Synthesis of Diferuloylspermines and Diferuloylspermidines Derivatives

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Master of Science Thesis in Organic Chemistry

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Thesis Title	Synthesis of Diferuloylspermines and Diferuloylspermidines Derivatives
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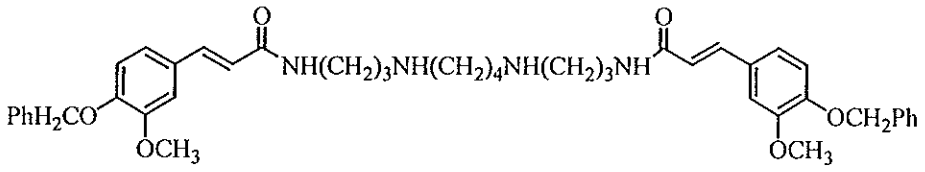
ABSTRACT

Diferuloylspermines and diferuloylspermidines derivatives (167-170) could be synthesized from the commercially available spermine and spermidine.



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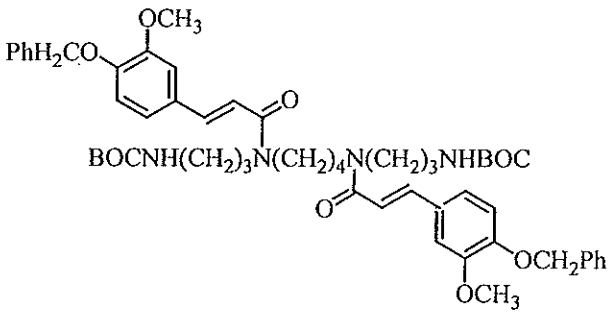

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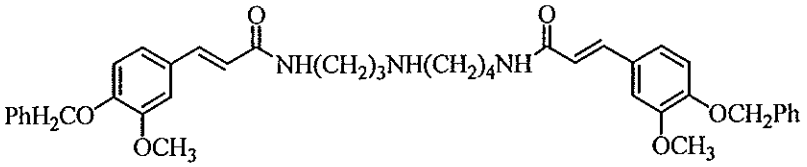

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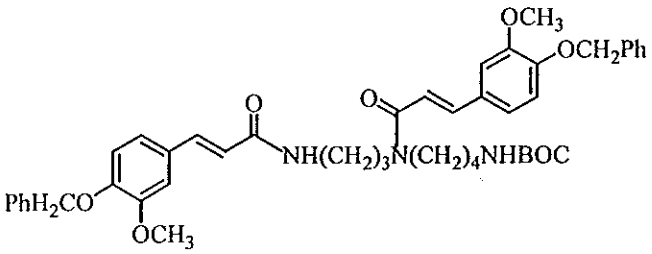

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ชื่อวิทยานิพนธ์	การสังเคราะห์อนุพันธ์ของสารประกอบไดเฟอรูโลอิลสเปอร์มีน และ ไดเฟอรูโลอิลสเปอร์มิดีน
ผู้เขียน	นางสาวรัตนา วรยุทธการ
สาขาวิชา	เคมีอินทรีย์
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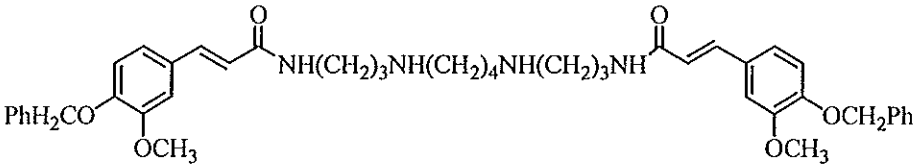
บทคัดย่อ

สังเคราะห์อนุพันธ์ของสารประกอบไดเฟอรูโลอิลสเปอร์มีน และ ไดเฟอรูโล-อิลสเปอร์มิดีน (167-170) ได้จากสเปอร์มีนและ สเปอร์มิดีนซึ่งสามารถหาซื้อได้



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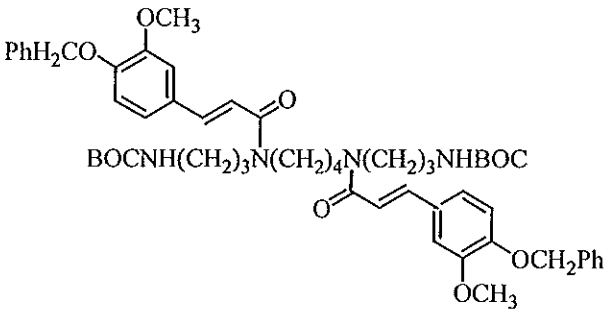

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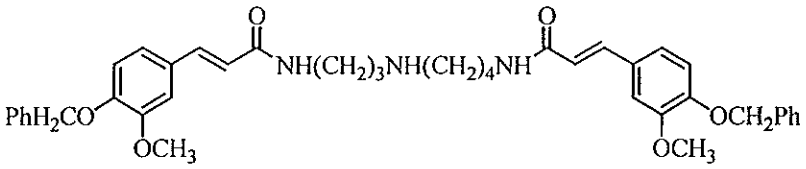
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O
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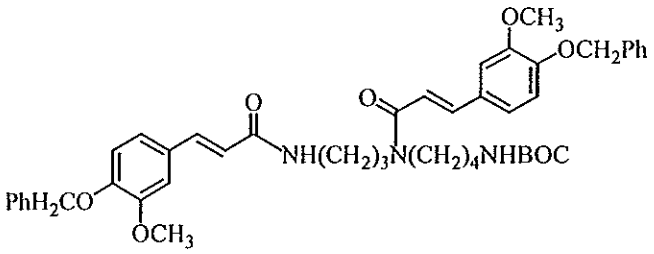
OCH3
OCH2Ph
OCH3

(169)


OCH3
OCH2Ph
O
O
NH(CH2)3N(CH2)4NHBOC
PhH2CO
OCH3

(170)

ACKNOWLEDGEMENT

I wish to express my gratitude to my supervisor, Assistant Professor Dr. Chatchanok Karalai and my co-supervisors, Associate Professor Dr. Kan Chantrapromma and Assistant Professor Chanita Ponglimanont for their advice, encouragement and guidance throughout this work.

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Rattana Worayuthakarn

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ABBREVIATIONS AND SYMBOLS

DCC	=	*N,N'*-dicyclohexylcarbodiimide
DMAP	=	4-dimethylaminopyridine
$(BOC)_2O$	=	di-*tert*-butyl-dicarbonate
BOC-ON	=	2-(*tert*-butoxycarbonyl-oxyimino)-2-phenylacetonitrile
THF	=	tetrahydrofuran
LAH	=	lithium aluminium hydride
HOBt	=	1-hydroxy-6-(trifluoromethyl) benzotriazole
Fmoc-Asn	=	*N*-(9-fluorenylmethoxycarbonyl)-L-asparagine
EDC.HCl	=	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
TFA	=	trifluoroacetic acid
TMSCl	=	trimethylsilyl chloride
MsCl	=	methanesulfonyl chloride
PCTr-Cl	=	polyamine conjugates on a 2-chlorotrityl resin
DIC	=	*N,N'*-diisopropylcarbodiimide
BzCl	=	benzoyl chloride
DIEA	=	*N,N'*-diisopropylethylamine
BOP	=	benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate
TBSCl	=	tetrabutylsilyl chloride
L-(+)-DET	=	(+)-Diethyl L-tartrate
LDP	=	lithium diphenylphosphide
TBAF	=	tetrabutylammonium fluoride

CHAPTER 1

INTRODUCTION

Polyamines occur in the plant and animal kingdoms as free bases (biogenic amines) as well as derivatives. The derivatives can be divided into several groups: one contains the di- or polyamine as part of a peptide [e.g., glutathionylspermidine, γ-glutamyl-cysteinylglycylspermidine] or as part of an amino acid [e.g., putreanine, $NH_2(CH_2)_4NH(CH_2)_2$ COOH]. Further, some antibiotics are known which contain a di- or polyamine. These include fatty acids and cinnamic acid conjugates as well as the simple, methylated compounds.

In addition to the well-known compounds putrescine, spermidine, spermine, and cadaverine, many other simple di-, tri- and tetraamine compounds are known in nature. Those that have been isolated as such from plants, animals or microorganisms are presented in **Table 1.**

Table 1. Naturally Occurring Di-, Tri- and Tetraamines.

	Trivial or systematic name
$H_2N(CH_2)_3NH_2$	1,3-Diaminopropane
$H_2N(CH_2)_4NH_2$	Putrescine
$H_2N(CH_2)_5NH_2$	Cadaverine
$H_2NCH_2\underset{\displaystyle OH}{\underset{\vert}{C}}H(CH_2)_2NH_2$	2-Hydroxyputrescine
$H_2N\diagdown\!\!\diagup HN{=}CNH(CH_2)_4NH_2$	Agmatine
$H_2N\diagdown\!\!\diagup HN{=}CNH(CH_2)_4NH\overset{\displaystyle NH_2}{\underset{\displaystyle NH}{\diagdown\!\!\diagup}}$	Arcaine
$H_2N\diagdown\!\!\diagup HN{=}CNH(CH_2)_5NH_2$	Homoagmatine
$H_2N\diagdown\!\!\diagup HN{=}CNH(CH_2)_5NHC\overset{\displaystyle NH_2}{\underset{\displaystyle NH}{\diagdown\!\!\diagup}}$	Audouine
$H_2N(CH_2)_3NH(CH_2)_3NH_2$	*sym*-Norspermidine
$H_2N(CH_2)_3NH(CH_2)_4NH_2$	Spermidine
$H_2N(CH_2)_3NH(CH_2)_5NH_2$	*N*-(3-Aminopropyl)-1,5-diaminopentane
$H_2N(CH_2)_4NH(CH_2)_4NH_2$	*sym*-Homospermidine
$H_2N\diagdown\!\!\diagup HN{=}CNH(CH_2)_3NH(CH_2)_4NH_2$	*N*-(3-Guanidinopropyl)-1,4-diaminobutane
$H_2N\diagdown\!\!\diagup HN{=}CNH(CH_2)_3NH(CH_2)_4NHC\overset{\displaystyle NH_2}{\underset{\displaystyle NH}{\diagdown\!\!\diagup}}$	Hirudonine
$H_2N(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$	*sym*-Norspermine (thermine)
$H_2N(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$	Thermospermine
$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	Spermine

These four aliphatic bases constitute the principal members of an ubiquitous family of natural products.

$NH_2(CH_2)_4NH_2$	putrescine	(1)
$NH_2(CH_2)_5NH_2$	cadaverine	(2)
$NH_2(CH_2)_3NH(CH_2)_4NH_2$	spermidine	(3)
$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	spermine	(4)

Polyamines have also been implicated as useful diagnostic markers in a number of diseases, such as cysticfibrosis (Rennert *et al.*, 1973) and in human malignancies. For instance, Russell has reported a dramatic elevation (up to fifty-fold) in the level of urinary polyamines in human subjects with various types of solid tumors or leukemia. Following surgical tumor removal, polyamines levels in the urine returned to near-normal levels. Bachrach has pointed out that a simple polyamine urinalysis may eventually constitute a routine diagnostic test for malignant tumors and for chemotherapy evaluation (Bachrach, 1973). Research on polyamines has been the subject of numerous monographs and reviews since 1970s.

Besides their presence in native form as free aliphatic bases, the common polyamines often occur conjugated with sugars, steroids, phospholipids, and peptides and also as substructural units within numerous families of plant alkaloids. Many of these more elaborate natural products exhibit remarkable biochemical and pharmacological profiles in their own right including antibiotic, antiviral, tumor-inhibitory and antihypertensive activities.

Classification of Polyamines

Polyamines can be classified into three groups on the basis of their nitrogen-containing structural features :

1. putrescine type (1)
2. spermidine type (3)
3. spermine type (4)

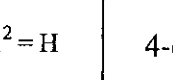
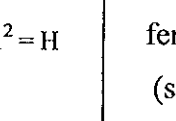
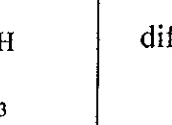
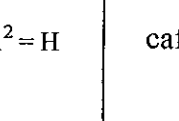
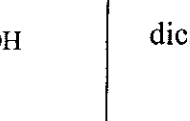
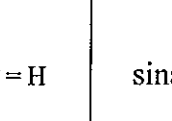
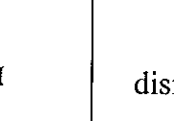
As already mentioned, these three bases belong to the biogenetic amines, but their derivatives (mostly containing fatty acid or cinnamic acid residues) are considered to be polyamines.

1. Putrescine Type

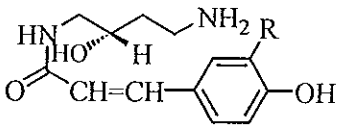
1.1 Simple Derivatives of Putrescine

A number of putrescine derivatives have been detected in nature containing one or two cinnamic acid derivatives attached through amide linkages. Paucine (9) is one of the first diaminoalkane alkaloids, which has been known since 1894 as a component of the seeds of *Pentaclethra macropylla.* Its structure was deduced from spectroscopic data (Guggisberg and Hesse, 1983). Other derivatives of putrescine are shown in **Table 2.** Several compounds were synthesized and compared to those obtained as natural products (Stoessl, 1965; Bird and Smith, 1981).

Table 2. Naturally occurring putrescine alkaloids.

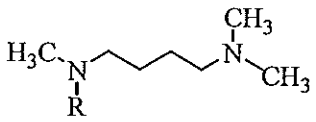
R^1-NH$(CH_2)_4$NH-R^2	NAME
R^1 = CO-CH$\overset{E}{=}$CH-  -OH, R^2 = H	4-coumaroyl putrescine (5)
$R^1 = R^2$ = CO-CH$\overset{E}{=}$CH-  -OH	di-4-coumaroyl putrescine (6)
R^1 = CO-CH$\overset{E}{=}$CH-  -OH, R^2 = H OCH_3	feruloyl putrescine (7) (subaphyline)
$R^1 = R^2$ = CO-CH$\overset{E}{=}$CH-  -OH OCH_3	diferuloyl putrescine (8)
R^1 = CO-CH$\overset{E}{=}$CH-  -OH, R^2 = H OH	caffeoyl putrescine(paucine) (9)
$R^1 = R^2$ = CO-CH$\overset{E}{=}$CH-  -OH OH	dicaffeoyl putrescine (10)
OCH_3 R^1 = CO-CH$\overset{E}{=}$CH-  -OH, R^2 = H OCH_3	sinapoyl putrescine (11)
OCH_3 $R^1 = R^2$ = CO-CH$\overset{E}{=}$CH-  -OH OCH_3	disinapoyl putrescine (12)

Two natural derivatives of 2-hydroxyputrescine have been found in wheat: N-(4-coumaroyl)- and N-feruloyl-2-hydroxyputrescine (13 and 14, respectively) and the synthesis of N-(4-coumaroyl)-2-hydroxyputrescine (13) has been reported by Mizusaki and co-workers (Guggisberg and Hesse, 1983).

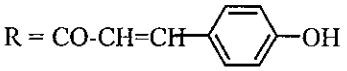


R = H	N-(4-coumaroyl)-2-hydroxyputrescine	(13)
R = OCH_3	N-(feruloyl)-2-hydroxyputrescine	(14)

Three methylated derivatives of putrescine are also known. Tetramethyl putrescine (15) was the first methylated derivative to be isolated from *Hyoscyamus muticus* in 1907. The comparison of the synthetic sample to the quarternized natural products proved the identity of both tetramethylene, 4-ditrimethyl ammonium diiodides (Guggisberg and Hesse, 1983). N,N,N′-Trimethyl-N′-(4-hydroxy-2-cinnamoyl) putrescine (16) and N,N,N′-Trimethyl-N′-(4-methoxy-2-cinnamoyl) putrescine (17) were isolated from three *Kniphofia* species (Guggisberg and Hesse, 1983).



R = CH_3 (15)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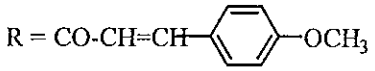
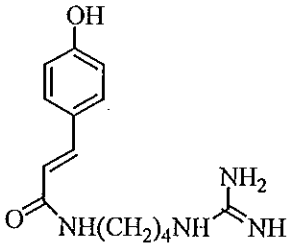

(17)

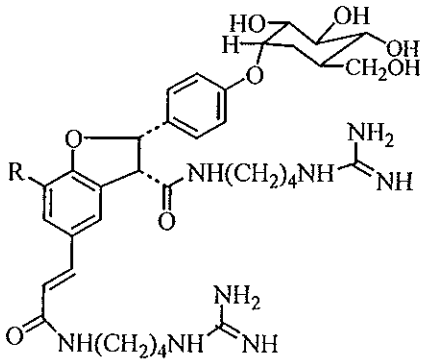
1.2 Agmatine Derivatives

Several agmatine derivatives have been isolated from barley seedlings. All are conjugates of coumaric acid, for example 4-coumaroylagmatine (18) and possess antifungal activity (Stoessl, 1966).



(18)

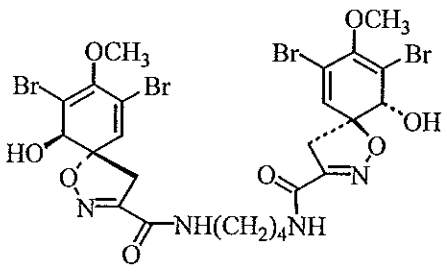
The other compounds are hordatine A (19) and hordatine B (20). The mixture of hordatine A and B glucosides called hordatine M has not been separated (Stoessl, 1966, 1967).



R = H	hordatine A	(19)
R = OCH_3	hordatine B	(20)

1.3 Aerothionine

Aerothionine, a tetrabromo derivative, has been isolated from sponges *Aplysina aerophoba* and *Verongia thiona.* The proposed structure is (21) (Fattorusso *et al.*, 1970).



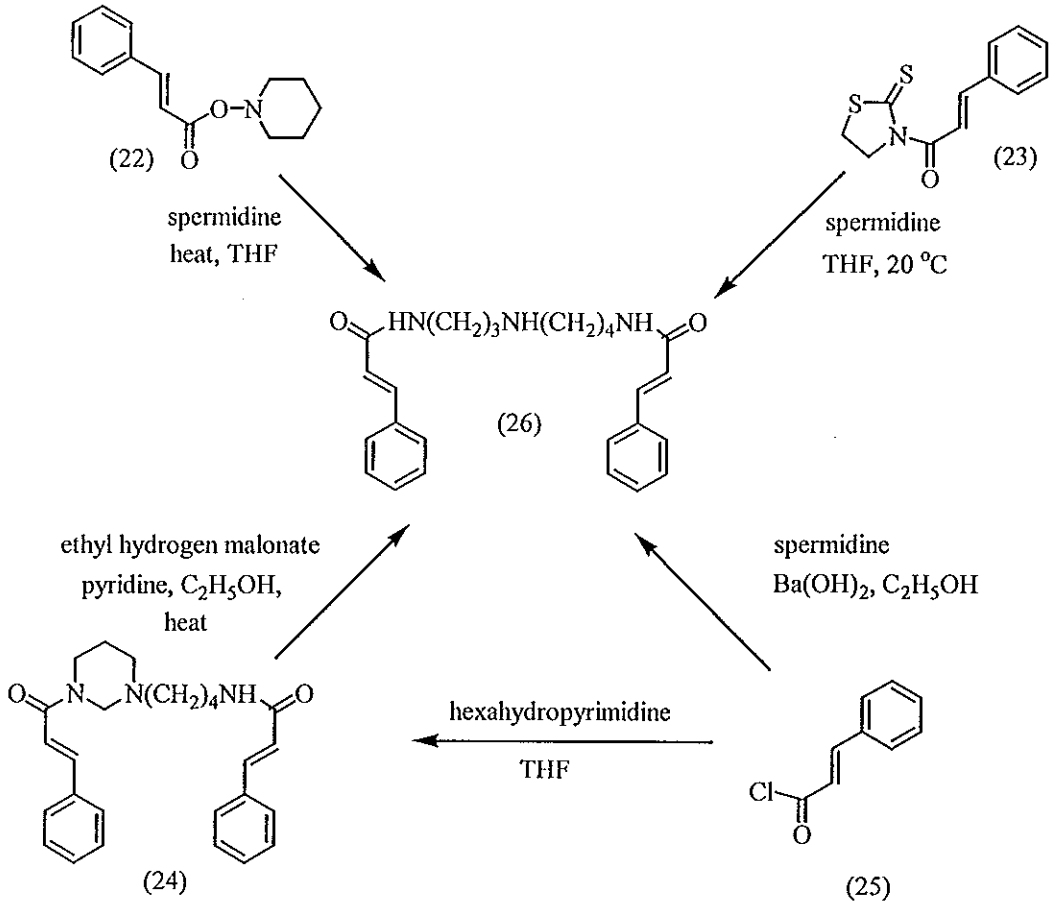
(21)

2. Spermidine Type

2.1 The Simple Open-Chain Spermidine Derivatives of Natural Origin

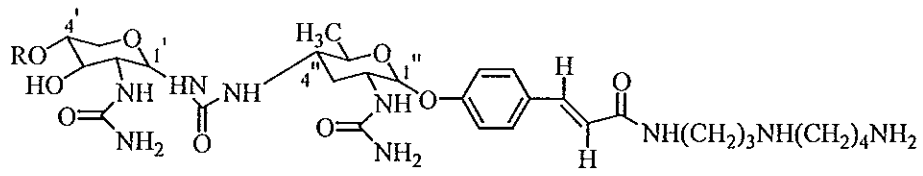
Spermidines substituted with cinnamic acid derivatives seem to be widely distributed in the plant kingdom. Cinnamic acid (alkaloid maytenine), caffeic acid (caffeoylspermidine, dicaffeoylspermidine), coumaric acid (coumaroylspermidine, dicoumaroylspermidine, tricoumaroylspermidine), ferulic acid (feruloylspermidine, diferuloylspermidine and sinapic acid (sinapoylspermidine, disinapoylspermidine) are known as aromatic amide substituted of spermidine. Several compounds were synthesized in the past (Bergeron *et al.*, 1980, 1981; Fujita *et al.*, 1980).

Maytenine (26) was the first of these so-called simple alkaloids to be isolated and identified structurally by the use of MS, NMR and UV spectroscopy (Englert *et al.*, 1979). Several syntheses of (26) have been reported as shown in **Scheme 1.**

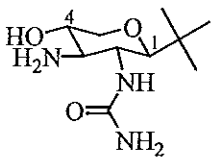


Scheme 1. Syntheses of maytenine (26)

The most fascinating compounds are the glycocinnamoyl spermidines LL-BM123 β, γ_1 and γ_2 (27-29) which were isolated from an unidentified species of *Nocardia* (Broschard *et al.*, 1978). The γ_1, and γ_2 components are of special interest in view of their potent activity against gram-negative organisms and their protective effects against infection.



R =



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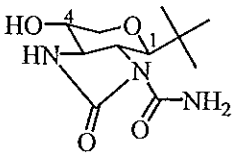

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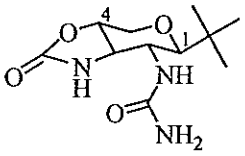
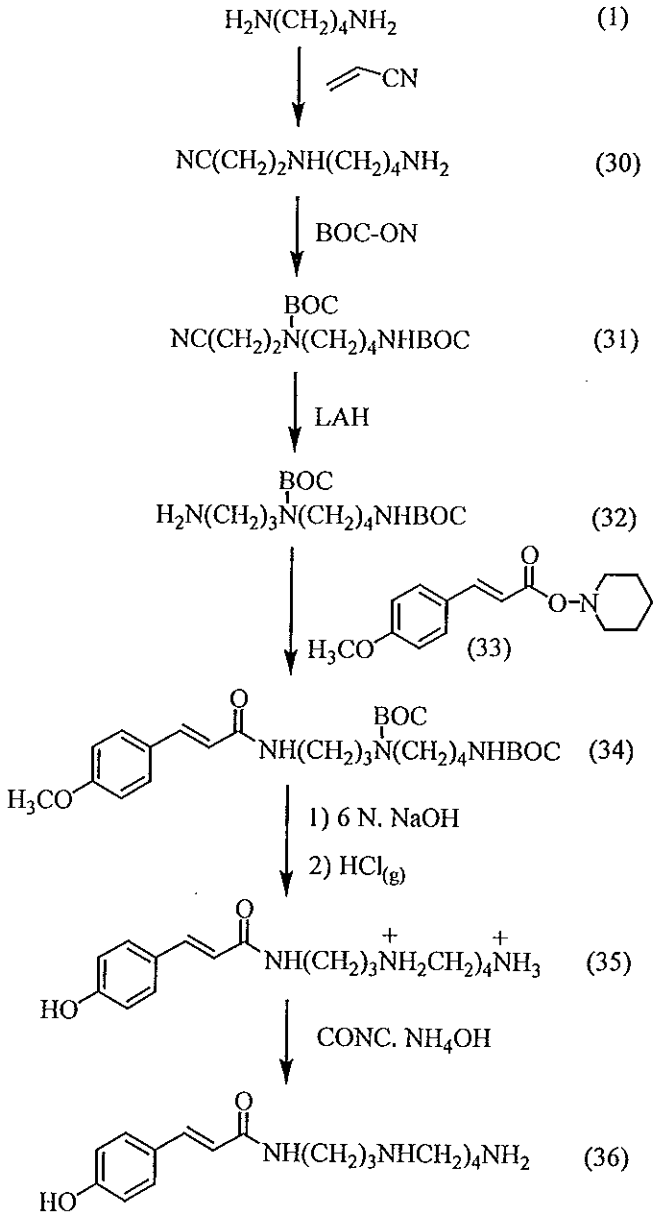


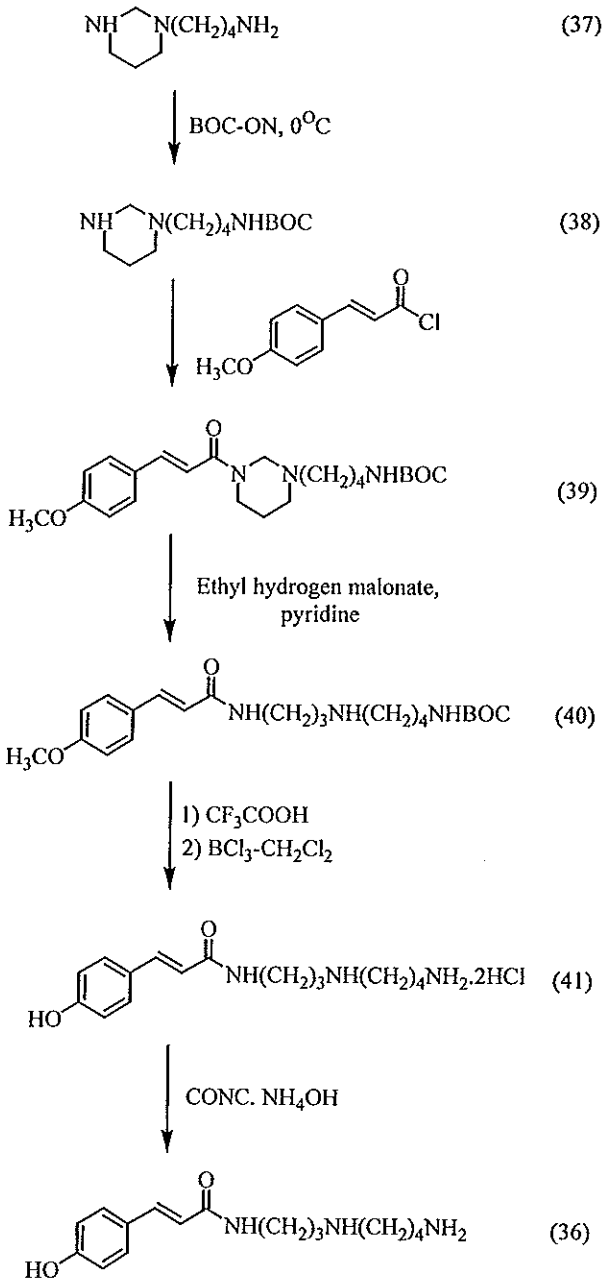
(29)

The first synthesis of the aglycone LL-BM 123 (36) was reported by Michael Humora and James Quick (Humora and Quick, 1979). The synthetic approach is depicted in **Scheme 2** .



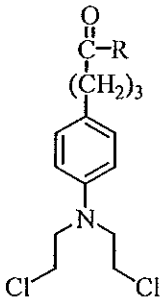
Scheme 2. Synthesis of the aglycone LL-BM 123 (36) (Hamura and Quick, 1979)

Chantrapromma and co-workers have prepared the agylcone LL-BM 123 (36) from hexahydropyrimidine (37) (Chantrapromma *et al.*, 1990). The synthetic route to the aglycone LL-BM 123 is shown in **Scheme 3.**



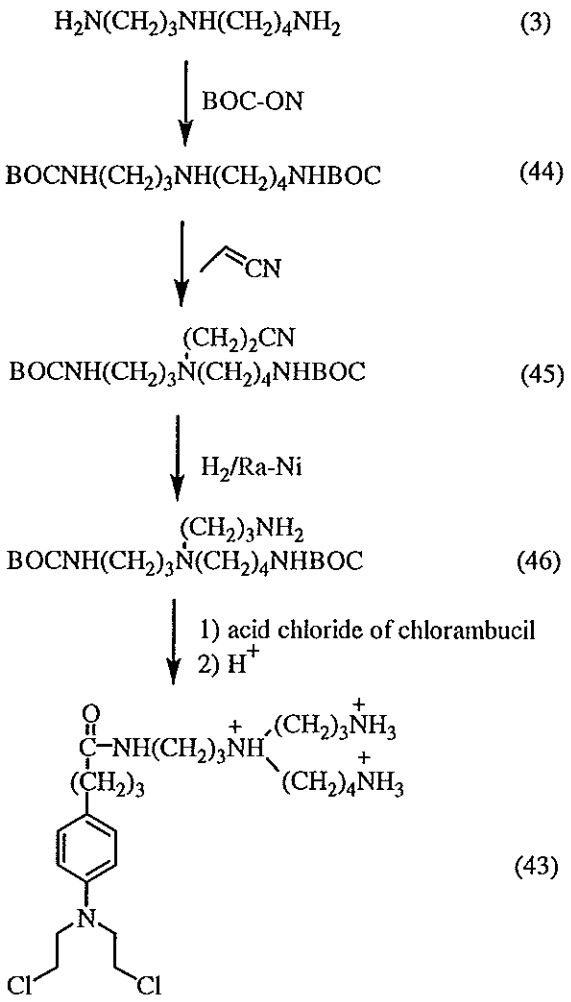
Scheme 3. Synthesis of the aglycone LL-BM 123 (36) (Chantrapromma *et al.*, 1990)

Gerald M. Cohen and co-workers have published the synthesis of chlorambucil spermidine conjugated (43) from spermidine (3) via N^1,N^8-bis-Boc-spermidine (44) as shown in **Scheme 4.** The compound (43) has been shown to crosslink DNA 10^4 times more efficiently than chlorambucil (42), a well known aromatic nitrogen widely used in the treatment of chronic lymphocytic leukemia, lymphomas and ovarian carcinoma (Cohen *et al.*, 1992).



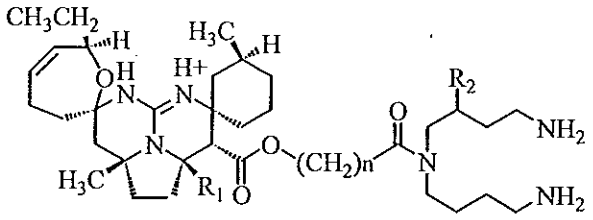
R = OH chlorambucil (42)

R = $NH(CH_2)_3\overset{+}{N}H_2(CH_2)_4\overset{+}{N}H_3$ chlorambucil spermidine conjugated (43)



Scheme 4. Synthesis of chlorambucil spermidine conjugated (43)

Ponasik and Ganem have published the synthesis of 3-hydroxyspermidine, an unusual polyamine constituent of cytotoxic marine compounds (Ponasik and Ganem, 1995) . This synthesis conducted as part of the characterization of the crambescidin family of antiviral and cytotoxic guanidines (47-51) from the red sponge *Crambe crambe* led to the identification of 3-hydroxyspermidine (52) (Jares-Erijman *et al.*, 1991). The synthetic route to 3-hydroxyspermidine (52) is shown in **Scheme 5.**



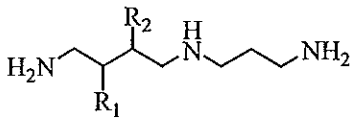
$R_1 = R_2 = OH$; n = 15 (47)

$R_1 = R_2 = OH$; n = 16 (48)

$R_1 = R_2 = OH$; n = 17 (49)

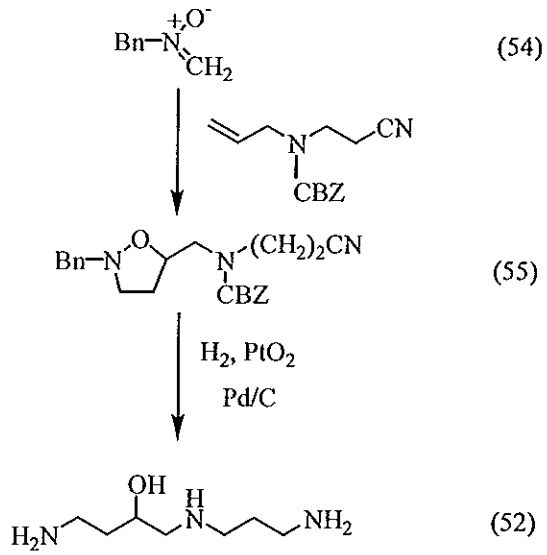
$R_1 = R_2 = H$; n = 15 (50)

$R_1 = H$, $R_2 = OH$; n = 15 (51)



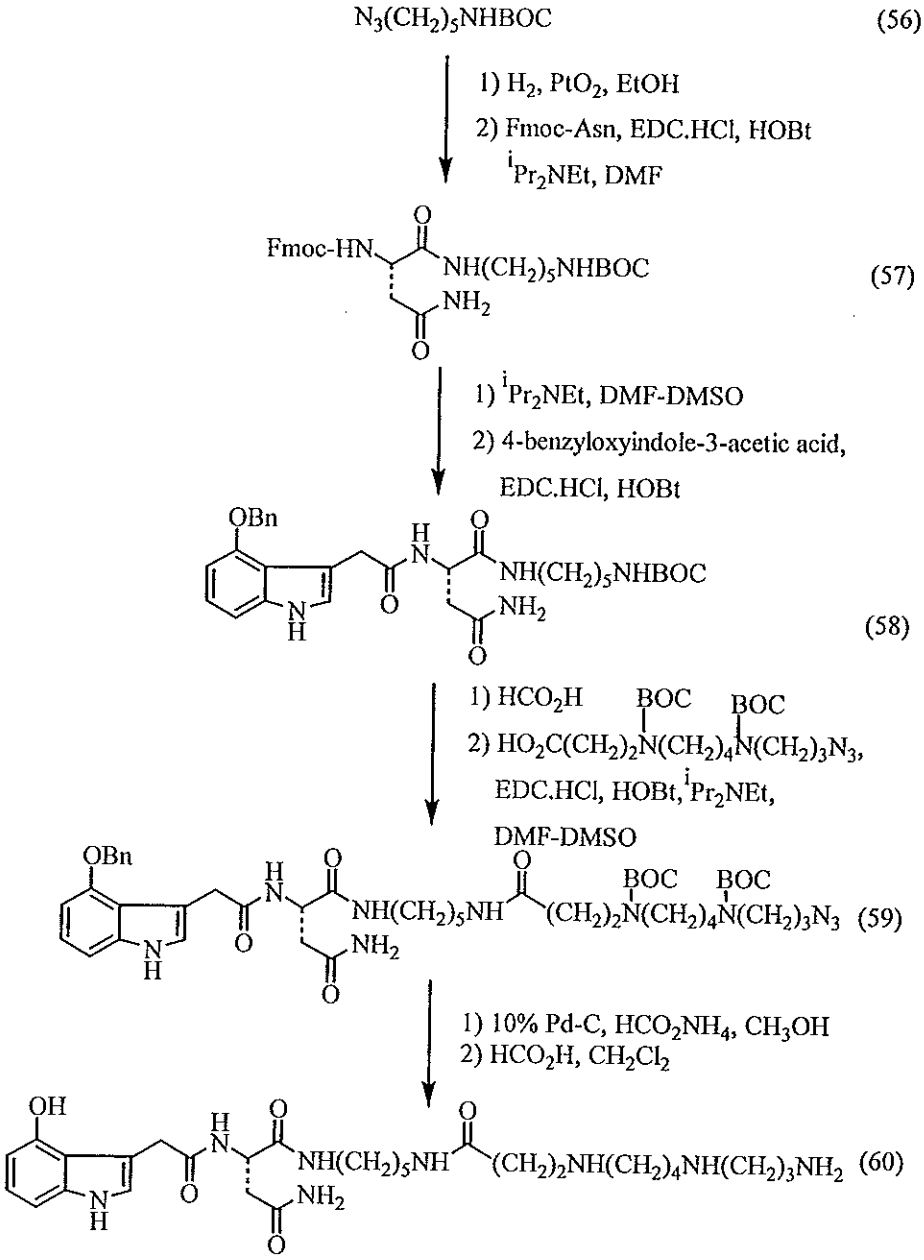
$R_1 = H, R_2 = OH$ (52)

$R_1 = OH, R_2 = H$ (53)



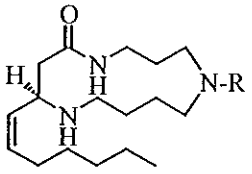
Scheme 5. Synthesis of 3-hydroxyspermidine (52) (Ponasik and Ganem, 1995)

Miyashita and co-workers have prepared nephilatoxin-1 (NPTX-1), a Joro spider (*Nephila clavata*) toxin having a 4-hydroxyindole nucleus via two key azide intermediates (Miyashita *et al.*, 1997). The first total synthesis of nephilatoxin-1(NPTX-1) (60) is shown in **Scheme 6.**

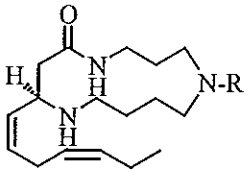


Scheme 6. Synthesis of nephilatoxin-1 (NPTX-1) (60)

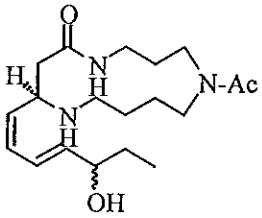
Spermidine alkaloids (+)-loesenerine (61), (+)-17,18-didehydro-loesenerine (62) and (+)-16,17-didehydroloesenerin-18-ol (63) were isolated from *Maytenus loeseneri* Urb. (Celastraceae). At the same time, (+)-myricoidine (64) and (+)-dihydromyricoidine (65) were reported as constituents of *Clerodendrum myricoides* Vatke (Verbenaceae). The synthesis of (+)-(2S)-dihydromyricoidine (65) was done in analogy to the synthesis of (-)-(2R)-dihydromyricoidine. The synthetic route to (+)-(2S)-myricodine (64) (Hausermann and Hesse, 1998) is shown in **Scheme 7.**



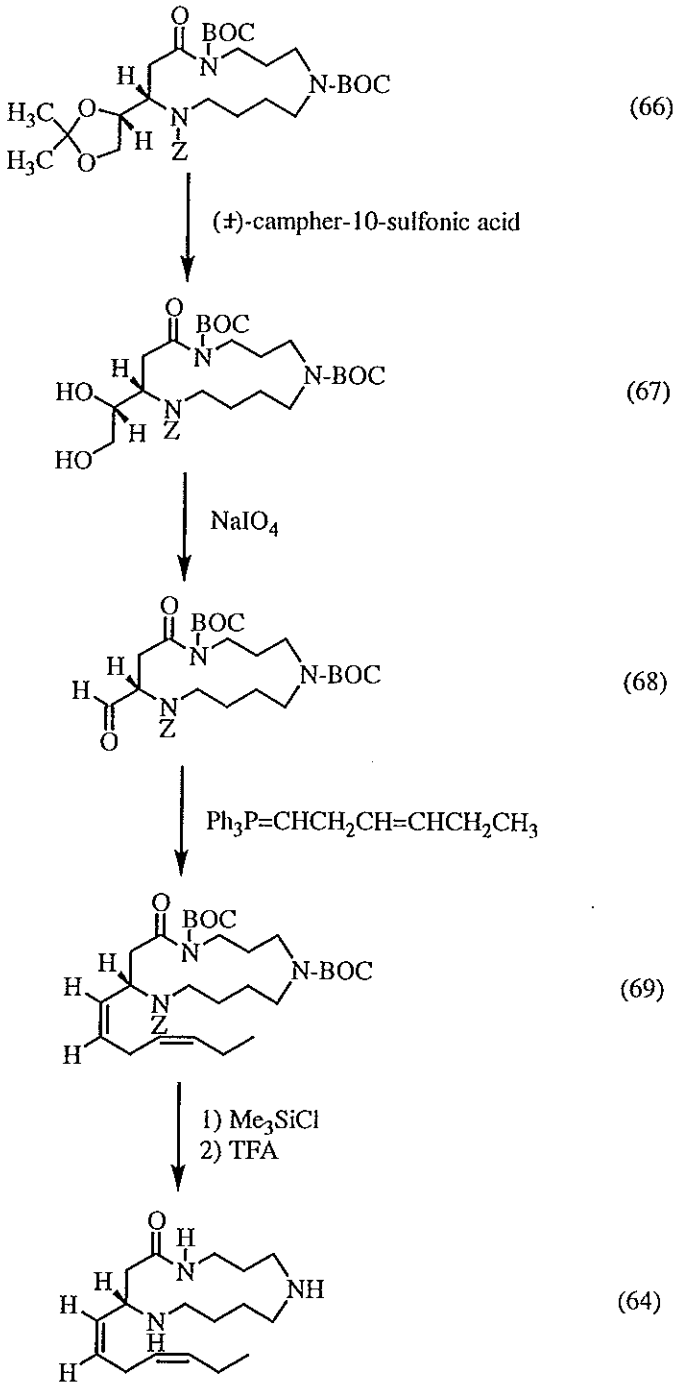
R = Ac (*R*)-loesenerine (61)
R = H (*R*)-dihydromyricoidine (65)



R = Ac (*R*)-17,18-didehydroloesenerine (62)
R = H (*R*)-myricoidine (64)



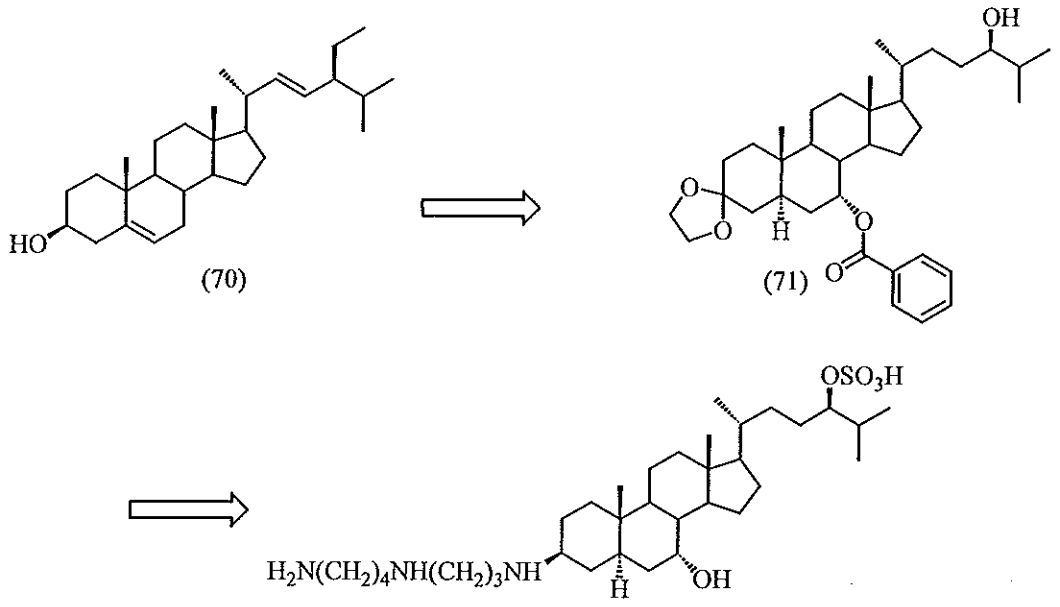
(R)-16,17-didehydroloesenerine-18-ol (63)



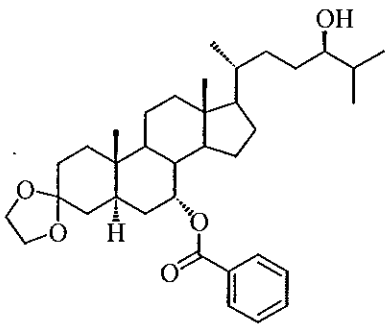
Scheme 7. Synthesis of (+)-(2S)-myricoidine (64)

Squalamine (76), a natural aminosterol, was isolated from the tissues of the dogfish shark. This compound has shown antimicrobial activity. More recently, squalamine was found to be antiangiogenic in that it inhibited endothelial cell function and retarded the growth of solid tumor. The syntheses of this compound have been reported (Moriarty *et al.,* 1994, 1995; Pechulis *et al.*, 1995).

The fifteen steps synthesis of squalamine were described by Zhang and co-workers (Zhang *et al.*, 1998). The first ten steps of this route have been reported (70 to 71) (Rao *et al.*, 1997; Jones *et al.*, 1998). The last five steps by Zhang and co-workers is shown in **Scheme 8.**

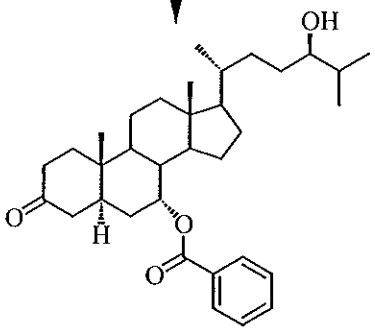


(70) (71) (76)



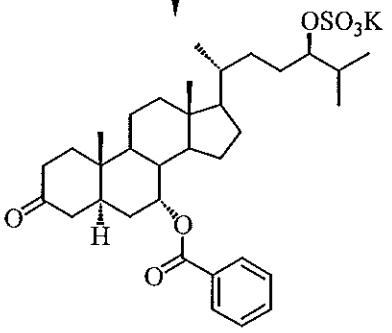
(71)

Amberlyst 15 ion exchange resin/ acetone



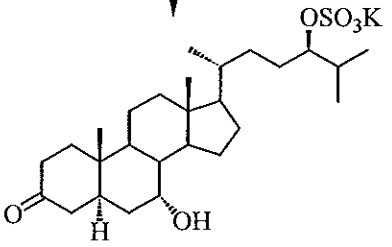
(72)

SO_3-py, pyridine, 80°C



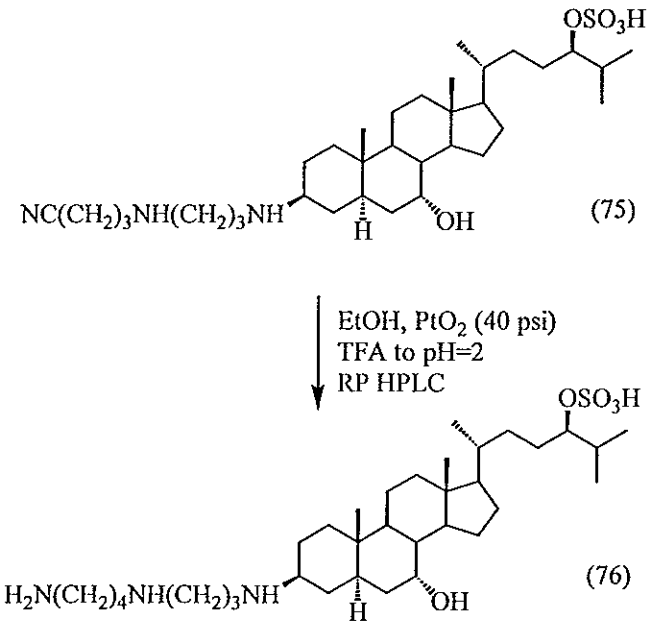
(73)

KOH, CH_3OH



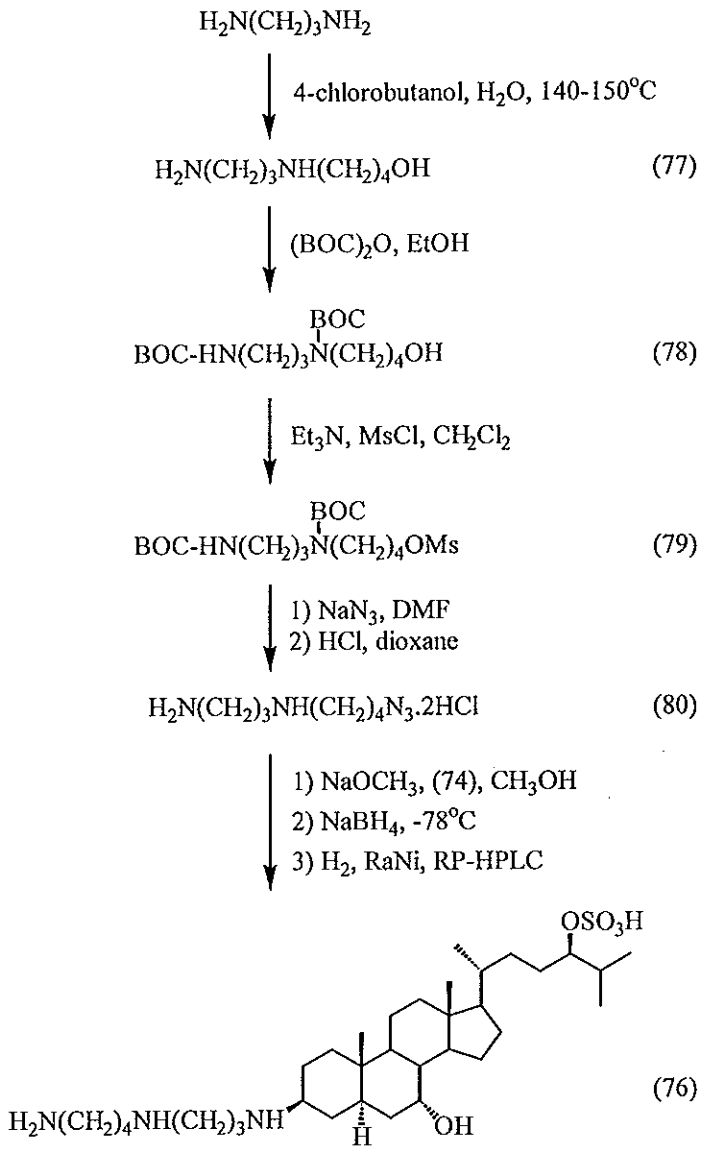
(74)

$NC(CH_2)_3NH(CH_2)_3NH_2$
trimethyl orthoformate
$NaBH_4$, CH_3OH



Scheme 8. Synthesis of squalamine (76) (Zhang *et al.*, 1998)

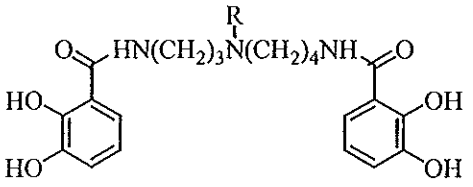
Recently Alexander L. Weis and co-workers have published the synthesis of squalamine (76) from 1,3-diaminopropane via azido spermidine equivalent (80) as shown in **Scheme 9.**



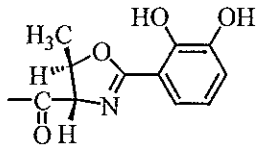
Scheme 9. Synthesis of squalamine (76) (Weis *et al.*, 1999)

2.2 Siderophore

Three siderophores (microbial iron-transport agents) belonging to the class of spermidine alkaloides: agrobactine (81) was isolated from *Agrobacterium tumefaciens* and both parabactine (82) and 1,8-bis(2,3-dihydroxybenzamido)-4-azaoctane (83) were isolated from *Paracoccus denitrificans* (Neilands *et al.*, 1979; Tait, 1975). The syntheses of these compounds have been reported (Bergeron *et al.*, 1980, 1981; Fujita *et al.*, 1980).

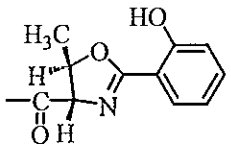


R =



agrobactine (81)

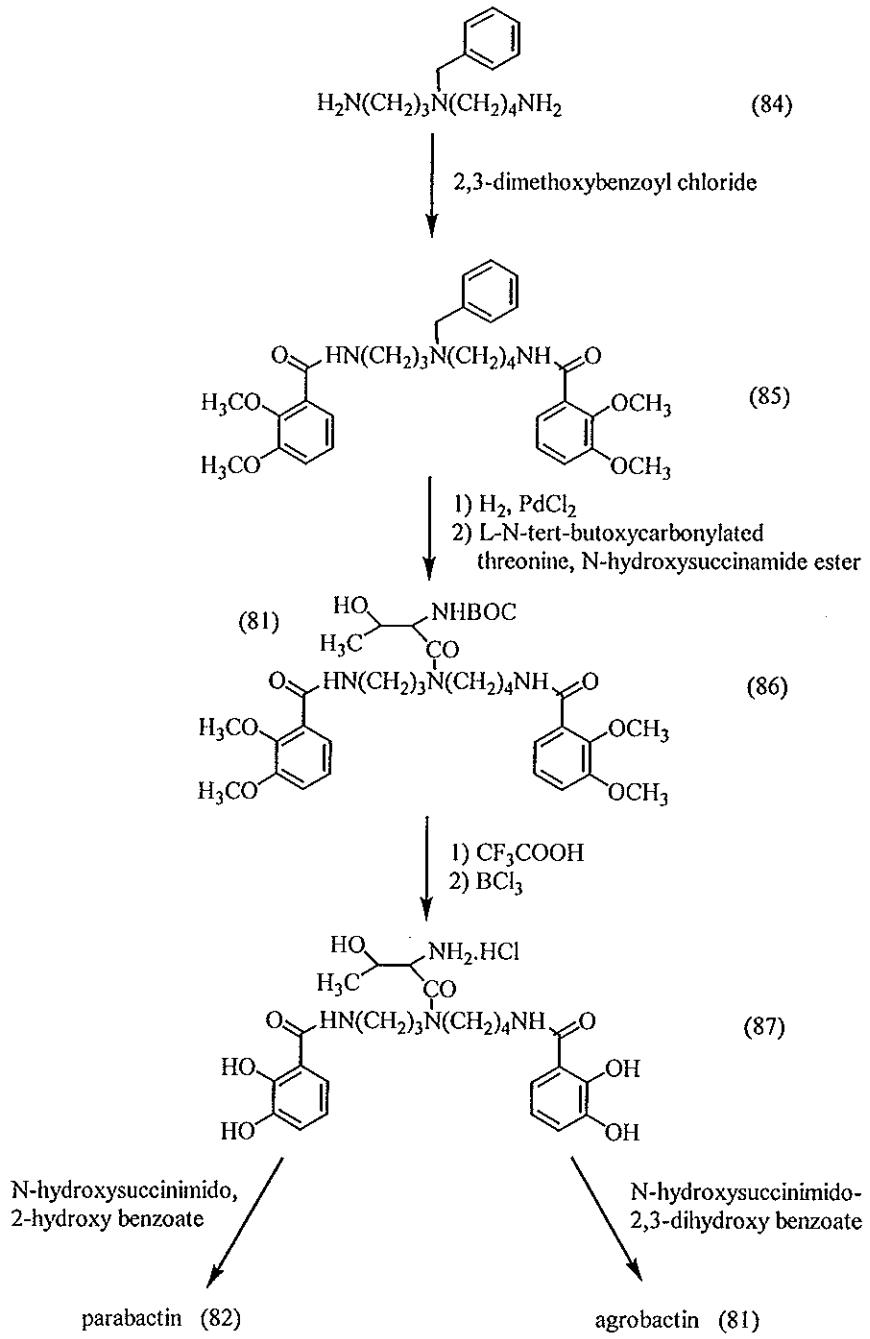
R =



parabactine (82)

R = H 1,8-bis(2,3-dihydroxybenzamido)-4-azaoctane (83)

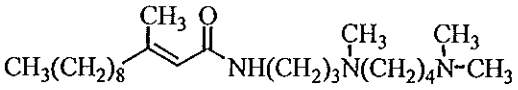
The synthesis of two polyamine catecholamide iron chelators, including parabactine (82) and agrobactine (81) is outlined in **Scheme 10** (Bergeron *et al.*, 1980, 1981).



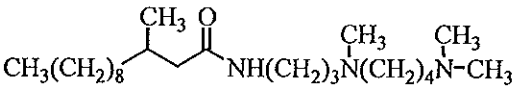
Scheme 10. Synthesis of parabactine (82) and agrobactine (81)

2.3 Spermidine of Soft Corals

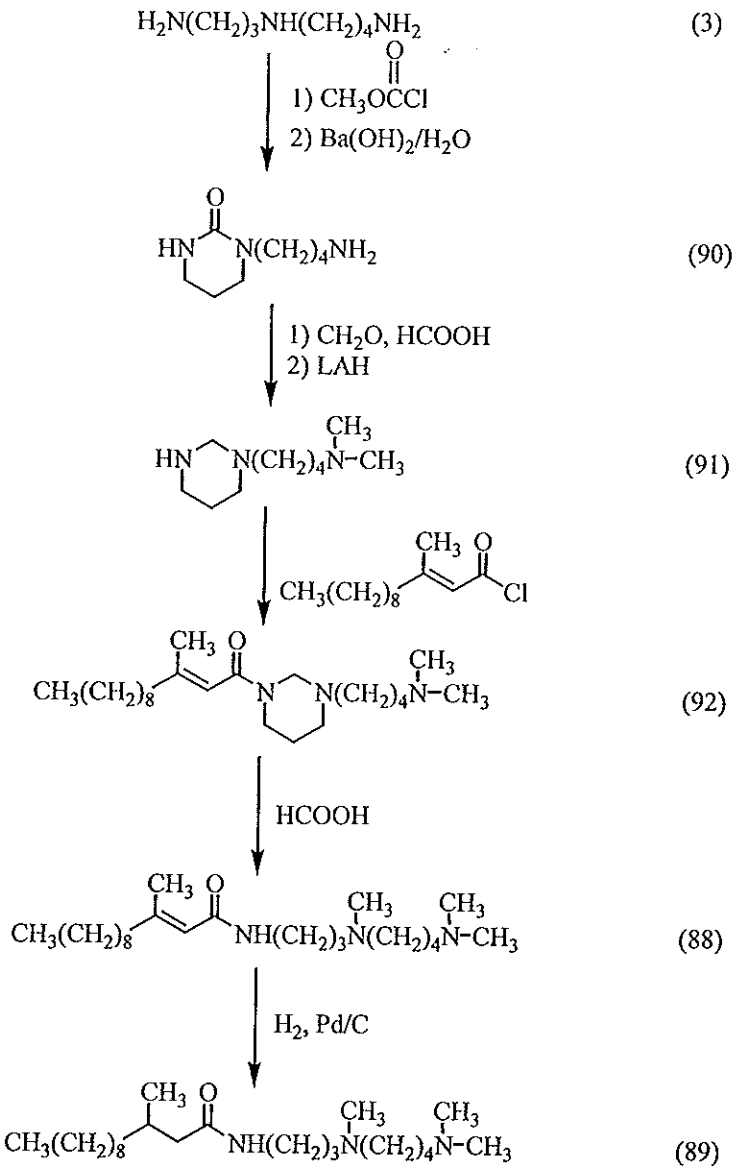
Two cytotoxic spermidine derivatives were isolated as a mixture from the Pacific soft coral *Sinularia brongersmai*: 5,12-dimethyl-1-dimethylamino-5,9-diazaheneicos-11-en-10-one (88) and its 11,12-dihydro derivative (89) (ratio 9:1) (Hollenbeak *et al.*, 1979). The synthesis of (88) and (89) was accomplished according to **Scheme 11** (Chantrapromma *et al.*, 1980).



(88)

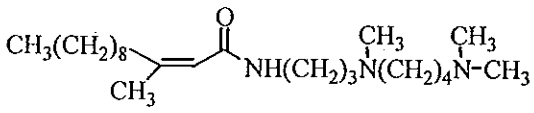


(89)

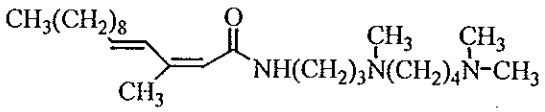


Scheme 11. Synthesis of 5,12-dimethyl-1-dimethylamino-5,9-diazaheneicos-11-en-10-one (88) and its 11,12-dihydro derivative (89)

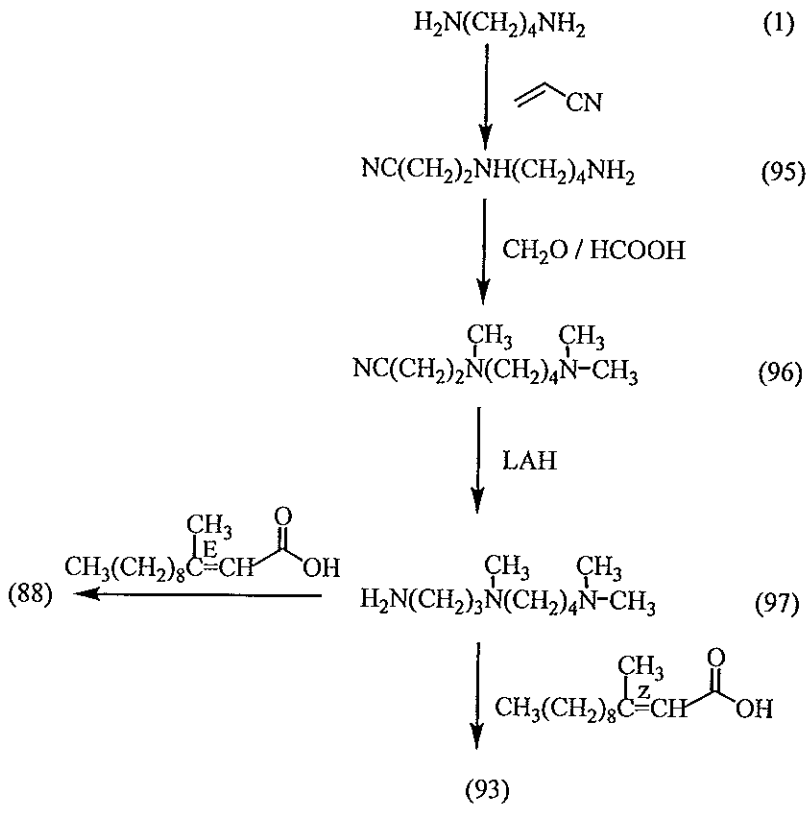
Two N-methylated spermidines (93) and (94) were isolated from a soft coral, *Sinularia sp* (Kazlauskas *et al.*, 1982). These compounds have shown potent activity both *in vitro* and *in vivo* against a human pathogenic bacterium *Pseudomonus aeruginosa.* The alternative synthesis of (88) and (93) was reported (Kazlauskas *et al.*, 1982) as indicated in **Scheme 12.**



(93)



(94)



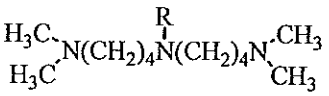
Scheme 12. Synthesis of spermidine derivatives (88) and (93)

2.4 Derivatives of Spermidine Lengthened by one Methylene Group (Homospermidine)

Homospermidine (Bachrach, 1973) is a type of polyamines, and the name is commonly used for the symmetric triamine (98) but once was also used for its unsymmetric isomer (99). Both skeletons can be found in derivatives of natural origin.

$H_2N(CH_2)_4NH(CH_2)_4NH_2$	symmetrical homospermidine	(98)
$H_2N(CH_2)_3NH(CH_2)_5NH_2$	unsymmetrical homospermidine	(99)

From plants belonging to the family Solanaceae, five alkaloids were isolated containing symmetrical homospermidine as the basic backbone: solamine (100), solapalmitine (101), solapalmitenine (102), solacaproine (103) and solaurethine (104). The common characteristic features of these compounds are the terminal bisdimethylamino group and the central nitrogen atom in the form of an amide (except for 100).

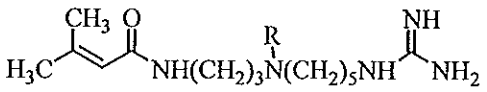


R = H	solamine	(100)
R = $CH_3(CH_2)_{14}CO$	solapalmitine	(101)
R = $CH_3(CH_2)_{12}CH{=}CHCO$	solapalmitenine	(102)
R = $CH_3(CH_2)_6CO$	solacaproine	(103)
R = CH_3CH_2OCO	solaurethine	(104)

Solamine (100) was found to be the principal component of *Cyphomandra betacea*. The structure of solamine from natural products was confirmed by direct comparison with synthetic material. Treatment of solamine (100) with palmitoyl chloride, trans-2-hexadecenoyl chloride, n-hexanoyl chloride, and ethyl chloroformate

produced solapalmitine (101), solapalmitenine (102), solacaproine (103), and solaurethine (104), respectively (Barboutis *et al.*, 1967, 1969; Evans *et al.*, 1972, 1977). Solapalmitine (101) and solapalmitenine (102), two tetramethylated acyl derivatives of solamine (100) studied by Barboutis and co-workers, possess significant tumor-inhibitory activity (Barboutis *et al.*, 1967, 1969).

A trio of marine natural products, a family of trifunctional unsymmetrical homospermidine known as the acarnidines (105), (106) and (107), was isolated in 1978 by Carter and Rinehart from the red-orange encrusting sponge *Acarnus erithacus* (de Laubenfels). The compouds were reported to have mild activity against *Herpes simplex virus* type I, as well as broad spectrum antimicrobial activities (Carter and Rinehart, 1978).



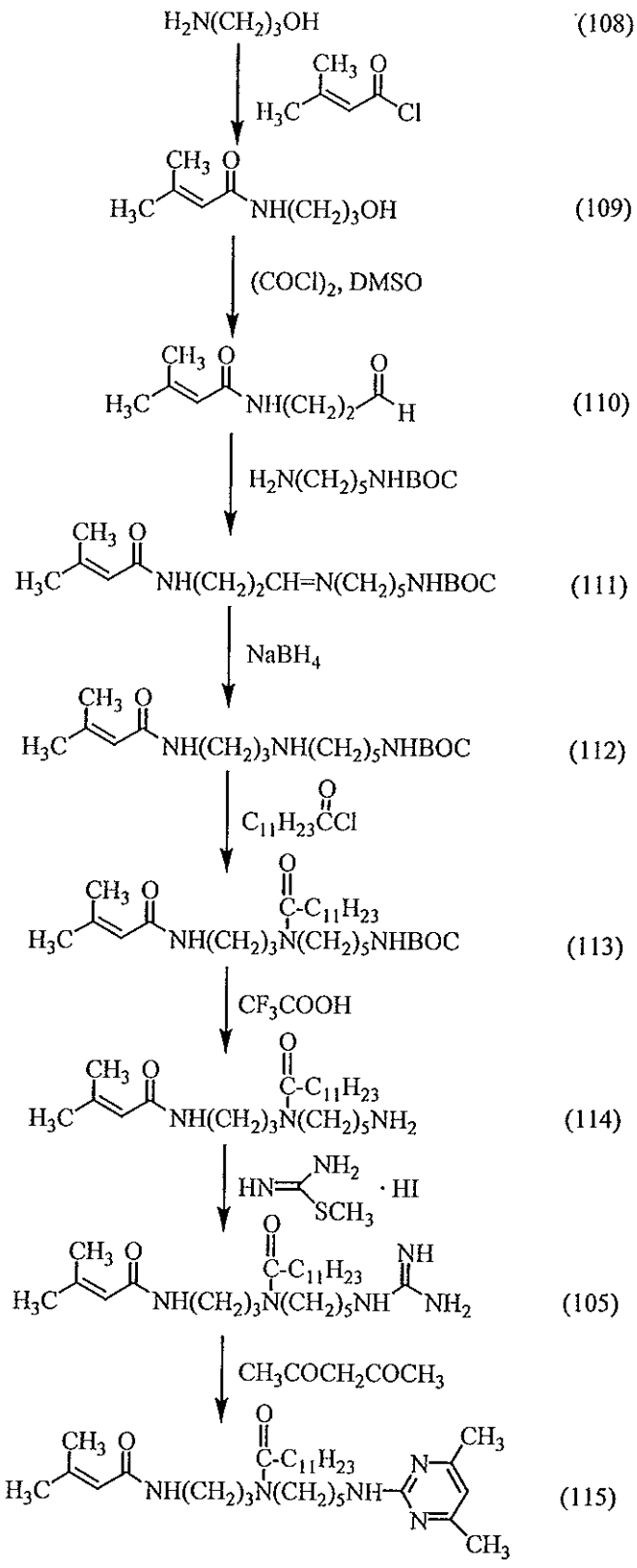
acarnidines

$R = CO(CH_2)_{10}CH_3$ (105)

$R = CO(CH_2)_3CH^{z}{=}CH(CH_2)_5CH_3$ (106)

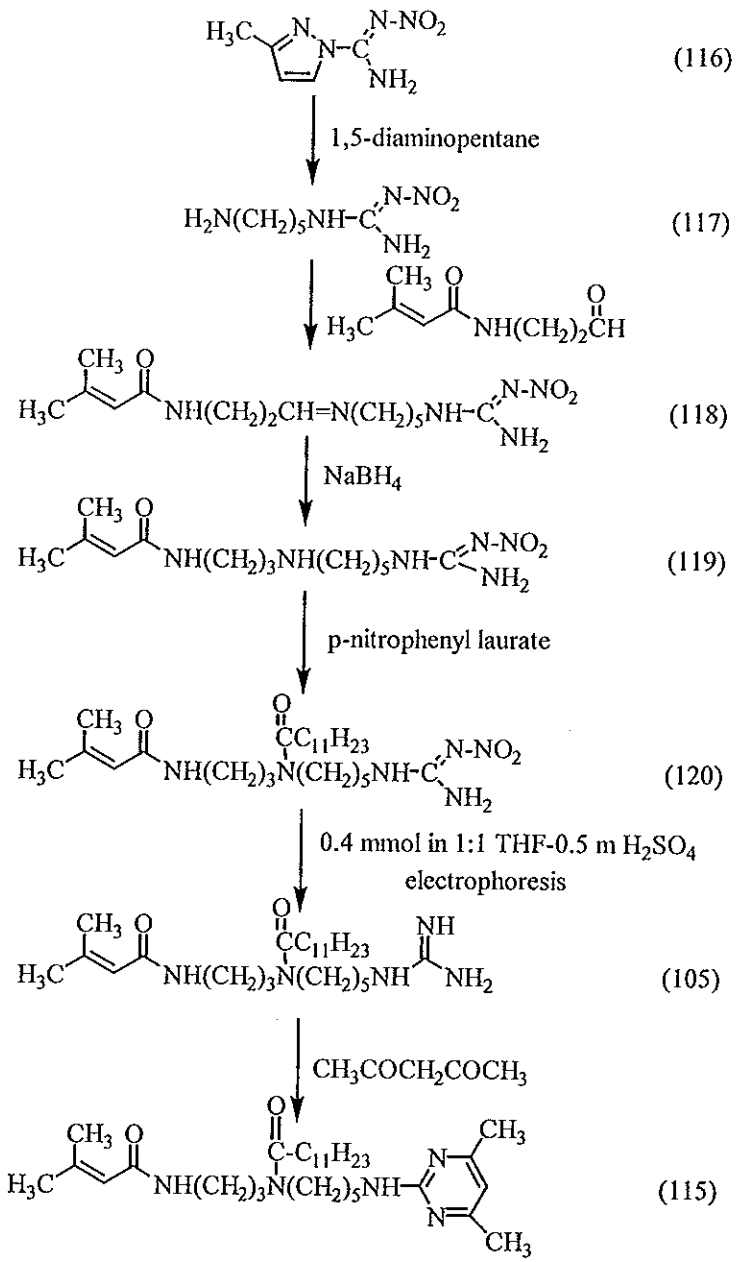
$R = COC_{13}H_{21}$ (107)

The first synthesis of acarnidine (105) was reported by Blunt and co-workers (Blunt *et al.*, 1982, 1986) as shown in **Scheme 13.**



Scheme 13. Synthesis of acarnidine (105) and 4,6-dimethylpyrimidine derivative (115) (Blunt *et al.*, 1986)

The synthesis of acarnidine (105) has also been reported by Boukouvalas and co-workers (Boukouvalas *et al.*, 1983) as shown in **Scheme 14.**



Scheme 14. Synthesis of acarnidine (105) and 4,6-dimethylpyrimidine derivative (115) (Boukouvalas *et al.*, 1983)

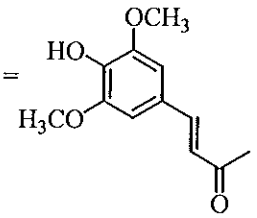
3. Spermine Type

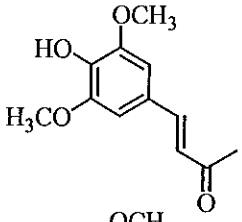
3.1 Simple Spermine Derivatives

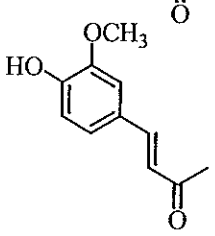
The structural elucidation of three simple spermine compounds sinapoylspermine(121), disinapoylspermine(122), and diferuloylspermine (123) was performed on the basis of chromatographic identification of their hydrolyzed products (Cabanne *et al.*, 1978).

$R^1NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NHR^2$

$R^1 = R^2 = H$ spermine (4)

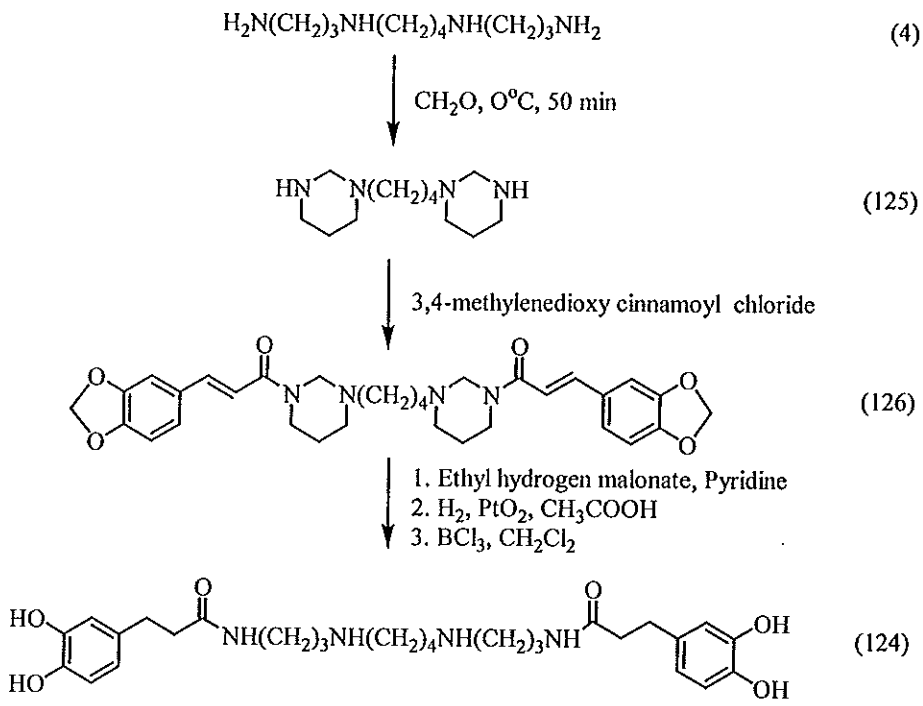
$R^1 =$  , $R^2 = H$ sinapoylspermine (121)

$R^1 = R^2 =$  disinapoylspermine (122)

$R^1 = R^2 =$  diferuloylspermine (123)

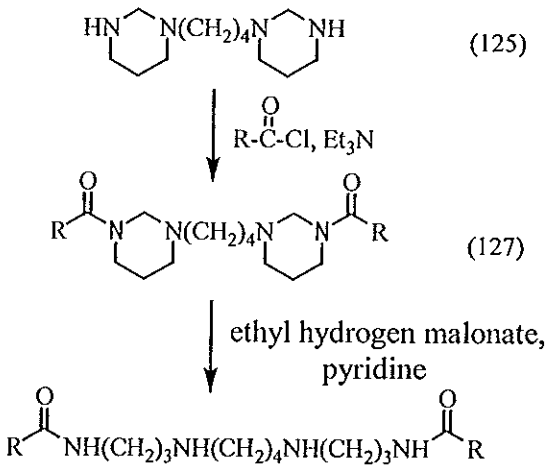
Kukoamine A (124) is a spermine alkaloid isolated from the crude drug " jikoppi ", which is prepared from *Lycium chinense* (Solanaceae) (Funayama *et al.*, 1980). This compound has shown hypotensive activity (Funayama *et al.*, 1980) and potent and selective inhibition of trypanothione reductase (Ponasik *et al.*, 1995).

In the Kukoamine A (124) synthesis, formaldehyde was used to form an aminal with the 1,3-diaminopropane part of spermine. The 1,4-diaminobutane portion of the base did not react under these conditions; the central amino group was, therefore, protected by formaldehyde. The two secondary amino groups were free to react with 3,4-methylenedioxy cinnamoyl chloride. After deprotection and catalytic hydrogenation, kukoamine A (124) was isolated in 62 % overall yield (**Scheme 15**) (Chantrapromma and Ganem, 1981).



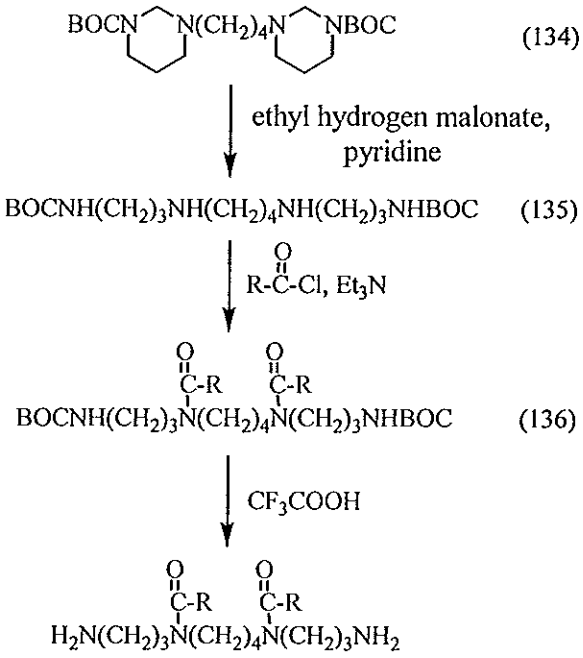
Scheme 15. Synthesis of kukoamine A (124) (Chantrapromma and Ganem, 1981)

Some kukoamine A derivatives (128-103) and (137-142) have also been prepared in high yield (Chantrapromma *et al.*, 1990) as shown in **Schemes 16** and **17.**



R =	CH_3	(128)
R =	Ph	(129)
R =	CH=CHPh	(130)
R =	CH_2CH_2Ph	(131)
R =	$CH=CH(C_6H_4)(OH)$	(132)
R =	$CH=CH(C_6H_3)(OH)_2$	(133)

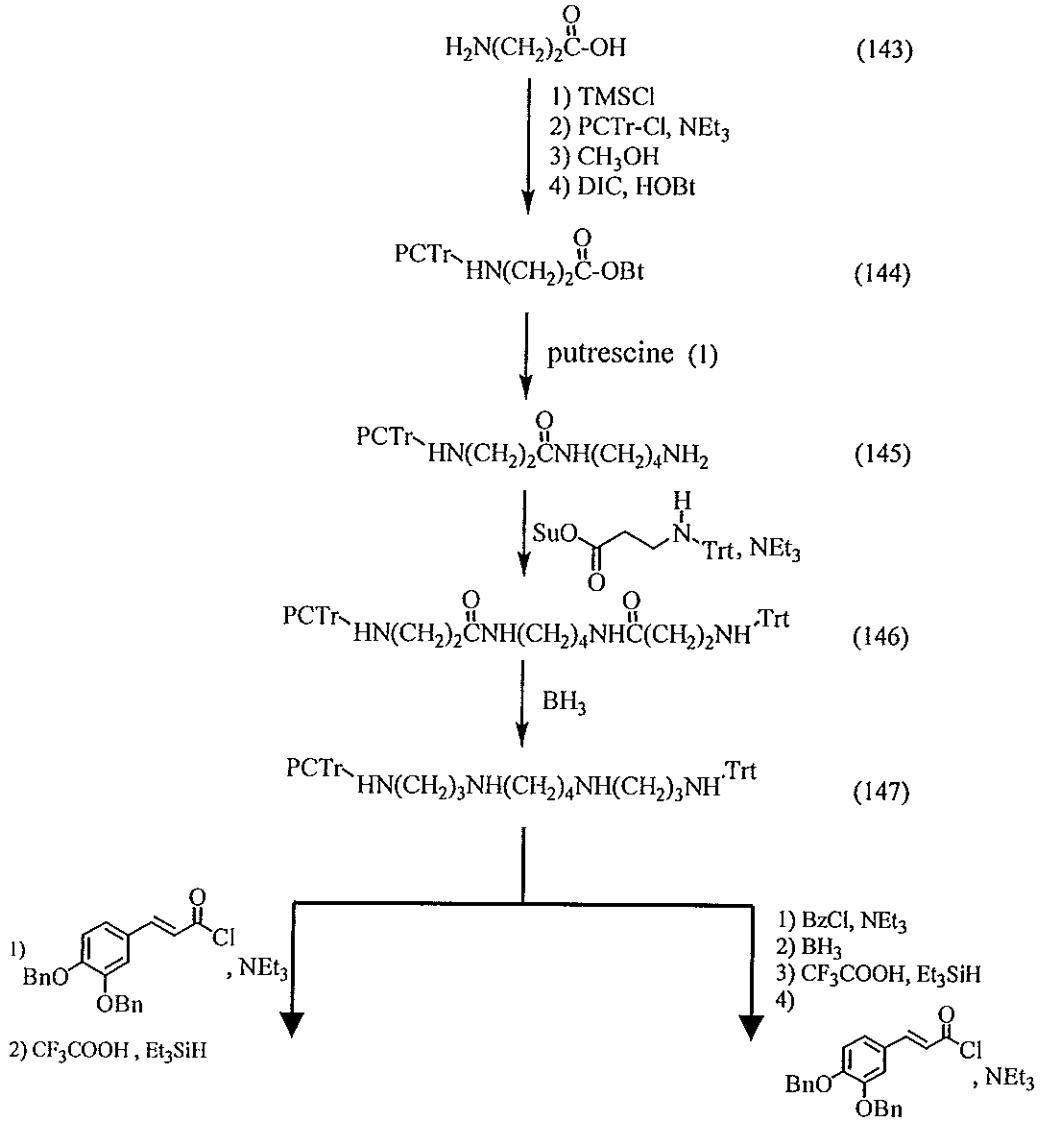
Scheme 16. Synthesis of kukoamine A derivatives (128-133) (Chantrapromma *et al.*, 1990)

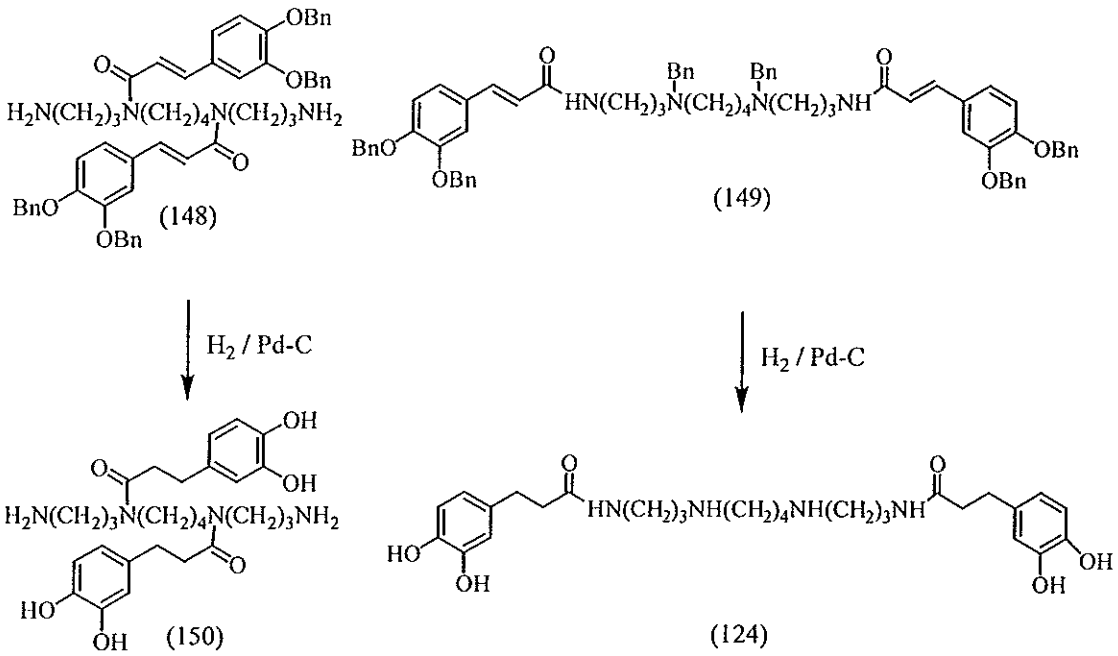


R =	CH_3	(137)
R =	Ph	(138)
R =	CH=CHPh	(139)
R =	CH_2CH_2Ph	(140)
R =	$CH=CH(C_6H_4)$ (OH)	(141)
R =	$CH=CH(C_6H_3)$ $(OH)_2$	(142)

Scheme 17. Synthesis of kukoamine A derivatives (137-142) (Chantrapromma *et al.*, 1990)

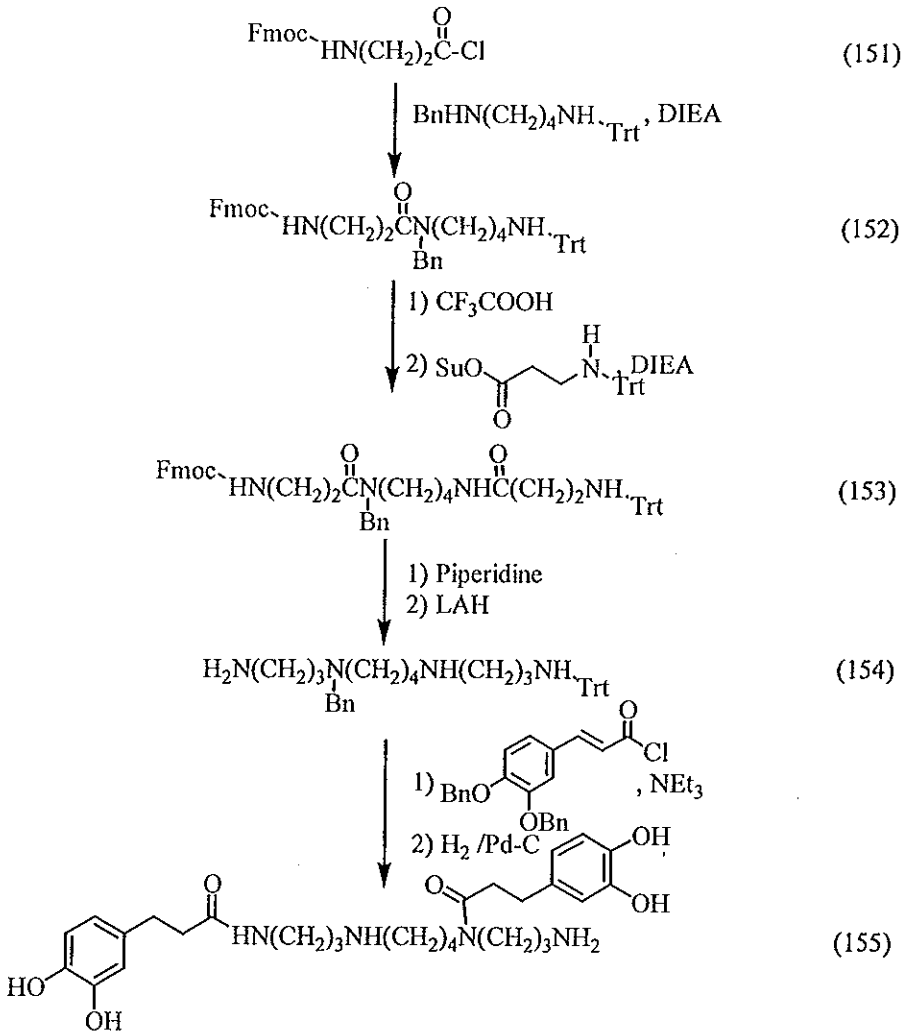
Funayama and co-workers isolated kukoamine B (155) from *Lycium chinense* and identified the structures by the use of FAB-MS, ^{13}C NMR, ^{1}H NMR and UV spectroscopy (Funayama *et al.*, 1995). More recently, Karigiannis and co-workers have published the synthesis of all four regioisomers of kukoamine. The synthetic routes to kukoamine A (124) and kukoamine C (150) are shown in **Scheme 18** (Karigiannis *et al.*, 1998).



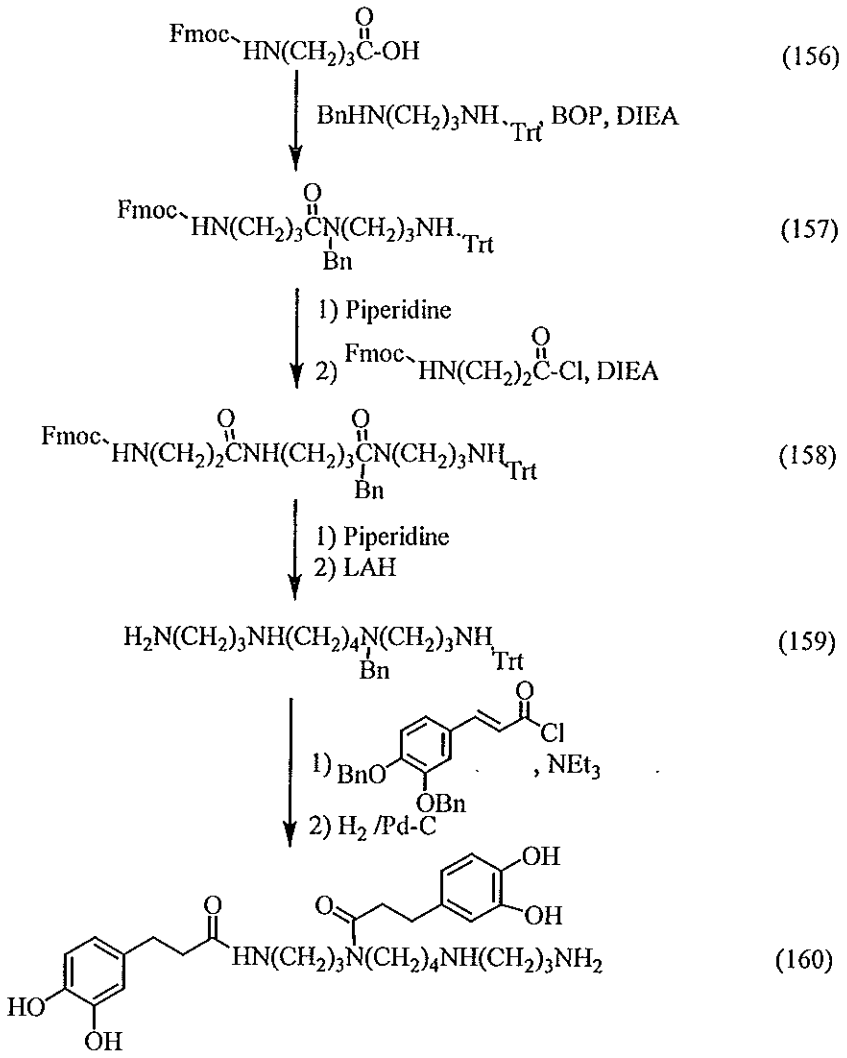


Scheme 18. Synthesis of kukoamine A (124) and kukoamine C (150) (Karigiannis *et al.*, 1998)

Furthermore, kukoamine B (155) and kukoamine D (160) have also been prepared (Karigiannis *et al.*, 1998) as shown in **Schemes 19** and **20**, respectively.



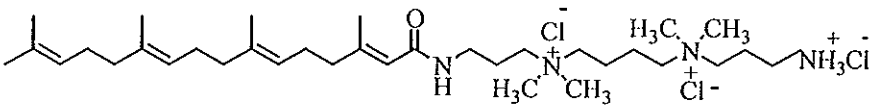
Scheme 19. Synthesis of kukoanime B (155) (Karigiannis *et al.*, 1998)



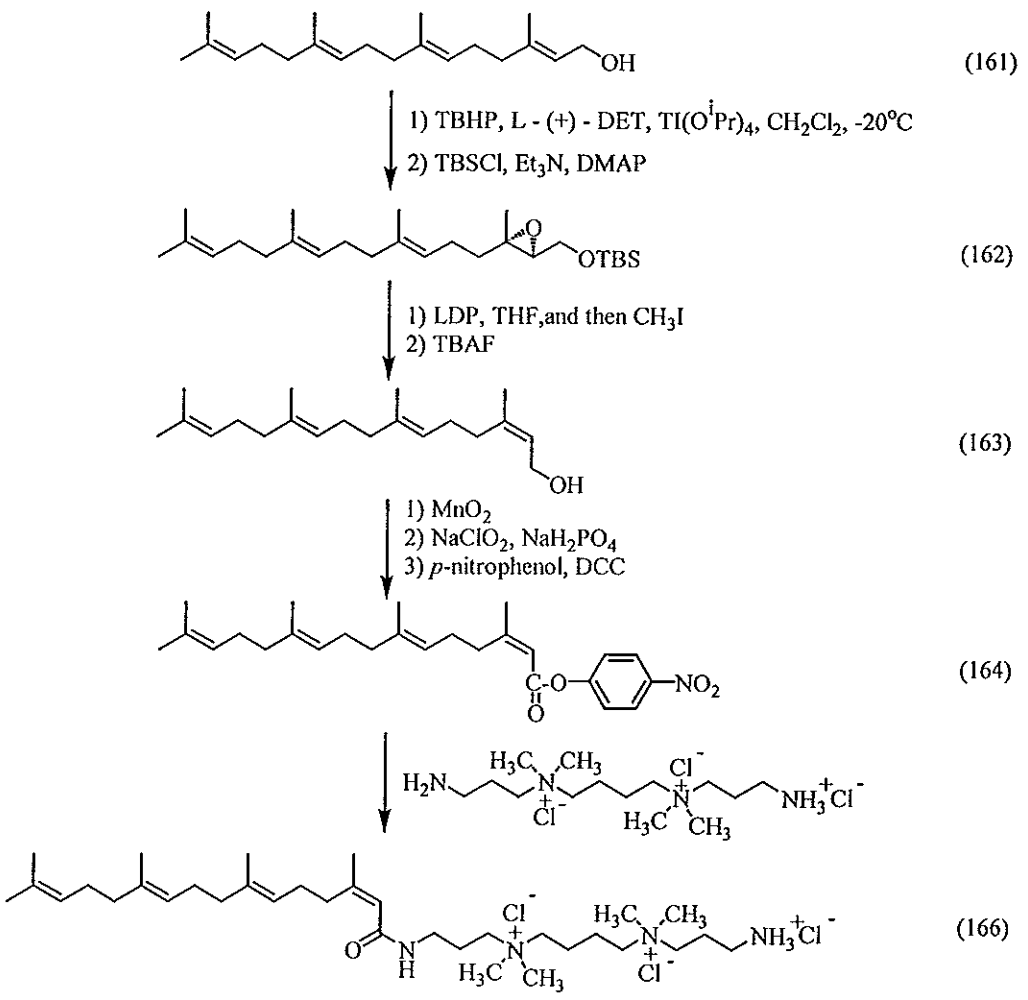
Scheme 20. Synthesis of kukoamine D (160) (Karigiannis *et al.*, 1998)

3.2 Spermine of Soft Corals.

Sinulamide (165), the first acylated spermine derivative was isolated from the soft coral *Sinularia sp.* (Sata *et al.*, 1999) as an H,K-ATPase inhibitor and cytotoxic against L1210 and P388. The structure was assigned on the basis of spectroscopic data and confirmed by a total synthesis. The synthetic route to trans isomer of the sinulamide (166) is shown in **Scheme 21.**

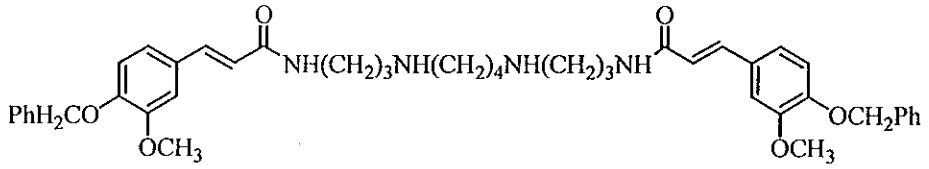


(165)

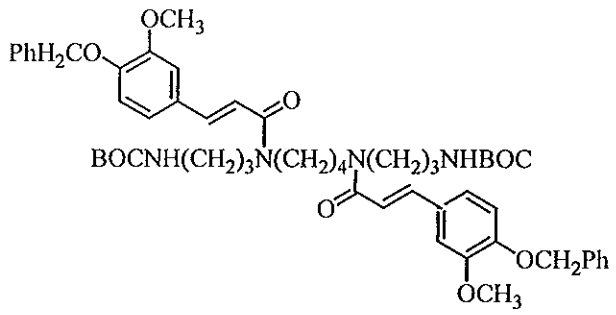


Scheme 21. Synthesis of sinulamide (166)

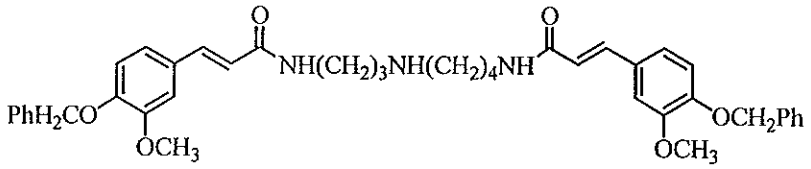
Hydroxycinnamic acid amide have been identified as the main phenolic constituents in the reproductive organs of a range of flowering plants. Diferuloylspermines and diferuloylspermidines have been isolated and identified from *Ananas comosus* (Bromeliaceae) (Martin-Tanguy *et al.,* 1978). This Thesis described the synthesis of the diferuloylspermines and diferuloylspermidines derivatives (167-170).



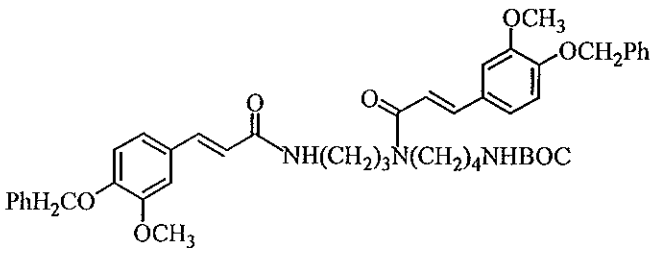
(167)



(168)



(169)



(170)

Objective

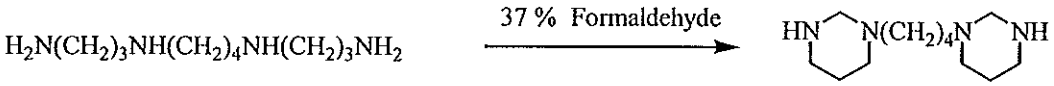
The objective of this work is aimed to synthesize diferuloylspermines and diferuloylspermidines derivatives (167-170) for biological testings.

CHAPTER 2

EXPERIMENTAL

Melting points were measured on an Electrothermal melting point apparatus and a digital Electrothermal melting point apparatus 9100; and were recorded in °C. Proton nuclear magnetic resonance spectra were recorded on a JEOL-PM_X 60 spectrometer and on FT-NMR 500 MHz Varian UNITY INOVA spectrometer with tetramethylsilane (TMS) as internal standard . Chemical shifts were expressed in ppm (δ); multiplicity, *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *quint* = quintet, *m* = multiplet. Infrared spectra were recorded on a Perkin-Elmer IR 783 and FTIR spectrophotometer; and were recorded in cm^{-1}. LC mass spectra were recorded on Bruker Data Analysis Esquire-LC spectrometer. Analytical and preparative thin layer chromatography (TLC) was carried out on glass plates coated with silica gel 60 GF_{254} (10-30 μm particle size). Column chromatography was performed with silica gel 60 (63-200 μm particle size). Flash column chromatography referred to the method described by Still, W. Clark., Kahn, Michael. and Mitra, Abhijit. (1978) using silica gel 60 (40-63 μm particle size). All silica gel were purchased from E. MERCK. Spots in TLC plates were located under ultraviolet light at 254 nm and by exposure to iodine vapour. The polyamine spots were visualized violet-blue by Schlittler reagent.

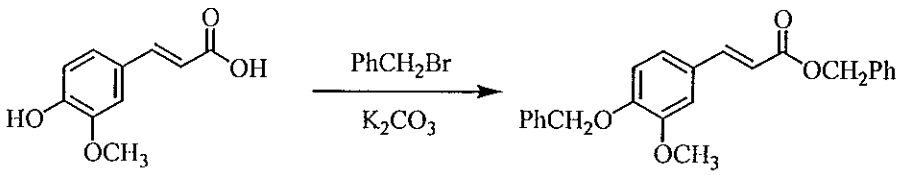
bis-Hexahydropyrimidine (125)



(4) (125)

Spermine (4) (1.00 g, 4.95 mmol) was dissolved in distilled water (70 ml), and the solution was cooled to 0°C under nitrogen. Formaldehyde (0.67 ml, 8.91 mmol) was slowly added to the cold solution, the reaction mixture was stirred at 0°C for 30 min and then stirred at room temperature for 2 h. The aqueous layer was saturated with solid sodium chloride and extracted with chloroform (6 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to afford bis-hexahydropyrimidine (125) (1.00 g, 89 %) as a nearly pure waxy white solid. **IR** (film) ν_{max}: 3260, 2930 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 60 MHz) δ: 1.30-1.82 (8H, *m*, 4 x C-CH_2-C), 2.09-3.03 (12H, *m*, 6 x CH_2-N), 3.35 (4H, *s*, 2 x N-CH_2-N).

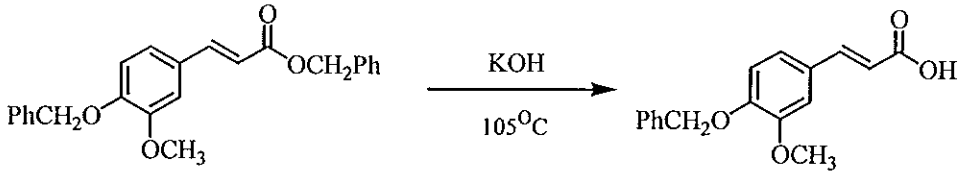
Benzyl (4-benzyloxy-3-methoxy) cinnamate (172)



(171) (172)

A mixture of the ferulic acid (171) (2.50 g, 12.8 mmol), benzyl bromide (4.6 ml, 38.4 mmol) and anhydrous potassium carbonate (12.38 g, 89.6 mmol) in dry dimethyl sulfoxide (30 ml) was stirred under nitrogen at room temperature for 24 h. The reaction mixture was poured into cold dilute hydrochloric acid (3N), and extracted with ether. The ether layer was washed with water (3 x 20 ml) and saturated brine (3 x 20 ml), dried over anhydrous sodium sulfate and evaporated to afford a yellow viscous oil. The product was further purified by column chromatography on silica gel, eluted with 20 % chloroform in hexane to afford benzyl (4-benzyloxy-3-methoxy) cinnamate (172) (4.05 g, 84 %) as a white solid, m.p. 70-71°C. **IR** (film) ν_{max} : 3060, 2960, 1715, 1640, 1270, 740, 700 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 60 MHz) δ: 3.87 (3H, *s*, OCH_3), 5.12 (2H, *s*, OCH_2) , 5.17 (2H, *s*, OCH_2), 6.24 (1H, *d*, J = 15 Hz, =CH-CO), 6.83-7.07 (3H, *m*, Ar-H), 7.13-7.40 (10H, *m*, 2 x Ph), 7.57 (1H, *d*, J = 15 Hz, CH=C-CO).

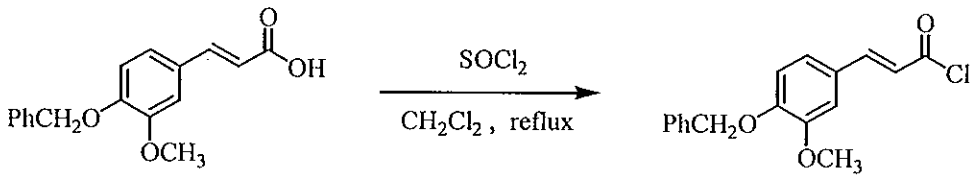
4-Benzyloxy-3-methoxycinnamic acid (173)



(172) (173)

To a solution of potassium hydroxide (11.65 g, 207.68 mmol) in distilled water (15 ml) was added benzyl (4-benzyloxy-3-methoxy) cinnamate (172) (9.70 g, 25.96 mmol) and dimethyl sulfoxide (40 ml) and the reaction mixture was brought to reflux at 105°C for 10 h. The solution was then cooled and poured into cold dilute hydrochloric acid (3N) and extracted with ethyl acetate. The combined organic extract was washed with water (3 x 20 ml), saturated brine (3 x 20 ml), dried over anhydrous sodium sulfate and evaporated to afford 4-benzyloxy-3-methoxycinnamic acid (173) (6.64 g, 90 %) as a white solid, m.p. 192-193°C. **FTIR** (KBr) ν_{max}: 3364-2610, 1667, 1467, 1260, 1163, 749, 700 cm^{-1}; **^{1}H NMR** ($CDCl_3$ + CD_3OD, 60 MHz) δ: 3.85 (3H, *s*, OCH_3), 5.09 (2H, *s*, OCH_2), 6.20 (1H, *d*, J = 15 Hz, =CH-CO), 6.97-7.10 (3H, *m*, Ar-H) , 7.13-7.39 (5H, *m*, Ph) , 7.52 (1H, *d*, J = 15 Hz, CH=C-CO).

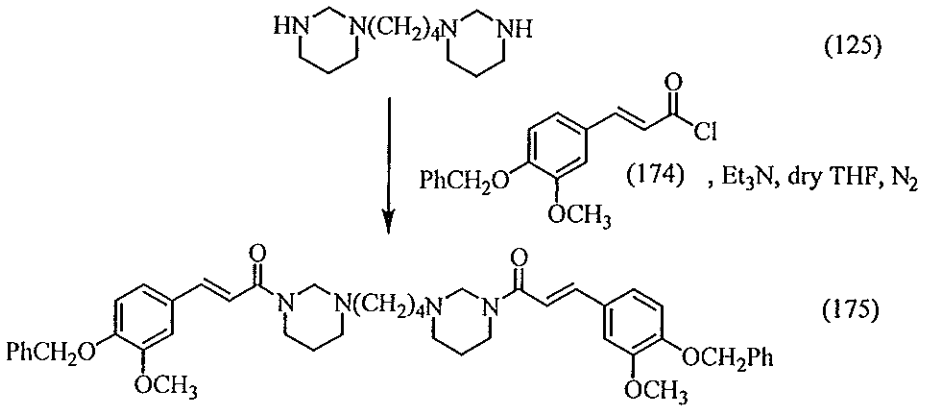
4-Benzyloxy-3-methoxycinnamoyl chloride (174)



(173) (174)

To a warm solution of 4-benzyloxy-3-methoxycinnamic acid (173) (1.88 g, 6.63 mmol) in dry dichloromethane (25 ml) was added thionyl chloride (1.46 ml, 19.89 mmol). Refluxing was continued for 6 h. After distilling off the solvent and the excess of thionyl chloride by rotatory evaporator, the 4-benzyloxy-3-methoxycinnamoyl chloride (174) was obtained as a crude yellow solid , and was used directly in the next step without further purification.

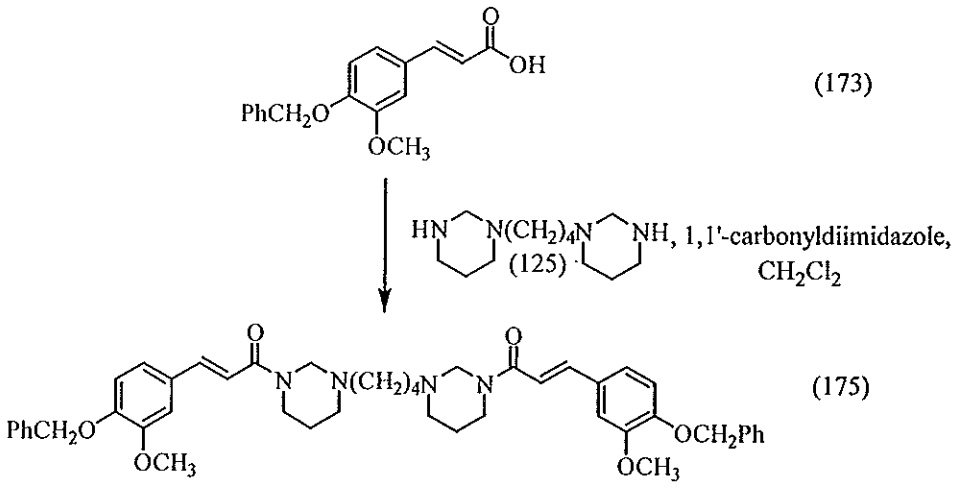
N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175)



A solution of 4-benzyloxy-3-methoxycinnamoyl chloride (174) (2.00 g, 6.63 mmol) in dry tetrahydrofuran (15 ml) was slowly added to a cooled solution of bis-hexahydropyrimidine (125) (500 mg, 2.21 mmol) and triethylamine (3.76 ml, 26.52 mmol) in dry tetrahydrofuran (20 ml) at 0°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 19 h. The solvent was then evaporated, and the residue was dissolved in chloroform (30 ml), washed with 5 % $NaHCO_3$ (3 x 20 ml), saturated brine (3 x 20 ml), water (3 x 20 ml), dried over anhydrous sodium sulfate and evaporated to afford a yellow viscous oil. The product was further purified by quick column chromatography on silica gel, eluted with 3 % methanol in dichloromethane to afford N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175) (1.30 g, 80 %).

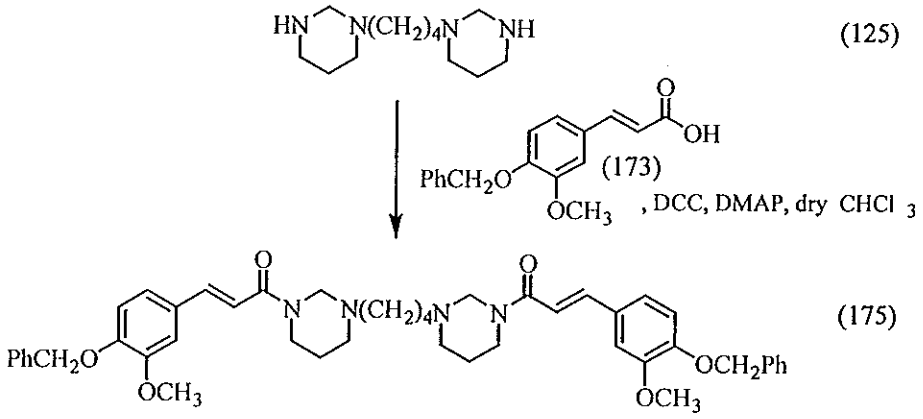
IR (film) ν_{max}: 3015, 2960, 1655, 1610, 1520, 1270, 1145, 760, 700 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 60 MHz) δ: 1.43-1.86 (8H, *m*, 4 x C-CH_2-C), 2.23-2.86 (8H, *m*, 4 x CH_2-N), 3.46-3.73 (4H, *m*, 2 x CH_2-N-CO), 3.83 (6H, *s*, 2 x OCH_3), 4.23 (4H, *s*, 2 x N-CH_2-N), 5.08 (4H, *s*, 2 x OCH_2), 6.62 (2H, *d*, J = 15 Hz, 2 x =CH-CO), 6.80-7.06 (6H, *m*, 2 x Ar-H), 7.20-7.36 (10H, *m*, 2 x Ph), 7.52 (2H, *d*, J = 15 Hz, 2 x CH=C-CO); **LC-Ms**: *m/z* 781 ($[M+Na]^+$).

N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175)



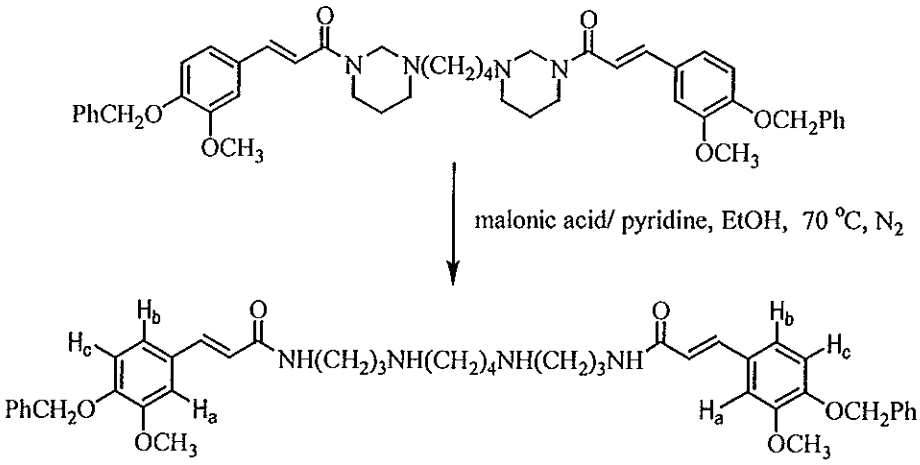
To a stirred suspension of 4-benzyloxy-3-methoxycinnamic acid (173) (942.8 mg, 3.32 mmol) in dry dichloromethane (20 ml) was added 1,1′-carbonyldiimidazole (538.3 mg, 3.32 mmol) in one portion. The suspension was stirred at room temperature under nitrogen for 1 h. A solution of bis-hexahydropyrimidine (125) (300 mg, 1.33 mmol) dissolved in dry dichloromethane (15 ml) was added. The resulting reaction mixture was kept overnight. The solvent was then evaporated, and the residue was dissolved in dichloromethane (30 ml). The resulting mixture was washed with saturated $NaHCO_3$ (3 x 20 ml), dried over anhydrous sodium sulfate and evaporated to afford a yellow viscous oil. The oily residue was further purified by quick column chromatography on silica gel, eluted with 3 % methanol in dichloromethane to afford N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175) (859.9 mg, 85 %). The spectroscopic data of this compound is on page 52.

N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175)



A solution of DCC (420.6 mg, 2.04 mmol) in dry chloroform (11 ml) was added to a solution of bis-hexahydropyrimidine (125) (203.2 mg, 0.89 mmol), 4-benzyloxy-3-methoxycinnamic acid (173) (543.4 mg, 1.91 mmol) and DMAP (242.4 mg, 1.98 mmol) in dry chloroform (9 ml) at 0°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The solid was filtered off and washed with chloroform. The chloroform wash was combined with the filtrate and more chloroform was added (30 ml). The combined solution was washed with 5 % $NaHCO_3$ (2 x 20 ml), water (2 x 20 ml), dried over anhydrous sodium sulfate and evaporated to afford an oil. The oily residue was further purified by preparative thin-layer chromatography on silica gel, eluted with 4 % ethanol in chloroform to afford N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175) (394.1 mg, 52 %). The spectroscopic data of this compound is on page 52.

N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)



(175)

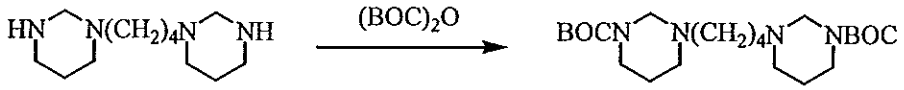
(167)

To a solution of N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175) (181.6 mg, 0.24 mmol) in dry ethanol (4 ml) were added dry pyridine (0.12 ml, 1.44 mmol) and solution of malonic acid (249.2 mg, 2.39 mmol) in dry ethanol (5 ml) at room temperature under nitrogen and then the reaction mixture was brought to reflux for 2 h. The solution was concentrated *in vacuo*, water (5 ml) was added and the pH was adjusted to 11 with 10 % NaOH and extracted with dichloromethane (5 x 15 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to afford N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167) (147 mg, 84 %) as a yellow solid, m.p. 152-154°C.

FTIR (film) ν_{max}: 3285, 3014, 2936, 1656, 1613, 1599, 1511, 1260, 1162, 1138, 755, 697 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 1.53 (4H, *m*, 2 x C-CH_2-C), 1.68 (4H, *quint*, J = 6 Hz, 2 x C-CH_2-C), 2.59 (4H, *t*, J = 6 Hz, 2 x CH_2-N), 2.68 (4H, *q*, J = 6.5 Hz, 2 x CH_2-N), 3.42 (4H, *t*, J = 6 Hz, 2 x CH_2-N-CO), 3.84 (6H, *s*, 2 x OCH_3), 5.12 (4H, *s*, 2 x OCH_2), 6.28 (2H, *d*, J = 15.5 Hz, 2 x =CH-CO), 6.81 (2H, *d*, J = 8.5 Hz, 2 x Ar-H_c), 6.97 (2H, *dd*, J = 8.5, 2 Hz, 2 x Ar-H_b), 7.00 (2H, *d*, J = 2 Hz, 2 x Ar-H_a), 7.27-7.40 (10H, *m*, 2 x Ph)*, 7.48 (2H, *d*, J = 15.5 Hz, 2 x CH=C-CO); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ: 27.53, 28.68, 38.47, 47.73, 49.37, 55.89, 70.90, 110.87, 113.89, 119.61, 121.70, 127.54, 128.27, 128.73, 128.88, 137.04, 140.47, 150.05, 150.14, 166.81; **LC-MS**: *m/z* 735 ($[M+H]^+$).

* The signal multiplicity of the protons from δ 727 to 7.40 were shown in **Table 7**.

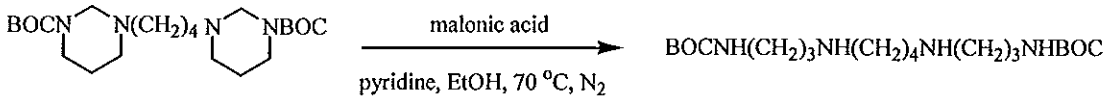
N^1,N^{12}-di-(*tert*-butoxycarbonyl) bis-hexahydropyrimidine (134)



(125) (134)

A solution of di-*tert*-butyl-dicarbonate (725 mg, 3.45 mmol) in dry tetrahydrofuran (10 ml) was slowly added dropwise to a solution of bis-hexahydropyrimidine (125) (313 mg, 1.38 mmol) in dry tetrahydrofuran (30 ml) at 0°C under nitrogen. After the addition was completed, the ice bath was removed and the reaction mixture was stirred for 12 h. The solvent was then evaporated, the residue was dissolved in dichloromethane (30 ml), washed with 10 % NaOH (3 x 20 ml) and saturated brine (3 x 20 ml), dried over anhydrous sodium sulfate and evaporated to afford a viscous oil. The oily residue was further purified by column chromatography on silica gel, eluted with 4 % ethanol in chloroform to obtain N^1,N^{12}-di-(*tert*-butoxycarbonyl) bis-hexahydropyrimidine (134) (482 mg, 86%) as a colorless oil. **IR** (film) ν_{max}: 2960, 1700, 1425, 1368, 1270, 1160 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 60 MHz) δ: 1.47 (18H, *s*, 6 x CH_3-C-O), 1.50-1.77 (8H, *m*, 4 x C-CH_2-C), 2.36-2.66 (8H, *m*, 4 x CH_2-N), 3.38 (4H, *t*, J = 6 Hz, 2 x CH_2-NH-BOC), 4.00 (4H, *s*, 2 x N-CH_2-N).

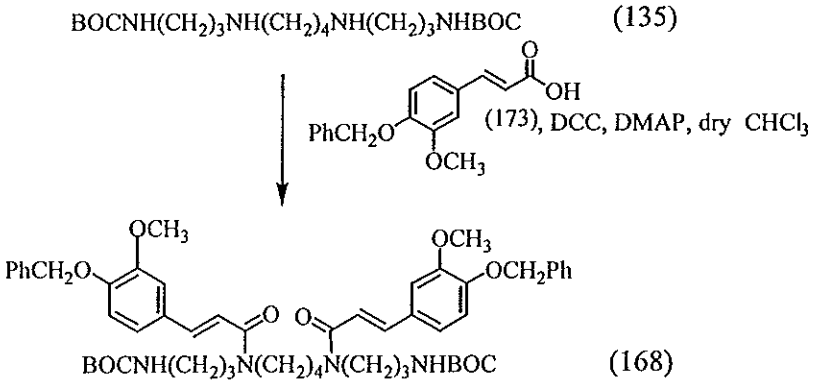
N^1,N^{12}-di-(*tert*-butoxycarbonyl) spermine (135)



(134) (135)

To a solution of N^1,N^{12}-di-(*tert*-butoxycarbonyl) bis-hexahydro-pyrimidine (134) (833.5 mg, 1.96 mmol) in dry ethanol (20 ml) under nitrogen were added dry pyridine (0.95 ml, 11.74 mmol) and solution of malonic acid (2.03 g, 19.56 mmol) in dry ethanol (10 ml) and then the reaction mixture was brought to reflux for 2 h. The solution was concentrated *in vacuo*, water (10 ml) was added and the pH was adjusted to 11 with 10 % NaOH and extracted with dichloromethane (5 x 20 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to afford N^1,N^{12}-di-(*tert*-butoxycarbonyl) spermine (135) (607.8 mg, 77 %) as a yellow solid, m.p. 84-86°C. **IR** (film) ν_{max}: 3330, 2930, 1700, 1515, 1395, 1370, 1265, 1170 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz) δ: 1.39 (18H, *s*, 6 x CH_3-C-O), 1.47 (4H, *m*, 2 x C-CH_2-C), 1.60 (4H, *quint*, J = 6.5 Hz, 2 x C-CH_2-C), 2.59 (4H, *t*, J = 6.5 Hz, 2 x CH_2-N), 2.62 (4H, *t*, J = 6.5 Hz, 2 x CH_2-N), 3.15 (4H, *m*, 2 x CH_2-NH-BOC), 5.25 (2H, *br.s*, 2 x NH-BOC); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ: 27.86, 28.45, 29.93, 39.25, 47.77, 49.79, 78.85, 156.13; **LC-Ms**: *m/z* 403 ($[M+H]^+$).

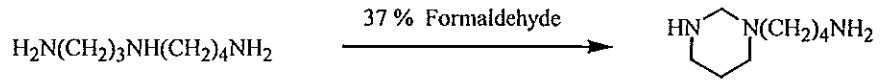
N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxy-cinnamoyl) spermine (168)



A solution of DCC (67.7 mg, 0.33 mmol) in dry chloroform (4 ml) was added to a solution of N^1,N^{12}-di-(*tert*-butoxycarbonyl) spermine (135) (54.2 mg, 0.13 mmol), 4-benzyloxy-3-methoxycinnamic acid (173) (95.1 mg, 0.34 mmol) and DMAP (40.9 mg, 0.34 mmol) in dry chloroform (5 ml) at 0°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 7 days. The solid was filtered off and washed with chloroform. The chloroform wash was combined with the filtrate and more chloroform added (20 ml). The combined solution was washed with 5 % $NaHCO_3$ (2 x 15 ml), water (2 x 15 ml), dried over anhydrous sodium sulfate and evaporated to afford an oil. The oily residue was further purified by column chromatography on silica gel, eluted with 3 % ethanol in chloroform to afford N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168) (94.7 mg, 75 %).

FTIR (film) ν_{max} : 3019, 2980, 1702, 1644, 1596, 1510, 1215, 1164, 755, 668 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 60 MHz)δ: 1.43 (18H, *s*, 6 x CH_3-C-O), 1.43-1.60 (8H, *m*, 4 x C-CH_2-C), 2.95-3.30 (12H, *m*, 4 x CH_2-N, 2 x CH_2-NH-BOC), 3.80 (6H, *s*, 2 x OCH_3), 4.67 (2H, *br.s*, 2 x NHBOC), 5.05 (4H, *s*, 2 x OCH_2), 6.18-7.03 (8H, *m*, 2 x =CH-CO, 2 x Ar-H), 7.17-7.33 (10H, *m*, 2 x Ph), 7.52 (2H, *d*, *J* = 15 Hz, 2 x CH=C-CO); **LC-Ms**: *m/z* 958 ($[M+H+Na]^+$).

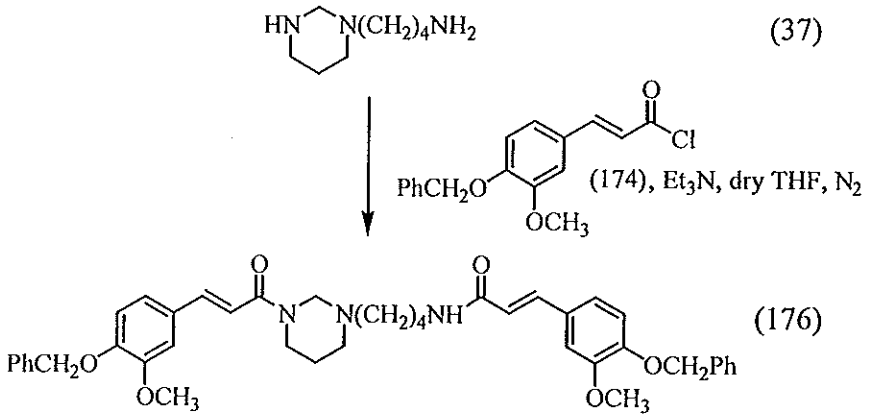
Hexahydropyrimidine (37)



$H_2N(CH_2)_3NH(CH_2)_4NH_2$ (3) → (37)

Spermidine (3) (1.11 g, 7.64 mmol) was dissolved in distilled water (30 ml), and the solution was cooled to 0°C under nitrogen. Formaldehyde (0.62 ml, 8.41 mmol) was slowly added to the cold solution, the reaction mixture was stirred at 0°C for 30 min and then stirred at room temperature for 2 h. The aqueous layer was saturated with solid sodium chloride and extracted with chloroform (6 x 30 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to afford hexahydropyrimidine (37) (1.18 g, 92 %) as a nearly pure waxy white solid. **IR** (film) ν_{max}: 3300, 2960 cm^{-1}; **^{1}H NMR**($CDCl_3$, 60 MHz) δ: 1.33-1.83 (6H, *m*, 3 x C-CH_2-C), 2.05-2.30 (3H, *m*, 3 x NH), 2.47-2.90 (8H, *m*, 4 x CH_2-N), 3.37 (2H, *s*, N-CH_2-N).

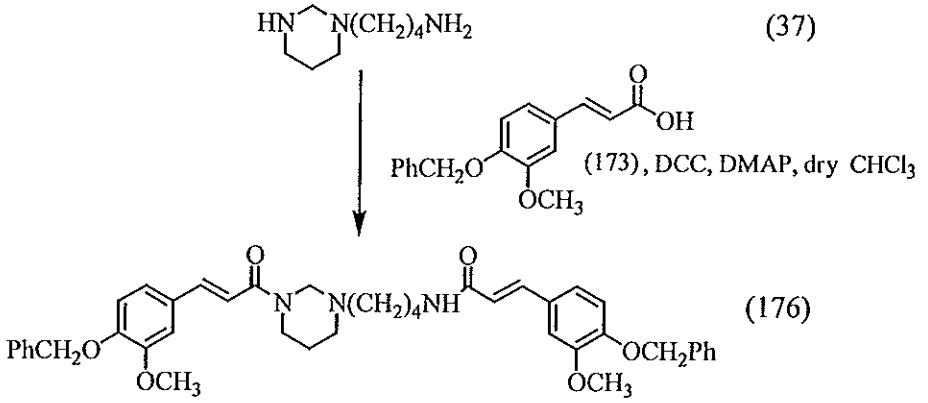
N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) hexahydropyrimidine (176)



A solution of 4-benzyloxy-3-methoxycinnamoyl chloride (174) (477.0 mg, 1.58 mmol) in dry tetrahydrofuran (9 ml) was slowly added to a cooled solution of hexahydropyrimidine (37) (100 mg, 0.63 mmol) and triethylamine (0.50 ml, 34.75 mmol) in dry tetrahydrofuran (12 ml) at 0°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 17 h. The precipitate was removed by filtration and the solvent was then evaporated. The residue was dissolved in chloroform (20 ml), washed with 5 % $NaHCO_3$ (3 x 15 ml), saturated brine (2 x 15 ml), dried over anhydrous sodium sulfate and evaporated to afford a yellow viscous oil. The product was further purified by flash column chromatography on silica gel, eluted with 2 % ethanol in chloroform to afford N^1,N^8-di-(4-benzyloxy-3-methoxy-cinnamoyl) hexahydropyrimidine (176) (224.7 mg, 52 %).

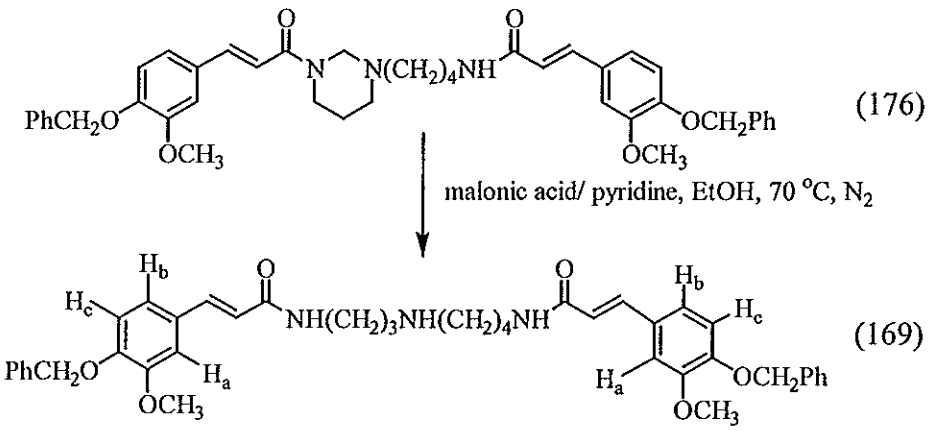
IR (film) ν_{max}: 3300, 3060, 2950, 1650, 1605, 1515, 1270, 745, 700 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 60 MHz) δ: 1.43-1.90 (6H, *m*, 3 x C-CH_2-C), 2.26-3.75 (8H, *m*, 4 x CH_2-N), 3.80 (6H, *s*, 2 x OCH_3), 4.26 (2H, *s*, N-CH_2-N), 5.05 (4H, *s*, 2 x OCH_2), 6.41 (2H, *m*, 2 x =CH-CO), 6.70-7.36 (6H, *m*, 2 x Ar-H), 7.15-7.36 (10H, *m*, 2 x Ph), 7.50 (2H, *m*, 2 x CH=C-CO); **LC-Ms**: *m/z* 712 ($[M+Na]^+$).

N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) hexahydropyrimidine (176)



A solution of DCC (564.6 mg , 2.73 mmol) in dry chloroform (8 ml) was added to a solution of hexahydropyrimidine (37) (230.0 mg, 1.46 mmol), 4-benzyloxy-3-methoxycinnamic acid (173) (903.9 mg, 3.18 mmol) and DMAP (357.0 mg, 2.92 mmol) in dry chloroform (10 ml) at 0°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The solid was filtered off and washed with chloroform. The chloroform wash was combined with the filtrate and more chloroform was added (30 ml). The combined solution was washed with 5 % $NaHCO_3$ (2 x 20 ml), water (2 x 20 ml), dried over anhydrous sodium sulfate and evaporated to afford an oil. The oily residue was further purified by preparative thin-layer chromatography on silica gel, eluted with 4 % ethanol in chloroform to afford N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) hexahydropyrimidine (176) (573.5 mg, 57 %). The spectroscopic data of this compound is on page 63.

N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)



(176)

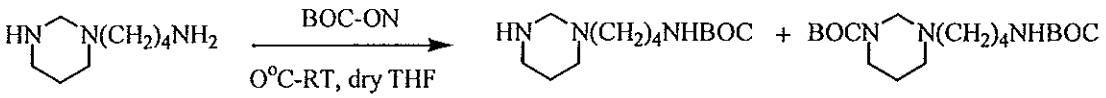
(169)

To a solution of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) hexahydropyrimidine (176) (296.7 mg, 0.44 mmol) in dry ethanol (7 ml) were added dry pyridine (0.10 ml, 1.31 mmol) and solution of malonic acid (225.5 mg, 2.16 mmol) in dry ethanol (6 ml) at room temperature under nitrogen and then the reaction mixture was brought to reflux for 2 h. The solution was concentrated *in vacuo*, water (5 ml) was added and the resulting aqueous solution was basidified to pH 11 with 10 % NaOH and extracted with dichloromethane (5 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to give yellow solid (169) (275.3 mg, 94 %) as a yellow solid, m.p. 182-183°C. **FTIR** (KBr) ν_{max} : 3295, 3055, 2937, 1650, 1615, 1536, 1512, 1253, 1134, 742, 696 cm^{-1}; **^{1}H NMR** ($CDCl_3$ + CD_3OD, 500 MHz) δ: 1.55-1.61 (4H, *m*, 2 x C-CH_2-C), 1.74 (2H, *quint*, J = 6.5 Hz, C-CH_2-C), 2.62 (2H, *t*, J = 6.5 Hz, CH_2-N), 2.65 (2H, *t*, J = 6.5 Hz, CH_2-N), 3.33 (2H, *t*, J = 6.5 Hz, CH_2-NH-CO), 3.38 (2H, *t*, J = 6.5 Hz, CH_2-NH-CO), 3.89 (6H, *s*, 2 x OCH_3), 5.15 (4H, *s*, 2 x OCH_2), 6.34 (1H, *d*, J = 15.5 Hz, =CH-CO), 6.36 (1H, *d*, J = 15.5 Hz, =CH-CO), 6.86 (2H, *dd*, J = 8.5, 2 Hz, 2 x Ar-H_c), 7.02 (2H, *dd*, J = 8.5,

2 Hz, 2 x Ar-H_b), 7.06 (2H, *d*, J = 2 Hz, 2 x Ar-H_a), 7.29-7.43 (10H, *m*, 2 x Ph)*, 7.48 (2H, *dd*, J = 15.5, 2 Hz, 2 x CH=C-CO); **^{13}C NMR** ($CDCl_3$ + CD_3OD, 125 MHz) δ: 26.34, 26.68, 28.69, 37.13, 39.01, 46.55, 48.65, 55.90, 70.97, 110.84, 113.93, 118.95, 119.11, 121.92, 121.97, 127.61, 128.32, 128.64, 128.75, 128.90, 136.99, 137.02, 140.85, 140.98, 150.09, 150.10, 150.11, 150.15, 167.64, 167.81; **LC-Ms**: *m/z* 678 ($[M+H]^+$).

* The signal multiplicity of the protons from δ 7.29 to 7.43 were shown in **Table 14.**

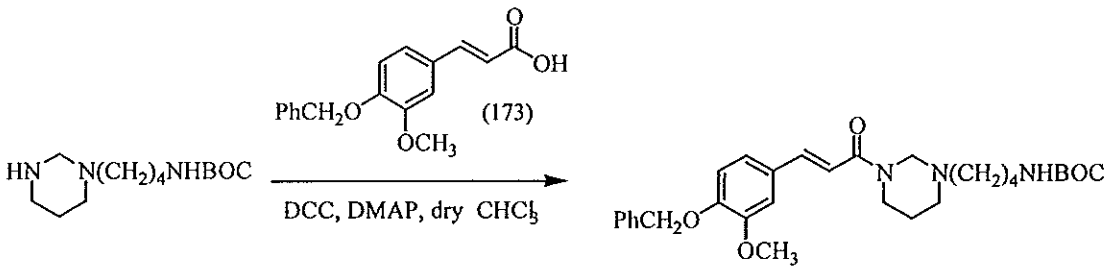
N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine (38)



(37) (38) (177)

A solution of BOC-ON (325 mg, 1.32 mmol) in dry tetrahydrofuran (5 ml) was slowly added dropwise to a solution of hexahydropyrimidine (37) (276.7 mg, 1.75 mmol) in dry tetrahydrofuran (15 ml) at 0°C under nitrogen. After the addition was completed, the ice bath was removed and the reaction mixture was stirred for 12 h. The solvent was then evaporated, and the residue was dissolved in dichloromethane (30 ml), washed with 10 % NaOH (3 x 20 ml), dried over anhydrous sodium sulfate and evaporated to afford a viscous oil. The oily residue was further purified by quick column chromatography on silica gel, eluted with ethyl acetate to afford N^1,N^8-di-(*tert*-butoxy-carbonyl) hexahydropyrimidine (177) (73 mg, 7 %); **FTIR** (film) ν_{max}: 2976, 1689, 1509, 1367, 1216, 1161 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 60 MHz) δ: 1.40-1.80 (24H, 6 x CH_3-C-O, 3 x C-CH2-C), 2.19-3.53 (8H, *m*, 4 x CH_2-N), 4.02 (2H, s, N-CH_2-N), 4.70-5.10 (1H, *br.s*, NH-BOC) and further eluted with 10 % methanol in ethyl acetate to afford N^1,N^8-di-(*tert*-butoxycarbonyl) hexahydropyrimidine (38) (460 mg, 61%). **IR** (film) ν_{max} : 3320, 2940, 1700, 1510, 1390, 1370, 1265, 1170 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 60 MHz) δ: 1.40-1.80 (15H, *m*, 3 x CH_3-C-O, 3 x C-CH_2-C), 2.15-3.25 (8H, *m*, 4 x CH_2-N), 3.35 (2H, *s*, N-CH_2-N), 5.53-5.86 (1H, *br.s*, NH-BOC).

N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine (178)

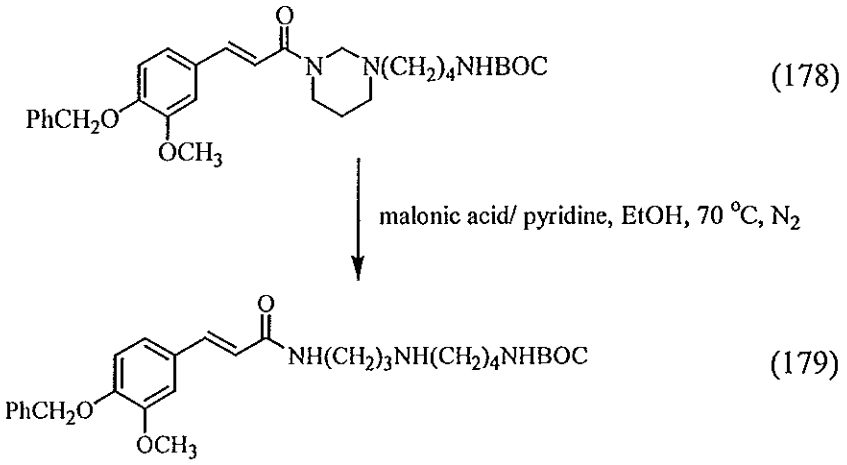


(38) (178)

A solution of DCC (460.2 mg, 2.23 mmol) in dry chloroform (8 ml) was added to a solution of N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine (38) (246.2 mg, 0.96 mmol), 4-benzyloxy-3-methoxycinnamic acid (173) (611.7 mg, 2.15 mmol) and DMAP (270.1 mg, 2.21 mmol) in dry chloroform (5 ml) at 0°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The solid was filtered off and washed with chloroform. The chloroform wash was combined with the filtrate and more chloroform was added (30 ml). The combined solution was washed with 5 % $NaHCO_3$ (2 x 20 ml), water (2 x 20 ml), dried over anhydrous sodium sulfate and evaporated to afford an oil. The oily residue was further purified by column chromatography on silica gel, eluted with 2 % ethanol in chloroform to afford N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine (178) (375.6 mg, 75 %).

IR (film) ν_{max}: 3350, 3060, 2950, 1710, 1650, 1515, 1270, 1140, 740, 700 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 60 MHz) δ: 1.43-1.83 (15H, *m*, 3 x CH_3-C-O, 3 x C-CH_2-C), 2.23-3.76 (8H, *m*, 4 x CH_2-N), 3.85 (3H, *s*, OCH_3), 4.23 (2H, *s*, N-CH_2-N), 4.63-4.93 (1H, *br.s*, NH-BOC), 5.07 (2H, *s*, OCH_2), 6.59 (1H, *d*, J = 15 Hz, =CH-CO), 6.80-7.03 (3H, *m*, Ar-H), 7.22-7.33 (5H, *m*, Ph), 7.49 (1H, *d*, J = 15 Hz, CH=C-CO).

N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179)



(178)

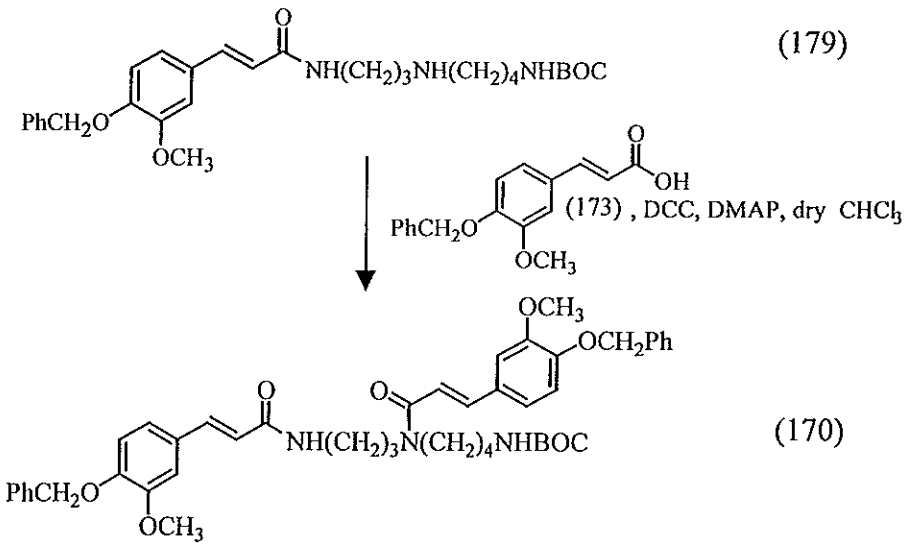
(179)

To a solution of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine (178) (168.1 mg, 0.32 mmol) in dry ethanol (4 ml) were added dry pyridine (78 µl, 0.96 mmol) and solution of malonic acid (167.0 mg, 1.60 mmol) in dry ethanol (2 ml) at room temperature under nitrogen and then the reaction mixture was brought to reflux for 2 h. The solution was concentrated *in vacuo*, water (5 ml) was added and the pH was adjusted to 11 with 10 % NaOH and extracted with dichloromethane (5 x 15 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to afford N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179) (151.0 mg, 92 %) as a yellow solid, m.p. 90-92°C.

IR (film) ν_{max}: 3330, 3040, 2960, 1715, 1675, 1525, 1270, 1174, 1145, 765, 700 cm^{-1}; **^{1}H NMR** ($CDCl_3$, 500 MHz)δ: 1.43 (9H, *s*, 3 x CH_3-C-O), 1.54 (4H, *m*, 2 x C-CH_2-C), 1.74 (2H, *m*, C-CH_2-C), 2.63 (2H, *t*, *J* = 6.5 Hz, CH_2-N), 2.73 (2H, *t*, *J* = 6.5 Hz, CH_2-N), 3.12 (2H, *m*, CH_2-NH-CO), 3.47 (2H, *q*, *J* = 6 Hz, CH_2-NH-CO), 3.89 (3H, *s*, OCH_3), 5.17 (2H, *s*, OCH_2), 6.28 (1H, *d*, *J* = 15.5 Hz, =CH-CO), 6.86 (1H, *d*, *J* = 8.5 Hz, Ar-H_c), 7.01 (1H, *dd*, *J* = 8.5, 2 Hz, Ar-H_b), 7.04 (1H, *d*, *J* = 2 Hz, Ar-H_a), 7.29-7.45 (5H, *m*, Ph)*, 7.52 (1H, *d*, *J* = 15.5 Hz, CH=C-CO); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ: 27.17, 27.76, 28.37, 28.89, 38.69, 40.32, 47.97, 49.26, 55.91, 70.79, 79.14, 110.33, 113.49, 119.13, 121.42, 127.14, 127.89, 128.31, 128.53, 136.64, 140.11, 149.49, 149.59, 166.10, 166.17; **LC-Ms**: *m/z* 512 ($[M+H]^+$).

* The signal multiplicity of the protons from δ 7.29 to 7.45 were shown in **Table 20.**

N^1,N^4-di-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170)



A solution of DCC (24.1 mg, 0.12 mmol) in dry chloroform (1 ml) was added to a solution of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179) (37.9 mg, 0.07 mmol), 4-benzyloxy-3-methoxycinnamic acid (173) (31.5 mg, 0.11 mmol) and DMAP (14.4 mg, 0.12 mmol) in dry chloroform (2 ml) at 0°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The solid was filtered off and washed with chloroform. The chloroform wash was combined with the filtrate and more chloroform was added (20 ml). The combined solution was washed with 5 % $NaHCO_3$ (2 x 15 ml), water (2 x 15 ml), dried over anhydrous sodium sulfate and evaporated to afford an oil. The oily residue was further purified by preparative thin-layer chromatography on silica gel, eluted with 3 % ethanol in chloroform to afford N^1,N^4-di-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170) (40.1 mg, 70 %) as a colourless oil.

IR (film) ν_{max}: 3320, 3015, 2950, 1710, 1655, 1610, 1520, 1265, 1145, 760, 700 cm^{-1}; ^{1}**H NMR** ($CDCl_3$, 60 MHz) δ: 1.43-1.96 (15H, *m*, 3 x CH_3-C-O, 3 x C-CH_2-C), 3.00-3.65 (8H, *m*, 4 x CH_2-N), 3.90 (6H, *s*, 2 x OCH_3), 5.13 (4H, *br.s*, 2 x OCH_2), 6.32 (2H, *m*, 2 x =CH-CO), 6.75-7.02 (6H, *m*, 2 x Ar-H), 7.25-7.35 (10H, *m*, 2 x Ph), 7.55 (2H, *m*, 2 x CH=C-CO); **LC-Ms** : *m/z* 801 ($[M+H+Na]^+$).

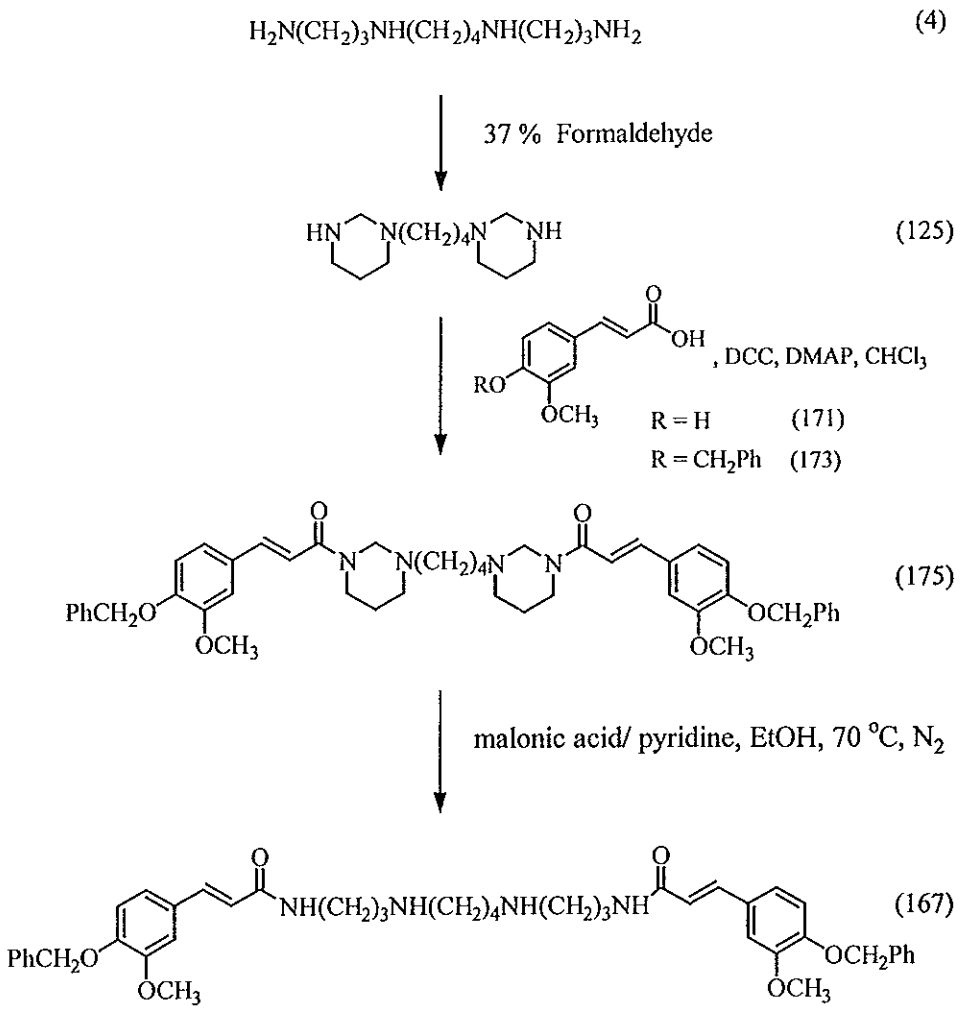
CHAPTER 3

RESULTS AND DISCUSSIONS

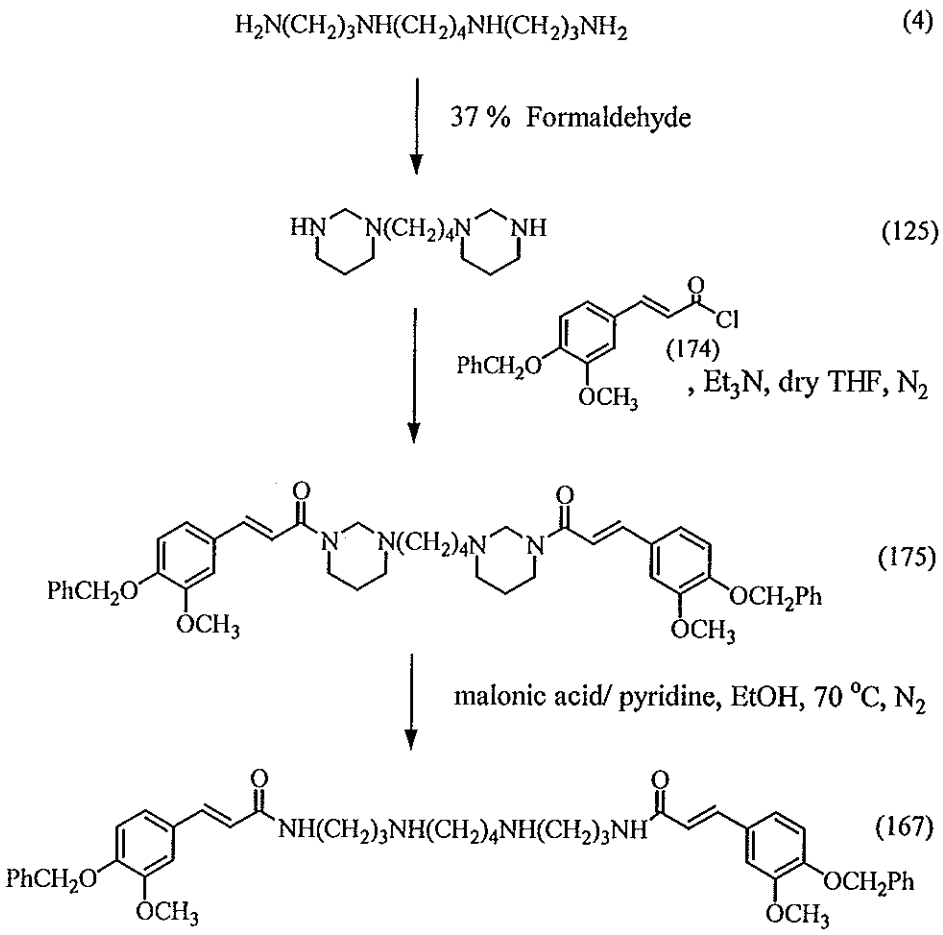
2.1 Synthesis of N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167) could be prepared via bis-hexahydropyrimidine (125) generated from spermine (4). Compound (125) was obtained from the reaction of spermine (4) with formaldehyde solution at 0°C for 30 min and then at room temperature for 2 h (Na Phatthalung, 1994). Several attempts to acylate the secondary amine nitrogen of (125) with 4-hydroxy-3-methoxycinnamoyl chloride in the presence of triethylamine in dry tetrahydrofuran or 4-hydroxy-3-methoxycinnamic acid (171) in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) and 4-dimethyl-aminopyridine (DMAP) in dry chloroform (Na Phatthalung, 1994) resulted in unidentified products and recovered starting materials. The expected product (175) was not detected. This may be due to high reactivity of free phenolic group of this compound under the performed condition. Thus, the phenolic group of acid (171) was protected by the use of benzyl bromide. Subsequently, N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175) was acquired in 52 %, 80 % or 85 % yield (**Table 3**) by condensation of (125) with various reagents and conditions: 4-benzyloxy-3-methoxycinnamic acid (173) in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC)

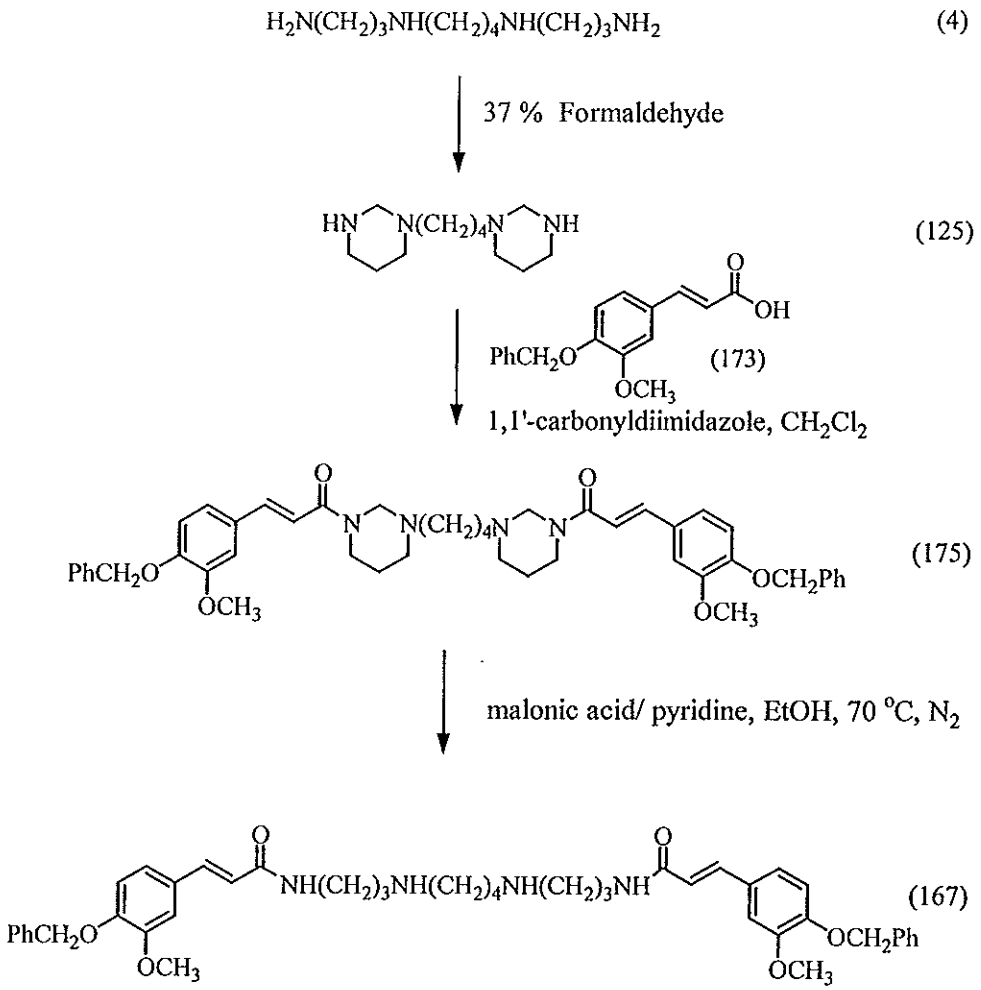
and 4-dimethylaminopyridine (DMAP) in dry chloroform (**Scheme 22**) or 4-benzyloxy-3-methoxycinnamoyl chloride (174) in the presence of triethylamine in dry tetrahydrofuran (**Scheme 23**) or 4-benzyloxy-3-methoxycinnamic acid (173) in the presence of 1,1′-carbonyldiimidazole in dry dichloromethane (**Scheme 24**) (Ponasik *et al.*, 1995). The methylene bridge of the bis-hexahydropyrimidine (175) was selectively removed by the Knoevenagel-like reaction using malonic acid and pyridine in hot ethanol to afford N^{1},N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167) in 84 % yield (Ganem and Nagarijan, 1985).



Scheme 22. Synthesis of N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)



Scheme 23. Synthesis of N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)



Scheme 24. Synthesis of N^{1},N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

Table 3. Reaction of bis-hexahydropyrimidine (125) with various reagents and conditions.

Entry	Reagents/conditions	% yield of product (175)
1	4-benzyloxy-3-methoxycinnamic acid, DCC, DMAP, dry chloroform, 0°C to rt for 24 h	52
2	4-benzyloxy-3-methoxycinnamoyl chloride, Et_3N, dry tetrahydrofuran, 0°C to rt for 19 h	80
3	4-benzyloxy-3-methoxycinnamic acid, 1,1′-carbonyldiimidazole, dry dichloromethane, rt for 24 h	85

The 125 MHz ^{13}C NMR spectrum of compound 167 in $CDCl_3$ (see **Table 4**) exhibited 20 signals for 44 carbon atoms. Analysis of the DEPT spectra of this compound (see **Table 4**) suggested the presence of one methoxy signal for two methoxy carbon atoms [δ 55.89 (2 x OCH_3)], six methylene signals for twelve methylene carbon atoms [δ 70.90 (2 x OCH_2), 49.37 (2 x CH_2), 47.73 (2 x CH_2), 38.47 (2 x CH_2), 28.68 (2 x CH_2) and 27.53 (2 x CH_2)], eight methine signals for twenty methine carbon atoms [δ 140.47 (2 x CH), 128.88 (4 x CH), 128.27 (2 x CH), 127.54 (4 x CH), 121.70 (2 x CH), 119.61 (2 x CH), 113.89 (2 x CH) and 110.87 (2 x CH)] and five quaternary signals for ten quaternary carbon atoms [δ 166.81 (2 x C=O), 150.14 (2 x C), 150.05 (2 x C), 137.04 (2 x C) and 128.73 (2 x C).

The structure of compound 167 was deduced from the detailed analysis of the DEPT (**Table 4**), ^{1}H-^{1}H COSY (**Table 5**)(see **Figure 1**), ^{13}C-^{1}H correlation (**Table 6**), ^{13}C and ^{1}H spectra. The complete assignment of ^{13}C and ^{1}H signals are shown in **Table 7**. The ^{13}C-^{1}H correlation by long-range coupling (HMBC) shown in **Table 8** and **Figure 2** confirmed some of the assignments. The LC mass spectrum showed the molecular ion peak at *m/z* 735 ($[M+H]^+$). The IR spectrum suggested the presence of amine groups, aromatic groups and amide groups (3285, 3014, 1656, 1613, 1599 and 1511 cm^{-1}).

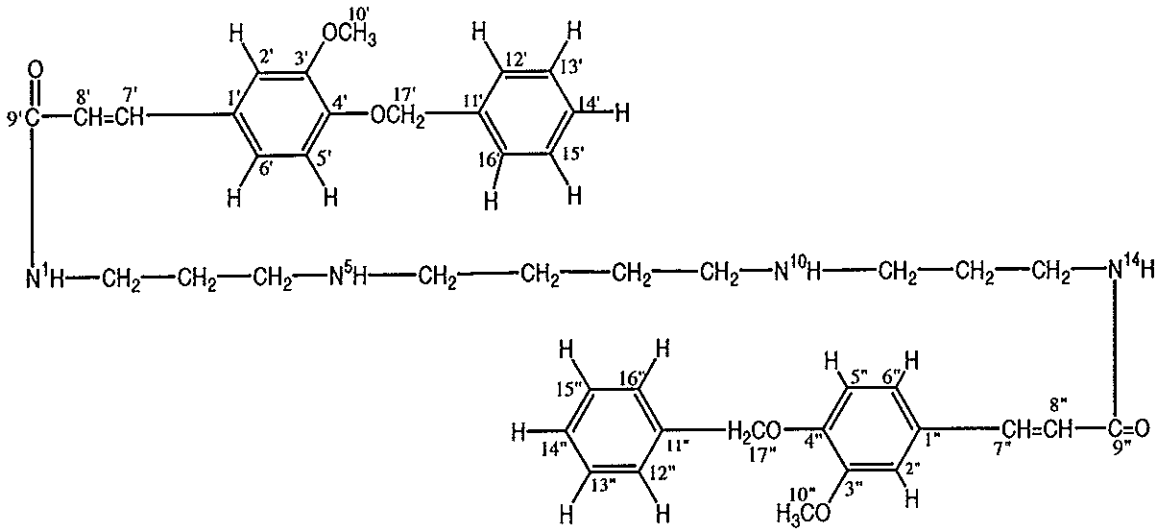


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Table 4. ^{13}C and DEPT NMR spectral data of compound 167

δ_C (ppm)	DEPT	C-type
166.81 (2 x C)		C=O
150.14 (2 x C)		C
150.05 (2 x C)		C
140.47 (2 x CH)	140.47	CH
137.04 (2 x C)		C
128.88 (4 x CH)	128.88	CH
128.73 (2 x C)		C
128.27 (4 x CH)	128.27	CH
127.54 (2 x CH)	127.54	CH
121.70 (2 x CH)	121.70	CH
119.61 (2 x CH)	119.61	CH
113.89 (2 x CH)	113.89	CH
110.87 (2 x CH)	110.87	CH
70.90 (2 x OCH_2)	70.90	OCH_2
55.89 (2 x OCH_3)	55.89	OCH_3
49.37 (2 x CH_2)	49.37	CH
47.73 (2 x CH_2)	47.73	CH_2
38.47 (2 x CH_2)	38.47	CH_2
28.68 (2 x CH_2)	28.68	CH_2
27.53 (2 x CH_2)	27.53	CH_2

Table 5. 500 MHz ^{1}H-^{1}H COSY correlation among some protons of compound 167

δ_H (ppm)		proton correlated with δ_H (ppm)
7.48 (H-7′,-7″)	⟺	6.28 (H-8′,-8″)
7.39-7.41 (H-12′,-12″,-16′,-16″)	⟺	7.32-7.35 (H-13′,-13″,-15′,-15″)
7.32-7.35 (H-13′,-13″,-15′,-15″)	⟺	7.39-7.41 (H-12′,-12″,-16′,-16″) , 7.26-7.29 (H-14′,-14″)
7.26-7.29 (H-14′,-14″)	⟺	7.32-7.35 (H-13′,-13″,-15′,-15″)
6.97 (H-6′,-6″)	⟺	6.81 (H-5′,-5″)
6.81 (H-5′,-5″)	⟺	6.97 (H-6′,-6″)
6.28 (H-8′,-8″)	⟺	7.48 (H-7′,-7″)
3.42 (H-2,-13)	⟺	1.68 (H-3,-12)
2.68 (H-4,-11)	⟺	1.68 (H-3,-12)
2.59 (H-6,-9)	⟺	1.53 (H-7,-8)
1.68 (H-3,-12)	⟺	2.68 (H-4,-11) , 3.42 (H-2,-13)
1.53 (H-7,-8)	⟺	2.59 (H-6,-9)

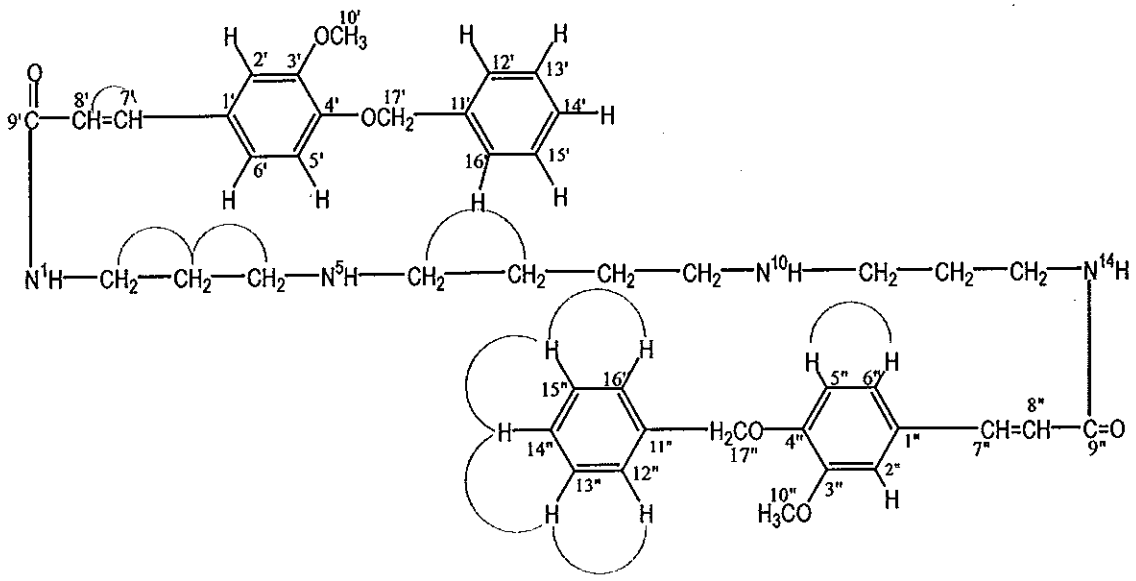


Figure 1. ^{1}H-^{1}H COSY of compound 167

Table 6. ^{13}C-^{1}H correlation 2D NMR spectral data of compound 167

δ_C (ppm)	δ_H (ppm)	assignment
140.47	7.48	CH-7′,-7″
128.88	7.32-7.35	CH-13′,-13″,-15′,-15″
128.27	7.26-7.29	CH-14′,-14″
127.54	7.39-7.41	CH-12′,-12″,-16′,-16″
121.70	6.97	CH-6′,-6″
119.61	6.28	CH-8′,-8″
113.89	6.81	CH-5′,-5″
110.87	7.00	CH-2′,-2″
70.90	5.12	CH_2-17′,-17″
55.89	3.84	CH_3-10′,-10″
49.37	2.59	CH_2-6,-9
47.73	2.68	CH_2-4,-11
38.47	3.42	CH_2-2,-13
28.68	1.68	CH_2-3,-12
27.53	1.53	CH_2-7,-8

Table 7. ^{13}C and ^{1}H NMR (HMQC) spectral data of compound 167

Positions	δ_C*	δ_H, *mult*, *J* (Hz)
2,13	38.47 (CH_2)	3.42 (2H, *q*, 6)
3,12	28.68 (CH_2)	1.68 (2H, *quint*, 6)
4,11	47.73 (CH_2)	2.68 (2H, *t*, 6.5)
6,9	49.37 (CH_2)	2.59 (2H, *t*, 6)
7,8	27.53 (CH_2)	1.53 (2H, *m*)
1′,1″	128.73 (C)	
2′,2″	110.87 (CH)	7.00 (1H, *d*, 2)
3′,3″	150.14 (C)	
4′,4″	150.05 (C)	
5′,5″	113.89 (CH)	6.81 (1H, *d*, 8.5)
6′,6″	121.70 (CH)	6.97 (1H, *dd*, 8.5, 2)
7′,7″	140.47 (CH)	7.48 (1H, *d*, 15.5)
8′,8″	119.61 (CH)	6.28 (1H, *d*, 15.5)
9′,9″	166.81 (C)	
10′,10″	55.89 (OCH_3)	3.84 (3H, *s*)
11′,11″	137.04 (C)	
12′,12″,16′,16″	127.54 (CH)	7.39-7.41 (2H, *m*)
13′,13″,15′,15″	128.88 (CH)	7.32-7.35 (2H, *m*)
14′,14″	128.27 (CH)	7.26-7.29 (1H, *m*)
17′,17″	70.90 (OCH_2)	5.12 (2H, *s*)

The new numbering system (positions of C and N atoms) is especially assigned for the consideration of ^{13}C-^{1}H correlation.

* Carbon type deduced from DEPT experiments.

Table 8. ^{13}C-^{1}H correlation by long-range coupling (HMBC) spectral data of compound 167

δ_C (ppm)		proton correlation with δ_C (ppm)
166.81 (C-9′,-9″)	⟺	3.42 (H-2,-13), 6.28 (H-8′,-8″), 7.48 (H-7′,-7″)
150.14 (C-3′,-3″)	⟺	3.84 (H-10′,-10″)
150.05 (C-4′,-4″)	⟺	5.12 (H-17′,-17″)
140.47 (C-7′,-7″)	⟺	6.97 (H-6′,-6″), 7.00 (H-2′,-2″)
137.04 (C-11′,-11″)	⟺	5.12 (H-17′,-17″), 7.32-7.35 (H-13′,-13″,-15′,-15″)
128.73 (C-1′,-1″)	⟺	6.28 (H-8′,-8″), 6.81 (H-5′,-5″), 7.48 (H-7′,-7″)
128.27 (C-14′,-14″)	⟺	7.39-7.41 (H-12′,-12″,-16′,-16″)
127.54 (C-12′,-12″,-16′,-16″)	⟺	7.26-7.29 (H-14′,-14″)
121.70 (C-6′,-6″)	⟺	6.81 (H-5′,-5″), 7.00 (H-2′,-2″), 7.48 (H-7′,-7″)
119.61 (C-8′,-8″)	⟺	7.48 (H-7′,-7″)
113.89 (C-5′,-5″)	⟺	6.97 (H-6′,-6″)
110.87 (C-2′,-2″)	⟺	6.97 (H-6′,-6″), 7.48 (H-7′,-7″)
49.37 (C-6,-9)	⟺	1.53 (H-7,-8), 2.68 (H-4,-11)
47.73 (C-4,-11)	⟺	1.68 (H-3,-12), 2.59 (H-6,-9)
38.47 (C-2,-13)	⟺	1.68 (H-3,-12), 2.68 (H-4,-11)
28.68 (C-3,-12)	⟺	2.68 (H-4,-11)
27.53 (C-7,-8)	⟺	2.59 (H-6,-9)

Total protons are not assigned in this table.

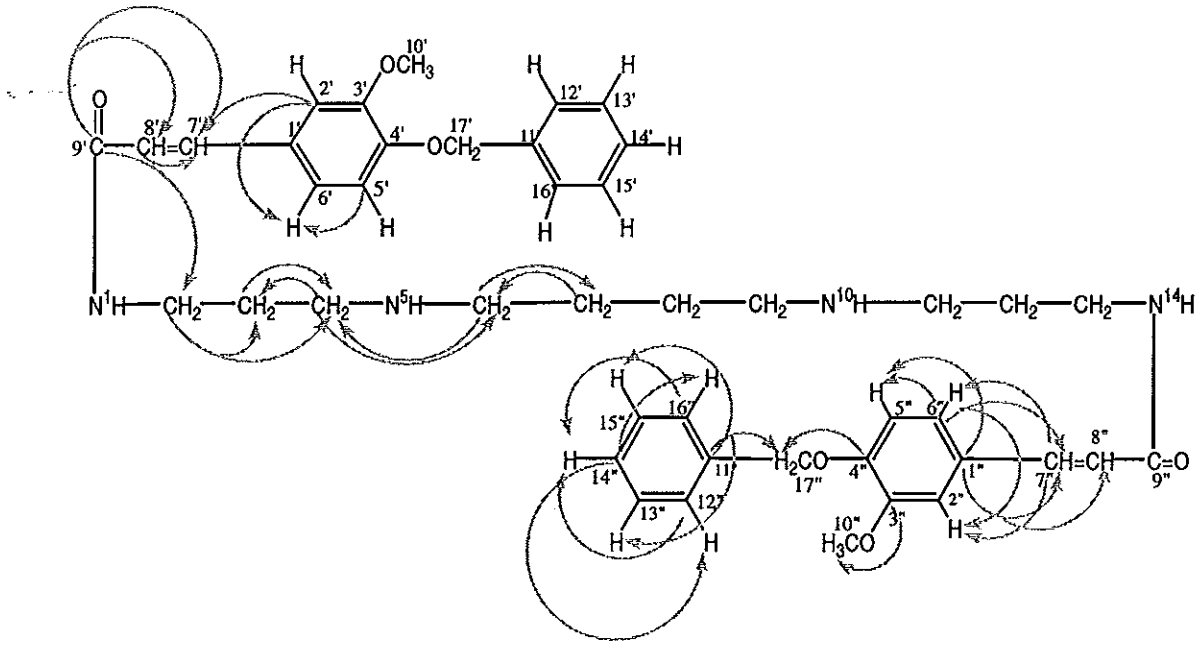


Figure 2. HMBC of compound 167

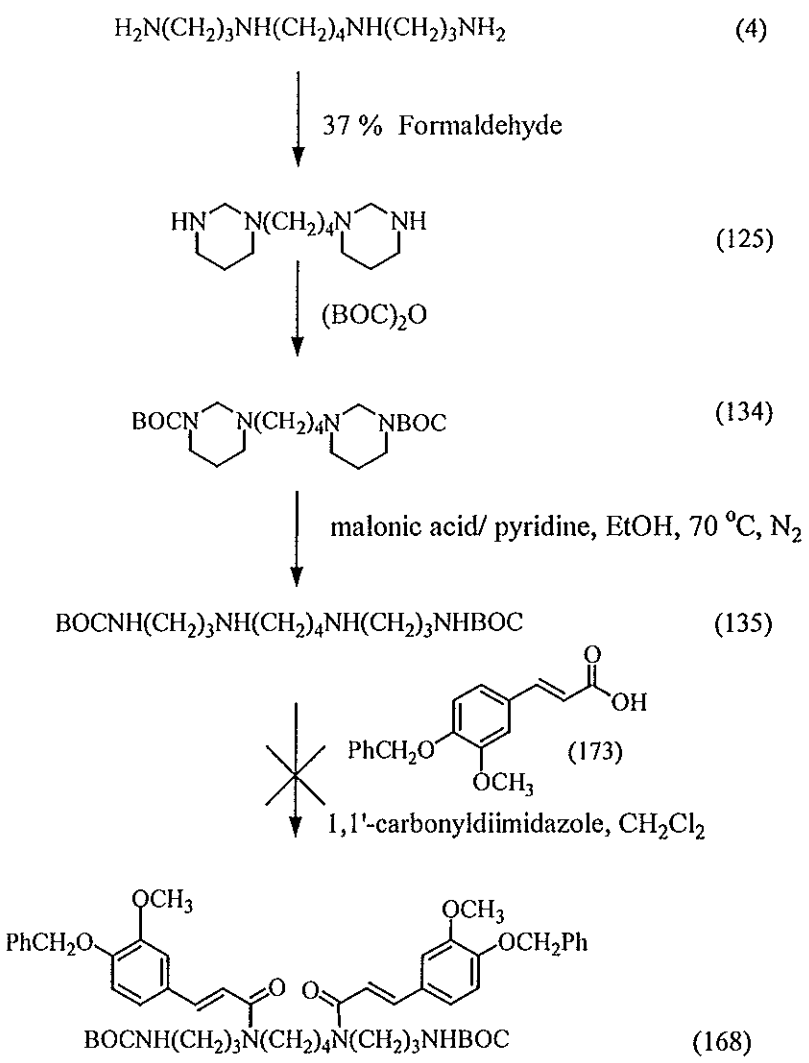
The arrows represent ^{13}C-^{1}H correlation (^{13}C-^{1}H long-range coupling) which were partially shown.

2.2 Synthesis of N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168)

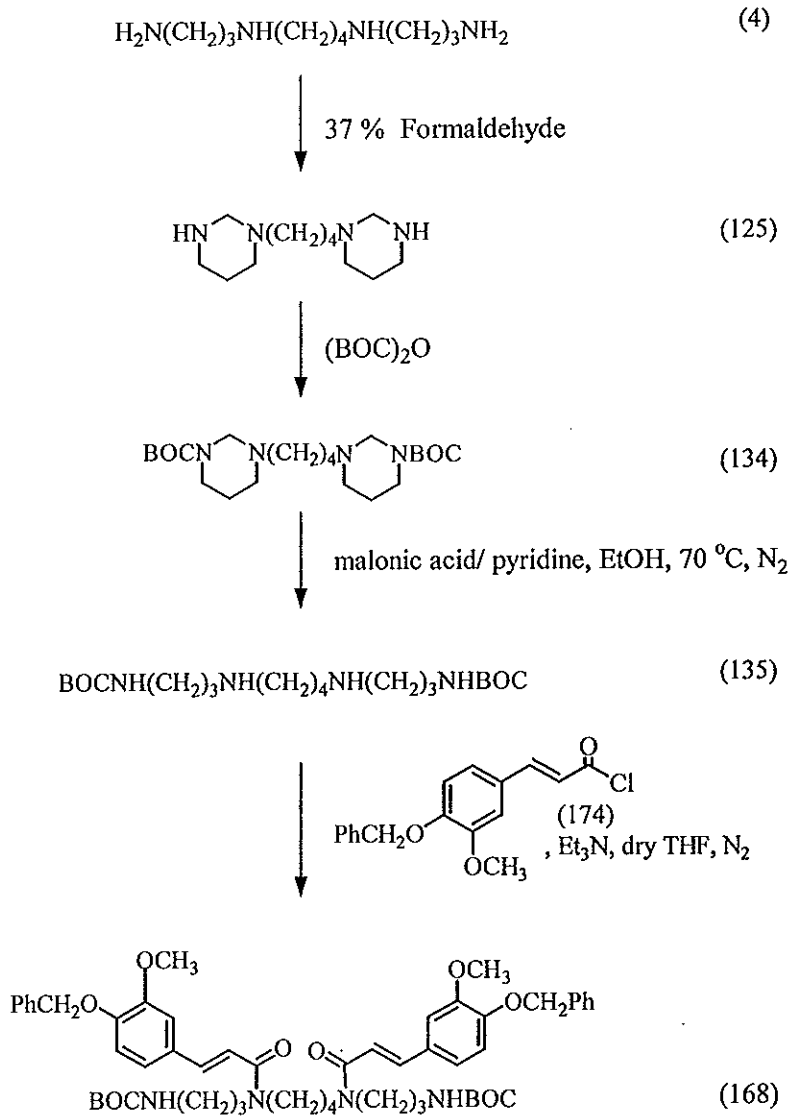
Synthesis of N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168) could be started from bis-hexahydropyrimidine (125). The next step was the protection of N^1 and N^{12} in the form of BOC groups (134, 86 %) which could be achieved by the reaction of bis-hexahydropyrimidine (125) with di-*tert*-butyldicarbonate ($(BOC)_2O$) in dry tetrahydrofuran (Tanikkul, 1990). The N,N-methylene bridge of N^1,N^{12}-di-(*tert*-butoxycarbonyl) bis-hexahydropyrimidine (134) was removed by the reaction with malonic acid and pyridine in ethanol under reflux to obtain N^1,N^{12}-di-(*tert*-butoxycarbonyl) spermine (135) (77 % yield) (Ganem and Nagarijan, 1985).

The reaction of spermine (135) with 4-benzyloxy-3-methoxy-cinnamic acid (173) in the presence of 1,1′-carbonyldiimidazole (Ponasik *et al.*, 1995) failed to give N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168) whereas the percent yield of spermine (168) was increased from 20 to 75 % (**Table 9**) when treated spermine (135) with corresponding acid chloride (174) and carboxylic acid (173) in the presence of DCC and DMAP as summarized in **Schemes 25, 26, 27**, respectively. The IR spectrum of this compound (168) after purification showed the following bands: 3019, 2000-1800, 1702, 1644, 1596, 1510 cm^{-1} and NMR spectrum of this compound showed the following characteristic protons : 3.80 (*s*, 2 x OCH_3), 5.05 (*s*, 2 x OCH_2Ph), 6.69-7.03 (*m*, 2 x =CH-CO, 2 x Ar-H) and 7.52 (*d*, J = 15 Hz, 2 x CH=C-CO) which indicated the presence of

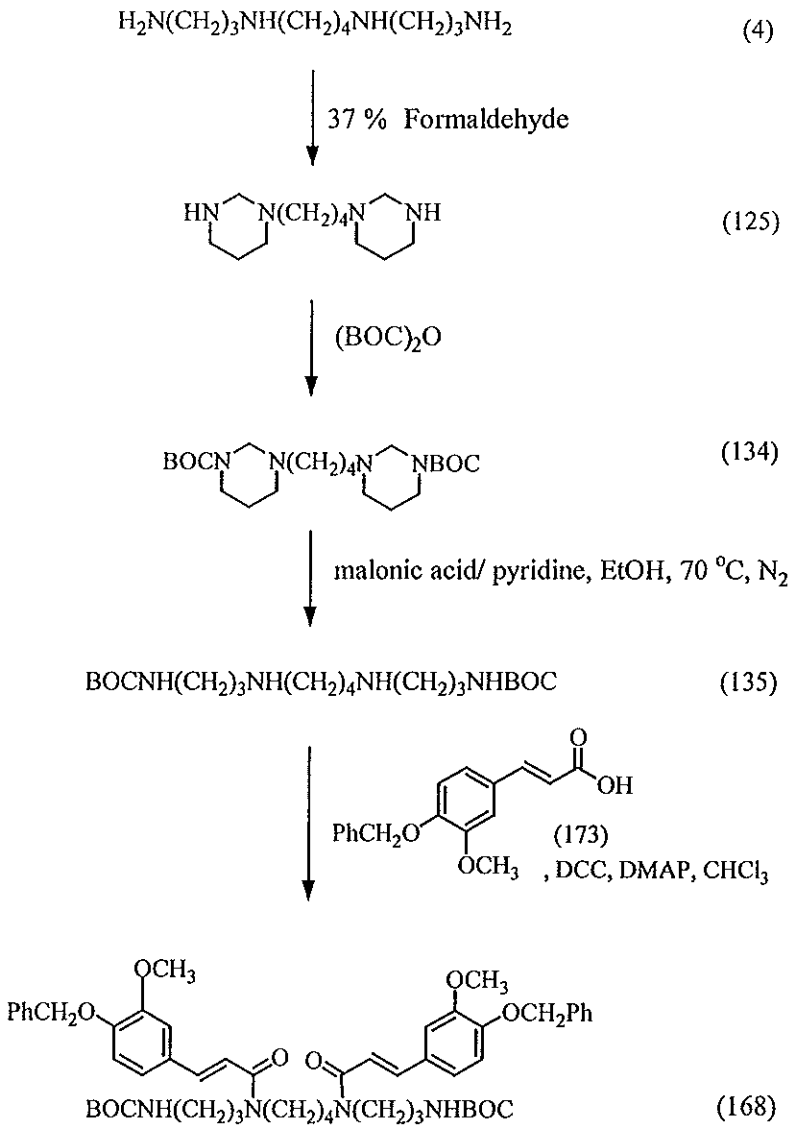
cinnamoyl groups in the molecule. Thus, the possible structure should be N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168).



Scheme 25. Synthesis of N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168)



Scheme 26. Synthesis of N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168)



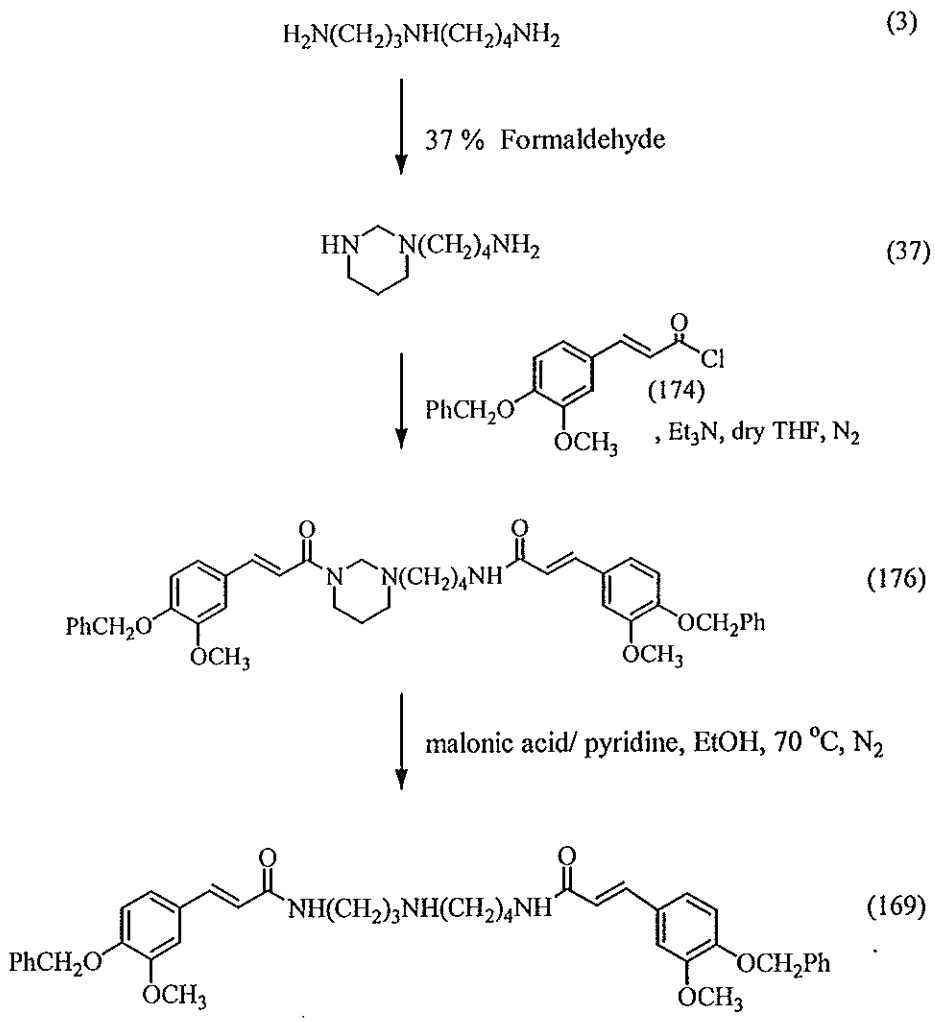
Scheme 27. Synthesis of N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168)

Table 9. Reaction of N^1,N^{12}-di-(*tert*-butoxycarbonyl) spermine (135) with various reagents and conditions.

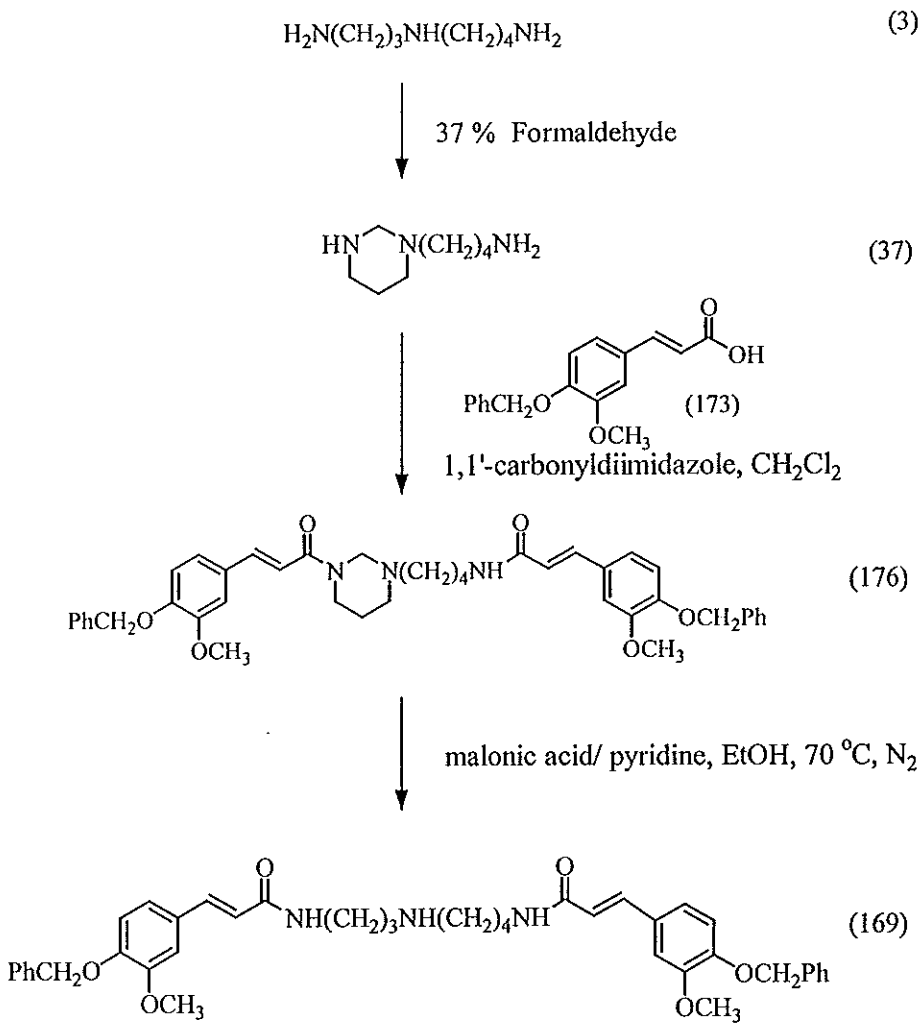
Entry	Reagents/conditions	% yield of product (168)
1	4-benzyloxy-3-methoxycinnamic acid, 1,1′-carbonyldiimidazole, dry dichloromethane, rt for 24 h	-
2	4-benzyloxy-3-methoxycinnamoyl chloride, Et_3N, dry tetrahydrofuran, 0°C to rt for 12 h	20
3	4-benzyloxy-3-methoxycinnamic acid, DCC, DMAP, dry chloroform, 0°C to rt for 24 h	75

2.3 Synthesis of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)

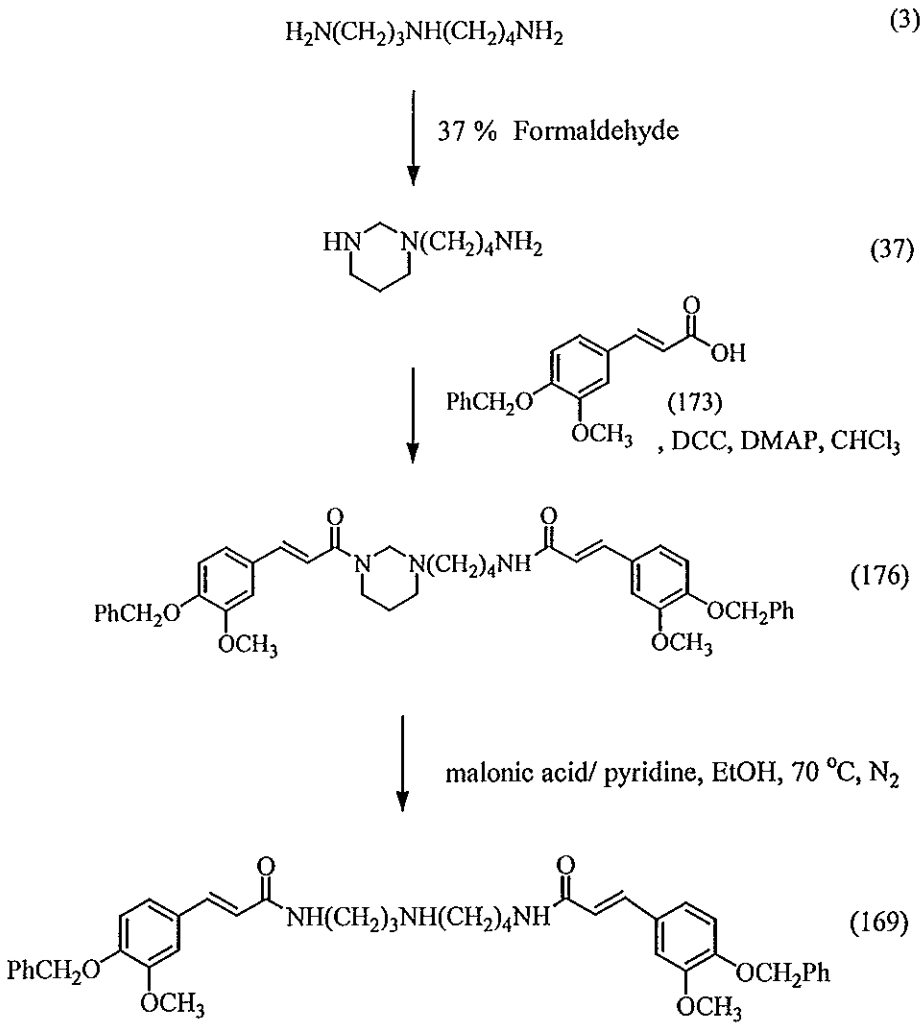
N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169) could be prepared via hexahydropyrimidine (37) generated from spermidine (3). In the first step, spermidine (3) reacted with formaldehyde solution at 0°C to produce a 92 % yield of the hexahydropyrimidine (37) (Chantrapromma *et al.*, 1990). Condensation of hexahydropyrimidine (37) with various reagents and conditions: 4-benzyloxy-3-methoxycinnamoyl chloride (174) in the presence of triethylamine in dry tetrahydrofuran or with 4-benzyloxy-3-methoxycinnamic acid (173) in the presence of 1,1′-carbonyldiimidazole in dry dichloromethane (Ponasik *et al.*, 1995) or with 4-benzyloxy-3-methoxycinnamic acid (173) in the presence of DCC and DMAP in dry chloroform produced N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) hexahydropyrimidine (176) according to **Schemes 28, 29, 30,** respectively. The percentage yields of these reactions were shown in **Table 10**. The gemdiamine protecting group of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) hexahydropyrimidine (176) was selectively removed by the Knoevenagel-like reaction using malonic acid and pyridine in hot ethanol to afford N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169) in 94 % yield (Ganem and Nagarijan, 1985).



Scheme 28. Synthesis of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)



Scheme 29. Synthesis of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)



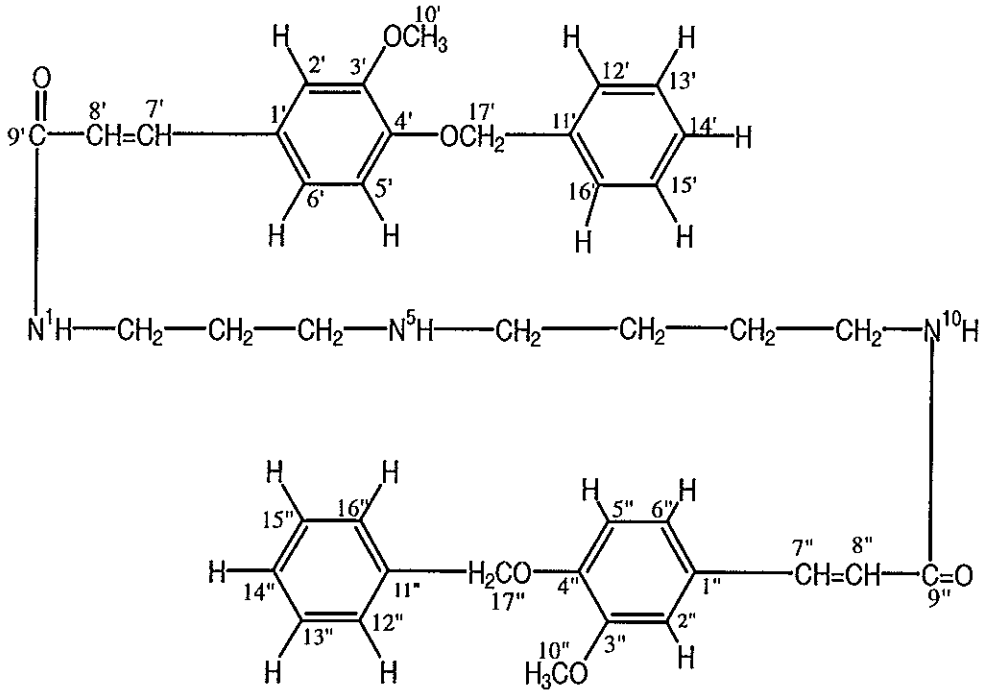
Scheme 30. Synthesis of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)

Table 10. Reaction of hexahydropyrimidine (37) with various reagents and conditions.

Entry	Reagents/conditions	% yield of product (176)
1	4-benzyloxy-3-methoxycinnamoyl chloride, Et_3N, dry tetrahydrofuran, 0°C to rt for 17 h	52
2	4-benzyloxy-3-methoxycinnamic acid, 1,1′-carbonyldiimidazole, dry dichloromethane, rt for 24 h	18
3	4-benzyloxy-3-methoxycinnamic acid, DCC, DMAP, dry chloroform, 0°C to rt for 24 h	57

The 125 MHz ^{13}C NMR spectrum of compound 169 in $CDCl_3$ (see **Table 11**) exhibited 30 signals for 41 carbon atoms. Analysis of the DEPT spectra of this compound (see **Table 12**) suggested the presence of one methoxy signal for two methoxy carbon atoms [δ 55.90 (2 x OCH_3)], eight methylene signals for nine methylene carbon atoms [δ 70.97 (2 x OCH_2), 48.65, 46.55, 39.01, 37.13, 28.69, 26.68, 26.34], eleven methine signals for twenty methine carbon atoms [δ 140.98, 140.85, 128.90 (4 x CH), 128.32 (2 x CH), 127.61 (4 x CH), 121.97, 121.92, 119.11, 118.95, 113.93 (2 x CH), 110.84 (2 x CH)] and ten quaternary carbon atoms [δ 167.81 (C=O), 167.64 (C=O), 150.15, 150.11, 150.10, 150.09, 137.02, 136.99, 128.75, 128.64].

The structure of compound 169 was deduced from the detailed analysis of the DEPT (**Table 11**), ^{1}H-^{1}H COSY (**Table 12**)(see **Figure 3**), ^{13}C-^{1}H correlation (**Table 13**), ^{13}C and ^{1}H spectra. The complete assignment of ^{13}C and ^{1}H signals are shown on **Table 14.** The ^{13}C-^{1}H correlation by long-range coupling (HMBC) shown in **Table 15** and **Figure 4** confirmed some of the assignments. The LC mass spectrum showed the molecular ion peak at *m/z* 678 ($[M+H]^+$). The IR spectrum suggested the presence of amine groups, aromatic groups and amide groups (3295, 3055, 1650, 1615, 1536, 1512 cm^{-1})



169

Table 11. ^{13}C and DEPT NMR spectral data of compound 169

δ_C (ppm)	DEPT	C-type
167.81		C=O
167.64		C=O
150.15		C
150.11		C
150.10		C
150.09		C
140.98	140.98	CH
140.85	140.85	CH
137.02		C
136.99		C
128.90 (4 x CH)	128.90	CH
128.75		C
128.64		C
128.32 (2 x CH)	128.32	CH
127.61 (4 x CH)	127.61	CH
121.97	121.97	CH
121.92	121.92	CH
119.11	119.11	CH
118.95	118.95	CH
113.93 (2 x CH)	113.93	CH
110.84 (2 x CH)	110.84	CH
70.97 (2 x OCH_2)	70.97	OCH_2
55.90 (2 x OCH_3)	55.90	OCH_3
48.65	48.65	CH_2
46.55	46.55	CH_2
39.01	39.01	CH_2
37.13	37.13	CH_2
28.69	28.69	CH_2
26.68	26.68	CH_2
26.34	26.34	CH_2

Table 12. 500 MHz ^{1}H-^{1}H COSY correlation among some protons of compound 169

δ_H (ppm)		proton correlated with δ_H (ppm)
7.48 (H-7′)	⟺	6.36 (H-8′)
7.48 (H-7″)	⟺	6.34 (H-8″)
7.41-7.44 (H-12′,-12″,-16′,-16″)	⟺	7.35-7.39 (H-13′,-13″,-15′,-15″)
7.35-7.39 (H-13′,-13″,-15′,-15″)	⟺	7.41-7.44 (H-12′,-12″,-16′,-16″)
		7.30 (H-14′,-14″)
7.30 (H-14′,-14″)	⟺	7.35-7.39 (H-13′,-13″,-15′,-15″)
7.02 (H-6′,-6″)	⟺	6.86 (H-5′,-5″)
6.86 (H-5′,-5″)	⟺	7.02 (H-6′,-6″)
6.36 (H-8′)	⟺	7.48 (H-7′)
6.34 (H-8″)	⟺	7.48 (H-7″)
3.38 (H-2)	⟺	1.74 (H-3)
3.33 (H-9)	⟺	1.59 (H-8)
2.65 (H-4)	⟺	1.74 (H-3)
2.62 (H-6)	⟺	1.55 (H-7)
1.74 (H-3)	⟺	3.38 (H-2), 2.65 (H-4)
1.55 (H-7)	⟺	2.62 (H-6)
1.59 (H-8)	⟺	3.33 (H-9)

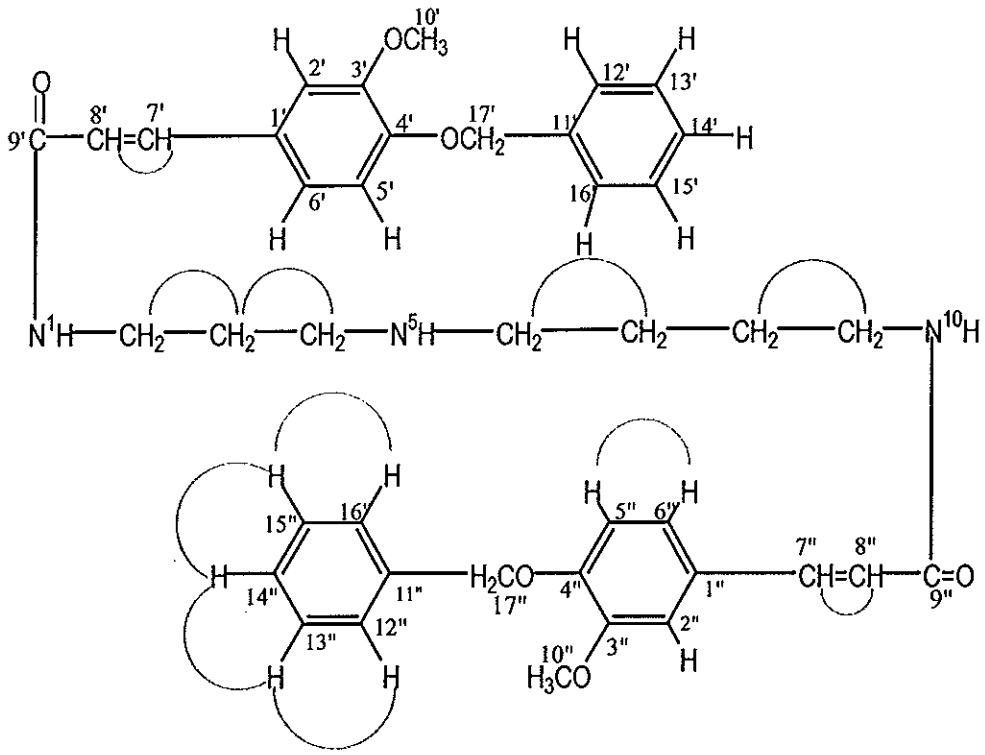


Figure 3. ^{1}H-^{1}H COSY of compound 169

Table 13. ^{13}C-^{1}H correlation 2D NMR spectral data of compound 169

δ_C (ppm)	δ_H (ppm)	assignment
104.98 – 140.85	7.48	CH-7′,-7″
128.90 (4 x CH)	7.35-7.39	CH-13′,-13″,-15′,-15″
128.32 (2 x CH)	7.30	CH-14′,-14″
127.61 (4 x CH)	7.41-7.44	CH-12′,-12″,-16′,-16″
121.97 – 121.92	7.02	CH-6′,-6″
119.11	6.36	CH-8′
118.95	6.34	CH-8″
113.93 (2 x CH)	6.86	CH-5′,-5″
110.84 (2 x CH)	7.06	CH-2′,-2″
70.97 (2 x OCH_2)	5.15	CH_2-17′,-17″
55.90 (2 $XOCH_3$)	3.89	CH_3-10′,-10″
48.65	2.62	CH_2-6
46.55	2.65	CH_2-4
39.01	3.33	CH_2-9
37.13	3.38	CH_2-2
28.69	1.74	CH_2-3
26.68	1.55	CH_2-7
26.34	1.59	CH_2-8

Table 14. ^{13}C and ^{1}H NMR (HMQC) spectral data of compound 169

Positions	δ_C*	δ_H, *mult*, *J* (Hz)
2	37.13 (CH_2)	3.38 (2H, *t*, 6.5)
3	28.69 (CH_2)	1.74 (2H, *quint*, 6.5)
4	46.55 (CH_2)	2.65 (2H, *t*, 6.5)
6	48.66 (CH_2)	2.62 (2H, *t*, 6.5)
7	26.68 (CH_2)	1.55 (2H, *m*)
8	26.34 (CH_2)	1.59 (2H, *m*)
9	39.01 (CH_2)	3.33 (2H, *t*, 6.5)
1′,1″	128.75, 128.64 (C)	
2′,2″	110.84 (2 x CH)	7.06 (2H, *d*, 2)
3′,3″	150.15, 150.11 (C)	
4′,4″	150.10, 150.09 (C)	
5′,5″	113.93 (2 x CH)	6.86 (2H, *dd*, 8.5, 2)
6′,6″	121.97, 121.92 (CH)	7.02 (2H, *dd*, 8.5, 2)
7′,7″	140.98, 140.85 (CH)	7.48 (2H, *dd*, 15.5, 2)
8′	119.11 (CH)	6.36 (1H, *d*, 15.5)
8″	118.95 (CH)	6.34 (1H, *d*, 15.5)
9′	167.81 (C=O)	
9″	167.64 (C=O)	
10′,10″	55.90 (2 x OCH_3)	
11′,11″	136.99, 137.02 (C)	
12′,12″,16′,16″	127.61 (4 x CH)	7.41-7.44 (4H, *m*)
13′,13″,15′,15″	128.90 (4 x CH)	7.35-7.39 (4H, *m*)
14′,14″	128.32 (2 x CH)	7.30 (2H, *m*)
17′,17″	70.97 (2 x OCH_2)	

The new numbering system (positions of C and N atoms) is especially assigned for the consideration of ^{13}C-^{1}H correlation.

* Carbon type deduced from DEPT experiments

Table 15. ^{13}C-^{1}H correlation by long-range coupling (HMBC) spectral data of compound 169

δ_C (ppm)		proton correlation with δ_C (ppm)
167.81 (C-9′)	⟺	7.48 (H-7′), 6.36 (H-8′), 3.38 (H-2)
167.64 (C-9″)	⟺	7.48 (H-7″), 6.34 (H-8″), 3.33 (H-9)
150.15, 150.11 (C-3′,-3″)	⟺	3.89 (H-10′,-10″)
150.10, 150.09 (C-4′,-4″)	⟺	5.15 (H-17′,-17″)
140.98, 140.85 (C-7′,-7″)	⟺	7.06 (H-2′,-2″), 7.02 (H-6′,-6″)
137.02, 136.99 (C-11′,-11″)	⟺	7.35-7.39 (H-13′,-13″,-15′,-15″), 5.15 (H-17′,-17″)
128.75, 128.64 (C-1′,-1″)	⟺	7.48 (H-7′,-7″), 6.86 (H-5′,-5″), 6.36 (H-8′), 6.34 (H-8″)
128.32 (C-14′,-14″)	⟺	7.41-7.44 (H-12′,-12″,-16′,-16″)
127.61 (C-12′,-12″,-16′,-16″)	⟺	7.30 (H-14′,-14″)
121.97, 121.92 (C-6′,-6″)	⟺	7.48 (H-7′,-7″), 7.06 (H-2′,-2″), 6.86 (H-5′,-5″)
119.11 (C-8′)	⟺	7.48 (H-7′,-7″)
118.95 (C-8″)	⟺	7.48 (H-7′,-7″)
113.93 (C-5′,-5″)	⟺	7.02 (H-6′,-6″)
110.84 (C-2′,-2″)	⟺	7.48 (H-7′,-7″), 7.02 (H-6′,-6″)
48.65 (C-6)	⟺	2.65 (H-4)
46.55 (C-4)	⟺	3.38 (H-2), 2.62 (H-6), 1.74 (H-3)
37.13 (C-2)	⟺	2.65 (H-4), 1.74 (H-3)
28.69 (C-3)	⟺	3.38 (H-2), 2.65 (H-4)
26.68 (C-7)	⟺	3.33 (H-9), 2.62 (H-6)
26.34 (C-8)	⟺	3.33 (H-9), 2.62 (H-6)

Total protons are not assigned in this table.

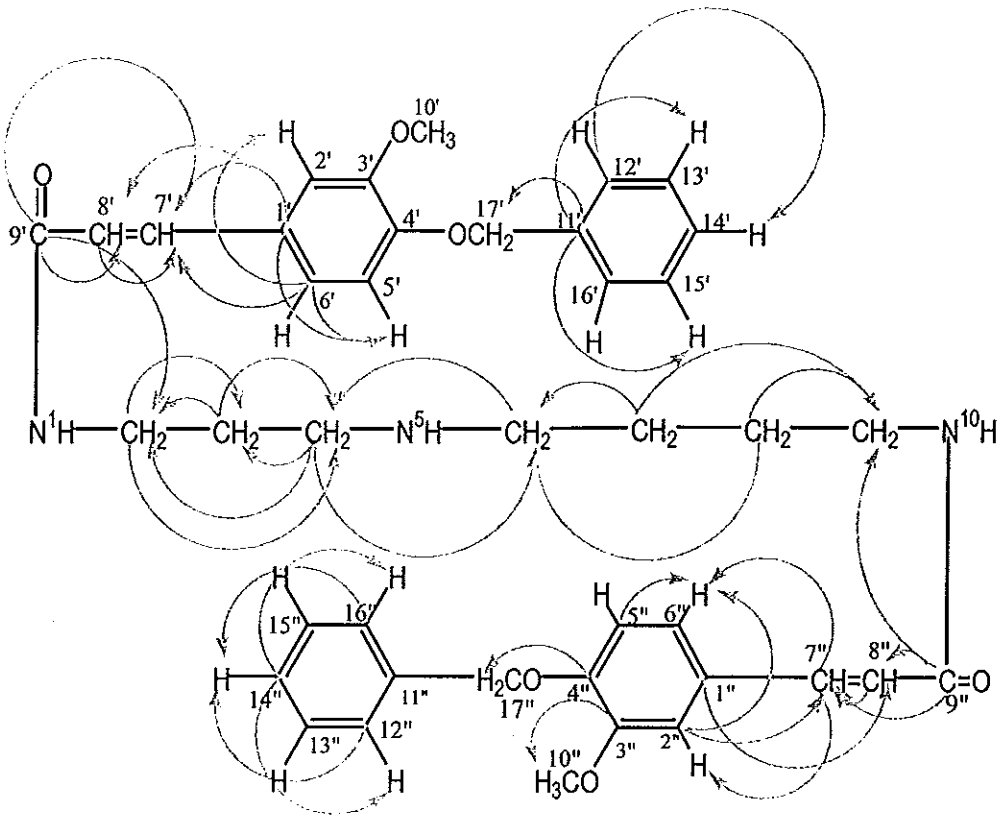


Figure 4. HMBC of compound 169

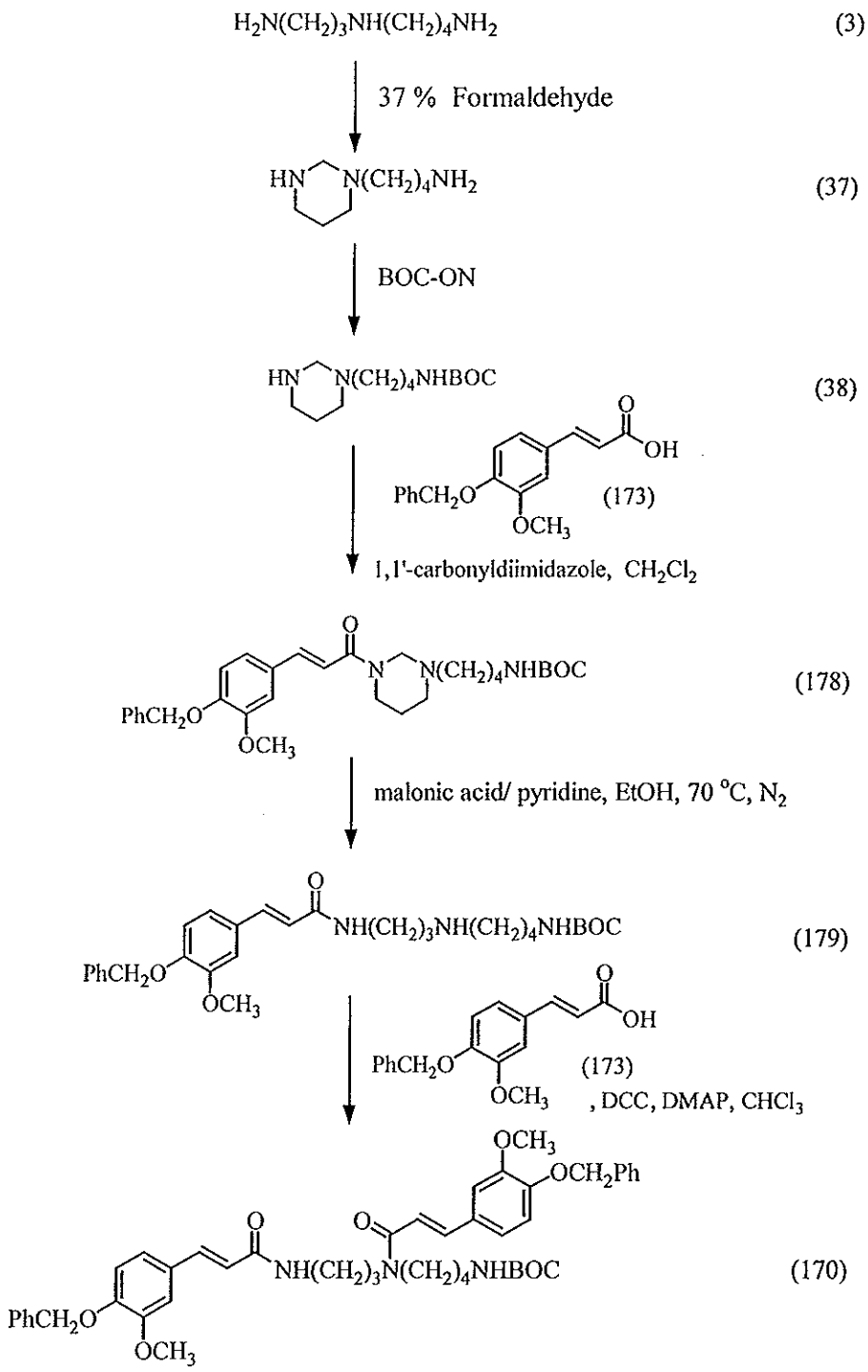
The arrows represent ^{13}C-^{1}H correlation (^{13}C-^{1}H long-range coupling) which were partially shown.

2.4 Synthesis of N^1,N^4-di-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170)

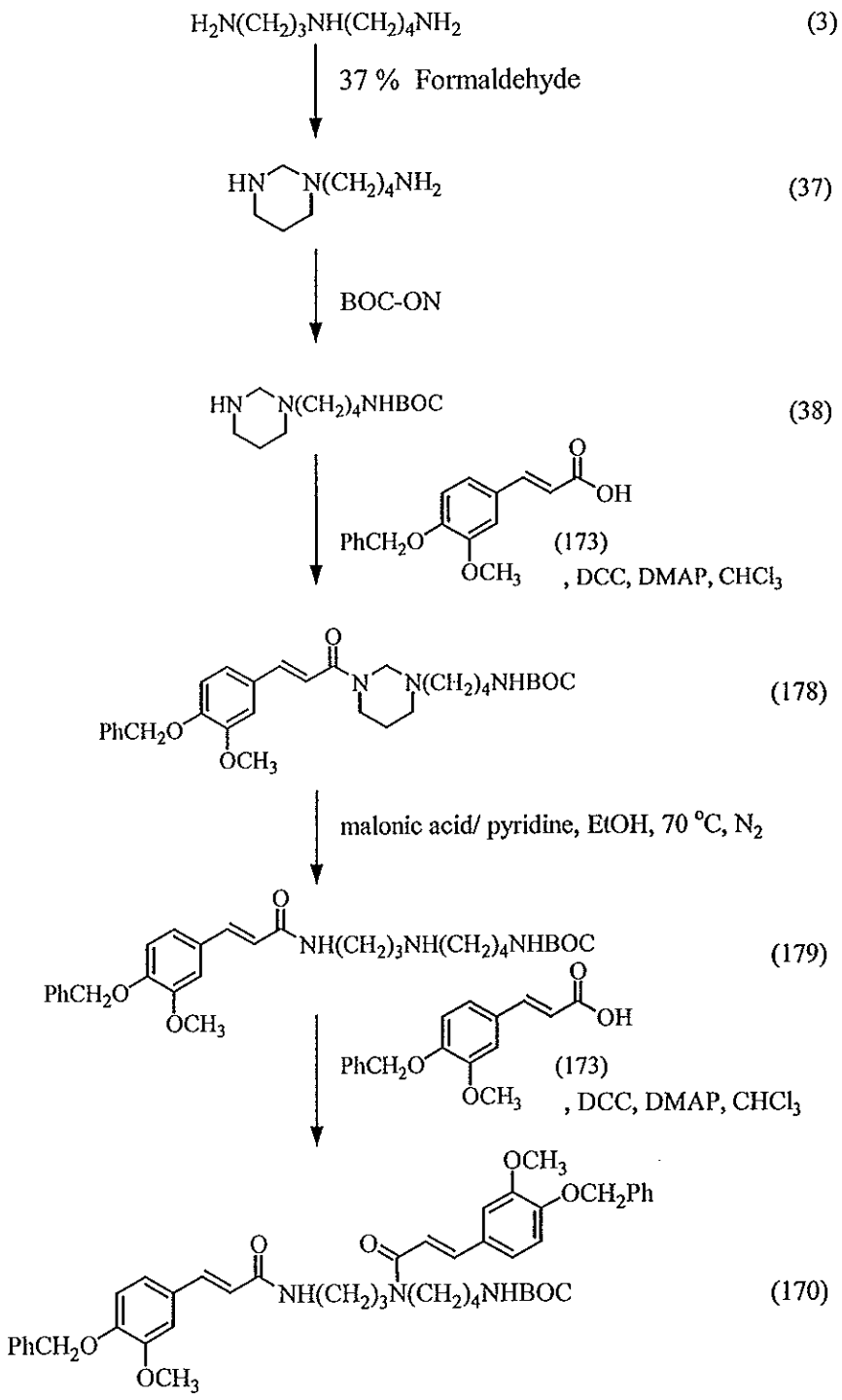
N^1,N^4-di-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170) is a triamine in which all three nitrogen atoms of spermidine backbone have been fully protected, two with the same groups and the third nitrogen atom with different group. This compound could be synthesized from hexahydropyrimidine (37). The reaction of hexahydropyrimidine (37) with 2[[(*tert*-butoxycarbonyl)-oxy]imino]-2-phenylacetonitrile (BOC-ON) in tetrahydrofuran at 0°C afforded N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine (38). Treatment of compound (38) with 4-benzyloxy-3-methoxycinnamic acid (173) and 1,1′-carbonyldiimidazole gave N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine (178)(35 %)(**Scheme 31**). The yield of this step could be improved up to 75 % (**Scheme 32**) with 4-benzyloxy-3-methoxycinnamic acid (173), DCC and DMAP (**Table 16**). The gem-diamine protecting group of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine(178) was removed by reaction with malonic acid and pyridine in hot ethanol to afford yellow solid (179). The 125 MHz ^{13}C NMR spectrum of this compound (179) in $CDCl_3$ (see **Table 17**) exhibited 25 signals for 29 carbon atoms. Analysis of the DEPT spectra of this compound (see **Table 17**) suggested the presence of one methoxy carbon atom (δ 55.91), one methyl carbon atom (δ 28.37), eight methylene carbon atoms (δ 70.79, 49.26, 47.97, 40.32, 38.69, 28.89, 27.76 and 27.17), eight methine signals for ten methine carbon atoms [δ 140.11, 128.53 (2 x CH), 127.89, 127.14 (2 x CH), 121.42, 119.13, 113.49 and

110.33)] and seven quaternary carbon atoms (δ 166.17, 166.10, 149.59, 149.49, 136.64, 128.31 and 79.14).

The structure of compound 179 was deduced from the detailed analysis of the DEPT (**Table 17**), ^{1}H-^{1}H COSY (**Table 18**) (see **Figure 5**), ^{13}C-^{1}H correlation (**Table 19**), ^{13}C and ^{1}H spectra. The complete assignment of ^{13}C and ^{1}H signals are shown in **Table 20**. The ^{13}C-^{1}H correlation by long-range coupling (HMBC) shown in **Table 21** and **Figure 6** confirmed some of the assignments. The LC mass spectrum showed the molecular ion peak at *m/z* 512 [$(M+H)^{+}$]. The IR spectrum suggested the presence of amine group, aromatic group, ester group and amide group (3330, 3040, 1715, 1675 and 1525 cm^{-1}).



Scheme 31. Synthesis of N^1,N^4-di-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170)



Scheme 32. Synthesis of N^1,N^4-di-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170)

Table 16. Acylation reaction of N^8-monoprotected BOC hexahydropyrimidine reagent (38) with various reagents and conditions.

Entry	Reagents/conditions	Equiv. of reagents	% yield of product (178)
1	4-benzyloxy-3-methoxycinnamic acid, 1,1′-carbonyldiimidazole, dry dichloromethane, rt for 24 h	1.1	35
2.1	4-benzyloxy-3-methoxycinnamic acid, DCC, DMAP, dry chloroform, 0°C to rt for 24 h	1.1	50
2.2	4-benzyloxy-3-methoxycinnamic acid, DCC, DMAP, dry chloroform, 0°C to rt for 24 h	2.2	75

Table 17. ^{13}C and DEPT NMR spectral data of compound 179

δ_C (ppm)	DEPT	C-type
166.17		C=O
166.		C=O
149.59		C
149.49		C
140.11	140.11	CH
136.64		C
128.53 (2 x CH)	128.53	CH
128.31		C
127.89	127.89	CH
127.14 (2 x CH)	127.14	CH
121.42	121.42	CH
119.13	119.13	CH
113.49	113.49	CH
110.33	110.33	CH
79.14		C
70.79	70.79	OCH_2
55.91	55.91	OCH_3
49.26	49.26	CH_2
47.97	47.97	CH_2
40.32	40.32	CH_2
38.69	38.69	CH_2
28.89	28.89	CH_2
28.37	28.37	CH_3
27.76	27.76	CH_2
27.15	27.15	CH_2

Table 18. 500 MHz ^{1}H-^{1}H COSY correlation among some protons of compound 179

δ_H (ppm)		proton correlated with δ_H (ppm)
7.52 (H-7′)	⟺	6.28 (H-8′)
7.43-7.45 (H-12′,-16′)	⟺	7.35-7.39 (H-13′,-15′)
7.35-7.39 (H-13′,-15′)	⟺	7.29-7.33 (H-14′), 7.43-7.45 (H-12′,-16′)
7.29-7.33 (H-14′)	⟺	7.35-7.39 (H-13′,-15′)
7.01 (H-6′)	⟺	6.86 (H-5′)
6.86 (H-5′)	⟺	7.01 (H-6′)
3.47 (H-2)	⟺	1.74 (H-3)
3.12 (H-9)	⟺	1.54 (H-8)
2.73 (H-4)	⟺	1.74 (H-3)
2.63 (H-6)	⟺	1.54 (H-7)
1.74 (H-3)	⟺	2.73 (H-4), 3.47 (H-2)
1.54 (H-7,-8)	⟺	2.63 (H-6), 3.12 (H-9)

Chemical shift of H-7 and H-8 were superimposed.

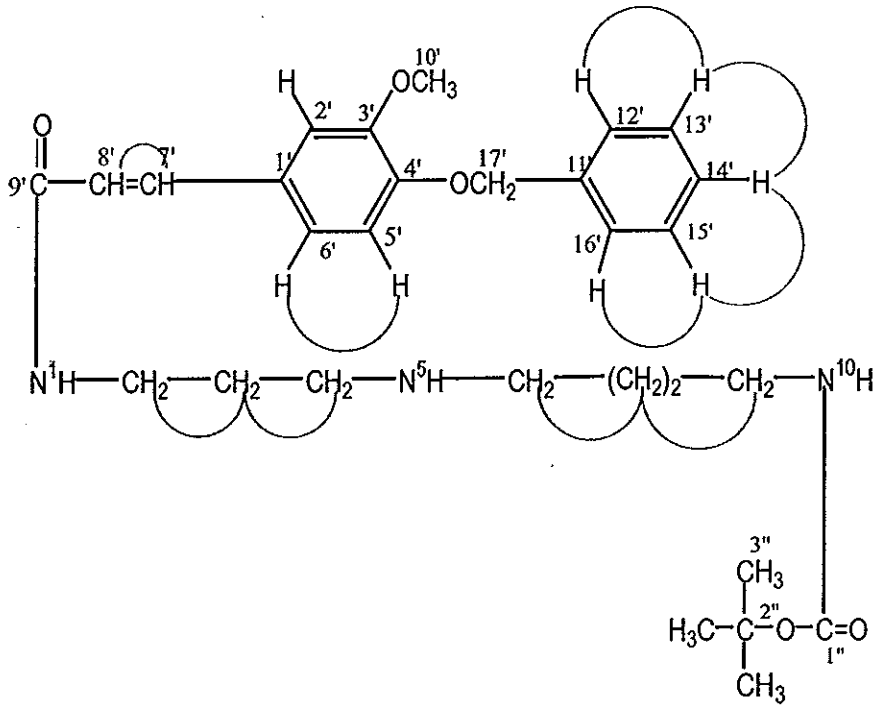


Figure 5. ^{1}H-^{1}H COSY of compound 179

Table 19. ^{13}C-^{1}H correlation 2D NMR spectral data of compound 179

δ_C (ppm)	δ_H (ppm)	assignment
140.11	7.52	CH-17′
128.53 (2 x CH)	7.35-7.39	CH-13′,-15′
127.89	7.29-7.33	CH-14′
127.14 (2 x CH)	7.43-7.45	CH-12′,-16′
121.42	7.01	CH-6′
119.13	6.28	CH-8′
113.49	6.86	CH-5′
110.33	7.04	CH-2′
70.79	5.17	CH_2-17′
55.91	3.89	CH_3-10′
49.26	2.63	CH_2-6
47.97	2.73	CH_2-4
40.32	3.12	CH_2-9
38.69	3.47	CH_2-2
28.89	1.74	CH_2-3
28.37	1.43	CH_3-3″
27.76 – 27.15	1.54	CH_2-7,-8

Table 20. ^{13}C and ^{1}H NMR (HMQC) spectral data of compound 179

Positions	δ_C*	δ_H, *mult*, *J* (Hz)
2	38.69 (CH_2)	3.47 (2H, *q*, 6)
3	28.89 (CH_2)	1.74 (2H, *m*)
4	47.97 (CH_2)	2.73 (2H, *t*, 6.5)
6	49.26 (CH_2)	2.63 (2H, *t*, 6.5)
7,8	27.15 (CH_2)	1.54 (2H, *m*) } superimpose
	27.76 (CH_2)	1.54 (2H, *m*)
9	40.32 (CH_2)	3.12 (2H, *m*)
1′	128.31 (C)	
2′	110.33 (CH)	7.04 (1H, *d*, 1.5)
3′	149.59 (C)	
4′	149.49 (C)	
5′	113.49 (CH)	6.86 (1H, *d*, 8.5)
6′	121.42 (CH)	7.01 (1H, *dd*, 8.5, 2)
7′	140.11 (CH)	7.52 (1H, *d*, 15.5)
8′	119.13 (CH)	6.28 (1H, *d*, 15.5)
9′	166.17 (C=O)	
10′	55.91 (OCH_3)	3.89 (3H, *s*)
11′	136.64 (C)	
12′,16′	127.14 (2 x CH)	7.43-7.45 (2H, *m*)
13′,15′	128.53 (2 x CH)	7.35-7.39 (2H, *m*)
14′	127.89 (CH)	7.29-7.33 (1H, *m*)
17′	70.79 (OCH_2)	5.17 (2H, *s*)
1″	166.17 (C=O)	
2″	79.14 (C)	
3″	28.37 (CH_3)	1.43 (9H, *s*)

The new numbering system (positions of C and N atoms) is especially assigned for the consideration of ^{13}C-^{1}H correlation.

* Carbon type deduced from DEPT experiments.

Table 21. ^{13}C-^{1}H correlation by long-range coupling (HMBC) spectral data of compound 179

δ_C (ppm)		proton correlated with δ_C (ppm)
166.17 (C-9′)	⟺	7.52 (H-7′), 3.47 (H-2)
149.59 (C-3′)	⟺	6.86 (H-5′)
149.49 (C-4′)	⟺	7.04 (H-2′), 7.01 (H-6′)
136.64 (C-11′)	⟺	7.35-7.39 (H-13′,-15′)
128.31 (C-1′)	⟺	7.52 (H-7′), 6.86 (H-5′)
127.89 (C-14′)	⟺	7.43-7.45 (H-12′,-16′)
127.14 (C-12′,-16′)	⟺	7.29-7.33 (H-14′)
121.42 (C-6′)	⟺	7.52 (H-7′), 7.04 (H-2′), 6.86 (H-5′)
119.13 (C-8′)	⟺	7.52 (H-7′)
113.49 (C-5′)	⟺	7.01 (H-6′)
110.33 (C-2′)	⟺	7.52 (H-7′), 7.01 (H-6′)
79.14 (C-2″)	⟺	1.43 (H-3″)
49.26 (C-6)	⟺	2.73 (H-4), 1.54 (H-7,-8)
47.97 (C-4)	⟺	3.47 (H-2), 2.63 (H-6), 1.74 (H-3)
40.32 (C-9)	⟺	1.54 (H-7,-8)
38.69 (C-2)	⟺	2.73 (H-4), 1.74 (H-3)
28.89 (C-3)	⟺	3.47 (H-2), 2.73 (H-4)

Total protons are not assigned in this table.

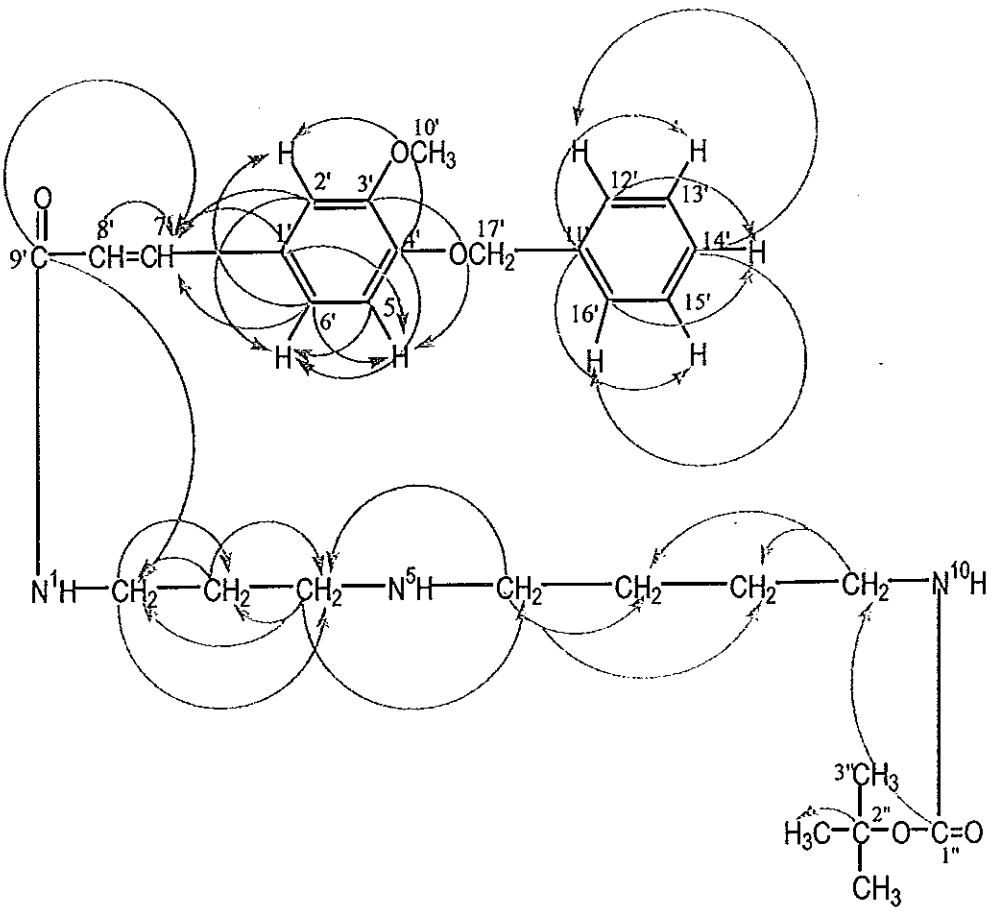


Figure 6. HMBC of compound 179

The arrows represent ^{13}C-^{1}H correlation (^{13}C-^{1}H long-range coupling)

From the above spectroscopic data , the possible structure should be N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179) which was obtained in 92 % yield (Ganem and Nagarijan, 1985). The reaction of N^1-(4-benzyloxy-3-methoxy-cinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179) with 4-benzyloxy-3-methoxycinnamic acid (173) in the presence of DCC and DMAP in dry chloroform gave yellow oil, which spontaneously

decomposed on TLC plate during the chromatographic separation. The NMR spectrum of this collected band showed the presence of *tert*-butoxy protons, methoxy protons, methyleneoxy protons but the integration of total aromatic protons were more than that expected from the product. This may be due to contamination with decomposed product. However, total NMR signals pattern indicated possible structure of N^1,N^4-di-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170).

CHAPTER 4

CONCLUSION

The synthesis of diferuloylspermines and diferuloylspermidines derivatives (167-170) could be started from the commercially available spermine and spermidine which were converted to bis-hexahydropyrimidine and hexahydropyrimidine, respectively. These prepared compounds were a good simple model for the studies of selective functionalization especially on nitrogen atoms because the molecule composed of different type of aliphatic amines (primary, secondary and tertiary amine). Various sequential treatments of the acylating agents yielded different products. Several attempts to acylate the primary and secondary amine nitrogen of the intermediate with unprotected hydroxycinnamic acid failed to give the required products (167-170). This may be due to high reactivity of free phenolic group of the products. Thus, the phenolic group of hydroxycinnamic acid was protected by the use of benzyl bromide. Finally, high yield of the required diferuloylspermines and diferuloylspermidines derivatives (167-170) were successfully synthesized by acylation of the primary and secondary amine nitrogen of the intermediate with protected hydroxycinnamic acid.

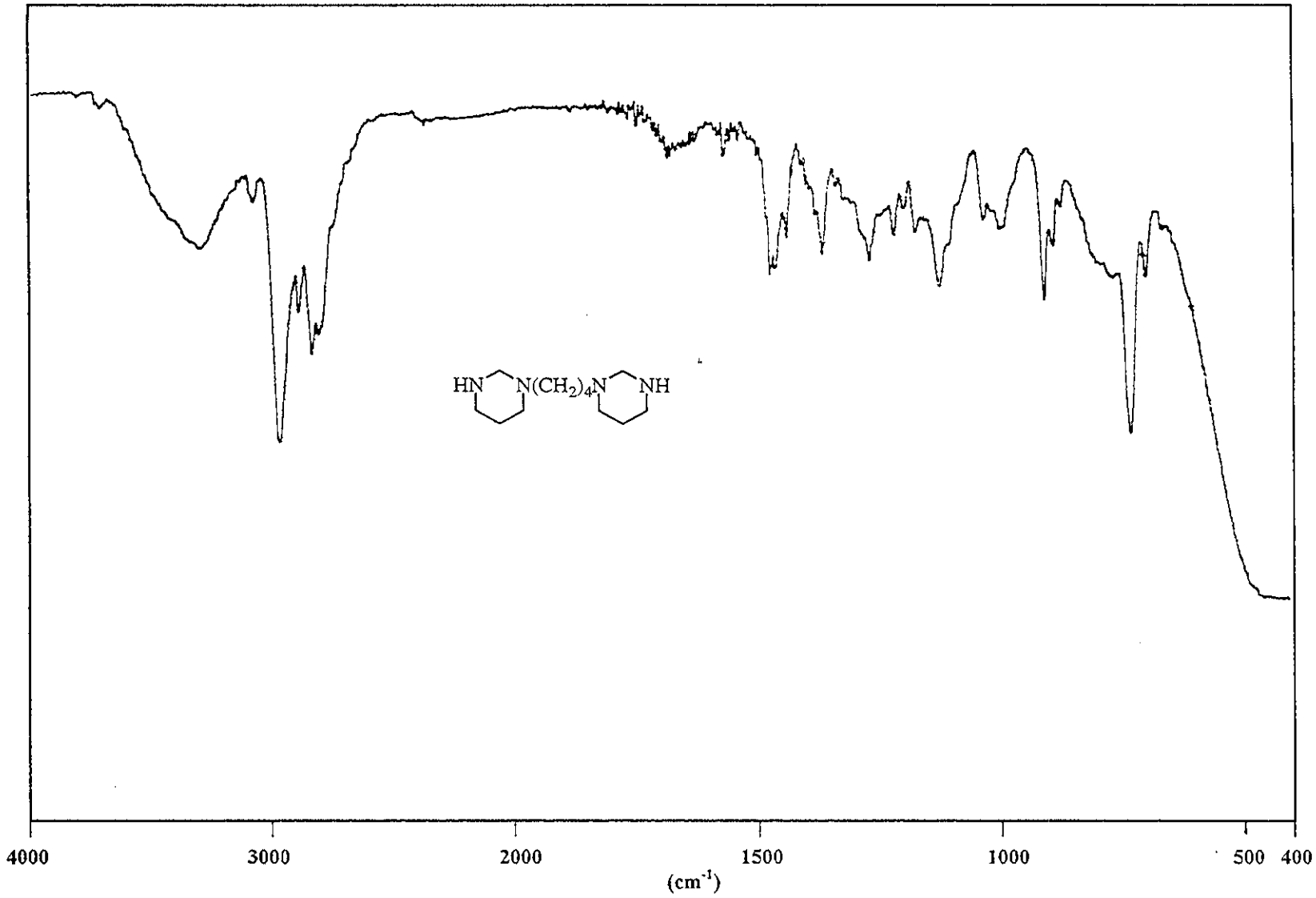


Figure 7. IR (film) spectrum of bis-Hexahydropyrimidine (125)

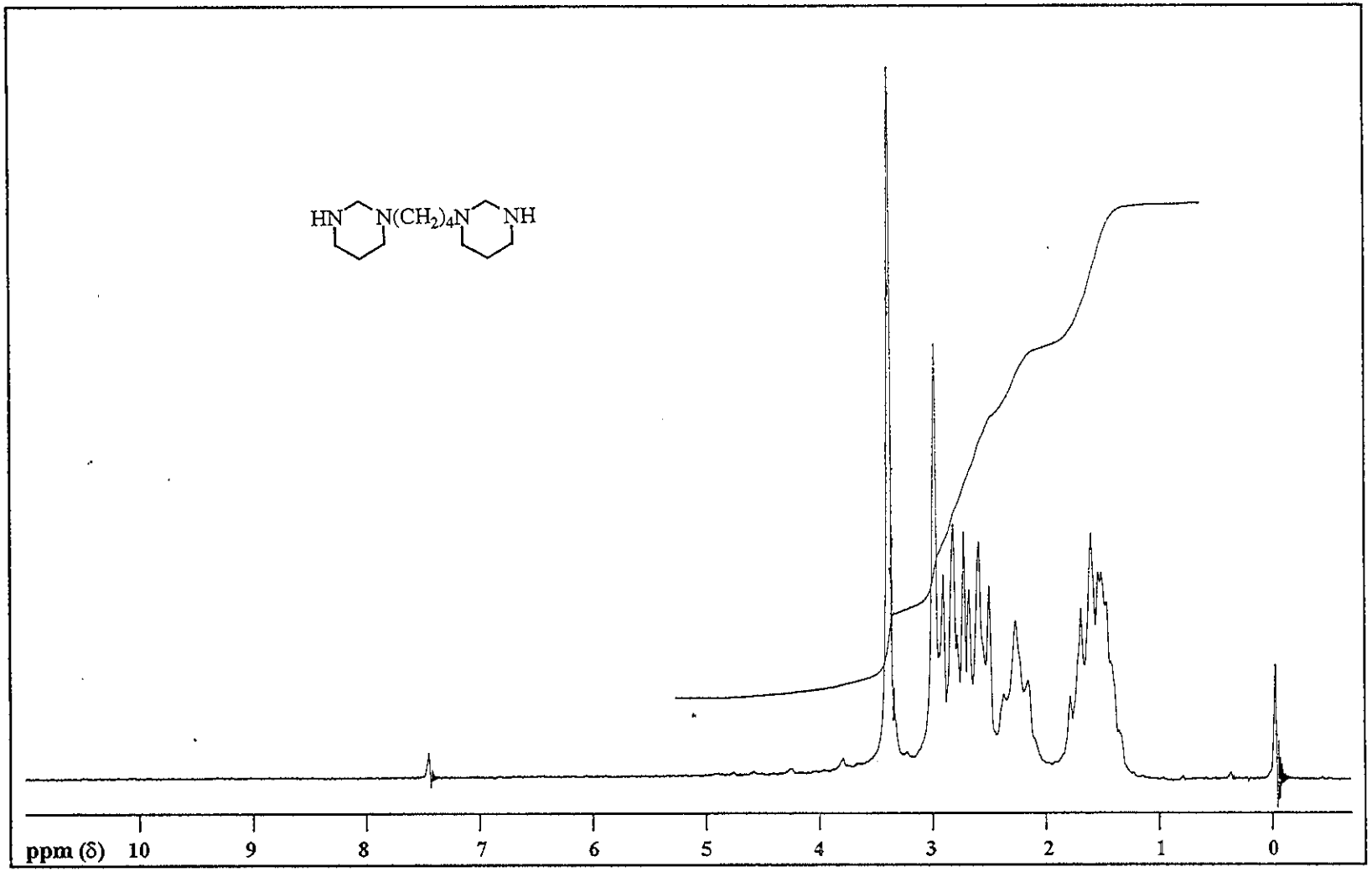


Figure 8. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of bis-Hexahydropyrimidine (125)

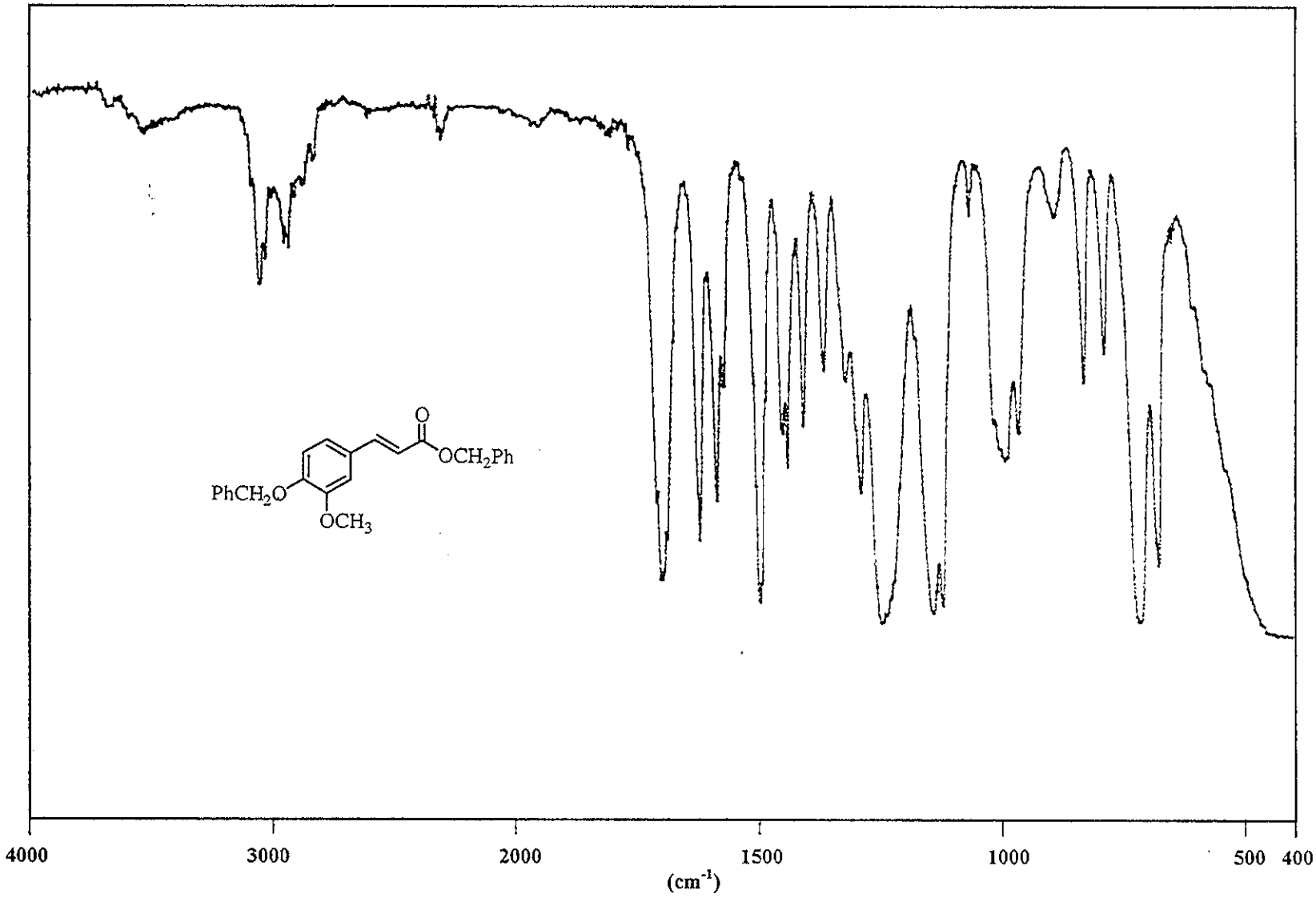


Figure 9. IR (film) spectrum of Benzyl (4-benzyloxy-3-methoxy) cinnamate (172)

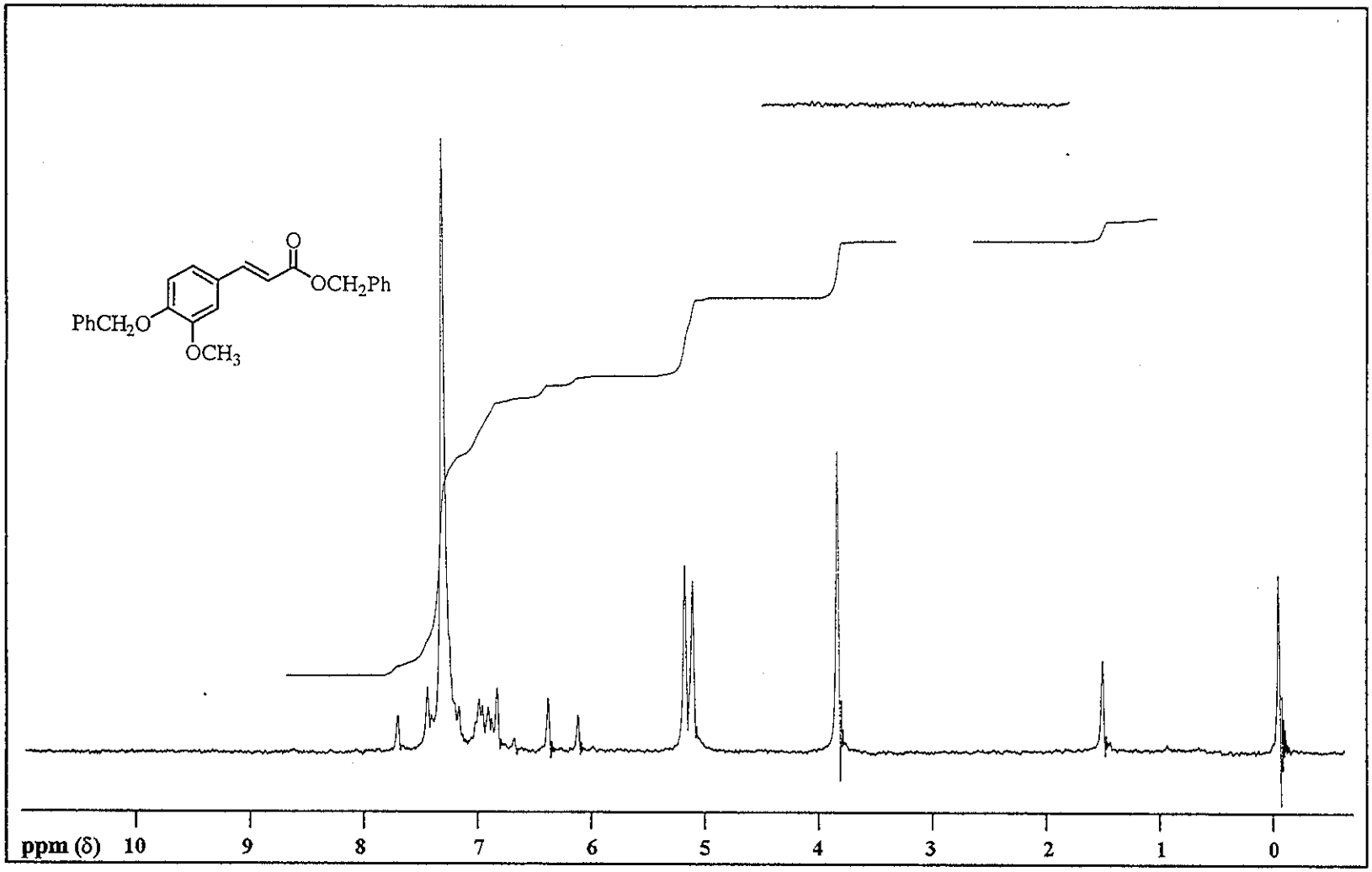


Figure 10. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of Benzyl (4-benzyloxy-3-methoxy) cinnamate (172)

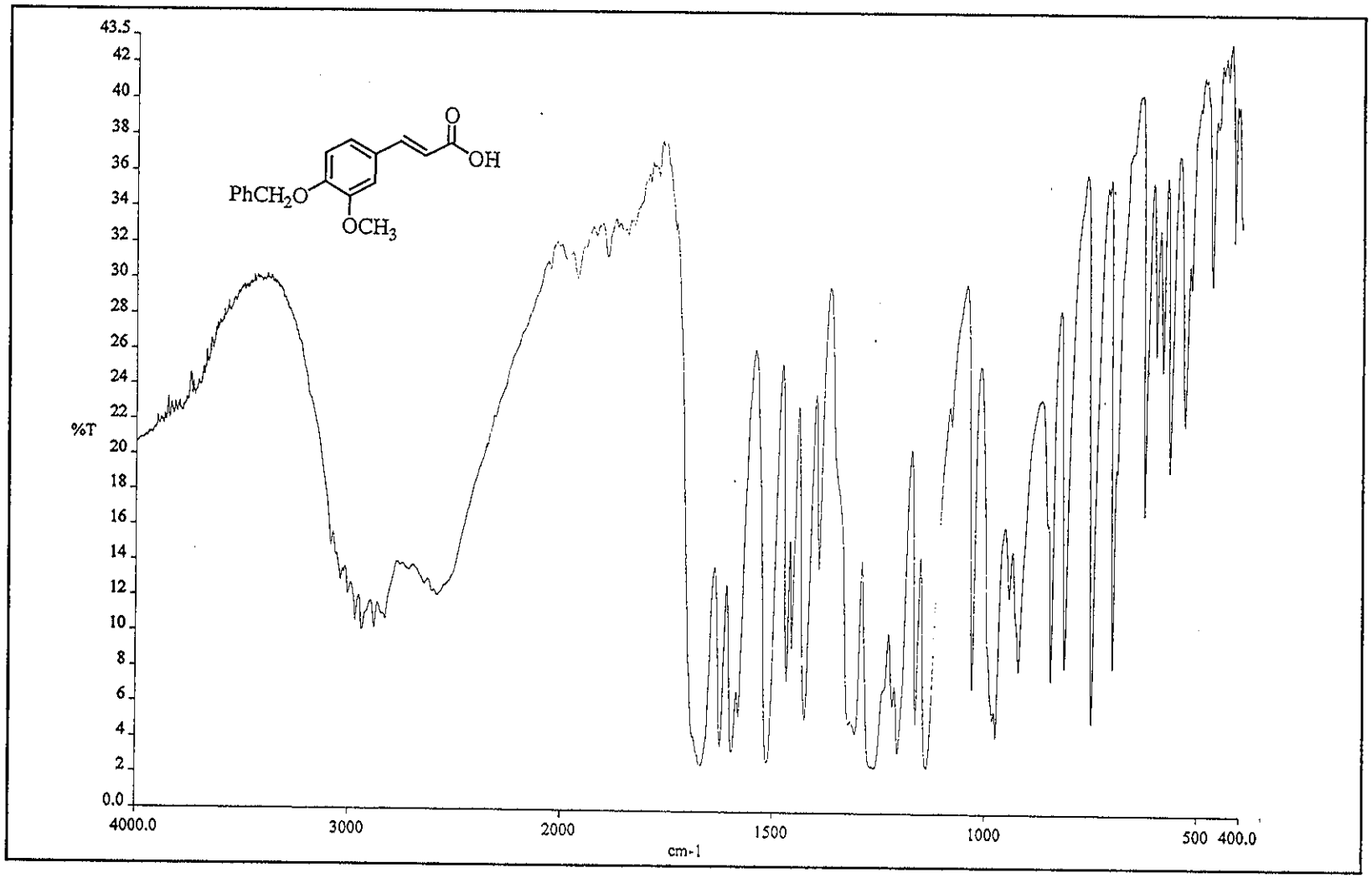


Figure 11. FTIR (KBr) spectrum of 4-Benzyloxy-3-methoxycinnamic acid (173)

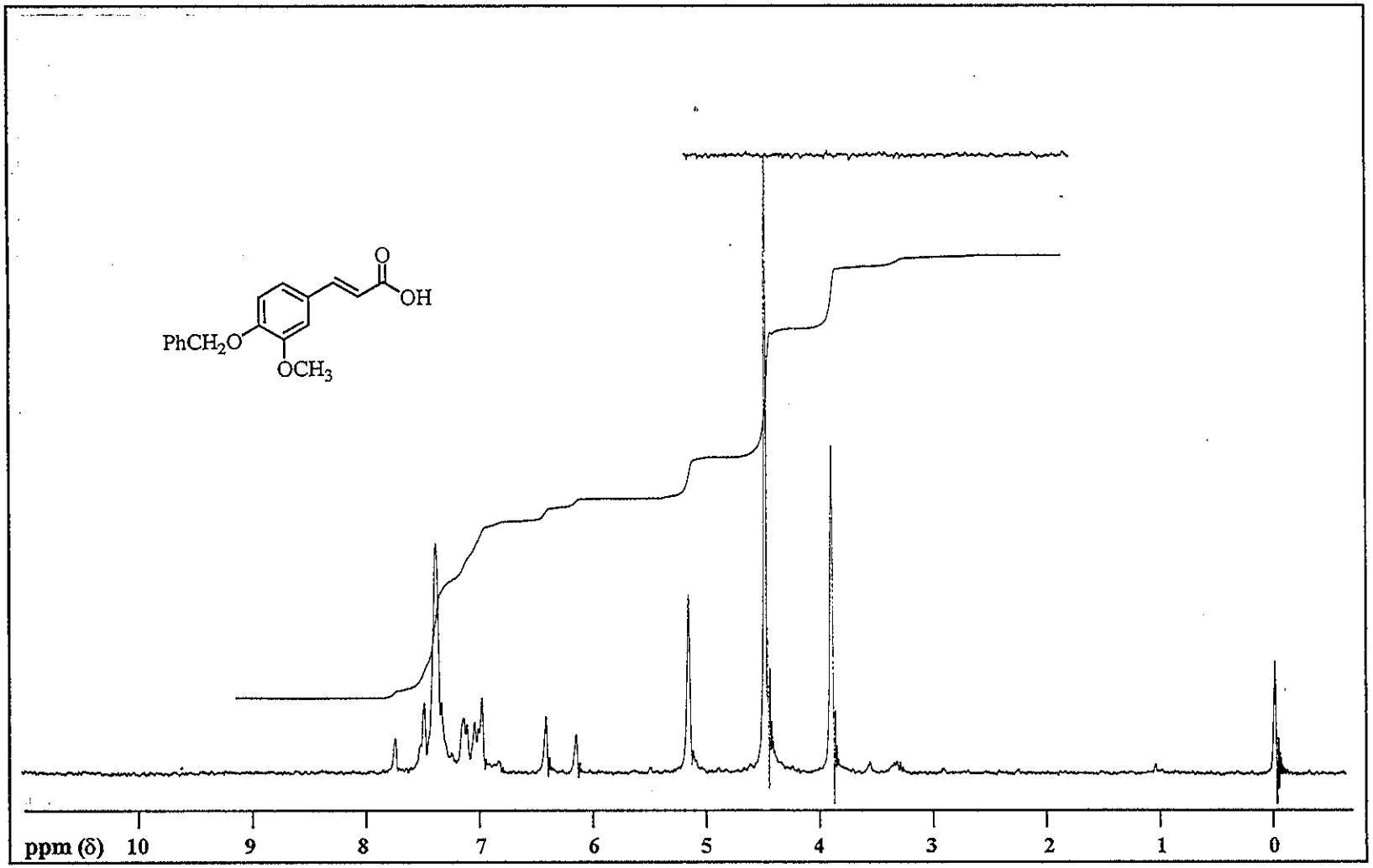


Figure 12. ^{1}H NMR ($CDCl_3$+CD_3OD, 60 MHz) spectrum of 4-Benzyloxy-3-methoxycinnamic acid (173)

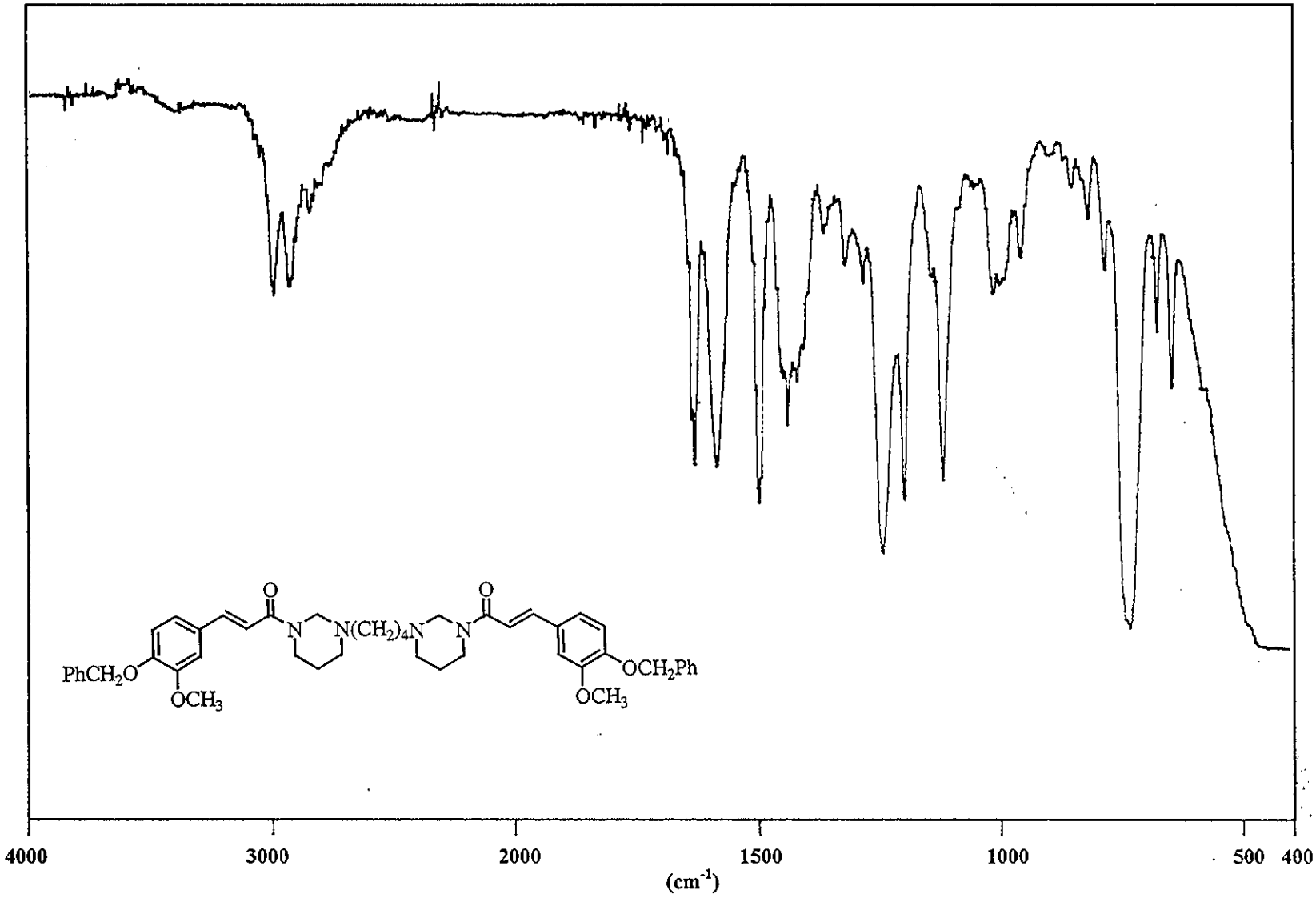


Figure 13. IR (film) spectrum of N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175)

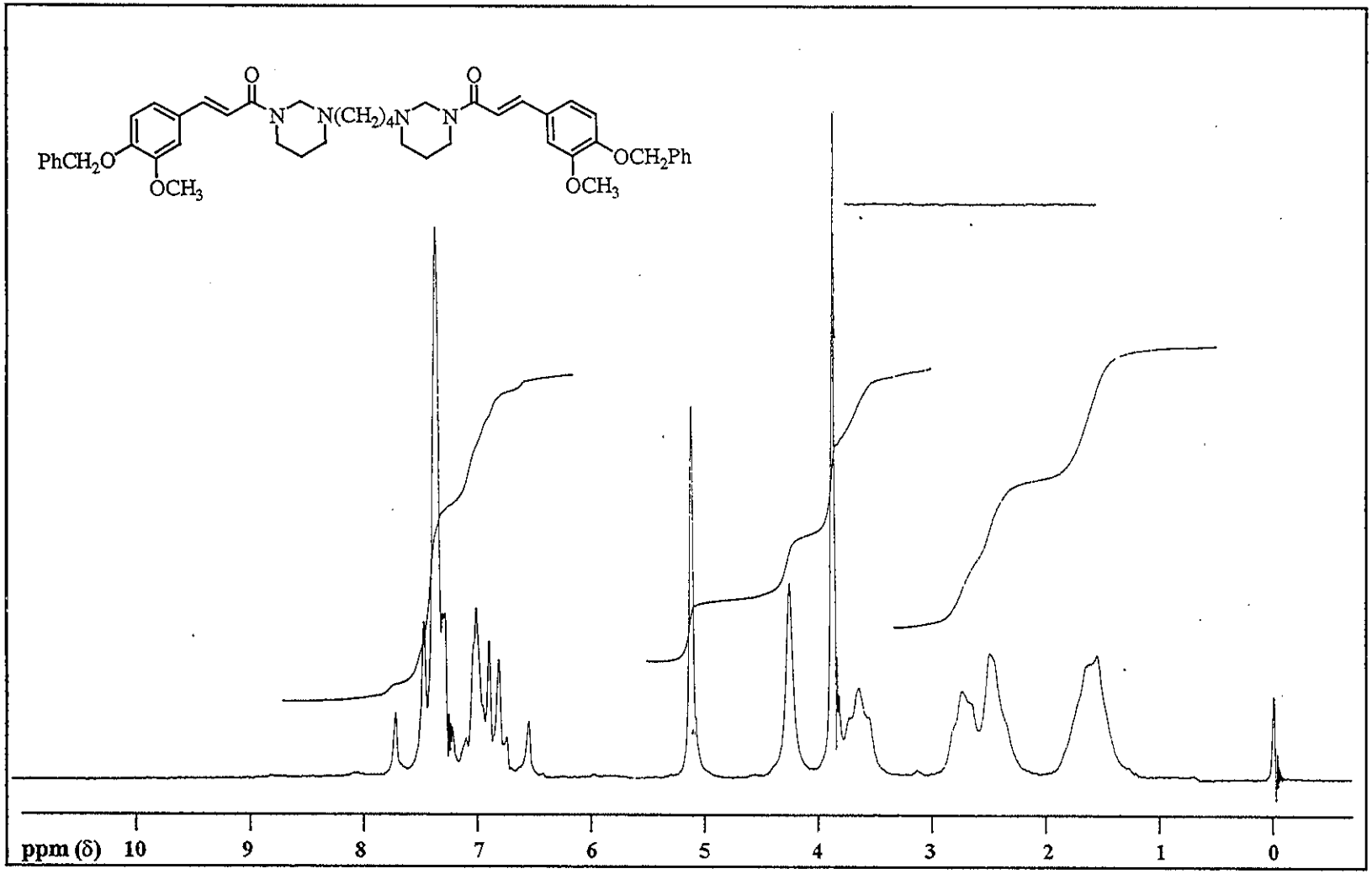


Figure 14. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of N^{1},N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175)

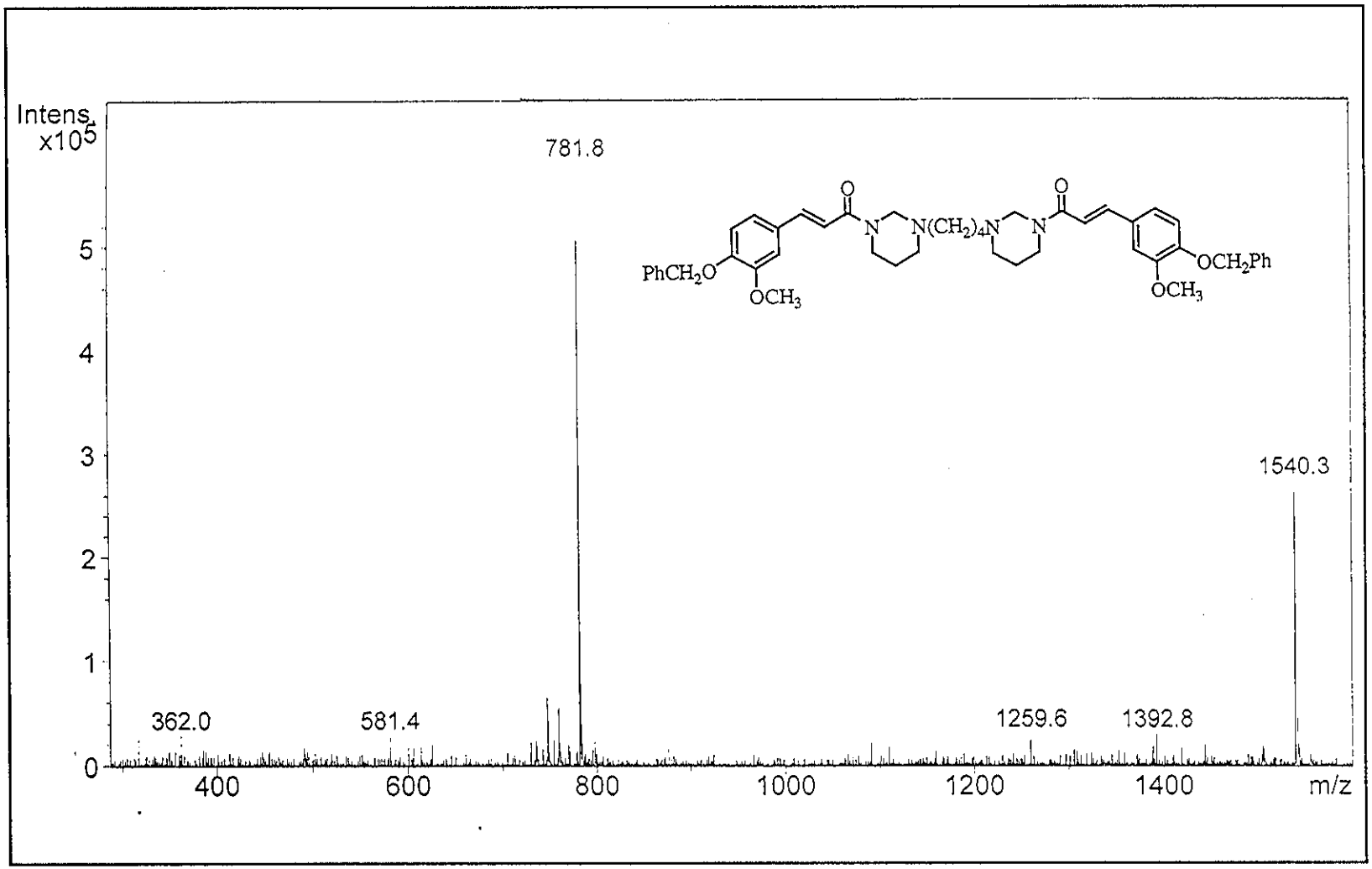


Figure 15. Mass spectrum (LC-MS) of N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) bis-hexahydropyrimidine (175)

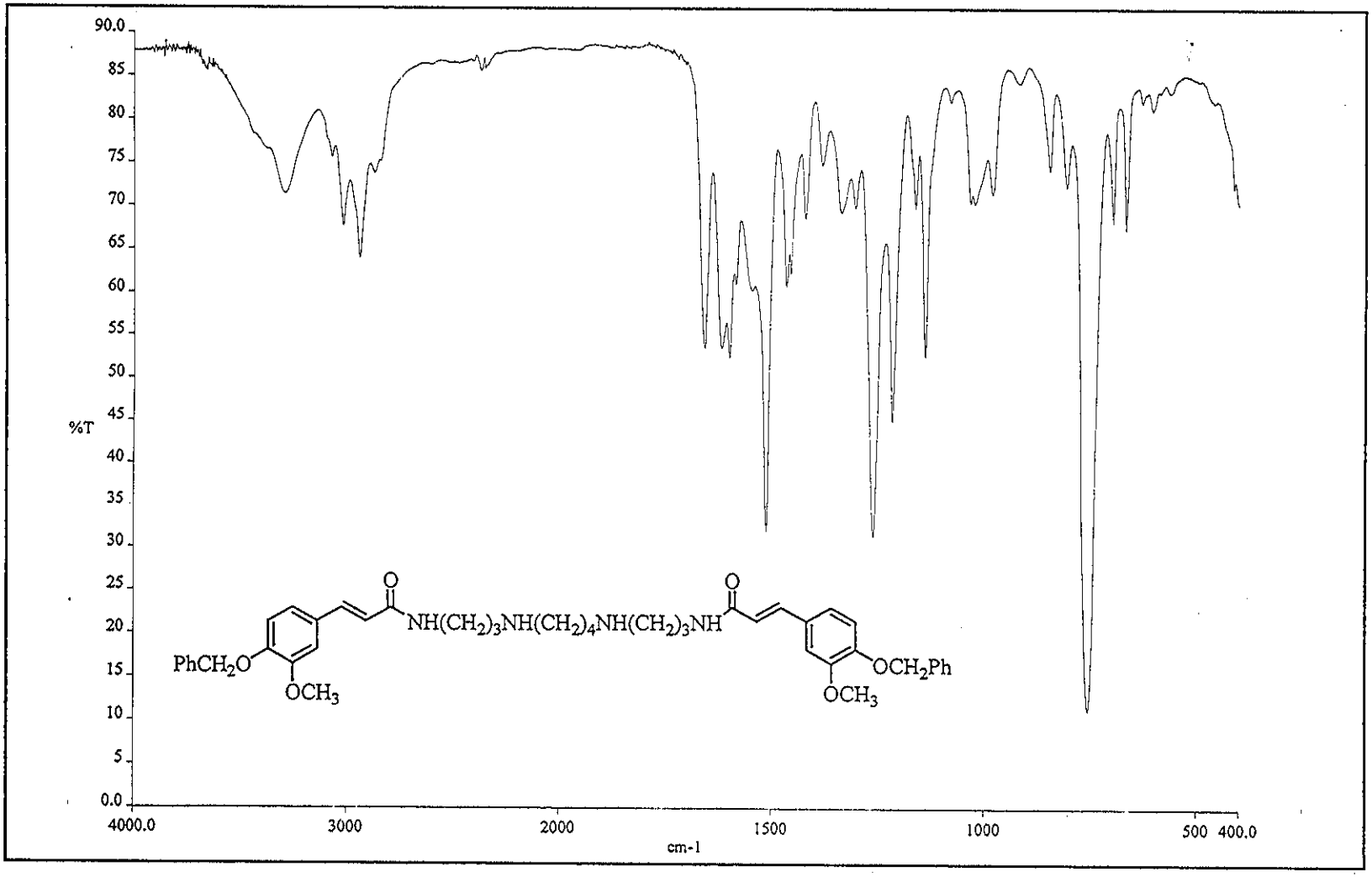


Figure 16. FTIR (film) spectrum of N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

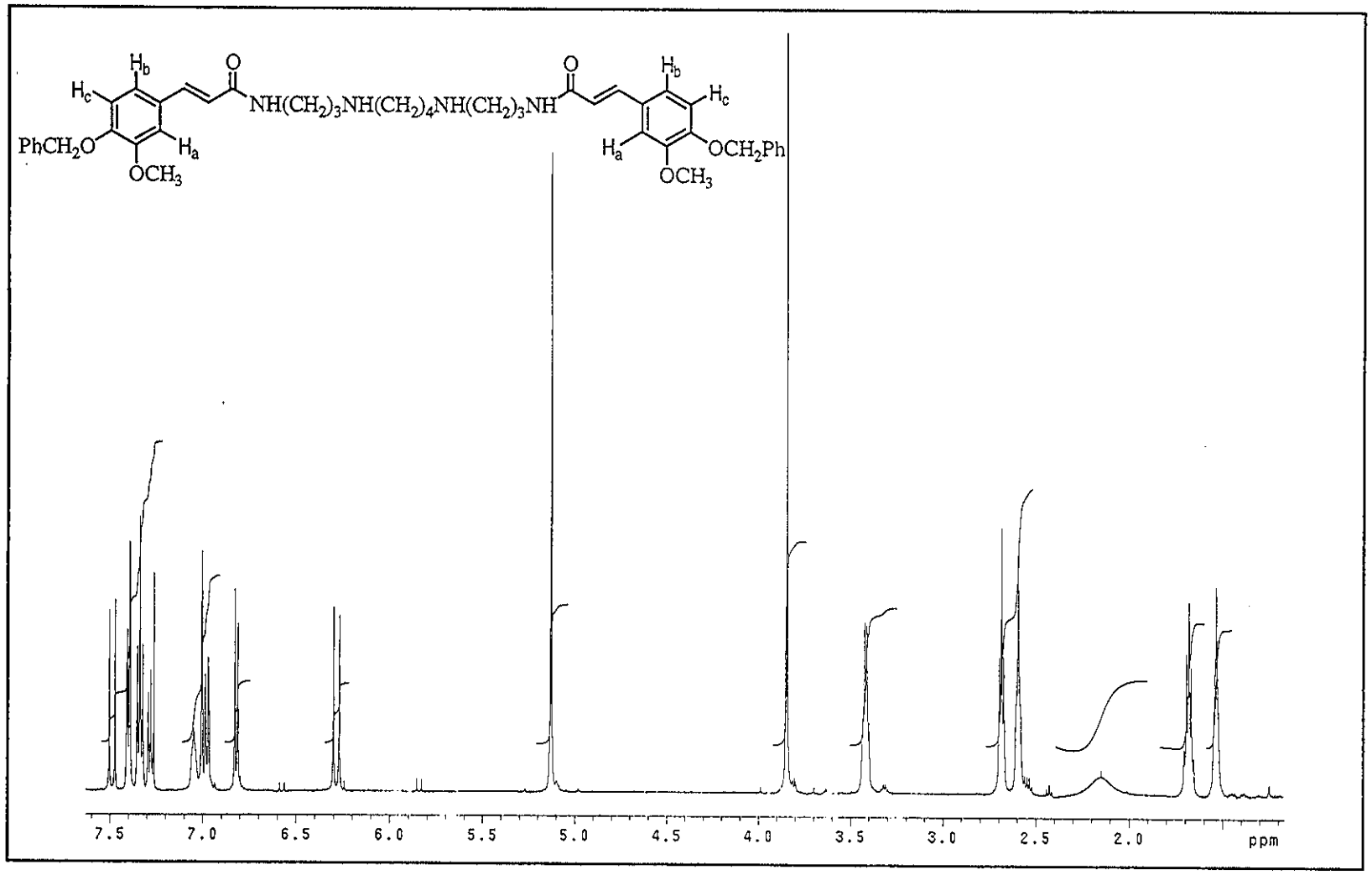


Figure 17. ^{1}H NMR ($CDCl_3$, 500 MHz) spectrum of N^{1},N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

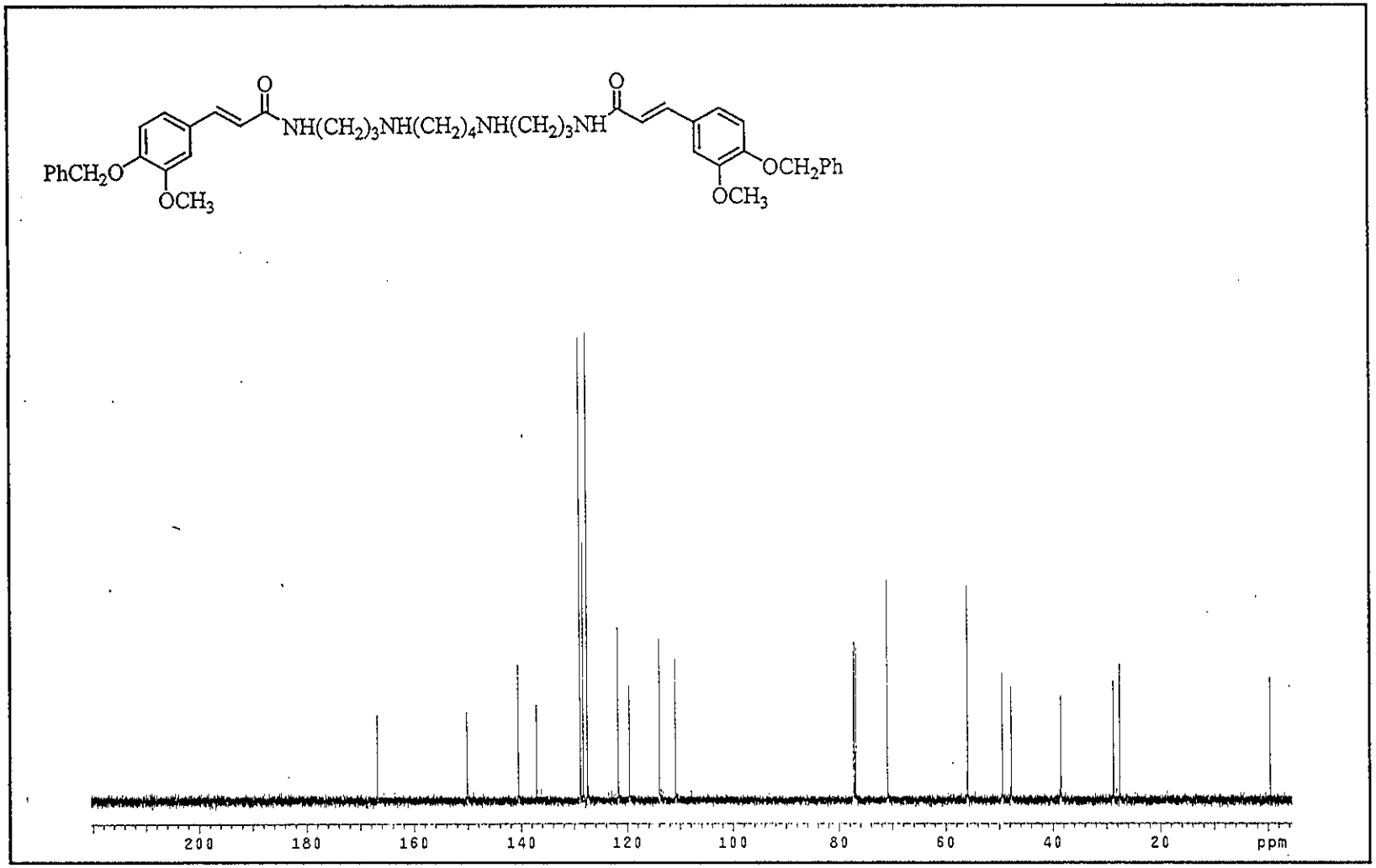


Figure 18. ^{13}C NMR ($CDCl_3$, 125 MHz) spectrum of N^1,N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

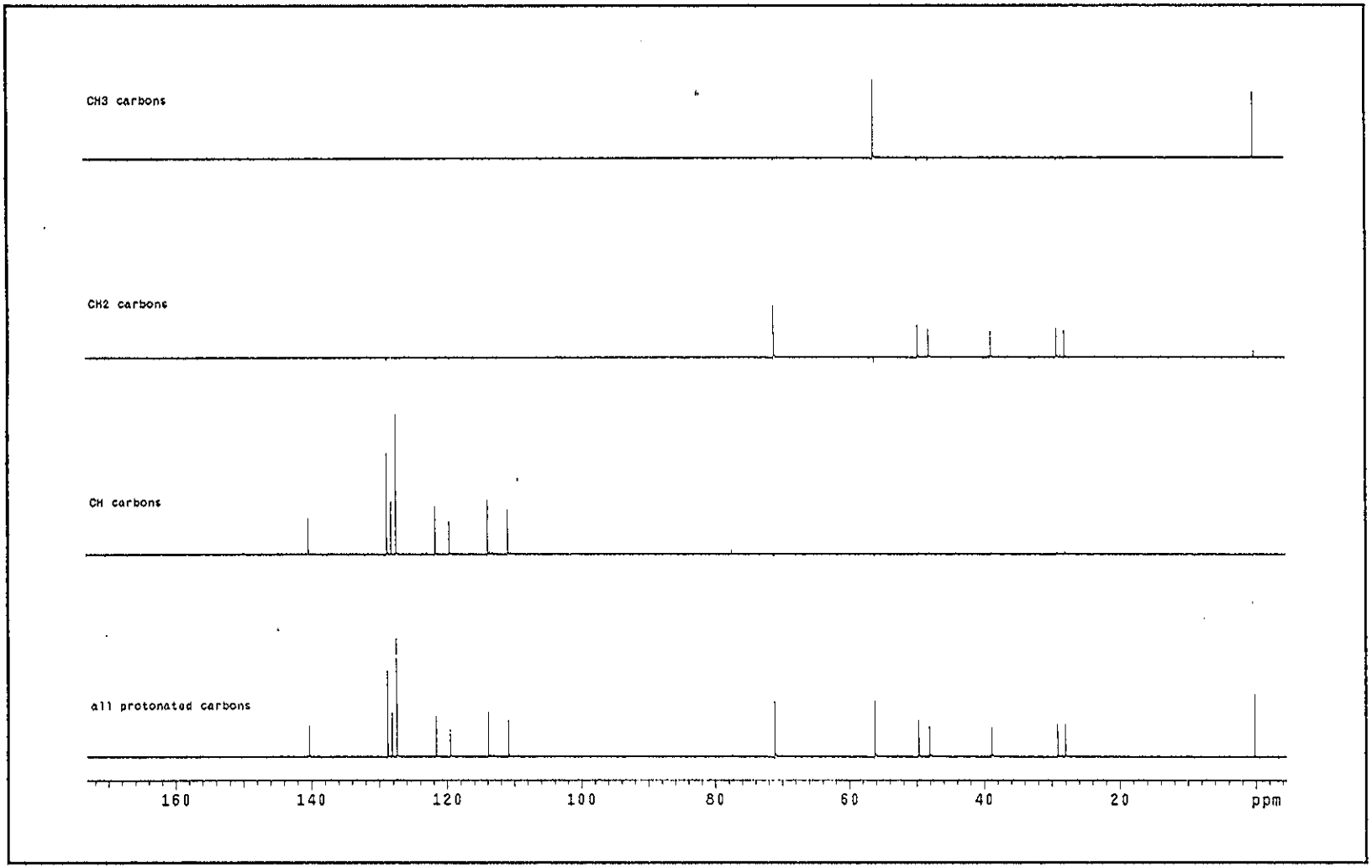


Figure 19. DEPT spectrum of N^{1},N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

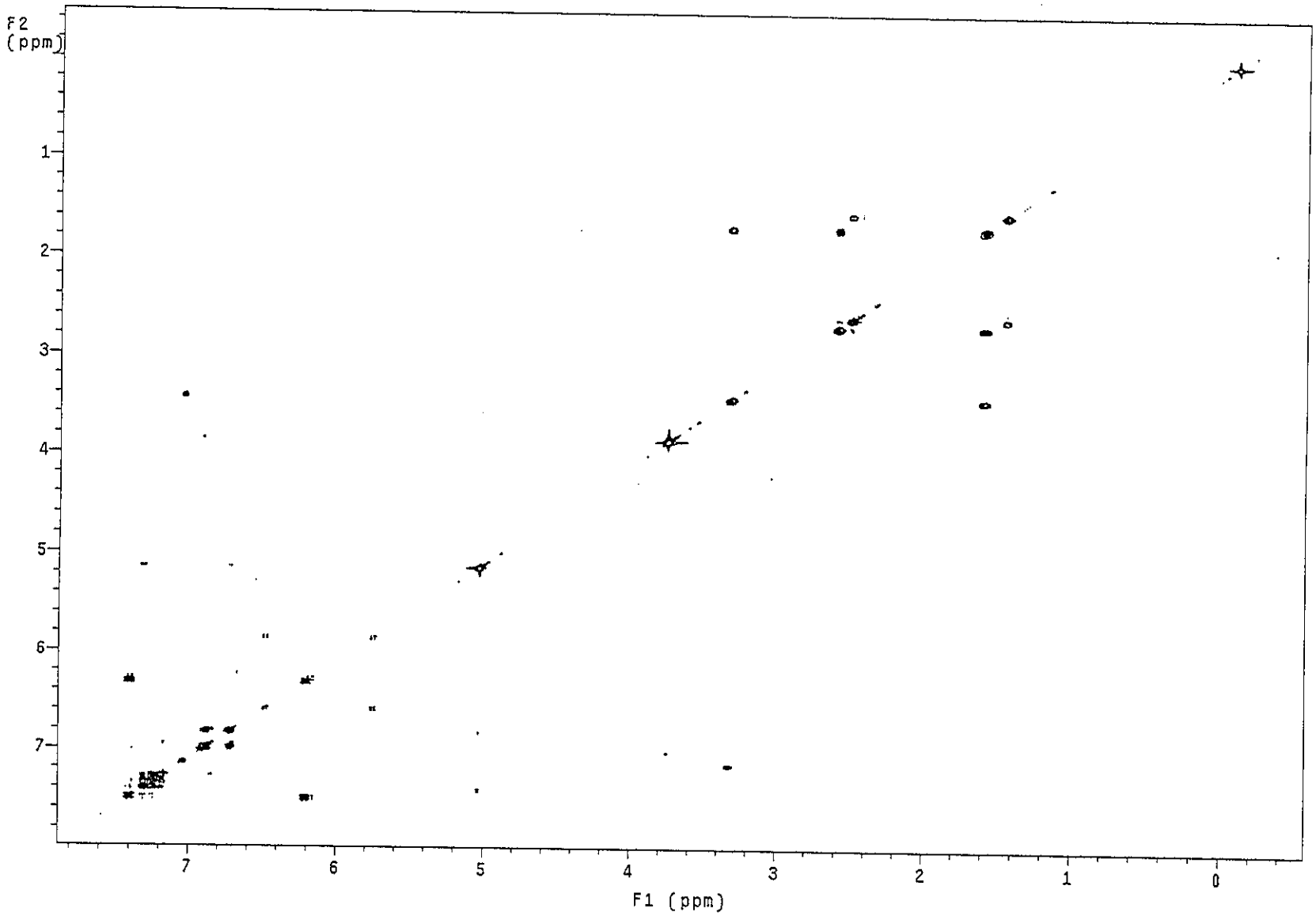


Figure 20. ^{1}H-^{1}H COSY spectrum of N^{1},N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

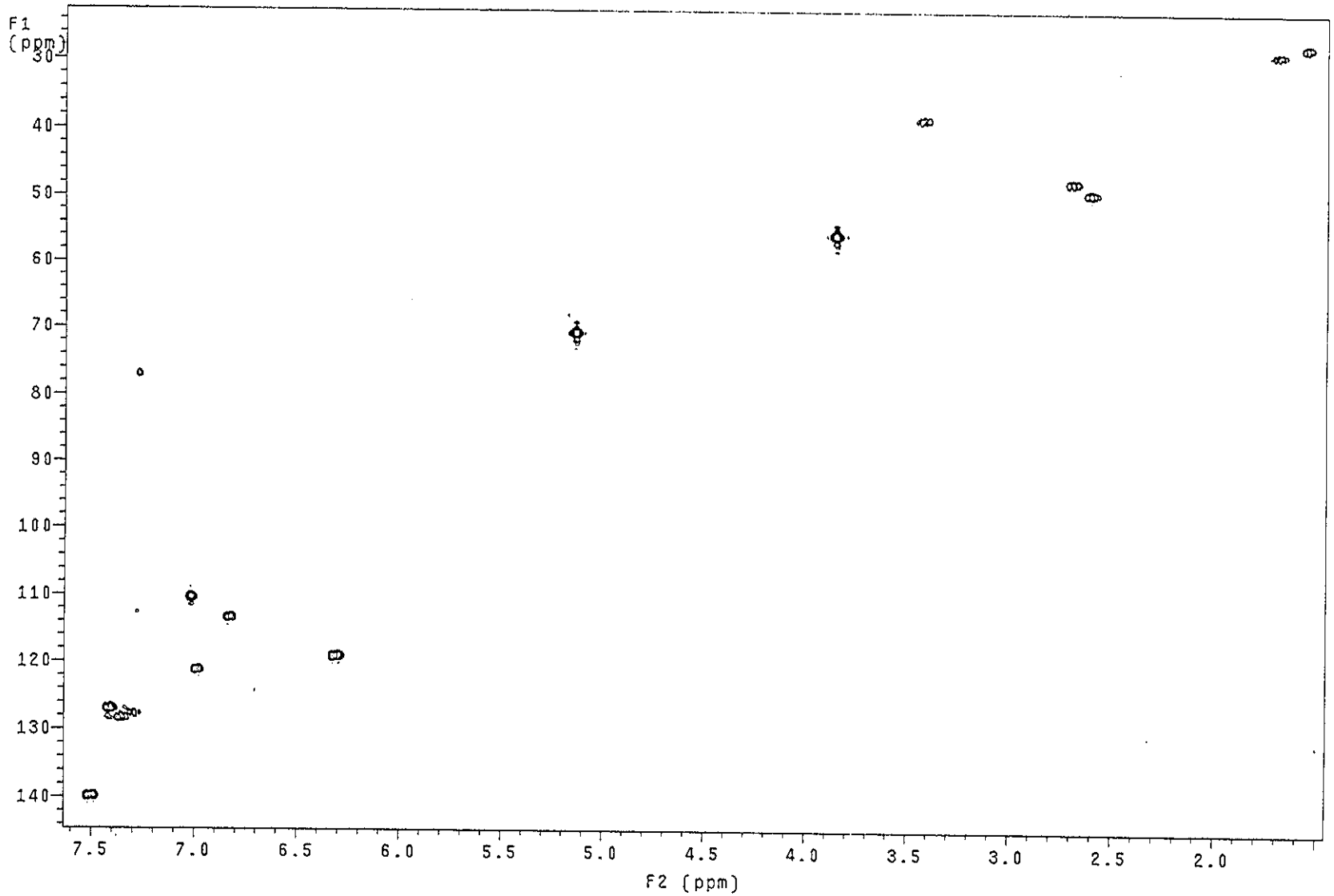


Figure 21. HMQC spectrum of N^{1},N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

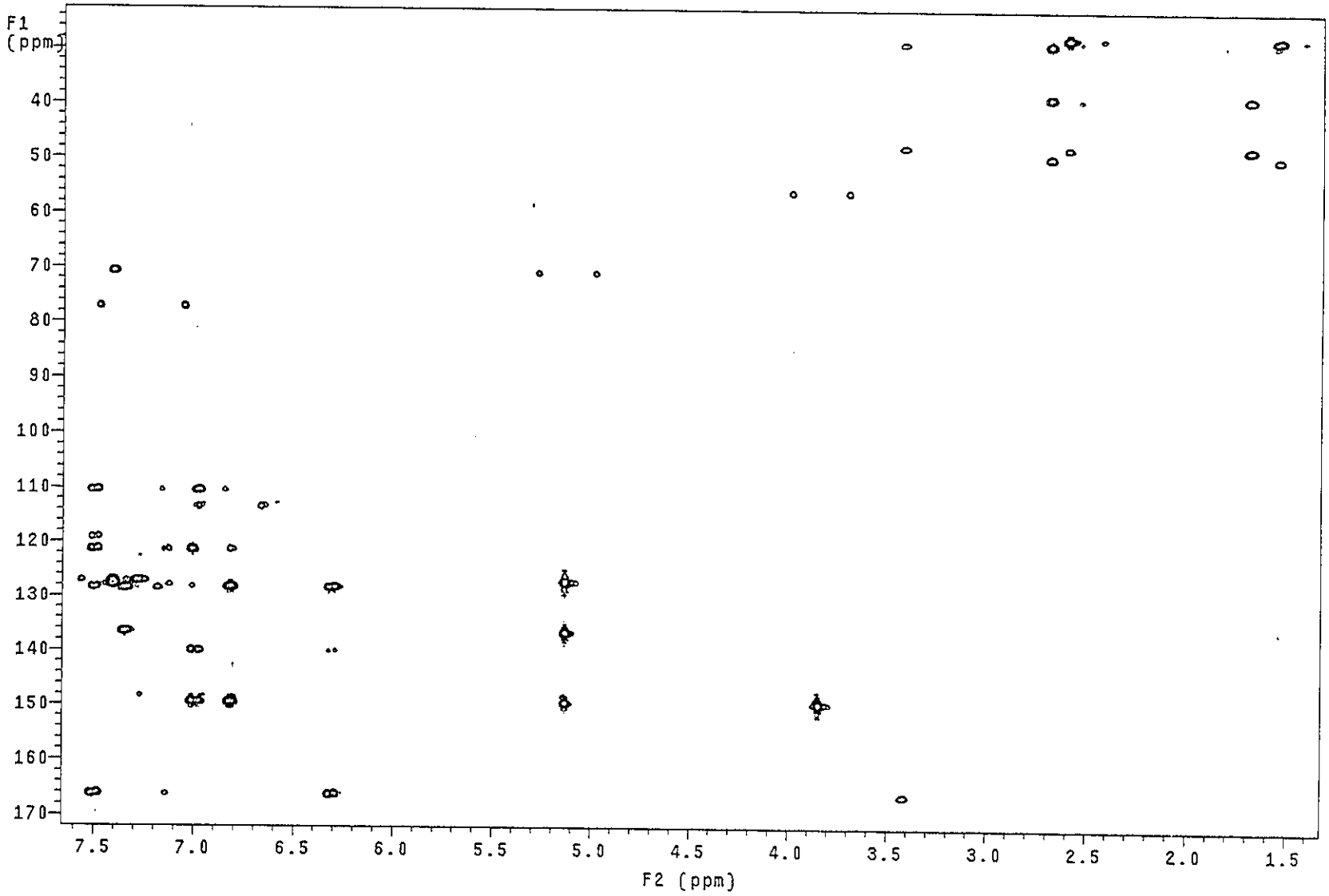


Figure 22. HMBC spectrum of N^{1},N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

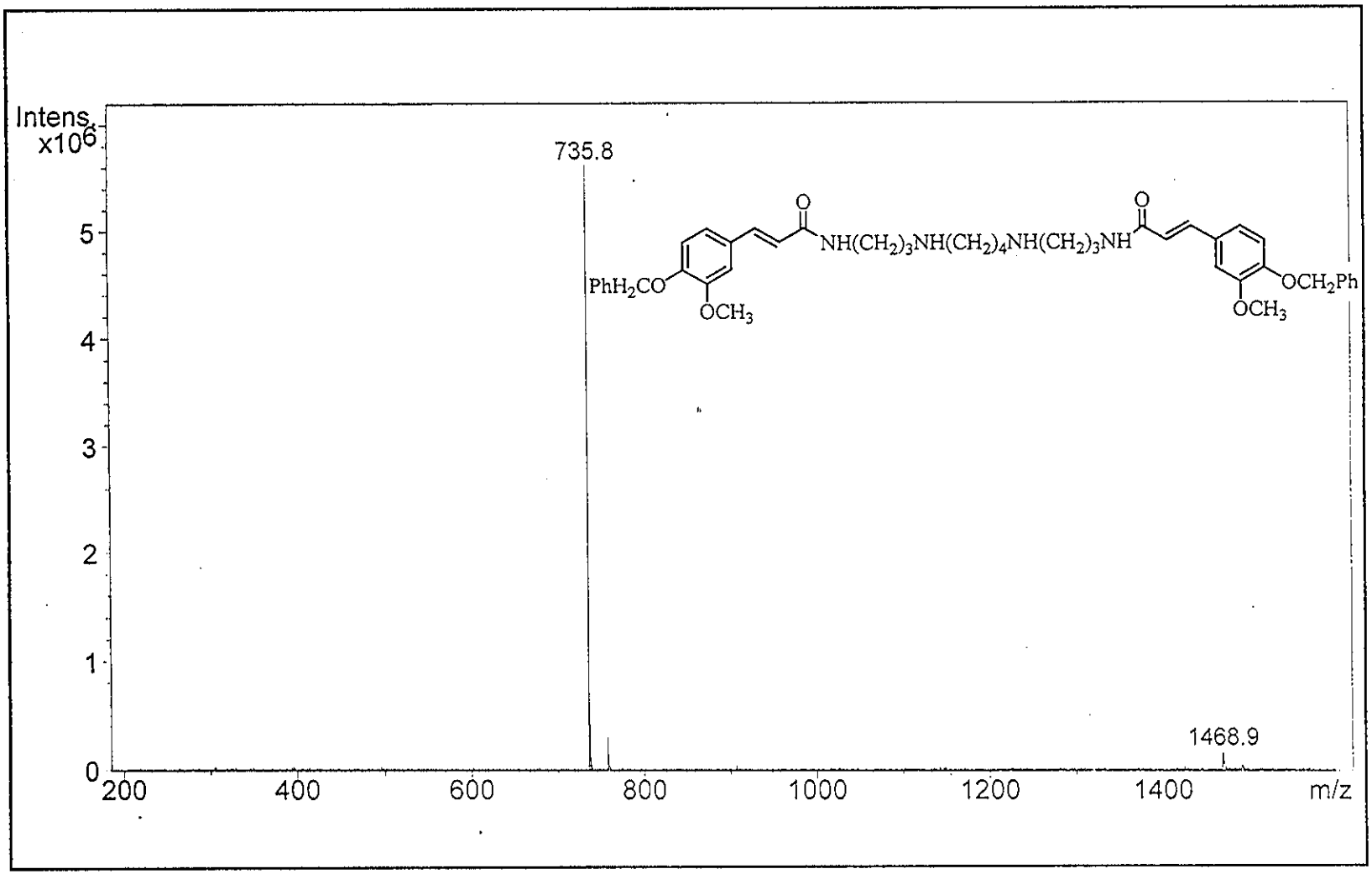


Figure 23. Mass spectrum (LC-MS) of N^{1},N^{12}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (167)

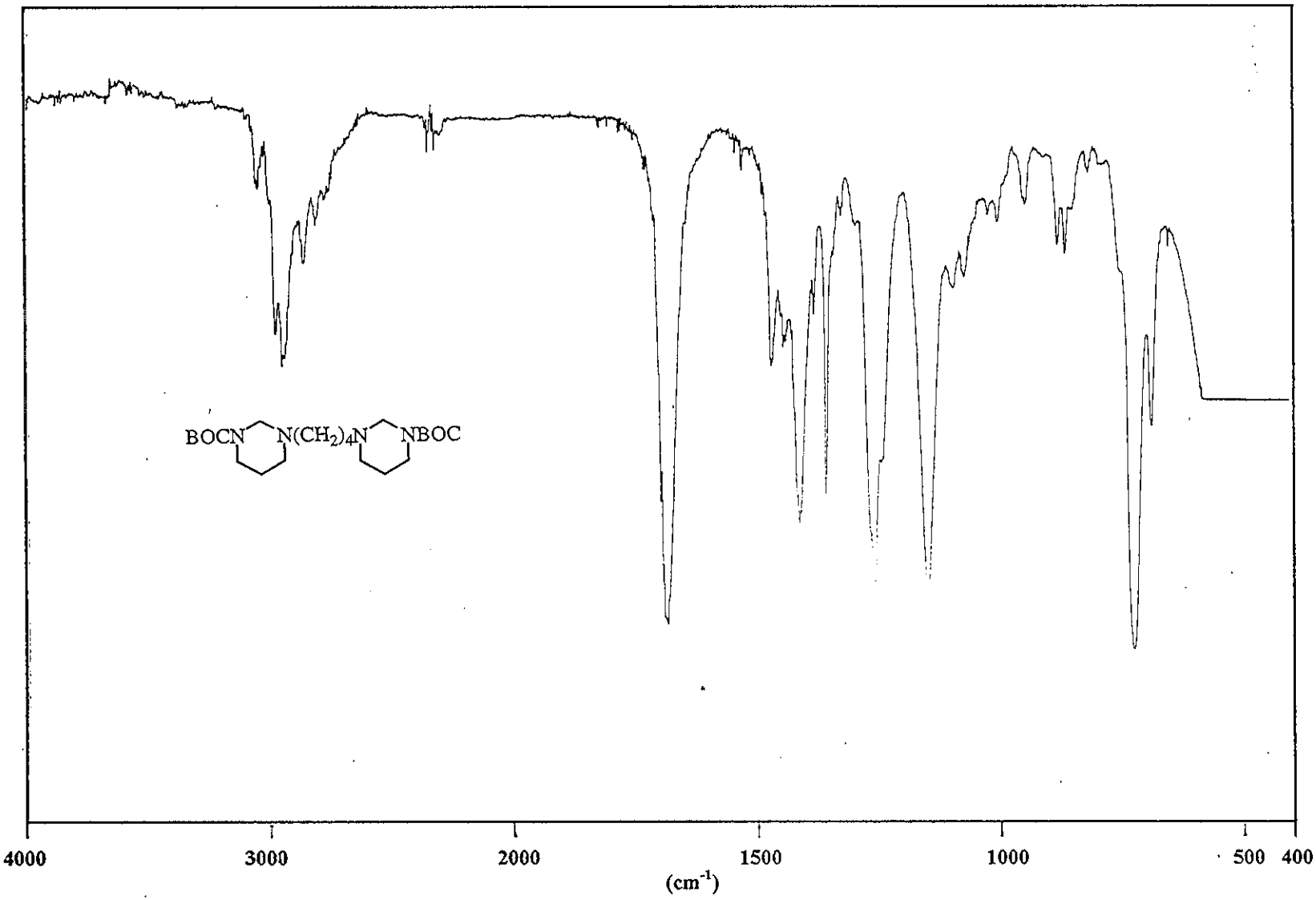


Figure 24. IR (film) spectrum of N^1,N^{12}-di-(*tert*-butoxycarbonyl) bis-hexahydropyrimidine (134)

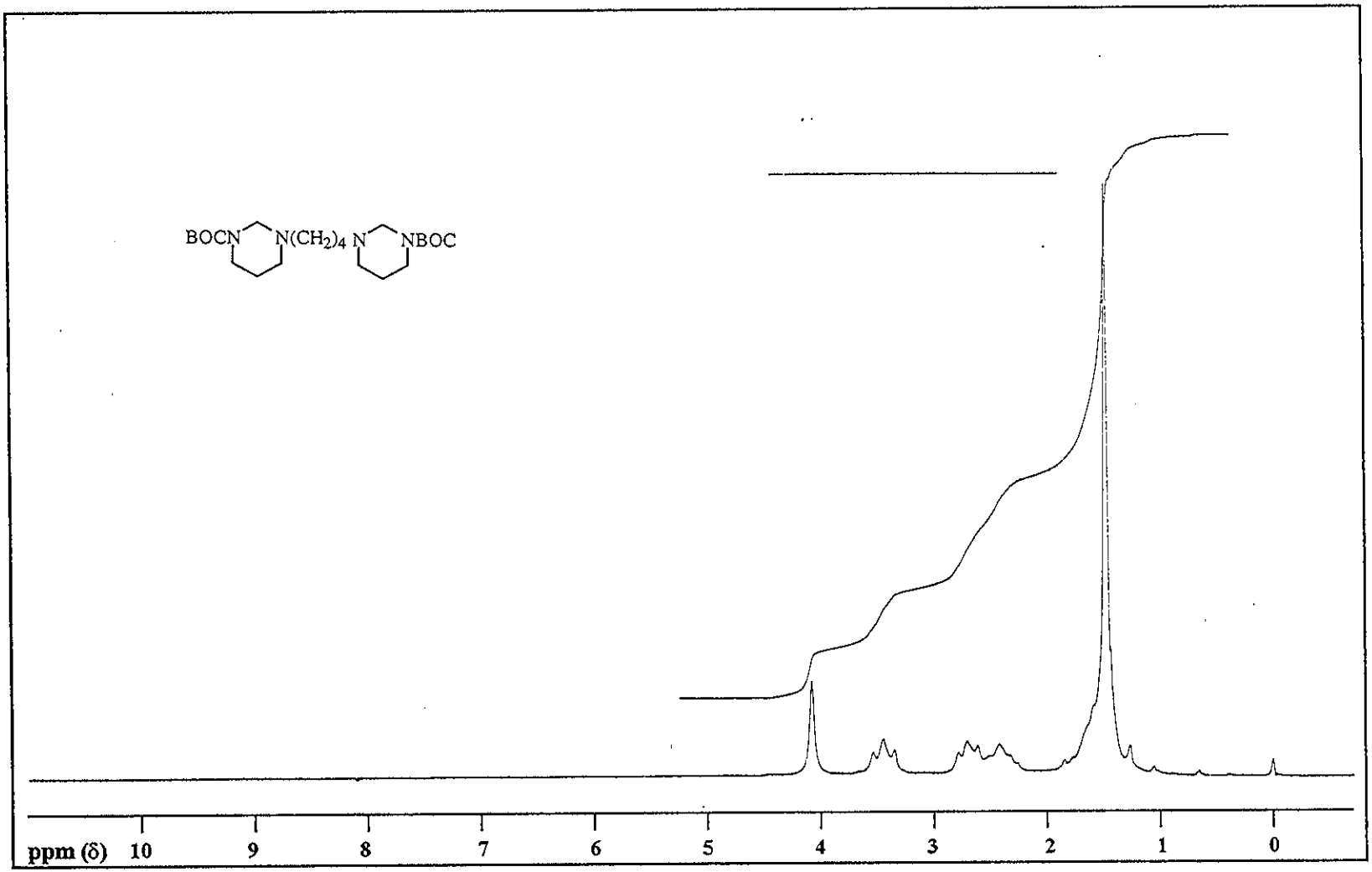


Figure 25. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of N^{1},N^{12}-di-(*tert*-butoxycarbonyl) bis-hexahydropyrimidine (134)

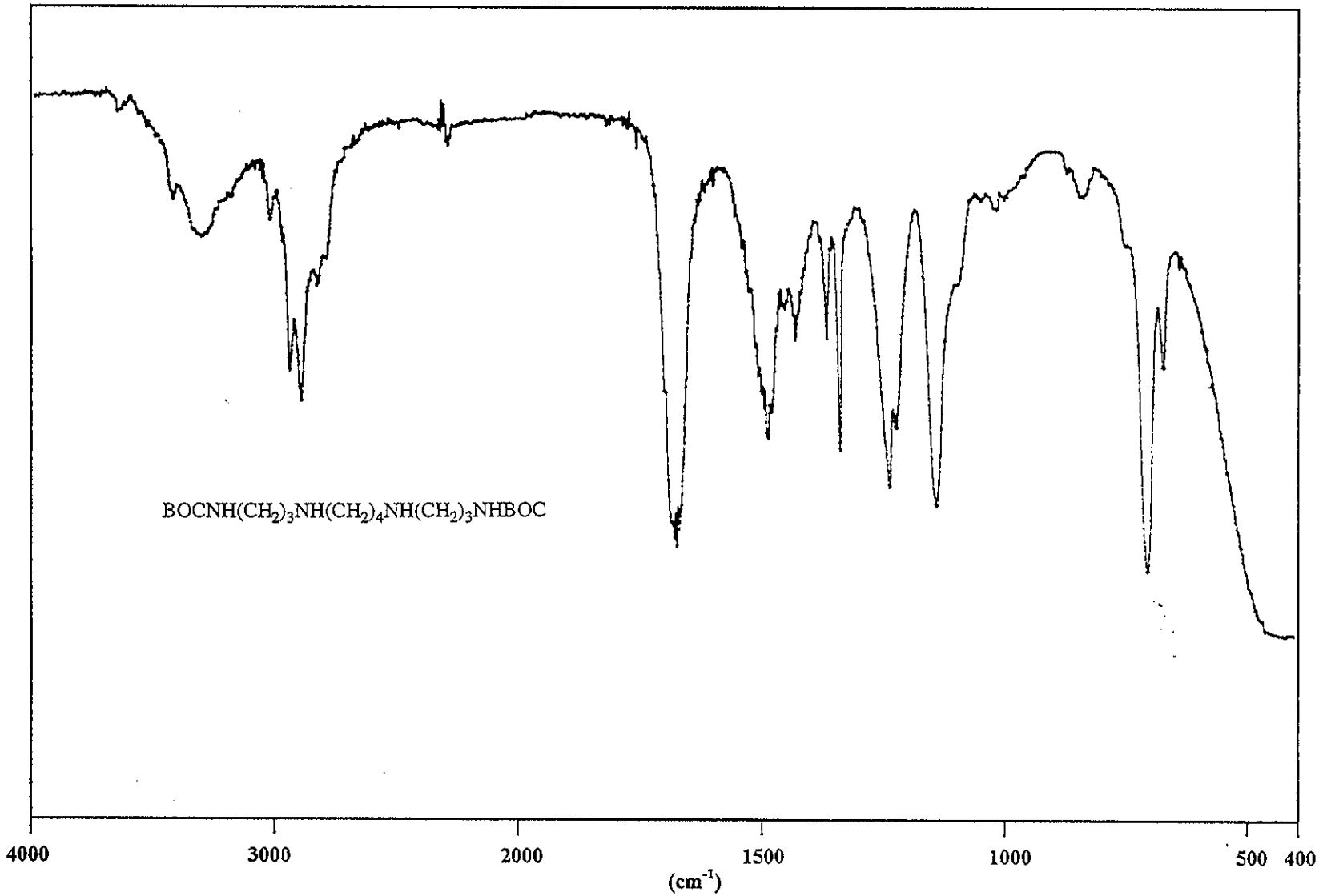


Figure 26. IR (film) spectrum of N^1,N^{12}-di-(*tert*-butoxycarbonyl) spermine (135)

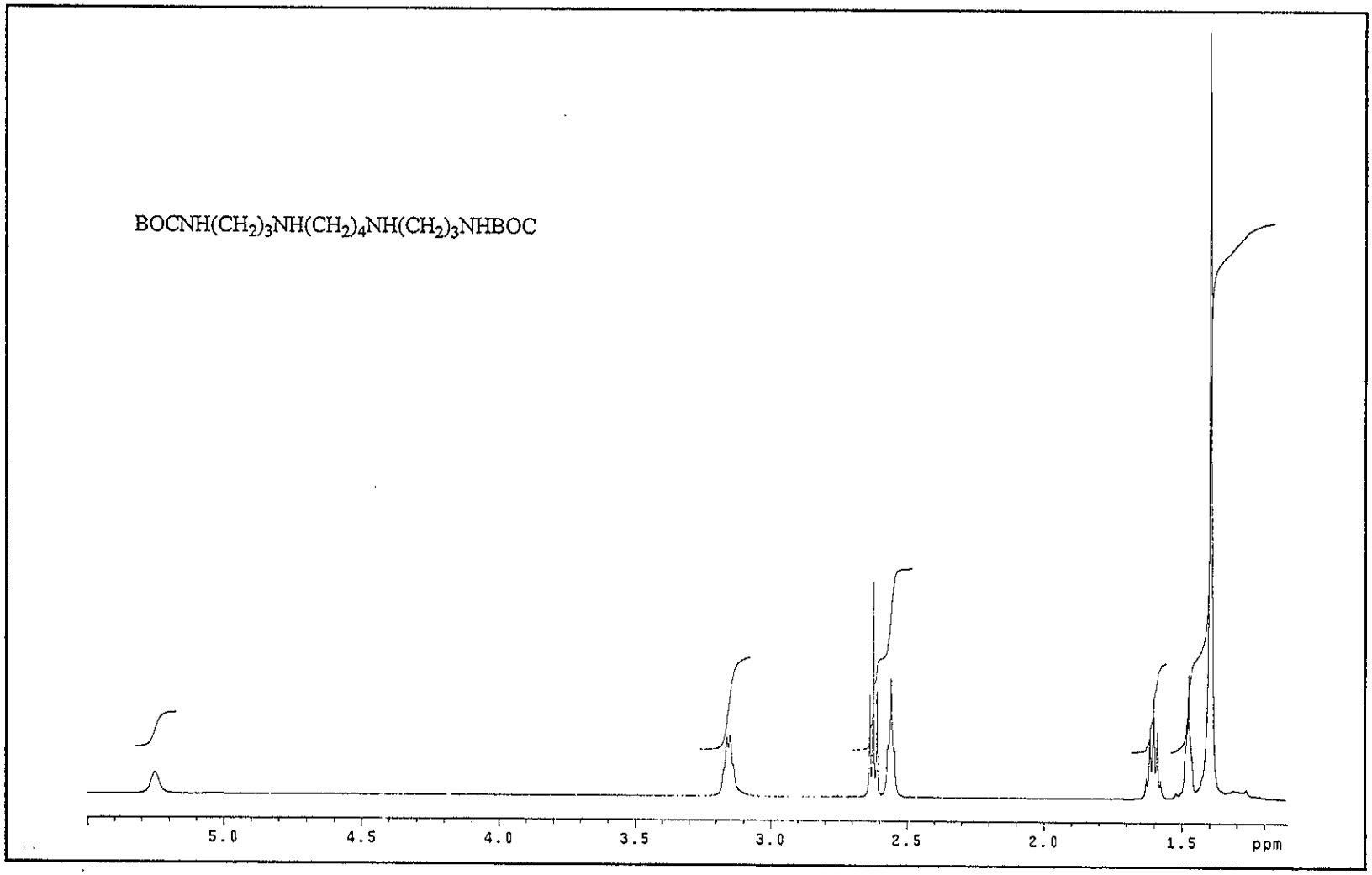


Figure 27. ^{1}H NMR ($CDCl_3$, 500 MHz) spectrum of N^{1},N^{12}-di-(*tert*-butoxycarbonyl) spermine (135)

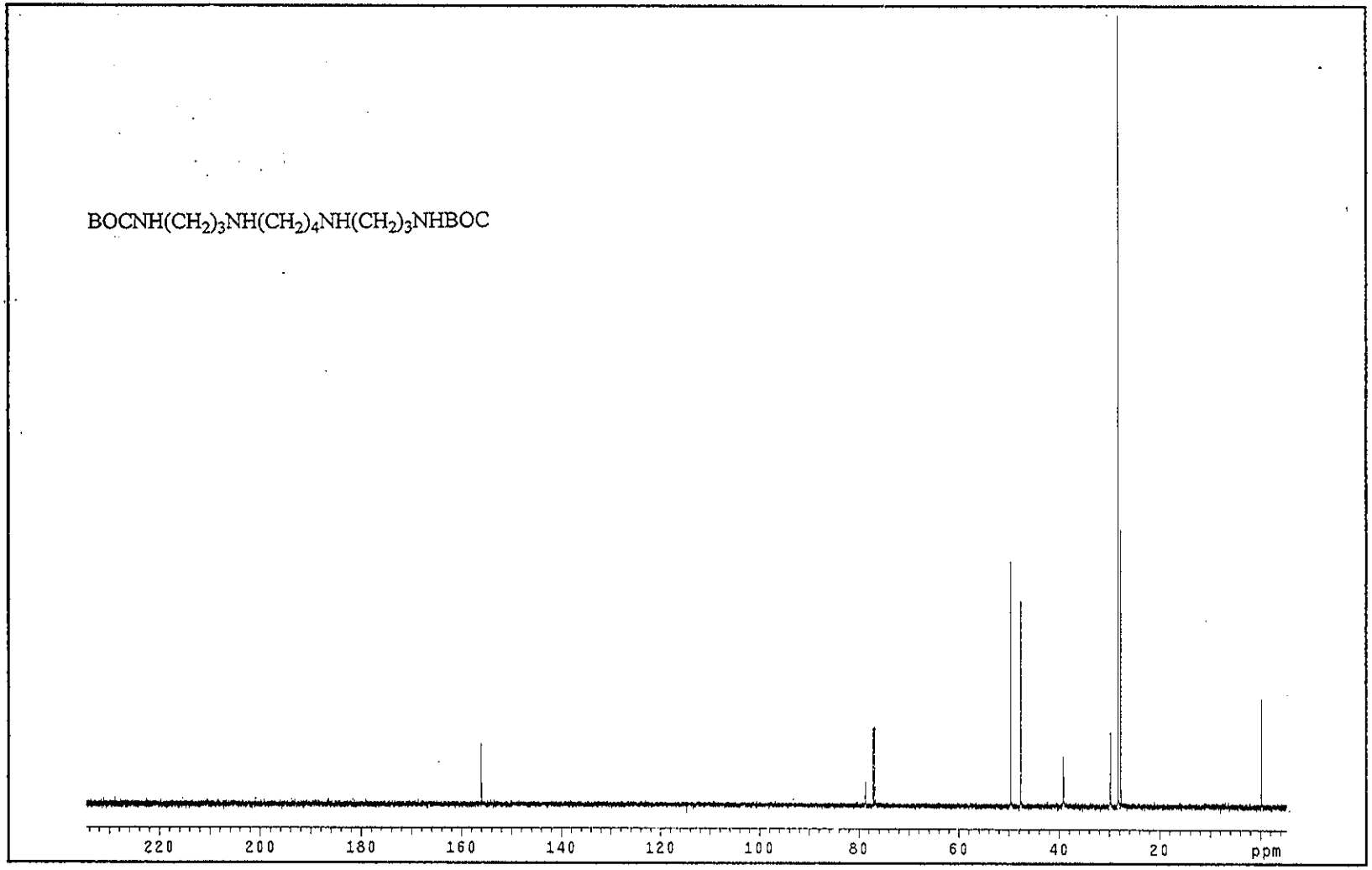


Figure 28. ^{13}C NMR ($CDCl_3$, 125 MHz) spectrum of N^1,N^{12}-di-(*tert*-butoxycarbonyl) spermine (135)

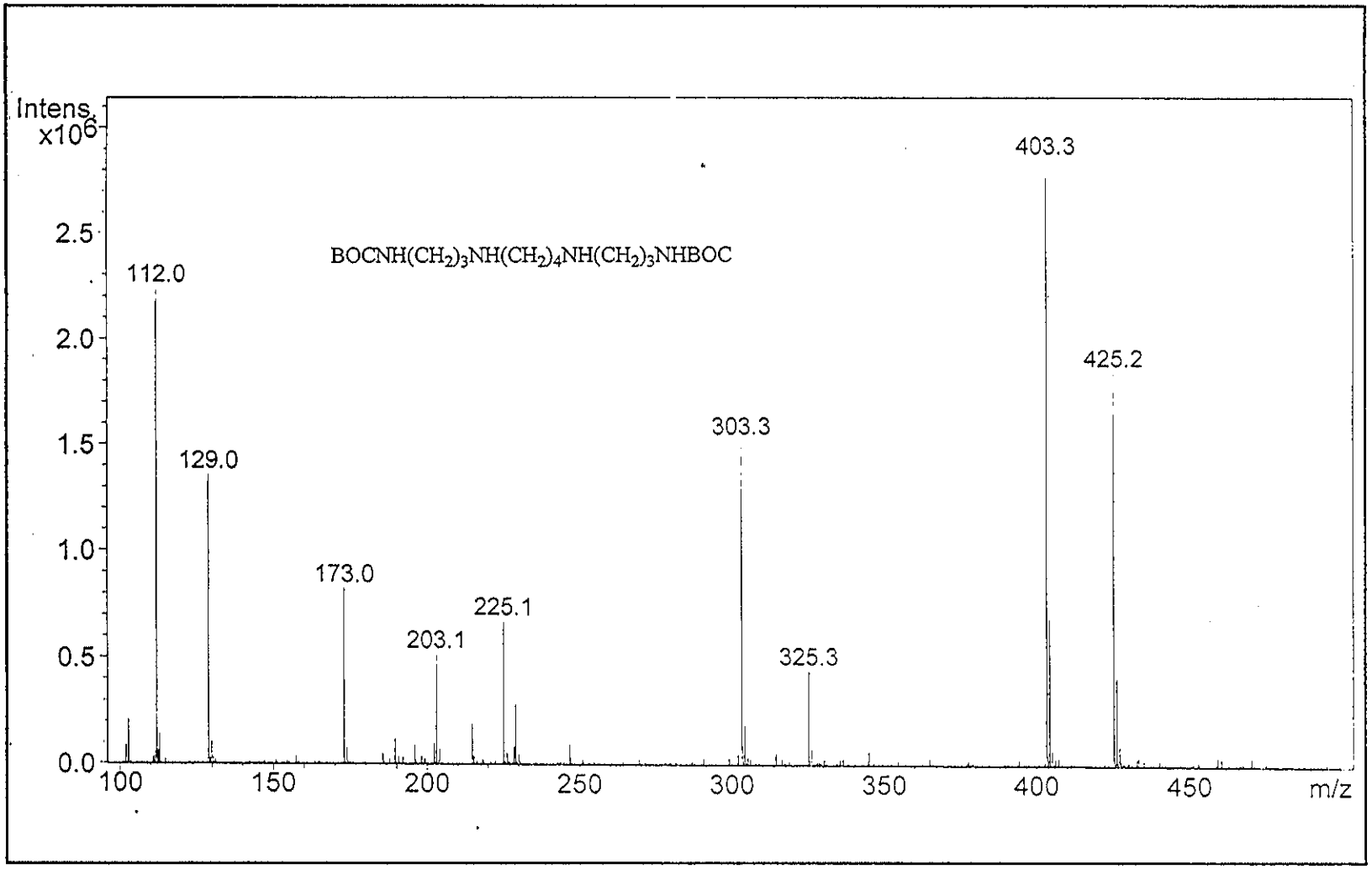


Figure 29. Mass spectrum (LC-MS) of N^1,N^{12}-di-(*tert*-butoxycarbonyl) spermine (135)

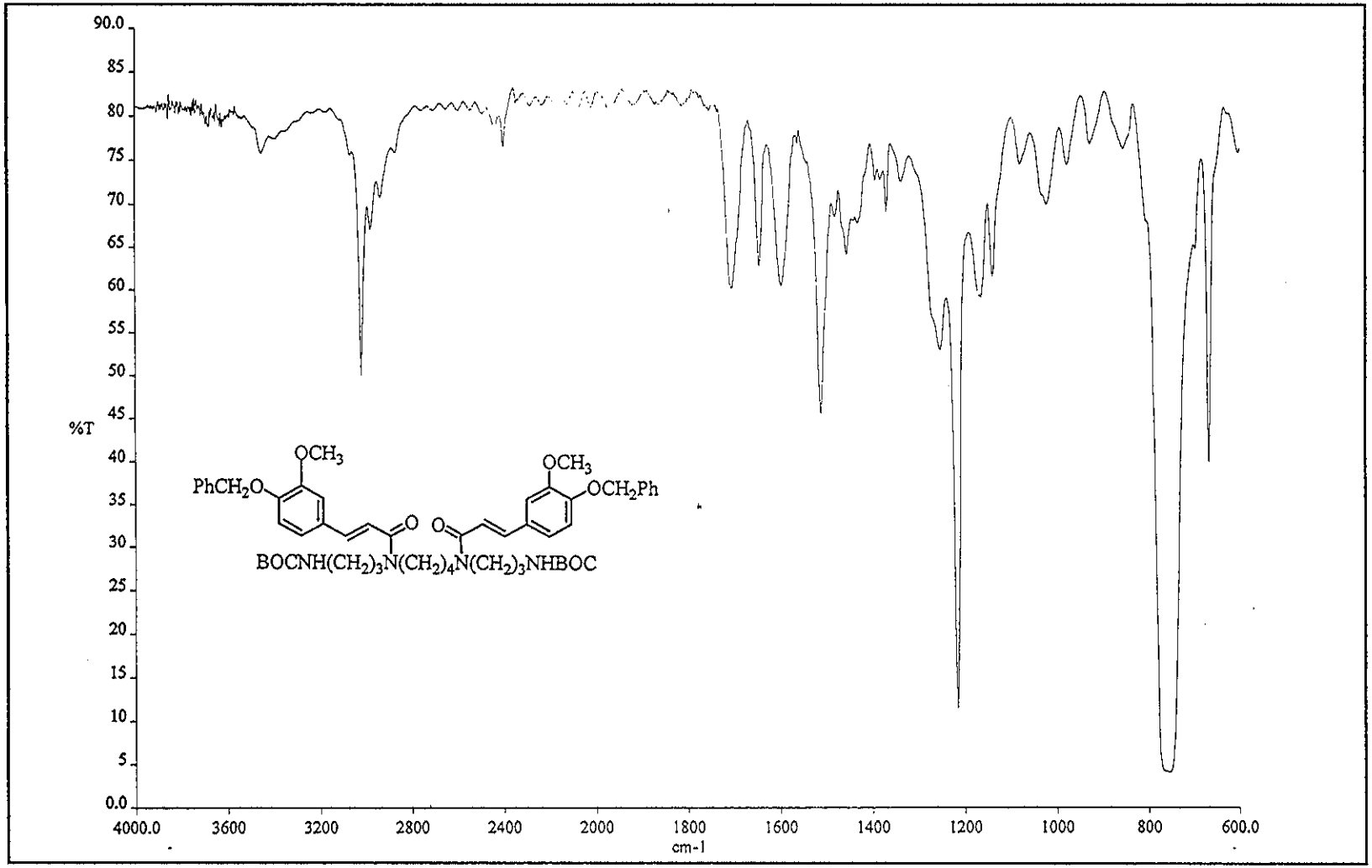


Figure 30. FTIR (film) spectrum of N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168)

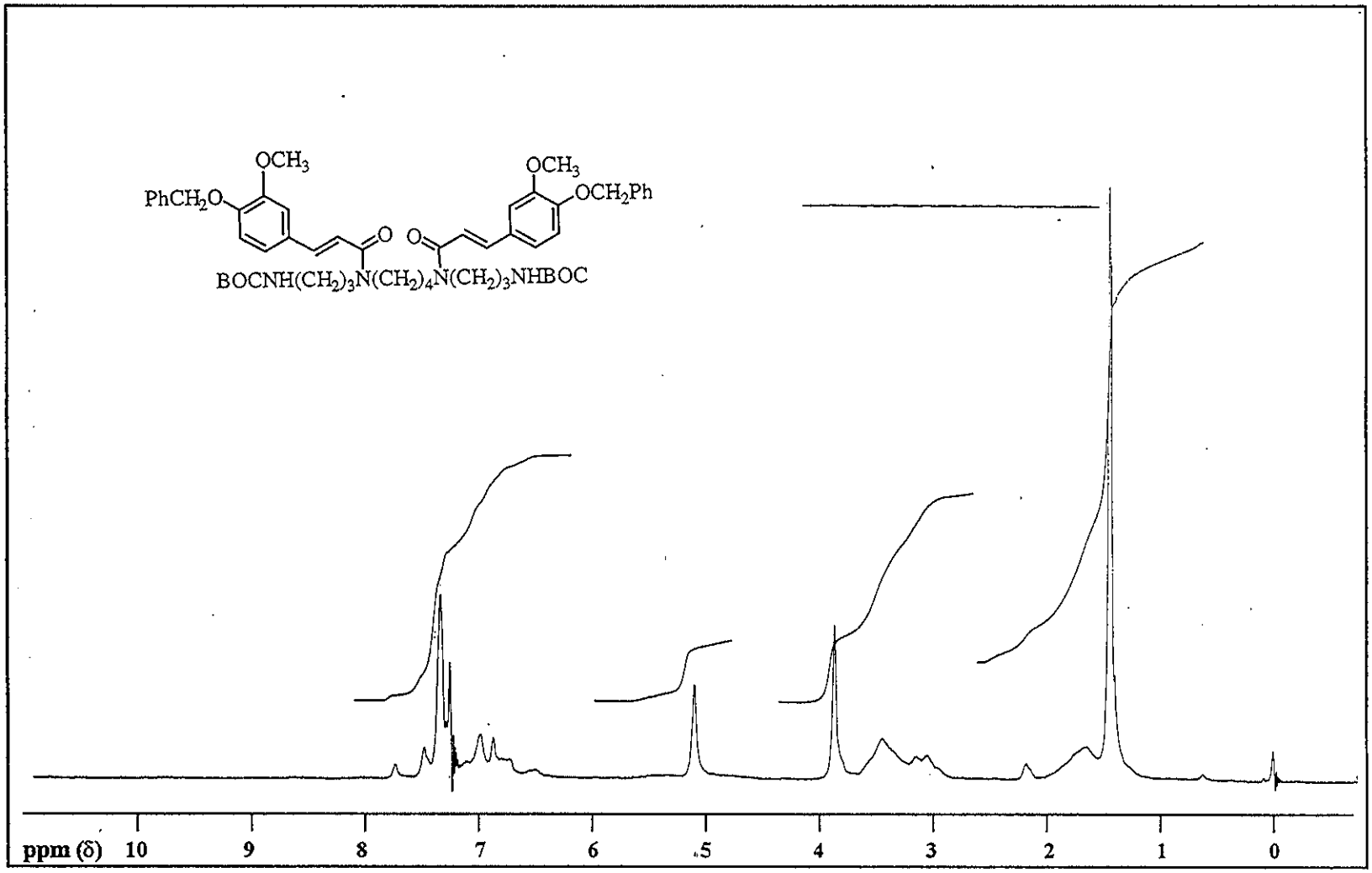


Figure 31. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of N^{1},N^{12}-di-(*tert*-butoxycarbonyl)-N^{4},N^{8}-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168)

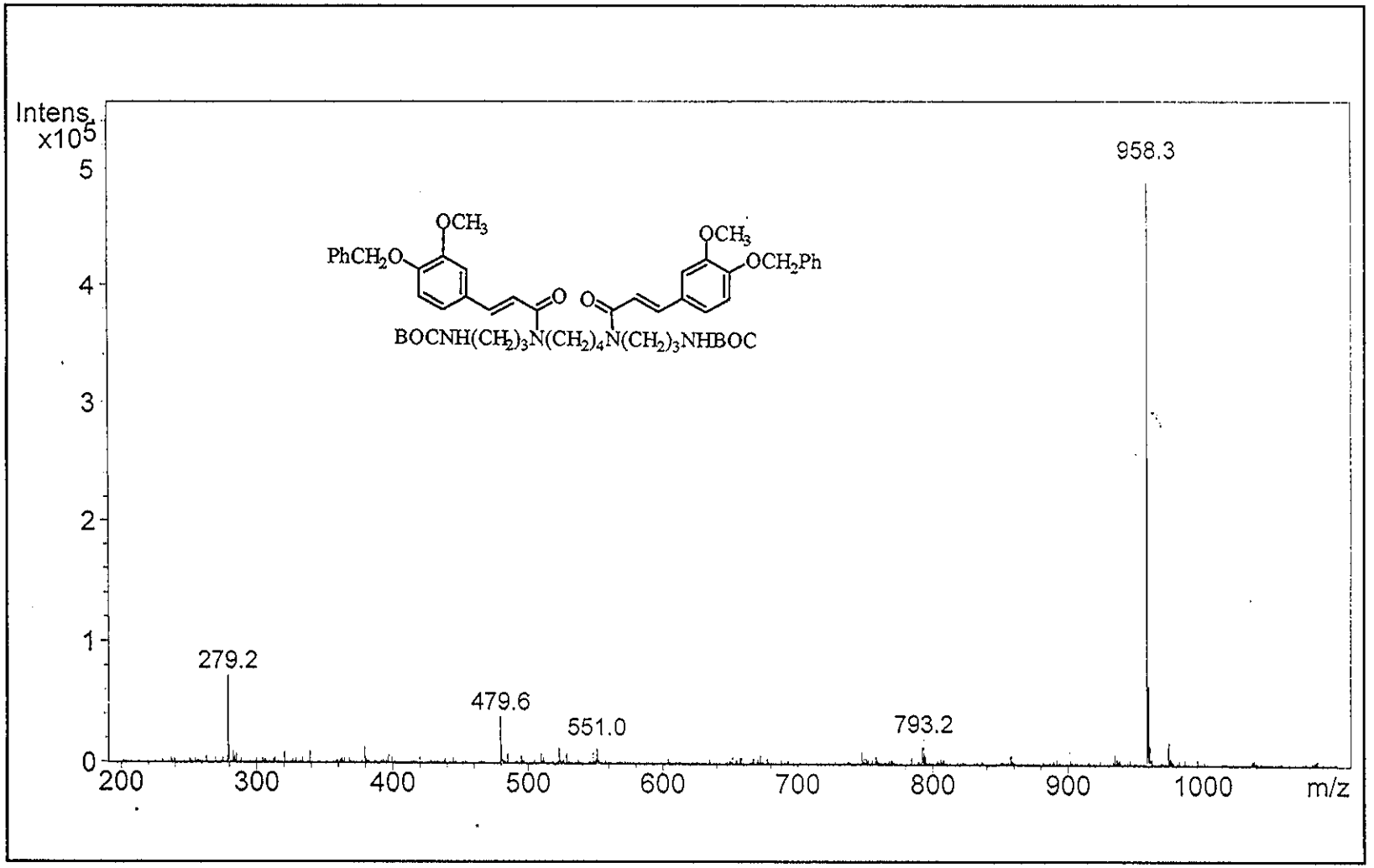


Figure 32. Mass spectrum (LC-MS) of N^1,N^{12}-di-(*tert*-butoxycarbonyl)-N^4,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermine (168)

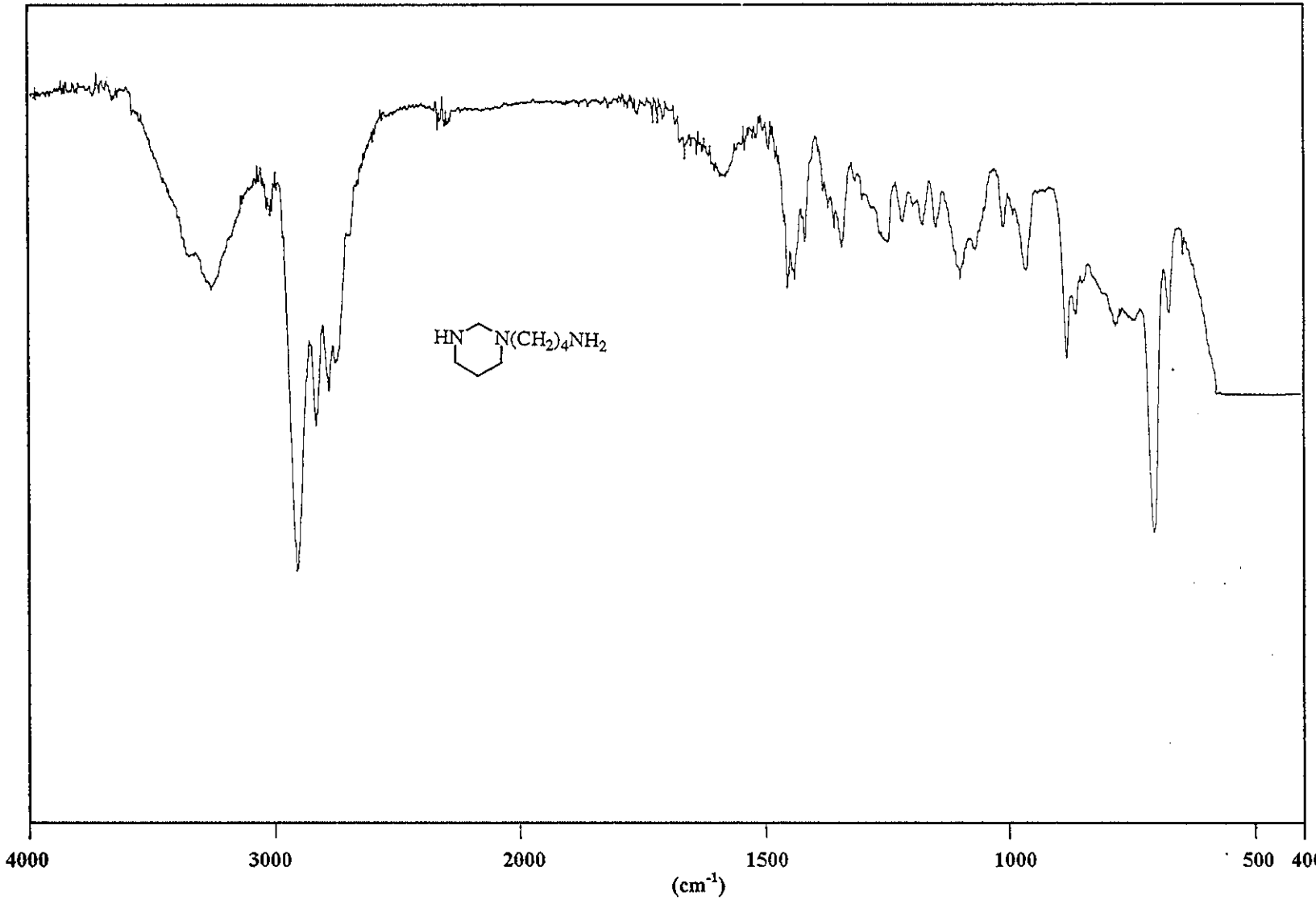


Figure 33. IR (film) spectrum of Hexahydropyrimidine (37)

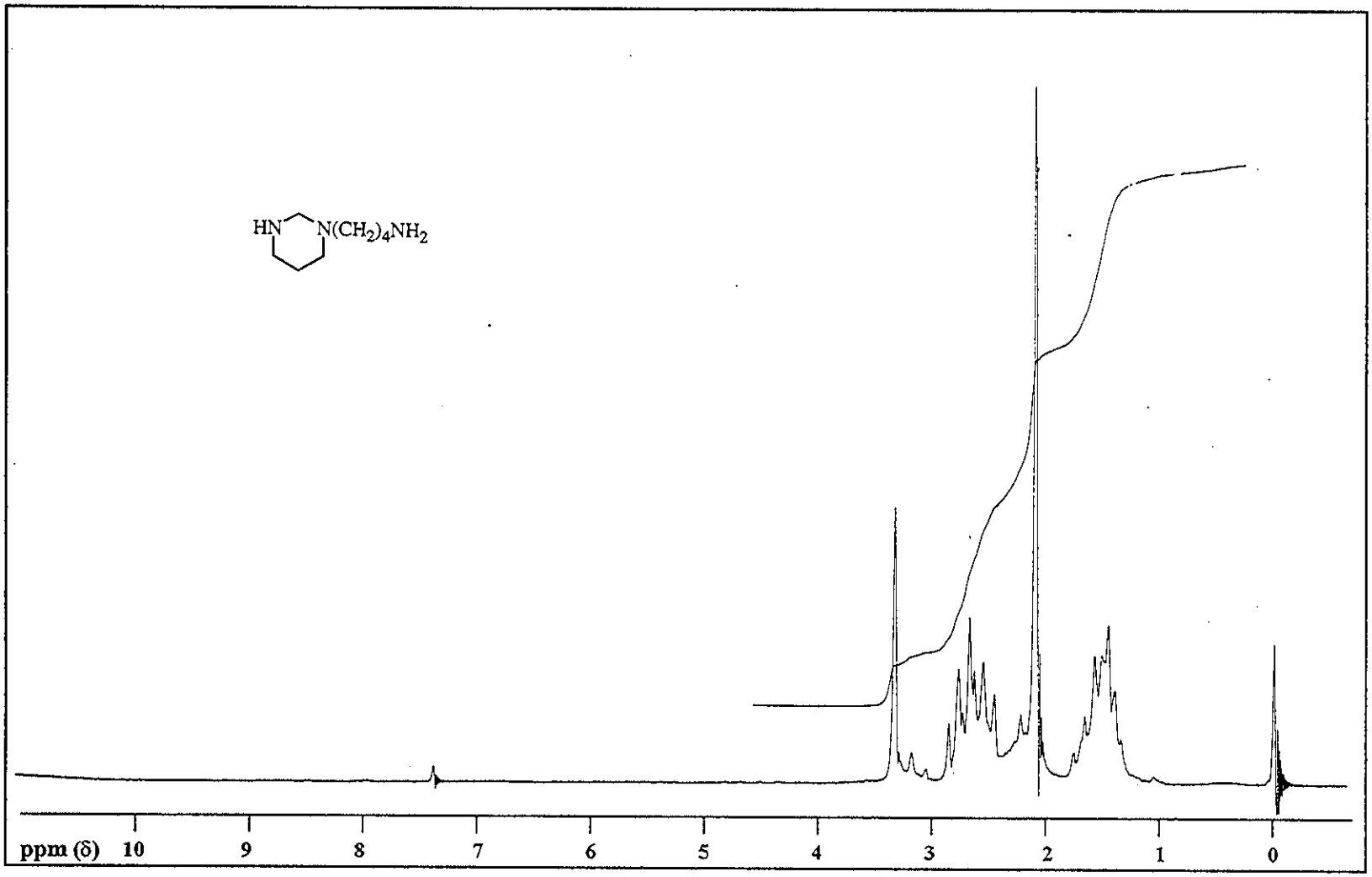


Figure 34. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of Hexahydropyrimidine (37)

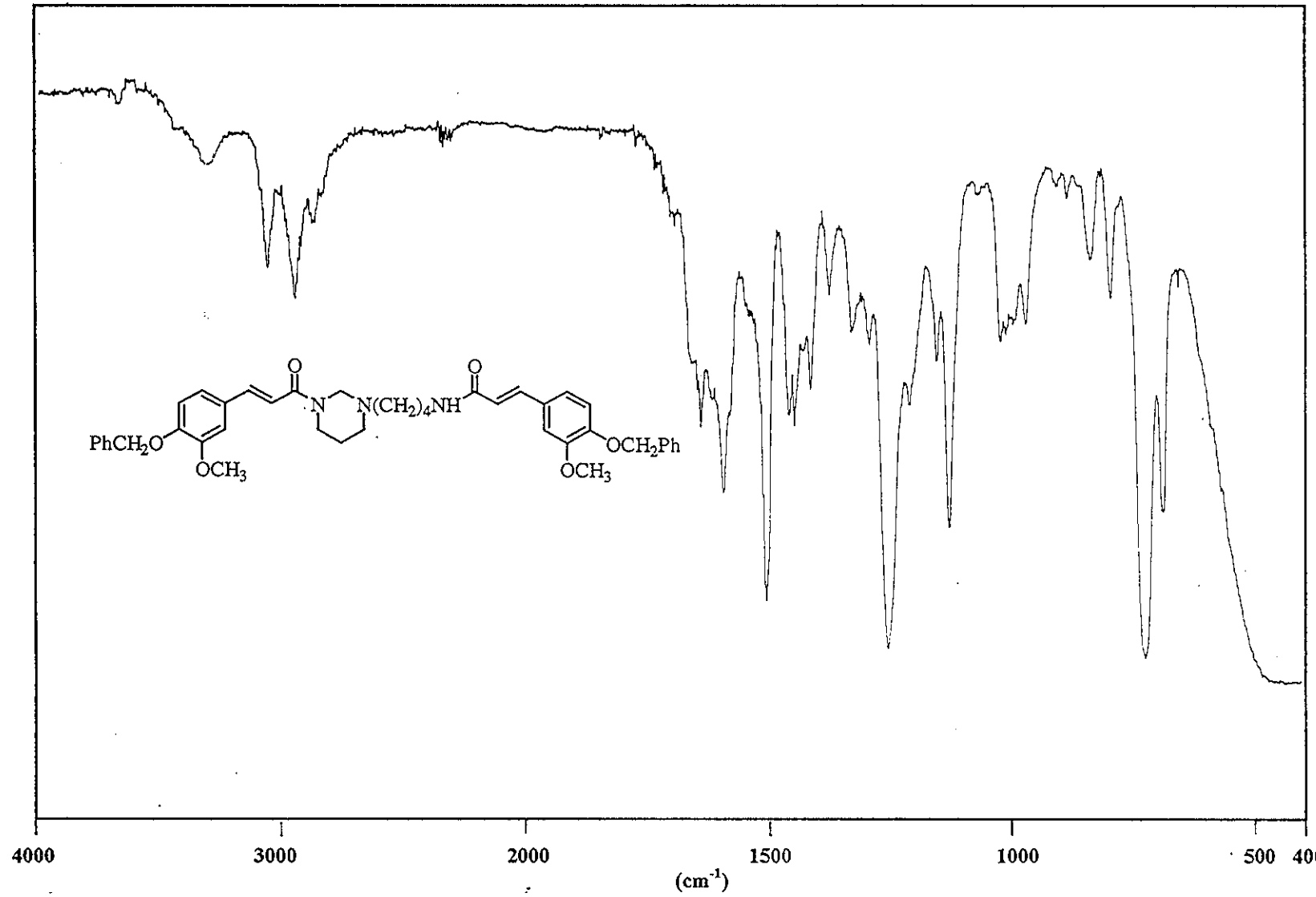


Figure 35. IR (film) spectrum of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) hexahydropyrimidine (176)

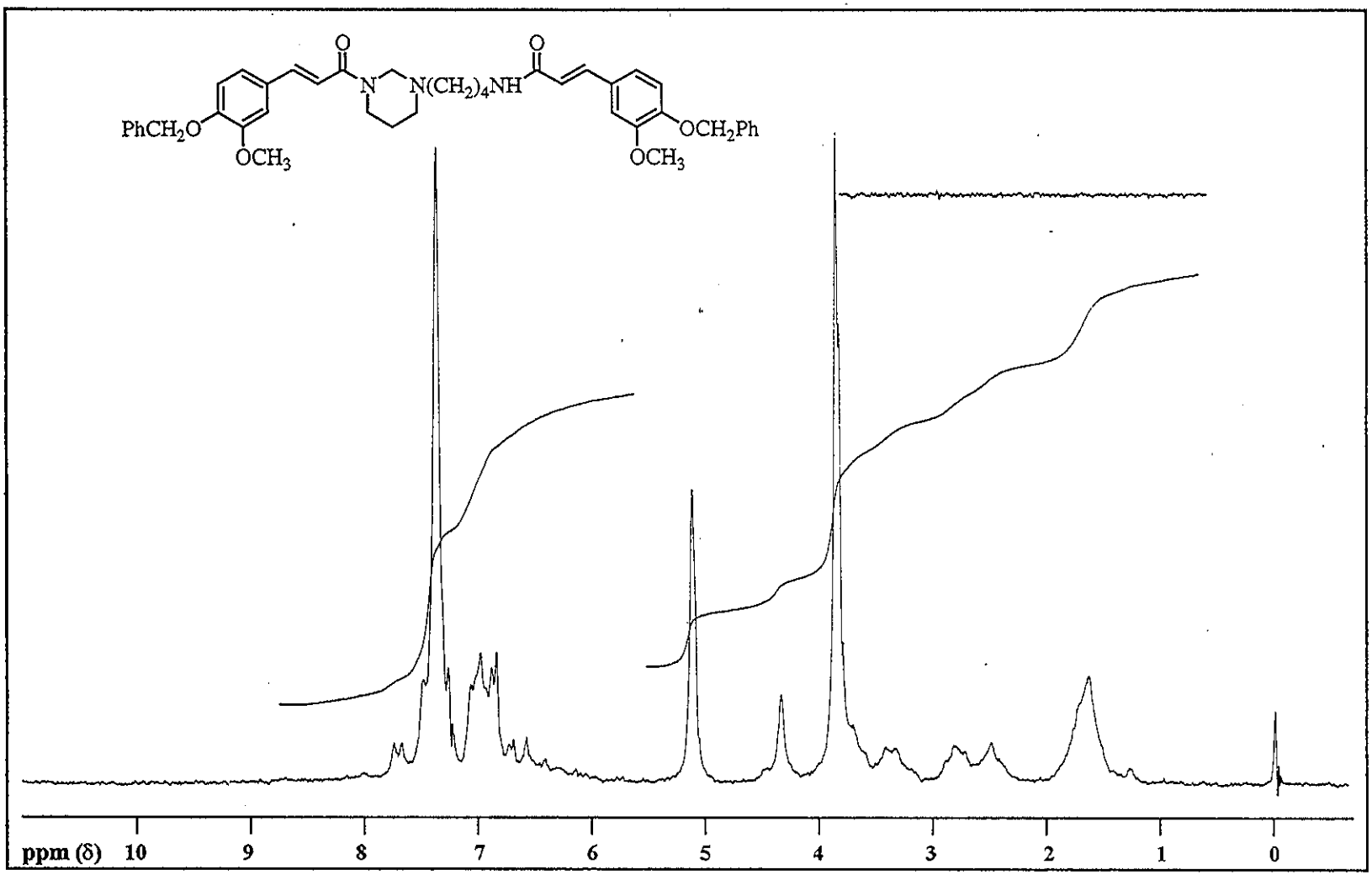


Figure 36. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) hexahydropyrimidine (176)

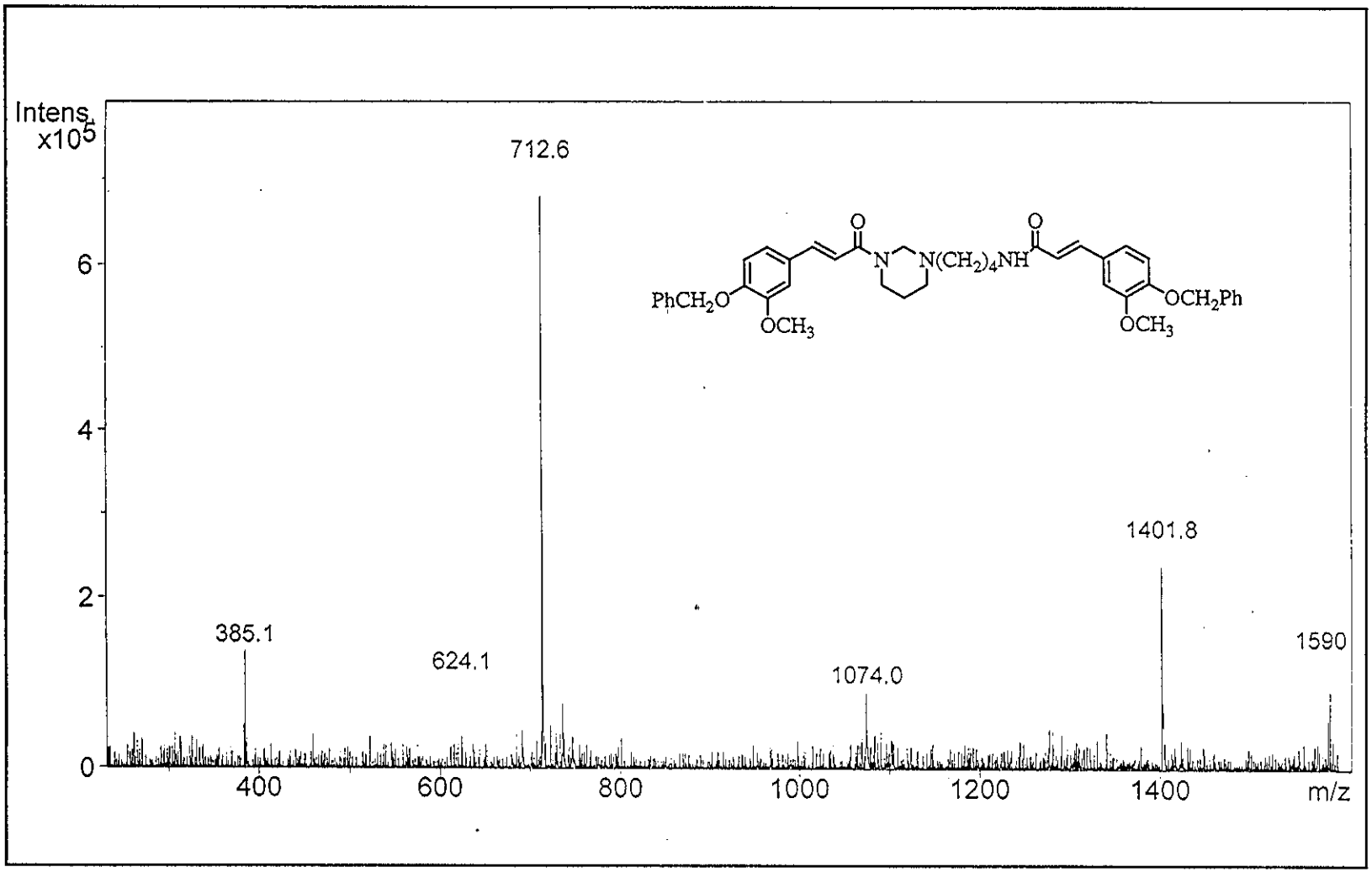


Figure 37. Mass spectrum (LC-MS) of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) hexahydropyrimidine (176)

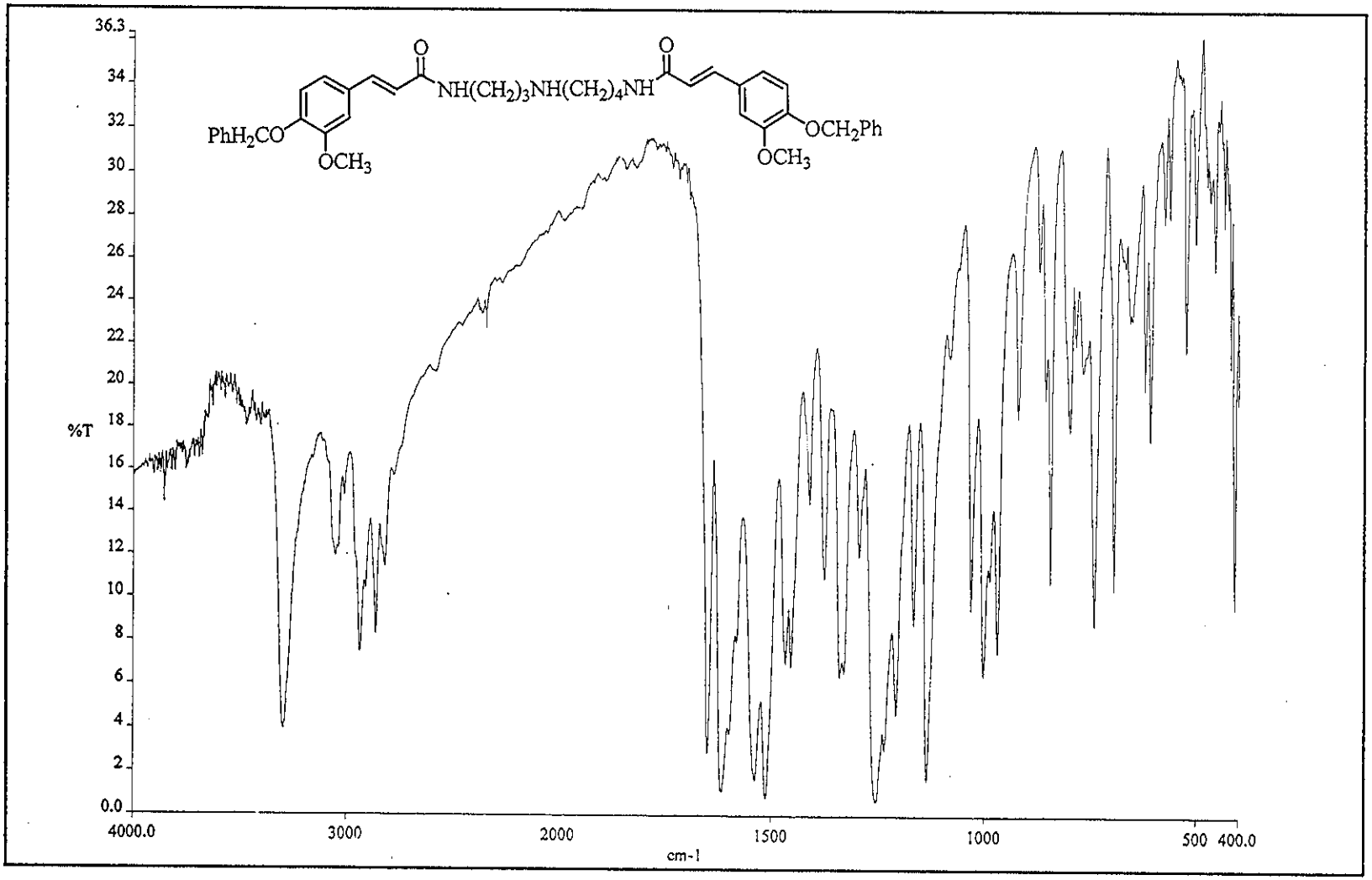


Figure 38. FTIR (KBr) spectrum of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)

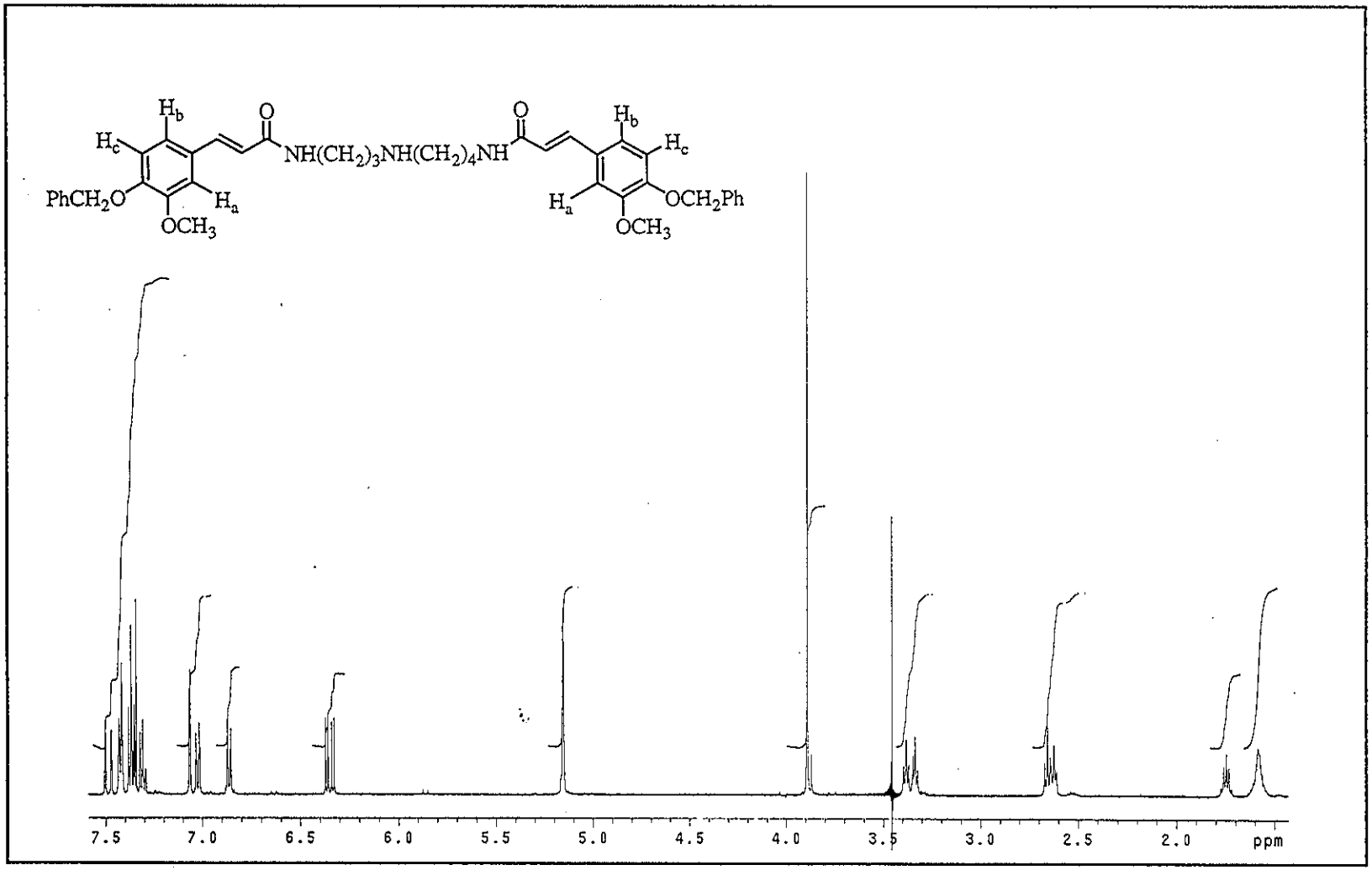


Figure 39. ^{1}H NMR ($CDCl_3$+CD_3OD, 500 MHz) spectrum of N^{1},N^{8}-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)

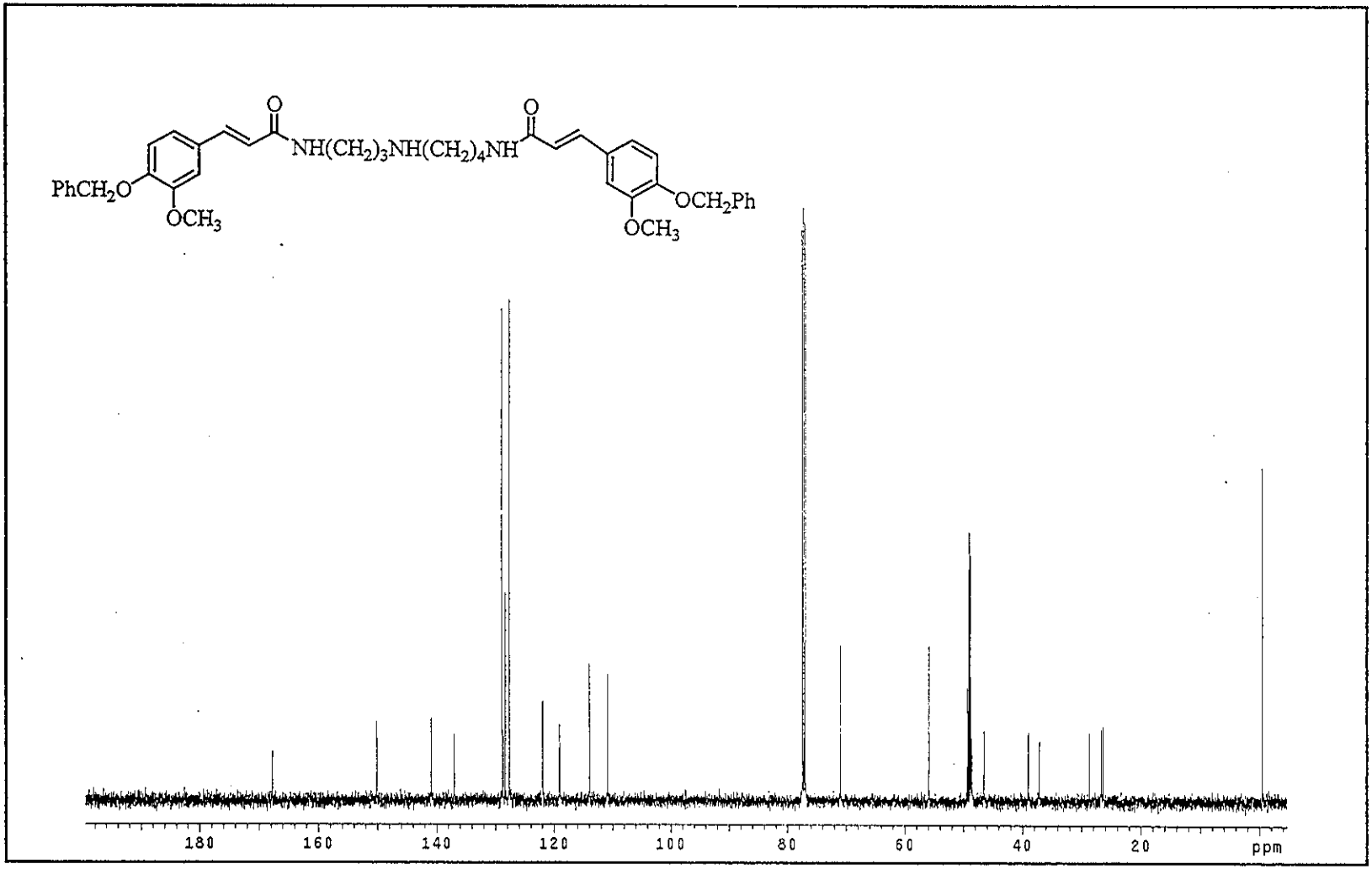


Figure 40. ^{13}C NMR ($CDCl_3$+CD_3OD, 125 MHz) spectrum of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169).

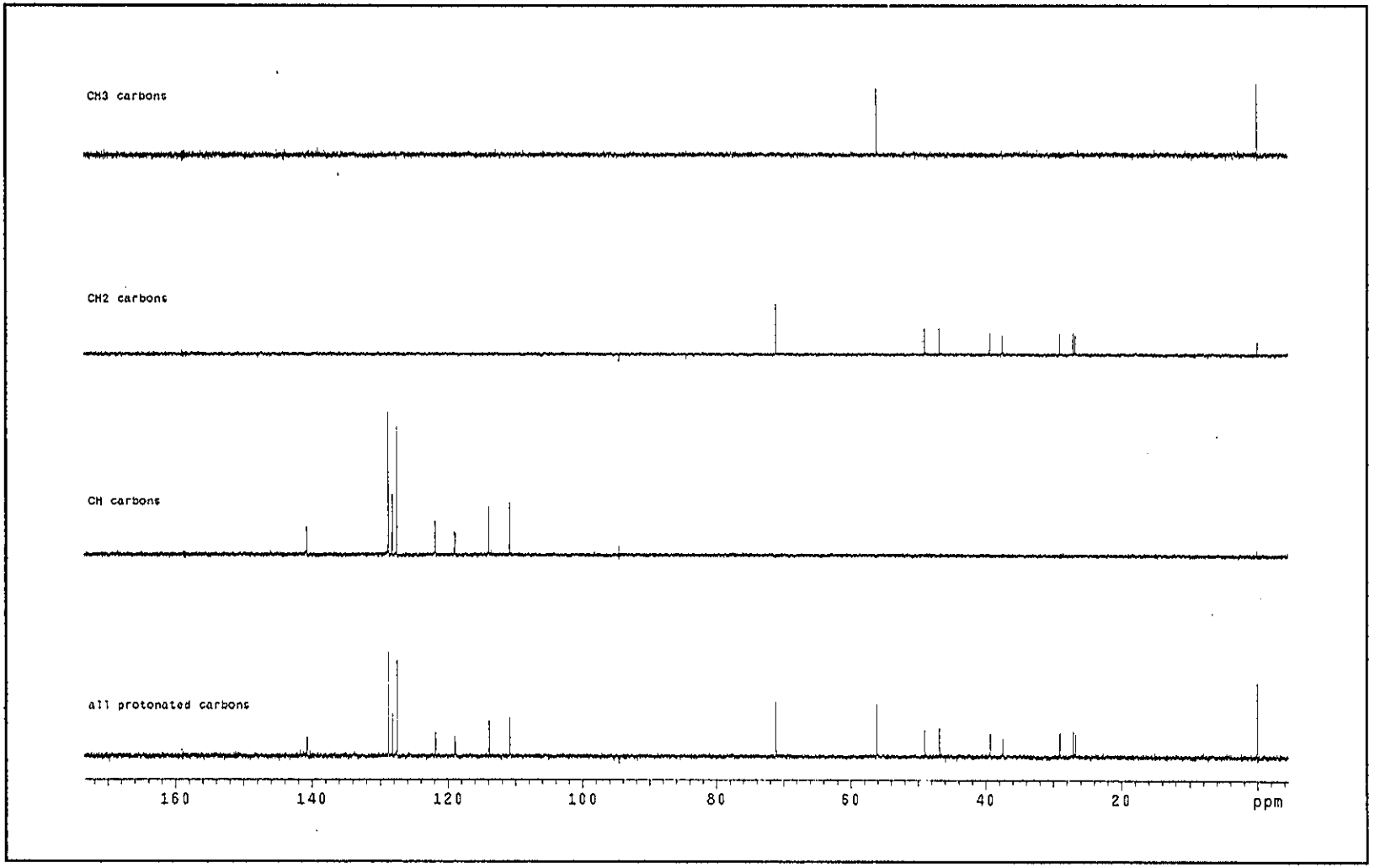


Figure 41. DEPT spectrum of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)

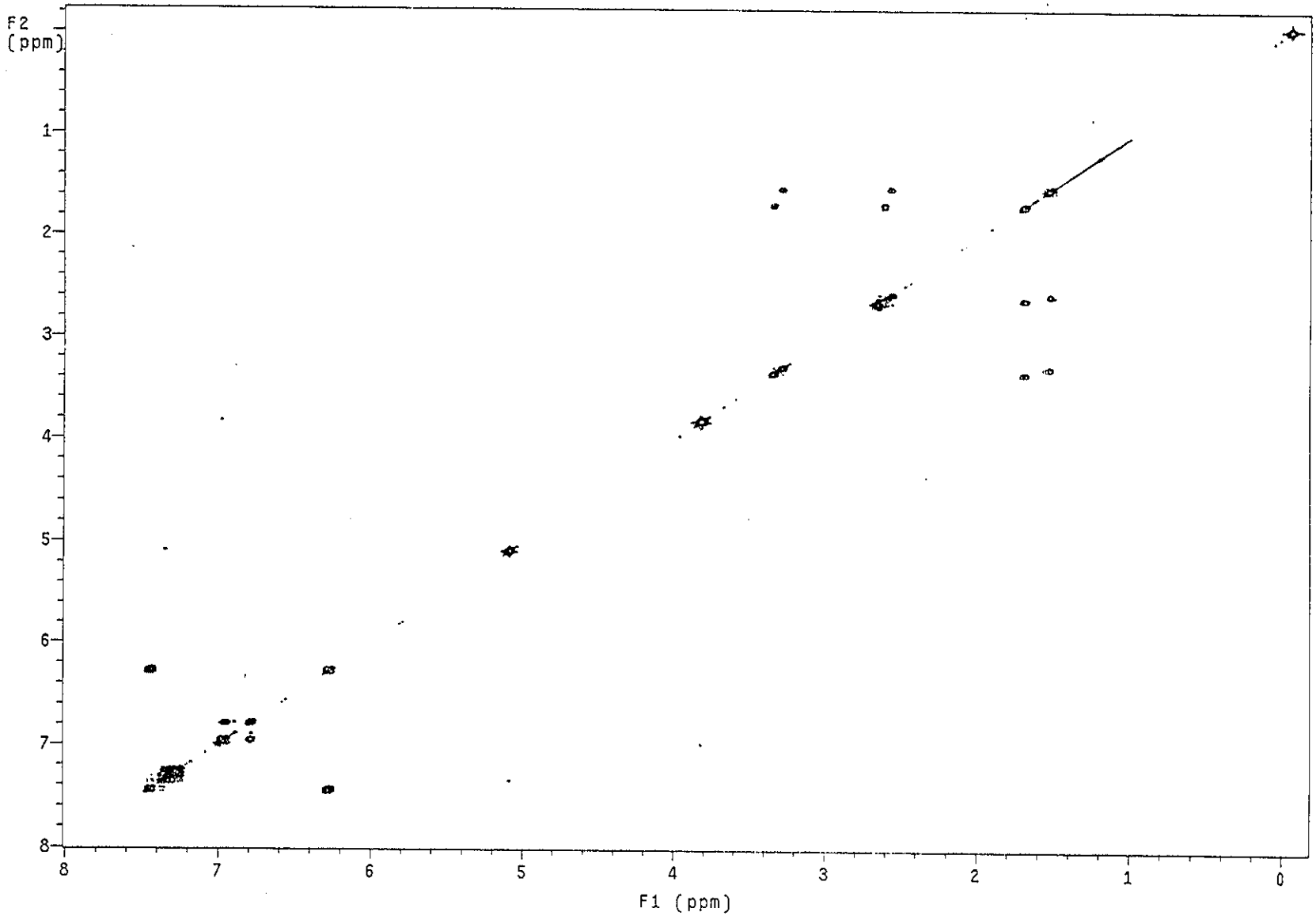


Figure 42. ^{1}H-^{1}H COSY spectrum of N^{1},N^{8}-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)

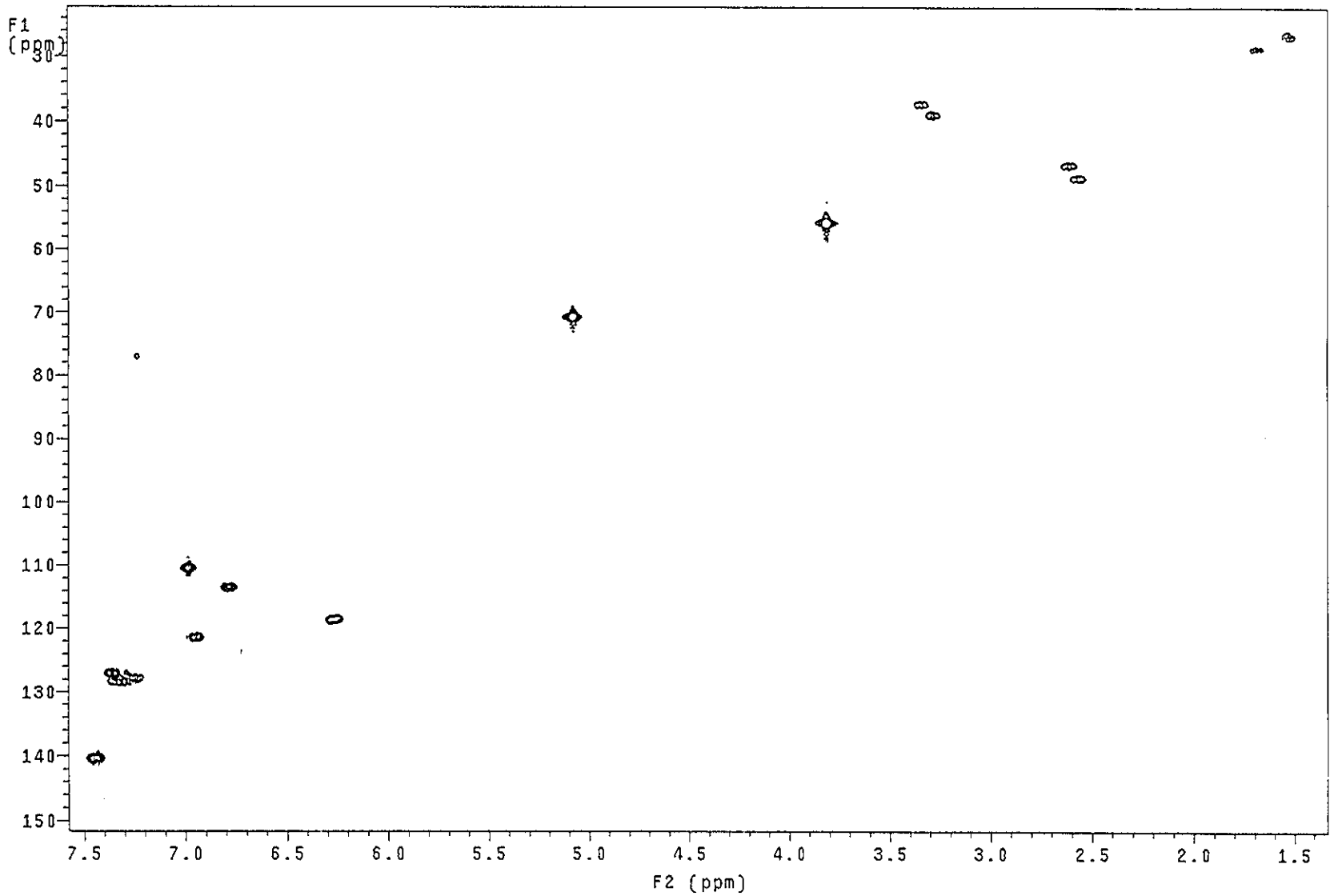


Figure 43. HMQC spectrum of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)

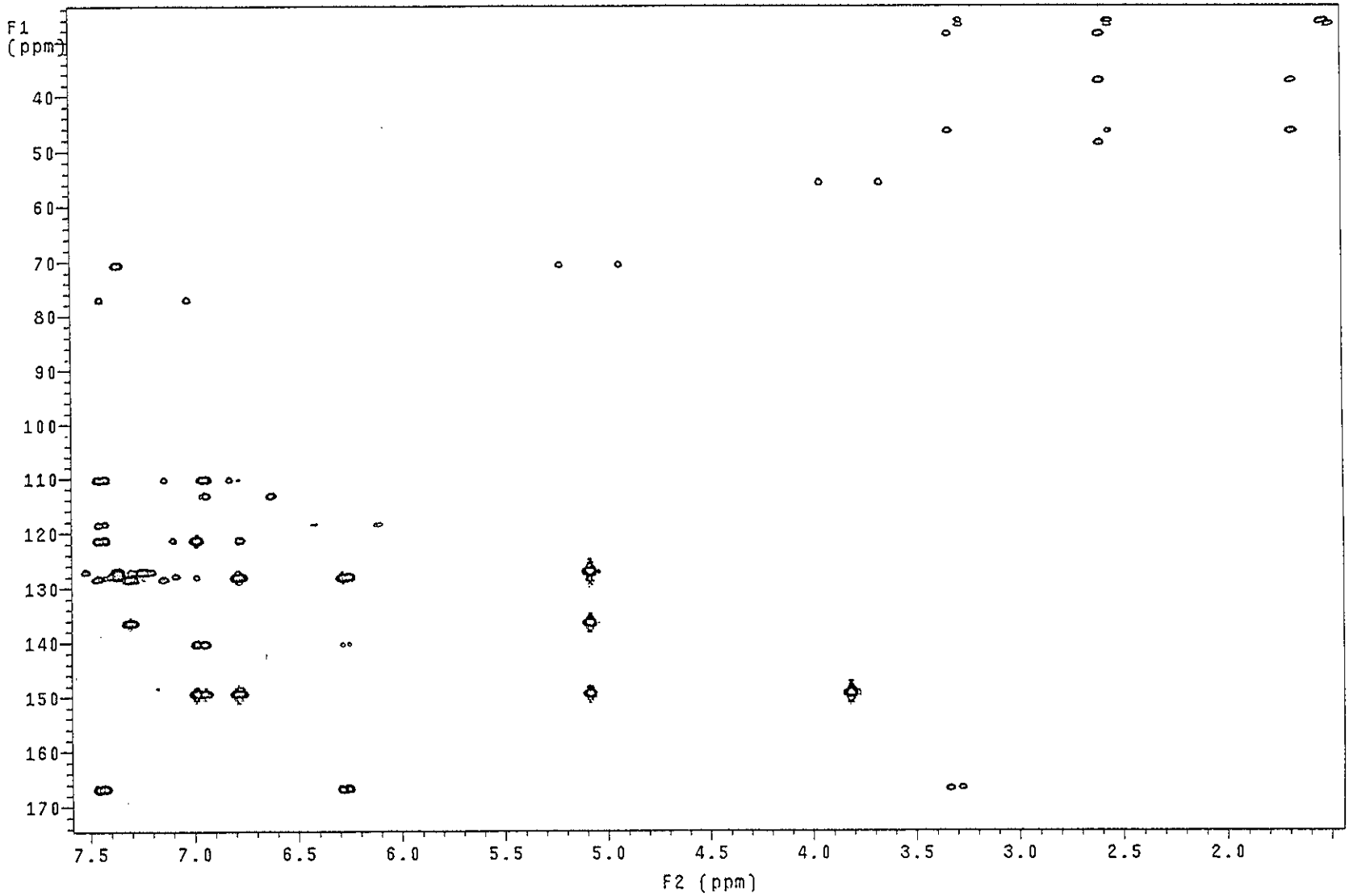


Figure 44. HMBC spectrum of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)

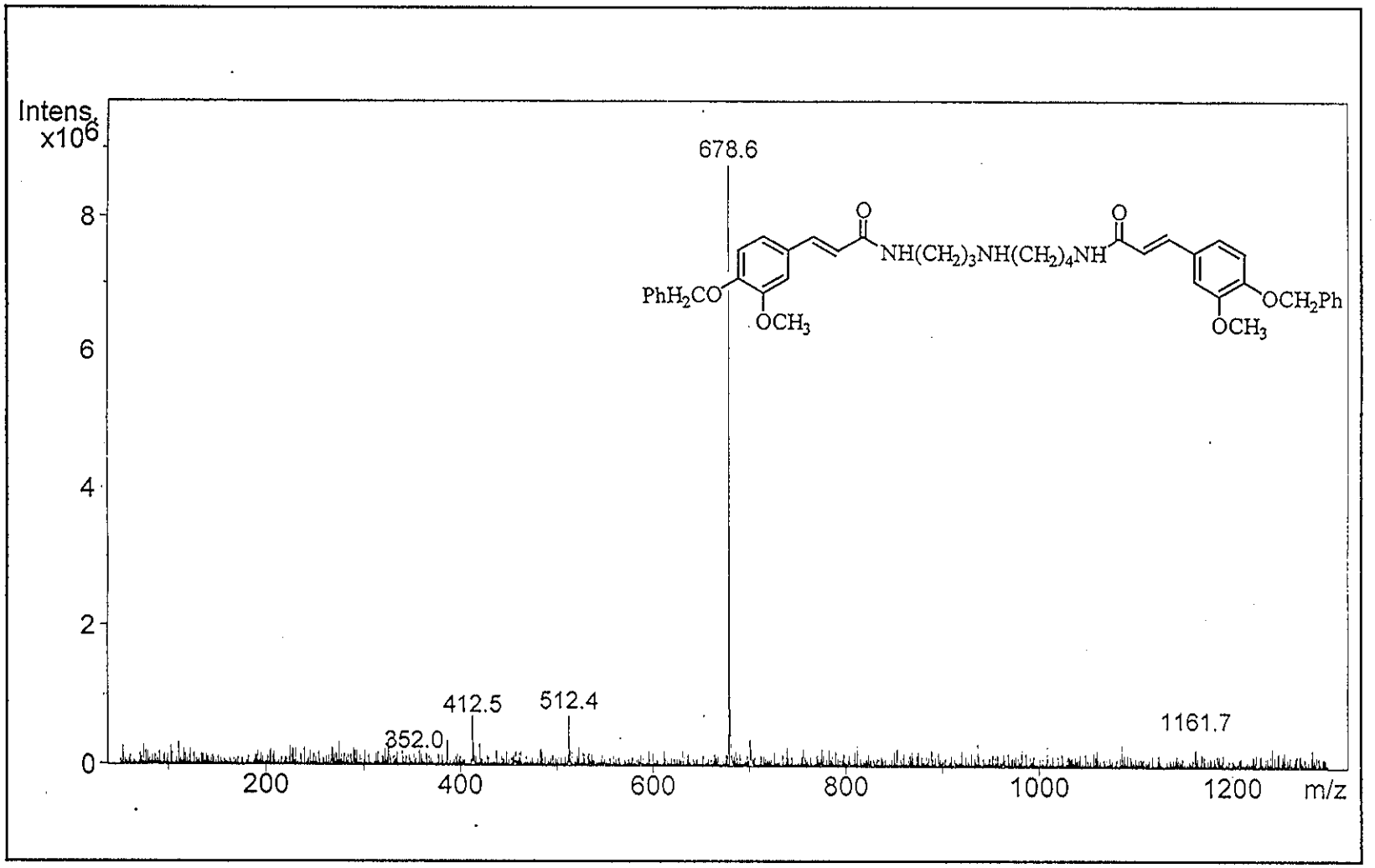


Figure 45. Mass spectrum (LC-MS) of N^1,N^8-di-(4-benzyloxy-3-methoxycinnamoyl) spermidine (169)

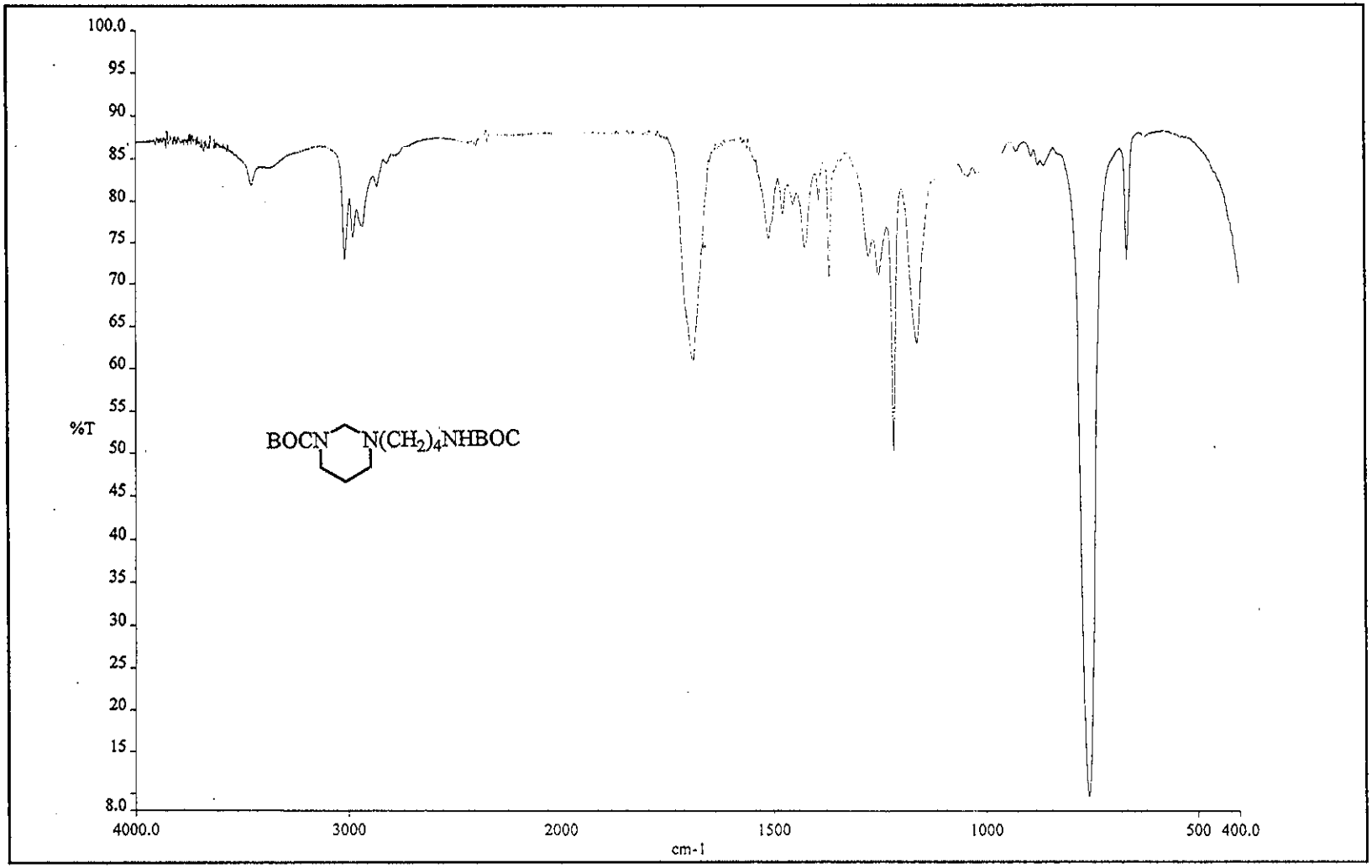


Figure 46. FTIR (film) spectrum of N^1,N^8-di-(*tert*-butoxycarbonyl) hexahydropyrimidine (177)

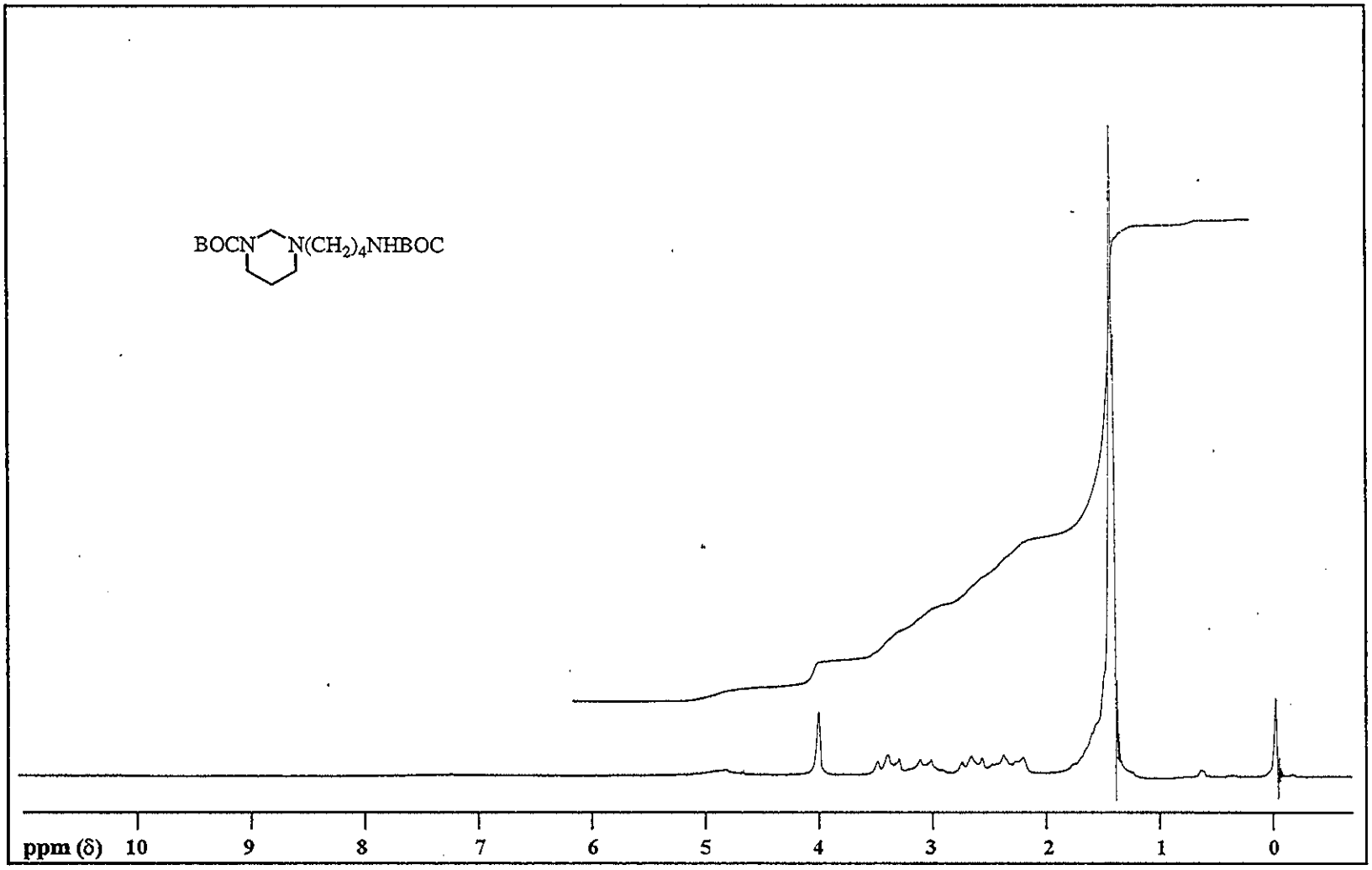


Figure 47. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of N^1,N^8-di-(*tert*-butoxycarbonyl) hexahydropyrimidine (177)

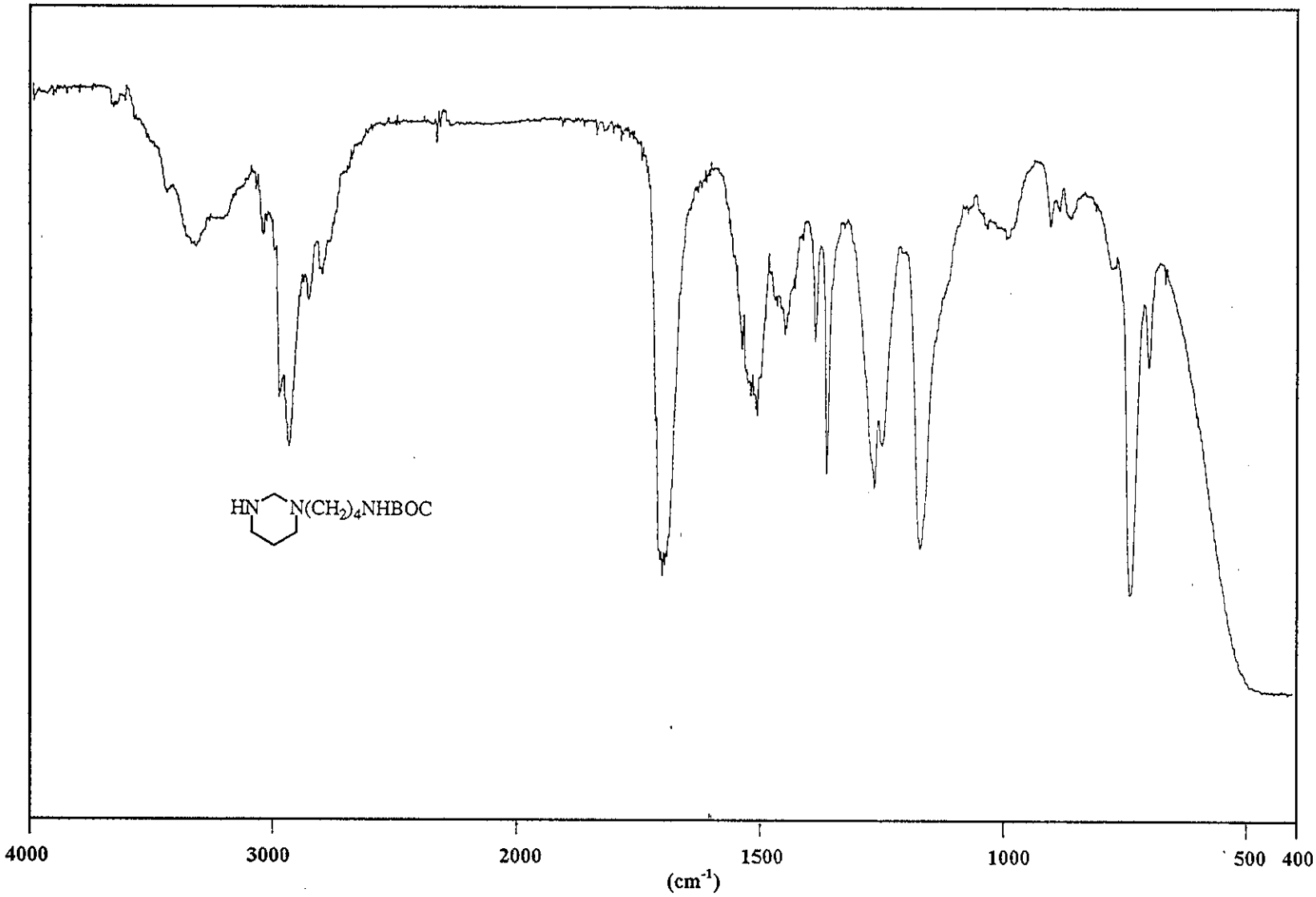


Figure 48. IR (film) spectrum of N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine (38)

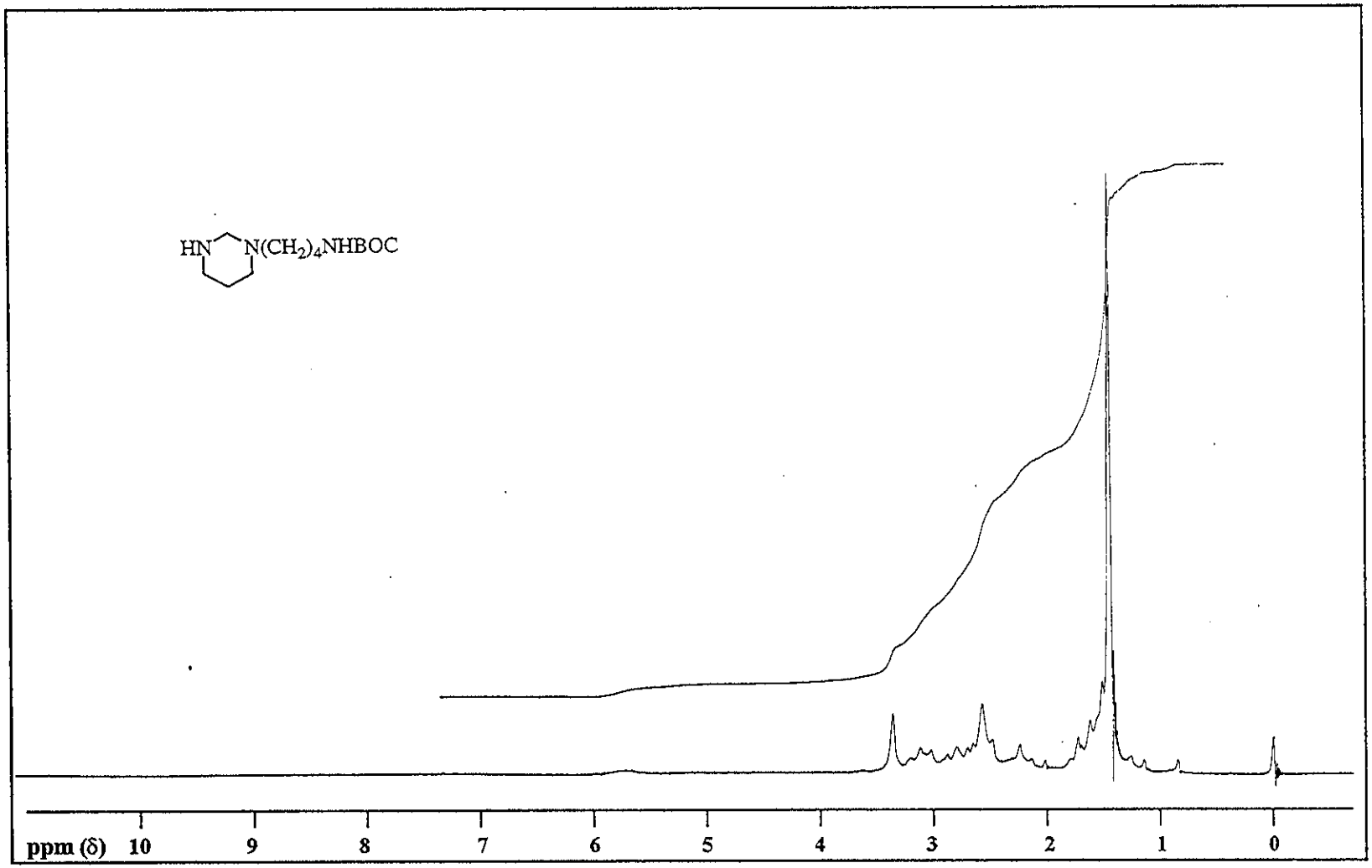


Figure 49. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of N^{δ}-(*tert*-butoxycarbonyl) hexahydropyrimidine (38)

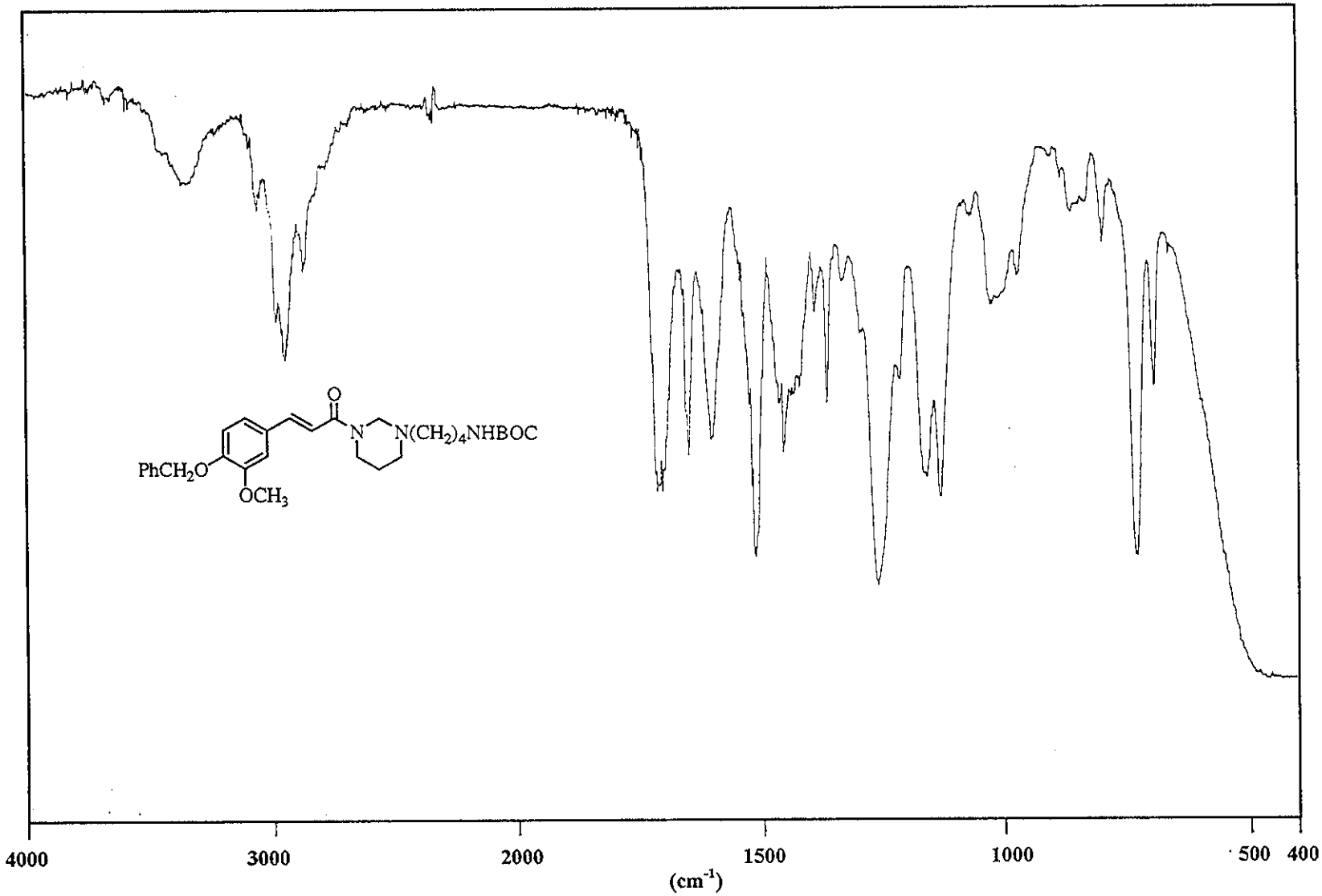


Figure 50. IR (film) spectrum of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) hexahydropyrimidine (178)

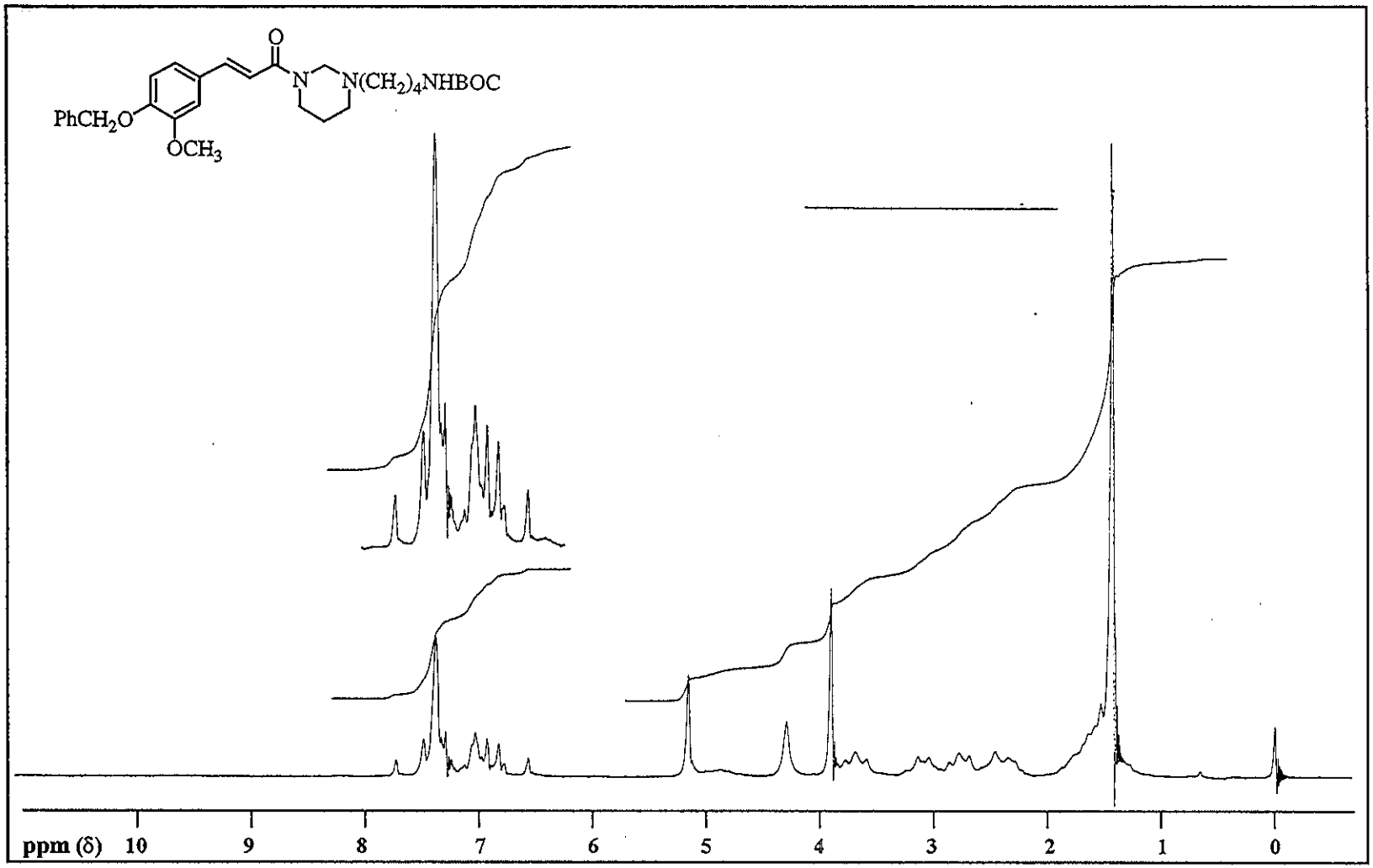


Figure 51. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of N^{1}-(4-benzyloxy-3-methoxycinnamoyl)-N^{8}-(*tert*-butoxycarbonyl) hexahydropyrimidine (178)

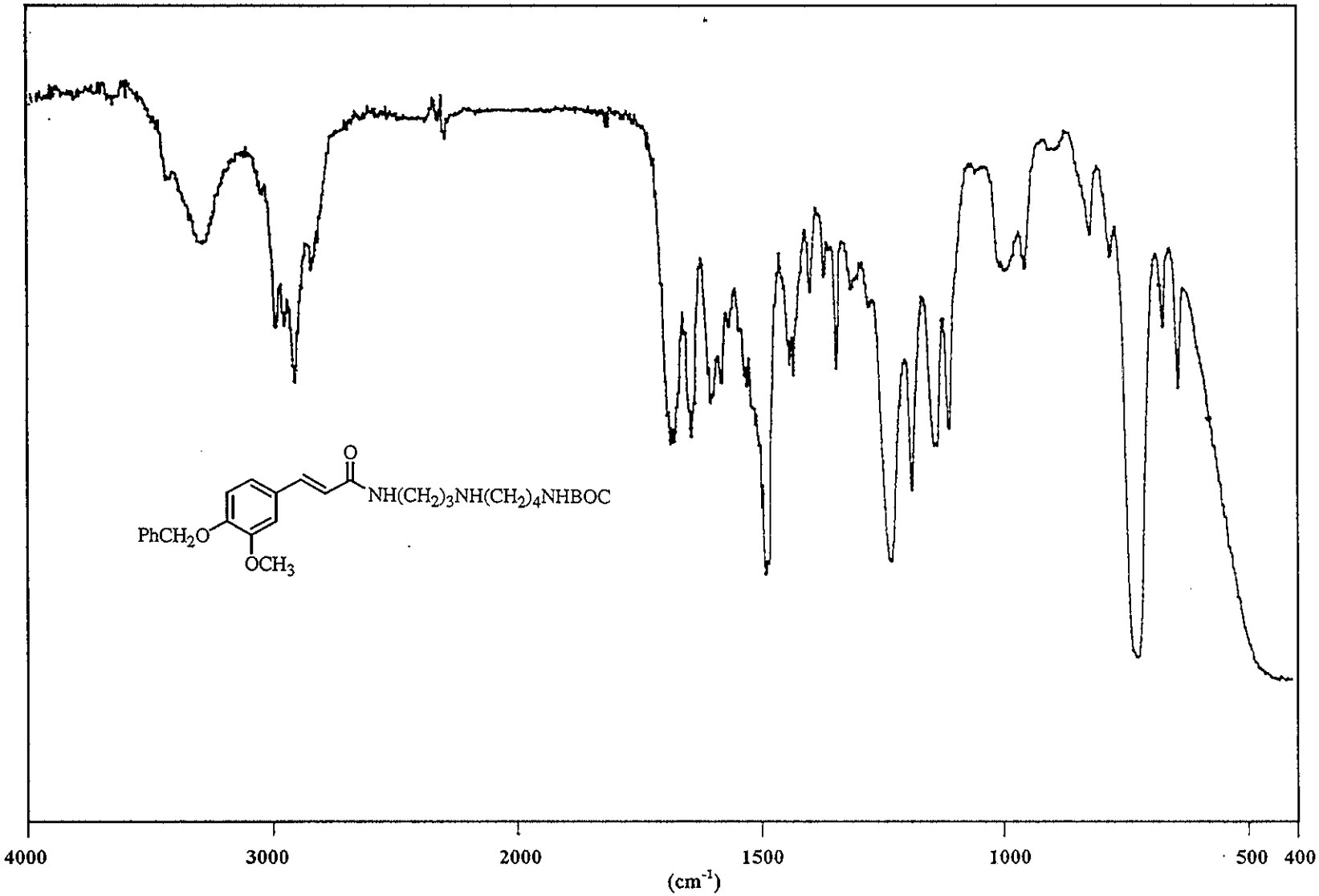


Figure 52. IR (film) spectrum of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179)

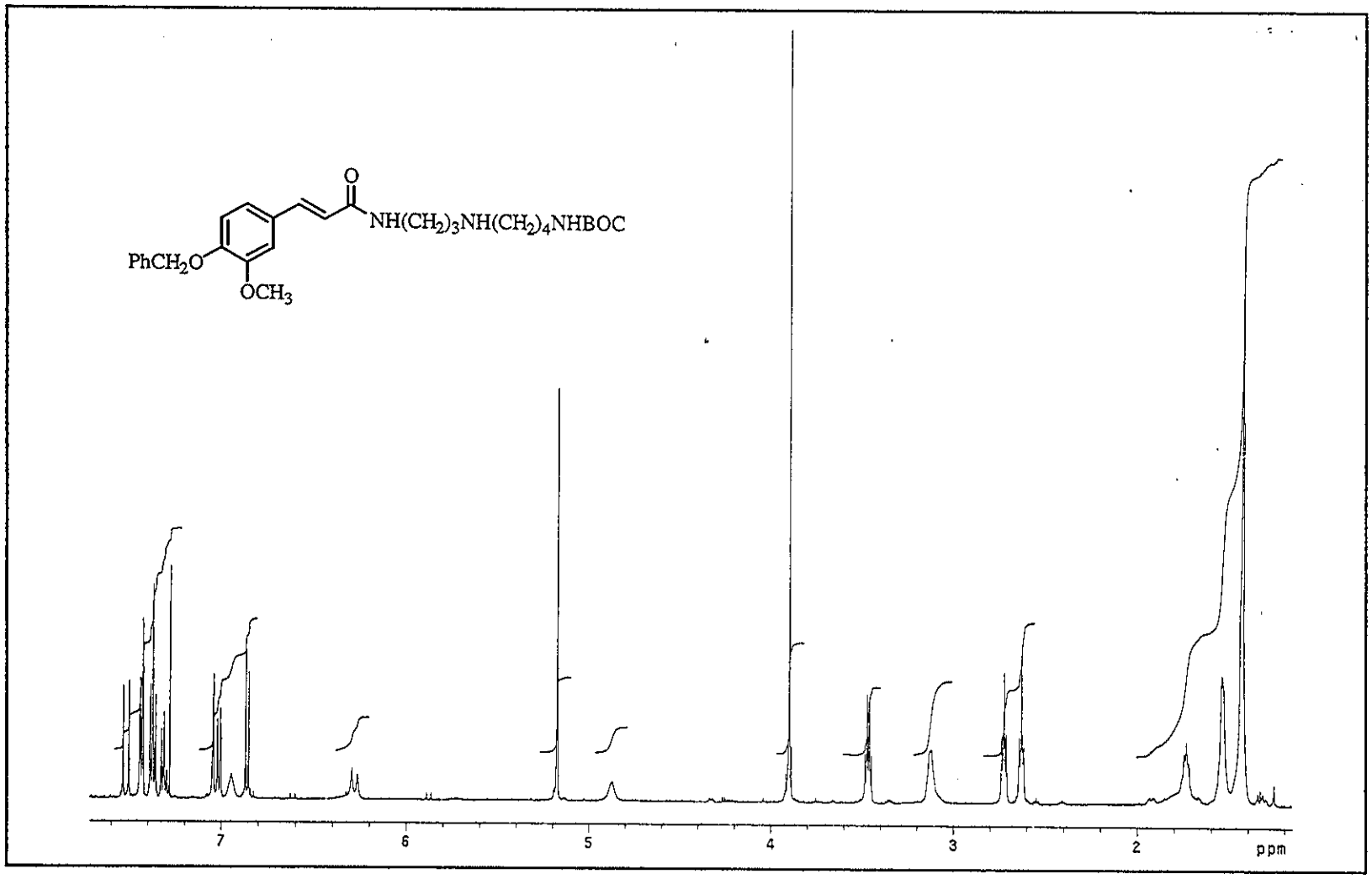


Figure 53. ^{1}H NMR ($CDCl_3$, 500 MHz) spectrum of N^{1}-(4-benzyloxy-3-methoxycinnamoyl)-N^{8}-(*tert*-butoxycarbonyl) spermidine (179)

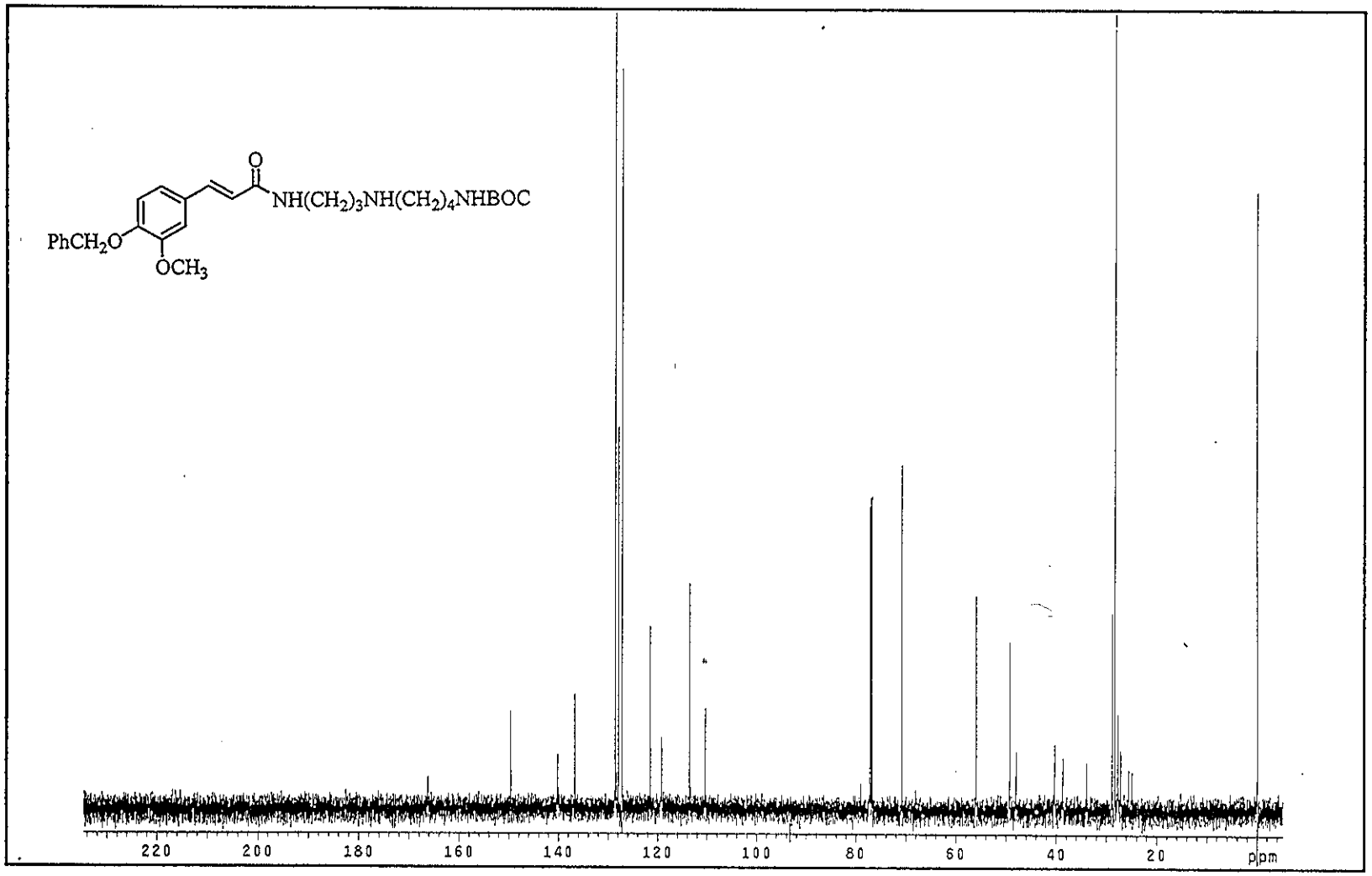


Figure 54. ^{13}C NMR ($CDCl_3$, 125 MHz) spectrum of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179)

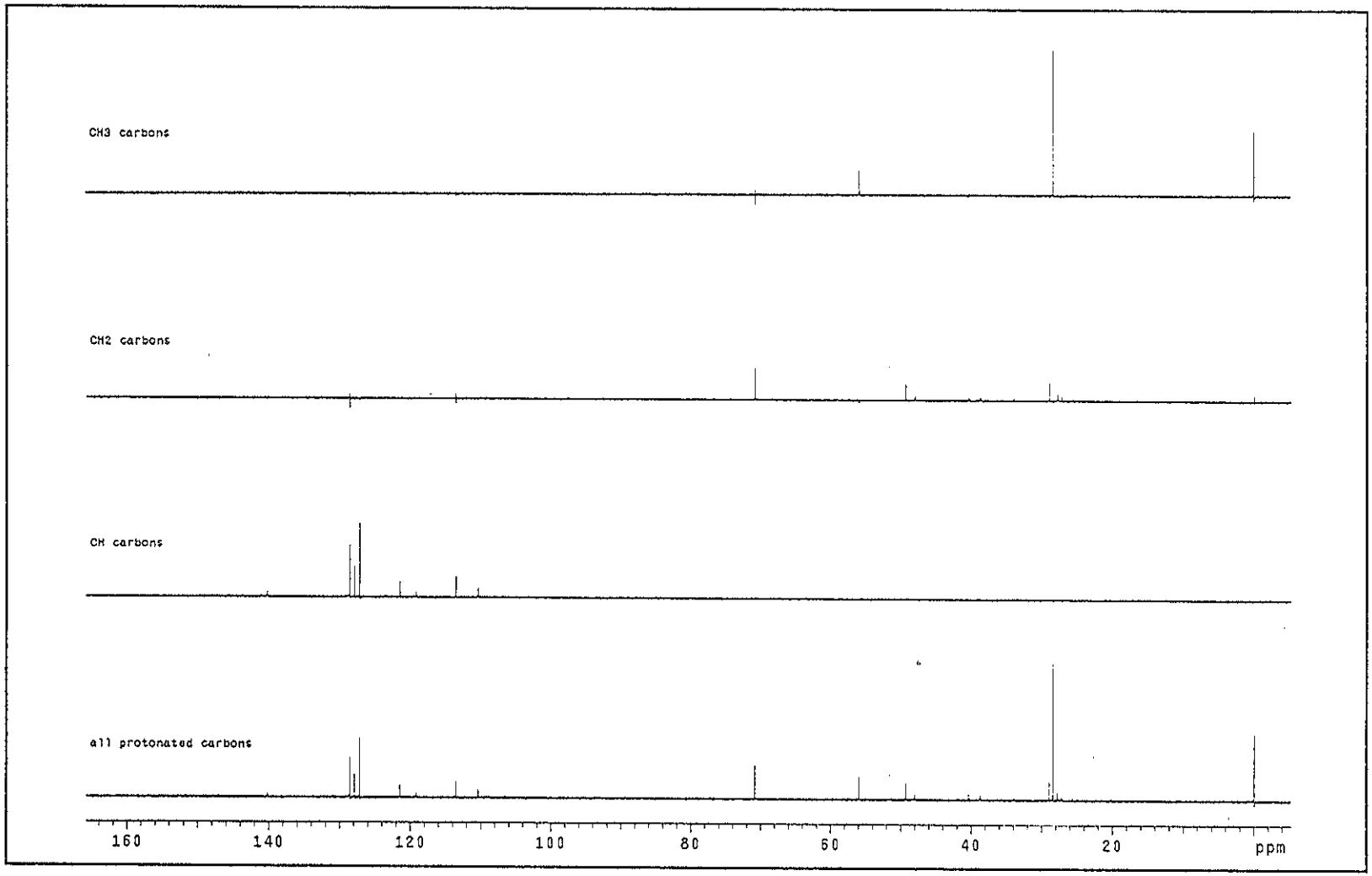


Figure 55. DEPT spectrum of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179)

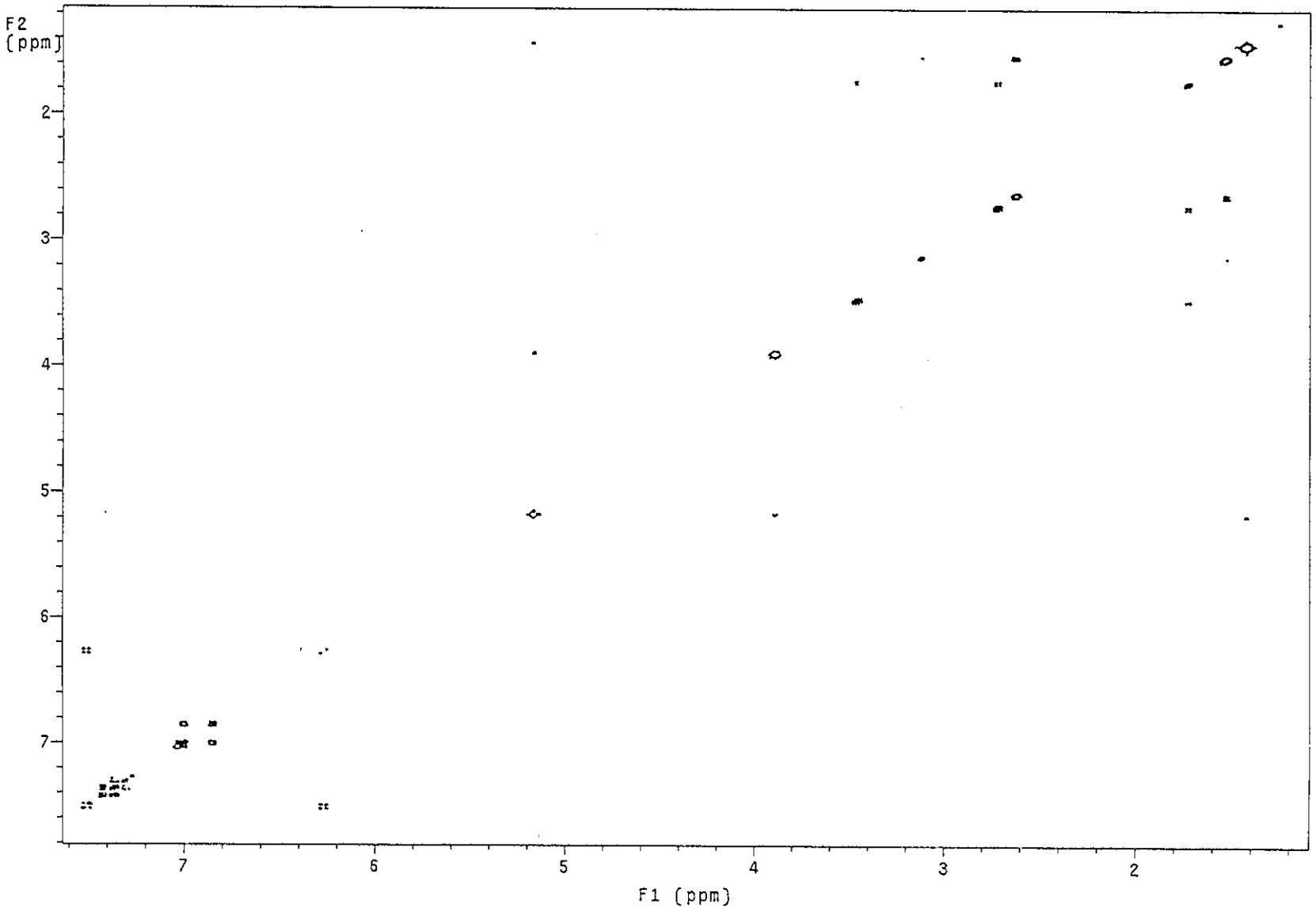


Figure 56. ^{1}H-^{1}HCOSY spectrum of N^{1}-(4-benzyloxy-3-methoxycinnamoyl)-N^{8}-(*tert*-butoxycarbonyl) spermidine (179)

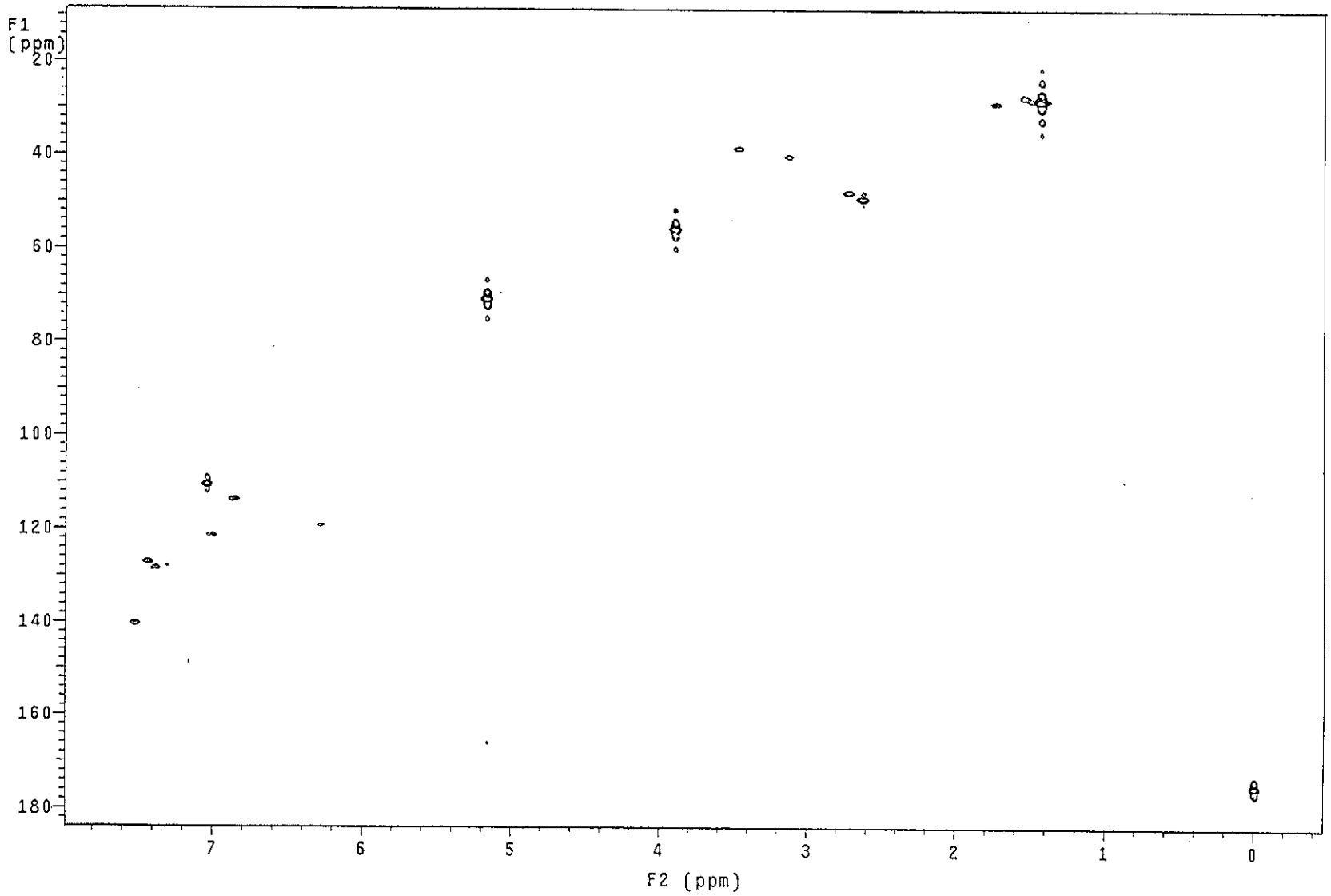


Figure 57. HMQC spectrum of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179)

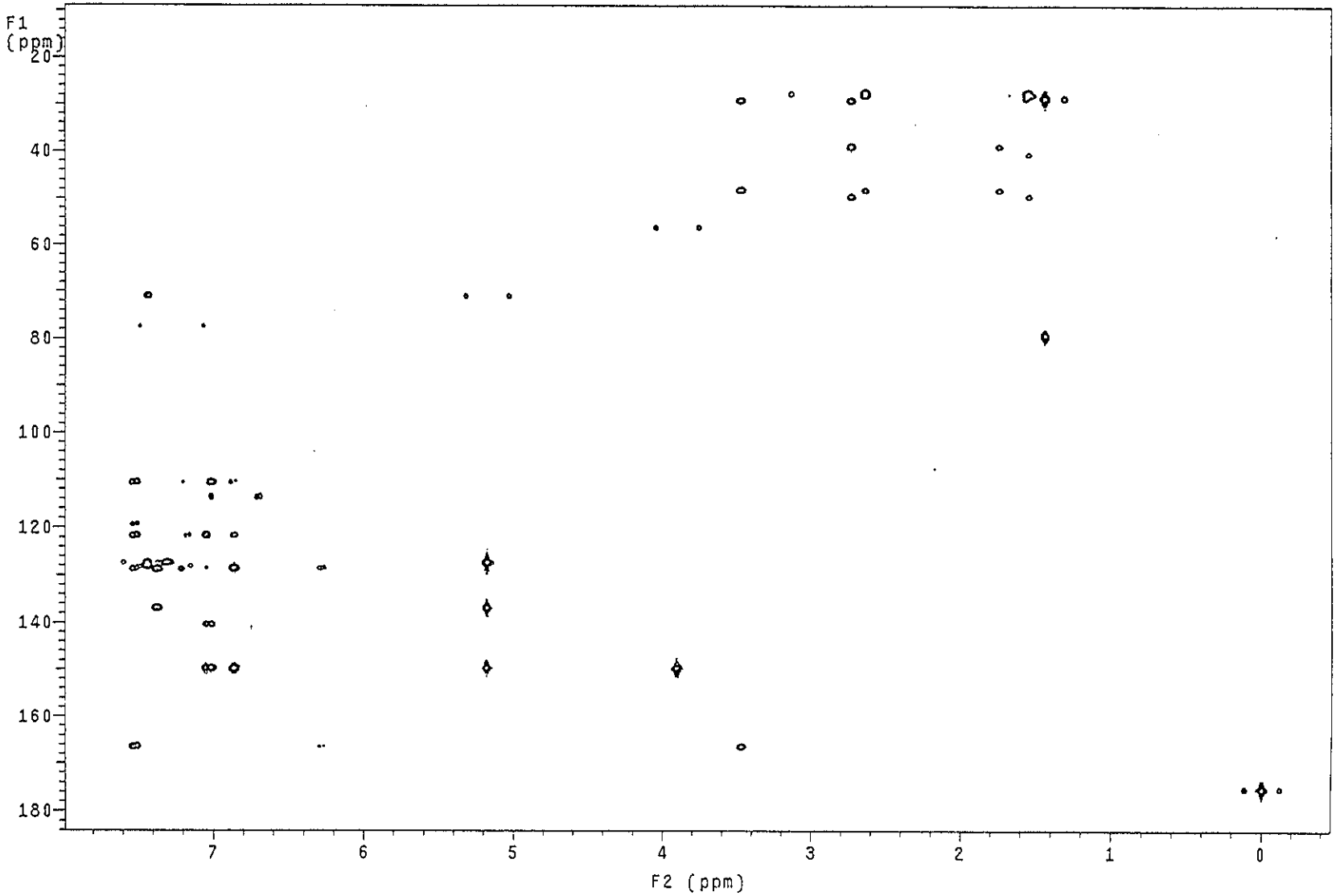


Figure 58. HMBC spectrum of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179)

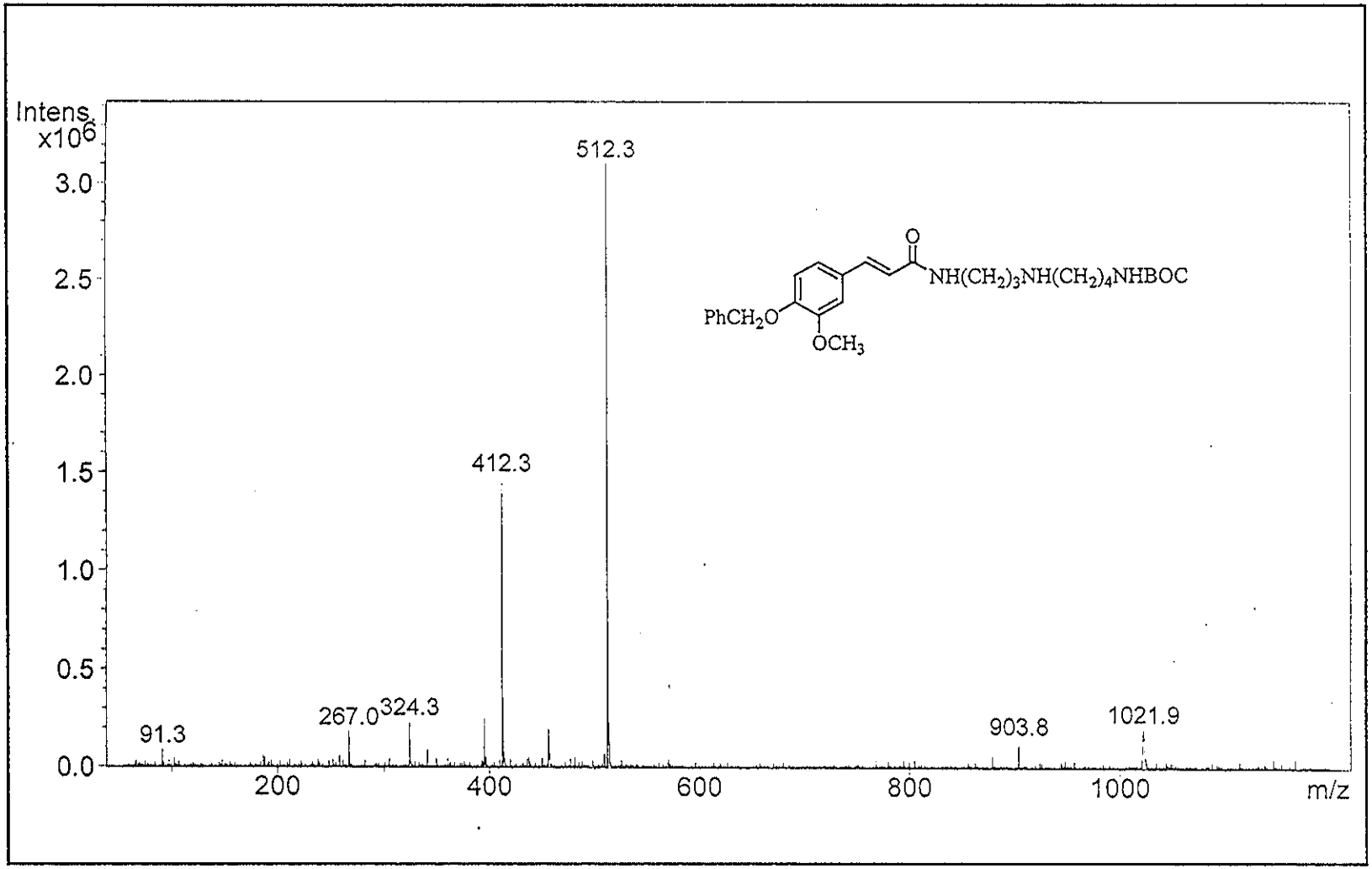


Figure 59. Mass spectrum (LC-MS) of N^1-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (179)

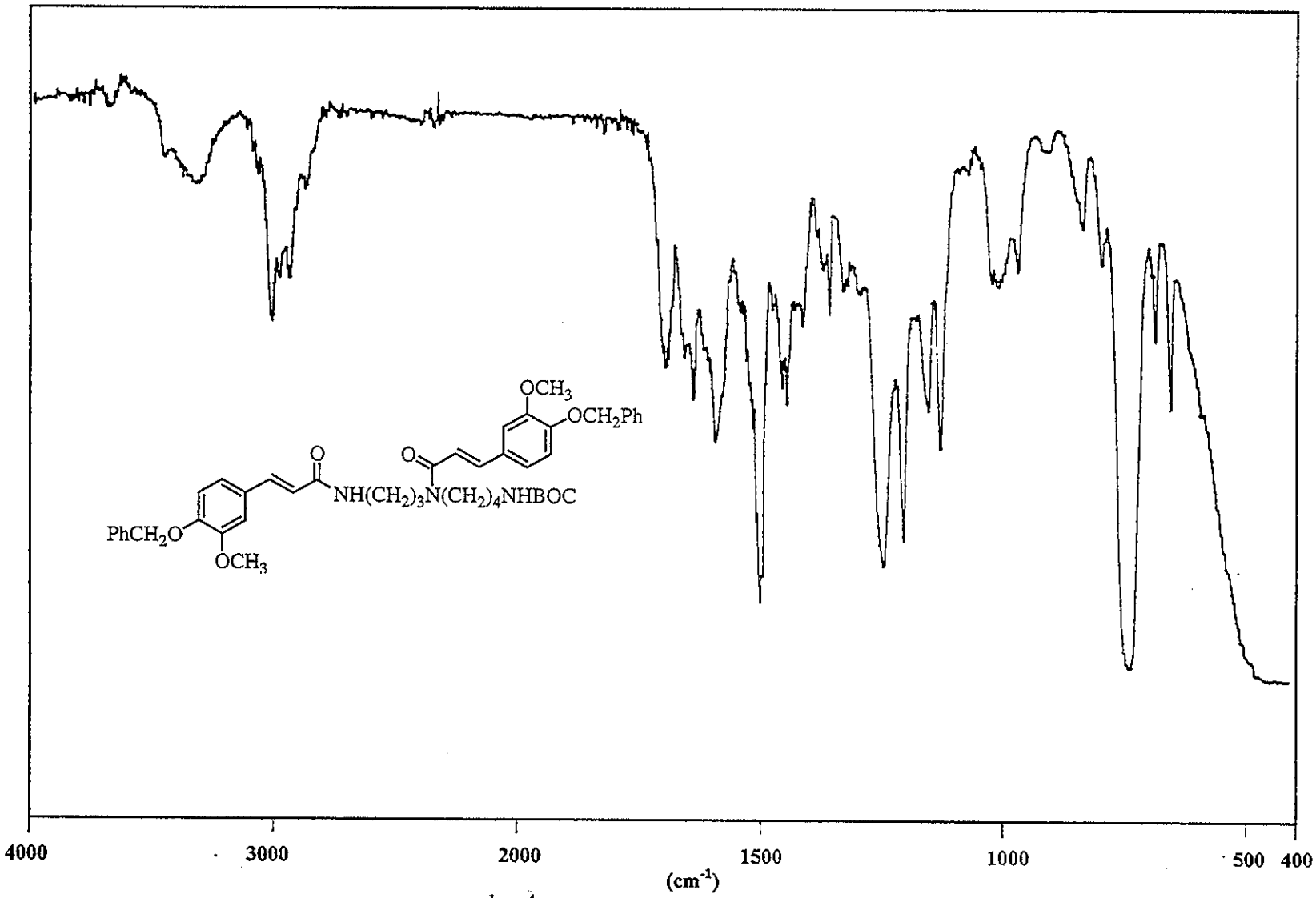


Figure 60. IR (film) spectrum of N^1,N^4-di-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170)

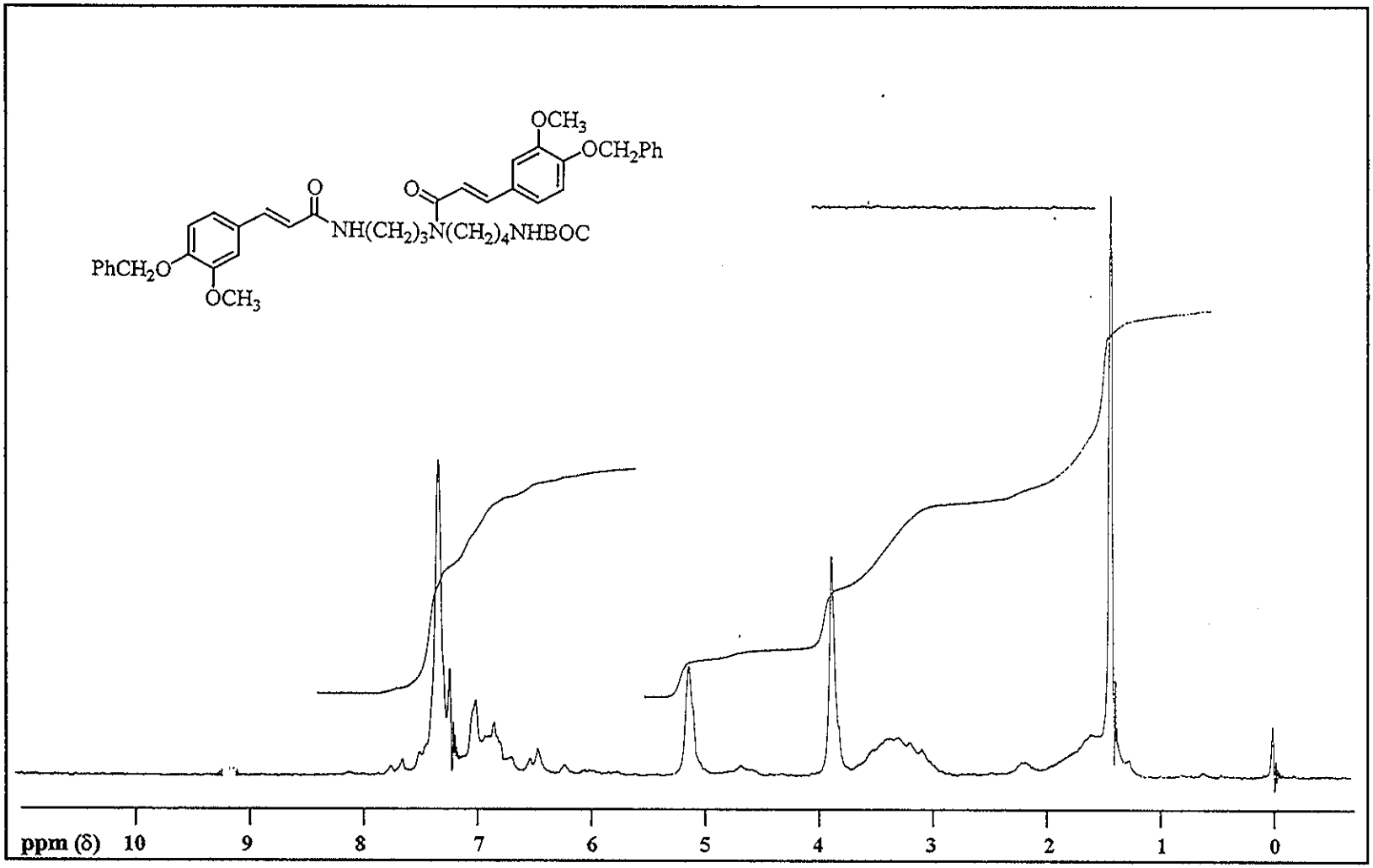


Figure 61. ^{1}H NMR ($CDCl_3$, 60 MHz) spectrum of N^1,N^4-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170)

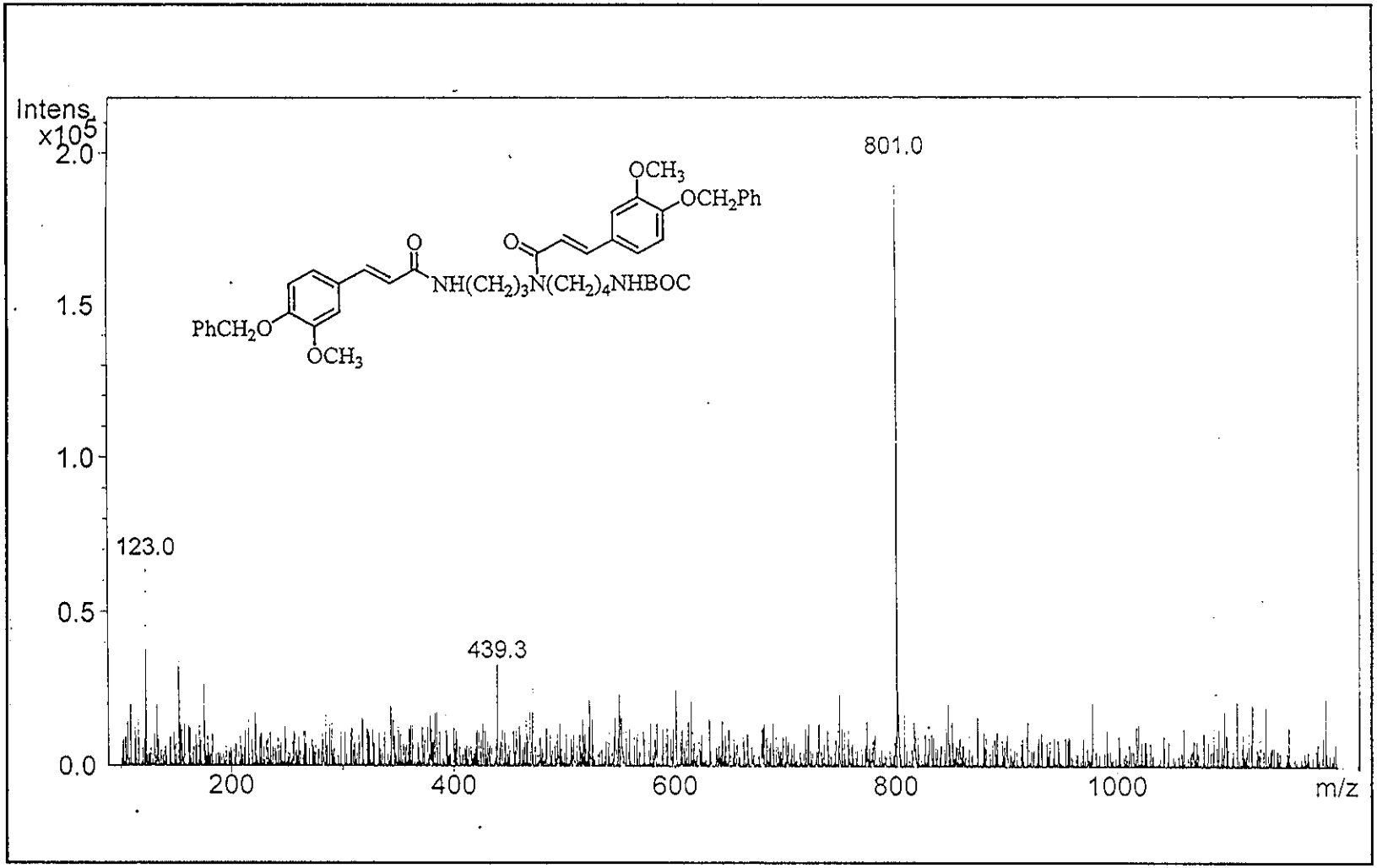


Figure 62. Mass spectrum (LC-MS) of N^1,N^4-(4-benzyloxy-3-methoxycinnamoyl)-N^8-(*tert*-butoxycarbonyl) spermidine (170)

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