SYNTHETIC STUDIES ON THE CYCLODEPSIPEPTIDE PORTION OF ANTITUMOUR ANTIBIOTIC A83586C

by

Vern Michael Delisser

A thesis submitted for the degree of Doctor of Philosophy

in the Faculty of Science at the University of London.

January 1995

University College London,

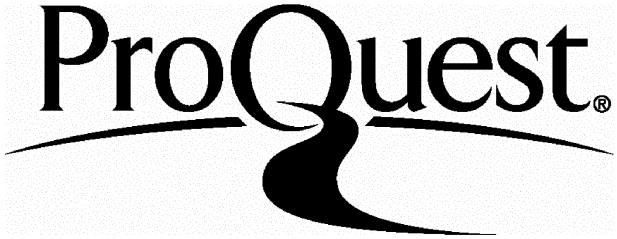
Chemistry Department,

Christopher Ingold Laboratories,

20 Gordon Street,

London,

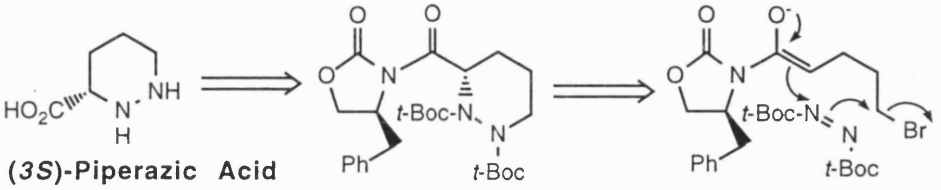
WC1H OAJ.



This thesis is dedicated to my beloved mother.

Abstract

Chapter 1 discusses the asymmetric synthesis of (*3S*)-hexahydropyridazine-3-carboxylic acid [(*3S*)-piperazic acid]. Two procedures were developed, firstly an electrophilic hydrazination of a chiral bromovaleryl carboxymide enolate with di-*tert*-butylazodicarboxylate, followed by intramolecular S_N2 displacement of the bromide by the resulting aza anion. Subsequent hydrolysis and acidolysis gave (*3S*)-piperazic acid in an enantiomeric excess greater than 96%.

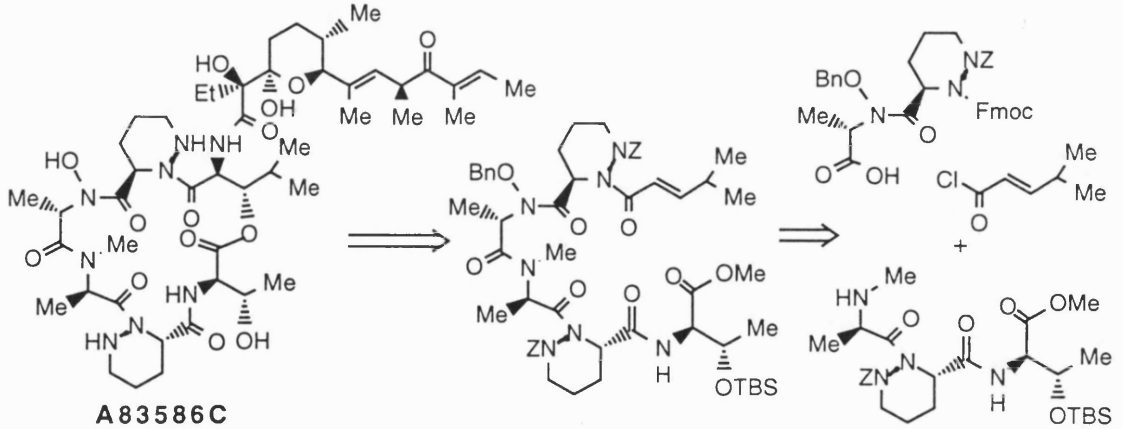


(*3S*)-Piperazic Acid

The second procedure was based on the formation of a titanium enolate from the chiral bromovaleryl carboximide at 0°C to form a hydrazine which was subsequently cyclised with the use of sodium hydride. Hydrolysis and acidolysis gave (*3S*)-piperazic acid in an enantiomeric excess of 78%.

Chapter 2 is a concise review of the synthesis of natural cyclodepsipeptides between the years 1960 and 1994.

Chapter 3 describes the evolution of a synthetic strategy for the cyclodepsipeptide portion of A83586C. A hexapeptide precursor has been prepared via a 3+2+1 fragment condensation.



A83586C

Acknowledgments

I would like to take this time to thank my supervisor Dr. K.J. Hale for the endless support and advice he has given me throughout this project. I would also like to thank my colleagues Gurpreet, John, Kuan, Soraya , Dr. S.A. Peak and Karen. Special thanks to Gill Maxwell, Steve Corker and Alan Stone for their analytical services during the course of my research. Many thanks to the ULIRS Mass Spectrometry Service at the London School of Pharmacy. Special thanks to Dr. P. Durette and Dr. C. Caldwell (Merck, Sharpe and Dohme, Rahway, USA) for supplying us with their procedure for the synthesis of (*3S*, *3R*)-piperazic acid. Thanks are also due to Dr. S. Redshaw (Roche Products, Welwyn Garden City) for presenting us with an authentic sample of (*3R*)-piperazic acid.

CONTENTS

Chapter 1: Synthesis of (*3S*)-Hexahydropyridazine-3-carboxylic acid (*3S*)-Piperazic acid

1.0 Introduction

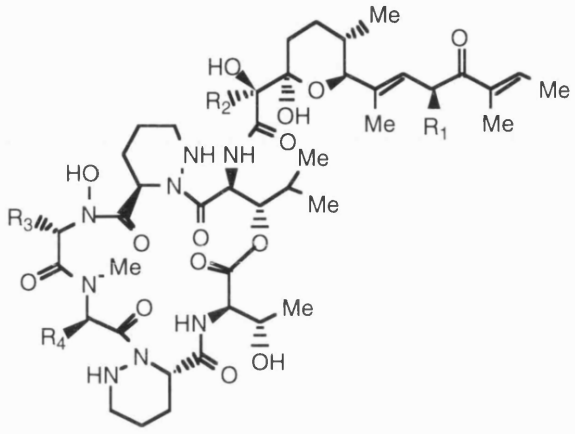
1.1 The Azinothricin Family of Antibiotics: Isolation, Structure and Biological Properties

In 1986, Maehr and his coworkers at Hoffmann La-Roche isolated a powerful new antibiotic from the fermentation broths of *Streptomyces* X-14950 in crystalline form. It had an MIC values of < 0.008 μg/ml against two strains of *Streptococcus pneumoniae*, and < 0.008-0.016 μg/ml against 31 strains of *Staphylococcus Aureus.* It was also active at 0.001 μg/ml against *Clostridium Septicum* and *Clostridium Histalyticum.* They named this compound Azinothricin **1**; its structure was determined by a series of chemical degradations and single crystal X-ray analysis. Azinothricin contains of a pyran side chain appended onto a 19-membered cyclohexadepsi-peptide ring linked at the C-(28) amide bond. The cyclohexadepsipeptide ring contains six unusual amino acids: *erythro*-3-hydroxy-L-leucine, D-threonine, L-piperazic acid, *N*-methyl D-alanine, *N*-hydroxy-*O*-methyl-serine and D-piperazic acid. A lactone bond links the carboxyl of the D-threonine residue and the hydroxyl group of the *erythro*-3-hydroxy-L-leucine moiety. The carbonyl stretch at 1745 cm^{-1} in the infrared spectrum of **1** was highly diagnostic of the lactone linkage.[1]

A83586C **2** a closely related antibiotic to Azinothricin was isolated two years later by workers at Eli Lilly from culture filtrates of *Steptomyces karnatakensis.* The structure was also determined by chemical degradation and single X-ray analysis. It was active against many Gram-positive bacteria and retarded the growth of a CCRF-CEM human T-cell leukaemia line with an IC_{50} of 0.0135 μg/ml. The only differences between **1** and **2** are the presence of an *N*-hydroxy-L-alanine in **2** instead of an *N*-hydroxy-*O*-methyl-L-serine, and a methyl instead of an ethyl group at C-37.[2]

In 1990, Japanese workers isolated *Streptomyces flavidovirens* from a soil sample in Brazil and found it produced an antibiotic with potent cytotoxicity which they named Citropeptin **3**. The structure of **3** was elucidated on the basis of NMR spectral comparison with **1** and **2**. The peptide

ring in **3** consists of a threonine, two piperazic acid residues, *N*-methylleucine, *O*-methyl-serine and 3-hydroxyleucine. The stereochemistry and absolute configuration of Citropeptin **3** is thought to be identical to that in Azinothricin and A83586C, as the specific rotation and ^{13}C chemical shifts are similiar. Its biological activity is quite remarkable, it possessing an IC_{50} of 0.02 μg/ml against murine P388 leukaemia cells and 0.1 μg/ml against B16 melanoma cells; it also prolongs the life of mice bearing the P388 tumour at doses of 2 mg/kg/day.[3]

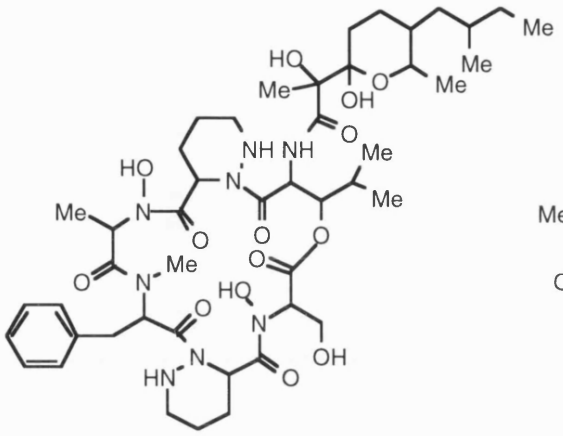


(1) Azinothricin, $R_1 = R_2 = Et$, $R_3 = CH_2OMe$, $R_4 = Me$.
(2) A83586C, $R_1 = R_2 = R_4 = Me$, $R_2 = Et$.
(3) Citropeptin, $R_1 = R_2 = Me$, $R_3 = CH_2OMe$, $R_4 = CH_2CH(Me)_2$.

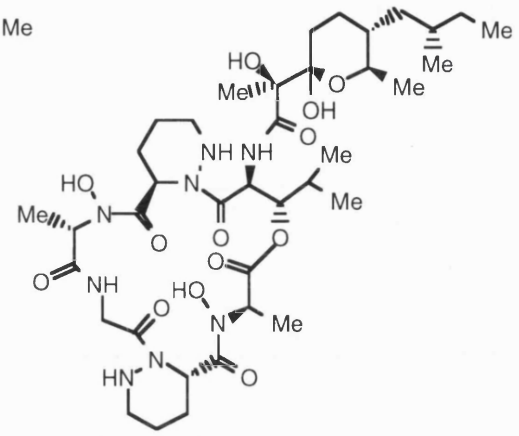
Another member of the Azinothricin family is Variapeptin **4**, isolated from *Streptomyces variabilis* in 1990. Its structure was determined by NMR analysis using a variety of 2D techniques and by spectral comparison with citropeptin **3**. This revealed the presence of *N*-methylphenyl-alanine, *N*-hydroxyalanine, 3-hydroxyleucine, serine and two piperazic acid residues. It showed potent *in vivo* activity against Gram positive bacteria but not against Gram negative bacteria or fungi. It had an IC_{50} value of 0.01 μg/ml against P388 leukaemia cells. When administered intra-peritoneally, it was toxic to mice at 5 mg/kg, but inactive *in vivo* against P388 lymphocytic leukemia at the highest non-toxic dose.[3,4]

L-156, 602 (**5**) is a compound resembling variapeptin that was isolated from a culture of *Streptomyces spp.* MA-6348, obtained from a Japanese plant rhizosphere soil sample. The structure of L-156,602 was determined by NMR studies and X-ray diffraction. The NMR studies, in conjunction with GC-MS analysis of the trimethylsilyl derivatives of the acid hydrolysate, indicated the presence of one glycine, one hydroxyleucine, two moles of piperazic acid and two moles of *N*-hydroxyalanine. L-156, 602 is a competitive inhibitor of the binding of anaphylatoxin C5a to its

receptor on human polymorphonuclear leukocytes, and as such it may be of therapeutic value for the treatment of a variety of inflammatory diseases.[5,6]

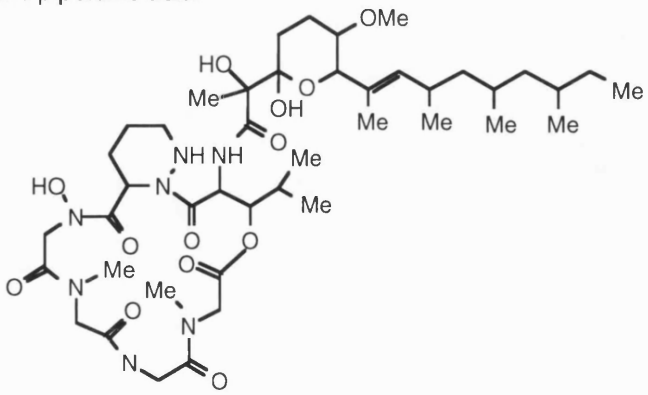


(4) Variapeptin



(5) L-156,602

In 1993, Japanese workers isolated a new antibiotic from *Actinomadura verrucosospora* Q886-2 which they named verucopeptin **6**. This substance showed potent *in vitro* cytotoxicity and specific *in vivo* activity against mouse B16 melanoma. It was found to inhibit the biosynthesis of DNA and RNA, having IC_{50} values of 0.26 and 0.29 μg/ml respectively, but its inhibition of protein synthesis was only marginal at 1.0 μg/ml. Its inhibitory action against *Strepto-coccus faecalis* and *Bacillus subtilis* was low, it showing MIC values around 100 μg/ml. Veruco-peptin showed no activity against other Gram-positive, Gram-negative, or anaerobic bacteria and fungi at 100 μg/ml.[7] The structure of **6** was determined primarily by spectroscopic analysis and chemical degradation, which revealed a 19-membered cyclodepsipeptide similar in structure to the azinothricin family of antibiotics. However, it differed from this clan in that it contained glycine, sarcosine and only one piperazic acid.[8]



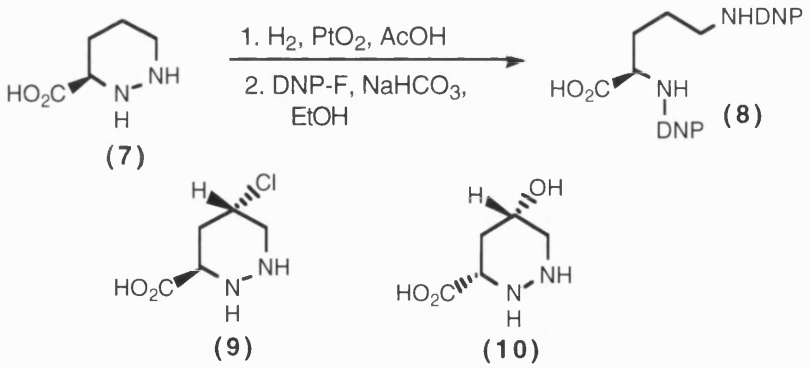
(6) Verucopeptin

It is interesting that the position and stereochemistry of the 3-hydroxy-leucine, D-piperazic acid and pyran region is conserved throughout all these compounds, and this could be a clue to the shape of the receptor sites.

1.2 Isolation and Structure determination of Piperazic Acid

Whilst investigating the structure of the monamycin family of cyclodepsipeptide antibiotics, Hassall and coworkers treated a complex mixture of monamycins with boiling 6N-hydrochloric acid and separated the hydrolysate by ion exchange chromatography on Amberlite C.G. 120. From the hydrolysate mixture, three closely related α-hydrazino acids were isolated having empirical formulae of $C_5H_{10}N_2O_2$, $C_5H_9ClN_2O_2$ and $C_5H_{10}N_2O_3$ respectively. In order to determine their structures, they were each hydrogenated over platinum oxide and subsequently treated with 1-fluoro-2,4-dinitrobenzene (DNP-F). In one of the cases, compound **8** was obtained, and shown to be identical to an authentic sample of N^{α},N^{δ}-bis(DNP)-D-ornithine (Scheme 1). This suggested that the structure of one of these three α-hydrazino acids was compound **7**; it was given the name D-piperazic acid by Hassall and coworkers.[9]

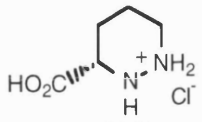
Scheme 1



The other two α-hydrazino acids were shown to be (*3R*,*5S*)-5-chloropiperazic acid **9** and (*3S*,*5S*)-5-hydroxypiperazic acid **10**, respectively, by their conversion to ornithine derivatives.[10,11]

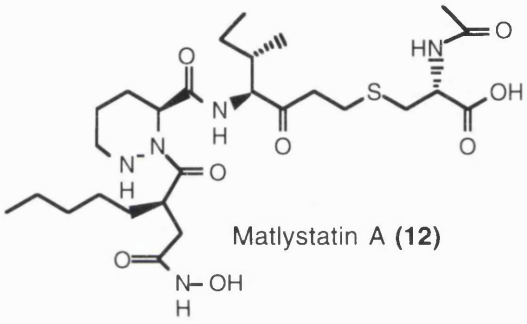
1.3 Occurence and Biological Properties of Piperazic acid

(*3S*)-Piperazic acid **11** has been shown to be a potent inhibitor of γ-aminobutyric acid (GABA) uptake in rat brain slices, it being 25 times more active than its (*3R*)-enantiomer **7**. Thus (*3S*)-piperazic acid is useful for the treatment of audiogenic seizures.[12]

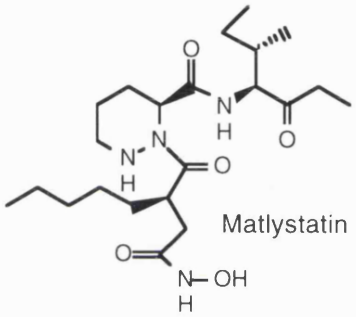


(11)

Piperazic acid is present in a number of pharmacologically active natural products that include the Matlystatins. These are isolated from *Actinomadura atramentaria* and are potent inhibitors of type IV collagenases.[13] Their mode of action is believed to arise through their binding to the zinc atom at the active site of these enzymes which prevents them from functioning in their normal capacity. Inhibitors of type IV collagenases may prove useful for preventing tumour cell invasion and metastasis. The structure of Matlystatin A **12**[14] and B **13**[15] has been confirmed by total synthesis.

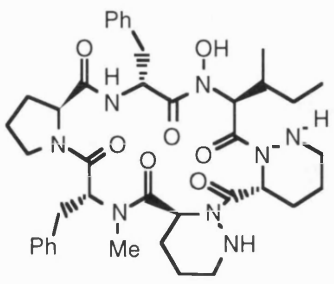


Matlystatin A **(12)**

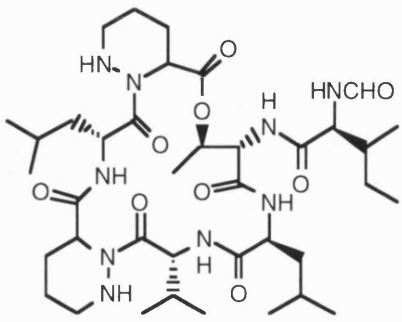


Matlystatin B **(13)**

L-156,373 **14** is a cyclic depsipeptide that contains both (*3S*) and (*3R*)-piperazic acids. It possesses antagonistic properties against oxytocin/arginine vasopressin, and as a result, may prove useful for the prevention of pre-term labour, and disturbances in water balance.[16,17] Piperazic acid is also present in several non-ribosomal, biologically-active depsipeptides typified by Depsidomycin **15**,[18] which functions as an immunosuppressive agent, and in the Azinothricin family of antibiotics.[1-8]

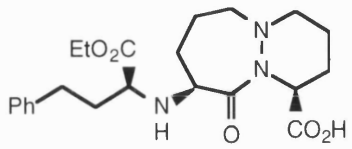


L-156,373 **(14)**



Depsidomycin **(15)**

An annulated (*3S*)-piperazic acid is also to be found in the commercial drug Cliazapril **16** which is currently used for the treatment of congestive heart failure and hypertension.[19]



(16)

1.4 Synthesis of α-Hydrazino acids

α-Hydrazino acids have aroused considerable synthetic interest in recent years due to their resemblance to α-amino acids, and their potential as pharmacologically active mimetics of these molecules.[20a-c]

Several methods have therefore been developed for their asymmetric synthesis. Gennari and coworkers developed a protocol for the synthesis of α-hydrazino acids that was based on the electrophilic amination of a silyl ketene acetal.[21] Enolisation of the *N*-methyl ephedrine ester **17** with lithium diisopropylamide (LDA) and subsequent trapping with trimethylsilyl chloride gave silyl ketene acetal **18**. Addition of the ketene acetal to a -80 °C solution of titanium tetrachloride and di-*tert*-butylazodicarboxylate (DBAD) in dichloromethane gave the protected hydrazino ester **19** as essentially a single diastereoisomer (Scheme 2). Subsequent treatment with trifluoroacetic acid then excised the *N*-*t*-Boc protecting groups, while hydrolysis with aqueous lithium hydroxide in THF gave α-hydrazino acid **20**, in good overall yield and ee (Table 1).

The electrophilic amination of enolates derived from chiral *N*-acylated oxazolidinones with DBAD was a technique for making α-hydrazino acids that was introduced by Evans [22a,b] and Vederas.[23] This procedure involves treating *N*-acylated oxazolidinones such as **21** with lithium diisopropylamide (LDA) at low temperature and trapping the resulting chiral enolates with DBAD; it

Scheme 2

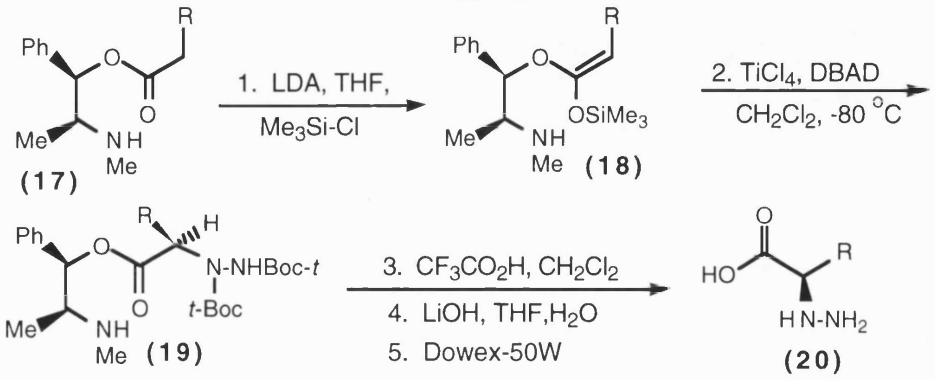
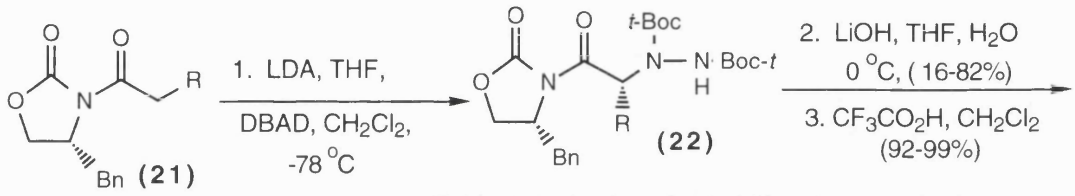


Table 1. Stereoselective " Amination" of ephedrine enolates

R	**19**, % yield	**20**, % yield	% ee of **20** (after crystallisation)	absolute config.
CH_3	70	78	> 98	R
CH_2Ph	45	81	> 98	R
$CH_2CH(CH_3)_2$	70	81	> 98	R
$(CH_2)_3CH_3$	45	78	> 98	R

leads to protected α-hydrazino adducts such as **22** in good yield. Subsequent removal of the auxiliary and acidolysis of the nitrogen protecting groups delivers the chiral α-hydrazino acids **20** in ee's greater than 98% and in excellent yield (Scheme 3).

Scheme 3



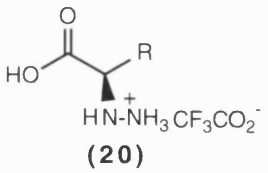
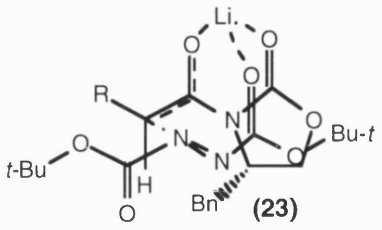


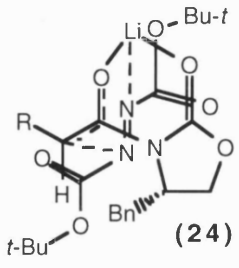
Table 2. Amination of chiral *N*-acyl-oxazolidinone

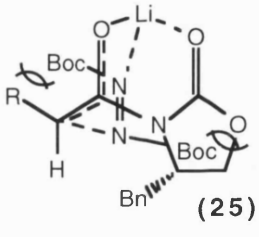
R	**22**, % yield	% ee of **20**	absolute config.
CH_3	92	> 98	R
CH_2Ph	91	>97	R
$CH(CH_3)_2$	95	> 98	R

Evans and coworkers rationalise the high levels of diastereoselectivity observed in these electrophilic aminations by invoking the highly organised, chelated pericyclic, 8-membered transition state such as the one shown in **23**. Transition states **24** and **25**, in which the azo-N atoms are also involved in chelation with the lithium enolate, were considered less likely due to the unfavourable steric interactions between the chiral enolate and DBAD Boc groups (Figure 1).

Figure 1







Oppolzer[25a,b] and Guanti[26] have also developed methods for the asymmetric synthesis of α-hydrazino acids that are based on the electrophilic *C*-amination of enolates derived from *N*-acylated 9,10-bornyl sultams and dianions of β-hydroxy esters respectively.

An alternative approach to the synthesis of α-hydrazino acids is to construct the N-N bond from an α-amino acid. The first such procedure to be developed was that of Yamada and coworkers. They nitrosated *N*-benzylated α-amino ester **26** with nitrous acid and reduced the resulting *N*-nitroso derivative with zinc in acetic acid to obtain the N^{β}-acyl derivative **27**. Hydrogenolytic removal of the N^{2}-benzyl group in the presence of *p*-toluenesulfonic acid gave hydrazine **28**, which was then acid hydrolysed to remove the N^{1}-acetyl and the ester group simultaneously to furnish α-hydrazino acid **20** (Scheme 4).[27] Unfortunately, this procedure was quite laborious, inefficient, and caused a loss in optical purity.

Scheme 4

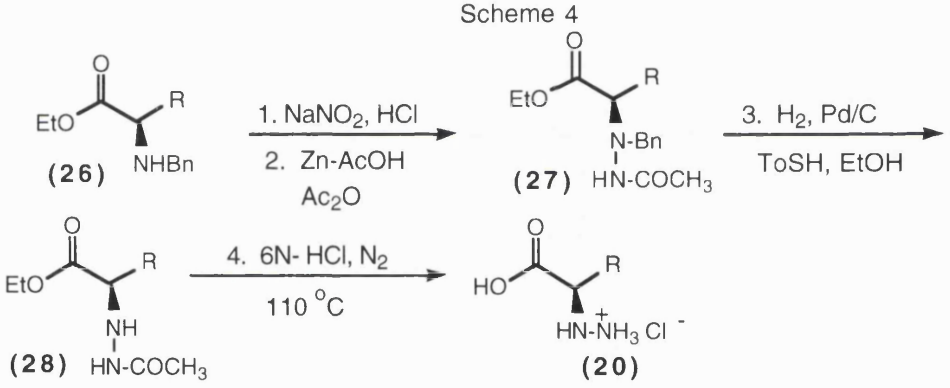
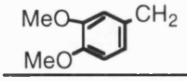


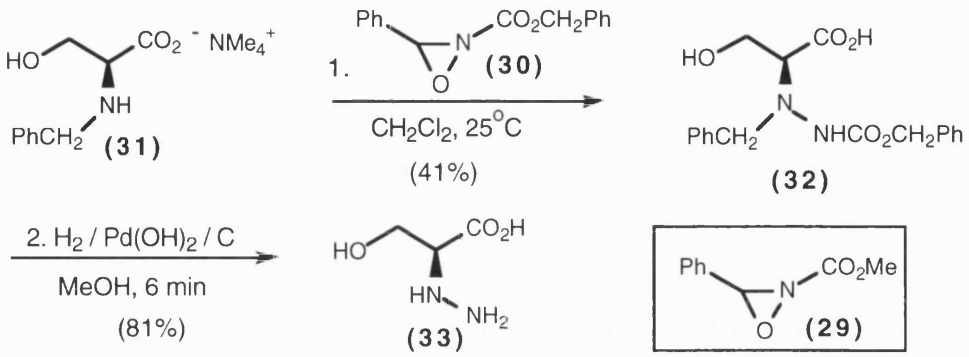
Table 3. Nitrosation of α-amino acid derivatives

R	**27**, % yield	**28**, % yield	**20**, % yield
$PhCH_2$	80%	85	87
$(Me)_2CH$		65	83
		71	80

Several years later, Collet and coworkers developed an approach that was based on the *N*-amination of α-amino acids with *N*-methoxycarbonyl-3-phenyloxaziridine **29**.[28, 29] However, due to the forcing conditions required for the removal of the *N*-methoxycarbonyl group an alternative oxaziridine method was developed by Vederas and coworkers, which utilised *N*-benzyloxycarbonyl-3-phenyloxaziridine **30**. Thus, treatment of the protected serine derivative **31**

with **30** in dichloromethane gave the protected α-hydrazino acid **32** in 41-55% yield. Hydrogenolysis with Pd catalysis furnished α-hydrazino serine **33** in 81% yield (Scheme 5).[30]

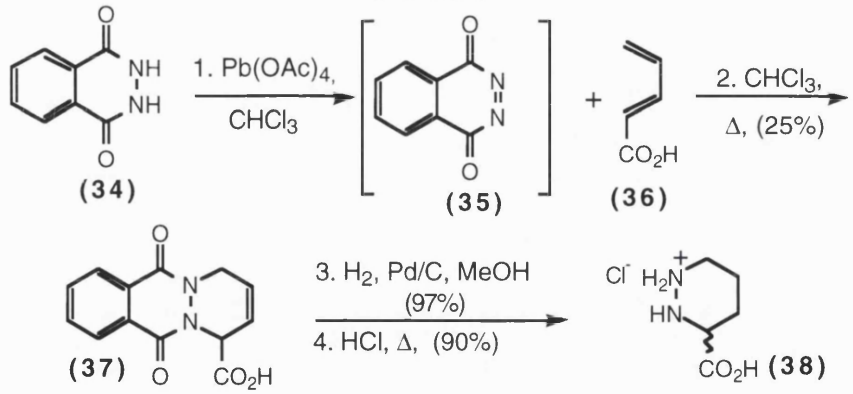
Scheme 5



1.5 Synthesis of (*3S,3R*) Piperazic Acid

The first published synthesis of piperazic acid was in racemic form by Bevan and coworkers whilst working on the structure determination of monamycin.[9] Their route was predicated on a hetero-Diels Alder reaction between the dienophile phthalazinedione **35**, (generated *in situ* by the oxidation of **34** with lead tetraacetate) and penta-2,4-dienoic acid **36** which resulted in the cycloadduct **37**. Hydrogenation of this adduct and subsequent acid hydrolysis produced (*3R,3S*)-piperazic acid as its hydrochloride salt **38** (Scheme 6).

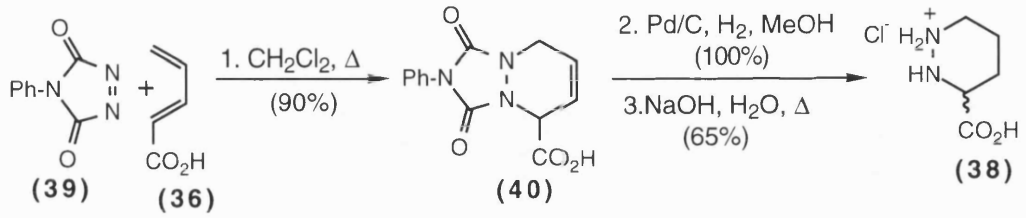
Scheme 6



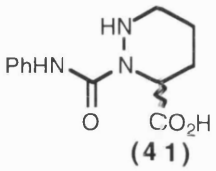
The formation of cycloadduct **37** was low yielding using lead tetraacetate as the oxidant for forming the dienophile. Another problem that led to the low yield was the inherent instability of dienophile **35**. Thus, a more stable and reactive dienophile was employed in the synthesis by Davies and Davies. The dienophile chosen was 4-phenyl-1,2,4-triazoline-3,5-dione **39**, prepared by oxidation of 4-phenylurazole with *t*-butyl hypochlorite. Its Diels-Alder reaction with penta-2,4-dienoic acid **36** yielded cycloadduct **40** in 90% yield (Scheme 7). Hydrogenation of the adduct

using palladium on carbon as catalyst, followed by hydrolysis with aqueous sodium hydroxide at reflux resulted in (*3S,3R*)-piperazic acid **38** after acidification in 65%.[31]

Scheme 7



Whilst using this prodecure Adams and coworkers noted that the hydrolysis of the hydrogenated cycloadduct with the sodium hydroxide solution at reflux only gave a low yield of the desired product, with a large quantity of the partially hydrolysed product **41** being formed.

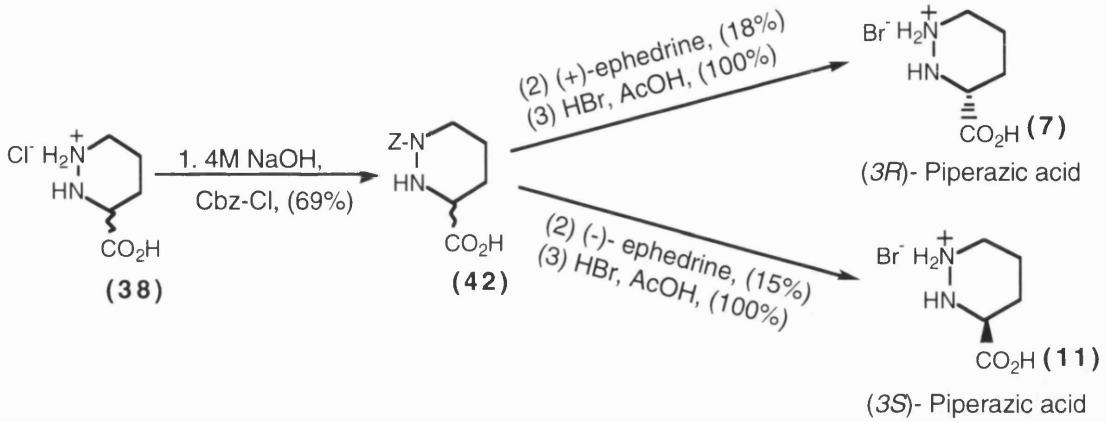


In order to obtain a good conversion to the target compound they executed the hydrolysis with 5.5 molar equivalents of a 85% potassium hydroxide solution in *n*-butanol at reflux.[32]

1.6 Resolution of (*3S,3R*)-Piperazic Acid

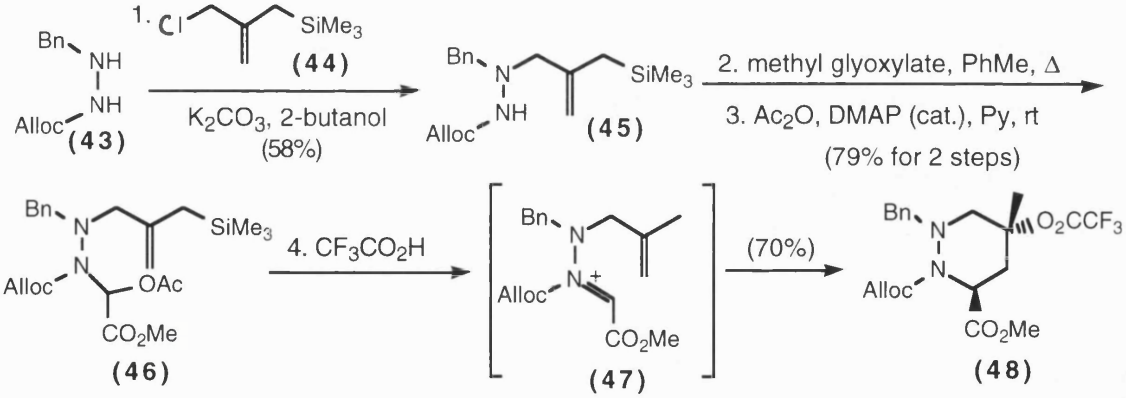
All the aforementioned procedures resulted in racemic (*3R,3S*)-piperazic acid **38**. However, to obtained a pure sample of each enantiomer, a resolution had to be carried out (Scheme 8).[33] This was achieved by initially forming the N^1-benzyloxycarbonyl (Z) derivative **42** using benzyl chloroformate with aqueous sodium hydroxide;[34] the product (**42**) was then recrystallised from ethyl acetate in the presence of (-)-ephedrine hydrate[35] to give pure (*3S*)-N^1-(Z)-piperazic acid (-)-ephedrine salt after several recrystallisations. A similar treatment of the mother liquors with (+)-ephedrine hydrate resulted in the opposite enantiomeric salt being isolated. Acidolysis with aqueous hydrochloric acid and subsequent deprotection of the benzyloxy-urethane group with hydrogen bromide in acetic acid gave pure (*3R*) **7** and (*3S*)-piperazic acid **11** hydrobromide salts in approximately 1.5% overall yield.

Scheme 8



Several workers have developed synthetic strategies to racemic piperazic acid derivatives. Speckman have devised an approach that is based on *N*-acyl-hydrazonium ion intermediates. Thus, hydrazine **43** was alkylated with 2-(chloromethyl)-3-trimethylsilyl)propene **44** to give **45** in 58% yield. This was then treated with anhydrous methyl glyoxylate (20 equiv), and subsequent acylation gave the cyclisation precursor **46** in a yield of 79%. Treatment of **46** with trifluoroacetic acid resulted in the piperazic acid derivative **48** in a yield of 70% via the acylhydrazonium ion **47** (Scheme 9).[36] This methodology suffered a major disadvantage in that the protecting groups could not be readily removed.

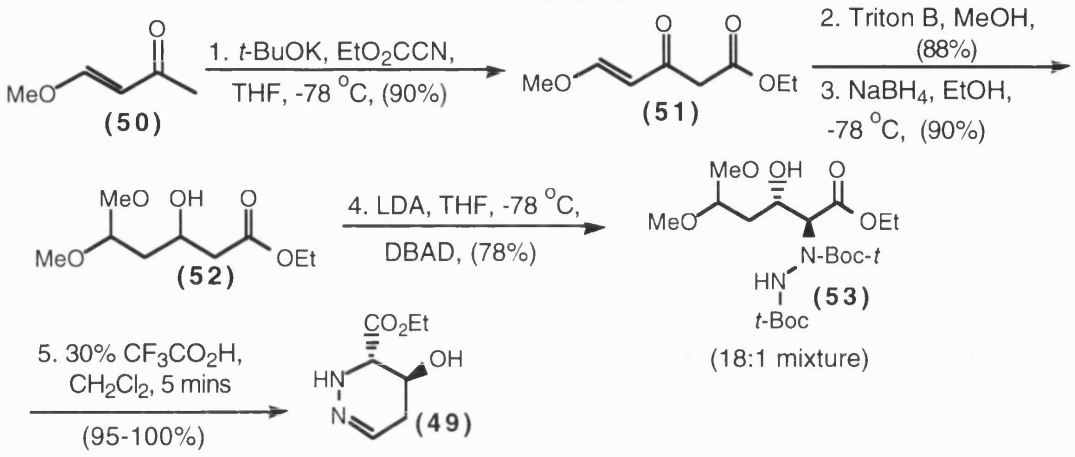
Scheme 9



Ciufolini and Xi, in1994, published a synthesis of (*3S*)-carboxy-(*4S*)-hydroxy-2,3,4,5-tetrahydropyridazine ethyl ester **49**,[37] an analogue of piperazic acid which is found in luzopeptin A .[38] Thus, the potassium enolate generated from **50** reacted with Mander's reagent to give β-keto ester **51** in 90% yield. Diacetal **52** was then formed by the conjugate addition of methoxide ion to **51**, followed by reduction with sodium borohydride. Dianion formation from **52** with lithium diisopropylamide and trapping of the ester enolate with DBAD led to hydrazine **53** as the major

component of an 18:1 mixture. Cyclisation to **49** ensued upon exposure to trifluoroacetic acid (Scheme 10).[37]

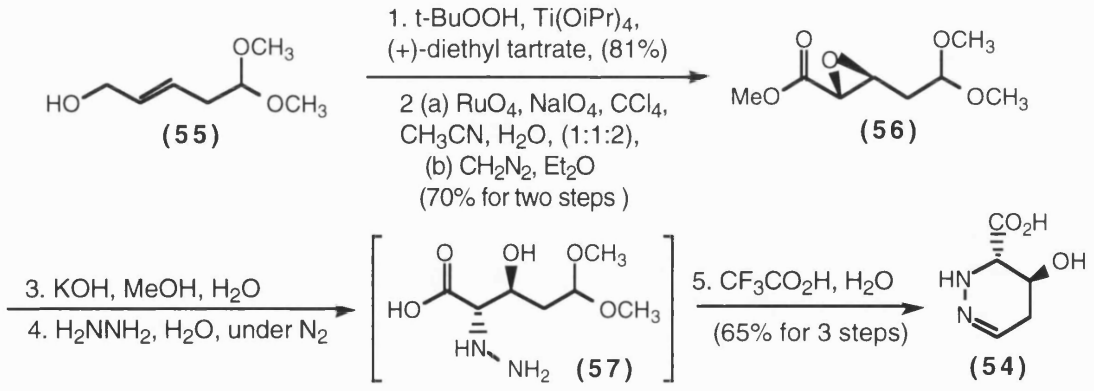
Scheme 10



1.7 Asymmetric Synthesis of Piperazic Acid Derivatives

The first asymmetric synthesis of a piperazic acid derivative was that of (*3S*)-carboxy-(*4S*)-hydroxy-2,3,4,5-tetrahydropyridazine **54** by Hughes and Clardy.[39] Sharpless asymmetric epoxidation of allylic alcohol **55**, subsequent oxidation with ruthenium tetraoxide, and esterification with diazomethane gave epoxy ester **56**. This was then transformed into **57** in a one-pot reaction, involving hydrolysis of the ester with potassium hydroxide in aqueous methanol, and epoxide opening with hydrazine. This gave the α-hydrazino acid **57**, which cyclised upon acidification to pH 1.3 with trifluoroacetic acid to give **54** in 65% yield (Scheme 11).

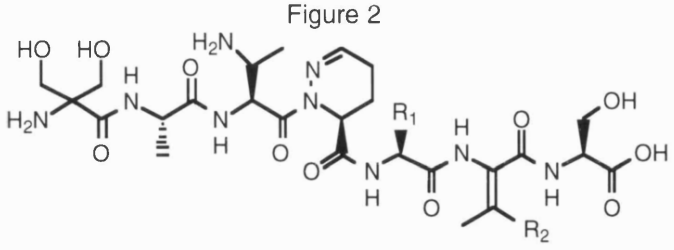
Scheme 11



(*3S*)-2,3,4,5-Tetrahydropyridazine-3-carboxylic acid (Pya) **67** is found in Antrimycin A **58** and Cirratiomycin A **59** (Figure 2). The former was isolated from *Streptomyces xanthocidicus* [40]

and the latter from *Strepomyces cirratus* [41]. Both are of interest on account of their marked tuberculostatic properties.

Figure 2

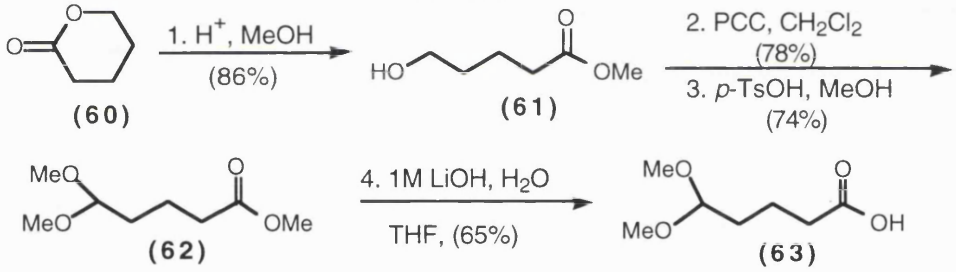


Antrimycin A (58)
R_1=Me R_2=Et

Cirratiomycin A (59)
R_1=*i*-Bu; R_2=Et

Nakamura and Shin developed an asymmetric synthesis of **67** that was based on Evans electrophilic amination of a *N*-acylated oxazolidinone.[42] The starting 5-dimethoxypentanoic acid **63** was synthesised in four steps from valerolactone **60**, by methanolysis of **60**, oxidation of the resulting alcohol **61**, and protection of the aldehyde as its dimethyl acetal. Hydrolysis of the methyl ester with lithium hydroxide furnished acid **63** in 56% yield (Scheme 12).

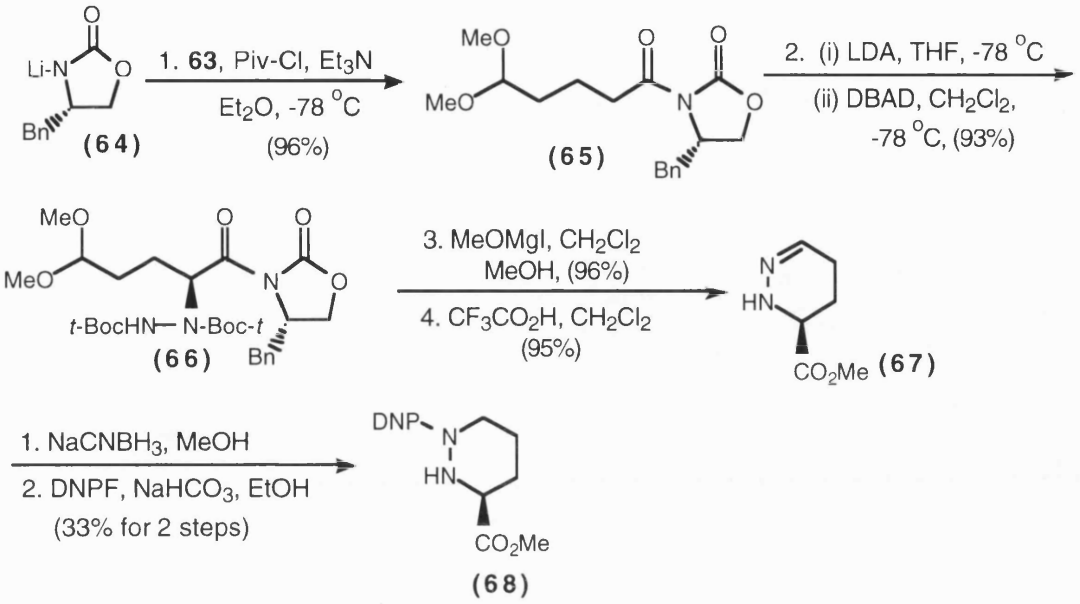
Scheme 12



N-Acyl-oxazolidinone **65** was prepared in 96% yield by addition of the mixed pivalic acid anhydride of **63** to the *N*-lithiated oxazolidinone **64**. Subsequent enolisation with lithium diisopropylamide (LDA) and conjugate addition to di-*tert*-butylazodicarboxylate (DBAD) resulted in α-hydrazino adduct **66** in 93% yield. After removal of the oxazolidinone auxiliary with magnesium methoxide, subsequent acidolysis cleaved the acetal and the two *tert*-butyloxycarbonyl (*t*-Boc) groups simultaneously to give the cyclised product **67** in 95% yield (Scheme 13).

In order to determine the enantiomeric purity of **67**, and assign its absolute configuration, it was converted to the known methyl-1-(2,4-dinitrophenyl)-(*3S*)-piperazate (Scheme 13). This was accomplished by reduction of **67** with sodium cyanoborohydride in methanol followed by the reaction with 2,4-dinitrophenyl fluoride (DNPF); methyl DNP-piperazate **68** was isolated in 33% overall yield for the 3 steps and in 98% ee .

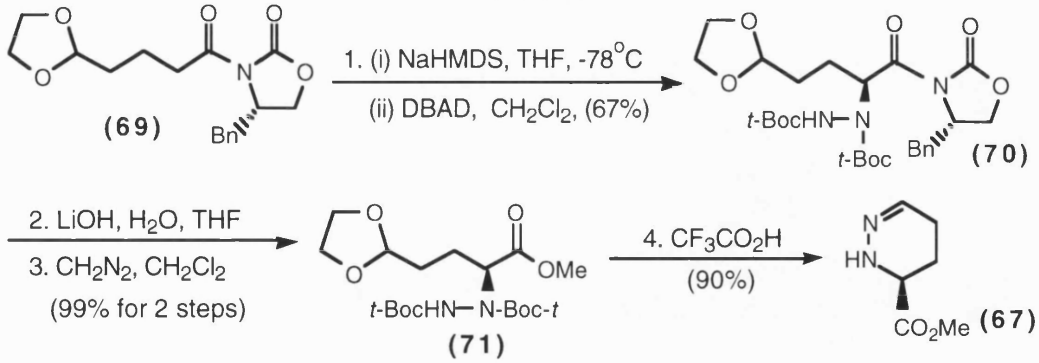
Scheme 13



The specific rotation of **68** was measured and found to be opposite in sign and value to the previously prepared (*3R*) isomer. This meant that the configuration of the synthesised derivative had to be (*3S*).

Schmidt and Riedl used a similar tactic to obtain **68**. They enolised the protected formyl-butyric derivative **69** with sodium hexamethyldisilazide (NaHMDS) and also hydrazinated with DBAD, but this time adduct **70** was the product isolated in 67% yield. Hydrolysis and reesterification afforded methyl ester **71** which underwent cyclisation when subjected to trifluoroacetic acid to give **67** in 90% yield (Scheme 14).[43a,b]

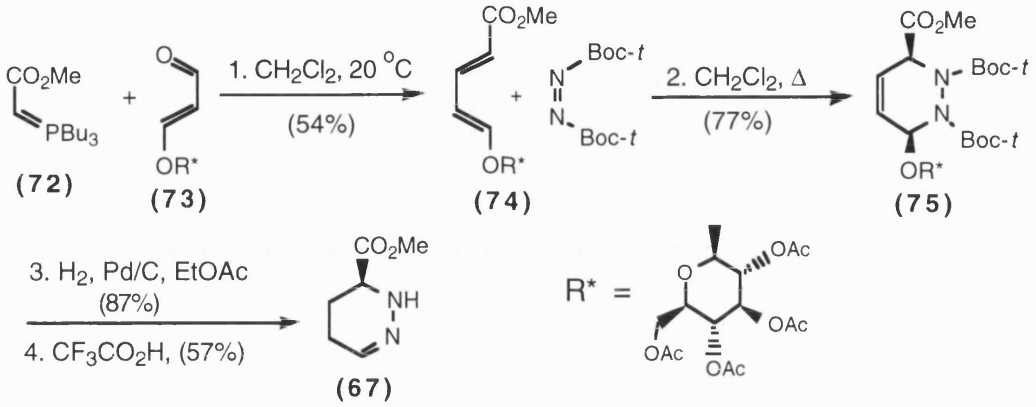
Scheme 14



Stoodley and coworkers have recently disclosed an asymmetric route to the Pya derivative **67** where the key step involved an asymmetric hetero Diels-Alder reaction with the chiral diene **74**.[44] The latter was obtained by a Wittig reaction between (*E*)-1-(2',3',4',6'-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-propenal **73** and stabilsed ylide **72**. Heating diene **74** with di-*tert*-butyl-

azodicarboxylate in dichloromethane at reflux led to cycloadduct **75** being isolated as a single diastereoisomer in 77% yield. Hydrogenation with palladium gave the piperazine which was converted to **67** by reaction with trifluoroacetic acid (Scheme 15).

Scheme 15



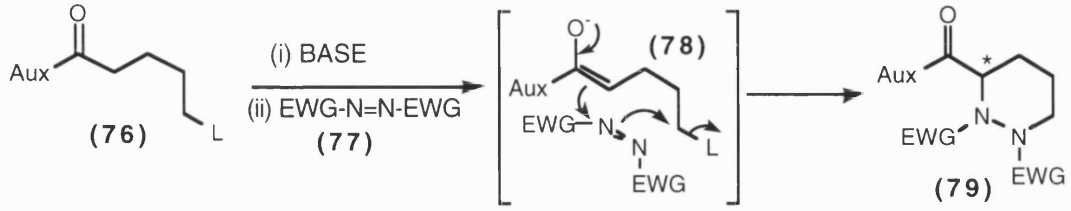
1.8 Discussion

For our projected total synthesis of antibiotic A83586C, we required a synthetic method that would furnish us with (*3R*) and (*3S*) piperazic acid in large quantity and high ee. When we set out on this project in 1991, the only method for obtaining chiral piperazic acid was the resolution procedure of Hassall and coworkers.[17] This, however, is very uneconomical to carry out on large scale, leading to each enantiomer in an overall yield of 1.5%. Development of an efficient route to these α-hydrazino acids, therefore, became an important objective in our synthetic investigations on the molecule A83586C. In the coming section, a new high-yielding synthesis of (*3S*)-piperazic acid will be presented.

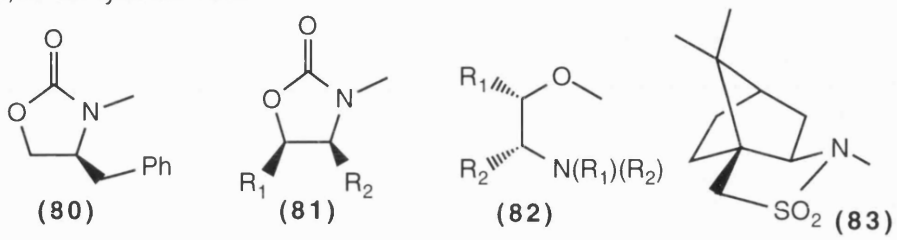
1.8.1 Retrosynthetic Analysis

Several routes were contemplated for the synthesis of (*3S*)-piperazic acid. However, the most attractive one was based on a tandem electrophilic hydrazination-nucleophilic displacement of a chiral valeryl carboximide enolate of type **78** with an azodicarboxylate **77** (Scheme 16).

Scheme 16



The chiral auxiliary which would direct the stereochemical course of the electrophilic hydrazination could potentially be either of oxazolidinones **80** and **81**, the *N*-alkylated-ephedrine **82**, or a 9,10-bornylsultam **83**.

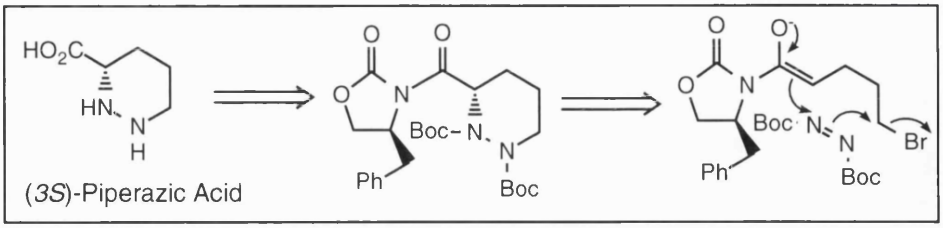


Treatment of **76** with a sterically hindered strong base was expected to yield the enolate **78**, which we hoped would then undergo an intermolecular conjugate Michael addition to the azo compound **77** to generate the N^1-aza anion. The latter could then displace a suitable leaving group (L) to form **79**. We felt that the leaving group L could be either a sulfonate ester, or a chloro, bromo, or iodo group. Subsequent removal of the auxiliary and the nitrogen protecting groups from **79** should then result in chiral piperazic acid.

1.8.2 Results

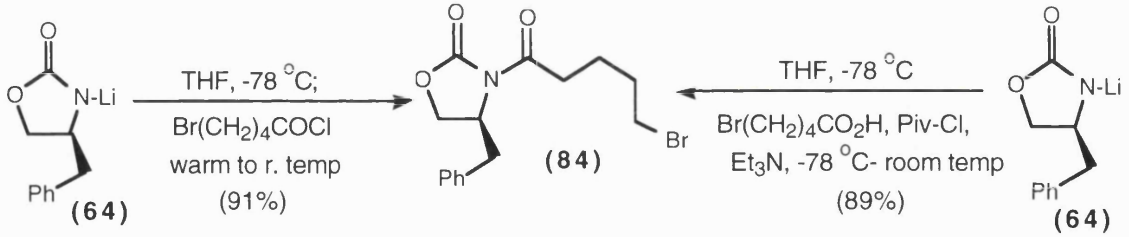
In light of the fact that Evans and Vederas had successfully α-hydrazinated chiral enolates derived from *N*-acyl oxazolidinones with di-*tert*-butylazodicarboxylate, we thought that a suitable chiral auxiliary for preparing (*3S*)-piperazic acid would be (*4S*)-(phenylmethyl)-2-oxazolidinone (**80**). We also believed that the best leaving group in Scheme 16 would be a bromide since 5-bromovaleric acid and its acid chloride were commercially available. With these considerations in mind, the initial retrosynthetic concepts enunciated in Scheme 16 were reduced to the retro-synthetic plan shown in Scheme 17.

Scheme 17



(*4S*)-(Phenylmethyl)-2-oxazolidinone **80** was synthesised from (*S*)-phenylalanine by the procedure of Evans and Weber.[45] The first step in our sequence to **89** entailed formation of the 5-bromovaleryl acylated-oxazolidinone **84**. This was accomplished by deprotonation of the oxazolidinone **80** with *n*-butyllithium at -78 °C, and addition of 5-bromovaleryl chloride. After warming the reaction mixture to room temperature and extractive work-up, bromide **84** was isolated in 91% yield by crystallisation of the crude reaction mixture from hexanes and ether at 0 °C. Bromide **84** could also be obtained from 5-bromovaleric acid in 89% yield by forming a mixed anhydride with pivaloyl chloride in the presence of triethylamine in ether and reacting it with the lithiated oxazolidinone **64** at -78 °C (Scheme 18).

Scheme 18



The infrared spectrum of **84** contained two intense absorptions at 1792 and 1701 cm^{-1} which corresponded to the carbonyl group stretching frequencies of the amide and the carbamate respectively. The structure of **84** was also apparent after inspection of the ^{13}C NMR spectrum in $CDCl_3$ at 100 MHz, since there were two resonances at δ 172.5 and 153.3 which were highly characteristic of the carbonyl groups in an *N*-acyloxazolidinone. Further structural proof was provided by the presence of two roughly equal $(M+H)^+$ ions at m/e 340 and 342, which arose due to the ^{79}Br and ^{81}Br isotopes respectively. The high resolution mass spectrum of **84** contained an $(M+H)^+$ ion peak at m/e 340.0544 (Calcd. for $C_{15}H_{19}O_3NBr$ $(M+H)^+$ 340.0548). In addition, compound **84** gave a satisfactory combustion microanalysis for $C_{15}H_{18}NO_3Br$.

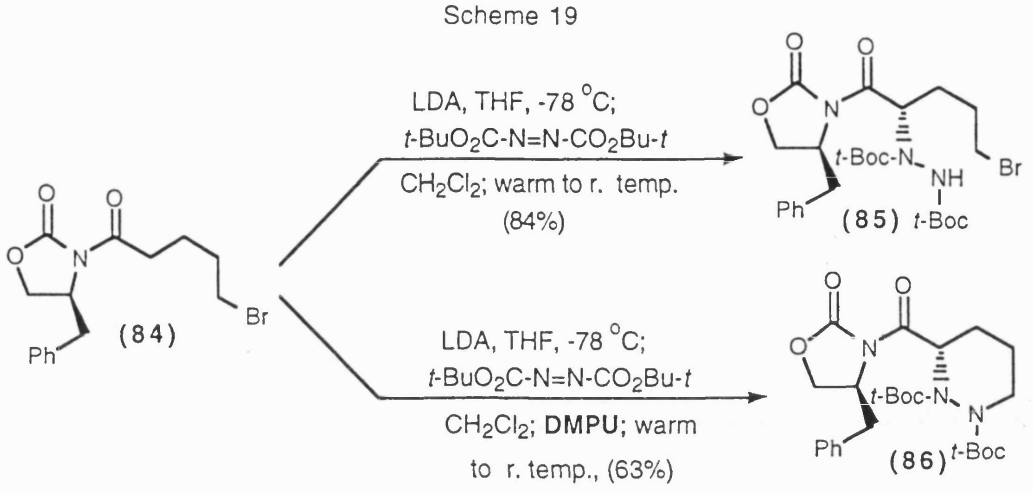
Electrophilic amination of **84** was carried out according to the procedure set out by Evans and coworkers.[22] Thus, a precooled solution of bromide **84** at -78 °C in dry THF was added to a

freshly prepared and cooled solution of lithium diisopropylamide (LDA) at - 78 °C in dry THF. The resulting yellow enolate solution was then stirred at -78 °C for 40 minutes prior to adding a precooled (-10 °C) solution of DBAD[46] in CH_2Cl_2 via cannula. The mixture was then allowed to warm to room temperature, but only a small amount of the desired cyclised product **86** could be detected after quenching with glacial acetic acid. The major product was the hydrazino adduct **85** which was isolated in 84% yield (Scheme 19). The infrared spectrum of **85** contained the expected NH stretching frequency at 3360 cm^{-1} and also contained two carbonyl stretching absorptions at 1790 and 1697 cm^{-1}. The 400 MHz ^1H NMR spectrum in DMSO-d_6 at 100 °C revealed a broad resonance between δ 8.30 and 8.00 corresponding to the NH proton and two singlets at δ 1.44 and 1.43 due to the two *t*-Boc groups. The structure of **85** was further confirmed by its high resolution mass spectrum, which contained an $(M+Na)^+$ ion at m/e 592.1642 (Calcd. for $C_{25}H_{36}N_3O_7BrNa$, $(M+Na)^+$ 592.1634). We reasoned that the desired cyclised product was not obtained because the aza anion intermediate was tightly coordinated to the lithium counterion, which considerably reduced its nucleophilicity for the subsequent intramolecular S_N2 displace-ment. In order to overcome this problem, we decided to add a dipolar aprotic solvent such as hexamethylphosphoric triamide (HMPA) or 1,3-dimethyl-3,4,5,6-tetrahydro-2(*1H*)-pyrimidinone (DMPU) which would solvate the cation, and generate a "naked" aza anion. This would then facilitate the displacement of the bromide atom by the aza anion. In view of the known carcino-genicity of HMPA, we chose to utilise DMPU for the cyclisation step. Thus, DMPU was added 30 minutes after the DBAD at -78 °C. The DMPU addition was done slowly over 40 minutes and when complete, the reaction mixture became frozen. As the reaction mixture warmed to room temperature it melted, and as this happened, cyclisation of the *N*-aza anion took place. The desired cyclisation product **86** was then isolated in 68% yield after extractive work-up with KH_2PO_4 (1.25 M) and ether (Scheme 19).

The 400 MHz ^1H NMR spectrum of **86** in DMSO-d_6 at 25 °C was extensively broadened, making it difficult to assign specific proton resonances. This broadening was due to restricted rotation of the Boc groups about the respective nitrogen atoms enabling several rotamers to be observed. However, when recorded at 125 °C in DMSO-d_6 the spectrum became sharp as the

time-averaged spectrum was observed (Figure 3). It was clear from the ^1H NMR spectrum of **86** at 125 °C that only one diastereomer had been formed.

Scheme 19



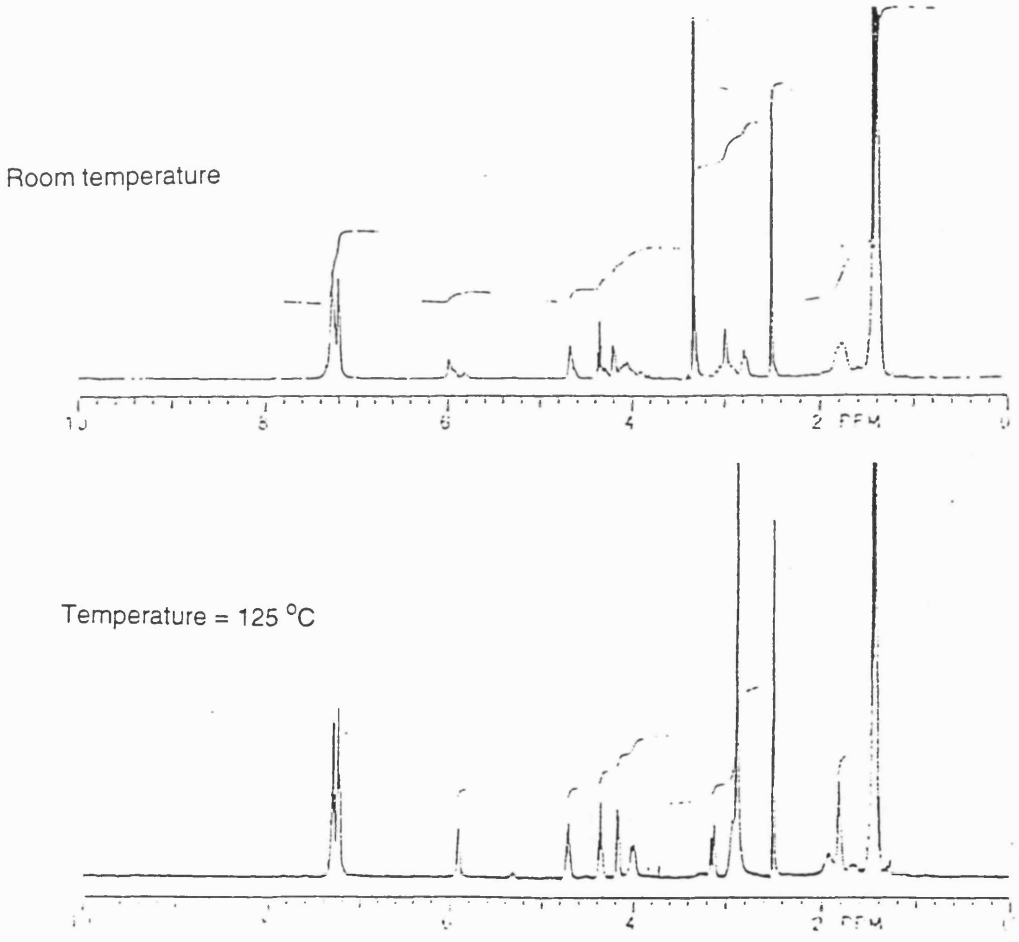


Figure 3: 400 MHz ^1H NMR spectra of cycloadduct **86** in DMSO-d_6 at room temperature and at 125 °C

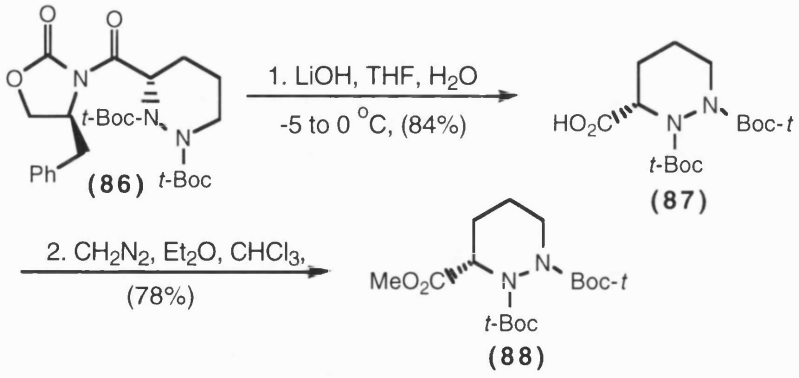
The high resolution mass spectrum of **86** contained an $(M+H)^+$ peak at m/e 490.255 (Calcd. for $C_{25}H_{36}N_3O_7$, $(M+H)^+$ 490.255). In addition, compound **86** gave a satisfactory C, H and N com-

bustion microanalysis for $C_{25}H_{35}N_3O_7$ (Calcd.: C, 61.23; H, 7.21; N, 8.59%. Found: C, 60.90; H, 7.35; N, 8.59%). It must be noted that a small amount of hydrolysis of oxazolidinone **86** occurs during quenching of the reaction (1-4%).

Hydrolysis of **86** with lithium hydroxide (2.3 eq) in THF/H_2O at -10 ^oC gave acid **87** in 84% yield after acidification; oxazolidinone **86** was recovered in 67% yield (Scheme 20). This hydrolysis is non-destructive as the hydrolxide ion attacks selectively at the exocyclic carbonyl rather than at the endocyclic carbamate carbonyl. The 400 MHz ^1H NMR spectrum of **87** in $CDCl_3$ contained a broad singlet between δ 11.50-10.00 which was attributable to the carboxylic acid proton. The absence of any aromatic proton signals between δ 7.34-7.20 also confirmed that the oxazolidinone had been removed. The infrared spectrum of **87** contained a broad absorption at 3204 cm^{-1} which was due to the acid OH stretching absorption; it also contained absorptions at 1737, 1704, and 1670 cm^{-1} due to the remaining carbonyl groups.

In order to assess the diastereoselectivity of the electrophilic amination step, the crude acid **87** was treated with excess ethereal diazomethane to give the methyl ester **88** in 78% yield (Scheme 20). The 400 MHz ^1H NMR spectrum in $CDCl_3$ contained a methoxy singlet at δ 3.67. The resonance at δ 51.9 and the carbonyl signal at δ 170.4 in the 100 MHz ^{13}C NMR spectrum in $CDCl_3$ also supported the presence of a methyl ester group.

Scheme 20



Compound **88** was then applied to a Chiracel-OD high performance analytical chiral column eluting with 1.4% *i*-propanol in hexane at a flow rate of 1ml/min and its purity checked with the use of a refractive index detector. A comparison of the the HPLC trace of the two separated enantiomers for the racemic (*3R,3S*) methyl ester **93** (prepared according to Scheme 25) with that obtained for compound **88** showed that the latter had been obtained in 96% ee (Figure 4).

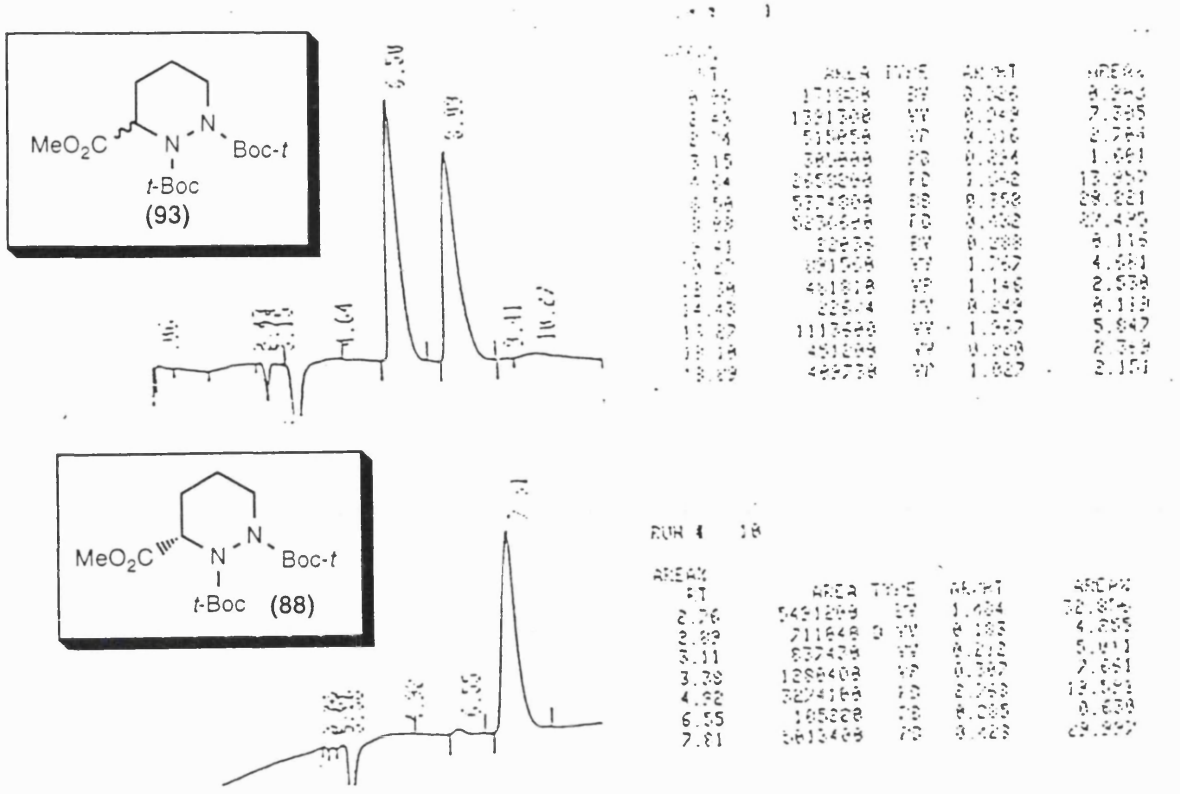
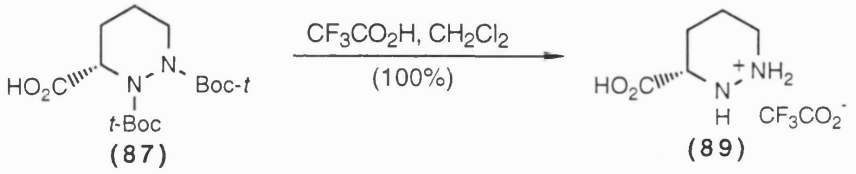


Figure 4: HPLC traces of racemic methyl ester **93** and the asymmetric derived compound **88**.

At this juncture, both *tert*-butyloxycarbonyl groups were removed from **87** with trifluoroacetic acid in dichloromethane,[47] providing the required piperazic acid trifluoroacetic acid salt **89** in 100% yield after recrystallisation from ethyl acetate/ethanol (Scheme 21).

Scheme 21

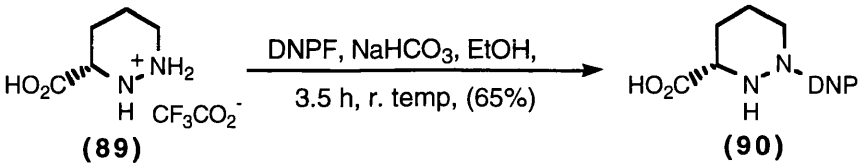


Compound **89** gave a microanalysis that was fully in accord with an empirical formula of $C_7H_{11}N_2O_4F_3$ (Calcd.: C, 34.43; H, 4.54; N, 11.47%. Found: C, 34.51; H, 4.55; N, 11.49%). In addition, the 400 MHz ^1H NMR spectrum of **89** in D_2O contained only seven proton resonances between δ 3.80 and 1.50 as would be expected.

1.8.3 Determination of the Configuration and Enantiomeric Excess

In order to determine the configuration of the synthesised piperazic acid **89** the known 2,4-dinitrophenyl derivatives **90** and **68** were synthesised.[9] Thus, **89** was treated with 2,4-dinitrophenyl fluoride and sodium bicarbonate in ethanol to give N^1-(2,4-dinitrophenyl) piperazic acid **90** in a yield of 65% (Scheme 22). The infrared spectrum of **90** contained an NH stretching absorption at 3200 cm^{-1}, a carbonyl stretching absorption at 1715 cm^{-1}, and an NO_2 stretch absorption at 1608 cm^{-1}. The 400 MHz ^1H NMR spectrum of **90** in $CDCl_3$ consisted *inter alia* of resonances at δ 8.40 (d, J = 2.6 Hz, 1H), 8.15 (dd, J = 2.6, 9.3 Hz, 1H) and at 6.95 (d, J = 9.3 Hz, 1H), which are characteristic of an *o,p*-disubstituted aromatic ring.

Scheme 22

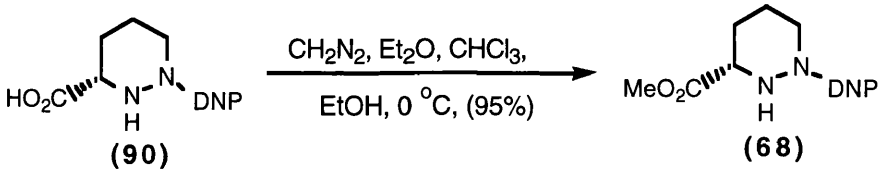


(89) **(90)**

The specific rotation of **90** was measured and found to be $[\alpha]_D$ -345° (*c* 1.0 in MeOH); the literature value for (*3R*) -N^1-DNP-piperazic acid is $[\alpha]_D$ +324.6° (*c* 1.0 in MeOH).[9] Since the specific rotation of **90** was of opposite sign to that for the authentic (*3R*)-enantiomer, this confirmed that (*3S*)-N^1-DNP-piperazic acid had been prepared. The higher $[\alpha]_D$ value suggested that the piperazic acid obtained via the above route was of high enantiomeric purity than that obtained by the resolution procedure of Hassall and coworkers.[33]

Methyl N^1-(2,4-dinitrophenyl) piperazate **68** was synthesised by treating the acid **90** with excess ethereal diazomethane (Scheme 23). The 400 MHz ^1H NMR spectrum in C_6D_6 contained a three proton singlet at δ 3.17 which was clearly due to the MeO group of the methyl ester. The presence of the MeO was also apparent from the 100 MHz ^{13}C NMR spectrum which now contained a resonance at δ 51.5 ppm due to this group. A satisfactory microanalysis was obtained for **68** which supported the empirical formula of $C_{12}H_{14}N_4O_6$ (Calcd.: C, 46.44; H, 4.55; N, 18.06%. Found: C, 46.05; H, 4.41; N, 17.77%).

Scheme 23



(90) **(68)**

The racemic derivative **94** (prepared according to Scheme 25) was applied to a Chiracel-OD high performance analytical column eluting with 25% isopropanol in hexane at a flow rate of 1ml/min. UV detector set at 254 nm. This system clearly separated both enantiomers. The ee of **68** was judged to be greater than 98% (Figure 5). Thus, we have developed a novel asymmetric synthesis of (*3S*)-piperazic acid in a high enantiomeric excess.

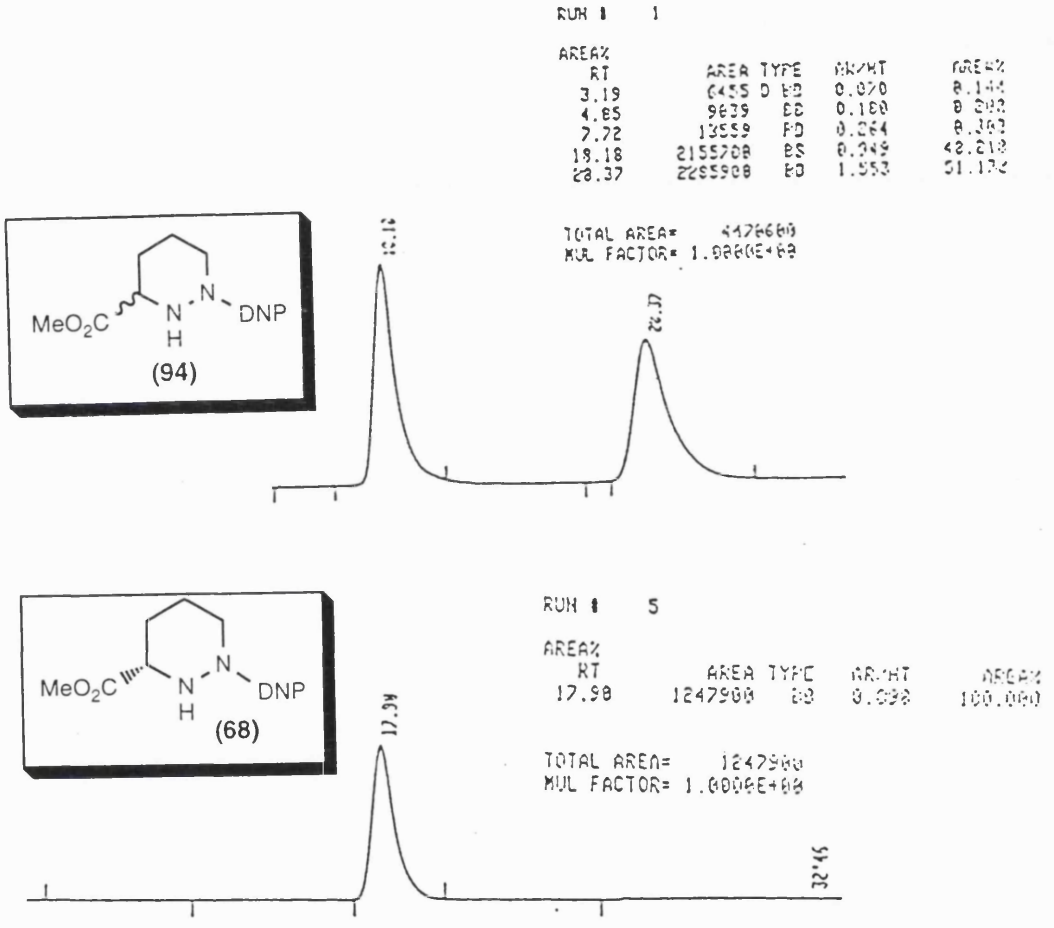


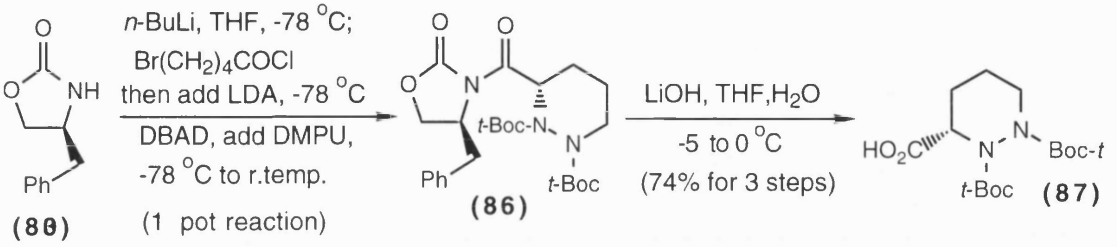
Figure 5: HPLC traces of racemic ester **94** and compound **68**

1.8.4 One-Pot Synthesis (*3S*)-N^1,N^2-bis-(*t*-butoxycarbonyl)hexahydropyridazine -3-carboxylic acid, (87)

The acylation of oxazolidinone **80** and subsequent cyclisation can be carried out in one-pot, using bromovaleryl chloride as the acylating agent. In this modified procedure 5-bromovaleryl chloride was added to the lithiated oxazolidinone in THF at -78 ^{O}C, the reaction mixture then stirred at room temperature, recooled to -78 ^{O}C, and then added to a cold solution of LDA in THF at -78 ^{O}C. Subsequent addition of DBAD followed by DMPU resulted in the cyclised product **86**

after aqueous work-up. Hydrolysis of the crude cyclised product with lithium hydroxide at 0 °C resulted in acid **87** being formed in 74% yield for the three steps (Scheme 24). The methyl (*3S*)-N^1-(2,4-dinitrophenyl)-piperazate was then synthesised as above and the ee was determined and found to be 95% by the chiral HPLC on a Chiracel-OD column.

Scheme 24



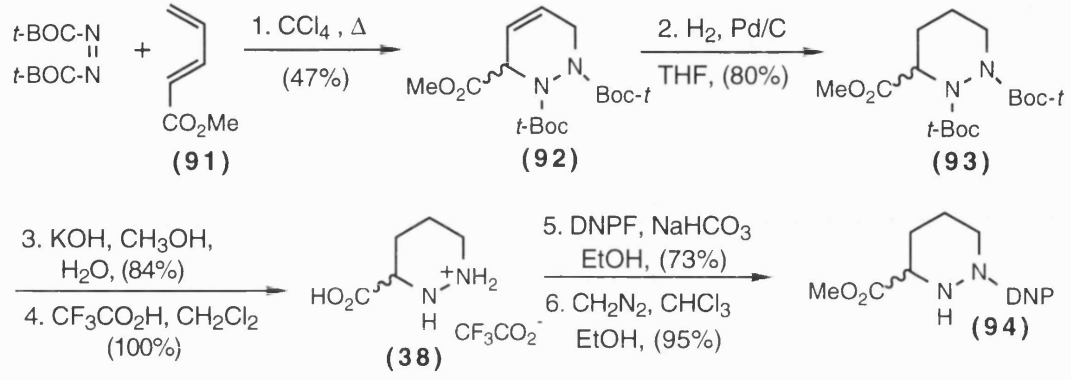
This synthetic scheme can be carried out without any purification of intermediates with an overall yield of 68% for the four steps and without any loss of ee in the resulting piperazic acid.[48]

1.8.5 Synthesis of Racemic (*3S,3R*)-Piperazic acid Derivatives

In order to independently confirm the structure of the newly synthesised (*3S*)-piperazic acid derivative, methyl (*3R,3S*)-N^1-(2,4-dinitrophenyl) piperazate **94** was synthesised according to the unpublished procedure supplied to us by Drs. Durette and Caldwell at Merck, Sharp and Dohme, Rahway (Scheme 25).[49] Thus di-*tert*-butylazodicarboxylate was reacted with methyl penta-2,4-dienoate **91** in chloroform at reflux to give the tetrahydropyridazine derivative **92** in 47% yield. The 400 MHz ^{1}H NMR spectrum in $CDCl_3$ contained a broad singlet at δ 5.87 integrating to two protons which was indicative of the olefinic protons, and a singlet at δ 1.48 which integrated to eighteen protons and which was due to the two *N*-Boc groups that were present. The 100 MHz ^{13}C NMR spectrum of **92** in $CDCl_3$ contained a resonance at δ 169.4 due to the ester C=O, and a signal at δ 154.8 due to the Boc-carbonyl group. There were also resonances at δ 125.4 and 122.2 which indicated the presence of two olefinic carbons. The infrared spectrum contained two intense carbonyl absorptions at 1744 and 1707 cm^{-1} due to the ester and carbamate carbonyl groups respectively. Hydrogenation of **92** over a palladium on carbon catalyst gave the protected piperazic methyl ester **93** in a yield of 80%. The 400 MHz ^{1}H NMR spectrum in $CDCl_3$ now showed the absence of the two olefinic protons at δ 5.87. In addition, the olefinic resonances at δ 125.4 and 122.2 were absent, there now being two new

aliphatic resonances at δ 20.0 and δ 24.8 in the 100 MHz ^{13}C NMR spectrum in $CDCl_3$. Subsequent hydrolysis of **93** with potassium hydroxide and removal of the two *tert*-butyloxycarbonyl groups with trifluoroacetic acid resulted in (*3S,3R*)-piperazic acid trifluoroacetic acid salt **38** in 100% yield. Compound **38** was transformed into **94** as discussed previously.

Scheme 25

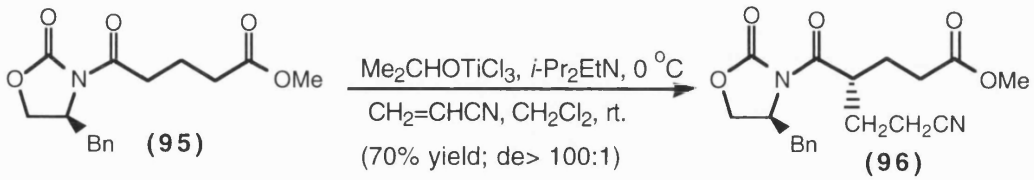


1.8.6 Synthesis of (*3S*)-Piperazic acid via a Titanium enolate

As we were hoping to develop a procedure for the synthesis of (*3R*) and (*3S*)- piperazic acids on a process plant scale, the original route was not considered commercially viable since low temperatures were required for the electrophilic hydrazination step.

Evans has recently found that chlorotitanium enolates from chiral *N*-acylated oxazolidinones react with Michael acceptors such as acrylonitrile with excellent levels of stereocontrol and in good yield (Scheme 26). [50]

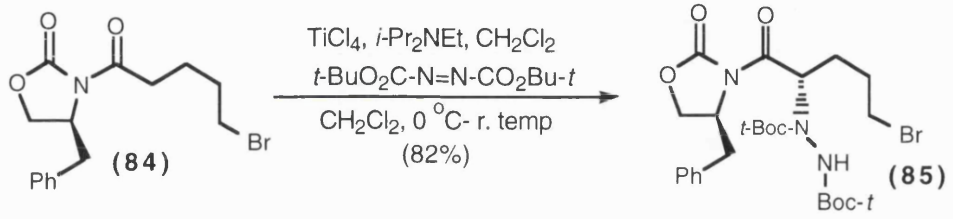
Scheme 26



Since, the electrophilic hydrazination step in our synthesis of (*3S*) piperazic acid can also be considered as a Michael type addition onto DBAD, we decided to reinvestigate this step at -10 °C using a chlorotitanium enolate rather than the lithium enolate of *N*-bromovaleryl oxazolidinone **84**. Addition of titanium tetrachloride to bromide **84** at -10 °C in dichloromethane followed by diisopropylethylamine after 5 min, resulted in a dark red solution of the titanium enolate being formed. When a cooled solution of DBAD in CH_2Cl_2 at -5 °C was then added and the mixture

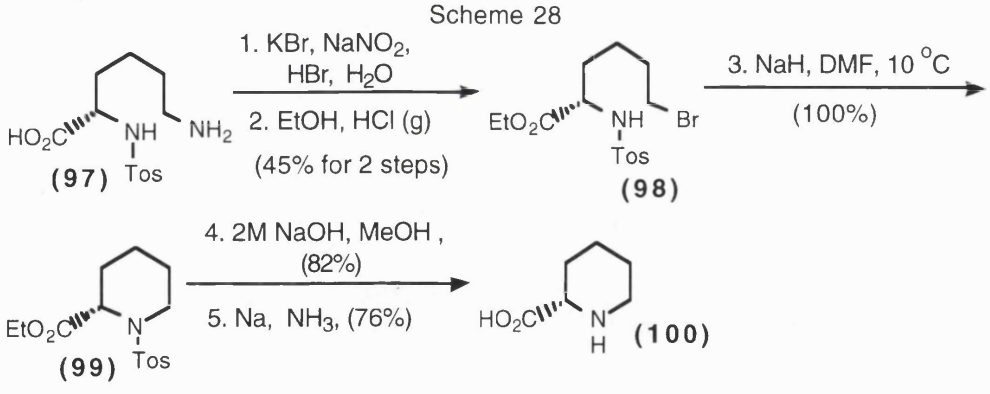
stirred for 3 hours at 0 °C, a single product **85** was formed in 82% yield (Scheme 27). The 400 MHz ^{1}H NMR spectrum of **85** at 25 °C in DMSO-d_6 was broad and largely unassignable. However at 100 °C the spectrum became sharp and revealed a broad resonance around δ 8.17 corresponding to the NH proton, a five proton multiplet between δ 7.38-7.21 due to the aromatic ring, and two singlets at δ 1.44 and 1.43 which integrated to eighteen hydrogens and which corresponded to the two *t*-butyl groups of the *t*-Boc protecting groups in **85**. The infrared spectrum of **85** revealed an NH stretch at 3360 cm^{-1} and carbonyl stretching absorptions at 1790 and 1697 cm^{-1}. Compound **85** gave a satisfactory microanalysis for $C_{25}H_{36}N_3O_7Br$ (Calcd.: C, 52.63; H, 6.36; N, 7.37; Br,14.01%. Found: C, 52.43; H, 6.3; N, 7.32; Br, 14.25%).

Scheme 27



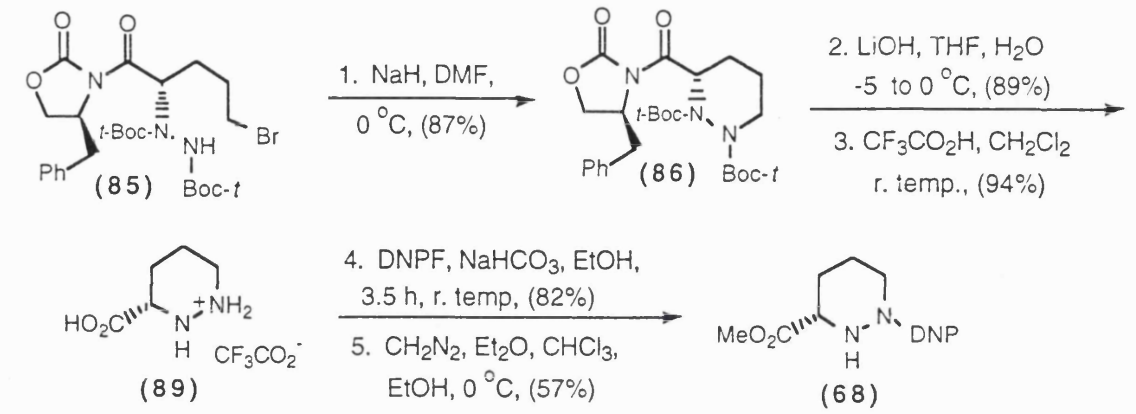
Presumably, cyclisation of the aza-anion did not occur because of the very strong affinity of the chlorotitanium ion for the aza anion, which leads to formation of a very strong ion pair. We therefore elected to attempt deprotonation of hydrazide **85** with sodium hydride in DMF in the hope that intramolecular cyclisation reaction would then take place. A literature precedent for this type of cyclisation can be found in the synthesis of L-pipecolic acid **100** by Fujii and Miyoshi (Scheme 28).[51]

Scheme 28



Treatment of adduct **85** with sodium hydride (1.1 eq) in dry DMF under nitrogen at 0 °C resulted in cycloadduct **86** being formed in 87% yield (Scheme 29). Compound **86** was then converted into methyl-(*3S*)-*N*^{1}-(2,4-dinitrophenyl) piperazate **68** as previously and the ee judged to be 78% by HLPC analysis on a Chiracel OD-column (Figure 6).

Scheme 29



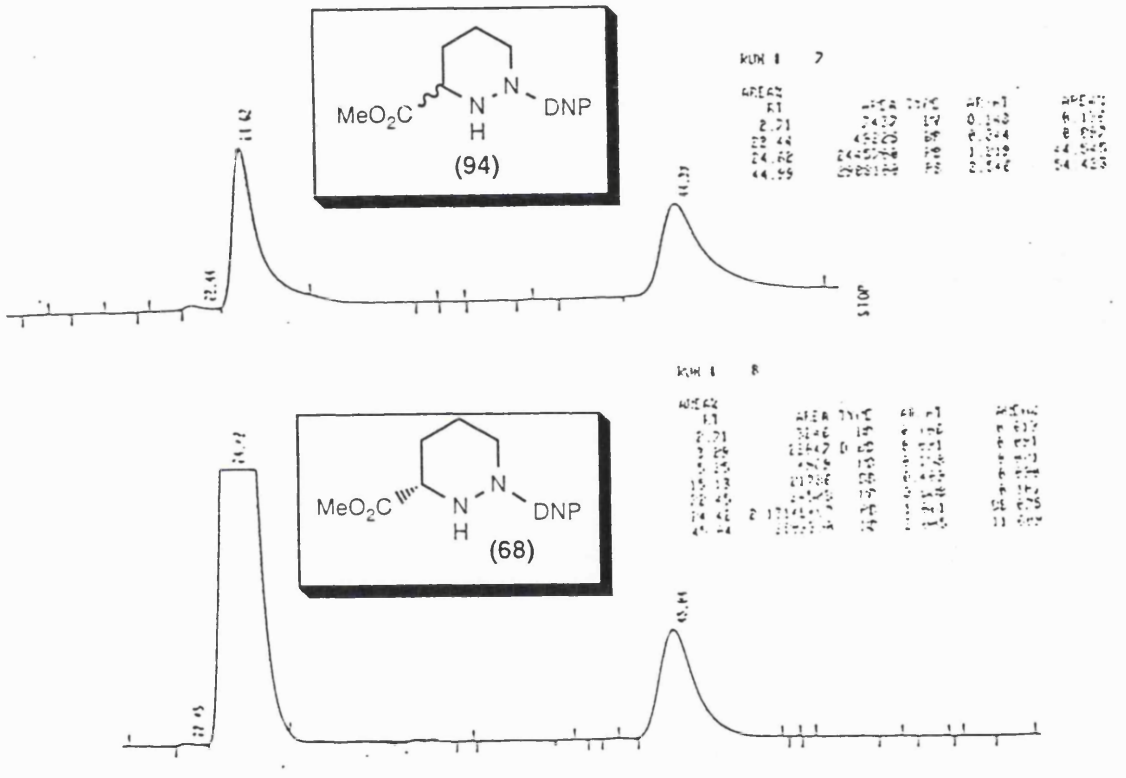


Figure 6: HPLC traces of racemic methyl ester **94** and the asymmetric derived compound **68**.

The lower ee value is presumably the result of some partial racemisation occurring in the base-induced cyclisation step. Nevertheless, this ee value is still high enough to be useful in a future large scale industrial synthesis of (*3S*)-piperazic acid.

1.9 References

(1) Maehr, H.; Liu, C. M.; Palleroni, N. J.; Smallheer, J.; Todaro, L.; Williams, T. H.; Blount, J. F., *J. Antibiotics,* **1986**, *39*, 17.

(2) Smitka, T. A.; Deeter, J. B.; Hunt, A. H.; Mertz, F. P.; Ellis, R. M.; Boeck, L. D.; Yao, R. C., *J. Antibiotics,* **1988**, *41*, 726.

(3) Nakagawa, M.; Hayakawa, Y.; Furihata, K.; Seto, H., *J. Antibiotics,* **1990**, *43*, 477.

(4) Nakagawa, M.; Hayakawa, Y.; Adachi, K.; Seto, H., *Agric. Biol. Chem.,* **1990**, *54*, 791.

(5) Hurley, T. R.; Bunge, R. H.; Willmer, N. E.; Hokanson, G. C.; French, J. C., *J. Antibiotics,* **1986**, *39*, 1651.

(6) Hensens, O. D.; Borris, R. P.; Koupal, L. R.; Caldwell, C. G.; Currie, S. A.; Haidri, A. A.; Homnick, C. F.; Honeycutt, S. S.; Lindenmayer, S. M.; Schwartz, C. D.; Weissberger, B. A.; Woodruff, H. B.; Zink, D. L.; Zintano, L.; Fieldhouse, J. M.; Rollins, T.; Springer, M. S.; Springer, J. P., *J. Antibiotics,* **1991**, *44*, 249.

(7) Sugawara, K.; Toda, S.; Moriyama, T.; Konishi, M.; Oki, T., *J. Antibiotics*, **1993**, *46,* 921.

(8) Nishiyama, Y.; Sugawara, K.; Tomita, K.; Yamamoto, H.; Kamei, H.; Oki, T., *J. Antibiotics*, **1993**, *46,* 928.

(9) Bevan, K.; Davies, J.S.; Hassall, C.H.; Morton, R.B.; Phillips, D.A.S., *J. Chem. Soc. (C)*, **1971**, 514.

(10) Hassall, C.H.; Morton, R.B.; Ogihara, Y.; Thomas, W.A., *Chem. Commun.,* **1969**, 1079.

(11) Hassall, C.H.; Ogihara, Y.; Thomas, W.A., *J. Chem. Soc. (C)*, **1971**, 522.

(12) Johnston, G.A.R.; Stephanson, A.L.; Twitchin, B., *J. Pharmacol.*, **1977**, 29, 240.

(13) Ogita, T.; Sato, A.; Enokita, R.; Suzuki, K.; Ishi, M.; Negishi, T.; Okazaki, T.; Tamoki, K.; Tanzawa, K., *J. Antibiotics*, **1992**, *45*, 1723.

(14) Tamaki, K.; Kurihara, S.; Sugimura, Y., *Tetrahedron Lett.*, **1993**, *34*, 8477.

(15) Tamaki, K.; Ogita, T.; Tanzawa, K.; Sugimura, Y., *Tetrahedron Lett.*, **1993**, *34*, 683.

(16) Pettibone, D.J.; Clineschimdt, B.V.; Anderson, P.S.; Friedinger, R.M.; Lundell, G.F.; Koupal, L.R.; Schwartz, C.D.; Williamson, J.M.; Goetz, M.A.; Hensens, O.D.; Liesch, J.M.; Springer, J.P., *Endocrinology*, **1989**, *125*, 217.

(17) Bock, M.G.; Dipardo, R.M.; Williams, P.D.; Pettibone, D.J.; Clineschimdt, B.V.; Ball, R.G.; Veber, D.F.; Friedinger, R.M., *J. Med. Chem.*, **1990**, *33*, 2321.

(18) Isshiki, K.; Sawa, T.; Naganawa, H.; Lizumi, Y.; Matsuda, N.; Hamada, M.; Takeuchi, T.; Iijima, M.; Osono, M.; Masuda, T.; Ishizuka, M., *J. Antibiotics*, **1990**, *43*, 1195.

(19) Attwood, M.R.; Hassal, C.H.; Krohn, A.; Lawton, G.; Redshaw, S., *J. Chem. Soc. Perkin Trans. 1*, **1986**, 1011.

(20) (a) Hassall, C.H.; Krohn, A.; C.J.; Thomas, W.A., *J. Chem. Soc., Perkin Trans. 1*, **1984**, 155; (b) Indelicato, J.M.; Pasini, C.E., *J. Med. Chem.*, **1988**, *31*, 1227; (c) Jungheim, L.N.; Boyd, D.B.; Indelicato, J.M.; Pasini, C.E.; Preston, D.A.; Alborn, W.E., *J. Med. Chem.*, **1991**, *34*, 1732.

(21) Gennari, C.; Colombo, L.; Bertolini, G., *J. Amer. Chem. Soc.*, **1986**, *108*, 6394.

(22) (a) Evans, D.A.; Britton, T.C.; Dorow, R.L.; Dellaria, J.F., *J. Amer. Chem. Soc,* **1986**, *108*, 6395. (b) Evans, D.A.; Britton, T.C.; Dorow, R.L.; Dellaria, J.F, *Tetrahedron*, **1988**, *44*, 5525.

(23) Trimble, L.A.; Vederas, J.C., *J. Amer. Chem. Soc.*, **1986**, *108*, 6397.

(24) Heathcock, C.E. in *Asymmetric Synthesis,* Morrison, J.D., Ed.; Academic: New York, **1984**, *3*; Chapter 2.

(25) (a) Oppolzer, W.; Moretti, R., *Helv. Chim. Acta.*, **1986**, *69*, 1923. (b) Oppolzer, W.; Moretti, R., *Tetrahedron*, **1988**, *44*, 5541.

(26) Guanti, G.; Banfi, J.; Narisano, E., *Tetrahedron*, **1988**, *44*, 5553.

(27) Achiwa, K.; Yamada, S.I., *Tetrahedron Lett.*, **1975**, *31*, 2701.

(28) Vidal, J.; Drouin, J.; Collet, A., *J. Chem. Soc., Chem. Commun.*, **1991**, 435.

(29) A review of amination with oxaziridines : Andreae, S.; Schmitz, E., *Synthesis*, **1991**, 327.

(30) Niedrere, D.A.; Kapron, J.T.; Vederas, J.C., *Tetrahedron Lett.*, **1993**, *34*, 6859.

(31) Davies, C.R.; Davies, J.S., *J. Chem. Soc., Perkin Trans. 1,* **1976**, 2390.

(32) Adams, C.E.; Aguilar, D.; Hertel, S.; Knight, W.H.; Paterson, J., *Syn.Comm.*, **1988**, *18* (*18*), 2225.

(33) Hassall, C.H.; Johnson, W.H.; Theobold, C.J., *J. Chem. Soc. Perkin Trans 1*, **1979**, 1451.

(34) Hall, M.J.; Handford, B.O.; Hassall, C.M.; Phillips, D.A.S.; Rees, A.V., *Antimicrob. Chemotherapy*, **1973**, *3*, 380.

(35) Oki, K.; Suzuki, K.; Tuchida, S.; Saito, T.; Kotake, H., *Bull. Chem. Soc. Jpn.*, **1970**, *43*, 2554.

(36) Rutjes, F.P.J.T.; Teerhuis, N.M.; Hiemstra, H.; Speckamp, W.N., *Tetrahedron*, **1993**, *49*, 8605.

(37) Ciufolini, M.A.,; Xi, N., *J. Chem. Soc., Chem. Commun.*, **1994**, 1867.

(38) Scattergood, A.; Maclean, A.L., *J. Amer. Chem. Soc.*, **1949**, *71*, 4153.

(39) Hughes, P.; Clardy, J.; *J. Org. Chem.*, **1989**, *54*, 3260.

(40) Christensen, H.N.; Aspen, A.J.; Rice, E.G., *J. Biol. Chem.*, **1956**, 220, 288.

(41) Shiroza, T.; Ebisawa, N.; Furihata, K.; Endo, T.; Seto, H.; Otake, N., *Agric. Biol. Chem.*, **1982**, *46* (*7*), 1891.

(42) Nakamura, Y.; Shin, C., *Chem. Lett.*, **1991**, 1953.

(43) (a) Schimdt, U.; Riedl, B., *J. Chem. Soc., Chem. Commun.*, **1992**, 1186; (b) Schimdt, U.; Riedl, B.,*Synthesis*, **1993**, 809.

(44) Aspinall, I.H.; Cowley, P.M.; Mitchell, G.; Stoodley, R.J., *J. Chem. Soc., Chem. Commun.*, **1993**, 1179.

(45) Evans, D.A.; Weber, A.E., *J. Amer. Chem. Soc.*, **1986**, *108*, 6757.

(46) Preparation of di-*tert*-butylazodicarboxylate; Carpino, L.A.; Terry, P.H.; Crowley, P.J., *J. Org. Chem.*,**1961**, *26*, 4336.

(47) Kappeler, H.; Schwyzer, R., *Helv. Chim. Acta.*, **1960**, *43*, 1453.

(48) Hale, K.; Delisser, V.M., Manaviazar, S., *Tetrahedron Lett.*, **1992**, *33*, 7613.

(49) Durette, P.L.; Baker, F.; Barker, P.L.; Boger, J.; Bondy, S.S.; Hammond, M.L.; Lanza, T.L.; Pessolano, A.A.; Caldwell, C.G., *Abs. 198th ACS National Meeting*, Miami Beach, Florida, September 10-15, **1990**, Abstract No. 119.

(50) Evans, D.A.; Urpi, F.; Somers, T.C.; Clark, J.S.; Bilodeau, M.T., *J. Amer. Chem. Soc.*, **1990**, *112*, 8215.

(51) Fujii, T.; Miyoshi, M., *Bull. Chem. Soc. Jpn.*, **1975**, *48* (*4*), 1341.

Chapter 2 A Review on the Synthesis of Naturally-Occurring Cyclodepsipeptides

2.1 Introduction

The purpose of this chapter is to give the unfamiliar reader a background on the problems associated with the synthesis of cyclodepsipeptides. It will also illustrate the various protecting group strategies and coupling reagents that are used in the synthesis of cyclodepsipeptides.

Depsipeptides are linear peptides that possess at least one ester linkage. Cyclodepsipeptides are cyclic peptides that contain α-amino acids and α-hydroxy acids and which possess a lactone bond in the sequence. Most naturally-occurring cyclodepsipeptides are neutral and insoluble in water; the majority, however, are soluble in organic solvents and tend to be crystalline. Their structures are usually determined by a combination of NMR measurements, X-ray and mass spectral analysis, and also through quantitative amino-acid analysis. The infrared spectra of cyclodepsipeptides are distinctive in that they show strong absorptions due to the ester (1755-1715 cm^{-1}) and the amide (1680-1635 cm^{-1}) carbonyl groups. Moreover the absence of an amide II band between 1575-1500 cm^{-1} and an N-H stretching band between 3360-3260 cm^{-1} is usually indicative of all the amino acid residues being *N*-alkylated. An earlier review on cyclodepsipeptides was published in 1964 by Losse and Bachmann, in which they discussed the chemistry, classification, and nomenclature of all the then known compounds.[1] D.W. Russell also published a concise review on the occurrence, structure determination, and synthesis of several cyclodepsipeptides.[2]

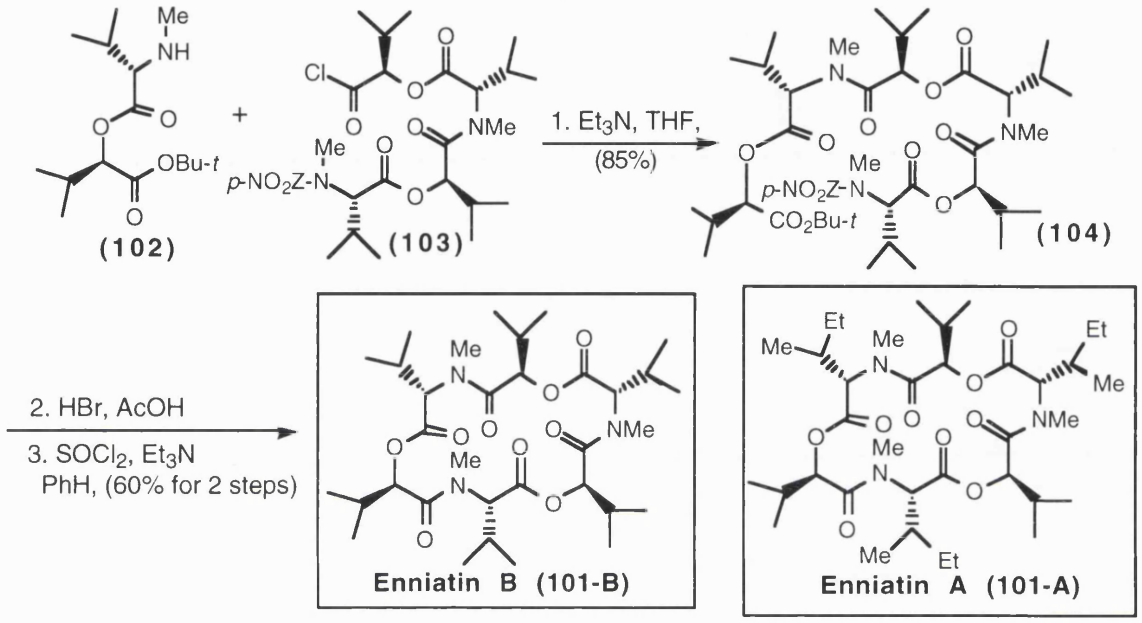
2.2 Synthesis

Enniatins

The first family of cyclodepsipeptides to be isolated were enniatins A (**101-A**) and B (**101-B**). They were discovered in the culture filtrates of various *Fusarium* species.[3] The synthesis of enniatin B **101-B** was achieved by Shemyakin and coworkers using a 4+2 fragment condensation strategy. This involved coupling of depsipeptide **102** with tetradepsipeptide **103** using acid chloride technology (PCl_5, Et_3N, THF). This led to protected hexadepsipeptide **104** in 85% yield. The *p*-nitrobenzyloxycarbonyl and *t*-butyl ester protecting groups were removed

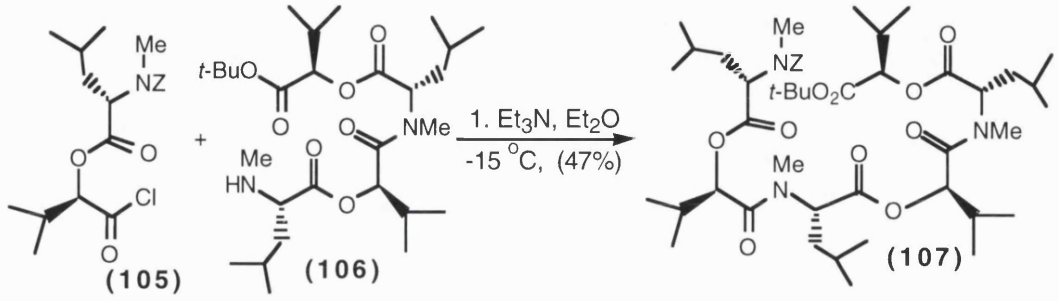
with hydrogen bromide in acetic acid and the ring subsequently cyclised via the acid chloride to give **101** in 60% yield (Scheme 1).[4a] Vogler and coworkers[4b] have also synthesised enniatin B in an analogous way to Shemyakin, and found their synthesised material had identical antimicrobial properties to the natural compound. The enniatin A (**101-A**) structure was also confimed by Vogler who synthesised it in a yield of 36% by a similar route.[5]

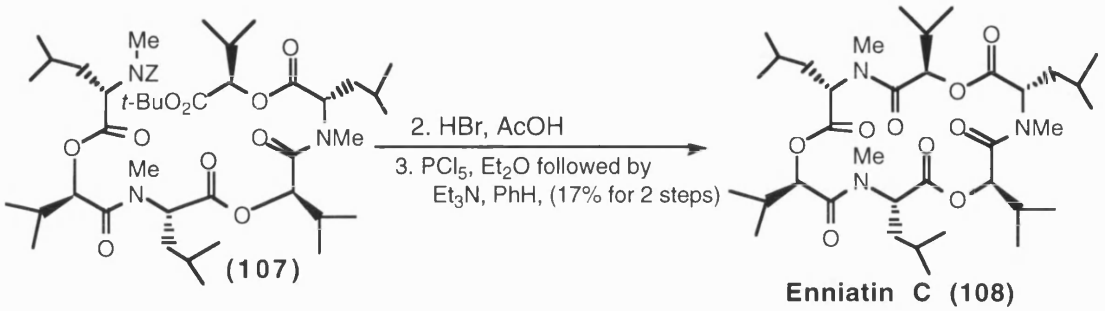
Scheme 1



Enniatin C[6a] (**108**), a natural analogue of enniatin B, was synthesised by Kanaoka and coworkers by coupling acid chloride **105** with tetradepsipeptide **106** in ether and triethylamine at -15 °C to give **107** in 47% yield. Simultaneous removal of the benzyloxycarbamate and the *t*-butyl ester from **107** with hydrogen bromide in acetic acid and subsequent cyclisation via the acid chloride method gave **108** in 17% yield (Scheme 2).[6b]

Scheme 2

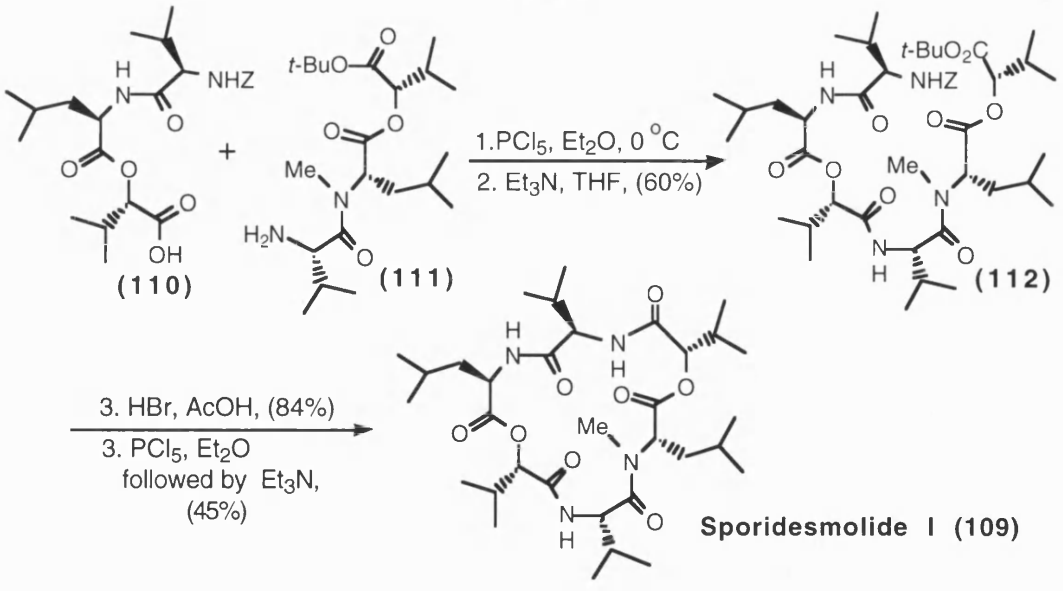




Sporidesmolides

Sporidesmolides are produced by the toxic pasture fungus *Pithomyces chartatum* and are the causative agent of facial eczema in ruminants. The structure of sporidesmolide I (**109**) was confirmed through total synthesis by Shemyakin and coworkers in 1963.[7] Thus, the acid chloride of tridepsipeptide **110** was coupled to **111** to give linear protected hexadepsipeptide **112** in 60% yield. Treatment of **112** with hydrogen bromide in glacial acetic acid gave the free hexadepsipeptide which was subsequently cyclised by the acid chloride protocol to give **109** in 45% yield (Scheme 3). Sporidesmolide II was synthesised in a similiar fashion, after substituting D-isoleucine for the D-valine residue.[8]

Scheme 3

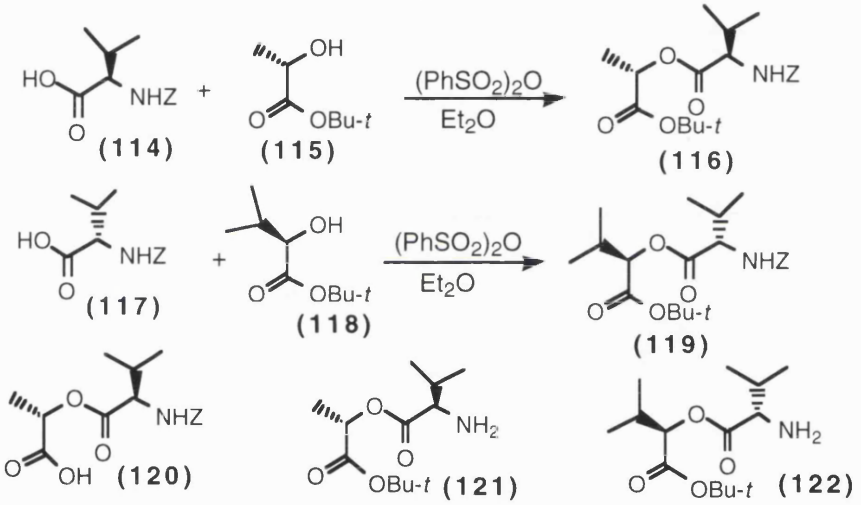


Valinomycin

Valinomycin (**113**) was isolated from *Streptomyces fulvissimus* and showed antibiotic properties.[9] Several structures were proposed by Brockman and coworkers, but these were subsequently found to be incorrect after total synthesis.[10] Brockman later revised the structure to be that of dodecadepsipeptide **113**. Valinomycin functions as an antibiotic by altering the

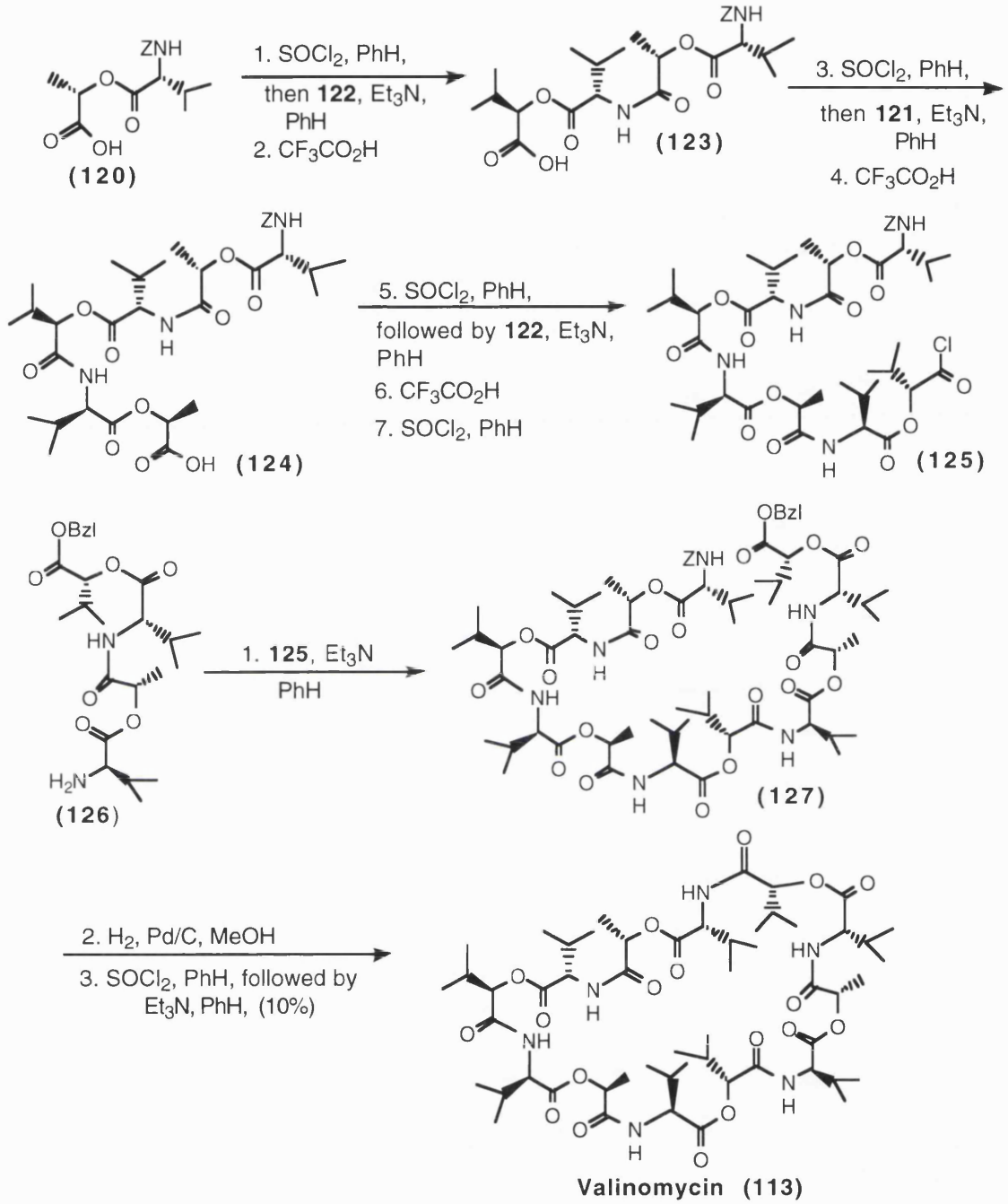
permeability of biological and artificial lipid membranes to monovalent cations[11,12] showing selectivity for potassium ions compared to sodium ions, in the membranes of mitochondria,[13] red-blood cells[14] and in lipid bilayers. The structure of valinomycin was confirmed by total synthesis in 1963 by Shemyakin and coworkers.[15] Their synthesis was initiated by the esterification of **114** with **115**, and **117** with **118** to give depsipeptides **116** and **119** respectively, via the mixed benzenesulfonic acid anhydride method (Scheme 4). Compound **116** was treated either with trifluoroacetic acid to reveal acid **120** or hydrogenated with palladium on carbon to give amine **121.** Depsipeptide **119** was hydrogenated with palladium on carbon to give amine **122** quantitatively (Scheme 4).

Scheme 4



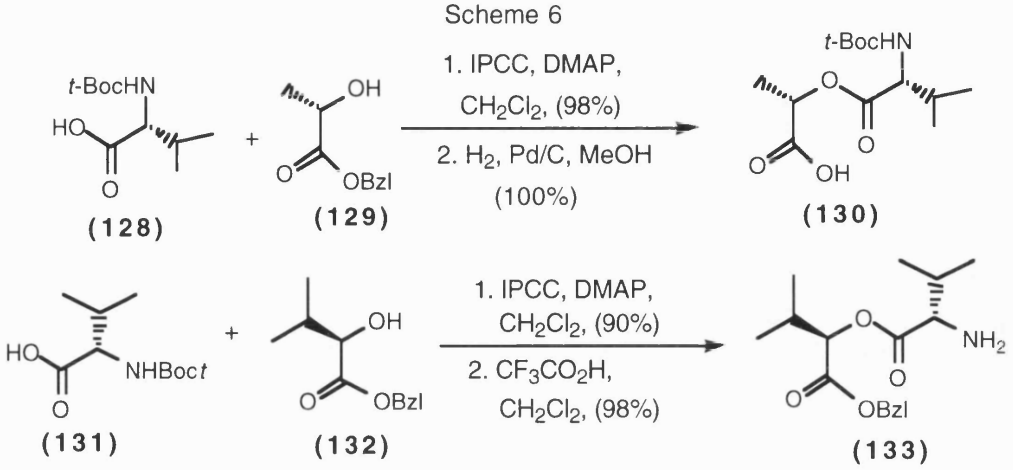
Coupling of the acid chloride from **120** with amine **122**, mediated by triethylamine, gave tetradepsipeptide **123** after acidolysis of the *t*-butyl ester with CF_3CO_2H. Acid **123** was then coupled to amine **121** via the acid chloride and removal of the ester group gave hexadepsipeptide **124**, to this was coupled **122** to form the octadepsipeptide which was then converted to acid chloride **125** (Scheme 5). Condensation of **125** with tetradepsipeptide **126** in the presence of triethylamine gave the linear dodecadepsipeptide **127**. Hydrogenation and subsequent cyclisation by the acid chloride protocol at high dilution gave valinomycin **113** in 10% yield (Scheme 5).

Scheme 5



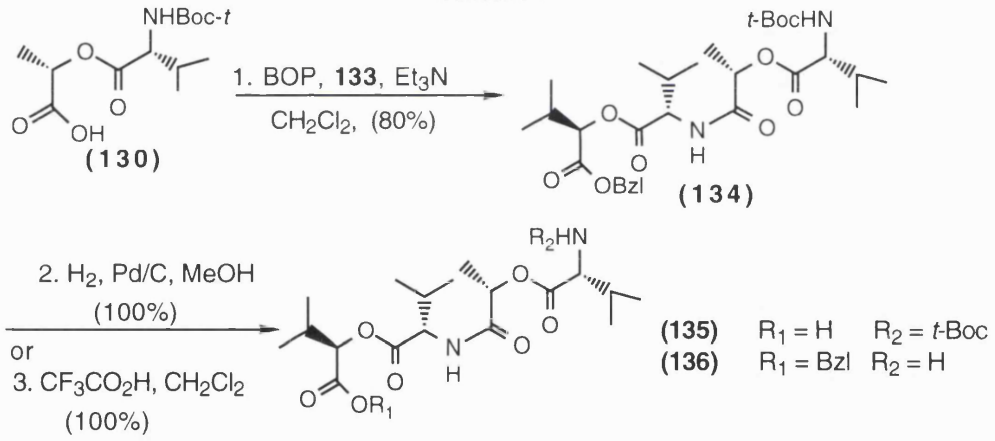
The synthesis of **113** by Jouin was based on the formation of depsipeptides **130** and **133** via the isopropenyl chlorocarbonate (IPCC) activation protocol.[16] Thus esterification of *N*-Boc-D-valine **128** with the lactate derivative **129** gave the ester in 98% yield, subsequent removal of the benzyl ester by hydrogenation gave acid **130** in a quantitative yield. *N*-Boc-L-valine **131** was coupled to the hydroxyvaleric acid derivative **132** via IPCC activation to furnish after the cleavage of the Boc group, amine **133** in 98% yield (Scheme 6).

Scheme 6



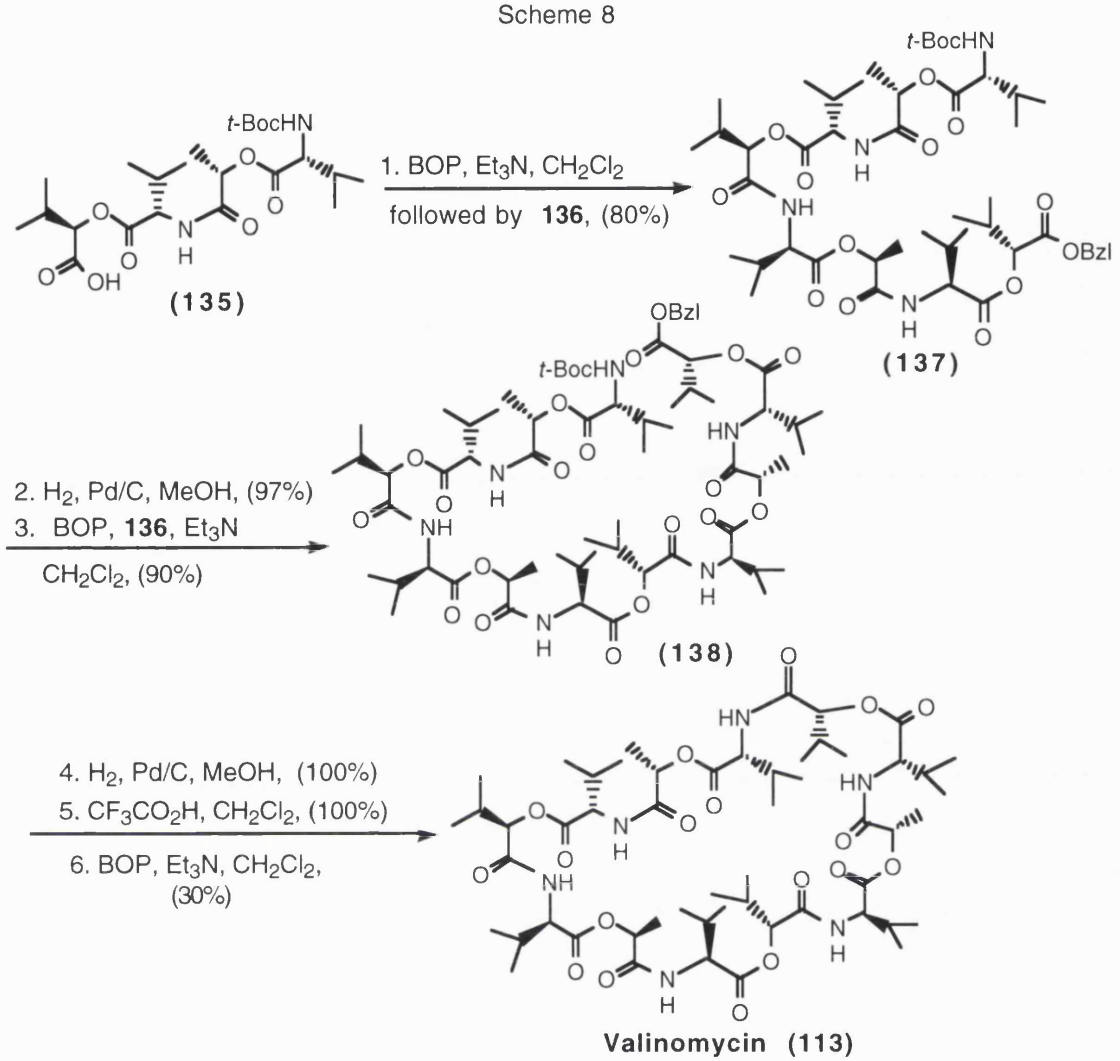
Condensation of **130** with **133** was effected by the benzotriazol-1-yloxytris(dimethyl-amino)phosphonium hexafluorophosphate (BOP) activated ester protocol[17] and gave **134** in 80% yield. Tetradepsipeptide **134** was either hydrogenated to give acid **135** or hydrolysed to give amine **136** respectively in quatitative yield (Scheme 7).

Scheme 7



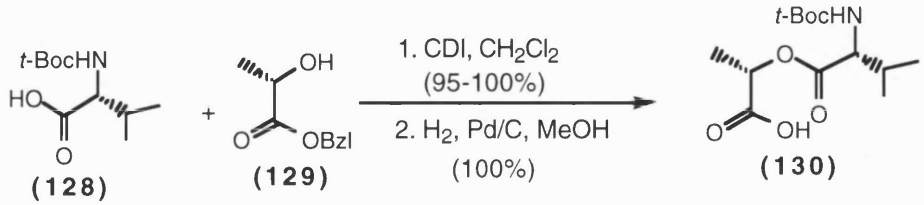
Coupling of **135** and **136** by the BOP method gave the linear octadepsipeptide **137** in 80% yield. Deprotection of the acid by hydrogenation and condensation with **136** gave the dodecadepsipeptide **138** in 90% yield. Deprotection of **138** by hydrogenation and acidolysis gave the free dodecadepsipeptide which was cyclised by the BOP procedure to form the hydroxyvaline-valine bond of valinomycin **113** in 30% yield (Scheme 8).

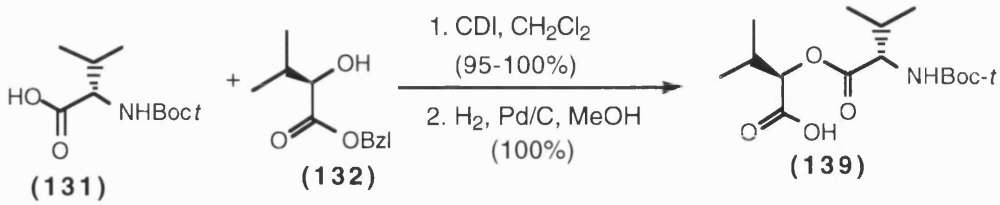
Scheme 8



A solid-phase approach to **113** has been developed by Merrifield that is based on the coupling of depsipeptides **130** and **139** alternately.[18] The depsipeptide **130** was synthesised by coupling lactate **129** and amino acid **128** with *N,N'*-carbonyldiimidazole (CDI).[19] Subsequent debenzylation by hydrogenolysis revealed acid **130**. Depsipeptide **139** was synthesised by the same method (Scheme 9).

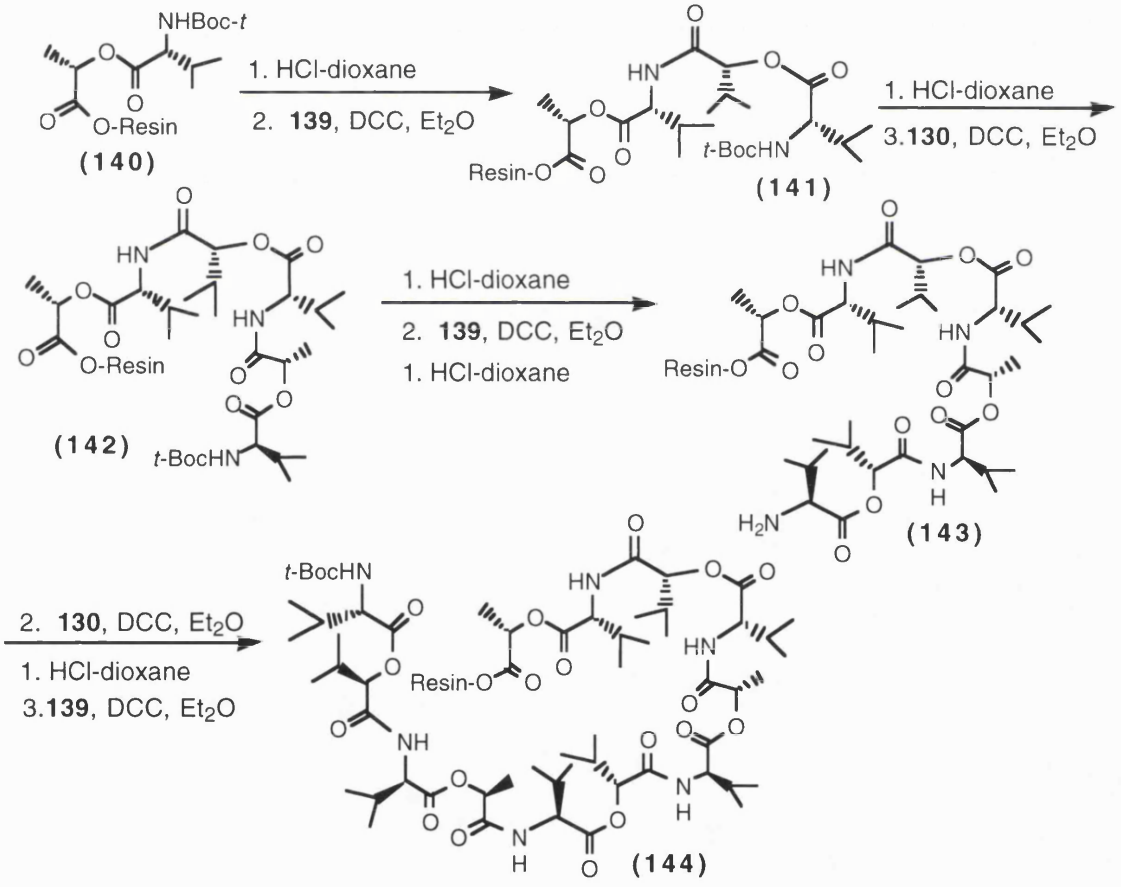
Scheme 9





Depsipeptide **130** was initially coupled to the chloromethylated stryrene-divinylbenzene resin[20] to give the depsipeptide resin **140**. This was then chain extended using the automated instrument. The key steps entailed removal of the *t*-Boc group with HCl in dioxane and coupling to the appropriate depsipeptide unit using *N,N*-dicyclohexylcarbodiimide (DCC)[21] as the activating agent. After five cycles the protected linear dodecadepsipeptide resin **144** was obtained (Scheme 10).

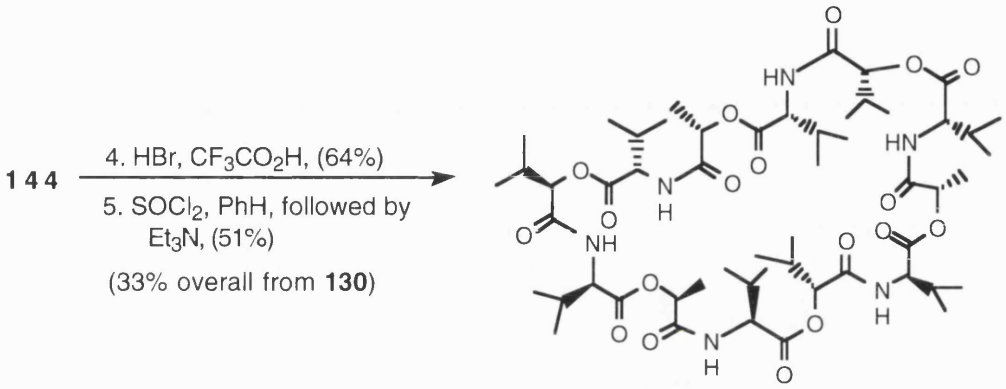
Scheme 10



After each coupling step a sample of the peptide-resin was cleaved with hydrogen bromide in acetic acid and the peptide was then eluted on a silica gel TLC plate in order to assess its homogeneity. Apart from the tetradepsipeptide of **141** which was contaminated with 5% of H-D-Val-L-Lac-OH, the other coupling products were homogeneous. In order to complete the

synthesis of **113** the dodecadepsipeptide-resin **144** was treated with hydrogen bromide in trifluoroacetic acid to give the free dodecadepsipeptide in 64% yield. This was cyclised to valinomycin **113** in 51% yield by the acid chloride method of Shemyakin[15] at high dilution (Scheme 11). This particular macrolactamisation went in a higher yield than that of Shemyakin since it was attempted at the D-Lac-D-Val bond rather than the more hindered D-hydroisovaleric (Hyv)-D-Val bond.

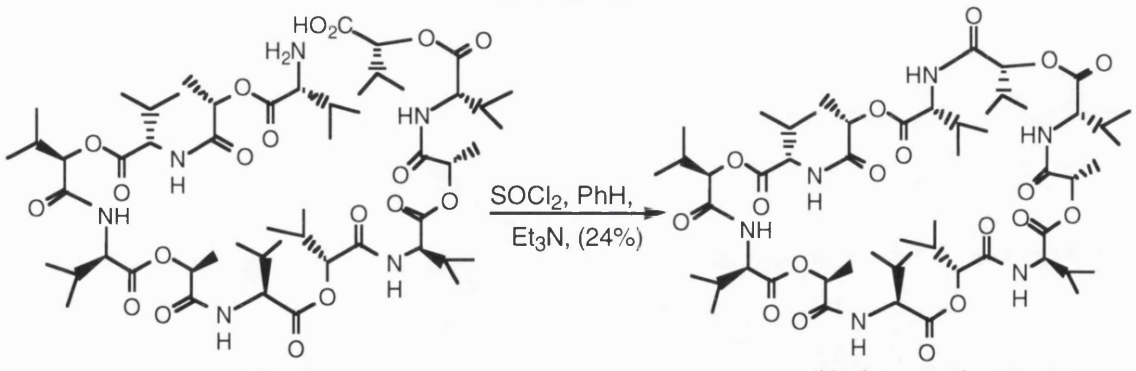
Scheme 11



Valinomycin (113)

The Losse and Klengel[22] synthesis of valinomycin (**113**) was based on the formation of linear dodecadepsipeptide **145**. Cyclisation was accomplished by the acid chloride/triethylamine protocol at high dilution to furnish valinomycin in 24% yield (Scheme 12).

Scheme 12

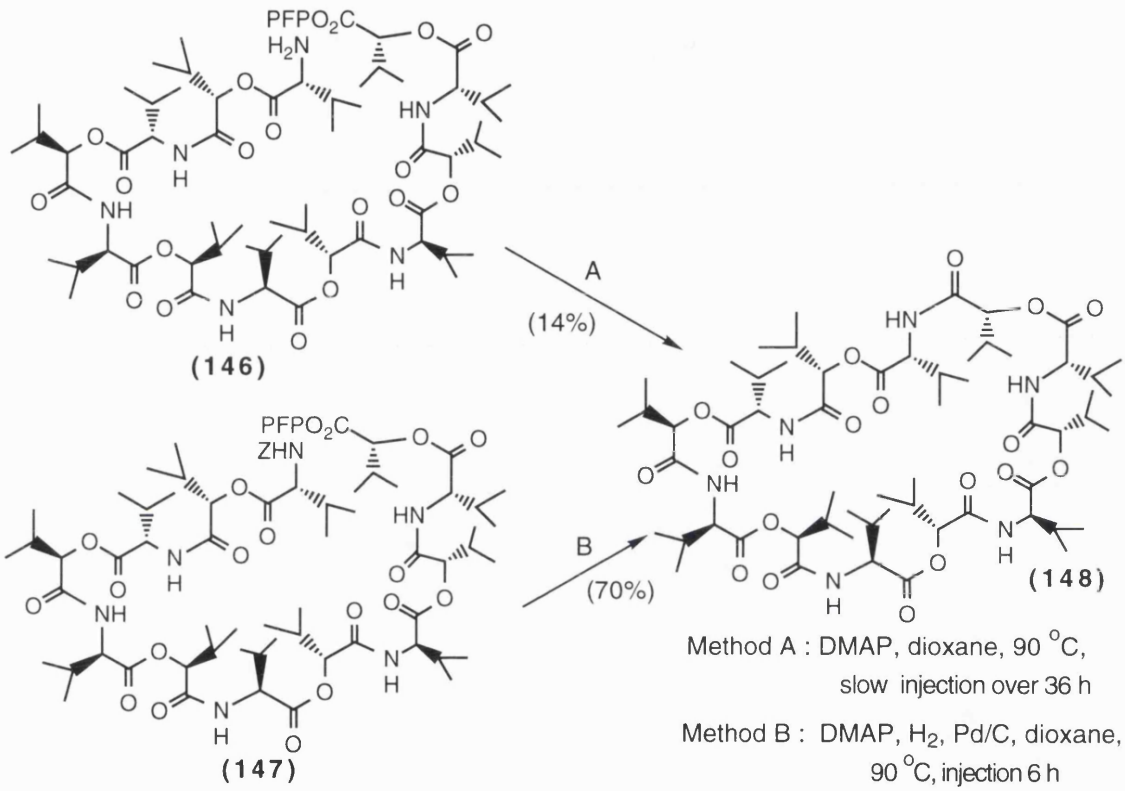


(145) **Valinomycin (113)**

Mellor and coworkers have developed an efficient cyclisation procedure for the synthesis of valinomycin anologues that utilises activated pentafluorophenyl esters. Slow injection of the trifluoroacetic acid salt of the pentafluorophenyl ester **146** over 36 hours to a solution of 4-(dimethylamino)pyridine (DMAP) in dioxane at 90 ^{o}C gave the desired cyclised product **148** in 14% yield (method A). In order to increase the yield of the product, **146** was generated *in situ*

from the corresponding benzyloxycarbonyl protected ester **147**. Thus **147** was slowly injected over 6 hours to a solution of dioxane at 90 °C containing of DMAP and palladium on charcoal, whilst bubbling hydrogen through the solution to give the desired product **148** in 70% yield (method B). This procedure minimises the formation of polymeric products (Scheme 13).[23]

Scheme13



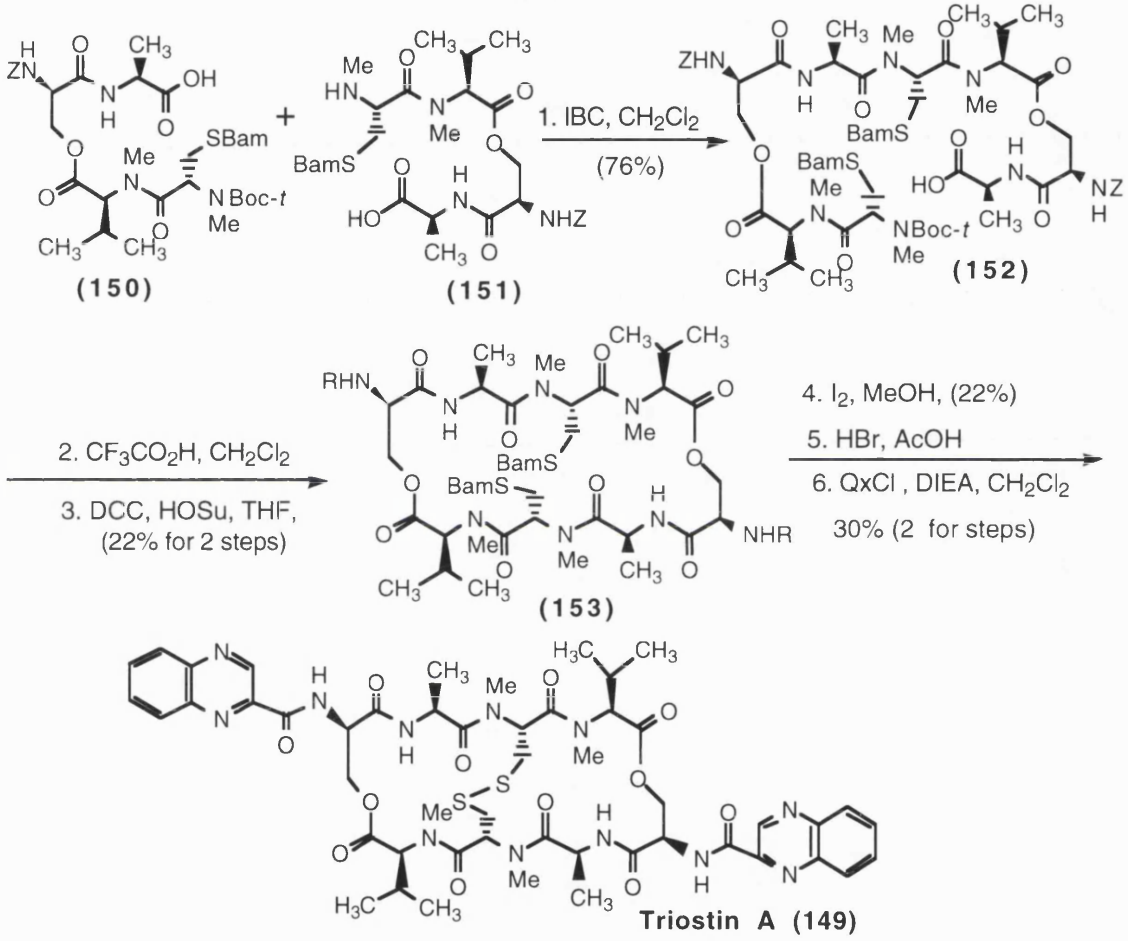
Triostin A

Triostin A (**149**) is a dimeric cyclic octadepsipeptide isolated from *Streptomyces aureus* that has antibacterial and cytotoxic activity. It belongs to the quinoxaline family of antibiotics, and as such binds to DNA as a bifunctional intercalating agent, thus inhibiting RNA synthesis. Triostin A contains D-serine, L-alanine, *N*-methyl-L-valine and *N,N* '-dimethyl-L-cystine linked together by a disulfide cross-bridge.[24]

The first synthesis of triostin A (**149**) was accomplished by Olsen and Chakravorty,[25] and was based on the coupling of acid **150** with amine **151** via the mixed anhydride formed from treatment with isobutylchloroformate (IBC)[26]; this gave **152** in 76% yield (Scheme 14). After removal of the *N*-methyl-Boc group of **152** with trifluoroacetic acid, cyclisation was effected by the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (HOSu) method

at high dilution (0.0025 M). Cyclodepsipeptide **153** was formed in 22% yield, and was converted into triostin A (**149**) in 30% yield by a further three operation. These involved oxidation with iodine in methanol[27,28] to simultaneously remove the benzamidomethyl (Bam)[29] group and form the disulfide bond, hydrogenolysis with palladium on carbon to excise the benzyloxycarbonyl protecting group of the serine amine, and acylation with 2-quinoxalinylcarbonyl chloride (Qxc-Cl).[30]

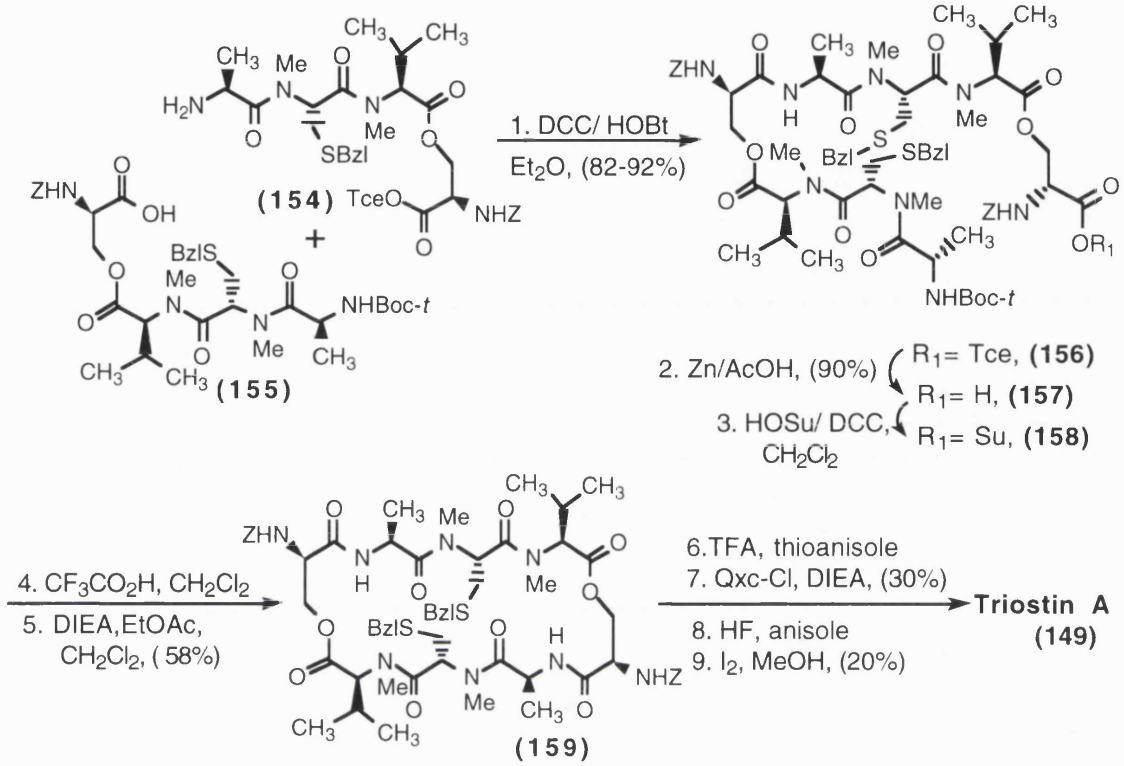
Scheme 14



Shin published an alternative route[31] to **149** in which the the key intermediates **154** and **155** were coupled using the DCC and HOBt protocol[21] to give the linear octadepsipeptide **156** in 82-92% yield. Removal of the 2,2,2- trichloroethyl ester (Tce) group with zinc in acetic acid[32] led to acid **157** which was then converted to its succinimide activated ester **158** with DCC and HOSu.[33] Deprotection of the Boc group with trifluoroacetic acid, followed by treatment with *N,N*-diisopropylethylamine (DIEA) in ethyl acetate at high dilution (2.0×10^{-4} M) gave the cyclic octadepsipeptide **159** in 58% yield. The yield of the cyclisation step was greater than that in the

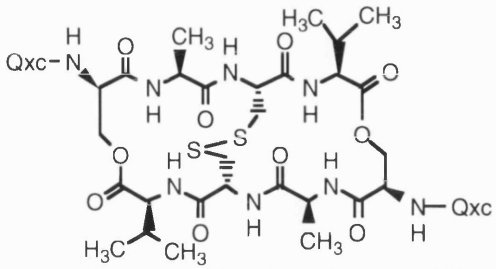
original synthesis of Olsen[25] since the lactamisation was attempted between the serine carboxyl and the alanine residue; the latter is less crowded than the alanine-*N* methylcystine amide bond. Compound **159** was then converted into Trisotin A **149** in four steps (Scheme 15).

Scheme 15

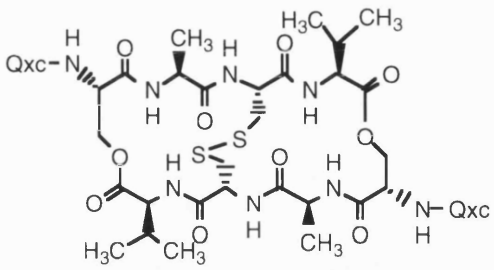


Several analogues of Triostin A have been synthesised and their mode of biological action determined. These include des-*N*-tetramethyltriostin A **160** which lacks the *N*-methyl groups on the valine and cysteine residues,[34,35] bis-L-seryl-des-*N*-tetramethyltriostin A **161**, which possess the L- rather than the D-serine residue[34] and [Lac^2, Lac^6] Tandem **162** which is an analogue of nortriostin that possesses L-lactic acid instead of the normal L-alanine residue.[36] The former was found to bind to DNA as a bifunctional intercalating agent while retaining the activity of the parent molecule. However, the latter two molecules were found to be inactive as intercalating agent (Figure 1).

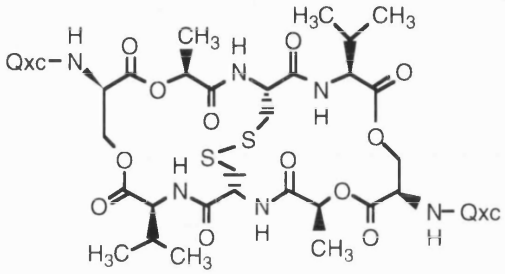
Figure 1



Des-*N*-tetramethyltriostin A (160)



Bis-L-seryl-des-*N*-tetramethyltriostin A (161)

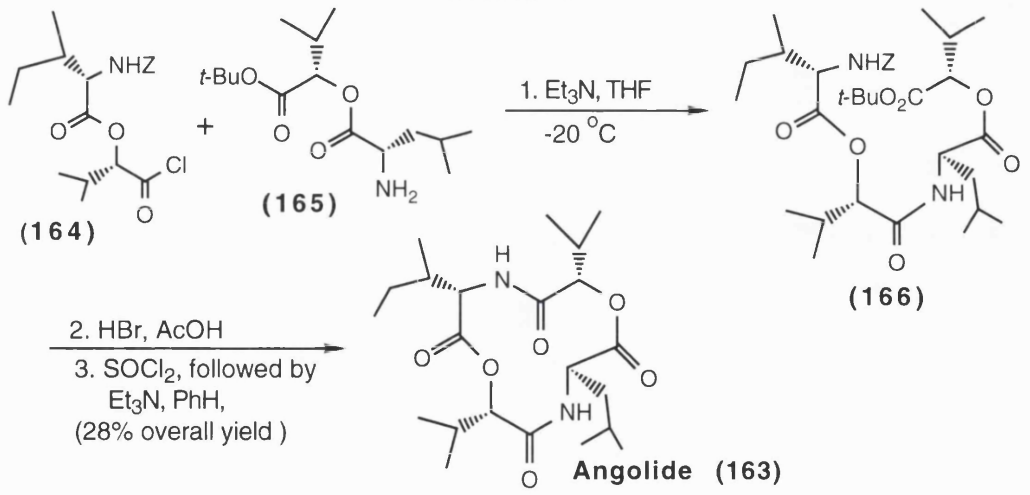


[Lac^2, Lac^6}-Tandem (162)

Angolide

Angolide (**163**) is a metabolic product of a *Pithomyces* fungus species, that was first synthesised by Shemyakin and coworkers utilising a coupling between the acid chloride **164** and depsipeptide **165** as a key step. Simultaneous removal of the protecting groups from the product **166** with hydrogen bromide in acetic acid and macrolactamisation by the acid chloride method in benzene gave angolide **163** in an overall yield of 28% (Scheme 16).[37]

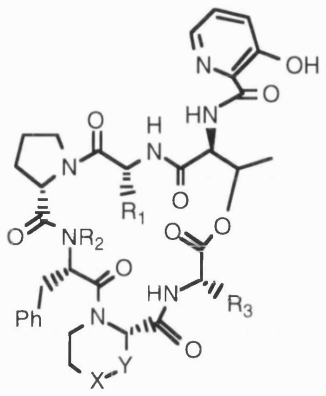
Scheme 16



Streptogramin

Kessler and coworkers[38] have devised a synthesis of streptogramin **172**. This is an analogue of virginiamycin S_1 **166-S_1** (Fig. 2), that contains a 19-membered cyclodepsipeptide ring in which the threonine and 4-oxopipecolic acid residues are replaced with *N*-Boc-serine and pipecolic acid respectively.

Figure 2

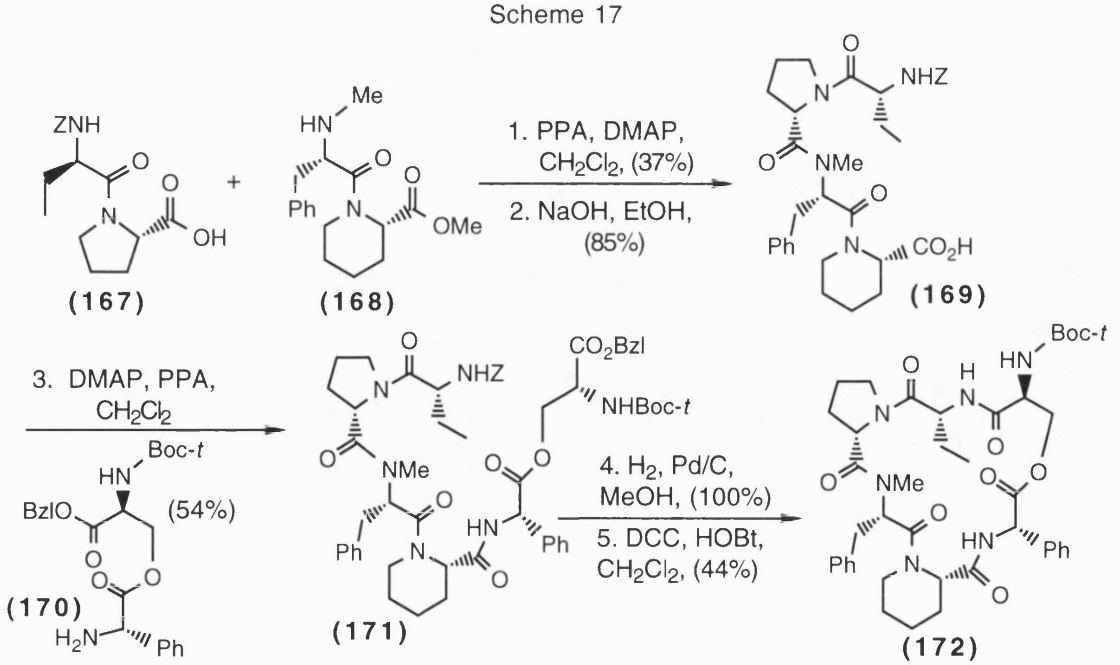


Virginiamycin (166)

	R_1	R_2	X	Y	R_3
S_1	C_2H_5	CH_3	CO	CH_2	C_6H_5
S_2	C_2H_5	H	CHOH	CH_2	C_6H_5
S_3	C_2H_5	CH_3	CO	CHOH	C_6H_5
S_4	CH_3	CH_3	CO	CH_2	C_6H_5
S_5	C_2H_5	CH_3	CHOH	CH_2	CH_3

Initially, dipeptides **167** and **168** were condensed using *N*-propylphosphonic anhydride (PPA)[39,40] and 4-(dimethylamino)pyridine (DMAP) to give the tetrapeptide **169** in 37% yield after saponification. Acid **169** was coupled to depsipeptide **170** using the PPA/DMAP reagent combination to produce hexadepsipeptide **171** in 54% yield (Scheme 17).

Scheme 17

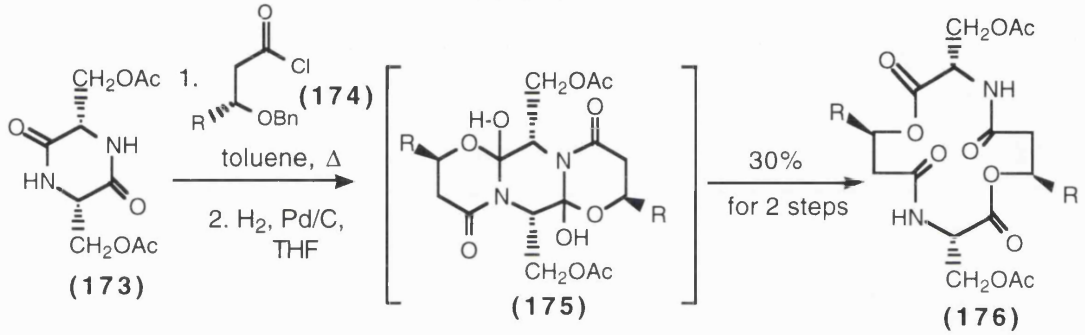


Hydrogenation of **171** removed both benzyl protecting groups to deliver the ω-amino acid which was ring-closed with DCC/HOBt to afford **172** in 44% yield (Scheme 17).

Serratamolide

Serratamolide is a metabolite of *Serratia marcescens*, which possesses activity against bacteria, yeasts and pathogenic fungi. Its structure was determined by chemical degradation which revealed it to be a symmetrical cyclodepsipeptide containing L-serine and D-β–hydroxy-decanoic acid.[41] The first synthesis of a serratamolide analogue **176** was achieved by Shem-yakin using a procedure that was based on a intriguing retro-aldol type reaction[42] to simulta-neously install the ester and amide linkages in **176** via the intermediate **175** in 30% yield (Scheme 18).[43]

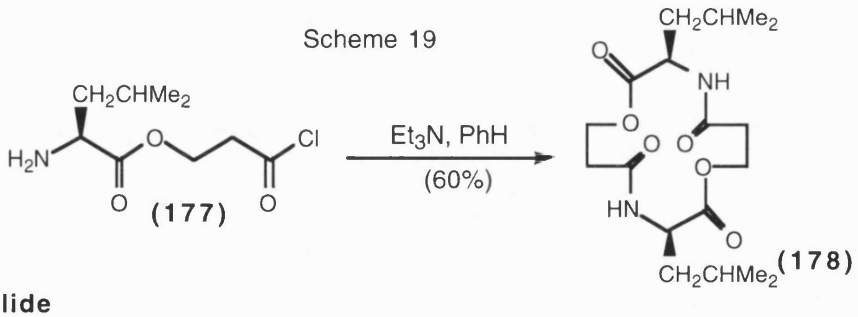
Scheme 18



R =-$(CH_2)_6CH_3$

Hassall also synthesised a serratamolide analogue (**178**) via a cyclodimerisation of two molecules of β-L-leucyloxypropionyl chloride **177**. This resulted in **178** being formed in 60% yield when carried out at high dilution with triethylamine in benzene (Scheme 19).[44]

Scheme 19

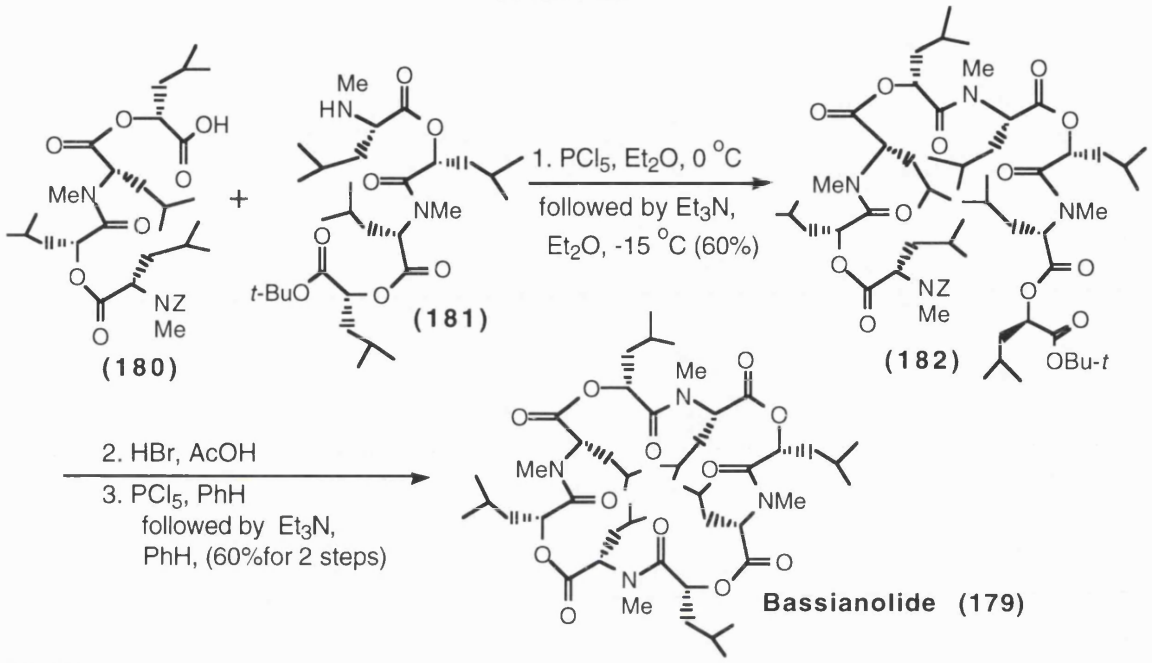


Bassianolide

Bassianolide (**179**) is an insecticidal cyclodepsipeptide produced by the entomopatho-genic fungi *Beauveria bassiana* and *Verticillium leccanii*.[45] The synthesis of this C_4-symmetrical structure developed by Kanaoka and coworkers employed a coupling of the tetradepsipeptides **180** and **181** using the acid chloride protocol to give **182** (Scheme 20). Global deprotection of

182 with hydrogen bromide in acetic acid and subsequent cyclisation via the acid chloride in benzene at high dilution gave bassianolide **179** in 60% yield.[6b] This approach is noteworthy since it is one of the few macrocyclisations to be carried out at an *N*-methylated nitrogen atom.

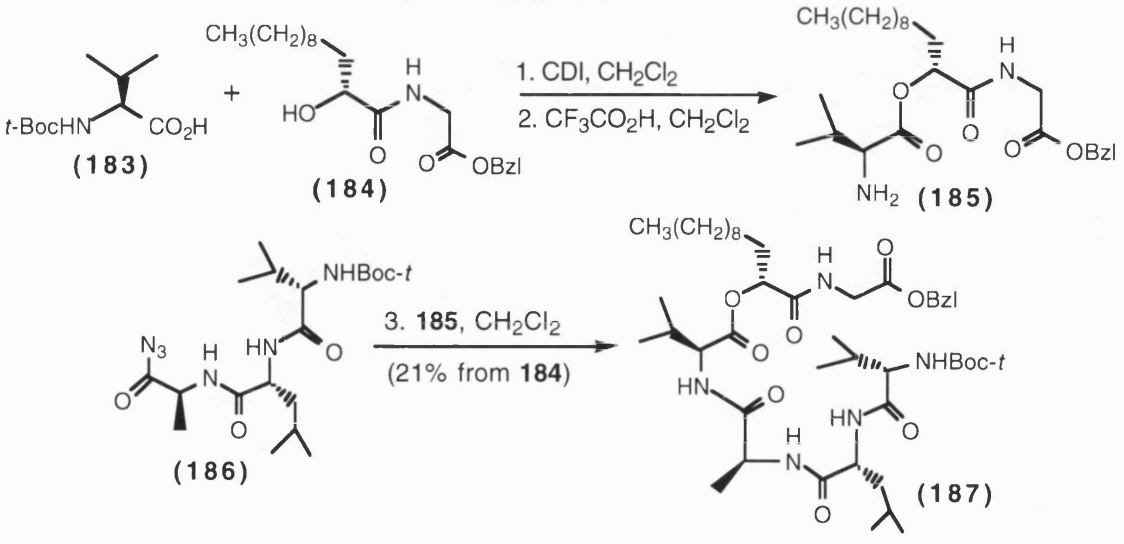
Scheme 20



Isariin

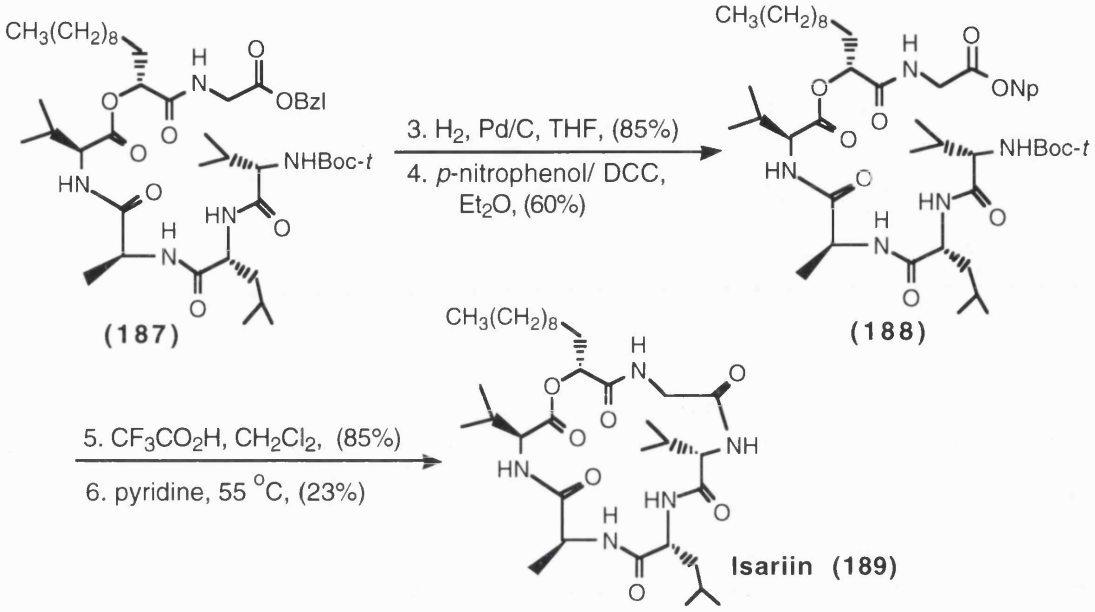
The structure of Isariin (**189**), a peptide metabolite of the fungus *Isaria cretacea,*[46] was confirmed after its total synthesis by Okada and coworkers.[47] Amino acid **183** was coupled to **184** using 1,1-carbonyldimidazole (CDI)[19] to give tridepsipeptide **185** after acidolysis. Condensation of **185** with acyl azide **186** afforded linear hexadepsipeptide **187** in an overall yield of 21% (Scheme 21).

Scheme 21



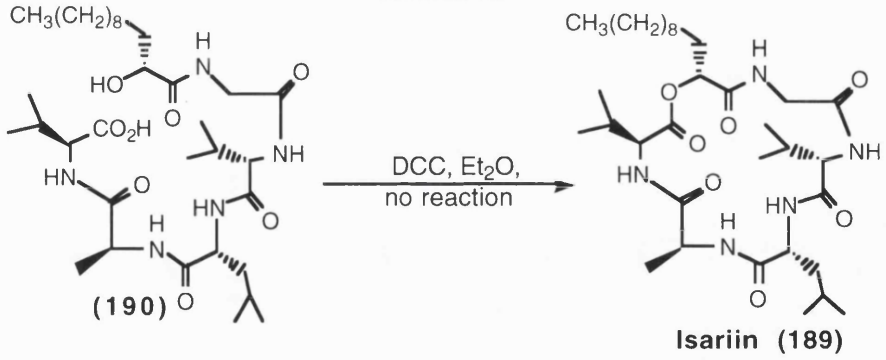
Hydrogenation of **187** and reesterification with *p*-nitrophenol[48] gave activated ester **188** in 60% yield. Deprotection of the Boc group followed by cyclisation at high dilution at 55 °C in pyridine gave isariin **189** in a yield of 23% (Scheme 22).

Scheme 22



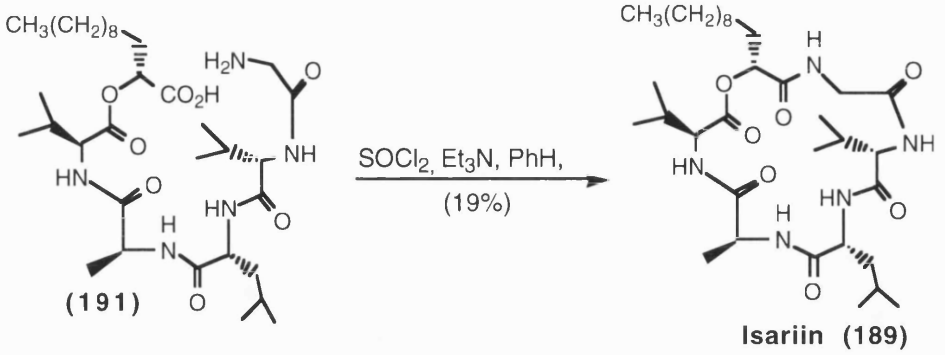
Rydon's studies on the synthesis of isariin **189** initially led to the assembly of linear pentadepsipeptide **190**. However, macrolactonisation with DCC was unsuccessful possibly due to steric hindrance around the carboxyl terminus and the hydroxy function (Scheme 23).

Scheme 23



As a result linear pentadepsipeptide **191** was next selected as the cyclisation precursor, since the glycine residue was less hindered and its amine therefore more nucleophilic. Ring closure was achieved, albeit in 19% yield, by the acid chloride procedure. Isariin **189** was obtained pure after reverse phase HPLC (Scheme 24).[49]

Scheme 24

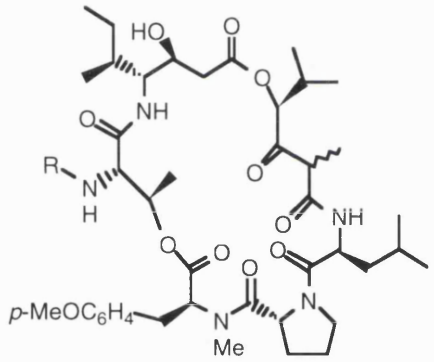


A solid phase synthesis of isariin has also been developed by Okada and coworkers.[50]

Didemnins

Three congeners of didemnin were isolated from the tunicate *Tridemnum solidum,*[51] of which didemnin B (**193**), displays strong cancerstatic and immunosuppressive properties, and is currently undergoing human clinical trials (Figure 3).[52]

Figure 3

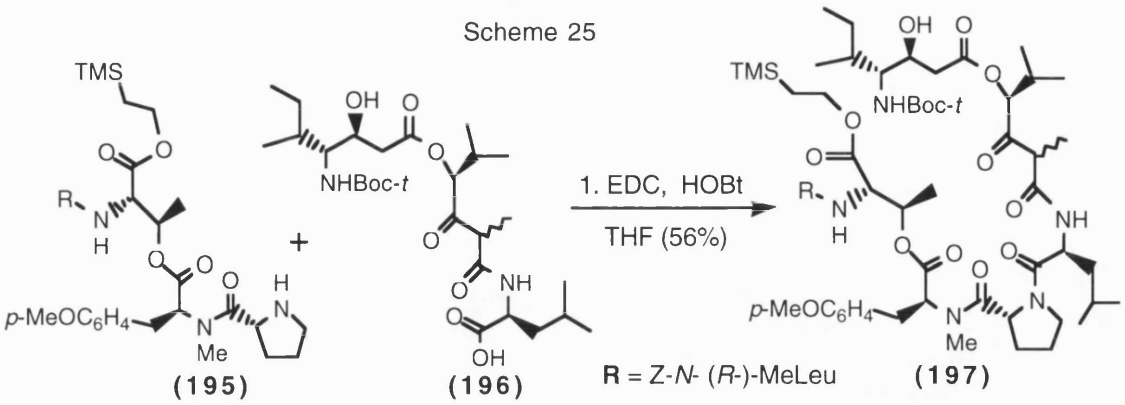


(192) Didemnin A; R = H-(*R*)-MeLue
(193) Didemnin B; R= H-(*S*)-Lac- (*S*)-Pro-(*R*)-MeLeu
(194) Didemnin C; R= H-(*S*)-Lac-(*R*)-MeLeu

The structure of didemnin B has been revised several times from the original structure proposed by Rinehart.[51] These revisions include the confirmation that the *N*-methyl-leucine has (*R*)-stereochemistry,[53] and that the hydroxyisovalerylpropionic acid has the (*2S, 4R*) configuration.[54] In addition a (*3S, 4R, 5S*)-isostatine residue is present rather than statine as previously thought.[55]

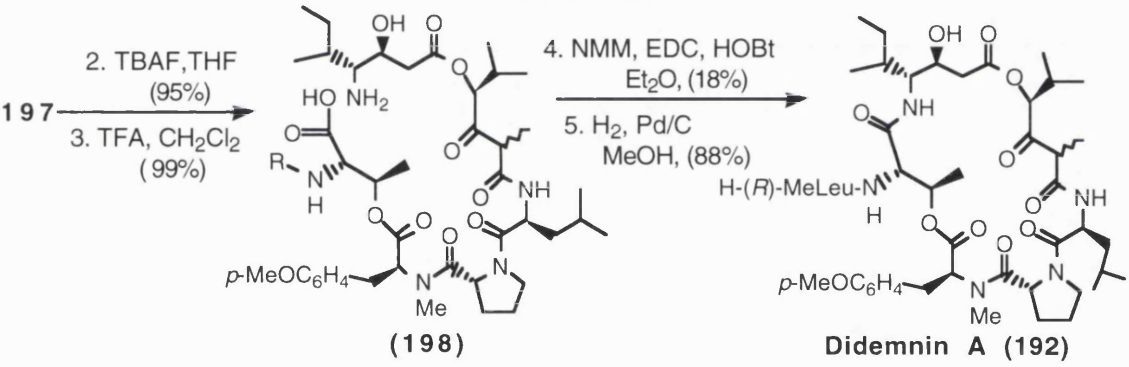
The first synthesis of didemnin A **192** was accomplished after a coupling of pentadepsipeptide **195** with tripeptide **196** using the 1-ethyl-3-(3-dimethylaminopropyl)carbodimide (EDC) /HOBt method to obtain linear octadepsipeptide **197** in 56% yield (Scheme 25).

Scheme 25



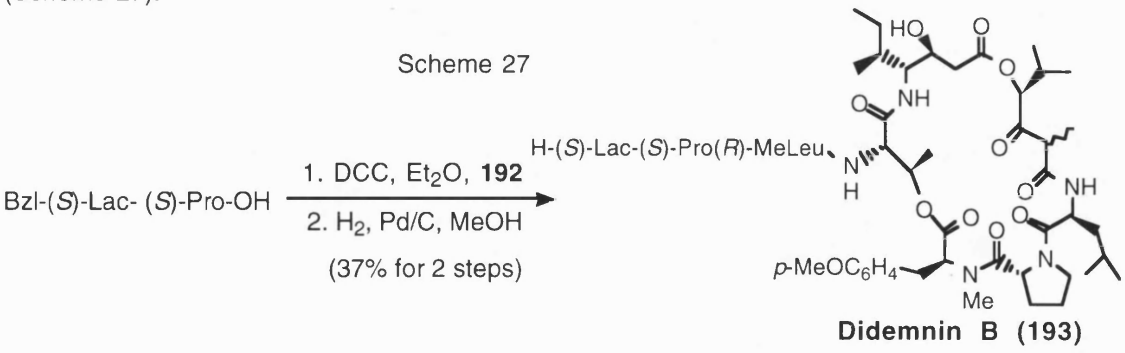
Subsequent removal of the β-trimethylsilylethyl ester group with tetra-*n*-butylammonium fluoride (TBAF), and the *N*-Boc group with trifluoroacetic acid in **197** revealed the cyclised precursor **198**. Cyclisation with EDC/HOBt furnished the cyclised product in a rather modest 18% yield. Hydrogenolysis of the benzyloxycarbonyl (Z) group of the *N*-methyl leucine side chain finally afforded didemnin A (**192**) in 88% yield (Scheme 26).[56]

Scheme 26



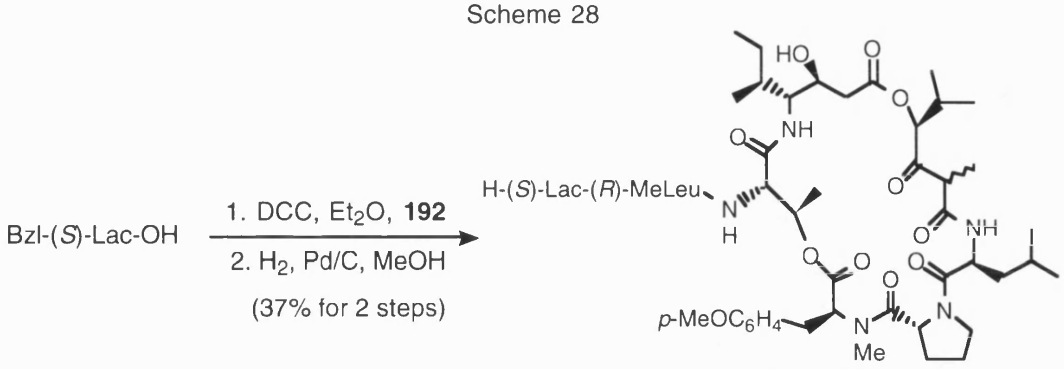
Didemnin B (**193**) was synthesised by coupling didemnin A (**192**) with Bzl-(*S*)-Lac-(*S*)-Pro-OH in the presence of EDC, subsequent debenzylation by hydrogenolysis gave didemnin B **193** (Scheme 27).

Scheme 27



Didemnin A was coupled to *O*-benzyl-L-lactic acid with DCC, and the benzyl ether removed by hydrogenation to furnish didemnin C**194** in an overall yield of 37% yield (Scheme 28).[56]

Scheme 28

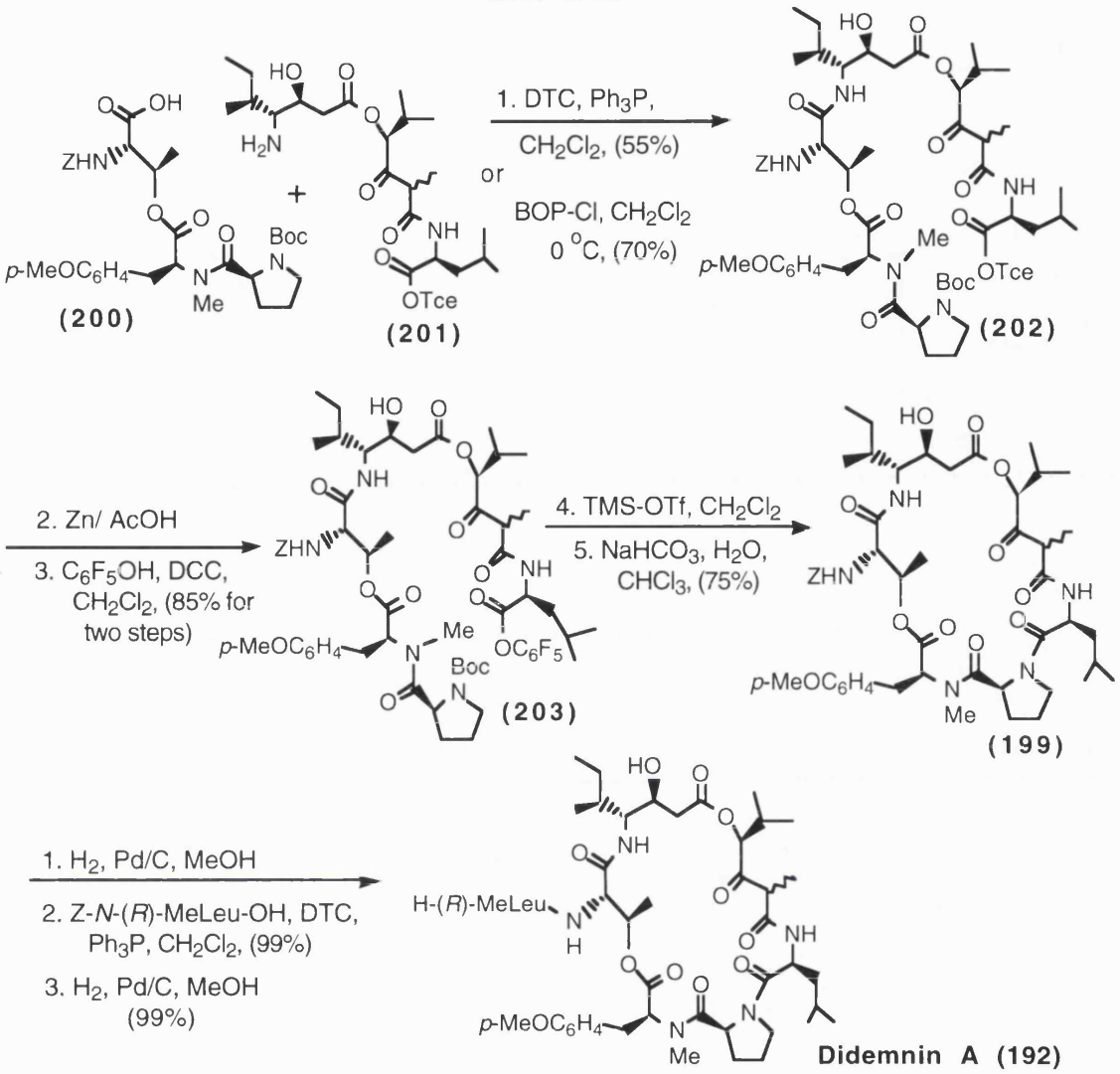


Didemnin C (194)

The Schmidt synthesis of the didemnin depsipeptide ring **199**[57] rested on a coupling of the tridepsipeptides **200** and **201** using the 4,6-dimethyl-2-thiopyridone-3-carbonitrile (DTC) method;[58,59] the linear hexadepsipeptide **202** was isolated in 55% yield. The trichloroethyl ester was then detached using zinc in acetic acid and the resulting acid converted to its pentafluorophenyl ester by DCC activation. This led to **203** whose Boc group was cleaved with trimethylsilyl triflate.[60] Once the amine in tridepsipeptide **201** was unmasked, the molecule became unstable due to the readiness with which the isostatine residue undergoes γ-lactam-isation. As a result, a powerful method for activating the acid in **200** was needed in order to couple it to **201**, without causing lactamisation. Several reagents were tested for this coupling;[61] these included the pentafluorophenyl ester (33%), the pivaloyl mixed anhydride (21%) and the diphenylphosphoryl azide (45%). Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[62,63] proved to be the reagent of choice, it leading to **202** in 70% yield (Scheme 29).

Cyclisation was then accomplished in the presence of aqueous sodium bicarbonate in the two phase system of $H_2O/CHCl_3$,[64] to give cyclodepsipeptide **199** as a 2.5:1 (*S*: *R*) mixture of epimers at the 2-position of the Hip residue (Scheme 30). The required (*S*)-isomer could be isolated by MPLC. The (*R*)-epimer was found to rearrange under acid or base catalysis to the thermodynamically more stable (*S*)-epimer.[67] In order to complete the synthesis of **192**, cyclodepsipeptide **199** was hydrogenated and coupled to the thiol ester of Z-*N*-methyl-(*R*)-leucine with 4,6-dimethyl-2-thiopyridone-3-carbonitrile (DCT). Subsequent hydrogenolysis afforded didemnin A **192** in 99% yield (Scheme 29).[61]

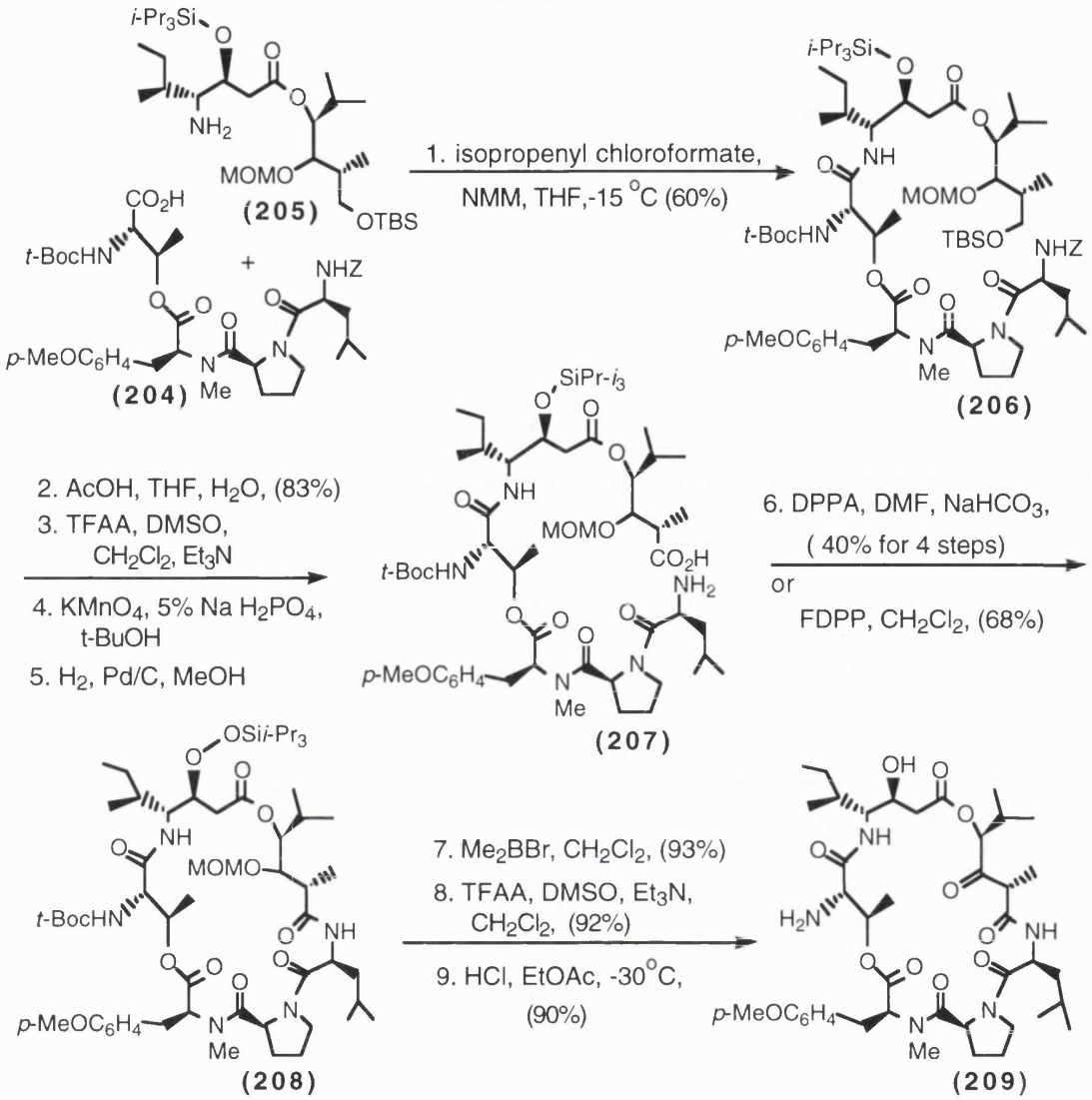
Scheme 29



Acylation of didemnin A **192** with the acid chloride of *Z*-*N*-lactyl-(*S*)-proline and subsequent hydrogenolysis gave didemnin B **193** in 85% yield. Didemnin C **194** was synthesised by the coupling of didemnin A **192** with the acid chloride of *O*-benzyloxycarbonyl-lactic acid, followed by hydrogenolysis in 80% yield.

Joullie and coworkers have devised a novel synthesis of the didemnins.[65] Acid **204** was condensed with amine **205** using isopropenyl chloroformate[66] to provide **206** in 60% yield. In order to accomplish the macrolactamisation, the primary alcohol was oxidised to the acid. This was achieved by cleaving primary silyl ether with aqueous acetic acid in THF and oxidising using the two-stage procedure developed by Masamune.[67] Hydrogenation then revealed the ω–amino acid **207** (Scheme 30).

Scheme 30



Subsequent lactamisation using diphenylphosphoryl azide (DPPA) and sodium bicarbonate at 0.001 M concentration[68] gave **208** in 40% overall yield. In order to install the isostatine residue, the MOM ether group in **208** was removed with dimethylboron bromide and the alcohol oxidised to the ketone in 92% yield. Global deprotection with hydrochloric acid in ethyl acetate then gave amine **209** in 90% yield. Didemnin A **192** was then synthesised by condensing **209** with *Z*-*N*-Me-(*R*)-Leu-OH using BOP as the activating reagent. Subsequent removal of the Z group by hydrogenation gave **192** in 85% yield. Didemnin B **193** was synthesised in 59% yield by coupling the tridepsipeptide, (*S*)-Lac-(*S*)-Pro-(*R*)-MeLeu-OH with **209** using the BOP procedure. Didemnin C was synthesised by condensing *O*-TBS-(*S*)-Lac-(*R*)-MeLeu-OH with **209** by the BOP protocol, followed by desilylation with hydrofluoric acid in acetonitrile to afford **194** in 89% yield.

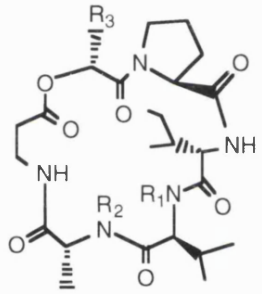
The macrolactamisation of **207** was reinvestigated by Joullie and coworkers[69] with other activating agents such as pentafluorophenyl diphenylphosphinate (FDPP),[70] 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTu)[71] and isobutyl chloroformate.[71] FDPP gave the best result of the reagents tried, 68% yield (Scheme 30).

An analogue of didemnin A containing statine in place of the isostatine residue was synthesised by Shioiri and coworkers. In his synthesis the depsipeptide ring was constructed at the *N*-atom of *N,O*-dimethyl-tyrosine unit.[72]

Destruxins

The destruxin family of cyclodepsipeptides (Figure 4) were isolated from the culture filtrates of *Metarrhizium anisopliae* and were shown to possess insecticidal, antiviral, and cytotoxic properties.[73a,b]

Figure 4

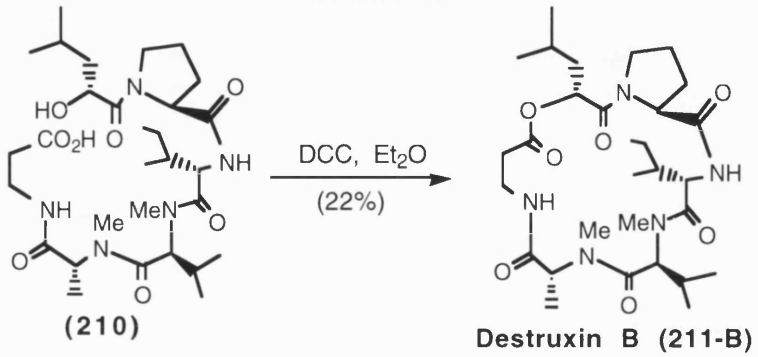


Destruxin (211)

$R_1 = CH_3$	$R_2 = CH_3$	$R_3 = CH_2{=}CHCH_2$-	**A**
$R_1 = CH_3$	$R_2 = CH_3$	$R_3 = (CH_3)_2CHCH_2$-	**B**
$R_1 = CH_3$	$R_2 = CH_3$	$R_3 = HOH_2C(CH)CH_3CH_2$-	**C**
$R_1 = CH_3$	$R_2 = CH_3$	$R_3 = HO_2C(CH)CH_3CH_2$-	**D**
$R_1 = H$	$R_2 = CH_3$	$R_3 = (CH_3)_2CHCH_2$-	Desmethyl destruxin
$R_1 = H$	$R_2 = H$	$R_3 = (CH_3)_2CHCH_2$-	Protodestruxin, **212**

To date, only two of the destruxins have been synthesised, namely, destruxin B **211-B** and protodestruxin **212**, which is a natural analogue of destruxin B containing no *N*-methyl groups. The synthesis of destruxin B **211-B** is noteworthy as it is one of the first examples of a macrolactonisation being employed for the preparation of a cyclodepsipeptide. Thus, hexapeptide **210** was cyclised with DCC to give **211-B** in 22% yield (Scheme 31). [74]

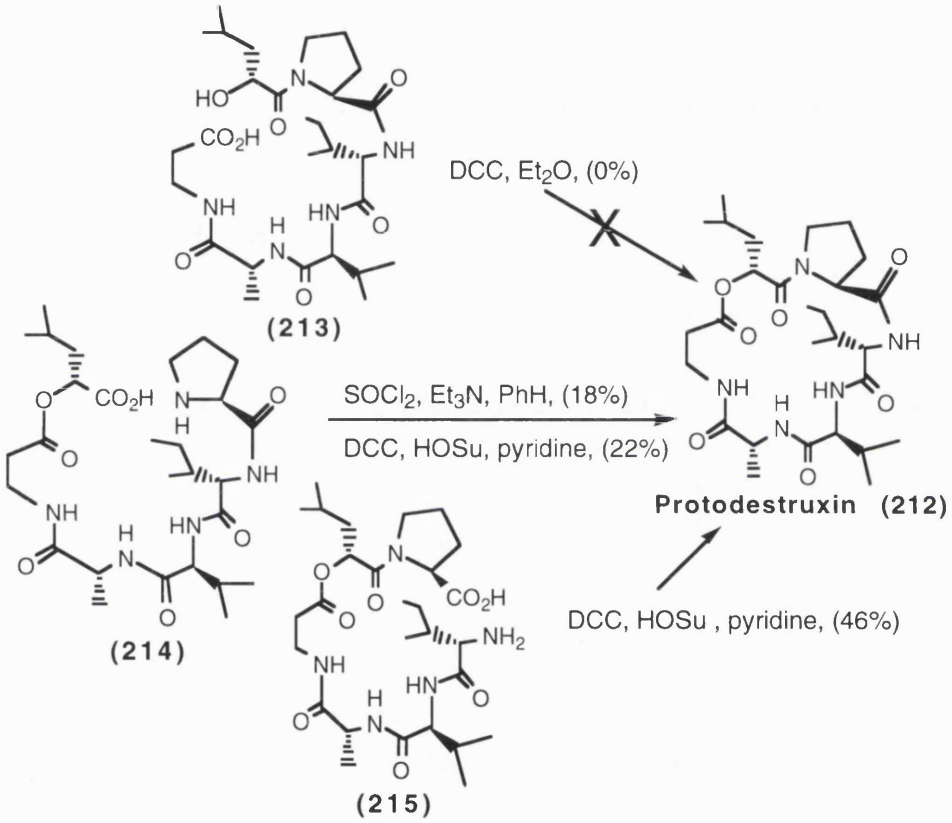
Scheme 31



(210) **Destruxin B (211-B)**

The synthesis of protodestruxin **212** was attempted through three different routes. Surprisingly, the cyclisation of *seco*-acid **213** with DCC was unsuccessful. However, cyclisation via the D-Hmp-Pro bond in **214** was successful using either the acid chloride method (18%) or the succinimide activated ester[75] (22%). Cyclisation through the formation of the Pro-Ile bond in **215** delivered **212** in an improved yield of 46% when the succinimide activated ester was used (Scheme 32).[76]

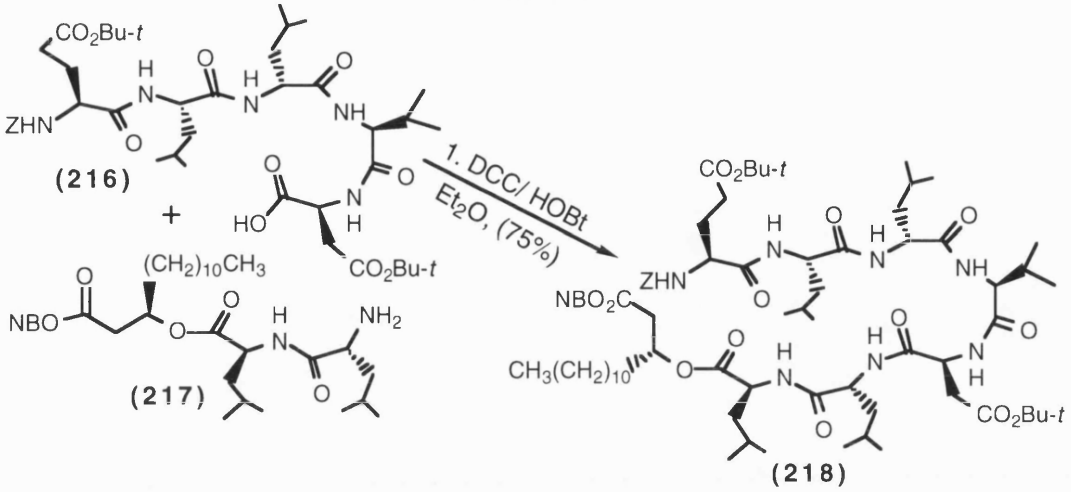
Scheme 32



Norsurfactin

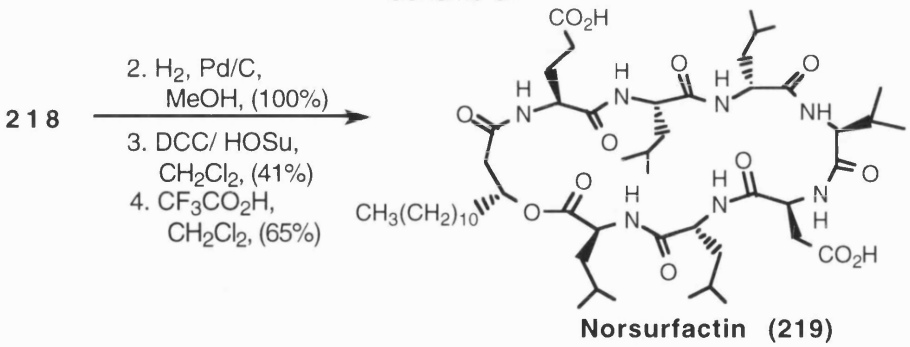
Morrison and coworkers have devised a synthesis of norsurfactin **219**, an analogue of surfactin, which lacks a 3-methyl group in the β-hydroxy acid side chain. It possesses haemolytic and anticoagulant properties. Their synthesis involved coupling pentapeptide **216** with depsi-peptide **217** using the *N,N*-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) protocol;[21] this gave **218** in 75% yield (Scheme 33).

Scheme 33



Hydrogenolysis of the terminal protecting groups in **218** and subsequent cyclisation in the presence of DCC and *N*-hydroxysuccinimide (HOSu)[77] at high dilution (1.8×10^{-3} M peptide) in dichloromethane/*N*,*N*-dimethylformamide (34:1v/v) for three days gave the cyclodepsipeptide in 41% yield. Subsequent treatment with trifluoroacetic acid gave norsurfactin **219** in 65% yield (Scheme 34).[78]

Scheme 34

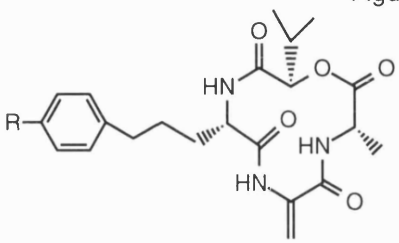


Norsurfactin (219)

AM-Toxins

AM-Toxins are host-specific phytotoxic metabolites produced by *Alternaria mali* which cause veinal necrosis on apple leaves.[79] Each of the three congeners was shown to be a cyclotetradepsipeptide (Figure 5), containing dehydroalanine, 2-hydroxy-3-methylbutanoic acid, L-alanine and either L-2-amino-5-(*p*-methoxyphenyl) pentanoic acid (AM-Toxin I, **220**),[80] L-2-amino-5-phenyl pentanoic acid (AM-Toxin II, **221**)[80] or L-2-amino-5-(*p*-hydroxyphenyl) pentanoic acid (AM-Toxin III, **222**).[81]

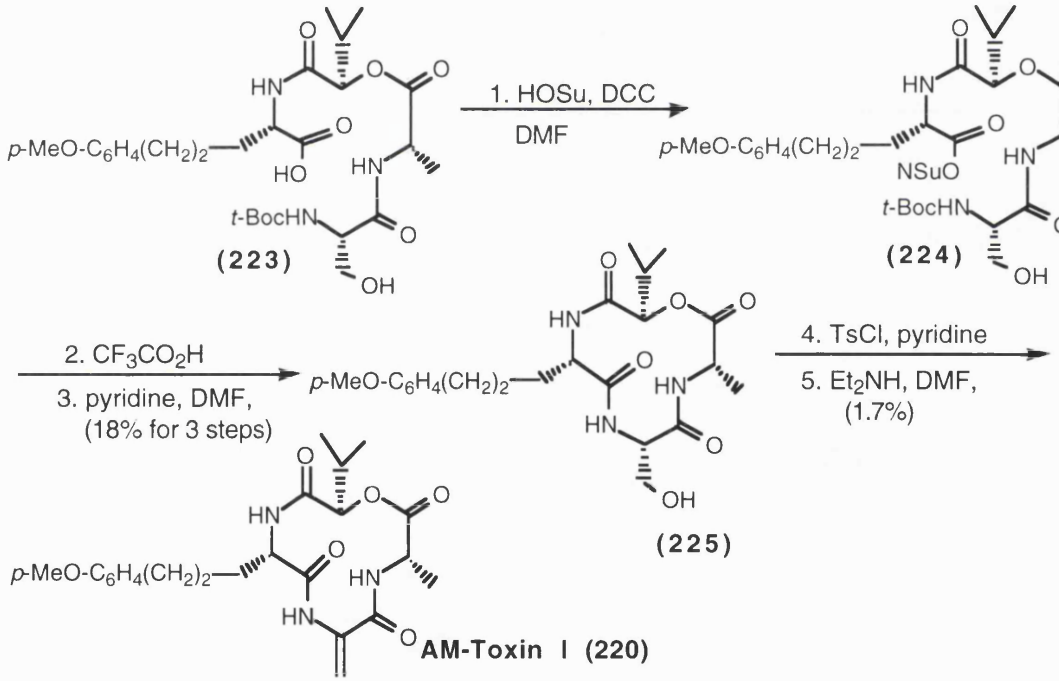
Figure 5



220 AM-Toxin I R = CH_3O
221 AM-Toxin II R = H
222 AM-Toxin III R = OH

The synthesis of AM-Toxin I **220** was based on the formation of tetradepsipeptide **223** by a linear approach, followed by transformation into the activated succinimide ester **224** using DCC and HOSu.[33] The Boc group in activated ester **224** was removed with TFA, and the residue cyclised by the addition of pyridine in DMF to give **225** in 18% yield after fractional recrystallisation from ethyl acetate. The serine residue was then converted into the dehydroalanine moiety by dehydration via the tosylate, with **220** being isolated in 1.7% yield (Scheme 35).[82,83]

Scheme 35

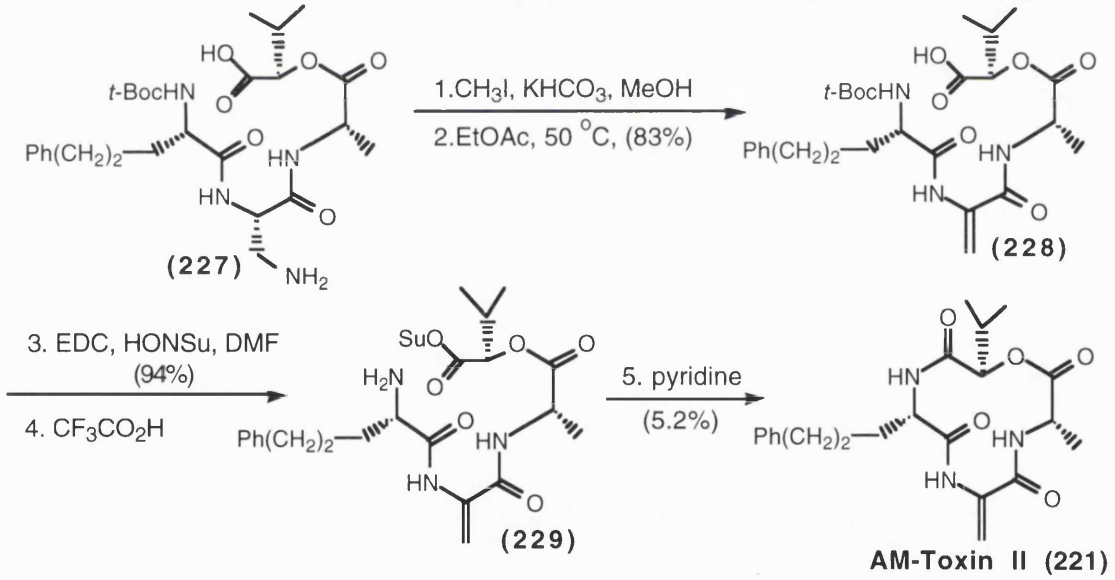


The cyclodepsipeptide of AM-Toxin II **221** was synthesised in a manner similar to that of AM-toxin I, substituting L-2-amino-5-phenylpentanoic acid for L-2-amino-5-(*p*-methoxyphenyl) pentanoic acid. However, dehydration of **226** was this time accomplished using methanesulfonyl chloride containing sulfur dioxide, followed by treatment with triethylamine (Scheme 36).[84]

Scheme 36

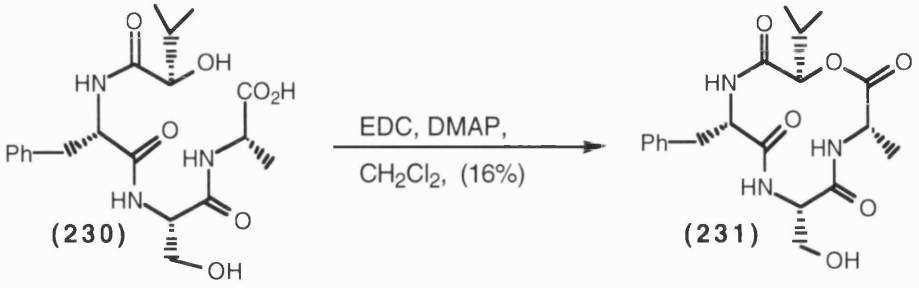
AM-Toxin II **221** was also synthesised by cyclisation of a linear tetradepsipeptide containing the dehydroalanine residue.[85] Dehydration of tetradepsipeptide **227** was achieved by the Hoffmann elimination procedure[86] to give **228** in 83% yield. Acid **228** was then transformed to the activated succinimide ester, and the Boc protecting group excised with trifluoroacetic acid to afford amine **229**. Cyclisation with pyridine at 0.003 M concentration resulted in AM-Toxin II **221** in a modest yield of 5.2% after preparative TLC chromatography (Scheme 37).

Scheme 37



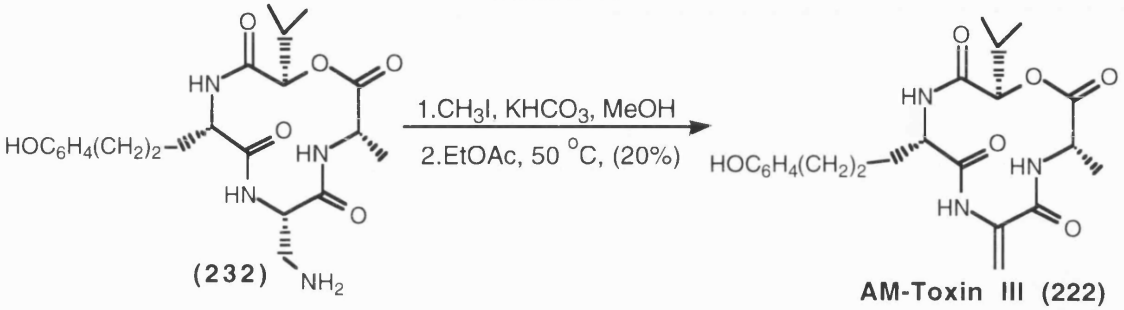
All the forementioned procedures for the synthesis of the AM-Toxins involved a macrolactamisation. An alternative synthesis of an AM-Toxin II analogue has been achieved using a macrolactonisation. Thus *seco*-acid **230** was cyclised in the presence of EDC and DMAP in CH_2Cl_2 at 0.01 M concentration to deliver **231** in 16% yield; unfortunately, however, a small amount of racemization occured (Scheme 38).[87]

Scheme 38



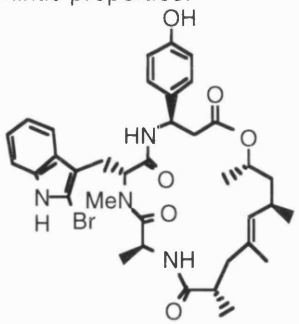
The key step in the synthesis of AM-Toxin III **222** was the formation of the dehydroalanine residue, by Hoffmann degradation[86] of cyclodepsipeptide **232** (Scheme 39).[88]

Scheme 39



(+)-Jasplakinolide

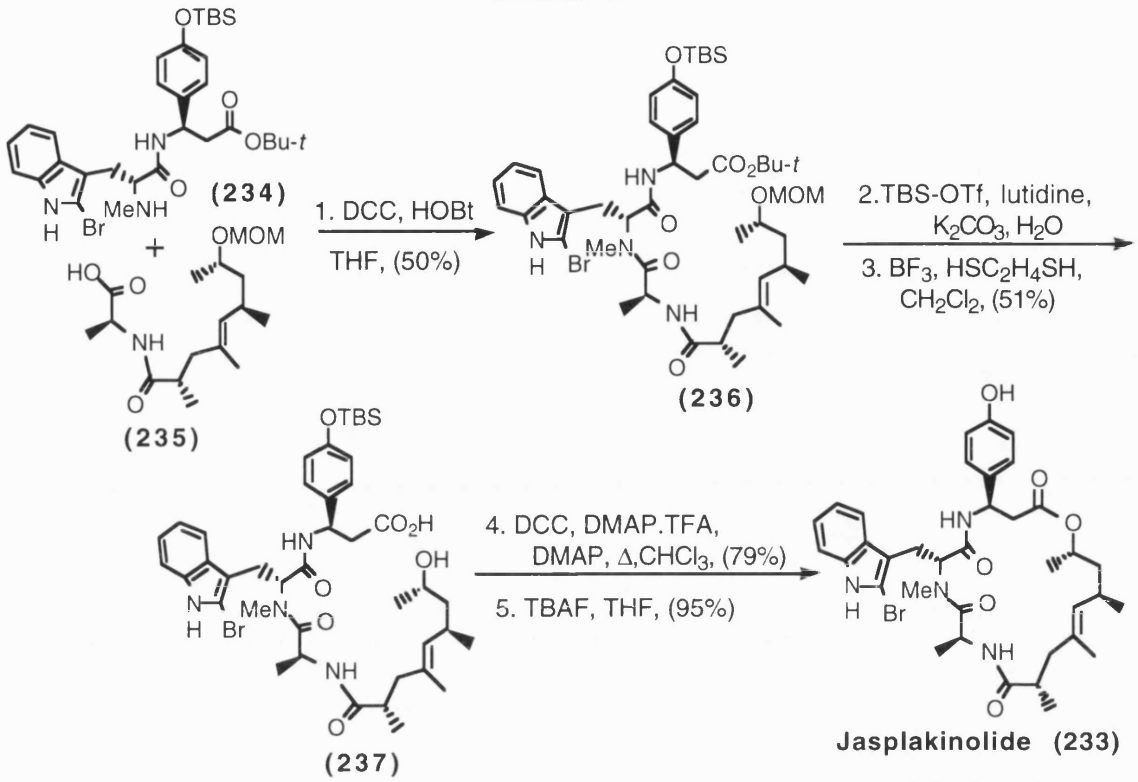
(+)-Jasplakinolide **233** was isolated from a soft-bodied sponge, *Jaspis sp* and possessed insecticidal, antifungal, and anthelmintic properties.[89,90]



Jasplakinolide (233)

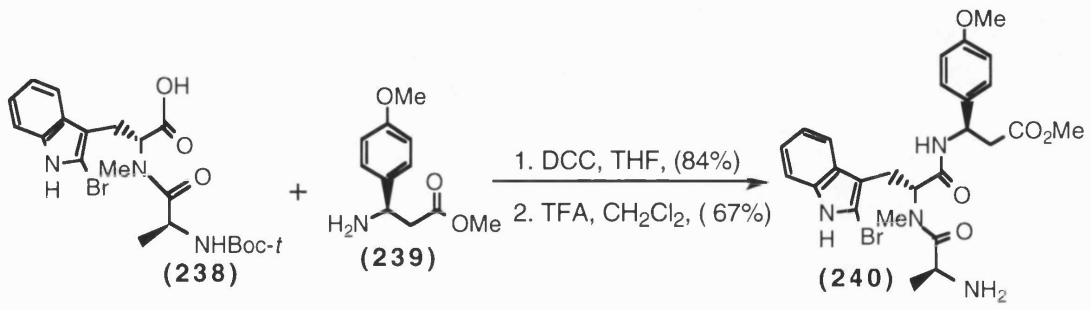
Its first synthesis was developed by Grieco and coworkers using a 2+2 fragment condensation approach, between dipeptide **234** and fragment **235** using the DCC/ HOBt protocol. Selective removal of the *t*-butyl ester group from **236** in the presence of the acid sensitive silyl ether was achieved using TBDMSOTf and 2,6-lutidine,[91] while the MOM group was excised with boron trifluoride and ethanedithiol; this resulted in the *seco*-acid **237**. Cyclisation of the latter with DCC, DMAP·TFA and DMAP [92] in refluxing chloroform resulted in the lactonised product in 79% yield, while *O*-desilylation with TBAF gave (+)- jasplakinolide **233** in 95% yield (Scheme 40).[93]

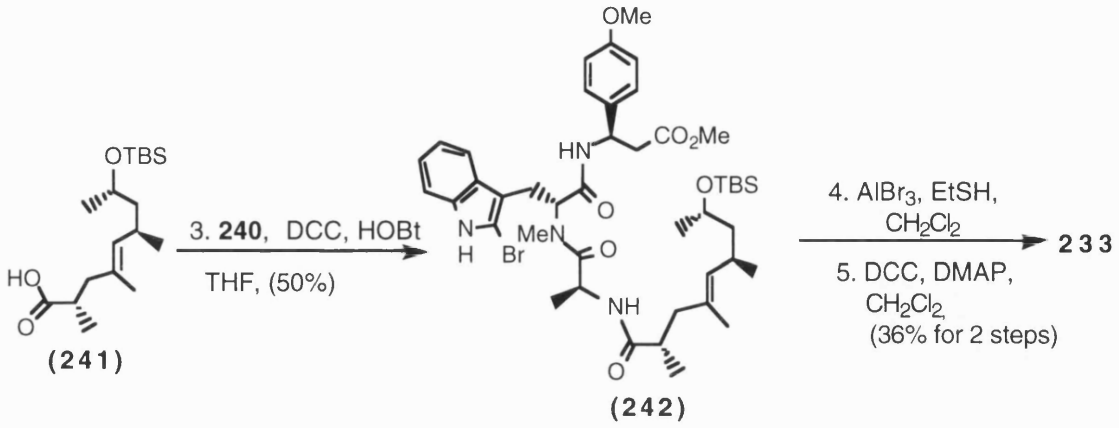
Scheme 40



The synthesis of (+)-jasplakinolide **233** by Konopelski and coworkers was based on a linear approach. Dipeptide **238** was coupled to **239** with DCC to give, after removal of the Boc group, the free amine **240** in 67% yield. Coupling of **240** with the lipophilic acid **241** using DCC afforded **242** in 50% yield. In order to complete the synthesis of **233**, **242** was globally deprotected with aluminium tribromide and ethanethiol and the product cyclised with DCC and DMAP. Compound **233** was obtained in an overall yield of 36% (Scheme 41).[94] A similar route was used by Rao and coworkers for their synthesis of (+)-jasplakinolide. However, macrolactonisation was achieved using the DCC, DMAP.TFA, DMAP combination in refluxing chloroform (22%).[92]

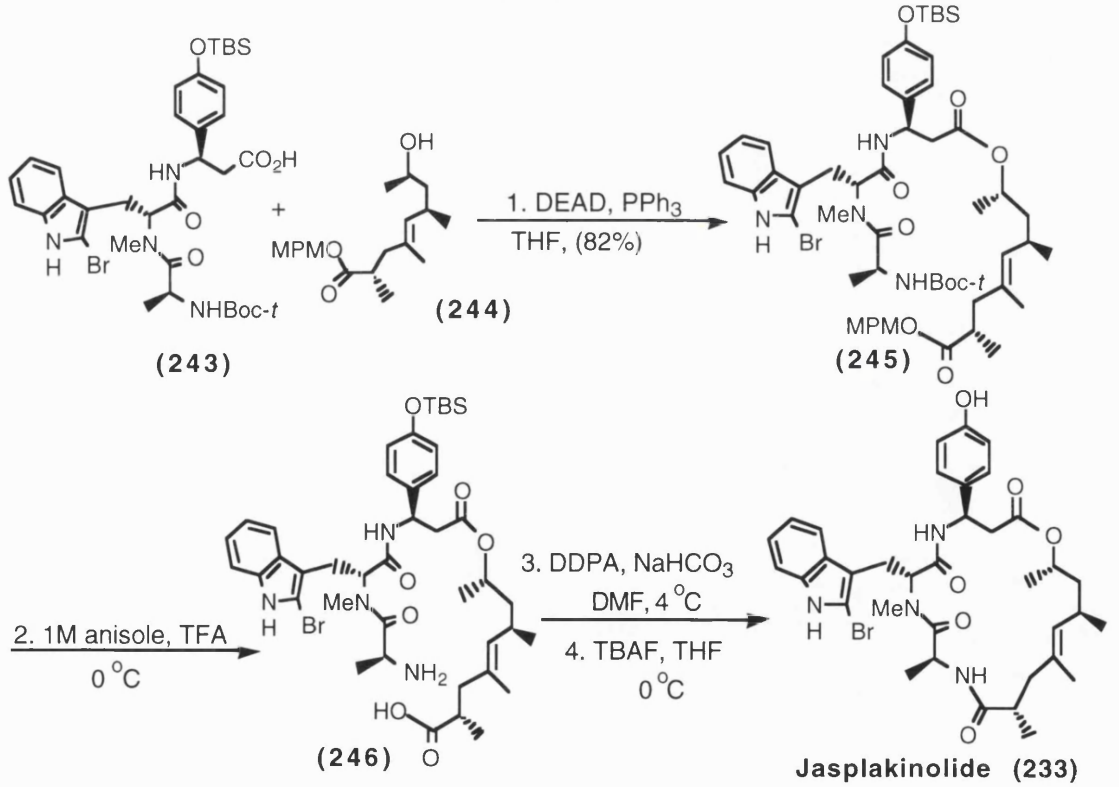
Scheme 41





(+)-Jasplakinolide **233** was synthesised by Imaeda via an amide macrolactamisation.[96] Thus, esterification of tripeptide **243** with alcohol **244** using the Mitsunobu reaction (DEAD, PPh_3)[97]produced **245** in 82% yield. Treatment of **245** with anisole (1 M) in trifluoroacetic acid cleaved the *N*-Boc group and the *p*-methoxyphenylmethyl (MPM) ester to afford **246** which was macrolactamised with diphenyl phosphorazidate (DPPA).[98] This gave (+)-jasplakinolide after desilylation with TBAF (Scheme 42).

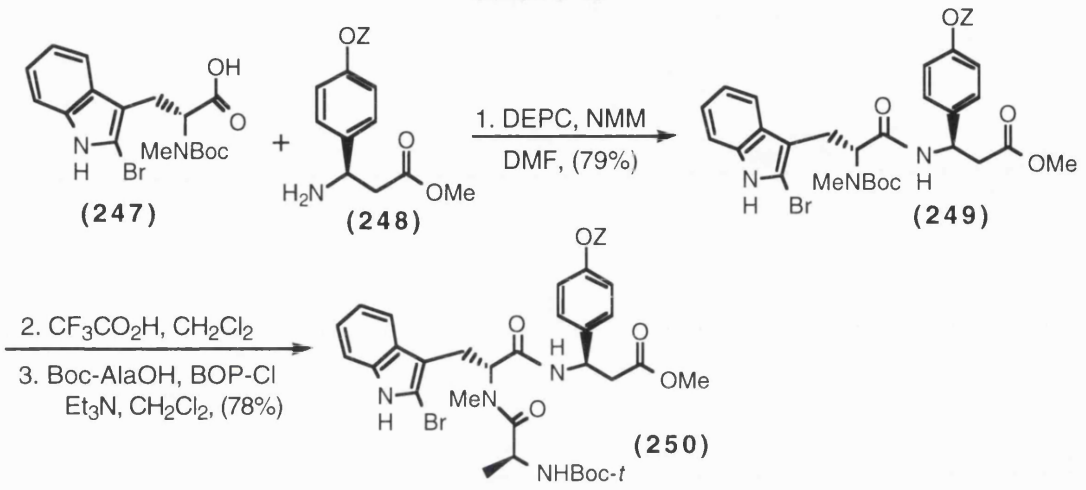
Scheme 42



An efficient synthesis of the peptide portion of (+)- jasplakinolide has been developed by Shioiri and coworkers.[99] The tyrosine derivative **248** was prepared in eight steps from *p*-*O*-

benzyloxycarbonyl-benzaldehyde and obtained in an optically pure form via a resolution of the menthyl ester by recrystallisation. Thus, acid **247** was coupled to amine **248** using diethylphosphorocyanidate (DEPC)[100] as the activating agent to give the dipeptide **249** in 79% yield. Removal of the Boc group with TFA and subsequent condensation with Boc-alanine in the presence of BOP-Cl[64] gave the required tripeptide **250** in 78% yield (Scheme 43).

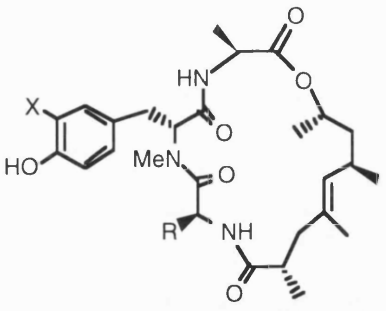
Scheme 43



Geodiamolides

Geodiamolides are isolated from the Carribean sponge *Geodia sp.*[101] and are similiar to (+)- jasplakinolide in structure and biological activity (Figure 6).

Figure 6



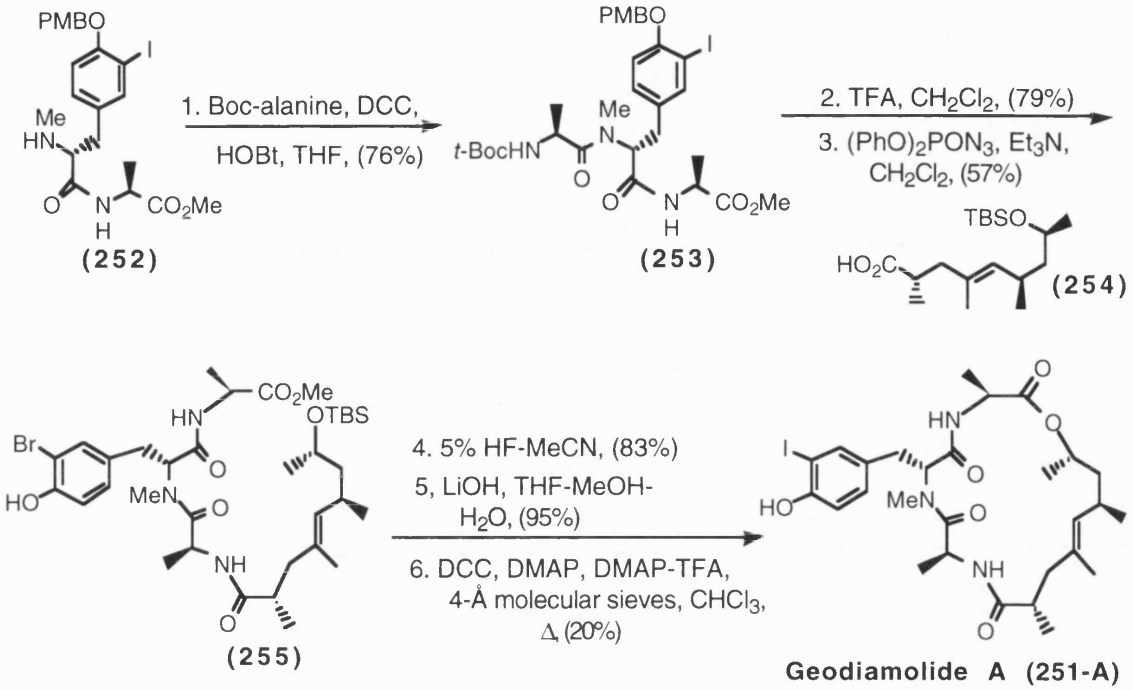
Geodiamolide (251)

A X = I, R = Me
B X = Br, R = Me
C X = Cl, R = Me
D X = I, R = H
E X = Br, R = H
F X = Cl, R = H

The synthesis of geodiamolide A **251-A** achieved by White and coworkers followed a linear strategy.[102] Dipeptide **252** was treated with Boc-L-alanine in the presence of DCC and HOBt to give the tripeptide **253** in 76% yield. Simultaneous removal of the Boc and *p*-methoxybenzyl ether groups with trifluoroacetic acid and subsequent coupling with the liphophilic acyl azide derived from **254** gave **255** in 57% yield. Removal of the silyl ether with hydrofluoric acid in

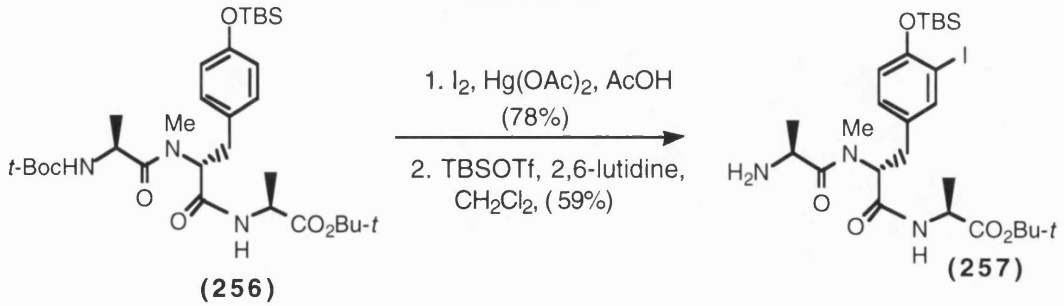
acetonitrile and saponification of the methyl ester with aqueous lithium hydroxide gave the free *seco*-acid which was finally lactonised using the DCC/ DMAP, DMAP-TFA procedure[92] to give **251-A** in 20% yield (Scheme 44).

Scheme 44



The Hirai synthesis[103] of geodiamolide A (**251-A**) was based on the halogenation of the tripeptide **256** with iodine and mercury diacetate in acetic acid and the selective removal of the Boc group in the presence of the silyl ether. The latter step was accomplished using TBSOTf and 2,6-lutidine[91] and gave amine **257** in 59% yield (Scheme 45).

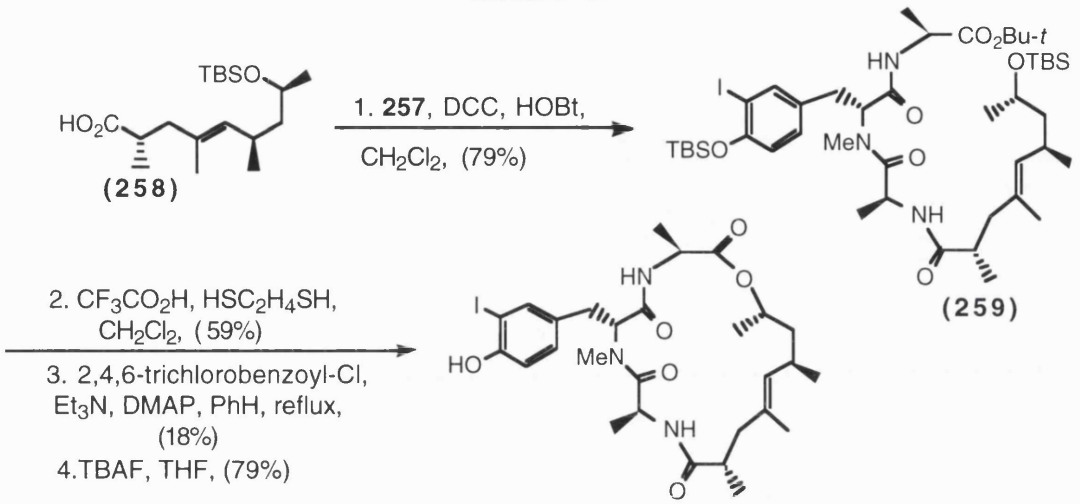
Scheme 45



Coupling of amine **257** with the aliphatic acid **258** using the DCC/HOBt protocol produced **259** in79% yield. This was then deprotected with trifluoroacetic acid and ethanedithiol to give the free tetrapeptide, which was lactonised in 18% yield by the formation of a mixed 2,4,6-trichlorobenzoyl

anhydride in refluxing benzene. Desilylation with TBAF led to **251-A** in 79% yield (Scheme 46). Geodiamolide B **251-B** was synthesised by a similar procedure.[103]

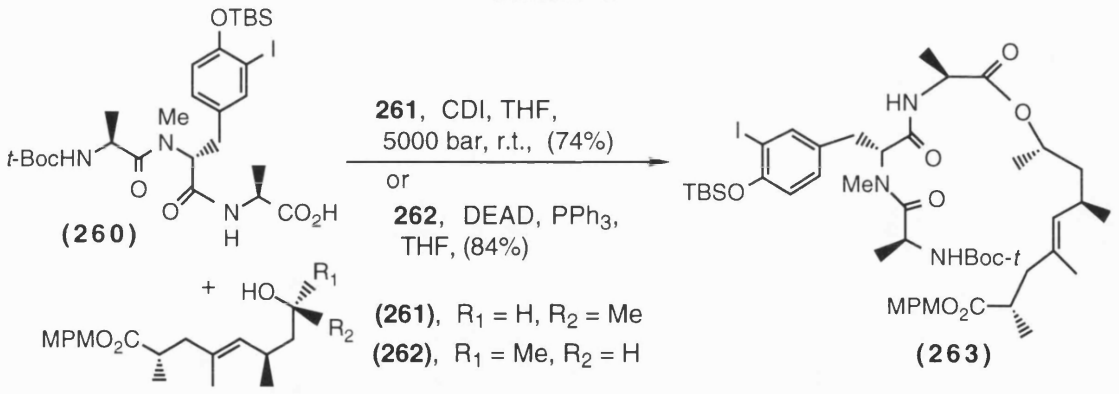
Scheme 46



Geodiamolide A (251-A)

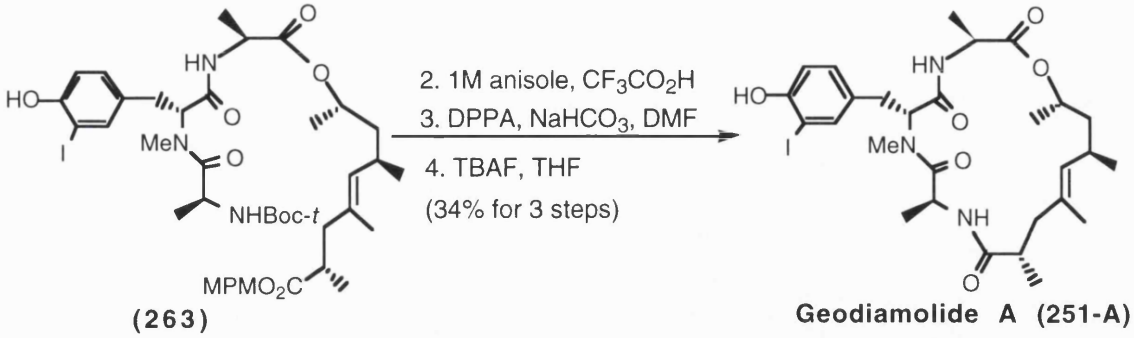
In 1994, Imaeda and coworkers published an efficient synthesis of geodiamolide A (**251-A**), in which the macrolactone ring was assembled by macrolactamisation at the amine residue of the (*S*)-alanine moiety.[96]The ester linkage of **263** was constructed using two novel approaches. Either the carboxyl group of **260** was activated as its acyl imidazolide, and this condensed with alcohol **261** under high pressure (74%), or alternatively a Mitsunobu reaction[96] between **260** and alcohol **262** (84%) could be employed for obtaining **263** (Scheme 47).

Scheme 47



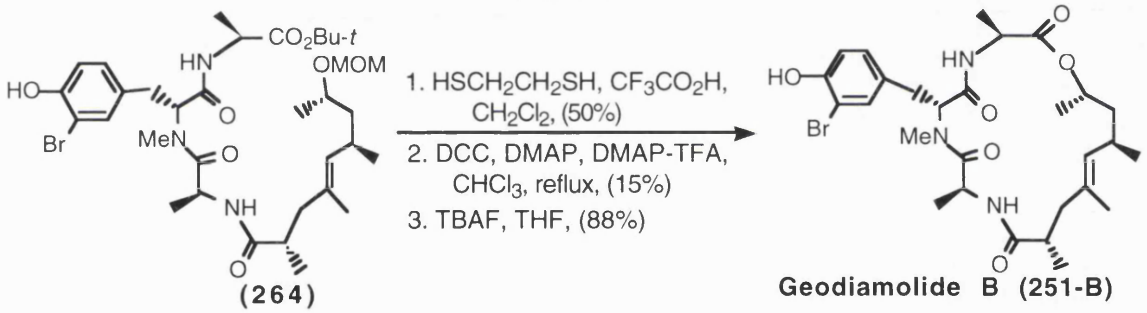
To complete the synthesis of **251-A**, **263** was treated with anisole in trifluoroacetic acid, and macrolactamisation preformed with diphenylphosphoryl azide (DPPA)[98] to give geodiamolide A **251-A** in 34% yield after desilylation with TBAF (Scheme 48).

Scheme 48



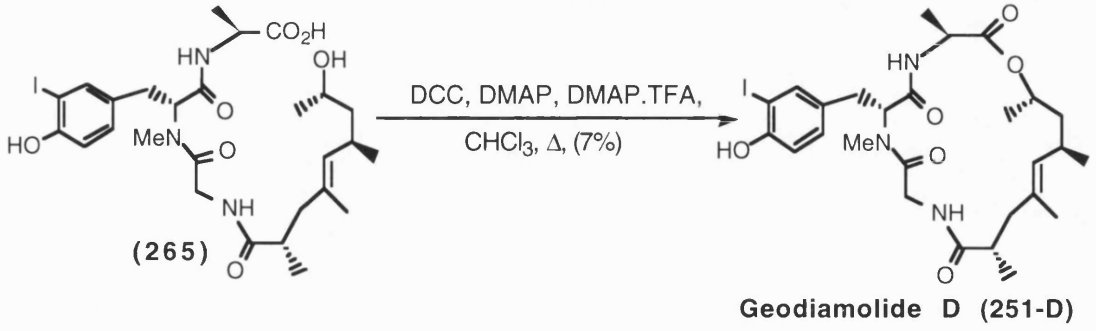
Grieco and coworkers synthesised of geodiamolide B **251-B** was based on the formation of linear tetrapeptide **264**. Simultaneous removal of the methoxymethyl ether (MOM) and the *tert*-butyl ester group in **264** using excess ethanedithiol and excess trifluoroacetic acid in dichloromethane resulted in the *seco*-acid, which was lactonised using the Keck procedure (DCC, DMAP and DMAP-TFA) in refluxing chloroform[92] in a yield of 15%. Desilylation with TBAF gave geodiamolide B **251-B** in 88% yield (Scheme 49).[104]

Scheme 49



Rao's group have achieved a total synthesis of geodiamolide D **251-D**, in which the key cyclisation step was also achieved by macrolactonisation using the Keck procedure in a low 7% yield (Scheme 50).[95]

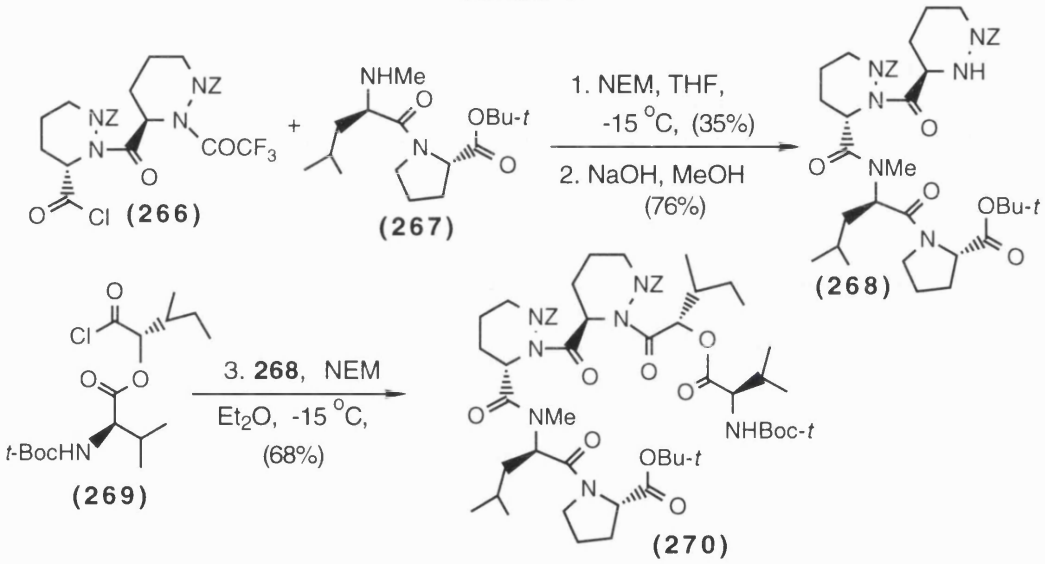
Scheme 50



Monamycin- X

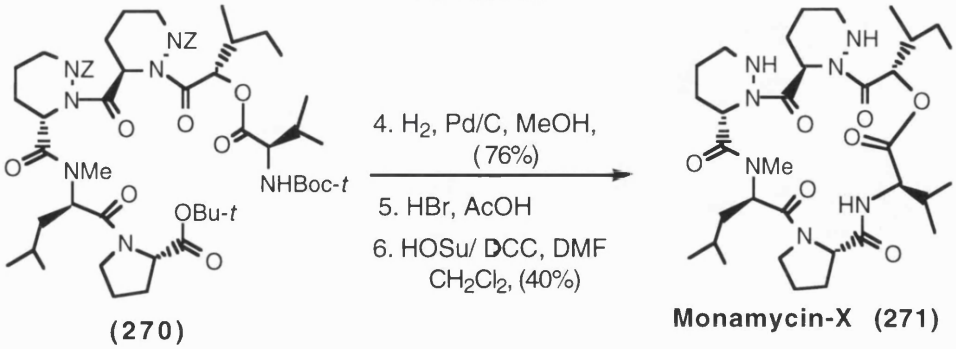
The monamycins are a family of cyclodepsipeptides having antibacterial properties. An analogue of monamycin-B_3 containing (*S*)-piperazic acid in place of (*3S,5S*)-5-hydroxypiperazic acid has been synthesised by Hassall and coworkers utilising a 2+2+2 fragment condensation strategy.[105] Initially, dipeptides **266** and **267** were unified employing *N*-ethyl-morpholine (NEM) as base. The trifluoroacetyl group protecting the (*R*)-piperazic residue in this tetrapeptide was then hydrolysed with sodium hydroxide in methanol to produce **268**. Acid chloride **269** was then coupled to **268** to produce linear protected hexadepsipeptide **270** in 68% yield (Scheme 51).

Scheme 51



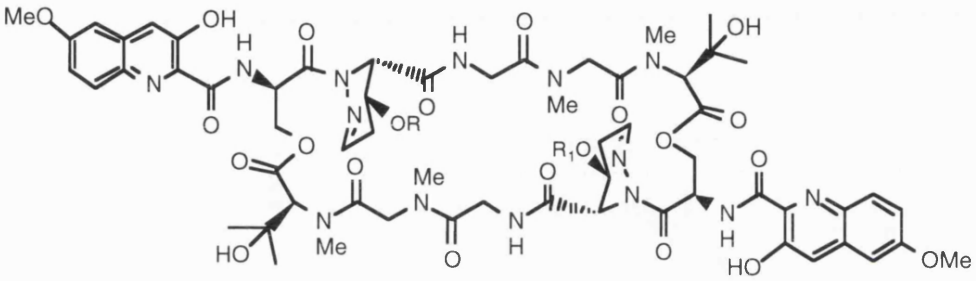
Compound **270** was hydrogenated and treated with hydrogen bromide in acetic acid. The linear hexadepsipeptide was cyclised by the DCC/HOSu method to give monamycin-X (**271**) in 40% yield (Scheme 52).

Scheme 52



Luzopeptin

The luzopeptin antibiotics (**272, A-C**) were isolated from *Actinomadura luzonesis*.[106] Their structures were determined by a combination of chemical degradation studies by Konishi and coworkers[107] and single X-ray diffraction by Clardy and coworkers.[108] They are dimeric cyclic decadepsipeptides, containing the unusual amino acid, 2-(*S*)-carboxy-3-(*S*)-hydroxy-2,3,4,5-tetrahydropyridazine and a substituted quinoline-2-carbonyl moiety attached to the depsipeptide ring. Luzopeptin A is a bis-intercalating agent towards DNA[109,110] and displays antitumour properties against several tumors.[111,112]

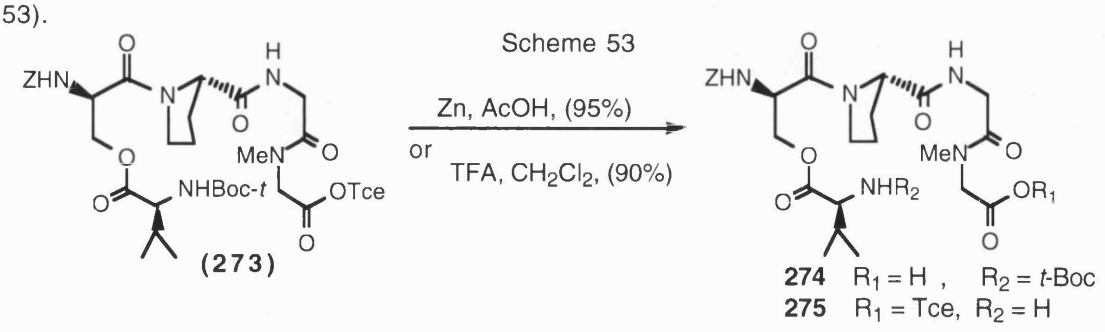


Luzopeptin, (272)

A, $R = R_1 = Ac$
B, $R = Ac$, $R_1 = H$
C, $R = R_1 = H$

Olsen's group have devised a synthesis of a luzopeptin A analogue **279** that contains an L-proline unit *in lieu* of the tetrapyridazine moiety. They also installed an L-valine in place of the *N*-methyl-β-hydroxyl-L-valine unit, and replaced the quinoline unit with an unsubstituted quinoline-2-carbonyl moiety.[113] Analogue **279** was synthesised from **273** by two separate routes. Pentadepsipeptide **273** was itself prepared in a stepwise fashion from *p*-chlorophenacyl *N*-(benzyloxycarbonyl)-D-serinate utilising the DCC/ HOBt coupling protocol. In the first route, **273** was treated with zinc in acetic acid to remove the 2,2,2-trichloroethyl ester (Tce) and reveal acid **274**. A sample of **273** was also treated with trifluoroacetic acid to obtain amine **275** (Scheme 53).

Scheme 53

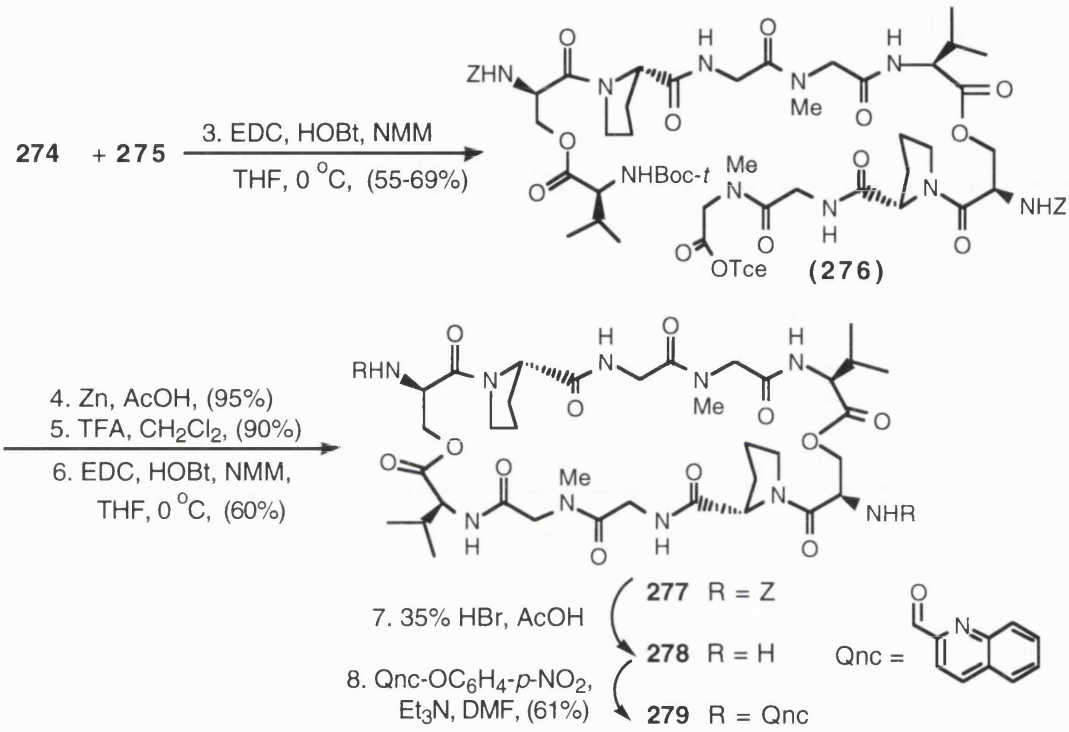


(273)

274 $R_1 = H$, $R_2 = t$-Boc
275 $R_1 = Tce$, $R_2 = H$

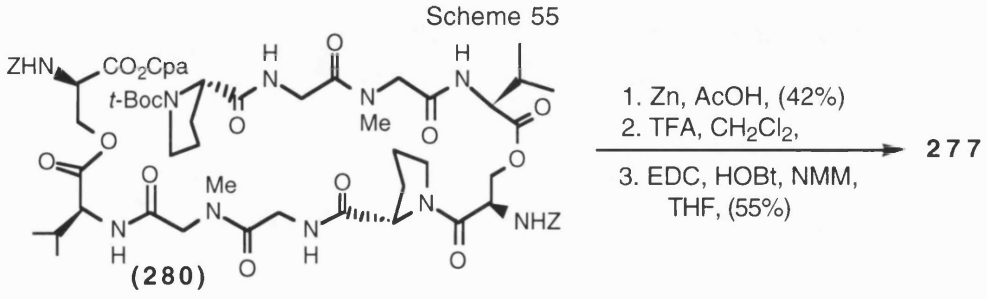
Subsequent coupling of **274** and **275** using EDC/HOBt afforded the linear decadepsipeptide **276** in 80% yield. Removal of the Tce and Boc protecting groups and subsequent cyclisation via the sarcosine-valine amide bond mediated by EDC and HOBt at high dilution ($6.7x10^{-4}$ M in THF) gave **277** in 66% yield. The synthesis of **279** was completed by the removal of the benzyloxy-carbonyl group and acylation with *p*-nitrophenyl quinoline-2-carboxylate (Scheme 54).[113]

Scheme 54



The second route (Scheme 55) to **279** was based on a macrolactamisation at the serine-proline bond. The removal of the *p*-chlorophenacyl ester of linear decadepsipeptide **280** with zinc in acetic acid[34,35] proved problematic, giving only a modest yield (42%) of the product along with recovered starting material. Removal of the *N*-Boc group from the prolyl amino function and cyclisation with EDC/HOBt gave the cyclic peptide **277** in 55% yield, which was subsequently transformed into **279** as shown in Scheme 54.

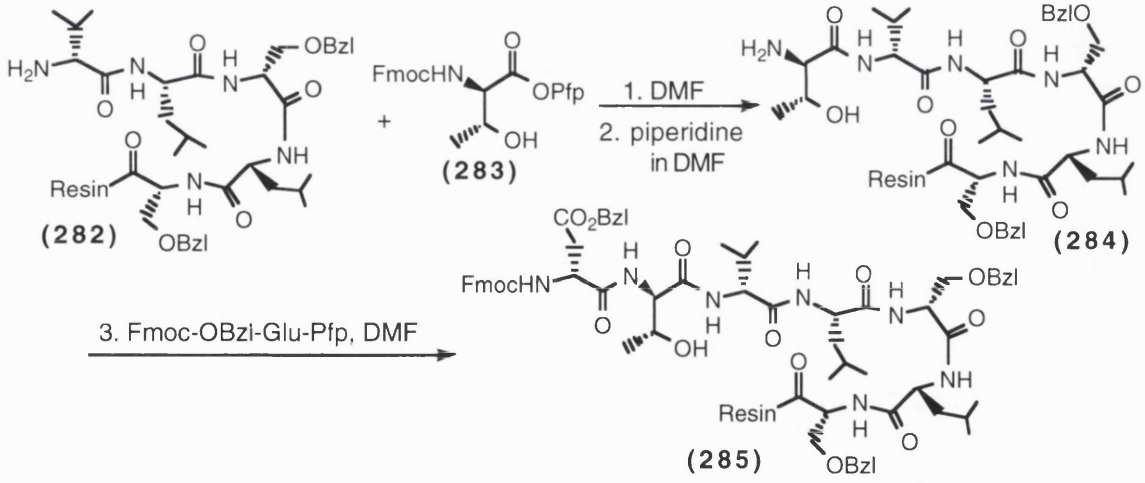
Scheme 55



Viscosin

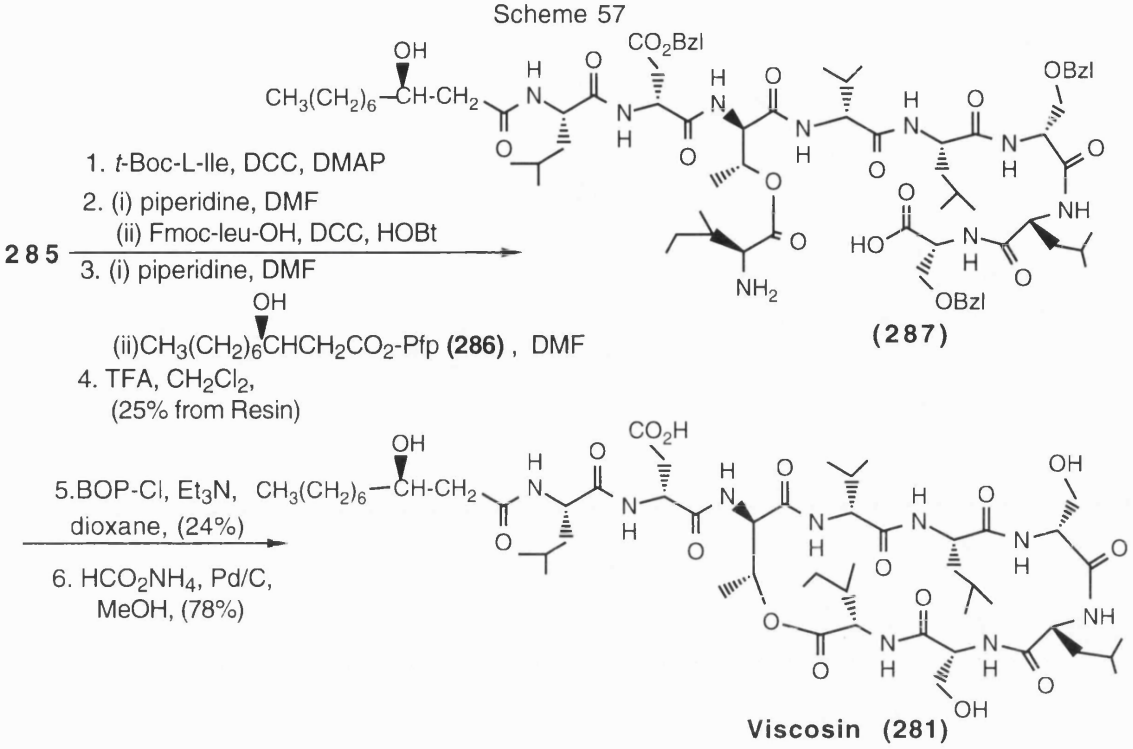
Viscosin **281** was isolated from a culture of *Pseudomonas viscosa* and was found to have antiviral and antimicrobial activity against various mycobacteria.[114] Its structure was confirmed through total synthesis by Burke and coworkers.[115] Their solid-phase synthesis relied on fluorenylmethoxycarbonyl (Fmoc) and Boc amine protecting groups and adopted the acid-sensitive alkoxy-benzyl alcohol resin of Wang. After coupling the Fmoc-OBzl-Ser to the resin with DCC/DMAP in DMF, the synthesis of the requisite amino acid sequence was achieved by the following strategy. The Fmoc protecting group was detached with piperidine and the resulting amine coupled with another appropriate Fmoc-amino acid activated by DCC/HOBt[21] in DMF. This approach was repeated to give **282**. Subsequent coupling of **282** with the pentafluorophenyl activated ester (Pfp) **283** furnished alcohol **284** after removal of the Fmoc group. Use of the pentafluorophenyl activated ester dispensed with the need for protecting of the OH group in the threonine residue of **284** since these esters only react with amines (Scheme 56).

Scheme 56



Esterification of **285** with Boc-Ile-OH in the presence of DCC and HOBt, and subsequent deblocking and coupling to the D-3-hydroxydecanoyl activated ester **286** gave the fully assembled depsipeptide resin. The peptide was cleaved from the resin with trifluoroacetic acid; this also removed the Boc group to give the cyclised precursor **287** in an overall yield of 25% based on the resin. Cyclisation was then achieved using BOP-Cl[63,64] and gave the desired cyclic product in 24% yield. Hydrogenolysis with ammonium formate and Pd/C in methanol gave viscosin **281** in 78% yield (Scheme 57).

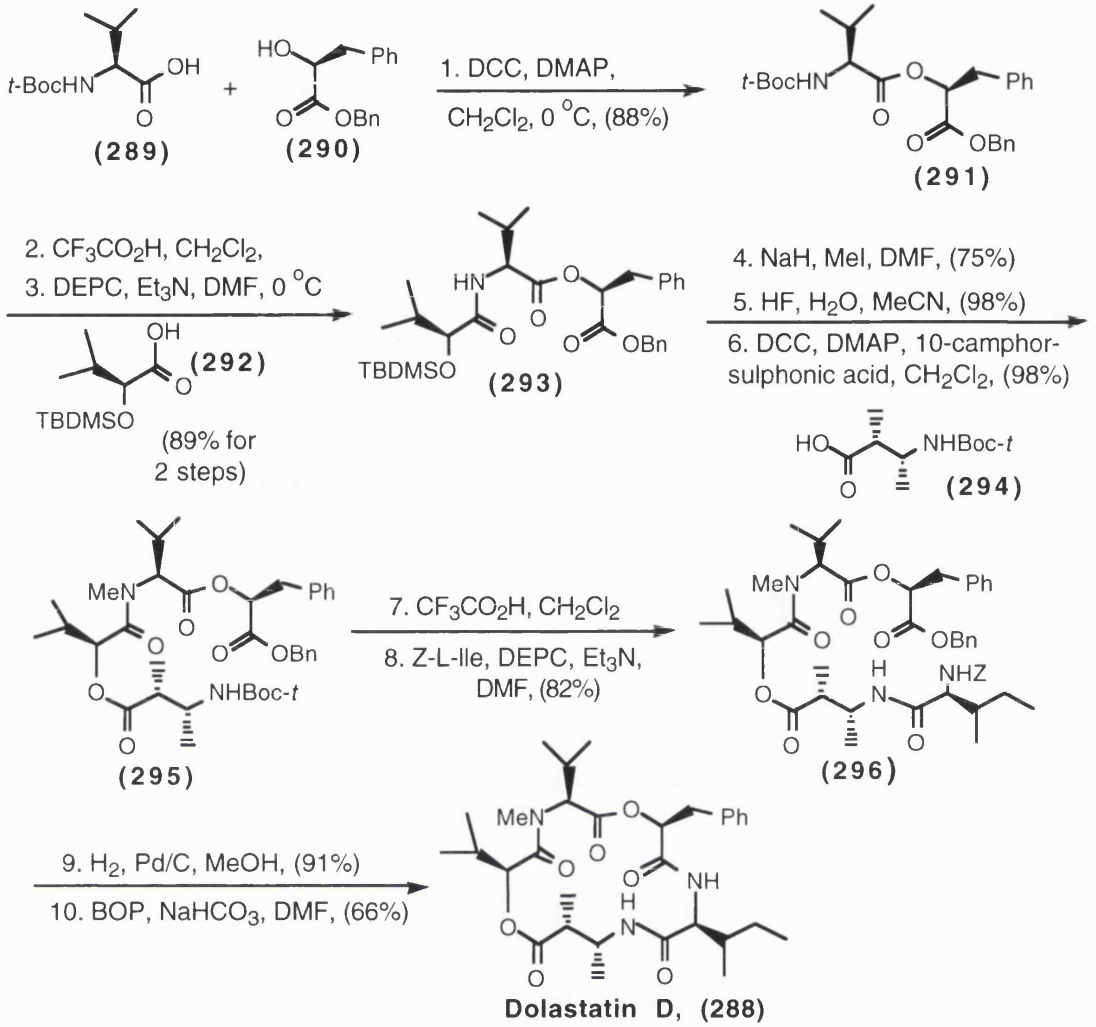
Scheme 57



Dolastatin D

Dolastatin D **288** is a cyclodepsipeptide isolated from the Japanese sea hare *Dolabella auricularia*; it was found to have antineoplastic and cytostatic properties.[116] A linear synthesis of **288** has been developed by Japanese workers.[116] This involved esterifying **289** with **290** using DCC to obtain depsipeptide **291** in 88% yield. Deprotection of the *N*-Boc protecting group of **291** with trifluoroacetic acid revealed the amine which was condensed with acid **292** using diethylphosphorocyanidate (DEPC).[100] Tridepsipeptide **293** was obtained in 89% yield. The latter underwent *N*-methylation in 75% yield, with sodium hydride and iodomethane, *O*-desilylation, and esterification with **294** to give tetradepsipeptide **295** in 98% yield. After removal of the Boc group from **295** and coupling with Z-Ile using DEPC, pentadepsipeptide **296** was obtained in 82% yield. Deprotection of the amino and carboxy termini was achieved by hydrogenation, and cyclisation to **288** subsequently investigated with various activating agents. These included bis(2-oxo-3-oxazolidinyl) phosphinic chloride [BOP-Cl] (13%), *N*-hydroxysuccinimide (41%), diphenylphosphoryl azide (47%). However, benzotriazol-1-yloxy-tris (dimethylamino)phosphonium hexafluorophosphate (BOP) gave the best result, it proceeding in 66% yield (Scheme 58).

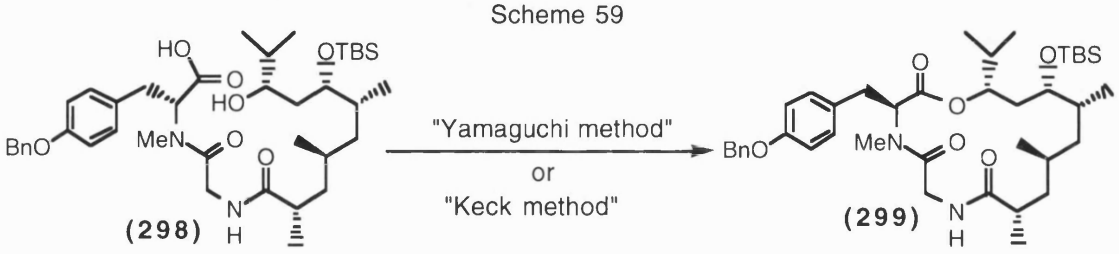
Scheme 58



Doliculide

Doliculide **297** was isolated from the Japanese sea hare *D. auricularia* and observed to have potent cytotoxic activity against HeLa-S_3 cells with an IC_{50} of 0.001μg/ml.[117] Its synthesis was completed by Yamada and coworkers in 1994. Their initial plan was to instigate macrolactonisation of **298** with the Yamaguchi (2,4,6-trichlorobenzoyl chloride, DMAP)[118] or Keck (DCC, DMAP)[92] reagents. However, both methods caused complete epimerisation in the tyrosine moiety and instead led to **299** being isolated (Scheme 59).

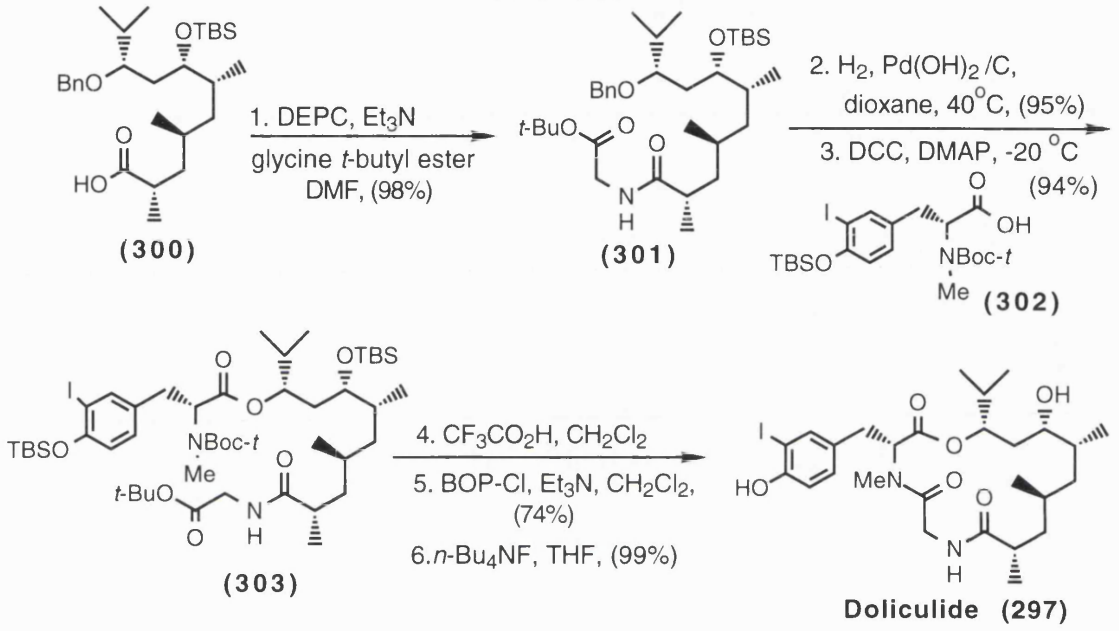
Scheme 59



These workers therefore investigated an alternative route to **297** which featured a

macrolactamisation step. Aliphatic acid **300** was coupled to glycine *t*-butyl ester with DEPC to give peptide **301**. After hydrogenation of the benzyl ether, esterification with the tyrosine derivative **302** was accomplished with DCC/DMAP to deliver linear tridepsipeptide **303** in 94% yield. Simultaneous removal of the *N*-Boc and *t*-butyl ester groups with TFA gave the free tridepsipeptide which was lactamised in 74% using BOP-Cl. The final step involved *O*-desilylation with TBAF to provide doliculide **297** in 99% yield (Scheme 60).[119]

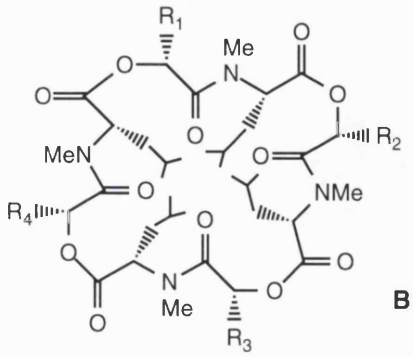
Scheme 60



PF1022A

PF1022, (**A-E**) was discovered in the mycelial cake of *Mycelia sterilia* and has a structure similiar to that of bassianolide (Figure 7). PF1022A **304-A** displays anthelmintic properties, it completely eradicating *Ascaridia galli* in chickens at a dosage of 2 mg/Kg.[120]

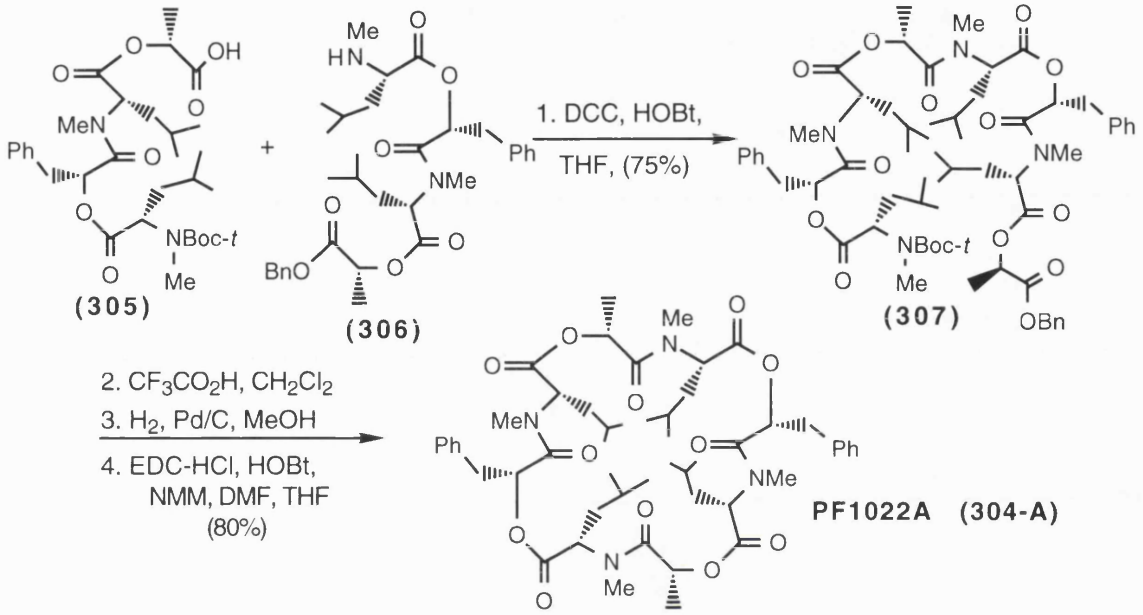
Figure 7



	R_1	R_2	R_3	R_4
A	CH_3	CH_2Ph	CH_3	CH_2Ph
B	CH_2Ph	CH_2Ph	CH_2Ph	CH_2Ph
C	CH_2Ph	CH_3	CH_3	CH_2Ph
D	CH_3	CH_3	CH_3	CH_2Ph
E	CH_3	$CH_2C_6H_4OH$	CH_3	CH_2Ph
Bassianolide	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)_2$

The synthesis of PF1022A **304-A** (Scheme 61) was achieved by coupling tetradepsipeptides **305** and **306** with DCC/HOBt to give the linear octadepsipeptide **307** in 75% yield. Standard deprotections led to an intermediate which cyclised to PF1022A **304-A** in 80% yield when reacted with EDC-HCl and HOBt.[121]

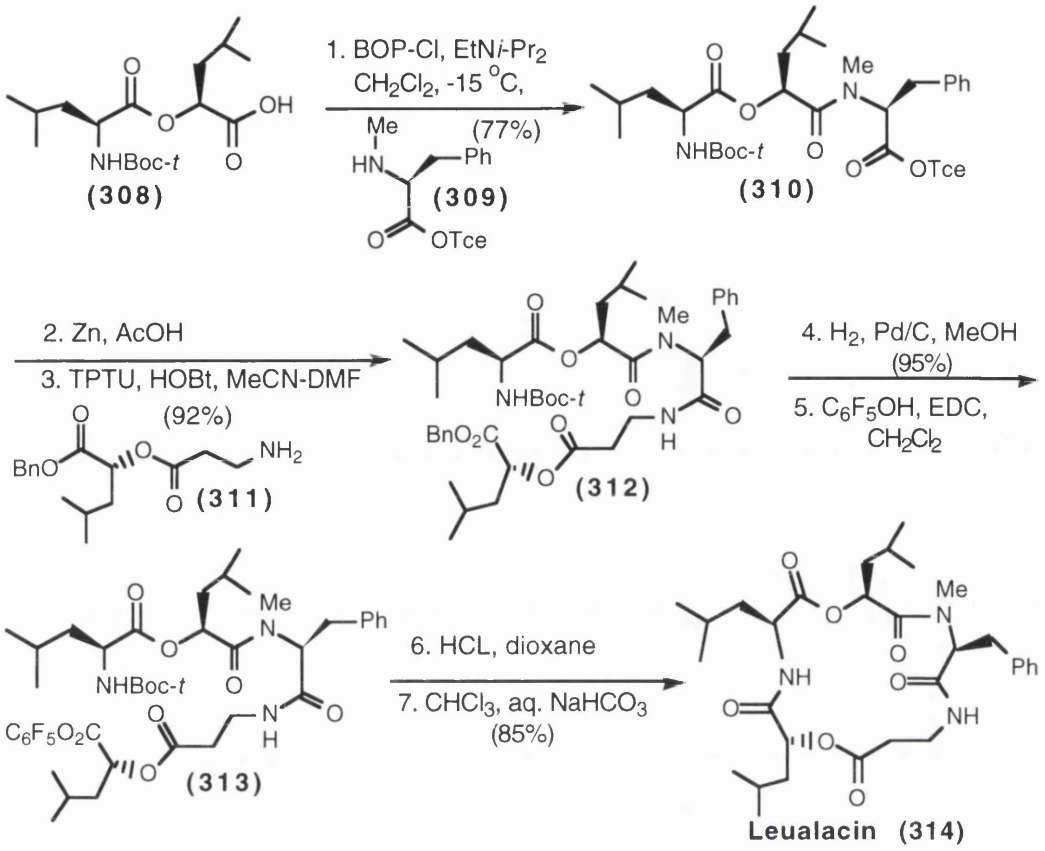
Scheme 61



Leualacin

Leualacin **314** was isolated from *Hapsidospora irregularis* and was found to inhibit the specific binding of nitrendipine to porcine heart microsomes, thus blocking calcium uptake.[122] The synthesis of **314** was initiated by the coupling of depsipeptide **308** and amine **309** using BOP-Cl to furnish the tridepsipeptide **310** in 77% yield. The 2,2,2-trichloroethyl ester was excised with zinc in acetic acid and the resulting acid condensed with **311** using *O*-(1,2-dihydro-2-oxo-1-pyridyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TPTU) and HOBt to provide linear depsipeptide **312** in 92% yield (Scheme 62). The *N*-Boc and benzyl ester protecting groups were removed and macrolactamisation attempted with various activating reagents that included *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluroborate[123] (50%), and pentafluorophenyl diphenylphosphinate (60%). However, cyclisation via the pentafluorophenyl ester **313** in the two-phase system of chloroform and aqueous sodium bicarbonate gave **314** in an optimium yield of 85% (Scheme 62).[124]

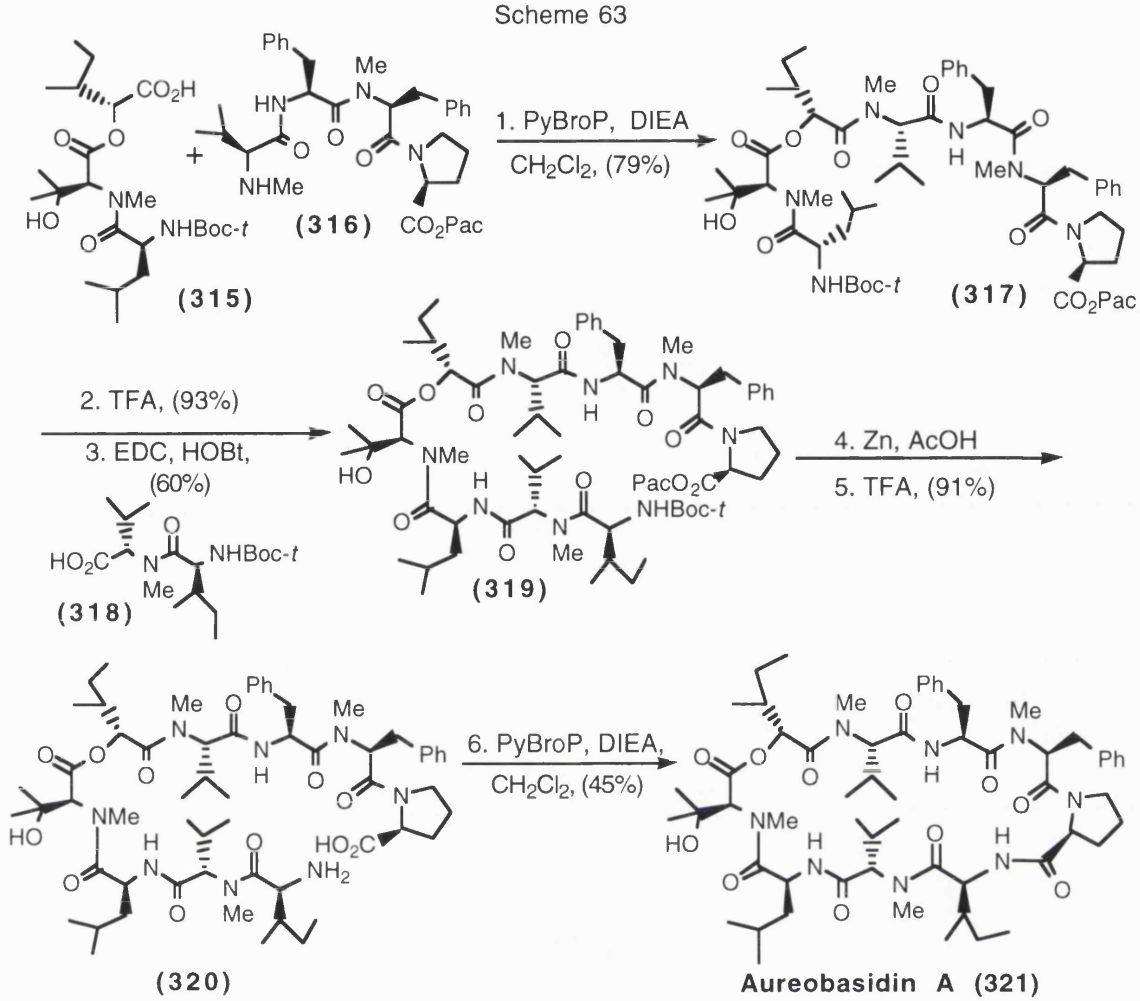
Scheme 62



Aureobasidin A

Aureobasidin A **321** is the major component of the culture medium of *Aureobasidium pullulans* R106. It exhibits potent antifungal activity against pathogenic fungi such as *Canidida albicans* and *Cryptococcus neoformans*.[125] A synthesis has been achieved that is based on a 4+3+2 fragment condensation strategy (Scheme 63). Thus tridepsipeptide **315** was coupled to tetradepsipeptide **316** using bromotris(pyrrolidino)phosphonium hexafluorophosphate (Py-BroP)[126] as the activating agent giving heptadepsipeptide **317** in 79% yield. Acidolysis of **317** with trifluoroacetic acid and subsequent coupling with dipeptide **318**, followed by deprotection of the phenylacyl ester (Pac) with zinc in acetic acid, and the *t*-Boc group with trifluoroacetic acid gave the free linear nanodepsipeptide **320**. Cyclisation with PyBroP in dichloromethane at high dilution (10^{-3} M) led to **321** in 45% yield (Scheme 63).[127]

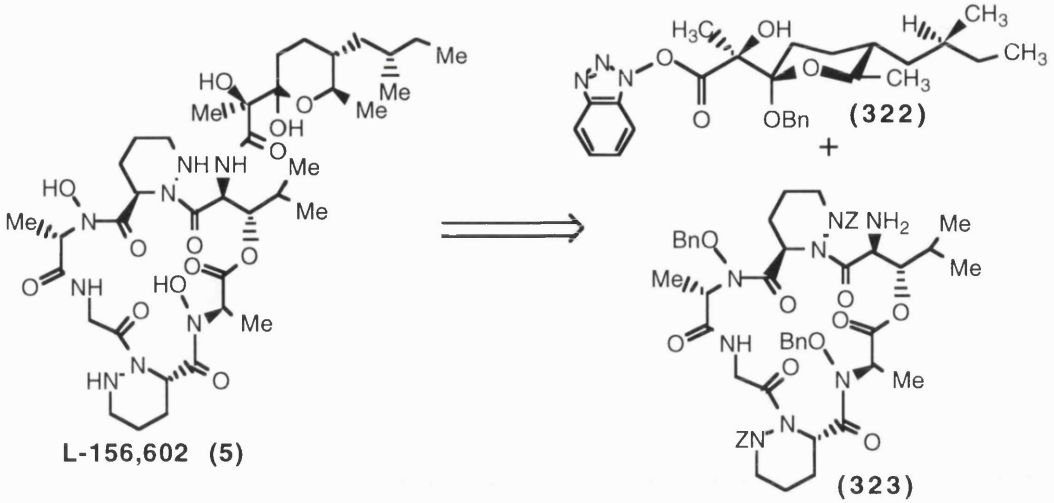
Scheme 63



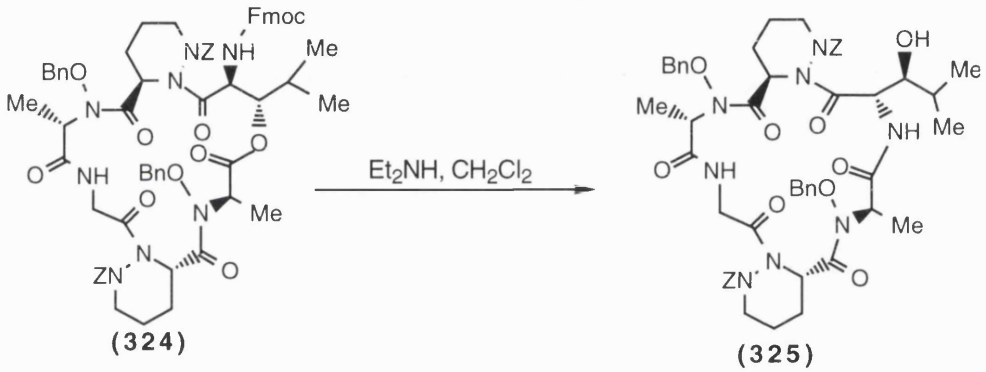
L-156,602

In 1990 workers at Merck Sharp & Dohme developed the first total synthesis of a member of the Azinothricin family namely, L-156,602, **5**.[128] Their initial strategy was based on the coupling of activated ester **322** with protected cyclodepsipeptide **323** (Scheme 64). However, this strategy was not successful, as removal of the Fmoc group from the protected cyclohexadepsipeptide **324** resulted in an *O,N*-acyl migration to give **325**, rather than the required depsipeptide **323** (Scheme 65).[129]

Scheme 64

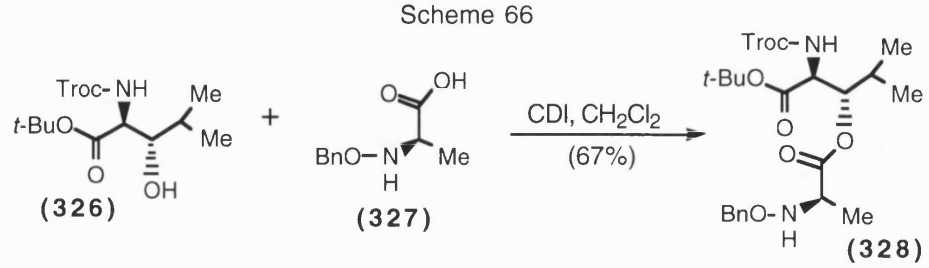


Scheme 65



An alternative synthetic plan was therefore developed which was based on a 2+2+2 fragment condensation strategy. Partially protected (*2S, 3S*)-hydroxyleucine **326** was coupled to *O*-benzylhydroxy-(*S*)-alanine **327** using 1,1-carbonyldiimidazole[19] to produce depsipeptide **328** in 67% yield. It should be noted that this coupling was accomplished without protecting the amine group in **327**, it being less nucleophilic than the hydroxyl group in **326** due to the -I effect of the adjacent benzyloxy group (Scheme 66).

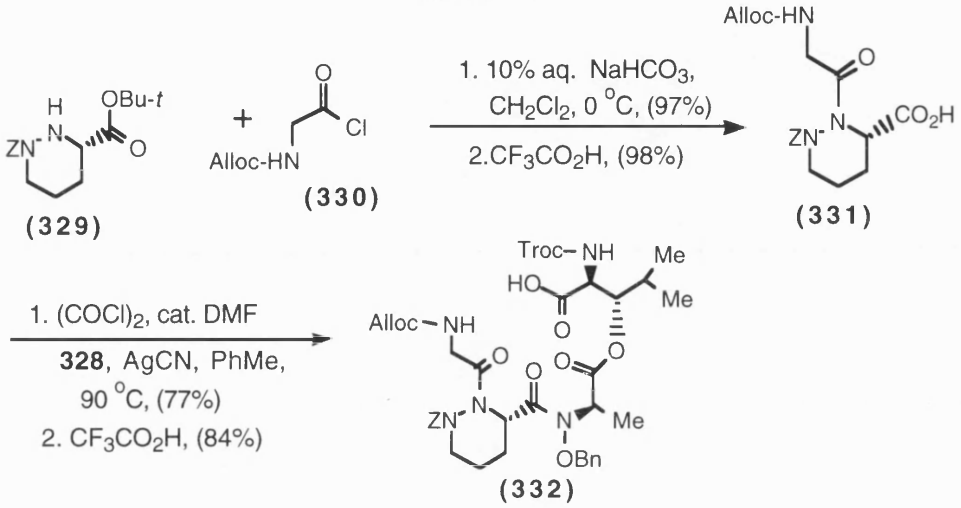
Scheme 66



Dipeptide **331** was accessed in 98% overall yield by joining *t*-butyl-(*3S*)-N^1-benzyloxy-carbonyl-piperazate **329** with the acid chloride **330** obtained from *N*-Alloc-glycine, and cleaving

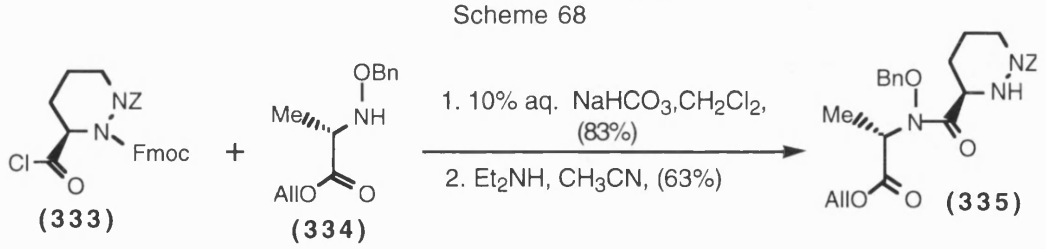
the *t*-butyl ester group with trifluoroacetic acid. Acid **331** was then converted to its acid chloride and this condensed with depsipeptide **328** in the presence of silver cyanide[130,131] in toluene at 90 °C to give the tetradepsipeptide in 77% yield. Subsequent cleavage of the *t*-butyl ester gave acid **332** in a yield of 84% (Scheme 67).

Scheme 67



Dipeptide **335** was synthesised from the N^1-fluorenylmethoxycarbonyl-(*3R*)-piperazic acid chloride **333** and the allyl ester derivative of *N*-benzyloxy-(*S*)-alanine **334** under the Schotten-Bauman conditions to give the dipeptide in 83% yield. Removal of the Fmoc group with diethylamine resulted in the partially protected hydrazine **335** in 63 % yield (Scheme 68).

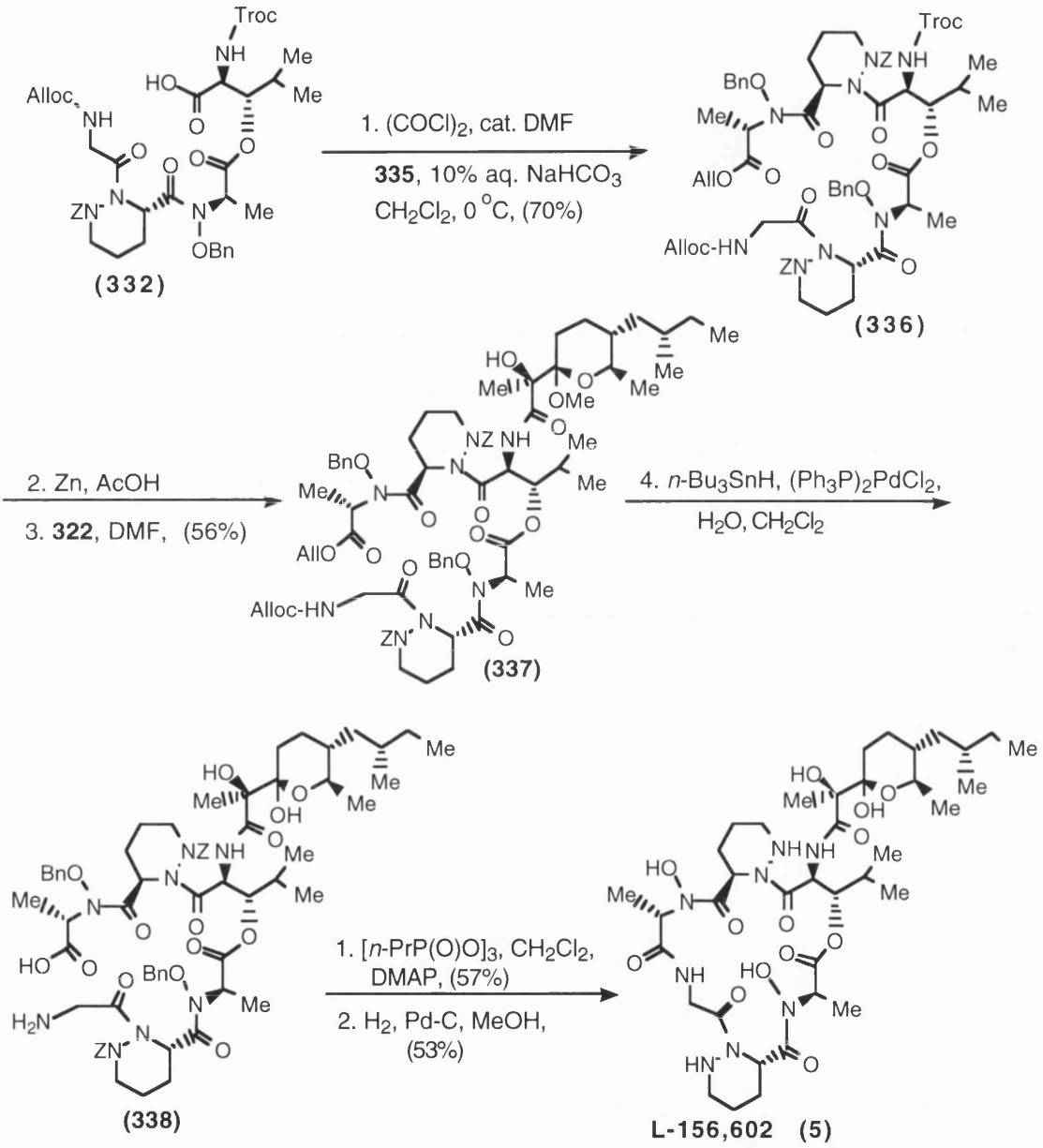
Scheme 68



The acid chloride of tetradepsipeptide **332** and the partially-protected hydrazine **335** were coupled using 10% aqueous sodium bicarbonate as base to give hexapeptide **336**. This product was treated with zinc in acetic acid to remove the Troc group and coupled to the benzotriazole activated ester **322**[132] in DMF to furnish **337** in 56% yield. The product was subsequently deprotected at the N and C termini using palladium(0)-catalysed hydrostannolysis.[133] These conditions also caused hydrolysis of the methyl pyranoside and led to the cyclised precursor **338.** To complete the synthesis, **338** was macrolactamised by the mixed phosphonic anhydride

method[39] in 57% yield and the product hydrogenated in methanol to give L-156,602 (**5**) in 53% yield (Scheme 69).

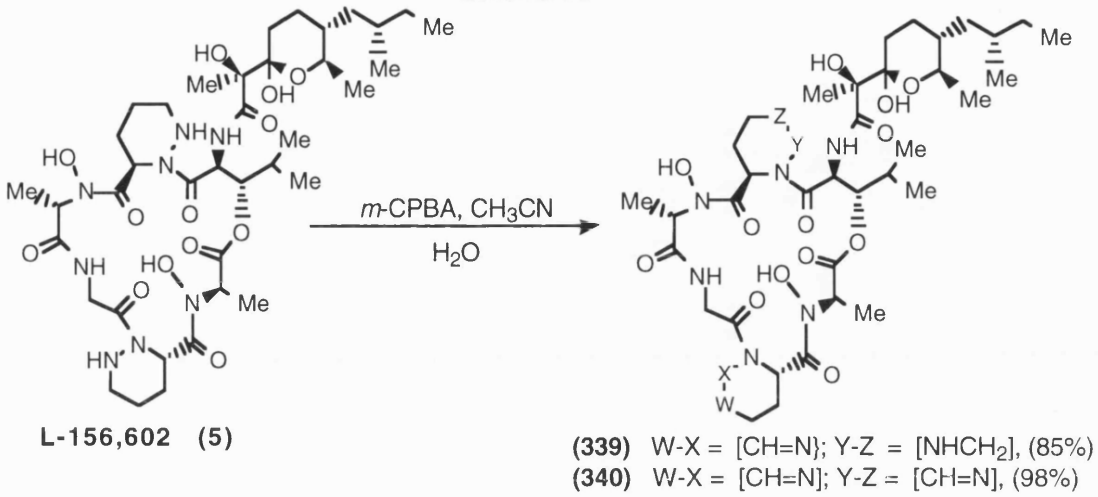
Scheme 69



Having completed a total synthesis of L-156,602 (**5**), the Merck group embarked on a programme of investigating the selective chemical modification of this natural product.[134] It was observed that the piperazic moiety could be oxidised to the mono-dehydro **339** or the bis-dehydro product **340** using 1.2-2.0 eqs. and 4.0 eqs. of *m*-chloroperbenzoic acid (*m*-CPBA) respectively. The N^1-piperazic acids atoms in **5** could not be acylated even under forcing

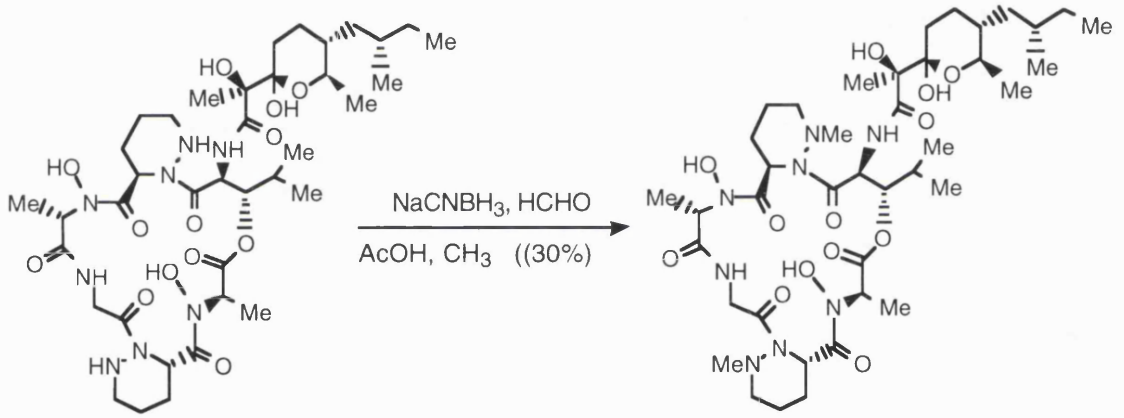
conditions. However, they could be methylated under the Borch conditions ($NaCNBH_3$, HCHO)[135] to give **341** (Scheme 70).

Scheme 70



L-156,602 (5)

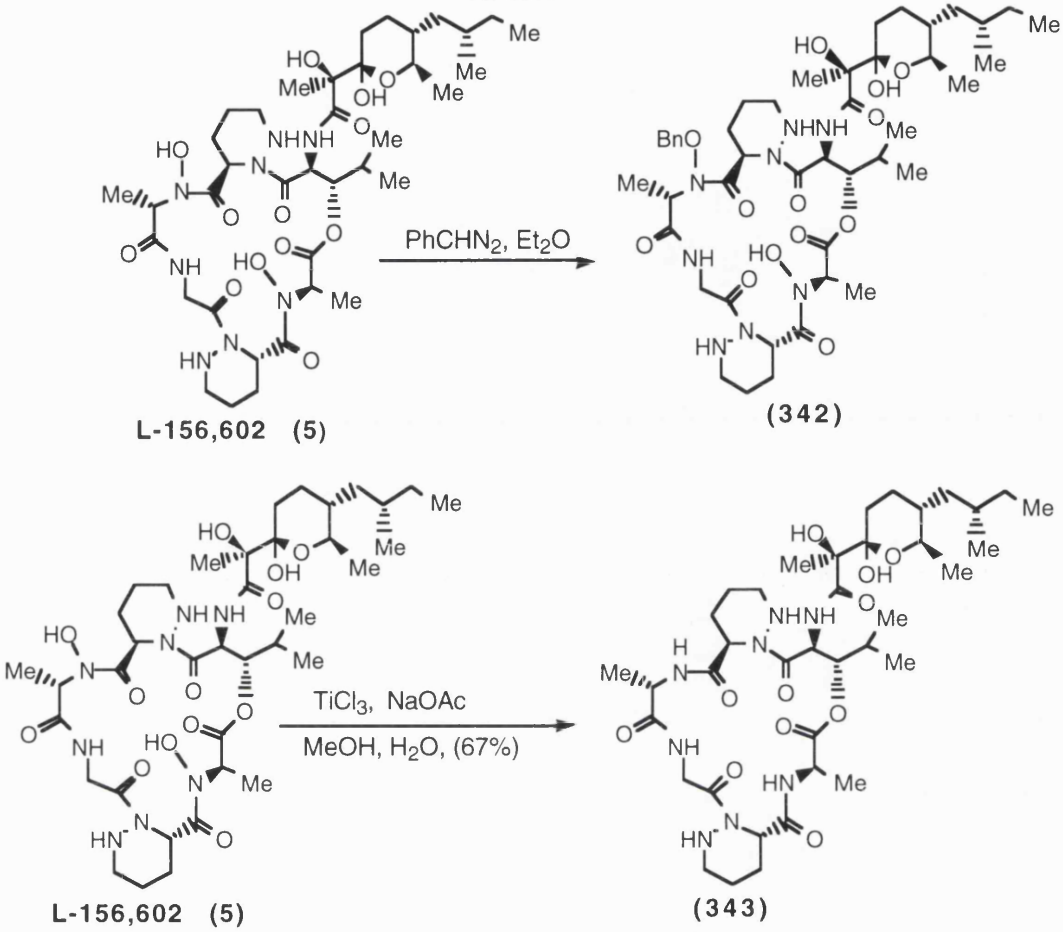
(339) W-X = [CH=N}; Y-Z = [$NHCH_2$], (85%)
(340) W-X = [CH=N]; Y-Z = [CH=N], (98%)



L-156,602 (5) **(341)**

Treatment of **5** with phenyldiazomethane resulted in **342** chemoselectively. This selective *O*-alkylation was attributed to hydrogen bonding between the *N*-OH of the (*R*)-*N*-OHAla and the hydroxy group of the lactic acid side chain. The latter resides under the peptide ring and the glycine amide carbonyl group, and thus shields the *N*-hydroxy group. Reducing agents such as titanium trichloride[136] deoxygenated both *N*-OH alanine residues to give the bis-alanine derivative **343** (Scheme 71).

Scheme 71



2.3 Concluding Remarks

There are several new cyclodepsipeptides whose structures have recently been determined by NMR methods. However, confirmation of these structures by total synthesis has not yet been achieved. These include the discokiolides[137] and theonellapeptolides[138] which are isolated from sponges. A83586C, a member of the Azinothricin family of antibiotics has also been the subject of synthetic investigation by the Hale group.[139-141]

2.4 References

(1) Losse, G.; Bachmann, G., *Z. Chem.*, **1964**, *4*, 204, 241.

(2) Russell, D. W., *Quart. Rev.*, **1966**, *20*, 559.

(3) (a) Plattner, Pl. A.; Nager, U., *Helv. Chim. Acta.*, **1948**, *31*, 2192. (b) Plattner, Pl. A.; Nager, U., *Helv. Chim. Acta.*, **1948**, *31*, 665.

(4) (a) Shemyakin, M. M.; Ovchinnikov, Y. O.; Ivanov, V. T.; Kiryushkin, A. A.,*Tetrahedron Lett.*, **1963**, *14*, 885. (b) Plattner, Pl. A.; Vogler, K.; Studer, P. O.; Quitt, P.; Keller-Schlierlei, W., *Helv. Chim. Acta.*, **1963**, *46*, 927.

(5) Quitt, P.; Studer, R.O.; Vogler, K., *Helv. Chim., Acta.*, **1963**, *46*, 1715.

(6) (a) Mikhaleva, I. I.; Ryabova, I. D.; Romanova, T. A.; Tarasova, T. I.; Ivanov, V. T.; Ovchinnikov, Y. A.; Shemyakin, M. M., *Zh. Obsch. Khim.*, **1968**, *38*, 1229. (b) Kanaoka, M.; Isogai, A.; Suzuki, A., *Agric. Biol. Chem.*, **1979**, *43 (5)*, 1079.

(7) Shemyakin, M. M.; Ovchinnikov, Y. A.; Ivanov, V. T.; Kiryushkin, A. A., *Tetrahedron,* **1963**, *19*, 995.

(8) Shemyakin, M. M.; Ovchinnikov, Y. A.; Ivanov, V. T.; Kiryushkin, A. A. ,*Tetrahedron Lett.*,**1963**, *4*, 1927.

(9) Brockmann, K.; Schmidt-Kastner, G.,*Chem. Ber.*,**1955**, *88*, 57.

(10) Shemyakin, M. M.; Vinogradova, E. I.; Feigina, M. Y.; Aldonova, N. A. ,*Tetrahedron Lett.*, **1963**, *4,* 351.

(11) Mueller, P.; Rudin, D. O., *Biochem. Biophys. Res. Commun.*, **1967**, *26*, 398.

(12) Tosteson, D. C.; Andreoli, T. E.; Tieffenberg, M.; Cook, P., *J. Gen. Physiol.*,**1968**, *51*, 373.

(13) Moore, C.; Pressmann, B. C. , *Biochem. Biophys. Res. Commun.*,**1964**, *15*, 562.

(14) Tosteson, D. C.; Andreoli, T. E.; Tieffenberg, M.; Cook, P., *J. Gen. Physiol.*, **1967**, *50*, 2513.

(15) Shemyakin, M. M.; Aldanova, N. A.; Vinogradova, E. I.; Feigina, M. Y., *Tetrahedron Lett.*, **1963**, *4*, 1921.

(16) Zeggaf, C.; Poncet, J.; Jouin, P.; Dufour, M. N.; Castro, B.,*Tetrahedron,* **1989**, *45*, 5039.

(17) Castro, B.; Dormoy, J.; Evin, G.; Selve, C.,*Tetrahedron Lett.* , **1975**, *14* 1219.

(18) Gisin, B. F.; Merrifield, R. B.; Tosteson, D. C., *J. Am. Chem. Soc.,* **1969**, 2691.

(19) Staab, H. A., *Justus Liebigs Ann. Chem.,* **1957**, *75*, 609.

(20) Merrifield, R. B.,*J. Amer. Chem. Soc.,* **1964**, *86*, 304.

(21) Konig, W.; Geiger, R.,*Chem. Ber.,***1970**, *103*, 788.

(22) Losse, G.; Klengel, H., *Tetrahedron* ,**1971**, *27*, 1423.

(23) Dory, Y. L.; Mellor, J. M.; McAleer, J. F., *Tetrahedron Lett.,* **1989**, *30*, 1695.

(24) Kuroya, M.; Ishida, N.; Katogiri, K.; Shaji, J.; Yashida, T.; Mayama, M.; Sato, K.; Matsura, S.; Niinomi, Y.; Shiratori, O., *J. Antibiotics,* **1961**, *A14*, 324.

(25) Chakravorty, P. K.; Olsen, R.,*Tetrahedron Lett.,***1978**, *19*, 1613.

(26) Anderson, G. W.; Zimmerman, J. E., *J. Amer. Chem. Soc.,* **1967**, *89*, 5012.

(27) Kamber, B., *Helv. Chim. Acta.,* **1972**, *55*, 2052.

(28) Ludescher, U.; Schwyzer, R., *Helv. Chim. Acta.,* **1971**, *54,* 927.

(29) Chakravorty, P. K.; Olsen, R. K.,*J. Org. Chem.,* **1978**, *43*, 1270.

(30) Koppel, H. C.; Honigberg, I. L.; Springer, R. H.; Chen, C. C., *J. Org. Chem.,***1963**, *28*, 1119.

(31) Shin, M.; Inouye, K.; Otsuka, H.,*Bull. Chem. Soc. Jpn.,* **1984**, *57*, 2203.

(32) Marinier, B.; Kim, Y. C.; Navarre, J. M., *Can. J. Chem.,***1973**, *51*, 208.

(33) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M., *J. Amer. Chem. Soc.,* **1963**, *85*, 3039.

(34) Ciardelli, T. L.; Chakravarty, P. K.; Olsen, R. K., *J. Am. Chem. Soc.,* **1978**, *100*, 7684.

(35) Dhaon, M. K.; Gardner, J. H.; Olsen, R. K., *Tetrahedron* ,**1982**, *38,* 57.

(36) Olsen, R. K.; Ramasamy, K.; Bhat, K. L.; Low, L. C. M.; Waring, M. J., *J. Am. Chem. Soc.,* **1986**, *108*, 6032.

(37) Kiryushkin, A.; Ovchinnikov, Y.; Shemyakin, M.,*Tetrahedron Lett.,* **1964**, *5*, 3313.

(38) Kessler, H.; Kutscher, B.; Mager, G.; Grell, E., *Liebigs Ann. Chem.,* **1983**, 1541.

(39) Wissmann, H.; Kleiner, H., *Angew. Chem., Int. Ed., Engl.,***1980**, *19*, 133.

(40) Wissmann, H.; Kleiner, H., *Angew. Chem.,***1980**, *92*, 129.

(41) Wasserman, H. H.; Keggi, J. J.; McKeon, J. E.,*J. Am. Chem. Soc.,* **1962**, *84*, 2978.

(42) Shemyakin, M. M.; Antonov, V.; Shkrob, A.; Shchelokov, V. I., *Tetrahedron,* **1965**, *21*, 3537.

(43) Shemyakin, M. M.; Ovchinnikov, Y. A.; Antonov, V. K.; Kiryushkin, A. A.; Ivanov, V. T.; Schelokov, V. I.; Shkrob, A. M.,*Tetrahedron Lett.,* **1964**, *5,* 47.

(44) Hassall, C. H.; Martin, T. G.; Schofield, J. A.; Thomas, J. O., *J. Chem. Soc. (C),* **1967**, 997.

(45) Suzuki, A.; Kanaoka, M.; Isogai, A.; Murakoshi, S.; Ichinoe, M.; Tamura, S.,*Tetrahedron Lett.,* **1977**,*18*, 2167.

(46) Vining, L. C.; Taber, W. A., *Can. J. Chem.,* **1962**, *40*, 1579.

(47) Okada, K.; Kurosawa, Y.; Hiramoto, M.,*Tetrahedron Lett.,***1972**, *13*, 2693.

(48) Bodanszky, M.; Vigneaud, V.,*J. Amer. Chem. Soc.,* **1959**, *81*, 5688.

(49) Hardy, P. M.; Prout, R. A.; Rydon, H. N. , *J. Chem. Soc., Perkin Trans. I ,* **1974**, 802.

(50) Okada, K.; Kurosawa, Y.; Hiramato, M. , *Chem. Pharm. Bull.,* **1974**, *22 (9)*, 2136.

(51) Rinehart, K. L.; Gloer, J. B.; Cook, J. C., *J. Amer. Chem. Soc.,* **1981**, *103*, 1857.

(52) Chun, H. G.; Davies, B.; Hogh, D.; Suffness, M.; Plowman, J.; Flora, K.; Grieshaber, C.; Leyland-Jones, B., *New Drugs,* **1986**, *4*, 279.

(53) Rinehart, K. L., *Anal. Chem. Symp. Ser.,* **1985**, *24*, 119.

(54) Ewing, W. R.; Bhat, K. L.; Joullie, M. M., *Tetrahedron,* **1986**, *42*, 5863.

(55) Castro, B., *10th Amer. Peptide Symp.,* **1987**, *St. Louis, MO*, Paper P-292.

(56) Rinehart, K. L.; Kishore, V.; Nagarajan, S.; Lake, R. J.; Gloer, J. B.; Bozich, F. A.; Li, K. M.; Maleczka, R. E.; Todsen, W. L.; Munro, M. H. G.; Sullins, D. W.; Sakai, R., *J. Am. Chem. Soc.,***1987**, *109*, 6846.

(57) Schmidt, U.; Kroner, M.; Griesser, H., *Tetrahedron Lett.,* **1988**, *29*, 3057.

(58) Schmidt, U.; Schefenacker, K., *Liebigs Ann. Chem.,* **1985**, 1254.

(59) Schmidt, U.; Potzolli, B., *Liebigs Ann. Chem.,* **1987**, 935.

(60) Fujii, N.; Otaka, A.; Ikemura, O.; Akaji, K.; Funakoshi, S.; Hayashi, Y.; Kurada, Y.; Yajima, H., *J. Chem. Soc., Chem. Commun.,***1987**, 274.

(61) Schmidt, U.; Kroner, M.; Griesser, H. , *Synthesis,* **1991**, 294.

(62) Diago-Meseguer, J.; Palomo-Coll, A. L.; Fernandez-Lizarde, J. R.; Zugaza-Bilbao, A., *Synthesis,* **1980**, 547.

(63) Tung, R. D.; Rich, D. H., *J. Am. Chem. Soc.,* **1985**, *107*, 4342.

(64) Schimdt, U.; Lieberknecht, A.; Griesser, H.; Talbiersky, J., *J. Org. Chem.,* **1982**, *47*, 3261.

(65) Li, W.-R.; Ewing, W. R.; Harris, B. D.; Joullie, M. M., *J. Amer. Chem. Soc.,* **1990**, *112*, 7659.

(66) Chen, F. M. F.; Lee, Y.; Steinaver, R.; Benoiton, N. L., *Can. J. Chem.,* **1987**, *65*, 613.

(67) Abiko, A.; Roberts, J. C.; Takemasa, T.; Masamune, S., *Tetrahedron Lett.,* **1986**, *27*, 4537.

(68) Brady, S. F.; Freidinger, R. M.; Paieveda, W. J.; Cotton, C. D.; Homnick, C. F.; Whittier, W. L.; Curley, P.; Nutt, R. F.; Veber, D. F., *J. Org. Chem.,* **1987**, *52*, 764.

(69) Dudash, J.; Jiang, J.; Mayer, S. C.; Joullie, M. M., *Syn. Comm.,* **1993**, *23*, 349.

(70) Chen, S.; Xu, J., *Tetrahedron Lett.,* **1991**, *32*, 6711.

(71) Knorr, R.; Trzeciak, A.; Bennworth, W.; Gillessen, D., *Tetrahedron Lett.,* **1989**, *30,* 1927.

(72) Shioiri, T., *Jpn. Symp. Peptide Chem.,* **1987**, *Kobe, Japan*, 111.

(73) (a) Destruxin A and B: Tamura, S.; Kuyama, S.; Kodaira, Y.; Higashikawa, S. , *Agric. Biol. Chem.,* **1964**, *28*, 137. (b) Destruxins C, D and desmethyldestruxin: Suzuki, A.; Taguchi, H.; Tamura, S., *Agric. Biol. Chem.*, **1970**, *34*, 813.

(74) Tamura, S.; Kuyama, S.,*Agric. Biol. Chem.,* **1965**, *29*, 168.

(75) Lee, S.; Izumiya, N.,*Tetrahedron Lett.,***1975**, *16,* 883.

(76) Lee, S.; Izumiya, N.,*Int. J. Peptide Protein Res.,* **1977**, *10 (3)*, 206.

(77) Wunsch, E.; Dress, F.,*Chem. Ber.,***1966**, *99*, 110.

(78) Morrison, J. D.; Ciardelli, T. L.; Husman, J. R.,*Tetrahedron Lett.,* **1976**, *17*, 1773.

(79) Okuno, T.; Ishita, Y.; Sawai, K.; Matsumoto, T.,*Chemistry Lett.,* **1974**, 635.

(80) Ueno, T.; Nakashima, T.; Hayashi, Y.; Fukami, H., *Agric. Biol. Chem.,***1975**, *39*, 1115.

(81) Ueno, T.; Nakashima, T.; Hayashi, Y.; Fukami, H. , *Agric. Biol. Chem.,* **1975**, *39*, 2081.

(82) Lee, S.; Aoyagi, H.; Shimohigashi, Y.; Izumiya, N.,*Tetrahedron Lett.,* **1976**, *17,* 843.

(83) Shimohigashi, Y.; S., L.; Aoyagi, H.; Kato, T.; Izumiya, N., *Int. J. Peptide Protein Res,* **1977**, *10*, 323.

(84) Shimohigashi, Y.; Izumiya, N., *Int. J. Peptide Protein Res.,***1978**, *12*, 7.

(85) Kozono, T.; Mihara, H.; Aoyagi, H.; Kato, T.; Izumiya, N., *Int. J. Peptide Protein Res.,* **1984**, *24*, 402.

(86) Nomoto, S.; Sano, A.; Shiba, T., *Tetrahedron Lett.,* **1979**, *20*, 521.

(87) Ueda, K.; Waki, M.; Izumiya, N., *Int. J. Peptide Protein Res.,* **1987**, *30*, 33.

(88) Kanmera, T.; Izumiya, N., *Int. J. Peptide Protein Res.,* **1982**, *19*, 79.

(89) Crews, P.; Manes, L. V.; Boehler, M., *Tetrahedron Lett.,***1986**, *27*, 2797.

(90) Zabriskie, T. M.; Klocke, J. A.; Ireland, C. M.; Marcus, A. H.; Molinski, T. F.; Faulkner, D. J.; Xu, C.; Clardy, J. C., *J. Amer. Chem. Soc.,* **1986**, *108*, 3123.

(91) Ohfune, Y.; Sakaitani, M., *Tetrahedron Lett.,***1985**, *26*, 5543.

(92) Boden, E. P.; Keck, G. E., *J. Org. Chem.,* **1985**, *50*, 2394.

(93) Grieco, P. A.; Hon, Y. S.; Perez-Medrano, A., *J. Am. Chem. Soc.,* **1988**, *110*, 1630.

(94) Chu, K. S.; Negrete, G. R.; Konopolski, J. P., *J. Org. Chem.,* **1991**, *56*, 5196.

(95) Rao, R. A. V.; Gurjar, M. K.; Nallaganchu, B. R.; Bhandari, A.,*Tetrahedron Lett.,* **1993**, *34*, 7085.

(96) Imaeda, T.; Hamada, Y.; Shioiri, T.,*Tetrahedron Lett.,* **1994**, *35*, 591.

(97) Mitsunobu, O.,*Synthesis* ,**1980**, 1.

(98) Shioiri, T.; Ninomiya, K.; Yamada, S., *J. Amer. Chem. Soc.,* **1972**, *94*, 6203.

(99) Kato, S.; Hamada, Y.; Shioiri, T.,*Tetrahedron Lett.,* **1988**, *29*, 6465.

(100) Shioiri, T.; Yokoyama, Y.; Kasai, Y.; Yamada, S., *Tetrahedron* ,**1976**, *32*, 2211.

(101) Chan, W. R.; Tinto, W. F.; Manchand, P. S.; Todaro, L. J., *J. Org. Chem.,* **1987**, *52*, 3091.

(102) White, J. D.; Amedio, J. C., *J. Org. Chem.,* **1989**, *54*, 736.

(103) Hirai, Y.; Yokata, K.; Yamazaki, T.; Mamase, T., *Heterocycles,* **1990**, *30*, 1101.

(104) Grieco, P. A.; Perez-Medrano, A., *Tetrahedron Lett.,* **1988**, *29*, 4225.

(105) Hassall, C. H.; Johnson, W. H.; Theobald, C. J., *J. Chem . Soc., Perkin Trans I* ,**1979**, 1451.

(106) Konishi, M.; Ohkuma, H.; Sakai, F.; Tsuno, T.; Koshiyama, H.; Naito, T.; Kawaguchi, H., *J. Antibiotics,* **1981**, *34*, 148.

(107) Konishi, M.; Ohkuma, H.; Sakai, F.; Tsuno, T.; Koshiyana, H.; Naito, T.; Kawaguchi, H., *J. Amer. Chem. Soc.,* **1981**, *103*, 1241.

(108) Arnold, E.; Clardy, J., *J. Amer. Chem. Soc.,* **1981**, *103*, 1243.

(109) Huang, C.-H.; Mong, S.; Crooke, S. T., *Biochemistry,* **1980**, *19*, 5537.

(110) Huang, C.-H.; Prestayko, A. W.; Crooke, S. T., *Biochemistry,* **1982**, *21*, 3704.

(111) Okuma, H.; Sakai, F.; Nishiyama, Y.; Ohbayashi, M.; Imanishi, H.; Konishi, M.; Miyaki, T.; Koshiyama, H.; Kawaguchi, H., *J. Antibiotics,* **1980**, *33*, 1087.

(112) Rose, W. C.; Schurig, J. E.; Huftalen, J. B.; Bradner, W. T., *Cancer Res.,* **1983**, *43*, 1504.

(113) Olsen, R. K.; Apparao, S.; Bhat, K. L., *J. Org. Chem.,* **1986**, *51*, 3079.

(114) Groupe, V.; Pugh, L. H.; Weiss, D.; Kochi, M., *Proc. Soc. Exptl. Biol. Med.,* **1951**, *78*, 354.

(115) Burke, T. R.; Knight, M.; Chandrasekhar, B., *Tetrahedron Lett.,* **1989**, *30*, 519.

(116) Sone, H.; Nemoto, T.; Ishiwata, H.; Ojika, M.; Yamada, K., *Tetrahedron Lett.,* **1993**, *34*, 8449.

(117) Ishiwata, H.; Nemoto, T.; Ojika, M.; Yamada, K., *J. Org. Chem.,* **1994**, *59*, 4710.

(118) Inanoga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M., *Bull. Chem. Soc. Jpn.,* **1989**, *52*, 1979.

(119) Ishiwata, H.; Sone, H.; Kigoshi, H.; Yamada, K., *J. Org. Chem.,* **1994**, *59*, 4712.

(120) Sasaki, T.; Takagi, M.; Yaguchi, S.; Miyadoh, S.; Okada, T.; Koyama, M., *J. Antibiotics ,* **1992**, *45*, 692.

(121) Ohyama, M.; Iinuma, K.; Isogai, A.; Suzuki, A., *Biosci. Biotech. Biochem.,***1994**, *58 (6)*, 1193.

(122) Hamano, K.; Kinoshita, M.; Furuya, K.; Miyamato, M.; Takamatsu, Y.; Hemmi, A.; Tanzawa, K., *J. Antibiotics,* **1992**, *45*, 899.

(123) Carpino, L. A., *J. Amer. Chem. Soc.,* **1993**, *115*, 4397.

(124) Schmidt, U.; Langner, J., *J. Chem. Soc., Chem. Commun.,* **1994**, 2381.

(125) Takesako, K.; Ikai, K.; Huruna, F.; Endo, M.; Shimanaka, K.; Sono, E.; Nakamura, T.; Kato, I.; Yamaguchi, H., *J. Antibiotics,* **1991**, *44*, 919.

(126) Coste, J.; Frerot, E.; Jouin, P.; Castro, B., *Tetrahedron Lett.,* **1991**, *32*, 1967.

(127) Kurome, T.; Inami, K.; Inoue, T.; Ikai, K.; Takesako, K.; Kato, I.; Shiba, T.,*Chem. Lett.,* **1993**, 1873.

(128) Durette, P. L.; F., Baker, F; P.L, Boger, J.; Bondy, S. S.; Hammond, M. L.; Pessolano, A. A.; Caldwell, C. G., *Tetrahedron Lett.,***1990**, *31*, 1237.

(129) Durette, P. L.; Baker, F.; Barker, P. L.; Boger, J.; Bondy, S. S.; Hammond, M. L.; Lanza, T. L.; Pessolano, A. A.; Caldwell, C. G., *Abs. 198th ACS National Meeting* **1989**, *Miami Beach, Florida*, September 10-15, Abstract No.119.

(130) Tung, R.; Dhaon, M. K.; Rich, D. H., *J. Org. Chem.,* **1986**, *51*, 3350.

(131) Williams, E.A., *J. Med. Chem.,* **1992**, *35*, 3905.

(132) Caldwell, C. G.; Ruprecht, K. M.; Bondy, S. S.; Davis, A. A., *J. Org. Chem.,* **1990**, *55*, 2355.

(133) Dangels, O.; Guibe, F.; Balavoine, G.; Laveielle, S.; Marquet, A., *J. Org. Chem.,* **1987**, *52*, 4984.

(134) Kopka, I. E., *Tetrahedron Lett.,* **1990**, *31*, 4711.

(135) Borch, R. F.; Dernstein, M. D.; Durst, H., D., *J. Amer. Chem. Soc.,***1971**, *93*, 2897.

(136) Mattingly, P. G.; Miller, M. J., *J. Org. Chem.,* **1980**, *45*, 410.

(137) Tada, H.; Tozyo, T.; Terui, Y.; Hayashi, F., *Chem. Lett.*, **1992**, 431.

(138) Kitagawa, I.; Lee, N.K.; Kobayashi, M.; Shibuya, H., *Tetrahedron*, **1991**, 47, 2169.

(139) Hale, K.J., Delisser, V.M.; Manaviazar, S., *Tetrahedron Lett.*, **1992**, 33, 7613.

(140) Hale, K.J., Bhatia, G.S.; Peak, S.A.; Manaviazar, S., *Tetrahedron Lett.*, **1993**, 34, 5343.

(141) Hale, K.J.; Delisser, V.M.; Yeh, L-K, Peak, S.A., Manaviazar, S.; Bhatia, G.S.,*Tetrahedron Lett.*, **1994**, 35, 7685.

Chapter 3. Synthetic Studies on the Hexadepsipeptide Portion of A83586C.

3.1 Introduction

Aware of these synthetic findings on L-156,602, we decided to embark on the total synthesis of the antitumour antibiotic, A83586C (**2**). Our main reason for initiating this venture was to use the total synthesis to create a library of analogues that could be used to probe the biological mechanism of antitumour action. We also hoped to identify simplified analogues that might have reduced toxicity compared to A83586C itself, and thus be of greater potential use as new anticancer treatments.

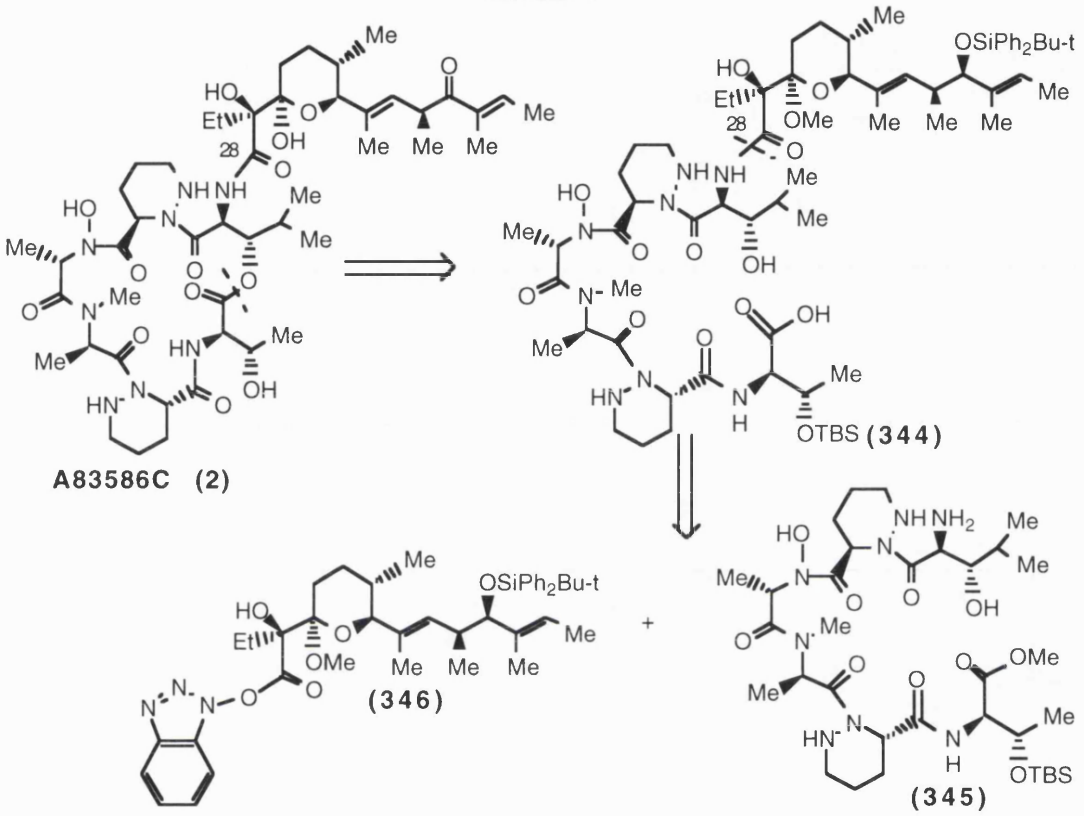
A83586C (**2**) is a very sensitive molecule towards the action of strong nucleophiles such as thiols and amines, since conjugate addition can occur on the isolated α, β unsaturated ketone present in the pyran side chain. These nucleophiles, along with bases, could also potentially cleave the lactone bond, and thus destroy the cyclodepsipeptide ring. Under strongly basic or acidic conditions the C(35) and C(36) double bond also runs the risk of moving into conjugation with C(38) ketone, to create a new stereocentre at C(35) while destroying one at C(37). Another possibility under strong acid conditions is a retro-aldoi cieavage in the threonine and the hydroxyleucine residues. Thus, when considering a potential retrosynthetic analysis of **2** it is critical that a correct choice of protecting groups is made. in our view, the protecting groups required for a synthesis of A83586C will need to be capable of being cleaved under mildly acidic or preferably neutral conditions.

3.2 Retrosynthetic Analysis of A83586C

A key step in our retrosynthetic analysis of A83586C is the ring closure of *seco*-acid **344** (Scheme 1). Several methods are available for this transformation, most notably those due to Corey ($(PyS)_2$, Ph_3P),[1,2] Mitsunobu (DEAD, Ph_3P),[3] Masumune (DCC, PPTS, Py)[4,5] and Mukaiyama (2-chloro-6-methyl-1,3-diphenylpyridinium tetrafluoroborate,2,4,6-triphenylpyridine).[6] Global deprotection of the resuling macrolactone with mild acid and subsequent selective oxida-tion of the allylic alcohol at C(38) with DDQ or O_2/Pt black[7] should then lead to the natural product. Our next disconnection is across the C(28)-amide bond in **344**; it potentially, could arise from a condensation of amine **345** with the hydroxybenzotriazole activated ester **346**. We opted for a

strategy proceeding via the linear hexapeptide **345**, rather than one utilising a fully-elaborated cyclodepsipeptide, bearing a free amine at C(18), so as to minimise the risk of macrolactone destruction through intramolecular *N*-acyl migration as was encountered in the Merck synthesis of L-156,602.[8]

Scheme 1

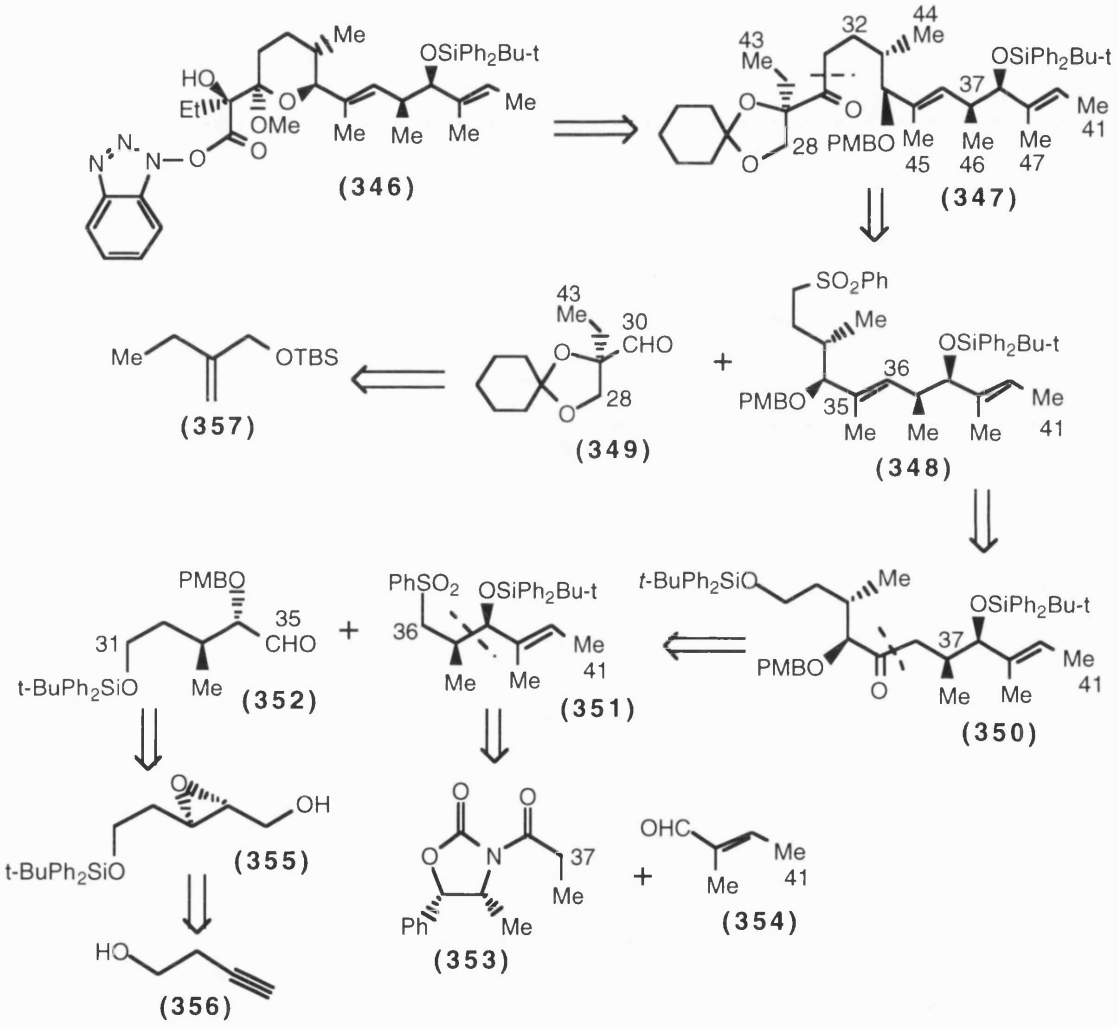


Next we hoped to excute a chemoselective coupling between amine **345** and activated ester **346** to establish the C(28) amide linkage. The C(18)-amine of hexapeptide **345** was considered to be the most nucleophilic site in this molecule, since the piperazic acid N^1-atoms and the hydroxamic acid hydroxyl would be deactivated by virtue of being attached to electron-withdrawing groups. They would also be sterically hindered.

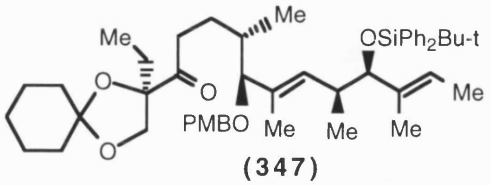
Activated ester **346** was potentially derivable from ketone **347**, by debenzylation, Fischer glycosidation with methanol and PPTS, oxidation of the primary alcohol to the acid, and treatment with benzotriazolyl-*N*-oxytridimethylaminophosphonium hexafluorophosphate (BOP). Ketone **347** could arise from a condensation between the α-phenylsulfonyl anion of **348** and aldehyde **349**. We were hoping to control the (*E*)-stereochemistry in the C(35)-C(36) trisubstituting olefin of **348** by a chelation-controlled Grignard addition of MeMgBr to **350** followed by dehydration. Disconnection of **350** led us to sulfone **351** and aldehyde **352** as sub-targets.

The stereocentre bearing the C(37) methyl group in **351** could be set by an Evans asymmetric aldol reaction [9,10] between the (Z)-di-*n*-butylboron enolate of **353** and tiglic aldehyde **354**. The stereocentres at C(33) and C(34) in **352** could be created by a chelation-controlled ring opening of the 2,3-epoxy alcohol **355** with trimethylaluminium, since there were no electron-withdrawing substituents adjacent to the C(3)-position of the epoxide.[11] A concise genesis of aldehyde **349** was envisaged to be from asymmetric dihydroxylation of alkene **357** wih AD-mix-α[12] (Scheme 2).

Scheme 2

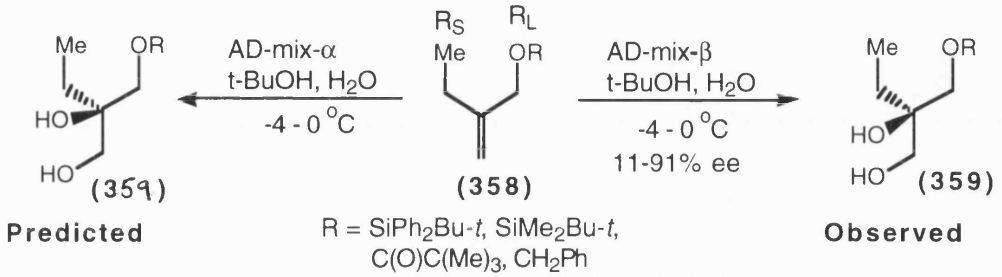


Since this initial retrosynthetic analysis an asymmetric synthesis of ketone **347** has been developed by the Hale group.[13] The results are discussed more fully by Bhatia[14] and Manaviazar.[15]



Interestingly, whilst synthesising aldehyde **349** via asymmetric dihydroxylation, it was observed that alkene **357** did not conform to the Sharpless mnemonic device in the AD reaction with AD-mix-α, and led to a product that had opposite stereochemistry to that predicted. Several other 1,1-disubstituted alkenes were examined and observed to give the opposite stereochemistry to that predicted (Scheme 3).[16]

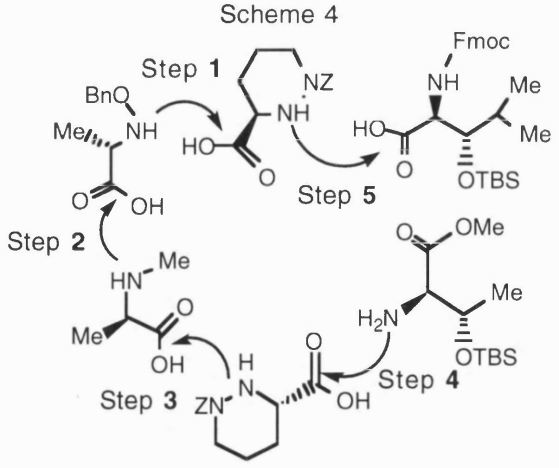
Scheme 3



3.3 Results and Discussion

From here on I shall be discussing our synthetic studies on the cyclodepsipeptide portion of A83586C. The first attempt at constructing hexapeptide **345** was based on the linear app-roach shown in Scheme 4. The numbers represents the order of the proposed couplings of the suitably protected α-amino and α-hydrazino acids.

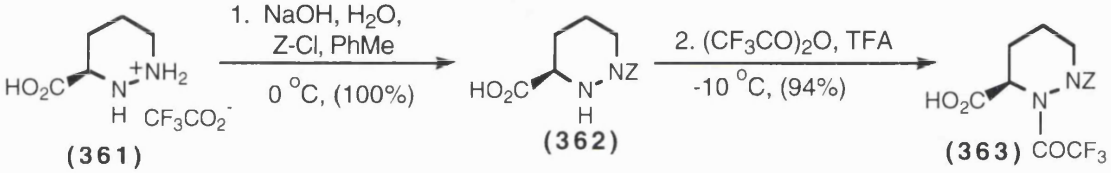
Scheme 4



In order to accomplish the first coupling (*3R*)-piperazic acid trifluoroacetic acid salt, **361**[17] was protected as its N^1-benzyloxycarbonyl derivative by treatment with benzyl chloroformate in toluene under the Schotten-Bauman conditions to give **362** in 100% yield. The infrared spectrum of **362** contained a sharp absorption at 3262 cm^{-1} which suggested the presence of a

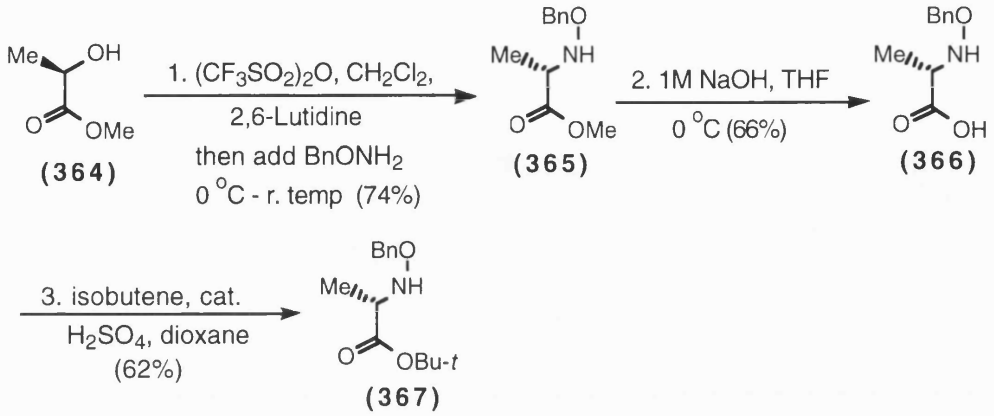
free NH group. There was also a broad acid carbonyl stretch at 1751 cm^{-1} and a carbamate carbonyl stretch at 1691 cm^{-1}. The structure of the product was further confirmed by the presence of a five proton multiplet between δ 7.38-7.29 in the 400 MHz ^1H NMR spectrum in $CDCl_3$, which was due to the aromatic protons. By keeping the reaction at 0 °C and the reaction time below 2 h, the yield of the bis protected piperazic acid could be reduced to a negligible amount. The N^2 of the N^1-*Z*- piperazic acid **361** was also protected prior to its coupling. Thus, treating **362** with trifluoroacetic anhydride in trifluoroacetic acid resulted in **363** in a yield of 94% (Scheme 5).[18] The absence of an NH stretch at 3262 cm^{-1} and the appearance of an additional carbonyl stretch at 1827 cm^{-1} corroborated the presence of a trifluoroacetyl group. The meas ured accurate mass was in accord with the structure calculated for $C_{15}H_{16}O_5N_2F_3$ $(M+H)^+$, 361.1011; Found: 361.1024.

Scheme 5



N-Hydroxy alanine derivative **367** was synthesised by the procedure of Ottenheijm and coworkers.[19] Methyl-D-lactate **364** was converted to its triflate ester and this displaced with *O*-benzylhydroxylamine in an S_N2 reaction to give **365** in 74% yield (Scheme 6). The structure of the product was apparent from its 400 MHz ^1H NMR spectrum in $CDCl_3$ since there was a multiplet between δ 7.24-7.35 which integrated to five protons, and which was due to the aromatic hydrogens; in addition, there was a methyl ester singlet at δ 3.65. The infrared spectrum of **365** contained a weak but sharp absorption at 3262 cm^{-1} which corresponded to the NH group. Hydrolysis of **365** with 1 M aqueous sodium hydroxide in THF at 0 °C gave acid **366** in 66% yield after acidification (Scheme 6). The absence of a singlet at δ 3.65 confirmed that the methoxy group was now absent from product **366**. The infrared spectrum of **366** also possessed a broad absorption between 3200 and 2000 cm^{-1} which was indicative of a carboxylic acid group. This observation was further consolidated by a shift of the carbonyl absorption from 1742 cm^{-1} for the methyl ester **365** to 1574 cm^{-1} in **366** which again was indicative of a carboxylate ion.

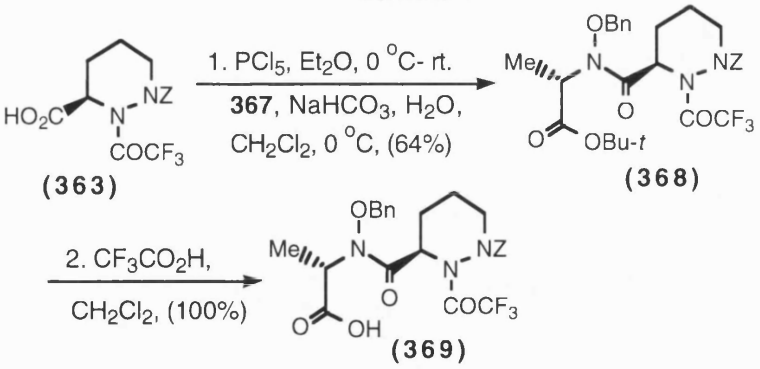
Scheme 6



Reesterification of **366** with isobutene and a catalytic amount of concentrated sulfuric acid in dioxane resulted in the *t*-butyl ester **367** being formed in a yield of 62 %. The structure of **367** was apparent from the nine proton singlet at δ 1.46. Furthermore, the high resolution mass spectrum of **367** contained an $(M+H)^+$ peak at m/e 252.1608 (Calcd. for $C_{14}H_{22}O_3N$, $(M+H)^+$ 252.1600) which was in accord with the postulated structure.

The bis-protected piperazic acid **363** was now converted to its acid chloride by treatment with phosphorus pentachloride in ether, and coupled to α-hydroxamic acid derivative **367** in a mixture of dichloromethane and 12% aqueous sodium bicarbonate. This furnished dipeptide **368** in 64% yield (Scheme 7).

Scheme 7

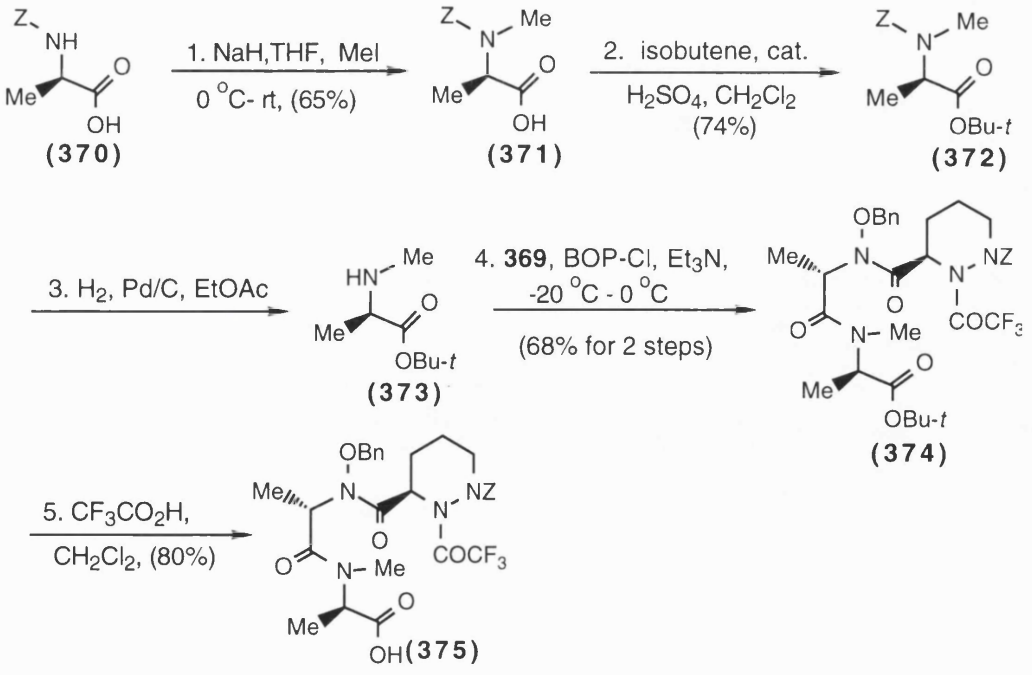


The infrared spectrum of **368** contained carbonyl absorptions at 1738, 1724 and 1674 cm^{-1} the latter clearly being due to an amide C=O stretch. The 400 MHz ^1H NMR spectrum of **368** at 135 °C in DMSO-d_6 consisted *inter alia* of a multiplet between δ 7.49 and 7.29 which corresponded to the benzyl groups of the benzyloxycarbonyl and the benzyl ether of the *O*-benzylhydroxyalanine derivative. The presence of two singlets at δ 1.43 and 1.42 due to the *t*-butyl ester group and the

doublet at δ 1.38 (J = 7.2 Hz) due to the methyl group of the α-hydroxamic residue supported the postulated structure. Quantitative cleavage of the *t*-butyl ester of **368** with trifluoroacetic acid resulted in acid **369** (Scheme 7). The absence of the *t*-butyl resonances at δ 1.43 and 1.42 in the 400 MHz ^{1}H NMR spectrum in $CDCl_3$ and the presence of a broad singlet at δ 12.90 was good evidence for formation of the acid. Further confirmation came from the broad absorption observed between 3478 and 3219 cm^{-1} in the IR spectrum of **369**.

N-Methyl-D-alanine **371** was synthesised by the chemoselective methylation method of Benoiton and coworkers[20,21] starting from *N*-benzyloxycarbonyl-D-alanine **370**. Subsequent esterification with isobutene and a catalytic amount of concentrated sulfuric acid resulted in the *t*-butyl ester **372** being formed in 74% yield. The infrared spectrum of **372** contained two carbonyl stretches at 1735 and 1708 cm^{-1} due to the ester and the carbamate carbonyl groups. The 400 MHz ^{1}H NMR spectrum of **372** in $CDCl_3$ revealed two singlets at δ 2.88 and 2.85 which were assignable to the *N*-methyl protons, and two singlets at δ 1.41 and 1.37 due to the *t*-butyl ester group. In order to complete the synthesis of the tripeptide **375**, **372** was hydrogen-ated in the presence of 10% palladium on carbon in ethyl acetate and the resulting amine **373** concentrated *in vacuo* at 25 °C before being diluted with dry dichloromethane (Scheme 8). The amine

Scheme 8

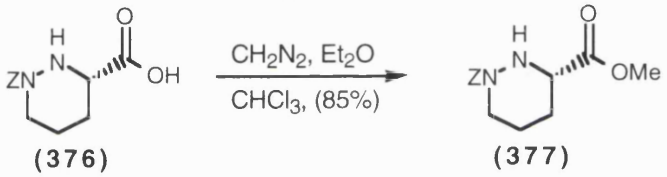


solution was then added to a solution of the mixed phosphinic acid anhydride of dipeptide **369** formed after reaction with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[22-24] and triethylamine in dichloromethane at -20 °C; compound **374** was isolated in 68% yield (Scheme 8).

The constitution of **374** was suggested by its high resolution mass spectrum which contained an $(M+H)^+$ peak at m/e 679.2959 (Calcd. for $C_{33}H_{42}N_4O_8F_3$, $(M+H)^+$ 679.2955). Cleavage of the *t*-butyl ester from **374** with trifluoroacetic acid gave **375** in 80% yield (Scheme 8). The infrared spectrum of **375** contained a broad absorption between 3690 and 2400 cm^{-1} which was indica-tive of an acidic OH group. Structure **375** was further proven by its high resolution mass spectrum which contained an $(M)^+$ peak at m/e 622.2243 (Calcd. for $C_{29}H_{33}N_4O_8F_3$, (M^+) 622.2250).

At this point, its coupling partner (*3S*)-N^1-Z-piperazic acid methyl ester **377** was prepared from **376** by treatment with ethereal diazomethane in chloroform (Scheme 9).

Scheme 9

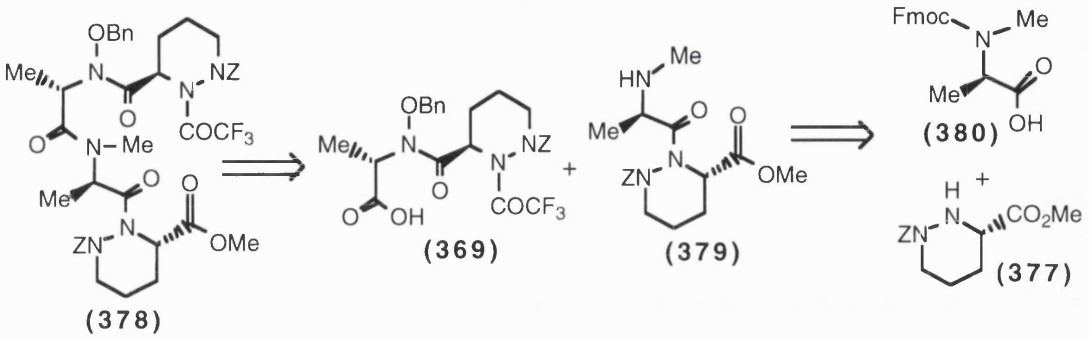


(376) **(377)**

With tripeptide **375** in hand, we next attempted to couple it with **377**, to form tetrapeptide **378** (Scheme 10). A range of methods for carboxyl activation were evaluated for obtaining tetrapeptide **378** from acid **375**. These included the acid chloride[25] and acid fluoride[26] procedures. A variety of bases were used for the coupling of **377** with the acid chloride from **375**; these included *N*-methylmorpholine, diisopropylethylamine, and 10% aqueous $NaHCO_3$ in dichloromethane. Silver cyanide in toluene at 90 °C was also tried, unsuccessfully.[27] Direct coupling of tripeptide **375** to **377** using reagents such as 2,2-dipyridyldisulfide/triphenylphosphine,[28] the Steglich reagent,[29] dicyclohexylcarbodiimide/hydroxybenzotriazole,[30] and 1,1-carbonyldiimidazole[31,32] were also futile. The failure of these reactions was presumably due to the poor nucleophilcity of the *N*(2)-atom in N^1acylated α-hydrazino acid derivatives, which is caused by the strong electron-withdrawing effect of the *N*(1)-acyl residue and the sterically hindered enviroment it creates around the *N*(2)-atom. The bulkiness of acid 375 may also be a reason for the failure of this coupling.

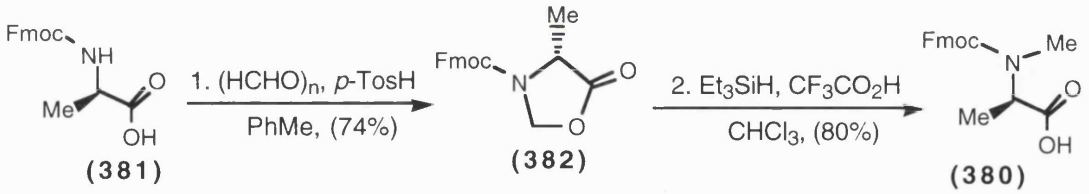
In light of this set-back, we elected to follow a [2+2] fragment condensation strategy between **369** and **379** to obtain **378** (Scheme 11). We envisaged that **379** would be formed by a coupling of **380** with **377**, followed by removal of the Fmoc protecting group.

Scheme 11



Acid **380** is a known compound that was prepared according to the literature method developed by Freidinger and coworkers (Scheme 12).[33] This involved treatment of Fmoc-D-alanine **381** with paraformaldehyde to obtain the oxazolidinone[34] **382** in 74% yield. Ionic reduction[35] of **382** with triethylsilane in trifluoroacetic acid gave the required acid **380** in 80% yield.

Scheme 12



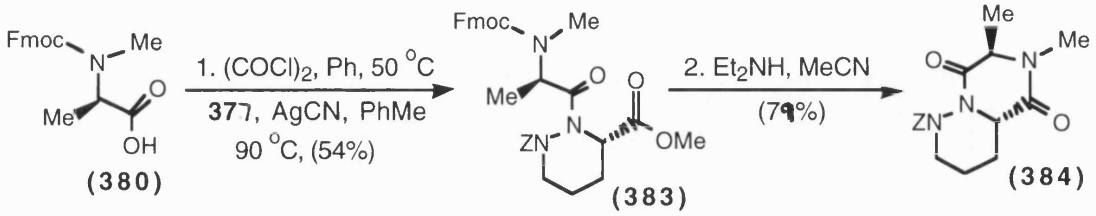
$[\alpha]_D$ +29° (*c* 1, CH_2Cl_2)

Lit[33] for L-enantiomer $[\alpha]_D$ -21.4° (*c 1*, CH_2Cl_2)

The acid chloride of **380** was formed by treatment with oxalyl chloride in benzene at 50 °C for 1 h, and concentration *in vacuo*. The crude acid chloride was used immediately for the coupling with methyl (*3S*)-Z-piperazate **377** mediated silver cyanide[36,37] in toluene at 90 °C for 1 h; this resulted in the desired dipeptide **383** being formed in 54% yield. The structure of **383** was apparent from its 400 MHz ^1H NMR spectrum in $CDCl_3$ since there were a pair of sharp singlets at δ 3.74 and 3.73 which strongly supported the presence of a methyl ester. There were also two broad singlets at δ 2.87 and 2.83 which were attributable to the *N*-methyl hydrogens. The infrared spectrum of **383** contained carbonyl stretches at 1740 and 1687 cm^{-1}. The high resolution mass spectrum displayed an $(M+H)^+$ peak at m/e 586.2559 (Calcd. for $C_{33}H_{36}O_7N_3$, $(M+H)^+$ 586.2553). The Fmoc group was cleaved[38,39] from **383** by reaction with diethylamine in

acetonitrile at room temperature. Unfortunately, this did not lead to the desired amine **379**, but rather, to a cyclised product **384** within about five minutes (Scheme 13).

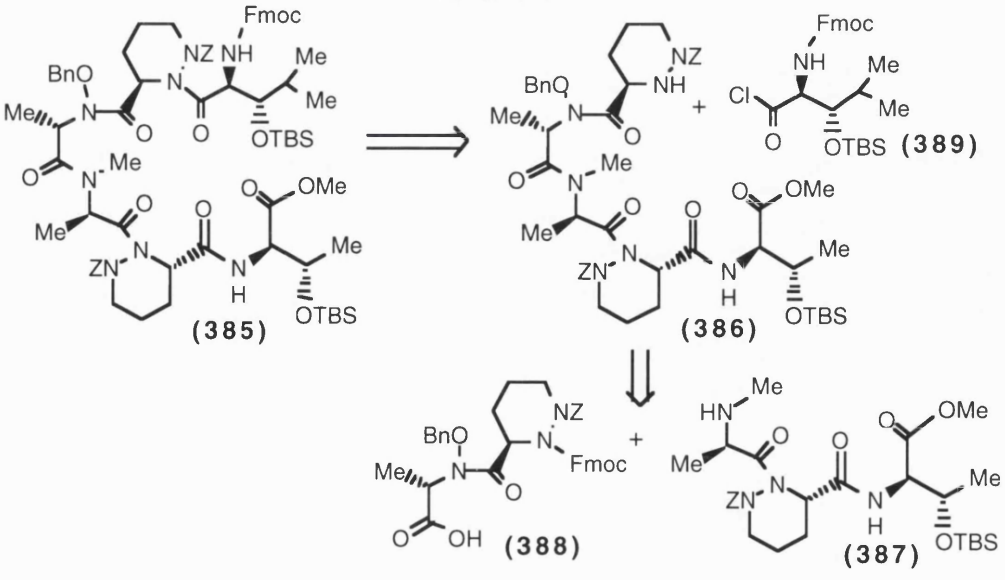
Scheme 13



The disappearance of the methyl ester singlets at δ 3.74 and 3.73 in the 400 MHz ^{1}H NMR spectrum of **384** in $CDCl_3$ strongly pointed to the cyclised product. Moreover, the infrared spectrum showed no NH stretch in the region of 3200 cm^{-1} and the presence of two tertiary amide carbonyl stretches at 1694 and 1661 cm^{-1} which lended additional support to this proposal. The high resolution mass spectrum of **384** further confirmed the proposed structure since there was an $(M+H)^+$ peak at m/e 332.1615 (Calcd. for $C_{17}H_{22}O_4N_3$, 332.1610). In our view, **384** was likely to be formed whatever amine protecting group was selected for the *N*-methyl alanine residue, and so the 2+2 fragment condensation route to tetrapeptide **378** was abandoned.

Our next strategy for obtaining **385** was through a 3+2+1 fragment condensation between **387**, **388** and **389** (Scheme 14).

Scheme 14



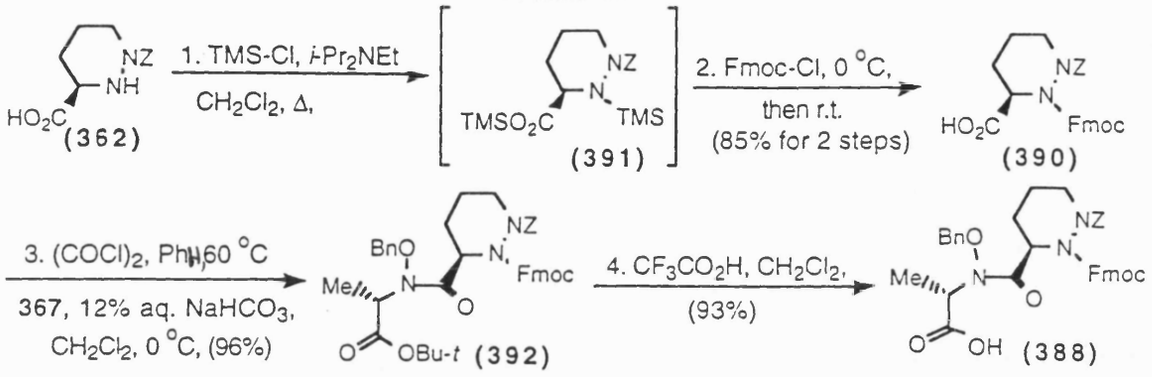
The synthesis of dipeptide **388** commenced with the synthesis of N^1-benzyloxycarbonyl -N^2-fluorenymethoxycarbonyl-(*3R*)-piperazic acid **390**. This was achieved by reacting **362** with chlorotrimethysilane (2 equiv, TMS-Cl) and diisopropylethylamine in dichloromethane at reflux to form **391**. The formation of **391** serves two functions. Firstly, it prevents the formation of any dipeptide impurities by preventing mixed anhydride formation. Secondly, preparing the *N*-silyl amine increases the reactivity of the amine due to the silicon moiety donating electron density towards the nitrogen. Without isolation, 9-fluorenylmethyl chloroformate (Fmoc-Cl)[40,41]was added to **391** at 0 °C to obtain **390** in 85% yield (Scheme 15).[42] The 400 MHz ^{1}H NMR spectrum of **390** in DMSO-d_6 at 100 °C contained apparent doublets at δ 7.80 (J = 7.6 Hz) and 7.60 (J = 7.1 Hz) due to the Fmoc aromatic protons. The absence of the sharp NH stretch at 3262 cm^{-1} in the infrared spectrum of **390** was taken as further evidence that acylation of the N^1-atom of **362** had taken place. The structure of **390** was confirmed by its high resolution mass spectrum, which revealed an $(M+H)^+$ peak at m/e 487.1853 (Calcd. for $C_{28}H_{27}N_2O_6$, $(M+H)^+$ 487.1869).

Compound **390** was converted to its acid chloride with oxalyl chloride (10 equiv) in benzene at 60 °C and coupled to α-hydroxamic acid derivative **367**, using the Carpino two-phase aqueous $NaHCO_3/CH_2Cl_2$ conditions[25] to deliver dipeptide **392** in 96% yield (Scheme 15). This compound had previously been prepared by Durette and Caldwell[8] but no spectra were available for comparision. It was apparent from the 400 MHz ^{1}H NMR spectrum of **392** at 125 °C in DMSO-d_6 that the coupling had given the correct product since there were resonances between δ 7.88-7.15 that integrated to eighteen protons, which were due to the aromatic protons. Moreover, there was a singlet superimposed on a multiplet between δ 1.41-1.36 which was attributable to the *t*-butyl ester group in the structure. HPLC analysis showed that the product was 99% pure (Figure 1).

Treatment of **392** with trifluoroacetic acid in dichloromethane afforded acid **388** in 93% yield (Scheme 15). The absence of the *t*-butyl singlet in the region δ 1.41-1.36 and the appearance of a broad singlet at δ 9.65 in the 400 MHz ^{1}H NMR spectrum of **388** in $CDCl_3$ confirmed that deprotection of the *t*-butyl ester group had occurred. The IR spectrum of **388** contained a broad absorption between 3431 and 3034 cm^{-1} which pointed to a carboxylic group.

Acid **388** gave a satisfactory C, H and N combustion microanalysis for empirical formula $C_{38}H_{37}N_3O_8$ (Calcd.: C, 68.77; H, 5.62; N, 6.33%. Found: C, 68.89; H, 5.55; N, 6.17%).

Scheme 15



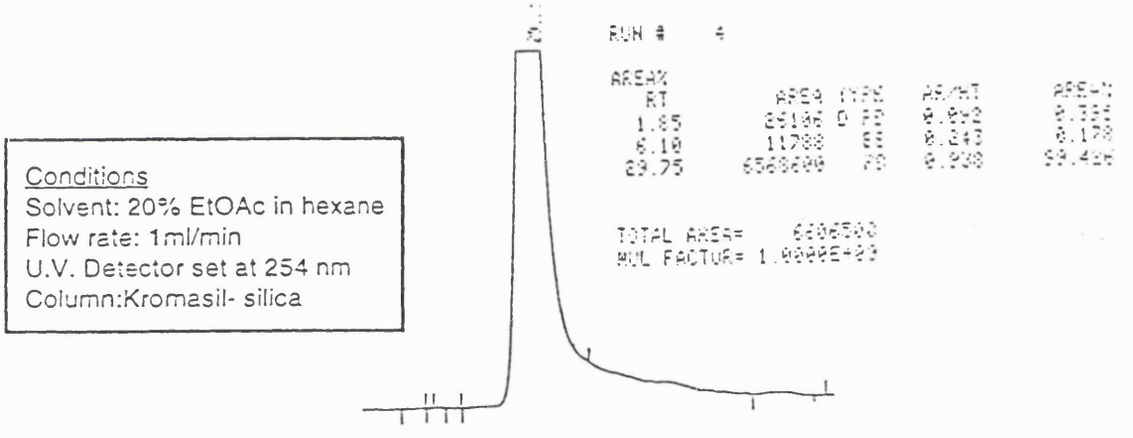
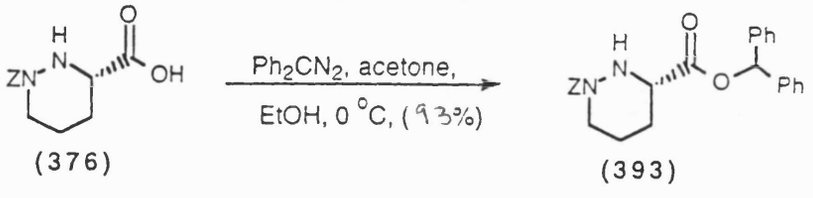


Figure 1: HPLC trace of dipeptide **392**.

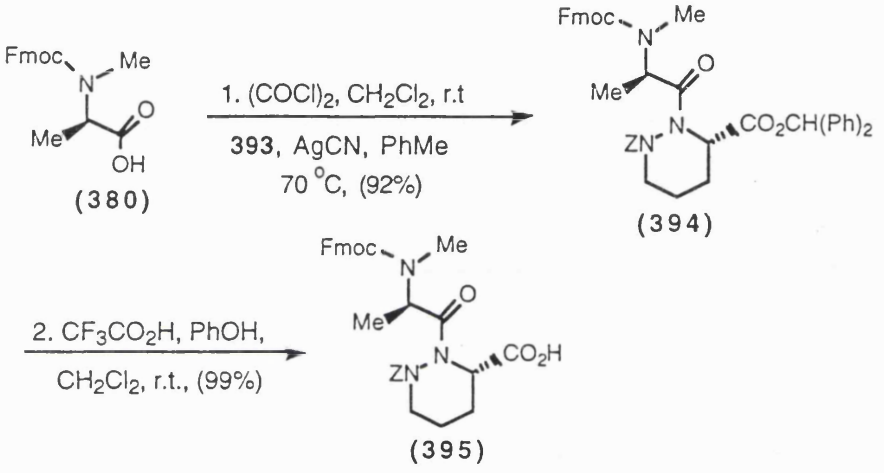
The synthesis of tripeptide **387** was initiated by preparing diphenylmethyl ester **393**. Thus, treatment of **376** with excess diphenyldiazomethane[43,44] in acetone gave **393** in 93% yield (Scheme 16). Evidence for the structure of **393** was provided by the 400 MHz ^{1}H NMR spectrum in $CDCl_3$ which displayed a multiplet from δ 7.36-7.24 that integrated to the fifteen aromatic protons, and a singlet at δ 6.92 which was due to the tertiary benzylic proton of the diphenylmethyl ester.

Scheme 16



Acid **380** was converted to its acid chloride with oxalyl chloride (10.0 equiv) in dichloromethane at 25 °C for 2 h. The acid chloride was then treated with a solution of **393** in toluene and silver cyanide (1.61 equiv.) at 70 °C to give dipeptide **394** in 92% yield after 1 h (Scheme 17). The 400 MHz ^{1}H NMR spectrum of **394** in $CDCl_3$ contained resonances for twenty three aromatic protons and showed the expected singlet at δ 6.90 for the tertiary proton of the diphenylmethyl ester. It also exhibited two singlets at δ 2.74 and 2.57 due to the *N*-methyl protons. HPLC analysis showed that coupling had occurred with minimal racemisation, compound 394 being obtained with 99% purity (Figure 2).

Scheme 17



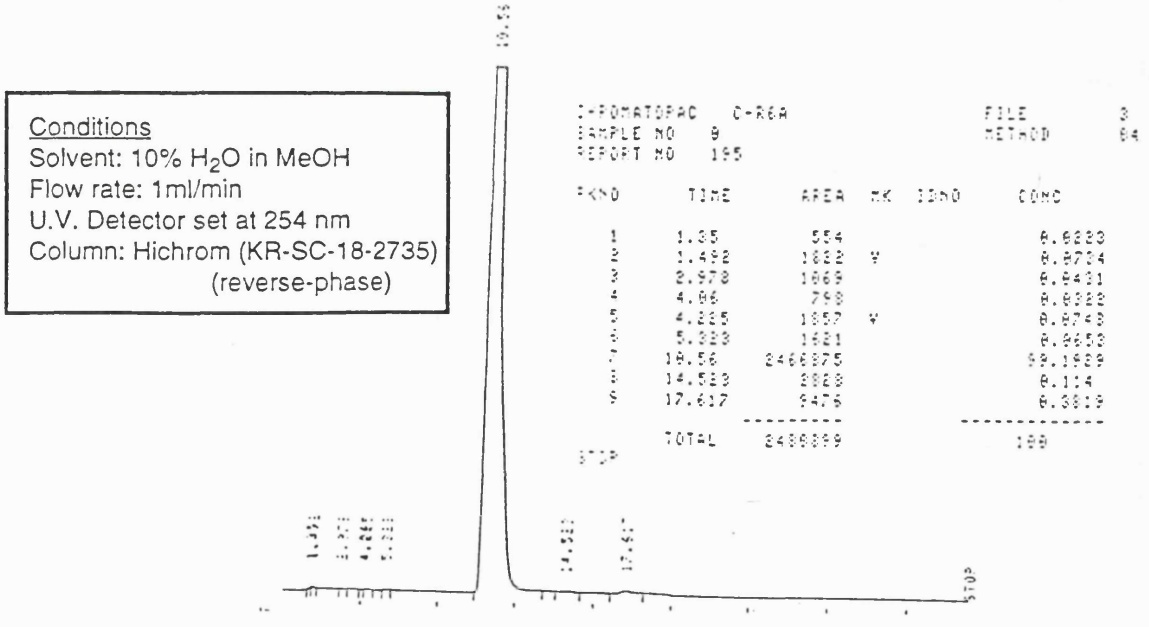


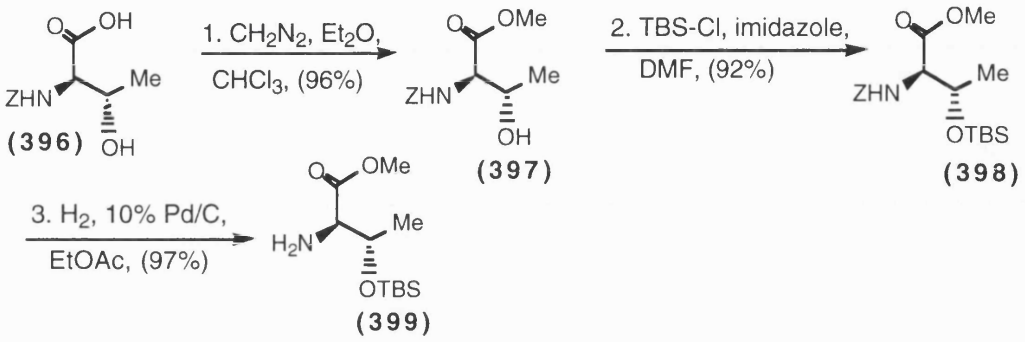
Figure 2: HLPC trace of dipeptide **394**

Removal of the diphenylmethyl ester from **394** was accomplished readily with trifluoro-acetic acid (12.5 equiv) and phenol (2.34 equiv) in dichloromethane[45] producing acid **395** in 99% yield.

The absence of a singlet at δ 6.90 in the 400 MHz ^{1}H NMR spectrum of **395** in DMSO-d_6 at 100 °C and the presence of a broad singlet at δ12.20 confirmed the identity of acid **395** (Scheme 17).

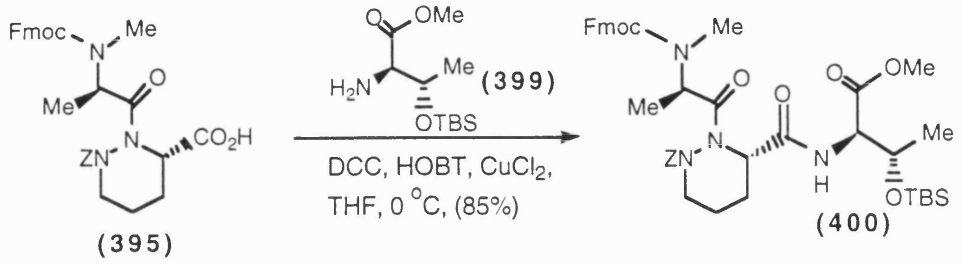
The D-threonine derivative **399** was synthesised in 3 steps starting from *N*-benzyloxy-carbonyl-D-threonine **396**. The sequence involved esterification with diazomethane, *O*-silylation with *t*-butyldimethyl-silyl chloride, and hydrogenation. This provided amine **399** in an overall yield of 94% as shown in Scheme 18.

Scheme 18



Several coupling protocols were investigated for the synthesis of tripeptide **400** from dipeptide **395** and threonine derivative **399**. These included the oxidation-reduction condensation of Mukaiyama with triphenylphosphine and 2,2'-dipyridyldisulphide,[28] the diphenylphosphinic mixed anhydride protocol of Kenner[46,47] and the *N,N*-diisopropylcarbodiimide method. However, the best results were obtained when dicyclohexylcarbodiimide (1.1 equiv), *N*-hydroxybenzotriazole (2.1 equiv) and cupric chloride (0.11 equiv) were employed in THF;[48] this regimen furnished **400** in 85% yield as a foam (Scheme 19). Reverse-phase HPLC showed that racemisation was negligible and the desired product was obtained in 98% purity (Figure 3). The IR spectrum of **400** contained an absorption at 3400 cm^{-1} which was reminiscent of a secondary amide NH stretch and one at 1531 cm^{-1} which was indicative of a secondary amide carbonyl group. It was apparent from the 400 MHz ^{1}H NMR spectrum of **400** in DMSO-d_6 at 75 °C that the tripeptide had been prepared since there were singlets at δ 3.64 and 3.55 attributable to the methyl ester, a broad singlet at δ 2.67 due to the *N*-methyl protons, and singlets at δ 0.81, 0.00 and -0.02 from the *t*-butyldimethylsilyl ether. Further proof of the structure was provided by the high resolution mass spectrum of **400**, which contained an $(M+Na)^+$ peak at m/e 823.3709 (Calcd. for $C_{43}H_{56}N_4O_9SiNa$, $(M+Na)^+$ 823.3714).

Scheme 19



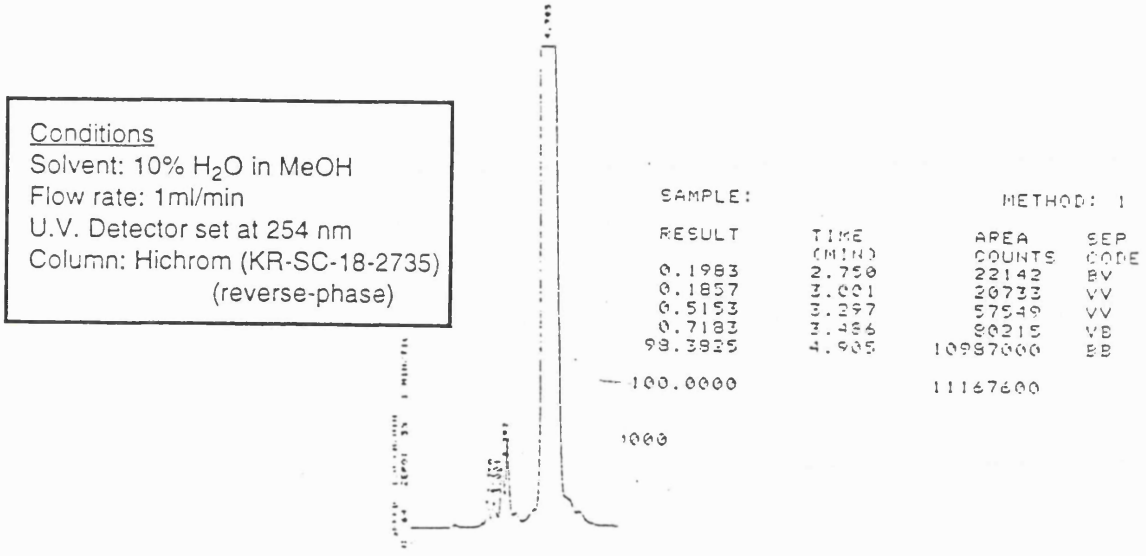
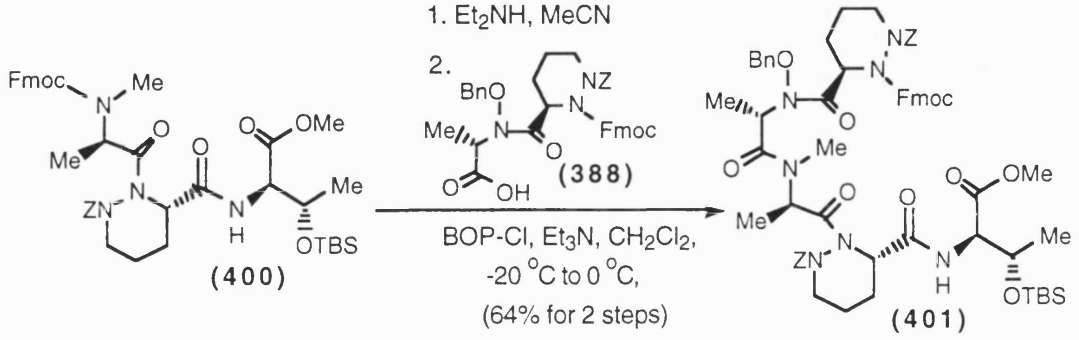


Figure 3: HPLC trace of tripeptide **400**.

Removal of the Fmoc group in **400** with the use of diethylamine in acetonitrile[39] gave amine **387**, which was then coupled with dipeptide **388** using *freshly recrystallised* bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[24] and triethylamine in dichloromethane at -20 °C (Scheme 20). Compound **401** gave a satisfactory C, H and N combustion microanalysis for $C_{66}H_{81}N_7O_{14}Si$ (Calcd.: C, 64.74; H, 6.67; N, 8.01%. Found: C, 64.41; H, 6.74; N, 7.82%). HPLC analysis of **401** indicated it to be of 95% purity (Figure 4).

Scheme 20



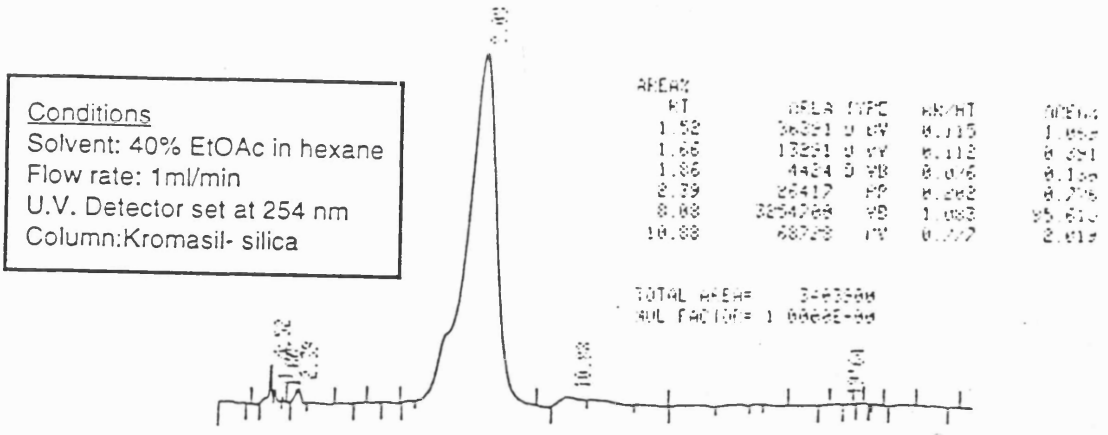
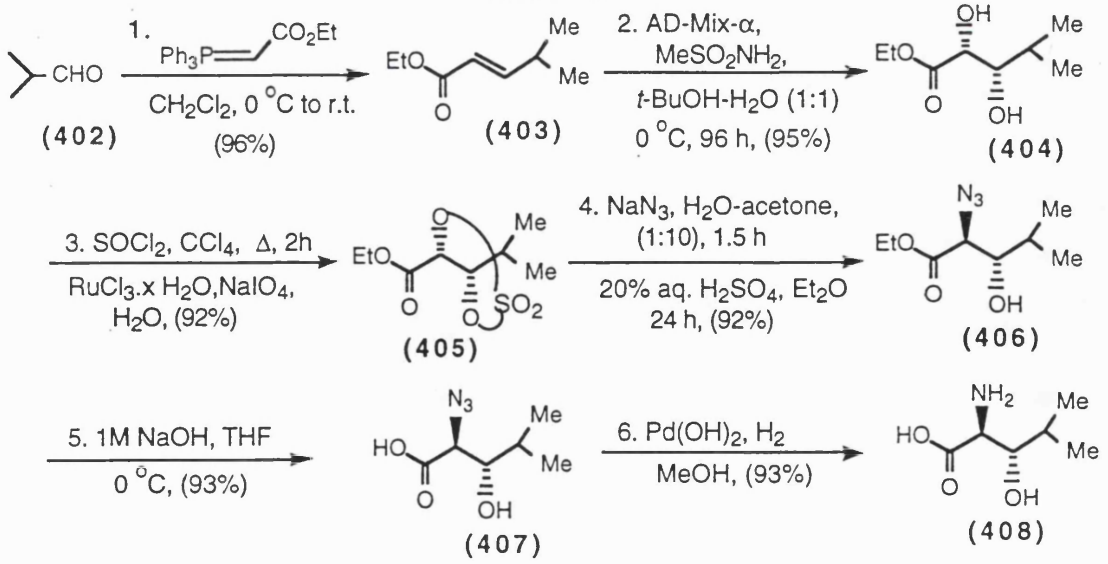


Figure 4: HPLC trace of pentapeptide **402**.

In order to complete the synthesis of the linear hexapeptide **385** we needed to synthesise the protected (*2S,3S*)-3-hydroxyleucine acid chloride **389**. Several elegant asymmetric synthesis of (*2S,3S*)-3-hydroxyleucine acid **408** has been reported over the years.[49-53] However, the most convenient route currently available to **408** is the one was developed in these laboratories.[54] The key steps in this new route are Sharpless asymmetric dihydroxylation[12] of α,β-unsaturated ester **403**, formation of cyclic sulphate **405** and S_N 2 displacement with sodium azide to form the α-azido ester **406**.[12,55] Hydrolysis of the ethyl ester with aqueous sodium hydroxide and subsequent hydrogenolysis with Pearlman's catalyst[56] typically furnish *erythro*-3-hydroxyleucine **408** in an overall yield of 57-67% and in an enantiomeric excess of 97% (Scheme 21).

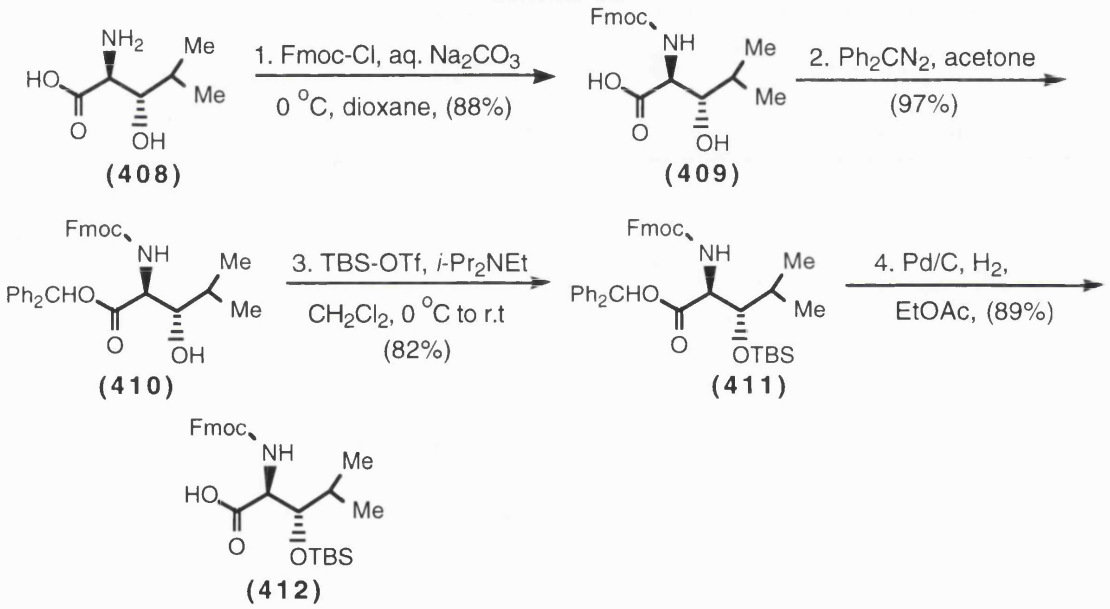
Scheme 21



(*2S,3S*)-3-Hydroxyleucine **408** was protected as its *N*-fluorenylmethoxycarbonyl derivative **409** by treatment with Fmoc-Cl and aqueous sodium carbonate in dioxane in 88% yield. The

400 MHz ^{1}H NMR spectrum of **409** in DMSO-d_6 contained a multiplet between δ 7.88-7.23 which was assignable to the Fmoc aromatic protons. The 100 MHz ^{13}C NMR spectrum of **409** in DMSO-d_6 contains two carbonyl resonances at δ 172.8 and 155.8 ppm. The accurate mass spectrum of **409** contained an $(M+H)^+$ peak at 370.1666 (Calcd. for $C_{21}H_{24}O_5N$, $(M+H)^+$ 370.1654). Silylation of **409** to obtain **412** directly was unsuccessful when *t*-butyldimethysilyl-chloride and imidazole or *N*-methylimidazole were employed.[57] Possibly, this may due to steric hindrance around the hydroxyl group from the *i*-propyl group. In order to *O*-silylate this hydroxy group, we had to first protect the acid as its diphenylmethyl ester **410** (Scheme 22). The presence of a singlet at δ 6.95 in the 400 MHz ^{1}H NMR spectrum of **410** in $CDCl_3$ confirmed its identity.

Scheme 22

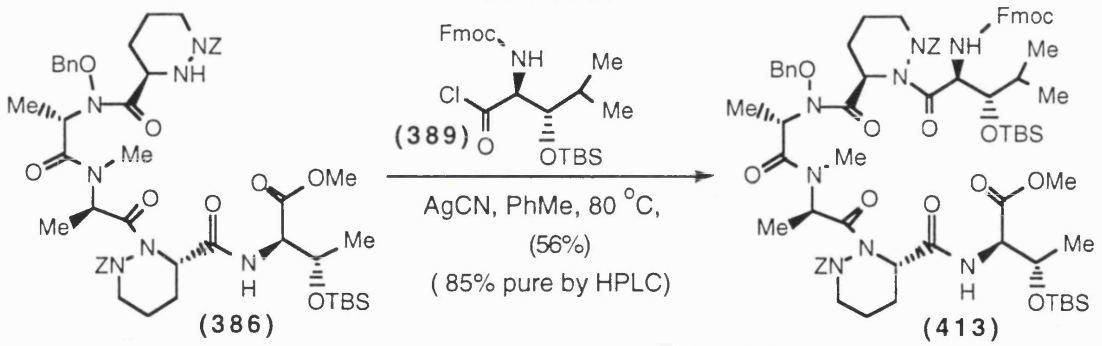


Silylation was then achieved in 82% yield by treatment of alcohol **410** with *t*-butyldimethylsilyl trifluromethanesulfonate (TBS-OTf, 1.2 equiv) and diisopropylethylamine (1.2 equiv) in dichloro-methane (Scheme 22). The structure of **411** was apparent from the 400 MHz ^{1}H NMR spectrum in $CDCl_3$, there now being resonances at δ 0.86 (s, 9H), 0.10 (s, 3H) and 0.07 (s, 3H) which were attributable to the silyl ether group. The 100 MHz ^{13}C NMR spectrum reveal *inter alia* resonances at δ -3.97 and -4.34 which were also due to the silyl ether group. The cleavage of the diphenyl-methyl ester by hydrogenolysis was of concern as the stability of the Fmoc group under these conditions can sometimes be a problem.[58,59] However, hydrogenation of **411** with 10% palladium on carbon in ethyl acetate gave the required acid **412** in a yield of 89% after column

chromatography. The absence of the singlet at δ 6.95 and the presence of a broad singlet at δ 10.30 in the 400 MHz ^{1}H NMR spectrum of **412** in $CDCl_3$ indicated the carboxylic acid unit had been unmasked. The high resolution mass spectrum of **412** contained an $(M+H)^+$ peak at m/e 484.2525 (Calcd. for $C_{27}H_{38}N_1O_5Si$, $(M+H)^+$ 484.2519). Acid **414** was then treated with oxalyl chloride in benzene at room temperature to give the acid chloride **389** (Scheme 22).

The desired hexapeptide **413** was synthesised in 56% yield by reacting the partially exposed acyl hydrazine **386** with acid chloride **389**, mediated by silver cyanide[36] in toluene at 80 °C (Scheme 23). However, this product was shown to be only 85% pure after reverse-phase preparative HPLC, eluting with 10% water in methanol (Figure 5). From the 400 MHz ^{1}H NMR spectrum in $CDCl_3$ the gross structure of **413** could be identified. There was a multiplet around δ 7.85 attributable to the Fmoc group, a singlet at δ 3.65 for the methyl ester, and singlets at δ 0.9 and 0.1 due to the silyl protecting groups. The structure of hexapeptide **413** was further confirmed by its high resolution mass spectrum, which contained an $(M+Na)^+$ peak at m/e 1489.7155 (Calcd. for $C_{78}H_{106}O_{16}N_8Si_2Na$, $(M+Na)^+$ 1489.7163).

Scheme 23



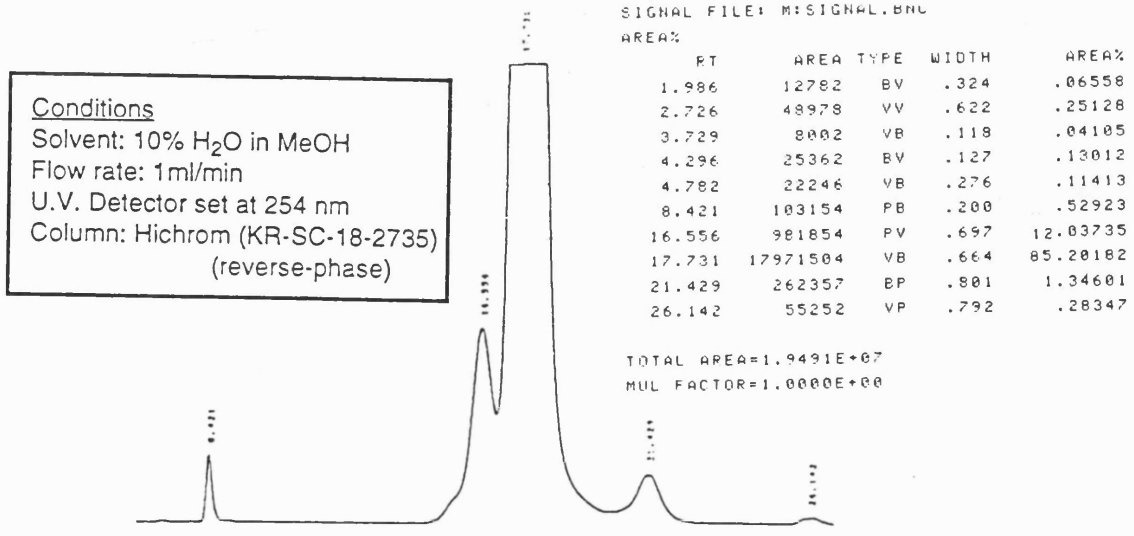
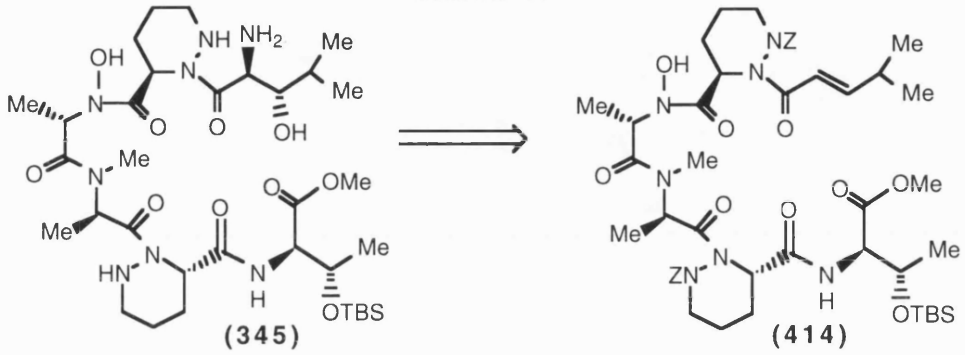


Figure 5: HPLC trace of hexapeptide **413**.

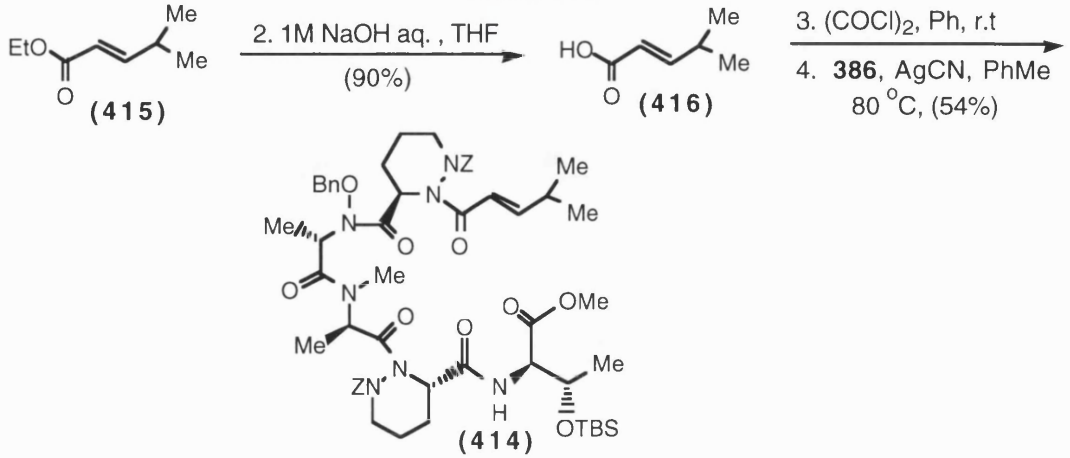
Due to our inability to completely purify **413**, we decided to investigate an alternative route to **345** commencing from alkene **414**. We hoped to dihydroxylate **414** with AD-mix-α, convert the resulting diol to its cyclic sulfate, displace with azide ion α- to the amide carbonyl, and hydrogenate the azide to obtain amine **345** (Scheme 24).

Scheme 24



Acid **416** was converted to its acid chloride by treatment with oxalyl chloride (10 equiv) in benzene at room temperature for 1 h. This was then reacted with the pentapeptide acyl hydrazine **386** in the presence of silver cyanide (2 equiv) in toluene at 80 °C[36] to give **414** in 53% yield after reverse-phase HPLC (Scheme 25). The 400 MHz ^{1}H NMR spectrum of **414** in DMSO-d_6 at 125 °C contained two double-doublets at δ 6.80 (J = 6.6, 15.5 Hz) and 6.13 (J =15.5, 1.0 Hz) which supported the presence of an (*E*)-α,β-unsaturated amide linkage. From the reverse-phase HPLC trace, compound **414** was 96.7% pure (Figure 6). Compound **414** also gave a satisfactory C, H and N combustion microanalysis for $C_{57}H_{79}N_7O_{13}Si$ (Calcd.: C, 62.33; H, 7.25; N, 8.93%. Found: C, 62.07; H, 7.29; N, 8.86%), thus confirming the structure of the hexapeptide precursor **414**.[60]

Scheme 25



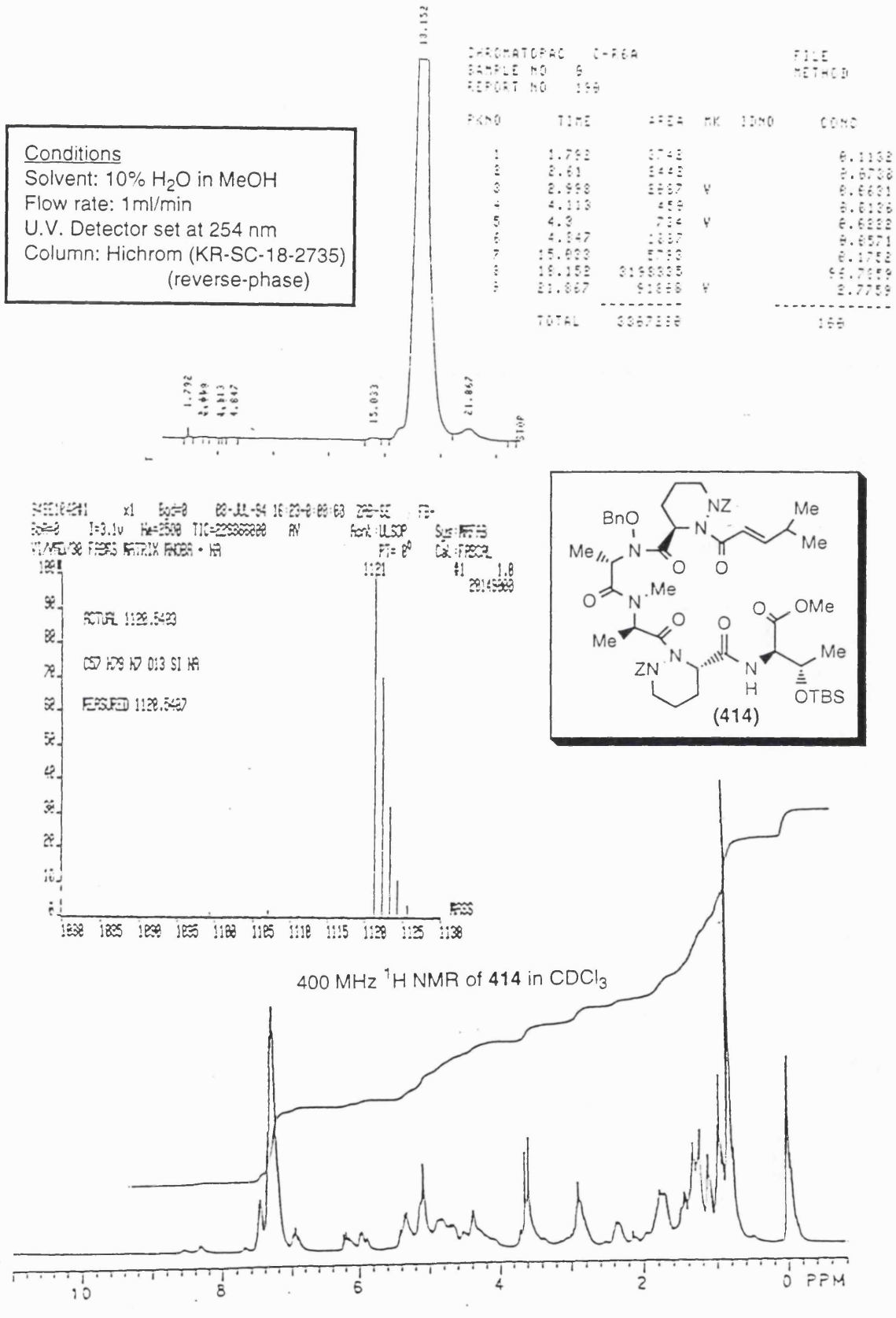


Figure 6: HPLC trace, accurate mass and 400 MHz ^{1}H NMR spectra of hexapeptide precursor **414**

In conlusion, we have completed an efficient enantiospecific synthesis of an advance hexapep-tide precursor for A83586C. Other workers in the group are currently attempting to convert **414** into amine **345** with a view to completing the synthesis of A83586C.

3.4 References

(1) Corey, E. J.; Nicolaou, K. C., *J. Amer. Chem. Soc.*, **1974**, *96*, 5614.

(2) Corey, E. J.; Brunelle, D. J., *Tetrahedron Lett.*,**1976**, 3409.

(3) Kurihara, T.; Nakajima, Y.; Mitsunobu, O., *Tetrahedron Lett.*, **1976**, 2455.

(4) Masamune, S., *Aldrichimica Acta.*, **1978**, *11*, 23.

(5) Kageyama, M.; Tamura, T.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D.; Masamune, S., *J. Amer. Chem. Soc.*, **1990**, *112*, 7407.

(6) Mukaiyama, T., *Angew. Chem. Int. Ed. Engl.*, **1979**, *18*, 707.

(7) Heyns, K.; Paulsen, H., *Adv. Carbohydrate Chem.*, **1962**, *17*, 169.

(8) Durette, P. L.; Baker, F.; Barker, P. L.; Boger, J.; Bondy, S. S.; Hammond, M. L.; Lanza, T. L.; Pessolano, A. A.; Caldwell, C. G. , *Abs. 198th ACS National Meeting* **1989**, *Miami Beach, Florida*, September 10-15, Abstract No.119.

(9) Evans, D. A.; Bartroli, J.; Shih, T. L., *J. Amer. Chem. Soc.*, **1981**, *103*, 2127.

(10) Baker, R.; Castro, J. L., *J. Chem. Soc., Perkin Trans. 1*, **1990**, 47.

(11) Takano, S.; Akiyama, M.; Sato, S.; Ogasawara, K., *Chem. Lett.*, **1983**, 1593.

(12) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-W.; Xu, D.; Zhang, X.-L., *J. Org. Chem.*, **1992**, *57*, 2768.

(13) Hale, K. J.; Bhatia, G. S.; Peak, S. A.; Manaviazar, S., *Tetrahedron Lett.*, **1993**, *34*, 5343.

(14) Bhatia, G. S. *Ph.D Thesis,* **1995**, University of London.

(15) Manaviazar, S. *Ph.D Thesis,* **1995**, University of London.

(16) Hale, K. J.; Manaviazar, S.; Peak, S. A., *Tetrahedron Lett.*, **1994**, *35*, 425.

(17) Hale, K. H.; Delisser, V. M.; Manaviazar, S., *Tetrahedron Lett.*, **1992**, *33*, 7613.

(18) Hassall, C. H.; Johnson, W. H.; Theobald, C. J., *J. Chem . Soc., Perkin Trans I,* **1979**, 1451.

(19) Feenstra, R. W.; Stokkingreef, E. H. M.; Nivard, R. J. F.; Ottenheijm, H. C. J., *Tetrahedron Lett.,* **1987**, *28*, 1215.

(20) McDermott, J. R.; Benoiton, N. L., *Can. J. Chem.,* **1973**, *51*, 1915.

(21) Chen, F. M. F.; Benoiton, N. L., *Can. J. Chem.,* **1977**, *55*, 906.

(22) Diago-Meseguer, J.; Palomo-Coll, A. L.; Fernandez-Lizarde, J. R.; Zugaza-Bilbao, A., *Synthesis,* **1980**, 547.

(23) Tung, R. D.; Rich, D. H., *J. Am. Chem. Soc.,* **1985**, *107*, 4342.

(24) Auwera, C. V.; Anteunis, M. J. O., *Int. J. Peptide Protein Res.,* **1987**, *29*, 574.

(25) Carpino, L. A.; Cohen, K. E.; Stephens, K. E.; Sadat-Aalaee, S. Y.; Tien, J.-H.; Langridge, D. C., *J. Org. Chem.,* **1986**, *51*, 3732.

(26) Carpino, L. A.; El-Sayed, M. E.; Sadat-Aalaee, D.; Sadat-Aalaee, M., *J. Org. Chem.,* **1991**, *56*, 2611.

(27) Tung, R.; Dhaon, M. K.; Rich, D. H., *J. Org. Chem.,* **1986**, *51*, 3350.

(28) Mukaiyama, T.; Matesueda, R.; Suzuki, M., *Tetrahedron Lett.,* **1970**, 1901.

(29) Kirtgen, R.; Sheppard, R. C.; Steglich, W., *J. Chem. Soc. Chem. Commun.,* **1987**, 1870.

(30) Konig, W.; Geiger, R., *Chem. Ber.,* **1970**, *103*, 788.

(31) Staab, H. A., *Justus Liebigs Ann. Chem.,* **1957**, *75*, 609.

(32) Staab, H. A., *Angew. Chem.,* **1959**, *71*, 194.

(33) Freidinger, R. M.; Hinkie, J. S.; Perlow, D. S.; Arison, B. H., *J. Org. Chem.,* **1983**, *48*, 77.

(34) Ben-Ishai, D., *J. Amer. Chem. Soc.,* **1957**, *79*, 5736.

(35) Auerbach, J.; Zamore, M.; Weinred, S. M., *J. Org. Chem.,* **1976**, *41*, 725.

(36) Williams, P. D.; Bock, M. G.; Tung, R. D.; Garsky, V. M.; Perlow, D. S.; Erb, J. M.; Lundell, G. F.; Gould, N. P.; Whitter, W. L.; Hoffman, J. B.; Kaufman, M. J.; Clineschmidt, B. V.; Pettibone, D. J.; Freidinger, R. M.; Veber, D. F., *J. Med. Chem.,* **1992**, *35*, 3905.

(37) Durette, P. L.; Baker, F.; Barker, P.L.; Boger, J.; Bondy, S.; Hammond, M.L.; Lanza, T. J.;Pessolano, A. A.; Caldweli, C. G., *Tetrahedron Lett.,* **1990**, *31*, 1237.

(38) Carpino, L. A.; Han, G. Y., *J. Amer. Chem. Soc.,* **1970**, *92*, 5478.

(39) Carpino, L. A.; Han, G. Y., *J. Org. Chem.,* **1973**, *38*, 4318.

(40) Brown, W. G.; Bluestein, B. A., *J. Amer. Chem. Soc.,* **1943**, *65*, 1082.

(41) Carpino, L. A.; Han, G. Y., *J. Org. Chem.,* **1972**, *37*, 3404.

(42) Bolin, D. R.; Sytwu, I. I.; Humiec, F.; Meienhofer, J., *Int. J. Peptide Protein Res.,* **1989**, *33*, 353.

(43) Smith, C. I.; Howard, K. L., *Org. Synthesis III* , **1943**, 351.

(44) Miller, J. B., *J. Org. Chem.,* **1959**, *24*, 560.

(45) Stelakatos, G. C.; Paganou, A.; Zervas, L., *J. Chem. Soc. (C),* **1966**, 1191.

(46) Galpin, I. J.; Hancock, F. E.; Handa, R. B.; Jackson, A. G.; Kenner, G. W.; McDowell, P.; Noble, P.; Ramage, R., *Tetrahedron,* **1981**, *37*, 979.

(47) Jackson, A. G.; Kenner, G. W.; Moore, G. A.; Ramage, R.; Thrope, W. D., *Tetrahedron Lett.,***1976**, *17,* 3627.

(48) Miyazawa, T.; Otomatsu, T.; Fukui, Y.; Yamada, T.; Kuwata, S., *Int. J. Peptide Protein Res.,* **1992**, *39*, 237.

(49) Jung, M. E.; Jung, Y. H., *Tetrahedron Lett.,* **1989**, *30*, 6637.

(50) Caldwell, C. G.; Bondy, S. S., *Synthesis,* **1990**, 34.

(51) Corey, E. J.; Lee, D.-H.; Choi, S., *Tetrahedron Lett.,* **1992**, *33*, 6735.

(52) Sunazuka, T.; Nagamitsu, T.; Tanaka, H.; Omura, S.; Sprengeler, P. A.; Smith, I., A.B., *Tetrahedron Lett.,* **1993**, *34*, 4447.

(53) Kanemasa, S.; Mori, T.; Tatsukawa, A., *Tetrahedron Lett.,* **1993**, *34*, 8293.

(54) Hale, K. J.; Manaviazar, S.; Delisser, V. M. ,*Tetrahedron,* **1994**, *50*, 9181.

(55) Oi, R.; Sharpless, K. B., *Tetrahedron Lett.,* **1991**, *32*, 999.

(56) Pearlman, W. M., *Tetrahedron Lett.,* **1967**, 1663.

(57) Fischer, P. M., *Tetrahedron Lett.,* **1992**, *33*, 7605.

(58) Atherton, E.; Bury, C.; Sheppard, R. C.; Williams, B. J., *Tetrahedron Lett.,* **1979**, 3041.

(59) Martinez, J.; Tolle, J. C.; Bodanszky, M., *J. Org. Chem.,* **1979**, *44*, 3596.

(60) Hale, K. J.; Delisser, V. M.; Yeh, L.-K.; Peak, S. A.; Manaviazar, S.; Bhatia, G. S., *Tetrahedron Lett.,* **1994**, *41*, 7685.

Chapter 4. Experimental

Apparatus and Materials

The reactions were carried out under an inert atmosphere (N_2) with freshly distilled solvents which were dried with CaH_2 under nitrogen. Reaction progress was monitored with precoated silica gel plates (250μm) with a fluorescent indicator (E. Merck). Flash chromatography was carried out with Sorbsil C60 40/60A (230-400 mesh) silica gel. Melting points were recorded on a Kofler hot stage melting point apparatus and are not corrected. The infrared spectra were recorded on a Nicolet model 205 FT-IR spectrometer and absorptions were recorded in terms of frequency (ν_{max}) in cm^{-1}.

Proton and carbon-13 nuclear magnetic resonance (NMR) spectra were recorded on a Varian AX-400 (400 MHz) spectrometer and are reported in δ values relative to tetramethylsilane. The signals were assigned as follows singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) and double doublet (dd). The spectra were recorded in deuterochloroform ($CDCl_3$), deuterated dimethylsulphoxide (DMSO-d_6) or deuterated benzene (C_6D_6) solution. High resolution mass spectra were measured at the London School of Pharmacy on a V.G. 7070H or VG-ZAB with a Finnigan Incos II data system.

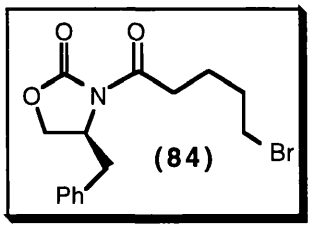
Optical rotations were measured on either an Optical Activity AA10 automatic polarimeter or a Perkin Elmer 141 polarimeter. Microanalysis were carried out on a Perkin Elmer 2400 CHN Elemental Analyser in the Microanalytical Laboratory at University College London.

High pressure liquid chromatography (HPLC) was performed on a Gilson analytical chromatograph equipped with a Gilson 303 and 305 pump system, a Gilson 811b dynamic mixer, a Gilson 805s manometric module and a Gilson 115 U.V. absorbance detector set at 254 nm, or a refractive index detector. The columns used are either Chiracel-OD (chiral),a Hichrom (KR-SC18-2735) (reverse-phase) or a Kromasil-silica.

1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) was dried over activated 4A molecular sieves and stored under nitrogen.

Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) was recrystallised from dry MeCN prior to use for coupling reactions.

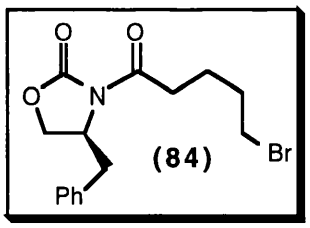
(*4S*)-5-(5-Bromovaleryl)-4-(phenylmethyl)-2-oxazolidinone 84



(84)

To a stirred solution of (*4S*)-4-(phenylmethyl)-2-oxazolidinone **80** (15.0 g, 84.8 mmol) in dry THF (150.0 ml) under nitrogen at -78 °C was added *n*-butyllithium (58.3 ml, 1.6 M in hexanes, 93.2 mmol) dropwise over 5 min and the resulting yellow solution stirred for 40 min. 5-Bromovaleryl chloride (14.7 ml, 110 mmol) was then added dropwise over 10 min. After 15 min, the cooling bath was removed and the reactants allowed to warm to room temperature and stirred for a further 2 h. The mixture was then diluted with EtOAc (100 ml) and saturated aqueous ammonium chloride and the EtOAc layer removed. The aqueous layer was extracted with EtOAc (3 x 80 ml) and the combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The syrupy residue crystallised from cold hexanes when left overnight at 4 °C to give **84** (26.0 g, 91%) as a white solid. An analytical sample of **84** was obtained by recrystallisation from hexanes/ EtOAc; m.p. 66-67 °C; $[\alpha]_D$ + 88° (*c* 1, MeOH); IR (KBr): 3552 (w), 3323 (w), 3071 (w), 3032 (w), 2956 (w), 1792 (s), 1701 (s), 1441 (m), 1377 (s), 1307 (m), 1244 (s), 1248 (s), 1202 (s), 1144 (m), 1100 (m), 1052 (m), 1020 (m), 951 (m), 761 (w), 750 (m), 544 (m), 488 (w) cm^{-1}; 400MHz ^1H NMR ($CDCl_3$): δ 7.35-7.16 (m, 5H), 4.68-4.62 (m, 1H), 4.22-4.14 (m, 2H), 3.43 (dd, *J* = 6.4, 13.1 Hz, 2H), 3.25 (dd, *J* = 3.2,13.2 Hz, 1H), 3.03-2.87 (m, 2H), 2.75 (dd, *J* = 9.61, 3.48 Hz, 1H), 1.98-1.82 (m, 4H); 100 MHz ^{13}C NMR ($CDCl_3$): δ 172.5, 153.3, 135.1, 129.3, 128.9, 127.3, 66.3, 55.0, 37.8, 34.5, 33.1, 31.9, 22.7; Acc. Mass Calcd. for $C_{15}H_{19}NO_3Br$ $(M+H)^+$ 340.0548; Found: 340.0544; Anal. Calcd. for $C_{15}H_{18}NO_3Br$: C, 52.95; H, 5.33; N, 4.12; Br, 23.49%. Found: C, 52.78; H, 5.16; N, 3.95; Br, 23.27%.

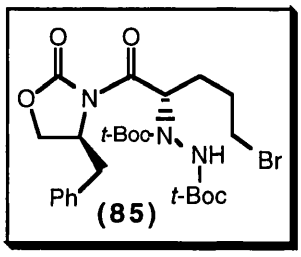
(*4S*)-3-(5-Bromovaleryl)-4-(phenylmethyl)-2-oxazolidinone 84 via a mixed anhydride



(84)

To a stirred solution of 5-bromovaleric acid (6.0 g, 36.4 mmol) in dry Et_2O (150 ml) under nitrogen was added dry triethylamine (5.1 ml, 36.0 mmol). The mixture was cooled to -78 °C and trimethylacetyl chloride (4.4 ml, 36.0 mmol) added dropwise over 4 min. The cooling bath was removed and the white suspension allowed to warm to room temperature with vigorous stirring for 1 h. In a separate flask charged with a solution of (*4S*)-4-(phenylmethyl)-2-oxazolidinone **80** (5.32 g, 30.0 mmol) in dry THF (40 ml) at -78 °C was added dropwise *n*-BuLi (21.0 ml, 1.6 M in hexanes, 33.0 mmol) and the reaction mixture stirred at -78 °C for 40 min. The lithiated oxazolidinone solution was then added to the recooled (-78 °C) mixed anhydride solution via cannula. The reaction mixture was warmed to room temperature, stirred for 1 h, and then quenched with saturated aqueous ammonium chloride and extracted with EtOAc (3 x 60 ml). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* The syrupy residue crystallised from cold hexanes/EtOAc when left overnight in the refrigerator to give **84** (9.0 g, 89%) as a white solid.

Bromovaleryl Hydrazide 85

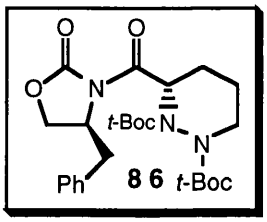


(85)

To a stirred solution of *N,N*-diisopropylamine (0.44 ml, 3.0 mmol) in dry THF (5 ml) under nitrogen at -60 °C was added *n*-BuLi (1.24 ml, 1.6 M in hexanes, 3.0 mmol) dropwise over 5 min and the solution stirred for 1 h. The resulting LDA solution was then cooled to -78 °C whereupon a precooled solution of bromide **84** (1.0 g, 3.0 mmol) in dry THF (5 ml) at -78 °C was added via cannula over 5 min. After 30 min at -78 °C, a precooled solution of di-*tert*-butylazodicarboxylate

(820 mg, 3.5 mmol) in CH_2Cl_2 (8 ml) at -10 °C was added via cannula to the enolate solution at -78 °C over 10 min. The mixture was stirred for 40 min at -78 °C and then quenched with glacial acetic acid (0.45 ml, 7.8 mmol). The mixture was partitioned between Et_2O (75 ml) and 1.25 M aqueous KH_2PO_4 (50 ml), the Et_2O layer was removed, and the aqueous layer,extracted with Et_2O (3x, 40 ml). The combined ethereal extracts were washed successively with H_2O, and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography using Et_2O/ hexanes (3:2) gave **85** (1.43 g, 84%) as a foam; IR (KBr): 3360 (br m), 2979 (s), 2933 (s), 1790 (br s), 1697 (br s), 1479 (m), 1455 (m), 1392 (br s), 1354 (br s), 1110 (m), 1052 (m), 1012 (w), 853 (w), 762 (m), 704 (m) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6, at 100 °C): δ 8.34-8.00 (br s, 1H, NH), 7.38-7.21 (m, 5H, ArH), 5.67-5.54 (m, 1H), 4.71-4.61 (m, 1H), 4.35 (t, *J* = 8.2 ,16.9 Hz, 1H), 4.18 (dd, *J* = 3.5, 5.7 Hz,1H), 3.53-3.50 (m, 2H), 3.19-3.04 (m, 2H), 2.96-2.87 (m, 1H), 2.08-1.78 (m, 3H), 1.44 (s, 9H), 1.43 (s, 9H); Acc. Mass Calcd. for $C_{25}H_{36}N_3O_7BrNa$ $(M+Na)^+$, 592.1634; Found: 592.1642.

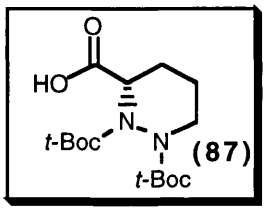
(*4S*)-3-(*3S-N,N'*-bis-(*t*-butoxycarbonyl)hexahydropyridazine-3-carboxy)-4-phenylmethyl)-2-oxazolidinone 86



To a stirred solution of *N,N*-diisopropylamine (8.69 ml, 60.0 mmol) in dry THF (100 ml) under nitrogen at -60 °C was added *n*-BuLi (24.8 ml, 1.6 M in hexanes, 60.0 mmol) dropwise over 5 min and the solution stirred for 1 h. The resulting LDA solution was then cooled to -78 °C whereupon a precooled solution of bromide **84** (20.0 g, 60.0 mmol) in dry THF (100 ml) at -78 °C was added via cannula over 5 min. After 30 min at -78 °C, a precooled solution of di-*tert*-butylazodicarboxylate (16.3 g, 70.0 mmol) in CH_2Cl_2 (150 ml) at -10 °C was added via cannula to the enolate solution at -78 °C over 10 min. The mixture was then stirred for 40 min at -78 °C whereupon 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) (150 ml) was added dropwise over 1 h. By the time the DMPU addition was complete, the reaction mixture had become frozen. The frozen reactants were then allowed to warm to room temperature and stirred

for an additional 2 h before being poured into a mixture of Et_2O and a 1.25 M solution of KH_2PO_4. The Et_2O layer was removed and the aqueous layer extracted with Et_2O (4 x 100 ml). The com-bined ethereal extracts were washed successively with H_2O (3x, 70 ml) and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* Purification of the residue by flash chromatography with hexanes/ Et_2O (2:3) gave **86** (17.9 g, 63%) as a foam; $[\alpha]_D$ +35.8° (*c* 0.5, MeOH); IR (KBr): 2979 (m), 2943 (w), 2933 (w), 1784 (s), 1700 (s), 1477 (w), 1450 (w), 1393 (s), 1367 (m),1254 (w), 1165 (m), 1102 (m), 720 (w), 657 (w) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6 at 125 °C): δ 7.34-7.20 (m, 5H, ArH), 5.91 (br s, 1H), 4.73-4.68 (m, 1H), 4.38-4.27 (m, 1H), 4.22-4.10 (m, 1H), 4.08-3.80 (br s, 1H), 3.14-3.05 (br d, 1H), 3.00-2.81 (m, 2H), 2.01-1.72 (br m, 3H) 1.68-1.51 (br m, 1H) 1.47 (s, 9H), 1.43 (s, 9H); Acc. Mass Calcd. for $C_{25}H_{36}N_3O_7$ $(M+H)^+$, 490.255; Found: 490.255; Anal. Calcd. for $C_{25}H_{35}N_3O_7$: C, 61.23; H,7.21; N, 8.59 %; Found: C, 60.90; H, 7.35; N, 8.59%.

(*3S*)-*N*,*N'*-Bis-(*t*-butoxycarbonyl)hexahydropyridazine-3-carboxylic acid 87

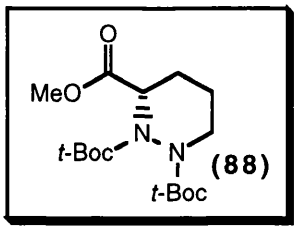


(87)

To a solution of **86** (15.2 g, 30.0 mmol) in THF (123.0 ml) at -10 ° was added via a pipette a chilled (-5 °C) suspension of lithium hydroxide monohydrate (3.0 g, 70.0 mmol) in H_2O (61 ml) over 7 min. The mixture was vigorously stirred between -10 °C and 0 °C for 2 h. The aqueous layer was then extracted with Et_2O (4 x 80 ml) and the combined organic layers washed with H_2O (2 x 15 ml) dried over $MgSO_4$, filtered, concentrated *in vacuo* and the oxazolidinone was recovered after flash chromatography and recrystallisation (67%). All the aqueous fractions containing **87** as its lithium carboxylate salt were combined, cooled in an ice bath, and acidified with aqueous $NaHSO_4$ (1 M) to pH 2. Compound **87** was extracted with EtOAc (3 x 100 ml). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **87** (8.6 g, 84%) as a white solid. An analytical sample was obtained by recrystallisation with hexanes/EtOAc; m.p. 112-115 °C; $[\alpha]_D$-13° (*c* 1, MeOH); IR (KBr): 3204 (br m), 2986 (w), 1737 (br m), 1704 (br m), 1670 (br m), 1456 (br m), 1431 (br w), 1394 (br w), 1371

(w), 1153 (br m), 1136 (br m), 1090 (m), 880 (s), 753 (m), 738 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 11.50-10.0 (br s, 1H, CO_2**H**), 5.21-6.64 (m, 1H), 4.17- 3.91 (m, 1H), 3.38-2.78 (br m, 1H), 2.43-1.45 (complex m, 22H); 100 MHz ^{13}C NMR ($CDCl_3$): δ 171.8, 170.5, 152.4, 83.6, 83.59, 83.2, 44.15, 42.1, 28.5, 28.4, 28.1, 28.06, 28.0, 23.8, 20.63, 20.6, 20.55, 20.2; Acc. Mass Calcd. for $C_{15}H_{27}N_2O_6$ $(M+H)^+$, 331.1869; Found: 331.1868; Anal. Calcd. for $C_{15}H_{26}N_2O_6$: C, 54.53; H, 7.93; N, 8.48%; Found: C, 54.5, 8.07; N, 8.37%.

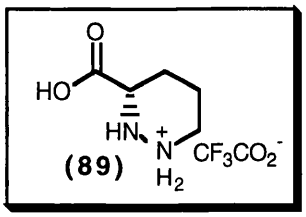
(*3S*)-*N*,*N'*-Bis-(*t*-butoxycarbonyl)hexahydropyridazine-3-carboxylic acid methyl ester 88



(88)

To a solution of **87** (200.0 mg, 0.6 mmol) in chloroform (4 ml) at 0 °C was added dropwise ethereal diazomethane until the reaction mixture went pale yellow. The mixture was then purged with nitrogen and concentrated *in vacuo*. The oily residue was purified by flash chromatography with hexanes/EtOAc (7:1) to give **88** (160.0 mg, 78%) as a clear oil; $[\alpha]_D$ -35.1° (*c* 1, CH_2Cl_2); IR (neat film): 2979 (s), 2934 (w), 1739 (s), 1703 (s), 1478 (w), 1456 (w), 1394 (m), 1367 (m), 1315 (w), 1280 (w), 1253 (m), 1169 (m), 1129 (m), 1086 (m), 880 (m), 750 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 5.02-4.85 (br m), 4.80-4.67 (br s), 4.17-3.84 (br m), 3.67 (br s), 2.98-2.69 (br m, 1H), 2.14-1.62 (complex m), 1.44 (s), 1.41 (s); 100 MHz ^{13}C NMR ($CDCl_3$): δ 170.4, 154.5, 81.6, 80.2, 54.4, 51.9, 42.6, 28.3, 27.9, 24.8, 20.0; Acc. Mass Calcd. for $C_{16}H_{29}N_2O_6$ $(M+H)^+$ 345.2026; Found, 345.2021; Anal. Calcd. for $C_{16}H_{28}N_2O_6$: C, 55.80; H, 8.19; N, 8.13%; Found: C, 55.58; H, 8.10; N, 8.08%.

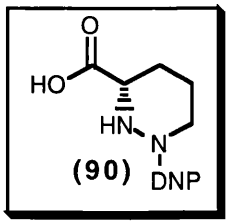
(*3S*)-Hexahydropyridazine-3-carboxylic acid trifluoroacetic acid salt 89



(89)

To a solution of acid **87** (8.57 g, 30.0 mmol) in dry CH_2Cl_2 (86 ml) under nitrogen was added CF_3CO_2H (86 ml, 1.11 mol) via syringe and the mixture stirred for 2 h. The reaction mixture was then concentrated *in vacuo*. Et_2O was then added to the solid residue and the resulting suspension placed in the fridge for 2 h. Filtration and washing of the precipitate with cold Et_2O gave **89** (6.30 g, 100%) as a white solid. An analytical sample was obtained by recrystallisation from EtOAc/EtOH; m.p. 139-141 ^oC; $[\alpha]_D$ +18.7^o (*c* 0.48, MeOH); IR (KBr): 3634-2250 (br), 3464 (br), 3178 (m), 2980 (m), 2914 (w), 2751 (w), 1722 (s), 1666 (s), 1590 (m), 1514 (m), 1422 (m), 1205 (br m), 1133 (w), 1061 (w), 934 (w), 900 (m), 844 (m), 785 (m), 723 (w), 607 (w), 523 (w) cm^{-1} ; 400 MHz ^1H NMR (D_2O): δ 3.75-3.66 (m, 1H), 3.14-3.02 (m, 1H), 2.98-2.86 (m, 1H), 1.97-1.87 (m,1H), 1.78-1.55 (m, 3H); 100 MHz ^{13}C NMR (D_2O): δ 176.5, 58.4, 47.3, 26.8, 21.9; Anal. Calcd. for $C_7H_{11}N_2O_4F_3$: C, 34.43; H, 4.54; N, 11.47%; Found: C, 34.51; H, 4.55; N, 11.49%; Acc. Mass Calcd. for C_5H_{11}-O_2N_2 $(M - CF_3CO_2H)^+$ 131.0821; Found: 131.0830.

(*3S*)-*N*^{1}-(2,4-dinitrophenyi)-hexahydropyridazine-carboxylic acid 90

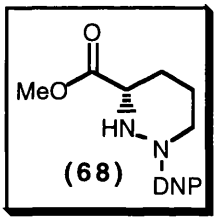


(90)

To a solution of *(3S)* piperazic acid trifluoroacetic acid salt **89** (650 mg, 3.0 mmol) in ethanol (13.5 ml) was added sodium bicarbonate (1.30 g, 15.0 mmol) followed by 1-fluoro-2,4-dinitrobenzene (1.3 ml, 13.0 mmol) and the mixture stirred for 12 h at room temperature. The reaction mixture was then diluted with H_2O and extracted with CH_2Cl_2 (3 x 25 ml). The aqueous layer was then cooled in an ice bath and acidified with 10% hydrochloric acid to pH 2 and then extracted with EtOAc (4 x 30 ml). The combined organic layers were dried over $MgSO_4$, filtered,

and concentrated *in vacuo* to give **90** (530 mg, 65%) as a yellow solid. An analytical sample was obtained by recrystallistion from toluene; m.p. 151-152 °C, Lit.[1] m.p.150.5-151.5 °C; $[\alpha]_D$ -345° (*c* 1, MeOH), Lit.[1] $[\alpha]_D$ +324.6 (*c* 1, MeOH); IR (KBr): 3622-2950 (br), 3200 (w), 2963 (w), 2909 (w), 2825 (w), 1715 (s), 1608 (s), 1527 (m), 1447 (w), 1337 (s), 1267 (m), 1127 (m), 903 (m), 861 (m), 800 (m), 725 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 8.40 (d, *J* = 2.6 Hz, 1H), 8.15 (dd, *J* = 2.6, 9.3 Hz,1H), 6.95 (d, *J* = 9.3 Hz, 1H), 6.01-3.9 (br s, 2H), 3.85 (ddd, *J* = 3.7, 7.4, 12.5 Hz, 1H), 3.72 (dd, *J* = 3.5, 11.2 Hz, 1H), 3.15 (ddd, *J* = 3.4,12.1, 12.1 Hz, 1H), 2.20 (m, 1H), 2.00 (m, 2H), 1.65 (m, 1H); 100 MHz ^{13}C NMR ($CDCl_3$) δ: 175.5, 147.4, 138.4, 127.4, 122.3, 115.0, 114.7, 57.3, 47.6, 27.6, 22.8; Acc. Mass Cacld. for $C_{11}H_{13}N_4O_6$ $(M+H)^+$ 297.08351; Found: 297.08348.

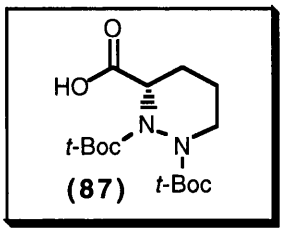
Methyl (*3S*)-*N'* -(2,4-dinitrophenyl)-hexahydropyridazine-3-carboxylate 68



(68)

To a solution of acid **90** (50.0 mg, 0.17 mmol) in chloroform (2 ml) was added ethereal diazomethane until the reaction was judge to be complete by TLC. nitrogen gas was then bubbled through the reaction mixture for 15 min. Concentration *in vacuo* and trituration with Et_2O gave **68** (49.0 mg, 95%) as a yellow solid. An analytical sample was obtained by recrystallisation from toluene; m.p. 96-97 °C [Lit[2] m.p. 96-97 °C]; $[\alpha]_D$ -289.2° (*c* 1, $CHCl_3$), Lit.[2] $[\alpha]_D$ -296.3° (*c* 0.3, $CHCl_3$); IR (KBr): 3232 (w), 3100 (w), 2952 (w), 1742 (s), 1609 (s), 1581 (m), 1541 (s), 1489 (w), 1481 (w), 1367 (w), 1331 (s), 1320 (s), 1259 (w), 1227 (w), 1150 (w), 1147 (w), 1050 (m), 840 (w), 751 (w) cm^{-1}; 400 MHz ^1H NMR (C_6D_6): δ 8.12 (d, *J* =2.7 Hz, 1H), 7.65 (dd, *J* = 2.7, 9.3 Hz, 1H), 5.84 (d, *J* = 9.3 Hz, 1H), 3.42 (td, *J* = 3.4, 11.3 Hz, 1H), 3.17 (s, 3H), 3.14 (d, *J* = 11.5 Hz, 1H), 2.63 (dt, *J* = 3.9, 12.6 Hz, 1H), 1.85 (m, 1H), 1.46 (m, 1H), 1.13-0.85 (complex m, 3H); 100 MHz ^{13}C NMR (C_6D_6): δ 171.2, 147.0, 139.5, 127.8, 127.6, 126.5, 122.1,114.5, 57.4, 51.5, 46.6, 27.5, 22.3; Acc. Mass Calcd. for $C_{12}H_{15}N_4O_6$ $(M+H)^+$ 311.0992; Found: 311.0990; Anal. Calcd. for $C_{12}H_{14}N_4O_6$: C, 46.44; H, 4.55; N, 18.06%. Found: C, 46.05; H,4.41; N, 17.77%.

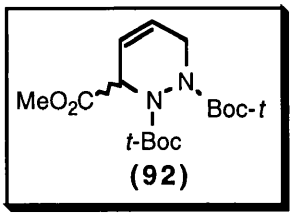
One-pot synthesis of compound 87



(87)

To a solution of (*4S*)-4-(phenylmethyl)-2-oxazolidinone **80** (2.0 g, 11.0 mmol) in dry THF (20 ml) under nitrogen at -78 °C was added *n*-BuLi (4.80 ml, 2.5 M in hexanes, 12.0 mmol) and the mixture stirred for 40 min. 5-Bromovaleryl chloride (1.56 ml, 12.0 mmol) was then added over 10 min and the reaction mixture warmed to room temperature for 1 h. The reaction mixture was then recooled to -78 °C and added to a freshly prepared solution of lithium diisopropylamide (13 mmol) in dry THF (10 ml) at -78 °C via a cannula. After 45 min, a precooled solution of di-*tert*-butylazodicarboxylate (3.22 g, 14.0 mmol) in dry CH_2Cl_2 (20 ml) at -10 °C was added into the enolate solution at -78 °C via cannula and the reactants stirred for 30 min. DMPU (80 ml) was then added dropwise over 40 min. The resulting frozen solution was allowed to melt by warming to room temperature. After stirring for 1 h, it was poured into a mixture containing Et_2O (80 ml) and KH_2PO_4 (1.25 M, 25 ml) and the aqueous layer extracted with Et_2O (4 x 75 ml). The combined ethereal layers were washed sequentially with H_2O (3 x 40 ml), brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The crude cyclised product **86** was dissolved in THF (45 ml), cooled to -5 °C and added to a chilled (0 °C) suspension of lithium hydroxide monohydrate (1.10 g, 26 mmol) in H_2O (22 ml) and the mixture stirred at -5 °C for 1.5 h. The reaction mixture was then diluted with H_2O (20 ml) and extracted with Et_2O. The combined organic layers were washed with H_2O (2x) and the aqueous layers combined, cooled in an ice bath, acidified to pH 2 using aqueous $NaHSO_4$ (1.0 M), and extracted with CH_2Cl_2 (5 x 60 ml). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give **87** (2.67g, 74% for 3 steps) as a white solid.

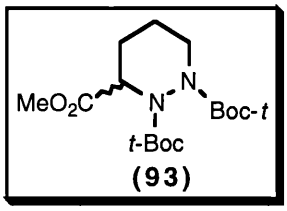
(*3S,3R*)-*N,N'*-Bis-(*t*-butoxycarbonyl)-1,2,3,6-tetrahydropyridazine-3-carboxylic acid methyl ester 92



(92)

The Diels Alder reaction between methyl penta-2,4-dieneoate **91** (3.0 g, 30.0 mmol) and di-*tert*-butylazodicarboxylate (3.0 g, 13.0 mol) gave **92** (2.10 g, 47%) as white crystals;[3] m.p. 79-80 °C; IR (KBr): 3004 (w), 2979 (m), 2932 (w), 1744 (s), 1707 (s), 1479 (m), 1441 (m), 1415 (m), 1396 (w), 1363 (w), 1316 (w), 1200 (w), 1178 (m), 1127 (w), 1077 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 5.87 (br s, 2H, =CHC**H$_2$**N), 5.34-5.04 (br d, 1H, C(O)CHC**H**=CH), 4.46-4.20 (br d, 1H, CH_2C**H**=CH), 3.81-3.48 (br s, 4H, C**H**CO_2 C**H$_3$**), 1.48 (s, 18H, 2x *t*-Boc); 100 MHz ^{13}C NMR ($CDCl_3$): δ 169.4, 154.8, 125.4, 122.2, 81.9, 80.6, 55.4, 52.2, 41.3, 28.1; Acc. Mass Calcd. for $C_{16}H_{27}N_2O_6$ $(M+H)^+$ 343.1869; Found: 343.1865.

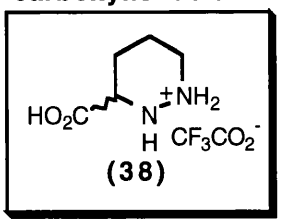
(*3S,3R*)-*N,N'*-Bis-(*t*-butoxycarbonyl)hexahydropyridazine-3-carboxylic acid methyl ester 93



(93)

Hydrogenation of **92** (1.6 g, 5.0 mmol) in THF (4 ml) gave **93** (1.4 g , 80%) as a clear oil.[3] An analytical sample was obtained by flash chromatography eluting with hexanes/EtOAc (6:1); 400 MHz ^1H NMR ($CDCl_3$): δ 5.02-4.85 (br m), 4.80-4.67 (br s), 4.17-3.84 (br m), 3.67 (br s), 2.98-2.69 (br m, 1H), 2.14-1.62 (complex m), 1.44 (s), 1.41 (s); 100 MHz ^{13}C NMR ($CDCl_3$): δ 170.4, 154.5, 81.6, 80.2, 54.4, 51.9, 42.6, 28.3, 27.9, 24.8, 20.0; Acc. Mass Calcd. for $C_{16}H_{29}N_2O_6$ $(M+H)^+$ 345.2026; Found: 345.2021.

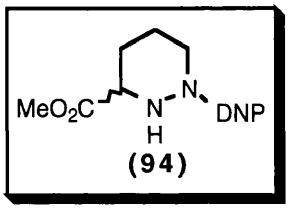
(*3S, 3R*)-Hexahydropyridazine-3-carboxylic acid trifluoroacetic acid salt 38



(38)

Saponification of **93** (1.0 g, 3.0 mmol) in THF (3 ml) with potassium hydroxide and subsequent acidolysis with CF_3CO_2H gave **38** (461mg, 64% for 2 steps) as a white solid; m.p. 142-145 ^oC; 400 MHz ^1H NMR (D_2O): δ 3.75-3.66 (m, 1H), 3.14-3.02 (m, 1H), 2.98-2.86 (m, 1H), 1.97-1.87 (m,1H), 1.78-1.55 (m, 3H); 100 MHz ^{13}C NMR (D_2O): δ 176.5, 58.4, 47.3, 26.8, 21.9; Acc. Mass Calcd. for $C_5H_{11}O_2N_2$ $(M - CF_3CO_2H)^+$131.082; Found: 131.0825.

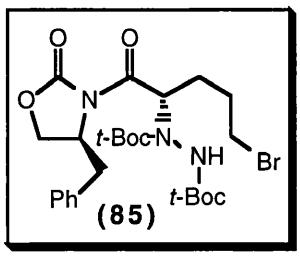
Methyl (*3S,3R*)-*N'* -(2,4-dinitrophenyl)-hexahydropyridazine-3-carboxylate 94



(94)

The compound was prepared from acid **38** (300.0 mg, 1.24 mmol) as described for **68**, to give **94** (232 mg, 60% for 2 steps) as a yellow solid; m.p. 145-146 ^oC; 400 MHz ^1H NMR (C_6D_6): δ 8.12 (d, *J* =2.7 Hz, 1H), 7.65 (dd, *J* = 2.7, 9.3 Hz, 1H), 5.84 (d, *J* = 9.3 Hz, 1H), 3.42 (td, *J* = 3.4, 11.3 Hz, 1H), 3.17 (s, 3H), 3.14 (d, *J* = 11.5 Hz, 1H), 2.63 (dt, *J* = 3.9, 12.6 Hz, 1H), 1.85 (m, 1H), 1.46 (m, 1H), 1.13-0.85 (complex m, 3H); 100 MHz; Acc. Mass Calcd. for $C_{12}H_{15}N_4O_6$ $(M+H)^+$ 311.0992; Found: 311.0996.

Bromovaleryl Hydrazide 85

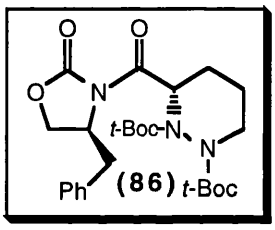


(85)

To a stirred solution of bromide **84** (3.13 g, 9.0 mmol) in dry CH_2Cl_2 (22 ml) at -10 ^oC under nitrogen was added titanium tetrachloride (10.0 ml, 1 M in CH_2Cl_2, 10.0 mmol). After 10 min, diisopropylethylamine (1.8 ml, 10.0 mmol) was added and the resulting deep red solution stirred at

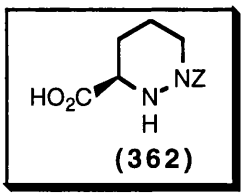
-10 °C for 1.5 h. A precooled solution of di-*tert*-butylazodicarboxylate (3.18 g, 14.0 mmol) in dry CH_2Cl_2 (10 ml) at -5 °C was added via cannula over 10 min and the mixture then stirred for 3 h at 0 °C. The reaction mixture was poured into a mixture of Et_2O (40 ml) and KH_2PO_4 (20 ml, 1.25 M) and the aqueous layer extracted with Et_2O (3 x 60 ml). The combined ethereal layers were washed with H_2O (2 x 15 ml), brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Purification of the residue by flash chromatography with hexanes/CH_2Cl_2 (2:1) gave **85** (4.32 g, 82%) as a foam; $[\alpha]_D$ +59.7° (*c* 1, MeOH); Anal. Calcd. for $C_{25}H_{36}N_3O_7Br$: C, 52.63; H, 6.36; N, 7.37; Br, 14.01%. Found: C, 52.43; H, 6.3; N, 7.32; Br, 14.25%. Spectral data for **85** matched those obtained previously.

(*4S*)-3-(*3S*-*N*,*N'*-bis-(*t*-butoxycarbonyl)hexahydropyridazine-3-carboxy)-4-phenylmethyl)-2-oxazolidinone 86



To a stirred solution of **85** (3.96 g, 7.0 mmol) in dry DMF (15 ml) under nitrogen at 0 °C was added sodium hydride (300.0 mg, 60% in mineral oil, 8.0 mmol) and the mixture maintained at 0 °C for 3 h. The reaction mixture was then carefully poured into a mixture of Et_2O (25 ml) and 10% HCl (10 ml). The ethereal layer was separated and the aqueous layer extracted with Et_2O (4x, 70 ml). The combined organic layers were washed with H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Purification of the residue by flash chromatography with Et_2O/hexanes (3:2) gave **86** (2.94 g, 87%) as a foam. The spectral data for **86** match those obtained previously.

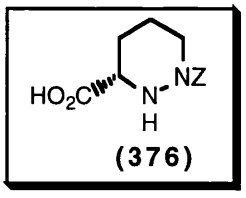
(*3R*)-*N*^{1}-Benzyloxycarbonylpiperazic acid 362



(362)

A stirred solution of (*3R*)-piperazic trifluoroacetic acid salt **361** (7.0 g, 30.0 mmol) in H_2O (10 ml) at 0 °C was neutralised with sodium hydroxide (14.5 ml, 2.0 M in H_2O, 30.0 mmol)). To this mixture at 0 °C was simultaneously added dropwise over 45 min a solution of benzyl chloroformate (4.8 ml, 30.0 mmol) in toluene (11 ml) and sodium hydroxide (14.5 ml, 2.0 M in H_2O, 30.0 mmol) with stirring. After the additions were complete the biphasic mixture was stirred for a further 2 h at 0 °C. The reaction mixture was then extracted with Et_2O and the aqueous layer removed and cooled in an ice bath, where it was brought to pH 2 with concentrated hydrochloric acid. The white suspension that formed was then left at 0 °C for 2 h, filtered, and washed with a cold solution of Et_2O/ acetone (1:1) to give **362** (7.90 g, 100%) as a white solid. An analytical sample of **362** was obtained by recrystallisation from EtOAc/MeOH; $[\alpha]_D$ +32.8° (*c* 1, CH_3OH), Lit[1] $[\alpha]_D$ +35° (*c* 0.5, CH_3OH); IR (KBr): 3445 (br w), 3262 (m), 2966 (m), 2947 (m), 2919 (m), 2858 (m), 1751 (s), 1691 (s), 1498 (m), 1416 (s), 1368 (m), 1262 (s), 1236 (m), 1193 (s), 1117 (m), 755 (m), 696 (m) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6): δ 7.38-7.29 (m, 5H, Ph), 5.09 (apparent s, 2H), 4.90-4.00 (br 1H), 3.83 (apparent br d, 1H), 3.38 (apparent br d, 1H), 3.05 (br m, 1H),1.89 (m, 1H), 1.67 (m, 1H), 1.60-1.42 (m, 2H); 100 MHz ^{13}C NMR (DMSO-d_6): δ 173.2, 155.6, 137.5, 128.9, 128.3, 128.1, 66.8, 58.4, 44.6, 44.5, 27.6, 23.4; Acc. Mass Calcd. for $C_{13}H_{17}N_2O_4$, $(M+H)^+$ 265.1188; Found: 265.1180

(*3S*)-*N*^{1}-Benzyloxycarbonylpiperazic acid 376

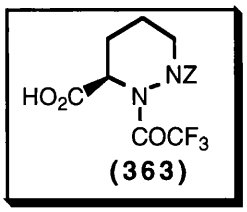


(376)

Procedure as above

$[\alpha]_D$ -32° (*c* 1, CH_3OH)

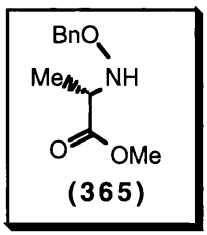
N^1-Benzyloxycarbonyl-N^2-trifluoroacetyl (*3R*)-piperazic acid 363



(363)

To a stirred solution of (*3R*)-Z-piperazic acid **362** (810 mg, 3.10 mmol) in trifluoroacetic acid (6 ml) under nitrogen at -10 °C was added trifluoroacetic anhydride (0.51 ml, 3.60 mmol) and the mixture maintained at that temperature for 1 h. The reaction mixture was then concentrated *in vacuo* and the residue diluted with Et_2O (20 ml), washed with H_2O (3 x 6 ml), dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The residue was purified by flash chromatography with hexanes/ EtOAc (6:**1**) as eluent to give **363** (1.04 g, 94%) as an oil; $[\alpha]_D$ +80° (*c* 1 CH_3OH), Lit[1] $[\alpha]_D$ +72° (*c* 1, CH_3OH); IR (neat film): 3204 (br s), 2966 (s), 2650 (br w), 1827 (w), 1721 (br s), 1588 (w), 1497 (w), 1454 (s), 1405 (s), 1349 (s), 1264 (s), 1187 (br s), 1026 (m) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6): δ 7.42-7.25 (m), 5.22 (m), 4.94 (m), 4.12 (br d), 3.70-3.00 (br), 1.9 (m, 2H), 2.08-1.43 (m); 100 MHz ^{13}C NMR (DMSO-d_6): δ 169.7, 157.0, 155.4, 135.5, 128.5, 128.4, 128.2, 127.9, 127.7, 117.2, 114.3, 68.7, 68.0, 52.9, 40.1 (br), 38.9, 23.7, 18.0 (br); Acc. Mass Calcd. for $C_{15}H_{16}O_5$-N_2F_3 $(M+H)^+$ 361.1011; Found: 361.1024.

(*S*)-*N* -Benzyloxyalanine methyl ester 365

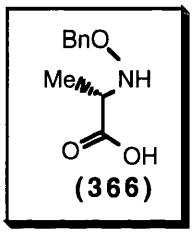


(365)

To a stirred solution of *R* (+)-methyl lactate **364** (2.33 ml, 24.0 mmol) in dry CH_2Cl_2 (73 ml) under nitrogen at 0 °C was added trifluoromethanesulfonic acid anhydride (4.44 ml, 26.0 mmol) in one portion and the resulting mixture stirred for 10 min 2,6-Lutidine (3.22 ml, 0.028 mol) was then added in one portion. After 10 min, a solution of *O*-benzylhydroxylamine (6.0 g, 49.0 mmol) in dry CH_2Cl_2 (35 ml) was added dropwise over 7 min and the now yellow-coloured reaction mixture stirred for 5 min at 0 °C before warming to room temperature for 1 h. The residue was then

concentrated *in vacuo* and purified by flash chromatography eluting with hexanes/Et_2O (4:1) as eluent. Compound **365** was obtained (3.70 g, 74%) as a pale yellow oil; $[\alpha]_D$ -57.4° (*c* 1, CH_2Cl_2), Lit.[4] $[\alpha]_D$ -30.15° (*c* 2, EtOH); IR (neat film): 3262 (w), 3030 (m), 2994 (m), 2952 (s), 2910 (m), 1742 (s), 1454 (s), 1436 (s), 1215 (s), 1172 (s), 1038 (m), 735 (s), 600 (s) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.24-7.35 (m, 5H, **Ph**), 5.95 (br s, 1H, **NH**), 4.61 (s, 2H, **CH_2**Ph), 3.65 (s, 3H, OMe), 3.61 (q, *J* = 7.1 Hz, 1H, C**H**CH_3), 1.11 (d, *J* = 7.1 Hz, 3H, CH**CH_3**); 100 MHz ^{13}C NMR ($CDCl_3$): δ 174.7, 137.7, 128.33, 128.31, 127.8, 76.3, 58.9, 58.8, 52.12, 52.1, 14.9; Acc. Mass Calcd. for $C_{11}H_{15}O_3N$ $(M)^+$ 209.10519; Found: 209.10510.

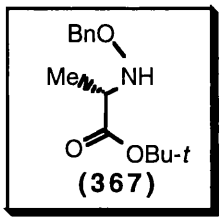
(*S*)-*N*-Benzyloxyalanine 366



(366)

To a vigorously stirred solution of methyl ester **365** (2.42 g, 12.0 mmol) in THF (19 ml) at 0 °C was added sodium hydroxide (13.2 ml, 1M in H_2O, 13.2 mmol). After 2 h, the reaction mixture was then extracted with Et_2O (3 x 25 ml) and the aqueous layer separated and cooled to 0 °C, where-upon it was adjusted to pH 2 with concentrated hydrochloric acid. The white suspension was placed in the fridge overnight, the precipitate removed by filtration and washed with cold Et_2O to give **366** (1.56 g, 66%) as a white solid. An analytical sample was obtained by recrystallisation from hexanes/EtOAc; m.p. 112-114 °C, Lit.[5] 113-114 °C; $[\alpha]_D$ -36.7° (*c* 1, CH_3OH), Lit.[5] $[\alpha]_D$ -25.1° (*c* 2, EtOH); IR (KBr): 3200- 2000 (br m), 2945 (m), 2727 (m), 1574 (br s), 1392 (s), 1358 (s), 1271 (m), 1096 (m), 1009 (m), 963 (m), 916 (m), 857 (m), 759 (s), 702 (s), 673 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.37-7.26 (m, 5H, **Ph**), 4.71 (s, 2H, C**H_2**Ph), 3.72 (q, *J* = 7.1 Hz, 1H, C**H**CH_3), 1.25 (d, *J* = 7.14 Hz, 3H, CH**CH_3**); 100 MHz ^{13}C NMR ($CDCl_3$): δ 179.1, 137.3, 128.5, 128.4, 128.0, 76.4, 58.7, 14.7; Acc. Mass Calcd. for $C_{10}H_{14}NO_3$ $(M+H)^+$ 196.0974; Found: 196.0982.

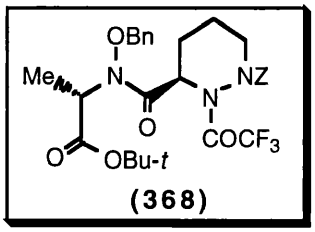
(*S*)-*N*-Benzyloxyalanine *t*-butyl ester 367



(367)

To a stirred solution of acid **366** (10.72 g, 55.0 mmol) in dioxane (100 ml) at -20 °C was added concentrated sulfuric acid (5 ml) followed by isobutene (80 ml) and the reaction vessel then securely sealed with a rubber septum and stirred at room temperature for 5 days. The reaction mixture was then recooled to -20 °C and the septum removed, allowing the excess isobutene to escape as the reaction mixture warmed to room temperature. The reaction mixture was then adjusted to pH 9 with saturated aqueous sodium bicarbonate, and extracted with EtOAc (4 x 80 ml). The combined organic layers were then washed successively with H_2O, brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The residue was purified by flash chromatography eluting with hexanes/Et_2O (10:1) to give **367** (8.54 g, 62%) as a clear oil; $[\alpha]_D$ -49.4° (*c* 1, CH_2Cl_2), Lit.[4] $[\alpha]_D$ -26.8° (*c* 2, EtOH); IR (neat film): 3451(br w), 3254 (w), 3030 (w), 2980 (s), 2938 (m), 1728 (s), 1447 (s), 1370 (s), 1225 (s), 1156 (s), 1098 (m), 1029 (m), 849 (m), 745 (m), 698 (s) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.33-7.24 (m, 5H, Ph), 4.70 (s, 2H, CH_2Ph), 3.58 (q, *J* = 7.1 Hz, $CHCH_3$, 1H), 1.46 (s, 9H, ***t*-Bu**), 1.16 (d, *J* =7.4 Hz, 3H, $CHCH_3$); 100 MHz ^{13}C NMR ($CDCl_3$): δ 173.5, 137.9, 128.3, 128.2, 127.7, 81.2, 76.1, 59.5, 28.0, 14.9; Acc. Mass Calcd. for $C_{14}H_{22}N_1O_3$ $(M+H)^+$ 252.1600; Found: 252.1608.

N^1-Benzyloxycarbonyl-N^2-trifluoroacetyl-(*3R*)-piperazyl-(*S*)-*N*-benzyloxyalanine *t*-butyl ester 368

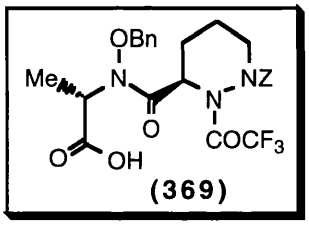


(368)

To a solution of acid **363** (1.55 g, 4.30 mmol) in dry Et_2O (7 ml) at 0 °C under nitrogen was added phosphorus pentachloride (1.00 g, 4.70 mmol) and the mixture stirred for 30 min. The reaction mixture was then stirred for a further 40 min at room temperature, then concentrated *in*

vacuo. The residue was diluted with dry CH_2Cl_2 (3 ml) and added dropwise over 5 min to a mixture of (*S*)-*N*-benzyloxyalanine *t*-butyl ester **367** (1.06 g, 4.20 mmol) and sodium bicarbonate (780.0 mg, 9.30 mmol) in a mixture of CH_2Cl_2 (10 ml) and H_2O (7 ml) at 0 °C. After 2 h, the reaction mixture was extracted with CH_2Cl_2 (3 x 50 ml) and the combined organic layers washed with saturated aqueous sodium bicarbonate, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Purification of the residue by flash chromatography eluting with hexanes/Et_2O (3:1) gave **368** (1.61 g, 64%) as an oil; IR (neat film): 3037 (w), 2981 (w), 2931 (w), 1738 (s), 1724 (s), 1674 (m), 1497 (w), 1455 (m), 1256 (m), 1211 (m), 1161 (s), 1124 (m), 745 (w), 688 (w) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6 at 135 °C): δ 7.49-7.29 (m), 5.48 (m), 5.24- 4.94 (m), 4.83 (m), 4.58 (m), 4.22 (m), 3.44 (m), 3.20 (m), 2.21 (m), 2.02 (m), 1.89 (m), 1.75 (m), 1.70- 1.50 (m), 1.43 (s), 1.42 (s), 1.38 (d, *J* = 7.2 Hz); Acc. Mass Calcd. for $C_{29}H_{35}N_3O_7F_3$ $(M+H)^+$ 594.2427; Found: 594.2416.

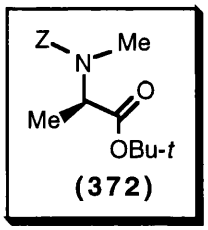
N^1-Benzyloxycarbonyl-N^2trifluoroacetyl-(*3R*)-piperazyl-(*S*)-*N*-benzyloxyalanine 369



(369)

To *t*-butyl ester **368** (1.61 g, 2.70 mmol) under nitrogen was added trifluoroacetic acid (10 ml). The mixture was stirred for 2 h and then concentrated *in vacuo.* Purification of the residue by flash chromatography with hexanes/EtOAc (3:1) as eluent gave **369** (1.44 g, 100%) as a colourless oil; IR (neat film): 3478-3219 (br w), 3030 (w), 2948 (w), 1714 (br s), 1676 (m), 1673 (s), 1454 (m), 1400 (m), 1350 (m), 1312 (w), 1270 (m), 1208 (s), 1129 (w), 1116 (s), 913 (w), 789 (m), 753 (m), 699 (m) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6): δ 12.90 (br s), 7.64-7.19 (m), 5.70-4.40 (complex m), 4.18 (m), 3.20- 2.98 (m), 2.24-1.05 (m); Acc. Mass Calcd. for $C_{25}H_{26}N_3O_7F_3$ $(M)^+$ 537.1723; Found: 537.1724.

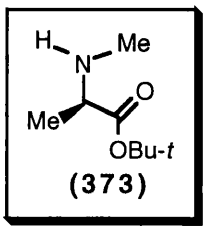
N-Benzyloxycarbonyl-N-methyl-(R)-alanine t-butyl ester 372



(372)

To a 0 °C solution of benzyloxycarbonyl-*N*-methyl-(*R*)-alanine **371** (1.58 g, 7.03 mmol) in CH_2Cl_2 (15 ml) was added concentrated sulfuric acid (0.5 ml) followed by isobutene (12 ml). The reaction flasked was then securely sealed with a rubber septum and allowed to stir at room temperature for 5 days. The flask was then cooled to -20 °C and the septum removed. The excess isobutene was then allowed to escape at room temperature and the mixture adjusted to pH 10 by adding it to10% aqueous sodium carbonate. The solvent was removed *in vacuo* and the residue diluted with EtOAc (25 ml), washed successively with aqueous 1M sodium bicarbonate, H_2O, brine, and dried over $MgSO_4$. After filtration and concentration *in vacuo*, the residue was purified by flash chromatography eluting with hexanes/Et_2O (8:1) as eluent. This gave **372** (1.45 g, 74%) as a pale yellow oil; IR (neat film): 3066 (w), 2980 (m), 2938 (w), 1735 (s), 1708 (s), 1455 (m), 1369 (m), 1310 (m), 1154 (s), 1096 (w), 849 (m), 749 (m), 698 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.3-7.24 (m, Ph), 5.18 (1/2ABq, *J* = 12.6 Hz), 5.13 (s), 5.06 (1/2ABq, *J* = 121.6 Hz), 4.77 (q, *J* = 7.5 Hz), 4.57 (q, *J* = 7.3 Hz), 2.88 (s), 2.85 (s), 1.41 (s), 1.37 (s), 1.34 (d, *J* = 7.3 Hz); 100 MHz ^{13}C NMR ($CDCl_3$): δ 171.2, 136.7, 136.6, 128.4, 127.9, 127.7, 81.3, 67.2, 54.8, 54.7, 30.6, 30.1, 27.9, 15.1, 14.7; Acc. Mass Calcd. for $C_{16}H_{24}O_4N$ (M+H)$^+$ 294.1705; Found: 294.1715.

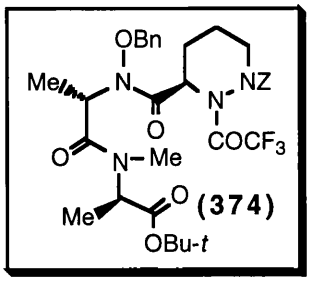
N-Methyl-(R)-alanine t-butyl ester 373



(373)

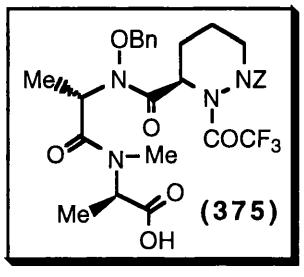
A solution of compound **372** (1.46 g, 5.20 mmol) in EtOAc (12 ml) was hydrogenated for 2 h in the presence of 10% Pd/C (70 mg) in EtOAc (12 ml). The catalyst was then removed by filtration and the solvent removed *in vacuo*. The crude *N*-methyl amine **373** was then used directly for the next step.

N^1-Benzyloxycarbonyl-N^2-trifluoroacetyl-(*3R*)-piperazyl-(*S*)-*N*-benzyloxyalanyl-*N*-(methyl)-(*R*)-alanine *t*-butyl ester 374



To a stirred solution of acid **369** (1.4 g, 2.60 mmol) in dry CH_2Cl_2 (10 ml) at -20 °Cunder nitrogen was added triethylamine (0.44 ml, 3.10 mmol) followed by bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (800 mg, 3.10 mmol) and the reaction mixture stirred for 1 h. Crude amine **373** in CH_2Cl_2 (2 ml) was then added to the mixed anhydride solution dropwise over 5 min and the reaction mixture maintained at -20 °C for a further 1 h before being warmed to 0 °C for a further 2 h. The reaction mixture was then washed successively with 10% sodium bicarbonate solution, 10% hydrochloric acid solution, brine, dried over $MgSO_4$ and filtered. The filtrate was concen-trated *in vacuo*, and the residue purified by flash chromatography with hexanes/EtOAc (5:1) as eluent. This gave **374** (1.20 g, 68%) as a foam; IR (KBr): 2981(br w), 2938 (m), 2827 (s), 1734 (br s), 1717 (br s), 1658 (m), 1456 (m), 1396 (w), 1264 (m), 1248 (m), 1214 (w), 1163 (w), 1084 (m), 789 (s), 754 (s) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6 at 70 °C): δ 7.50-7.25 (m), 5.58 (br), 5.38- 5.10 (complex m), 5.08- 4.65 (m), 4.2- 4.08 (m), 3.45 (m), 3.20 (m), 2.99 (s), 2.90-2.65 (br m), 2.10 (br), 2.00 (br), 1.88 (br), 1.76 (br), 1.65 (br), 1.55 (br), 1.40-1.10 (complex m); Acc. Mass Calcd. for $C_{33}H_{42}N_4O_8F_3$ $(M+H)^+$ 679.2955; Found: 679.2959.

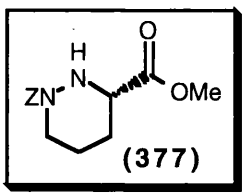
N^1-Benzyloxycarbonyl-N^2-trifluoroacetyl-(*3R*)-piperazyl-*N*-benzyloxy-(*S*)-alanyl-*N*-(methyl)-(*R*)-alanine 375



A solution of the *t*-butyl ester **374** (980 mg, 1.40 mmol) in CF_3CO_2H (5 ml) was stirred for 2 h under a nitrogen atmosphere and the solvent removed *in vacuo*. The residue was then diluted with Et_2O (20 ml) and washed with H_2O (3 x 7 ml), dried over $MgSO_4$, filtered and

concentrated *in vacuo*. The residue was purified by flash chromatography eluting with hexanes/ EtOAc (3:1) to give **375** (720 mg, 80%) as a foam; IR (KBr): 3690-2400 (br), 3030 (w), 2943 (m), 1745 (s), 1717 (s), 1659 (s), 1503 (w), 1456 (m), 1404 (m), 1349 (w), 1266 (m), 1249 (m), 1205 (s), 1163 (s), 1082 (w), 752 (m), 702 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.50- 7.23 (complex m), 5.80- 5.63 (m), 5.42- 4.65 (complex m), 4.56- 4.06 (m), 3.55 (br m), 3.24- 2.40 (complex m), 2.22- 1.15 (complex m); Acc. Mass Calcd. for $C_{29}H_{33}N_4O_3F_3$ $(M)^+$ 622.2250; Found: 622.2243.

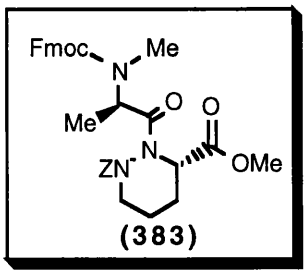
Methyl (*3S*)-*N*^{1}-benzyloxycarbonylpiperazate 377



(377)

To a solution of **376** (360 mg, 1.40 mmol) in $CHCl_3$ (5 ml) and EtOH (5 ml) at 0 °C was added a solution of diazomethane in Et_2O until the reaction mixture became pale yellow. Excess diazomethane was then expelled with nitrogen and the solvent removed *in vacuo* to give **377** (330 mg, 85%) as an oil. An analytical sample was obtained by flash chromatography eluting with hexanes/EtOAc (4:1); IR (neat film): 3472 (br w), 3297 (w), 3037 (w), 2952 (s), 2860 (w), 1750 (s), 1704 (s), 1507 (m), 1452 (s), 1403 (s), 1357 (s), 1332 (m), 1262 (s), 1173 (s), 1108 (m), 1028 (m), 987 (m), 755 (m), 700 (s) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.34- 7.23 (m, 5H, Ph), 5.15 (s, 2H, **CH_2**Ph), 3.97 (br m, 1H), 3.70 (s, 3H, **OCH_3**), 3.5 (br m, 1H), 3.10 (br m, 1H), 2.04 (m, 1H), 1.8- 1.50 (m, 3H); 100 MHz ^{13}C NMR ($CDCl_3$): δ 171.4 155.2, 153.8, 136.4, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 67.6, 58.3, 52.1, 44.7, 33.6, 27.4, 23.3; Acc. Mass Calcd. for $C_{14}H_{19}N_2O_4$ (M^+) 279.1345; Found: 279.1345.

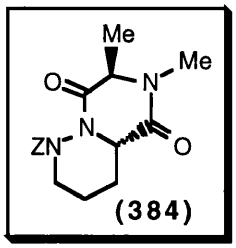
_N_-Fluorenymethoxycarbonyl-_N_-methyl-(_R_)-alanyl-N^1-benzyloxycarbonyl-(_S_)-piperazic acid methyl ester 383



(383)

To a stirred solution of **380** (1.90 g, 4.60 mmol) in C_6H_6 (5 ml) under nitrogen was added oxalyl chloride (4.0 ml, 46.0 mmol) and the mixture stirred in an oil bath at 50 °C for 1 h. The solvents were then removed *in vacuo* and the residue coevaporated with C_6H_6 (2 x 6 ml). A solution of **377** (860.0 mg, 3.10 mmol) in toluene (3 ml) was then added to the acid chloride under nitrogen followed by silver cyanide (950.0 mg, 7.13 mmol). The flask was heated in darkness at 90 °C for 1.5 h. The reaction mixture was then cooled to room temperature, filtered through Celite, and concentrated *in vacuo.* The residue was diluted with Et_2O (30 ml) washed successively with 10% aqueous sodium bicarbonate solution, H_2O, brine, and then dried over $MgSO_4$. It was then filtered and concentrated *in vacuo.* The crude residue was purified by flash chromatography eluting with hexanes/EtOAc (4:1) to give **383** (980 mg, 54%) as a foam; IR (KBr): 3072 (w), 3037 (w), 2952 (s), 1740 (s), 1687 (s), 1451 (m), 1400 (m), 1263 (m), 1245 (m), 1156 (m), 788 (s), 759 (s), 741 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.73 (m), 7.55 (complex m), 7.45- 7.00 (complex m), 5.45- 4.80 (m), 4.65 (br), 4.50- 3.85 (complex m), 3.74 (s), 3.73 (s), 3.69 (s), 2.87 (br s), 2.83 (br s), 2.24- 1.10 (complex m), 1.31 (d, superimposed on m, J = 7.0 Hz), 1.02 (d, J = 6.8 Hz); Acc. Mass Calcd. for $C_{33}H_{36}O_7N_3$ $(M+H)^+$ 586.2553; Found: 586.2559.

Diketopiperazine 384

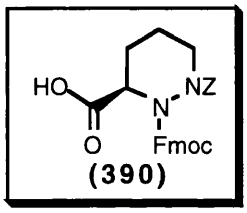


(384)

To a solution of **383** (910.0 mg, 1.60 mmol) in acetonitrile (8 ml) at room temperature was added diethylamine (8 ml) and the reaction mixture stirred for 5 min. It was then concentrated *in*

vacuo, and the residue purified by flash chromatography eluting with hexanes/EtOAc (1:2) to give **384** (420.0 mg, 79%) as an oil; IR (neat film): 2931 (w), 2854 (w), 1720 (s), 1694 (s), 1661 (s), 1490 (w), 1453 (m), 1404 (m), 1259 (m), 1186 (m), 1131 (w), 1040 (w) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6): δ 7.42- 7.18 (complex m), 5.29-5.04 (m), 4.33-3.78 (complex m) 3.15-2.75 (br m), 2.96 (s), 2.87 (s), 1.89-1.51 (br m), 1.57 (d, *J* = 6.8 Hz), 1.17 (d, *J* = 6.7 Hz); 100 MHz ^{13}C NMR ($CDCl_3$): δ 164.7, 164.3, 154.0, 128.5, 128.4, 128.3, 68.7, 68.6, 58,6, 57.7, 57.5, 46.0, 44.4, 32.2, 29.9, 29.4, 23.7, 23.2, 19.2, 19.0; Acc Mass Calcd. for $C_{17}H_{22}O_4N_3$ $(M+H)^+$ 332.1610; Found: 332.1615.

N^1-Benzyloxycarbonyl-N^2-9-fluorenylmethoxycarbonyl-(*3R*)-piperazic acid 390

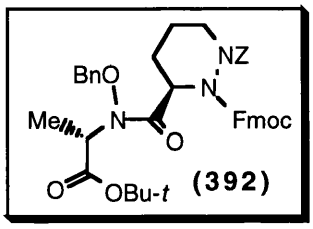


(390)

To a suspension of (*3R*) Z-piperazic acid **362** (7.0 g, 26.0 mmol) in dry CH_2Cl_2 (60 ml) under nitrogen was added diisopropylethylamine (7.90 ml, 45.0 mmol) followed by chlorotrimethylsilane (6.6 ml, 52.0 mmol). The resulting solution was heated at reflux for 2 h. The mixture was then cooled to 0 °C and with stirring 9-fluorenylmethyl chloroformate (8.75 g, 34.0 mmol) was added in one portion. After 30 min, the reaction mixture was allowed to warm to room temperature and stirred for 12 h. The mixture was then concentrated *in vacuo* and diluted with Et_2O (60 ml). The slurry was then extracted with 2.5% aqueous sodium bicarbonate (3 x 40 ml) and the combined aqueous layers were washed with Et_2O. The aqueous layer was then acidified to pH 2 with concentrated hydrochloric acid and extracted with EtOAc. The combined EtOAc extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by flash chromatography eluting with hexanes/EtOAc (4:1) gave **390** (10.74 g, 85%) as a foam; $[\alpha]_D$ +2.5° (*c* 1, CH_2Cl_2); IR (KBr): 3400- 2400 (br), 3067 (m), 3039 (m), 2956 (m), 1733 (s), 1712 (s), 1451 (s), 1423 (s),1359 (m), 1306 (s), 1252 (s), 1193 (m), 1129 (m), 1090 (m), 1048 (m),758 (s), 742 (s), 698 (w) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6 at 100 °C): δ 7.80 (apparent d, *J* = 7.6 Hz), 7.60 (apparent d, *J* = 7.1 Hz), 7.41- 7.19 (complex m), 5.03 (1/2 ABq, *J* = 12.7 Hz),

4.98 (1/2ABq, J = 12.8 Hz), 4.76 (br s), 4.58- 4.28 (m), 4.20 (br s), 3.95 (br d, J = 12.7 Hz), 2.78 (br m), 1.89-1.41 (complex m); Acc. MAss Calcd. for $C_{28}H_{27}N_2O_6$ $(M+H)^+$ 487.1869; Found: 487.1853.

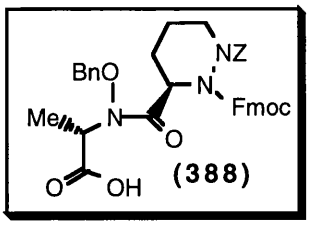
N^1-Benzyloxycarbonyl-N^2-9-fluorenymethoxycarbonyl-(*3R*)-piperazyl-(*S*)-*N*-benzyloxyalanine *t*-butyl ester 392



(392)

To a solution of acid **390** (9.48 g, 20.0 mmol) in C_6H_6 (144 ml) under nitrogen was added oxalyl chloride (17.1 ml, 200.0 mmol) and the mixture stirred for 1 h at 50 °C. The mixture was then concentrated *in vacuo* and the residue coevaporated twice with C_6H_6 before being diluted with CH_2Cl_2 (30 ml). The acid chloride solution was then added dropwise to a mixture of amine **367** (4.44 g, 18.0 mmol) and sodium bicarbonate (3.22 g, 39.0 mmol) in CH_2Cl_2/H_2O (57:19 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h. CH_2Cl_2 (100 ml) was then added and the mixture was washed with saturated aqueous sodium bicarbonate and dried over $MgSO_4$. Filtration, followed by concentration *in vacuo* and flash chromatogarphy with hexanes/EtOAc (5:1) gave **392** (12.22 g, 96%) as a foam; $[\alpha]_D$ -6.1° (*c* 1, CH_2Cl_2); IR (KBr): 3065 (w), 3044 (w), 2976 (m), 2946 (m), 1736 (s), 1710 (s), 1676 (s), 1452 (s), 1406 (s), 1367 (s), 1296 (s), 1254 (s), 1156 (s), 741 (s), 698 (m) cm^{-1}; 400 MHz ^1H NMR (DMDO-d_6 at 125 °C): δ 7.88-7.15 (complex m, 18H), 5.20 (br s, 1H), 5.10 (br d, 1H), 4.99 (br d, 1H), 4.91 (br s, 2H), 4.51 (apparent br d, 3H), 4.19 (apparent t, J = 5.9 Hz, 1H), 4.00 (br d, 1H), 2.85 (br m, 1H), 2.10 (br m, 1H), 1.80 (br, 1H), 1.65 (br, 1H), 1.41-1.36 (s superimposed on m, 13 H); Acc. Mass Calcd. for $C_{42}H_{46}N_3O_8$ $(M+H)^+$ 720.3285; Found: 720.3280; Anal. Calcd. for $C_{42}H_{45}N_3O_8$: C, 70.08; H, 6.30; N, 5.84%. Found: C, 69.96; H, 6.17; N, 5.78%.

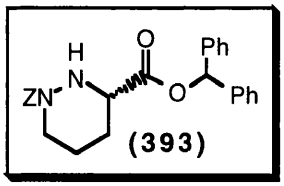
N^1-Benzyloxycarbonyl-N^2-9-fluorenymethoxycarbonyl-(*3R*)-piperazyl-(*S*)-*N*-benzyloxyalanine 388



(388)

To a solution of *t*-butyl ester **392** (2.0 g, 3.0 mmol) in CH_2Cl_2 (4.5 ml) under nitrogen was added CF_3CO_2H (3.2 ml, 42.0 mmol) and the mixture stirred for 1.5 h. After concentration *in vacuo* the residue was diluted with Et_2O (25 ml), washed with H_2O (3 x 10 ml) , dried over $MgSO_4$, filtered and concentrated *in vacuo.* Purification of the residue by flash chromatography with hexanes/ EtOAc (3:1) gave **388** (1.85 g, 93%) as a foam; $[\alpha]_D$ -24.5° (*c* 1, CH_2Cl_2); IR (KBr): 3431 (br w), 3191 (br m), 3065 (m), 3034 (m), 2949 (m), 1711 (br s), 1680 (s), 1452 (s), 1452 (s), 1409 (s), 1295 (s), 1255 (s), 1195 (br s), 741 (s), 698 (m) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6): δ 9.65 (br s), 7.8- 7.0 (complex m), 5.49 (br), 5.3- 3.80 (complex br m), 2.85 (br), 2.40- 1.00 (complex br m); Acc. Mass Calcd. for $C_{38}H_{38}N_3O_8$ $(M+H)^+$ 664.2659; Found: 664.2653; Anal. Calcd. for $C_{38}H_{37}N_3O_8$: C, 68.77; H, 5.62; N, 6.33%. Found: C, 68.89; H, 5.55; N, 6.17%.

N^1-Benzyloxycarbonyl-(*3S*)-piperazic acid diphenyimethyl ester 393

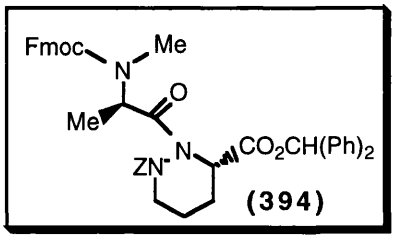


(393)

To a stirred solution of (*3S*)-N^1- Z-piperazic acid **376** (4.40 g, 16.7 mmol) in acetone (80 ml) and ethanol (25 ml) at room temperature was added dropwise a solution of diphenyldiazomethane (6.00 g, 40.0 mmol) in acetone (42 ml) and the resulting purple solution stirred for 3 h. The mixture was then concentrated *in vacuo* and the residue purified by flash chromatography with hexanes/EtOAc (6:1) to give **393** (6.67 g, 93%) as a white solid; m.p. 76-77 °C; $[\alpha]_D$ -21° (*c* 0.5, CH_2Cl_2); IR (KBr): 3242 (m), 3033 (w), 2948 (w), 2921 (w), 2854 (s), 1738 (s), 1669 (s), 1524 (m), 1496 (m), 1447 (m), 1410 (s), 1288 (m), 1272 (s), 1172 (s), 1147 (s), 755 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.36-7.24 (m, 15H), 6.92 (s, 1H), 5.60-4.20 (very br, 1H) 5.16 (s, 2H),

3.98 (br d, 1H), 3.64 (br d, 1H), 3.10 (br, 1H), 2.13 (m, 1H), 1.9-1.48 (m, 3H); 100 MHz ^{13}C NMR ($CDCl_3$): δ 170.0, 155.2., 139.6, 139.5, 136.3, 128.5, 128.0, 127.9, 127.1, 126.9, 77.4, 67.5, 58.3, 44.7, 27.3, 23.8; Acc. Mass Calcd. for $C_{26}H_{26}O_4N_2Na$ $(M+Na)^+$ 453.1790; Found: 453.1793; Anal Calcd. for $C_{26}H_{26}O_4N_2$: C, 72.54; H, 6.09; N, 6.51%. Found: C, 72.32; H, 6.18; N, 6.48%.

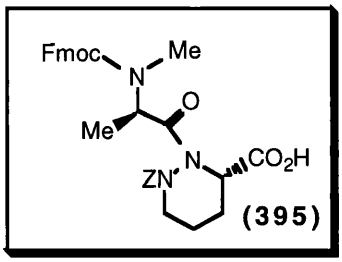
_N_-Fluorenymethoxycarbonyl-_N_-methyl-(_R_)-alanyl-_N_^{1}-benzyloxycarbonyl-(_S_)-piperazic acid diphenylmethyl ester 394



(394)

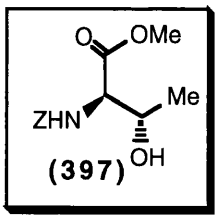
To a stirred solution of acid **380** (1.8 g, 6.0 mmol) in dry CH_2Cl_2 (18 ml) under nitrogen was added oxalyl chloride (4.7 ml, 60.0 mmol) and the mixture maintained at room temperature for 2 h. The mixture was then concentrated *in vacuo* and the residue coevaporated twice with C_6H_6. To this crude acid chloride was added a solution of **393** (1.8 g, 4.0 mmol) in dry toluene (18 ml) under nitrogen, followed by silver cyanide (880.0 mg, 6.6 mmol). The reaction vessel was then wrapped in foil and placed in an oil bath at 80 °C for 2 h. The mixture was then cooled to room temperature and filtered through Celite and the solvent removed *in vacuo*. The residue was then diluted with EtOAc (30 ml) and washed sequentially with saturated aqueous sodium bicarbonate and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash chromatography eluting with CH_2Cl_2/EtOAc (40:1) gave **394** (2.80 g, 91%) as a foam; $[\alpha]_D$ +11° (*c* 1, CH_2Cl_2); IR (KBr): 3065 (w), 3030 (w), 2945 (w), 1736 (s), 1726 (s), 1687 (s), 1496 (w), 1452 (s), 1399 (s), 1309 (s), 1245 (s), 1167 (s), 1126 (m),1079 (m), 759 (s), 740 (s), 699 (s) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.82- 7.00 (complex m), 6.90 (s), 6.50 (br), 5.43-3.70 (complex m), 3.30-2.85 (complex m), 2.74 (br s), 2.57 (br s), 2.25-0.90 (complex m), 1.29 (d, *J* = 7.0 Hz); Acc. Mass Calcd. for $C_{45}H_{44}O_7N_3$ $(M+H)^+$ 738.3179; Found: 738.3174; Anal. Calcd. for $C_{45}H_{43}O_7N_3$: C, 72.35; H, 6.07; N, 5.89%. Found: C, 72.08; H, 5.89; N, 5.64%.

_N_-Fluorenymethoxycarbonyl-_N_-methyl-(_R_)-alanyl-N^1-benzyloxycarbonyl-(_S_)-piperazic acid 395



To a stirred solution of ester **394** (3.76 g, 5.1 mmol) and phenol (1.13 g, 12.0 mmol) in CH_2Cl_2 (60 ml) at room temperature was added in one portion CF_3CO_2H (4.9 ml, 64.0 mmol) and the resulting solution stirred for 20 min. The mixture was then concentrated *in vacuo* and the residue purified by flash chromatography with hexanes/EtOAc (2:1) to give **395** (2.90 g, 99%) as a foam; $[\alpha]_D$ + 40° (*c* 0.5, CH_2Cl_2); IR (KBr): 3800-2600 (br), 3466 (br m), 3954 (m), 1742 (s), 1689 (s), 1473 (m), 1404 (m), 1357 (s), 1314 (m), 1249 (m), 1158 (m), 759 (s), 741 (s) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6 at 100 °C): δ 12.20 (br s, 1H), 7.86 (d, *J* = 7.6 Hz), 7.57 (d, *J* = 6.9 Hz), 7.41-7.20 (complex m), 5.17 (br m), 4.95 (br s), 4.50-3.80 (complex m), 3.05 (br m), 2.6 7 (br s), 2.61 (br s), 2.00-1.23 (complex m), 1.13 (d, *J* = 5.65 Hz), 1.07 (d, *J* = 5.5 Hz); Acc. Mass Calcd. for $C_{32}H_{34}O_7N_3$ $(M+H)^+$ 572.2397; Found: 572.2393; Anal. Calcd. for $C_{32}H_{33}O_7N_3$: C, 67.24; H, 5.82; N, 7.35%. Found: C, 66.93; H, 5.83; N, 7.18%.

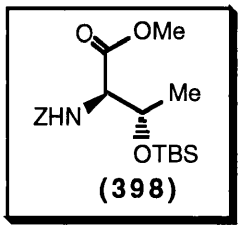
_N_-Benzyloxycarbonyl-(_R_)- threonine methyl ester 397



To a cool solution of acid **396** (2.00 g, 8.0 mmol) in $CHCl_3$ (15 ml) at 0 °C was added ethereal diazomethane until the solution became pale yellow. The reaction mixture was then purged with nitrogen gas and concentrated *in vacuo* to give **397** (2.06 g, 96%) as a white solid. An analytical sample was obtained by recrystallisation from hexanes/EtOAc; m.p. 84-85 °C; IR (KBr): 3473 (s), 3315 (s), 3041 (m), 2945 (m), 1719 (s), 1651 (s), 1546 (m), 1441 (w), 1336 (m), 1282 (w), 1247 (m), 1075 (m), 752 (m), 698 (s) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.34- 7.28 (m, 5H, Ph), 5.67 (d, *J* = 8.6 Hz, 1H), 5.10 (s, 2H), 4.30 (d, *J* = 7.4 Hz, 2H), 3.73 (s, 3H, OMe), 2.37 (br

s, 1H), 1.21 (d, J = 6.2 Hz, 3H); 100 MHz ^{13}C NMR ($CDCl_3$): δ 171.7, 156.7, 136.1, 128.5, 128.2, 128.0, 67.9, 67.2, 59.1, 52.6, 52.57, 19.8; Acc. Mass Calcd. for $C_{13}H_{18}NO_5$ $(M+H)^+$ 268.1185; Found: 268.1180.

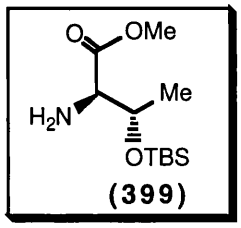
_N_-Benzyloxycarbonyl-_O_- _t_-butyldimethylsilyl-(_R_)-threonine methyl ester 398



(398)

To a solution of alcohol **397** (2.00 g, 7.50 mmol) in dry DMF (6 ml) under nitrogen was added imidazole (620.0 mg, 9.0 mmol) and _t_-butyldimethylsilyl chloride (1.36 g, 9.0 mmol) and the reaction mixture stirred for 5 h. The reaction mixture was then diluted with Et_2O (50 ml) and saturated aqueous sodium bicarbonate. The Et_2O layer was removed and the aqueous phase further extracted with Et_2O (4 x 50 ml). The combined ethereal layers were washed with H_2O, brine, dried over $MgSO_4$, filtered, and concentrated _in vacuo_. The residue was purified by flash chromatography eluting with hexanes/Et_2O (7:1). Compound **398** (2.64 g, 92%) was obtained as an oil; IR (neat film): 3452 (w), 3353 (w), 2952 (m), 2924 (m), 1755 (s), 1731 (s), 1508 (s), 1321 (m), 1258 (m), 1208 (m), 1100 (m), 1071 (s), 963 (m), 838 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.37- 7.25 (m, 5H, Ph), 5.43 (br d, 1H), 5.12 (s, 2H), 4.12 (m, 1H), 4.25(dd, J = 1.8 and 9.8 Hz, 1H), 3.71 (s, 3H, OMe), 1.18 (d, J = 6.3 Hz, 3H), 0.81 (s, 9H, 0.01 (s), -0.04 (s, 3H); 100 MHz ^{13}C NMR ($CDCl_3$): δ 171.2, 156.7, 136.2, 128.5, 128.1, 68.7, 67.1, 59.9, 52.3, 25.6, 20.8, 17.8, -4.5, -5.4; Acc. Mass Calcd. for $C_{19}H_{32}NO_5Si$ $(M+H)^+$ 382.2050; Found: 382.2046.

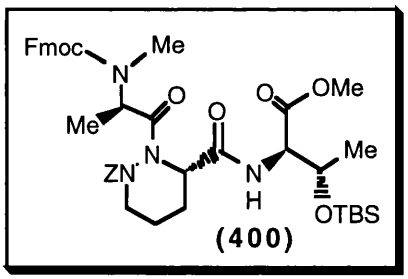
O- *t*-Butyldimethylsilyl-(*R*)-threonine methyl ester 399



(399)

A solution of **398** (2.64 g, 7.0 mmol) in THF (15 ml) was hydrogenated with 10% palladium on charcoal (67.0 mg) for 12 h. The reaction mixture was then filtered the catalyst washed with EtOAc and the filtrate concentrated *in vacuo* to give **399** (1.67g, 97%) as an oil. An analytical sample of **75** was obtained by flash chromatography eluting with hexanes/ Et_2O (6:1); IR (neat film): 3395 (w), 3332 (w), 2956 (s), 2932 (s), 2858 (s),1748 (s), 1602 (w), 1472 (m), 1375 (m), 1254 (s), 1160 (s), 1077 (s) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 4.3 (qd, J = 2.7, 6.3 Hz, 1H), 3.70 (s, 3H, OMe), 3.27 (d, J = 2.6 Hz, 1H), 1.70 (s, 2H), 1.23 (d, J = 6.3 Hz, 3H), 0.83 (s, 9H), 0.02 (s, 3H), -0.03 (s, 3H); 100 MHz ^{13}C NMR ($CDCl_3$): δ 174.8, 69.4, 60.7, 51.8, 25.6, 20.9, 17.8, -4.4, -5.3; Acc. Mass Calcd. for $C_{11}H_{26}NO_3Si$ $(M+H)^+$ 248.1682; Found: 248.1675.

N-Fluorenymethoxycarbonyl-*N*-methyl-(*R*)-alanyl-N^1-benzyloxycarbonyl-(*3S*)-piperazyl-(*O*-*t*-butyldimethylsilyl)- (*R*)-threonine methyl ester 400

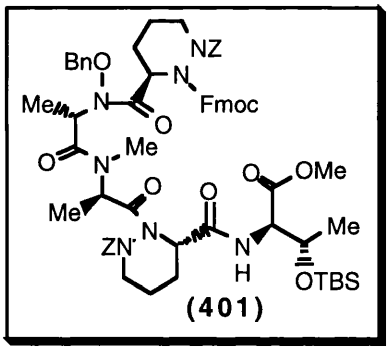


(400)

To a stirred solution of acid **395** (1.00 g, 1.80 mmol) and amine **399** (440.0 mg, 1.80 mmol) in dry THF (7 ml) at 0 °C was added 1-hydroxybenzotriazole hydrate (260.0 mg, 1.80 mmol), copper (II) chloride (10.0 mg, 1.80 mmol) and 1,3-dicyclohexylcarbodiimide (430.0 mg, 1.80 mmol). The reaction mixture was then stirred at 0 °C for 5 h. The reaction mixture was then filtered through Celite and the solvent evaporated *in vacuo.* The residue was diluted with EtOAc (25 ml) and washed sequentially with saturated aqueous sodium bicarbonate, H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Purification of the residue by flash

chromatography with hexanes/EtOAc (3:1) gave **400** (1.20 g, 85%) as a foam; $[\alpha]_D$ +21.6° (*c* 0.25, CH_2Cl_2); IR (KBr): 3400 (br w), 3325 (m), 2952 (m), 2924 (m), 1753 (m), 1720 (s), 1688 (s), 1531 (m), 1451 (m), 1404 (m), 1249 (s), 1156 (m), 1091 (m), 838 (m), 740 (m) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6 at 75 °C): δ 8.24 (s), 7.85 (d), 7.79-7.22 (complex m), 5.00 (br), 4.75 (br), 4.55-4.15 (br), 3.96 (br), 3.64 (small s, OMe), 3.55 (s, OMe), 2.67 (br s, NMe), 1.96-1.30 (br), 1.3-0.94 (br), 1.05 (d, *J* = 6.2 Hz, Me), 0.82 (d, Me) partially superimposed on 0.81 (s, 1), 0.00 (s, TBS-Me), -0.02 (s, TBS-Me); Acc. Mass Calcd. for $C_{43}H_{56}N_4O_9SiNa$ $(M+Na)^+$ 823.3714; Found: 823.3709; Anal Calcd. for $C_{43}H_{56}N_4O_9Si$: C, 64.48; H, 7.05; N, 6.99%. Found: C, 64.22; H, 7.10; N, 6.90%.

N^1Benzyloxycarbonyl-N^2-9-fluorenymethoxycarbonyl-(*3R*)-piperazyl-*N*-benzyl-oxyalanyl-*N*-methyl-(*R*)-alanyl-N^1-benzyloxycarbonyl-(*3S*)-piperazyl-(*O-t*-butyldimethylsilyl)-(*R*)-threonine methyl ester 401

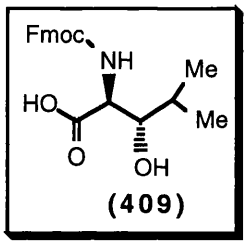


(401)

A solution of tripeptide **400** (300.0 mg, 0.38 mmol) in acetonitrile (4.5 ml) was added diethylamine (3 ml) and the mixture stirred at room temperature for 1.5 h. The mixture was then concentrated *in vacuo* and the residue coevaporated twice with benzene before being diluted with dry CH_2Cl_2 (1 ml). In a separate flask charged with the dipeptide **388** (270.0 mg, 4.50 mmol) in dry CH_2Cl_2 (4 ml) at -20 °C was added dry triethylamine (0.06 ml, 0.45 mmol) followed by bis(2-oxo-3-oxazolidinyl)phosphinic chloride (120.0 mg, 4.50 mmol) in one portion and the solution stirred for 1 h. The amine solution then added to this solution of the mixed anhydride, followed by triethylamine (0.06 ml, 0.45 mmol) and the resulting mixture stirred for 1 h at -20 °C then at 0 °C for 4 h. The mixture was then diluted with EtOAc (20 ml) and washed successively with a (1M) aqueous solution of hydrochloric acid, H_2O, 10% sodium bicarbonate solution and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the

residue by flash chromatography with hexanes/EtOAc (2:1) gave **401** (290.0 mg, 64%) as a foam; $[\alpha]_D$ -37.8° (*c* 0.4, CH_2Cl_2); IR (KBr): 3395 (br w), 3318 (w), 3072 (w), 3030 (w), 2952 (m), 2924 (m), 2854 (w), 1722 (br s), 1673 (s), 1652 (s), 1525 (w), 1447 (m), 1405 (m), 1356 (m), 1349 (m), 1286 (m), 1244 (s), 1194 (m), 1131 (m), 1082 (m), 1040 (m), 836 (w), 751 (m), 737 (m), 688 (m) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 8.65 (m), 8.25 (br m), 7.80-7.00 (complex m)), 5.60-3.90 (complex m), 3.80-3.30 (complex m), 3.30-1.00 (complex m), 0.08 (s), 0.05 (s), 0.04 (s), 0.03 (s), 0.02 (s); Acc. Mass Calcd. for $C_{66}H_{81}N_7O_{14}SiNa$ $(M+Na)^+$ 1246.5508; Found: 1246.5504; Anal. Calcd. for $C_{66}H_{81}N_7O_{14}Si$: C, 64.74; H, 6.67; N, 8.01%. Found: C, 64.41; H, 6.74; N, 7.82%.

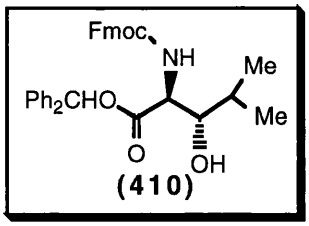
N-9-Fluorenymethoxycarbonyl-(*2S,3S*)-3-hydroxyleucine 409



(409)

To a stirred solution of (*2S,3S*)-3-hydroxyleucine **408** (330.0 mg, 2.31 mmol) in 10% aqueous sodium carbonate solution (6 ml) at 0 °C was added a solution of 9-fluorenylmethyl chloroformate (660.0 mg, 2.55 mmol) in dioxane (4 ml) over 5 min. When the addition was complete, the ice bath was removed and the mixture stirred for 2 h at room temperature. The mixture was then extracted with Et_2O (2 x 15 ml), acidified to pH 2 with concentrated hydrochloric acid, and extracted with EtOAc (3 x 35 ml). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **409** (750.0 mg, 88%) as a white solid. An analytical sample was obtained by recrystallisation with hexanes/EtOAc; IR (KBr): 3395 (m), 3376 (m), 2965 (w), 1759 (s), 1740 (w), 1690 (s), 1514 (s), 1448 (m), 1321 (m), 1297 (m), 1212 (s), 1129 (m), 1064 (m), 1040 (m), 761 (m), 741 (s), 538 (w) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6): δ 12.43 (br s), 7.88 (d, *J* = 7.4 Hz), 7.71 (d, *J* = 7.4 Hz), 7.50 (d, *J* = 8.8 Hz), 7.42-7.23 (m), 4.88 (br s), 4.39-4.19 (m), 4.03 (m), 3.45 (m),1.80 (m), 0.89 (d *J* = 6.7 Hz), 0.80 (d, *J* = 6.6 Hz); 100 MHz ^{13}C NMR (DMSO-d_6): δ 172.8, 155.8, 143.8, 140.7, 127.7, 127.0, 125.3, 120.1, 75.1, 65.7, 57.0, 46.7, 29.2, 19.7, 16.6; Acc. Mass Calcd. for $C_{21}H_{24}O_5N$ $(M+H)^+$ 370.1654; Found: 370.1666.

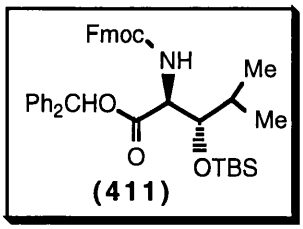
_N_-9-Fluorenymethoxycarbonyl-(*2S,3S*)-3-hydroxyleucine diphenylmethyl ester 410



(410)

To a solution of **409** (200.0 mg, 0.54 mmol) in acetone (1.5 ml) at room temperature was added a solution of diphenyldiazomethane (150.0 mg, 0.77 mmol) in acetone (2 ml) and the resulting purple solution stirred for 1 h. The reaction mixture was then concentrated *in vacuo* and the residue purified by flash chromatography eluting with hexanes/EtOAc (5:1) to give **410** (280.0 mg, 97%) as a white solid; IR (KBr): 3634 (w), 3585 (w), 3332 (m), 3030 (w), 2966 (m), 2875 (w), 1724 (s), 1537 (s), 1450 (m), 1317 (m), 1249 (s), 1221 (m), 1001 (s), 757 (s), 743 (s), 703 (s) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.76 (d, *J* = 7.3 Hz), 7.58 (d, *J* = 6.9 Hz), 7.45-7.19 (complex m), 6.95 (s), 5.85 (d, *J* = 7.9 Hz), 4.66 (m), 4.40 (m), 4.19 (m), 3.52 (br m), 2.52 (d, *J* = 7.9 Hz), 1.66 (m), 0.93 (m); 100 MHz ^{13}C ($CDCl_3$): δ 170.1, 156.1, 143.7, 143.6, 141.2, 139.2, 128.5, 128.2, 128.0, 127.7, 127.4, 127.0, 126.8 125.0, 119.9, 78.7, 78.4, 67.2, 56.7, 47.1, 31.0, 19.1, 18.6; Acc. Mass Calcd. for $C_{34}H_{33}O_5N$ (M^+) 535.2359; Found: 535.2354.

_N_-9-Fluorenymethoxycarbonyl-_O_-_t_-butyldimethylsilyl-(*2S,3S*)-3-hydroxyleucine diphenylmethyl ester 411

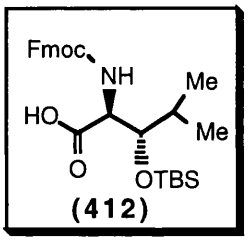


(411)

To a solution of **410** (250.0 mg, 0.47 mmol) in dry CH_2Cl_2 (1.5 ml) under nitrogen was added diisopropylethylamine (0.10 ml, 0.57 mmol) followed by *t*-butyldimethylsilyl trifluoromethanesulfonate (0.13 ml, 0.57 mmol) and the mixture stirred at room temperature for 12 h. The reaction mixture was then quenched with saturated aqueous sodium bicarbonate and the product extra-cted with CH_2Cl_2 (4 x 25 ml). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The residue was then purified by flash chromatography eluting with hexanes/Et_2O (10:1) to give **411** (250.0 mg, 82%) as an oil; IR (neat

film): 3445 (w), 3374 (br w), 3072 (w), 3030 (w), 2959 (m), 2931(m), 1725 (s), 1498 (s), 1451(m), 1362 (m), 1254 (s), 1196 (m), 1082 (s), 1057 (m), 838 (m), 778 (s), 740 (s), 699 (s) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.61 (m, 2H), 7.45-7.24 (m, 14H), 6.99 (s, 1H), 5.70 (d, *J* = 7.6 Hz, 1H), 4.72 (dd, *J* = 2.2 and 7.6 Hz, 1H), 4.40 (m, 2H), 4.24 (dd, *J* =7.0, 7.0 Hz, 1H), 3.66 (dd, *J* = 2.0, 8.4 Hz, 1H), 2.07 (m, 1H), 1.04 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.86 (s, 9H), 0.10 (s, 3H), 0.07 (s, 3H); 100 MHz ^{13}C NMR ($CDCl_3$): δ 169.1, 155.4, 143.9, 143.6, 141.3, 139.5, 139.4, 128.6, 128.4, 128.1, 128.0, 127.7, 127.4, 127.1, 125.2, 125.0, 120.0, 80.5, 78.3, 67.0, 57.6, 47.1, 31.5, 25.9, 19.7, 19.5, 18.2 -3.97, -4.34; Acc Mass Calcd. for $C_{40}H_{48}O_5NSi$ $(M+H)^+$ 650.3302; Found: 650.3311.

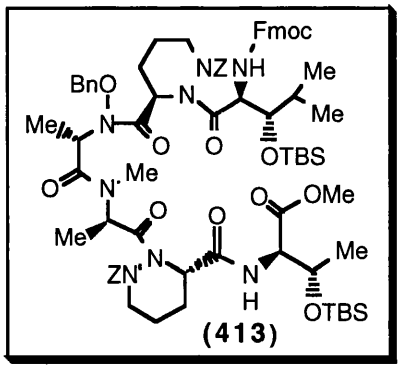
_N_-9-Fluorenymethoxycarbonyl-_O_-_t_-butyldimethylsilyl-(_2S,3S_)-3-hydroxyleucine 412



(412)

A mixture of **411** (250.0 mg, 0.38 mmol) and 10% palladium on carbon (21 mg) in EtOAc (5 ml) was stirred under an atmosphere of hydrogen for 12 h. The catalyst was then removed by filtration, and the filtrate concentrated *in vacuo*. The residue was purified by flash chromatography with hexanes/EtOAc (3:1) to give **412** (160.0 mg, 89%) as a foam; IR (KBr): 3600- 2300 (br), 3445 (w), 3058 (w), 2959 (s), 2932 (s), 2858 (m), 1721 (br s), 1513 (m), 1448 (m), 1473 (m), 1255 (s), 1213 (m), 1255 (s), 1082 (s), 1058 (s), 838 (s), 776 (s), 739 (s) cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 10.30 (br s, 1H), 7.74 (d, *J* = 7.6 Hz, 2H), 7.58 (m, 2H), 7.38 (m, 2H), 7.29 (m, 2H), 5.50 (d, *J* = 8.2 Hz, 1H), 4.58 (dd, *J* = 2.8, 8.0 Hz, 1H), 4.39 (m, 2H), 4.21 (dd, *J* = 7.0, 7.1 Hz, 1H), 3.60 (m, 1H), 1.99 (m, 1H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.87 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); 100 MHz ^{13}C NMR ($CDCl_3$): δ 175.4, 155.53, 143.8, 143.6, 141.3, 127.7, 127.0, 125.1, 125.0, 120.0, 79.9, 67.1, 57.2, 47.1, 31.4, 25.9, 19.5, 19.4, 18.2, -4.1, -4.2; Acc Mass Calcd. for $C_{27}H_{38}O_5NSi$ $(M+H)^+$ 484.2519; Found: 484.2525.

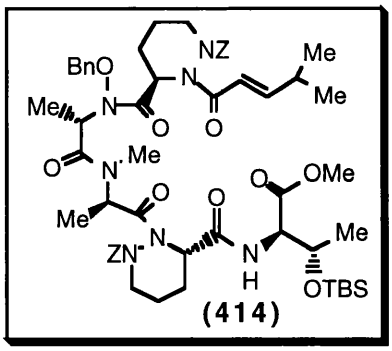
_N_-9-Fluorenymethoxycarbonyl-_O_-_t_-butyldimethylsilyl-(_2S,3S_)-3-hydroxyleucyl-N^1-benzyloxycarbonyl-(_3R_)-piperazyl-_N_-benzyloxyalanyl-_N_-methyl-(_R_)-alanyl-N^1-benzyloxycarbonyl-(_3S_)-piperazyl-(_O_-_t_-butyldimethylsilyl)-(_R_)-threonine methyl ester 413



(413)

To a stirred solution of pentapeptide **401** (82.0 mg, 0.067 mmol) in acetonitrile (2 ml) was added diethylamine (1 ml) and the mixture stirred at room temperature for 1.5 h. The mixture was then concentrated *in vacuo* and the residue coevaporated with dry benzene (2x). The crude acyl hydrazine **386** was then diluted with toluene (1 ml) and added to acid chloride **389** [prepared from **412** (49.0 mg, 0.1 mmol) by treatment with oxalyl chloride (0.1 ml, 1.0 mmol) in C_6H_6 (1 ml) for 1.5 h] followed by silver cyanide (18 mg, 0.13 mmol). The reaction flask was then covered with foil and placed in an oil bath at 80 ^oC for 40 min. The reaction mixture was cooled to room tempera-ture and filtered through Celite. The filtrate was washed with 10% aqueous sodium bicarbonate, brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* Purification of the crude residue by flash chromatography with hexanes/EtOAc (6:1) as eluent gave **413** (55.0 mg, 56%) as a foam; HPLC analysis indicated **413** was 85% pure; Acc. Mass Calcd. for $C_{78}H_{106}O_{16}N_8Si_2Na$ $(M+Na)^+$ 1489.7163; Found: 1489.7155.

Hexapeptide Precursor 414



(414)

To a stirred solution of pentapeptide **401** (120.0 mg, 0.1 mmol) in acetonitrile (1.5 ml) was added diethylamine (0.90 ml) and the mixture stirred at room temperature for 1.5 h. The mixture was then concentrated *in vacuo* and the residue coevaporated twice with dry benzene. The crude acyl hydrazine **386** was then diluted with toluene (1 ml) and added to the acid chloride [prepared from **417** (22.0 mg, 0.2 mmol) by treatment with oxalyl chloride (0.17 ml, 2.0 mmol) in C_6H_6 (1.5 ml) at room temperature for 1 h] followed by silver cyanide (10 mg, 0.2 mmol). The reaction flask was then covered with foil and placed in an oil bath at 90 °C for 1 h The reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was washed with 10% aqueous sodium bicarbonate, brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* Purification of the crude residue by flash chromatography with hexanes/EtOAc (2:1) as eluent gave **414** (58.0 mg, 54%) as a foam; $[\alpha]_D$ -55° (*c* 0.2, CH_2Cl_2); IR (KBr): 3325 (s), 2959 (m), 2933 (m), 2858 (m), 1724 (s), 1664 (s), 1523 (m), 1500 (m), 1457 (m), 1403 (m), 1359 (m), 1254 (s), 1195 (m), 838 (s), 698 (s) cm^{-1}; 400 MHz ^1H NMR (DMSO-d_6 at 125 °C): δ 7.85 (br), 7.43-7.30 (complex m, Ph), 6.80 (2 x dd, *J* = 15.5, 6.6 Hz), 6.71 (smaller dd), 6.13 (dd, *J* = 15.5, 1.0 Hz, superimposed on small d), 5.76 (br m), 5.4-4.64 (complex br m), 4.47 (br m), 4.37 (br m), 4.34-3.74 (complex br m), 3.62 (s, OMe), 3.58 (s, OMe), 3.06 (br m), 2.93 (s), 2.4 (m), 2.08 (br), 1.82 (br m), 1.73 (br m), 1.46 (br m), 1.31 (br), 1.22 (d, *J* = 7.0 Hz), 1.10 (d, *J* = 6.0 Hz), 1.04 (d, *J* = 6.2 Hz), 0.96 (apparent t), 0.86 (large s, Bu-*t*), 0.04 (large s, TBS-Me), 0.03 (small s, TBS-Me), -0.02 (small s, TBS-Me); Acc. Mass Calcd. for $C_{57}H_{79}N_7O_{13}SiNa$ $(M+Na)^+$ 1120.5403; Found: 1120.5400; Anal. Calcd. for $C_{57}H_{79}N_7O_{13}Si$: C, 62.33, H, 7.25; N, 8.93%. Found: C, 62.07; H, 7.29; N, 8.86%.

4.2 References

(1) Hassall, C.H.; Johnson, W.H.; Theobald, C.J., *J.Chem. Soc., Perkin Trans. I,* **1979**, 1451.

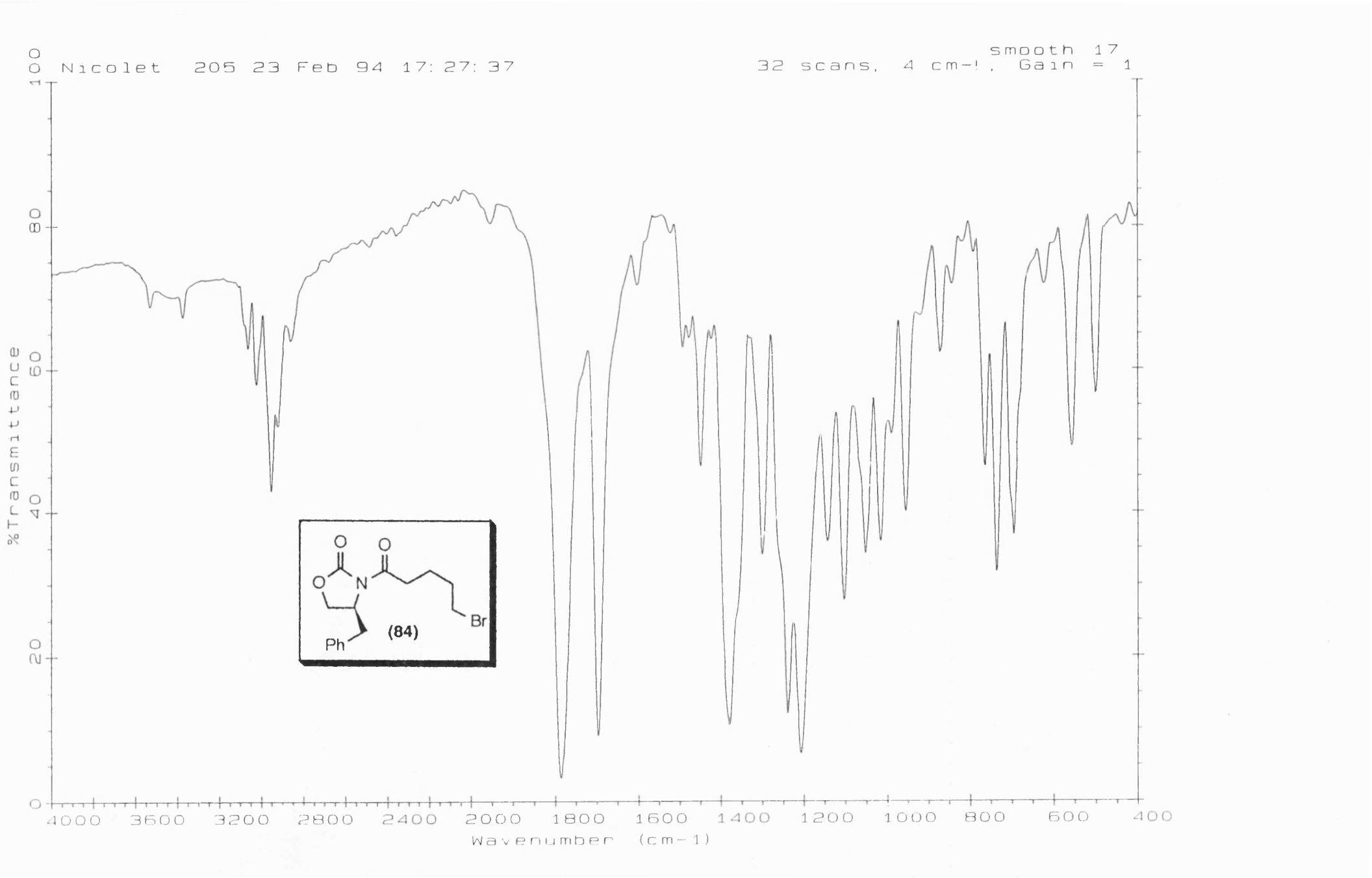
(2) Nakamura, Y.; Shin, C., *Chem. Lett.*, **1991**, 1953.

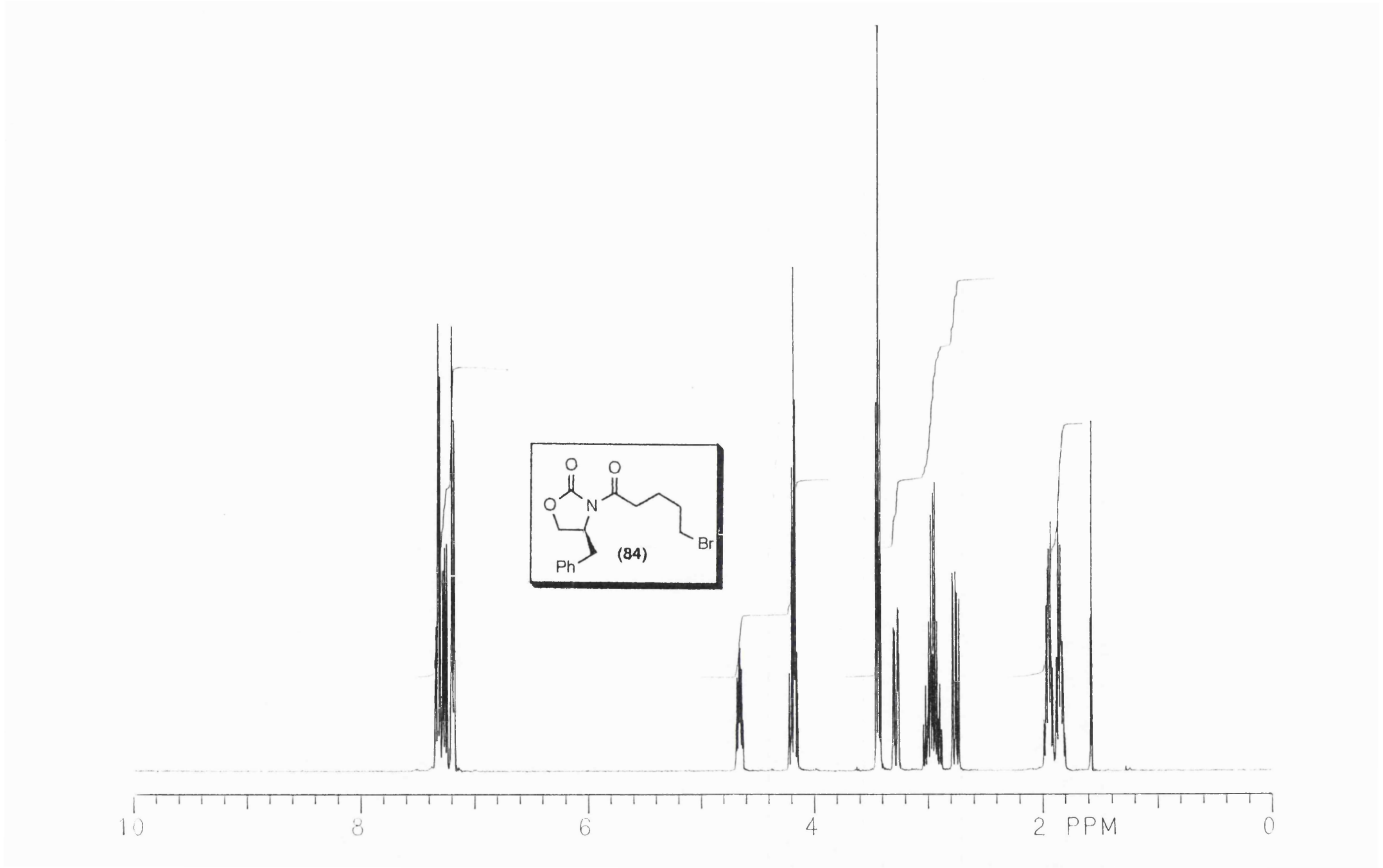
(3) Durette, P. L.; Baker, F.; Barker, P. L.; Boger, J.; Bondy, S. S.; Hammond, M. L.; Lanza, T. L.; Pessolano, A. A.; Caldwell, C. G. , *Abs. 198th ACS National Meeting,* **1989**, *Miami Beach, Florida*, September 10-15, Abstract No.119.

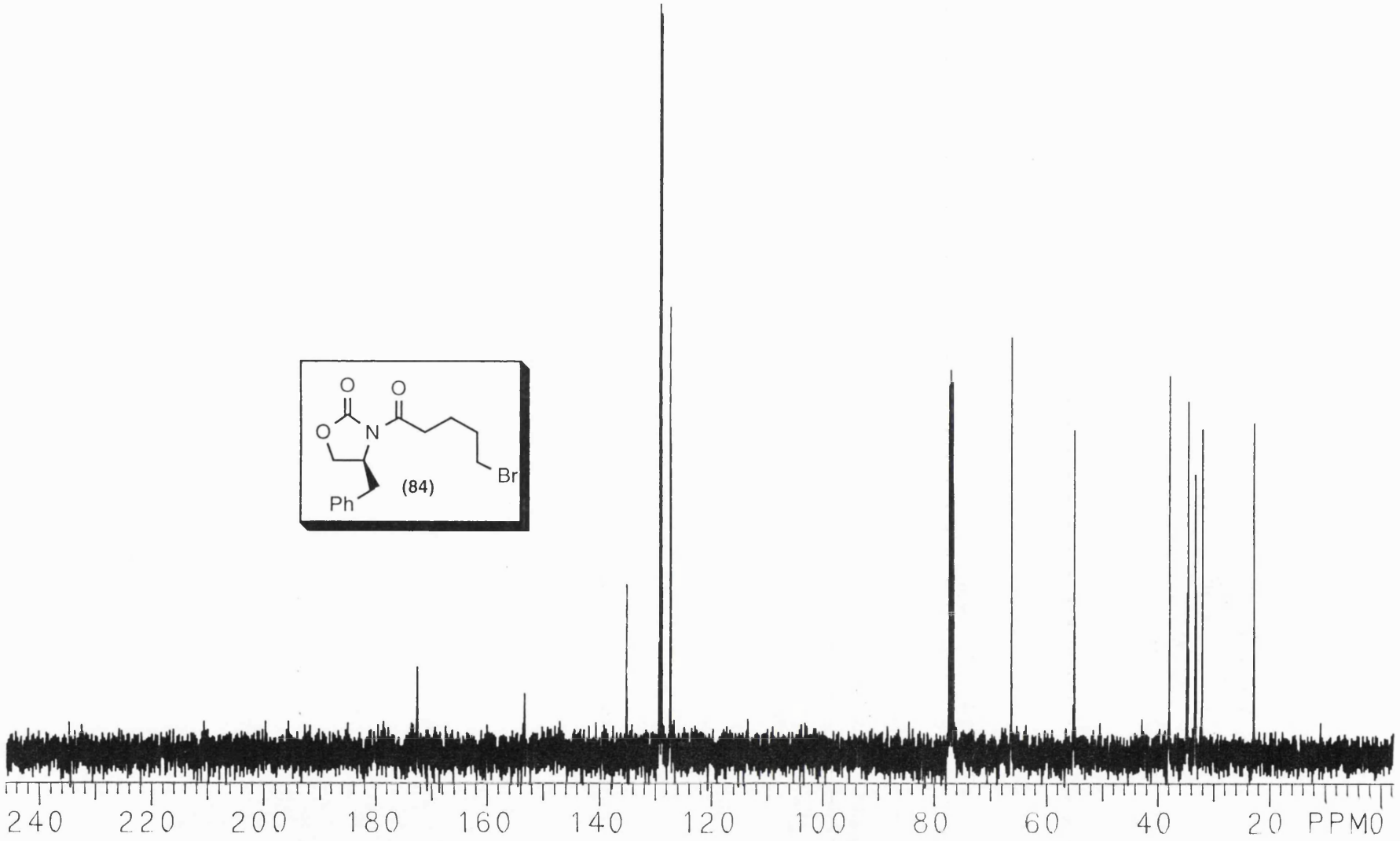
(4) Kolasa, T.; Chimiak, A.; Kitowska, A., *J. Prakt. Chemie.,* **1975**, 252.

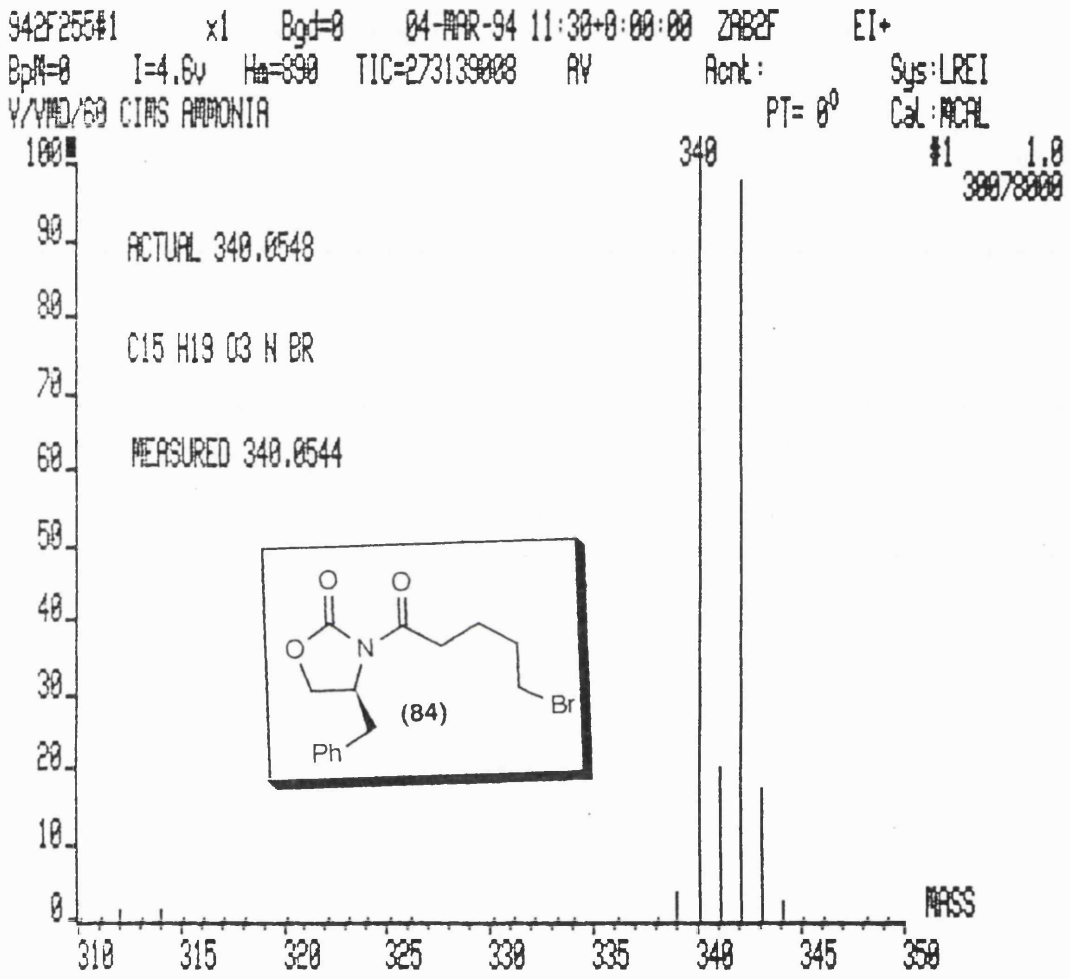
(5) Kolasa, T.; Chimiak, A. *Tetrahedron,* **1975**, *30*, 3591.

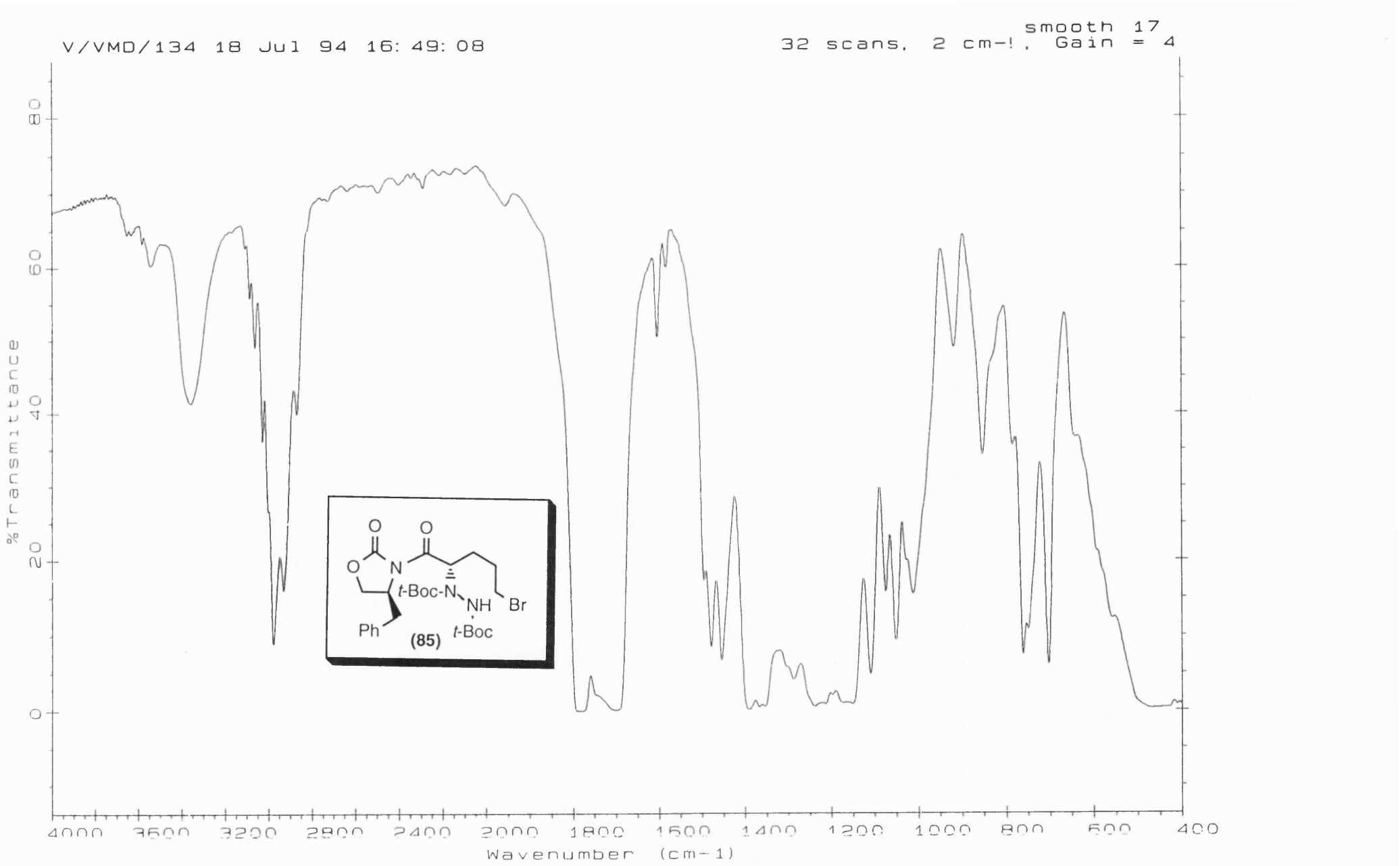
Appendix 1: Selected Spectral Data

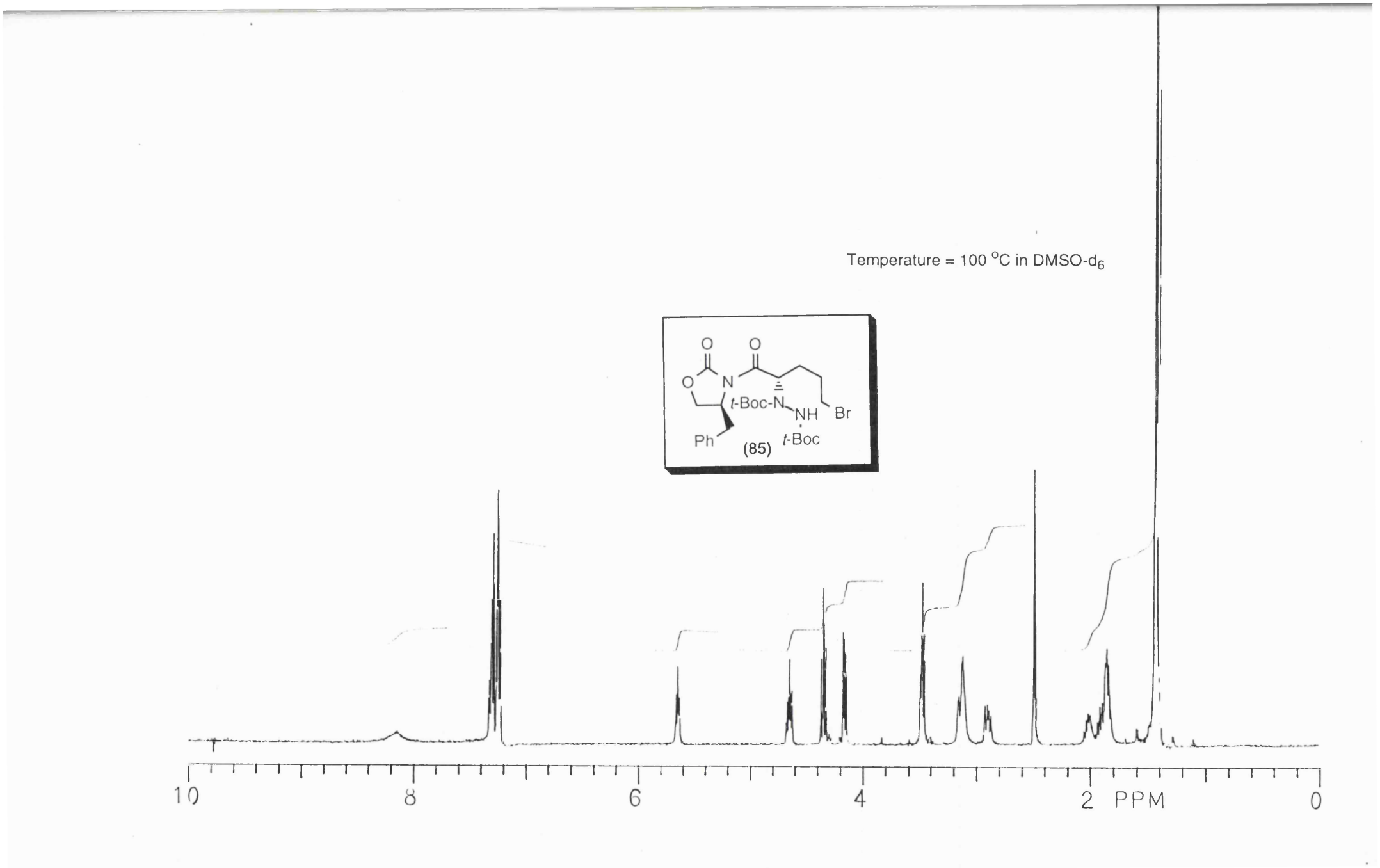

Nicolet 205 23 Feb 94 17:27:37
smooth 17
32 scans, 4 cm-1, Gain = 1
%Transmittance
100
80
60
40
20
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
O
O
O
N
Br
Ph
(84)

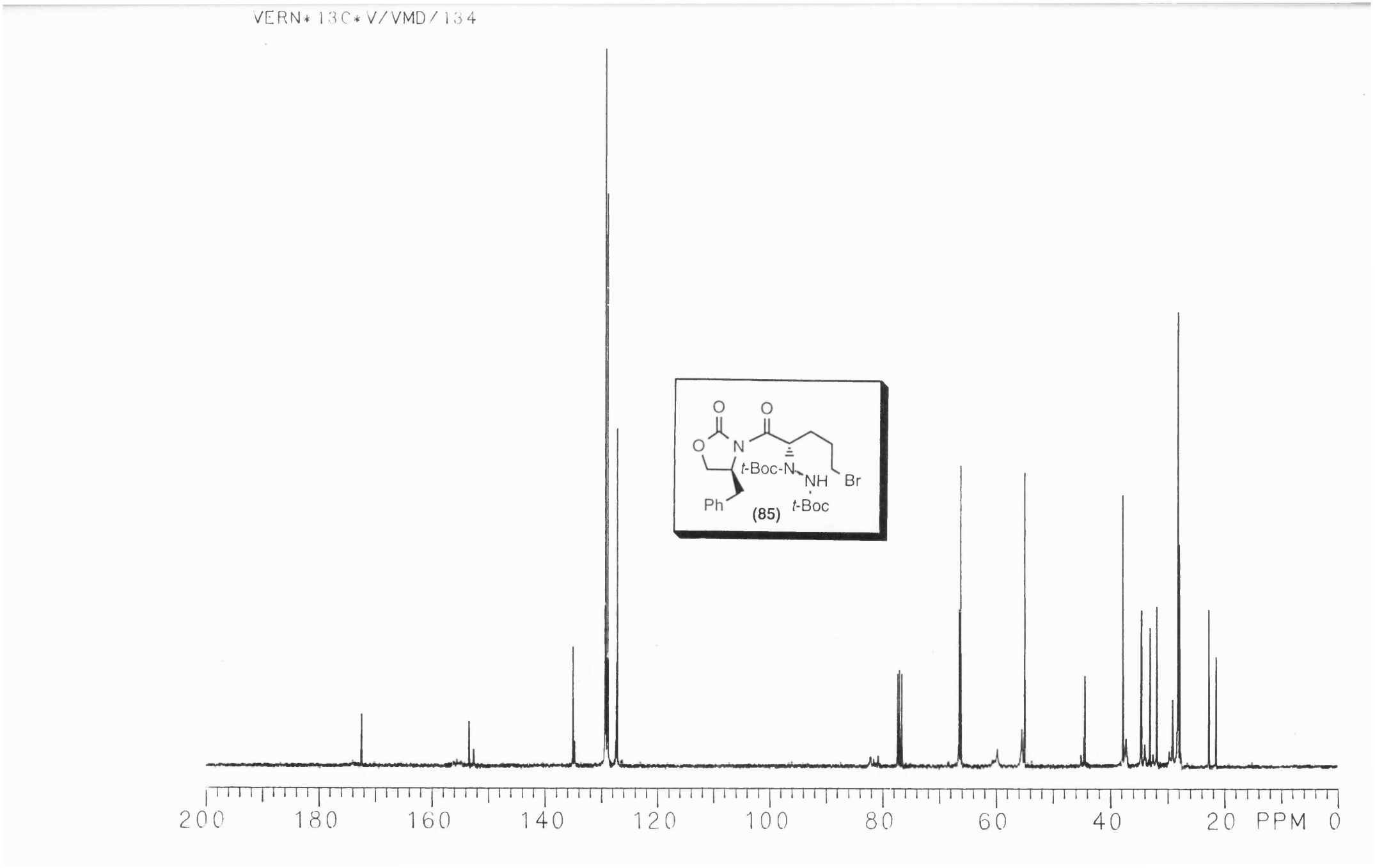

O
O
O
N
Br
Ph
(84)
10
8
6
4
2 PPM
0

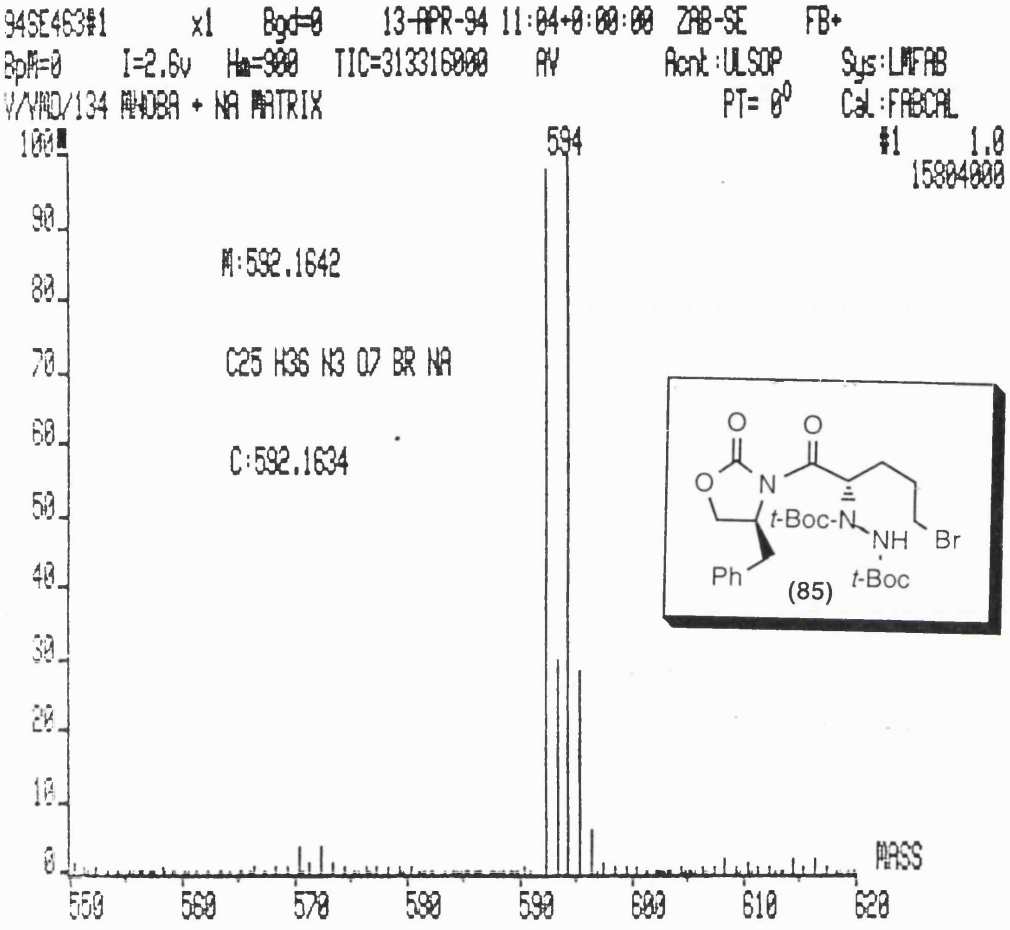

O
O
O
N
Br
Ph
(84)
240
220
200
180
160
140
120
100
80
60
40
20
PPM0

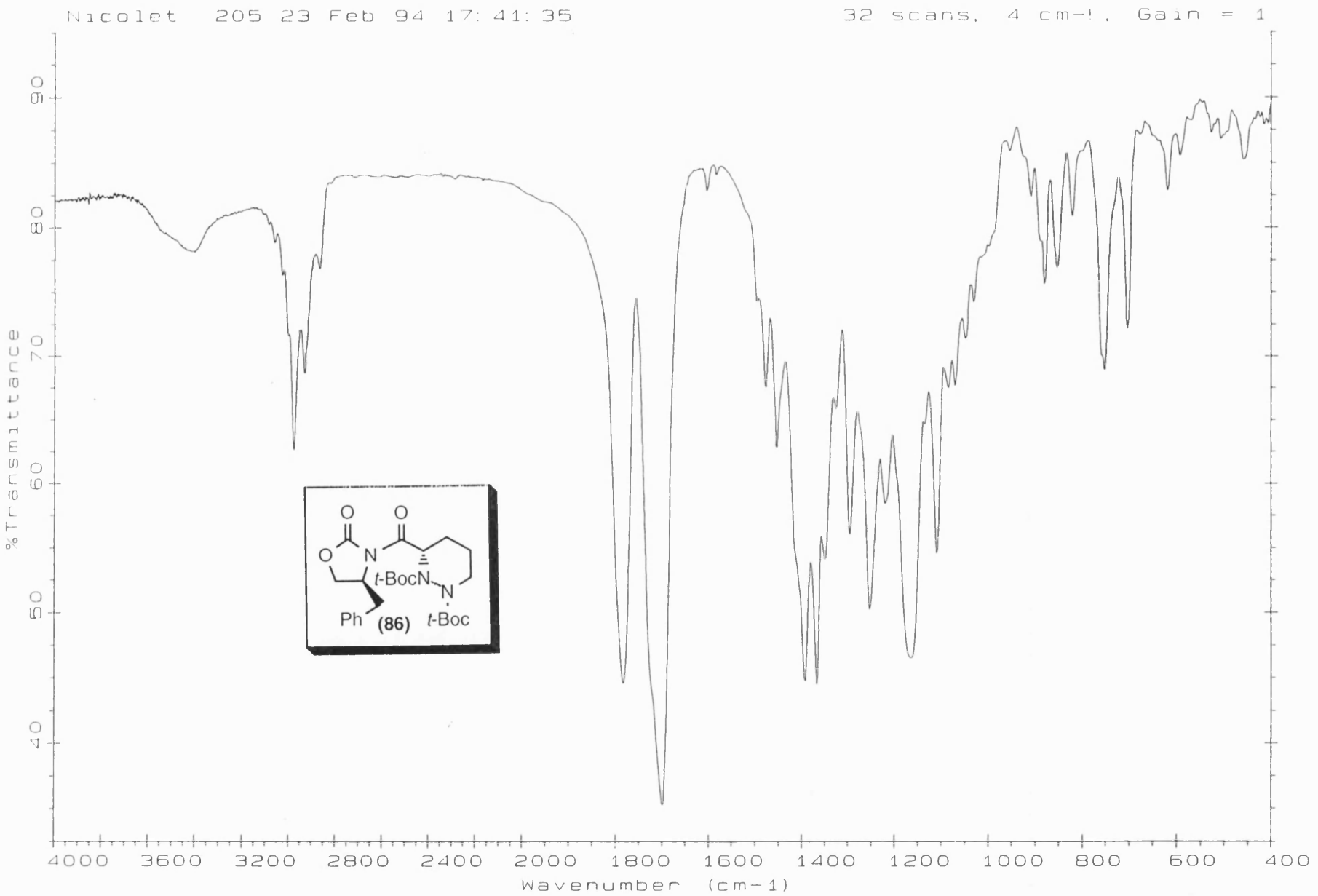

942F255#1 x1 Bgd=0 04-MAR-94 11:30+0:00:00 ZAB2F EI+
BpM=0 I=4.6v Ma=600 TIC=273139008 AV Acnt: Sys:LREI
V/VMD/60 CIMS AMMONIA PT= 0° Cal:MCAL
ACTURAL 340.0548
C15 H19 O3 N BR
MEASURED 340.0544
(84)
Ph
Br
340
#1 1.0
300780000
MASS
100
90
80
70
60
50
40
30
20
10
0
310
315
320
325
330
335
340
345
350

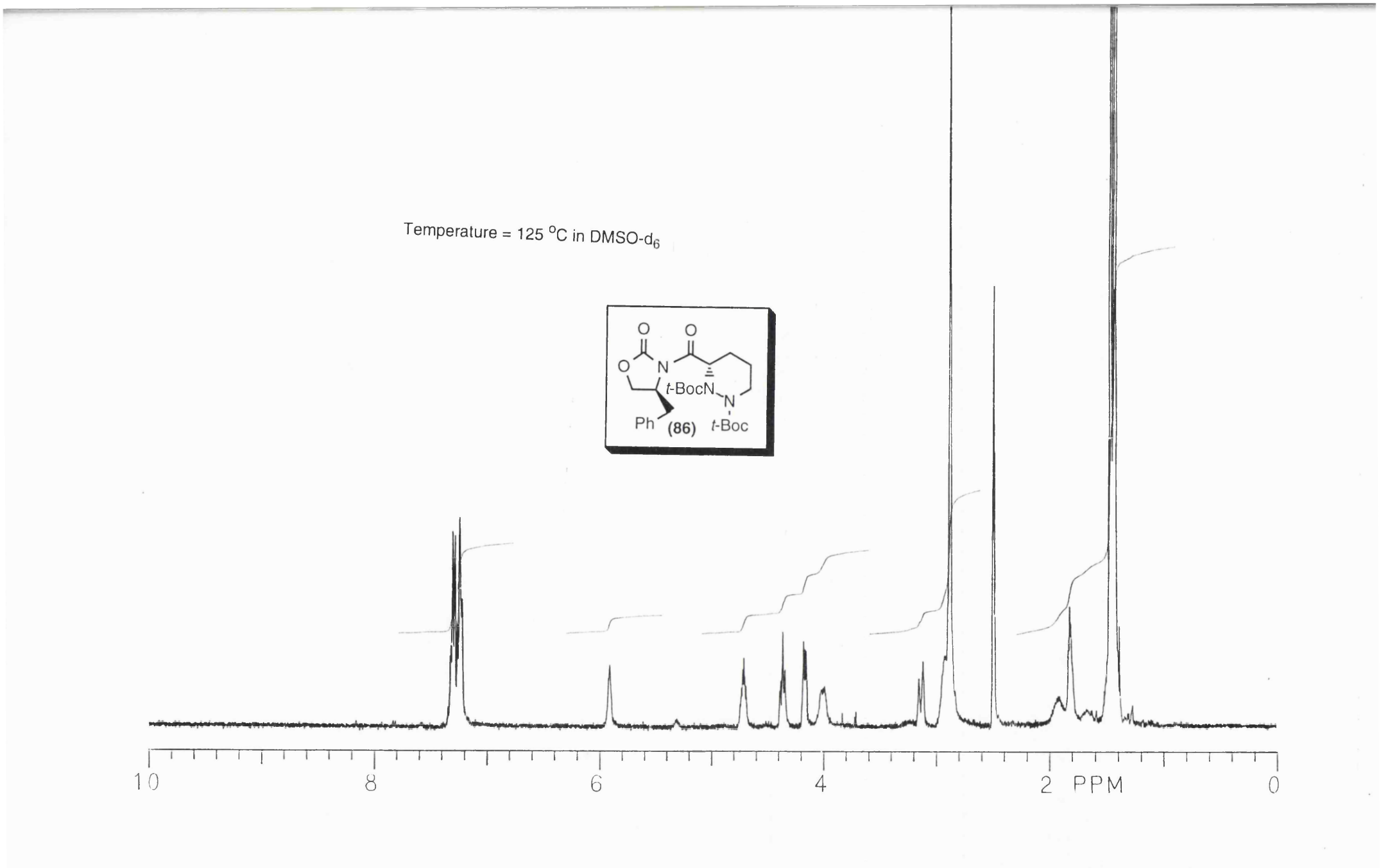

V/VMD/134 18 Jul 94 16: 49: 08
smooth 17
32 scans, 2 cm-!, Gain = 4
%Transmittance
80
60
40
20
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
O
O
O
N
t-Boc-N
NH
Br
Ph
t-Boc
(85)

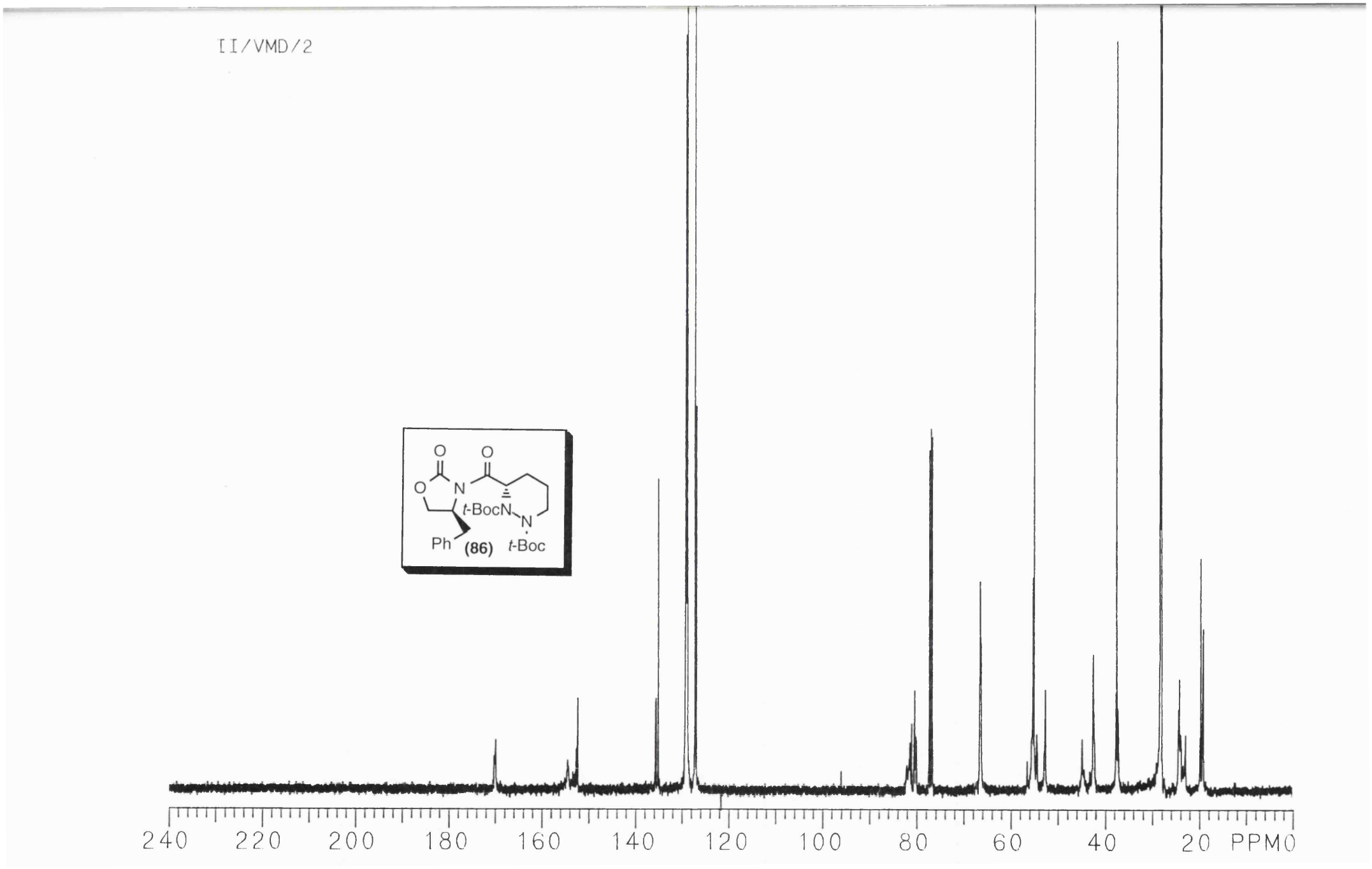

Temperature = 100 °C in DMSO-d6
O
O
O
N
t-Boc-N
NH
Br
Ph
t-Boc
(85)
10
8
6
4
2 PPM
0

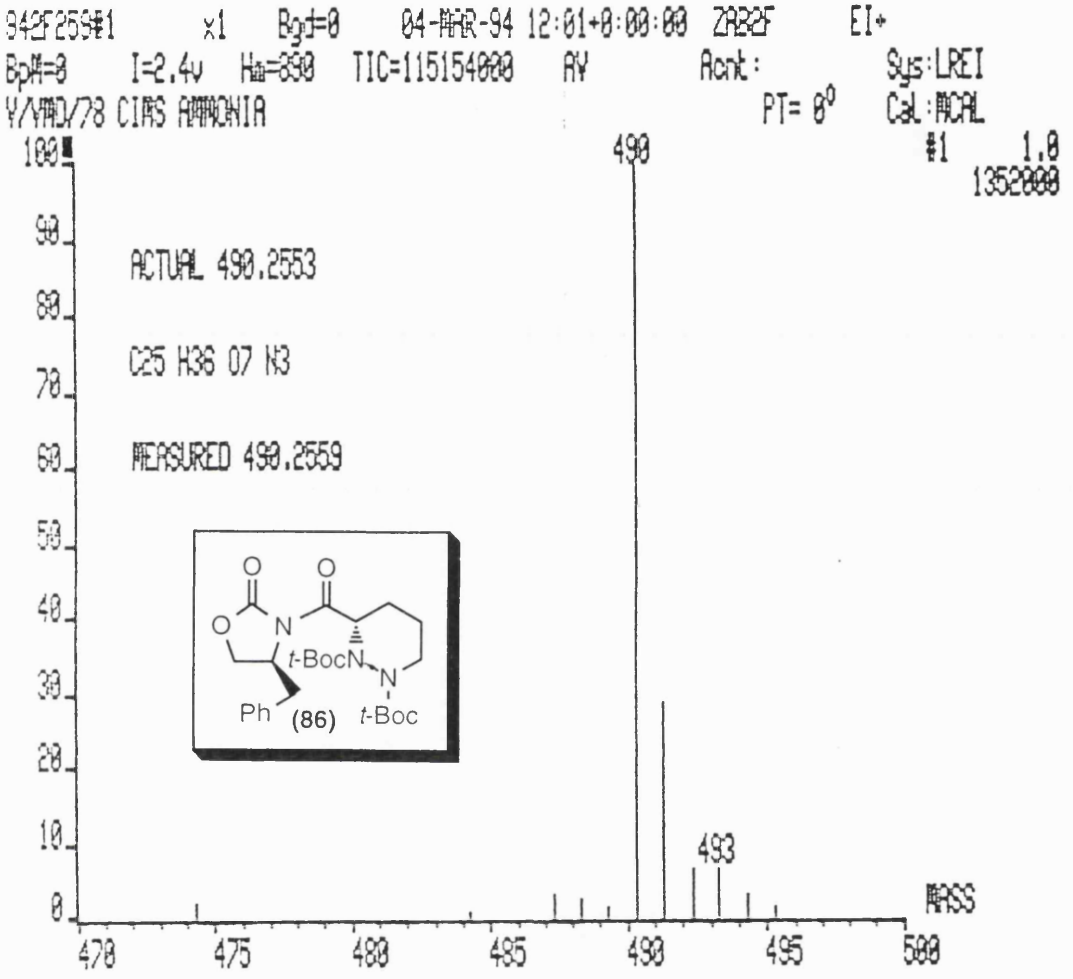

VERN* 13C* V/VMD/134
O
O
O
N
t-Boc-N
NH
Br
Ph
t-Boc
(85)
200
180
160
140
120
100
80
60
40
20
PPM
0

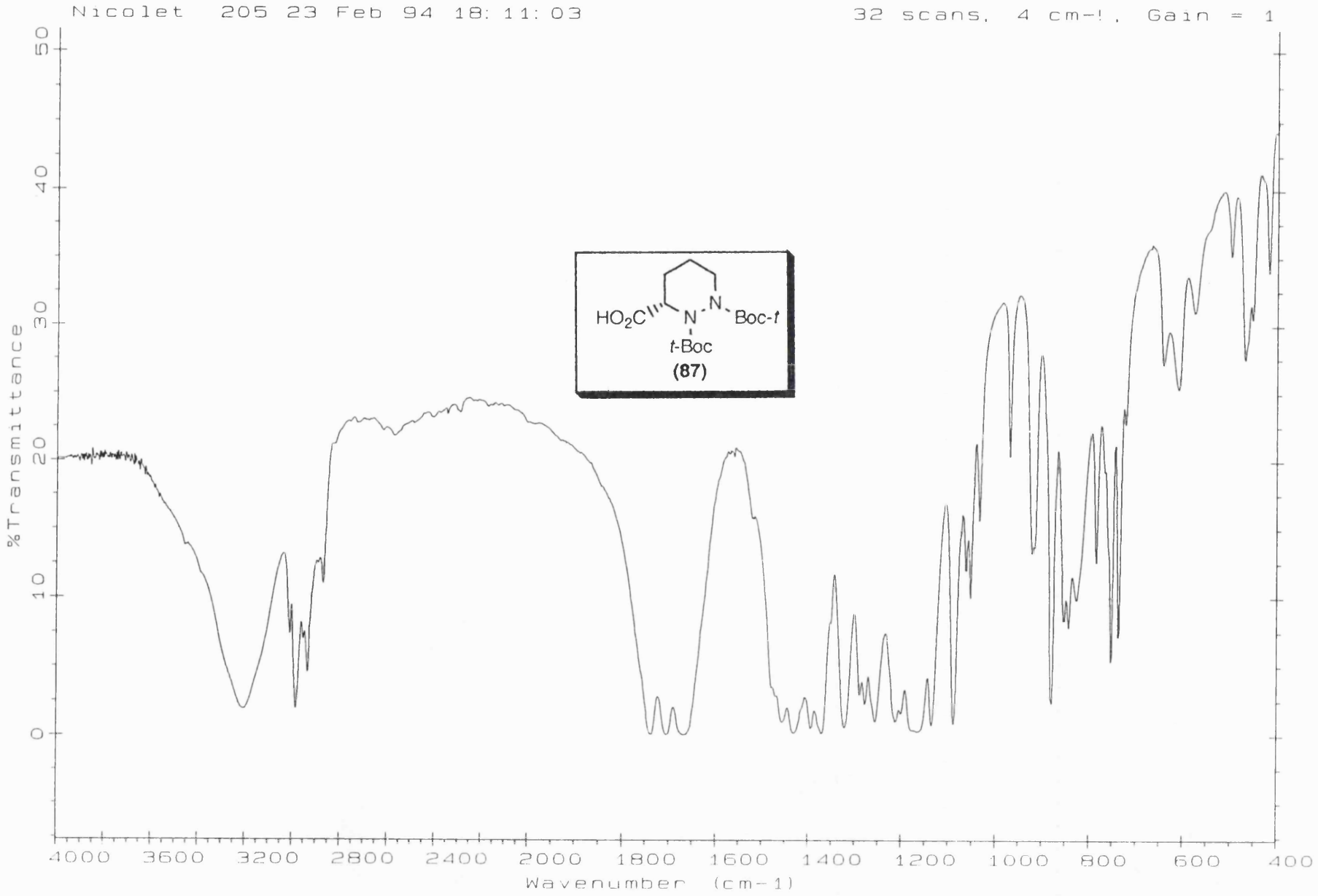

94SE463#1 x1 Bgd=0 13-APR-94 11:04+0:00:00 ZAB-SE FB+
BpM=0 I=2.6v Hm=900 TIC=313316000 AV Acnt:ULSOP Sys:LMFAB
V/YMD/134 MNOBA + NA MATRIX PT= 0° Cal:FABCAL
#1 1.0
15804000
594
M:592.1642
C25 H36 N3 O7 BR NA
C:592.1634
O
O
O
N
t-Boc-N
NH
Br
Ph
t-Boc
(85)
100
90
80
70
60
50
40
30
20
10
0
MASS
550
560
570
580
590
600
610
620

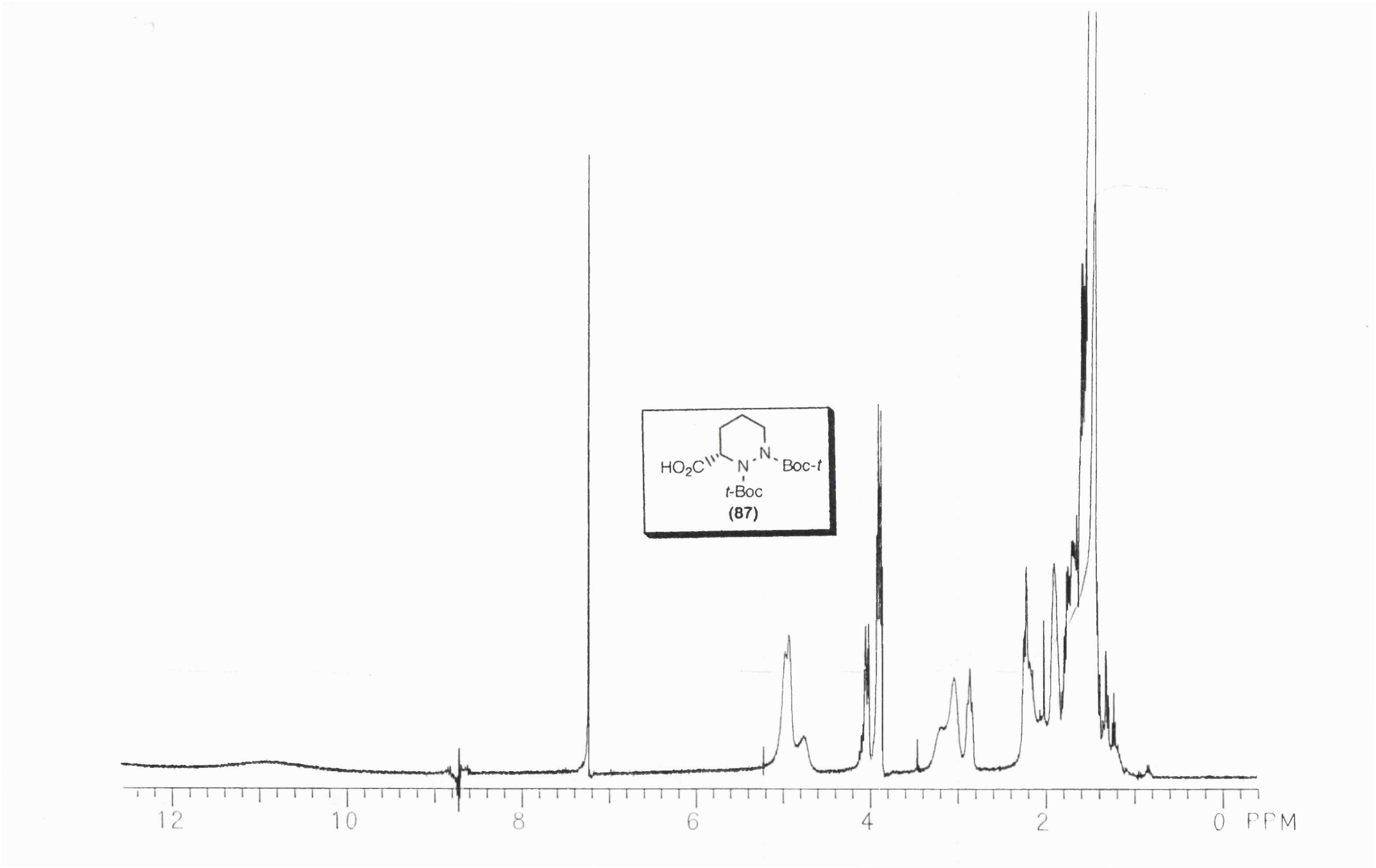

Nicolet 205 23 Feb 94 17:41:35
32 scans, 4 cm-1, Gain = 1
%Transmittance
90
80
70
60
50
40
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
O
O
O
N
t-BocN
N
Ph
(86)
t-Boc

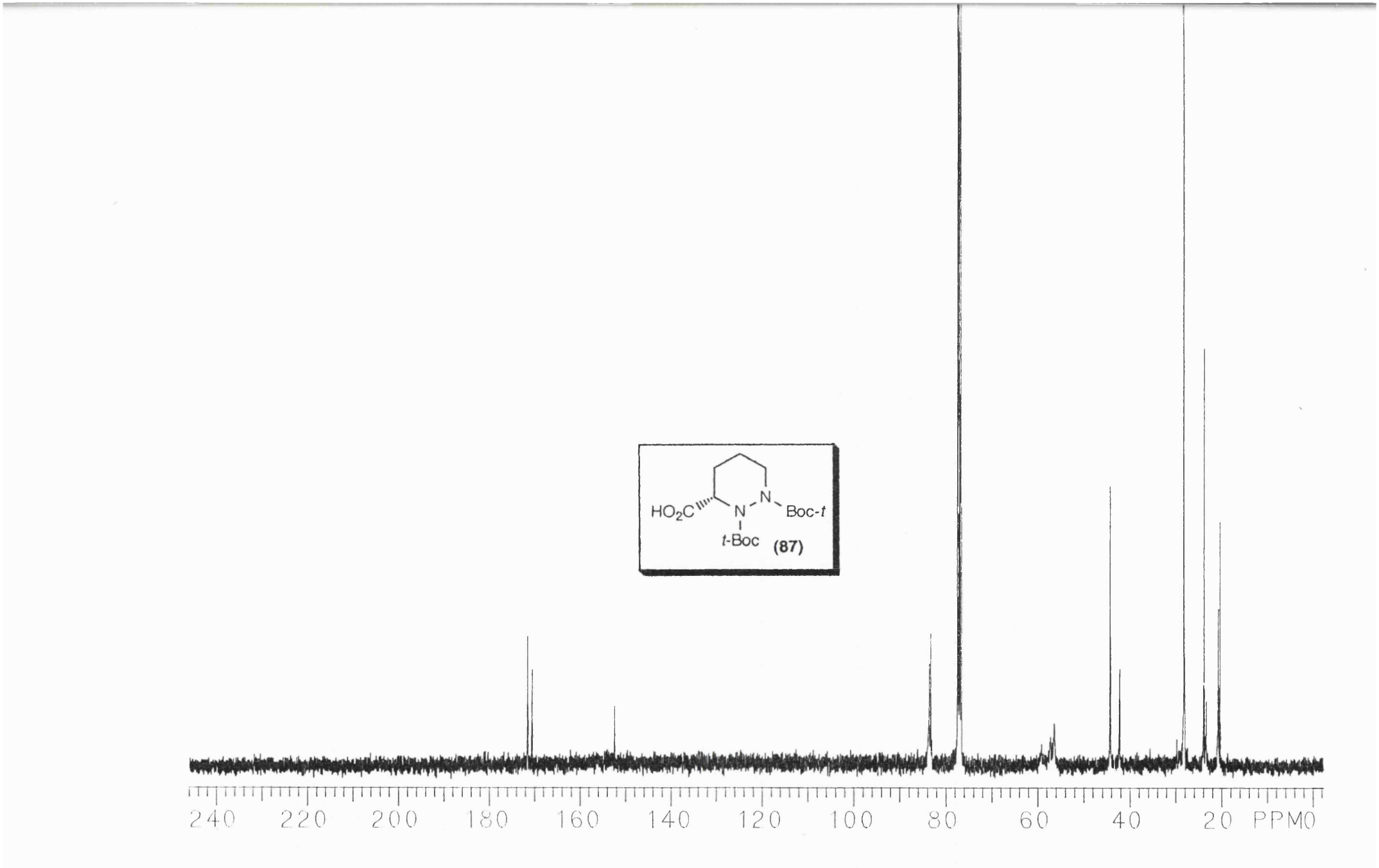

Temperature = 125 °C in DMSO-d6
O
O
O
N
t-BocN
N
Ph
(86)
t-Boc
10
8
6
4
2 PPM
0

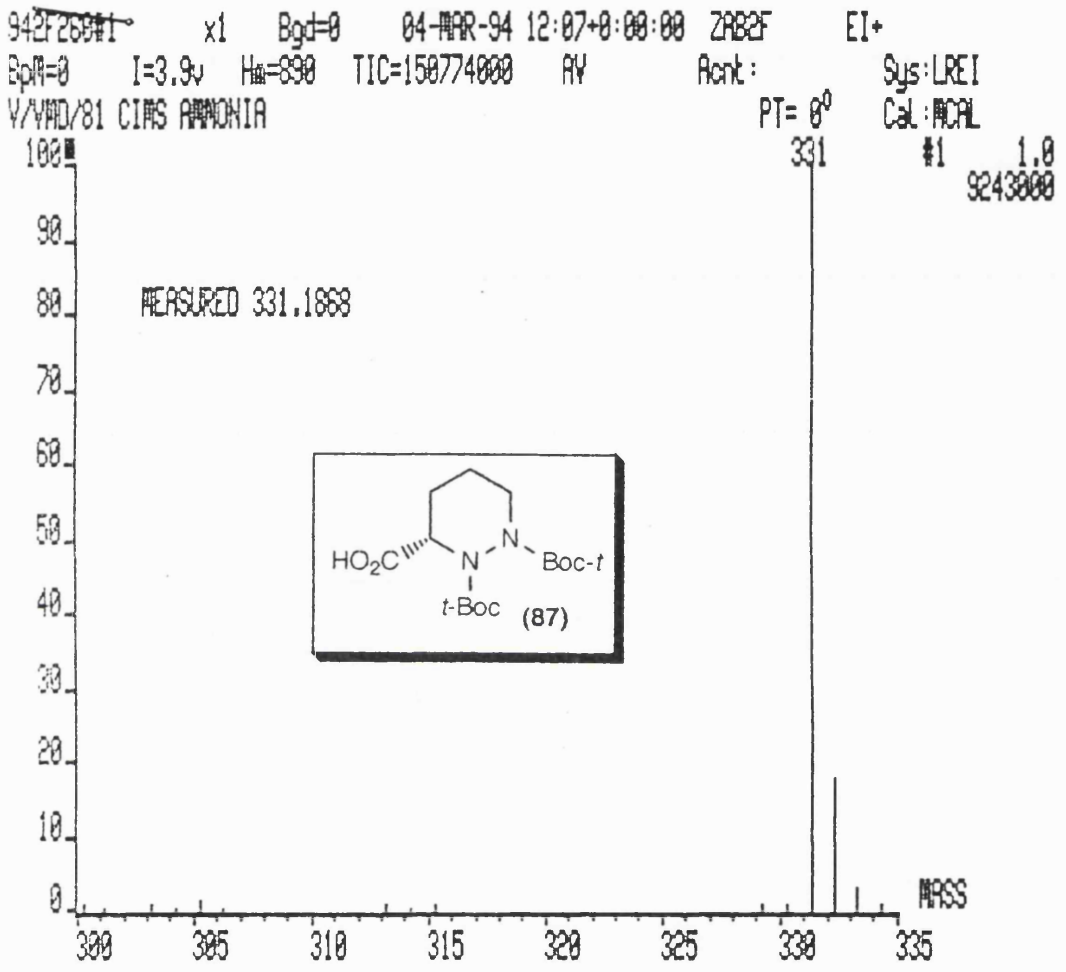

II/VMD/2
O
O
O
N
t-BocN
N
Ph
(86)
t-Boc
240
220
200
180
160
140
120
100
80
60
40
20
PPM0

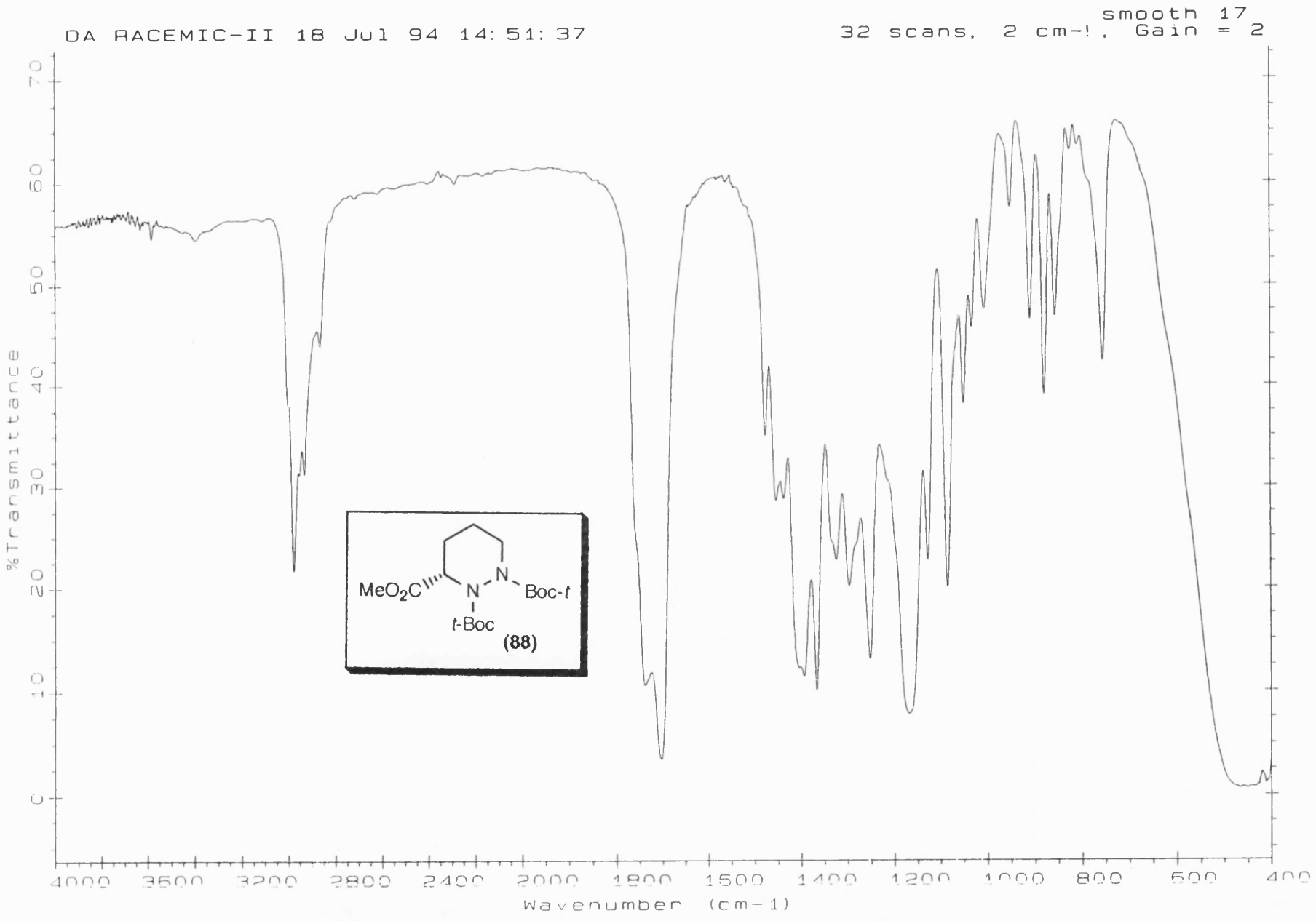

942F259#1 x1 Bgd=0 04-MAR-94 12:01+0:00:00 ZR82F EI+
BpM=0 I=2.4v Ma=850 TIC=115154000 AV Acmt: Sys:LREI
Y/YMD/78 CIMS AMMONIA PT= 0° Cal:MACAL
#1 1.0
1352000
ACTUAL 490.2553
C25 H36 O7 N3
MEASURED 490.2559
(86)
Ph
t-Boc
t-BocN
490
493
MASS
100
90
80
70
60
50
40
30
20
10
0
470
475
480
485
490
495
500

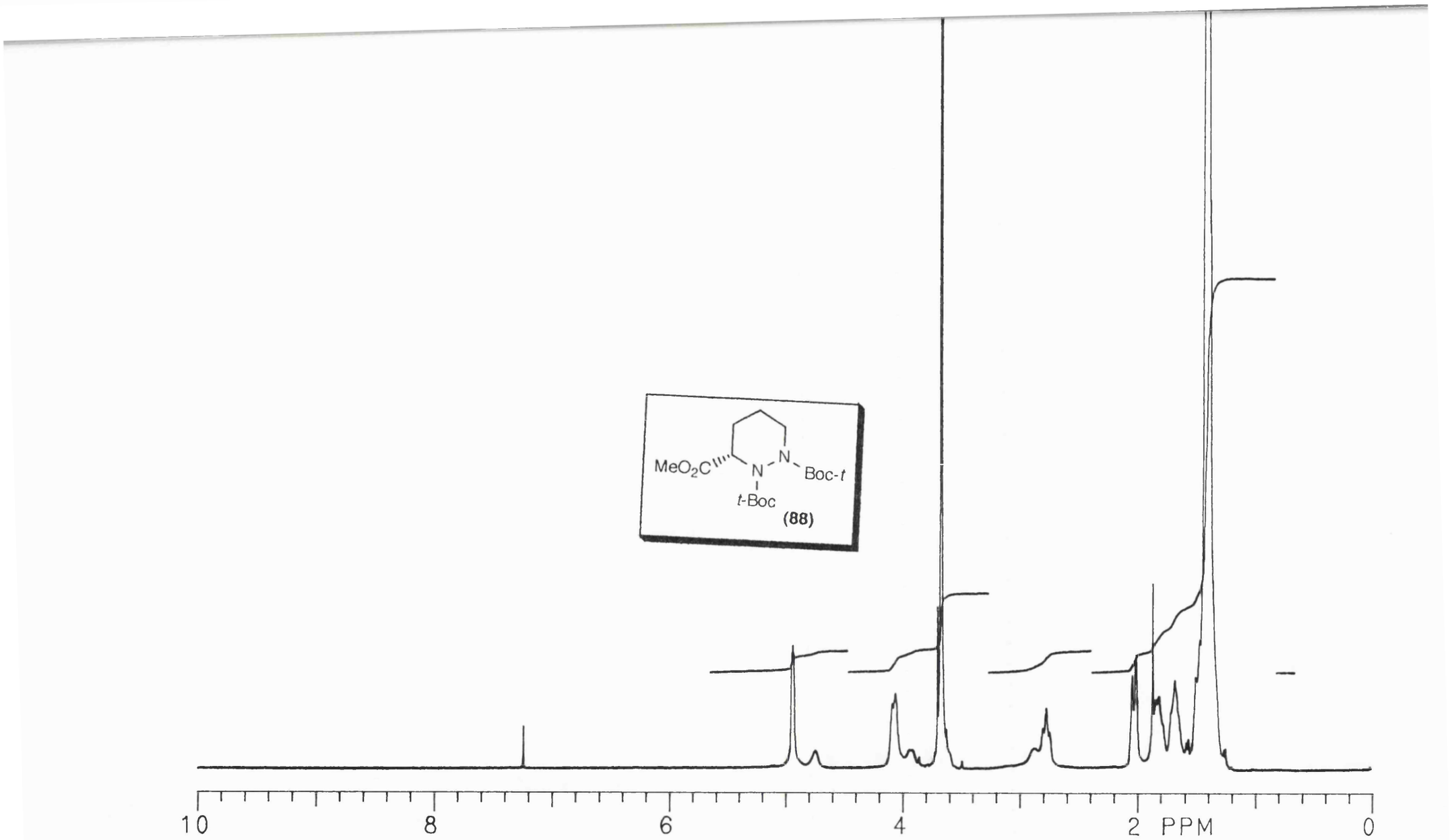

Nicolet 205 23 Feb 94 18:11:03
32 scans, 4 cm-1, Gain = 1
%Transmittance
50
40
30
20
10
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
HO2C
N
N
Boc-t
t-Boc
(87)

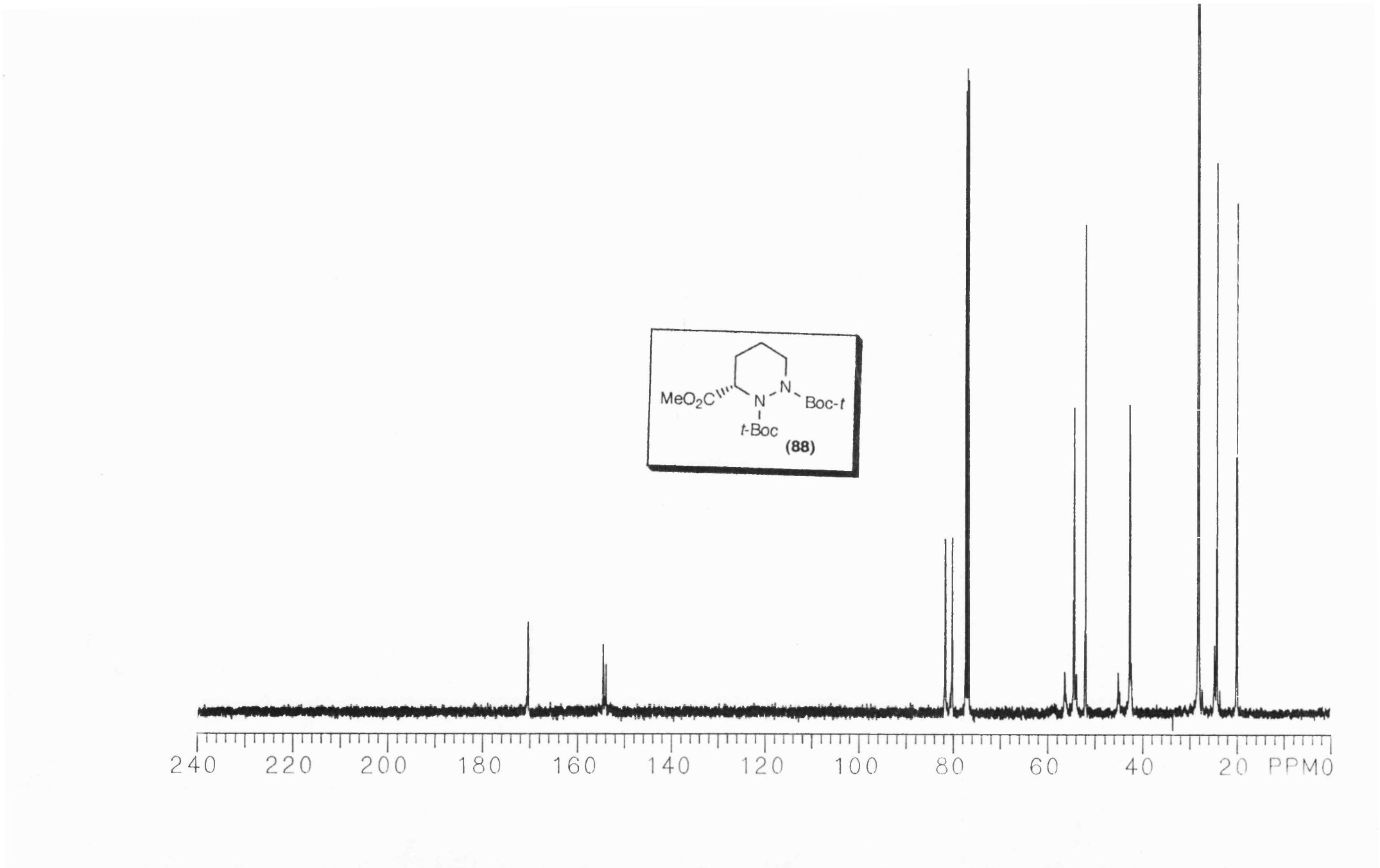

HO2C
N
N
Boc-t
t-Boc
(87)
12
10
8
6
4
2
0 PPM

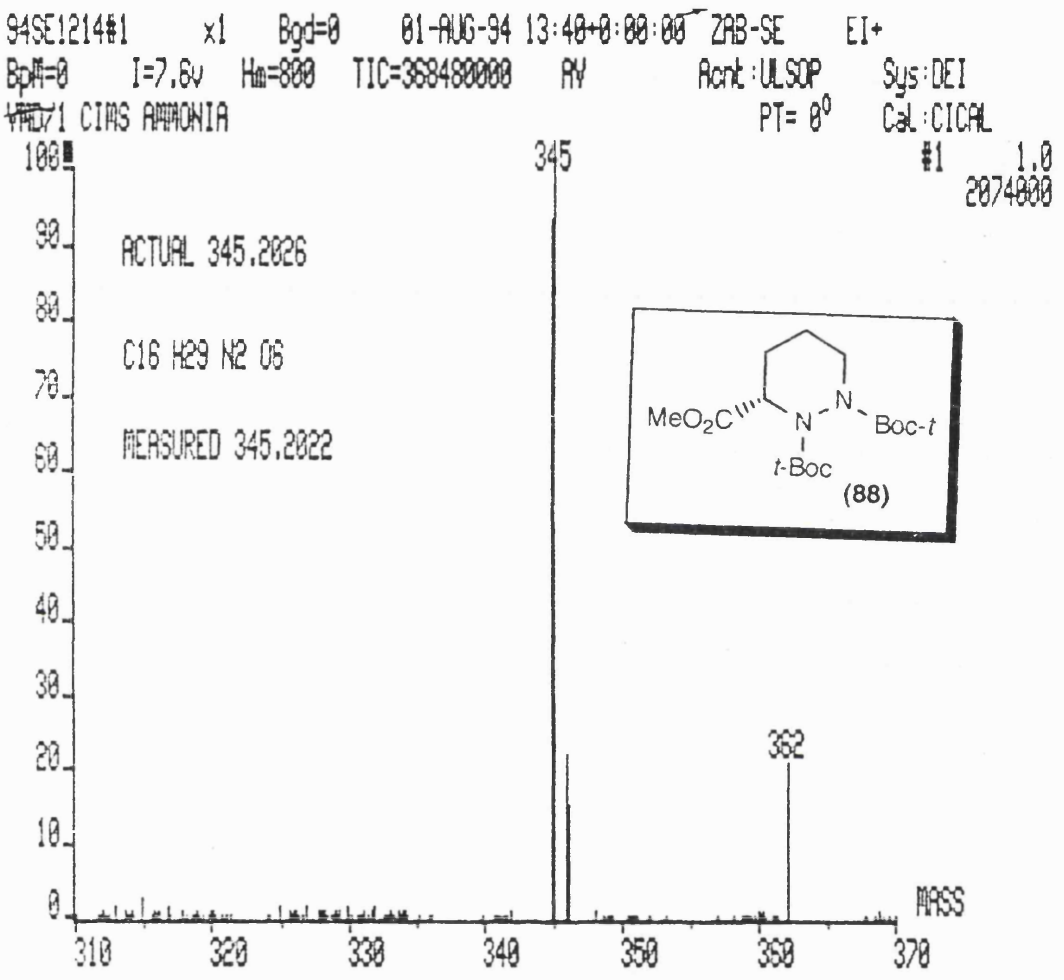

HO2C
N
N
Boc-t
t-Boc
(87)
240
220
200
180
160
140
120
100
80
60
40
20
PPM0

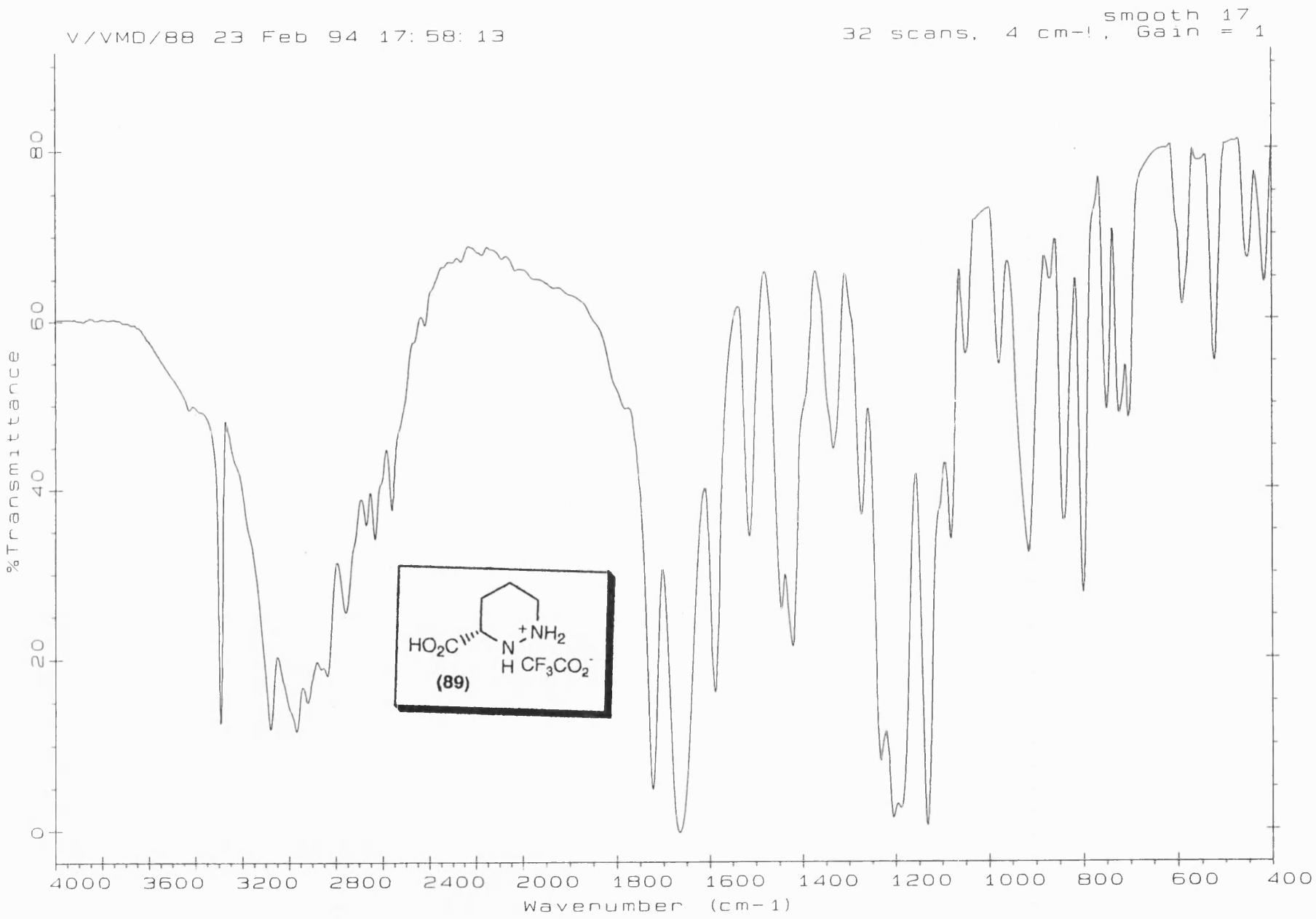

x1 Bgd=0 04-MAR-94 12:07+0:00:00 ZAB2F EI+
I=3.9v Hm=890 TIC=150774000 AV Rcnt: Sys:LREI
V/VMD/81 CIMS AMMONIA PT= 0° Cal:MCAL
331 #1 1.0
92430000
MEASURED 331.1883
HO2C
N
N
Boc-t
t-Boc
(87)
MASS
100
90
80
70
60
50
40
30
20
10
0
300
305
310
315
320
325
330
335

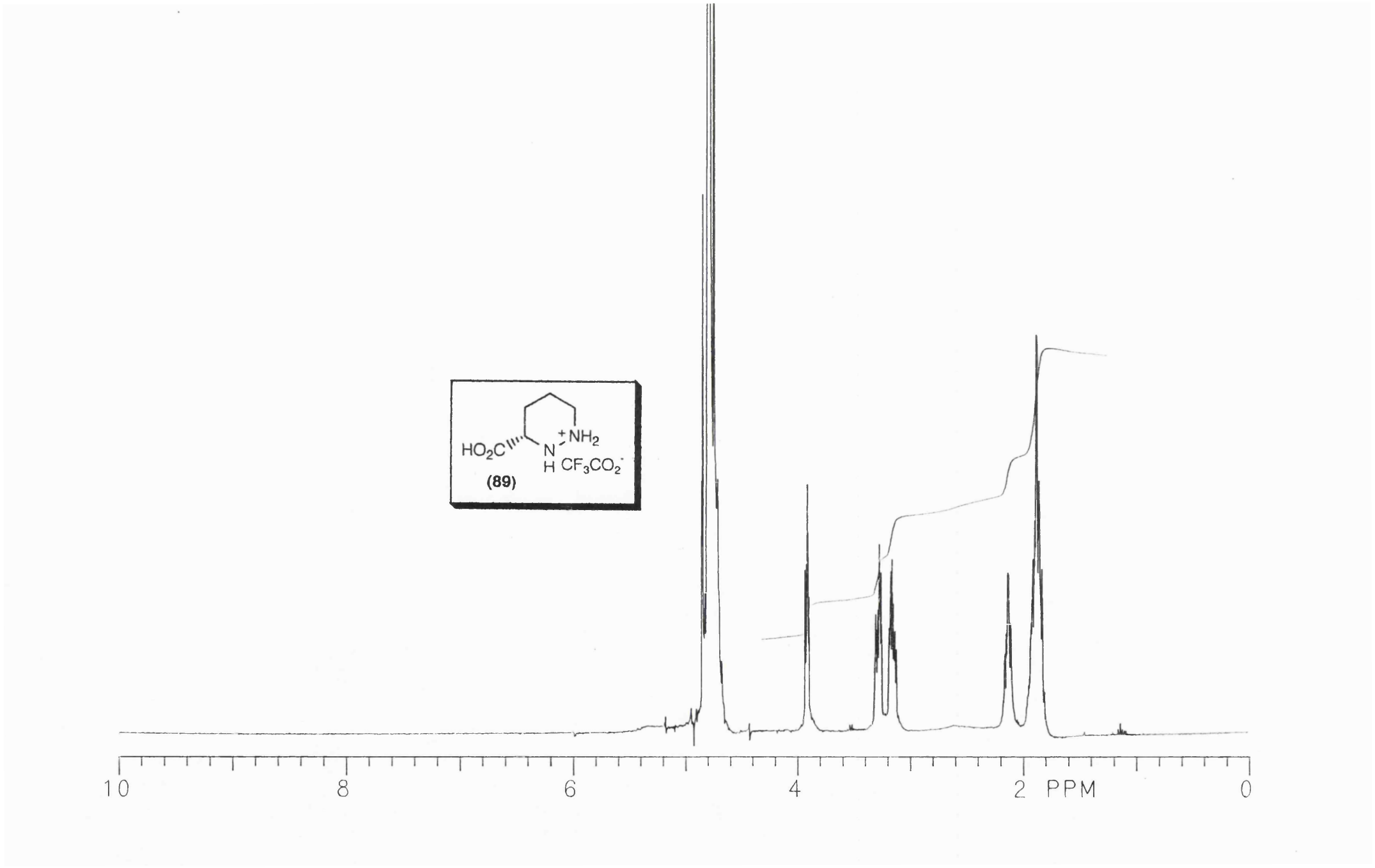

DA RACEMIC-II 18 Jul 94 14:51:37
smooth 17
32 scans, 2 cm-!, Gain = 2
%Transmittance
70
60
50
40
30
20
10
0
4000
3500
3200
2800
2400
2000
1800
1500
1400
1200
1000
800
600
400
Wavenumber (cm-1)
MeO2C
N
N
Boc-t
t-Boc
(88)

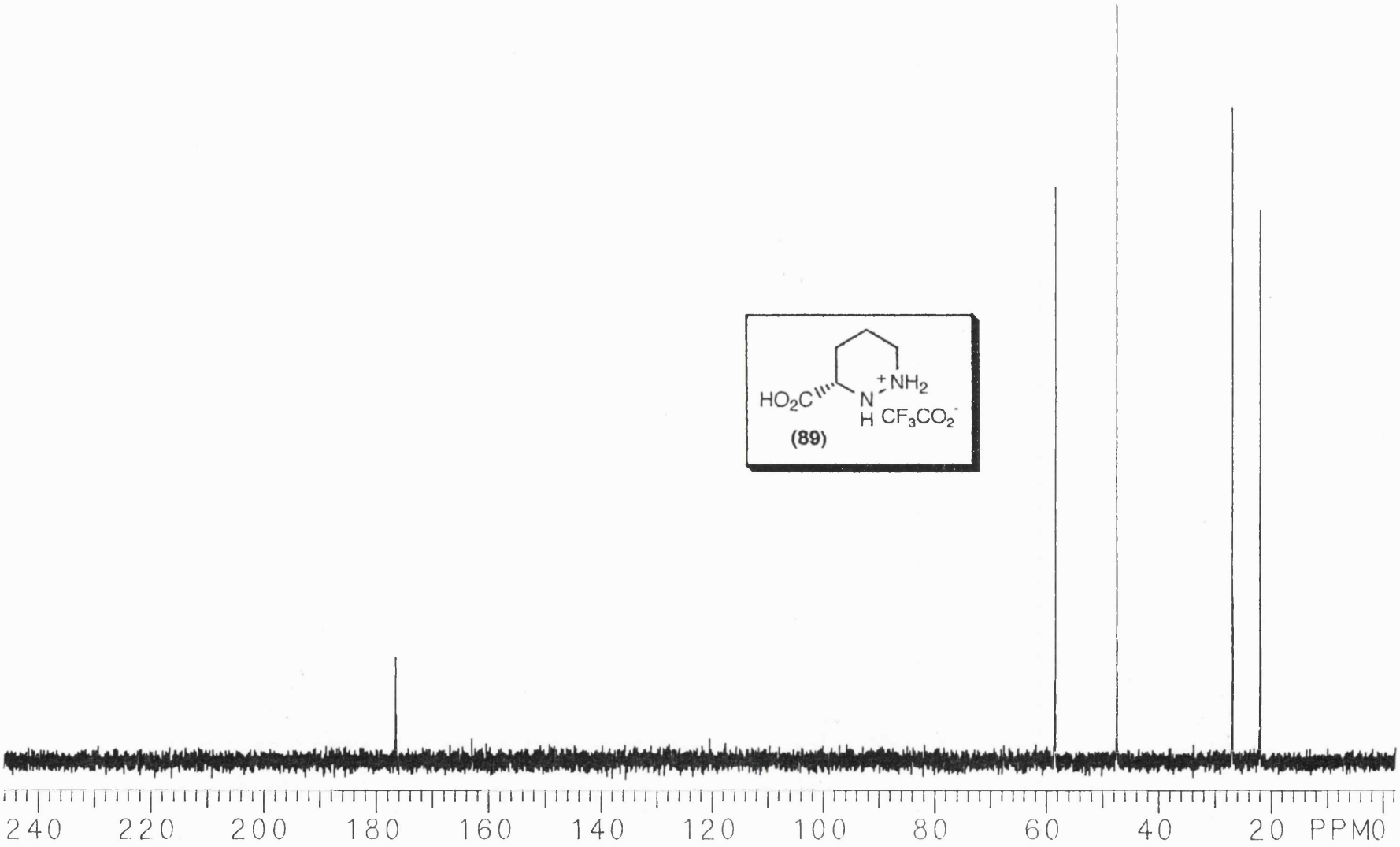

MeO2C
N
N
Boc-t
t-Boc
(88)
10
8
6
4
2 PPM
0

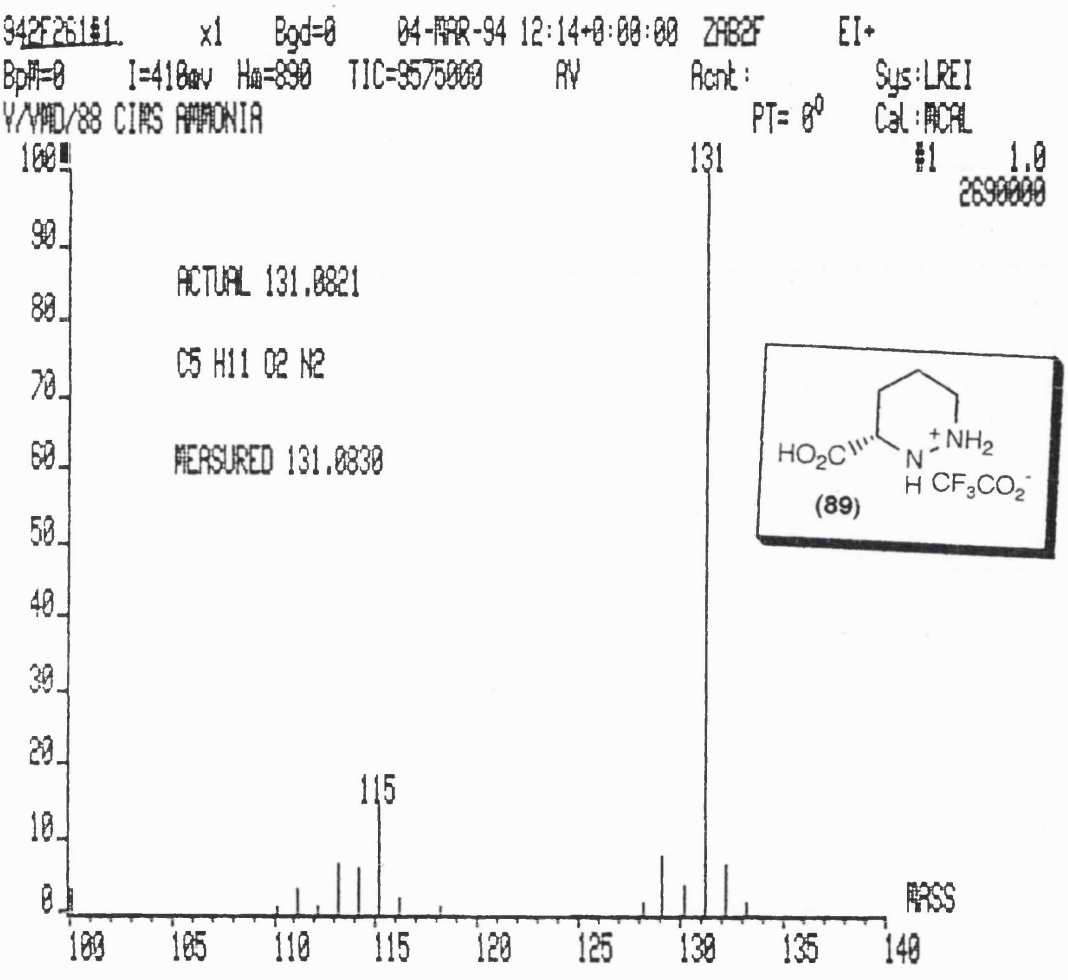

MeO2C
N
N
Boc-t
t-Boc
(88)
240
220
200
180
160
140
120
100
80
60
40
20
PPM0

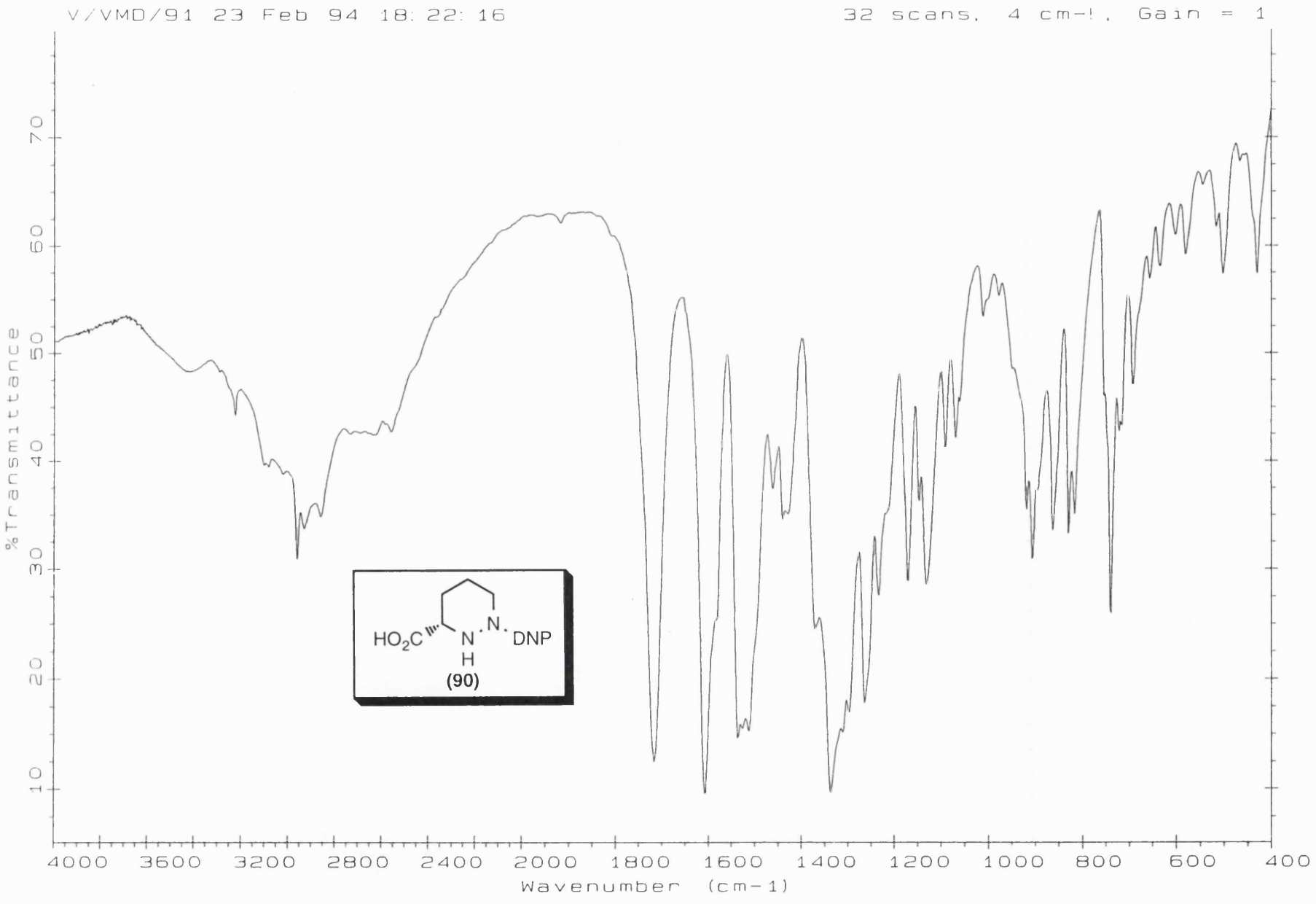

94SE1214#1 x1 Bgd=0 01-AUG-94 13:40+0:00:00 ZAB-SE EI+
BpM=0 I=7.6v Hm=800 TIC=3684800000 AV Acmt:ULSOP Sys:DEI
VMED/1 CIMS AMMONIA PT= 0° Cal:CICAL
#1 1.0
2074000
345
362
ACTUAL 345.2026
C16 H29 N2 O6
MEASURED 345.2022
MeO2C
N
N
Boc-t
t-Boc
(88)
MASS
100
90
80
70
60
50
40
30
20
10
0
310
320
330
340
350
360
370

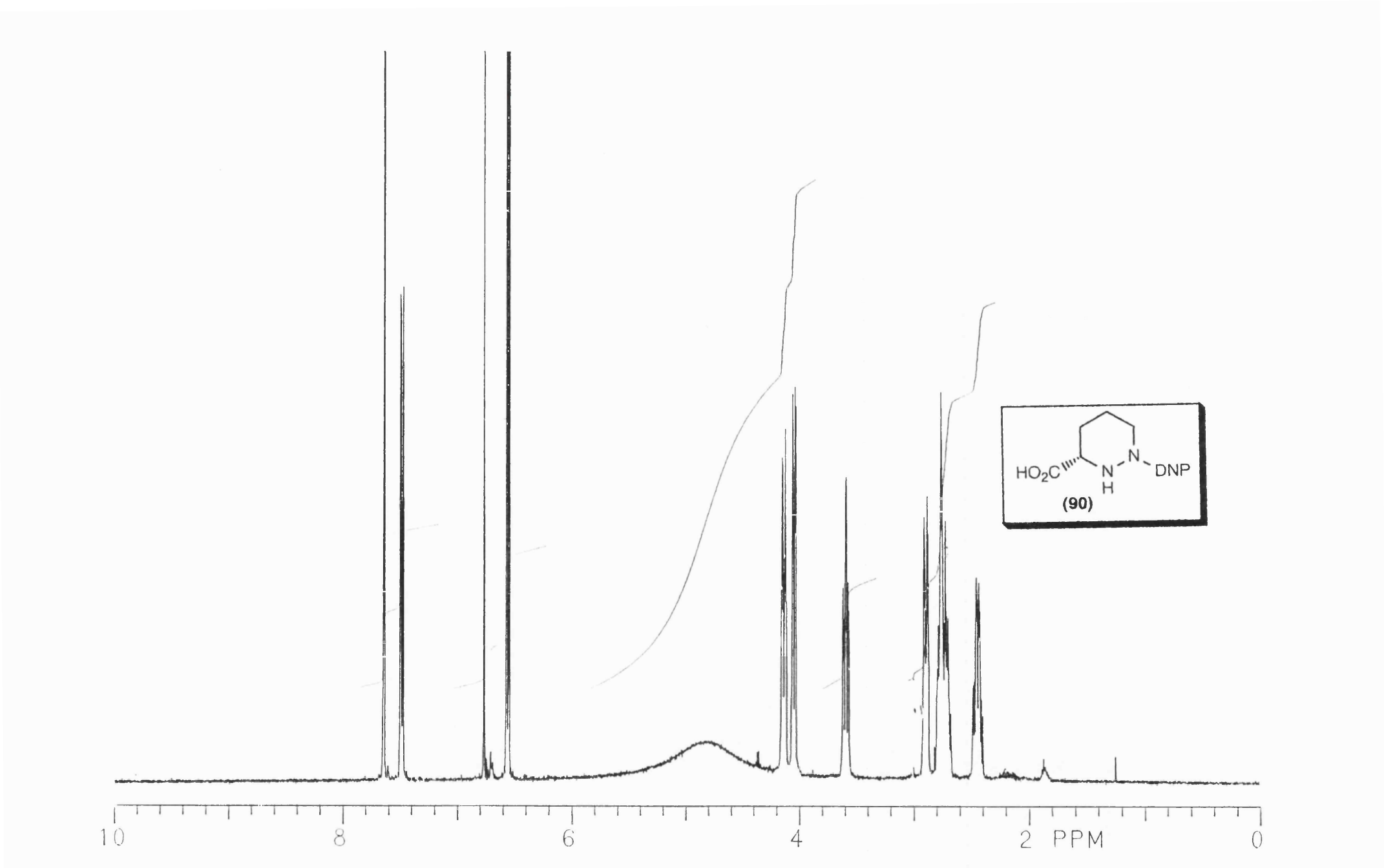

V/VMD/88 23 Feb 94 17:58:13
smooth 17
32 scans, 4 cm-1, Gain = 1
%Transmittance
0
20
40
60
80
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
HO2C
N
H
+NH2
CF3CO2-
(89)

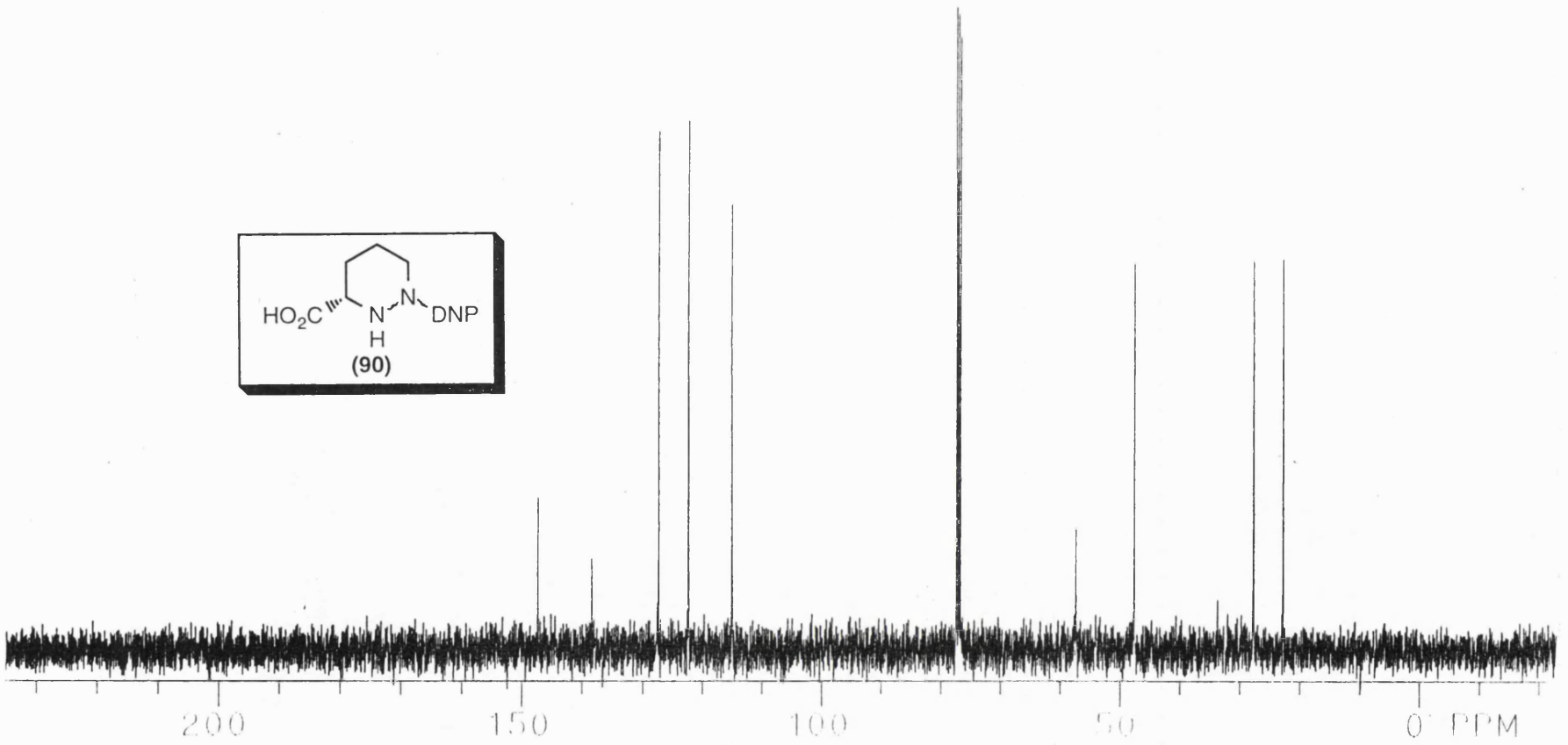

HO2C
+NH2
N
H CF3CO2-
(89)
10
8
6
4
2 PPM
0

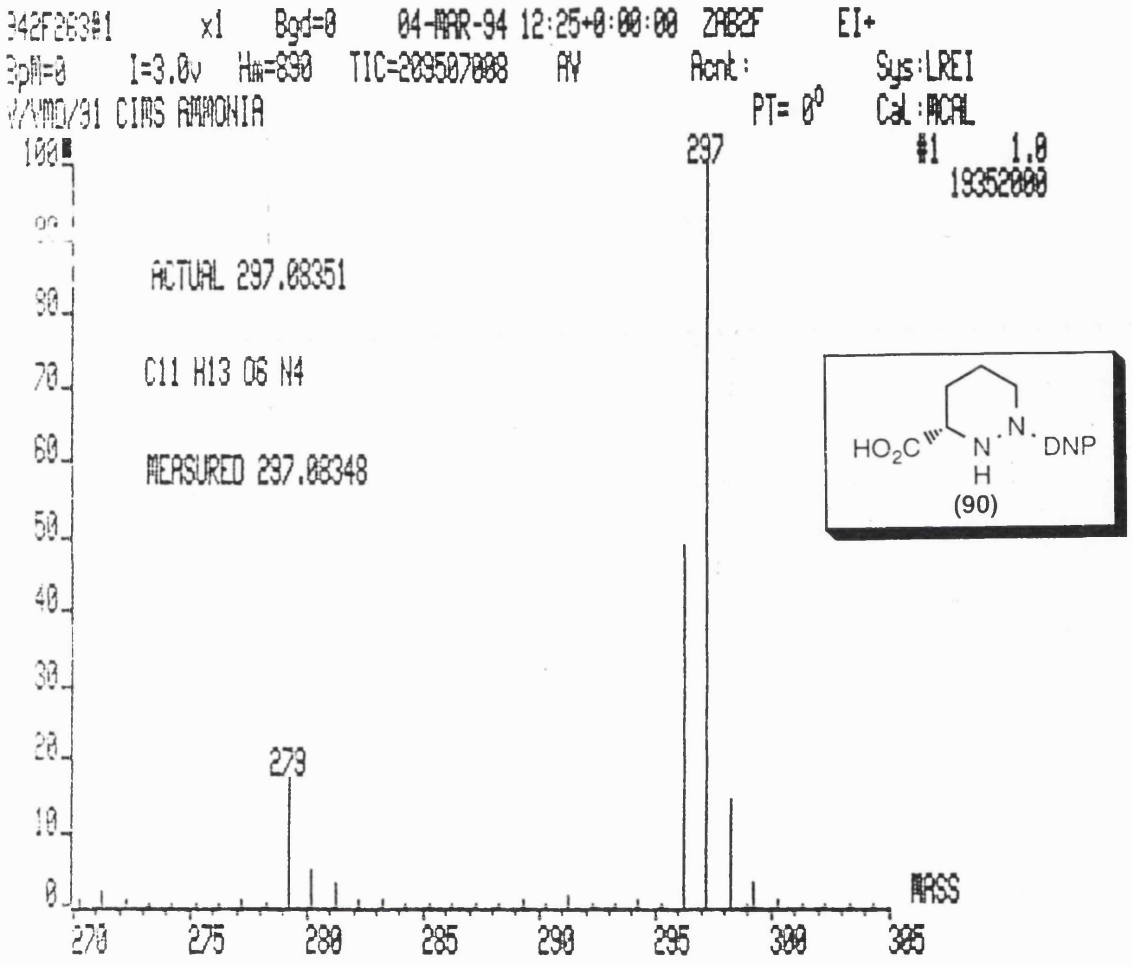

HO2C
+NH2
N
H CF3CO2-
(89)
240
220
200
180
160
140
120
100
80
60
40
20
PPM0

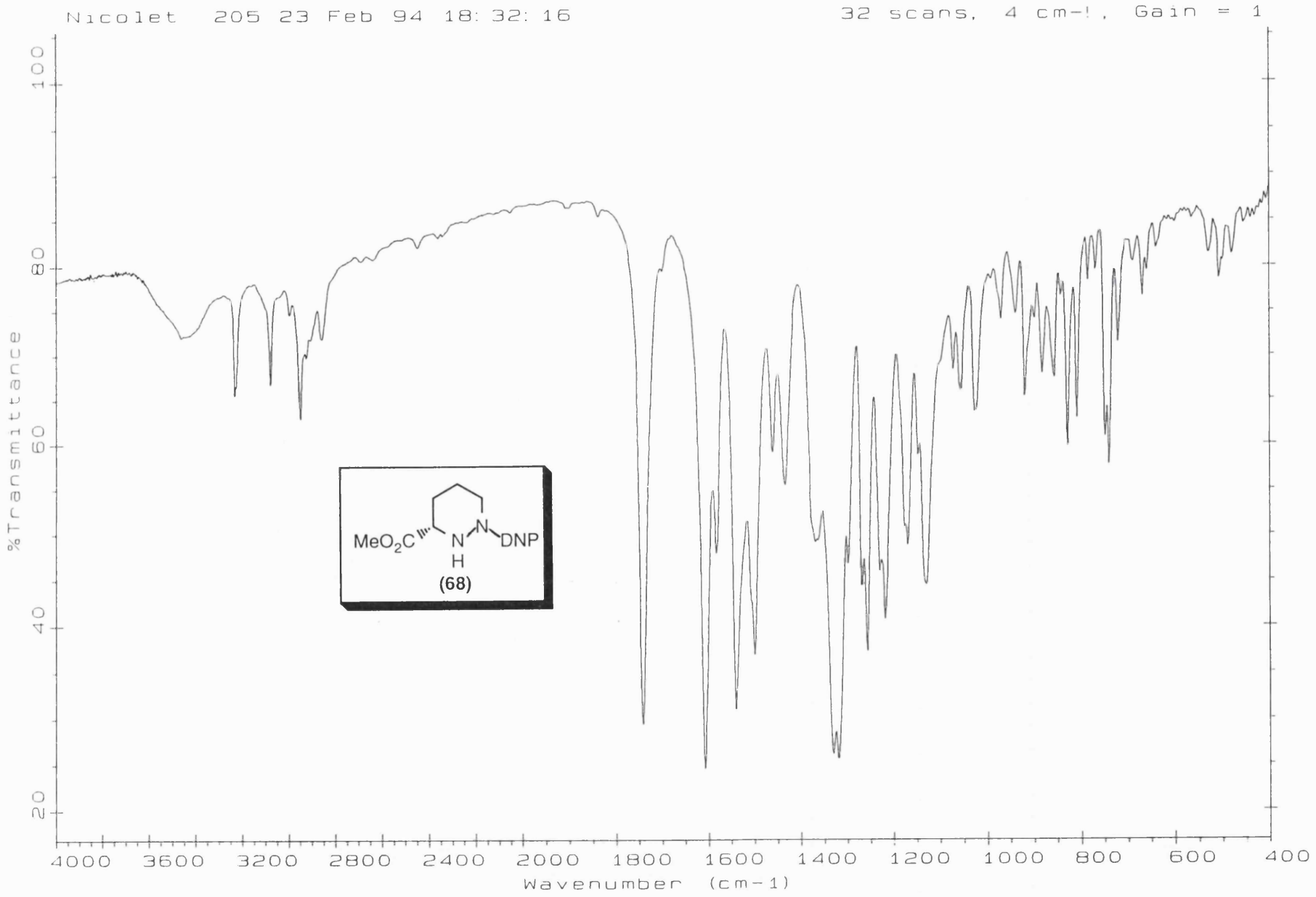

942F261#1. x1 Bgd=0 04-MAR-94 12:14+0:00:00 ZAB2F EI+
BpM=0 I=418mv Hm=8890 TIC=95750000 RV Rcnt: Sys:LREI
Y/YMD/88 CIMS AMMONIA PT= 0° Cal:MCAL
#1 1.0
26900000
131
ACTUAL 131.0821
C5 H11 O2 N2
MEASURED 131.0830
115
MASS
100 105 110 115 120 125 130 135 140
(89)

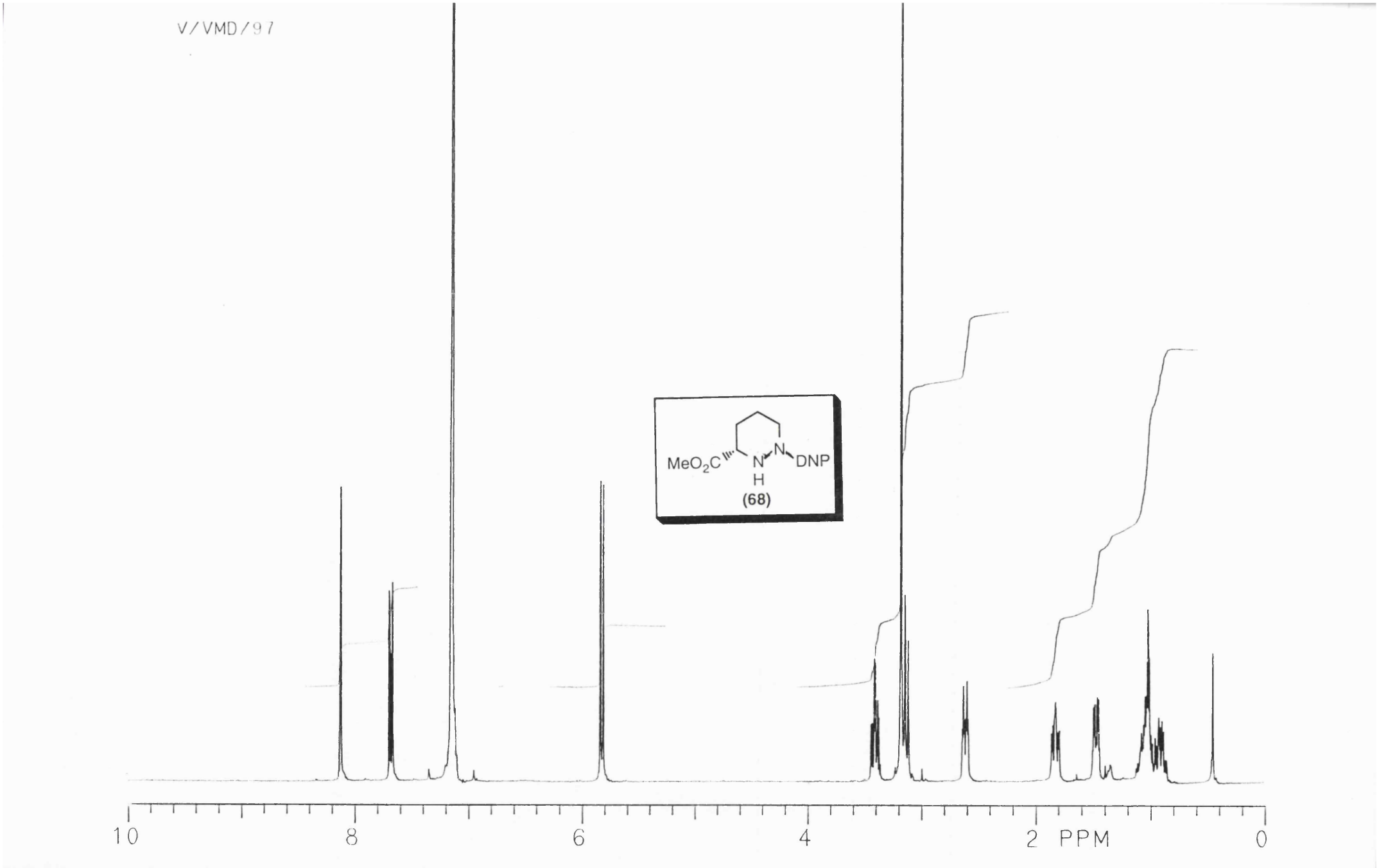

V/VMD/91 23 Feb 94 18:22:16
32 scans, 4 cm-1, Gain = 1
%Transmittance
70
60
50
40
30
20
10
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
HO2C
N
N
DNP
H
(90)

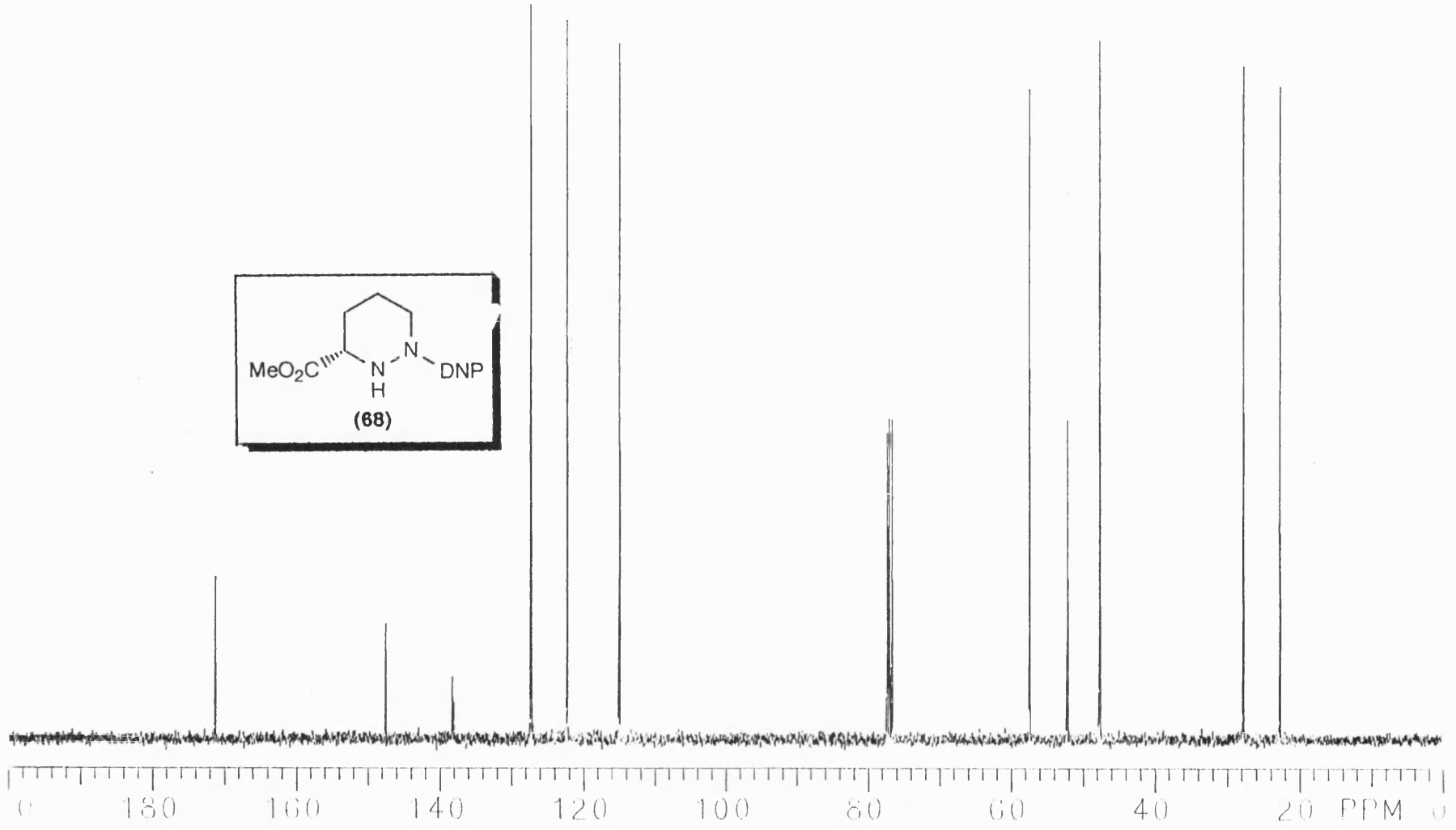

HO2C
N
H
N
DNP
(90)
10
8
6
4
2 PPM
0

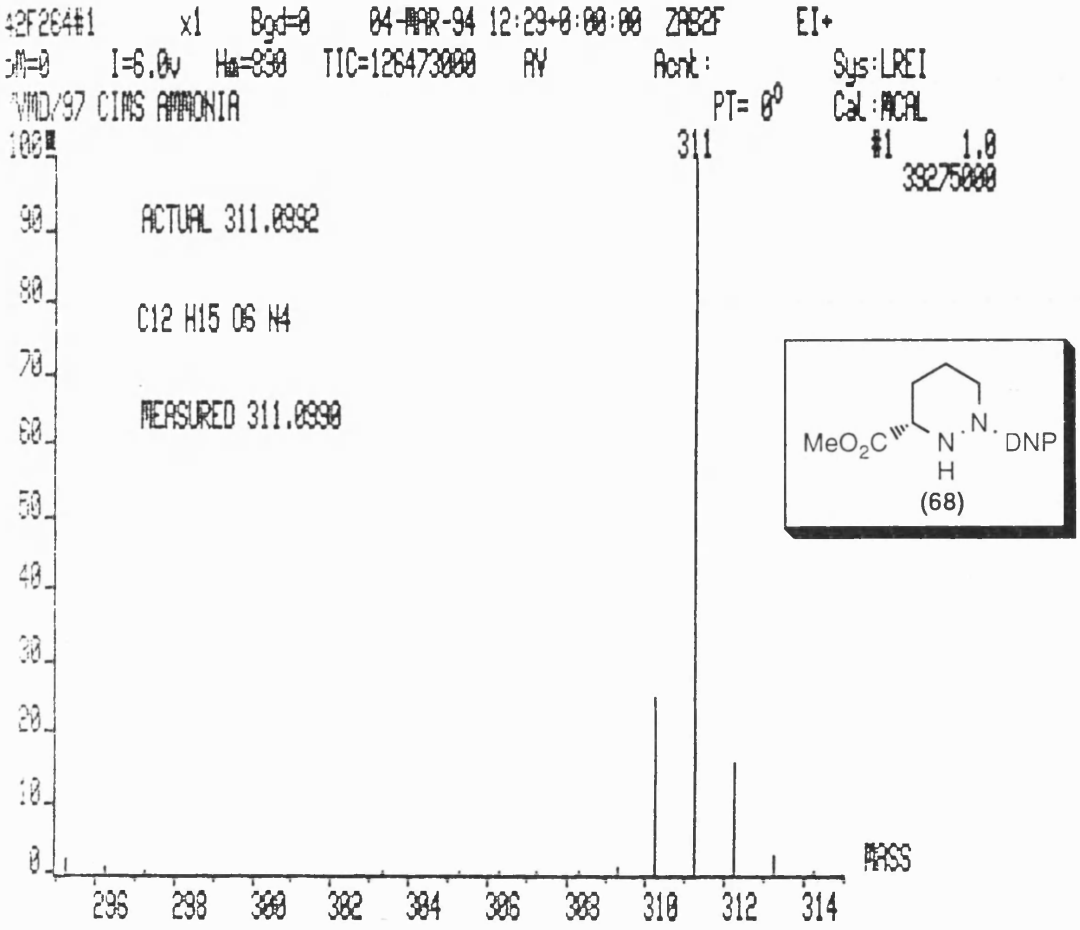

HO2C
N
N
DNP
H
(90)
200
150
100
50
0 PPM

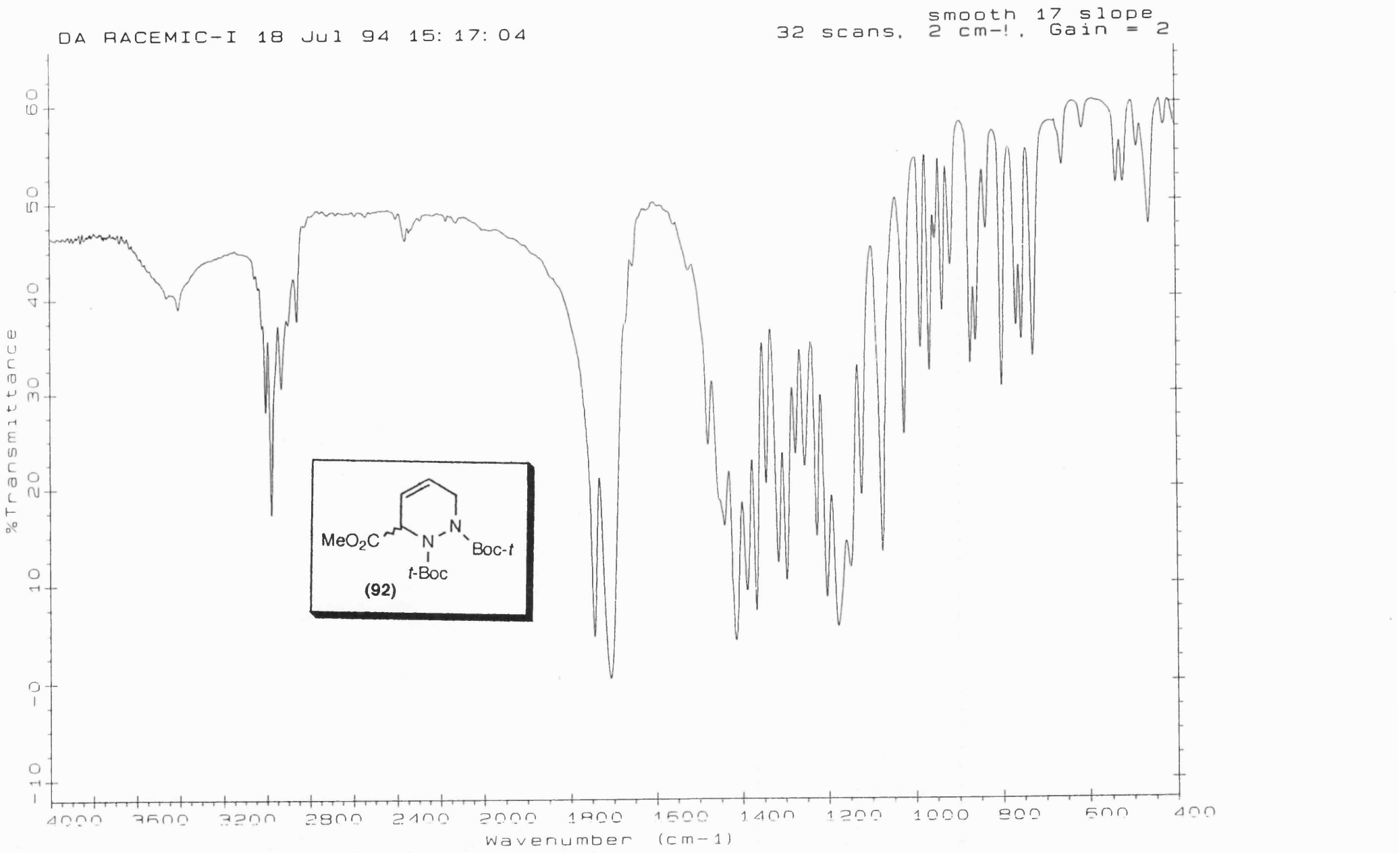

342F2B3#1 x1 Bgd=0 04-MAR-94 12:25+0:00:00 ZAB2F EI+
BpM=0 I=3.0v Hw=890 TIC=2095070080 AV Acnt: Sys:LREI
V/VMS/31 CIMS AMMONIA PT= 0° Cal:MCAL
#1 1.0
19352000
297
ACTUAL 297.08351
C11 H13 O6 N4
MEASURED 297.08348
279
HO2C
N
N
H
DNP
(90)
MASS
100
90
80
70
60
50
40
30
20
10
0
270
275
280
285
290
295
300
305

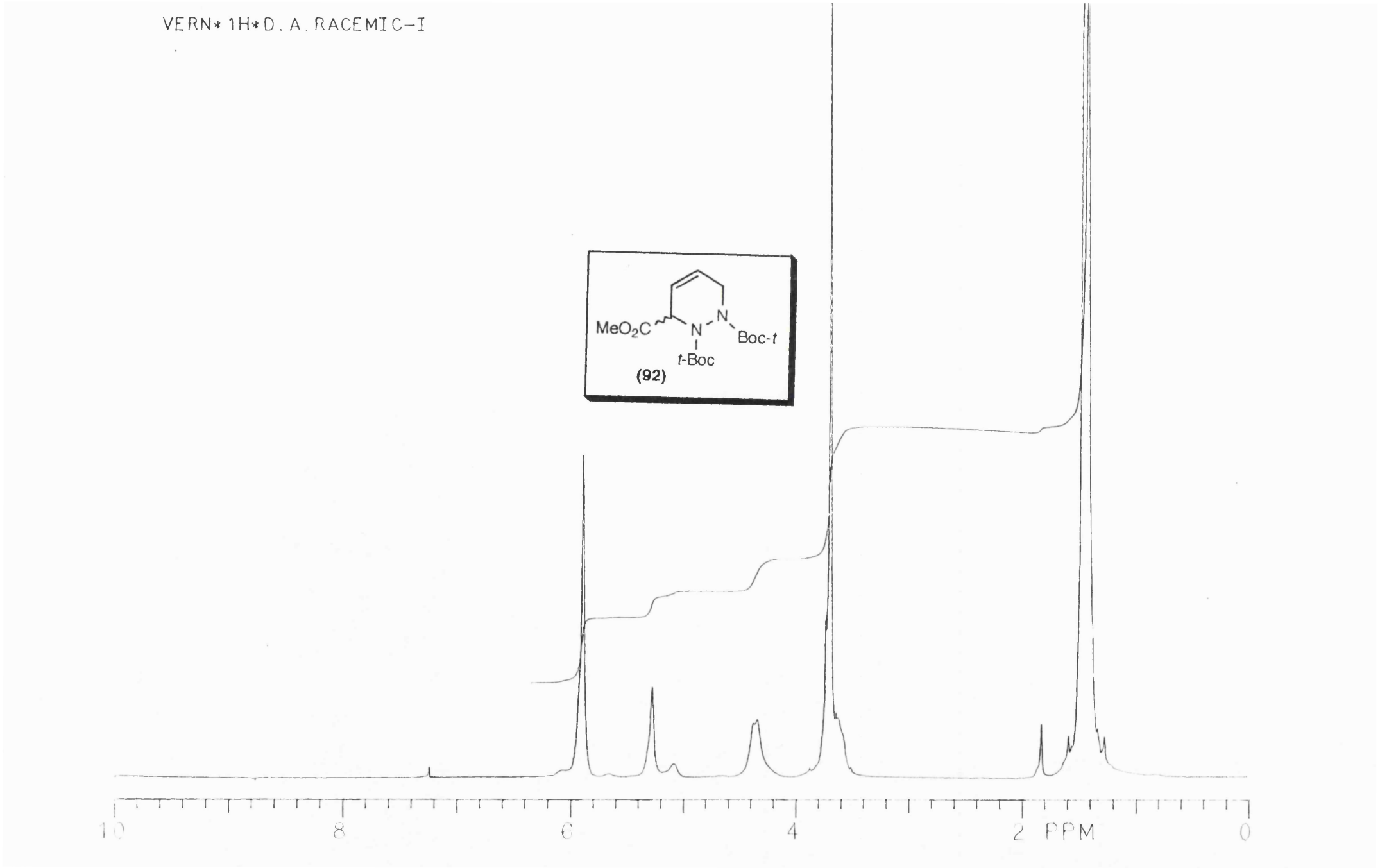

Nicolet 205 23 Feb 94 18: 32: 16
32 scans, 4 cm-!, Gain = 1
%Transmittance
100
80
60
40
20
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
MeO2C
N
H
N
DNP
(68)

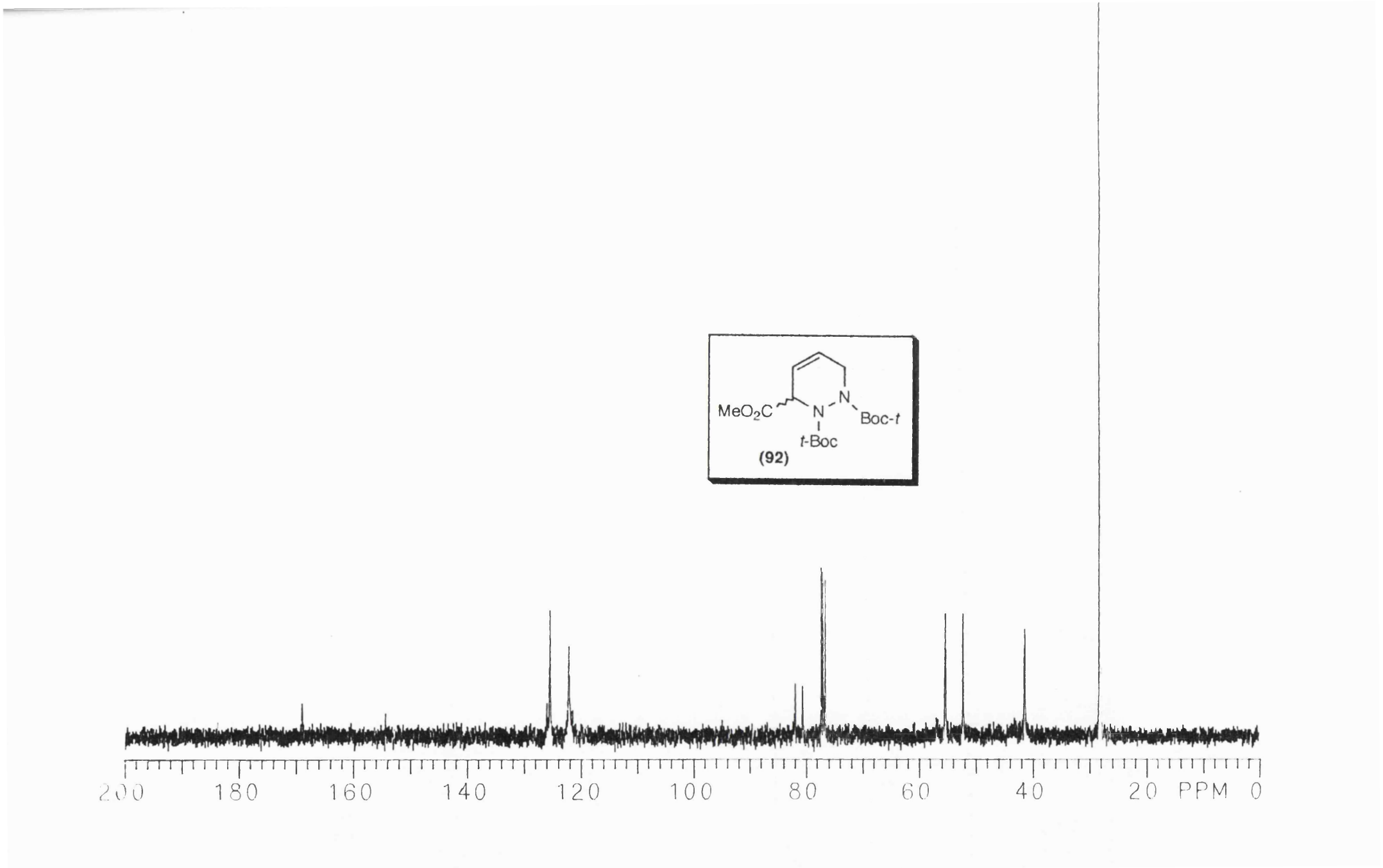

V/VMD/97
MeO2C
N
N
H
DNP
(68)
10
8
6
4
2 PPM
0

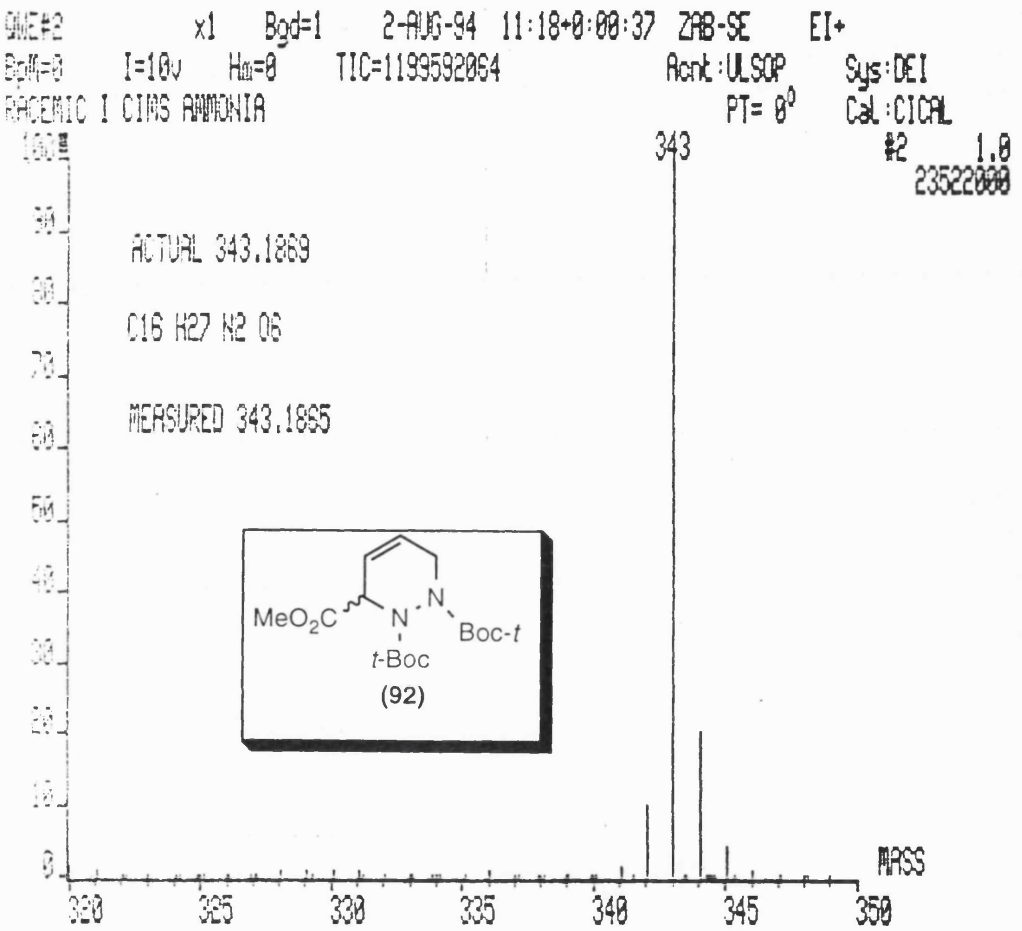

MeO2C
N
H
N
DNP
(68)
180
160
140
120
100
80
60
40
20
PPM

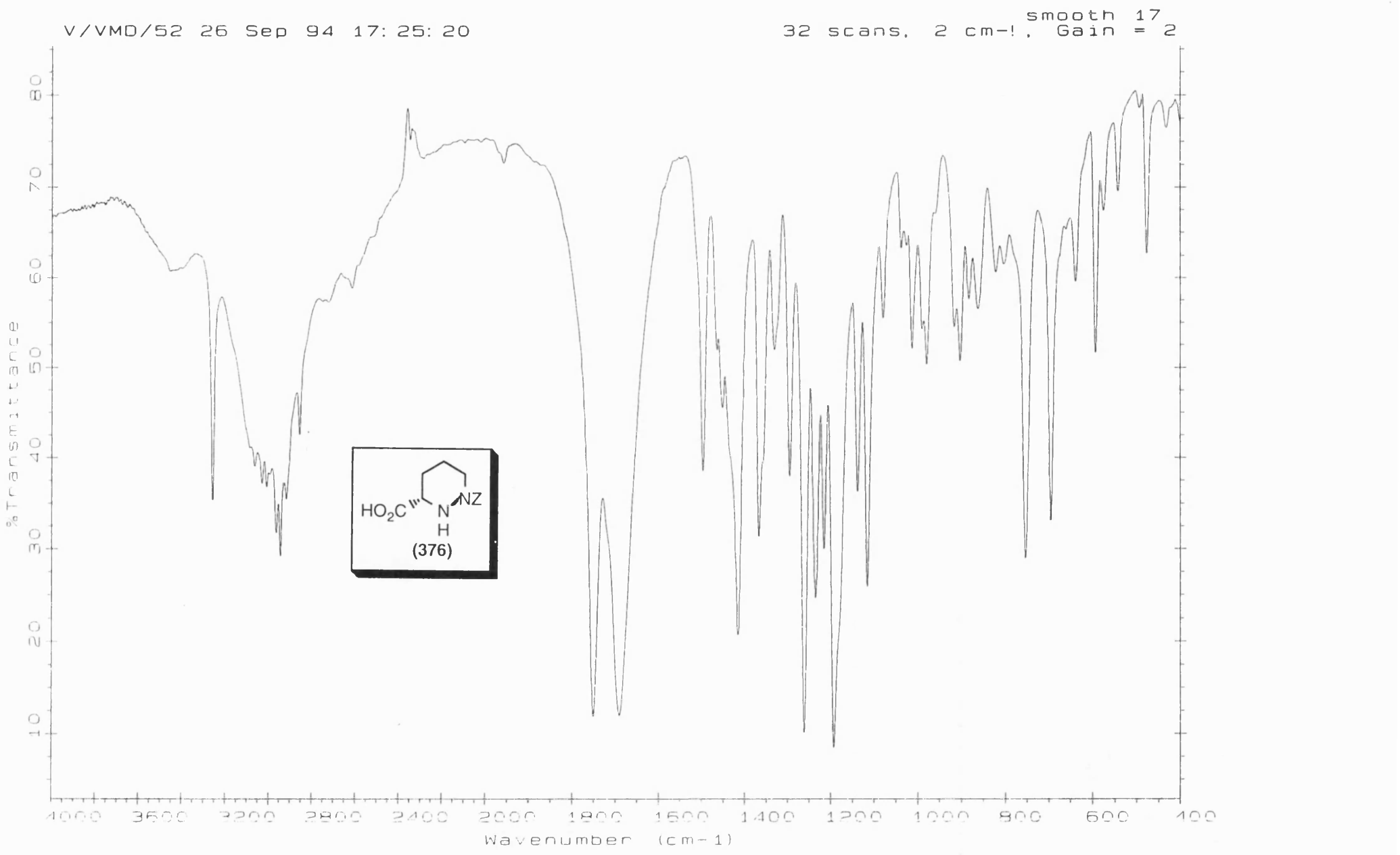

42F264#1 x1 Bgd=0 04-MAR-94 12:29+0:00:00 ZAB2F EI+
JM=0 I=6.0v Ma=830 TIC=126473000 AV Acnt: Sys:LREI
VMD/97 CIMS AMMONIA PT= 0° CaL:MCAL
311 #1 1.0
392750000
ACTUAL 311.0992
C12 H15 O6 N4
MEASURED 311.0990
MeO2C
N
H
N
DNP
(68)
MASS
100
90
80
70
60
50
40
30
20
10
0
296 298 300 302 304 306 308 310 312 314

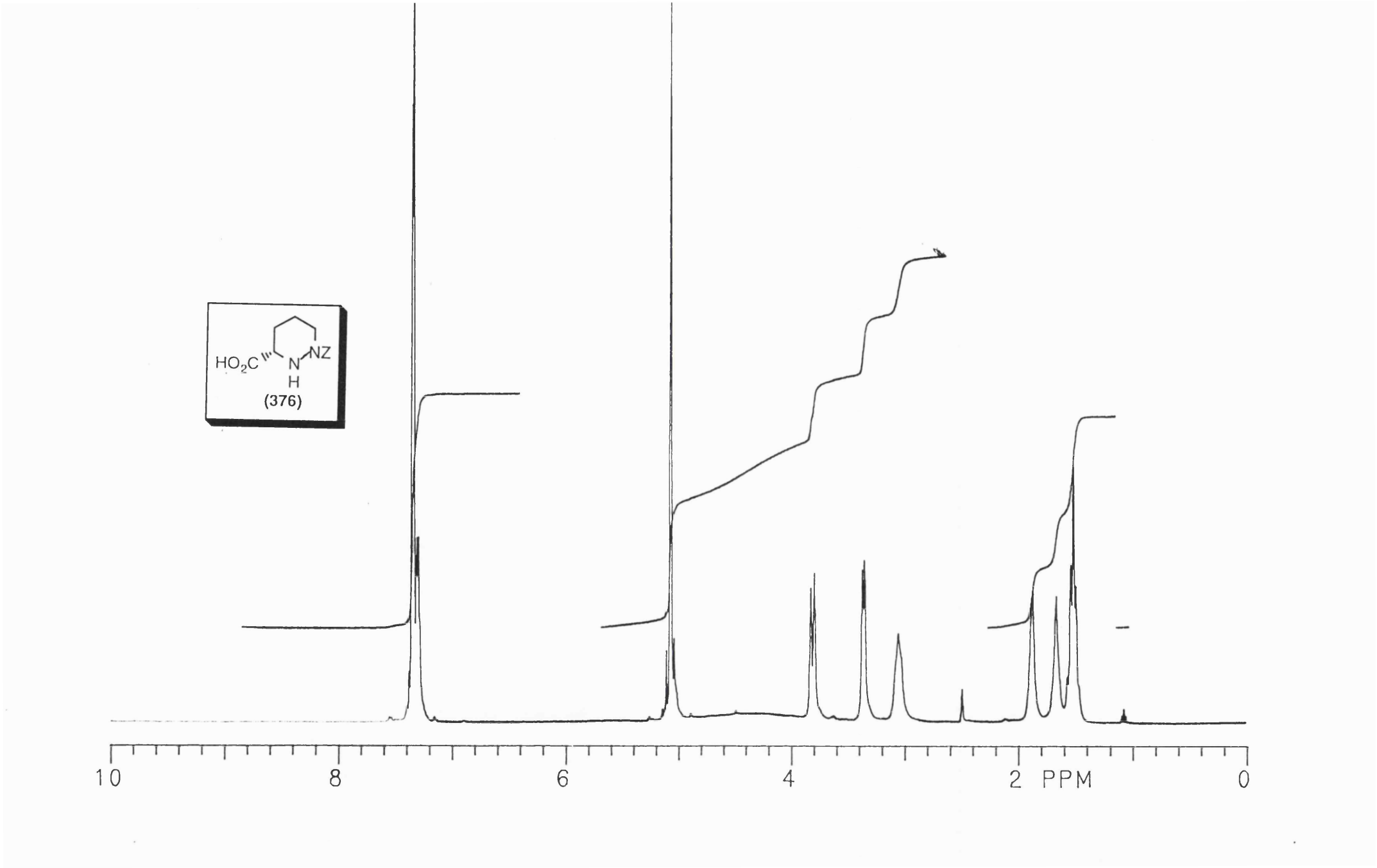

DA RACEMIC-I 18 Jul 94 15: 17: 04
smooth 17 slope
32 scans, 2 cm-!, Gain = 2
%Transmittance
60
50
40
30
20
10
-0
-10
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
MeO2C
N
N
Boc-t
t-Boc
(92)

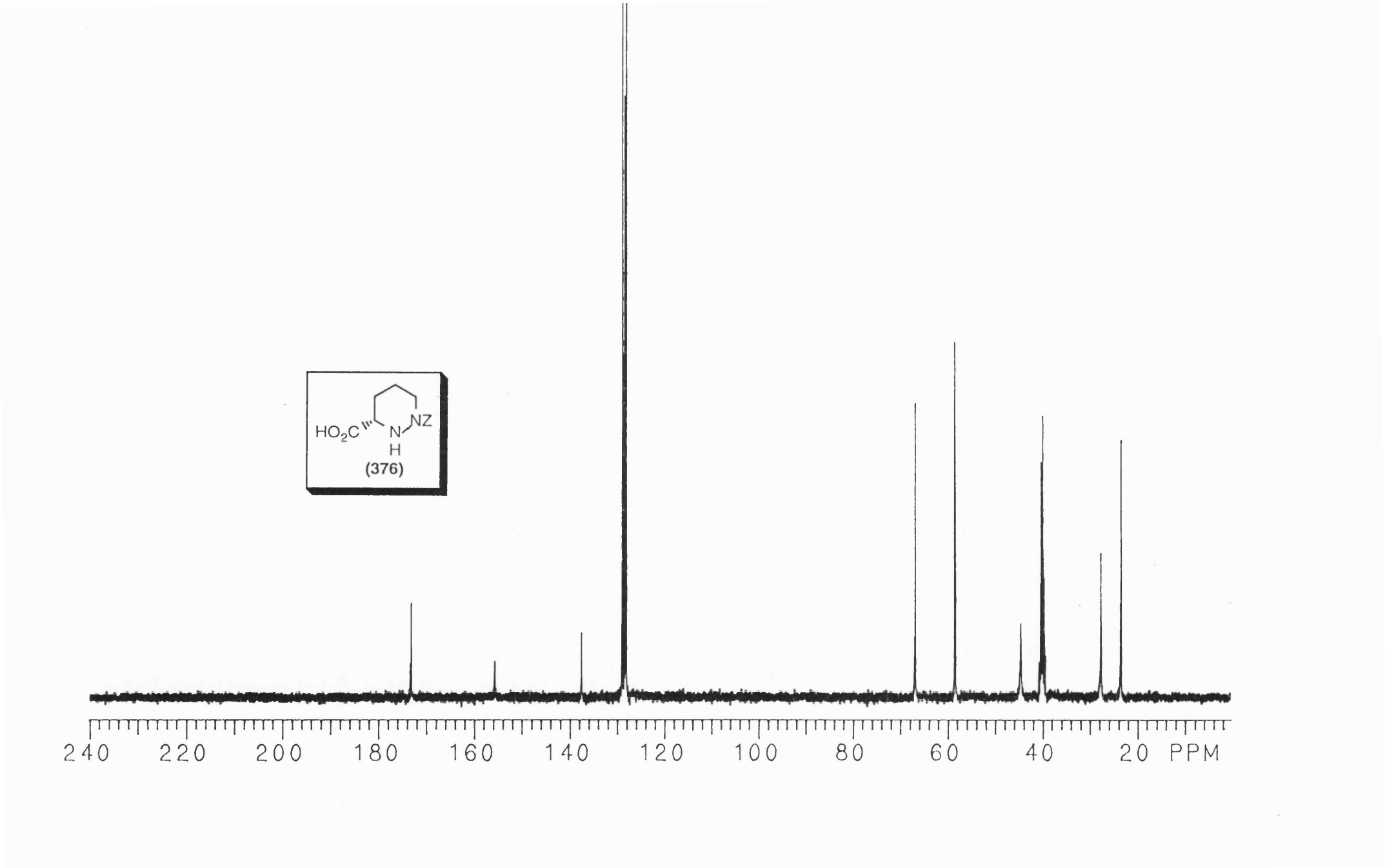

VERN* 1H* D. A. RACEMIC-I
MeO2C
N
N
Boc-t
t-Boc
(92)
10
8
6
4
2 PPM
0

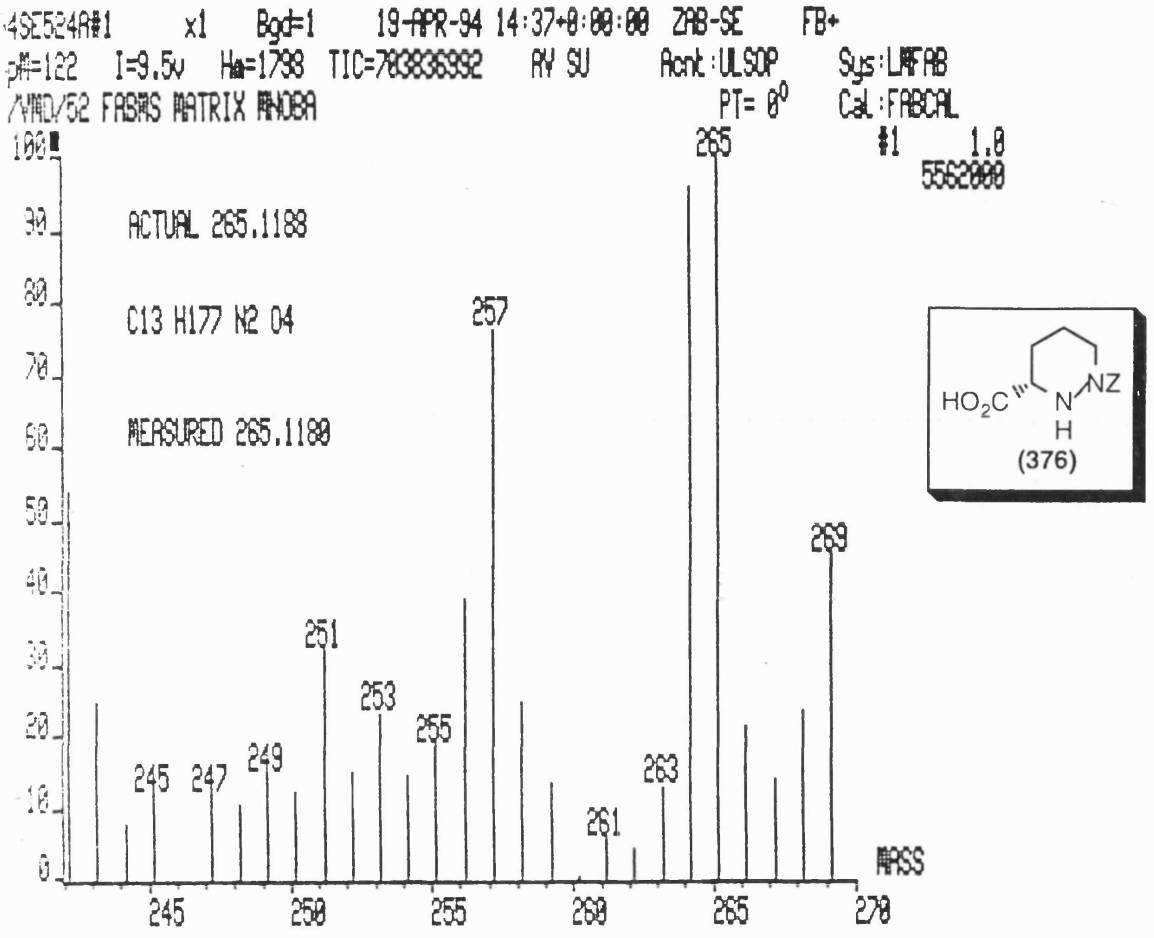

MeO2C
N
N
Boc-t
t-Boc
(92)
200
180
160
140
120
100
80
60
40
20
PPM
0

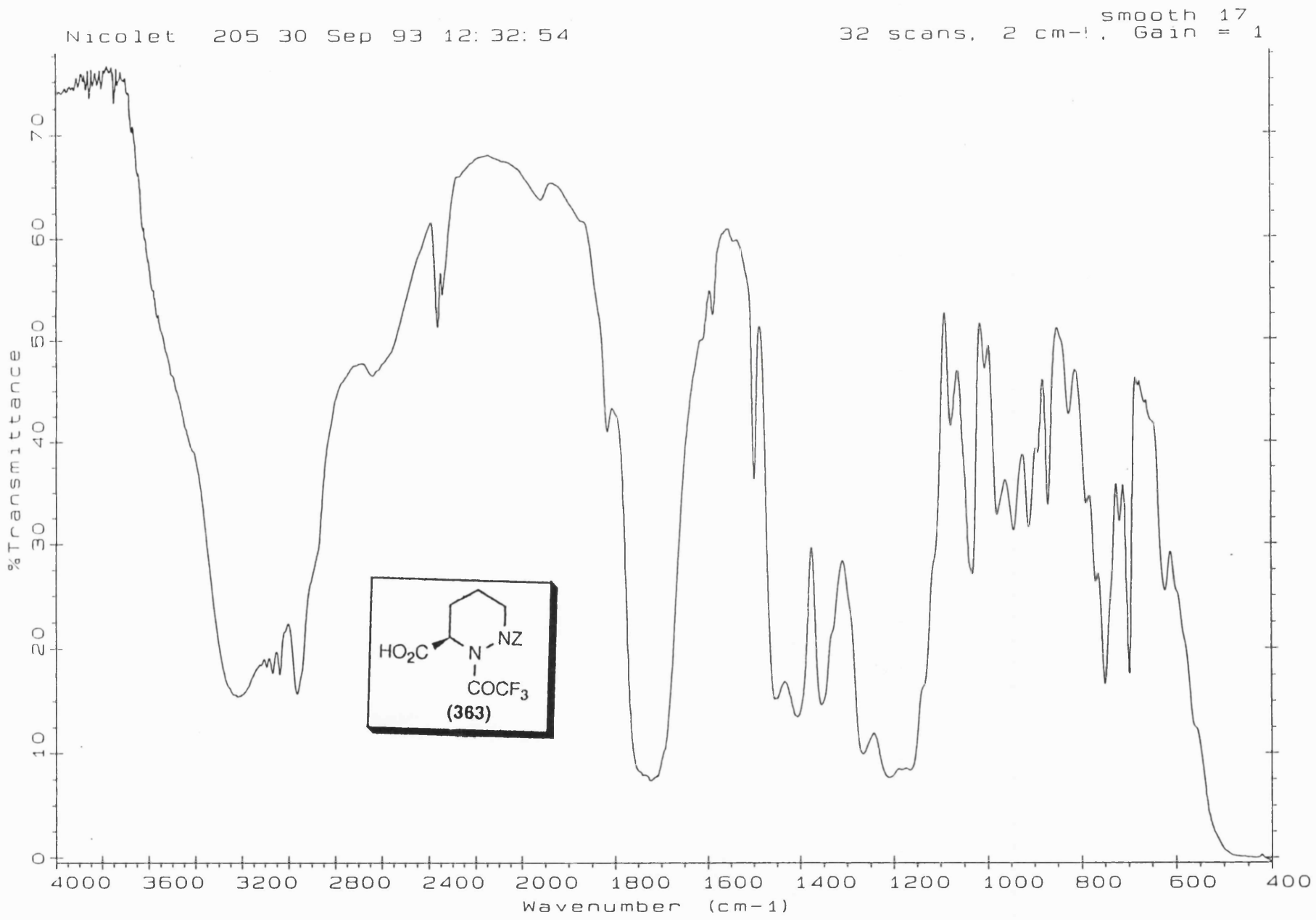

SAMPLE#2 x1 Bgd=1 2-AUG-94 11:18+0:00:37 ZAB-SE EI+
BgM=0 I=10v Hm=0 TIC=1199592064 Acmt:ULSOP Sys:DEI
RACEMIC I CIMS AMMONIA PT= 0° Cal:CICAL
343
#2 1.0
23522000
ACTUAL 343.1869
C16 H27 N2 O6
MEASURED 343.1865
MeO2C
N
N
Boc-t
t-Boc
(92)
MASS
320
325
330
335
340
345
350

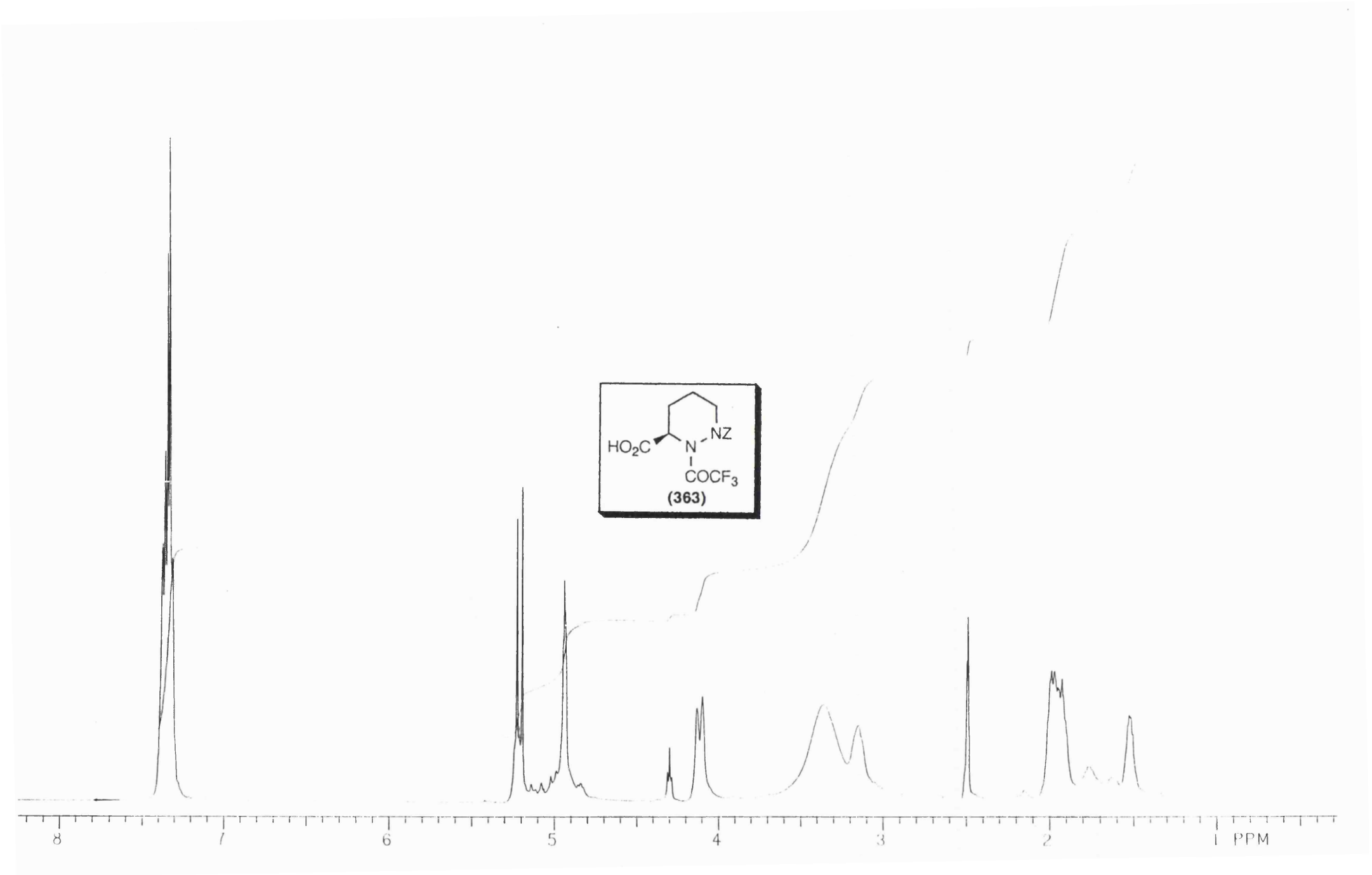

V/VMD/52 26 Sep 94 17: 25: 20
smooth 17
32 scans, 2 cm-!, Gain = 2
%Transmittance
80
70
60
50
40
30
20
10
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
HO2C
N
H
NZ
(376)

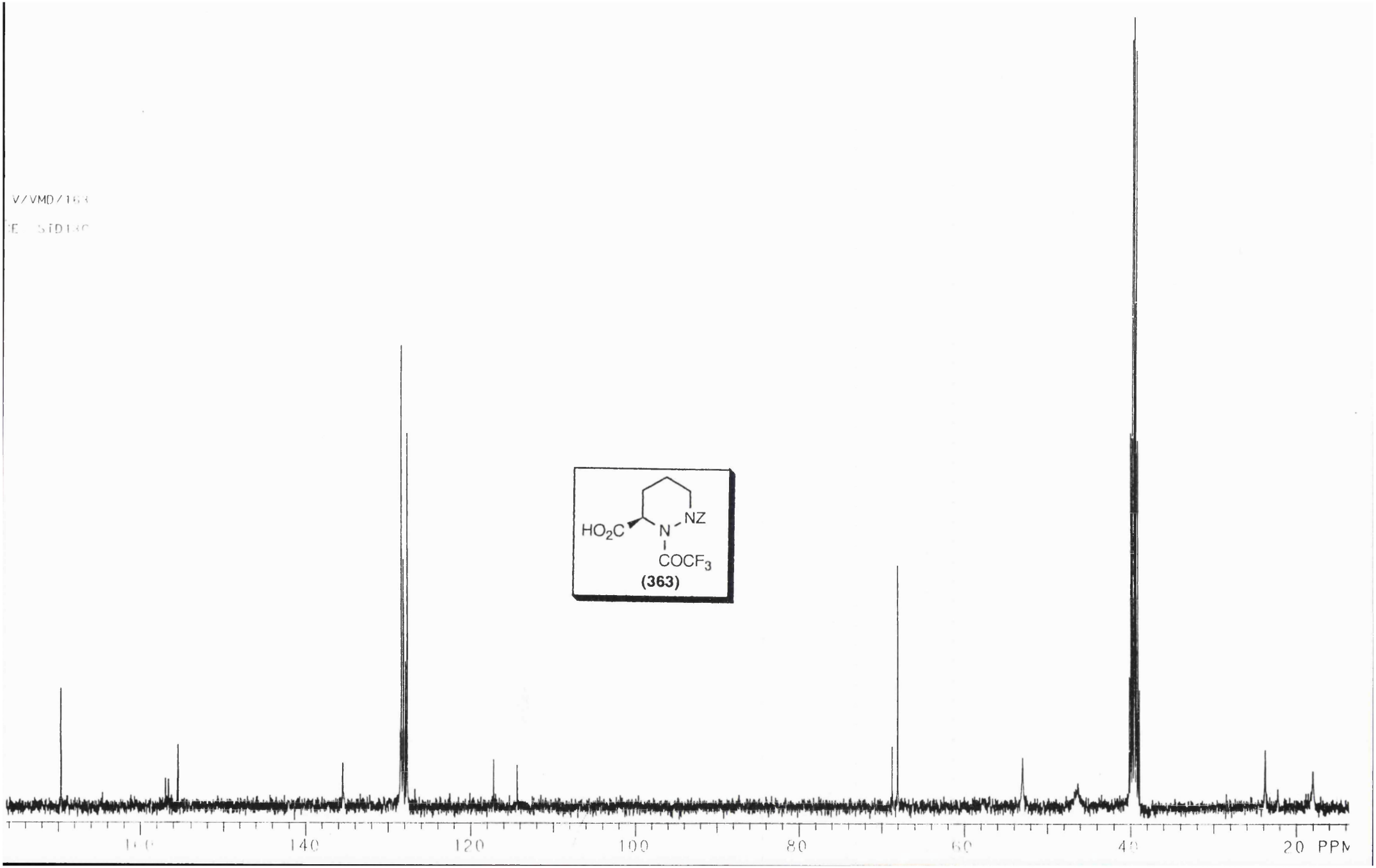

HO2C
N
NZ
H
(376)
10
8
6
4
2 PPM
0

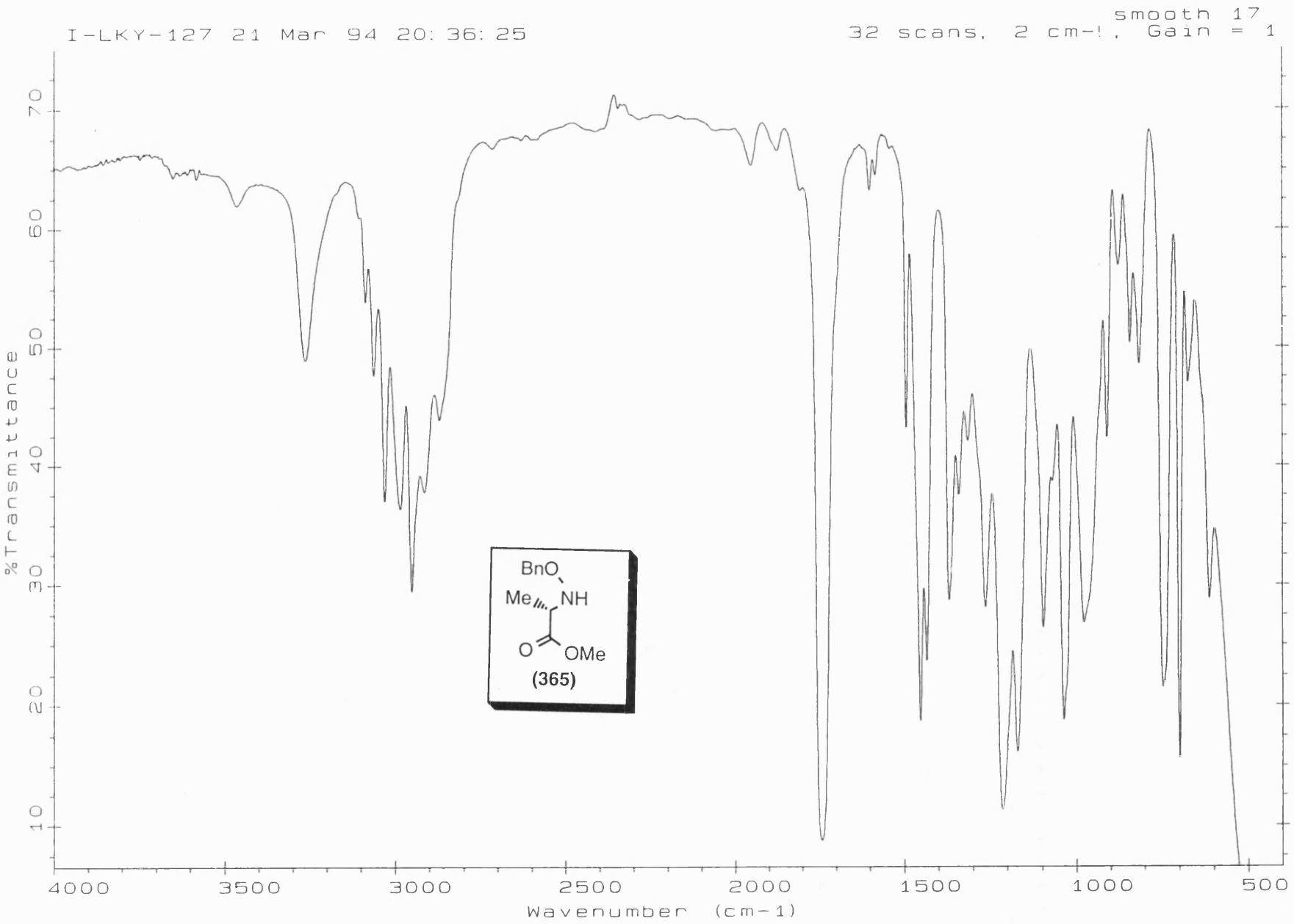

HO2C
N
NZ
H
(376)
240
220
200
180
160
140
120
100
80
60
40
20
PPM


4SE524A#1 x1 Bgd=1 19-APR-94 14:37+0:00:00 ZAB-SE FB+
pM=122 I=9.5v Ma=1798 TIC=703836992 RV SU Acnt:ULSOP Sys:LMFAB
/VMD/52 FABMS MATRIX MNOBA PT= 0° Cal:FABCAL
#1 1.0
5562000
ACTURAL 265.1188
C13 H17 N2 04
MEASURED 265.1180
265
257
269
251
253
255
245
247
249
263
261
MASS
100
90
80
70
60
50
40
30
20
10
0
245
250
255
260
265
270
HO2C
N
NZ
H
(376)

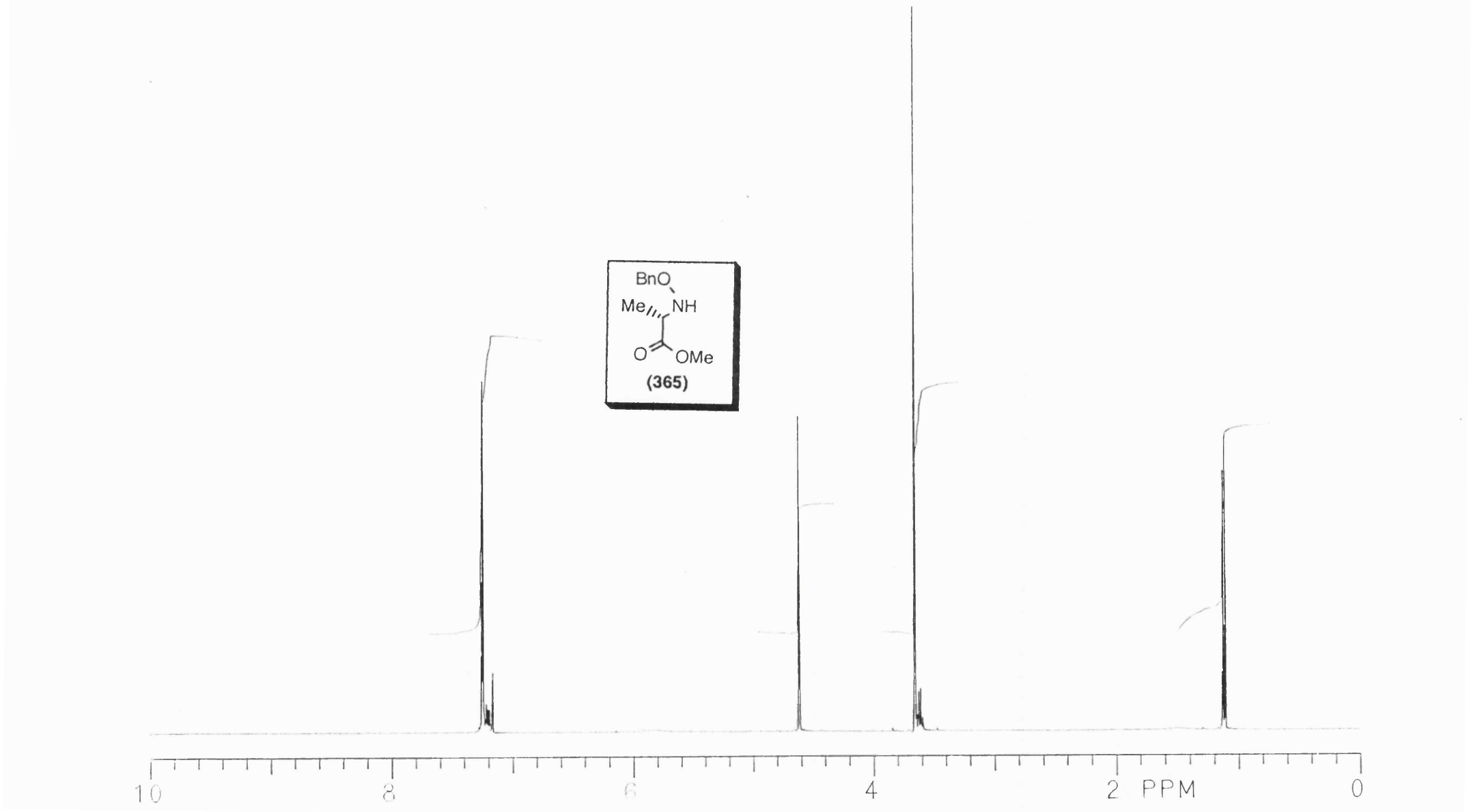

Nicolet 205 30 Sep 93 12:32:54
smooth 17
32 scans, 2 cm-1, Gain = 1
%Transmittance
0
10
20
30
40
50
60
70
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
HO2C
N
NZ
COCF3
(363)

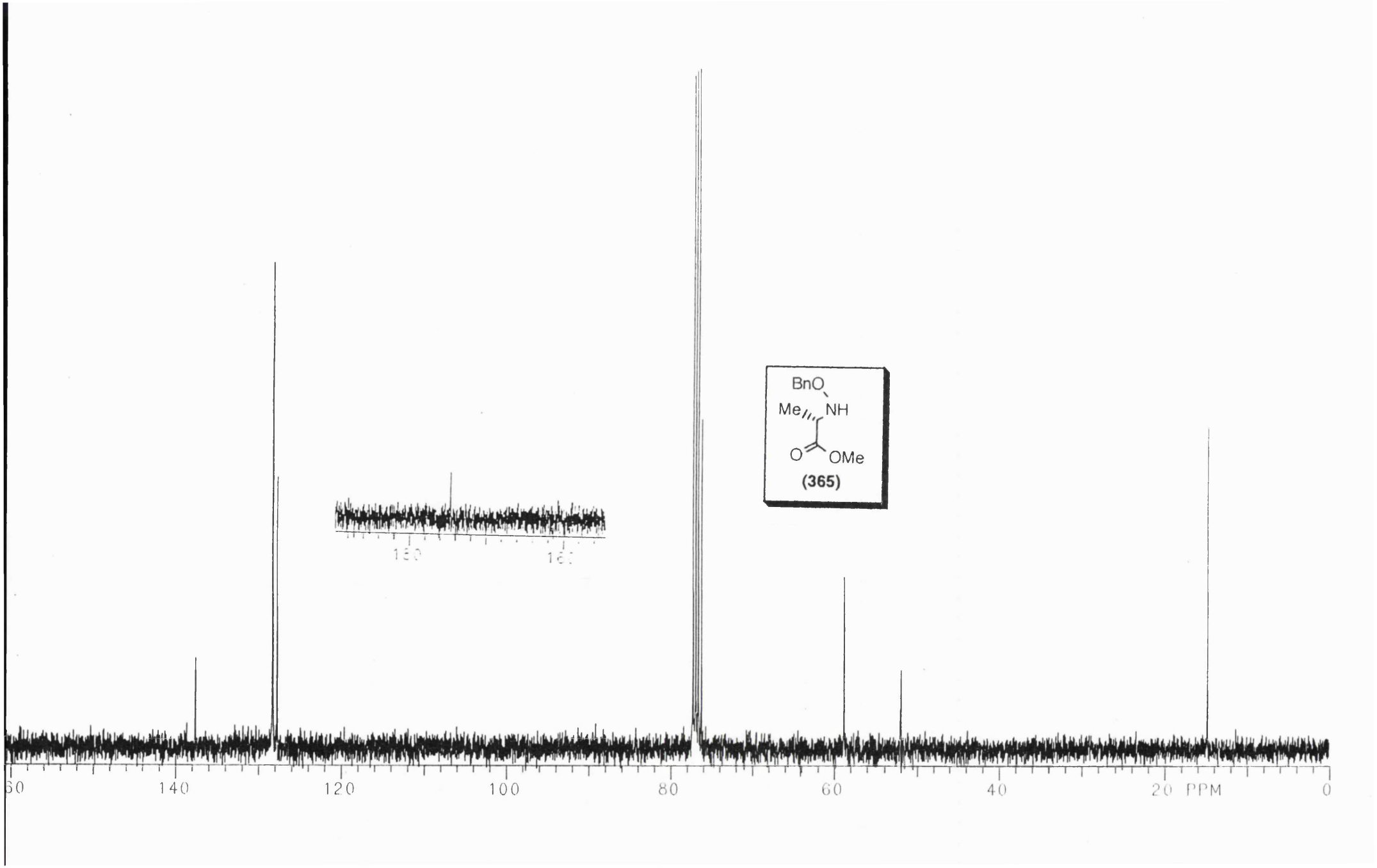

HO2C
N
NZ
COCF3
(363)
8
7
6
5
4
3
2
1 PPM

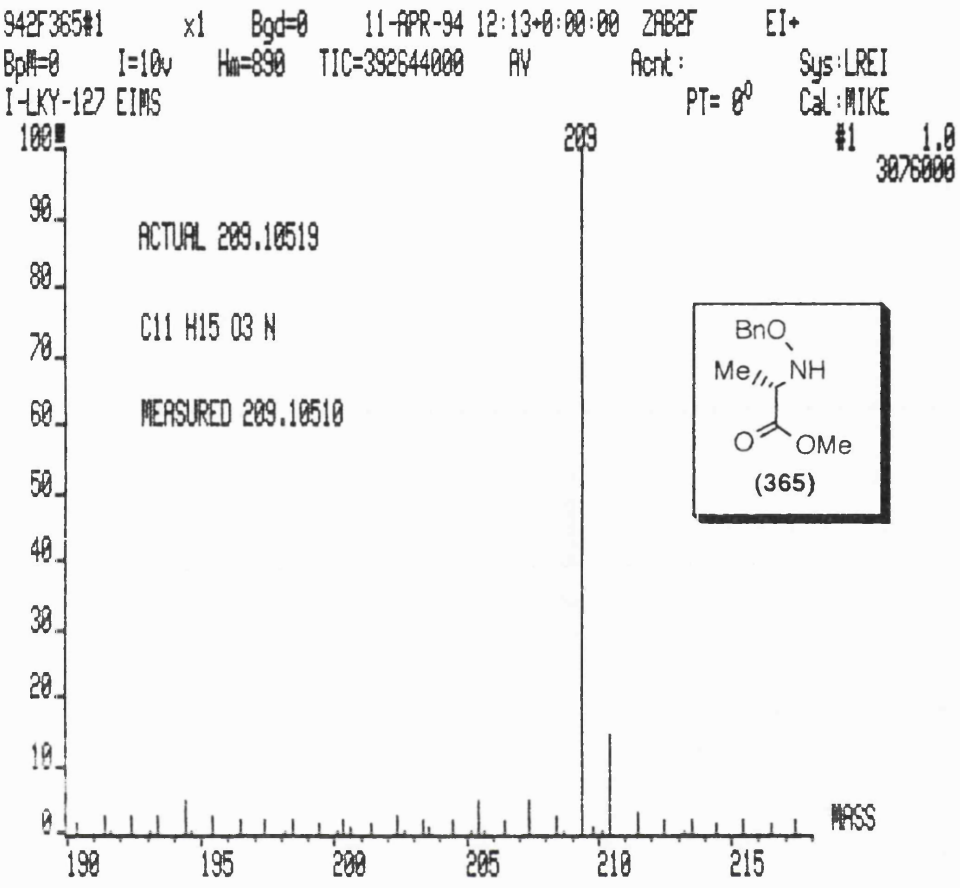

V/VMD/163
HO2C
N
NZ
COCF3
(363)
140
120
100
80
60
40
20 PPM

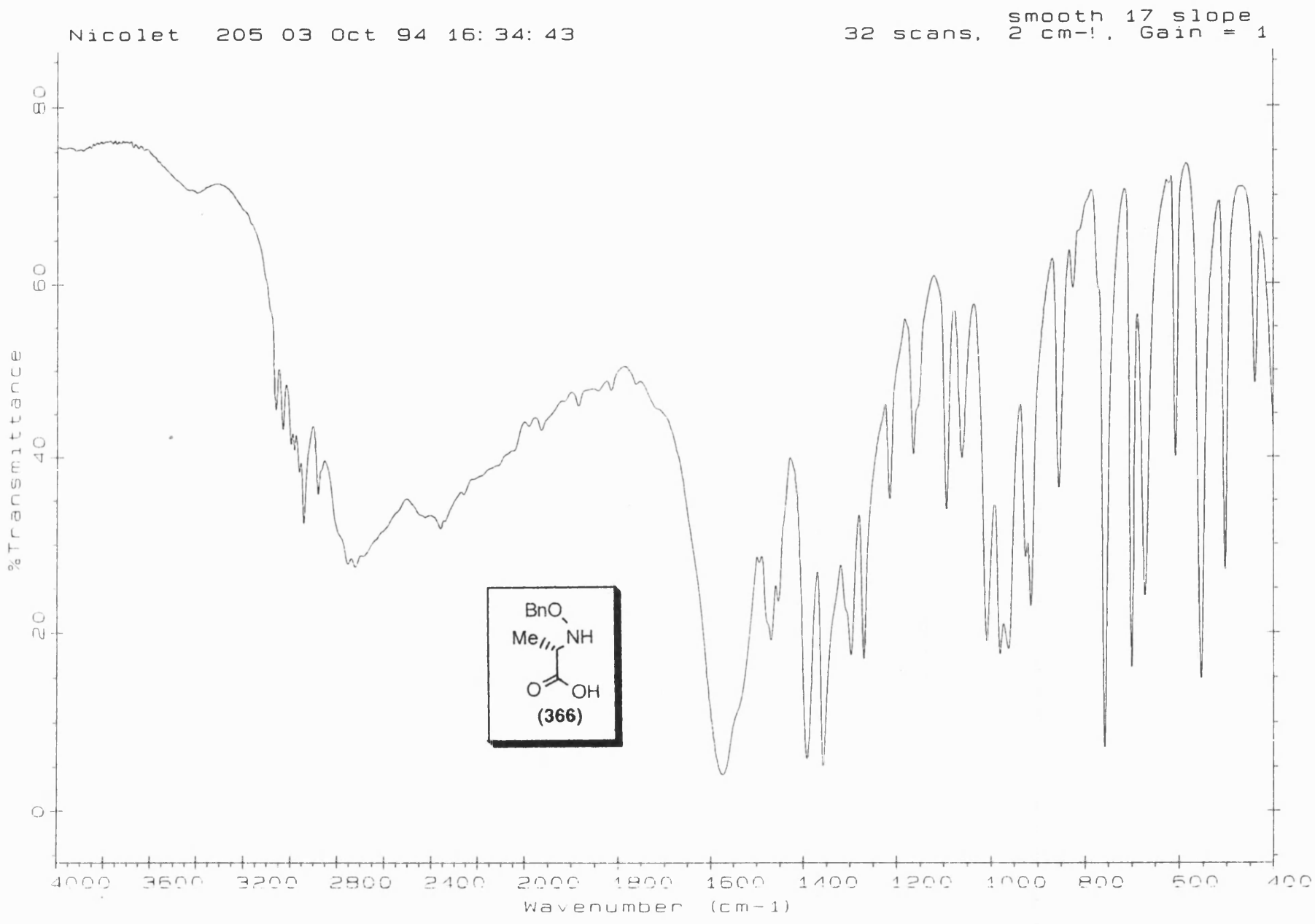

I-LKY-127 21 Mar 94 20:36:25
smooth 17
32 scans, 2 cm-1, Gain = 1
%Transmittance
70
60
50
40
30
20
10
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)
BnO
Me
NH
O
OMe
(365)

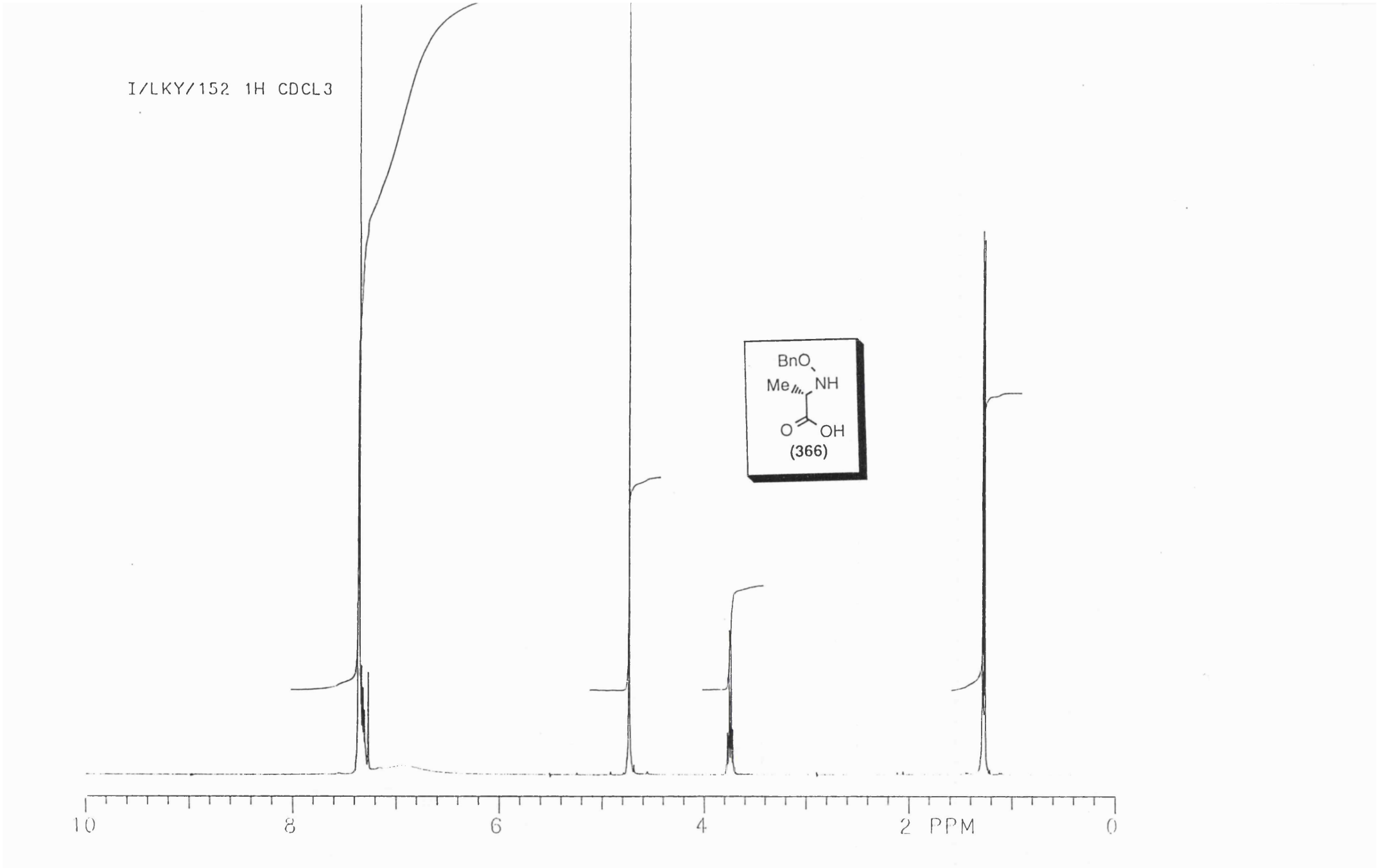
I-LKY-127

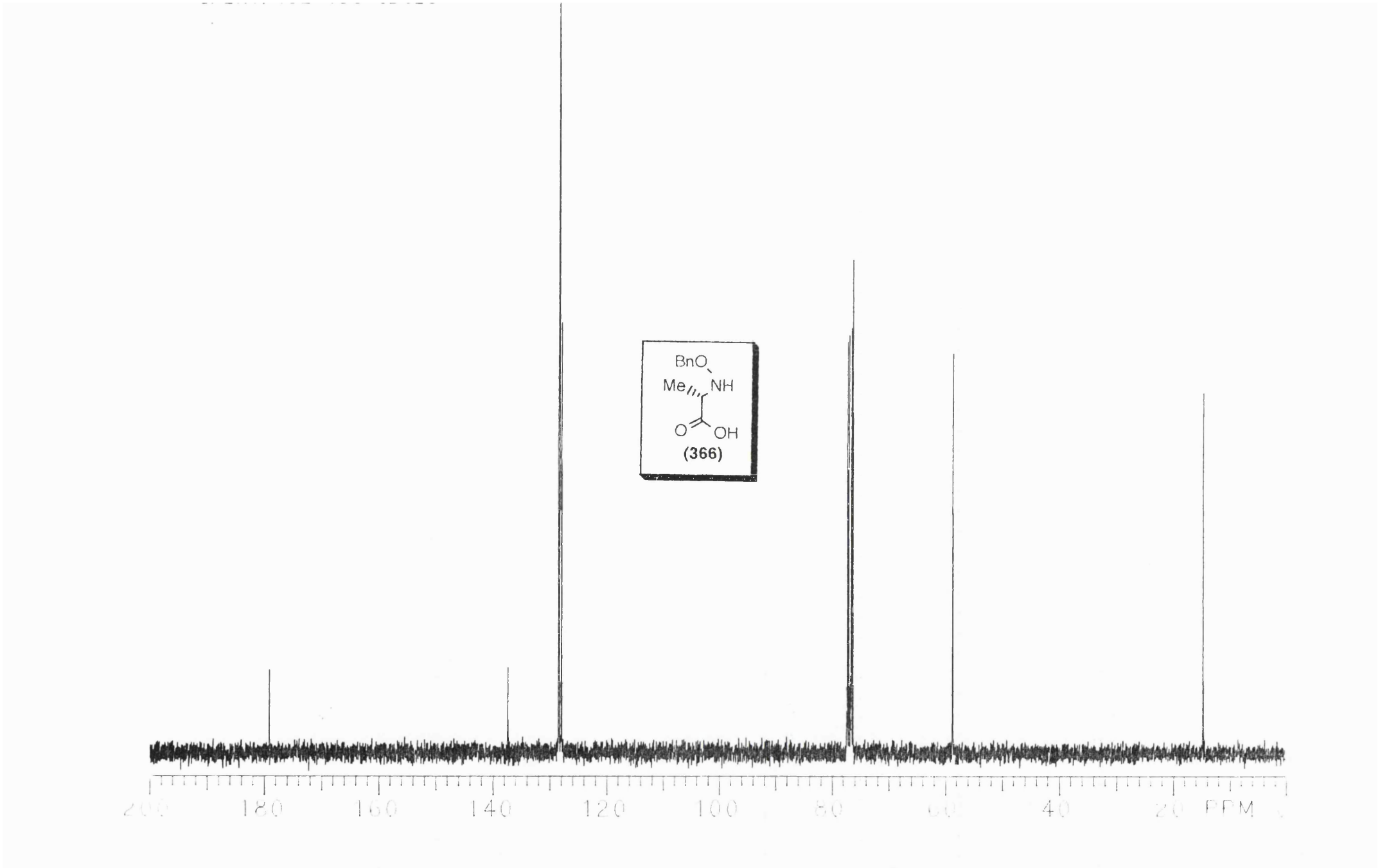


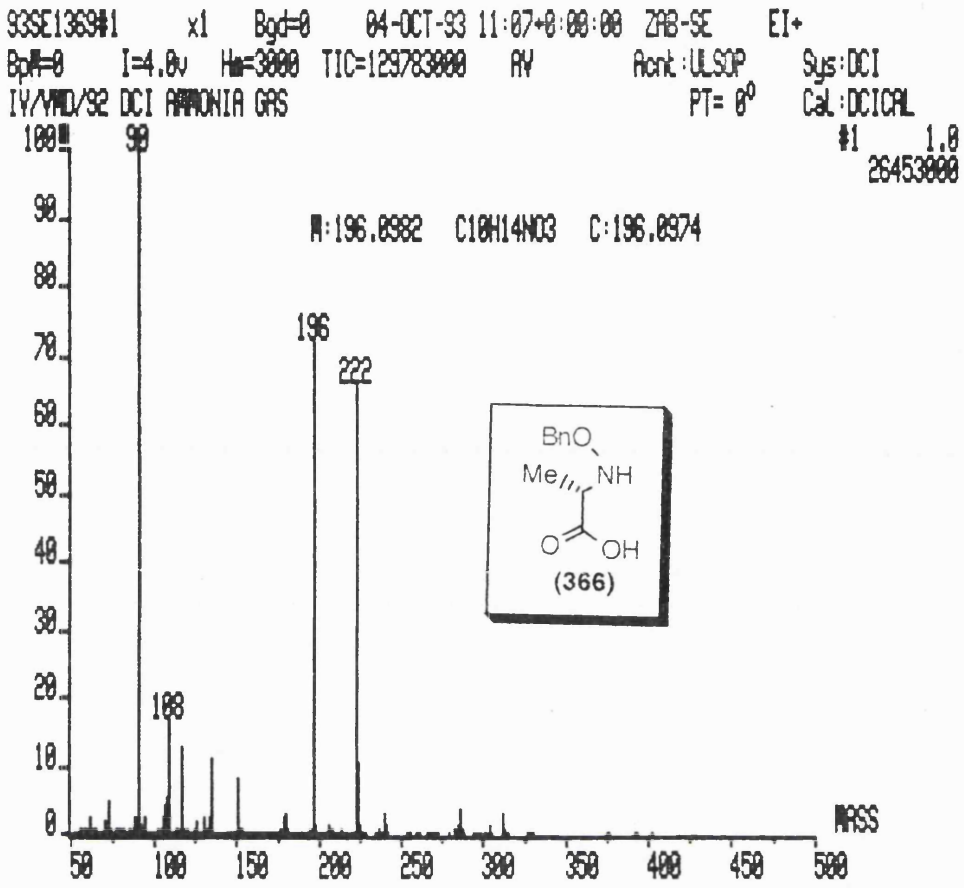

BnO
Me
NH
O
OMe
(365)
160
140
120
100
80
60
40
20 PPM
0

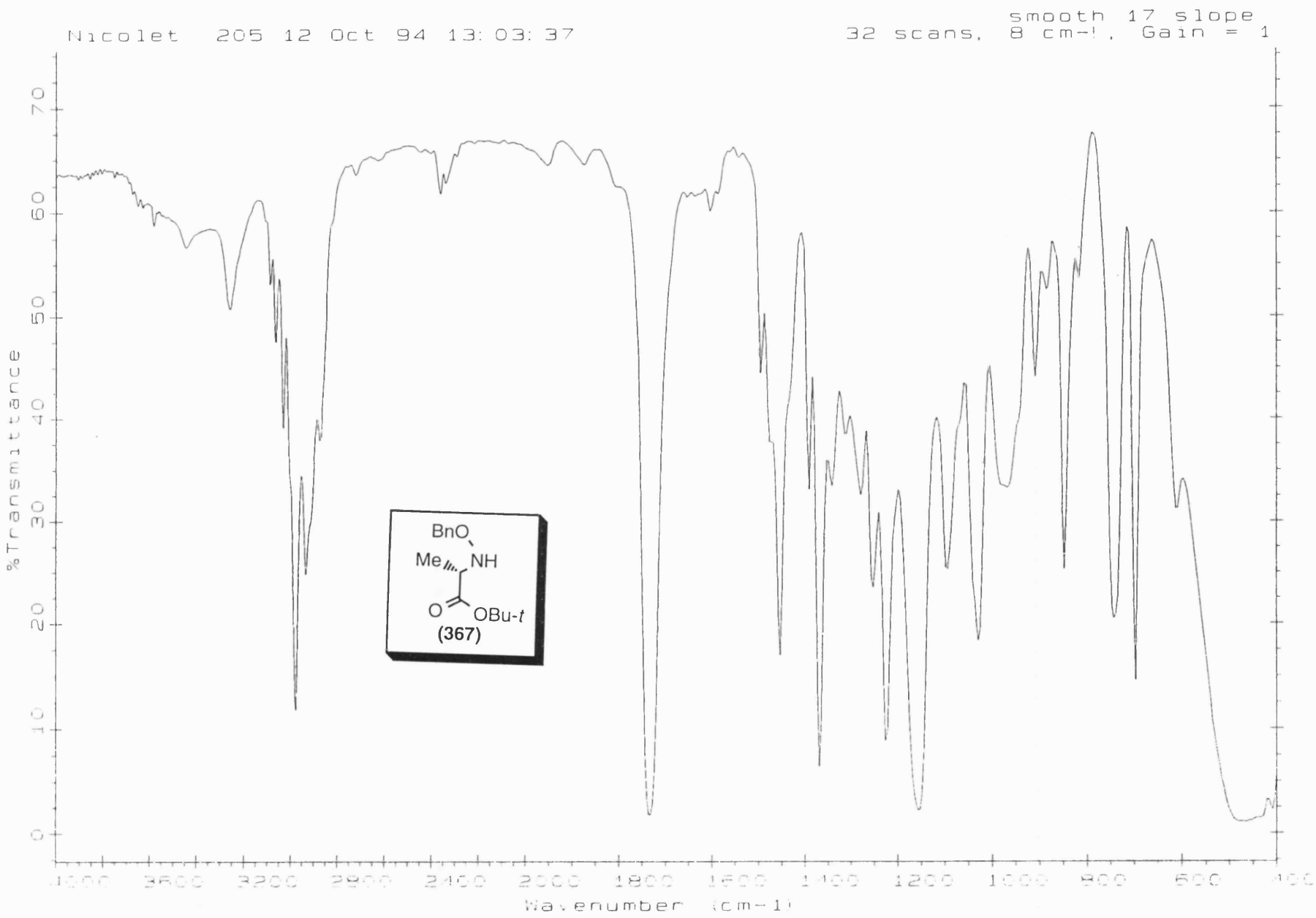

942F365#1 x1 Bgd=0 11-APR-94 12:13+0:00:00 ZAB2F EI+
BpM=0 I=10v Hm=890 TIC=392644000 AV Acnt: Sys:LREI
I-LKY-127 EIMS PT= 0° Cal:MIKE
#1 1.0
3076000
209
ACTURAL 209.10519
C11 H15 O3 N
MEASURED 209.10510
BnO
Me
NH
O
OMe
(365)
MASS
100
90
80
70
60
50
40
30
20
10
0
190
195
200
205
210
215

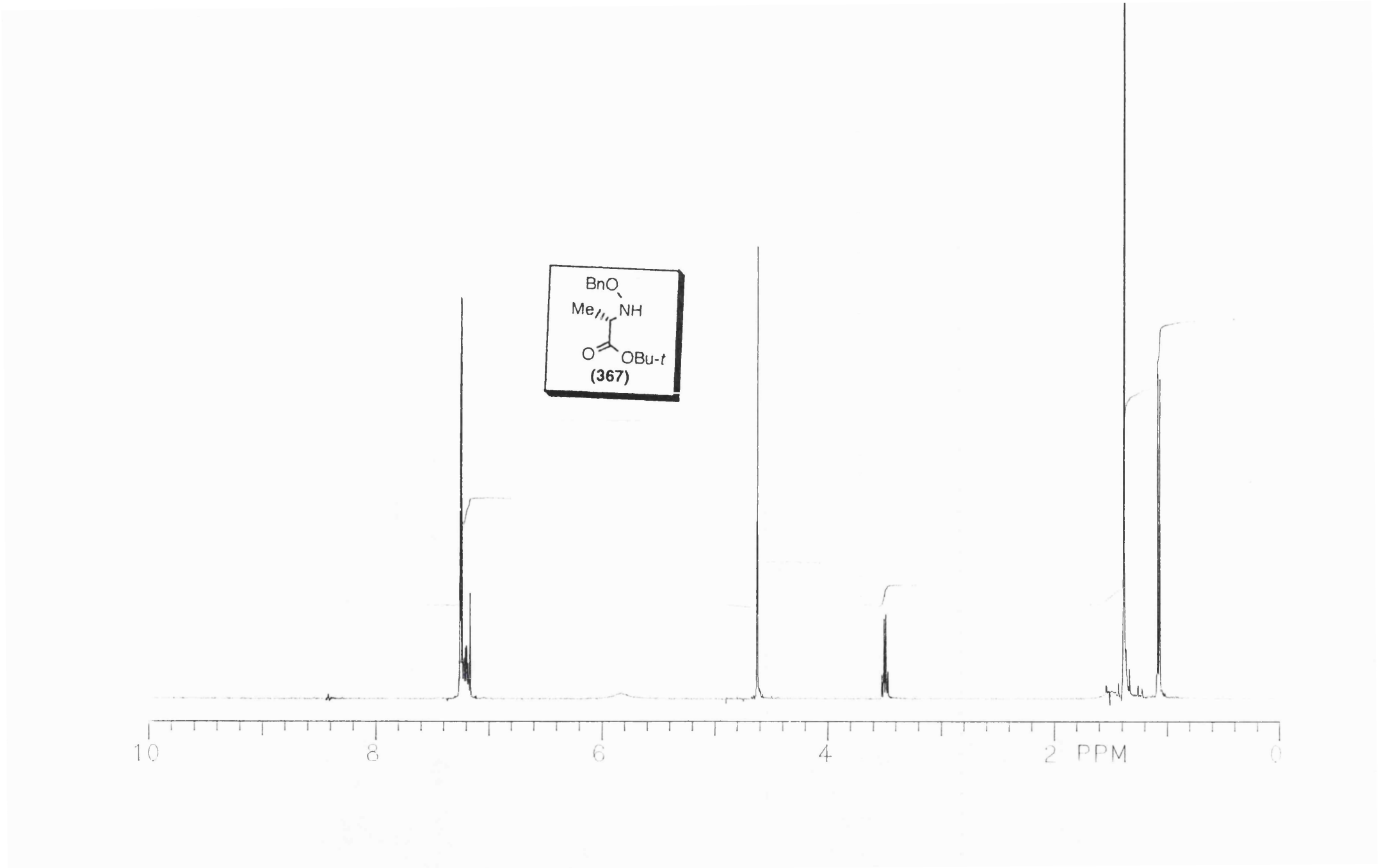

Nicolet 205 03 Oct 94 16:34:43
smooth 17 slope
32 scans, 2 cm-!, Gain = 1
%Transmittance
80
60
40
20
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
BnO
Me
NH
O
OH
(366)

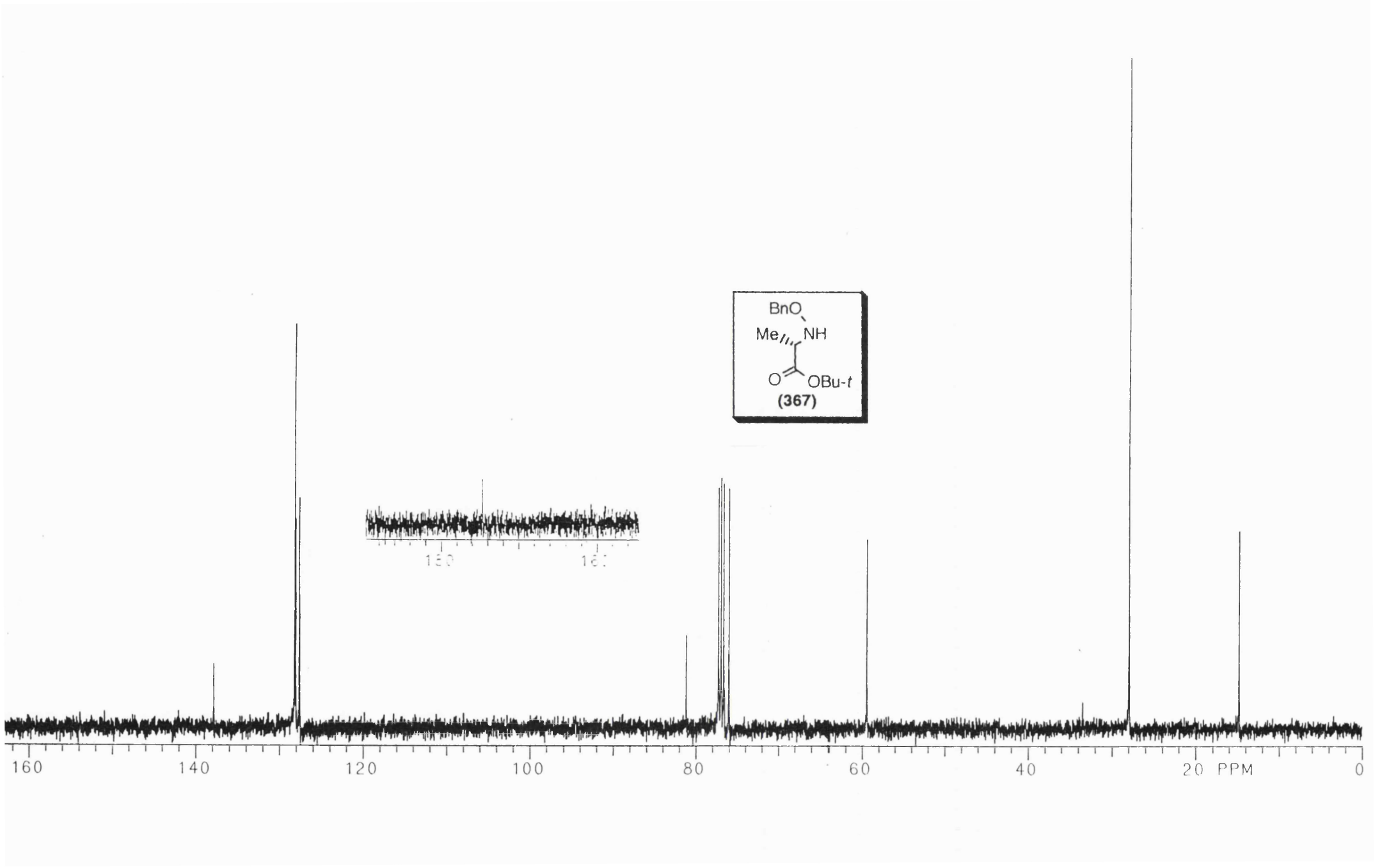

I/LKY/152 1H CDCL3
BnO
Me
NH
O
OH
(366)
10
8
6
4
2 PPM
0

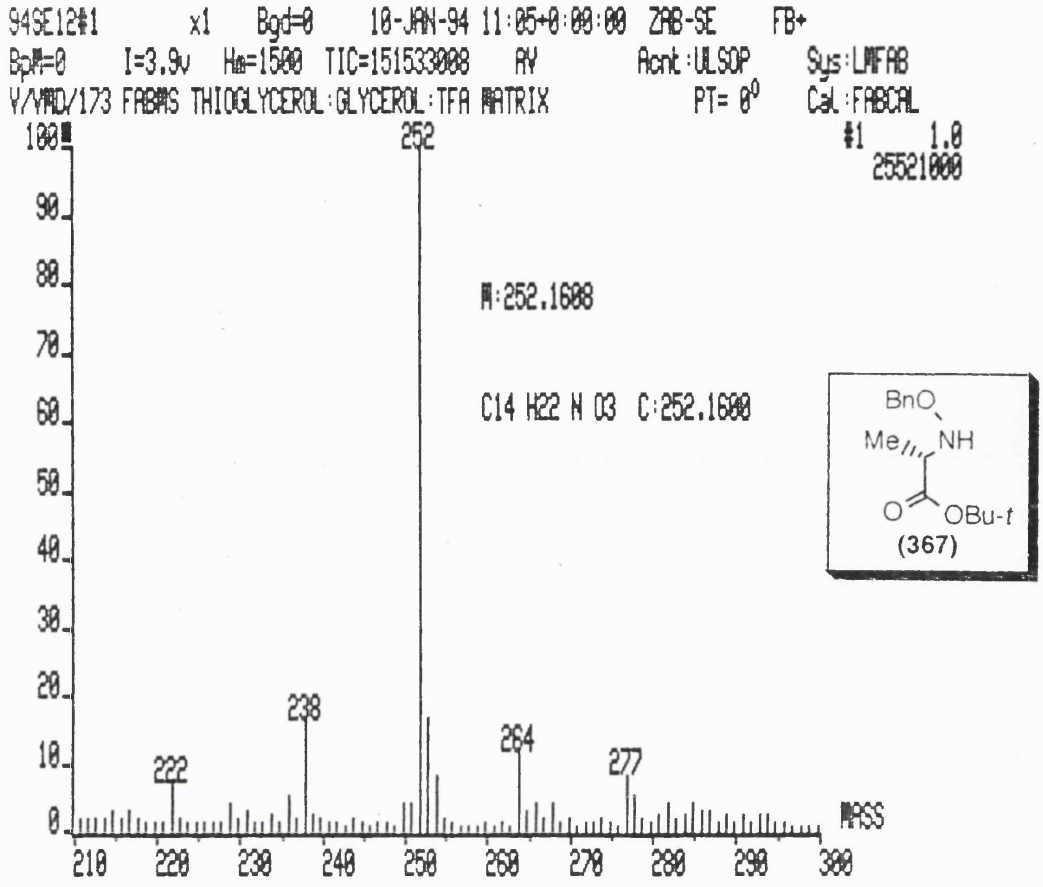

BnO
Me
NH
O
OH
(366)
180
160
140
120
100
80
40
20
PPM

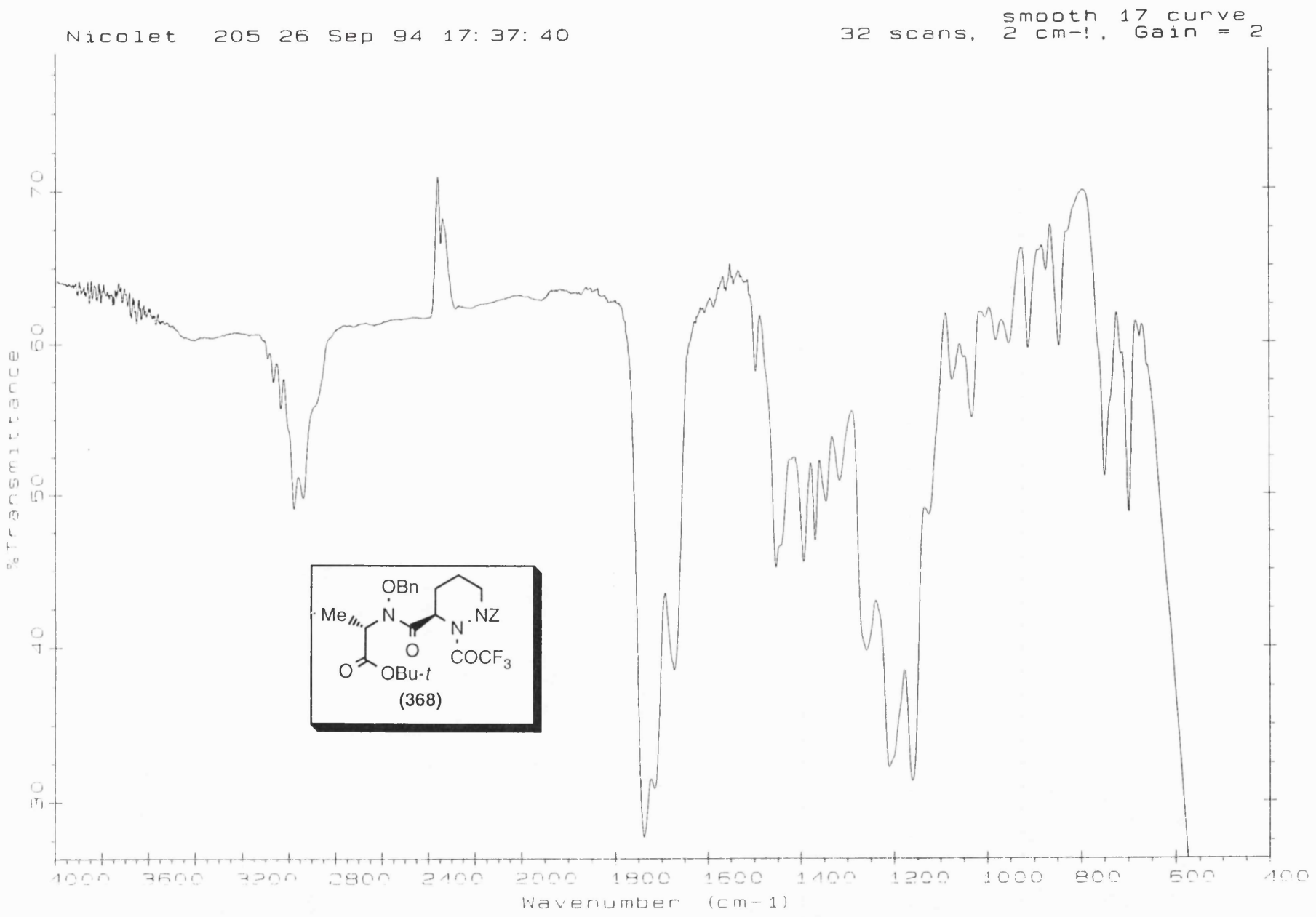

93SE1363#1 x1 Bgd=0 04-OCT-93 11:07+0:00:00 ZAB-SE EI+
BpM=0 I=4.0v Hm=3000 TIC=129783000 AV Acnt:ULSOP Sys:DCI
IV/YMD/92 DCI AMMONIA GRS PT= 0° CaL:DCICAL
#1 1.0
26453000
M:196.0982 C10H14NO3 C:196.0974
98
196
222
168
BnO
Me
NH
O
OH
(366)
MASS
50 100 150 200 250 300 350 400 450 500


Nicolet 205 12 Oct 94 13:03:37
smooth 17 slope
32 scans, 8 cm-1, Gain = 1
%Transmittance
70
60
50
40
30
20
10
0
BnO
Me
NH
O
OBu-t
(367)
Wavenumber (cm-1)


BnO
Me
NH
O
OBu-t
(367)
10
8
6
4
2 PPM
0

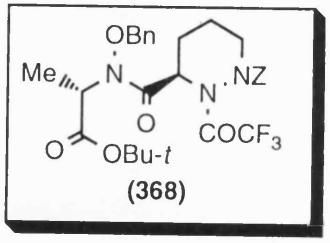

BnO
Me
NH
O
OBu-t
(367)
160
140
120
100
80
60
40
20 PPM
0


94SE12#1 x1 Bgd=0 10-JAN-94 11:05+0:00:00 ZAB-SE FB+
BpM=0 I=3.9v Hm=1500 TIC=151533008 RV Acmt:ULSOP Sys:LMFAB
Y/YMD/173 FABMS THIOGLYCEROL:GLYCEROL:TFA MATRIX PT= 0° Cal:FABCAL
#1 1.0
25521000
252
M:252.1608
C14 H22 N O3 C:252.1600
BnO
Me
NH
O
OBu-t
(367)
238
222
264
277
MASS
100
90
80
70
60
50
40
30
20
10
0
210
220
230
240
250
260
270
280
290
300

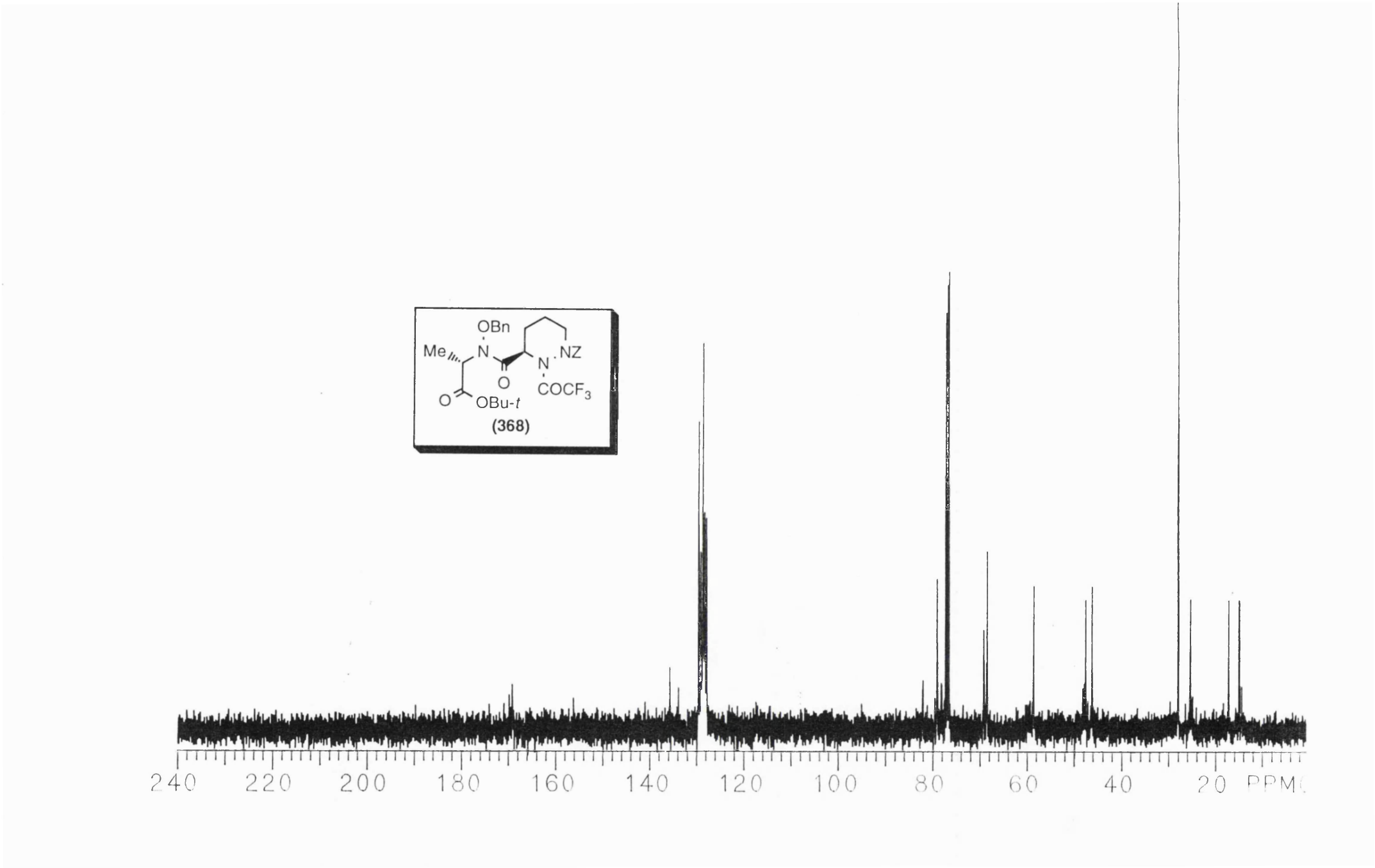

Nicolet 205 26 Sep 94 17:37:40
smooth 17 curve
32 scans, 2 cm-!, Gain = 2
%Transmittance
70
60
50
40
30
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
OBn
Me
N
NZ
O
N
COCF3
O
OBu-t
(368)

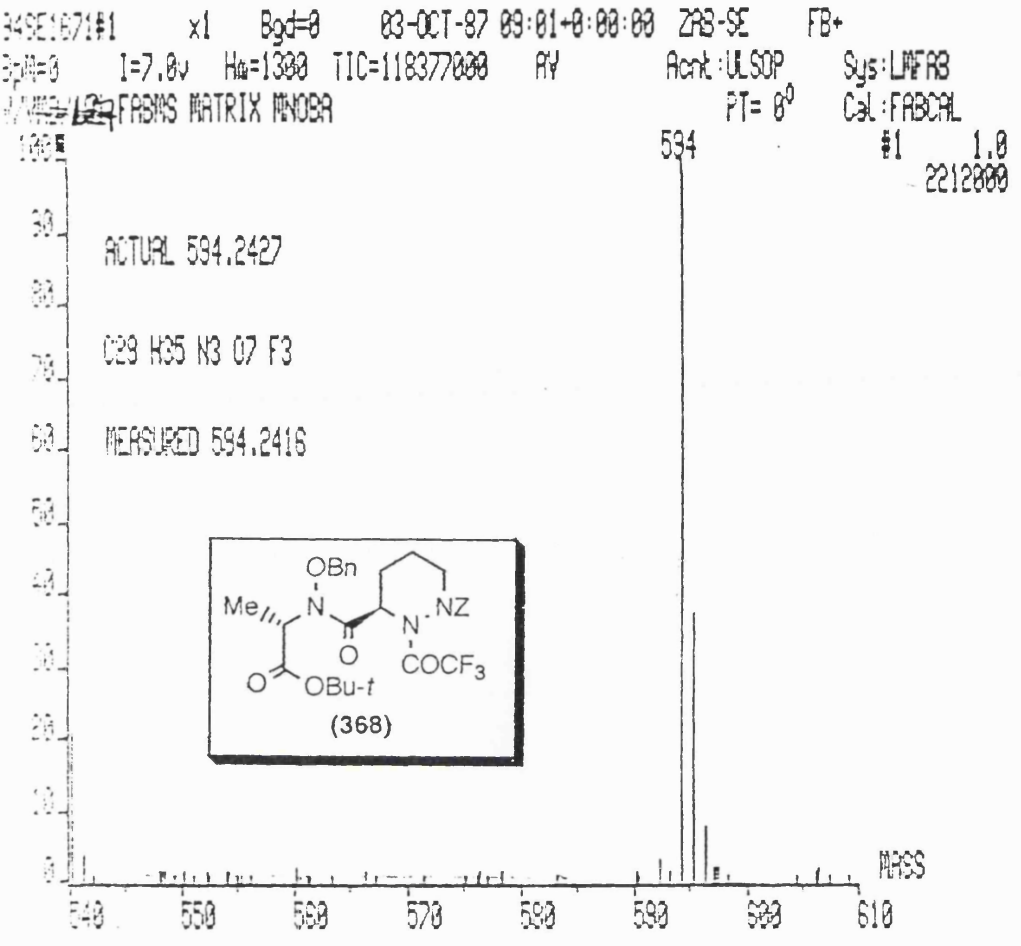
STD1H

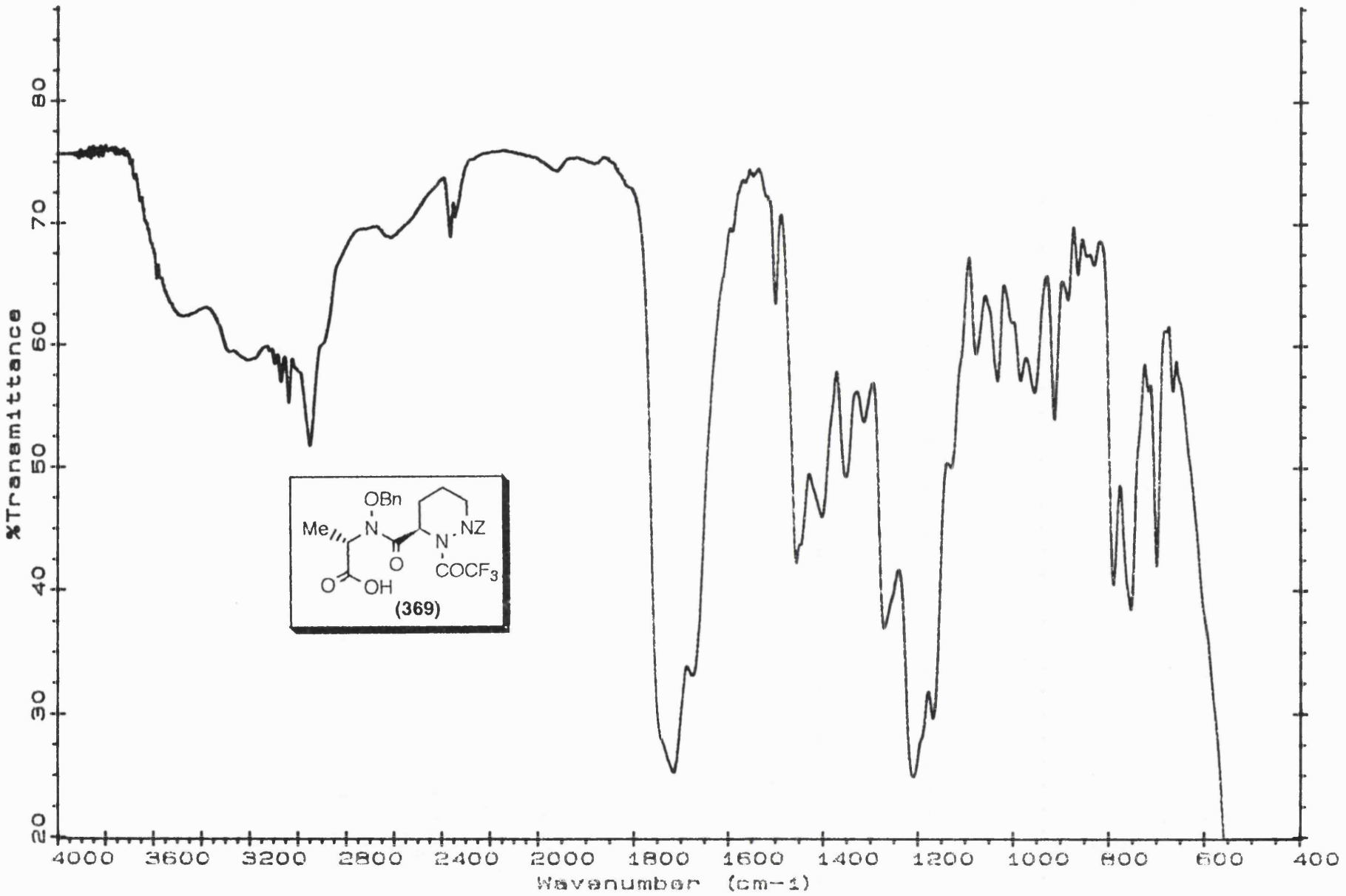
Temperature = 135 °C in DMSO-d_6

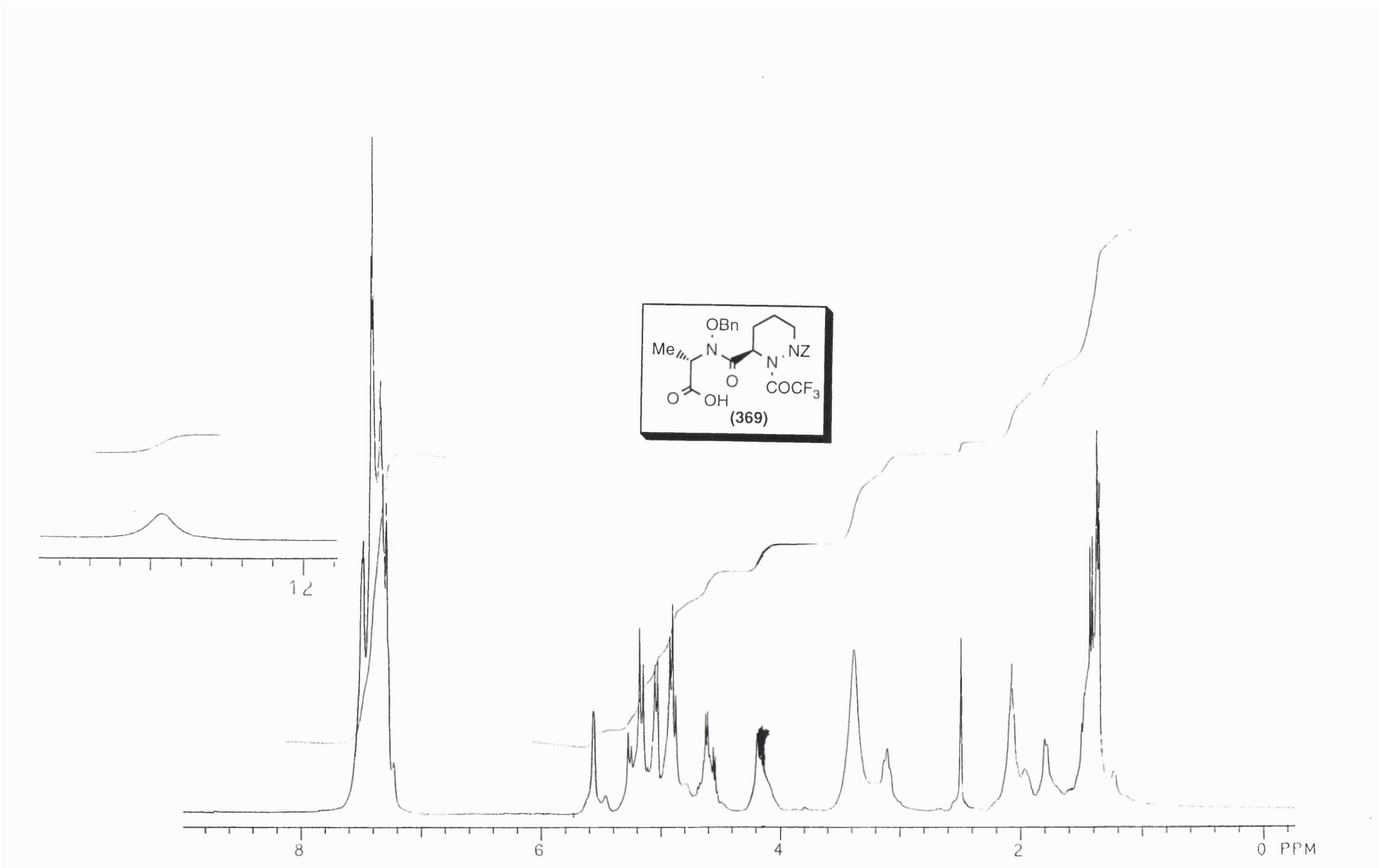


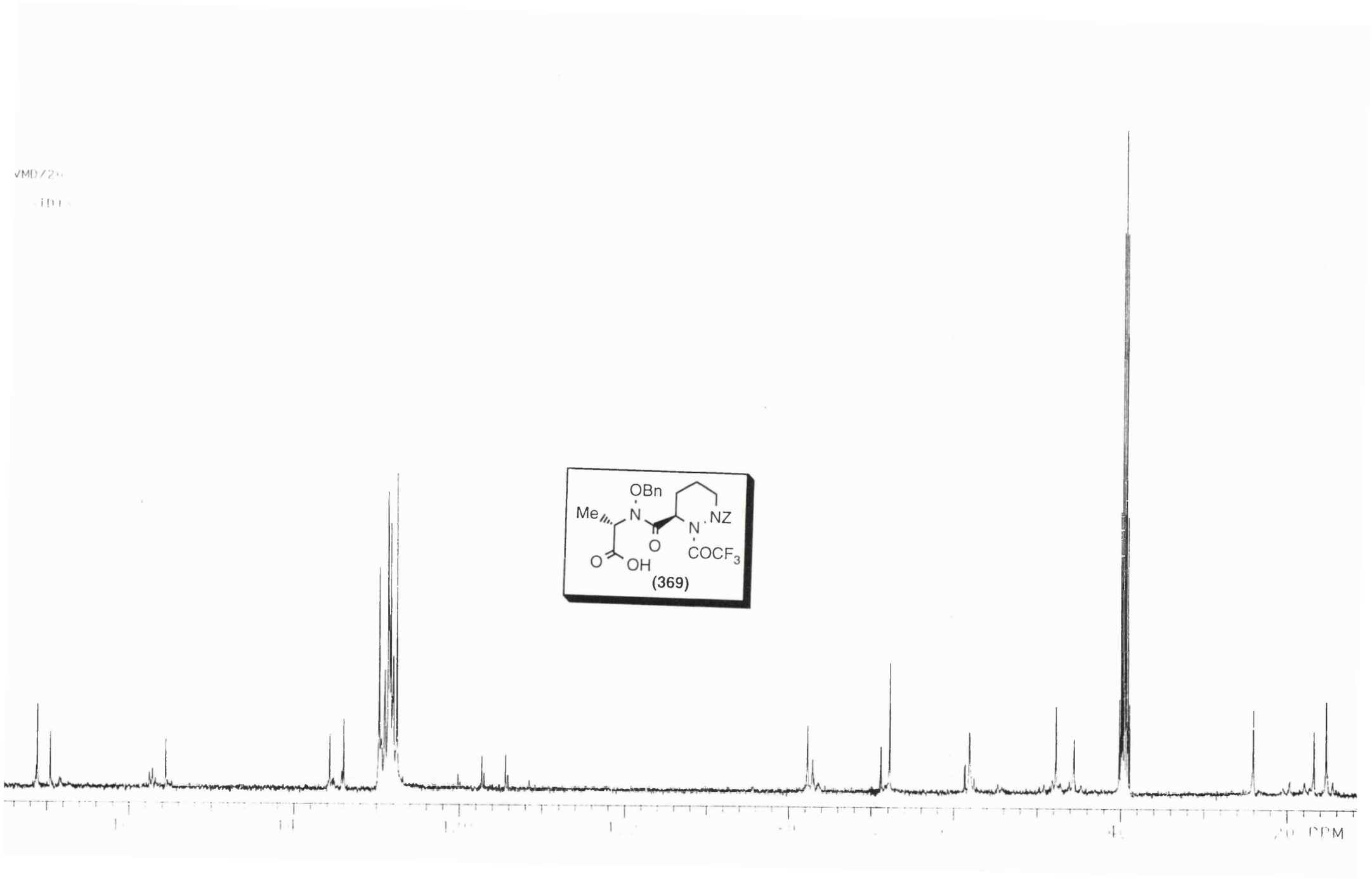
(368)

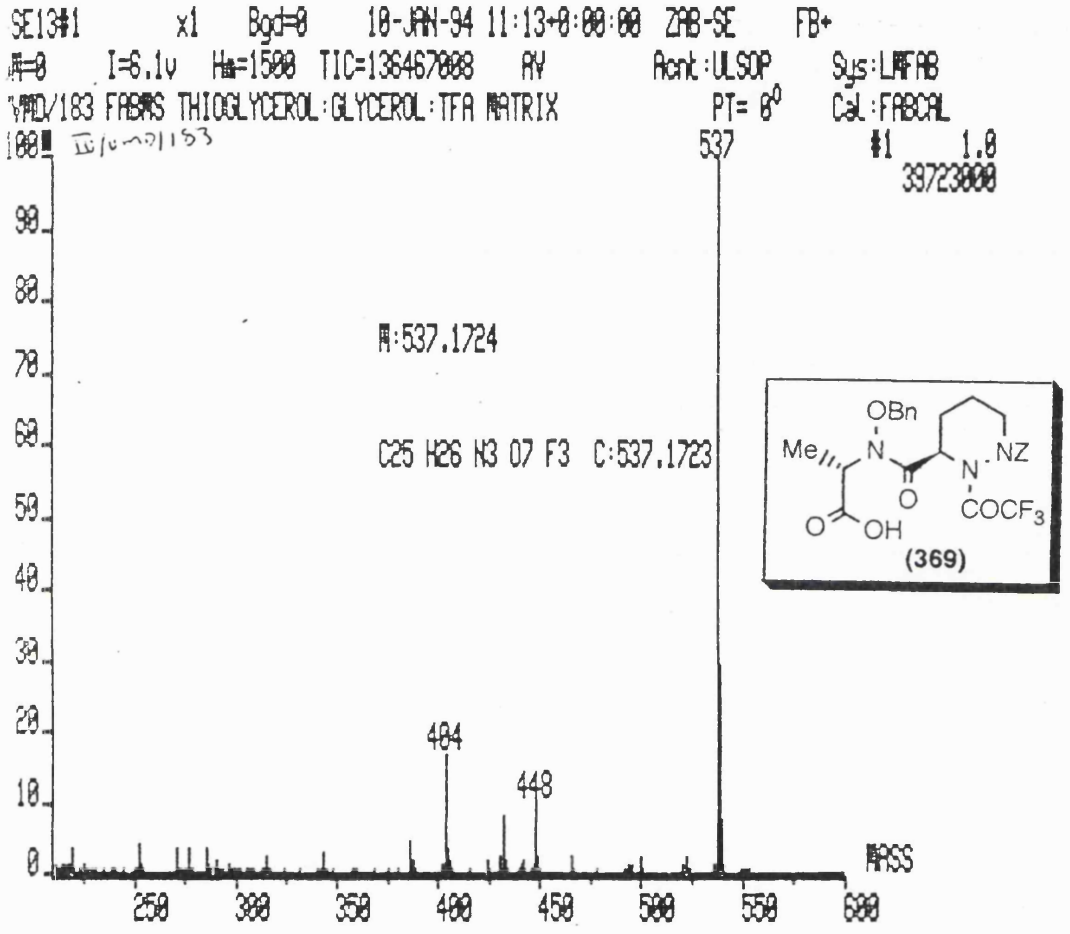

OBn
Me
N
NZ
N
O
COCF3
O
OBu-t
(368)
240
220
200
180
160
140
120
100
80
60
40
20
PPM

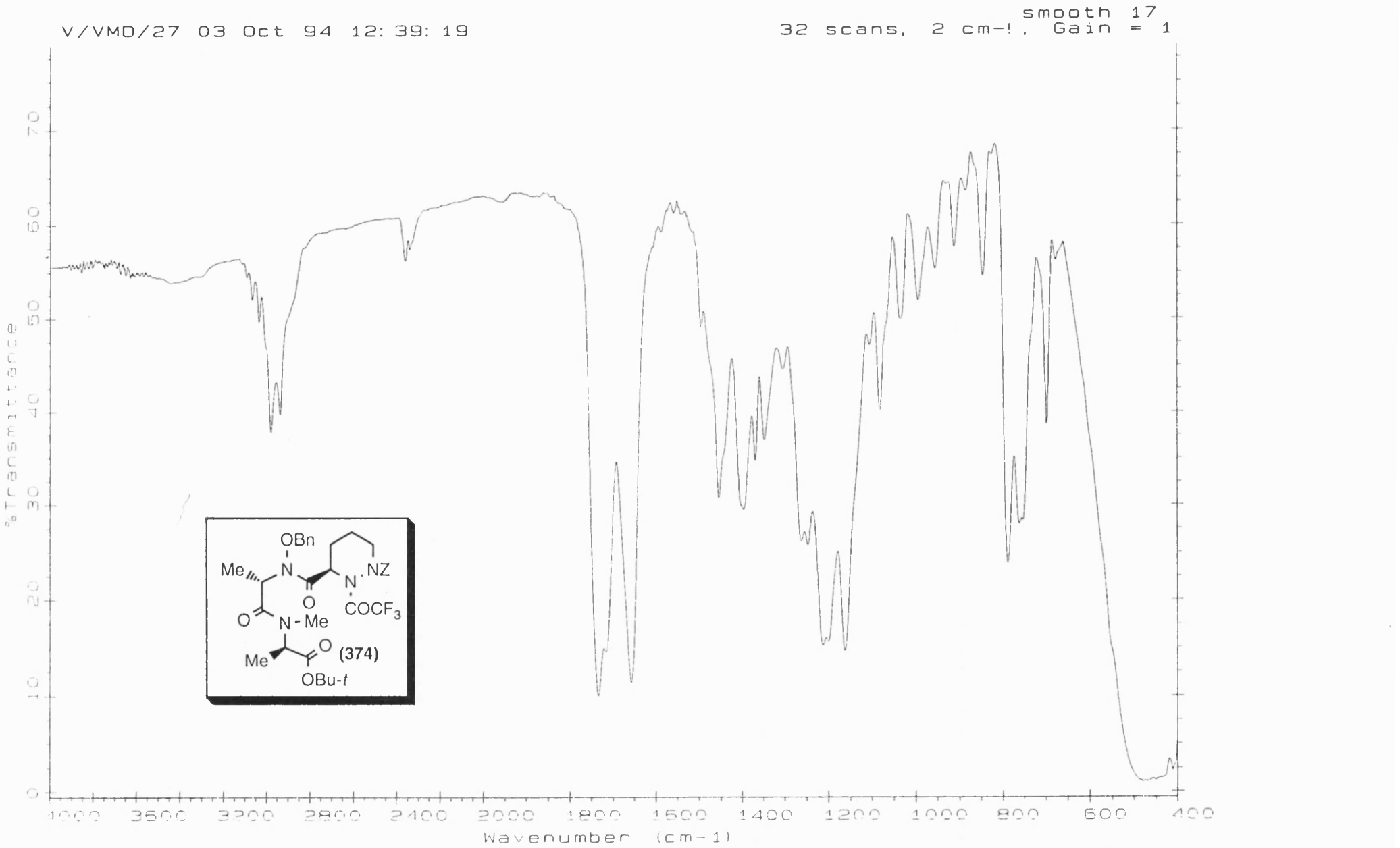

FABMS MATRIX MNOBA
594
#1 1.0
2212000
ACTURL 594.2427
C29 H35 N3 O7 F3
MEASURED 594.2416
OBn
Me
N
NZ
N
O
O
OBu-t
COCF3
(368)
MASS
540
550
560
570
580
590
600
610

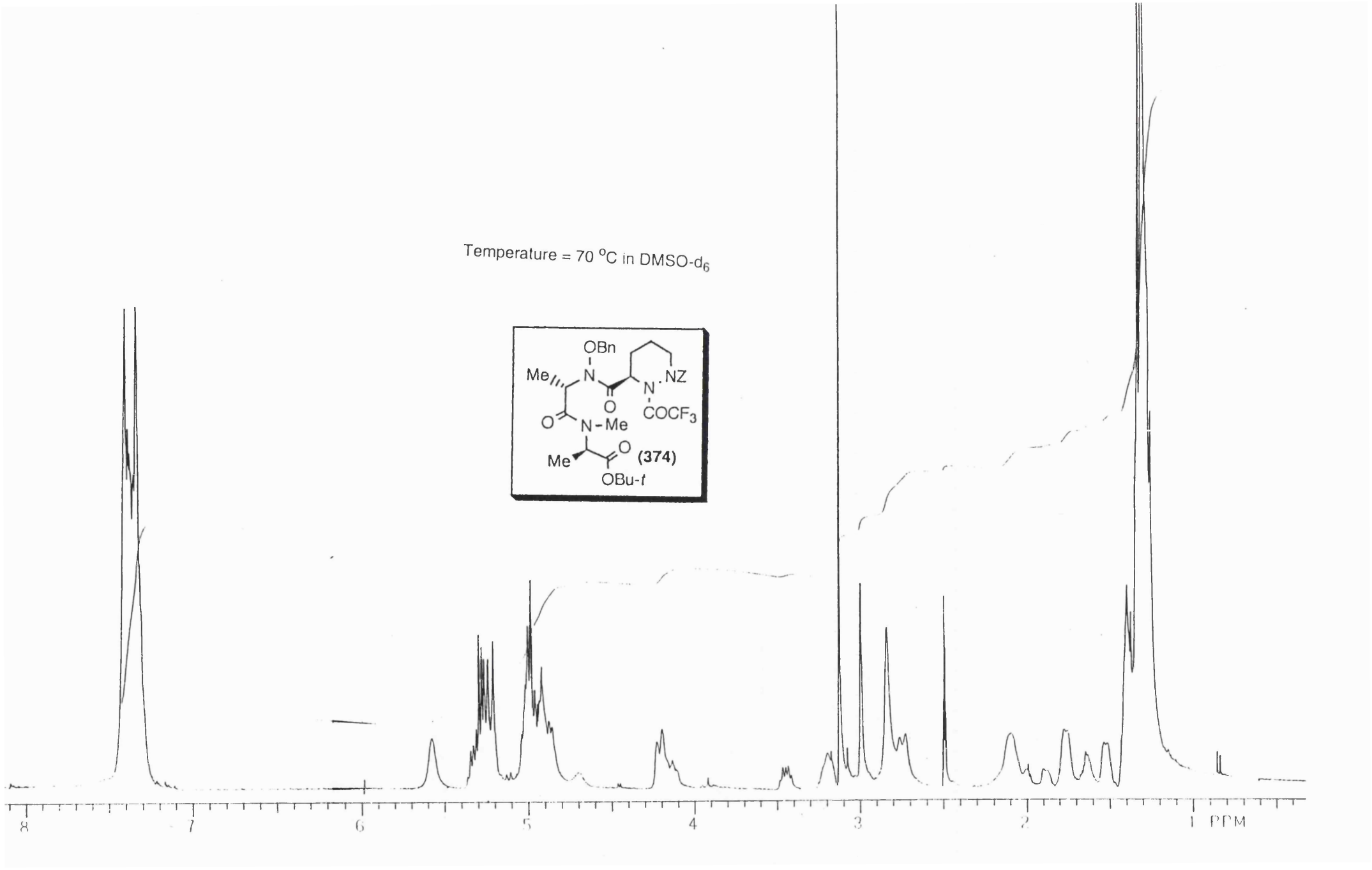

%Transmittance
80
70
60
50
40
30
20
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
OBn
Me
N
NZ
O
N
COCF3
O
OH
(369)

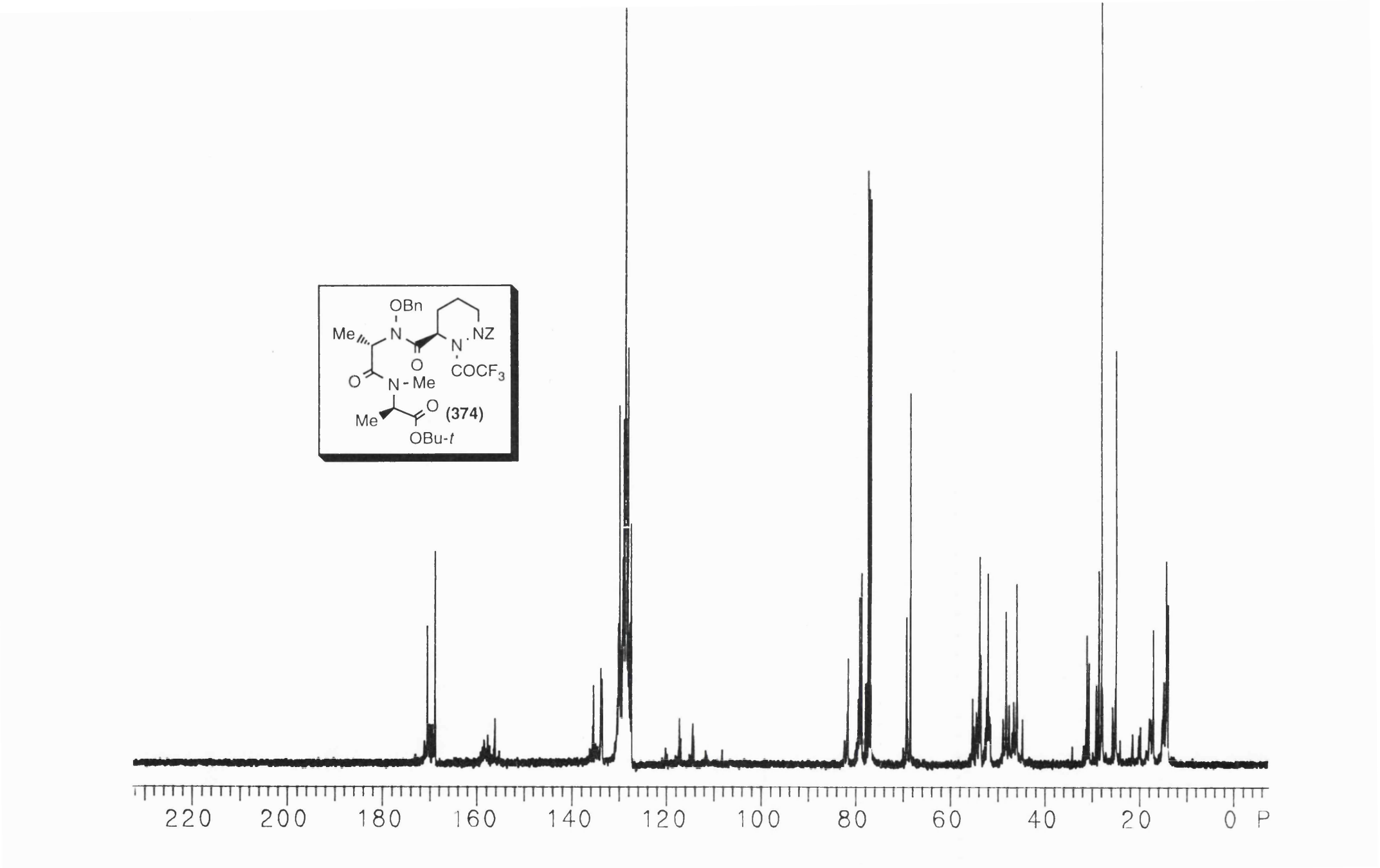

OBn
Me
N
NZ
N
O
COCF3
O
OH
(369)
12
8
6
4
2
0 PPM

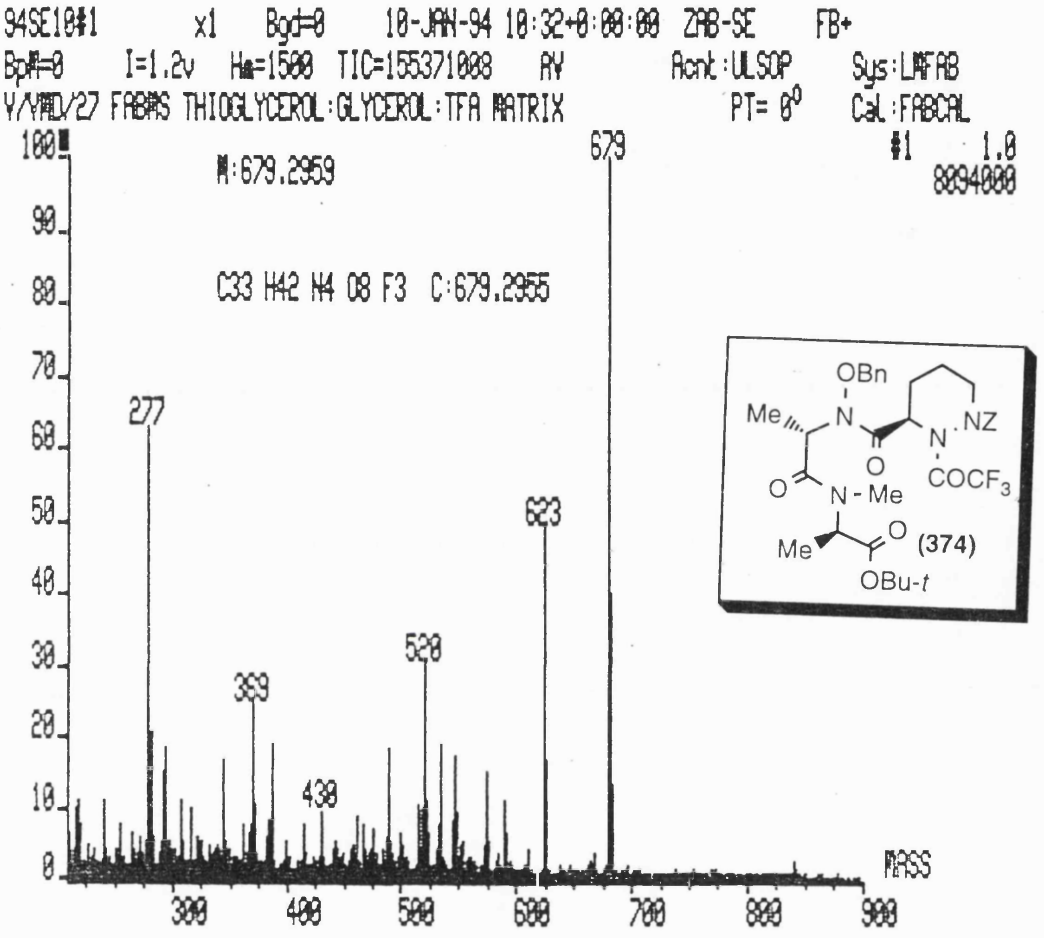

OBn
Me
N
NZ
N
O
O
OH
COCF3
(369)

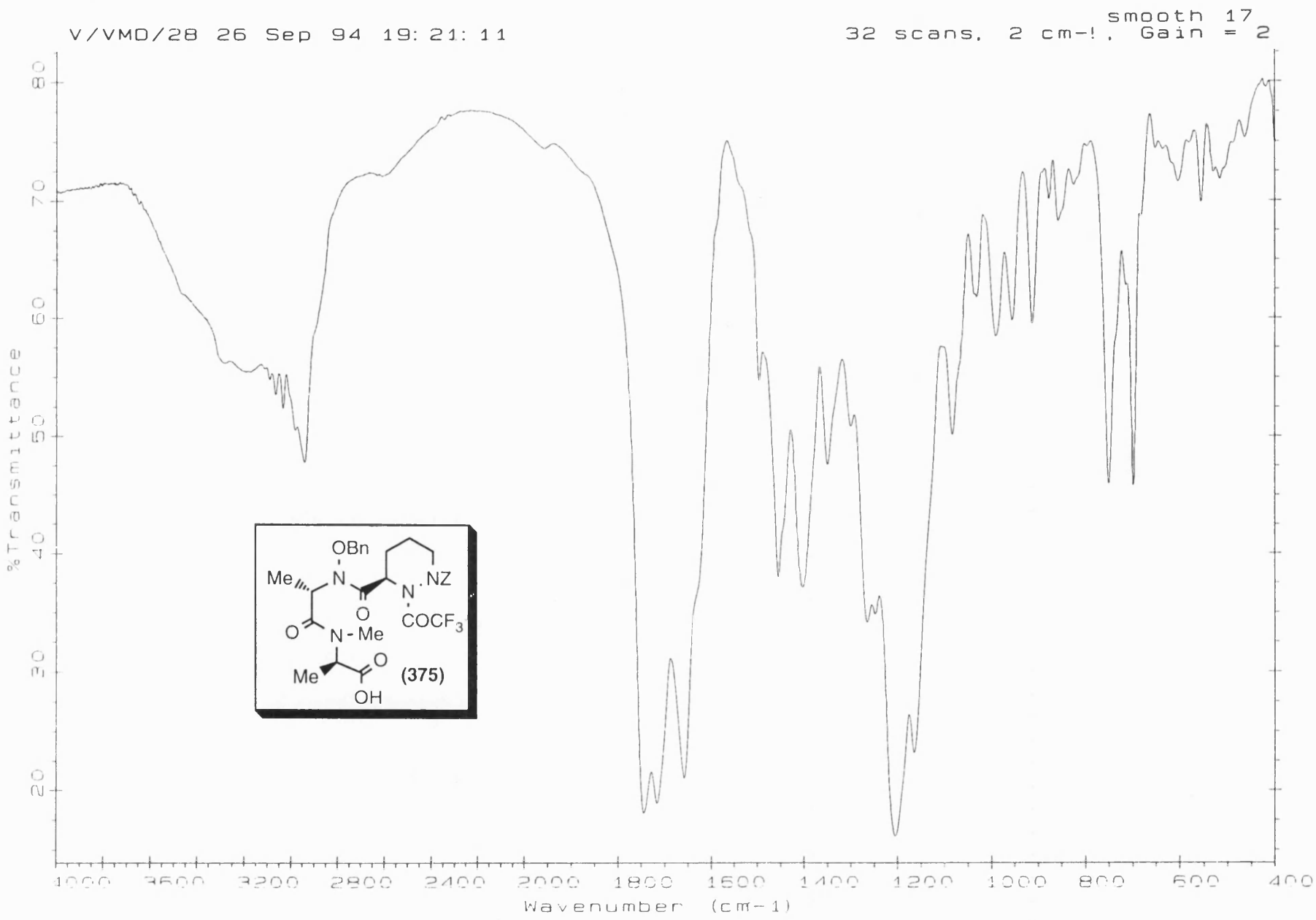

SE13#1 x1 Bgd=0 10-JAN-94 11:13+0:00:00 ZAB-SE FB+
I=6.1v Ha=1500 TIC=136467008 AV Acnt:ULSOP Sys:LAFAB
PT= 6° Cal:FABCAL
537
#1 1.0
39723008
M:537.1724
C25 H26 N3 O7 F3 C:537.1723
OBn
Me
N
NZ
O
OH
COCF3
(369)
484
448
MASS
250
300
350
400
450
500
550
600

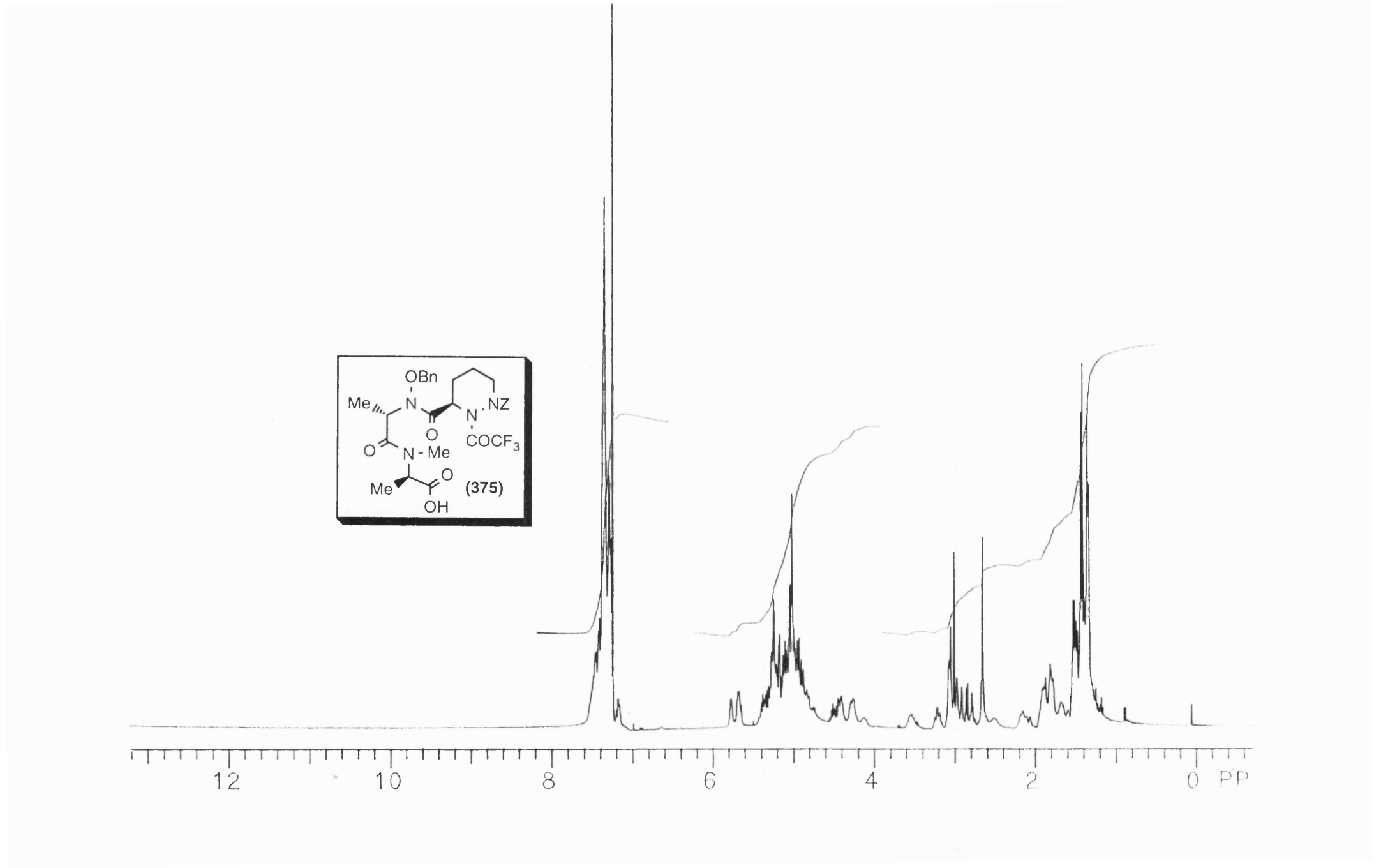

V/VMD/27 03 Oct 94 12: 39: 19
smooth 17
32 scans, 2 cm-1, Gain = 1
%Transmittance
0
10
20
30
40
50
60
70
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
OBn
Me
N
NZ
O
N
COCF3
O
N-Me
Me
O
(374)
OBu-t

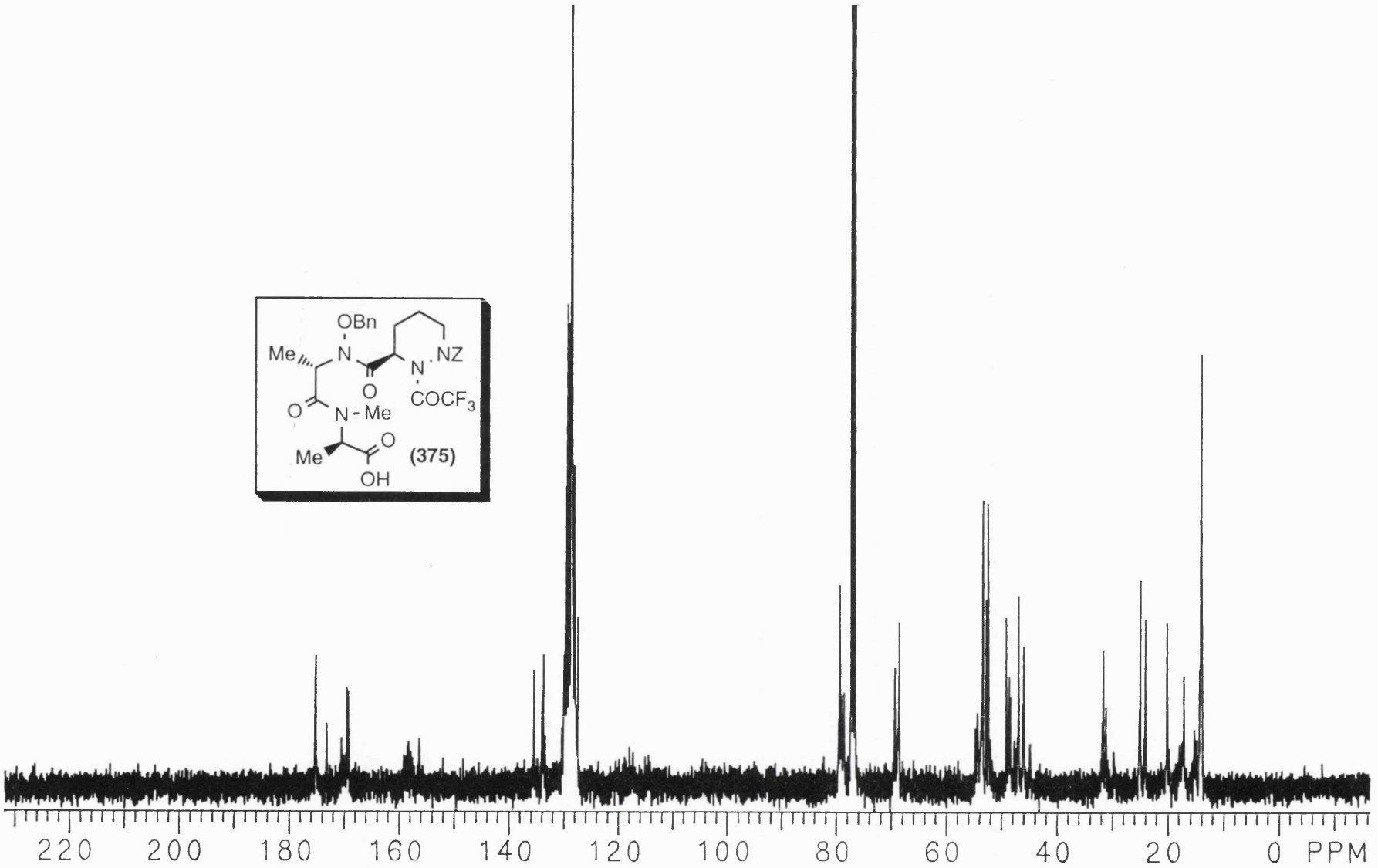

Temperature = 70 °C in DMSO-d6
OBn
Me
N
NZ
O
N
COCF3
O
N-Me
Me
O
(374)
OBu-t
8
7
6
5
4
3
2
1 PPM

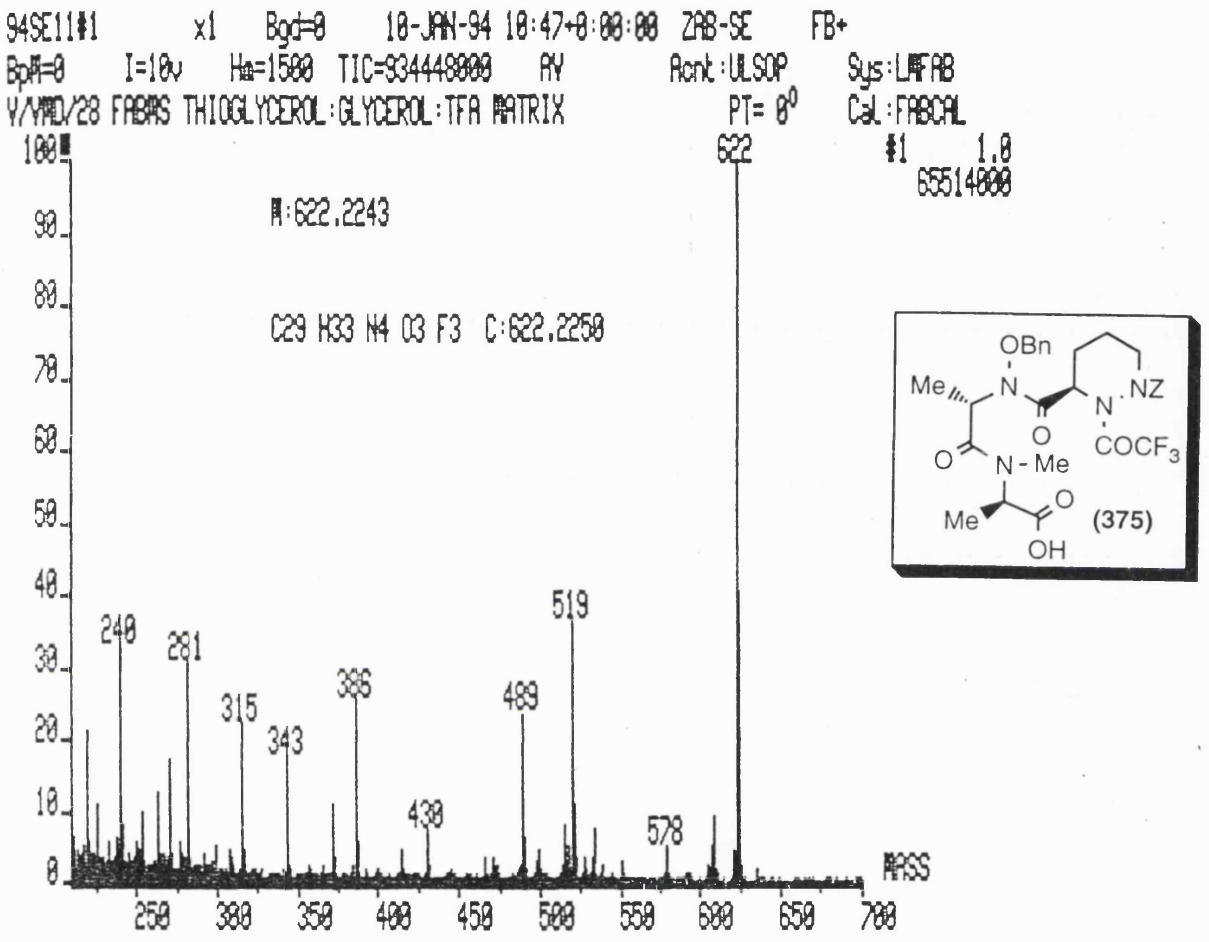

OBn
Me
N
NZ
N
O
COCF3
O
N - Me
Me
O
(374)
OBu-t
220
200
180
160
140
120
100
80
60
40
20
0
P

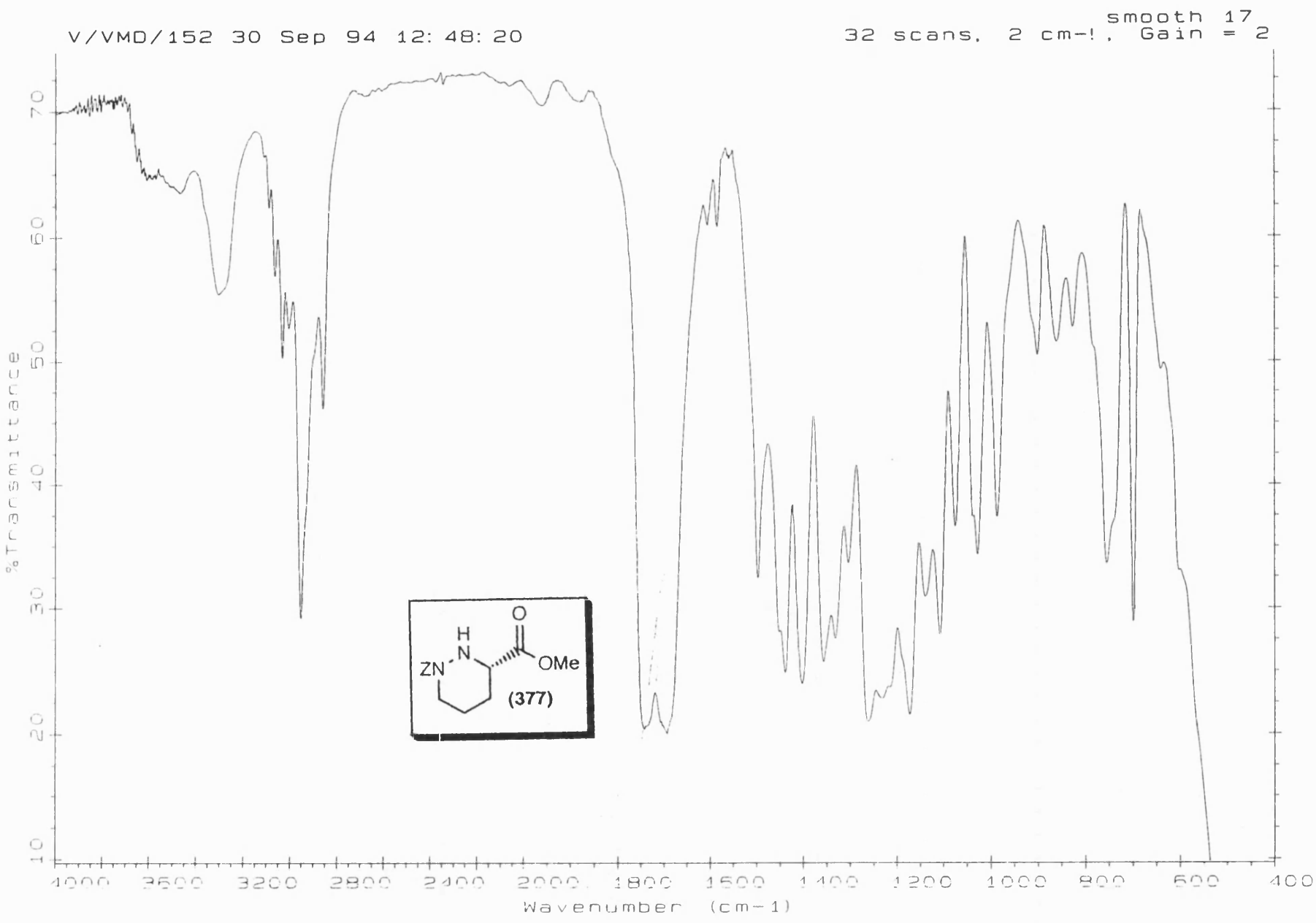

94SE10#1 x1 Bgd=0 10-JAN-94 10:32+0:00:00 ZAB-SE FB+
BpM=0 I=1.2v Ma=1500 TIC=155371008 AV Acnt:ULSOP Sys:LMFAB
Y/YMD/27 FABMS THIOGLYCEROL:GLYCEROL:TFA MATRIX PT= 0° CaL:FABCAL
#1 1.0
88894000
M:679.2959
C33 H42 N4 O8 F3 C:679.2955
679
623
528
369
438
277
MASS
100
90
80
70
60
50
40
30
20
10
0
300
400
500
600
700
800
900
OBn
Me
N
NZ
O
N - Me
COCF3
Me
O
(374)
OBu-t

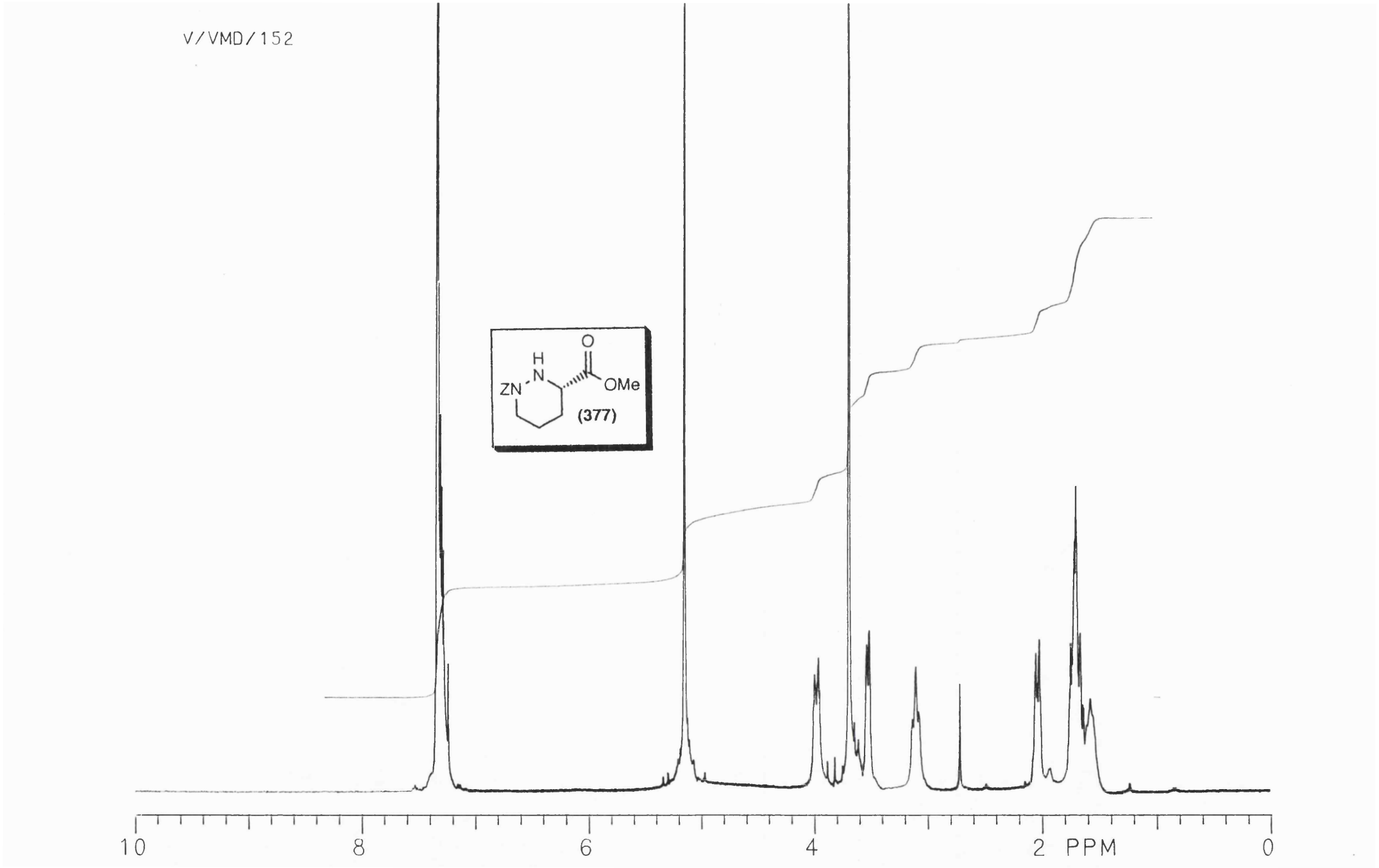

V/VMD/28 26 Sep 94 19: 21: 11
smooth 17
32 scans, 2 cm-!, Gain = 2
%Transmittance
80
70
60
50
40
30
20
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
OBn
Me
N
NZ
O
N
COCF3
O
N-Me
Me
O
OH
(375)


OBn
Me
N
NZ
O
N
COCF3
O
N - Me
Me
O
OH
(375)
12
10
8
6
4
2
0 PP

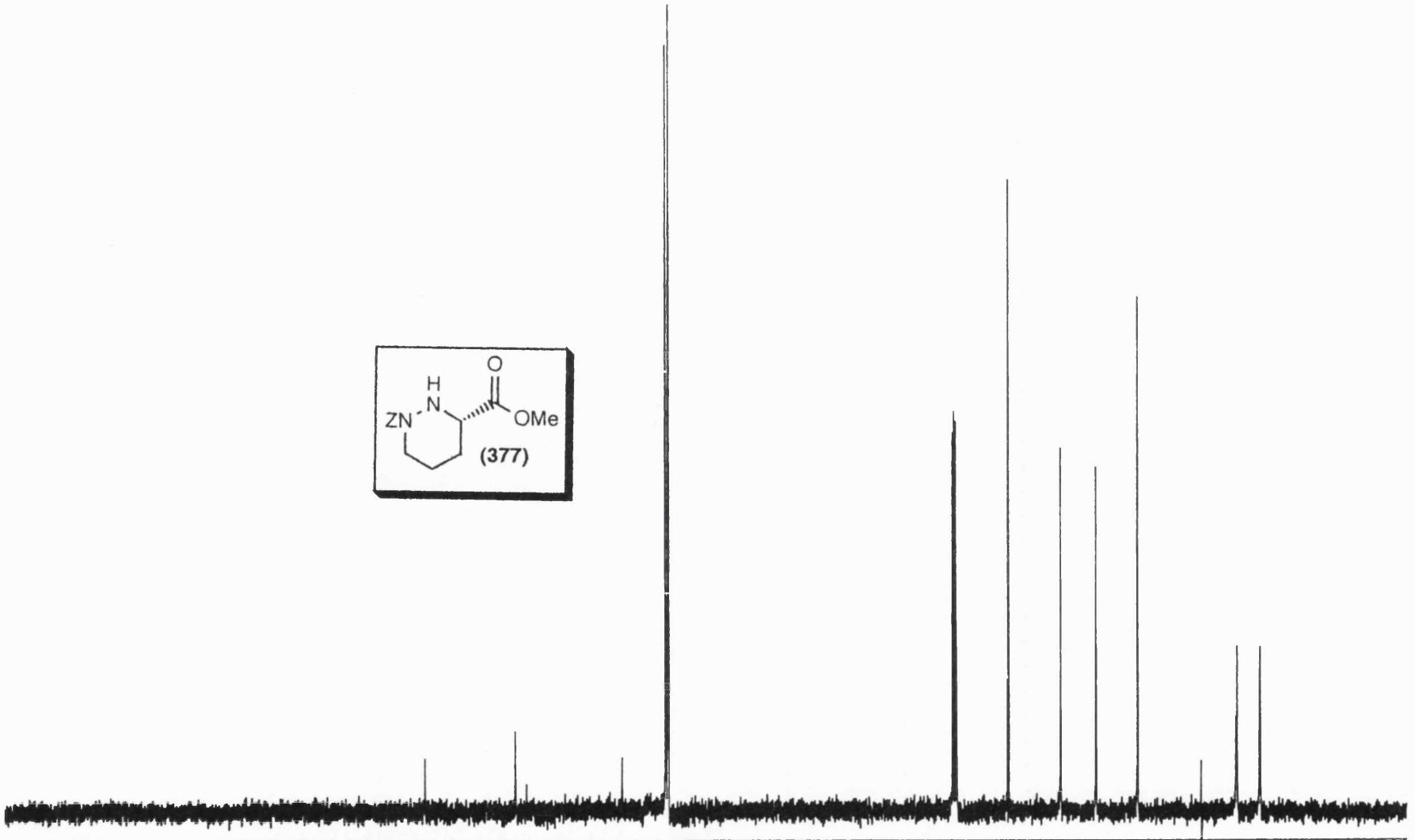

OBn
Me
N
NZ
N
O
COCF3
O
N - Me
Me
O
OH
(375)
220
200
180
160
140
120
100
80
60
40
20
0
PPM

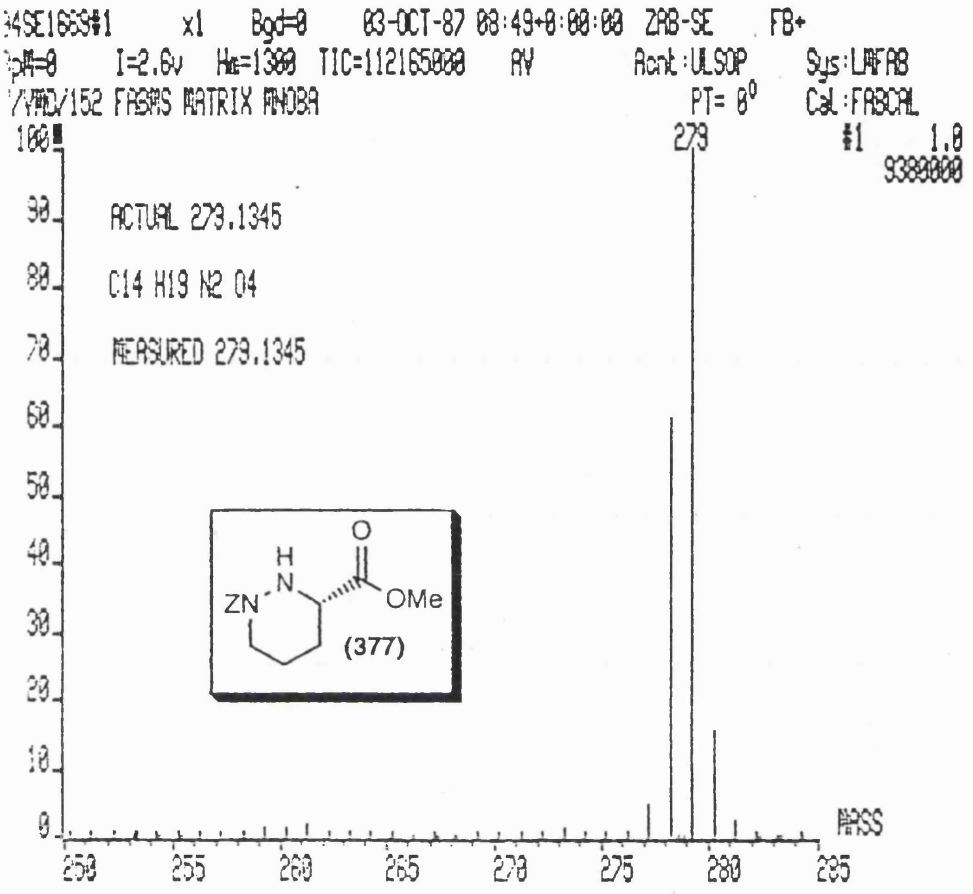

94SE11#1 x1 Bgd=0 10-JAN-94 10:47+0:00:00 ZAB-SE FB+
BpM=0 I=10v Ma=1500 TIC=3344480000 AV Acnt:ULSOP Sys:LMFAB
Y/YMD/28 FABMS THIOGLYCEROL:GLYCEROL:TFA MATRIX PT= 0° Cal:FABCAL
M:622.2243
C29 H33 N4 O3 F3 C:622.2250
622
#1 1.0
655140000
519
489
386
315
343
281
240
430
578
MASS
OBn
Me
N
NZ
O
COCF3
N - Me
Me
OH
(375)

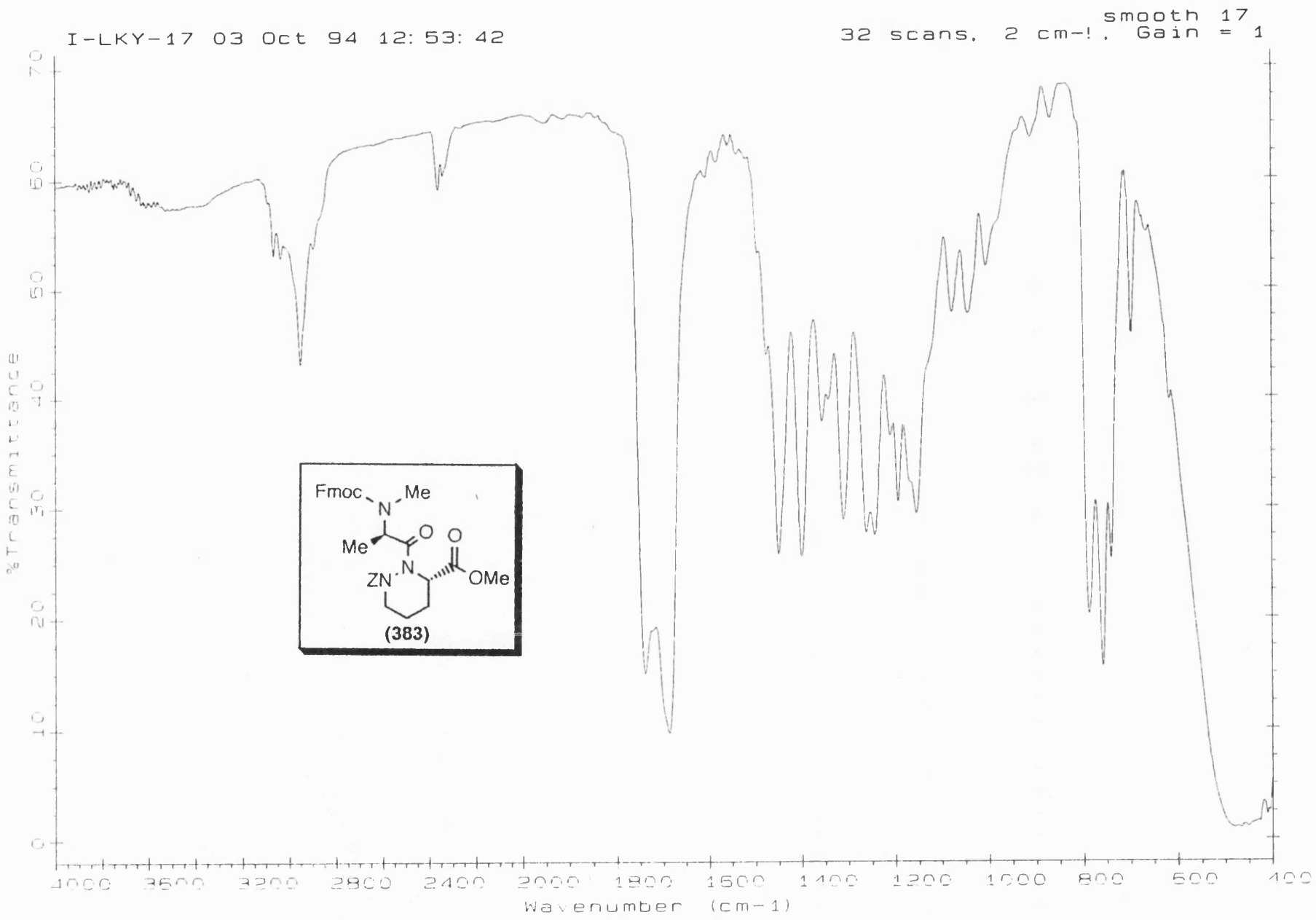

V/VMD/152 30 Sep 94 12: 48: 20
smooth 17
32 scans, 2 cm-!, Gain = 2
%Transmittance
70
60
50
40
30
20
10
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
H
N
O
ZN
OMe
(377)

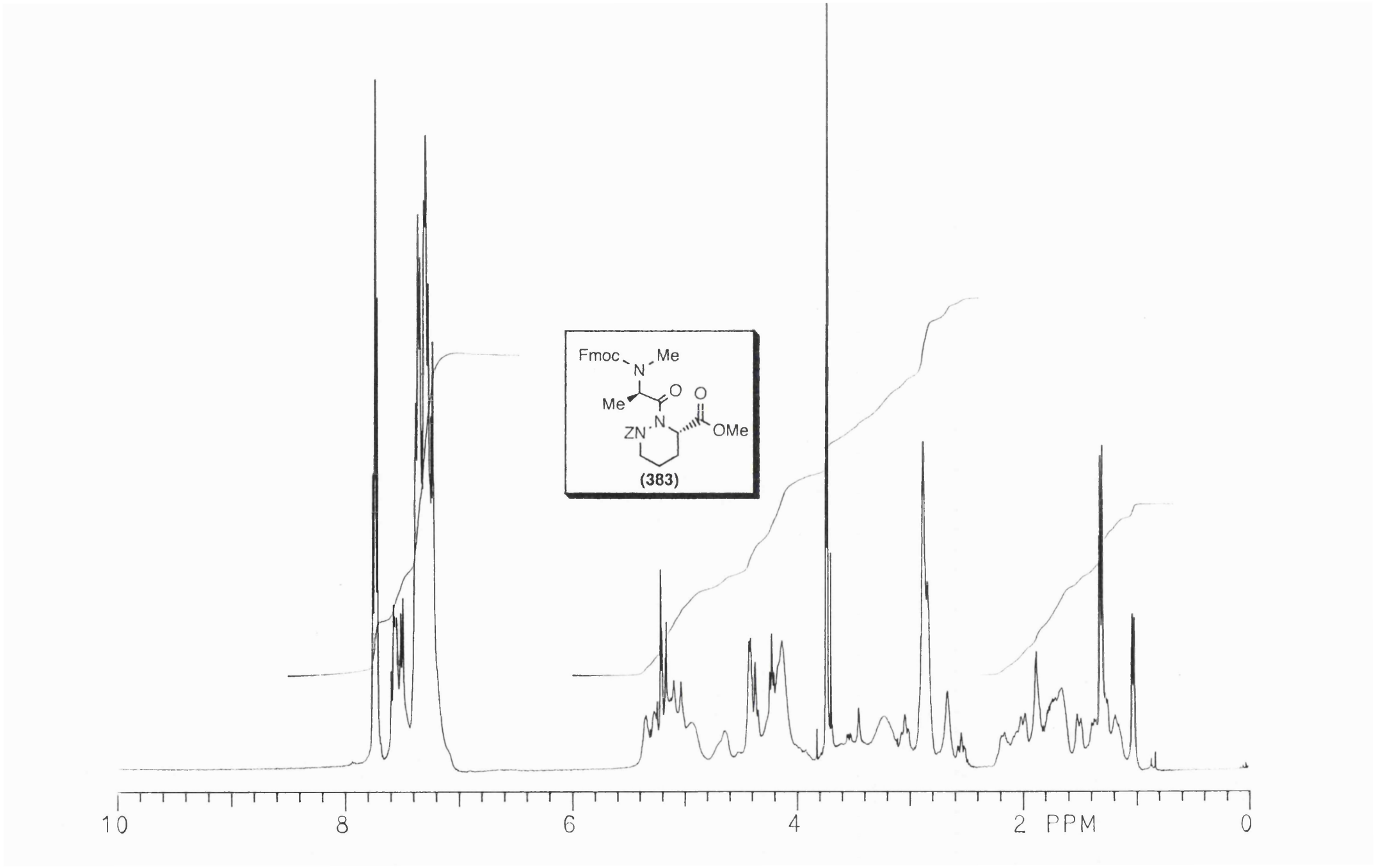

V/VMD/152
H
N
O
ZN
OMe
(377)
10
8
6
4
2 PPM
0

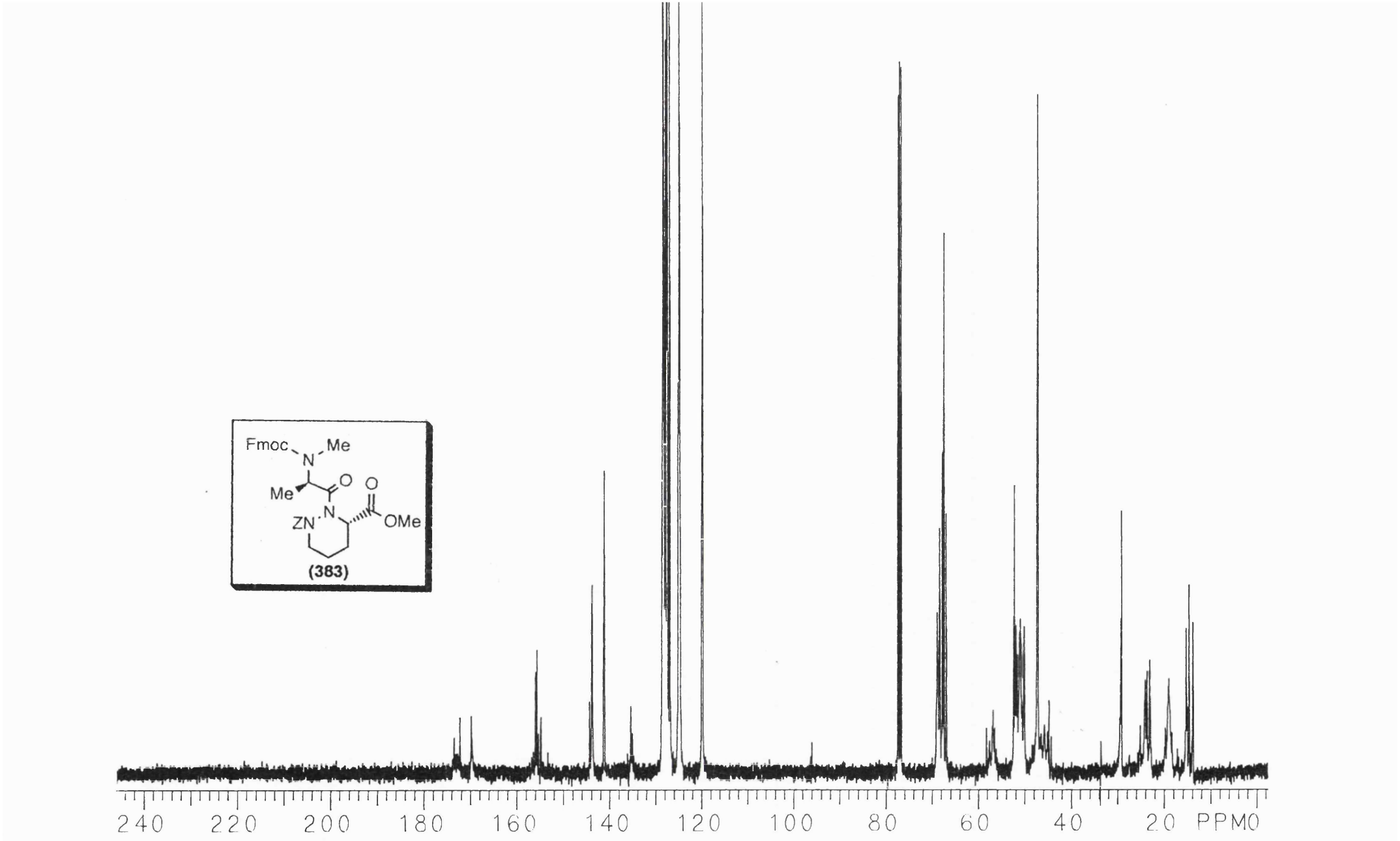
V/VMD/152

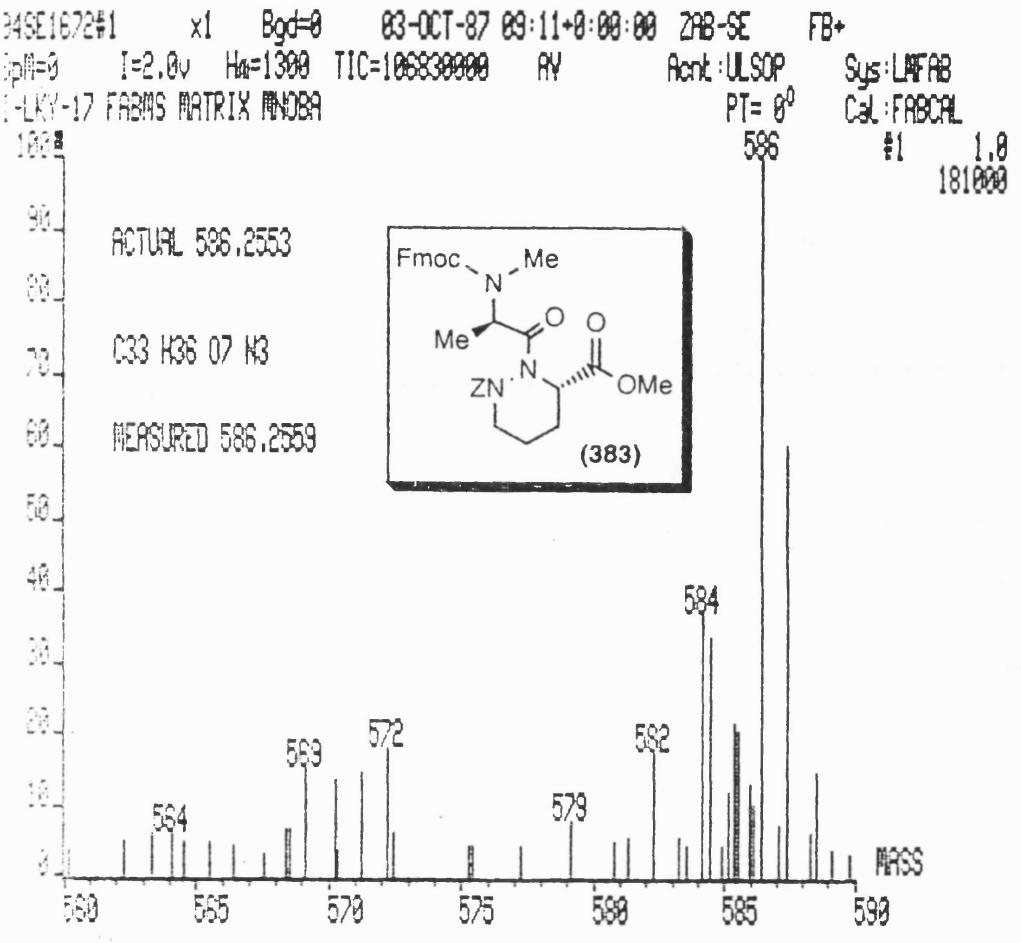


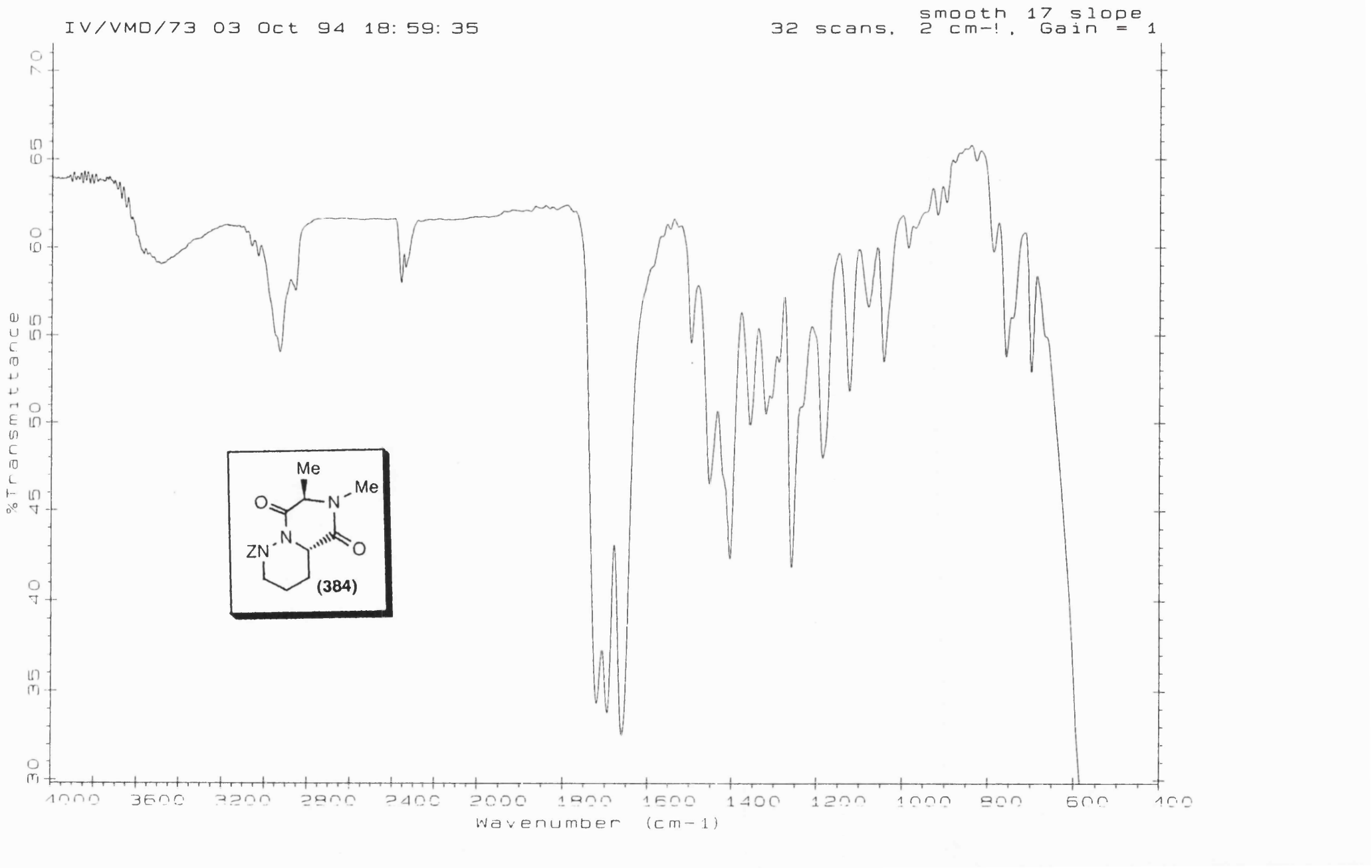

ACTUAL 279.1345
C14 H19 N2 O4
MEASURED 279.1345
279
#1 1.0
9380000
MASS
(377)

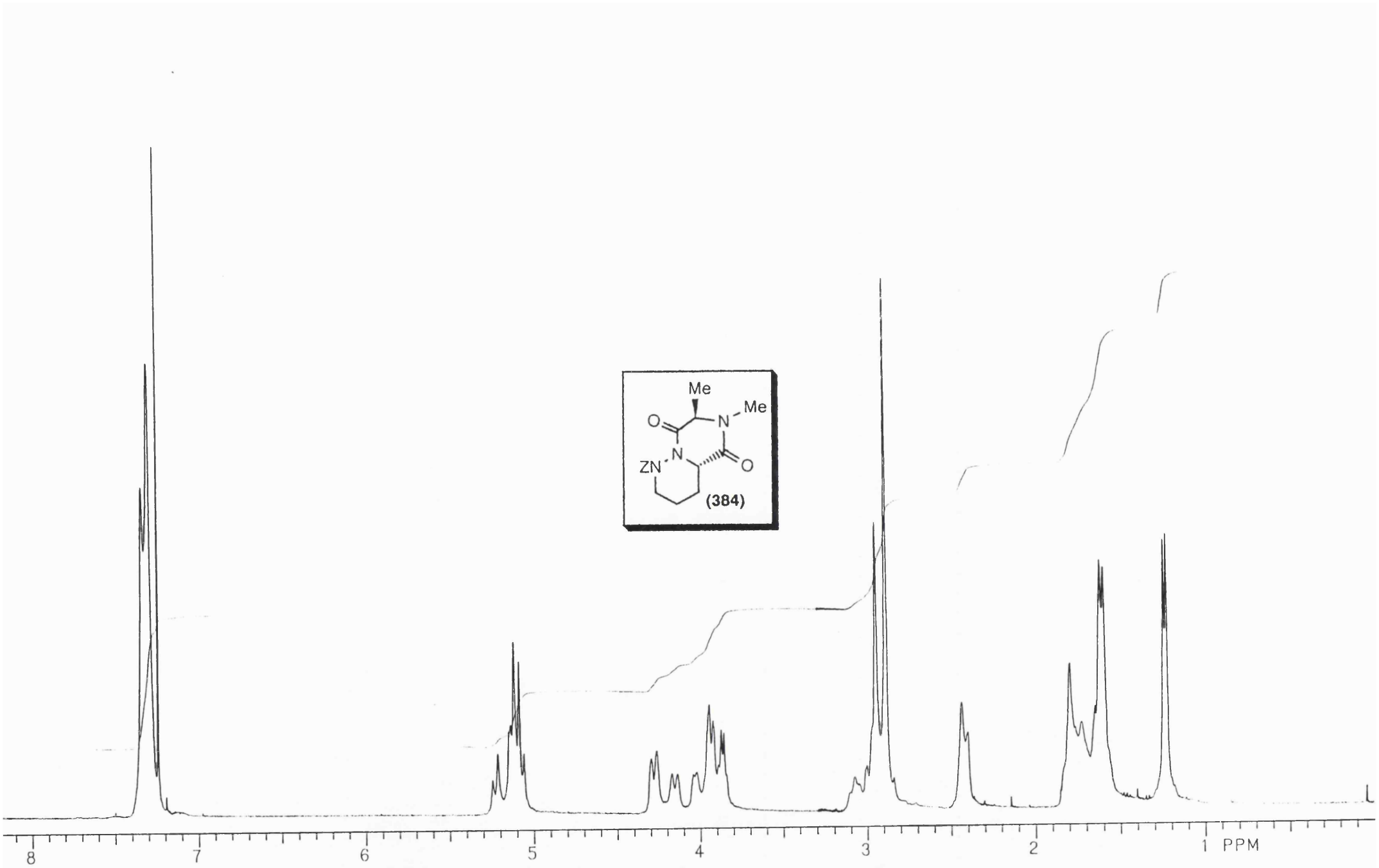

I-LKY-17 03 Oct 94 12:53:42
smooth 17
32 scans, 2 cm-!, Gain = 1
%Transmittance
0
10
20
30
40
50
60
70
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
Fmoc
N
Me
Me
O
O
ZN
N
OMe
(383)

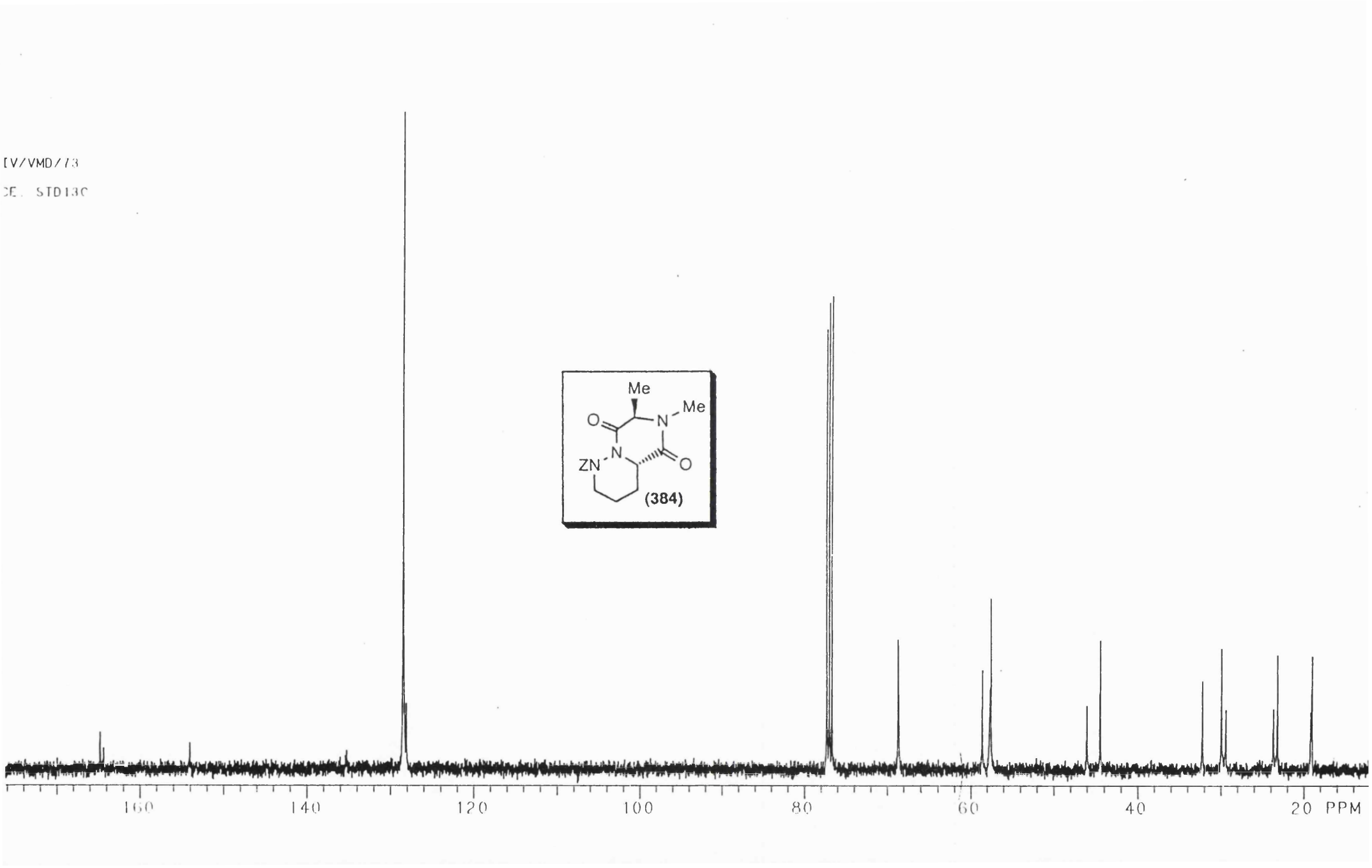

Fmoc
N
Me
Me
O
O
ZN
N
OMe
(383)
10
8
6
4
2 PPM
0

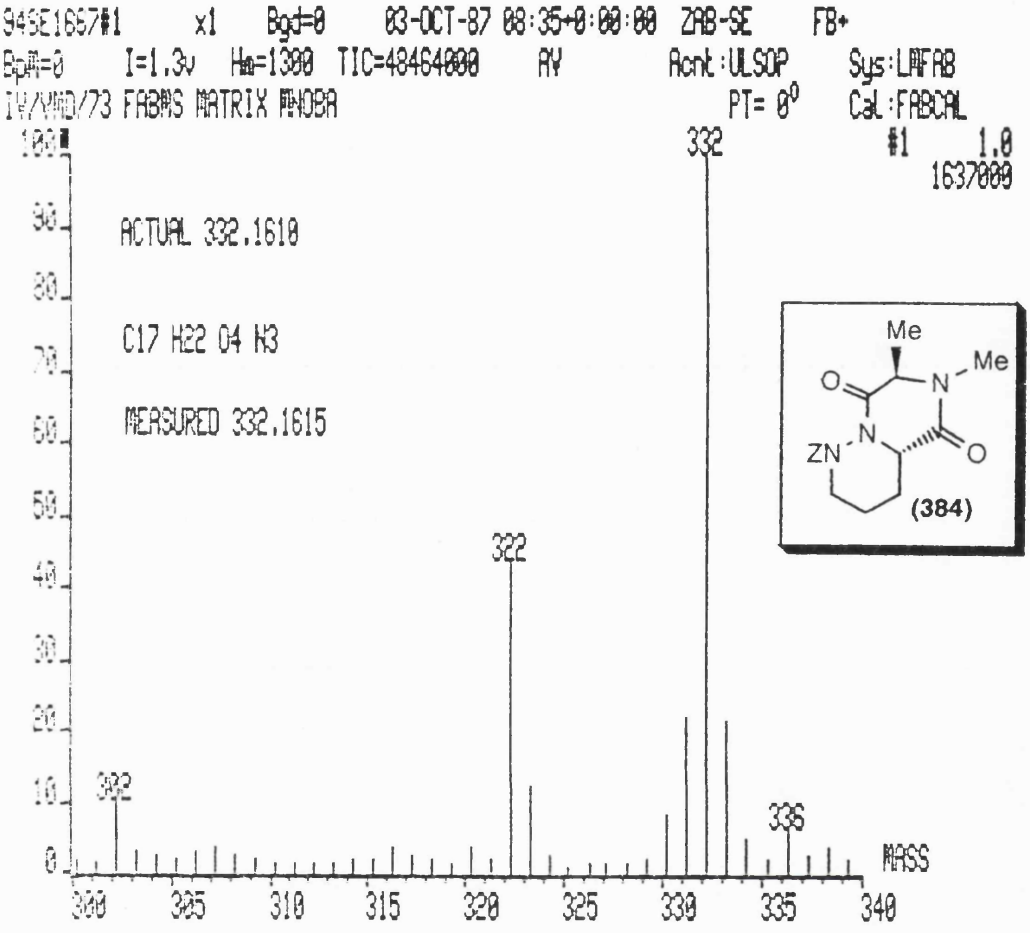

Fmoc
N
Me
Me
O
O
N
ZN
OMe
(383)
240
220
200
180
160
140
120
100
80
60
40
20
PPM0

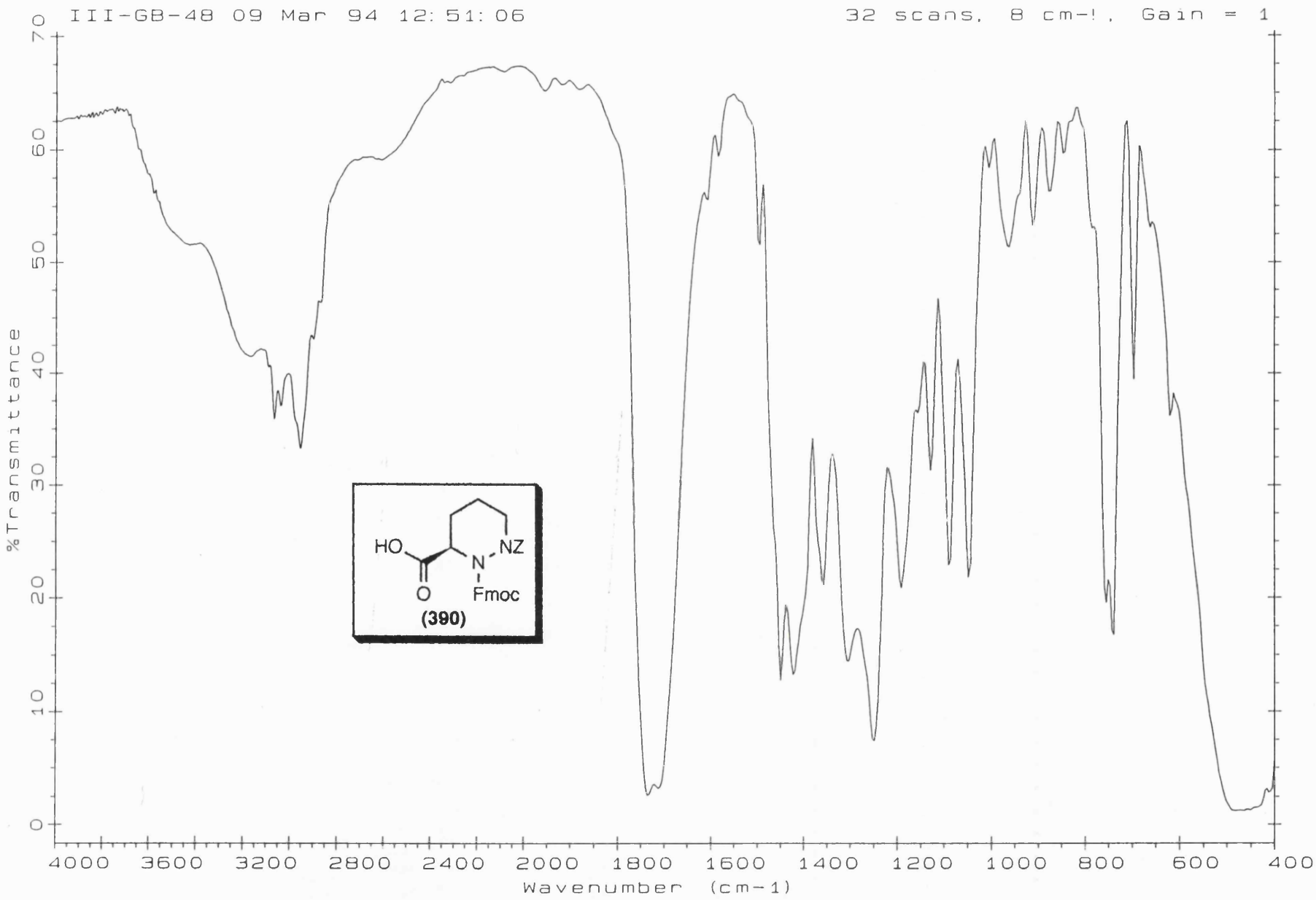

34SE1672#1 x1 Bgd=0 03-OCT-87 09:11+0:00:00 ZAB-SE FB+
Cp#=0 I=2.0v Hw=1300 TIC=106830000 AV Acnt:ULSOP Sys:LMFAB
-LKY-17 FABMS MATRIX MNOBA PT= 0° Cal:FABCAL
#1 1.0
181000
ACTUAL 586.2553
C33 H36 O7 N3
MEASURED 586.2559
Fmoc
N
Me
Me
O
O
ZN
N
OMe
(383)
586
584
572
569
582
564
579
MASS
560
565
570
575
580
585
590

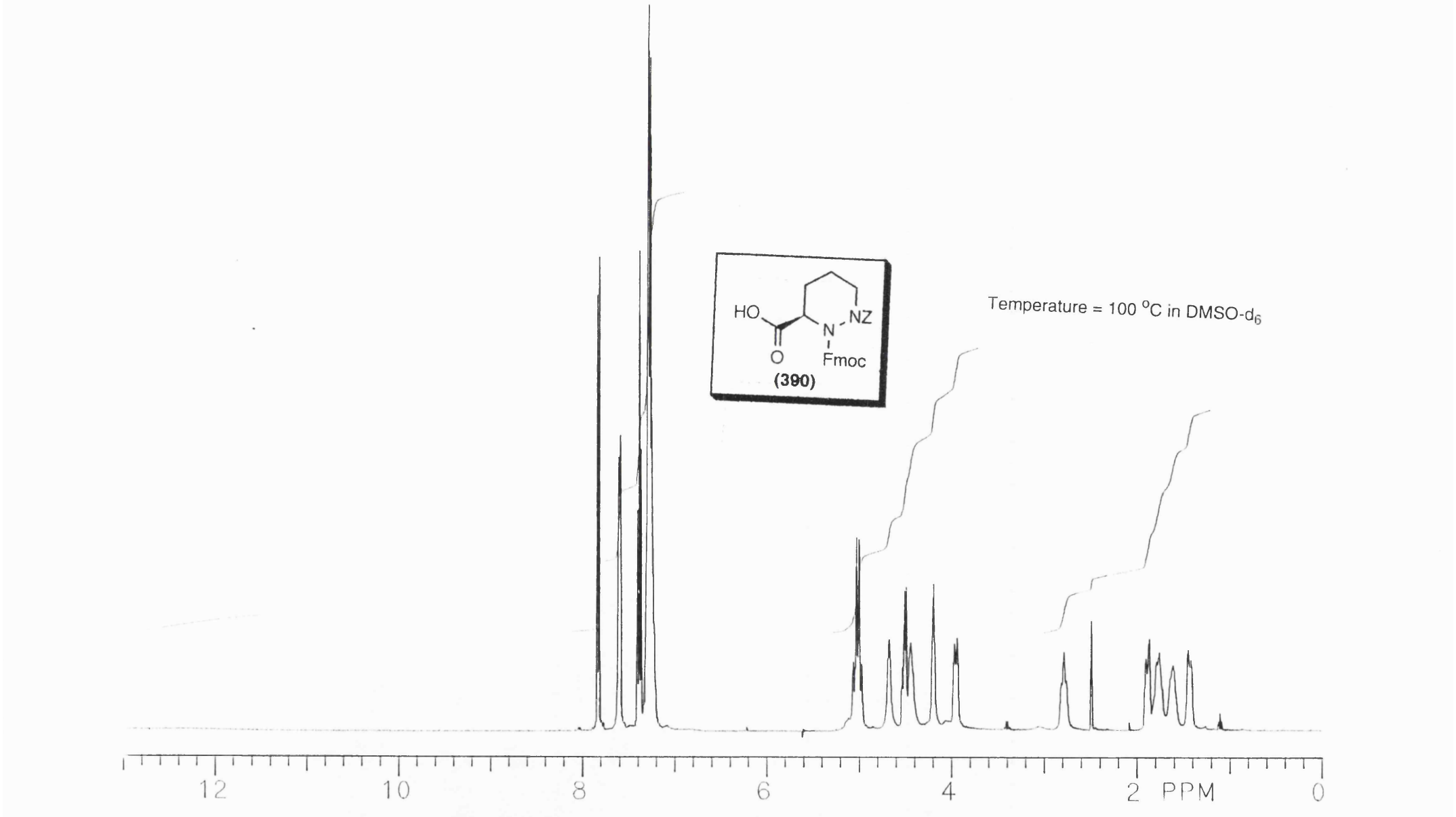

IV/VMD/73 03 Oct 94 18:59:35
smooth 17 slope
32 scans, 2 cm-!, Gain = 1
%Transmittance
70
65
60
55
50
45
40
35
30
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
Me
Me
O
N
N
ZN
O
(384)

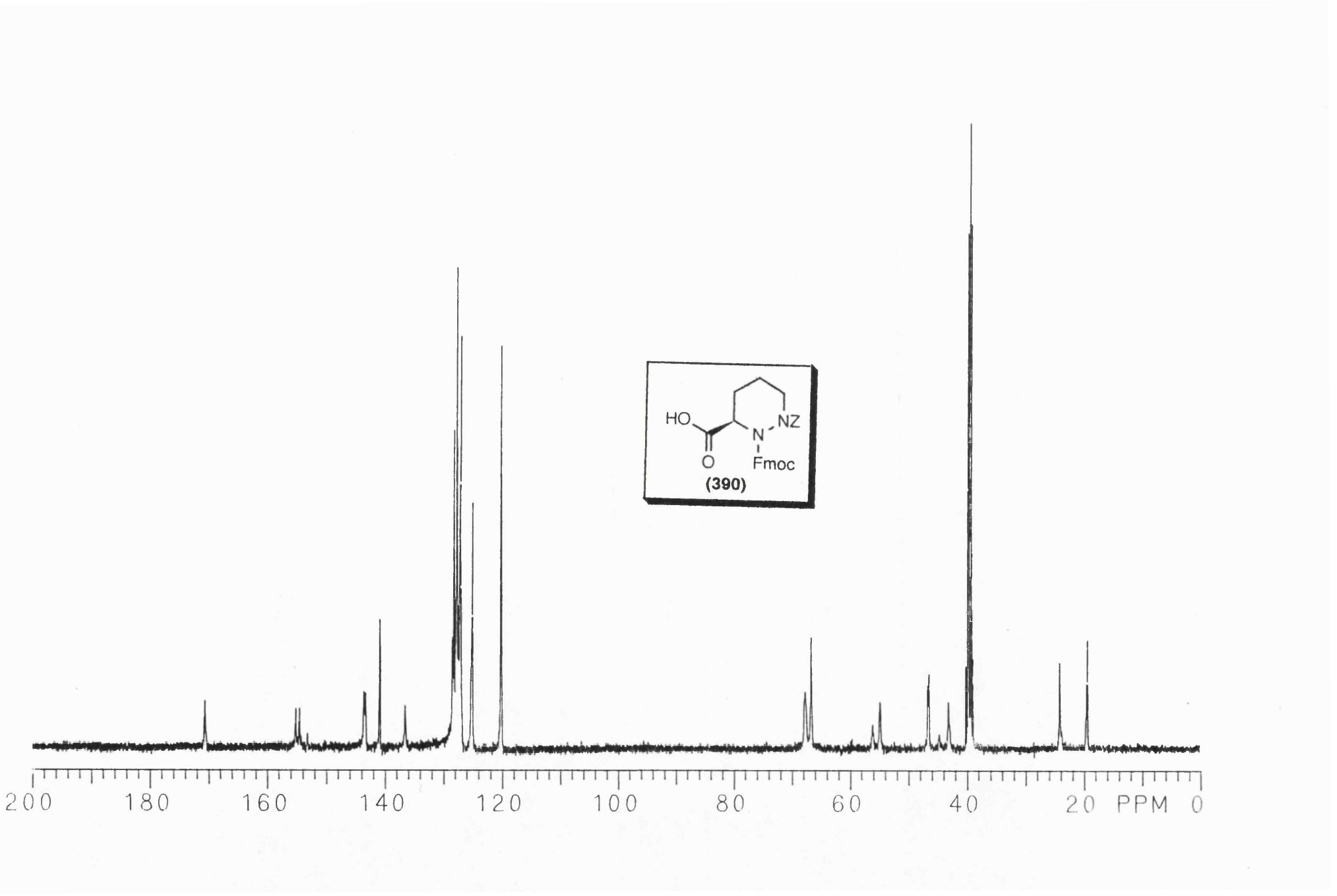

Me
Me
O
N
ZN
N
O
(384)
8
7
6
5
4
3
2
1 PPM

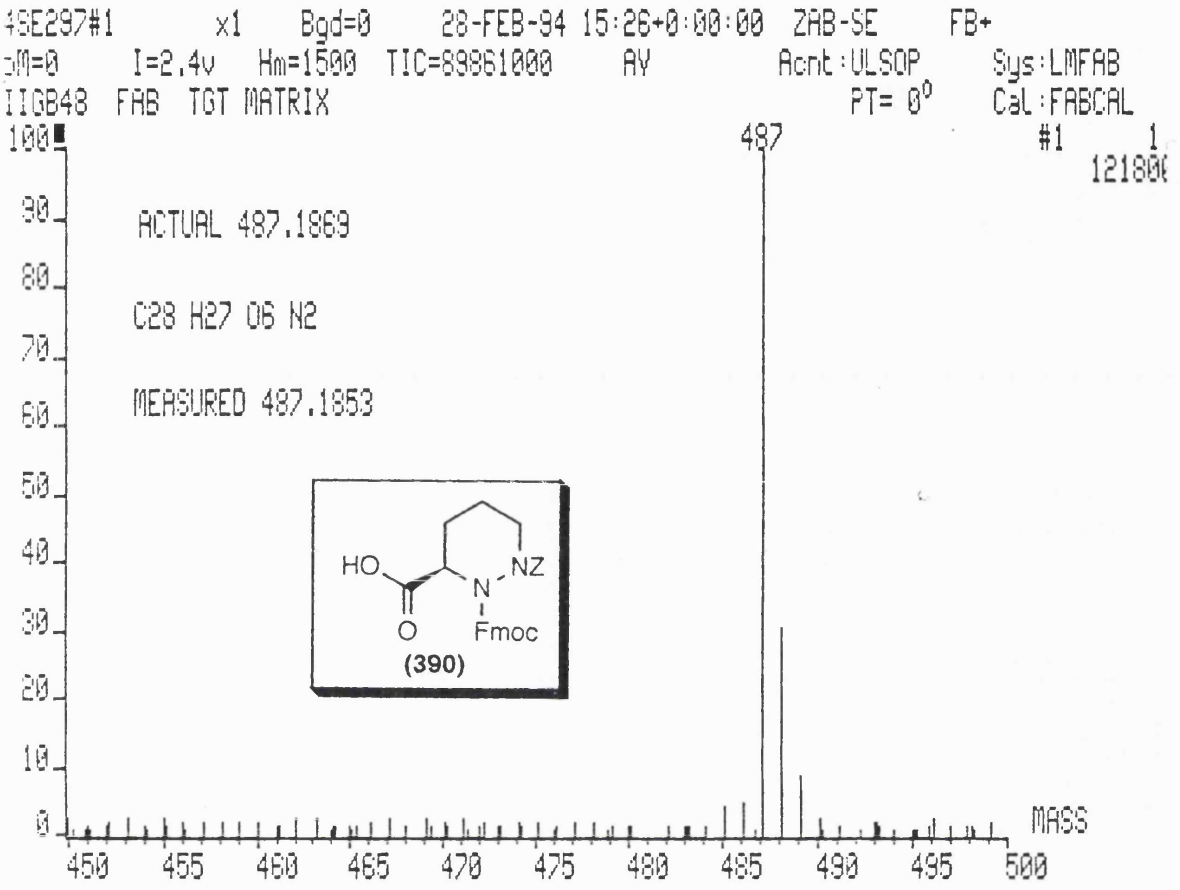

[V/VMD/73
CE. STD13C
Me
Me
N
O
N
ZN
O
(384)
160
140
120
100
80
60
40
20 PPM

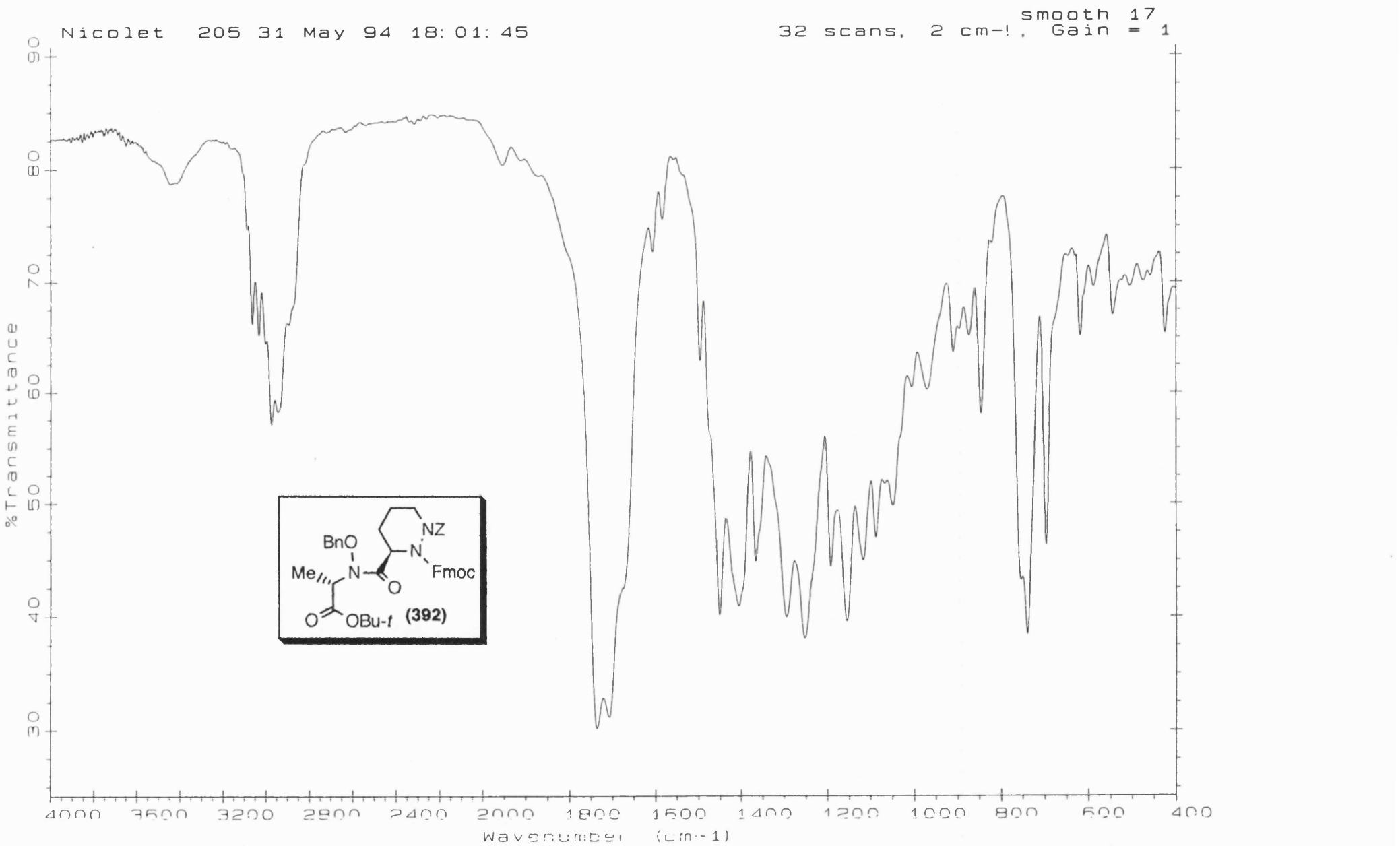

946E1657#1 x1 Bgd=0 03-OCT-87 08:35+0:00:00 ZAB-SE FB+
BpM=0 I=1.3v Hm=1300 TIC=48464000 AV Acmt:ULSOP Sys:LMFAB
IV/VMD/73 FABMS MATRIX MNOBA PT= 0° CaL:FABCAL
#1 1.0
1637000
ACTURAL 332.1610
C17 H22 O4 N3
MERSURED 332.1615
332
322
302
336
MASS
100
90
80
70
60
50
40
30
20
10
0
300
305
310
315
320
325
330
335
340
Me
Me
N
O
ZN
N
O
(384)

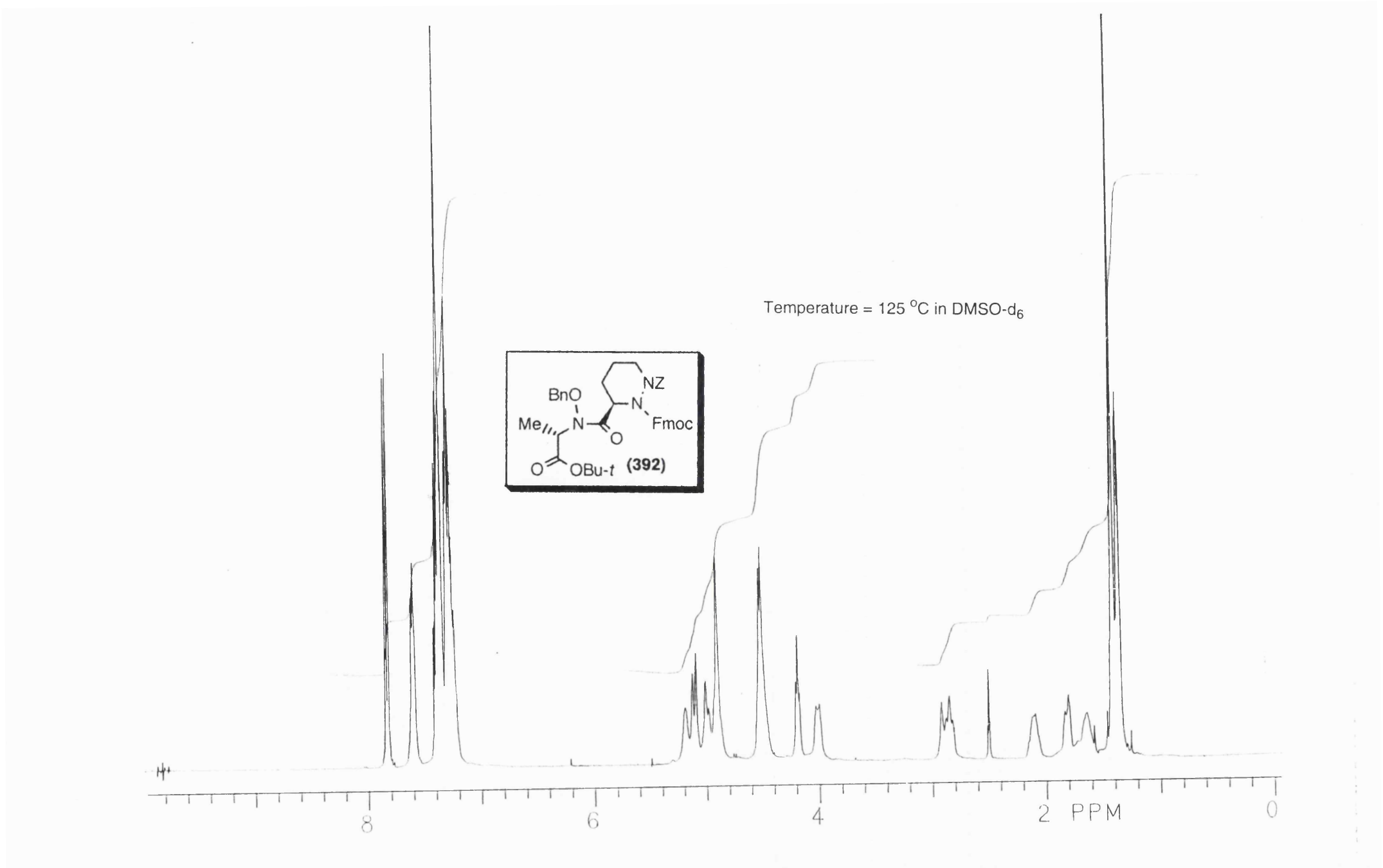

III-GB-48 09 Mar 94 12:51:06
32 scans, 8 cm-1, Gain = 1
%Transmittance
70
60
50
40
30
20
10
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
HO
NZ
N
O
Fmoc
(390)

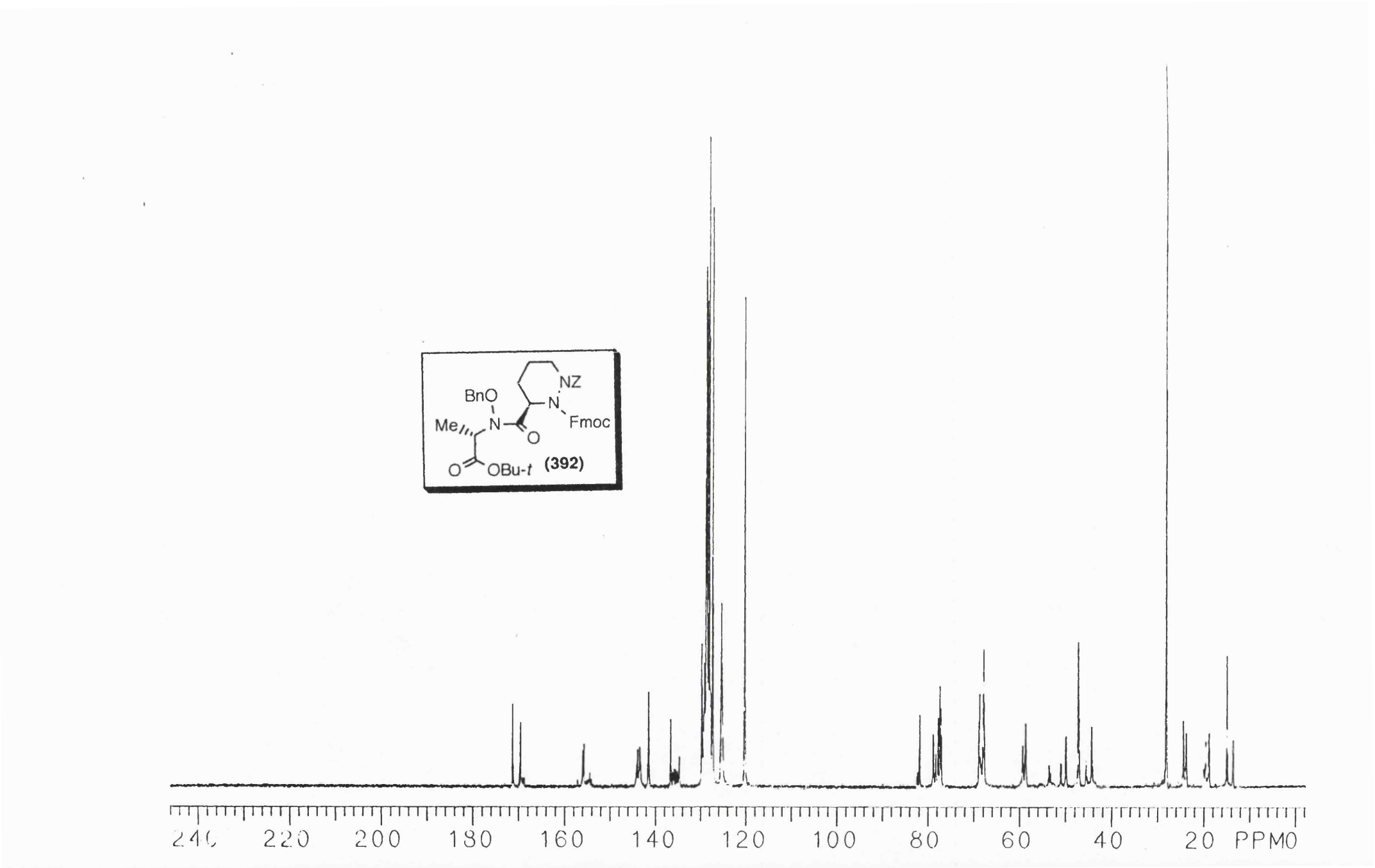

HO
NZ
N
O
Fmoc
(390)
Temperature = 100 °C in DMSO-d6
12
10
8
6
4
2 PPM
0

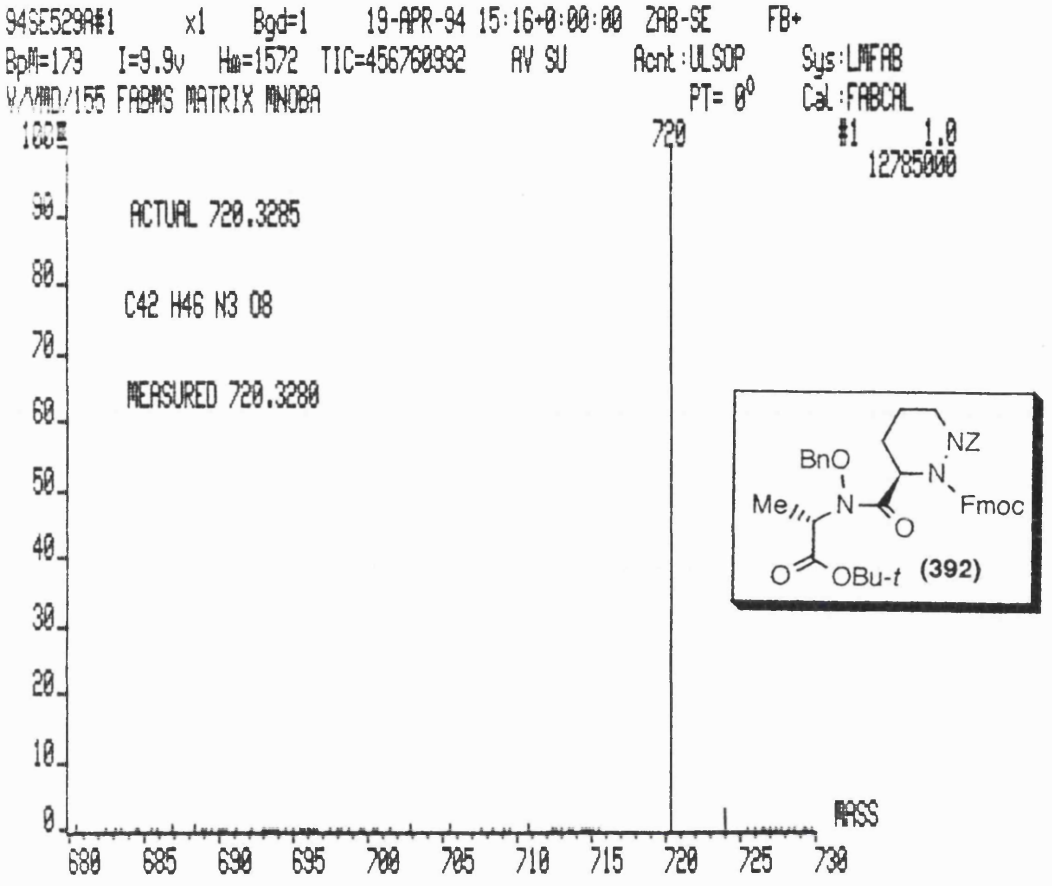

HO
O
N
NZ
Fmoc
(390)
200
180
160
140
120
100
80
60
40
20
PPM
0

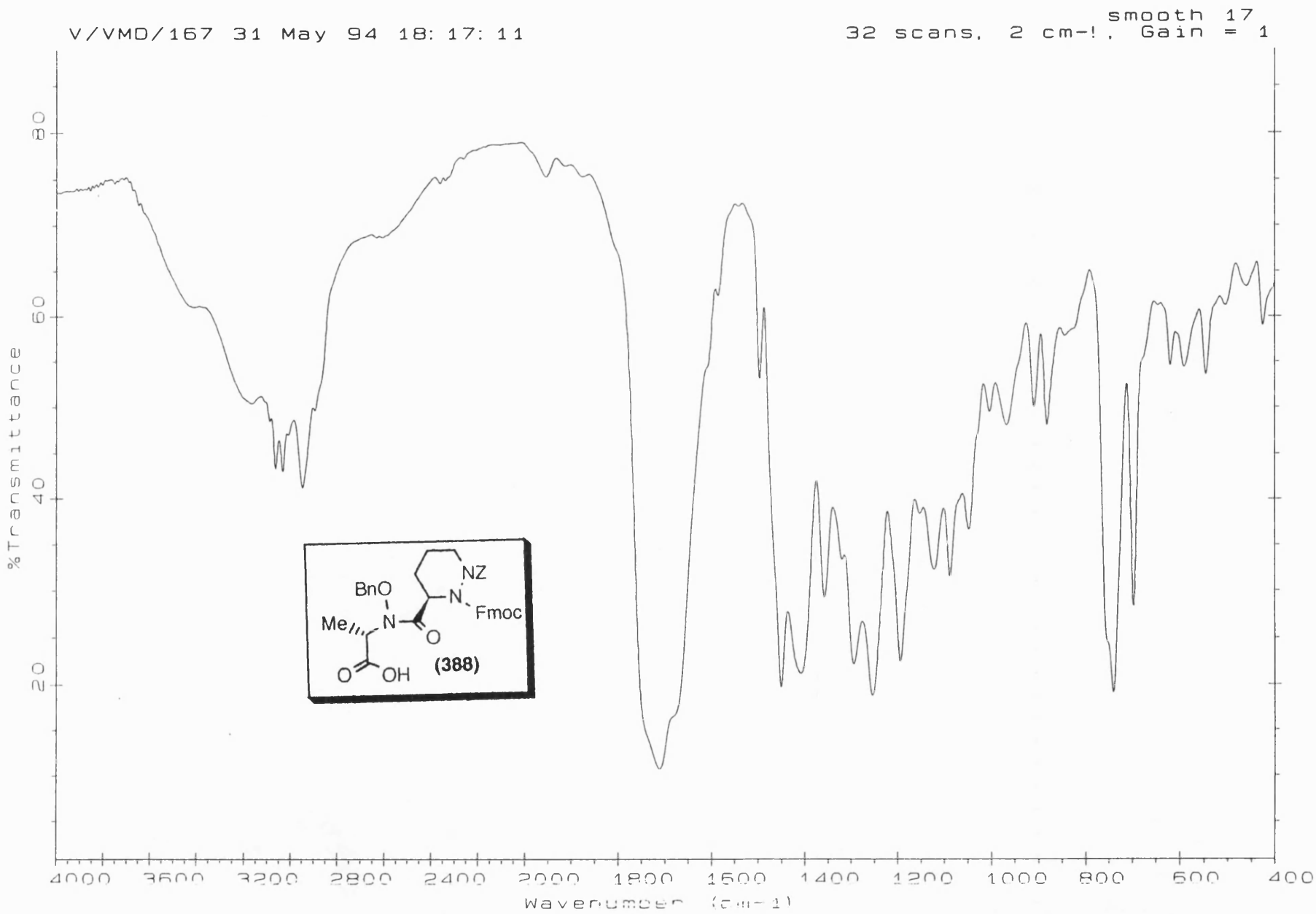

4SE297#1 x1 Bgd=0 28-FEB-94 15:26+0:00:00 ZAB-SE FB+
cM=0 I=2.4v Hm=1500 TIC=8986000 AV Acnt:ULSOP Sys:LMFAB
IIGB48 FAB TGT MATRIX PT= 0° Cal:FABCAL
487
#1
ACTUAL 487.1869
C28 H27 O6 N2
MEASURED 487.1853
HO
NZ
N
O
Fmoc
(390)
MASS
100
90
80
70
60
50
40
30
20
10
0
450
455
460
465
470
475
480
485
490
495
500

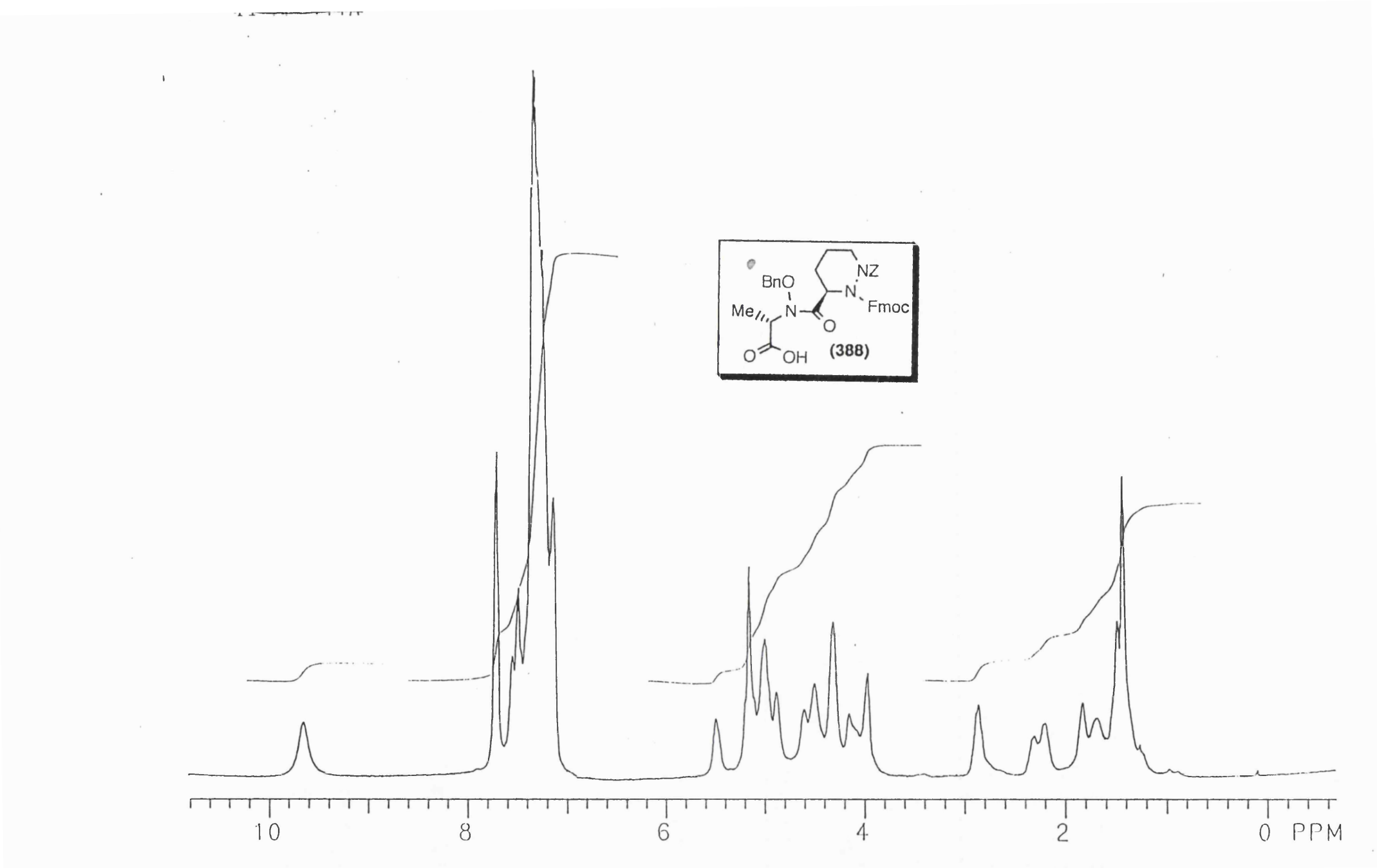

Nicolet 205 31 May 94 18:01:45
smooth 17
32 scans, 2 cm-1, Gain = 1
%Transmittance
90
80
70
60
50
40
30
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
BnO
N
Me
N
NZ
Fmoc
O
O
OBu-t
(392)


Temperature = 125 °C in DMSO-d6
BnO
NZ
N
Me
N
Fmoc
O
O
OBu-t
(392)
8
6
4
2 PPM
0

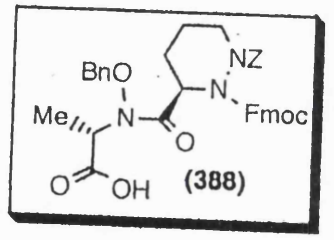

BnO
N
NZ
N
Me
Fmoc
O
O
OBu-t
(392)
240
220
200
180
160
140
120
100
80
60
40
20
PPM0


94SE529R#1 x1 Bgd=1 19-APR-94 15:16+0:00:00 ZAB-SE FB+
BpM=179 I=9.9v Hm=1572 TIC=456760992 AV SU Acnt:ULSOP Sys:LMFAB
V/AMD/155 FABMS MATRIX MNOBA PT= 0° CaL:FABCAL
720 #1 1.0
12785000
ACTUAL 720.3285
C42 H46 N3 O8
MEASURED 720.3280
MASS
680 685 690 695 700 705 710 715 720 725 730
BnO
NZ
N
Me
N
Fmoc
O
O
OBu-t
(392)


V/VMD/167 31 May 94 18: 17: 11
smooth 17
32 scans, 2 cm-!, Gain = 1
%Transmittance
80
60
40
20
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
BnO
N
Me
O
OH
NZ
N
Fmoc
O
(388)

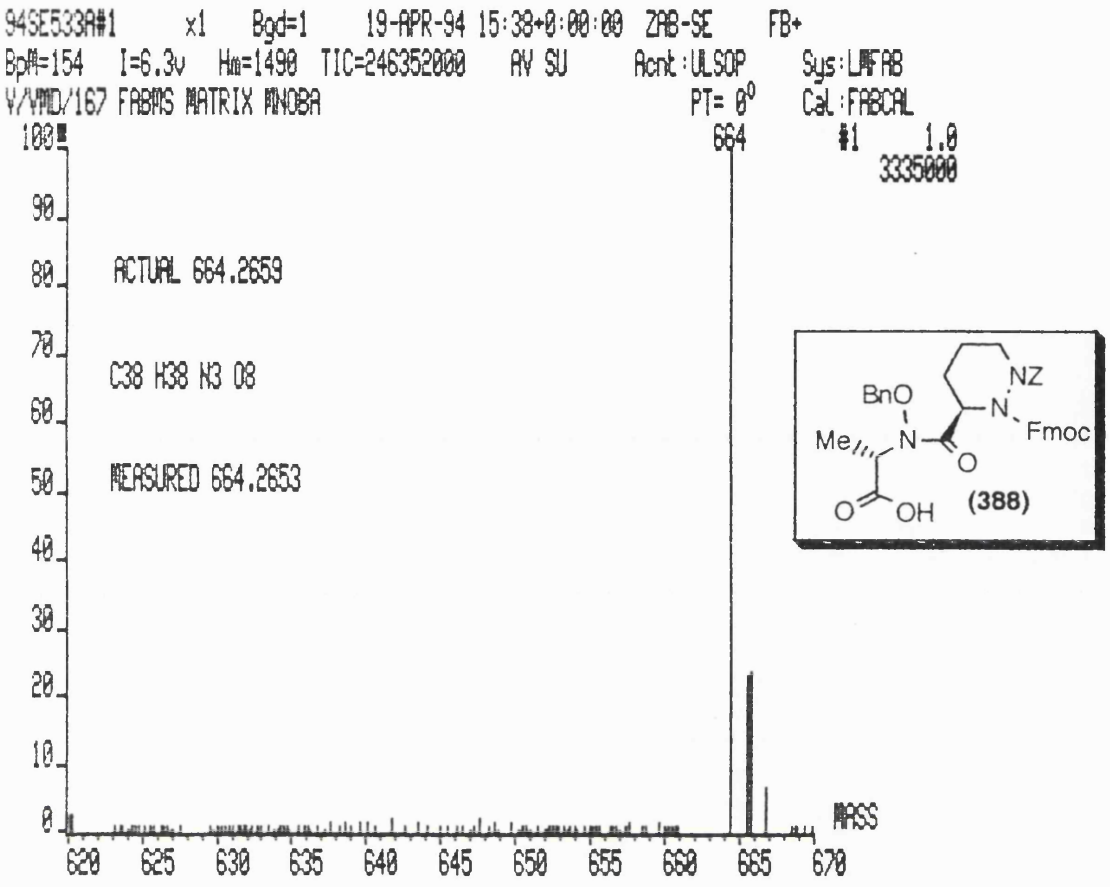

BnO
N
Me
N
O
O
OH
NZ
N
Fmoc
(388)
10
8
6
4
2
0 PPM

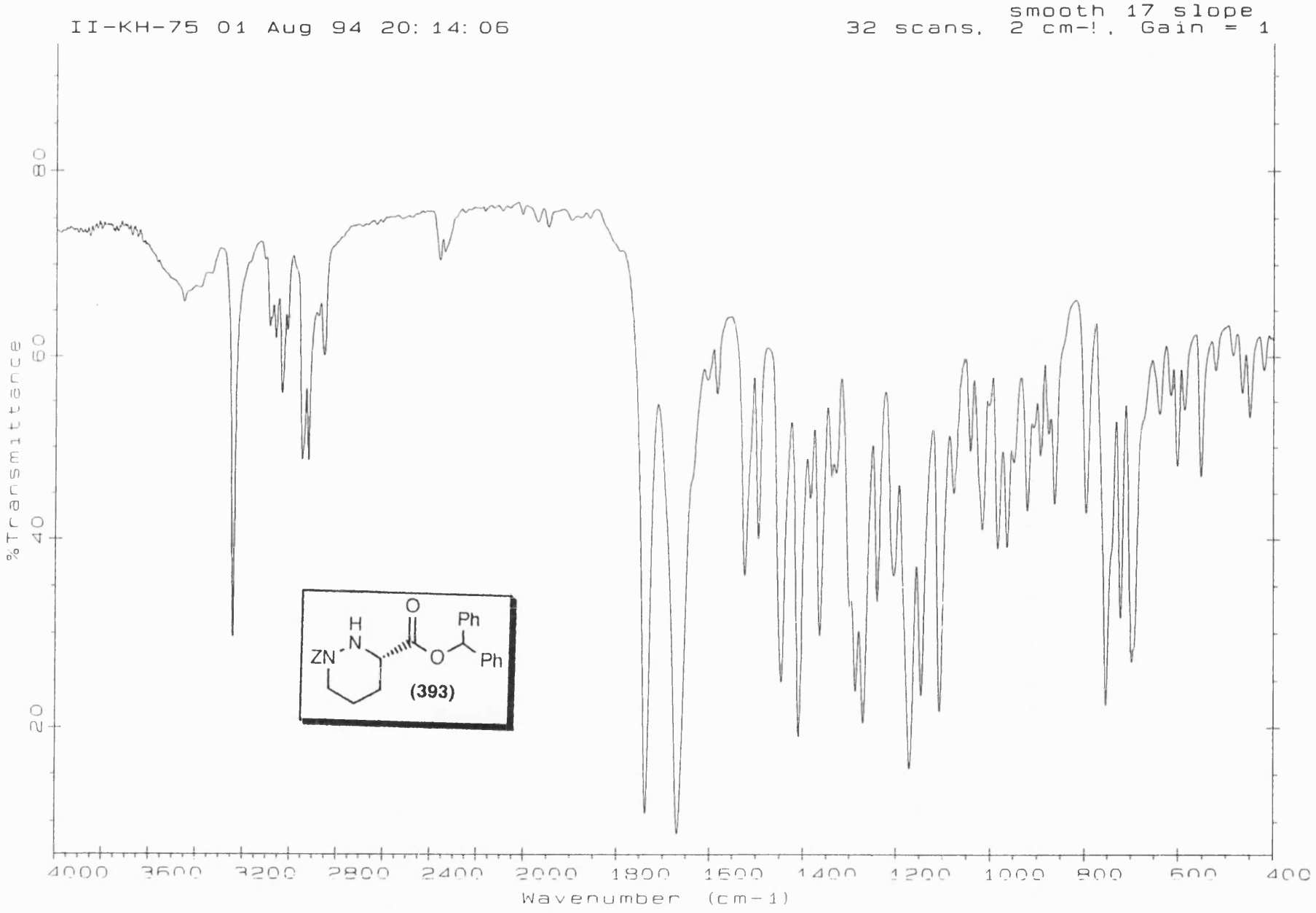
V/VMD/167

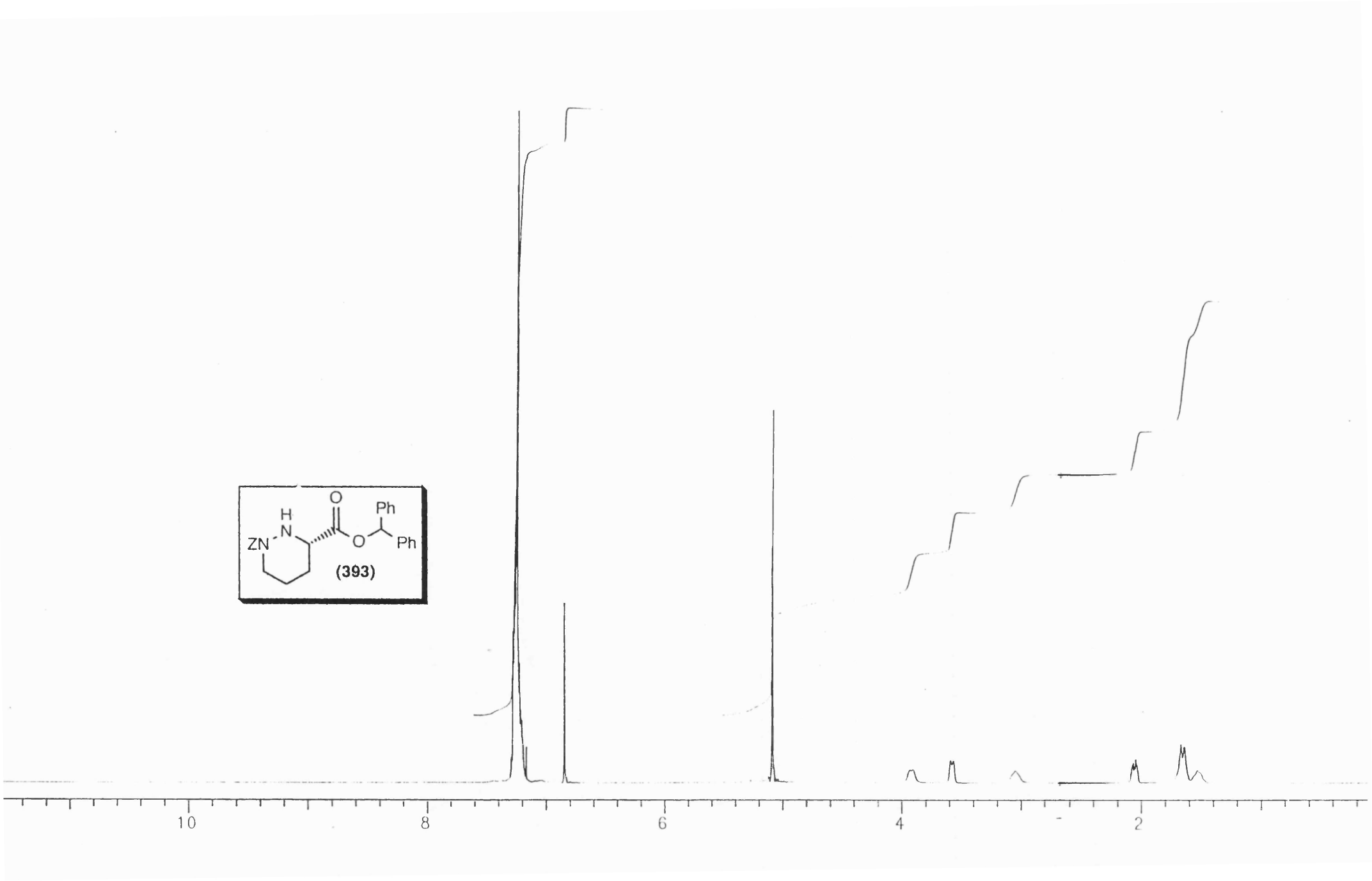


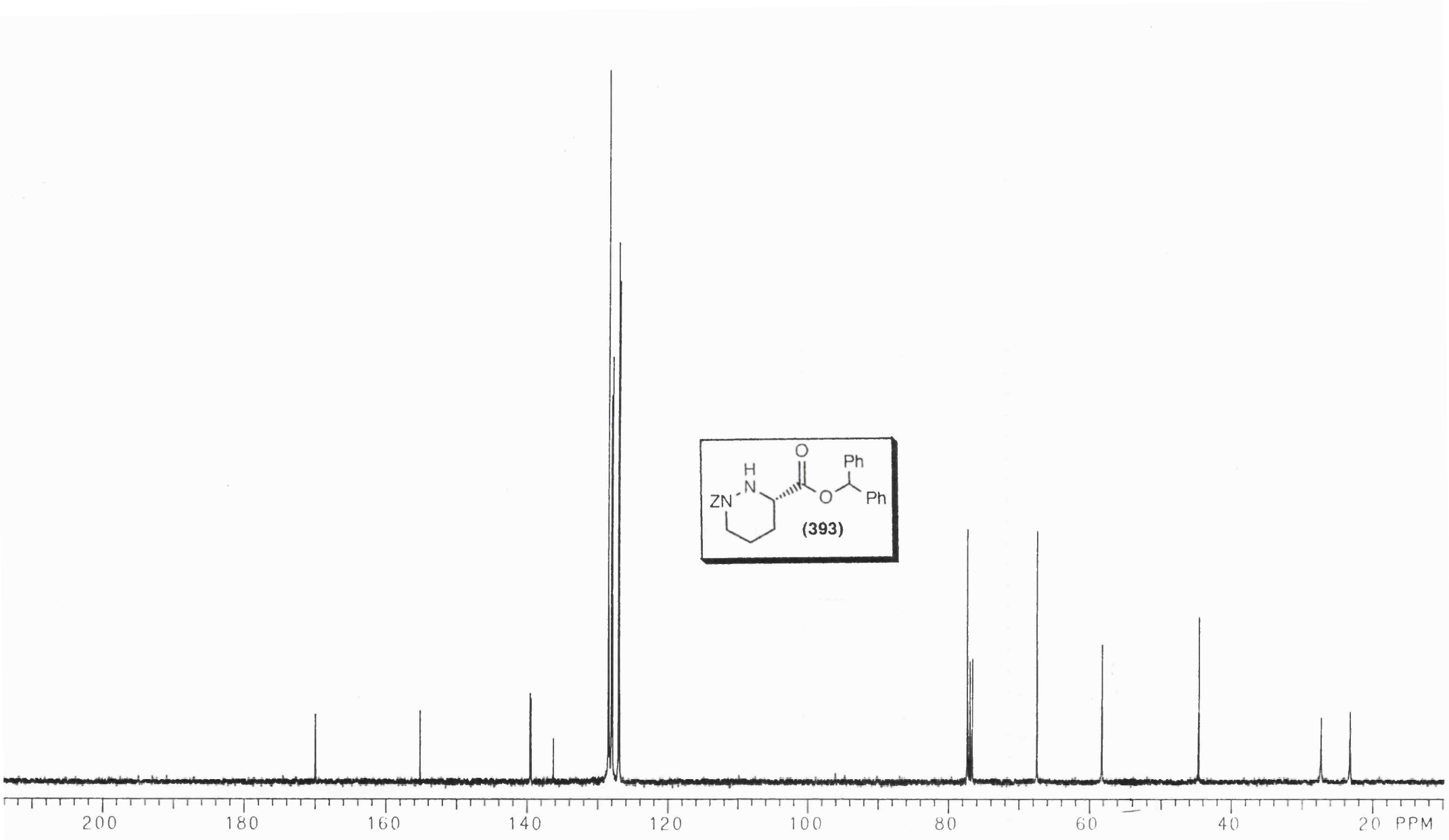
(388)

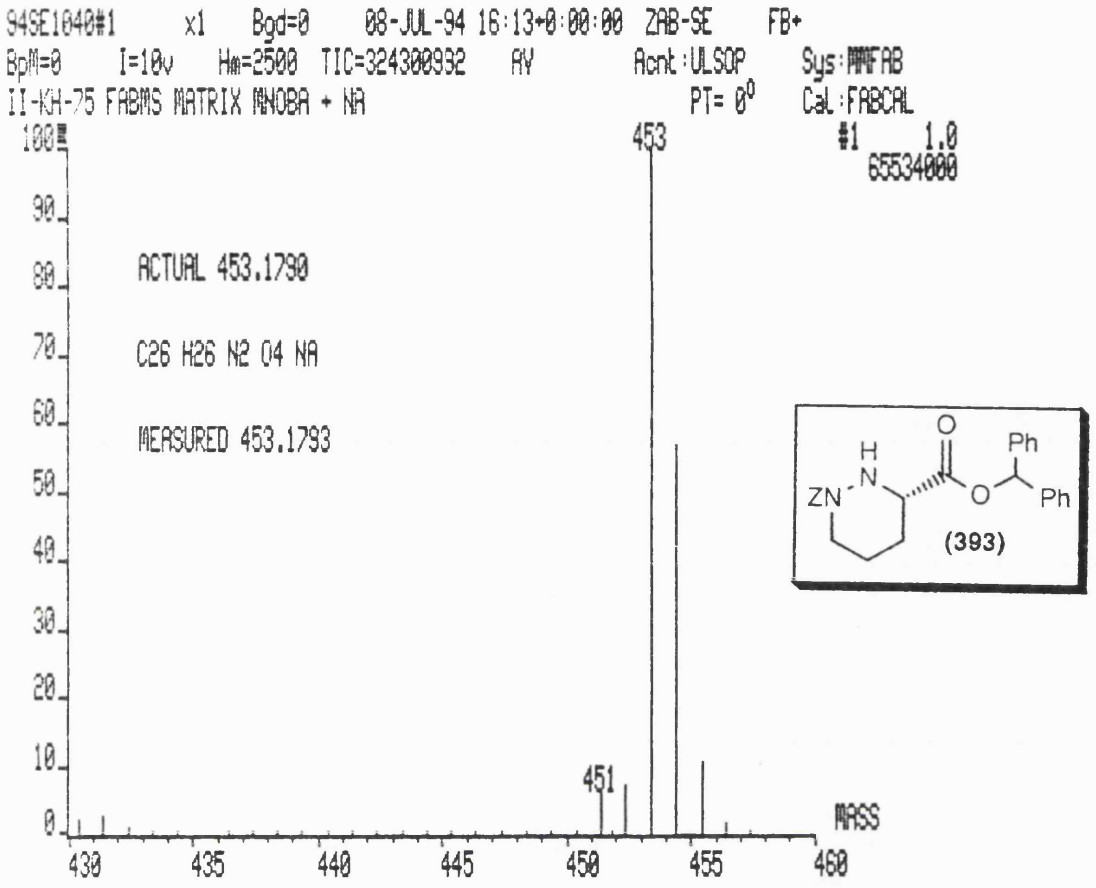
250 200 150 100 50 0 PPM

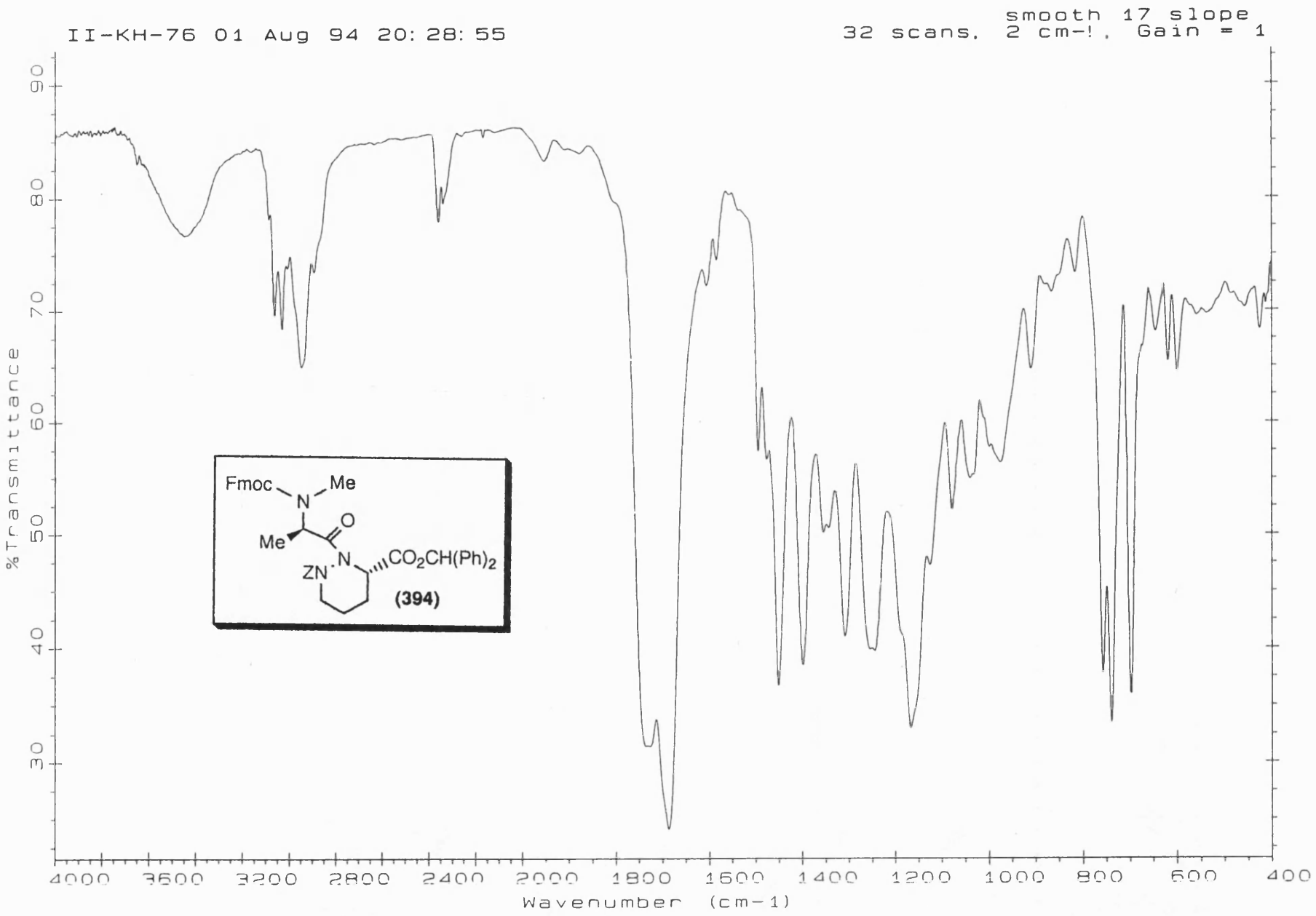

94SE533A#1 x1 Bgd=1 19-APR-94 15:38+0:00:00 ZAB-SE FB+
Bp#=154 I=6.3v Hm=1490 TIC=246352000 AV SU Acnt:ULSOP Sys:LMFAB
V/VMD/167 FABMS MATRIX MNOBA PT= 0° Cal:FABCAL
664 #1 1.0
3335000
ACTUAL 664.2659
C38 H38 N3 O8
MEASURED 664.2653
MASS
100
90
80
70
60
50
40
30
20
10
0
620 625 630 635 640 645 650 655 660 665 670
BnO
NZ
N
Fmoc
Me
N
O
O
OH
(388)

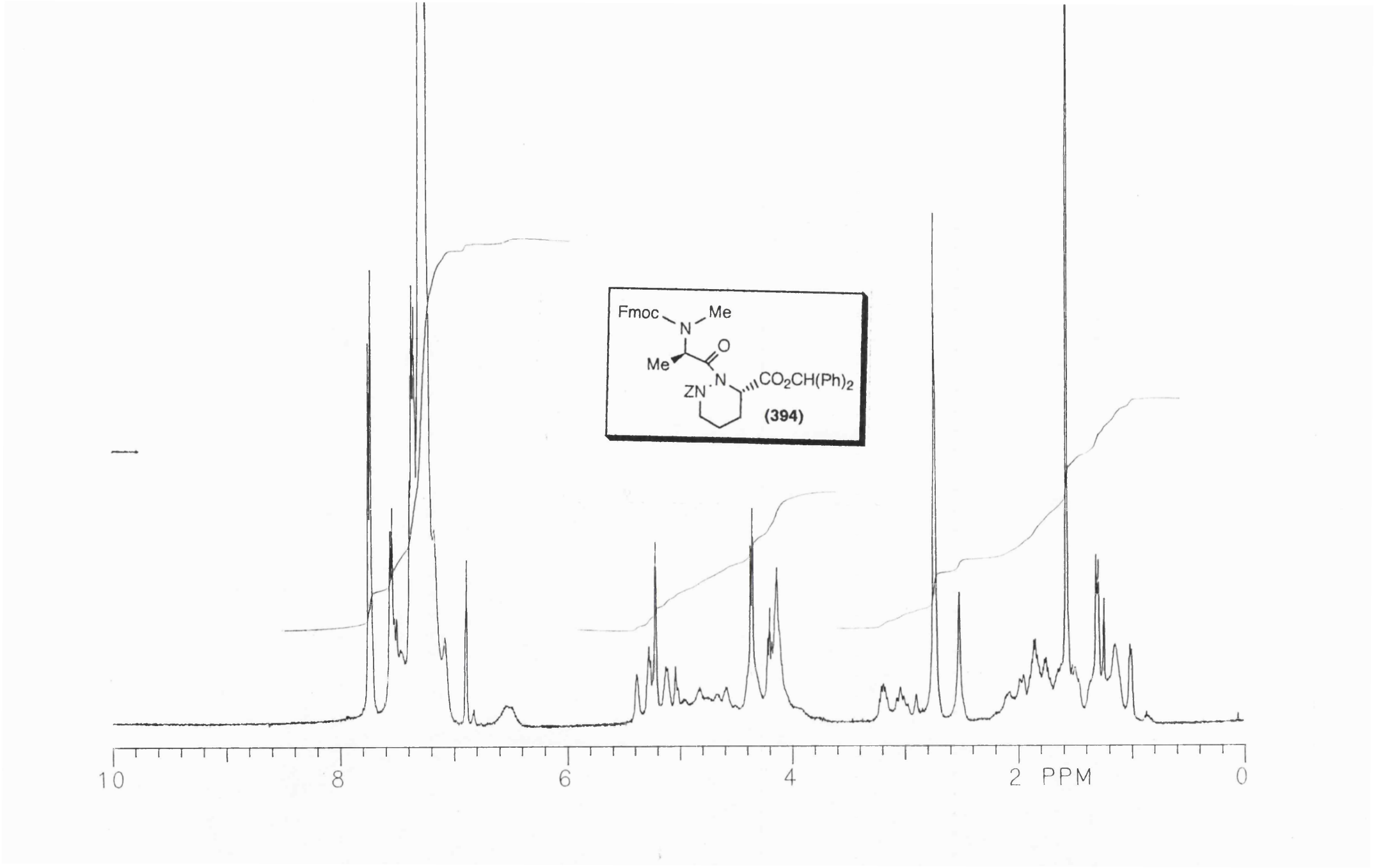

II-KH-75 01 Aug 94 20: 14: 06
smooth 17 slope
32 scans, 2 cm-!, Gain = 1
%Transmittance
80
60
40
20
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
ZN
H
N
O
O
Ph
Ph
(393)

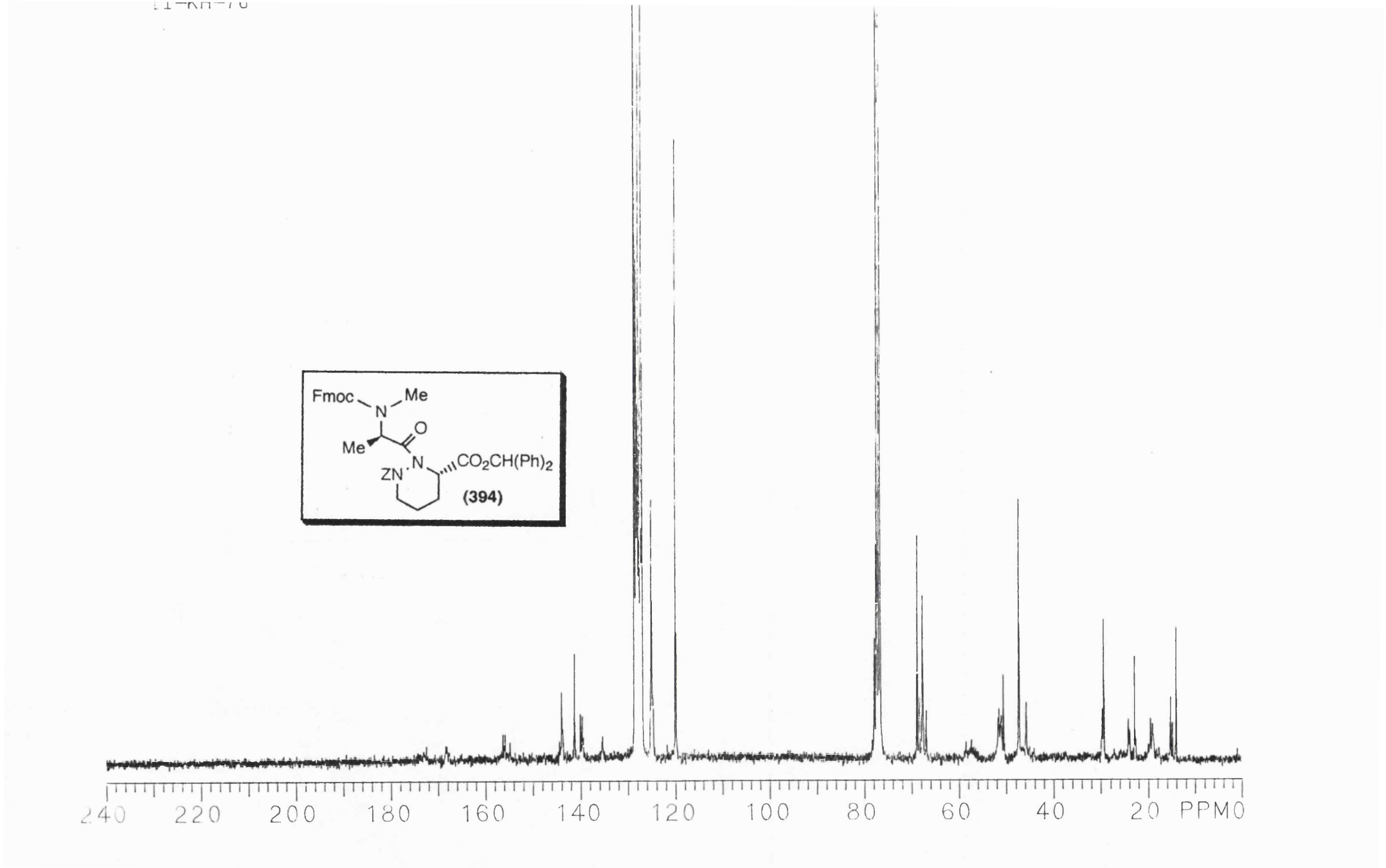

O
H
N
Ph
ZN
O
Ph
(393)
10
8
6
4
2

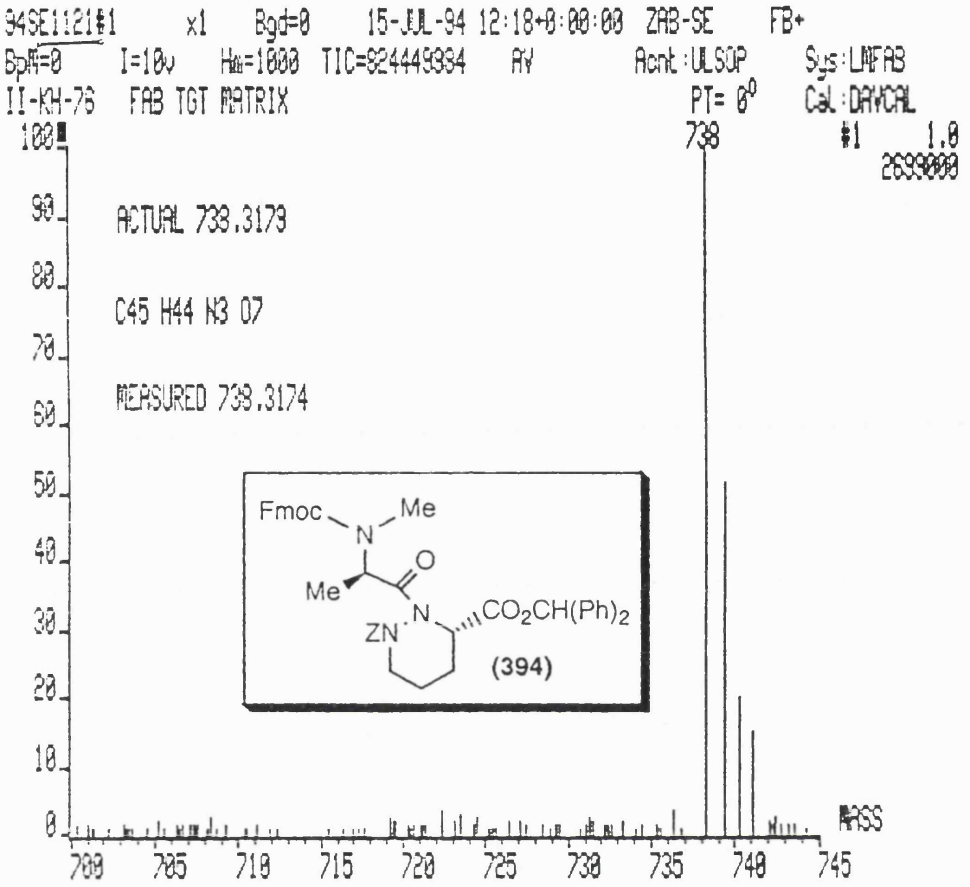

O
H
N
ZN
O
Ph
Ph
(393)
200
180
160
140
120
100
80
60
40
20 PPM

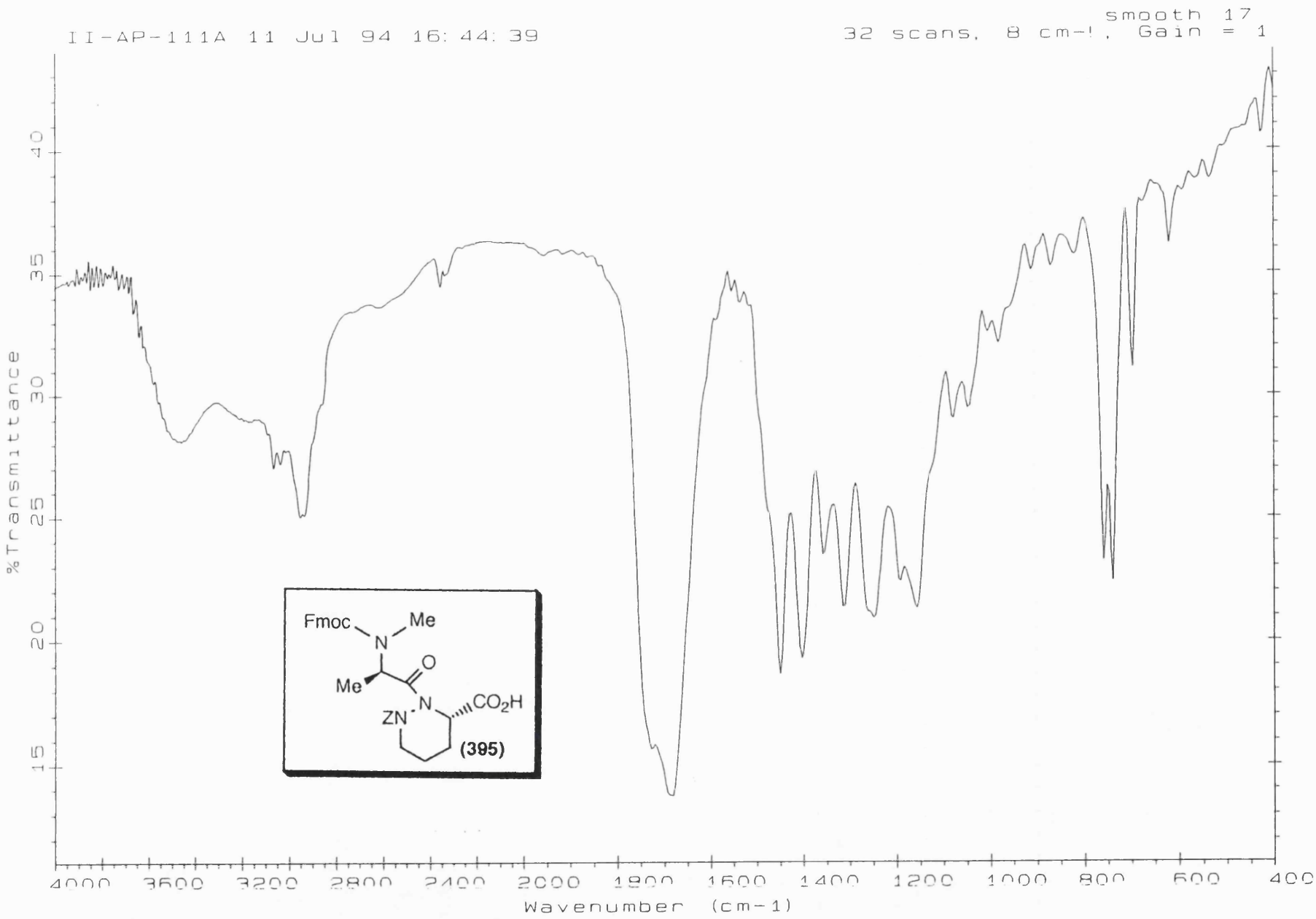

94SE1040#1 x1 Bgd=0 08-JUL-94 16:13+0:00:00 ZAB-SE FB+
BpM=0 I=10v Hm=2500 TIC=324300932 AV Acnt:ULSOP Sys:MMFAB
II-KH-75 FABMS MATRIX MNOBA + NA PT= 0° CaL:FABCAL
#1 1.0
65534000
453
ACTUAL 453.1730
C26 H26 N2 O4 NA
MEASURED 453.1793
451
MASS
100
90
80
70
60
50
40
30
20
10
0
430
435
440
445
450
455
460
O
Ph
H
N
ZN
O
Ph
(393)

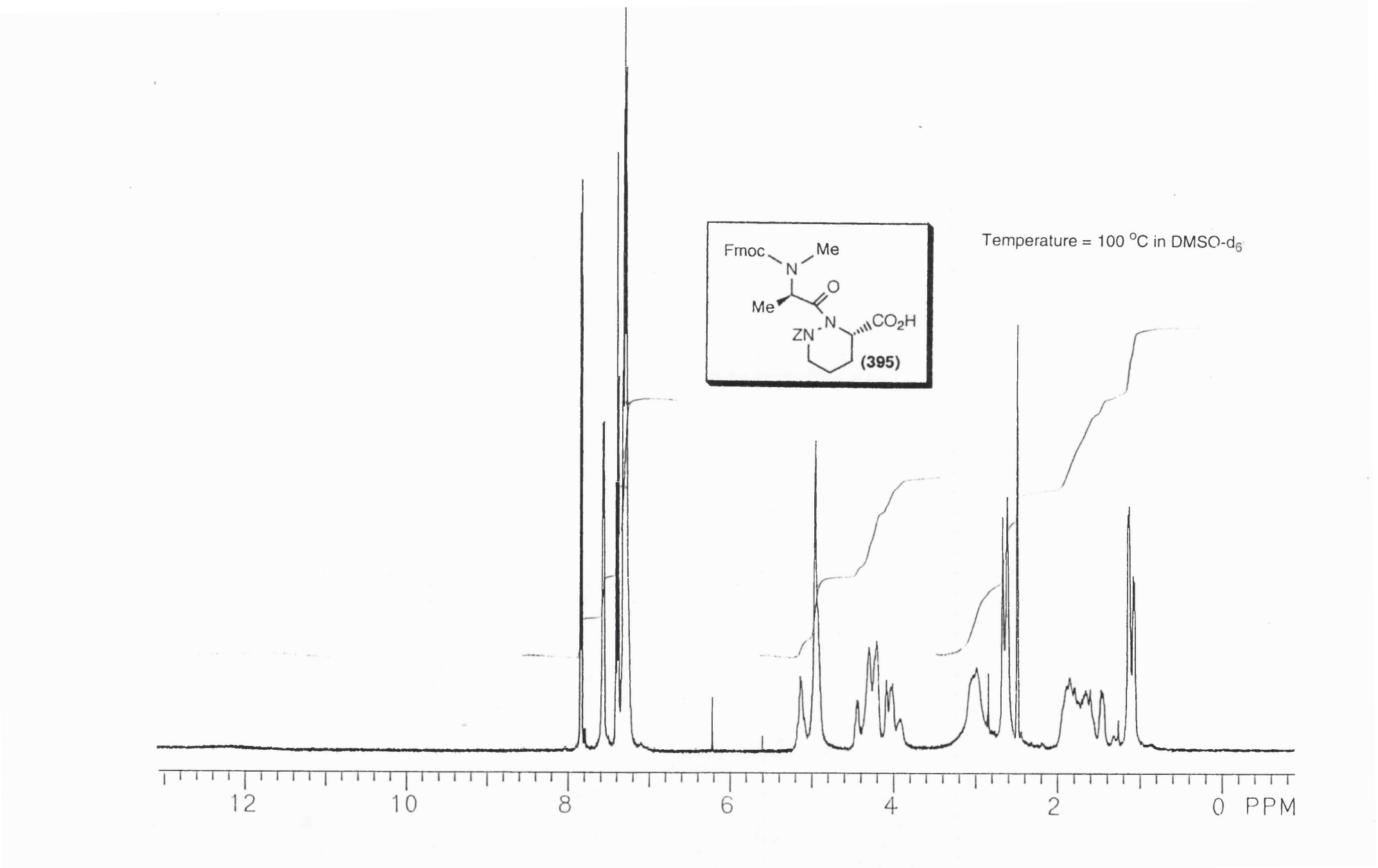

II-KH-76 01 Aug 94 20:28:55
smooth 17 slope
32 scans, 2 cm-!, Gain = 1
%Transmittance
90
80
70
60
50
40
30
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
Fmoc
N
Me
Me
O
ZN
N
CO2CH(Ph)2
(394)


Fmoc
N
Me
Me
O
N
ZN
$CO_2CH(Ph)_2$
(394)
10
8
6
4
2 PPM
0

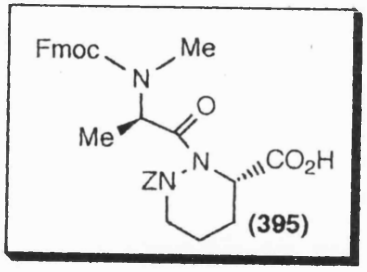

Fmoc
N
Me
Me
O
ZN
N
CO2CH(Ph)2
(394)
240
220
200
180
160
140
120
100
80
60
40
20
PPM0


94SE1121#1 x1 Bgd=0 15-JUL-94 12:18+0:00:00 ZAB-SE FB+
BpM=0 I=10v Hm=1000 TIC=824449334 AV Acnt:ULSOP Sys:LMFAB
II-KH-76 FAB TGT MATRIX PT= 0° Cal:DAVCAL
738 #1 1.0
ACTUAL 738.3173
C45 H44 N3 O7
MEASURED 738.3174
Fmoc
N
Me
Me
O
N
ZN
CO2CH(Ph)2
(394)
MASS
100
90
80
70
60
50
40
30
20
10
0
700
705
710
715
720
725
730
735
740
745


II-AP-111A 11 Jul 94 16:44:39
smooth 17
32 scans, 8 cm-1, Gain = 1
%Transmittance
40
35
30
25
20
15
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
Fmoc
N
Me
O
Me
ZN
N
CO2H
(395)

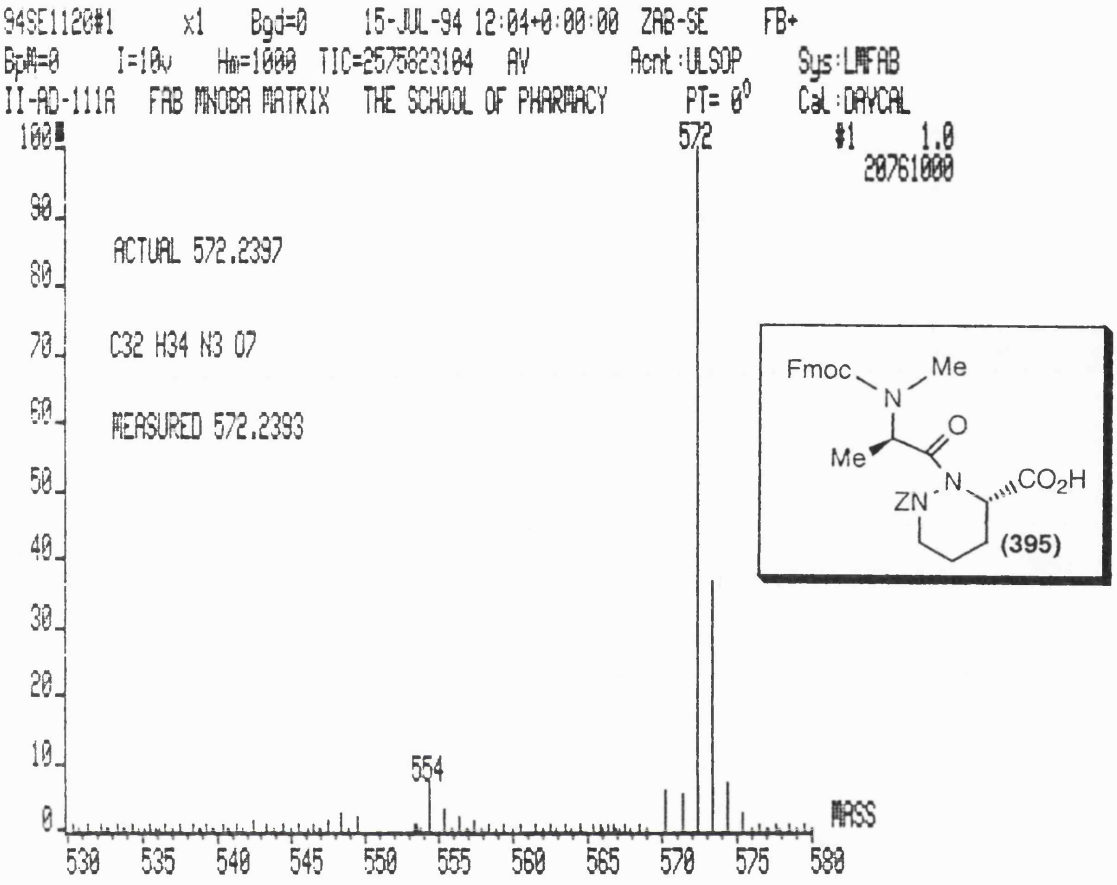

Fmoc
N
Me
Me
O
N
CO2H
ZN
(395)
Temperature = 100 °C in DMSO-d6
12
10
8
6
4
2
0 PPM

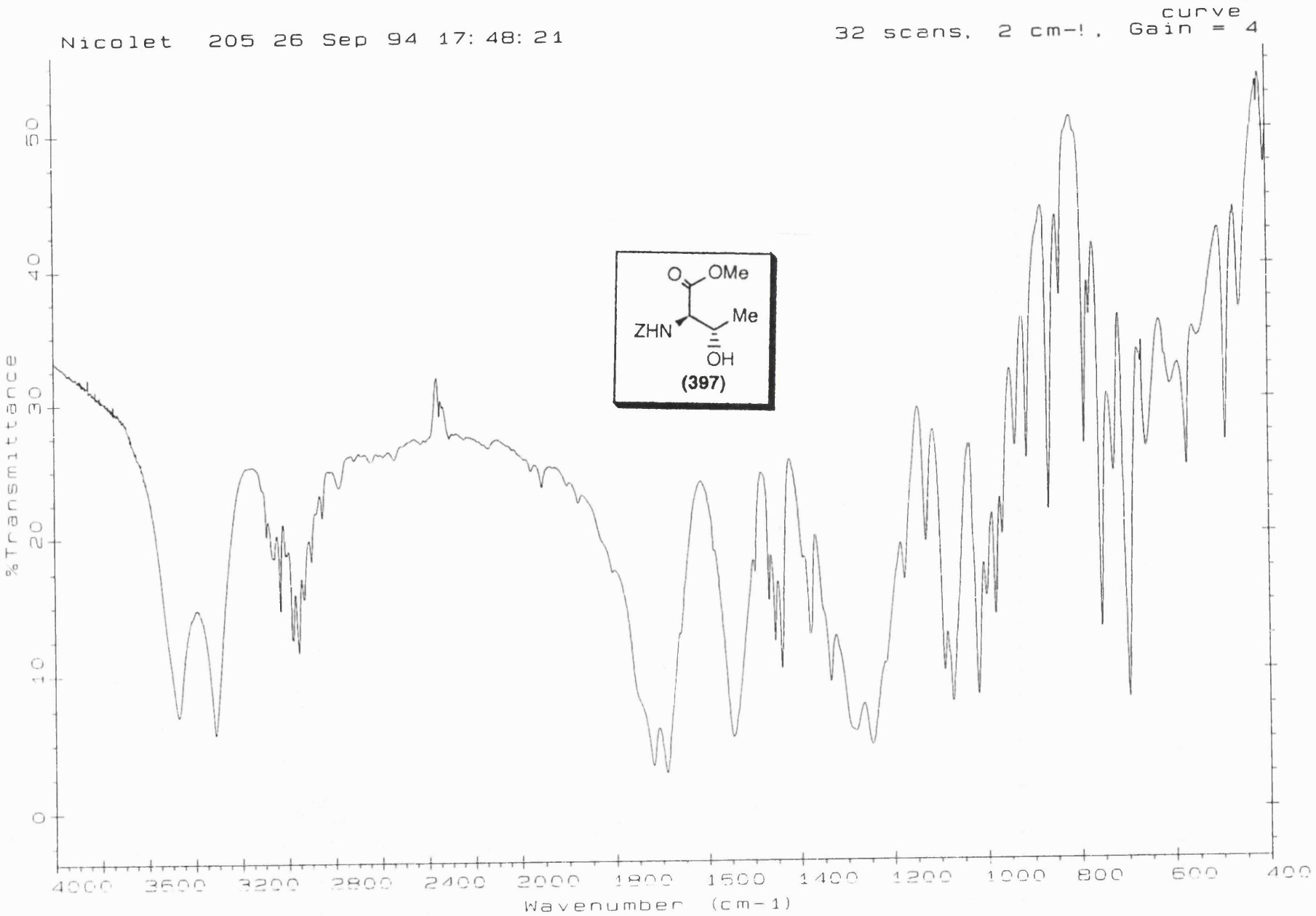
II-AP-111A

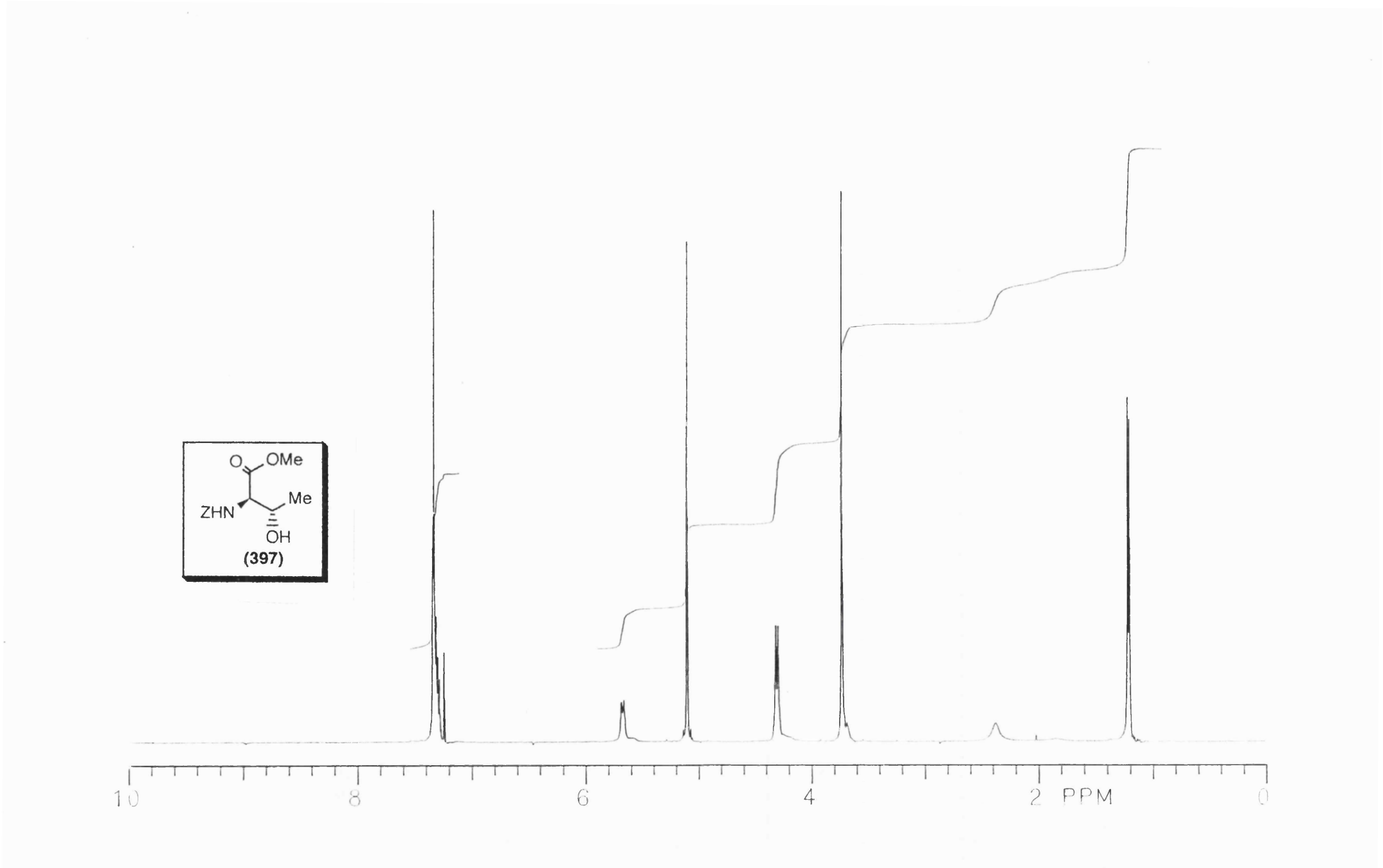


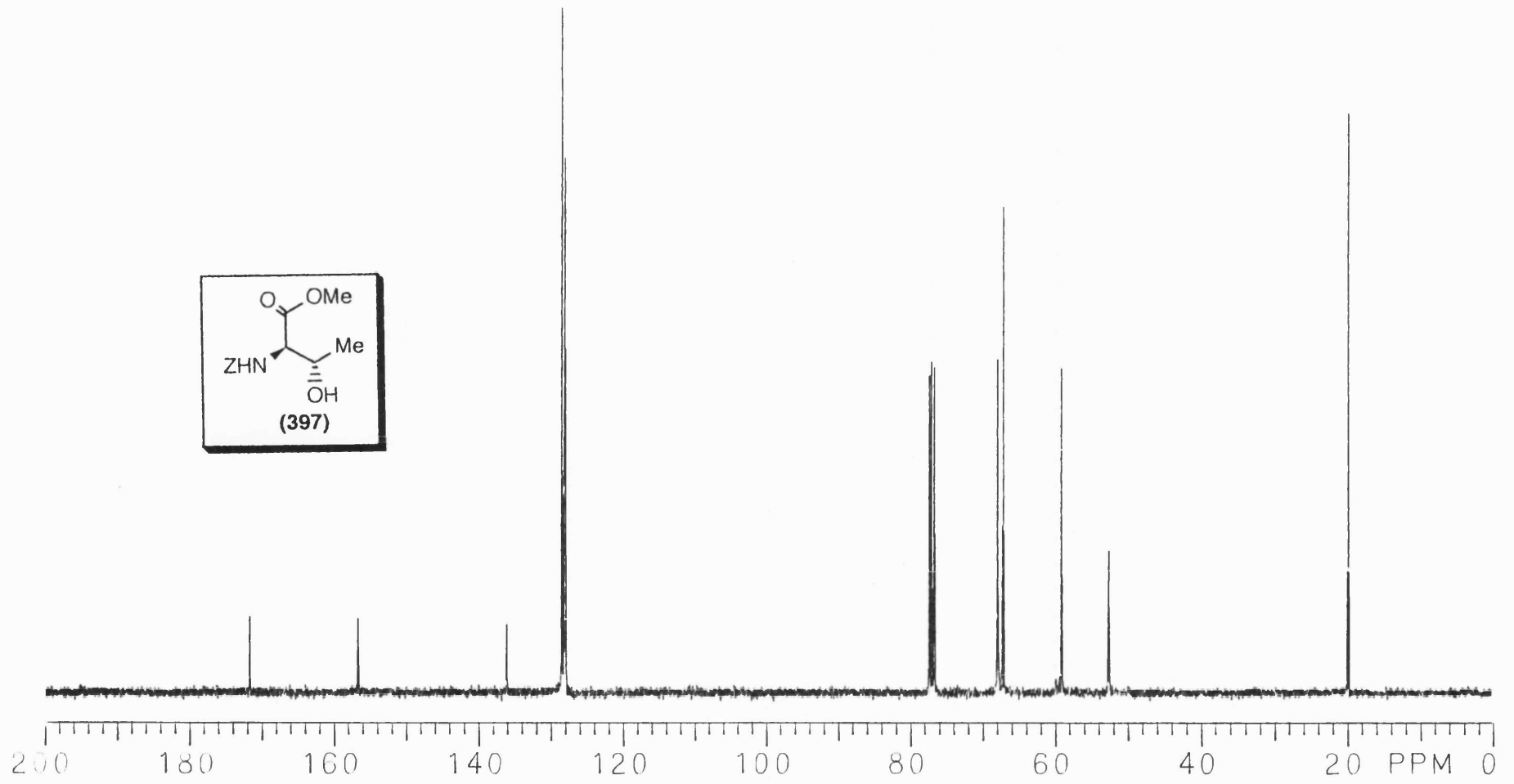
(395)

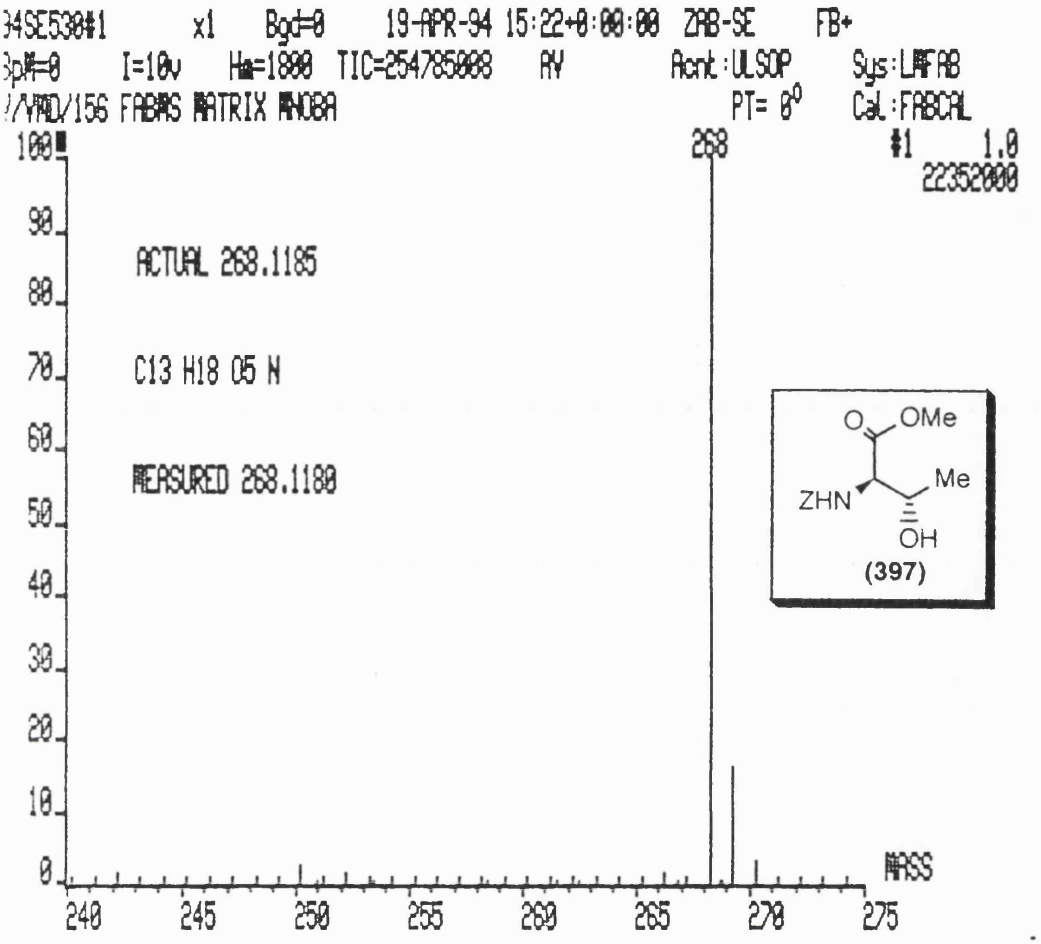
240 220 200 180 160 140 120 100 80 60 40 20 PPM0

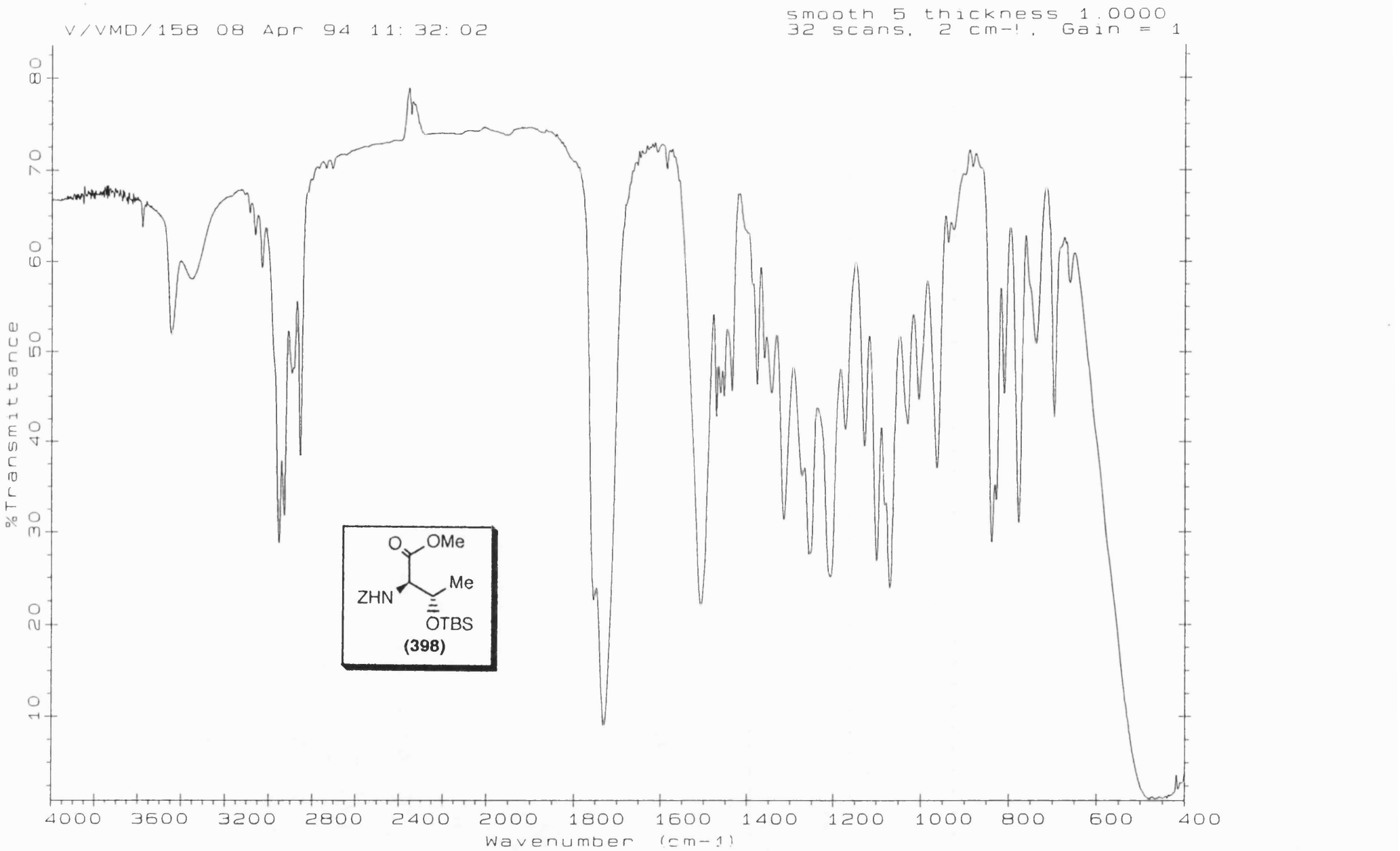

94SE1120#1 x1 Bgd=0 15-JUL-94 12:04+0:00:00 ZAB-SE FB+
BpM=0 I=10v Hm=1000 TIC=2575823104 AV Acmt:ULSOP Sys:LMFAB
II-AD-111A FAB MNOBA MATRIX THE SCHOOL OF PHARMACY PT= 0° CaL:DAYCAL
572
#1 1.0
20761000
ACTUAL 572.2397
C32 H34 N3 O7
MEASURED 572.2393
554
MASS
100
90
80
70
60
50
40
30
20
10
0
530 535 540 545 550 555 560 565 570 575 580
Fmoc
Me
N
O
Me
N
ZN
CO2H
(395)

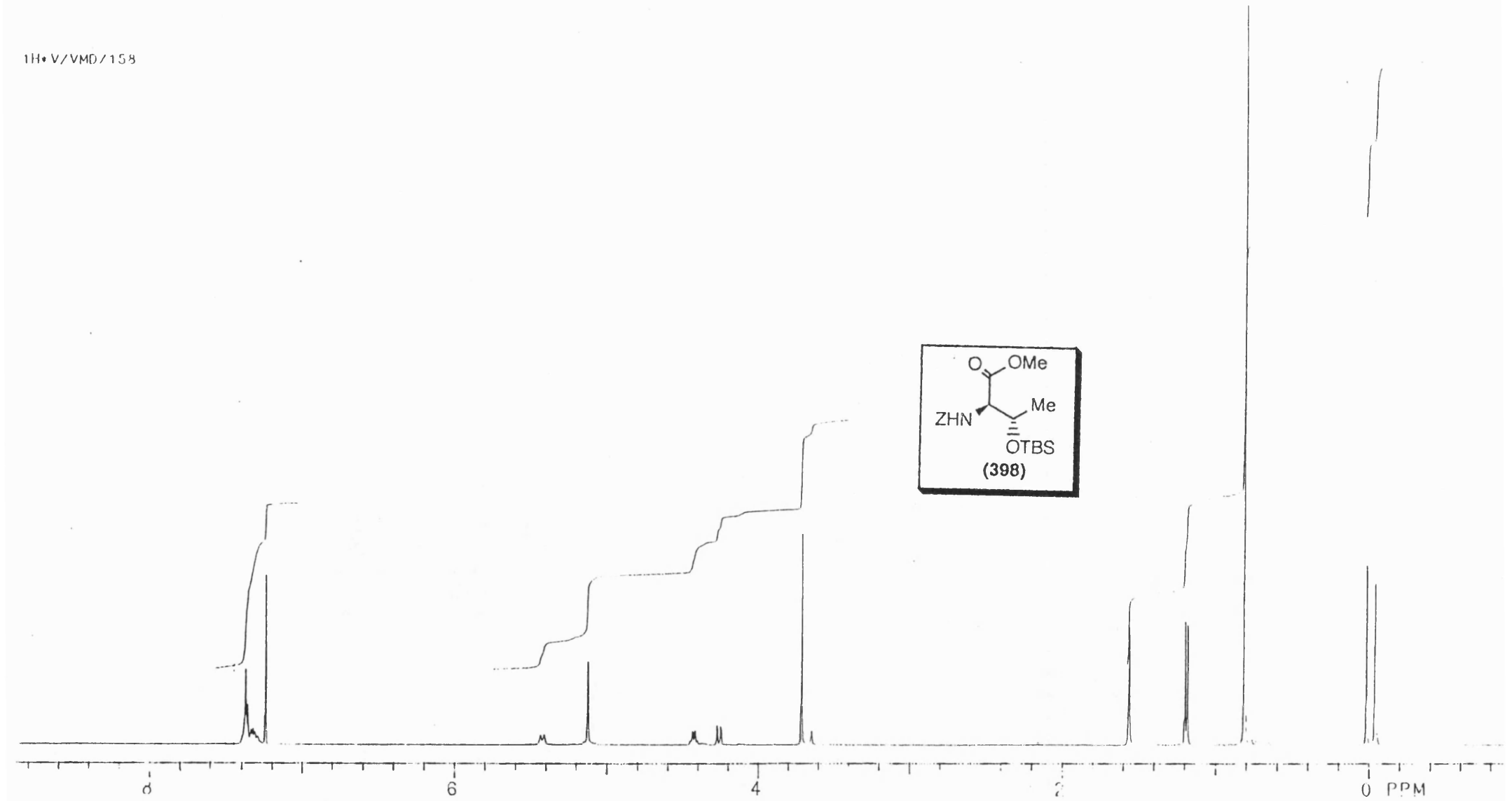

Nicolet 205 26 Sep 94 17:48:21
32 scans, 2 cm-!, Gain = 4
curve
%Transmittance
0
10
20
30
40
50
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
OMe
O
ZHN
Me
OH
(397)

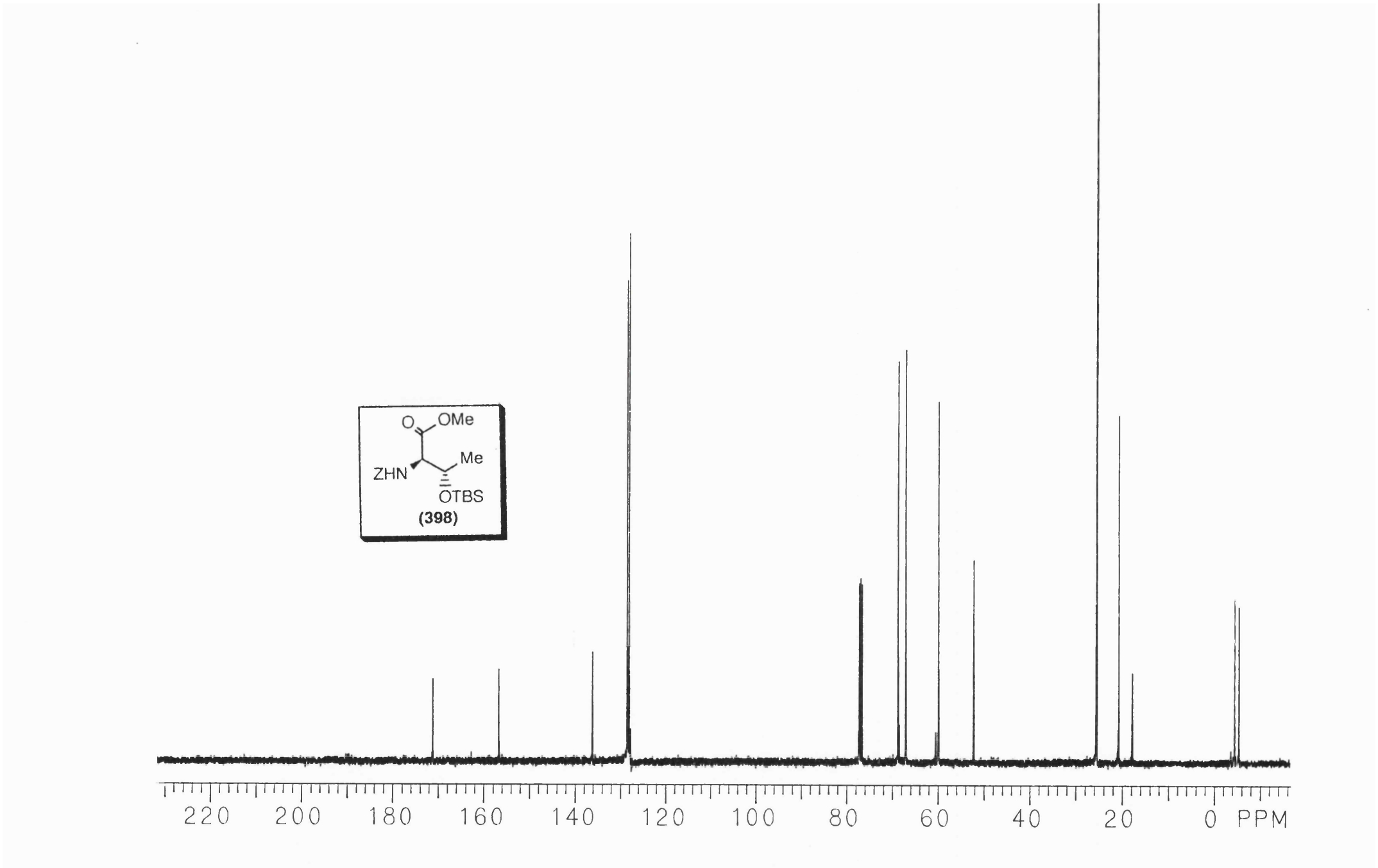

O
OMe
Me
ZHN
OH
(397)
10
8
6
4
2 PPM
0

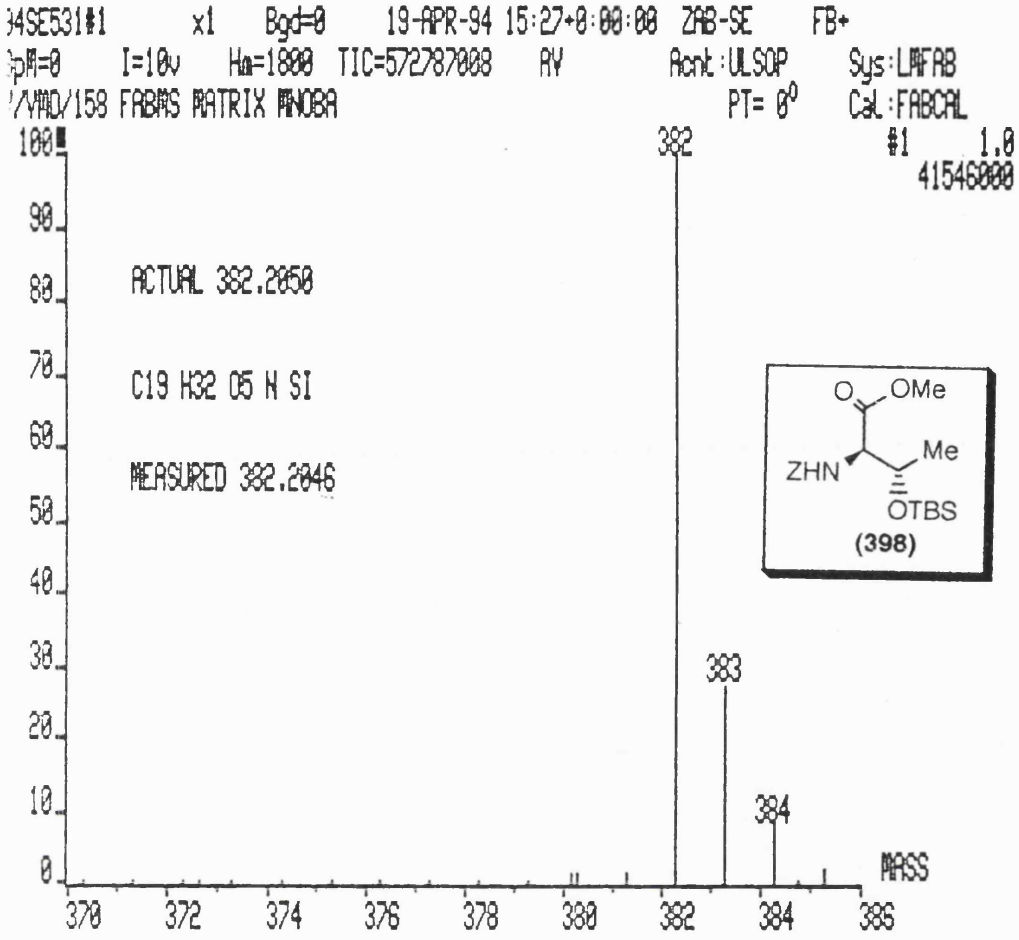

O
OMe
Me
ZHN
OH
(397)
200
180
160
140
120
100
80
60
40
20
PPM
0

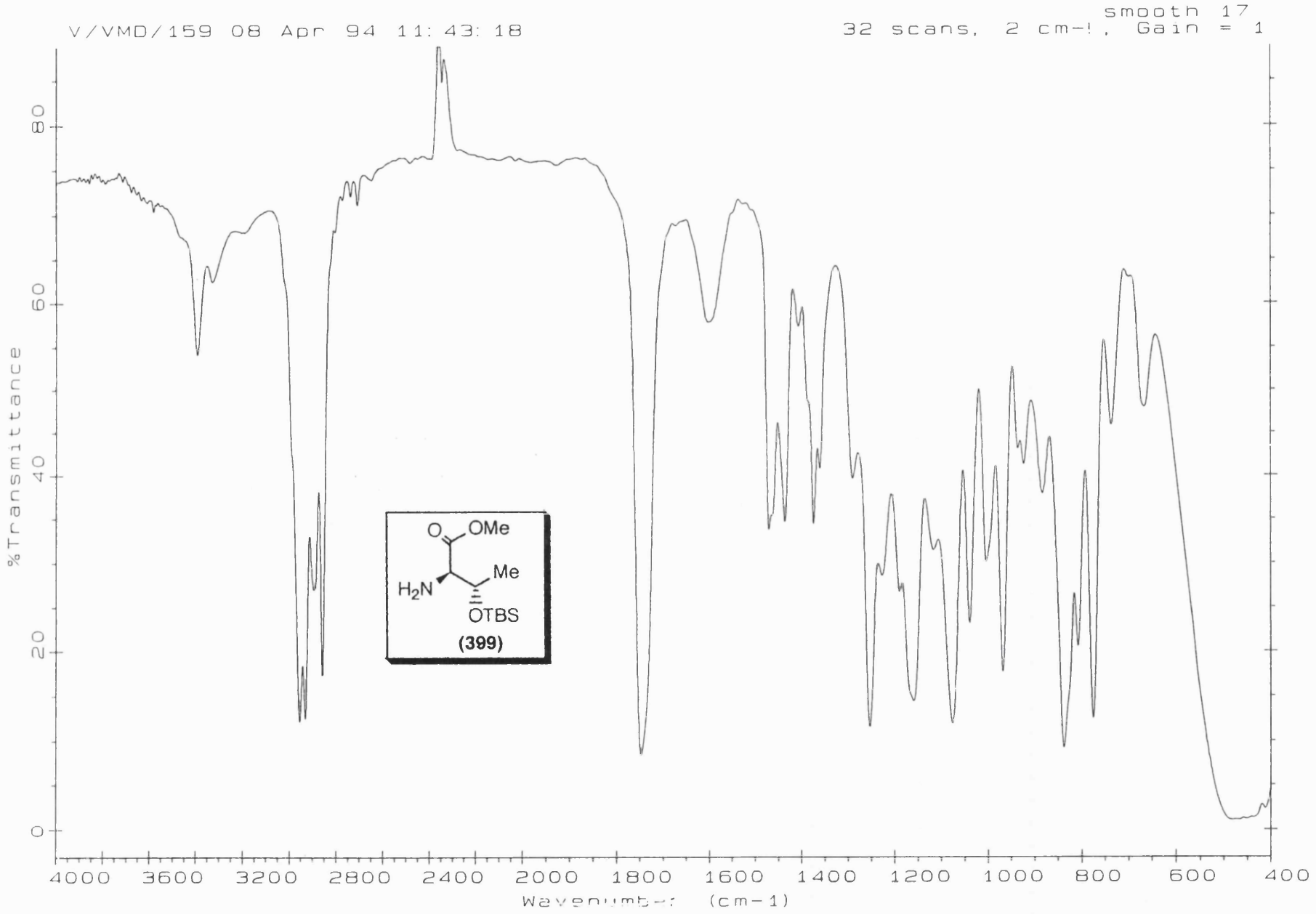

34SE538#1 x1 Bgd=0 19-APR-94 15:22+0:00:00 ZAB-SE FB+
Sp#=0 I=10v Ma=1800 TIC=2547850008 AV Acnt:ULSOP Sys:LMFAB
//YMD/156 FABMS MATRIX MNOBA PT= 0° Cal:FABCAL
100
90
80
70
60
50
40
30
20
10
0
268
#1 1.0
22352000
ACTUAL 268.1185
C13 H18 O5 N
MEASURED 268.1180
OMe
Me
ZHN
OH
(397)
MASS
240
245
250
255
260
265
270
275

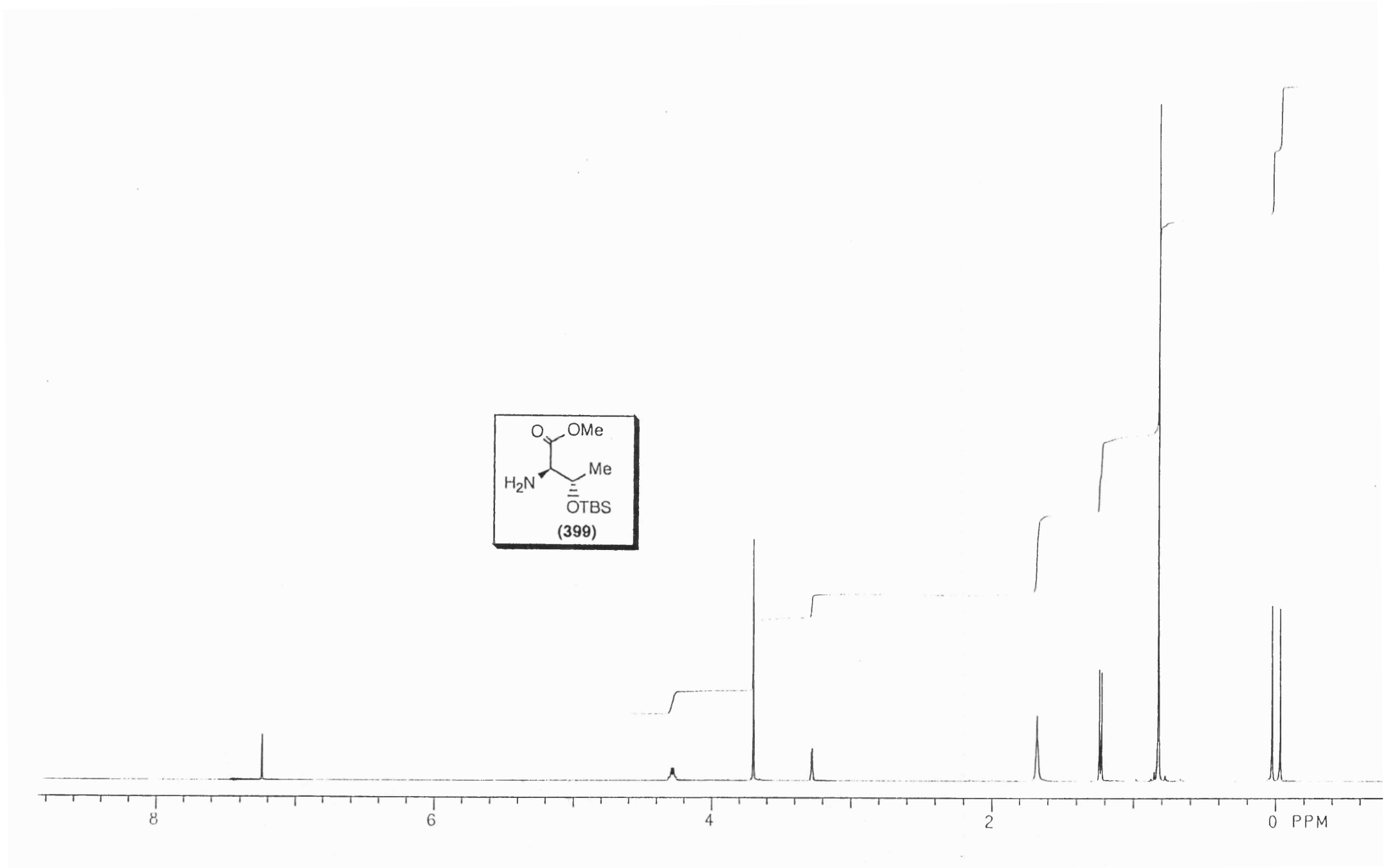

V/VMD/158 08 Apr 94 11:32:02
smooth 5 thickness 1.0000
32 scans, 2 cm-1, Gain = 1
%Transmittance
80
70
60
50
40
30
20
10
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
O
OMe
ZHN
Me
OTBS
(398)

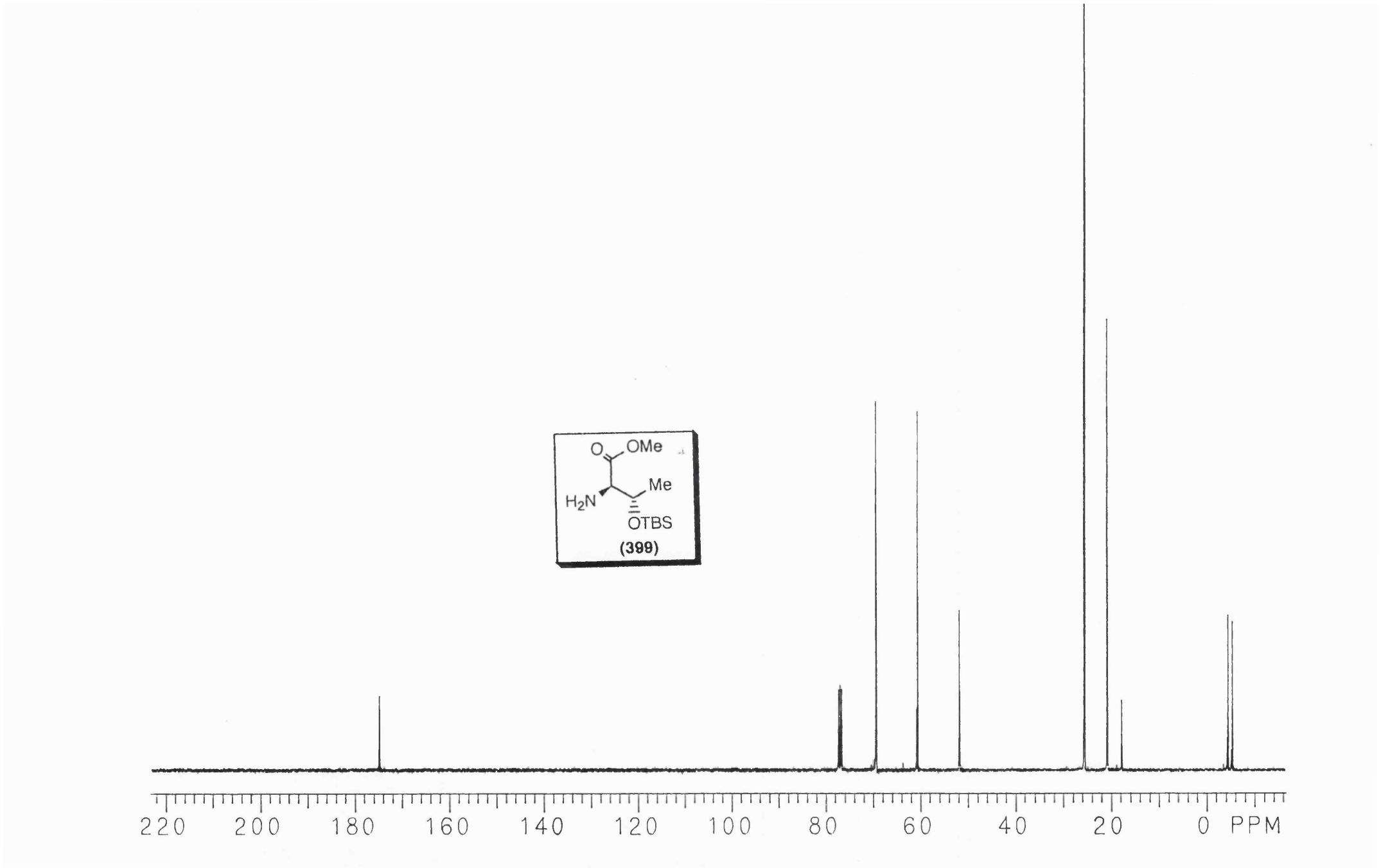

1H+V/VMD/158
O
OMe
ZHN
Me
OTBS
(398)
6
4
0 PPM

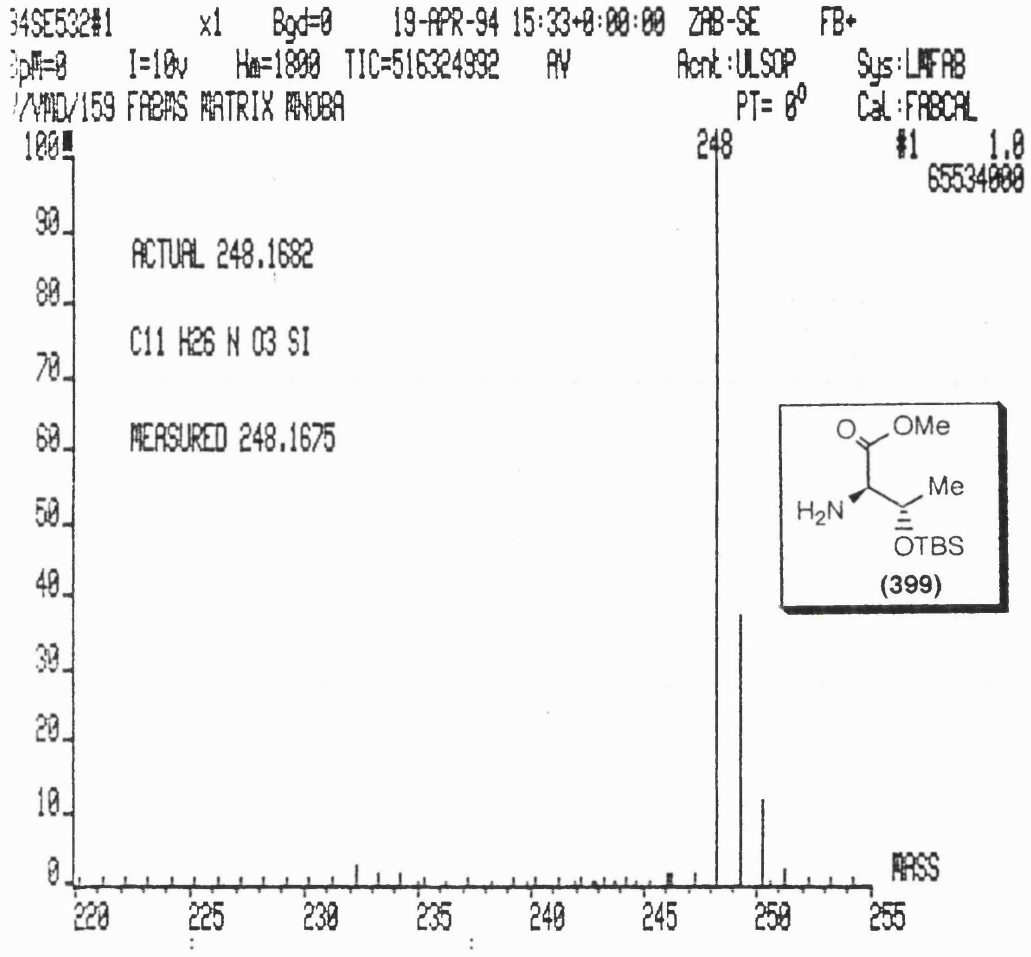

O
OMe
Me
ZHN
OTBS
(398)
220
200
180
160
140
120
100
80
60
40
20
0
PPM

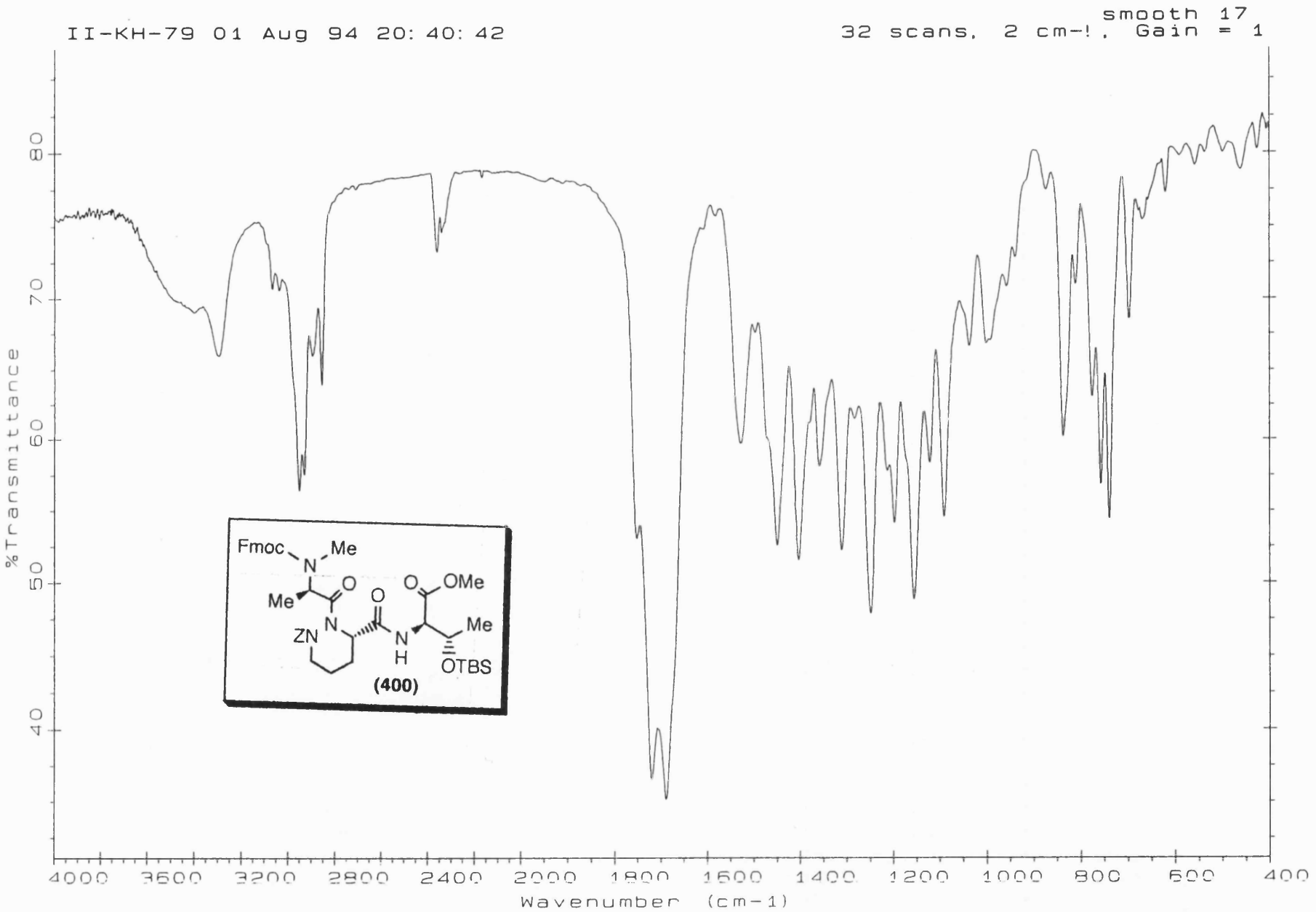

34SE531#1 x1 Bgd=0 19-APR-94 15:27+0:00:00 ZAB-SE FB+
SpM=0 I=10v Hm=1800 TIC=572787008 RY Acnt:ULSOP Sys:LMFAB
/VMD/158 FABMS MATRIX MNOBA PT= 0° Cal:FABCAL
#1 1.0
415460000
ACTURAL 382.2650
C19 H32 O5 N SI
MEASURED 382.2646
382
383
384
MASS
100
90
80
70
60
50
40
30
20
10
0
370
372
374
376
378
380
382
384
386
O
OMe
Me
ZHN
OTBS
(398)


V/VMD/159 08 Apr 94 11:43:18
smooth 17
32 scans, 2 cm-1, Gain = 1
%Transmittance
80
60
40
20
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
O
OMe
Me
H2N
OTBS
(399)

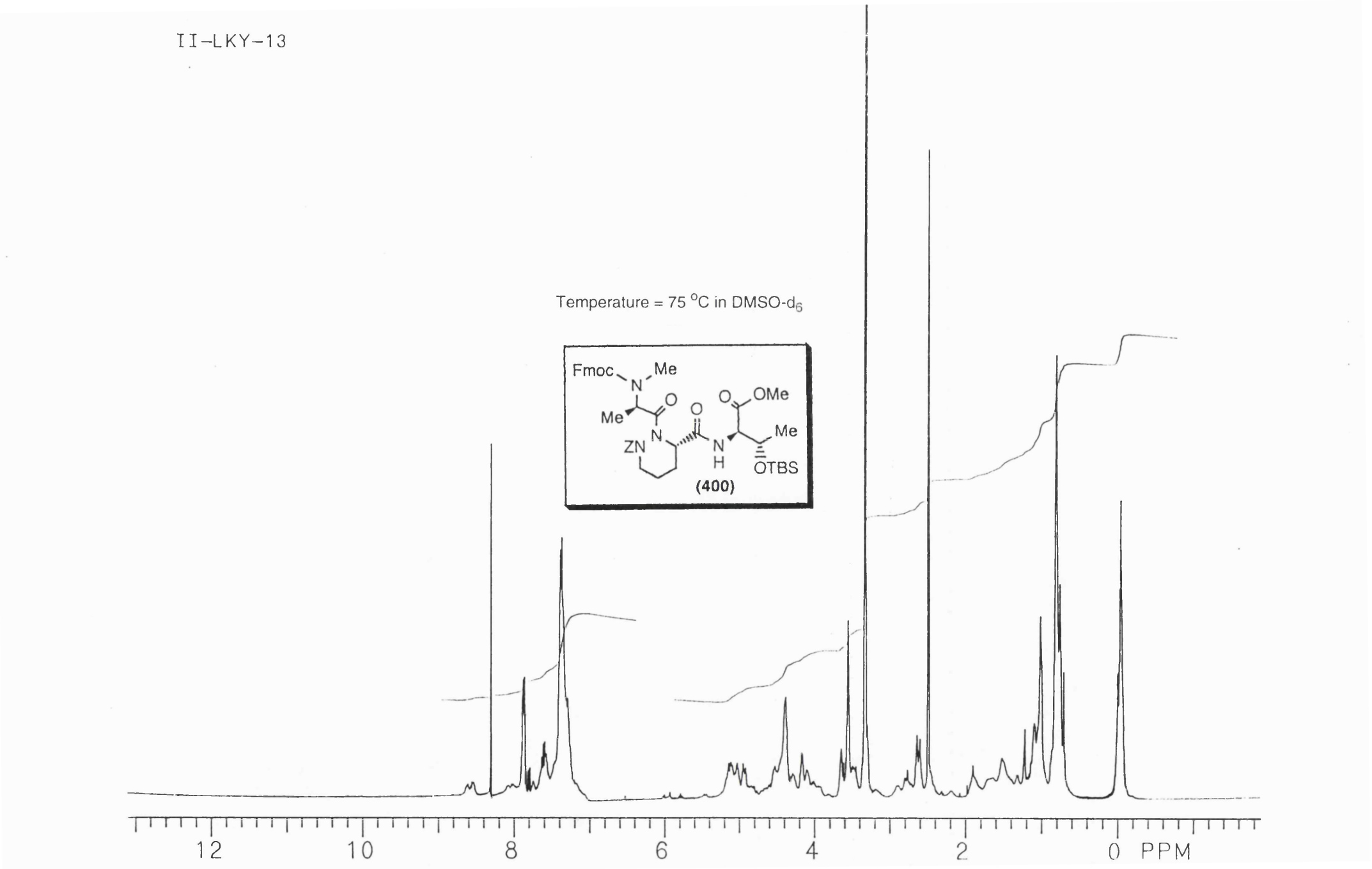

O
OMe
Me
H_2N
OTBS
(399)
8
6
4
2
0 PPM


O
OMe
Me
H_2N
OTBS
(399)
220
200
180
160
140
120
100
80
60
40
20
0
PPM

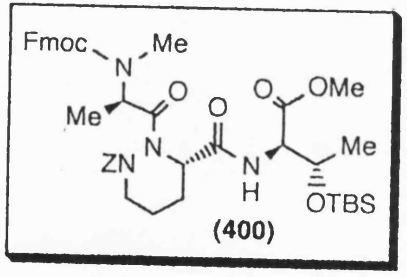

34SE532#1 x1 Bgd=0 19-APR-94 15:33+0:00:00 ZAB-SE FB+
SpM=0 I=10v Hm=1800 TIC=516324992 AV Acnt:ULSOP Sys:LMFAB
/VMD/159 FABMS MATRIX MNOBA PT= 0° Cal:FABCAL
248 #1 1.0
655340000
ACTUAL 248.1682
C11 H26 N O3 SI
MEASURED 248.1675
OMe
Me
H2N
OTBS
(399)
MASS
100
90
80
70
60
50
40
30
20
10
0
220
225
230
235
240
245
250
255


II-KH-79 01 Aug 94 20: 40: 42
smooth 17
32 scans, 2 cm-!. Gain = 1
%Transmittance
80
70
60
50
40
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
Fmoc
N
Me
Me
O
O
N
ZN
O
N
H
O
OMe
Me
OTBS
(400)

II-LKY-13

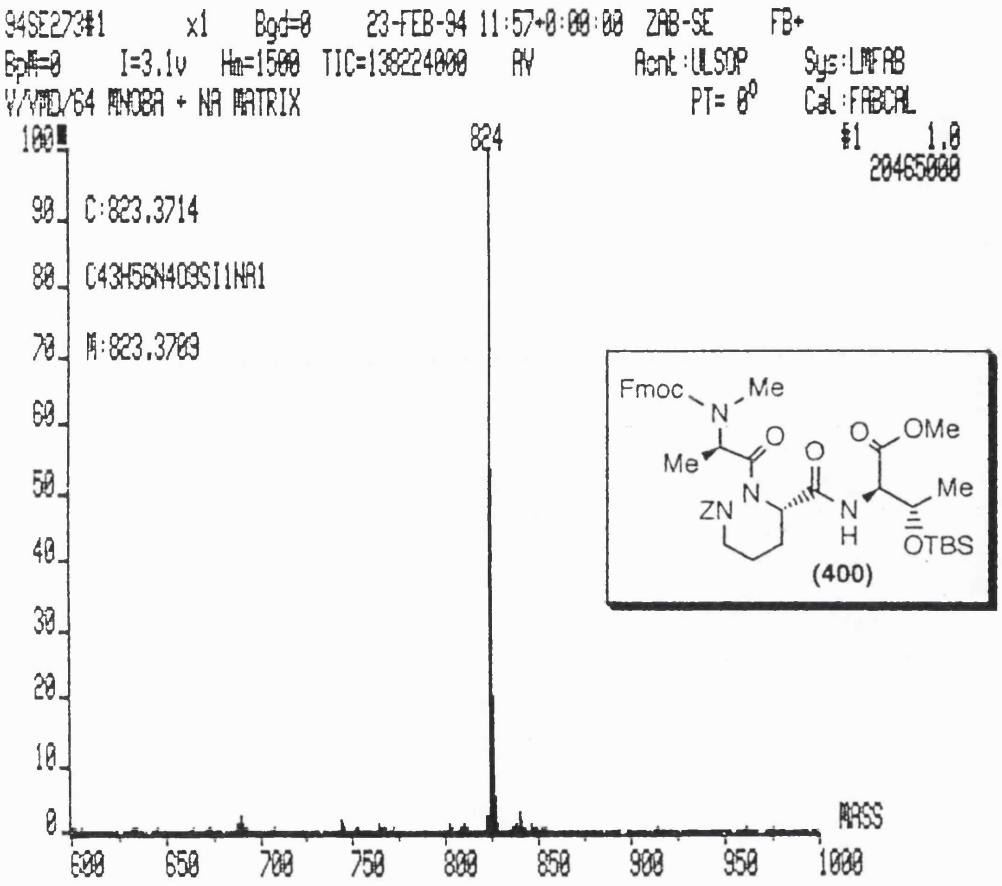

Temperature = 75 °C in DMSO-d6
Fmoc
N
Me
Me
O
O
N
ZN
O
OMe
Me
N
H
OTBS
(400)
12
10
8
6
4
2
0 PPM

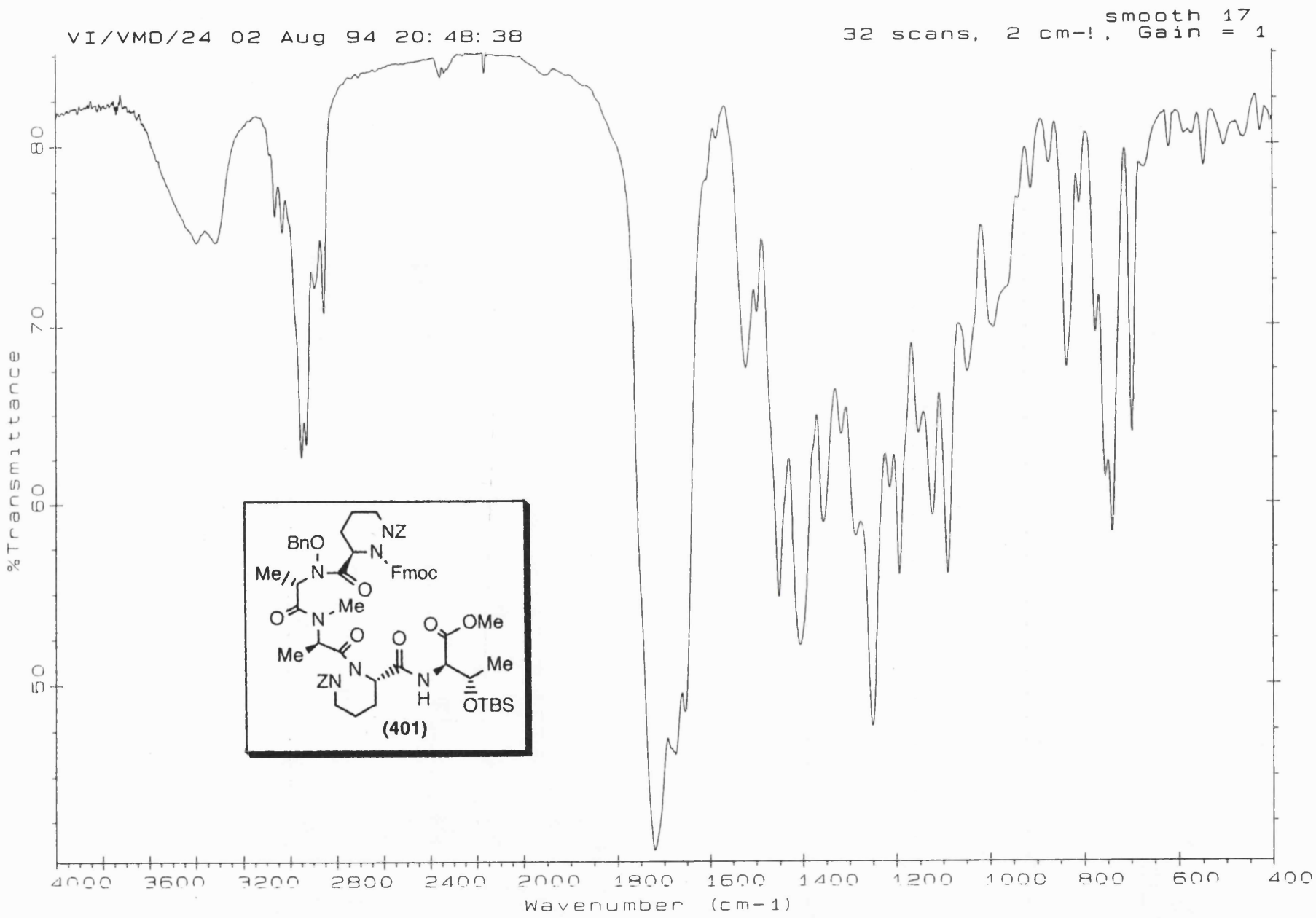
II-KH-79

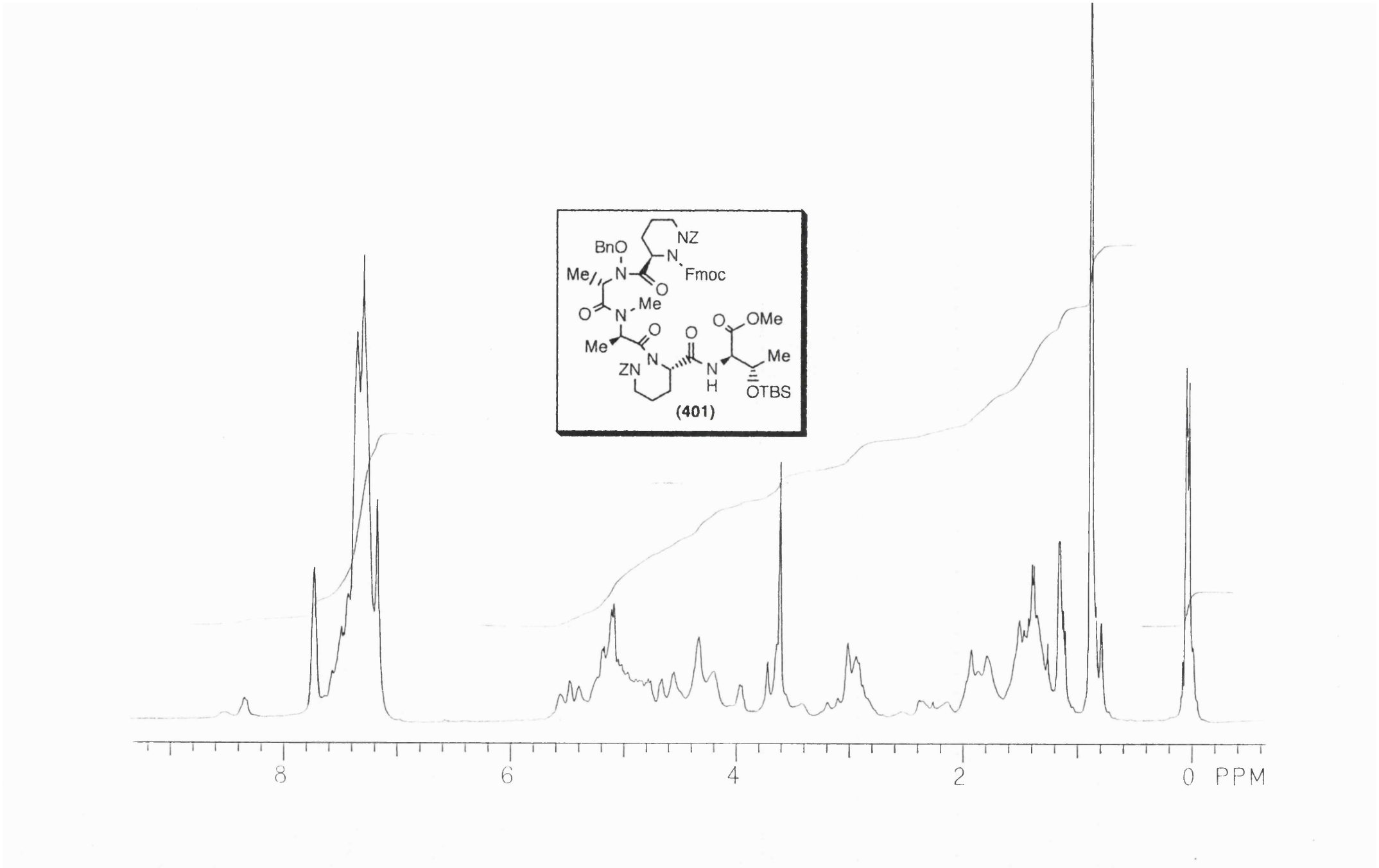


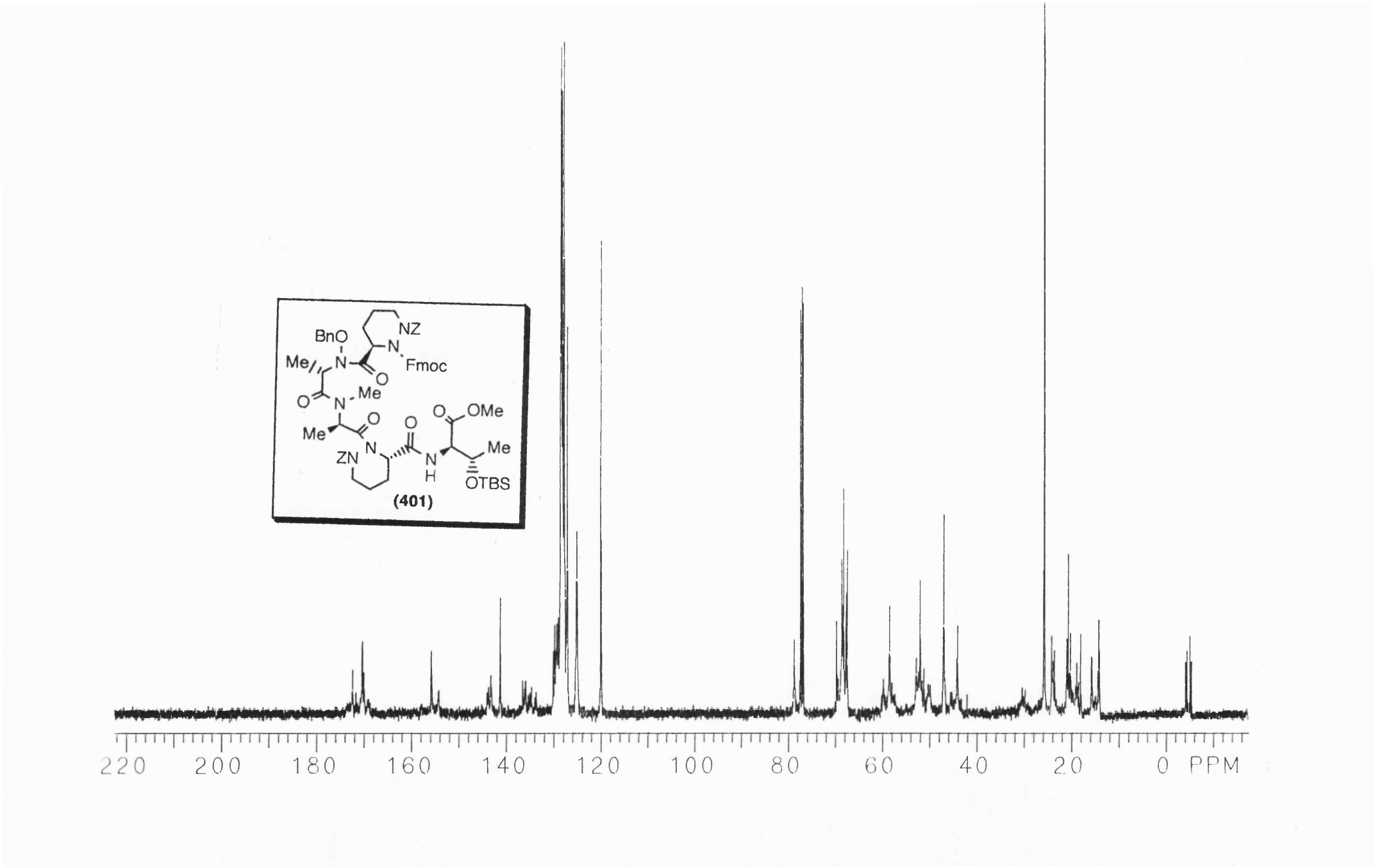
(400)

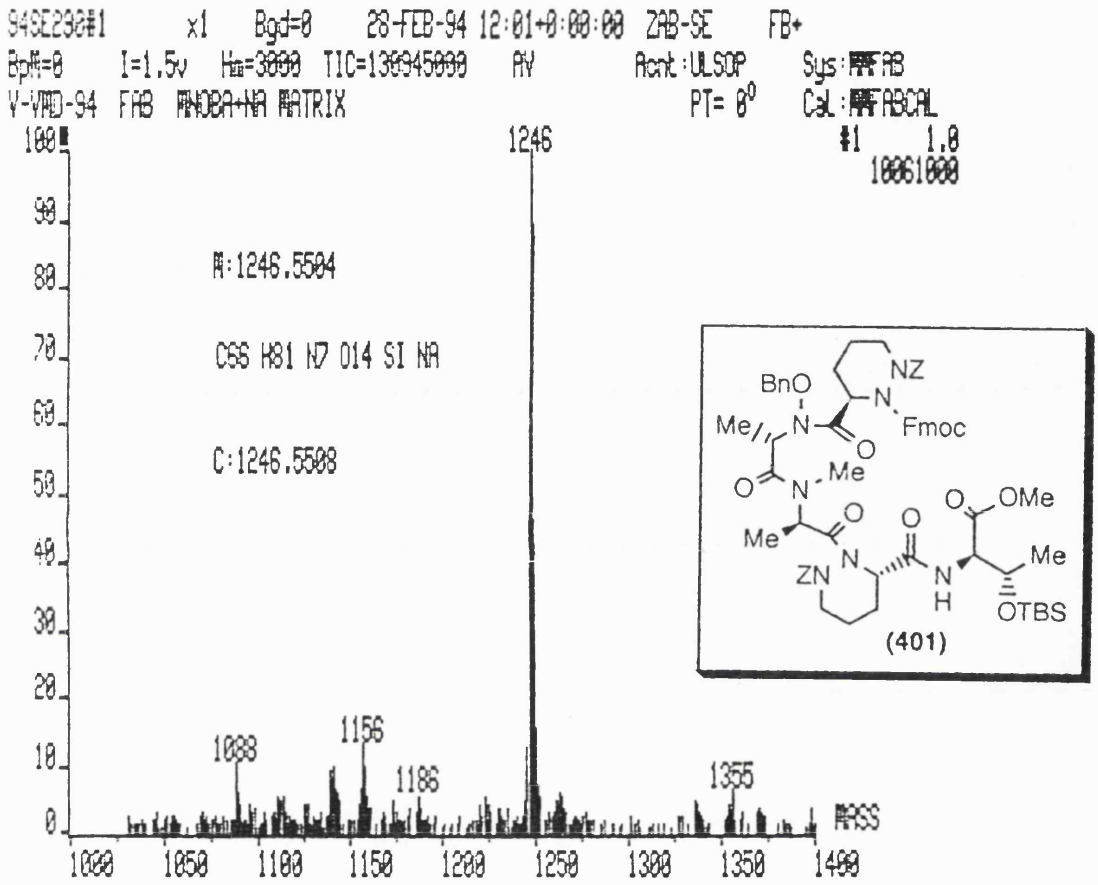
220 200 180 160 140 120 100 80 60 40 20 0 PPM

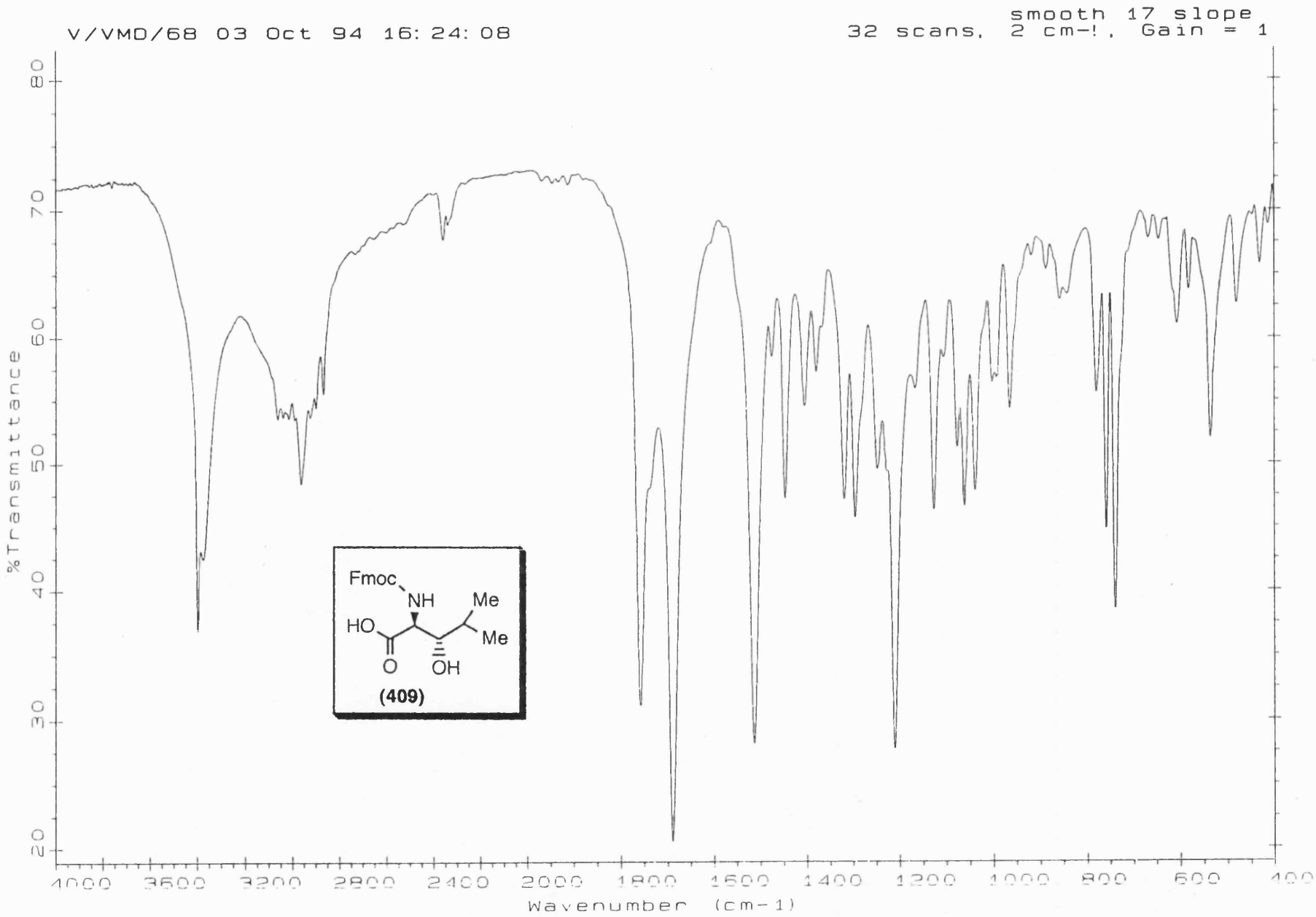

94SE273#1 x1 Bgd=0 23-FEB-94 11:57+0:00:00 ZAB-SE FB+
Bpk=0 I=3.1v Hm=1500 TIC=138224000 RV Acnt:ULSOP Sys:UMFAB
V/VMEL/64 MNOBA + NA MATRIX PT= 0° Cal:FABCAL
824
#1 1.0
20465000
C:823.3714
C43H56N4O9SI1NA1
M:823.3709
Fmoc
N
Me
Me
O
O
N
ZN
O
N
H
O
OMe
Me
OTBS
(400)
MASS
100
90
80
70
60
50
40
30
20
10
0
600
650
700
750
800
850
900
950
1000

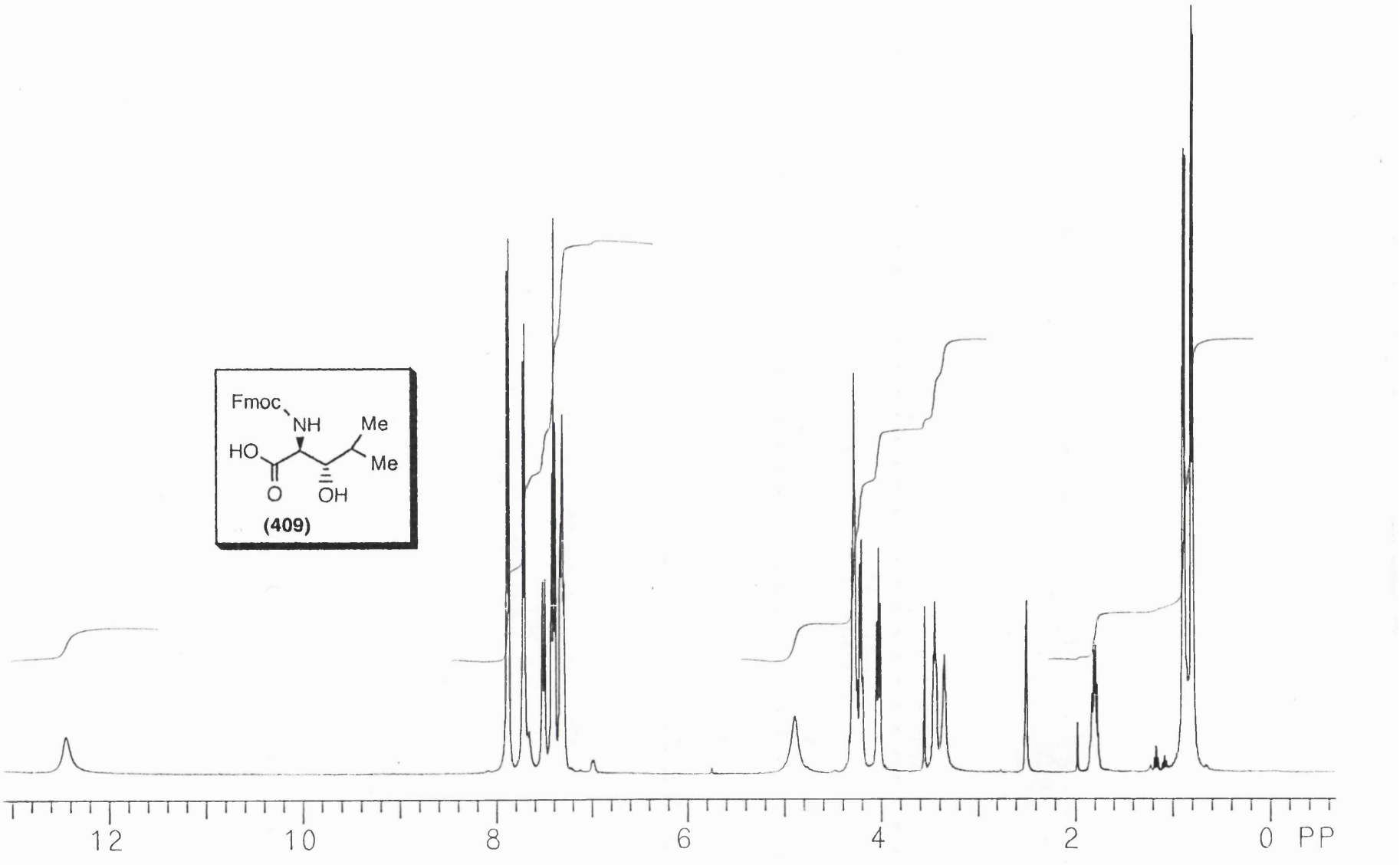

VI/VMD/24 02 Aug 94 20: 48: 38
smooth 17
32 scans, 2 cm-!, Gain = 1
%Transmittance
80
70
60
50
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
BnO
NZ
N
Fmoc
Me
N
O
O
N
Me
Me
O
O
O
OMe
Me
ZN
N
N
H
OTBS
(401)

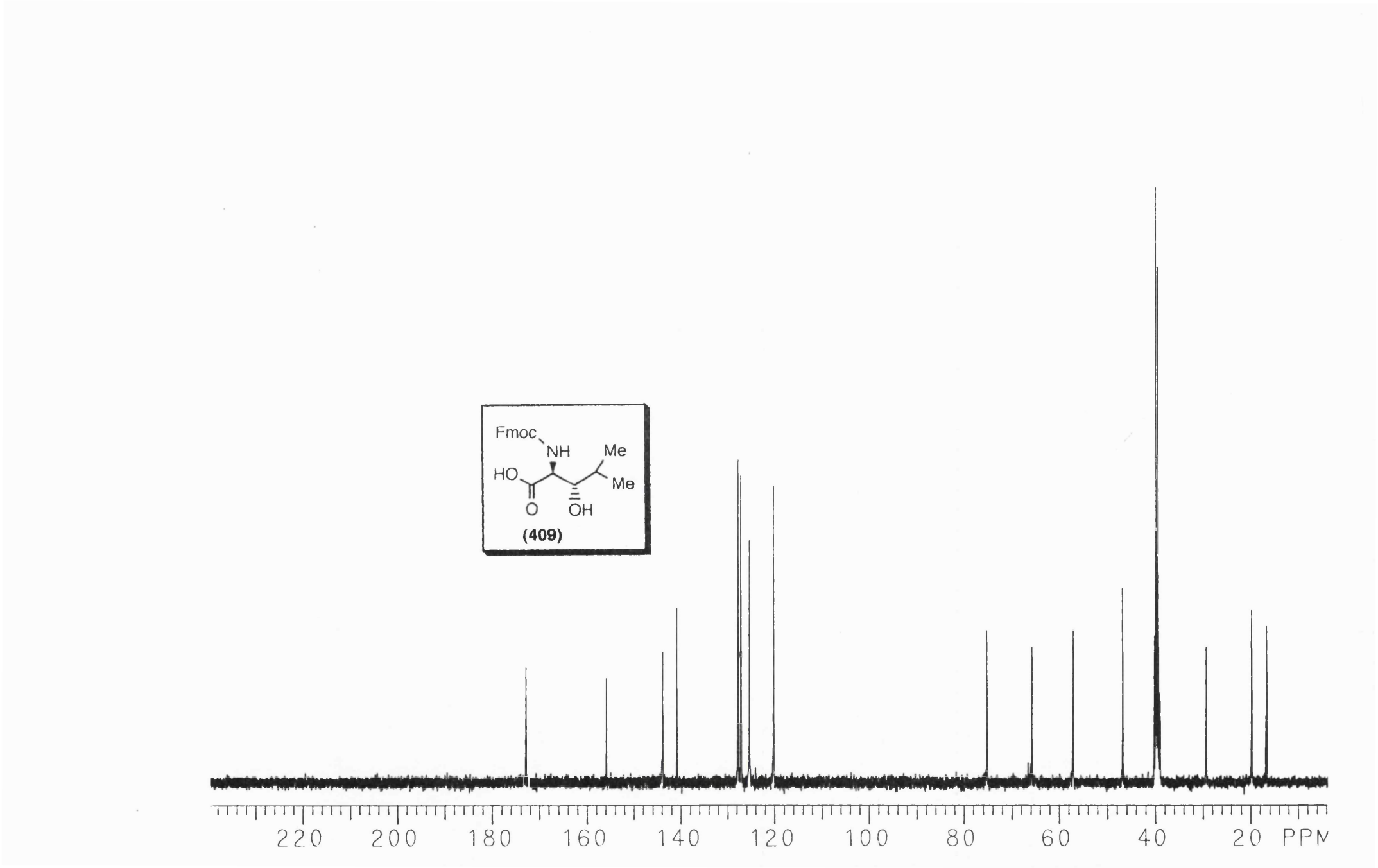

BnO
NZ
N
Me
N
Fmoc
O
O
N
Me
O
O
OMe
Me
O
O
Me
N
N
ZN
H
OTBS
(401)
8
6
4
2
0 PPM

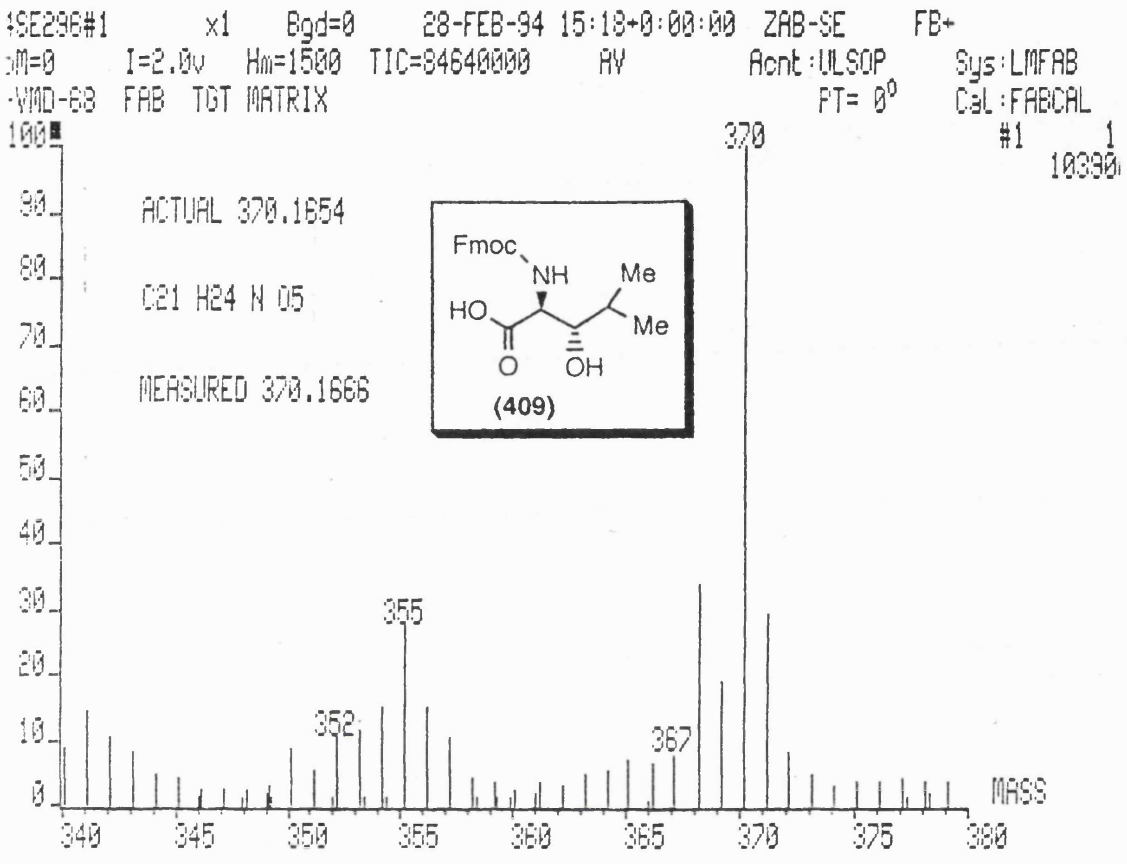

BnO
NZ
N
Fmoc
Me
N
O
O
N
Me
Me
O
O
OMe
O
N
N
Me
ZN
H
OTBS
(401)
220
200
180
160
140
120
100
80
60
40
20
0
PPM

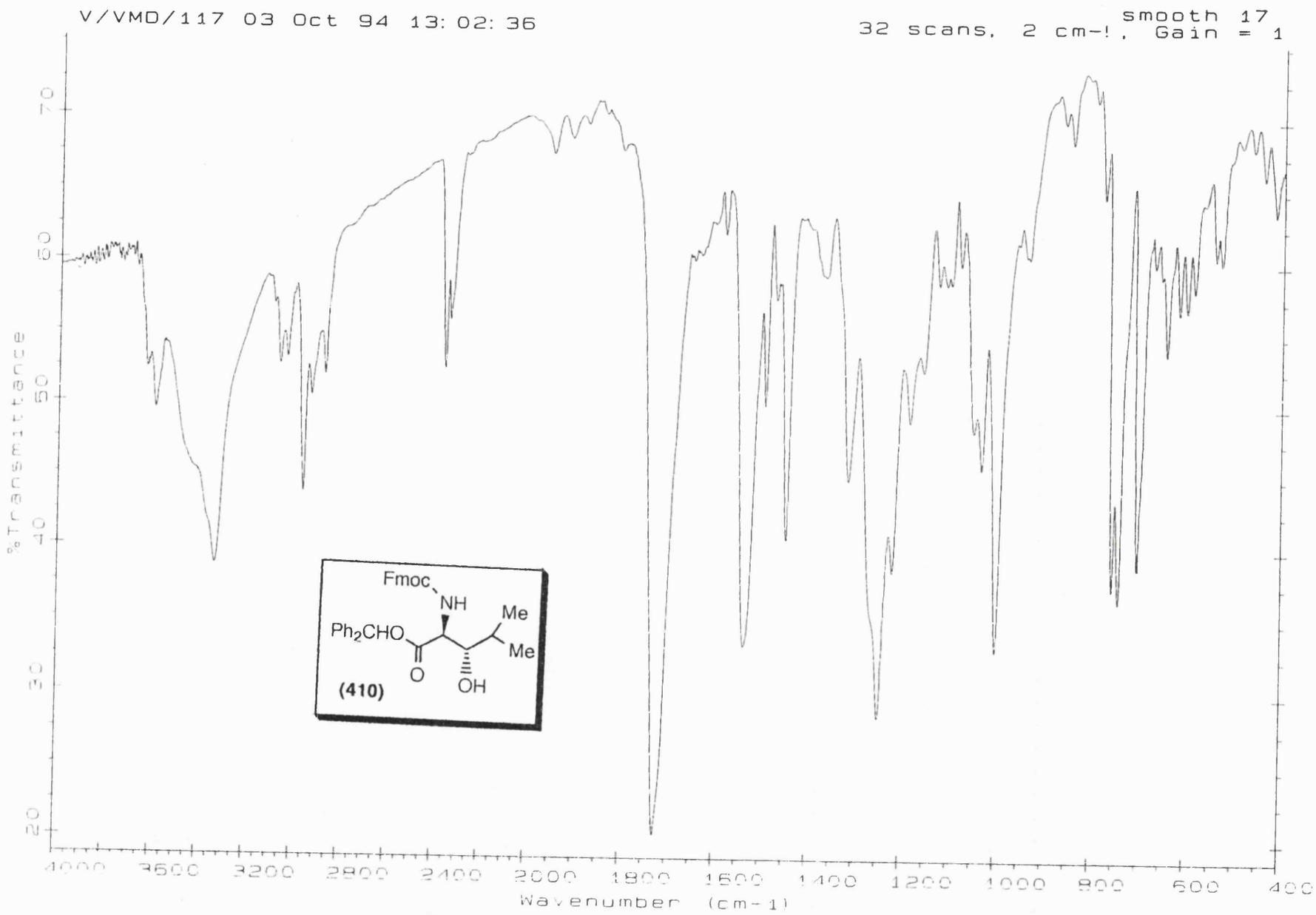

94SE230#1 x1 Bgd=0 28-FEB-94 12:01+0:00:00 ZAB-SE FB+
Bp#=0 I=1.5v Hm=3000 TIC=136945000 AV Acnt:ULSOP Sys:
V-VMD-94 FAB MNOBA+NA MATRIX PT= 0° Cal:
#1 1.0
100061000
1246
M:1246.5504
C66 H81 N7 O14 SI NA
C:1246.5508
1088
1156
1186
1355
MASS
100
90
80
70
60
50
40
30
20
10
0
1000
1050
1100
1150
1200
1250
1300
1350
1400
BnO
NZ
N
Fmoc
Me
N
O
O
N
Me
O
OMe
Me
O
Me
N
O
ZN
N
H
OTBS
(401)

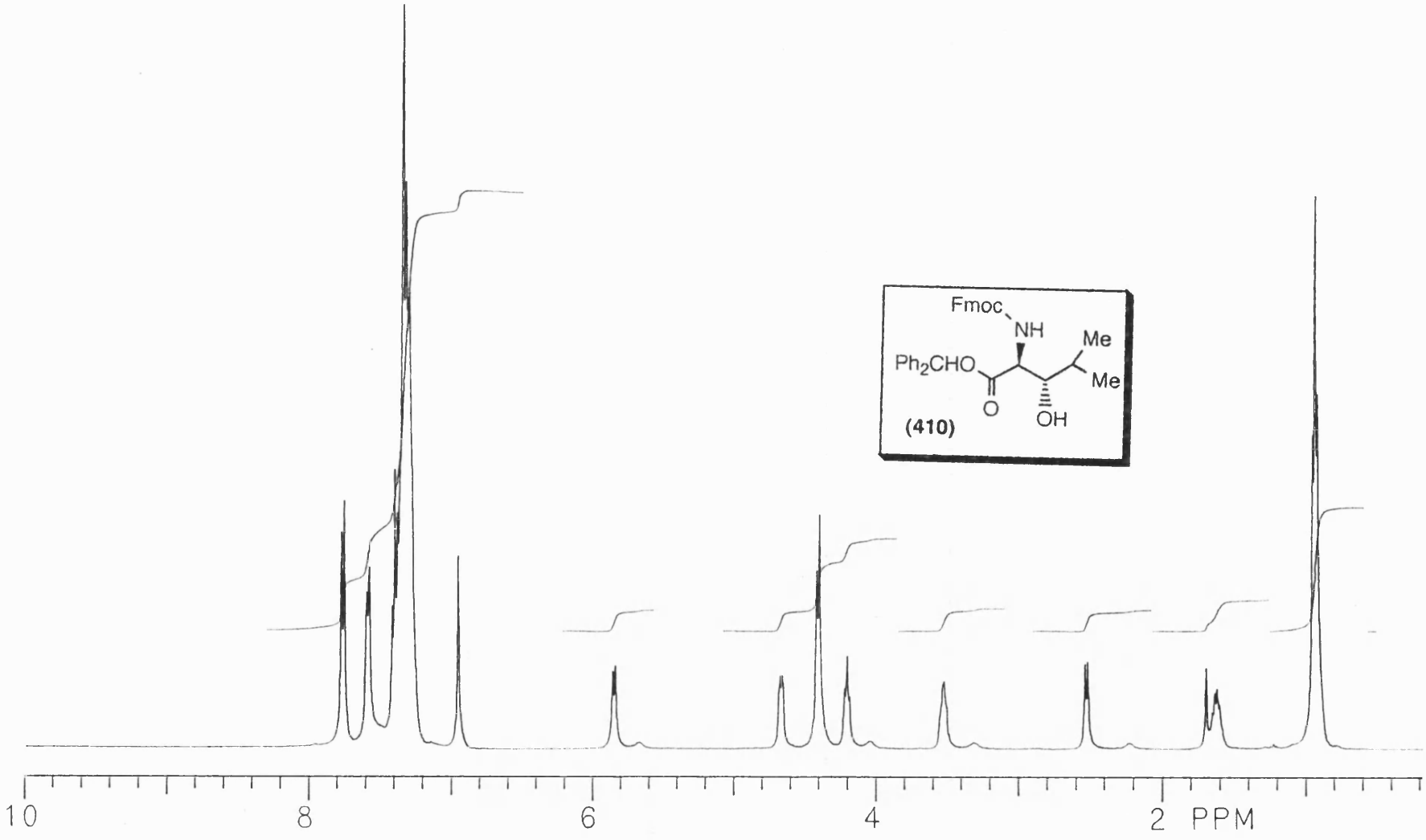

V/VMD/68 03 Oct 94 16: 24: 08
smooth 17 slope
32 scans, 2 cm-!, Gain = 1
%Transmittance
80
70
60
50
40
30
20
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
Fmoc
NH
Me
HO
Me
O
OH
(409)

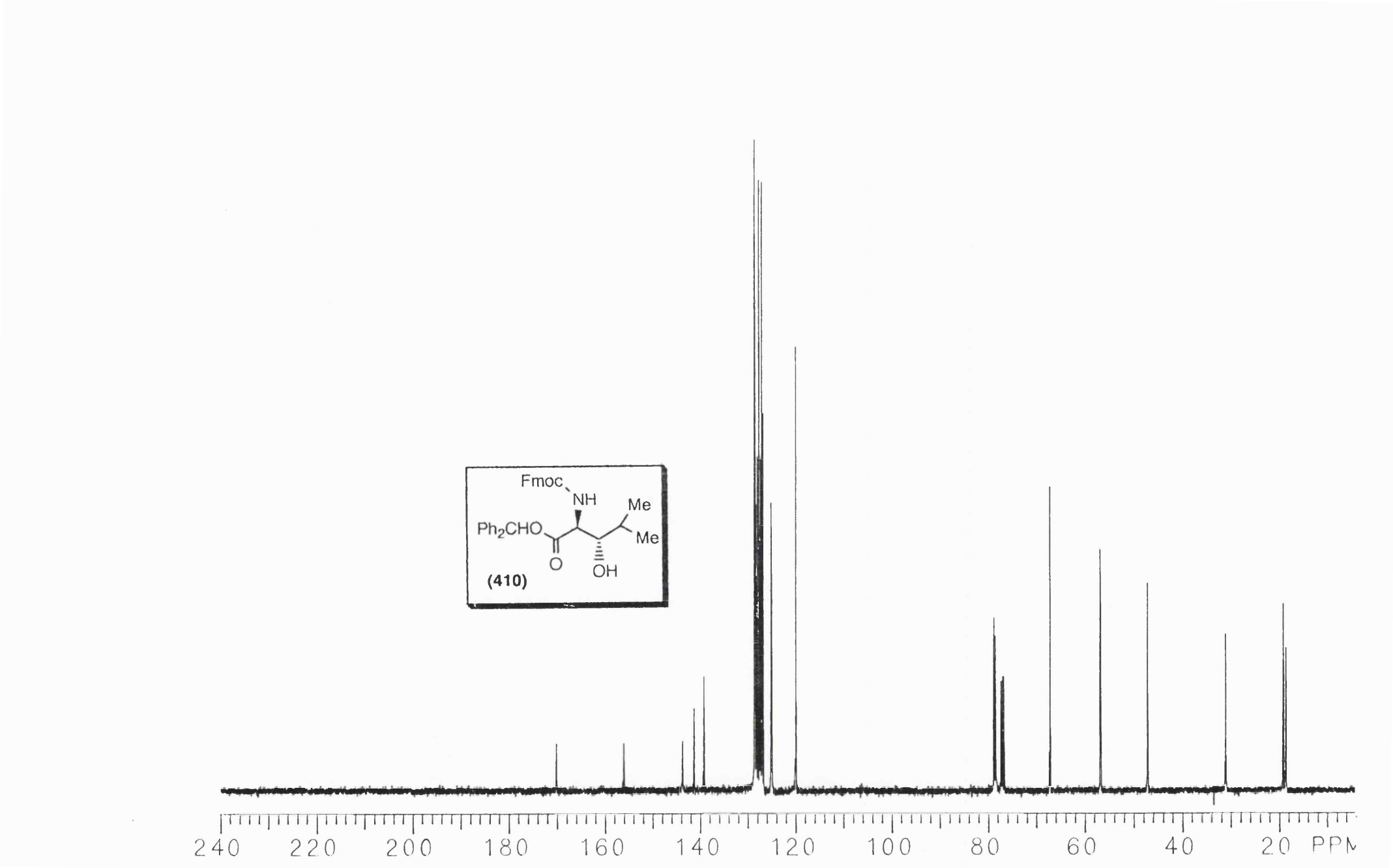

Fmoc
NH
Me
HO
Me
O
OH
(409)
12
10
8
6
4
2
0
PP

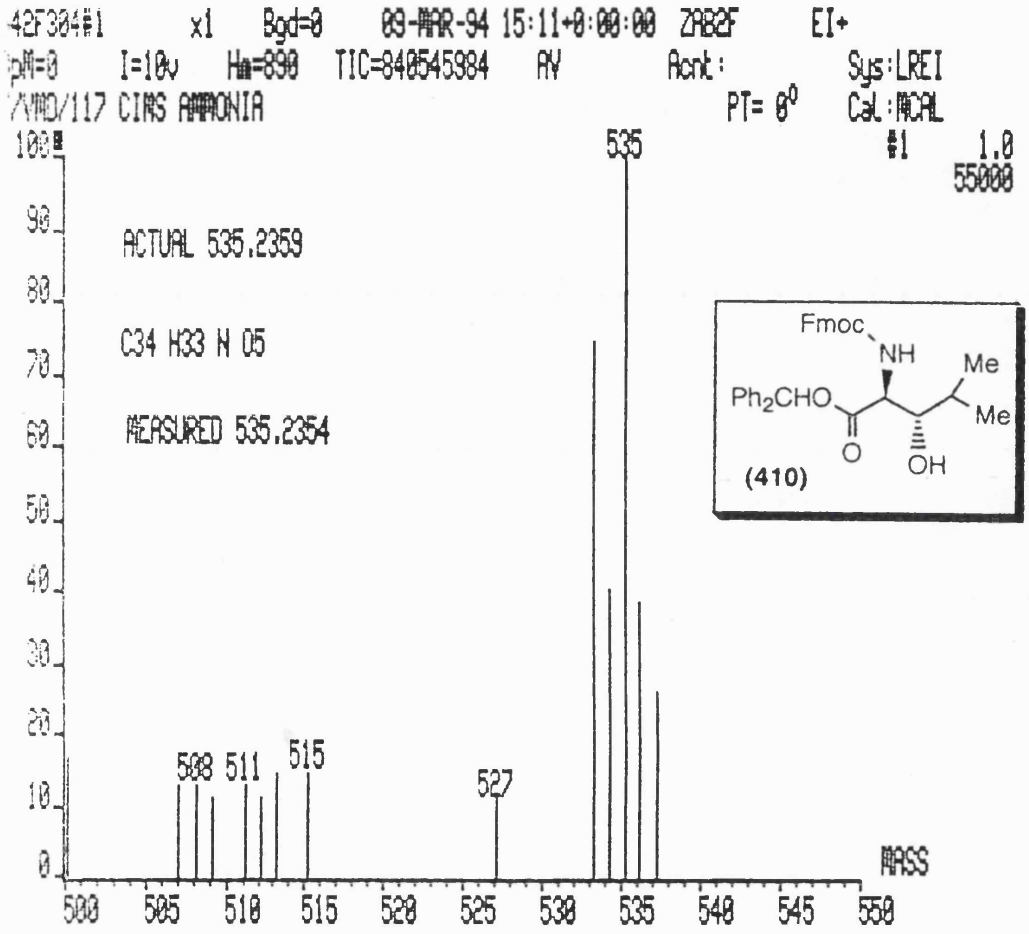

Fmoc
NH
Me
HO
Me
O
OH
(409)
220
200
180
160
140
120
100
80
60
40
20
PPM

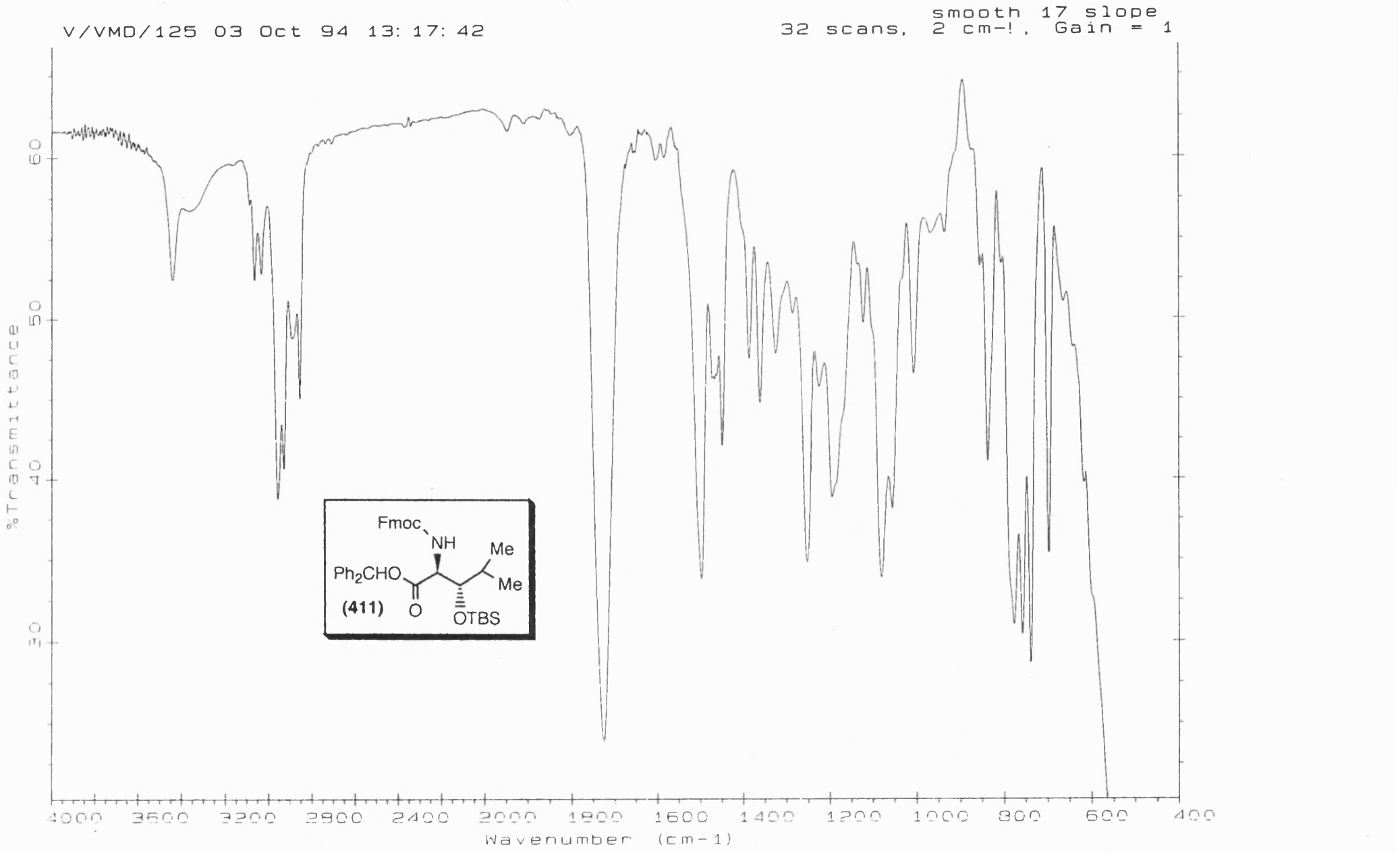

SE296#1 x1 Bgd=0 28-FEB-94 15:18+0:00:00 ZAB-SE FB+
M=0 I=2.0v Hm=1500 TIC=84640000 AV Acnt:ULSOP Sys:LMFAB
VMD-68 FAB TGT MATRIX PT= 0° Cal:FABCAL
100
90
80
70
60
50
40
30
20
10
0
370
#1 1
10390
ACTUAL 370.1654
C21 H24 N O5
MEASURED 370.1666
Fmoc
NH
Me
HO
Me
O
OH
(409)
355
352
367
MASS
340
345
350
355
360
365
370
375
380

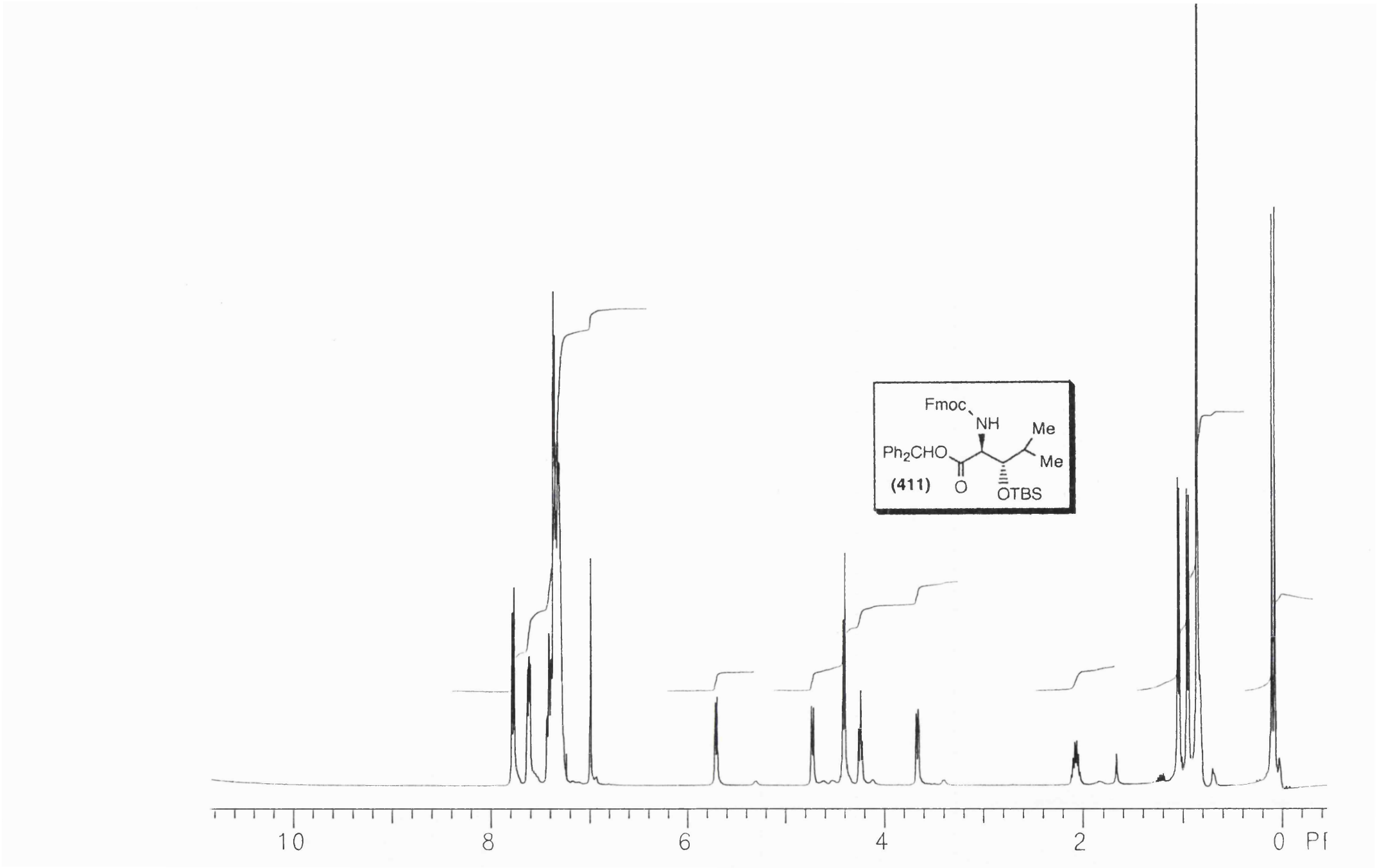

V/VMD/117 03 Oct 94 13: 02: 36
smooth 17
32 scans, 2 cm-!, Gain = 1
%Transmittance
70
60
50
40
30
20
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
Fmoc
NH
Me
Ph2CHO
Me
O
OH
(410)

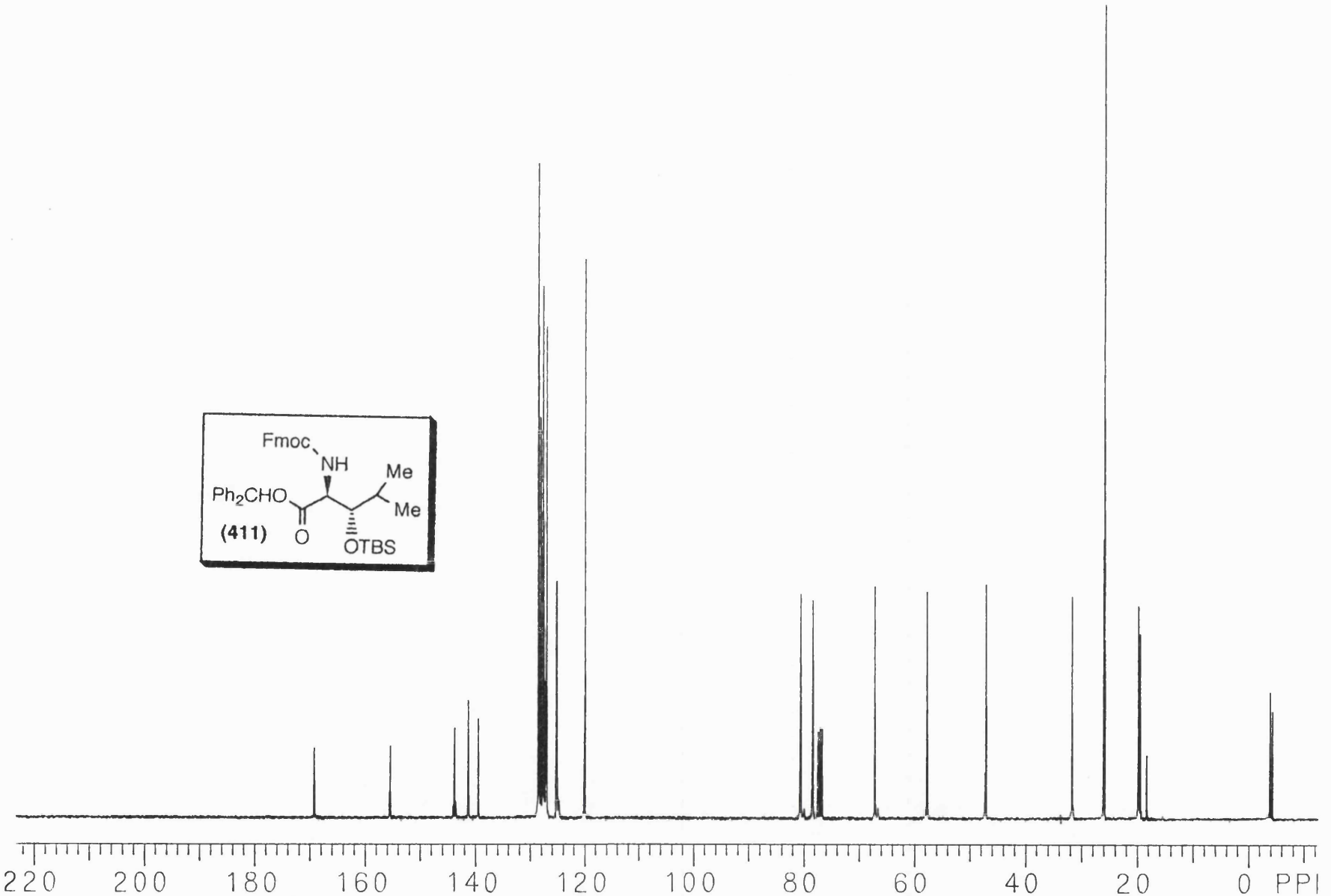

Fmoc
NH
Me
Ph2CHO
Me
O
OH
(410)
10
8
6
4
2 PPM

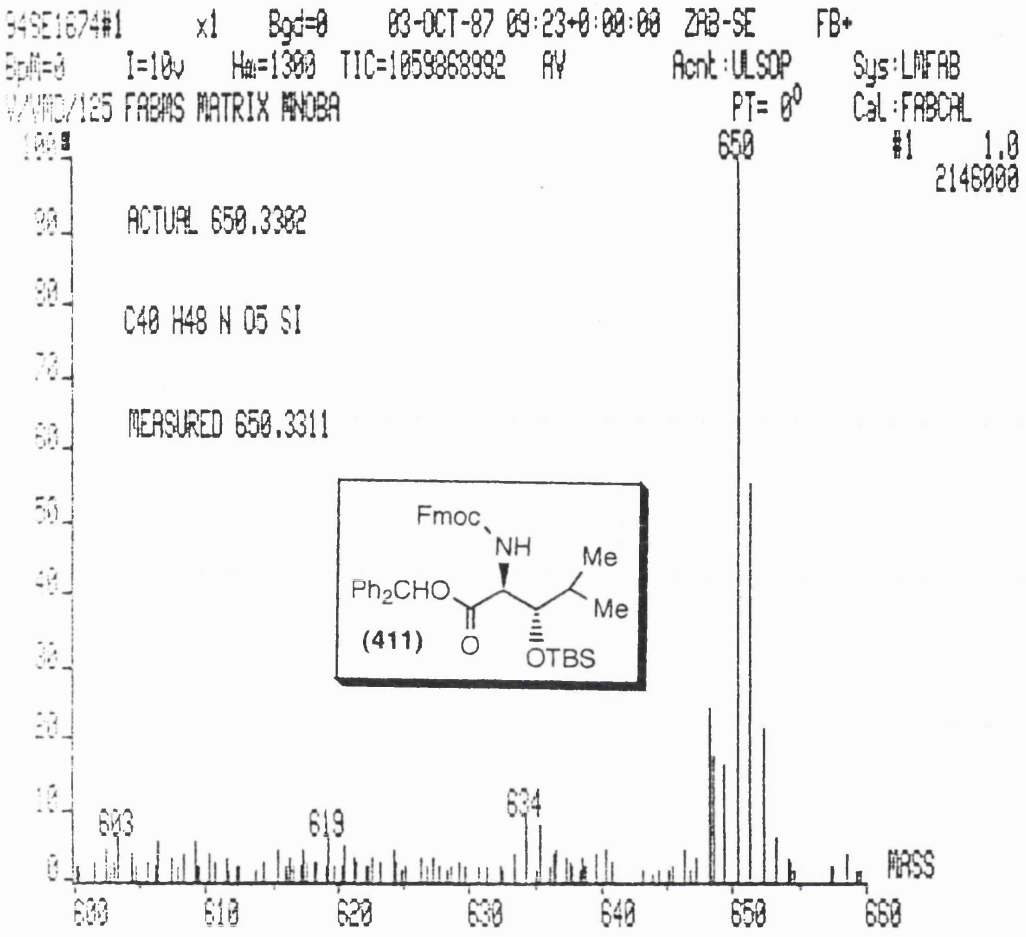

Fmoc
NH
Me
Me
Ph2CHO
O
OH
(410)
240
220
200
180
160
140
120
100
80
60
40
20
PPM

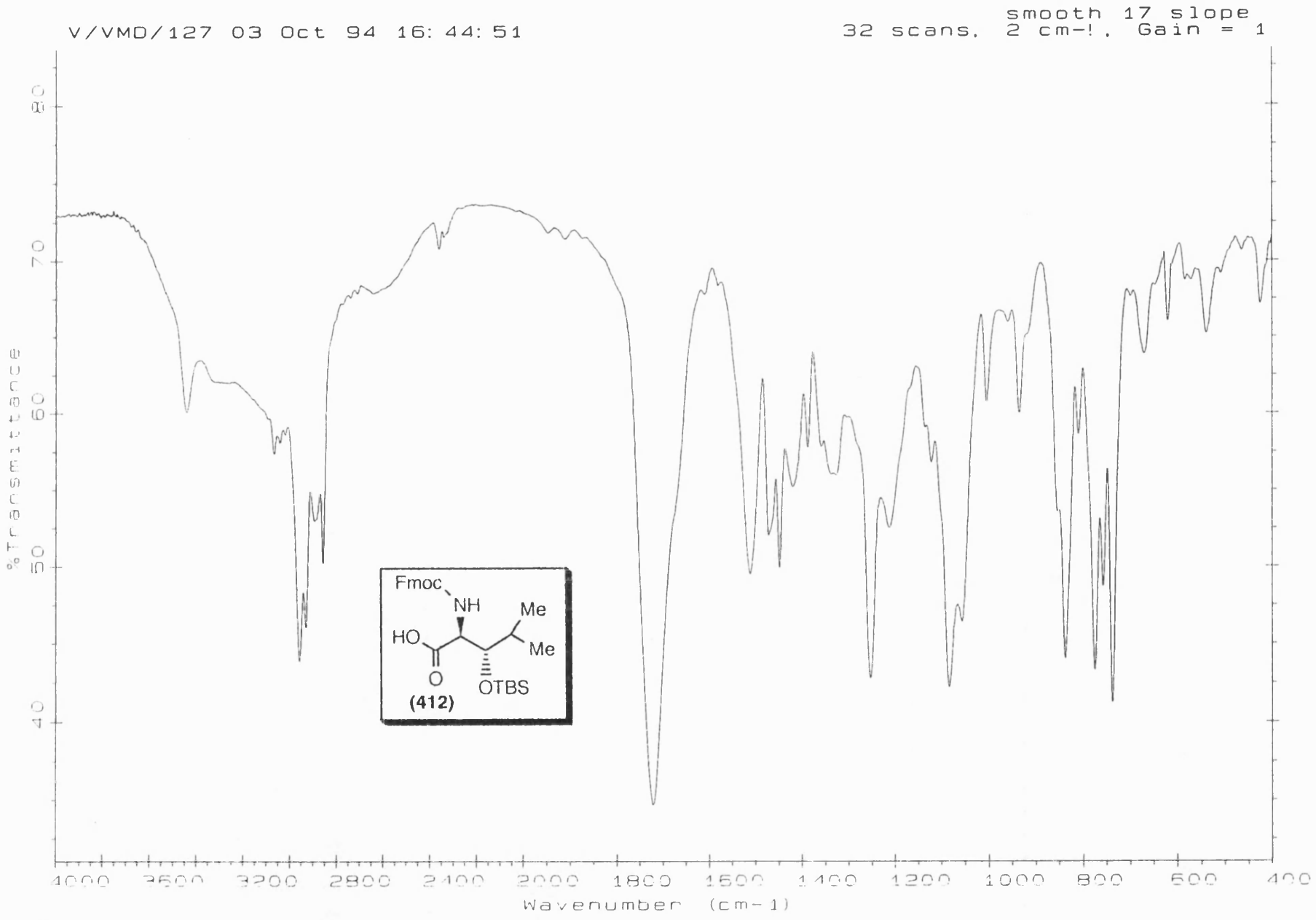

42F304#1 x1 Bgd=0 09-MAR-94 15:11+0:00:00 ZAB2F EI+
pM=0 I=10v Hm=800 TIC=840545984 AV Acnt: Sys:LREI
/AMD/117 CIMS AMMONIA PT= 0° Cal:MCAL
#1 1.0
55000
ACTURAL 535.2359
C34 H33 N 05
MEASURED 535.2354
535
508 511 515
527
MASS
100
90
80
70
60
50
40
30
20
10
0
500 505 510 515 520 525 530 535 540 545 550
Fmoc
NH
Me
Ph2CHO
Me
O
OH
(410)

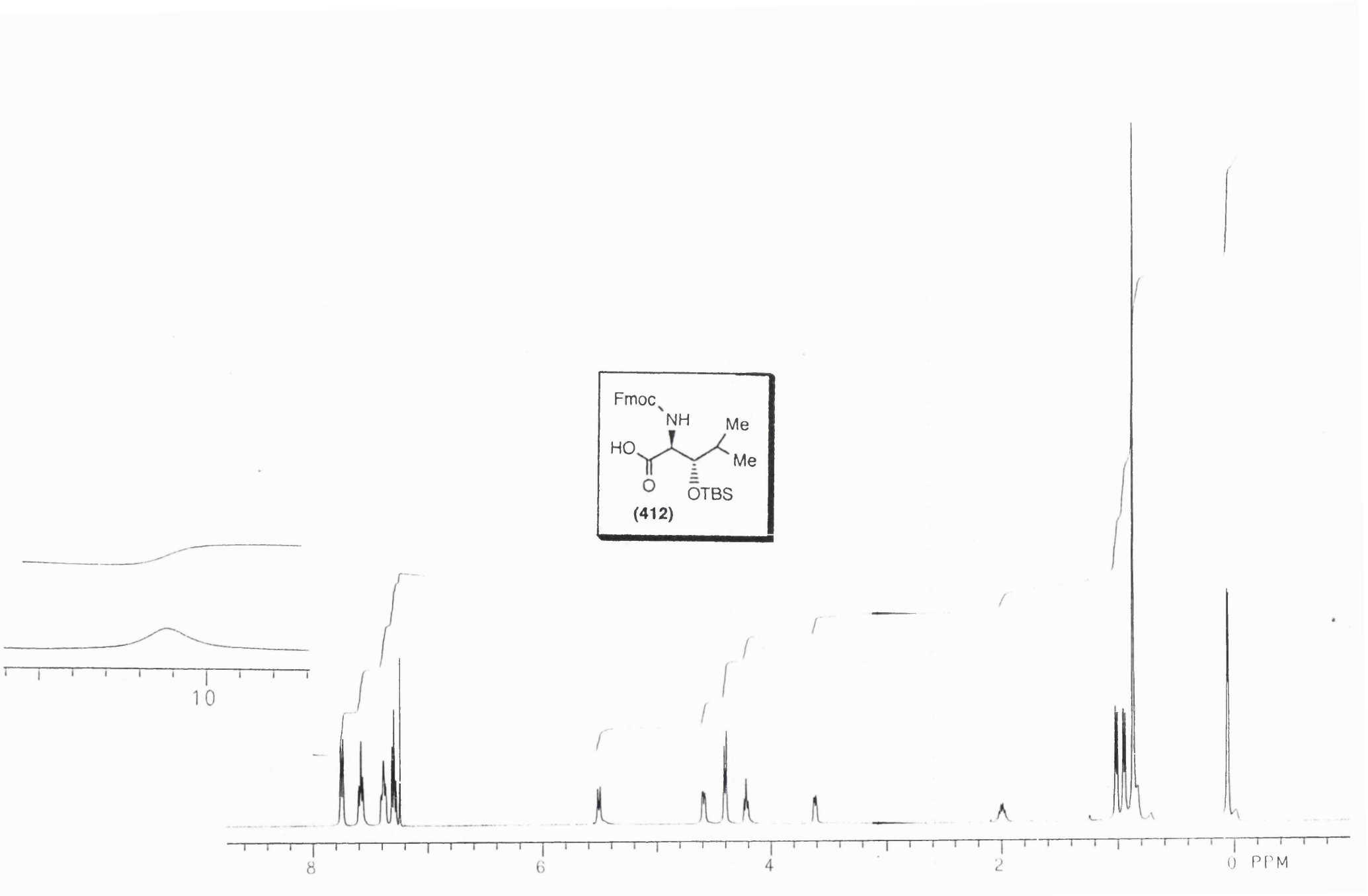

V/VMD/125 03 Oct 94 13: 17: 42
smooth 17 slope
32 scans, 2 cm-!, Gain = 1
%Transmittance
60
50
40
30
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
Fmoc
NH
Me
Me
Ph2CHO
(411)
O
OTBS

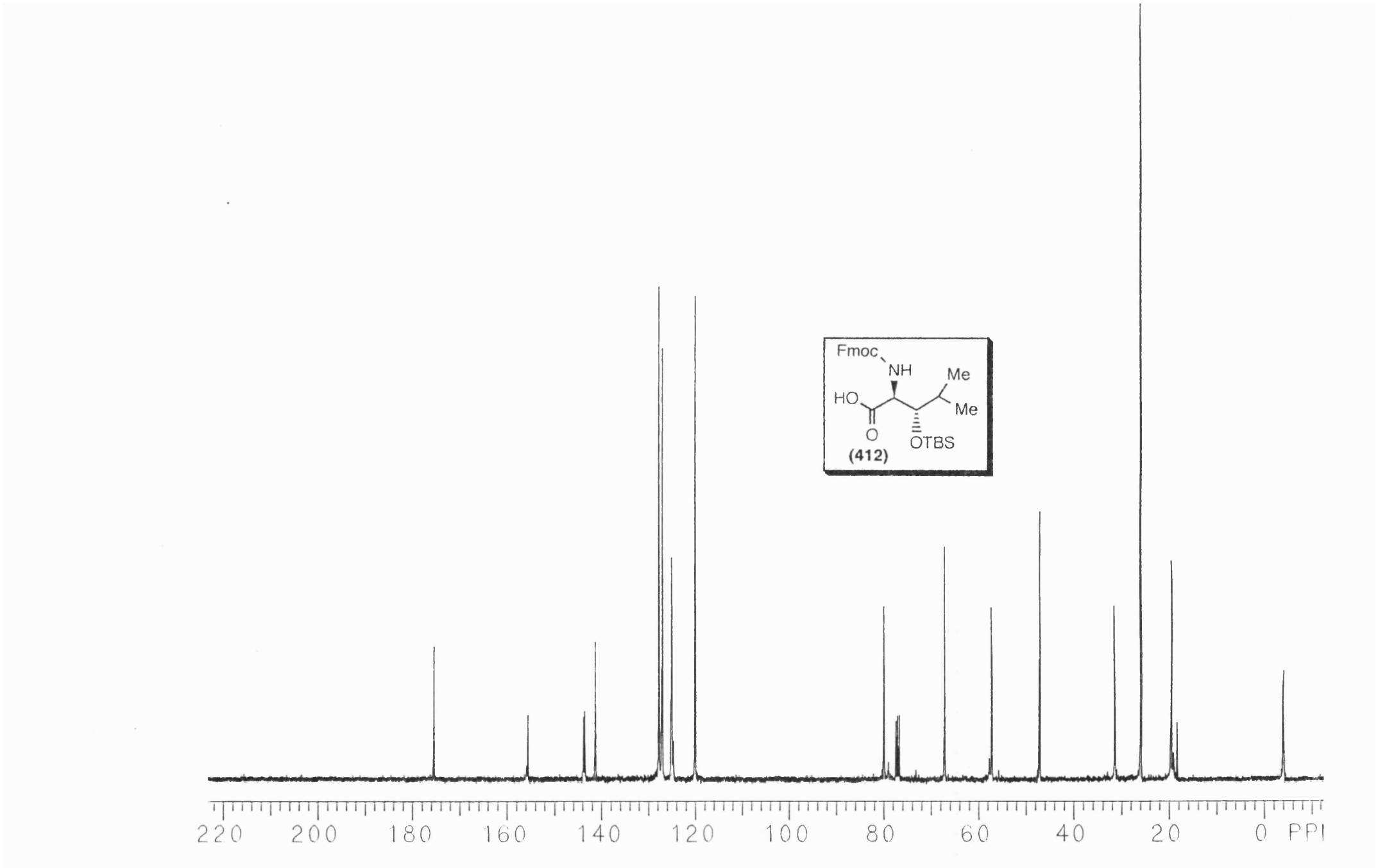

Fmoc
NH
Me
Me
Ph2CHO
(411)
O
OTBS
10
8
6
4
2
0

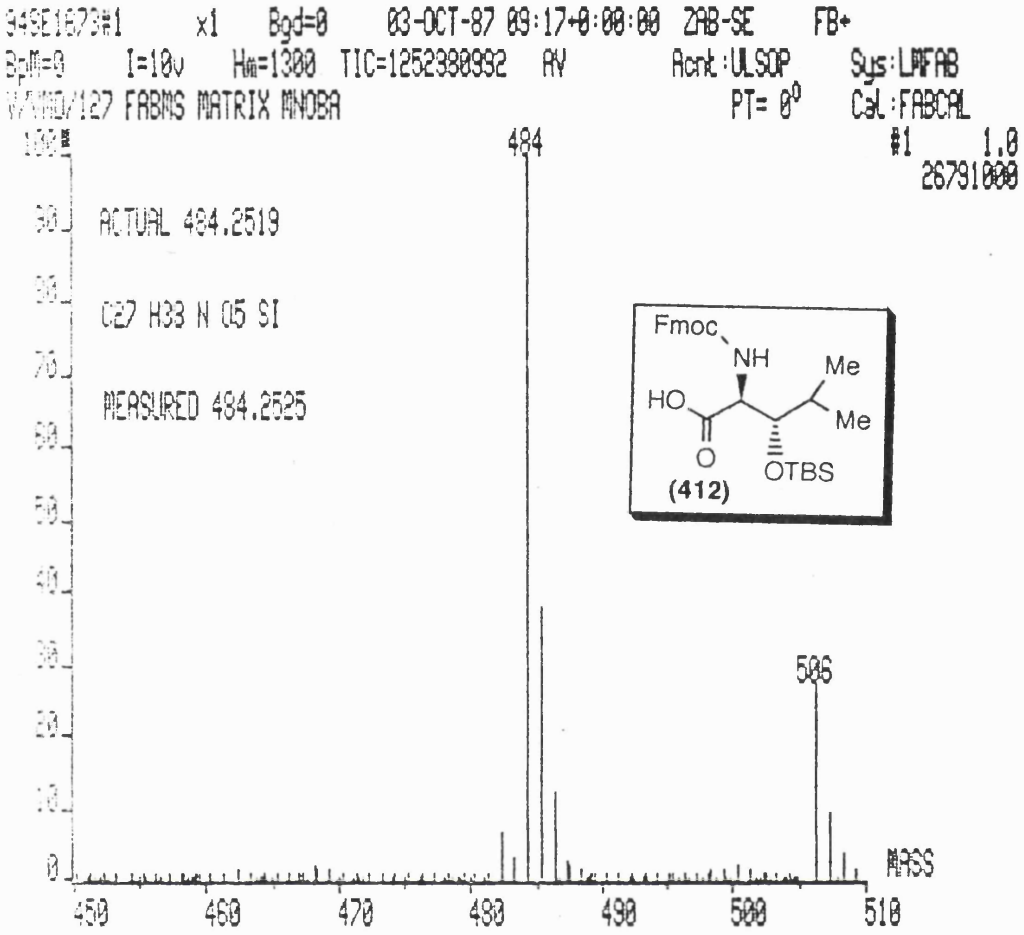

Fmoc
NH
Me
Me
Ph2CHO
(411)
O
OTBS
220
200
180
160
140
120
100
80
60
40
20
0
PPI

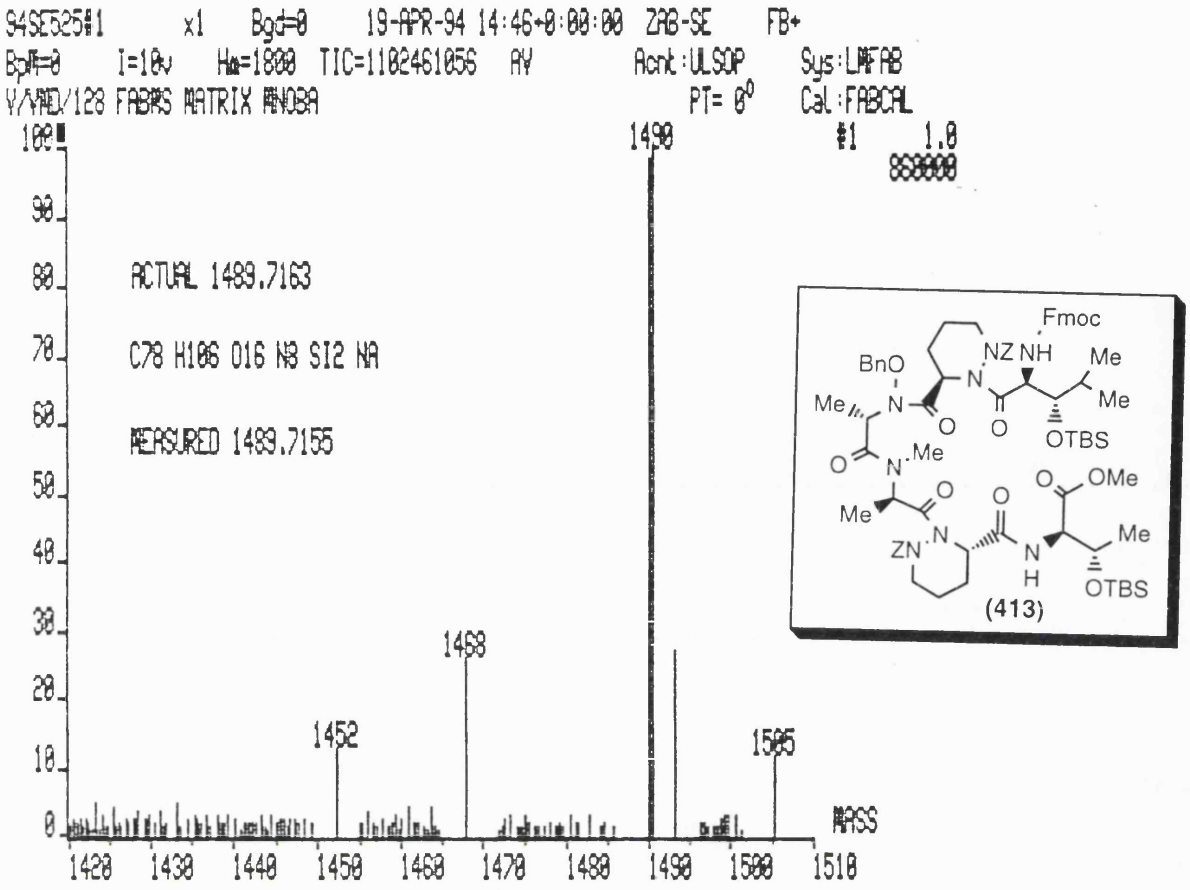

ACTURAL 650.3382
C40 H48 N 05 SI
MEASURED 650.3311
Fmoc
NH
Me
Me
Ph2CHO
(411)
O
OTBS
603
619
634
650
MASS

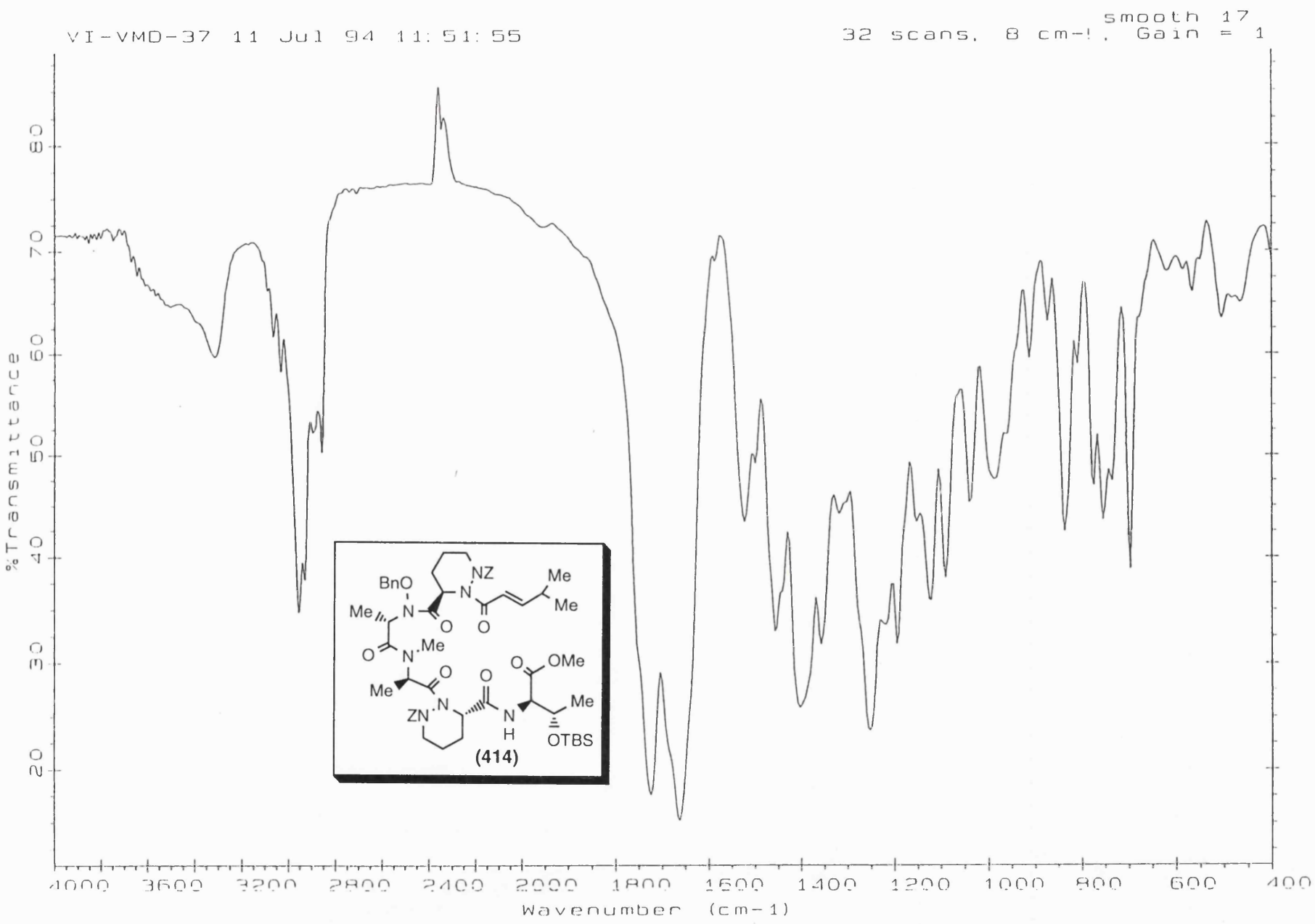

V/VMD/127 03 Oct 94 16: 44: 51
smooth 17 slope
32 scans, 2 cm-!, Gain = 1
%Transmittance
80
70
60
50
40
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
Fmoc
NH
Me
HO
Me
O
OTBS
(412)

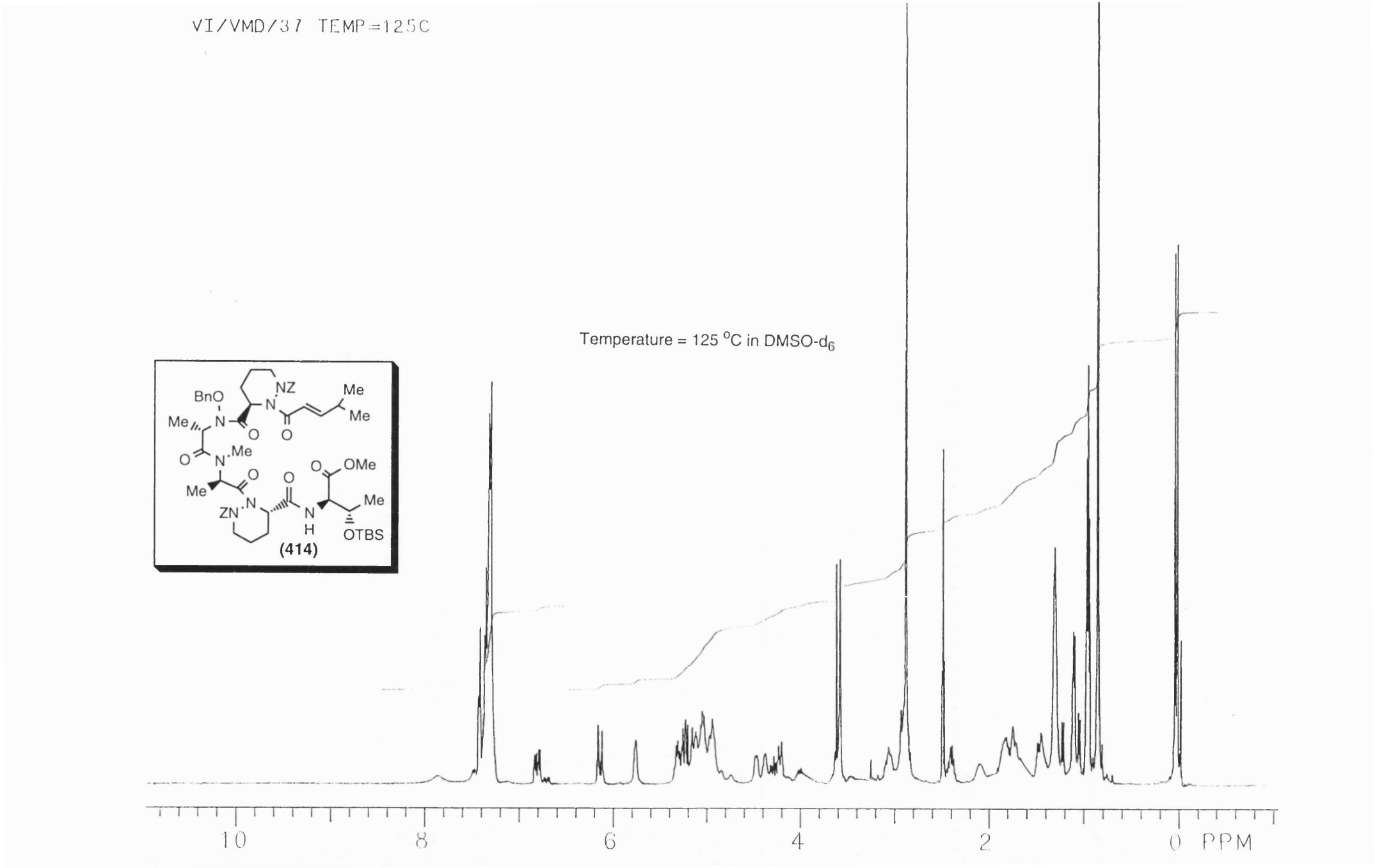

Fmoc
NH
Me
HO
Me
O
OTBS
(412)
10
8
6
4
2
0 PPM


Fmoc
NH
Me
HO
Me
O
OTBS
(412)
220
200
180
160
140
120
100
80
60
40
20
0
PPI

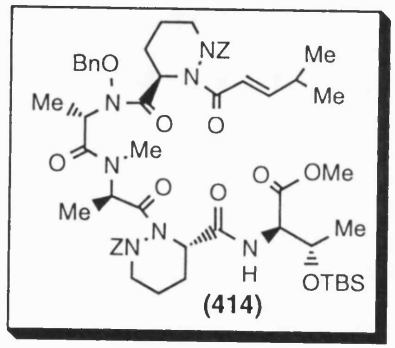

94SE1673#1 x1 Bgd=0 03-OCT-87 09:17+0:00:00 ZAB-SE FB+
BpM=0 I=10v Hm=1300 TIC=1252380992 AV Acnt:ULSOP Sys:LMFAB
PT= 0° Cal:FABCAL
#1 1.0
26791000
484
ACTUAL 484.2519
C27 H33 N O5 Si
MEASURED 484.2525
Fmoc
NH
Me
HO
Me
O
OTBS
(412)
506
MASS
450
460
470
480
490
500
510


94SE525#1 x1 Bgd=0 19-APR-94 14:46+0:00:00 ZAB-SE FB+
BpM=0 I=10v Hm=1000 TIC=1102461056 AV Acnt:ULSOP Sys:LMFAB
V/VMD/128 FABMS MATRIX MNOBA PT= 0° CaL:FABCAL
#1 1.0
ACTUAL 1489.7163
C78 H106 O16 N8 SI2 NA
MEASURED 1489.7155
1490
1468
1452
1505
MASS
1428 1430 1440 1450 1460 1470 1480 1490 1500 1510
Fmoc
NZ NH
BnO
Me
Me
OTBS
Me
Me
OMe
Me
OTBS
(413)


VI-VMD-37 11 Jul 94 11:51:55
smooth 17
32 scans, 8 cm-1, Gain = 1
%Transmittance
80
70
60
50
40
30
20
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Wavenumber (cm-1)
BnO
NZ
Me
Me
Me
N
O
O
O
N
Me
O
OMe
Me
Me
ZN
N
H
OTBS
(414)

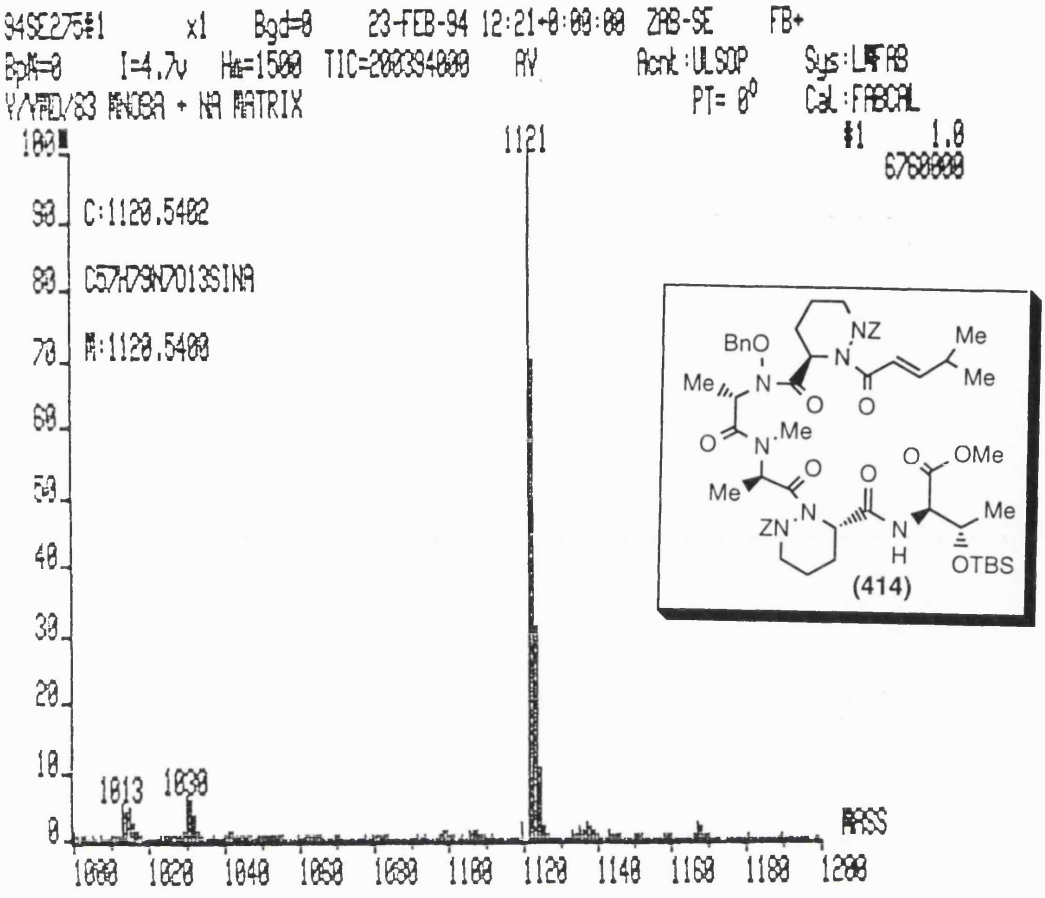

VI/VMD/37 TEMP=125C
Temperature = 125 °C in DMSO-d6
BnO
NZ
Me
Me
Me
OMe
Me
ZN
N
H
OTBS
(414)
10
8
6
4
2
0 PPM

VI/VMD/37 RT C



(414)

200 150 100 50 0 PPM


C:1120.5402
C57H79N7O13SINa
M:1120.5400
1121
1013
1030
MASS
(414)