Université de Montréal

Alkyl-Branched Indolizidinone Amino Acids as Scaffolds for Solid-Phase Synthesis of Peptide Hormone Mimics

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Abstract

Methodology was developed for synthesizing chemical libraries for discovering new peptide hormone receptor ligands. In particular, a combinatorial strategy was used for library synthesis of neruokinin and somatostatin receptor ligand candidates. This strategy was based on the design and synthesis of indolizidin-2-one amino acid scaffolds and their subsequent incorporation into a series of amido amides (**88-140**) using a novel solid-phase approach.

Dipeptide mimic 7-benzylindolizidinone amino acid **48** was synthesized on a gram scale by a literature procedure and then introduced into a library of candidates (**88-140**) to discover new somatostatin and neruokinin receptor ligands. A combinatorial strategy was employed for their synthesis which featured oxime resin as solid support. 7-Benzylindolizidinone *N*-(BOC) amino acid **48** was coupled to oxime resin. The library members were then obtained by a sequence featuring deprotection of the BOC group, free basing and coupling to the amine with 5 different carboxylic acids, followed by displacement with 9 different primary amines to give amido amide targets.

A second scaffold was conceived and synthesized in the second part of the thesis. Enantiopure *N*-(BOC)amino-7-[3-azidopropyl]indolizidin-2-one acid **141** has been synthesized by displacement of the methanesulfonate of its 7-hydroxypropyl counterpart **151** with sodium azide and subsequent ester hydrolysis. *N*-(BOC)Amino-7-[3-hydroxypropyl]indolizidin-2-one ester **151** was obtained from a sequence commencing with the alkylation of (2*S*,8*S*)-di-*tert*-butyl 5-oxo-2,8-di-[*N*-(PhF)amino]azelate **75** (PhF = 9-(9-phenylfluorenyl)). Stereoselective allylation of **75**, regioselective olefin hydroboration, selective primary alcohol protection as a silyl ether and oxidation of the secondary alcohol gave (2*S*,4*R*,8*S*)-di-*tert*-butyl 4-[3-*tert*-butyldimethylsiloxypropyl]-5-oxo-2,8-di-

[*N*-(PhF)amino]azelate **149** as a pure diastereomer in 33% overall yield. Linear ketone **149** was then converted into the indolizidinone heterocycle by a route featuring reductive amination, lactam cyclization and isolation by way of a silyl ether which provided the (6*S*,7*R*)-isomer of **151**. Enantiopure *N*-(BOC)amino-7-(3-azidopropyl)indolizidin-2-one acid **141** was also achieved by converted **151** to its respective methanesufonate followed by displacement with sodium azide.

In summary, this thesis provides first an introduction into the importance of the peptide hormones Substance P and somatostatin. The combinatorial strategy is then illustrated by the introduction of 7-benzylindolizidin-2-one **48** into a focused library. Finally, methodology is presented for preparing alternative 7-alkylindolizidin-2-one amino acid scaffolds.

SOMMAIRE

Une méthodologie a été développée pour la synthèse d'une librairie de molécules dans le but de mettre au point de nouveaux ligands pour les récepteurs d'hormones peptidiques. En particulier, une stratégie combinatoire a été utilisée dans la synthèse d'une librairie de ligands potentiels aux récepteurs de la neurokinine et de la somatostatine. Cette stratégie est basée sur le design et la synthèse des acides aminés indolizidin-2-one comme squelettes et leur incorporation dans une série d'amido amides utilisant une nouvelle approche sur support solide.

Le peptidomimétique 7-benzylindolizidinone **48** a été synthétisé sur une échelle multigramme selon un protocole de la littérature et a ensuite été incorporé dans une librairie de ligands potentiels aux récepteurs de la somatostatine et de la neurokinine. L'approche combinatoire utilisée pour la synthèse fait appel à la résine d'oxime comme support solide. L'acide aminé 7-benzylindolizidinone *N*-(BOC) **48** a été couplé à la résine. La librairie moléculaire a ensuite été obtenue par la déprotection du groupe BOC, l'obtention de la base libre et le couplage de l'amine avec cinq différents acides carboxyliques, suivi du déplacement avec neuf amines primaires différentes pour donner les amido amides ciblés.

Un deuxième squelette a été conçu et synthétisé dans la deuxième partie du projet. L'acide *N*-(BOC)amino-7-[3-azidopropyl]indolizidin-2-one énantiopur **141** a été synthétisé par le déplacement du méthanesulfonate du 7-hydroxypropyl **151** avec l'azoture de sodium suivi de l'hydrolyse de l'ester. L'ester *N*-(BOC)amino-7-[3-hydroxypropyl]indolizidin-2-one **151** fût obtenu par une séquence de réactions commençant par l'alkylation du (2*S*, 8*S*)-di-*tert*-butyl-5-oxo-2,8-di-[*N*-(PhF)amino]azélate **75** (PhF = 9-(9-phénylfluorényl)). L'allylation stéréosélective de **75** , suivi de l'hydroboration régiosélective de l'oléfine, de la

protection sélective de l'alcool primaire sous forme d'éther silylé puis de l'oxidation de l'alcool secondaire a donné le (2*S*, 4*R*, 8*S*)-di-*tert*-butyl-4-[3-*tert*-butyldiméthylsiloxypropyl]-5-oxo-2,8-di-[*N*-(PhF)amino]azélate **149** comme seul diastéréoisomère dans un rendement global de 33%. La transformation de la cétone **149** en hétérocycle indolizidinone a par la suite été réalisée par une amination réductive, une cyclisation à la lactame et l'isolation sous forme d'éther silylé pour donner l'isomère (6*S*, 7*R*) de **151**. L'acide *N*-(BOC)amino-7-[3-azidopropyl]indolizidin-2-one **141** énantiopure a aussi été synthétisé par la conversion de **151** au méthanesulfonate correspondant suivi du déplacement avec l'azoture de sodium.

En résumé, ce mémoire donne premièrement une introduction à l'importance des hormones peptidiques que sont la substance P et la somatostatine. La stratégie utilisée est ensuite illustrée par l'introduction du 7-benzylindolizidin-2-one **48** dans une librairie de molécules. Finalement, la méthodologie pour préparer différents acides aminés 7-alkylindolizidin-2-one est présentée.

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Abbreviations

[α]	Specific rotation
δ	Chemical shift
Ac	Acetyl
9-BBN	9-Borabicyclo[3.3.1]nonane
BOC	*tert*-butoxycarbonyl
COSY	Correlated spectroscopy
DCC	dicyclohexylcarbodiimide
DIC	diisopropylcarbodiimide
DIEA	Diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	*N,N*-dimethylformamide
EACNOx	ethyl-2-(hydroxyimino)-2-cyanoacetate
Et	Ethyl
Fmoc	9-(fluorenyl)methoxycarbonyl
HATU	*O*-(7-Azabenotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HMPA	Hexamethylphosphoramide
HOBt	1-hydroxybenzotriazole hydrate
HRMS	High resolution mass spectrum
Hz	Hertz
IR	Infrared spectroscopy
KHMDS	potassium bis(trimethylsil)amide
Ms	Mass spectroscopy
NaHMDS	sodium bis(trimethylsil)amide
NK	neurokinin
NOESY	Two-dimensional nuclear Overhauser effcet
PhF	9-(9-phenylfluorenyl)
PyBOP	benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluoro-phosphate

SP	substance P
TBAF	tetrabutylammoiumfluoride
TBDMS	*tert*-butyldimethylsil
TBTU	benzotriazolyl-1,1,3,3-tetramethylaminium tetraflouorborate
TCEP	tris(2-carboxyethyl)phosphine
TFA	trifluoroacetic acid
THF	tetrahydrofuran

CHAPTER 1

Introduction and Objectives

1. Introduction and Objectives

1.1 Substance P and Somatostatin

Peptide hormones are an important class of molecules in biochemistry, medicinal chemistry and physiology, because of their activity and distribution in the central and peripheral nervous systems. Two representative examples of biologically relevant peptides hormones are substance P and somatostatin. Recently discovered, these peptide hormones have stimulated an intense effort to understand their physiology and to harness their activity for medicinal applications.

Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	**1**
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2	**2**
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2	**3**

Figure 1. Mammalian Tachykinins Sequences

Substance P was isolated from equine brain and intestine[1] and shown to elicit smooth muscle contractions and to lower blood pressure.[2] Substance P is an endogenous undecapeptide belonging to the neurokinin family of mammalian peptides (Figure 1).[3] Three neurokinin receptor subtypes have been characterized: NK-1, NK-2 and NK-3 receptors which prefer respectively the agonists: substance P (SP) **1**, neurokinin A (NKA) **2** and neurokinin B (NKB) **3**. The neurokinins share a common C-terminal sequence consisting of Phe-Xaa-Gly-Leu-Met-NH_2 (Xaa = Phe, Tyr, Ile, or Val).[4] Phenylalanine is conserved at the beginning of this sequence in all neurokinins (position 7 in SP) and appears to be an essential residue, probably involved in receptor binding. Replacement of Phe^7 in SP by Ala, Leu or Ile decreased hormone potency by 50- to 500-fold.[5]

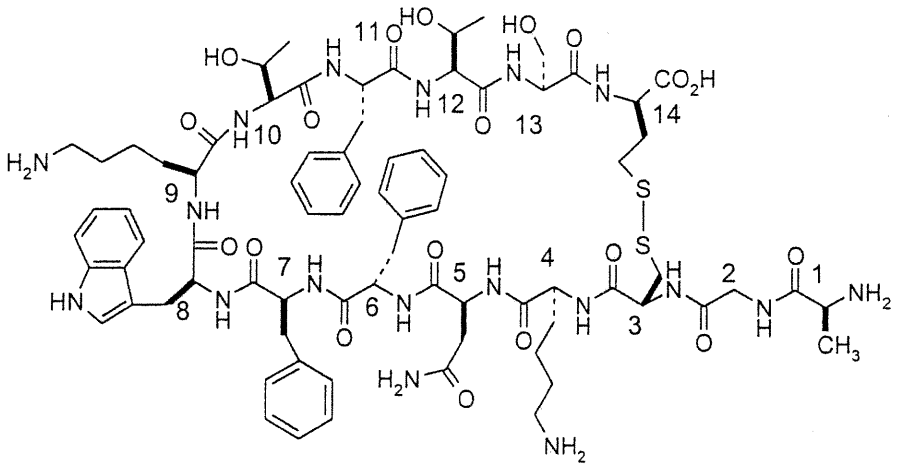

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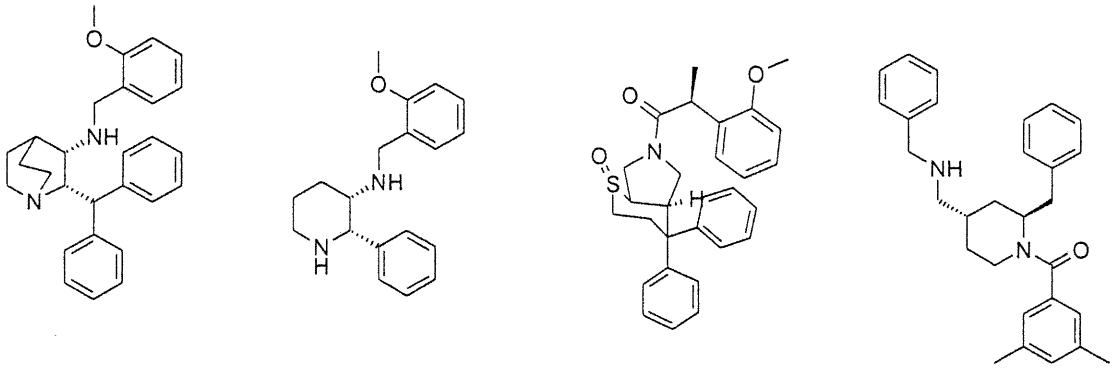
Figure 2. Somatostatin

Somatostatin (Somatotripin release inhibiting factor SRIF, **4**, Figure 2) is a cyclic tetradecapeptide first isolated form sheep hypothalami which exhibits a diverse range of physiological activities including the inhibition of secretion of hormones and digestive enzymes from the pituitary, pancreas and gastrointestinal tract.[6] In the central nervous system, somatostatin is widely distributed and functions as a neurotransmitter and neuromodulator that regulates neuronal firing.[7] Because somatostatin exhibits anti-proliferative effects *in vitro* and *in vivo*, it has been considered as a regulator of growth and development.[8]

In spite of their important biological activities, the application of these and other peptide hormones as drugs has been limited because of the rapid metabolism of their amide backbone. For example, the half-life of somatostatin is less than 3 min *in vivo*.[9] Furthermore, peptides tend to have poor bioavailability.[10] Finally, their conformational flexibility can manifest itself in non-selective effects at multiple receptors.[11] One method to increase metabolic stability involves replacement of L-amino acids by their D-enantiomers in the peptide chain.[12] In the case of somatostatin, for example, the replacement of L-Trp by D-Trp at the 8 position resulted in an increased metabolic stability.[10]

1.2 Neurokinin Antagonists

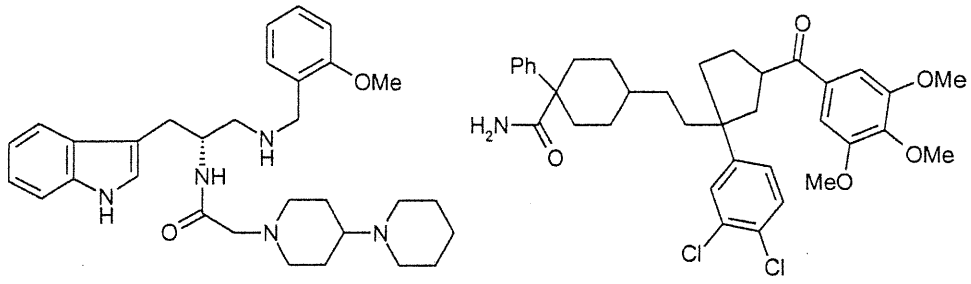
The availability of antagonists that specifically block the action of peptide hormones at their receptors is of crucial importance for the elucidation of their physiological function. Such antagonists may also possess desirable therapeutic properties. Two completely different ways have generally been used to develop peptide hormone antagonists. One has been based on random screening of non-peptide structures. The second approach has been to modify the structure of the peptide synthetically to yield a competitive antagonist. For example, insertion of D-amino acids at crucial positions in the sequence of substance P gave [D-Pro^2, D-Phe^7, D-Trp^9] substance P, a competitive antagonist of substance P.[13]



CP96,345 (**6**) CP99,994 (**7**) RP73,3467 (**8**) CGP 47,889 (**9**)

Figure 3. Nonpeptide NK-1 Antagonists

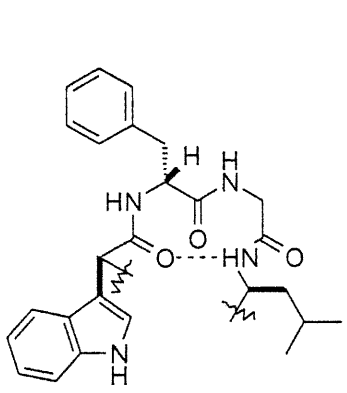
The first nonpeptide receptor antagonist selective for the NK-1 receptor, CP96,345 (**6,** Figure 3)[14] was discovered by random screening. Subsequent modification of **6** led to the discovery of CP99,994 (**7**)[15] which exhibited greater selectivity. Later, RP73,467 (**8**)[16] and CGP47,899 (**9**)[17] were also identified by random screening. All of these compounds have similar structural elements, such as two or more aromatic rings linked to a heterocyclic central core.



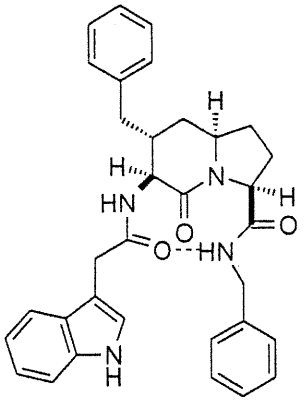
LY303,870 (**10**) MDL105,212 (**11**)

Figure 4. Selective Nonpeptide NK1 Antagonists

A tryptophan derived antagonist, LY303,870 (**10**, Figure 4)[18] was similarly identified and shown to have high affinity and selectivity for the NK-1 receptor, yet no ion channel activity. The class of compounds represented by MDL105,212 (**11**)[19] has attracted interest, because of their significant affinity for both the NK-1 and NK-2 receptors, as well as their potential to be converted to selective receptor antagonists in some cases.[20]



Partial structure of a cyclic hexapeptide SP agonist (Ref. 20)



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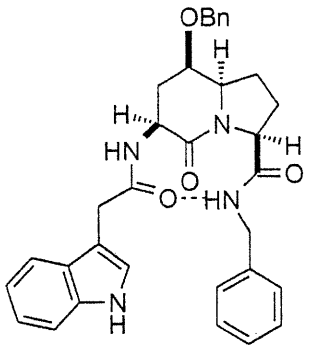

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Figure 5. Selective Nonpeptide NK-2 Antagonists

Conformational studies in solution of a potent cyclic hexapeptide agonist of substance P by NMR spectroscopy revealed information supporting a β-turn involving the Trp-Phe-Gly-Leu residues (Figure 5).[21] Based on this observation, Hanessian and coworkers designed and synthesized two indolizidinone analogues **12** and **13** that acted as antagonists at the NK-2 receptor.[22a,b] Although their activity was low (IC_{50} = 1-3 μM), they represented a new type of tachykinin receptor antagonist selective for the NK-2 receptor.

1.3. Peptide Somatostatin Agonists

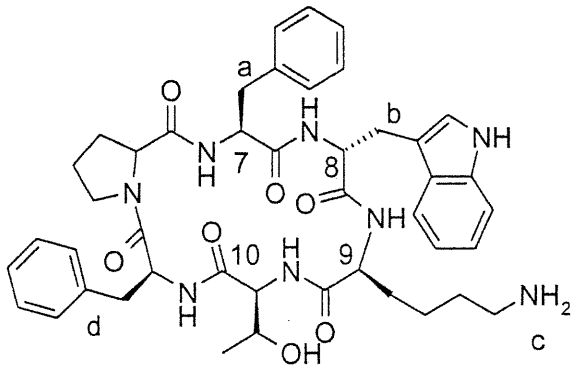


Figure 6. Somatostatin analogue L-363,301 (**14**), numbering corresponds to residues in native somatostatin

Towards the development of somatostatin analogues possessing an increased metabolic stability, a cyclic hexapeptide L-363,310 (**14**, Figure 6)[23] was synthesized that exhibited activity similar to the parent 14 amino acid peptide. The potency of L-363,301 demonstrated the importance of residues 7 to 10 and illustrated that the disulfide bridge served for organizing these amino acids for activity. Furthermore, the C-terminal amino acids were shown to be unnecessary for bioactivity. The conformation of **14** has been studied in solution by proton NMR spectroscopy.[24] Characteristics supposed to be important for the biological activity of **14** included:

1) The presence of a type VI β-turn centered at the Phe-Pro residue with a prolyl *cis* amide bond.

2) The presence of a type II ' β-turn centered at D-Trp^{8}-Lys^{9}.

3) A small distance between the D-Trp^{8} and Lys^{9} side-chains. The distances between labeled atoms in peptide **14** have been determined by computational analysis based on NMR studies in solution (Table 1).[25]

Table 1. Distances Between Labeled Atoms (Å) in L-363,301 (**14**, As Depicted in Figure 6)

a-b	a-c	a-d	b-c	b-d	c-d
7.1	11.3	9.2	7.3	9.2	14.1

Based on the structure of L-363,301, a series of related cyclic hexapeptides were synthesized possessing α- and β- methyl amino acids residues (Table 2).[26]

Table 2. Examples of α- and β-Methylated Somatostatin-Related Cyclic Hexapeptides

L-363,310 (**14**)	*c*[-Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-Phe^{11}-]	IC_{50} = 1 nM
15	*c*[-Pro^6-Phe^7-D-Trp^8-Lys^9-(*S*)-α-MeVal^{10}-Phe^{11}-]	IC_{50} = 1000 nM
16	*c*[-Pro^6-Phe^7-(2*R*,3*R*)-β-MeTrp^8-Lys^9-Thr^{10}-Phe^{11}-]	IC_{50} < 1 nM
17	*c*[-Pro^6-Phe^7-(2*R*,3*S*)-β-MeTrp^8-Lys^9-Thr^{10}-Phe^{11}-]	IC_{50} > 1000 nM
18	*c*[-Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-(2*R*,3*R*)-β-MePhe^{11}-]	IC_{50} = 50 nM
19	*c*[-Pro^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}-(2*R*,3*S*)-β-MePhe^{11}-]	IC_{50} > 1000 nM

The biological activity of the methylated analogues combined with their conformational analysis by NMR spectroscopy in solution were used to develop a refined model for the active conformation of somatostatin at its receptor. The design of -methylated residues were based on two considerations:

1) a topological effect of the β-methyl group which reduced the flexibility of the side chain to prefer a particular rotamer.

2) An effect of the chirality of the β-methyl center which may have different steric interactions at the receptor surface.

Both effects became clear in the results of the binding affinities of the analogues. Switching from 3*S*- to 3*R*-stereochemistry at β-MeTrp^8 reduced binding affinity more than 1000–fold (**16** to **17**). The modification from 3*S*- to *3R*-stereochemistry at β-MePhe^{11} reduced binding affinity more than 20– fold (**18** to **19**). The effects of side-chain methylation on structure were studied by NMR and modeling with the following results.[25a,b]

1) The side chains of Trp^8 and Lys^9 were shown to be close in the active analogue.

2) Active somatostatin analogues were suggested to bind at a receptor "pocket" through the side chains of Trp^8, Lys^9 and Phe^{11} arranged about a folded backbone conformation (Figure 7).

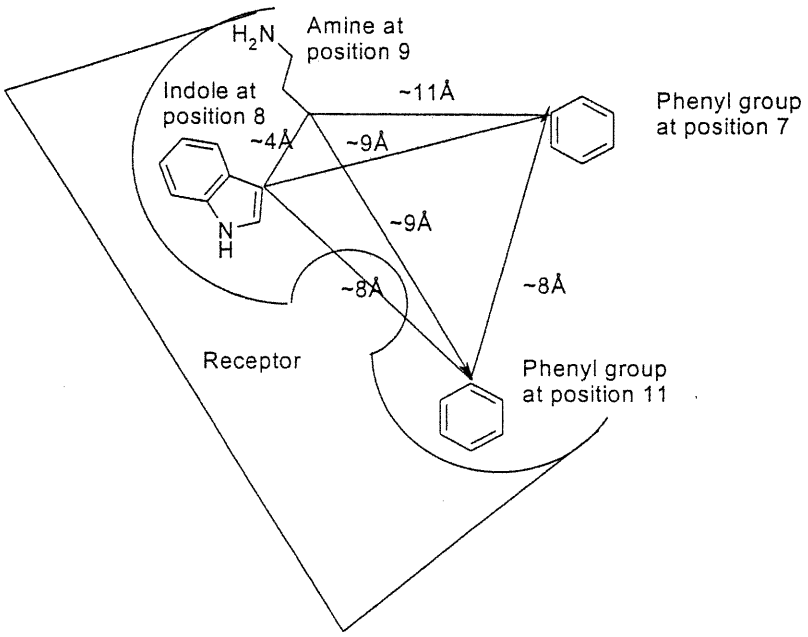
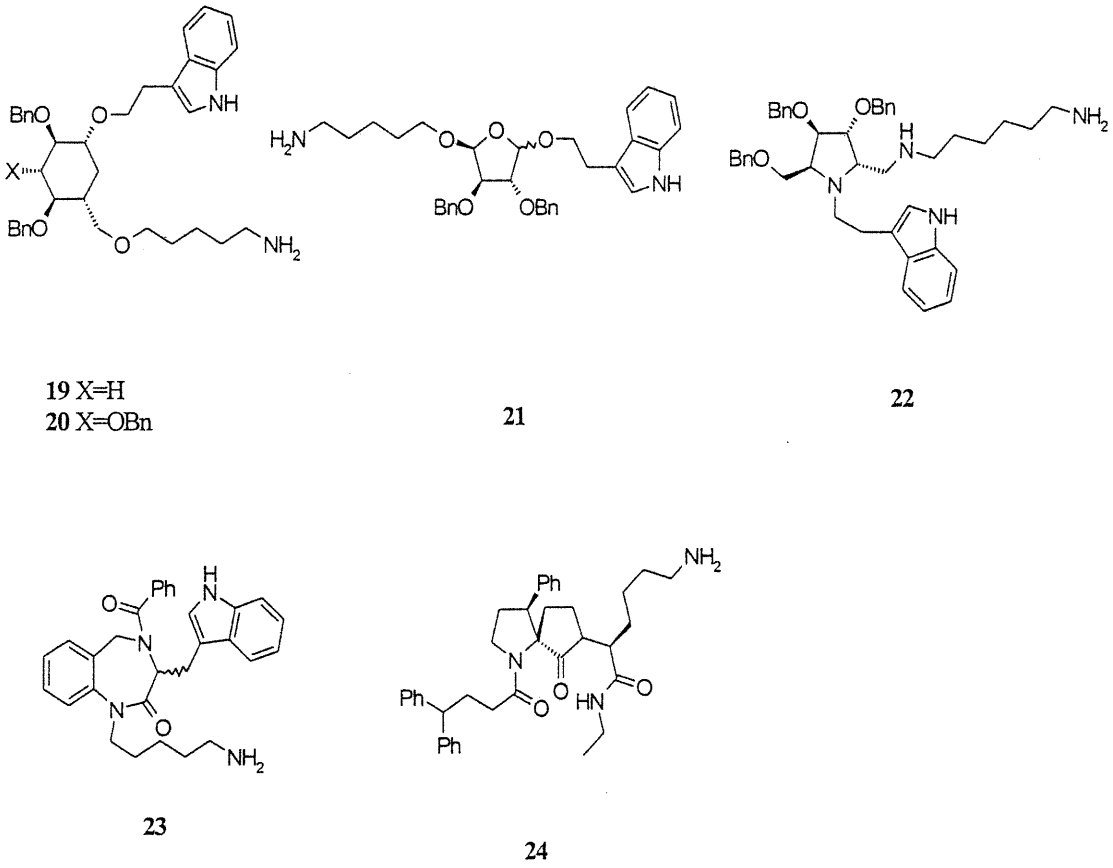


Figure 7. Model for Somatostatin Pharmacophores in the Active Receptor-Bound Conformation[25]

In the bioactive conformation, the distance between each functional group was predicted by analysis of the spectral data of L-363,301 (**14**) and its - and - methylated analogues (Figure 7). Analysis of the bioactive conformation **14** using methylated amino acid residues, NMR spectroscopy and computational analysis suggested the application of this approach for studying various peptide hormones.[25]

1.4 Nonpeptide Somatostatin Agonists



19 X=H
20 X=OBn

21

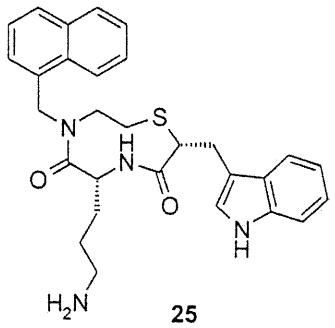
22

23

24

Figure 8. Nonpeptide Somatostatin Analogues

Among the nonpeptide somatostatin receptor ligands, sugar analogues were synthesized (Figure 8, **19** to **22**) based on a molecular modeling study suggesting a means to direct the Trp[8] and Lys[9] side chain in a β-turn-like orientation as in the active cyclic peptide conformation.[27] Compounds **21** (IC_{50} = 23 μM)[28] and **22** (IC_{50} = 14 μM)[29] showed potent agonist activity. Benzodiazepine **23** (IC_{50} = 7 μM, Figure 8) also exhibited weak agonist bioactivity.[30] A spirocyclic proline lactam **24** (IC_{50} = 11-22 μM, Figure 8) was designed as a β-turn peptide mimic and demonstrated weak agonist bioactivity.[31]



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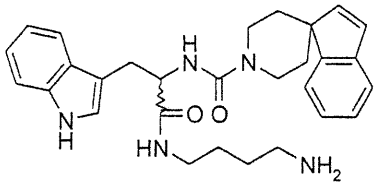

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Figure 9. Selective Nonpeptide Somatostatin Agonists Obtained from Screening of Chemical Libraries

A series of potent, subtype-selective small molecule somatostatin agonists have been discovered by screening chemical libraries. Compound **25** (Figure 9) was designed based on a β-turn structure having three side chains displayed on a central heterocyclic scaffold,[32] and exhibited agonist activity at the somatostatin receptor of subtype 5.

Based on molecular modeling of cyclic peptide somatostatin agonists, Merck group designed, synthesized and screened an approximately 130,000 compound chemical library.[33] Compound **26**, an original lead for a somatostatin subtype 2 receptor agonist, was an example from this library.

In summary, among the nonpeptide somatostatin agonists, structural features include multiple aromatic rings including heterocycles, as well as at least one primary amine side chain.

1.5 Objectives

Combinatorial chemistry has emerged as a powerful tool for the discovery of novel bioactive lead compounds.[34] Towards the discovery of new ligands for the somatostatin and neurokinin receptors, we designed a small library based on the structure of nonpeptide somatostatin agonists and nonpeptide neurokinin antagonists. The library members were composed of a 7-alkylindolizidinone amino acid which may serve as a β-turn mimic scaffold. Attachment of different pharmacophores onto this core structure by a combinatorial approach has been used to generate a series of candidate compounds that were examined for neruokinin- and somatostatin-like activities.

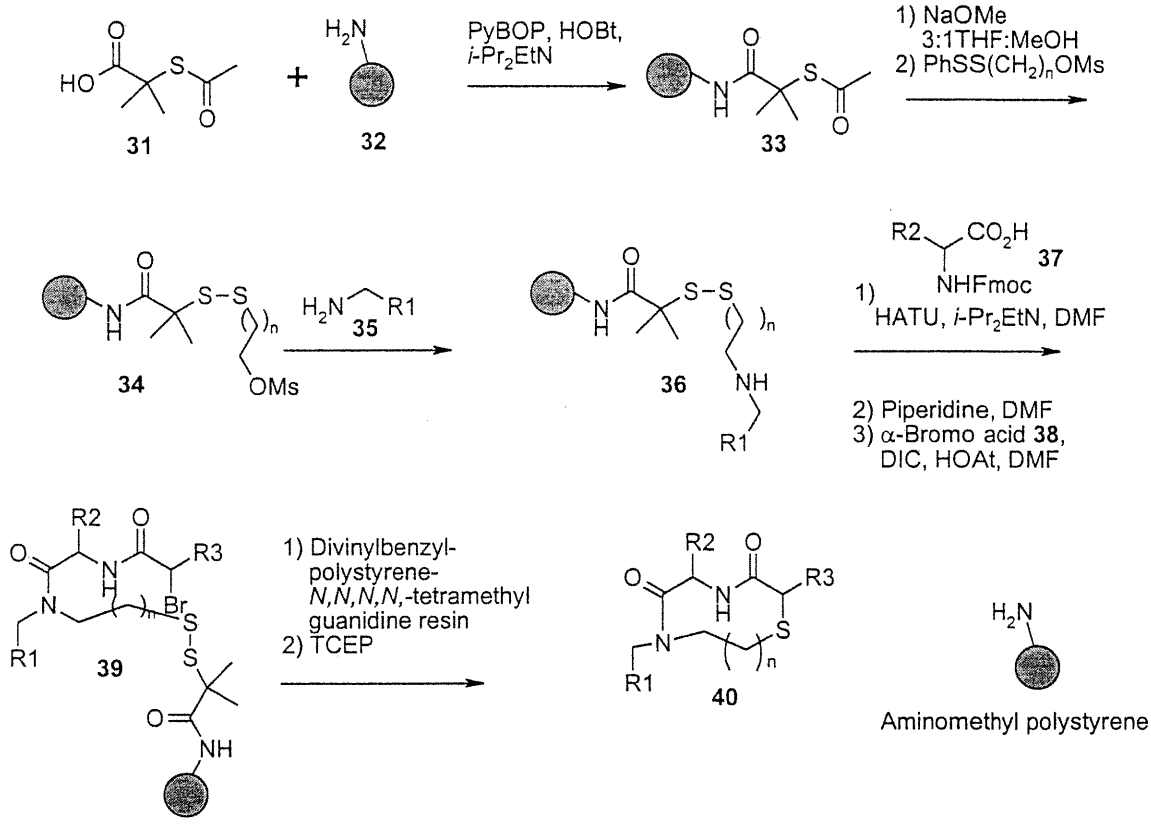
Chapter 2

Synthesis of 7-Benzylindolizidinone Amido Amide Library

2. Synthesis of 7-Benzylizidinone Amido Amide Library

2.1 Introduction

The rapid preparation of chemical libraries for drug discovery has been revolutionized by combinatorial synthesis techniques.[35]

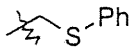
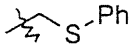
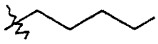
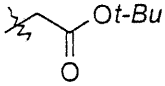
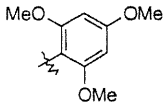
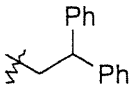
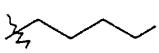
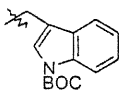
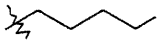
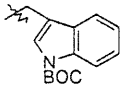


Scheme 1. Synthesis of Heterocycle Library

As mentioned in Chapter 1, combinatorial chemistry has been used to prepare libraries of ligands for the subtype-selective somatostatin receptor (Figure 9).[33] For example, a solid-phase method was developed to make 9-membered lactams of the type represented by structure **40** (Scheme 1). Intermolecular thioalkylation gave high yields in the macrocyclization without dimer formation. Linker **33** was first prepared

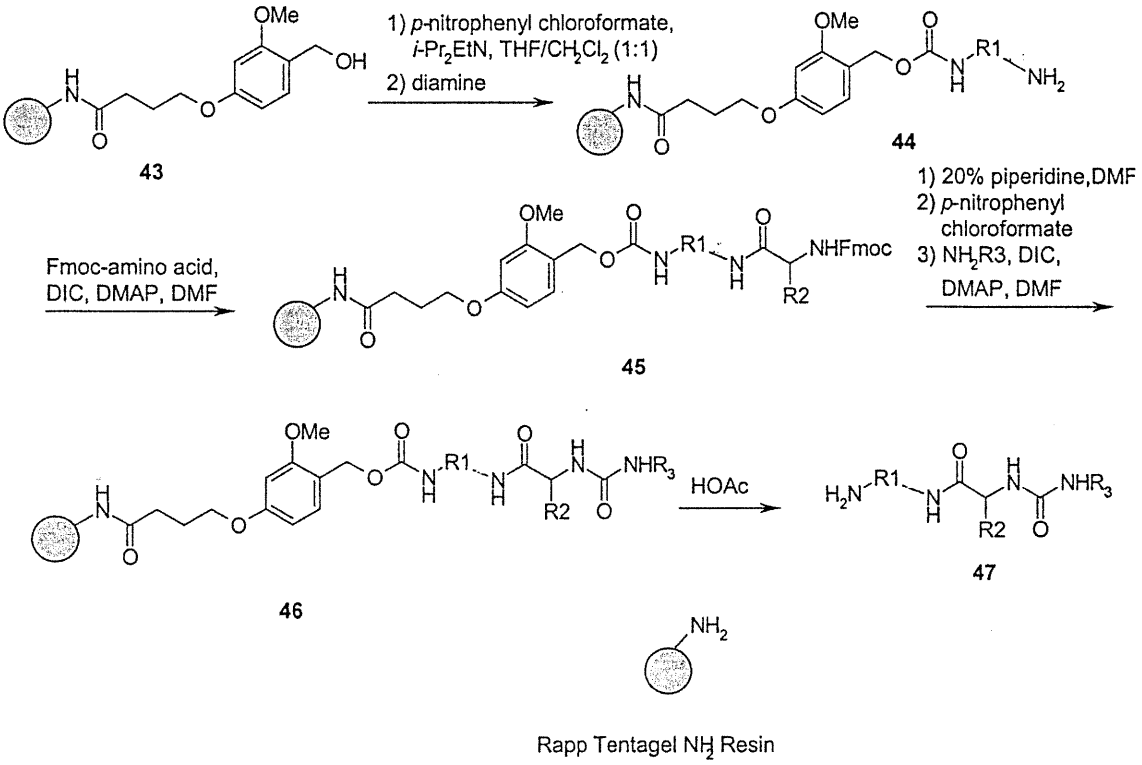
by coupling *S*-acetyl 2-mercapto 2-methylpropionic acid **31** to aminomethyl polystyrene **32** using benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluoro-phosphate (PyBOP), HOBt and DIEA in DMF at room temperature. Linker **33** was then treated with sodium methoxide in a 3:1 THF:MeOH solution under argon to remove the acetyl group, followed by benzothiazolyldisulfide mesylate [$PhSS(CH_2)_2OMs$] in dichloromethane for 12 h to provide disulfide **34**. The linear intermediate **41** was then prepared by treatment of **34** with different amines (**35**, Table 3) in DMF, followed by a series of peptide couplings. First *N*-(Fmoc)amino acids **37** were added to secondary amine **36** using HATU and DIEA in DMF. After Fmoc deprotection with 20% piperidine in DMF, various -bromo acids **38** were next coupled to the resin using diisopropylcarbodiimide (DIC), HOAt and DIEA in DMF to furnish **39**. The heterocycles **40** were then obtained on treatment of **39** with tris(2-carboxyethyl)phosphine (TCEP) to reduce the disulfide and *N,N,N,N*-tetramethylguanidine resin to affect intramolecular cyclization.

Table 3. Synthesis of Heterocycle Library

	R1	R2	R3	n	Purity (crude)	Yield (purified)
40a				1	92	60
40b				1	94	64
40c				1	86	56
40d				1	93	61
40e				2	94	51
40f				1	84	59
40g				1	93	59

Heterocycles **40(a-g)** were prepared to demonstrate the generality of the synthesis sequence. High levels of purity (84-89%) were observed for the crude products obtained directly from cleavage of the resin as determined by HPLC and proton NMR spectroscopy. Good yields (59-64% based on initial loading) of final product were obtained after purification (Table 3).

Another example in which a combinatorial approach was used for the discovery of nonpeptide subtype-selective somatostatin receptor ligands used a mix and split strategy.[36] A library was prepared in a set of 79 receptor wells each containing 20 diamines (NH_2R1NH_2) and 20 amino acids ($R2CHNH_2CO_2H$) to provide a total library composed of 20 X 20 X 79 or 31600 compounds (Scheme 2).



Scheme 2. Synthesis of Nonpeptide Subtype-Selective Somatostatin Receptor Ligands

For the synthesis of the library, HMPB resin **43**[37] was treated with *p*-nitrophenyl chloroformate and DIEA in a THF/CH_2Cl_2 (1:1) solution followed by different diamines in DMF to provide a series of carbamates **44**. The carbamates were mixed and split into 20 wells, that were treated with different Fmoc-amino acids, DIC and 3% DMAP in DMF to provide resin **45**. After mixing and splitting into 79 wells the Fomc groups were removed from resin **45** with 20% piperidine in DMF. Ureas **46** were then prepared by acylation with *p*-nitrophenyl chloroformate

and DIEA, followed by reaction with one of 79 amines. Final compound mixtures **47** were obtained after cleavage from the resin by glacial acetic acid. As mentioned in Chapter 1, compound **25** was found from this library and exhibited high activity at the somatostatin subtype 2 receptor (K_i = 200 nM, Figure 9).

2.2 Strategy for the Synthesis of 7-Benzylindolizidinone Amido Amide Library

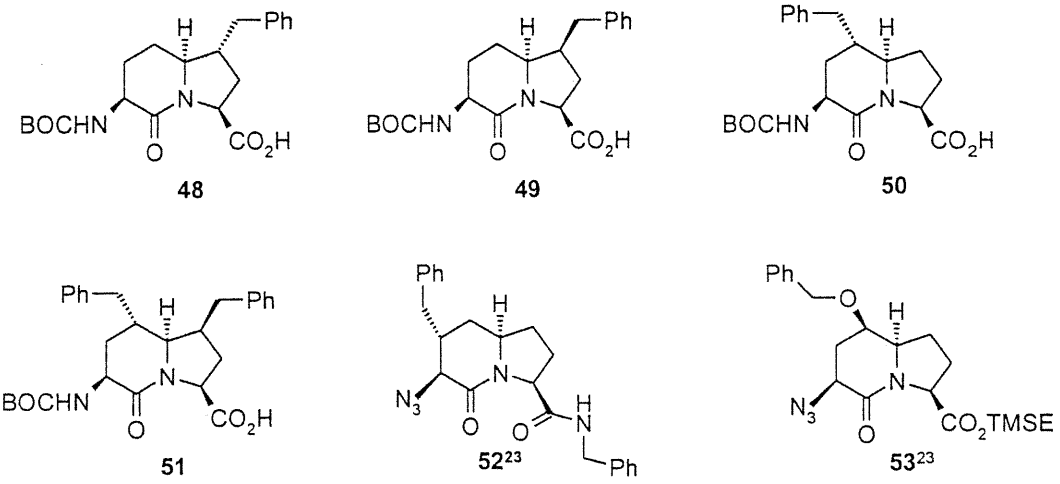
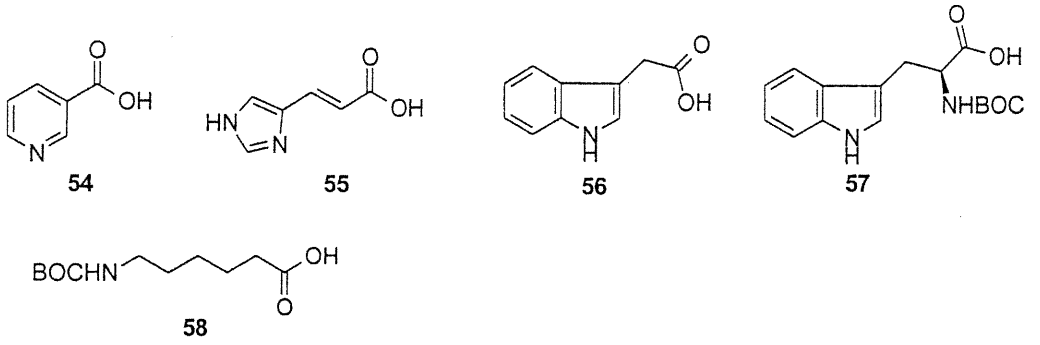


Figure 10. Benzylindolizidinone Scaffolds **48-51** and Neurokinin Receptor Ligands **52** and **53**

Considering the nonpeptide somatostatin agonists and neurokinin antagonists, we designed a small library using the (7*S*)-7-benzylindolizidinone *N*-(BOC)amino acid **48** as a scaffold. As mentioned in Chapter 1 benzylindolizidinone **52** and **53** have exhibited antagonist activity at the NK2 receptor. Furthermore, 5- and 7-benzyl indolizidin-2-ones **48-51** have been synthesized by a general strategy.[38] For our library synthesis, 7-benzylindolizidinone amino acid **48** (Figure 10) was selected as the central core for several reasons. The scaffold possessed a benzyl side-chain on the bicycle, as well as carboxylate and amino groups for subsequent modification by combinatorial chemistry. Indolizidinone **48** was the easiest analogue to synthesize in high yield among scaffolds **48-51**. The consequence of the 7-position substituent on the yields of our combinatorial approach was of general interest to explore. Finally, the activity of benzylindolizidin-2-one possessing a 7-position benzyl substituent had never been studied.

Electrophilic $R1CO_2H$



Nucleophilic H_2NR2

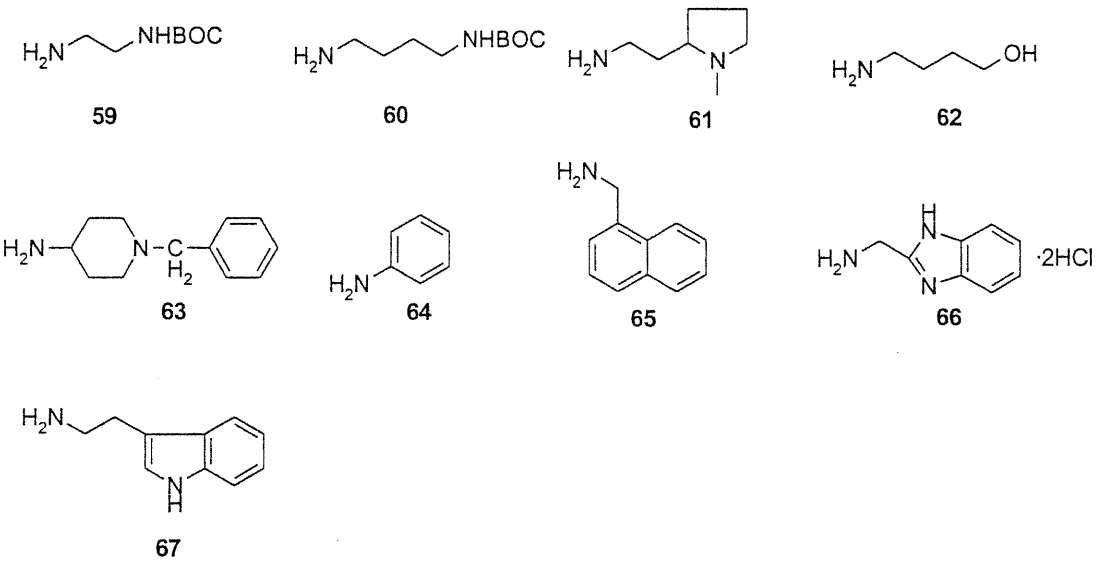
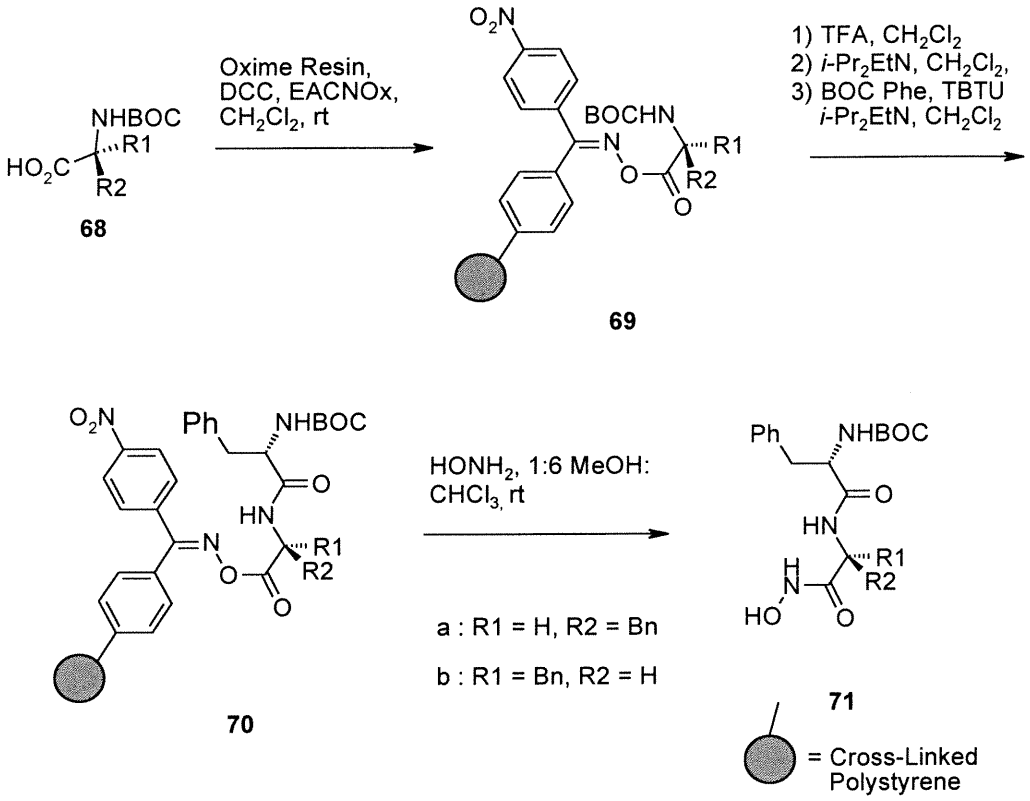


Figure 11. Electrophiles and Nucleophiles for Library

A library was prepared using 5 electrophilic and 9 nucleophilic reagents (Figure 11). Because somatostatin and neurokinin analogues have some similar structural elements such as aromatics side-chain and hetereocycles, the electrophilic reagents **54–57** and nucleophilic reagents **63-67** were selected to mimic these aromatic side-chain. The electrophilic reagents **58** and nucleophilic reagents **59-62** were mimics of the primary amine side-chain in somatostatin.

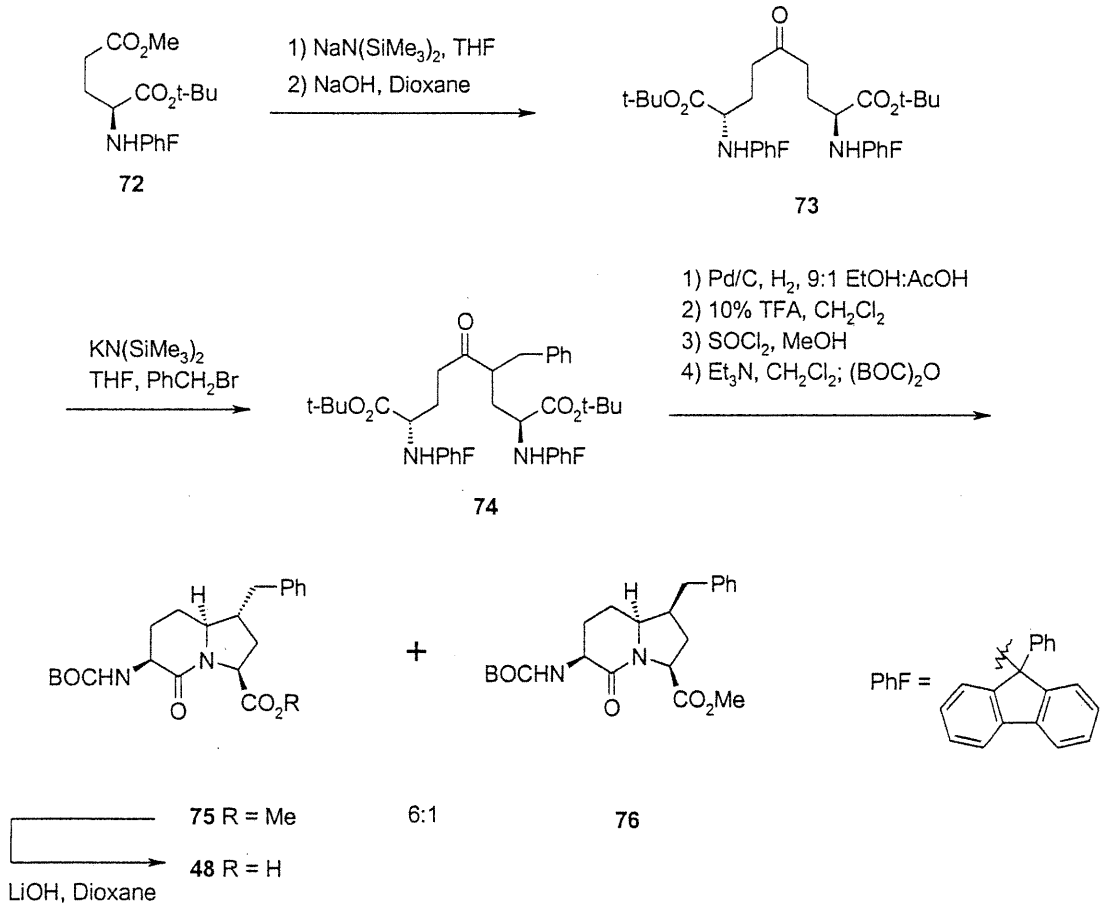
p-Nitrobenzophenone cross-linked polystyrene oxime resin was selected as the solid–phase because of its effective use in peptide synthesis and combinatorial chemistry.[39] For example, in the synthesis of somatostatin analogues **15-19** possessing α- and β- methylated amino acid residues, oxime resin was used for peptide elongation and macro-cyclization to afford the cyclic analogues.[25] In our lab, oxime resin has recently been used for the parallel synthesis of a series of hydroxamic acids (Scheme 3).[40]



Scheme 3. Synthesis of Hydroxamic Acid **71** using Oxime Resin as Solid-Support

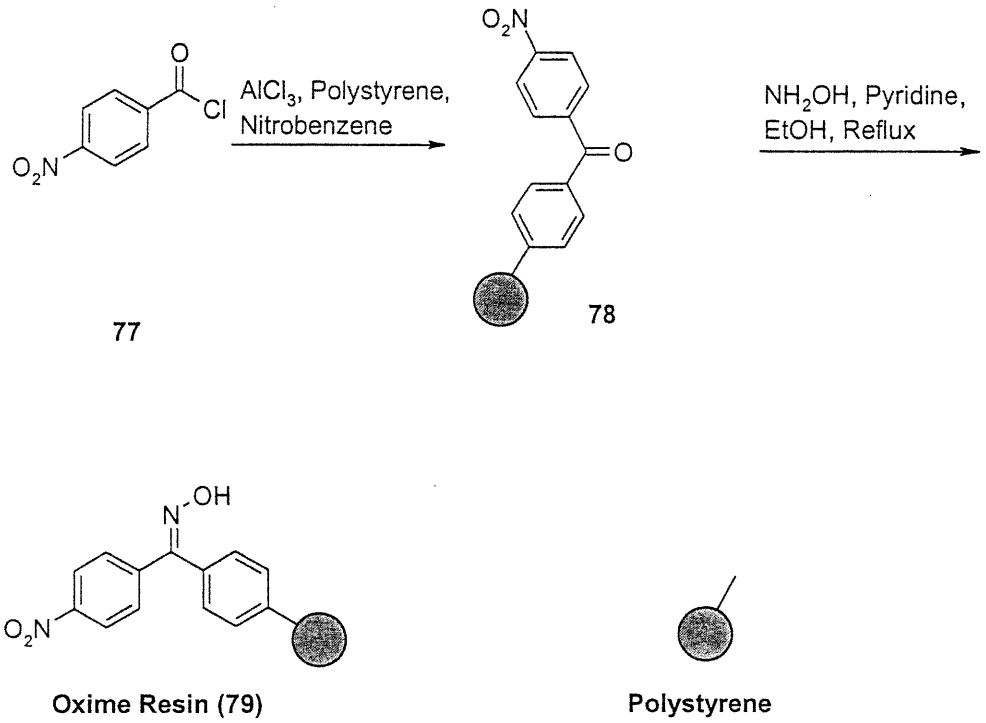
Using this approach, the *N*-(BOC)amino acid **68** was first anchored onto the oxime resin with dicyclohexylcarbodiimide (DCC) and ethyl-2-(hydroxyimino)-2-cyanoacetate (EACNOx) in dichloromethane at room temperature to give **69.** Treatment of **69** with TFA in dichloromethane removed the BOC group. Washing of the resin with DIEA gave the free base which was coupled to a second *N*-(BOC)amino acid using TBTU in DMF. Hydroxamic acid **71** was then obtained from treatment of **70** with hydroxylamine in methanol/chloroform (1:6) which produced diastereomerically pure product (>99% de).

2.3 Preparation of the Components for Library Synthesis



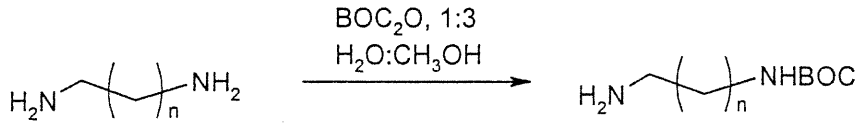
Scheme 4. Synthesis of 7-Benzylindolizidin-2-one Amino Acid **48**

For the preparation of indolizidinone amido amide libraries, 7-benzyl indolizidinone *N*-(BOC)amino acid **48** was first synthesized in gram quantities (Scheme 4).[47] *N*-(PhF)Glutamate **72** was synthesized in >330 mmol scale in two steps from γ-methyl glutamate (PhF = 9-(9-phenylfluorenyl)). Exposure of glutamate **72** to NaHMDS at –40 °C in THF, followed by decarboxylation of the resulting β-keto ester with NaOH in dioxane gave symmetrical ketone **73**.[37] 4-Benzyl ketone **74** was obtained by alkylation of ketone **73** with KHMDS and BnBr in THF at –78 °C with warming to room temperature. 7-Benzylindolizidin-2-one **75** was then prepared by the reductive amination protocol on 4-benzyl ketone **74** featuring hydrogenation with palladium-on-carbon as catalyst under 11 atm of hydrogen, *tert*-butyl ester hydrolysis with TFA, esterification with methanol and $SOCl_2$, lactam formation with triethylamine in dichloromethane, and protection with di-*tert*-butyldicarbonate. A 6:1 ratio of (7*S*)- : (7*R*)-7-benzylindolizidinone *N*-(BOC)amino esters was obtained, and the diastereomers were separated by chromatography. Methyl ester **75** was subsequently hydrolysed with LiOH in dioxane to give 7-benzylindolizinone *N*-(BOC)amino acid **48.**



Scheme 5. Synthesis of Oxime Resin

According to the literature method,[41] oxime resin was synthesized from polystyrene (Biobeads Sx1^{TM}) by acylation with *p*-nitrobenzoyl chloride **77** and trichloroaluminum in nitrobenzene at room temperature for 48 h (Scheme 5). Treatment of the resulting ketone **78** with hydroxylamine and pyridine in ethanol at reflux for 14 h gave oxime resin **79** which exhibited an IR spectrum having strong absorbances at 3534, 1525, and 1312 cm^{-1}.

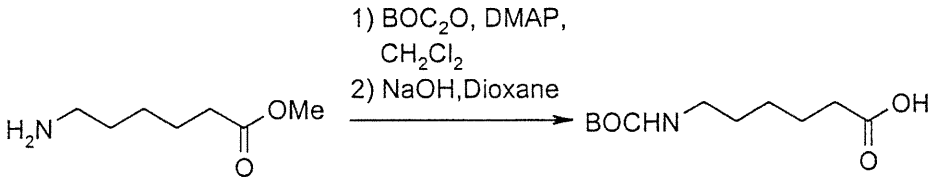


80 n = 1
81 n = 2
82 n = 3

59 n = 1
83 n = 2
60 n = 3

Scheme 6. Synthesis of Mono-Protected BOC Diamines

Mono-protected α,ω-diamines were synthesized by an optimized literature procedure (Scheme 6),[42] featuring treatment of the diamines (200 mol%) with BOC_2O (100 mol%) in 1:3 water:methanol at room temperature for 8 h.

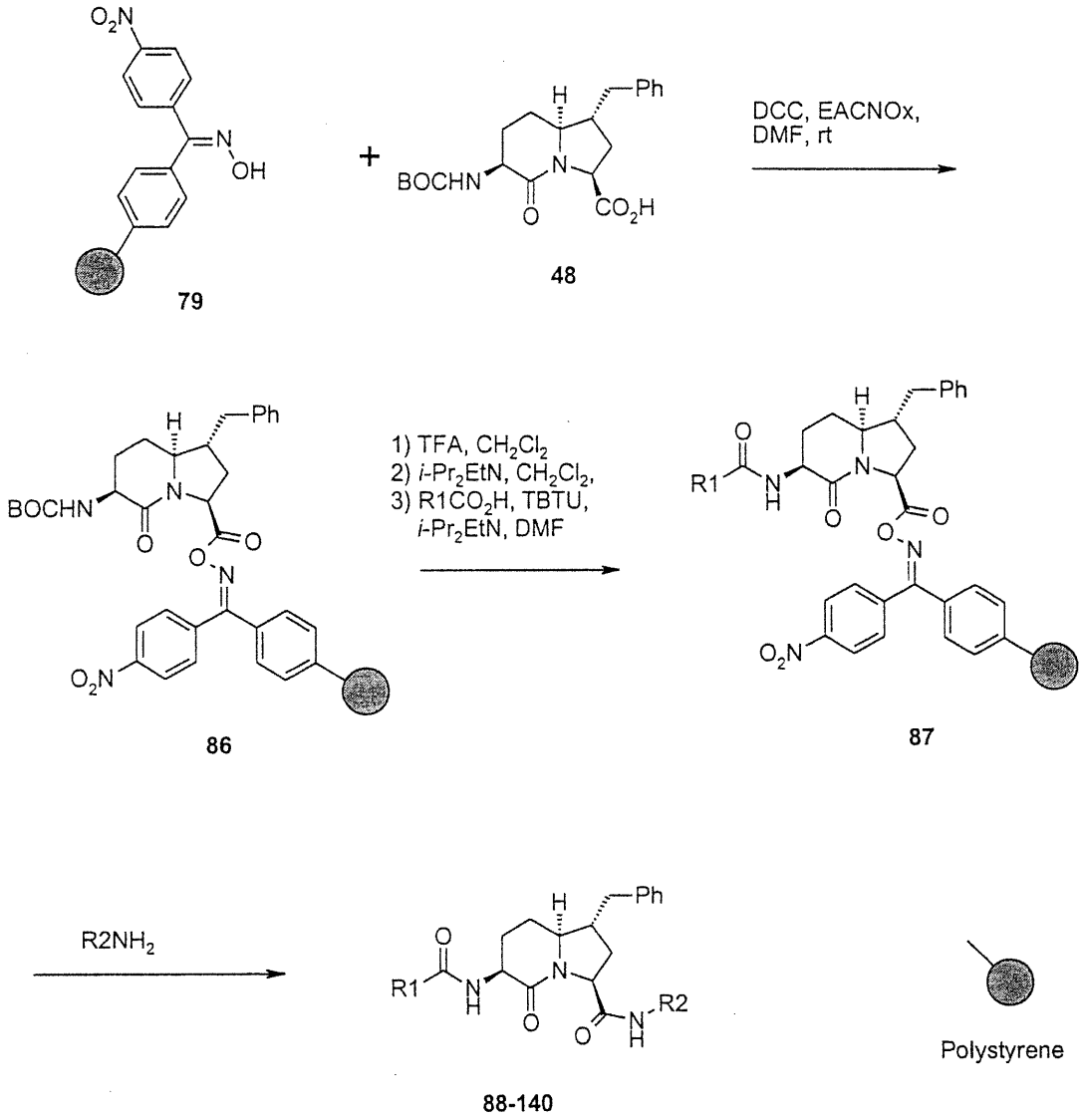


84 **85**

Scheme 7. Protection of 6-Aminocaproic Acid

6-*N*-(BOC)Aminocaproic acid (**85**) was obtained on treatment of 6-aminocaproate (**84**) with BOC_2O and DMAP in dichloromethane at room temperature for 4 h, followed by hydrolysis with NaOH in water/dioxane. The acid was isolated in 86% yield (Scheme 7).

2.4 Library Synthesis

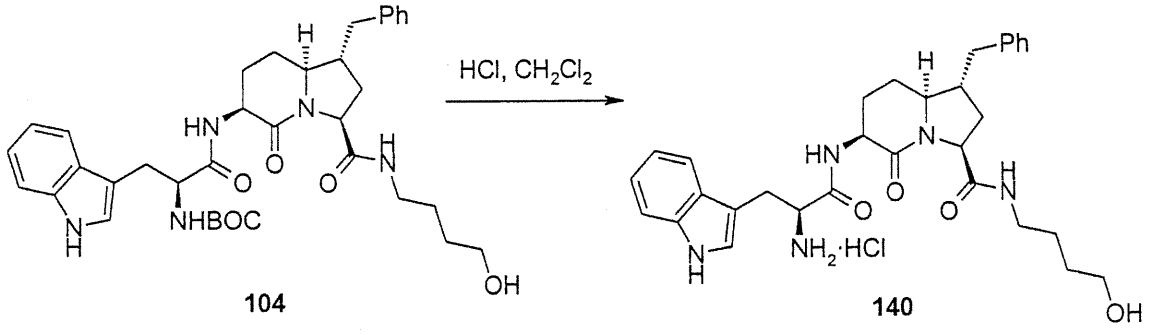


Scheme 8. Synthesis of 7-Benzylindolizidinone Amido Amide Library

The introduction of the indolizidin-2-one amino acid **48** into the library of receptor ligand candidates was pursued using a solid phase strategy consisting of three fundamental steps. First scaffold **48** was linked to oxime resin. The BOC group was removed and various electrophilic reagents were coupled to the amine. Finally, the scaffold was removed from the oxime resin using various amine nucleophiles to produce amido 7-benzylindolizidinone amides **88-140** (Scheme 8 and Table 4).

The (7*S*)-7-benzyl indolizidinone *N*-(BOC)amino acid **48** was coupled to oxime resin **79** using DCC and EACNOx in DMF at room temperature for 18 h to give **86**. The loading of resin **86** was determined by displacement of *N*-(BOC)amino indolizidinone *N'*-*n*-butylamide from the resin with *n*-butylamine in chloroform. Subsequent measurement of the purity and weight of displaced indolizininone amide demonstrated the loading of **86** to be in the range of 0.56-0.58 mmol/g. The BOC group was then removed with 20% TFA in dichloromethane and the amine was treated by washing the resin with DIEA in dichloromethane. Carboxylic acids **54–58** (Figure 11) were then coupled to the amine using TBTU, HOBt and DIEA in DMF to give **87**. Couplings were performed until a negative ninhydrin test was observed.[43] The final amido amides **88-140** were obtained on displacement of the resin with amines **59–67** (Figure 11).

In the nucleophilic displacement, *N*-(benzyl)piperidinyl-4-amine **63** attacked oxime ester **87** to give amide **88** in less than 20% yield (determined by weight). By using acetic acid as an additive, the yield could be augmented to 85%. 2-(Aminomethyl)benzimidazole **65** was obtained as its hydrochloride salt that was treated with triethylamine in chloroform, prior to addition to oxime ester **86** and samples (**117-121** and **127**) prepared with **86** were often contaminated with triethylamine.



Scheme 9 Example of the deprotection of the BOC group of a library member

In cases where the electrophilic and nucleophilic reagents possessed an amino group protected with a BOC group, the BOC group was removed with dry HCl gas in dichloromethane to furnish the library member. For example, treatment of compound **104** with HCl gas in dichloromethane gave compound **140** as its hydrochloride salt (Scheme 9).

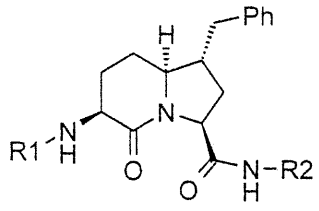
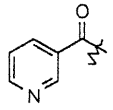
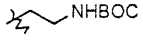
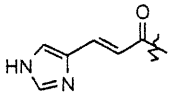
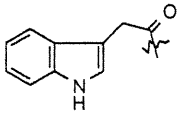
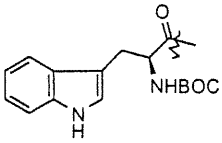
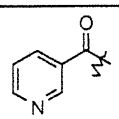
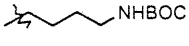
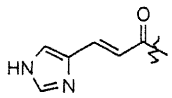
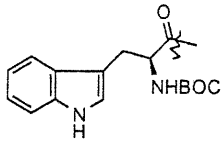
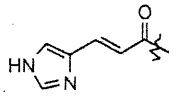
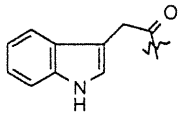
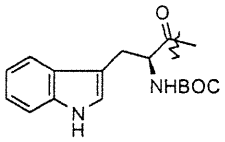
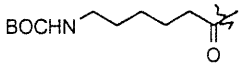
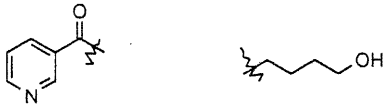
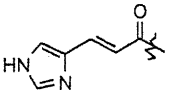
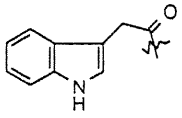
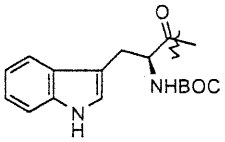
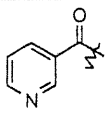
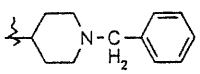
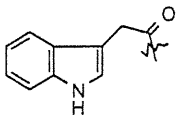
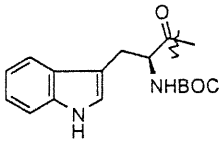
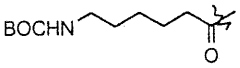
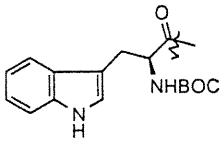
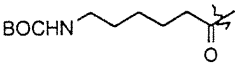
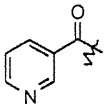
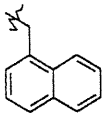
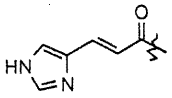
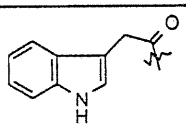
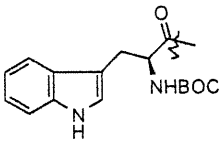
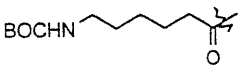
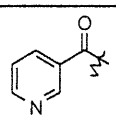
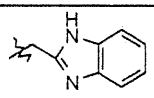
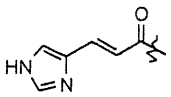
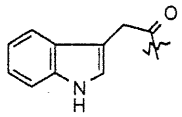
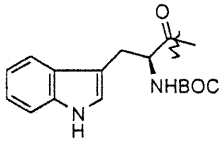
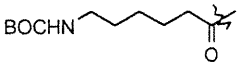
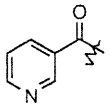
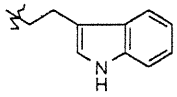
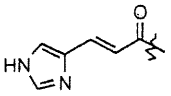
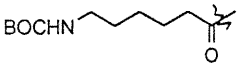
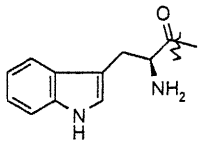
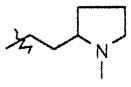
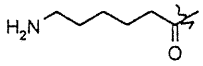
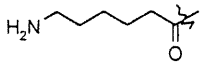
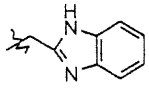


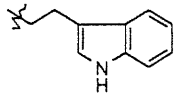
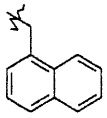
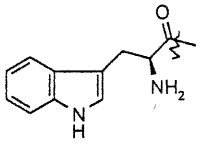
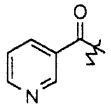
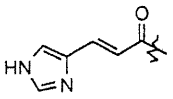
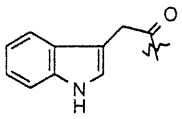
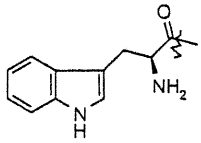
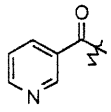
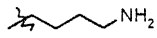
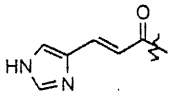
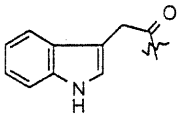
Table 4 7-Benzylindolizidinone amido amides of Library[a]

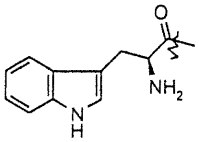
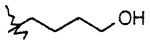
	R1	R2	**LRMS** **m/z**	**HRMS** Calcd. Found	**NMR Spectral Quality**
88			536 (MH^+, 10%) 57 (100%)	536.2873 536.2891	VG
89		"	551 (MH^+, 8%) 154 (100%)	551.2982 551.3000	VG
90		"	588 (MH^+, 20%) 57 (100%)	588.3186 588.3170	VG
91		"	717 (MH^+, 12%) 57 (100%)	717.3976 717.3953	VG
92			546 (MH^+, 6%) 57 (100%)	564.3186 564.3203	G

93		"	579 (MH^+, 20%) 57 (100%)	579.3295 579.3311	G
95		"	745 (MH^+, 40%) 154 (100%)	745.4289 745.4269	G
96			504 (MH^+, 12%) 55 (100%)	504.2974 504.2954	VG
97		"	519.6 (MH^+, 70%) 84.1 (100%)	519.3084 519.3099	VG
98		"	556 (MH^+, 30%) 154 (100%)		VG
99		"	685.5 (MH^+, 14%) 55.1 (100%)	685.4077 685.4103	VG
100		"	612 (MH^+, 24%) 84 (100%)	612.4125 612.4146	VG
101			465 (MH^+, 22%) 154 (100%)	465.2502 465.2484	VG
102		"	480 (MH^+, 70%) 154 (100%)		VG
103		"	517 (MH^+, 12%) 154 (100%)	517.2815 517.2796	VG
104		"	646 (MH^+, 20%) 154 (100%)	646.3605 646.3629	VG

105			556.4 (MH^+, 15%) 154.1 (100%)		VG
106		"	618 (MH^+, 24%) 154 (100%)	618.5612 618.5686	VG
107		"	747.5 (MH^+, 30%) 154.0 (100%)	747.4234 747.4261	VG
108		"	674.5 (MH^+, 36%) 154.1 (100%)	674.4282 674.4250	VG
110			650 (MH^+, 7%) 154 (100%)	650.3342 650.3370	I
111		"	577 (MH^+, 12%) 154 (100%)	577.3390 577.3371	G
112			533 (MH^+, 12%) 141 (100%)	533.2553 533.2574	G
113		"	548 (MH^+, 14%) 141 (100%)	548.2662 548.2689	I
114		"	585 (MH^+, 5%) 154 (100%)	585.2866 585.2844	VG
115		"	714.6 (MH^+, 10%) 141.1 (100%)	714.3655 714.3632	G
116		"	614 (MH^+, 16%) 141 (100%)		G

117				523.2458 523.2433	I[b]
118		"	538 (MH^+, 8%) 102 (100%)	538.2566 538.2583	I[b]
119		"	575 (MH^+, 12%) 102 (100%)	575.2770 575.2786	I[b]
120		"	704 (MH^+, 18%) 102 (100%)	704.3560 704.3589	I[b]
121		"	631 (MH^+, 14%) 154 (100%)	631.3608 631.3590	I[b]
122			536 (MH^+, 6%) 154 (100%)	536.2662 536.2689	G
123		"	551(MH^+, 3%) 154 (100%)		VG
124		"	666 ($M+Na^+$, 1%) 57 (100%)		G
125			585 (MH^+, 0.5%) (100%)	585.3523 585.3535	I
126		"	512.3 (MH^+, 95%) 501.3 (100%)	512.3601 512.3561	I
127			531.4 (MH^+, 3%) 154.1 (100%)	531.3084 531.3062	I[b]

			477.3 (MH^+, 6%)	477.2866	VG
128	"		154.1 (100%)	477.2881	
			544.4 (MH^+, 2%)	544.3287	P
129	"		154.1 (100%)	544.3309	
			541.4(MH^+, 2%)	541.3179	G
130	"		154.1 (100%)	541.3200	
			614.4 (MH^+, 3%)	614.3131	I
131		"	154.1 (100%)	614.3145	
			436 (MH^+, 3%)		P
132			154 (100%)		
			451.3 (MH^+, 3%)		I
133		"	(100%)		
			488.2 (MH^+, 6%)	488.2662	G
134		"	154.0 (100%)	488.2676	
			517.3 (MH^+, 30%)	517.2927	
135		"	154.1 (100%)	517.2905	
			479.3 (MH^+, 70%)	479.2771	G
136			307 (100%)	479.2791	
			464.3 (MH^+, 25%)	464.2662	I
137		"	154.1 (100%)	464.2649	
			516.4 (MH^+, 4%)	561.2975	G
138		"	154.1 (100%)	516.2960	

139		"	545.4 (MH^+, 40%) 154.1 (100%)	545.3240 545.3266	G
140	"		546.3 (MH^+, 60%) 154.1 (100%)		VG

[a] Vg: =very good, estimated purity >80%, G: =good, estimated purity >65%, I: =impure, presence of a significant impurity, P: =poor, trace amount of sample in spectrum. [b] triethyl amine was major contaminant.

All of the library members were obtained on 3-10 mg scales. The compounds **88-140** were characterized by LRMS, HRMS and proton NMR spectroscopy (see supporting information). Compounds **98**, **106**, **111** and **121** were respectively shown to be of 85%, 86%, 50% and 62% purities as determined by HPLC analysis. Compound **121** contained triethylamine as major contaminant.

Compounds **98, 105-107, 110, 112, 113-115, 117-120, 122** and **123** all possess more than two aromatic or heteroaromatic side-chains. They were tested for bioactivity at the neruokinin receptors by Dr R. Couture at l'Université de Montréal. Compounds **98, 100, 106, 126-129** and **132-140** which contained one primary amine or amine mimic side-chain were tested for bioactivity at somatostatin receptors by Biomeasure Inc. Unfortunately, none of the library members have exhibited significant biological activity at this time.

Chapter 3

Synthesis of Enantiopure 7-[3-Azidopropyl]indolizidin-2-one Amino Acid

3. Synthesis of Enantiopure 7-[3-Azidopropyl]indolizidin-2-one Amino Acid

3.1 Introduction

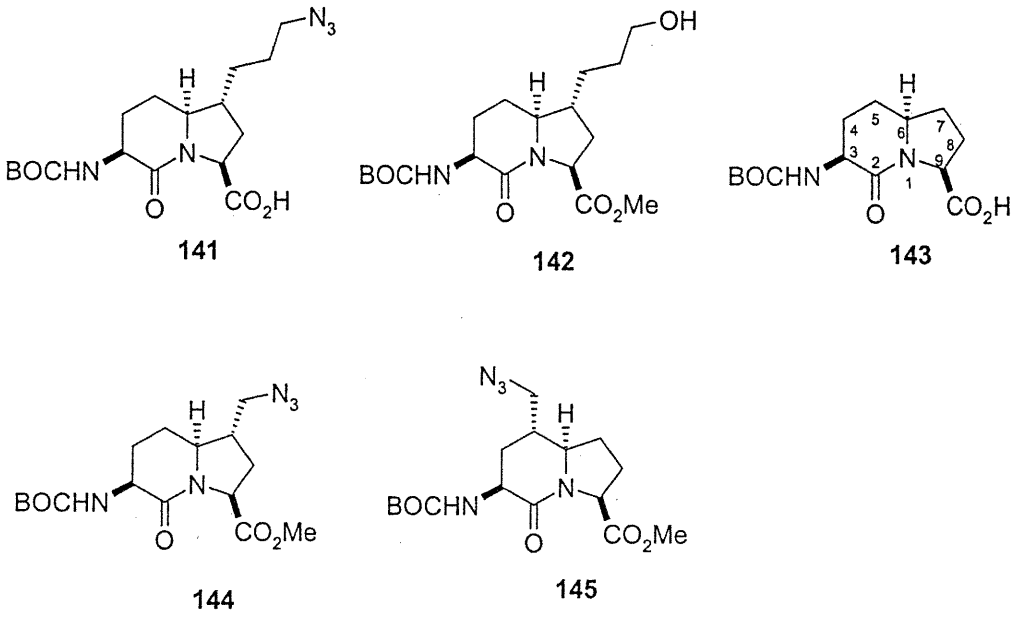
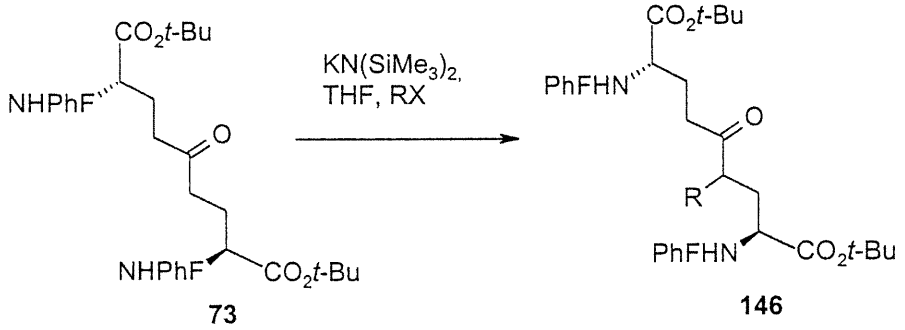


Figure 12. Indolizidin-2-one Amino Acid **143** and Related Orthogonally Protected Indolizidinone Diamino Carboxylates **141**, **142**, **144** and **145**.

Constrained surrogates of Orn, Lys and Arg residues are interesting targets because of their potential for probing various recognition events in protein chemistry and biology.[44 45] The orthogonally protected versions of these α,β-diamino acids are also interesting as inputs for library synthesis in medicinal chemistry, because they can serve for orchestrating three different pharmacophores in a geometrically defined display.[46] For respective applications as constrained Orn-Pro and Ala-Orn surrogates, 3-*N*-(BOC)amino 5- and 7-azidomethylindolizidin-2-one 9-carboxylates **144** and **145** were synthesized by a methanesulfonate displacement / lactam cyclization

also interesting as inputs for library synthesis in medicinal chemistry, because they can serve for orchestrating three different pharmacophores in a geometrically defined display.[46] For respective applications as constrained Orn-Pro and Ala-Orn surrogates, 3-*N*-(BOC)amino 5- and 7-azidomethylindolizidin-2-one 9-carboxylates **144** and **145** were synthesized by a methanesulfonate displacement / lactam cyclization sequence.[47] By exploring an alternative sequence, we have now synthesized *N*-(BOC)amino-7-(3-azidopropyl)indolizidin-2-one acid **141** which may serve as a constrained Ala-Lys mimic as well as a scaffold for the synthesis of libraries directed towards the discovery of somatostatin agonists and neruokinin antagonists (Figure 12.).

3.2 Alkylation of Ketone 73



73 146

Scheme 10. Study of Alkylation Symmetric Ketone **73**

Table 5. Alkylation of Ketone 73

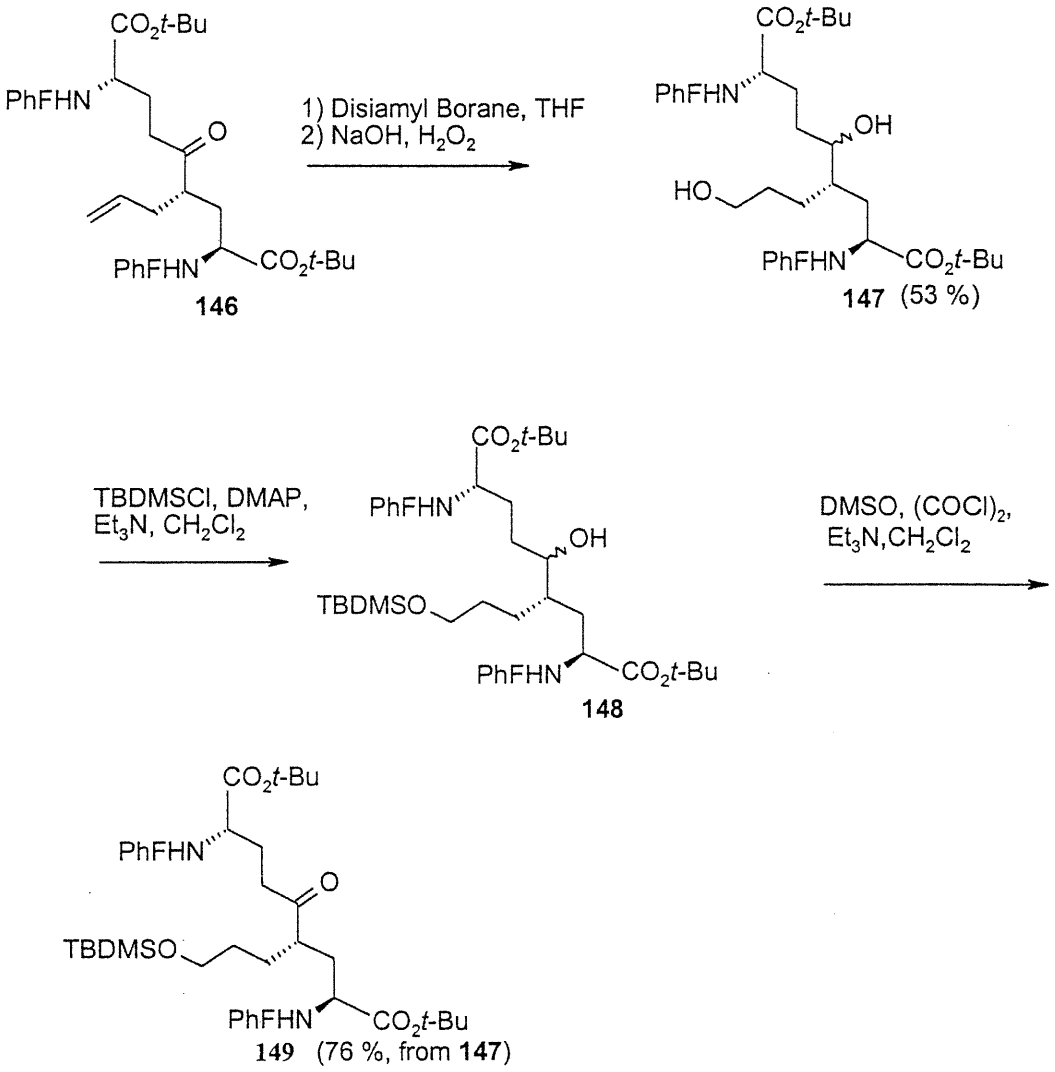
	RX (mol%)	KHMDS mol%	Conversion % (4*S*)-**146** : (4*R*)-**146**[a]	Isolated Yield (4*S*)-**146** : (4*R*)-**146**	
1	$PhCH_2Br$ (170)	130	82 (1:8~10)		
2	$PhCH_2Br$ (110)	160	>95 (1:7)	7	60[b]
3	$PhCH_2Br$ (105)	400	>95 (2:1)		
4	CH_2=$CHCH_2Br$ (105)	150	>95 (1:3)		
5	CH_2=$CHCH_2I$ (105)	150	>95 (1:5)		
6	CH_2=$CHCH_2I$ (105)	80 + 60[c]	>95 (<1:10)		81[d]
7	$Me_3SiO_2CCH_2CH_2I$ (200)	160	~60 (1:1)		
8	$HO_2CCH_2CH_2Br$ (160)	160	NR[e]		
9	Et O_2CCH=$CHCH_2Br$(160)	160	NR		

[a]Determined by integration of the *tert*-butyl ester singlets in the proton NMR spectrum of crude product. [b]Performed on 5 g of **146**. In addition 10% of a mixture of (4*S*)- and (4*R*)-**146** (2:1) was also isolated. [c]Initially 80 mol% of KHMDS was added, followed by allyl iodide and a second 60 mol% of KHMDS. [d]Performed 21 mmol on **146**. [e]NR: no reaction

Indolizidinone diamino acid **141** was synthesized by a route involving alkylation of (2*S*, 8*S*)-di-*tert*-butyl 5-oxo-2,8-di-[*N*-(PhF)amino]azelate **73** (PhF = 9-(9-phenylfluorenyl)) with allyl iodide. δ-Keto azelate **73** was previously alkylated with allyl bromide using potassium bis(trimethylsilyl)amide (KHMDS) in a 2:5 toluene/THF solution at –78 °C with warming to –20 °C over 1-2 h.[47] Diastereoselectivity was typically low using these conditions which provided a 3:1 mixture of (4*R*)- and (4*S*)-alkyl-branched ketones **146**. Improved diastereoselectivity (6:1) was attained upon switching to allyl iodide as electrophile. After examination of several conditions, a <1:10 ratio of (4*S*)- and (4*R*)- isomers was

Alkylation with benzyl bromide was also studied and the selectivity of the alkylation was found to depend on KHMDS (Table 5). High diastereoselectivity and good conversion was obtained using 160 mol% of KHMDS and 110 mol% of benzyl bromide in THF at –78 °C to room temperature. Among the other electrophiles tried, alkylation with 3-iodopropionic acid trimethyl silyl ester gave 60% conversion as shown by proton NMR and 3-bromopropionic acid and ethyl 4-bromocrotonate gave no reaction.

3.3 Synthesis of 4-(3-*tert*-butyldimethylsilyloxypropyl)-azelate 147



Scheme 11. Synthesis of 4-(3-*tert*-butyldimethylsiloxypropyl)azelate **149**

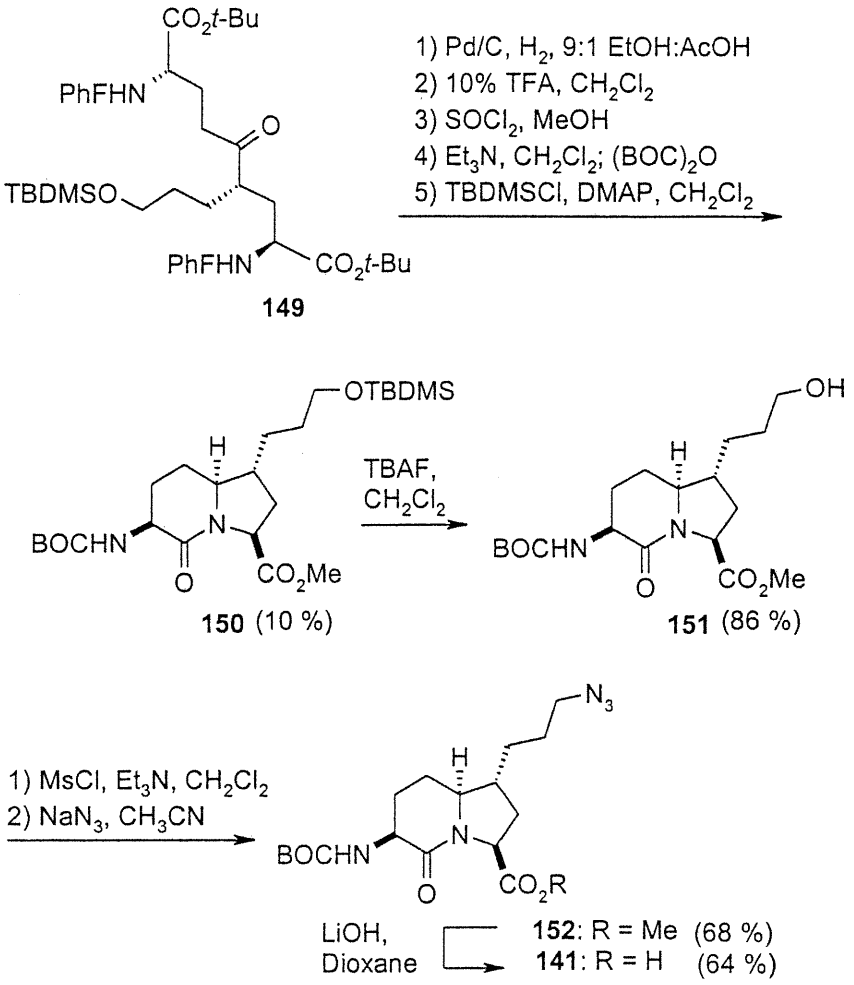
(2*S*,4*R*,8*S*)-Di-*tert*-butyl 4-allyl-5-oxo-2,8-di-[*N*-(PhF)amino]azelate (**146**) was hydroborated initially using a borane dimethylsulfide complex in THF followed by oxidation with an alkaline peroxide solution;[48] however, these conditions were not regioselective and the desired diol **147** was isolated in low yield (35%) after

separation from diol possessing two secondary alcohols by chromatography. Selective formation of diol **147** was achieved using 9-BBN in THF at room temperature;[51b,49] however, the cyclooctane diol by-product, produced in the oxidation step, was difficult to remove from diol **147**. Disiamyl borane proved more effective for producing pure diol **147** albeit in 53% yield after chromatography as a mixture of 5-position diastereomers.[50]

Ketone **89** was synthesized in two steps from diol **147**. First, the primary alcohol was protected selectively as its *tert*-butyldimethylsilyl ether using the respective chlorosilane, triethylamine and DMAP in dichloromethane.[51] Finally, oxidation of the secondary alcohol with oxalyl chloride and DMSO in dichloromethane gave, after chromatography, ketone **149** in 76% overall yield from **147**.[52]

3.4 Synthesis of 7-(3-Azidopropyl)indolizidin-2-one Amino Acid

7-(3-*tert*-Butyldimethylsiloxypropyl)indolizidinone amino ester **150** was synthesized from ketone **149** using our reductive amination / lactam cyclization protocol[10] as developed previously for the synthesis of its 7-benzylindolizidinone counter part.[4] Hydrogenation of a solution of ketone **149** in 10:1 EtOH:AcOH with palladium-on-carbon under 10 atm of H_2 caused cleavage of the PhF groups, intramolecular imine formation and reduction to a 5-alkylproline. Indolizidinone *N*-(BOC)amino ester **150** was then obtained after *tert*-butyl ester and silyl ether removal with TFA in dichloromethane, esterification with thionyl chloride in methanol, lactam cyclization on treatment with triethylamine in dichloromethane, and amine protection with di-*tert*-butyl dicarbonate. (3*S*,6*S*,7*R*,9*R*)-7-(3-*tert*-Butyldimethylsiloxypropyl) indolizidinone *N*-(BOC)amino ester (**151**) was isolated in 10% overall yield after this multiple step sequence by alcohol protection with TBDMSCl, triethylamine and DMAP in dichloromethane and chromatography on silica gel.



Scheme 12 Synthesis of 7-(3-Azidopropyl)indolizidinone Amino Acid **141**

7-(3-Azidopropyl)indolizidinone amino acid **141** was prepared by a sequence commencing with silyl ether **150** which was first deprotected using TBAF in THF to afford alcohol **151**. Activation of alcohol **151** as its methanesulfonate using methanesulfonyl chloride and triethylamine in dichloromethane and subsequent nucleophilic displacement with sodium azide in acetonitrile at room temperature gave azide **152**. Orthogonally protected diamino acid **141** was then isolated in 44% overall yield from **150** after hydrolysis of methyl ester **152** with 1 M aqueous LiOH in dioxane.

3.5 Relative Stereochemistry and Enantiomeric Purity of Diamino Indolizidinone Carboxylate 150.

Stereochemical assignments at the indolizidinone ring-fusion and alkyl-branched centers were made based on a series of two dimensional NMR experiments on siloxypropyl analogue **150**. Initially, COSY spectroscopy was used to locate the alkyl-branch at the 7-position. In a subsequent NOESY experiment, a transfer of magnetization was observed between the ring-fusion proton (3.18 ppm) and the protons of the peptide backbone at C-3 (4.02 ppm) and C-9 (4.41 ppm) that confirmed the concave indolizidinone geometry. The 7*R* stereochemical assignment was made based on observation of a greater nuclear Overhauser effect between the side-chain methylene protons (1.24 ppm and 1.35 ppm) with the C-8α proton (1.73 ppm) relative to the C-8β proton (2.08 ppm). Further support for this stereochemical assignment came from the strong nuclear Overhauser effect between the side-chain methylene and C-6 protons (Figure 13).

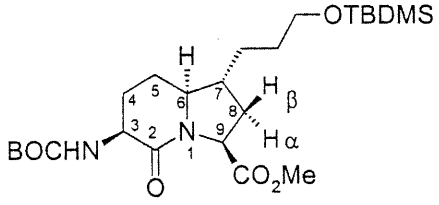
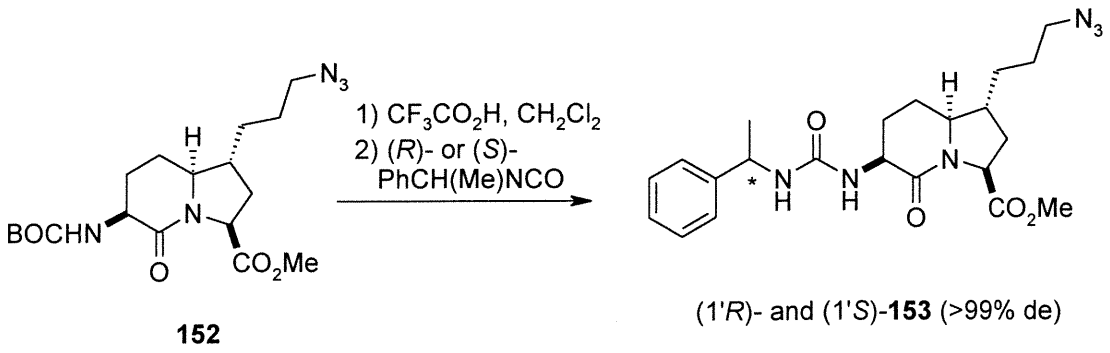


Figure 13 Diamino indolizidinone carboxylate 150

A predominance of the (6*S*, 7*R*)-stereoisomer had previously been observed in the 7-benzylindolizidinone products obtained from the reductive amination / lactam cyclization sequence using either the (4*S*)- or (4*R*)-isomer of the relate 4-benzyl-5-oxo-2,8-di-[*N*-(PhF)amino]azelate and was ascribed earlier to an imine-enamine tautomerization during the hydrogenation step.[42] Although the stereochemistry at the alkyl-branch may arise from a retention of configuration, a similar tautomeric equilibrium may play a role in the production of the (7*R*)-siloxypropylindolizidinone isomer **150**.



Scheme 13 Enantiomeric purity of 7-(3-azidopropyl)indolizidinone **152**

The enantiomeric purity of diamino indolizidinone carboxylate **152** was ascertained after conversion to diastereomeric ureas (1'*R*)- and (1'*S*)-**153**.[53] Cleavage of carbamate **152** with TFA in dichloromethane followed by treatment with triethylamine and acylation respectively with (*R*)- and (*S*)-α-methylbenzylisocyanate in THF at room temperature gave α-methylbenzyl ureas **153** that were directly examined by 400 MHz NMR spectroscopy in $CDCl_3$. Measurement of the diastereomeric methyl ester singlets at 3.72 and 3.73 ppm demonstrated the ureas diastereomeric excess. The limite was determined by adding (1'*S*)-**153** to (1'*R*)-**153**, until (1'*S*)-**153** can be tested by NMR. That showed to be of > 99% diastereomeric excess. Hence, 7-(3-hydroxpropyl)indolizidinone **151** and diamino indolizidinone carboxylates **152** and **141**, all are presumed to be of >99% enantiomeric excess.

Chapter 4
Conclusion

4 Conclusion

4.1 Conclusion

Methodologly was developed for synthesizing chemical libraries for discovering new peptide hormone receptor ligands. In particular, a combinatorial strategy was used in the library synthesis of neurokinin and somatostatin receptor ligands. The utility of oxime resin was demonstrated by solid support conversion of (3*S*,6*S*,7*S*,9*S*)-7-benzylindolizidin-2-one *N*-(BOC)amino acid **48** into its respective *N*-acylamino *N'*-amides using 5 carboxylic acids and 9 amines to furnish a 7-benzylindolizinone library. To our knowledge, this is the first time 7-benzylindolizidinone amino acids have been used as scaffolds for exploring the neruokinin and the somatostatin receptors. Unfortunately, no significantly active compounds were identified from examination of our library.

Towards the design of indolizidin-2-one scaffolds possessing heteroatomic side-chains , we have synthesized 7-(3-hydroxypropyl)indolizidinone amino ester **151** from azelate **73** by a strategy featuring selective allylation, hydroboration, reductive amination and lactam cyclization. The alcohol of **151** can be converted to other heteroatomic side-chains, as demonstrated by the preparation of enantiopure 7-(3-azidopropyl)indolizidinone amino acid **141** via displacement of its respective methanesulfonate with sodium azide. Complementing the related recently synthesized 5- and 7-azidomethylindolizidinones **142** and **143**,[47] orthogonally protected diamino indolizidinone acid **141** expands a unique series of a constrained dipeptide surrogates for exploring recognition events in peptide science involving α,ω-diamino acid residues.

4.2 Further Work

Alternative regio- and stereochemical isomers of the scaffolds may be used to synthesize somatostatin and neruokinin receptor ligands having better potency. For example, scaffold **49** may be studied next for library synthesis, because its different stereochemistry at the 7-position may favour a geometry that will favour binding at the somatostatin receptor according to the model described in the Introduction. Alternatively the influence of the aromatic side-chain at a different position of the scaffold could be explored by the application of scaffold **50** for the receptor ligands. Scaffolds **141-143** which possess amine–bearing side-chains on indolizidinone may be studied to generate alternative somatostatin receptor ligand candidates. In summary, both a combinatorial approach as well as a novel synthesis strategy have been elaborated for making and using 7-alkylindolizidinone amino acid in the generation of ligands for peptide hormone mimicry.

Chapter 5

Experimental Section

General. Unless otherwise noted all reactions were run under nitrogen atmosphere and distilled solvents were transferred by syringe. Tetrahydrofuran (THF) and ether were distilled from sodium/benzophenone immediately before use; 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and CH_2Cl_2 were distilled from CaH_2; Et_3N was distilled from BaO. Final reaction mixture solutions were dried over Na_2SO_4. Chromatography was on 230-400 mesh silica gel; TLC on aluminum-backed silica plates. Melting points are uncorrected. Mass spectral data, HRMS (EI and FAB), was obtained by the Université de Montréal Mass Spec. facility. ^1H NMR (300/400 MHz) and ^{13}C NMR (75/100 MHz) were recorded in $CDCl_3$. Chemical shifts are reported in ppm (δ unit) downfield of internal tetramethylsilane ($(CH_3)_4Si$). Coupling constants are given in Hz. Chemical shifts for aromatic PhF carbons are not reported.

General Procedure for *N*-(Boc)-α,ω-Alkanediamine.[42] A solution of α,ω-alkanediamine (5 mmol 200 mol%) in methanol/water (3:1, 50 mL) was treated with di-*tert*-butyldicarbonate (2.5 mmol, 100 mol%) at room temperature, and stirred for 18 h. The reaction mixture was evaporated to 60% of its original volume, diluted with 1 M KH_2PO_4 (15 mL) and extracted with EtOAc (2x15 mL). The aqueous phase was adjusted with 5 M NaOH to pH 12, then extracted with EtOAc (2x30 mL). The combined organic phase was dried and evaporated to furnish the mono-protected diamine.

***N*-(Boc)-1,2-Diaminoethane (59).** Yield 85 %: ^{13}C NMR δ 27.6, 38.8, 41.6, 78.9, 155.7.

***N*-(Boc)-1,3-Diaminopropane (83).** Yield 83%: ^{13}C NMR δ 28.3, 31.2, 40.6, 42.1, 78.8, 155.6.

***N*-(Boc)-1,4-Diaminobutane (60).** Yield 87%: ^{13}C NMR δ 27.4, 28.4, 30.9, 40.4, 41.8, 78.9 155.6.

6-*N*-(BOC)Aminocaproic acid (85). Methyl 6-aminocaproic ester (**84**, 120 mg, 0.83 mmol, 100 mol%) was dissolved in 10 mL of CH_2Cl_2, treated with BOC_2O (360 mg, 1.65 mmol, 200 mol%) and DMAP (10 mg, 0.08 mmol, 10 mol%), After stirring for 4 h at room temperature, the reaction mixture was treated with a 1M solution of NaH_2PO_4 (10 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2x10 mL). The combined organic phases was washed with brine (10 mL), dried filtered and evaporated to a residue. The residue was dissolved in dioxane (5 mL), treated with a solution of 2M NaOH (5 mL), stirred for 1 h at room temperature, acidified with a solution of 3M H_3PO_4 to pH ~4 and diluted with CH_2Cl_2 (20 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2x20 mL). The combined organic phases were washed with brine (20 mL), dried, filtered and evaporated to provide 6-*N*-(BOC)aminocaproic acid as an oil (165.6 mg, 86%): ^{13}C NMR δ 24.4, 26.1, 28.2, 29.6, 33.7, 51.2, 78.9, 155.8, 173.8.

Oxime Resin (79). Polystyrene (Biobead Sx1 Bio-Rad Lab) 300 g was washed with 500 mL each of 1M NaOH, 1 M HCl and water at 60°C for 60 min. The resin was then washed with 0.5 L each of DMF, 2M NaOH/dioxane (1:2), and 2M HCl/dioxane (1:2) at 60°C for 10 min, followed by 500 mL each of 2M HCl in methanol, water, methanol, methanol/dichloromethane (1:3) and methanol/dichloromethane (1:10) at room temperature and dried under vacuum pump over night. The washed polystyrene (35 g) was converted into oxime resin **79** according to the literature method[43] to provide 40.1 g of product (IR spectrum having strong absorbances at 3534, 1525, and 1312 cm^{-1}).

General Procedure for library synthesis.

(3*S*,6*S*,7*S*,9*S*)-2-Oxo-3-[*N*-(BOC)amino]-7-benzyl-1-azabicyclo[4.3.0]-nonane-9-carboxylic acid (48) attachment to oxime resin. Oxime resin (**49**, 2.5 g) in a 500 mL round-bottom flask was swelled with 170 mL of DMF and treated with 7-benzylindolizidinone **48** (0.735 g, 1.9 mmol, 110 mol%), DCC (0.425 g, 2.09 mmol, 120 mol%) and $EACNO_x$ (0.539 g, 3.8 mmol, 225 mol%). The flask was agitated by shaken for 24 h at room temperature. After filtration, the resin **86** was washed with 2x200 mL each of CH_2Cl_2, DMF, CH_2Cl_2, CH_2Cl_2/isopropanol (1:1) and CH_2Cl_2 then dried under vacuum pump overnight to give the resin **86** (3.12g).

N-Terminal diversification. The resin **86** (3.1 g, 0.57 mmol/g) was treated with CH_2Cl_2/TFA (3:1 200 mL) at room temperature for 2x20 min to remove the BOC group. The resin was washed with 2x200 mL each of CH_2Cl_2, CH_2Cl_2/diisopropyl ethyl amine (9:1), dichloromethane, isopropanol and CH_2Cl_2, and dried under vacuum overnight to give the resin (2.91 g) that was split into 5 portions. Each portion (390 mg, 0.23 mmol, 100 mol%) was placed into a reaction vessel and treated with a carboxylic acid (**54-58,** 0.3 mmol, 130 mol%), TBTU (96 mg, 0.3 mmol, 130 mol%), HOBt (40.5 mg, 0.23 mmol, 100 mol%) and DIEA (37.8 mg, 50 L, 0.3 mmol, 130 mol%) in 5 mL of DMF. After shaking at room temperature for 24 h, the reaction vessels were drained and the resins were washed with 2x5 mL each of DMF and CH_2Cl_2. Each of the resins was dried under vacuum for 24 h and evaluated by quantitative ninhydrin test.[54] The resin was retreated under the same conditions until a negative ninhydrin test was obtained.

Resin displacement. Each resins **87** was split into a series of reaction vessels, treated with a 1M solution of primary amine (**59-67,** 100 mol%) in chloroform (0.32-0.45 mmol/g of resin) and agitated by shaking overnight. Each tube was filtered and the resin was washed with 1 mL each of CH_2Cl_2 and CH_2Cl_2:methanol 1:1. The filtrate and washings were combined and evaporated to a

crude product which was characterized by proton NMR spectroscopy, LRMS and HRMS (table 4).

Final deprotection of BOC protected amido amides. (3S,6S,7S,9S,2'S)-2-oxo-3-[*N*'-(BOC)tryptophanamido]-7-benzyl-1-azabicyclo[4.3.0]nonane-9-*N*'-(4'-hydoxyl)butylamide (**104**) was dissolved in CH_2Cl_2 (2 mL), treated with dry hydrogen chloride gas bubbles for 20 min and evaporated to a residue to give (3S,6S,7S,9S,2'S)-2-oxo-3-tryptophanamido-7-benzyl-1-azabicyclo[4.3.0]nonane-9-*N*'-(4'-hydoxyl)butylamide (**140**). ^{1}H NMR δ 1.2-1.6 (m, 7H), 1.8-1.9 (m, 2H), 1.9-2.0 (m, 1H), 2.2 (m, 1H), 2.6 (m, 1H), 2.75 (t, 1H J = 6.2), 2.8-3.2 (m, 4H), 3.54 (t, 2H J = 5.5), 3.63 (t, 1H J = 5.8), 4.13 (m, 2H), 4.32 (d, 1H J = 9.2) 7.05 -7.68 (m, 10H).

Determination of resin loading

Resin **86** (39.7 mg) was treated with a 4 M solution of *n*-butylamine in chloroform (2 mL) and agitated by shaking overnight. The reaction mixture was filtrered and the resin was washed with 1 mL each of CH_2Cl_2 and CH_2Cl_2/methanol (1:1). The filtrate and washings were combined and evaporated to provide *N*-(BOC)amino indolizidinone *n*-butylamide that was calculated by weight to give a resin loading of 0.56-0.58 mmol/g.

(2*S*,4*R*,8*S*)-Di-*tert*-butyl 4-allyl-5-oxo-2,8-di-[*N*-(PhF)amino]azelate (146). Ketone **73** (18 g, 21.8 mmol, 100 mol %) in THF (44 mL) was treated with a 0.5 M solution of KHMDS in toluene (35 mL, 17.5 mmol, 80 mol %) stirred for 30 min, and treated with allyl iodide (2.0 mL, 22.9 mmol, 105 mol %). After stirring for 0.5 h at –78 °C, the reaction mixture was treated with a second 0.5 M solution of KHMDS in toluene (26.2 mL, 13.1 mmol, 60 mol%), stirred for an additional 2 h at –78 °C then partitioned with agitation between 1 M KH_2PO_4 (400 mL) and EtOAc (400 mL). The aqueous layer was extracted with EtOAc (2x400 mL). The combined organic layers were washed with brine, dried, and evaporated to a residue that was analyzed by proton NMR spectroscopy which showed a 15:1 ratio of diastereomeric *tert*-butyl

ester signals. Chromatography of the residue on silica gel using an eluant of hexane to hexane/EtOAc (95:5) gave first a 1:1 mixture of (4*S*)- and (4*R*)-**146** (1.3 g, 7 %), followed by pure (4*R*)-**146** (15.3 g, 81 %): mp 131-133 °C, $[\alpha]_D$ –175.1° (*c* 0.74, $CHCl_3$)*, lit.[47] $[\alpha]_D^{20}$ –74.0° (*c* 1.5, $CHCl_3$); the spectral data for 146 were identical with values reported in reference. (* The $[\alpha]_D$ is different that is probably coming from different temperature and concentration or different instrument.)

(2*S*,4*R*,8*S*)-Di–*tert*-butyl 4-(3-*tert*-Butyldimethylsiloxypropyl)-5-oxo-2,8-di-[*N*-(PhF)amino]azelate (149). A 0 °C solution of olefin **146** (3.2 g, 3.7 mmol, 100 mol %) in THF (200 mL) was treated drop-wise with a 0.5 M solution of disiamyl borane in THF (28 mL, 380 mol %) over 20 min. The reaction mixture was warmed to room temperature and stirred for 8 h, cooled to 0 °C and quenched with 5 mL of ethanol. The reaction mixture was treated with 3 M NaOH (5.5 mL, 450 mol%) and aqueous H_2O_2 (5.5 mL, 30% w/w%), heated at a reflux for 90 min, cooled to room temperature, and concentrated to ~40% of its original volume. The mixture was extracted with ether (200 mL). The aqueous phase was diluted with water (150 mL), adjusted to pH ~7 with 3 M H_3PO_4, and extracted with EtOAc (3x200 mL). The combined organic phase was dried and evaporated. The residue was chromatographed using 10 g of silica gel for each gram of residue with an eluant of hexane to EtOAc/hexane (1:9) to remove the higher R_f products. The column was then washed with pure hexane. The desired alcohols were eluted using a gradient of CH_2Cl_2 to CH_2Cl_2/EtOAc (6:4) in order to afford **147** as a mixture of diastereomeric diols 1.7 g (53%). To obtain analytical samples of pure diastereomeric diols **147**, 100 mg of the mixture was chromatographed on silica gel using a gradient of CH_2Cl_2 to CH_2Cl_2:EtOAc (6:4). The first diastereomer to elute: $[\alpha]_D^{20}$ –119.0 (*c* 0.42, $CHCl_3$); ^{1}H NMR δ 1.18 (s, 9H), 1.19 (s, 9H), 1.21-1.41 (m, 7H), 1.50-1.65 (m, 5H), 2.35 (m, 1H), 2.49 (m, 1H), 3.40 (t, 2H), 3.48 (m, 1H), 7.13-7.68 (m, 26H); ^{13}C NMR δ 27.2, 28.08, 28.11, 29.0, 32.7, 35.7, 40.4, 55.6, 56.7, 63.1, 72.9, 73.2, 73.3, 80.8, 80.9, 175.3, 175.8. The second diastereomer to elute: $[\alpha]_D^{20}$ –132.1 (*c* 0.84, CHCl3); ^{1}H NMR δ 1.17 (s, 9H), 1.22 (s, 9H), 1.25-1.38 (m, 7H), 1.46-1.64 (m, 5H), 2.33 (m,

1H), 2.57 (m, 1H), 3.39 (t, 2H), 7.13-7.68 (m, 26H); ^{13}C NMR δ 27.0, 27.9, 29.5, 30.1, 37.0, 40.0, 55.3, 56.0, 62.8, 72.9, 73.1, 73.3, 80.8, 175.2, 175.6.

A solution of diastereomeric diols **147** (1.5 g 1.5 mmol, 100 mol%) in 200 mL of CH_2Cl_2 at room temperature was treated with TBDMSCl (270 mg, 1.8 mmol, 105 mol%), DMAP (8 mg, 0.06 mmol, 4 mol%) and triethylamine (255 μL, 105 mol%), stirred for 18 h and evaporated. The residue was dissolved in EtOAc (200 mL) and washed with 1M KH_2PO_4 (50 mL). The aqueous phase was extracted with EtOAc (2 x 30 mL). The combined organic phase was dried and evaporated to a crude residue containing alcohol **148** which was used without further purification.

A –78 °C solution of oxalyl chloride (220 μl, 2.6 mmol, 150 mol%) in CH_2Cl_2 (20 mL) was treated with DMSO (322 μL, 2.4 mmol, 200 mol%), stirred for 30 min, warmed to –30 °C, cooled to –78 °C, and then treated drop-wise with a solution of the protected alcohol **148** (1.7 g, 1.7 mmol, 100 mol%) in CH_2Cl_2 (20 mL) followed by triethylamine (360 μL, 5.1 mmol, 300 mol %). The reaction mixture was stirred at –78 °C for 1 h, warmed to room temperature over 1h and quenched with a solution of 1M aqueous NaH_2PO_4 (200 mL), and the phases were separated. The aqueous phase was extracted with EtOAc (3x200 mL). The combined organic phase was dried and evaporated. Chromatography of the residue on silica gel (0-10% EtOAc in hexane) gave ketone **149** (1.3 g, 76 %): $[\alpha]_D^{20}$ 137.4 (c 0.39, $CHCl_3$); ^{1}H NMR δ 0.01 (s, 6H), 0.91 (s, 9H), 1.18 (s, 9H), 1.23 (s, 9H), 1.26-1.38 (m, 5H), 1.69-1.74 (m, 3H), 2.45-2.53 (m, 3H), 2.71-2.80 (m, 2H), 3.01-3.10 (br, 2H), 3.39 (t, 2H), 7.27-7.73 (m, 26H); ^{13}C NMR δ –5.1, 18.5, 27.4, 28.0, 28.1, 29.5, 30.4, 37.0, 38.6, 48.1, 54.5, 55.5, 63.2, 73.1, 73.2, 80.7, 175.3, 175.5, 213.5. FAB m/z 997.5 (M+1, 17%), 241.0 (100%).

(3*S*,6*S*,7*R*,9*S*)-Methyl 2-Oxo-3-*N*-(BOC)amino-7-(3-*tert*-butyldimethylsiloxypropyl)-1-azabicyclo[4.3.0]nonane-9-carboxylate (150). A hydrogenation vessel containing a solution of ketone **149** (1.57 g, 1.57 mmol, 100

mol%) in anhydrous EtOH (100 mL) and AcOH (10 mL) was charged with 0.16 g of palladium-on-carbon (10 wt %), then filled, vented and refilled three times with hydrogen. After stirring for 24 h under 10 atm of hydrogen, the reaction mixture was filtered onto Celite™ and washed with EtOAc (100 mL). The combined organic solution was evaporated to dryness and the residue was digested in EtOH/H_2O (85:15, 50 mL), then filtered. The filter cake was washed with EtOH/H_2O (85:15, 50 mL). The combined filtered solution was evaporated to dryness. The crude product was dissolved in a solution of 10 % TFA in CH_2Cl_2 (15 mL) and stirred overnight. Evaporation of the volatiles gave a residue which was dissolved in methanol (30 mL), cooled to 0 °C, treated with $SOCl_2$ (300 mol %), stirred at 0 °C for 2 h, at room temperature overnight and then evaporated. The residue was dissolved in CH_2Cl_2 (60 mL) treated with Et_3N (500 mol %), stirred at room temperature for 36 h, treated with di-*tert*-butyl dicarbonate (500 mol %), and stirred at room temperature for 4 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with a 1 M solution of NaH_2PO_4 (10 mL) and brine (10 mL), dried and evaporated. The residue was dissolved in EtOAc (5 mL), filtered through a plug of silica gel, washed first with hexane/EtOAc (1:1) to remove higher R_f products, and washed next with EtOAc/ methanol (10:1) to obtain the alcohol product. The collected fractions were evaporated. The residue was dissolved in CH_2Cl_2 (20 mL), treated with TBDMSCl (0.25 g, 1.65 mmol, 105 mol%), DMAP (6.4 mg, 4 mol%) and Et_3N (100 mol %), stirred at room temperature overnight, diluted with CH_2Cl_2 (20 mL), then washed with 1M NaH_2PO_4 (10 mL) and brine (10 mL), dried and evaporated. The residue was chromatographed with 3:7 hexane: EtOAc as eluant. Evaporation of the collected fractions gave **150** (60 mg, 10% from **149**): $[\alpha]_D^{20}$ –18.0 (*c* 0.15 CH_2Cl_2); ^{1}H NMR δ 0.06 (s, 6H), 1.18 (s, 9H), 1.20-1.24 (m, 1H), 1.34 (s, 9H), 1.34-1.38 (m, 3H), 1.42-1.59 (m, 2H), 1.61-1.63 (m, 2H), 1.70-1.90 (m, 1H), 1.95-1.99 (m, 1H), 2.08 (dd, 1H, J = 6, 10), 2.34 (m, 1H), 3.18 (m, 1H), 3.51 (t, 2H, J = 6), 3.64 (s, 3H); 4.02 (m, 1H), 4.41 (d, 1H, J = 6), 5.40 (bs, 1H); ^{13}C NMR δ –5.1, 18.5, 21.8, 26.1, 26.2, 27.3, 28.6, 31.2, 35.4, 45.2, 50.3, 52.6, 58.0, 61.8, 63.0, 79.7, 156.0, 169.4,

172.4. HRMS cacld for $C_{24}H_{45}SiO_6N_2$ (MH^+) 485.3047; found 485.3080.

(3*S*,6*S*,7*R*,9*S*)-Methyl 2-Oxo-3-*N*-(BOC)amino-7-(3-hydroxypropyl)-1-azabicyclo [4.3.0]nonane-9-carboxylate (151). Methyl ester **150** (60 mg, 0.15 mmol) in 10 mL of CH_2Cl_2 was treated with TBAF (80 mg, 0.3 mmol, 200 mol%) and stirred overnight at room temperature. The reaction mixture was diluted with 10 mL of CH_2Cl_2 and washed with 1M NaH_2PO_4 (5 mL) and brine (5 mL), dried and evaporated. The residue was chromatographed using a gradient of EtOAc to ethanol/EtOAc (1:20) as eluant. Evaporation of the collected fractions gave the alcohol **151** (48 mg, 86%): $[\alpha]_D^{20}$ –18.3 (*c* 0.11 CH_2Cl_2); ^{1}H NMR δ 1.21-1.24 (m, 1H), 1.33 (s, 9H), 1.34-1.40 (m, 3H), 1.45-1.61 (m, 2H), 1.63-1.65 (m, 2H), 1.72-1.93 (m, 1H), 1.96-2.01 (m, 1H), 2.11 (dd, 1H, *J* = 6), 2.37 (m, 1H), 3.35 (t, 2H, *J* = 6), 3.64 (s, 3H), 4.02 (m, 1H), 4.41 (d, 1H, *J* = 6), 5.40 (bs, 1H); ^{13}C NMR δ 25.1, 26.2, 27.3, 31.2, 35.4, 39.8, 45.2, 50.3, 52.6, 58.0, 62.8, 64.2, 79.7, 156.0, 169.4, 172.4; HRMS cacld for $C_{18}H_{31}O_6N_2$ (MH^+) 371.2182; found 371.2169.

(3*S*,6*S*,7*R*,9*S*)-Methyl 2-Oxo-3-*N*-(BOC)amino-7-(3-azidopropyl)-1-azabicyclo [4.3.0]nonane-9-carboxylate (152). Alcohol **151** (35 mg, 0.07 mmol) in 15 mL of CH_2Cl_2 was treated with methanesulfonyl chloride (11 μL, 200 mol%), and Et_3N (24 μL, 250 mol%), and stirred at 0 °C for 1 h. The ice bath was removed and the reaction mixture was stirred an additional 1 h at room temperature. The solution was dilute with CH_2Cl_2 (15 mL) and washed with 1M NaH_2PO_4 (10 mL) and brine (10 mL), dried and evaporated. The crude residue was dissolved in acetonitrile (10 mL), treated with NaN_3 (30 mg, 300 mol%), stirred at room temperature for 18 h and evaporated. The crude residue was dissolved in EtOAc (10 mL), washed with 1M NaH_2PO_4 (5 ml) and brine (5 mL), dried and evaporated. The crude product was chromatographed using a gradient of 20-50% EtOAc in hexane. Evaporation of the collected fractions gave **152** (19.2 mg, 68% yield): $[\alpha]_D^{20}$ –18.3 (*c* 0.19 CH_2Cl_2); IR (KBr), 2093 cm^{-1}; ^{1}H NMR δ 1.36-1.39 (m, 1H), 1.44 (s, 9H), 1.57-1.59 (m, 3H),

1.70-1.75 (m, 3H), 1.82-1.86 (m, 1H), 1.90-2.10 (m, 1H), 2.10-2.16 (m, 1H), 2.18 (dd, 1H, J = 6), 2.35-2.46 (m, 1H), 3.29 (t, 2H, J = 6), 3.75 (s, 3H), 4.13-4.19 (m, 1H), 4.53 (d, 1H), 5.50 (br, 1H); ^{13}C NMR δ 26.2, 27.3, 27.5, 28.6, 29.3, 35.2, 45.3, 50.3, 51.5, 52.7, 58.0, 61.6, 79.8, 155.9, 169.4, 172.3. HRMS cacld for $C_{18}H_{30}O_6N_5$ (MH^+) 396.2247 found 396.2255.

(3*S*,6*S*,7*R*,9*S*)-2-Oxo-3-*N*-(BOC)amino-7-(3-azidopropyl)-1-azabicyclo[4,3,0]nonane-9-carboxylic Acid (141). Methyl ester **152** (5.6 mg, 0.014 mmol) in 2 mL of dioxane was treated with 1 M aqueous LiOH (0.03 mL, 200 mol%) and stirred at room temperature for 1.5 h and treated with 1 M NaH_2PO_4 (5 mL). The pH was adjusted to pH ~2 using H_3PO_4 and the mixture was extracted with EtOAc (3x15 mL). The organic solutions were combined, dried and evaporated to a residue that was chromatographed using a gradient of 0-5% AcOH in EtOAc to give **141** (3.4 mg, 0.009 mmol, 64 %): $[\alpha]_D^{20}$ –17.1 (*c* 0.032 CH_2Cl_2); ^{1}H NMR δ 1.46 (s, 9H), 1.52-1.57 (m, 3H), 1.61-1.69 (m, 3H), 1.80-1.85 (m, 1H), 1.91-2.36 (m, 3H), 2.35-2.46 (m, 1H), 3.29-3.36 (m, 2H), 3.72-3.75 (m, 1H), 4.28-4.30 (m, 1H), 4.58 (d, 1H, J = 7.7), 5.50 (br, 1H); ^{13}C NMR δ 25.9, 27.2, 27.6, 28.4, 29.1, 35.0, 45.8, 51.4, 52.7, 58.0, 61.5, 79.7, 154.7, 169.4, 174.8. FAB m/z 404.1 (M+Na 10%), 382.2 (M+1, 17%), 154.0 (90%).

Enantiomeric Purity of (3*S*,6*S*,7*R*,9*S*)-Methyl 2-Oxo-3-*N*-(BOC)amino-7-(3-azidopropyl)-1-azabicyclo[4,3,0]nonane-9-carboxylate (152). A solution of (3*S*, 6*S*, 7*R*, 9*S*)-**12** (7.8 mg, 0.025 mmol) in CH_2Cl_2 (1 mL) was treated with TFA (0.3 mL), stirred for 5 h at room temperature, and the volatiles were removed under vacuum to provide a residue: ^{1}H NMR δ 1.28-1.37 (m 1H), 1.56-1.66 (m, 3H), 1.71-1.77 (m, 1H,), 1.88-2.05 (m, 3H), 2.13-2.20 (m, 2H) 2.40-2.43 (m, 1H), 3.30 (t, 2H J = 6.3), 3.71 (s, 3H), 4.01 (m, 1H), 4.49 (d, 1H J = 8.5). Without further purification, the residue was dissolved in CH_2Cl_2 (2 mL), treated with either (*R*)- or (*S*)-1-phenylethylisocyanate (7.5 mg, 0.05 mmol, 200 mol%) and Et_3N (7 μL, 0.05 mmol,

200 mol%), and stirred at room temperature for 5 h. The volatiles were removed under vacuum and the residue was directly examined by proton NMR spectroscopy. The limits of detection were determined by measuring the diastereomeric methyl ester singlets at 3.73 and 3.72 ppm in $CDCl_3$ in the 400 MHz ^1H NMR spectra. By adding urea (1'*S*)-**153** to urea (1'*R*)-**153** until urea (1'*S*)-**153** methyl ester singlets can be detected. The diastereomeric excess was more than 99%.

Urea (1' *R*)-153: ^1H NMR δ 1.28-1.37 (m, 1H), 1.56-1.78 (m, 6H), 1.71-1.77 (m, 1H), 1.88-2.05 (m, 3H), 2.13-2.20 (m, 2H), 2.40-2.43 (m, 1H), 3.30 (m, 3H), 3.73 (s, 3H), 4.15-4.26 (m, 1H), 4.44 (d, 1H J = 8.9), 4.83 (t, 1H, J = 7.2), 5.09-5.14 (br, 1H), 5.47 (br, 1H), 7.26-7.40 (m, 5H).

Urea (1'*S*)-153: ^1H NMR δ 1.28-1.37 (m, 1H), 1.56-1.78 (m, 6H), 1.71-1.77 (m, 1H), 1.88-2.05 (m, 3H), 2.13-2.20 (m, 2H), 2.40-2.43 (m, 1H), 3.30 (m, 3H), 3.72 (s, 3H), 4.15-4.26 (m, 1H), 4.44 (d, 1H J = 8.9), 4.84 (t, 1H, J = 7.2), 5.09-5.14 (br, 1H), 5.47 (br, 1H), 7.26-7.40 (m, 5H).

Chapter 6
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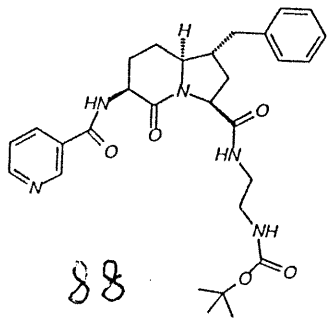
FENG 56

EfN36



88

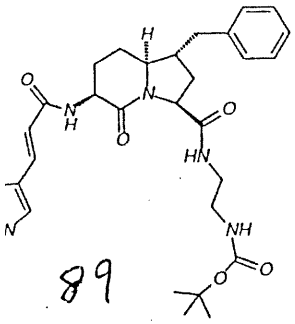
Molecular Weight = 535.65
Molecular Formula = C29H37N5O5

Annexe I

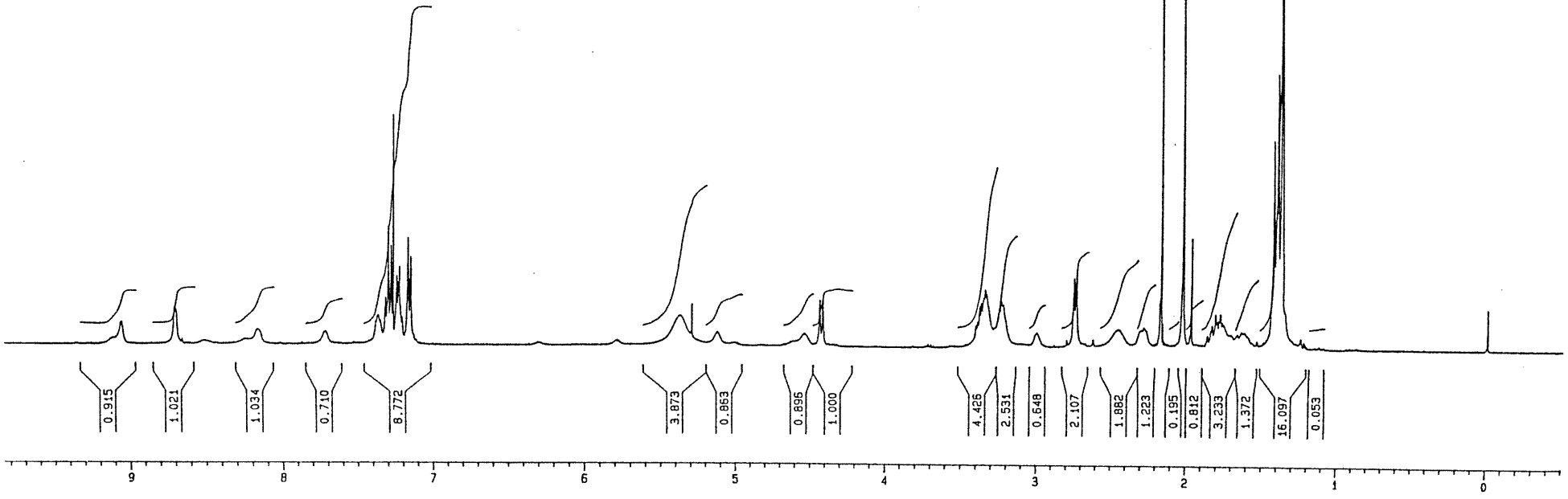
Annexe II

?N36

ENG 56

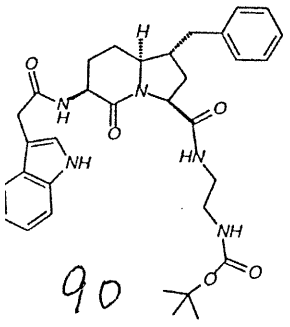


Molecular Weight = 550.66
Molecular Formula = C29H38N6O5



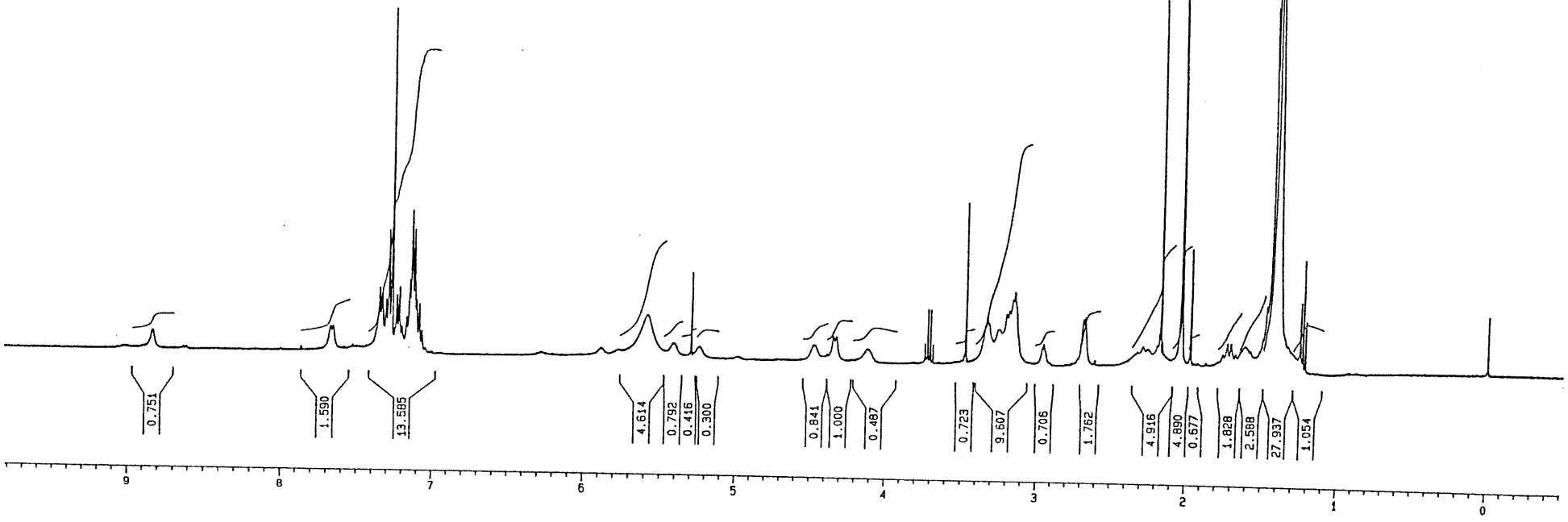
f3 N36

FENG55



90

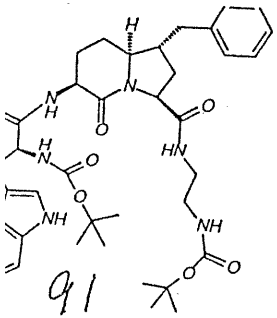
cular Weight = 587.73
cular Formula = C33H41N5O5



Annexe III

ENG 57

Ef1N36

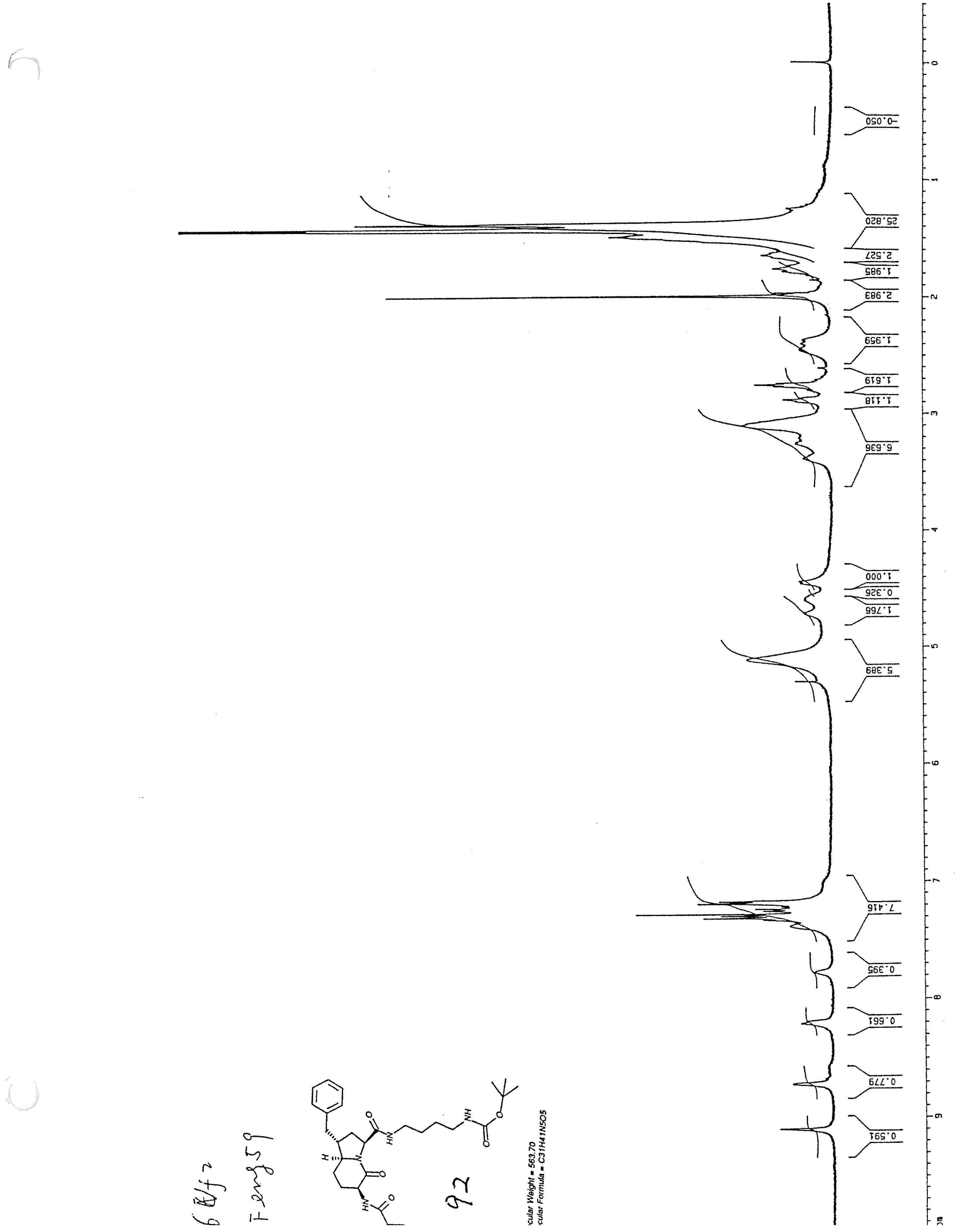


91

lecular Weight = 716.89
lecular Formula = C39H52N6O7

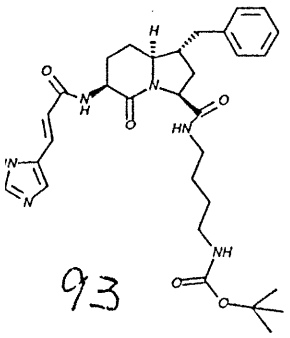
Annexe IV

Annexe V


-0.050
25.820
2.527
1.985
2.983
1.959
1.619
1.118
6.636
1.000
0.326
1.766
5.389
7.416
0.395
0.661
0.779
0.591
0
1
2
3
4
5
6
7
8
9
cular Weight = 563.70
cular Formula = C31H41N5O5
92

FENG 58

Eq N_{f_2}



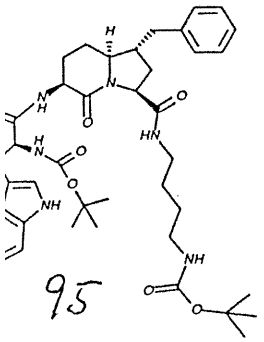
93

Molecular Weight = 578.72
Molecular Formula = C31H42N6O5

Annexe VI

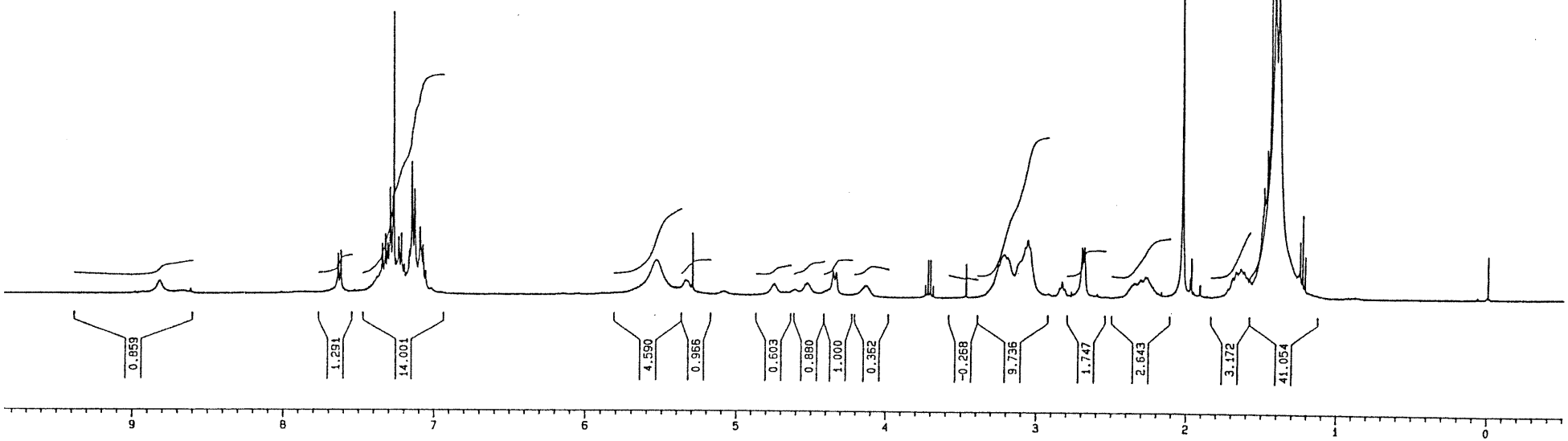
Ef3 Nf2

FENG 60

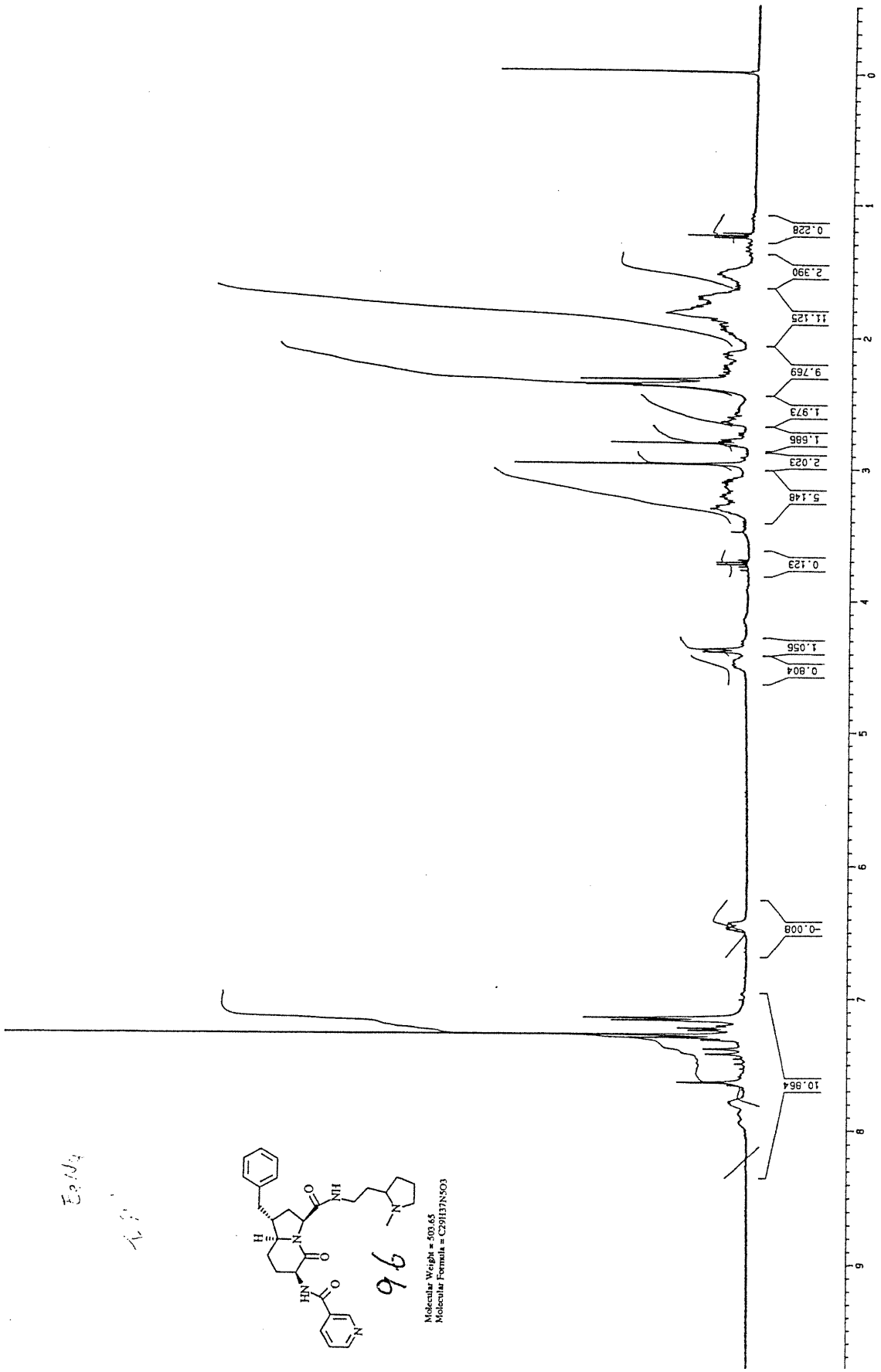


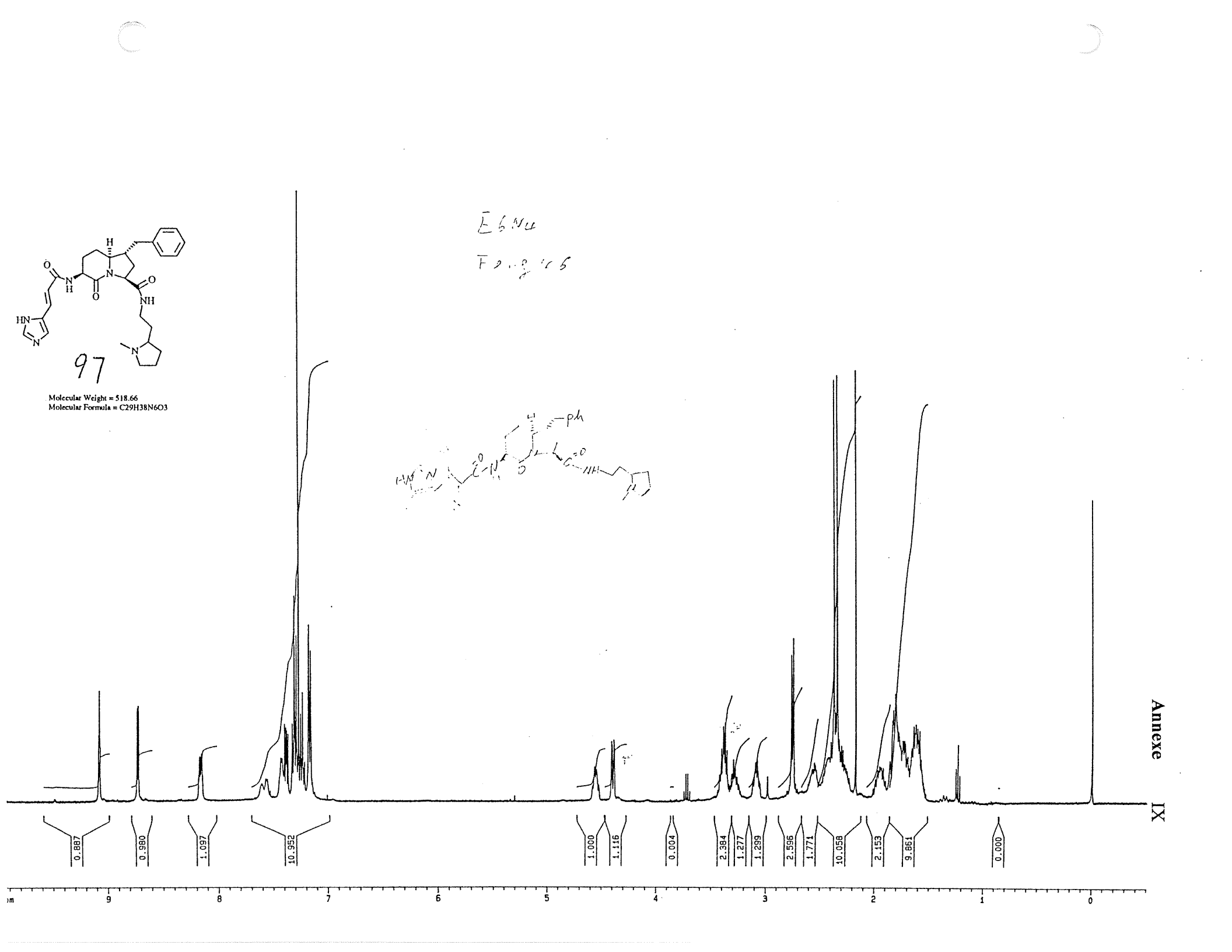
95

lar Weight = 744.94
lar Formula = C41H56N6O7



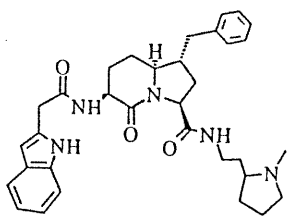
Annexe VII


0.228
2.390
11.125
9.769
1.973
1.686
2.023
5.148
0.123
1.056
0.804
-0.008
10.864
Molecular Weight = 503.65
Molecular Formula = C29H37N5O3
96


97
Molecular Weight = 518.66
Molecular Formula = C29H38N6O3
0.887
0.980
1.097
10.952
1.000
1.116
0.004
2.384
1.277
1.299
2.596
1.771
10.058
2.153
9.861
0.000
9
8
7
6
5
4
3
2
1
0

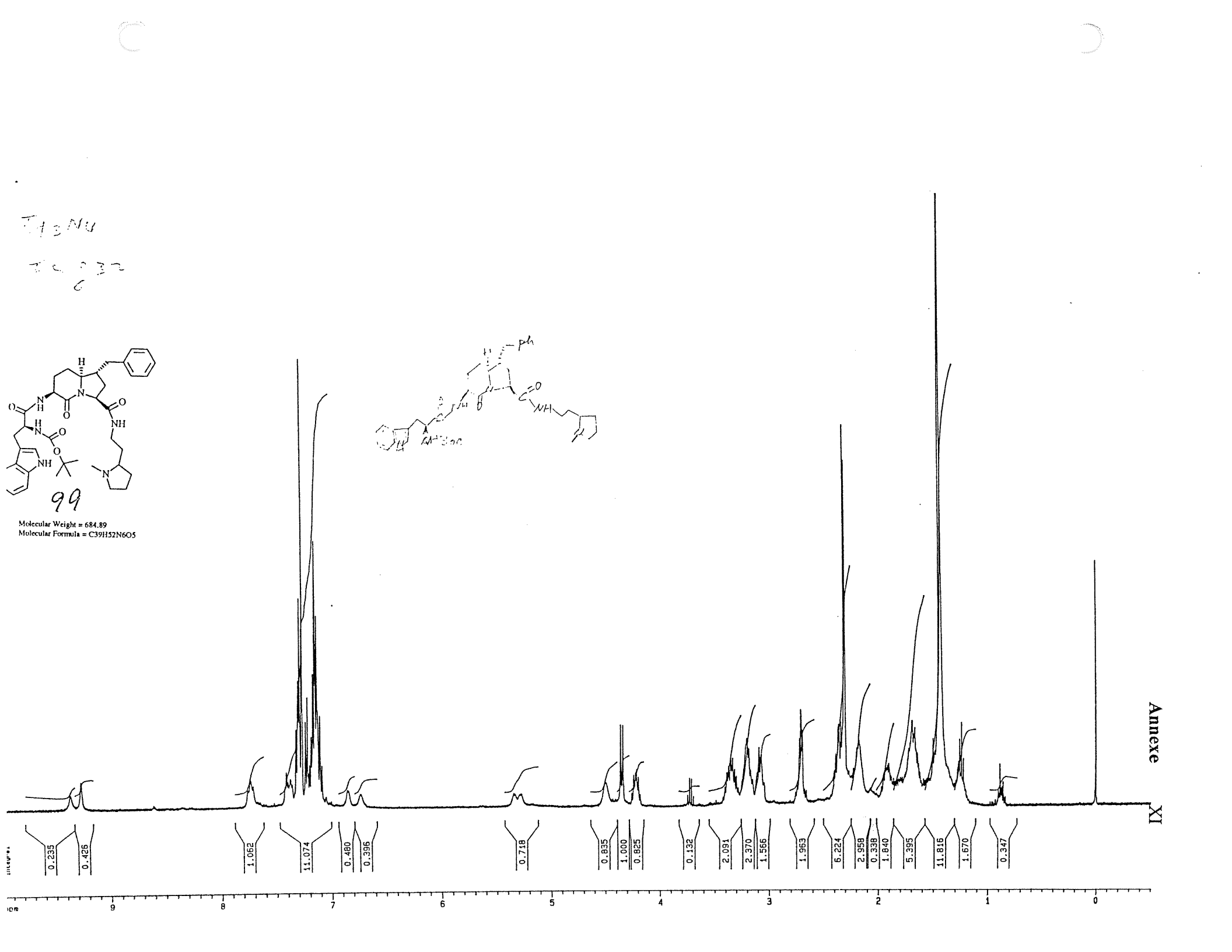
Annexe IX

Et2 N4

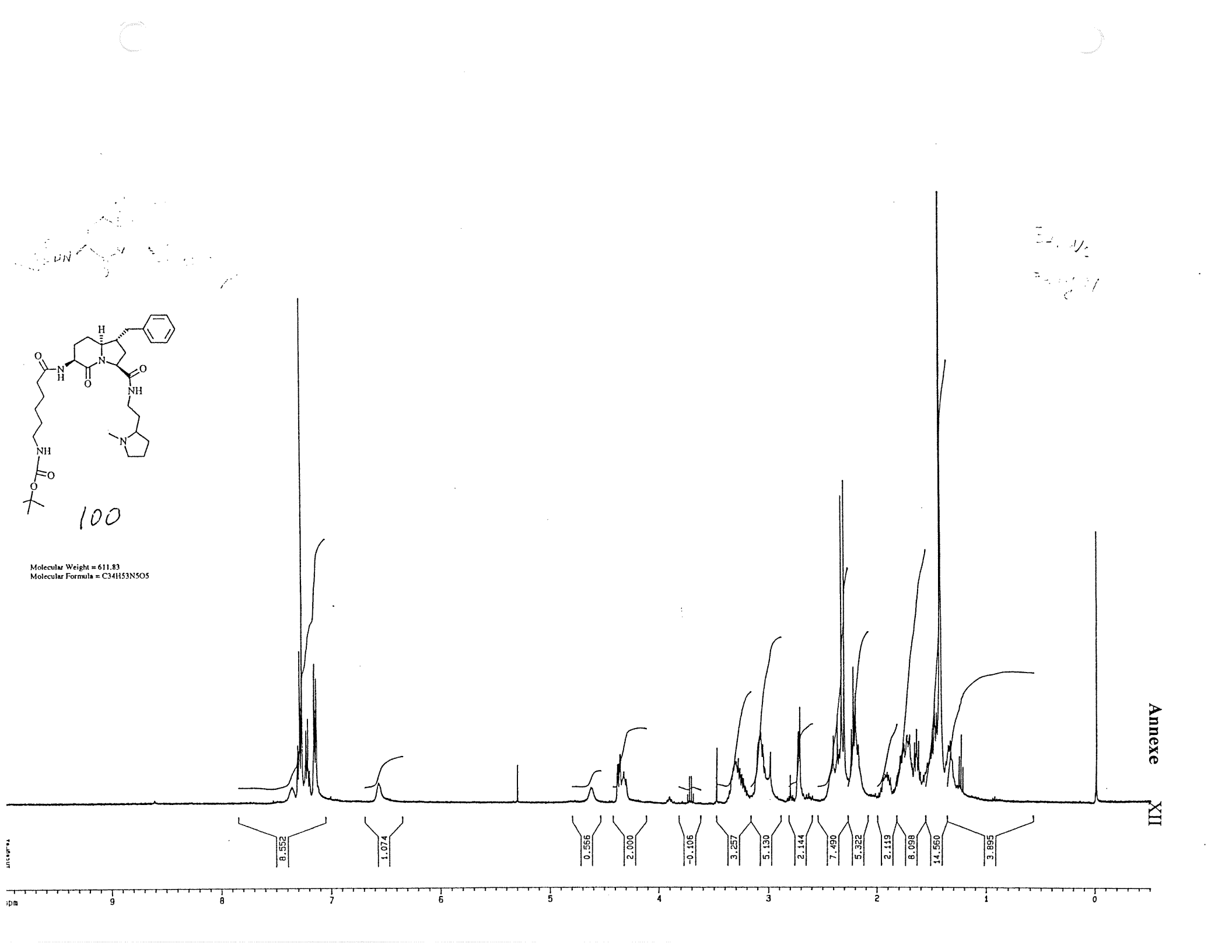


98

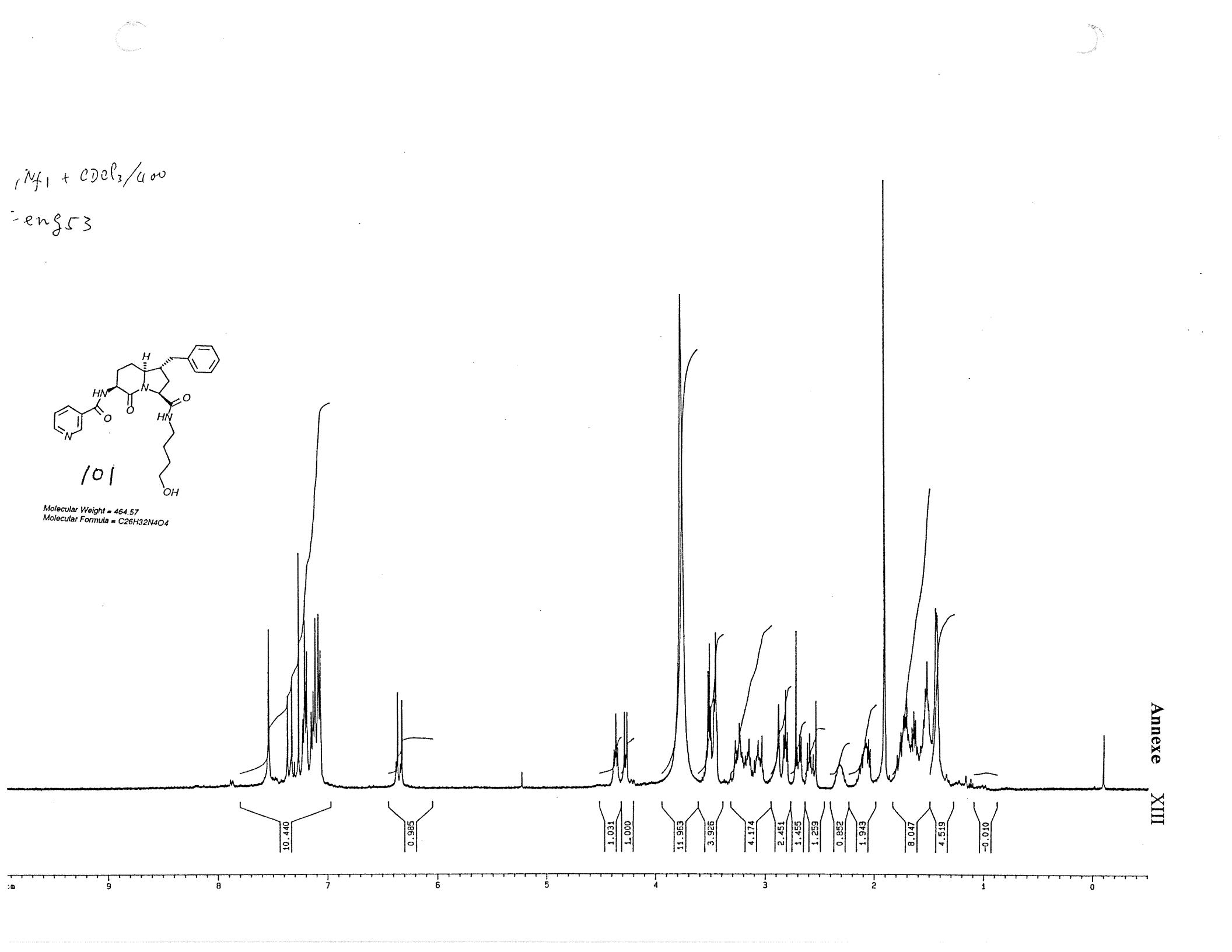
Annexe X


99
Molecular Weight = 684.89
Molecular Formula = C39H52N6O5
0.235
0.426
1.062
11.074
0.480
0.395
0.718
0.835
1.000
0.825
0.132
2.091
2.370
1.566
1.963
6.224
2.958
0.338
1.840
5.395
11.816
1.670
0.347
9
8
7
6
5
4
3
2
1
0

Annexe XI

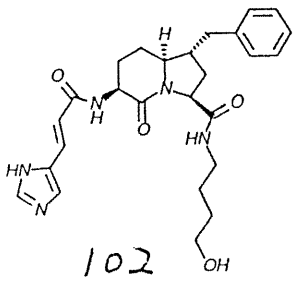

100
Molecular Weight = 611.83
Molecular Formula = C34H53N5O5
8.552
1.074
0.566
2.000
-0.106
3.257
5.130
2.144
7.490
5.322
2.119
8.098
14.560
3.895
ppm
9
8
7
6
5
4
3
2
1
0

Annexe XII


Molecular Weight = 464.57
Molecular Formula = C26H32N4O4
10.440
0.985
1.031
1.000
11.963
3.926
4.174
2.451
1.455
1.259
0.852
1.943
8.047
4.519
-0.010

Annexe XIII

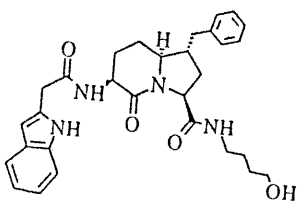
Feng 51



102

Molecular Weight = 479.58
Molecular Formula = C26H33N5O4

Annexe XIV



103

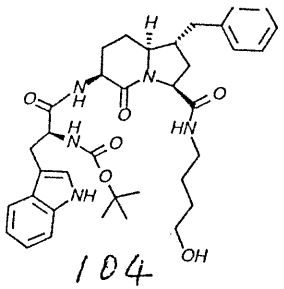
$C_{30}H_{36}N_4O_4$

516.64

Et2 N41

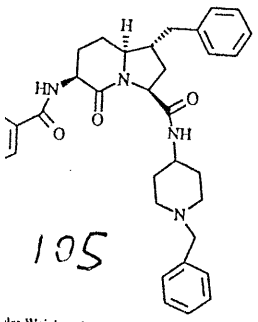
Fig 52

Ef3 N4



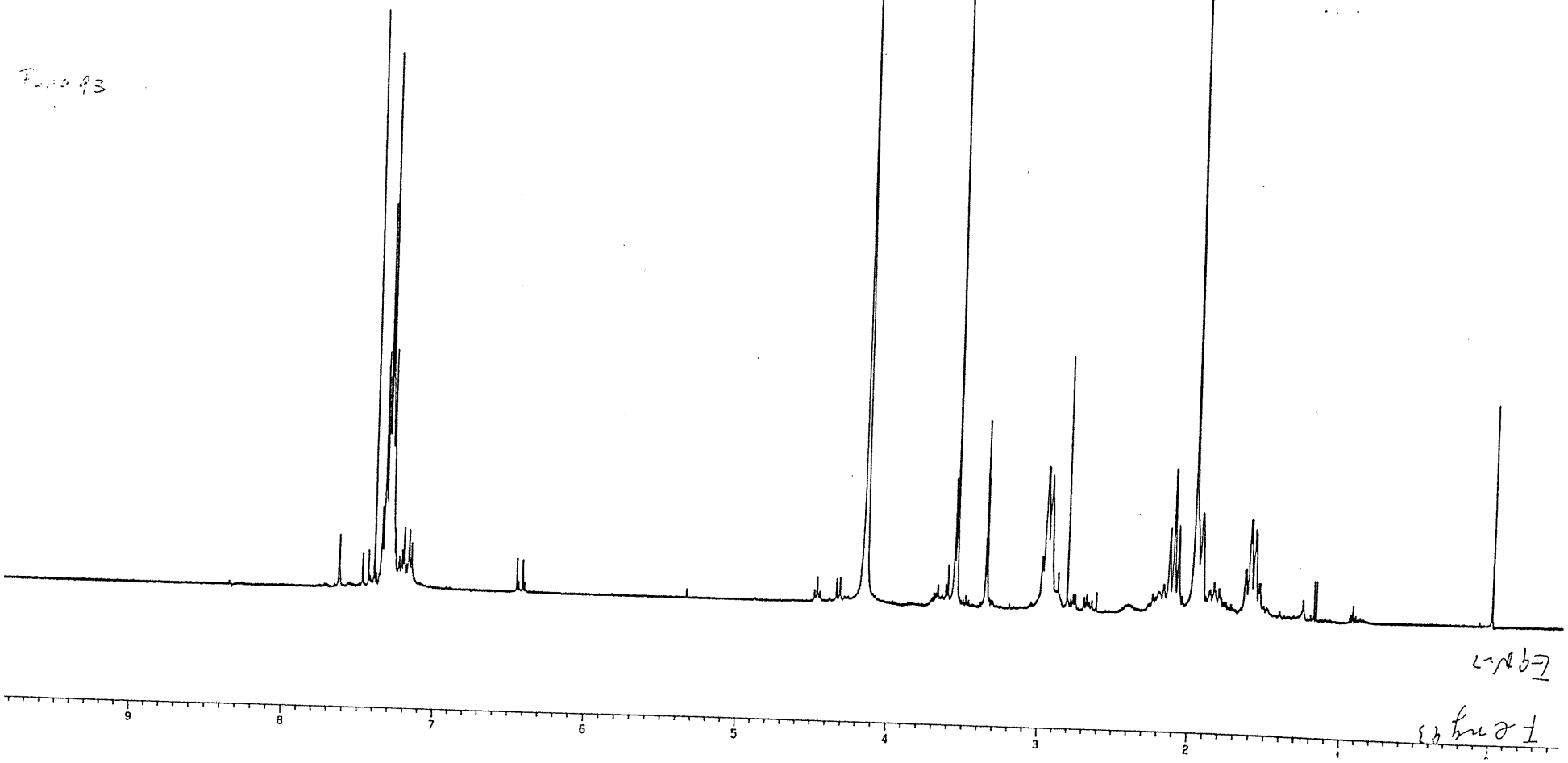
104

Molecular Weight = 645.81
Molecular Formula = C36H47N5O6

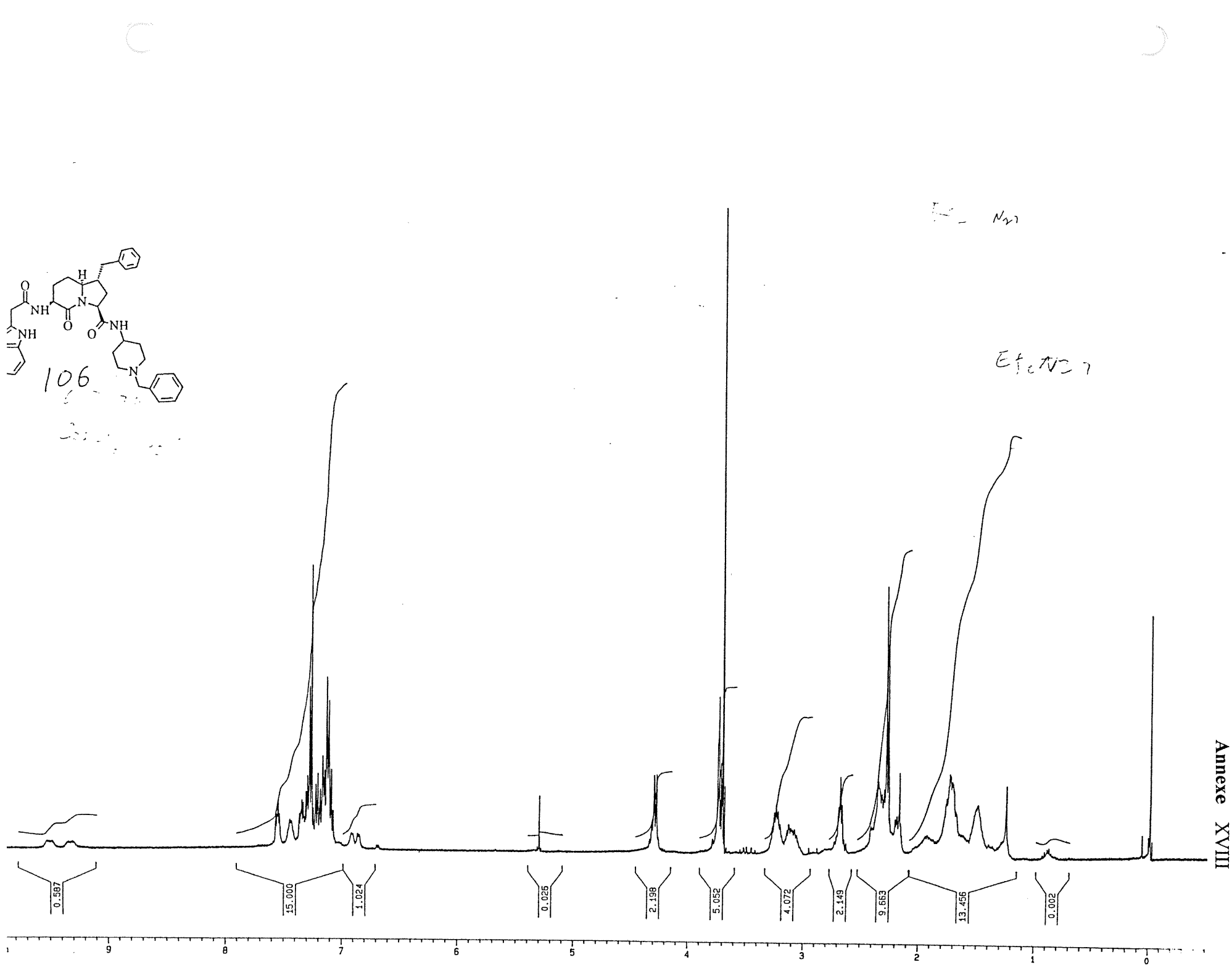


105

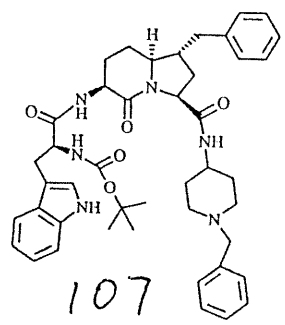
lar Weight = 565.72
lar Formula = C34H39N5O3



Annexe XVII



Annexe XVIII



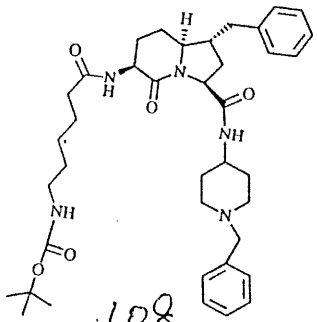
107

Molecular Weight = 746.96
Molecular Formula = C44H54N6O5

Annexe XIX

Fig 97

Ef1N27



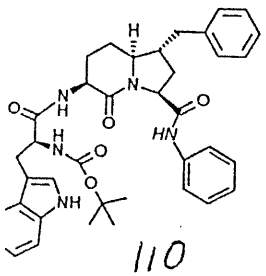
108

EF1N27
Molecular Weight = 673.90
Molecular Formula = C39H55N5O5

Annexe XX

Fey 100

Ef3 N8



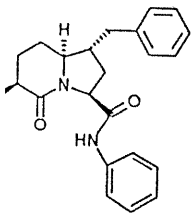
110

EF3N8
Molecular Weight = 649.80
Molecular Formula = C38H43N5O5

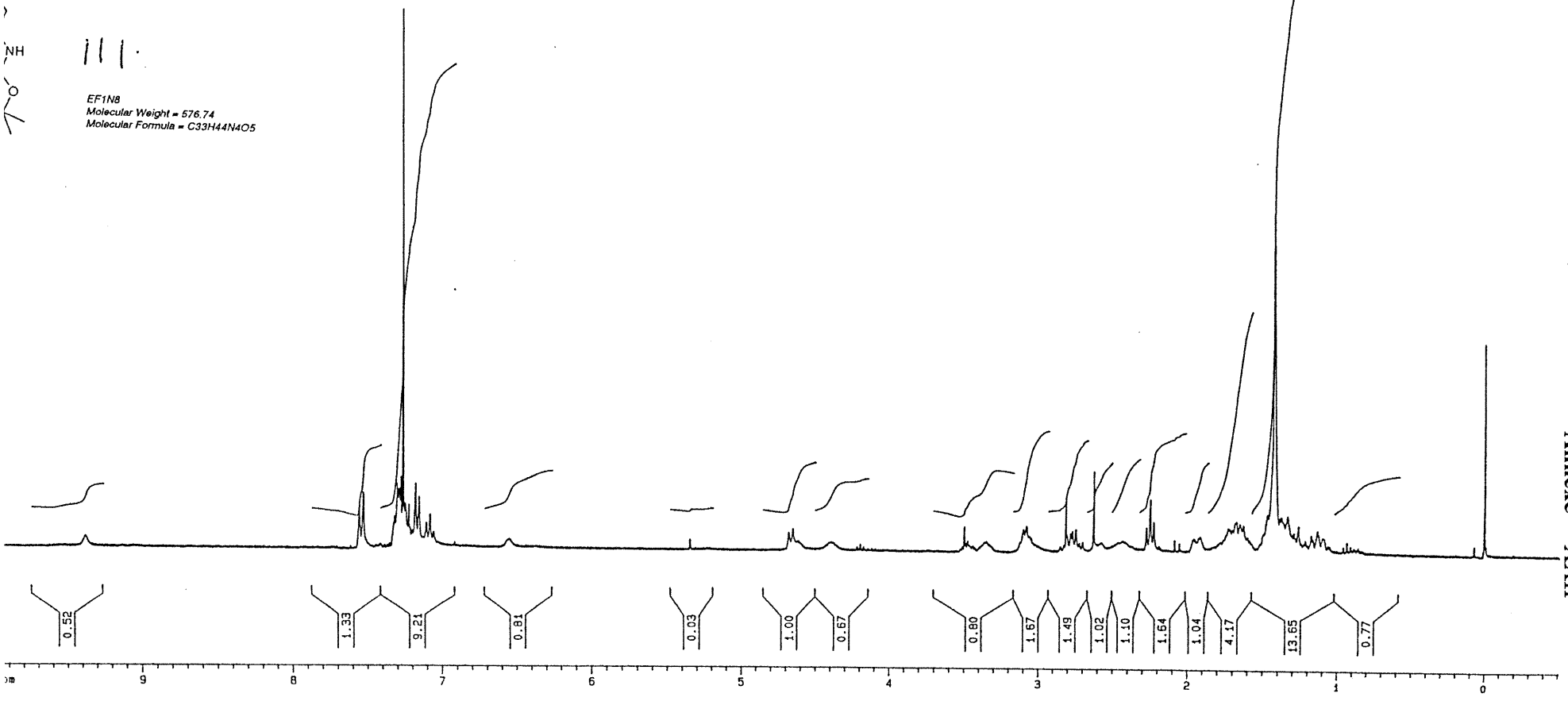
Annexe XXI

eng 99

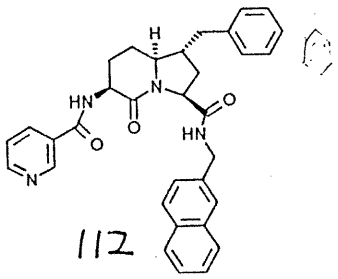
Ef1 N8



EF1N8
Molecular Weight = 576.74
Molecular Formula = C33H44N4O5



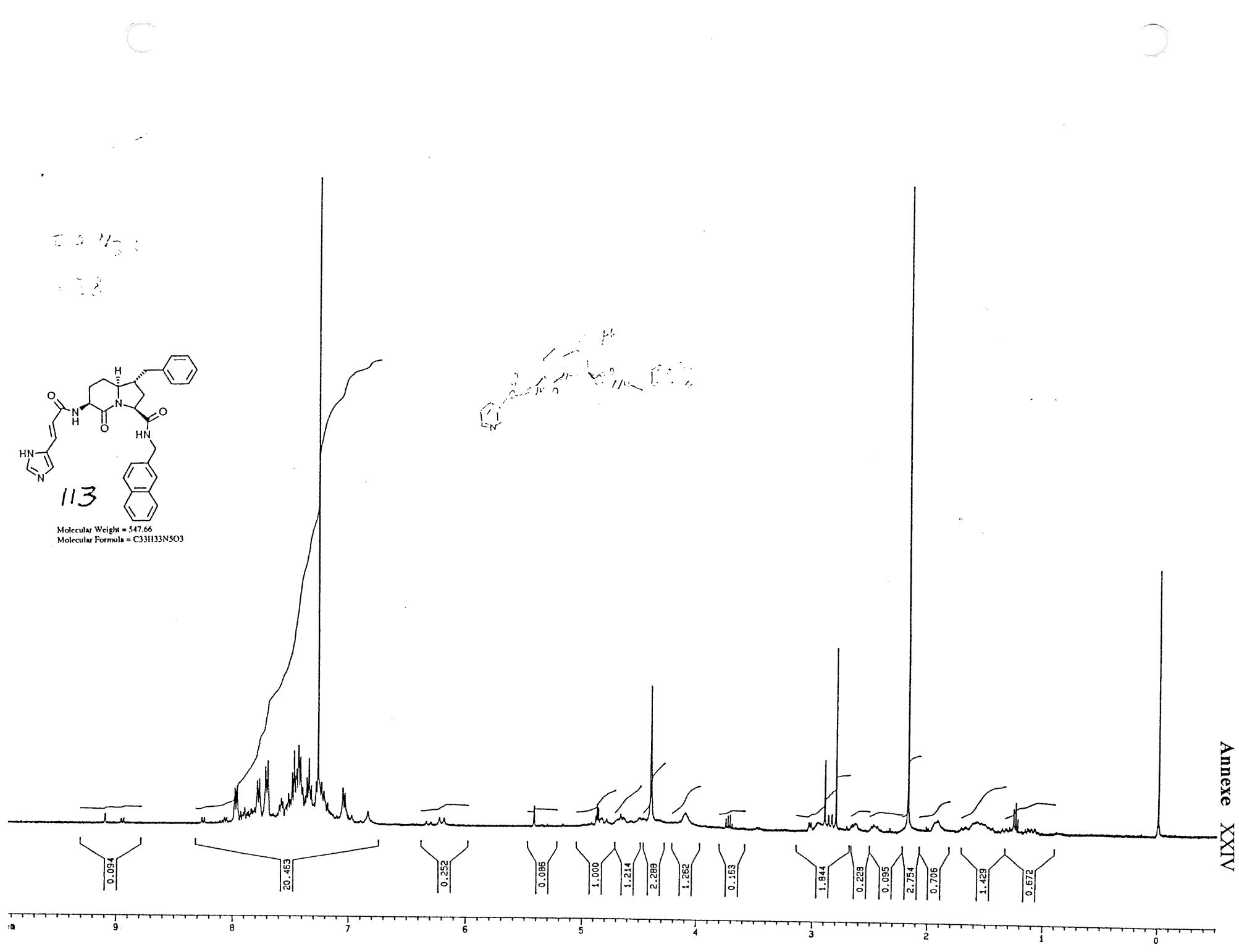
Annexe XXII



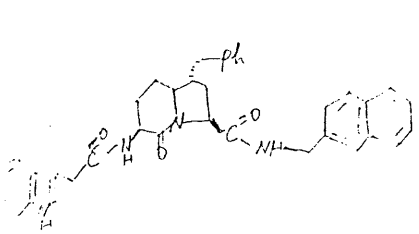
112

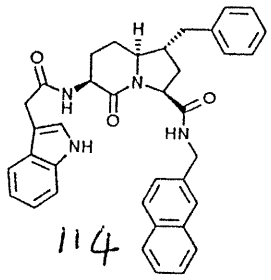
Molecular Weight = 532.65
Molecular Formula = C33H32N4O3

Annexe XXIII


113
Molecular Weight = 547.66
Molecular Formula = C33H33N5O3
0.094
20.463
0.252
0.086
1.000
1.214
2.288
1.262
0.163
1.844
0.228
0.095
2.754
0.706
1.429
0.672
9
8
7
6
5
4
3
2
1
0

Annexe XXIV

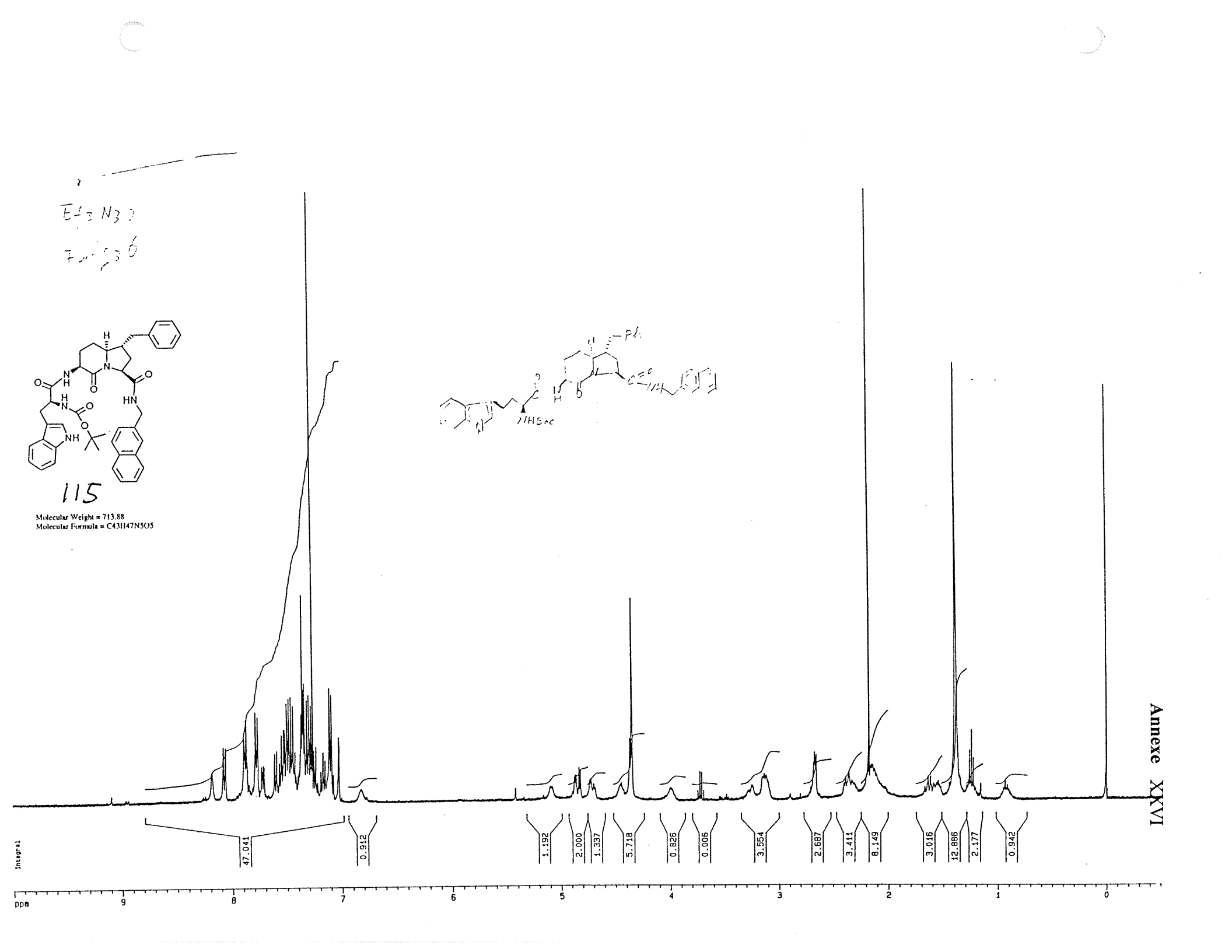




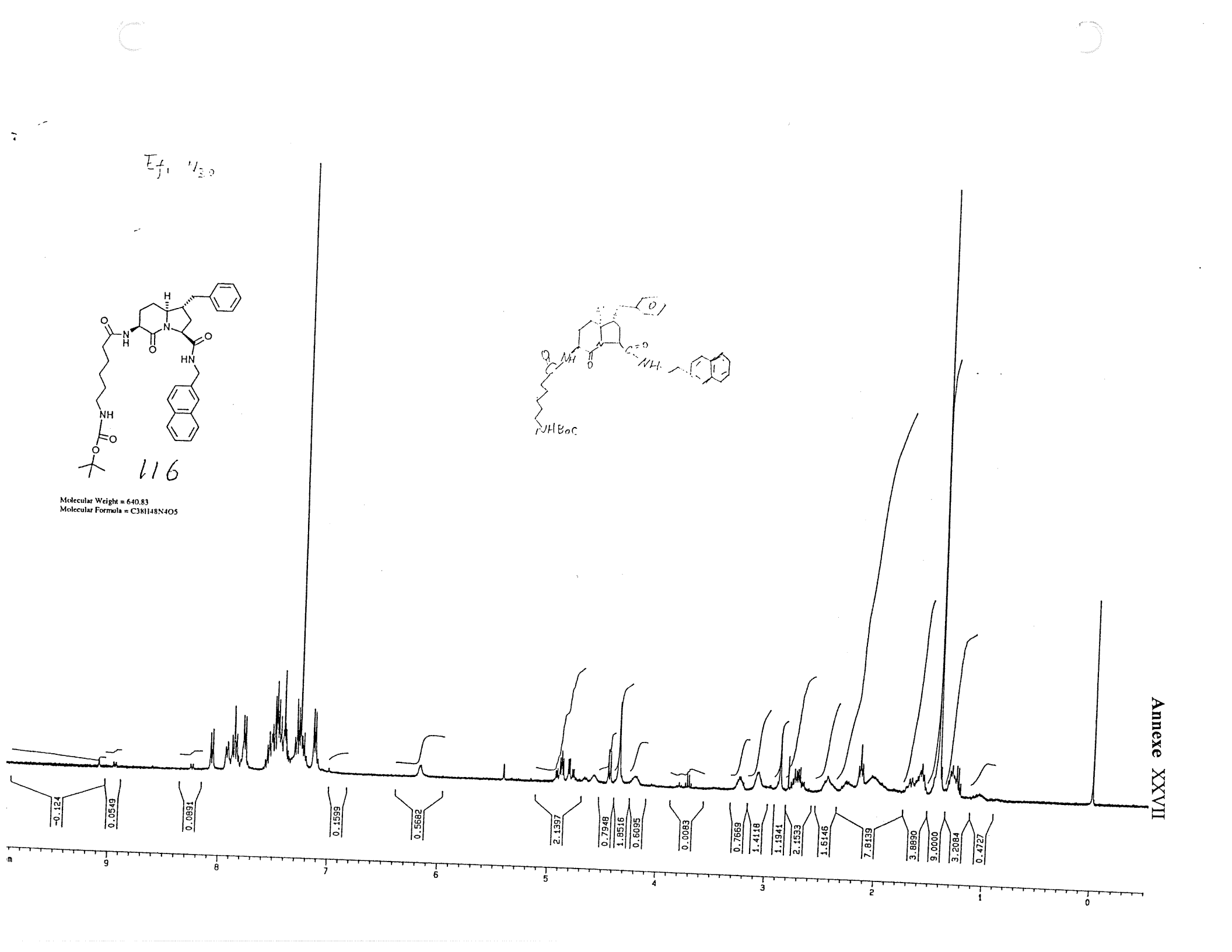
114

Molecular Weight = 584.72
Molecular Formula = C37H36N4O3

Annexe XXV

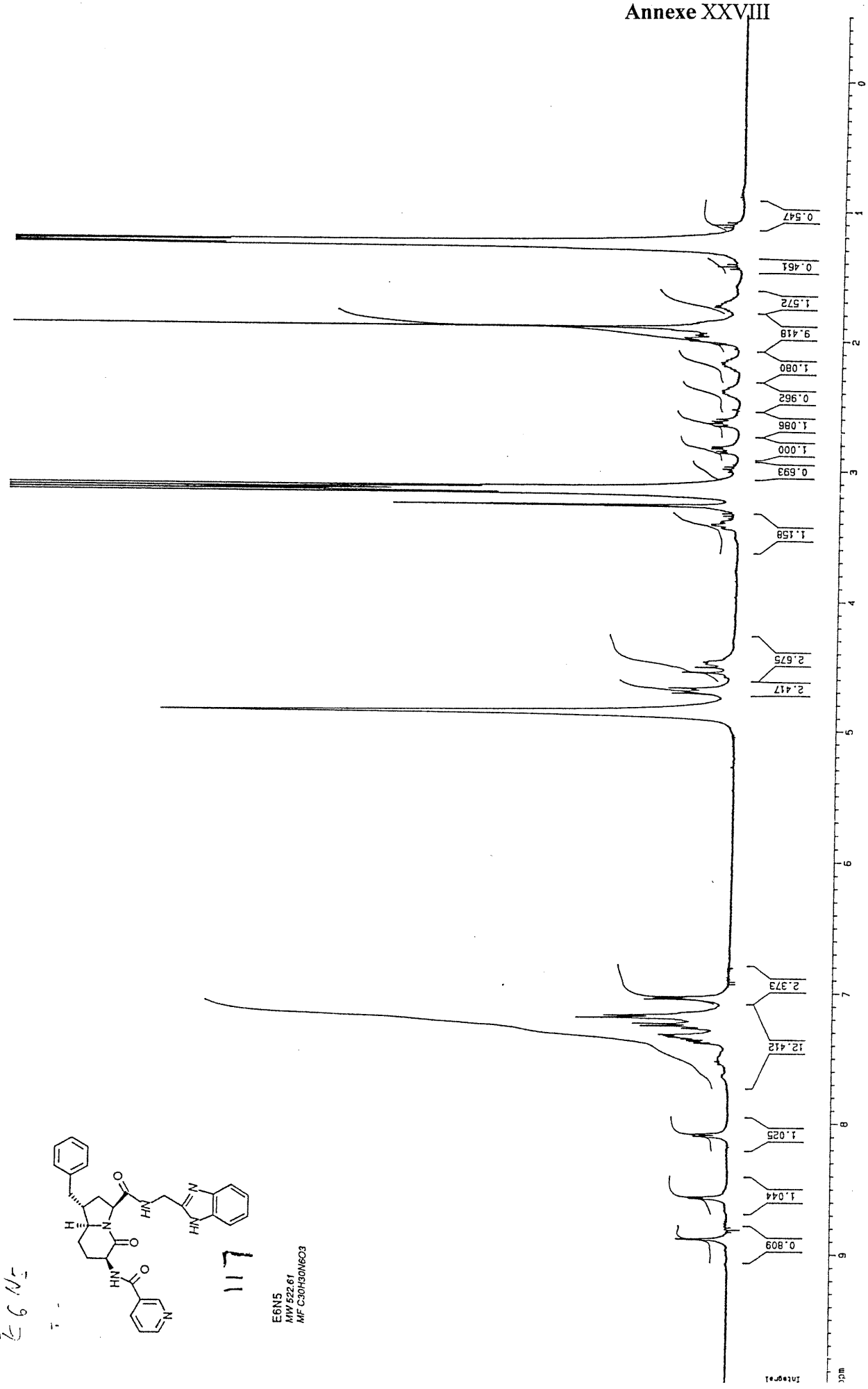

115
Molecular Weight = 713.88
Molecular Formula = C43H47N5O5
47.041
0.912
1.192
2.000
1.337
5.718
0.826
0.006
3.554
2.687
3.411
8.149
3.016
12.886
2.177
0.942
ppm
9
8
7
6
5
4
3
2
1
0

Annexe XXVI

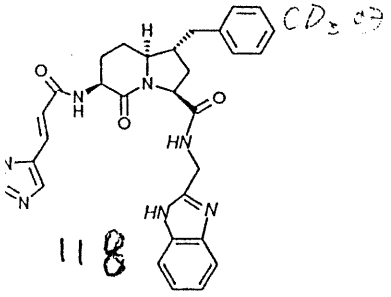


Annexe XXVII

Annexe XXVIII



Fery 100

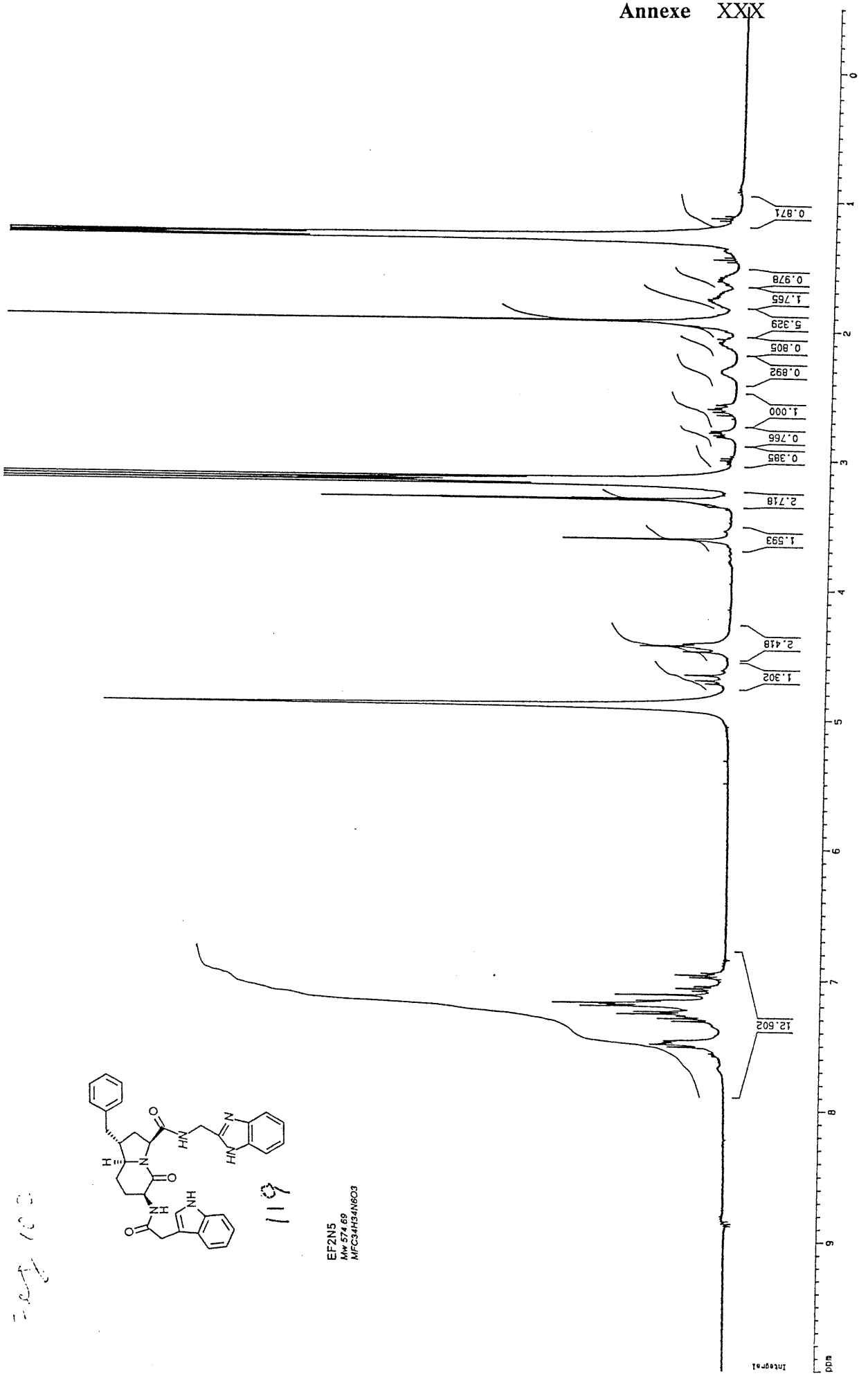


118

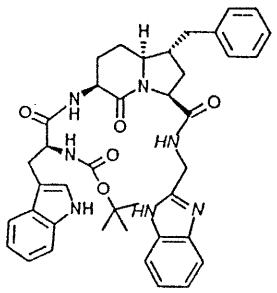
E9N5
MW 537.63
MF C30H31N7O3

Annexe XXIX

Annexe XXIX


0.871
0.978
1.765
5.329
0.805
0.892
1.000
0.766
0.385
2.718
1.593
2.418
1.302
12.602
ppm
EF2N5
Mw 574.69
MFC34H34N6O3

Ef3N5



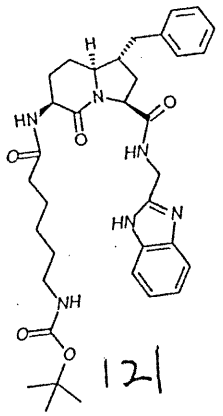
120

EF3N5
MW 703.85
MF C40H45N7O5

Annexe XXXI

Ef1N5

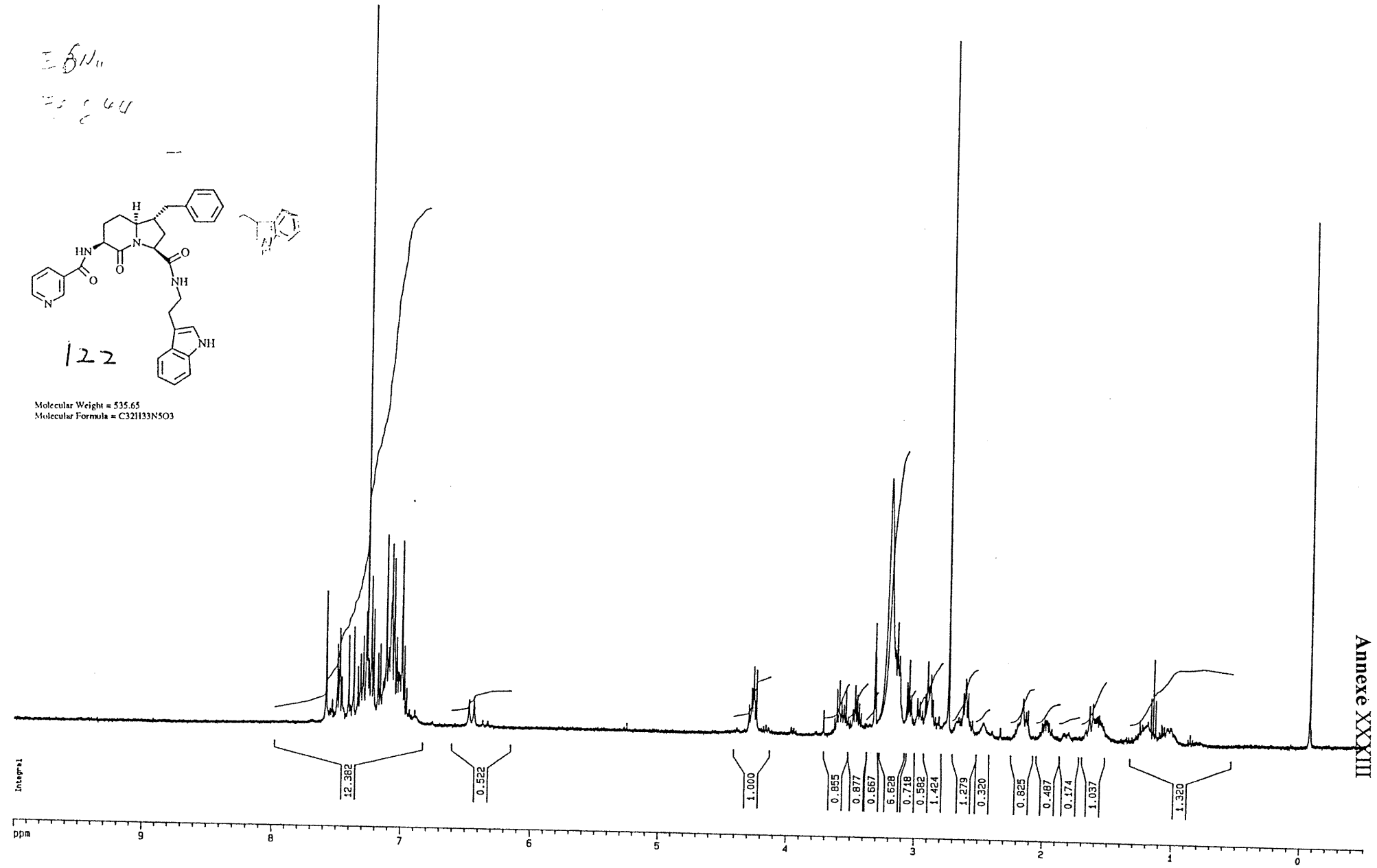
FENG62



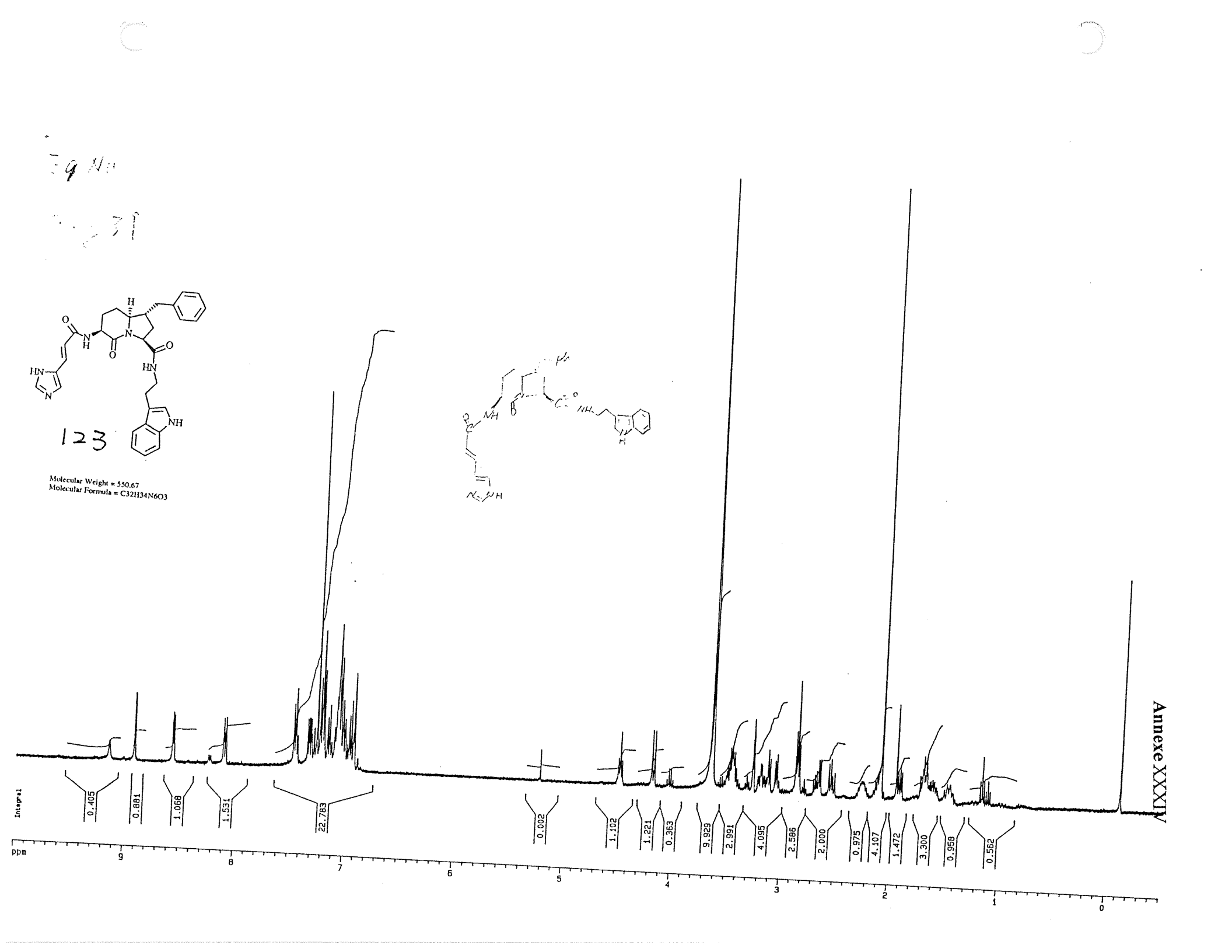
121

EF1N5
MW 630.79
MF C35H46N6O5

Annexe XXXIII

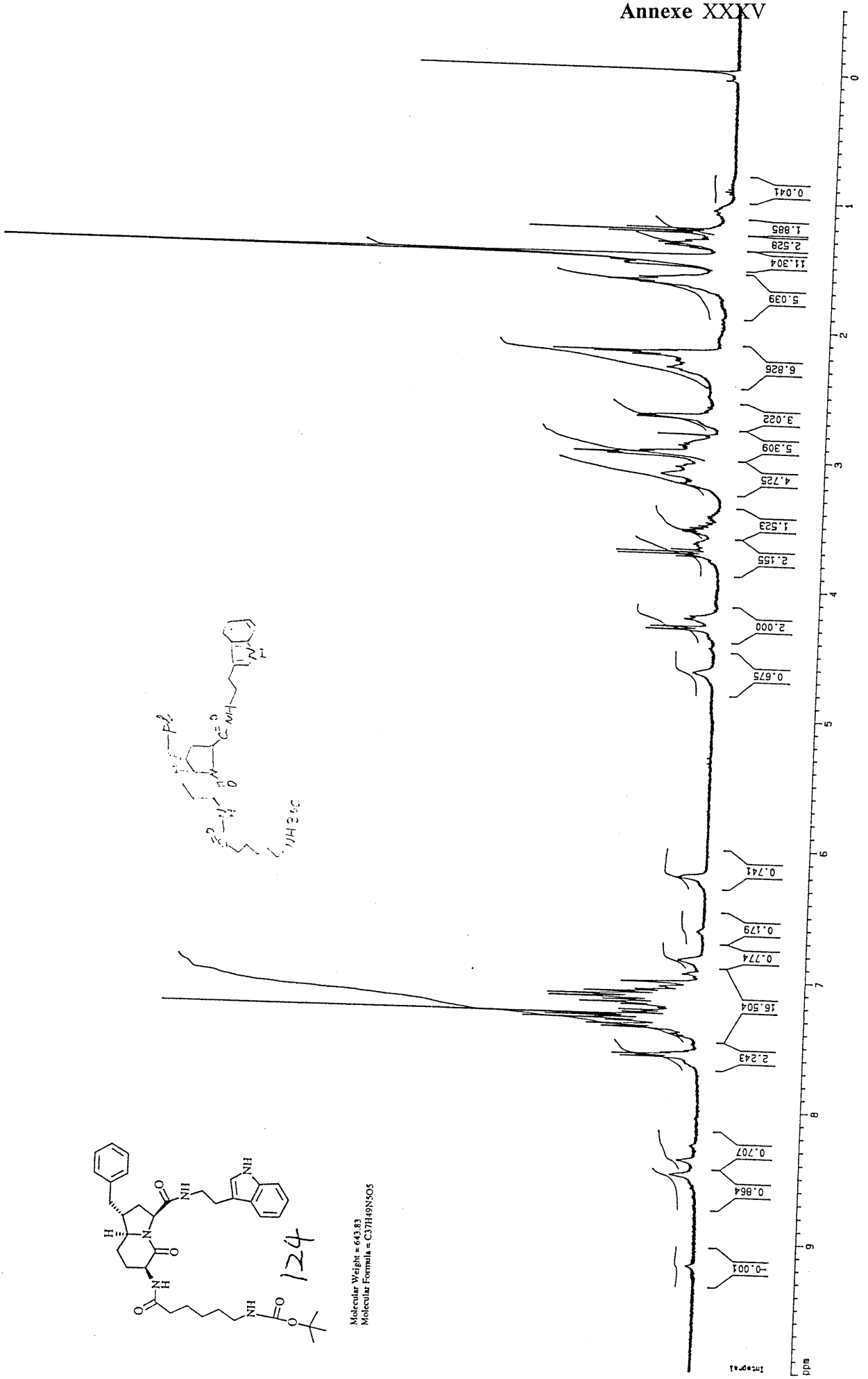


Annexe XXXIII



Annexe XXXIV

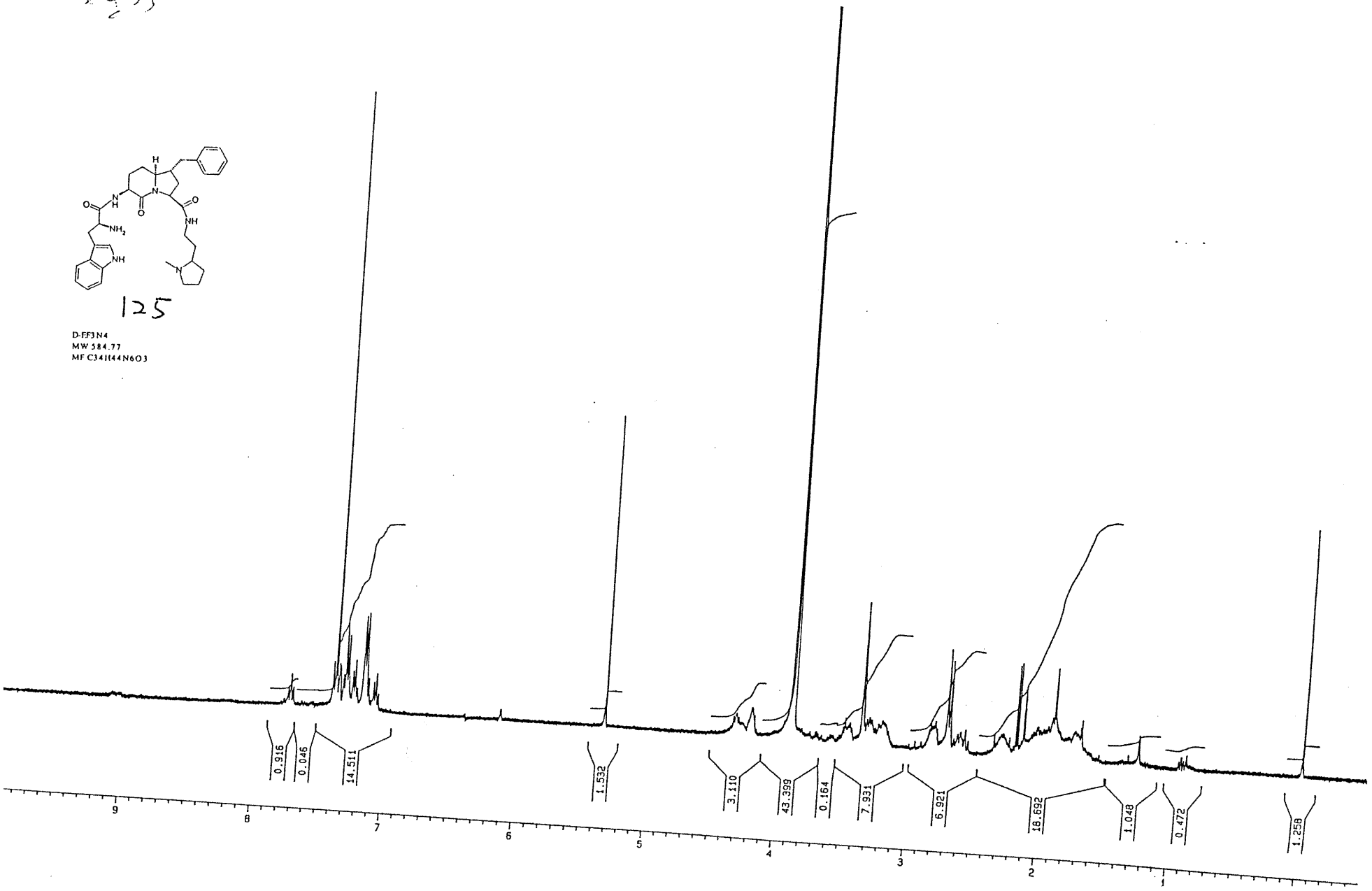
Annexe XXXV


Molecular Weight = 643.83
Molecular Formula = C37H49N5O5
124
0.041
1.885
2.528
11.304
5.039
6.826
3.022
5.309
4.725
1.523
2.155
2.000
0.675
0.741
0.179
0.774
16.504
2.243
0.707
0.864
-0.001
Integral
ppm

Ef3-N4

125

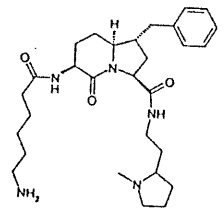
D-FF3N4
MW 584.77
MF C34H44N6O3



Annexe XXXVI

Fig 83

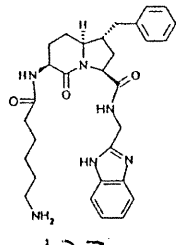
Fig. N4



126

D-EF1N4
MW 511.71
MF C29H45N5O3

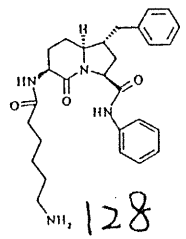
Annexe XXXVII



127

D-EFLN5
MW 530.68
MF C30H38N6O3

Annex XXXVIII

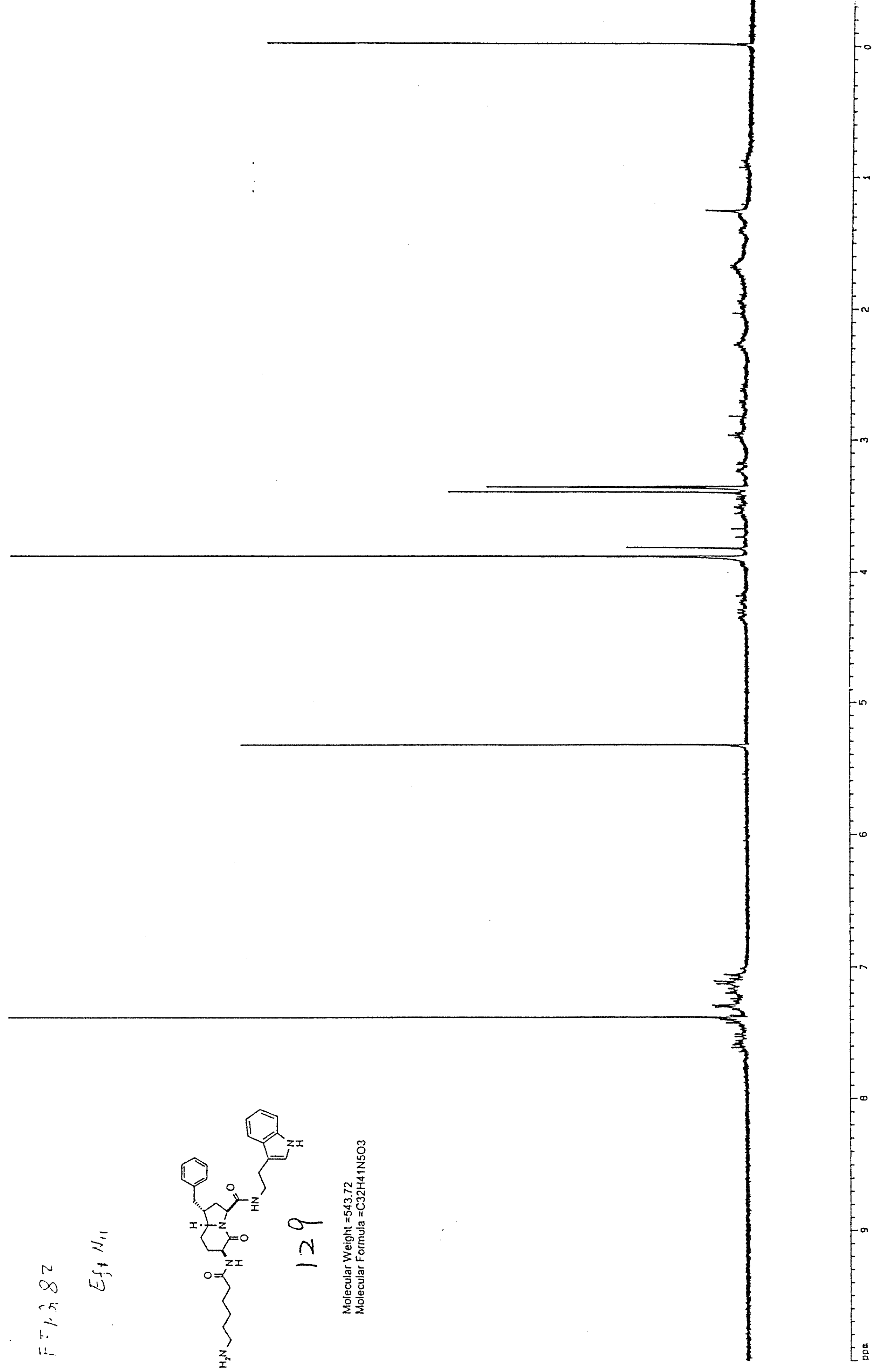


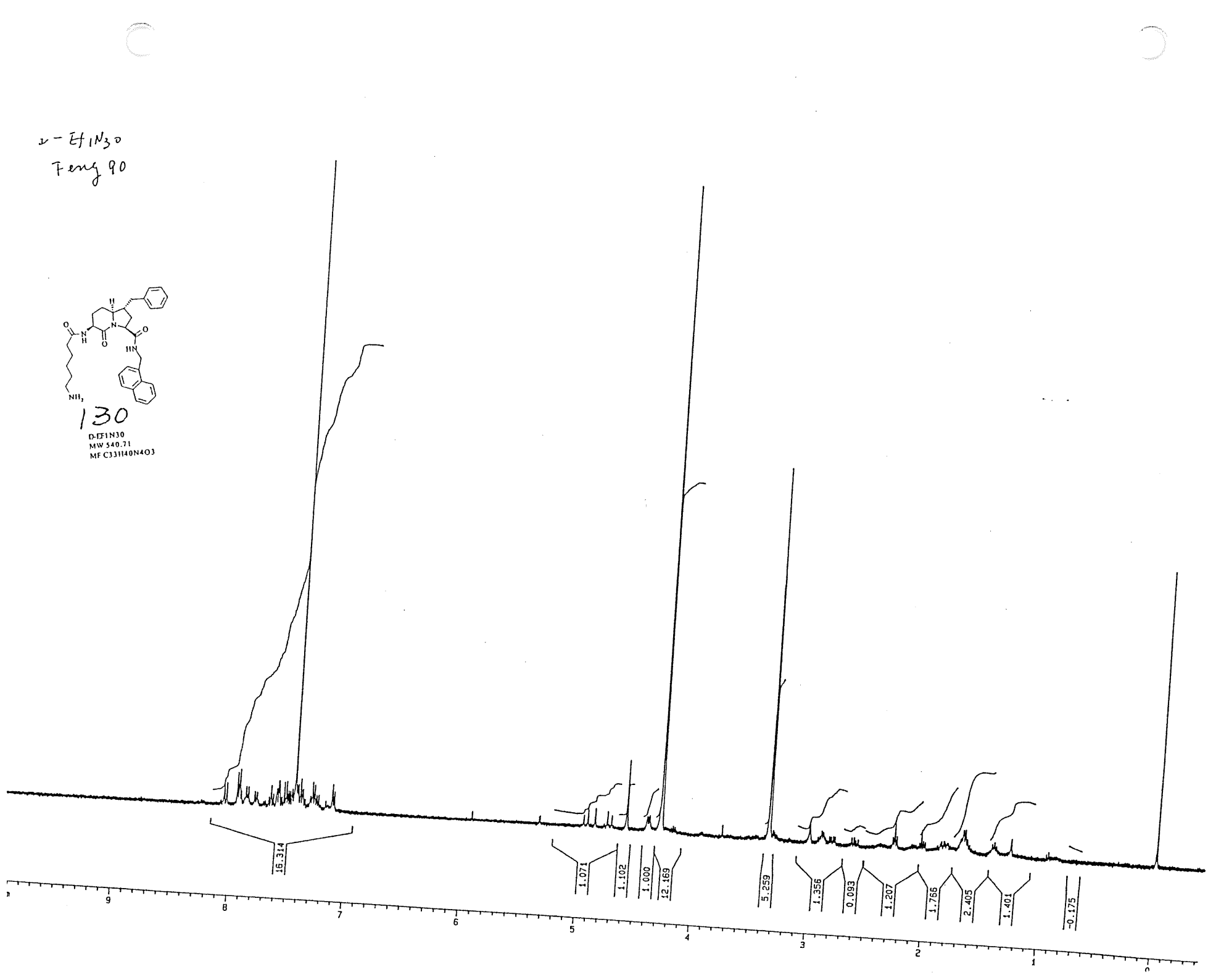
128

D-EF1N8
Molecular Weight = 476.62
Molecular Formula = C28H36N4O3

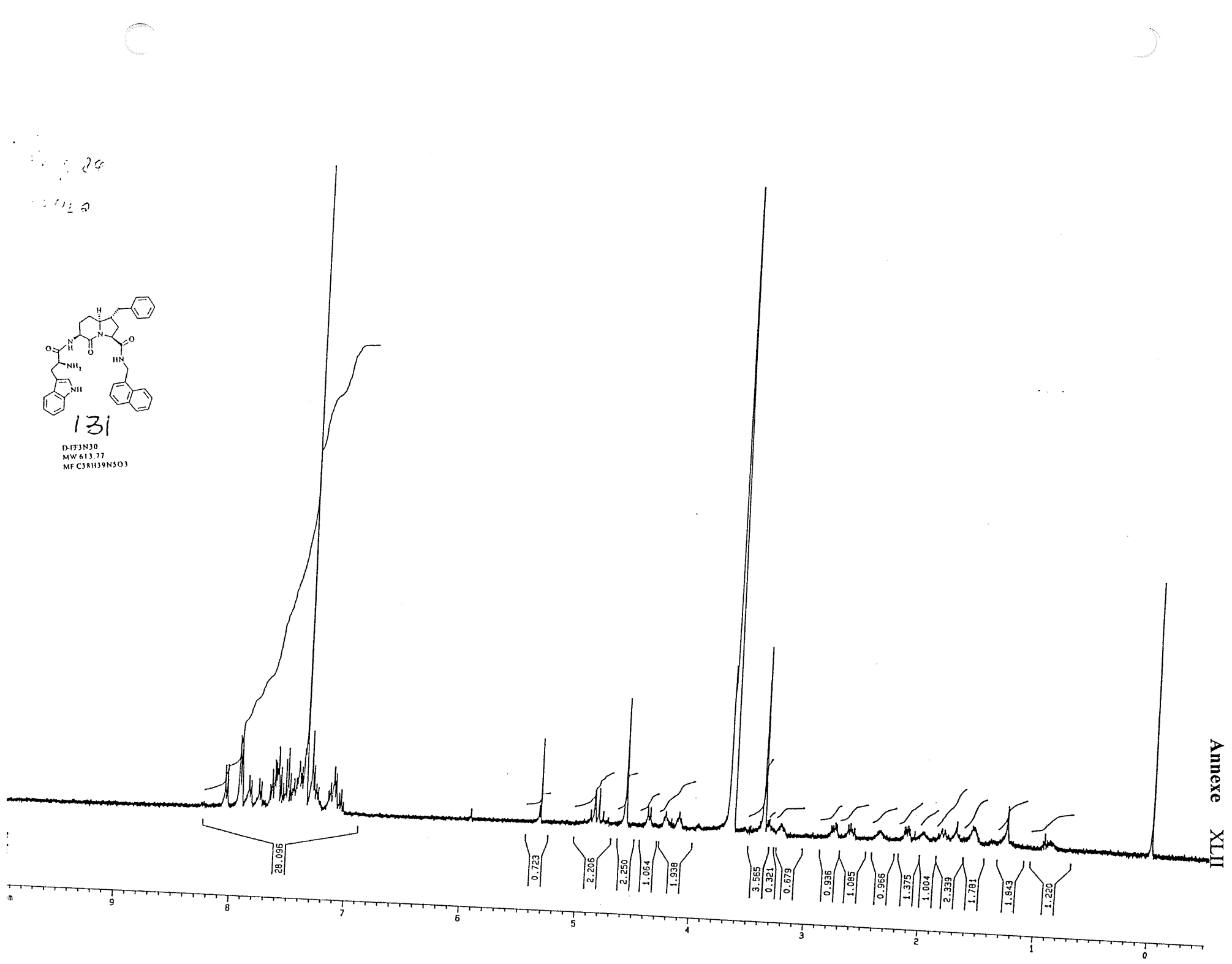
Annexe XXXIX

Annexe XL


0
1
2
3
4
5
6
7
8
9
ppm
FT 1.3.82
129
Molecular Weight =543.72
Molecular Formula =C32H41N5O3

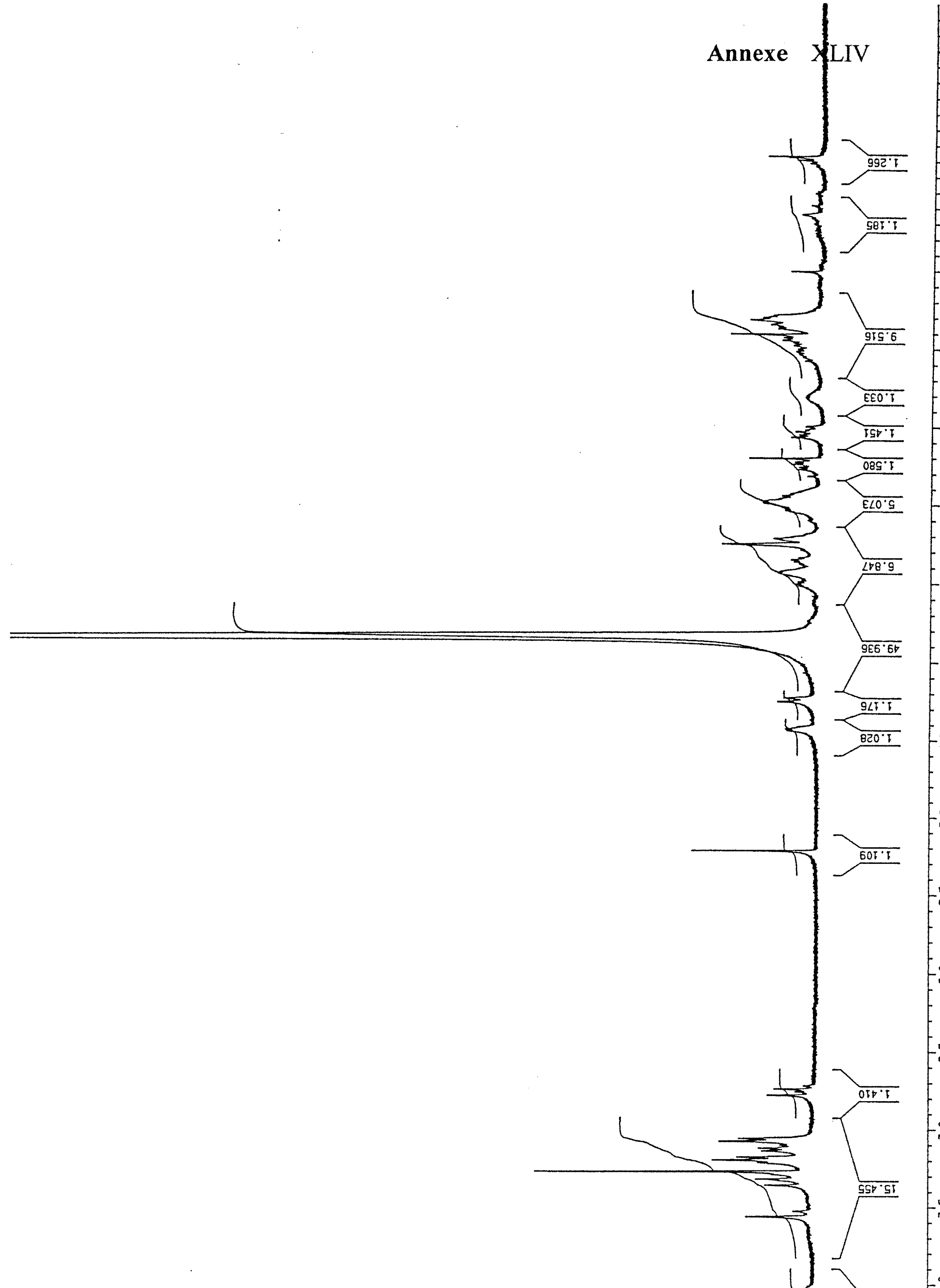

D-EF1N30
MW 540.71
MF C33H40N4O3
130
16.314
1.071
1.102
1.000
12.169
5.259
1.356
0.093
1.207
1.766
2.405
1.401
-0.175

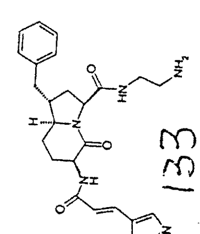
Annexe XLI


131
D-FF3N30
MW 613.77
MF C38H39N5O3
28.096
0.723
2.206
2.250
1.064
1.938
3.565
0.321
0.679
0.936
1.085
0.966
1.375
1.004
2.339
1.781
1.843
1.220
9
8
7
6
5
4
3
2
1
0

Annexe XLII

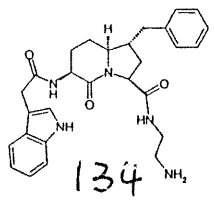
Annexe XLIV





133

D-E9N36
MW 450.55
MF C24H30N6O3



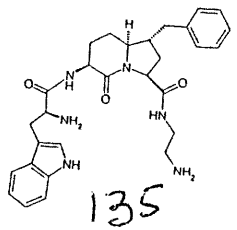
134

D-EF2N36
MW 487.61
MF C28H33N5O3

Annexe XLV

Exp > 6

Ef3 1136



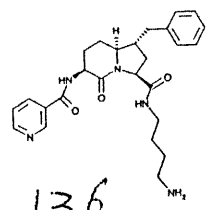
135

D-EF3N36
MW 516.65
MF C29H36N6O3

Annexe XLVI

Fang 72

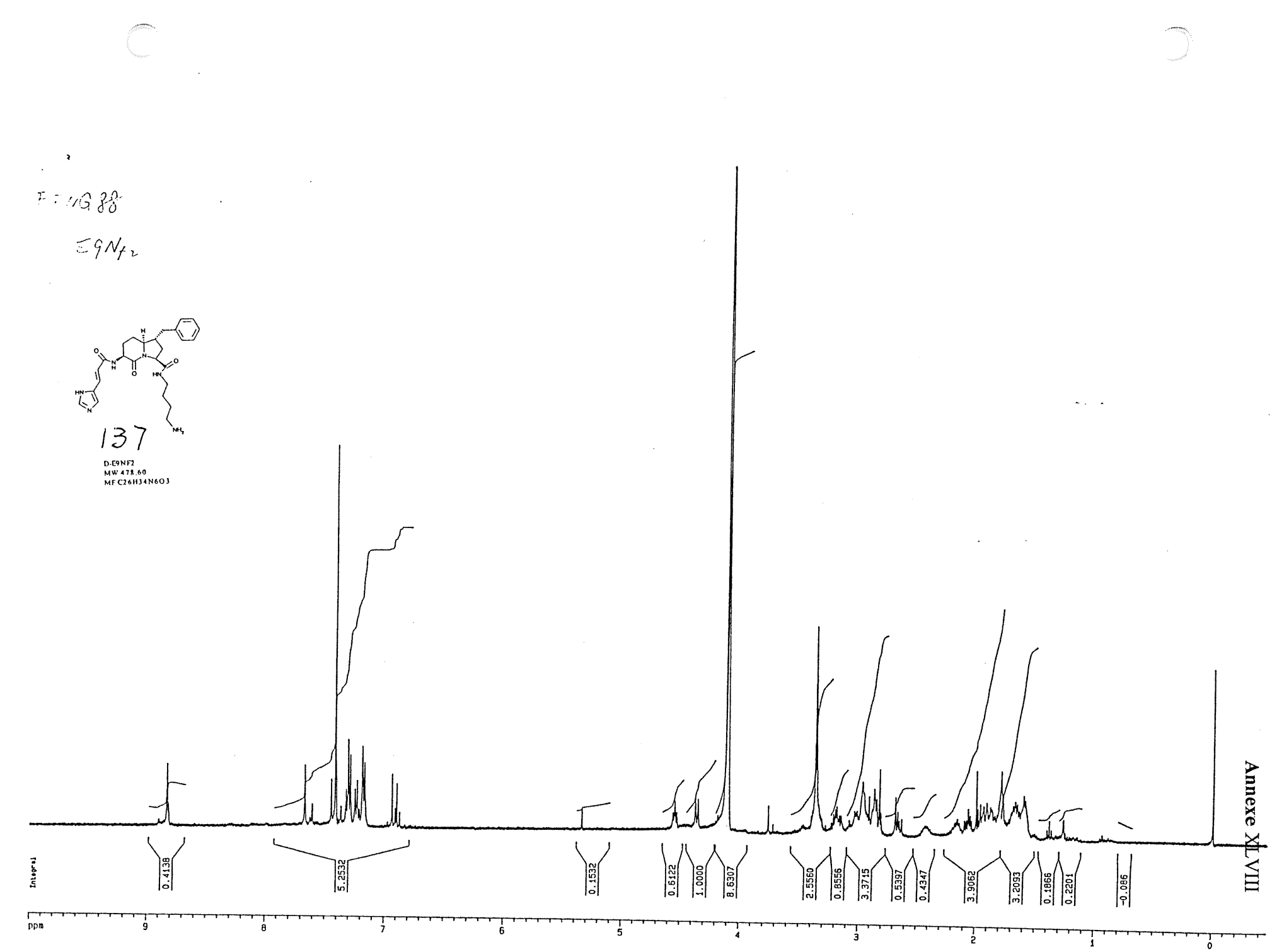
D-E6 Nf2



136

D-E6NF2
MW 463.58
MF C26H33N5O3

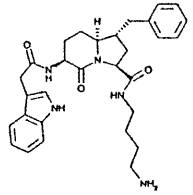
Annexe XII/VII



Annexe XLVIII

, 2Nf2

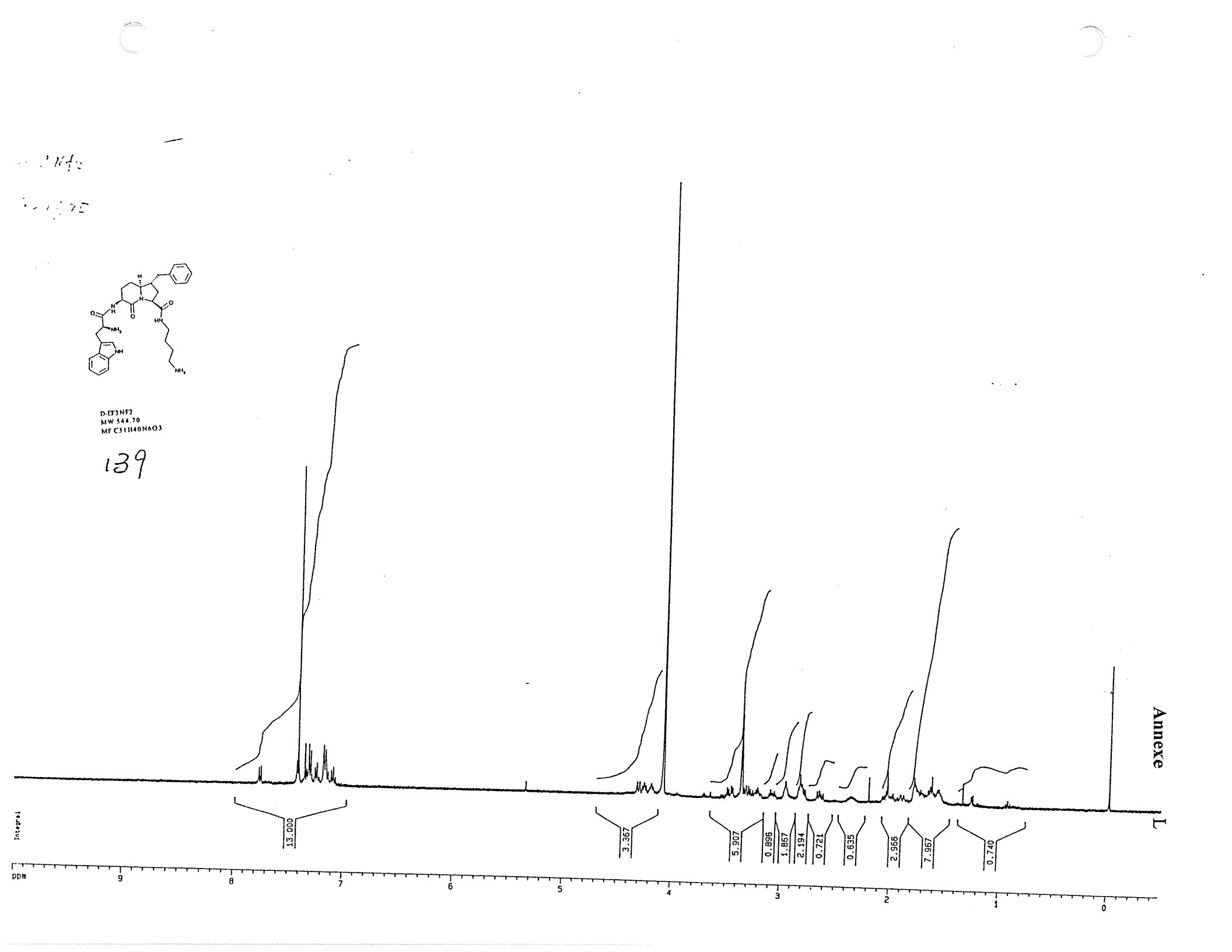
Feng 88

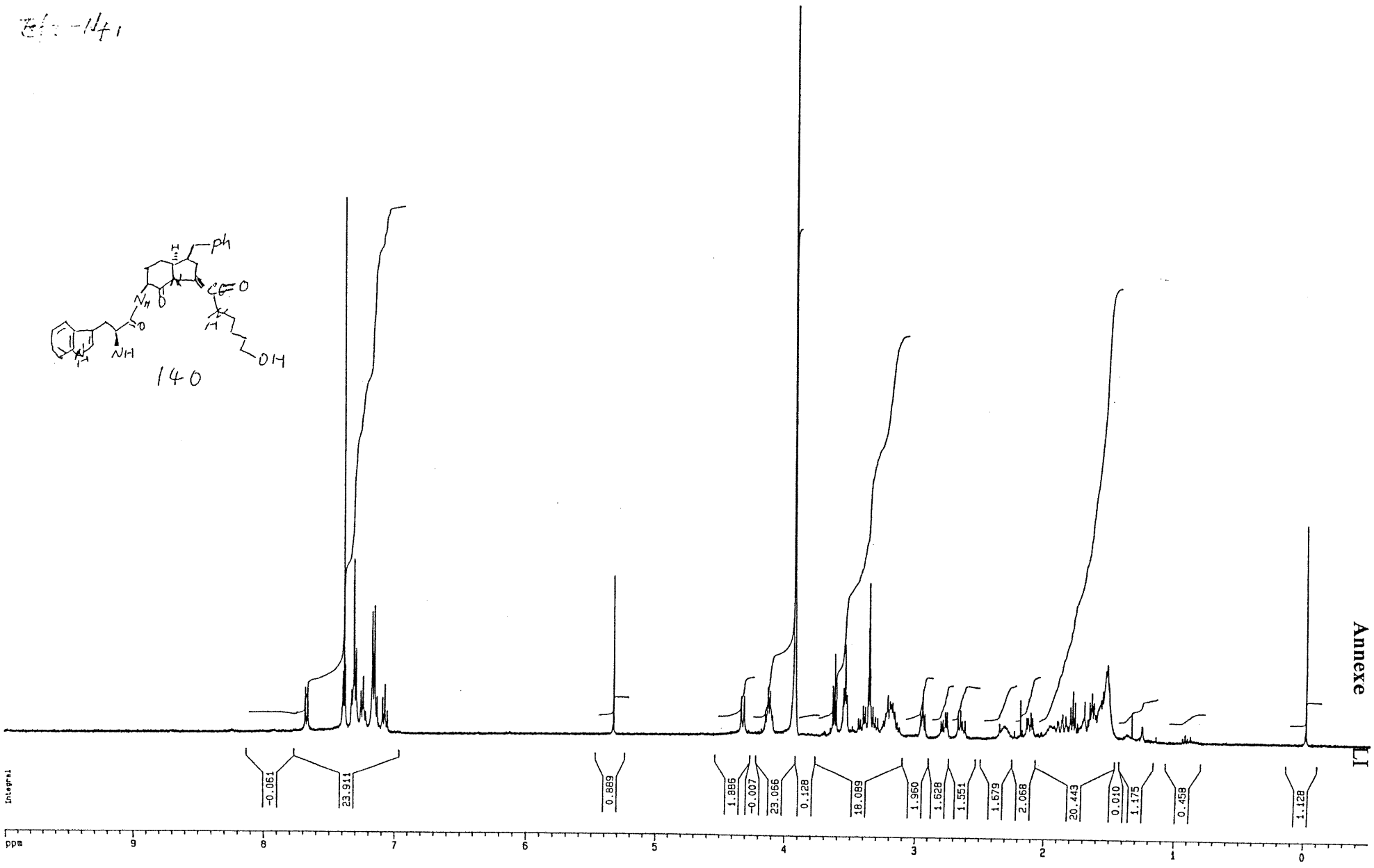


D-EF2NF2
MW 515.66
MF C30H37N5O3

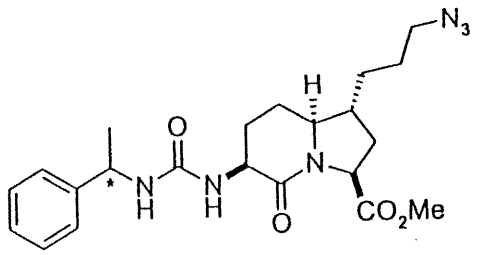
138

Annexe XLIX


D-TF3NF2
MW 544.70
MF C31H40N6O3
139
13.000
3.367
5.907
0.896
1.867
2.194
0.721
0.635
2.966
7.967
0.740
Integral
ppm
9
8
7
6
5
4
3
2
1
0
Annexe
L

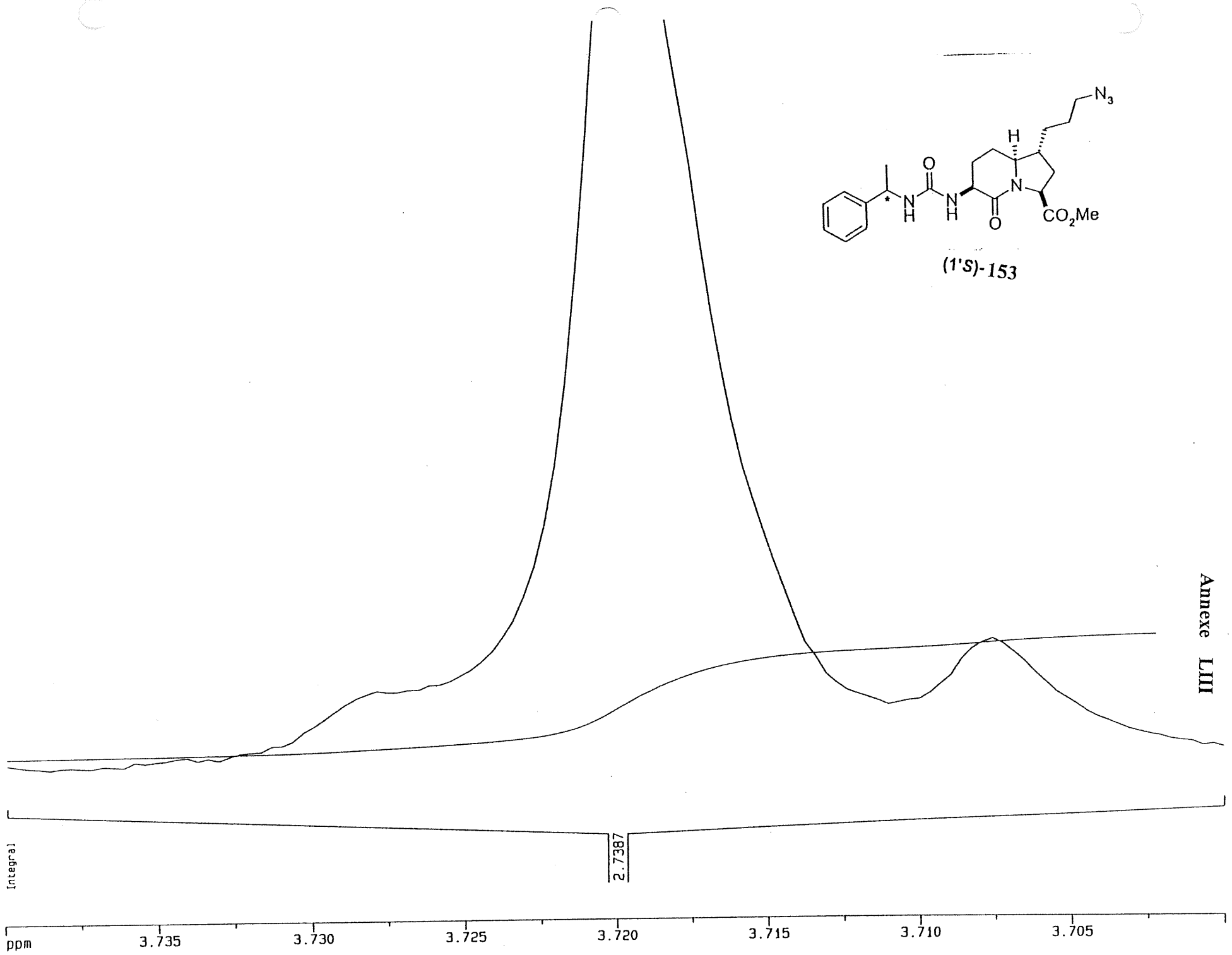

Ph
O
NH
OH
140
Integral
-0.061
23.911
0.889
1.886
-0.007
23.066
0.128
18.089
1.960
1.628
1.551
1.679
2.068
20.443
0.010
1.175
0.458
1.128
ppm
9
8
7
6
5
4
3
2
1
0

Annexe LI

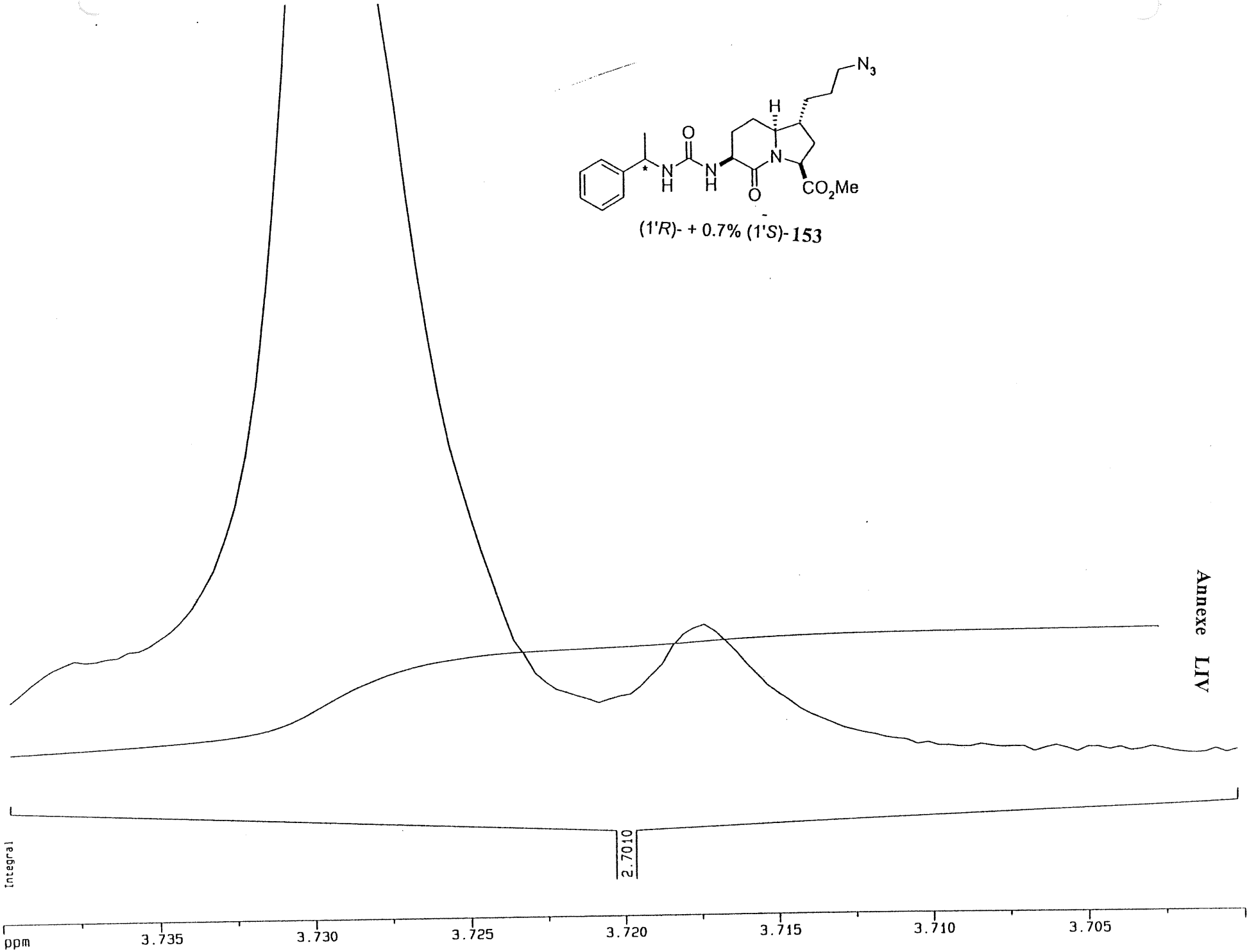

N3
H
O
N
H
N
H
O
N
CO2Me

(1'*R*)-153

Integral
2.6214
ppm
3.735
3.730
3.725
3.720
3.715
3.710
3.705


N3
H
O
N
H
N
H
O
N
CO2Me
(1'S)-153
Integral
2.7387
ppm
3.735
3.730
3.725
3.720
3.715
3.710
3.705

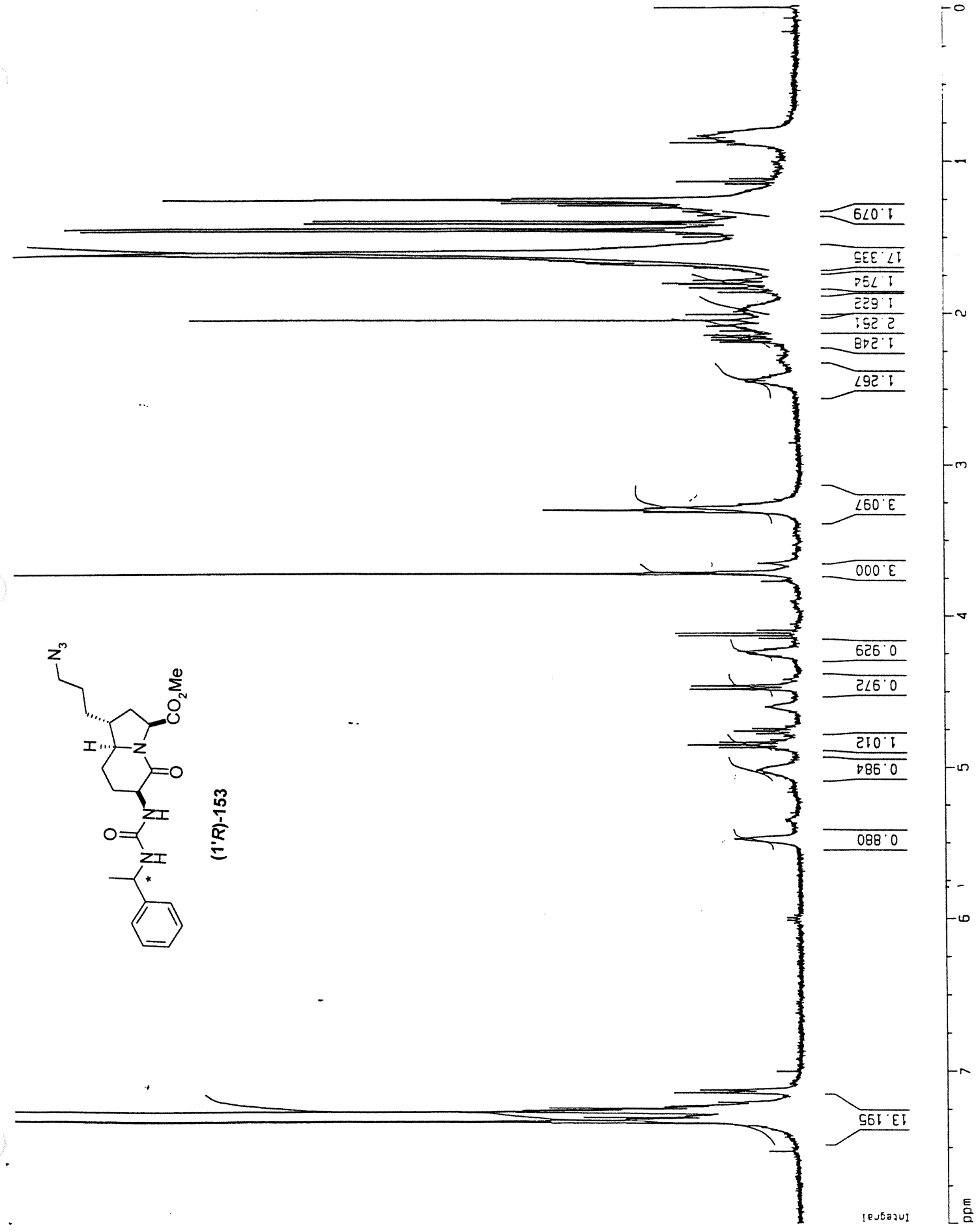
Annexe LIII



(1'*R*)- + 0.7% (1'*S*)-**153**

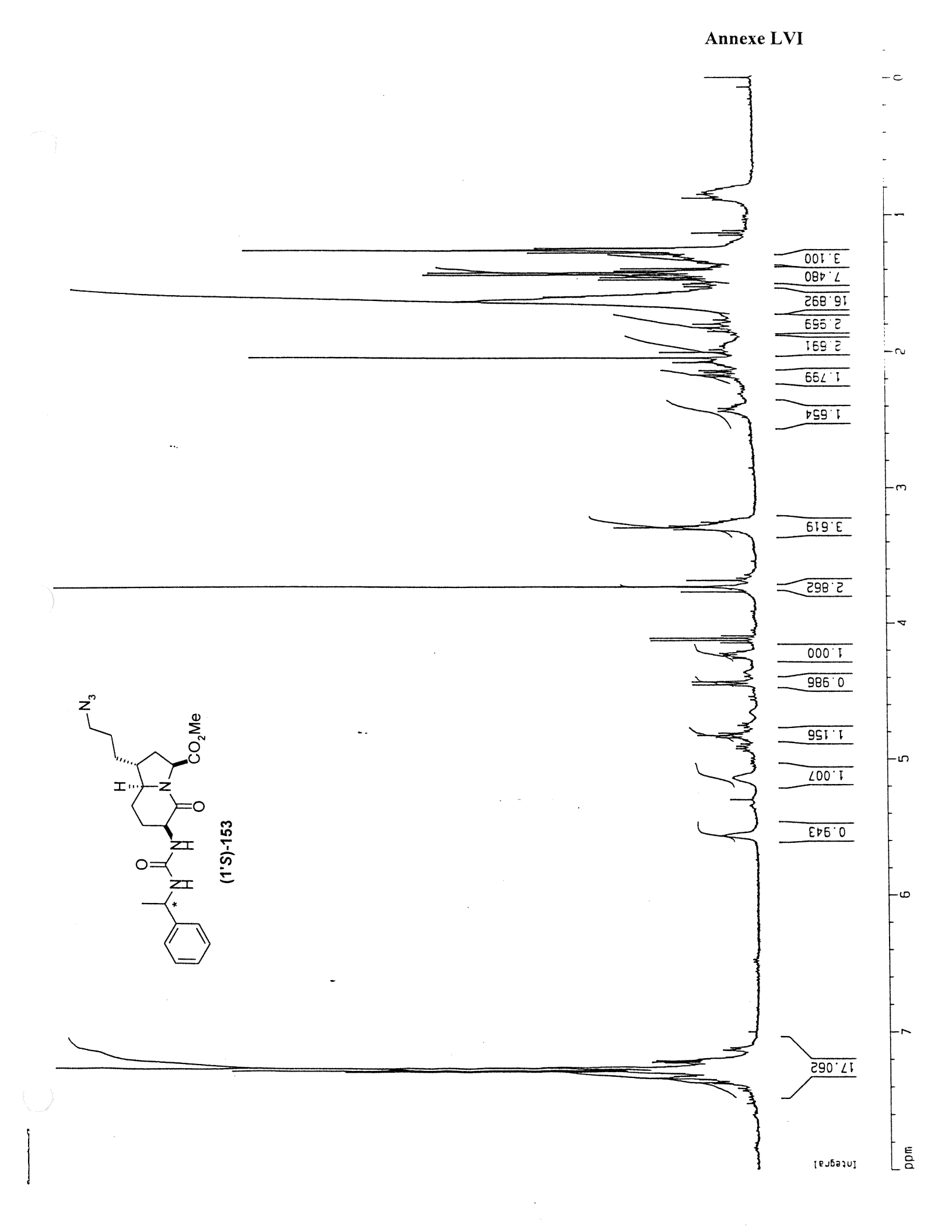
Annexe LIV

Annexe LV



(1'*R*)-153

Annexe LVI


(1'S)-153
Integral
ppm
17.062
0.943
1.007
1.156
0.986
1.000
2.862
3.619
1.654
1.799
2.691
2.569
16.892
7.480
3.100