gel, methylene chloride:EtOAc:MeOH, 4:1:0.3) to give 84 mg of **82** as an oil.

HRMS(FAB): Calcd. for ($C_{36}H_{41}N_6O_8$ + H) = 700.3095. Found: (M + H) = 700.3112.

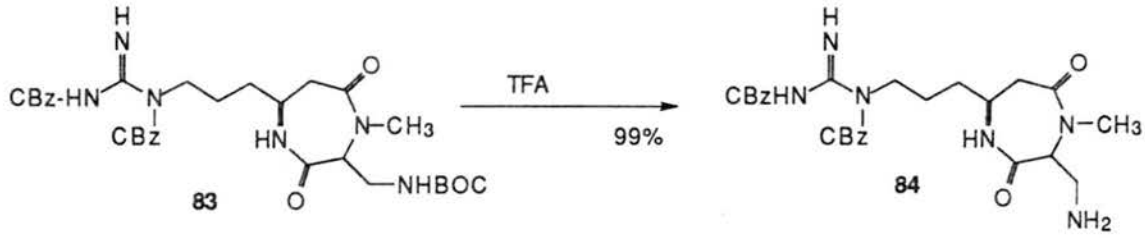
To a mixture of **82** in H_2O/dioxane(1 mL, 1:1) and Et_3N(217 uL, 1.5 mmol, 10 eq) was added BOC-on(106 mg, 0.45 mmol, 3.0 eq). The mixture was stirred overnight at room temperature and extracted with EtOAc (2x100 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated and purified on column chromatography (silica gel, eluted with CH_2Cl_2:MeOH, 9:1) to give 35 mg (38%) of **83** as a semi-solid.

^1H NMR (300 MHz, CD_3OD): δ 1.42(9H, s); 1.47(2H, m); 1.66(2H, m); 2.63(1H, m); 2.85(1/2H, m); 2.90/2.93(3H, s); 3.05(1/2H, m); 3.52(2H, m); 3.72(1H, m); 3.92(2H, m); 4.25(1H, m); 5.11(2H, s); 5.26/5.27(2H, s); 7.35(10H, m)ppm.

IR (NaCl, film): 3386, 3289, 2934, 1716, 1652, 1609, 1507, 1456, 1366, 1252, 1175, 1095, 1006, 910, 734, 698cm-^{1};

$[\alpha]_D^{25}$ = - 22.4 (c 1.1, CH_2Cl_2);

HRMS(FAB): Calcd. for ($C_{32}H_{42}N_6O_8$ + H) = 639.3142. Found: (M + H) = 639.3157.



Amine 84.

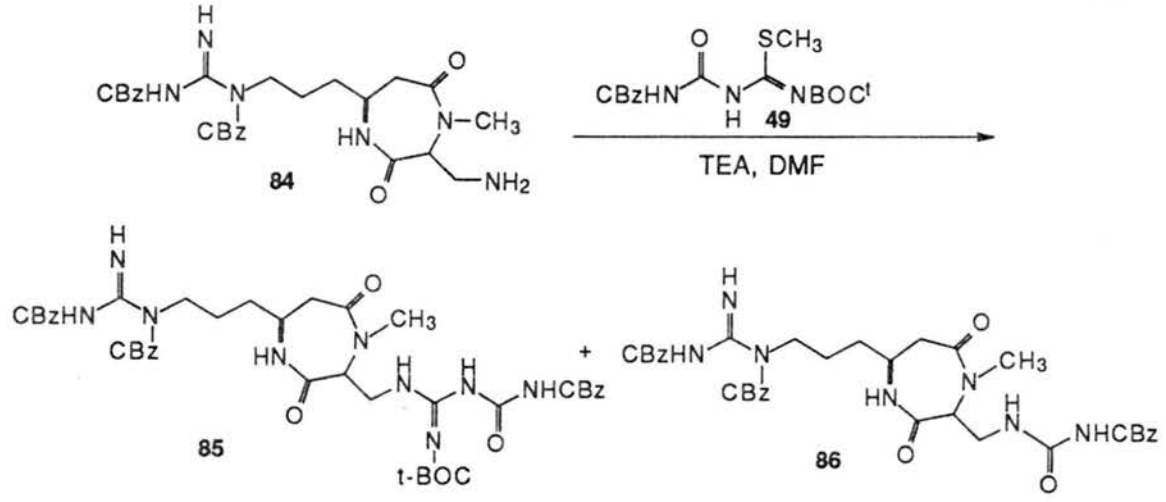
The **83** (33 mg, 0.052 mmol) was treated with anisole (0.1 mL) and TFA (1.0 mL) at 0 °C. The mixture was stirred for 1 h at room temperature, concentrated and triturated in dry ether to give 32 mg (99%) of **84** as an oil.

^{1}H NMR (300 MHz, CD_3OD): δ 1.49(2H, m); 1.69(2H, m); 2.60(1H, dd, J = 3.9, 6.6 Hz); 2.90(3H, s); 3.37(1H, dd, J = 3.0, 3.0 Hz); 3.35(1H, m); 3.59(2H, m); 3.88(2H, m); 4.61(1/2H, t, J = 7.2 Hz); 4.73(1/2H, t, J = 7.2 Hz); 5.20(2H, s); 5.29(2H, s); 7.37(10H, m)ppm.

IR (NaCl, film): 3387, 3274, 2926, 1718, 1652, 1610, 1498, 1456, 1380, 1254, 1207, 1108, 1005cm-^{1};

$[\alpha]_D^{25}$ = - 7.7 (c 1.1, CH_2Cl_2);

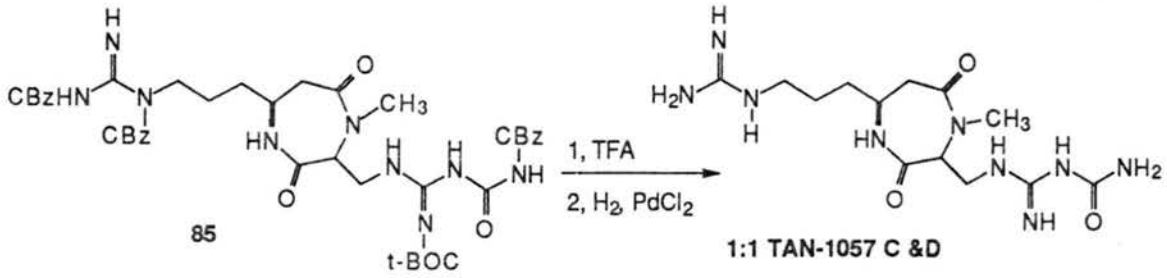
HRMS(FAB): Calcd. for ($C_{27}H_{34}N_6O_6$ + H) = 539.26205. Found: (M + H) = 539.2597.



Condensation Product 85.

The solution of **84** (30 mg, 0.046 mmol, 1.0 eq), triethylamine (20 uL, 0.14 mmol, 3.0 eq) and **49** (37 mg, 0.092 mmol, 2.0 eq) in CH_2Cl_2 (1.0 mL) was stirred 4 h at room temperature. The resulting mixture was poured into EtOAc, washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated and separated on PTLC (silica gel, CH_2Cl_2: EtOAc, 7:3) to give 12 mg (30%) of **85** as a semi-solid and 13 mg (39%) of **86** as an oil. ^1H NMR (300 MHz, CD_3OD): δ 1.42 (2H, m); 1.48 (9H, s); 1.67 (2H, m); 2.70 (1H, m); 2.83/2.89 (3H, s); 2.98 (1H, m); 3.62 (2H, m); 3.91 (3H, m); 4.67 (1H, m); 5.10 (2H, s); 5.14 (2H, s); 5.26 (2H, s); 7.35 (15H, m) ppm. IR (NaCl, film): 3384, 3293, 2932, 1716, 1722, 1651, 1491, 1254, 1144, 1008 cm^{-1}; $[\alpha]_D^{25}$ = - 28.2 (c 0.5, CH_2Cl_2); HRMS(FAB): Calcd for ($C_{42}H_{51}N_9O_{11}$ + H) = 858.3786. Found: (M + H) = 858.3763.

The by-product **86**: ^1H NMR (300 MHz, CD_3OD): δ 1.45(2H, m); 1.67(2H, m); 2.73(1H, m); 2.82(1H, m); 2.85/2.91(3H, s); 3.54(1H, m); 3.70(1H, m); 3.91(3H, m); 4.44(1H, t, J = 9 Hz); 5.10(2H, m); 5.14(2H, m); 5.27(2H, m); 7.35(15H, m)ppm. IR (NaCl, film): 3385, 3272, 2931, 1715, 1651, 1494, 1455, 1379, 1241, 1092cm^{-1}; $[\alpha]_D^{25}$ = - 25.6 (c 0.65, CH_2Cl_2); HRMS (FAB): Calcd. for ($C_{36}H_{41}N_7O_9$ + H) = 716.3044. Found: (M + H) = 716.3021.



[[[[5-[3-[(aminoiminomethyl)amino]propyl]hexahydro-(2S/R,5S)-1-methyl-3,7-dioxo-1H-1,4-diazepin-2-yl]methyl]amino]iminomethyl]urea

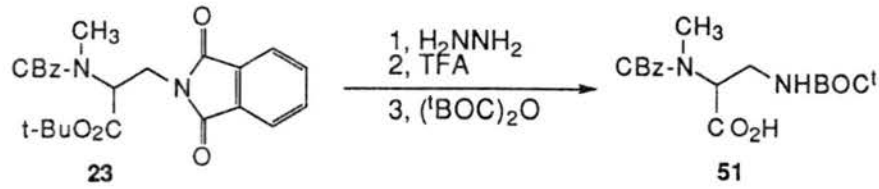
The **85** (10 mg, 0.012 mmol) was treated with anisole (0.1 mL) and TFA (1.0 mL) at 0 °C. The mixture was stirred for 1 h at room temperature, concentrated and purified on PTLC (silica gel, CH_2Cl_2:MeOH, 6:1) to give 6.0 mg of the intermediate as a semi-solid.

To a solution of the intermediate (6 mg, 0.0066 mmol, 1.0 eq) in MeOH (1.0 mL)/CH_2Cl_2 (0.5 mL) was added $PdCl_2$ (8.0 mg). After degassed with N_2, the reaction mixture was charged with H_2 (1 atm.) and the mixture was hydrogenated for 10 min. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **TAN-1057 C/D** (1:1, 3 mg, 100% yield) as an amorphous solid.

^{1}H NMR (300 MHz, DMSO-d6 vs TMS): δ 1.51(4H, m); 2.82(3H, s); 2.70-2.90(2H, m); 3.09(2H, m); 3.43(1H, m); 3.79(2H, m); 4.66(1/2H, m); 4.75(1/2H, m); 7.26(6H, m); 7.86(1H, br); 8.06(1H, br); 8.70(2H, br); 9.15(1H, br); 10.35(1H, br)ppm

MS(FAB): Calcd. for ($C_{13}H_{25}N_9O_3$ + H) = 356.2. Found: (M + H) = 356.3; $(M + 2H)^{++}$ = 178.7.

HPLC: Rt = 8.96, 13.01 min (3 mL/min); Lit. Rt = 11.5, 16.7 min (2 mL/min)



N^2-CBz-N^2-methyl-N^3-t-BOC-2,3-diaminopropionic acid 51:

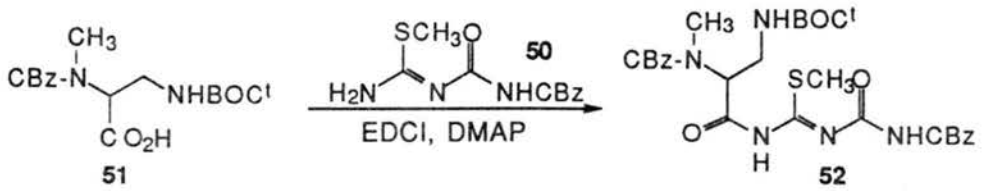
To a solution of **23** (1.35 g, 3.0 mmol, 1.0 eq) in MeOH (30 mL) was added hydrazine (480 uL, 15 mmol, 5.0 eq). The resulting mixture was stirred for 24 h, concentrated, and dried on *vacuo* to give a white solid. The white solid was treated with CH_2Cl_2/sat. $NaHCO_3$ (500 mL, 1:1). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated to give an oily residue. This crude amine was treated with TFA (10 mL) and stirred for 2 h. After evaporation of TFA, the residue was dissolved in H_2O/t-BuOH (50 mL, 1:1). To this solution were added $(t\text{-BOC})_2O$ (1.20 g, 5.56 mmol, 1.85 eq) and 2 N NaOH solution (20 mL). The resulting mixture was stirred for 16 h, diluted with water (200 mL) and extracted with Et_2O. The aqueous layer was acidified to pH 4 by 1N HCl solution and extracted with CH_2Cl_2 (2x30 mL). The CH_2Cl_2 extracts were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification via column chromatography (silica gel, 9:1 CH_2Cl_2:MeOH) provided 310 mg (30%) of **51** as an oil.

^1H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.42 (9H, s); 2.97(3H, s); 3.66(2H, m); 4.54(1H, t, J = 6.9 Hz); 4.96(1H, br, D_2O exchanged); 5.14(2H, s); 7.31(5H, m); 10.13(1H, br, D_2O exchanged)ppm.

IR (NaCl, film): 3333, 2977, 1699, 1521, 1456, 1366, 1251, 1168, 1026cm^{-1};

$[\alpha]_D^{25}$ = + 11.3 (c 1.1, CH_2Cl_2);

HRMS: Calcd. for ($C_{17}H_{24}N_2O_6$ + H) = 353.1713. Found: (M+ H) = 353.1707.



Coupling Product 52.

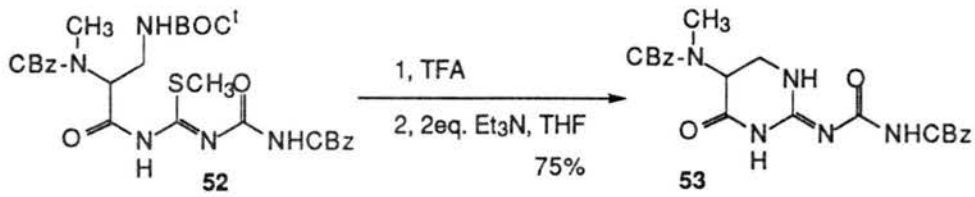
To a solution of **51** (246 mg, 0.7 mmol, 1.0 eq), DMAP (171 mg, 1.4 mmol, 2.0 eq) and EDCI (148 mg, 0.77 mmol, 1.1 eq) in CH_2Cl_2(2.0 mL) was added **50** (300 mg, 0.84 mmol, 1.2 eq). The resulting mixture was stirred overnight at room temperature, diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification on column chromatography (silica gel, 4:1:0.1 CH_2Cl_2: EtOAc:MeOH) provided 172 mg (41%) of **52** as a semi-solid.

^{1}H NMR (300 MHz, CD_3COOD vs TMS, 333K): δ 1.45 (9H, s); 2.34(3H, s); 3.06(3H, s); 3.65(1H, m); 3.81(1H, dd, J = 5.4, 5.6 Hz); 4.72(1H, m); 5.19(2H, s); 5.26(2H, s); 7.38(10H, m)ppm.

IR (NaCl, film): 3357, 3184, 2976, 1769, 1713, 1534, 1486, 1456, 1403, 1315, 1164, 1066, 1002, 733cm^{-1}.

$[\alpha]_D^{25}$ = 0 (c 1.0, CH_2Cl_2);

HRMS: Calcd. for ($C_{28}H_{35}N_5O_8S$ + H) = 602.2285. Found: (M+ H) = 602.2285.



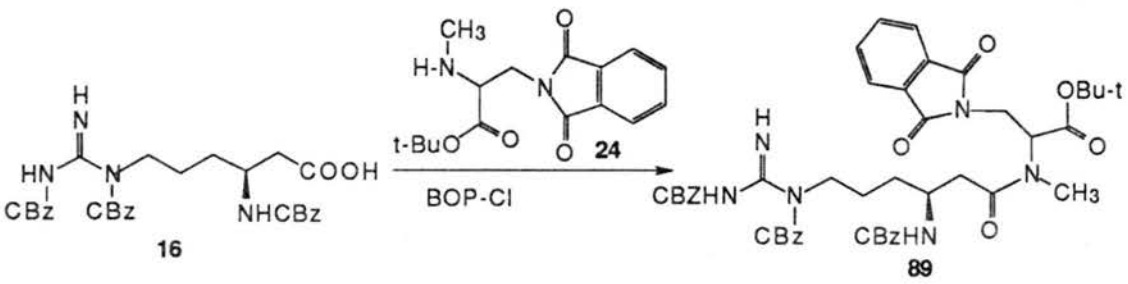
Cyclization Product 53.

The **52** (30 mg, 0.05 mmol.) was treated with anisole (0.1 mL) and TFA (2.0 mL) at 0 °C. The mixture was stirred for 5 h at room temperature, concentrated and dissolved in THF (1.0 mL). To this solution was added Et_3N (14 uL, 0.1 mmol, 2.0 eq). The mixture was stirred for 4.5 hr at room temperature, and poured into CH_2Cl_2/brine. The organic phase was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated and triturated with ether to give 21 mg (75%) of **53** as a semi-solid.

^1H NMR (300 MHz, DMSO-d6 vs TMS): δ 2.83/2.88(3H, s); 3.56(1H, m); 3.70(1H, q, J =12.2 Hz); 4.80(1H, m); 5.11(4H, s); 7.37(10H, m)ppm.

IR (NaCl, film): 3226, 2952, 1766, 1662, 1635, 1542, 1498, 1455, 1390, 1324, 1249, 1198, 1071cm^{-1}. $[\alpha]_D^{25}$ = + 6.0 (c 1.0, CH_2Cl_2/CH_3OH);

HRMS: Calcd. for ($C_{22}H_{23}N_5O_6$ + H) = 454.1727. Found: (M+ H) = 454.1730.



Peptide 89:

To a mixture of the acid **16** (1.50 g, 2.54 mmol, 1.0 eq) and NMM (464 uL, 1.2 mmol, 1.3 eq) in CH_2Cl_2 (3 mL) was added BOP-Cl (306 mg, 1.2 mmol, 1.2 eq) at 0 °C, and the amine **24** (970 mg, 3.19 mmol, 1.26 eq) in CH_2Cl_2 (3 mL) at 10 min later. The resulting mixture was stirred overnight, diluted with CH_2Cl_2 (200 mL.), washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, methylene chloride:EtOAc, 8:2) provided 1.22 g (55%) of **89** as an oil.

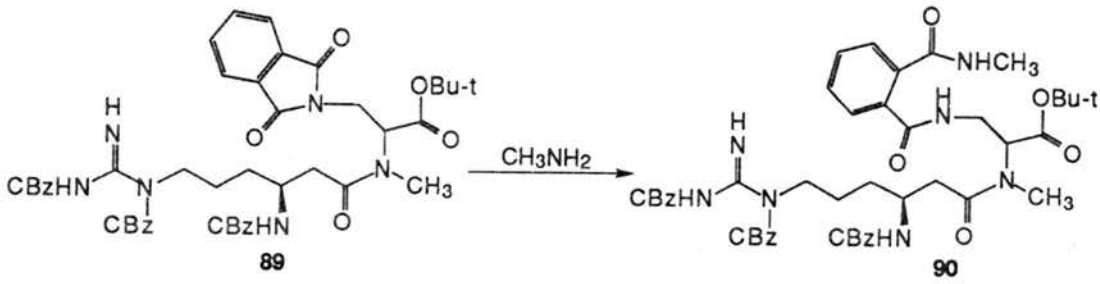
89: ^{1}H NMR (300 MHz, CD_3OD): δ 1.20(2H, m); 1.39(2H, m); 1.43(9H, s); 2.21(1H, m); 2.53(1H, m); 2.92(3H, s); 3.72(3H, m); 4.67(2H, d, J = 7.8 Hz); 4.95(2H, s); 5.08(2H, s); 5.20(2H, s); 5.22(1H, m); 7.33(15H, m); 7.64(2H, m); 7.74(2H, m)ppm.

IR (NaCl, film): 3389, 2936, 1774, 1716, 1646, 1609, 1505, 1395, 1370, 1251, 1100, 1008, 722 cm^{-1}; $[a]_D^{25}$ = - 9.7 (c 0.7, CH_2Cl_2); Anal. Calcd. for $C_{47}H_{52}N_6O_{11}$: C, 64.37; H, 5.98; N, 9.58. Found: C, 63.38; H, 6.08; N, 9.60.

89a: ^{1}H NMR (300 MHz, CD_3CO_2D, 373K): δ 1.46(9H, s); 1.60(4H, m); 2.40(1H, m); 2.60(1H, m); 2.98(3H, s); 3.89(3H, m); 4.14(2H, m); 5.04(2H, s); 5.16(2H, s); 5.23(1H, m); 5.28(2H, s); 7.29(15H, m); 7.69(2H, m); 7.81(2H, m)ppm.

$[\alpha]_D^{25}$ = - 36.1 (c 0.7, CH_2Cl_2);

HRMS: Calcd. for $C_{47}H_{52}N_6O_{11}$: (M+ H) = 877.3772. Found: (M+ H) = 877.3768.



Peptide 90:

To a solution of **89** (500 mg, 0.57 mmol, 1.0 eq) in CH_2Cl_2 (10 mL) was added 2 N methylamine in MeOH (5.0 mL). The mixture was stirred for 5 min and concentrated. Purification via column chromatography (silica gel, methylene chloride: EtOAc: MeOH, 4:1:0.3) provided 500 mg (97%) of **90** as an oil.

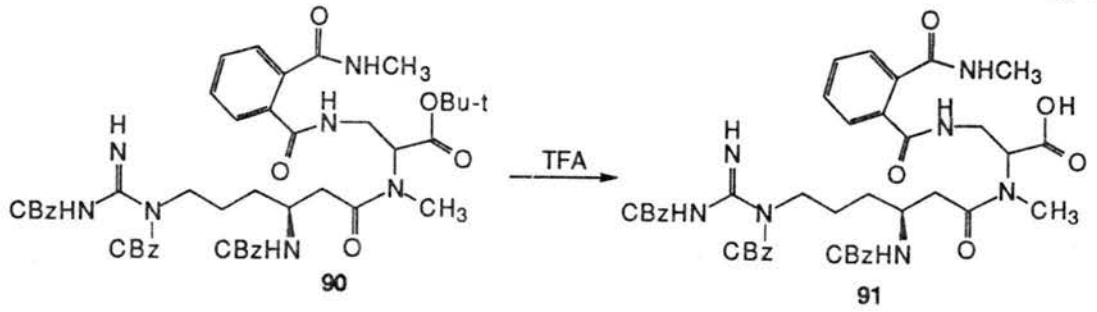
90: ^1H NMR (300 MHz, CD_3OD): δ 1.43(9H, s); 1.61(4H, m); 2.55(1H, m); 2.64(1H, m); 2.85(3H, m); 3.03/3.04(3H, s); 3.70(1H, m); 3.86(4H, m); 4.94(3H, m); 5.09(2H, s); 5.21(2H, s); 7.37(19H, m)ppm.

IR (NaCl, film): 3383, 3280, 3067, 2935, 1717, 1646, 1507, 1456, 1373, 1252, 1098, 697cm^{-1}.

$[\alpha]_D^{25}$ = -3.1 (c 3.0, CH_2Cl_2);

Anal. Calcd. for $C_{48}H_{57}N_7O_{11}$: C, 63.49; H, 6.33; N, 10.80. Found: C, 63.51; H, 6.38; N, 10.81.

90a: $[a]_D^{25}$ = - 16.4 (c 1.3, CH_2Cl_2);



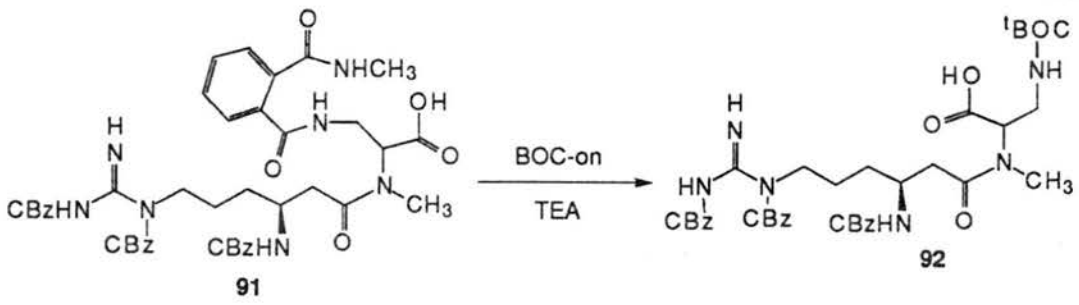
Acid 91:

The **90** (500 mg, 0.55 mmol, 1.0 eq) was treated with anisole (0.2 mL) and TFA (5.0 mL) at 0 °C. The resulting mixture was stirred for 1 h at room temperature, concentrated and triturated in dry ether to give **91** (437 mg, 93%) as an oil.

91: ^{1}H NMR (300 MHz, CD_3OD): δ 1.59(4H, m); 2.53(1H, m); 2.80(1H, m); 2.84(3H, s); 3.03/3.05(3H, s); 3.87(5H, m); 4.94(3H, m); 5.13(2H, s); 5.22(2H, s); 7.34(19H, m)ppm. IR (NaCl, film): 3396, 3290, 2945, 1717, 1684, 1615, 1540, 1378, 1254, 1099cm^{-1}. $[\alpha]_D^{25} = 0$ (c 1.0, CH_2Cl_2).

MS (ES^+): Cacld for ($C_{44}H_{49}N_7O_{11}$ + H) = 852.3, Found (M + H) = 852.3.

Anal. Calcd. for ($C_{44}H_{49}N_7O_{11}\cdot 3H_2O$): C, 58.33; H, 6.12; N, 10.82. Found: C, 58.22; H, 5.93; N, 10.85.



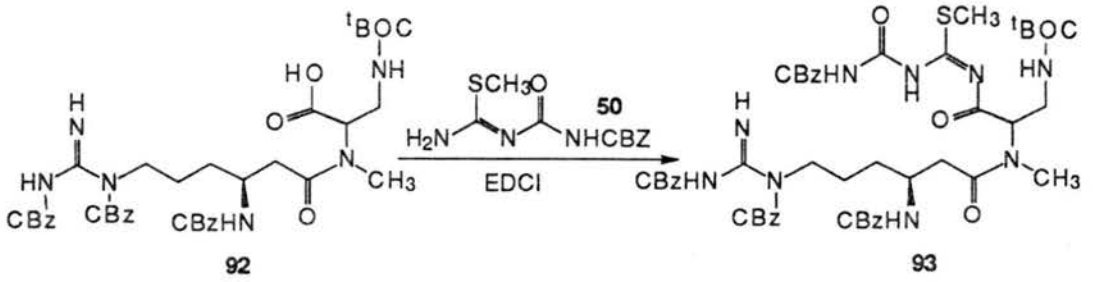
Acid 92:

To a solution of **91** (437 mg, 0.51 mmol, 1.0 eq) in H_2O/dioxane (8 mL, 1:1) and Et_3N (715 uL, 5.1 mmol, 10 eq) was added BOC-on (376 mg, 1.53 mmol, 3.0 eq). The mixture was stirred overnight and extracted with EtOAc(2x100 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2: MeOH, 9:1) provided 320 mg (79%) of **92** as a semi-solid.

92: ^1H NMR (300 MHz, CD_3OD): δ 1.35(9H, s); 1.65(4H, m); 2.50(2H, m); 2.86(3H, m); 3.60(3H, m); 3.95(3H, m); 5.00(2H, m); 5.10(2H, s); 5.24(2H, s); 7.32(15H, m)ppm. IR (NaCl, film): 3388, 2927, 1714, 1609, 1513, 1454, 1382, 1253, 1173, 1098, 1006, 697cm^{-1}. $[\alpha]_D^{25}$ = - 1.0 (c 1.0, CH_2Cl_2).

HRMS (FAB): Cacld for ($C_{40}H_{50}N_6O_{11}$ + H) = 791.3616, Found (M + H) = 791.3616.

92 (a/b = 3:1): $[a]_D^{25}$ = - 17.5 (c 1.0, CH_2Cl_2).



S-methylisothiourea 93:

To a solution of **92** (70 mg, 0.066 mmol, 1.0 eq), DMAP(25 mg, 0.13 mmol, 2.0 eq) and EDCI.HCl (21 mg, 0.07 mmol, 1.1 eq) in CH_2Cl_2 (0.5 mL) was added **50** (45 mg, 0.1 mmol, 1.5 eq). The resulting mixture was stirred overnight at room temperature, diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2: EtOAc: MeOH, 4:1:0.1) provided 36 mg (52%) of **93** as a semi-solid.

^{1}H NMR (300 MHz, CD_3OD): δ 1.39(9H, s); 1.64(4H, m); 2.28(3H, s); 2.60(1H, m); 2.75(1H, m); 3.05(3H, m); 3.47(1H, m); 3.69(1H, m); 3.91(3H, m); 4.94(3H, m); 5.08(2H, s); 5.20(2H, s); 5.23(2H, s); 7.31(20H, m)ppm.

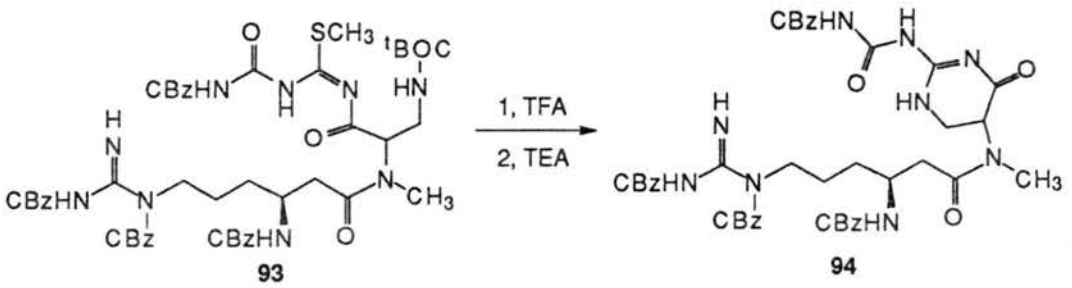
IR (NaCl, film): 3388, 2969, 1770, 1713, 1647, 1609, 1499, 1251, 1175, 1099cm^{-1}.

$[\alpha]_D^{25}$ = + 1.0 (c 1.6, CH_2Cl_2).

MS (ES^+): Cacld for ($C_{51}H_{61}N_9O_{13}S$ + H) = 1040.4, Found: (M+ H) = 1040.4.

Anal. Calcd. for $C_{51}H_{61}N_9O_{13}S$: C, 58.89; H, 5.91; N, 12.12. Found: C, 59.03; H, 6.12; N, 12.19.

93a: $[\alpha]_D^{25}$ = - 6.6 (c 0.7, CH_2Cl_2).



93 94

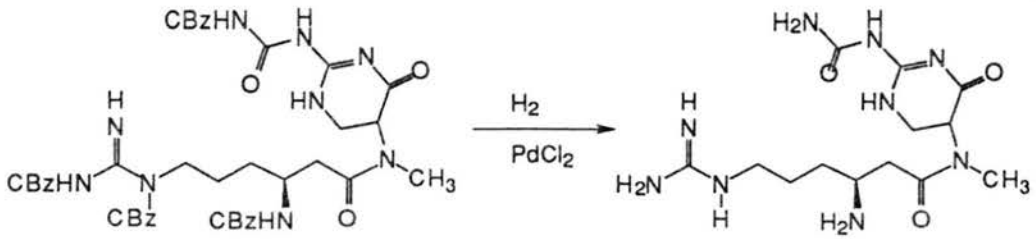
Cyclization product 94:

To a mixture of **93** (70 mg, 0.067 mmol, 1.0 eq) and anisole (0.1 mL) was added TFA (1.0 mL). The resulting mixture was stirred for 15 min at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on vacuo for 2 h and triturated with ethyl ether to give a white solid. This white solid was dissolved in THF (1.5 mL). To this solution was added triethylamine (20 uL, 0.14 mmol, 2.0 eq). Ten min later, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.5) immediately to give 40 mg (67%) of **94** as a white solid. This product was unstable with tendency to form a bicycle by-product ($t_{1/2}$ is about 1 day) and was used for next step immediately.

^{1}H NMR (300 MHz, CD_3Cl/D_2O vs TMS): δ 1.62(4H, m); 2.45(2H, m); 2.76/2.78(3H, s); 3.32(1H, m); 3.70(1H, m); 3.93(4H, m); 5.17(8H, m); 7.32(20H, m)ppm.

IR (NaCl, film): 3381, 3258, 2934, 1765, 1713, 1646, 1608, 1504, 1452, 1252, 1186, 1096, 1063cm^{-1}.

MS (ES^+): Cacld for ($C_{45}H_{49}N_9O_{11}$ + H) = 892.4. Found (M + H) = 892.4; (M + H - 108) = 784.3.



94 1:1 TAN-1057A/B

3S,5'S/R-3-amino-6-[(aminoiminomethyl)amino]-N-[2-[(aminocarbonyl) amino]-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-N-methyl-hexanamide, TAN-1057 A/B:

To a solution of **94** (40 mg, 0.045 mmol, 1.0 eq) in MeOH (1.5 mL)/CH_2Cl_2 (0.5 mL) was added $PdCl_2$ (40 mg). The reaction flask was degassed and charged with H_2 (1 atm). The mixture was stirred for 30 min. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **TAN-1057 A/B** (1:1, 20 mg, 100% yield) as an amorphous solid. This product was identical in mobility by HPLC and antibiotic activity by bioassay to authentic TAN-1057A/B.

^{1}HNMR (300 MHz, D_2O): δ 1.77(4H, m); 2.85(1H, dd, J = 18, 9.3 Hz); 3.00(1H, dd, J = 18, 4.0 Hz); 3.17(3H, s); 3.27(2H, t, J = 6.0 Hz); 3.70(1H, m); 3.99(2H, m); 5.14(1H, dd, J = 12, 8.5 Hz)ppm;

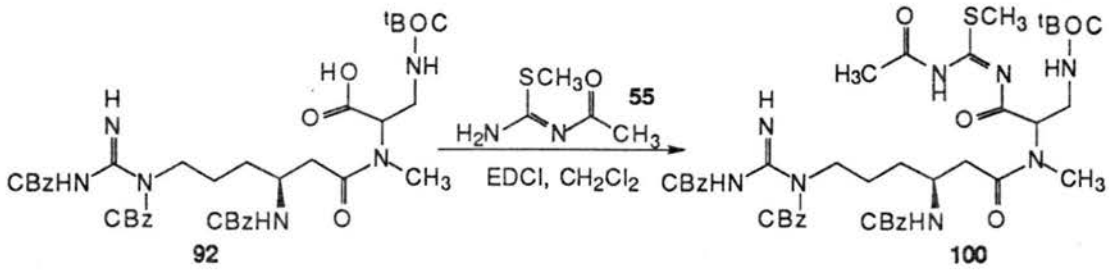
IR (KBr pellet): 3350, 3179, 2955, 1696, 1622, 1395, 1205, 1135cm^{-1}.

$[\alpha]_D^{25}$ = + 5.0 (c 0.7, H_2O);

MS (ES^+): Cacld. ($C_{13}H_{25}N_9O_3$ + H) = 356.2, Found (M + H) = 356.4.

HPLC: Rt = 11.98, 12.81 min (flow rate, 1.0 mL/min);

Lit.[2b] TAN-1057 A (2HCl): ^{1}HNMR (300 MHz, D_2O): δ 1.76(4H, m); 2.84(1H, dd, J = 18, 8.5 Hz); 2.99(1H, dd, J = 18, 4.0 Hz); 3.14(3H, s); 3.25(2H, t, J = 6.0 Hz); 3.68(1H, m); 3.93(2H, m); 5.11(1H, dd, J = 12, 9 Hz)ppm;



S-methylisothiourea 100:

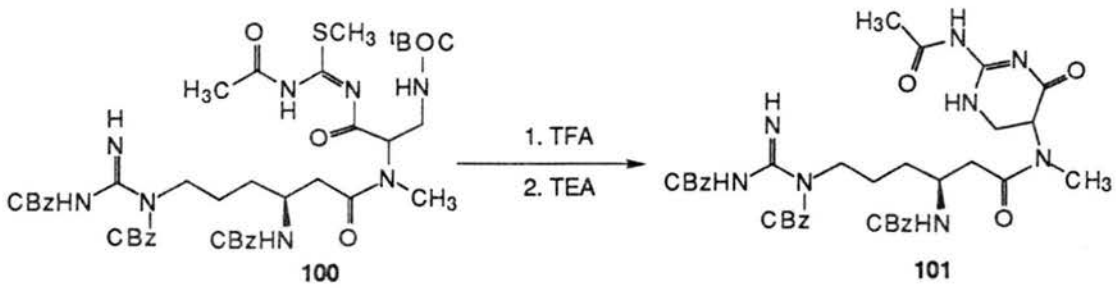
To a solution of **92** (278 mg, 0.35 mmol, 1.0 eq), DMAP(115 mg, 0.95 mmol, 2.7 eq) and EDCI.HCl (81 mg, 0.42 mmol, 1.2 eq) in CH_2Cl_2 (2.0 mL) was added **55** (130 mg, 0.53 mmol, 1.5 eq). The resulting mixture was stirred overnight at room temperature. Then, diluted with CH_2Cl_2 and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 7:3) provided 167 mg (52%) of **100** as a semi-solid.

^{1}H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.42(9H, s); 1.71(4H, m); 2.11(3H, s); 2.27(3H, s); 2.52(2H, m); 2.95(3H, s); 3.48(1H, m); 3.71(1H, m); 3.95(3H, m); 4.39(1H, br, D_2O exchanged); 5.04(2H, s); 5.07(1H, m); 5.10(2H, s); 5.22(2H, s); 7.36(15H, m); 5.88(1H, d, J = 5.7 Hz, HNCBz); 9.28(1H, br, D_2O exchanged); 9.43(1H, br, D_2O exchanged); 12.06/12.19(1H, br, D_2O exchanged)ppm.

IR (NaCl, film): 3382, 2974, 2931, 1713, 1615, 1538, 1503, 1387, 1250, 1096, 1012 cm^{-1}.

$[\alpha]_D^{25}$ = + 3.0 (c 1.1, CH_2Cl_2);

HRMS: Calcd. for ($C_{44}H_{56}N_8O_{11}S$ + H) = 905.3868. Found: (M + H) = 905.3901.



100 101

Cyclization product 101:

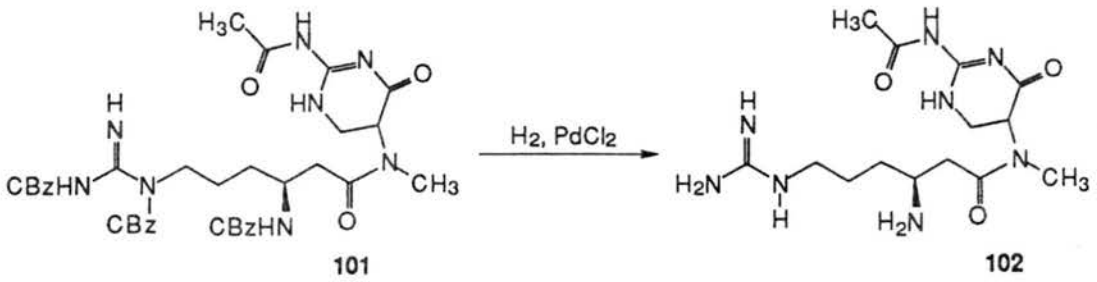
To a mixture of **100** (90 mg, 0.103 mmol, 1.0 eq) in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL). The resulting mixture was stirred for 15 min at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on vacuo for 2 h and triturated with ethyl ether to give a white solid. This white solid was dissolved in THF (1.5 mL). To this solution was added triethylamine (30 uL, 0.206 mmol, 2.0 eq). After stirring the solution for 10 min, the solvent was evaporated. Separation via PTLC (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.5) provided 26 mg (32 %) of **101** as a colorless oil.

^{1}H NMR (300 MHz, CD_3OD): δ 1.60(4H, m); 2.18(3H, s); 2.55(1.5H, m); 2.83(1/2H, d, J = 22 Hz); 2.91(3H, s); 3.28(1H, m); 3.46(2H, m); 3.95(3H, m); 5.07(2H, m); 5.12(2H, s); 5.23(2H, s); 6.11(1H, d, J = 9.0 Hz, H-N); 7.35(15H, m)ppm.

IR (NaCl, film): 3385, 3262, 2936, 1713, 1612, 1555, 1501, 1377, 1251, 1098cm^{-1}.

$[\alpha]_D^{25}$ = - 32.0 (c 0.4, CH_2Cl_2);

HRMS: Calcd. for ($C_{38}H_{44}N_8O_9$ + H) = 757.3309. Found: (M + H) = 757.3299.

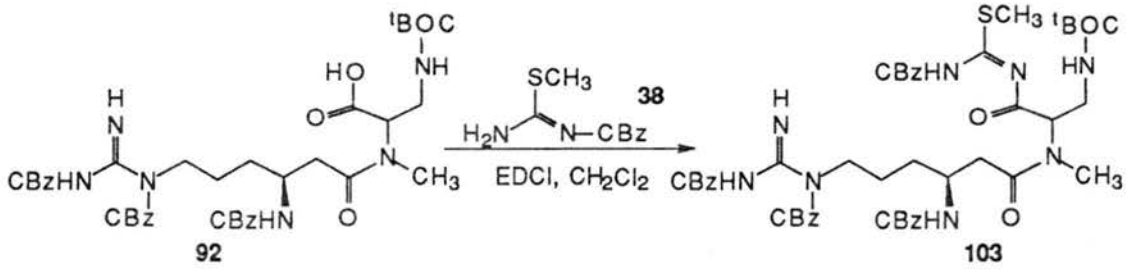


101 102

3S,5'S/R-3-amino-6-[(aminoiminomethyl)amino]-N-(2-acetylamino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-N-methyl-hexanamide 102:

To a solution of **101** (13 mg, 0.016 mmol, 1.0 eq) in MeOH (0.5 mL)/CH_2Cl_2 (0.1 mL) was added $PdCl_2$ (13 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm. of H_2 for 15 min. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **102** (6 mg, 100% yield) as an amorphous solid.

^{1}HNMR (300 MHz, D_2O vs DOH): δ 1.77(4H, m); 2.31(3H, s); 2.87(1H, m); 3.02(1H, m); 3.14(3H, s); 3.27(2H, t, J = 6.3 Hz); 3.70(1H, m); 3.97(2H, m); 5.16(1H, m)ppm;
IR (KBr pellet): 3394, 3156, 2913, 1737, 1651, 1591, 1365, 1203, 1138cm^{-1}.
$[\alpha]_D^{25}$ = + 10.7 (c 0.6, CH_3OH);
HRMS (FAB): Cacld for ($C_{14}H_{26}N_8O_3$ + H) = 355.2206, Found (M + H) = 355.2204.



S-methylisothiourea 103:

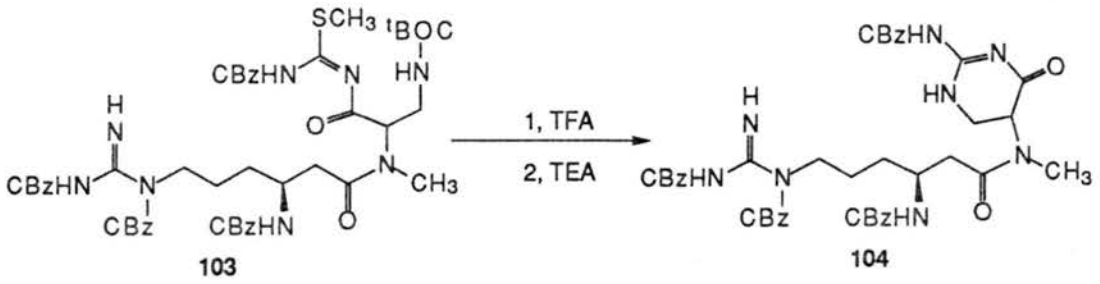
To a solution of **92** (120 mg, 0.15 mmol, 1.0 eq), DMAP(37 mg, 0.3 mmol, 2.0 eq) and EDCI.HCl (32 mg, 0.16 mmol, 1.1 eq) in CH_2Cl_2 (0.5 mL) was added **38** (50 mg, 0.23 mmol, 1.5 eq). The resulting mixture was stirred overnight at room temperature. Then, diluted with CH_2Cl_2 and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 7:3) provided 70 mg (52%) of **103** as a semi-solid.

^{1}H NMR (300 MHz, CD_3OD): δ 1.40(9H, s); 1.61(4H, m); 2.32(3H, s); 2.51(1H, m); 2.77(1H, m); 3.02/3.04(3H, s); 3.40(1H, m); 3.68(1H, m); 3.91(3H, m); 4.61(1/2H, m); 4.74(1/2H, m); 4.99/5.00(2H, s); 5.08(4H, s); 5.21(2H, s); 7.27(20H, m)ppm.

IR (NaCl, film): 3388, 2976, 1715, 1650, 1609, 1505, 1416, 1254, 1172, 1100cm^{-1}.

$[\alpha]_D^{25}$ = + 3.4 (c 0.5, CH_2Cl_2);

HRMS(FAB): Calcd for ($C_{50}H_{60}N_8O_{12}S$ + H) = 997.4146. Found: (M + H) = 997.4111.



103 104

Cyclization product 104:

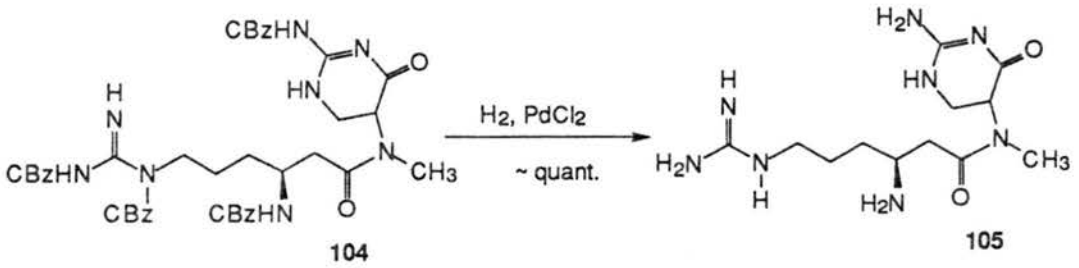
To a mixture of **103** (40 mg, 0.040 mmol, 1.0 eq) and anisole (20 uL) in CH_2Cl_2 (1.0 mL) was added TFA (1.0 mL). The resulting mixture was stirred for 20 min at 0 °C. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on vacuo for 2 h and triturated with ethyl ether to give a white solid. This white solid was dissolved in CH_2Cl_2 (1.0 mL). To this solution was added triethylamine (12 uL, 0.04 mmol, 2.0 eq). After stirring the solution for 10 min, the solvent was evaporated. Separation via PTLC (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.5) provided 17 mg (50 %) of **104** as a colorless oil.

^1H NMR (300 MHz, CD_3OD): δ 1.52(4H, m); 2.54(2H, m); 2.98(3H, s); 3.56(2H, m); 3.92(3H, m); 5.05(3H, m); 5.11(2H, s); 5.15(2H, s); 5.25(2H, s); 7.31(20H, m)ppm.

IR (NaCl, film): 3384, 3272, 2925, 1722, 1645, 1514, 1503, 1254, 1084cm^{-1}.

$[\alpha]_D^{25}$ = - 3.9 (c 0.85, CH_2Cl_2);

HRMS(FAB): Calcd. for ($C_{44}H_{48}N_8O_{10}$ + H) = 849.3571. Found: (M + H) = 849.3571.

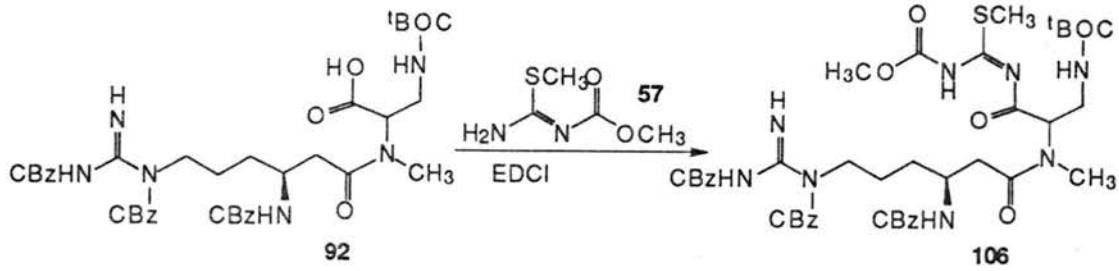


104 105

3S,5'S/R-3-amino-6-[(aminoiminomethyl)amino]-N-(2-amino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-N-methyl-hexanamide 105:

To a solution of **104** (12 mg, 0.014 mmol, 1.0 eq) in MeOH (1.0 mL)/CH_2Cl_2 (0.2 mL) was added $PdCl_2$ (10 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm. of H_2 for 20 min. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 3HCl salt of **105** (6 mg, 100% yield) as an amorphous solid.

^{1}HNMR (300 MHz, D_2O vs DOH): δ 1.76(4H, m); 2.83(1H, m); 3.01(1H, m); 3.13(3H, s); 3.26(2H, t, J = 5.1 Hz); 3.69(1H, m); 3.78(1H, m); 3.87(1H, m); 5.06(1H, m)ppm;
IR (KBr pellet): 3367, 3167, 2922, 1728, 1711, 1650, 1500, 1217, 1156cm^{-1}.
$[\alpha]_D^{25}$ = + 18.6 (c 0.6, CH_3OH);
HRMS (FAB): Cacld. ($C_{12}H_{24}N_8O_2$ + H) = 313.2094, Found (M + H) = 313.2100.



Coupling Product 106.

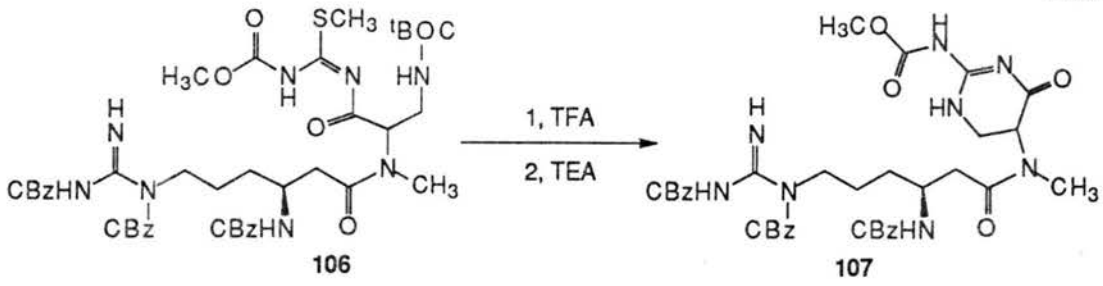
To a solution of **92** (320 mg, 0.40 mmol, 1.0 eq), DMAP(146 mg, 1.2 mmol, 3.0 eq) and EDCI.HCl (96 mg, 0.5 mmol, 1.2 eq) in CH_2Cl_2 (2 mL) was added **57** (131 mg, 0.5 mmol, 1.2 eq). After stirring overnight at room temperature, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 7:3) provided 141 mg (38%) of **106** as a semi-solid.

^{1}HNMR (300 MHz, CD_3OD): δ 1.44(9H, s); 1.63(4H, m); 2.31(3H, s); 2.60(2H, m); 3.04(3H, s); 3.42(1H, m); 3.66/3.67(3H, s); 3.70(1H, m); 3.93(3H, m); 4.67(1H, m); 5.01(2H, s); 5.10(2H, s); 5.24(2H, s); 7.31(15H, m)ppm;

IR (NaCl, CH_2Cl_2): 3389, 2959, 1715, 1254, 1171, 1099cm^{-1}.

$[\alpha]_D^{25}$ = + 2.2 (c 0.86, CH_2Cl_2);

HRMS (FAB): Cacld. ($C_{44}H_{56}N_8O_{12}S$ + H) = 921.3817. Found (M + H) = 921.3834.



106 107

Cyclization product 107:

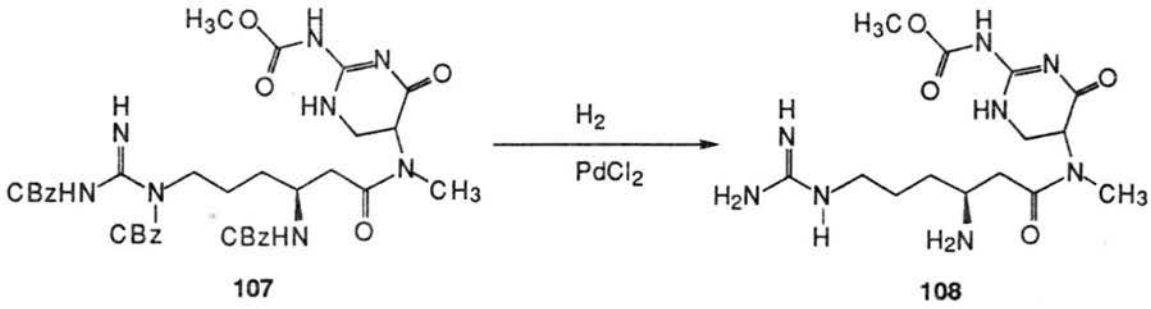
To a mixture of **106** (46 mg, 0.05 mmol, 1.0 eq) in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL). The resulting mixture was stirred for 15 min. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on vacuo for 2 hours and triturated with dry ether to give a white solid. This white solid was dissolved in THF (1.0 mL). To this solution was added triethylamine (15 uL, 0.1 mmol, 2.0 eq). After stirring for 10 min, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.5) to give 9 mg (22%) of **107** as a colorless oil.

^{1}HNMR (300 MHz, CD_3OD): δ 1.60 (4H, m); 2.48 (1H, m); 2.57 (1H, m); 2.87/2.93 (3H, s); 3.02 (1H, m); 3.27 (1H, m); 3.31 (3H, s); 3.94 (3H, m); 4.82 (1H, m); 5.02 (2H, s); 5.11 (2H, S); 5.25 (2H, S); 7.30 (15H, m)ppm;

IR (NaCl, film): 3377, 2917, 1722, 1642, 1512, 1256, 1092cm^{-1}.

$[\alpha]_D^{25} = 0$ (c 0.6, CH_2Cl_2);

HRMS (FAB): Cacld. ($C_{38}H_{44}N_8O_{10}$ + H) = 773.3266, Found (M + H) = 773.3259.



3S,5'S/R-3-amino-6-[(aminoiminomethyl)amino]-N-(2-methoxycarbonylamino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-N-methyl-hexanamide 108.

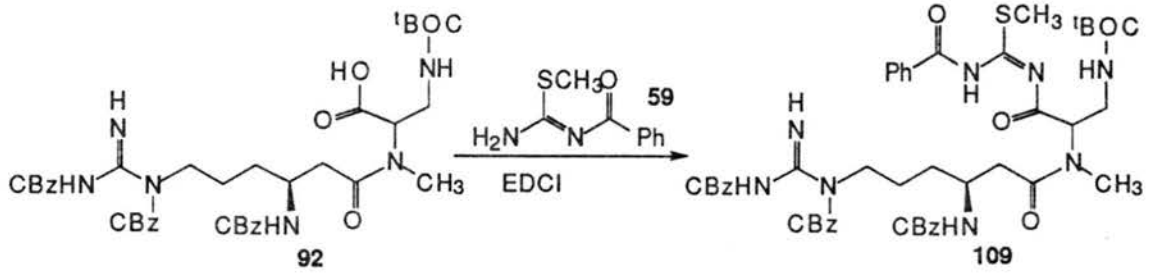
To a solution of **107** (9 mg, 0.012 mmol, 1.0 eq) in MeOH (0.5 mL)/CH_2Cl_2 (0.1 mL) was added $PdCl_2$ (10 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm. of H_2 for 15 min. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **108** (5 mg, 97%) as a white armorphous solid.

^{1}HNMR (300 MHz, D_2O): δ 1.76 (4H, m); 2.85 (1H, m); 3.01 (1H, m); 3.15 (3H, s); 3.26 (2H, t, J = 6 Hz); 3.69 (1H, m); 3.86 (3H, s); 3.98 (2H, m); 5.13 (1H, m) ppm;

IR (KBr, pellet): 3430, 3379, 2948, 1762, 1642, 1213, 1134cm^{-1}.

$[\alpha]_D^{25}$ = + 4.9 (c 0.35, CH_3OH);

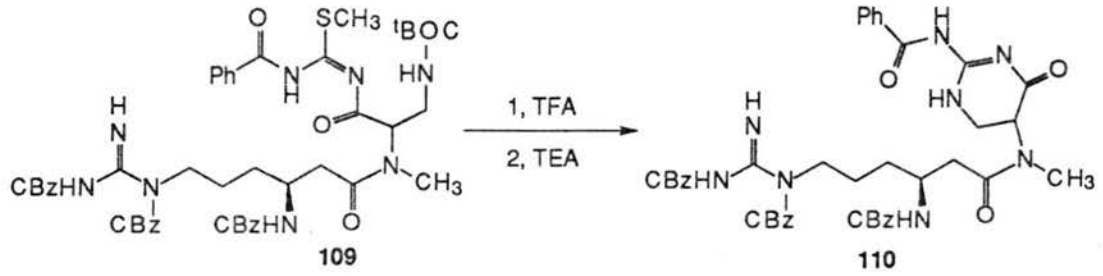
HRMS (FAB): Cacld. ($C_{14}H_{26}N_8O_4$ + H) = 371.2155, Found (M + H) = 371.2170.



Coupling Product 109.

To a solution of **92** (158 mg, 0.20 mmol, 1.0 eq), DMAP(73 mg, 0.6 mmol, 3.0 eq) and EDCI.HCl (46 mg, 0.24 mmol, 1.2 eq) in CH_2Cl_2 (2 mL) was added **59** (93 mg, 0.5 mmol, 1.2 eq). After stirred overnight at room temperature, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 7:3) provided 39 mg (39%) of **109** as a semi-solid.

^{1}HNMR (300 MHz, CD_3OD): δ 1.42 (9H, s); 1.58 (4H, m); 2.38-2.81 (2H, m); 2.52 (3H, s); 3.11 (3H, s); 3.49 (1H, m); 3.86 (4H, m); 4.70 (1H, m); 4.95 (2H, m); 5.07 (2H, s); 5.16 (2H, m); 7.34 (15H, m); 7.51 (2H, m); 7.88 (1H, m); 8.21 (2H, m) ppm;
IR (NaCl, film): 3388, 2974, 1715, 1608, 1538, 1454, 1392, 1249, 1171, 1099cm^{-1}.
$[\alpha]_D^{25}$ = + 0.6 (c 1.2, CH_2Cl_2);
HRMS (FAB): Cacld. ($C_{49}H_{58}N_8O_{11}S$ + H) = 967.4024, Found (M + H) = 967.4051.



Cyclization product 110:

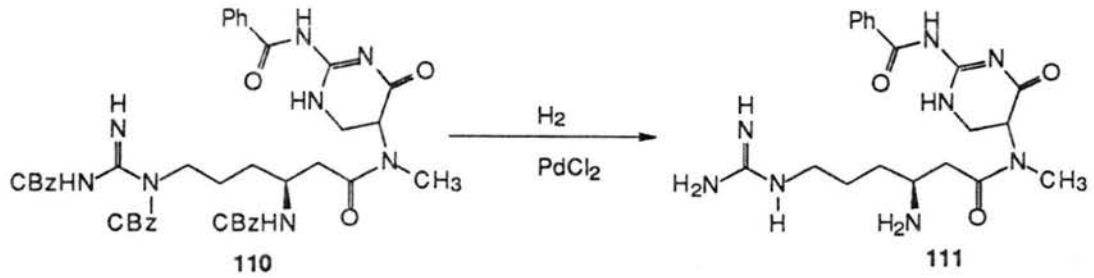
To a mixture of **109** (45 mg, 0.042 mmol, 1.0 eq) in CH_2Cl_2 (0.5 mL) is added TFA (0.5 mL). The resulting mixture was stirred for 15 min at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on vacuo for 2 hours and triturated with dry ether to give a white solid. This white solid was taken into THF (1.0 mL). To this solution was added triethylamine (20 uL, 0.136 mmol, 4.0 eq). After stirring the solution for 10 min, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.5) to give 4 mg (10%) of **110** as a colorless oil.

^{1}HNMR (300 MHz, CD_3OD): δ 1.62 (4H, m); 2.56 (2H, m); 3.02 (3H, s); 3.37 (1H, m); 3.70 (1H, m); 3.93 (3H, m); 5.02 (3H, m); 5.12 (2H, s); 5.25 (2H, s); 7.32 (18H, m); 8.15 (2H, d, J = 7.2 Hz)ppm;

IR (NaCl, film): 3385, 3263, 3056, 2927, 1720, 1633, 1505, 1454, 1378, 1322, 1253, 1094, 1063cm^{-1}.

$[\alpha]_D^{25}$ = + 3.0 (c 0.2, CH_2Cl_2);

HRMS (FAB): Cacld. ($C_{43}H_{46}N_8O_9$ + H) = 819.3466, Found (M + H) = 819.3436.



110 111

3S,5'S/R-3-amino-6-[(aminoiminomethyl)amino]-N-(2-benzoylamino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-N-methyl-hexanamide 111:

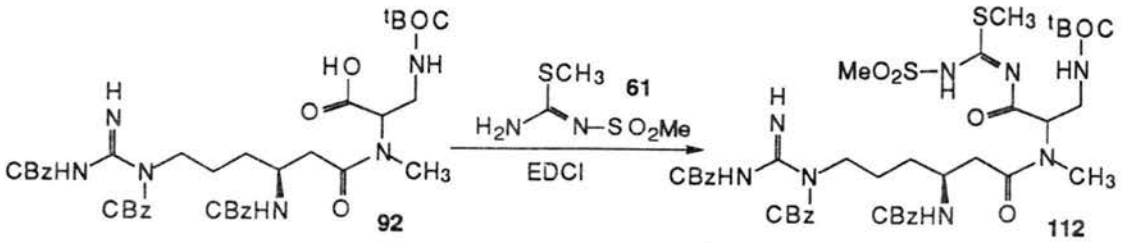
To a solution of **110** (3 mg, 0.003 mmol, 1.0 eq) in MeOH (0.5 mL)/CH_2Cl_2 (0.1 mL) was added $PdCl_2$ (5 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm. of H_2 for 15 minutes. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **111** (2 mg, 100%) as a colorless armorphous solid.

^{1}HNMR (300 MHz, D_2O): δ 1.76(4H, m); 2.85(1H, m); 3.02(1H, m); 3.18(3H, s); 3.26(2H, t, J = 5.4 Hz); 3.69(1H, m); 4.05(2H, m); 5.18(1H, dd, J = 8.7 Hz); 7.62(2H, t, J = 7.2 Hz); 7.77(1H, t, J = 6.6 Hz), 7.98(1H, d, J = 7.2 Hz)ppm;

IR (NaCl, film): 3398, 2935, 1732, 1650, 1411, 1259cm^{-1}.

$[\alpha]_D^{25}$ = + 8.3 (c 0.2, CH_3OH);

HRMS (FAB): Cacld. ($C_{19}H_{28}N_8O_2$ + H) = 417.2363, Found (M + H) = 417.2371.



Coupling Product 112.

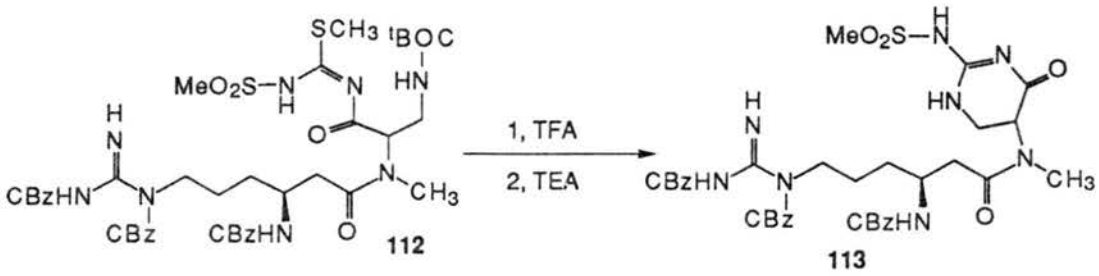
To a solution of **92** (237 mg, 0.30 mmol, 1.0 eq), DMAP(110 mg, 0.9 mmol, 3.0 eq) and EDCI.HCl (61 mg, 0.36 mmol, 1.2 eq) in CH_2Cl_2 (2 mL) was added **61** (102 mg, 0.5 mmol, 1.2 eq). After stirred overnight at room temperature, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 7:3) provided 97 mg (34%) of **112** as a semi-solid.

^{1}HNMR (300 MHz, CD_3OD): δ 1.39 (9H, s); 1.64 (4H, m); 2.29 (3H, s); 2.51-2.76 (2H, m); 3.00 (6H, s); 3.40 (1H, m); 3.64 (1H, m); 3.93 (3H, m); 4.55 (1H, m); 5.01 (2H, s); 5.11 (2H, s); 5.24 (2H, s); 7.29 (15H, m) ppm;

IR (NaCl, film): 3388, 2976, 2498, 1714, 1688, 1504, 1416, 1251, 1113 cm^{-1}.

$[\alpha]_D^{25}$ = + 4.3 (c 0.8, CH_2Cl_2);

HRMS (FAB): Cacld for ($C_{43}H_{56}N_8O_{12}S_2$ + H) = 941.3537, Found (M + H) = 941.3533.



Cyclization product 113:

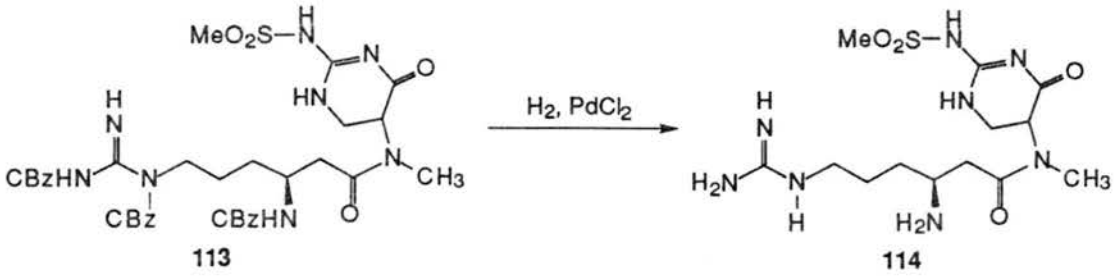
To a mixture of **112** (38 mg, 0.04 mmol, 1.0 eq) in CH_2Cl_2 (1 mL) was added anisole (10 uL) and TFA (1 mL). The resulting mixture was stirred for 15 min. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on vacuo for 2 h and triturated with dry ether to give a white solid. This white solid was taken into THF (1.0 mL). To this solution was added triethylamine (22 uL, 0.16 mmol, 4.0 eq). After stirring the solution for 10 min, the solvent was evaporated and the resulting residue was purified on PTLC(silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.5) to give 28 mg (88%) of **113** as a colorless oil.

^{1}HNMR (300 MHz, $CD_3OD/CDCl_3$ vs TMS): δ 1.50 (2H, m); 1.63 (2H, m); 2.52 (2H, m); 2.95 (3H, s); 2.97 (3H, s); 3.25 (1H, m); 3.57 (1H, m); 3.90 (3H, m); 4.80 (1H, m); 5.02 (2H, m); 5.09 (2H, m); 5.22 (2H, s); 7.29 (15H, m) ppm;

IR (NaCl, film): 3392, 3286, 2940, 1846, 1716, 1506, 1456, 1377, 1254, 1108, 1003, 907cm^{-1}.

$[\alpha]_D^{25}$ = + 1.3 (c 1.4, CH_2Cl_2);

HRMS (FAB): Cacld. ($C_{37}H_{44}N_8O_{10}S$ + H) = 793.2979, Found (M + H) = 793.3012.



113 114

3S,5'S/R-3-amino-6-[(aminoiminomethyl)amino]-N-(2-methylsulfonyl amino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-N-methyl-hexanamide 114.

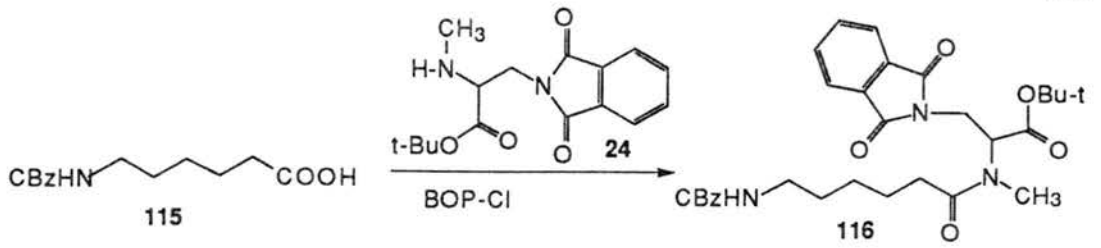
To a solution of **113** (28 mg, 0.035 mmol, 1.0 eq) in MeOH (1 mL)/CH_2Cl_2 (0.3 mL) was added $PdCl_2$ (12 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm. of H_2 for 15 min. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **114** (16 mg, 99%) as a colorless semi-solid.

^{1}HNMR (300 MHz, D_2O): δ 1.76 (4H, m); 2.84 (1H, m); 3.00 (1H, m); 3.12 (3H, s); 3.13 (3H, s); 3.26 (2H, t, J = 5.7 Hz); 3.69 (1H, m); 3.76 (1H, m); 3.86 (1H, m); 5.01 (1H, m) ppm;

IR (KBr pellet): 3411, 3156, 2933, 1733, 1639, 1373, 1261, 1100, 994 cm^{-1}.

$[\alpha]_D^{25}$ = + 15.7 (c 0.75, CH_3OH);

HRMS (FAB): Cacld for ($C_{13}H_{26}N_8O_4$ + H) = 391.1876, Found (M + H) = 391.1885.



Peptide 115:

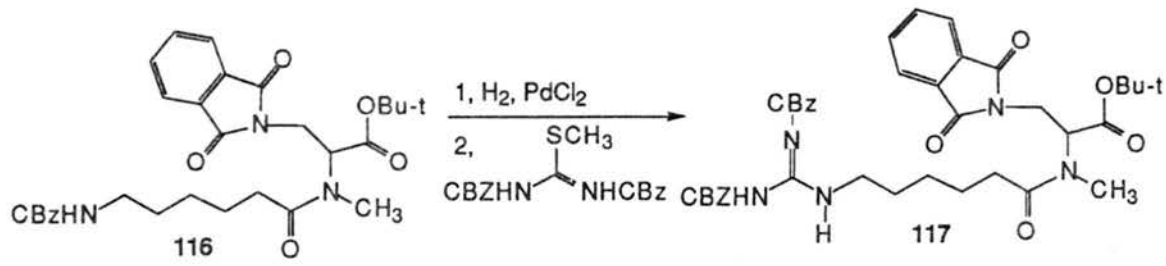
To a mixture of the acid **115** (530 mg, 2.0 mmol, 1.0 eq) and NMM (286 uL, 2.6 mmol, 1.3 eq) in CH_2Cl_2 (2 mL) was added BOP-Cl (664 mg, 2.6 mmol, 1.3 eq) at 0 °C, and amine **24** (970 mg, 3.19 mmol, 1.26 eq) in CH_2Cl_2 (3 mL) at 10 min later. The resulting mixture was stirred overnight, diluted with CH_2Cl_2 (200 mL.), washed with brine, dried over anhydrous Na_2SO_4, and concentrated. Purification via column chromatography (silica gel, methylene chloride:EtOAc, 8:2) provided 462 mg (42%) of **116** as an oil.

^{1}HNMR (300 MHz, CD_3OD): δ 1.10 (2H, m); 1.37 (4H, m); 1.47 (9H, s); 2.22 (2H, t, J = 7.2 Hz); 2.95(2H, m); 2.98 (3H, s); 4.09 (2H, m); 5.04 (2H, s); 5.26 (1H, dd, J = 10.2, 4.5 Hz); 7.32 (5H, m); 7.75 (4H, m) ppm;

IR (NaCl, film): 3344, 2935, 1774, 1745, 1650, 1530, 1395, 1369, 1300. 1249, 1159, 1019 cm^{-1}.

$[\alpha]_D^{25}$ = - 11.2 (c 0.5, CH_2Cl_2);

HRMS (FAB): Cacld. ($C_{30}H_{37}N_3O_7$ + H) = 552.2710, Found (M + H) = 552.2711.



Peptide 117:

To a solution of **116** (280 mg, 0.51 mmol, 1.0 eq) in THF (10 mL) was added $PdCl_2$ (50 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm. of H_2 for 2.5 h. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a crude amine as a yellowish solid.

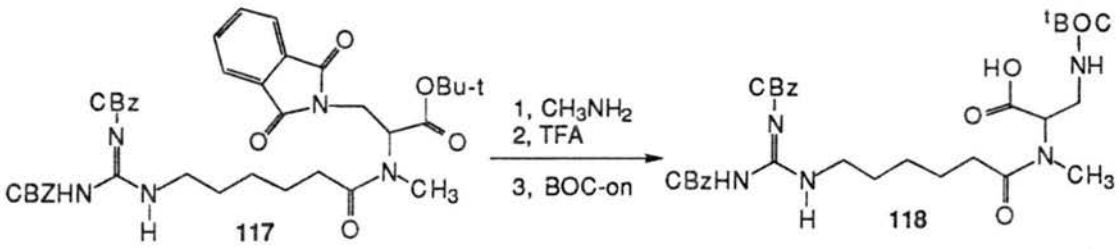
To a solution of the crude amine in CH_2Cl_2 (6 mL) was added N,N'-diCBz-S-methylthiourea (230 mg, 1.02 mmol, 2.0 eq) and TEA (286 uL, 2.04 mmol, 4.0 eq). The mixture was stirred for 2 h at room temperature, concentrated and purified on column chromatography (silica gel, CH_2Cl_2:EtOAc, 8:2) to give 270 mg (73%) of **117** as a semi-solid.

^{1}HNMR (300 MHz, CD_3OD): δ 1.12 (2H, m); 1.36 (4H, m); 1.46 (9H, s); 2.23 (2H, t, J = 7.2 Hz); 2.96 (3H, s); 3.23 (2H, m); 4.09 (2H, m); 5.10 (2H, s); 5.22 (2H, m); 5.25 (1H, m); 7.36 (10H, m); 7.72 (2H, m), 7.80 (2H, m) ppm;

IR (NaCl, film): 3339, 2937, 1773, 1718, 1638, 1569, 1394, 1321, 1266, 1136, 1049 cm^{-1}.

$[\alpha]_D^{25}$ = - 9.0 (c 1.0, CH_2Cl_2);

HRMS (FAB): Cacld for ($C_{39}H_{45}N_5O_9$ + H) = 728.3295, Found: (M + H) = 728.3272.



Acid 118:

To a solution of **117** (200 mg, 0.275 mmol, 1.0 eq) in CH_2Cl_2 (1.0 mL) was added 2.0 M methylamine/CH_3OH (1.5 mL). The mixture was stirred for 7 min at room temperature, concentrated and separated on column chromatography (silica gel, methylene chloride:EtOAc:MeOH, 4:1:0.3) to give 183 mg of product as an oil.

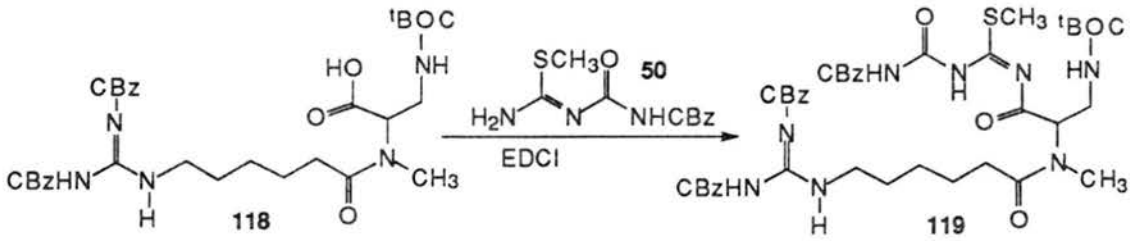
Then, this crude product was treated with anisole (0.1 mL) and TFA (1.0 mL) at 0 °C. The mixture was stirred for 30 min at room temperature, concentrated and triturated in dry ether to give 210 mg of solid. This crude solid was taken into H_2O/dioxane (1 mL, 1:1). To this mixture was added BOC-on (221 mg, 0.9 mmol, 3.0 eq) and TEA (421 uL, 3.0 mmol, 10 eq). The mixture was stirred overnight and treated with ethyl acetate/sat. NaH_2PO_4 aqueous solution (100 mL, 1:1). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:MeOH, 9:1) provided 70 mg (36%) of **118** as a semi-solid.

^{1}HNMR (300 MHz, CD_3OD): δ 1.01 (1H, m); 1.38 (9H, s); 1.46 (2H, m); 1.62 (3H, m); 2.18 (1H, m); 2.40 (1H, m); 2.89 (3H, m); 3.19 (1H, m); 3.40 (1H, m); 3.58 (1H, m); 4.11 (1H, m); 4.45 (1/2H, m); 5.13 (2H, m); 5.23 (2H, m); 5.49 (1/2H, m); 7.37 (8H, m); 7.70 (1H, m); 7.78 (1H, m) ppm;

IR (NaCl, film): 3340, 2936, 1716, 1636, 1624, 1424, 1386, 1325, 1262, 1208, 1137, 1049 cm^{-1}.

$[\alpha]_D^{25}$ = - 5.4 (c 0.35, CH_2Cl_2);

HRMS (FAB): Cacld for ($C_{32}H_{43}N_5O_9$ + Na) = 664.2958, Found $(M + Na)^+$ = 664.2982.



Coupling Product 119:

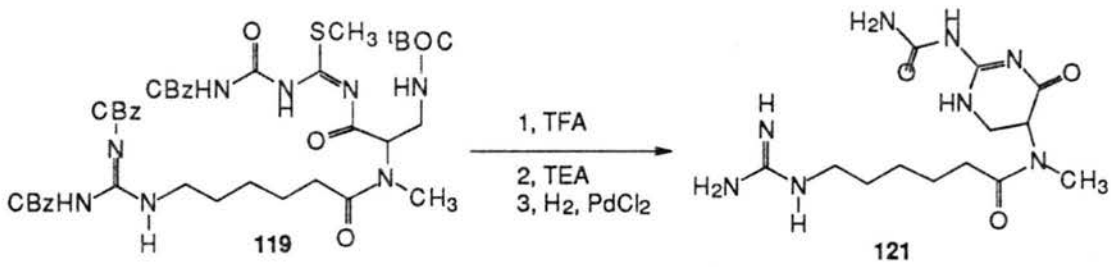
To a solution of **118** (70 mg, 0.11 mmol, 1.0 eq), DMAP (40 mg, 0.33 mmol, 3.0 eq) and EDCI.HCl (32 mg, 0.165 mmol, 1.1 eq) in CH_2Cl_2(0.5 mL) was added **350** (61 mg, 0.17 mmol, 1.5 eq). After stirred overnight at room temperature, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 7:3) provided 25 mg (25%) of **119** as a colorless oil.

^{1}HNMR (300 MHz, CD_3OD): δ 1.42 (9H, s); 1.45 (2H, m); 1.61 (4H, m); 2.35 (3H, s); 2.50 (2H, m); 3.07/3.17 (3H, s); 3.70 (1H, m); 3.56 (2H, m); 3.66 (2H, m); 4.53 (1H, m); 5.10 (2H, s); 5.15 (2H, s); 5.21 (2H, s); 7.35 (15H, m) ppm.

IR (NaCl, film): 3340, 2947, 1728, 1644, 1574, 1455, 1319, 1243, 1207, 1167, 1051 cm^{-1}.

$[\alpha]_D^{25}$ = 0 (c 1.2, CH_2Cl_2);

HRMS (FAB): Cacld for ($C_{43}H_{54}N_8O_{11}S$ + H) = 891.3711, Found (M + H) = 891.3755.



5'S/R-6-[(aminoiminomethyl)amino]-N-(2-[aminocarbonyl]amino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-N-methyl-hexanamide 121.

To a mixture of **119** (20 mg, 0.022 mmol, 1.0 eq) in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL). The resulting mixture was stirred for 15 min at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on *vacuo* for 2 h and triturated with dry ether to give a white solid. This white solid was taken into THF (1.0 mL). To this solution was added triethylamine (8.0 uL, 0.044 mmol, 2.0 eq). After stirring the solution for 10 min, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.5) to give 7 mg (43%) of cyclic intermediate **120** as a colorless oil. Compound **120** was unstable and was used in next step immediately.

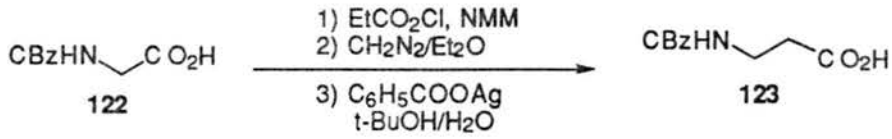
To a solution of **120** (4.5 mg, 0.006 mmol, 1.0 eq) in MeOH (0.5 mL)/CH_2Cl_2 (0.5 mL) was added $PdCl_2$ (4 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm. of H_2 for 15 min. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **121** (2.5 mg, 100%) as a semi-solid.

^{1}HNMR (300 MHz, D_2O): δ 1.40 (2H, m); 1.62 (4H, m); 2.51 (2H, t, J = 7.5 Hz); 3.17 (3H, s); 3.20 (2H, m); 3.93 (2H, d, J = 10.2 Hz); 4.97 (1H, t, J = 10 Hz) ppm;

IR (KBr, pellet): 3491, 2958, 1720, 1651, 1493, 1371, 1200 cm^{-1}.

$[\alpha]_D^{25} = 0$ (c 0.25, CH_2Cl_2);

HRMS (FAB): Cacld. ($C_{13}H_{24}N_8O_3 + NH_4^+$) = 358.2315, Found ($M+ NH_4$)$^+$ = 358.2334



N-CBz-β-homoglycine 123:

To a solution of **122** (1.05 g, 5.0 mmol, 1.0 eq) in THF (30 mL) was added NMM (604 uL, 5.5 mmol, 1.1 eq) and ethyl chloroformate (526 uL, 5.5 mmol, 1.1 eq) at 0 °C. The resulting mixture was stirred for 1h at 0 °C. Then the precipitated amine hydrochloride was rapidly filtered off in the cold. To this clear solution was added CH_2N_2/ether solution (generated from MNNG).The solution was stirred overnight at room temperature and concentrated to give an oily diazoketone.

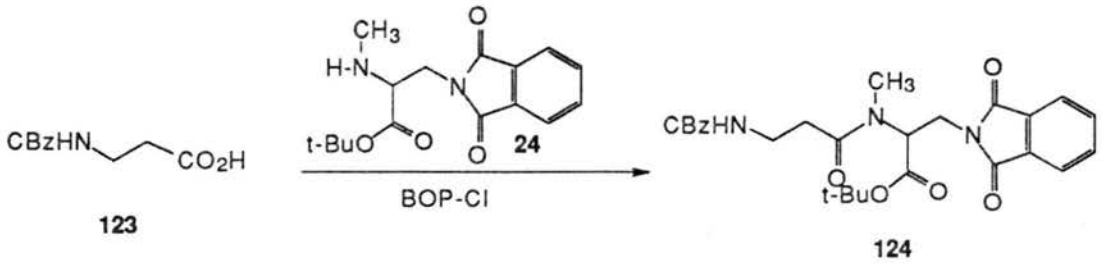
The oily diazoketone was taken into t-BuOH/H_2O (40 mL, 1:1), and to this solution was added silver benzoate (500 mg) and triethyl amine (4.0 mL). The resulting mixture was stirred overnight in the dark and then concentrated *in vacuo*. The residue was dissolved in ethyl acetate/sat. NaH_2PO_4 aq. and the organic layer was separated, dried over anhydrous sodium sulfate. After filtration, the solvents were evaporated and the crude product was recrystalyzed in EtOAc/Hexane (1:1) to give 1.30 g (58%) of **123** as a white solid.

^{1}H NMR (300 MHz, CD_3OD): δ 2.49(2H, t, J = 6.9 Hz); 3.36(2H, m); 5.06(2H, s); 7.32(5H, m) ppm.

IR (NaCl, film): 3332, 3026, 2911, 1694, 1684, 1650, 1538, 1422, 1322, 1220, 1094, 1032 cm^{-1}.

mp: 104-5 °C

HRMS (DCI): Cacld. ($C_{11}H_{13}NO_4$ + H) = 224.0923, Found (M + H) = 224.0928.



Peptide 124:

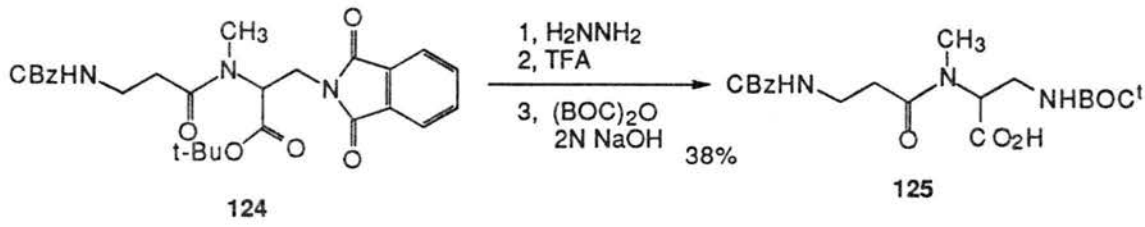
To a mixture of the acid **123** (200 mg, 0.9 mmol, 1.0 eq) and NMM (128 uL, 1.17 mmol, 1.3 eq) in CH_2Cl_2 (2 mL) was added BOP-Cl (300 mg, 1.17 mmol, 1.3 eq) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C. Then, to the resulting mixture was added amine **24** (270 mg, 0.89 mmol, 1.0 eq) in CH_2Cl_2 (3 mL). The mixture was stirred overnight at room temperature, diluted with CH_2Cl_2 (200 mL), washed with brine, dried over anhydrous Na_2SO_4. After concentration, the residue was separated on column chromatography (silica gel, eluted with methylene chloride:EtOAc:MeOH, 8:2:0.02) to give 290 mg (64%) of **124** as an oil

^1H NMR (300 MHz, $CDCl_3$): δ 1.46 (9H, s); 2.39 (2H, m); 2.93 (3H, s); 3.31 (2H, m); 4.14 (2H, m); 5.02 (2H, m); 5.26 (1H, m); 5.28 (br, D_2O exchanged); (7.33 (5H, m); 7.55 (2H, m); 7.78 (2H, m) ppm.

IR (NaCl, film): 3390, 2978, 1774, 1715, 1650, 1396, 1369, 1250, 1158, 1010cm^{-1}.

$[\alpha]_D^{25}$ = -12.8 (c 1.7, CH_2Cl_2);

HRMS (DEI): Cacld for $C_{27}H_{31}N_3O_7$ = 509.2161, Found M^+ = 509.2157.



124

125

Acid 125:

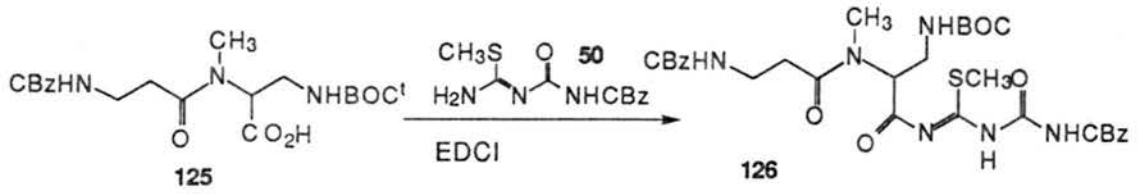
To a solution of **124** (90 mg, 0.18 mmol, 1.0 eq) in MeOH (2.0 mL) was added hydrazine (55 mg, 1.8 mmol, 10 eq). The resulting mixture was stirred for 3 h, concentrated, and dried on *vacuo* overnight to give a white solid. The white solid was treated with CH_2Cl_2/sat. $NaHCO_3$ (50 mL, 1:1). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated to give an oily residue. This crude amine was treated with TFA (1.0 mL) and stirred for 2 h. After evaporation of TFA, the residue was taken into H_2O/t-BuOH (1.0 mL, 1:1). To this solution were added $(t\text{-BOC})_2O$ (90 mg, 0.41 mmol, 2.3 eq) and 2 N NaOH solution (300 uL). The resulting mixture was stirred for 16 h, diluted with water (20 mL) and extracted with Et_2O. The aqueous layer was acidified to pH 4 by 1N HCl solution and extracted with CH_2Cl_2 (2x30 mL). The CH_2Cl_2 extracts were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:MeOH, 9:1) provided 29 mg (38%) of **125** as an oil.

^1H NMR (300 MHz, CD_3OD): δ 1.40(9H, s); 2.55(2H, m); 2.81/2.93(3H, s); 3.36(1H, m); 3.42(2H, m); 3.60(1H, m); 4.44(1/2H, m); 4.96(1/2H, m); 5.06(2H, s); 7.33(5H, m)ppm.

IR (NaCl, film): 3338, 2976, 1697, 1622, 1520, 1392, 1254, 1171, 1068cm^{-1}.

$[\alpha]_D^{25}$ = - 6.1 (c 0.9, CH_2Cl_2);

HRMS (FAB): Cacld for ($C_{20}H_{29}N_4O_7$ + H) = 424.2084, Found (M + H) = 424.2087.



Coupling Product 126.

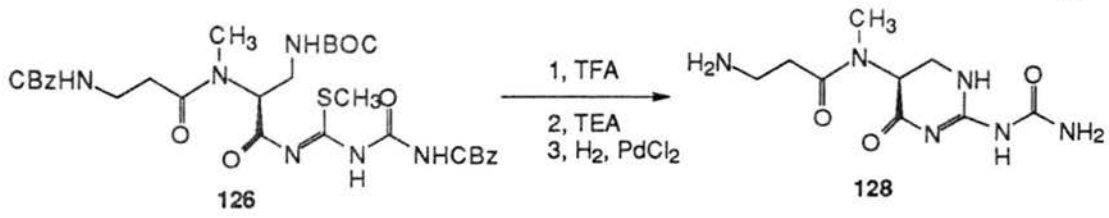
To a solution of **125** (43 mg, 0.10 mmol, 1.0 eq), DMAP (15 mg, 0.12 mmol, 1.2 eq) and EDCI.HCl (23 mg, 0.12 mmol, 1.2 eq) in CH_2Cl_2 (0.5 mL) was added **50** (44 mg, 0.12 mmol, 1.2 eq). After stirred overnight at room temperature, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 7:3) provided 26 mg (39%) of **126** as a semi-solid.

^{1}HNMR (300 MHz, CD_3OD): δ 1.45 (9H, s); 2.32//2.36 (3H, s); 2.72 (2H, m); 3.07/3.12 (3H, s); 3.42 (2H, m); 3.63 (2H, m); 4.59 (1H, m); 5.03 (2H, m); 5.21 (2H, m); 7.31 (10H, m) ppm;

IR (NaCl, film): 3356, 2944, 1702, 1646, 1532, 1468, 1418, 1379, 1197, 1158, 1066, 1000 cm^{-1}.

$[\alpha]_D^{25} = 0$ (c 0.6, CH_2Cl_2);

HRMS (FAB): Cacld. ($C_{31}H_{40}N_6O_9S$ + H) = 673.2656, Found (M + H) = 673.2680.



5'S/R-3-amino-N-(2-[aminocarbonyl]amino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-N-methyl-propanamide 128:

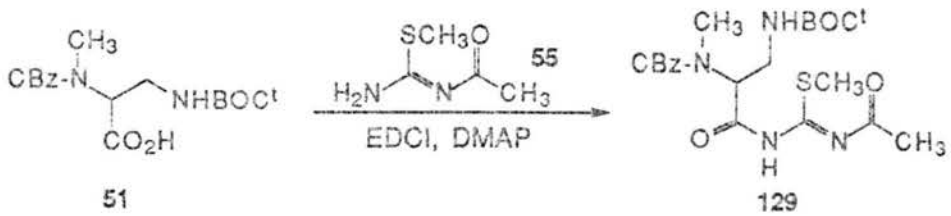
To a mixture of **126** (16 mg, 0.024 mmol, 1.0 eq) in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL). The resulting mixture was stirred for 15 min at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on *vacuo* for 2 h and triturated with dry ether to give a white solid. This white solid was taken into THF (1.0 mL). To this solution was added triethylamine (8.0 uL, 0.048 mmol, 2.0 eq). After stirring the solution for 10 min, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.5) to give 4 mg of cyclic product as a colorless oil. This oily cyclic compound was taken into MeOH (0.5 mL)/CH_2Cl_2 (0.5 mL). To the resulting solution was added $PdCl_2$ (4 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm. of H_2 for 15 min. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **128** (2 mg, 25%) as a colorless armorphous solid.

^{1}HNMR (300 MHz, D_2O): δ 2.94 (2H, m); 3.14 (3H, s); 3.28 (2H, t, J = 6.0 Hz); 3.96 (2H, m); 5.11 (1H, dd, J = 11.9, 8.7 Hz) ppm;

IR (KBr, pellet): 3450, 3198, 2976, 1743, 1620, 1511, 1423, 1157 cm^{-1}.

$[\alpha]_D^{25} = 0$ (c 0.2, CH_3OH);

HRMS (FAB): Cacld for ($C_9H_{16}N_6O_3$ + H) = 257.1283, Found (M + H) = 257.1351.



Coupling Product 129:

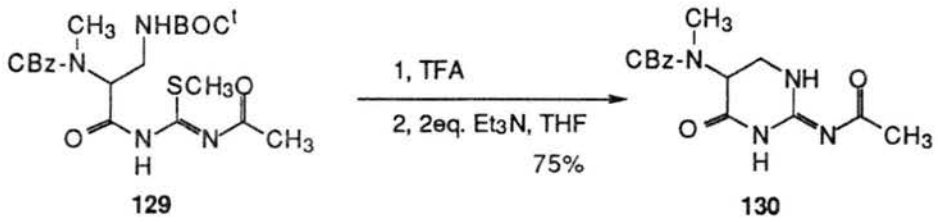
To a solution of **51** (85 mg, 0.24 mmol, 1.0 eq), DMAP (88 mg, 0.72 mmol, 3.0 eq) and EDCI.HCl (56 mg, 0.42 mmol, 1.2 eq) in CH_2Cl_2 (1.0 mL) was added **55** (71 mg, 0.29 mmol, 1.2 eq). The resulting mixture was stirred overnight at room temperature. Then, diluted with CH_2Cl_2 and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc, 7:3) provided 38 mg (34%) of **129** as a semi-solid.

^{1}HNMR (300 MHz, $CDCl_3$ vs TMS): δ 1.42 (9H, s); 2.12/2.18 (3H, s); 2.28 (3H, s); 3.02 (3H, s); 3.48 (1/2H, m); 3.62 (1/2H, m); 3.80 (1H, m); 4.42 (1H, m); 5.0 (1H, br, D_2O exchanged); 5.14/5.16 (2H, s); 7.31 (5H, m); 12.48 (1/2H, br, D_2O exchanged); 12.68 (1/2H, br, D_2O exchanged) ppm;

IR (NaCl, film): 3364, 2975, 2929, 1701, 1625, 1542, 1400, 1314, 1248, 1162 cm^{-1}.

$[\alpha]_D^{25} = 0$ (c 1.0, CH_2Cl_2);

HRMS(DCI): Calcd for ($C_{21}H_{30}N_4O_4S$ + H) = 447.1981. Found: (M + H) = 447.1969.



Cyclization product 130.

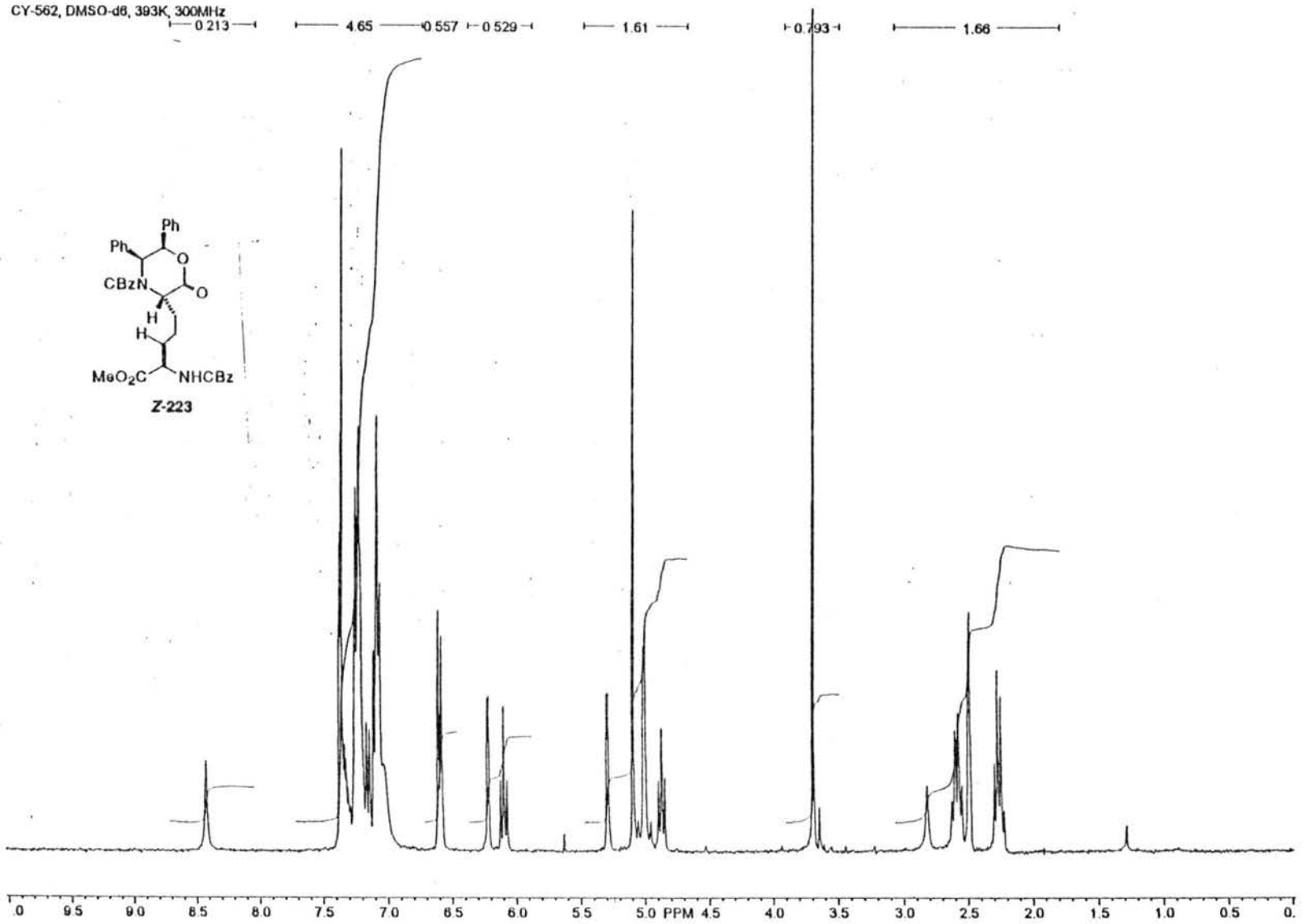
To a mixture of **129** (8.0 mg, 0.017 mmol, 1.0 eq) anisole (10 uL) was added TFA (1.0 mL). The resulting mixture was stirred for 20 min at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on vacuo for 2 h, triturated with dry ether to give a white solid. This white solid was dissolved in THF (1.5 mL). To this solution was added triethylamine (7.0 uL, 0.05 mmol, 3.0 eq). The resulting mixture was stirred for 10 min, concentrated and purified on PTLC (eluted with CH_2Cl_2:EtOAc:MeOH, 4:1:0.5) to give 4.0 mg (74 %) of **130** as a semi-solid.

^{1}HNMR (300 MHz, CD_3OD): δ 2.14 (3H, s); 2.94 (3H, s); 3.71 (2H, m); 4.80 (1H, m); 5.12 (2H, m); 7.33 (5H, m) ppm;

IR (NaCl, film): 3458, 2964, 1643, 1477, 1270, 1155, 1028 cm^{-1}.

$[\alpha]_D^{25}$ = - 7.0 (c 0.3, CH_3OH);

HRMS: Calcd. for $C_{15}H_{18}N_3O_4$: (M + H) = 319.1406. Found: (M + H) = 319.1404.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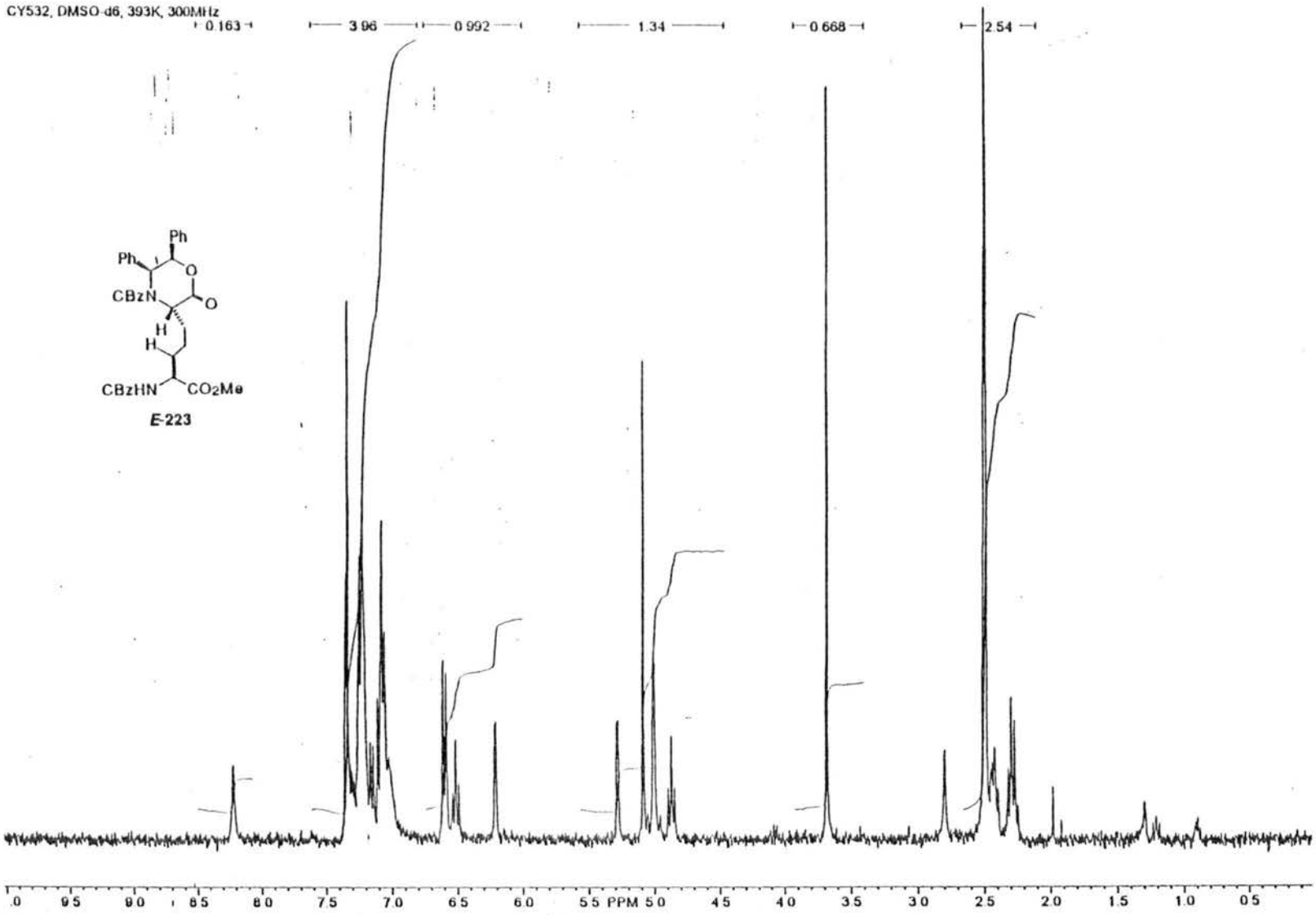
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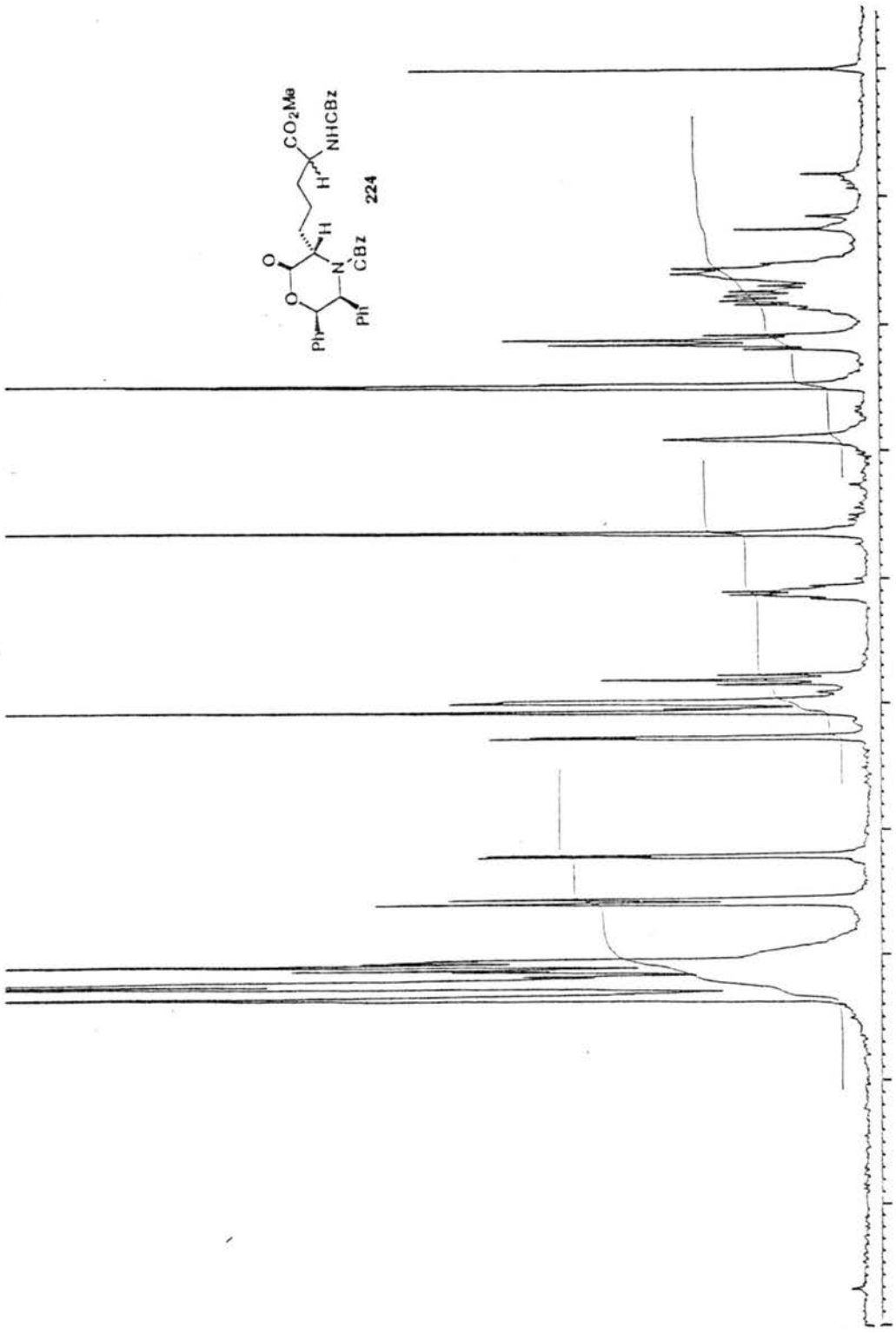
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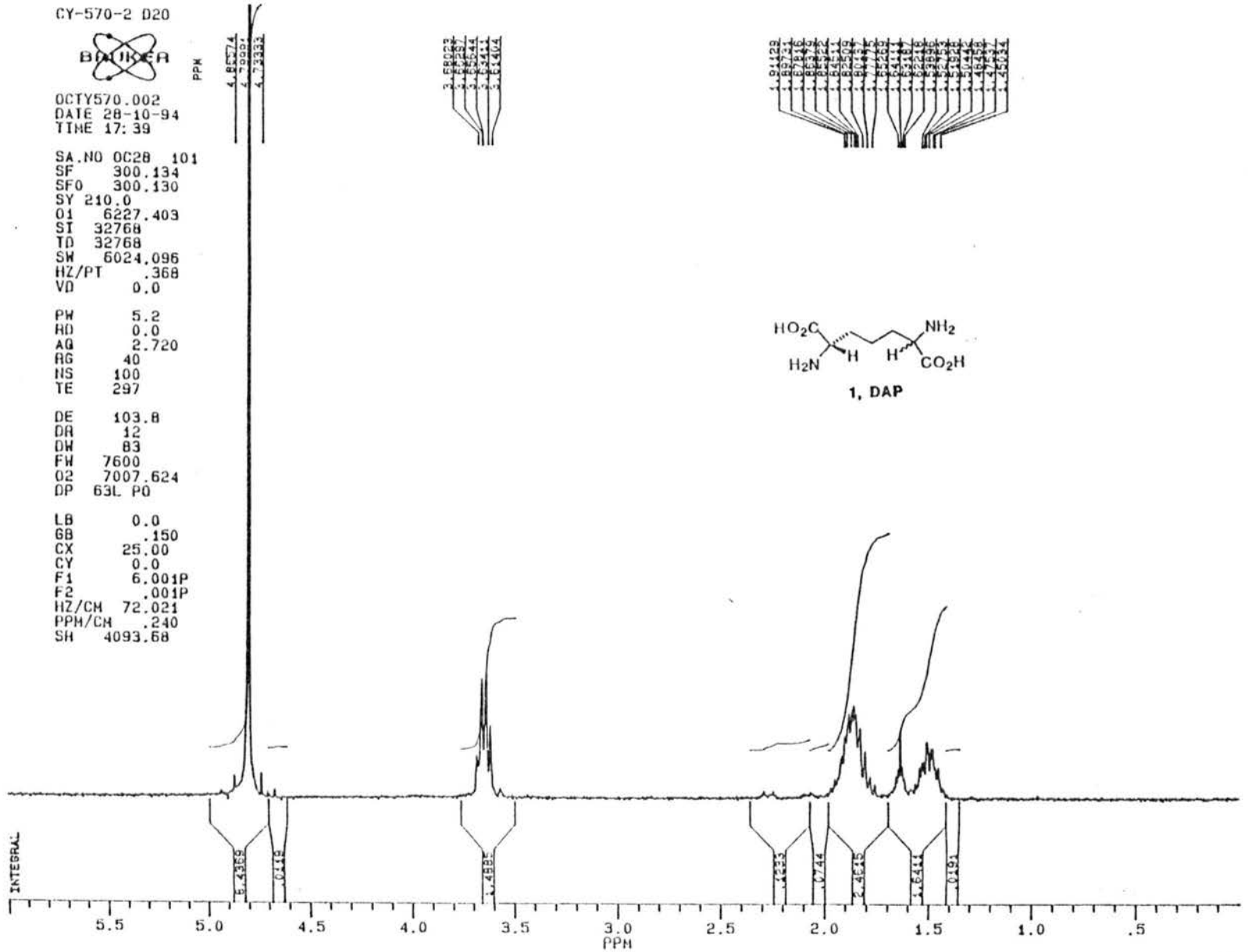
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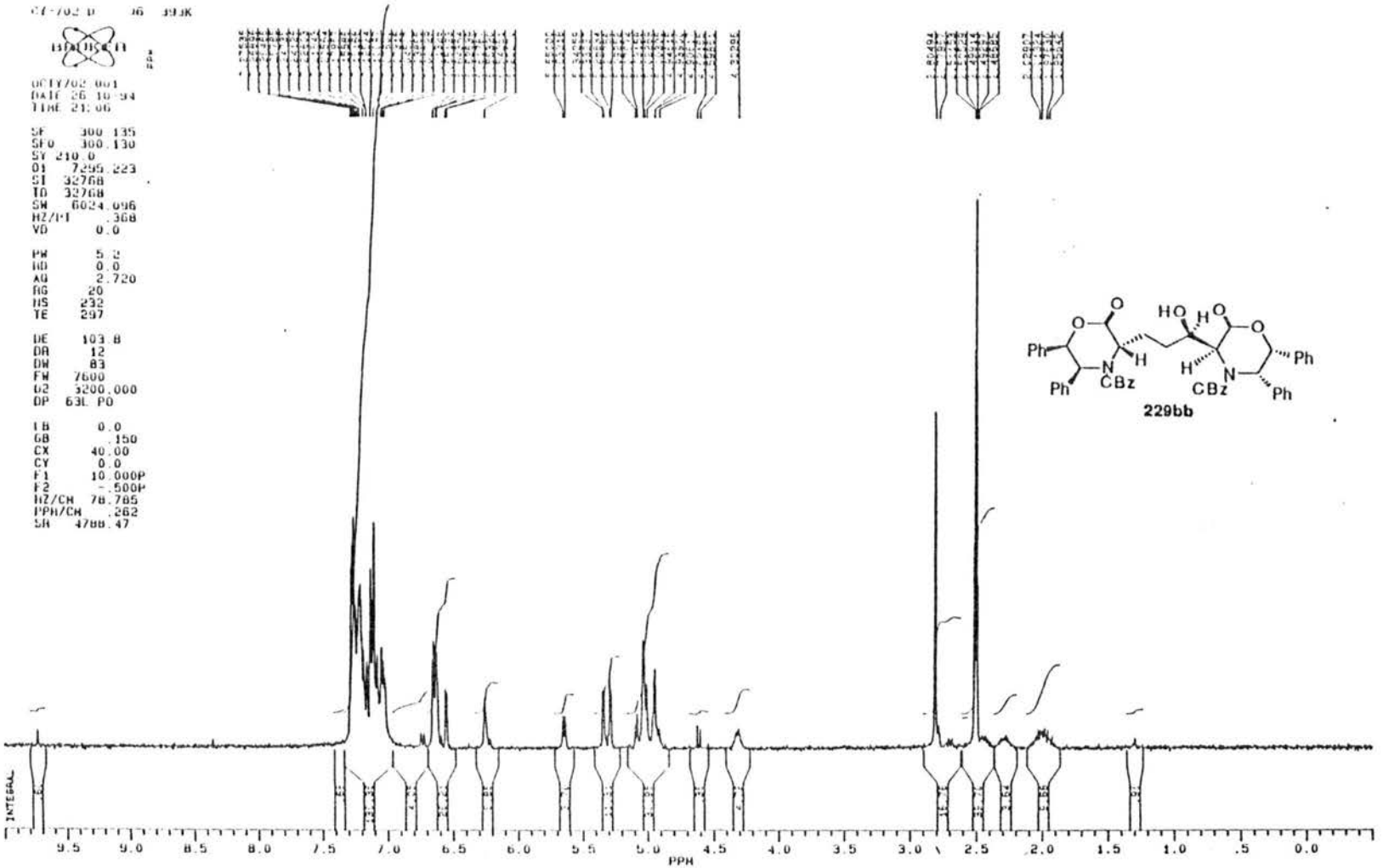
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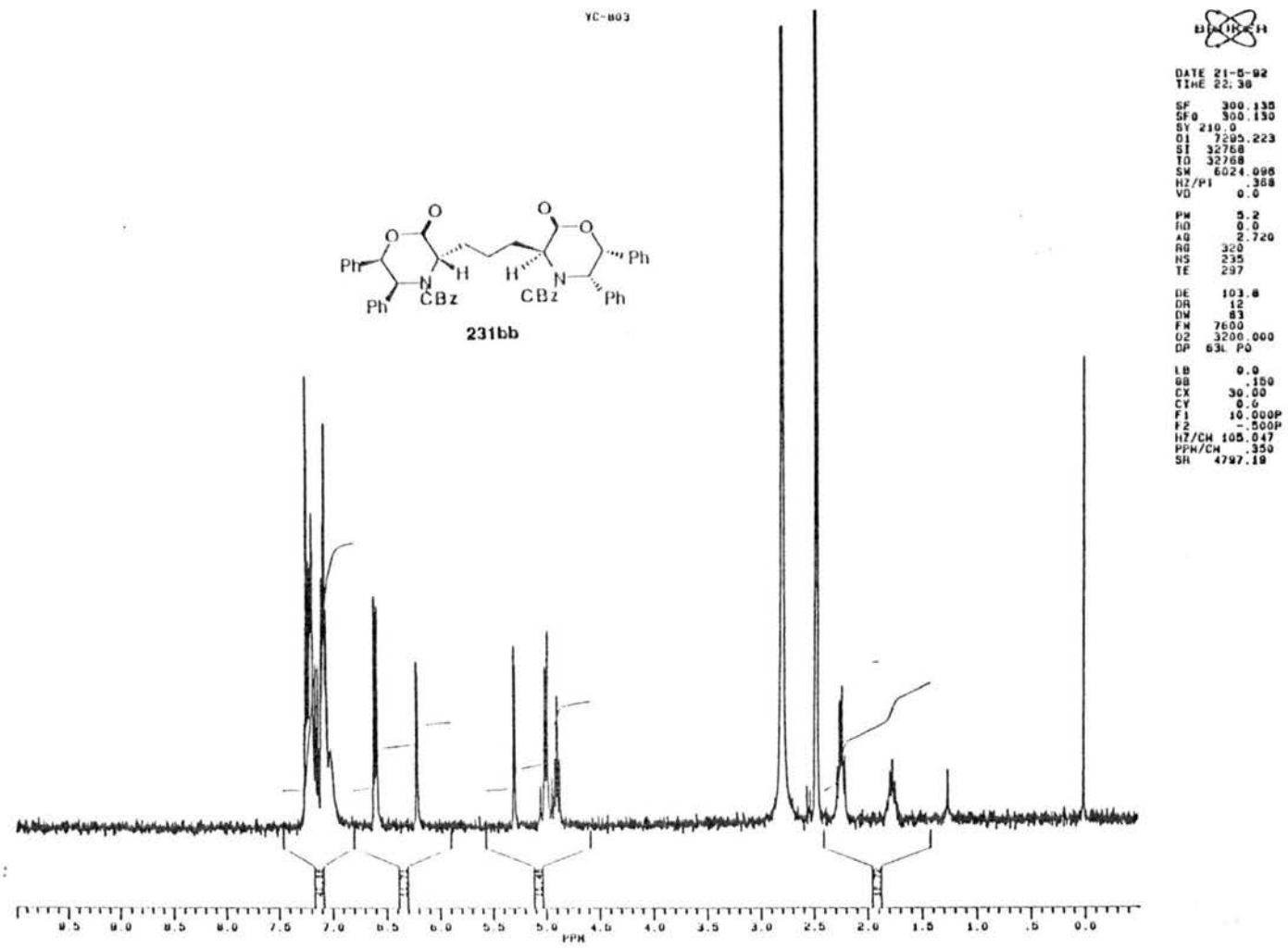
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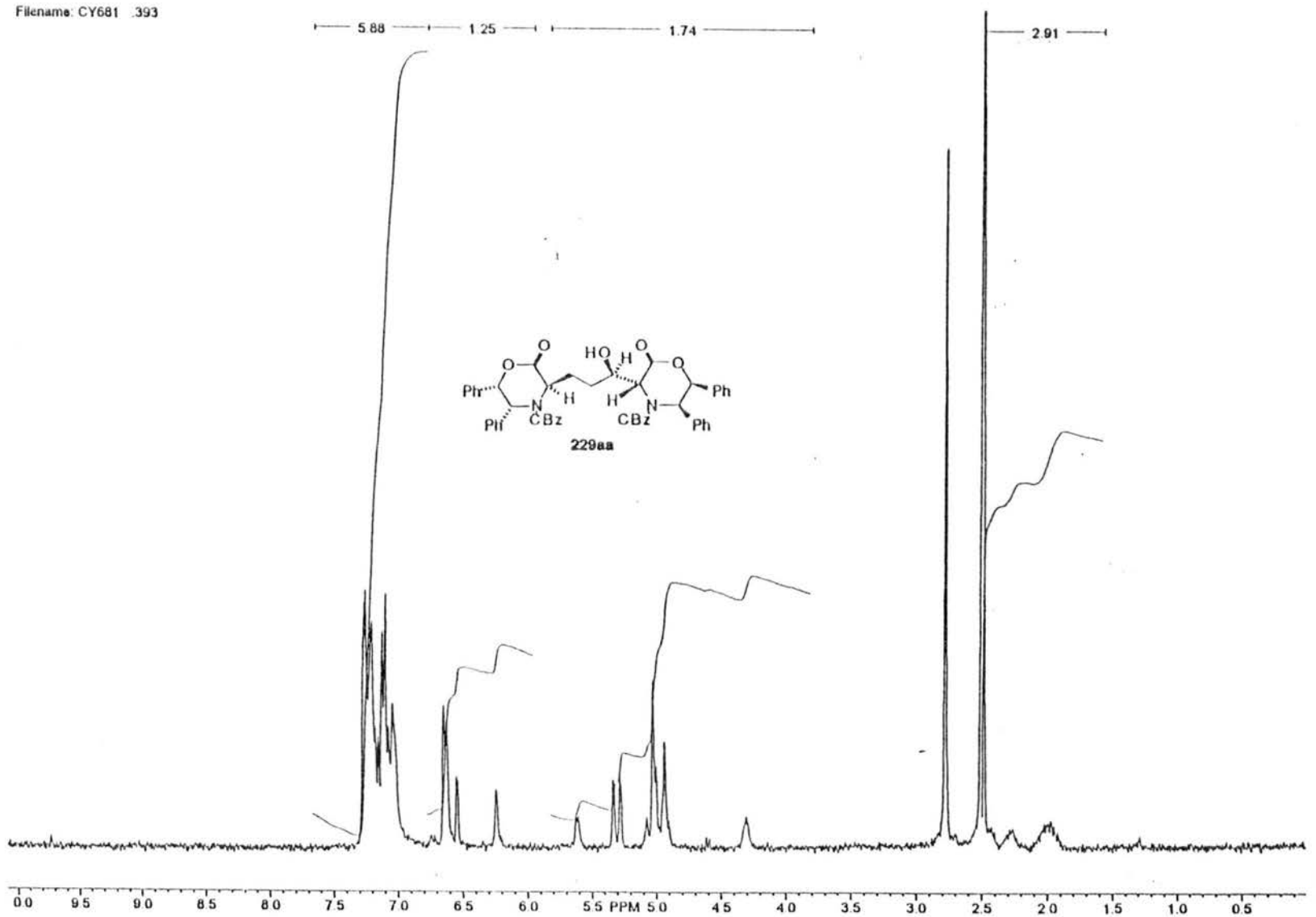
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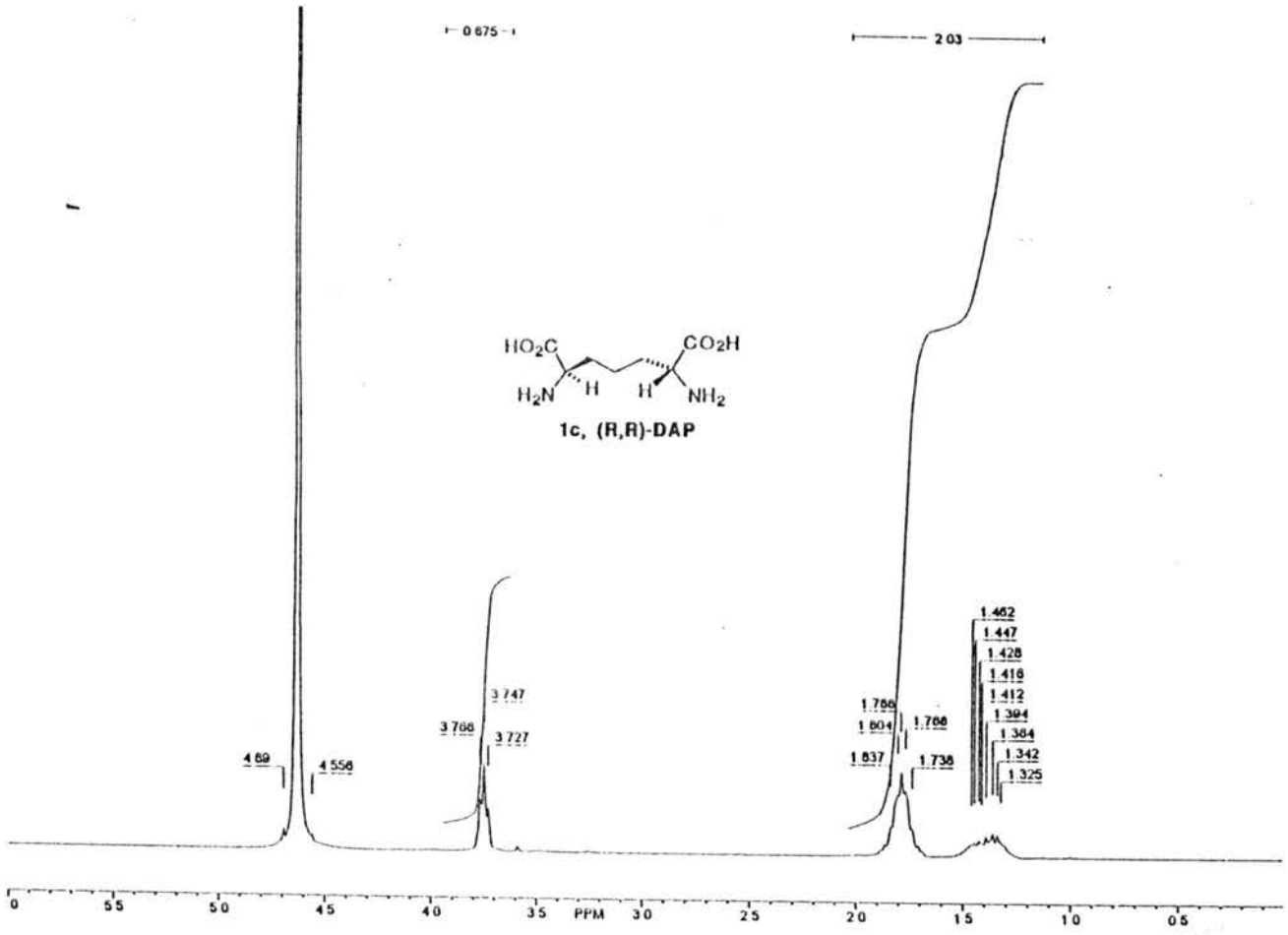
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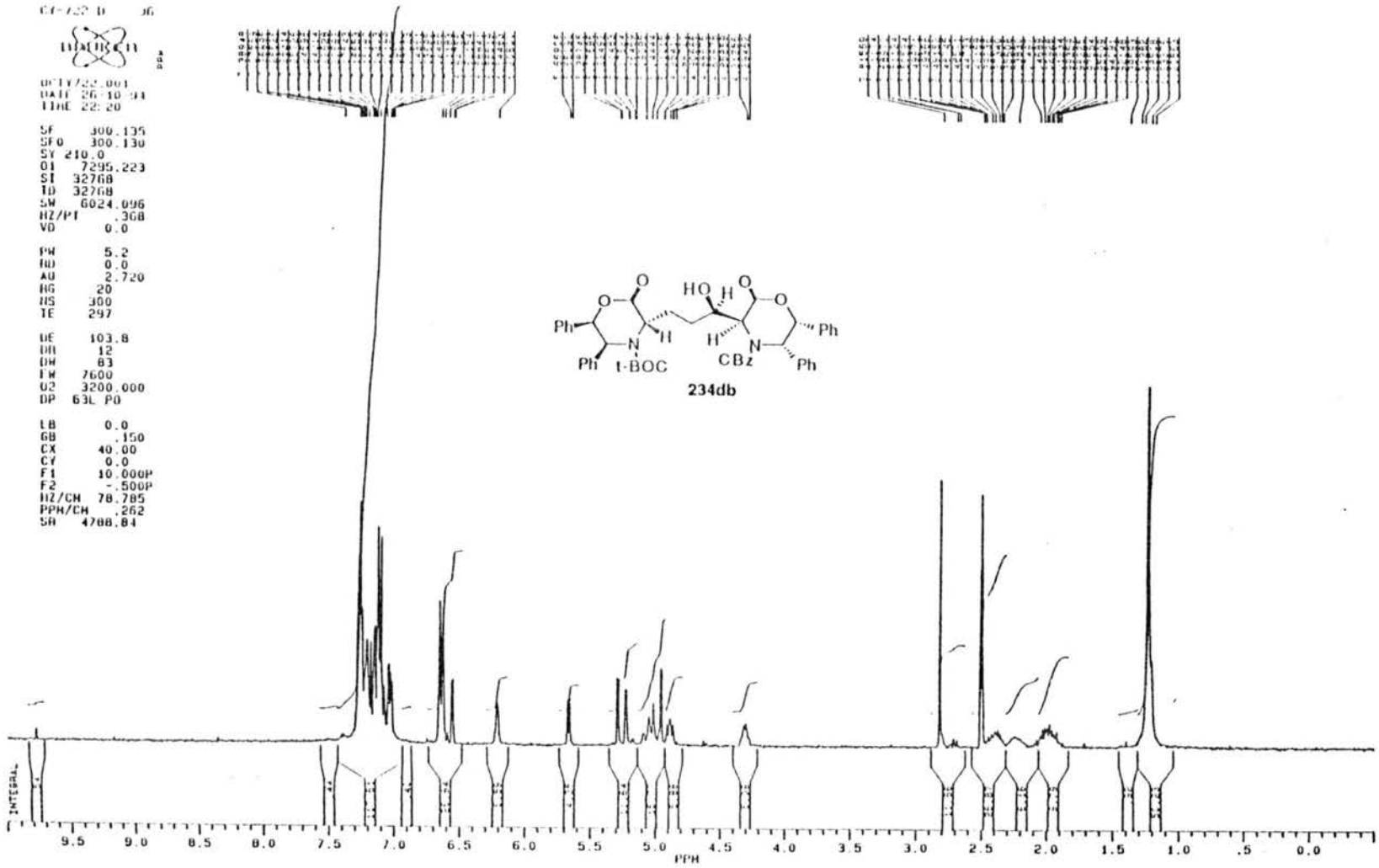
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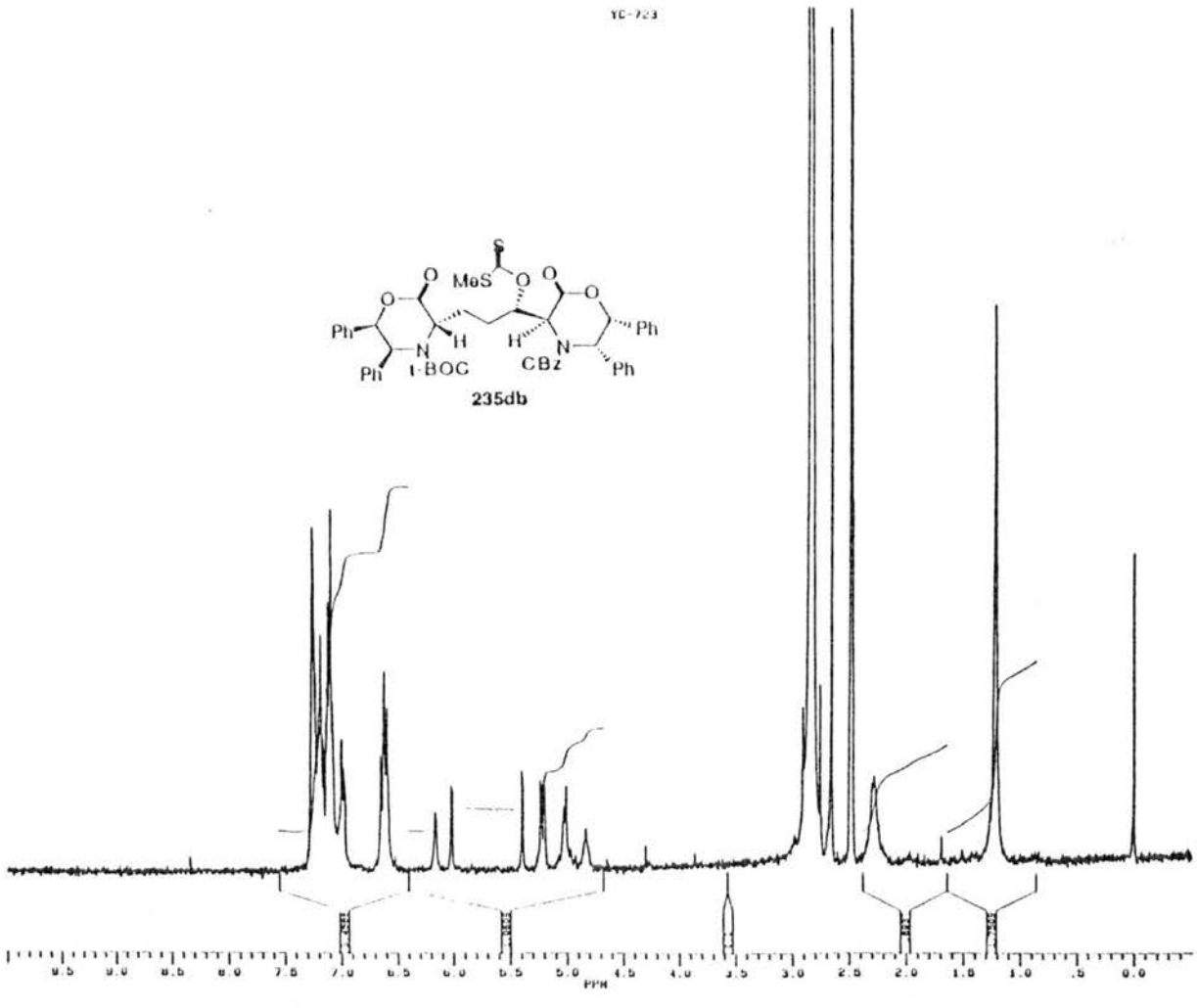
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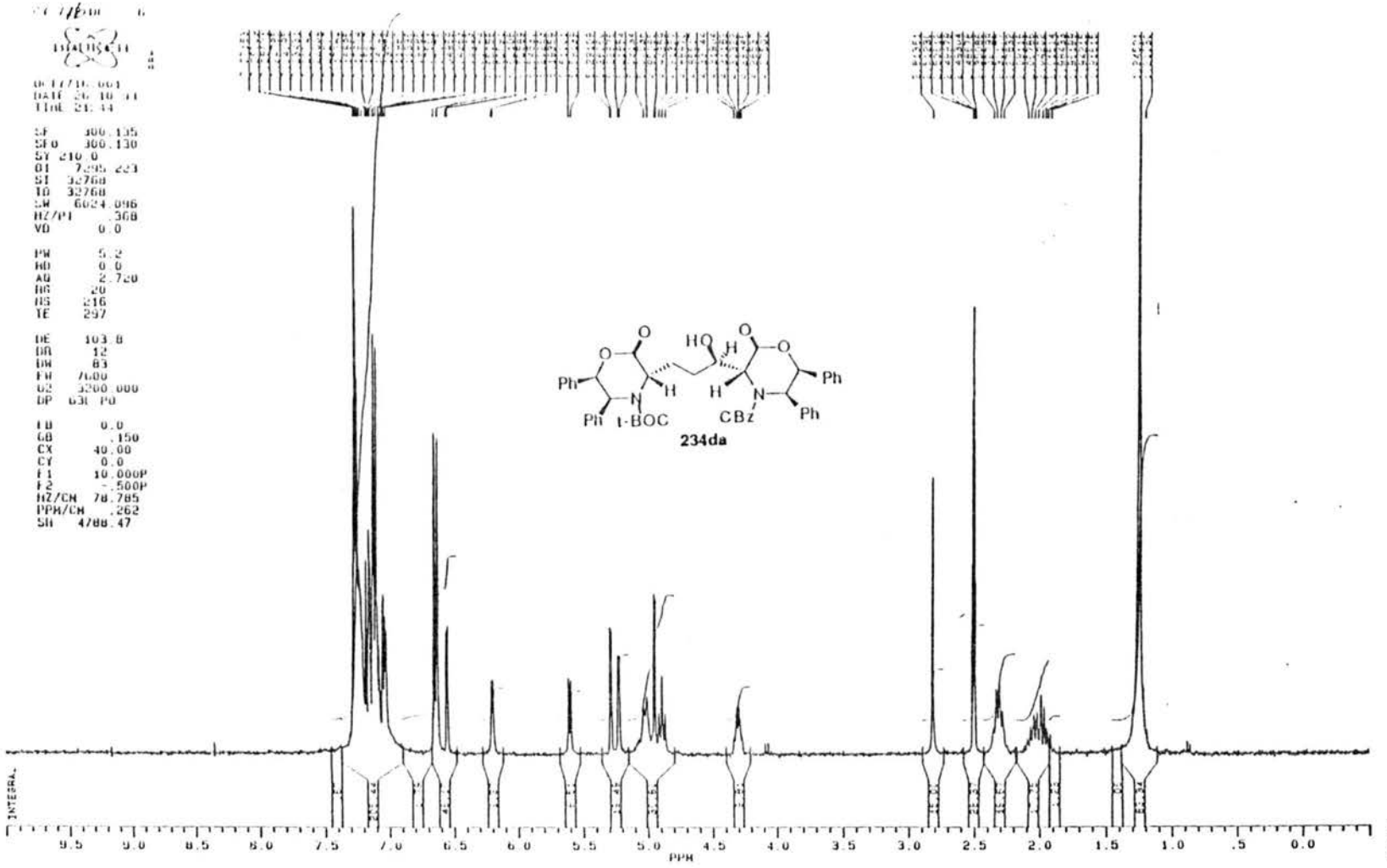
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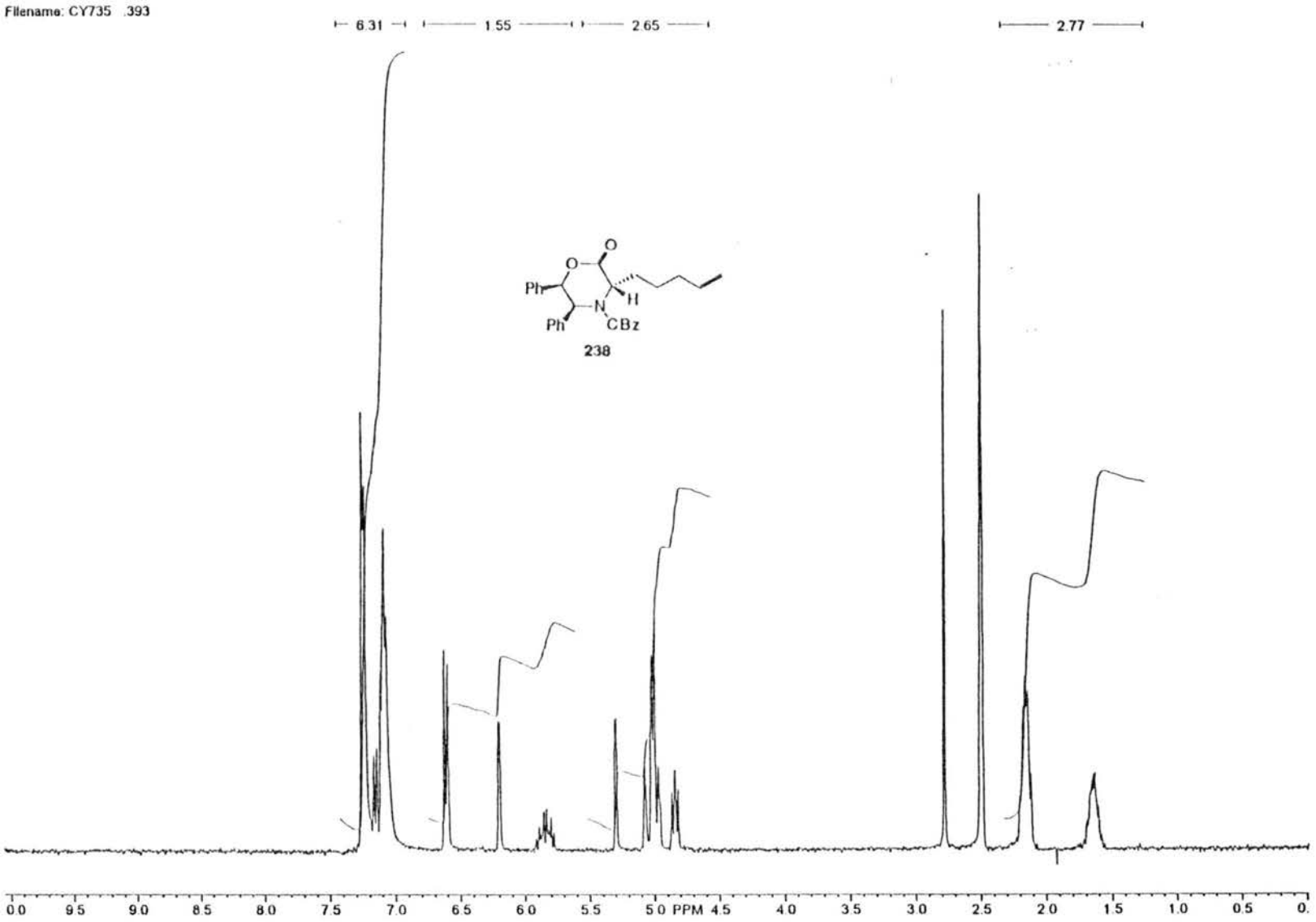
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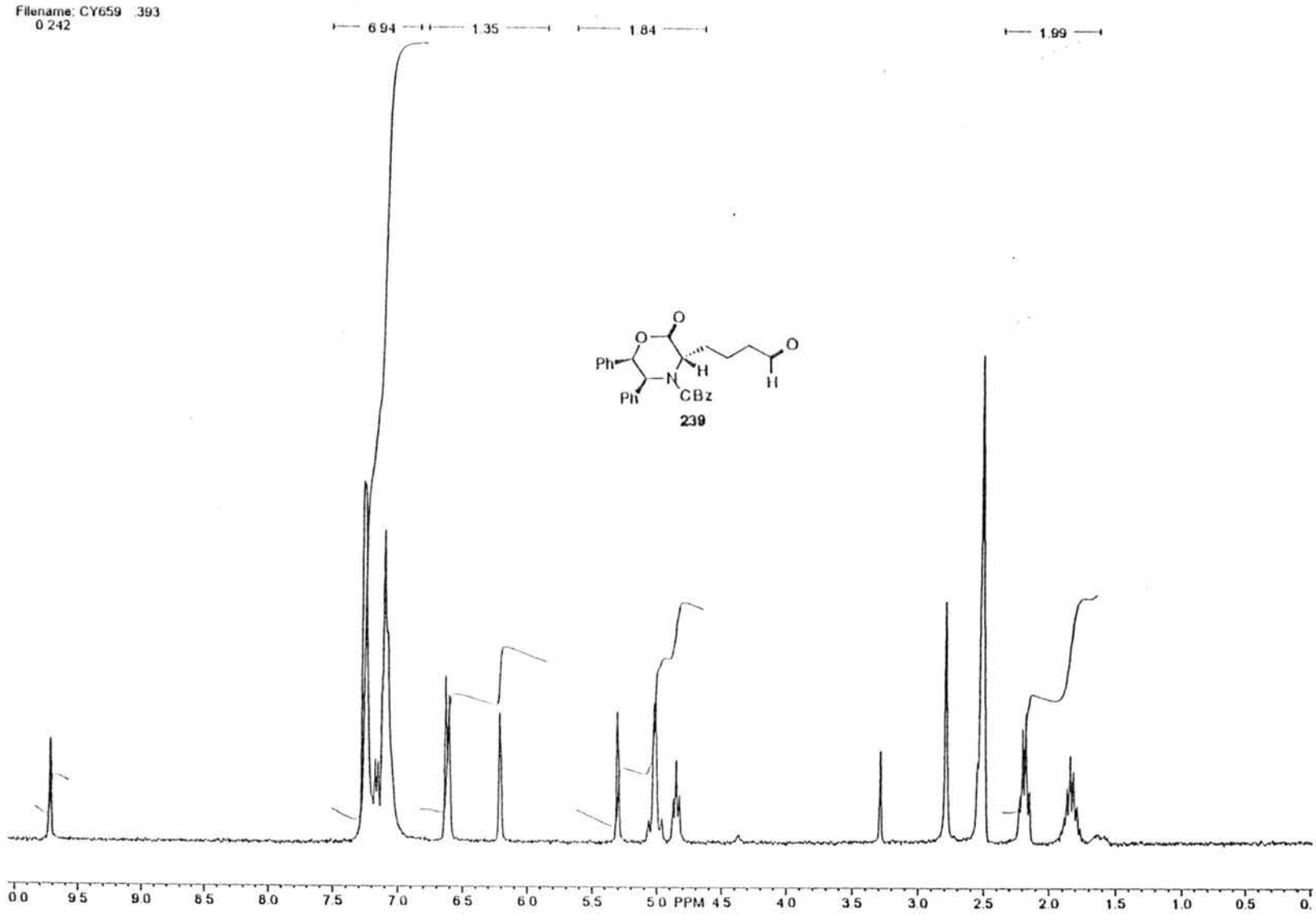
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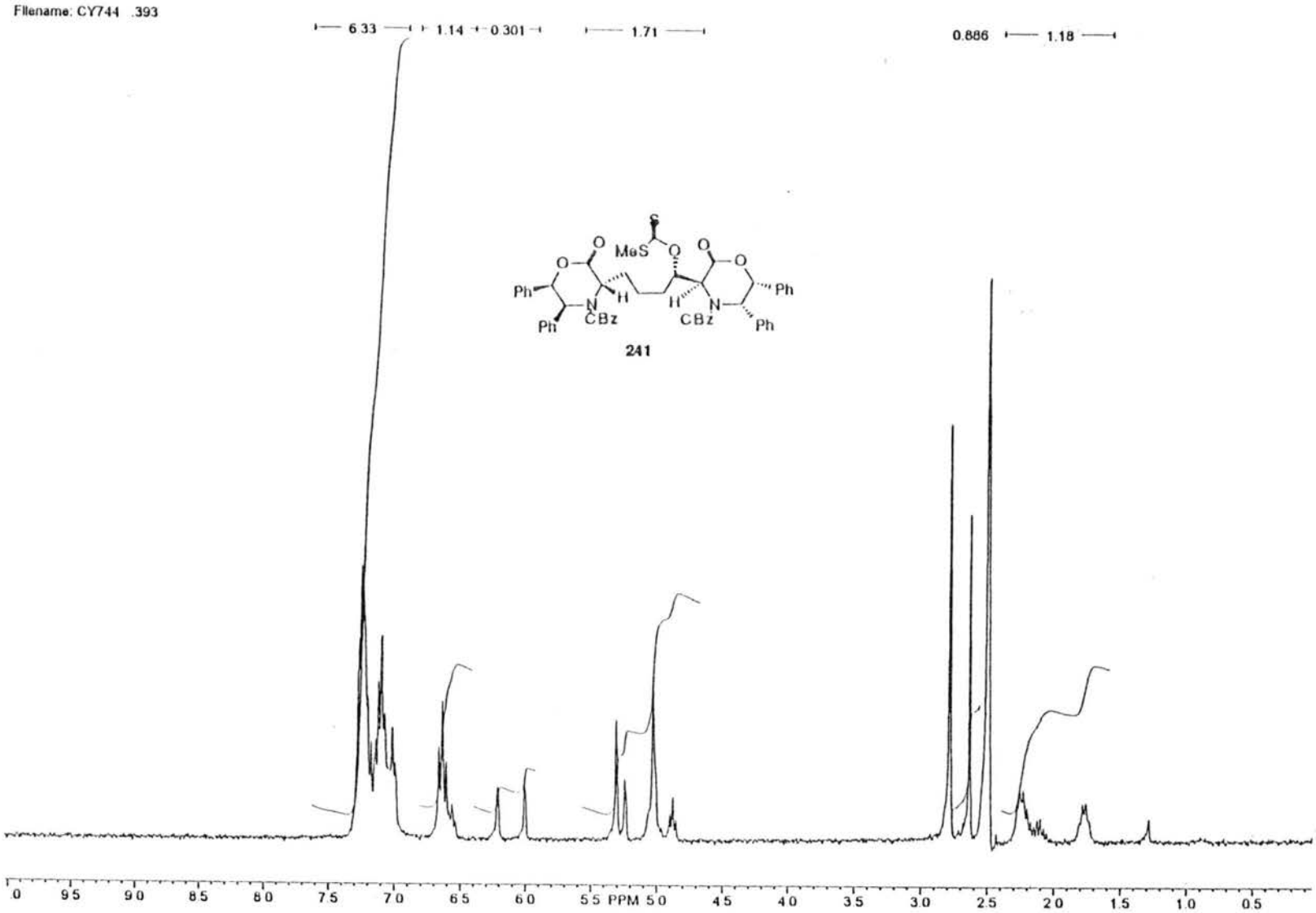
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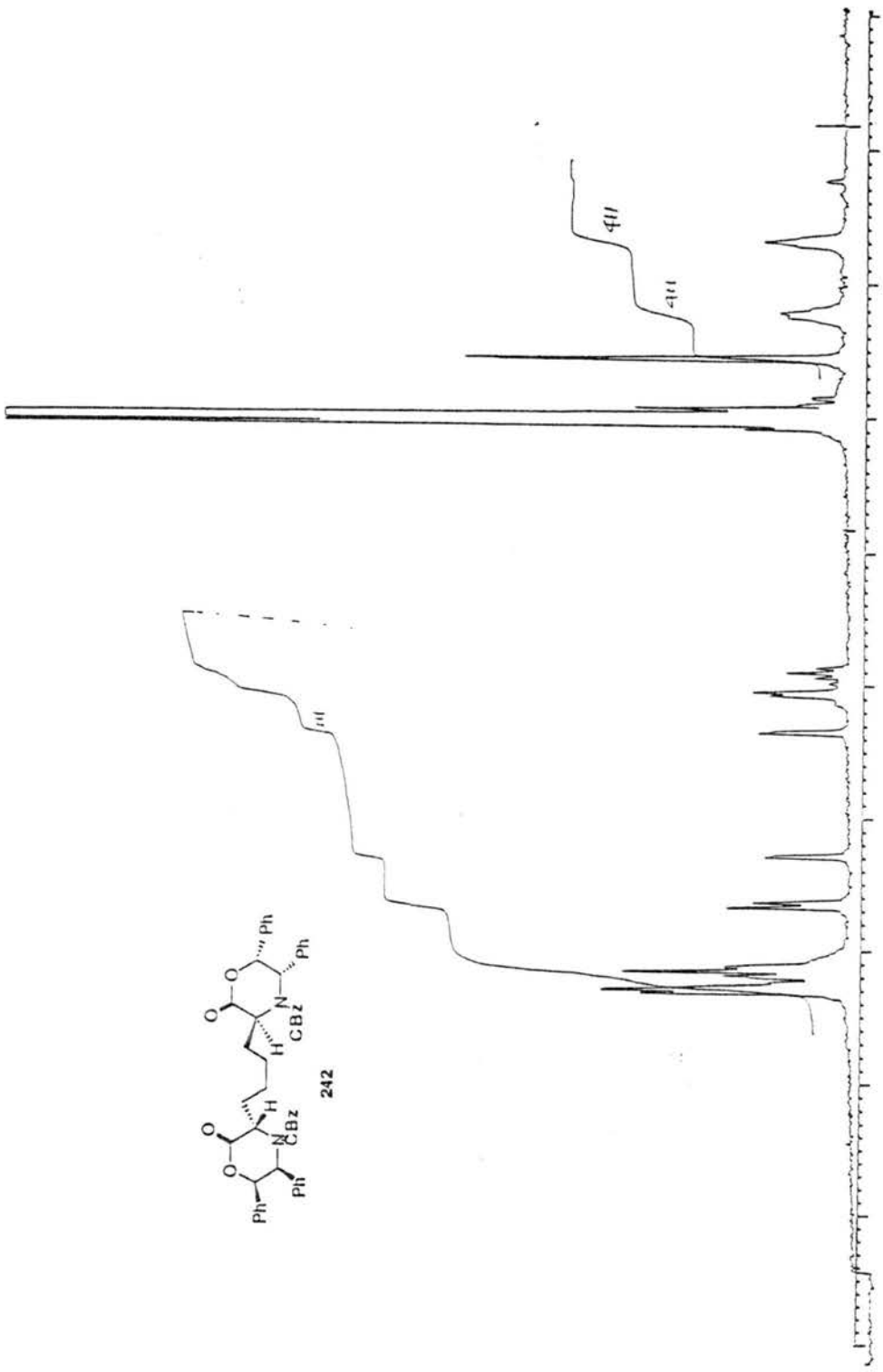
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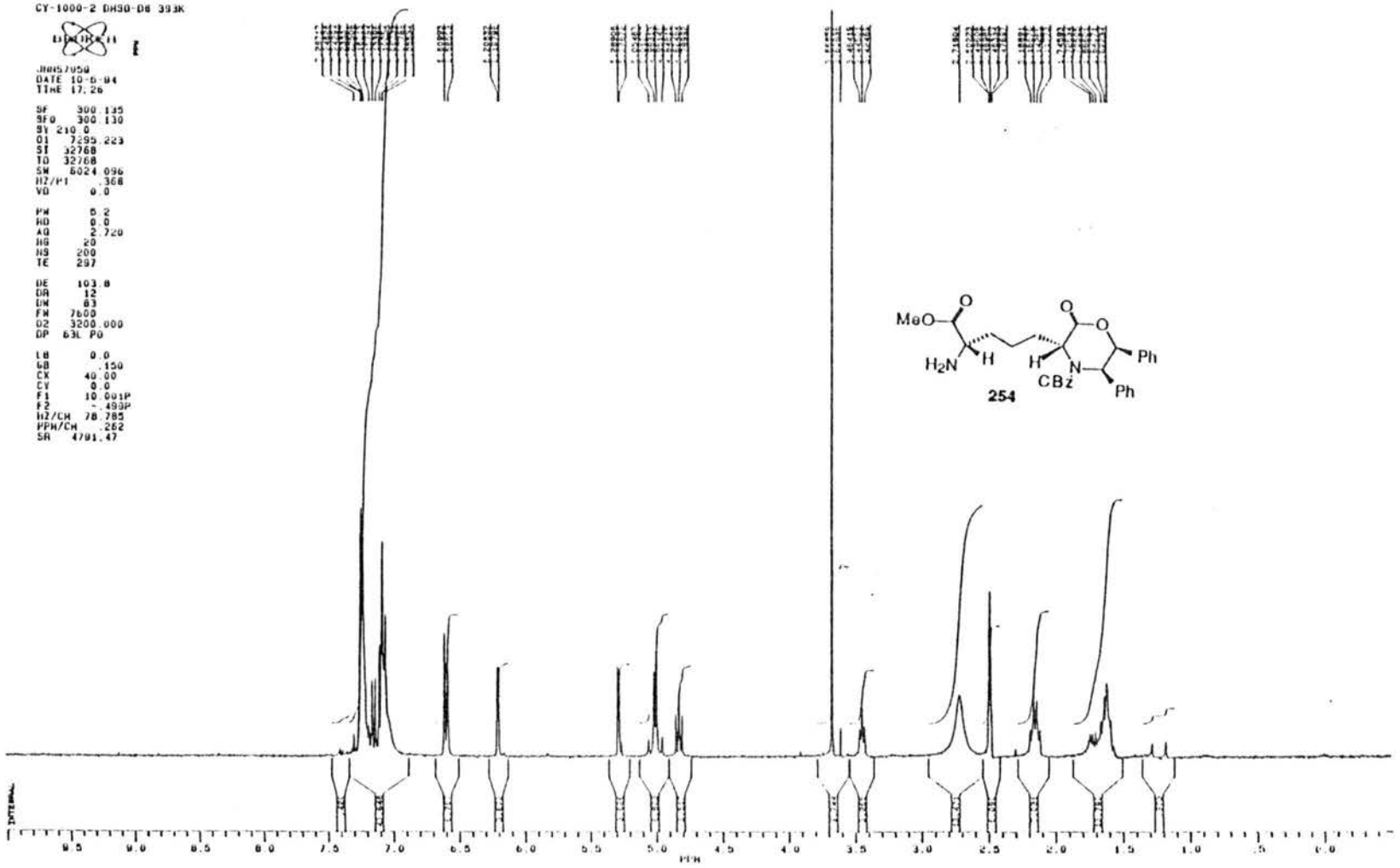
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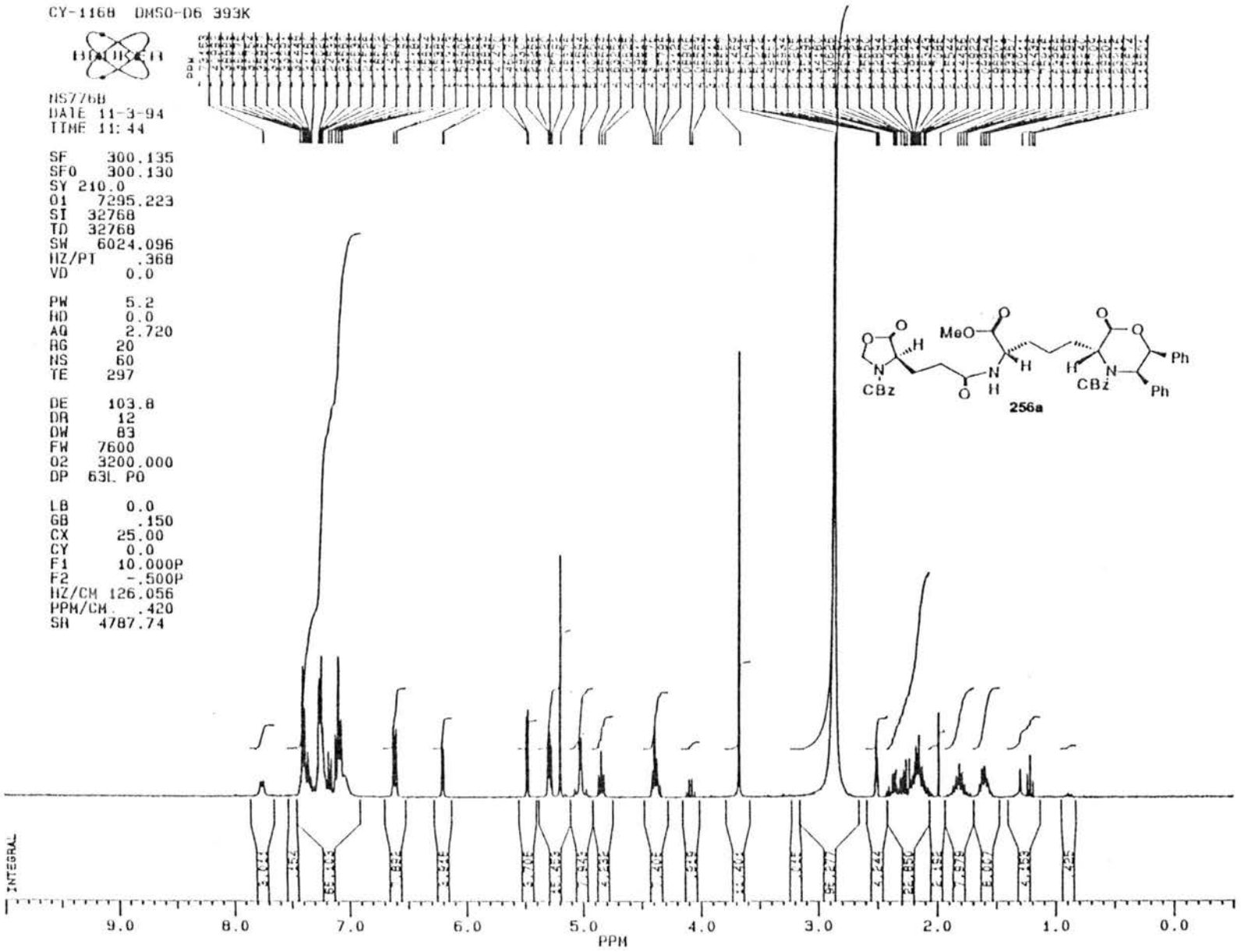
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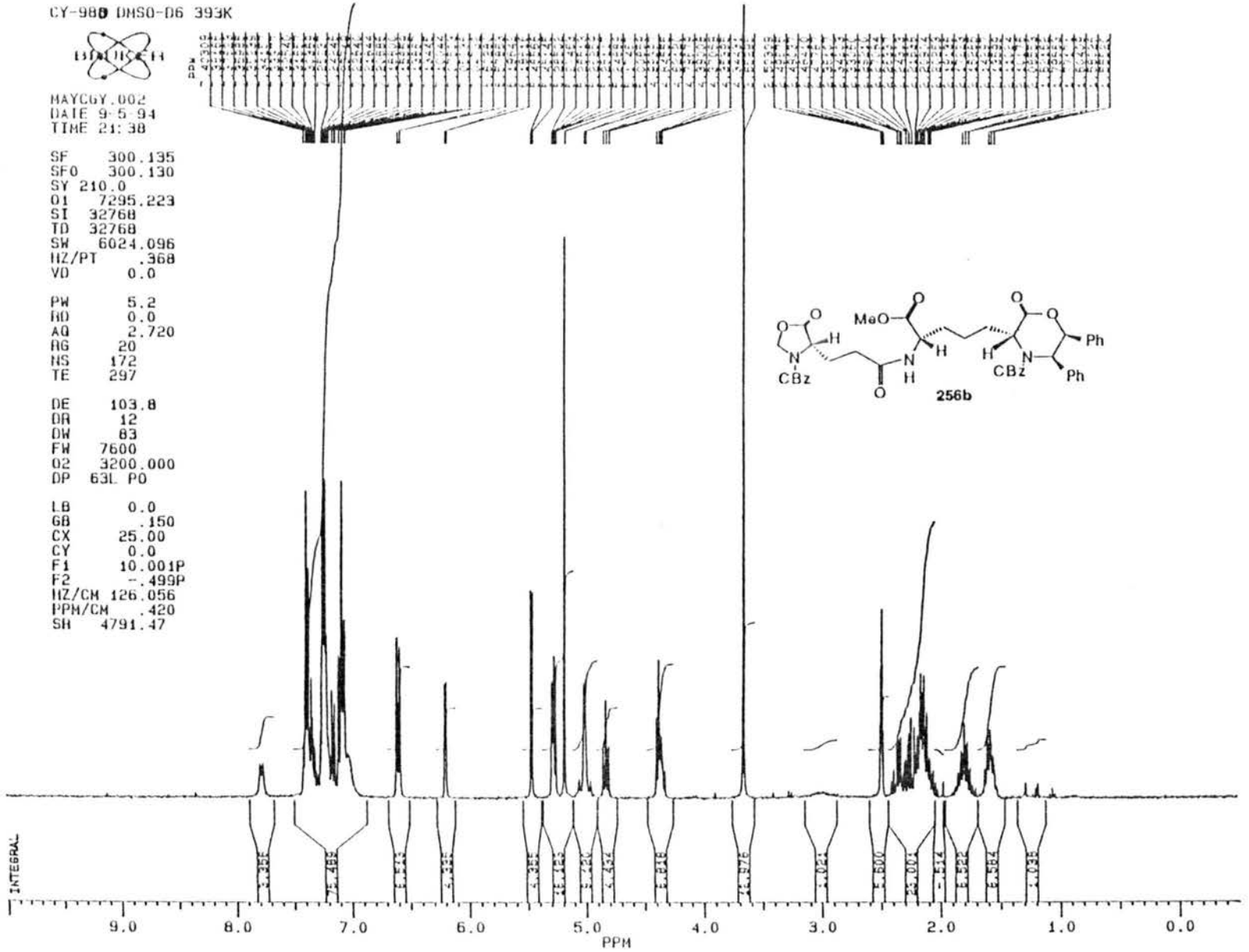
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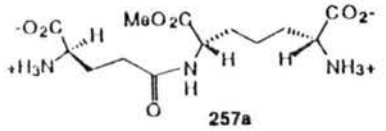

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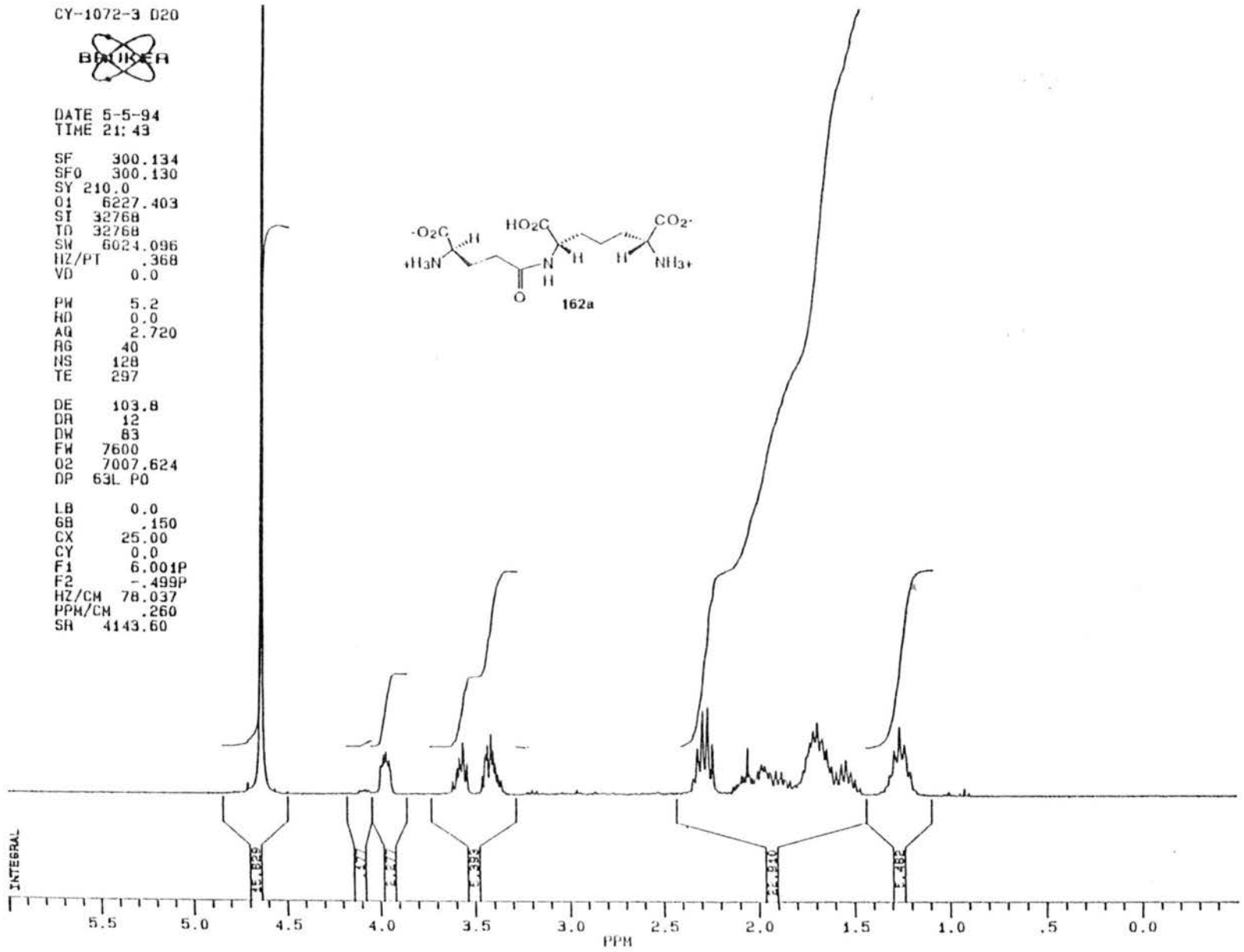
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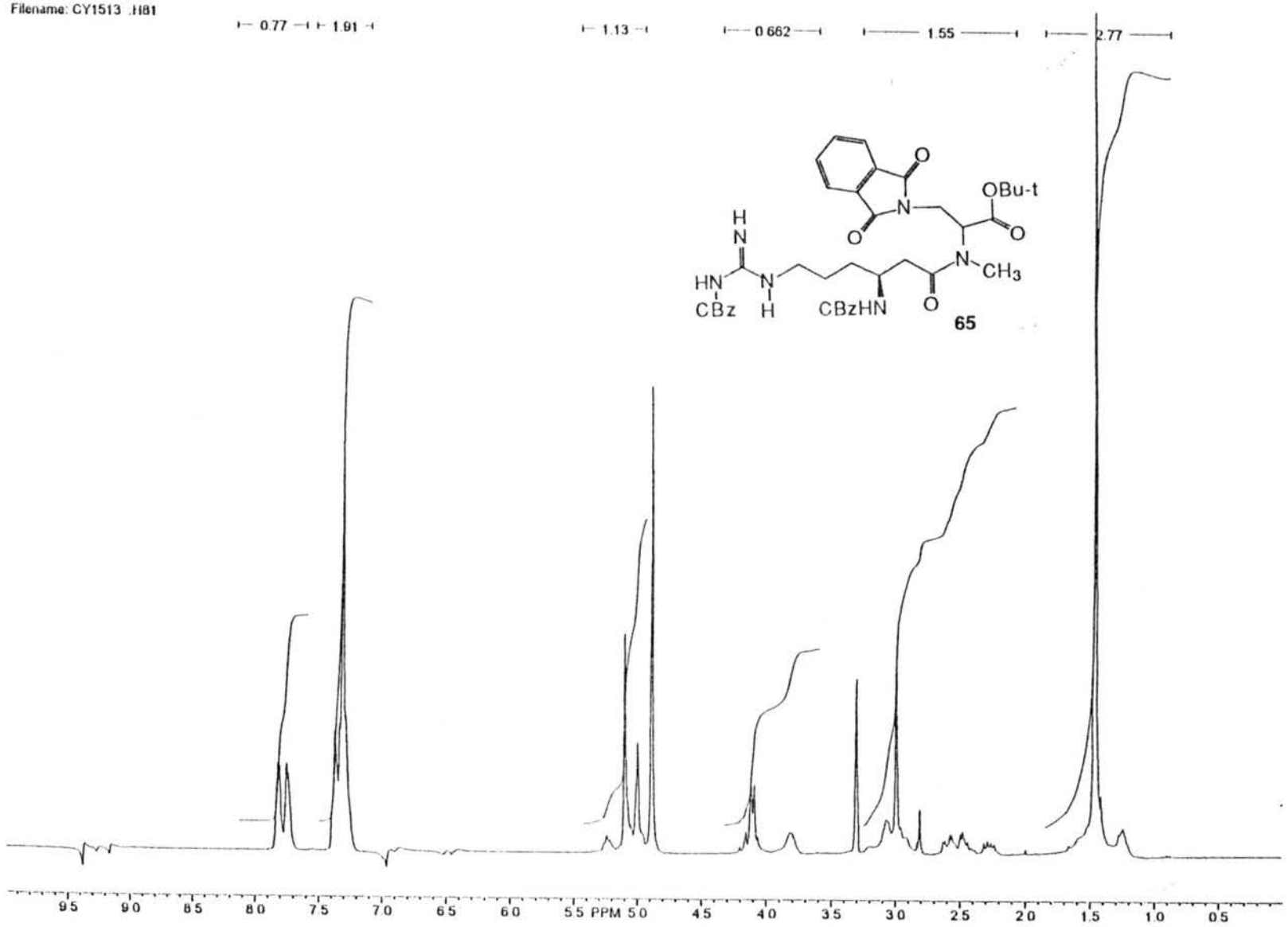
SW 6024.096
HZ/PT .368
VD 0.0
PW 5.2
RD 0.0
AQ 2.720
RG 40
NS 128
TE 297
DE 103.8
DA 12
DW 83
FW 7600
O2 7007.624
DP 63L PO
LB 0.0
GB .150
CX 25.00
CY 0.0
F1 6.001P
F2 -.499P
HZ/CM 78.037
PPM/CM .260
SR 4143.60
-O2C
+H3N
HO2C
CO2-
NH3+
162a
INTEGRAL
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
0.0
PPM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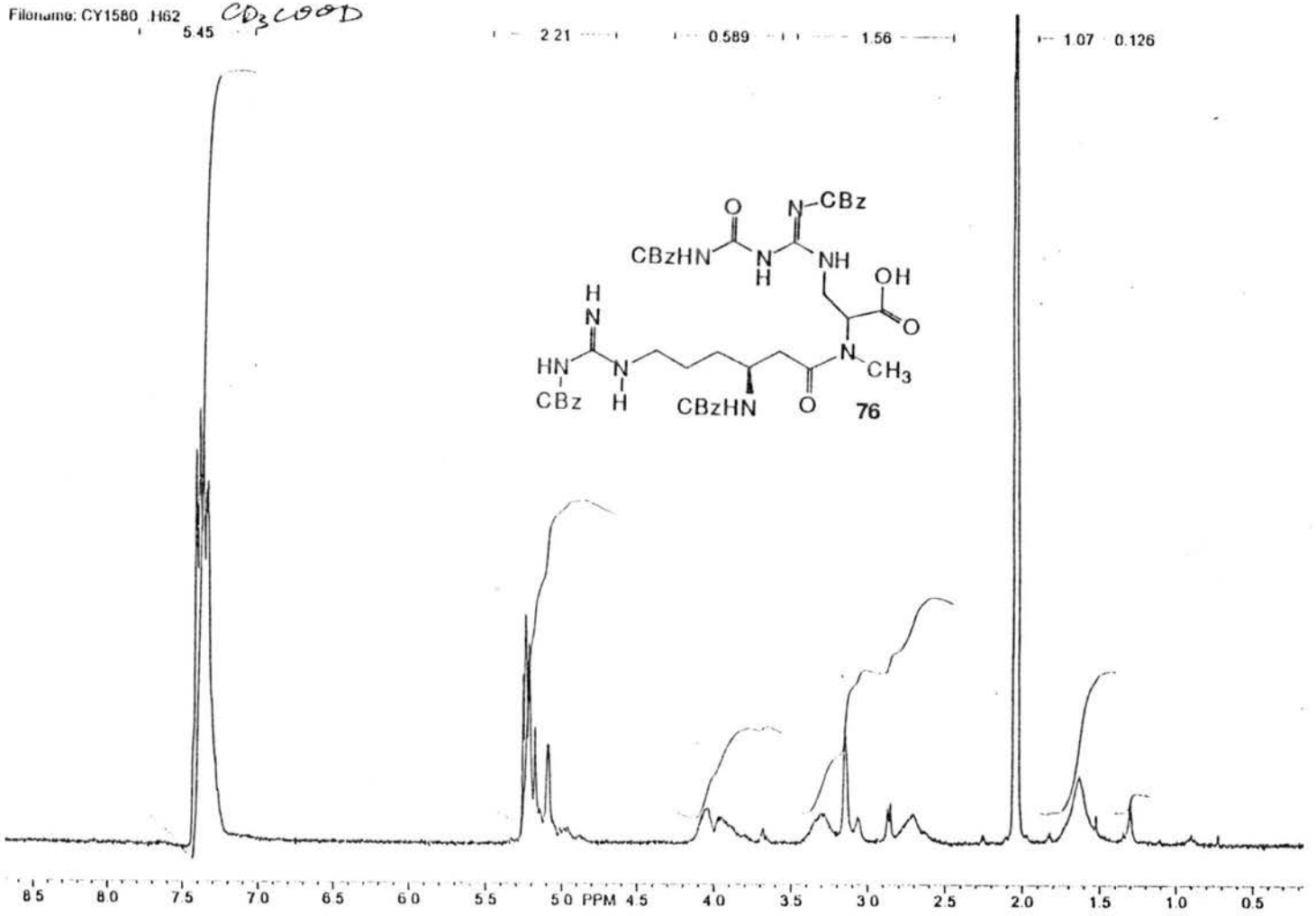
0.77
1.91
1.13
0.662
1.55
2.77
OBu-t
HN
CBz
H
N
H
CBzHN
N
CH3
65
95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 1.5 1.0 0.5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CD_3COOD
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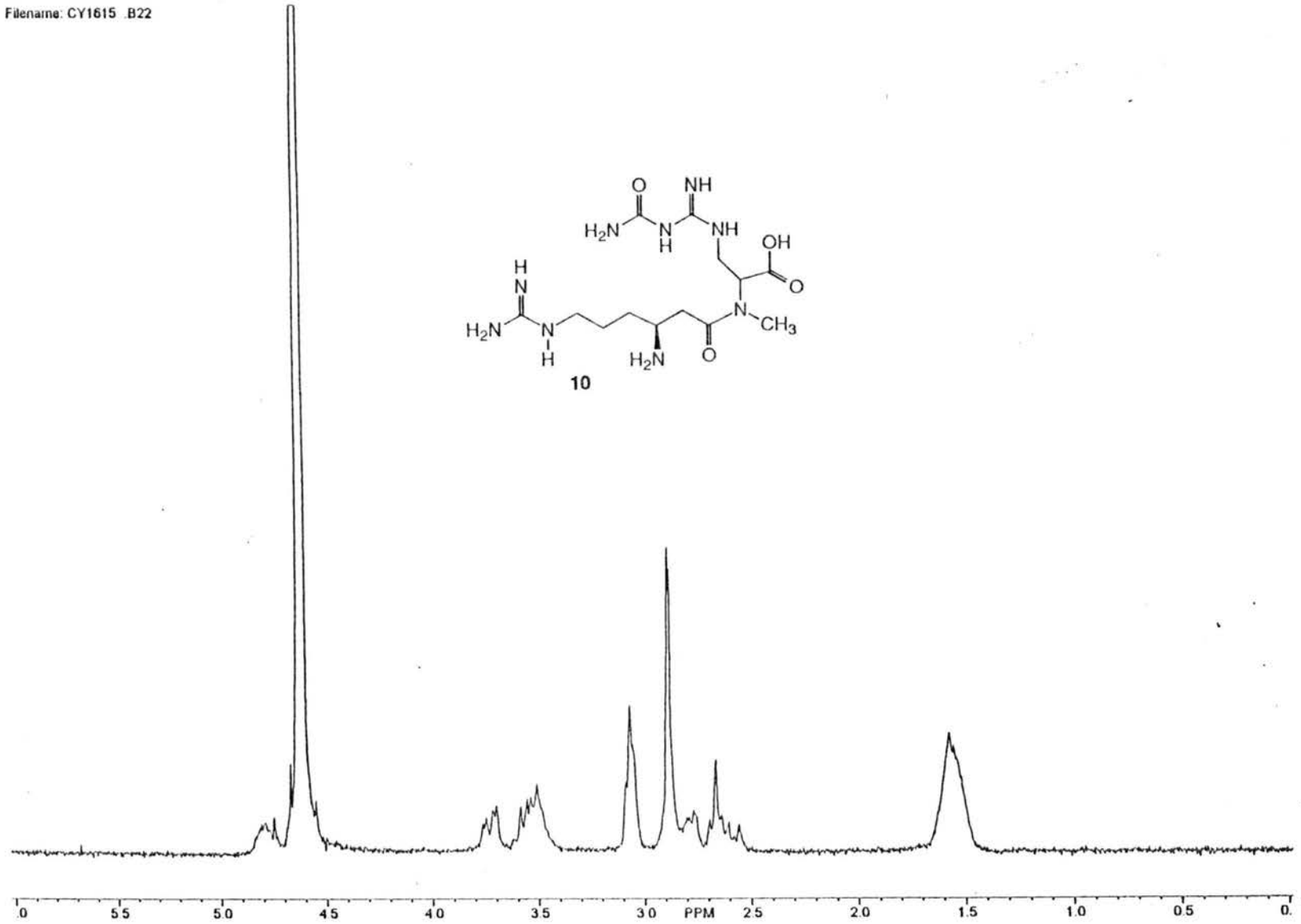
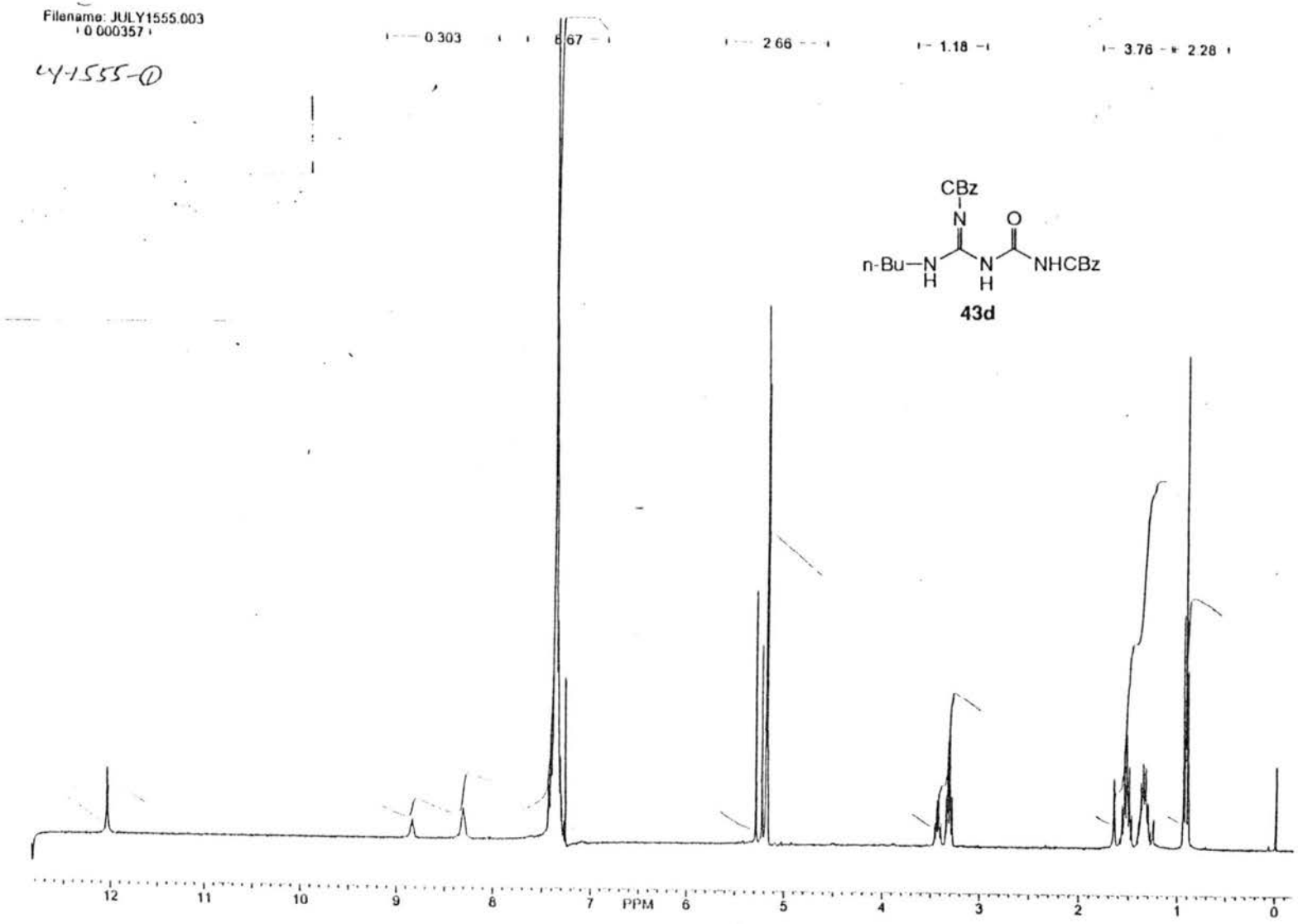
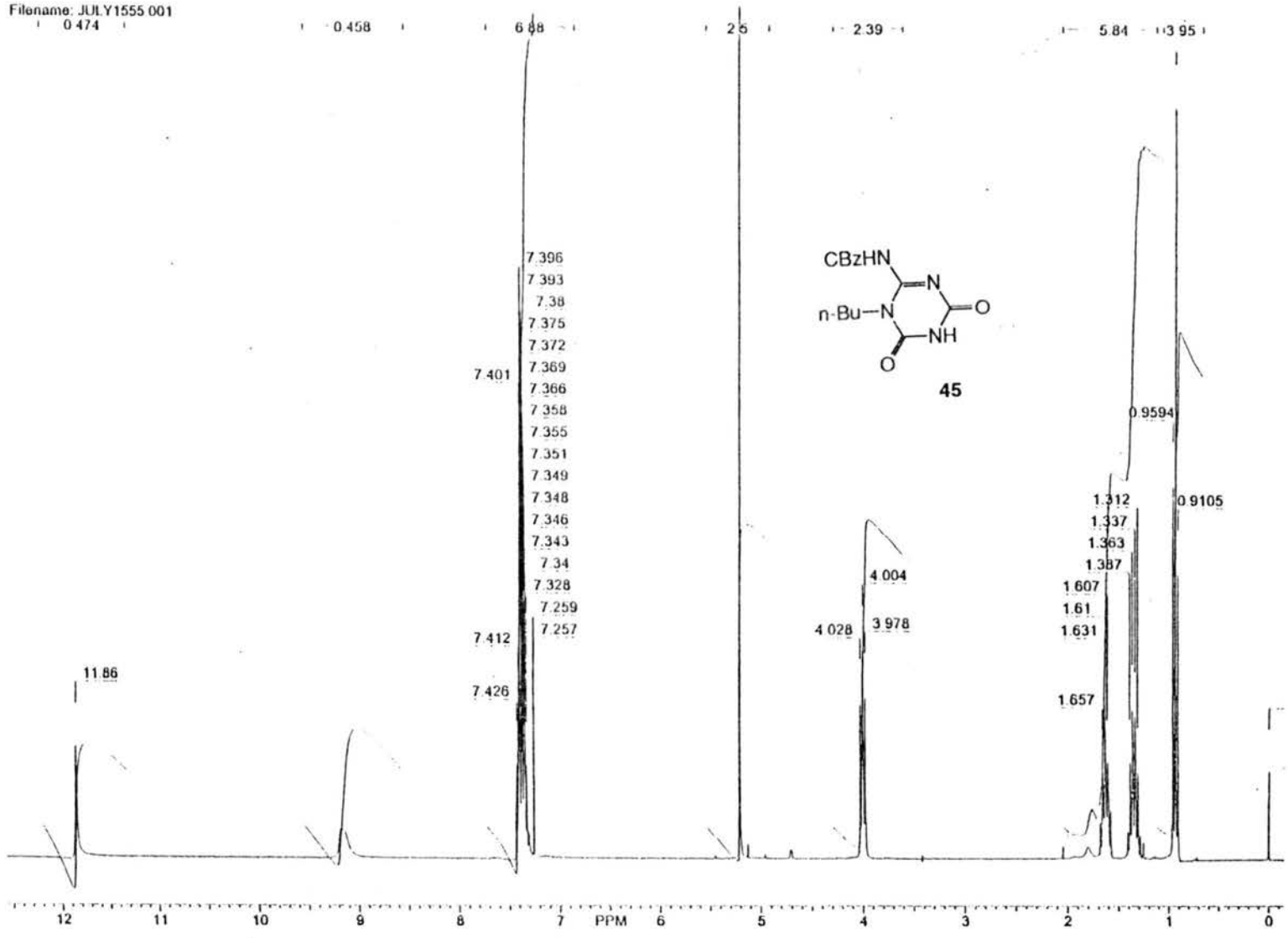
2.21
0.589
1.56
1.07
0.126
CBzHN
N–CBz
NH
OH
HN
CBz
CBzHN
CH_3
76
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0 PPM 4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5


Filename: CY1615 .B22
10
.0
5.5
5.0
4.5
4.0
3.5
3.0
PPM
2.5
2.0
1.5
1.0
0.5
0.


Filename: JULY1555.003
0.000357
0.303
8.67
2.66
1.18
3.76
2.28
CY-1555-①
CBz
N
O
n-Bu—N
H
N
H
NHCBz
43d
12
11
10
9
8
7
PPM
6
5
4
3
2
1
0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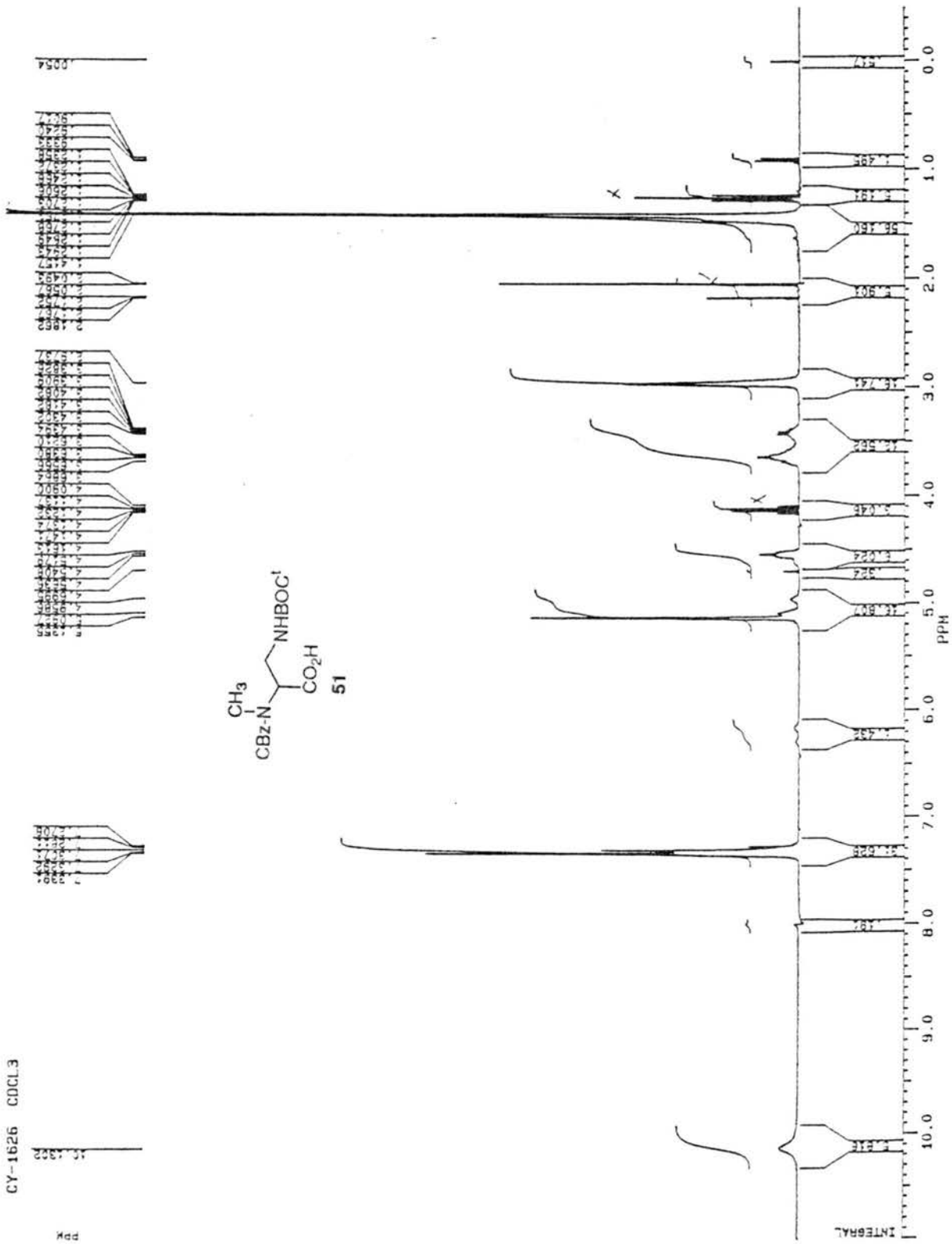
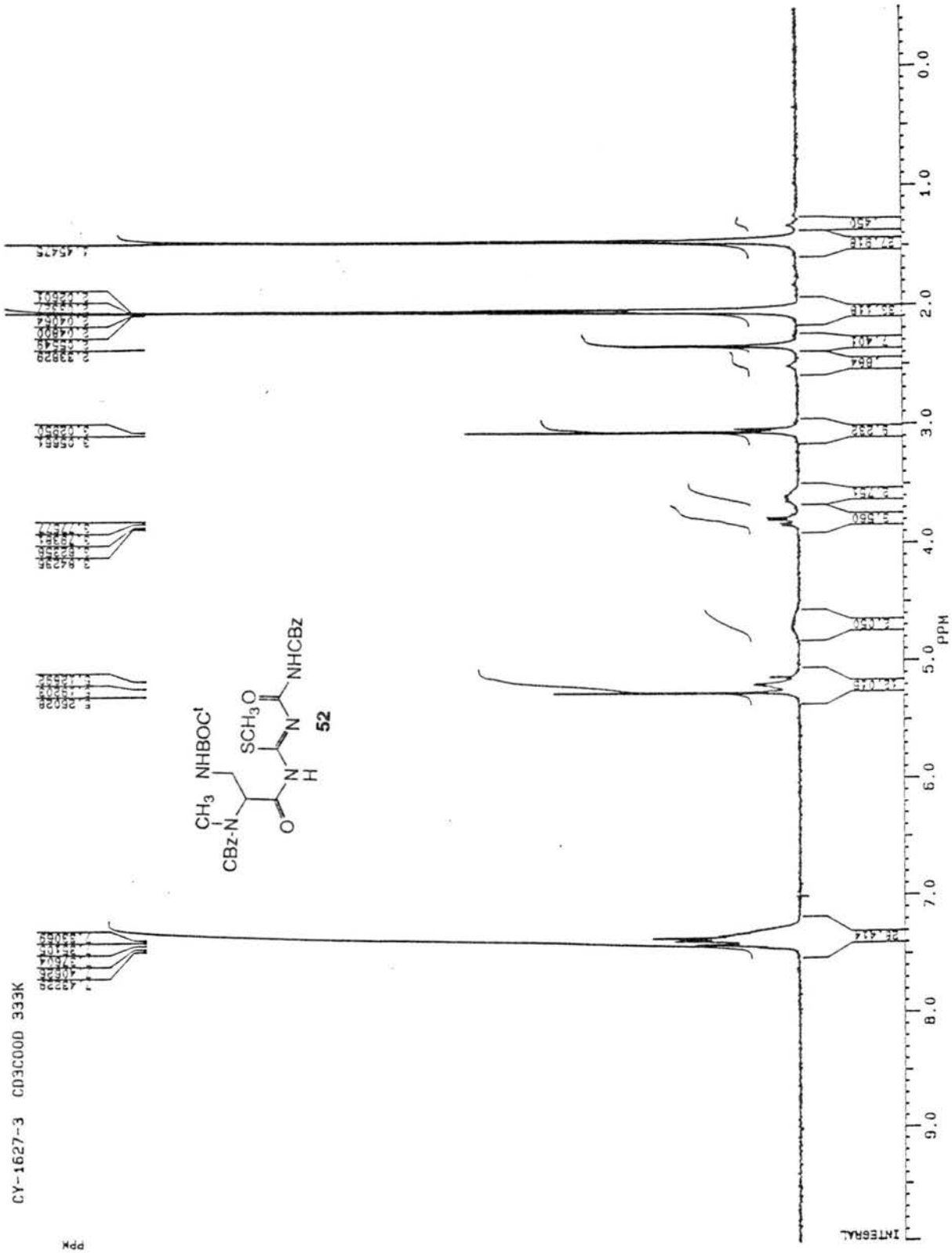
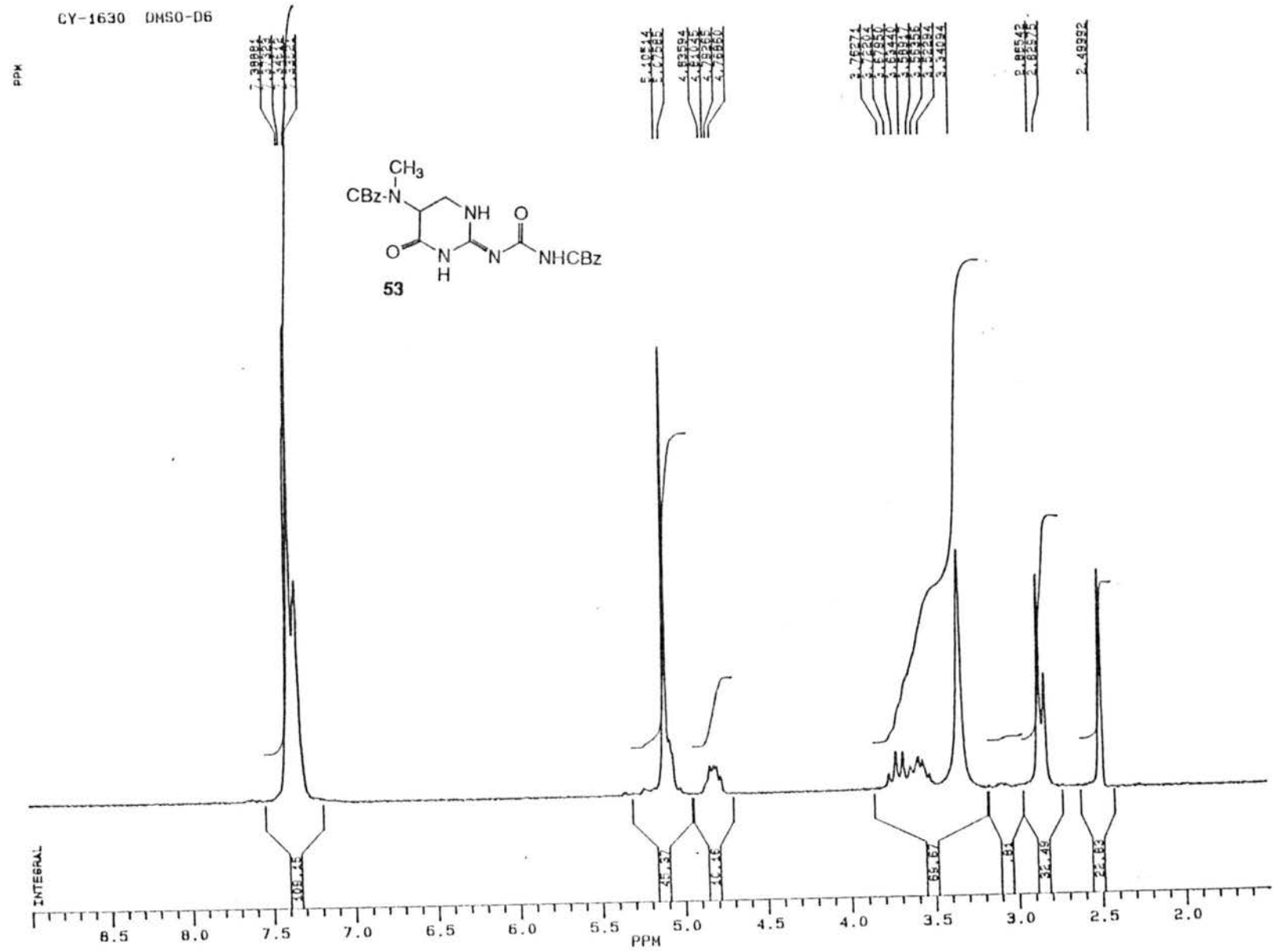
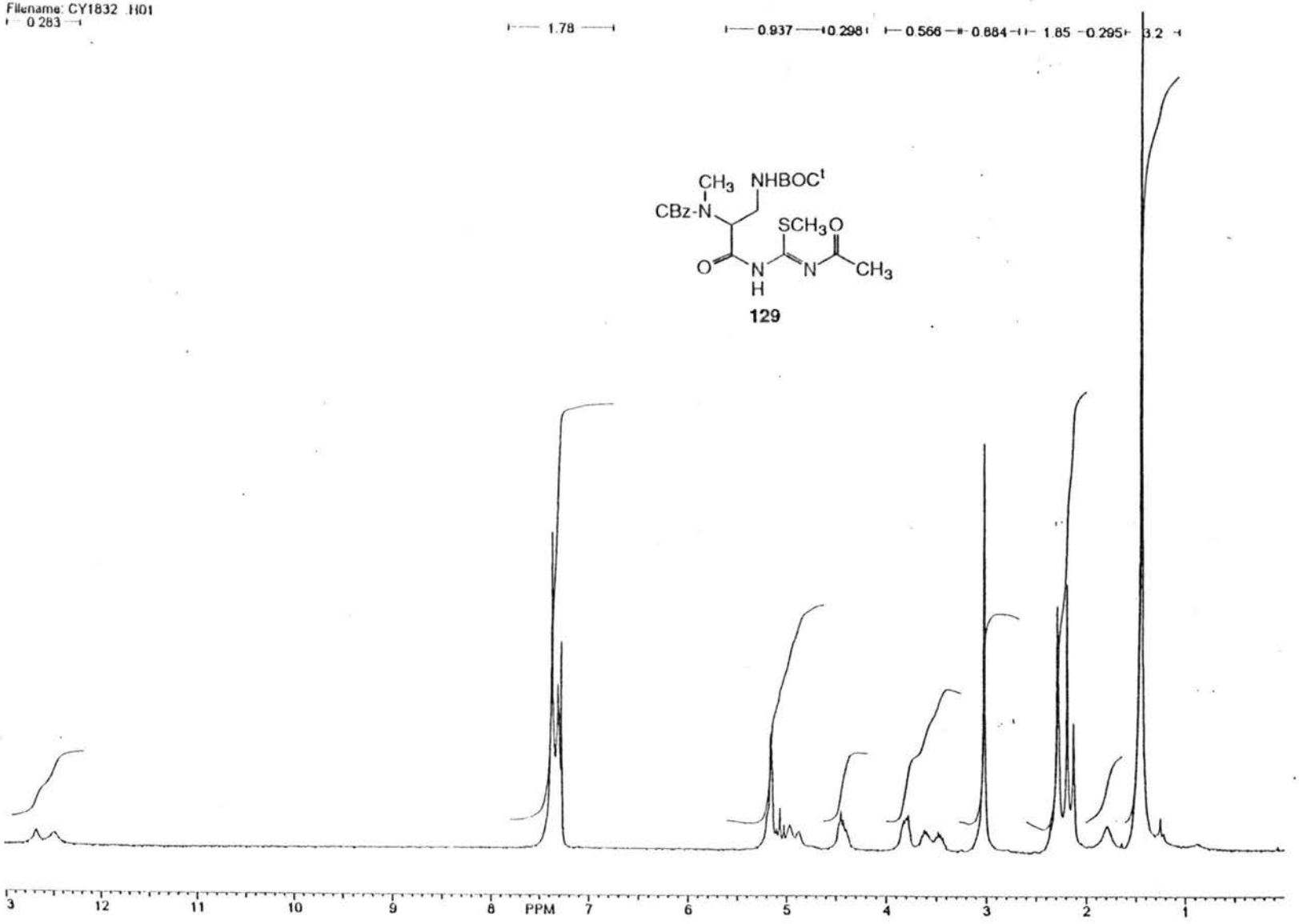
0.458
6.08
2.6
2.39
5.84
3.95
7.396
7.393
7.38
7.375
7.372
7.369
7.401
7.366
7.358
7.355
7.351
7.349
7.348
7.346
7.343
7.34
7.328
7.259
7.257
7.412
7.426
11.86
4.004
4.028
3.978
1.312
1.337
1.363
1.387
1.607
1.61
1.631
1.657
0.9594
0.9105
CBzHN
N
n-Bu—N
O
NH
O
45
12
11
10
9
8
7
PPM
6
5
4
3
2
1
0


CY-1626 CDCL3
PPM
INTEGRAL
PPM
CBz-N
CH3
NHBOC'
CO2H
51


CY-1627-3 CD3COOD 333K
ppm
INTEGRAL
NHBOC'
CH3
CBz-N
SCH3O
NHCBz
N
H
O
52


CY-1630 DMSO-D6
PPM
CBz-N
CH3
NH
O
N
H
N
NHCBz
53
INTEGRAL
6.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
PPM
4.5
4.0
3.5
3.0
2.5
2.0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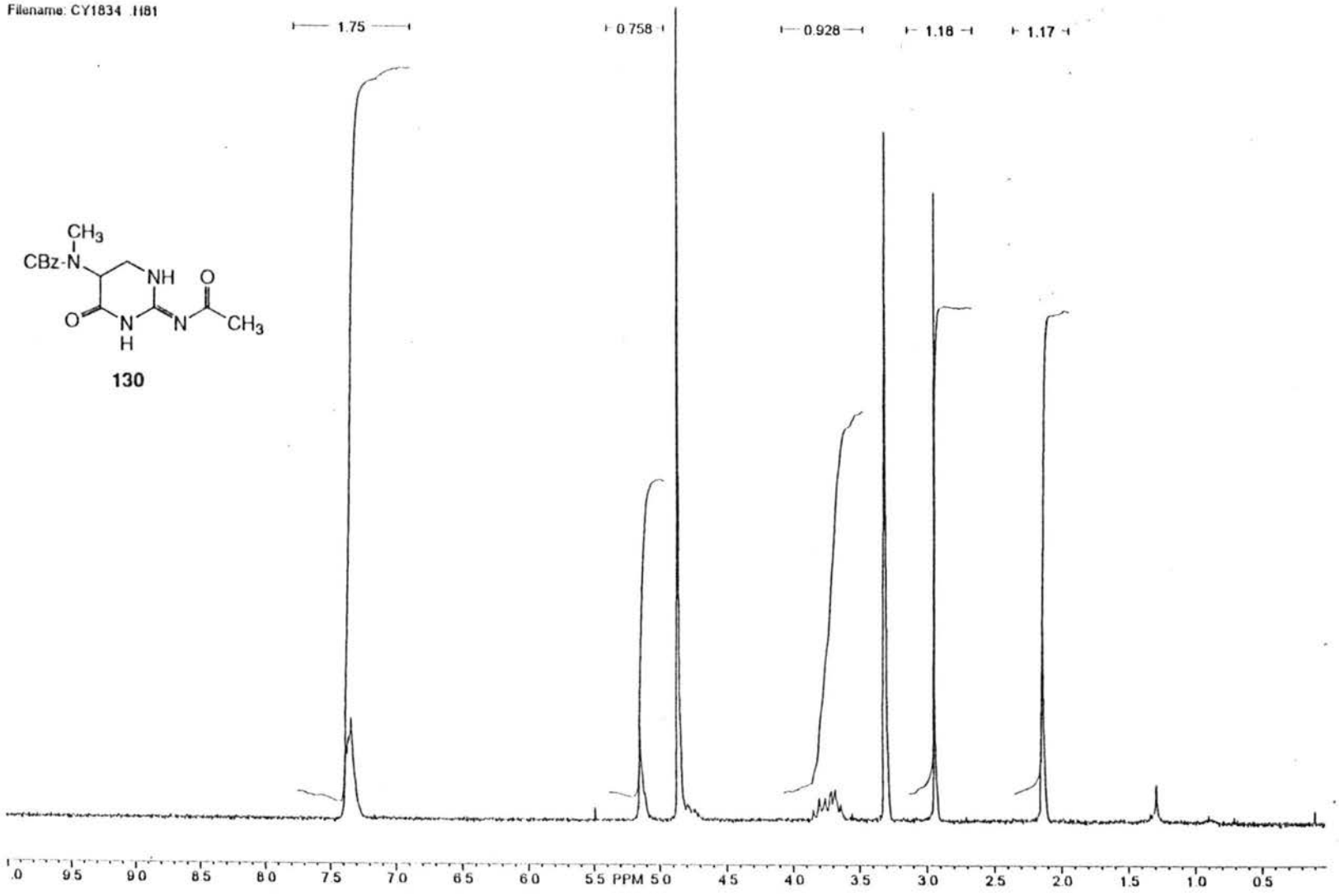
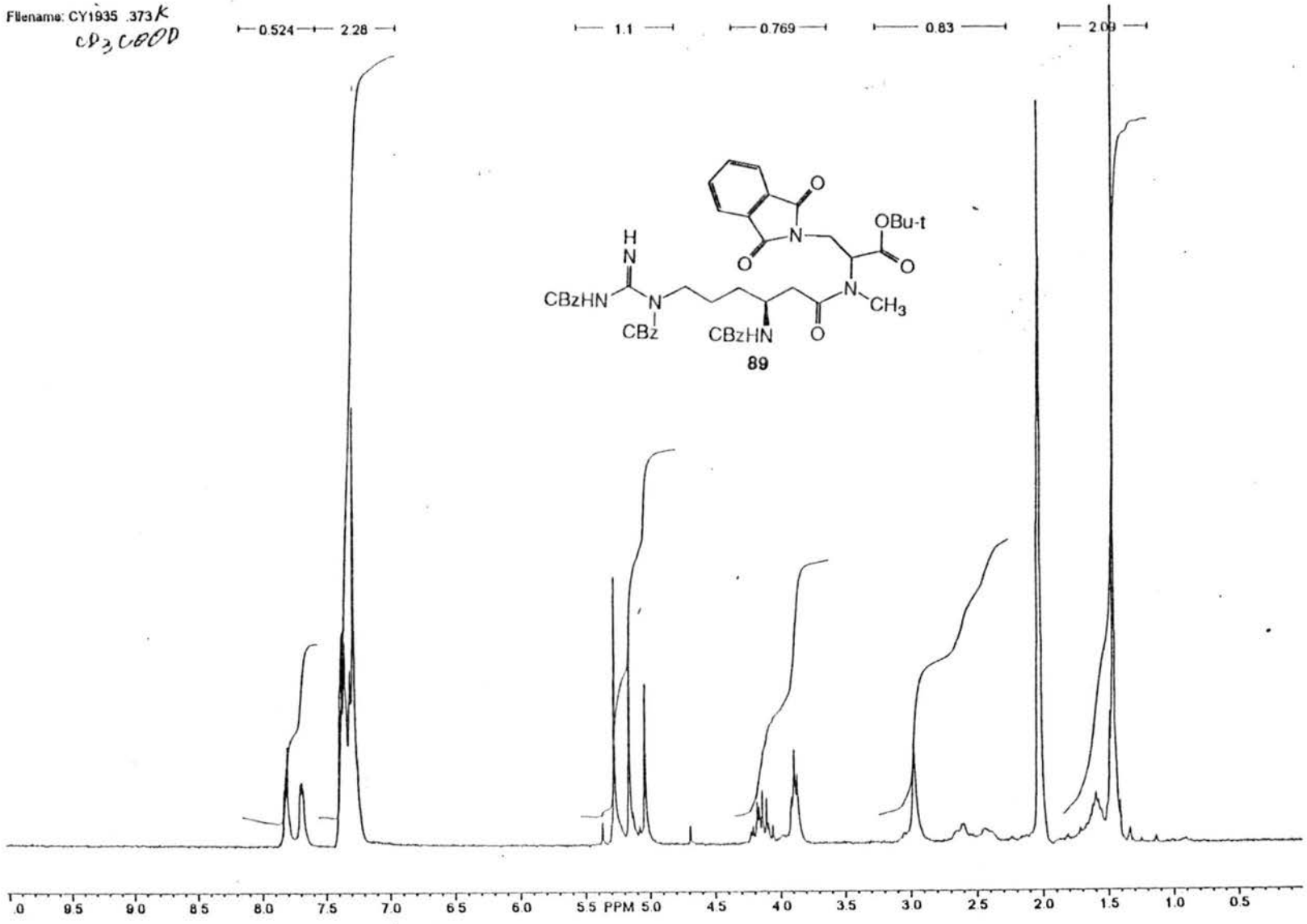
1.78
0.937
0.298
0.566
0.884
1.85
0.295
3.2
CH3
NHBOCt
CBz-N
SCH3
O
O
N
H
N
CH3
129
3
12
11
10
9
8
PPM
7
6
5
4
3
2
1


Filename: CY1834 .1H1
1.75
0.758
0.928
1.18
1.17
CH3
CBz-N
NH
O
O
N
H
N
N
CH3
130
.0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 10 05


Filename: CY1935 .373K
CD3OOD
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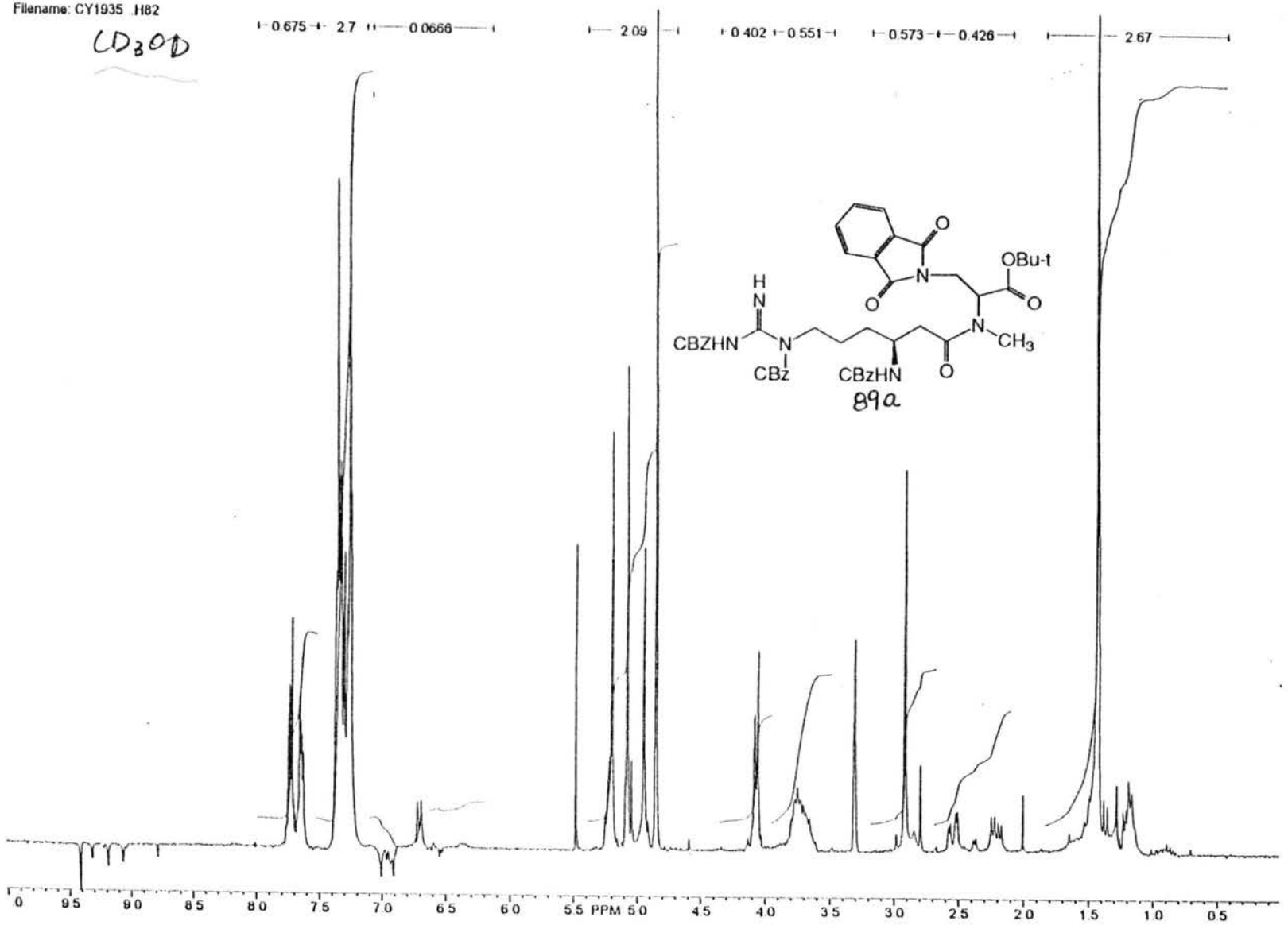
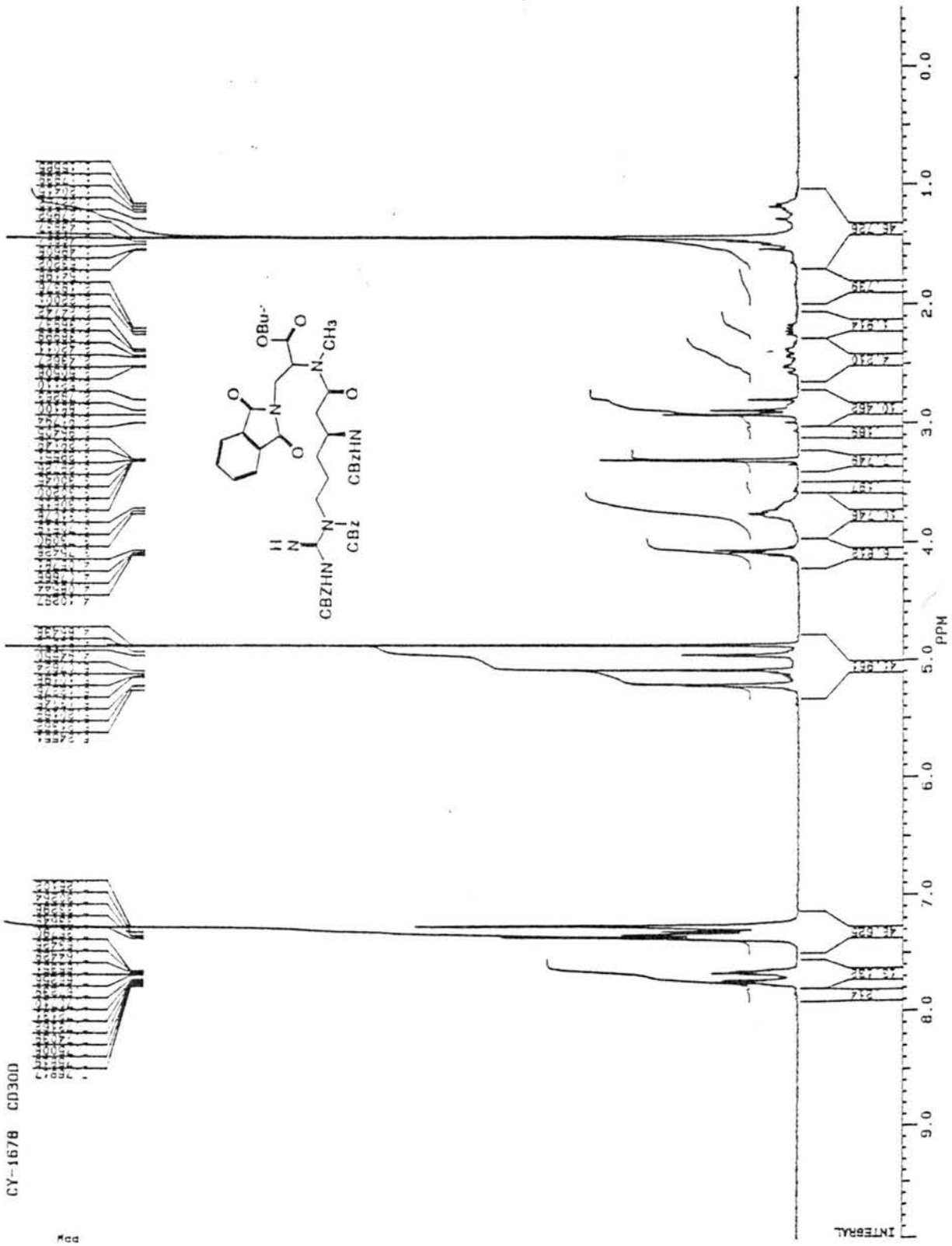
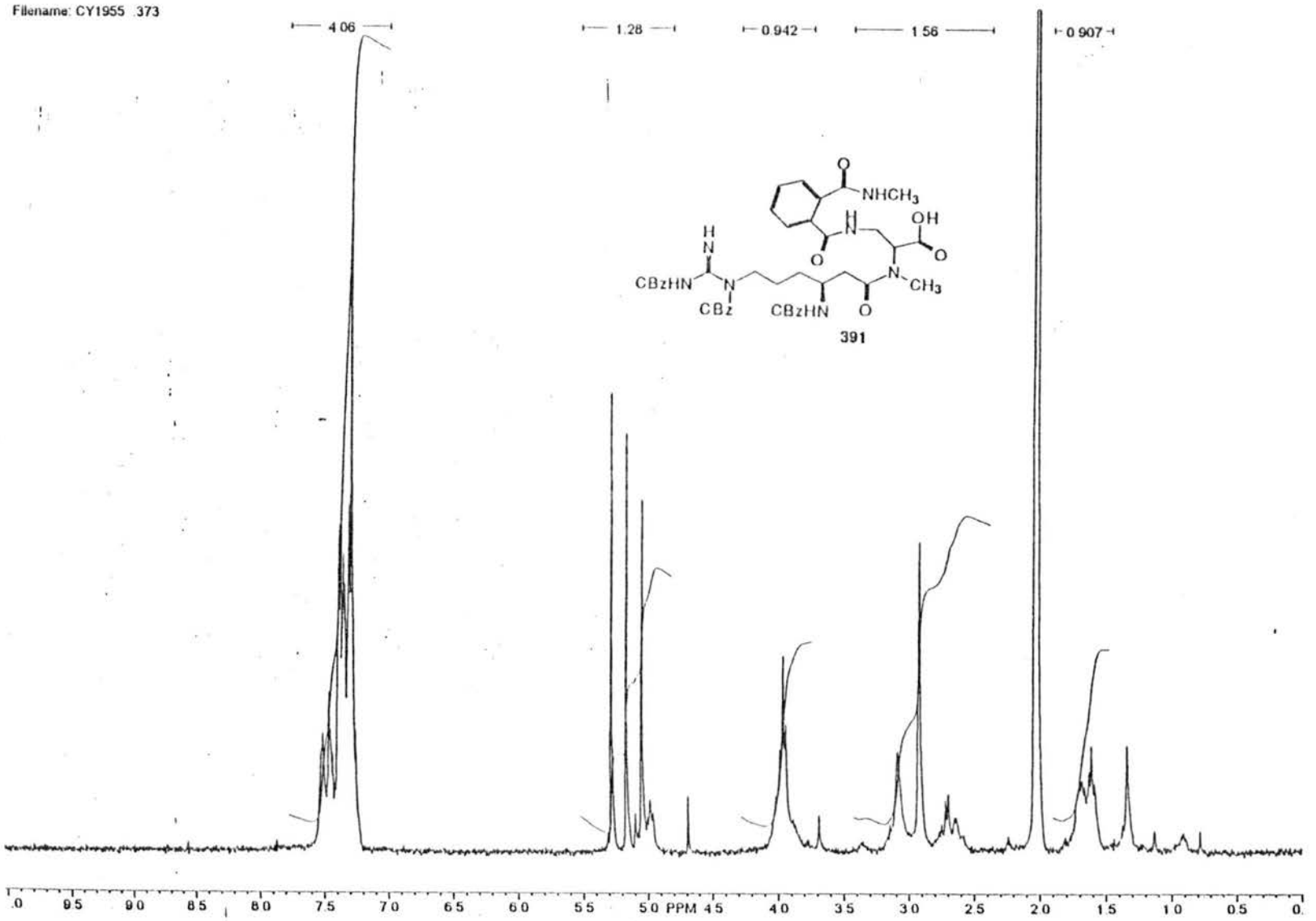
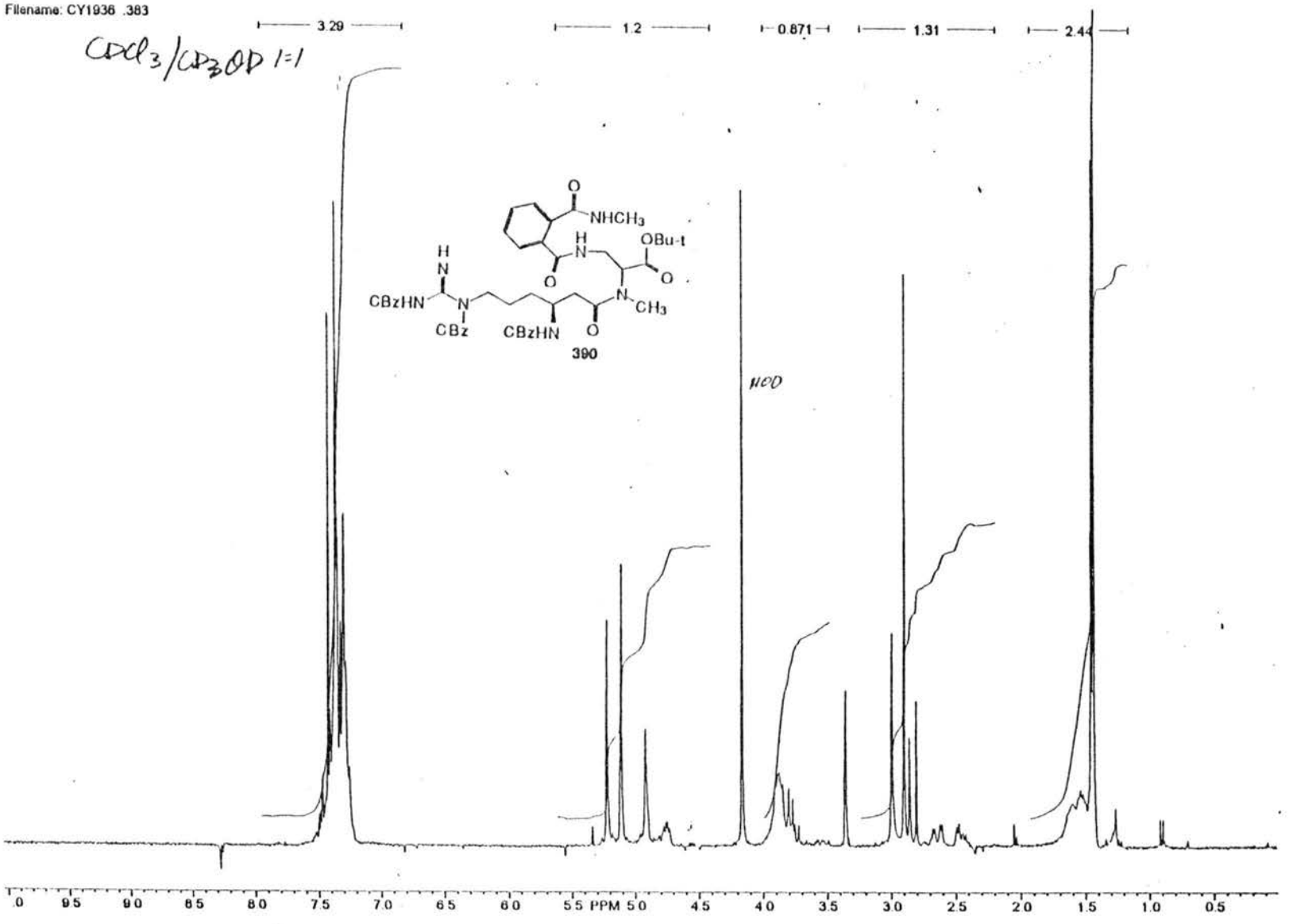
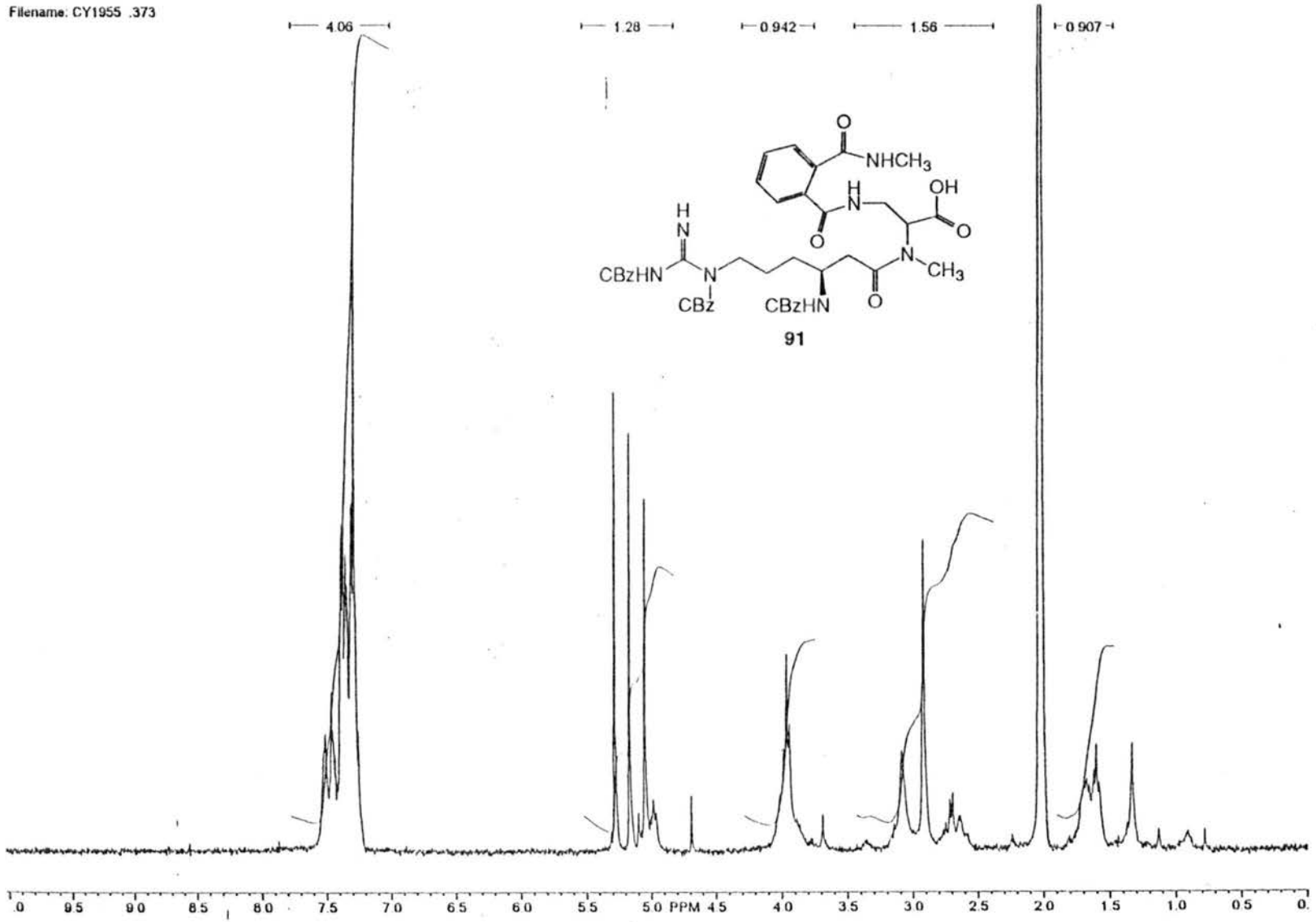
2.28
1.1
0.769
0.83
2.09
OBu-t
CBzHN
CBz
CBzHN
CH3
89
.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 PPM 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5


Filename: CY1935 .H82
CD3OD
0.675
2.7
0.0666
2.09
0.402
0.551
0.573
0.426
2.67
OBu-t
CBZHN
CBz
CBzHN
CH3
89a
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 10 05


CY-1678 CD30D
OBu-t
CH3
CBzHN
CBZHN
CBz
INTEGRAL
PPM


Filename: CY1955 .373
4.06
1.28
0.942
1.56
0.907
NHCH3
OH
CBzHN
CBz
CBzHN
CH3
391
.0 95 90 85 80 75 70 65 60 55 50 PPM 45 40 35 30 25 20 15 10 05 0.


Filename: CY1936 .383
3.29
1.2
0.871
1.31
2.44
CDCl3/CD3OD 1:1
NHCH3
OBu-t
CBzHN
CBz
CBzHN
CH3
390
MeOD
.0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 1.5 1.0 0.5


Filename: CY1955 .373
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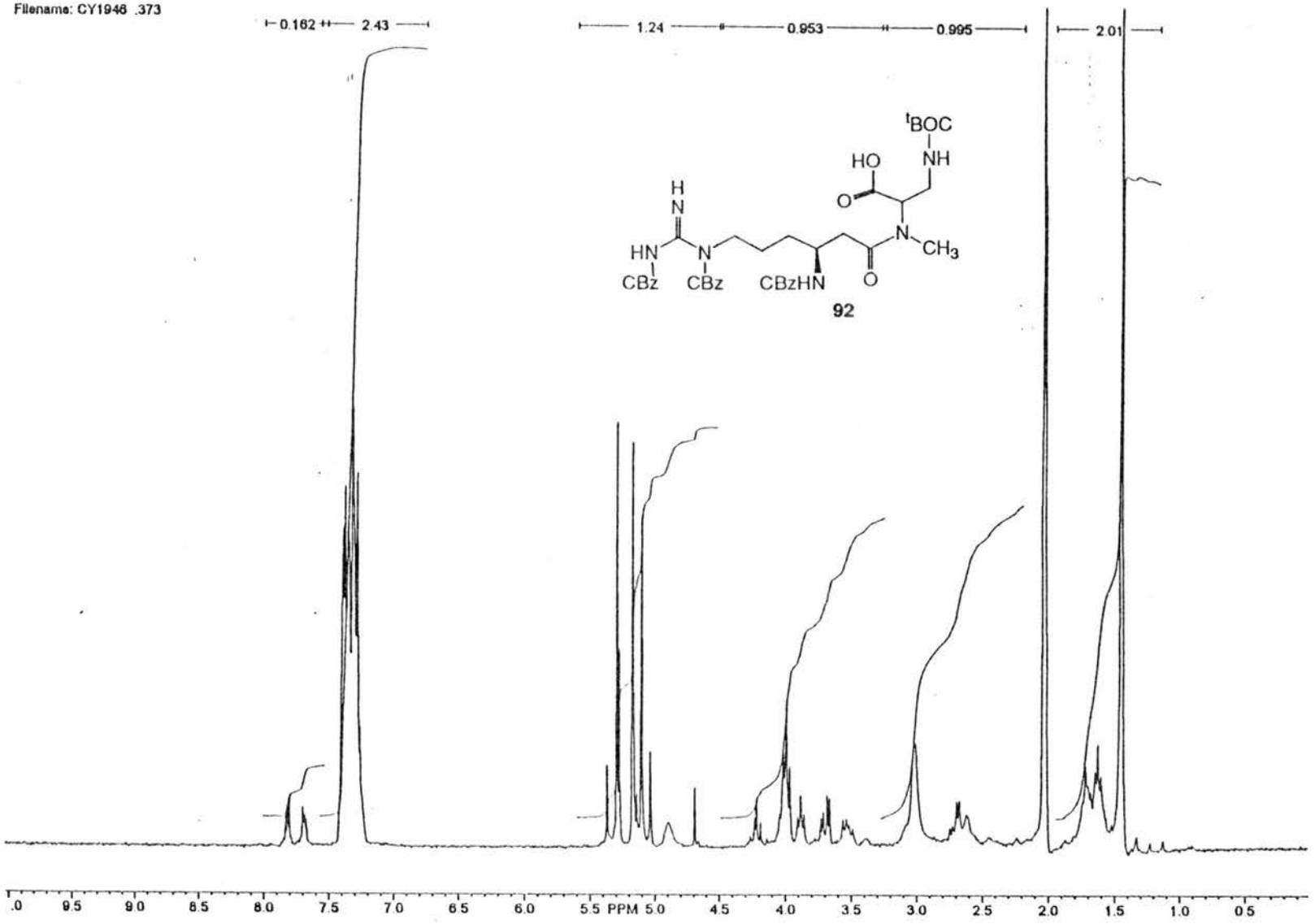
1.28
0.942
1.56
0.907
NHCH3
OH
CBzHN
CBz
CBzHN
CH3
91
PPM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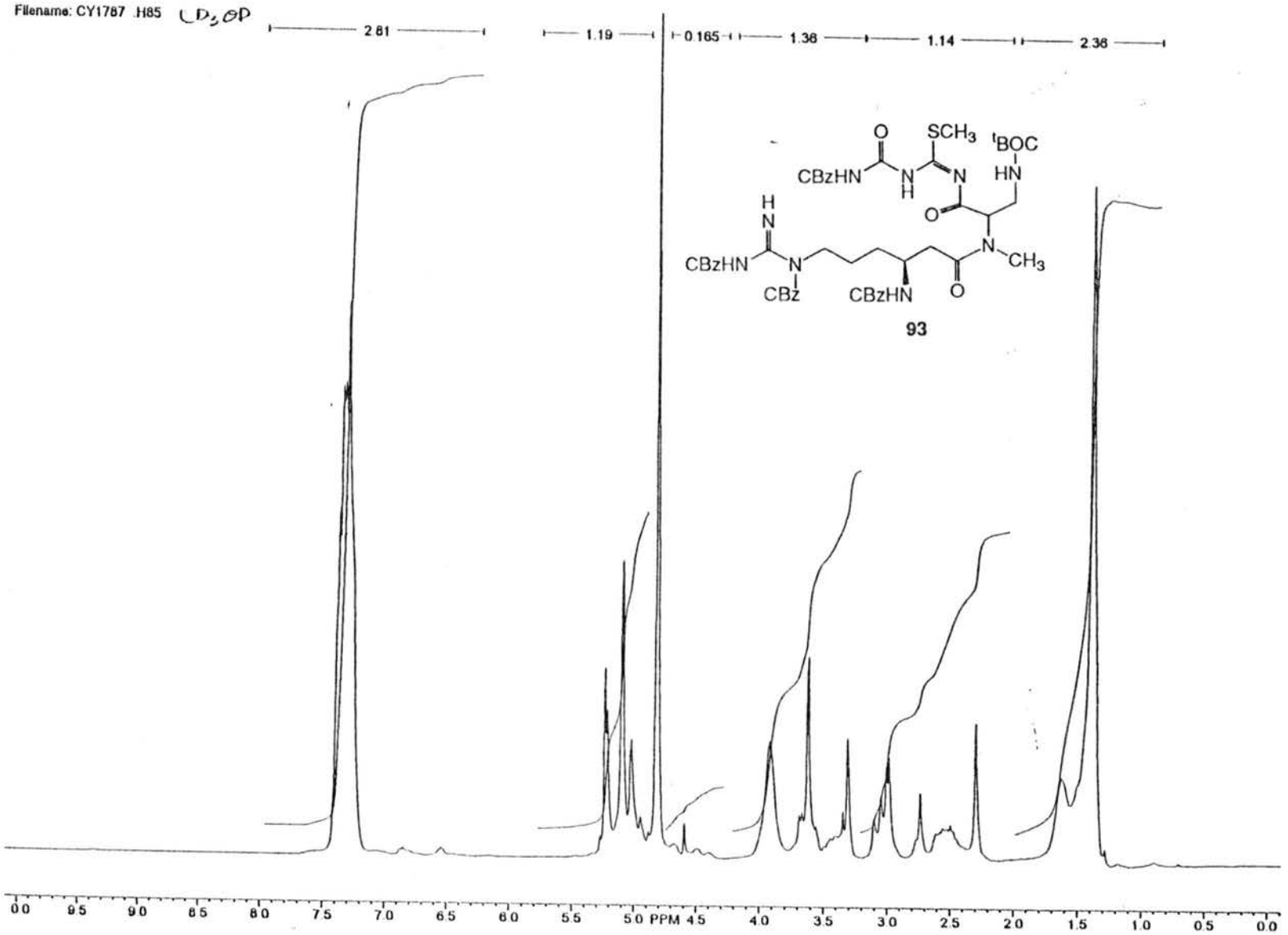
2.43
1.24
0.953
0.995
2.01
tBOC
NH
HO
O
H
N
HN
N
N
CH3
CBz
CBz
CBzHN
O
92
.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5 PPM 5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5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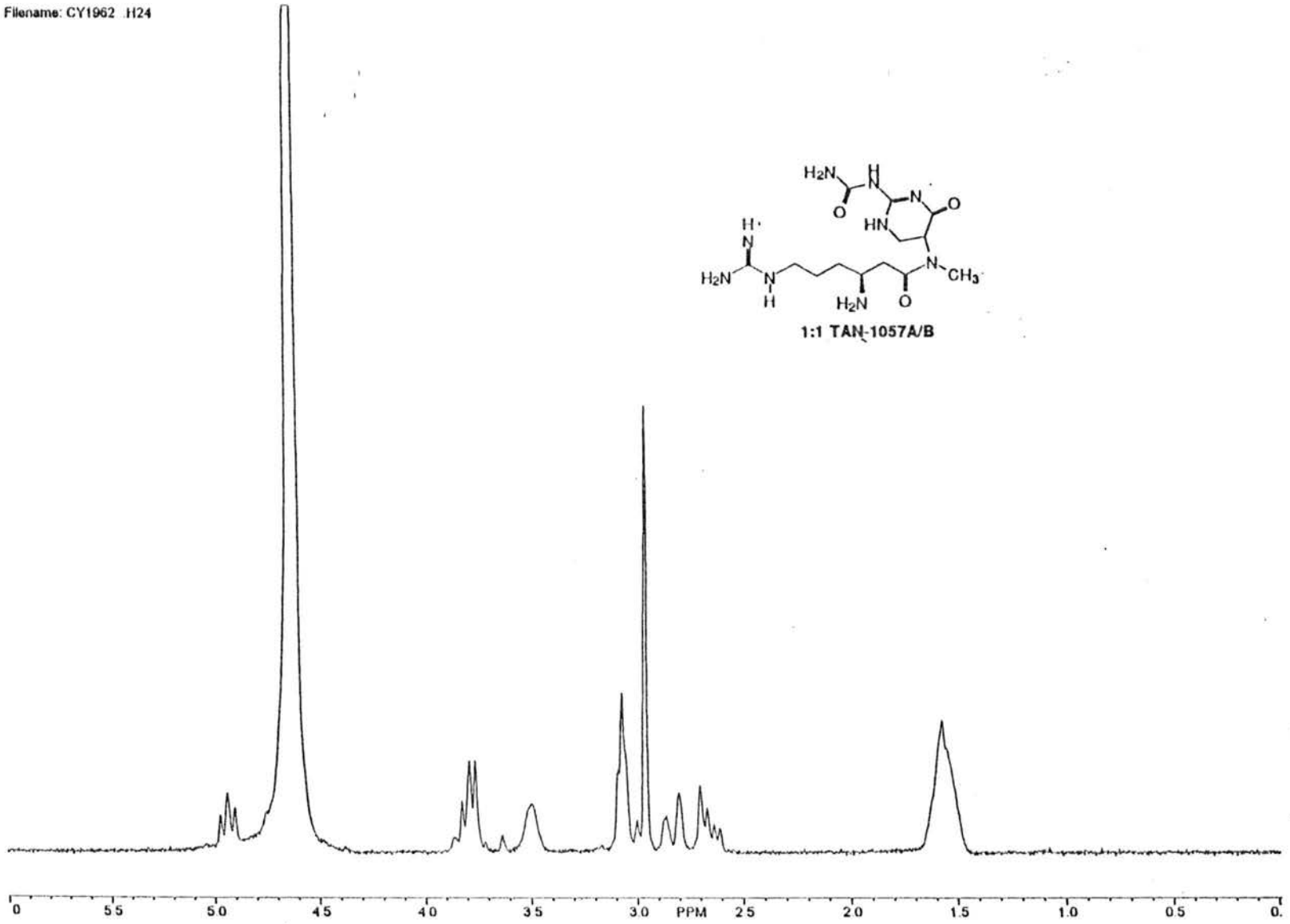
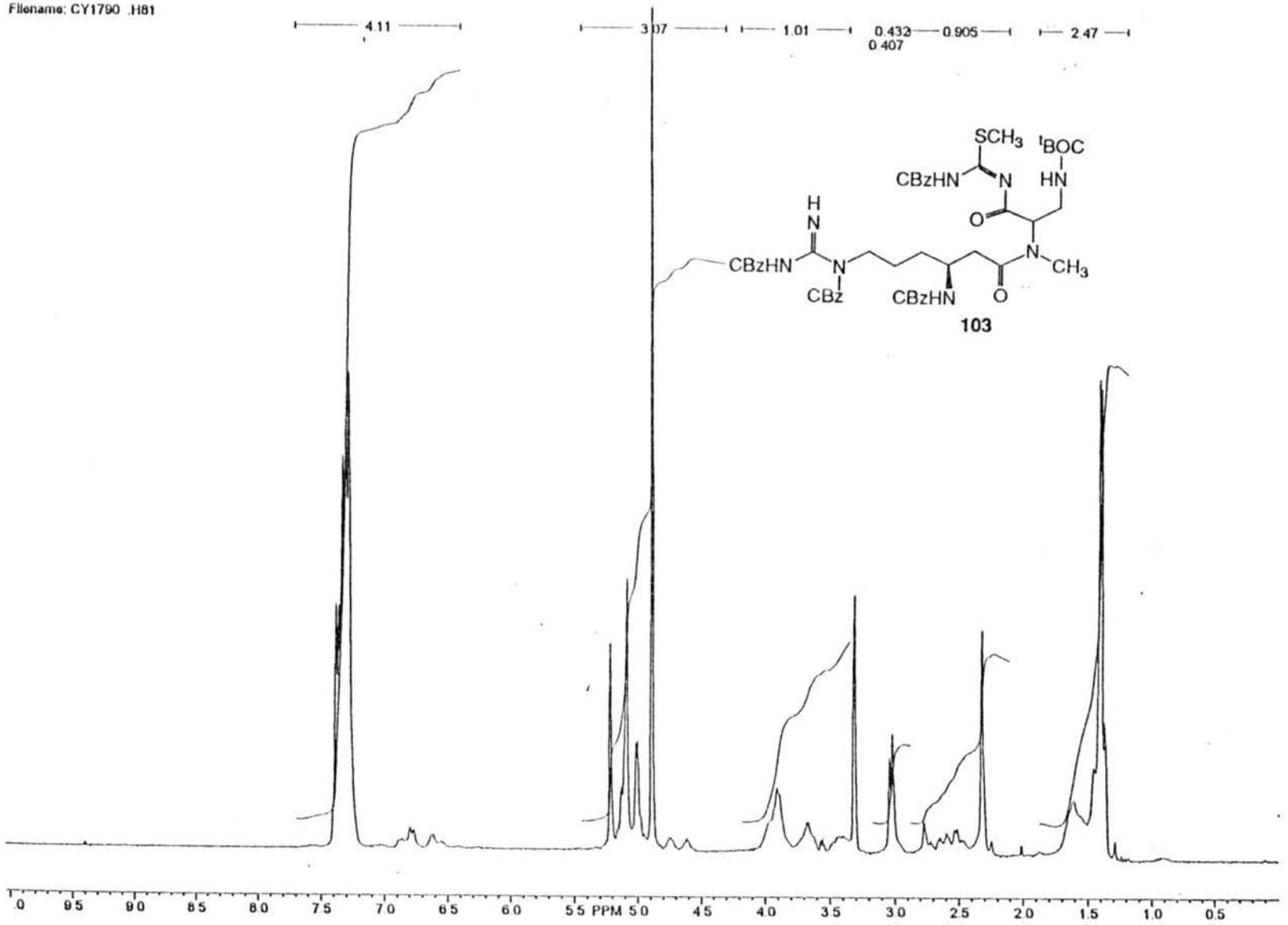
2.81
1.19
0.165
1.38
1.14
2.38
93
PPM


Filename: CY1962 H24
1:1 TAN-1057A/B
0
55
50
45
40
35
30
PPM
25
20
15
10
05
0.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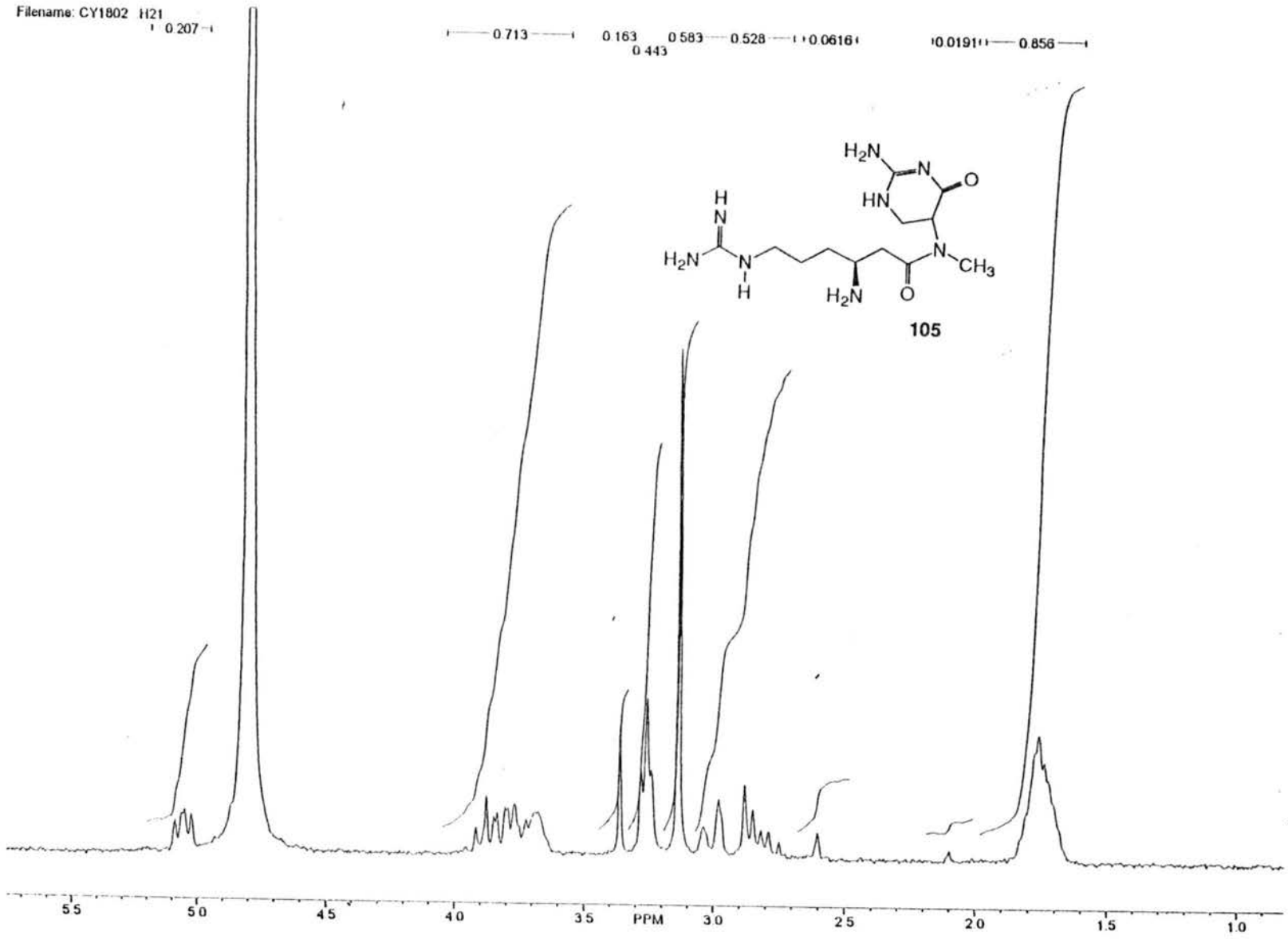
4.11
3.07
1.01
0.432
0.407
0.905
2.47
SCH3
tBOC
CBzHN
N
HN
O
H
N
CBzHN
N
CH3
CBz
CBzHN
O
103
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 10 05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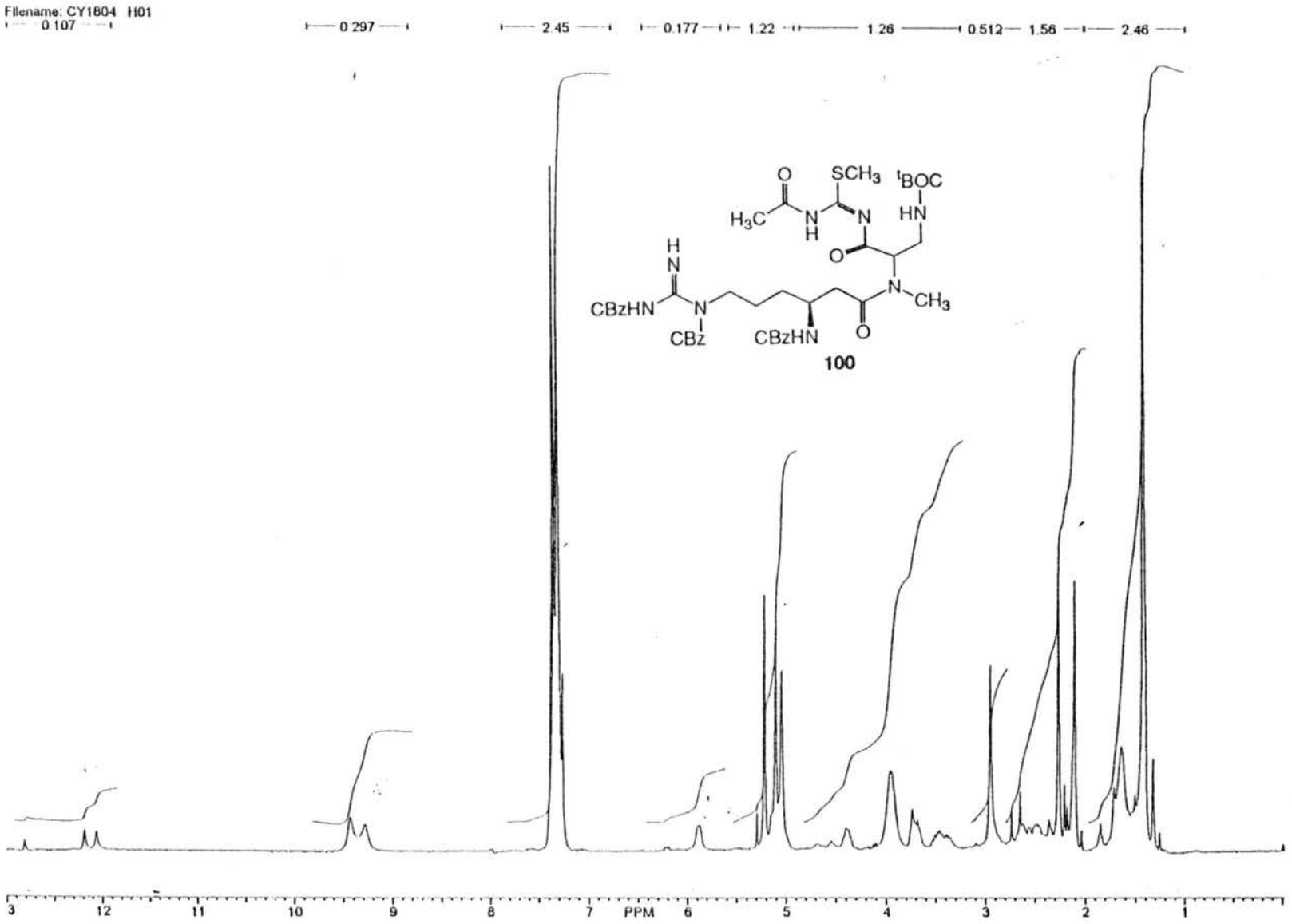
0.207
0.713
0.163
0.443
0.583
0.528
0.0616
0.0191
0.856
105
55
50
45
40
35
PPM
30
25
20
15
10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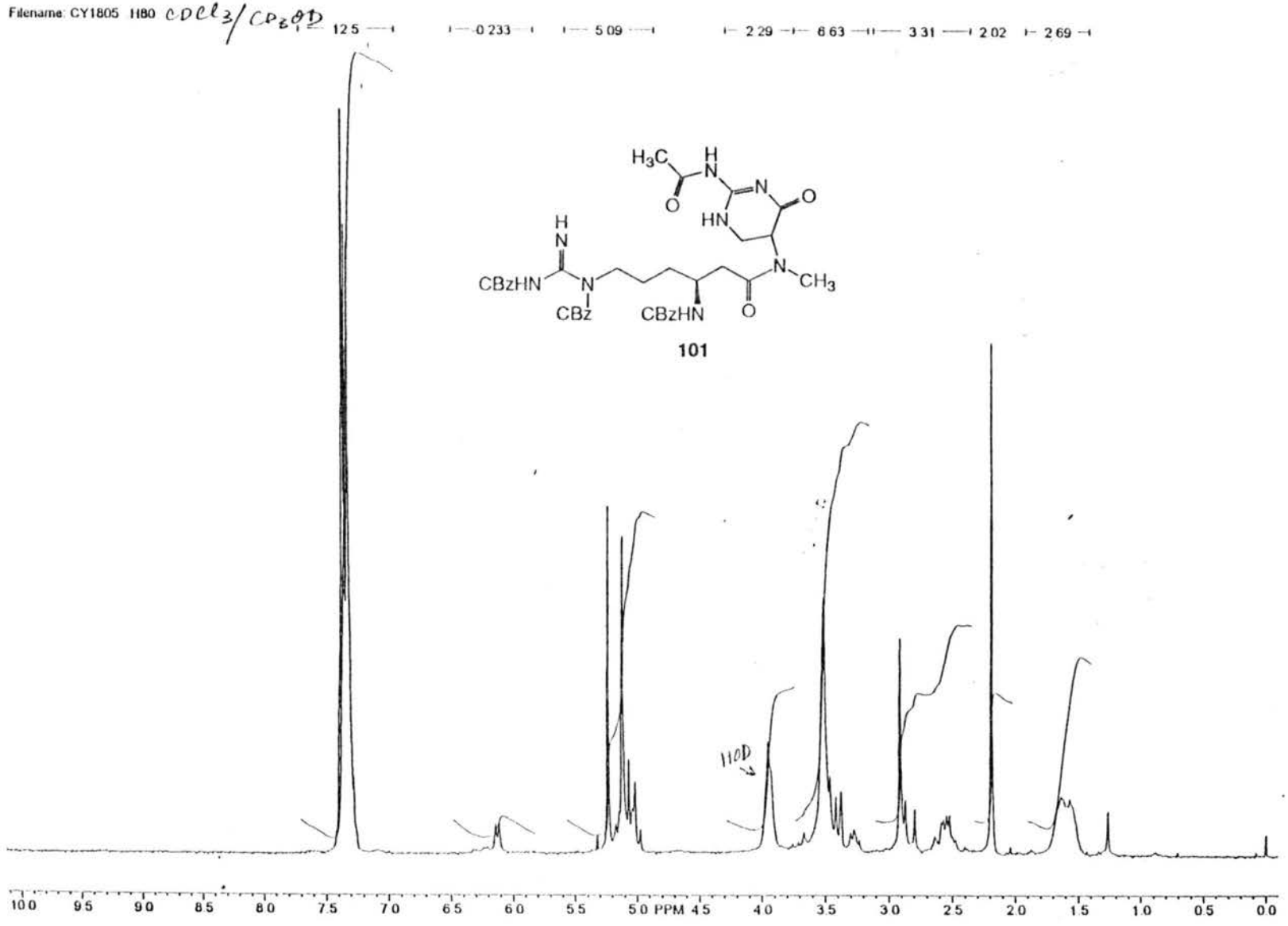
0.107
0.297
2.45
0.177
1.22
1.26
0.512
1.56
2.46
SCH3
tBOC
H3C
N
H
N
HN
O
CBzHN
CBz
CBzHN
CH3
100
PPM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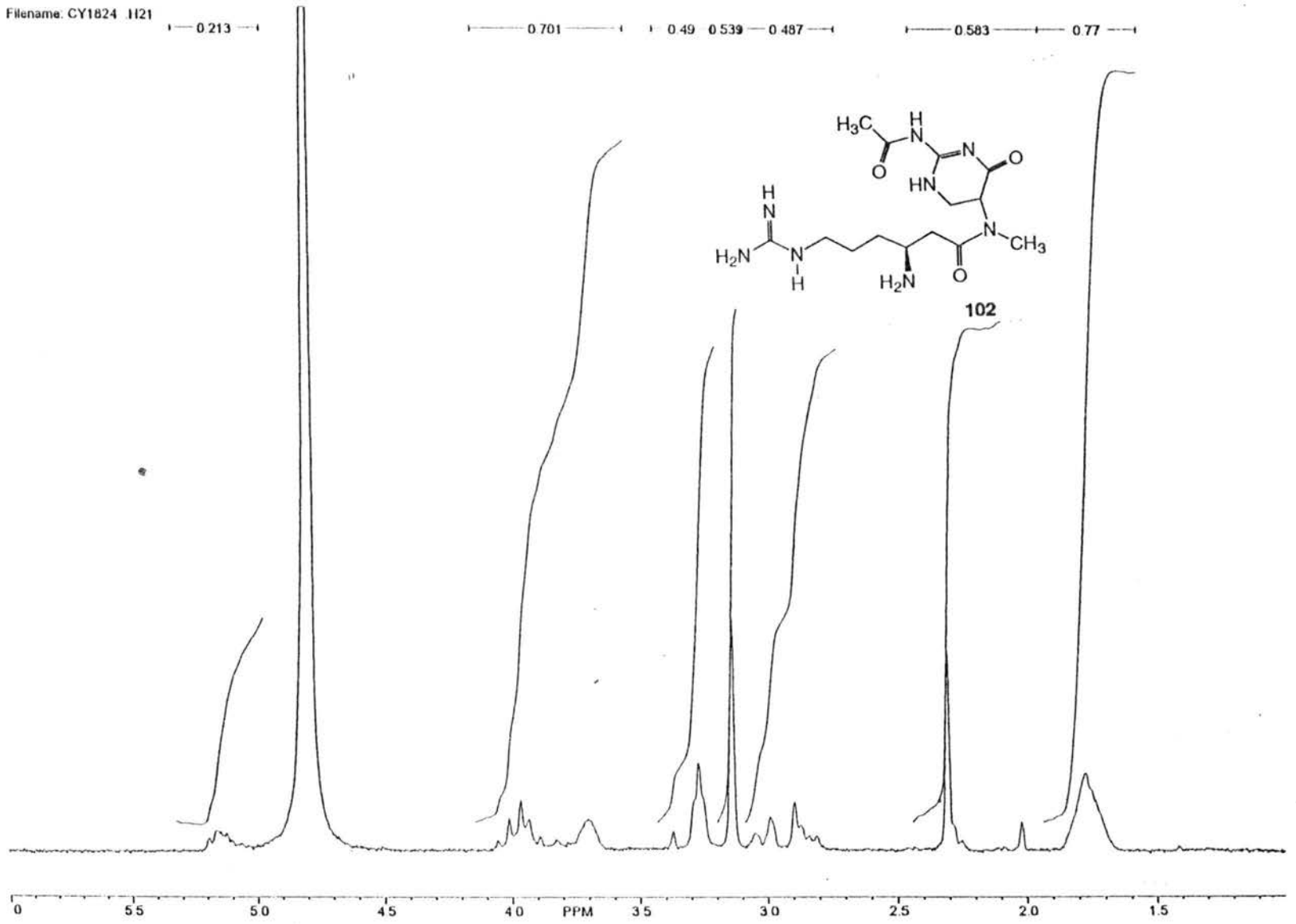
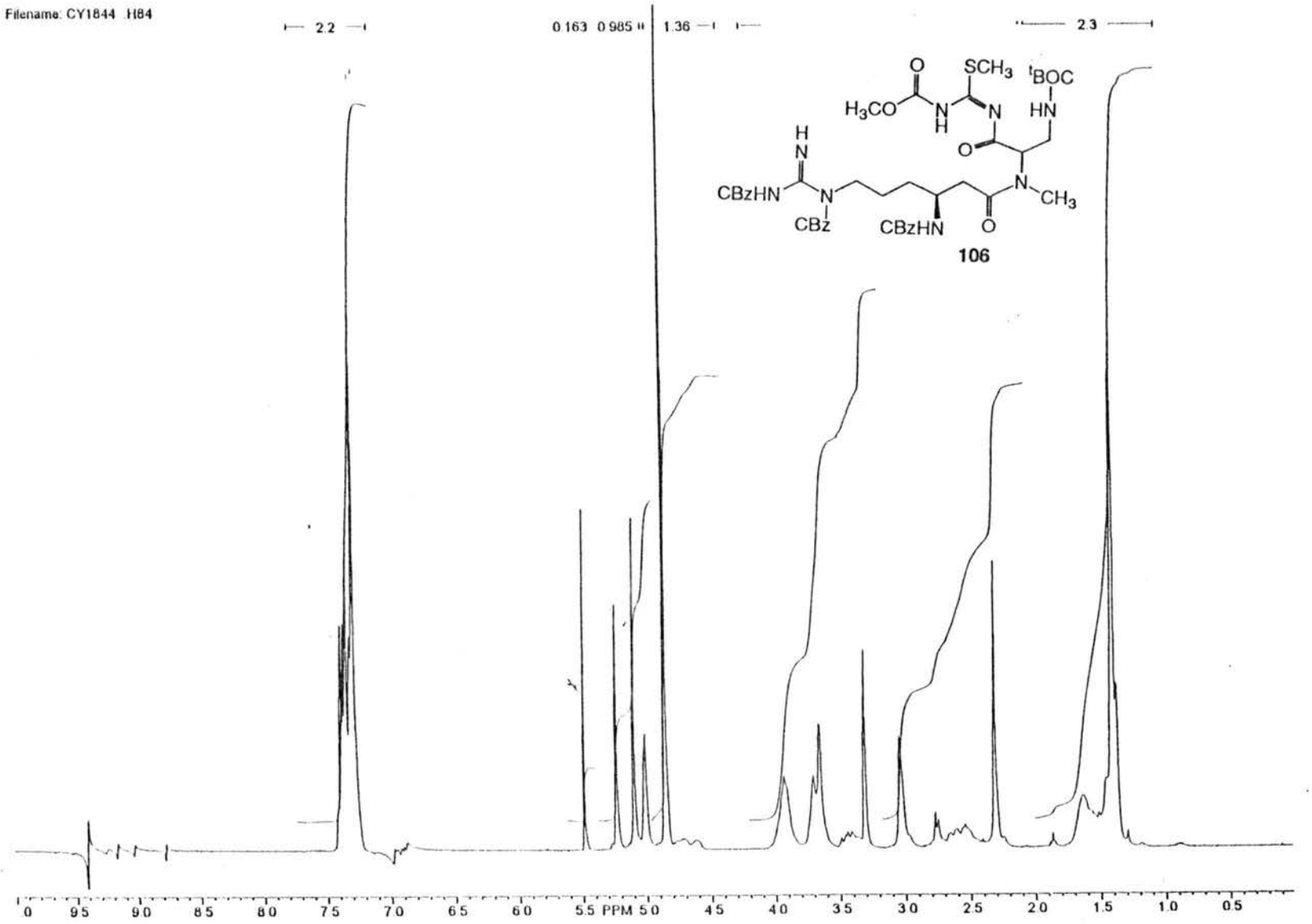
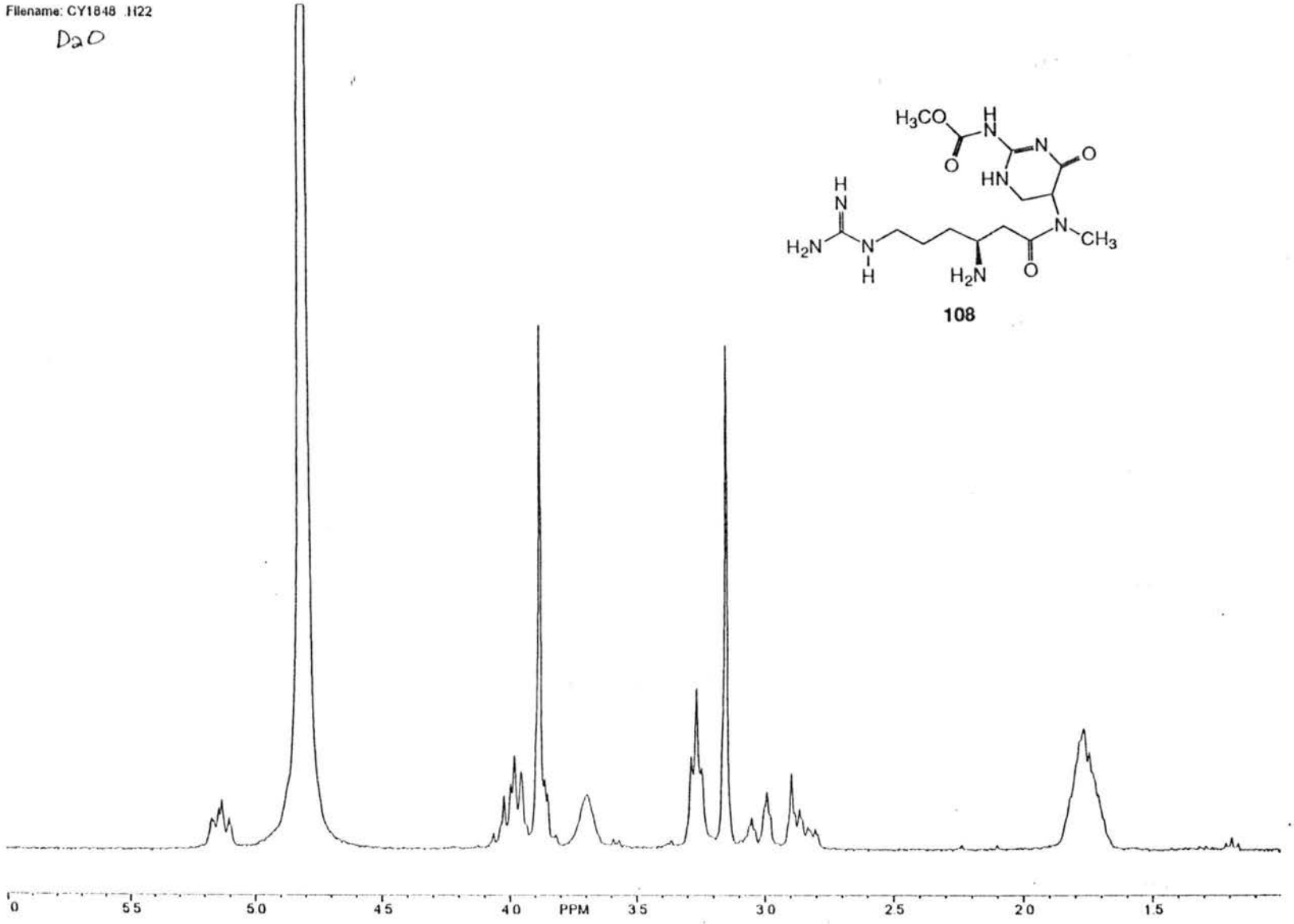
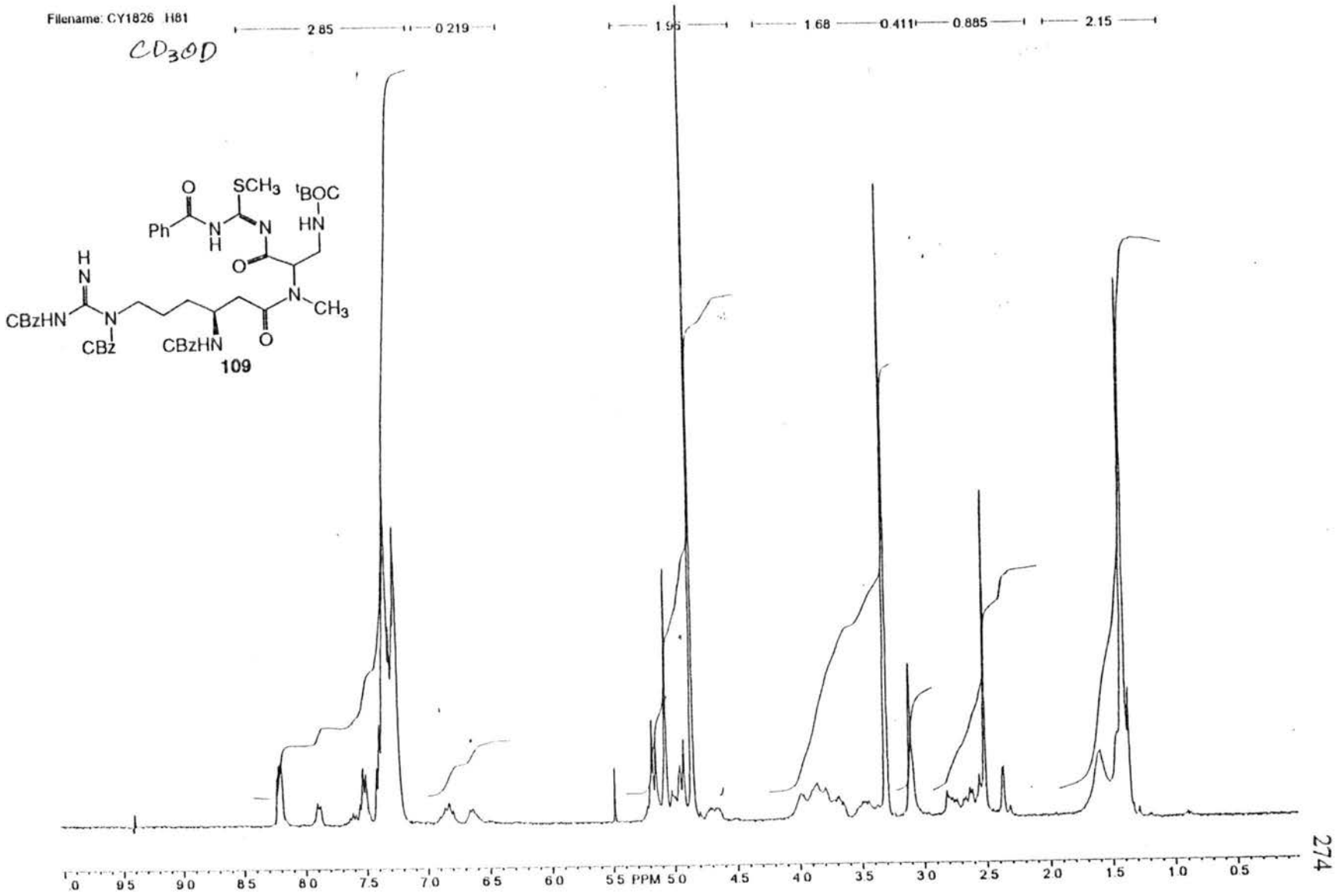
12.5
0.233
5.09
2.29
6.63
3.31
2.02
2.69
H3C
H
N
O
N
O
HN
H
N
CBzHN
N
CBz
CBzHN
O
N
CH3
101
H2O
100 95 90 85 80 75 70 65 60 55 50 PPM 45 40 35 30 25 20 15 10 05 00


Filename: CY1824 1121
0.213
0.701
0.49
0.539
0.487
0.583
0.77
102
0
5.5
5.0
4.5
4.0
PPM
3.5
3.0
2.5
2.0
1.5


Filename: CY1844 H84
2.2
0.163
0.985
1.36
2.3
106
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 1.5 1.0 0.5



108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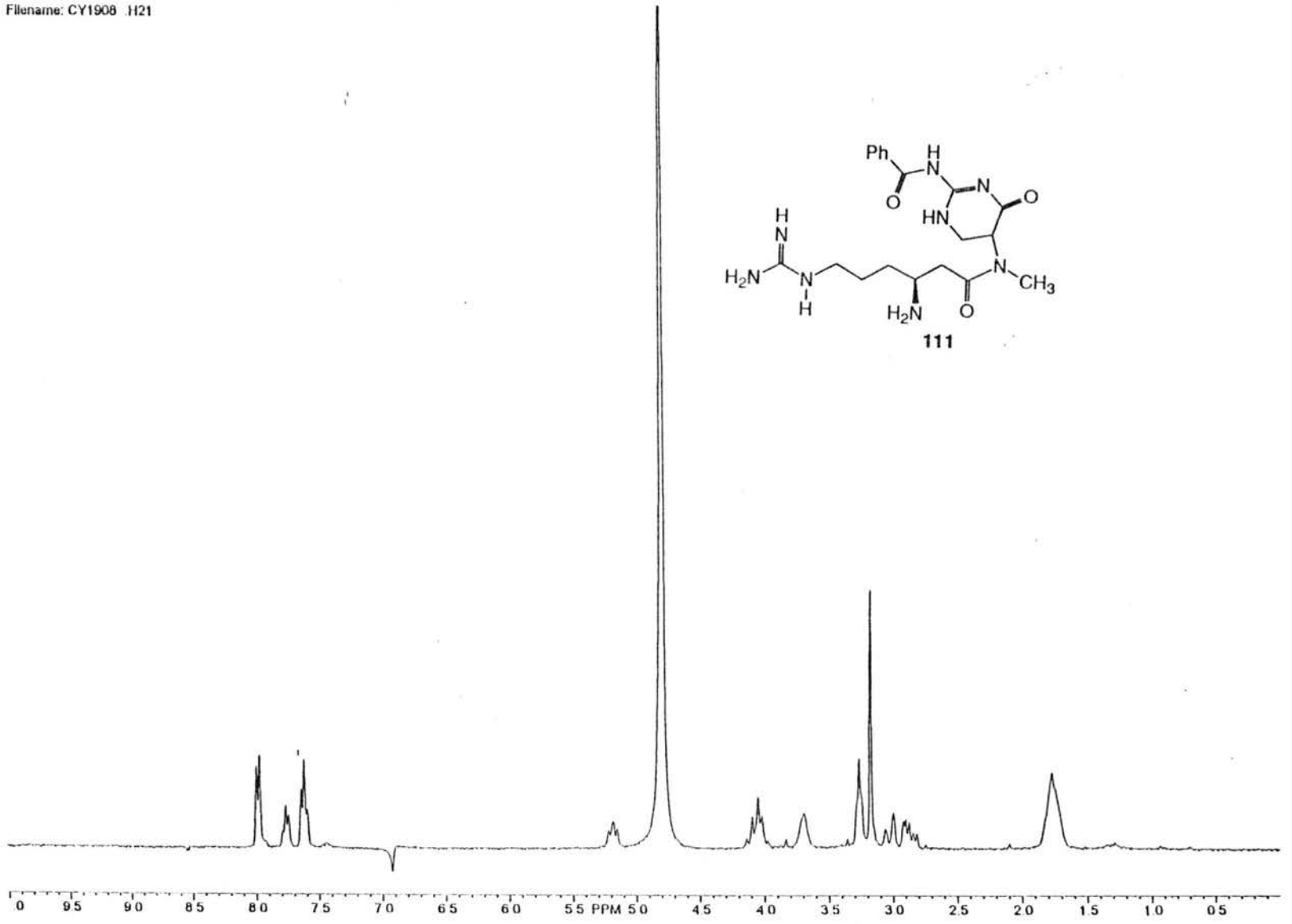
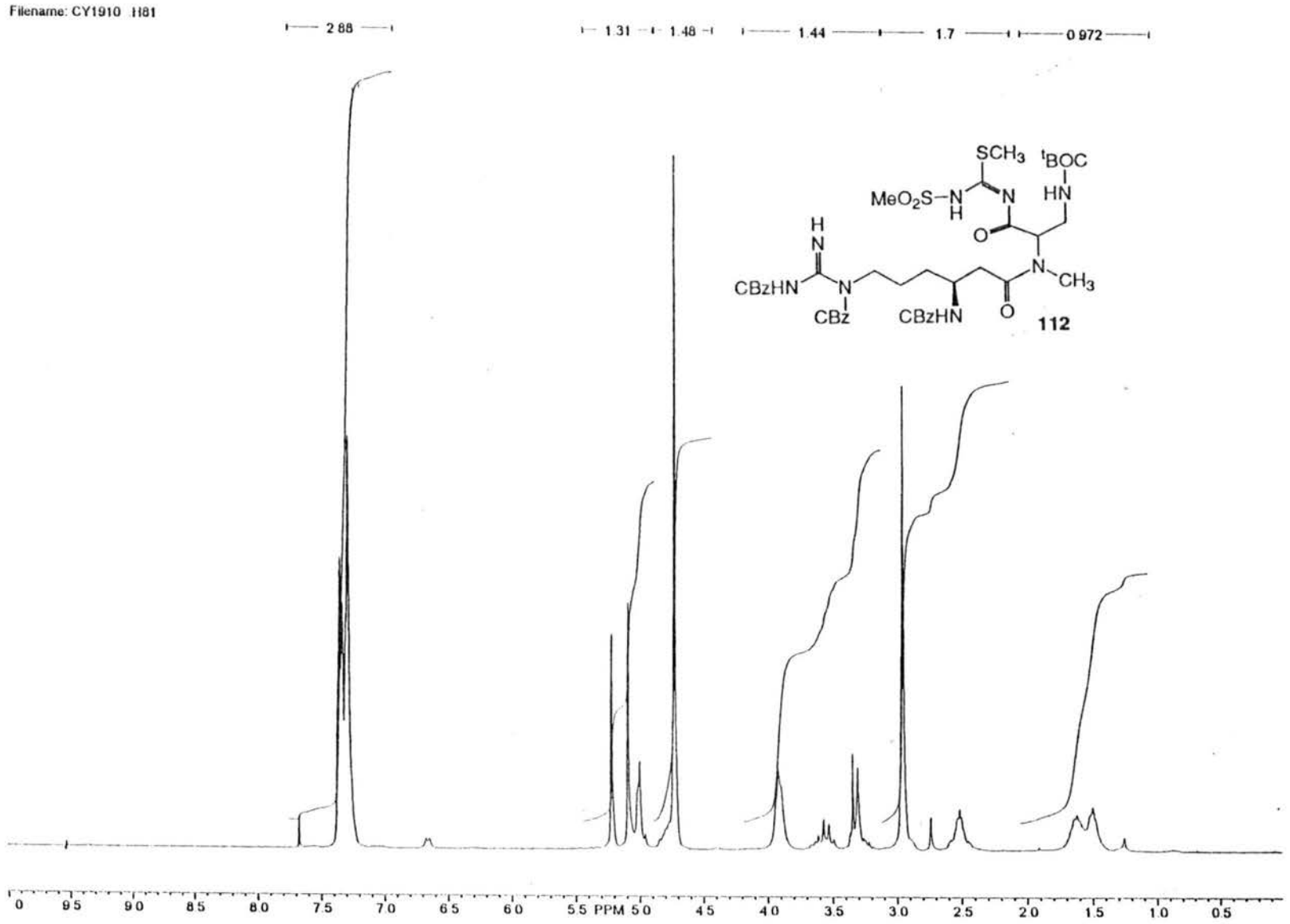
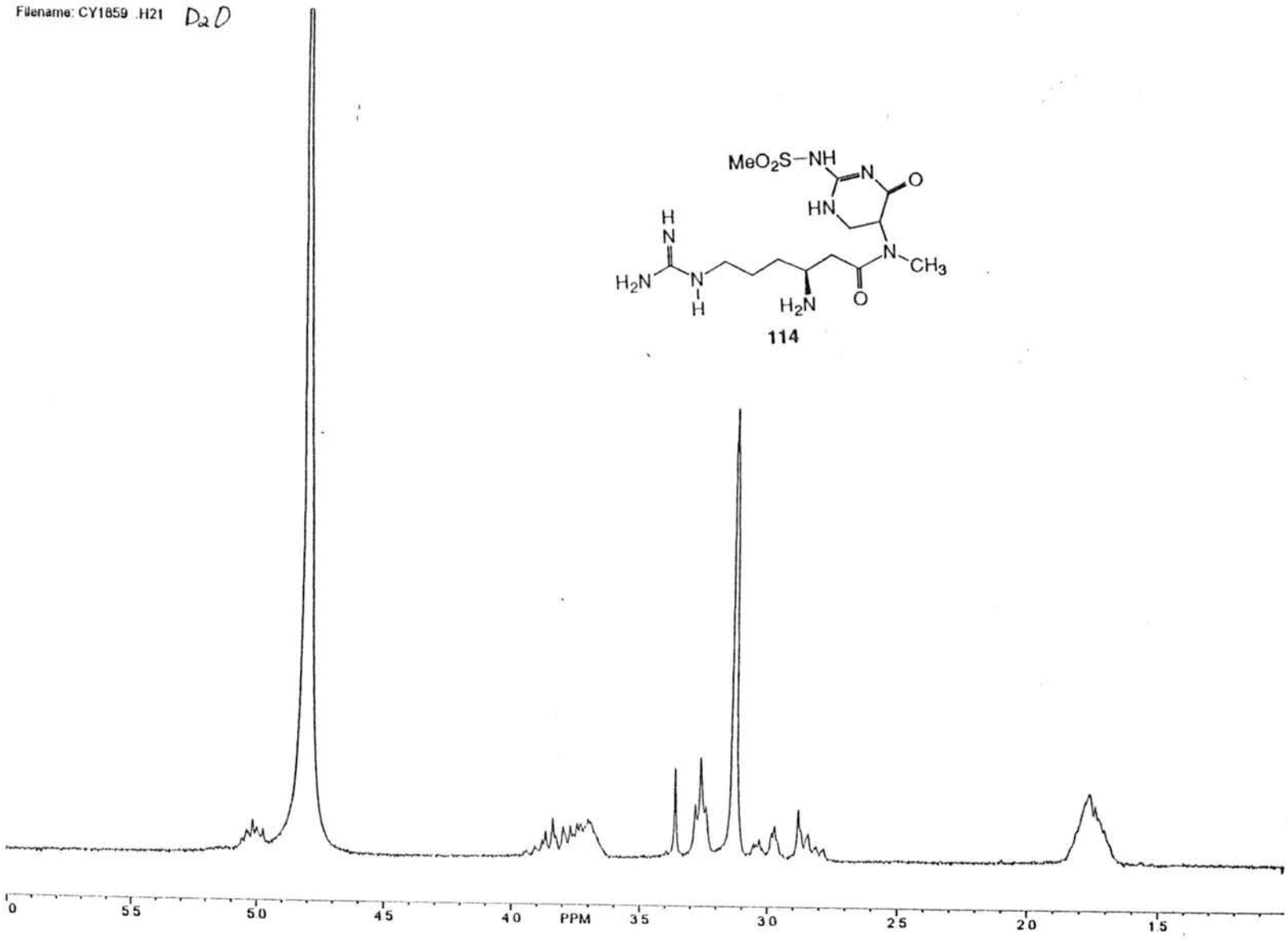
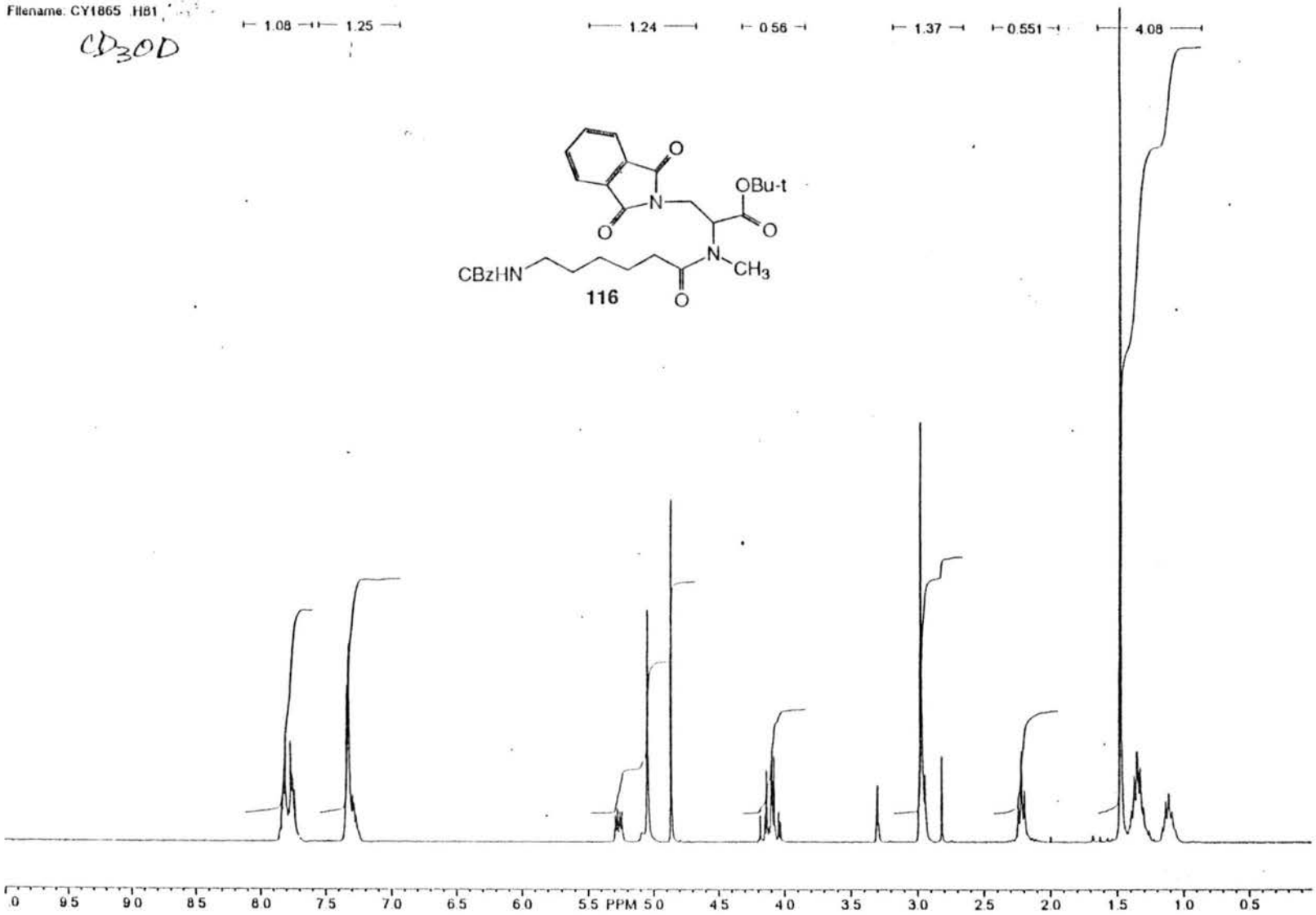
CD3OD
2.85
0.219
1.96
1.68
0.411
0.885
2.15
109
PPM


Filename: CY1908 .H21
Ph
H
N
O
N
O
HN
H
N
H2N
N
H
N
CH3
H2N
O
111
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 10 05


Filename: CY1910 H81
2.88
1.31
1.48
1.44
1.7
0.972
SCH3
tBOC
MeO2S
N
H
HN
O
CBzHN
CBz
CBzHN
CH3
112
0
95
90
85
80
75
70
65
60
55 PPM 50
45
40
35
30
25
20
15
10
05


Filename: CY1859 .H21
D2O
MeO2S–NH
HN
N
O
H
N
H2N
N
H
H2N
N
CH3
O
114
0
55
50
45
40
PPM
35
30
25
20
15


Filename: CY1865 H81
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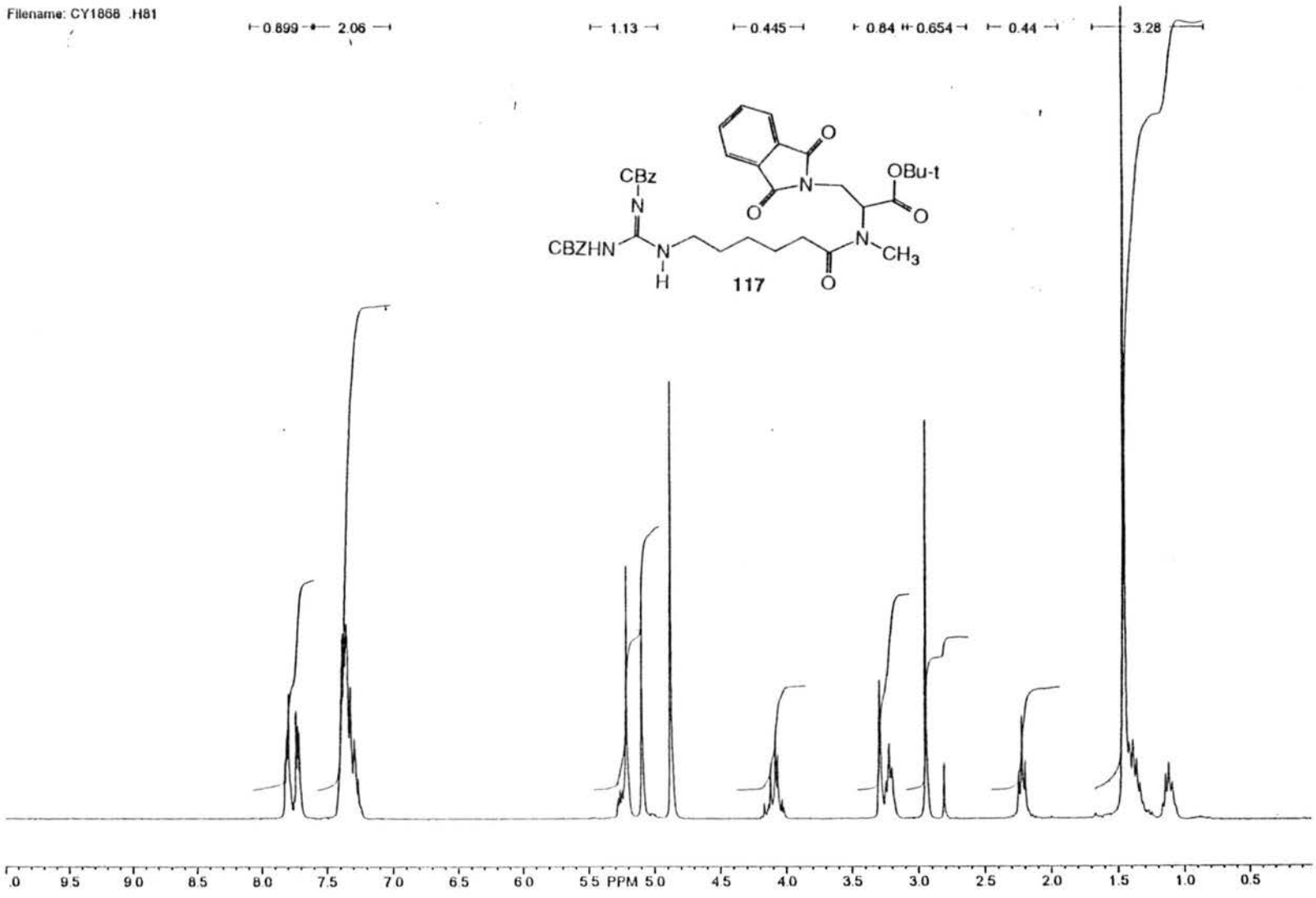
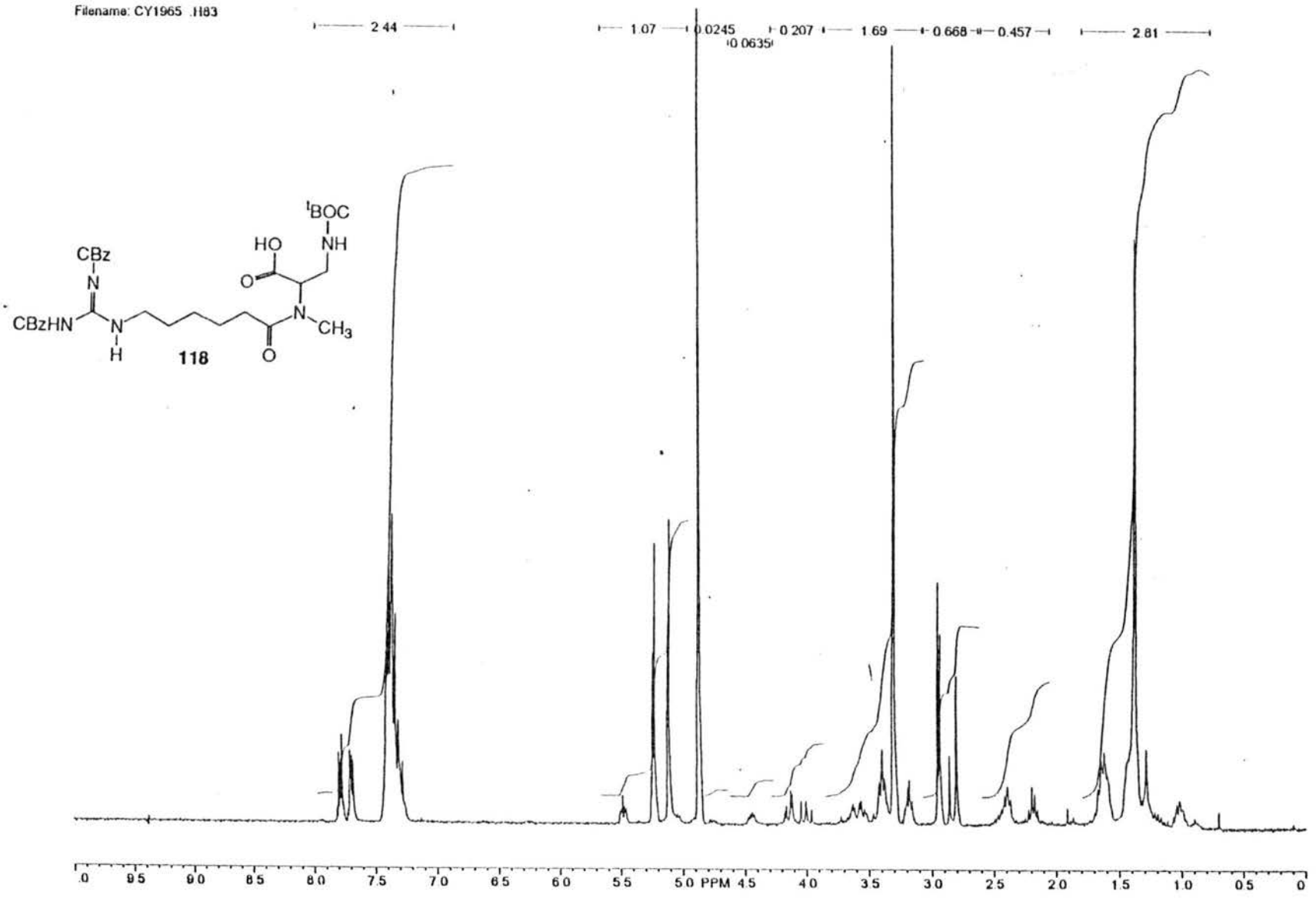
1.25
1.24
0.56
1.37
0.551
4.08
OBu-t
CBzHN
116
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 10 05


Filename: CY1868 .H81
0.899
2.06
1.13
0.445
0.84
0.654
0.44
3.28
CBz
N
CBZHN
N
H
OBu-t
O
N
CH3
117
PPM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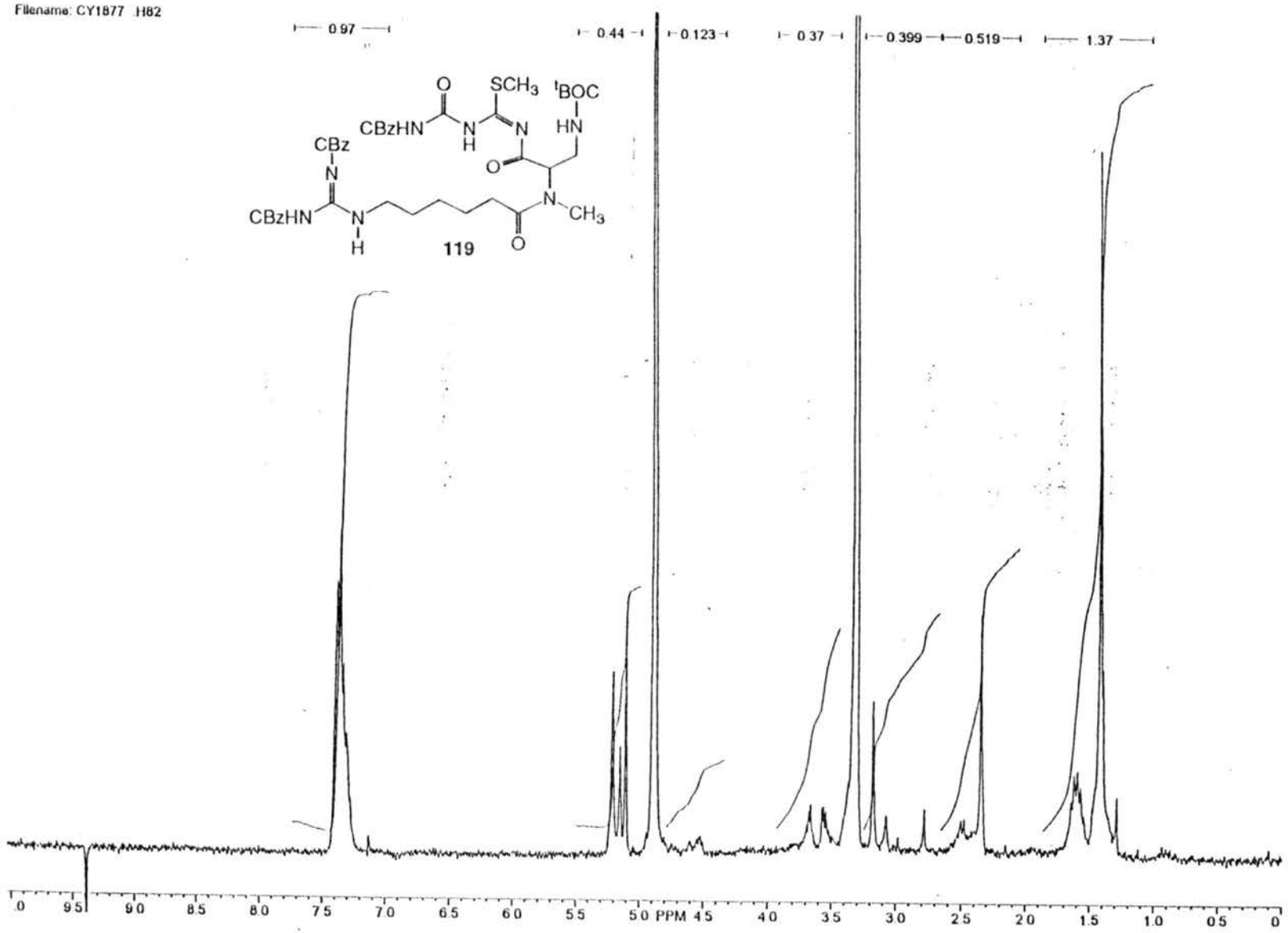
0.0635
0.207
1.69
0.668
0.457
2.81
CBz
N
CBzHN
N
H
HO
O
NH
tBOC
N
CH3
O
118
PPM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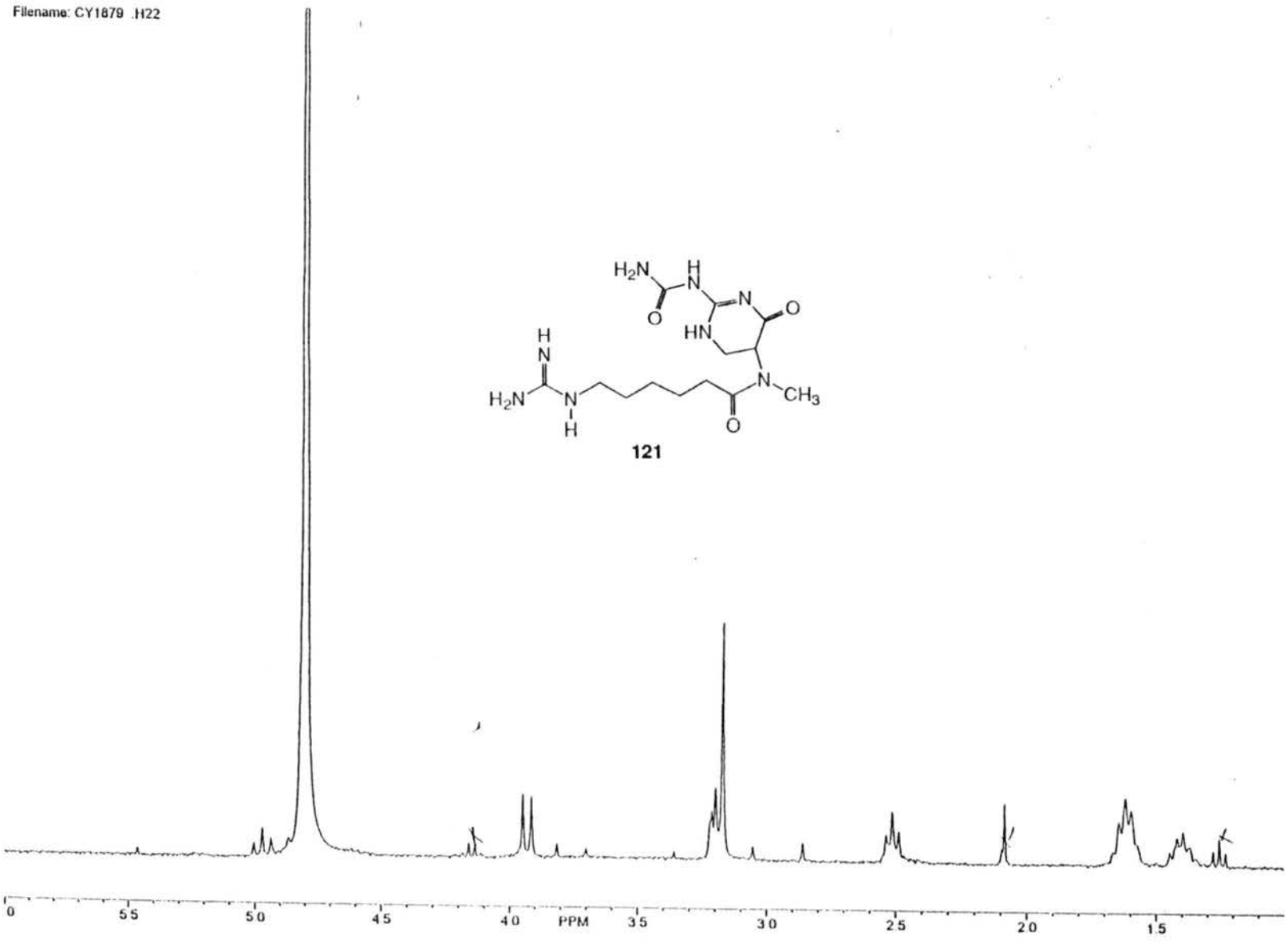
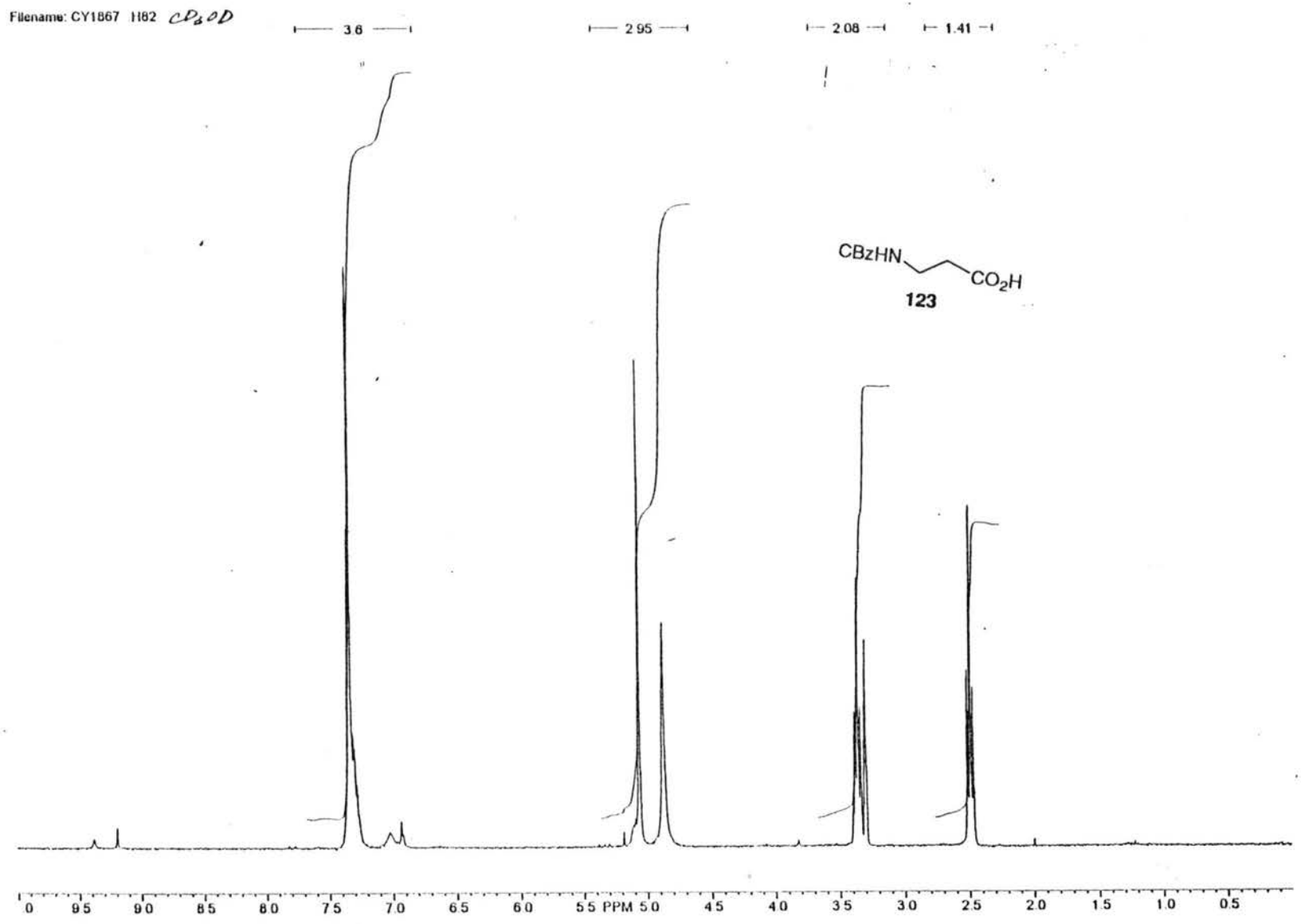
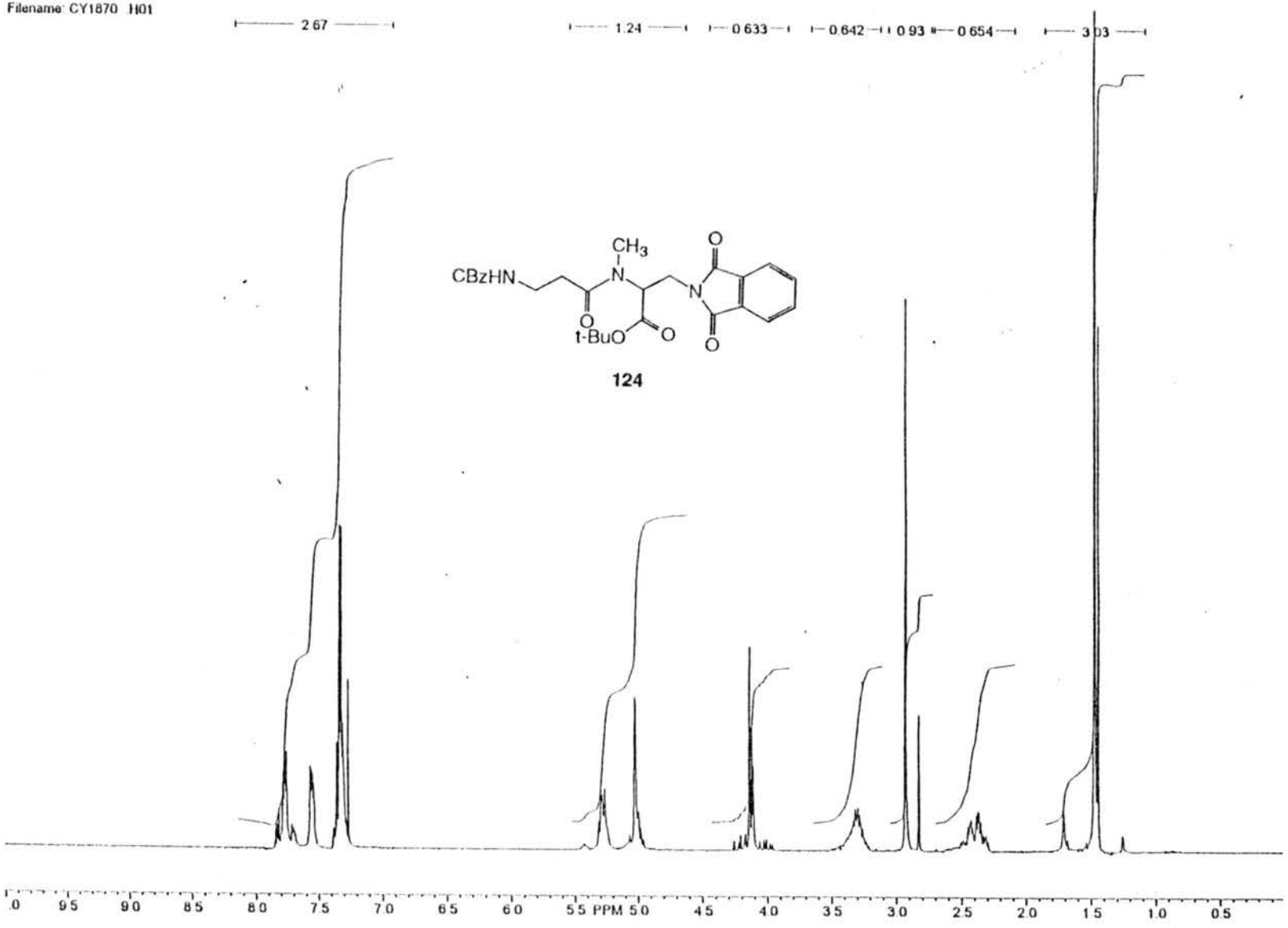
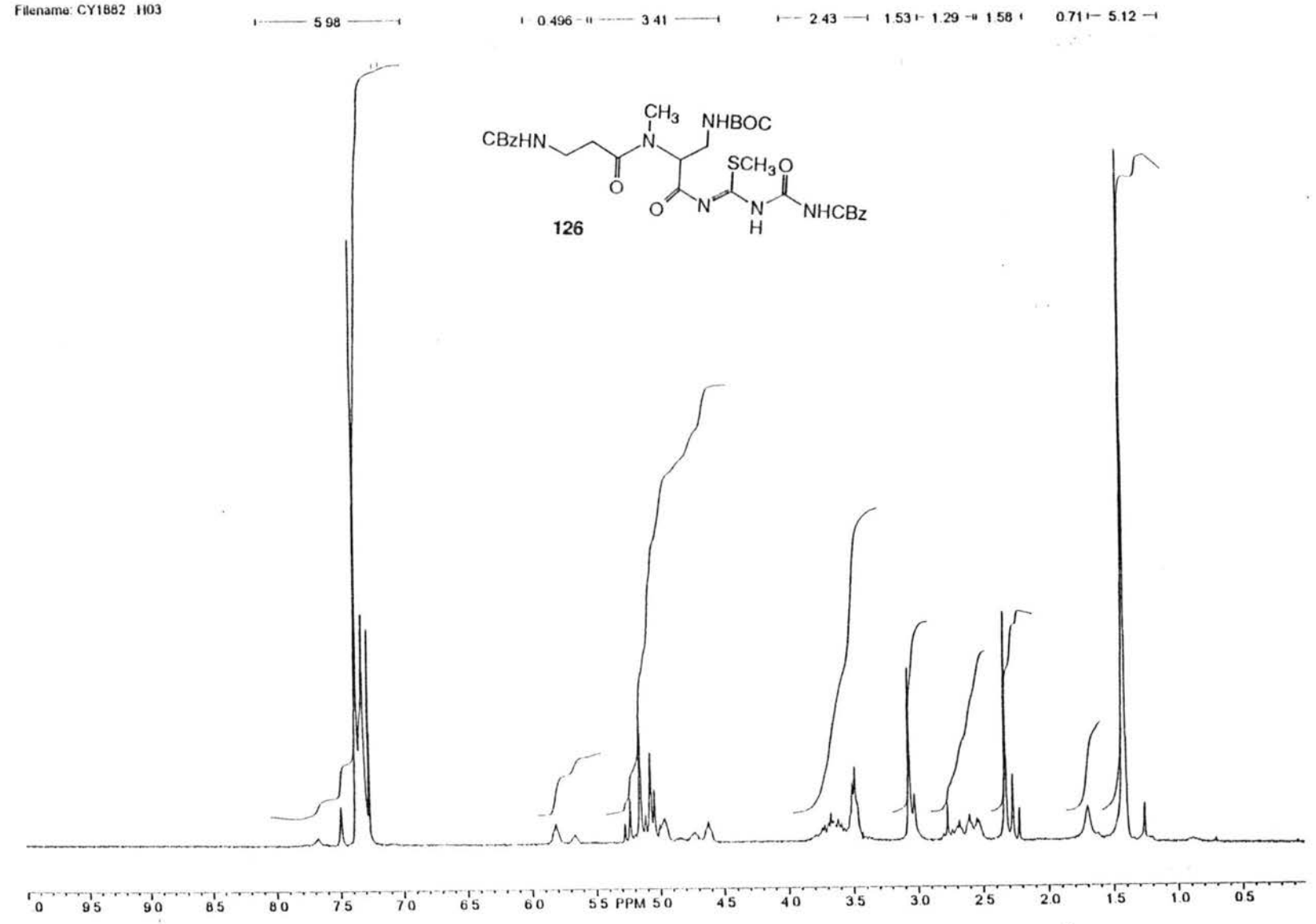
0.44
0.123
0.37
0.399
0.519
1.37
CBzHN
CBz
N
CBzHN
N
H
O
SCH3
N
O
CBzHN
N
H
tBOC
HN
N
CH3
O
119
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0 PPM 4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0

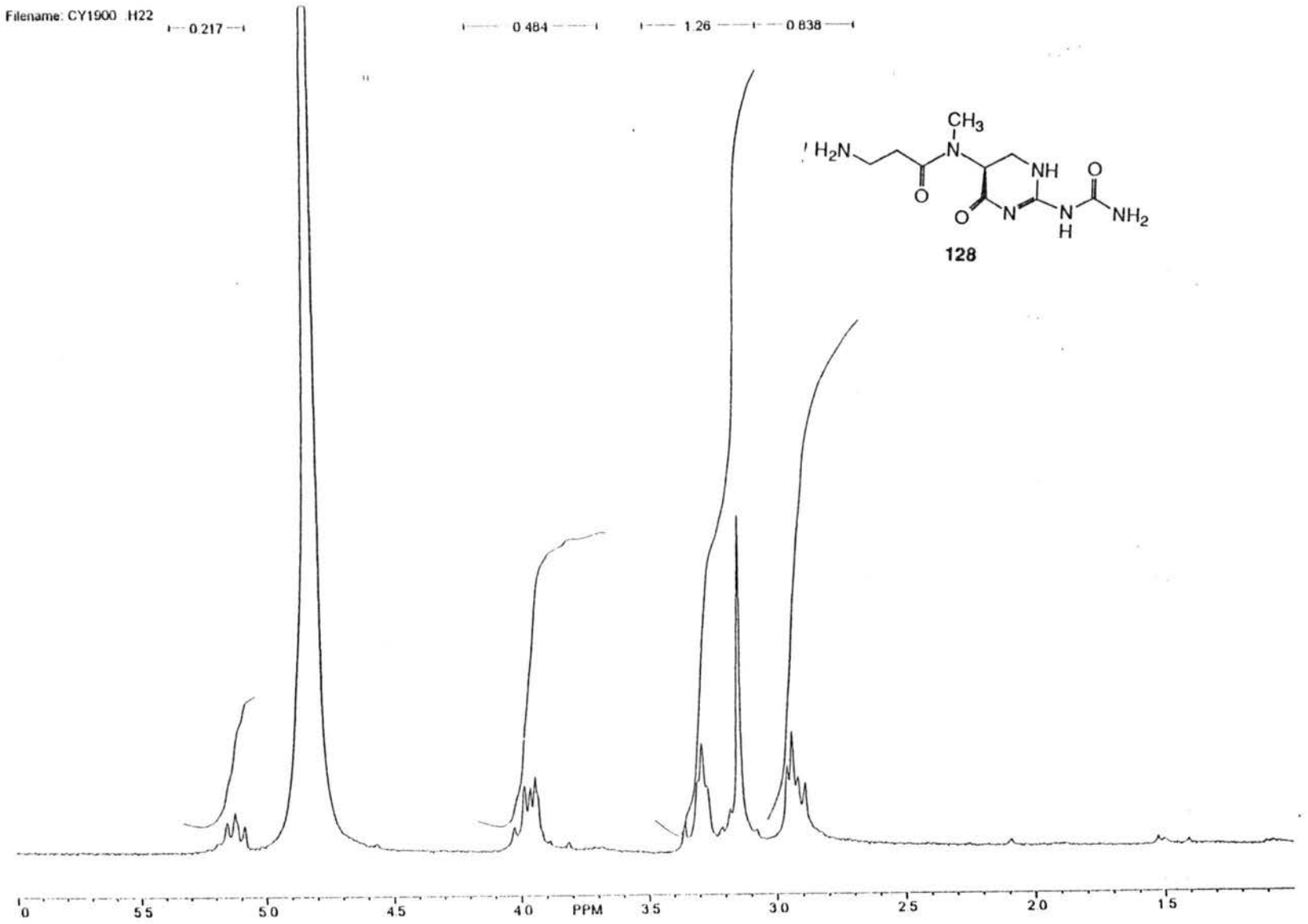


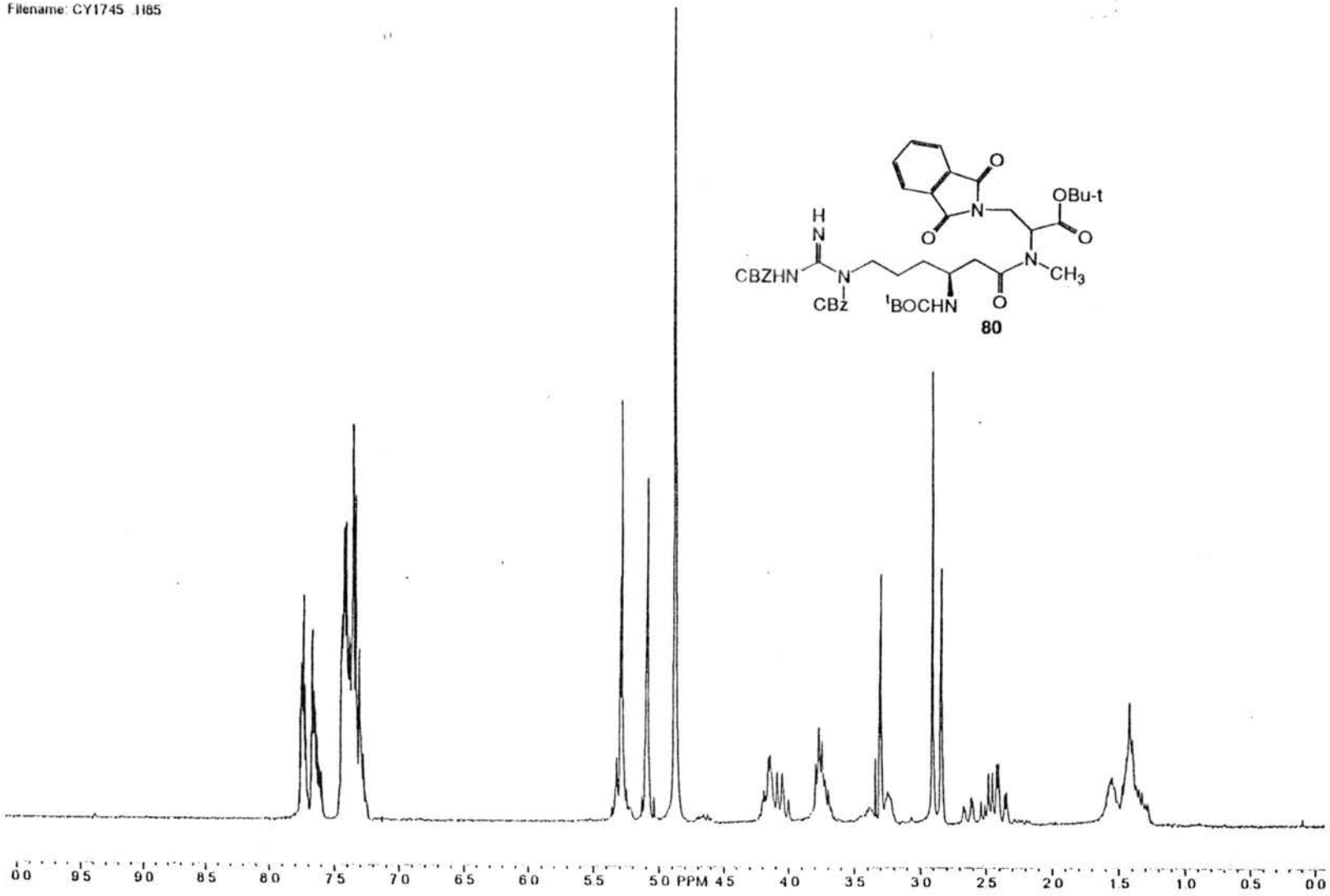
121

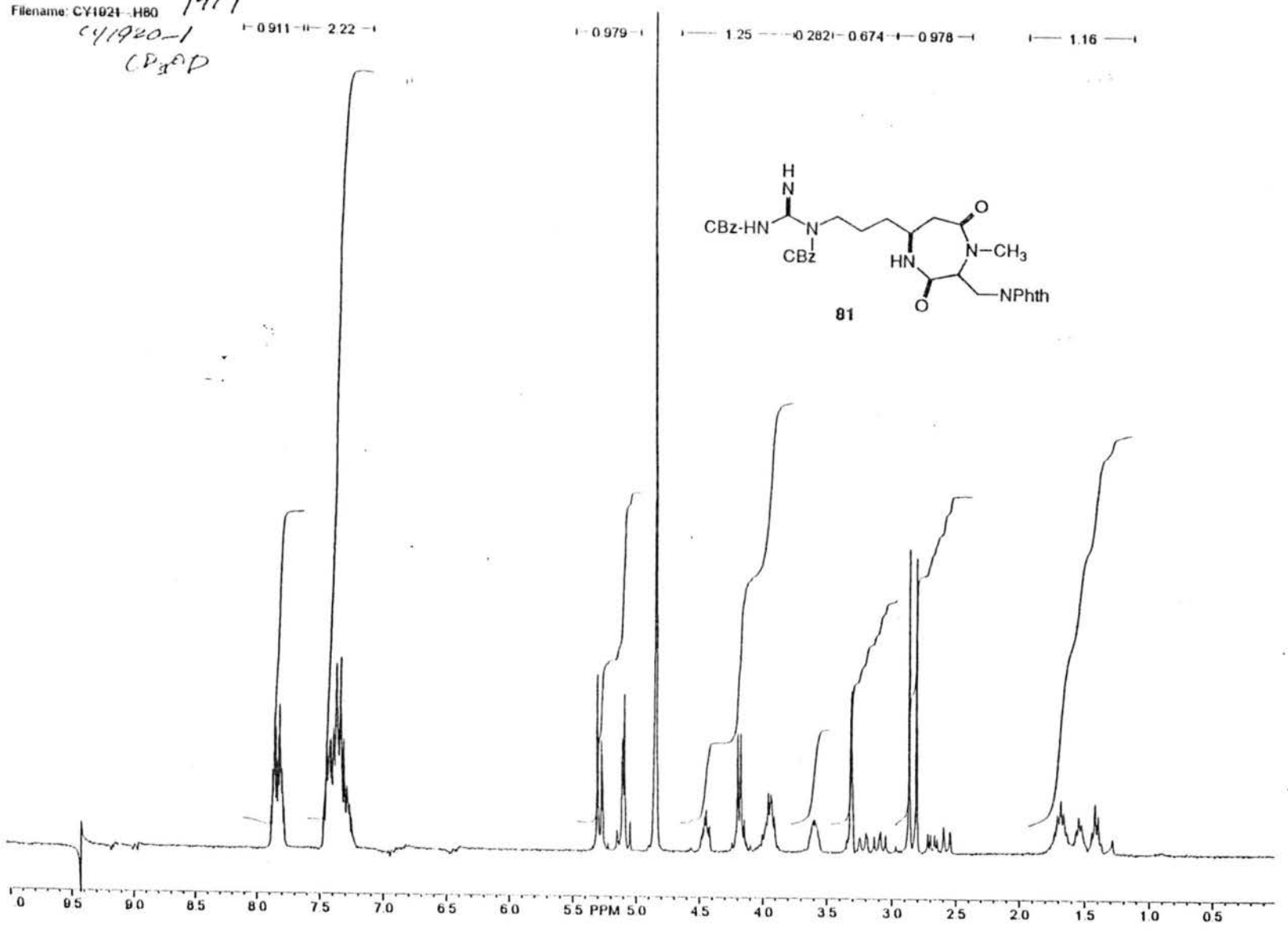

Filename: CY1867 H82 CD_3OD
3.6
2.95
2.08
1.41
CBzHN
CO_2H
123
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 1.0 0.5


Filename: CY1870_H01
2.67
1.24
0.633
0.642
0.93
0.654
3.03
CBzHN
CH3
N
O
O
t-BuO
O
N
O
O
124
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 1.0 05

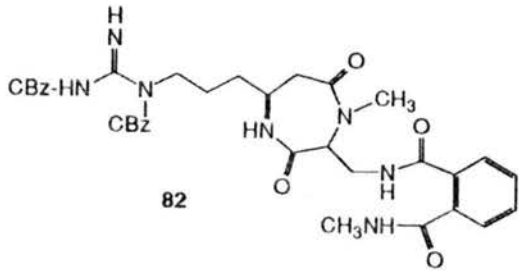

Filename: CY1882 H03
5.98
0.496
3.41
2.43
1.53
1.29
1.58
0.71
5.12
CBzHN
CH3
N
NHBOC
O
SCH3
O
O
N
N
H
NHCBz
126
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 1.0 0.5


Filename: CY1900 H22
0.217
0.484
1.26
0.838
CH3
H2N
N
NH
O
O
N
N
H
NH2
128
0
55
50
45
40
PPM
35
30
25
20
15

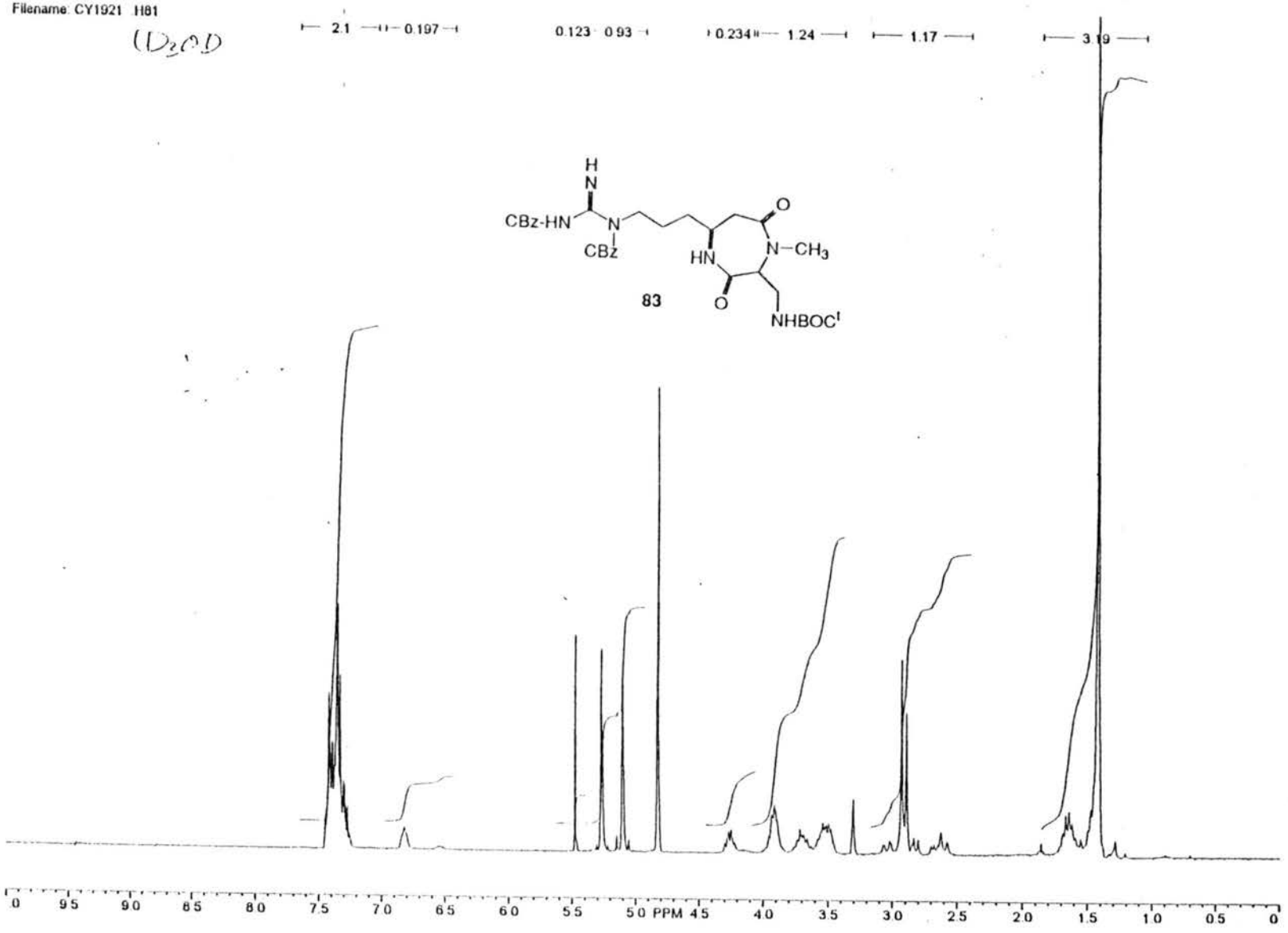

Filename: CY1745 1185
OBu-t
H
N
CBZHN
N
CBz
'BOCHN
N
CH3
80
00 95 90 85 80 75 70 65 60 55 50 PPM 45 40 35 30 25 20 15 10 05 00

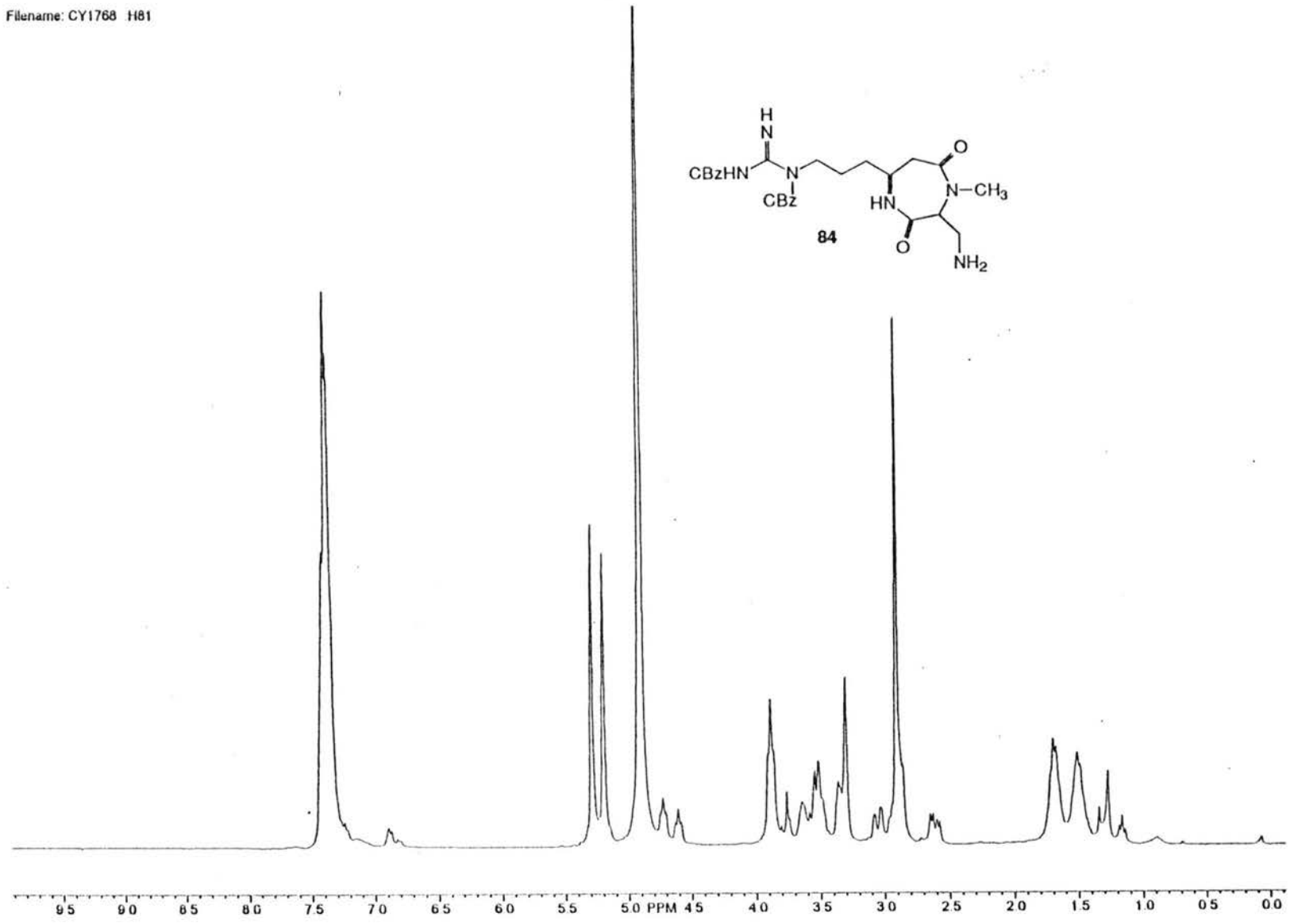

Filename: CY1021-H80
1917
CY1920-1
CD3OD
0.911
2.22
0.979
1.25
0.282
0.674
0.978
1.16
CBz-HN
N
CBz
H
N
HN
O
N–CH3
NPhth
81
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 10 05

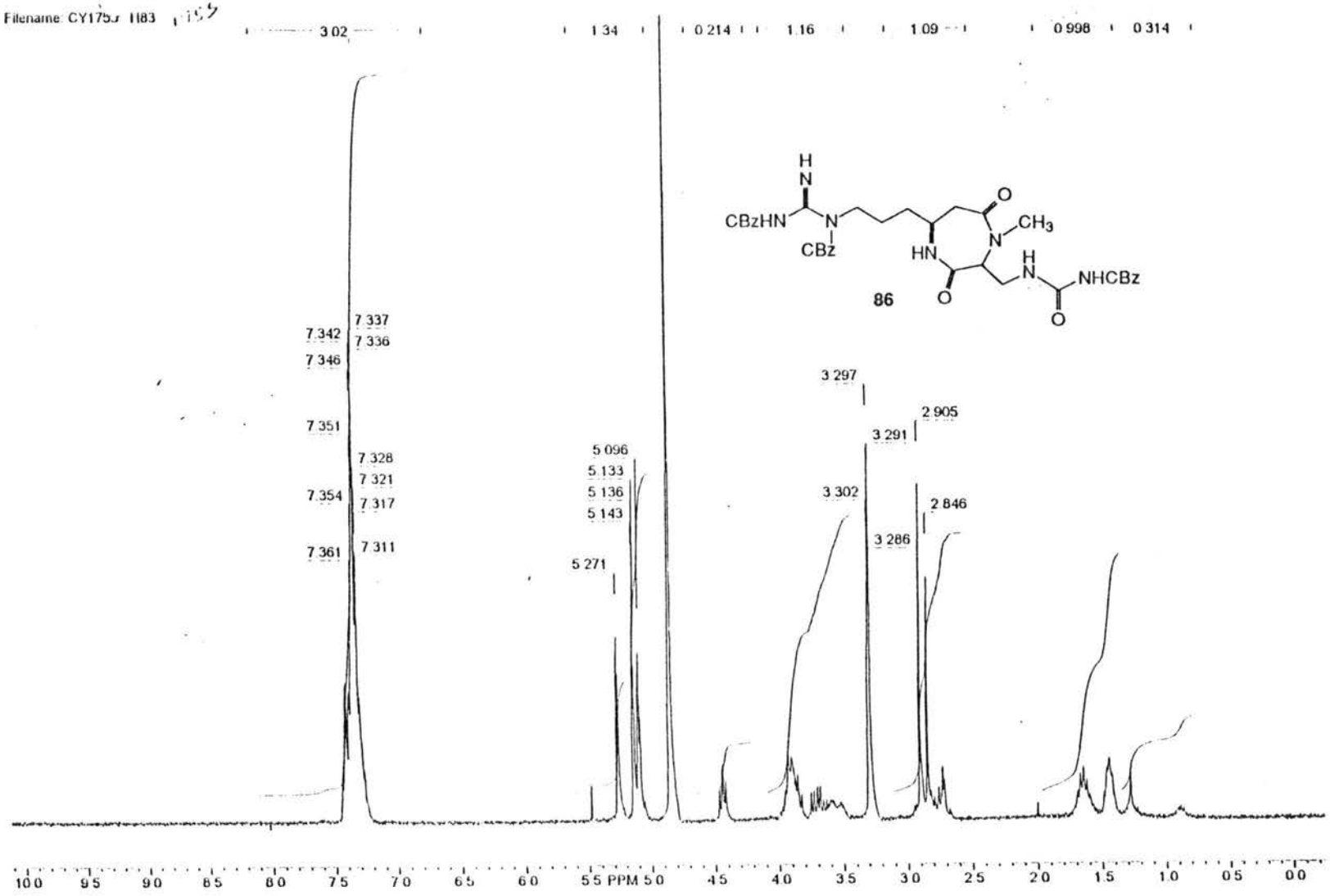
Filename: CY1760 .H85



82


Filename: CY1921 H81
(D2O)
2.1
0.197
0.123
0.93
0.234
1.24
1.17
3.19
CBz-HN
N
CBz
HN
N–CH3
O
O
NHBOC'
83
0
95
90
85
80
75
70
65
60
55
50 PPM 45
40
35
30
25
20
15
10
05
0


Filename: CY1768 H81
CBzHN
N
H
N
CBz
HN
O
N–CH3
O
NH2
84
95 90 85 80 75 70 65 60 55 50 PPM 45 40 35 30 25 20 15 10 05 00


Filename: CY175J 1183
3.02
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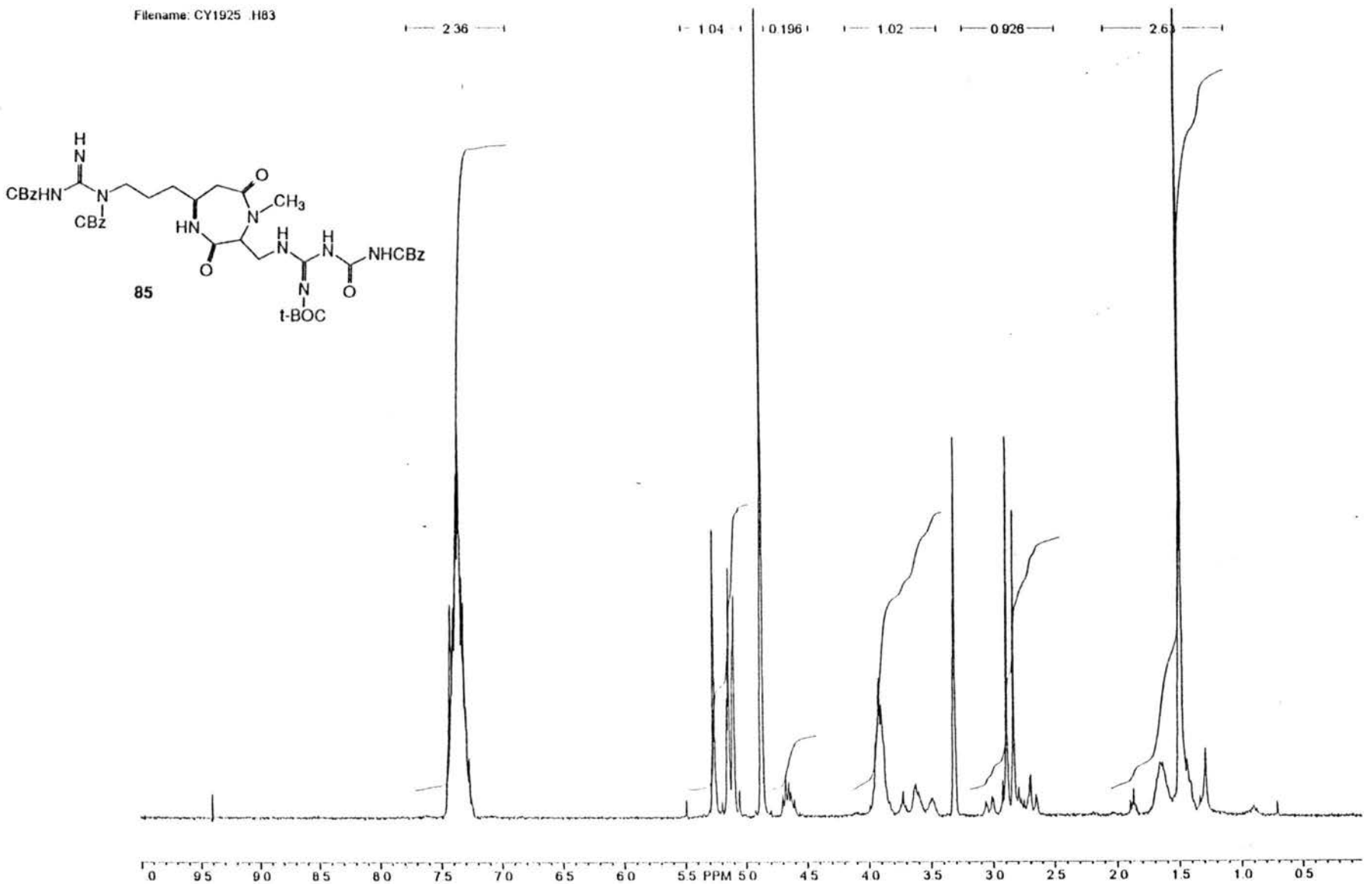
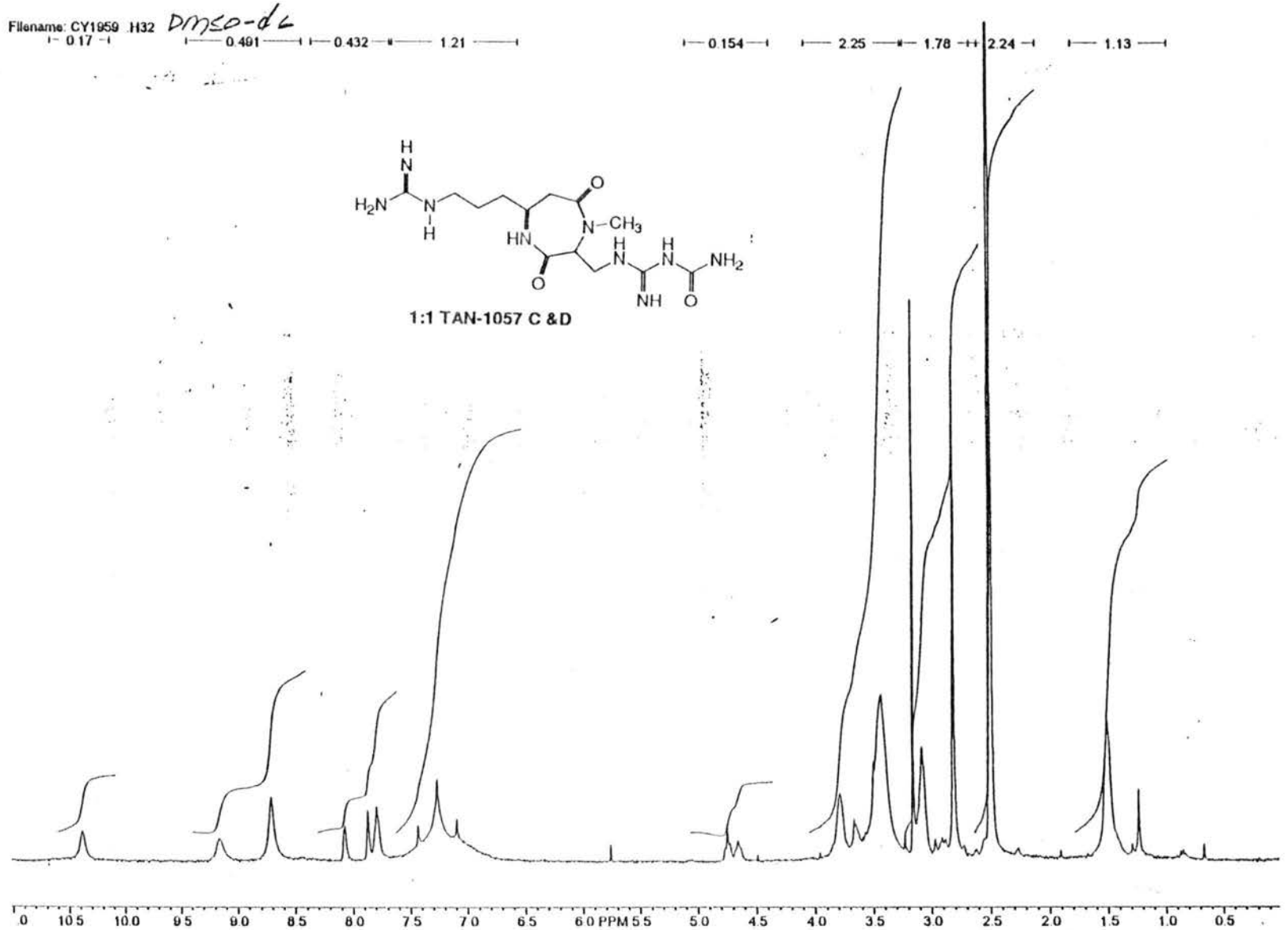
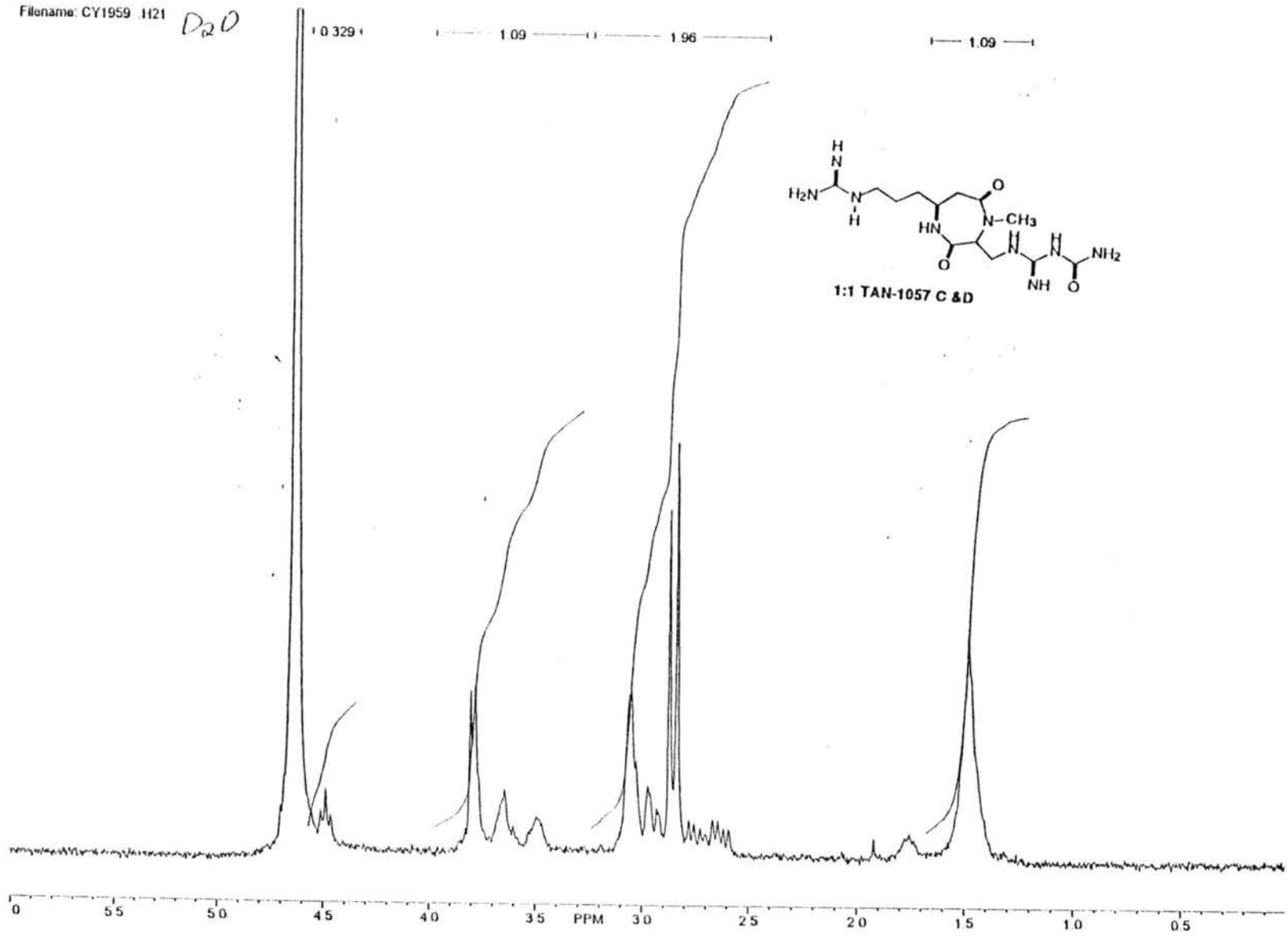
0.214
1.16
1.09
0.998
0.314
7.342
7.337
7.336
7.346
7.351
7.328
7.321
7.354
7.317
7.361
7.311
5.271
5.096
5.133
5.136
5.143
3.297
3.291
2.905
3.302
2.846
3.286
CBzHN
CBz
HN
CH3
NHCBz
86
100 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 10 05 00


Filename: CY1925 .H83
2.36
1.04
0.196
1.02
0.926
2.63
CBzHN
CBz
HN
CH3
NHCBz
t-BOC
85
0 95 90 85 80 75 70 65 60 55 PPM 50 45 40 35 30 25 20 15 1.0 05


Filename: CY1959 H32
DMSO-d6
0.17
0.491
0.432
1.21
0.154
2.25
1.78
2.24
1.13
1:1 TAN-1057 C &D
PPM


Filename: CY1959 H21
D2O
0.329
1.09
1.96
1.09
1:1 TAN-1057 C &D
0
55
50
45
40
35
PPM
30
25
20
15
10
05